PHYSICAL AND NUTRITIONAL QUALITY OF EXTRUDED HIGH-MOISTURE MEAT ANALOGUES MADE FROM SUNFLOWER MEAL AND PEA PROTEIN BLENDS

By

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ABSTRACT

Introduction: Incorporating protein-rich food industry by-products, such as sunflower meal (SFM), into foods aligns with United Nations Sustainable Development goals, as it offers environmental-friendly food alternatives for a secure food future. SFM is rich in protein and indispensable amino acids, making it a promising candidate for enhancing the nutritional quality of novel foods such as plant-based meat analogues. This study investigated the production of high-moisture meat analogues (HMMAs) using blends of expeller-pressed SFM and pea protein isolate (PPI).

Methods: Defatted SFM was produced using a laboratory-scale expeller press, which extracted oil from dehulled sunflower seeds at a controlled temperature of 70°C. The blends for extrusion contained 40% SFM-60% PPI and 50% SFM-50 % PPI to achieve a balanced amino acid composition and high protein quality. During extrusion, the cooling die temperature was maintained at 80-50°C, with a screw speed of 200 rpm and a feed rate of 0.5 kg/h (d.b.). The effects of feed moisture content (FMC) (48% and 58%, w.b.), extruder barrel temperature profile (60-80-115-125°C, 80-100-125-135°C, and 100-120-135-145°C), and SFM concentration (40% and 50%) on the physical (texture and color) and nutritional (protein quality and anti-nutritional factors) properties of the resulting HMMAs were examined.

Results: Generally, the HMMAs produced in this study had comparable protein quality to real meat, with 70-74% (d.b.) protein content and 86-89% *in-vitro* protein digestibility, while tryptophan was the first limiting amino acid. The texture and color analysis showed that the meat analogues were harder and darker than cooked chicken. The HMMAs became softer and lighter in color as FMC increased and extrusion temperature decreased. Interestingly, the color of the HMMAs produced at some extrusion conditions was unstable during the storage of 40 days, attributed to the oxidation of chlorogenic acid (CGA), an anti-nutritional factor, that can form colored compounds. Moreover, extrusion processing significantly ($p < 0.05$) reduced the levels of anti-nutritional compounds including phytic acids, trypsin inhibitors, and chlorogenic acid.

Conclusion: This research offered valuable insights into the potential of replacing PPI with SFM to produce HMMAs, demonstrating a sustainable way to add value to an underutilized by-product in the food industry. The understanding of the effect of extrusion conditions on the physical and nutritional properties of SFM-PPI blend-based meat analogues is important for the development of high-quality plant-based protein products.

Keywords: Sunflower meal, Meat analogues, High-moisture extrusion, Protein quality, Anti-nutritional factors.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAA – Aromatic Amino Acids

AACC – American Association of Cereal Chemists

AAS – Amino Acid Score

ANF – Anti-Nutritional Factor

AOAC – American Association of Analytical Chemists

CGA – Chlorogenic Acid

FAO – Food and Agriculture Organization

FMC – Feed Moisture Content

HIS – Histidine

HMMA – High-Moisture Meat Analogue

ILE – Isoleucine

IVPD – *in-vitro* Protein Digestibility

IVPDCAAS – *in-vitro* Protein Digestibility Corrected Amino Acid Score

LEU – Leucine

LYS – Lysine

PA – Phytic Acid

PPI – Pea Protein Isolate

SAA – Sulfur Amino Acids

SFM – Sunflower Meal

SME – Specific Mechanical Energy

THR – Threonine

TIA – Trypsin Inhibitor activity

TRP – Tryptophan

TPC – Total Phenolic Compounds

VAL – Valine

WHO – World Health Organization

CHAPTER 1: INRODUCTION

The global demand for sustainable and plant-based protein sources has driven the rapid growth of the plant-based meat alternatives market when issues surrounding the negative environment impact, human health, and animal welfare impacts of animal agriculture (Safdar et al., 2022). Meat alternatives are designed to mimic the texture, flavor, and appearance of conventional meat, as well as providing nutritional values to humans (Zhang et al., 2022). High-moisture extrusion is an advanced processing method used to produce texturized meat analogues with fibrous structures, also known as high-moisture meat analogues (HMMAs). HMMAs utilize various plant-based protein sources, including those from legumes, cereals, and novel ingredients (Kyriakopoulou et al., 2021). Because of the high protein and low fat content of plant-based protein-rich ingredients, meat analogues are believed to reduce the risk of chronic diseases (Safdar et al., 2022). However, it is challenging to achieve both the meat-like texture and high nutritional values when attempting to completely replace real meat (Barnés-Calle et al., 2024). Researchers are still innovating to formulate and process to replicate the sensory appeal and nutritional properties of real meat.

Pea and sunflower proteins are considered alternative protein sources to commonly used ingredients, i.e., soy and wheat proteins, for the production of HMMAs (Kyriakopoulou et al., 2021). Pea protein isolate-based meat analogues with similar texture to cooked chicken was obtained by Barnés-Calle et al. (2024). Pea protein has less antinutritional factors and lower allergenic potential when compared to those from soy and wheat (Osen et al., 2014). Sunflower meal (SFM) is the by-product of its oil production, which is identified as a potential protein source for HMMAs due to its similar functional properties to soy protein (Filho & Egea, 2021), while its application is limited mainly to bakery foods production (Wildermuth, Young, & Were, 2016; Liang & Were, 2018; Pepra-Ameyaw et al., 2023). Compared to other oilseeds meals, like rapeseeds and hemp seeds, SFM has less antinutritional factors, and higher phenolic compounds (Filho & Egea, 2021). However, phenolic compounds in SFM, such as chlorogenic acid, can bind with proteins, reduce the protein digestibility and form green color to lower the consumer acceptance of end-products (Pöri et al., 2023). Sunflower protein is rich in sulfur-containing amino acids, while pea protein provides lysine, making their combination nutritionally complementary (Öztürk et al., 2024).

The utilization of by-products can effectively reduce the waste, carbon footprint and resource requirements (Singh et al., 2024), contributing to a sustainable and circular economy of sunflower seeds. However, the presence of antinutritional factors (ANF) in plants typically results in plant-based foods having lower protein quality compared to meat (Nikmaram et al., 2017). Protein quality of foods is determined by their amino acid composition and protein digestibility (Sá et al., 2020). Among different methods, *in-vitro* protein quality assessment offers a fast and economical evaluation method (Krul et al., 2024). Previous studies have already proven that extrusion is effective in reducing ANFs, while simultaneously improving the *in-vitro* protein digestibility of plant proteins (Vidal et al., 2022). However, extrusion with high temperature may cause protein denaturation, altering protein structure and influencing their digestibility (Zhang et al., 2022). Some heat-sensitive amino acids, such as lysine, can also be destroyed under such conditions (Pismag et al., 2024). The impact of extrusion on protein quality largely depends on extrusion conditions, e.g., feed moisture, temperature, screw feed (Öztürk et al., 2024), while research on this area remains limited. To date, only a few studies have investigated the physical and nutritional quality of sunflower protein-based meat analogues produced with high-moisture extrusion. These studies include meat analogues made with soybean protein isolate and SFM (Singh et al., 2024), meat analogues made with sunflower protein concentrate and pea protein isolate (Öztürk et al., 2024), and meat analogues made with fermented sunflower protein (Pöri et al., 2023). Even so, there is no research on the effect of extrusion temperature on the physical and nutritional quality of SFM-based meat analogues. Additionally, the effect of high-moisture extrusion on ANFs in SFM-based meat analogues has not been investigated to date.

This study aimed to utilize SFM with pea protein isolate (PPI) in high-moisture extrusion to produce texturized meat analogues with high protein quality. The objectives were to evaluate the physical properties (texture profile, cutting strengths and color properties) and nutritional quality (focus on protein quality and antinutritional factors) of meat analogues made from SFM and PPI in two blending ratios under various extrusion temperatures and feed moisture contents.

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CHAPTER 2: LITERATURE REVIEW

2.1 Processing methods for the production of meat analogues

Meat analogues have gained popularity in recent years due to various reasons, including (1) contributing to the environmental sustainability by using less resources, (2) health concerns about traditional red meat-based diets, and (3) ethical considerations about animal welfare (Safdar et al., 2022). Currently, there are mainly 3 processing methods, shown in Table 2.1, being used to produce meat analogues, including microbial mycelium protein meat analogues, texturized plant protein meat analogues and *in-vitro* cultured meat (Zhang et al., 2022). Extrusion is an integrated technique with mixing, heating, melting and cooling actions for plant protein restructure and fibrous texture formation (Baig et al., 2024). When considering the cost and input of meat analogues production, texturized plant protein meat analogues appear to be the most ideal alternative for animal meat due to the easy access to the raw materials and the rich fibrous structures of the end-products (Zhang et al., 2022). In previous studies, it has been shown that meat extenders produced by low-moisture extrusion generally need further processing into emulsion type products, such as sausages, frankfurters, or ground meat products, such as burgers, patties, nuggets (Kyriakopoulou et al., 2021). Because texturized vegetable proteins cannot form coherent products alone, their structure is not exactly like real animal meat. However, high-moisture meat analogues (HMMAs) are characterized by the presence of a fibrous and layered structure, which is similar to chicken breast and have potential to mimic beef steak-like products if processing innovations allow for incorporation of fats into them (Kyriakopoulou et al., 2021). Raw materials used in high-moisture extrusion are usually defatted in advance, HMMAs are considered to have higher protein content, but lower fat content than animal meat. In previous research, many plant proteins, such as wheat gluten, potato, green bean, and rice proteins have been shown to form fibrous texture of end-products and enhance the nutritional properties by increasing the ratio of protein (Safdar et al., 2022). Currently, the use of high-moisture extrusion on these ingredients still needs more research and have potential to expand the meat analogues market (Baig et al., 2024).

Table 2.1 Comparison of three types of manufacturing methods for meat analogues

Processing Methods	**Microbial mycelium protein**	**Texturized plant protein meat analogues**		***In-vitro* cultured meat**
		Low-moisture extrusion (LME)	**High-moisture extrusion (HME)**	
Media	Media for microbial growth	Single or twin-screw extruder, moisture content: 20-40%	Twin-screw extruder, moisture content: 40-80%	Nutrient media
Raw Materials	Microbial mycelium	Plant flour, protein concentrates	Protein isolates, defatted plant meal	Animal cell
Final Product Features	Texture is soft, taste is similar to meat	A sponge-like structure is formed, but differs from animal meat	A meat-like fibrous texture is formed	Simulated raw animal meat
Technical Development	Mature	Mature	Immature	Immature
References	Zhang et al. (2022)	Lin et al. (2022); Kyriakopoulou et al. (2021)	Kyriakopoulou et al. (2021); Palanisamy et al. (2019)	Zhang et al. (2022)

2.2 Plant protein sources used for HMMAs

Animal meat is a good source of protein. Properly selecting plant-based proteins as alternatives to animal meat is critical for nutritional quality of HMMAs. Protein content, nutritional quality and functional properties of raw ingredients should be taken into consideration for HMMAs production (Kyriakopoulou et al., 2021). Some of the commonly used protein sources for the development of meat analogues are listed in Table 2.2.

Soybean

Soybean is one of the most widely used plant-based protein source for the production of plant-based meat-like products (e.g., tofu) (Benković et al., 2023). Soybean is a good protein source with high protein content and all nine essential amino acid. The protein digestibility corrected amino acid score (PDCAAS) of soybean flour and meal are both about 81%, and soy protein isolate can have a PDCAAS value as high as 87.2% (Vandenberg et al., 2022). Soy protein is widely used in high-moisture extruded meat analogues production because of good gelling and water holding properties that help form a fibrous structure like meat during extrusion (Kyriakopoulou et al., 2021). Soy protein showed better texture properties with a sponge-like structure compared to other protein sources when texturized through extrusion (Samard & Ryu, 2019). However, the use of soy protein is limited due to allergenicity concerns and its low content of sulfur amino acids compared to cereals (Samard & Ryu, 2019). Additionally, the use of soybean in food products can be controversial due to concerns about genetically modified organisms (Benković et al., 2023).

Wheat gluten

Wheat gluten is another frequently used protein source in both low- and high-moisture extrusion to produce plant-based meat alternatives (Kyriakopoulou et al., 2021). Gluten protein, with its viscoelastic properties can form a 3D network through disulfide bonds, helping form fibrous structures in HMMAs (Kurek et al., 2022). However, the use of wheat proteins may require consideration of protein quality. The density of fibrous structures can affect the protein digestibility of the texturized wheat protein-based meat analogues by lowering the degree of proteolysis in digestion (Lin et al., 2022). Complementary proteins to wheat gluten, such as those of legumes, are usually needed to balance the amino acid profile of the end-products due to wheat being deficient in lysine (Kurek et al., 2022). Besides, gluten intolerance is another issue for the application of wheat proteins in HMMAs (Benković et al., 2023).

Legumes

Apart from soybean, other legumes, such as pea (Osen et al., 2014), lentil (Guo et al., 2024), lupin (Palanisamy et al., 2019), have been proven feasible to produce meat analogues using high-moisture extrusion. Compared to soy protein, their gelling ability is relatively lower, leading to difficulties in texturization (Benković et al., 2023). However, legume proteins are rich in lysine, which help improve the amino acid balance in the end-products when mixed with other protein sources, especially those from cereals (Kurek et al., 2022).

Pea protein is an alternative ingredient to soy protein, which has gained interest due to its nutritional value and low allergic potential (Osen et al., 2014). Although the texturized structure formed by pea protein is weaker than soy protein (Kyriakopoulou et al., 2021), pea protein-based meat analogues have comparable texture and sensory properties to cooked chicken breast (Barnés-Calle et al., 2024).

Legumes are promising protein ingredients for HMMAs production, but the anti-nutritional factors (ANF), such as phytic acid, trypsin inhibitors and tannin present in legumes, can reduce the nutritional quality of the end-products made from those ingredients (Rathod & Annapure, 2016). Extrusion cooking can effectively reduce the levels of antinutritional compounds, while the extent of reduction depends largely on extrusion parameters (Kaur et al., 2015).

Oilseeds

Novel protein sources from oilseeds are also considered potential ingredients for meat analogues production because of their functional properties (Kurek et al., 2022). Flaxseed protein concentrate (Riazi et al., 2023), hemp protein concentrate (Zahari et al., 2020) and rapeseed protein concentrate (Zahari et al., 2021) have been incorporated with other protein sources for the development of plant-based meat analogues. However, the presence of antinutritional factors in oilseeds limit their food applications (Kyriakopoulou et al., 2021).

