UNIVERSITÀ DEGLI STUDI DI PADOVA

Dip. di Agronomia Animali Alimenti Risorse Naturali e Ambiente

Corso di laurea magistrale in Scienze e Tecnologie Alimentari

Use of whey protein in meat analogues produced by high moisture extrusion: impact on texture, physicochemical properties, and sensory attributes

Relatore
Prof. Giovanna Lomolino

Laureanda
Sofia Degrasi
Matricola n. 2056014

ANNO ACCADEMICO 2024 – 2025

INDEX

ABSTRACT **5**

RIASSUNTO **5**

THESIS OBJECTIVES **7**

CHAPTER 1: INTRODUCTION **7**

1.1 Production of meat analogues **7**

1.1.1 Key ingredients and functional role 9

1.1.1.1 Oat Fiber Concentrate *10*

1.1.1.2 Pea Protein Isolate *11*

1.1.1.3 Potato Protein Isolate *13*

1.1.1.4 Whey Protein Isolate *14*

1.2 Extrusion of meat analogues **15**

1.2.1 Types of extrusion: HME and LME 15

1.2.2 Mechanisms of texture formation in high moisture extrusion 16

1.2.3 Benefits of HME in meat analogues production 18

1.2.4 Limitations of HME in meat analogues production 19

1.3 Sensory study of meat analogues **21**

1.3.1 Challenges in replicating sensory qualities of meat in plant-based analogues 22

1.3.1.1 Taste and flavor *22*

1.3.1.2 Texture and mouthfeel *23*

1.3.1.3 Appearance *24*

1.4 Physicochemical study of meat analogues **26**

1.4.1 Texture profile analysis 27

1.4.2 Aw 28

1.4.3 Water content 29

1.4.4 Color 30

CHAPTER 2: MATHERIALS AND METHODS 33

2.1 Sample preparation 33

2.2 Extrusion 34

2.3 Determination of mechanical and physicochemical properties 35

2.3.1 TPA 35

2.3.2 Color 36

2.3.3 Moisture content 36

2.4 Sensory evaluation 37

2.5 Statistical analysis 39

CHAPTER 3: RESULTS 41

3.1 First sensory study 41

3.1.1 Demographics 41

3.1.2 Pleasantness 42

3.1.3 CATA 44

3.1.3.1 CATA taste *45*

3.1.3.1 CATA Flavor *46*

3.1.3.3 CATA Texture *48*

3.2. Second sensory study 50

3.2.1 Demographics 50

3.2.2 Pleasantness 50

3.2.3 CATA 53

3.2.3.1 CATA taste *54*

3.2.3.2 CATA flavor *55*

3.2.3.3 CATA texture *57*

3.3 Physicochemical studies 59

3.3.1 Photos 59

3.3.2 Color 63

3.3.3 TPA 65

3.3.4 Aw 67
3.3.5 Water content 69

CHAPTER 4: VARIATION OF SENSORY ATTRIBUTES BETWEEN THE FIRST AND THE SECOND SENSORY STUDY 71

4.1 Pleasantness 71

4.2 CATA 75

4.2.1 CATA taste 75
4.2.2 CATA flavor 76
4.2.3 CATA texture 78

CHAPTER 5: CONCLUSIONS 81

REFERENCES 83

ABSTRACT

Hybrid meat analogues containing both animal and plant-based ingredients may help omnivores in transition to more sustainable diets. The aim of the study was to investigate sensory appeal of hybrid meat analogues including whey protein isolate (WPI) as an ingredient. We studied four meat analogues produced by high-moisture extrusion: Sample A included 50% pea protein isolate (PPI, commonly used in meat analogs), sample B included 25% WPI + 25% PPI, sample C included 25% WPI + 25% potato protein isolate (PoPI), and sample D included 50% WPI. In addition, all samples contained 50% OFC, oat fiber concentrate (rich in beta-glucan). The samples were subjected to two sensory studies, the first one with raw samples, the second one with samples deep fried in canola oil. The formulations were evaluated for pleasantness (of appearance, smell, taste, texture, and overall) using a 9-point scale), perceived flavor and texture attributes (using CATA), and ranking by an untrained panel (N1 = 100 panelists, N2 = 80 panelists). Pleasantness of all samples was evaluated, on average, around the middle of the scale (mean 4.1–5.9, on a scale 1-9) and by 49-60% of the panelists as 'mild'. Importantly, bitterness, a common challenge in plant protein ingredients, was rarely cited. The samples '50% PPI' and '25% WPI + 25% PPI' were rated as more pleasant in taste and texture than the sample '25% WPI + 25% PoPI', that was regarded by 80% of the panelists as 'dry' and 'floury'.

The samples were also subjected to physicochemical analyses, in particular color, TPA, water activity (aw), and moisture content.

WPI addition improved cohesion and fibrous structure, with the most notable benefits observed when combined with PPI rather than PoPI. In conclusion, our results suggest that WPI is a promising protein ingredient with mild flavor for meat analogues when used with PPI and oat fiber concentrate.

RIASSUNTO

I prodotti proteici analoghi della carne, contenenti sia ingredienti di origine animale che vegetale, rappresentano una soluzione per supportare i consumatori onnivori nella transizione verso diete più sostenibili. Lo scopo dello studio era quello di investigare il gradimento dal punto di vista sensoriale di prodotti analoghi della carne addizionati di isolato proteico di siero di latte (WPI). Le 4 formulazioni approfondite, prodotte tramite estrusione ad alta umidità, sono: campione A (50% PPI, isolato di proteine di pisello), campione B (25%

WPI + 25% PPI), campione C (25% WPI + 25% PoPI, isolato di proteine di patata), campione D (50%WPI). Tutti i campioni inoltre contenevano il 50% di OFC, concentrato di farina di avena, ricca in beta-glucani.

Sono stati effettuati due test sensoriali, il primo con i campioni tal quali, mentre il secondo studio è stato condotto utilizzando i campioni fritti in olio di canola.

Le formulazioni sono state valutate per "pleasantness" (gradevolezza di aspetto, odore, sapore, texture e generale) usando una scala a 9 punti, e per attributi di texture e sapore percepito (attraverso test CATA). Il panel era costituito da N1 = 100 partecipanti nel primo studio, N2 = 81 nel secondo.

La gradevolezza dei campioni è stata valutata, in media, nei pressi del centro della scala (4,1-5,9 su una scala da 1 a 9), e il 49-60% dei partecipanti ha indicato i campioni come "mild", senza un sapore dominante. Un dato significativo è che l'attributo "amaro" è stato raramente selezionato. Questo è un fattore cruciale, dato che un sapore amaro negli analoghi della carne spesso riduce l'accettazione da parte del consumatore.

I campioni contenenti 50% PPI e 25% WPI + 25% PPI sono stati valutati come più piacevoli per gli attributi sapore e texture, rispetto al campione 25% WPI + 25% PoPI, che l'80% dei partecipanti ha descritto come secco e farinoso.

I campioni sono stati inoltre sottoposti ad analisi chimico-fisiche, in particolare per colore, TPA, attività dell'acqua e contenuto di umidità.

L'addizione di WPI nelle formulazioni ha migliorato la coesione e la formazione di una struttura fibrosa, in particolare nei campioni contenenti PPI rispetto a quelli contenenti PoPI.

In conclusione, i risultati suggeriscono che WPI è un ingrediente promettente che non apporta sapore deciso alle formulazioni, e migliora la struttura dei prodotti a cui è aggiunto, soprattutto in combinazione con isolato di proteine di pisello e concentrato di farina di avena.

THESIS OBJECTIVES

The aim of this thesis is to investigate the effect of the addition of whey protein isolate (WPI) on texture, physicochemical properties, and sensory attributes of meat analogues produced through high-moisture extrusion (HME). By formulating and testing different protein blends, including pea protein isolate (PPI), potato protein isolate (PoPI), oat fiber concentrate (OFC), and WPI, this study seeks to identify how protein combinations influence product structure, quality, and consumer acceptance. The findings aim to contribute to the development of high-quality, nutritious, and appealing plant-based meat alternatives.

CHAPTER 1: INTRODUCTION

1.1 Production of meat analogues

The world population is expected to reach 9.8 billion by 2050 (Falcon et al., 2022), causing, together with the change of diet habits, an increase of protein demand between 32% and 43% in the next 25 years (Henchion et al., 2017). The demand is influenced not only by the growth of population but also by rising incomes, particularly in developing countries, where protein consumption tends to increase with economic growth. As more consumers adopt protein-rich diets and global demand rises, concerns about the sustainability of current protein sources become even more important.

Meat and dairy products are the primary sources of protein for the majority of the population of developed countries, with their consumption increasing globally. However, meat consumption is linked to environmental, ethical, and health-related issues.

From an environmental perspective, high levels of meat production demand a lot of resources, resulting in a significant carbon footprint and a decrease of natural resources. Animal agriculture contributes to a large share of global greenhouse gas (GHG) emissions, deforestation for the conversion of forests into arable land, biodiversity loss and water usage. Specifically, producing one kilogram of beef requires approximately 15,000 liters of water, a high amount compared to plant-based protein sources, which generally require far less water to produce the same nutritional content: for example, soybeans require around 2000 litres of water per kg of product (Mekonnen et al., 2012). The deforestation of areas to expand grazing areas contributes to the degradation of the ecosystem and is a threat to endangered species (Godfrayet al., 2018).

From an ethical point of view, reducing consumption of red meat is tied to concerns about animal welfare and the intensive farming practices used in modern meat production: animals are often overcrowded, stressed and subjected to unnatural living conditions, raising ethical concerns about how animals are treated to produce food.

Consumers, especially in developed countries, are becoming aware of these ethical issues, with the growth of movements toward "cruelty-free" and sustainable food practices. Public awareness and advocacy from animal welfare organizations have driven some individuals to adopt plant-based diets as a way to reduce their ecological and ethical footprint.

From a health perspective, reducing meat intake can have notable benefits. Clinical evidence indicates that adopting a plant-based diet, particularly one focused on minimally processed foods, may help lower health risks and support overall well-being in the long term. By

incorporating a variety of vegetables, legumes, and whole grains, plant-based diets provide essential nutrients, like fiber and antioxidants, that support immunity, reduce inflammation, and improve digestive health (Satiya et al., 2018).

The environmental, ethical, and health-related topics for reducing meat intake are strong and supported by a wide body of scientific literature: for all these reasons, reducing meat consumption is increasingly recognized as an essential step toward achieving a more sustainable, ethical, and nutritionally balanced diet, especially in the context of a growing global population. More people are embracing the idea of a transition from animal-based proteins to sustainable alternatives as a way to meet protein demands and address ecological and ethical concerns.

The growing demand for protein sources highlights the need for sustainable solutions, including plant-based proteins, algae, and insects. The objective is to reduce ecological effect while meeting nutritional needs; these alternatives contribute to food security by providing nutritious options that require less natural resources. Algae is a renewable resource, while insect protein has high nutritional value with minimal environmental impact.

Plant-based protein alternatives are well-established in the market, primarily sourced from soy (such as tofu and tempeh) and wheat gluten (like seitan): meat analogues are formulated to replicate sensory attributes, structure and functionality of traditional meat. Textural and sensorial properties are the key targets for the consumers, which require specific product characteristics. In recent years, the demand for plant-based meat substitutes has expanded beyond the vegetarian and vegan markets to include flexitarians and omnivores who are interested in reducing meat consumption for health or environmental reasons. Making plant-based meat alternatives with realistic textures and flavors is important to meet the expectations of different consumers. The growing interest in alternative protein sources has led to major progress in creating meat analogues: this trend reflects the need for more sustainable protein options and innovative solutions to keep up with the changes in the global food system.

Researchers and industries are working together to optimize sensory properties, protein quality, and sustainability in plant-based meat analogues. Techniques like high moisture Extrusion (HME), are becoming popular to create a fibrous, meat-like texture in these products**.** The growing demand for meat alternatives supports the shift towards plant-based diets or consuming meat in a more sustainable way (Malav et al., 2015); these alternatives offer vegetarians and to some extent vegans, a nutritious option for their meals (McClements et al., 2021). However, the limited sensory appeal and nutritional composition are still

challenges, because they can discourage consumers used to the taste and texture of traditional meat. Plant-based meat alternatives often have distinct flavor profiles due to plants production of flavor-influencing compounds (Wang et al., 2022). Researchers are working to mitigate these issues through flavor engineering and the use of natural flavoring agents. Another challenge is making sure that plant-based products provide enough nutrients, such as complete amino acid profile and similar nutrient density to animal meat.

Acceptability, cost-effective, reliable production, and consistent quality are essential for these products, but the top priority remains product safety, especially considering large-scale production. To gain consumer trust and meet legal requirements, production must ensure proper safety measures. At the same time, new regulations need to be developed to support the safe growth of alternative protein products in the global market.

1.1.1 Key ingredients and functional role

Texturised plant proteins are texturates formed from water and plant-based protein sources that have undergone a process to change them from powder-like materials to structured materials. Usually, plant-based meat analogues contain also flavourings, oils, binding agents and colouring agents: however, most of the used ingredients have to undergo a process of raffination, making them perceived as artificial products (Kyriakopoulou., 2021).

There are two main types of Texturised Vegetable Proteins (TVP) available in the market: low moisture TVP (LM-TVP) has a spongy texture, while high moisture TVP (HM-TVP) has a fibrous structure that resembles muscle meat.

Plant-based proteins, such as protein concentrates and isolates, are key ingredients in the formulation of meat analogues. Protein isolates are obtained through wet fractionation and consist of 75–90% protein, while protein concentrates are obtained through dry fractionation and have a protein content of 48–65%. Different methods of production lead to variations in the techno-functional properties and affect how these proteins perform when used as ingredients (Vatansever et al., 2020).

Traditionally, plant protein isolates are produced through wet extraction methods, which involve water and chemical intensive steps (such as extraction, centrifugation, and drying) to separate proteins from raw materials. While wet extraction produces high-purity protein isolates with the functional characteristics needed for HME, it is resource-intensive because of significant consumption of water, chemicals, and energy. Dry separation has recently

become popular as a more sustainable method, using physical processes like ultrafine milling and air or electrostatic classification. This method requires less energy and water, avoids chemical use, making it effective for producing protein concentrates from various legumes like peas, beans, and lentils (Zhu et al., 2021). Although dry fractionation yields protein concentrates of lower purity than isolates, the process does not involve the use of water or heating stages, preserving the native state of proteins. This results in improved functionality of the proteins such as solubility, foaming capacity, emulsion stability and gelling properties. Therefore, dry fractionation is increasingly regarded as a viable and environmentally friendly option for producing functional proteins for HME (Schutyser et al., 2015).

Traditionally, soy protein has been the primary ingredient used in HME due to its favorable texturizing properties, high protein content, and availability. However, as consumers have discovered the environmental impacts of soy production (such as deforestation and biodiversity loss in regions like the Amazon rainforest) there has been a shift toward alternative plant proteins. Examples are pea protein, fava bean protein, and more regionally sourced protein crops, that can help mitigate deforestation and align with consumers' sustainability expectations (Heusala et al., 2020).

Even though the choice of plant protein constituents forms the primary foundation for new product development, the specific choice is typically influenced by the availability of the protein, crop production, and protein extraction capability. In this study in particular, the main plant proteins used are oat fiber concentrate (OFC), pea protein isolate (PPI), potato protein isolate (PoPI), mixed in different blends to create different meat analogues. Whey protein isolate (WPI) was also included in some of the samples, to assess whether this component would enable the formation of an improved fibrous structure.

1.1.1.1 Oat Fiber Concentrate

Oat fiber concentrate is increasingly utilized in food applications, especially in the production of meat analogues, for its notable functional properties and nutritional benefits. This ingredient plays a key role in water retention, essential for keeping product moisture and enhancing their juiciness during cooking. The high water-holding capacity of oat fiber stabilizes meat analogue formulations by reducing moisture loss, improving texture and mouthfeel (Benkovic et al., 2023). This attribute is useful in high-moisture extrusion processes, where water retention aids in forming textures that resemble real meat.

Oat fiber improves the texture of meat analogues by adding volume and helping to create a denser, more cohesive structure: this improves the chewiness and elasticity of the final product. The addition of oat fiber is a great tool for reducing fat and calorie content, since it adds bulk with very few calories. This allows manufacturers to reduce fat content without losing the texture consumer expect (Leszczynska et al., 2023). These qualities make oat fiber a valuable ingredient for developing healthier, low-calorie products, that meet consumer demands for nutritious plant-based alternatives.

From a nutritional perspective, oat is a cereal grain that would potentially bring the health benefits of β-glucan to the final product. Oat fiber is rich in β-glucan, soluble fiber associated with cholesterol reduction and improved cardiovascular health (Rebello et al., 2016).

The viscosity of β-glucan, resulting from its degree of solubility and extractability in water, explains the glucose-attenuating role of β-glucan: this process of glycemic control can make fiber-fortified meat analogues more appealing to health-conscious consumers (Cloetens et al., 2015).

Oat fiber also supports digestive health thanks to its insoluble fiber content, which helps maintain gut health by adding bulk to stools and promoting regular digestion.

Furthermore, oat fiber provides important micronutrients such as B vitamins, magnesium, and iron, enhancing the overall nutritional profile of meat analogues (Chen et al., 2020). The inclusion of oat fiber concentrate in these products not only improves their structure, it also aligns with the growing trend for functional foods, which combines taste and health benefits.

1.1.1.2 Pea Protein Isolate

Pea protein isolate (PPI) has gained prominence as a functional protein source in meat analogue formulations due to its textural properties and nutritional profile: it presents an opportunity to develop an alternative plant protein source that can fulfill nutritional requirements in a cost-effective and sustainable way. As a protein source derived from yellow peas (*Pisum sativum*), PPI is highly valued for its ability to undergo textural transformations during processes like high-moisture extrusion, resulting in a fibrous, meat-like structure.

Pea protein can be used in food applications as an isolate (protein content higher than 80%) or in concentrate form (protein content lower than 80%): higher contents of pea protein isolate (up to 17%) increased the hardness and chewiness of meat analogues due to the creation of a more extensive network of protein cross-links A notable characteristic of

extruded meat analogues made from plant proteins is the compact nature of their protein/protein-based fibrous structures, which can lead to diminished juiciness and moisture retention (Sha et al., 2022).

The primary protein fractions in PPI**,** legumin and vicilin play a crucial role in texture formation. During thermal and mechanical processing, these proteins undergo denaturation and cross-linking**,** enabling the formation of a layered and aligned protein matrix that resembles the structure of animal muscle fibers. The protein alignment is reinforced by hydrogen bonds and disulfide linkages and contributes to the elastic and chewy textures desired in plant-based meat products (Peng et al., 2022).

From a nutritional point of view, PPI is advantageous due to its low glycemic index, high protein content (approximately 80-85%) and well-balanced amino acid profile, which is rich in essential amino acids, in particular branched-chain amino acids such as leucine, isoleucine, and valine. These amino acids are important for muscle health and metabolism, so products containing PPI are a good choice for consumers that want high-quality proteins without meat. PPI has a low content of certain amino acids such as methionine, but a nutritionally complete product can be created by combining PPI with other protein sources (Leterme et al., 1990).

Positive effects on cardiovascular health have been shown by pea protein because it is associated with lowering blood pressure and cholesterol levels due to the presence of bioactive peptides (Roy et al., 2010). Many studies suggest that incorporating peas into a balanced diet can help lower the risk of multiple cancers, such as breast, pancreatic, prostate, lung, and colon cancers, as well as leukemia. (Patel et al., 2024).

PPI is also hypoallergenic, making it a safe alternative for consumers with soy or dairy allergies. Additionally, PPI is a sustainable protein source: peas have a low environmental footprint, require minimal fertilizers, and can fix nitrogen in the soil; they enhance soil health and reduce the environmental impact of agriculture (Cellura et al., 2022). As a result, PPI is a top ingredient thanks to its structure-forming characteristics and its contribution to a balanced and nutritious diet. For these reasons, it is one of the preferred choices in plant-based protein formulations.

1.1.1.3 Potato Protein Isolate

Potato protein isolate (PoPI) is a functional ingredient in the high-moisture extrusion of meat analogues thanks to its ability to create stable emulsions between water and oil phases, and its positive impact on mouthfeel.

The emulsification effect allows PoPI to disperse effectively fat particles, maintaining even distribution of fat and moisture in the matrix: this creates a juicier and cohesive texture (preventing phase separation) that better replicates the sensory qualities of meat. As a result, the product not only retains moisture but also provides a resilient, mouth-coating texture similar to traditional meat (Schmidt et al., 2018). PoPI supports the creation of a tender, cohesive structure within the extruded product, leading to an enhanced mouthfeel and texture that closely mimics the juiciness and succulence of conventional meat products.

The sensory properties of potato protein, particularly its influence on mouthfeel, make it highly suitable for use in HME meat analogues. PoPI enhances the perceived juiciness and elasticity of the product, addressing two critical sensory qualities that are often challenging to replicate in plant-based meats. By improving the product's water-binding ability, PoPI contributes to a moist and tender mouthfeel that resembles muscle meat, enhancing consumer acceptance. Additionally, its gel-forming properties provide a resilient texture that holds up well to both cooking and mastication, offering a satisfying bite similar to that of traditional meat. (Kyriakopoulou et al., 2021).

