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OYSTER MUSHROOM AS A MEAT SUBSTITUTE IN MEAT PRODUCTS

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LIST OF ABBREVIATIONS

*a** - CIELab: Redness

ANOVA – Analysis of Variance

AOAC – Association of Official Analytical Chemists

*b** - CIELab: Yellowness

CIE – Commission Internationale de l'Eclairage

HHP – High Hydrostatic Pressure

*L** - CIELab: Lightness

LAB – Lactic Acid Bacteria

MDA – Malondialdehyde

PUFA – Polyunsaturated fatty acids

TBA – Thiobarbituric Acid

TBARS – Thiobarbituric Acid Reactive Substances

TCA –Trichloroacetic acid

TPA – Texture Profile Analysis

WBSF- Warner Bratzler Shear Force

WHC – Water Holding Capacity

ΔE – Total color difference (Empfindung)

1. INTRODUCTION

A major challenge confronting modern society is the development of sustainable food systems that can provide healthy diets for the rapidly expanding global population. Meat and meat products have been widely consumed around the world serving as important sources of high-quality proteins, essential vitamins (mainly B6, B9 and B12), and minerals (iron, zinc, selenium). However, meat products often lack key nutrients such as vitamin Control, calcium, dietary fiber, and antioxidants (Das et al., 2021). Despite being more nutrient-dense than many plant-based foods, red and processed meats are frequently recommended for limited consumption due to their high levels of fat, saturated fatty acids, cholesterol, calories, and synthetic additives. Excessive intake of these components has been linked to non-communicable diseases, including obesity, type 2 diabetes, cardiovascular diseases, and certain cancers (Saldaña et al., 2021). These concerns underscore the necessity of shifting toward healthier options in processed meat products.

In addition to health concerns, there is a growing awareness among consumers about the ethical and environmental implications of meat production. The complex challenges surrounding animal meat production have led many consumers to decrease their consumption of muscle-based foods. Key factors driving this shift include the relatively low efficiency of animal meat production, the environmental problems caused by animal farming, and issues related to animal welfare (Sha & Xiong, 2020). Recognizing these challenges, the Food and Agriculture Organization (FAO) of the United Nations has emphasized the need for alternative protein sources to adequately feed the growing global population. This has led to increased research efforts aimed at developing meat alternatives and enhancing the nutritional content of traditional meat products by incorporating sustainable and potentially health-promoting ingredients.

In this context, mushrooms have emerged as a promising substitute in various meat products, either as replacements or as innovative ingredients. Edible mushrooms, a diverse group of fast-growing macrofungi, are popular food items across the globe. They have been studied and consumed for their nutritional, medicinal, economic, and sustainable contributions, positioning them as future-oriented healthy foods (Kumar et al., 2022). They offer benefits beyond their unique flavor and health-promoting properties, acting as a valuable source of essential nutrients, dietary fibers, antioxidants and low levels of lipids (Perez-Montes et al., 2021). Extensive reviews have highlighted the use of various mushrooms as alternatives to meat, fat, salt, and other additives in a broad range of meat products.

Oyster mushrooms (*Pleurotus ostreatus*), known as the "poor man's meat," (Torres-Martínez et al., 2022) are widely consumed edible fungi recognized for their low fat, calorie, and cholesterol content. They are capable of growing on a wide range of agricultural wastes within a short period and can be cultivated with ease and at a low cost, as they do not demand precise environmental control (El-Ramady et al., 2022). Besides containing all the essential amino acids, their umami flavor and fibrous, meat-like texture make them an ideal meat substitute that blends well with meat products (Das et al., 2021). Thus, they can be utilized to produce meat-substituted products with enhanced health benefits and more sustainable attributes, serving as a valuable reference for the food industry.

Fresh mushrooms have a limited shelf life, lasting only about three days under ambient conditions, due to their high water content (87–95%), high metabolic rate, active enzymes, and susceptibility to bacterial contamination (Nketia et al., 2020). Various preservation methods, including drying, cooking, frying, irradiation, and fermentation, have been employed to extend their longevity. Although extensive research has been conducted on the effects of various processing techniques on mushroom quality, the primary emphasis has been on drying technologies. Mushrooms are typically utilized in meat products in the forms of dried powder, fresh ground, or extracts. There is limited research on the use of emerging technologies—such as microwave, high hydrostatic pressure, and ultraviolet light treatment—and their comparative effects on mushroom quality compared to traditional methods. Also, the use of fermentation processes in the production of meat alternatives remains rare, despite its potential to enhance functionality, improve nutritional content, and create appealing aromas. To our knowledge, there have been no studies that investigate the effects of different pretreatments and fermentation of oyster mushrooms, and their incorporation in sausage formulations as meat replacers.

2. OBJECTIVES

The overall objective of the study was to investigate the incorporation of oyster mushrooms as meat substitutes in sausage formulations. The specific aims of the study were:

- To investigate different quality attributes of sausages under the substitution of meat with fresh oyster mushrooms up to 100%, to ascertain how the replacement ratio affected the moisture content, pH, color, texture characteristics and protein denaturation of the final product

- To compare the effect of different pretreatments (Blanching, Steaming, Oven Cooking, Microwave, HHP and UV Light) and fermentation on physicochemical and textural properties of oyster mushrooms. Are there differences in the effects of conventional and alternative pretreatment methods?

- To investigate the effect of different pretreatments (Blanching, Steaming, Oven Cooking, Microwave, HHP and UV Light) and fermentation on nutritional quality (total amino acid, essential amino acid, free amino acid and biogenic amine contents) of oyster mushrooms

- To compare the sausage quality under the partial substitution of meat with fermented oyster mushrooms. How does the use of different pretreatment technologies before the fermentation of the mushrooms affect the quality characteristics of the sausages? Is the effect of pretreatment methods significant?

- To assess the sausage quality under the partial substitution of meat with fermented oyster mushrooms. How does the mushroom ratio affect the quality characteristics of the sausages, when the mushrooms were identically pretreated. Is mushroom ratio replacing meat in the sausage formulation decisive?

- To examine the storage stability of sausages under the partial substitution of meat with fermented oyster mushrooms. Are the alternative pretreatment methods result in similar or better quality parameters of the sausages in comparison to the conventionally pretreated ones?

3. LITERATURE REVIEW

3.1. Meat consumption

Meat has been a fundamental component of human diets throughout history, and our bodies have adapted to efficiently digest and metabolize it, allowing us to absorb its wide range of nutrients. Meat is a rich source of high-quality proteins and provides essential minerals such as iron, zinc, and selenium, as well as key vitamins like B6 and B12 (Font-i-Furnols, 2023). Certain nutrients, like vitamin B12, are exclusively found in animal-based foods, and others, such as iron and zinc, are more readily absorbed from meat than from plant sources. Meat is particularly valued for its protein content, which supplies essential amino acids like lysine, threonine, and methionine that are often lacking in plant-based diets. Additionally, meat is a primary source of long-chain omega-3 fatty acids, such as EPA and DHA. Furthermore, meat contains several bioactive compounds, including taurine, creatine, carnosine, conjugated linoleic acid, carnitine, choline, ubiquinone, and glutathione. These compounds can provide significant health benefits, particularly in supporting cognitive function and overall development (Leroy & Cofnas, 2020).

Meat and processed meat products are widely consumed around the globe and highly valued by consumers, not only for their nutritional benefits but also for their flavor and texture. Over the past few decades, there has been a significant rise in global meat consumption. This growth has been noticeable across all regions of the world, with particularly substantial increases in Asia, Latin America, and Africa. When looking at specific types of meat, pork and poultry are currently the most consumed worldwide, and they have experienced the greatest surge in consumption. The rise in pork consumption is largely attributed to China and several other Southeast Asian countries (Parlasca & Qaim, 2022). Based on food balance sheet data, the average global meat consumption is estimated to be 122 grams per day. Of this amount, approximately one-third comes from pork, another third from poultry, about a fifth from beef, and the rest from sheep, goats, and other animals (Godfray et al., 2018). Around 190 million metric tons of red meat are produced worldwide annually, while meat demand is anticipated to exceed 376 million metric tons by 2030. A major factor influencing global meat consumption trends is population growth. Additionally, rising income levels play a crucial role, leading to increased per capita meat consumption. Nearly half of the produced meat is being consumed by less than a quarter of the population residing in developed countries, where the average adult consumes roughly 200 to 250 grams of meat per day. Conversely, in developing countries, the intake of animal protein is quite low, averaging between 7 and 17 grams per person per day, and contributing to less than 2% of their total caloric intake. It has been proposed that individuals should

aim to consume 90 grams of meat per day, with no more than half of this amount coming from red meat sources, minimizing processed meat (Hicks et al., 2018; McMichael et al., 2007).

3.1.1. Health Concerns

A larger proportion of the meat consumed today is processed before it reaches consumers. In high-income Western countries, extensive prospective studies and meta-analyses generally reveal that individuals who consume large amounts of red and processed meat have slightly increased overall mortality rates compared to those with lower meat consumption (Godfray et al., 2018). In October 2015, the International Agency for Research on Cancer (IARC) released a statement on its assessment of the cancer risks associated with red and processed meat consumption. After reviewing the existing scientific literature, the IARC classified red meat as "probably carcinogenic to humans" and processed meat as "carcinogenic to humans" (Domingo & Nadal, 2017). The IARC reported that the strongest evidence linking red meat consumption to cancer was for colorectal cancer. However, they also noted evidence suggesting associations with pancreatic and prostate cancers and between consumption of processed meat and stomach cancer (Bouvard et al., 2015). The mechanisms by which high consumption of processed or red meat increases the risk of colorectal cancer are not fully understood. Potential carcinogenic factors in meat include heme iron, N-nitroso compounds commonly found in many processed meats, and heterocyclic aromatic amines and polycyclic aromatic hydrocarbons, which are produced when meat is cooked at high temperatures (Godfray et al., 2018). Besides, growing scientific evidence suggests that a high intake of red meat, particularly processed meat, may be linked to a higher risk of developing major chronic diseases, such as obesity, type 2 diabetes mellitus and cardiovascular disease as well as an increased risk of mortality (Beriain et al., 2018; Wolk, 2017). Red meat and processed meats may raise health risks primarily due to their high levels of saturated fatty acids, which can boost low-density lipoprotein cholesterol. Processed meats, in addition to containing synthetic additives, are frequently high in salt, which might also lead to increased blood pressure. Additionally, the formation of trimethylamine N-oxide from L-carnitine present in meat could be another contributing factor (Godfray et al., 2018).

Beyond nutritional considerations, livestock are crucial in the transmission and spread of diseases from animals to humans. In these cases, wild animals typically serve as the primary hosts, while farm animals act as amplifying hosts, facilitating the spread of the pathogens. The extensive use of antibiotics in intensive meat production systems is linked to the risk of developing antibiotic resistance in pathogens, which can diminish the effectiveness of these drugs in medical treatments (Parlasca & Qaim, 2022).

3.1.2. Environmental Concerns

In 2015, every United Nations Member State committed to "The 2030 Agenda for Sustainable Development" (UN, 2015), which outlines 17 Sustainable Development Goals to be met by 2030. A central component of this agenda is the promotion of sustainable food production systems that ensure food is not only sufficient, safe, and affordable but also nutritious and produced in a sustainable way (Pintado & Delgado-Pando, 2020). Meat production has a significantly greater environmental and climate impact compared to plant-based foods. The production of red meat comes with many environmental costs, contributing to greenhouse gas emissions, high fossil fuel consumption, and extensive land and water usage. The livestock industry occupies about 30% of the Earth's ice-free land area, both through grazing and the cultivation of feed crops. Moreover, livestock are a major contributor to land-based pollution, discharging nutrients, organic matter, pathogens, and drug residues into rivers, lakes, and coastal waters. Concentrated livestock operations can severely impact water quality, often becoming major sources of water pollution due to the release of animal waste, fertilizers, and pesticides (Steinfeld et al., 2006; Wolk, 2017). The livestock industry is also a major contributor to biodiversity loss in certain regions and is responsible for a substantial portion of agricultural greenhouse gas emissions. (Parlasca & Qaim, 2022; Xu et al., 2021). The agriculture sector accounts for about 22% of global greenhouse gas emissions, a level that matches that of the industrial sector and surpasses the emissions from transportation. Nearly 80% of these agricultural emissions are due to livestock production, which includes the transport of animals and their feed (McMichael et al., 2007).

Additionally, some animal husbandry systems are linked to serious animal welfare concerns. The majority farm animals are kept in intensive farming systems, usually housed in cages or confined spaces with no access to the outdoors. Europe is frequently seen as the world leader in animal welfare legislation, however, several painful practices are legal and prevalent across European countries (Bonnet et al., 2020). The source and sustainability of meat are important quality considerations for consumers. Previous research has indicated that attitudes towards reducing meat consumption are significantly influenced by beliefs related to environmental concerns and animal welfare (Seffen & Dohle, 2023). Prioritizing animal welfare in agricultural policy is strongly connected to ethical considerations and individual values (Ammann et al., 2023). As awareness and concerns about sustainability increase, these factors are expected to become even more significant in consumer decision-making (Resare Sahlin et al., 2020).

3.1.3. Changing dietary habits

Meat consumption has accompanied human survival for centuries, but it can no longer be taken for granted in modern diets. Research has indicated that decreasing meat consumption and shifting towards more plant-based diets will be essential for meeting both health and environmental sustainability challenges. The importance and privilege of meat consumption is being challenged by the increasing number of people who identify as vegans, vegetarians and flexitarians (Hicks et al., 2018). A common strategy for reducing meat consumption is vegetarianism, which totally excludes meat from the diet. Although a vegetarian diet inherently eliminates meat consumption, many people find it challenging to adopt and sustain this lifestyle. Therefore, numerous advocacy groups have been promoting flexitarian diets, which involve decreasing meat consumption without entirely eliminating it (Dakin et al., 2021). "Flexitarianism" is a relatively new term that has recently gained traction in both scientific and public discourse. The word "flexitarian," added to the Oxford English Dictionary in 2014, is a blend of "flexible" and "vegetarian." It describes someone who primarily follows a vegetarian diet but occasionally consumes meat or fish (Derbyshire, 2017). The flexitarian diet values meat for its nutritional benefits but also emphasizes addressing environmental and ethical issues related to farming practices and animal welfare.

Despite the difficulties associated with changing dietary habits, a growing number of consumers are choosing to reduce their meat consumption. Some people are embracing meat-free or meat-reduced days each week, replacing meat with alternative protein sources. This rising trend among flexitarians holds significant potential for altering societal patterns of meat consumption (Moreira et al., 2022). Since reducing meat intake is generally more feasible for individuals than complete abstinence, flexitarian diets may be more widely adopted. Encouraging such dietary changes could lead to substantial reductions in meat consumption at the population level (Spencer et al., 2018).

Several national authorities have started to incorporate recommendations for lower meat consumption into their dietary guidelines. This trend is reflected in the dietary recommendations from Sweden, the United Kingdom, the Netherlands, and the United States of America (Mullee et al., 2017). Additionally, the Food and Agriculture Organization of the United Nations (FAO) highlights the importance of utilizing alternative protein sources to ensure adequate nutrition for the growing global population (Stephan et al., 2018).

3.2. Meat Alternatives

Meat alternatives are commonly used as dietary protein sources by vegetarians, vegans, individuals adhering to religious or cultural dietary restrictions, and their appeal has broadened to include non-vegetarians and flexitarians. Meat alternatives are not exclusively plant-based; some products incorporate animal-derived proteins like milk, eggs, or insect components. However, they are predominantly plant-based products created from vegetarian or vegan ingredients to serve as substitutes for meat in the diet (Andreani et al., 2023; Hartmann et al., 2022). Animal proteins, found in foods like meat, eggs, and milk, are considered complete (high quality) proteins because they supply all 9 essential amino acids our bodies require and offer good digestibility. The EFSA recommendations specify the daily intake of essential amino acids for an average adult in mg per kg of body weight per day. Histidine is recommended at 10 mg/kg, isoleucine at 20 mg/kg, leucine at 39 mg/kg, lysine at 30 mg/kg, and the combined methionine and cysteine at 15 mg/kg. Similarly, phenylalanine and tyrosine together are recommended at 25 mg/kg, threonine at 15 mg/kg, tryptophan at 4 mg/kg, and valine at 26 mg/kg (EFSA, 2012). Most proteins derived from animal sources have high bioavailability, ensuring efficient absorption and utilization by the body (Wu et al., 2013). In contrast, proteins derived from plant sources typically have lower bioavailability due to the presence of antinutritive factors such as tannins, lectins, and protease inhibitors, which necessitate varying degrees of food processing to mitigate their adverse effects. Moreover, plant proteins are less accessible because their cell walls are only partially digestible in the human gastrointestinal tract (Elmadfa & Meyer, 2017). While some plant-based foods provide a complete source of protein (such as quinoa and soybeans), many are deficient or limited in one or more essential amino acids, particularly methionine and lysine. A beneficial approach when designing a meat alternative would be to combine different protein sources to ensure a complete amino acid profile (Bohrer, 2019). On the other hand, most meat substitutes are cholesterol-free and typically low in saturated fat and calories. They also provide a good source of fiber, folate, and manganese. However, to match the nutritional quality of meat products, their nutritional profiles should be enhanced by adding iron, calcium, zinc, and B12 vitamins (Andreani et al., 2023).

Meat alternative products are known by various names, including meat substitutes, meat replacers, meat analogs, meat surrogates, fake meat, faux meat, mock meat, and imitation meat, depending on the context and specific characteristics. These alternatives can be designed to replicate specific attributes of various meats, including texture, flavor, appearance, or chemical properties (Thavamani et al., 2020). Meat alternatives are broadly categorized into three main types: plant-based

(such as those made from soy, pea, or gluten), cell-based (including in vitro or cultured meat), and fermentation-based (such as mycoproteins). Some examples of meat alternatives include:

- **Tofu**: Produced from coagulated soy milk
- **Tempeh:** Made from fermented soybeans pressed into blocks
- **Seitan:** Extracted gluten protein from wheat flour
- **Textured Vegetable Protein (TVP):** Processed from soybeans and vegetables
- **Pea Protein:** Isolated protein from peas
- **Mycoprotein:** Protein derived from fungi through fermentation (Finnigan et al., 2019)
- **Cultured meat (in vitro):** Laboratory-grown meat using animal stem cells (Zhang et al., 2020)
- **Microalgae proteins:** Extracted protein from Spirulina (Espinosa-Ramírez et al., 2023)
- **Insect protein:** Isolated protein from insects (Caparros Megido et al., 2016)
- **Nuts, seeds, legumes and pulses:** rapeseed, lentil, chickpea
- **Mushrooms:** various types in different forms (fresh, dried, extract)

Some of the earliest meat alternatives, such as tofu and tempeh made from soy, and seitan derived from wheat gluten, have long been produced and consumed in Asian countries. Thanks to its rheological and viscoelastic properties, wheat protein has been used for many years in meat analogues, helping to create a fibrous texture in the finished products as binder and extender (Samard et al., 2019). Today, soy remains a primary ingredient in the production of many meat alternatives due to its advantageous properties, such as solubility and gelling abilities with a balanced amino acid profile and a protein digestibility-corrected amino acid score (PDCAAS) of 1.00, making it comparable to meat (Boukid, 2021). However, concerns about GMOs, allergies, climate impact, and biodiversity have driven research towards other protein sources. Despite this, high-protein isolates (such as pea), typically above 75% protein content, continue to be the primary raw materials used in meat alternatives (Andreani et al., 2023). Cereal proteins, including those from rice, barley, and oats, are frequently listed as ingredients in today's meat analogue products. Also, legume proteins such as pea, lentil, lupine, chickpea, and mung bean have been increasingly used by meat analogue producers (Bohrer, 2019).

3.2.1. Meat Analogues

The term "meat alternatives" broadly encompasses various products designed to replace meat. Within this category, a sub-group known as "meat analogues" has emerged, which are specifically formulated to replicate the functionality of meat. These products closely mimic mainly the texture, flavor, and appearance of meat, and include restructured items that resemble processed meats such as burgers, patties, sausages, nuggets, chunks, strips, and crumbles. These products can be cooked and handled by consumers just like traditional meat. A range of non-animal protein sources, including cereals, vegetables, legumes, microalgae, and fungi, are utilized to replace animal proteins in the creation of diverse meat-free but meat-looking products (Sha & Xiong, 2020). To achieve a meat-like appearance, red pigments can be added, and a range of vitamins and minerals are included to match the nutritional content of traditional meat.

The fundamental approach to creating plant-based meat analogues involves converting globular plant proteins into linear, muscle-like textured proteins, achieved through various processing techniques (Boukid, 2021). The development of meat-like fibrous textures involves several intensive processing techniques such as spinning, extrusion, shear cell technology, cross-linking, freeze structuring and mixing plant proteins and hydrocolloids. These methods modify the proteins' native structures by unfolding and denaturing them, which enhances their ability to interact with carbohydrate polymers (Yuliarti et al., 2021). Mycoprotein, cultured meat and bioprinting (3D printing technology) are also emerging technologies for the production of meat alternatives.

3.2.2. Structuring techniques

In contrast to myosin or actomyosin found in muscle tissue, the molecular arrangement and structural alignment of plant globular proteins do not naturally provide the fibrous texture or water-binding properties characteristic of muscle cells (Boukid, 2021). Different techniques utilize either a bottom-up or a top-down strategy to develop the fibrous structure of meat analogues. The bottom-up method involves starting with basic components, where individual elements are first created and then assembled into larger products, following the structural organization of muscle. On the other hand, the top-down method involves focusing on replicating structures at larger length scales. As a result, while these methods produce structures that resemble meat, they do not fully replicate its hierarchical architecture (Dekkers et al., 2018).

The fiber (wet) spinning technique emerged as an early method for creating simulated meat. This approach involved extruding an alkaline protein solution through spinnerets into an acidic coagulating bath, which resulted in the formation of protein filaments. These filaments were

subsequently combined into meat analogue products using various binding agents (Sun et al., 2021). In the electrospinning process, a biopolymer solution is exposed to a high electric field as it passes through a hollow needle or spinneret, leading to the formation of thin fibers due to surface charge.