After oil extraction, the remaining part of the oilseeds, i.e., cakes and meals as oil extraction by-products, is rich in protein. For example, dehulled sunflower meal can have up to 44% protein content (González-Pérez, 2015). Oil extraction by-products are mainly used for animal feed but have become increasingly popular for their potential in human diet due to their high protein content and low cost (Nevara et al., 2022). However, the presence of phenolic compounds is the major challenge for oilseed meals or cakes, negatively affecting the taste and appearance of the end-products made with sunflower seed protein (Benković et al., 2023). Additionally, to reduce the levels of ANF that negatively affect sunflower seed nutritional quality, pre-treatments, such as ethanol washing, fermentation and enzymatic treatment, are needed (Jia et al., 2022). Given that low-moisture extrusion can effectively remove phenolic compounds and improve the quality of sunflower protein (Vidal et al., 2022), extrusion at high moisture levels to produce HMMAs may provide a highly efficient and low-cost method for oilseeds proteins in meat analogue applications.

Table 2.2 Common plant-based ingredients for meat analogues

Plant-based Protein Sources	Protein Ingredients	References
Soybean	Soy isolate, soy concentrate, soy flour, defatted soy meal	Vandenberg et al. (2022); Kyriakopoulou et al. (2021)
Wheat	Wheat gluten	Lin et al. (2022)
Legumes (other than soybean)	Legume proteins from pea, lentil, lupin, chickpea, faba bean, mung bean and other types of beans	Palanisamy et al. (2019)
Oilseeds	Hemp, rapeseed/canola, flaxseed, sunflower, peanut	Kyriakopoulou et al. (2021)
Other plant proteins	Algae, Potato, Zein	Zahari et al. (2020); Kyriakopoulou et al. (2021)

2.3 Considerations of novel plant proteins in HMMAs production

With the development of novel protein extraction techniques, the study of novel plant protein sources for HMMAs production have been a topic for researchers (Benković et al., 2023). While the study of traditional proteins, i.e., soy proteins, provides a foundation of HMMAs production, current research focuses on incorporating novel ingredients to improve physical properties, enhance nutritional values (Palanisamy et al., 2019), as well as produce desired taste for better consumer acceptances (Benković et al., 2023). One critical aspect influencing the quality of

HMMAs is the formulation, as the selection and mixing ratios of different protein sources affect texture, nutritional value and sensory properties (Zahari et al., 2022). When selecting plant protein sources for extrusion, the proteins should have good amino acid profiles, good function-technological properties, neutral taste and low allergenicity, such as sunflower protein (Filho & Egea, 2021; Benković et al., 2023), as discussed in Section 2.4.

Furthermore, extrusion processing parameters, e.g., feed moisture content, temperature, and screw speed, can also affect the overall quality of the final meat analogues (Palanisamy et al., 2019). Properly controlling those parameters and investigating the effects of process parameters can help optimize the quality of the final products and avoid undesirable properties (Schmid et al., 2022), as detailed in Section 2.5.1.

Regarding the nutritional value, animal proteins have high protein digestibility and excellent amino acid profile (Azizi et al., 2024), while plant-based meat analogues usually have lower protein digestibility than animal meat because the presence of ANF affecting the protein digestion and absorption (Xie et al., 2022; Baig et al., 2024). The quality of the HMMAs largely depends on the quality of the plant protein sources (Cutroneo et al., 2023). Additionally, extrusion conditions may affect protein digestibility of the HMMAs by forming different protein microstructures (Azizi et al., 2024). The effects of extrusion conditions on the protein quality and ANF are explored in Section 2.5.2 and 2.5.3, respectively.

2.4 The potential of sunflower meal in meat analogues production

2.4.1 The value of sunflower meal

Sunflower seeds are mainly used for oil production, which accounts for ~10% of the global plant-derived oil production (Filho & Egea, 2021). With the increasing demand for oil extracted from seeds, many environmental, health and economic issues are caused by the disposal of its by-product, i.e., cake or meal (Nevara et al., 2022). However, these by-products of oil production are low-cost, but valuable materials (Pedroche, 2015). Because most oil is removed during oil extraction, the proportion of protein in the by-product is increased. The protein content of sunflower meal (SFM) can range from 40% to 66%, depending on the treatment during oil extraction. If the sunflower seeds are dehulled before oil extraction, the protein content can be further increased as dietary fiber is removed during dehulling (González-Pérez, 2015). Currently, SFM is typically used for livestock feeding as a nitrogen source, but it has the potential for use in food products as well.

In addition to its high protein content, SFM has all essential amino acids. However, lysine is the first limiting amino acid in SFM, which is also limiting in most cereal proteins (Suloma et al., 2022). Therefore, SFM is usually utilized with legume flours or proteins to achieve a balanced amino acid composition. In previous studies, SFM was used as a partial replacement for soybean meal to improve the protein digestibility of animal feeds (Suloma et al., 2022). Furthermore, the addition of SFM into corn flour improved the nutritional value of ready-to-eat breakfast cereals by increasing the overall amino acid score of the end-product, while lysine was still the first limiting amino acid (Filipović et al., 2017).

2.4.2 Sunflower meal in HMMAs production

Based on the functional and nutritional qualities of SFM, they have great potential in development of novel foods which can increase their values (Filho & Egea, 2021). Current food applications of SFM include bakery, beverage, energy bars, pasta and plant-based meat products (Liang & Were, 2018; Pepra-Ameyaw et al., 2023; Nevara et al., 2022). Several studies have been completed to produce plant-based meat products with oilseed-based protein sources. Hemp protein concentrates, hemp flour, chia seed meals and poppy seed meals were successfully applied in burger patties, chopped meat or texturized meat productions (Nevara et al., 2022). Among oilseed protein sources, SFM also has great potential to be incorporated into HMMAs production because of its high protein content and low cost, while the research is limited. Additionally, SFM protein has good functional properties, such as emulsification, water holding and gelation, which make it well-suited for use in HMMAs production. For example, sunflower protein concentrate has moderate water-holding capacity value similar to commercial soy protein isolate and can be used as a thickening agent (Kyriakopoulou et al., 2021). Sunflower protein concentrate successfully formed fibrous structures when texturized using shear cell technology (Jia et al., 2022), proving the potential of SFM in HMMA production. Furthermore, previous study investigated the production of HMMAs using blends of SFM and soy protein isolate, evaluating the effect of feed moisture content during high-moisture extrusion on the physical and protein qualities (Singh et al., 2024). High-moisture extrusion can improve the protein digestibility of HMMAs produced from sunflower protein concentrate and pea protein isolate, achieving levels comparable to beef (Öztürk et al., 2024).

2.4.3 Challenges associated with sunflower meal in HMMA formulations

Despite the potential of SFM as a protein source for food applications, several challenges limit its practical utilization. One of the primary limitations of SFM in food formulations is the presence of ANF, such as phytic acid, trypsin inhibitors, and phenolic compounds, which can negatively impact nutrient bioavailability and protein digestibility (Nevara et al., 2022). In Canada, the predominant commercial oil extraction method is cold pressing, which is beneficial for retaining most nutrients (House et al., 2010). However, the processing temperature of oil extraction is insufficient for effectively reducing ANFs. Phytic acid can bind to minerals and proteins to form a complex with low solubility, reducing mineral absorption and protein digestibility (Vidal et al., 2022). Phenolic compounds can also bind with proteins, reducing protein digestibility (Pedroche, 2015). Trypsin inhibitors reduce the protein digestibility by affecting the enzymatic activity (Pismag et al., 2024).

Soaking, germination and fermentation are some common pretreatment methods used to remove ANF from plants (Abd El-Hady & Habiba, 2003). However, thermal processing methods, such as broiling, microwaving, autoclaving, and extrusion, are proven to be more effective in removing those compounds (Nevara et al., 2022). For example, extrusion with 17% feed moisture content was used to enhance the nutritional value of cold-pressed sunflower cake by decreasing the free phenolic compound content by 68% (Vidal et al., 2022). However, the effect of high-moisture extrusion on ANFs in SFM is still unknown.

Another challenge in producing high-quality HMMAs with SFM is the formation of undesired color and flavors in the final product. The interaction between proteins and phenolic compounds can cause dark color, which is usually green or brown (González-Pérez, 2015). For example, green color was observed for sunflower protein-based bakery foods due to the reaction of chlorogenic acid and proteins (Wildermuth et al., 2016). Additionally, chlorogenic acid was reported to be converted to bitter compounds for coffee beans (Jiang & Peterson, 2010), which could also be a concern for SFM in HMMAs application. Being rich in chlorogenic acid, development of HMMAs using SFM is still challenging in terms of consumer acceptance.

2.5 The effect of high-moisture extrusion on the final quality of HMMAs

2.5.1 Effect of high-moisture extrusion on the fibrous structure formation

High-moisture extrusion is an efficient technique for texturization of plant proteins for meat analogues production. During this process, various physicochemical reactions occur, including denaturation and aggregation of proteins, gelatinization and degradation of starch, lipid oxidation, and degradation of antinutrients (Zhang et al., 2022). The subjection of proteins to high temperature and shear force leads to the proteins unfolding and reaggregation, contributing to the fibrous structure formation of meat analogues (Baig et al., 2024). Controlling extrusion conditions is important for those chemical reactions and structure formation, which can have a significant dictate on the physical quality of final products (Sui et al., 2024).

Extrusion temperature and feed moisture content are two of the primary parameters that affect the texturization and physical quality (Schmid et al., 2022). Water performs an important role in extrusion by reducing the viscosity of melted materials and serving as a lubricant and plasticizer (Chen, Wei & Zhang, 2011). The feed moisture content during extrusion significantly influences the texture of meat analogues. For example, an increase in feed moisture content from 26% to 32% increased the hardness, springiness and degree of texturization of wheat protein-based meat analogues (Lin et al., 2022). HMMAs are produced at feed moisture contents between 40% and 70%, which allows protein cross-linking and contributes to the formation of a fibrous and layered structure that mimics that of meat, e.g., chicken breast (Schmid et al., 2022). An increase in feed moisture content can reduce cross-linking, leading to a softer texture and decreased hardness in HMMAs (Palanisamy et al., 2019; Lin, Huff, & Hsieh, 2000).

Furthermore, extrusion temperature is crucial for protein unfolding and denaturation, affecting the formation of fibrous structures (Wittek et al., 2021). For example, the fibrous structure was formed by high-moisture extrusion at temperature of 160°C for HMMAs made by wheat protein, rice protein and pea protein isolates, while this structure was not shown at extrusion temperature of 95°C (Nisov et al., 2022). Increasing extrusion temperature from 135°C to 155°C was reported to increase the level of proteins cross-linking, leading to better fibrous structure formation (Palanisamy et al., 2019). However, temperature's effect on protein texturization differs with respect to protein type. Previous research reported that the optimal extrusion temperature for peanut protein texturization was 140°C, while for soybean protein it was about 150°C (Zhang et al., 2022).

2.5.2 Effect of extrusion conditions on the protein quality

Protein quality refers to the relative contribution of amino acids to an individual's requirement and the digestibility of the protein for human growth, maintenance, and repair of tissues (House et al., 2010). Most meat analogues have a protein content comparable to that of traditional meat products, although their nutritional values and digestibility may not be as good as those of real meats (Schmid et al., 2022). High-moisture extrusion involves high temperature processing conditions, affecting the protein quality of meat analogues. Increasing the temperature up to 180°C during extrusion of soybean and SFM can cause a significant loss of lysine content because of the Maillard reaction (Suloma et al., 2022). The loss of lysine content may discourage the use of cereal and oilseed proteins in extrusion because lysine is the first limiting amino acid in these protein sources (González-Pérez, 2015). However, the loss of lysine during high-moisture extrusion was rarely reported because proteins is exposed to high temperature for a short time that the loss of amino acid is not significant (Schmid et al., 2022).

Apart from the loss of amino acids, protein digestibility of plant proteins, e.g., faba bean, navy bean and pinto, can be improved by extrusion processing when comparing with cooking and baking (Nosworthy et al., 2018). The temperature during extrusion typically exceeds protein denaturation temperature, which can change the native protein structure and make the protein more easily digested by digestive enzymes (Ohanenye et al., 2022). However, excessively high extrusion temperature (above 160°C) can decrease the protein digestibility for meat analogues made from soybean protein (Fu et al., 2024). The decrease of protein digestibility may be attributed to thermal cross-linking and increased protein aggregation (Palanisamy et al., 2019). Therefore, optimizing extrusion conditions is crucial to achieve optimal protein digestibility for HMMAs.

2.5.3 Effect of extrusion conditions on anti-nutritional factors

ANF are naturally presenting compounds that can impair nutrient absorption, digestibility, and utilization, leading to a reduction in the bioavailability of essential nutrients (Nikmaram et al., 2017). Conventional thermal techniques, e.g., cooking, baking and boiling, can reduce the levels of ANF, but they may also lead to the loss of lysine, tryptophan and sulfur amino acids (Baig et al., 2024). Compared with those treatments, extrusion is more efficient in reducing ANF and improving protein quality with low energy use and cost (Alonso, Orue & Marzo, 1998). It is reported that extrusion can reduce tannin content and trypsin inhibitor activity while improving *in-vitro* digestibility for legume seed proteins (Ohanenye et al., 2022).

For SFM, the major ANFs are phytic acid and phenolic compounds (González-Pérez, 2015). Extrusion (temperature of 130°C and 17% feed moisture content) can significantly reduce the levels of trypsin inhibitor and phenolic compounds by thermal degradation (Vidal et al., 2022). The inactivation of ANF through low-moisture extrusion also applied to cereals (Kaur et al., 2015), and legumes (Alonso, Orue and Marzo, 1998). A higher temperature, about 180°C, was more effective in reducing phytic acid than the lower temperature (140°C) (Abd El-Hady & Habiba, 2003), but there is a risk of amino acids loss. However, the high-water level for high-moisture extrusion may lead to milder conditions for ANF that the efficiency of ANF deactivation can be lower than low-moisture extrusion (Schmid et al., 2022). It should be noted that the effect of high-moisture extrusion conditions on ANFs in HMMAs is still not sufficiently investigated.

2.6 Evaluation methods of protein quality for meat analogues

Protein is an essential component of the human diet. During extrusion, protein content is not changed too much as reported in previous studies (Vidal et al., 2022; Singh et al., 2024; Zahari et al., 2021). However, the protein quality may be affected by extrusion conditions. There are several measures of protein quality, including the protein digestibility-corrected amino acid score (PDCAAS), the biological value (BV), and the protein efficiency ratio (PER). In North America, PDCAAS is a widely accepted method for protein quality evaluation that takes into account both the amino acid score (AAS) and digestibility of a protein (Singh et al., 2024; Boye, Wijesinha-Bettoni & Burlingame, 2012). However, the official methods used in Canada is PER (Sá et al., 2024).

The AAS is determined by the first limiting amino acid (House et al., 2010), and calculated based on the requirement for specific groups of people (FAO/WHO, 1991). Extrusion's impact on the amino acid composition of different protein sources depends on extrusion conditions. For example, extrusion at 129°C can cause a decrease in valine, phenylalanine and lysine contents and 142°C can cause a decrease in tryptophan content (Boye, Wijesinha-Bettoni & Burlingame, 2012). Given that, lysine is limited in wheat and sunflower proteins, whereas tryptophan is limited in some legume proteins, the AAS can reflect the sensitivity of specific amino acids to extrusion conditions, providing a method for evaluating protein quality in HMMAs.