Potato protein isolate is compatible with other plant proteins commonly used in HME, such as pea and soy proteins: this compatibility facilitates the formation of a cohesive protein matrix, allowing for better protein alignment and fibrous texture development during extrusion. By interacting with other protein isolates, PoPI supports the structural integrity of the extrudate, resulting in a final product that exhibits enhanced chewiness and structure while retaining a high moisture content (Lan Schut et al., 2020). PoPI's contribution to overall texture is particularly valuable in formulations aiming for complex, layered textures characteristic of meat, as it helps balance both resilience and softness within the matrix.

In summary, PoPI's emulsification properties, its impact on mouthfeel, and its compatibility with other plant proteins make it an effective component in the production of high-quality meat analogues via high-moisture extrusion. Its role in stabilizing water and fat, improving juiciness, and forming a cohesive texture matrix aligns with the sensory and structural requirements for realistic meat alternatives, making it a versatile and beneficial ingredient in plant-based formulations.

1.1.1.4 Whey Protein Isolate

Whey protein isolate (WPI) plays a key role in improving the texture, binding and structure of plant-based meat alternatives, especially in high-moisture extrusion processes. WPI has been discovered to be a useful component in plant-based formulations for simulating the fibrous and elastic structure of meat due to its high protein content, good solubility, and ability to form gels when heated. In HME, where plant proteins undergo a process of unfolding, aligning, and cross-linking to replicate meat's fibrous structure, WPI contributes significantly to texture development. Whey proteins unfold at high temperatures, exposing hydrophobic groups that interact with other proteins in the mixture, particularly under the high-shear conditions of extrusion. This protein alignment contributes to creating a layered structure that gives the appearance and mouthfeel of muscle fibers in meat (Wittek et al., 2021). The moist, fibrous matrix required for genuine meat textures is supported by WPI's capacity to improve water retention.

WPI facilitates the binding of plant protein components, resulting in a cohesive network that enhances the mechanical properties of the final product. When heated during extrusion, whey proteins form gels that contribute to elasticity and resilience in the extrudate: these are key qualities for replicating the chewiness and cohesiveness of meat. WPI's gelling ability stabilizes the product's structure and promotes the development of a solid yet flexible texture that can resist further processing and consumption without breaking or disintegrating (Lorenzen et al., 2006). Additionally, WPI's interaction with other proteins in the mixture contributes to the formation of intermolecular bonds, enhancing the texture and mouthfeel of meat analogues by increasing chewiness and structural integrity.

Functionally speaking, WPI's high solubility in water allows it to distribute uniformly throughout the mixture, guaranteeing consistent structural improvements throughout the product. This makes meat analogous more interesting to consumers by enhancing bite and juiciness, in addition to visual appeal. Furthermore, using WPI in HME formulations contributes valuable amino acids, complementing the nutritional profile of plant-based meat analogues (Wittek et al., 2021).

WPI enhances binding properties, improves texture and facilitates protein alignment, acting as a useful ingredient to create realistic, high-quality meat analogues through HME. The inclusion of WPI in plant-based protein formulations aligns with consumer demands regarding both sensory quality and nutritional value of alternative proteins.

1.2 Extrusion of meat analogues

Structuring is a crucial process for developing a desirable texture in plant-based meat analogues (Fu et al., 2023). To develop the fibrous texture necessary for realistic meat analogues, plant proteins undergo a series of structural modifications, including unfolding, cross-linking, and alignment, resulting in fibers that range from microscopic to macroscopic scale. Various structuring techniques such as extrusion, spinning, freeze structuring, 3D printing, and shear flow, have been applied to create the fibrous texture characteristics of plant-based meats (Ismail et al., 2020).

These methods use different mechanisms for generating fibrous textures in meat analogues: extrusion technology is considered one of the best techniques for creating textured plant protein meat analogues with a dense fibrous structure and elastic texture, that closely resembles animal muscle. In this process, ingredients undergo thermal and mechanical treatments that significantly change their physical and chemical nature, contributing to the texture and consistency desired for realistic meat analogues.

1.2.1 Types of extrusion: HME and LME

It is possible to differentiate between two types of processes for the extrusion of meat analogues: low-moisture extrusion (LME) for extrusions with a feed moisture content below 40%, and high-moisture extrusion (HME) for extrusions performed with a feed moisture content above 40% (Akdogan et al., 1999). Beyond moisture content, these two processes differ in the temperature of the extrusion, with LME performed at 120-150°C and HME at 140-170°C, influencing end product characteristics and their respective applications.

The product of LME consists of dry, small pellets of Textured Vegetable Proteins (TVP) with fibrous texture that can be easily rehydrated. These can be mixed with other ingredients to produce burgers, meatballs, and patties. The texture achieved is fibrous but lacks the dense, layered structure that characterizes whole muscle meats. Because of this, LME is more commonly applied to ground or minced-style products instead of whole cuts.

In contrast, HME produces larger, chunk-like structures that closely resemble muscle meat. Because of its high moisture content, HME produces non-expanded, fibrous products that are ideal for formulating chunk-type meat analogues, such as plant-based chicken breasts or beef strips. These products are well suited for direct use in meals that require whole-meat

textures, since they maintain the layered structure and fibrousness associated with animal meat. From the sensory point of view, high moisture extrusion provides better results in final product evaluation, mainly in term of fibrousness and finely layered meat-like texture. Therefore, HME remains a favored technique for manufacturers focused on creating realistic meat analogues (De Angelis et al., 2020).

1.2.2 Mechanisms of texture formation in high moisture extrusion

The most used technology for meat analogues production is high-moisture extrusion cooking, where plant proteins undergo considerable changes in the structure due to thermal and mechanical treatments. High-moisture extrusion technology can produce a fibrous, non-expanded product that closely replicates the texture and mouthfeel of traditional meat (Hood-Niefer et al., 2017).

The HME process involves several phases in the extruder: from a low-temperature mixing zone that hydrates proteins without changing their structure, to a moderate-temperature zone that achieves partial denaturation and culminating in a high-temperature melting zone where total denaturation of protein takes place. Later there is a zone for controlled cooling of the melt, that solidifies, facilitating phase separation and bond formation to finalize the fibrous structure.

The principle behind HME is that in a twin-screw extruder, under high-temperature and high-pressure conditions, the physical and chemical properties of plant proteins are modified. The high shear forces in the extrusion barrel align proteins in the direction of the flow. This alignment is the critical factor for achieving a multi-layered texture similar to animal muscles and gives meat analogues their typical bite and elasticity. The process not only transforms proteins into fibrous structures but also increases water retention, which contributes to juiciness, an important attribute for meat-like sensory qualities. The alignment of proteins, coupled with their denaturation and cross-linking, results in a fibrous structure that mimics the layered, muscle-like texture found in meat. High shear plays an important role, because it does not only stretch the protein molecules but also aligns them into a layered structure, giving the product its muscle-like appearance (Guyony et al., 2023).

The twin-screw extruder design is very important because it allows better control over shear forces, temperature distribution, and mixing compared to single-screw extruders. This makes

it possible to have greater flexibility in the processing of different plant protein sources, such as soy, pea, or wheat, and blending them to achieve desirable textures and functionality.

After the high shear phase**,** the extrudate enters a long cooling die, where temperature gradient and flow behaviour allow for gradual solidification while retaining aligned protein structure.

Controlled cooling is essential for the development of a dense, elastic texture that is resistant enough to be cut and chewed without collapsing, just like traditional meat (Schmid et al., 2022). As the product cools, the protein molecules retain their alignment to form a cohesive structure that then locks in the fibrous arrangement. The cooling die needs to be carefully regulated to avoid temperature gradients that will cause the rapid solidification of the product and can result in cracking or non-uniform texture in the final product. Maintaining consistent cooling parameters also ensures that the product can meet industry standards for mechanical strength and sensory quality. This process of intensive sharing, followed by gradual cooling, promotes unfolding and denaturation of protein structures, enhancing interaction between proteins and carbohydrates and resulting in the formation of fibrous meat-mimicking structures, that meet both sensory and visual expectations.

To achieve optimal results, high moisture extrusion needs specific designs for both the extruder and the die: their configurations must ensure perfect mixing and shearing in the screw and texturization and cooling in the die. This process also requires the use of concentrated protein sources, which include plant protein concentrates (70% proteins dry basis), plant protein isolate (90% dry basis protein), or gluten (80% dry basis protein) used individually or in blends. Additionally, additives such as hydrocolloids, oils, and natural flavors can be incorporated to improve functional properties and mouthfeel.

Unlike TVP, HME extrudates are highly perishable because of their moisture content and call for careful handling in hygienic conditions. After forming the extrudates, they are cooled, shaped and frozen to maintain quality (Guyony et al., 2023). The freezing step is important to maintain the structural integrity and texture of the product, especially during transportation and storage. Inadequate freezing or storing of the extrudate can result in texture degradation or microbial spoilage, making these steps essential for commercial viability.

1.2.3 Benefits of HME in meat analogues production

High-moisture extrusion helps to more closely achieve the characteristics of a meat product in both sensory and structural aspects. Because of this, it is considered an important technique in plant-based product development. One of these benefits is enhanced moisture retention, that creates a juicier, more appealing texture similar to real meat. This is explained by the stable interactions in the protein matrix, which can trap water molecules inside the structure and help prevent moisture loss during production and cooking. As a result, HME-based products retain qualities associated with animal meat, including a succulent bite and tender mouthfeel, two critical elements for achieving consumer acceptance (Hu et al., 2024). In addition to juiciness, HME also strengthens the structure of plant-based analogues through the creation of cohesive, fibrous textures similar in structure to muscle tissue found in meat. The high shear developed in this process aligns and cross-links the proteins to create a layered, muscle-like texture that reduces the need for additional binders. This makes the development of product formulations easier by using fewer ingredients, for a "cleaner" ingredient profile, keeping form and resilience in the product when prepared and cooked. This is ideal for whole-cut or chunk-style meat alternatives. The improved mouthfeel is an important feature of any product's appeal, and the fibrous texture enhances the sensory experience and makes HME products highly suitable for various ways of cooking (Sui et al., 2024).

Additionally, extrusion is very adaptable to a wide range of plant protein sources such as soy, pea, chickpea and faba bean, allowing product developers to create analogues with different nutritional profiles and textures. With this flexibility, products can be created to meet different dietary needs and sensory expectations. Accommodating various protein sources, HME allows for innovations in flavor, texture, and appearance; it broadens the range of high-quality meat analogues popular to the consumer base. Combining improved moisture retention, strong structural integrity, and ingredient versatility, HME has established itself as one of the leading technologies to make plant-based meats that accurately mimic the sensory attributes of traditional meat.

HME presents nutritional benefits in plant-based meat analogues, mainly by improving protein bioavailability and reducing antinutritional factors. Thermal and mechanical processes involved in HME decompose heat-sensitive antinutritional compounds, including phytic acid, trypsin inhibitors, and tannins. These interact negatively with nutrient absorption

and protein digestibility, but the high temperatures and shear forces involved in HME decrease their activity, enhancing the overall nutritional quality (Nikmaram et al., 2017). This benefit is advantageous in pulses and legumes, which are staple protein sources in plant-based analogues but naturally contain higher levels of antinutritional factors.

Besides, HME increases protein and starch digestibility, increasing the effectiveness of digestion and utilization of these nutrients by the body. Improved digestibility makes HME-processed products suitable as ready-to-eat options, being attractive to both underdeveloped regions, where protein availability is limited, and developed regions, which pay more attention to healthy nutrition. Research indicates that this process also conserves essential amino acids in food, boosting the nutritional profile of plant-based analogues and meeting dietary protein needs more efficiently (Huang et al., 2022). In addition, HME can retain essential vitamins and micronutrients, such as B vitamins and minerals, which could be lost in high-temperature processes. The potential to fortify extruded products with additional nutrients during processing also adds flexibility, allowing manufacturers to enhance the nutritional density of plant-based analogues based on regional dietary needs or specific health trends (Jang et al., 2024). As a result, HME supports nutrient retention and improves the overall health appeal of plant-based products, other that replicating meat's sensory qualities.

1.2.4 Limitations of HME in meat analogues production

The main limitations and considerations of high-moisture extrusion in plant-based meat analogues are related to sensory, structural, and nutritional qualities. While HME can create products with a fibrous texture that mimics animal meat, achieving a sensory profile comparable to conventional meat remains challenging. For instance, plant-based proteins generally lack specific flavor and aroma compounds characteristic of meat: because of that, an extensive use of flavor additives is often required, which may be perceived as overly processed by consumers (Wild et al., 2014). The use of natural flavor enhancers, such as yeast extracts or miso, is an emerging solution to improve sensory profiles without relying heavily on synthetic additives. These ingredients can boost umami flavors and create a more balanced taste profile. Additionally, due to the high temperature and pressure involved, certain ingredients may lose their functional properties during HME, which can limit the ability to achieve desired textural qualities consistently.

Structurally, while HME is effective in producing fibrous textures that resemble the layered appearance of muscle tissue, the extrusion process itself can lead to a rigid or dry texture if moisture is not carefully controlled. Because of that, binders or gums are often added in the formulation, which can detract from the natural composition of the product and affect mouthfeel (Fang et al., 2022). Alternatively, natural emulsifiers such as lecithin or pectin can be used to reduce dryness without compromising a more natural ingredient profile.

Moreover, since the fibrous network in plant proteins is different from that of animal muscle, plant-based analogues may lack the elasticity and bite of traditional meat, which can make for a less authentic eating experience (Osen et al., 2015).

From a nutritional point of view, studies have indicated that meat analogues may be nutritionally deficient compared to animal meat, lacking adequate levels of essential amino acids such as lysine and methionine, as well as key micronutrients like iron and zinc, which are more abundant in conventional meats (Guyony et al., 2023). While HME can reduce antinutritional factors (like phytic acid) that inhibit mineral absorption, it does not improve the aminoacidic profile or increase trace mineral contents. Moreover, the over-processing needed for creating flavor and textural profiles generally leads to high sodium content and sometimes genetically modified ingredients, which can concern consumers for health and environmental reasons (Schutyser et al., 2020). This makes it challenging to develop nutritionally comprehensive products with extrusion alone. Besides that, heavy reliance on highly processed ingredients and additives can create a negative perception among health-conscious consumers, and therefore further limit the market potential for these products in certain demographics.

The most promising strategy toward the value addition of nutritional aspects in HME-based products is pretreatment methods, such as soaking or sprouting that can enhance nutrient profile of raw materials before they undergo extrusion. Additionally, fermentation has shown potential to increase nutrient density and bioavailability in plant proteins by enhancing essential amino acid content and enriching flavors naturally (Luise et al., 2022). These methods, when combined with HME, offer a path forward for creating nutritionally improved plant-based products that better meet consumer expectations for a balanced meat alternative. As consumer demand evolves, integrating pretreatment methods and extrusion with clean label initiatives will be critical to ensure that products align with both nutritional and sustainability expectations.

1.3 Sensory study of meat analogues

Sensory analysis is a quality assessment method that uses human senses to evaluate the acceptability of products by measuring attributes such as color, taste, texture, and aroma (Younis et al., 2022).

Sensory analysis is essential for understanding preferences and acceptance of meat analogues of consumers. Sensory characteristics such as flavor, texture, and appearance are critical, consumer acceptance is also influenced by personal factors like ethical beliefs, environmental concerns, and familiarity with plant-based foods: these factors can encourage or discourage individuals from trying and liking meat analogues.

Plant-based products are often perceived as dry and lacking in juiciness. Another challenge involves replicating the taste and aroma of meat due to the complexity of the compounds associated with meat flavor (Li et al., 2020). Bitterness, astringency and off-flavors described as beany, green, vegetal, rancid, are commonly found in plant-based alternatives and are a limiting factor for the consumption of plant proteins (Murat et al., 2013).

These unwanted flavors result from the natural characteristics of the plant components or the conditions of processing and storage, like the oxidative deterioration of unsaturated fatty acids. Off-flavors present a challenge to consumer acceptance and are a key point for the development of plant-based meat analogues, despite significant progress in texture enhancements in the last years (He et al., 2020).

One significant challenge with novel foods like meat analogues is their unfamiliarity, which can lower consumer expectations and negatively impact sensory perception and overall enjoyment. Identifying which sensory attributes drive consumer liking is crucial, as is acknowledging that personal factors vary greatly among individuals. By combining sensory evaluation data with instrumental measurements like texture and color analysis, researchers can obtain a more comprehensive understanding of the physical and sensory qualities of meat analogues.

Before tasting, visual attributes such as shape, color, and overall appearance heavily influence consumer perceptions. Products that visually look like traditional meat are more likely to be accepted, even before consumers assess flavor and texture. Sensory evaluation plays a key role in exploring these attributes, offering insights into how sensory properties interact with other factors, such as cultural norms and dietary habits. This information can guide strategies to enhance product appeal and acceptance among different consumer groups.

Hedonic testing is one of the most commonly used methods in sensory evaluation. Also referred to as consumer acceptability tests, this method assesses how much people like or dislike a product based on its sensory characteristics. The test is performed by untrained participants that rate attributes like appearance, flavor, taste, and texture. The 9-point hedonic scale is widely used, ranging from "like extremely" to "dislike extremely." Another common method is the Check-All-That-Apply (CATA) test, where participants choose terms from a predefined list to describe each sample. These terms can be developed by trained experts or sourced from previous research.

In addition to hedonic testing, descriptive sensory analysis provides a deeper understanding of a product's sensory profile: it involves trained panelists who assess the intensity of individual attributes, such as how strong the flavor is or how firm the texture feels. Panelists undergo extensive training to ensure consistent and accurate evaluations. They rate sensory attributes on a defined scale, providing both qualitative and quantitative data. While consumer tests reveal preferences, descriptive analysis identifies specific attributes that need improvement to meet those preferences.

The two sensory evaluation methods have strengths and limitations. Consumer tests are useful for understanding overall liking and acceptance but are subjective and less precise. Instead, descriptive analysis provides detailed and measurable data but requires more time, resources, and trained personnel.

Mechanical analysis, such as TPA or cutting strength could be associated to sensory studies because they provide objective data on product properties such as hardness, elasticity, and chewability. However, these methods can be affected by variations in the shape or structure of the samples, which may lead to inaccuracies.

1.3.1 Challenges in replicating sensory qualities of meat in plant-based analogues

1.3.1.1 Taste and flavor

The development of taste and flavor in plant-based analogues is a primary challenge: the flavor of meat is complex and results from the combination of amino acids, fats, and reactions during cooking. Meat is rich in umami, due to the presence of amino acids like glutamate and nucleotides such as inosinate and guanylate, which create the savory taste typical of traditional meat (Toldrà et al., 2018). These compounds contribute to meat's

complex flavor profile and are difficult to mimic with plant-based ingredients, which lack the same amino acid composition and because of that require flavor additives or enhancers (Mouritsenet al., 2014).

Plant-based proteins, especially those derived from sources like pea, soy, and wheat, often have distinct, inherent flavors, such as beany or grassy notes, that can interfere with the perception of a meat-like taste (Aiking et al., 2011). These off-flavors such as aldehydes, ketones, and polyphenols derive from secondary metabolites in plants and can interfere with the appeal of the plant-based meat product. Flavor masking agents or processes like fermentation can be used to mitigate these undesired flavors: it's still difficult to obtain a balanced taste without altering texture or mouthfeel of the final product (Stone et al., 2015).

The Maillard reaction, a critical process during the cooking of meat, significantly enhances flavor by producing aromatic compounds such as browned, roasted flavors associated with cooked meat. However, plant proteins typically lack the same combination of reducing sugars and amino acids necessary for a comparable Maillard reaction, limiting the formation of similar notes during cooking (Naik et al., 2021). As a consequence, developers often incorporate reducing sugars or specific amino acids in formulations for plant-based analogues, although it is difficult to find the right balance to avoid excessive browning or unintended flavors (Li et al., 2020).

Developing a convincing meat flavor profile in plant-based analogues demands innovative strategies in flavor engineering, including the use of natural flavors, yeast extracts, and enzymatic treatments, which can mimic meat flavor (McClements et al., 2021). Achieving a stable, natural taste profile remains a key research focus, with the goal of creating analogues that are not only palatable but also nutritionally comparable to meat, without the overuse of additives.

1.3.1.2 Texture and mouthfeel

Texture and mouthfeel of meat in plant-based analogues are hard to replicate due to the unique structural and mechanical properties of animal muscle tissue. The texture of meat is made of fibrous arrangement of muscle proteins (myofibrils) and intramuscular fat: these components provide the elasticity, firmness, and juiciness that contribute to the typical mouthfeel of meat. This natural fibrous structure is not present in plant proteins like pea, soy, and wheat gluten, which makes it difficult to get the same level of elasticity and

chewiness in plant-based products without advanced processing methods (Dekkers et al., 2018).

One of the main textural challenges is the replication of a fibrous, layered structure that can mimic muscle tissue. High moisture extrusion is really popular: it applies high shear and pressure to align plant protein molecules into fibrous formations. This method creates a layered texture, giving plant-based products a structure that more closely resembles the bite and chewiness of meat. However, developing a consistent texture with different plant proteins is complex, because every protein source has its unique properties when treated under high-temperature and high-shear conditions. This can affect density, firmness, and cohesiveness of the final product (Cornet et al., 2022).

Moreover, mouthfeel is a very important factor to the overall sensory quality, and is defined by factors such as juiciness, fat distribution, and cohesiveness. Traditional meat gets much of its juiciness from intramuscular fat, naturally distributed throughout the muscle tissue, enhancing moisture retention and flavor release. Plant-based analogues, however, often rely on added fats and binders to replicate this effect, which may separate and not integrate uniformly throughout the product during the cooking process (Kyriakopoulou et al., 2019). Because of this, plant-based products can have drier or less cohesive texture, especially if they lack moisture-binding ingredients.