The extrusion method involves combining typically defatted plant proteins with other ingredients such as water, carbohydrates, salts etc. before being fed into a twin-screw extruder. This process occurs under high temperature and varying moisture conditions (< 35% low-moisture, 40–80% high-moisture extrusion), to develop a meat-like fibrous texture. During extrusion, changes in protein conformation such as denaturation, unfolding, crosslinking, and alignment are induced by shearing, heating, compression, and cooling, resulting in structured aggregates or fibrils that mimic the texture of meat (Kyriakopoulou et al., 2019). Thermo-extrusion is the leading processing technology due to its high efficiency, cost-effectiveness, adaptability, and energy-saving properties in the production of meat alternative products.

Shear cell technology, which utilizes flow-induced structuring, involves subjecting the analogue mixture to 15 minutes of consistent shearing and heating, using cone-on-cone and cylinder-in-cylinder devises. After this process, the mixture is cooled to 25 °C. The resulting products are then sealed in a plastic bag and left at room temperature for a minimum of 1 hour to ensure the formation of a structurally stable fiber (Sha & Xiong, 2020).

In freeze structuring process, proteins are mixed with other ingredients to form a homogeneous emulsion. The mixture is then molded, frozen to create layers of ice crystals, and subsequently dried through methods such as steaming, baking, or frying. High-temperature drying solidifies the porous, fibrous protein structure that closely mimics the texture of animal muscle meat (Yuliarti et al., 2021).

Mixing plant proteins with hydrocolloids is a patented approach for developing meat-like products. This process combines water, a vegetable oil or fat, and various proteins with hydrocolloids as sodium alginate and methylcellulose, to produce a stable emulsion and a colloidal solution with divalent metal cations. Fiber formation is then initiated by adding casein, which coagulates with the cations, helping to trap the anisotropic structures within the mixture (Boukid, 2021).

Mycoprotein is produced through the continues fermentation of a filamentous naturally occurring fungus, *Fusarium venenatum*, followed by steaming, chilling, and freezing of the RNA-reduced biomass. The filamentous structures of the fungus undergo controlled denaturation and are transformed into a meat-like texture with the addition of various additives. The resulting product is a

high-protein, high-fiber food is approved for human consumption by the UK's Ministry of Agriculture, Fisheries, and Food in 1984. Mycoprotein, marketed under the brand name Quorn, is available in 17 countries, including the United States (Finnigan et al., 2019; Thavamani et al., 2020).

Cultured meat, also known as cell meat or lab-grown meat uses the stem cells harvested from the skeletal muscle of an animal. This method involves isolating stem cells, placing them in a growth medium with essential nutrients for the cell growth and stimulating their proliferation, differentiation, and maturation. Once the cells have sufficiently developed, they are scaled up, harvested, and processed into meat products. Currently, cultured 'meat' is only available in Singapore, but it is anticipated that it will soon receive approval in other regions (Font-i-Furnols, 2023; Warner, 2019).

Bioprinting, a form of 3D printing, has been adapted to produce meat analogues, replicating muscle-like structures with precise control over plant protein incorporation. This process involves extruding a paste composed of plant proteins, water, fat, and polysaccharides through a fine nozzle to create multilayered structures. The paste's viscosity is crucial for achieving the desired texture, often requiring rheological modifiers to fine-tune its properties. After printing, the meat analogue is matured in a bioreactor under specific conditions to ensure structural integrity. This method is used for producing various meat substitutes, including beef steaks and other meat analogues by NovaMeat (Sha & Xiong, 2020).

3.2.3. Meat-reduced products

Reformulating meat products to create healthier and more sustainable options has been a strong strategy that aligns with the Sustainable Development Goals (UN, 2015). Ongoing research has been primarily following two approaches: reducing harmful components to safe levels and incorporating ingredients that may enhance health. The first approach targets the reduction of harmful saturated fatty acids, salt, cholesterol, and additives like nitrites and phosphates. The second approach involves adding "functional ingredients," such as fiber, specific types of vegetable proteins, monounsaturated and polyunsaturated fatty acids, antioxidants, and other similar components, that provide health benefits to the meat products (Pintado & Delgado-Pando, 2020). In addition to enhancing the nutritional profile of traditional meat products, there is an increasing emphasis on exploring new and healthy components to partially replace meat in various products aimed at reducing overall meat consumption (Spencer & Guinard, 2018).

Reducing meat content, rather than fully eliminating it, can be considered a practical and more sustainable strategy compared to relying entirely on processed meat substitutes. This approach is generally more acceptable to consumers (Lang, 2020). Initially developed as a cost-saving solution,

meat extenders—also known as meat substitutes or meat replacers—provide an opportunity to reduce the amount of meat in products by incorporating protein-rich non-meat ingredients. These extenders can modify key attributes of the final product, such as water-holding capacity (WHC), texture, taste, and appearance, offering not just economic but also potential nutritional and functional benefits (Mills, 2014; Pintado & Delgado-Pando, 2020). In addition to the non-meat proteins previously mentioned, edible mushrooms can serve as a viable alternative protein source to meat (Asgar et al., 2010). Recent studies (2019- 2024) on the use of mushrooms replacing meat in various meat products are summarized in Table 1.

Table 1. Recent studies on the use of mushrooms replacing meat in meat products

Product	Mushroom species / meat substitution ratio	Results	References
Beef patties	Fresh Button mushroom (*Agaricus bisporus)* / 10%, 20%, 30%, 40%, and 50%	The 20% mushroom formulation exhibited good overall appeal, including aroma, flavor, saltiness, and juiciness.	(Wong et al., 2019)
Pork sausages	Dried Shiitake mushroom *(Lentinula edodes)* / 25%, 50%, 75%, and 100%	Increased moisture, fiber, methionine, glutamic, cysteine and total phenolic content, cooking loss and antioxidant activity. Slight darkening of sausages.	(Wang et al., 2019a)
Burger Patties	Blanched Oyster Mushroom (*Pleurotus ostreatus*) / 25%, 50%, 75%	No significant differences in terms of appearance, aroma, taste, and texture compared to control samples.	(Tabaldo-Tucar & Solar, 2019)
Abon Quail shredded meat	Fresh ground White Oyster Mushroom (*Pleurotus ostreatus*) / 10%, 20% and 30%	Reduced product price and texture of quail. increased moisture and decreased protein content.	(Aritonang et al., 2019)
Frankfurter	*Cantharellus cibarius* / 25% mushroom decoction	No negative effects in sensory evaluation. Minor changes in pH, yield, hardness, color. antimicrobial effect was observed.	(Novakovic et al., 2019)
Frankfurter	Porcini mushroom decoction (*Boletus edulis*) / 25%	Color modification, Increased moisture and hardness. Antimicrobial effect, extended shelf life.	(Novakovic et al., 2020)
Goat meat nuggets	Dried Enoki mushroom (*Flammulina velutipes)* / 2%, 4% and 6%	Improved oxidative stability and shelf life without affecting sensory attributes	(Banerjee et al., 2020)
Chicken patty	Dried Oyster mushroom (*Pleurotus sapidus*) / 10%, 20%, 30%	Enhanced antioxidant activity. Increase in hardness and chewiness. 10% optimal formulation	(Wan-Mohtar et al., 2020)
Chicken nuggets	Grey oyster mushroom stems (*Pleurotus sajor-caju*) / 45%, 50%, 55% and 60%	Similar texture, lower fat, carbohydrate and protein content, higher water activity, and moisture	(Husain & Huda-Faujan, 2020)
Chicken sausages	Fresh ground oyster mushroom (*Pleurotus sajor-caju*) / 15%, 30%, 45%, 60%	Increased moisture and fiber but reduced the ash content, crude fat, and crude protein	(Yahya & Ting, 2020)
Sausage Analogue Elaborated with Edible Mushroom and Soy Protein Isolate	*Lentinus edodes*, *Pleurotus ostreatus*, *Coprinus comatus* / 15% to 100%	the extruded mushroom-based meat analogue prepared from *Coprinus comatus* (15% addition) and SPI exhibited close textural profiles to real beef.	(Yuan et al., 2022a)
Mushroom–Legume-Based Minced Meat	*Pleurotus sajor-caju* / 12.5% to 50%	PSC mushrooms to chickpea flour in a ratio of 37.5:12.5 had better textural properties, higher consumer acceptability with protein content up to 47%.	(Mazumder et al., 2023)
Carb meat burger	*Pleurotus ostreatus / %5 to 20%*	Increased nutritional value of a functional food character, significantly improved sensory qualities of the finished product. Antioxidant effect.	(Tokarczyk et al., 2023)
Beef patties	Pink oyster mushroom *(Pleurotus djamor)/ 5% to 7.5%*	Decreased moisture and increased fiber content. Did not affect the protein level, texture and sensorial parameters were significantly affected, lower microbial count observed.	(Bermúdez et al., 2023)
Emulsion-type sausages	*Agaricus bisporus* / %10, %30, %50, and 70%	Improved the ester, ketone and polyunsaturated fatty acid content of sausage. Delayed the lipid and protein oxidation, best results for 10% mushroom substitution.	(Fu et al., 2023)
Chicken meatball	*Pleurotus ostreatus* / %10, 20% and 30%	addition of oyster mushrooms significantly influenced moisture content and organoleptic characteristics.	(Meiliany, 2024)
Pork sausages	*Lentinula edodes* / *30% to 40%*	lower fat and saturated fatty acid content, reduced energy levels, and higher levels of amino acids and polyunsaturated fatty acids. antioxidant performance.	(Wang et al., 2024)

3.3. Quality characteristics of sausages

Sausage is a food product created by combining appropriate ingredients in specific proportions, along with a structured design and controlled processing. As a result, the quality of the final product is typically a direct reflection of both the condition of the raw materials and the processing methods used (Essien, 2003). Other than their nutritional content, the quality of meat products is generally determined by evaluating physico-chemical properties, technological performance, sensory characteristics and shelf life.

3.3.1. Moisture content

Moisture content in meat products is inversely proportional to their dry matter, making it economically significant for both producers and consumers. It serves as a direct indicator of the food product's economic value, stability, and overall quality. The moisture content is a critical factor in assessing quality of sausages, as it is connected to lipids that impact sensory qualities like flavor, juiciness, tenderness, color, and appearance. Additionally, microbial growth is significantly affected by the moisture content since micro-organisms utilize the water within the sausage for their biological functions. Moisture content also correlates strongly with parameters such as water activity, texture, water-holding capacity, all of which ultimately influence the sausage shelf life and quality assessment (Feng & Arai, 2023; Nollet & Toldra, 2008). Water-holding capacity refers to the ability of product to maintain its moisture when subjected to external forces, such as gravity, heating, or pressing. In terms of water holding capacity, beef generally exhibits the highest ability, followed by pork, while poultry has the lowest capacity for retaining water (Font-i-Furnols et al., 2015). The average moisture content of sausages typically ranges between 50% and 60%. However, this can vary depending on the type of sausage, ingredients, and processing methods used.

3.3.2. Water activity

Water activity (a_w) plays a crucial role in the preservation and quality of meat. It is defined as the ratio of the water vapor pressure of a food product to the vapor pressure of pure water at the same temperature. Water activity provides a more accurate prediction of food stability, safety, and other properties than moisture content alone. It specifically measures the unbound, free water in a food system that is not chemically or physically bound and can participate in biological and chemical reactions. Bacteria, enzymes, and other reactants interact with this free water at the microenvironmental level, with most bacteria needing a water activity between 0.9 and 1.0 to thrive at temperatures suitable for bacterial growth (Jamilah et al., 2008; Nollet & Toldra, 2008).

3.3.3. pH

The pH is considered a key factor influencing pork quality. Both initial and ultimate pH affect the degree of protein denaturation and pork quality traits such as color and water-holding capacity. These traits play a crucial role in determining processing yields, export suitability, consumer preferences, and the sensory qualities of fresh or processed pork products. The normal ultimate pH typically falls within the range of 5.5-5.8 for pork and beef. Muscle pH impacts quality in several ways: as pH moves further from its isoelectric point (5.0-5.1), water-binding capacity increases, and the meat appears darker. Increased water holding capacity enhances juiciness and texture, however this can also lead to a shorter shelf life due to increased spoilage risk and reduced suitability for drying. A lower ultimate pH leads to increased protein denaturation, reduced WHC and paler meat color and drip loss (Bidner et al., 2004; Font-i-Furnols et al., 2015). The pH of pork sausages typically ranges from 5.5 to 6.2, depending on various factors such as the processing methods, added ingredients, and fermentation conditions. In fermented pork sausages, the pH typically decreases during the fermentation process due to the production of lactic acid, leading to enhanced preservation and flavor development. pH significantly influences microbial growth, with neutral pH having an increased spoilage potential, foodborne illnesses and reduced shelf life. Maintaining an appropriate pH is crucial for ensuring the safety and shelf life of pork sausages, as most bacteria thrive around pH 7 and struggle at pH levels below 4 or above 9 (Hui, 2012; Walker & Betts, 2008).

3.3.4. Color

The color of meat and meat products is a key quality factor that significantly affects consumer acceptance. Consumers tend to favor fresh meat with a bright-red appearance, cooked meat with a brown-gray hue, and cured meat with a pink color. The appearance of fresh meat is determined by the concentration of pigments, particularly myoglobin, the chemical form of these pigments, and the physical characteristics of meat. The oxidation of heme pigments is influenced by the muscle's reducing ability, the availability of oxygen, and the rate of myoglobin autoxidation. Myoglobin is the primary protein responsible for determining meat color, though other heme proteins like hemoglobin and cytochrome Control can also contribute to the color of beef, lamb, pork, and poultry (Jo et al., 2000; Kim et al., 2010). Beef contains significantly more myoglobin than pork or lamb, which gives its more intense color. In raw meat, there is a dynamic cycle where, in the presence of oxygen, myoglobin, oxymyoglobin and metmyoglobin continuously interconvert, maintaining an equilibrium among the three pigment forms. Interconversion of meat pigments occurs through chemical changes. When myoglobin binds with oxygen, it becomes oxymyoglobin, which is bright red in color. Both myoglobin and oxymyoglobin can undergo oxidation, losing an electron to form metmyoglobin

which has a brownish color (Mancini & Hunt, 2005). Post harvest factors affecting the color of fresh meat include temperature, packaging conditions, pH and lipid oxidation during aging and consumer exposure. Temperature plays a crucial role in maintaining the stability of myoglobin's structure. Keeping temperatures low (below 4°C) is essential to minimize changes in the characteristic color of fresh meat. Lipid oxidation can lead to a shift from the bright cherry-red color of meat to brownish hues, resulting in discoloration of meat products. When the ultimate pH is higher than anticipated, the meat darkens; conversely, a lower than expected pH results in a paler appearance (Tomasevic et al., 2021).

The CIELAB system is the most commonly used method for color measurement, utilizing the coordinates *L**, *a**, and *b**. This color space is widely utilized for color assessment across various foods, including meat products. In particular, lightness (*L**) and redness (*a**) have been identified as key indicators of PSE (pale, soft, exudative) and/or DFD (dark, firm, dry) conditions in pork, as well as overall visual meat color (Font-i-Furnols et al., 2015; Kim et al., 2010).

3.3.5. Texture

Consumer acceptance of food products is significantly influenced by their textural properties and texture is a crucial factor in the quality assessment of meat and meat products. Texture is a key attribute in evaluating meat quality, representing the cross-sectional structure of the meat. It is influenced by factors such as muscle fiber diameter, the number of fibers in a bundle, the surface area of fiber bundles, and the thickness and amount of connective tissue. Texture can vary from tight to loose or fine to coarse, playing a critical role in determining tenderness and shear force (Migdał et al., 2020). Meat texture consists of several attributes, including hardness, springiness, chewiness, cohesiveness, and juiciness, while sensory tenderness specifically refers to hardness. The perception of texture is closely linked to the mechanical failure characteristics of the muscle structure. Variations in meat texture arise from inherent differences in the structure of raw meat or muscle tissue, which involve contractile proteins, connective tissue, lipids, and carbohydrates, as well as external factors like cooking methods, temperatures and sample handling (Font-i-Furnols et al., 2015; Solomon et al., 2008). Tenderness is the sensation experienced during biting and chewing and is measured by the resistance during chewing. These characteristics influence the suitability of meat for thermal processing. During thermal treatments such as cooking, roasting, and frying, significant changes occur in meat components, with the most notable alterations occurring in the protein fractions (Migdał et al., 2020). The Warner-Bratzler Shear Force (WBSF) test and texture profile analysis (TPA) are established instrumental techniques used to estimate meat tenderness and toughness. Texture profile analysis has become a widely used instrumental method for evaluating sausage texture, offering

insights into both deformation and fracture properties under significant strain. TPA characterizes textural attributes such as hardness, springiness, cohesiveness, and chewiness, which are derived from force–time curves (Dong et al., 2007). On the other hand, the Warner-Bratzler test is designed to replicate the forces exerted during biting and chewing. This test quantifies the maximum force (N) in relation to knife movement (mm) and the compression required to shear (cut) a meat sample. The outcome of this measurement indicates the hardness (toughness) of the meat product (Novaković & Tomašević, 2017). However, assigning a precise physical interpretation to this shear force is challenging, as it reflects a combination of shearing, compression, and tensile stresses, making it more indicative of overall quality attributes. Warner-Bratzler test is commonly employed to assess the texture of various meat products, particularly whole muscle items and sausages (Schreuders et al., 2021).

3.3.6. Oxidative stability

Lipids are essential compounds for human nutrition, as they not only supply energy for bodily functions but also provide key substances like essential fatty acids and fat-soluble vitamins that must be obtained through the diet. Additionally, lipids contribute to many of the desirable attributes of meats and meat products, influencing flavor while enhancing tenderness and juiciness. However, lipids are prone to degradation, with lipid oxidation being the primary non-microbial factor responsible for the decline in quality of meat and meat products (Domínguez et al., 2019). Muscle components that promote lipid oxidation comprise iron, myoglobin, hydrogen peroxide, and ascorbic acid. Processed meats are more vulnerable to oxidation as heat denatures the muscle fibers, exposing lipids to oxygen and prooxidants. Additionally, antioxidant enzymes are inactivated during a thermal process, further increasing susceptibility to oxidative changes (Huang & Ahn, 2019).

Lipid oxidation can degrade nutritional components such as essential fatty acids and vitamins (A, D, and E) generating a range of primary and secondary by-products such as hydroperoxides, aldehydes, oxysterols, cholesterol oxidation products. These by-products not only affect meat quality but also have potential health implications for consumers. The rancid smell and taste are direct consequences of lipid oxidation altering the sensory attributes of meat products. These undesirable off flavors arise from the reaction between lipids (primarily impacting fatty acids, especially polyunsaturated ones) and atmospheric oxygen, known as oxidative rancidity, or from hydrolytic reactions catalyzed by lipases from either food or microorganisms (Amaral et al., 2018; Fernández et al., 1997).

Lipid oxidation can be classified into three types as photo-oxidation, enzyme-catalyzed

oxidation, and free radical oxidation (autoxidation). The occurrence of photo-oxidation necessitates the presence of light, photosensitizers (as myoglobin or hemoglobin), and oxygen. Enzyme-catalyzed oxidation involves the actions of lipoxygenase and cyclooxygenase, and is essential for the synthesis of eicosanoids. On the other hand, free radical oxidation, commonly referred to as autoxidation, represents the most critical form of lipid oxidation in meat. The mechanisms of photo-oxidation and enzymatic oxidation differ from autoxidation primarily in the production of hydroperoxides during the initiation phase. The autoxidation leads to the formation of undesirable odors, the generation of harmful compounds (such as carcinogens), a decrease in nutritional value and functional properties, as well as changes in meat color (Huang & Ahn, 2019).

Autoxidation is the primary mechanism by which unsaturated fatty acids react with oxygen, progressing through initiation, propagation, and termination phases. It begins with initiation, where active oxygen species generate free radicals, primarily alkyl radicals. At this stage, free radicals first target the natural antioxidants in meat, temporarily protecting fatty acids. In the propagation phase, free radicals increase due to lipid-lipid interactions, while hydroperoxides, the primary oxidation products, break down into other reactive species like hydroxyl, alkoxy, and peroxyl radicals, contributing to the overall oxidative deterioration of meat. Termination involves the degradation or interaction of these reactive species to form non-reactive products, including secondary compounds like lipid alcohols, carbonyls, ketones, and aldehydes. These stable secondary compounds are responsible for rancid odors and flavors, reflecting the negative impact of oxidation on meat quality (Domínguez et al., 2019; Erickson, 2002; Frankel, 1984).

When oxidation occurs at an accelerated rate, such as in cooked meats and meat products, primary oxidation products quickly break down into stable secondary products. Therefore it is more appropriate to measure these secondary products as an indicator of lipid oxidation (Gray & Monahan, 1992). Malondialdehyde (MDA), is a stable secondary product and the key aldehyde formed during the secondary oxidation of polyunsaturated fatty acids, which plays a significant role in meat quality. MDA serves as an indicator of lipid peroxidation as it generates rancid aromas even at low concentrations. It is measured through the spectrophotometric TBARS test (Thiobarbituric Acid Reactive Substances), which is the most common method used to evaluate lipid oxidation in meat and meat products (Amaral et al., 2018). TBARS test measures the color of a red complex formed between TBA and MDA with an absorption maximum at 532-535 nm. However, although MDA is the main TBA-reactive substance, the reaction is not specific to MDA. Other aldehydes and various oxidation products can also react with TBA. As a result, the method is referred to as TBARS, to

account for all substances that interact with TBA (Domínguez et al., 2019). The results are expressed as milligrams of malonaldehyde equivalents per kilogram of sample. Studies have identified MDA levels of 2–2.5 mg/kg as the threshold below which rancidity is not detectable in meat and meat products (Amaral et al., 2018).

The incorporation of antioxidants can effectively inhibit or reduce oxidative processes. Although synthetic antioxidants have been widely utilized, there is an increasing demand for natural alternatives such as phenolic compounds obtained from plants (Huang & Ahn, 2019). The antioxidant properties of various compounds isolated from mushrooms, including phenolic compounds, vitamins, polysaccharides, peptides, proteins, organic acids, carotenoids, alkaloids, and nucleotides, and their use in meat products have been documented (Cheung & Cheung, 2005; Perez-Montes et al., 2021; Stajic et al., 2013).