Protein digestibility can be determined by *in-vitro* or *in-vivo* methods. *In-vitro* evaluation methods have been confirmed having strong correlation with the results obtained from *in-vivo* methods (Nosworthy et al., 2018). Due to its advantages of fast and low-cost analyses, *in-vitro* protein digestibility (IVPD) methods are widely performed (Singh et al., 2024). The value of PDCAAS is crucial for HMMAS to simulate nutritional quality of animal meats and compare with other plant-based meat analogues (Azizi et al., 2024).

2.7 Existing studies on the utilization of sunflower meal in extrusion applications

Extrusion technology can utilize undervalued by-products, such as SFM, incorporating them into HMMAs production to enhance both economic and nutritional value (Alam et al., 2016). An early study tested the texture and sensory properties of texturized meat extenders made with defatted SFM, which reported that the partially replacement of beef with sunflower meat extender did not result in significant differences in sensory quality compared with beef patties (Rossi, 1988). However, the protein quality was not evaluated in this study. The texturized sunflower protein was produced by low-moisture extrusion with 16% feed moisture content and extrusion temperature of 149°C (Bhise et al., 2015). Sunflower protein has good functional properties, which has potential for meat analogues application (Jia et al., 2022).

In recent years, oilseed proteins have been widely used in the production of HMMAs, including rapeseed protein concentrate (Jia et al., 2021), hemp protein concentrate (Zahari et al., 2020; Nasrollahzadeh et al., 2022). This has also led to an increase of attention for sunflower proteins. The texture and sensory properties of HMMAs made from fermented sunflower protein were tested by Pöri et al. (2023) and the protein quality of which was evaluated by Öztürk et al. (2024). However, they did not investigate the effect of extrusion conditions on the physical, nutritional and sensory quality of SFM-based HMMAs. Singh et al. (2024) tested the effect of extrusion feed moisture on the physical and protein quality of the meat analogues made by SFM and soy protein. The effects of temperature and the response of ANF to extrusion conditions remain unknown.

2.8 Conclusion

SFM is a low-cost protein source for meat analogues production. The utilization of SFM in extrusion can increase its nutritional value, and also contribute to a sustainable economy. Though the protein quality of SFM is not good due to the presence of ANF, extrusion is promising processing technology to reduce its ANF and improve its protein quality. However, the effect of extrusion on protein quality is not universal and depends on several factors such as extrusion conditions and the type of protein used. Besides, the investigation of the effect of high-moisture extrusion on ANF is still limited. Sunflower protein has been applied in the development of HMMAs, but further research is needed to optimize extrusion conditions and improve the physical, nutritional and sensory quality of the end-products.

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CHAPTER 3: HYPOTHESES & OBJECTIVES

Hypotheses

1) Extrusion cooking of sunflower meal and pea protein isolate can produce texturized meat analogues with fibrous structures that mimic the texture of animal meats.
2) The effects of extrusion temperature profile, feed moisture content and sunflower meal concentration in the formula during high-moisture extrusion can influence physical properties, including texture profile, cutting strengths and color.
3) High-moisture extrusion can effectively reduce the level of antinutritional factors, resulting in better physical properties and protein quality of the meat analogues.

Objectives

1) To produce texturized meat analogues made with sunflower meal and pea protein isolate by high-moisture extrusion under various temperature profiles and feed moisture contents.
2) To evaluate the effect of extrusion temperature profile, feed moisture and sunflower meal concentration on the physical properties (texture profile, cutting strength and color), protein quality (amino acid scores, *in-vitro* protein digestibility and *in-vitro* protein digestibility corrected amino acid scores) and antinutritional factors (total phenolic compounds, chlorogenic acid, phytic acid and trypsin inhibitor activity).

CHAPTER 4: MATERIALS AND METHODS

4.1 Materials

Dehulled sunflower seeds were purchased from Sunny Day Products Ltd. (Winkler, MB, Canada). A laboratory French oil screw press (L-250, French Oil Mill Machinery Company, OH, USA) with a steam mixer (temperature set at 70°C) was used to generate defatted sunflower meal. Defatted sunflower meal was then ground into fine particles (≤0.75 mm) for further processing and analysis using a Retsch SR200 mill (Retsch GmbH, Haan, Germany).

Pea protein isolate (NUTRALYS® F85M) was obtained from Roquette Ltd. (Portage la Prairie, MB, Canada). Both defatted sunflower meal (SFM) and pea protein isolate (PPI) were tested for dry matter, crude protein content (N × 6.25) and crude fat content using AACC International methods 44-19.01, 46-13.01 and 30-25.01, respectively (AACC, 1999). The dry matter and crude protein content were analyzed in triplicate, while crude fat content was analyzed in duplicate.

For phytochemical analyses, Folin-Ciocalteu phenol reagent (FCR), gallic acid, chlorogenic acid standard (CGA), phytic acid sodium salt hydrate (PA; 93% phosphorus, d.b.), ammonium iron (III) sulphate dodecahydrate, 2,2'-bipyridyl, thioglycolic acid, acetic acid, calcium chloride dehydrate, bovine trypsin (≥ 10,000 benzoyl-L-arginine ethyl ester (BAEE) units/mg protein), tris hydroxymethyl aminomethane, benzoyl-DL-arginine-p-nitroanalide hydrochloride (DL-BAPA), dimethyl sulfoxide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

4.2 High-moisture extrusion processing

SFM was blended with PPI in two formulations: 50%:50% and 40%:60% (SFM:PPI, w/w w.b.). A laboratory scale, twin-screw, co-rotating extruder (model MPF-19, APV Baker Ltd., Peterborough, UK) was used to produce high-moisture meat analogues (HMMAs) with two different feed moisture contents (FMC): 48% and 58% (w.b.). The blended dry ingredients were fed into extruder barrel at a fixed flow rate of 0.5 kg/h (d.b.), and screw speed was constant at 200 rpm. The screw profile was set following Singh et al. (2024).

Three different temperature profiles in the four temperature-controlled zones of the extruder barrel were used: (a) 60 °C, 80 °C, 115 °C, 125 °C; (b) 80 °C, 100 °C, 125 °C, 135 °C; and (c) 100 °C, 120 °C, 135 °C, 145 °C, respectively, from feeder to the die. The extruder barrel was fitted with a long cooling die (inside dimensions: 300×50×5 mm) attached to the end of the extruder barrel and the two temperature-controlled zones were set at 80 °C (close to the barrel) and 50 °C (far from the barrel).

The torque and die pressure were recorded in quintuplicate and specific mechanical energy (SME) was calculated based on the equation 1 where the max screw speed and motor power were 500 rpm and 2.2 kW, respectively, following Luo et al. (2020):

$$\text{SME (kWh/kg)} = \frac{\text{Actual screw speed (rpm)}}{\text{Max screw speed (rpm)}} \times \frac{\text{Torque (\%)}}{100} \times \frac{\text{Motor power (kW)}}{\text{Total mass flow rate (kg/h)}} \quad (1)$$

The blended ingredients were extruded at different FMC and temperature profiles in triplicate. HMMAs were collected as 10 cm long strips and stored in sealed plastic bags at 4 °C for physical properties analyses, and at -18 °C for other analyses. The frozen samples were then freeze-dried (Genesis XL-70, Virtis, Warminster, PA, USA) and ground (ZM200, Retsch, Haan, Germany) to particle size lower than 0.75 mm for protein quality analyses.

4.3 Physical property analysis

The physical properties of the HMMAs were evaluated 18 h after extrusion processing. The HMMAs were equilibrated back to room temperature and cut into 20×20 mm (5 mm thickness pieces) before the analysis.

4.3.1 Texture profile analysis

Texture attributes (hardness, springiness, gumminess and chewiness) of the HMMAs were measured using a texture analyzer (TA-XT-plus, Stable Micro Systems, Godalming, UK) equipped with a 38-mm cylindrical probe using a 30-kg load cell, following the method reported by Ramos Diaz et al. (2022). The HMMAs were cut into dimensions of 20×20 mm pieces before analysis (n = 6). The hardness was expressed as the peak force (N) in the first compression. Springiness, gumminess (N) and chewiness (N) were calculated based on equations 2,3 and 4, respectively, using the force vs. time graph obtained during the test:

$$\text{Springiness} = \frac{\text{Time to reach the peak force during the second compression}}{\text{Time to reach the peak force during the first compression}} \tag{2}$$

$$\text{Gumminess} = \frac{\text{Area during the second compression}}{\text{Area during the first compression}} \times \text{Hardness} \tag{3}$$

$$\text{Chewiness} = \text{Gumminess} \times \text{Springiness} \tag{4}$$

4.3.2 Cutting strengths and degree of texturization

A texture analyzer (TA-XT-plus, Stable Micro Systems, Godalming, UK) with a cutting probe (A/ECB), fitted with a 30-kg load cell was used to measure the longitudinal and transverse cutting force (n = 6 for each direction), following Singh et al. (2024). The HMMA pieces were cut to 75% of their original thickness. The peak positive force in either direction was determined as longitudinal cutting force (F_L, parallel to the melt flow direction in the barrel) or transverse cutting force (F_T, perpendicular to the melt flow direction in the barrel). The degree of texturization was calculated as the ratio of F_L to F_T.

4.3.3 Color properties

The color properties of the HMMAs were analyzed (n = 6) 1 day and 40 days after extrusion processing to evaluate the immediate and long-term effects during storage, using a color spectrophotometer (CM-3500d, Minolta, Osaka, Japan). Lightness-darkness (L*), greenness-redness (a*), and blueness-yellowness (b*) were measured, and raw blended ingredients were used as reference. The total color change (ΔE) was calculated based on equation 5:

$$\Delta E = \sqrt{(L_m - L_r)^2 + (a_m - a_r)^2 + (b_m - b_r)^2} \quad (5)$$

where L_m, a_m, b_m refer to the color attributes of the HMMAs, and L_r, a_r, b_r refer to the color attributes of the raw blended ingredients.

4.4 Protein quality analysis

4.4.1 Protein content and amino acid composition

The protein content of the HMMAs were determined in duplicate by a rapid N exceed® nitrogen and protein analyzer (Elementar, Langenselbold, Germany) from Richardson Centre for Food Technology and Research (Winnipeg, MB, Canada) using the AACC International method 46-13.01 (AACC, 1999), with 6.25 as conversion factor.

The regular amino acid contents of the raw materials and the HMMAs were determined by acid hydrolysis according to the AOAC method 982.30. Sulfur amino acids, methionine and cysteine, were determined by the performic acid oxidized hydrolysis procedure according to the

AOAC method 985.28 (AOAC International, 2012). The chromatography analysis was performed by a Shimadzu Nexera ultra-high performance liquid chromatography (UHPLC) system (Kyoto, Japan) equipped with a Waters Acc-Q C18 column (100 mm×2.1 mm, 1.7 µm). The column temperature was 51 ºC for regular amino acids and 40 ºC and 60 ºC for cysteine and methionine, respectively. The derivatives were measured by UV detection at 260 nm for regular amino acids, and by fluorescence with excitation at 266 nm and emission at 473 nm for sulfur amino acids.

For tryptophan, samples were hydrolyzed with barium hydroxide in an autoclave (110 ºC, 20 h, 1.5 times atmospheric pressure) following the ISO 13904:2005 method (ISO, 2005). The samples were injected onto a Phenomenex Luna C18 column (250 mm×4.6 mm, 3 µm) with a flow rate of 1 mL/min for chromatography analysis. The detection was tested by fluorescence with an excitation wavelength of 280 nm and an emission wavelength of 356 nm. Lab Solutions software (Shimadzu, Kyoto, Japan) was used to process the chromatographs and raw data from the UHPLC.

4.4.2 *In-vitro* protein digestibility

The static enzymatic pH drop method was used to evaluate the *in-vitro* protein digestibility (IVPD) according to Hsu, Vavak, Satterlee, & Miller (1977), with minor modifications (Tinus, Damour, van Riel, & Sopade, 2012). The protein from each HMMA was weighed to 62.5 ± 1 mg, mixed with 10 mL of Milli-Q water, and heated to 37 ºC, in triplicate (n = 3). The initial pH (pH_i) of sample solutions was adjusted to 8.0 using 0.1 N NaOH or 0.1 M HCl. A multienzyme solution containing 3.1 mg/mL chymotrypsin (from bovine pancreas, Type II, lyophilized powder, ≥ 40 units/mg protein), 1.6 mg/mL trypsin (from porcine pancreas, Type IX-S, lyophilized powder, 13,000-20,000 BAEE units/mg protein) and 1.3 mg/mL protease (from Streptomyces griseus, Type XIV, ≥3.5 units/mg solids) was heated to 37 ºC and adjusted to pH 8.0. After 1 h stabilization, 1 mL multienzyme solution was transferred to the sample solution. The final pH (pH_f) of the mixture was measured after 10 min. The IVPD of HMMAs was calculated based on the pH variation (ΔpH), as shown in equations 6 and 7:

$$\Delta pH = pH_f - pH_i \quad (6)$$

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH \quad (7)$$

4.4.3 *In-vitro* protein digestibility-corrected amino acid score

The amino acid score (AAS) was calculated based on the reference pattern for children aged from 2 to 5 (FAO/WHO, 1991), as shown in equation 8:

$$AAS = \frac{\text{mg of amino acid per gram of test protein}}{\text{mg of amino acid in reference pattern}} \times 100 \quad (8)$$

The essential amino acid with the lowest AAS was identified as the first limiting amino acid for HMMAs and was used to calculate the *in-vitro* protein digestibility-corrected amino acid score (IV-PDCAAS), following equation 9:

$$IV\text{-}PDCAAS\ (\%) = IVPD\% \times AAS \quad (9)$$

4.5 Phytochemical analyses

4.5.1 Total phenolic compounds content

The total phenolic compounds (TPC) content of HMMAs was analyzed calorimetrically according to Konieczny et al. (2020). The samples were extracted using 1% HCl in methanol (w:v = 1 g:5 mL) for three times. The supernatant after mixing (40 rpm, 2 h) and centrifugation (1050

× g, 10 min) was collected and diluted with 15 mL 1% HCl in methanol. 0.125 mL of final extract solution, in triplicate (n = 3), and transferred to a hydrolysis tube, mixed with 1.5 mL Milli-Q water and oxidized with 0.125 mL Folin-Ciocalteu reagent. After 5 min, 1.25 mL 7% Na_2CO_3 was added to neutralize the solutions. The mixture was incubated for 100 min and absorbance was measured at 750 nm by a UV/visible spectrophotometer (Evolution One, Thermo Fisher Scientific, USA). The results were expressed as mg gallic acid equivalents (GAE) per g of sample.