Achieving a balance between firmness and elasticity is challenging when using plant proteins. While wheat gluten offers some elasticity due to its glutenin and gliadin content, other plant proteins such as pea and soy may yield softer, less elastic textures, requiring binders and texture modifiers to achieve the desired firmness (Maningat et al., 2021). Developers are actively studying ingredient combinations and processing conditions to optimize mouthfeel and create plant-based analogues that resemble traditional meat.

1.3.1.3 Appearance

Appearance is a really important factor in consumer perception and acceptance of meat substitutes (Hoek et al., 2004). Traditional meat has a distinctive color that ranges from red to brown, due to the presence of myoglobin and its interactions with oxygen during cooking. This change of color, which signals the cooking state and flavor to the consumer, is difficult to replicate in plant-based products that lack myoglobin. As a result, developers often rely on natural or artificial colorants (beet juice, tomato extract, or heme-containing proteins) to mimic the color changes of meat during cooking (Joshi & Kumar, 2015). Achieving color

stability across cooking temperatures and storage conditions still have difficulties as plant-based colorants may degrade during shelf life (Wu et al., 2022).

In addition to color, another challenge is replicating the marbling and structural integrity of traditional meat. Marbling is the distribution of fat within muscle tissue: this contributes to meat's juiciness and flavor but also plays a role in visual appeal. Developing a marbled appearance in plant-based analogues requires innovative techniques to incorporate fat in the protein matrix, such as co-extrusion of protein and fat layers or encapsulation methods that prevent fat separation (Kyriakopoulou et al., 2019). Despite the help of these new techniques, plant-based products often have less uniform fat distribution, which can negatively impact consumer perception of authenticity.

The structural integrity of whole-muscle meat cuts (steaks or chicken breasts) is difficult to develop in meat analogues due to absence of fibrous and aligned muscle tissues (Dekkers et al., 2018). High moisture extrusion (HME) and other structuring technologies have helped to replicate some of these structural features by aligning plant proteins into fibrous formations: replicating the complex structure of whole-muscle meats is still limited in formulations (Schreuders et al., 2019). New advancements in extrusion and structuring methods are essential to more accurately mimic meat's appearance, enhancing the appeal and marketability of plant-based analogues.

1.4 Physicochemical study of meat analogues

For a complete evaluation of meat analogues, the best approach is combining sensory methods with instrumental techniques: this allows researchers to understand the quality and sensory characteristics of the product and guides the development of meat analogues that better meet consumer expectations.

Texture is one of the most important factors in evaluating meat analogues, as it determines how the product feels during chewing. Instrumental methods for measuring texture rely on mechanical tests, which analyze a product's response to external forces, such as compression, shearing, and cutting. Texture Profile Analysis (TPA) is commonly used to measure parameters like hardness, cohesiveness, and chewiness. Cutting tests, on the other hand, measure the force required to slice a sample, providing information about its firmness and fibrousness. These techniques are essential for evaluating if plant-based products can replicate the structural properties of animal-based meat (Samard et al., 2018).

In recent years, advanced techniques such as spectroscopy and imaging methods have also been introduced to study the texture and internal structure of meat analogues. These approaches complement mechanical tests by providing a deeper understanding of how processing methods and ingredient composition influence the product's final texture.

Moisture content, water activity (aw), and color are other parameters that affect sensory properties and shelf life of meat analogues. Moisture content is linked to juiciness and mouthfeel, while aw is related to microbial stability and overall freshness. Color, determined by instrumental techniques like colorimetry, is another important attribute, since it defines the appearance of the product before consumption (He et al., 2020).

Combining traditional mechanical tests with advanced analytical techniques, researchers can understand the quality attributes of plant-based meat analogues. This knowledge is essential for improving product formulations and ensuring consistency in texture, flavor, and appearance, with the aim of enhance consumer satisfaction.

1.4.1 Texture profile analysis

Texture Profile Analysis (TPA) is a method used to assess the mechanical and physical characteristics of food by simulating the chewing process. This technique involves a two-cycle compression of a sample between two parallel plates, to produce a deformation that mimics chewing. Quantitative data concerning different parameters of texture can be obtained through a force/time graph (Mabrouki et al., 2023).

Texture Profile Analysis is a sensible, objective and easy to operate method used to quantify the quality factors. The results of this method can be divided into primary and secondary results. Primary result are hardness (the force required to compress the food), cohesiveness (how well the food holds together), springiness (the ability of the food to return to its original shape after being compressed), adhesion, while secondary results are gumminess (the energy needed to disintegrate a semi-solid food), chewiness (the energy required to chew solid food until it is ready to swallow), resilience (Mateen et al., 2023). Each of these parameters is really important to understand how a food product will feel in the mouth during consumption.

For plant-based meat analogues, TPA is important because texture is one of the important factors in replicating the sensorial experience of traditional meat. Firmness, elasticity, and juiciness are really important parameters in the texture of meat, and they derive from the fibrous structure typical of animal meat. These factors contribute to mouthfeel and overall eating quality of meat (Dekkers et al., 2018).

Plant-based products are often made with proteins from peas, soy, wheat, that lack the natural fibrous structure of animal muscle. For this reason, to achieve a meat-like texture, complex processing is needed: an example is high-moisture extrusion, which aligns plant proteins into fibrous structures. TPA is really important in evaluating the efficiency of these processing techniques. The importance of texture in consumer acceptance cannot be overstated: consumers often judge the quality of plant-based meat analogues based on the textural similarity to traditional meat (Sha & Xiong, 2020). If a plant-based product is too tough, crumbly, or gummy, it can negatively affect the overall sensory experience and reduce consumer satisfaction. With analysis of texture, manufacturers can quantify and optimize the textural properties of their products to match consumer expectations. Parameters such as hardness and cohesiveness indicate that the product feels firm and fibrous, while springiness and chewiness reflect how satisfying the product is to bite and chew. It can also help the improvement of production processes and ensure the consistency of the final product. With

this analysis the effects of modifications (such as ingredient composition, moisture content, extrusion conditions) on texture can be measured and controlled, enabling developers to produce analogues with consistent quality.

TPA is a powerful and versatile method to help the sensory characteristics of meat be closely replicated in plant-based products. This method also serves as a way to deliver innovation and insights in the developing industry. Combining data from TPA, sensory analysis and consumer feedback, researchers and food producers can better engineer their products to closely mimic the experience of traditional meat, making them appeal to a wider audience (Bourne, 2002).

1.4.2 Aw

Water activity (aw) is the measure of free water in a food that can support chemical reactions and microbial growth. It is not equivalent to total moisture content, which is all the water contained in a product (including water bound to other molecules). Water activity deals with the portion of water that impacts food stability, quality, and safety.

Aw is calculated as the ratio of the vapor pressure of water in a substance to the vapor pressure of pure water under identical conditions. This parameter is measured on a scale from 0 to 1, and the higher the available water, the higher the values. Most of the hazard and spoilage microorganisms, such as bacteria, yeasts, and molds, grow at aw above 0.85: their control is crucial for food preservation and for determining shelf life of products (Tapia et al., 2020).

Maintaining the appropriate aw level is critical for microbiological and chemical stability when the product is stored. Low aw inhibits microbial growth, slows enzymatic activity, and reduces the rate of chemical reactions like lipid oxidation; however, if aw is too low, it can lead to over-drying, negatively affecting texture, taste, and consumer acceptability. It is very important to find the right aw for plant-based meat analogues, because water activity is linked to their texture and juiciness. Elevated aw value enhances succulence, which is essential to provide a juicy mouthfeel typical of conventional meat; however it increases the risk of microbial spoilage, especially if the product is stored in optimal conditions (Cornet, 2021). On the other hand, a very low aw can result in a dry, less palatable product that fails to meet consumer expectations. Plant proteins and fibers interact differently with water

compared to animal-based ingredients, making it challenging to achieve the right balance (Beuchat, 1981).

Control and measurement of aw in meat analogues are essential to improve shelf life without compromising quality. It requires a close understanding of the relationship between ingredients and water dynamics. For example, incorporation of humectants or alteration of processing conditions can make it easier to achieve the desired level of water activity. Research in this area highlights the importance of aw as a key factor in ensuring safe, stable food and in the overall product acceptability.

1.4.3 Water content

Water content (or moisture content) is the total amount of water contained in a food: it includes free and bound water and is usually expressed as a percentage of the total weight. Free water is not bound chemically to other molecules and can easily be lost or absorbed, while bound water is held within the structure of the food and is less available for chemical reactions or microbial growth. The distribution of water in food has an important impact on texture, taste and self-life.

Water content and water activity (aw) are not the same parameter: the first is a measure of the total amount of water contained in a food, the latter is a quantification of the water that is available for microbial growth and chemical reactions. Two foods with the same moisture content may differ in their water activity level, that will influence their shelf life. Aw is easier to quantify than moisture content, and the analysis is non-destructive.

Water influences food texture, juiciness, and overall sensory acceptability. It is a determinant of how fresh or appealing a product feels and affects its mouthfeel and palatability. Both water content and water activity must be controlled in order to maintain product quality and ensure safety.

In plant products, having the right water content is important to replicate the juiciness and tenderness of meat. Plant proteins tend to be different from animal proteins in water-binding capacities, and because of this the water content must be precisely regulated to obtain the desired texture. Additionally, water plays a fundamental role in food processing, especially in extrusion technology, which is extensively used in the production of plant-based meat analogues. The correct water balance promotes the correct dough developement, improves machinability, and helps create a fibrous, meat-like texture. Both water content and water

activity need to be understood and optimized in order to improve quality, self-life, and consumer acceptance of these products. Plant based meat analogues, with their high-water content, high protein levels and neutral pH, are susceptible to spoilage, making water activity control critical.

1.4.4 Color

Color is one of the first things consumers notice in food, and it strongly influences their perception and acceptance. Because of this, it is really important to replicate the color of traditional meat (both raw and cooked) when developing meat analogues, to make the product visually appealing. Analyses have shown that consumers may find meat analogues unattractive if their color does not closely resemble real meat, even if their taste and texture are satisfactory (Wu et al., 2024).

The main issue is that natural pigments in plant proteins have colors ranging from white to beige or yellowish-brown, which contrast with the red hue of raw meat. Food scientists have developed methods to create plant-based products which resemble actual meat. Food colors are obtained through natural plant extracts, such as beet juice, tomato powder, annatto, and caramel color. However, maintaining color stability is challenging, because these colorants can degrade when exposed to light, temperature, pH, and oxygen, resulting in color alterations that affect negatively product quality. Many natural pigments like anthocyanins, carotenoids, and chlorophylls, are sensitive to environmental conditions: if these pigments break down, the product may lose its expected appearance, reducing consumer appeal. Stable colorants should be selected for plant-based meat analogues because they need to maintain visual appeal throughout processing and storage.

The CIE Lab* system measures food color through L* indicating brightness (from black at zero to white at one hundred), a* showing green on the minus side and red on the positive side, and b* displaying blue on the minus side and yellow on the positive side. The CIE Lab* system provides both precise description and consistent appearance evaluation of food colors. (Markovic et al., 2011). The CIE Lab* system provides a quantitative basis for modifying color appearance in meat analogues to achieve authenticity compared to conventional animal meat products. Raw meat contains higher a* value (red) because of myoglobin pigment but cooking decreases myoglobin content and raises b* value (brown/yellow). To achieve the same color transition from raw to cooked state as real meat

plants need colorants which naturally or synthetically replicate this behavior during heating processes. Through the Lab system food scientists can adjust plant-based product formulations to create more realistic visual presentation which enhances consumer willingness to accept these products.

CHAPTER 2: MATHERIALS AND METHODS

2.1 Sample preparation

The samples consist of six blends made from four commercially available raw materials (Oat Fiber Concentrate, Potato Protein Isolate, Pea Protein Isolate, and Whey Protein Isolate). The names of the samples, their acronyms, and the companies they were purchased from are reported in Tab. 2.1.

Raw materials	**Acronym**	**Origin**
Oat Fiber Concentrate	OFC	Finnamyl Oy, Finland
Potato Protein Isolate	PoPI	Finnamyl Oy, Finland
Pea Protein Isolate	PPI	Finnamyl Oy, Finland
Whey Protein Isolate	WPI	Ingredia SA, France

Tab. 2.1 - Meat analogues ingredients: raw materials

The mixture compositions of our research are:

A. 50% PoPI + 50% OFC
B. 25% PoPI + 25% PPI + 50% OFC
C. 50% PPI + 50% OFC
D. 25% WPI + 25% PPI + 50% OFC
E. 25% WPI + 25% PoPI + 50% OFC
F. 50% WPI + 50% OFC

The mixtures were prepared by calculating the water content of raw materials, as the ratio between the different components of the mixtures must be based on their solid content, not the total weight. It's important to consider that each raw material contains about 5% water. The moisture content was calculated using the following formula:

The final mixture compositions, calculated considering the moisture content of the raw materials, are reported in Tab. 2.2.

Sample:	OFC (Kg)	PoPI (Kg)	PPI (Kg)	WPI (Kg)	Tot (Kg)
A	3,603	3,400	-	-	7
B	3,579	1,690	1,731	-	7
C	3,558	-	3,442	-	7
D	3,574	-	1,729	1,697	7
E	3,595	1,698	-	1,707	7
F	3,591	-	-	3,409	7

Tab. 2.2 – Compositions (kg) of the formulations of different samples.

Before the extrusion of each sample, feeding experiments were made to calculate the rate of the mixture going inside the extruder: this is done by setting the screw speed rate and measuring the quantity of flour that comes out in 1 minute. The measure is repeated two times, each time changing the screw speed rate.
Feeding experiments must be done every time before the extrusion because the rate depends on particle size and different electrostatic potentials of the flours.

2.2 Extrusion

A twin-screw laboratory extruder (Thermo Prism PTW24, Thermo Haake, Polylab System, Germany) and long cooling die (Flat Cooling Noozle FKD75, DIL Deutsches Institut für Lebensmitteltechnik, Quakenbrück, Germany) were used to produce the six extrudates. Only reverse osmosis water was supplied into the extruder as a liquid.
The total mass feed rate was 85 g/min, and the water content was 60%. The extruder barrel consisted of six temperature-controlled zones with the following temperature profile: 25°C, 40°C, 80°C, 100°C, 120°C and 150°C, respectively. The seventh zone had a temperature of 150°C and the temperature of the long cooling die was set at 40°C. 400 rpm was the chosen screw speed.
After extrusion, samples were manually cut into pieces of 20cm, 10cm, and 5cm, placed inside polyethylene zip-lock bags, and stored at –30 °C. When needed for further analysis, the samples were melted through a water bath of 30°C for 45 min.

2.3 Determination of mechanical and physicochemical properties

2.3.1 TPA

A Texture Analyzer TAXT2i (Stable Micro Systems, Surrey, England) was used to analyze the FMAs' mechanical characteristics. The samples were cut into specimens of 24 mm × 24 mm × 14 mm and placed on a flat aluminum sample holder. From each sample, five replicates were measured.

A cylindrical probe (Ø 36 mm) was used to compress the sample through two sequential vertical descents on every sample, while the corresponding data (force vs time) were registered. The settings were the same used by Ramoz-Diaz et al (Fibrous meat analogues containing oat fiber concentrate and pea protein isolate: Mechanical and physicochemical): load cell, 30 kg; pre-test speed, 1 mm/s; test speed, 1 mm/s; post-test speed, 5 mm/s; deformation distance, 7 mm (50% strain); resting time, 5 s; and trigger force, 5 g.

Each measurement resulted in a two-cycle force-time curve from which the parameters hardness, gumminess, springiness, and chewiness were calculated using the following equations and subsequently evaluated.

Gumminess: A2/A1 x Hardness
Springiness: L2/L1
Chewiness: Gumminess x Springiness

Hardness describes the maximum force of the first bite (A1).

Gumminess is calculated by dividing the area under the deformation curve of the second bite by the area under the deformation curve of the first bite.

Springiness is the ratio between the distance of the detected height during the second bite and the distance of the detected height during the first bite. L1 and L2 are the length of time corresponding to A1 or A2.

2.3.2 Color

The color of the samples was measured using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan). The color was defined as the L*, a*, and b* CIE-lab values. The sample's lightness is expressed by the parameter L* (0 indicates black and 100 indicates white). Positive values for the parameter a* express the sample's redness, whereas negative values express the sample's greenness. Positive values for the parameter b* express the sample's yellowness, whereas negative values express the sample's blueness. Each sample was measured ten times.

2.3.3 Moisture content

To calculate the moisture content of the samples, the initial weight of the sample before freeze-drying and the weight of the dried sample after the freeze-drying process are needed. Firstly, the samples were cut into cubes of 30 mm x 20 mm x 10 mm, weighed, put into plastic cups, and then covered with tin foil. Two replicates were made for each extrudate. Then the samples were placed in the freeze dryer (Lyovac GT 2 freeze dryer, Amsco Finn-Aqua GmbH, Hürt, Germany) at -60°C overnight, and the following morning the drying process was initiated.

After the freeze-drying process was completed, the samples were removed from the machine and put into a desiccator to allow them to reach room temperature without moisture absorption.

Lastly, the dried samples were weighed, and the moisture content was calculated through the following equation:

Moisture Content (%) = (Initial Weight - Dried Weight) / Initial Weight) x 100

2.4 Sensory evaluation

Two sensory studies were conducted to evaluate the samples before and after a deep-frying treatment with rapeseed oil. The sensory analyses were carried out with a set of untrained panelists, with 100 participants in the first study and 81 in the second. They were asked to evaluate the meat analogues based on the pleasantness of appearance, smell, taste, texture, and overall pleasantness, using a 1-9 point descriptive scale where 1 indicated "very unpleasant" and 9 indicated "very pleasant". The samples were also evaluated for perceived taste, flavor and texture attributes using CATA analysis. Tab. 1.3 provides all the sensory attributes, definitions, and scale anchors for the meat analogues used in the two sensory studies.

Panelists were asked to rank samples according to their preferences and provided information about their demographics and eating habits. To neutralize their sense of taste, panelists were instructed to drink water and eat snacks provided after evaluating each sample.

Before the sensory evaluation, the extrudates were melted and cut into pieces 1cm wide.

In sensory analysis 1, the extrudates were served without any additional treatment, while in sensory analysis 2 they were deep fried in rapeseed oil at 165°C for 3 minutes. The samples were then placed into plastic cups (with lids in the second study to prevent the absorption of moisture from the environment) and identified with a numeric code and arranged in a randomized order.

Out of the six extrudates produced, only four of them were submitted to the sensory study: sample A (50% PoPI + 50% OFC) and sample B (25% PoPI + 25% PPI + 50% OFC) were excluded based on a preliminary team evaluation, which found them to be too bitter to be included without risking of biasing the final results.

	ATTRIBUTE	DEFINITION	SCALE ANCHORS
Pleasantness	*Appearance*	Visual appeal of the product	1- very unpleasant; 9- very pleasant
	Smell	Appeal of aroma	
	Taste	Satisfaction of flavor	
	Texture	Satisfaction of the product in the mouth	
	Overall	General satisfaction and liking of the product as a whole	
Taste-related Attributes	*Sweet*	Association with sugar	X- percieved; Blank- not perceived
	Salty	Association with salt	
	Sour	Association with acidic taste	
	Bitter	Association with sharp or unpleasant taste	
	Umami	Association with savory, meaty taste	
Flavor-aroma descriptors	*Milk*	Creamy or dairy-like flavor or smell	
	Cereal	Grainy or oat-like flavor or smell	
	Pea	Earthy, plant-like flavor or smell	
	Potato	Starchy, nutty flavor or smell	
	Mild	Subtle and delicate flavor or smell	
Texture descriptors	*Dry*	Lack of moisture or juiciness	
	Juicy	Moist and succulent texture	
	Tough	Firm and resistant to chewing or cutting	
	Tender	Softness and easy to chew	
	Floury	Powderly texture	
	Gummy	Sticky and elastic texture	

Tab. 1.3 - Sensory attributes, definitions for meat analogues, and scale anchors for meat analogues (samples C, D, E, F).

2.5 Statistical analysis

Pleasantness data were analyzed using one-way ANOVA followed by Tukey's HSD (honestly significant difference) test. Significant differences were determined at $p \leq 0.05$ by SPSS (IBM Corp. Released 2021). IBM SPSS Statistic for Windows, Version 28.0. Armonk, NY: IBM Corp). CATA and physicochemical data were processed using means and standard deviations.

CHAPTER 3: RESULTS

3.1 First sensory study

As previously mentioned, only samples C, D, E, F were submitted to the sensory study. The first sensory evaluation was carried out with a set of 100 untrained panelists

Each of these formulations was assigned a 3 digits number:

Sample 1 -> 780 (50% PPI + 50% OFC)

Sample 2 -> 552 (25% WPI + 25% PPI + 50% OFC)

Sample 3 -> 223 (25% WPI + 25% PoPI + 50% OFC)

Sample 4 -> 451 (50% WPI + 50% OFC)

For simplicity, from this point onward, OFC will be omitted in the notation, as all samples contain 50% OFC.

An example of setting of the first sensory evaluation can be seen in Fig. 3.1.

Fig. 3.1 - Example of setting of the first sensory evaluation.

3.1.1 Demographics

The first sensory evaluation involved 100 panelists, their gender consisted of 72% females and 28% males. The age distribution was predominantly younger adults, with 58% aged between 18 and 29 years, followed by 22% between 30 and 39 years, 11% between 40 and 49 years, 6% between 50 and 59 years, and 3% aged 60 years or older.