3.4. Mushrooms

Macrofungi, commonly known as mushrooms, include species from the Basidiomycota and Ascomycota phyla within the kingdom Fungi (Zhao, 2023). Botanically, mushrooms are the fleshy fruiting bodies of large, filamentous, saprophytic macrofungi that rapidly develop above or belove the ground (Das et al., 2021). Mushrooms, among the oldest food products known, are highly regarded as a culinary asset due to their distinctive taste, texture, and significant nutritional benefits across the globe. Ancient Egyptian hieroglyphics from approximately 4600 years ago reveal that mushrooms were regarded as a plant of immortality and a divine gift. Consequently, they were reserved exclusively for royalty, with commoners prohibited from consuming them (El Sheikha & Hu, 2018). The Romans referred to mushrooms as the “Food of the Gods,” while in Chinese culture, they have long been cherished as a health food and considered an “elixir of life”. In many cultures, they are popularly referred to as “vegetable meat” due to their significant culinary and nutritional contributions. (Das et al., 2021; Valverde et al., 2015).

Mushrooms can be classified into several ecological groups, with saprophytes being the most prominent, feeding on decomposing organic material, and soil-based varieties. Other groupings are based on their growth habitats, including mycorrhizal, ignicolous, entomogenous, and coprophilous (Chang & Wasser, 2017). Mushrooms are categorized into four main types according to their use: edible, medicinal, poisonous, and those with properties that are not well-documented. Mushrooms can be cultivated or naturally grown in the wild, either above ground (epigeal) or entirely underground (hypogeal). Out of the 14000 known mushroom species, approximately 3000 are edible, only around 35 are currently cultivated and used commercially, while around 700 possess recognized medicinal

properties (Pérez-Montes et al., 2021). However, the distinction between edible and medicinal mushrooms is often minimal, as many species exhibit pharmacological properties and are used for both culinary and therapeutic purposes (Guillamón et al., 2010). Edible mushrooms are classified as macrofungi with prominent fruiting bodies that are "large enough to be seen by naked eye and collected by hand" and vary widely in size, shape, and color across different species. They consist of filamentous structures enclosed by cell walls and are immobile. Although mushroom cells have walls, they lack chlorophyll and are unable to carry out photosynthesis. Their cell walls contain chitin, they absorb oxygen and release carbon dioxide, making them functionally more similar to animal cells than plant cells (Chang & Wasser, 2017; Chang, 2008) Mushrooms reproduce through both sexual and asexual means via spores. Their development begins from extensive mycelia (hyphae) primarily found underground, which eventually produces the visible fruiting structures (Torres-Martínez et al., 2022).

Several species of edible mushrooms, including *Agaricus bisporus* (button mushroom), *Lentinus edodes* (shiitake), *Pleurotus* spp. (oyster mushrooms), *Auricula auricula* (wood ear mushroom), *Flamulina velutipes* (winter mushroom) and *Volvariella volvacea* (straw mushroom) are cultivated commercially around the world, expanding consumer markets with both fresh and processed mushroom products. *Agaricus bisporus* is the most cultivated species globally, with China leading as the largest producer, contributing to more than 80% of the world's total macrofungi production. Mushroom cultivation continues to rise in other parts of the world as well (Aida et al., 2009; Kumar et al., 2022).

Besides their notable organoleptic qualities, mushrooms are recognized as nutritious foods. They are low in calories, fat, cholesterol, and sodium, but rich in protein (including essential amino acids), dietary fiber (chitin, hemicellulose, mannans, and β-glucans), phenolic compounds, vitamins (B, Control, and D), and essential minerals like selenium, zinc, iron, and potassium (Das et al., 2021; Rangel-Vargas et al., 2021). Mushrooms are the sole vegetarian source of vitamin D (Cardwell et al., 2018). Although mushrooms contain a low fat content (2–6% on a dry basis), the predominant types of fat are unsaturated fatty acids, making up over 75% of the total fatty acids. The most prevalent of these are oleic and linoleic acids. Also, dietary fiber in mushrooms ranges from 27% to 44% on a dry basis, with approximately 10% of this fiber being soluble (Bach et al., 2017). Chitin and β-glucan are essential elements in mushroom cell walls, playing multiple physiological roles. The non-digestible, prebiotic β-glucans are key components of mushroom polysaccharides that reach the large intestine and promote the growth of beneficial microbiota (Kiss et al., 2021; Yadav & Negi, 2021). Diets

supplemented with β-glucan have been shown to enhance immunity against upper respiratory infections, seasonal allergies, and osteoarthritis (Vlassopoulou et al., 2021).

The protein content of edible mushroom ranges from 19% to 40% on a dry basis, however this can vary significantly depending on the mushroom species and the conditions under which they are grown (Rangel-Vargas et al., 2021). The amino acid profile of mushroom proteins is comparable to that of animal proteins, offering a valuable alternative to reduce reliance on animal-based food sources, particularly in developed countries (Ayimbila & Keawsompong, 2023; Guillamón et al., 2010). Edible mushroom proteins offer a full spectrum of amino acids, with the eight essential amino acids making up 25%–45% of the total. Research has shown that edible fungi are particularly rich in lysine and leucine, in contrast to their lower levels in grains (Quintieri et al., 2023; Zhang et al., 2021b). In recent years, numerous bioactive proteins and peptides (such as lectins and laccases) with notable biological activities have also been discovered and isolated from edible fungi. Additionally, it has been reported that thermal, freezing, acid, alkali, and dehydration treatments do not compromise mushroom protein quality, suggesting their potential for stable nutraceutical and functional food applications (Singh et al., 2023; Yadav & Negi, 2021).

Often regarded as next-generation health foods or functional foods, mushrooms are widely used in commercial nutraceuticals and pharmaceutical products due to their strong bioactivity. Studies have confirmed that edible fungi contain a diverse range of biologically active compounds, including polysaccharides, alkaloids, steroids, and polyphenols (Zhang et al., 2021b). Mushroom bioactive compounds have been found to enhance immune function and exhibit antitumor, antimicrobial, anti-inflammatory, hypoglycemic, hypocholesterolemic, and antioxidant properties (Lu et al., 2020; Yadav & Negi, 2021).

Additionally, mushrooms contribute to ecosystem by converting lignocellulosic biomass into food or other useful products and mushroom cultivation helps lowering environmental pollution levels. They play a vital role in bioremediation, where their mycelia absorb and break down pollutants through the biosorption process (Chang & Wasser, 2017).

3.4.1. Oyster mushroom *(Pleurotus ostreatus)*

The *Pleurotus* genus (Jacq. Fr.) P. Kumm. (Basidiomycota, Pleurotaceae), commonly referred to as “white rot fungi,” are characterized by their white mycelium and their typical cultivation on non-composted lignocellulosic substrates, such as agro-industrial by-products. *Pleurotus* spp. are native to tropical and subtropical rainforests and cultivated both on large and small scales as one of the most diversified medicinal and edible mushrooms. In 1917, Germany began large-scale

cultivation of *Pleurotus* mushrooms to combat food shortages During World War I. Today, mushrooms from the genus *Pleurotus* hold the second position after *Agaricus bisporus* in the global mushroom market sales and are the most widely consumed mushrooms in China, due to their shorter cultivation period relative to other mushrooms (Bellettini et al., 2019; Piska et al., 2016). These species feature a basidiocarp that is shell or oyster shaped and can be in a range of colors including white, cream, yellow, light brown, pink, and grey, leading to their common name, oyster mushrooms. (Sharma et al., 2021).

Due to their high adaptability, many *Pleurotus* species (in total around 200 saprophytic species) exhibit resistance to pests and diseases, and do not require special growth conditions. Numerous studies have highlighted the beneficial traits of the *Pleurotus* genus, confirming their value as low-cost industrial solutions for addressing ecological challenges (Carrasco-González et al., 2017; El-Ramady et al., 2022). They are esteemed for their exceptional nutritional and medicinal properties which include anticancer, anti-hypercholesterolemic, anti-inflammatory, antioxidant, immune-enhancing, and antiviral effects, as well as their ability to manage blood lipid and glucose levels (Melanouri et al., 2022). Among the *Pleurotus* genus, the most prevalent species include the grey abalone oyster *(P. sajor-caju),* which has recently been reassigned to the *Lentinus* genus; the pink oyster mushroom (*P. djamor*); the king oyster mushroom *(P. eryngii*); the golden oyster (*P. citrinopileatus*); the branched oyster mushroom (*P. cornucopiae*); the king tuber oyster mushroom (P. tuber-regium); the phoenix oyster (*P. pulmonarius*); the abalone mushroom (*P. cystidiosus*); the white ferula mushroom (*P. nebrodensis*); and the classic oyster mushroom (*P. ostreatus*) (Torres-Martínez et al., 2022).

Among the genus, *Pleurotus ostreatus*, or the oyster mushroom, is the most extensively consumed and researched species and is noted for its distinct taste, texture and aroma. It is characterized by having high moisture content, low calorie count, many nutraceutical benefits and bioactive compounds, with antibacterial and antioxidant activity. Their chitin-rich cell walls provide dietary fiber, and they contain vitamins (B1, B2, B12, Control, D, and E), various micro and macro elements, carbohydrates, secondary metabolites like betalains, alkaloids, glycoproteins, and polysaccharides, minimal fat, and nearly zero cholesterol (Bellettini et al., 2019; Torres-Martínez et al., 2022). *P. ostreatus* ranked among the top producers of Vitamin B12 out of 38 edible mushrooms and a concentration of 0.6 μg per 100 g dry weight was reported (Lavelli et al., 2018). In addition to its high protein content (7.3% to 53.3% dry weight), *P. ostreatus* serves as a significant vegan protein source thanks to its protein quality and its supply of 9 essential amino acids and nitrogen, vital for

diverse bodily functions. The Amino Acid Score (AAS) of *P. ostreatus* fulfills the nutritional needs for all essential amino acids in adults. *P. ostreatus* shows exceptional protein digestibility, with its Protein Digestibility-Corrected Amino Acid Score (PDCAAS) matching the quality of casein (100), eggs (100), and soy protein isolate (100) (Bach et al., 2017; Carrasco-González et al., 2017). Compared to other protein sources like mushrooms, beef jerky, whole milk, and black beans, *P. ostreatus* was found to have the highest ratio of protein to energy (Bakratsas et al., 2023). Amino acid profiling of *Pleurotus* spp. has revealed that it contains all essential amino acids, with leucine, aspartic acid, phenylalanine, and lysine being the most prevalent. Additionally, umami amino acids and non-essential amino acids like gamma-aminobutyric acid and ornithine were also detected (Mumpuni et al., 2017; Quintieri et al., 2023). *Pleurotus* spp. are recognized as some of the richest mushrooms in umami-tasting amino acids, which resembles the taste of meat. A trained sensory panel revealed that *P. ostreatus* demonstrates the highest equivalent umami concentration and umami flavor among 17 edible mushrooms (Lavelli et al., 2018). *P. ostreatus* effectively accumulates minerals in its fruiting body. The highest concentrations are found in potassium, followed by phosphorus, magnesium, sodium, calcium, iron, zinc, manganese, and copper (Carrasco-González et al., 2017).

The health benefits of *Pleurotus* spp. including prebiotic effects, anti-cholesterol, anticancer, and immunomodulatory properties are primarily linked to their fiber content, especially β-glucans, which are abundant in *P. ostreatus* (Carrasco-González et al., 2017; Sharma et al., 2021). One of the β-glucans found in *P. ostreatus,* pleuran, characterized as an immunomodulatory agent with potential applications in treating cancer, infections, and immune system disorders (Piska et al., 2016) is currently available on the market as a natural immunostimulant. Furthermore, *Pleurotus ostreatus* contains a notably high level of ergothioneine, potentially functioning as a physiological antioxidant, along with lovastatin, which inhibits the rate-limiting enzyme in cholesterol synthesis and has been demonstrated to lower the risk of coronary heart disease (Lavelli et al., 2018).

In this context, *Pleurotus ostreatus* mushrooms are particularly advantageous for replacing meat in traditional meat products due to their key characteristics such as high protein content with essential amino acids, their fibrous texture and rich umami flavor which closely resembles that of meat. In addition to having antioxidant properties, being low in fat (but rich in polyunsaturated fatty acids), calories, and cholesterol is addressing the negative aspects of conventional meat products. They also offer vitamin B12, typically sourced from animal-based foods, which helps mitigate the deficiency risk for vegans.

3.4.2. Fermentation of mushrooms

Fresh mushrooms have a limited shelf life at ambient temperatures, primarily due to their high moisture content (87–95%), fast metabolic processes, enzyme activity, and sensitivity to bacterial growth. This leads to a decline in quality, including moisture loss, discoloration, changes in texture and flavor, and reduced nutrient levels. To prolong shelf life, several preservation methods like drying, boiling, cooking, marinating, freezing, pickling, frying, gamma irradiation and fermentation have been investigated and applied (Nketia et al., 2020).

Fermentation can be regarded as a biological approach to preserving food. It is among the earliest methods of food preservation worldwide, imparting desirable sensory characteristics to food products, particularly in terms of flavor and texture. Originally valued for their extended shelf life, safety, and sensory qualities, fermented foods are now increasingly recognized for their enhanced nutritional and functional benefits. This is attributed to the transformation of substrates and the production of bioactive or bioavailable compounds during fermentation (Marco et al., 2017; Rhee et al., 2011). Microorganisms play a crucial role in the development of fermented foods, metabolizing certain compounds in food, leading to distinctive flavors in the fermented product and potentially offering various health benefits. Currently, the microorganisms most commonly utilized in vegetable fermentation include bacteria, notably lactic acid and acetic acid bacteria. Among these, *Lactiplantibacillus plantarum* is a frequently employed lactic acid bacterium in food fermentation, particularly foods from plant origin (Chen et al., 2021). Lactic acid fermentation is an anaerobic biological process in which glucose and other six-carbon sugars, including disaccharides like sucrose and lactose, are converted into cellular energy, lactic acid, and smaller amounts of other acids, resulting in a pH decrease (Liu et al., 2016). Lactic acid fermented vegetables are often categorized based on their ingredients and preparation methods. Sauerkraut, pickles (such as cucumber and table olive pickles), and kimchi are the most extensively studied varieties, primarily due to their commercial significance (Zheng et al., 2018). Lactic acid fermentation can spontaneously occur in raw vegetables and fruits when favorable conditions—such as proper moisture, water activity, temperature, and salt concentration—are present for the growth of native lactic acid bacteria. The addition of salt and sugar, or immersing vegetables in brine enhances this environment, promoting lactic acid bacteria (LAB) growth by protecting the vegetables from light and oxygen. Physical air exclusion and the depletion of oxygen by plant cells during the early stages of fermentation, helps establish the anaerobic conditions required for LAB to thrive (Di Cagno et al., 2015).

Lactic acid fermentation is a traditional method employed to preserve both wild and cultivated

edible mushrooms in various regions of the world such as Southeast Asia, Japan and India. Various fungi species have been salted and fermented by Eastern Slavs, Estonians, and Poles, serving as a primary method for preserving mushrooms during the winter months (Sõukand et al., 2015). The health-promoting properties of mushrooms are enhanced by the enrichment with metabolites produced by LAB and the presence of viable beneficial microflora. The majority of the dry matter in edible mushroom fruiting bodies consists of carbohydrates, making them a key component for utilizing mushrooms in the lactic acid fermentation. Due to their polysaccharide content, including chitin, hemicellulose, α- and β-glucans, mannans, xylans, and galactans, edible mushroom fruiting bodies are also recognized as prebiotic raw materials (Jabłońska-Ryś et al., 2019). Mushroom fermentation can help develop distinct sensory qualities, improve nutritional value by increasing vitamin and essential amino acid content, decrease anti-nutritional factors, and enhance health benefits through the release of bioactive compounds like immunomodulatory peptides (Molfetta et al., 2022). At present, fermented mushrooms are absent from the European market, as this preservation technology has been largely supplanted by alternative methods such as freezing, marinating and sterilization. While mushroom fermentation holds considerable promise for innovation, product diversification and sustainable development across multiple industries, research on the lactic acid fermentation of edible mushroom fruiting bodies remains relatively limited (Jabłońska-Ryś et al., 2019; Sangeeta et al., 2024).

3.4.2.1. Biogenic amines

The fermentation process enhances the safety of food by significantly reducing or eliminating anti-nutritional factors and toxins. Nevertheless, fermentation can lead to an increase in the concentration of biogenic amines (BAs) in the final product. Mushrooms should be considered as food products that may contain high levels of biogenic amines, with lactic acid fermentation playing a crucial role in influencing their concentration. BAs are small molecular weight nitrogenous compounds primarily produced through the enzymatic decarboxylation of free amino acids or the amination and transamination of aldehydes and ketones (Jabłońska-Ryś et al., 2022). BAs can be categorized based on their chemical structure into heterocyclic (histamine and tryptamine), aliphatic (putrescine and cadaverine), and aromatic (tyramine and phenylethylamine) groups. They can also be classified according to the number of amine groups they contain, with monoamines (tyramine and phenylethylamine) having one amine group and diamines (histamine, putrescine, and cadaverine) containing two (Fong et al., 2021). BAs can be formed during microbial, plant, and animal metabolism, as they perform a variety of physiological and pathophysiological roles, such as regulating blood pressure, controlling cell growth, and mediating allergic responses. As a result, small

amounts of BAs naturally occur in food products. However, their major origin is the decarboxylation of free amino acids during processes like fermentation, spoilage, or putrefaction. Consequently, foods that support microbial growth, biochemical activity, and contain proteins or free amino acids are most prone to BA formation (Costa et al., 2018). Free amino acids are naturally present in foods and can also be released through proteolysis during storage and processing, and they are the precursors of biogenic amines. The most significant biogenic amines found in foods include tyramine (precursor: tyrosine), histamine (precursor: histidine), spermidine and spermine (derived from putrescine), putrescine (precursors: arginine and agmatine), agmatine (precursor: arginine), and cadaverine (precursor: lysine) (Sarkadi, 2019).

In fermented foods, lactic acid bacteria, along with other decarboxylase-positive bacteria and yeasts typically generate a small amount of BAs. However, excessive accumulation of these compounds (mainly histamine) can pose serious health risks when consumed, potentially causing symptoms like rash and burning sensations, diarrhea, vomiting, heart palpitations, and headaches. BA accumulation in food is influenced by precursor availability, the presence of decarboxylase-positive microorganisms and free amino acids, and a variety of intrinsic, technological, and environmental factors that facilitate microbial growth and enzyme production (Bartkiene et al., 2022). Furthermore, maintaining proper hygienic conditions during the handling of raw materials and throughout the manufacturing or storage processes can help minimizing biogenic amine levels in foods (Makhamrueang et al., 2021). The presence of biogenic amines not only poses a risk for intoxications but also serves as an indicator of food quality. Specifically, cadaverine and putrescine are regarded as markers of freshness and overall food quality. Histamine and tyramine are among the most physiologically significant and most toxic biogenic amines and both are vasoactive substances. Histamine toxicity, commonly referred to as scombroid poisoning, occurs from consuming spoiled fish, whereas tyramine is linked to the “cheese reaction,” originally attributed to the intake of aged cheeses (Ruiz-Capillas & Herrero, 2019; Sarkadi, 2019). The Good Manufacturing Practice sets the histamine limit for fish and fish products at 100–200 mg/kg. While consuming 50 mg of histamine and 600 mg of tyramine is generally safe for healthy individuals, these amounts can cause severe poisoning or death in people with metabolic disorders or those on monoamine oxidase inhibitors (intake of antidepression medicines). Although histamine regulations exist for fish, specific limits for other biogenic amines in food are generally lacking, with some recommendations for tyramine (100–800 mg/kg) and 2-phenylethylamine (30 mg/kg). Non-toxic compounds like putrescine and cadaverine may increase the toxicity of other amines, including histamine. The no-observed-adverse-effect levels for putrescine, spermidine, and spermine are 180, 83, and 19 mg/kg body weight,

respectively (Commission Regulation, 2015; EFSA, 2011; Jabłońska-Ryś et al., 2020; Sarkadi, 2019). The overall biogenic amine content in food should remain below 750–900 mg/kg (Bartkiene et al., 2022).

The composition of food, microbial load, various additives, processing and storage conditions influence decarboxylase enzyme activity. Some investigated control methods, including emerging thermal and non-thermal techniques, consist of high hydrostatic pressure, irradiation, innovative packaging, ultrasound, microbial modeling, and the addition of amine-negative starter cultures or preservatives (Naila et al., 2010). The current market is shifting towards the creation of innovative products using novel ingredients and advanced processing techniques, which introduce conditions that may either promote or inhibit the formation of biogenic amines (Ruiz-Capillas & Herrero, 2019).

3.4.3. Pretreatment of mushrooms

The commercialization of mushrooms faces difficulties because of their extreme perishability, characterized by their delicate texture, rapid rate of deterioration, and the necessity for prompt and appropriate processing following harvest. During the post-harvest period, various changes can occur, including microbial spoilage, weight and moisture loss, surface discoloration (mainly browning), texture alterations and cap expansion. Pretreatment is a crucial phase in mushroom processing, significantly affecting the final product's quality. This phase include cleaning, sorting, washing, cutting, and blanching, serving as a comprehensive term for the various preliminary processes needed to prepare mushrooms for subsequent processing. Pretreatments usually starts with washing to remove dirt and contaminants and with blanching to inactivate mushroom enzymes (Ritota et al., 2023; Sangeeta et al., 2024). Enzymes play a significant role in altering the mushroom's color (polyphenol oxidase), smell and flavor (peroxidase and protease), and cause texture softening (cellulase and protease). To assess the quality of mushrooms based on color, indicators like the Browning Index and Yellowness Index are employed (Doroški et al., 2021). Common methods used to prevent mushroom browning such as soaking in anti-browning solutions, steaming and water blanching, often lead to substantial losses in weight and nutritional value, emphasizing the need for new industrial pretreatment approaches with higher yield and minimal effect on mushroom qualities (Eissa et al., 2009).