4.5.2 Chlorogenic acid

The measurement of chlorogenic acid (CGA) followed the method reported by Pedrosa et al. (2000), with modifications (Pepra-Ameyaw et al., 2023). CGA was extracted from 0.08 g samples using 1.6 mL 80% ethanol, in triplicate (n = 3). The solution was vortexed and mixed by a rotating shaker at 40 rpm for 30 min. The extract in supernatant was collected after 10 min centrifugation at 12,100 × g. The extraction procedure was repeated for three more times to minimize the CGA residue in samples. CGA extract was passed through 0.2 μm syringe filter to amber vials and stored in darkness at 4 °C before analysis.

The quantitative analysis of CGA was performed using a high-performance liquid chromatography (HPLC) system (Model 2695, Waters, USA) with a photodiode array detector (Model 2996, Waters, USA). Ten μL of samples were injected onto a Phenomenex Luna C18 (150mm × 4.6 mm, 5 μm) column at a flow rate of 1 mL/min. A wavelength of 320 ± 1.2 nm was selected for the PDA detector, at a constant sample temperature of 30 °C (Šilarová et al., 2019). The gradient program reported by Verde et al. (2022) was employed: 0 to 13 % B in 1 min, up to 27 % B at 8 min, up to 100 % B at 8.5 min, isocratic at 100 % B to 9.5 min, 100 % B returned to

0 % B at 9.6 min with 3 min post-time. A data acquisition software Empower Pro (Build 1154, Waters Co., USA) was used for data collection and processing. A 10-point standard curve from 0-0.5 mg/mL CGA (dissolved in 80% ethanol) was prepared for calculation of mg CGA per g sample.

4.5.3 Phytic acid

The phytic acid (PA) content of HMMAs was analyzed using the method by Haug, W., & Lantzsch, H. J. (1983), with minor modifications. Ferric solution was prepared by addition of 0.1 g ammonium iron (III) sulphate dodecahydrate in 50 mL 2M HCl (the final volume was adjusted to 500 mL with Milli-Q water). To prepare bipyridine solution, 5 g 2,2'-bipyridyl was dissolved in 5 mL thioglycolic acid, adjusted to 500 mL using Milli-Q water.

150 mg of HMMA samples were weighed and extracted with 5 mL 0.2M HCl for 3 h. 0.5 mL of solutions from the interface was transferred to a microcentrifuge tube (n = 2), mixed with 1 mL ferric solution and heated at 100 ºC for 30 min. The mixed content was centrifuged at 3000 × g for 30 min. 0.75 mL of bipyridine solution was added to the supernatant to start the reaction before analysis. The absorbance was measured using a spectrophotometer equipped with Insight Pro Software (Evolution One, Thermo Fisher Scientific, USA). The absorbance was measured at 519 nm after 50 s. The calibration curve was obtained with 10 measurements of serial-diluted reference standards, ranging from 3 – 30 μg/mL phytate phosphorus.

4.5.4 Trypsin inhibitor activity

Trypsin inhibitor activity (TIA) measurement was performed following Liu et al. (2021) with minor modifications. To prepare BAPA solution, 0.2 g DL-BAPA was dissolved in 5 mL dimethyl sulfoxide and diluted to 100 mL with Tris buffer. Tris buffer (0.05 M, pH 8.2) contained 6.05 g tris hydroxymethyl aminomethane and 2.94 g $CaCl_2$ in 1 L of Milli-Q water. The composition of HCl solution was 0.736 g $CaCl_2$ in 1 L Milli-Q water. For trypsin solution, 0.01 g bovine trypsin was dissolved in 50 mL HCl solution and then diluted to concentration of 20 μg/mL. Acetic acid solution was made up with 30 mL acetic acid in 70 mL Milli-Q water.

The samples were weighed for 0.5 g with 25 mL of 10 mM NaOH solution for extraction (mixed by multi-point magnetic stirrer at 500 rpm for 3 hours). An aliquot was prepared and diluted with Milli-Q water to control a ~50% trypsin inhibition. One mL of diluted extract was transferred to a hydrolysis tube, in duplicate (n = 2), where Milli-Q water was used for reference reading. The test tubes were kept in water bath (37 ± 0.5 °C), mixed with 2.5 mL prewarmed BAPA solution. For the sample set, 1 mL trypsin solution was added to start the reaction, and 0.5 mL acetic acid solution was added after 10 min as a reaction stoper. For the blank set, the order was reversed. The mixtures were centrifuged at 1500 × g for 5 min, and the supernatant was collected for measurement at 410 nm using a spectrophotometer (with Insight Pro Software, Evolution One, Thermo Fisher Scientific, USA). TIA was expressed as trypsin unit inhibited (TUI) per mg of sample, following equations 10 and 11:

$$\text{TUI/mg sample} = \frac{(\text{AbsR} - \text{AbsRB}) - (\text{AbsS} - \text{AbsSB})}{0.02 \times (\text{mg sample in the assay})} \tag{10}$$

$$\text{mg sample in the assay} = \frac{0.5\text{ g of sample} \times \text{Dilution factor}}{25\text{ mL of extract}} \tag{11}$$

Where AbsR, AbsRB, AbsS and AbsSB refer to the absorbance values for the reference, reference blank, sample and sample blank, respectively. One trypsin unit is defined as an increase of 0.02 absorbance unit at 410 nm for 5 mL testing solution (Liu et al., 2021).

4.6 Statistical analysis

Results were expressed as mean ± standard deviation on dry matter basis. One-way ANOVA with Tukey's test ($p<0.05$) was performed on R statistical program (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria) to determine the significant differences among different treatments.

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CHAPTER 5: RESULTS AND DISCUSSIONS

5.1 Proximate composition and protein nutritional quality of the raw materials

The dry matter, protein and fat contents of SFM, PPI, and two blended formulations are presented in Table 5.1. SFM contained 54.28% protein content (d.b.) and 17.05% fat content (d.b.), while PPI had 87.06% protein content (d.b.) and 0.45% fat content (d.b.). The proximate composition of the raw materials allowed a low enough overall fat content and a high enough protein content for texturization when blended. The extrusion feed blends were made by substituting 40% and 50% of PPI with SFM to achieve protein contents of 73.87% and 70.64% (d.b.), respectively. The calculated fat contents of the feed formulations, containing 40% and 50% SFM, were both lower than 10% (7.02% and 8.67%, d.b., respectively).

Table 5.1 Proximate composition of the raw materials

Raw materials	Dry matter (%)	Crude fat (%, d.b.)	Crude protein (%, d.b.)
Sunflower meal (SFM)	92.67 ± 0.01	17.05 ± 0.95	54.28 ± 0.00
Pea protein isolate (PPI)	93.98 ± 0.00	0.45 ± 0.00	87.06 ± 0.79
40% SFM and 60% PPI blend	93.69	7.02	73.87
50% SFM and 50% PPI blend	93.51	8.67	70.64

Data is expressed as mean ± standard deviation (n = 3 for dry matter and protein analysis, n = 2 for fat analysis). The data for the blends were calculated based on their mixing ratios.

The AAS, IVPD and IVPDCAAS values of the raw materials and the feed blends are presented in Table 5.2. According to the requirement for pre-school children (2 to 5 years) (FAO/WHO, 1991), the first limiting AA for PPI was SAA (methionine and cysteine), however, for SFM it was lysine. Because of the deficiency of lysine, SFM had a 41.81% IVPDCAAS value, even though its IVPD was 80.44%. Our results are in line with the literature that the addition of lysine is necessary to improve the protein quality of SFM-based foods in human food applications (Salgado & Chieus, 1988). Besides, the amino acid profiles reported in Table 5.2 indicate that SFM and PPI could be complementary proteins to each other because there was no deficiency of AA observed in the blends, and all indispensable AAS were higher than 1. With the substitution of 40% and 50% PPI with SFM, optimal protein quality with balanced amino acid composition and relatively high protein digestibility were obtained. The IVPDCAAS of the blends, containing 40% SFM and 50% SFM, were 86.93% and 86.10%, respectively. Compared with the results of individual raw materials, the initial protein quality was improved by blending SFM and PPI.

Table 5.2 Amino acid score (AAS) and *in-vitro* protein digestibility of the raw materials

Sample Description	HIS	ILE	LEU	LYS	SAA	AAA	THR	TRP	VAL	IVPD (%)	IVPDCAAS (%)
Pea protein isolate (PPI)	1.27	2.04	1.51	1.57	0.90	1.69	1.27	0.91	1.75	89.55	80.64
Sunflower meal (SFM)	1.17	1.12	0.72	0.52	1.46	0.84	0.79	1.25	1.07	80.44	41.81
40% SFM and 60% PPI blend	1.24	1.77	1.28	1.26	1.07	1.44	1.13	1.01	1.55	86.93	86.93
50% SFM and 50% PPI blend	1.23	1.69	1.21	1.17	1.12	1.36	1.09	1.04	1.49	86.10	86.10

The reference pattern used to determine the AAS was: HIS: 19, ILE: 28, LEU: 66, LYS: 58, SAA: 25, AAA: 63, THR: 34, TRP:11, VAL:35 for pre-school children (2 to 5 years) (FAO/WHO, 1991). Abbreviations: THR: threonine, VAL: valine, SAA: sulfur amino acids (methionine + cysteine), ILE: isoleucine, LEU: leucine, AAA: aromatic amino acids (phenylalanine +tyrosine), HIS: histidine, LYS: lysine, TRP: tryptophan, IVPD: *in-vitro* protein digestibility, IVPDCAAS: *in-vitro* protein digestibility corrected amino acid score. The underlined data represents the first limiting AA for the raw materials.

5.2 Torque, die pressure and specific mechanical energy (SME) input

Torque, die pressure and specific mechanical energy (SME) input values during extrusion are presented in Table 5.3. A significant ($p < 0.05$) reduction in the die pressure, torque, and SME was noted as the feed moisture content (FMC) increased from 48% to 58%, consistent with the findings of Singh et al. (2024), who studied extrusion of blends of sunflower meal and soy protein isolate. This trend aligns with the observations by Palanisamy et al. (2019) who attributed the reduction in torque, die pressure and SME input to the lower protein concentration, thus decreasing protein melt viscosity in the extruder caused by higher water content.

When the effect of extrusion temperature was considered, the torque and SME input significantly ($p < 0.05$) decreased with an increase of temperature from 125°C to 145°C. This can be attributed to the reduced melt viscosity in the extruder (Pismag et al., 2024; Chen et al., 2010), which lowers shear force and reduces the required SME input (Palanisamy et al., 2019). As the viscosity of the melt decreases, the die pressure can decrease because of the increase of mobility of the molecules (Pietsch et al., 2019). However, there was no general trend observed for die pressure when extrusion temperature was increased from 135°C to 145°C. The extent of non-covalent and/or covalent interaction between sunflower protein and pea protein may increase at extremely high extrusion temperature (e.g., 145°C), which can promote protein aggregation and improve the formation of a denser protein cross-linking network (Fang et al., 2014; Pietsch et al., 2019; Wittek et al., 2021). Besides, oil from SFM can decrease the melts viscosity and lubricate the extruder, resulting in a decrease in shear force and friction (Benković et al., 2023). These effects are cumulative and may outweigh by each other, resulting in the differences in die pressure.

Table 5.3 Effects of feed moisture content (FMC) and temperature on torque, die pressure and specific mechanical energy (SME) input during extrusion processing.

SFM concentration in the blend (%)	FMC (%)	Temperature (°C)	Torque (%)	Die pressure (kPa)	SME (Wh/kg)
40	48	125	10.0 ± 0.0[b]	500.0 ± 0.0[a]	91.3 ± 0.0[b]
		135	9.0 ± 0.0[d]	400.0 ± 0.0[c]	82.1 ± 0.0[e]
		145	9.5 ± 0.0[c]	450.0 ± 0.0[b]	86.7 ± 0.0[d]
	58	125	9.0 ± 0.0[d]	400.0 ± 0.0[c]	66.4 ± 0.0[g]
		135	8.5 ± 0.0[e]	200.0 ± 0.0[g]	62.6 ± 0.0[h]
		145	8.0 ± 0.0[f]	250.0 ± 0.0[f]	59.0 ± 0.0[i]
50	48	125	11.0 ± 0.0[a]	450.0 ± 0.0[b]	100.4 ± 0.0[a]
		135	10.0 ± 0.0[b]	400.0 ± 0.0[c]	91.2 ± 0.0[c]
		145	9.0 ± 0.0[d]	350.0 ± 0.0[d]	82.0 ± 0.0[f]
	58	125	9.0 ± 0.0[d]	300.0 ± 0.0[e]	66.4 ± 0.0[g]
		135	9.0 ± 0.0[d]	300.0 ± 0.0[e]	66.4 ± 0.0[g]
		145	8.0 ± 0.0[f]	300.0 ± 0.0[e]	59.0 ± 0.0[i]

Data is expressed as mean ± standard deviation (n = 3 for extrusion, n = 4 for torque, die pressure and SME values). Values followed by different letters in a column are significantly different ($p < 0.05$). SFM: sunflower meal.

5.3 Physical property analysis

5.3.1 Texture profile

The results of texture quality attributes are presented in Table 5.4. Significant ($p < 0.05$) changes were observed in most texture attributes, including hardness, gumminess, and chewiness, as a function of varying extrusion conditions. However, springiness, with values ranging from 0.89 to 0.95, was not significantly affected. The hardness of cooked chicken breast was reported to range from 24 N (boiled) to 130 N (convection oven cooked), and the chewiness of which ranged from 12 N (boiled) to 520 N (convection oven cooked) depending on the cooking method (Wang et al., 2022; Ergönül, 2017). Overall, the meat analogues produced in this study were harder compared to boiled and cooked chicken breast, while chewier than boiled chicken. These distinct textural characteristics suggest that SFM extrudates could have broader applications in hard meat snacks, for example, jerky type food and cured meats (e.g., bacon).

An increase of FMC from 48% to 58% significantly ($p < 0.05$) reduced hardness, gumminess, and chewiness, consistent with findings from studies on meat analogues made from pea protein (Dubey, Mateen, & Singh, 2024), soy protein (Chen et al., 2010), and blends of soy protein and sunflower meal (Singh et al., 2024). While water facilitates protein unfolding and alignment, higher feed moisture reduces protein concentration and decrease the extent of protein aggregation (Chen et al., 2010; Mateen, Mathpati & Singh, 2023). Therefore, compared to the meat analogues produced at 48% FMC, those produced at 58% FMC had relatively less protein concentration, resulting in a softer texture.

In terms of extrusion temperature, an increase of temperature from 125°C to 145°C significantly ($p < 0.05$) decreased hardness, gumminess and chewiness when FMC was constant at 48%. The similar trend was also observed for meat analogues made from soy protein (Chen et al., 2010) and pea protein (Wang et al., 2022). However, the texture properties of pea protein extrudates was reported to be less dependent on extrusion temperature (Wang et al., 2022). Compared to FMC, temperature appeared to have a smaller impact on texture properties, in line with other studies on high-moisture extrusion process (Lin, Huff, & Hsieh, 2000). Therefore, the effect of extrusion temperature on hardness, gumminess and chewiness was not significant when FMC was 58%.