Regarding dietary habits, 54% of participants were omnivores, 22% flexitarians, 20% vegetarians, and 4% vegans.

The frequency of plant-based meat analogue consumption varied among the panelists: 10% consumed them daily, 23% 3-6 times per week, 24% 1-2 times per week, 25% 1-3 times per month, while 18% consumed them more rarely.

3.1.2 Pleasantness

The attributes assessed include appearance, smell, taste, texture, and overall pleasantness, are reported in Tab. 3.1. Ratings are provided as mean values ± standard deviations. Superscripts (a, b, c) indicate statistical significance, meaning different letters mean significant differences between the samples.

Attribute	**PPI**	**WPI + PPI**	**WPI + PoPI**	**WPI**
Appearance	5,09±1,706^{ab}	5,28±1,518^{b}	4,64±1,618^{a}	5,38±1,662^{b}
Smell	5,16±1,619^{a}	5,03±1,766^{a}	5,15±1,617^{a}	5,41±1,870^{a}
Taste	5,08±1,978^{b}	5,15±1,828^{b}	4,09±1,670^{a}	4,64±1,872^{ab}
Texture	5,89±2,01^{c}	5,35±2,124^{bc}	4,30±2,003^{a}	4,68±2,059^{ab}
Overall	5,49±1,738^{c}	5,31±1,716^{bc}	4,37±1,649^{a}	4,80±1,627^{ab}

Tab. 3.1 - Results of pleasantness attributes in first sensory evaluation.

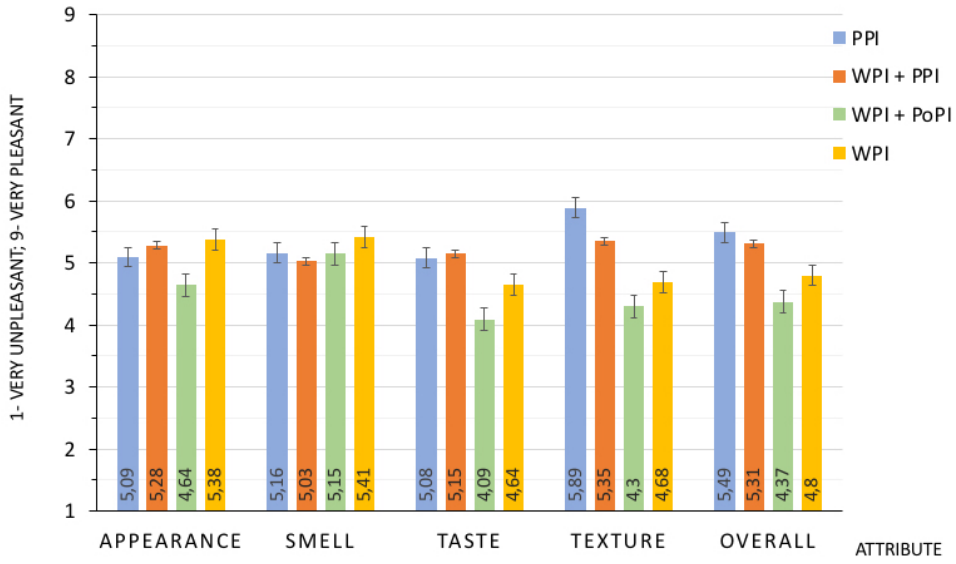

Fig. 3.2 - Pleasantness ratings of different attributes (appearance, smell, taste, texture, overall) in the first sensory evaluation. The parameters range from 1 (very unpleasant) to 9 (very pleasant).

The overall appearance of a product is important for priming consumers and developing expectations before consumption. Ratings of different attributes can be found in Fig. 3.2. In relation to appearance, WPI + PPI and WPI ranked the highest (5,28±1,518b and 5,38±1,662b), significantly higher than WPI + PoPI (4,64±1,618a). PPI was intermediate (5,09±1,706ab), not significantly different from WPI +PPI and WPI. This implies that the addition of potato protein may have a negative effect on the appearance of the product.

Concerning the smell, no basic differences existed among the samples (all 5,0-5,4), suggesting that the protein composition did not strongly influence the perceived aroma.

Taste results indicated that PPI and WPI + PPI were rated highest (5,08 ±1,978b – 5,15±1,828b), significantly better than WPI + PoPI (4,09±1,670a) which means that the addition of potato protein could have added negative taste attributes. WPI was rated in between, with no significant differences from the rest of the formulations.

Texture was an important differentiating attribute, and highest was scored by PPI (5,895,89±2,01c), much better than WPI + PoPI (4,30±2,003a). Intermediate were WPI + PPI and WPI again (5,35±2,124bc and 4,68±2,059ab), reinforcing the idea that the presence of potato protein negatively affected the texture.

Overall pleasantness followed the same direction, with PPI scoring the highest (5,49±1,738c), significantly above WPI + PoPI (4,37±1,649a). WPI + PPI and WPI came in

between (5,31±1,716bc and 4,8±1,627ab) and indicated that, while these formulations were acceptable, they did not match the sensory appeal of PPI alone.

PPI performed best in terms of sensory acceptance, particularly for texture and overall pleasantness. This means that pea protein isolate likely plays a key role in making the product more enjoyable. The WPI + PoPI formulation consistently scored the lowest, especially for taste, texture and overall appeal, indicating that the use of potato proteins may negatively impact key sensory attributes. WPI and WPI + PPI had intermediate values and WPI + PPI performed well in taste and appearance. Smell was unaffected by protein composition, suggesting that aroma is less impacted by protein type compared to other sensory traits.

3.1.3 CATA

The results of pleasantness attributes in the first sensory evaluation are reported in Tab. 3.2.

Attribute	**PPI**	**WPI + PPI**	**WPI + PoPI**	**WPI**
Sweet	16	16	7	6
Salty	22	16	4	8
Sour	6	7	6	3
Bitter	5	8	11	5
Umami	36	38	19	24
Milk	7	10	1	15
Cereal	44	38	61	47
Pea	45	32	26	17
Potato	20	24	26	19
Mild	55	49	58	60
Dry	43	52	84	75
Juicy	18	13	3	4
Tough	12	42	7	58
Tender	57	38	28	22
Floury	29	21	76	38
Gummy	18	37	8	32

Tab. 3.2 - Results of CATA attributes in first sensory evaluation.

3.1.3.1 CATA taste

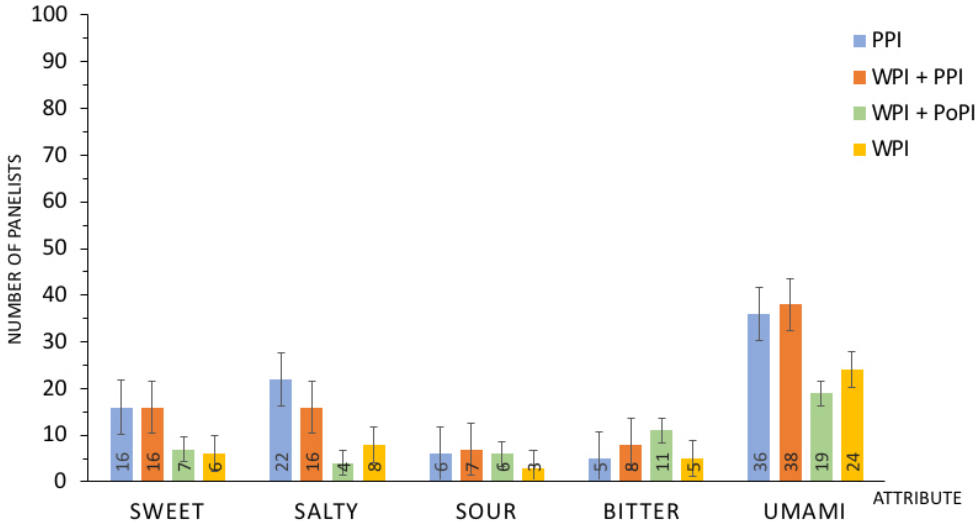

Fig. 3.3 - Number of panelists who selected given attributes regarding taste of 4 different formulations of meat analogues. The attributes considered for CATA Taste are sweet, salty, sour, bitter, umami.

The sweetness scores were very similar across all samples, with no significant differences in the percentage of participants selecting this attribute. The values were low for all samples: PPI (16%), WPI + PPI (16%), WPI + PoPI (7%), and WPI (6%). This indicates that none of the samples had a pronounced sweetness. Only a small minority of participants perceived any sweet taste, which is generally favorable in this product category where sweetness is not a desired trait. A visual representation of the data can be found in Fig. 3.3.

For saltiness, the sample with PPI was the most selected (22%), followed by WPI + PPI (16%) and WPI (8%). The WPI + PoPI formulation had the lowest saltiness (4%). This shows that adding potato protein might lower the perception of saltiness. The saltiness of the WPI + PoPI sample was markedly lower compared to the PPI sample, which could affect flavor intensity and overall consumer acceptance, given that saltiness is a key contributor to savory flavor in meat analogues.

Sourness was the least selected attribute across all samples PPI and WPI + PoPI both had 6% of participants selecting this attribute, WPI + PPI had 7%, and WPI had only 3%, indicating that none of the samples had a noticeable sour taste.

Bitterness, a common off note in plant proteins, was also selected infrequently. The WPI + PoPI sample had the highest bitterness score (11%), followed by WPI + PPI (8%), while both PPI and WPI samples had only 5% of participants associating them with a bitter taste. Although these numbers are relatively low, the slightly higher score for the WPI + PoPI blend suggests that the inclusion of potato protein may increase bitterness perception. However, the overall low selection frequency indicates that bitterness was not a major issue in any formulation.

Umami was the most prominent taste attribute across the board. WPI + PPI had the highest percentage of participants (38%) identifying this attribute, closely followed by PPI (36%). In contrast, the WPI + PoPI formulation scored significantly lower, with only 19% of participants selecting umami, while WPI alone had an intermediate score of 24%. These results suggest that adding PPI may enhance the savory quality of the product, whereas potato protein might actually diminish it.

To sum up the results of CATA taste, PPI and WPI + PPI performed better overall, particularly in saltiness and umami. The WPI + PoPI sample had lower scores in both saltiness and umami, suggesting that the addition of potato protein might negatively affect these taste attributes. All samples had very low sweetness, sourness, and bitterness, with no significant differences among them.

3.1.3.1 CATA Flavor

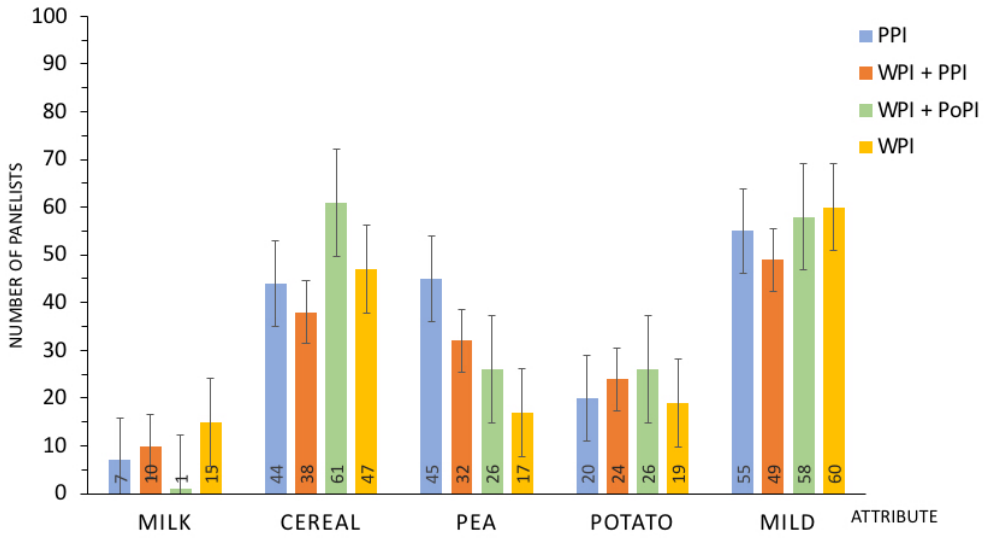

Fig. 3.4 - Number of panelists who selected given attributes regarding flavor of 4 different formulations of meat analogues. The attributes considered for CATA flavor are milk, cereal, pea, potato, and mild.

Milk flavor was generally weak across all samples, with no strong notes detected. The WPI formulation had the highest score (15%), followed by WPI + PPI (10%), PPI (7%), and WPI + PoPI (1%). While the values are low overall, the slightly higher score for WPI suggests a minor association between whey protein and milk-like notes. However, contrary to expectations based on the presence of potato protein, the WPI + PoPI sample had the lowest percentage, indicating that potato protein does not enhance the perception of milk flavor in this context. A visual representation of the data can be found in Fig. 3.4.

Cereal flavor showed more pronounced differences among the samples. WPI + PoPI had the highest score, with 61% of participants identifying this attribute, suggesting a strong cereal-like profile. WPI (47%) and PPI (44%) followed closely, while WPI + PPI had the lowest score (38%). These results indicate that WPI + PoPI stands out for its cereal flavor intensity, possibly due to synergistic effects between the two proteins and the oat fiber concentrate (OFC). The lower score for the WPI + PPI combination might suggest some masking or alteration of these notes when pea and whey proteins are blended.

As expected, pea flavor was associated with the PPI sample and was selected by 45% of participants.

The WPI + PPI formulation showed a lower score (32%), indicating that blending with whey protein reduces the intensity of pea-related notes. WPI + PoPI had an even lower value (17%), and WPI alone was at 26%. These results confirm that pea flavor is a key sensory marker of PPI and that its presence becomes less pronounced in blends, particularly when potato protein is used.

Perception of potato flavor was low across all samples, with no meaningful differences between them: WPI + PoPI and WPI + PPI both scored 24% and 26%, respectively, while PPI had 20% and WPI scored 19%. These minor differences suggest that potato protein, even when present in the formulation, does not strongly contribute to a distinct potato flavor. Additionally, the slight presence of this attribute in samples without added PoPI (such as PPI and WPI) implies that any potato-like flavor may also originate from other ingredients or processing effects rather than PoPI specifically.

All of the samples were rated similarly in terms of mildness, with none perceived as intense or overpowering. WPI scored the highest (60%), followed closely by WPI + PoPI (58%), PPI (55%), and WPI + PPI (49%). This is a really important result because it implies that all formulations maintained a generally mild flavor profile, regardless of protein composition.

This is an is often desirable in plant-based meat analogues intended to accommodate a variety of seasonings and applications

In terms of flavor attributes, WPI + PoPI stood out for its cereal and milk-like notes, while PPI contributed strongly to the pea flavor. Potato notes remained subtle, and all samples shared a generally mild profile. These results suggest that while specific proteins can shift certain flavor attributes, the overall taste remains balanced and not overly dominated by any single note.

3.1.3.3 CATA Texture

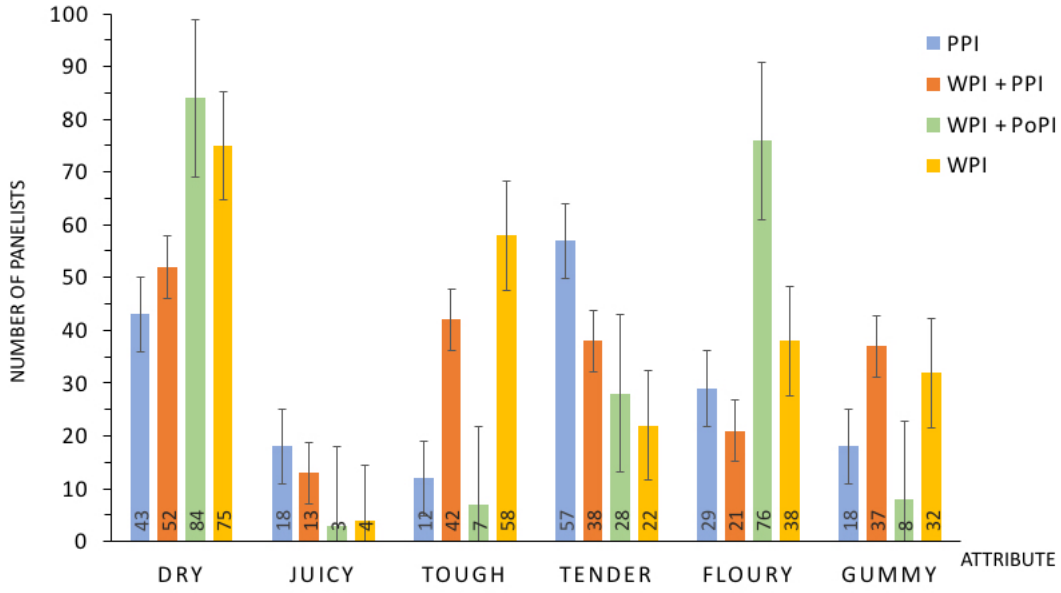

Fig. 3.5 - Number of panelists who selected given attributes regarding texture of 4 different formulations of meat analogues. The attributes considered for CATA texture are dry, juicy, tough, tender, floury, gummy.

Regarding dryness, samples WPI + PoPI scored higher dryness scores (84% and 75%) compared to PPI (43%) and WPI + PPI (52%), indicating a dryer texture when whey protein was included. Overall, all the four samples have high values of dryness. A visual representation of the data can be found in Fig. 3.5.

The juiciness scores, on the other hand, were generally low for all samples. PPI was perceived as the juiciest (18%), followed by WPI + PPI (13%). The lowest ratings were recorded in WPI + PoPI (3%) and WPI (4%) samples, reinforcing the idea that whey proteins may reduce perceived juiciness. This aligns with the dryness results, as the least juicy

samples were also the driest. In their study Sha and Xiong (2020), it suggested that the juiciness of meat analogues depends on how similar the protein network and the structure of animal tissues are. Cornet et al (2020) and coworkers showed that the presence of air pockets in meat analogues is related to juiciness of the final products.

For toughness, WPI scored the highest (58%), followed by WPI + PPI (42%). PPI (12%) and WPI + PoPI (7%) had lower scores, indicating a softer texture. The above results indicate that pea as well as potato proteins play a role in lowering toughness, while whey protein increases it.

Tenderness trended inversely: the PPI sample was the highest scoring (57%), highlighting the importance of PPI in contributing to a less hard and more tender mouthfeel. The remaining blends, WPI + PPI (38%), WPI + PoPI (28%), and WPI (22%) had significantly lower ratings.

Flouriness was strongest perceived in WPI + PoPI sample (76%), significantly greater than the others. PPI (29%) and WPI + PPI (21%) had lowest flouriness, and WPI (38%) was intermediate. This suggests a strong influence of PoPI in increasing floury texture perception.

As for gumminess, WPI + PPI combination was the most selected (37%), followed closely by WPI (32%) relatively high. In contrast, WPI + PoPI (8%) and PPI (18%) showed considerably lower values. This suggests that PoPI could decrease gumminess, while WPI tends to increase it.

To sum up the flavor results, the addition of PoPI increased dryness and flouriness but reduced juiciness and gumminess, indicating a drier, flourier texture overall. PPI added positively to tenderness and juiciness and lowered toughness and gumminess. WPI produced the toughest and one of the driest samples. Generally, PPI seems to add to textural attributes related to tenderness and juiciness, whereas PoPI has a greater effect on dryness and flouriness.

3.2. Second sensory study

As previously mentioned, only samples C, D, E, F were submitted to the sensory study. The second sensory evaluation was carried out with a set of 81 untrained panelists.
Each of these formulations was assigned a 3 digits number:
sample 1 -> 121 (50% PPI + 50% OFC)
Sample 2 -> 347 (25% WPI + 25% PPI + 50% OFC)
Sample 3 -> 669 (25% WPI + 25% PoPI + 50% OFC)
Sample 4 -> 460 (50% WPI + 50% OFC)
For simplicity, from this point onward, OFC will be omitted in the notation, as all samples contain 50% OFC.

3.2.1 Demographics

The second sensory evaluation involved 81 panelists, their gender consisted of 75,3% females, 23,5% males and 1,2% others. The age distribution was predominantly younger adults, with 44,4% aged between 18 and 29 years, followed by 30% between 30 and 39 years, 18,5% between 40 and 49 years, 4,9% between 50 and 59 years, and 2,5% aged 60 years or older.
Regarding dietary habits, 53,1% of participants were omnivores, 19,8% flexitarians, 26% vegetarians, and 1,2% vegans.
The frequency of plant-based meat analog consumption varied among the panelists: 8,6% consumed them daily, 16 % 3-6 times per week, 34,6% 1-2 times per week, 30,9% 1-3 times per month, while 9,9% consumed them more rarely.

3.2.2 Pleasantness

The attributes assessed include appearance, smell, taste, texture, and overall pleasantness, with ratings provided as mean values ± standard deviations. Superscripts (a, b, c) can be seen in Tab. 3.4, and indicate statistical significance, meaning different letters denote significant differences between the samples.

	Attribute	PPI	WPI + PPI	WPI + PoPI	WPI
PLEASANTNESS	Appearance	5,52±1,769^{bc}	4,43±1,843^{a}	6,16±1,721^{c}	5,05±1,731^{ab}
	Smell	6,11±1,796^{a}	5,81±1,621^{a}	6,36±1,996^{a}	5,72±1,712^{a}
	Taste	5,38±1,947^{b}	5,41±1,916^{b}	4,91±1,925^{a}	4,36±1,630^{a}
	Texture	5,37±1,959^{b}	5,62±1,855^{b}	4,23±1,964^{a}	5,05±1,936^{b}
	Overall	5,64±1,763^{b}	5,49±1,733^{ab}	5,11±1,732^{ab}	4,89±1,541^{a}

Tab. 3.4 - Results of pleasantness attributes in second sensory evaluation.