3.4.3.1. Blanching (in water)

Conventional hot water blanching is the most commonly used thermal pretreatment method in the food industry prior to canning, drying, freezing and fermentation. In general, blanching involves immersing mushrooms in boiling water (with or without added chemicals) for 2 to 5 minutes to

remove excess air, inactivate enzymes (polyphenol oxidase, peroxidase), minimize non-enzymatic browning reactions, decrease microbial load, improve texture, and enhance quality before further processing steps (Jabłońska-Ryś et al., 2019). Although fermented mushrooms subjected to water blanching have better qualities than not blanched ones, the process has notable drawbacks. It is often associated with the leaching of water-soluble nutrients such as vitamins, flavors, minerals, carbohydrates, sugars, proteins, electrolytes, secondary metabolites to the blanching water and partial biochemical changes that can alter aroma. Blanching can also reduce functional and sensory properties, negatively affecting key attributes such as texture and color (Jambrak et al., 2007; J. Li et al., 2014). In water blanching operations, large amounts of water and energy are consumed. Water must first be heated, and after a certain period, it needs to be replaced as it becomes saturated with nutrients leached from the products, leading to high production of wastewater and energy usage due to the reheating required (Xiao et al., 2017). To address the challenges associated with traditional hot water blanching, new energy efficient pretreatment/blanching technologies are being developed and implemented.

3.4.3.2. Steaming

Superheated steam is widely used as a pretreatment due to its high enthalpy, which efficiently transfers heat to the product. During the initial phase of steam blanching, steam condenses on the product surface, releasing significant latent heat due to the lower temperature of the product compared to the steam. As the product warms, it eventually reaches a temperature sufficient to deactivate enzymes and microorganisms (Xiao et al., 2017). Steam blanching is more cost-effective and retains more minerals and water-soluble components compared to water blanching, thanks to minimal nutrient leaching. It also generates fewer pollutant effluents and uses 75 to 90% less energy than hot water blanching. However, steam blanching can cause tissue softening and quality degradation if the heating time is too long, as steam transfers heat less efficiently than hot water. Also, it can lead to weight loss and the formation of a dried layer on the product's surface due to water evaporation (Reis, 2017).

3.4.3.3. Oven pretreatment

Although ovens are primarily designed for cooking, oven blanching as a thermal pretreatment was included in the research to compare its effects with microwave pretreatment. In this process, heated air is used to briefly treat food before further processing, primarily aiming to deactivate enzymes and prepare the food for subsequent steps like fermentation. This process involves simultaneous heat and mass transfer: heat is transferred from the hot air to the product, while moisture

is either absorbed into the product during high humidity cooking or evaporated from it during low humidity cooking (Murphy et al., 2001). Unlike water blanching, oven blanching can prevent nutrient loss through leaching, however, it can face challenges such as uneven heat distribution and longer processing times.

3.4.3.4. Microwave pretreatment

In microwave blanching, polar substances absorb microwave energy, which is transformed into heat through dielectric heating. A key feature of microwave blanching is its direct interaction between the electromagnetic field and food materials, which facilitates the generation of heat. It presents an appealing alternative due to its advantages such as fast volumetric heating, low nutrient loss, reduced processing time and enhanced heating efficiency. Additionally, as a dry technique, microwave blanching produces less wastewater and consequently reduce the loss of soluble nutrients compared to traditional wet blanching methods such as water and steam blanching (Ruiz-Ojeda & Peñas, 2013; Trilokia et al., 2019). The primary advantage of microwave blanching system is its ability to generate heat internally and achieve greater penetration depth. However, a key limitation in applying microwave heating to blanching processes is the heating uniformity. The temperature gradients that develop in the sample can lead to overheating in some regions while leaving colder regions where enzymes may not be fully inactivated. Decreased polyphenol oxidase activity was reported with the microwave blanching of *Agaricus bisporus* mushrooms at 1000 W, 2.45 GHz for 5 minutes (Bernaś & Jaworska, 2015; Ranjan et al., 2017).

3.4.3.5. High Hydrostatic Pressure pretreatment

High hydrostatic pressure (HHP) is an emerging non-thermal physical treatment that improves the safety and shelf life of a wide range of food products by deactivating enzymes and microorganisms. Due to the isostatic nature of HHP, pressure is applied uniformly and instantly to products, leading to changes in proteins, polysaccharides, and lipids. HHP can maintain the quality of fresh foods like mushrooms, preserve bioactive compounds, with minimal impact on the nutritional and sensory qualities of the food. However, the pressure conditions needed to inactivate enzymes or microorganisms may interfere with maintaining color and texture. To make HHP an effective preservation technique, it must inactivate polyphenol oxidase, the key enzyme responsible for browning in mushrooms (Matser et al., 2000; Sangeeta et al., 2024). Some authors reported that enzyme activity may increase under pressure treatments at 600 MPa, while complete inactivation typically requires pressures up to 950 MPa (Podolak et al., 2020). Mushrooms that undergo vacuum treatment before HHP retain better color compared to those treated with HHP alone, with color values

similar to conventionally blanched mushrooms. Research on the antioxidant properties of HHP-treated mushroom powders has shown enhanced free radical scavenging activity, improved chelating activity, and increased total antioxidant activity (Lv et al., 2014).

3.4.3.6. Ultraviolet Light (UV) pretreatment

UV treatment is an emerging non-thermal disinfection method widely used for specific applications such as treating food-contact surfaces and disinfecting water and air, inactivating microorganisms, including bacteria, yeasts, molds, viruses, algae, and protozoa. This process utilizes radiation from the electromagnetic spectrum ranging wavelengths from 100 to 400 nm (Riganakos et al., 2017). The effectiveness of the treatment mainly depends on treatment time, intensity and distance from the UV source. The use of UV light in food production is increasingly popular due to its lack of toxic by-products, minimal impact on organoleptic properties, and lower energy requirements compared to high hydrostatic pressure processes and thermal pasteurization (Soro et al., 2023). Applying UV light as a postharvest treatment for mushrooms can effectively inhibit microbial growth and prevent browning lesions. Inactivation of polyphenol oxidase (PPO) with UV light and oxidation and transformation of phenolic compounds, which are linked to browning in various mushroom tissues were reported (Lei et al., 2018). PPO was found to be the most UV-resistant enzyme whereas peroxidase (POD) was found to be the least resistant. Several studies examining the impact of UV-C processing on the enzymatic activity of PPO, POD and lipoxygenase (LOX) enzymes in whole fruits revealed that UV exposure times ranging from 15 to 100 minutes resulted in varying degrees of enzyme inactivation, from complete inactivation to negligible effects (Sampedro et al., 2014). A recent study indicated that UV-C pretreatment of mushrooms is a superior alternative to water blanching, as it effectively preserves phenolic compounds and antioxidant activity while causing minimal color changes (Popa et al., 2022).

4. MATERIAL AND METHODS

4.1. Sausage production with fresh oyster mushrooms (Preliminary study)

Fresh oyster mushrooms (*P. ostreatus*) and ground pork with 30% fat content were sourced from a local market in Budapest, Hungary. Sausage production took place at the pilot plant of the Department of Livestock Products and Food Preservation Technology at the Hungarian University of Agriculture and Life Sciences. The mushrooms were thoroughly cleaned after discarding any damaged and unwanted parts. The sausage emulsions were created by combining ground pork meat, fresh oyster mushrooms, sodium nitrate, sodium ascorbate, phosphate, and ice in a cutter (Robot-Coupe R202). Eleven sausage formulations were prepared, each with varying levels of oyster mushroom as a meat substitute. Ice was adjusted and added to each sample group based on the water content of the control sample. The moisture content of the control sample was calculated based on the water content of each individual ingredient. For example, the water content of meat was considered to be 70%, and similar calculations were applied to other ingredients accordingly. The total water content of the control sample was then determined by summing the contributions from all components. The sausage formulations were as follows: 0P (control) contained no mushroom, while the meat replacement levels ranged from 10% (1P) to 100% (10P), as outlined in Table 2. The sausage mixtures were then filled into artificial casings (NaloShape plastic casing, 30 mm) using a manual sausage filler. The sausages were cooked in an oven (Lainox VE051P, Lainox, Vittorio Veneto, Italy) with steam function at 80°C for 30 minutes, and the internal temperature was monitored using a thermometer (Testo 926 with a needle sensor 0628). After cooking, the sausages were rapidly cooled to below 5°C. Moisture content, water activity, pH, color, and texture properties of the sausages were assessed on the same day of production, and Differential Scanning Calorimetry (DSC) analysis was conducted on frozen samples. All treatments were performed in duplicate independently. Images of the sausage samples with mushroom replacing meat are shown in Figure 1.

Table 2. Sausage formulations with fresh oyster mushroom replacing meat

Ingredients	**0P (Control)**	**1P**	**2P**	**3P**	**4P**	**5P**	**6P**	**7P**	**8P**	**9P**	**10P**
Meat replacement ratio	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Meat (70/30)	500	450	400	350	300	250	200	150	100	50	0
Ice (g)	300	279	259	238.5	218	197.5	177	156.5	136	115.5	95
Sodium nitrate (g)	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67	4.67
Phosphate (g)	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
Sodium ascorbate (g)	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
Oyster Mushroom (g)	0	50	100	150	200	250	300	350	400	450	500

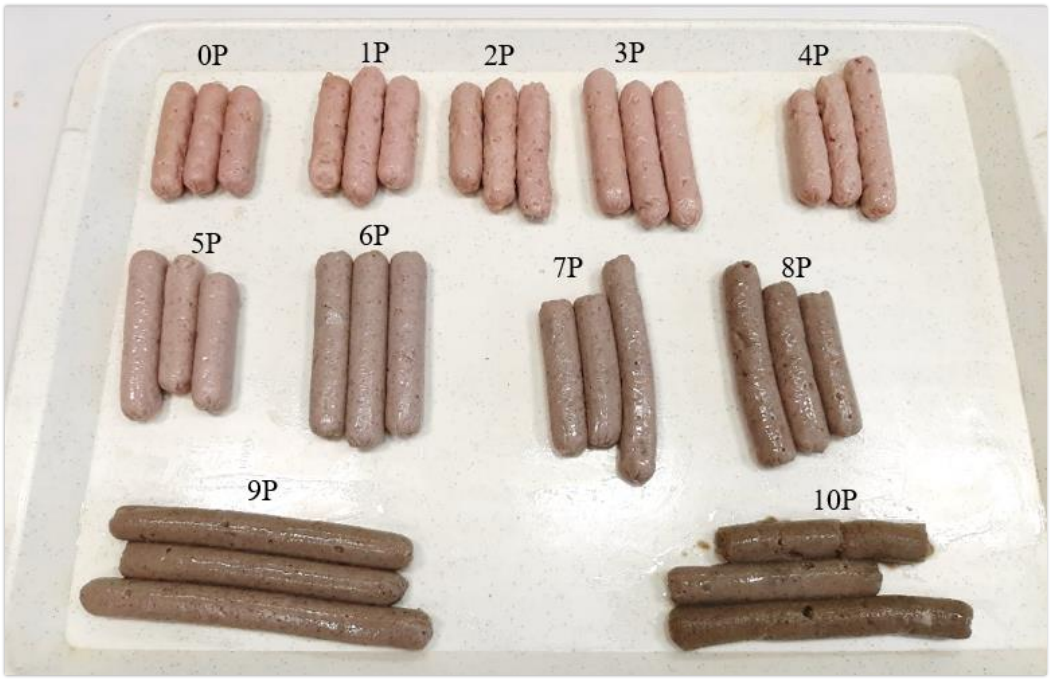


Figure 1. Images of the sausage samples from 0% (0P) to 100% (10P) mushroom replacement

4.2. Mushroom pretreatments and fermentation

Fresh oyster mushrooms were stored at 4 °C until their utilization. The pretreatment steps and fermentation were carried out at the pilot facility of the Department of Livestock Products and Food Preservation Technology, Hungarian University of Agriculture and Life Sciences. After discarding any damaged and unwanted parts of the mushrooms, the remaining were thoroughly cleaned, longitudinally sliced to be utilized for pretreatments and fermentation. For each pretreatment group, 2 kg of fresh oyster mushrooms were used. In addition to fresh oyster mushrooms (Fresh), six distinct pretreatment methods were applied prior to mushroom fermentation: blanching in water (Blanch), steaming (Steam), oven (Oven), microwave (MW), High Hydrostatic Pressure (HHP), and Ultraviolet Light treatment (UV). Fresh whole and sliced oyster mushrooms are shown in Figure 2.

Figure 2. Fresh whole and sliced oyster mushrooms

4.2.1. Blanching in water

Blanching was performed by immersing fresh longitudinally sliced oyster mushrooms in boiling water at 100°C for 3 minutes. After water blanching, they were drained to eliminate excess water and allowed to cool down to room temperature (21-22 °C) on stainless steel trays until further processing. Blanching of oyster mushrooms in water are shown in Figure 3.

Figure 3. Blanching of oyster mushrooms in water

4.2.2. Steaming

Steaming was performed at 100°C for 3 minutes in a multifunctional oven (Lainox VE051P, Lainox, Vittorio Veneto, Italy) using only the steam function. Pretreated mushrooms were let to cool down to room temperature (21-22 °C) on stainless steel trays until further processing. Steaming of oyster mushrooms are shown in Figure 4.

Figure 4. Steaming of oyster mushrooms

4.2.3. Oven pretreatment

Oven pretreatment was performed at 100°C for 3 minutes in a multifunctional oven (Lainox VE051P, Lainox, Vittorio Veneto, Italy) using only the oven cooking function without steam. Pretreated mushrooms were let to cool down to room temperature (21-22 °C) on stainless steel trays until further processing. Oven pretreatment of oyster mushrooms is shown in Figure 5.

Figure 5. Oven pretreatment of oyster mushrooms

4.2.4. Microwave pretreatment

For microwave pretreatment, mushroom samples were divided into 300 g portions and placed on porcelain plates. The mushrooms were then subjected to 85°C at 900 W, 2.45 GHz for 3 minutes using the A3 (vegetable) setting in a microwave oven (SHARP R722STWE, Sharp Electronics Europe Ltd., Middlesex, UK). Pretreated mushrooms were let to cool down to room temperature (21-22 °C) on stainless steel trays before further processing. Microwave pretreatment of oyster mushrooms is shown in Figure 6.

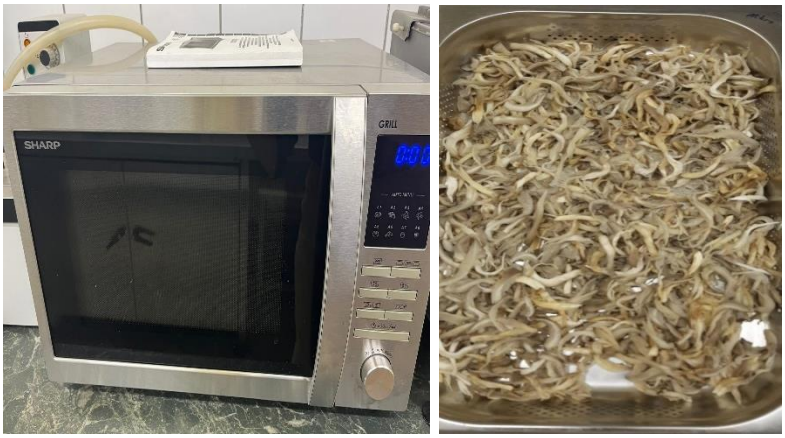


Figure 6. Microwave pretreatment of oyster mushrooms

4.2.5. HHP pretreatment

High Hydrostatic Pressure (HHP) treatment was applied at 20 °C and 300 MPa, with a holding time of 3 minutes (RESATO PU-100-2000, Resato International B.V., Assen, The Netherlands), in plastic sealed pouches (90 µm PA/PE poach, (20µm PA - 70µm PE, AMCO Kft., Hungary). HHP pretreatment of oyster mushrooms are shown in Figure 7.

Figure 7. HHP pretreatment of oyster mushrooms

4.2.6. UV Light pretreatment

During UV light pretreatment, mushrooms were irradiated with a 30 W UV light at 312 nm (VL-115.M, Vilber Lourmat, Marne La Vallee, France) for 15 minutes at 20°C. To ensure even irradiation, mushrooms were placed on an open shelf, 20 cm below the light source. UV Light pretreatment of oyster mushrooms are shown in Figure 8. Images of pretreated oyster mushrooms are given in Figure 9.

Figure 8. UV Light pretreatment of oyster mushrooms

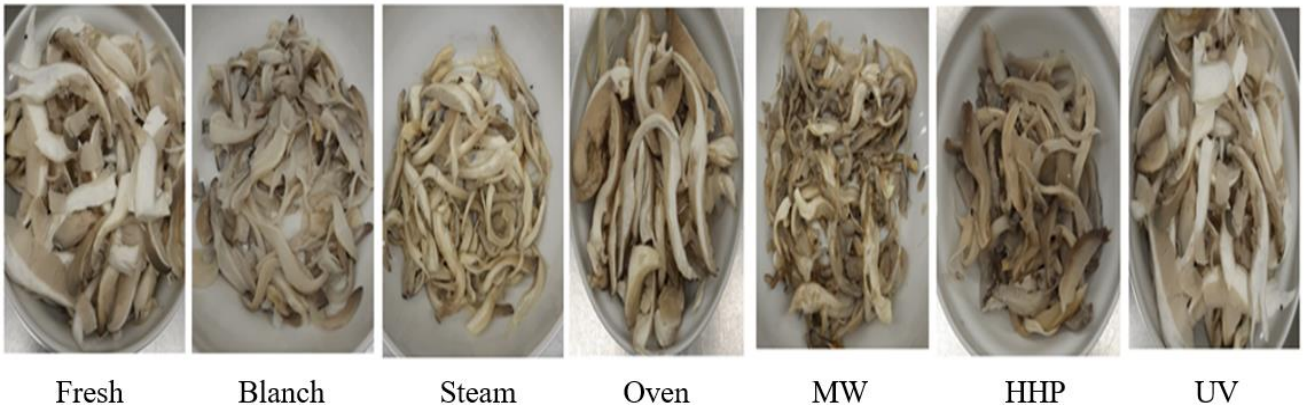


Figure 9. Images of the pretreated oyster mushrooms (Fresh – fresh; Blanch – blanched; Steam – steamed; Oven – oven pretreated; MW – microwave pretreated; HHP – HHP pretreated; UV – UV light pretreated)

4.2.7. Mushroom Fermentation

After applying the pretreatments, fermentation was carried out based on the method described by Jabłońska-Ryś et al. (2022), with some modifications. The mushrooms were subjected to an 8-day spontaneous fermentation at a temperature of 21–22 °C. This process took place in sealed pouches, each containing 2% (w/w) salt, 1% (w/w) sucrose, and 70 mL of a 2% salt solution. Upon completion of fermentation, the fermented mushrooms were stored at 4 °C for a week for maturation. Before the analyses, the sealed pouches were opened, and the mushrooms were drained to eliminate excess water. Images of the fermented oyster mushrooms are given in Figure 10.

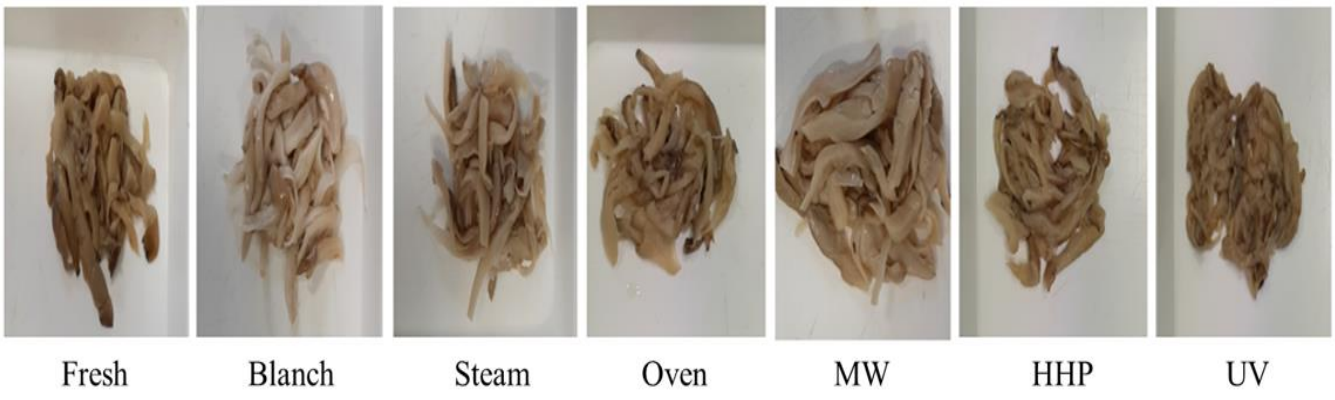


Figure 10. Images of the fermented oyster mushrooms (Fresh – fresh; Blanch – blanched; Steam – steamed; Oven – oven pretreated; MW – microwave pretreated; HHP – HHP pretreated; UV – UV light pretreated)

4.3. Sausage production with pretreated fermented oyster mushrooms

Sausage mixtures were obtained by blending meat (70/30), fermented oyster mushrooms, sodium nitrate, sodium ascorbate, phosphate, and ice in a cutter (Robot-Coupe R202, Robot-Coupe, Burgundy, France) according to the formulations given in Table 2. Sausage production took place at the pilot plant of the Department of Livestock Products and Food Preservation Technology at the Hungarian University of Agriculture and Life Sciences. A total of fifteen formulations were produced, involving six different pretreatment and three replacement ratios, as 0%, 25%, and 50% of the meat content. Table 3 provides details of the sample groups. The sausage mixtures were manually stuffed into Ø30 mm synthetic casings (NaloShape, ViskoTeepak, Lommel, Germany) and subsequently subjected to heat treatment at 80 °C for 30 minutes using the steam function of a Lainox VE051P oven (Lainox, Vittorio Veneto, Italy). The sausages obtained (around 20 per sample group) were then quickly chilled to below 5 °C. The images of the sausage samples on the production day after the heat treatment are given in Figure 11. Evaluations of moisture content, pH, color, texture properties, and lipid oxidation were performed on the production day and at storage intervals of 7, 14, 21, and 28 days.