Previous studies have shown that the incorporation of additional protein ingredients can alter texture properties of meat analogues made from faba and pea protein blends (Knaapila et al., 2024), wheat gluten and pea protein blends (Zhao et al., 2024), and sunflower meal and soy protein blends (Singh et al., 2024). The higher SFM concentration can lower the protein concentration and reduce the extent of protein cross-linking, leading to a softer texture (Singh et al., 2024). However, the increase of SFM concentration in present study did not significantly affect texture properties, likely due to the extrusion FMC which was lower than those in previous studies. Barnés-Calle et al. (2024) noted that higher FMC (> 60%) can delay protein-protein interactions, while the FMC of 58% was likely sufficient to allow protein interactions to happen, resulting in minimal changes in texture properties.

Table 5.4 Effects of feed moisture content (FMC) and die temperature on the texture profile analysis attributes of the meat analogues.

SFM concentration in the HMMAs (%)	FMC (%)	Temperature (°C)	Hardness (N)	Gumminess (N)	Chewiness (N)
40	48	125	252.78 ± 13.90[b]	195.46 ± 17.17[b]	182.39 ± 17.30[b]
		135	307.47 ± 18.23[a]	256.21 ± 22.39[a]	242.31 ± 18.24[a]
		145	312.21 ± 8.93[a]	266.55 ± 9.89[a]	242.00 ± 12.72[a]
	58	125	190.71 ± 8.16[d]	155.23 ± 7.06[c]	142.79 ± 7.42[c]
		135	195.20 ± 7.11[cd]	160.09 ± 5.87[c]	146.67 ± 6.29[c]
		145	211.54 ± 5.11[c]	174.94 ± 5.19[c]	161.74 ± 5.00[c]
50	48	125	255.54 ± 8.25[b]	201.91 ± 5.80[b]	185.96 ± 9.56[b]
		135	304.16 ± 7.88[a]	255.16± 9.20[a]	230.96 ± 4.40[a]
		145	301.82 ± 6.95[a]	253.99 ± 5.76[a]	232.99 ± 5.63[a]
	58	125	196.56 ± 5.73[cd]	159.76 ± 5.10[c]	145.56 ± 4.75[c]
		135	199.44 ± 10.4[cd]	164.21 ± 8.24[c]	146.80 ± 9.16[c]
		145	194.72 ± 2.59[cd]	159.47 ± 1.74[c]	145.46 ± 2.29[c]

Data is expressed as mean ± standard deviation (n = 6 for texture tests). Values followed by different letters in a column are significantly different ($p < 0.05$). SFM: sunflower meal.

5.3.2 Cutting strengths and degree of texturization

The results of longitudinal and transverse cutting force of HMMAs are presented in Figure 5.1(a) and (b), respectively. The ratio of longitudinal and transverse cutting force reflects the degree of texturization and provides insight into the direction of fiber structure formation in HMMAs. The degree of texturization results are presented in Figure 5.1(c). Similar to the texture profile analyses, increasing the FMC from 48% to 58%, while maintaining constant SFM concentration and temperature, significantly ($p < 0.05$) reduced both the longitudinal and transverse cutting strengths of the meat analogues. This reduction can be attributed to reduced protein concentration, and thus reduced protein interactions under higher moisture extrusion conditions (Schmid et al., 2022).

Moreover, the increase of extrusion temperature from 125°C to 145°C significantly ($p < 0.05$) increased the cutting strengths in both directions for 40% SFM-based meat analogues with 48% FMC, in agreement with previous high-moisture extrusion for lupin protein by Palanisamy et al. (2019). Higher extrusion temperature can promote protein denaturation, contributing to more efficient alignment of protein molecules in the cooling die and improved anisotropic structure formation (Wang et al., 2022). However, the cutting forces of the meat analogues were not affected by extrusion temperature at higher FMC (i.e., 58%). Extrusion temperature was found to have a minor effect on cutting strength of the meat analogues compared to FMC (Lin, Huff, & Hsieh, 2000). High moisture levels can reduce the impact of heating and the extent of protein denaturation, in turn leading to insufficient texturization of the molten materials in the cooling die (Schmid et al., 2022).

Interestingly, the increase of temperature from 135°C to 145°C significantly ($p < 0.05$) decreased the transverse cutting strength for 50% SFM-based meat analogues with 48% FMC, showing a different trend with the transverse cutting strength of those produced with 40% SFM at same extrusion conditions. It was reported that extrusion temperature has no significant effect on transverse strength of meat analogues made from pea protein isolate (Osen et al., 2014). The probable explanation would be the higher SFM concentration led to less protein content and more oil. The addition of oil during extrusion can negatively affect the protein denaturation and realignment of protein molecules in the cooling die (Kyriakopoulou et al., 2021), thus reducing the extent of fibrous structure formation and cutting strength.

The degree of texturization in this study ranged from 1.00 to 1.32, comparable to values reported by Riazi et al. (2023) for pea-flaxseed-based-meat analogues (1.08–1.12) and Singh et al. (2024) for SFM-soy-based meat analogues (1.06–1.13). Notably, all HMMAs in this study exhibited degree of texturization values greater than 1, indicating successful formation of fibrous structures in the direction of flow in the die (Singh et al., 2024). Additionally, the fibrous structures of the meat analogues displayed a parabolic pattern, shown in Figure A1, likely due to differences in solidification rates caused by the hotter core temperature of the melt compared to the cooler temperature near the cooling die wall. This pattern was also observed for meat analogues made from soy protein isolate (Wittek et al., 2021) and pea protein isolate (Osen et al., 2014).

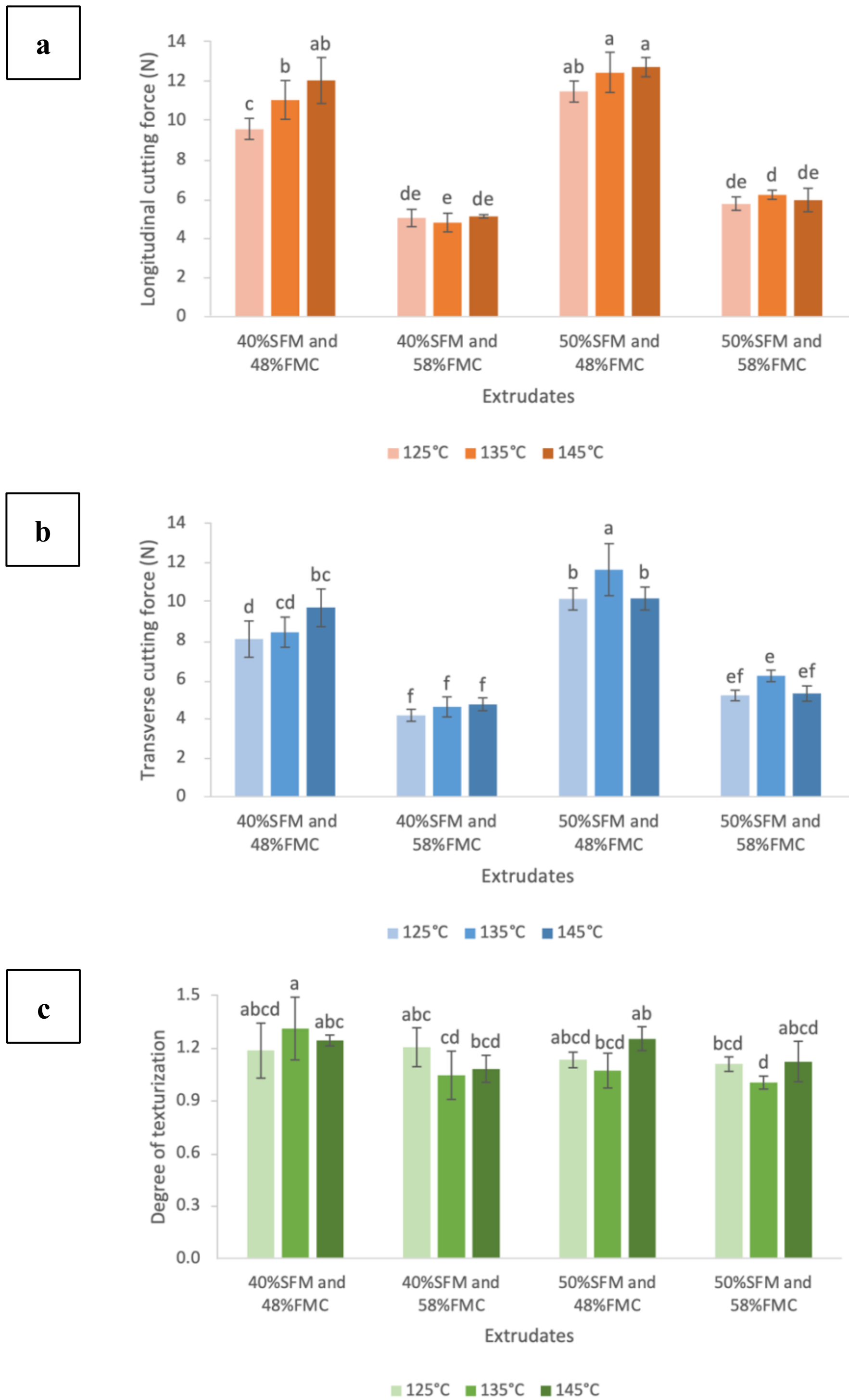


Figure 5.1 The effects of feed moisture content and die temperature on (a) longitudinal cutting force, (b) transverse cutting force, (c) degree of texturization of the meat analogues.

SFM: sunflower meal concentration in the meat analogues, FMC: feed moisture content for extrusion. Error bars represent ± standard deviation (n = 3 for extrusion, n = 6 for cutting forces and degree of texturization). Different letters above bars reflect statistically significant differences ($p < 0.05$).

5.3.3 Color analysis

Color attributes of HMMAs are summarized in Table 5.5. In general, extrusion processing resulted in a reduction of lightness (L*) and yellowness (b*), accompanied by an increase in redness (a*) in the meat analogues compared to their corresponding raw blends. The reduction in lightness is primarily attributed to the Maillard reaction, as also reported by Wang et al. (2022) for pea protein extrusion. The lightness of the meat analogues produced in this study is comparable to the meat analogues made from SFM and soy protein isolate by Singh et al. (2024), while darker than those made from pure pea protein isolate (Wang et al., 2022). This darker coloration may negatively impact consumer acceptance, as it can be challenging to be covered with food dyes (Zhao et al., 2024). In addition, the reactions, such as caramelization, phenolic compound degradation and oil oxidation, are also common causes of browning during the extrusion process (Zahari et al., 2020; Liang & Were, 2018).

The original yellowish color of pea protein isolate, due to pigments such as carotenoids and lutein, is more intense compared to sunflower meal powder (Marles et al., 2013). Previous study has shown that extrusion processing can reduce pigment content, as observed in cornflakes (Cueto et al., 2017). Consequently, the b* value of the meat analogues was significantly ($p < 0.05$) reduced by approximately 50% by extrusion compared to the raw formulations. The increase of SFM concentration significantly increased b* values for the meat analogues. The inclusion of SFM increased oil content during extrusion and oil oxidation could occur at high temperature (Lin, Hsieh & Huff, 1998). The products of lipid oxidation, namely aldehydes, were proven to be related to the yellow color formation (Geng, Liu & Zhang, 2023; Thanonkaew et al., 2006).

In terms of the effect of extrusion parameters on the color attributes of the meat analogues, the increase of FMC made the meat analogues significantly ($p < 0.05$) lighter. A higher level of feed moisture during extrusion processing can decrease protein concentration and reduce the residence time of the melt inside the barrel, thereby decreasing the color change caused by Maillard reaction (Dubey, Mateen & Singh, 2024; Pismag et al., 2024). Therefore, the total color change (ΔE) was significantly ($p < 0.05$) higher for the meat analogues with 48% FMC. However, the increase of temperature from 125°C to 145°C only resulted in significantly ($p < 0.05$) higher L* value at 48% FMC for any SFM concentration, which is consistent with the results of the meat analogues made from lupin protein (Palanisamy et al., 2019) and extrusion of maize grits (Ilo & Berghoder, 1999). This slight increase may be attributed to the decreased SME, as shown in Table 5.3. It was reported that higher SME can lead to darker color for meat analogues (Fang et al., 2014).

Furthermore, chlorogenic acid (CGA) is a type of phenolic compound present in sunflower meal. The formation of green color due to the reaction between CGA and protein is one of the challenges of using SFM in food applications (Wildermuth, Young, & Were, 2016). However, the green color was not observed for meat analogues in this study, in agreement with meat analogues made from SFM and soy protein isolate (Singh et al., 2024). Previous study has shown that the formation of green color primarily occurs in bakery products (e.g., cookies, breads) due to the addition of baking soda, which raises the pH during processing (Wang et al., 2022). The higher pH processing condition can promote the reaction between CGA and proteins, and facilitate the formation of green-colored compounds (Liang & Were, 2018). The pH of the meat analogues made with SFM is likely close to neutral as reported for sunflower containing meat analogues processed using shear cell technology (Jia et al., 2021). Additionally, processing at temperature above 100°C has been shown to effectively lower the concentration of CGA (Wildermuth, Young, & Were, 2016). The positive a* values of meat analogues indicated that the extrusion conditions used in this study were effective in preventing the reaction between CGA and proteins.