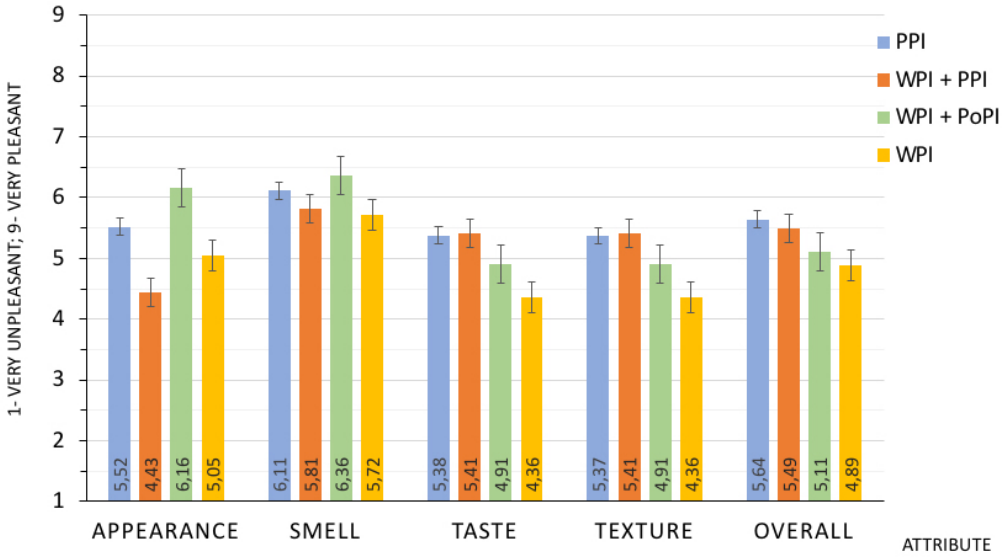

Fig. 3.7 - Pleasantness ratings of different attributes (appearance, smell, taste, texture, overall) in the second sensory evaluation. The parameters range from 1 (very unpleasant) to 9 (very pleasant).

The sensory analysis of different compositions of meat analogues showed variations in how each formulation was perceived. A visual representation of the data can be found in Fig. 3.7. For appearance, WPI + PoPI (6,16±1,721c) received the highest score, significantly higher than WPI + PPI (4,43±1,843a), which had the lowest rating. PPI (5,52±1,769bc) and WPI (5,05±1,731ab) were intermediate, indicating that potato protein may enhance visual appeal, while the combination of whey and pea protein led to a less favorable appearance.

Smell ratings were similar across all samples, with values ranging from 5,72±1,712a (WPI) to 6,36±1,996a (WPI + PoPI), showing no significant differences. This suggests that the type of protein used had little impact on aroma perception even in deep fried samples.

In terms of taste, PPI (5,38±1,947b) and WPI + PPI (5,41±1,916b) were rated highest, while WPI + PoPI (4,91±1,925a) and WPI (4,36±1,630a) scored significantly lower. This indicates that formulations containing pea protein were better received in terms of flavor, whereas the addition of potato protein or whey protein alone resulted in a less appealing taste.

For texture, WPI + PPI (5,62±1,855b) and PPI (5,37±1,59b) had the highest ratings, while WPI + PoPI (4,23±1,964a) had the lowest, suggesting that potato protein negatively affected the texture perception. WPI (5,05±1,936b) was intermediate, similar to PPI and WPI + PPI, showing that whey protein alone did not significantly improve or worsen texture perception.

Overall pleasantness was highest for PPI (5,64±1,763b), followed closely by WPI + PPI (5,49±1,733ab), while WPI (4.89±1,541a) had the lowest rating. WPI + PoPI (5,11) was intermediate but not significantly different from the other formulations. This indicates that while all samples were relatively acceptable, PPI performed the best in overall sensory appeal, whereas WPI alone had the least favorable reception.

These findings suggest that PPI is the most favorable protein source in terms of sensory perception, performing well across all attributes, particularly in overall pleasantness and taste. WPI + PPI also showed good sensory properties, especially in texture. Although WPI + PoPI had the highest score for appearance, it performed poorly in texture and taste, which may limit its suitability for meat analogue applications. WPI alone was the least preferred formulation overall, indicating that whey protein alone may not be ideal for optimizing the sensory properties of meat analogues.

3.2.3 CATA

The results of CATA attributes in the second sensory evaluation are reported in Tab. 3.5.

	Attribute	**PPI**	**WPI + PPI**	**WPI + PoPI**	**WPI**
CATA	Sweet	12	7	9	5
	Salty	25	32	7	15
	Sour	1	4	0	4
	Bitter	4	12	4	11
	Umami	40	47	28	17
	Milk	9	1	10	2
	Cereal	44	43	37	58
	Pea	25	44	19	23
	Potato	17	25	16	19
	Mild	62	49	59	63
	Dry	74	58	95	86
	Juicy	6	14	1	4
	Tough	36	20	64	5
	Tender	31	44	12	32
	Floury	36	23	33	79
	Gummy	14	11	12	2

Tab. 3.5 - Results of pleasantness attributes (sweet, salty, sour, bitter, umami, milk, cereal, pea, potato, mild, dry, juicy, tough, tender, floury and gummy). The number indicates the number of time every attribute was selected by a panel of 81 people.

3.2.3.1 CATA taste

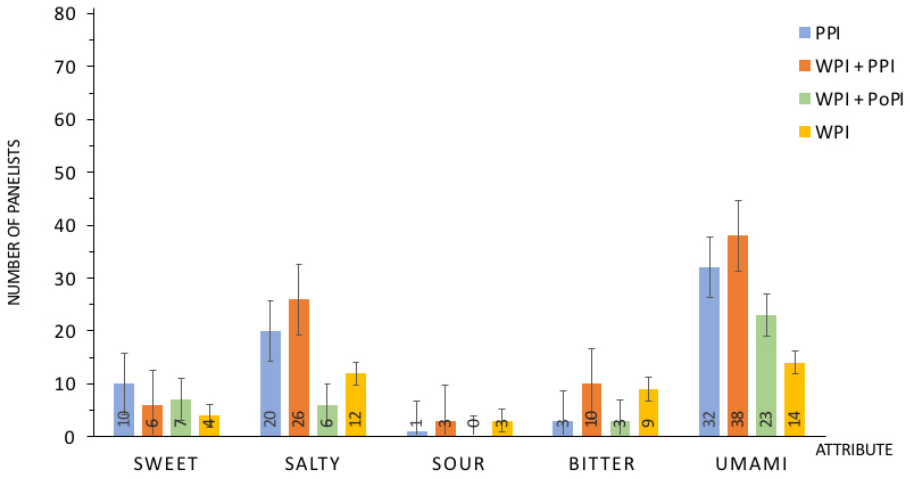

Fig. 3.8 - Number of panelists who selected given attributes regarding taste of 4 different formulations of meat analogues. The attributes considered for CATA Taste are sweet, salty, sour, bitter, umami and the panel consist of 81 people.

Sweetness was perceived as very low across all samples. The highest score was recorded for PPI formulation (12%), while WPI + PPI (7%) and WPI + PoPI (9%) showed similar intermediate values. WPI had the lowest score (5%). These small differences suggest that none of the formulations were sweet, and that sweetness is not a defining sensory characteristic of these protein blends. The slight elevation in PPI may be due to subtle flavor compounds inherent to pea protein, though the impact remains minimal. A visual representation of the data can be found in Fig. 3.8.

Saltiness perception varied more clearly across the samples. The WPI + PPI formulation was the most selected by the panelists (32%), followed by PPI (25%). WPI showed a moderate value (15%), while WPI + PoPI was the least selected (7%). These results suggest that adding pea protein, alone or in combination with whey, increases the perception of saltiness. In contrast, potato protein may reduce this perception when blended with whey, potentially due to a masking effect or interaction with salty taste receptors.

Sourness was the least selected across the board. WPI and WPI + PPI both reached 4%, while PPI had only 1% and WPI + PoPI was not selected by the panelists (0%). These values confirm that sour taste was not a prominent trait in any sample, and differences were too small to be considered meaningful. This uniform result across formulations suggests that the protein type has little to no effect on perceived sourness in this context.

Bitterness scores remained low for all samples, but slight differences were observed. WPI + PPI had the highest bitterness score at 12%, while WPI (11%) followed closely. Both PPI and WPI + PoPI were at 4%. These results indicate that blending whey and pea protein may enhance the perception of bitterness slightly more than when they are used individually. However, the overall low percentages suggest that bitterness was not a dominant sensory issue in any of the samples, and that protein blends remained generally palatable from a bitterness standpoint.

Umami emerged as the most differentiated taste attribute among the samples. WPI + PPI formulation was the most selected (47%), indicating a boost in savory flavor. PPI alone also scored relatively high (40%), while WPI + PoPI dropped to 28%. The WPI sample scored the lowest at just 17%. This suggests that pea protein plays a key role in enhancing umami perception, and its combination with whey protein further boosts this effect. On the other hand, potato protein may lower this savory note.

In conclusion, taste attributes revealed differences between the formulations. PPI and WPI + PPI stood out for their higher saltiness and umami perceptions, supporting their potential to deliver a more savory and flavorful profile. WPI + PoPI, on the other hand, was the least selected for these attributes, suggesting that potato protein might mask these notes. Sweetness, sourness, and bitterness remained minimal and relatively consistent across samples, indicating a generally balanced and non-intrusive taste base suitable for further flavor development.

3.2.3.2 CATA flavor

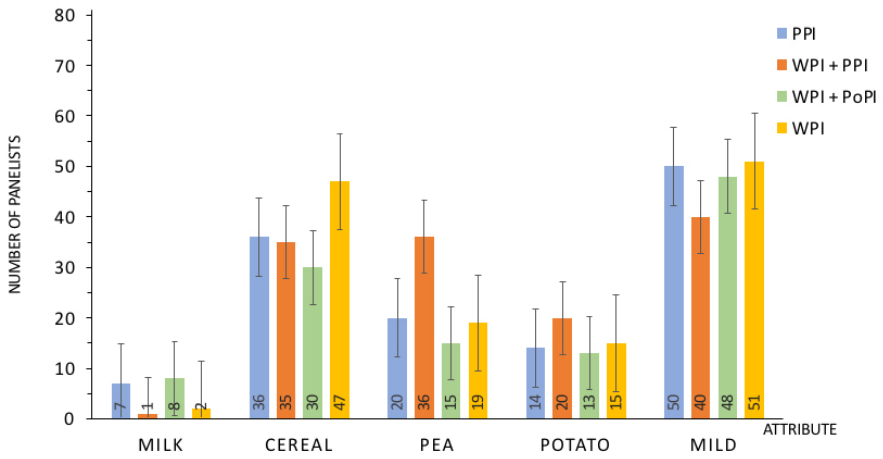

Fig. 3.9 - Number of panelists who selected given attributes regarding flavor of 4 different formulations of meat analogues. The attributes considered for CATA Flavor are milk, cereal, pea, potato, mild, and the panel consists of 81 people.

Milk flavor was weak across all samples, with low selection percentages. WPI + PoPI formulation received was the most perceived (10%), followed closely by PPI (9%). WPI and WPI + PPI were selected much lower, with only 2% and 1%, respectively. These results suggest that none of the formulations exhibited a strong milk-like flavor, although the presence of potato or pea protein might slightly enhance this note compared to WPI alone. A visual representation of the data can be found in Fig. 3.9.

Cereal flavor was moderately perceived across all samples, with more noticeable differences. The highest selection percentage was observed in the WPI sample (58%), followed by PPI (44%) and WPI + PPI (43%). WPI + PoPI had the lowest selection rate (37%). This suggests that samples containing WPI or WPI + PPI have stronger cereal notes, while the addition of potato protein may suppress this perception.

Pea flavor was most evident in the WPI + PPI sample (44%), as expected due to the presence of pea protein in the blend. The PPI sample also showed a relatively high selection rate (25%), confirming the natural presence of this note in isolated pea protein. On the other hand, WPI + PoPI (19%) and WPI (23%) were less selected by the panel.

Potato flavor was generally low across all samples, with only slight variations. WPI + PPI had a 25% selection score, followed by WPI (19%), PPI (17%), and WPI + PoPI (16%). Interestingly, the addition of potato protein did not increase the perception of potato-like flavor, suggesting that PoPI does not impart a distinctive potato character to the final product.

Mildness was similarly rated across all formulations, with high selection rates and no meaningful differences. WPI (63%) and PPI (62%) were perceived as more mild, followed by WPI + PoPI (59%) and WPI + PPI (49%). These findings indicate that all samples maintained a mild flavor profile.

In summary, the flavor attribute analysis shows that pea protein strongly contributes to vegetal notes, while potato protein has a limited impact on potato flavor. Cereal notes were enhanced by WPI, and all formulations shared a pleasant mildness. These results suggest that although individual proteins modulate certain flavor attributes, the overall sensory profile remains balanced and suitable for broad consumer acceptance.

3.2.3.3 CATA texture

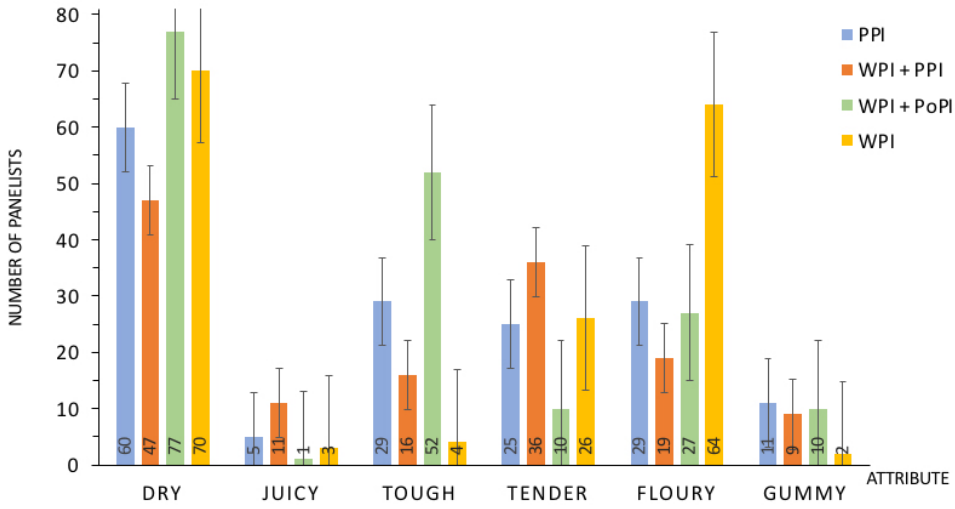

Fig. 3.10 - Number of panelists who selected given attributes regarding texture of 4 different formulations of meat analogues. The attributes considered for CATA Texture are dry, juicy, tough, tender, floury, gummy, and the panel consists of 81 people.

Regarding dryness, the WPI + PoPI formulation was the most selected (95%), followed by WPI (86%). PPI (74%) and WPI + PPI (58%) had a lower frequency of selection, indicating a less pronounced dry sensation. These results suggest that the inclusion of potato protein may contribute to increased dryness in the texture, while the addition of pea protein, either alone or in combination with WPI, may reduce this perception. A visual representation of the data can be found in Fig. 3.10.

Juiciness has a low selection rate on all formulations. The WPI + PPI sample showed the highest juiciness score (14%), whereas WPI + PoPI (1%), WPI (4%), and PPI (6%) were perceived as less juicy. This indicates that none of the formulations produced a distinctly juicy texture.

Toughness showed notable differences across the samples. WPI + PoPI had the highest toughness rating (64%), followed by PPI (36%) and WPI + PPI (20%). WPI alone had the lowest score (5%), suggesting a softer and more yielding texture. These findings imply that the presence of potato protein increases the perception of toughness, while WPI contributes to a more tender structure.

Tenderness results mirrored the toughness scores, with the WPI + PPI sample showing the highest tenderness (44%), followed by WPI (32%) and PPI (31%). As expected, the WPI +

PoPI sample had the lowest tenderness rating (12%), supporting the observation that potato protein may reduce tenderness and contribute to a firmer, less compliant texture.

Flouriness was most prominent in the WPI sample (perceived by 79% of panel), indicating a powdery or dry-mouth sensation. This attribute was less identified in PPI (36%), WPI + PoPI (33%), and WPI + PPI (23%). These results suggest that flouriness may be primarily associated with whey protein, and the inclusion of plant proteins tends to mitigate this sensory characteristic.

Gumminess was perceived at low levels in all samples. PPI (14%) and WPI + PPI (11%) had slightly higher selection rates than WPI + PoPI (12%) and WPI (2%). Although the values are overall low, the presence of pea protein may slightly increase the gumminess perception, while WPI alone contributes minimally to this attribute.

In summary, the texture-related attributes revealed distinct differences among the samples based on protein composition. The addition of potato protein isolate (PoPI) appeared to increase perceived dryness and toughness, while simultaneously reducing juiciness and tenderness, resulting in a firmer and less palatable texture. On the other hand, blends containing pea protein isolate (PPI), alone or combined with WPI, showed more tender and juicy profiles, with lower dryness and toughness selection rates. Flouriness was most pronounced in the WPI sample, suggesting that whey protein contributes to a dry or powdery mouthfeel. Gumminess remained a minor characteristic overall but was slightly more evident in formulations containing pea protein.

3.3 Physicochemical studies

3.3.1 Photos

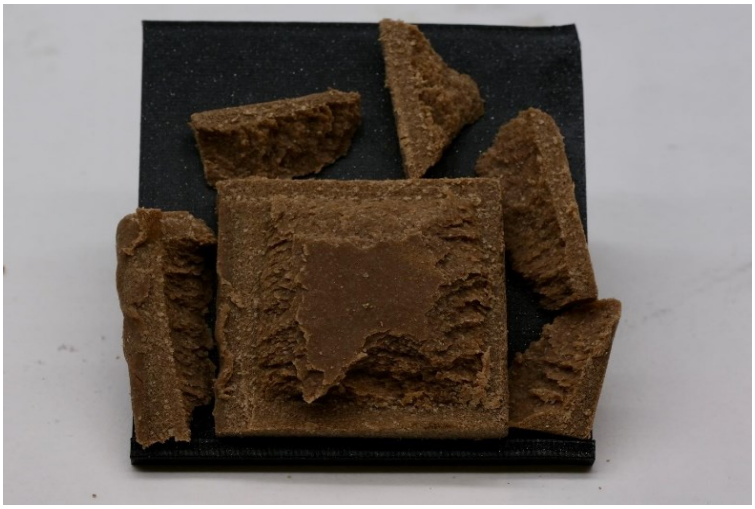

Fig. 3.11 - Cross-section of a meat analogue made from 50% Oat Fiber Concentrate and 50% Potato Protein Isolate.

A cross-section of a sample made with 50% OFC and 50% PoPI can be seen in Fig. 3.11. The sample appears crumbly and brittle. This suggests poor structural cohesion and limited elasticity, likely due to the weaker gel-forming ability of potato protein compared to other proteins like whey. The lack of visible fibrous layering indicates insufficient protein alignment during extrusion, possibly due to low melt viscosity or weak protein–fiber interactions.

Fig. 3.12 - Cross-section of a meat analogue made from 50% Oat Fiber Concentrate, 25% Potato Protein Isolate and 25% Pea Protein Isolate.

A cross-section of a sample made from 50% OFC, 25%PoPI, and 25% PPI can be seen in Fig. 3.18.

The product displays moderate fibrous structuring, with noticeable layering. This suggests that the extrusion process resulted in the formation of some level of protein alignment and matrix formation. The inclusion of pea protein has improved gel strength compared to the 50% PoPI sample, but the overall texture remains partially developed and heterogeneous.

Fig. 3.19 - Cross-section of a meat analogue made from 50% Oat Fiber Concentrate and 50% Pea Protein Isolate.

A cross-section of a sample made with 50% OFC and 50% PPI can be seen in Fig. 3.19.

The sample displays moderate fibrous alignment: the structure, however, appears loose and uneven in some parts, with areas showing tearing and internal gaps. This suggests that while pea protein contributes to better gel formation than potato protein, it still lacks the strong network-forming ability seen in other proteins like whey. Overall, the texture is partially structured but lacks tight cohesion.

Fig. 3.20 - Cross-section of a meat analogue made from 50% Oat Fiber Concentrate, 25% Whey Protein Isolate 25% Pea Protein Isolate.

A cross-section of a sample made with 50% OFC, 25% WPI, and 25% PPI can be seen in Fig. 3.12.

The sample shows a defined, uniform fibrous layering, indicating strong alignment between protein and fiber, and effective gelation during extrusion. The structure appears compact and elastic, suggesting improved binding capacity, likely due to the functional properties of WPI, such as gel and film-forming abilities.

The sample is visibly lighter in color than those with only plant proteins, reflecting the presence of WPI, which is naturally lighter than PPI or OFC.

Fig. 3.13 - Cross-section of a meat analogue made from 50% Oat Fiber Concentrate, 25% Whey Protein Isolate 25% Potato Protein Isolate.

A cross-section of a sample made with 50% OFC, 25% WPI, and 25% PoPI can be seen in Fig 3.13.