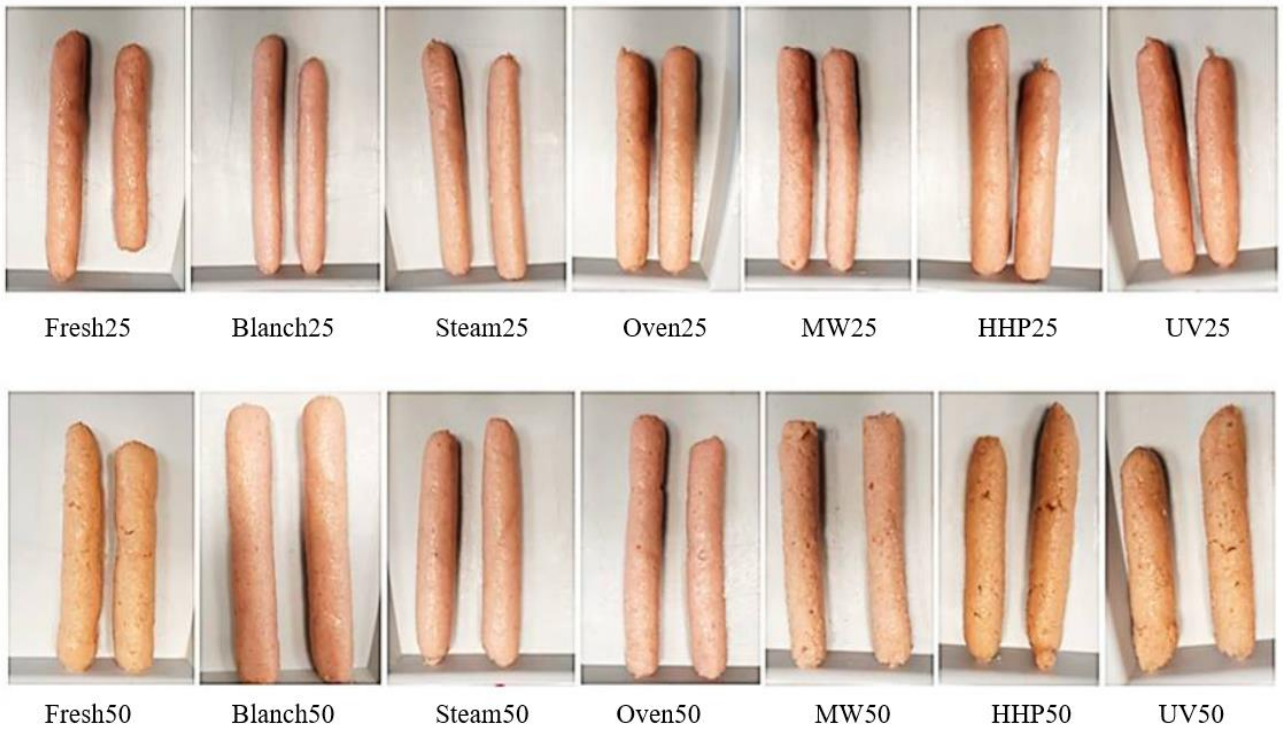


Figure 11. Images of the sausage samples with 25% and 50% meat replacement

Table 3. Description of the sample groups

Sample	Description
Control	Control sample, 100% ground pork sausage
Sausage samples with 25% meat replacement	
Fresh25	25% Fresh fermented oyster mushroom + 75% ground pork
Blanch25	25% Blanched fermented oyster mushroom + 75% ground pork
Steam25	25% Steamed fermented oyster mushroom + 75% ground pork
Oven25	25% Oven pretreated fermented oyster mushroom + 75% ground pork
MW25	25% Microwave pretreated fermented oyster mushroom + 75% ground pork
HHP25	25% HHP pretreated fermented oyster mushroom + 75% ground pork
UV25	25% UV pretreated fermented oyster mushroom + 75% ground pork
Sausage samples with 50% meat replacement	
Fresh50	50% Fresh fermented oyster mushroom + 50% ground pork
Blanch50	50% Blanched fermented oyster mushroom + 50% ground pork
Steam50	50% Steamed fermented oyster mushroom + 50% ground pork
Oven50	50% Oven pretreated fermented oyster mushroom + 50% ground pork
MW50	50% Microwave pretreated fermented oyster mushroom + 50% ground pork
HHP50	50% HHP pretreated fermented oyster mushroom + 50% ground pork
UV50	50% UV pretreated fermented oyster mushroom + 50% ground pork

4.4. Applied measurements

The measurements conducted for each product are summarized in Table 4. All measurements were conducted at the Department of Livestock Products and Food Preservation Technology other than the amino acid and biogenic amine analysis, which was carried out at the Department of Nutrition, Hungarian University of Agriculture and Life Sciences.

Table 4. The measurements conducted for each product

Measurements	Sausages with fresh oyster mushroom (Preliminary study)	Pretreated oyster mushrooms	Fermented oyster mushrooms	Sausages with pretreated fermented oyster mushroom
Moisture content (%)	+	+	+	+
a_w	+			
pH	+	+	+	+
Color attributes (*L, a*, b**)	+	+	+	+
Texture (Fmax, N)	+	+	+	+
DSC (Enthalpy, J/g)	+			
Yield (%)		+	+	
Lipid oxidation (mg MDA/kg)				+
NIR		+	+	+
Essential amino acid and proteinogenic (total) amino acid profile		+	+	+
Free amino acid profile		+	+	+
Biogenic amine profile		+	+	+
Sensory evaluation				+

4.4.1. Moisture content

The moisture content of the mushroom and sausage samples were determined in accordance with the AOAC 950.46 method (AOAC, 2005). For this purpose, around 3 g of each sample was dried in a convection oven (Labor Müszeripari Müvek, Budapest, Hungary) at 105 °C for 16 hours. Each measurement was performed in triplicates, using three separate samples and the moisture content (%) of the samples were calculated according to their initial and dried weight.

4.4.2. Water activity (a_w)

The water activity (a_w) of the sausage samples was measured with a a_w meter (Testo 0645) with three repetitions.

4.4.3. pH measurement

The pH levels of the mushroom and sausage samples were assessed using a calibrated pH meter (Testo SE, Titisee-Neustadt, Germany). The electrode was directly inserted into the samples. Each measurement conducted in triplicate.

4.4.4. Color measurement

The color properties of the mushroom and sausage samples were assessed using the CIELAB scoring system (CIE, 1986). In this system, Lightness (L^*) is typically represented along the vertical axis, ranging from 0 (dark) to 100 (white). The a^* value is plotted on the X-axis, reflecting the green/red opponent colors, with negative values indicating green and positive values representing red (scale: -60 for green to +60 for red). The b^* value is shown on the Y-axis and corresponds to the blue/yellow opponent colors, where negative values denote blue and positive values indicate yellow (scale: -60 for blue to +60 for yellow). Lightness (L^*), redness (a^*), and yellowness (b^*) were measured with an 8 mm aperture CR-410 colorimeter (Konika Minolta Sensing Inc., Osaka, Japan), utilizing a 2° observer and calibrated with illuminant Control against a standard white reflectance calibration plate (CRA43). Measurements were conducted at room temperature, with each sample undergoing nine parallel assessments. For the mushroom samples, The Browning Index, Yellowness Index, and Total Color Change were also computed using the obtained L^*, a^*, and b^* values according to the given equations on Figure 12.

$$\Delta E = \sqrt{(a^* - a_o^*)^2 + (b - b_o^*)^2 + (L^* - L_o^*)^2}$$

$$\mathrm{BI} = \frac{100\,(x - 0.31)}{0.17}, \quad \text{where } x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$$

$$\mathrm{YI} = 142.86 \cdot \frac{b^*}{L^*}$$

Figure 12. Browning Index (BI), Yellowness Index (YI) and Total Color Change (ΔE) equations (Doroški et al., 2021)

4.4.5. Texture measurement

The texture of the sausage samples was evaluated using a TA.XT Plus texture analyzer (Stable Micro System, Surrey, United Kingdom). For the shear force analysis, the samples were cut using a Warner-Bratzler shear blade with a flat end, at a speed of 2 mm/s both prior to and during the measurement, with a set distance of 30 mm. Force (N) was recorded as a function of time and distance. The maximum peak force (Fmax, N) observed on the graph represented the shear force, indicating the tenderness/firmness of the sausage sample. The area under the curve, from the start of the test to the target deformation distance, was used to calculate the work performed (mJ) during each test. Nine parallel measurements were conducted for each sausage sample.

For the textural evaluation of mushroom samples by shear force analysis, 60 g of mushroom samples were placed inside a Kramer cell. The measurement speed, both pre-test and during testing, was set at 2 mm/s, with a testing distance of 30 mm. A trigger force of 0.049 N was applied. The peak force (N) required to shear the mushroom samples was used to assess their firmness. Nine shear press values were obtained for each pretreatment group. All the measured data were processed using Texture Exponent 32 software for Windows (Stable Micro System). All measurements were conducted at room temperature.

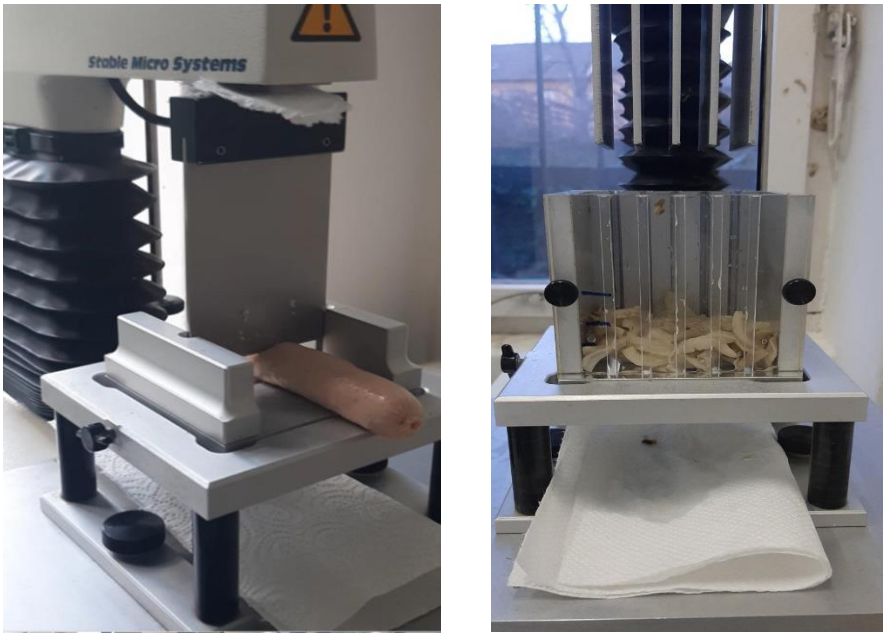


Figure 13. Texture measurement of sausages with Warner-Bratzler shear blade and mushroom samples with Kramer cell

4.4.6. Yield

The weights of the mushrooms were recorded both before and after the application of each pretreatment and fermentation. Subsequently, the yield for each group was calculated using the formula below. The initial weight refers to the weight of the mushrooms before any pretreatment was applied, and the final weight is the weight of the mushrooms after the pretreatments (Blanch, Steam, Oven, MW, HHP, UV) and fermentation. For each pretreatment group, the initial weight was standardized at 2 kg for consistency. After the application of each pretreatment and fermentation process, the final weight was recorded. This calculation was applied separately for each pretreatment and fermentation condition to assess the effect of the various treatments on the weight change.

Yield = Initial weight of the sample group / Final weight of the sample group x100

4.4.7. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is utilized to monitor temperature and heat flow over time within a controlled environment. The thermal denaturation and alterations in the state of the sample proteins were observed using a Micro DSC III (SETARAM, France). As reference substance, bi-distilled water (776.6 mg) was employed alongside each sample. Sausage samples were weighed (776.6 ± 5 mg) and placed in the stainless steel cylinder sample containers. The analysis began with a temperature stabilization phase at 20 °C for 2 minutes and followed by heating to 95 °C at a rate of 1 °C/min. Throughout this procedure, heat flow curves were captured. The DSC curves were then analyzed within the 35 to 90 °C range using the device's Calisto 7.6 software. The total denaturation

enthalpy (ΔH, [J/g]) for each sample was determined by calculating the area between the DSC curve and a linear baseline. The peak temperatures were also recorded. Each sample underwent two parallel measurements.

4.4.8. Lipid oxidation

The extent of lipid oxidation in sausages during storage was evaluated by measuring Thiobarbituric Acid Reactive Substances (TBARS) values, following the method outlined by Tarladgis et al. (1960). A 5 g sausage sample was homogenized in 20 mL of 5% trichloroacetic acid (TCA) for 2 minutes using a Digital Ultra-Turrax (Staufen, Germany), then centrifuged at 4500 rpm for 10 minutes at 4 °C. The supernatant was filtered through Whatman No. 1 filter paper, and 2 mL of the filtrate was combined with 2 mL of 0.08% (w/v) thiobarbituric acid (TBA) in a glass tube. The tubes were heated in a 95 °C water bath for 30 minutes, then cooled down to room temperature. The absorbance was measured at 532 nm against a blank solution (a mix of 2 mL of 5% TCA and 2 mL of 0.08% TBA) using a UV 2900 UV–visible spectrophotometer (Hitachi Ltd., Tokyo, Japan). The results were reported as milligrams of malondialdehyde (MDA equivalent) per kilogram of sausage.

Figure 14. Measurement of TBARS values of sausage samples

4.4.9. Sensory analysis

Sensory analysis of sausage samples with pretreated fermented mushrooms was performed for different sensory characteristics such as visual appearance, odor, texture, and overall characteristics at the end of the storage period. In the sensory test, 20 panelists were instructed to rank the samples considering given attributes, based on their liking. The sensory panel consisted of professors, researchers and students at Hungarian University of Agriculture and Life Sciences who were familiar with sensory analysis and briefly informed about the samples. The cooked samples were cut into 2-cm sections. All samples were assigned three-digit random codes and offered to the panelists in a

random order at room temperature.

4.4.10. Essential amino acids and proteinogenic (total) amino acids

The mushroom samples were freeze-dried using a Christ Alpha 2-4 lyophilizer at the Department of Bioengineering and Fermentation Technology, and the amino acid analysis was carried out at the Department of Nutrition, Hungarian University of Agriculture and Life Sciences. For the determination of proteinogenic (total) amino acid content, 0.1 g of powdered oyster mushroom sample was weighed accurately into hydrolysis tubes (KUTESZ, Budapest, Hungary). Ten milliliters of 6 MW hydrochloric acid were added to the samples, which were then bubbled with nitrogen for 30 seconds. The hydrolysis tubes were sealed with Teflon-lined caps and hydrolyzed at 110 °C for 24 hours in a block thermostat (FALC Instruments, Treviglio, Italy). After cooling, the samples were rinsed with distilled water into 25 mL volumetric flasks. The mixture was neutralized by adding 10 mL of 4 MW NaOH solution. The flasks were filled to the mark with distilled water. The solutions were filtered first through pleated filter paper and then through a 0.22 μm syringe filter (FilterBio® CA Syringe Filter). The homogenized samples were transferred to 1.5 mL Eppendorf tubes and stored in a deep freezer until further analysis.

4.4.11. Free amino acids and biogenic amines

For the analysis of free amino acids and biogenic amines, 0.5 g of powdered oyster mushroom sample was weighed with analytical precision into a 50.0 mL Erlenmeyer flask. 6 mL of 10% trichloroacetic acid were added, and the samples were extracted for 1 hour at 100 rpm using a Laboshake shaker (Gerhard). The extracts were filtered first through standard filter paper and then through a 0.22 μm syringe filter (FilterBio® CA Syringe Filter) into 1.5 mL Eppendorf tubes and stored frozen in a deep freezer until analysis. The analysis of amino acids and biogenic amines was carried out using an AAA 400 Automatic Amino Acid Analyzer (Ingos Ltd., Czech Republic). The device operates on the principle of ion-exchange column chromatography, with post-column derivatization using ninhydrin. Detection was performed at 570 nm, with an additional measurement at 440 nm for proline, using a flow-through cuvette detector. The measurement parameters of the device are detailed in Table 5.

Table 5. Measurement Parameters of the Automatic Amino Acid Analyzer

	Proteinogenic and free amino acids	**Biogenic amines**
Cation-exchange column type	IONEX OSTION LCP5020	OSTION LG ANB
Column size	200 mm x 3.7 mm	60 mm x 3.7 mm
Column temperature	55 °C and 65 °C	55 °C and 65 °C
Reactor temperature	121 °C	121 °C
Eluent flow rate	0.30 cm^3/min	0.30 cm^3/min
Ninhydrin flow rate	0.25 cm^3/min	0.25 cm^3/min
Sample volume injected onto the column	100 µL	100 µL
Detection wavelength	440 nm (proline) and 570 nm	440 nm and 570 nm
Buffers	Li-citrate buffers	Na/K-citrate buffers
Analysis time	200 minutes	97 minutes
Detection limit	0.5 µmol/dm^3	0.5 µmol/dm^3
Standard deviation	2-5%	2-5%

4.4.12. Near-Infrared Spectroscopy – NIR

The measurements were conducted using a Hungarian-developed METRIKA NIR device (METRINIR 10-17 PR), which operates within the near-infrared frequency range of 700–1700 nm. Spectra were recorded within this range in transflexion mode, at 2 nm intervals, using the device's own MetriNIR® software. The measurements were performed on the samples in grinded form. Each sample was examined with two independent loadings, with three rotations of the sample holder per loading. The sample holder was rotated 120° in the same direction after each measurement, resulting in six spectra recordings per sample for data analysis. The previously prepared samples were loaded into a 55 mm diameter sample holder in an even layer. Calibration of the device was performed before each sample series. Processing and evaluation of the NIR spectra took place in several steps. The recorded datasets were exported, followed by spectrum smoothing (using a Savitzky-Golay filter: 2–2 left-right) and generation of the second derivative spectrum using the Unscrambler software. This smoothing reduces spectral noise but does not impact the information content, as it only averages two neighboring points in each direction. The resulting spectral data were then evaluated with discriminant analysis (CDA), using IBM SPSS (Version 29, SPSS Inc., Chicago, IL, USA).

4.4.13. Statistical analysis

The experimental data were analysed with IBM SPSS (Version 29, SPSS Inc., Chicago, IL, USA) using multivariate analysis of variance (MANOVA) and principal component analysis (PCA). Levene’s test was employed to verify the homogeneity of variances. Tukey’s post hoc tests were

conducted to identify significant differences between the samples if the homogeneity of variances was satisfied, otherwise Games-Howell's method was used. Statistical significance was determined at $p < 0.05$. The obtained results were reported as mean values ± standard deviation. Discriminant Canonical Analysis with cross-validation was performed to evaluate the effects of different pretreatments and mushroom percentages on the classification of sausage samples.

5. RESULTS AND DISCUSSION

5.1. ASSESSMENT OF FRESH OYSTER MUSHROOM AS A MEAT SUBSTITUTE IN SAUSAGES (Preliminary study)

5.1.1. Moisture content and water activity of the sausage samples

The moisture content of the sausage samples is illustrated in Figure 15. Replacing increasing amounts of meat with fresh oyster mushrooms led to significant changes in moisture content ($p < 0.05$). The moisture content notably increased with higher levels of mushroom substitution ($p < 0.05$), where the 0P samples, containing no mushroom, exhibited the lowest moisture content (75.01 ± 1.20%), and the 10P samples, with complete mushroom replacement, showed the highest moisture content (88.35 ± 0.69%). This rise in moisture content of the sausage samples can be attributed to the high moisture (85-95%) and dietary fiber content of fresh oyster mushrooms (Nketia et al., 2020). Numerous studies have demonstrated that the porous and hydrophilic nature of dietary fiber promotes water retention, resulting in higher moisture levels. Additionally, the hydration capacity of dietary fiber increases with temperature, as its solubility improves when exposed to thermal processing, such as cooking (Cerón-Guevara et al., 2020; Elleuch et al., 2011). On the other hand, the moisture content of meat is influenced by the extent of thermal protein denaturation, which induces muscle fiber shrinkage. This shrinkage results in a reduction in volume and subsequent water loss, particularly at elevated cooking temperatures (Puolanne & Halonen, 2010).

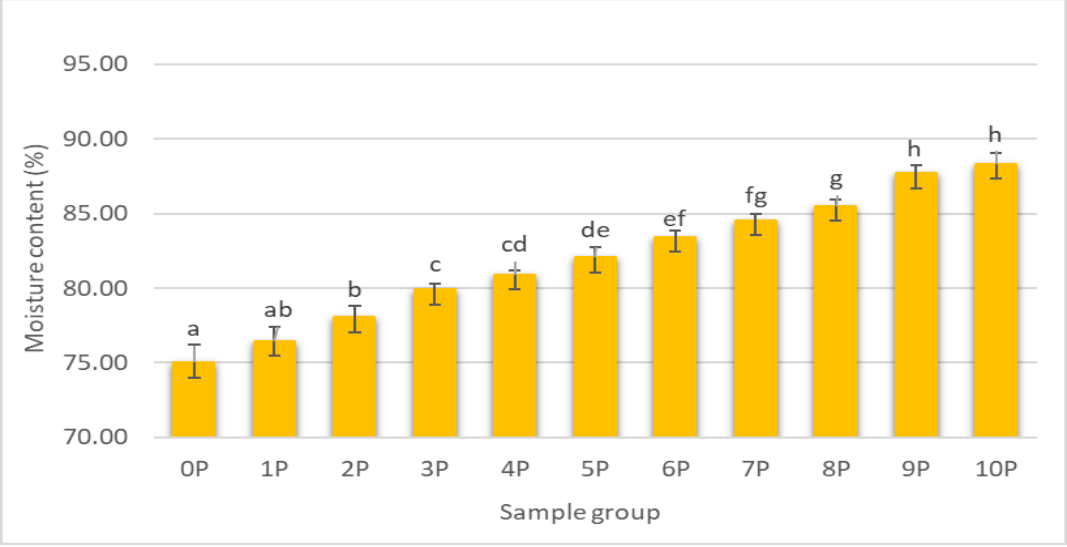


Figure 15. Moisture content of the sausage samples with fresh oyster mushroom substitution. a-h; Mean values labeled with different letters indicate statistically significant differences among the sausage samples. ($p < 0.05$).

Substituting pork meat with fresh oyster mushrooms in the sausage formulations did not lead to significant changes in water activity of the samples. The water activity values for all sample groups ranged between 0.94 and 0.95. These findings align with a previous study where *Lentinula edodes* was used to replace lean pork in sausages, and no differences in water activity were observed between the sample groups with varying level of mushroom substitution (Wang et al., 2019a). In their study, the water activity of the samples was reported as 0.98.