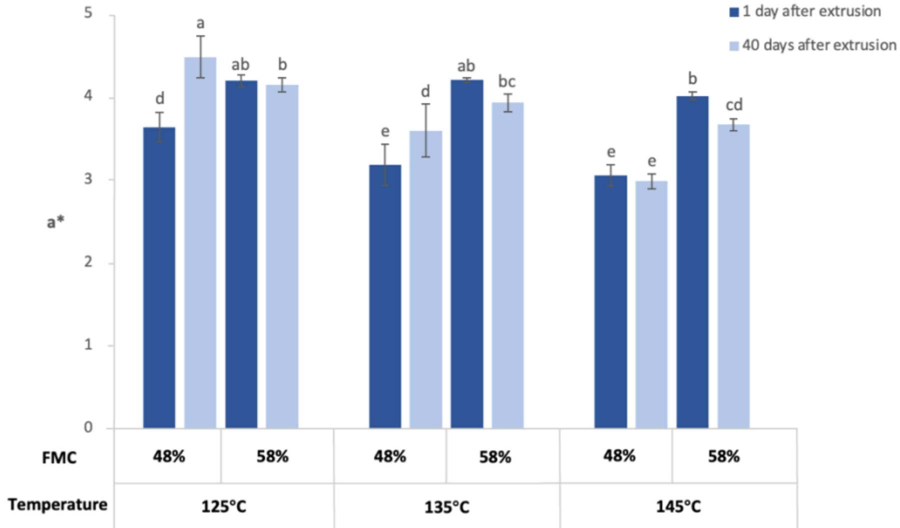
Shortly after sample collection, the color of meat analogues changed rapidly, as observed visually (shown in Figure A2). Color is an important physical parameter, related to quality and consumer acceptance (Zheng et al., 2020). Therefore, it is necessary to evaluate the color stability and development during long-term storage. For both blends, only the a* values of the meat analogues changed over time. The a* values after 40 days storage in the freezer are presented in Figure 5.2. For most meat analogues, the a* values were stable or tend to be slightly higher, with the exception for the meat analogues produced at 145°C and 58% FMC. Due to protein denaturation and subsequent aggregation during extrusion, a denser cross-linked structure is formed, resulting in fewer charged amino acid residues available for reaction with CGA (Sui et al., 2024; Wildermuth, Young, & Were, 2016). Because of the lack of amino acid residues, CGA can be oxidized to CGA-quinone (CGA-Q) which is unstable and react quickly with each other to form brown color (Yabuta et al., 2001). However, the a* values decreased for meat analogues produced at 145°C and 58% FMC, which probably can be attributed to intense extrusion conditions, i.e., high extrusion temperature and high feed moisture content, that help protein molecular unfolding and expose more reactive groups for the interaction with CGA (Chen et al., 2010; Zhang et al., 2023; Tarahi et al., 2024). Additionally, this interaction appears to be more favorable to high moisture storage conditions (Liang & Were, 2018; Wildermuth, Young, & Were, 2016). According to the results shown in Figure 5.2, some meat analogues showed no significant color change over time. Therefore, the management of temperature and FMC during extrusion is essential to minimize long-term color changes and improve product stability.

Table 5.5 Effects of feed moisture content (FMC) and die temperature on the color attributes of the meat analogues (1 day after extrusion).

SFM concentration in the HMMAs (%)	FMC (%)	Temperature (°C)	L*	a*	b*	ΔE
40	Raw	-	75.86 ± 0.42[a]	2.97 ± 0.05[f]	20.81 ± 0.05[a]	-
	48	125	32.93 ± 0.74[h]	3.63 ± 0.18[e]	8.91 ± 0.37[g]	44.55 ± 0.78[a]
		135	34.65 ± 1.32[fg]	3.18 ± 0.25[f]	9.30 ± 0.36[g]	42.79 ± 1.32[ab]
		145	34.56 ± 0.59[fg]	3.05 ± 0.13[f]	9.52 ± 0.76[g]	42.82 ± 0.67[a]
	58	125	40.22 ± 1.02[cd]	4.19 ± 0.07[bc]	11.01 ± 0.18[ef]	36.99 ± 1.01[d]
		135	40.43 ± 0.42[cd]	4.20 ± 0.03[bc]	10.99 ± 0.27[ef]	36.79 ± 0.46[d]
		145	39.91 ± 0.61[d]	4.01 ± 0.05[cd]	11.68 ± 0.53[d]	37.10 ± 0.71[d]
50	Raw	-	75.47 ± 0.07[a]	2.49 ± 0.04[g]	19.45 ± 0.14[b]	-
	48	125	34.03 ± 0.78[gh]	4.15 ± 0.04[bc]	10.32 ± 0.26[f]	42.47 ± 0.81[b]
		135	35.70 ± 0.23[ef]	3.92 ± 0.06[d]	10.68 ± 0.16[f]	40.76 ± 0.22[c]
		145	36.32 ± 0.48[e]	3.86 ± 0.06[d]	11.46 ± 0.26[de]	39.99 ± 0.51[c]
	58	125	42.54 ± 0.80[b]	4.51 ± 0.09[a]	12.50 ± 0.37[c]	33.72 ± 0.85[e]
		135	41.63 ± 0.23[bc]	4.47 ± 0.06[a]	12.67 ± 0.22[c]	34.58 ± 0.21[e]
		145	41.40 ± 0.65[bcd]	4.23 ± 0.04[b]	12.58 ± 0.17[c]	34.80 ± 0.66[e]

Data is expressed as mean ± standard deviation (n = 6 for color attributes). Values followed by different letters in a column are significantly different ($p < 0.05$). SFM: sunflower meal.

a



b

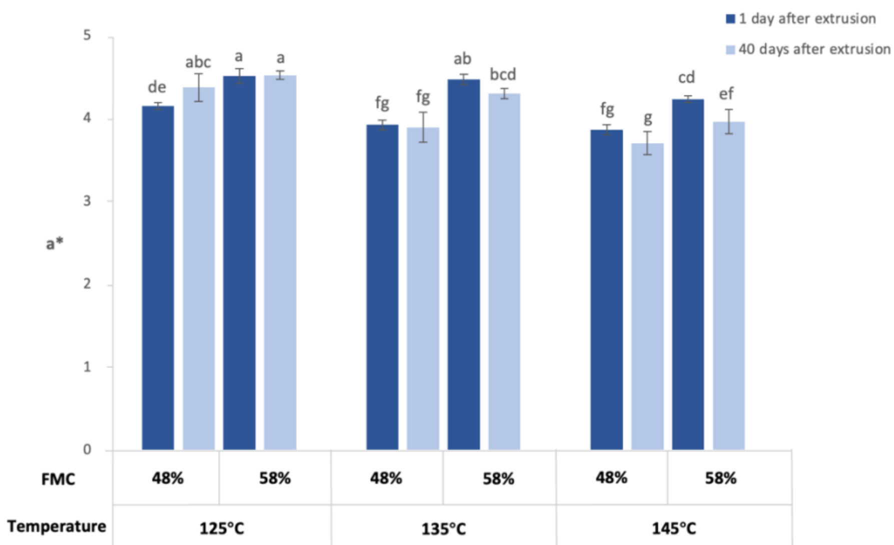


Figure 5.2 The green color intensity (a* value) changes during the storage (40 days after extrusion processing) of meat analogues extruded at different feed moisture contents (FMC) and temperatures, (a) 40%, and (b) 50% concentration of sunflower meal in the meat analogues.

Error bars represent ± standard deviation (n = 6 for color analysis). For each subfigure, different letters above bars reflect statistically significant differences ($p < 0.05$).

5.4 Protein quality analysis

5.4.1 Amino acid composition and scores

Among the 20 common amino acids, nine amino acids are identified as indispensable amino acids for humans (FAO, 2013). The one present in the lowest proportion relative to its reference pattern (to meet daily requirements for individuals) is called the limiting amino acid, and it is directly related to the protein quality of a food (Mansilla et al., 2020). The amino acid score (AAS) of indispensable amino acids for the meat analogues made from sunflower meal and pea protein are presented in Table 5.6. Sunflower meal protein and pea protein have been proven to be complementary to each other, as shown in Table 5.2, with pea protein rich in lysine and sunflower meal providing methionine and cysteine. The raw blends of SFM and PPI had a balanced amino acid composition for both formulations, meeting the requirement of amino acids for pre-school children (2 - 5 years) established by FAO/WHO (1991). However, for meat analogues, tryptophan was the first limiting amino acid, whose score ranged from 0.83 – 0.93. This was because pea protein isolate is deficient in both tryptophan and sulfur amino acids, in agreement with previous work on sunflower and pea protein by Öztürk et al. (2024).

It was also observed that the scores of lysine decreased after extrusion and were lower than 1 for some 50% SFM-based meat analogues. One reason could be that the addition of SFM lowered the initial lysine content of the blends, due to raw SFM having a lower concentration of lysine when compared to raw PPI. Furthermore, extrusion processing involves high temperature and pressure, which can cause amino acid losses (e.g., lysine) (Pismag et al., 2024). The decrease of lysine may contribute to the red color formation, resulting in the positive a* values for the meat analogues (shown in Table 5.5) as the reducing sugars from sunflower meal can react with the epsilon-amino group of lysine, leading to Maillard reaction during extrusion (Iwe et al., 2004). Thus, 50% SFM-based meat analogues had higher a* values, compared with 40% SFM-based meat analogues. However, despite its loss during extrusion, lysine was not the first limiting amino acid in the meat analogues, indicating that its loss was not substantial. High-moisture extrusion (FMC > 40%) was reported as a high-temperature, short-time processing method, having limited effect on the amino acid loss (Schmid et al., 2022). The scores of amino acids besides tryptophan and lysine did not decrease much after extrusion processing and could meet the amino acid requirement by FAO/WHO (1991), consistent with previous work on extrusion of rapeseed and pea protein by Zahari et al. (2021).

Table 5.6 Amino acid score (AAS) of indispensable amino acids for meat analogues.

Sample Description			HIS	ILE	LEU	LYS	SAA	AAA	THR	TRP	VAL
SFM concentration in the HMMAs (%)	**FMC (%)**	**Temperature (°C)**									
40		**Raw**	1.24	1.77	1.28	1.26	1.07	1.44	1.13	1.01	1.55
	48	**125**	1.34	1.59	1.13	1.07	1.10	1.37	1.05	<u>0.90</u>	1.40
		135	1.17	1.58	1.12	1.04	1.00	1.35	1.04	<u>0.87</u>	1.37
		145	1.21	1.56	1.11	1.02	0.95	1.37	1.03	<u>0.89</u>	1.37
	58	**125**	1.26	1.57	1.12	1.07	1.05	1.32	1.04	<u>0.86</u>	1.38
		135	1.22	1.57	1.12	1.05	1.04	1.35	1.03	<u>0.89</u>	1.37
		145	1.25	1.53	1.07	1.05	1.02	1.28	1.00	<u>0.84</u>	1.34
50		**Raw**	1.23	1.69	1.21	1.17	1.12	1.36	1.09	1.04	1.49
	48	**125**	1.19	1.53	1.07	0.98	1.02	1.29	1.02	<u>0.85</u>	1.35
		135	1.26	1.52	1.07	0.97	1.10	1.27	1.02	<u>0.90</u>	1.35
		145	1.29	1.53	1.06	0.98	1.10	1.27	1.01	<u>0.85</u>	1.34
	58	**125**	1.24	1.53	1.07	1.00	1.07	1.29	1.02	<u>0.91</u>	1.35
		135	1.23	1.51	1.06	1.00	1.07	1.25	1.02	<u>0.93</u>	1.34
		145	1.19	1.52	1.06	0.98	1.02	1.26	1.01	<u>0.83</u>	1.33

The reference pattern used to determine the AAS was: HIS: 19, ILE: 28, LEU: 66, LYS: 58, SAA: 25, AAA: 63, THR: 34, TRP:11, VAL:35 for pre-school children (2 to 5 years) (FAO/WHO, 1991). Abbreviations: SFM: Sunflower meal, FMC: Feed moisture content, THR: threonine, VAL: valine, SAA: sulfur amino acids (methionine + cysteine), ILE: isoleucine, LEU: leucine, AAA: aromatic amino acids (phenylalanine +tyrosine), HIS: histidine, LYS: lysine, TRP: tryptophan. The underlined data represents the first limiting AA for the meat analogues.

5.4.2 *In-vitro* protein digestibility (IVPD) and *in-vitro* protein digestibility-corrected amino acid score (IVPDCAAS)

Protein content, *in-vitro* protein digestibility (IVPD) and *in-vitro* protein digestibility corrected amino acid score (IVPDCAAS) are presented in Table 5.7. The protein content of the meat analogues ranged from 70.25% to 74.13% (d.b.), which is lower than chicken breast fillet (86.8%, d.b.) and beef steaks (72.3%-91.4%, d.b.), yet higher than common plant-based meat steaks made with soy, wheat or lupin proteins (46.4%-50.7%, d.b.) (Ng et al., 2024; Cutroneo et al., 2024). The commercial plant-based meat products typically contain additives, such as salt, oil and color agents, resulting in a reduction of the protein proportion (Guyony, Fayolle & Jury, 2023). However, despite the relatively higher lipid content of SFM, the meat analogues developed in this study provided higher protein contents compared to commercial plant-based meat analogues, further highlighting the potential of sunflower meal as a valuable protein source for such products.

During extrusion, digestibility of the proteins can be affected by the shear forces and heat (Schmid et al., 2022). In this study, IVPD of the meat analogues varied from 86.2% to 88.5%, showing no changes compared to the blends. In most cases, the increased temperature can help protein unfolding and improve protein digestibility (Pismag et al., 2024). However, very high temperature (e.g., 170°C) was reported to reduce protein digestibility for amaranth, bean and corn flour compared with those produced at lower temperature (e.g., 130°C) (Akande et al., 2017). Furthermore, in a static digestion model, the *in-vitro* digestibility of pork and beef proteins was reported to be around 80%, while the digestibility of plant-based meat analogues was around 60% (Xie et al., 2022). Similarly, chicken breast was reported to have 76% digestibility, in contrast to the vegetarian chicken strips with a 43% protein digestibility (Ng et al., 2024). The meat analogues produced in this study had a comparable protein digestibility with commercial meat, which can be attributed to high-temperature extrusion processing, during which proteins denature making more sites accessible to digestive enzymes and thus improving protein digestibility (Xie et al., 2024). Meanwhile, the pressure-, shear- and heat-sensitive anti-nutritional factors could be deactivated during extrusion, causing increase in protein digestibility (Azizi et al., 2024). Compared to a lab-made meat analogue with pea protein and wheat gluten, the digestibility of SFM-based meat analogues was higher, exceeding the 50% to 62% protein digestibility observed in the former (Zhao et al., 2024). Öztürk et al. (2024) argued that the meat analogues made from sunflower protein had a finer fiber structure, allowing easy accessibility to digestive enzymes, improving their IVPD and further confirming the potential advantage of sunflower meal protein in the production of meat analogues.

The IVPDCAAS of the meat analogues ranged from 72.3% to 81.4%. Compared to the IVPDCAAS of 84.8% to 90.8% for meat analogues made with sunflower meal and soy protein by Singh et al. (2024), the IVPDCAAS of the meat analogues produced in this study was lower. This can be explained by the fact that soy protein has better protein quality compared to pea protein, similar to the better protein quality of impossible burger (soy protein-based) than beyond burger (pea protein-based) (Fanelli et al., 2022).

Table 5.7 The protein content, *in-vitro* protein digestibility (IVPD) and *in-vitro* protein digestibility corrected amino acid score (IVPDCAAS) of the meat analogues.