The internal structure exhibits moderate fiber formation, with visible but less distinct alignment compared to samples containing pea protein. The texture appears slightly uneven, with small breaks and a rougher surface. The presence of WPI likely improves gelation and elasticity, though the weaker network-forming ability of potato isolate may limit structural integrity. The sample is lighter in color, which is attributable to the inclusion of WPI.

Fig. 3.14 - Cross-section of a meat analogue made from 50% Oat Fiber Concentrate and 50% Whey Protein Isolate.

A cross-section of a sample made with 50% OFC and 50% WPI can be seen in Fig. 3.14.

The sample shows clear fibrous layering, a sign of well-aligned anisotropic structure, which is a key quality trait for mimicking the texture of real meat. The combination of OFC and WPC supports the development of a cohesive matrix. The cut is clean, indicating good gel strength and elasticity from the whey protein.

The light beige color reflects minimal Maillard browning, likely due to moderate extrusion temperatures. Overall, this sample demonstrates a good structural integrity and fibrous definition.

3.3.2 Color

SAMPLE	L* AVERAGE	a* AVERAGE	b* AVERAGE
PoPI	46,645	5,629	17,529
PoPI + PPI	46,588	4,486	15,361
PPI	52,217	5,416	20,29
WPI + PPI	57,635	4,064	21,624
WPI + PoPI	50,541	5,298	18,06
WPI	63,878	2,849	20,684

Tab. 3.6 - Color parameters (L*, a*, b*) of the six meat analogue formulations.

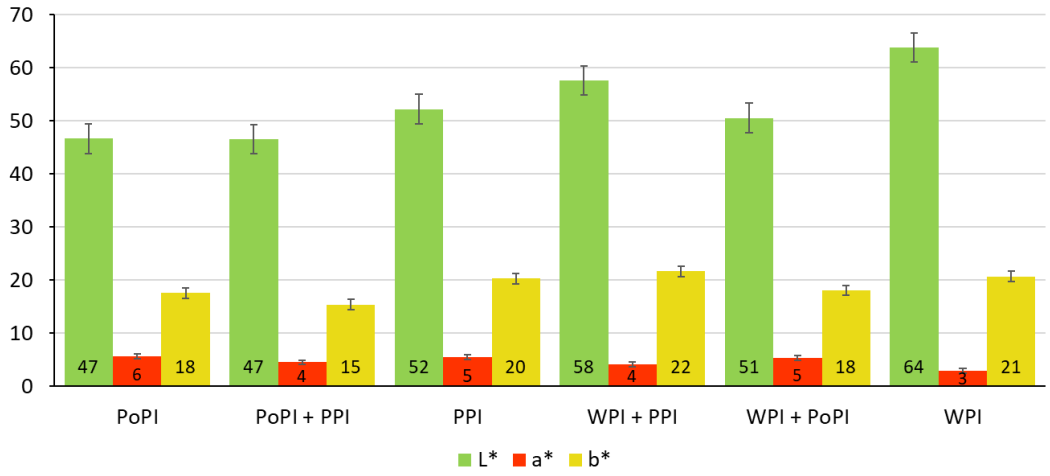

Fig. 3.15 - L*, a*, and b* color values of six meat analogue formulations based on different protein sources: all the samples contain 50% Oat Fiber Concentrate, while the other half is composed by PoPI, PoPI + PPI, PPI, WPI + PPI, WPI + PoPI, WPI. L* represents lightness (0 = black, 100 = white), a* represents redness-greenness (positive values = red), and b* represents yellowness-blueness (positive values = yellow). Error bars indicate standard deviation.

The results of color analysis can be found in Tab. 3.6, while Fig. 3.15 offers a visual representation of the data presented. The color of the different formulations of meat analogues is an important parameter that varies across the samples. The L*ab** values showed that samples containing WPI appeared generally lighter and more yellow than those based on PPI or PoPI. WPI had the highest lightness value (L* = 63,9), followed by WPI + PPI

(57,6). In contrast, PoPI and PoPI + PPI were the darkest samples, with L* values around 46,6. These results suggest that WPI contributes to a brighter appearance, while PoPI tends to darken the product. A similar effect of WPI on lightness was observed in other studies, where it improved the visual appeal of meat analogues by increasing brightness and reducing browning during processing (Kyriakopoulou et al., 2021; Dekkers et al., 2018).

Redness (a* value) was highest in PoPI (5,6) and PPI (5,4), and slightly lower in PoPI + PPI and WPI + PoPI (respectively 4,4 and 5,2). WPI had the lowest redness (a* = 2.8), confirming that whey protein produces a paler, less red color. This might be considered less meat-like by some consumers, as redness is often associated with raw or rare meat. However, a high red value is not always preferred, especially after cooking, when consumers expect a golden or brownish tone instead.

Yellow tones (b* values) were also influenced by protein composition. The highest b* values were found in WPI + PPI (21,6), WPI (20,7), and PPI (20,3), while PoPI + PPI had the lowest (15,4). The combination of whey and pea proteins seemed to enhance yellowness, which could be desirable in fried or baked products, where a golden surface is associated with doneness and better sensory acceptance (Dekkers et al., 2018). On the other hand, PoPI-based formulations resulted in duller, darker tones, which might be less appealing, especially if the product is meant to resemble lighter meats like chicken or fish.

In summary, WPI improved lightness and yellowness, contributing to a more uniform and bright appearance, while PoPI gave darker and redder shades. These findings are in line with previous research showing that protein type significantly affects color parameters in plant-based meat analogues (Sha & Xiong, 2020). Visual aspects are crucial for consumer expectations and can influence perceived freshness, doneness, and overall quality.

3.3.3 TPA

	GUMMINESS		SPRINGINESS		CHEWINESS	
	AVERAGE (Newton)	DEV. ST	AVERAGE	DEV. ST	AVERAGE (Newton)	DEV. ST
PoPI	100,538	7,392	0,684	0,039	57,203	5,117
PoPI + PPI	54,046	3,548	0,603	0,064	35,528	3,397
PPI	101,033	3,262	0,759	0,045	76,668	4,138
WPI + PPI	148,792	11,181	0,729	0,039	108,763	12,836
WPI + PoPI	150,356	18,921	0,785	0,049	117,451	10,704
WPI	77,626	21,462	0,735	0,055	57,650	18,585

Tab. 3.7 - Results of TPA on the six sample formulations (PoPI, PoPI+PPI, PPI, WPI+PPI, WPI+PoPI, WPI). Average values and standard deviations of gumminess, springiness, and chewiness are shown.

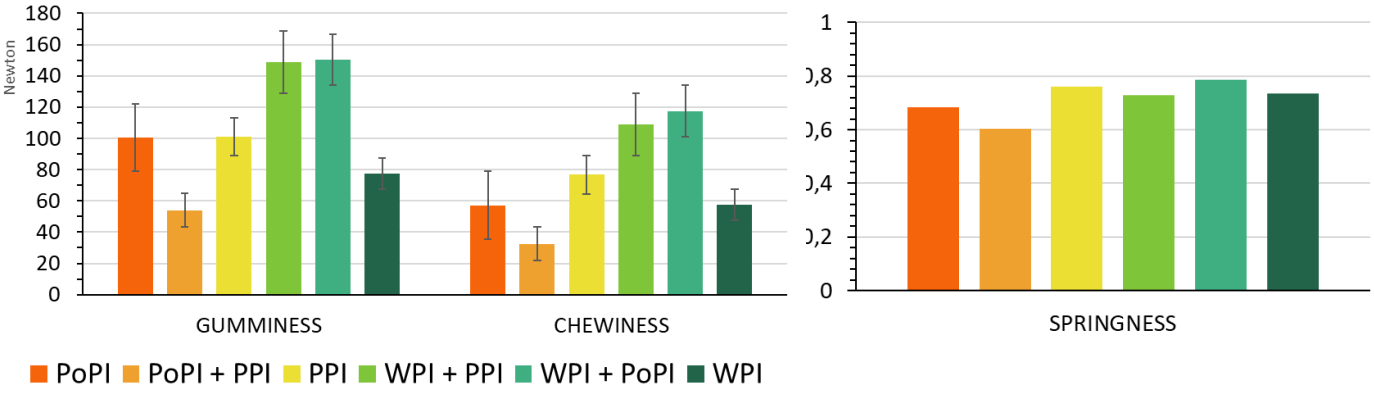

Fig. 3.16 - Gumminess, springiness, and chewiness for the six formulations (PoPI, PoPI+PPI, PPI, WPI+PPI, WPI+PoPI, WPI), calculated from the data presented in Tab. 3.7.

The textural analysis of the six different formulations, based on gumminess, springiness, and chewiness, highlights the significant impact of protein source and combinations on final

product consistency. The results of the measured textural attributes can be found in Tab. 3.7, while on Fig. 3.16 a graphical representation of the data can be found.

Among all formulations, WPI + PoPI showed the highest gumminess (150,356) and chewiness (117,451) values. This suggests a strong protein network formation, likely due to the synergy of whey and potato proteins: whey contributes to gel strength and elasticity, while potato protein may enhance water binding and structural integrity. Similarly, the WPI + PPI formulation also displayed elevated gumminess and chewiness values, reinforcing the idea that WPI plays a key role in improving mechanical resistance and elasticity in protein matrices. This is supported by Zhang et al. (2024), that in their study concluded that adding WP significantly improved the hardness, cohesiveness and gumminess of high moisture meat analogues.

On the other end the PoPI + PPI formulation had the lowest values of gumminess (54.046) and chewiness (35.528), indicating a significantly weaker texture. This could be attributed to the limited ability of plant protein blends, without the presence of WPI, to form strong gel networks. The weak structural characteristics of PoPI and PPI likely resulted in a less cohesive matrix.

Springiness values, while relatively close across all formulations, ranged from 0.603 to 0.785. The highest springiness was in the WPI + PoPI formulation, possibly due to the synergistic interaction between the proteins that resulted in enhancement of elasticity. As Godschalk-Broers et al. (2022) found out in their study, higher values of springness are related to samples with a more fibrous structure. Lower springiness in blends without the presence of whey reflects a less elastic structure due to weaker interactions between proteins.

In conclusion, formulations containing WPI consistently outperformed plant-only protein systems in terms of texture, particularly gumminess and chewiness. This suggests that WPI gelation properties play a critical role in developing desirable textural characteristics in the formulations of plant based meat analogues.

3.3.4 Aw

AW	1	2	AVERAGE	DEV ST
PoPI	0,916	0,929	0,9225	0,00250
PoPI + PPI	0,92	0,925	0,9225	
PPI	0,928	0,928	0,928	
WPI + PPI	0,928	0,927	0,9275	
WPI + PoPI	0,927	0,926	0,9265	
WPI	0,926	0,928	0,927	

Tab. 3.8 - Water activity values of six different meat analogue formulations. Values are reported as the means of two replicates.

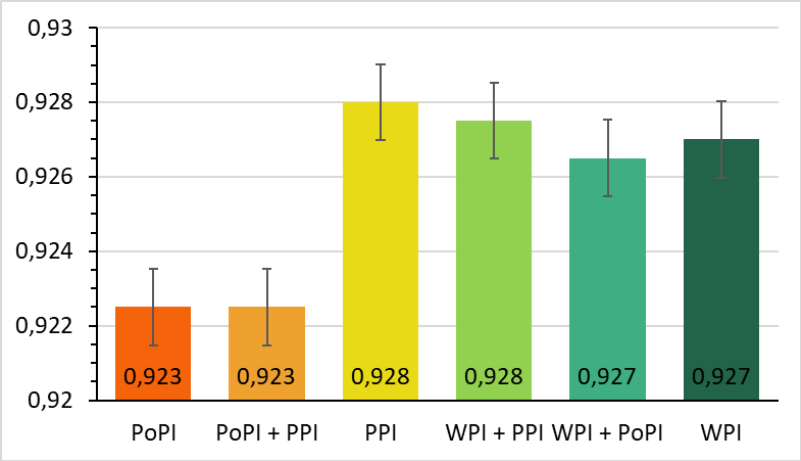

Fig. 3.17 - Average water activity (Aw) of the six meat analogue formulations. Error bars show the standard deviations of the data.

Water activity values measured across the six different formulations are summarized in Tab. 3.8. Aw plays a crucial role in determining the microbiological stability and shelf-life of food products, especially in high-moisture matrices such as meat analogues. In all samples, Aw values ranged between 0,9225 and 0,9280, indicating a relatively high availability of free water. A graphical representation of the data can be found in Fig. 3.17.

Among all formulations, the PoPI and PoPI + PPI samples showed the lowest Aw values (0,9225), which may be linked to the water-binding properties of potato protein. Potato proteins have been reported to exhibit good water retention capacity due to their high surface

hydrophilicity and molecular flexibility (Katzal et al., 2020). This could lead to slightly lower availability of free water in the matrix, which in turn may enhance microbial stability. On the other hand, the PPI sample showed the highest Aw (0,9280). This result suggests that pea protein may retain more free water in the system, possibly due to its less efficient water-holding ability compared to other proteins. Pea proteins generally form loose gels with lower water retention compared to whey or potato proteins, which may explain the observed difference.

Formulations containing WPI either alone or in combination, consistently showed intermediate Aw values (ranging from 0,9265 to 0,9275) and low variability.

Overall, the data suggests that PoPI may contribute slightly more to water immobilization in the matrix, while PPI alone results in higher Aw. The combinations involving WPI appeared to stabilize water activity at intermediate levels, possibly due to a more uniform protein network. This implies that the choice and ratio of protein sources can influence water mobility and, by extension, product shelf-life and microbial stability.

3.3.5 Water content

		INITIAL WATER CONTENT (g)	FINAL WATER CONTENT (g)	MOISTURE (%)	AVERAGE (%)	DEV ST INTRA GROUP	DEV ST BETWEEN GROUPS
PoPI	A	7,280	3,183	56,280	56,158	0,183	0,783
	B	7,077	3,118	55,947			
	C	7,127	3,118	56,248			
PoPI + PPI	A	6,794	3,031	55,393	55,310	0,145	
	B	6,962	3,105	55,396			
	C	6,872	3,083	55,142			
PPI	A	6,354	2,772	56,375	56,735	0,511	
	B	6,767	2,888	57,319			
	C	6,702	2,915	56,510			
WPI + PPI	A	6,793	3,047	55,143	55,206	0,068	
	B	6,845	3,067	55,197			
	C	6,480	2,898	55,279			
WPI + PoPI	A	7,063	3,120	55,821	55,856	0,038	
	B	7,010	3,095	55,851			
	C	7,005	3,090	55,897			
WPI	A	7,239	3,309	54,290	54,520	0,201	
	B	6,881	3,120	54,662			
	C	7,200	3,268	54,609			

Tab. 3.9 - Initial and final weights (g) and moisture content (%) for each formulation (PoPI, PoPI + PPI, PPI, WPI + PPI, WPI + PoPI, and WPI). Values are reported for three replicates (A, B, C). The average moisture content and standard deviation are calculated for each formulation.

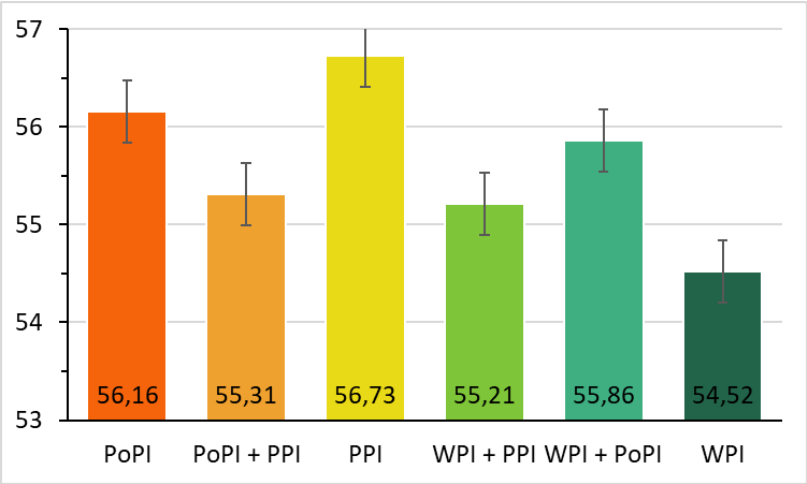

Fig 3.26 - Moisture content (%) of samples containing different protein combinations. Samples include PoPI, PoPI + PPI, PPI, WPI + PPI, WPI + PoPI, and WPI.

The results of the moisture content tests are reported in Tab. 3.9, while the average moistures are represented graphically in Fig. 3.26. The highest average moisture content was observed in the PPI treatment (56,74%), in accordance with the findings of CATA Texture of the first and second sensory evaluations, where the PPI sample was perceived as the juiciest and less dry. The lowest value was found in WPI sample (54,52%), while other combinations (PoPI + PPI or WPI + PoPI) had intermediate moisture levels. This suggests that PPI tends to retain more water, while WPI contributes to lower moisture retention.

The lowest intra-group standard deviation was observed in the WPI + PoPI combination (SD = 0.038), indicating a good repeatability across replicates. Instead, PPI alone displayed the highest variability (SD = 0.511), indicating less consistent measurements.

The standard deviation calculated across the means of all six formulations is 0.78. This value reflects the variation attributable to the differences in formulations, instead of to random variation within groups.

The relatively high between-group standard deviation compared to intra-group standard deviations confirms that the differences observed among treatments are meaningful and likely attributable to formulation effects rather than random measurement error.

As Godschalk et al. (2022) found out in their study, moisture is correlated with dryness and chewiness: the cooking process of meat analogues leads to a water loss that causes an increase of hardness and chewiness of the products.

CHAPTER 4: VARIATION OF SENSORY ATTRIBUTES BETWEEN THE FIRST AND THE SECOND SENSORY STUDY

4.1 Pleasantness

The sensory evaluation of the raw and deep-fried samples of the meat analogues formulations revealed both similarities and notable changes in how the samples were perceived after frying.

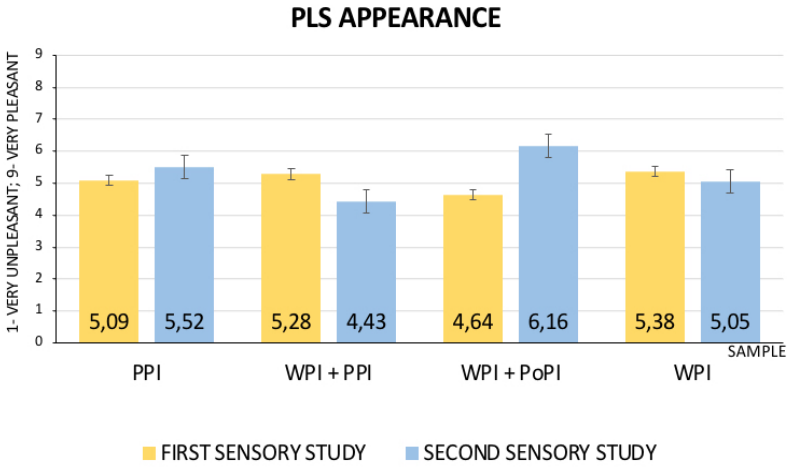

Fig. 4.1 - Mean appearance perception scores for different protein formulations evaluated in two sensory studies**.** The panel rated appearance on a 9-point hedonic scale (1 = extremely poor, 9 = extremely good). Formulations included PPI, WPI + PPI, WPI + PoPI, WPI. Yellow bars represent data from the first sensory study; blue bars from the second.

In terms of appearance, frying had mixed effects on the samples. A visual representation of the data can be found in Fig. 4.1. For example, PPI improved slightly from 5.09 to 5.52, while WPI + PoPI showed a large increase from 4.64 to 6.16, becoming the highest-rated in this attribute. The high score of PoPI may be due to browning and creating a more appetizing color. On the other hand, the other two samples containing WPI (WPI + PPI and WPI) decreased in appearance score (from 5,28 to 4,43 and from 5,38 to 5,05), possibly due to less favorable surface changes after cooking.

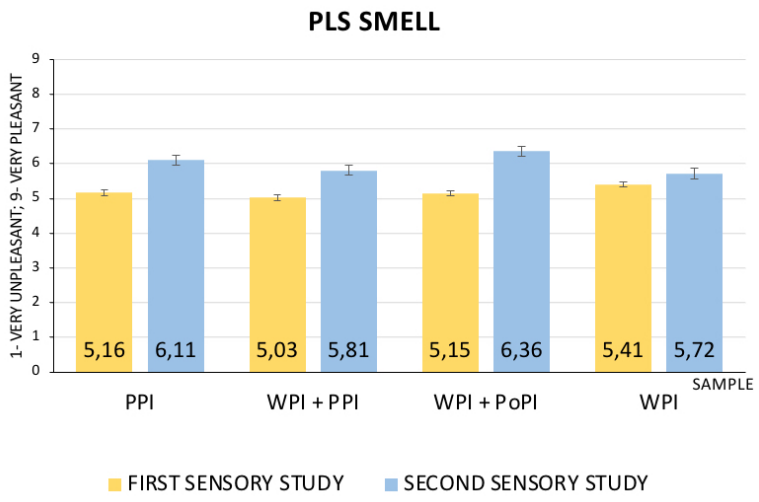

Fig 4.2 - Mean smell perception scores for different protein formulations evaluated across two sensory studies**.** Participants rated the smell on a 9-point hedonic scale (1 = extremely poor, 9 = extremely good). Formulations included PPI, WPI + PPI, WPI + PoPI, WPI. Yellow bars represent data from the first sensory study; blue bars from the second.