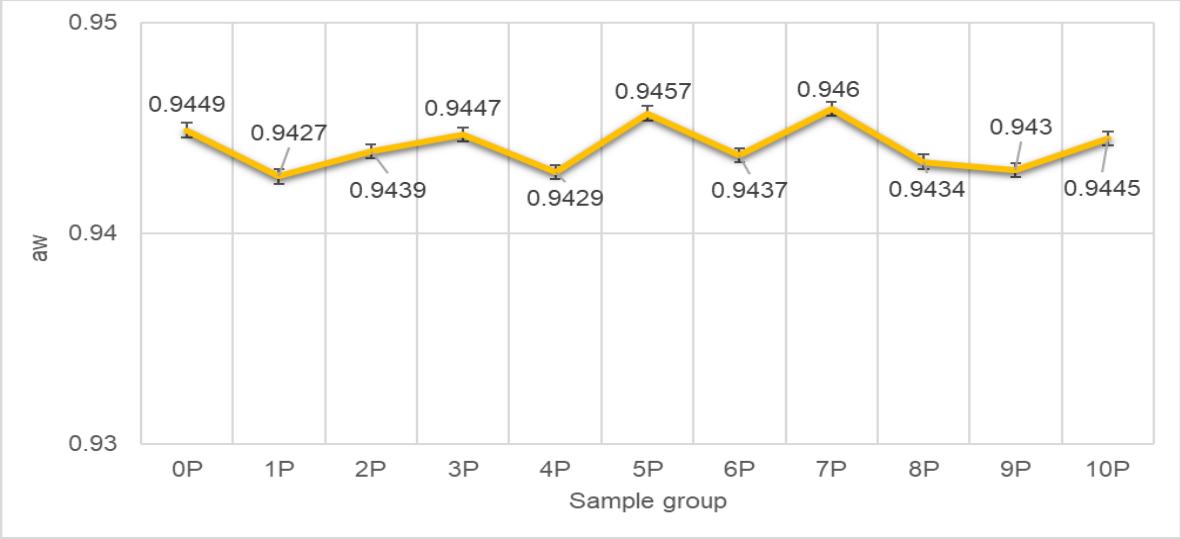


Figure 16. Water activity of the sausage samples with fresh oyster mushroom substitution

5.1.2. pH of the sausage samples

pH is a key factor in determining the quality of meat products, influencing their shelf life, color, and water-holding capacity. The fresh oyster mushrooms used in this research had an average pH of 6.35. As shown in Figure 17, the pH of the sausage samples did not significantly differ across different formulations ($p > 0.05$), indicating that the fresh oyster mushroom had no discernible effect on the samples' pH. The pH of the control sample (0P) was comparable to that of the 10P group, which had 100% mushroom substitution, with values of 6.85 ± 0.18 and 6.88 ± 0.21, respectively. These results align with those reported by (Patinho et al., 2021), where adding *Agaricus bisporus* mushrooms (pH 6.61) to burgers caused no significant change in pH values of the samples. Similarly, another study found that the inclusion of winter mushrooms in beef and fish meat slightly raised the pH due to the buffering effect of mushroom proteins, though the increase was not statistically significant (Bao et al., 2008). This could be attributed to the reduced overall protein buffering capacity of the samples, as mushrooms contain less protein than meat (Stephan et al., 2018).

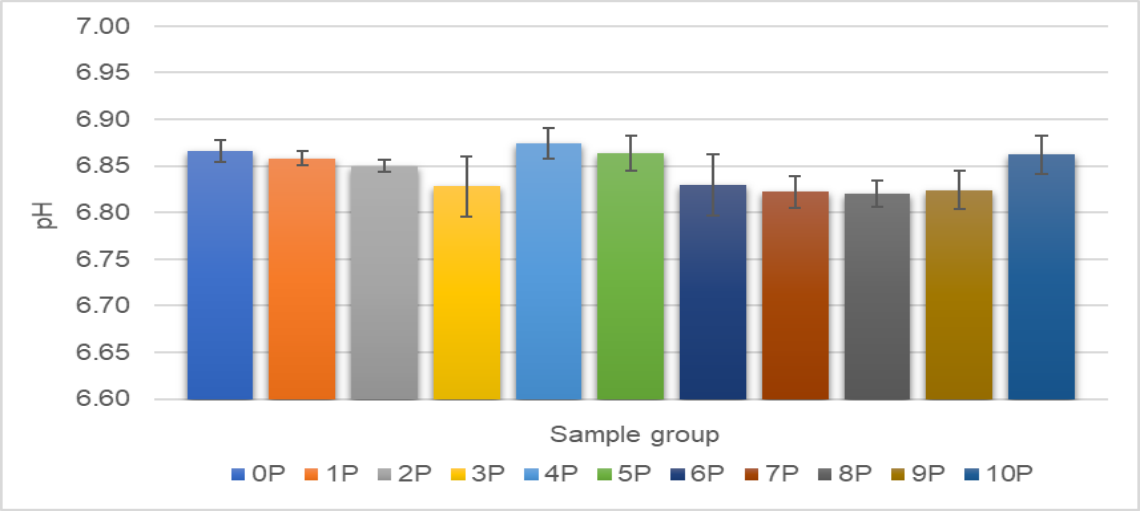


Figure 17. pH of the sausage samples with fresh oyster mushroom substitution

5.1.3. Color attributes of the sausage samples

Color is one of the primary factors that affect customer decisions and is a critical quality attribute influencing the sensory evaluation of meat products (García-Esteban et al., 2003). The lightness (*L**), redness (*a**), and yellowness (*b**) values of the sausage samples are presented in Table 6.

Table 6. Means ± standard deviations of color attributes of the sausages with fresh oyster mushroom substitution

Sample	*L**	*a**	*b**
0P	77.20 ± 2.4^{a}	9.99 ± 0.1^{a}	10.66 ± 0.3^{d}
1P	78.59 ± 1.2^{a}	8.83 ± 0.1^{b}	10.83 ± 0.3^{d}
2P	78.22 ± 1.2^{a}	8.24 ± 0.2^{b}	10.76 ± 0.6^{d}
3P	76.30 ± 0.8^{a}	8.44 ± 0.6^{b}	11.20 ± 1.4^{cd}
4P	74.55 ± 1.4^{ab}	7.37 ± 0.1^{c}	11.89 ± 0.5^{bcd}
5P	74.45 ± 1.3^{ab}	7.19 ± 0.1^{c}	11.41 ± 0.3^{bcd}
6P	71.79 ± 1.0^{b}	6.13 ± 0.1^{d}	12.58 ± 0.5^{abcd}
7P	64.17 ± 3.1^{c}	5.26 ± 0.4^{e}	13.03 ± 0.8^{abc}
8P	62.97 ± 0.3^{c}	5.08 ± 0.1^{e}	12.91 ± 0.3^{abc}
9P	56.52 ± 0.7^{d}	3.95 ± 0.1^{f}	13.78 ± 0.4^{ab}
10P	56.05 ± 3.6^{d}	2.28 ± 0.4^{g}	12.10 ± 1.5^{ab}

$^{a–g}$; Means within same column with different superscripts are different ($p < 0.05$).

Replacing meat with oyster mushrooms significantly influenced the *L** values of the sausages ($p < 0.05$). As the amount of oyster mushroom in the formulations increased, the *L** values declined, resulting in a darker product. Similar trends were observed in beef paste containing four edible mushrooms (Qing et al., 2021), frankfurters incorporating *Agaricus bisporus* and *Pleurotus ostreatus* mushrooms (Cerón-Guevara et al., 2020), and pork sausages where *Lentinus edodes* mushrooms were used as a meat substitute (Wang et al., 2019a). The reduction in *L** values was likely due to the inherent color of the added mushrooms. Different mushrooms have distinct colors, which could impact the product's color based on both the mushroom type and the concentration used. This could explain the decline in *L** values observed in our study, as increasing amounts of gray-colored mushrooms were added to the formulations.

The *a** values also significantly decreased as the substitution level increased, leading to a less red appearance in the sausages ($p < 0.05$). A similar reduction in *a** values was observed when mushroom flour (*Agaricus bisporus* and *Pleurotus ostreatus*) was incorporated into beef patties (Cerón-Guevara et al., 2020). This may be attributed to the decreased myoglobin content in the final product due to meat replacement. As a result, natural colorants like black carrot extract, beetroot extract, or peanut red pigment have been used to achieve the desired red color in meat products or alternatives (Stephan et al., 2018). For instance, a study with *Lentinula edodes* as a pork meat substitute in sausages demonstrated that adding peanut red pigment yielded redness and yellowness levels comparable to those of red meat (Wang et al., 2019a).

A significant increase in *b** values was noted ($p < 0.05$). Comparable results were found when porcini mushrooms (*Boletus edulis*) were added to frankfurters, with reformulated samples showing notably higher *b** values than the control group (Novakovic et al., 2020). Similarly, sausages containing *Flammulina velutipes* also exhibited a marked increase in *b** values compared to control samples. This increase was likely due to the high dietary fiber content of mushrooms, which tends to be lighter or yellower, thereby boosting the *b** values of the products (Henning et al., 2016; Wang et al., 2019c).

5.1.4. Texture properties of the sausage samples

Tenderness or firmness is a key sensory attribute of meat products and plays a crucial role in defining meat quality. The primary challenge in developing meat substitutes lies in replicating the texture and mouthfeel of meat, utilizing various techniques and ingredients (Dekkers et al., 2018; Sun et al., 2021). In this research, tenderness is assessed by measuring the shear force required to cut through the meat, reflecting the force needed by the teeth to compress and slice the product. The work value corresponds to the energy required to break down the sample. The results of the sausage samples' texture analysis

are illustrated in Figure 18.

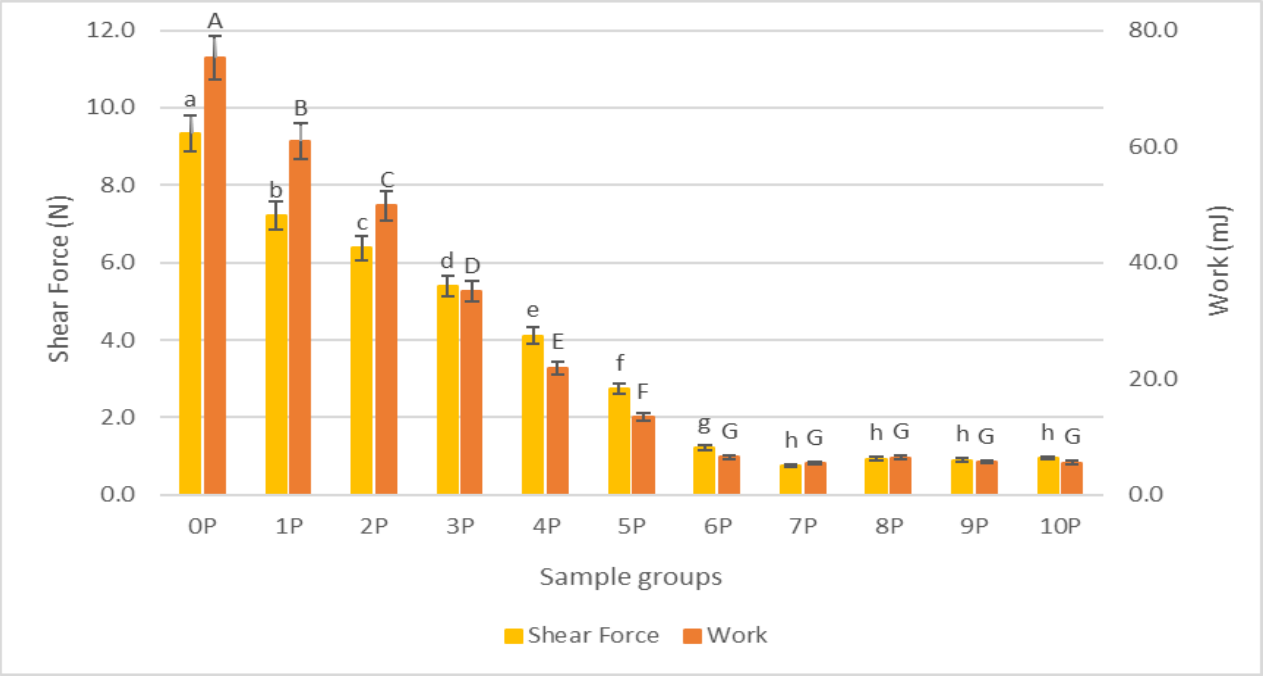


Figure 18. Texture properties of the sausage samples with fresh oyster mushroom substitution. a-h; Mean values with different letters differ significantly among shear force values; A-G; Mean values with different capital letters differ significantly among work values ($p < 0.05$)

The results revealed significant differences among the sample groups, with the control group (0P) exhibiting the highest shear force and work values ($p < 0.05$). Increasing the ratio of oyster mushroom up to 60% resulted in a reduction in both shear force and work values, enhancing tenderness and producing softer sausages. Interestingly, when the replacement exceeded 60%, this trend disappeared, and no significant differences were observed between the groups. Previous studies have similarly reported a softer texture in meat products supplemented with various mushrooms, as dietary fibers retain fluids within the product (Das et al., 2021; Rangel-Vargas et al., 2021). Additionally, research has shown that adding dietary fiber to meat products can interfere with the formation of protein-water or protein-protein gel networks, thereby weakening the gel strength (Wang et al., 2019c). Likewise, in the present research, the high dietary fiber content of oyster mushrooms may have contributed to the softer texture observed in the samples.

5.1.5. Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) has been employed to examine the thermal characteristics of complex food matrices and their components, such as water, proteins, and fats, in response to temperature changes (Kazemi et al., 2011). Monitoring and analyzing protein denaturation is essential,

as it significantly influences several quality attributes of the product. The results of the DSC analysis are displayed in Figures 19 and 20. In the thermograms, endothermic peaks indicate the heat absorbed during the thermal denaturation of proteins. Based on the DSC curves, the control sample (0P) shows three distinct peaks representing the primary meat proteins, as it consists solely of pork meat. The myosin protein group appears around 50-55°C, sarcoplasmic proteins and collagen near 60-65°C, and actin at 70°C.

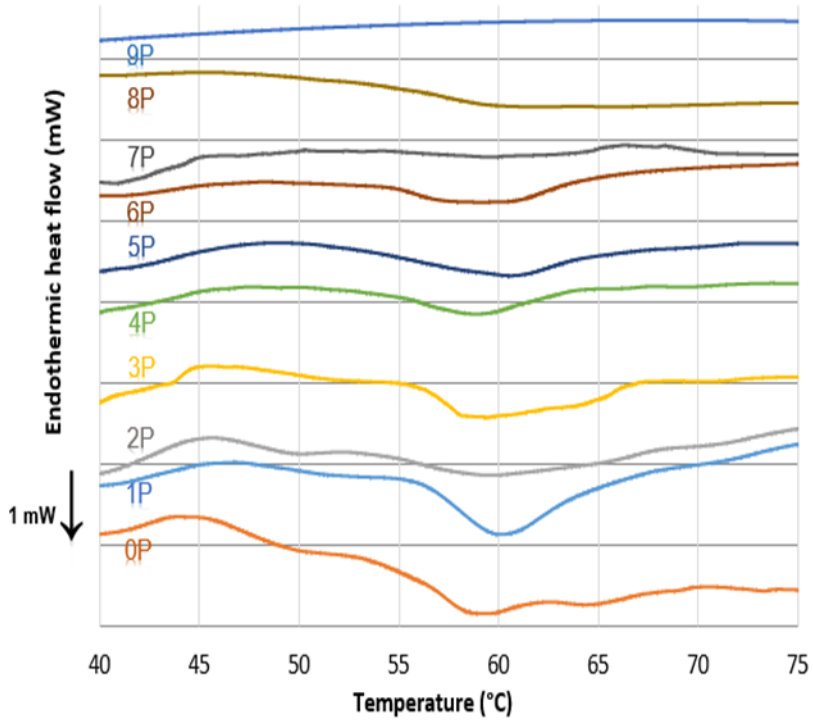


Figure 19. DSC thermograms of the sausage samples with fresh oyster mushroom substitution

As the proportion of meat replacement increased, the denaturation peaks became smaller and less distinct in the diagrams. The calculated enthalpy values and peak temperatures aligned with the visual trends observed. Peak temperatures, representing sarcoplasmic proteins and collagen, were recorded between 59°C and 60°C for all sample groups. The enthalpy values were 1.216 J/g for the 0P sample and 0.118 J/g for the 9P sample, with no data recorded for the 10P sample, where meat was fully replaced. This absence of data could be attributed to the lower protein content in fresh oyster mushrooms compared to meat. The reduced area under the endothermic peak indicates fewer proteins available for denaturation, highlighting the impact of meat substitution on protein denaturation. Consequently, less energy is required to unfold the denatured proteins, resulting in lower enthalpy values. Additionally, other researchers have noted that standard DSC instruments may lack the

sensitivity to detect minor thermal transitions and energy changes associated with the denaturation of small protein quantities. For this reason, it has been suggested to use higher protein concentrations, larger sample sizes, or DSC microcalorimeters, which are better equipped to detect protein denaturation in such food systems (McClements et al., 2021; Vu et al., 2022).

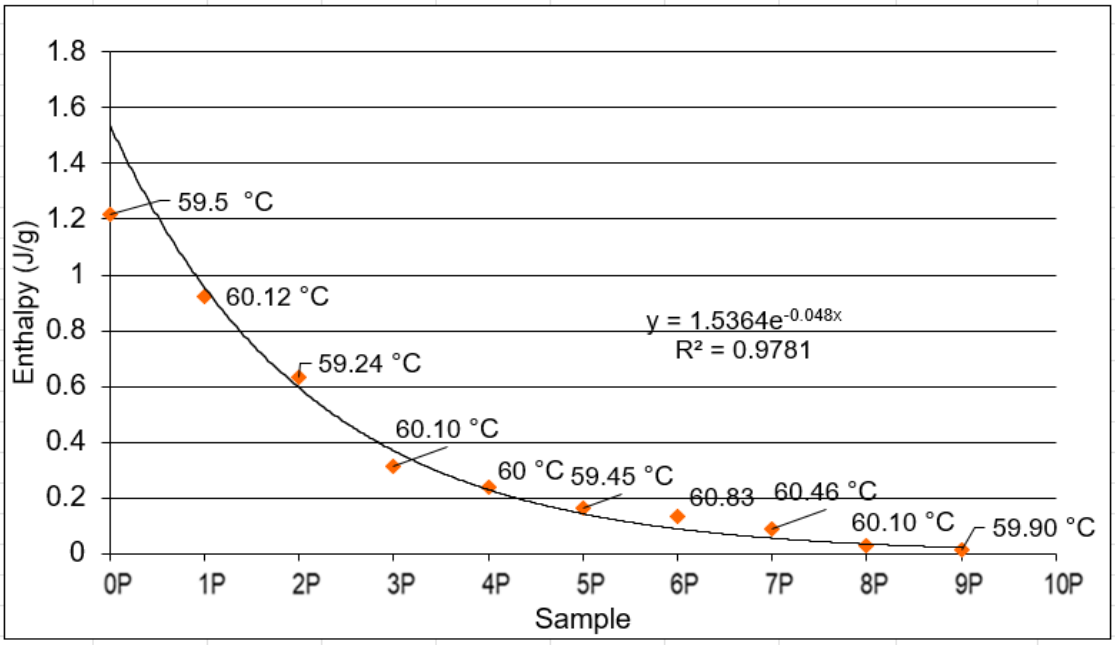


Figure 20. Enthalpy and peak temperatures of the sausage samples with fresh oyster mushroom substitution

The preliminary study demonstrated the potential of fresh oyster mushrooms as a meat substitute in sausages. However, challenges arose with higher substitution ratios, leading to undesirable changes in texture and color, such as increased softness and a darker, less red appearance (Boylu et al., 2023b). Based on these findings, the decision was made to continue research with the 25% and 50% substitution ratios, as they maintained acceptable product quality. Also, these limitations and the short shelf life of fresh oyster mushrooms highlighted the importance of optimizing mushroom processing techniques. To build upon this foundation, the main study focuses on the meat replacement with pretreated fermented oyster mushrooms at 25% and 50% substitution ratios. Before their incorporation into sausage formulations, a comprehensive quality analysis was performed on the mushrooms following their pretreatments and fermentation.

5.2. IMPACT OF ALTERNATIVE PRETREATMENT METHODS AND FERMENTATION ON THE QUALITY ATTRIBUTES OF OYSTER MUSHROOMS

5.2.1. Moisture content of the mushroom samples

The application of various pretreatments [fresh (Fresh), blanching (Blanch), steaming (Steam), oven (Oven), microwave (MW), High Hydrostatic Pressure (HHP), and Ultraviolet Light treatment (UV)] resulted in significant variations in the moisture contents of the mushrooms. Table 7 presents the moisture content of mushroom samples following pretreatment and fermentation processes. After pretreatment, Blanch, HHP, Oven, and UV samples exhibited comparable moisture contents, ranging from 88.90% to 90.47%, which is similar to that of fresh oyster mushrooms (89.66%) as reported by (Nketia et al., 2020). In contrast, MW samples exhibited the lowest moisture content at 83.91%, while Steam samples had a moisture content of 87.16%. Further significant differences in moisture content were noted after the fermentation of mushrooms. Samples Fresh and UV post-fermentation displayed the highest moisture content, both at 90%, while MW samples recorded the lowest at 85.56%. These findings are consistent with those reported by (Khaskheli et al., 2015) in their study on *Auricularia auricula* mushroom pickles. The elevated moisture content in UV samples can be attributed to the nature of UV treatment, which primarily affects the surface. Conversely, the reduced moisture content in MW samples may result from the rapid absorption of microwave energy by water molecules in the mushrooms, which is subsequently converted to heat, leading to swift evaporation (Zhang et al., 2018).