SFM concentration in the HMMAs (%)	FMC (%)	Temperature (°C)	Protein content (%, d.b.)	IVPD (%)	IVPDCAAS (%)
40	Raw		73.87 ± 0.50[ab]	86.9 ± 1.0[a]	86.9 ± 1.0[a]
	48	125	73.55 ± 0.09[ab]	88.2 ± 0.3[a]	79.3 ± 0.3[bc]
		135	74.13 ± 0.02[a]	86.8 ± 1.8[a]	75.5 ± 1.9[efg]
		145	73.81 ± 0.21[ab]	86.4 ± 0.7[a]	76.9 ± 0.8[cde]
	58	125	73.05 ± 0.04[b]	88.5 ± 0.7[a]	76.1 ± 0.7[def]
		135	73.40 ± 0.00[ab]	87.1 ± 0.9[a]	77.5 ± 0.9[cde]
		145	73.29 ± 0.04[ab]	86.3 ± 1.2[a]	72.5 ± 1.2[h]
50	Raw		70.64 ± 0.42[c]	86.1 ± 1.0[a]	86.1 ± 1.0[a]
	48	125	70.25 ± 0.14[c]	86.8 ± 0.1[a]	73.8 ± 0.2[fgh]
		135	70.46 ± 0.11[c]	87.2 ± 0.1[a]	78.5 ± 0.2[cd]
		145	70.54 ± 0.13[c]	86.2 ± 0.3[a]	73.3 ± 0.3[gh]
	58	125	70.65 ± 0.30[c]	87.2 ± 0.1[a]	79.4 ± 0.2[bc]
		135	70.44 ± 0.03[c]	87.5 ± 0.5[a]	81.4 ± 0.5[b]
		145	70.51 ± 0.21[c]	87.1 ± 0.6[a]	72.3 ± 0.6[h]

Data is expressed as mean ± standard deviation (n = 2 for protein content, n = 3 for IVPD analysis). Values followed by different letters in a column are significantly different ($p < 0.05$).

IVPDCAAS was calculated as IVPD × AAS, using amino acid scoring pattern for pre-school children (2 to 5 years) (FAO/WHO, 1991). Abbreviations: SFM: sunflower meal, FMC: feed moisture content, d.b.: dry basis, IVPD: *in-vitro* protein digestibility, IVPDCAAS: *in-vitro* protein digestibility corrected amino acid score

5.5 Total phenolic compounds (TPC) content and chlorogenic acid (CGA) content

Phenolic compounds are identified as anti-nutritional factors (ANF) because they can bind with proteins, affecting protein solubility, protein digestibility and the sensory properties of the final products (e.g., bitter flavor and dark color) (Vidal et al., 2022; Pismag et al., 2024). The total phenolic compounds (TPC) content of the raw blends and the meat analogues is shown in Table 5.8. TPC content of 40% SFM-based meat analogues varied from 9.68 - 10.02 mg gallic acid equivalent (GAE) per g (d.b.), with a significant decrease ($p < 0.05$) after extrusion from the raw blend (11.44 mg GAE/g, d.b.). The TPC content of the meat analogues with 50% SFM was in the range of 11.90 - 12.22 mg GAE/g (d.b.), with no significant changes observed when compared to their respective raw blend (12.33 mg GAE/g, d.b.). As reported in previous studies, sunflower cake has a higher TPC content (25.4 mg GAE/g, d.b.) than pea seed (0.27 – 1.95 mg GAE/g, d.b.) (Vidal et al., 2022; Zhao et al., 2020). Accordingly, incorporating more sunflower meal in the formula resulted in higher TPC contents for the meat analogues. There was no significant change of TPC content observed as a function of feed moisture content or barrel temperature profile, in agreement with the results reported for extruded snacks made with rice and red bean flours by Oliveira et al. (2017). While low-moisture (~20% FMC) and high intensity extrusion processing was reported to have positive effect on the reduction of phenolic compounds for legumes (Abd El-Hady & Habiba, 2003; Korus et al., 2007), high-moisture extrusion is a relatively gentler process, making the degradation of phenolic compounds less likely to depend on extrusion process parameters (Oliveira et al., 2017). High level of feed moisture was reported to preserve more phenolic compounds by promoting polymerization of phenolic compounds (Korus et al., 2007; Paucar-

Menacho et al., 2022). Hydrothermal treatment is a process with high pressure and high temperature, which is similar to high-moisture extrusion. During hydrothermal treatment, the presence of water and high temperature helped break the bonds between phenolic compounds and carbohydrates or proteins, further promoting the release of bound phenolic compounds, while only very high temperature (> 140°C) and long duration caused phenolic compounds degradation (Chao & Fan, 2023). In contrast, a number of studies have observed the increase of some phenolic compounds, e.g., quercetin, cinnamic acid and syringic acid, during extrusion and hydrothermal processing, which was attributed to the release of bound phenolic compounds (Vidal et al., 2022; Chao & Fan, 2023; Oliveira et al., 2017). Therefore, it is likely that the addition of SFM led to the release of more bound phenolic compounds, increasing the extractability of phenolic compounds (Oliveira er al., 2017) and resulting in no net changes in total phenolic content for 50% SFM-based meat analogues.

Table 5.8 Total phenolic compounds (TPC) content of the raw blends and the meat analogues.

SFM concentration (%)	FMC (%)	Temperature (°C)	TPC content (mg gallic acid equivalent / g, d.b.)
40	Raw		11.44 ± 0.08^{a}
	48	125	10.01 ± 0.64^{b}
		135	9.89 ± 0.70^{b}
		145	9.68 ± 0.55^{b}
	58	125	10.02 ± 0.05^{b}
		135	9.86 ± 0.62^{b}
		145	9.85 ± 0.25^{b}
50	Raw		12.33 ± 1.07^{a}
	48	125	12.21 ± 0.43^{a}
		135	12.09 ± 0.57^{a}
		145	11.90 ± 0.22^{a}
	58	125	12.22 ± 0.51^{a}
		135	12.07 ± 0.13^{a}
		145	12.06 ± 0.14^{a}

SFM: sunflower meal, FMC: feed moisture content, d.b.: dry basis. Data is expressed as mean ± standard (n = 3 for TPC measurement). Values followed by different letters in a column are significantly different ($p < 0.05$).

Chlorogenic acid (CGA) is a type of phenolic compound, representing around 70% of the total phenolic compounds in sunflower meal (Laguna et al., 2018). CGA content of the raw blends and the meat analogues is presented in Table 5.9. There was no CGA naturally present in the PPI used in this study. With the increase in SFM concentration, the CGA content was significantly ($p < 0.05$) increased for both the raw blends and the meat analogues. After extrusion processing, a significant ($p < 0.05$) reduction of CGA content was observed for all meat analogues, in agreement with a previous study on raw and extruded sunflower cake by Vidal et al. (2022). However, compared to the 60% reduction of CGA observed in low-moisture extrusion by Vidal et al. (2022), the efficiency of high-moisture extrusion in degrading CGA appeared to be lower in this study (15.5% – 30.9%), which can be attributed to the protection of water on CGA, promoting CGA polymerization and resistance to high temperature (Oliveira et al., 2017).

Regardless of the SFM concentration, the increase of temperature from 125°C to 145°C significantly ($p < 0.05$) reduced the CGA content. CGA is relatively heat-stable at the temperature lower than 100°C (Murakami et al., 2004), and can be fully destructed by 140°C, 30 mins thermal treatment (Chao & Fan, 2023). The extent of CGA degradation depends on the processing temperature and reaction time (Hwang et al., 2018). A longer heating time was reported to be more beneficial to reducing CGA in coffee beans (Perrone et al., 2010). However, high-moisture extrusion is a high-temperature and short-time processing method, which is relatively gentler than conventional thermal treatments (Schmid et al., 2022). For the meat analogues with 58% FMC, higher temperature was required to counteract the protective effect of water and promote the thermal degradation of CGA, as only an increase of temperature from 135°C to 145°C achieved a significant ($p < 0.05$) reduction in CGA content. Thus, it is necessary to adjust the temperature appropriately when increasing the feed moisture content during extrusion to achieve effective CGA reduction.

The formation of green color caused by CGA has frequently been reported in the literature for sunflower-based bakery foods (Liang & Were, 2018; Pepra-Ameyaw et al., 2023). However, the green color was not observed for the meat analogues in this study, in agreement with previous studies on the meat analogues made with fermented sunflower protein concentrate and pea protein (Pöri et al., 2023), and sunflower meal and soy protein isolate (Singh et al., 2024), indicating there was no interaction between sunflower protein and CGA (Jia et al., 2022). It was reported that CGA can be oxidized to unstable CGA-quinone (CGA-Q) at high temperature and react with the side chains of proteins (e.g., the ε-amino group of lysine and thiol group of cysteine) to produce green-colored compounds at alkaline conditions (Wildermuth, Young, & Were, 2016; Bongartz et al., 2016; Wang et al., 2022). When the pH is lower than 7, CGA-Q is prone to polymerization, leading to brown color formation (Bongartz et al., 2016). Therefore, the a* value was positive for all meat analogues in this study. The interaction between CGA and proteins cannot be completely ruled out, it is likely to occur at a low extent, especially for extrusion with high FMC, where reactive groups of proteins are exposed and CGA oxidation is more likely to take place (Zhang et al., 2023; Chao & Fan, 2023).

Phenolic compounds, especially CGA, appeared to have impact only on color attributes (i.e., a* value), with no influence on fibrous structure formation (Jia et al., 2022) and protein digestibility (Alexandrino et al., 2017).

Table 5.9 Chlorogenic acid (CGA) content of the raw blends and the meat analogues.

SFM concentration (%)	FMC (%)	Temperature (°C)	CGA content (mg / g, d.b.)
40	Raw		11.12 ± 0.34^{b}
	48	125	9.16 ± 0.08^{f}
		135	7.96 ± 0.05^{h}
		145	7.68 ± 0.09^{h}
	58	125	8.76 ± 0.13^{g}
		135	8.46 ± 0.01^{g}
		145	7.78 ± 0.03^{h}
50	Raw		13.90 ± 0.34^{a}
	48	125	11.75 ± 0.08^{c}
		135	10.90 ± 0.04^{d}
		145	10.47 ± 0.06^{e}
	58	125	10.83 ± 0.03^{d}
		135	11.00 ± 0.04^{d}
		145	10.44 ± 0.16^{e}

SFM: sunflower meal, FMC: feed moisture content, d.b.: dry basis. Data is expressed as mean ± standard deviation (n = 3 for CGA determination). Values followed by different letters in a column are significantly different ($p < 0.05$).

5.6 Phytic acid (PA)

Phytic acid (PA) is another bioactive compound commonly present in plants, e.g., cereals, legumes and oilseeds, binding with minerals and proteins to form insoluble complexes (Pei, Deng & Li, 2024; Wehrmaker et al., 2024). These complexes can be resistant to hydrolysis by digestive enzymes (i.e., proteases), leading to reduced protein digestibility for final products made from these protein sources (Shi, Arntfield & Nickerson, 2018). The phytic acid content of raw blends and meat analogues is presented in Table 5.10. The raw blends had approximately 15 mg PA/g dry sample, which is higher than the previously reported value for sunflower protein concentrate (8.8 mg/g) and raw pea seed (11.93 mg/g) (Eklund, 1975; Alonso et al., 1998). The phytic acid content can vary between the cultivars and species of the plant source and depends on the processing methods (Nikmaram et al., 2017). However, the PA content of pea and sunflower are relatively low compared to other plant sources (e.g., common beans) (Nikmaram et al., 2017; Vidal et al., 2022).

Compared with the raw blends, the meat analogues had significantly ($p < 0.05$) lower phytic acid contents. While phytic acid is resistant to conventional heating methods, such as roasting and autoclaving (Sathe & Venkatachalam, 2001). Extrusion temperature above 140°C could effectively reduce PA for lentils up to 99% (Rathod & Annapure, 2016), which can be attributed to the thermal degradation of PA (Nikmaram et al., 2017). Previous research showed low-moisture extrusion can reduce the level of PA for beans (Batista, Prudêncio & Fernandes, 2010), cereal brans (Kaur et al., 2015), lentil (Rathod & Annapure, 2016) and peas (Alonso et al., 1998). The highest PA reduction (83%) was observed in this study at 135°C with 58% FMC during extrusion, which is higher than the values (10% - 60%, at extrusion temperature around 140°C) reported by previous studies for low-moisture extrusion (Kaur et al., 2015; Abd El-Hady & Habiba, 2003).

The reduction in phytic acid can be considered one of the reasons for the improvement in protein digestibility for high-moisture meat analogues (Öztürk et al., 2024). Phytic acid can form complexes with protein and proteases in the intestinal tract, affecting the digestibility of proteins (Akande et al., 2017). These complexes can be destructed by extrusion, thereby exposing more enzyme binding sites and making proteins more susceptible to hydrolysis (Pei, Deng & Li, 2024; Alonso, Aguirre & Marzo, 2000). However, IVPD of the meat analogues in this study were similar to or higher than the IVPD of the raw blends (Table 5.7). The reduction of PA by extrusion may have some role in these similar or higher IVPD results for the HMMAs compared to their respective raw blends. However, it should be noted that the high level of feed moisture may reduce the residence time in the barrel and hinder the denaturation of proteins during extrusion (Pismag et al., 2024), offsetting the improvement by the reduction of PA.

Table 5.10 Phytic acid (PA) content of the raw blends and the meat analogues.

SFM concentration (%)	FMC (%)	Temperature (°C)	PA content (mg / g, d.b.)
40	Raw		15.83 ± 0.91^{a}
	48	125	5.04 ± 0.21^{bcd}
		135	4.23 ± 0.02^{cde}
		145	6.04 ± 0.12^{b}
	58	125	3.83 ± 0.12^{de}
		135	2.70 ± 0.09^{e}
		145	4.92 ± 0.11^{bcd}
50	Raw		15.38 ± 0.21^{a}
	48	125	5.71 ± 0.18^{bc}
		135	5.17 ± 0.08^{bcd}
		145	5.45 ± 0.13^{bc}
	58	125	4.59 ± 0.07^{bcd}
		135	3.89 ± 0.07^{de}
		145	4.71 ± 0.04^{bcd}

SFM: sunflower meal, FMC: feed moisture content, PA: phytic acid, d.b.: dry basis. Data is expressed as mean ± standard (n = 2 for phytic acid measurement). Values followed by different letters in a column are significantly different ($p < 0.05$).

5.7 Trypsin inhibitor activity (TIA)

The trypsin inhibitor activity (TIA) of the raw blends and the meat analogues is shown in Table 5.11. The range of TIA for the raw blends was from 1.79 to 1.89 TUI / mg (d.b.). Trypsin inhibitor can inhibit the activity of pancreatic enzymes (e.g., trypsin) by binding with dietary proteins and forming stable, indigestible complexes, leading to the reduction of digestion and absorption of proteins (Avilés-Gaxiola et al., 2018). The reduction level of TIA depends on the types of raw materials and the processing methods used (Nikmaram et al., 2017). Different materials have varying levels and types of trypsin inhibitors (Avilés-Gaxiola et al., 2018). Typically, sunflower meal is considered to carry little trypsin inhibitors (0.53 TUI / mg, d.b.) compared to other oilseeds (Filho & Egea, 2021; DellaGatta & Piergiovanni, 1996). Compared to legumes, pea also has low TIA, around 1.36 TUI / mg (d.b.) (Linsberger-Martin et al., 2013). Furthermore, there was a significant ($p < 0.05$) decrease of TIA after extrusion, which could be attributed to the thermal and mechanical energy input causing degradation of trypsin inhibitor during extrusion (Batista, Prudêncio & Fernandes, 2010). Trypsin inhibitor exhibits a certain degree of heat stability, however, the combination of high-temperature and moisture during extrusion processing can reduce its inhibitory activity (Batista, Prudêncio & Fernandes, 2010; Nikmaram et al., 2017). Extrusion above 140°C with FMC around 20% was reported to effectively reduce TIA up to 94.6% for peas (Alonso et al., 1998), 72.38% for cereal brans (Kaur et al., 2015) and 70% for beans (Batista, Prudêncio & Fernandes, 2010). However, the maximum reduction of TIA was 64.2% in this study, which was observed during extrusion at 145°C and 48% FMC for 50% SFM-based meat analogues. This may be attributed to the relatively gentle conditions at high-moisture extrusion

(Oliveira et al., 2017). Overall, the TIA was lower than 5 TUI/mg, which can be considered low, as it can be compensated by increasing trypsin production from body (Fagbenro, Adeparusi & Jimoh, 2010).