Smell ratings were consistently higher in the fried samples, ranging from 5,72 (WPI) to 6,36 (WPI + PoPI), compared to 5.03–5.41 in the raw samples. Smell was the highest scoring attribute out of the five categories. This indicates that the frying process enhanced aroma perception for all formulations, likely due to the formation of volatile compounds during cooking. Despite these increases, differences between formulations remained non-significant in both tests. A visual representation of the data can be found in Fig. 4.2.

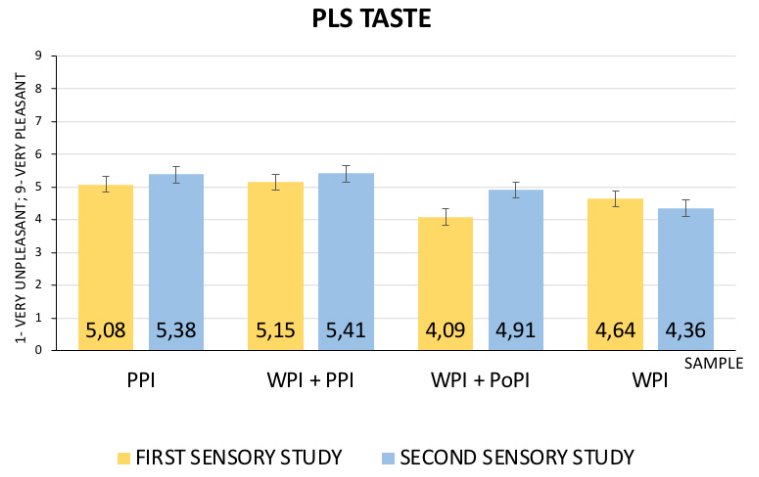

Fig. 4.3 - Mean taste perception scores for different protein formulations evaluated across two sensory studies. Participants rated taste on a 9-point hedonic scale (1 = extremely poor,

9 = extremely good). Formulations included PPI, WPI + PPI, WPI + PoPI, WPI. Yellow bars represent data from the first sensory study; blue bars from the second.

The taste attribute followed a more complex pattern: a visual representation of the data can be found in Fig. 4.3. In the raw test, WPI + PPI and PPI were rated higher (5,08-5,15), while WPI + PoPI (4,09) had the lowest score. After frying, taste ratings increased slightly for PPI (from 5,08 to 5,38) and WPI+PPI (from 5,15 to 5,41) and increased more for WPI + PoPI (from 4,09 to 4,91). The rating for WPI dropped (from 4,64 to 4.36), making it the least liked sample in terms of flavor. These results suggest that formulations with pea protein (PPI, WPI + PPI) retained better tasting qualities through both raw and cooked states, while the inclusion of potato or whey protein alone did not significantly improve flavor upon cooking.

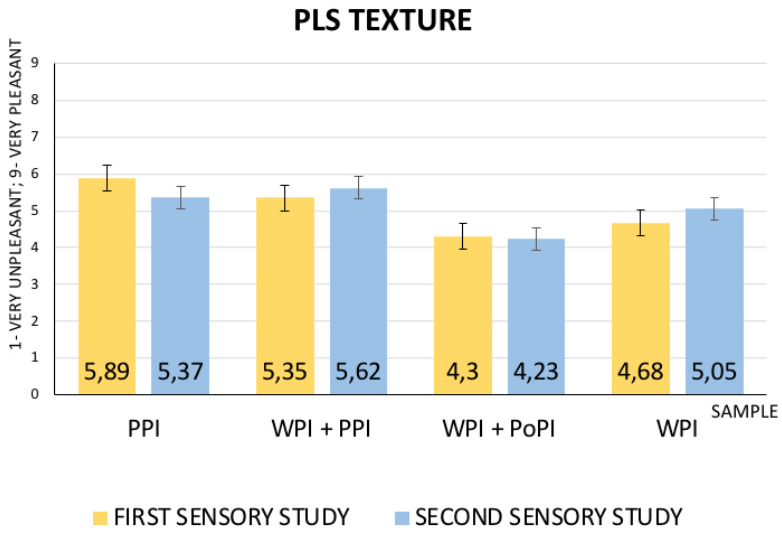

Fig. 4.4 - Mean texture perception scores for different protein formulations evaluated across two sensory studies**.** Participants rated texture on a 9-point hedonic scale (1 = extremely poor, 9 = extremely good). Formulations included PPI, WPI + PPI, WPI + PoPI, WPI. Yellow bars represent data from the first sensory study; blue bars from the second.

A visual representation of the data regarding pleasantness of texture in the first and second sensory studies can be found in Fig 4.4. Regarding texture perception, in the raw state, PPI had the highest texture score (5,89), followed by WPI + PPI (5,35) and WPI (4,68) while WPI + PoPI (4,30) was the lowest. After frying, WPI + PPI slightly improved (from 5,32 to 5,62), while PPI dropped from 5,89 to 5,37, possibly due to structural changes during cooking. WPI + PoPI remained the least favorable in texture (4,23), suggesting that its

structural properties may not withstand frying well. This observation is supported by Zhang et al. (2023), who found that pea protein content positively influenced the texture profile of meat analogues.

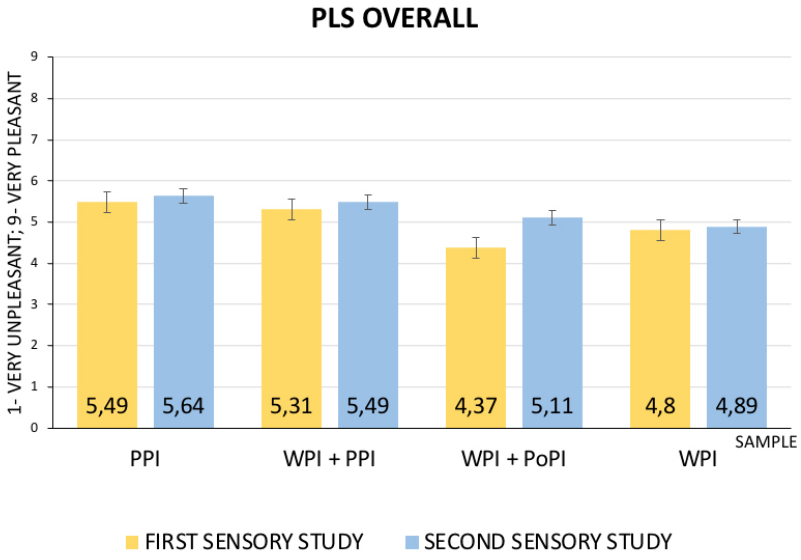

Fig. 4.5 - Mean overall perception scores for different protein formulations evaluated across two sensory studies**.** Participants rated the samples on a 9-point hedonic scale (1 = extremely poor, 9 = extremely good). Formulations included PPI, WPI + PPI, WPI + PoPI, WPI. Yellow bars represent data from the first sensory study; blue bars from the second.

Overall pleasantness improved slightly in all samples after frying: A visual representation of the data can be found in Fig. 4.5. PPI scored from 5,49 to 5,64, remaining the most liked and confirming its strong sensory appeal in both raw and deep fried forms, WPI+PPI scored from 5,31 to 5,49, WPI scored from 4,8 to 4,89. WPI + PoPI had the highest gap, going from 4,37 to 5,11. The superior performance of pea protein formulations is consistent with findings by Zhang et al. (2023), who highlighted the favorable sensory properties of pea protein in meat analogues.

These findings show that while frying generally enhanced aroma and appearance, it did not equally improve all other attributes. Formulations with pea protein (PPI and WPI + PPI) were consistently better rated across most sensory parameters, both raw and fried. The inclusion of potato protein improved appearance and smell after cooking but negatively affected texture. Whey protein was the least successful in maintaining favorable sensory characteristics after frying, indicating it may not be ideal for standalone use in meat analogue development.

4.2 CATA

4.2.1 CATA taste

Sweetness perception was consistently low across all samples in both raw and fried states. In the raw state, PPI and WPI + PPI each had 16% of participants perceiving sweetness, while WPI + PoPI and WPI were at 7% and 6%, respectively. After frying, PPI slightly decreased to 12%, WPI + PPI to 7%, WPI + PoPI increased to 9%, and WPI remained low at 5%. These minimal variations suggest that sweetness is not a defining characteristic in these formulations, which is desirable in savory products.

Saltiness perception showed more notable differences. In the raw state, PPI had the highest saltiness perception at 22%, followed by WPI + PPI at 16%, WPI at 8%, and WPI + PoPI at 4%. After frying, saltiness perception increased across all samples: WPI + PPI rose to 32%, PPI to 25%, WPI to 15%, and WPI + PoPI to 7%. The increase in saltiness perception post-frying, especially in formulations containing pea protein, may be attributed to the Maillard reaction enhancing savory flavors during cooking. This observation is supported by De Angelis et al. (2020), who reported that dry-fractionated pea protein contributes to a more intense odor profile, potentially enhancing saltiness perception in meat analogues.

Sourness was the least perceived taste attribute across all samples. In the raw state, WPI + PPI had 7% of participants perceived sourness, PPI and WPI + PoPI each had 6%, and WPI had 3%. After frying, sourness perception decreased: WPI and WPI + PPI were at 4%, PPI at 1%, and WPI + PoPI at 0%. These low values indicate that sourness is not a prominent trait in these formulations, and the decrease in post-frying suggests that cooking may further suppress any residual sour notes.

Bitterness, often associated with plant proteins, was perceived at low levels in all samples. In the raw state, WPI + PoPI had the highest bitterness perception at 11%, followed by WPI + PPI at 8%, and both PPI and WPI at 5%. After frying, WPI + PPI increased to 12%, WPI to 11%, while PPI and WPI + PoPI decreased to 4% each. The increase in bitterness perception in WPI + PPI and WPI formulations post-frying may be due to the formation of bitter compounds during cooking. De Angelis et al. (2020) in their study suggest that the presence of saponins and phenolic compounds in plant proteins, particularly in pea and potato proteins, has been linked to bitterness.

Umami was the most prominent taste attribute across all samples. In the raw state, WPI + PPI had the highest umami perception at 38%, followed by PPI at 36%, WPI at 24%, and WPI + PoPI at 19%. After frying, umami perception increased: WPI + PPI to 47%, PPI to 40%, WPI + PoPI to 28%, and WPI to 17%. The higher umami perception in formulations containing pea protein may be attributed to the presence of umami-enhancing compounds such as 5'-adenosine monophosphate (AMP) and 5'-uridine monophosphate (UMP) in pea protein isolates (Jiang et al., 2024).

The sensory analysis indicates that formulations containing pea protein (PPI and WPI + PPI) consistently show higher perceptions of saltiness and umami, both in raw and fried states, enhancing their savory profiles. Frying generally intensified these taste attributes, likely due to flavor-enhancing reactions such as the Maillard reaction. Bitterness remained low across all samples, with slight increases in some formulations post-frying, possibly due to the formation of bitter compounds during cooking. Sweetness and sourness were minimal and relatively unchanged, indicating a balanced taste profile suitable for savory applications.

These findings suggest that incorporating pea protein into meat analogue formulations can enhance desirable taste attributes, particularly saltiness and umami, while maintaining low levels of undesirable tastes such as bitterness and sourness. The addition of potato protein may contribute to a slight increase in bitterness, which should be considered in product development.

4.2.2 CATA flavor

Milk flavor was weak in all formulations, in both raw and fried samples. In the raw state, the WPI sample had the highest milk flavor perception (15%), followed by WPI + PPI (10%), PPI (7%), and WPI + PoPI (1%). After frying, the scores slightly shifted: WPI + PoPI reached 10%, PPI rose to 9%, and WPI and WPI + PPI dropped to 2% and 1%, respectively. These results suggest that milk-like notes are not dominant in any sample. The higher raw score for WPI might reflect whey protein's dairy background, while the increase in fried PPI and WPI + PoPI may relate to cooking-induced flavor changes. Previous studies show that heating can influence aroma development through Maillard reactions, but this does not necessarily enhance "milky" flavors (Wang et al., 2022).

The perception of the cereal flavor was more variable. In the raw state, WPI + PoPI scored highest (61%), followed by WPI (47%), PPI (44%), and WPI + PPI (38%). After frying, WPI

was perceived as the most cereal-like (58%), while PPI (44%) and WPI + PPI (43%) remained stable. WPI + PoPI was the sample that changed the most (from 61% to 37%). These findings suggest that frying enhances cereal notes in WPI, possibly due to heat-reactive compounds in whey protein. However, the sharp drop in WPI + PoPI implies that potato protein may slightly suppress cereal-like perceptions after cooking. These effects reflect known interactions between plant proteins and flavor carriers during thermal processing, reported by De Angelis et al. (2020).

As ipotized by Pasqualone et al. (2020) the incorporation of oat protein (all the samples are formulated at 50% with Oat Fiber Concentrate) might have contributed to smooth the odor intensity, improving the sensory characteristics, together with the favorable effect of the extrusion process.

Pea flavor was clearly associated with pea protein. In the raw state, PPI was selected by most panelists (45%), followed by WPI + PPI (32%), WPI (26%), and WPI + PoPI (17%). After frying, the pattern shifted: WPI + PPI became the most associated with pea notes (44%), while PPI dropped to 25%. WPI and WPI + PoPI scored similarly at 23% and 19%. These results confirm that pea protein brings a clear vegetal flavor, but this note can be reduced when blended with whey protein. The drop in the PPI sample after frying may be due to heat degradation of volatile compounds responsible for grassy or beany aromas, as shown in plant protein research (Zeeb et al., 2018).

Potato flavor remained low in all formulations, even the one containing potato protein. In the raw state, WPI + PPI (26%) and WPI + PoPI (24%) were selected more frequently, followed by PPI (20%) and WPI (19%). After frying, WPI + PPI again scored highest (25%), followed by WPI (19%), PPI (17%), and WPI + PoPI (16%). These small differences suggest that potato protein (PoPI) does not contribute to a strong potato-like flavor. The fact that formulations without PoPI also showed some potato notes supports the idea that these flavors may come from processing effects or shared flavor compounds, not PoPI itself.

Regarding mildness, the samples were perceived as mild in both states. In the raw state, WPI was the most selected (60%), followed closely by WPI + PoPI (58%), PPI (55%), and WPI + PPI (49%). In the second evaluation, values of fried samples were slightly higher across the board: WPI (63%), PPI (62%), WPI + PoPI (59%), and WPI + PPI remained at 49%. These consistently high values suggest that all formulations have a mild, non-overpowering flavor profile, which is highly desirable for meat analogues: this is important for further product development, since meat analogues are meant to carry additional seasoning or mimic various products. This aligns with the concept of sensory neutrality in alternative proteins,

as discussed by Schindler et al. (2012), which is important for consumer acceptability and versatility in product development.

To sum up CATA flavor results, the analysis shows that pea protein contributes with vegetal (pea) notes, while whey protein enhances cereal and sometimes milk-like attributes. Potato protein does not increase potato flavor and may slightly suppress other notes like cereal. All formulations remained mild in flavor, both raw and fried samples, which is a beneficial trait for flexible applications in plant-based meat alternatives. Cooking changed some flavor intensities, especially in pea and cereal notes, but no extreme flavors emerged, supporting the overall balanced taste profile of all formulations.

4.2.3 CATA texture

Dryness was a dominant texture attribute in all formulations, especially in those containing whey and potato proteins. In the raw samples, WPI + PoPI and WPI were perceived as the driest (84% and 75% respectively), followed by WPI + PPI (52%) and PPI (43%). After frying, dryness perception increased in all samples, with WPI + PoPI reaching 95%, WPI at 86%, PPI at 74%, and WPI + PPI at 58%. These results confirm that PoPI increases the sensation of dryness, particularly after cooking, as well as whey protein. Pea protein, on the other hand, consistently helped reduce this perception, possibly due to its higher water-binding capacity (Dekkers et al., 2018).

Juiciness was generally perceived low in all formulations, in both the first and the second sensory study. Raw PPI was perceived as the juiciest (18%), followed by WPI + PPI (13%). In WPI (4%) and WPI + PoPI (3%) the juiciness attribute was selected by less panelists. After frying, the selection of this attribute further declined. WPI + PPI remained the most selected (14%), while PPI (6%), WPI (4%), and WPI + PoPI (1%) were chosen less often. These results support that pea protein contributes to juiciness of meat analogues, while PoPI and WPI tend to reduce it, especially after heat treatment, that can cause water loss and tougher textures (Kyriakopoulou et al., 2021).

Toughness varied widely between samples. In the first sensory study WPI had the highest selection rat5e of this attribute (58%), followed by WPI + PPI (42%), with PPI (12%) and WPI + PoPI (7%) being perceived as much softer. After deep frying, the rankings changed: WPI + PoPI became the toughest (64%), followed by PPI (36%) and WPI + PPI (20%). WPI's toughness selection dropped to 5%. These results suggest that PoPI contributes to

toughness, especially when cooked, possibly due to heat-induced textural firming. On the other hand, WPI becomes softer after frying, possibly due to protein denaturation and moisture redistribution (Dekkers et al., 2021).

Tenderness selection rates showed an opposite pattern to toughness. In the raw samples, PPI was percieved as the most tender (57%), followed by WPI + PPI (38%), WPI + PoPI (28%), and WPI (22%). After frying, WPI + PPI led in tenderness (44%), followed by WPI (32%), PPI (31%), and WPI + PoPI (12%). These results confirm that PPI promotes this attribute, especially in raw blends, while PoPI reduces it after cooking, possibly because it interacts with water and becomes more compact during frying.

Flouriness was strongly influenced by formulation. In the first sensory study, WPI + PoPI was the sample where this attribute was selected the most (76%), followed by WPI (38%), with PPI (29%) and WPI + PPI (21%) being much lower. After frying, WPI had the flouriest perception (79%), while PPI (36%), WPI + PoPI (33%), and WPI + PPI (23%) were less affected. This shift suggests that PoPI drives flouriness in raw texture, but after frying, WPI is most responsible for dry and powdery mouthfeel. Studies suggest that the cooking processing may intensify the perception of drying proteins like WPI (Loveday, 2020).

Gumminess was not a major texture component in any formulation, but some differences were shown. In the raw samples, WPI + PPI had the highest gumminess selection (37%), followed by WPI (32%), PPI (18%), and WPI + PoPI (8%). In the second sensory study, values of gumminess of the deep fried samples were lower across the board: PPI (14%), WPI + PoPI (12%), WPI + PPI (11%), and WPI (2%). These findings suggest that WPI increases gumminess in raw form, but this effect is reduced by deep frying the samples. PoPI contributes the least to gumminess, possibly due to its granular and less cohesive structure.

Textural analysis of raw and deep-fried meat analogues formulations highlights distinct functional roles for each protein: Potato protein increases dryness, flouriness, and toughness, especially after cooking, resulting in a drier and firmer mouthfeel. Pea protein improves tenderness and juiciness, while lowering toughness and gumminess, making it beneficial for softer, more palatable textures. This observation is supported by Zhang et al. (2023), who found that pea protein content positively influenced the texture profile of meat analogues. Whey protein contributes to dryness, flouriness, and toughness in raw samples, but softens when fried. WPI + PPI blend showed balanced performance with moderate juiciness and tenderness, making them suitable for well-rounded textural outcomes.

These insights suggest that selection and combination of proteins in the development of plant-based meat analogues is key for optimizing texture. The interaction between plant and

dairy proteins shapes key sensory properties like dryness, juiciness, and flouriness, all of which impact consumer perception and product performance.

CHAPTER 5: CONCLUSIONS

This study aimed to evaluate how different combinations of plant proteins affect the sensory qualities and physicochemical characteristics of meat analogues produced through high-moisture extrusion. The goal was to better understand whether whey protein isolate can contribute to improving the structural and sensorial properties of meat analogues. The study also evaluated which ingredients contribute positively to appearance, taste, texture, and overall acceptance of plant-based meat alternatives. Four formulations containing 50% oat fiber concentrate (OFC) and various proportions of pea protein isolate (PPI), potato protein isolate (PoPI), whey protein isolate (WPI) were analysed through two sensory tests (on raw and fried samples) and a series of instrumental measurements.

The sensory results clearly showed that PPI played a central role in improving product acceptability. It was associated with higher scores in terms of taste, texture, and overall liking. Products containing only PPI were described as juicy, tender, and mild in flavor, attributes that are essential in mimicking real meat. The positive effects of PPI were mostly maintained after frying, suggesting good heat stability in terms of sensory performance.

The combination of WPI and PPI also gave good results, especially in terms of appearance and mechanical texture. WPI contributed to a more cohesive and elastic structure, enhancing gumminess and chewiness to its gelation properties. This was reflected in the texture profile analysis. Samples with WPI, either alone or in combination, showed higher values of gumminess, springiness, and chewiness, reflecting more cohesive structures. However, WPI alone was not enough to produce high pleasantness scores, confirming that it works best when combined with other proteins, particularly PPI.

In contrast, samples containing PoPI showed weaker performance. While PoPI improved some visual characteristics, especially after frying, it negatively affected the mouthfeel. Panelists described these samples as dry, floury, and tough, which was supported by instrumental data showing lower moisture and higher hardness. These traits made PoPI the least favourable ingredient in terms of consumer acceptance.

Physicochemical evaluations confirmed and supported the sensory findings: for example, higher water activity and moisture content were linked to better juiciness and mouthfeel. These values were highest in samples containing PPI and lowest in those with PoPI. Color values changed after frying, improving the visual appeal, especially in the samples containing WPI and PoPI. However, it is important to note that visual improvements were not always matched by better taste or texture.