Table 7. Moisture, pH and yield values of the mushroom samples after pretreatments and fermentation

Sample	After pretreatments			After fermentation		
	Moisture (%)	pH	Yield (%)	Moisture (%)	pH	Yield (%)
Fresh	89.66 ± 1.23^{c}	6.35 ± 0.04^{a}	100.00	90.21 ± 0.28^{F}	3.96 ± 0.07^{A}	88.28
Blanch	90.01 ± 0.35^{c}	7.60 ± 0.02^{e}	91.00	88.61 ± 0.48^{C}	4.50 ± 0.10^{DE}	92.32
Steam	87.16 ± 0.51^{b}	6.61 ± 0.09^{c}	92.33	86.27 ± 0.39^{B}	4.98 ± 0.25^{F}	89.59
Oven	88.90 ± 1.17^{c}	6.71 ± 0.02^{d}	94.12	89.13 ± 0.48^{D}	4.18 ± 0.25^{BC}	89.30
MW	83.91 ± 3.11^{a}	6.69 ± 0.04^{d}	80.00	85.56 ± 0.73^{A}	4.67 ± 0.26^{E}	95.46
HHP	89.88 ± 0.22^{c}	6.50 ± 0.03^{b}	98.65	89.69 ± 0.55^{E}	4.37 ± 0.46^{CD}	68.70
UV	90.47 ± 0.61^{c}	6.46 ± 0.09^{b}	99.71	90.37 ± 0.19^{F}	4.02 ± 0.12^{AB}	69.41

a–e, A-F; Mean values labeled with different superscripts in the same column indicate significant differences among the samples ($p < 0.05$).

5.2.2. pH of the mushroom samples

The pH level of fermented foods plays a vital role in their stability and microbiological safety. Consequently, achieving a rapid and substantial reduction in pH (and an increase in titratable acidity) is essential in the fermentation process. In our study, the fresh oyster mushrooms exhibited a mean pH of 6.35 ± 0.04. The pH values for the pretreated and fermented mushroom samples are presented in Table 7. The results indicated that different pretreatment methods and fermentation processes significantly influenced the pH of the mushroom samples. The control sample (Fresh) exhibited the lowest pH, while the Blanch samples showed the highest pH value of 7.60 ± 0.02, compared to the mean pH of 6.35 ± 0.04 for fresh mushrooms. All pretreated samples recorded a higher pH than the fresh oyster mushrooms. This finding aligns with Jambrak et al. (2007), who noted an increase in the pH of button mushrooms following a 3-minute water blanching treatment. As anticipated, all sample groups experienced a decrease in pH after fermentation. The Fresh samples achieved the lowest pH value post-fermentation at 3.96 ± 0.07, whereas the Steam samples had the highest pH at 4.98 ± 0.25. These results are in line with (Chen et al., 2021), who reported a reduction in the pH of Shiitake mushrooms from 6.5 to 5.0 after five days of fermentation. In another study involving oyster and chanterelle mushrooms, pH levels reached 3.5 after three to four days of fermentation with *L. plantarum* (Jabłońska-Ryś et al., 2016). Research indicates that the pH of various fermented mushroom species ranges from 3.3 to 4.6, which is influenced by factors such as fermentation temperature, carbohydrate content, and the chemicals utilized during the process (Jabłońska-Ryś et al., 2019). A significant negative correlation was observed between moisture content and pH of fermented mushroom samples ($R=-0.686$, $P < 0.01$). Meanwhile no significant correlation was observed for pretreated samples.

5.2.3. Yield of the mushroom samples

The yields resulting from the various pretreatment methods, both before and after fermentation, are shown in Table 7. For fresh mushrooms (Fresh), the yield was set at 100% since no pretreatment was applied, serving as the control. After pretreatment, the highest final weights were observed in the UV (99.71%) and HHP (98.65%) samples. In contrast, the lowest yield (80%) was recorded for mushrooms subjected to microwave treatment (MW). The reduction in weight for the MW, Steam, and Oven samples can be attributed to water loss, whereas the Blanch samples experienced yield loss due to leaching from mushroom tissues into the blanching water (Maray et al., 2018). Following fermentation, the highest yield was observed in MW samples (95.46%), followed by Blanch (92.32%) and Steam (89.59%) samples. The HHP and UV samples had the lowest yields post-fermentation, with 68.70% and 69.41%, respectively. These results underscore the significance of pretreatment methods in influencing the yield during fermentation.

5.2.4. Color attributes of the mushroom samples

Due to processing, microbial contamination, and enzymatic reactions, mushrooms are highly susceptible to browning and color changes. The applied pretreatments and fermentation processes markedly influenced the color attributes of the mushrooms, as shown in Table 8. Images of pretreated and fermented oyster mushrooms are also given in Figure 9 and Figure 10. All pretreated samples exhibited significantly lower L^* values than untreated samples (Fresh), except for UV samples ($p < 0.05$). This trend aligned with the Browning Index, suggesting that browning was most pronounced in Steam, MW, and Oven samples and least observed in UV samples. Similar findings were noted in another study regarding UV-C treatment, which minimized surface browning in oyster mushrooms (Wang et al., 2017). The Yellowness Index showed a consistent trend with the Browning Index, matching the b^* values. The lowest a^* values were observed in HHP and Oven samples. The Total Color Change (ΔE), which quantifies overall color changes in relation to a control sample (F, in this research), indicated the greatest color shifts in HHP and Steam samples. Likewise, marked darkening and Browning Index increases were noted for steam-blanched, canned oyster mushrooms. This phenomenon is attributed to enzymatic oxidation of polyphenols by polyphenol oxidase, leading to browning and reduced whiteness (Nketia et al., 2020). These findings align with Eissa et al. (2009), who reported lower BI values in water blanched mushrooms compared to steam blanched ones, as thermal pretreatments generally enhanced non-enzymatic browning, as seen in steamed, microwaved, and oven pretreated samples in this research.

Table 8. Means ± standard deviations of color attributes of the mushroom samples, Browning Index, Yellowness Index and Total Color Change (ΔE)

Sample	*L**	*a**	*b**	BI	YI	TCC (ΔE)
After Pretreatment						
Fresh	70.29±6.02^{b}	0.28±0.65^{d}	9.80±2.30^{a}	14.96	19.92	0.00
Blanch	62.70±5.32^{a}	0.47±0.64^{d}	10.84±1.84^{ab}	19.10	24.70	7.66
Steam	63.93±4.79^{a}	-0.07±0.61^{cd}	15.72±1.83^{d}	27.46	35.13	8.70
Oven	63.91±6.32^{a}	-0.97±0.76^{ab}	13.43±2.27^{c}	21.87	30.02	7.45
MW	64.65±4.57^{a}	-0.42±0.70^{bc}	15.02±2.12^{cd}	25.31	33.19	7.72
HHP	61.68±5.72^{a}	-1.09±0.80^{a}	11.65±1.64^{b}	19.10	26.98	8.91
UV	72.39±5.67^{b}	0.10±0.57^{cd}	9.68±1.74^{a}	14.12	19.10	2.11
After Fermentation						
Fresh	59.24±6.13^{A}	-0.25±1.56^{A}	16.23±1.87^{B}	30.90	39.14	0.00
Blanch	64.65±5.65^{C}	0.61±0.81^{B}	13.56±1.72^{A}	23.70	29.96	6.09
Steam	60.17±4.97^{AB}	-0.08±0.84^{AB}	16.69±1.71^{B}	31.58	39.63	1.05
Oven	57.53±4.70^{A}	-0.31±0.77^{A}	16.71±1.18^{B}	33.03	41.49	1.78
MW	63.83±5.71^{BC}	-0.50±0.81^{A}	16.63±1.68^{B}	28.84	37.22	4.61
HHP	58.31±4.59^{A}	-0.09±0.79^{AB}	21.32±2.10^{C}	44.08	52.23	5.18
UV	59.42±5.70^{A}	-0.21±1.01^{A}	19.79±2.63^{C}	39.14	47.58	3.56

a–d, A-C; Mean values with different superscripts in the same row differ significantly among samples ($p < 0.05$).

Following fermentation, notable differences in all color parameters were observed among the mushroom samples ($p < 0.05$). The highest *L** values were recorded for Blanch and MW samples, while the lowest values were seen in Fresh, UV, Oven, and HHP samples, suggesting that water blanching and microwave treatments may help reduce browning in mushrooms, as reflected in the BI results. Also, Blanch samples displayed the highest *a** values, followed by Steam and HHP samples, whereas Fresh, MW, UV, and Oven samples showed the most substantial decreases in redness. YI, consistent with *b** values, revealed that HHP samples had the greatest yellowness, while Blanch

samples exhibited the least. Additionally, fermentation led to a reduction in lightness and redness (except for Blanch samples) and significantly increased yellowness across all samples. Similar changes were detected in lactic-fermented oyster mushrooms, noting a comparable rise in *b** values and a darkening of the mushroom bodies (decreased *L** values), along with an increase in *a** values (Liu et al., 2016). A related study on fermentation of button mushrooms found increased *L** and *b** values alongside decreased *a** values (Jabłońska-Ryś et al., 2022). In their study, blanching led to a reduction in the *L** parameter, while both *a** and *b** values increased.

5.2.5. Texture evaluation of the mushroom samples

Mushroom texture is influenced by factors like time, moisture loss, injuries, mechanical damage, and heat treatments (Zhang et al., 2018). Pretreatment type caused significant differences in the mushrooms' texture ($p < 0.05$). Texture evaluation results are shown in Figure 21 and 22. Fresh (54.79 ± 4.86), UV (54.41 ± 3.10), and MW (52.41 ± 3.39) samples required notably higher shear force than other samples, while HHP (35.30 ± 4.39) samples required the least. These samples also required more shear work to cut through than HHP and Blanch samples.

Figure 21. Texture evaluation of the mushroom samples after pretreatments. a-d; Mean values with different letters differ significantly among shear force values; A-D; Mean values with different capital letters differ significantly among work values ($p < 0.05$)

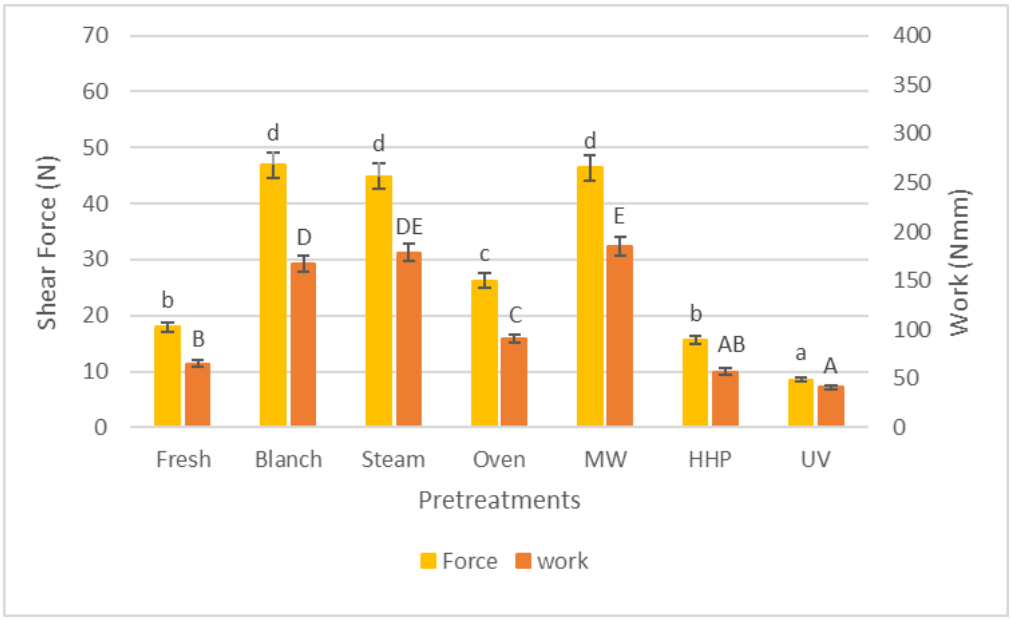


Figure 22. Texture evaluation of the pretreated mushroom samples after fermentation. a–d, Mean values with different letters differ significantly among shear force values; A-E; Mean values with different capital letters differ significantly among work values ($p < 0.05$)

In a study on *Agaricus bisporus* and *Boletus edulis* mushrooms, Kramer shear cell analysis showed that blanched *Agaricus* mushrooms required more shear force than fresh ones, whereas blanched *Boletus* mushrooms required less force compared to their fresh counterparts. Work values also indicated that fresh mushrooms required more work to shear than blanched ones (Jaworska et al., 2010). High blanching temperatures were attributed to these effects, as they cause protein denaturation, membrane destabilization, and reductions in weight or volume, leading to tissue softening in mushrooms (Zivanovic & Buescher, 2004). In this research, the HHP samples exhibited more substantial texture changes than even heat-treated samples, while UV and MW samples demonstrated greater structural integrity. This could be due to the distinct ways that heat treatments and HHP influence protein structures (Kenesei et al., 2017). Significant textural differences were found between samples post-fermentation (Fig 22), with Blanch (46.85 ± 4.29), MW (46.37 ± 4.85), and Steam (44.88 ± 5.35) samples requiring greater shear force compared to others, whereas UV (8.53 ± 1.12) samples needed the least force. Shear work values mirrored these results, with UV, HHP, and Fresh samples requiring less work post-fermentation. Other researchers observed similar reductions in firmness in fermented mushrooms, referred as a common issue among fermented vegetables (Jabłońska-Ryś et al., 2022). Ultimately, the blanched, steamed, and microwave pretreated mushrooms showed greater resistance to both pretreatment and fermentation, while fresh, UV pretreated, and HHP pretreated samples experienced the most pronounced textural changes

Pearson correlation coefficients revealed significant relationships between textural properties and other measured parameters of the mushroom samples. A significant positive correlation was found between shear force and work values (R= 0.813) and between lightness and work values (R= 0.449) in pretreated mushroom samples, suggesting that darker samples tend to have a softer texture. Conversely, a significant negative correlation was observed between pH and work ($R = -0.437$, $p < 0.01$). Shear force and work values show a stronger positive correlation in fermented samples ($R = 0.969$) compared to pretreated samples ($R = 0.813$), suggesting that fermentation enhances the textural consistency. Shear force values showed a significant negative correlation with b^* ($R = -0.534$) and moisture content ($R = -0.799$), while exhibiting a positive correlation with pH ($R = 0.617$) in fermented samples ($p < 0.001$). Similarly, work values showed a significant negative correlation with b^* ($R = -0.467$) and moisture content ($R = -0.827$), while exhibiting a positive correlation with pH ($R = 0.643$) in fermented samples ($p < 0.001$). This indicates that increased yellowness and moisture content, along with decreased pH, are strongly associated with softer textures of fermented mushroom samples.

5.2.6. Essential amino acid and proteinogenic amino acid profile of the mushroom samples

The total essential amino acid and proteinogenic amino acid contents of the pretreated mushroom samples are presented in Figure 23. The oyster mushroom samples contained 17 protein-building amino acids, with their total amounts ranging from 144.34 to 248.91 mg/g for the pretreated samples. The total amino acid content of the fresh oyster mushroom was 144.34 mg/g. All pretreatment methods caused a significant increase of essential amino acid and total amino acid content of the mushroom samples ($p < 0.05$). Among these, microwave pretreatment increased the amino acid concentration the most, (72.4%) reaching 248.91 mg/g. Similarly, the essential amino acid content showed the highest increase in microwaved samples, rising from 58.05 mg/g to 102.22 mg/g. The essential amino acid content (including arginine) constituted 35–44% of the total amino acids in the pretreated mushroom samples, indicating excellent protein quality.

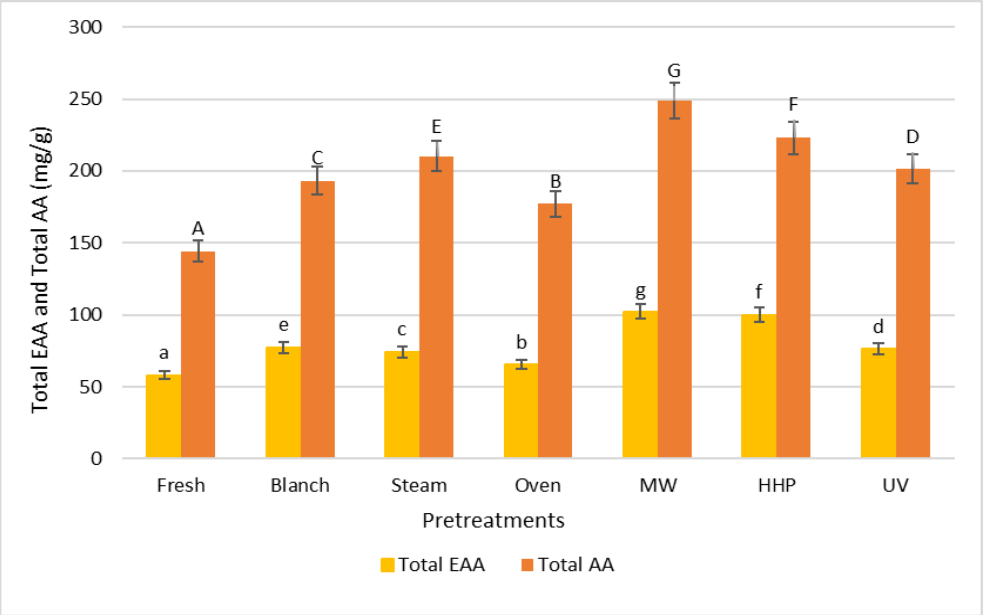


Figure 23. Total essential amino acid and total proteinogenic amino acid content of the mushroom samples after pretreatments. a–g, Mean values with different letters differ significantly among Total EAA values; A-G; Mean values with different capital letters differ significantly among Total AA values ($p < 0.05$)

The total essential amino acid and proteinogenic amino acid contents of the pretreated fermented mushroom samples are presented in Figure 24. In general, fermentation reduced the total amino acid content of the pretreated samples, except in the case of fresh fermented ones. The total amount of protein-building amino acids in the pretreated fermented samples ranged from 130.86 to 186.96 mg/g. Among these, fresh fermented oyster mushrooms had the highest total amino acid content at 186.96 mg/g, followed by blanched fermented samples at 184,36 mg/g, while steamed fermented samples showed the lowest at 130.86 mg/g. Fermentation affected the essential amino acid content of the mushroom samples differently, causing an increase for fresh, blanched and oven samples and decrease for others. Among these, blanched fermented samples showed the highest essential amino acid ratio (44.92%) reaching 82.81 mg/g. Notably, among all the samples, only the steamed fermented ones exhibited a lower essential amino acid content compared to both their pretreated counterpart and fresh oyster mushrooms. The essential amino acid content (including arginine) constituted 38–45% of the total amino acids in the pretreated fermented mushroom samples.

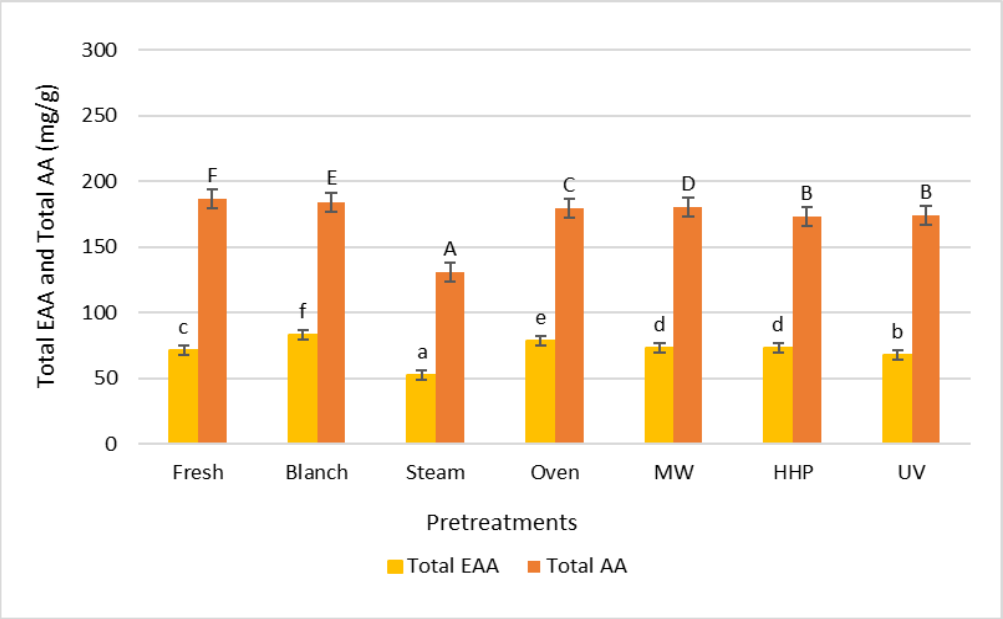


Figure 24. Total essential amino acid and total (proteinogenic) amino acid content of the pretreated mushroom samples after fermentation. a–f, Mean values with different letters differ significantly among Total EAA values; A-F; Mean values with different capital letters differ significantly among Total AA values ($p < 0.05$)

The total amino acid profile of mushroom samples is presented in annex 1 and annex 2. For all samples, aspartic acid (22.22-51.90 mg/g) and glutamic acid (20.40-52.45 mg/g) were the most abundant amino acids. Of the nine essential amino acids, eight were identified in the oyster mushroom samples, with tryptophan being the exception. In general, leucine had the highest concentration (10.41-21.26 mg/g) in the mushroom samples, followed by lysine (7.36-14.61 mg/g), while methionine (2.05-3.87 mg/g) was present in smaller quantities. Histidine content ranged from 2.89 to 6.76 mg/g across all samples, except for the HHP pretreated samples, which exhibited an exceptionally high level of 31.50 mg/g. Considering all the essential amino acids, microwave pretreated samples exhibited the highest levels of threonine (11.07 mg/g), leucine (21.15 mg/g), lysine (14.80 mg/g), phenylalanine (10.43 mg/g), and arginine (13.34 mg/g) compared to the other samples. A recent study investigated the amino acid composition of oyster mushrooms and identified 13 amino acids: alanine, aspartic acid, proline, serine, asparagine, hydroxyproline, cysteine, glutamine, leucine, threonine, methionine, phenylalanine, and lysine, five of which are essential. The total amino acid concentration was 632 mg/100 g, with aspartic acid constituting a significant portion (492.12 mg/100 g) and the total essential amino acid content was 67.83 mg/100g. Lysine (23.18 mg/100 g) was the second most abundant amino acid, while the remaining 11 amino acids ranged between 9 and 14 mg/100 g (Effiong et al., 2024). The amino acid profile of mushroom samples was evaluated with principal component analysis, as shown in Figure 25 and 26. The first two principal components accounted for 54.12% of

the variance, while the first three principal components accounted for 65.80% of the total variance, representing the majority of the dataset's variation.