Table 5.11 Trypsin inhibitor activity (TIA) of the raw blends and the meat analogues.

SFM concentration (%)	FMC (%)	Temperature (°C)	TIA (TUI / mg, d.b.)
40	**Raw**		1.89 ± 0.04^{a}
	48	**125**	1.05 ± 0.10^{cdef}
		135	0.79 ± 0.22^{ef}
		145	0.74 ± 0.06^{ef}
	58	**125**	1.33 ± 0.08^{cd}
		135	1.44 ± 0.16^{bc}
		145	0.96 ± 0.09^{def}
50	**Raw**		1.79 ± 0.05^{ab}
	48	**125**	0.83 ± 0.04^{ef}
		135	0.65 ± 0.18^{f}
		145	0.64 ± 0.08^{f}
	58	**125**	1.37 ± 0.04^{bcd}
		135	1.44 ± 0.15^{bc}
		145	1.12 ± 0.02^{cde}

SFM: sunflower meal, FMC: feed moisture content, TUI = trypsin unit inhibited, d.b.: dry basis. Data is expressed as mean ± standard (n = 2 for TIA measurement). Values followed by different letters in a column are significantly different ($p < 0.05$).

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CHAPTER 6: CONCLUSION

The objectives of this thesis were to: (1) produce texturized meat analogues made with sunflower meal and pea protein isolate by high-moisture extrusion under various temperature profiles and feed moisture contents, (2) evaluate the effect of extrusion temperature, feed moisture content and sunflower meal concentration in blends on the physical properties (texture profile, cutting strengths and color), protein quality (amino acid scores, *in-vitro* protein digestibility and *in-vitro* protein digestibility corrected amino acid scores) and antinutritional factors (total phenolic compounds, chlorogenic acid, phytic acid and trypsin inhibitor activity) of the meat analogues produced.

Defatted sunflower meal (SFM) obtained from a laboratory expeller press was blended with pea protein isolate (PPI) in two blending ratios (SFM:PPI = 40:60 and 50:50) to achieve a >70% (d.b.) protein content and <10% (d.b.) fat content for high-moisture extrusion. As expected, SFM was rich in sulfur amino acids, but limited in lysine, which can be complementary to pea protein isolate. The *in-vitro* protein digestibility of both raw ingredients was higher than 80%. Therefore, the blends had an improved protein quality with high IVPDCAAS values (more than 86%).

The blends were extruded with two feed moisture contents (FMC) of 48% and 58%, w.b., and three temperature profiles ((a) 60°C, 80°C, 115°C, 125°C, (b) 80°C, 100°C, 125°C, 135°C, and (c) 100°C, 120°C, 135°C, 145°C) The screw profile, screw speed and feed rate were kept constant. The meat analogues produced had visual fibrous structures in a parabolic pattern with the degree of texturization values ranging 1.00 to 1.32. Higher FMC significantly ($p < 0.05$) decreased both longitudinal and transverse cutting strengths, while die temperature had a minor effect on cutting strength compared to FMC. Regarding the texture profile of the meat analogues, the hardness ranged from 190.71 – 312.21 N, gumminess ranged from 155.23 – 266.55 N and chewiness ranged from 142.79 – 242.31 N. All these three texture attributes were significantly ($p < 0.05$) affected by the extrusion conditions (i.e., FMC and temperature), while springiness (0.89 – 0.95) was not. Increasing FMC caused a softer texture of the meat analogues, due to the reduced protein concentration. However, the effect of temperature on the texture was not as strong as the effect of FMC in high-moisture extrusion. The significant ($p < 0.05$) changes of texture attributes with temperature were only observed at meat analogues with 48% FMC when increasing temperature from 125°C to 145°C. Compared to meat analogues made from pea protein and cooked chicken, the meat analogues made with SFM and PPI had a harder and chewier texture. These harder SFM-based meat analogues resemble jerky-type foods available in the market, which may indicate their potential application in practice.

In general, extrusion processing led to a reduction of lightness and yellowness, while an increase of redness in meat analogues compared to their respective raw blends. Increasing FMC was able to significantly ($p < 0.05$) offset the darker colors likely caused by Maillard reaction. Although chlorogenic acid-caused green color formation was frequently reported in SFM-based bakery products, green color was not observed in the meat analogues in this study, likely because extrusion conditions vary from those of baking from the perspective of pH. During extrusion, pH was lower than 7 (result was not shown) which makes CGA-protein interaction difficult. Notably, the color of some meat analogues was not stable during the storage. The interaction between protein and CGA cannot be fully ruled out, which may occur at a low extent for meat analogues, especially those produced with 58% FMC during extrusion. The management of temperature and FMC during extrusion could be essential to minimize the long-term color change.

When talking about the nutritional quality of the SFM-based meat analogues, the protein content ranged from 70.25 to 74.38% (d.b.) which is slightly lower than chicken breast and beef steaks. However, IVPD of the meat analogues ranged from 86.23 to 88.53%, comparable to real meat and higher than commercial plant-based meat analogues. The negative effect of extrusion was partial amino acid loss. Lysine content for some meat analogues was decreased by extrusion which cannot meet the requirement for pre-school children (2 - 5 years) established by FAO/WHO (1991). While the first limiting amino acid for all meat analogues was tryptophan because the initial tryptophan content was low. Therefore, the overall IVPDCAAS of the meat analogues made by blending SFM and PPI was slightly lower than similar product made with soy protein and SFM. Besides, this study confirmed the effect of extrusion on the reduction of anti-nutritional factors (i.e., CGA, phytic acid and trypsin inhibitor). The reduction of CGA, phytic acid and trypsin inhibitor can reach up to 31%, 83% and 64%, respectively. High extrusion temperature reduced levels of chlorogenic acid, phytic acid and trypsin inhibitor, attributed to the thermal and mechanical input. However, total phenolic compounds content of the meat analogues varied from 9.68 to 12.22 GAE/g (d.b.), which was not affected by different extrusion conditions.

This research successfully incorporated SFM with PPI into high-moisture extrusion to produce texturized high-moisture meat analogues with comparable physical and nutritional quality to real meat. This study confirmed the potential of sunflower meal as a protein source for meat analogues production and provided insights into optimizing extrusion conditions. The utilization of SFM may increase its economic and nutritional value and the application of SFM in high-moisture meat analogues production may contribute to the circular economy for sunflower seeds in the future.

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CHAPTER 7: STUDY LIMITATIONS AND FUTURE DIRECTIONS

This study offered insight into the potential of mixing sunflower meal with pea protein in meat analogues production and a fundamental understanding of the effect of extrusion temperature and feed moisture content on the physical and nutritional quality of meat analogues. Among physical quality attributes, while the textural quality and color play a critical role in consumer acceptance of meat analogues, their flavor and sensory properties are also very important. Such sensory evaluation was not conducted in this study because of the lean formulas (i.e., only sunflower meal and pea protein isolate used). Additives, for example salt, oil and color agents, are necessary to improve the flavor and sensory properties of the meat analogues in the market (Guyony et al., 2023). The involvement of these additives may influence the rheological properties of the melt in the barrel of the extruder, resulting in the change of texturization (Kyriakopoulou et al., 2021). So, future studies should incorporate sensory analysis and consumer preference testing as well as texturization behavior to better understand the market potential of these formulations.

Extrusion processing in this study was performed using a lab-scale twin-screw extruder. For future studies, different types of extruders (e.g., barrel length, number of temperature zones, screw configuration) can be used to evaluate the effect of more combination of extrusion parameters (e.g., screw speed, feed rate, temperature profile) and determine the optimal conditions for high-quality meat analogues production. Besides, the response of sunflower proteins to the extrusion conditions may vary by their cultivars, post-harvest processing, growing and storage conditions. The investigation of the functional properties of various sunflower protein sources is needed to have a better understating on the use of sunflower meal.

For the meat analogues produced in present study, there were still some issues that need improvement. For example, the harder texture and darker color of the meat analogues was not desirable compared to lighter colored meat types like cooked chicken breast. In addition, CGA was not completely degraded, and it's oxidation led to color instability during storage, which can affect the final quality of the products. Further research could focus on addressing these existing issues and providing a more comprehensive understanding to help generate better products that would be more easily accepted by consumers.

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APPENDICES

Appendix 1 The direction of fibrous structure formation in meat analogues

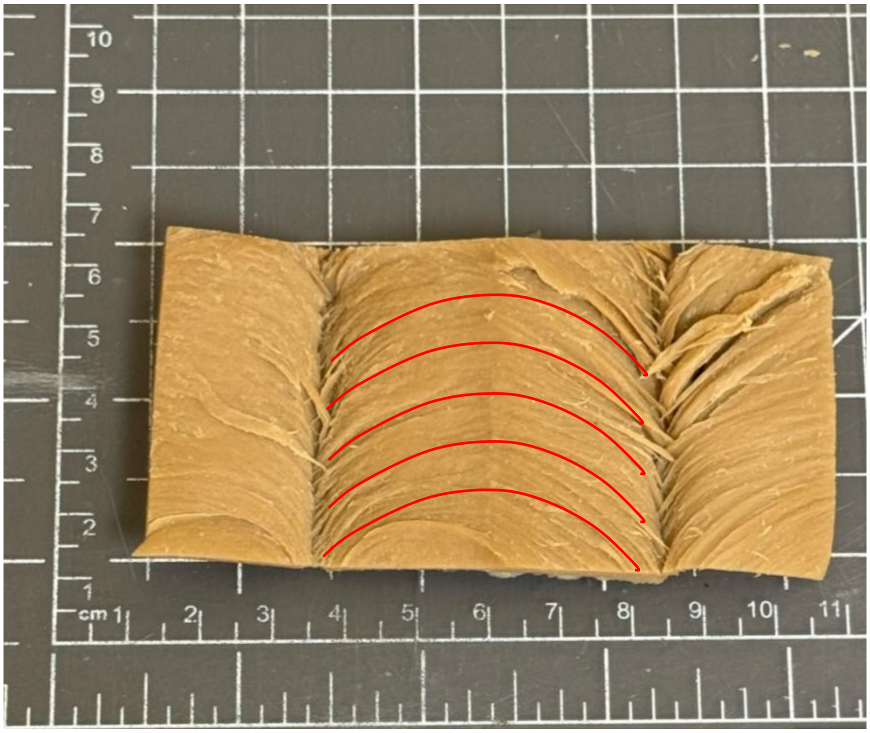

Figure A1. The parabolic pattern displayed in the meat analogues (the example shown here is the meat analogue produced with 40% SFM and 60% PPI, extruded at 125°C die temperature and 58% feed moisture content). The red curves are only placed to guide the eye.

Appendix 2 The change of color for meat analogues shortly after collection

Figure A2. Rapid color change of meat analogues shortly after collection (three replicates were collected one-minute interval, with the first (left) appearing darker and the third (right) becoming lighter).

Appendix 3 R scripts for statistical analysis

```
> #one-way aov
> Q1 <- read.csv("~/Desktop/Processed Data/Q1.csv")
> View(Q1)
> str(Q1)
'data.frame':	72 obs. of  9 variables:
 $ Blends      : chr  "40_60" "" "" "" ...
 $ Temp        : int  125 125 125 125 125 125 135 135 135 135 ...
 $ MC          : int  48 48 48 48 48 48 48 48 48 48 ...
 $ Name        : chr  "40_125_48" "40_125_48" "40_125_48" "40_125_48" ...
 $ Hardness    : num  242 243 236 262 266 ...
 $ Springiness : num  0.87 0.91 0.98 0.98 0.89 0.97 0.97 0.92 0.93 0.92 ...
 $ Cohesiveness: num  0.78 0.79 0.71 0.77 0.81 0.78 0.8 0.82 0.86 0.86 ...
 $ Gumminess   : num  189 192 167 202 215 ...
 $ Chewiness   : num  164 174 163 198 192 ...
> Q1$Name <- as.factor(Q1$Name)
> m1 <- lm(Q1$Hardness~Q1$Name, data = Q1)
> anova(m1)
Analysis of Variance Table

Response: Q1$Hardness
          Df Sum Sq Mean Sq F value    Pr(>F)    
Q1$Name   11 173524   15775  181.27 < 2.2e-16 ***
Residuals 60   5222      87                      
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> emm.name <- emmeans(m1, specs = "Name")
> emm.name
 Name      emmean   SE df lower.CL upper.CL
 40_125_48    253 3.81 60      245      260
 40_125_58    191 3.81 60      183      198
 40_135_48    307 3.81 60      300      315
 40_135_58    193 3.81 60      186      201
 40_145_48    312 3.81 60      305      320
 40_145_58    212 3.81 60      204      219
 50_125_48    254 3.81 60      246      261
 50_125_58    197 3.81 60      189      204
 50_135_48    304 3.81 60      297      312
 50_135_58    199 3.81 60      192      207
 50_145_48    302 3.81 60      294      309
 50_145_58    195 3.81 60      187      202

Confidence level used: 0.95
```

```
> library(multcomp)
> library(multcompView)
> cld(object = emm.name,
+     Letters = letters,
+     reversed = TRUE)
 Name      emmean   SE df lower.CL upper.CL .group
 40_145_48    312 3.81 60      305      320  a
 40_135_48    307 3.81 60      300      315  a
 50_135_48    304 3.81 60      297      312  a
 50_145_48    302 3.81 60      294      309  a
 50_125_48    254 3.81 60      246      261   b
 40_125_48    253 3.81 60      245      260   b
 40_145_58    212 3.81 60      204      219    c
 50_135_58    199 3.81 60      192      207    cd
 50_125_58    197 3.81 60      189      204    cd
 50_145_58    195 3.81 60      187      202    cd
 40_135_58    193 3.81 60      186      201    cd
 40_125_58    191 3.81 60      183      198     d

Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 12 estimates
significance level used: alpha = 0.05
NOTE: If two or more means share the same grouping symbol,
      then we cannot show them to be different.
      But we also did not show them to be the same.
```

(The script shown here is an example code used for statistical analysis of texture attributes.)