Overall, the best performing formulations were those that combined PPI and WPI, providing the best balance between solid structural properties and good sensory qualities that remained stable after deep-frying. PoPI may be used in small amounts to improve appearance, but its impact on texture and juiciness must be carefully managed.

In conclusion, this study highlights how the right protein blend can significantly influence consumer perception and product quality in meat analogues. The addition of whey protein can help the development of meat analogues that are both texturally satisfying and appealing to consumers.

Future research could focus on optimizing these formulations further, testing new ingredient combinations, or exploring the nutritional and shelf-life characteristics of the products. In particular, whey protein isolate deserves deeper investigation, as it showed promising results in terms of texture enhancement and structural stability. Its ability to form gels, retain water, and interact with plant proteins suggests that it could play an important role in improving the mouthfeel and mechanical properties of meat analogues. More focused studies on its interactions, sensory effects, and behavior during processing could help show how whey protein can be used to improve the quality of meat analogues.

REFERENCES

Aiking, H. (2011). Future Protein Supply. *Trends in Food Science & Technology*, *22* (2-3), 112–120. https://doi.org/10.1016/j.tifs.2010.04.005

Akdogan, H. (1999). High Moisture Food Extrusion. *International Journal of Food Science & Technology*, *34*(3), 195–207. https://doi.org/10.1046/j.1365-2621.1999.00256.x

Benković, M., Jurinjak Tušek, A., Sokač Cvetnić, T., Jurina, T., Valinger, D., Gajdoš Kljusurić, J. (2023). An Overview of Ingredients Used for Plant-Based Meat Analogue Production and Their Influence on Structural and Textural Properties of the Final Product. *Gels (Basel, Switzerland)*, *9*(12), 921. https://doi.org/10.3390/gels9120921

Beuchat, L. R. (1981). Combined effects of solutes and food preservatives on rates of inactivation of and colony formation by heated spores and vegetative cells of molds. *Applied and Environmental Microbiology*, *41*(2), 472–477. https://doi.org/10.1128/aem.41.2.472-477.1981

Cellura, M., Cusenza, M. A., Longo, S., Luu, L. Q., Skurk, T. (2022). Life Cycle Environmental Impacts and Health Effects of Protein-Rich Food as Meat Alternatives: a Review. *Sustainability*, *14*(2), 979. https://doi.org/10.3390/su14020979

Chen, O., Mah, E., Dioum, E., Marwaha, A., Shanmugam, S., Malleshi, N., Chu, Y. (2021). The Role of Oat Nutrients in the Immune System: A Narrative Review. *Nutrients*, *13*(4), 1048. https://doi.org/10.3390/nu13041048

Cloetens, L., Ulmius, M., Johansson-Persson, A., Åkesson, B., Önning, G. (2012). Role of dietary beta-glucans in the prevention of the metabolic syndrome. *Nutrition Reviews*, *70*(8), 444–458. https://doi.org/10.1111/j.1753-4887.2012.00494.x

Cornet, S. (2021). *Water binding and water release by plant-based meat analogues*. University of Wageningen. Primary supervisor van der Goot, A. J., Faculty of Philosophy, University of Wageningen.

Cornet, S. H. V., Snel, S. J. E., Schreuders, F. K. G., van der Sman, R. G. M., Beyrer, M., van der Goot, A. J. (2021). Thermo-mechanical Processing of Plant Proteins Using Shear Cell and high-moisture Extrusion Cooking. *Critical Reviews in Food Science and Nutrition*, *62*(11), 1–18.

https://doi.org/10.1080/10408398.2020.1864618

De Angelis, D., Kaleda, A., Pasqualone, A., Vaikma, H., Tamm, M., Tammik, M.-L. Summo, C. (2020). Physicochemical and Sensorial Evaluation of Meat Analogues Produced from Dry-Fractionated Pea and Oat Proteins. *Foods*, *9*(12), 1754. https://doi.org/10.3390/foods9121754

Dekkers, B. L., Boom, R. M., van der Goot, A. J. (2018). Structuring Processes for Meat Analogues. *Trends in Food Science & Technology*, *81*(November 2018), 25–36. https://doi.org/10.1016/j.tifs.2018.08.011

Falcon, W. P., Naylor, R. L., Shankar, N. D. (2022). Rethinking Global Food Demand for 2050. *Population and Development Review*, *48*(4). https://doi.org/10.1111/padr.12508

Fu, J.-L., Sun, C., Chang, Y., Li, S., Zhang, Y., Fang, Y. (2022). Structure Analysis and Quality Evaluation of Plant-based Meat Analogs. *Journal of Texture Studies*, *54*(3). https://doi.org/10.1111/jtxs.12705

Galanakis, C. M. (2019). *Sustainable meat production and processing* (pp. 103–126 (Chapter 6: Plant-based meat analogues)). London Academic Press.

Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., Jebb, S. A. (2018). Meat consumption, health, and the environment. *Science*, *361*(6399). https://doi.org/10.1126/science.aam5324

Godschalk-Broers, L., Sala, G., Scholten, E. (2022). Meat Analogues: Relating Structure to Texture and Sensory Perception. *Foods*, *11*(15), 2227. https://doi.org/10.3390/foods11152227

Grahl, S., Palanisamy, M., Strack, M., Meier-Dinkel, L., Toepfl, S., Mörlein, D. (2018). Towards more sustainable meat alternatives: How technical parameters affect the sensory properties of extrusion products derived from soy and algae. *Journal of Cleaner Production*, *198*, 962–971. https://doi.org/10.1016/j.jclepro.2018.07.041

Guyony, V., Fayolle, F., Jury, V. (2022). High Moisture Extrusion of Vegetable Proteins for Making Fibrous Meat analogs: a Review. *Food Reviews International*, *39*(7), 1–26. https://doi.org/10.1080/87559129.2021.2023816

He, J., Evans, N. M., Liu, H., Shao, S. (2020). A Review of Research on Plant-based Meat alternatives: Driving forces, history, manufacturing, and Consumer Attitudes. *Comprehensive Reviews in Food Science and Food Safety*, *19*(5), 2639–2656. https://doi.org/10.1111/1541-4337.12610

Henchion, M., Hayes, M., Mullen, A., Fenelon, M., Tiwari, B. (2017). Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods*, *6*(7), 53. https://doi.org/10.3390/foods6070053

Heusala, H., Sinkko, T., Sözer, N., Hytönen, E., Mogensen, L., Knudsen, M. T. (2020). Carbon footprint and land use of oat and faba bean protein concentrates using a life cycle assessment approach. *Journal of Cleaner Production*, *242*, 118376. https://doi.org/10.1016/j.jclepro.2019.118376

Hoek, A. C., Luning, P. A., Stafleu, A., de Graaf, C. (2004). Food-related Lifestyle and Health Attitudes of Dutch vegetarians, non-vegetarian Consumers of Meat substitutes, and Meat Consumers. *Appetite*, *42*(3), 265–272. https://doi.org/10.1016/j.appet.2003.12.003

Hu, A., Li, T., Zhou, H., Guo, F., Wang, Q., Zhang, J. (2024). Water Binding Ability Changes of Different Proteins during high-moisture Extrusion. *Food Hydrocolloids*, *152*(2024), 109935–109935. https://doi.org/10.1016/j.foodhyd.2024.109935

Huang, X., Liu, H., Ma, Y., Mai, S., Li, C. (2022). Effects of Extrusion on Starch Molecular degradation, Order–disorder Structural Transition and Digestibility—a Review. *Foods*, *11*(16), 2538. https://doi.org/10.3390/foods11162538

Ismail, I., Hwang, Y.-H., Joo, S.-T. (2020). Meat Analog as Future food: a Review. *Journal of Animal Science and Technology*, *62*(2), 111–120. https://doi.org/10.5187/jast.2020.62.2.111

Jang, J., Lee, D.-W. (2024). Advancements in Plant Based Meat Analogs Enhancing Sensory and Nutritional Attributes. *Npj Science of Food*, *8*(1). https://doi.org/10.1038/s41538-024-00292-9

Jiang, W., Yang, X., Li, L. (2024). Flavor of extruded meat analogs: A review on composition, influencing factors, and analytical techniques. *Current Research in Food Science*, *8*, 100747–100747. https://doi.org/10.1016/j.crfs.2024.100747

Joshi, V., Kumar, S. (2015). Meat Analogues: Plant Based Alternatives to Meat products- a Review. *International Journal of Food and Fermentation Technology*, *5*(2), 107. https://doi.org/10.5958/2277-9396.2016.00001.5

Katzav, H., Chirug, L., Okun, Z., Davidovich-Pinhas, M., Shpigelman, A. (2020). Comparison of Thermal and High-Pressure Gelation of Potato Protein Isolates. *Foods*, *9*(8), 1041. https://doi.org/10.3390/foods9081041

Kyriakopoulou, K., Dekkers, B., van der Goot, A. J. (2019). Plant-Based Meat Analogues. *Sustainable Meat Production and Processing*, *78*(6), 103–126. https://doi.org/10.1016/b978-0-12-814874-7.00006-7

Kyriakopoulou, K., Keppler, J. K., Goot, A. J. (2021). Functionality of Ingredients and Additives in Plant-Based Meat Analogues. *Foods*, *10*(3), 600. https://doi.org/10.3390/foods10030600

Lan Schut, J. L. (2020). Analyzing potato protein isolates for their potential in developing meat analogues. Primary Supervisor Tornberg E., Faculty of Engineering, University of Lund, Sweden

Leszczyńska, D., Wirkijowska, A., Gasiński, A., Dominika Średnicka-Tober, Trafiałek, J., Kazimierczak, R. (2023). Oat and Oat Processed Products—Technology, Composition, Nutritional Value, and Health. *Applied Sciences*, *13*(20), 11267–11267. https://doi.org/10.3390/app132011267

Leterme, P., Monmart, T., Baudart, E. (1990). Amino Acid composition of pea (Pisum sativum) proteins and protein profile of pea flour. *Journal of the Science of Food and Agriculture*, *53*(1), 107–110. https://doi.org/10.1002/jsfa.2740530112

Li, X., Li, J. (2020). The Flavor of Plant-Based Meat Analogues. *Cereal Foods World*, *65*(4). https://doi.org/10.1094/cfw-65-4-0040

Lorenzen, P. C., Schrader, K. (2006). A Comparative Study of the Gelation Properties of Whey Protein Concentrate and Whey Protein Isolate. *Le Lait*, *86*(4), 259–271. https://doi.org/10.1051/lait:2006008

Loveday, S. M. (2020). Plant protein ingredients with food functionality potential. *Nutrition Bulletin*, *45*(3), 321–327. https://doi.org/10.1111/nbu.12450

Malav, O. P., Talukder, S., Gokulakrishnan, P., Chand, S. (2013). Meat Analog: a Review. *Critical Reviews in Food Science and Nutrition*, *55*(9), 1241–1245. https://doi.org/10.1080/10408398.2012.689381

Maningat, C. C., Jeradechachai, T., Buttshaw, M. R. (2021). Textured Wheat and Pea Proteins for Meat Alternative Applications. *Cereal Chemistry*, *99*(1), 37–66. https://doi.org/10.1002/cche.10503

Markovic, I., Ilic, J., Markovic, D., Simonovic, V., Kosanic, N. (2011). *Color Measurement of Food Products Using CIE L*a*b* and RGB Color Space* (pp. 489–494). Journal of Hygienic Engineering and Design.

Mateen, A., Manoj Mathpati, Singh, G. (2023). A study on high moisture extrusion for making whole cut meat analogue: Characterization of system, process and product parameters. *Innovative Food Science & Emerging Technologies*, *85*(May 2023), 103315–103315. https://doi.org/10.1016/j.ifset.2023.103315

McClements, D. J., Grossmann, L. (2021a). A Brief Review of the Science behind the Design of Healthy and Sustainable plant-based Foods. *Npj Science of Food*, *5*(1). https://doi.org/10.1038/s41538-021-00099-y

McClements, D. J., Grossmann, L. (2021b). The Science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4). https://doi.org/10.1111/1541-4337.12771

Mekonnen, M. M., Hoekstra, A. Y. (2012). A Global Assessment of the Water Footprint of Farm Animal Products. *Ecosystems*, *15*(3), 401–415. https://doi.org/10.1007/s10021-011-9517-8

Mouritsen, O. G., Klavs Styrbæk. (2014). *Umami*. University of Columbia. https://doi.org/10.7312/mour16890

Murat, C., Bard, M.-H., Dhalleine, C., Cayot, N. (2013). Characterisation of Odour Active Compounds along Extraction Process from Pea Flour to Pea Protein Extract. *Food Research International*, *53*(1), 31–41. https://doi.org/10.1016/j.foodres.2013.03.049

Naik, R. R., Wang, Y., Selomulya, C. (2021). Improvements of Plant Protein Functionalities by Maillard Conjugation and Maillard Reaction Products. *Critical Reviews in Food Science and Nutrition*, *6*(25), 1–26. https://doi.org/10.1080/10408398.2021.1910139

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R, Roohinejad, S. (2017). Effect of Extrusion on the anti-nutritional Factors of Food products: An overview. *Food Control*, *79*(2017), 62–73. https://doi.org/10.1016/j.foodcont.2017.03.027

Pasqualone, A., Costantini, M., Coldea, T. E., Summo, C. (2020). Use of Legumes in Extrusion Cooking: a Review. *Foods*, *9*(7), 958.

https://doi.org/10.3390/foods9070958

Patel, L., La Vecchia, C., Negri, E., Mignozzi, S., Augustin, L., Levi, F., Alicandro, G. (2024). Legume intake and cancer risk in a network of case-control studies. *European Journal of Clinical Nutrition*, *78*(2024). https://doi.org/10.1038/s41430-024-01408-w

Peng, H., Zhang, J., Wang, S., Qi, M., Yue, M., Zhang, S. Ma, C. (2022). High moisture extrusion of pea protein: Effect of l-cysteine on product properties and the process forming a fibrous structure. *Food Hydrocolloids*, *129*(August 2022), 107633. https://doi.org/10.1016/j.foodhyd.2022.107633

Rebello, C. J., O'Neil, C. E., Greenway, F. L. (2016). Dietary fiber and satiety: the effects of oats on satiety. *Nutrition Reviews*, *74*(2), 131–147. https://doi.org/10.1093/nutrit/nuv063

Roy, F. R., Boye, J. I. B., Simpson, B. K. S. (2010). Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International*, *43*(2), 432–442. https://doi.org/10.1016/j.foodres.2009.09.002

Sabah Mabrouki, Brugiapaglia, A., Sara Glorio Patrucco, Tassone, S., Barbera, S. (2023). Texture Profile Analysis of Homogenized Meat and plant-based Patties. *International Journal of Food Properties*, *26*(2), 2757–2771. https://doi.org/10.1080/10942912.2023.2255758

Samard, S., Ryu, G. (2019). A Comparison of Physicochemical characteristics, texture, and Structure of Meat Analogue and Meats. *Journal of the Science of Food and Agriculture*, *99*(6), 2708–2715. https://doi.org/10.1002/jsfa.9438

Satija, A., Hu, F. B. (2018). Plant-based diets and cardiovascular health. *Trends in Cardiovascular Medicine*, *28*(7), 437–441. https://doi.org/10.1016/j.tcm.2018.02.004

Schindler, S., Zelena, K., Krings, U., Bez, J., Eisner, P., Berger, R. G. (2012). Improvement of the Aroma of Pea (Pisum sativum) Protein Extracts by Lactic Acid Fermentation. *Food Biotechnology*, *26*(1), 58–74. https://doi.org/10.1080/08905436.2011.645939

Schmid, E., Farahnaky, A., Adhikari, B., Torley, P. J. (2022). High Moisture Extrusion Cooking of Meat analogs: a Review of Mechanisms of Protein Texturization. *Comprehensive Reviews in Food Science and Food Safety*, *21*(6), 4573–4609. https://doi.org/10.1111/1541-4337.13030

Schmidt, J. M., Damgaard, H., Greve-Poulsen, M., Larsen, L. B., Hammershøj, M. (2018). Foam and Emulsion Properties of Potato Protein Isolate and Purified Fractions. *Food Hydrocolloids*, *74*(2018), 367–378. https://doi.org/10.1016/j.foodhyd.2017.07.032

Schreuders, F. K. G., Dekkers, B. L., Bodnár, I., Erni, P., Boom, R. M., van der Goot, A. J. (2019). Comparing Structuring Potential of Pea and Soy Protein with Gluten for Meat Analogue Preparation. *Journal of Food Engineering*, *261*(November 2019), 32–39. https://doi.org/10.1016/j.jfoodeng.2019.04.022

Schutyser, M. A. I., Pelgrom, P. J. M., van der Goot, A. J., Boom, R. M. (2015). Dry fractionation for sustainable production of functional legume protein concentrates. *Trends in Food Science & Technology*, *45*(2), 327–335. https://doi.org/10.1016/j.tifs.2015.04.013

Sha, L., Xiong, Y. L. (2020). Plant protein-based alternatives of reconstructed meat: Science, technology, and challenges. *Trends in Food Science & Technology*, *102*(August 2020), 51–61. https://doi.org/10.1016/j.tifs.2020.05.022

Steven H.V. Cornet, Edwards, D., Van der Goot, A. (2020). Water release kinetics from soy protein gels and meat analogues as studied with confined compression. *Innovative Food Science and Emerging Technologies*, *66* (December 2020), 102528–102528. https://doi.org/10.1016/j.ifset.2020.102528

Stone, A. K., Karalash, A., Tyler, R. T., Warkentin, T. D., Nickerson, M. T. (2015). Functional Attributes of Pea Protein Isolates Prepared Using Different Extraction Methods and Cultivars. *Food Research International*, *76*(2015), 31–38. https://doi.org/10.1016/j.foodres.2014.11.017

Sui, X., Zhang, T., Zhang, X., Jiang, L. (2024). High-Moisture Extrusion of Plant Proteins: Fundamentals of Texturization and Applications. *Annual Review of Food Science and Technology*, *15*(1), 125–149. https://doi.org/10.1146/annurev-food-072023-034346

Tapia, M. S., Alzamora, S. M., Chirife, J. (2020). Effects of Water Activity (aw) on Microbial Stability as a Hurdle in Food Preservation. *Water Activity in Foods*, *14*, 323–355. https://doi.org/10.1002/9781118765982.ch14

Toldrá, F., Reig, M., Aristoy, M-Concepción., Mora, L. (2018). Generation of Bioactive Peptides during Food Processing. *Food Chemistry*, *267*(2018), 395–404. https://doi.org/10.1016/j.foodchem.2017.06.119

Vatansever, S., Tulbek, M., Riaz, M. (2020). Low- and High-Moisture Extrusion of Pulse Proteins as Plant-Based Meat Ingredients: a Review. *Cereal Foods World*, *65*(4). https://doi.org/10.1094/cfw-65-4-0038

Wang, Y., Tuccillo, F., Lampi, A., Knaapila, A., Pulkkinen, M., Kariluoto, S., Katina, K. (2022). Flavor challenges in extruded plant-based meat alternatives: A review. *Comprehensive Reviews in Food Science and Food Safety*, *21*(3). https://doi.org/10.1111/1541-4337.12964

Wild, F., Czerny, Michael, M., (2014). The evolution of a plant-based alternative to meat: From niche markets to widely accepted meat alternatives. Agro Food Industry Hi-Tech. 25. 45-49.

Wittek, P., Karbstein, H. P., Emin, M. A. (2021). Blending Proteins in High Moisture Extrusion to Design Meat Analogues: Rheological Properties, Morphology Development and Product Properties. *Foods*, *10*(7), 1509. https://doi.org/10.3390/foods10071509

Wood, P. J. (2010). Review: Oat and Rye β-Glucan: Properties and Function. *Cereal Chemistry*, *87*(4), 315–330. https://doi.org/10.1094/cchem-87-4-0315

Wu, H., Sakai, K., Zhang, J., McClements, D. J. (2024). Plant-based Meat analogs: Color Challenges and Coloring Agents. *Food, Nutrition and Health.*, *1*(4). https://doi.org/10.1007/s44403-024-00005-w

Younis, K., Ashfaq, A., Ahmad, A., Anjum, Z., Yousuf, O. (2022). A Critical Review Focusing the Effect of Ingredients on the Textural Properties of Plant-based Meat Products. *Journal of Texture Studies*, *54*(3). https://doi.org/10.1111/jtxs.12704

Zeeb, B., Merve Yavuz-Düzgun, Dreher, J., Evert, J., Timo Stressler, Fischer, L., Weiss, J. (2018). Modulation of the bitterness of pea and potato proteins by a complex coacervation method. *Food & Function*, *9*(4), 2261–2269. https://doi.org/10.1039/c7fo01849e

Zhang, J., Yuan, J., Han, X., Li, Q., Liao, X., Zhao, J. (2024). Development and characterization of fibrous high moisture extrudates based on pea protein isolate and whey protein. *Journal of the Science of Food and Agriculture*, *105*(2). https://doi.org/10.1002/jsfa.13866

Zhang, Y., Ryu, G. (2023). Effects of pea protein content and extrusion types on physicochemical properties and texture characteristic of meat analogs. *JSFA Reports*, *3*(1), 30–40. https://doi.org/10.1002/jsf2.97

Zhu, H.-G., Tang, H.-Q., Cheng, Y.-Q., Li, Z.-G., Tong, L.-T. (2021). Potential of preparing meat analogue by functional dry and wet pea (Pisum sativum) protein isolate. *LWT*, *148*, 111702. https://doi.org/10.1016/j.lwt.2021.111702