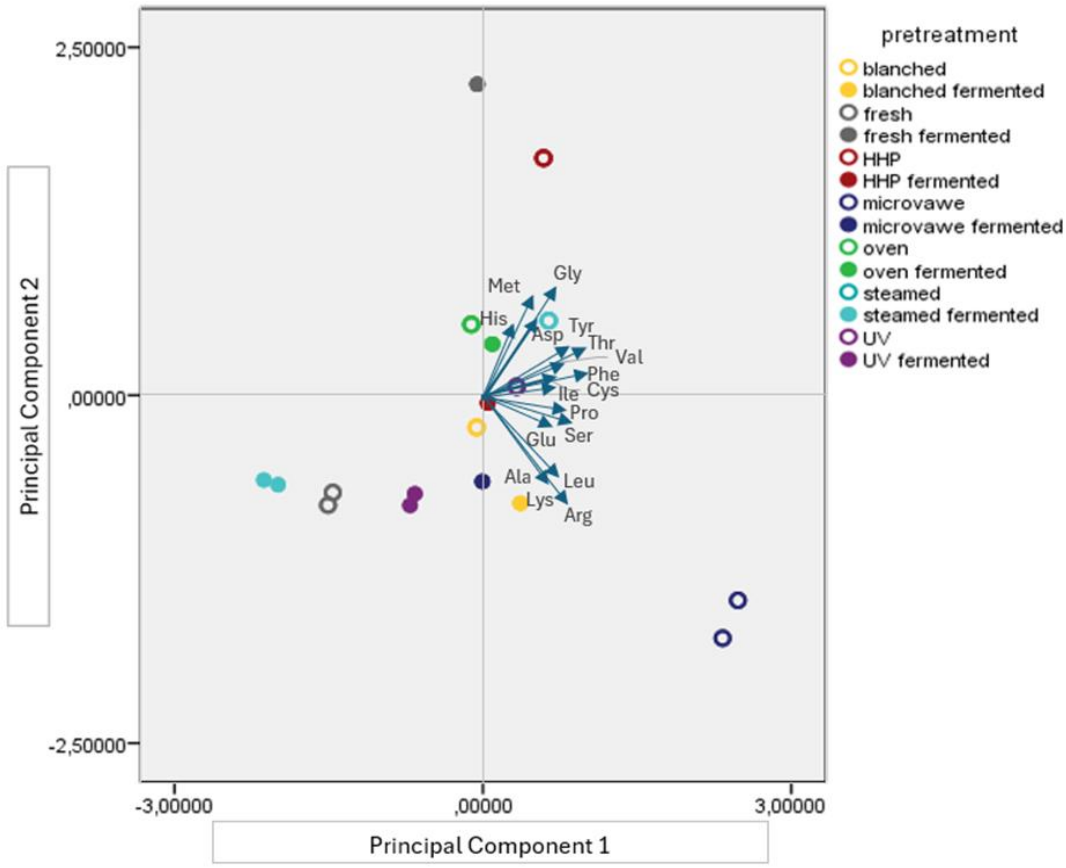


Figure 25. Principal Component Analysis plot (Component 1 vs. Component 2) of total amino acid profile of mushroom samples

In Figure 25, the upper right quadrant contains markers corresponding to four samples: HHP, steamed, UV pretreated and oven fermented mushrooms. The lower right quadrant features markers for two samples: microwave and blanched fermented mushrooms. The lower left quadrant contains four samples: fresh, blanched, steamed fermented and UV fermented mushrooms. The upper left quadrant contains oven pretreated and fresh fermented mushrooms. Samples located in the same quadrant indicate similarities in their amino acid profiles, with the degree of similarity being reflected by their proximity to one another, forming distinct clusters. Among the samples, fresh fermented, HHP pretreated, microwave pretreated and steamed fermented samples were the furthest from the origin, indicating that their amino acid profile differs significantly from the others. HHP fermented samples are located in the center of the plot, close to both blanched and UV pretreated samples, indicating that their amino acid profile is similar to the overall average of the dataset. Most fermented samples are farther from the origin compared to their pretreated counterparts, demonstrating that fermentation

generally increases variability in amino acid profiles. The arrows represent the amino acids' contribution to the principal components. Longer arrows (e.g. Gly, Arg) indicate stronger influence on the variance, while shorter arrows (e.g. Glu, Ile) contribute less. Glycine and arginine point in opposite directions on the PCA plot, indicating that these amino acids are abundant in distinct clusters. Glycine is present in relatively higher concentrations in fresh fermented, HHP, steamed, UV-pretreated, and oven-fermented mushrooms, whereas arginine is more concentrated in microwave and blanched fermented mushrooms. On the other hand, principal component 3 accounts for additional variability not captured by the first two components, revealing further differences among the samples. (Figure 26). For instance, in the first plot (PC1 vs. PC2), HHP fermented sample remains closer to the origin, indicating minimal separation along these components, while in the second plot (PC1 vs. PC3), it is strongly separated along PC3, highlighting distinct amino acid changes due to high hydrostatic pressure and fermentation interaction. Proline and serine are significant contributors to the variations captured by this plot. Multivariate analysis confirmed that pretreatments, fermentation, and their interaction had significant effects on the amino acid profile of the mushroom samples ($p < 0.05$).

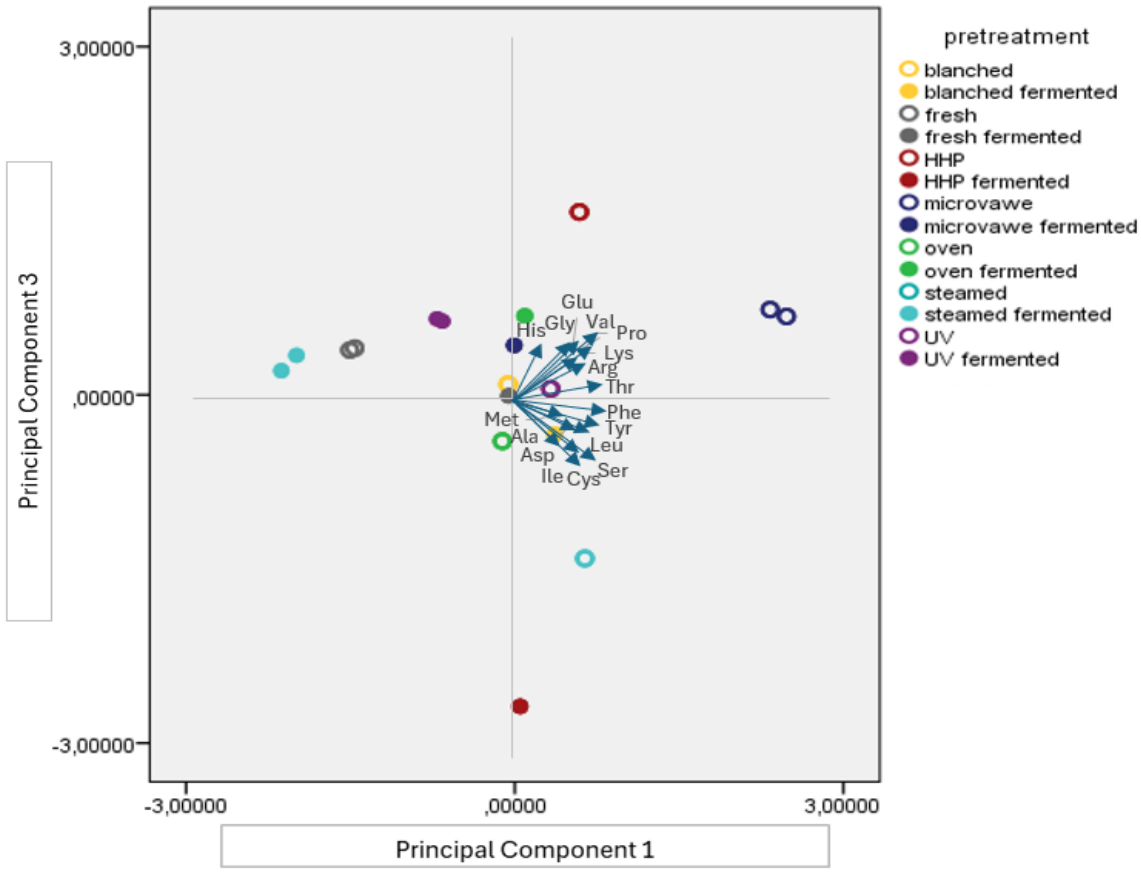


Figure 26. Principal Component Analysis plot (Component 1 vs. Component 3) of total amino acid profile of mushroom samples

5.2.7. Free amino acid profile of the mushroom samples

The total free amino acid contents of the pretreated mushroom samples are presented in Figure 27. The oyster mushroom samples contained 22 free amino acids, with their total amounts ranging from 25.95 to 55.68 mg/g for pretreated samples. The total free amino acid content of the fresh oyster mushroom was 34.07 mg/g. Blanching (23.8%) and microwave (14.9%) pretreatments caused a significant decrease in free amino content of the mushroom samples, while HHP (63.4%), oven (61.9%), UV (60.1%) and steam (8.0%) pretreatments caused an increase ($p < 0.05$). Among these, HHP pretreatment increased the free amino acid concentration the most, reaching 55.68 mg/g.

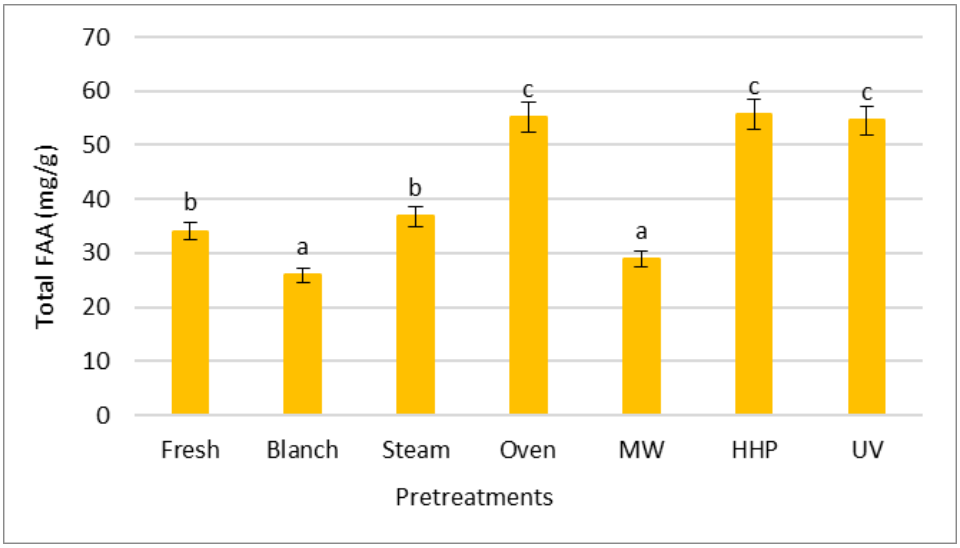


Figure 27. Total free amino acid content of the mushroom samples after pretreatments. a–c, Mean values with different letters differ significantly among Total FAA values after pretreatments ($p < 0.05$)

In the fermented sample group (Figure 28), the fresh fermented samples exhibited the highest free amino acid content at 53.64 mg/g, while blanched fermented resulted in the lowest content at 26.23 mg/g. This suggests that fermentation alone significantly increases the total free amino acid content, underscoring the importance of pretreatments before fermentation. Both blanching, steaming, and microwave pretreatments followed by fermentation led to lower free amino acid contents compared to not only the other pretreatments but also the fresh oyster mushrooms themselves ($p < 0.05$). However, oven, HHP and UV fermented samples retained their high free amino acid contents with a slight reduction compared to their pretreated counterparts.

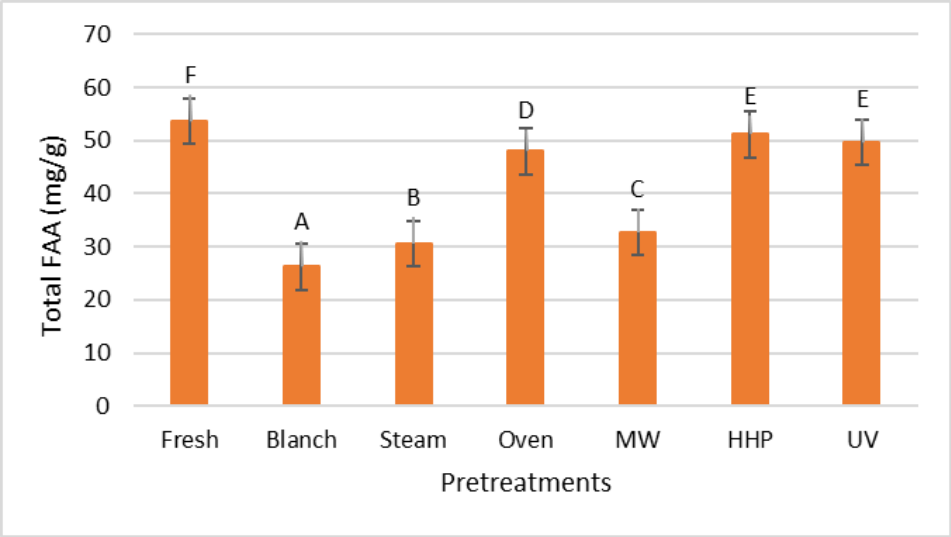


Figure 28. Total free amino acid content of the pretreated mushroom samples after fermentation. A–F, Mean values with different letters differ significantly among Total FAA values after pretreatments ($p < 0.05$)

The free amino acid profile of mushroom samples is presented in annex 3 and 4. For fresh oyster mushrooms, the predominant free amino acids were alanine (3.8 mg/g), asparagine (3.64 mg/g), and glutamic acid (3.62 mg/g). In pretreated samples, glutamic acid, glutamine, asparagine, and alanine were the most abundant, with concentrations ranging from 2.10 to 8.6 mg/g. Among the pretreated samples, HHP pretreated mushrooms had the highest levels of alanine (6.84 mg/g), asparagine (6.56 mg/g), and leucine (5–5.5 mg/g). Within the fermented sample group, fresh fermented, UV fermented, and HHP fermented mushrooms exhibited elevated levels of alanine (5–6 mg/g), glutamic acid (6–7 mg/g), and leucine (5.36 mg/g). Notably, in blanched fermented samples, the most abundant free amino acid was aspartic acid (2.19 mg/g). Gamma-aminobutyric acid (4.85 mg/g) was predominant in steamed fermented mushrooms and was also present in high amounts in UV fermented samples (4.26 mg/g). According to another study on oyster mushrooms, fermentation significantly influenced the free amino acid content. A total of 18 free amino acids were identified in both non-fermented and fermented samples, with 12 amino acids exhibiting significantly higher concentrations in the fermented samples. Among these, alanine was the most abundant (Ogidi & Agbaje, 2021). The free amino acid profile of mushroom samples was evaluated with principal component analysis, as shown in Figure 29. The first two principal components accounted for 66.3% of the variance, representing the majority of the dataset's variation.

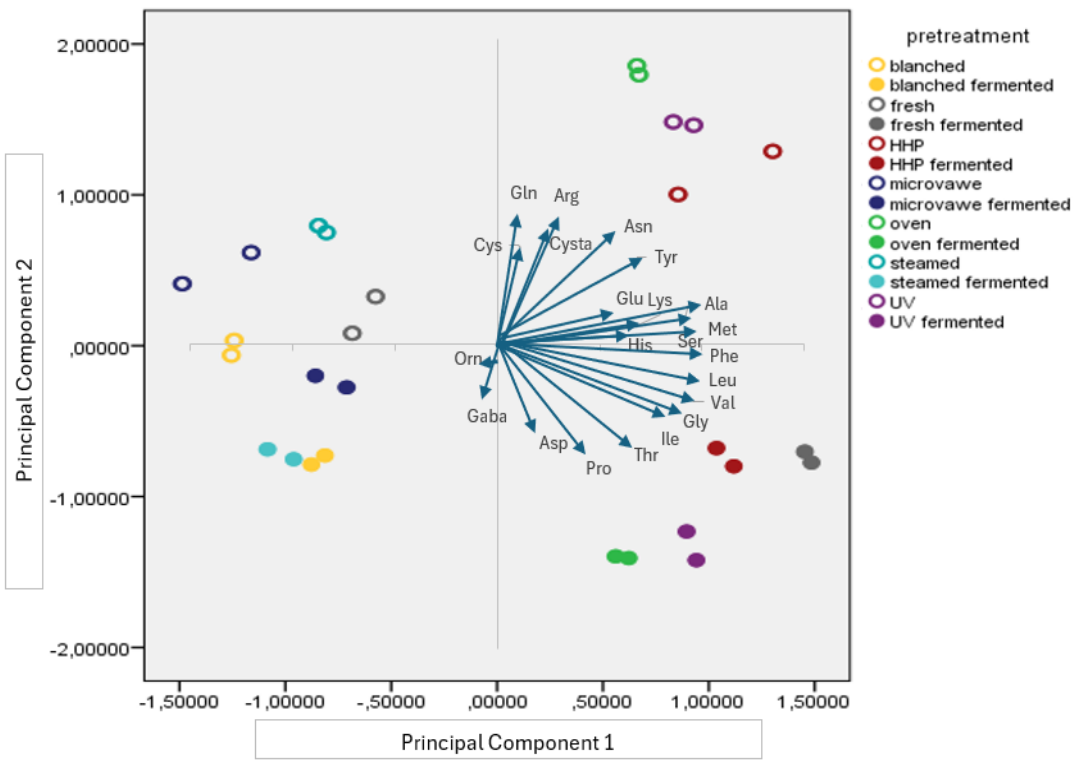


Figure 29. Principal Component Analysis plot (Component 1 vs. Component 2) of free amino acid profile of mushroom samples

In Figure 29, the upper right quadrant contains markers corresponding to three samples: oven, UV and HHP pretreated mushrooms, while lower right quadrant features markers for these samples' fermented counterparts and fresh fermented samples. The upper left quadrant contains four samples: microwave, steamed, fresh and blanched mushrooms, while the lower left quadrant contains their fermented counterparts. The clear separation of pretreated and fermented samples along principal component 2 highlights the significant impact of fermentation on the free amino acid profiles of the samples. Additionally, principal component 1 distinctly differentiates the various pretreatments. Samples located in the same quadrant (oven-UV-HHP and microwave-blanched-steamed) indicate similarities in their amino acid profiles, with the degree of similarity being reflected by their proximity to one another, forming distinct clusters. The greater distance between fermented samples and their pretreated counterparts in the oven, UV, and HHP groups suggests that these samples underwent more substantial changes in their free amino acid composition compared to the other samples. Notably, the fresh mushroom samples exhibited the most significant change in composition, shifting from the upper left quadrant to the lower left quadrant upon fermentation, indicating that the application of fermentation without any pretreatments causes a pronounced alteration in the free amino acid profile.

The arrows represent the free amino acids' contribution to the principal components. Fresh, HHP and UV fermented samples positioned further along the positive PC1 axis, suggesting high levels of free amino acids such as Glycine, Valine, Leucine, and Isoleucine. Glutamine, arginine, and cysteine arrows point upward, indicating that these amino acids are the most influential along principal component 2, affecting the oven pretreated samples. Although present in relatively low amounts, ornithine was most abundant in microwave-pretreated samples, which was reflected in their position on the plot.

5.2.8. Biogenic amine profile of the mushroom samples

The total biogenic amine contents formed from the free amino acids of the pretreated mushroom samples are presented in Figure 30. The oyster mushroom samples contained 7 biogenic amines. In the pretreated sample group, the total biogenic amine content ranged from 0.17 to 0.34 mg/g, being under the overall biogenic amine content limits (0.75-0.9 mg/g food). The lowest biogenic amine content was observed in fresh samples (0.17 mg/g), while the highest was in UV pretreated (0.34 mg/g) samples. Compared to the fresh sample, all pretreated samples showed increased biogenic amine levels: UV pretreated samples by 95.7%, oven pretreated by 77.7%, HHP pretreated by 70.6%, steamed by 50.2%, microwave pretreated by 45.6%, and blanched by 39.6%. However, this increase was not significant for blanched and microwaved samples ($p > 0.05$).

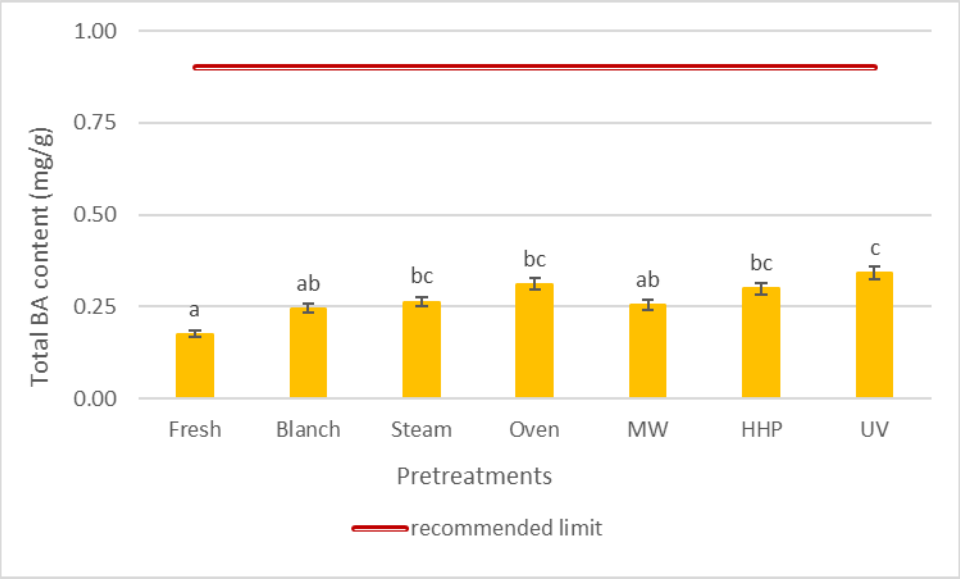


Figure 30. Total biogenic amine content of the mushroom samples after pretreatments. a–c, Mean values with different letters differ significantly among Total BA values after pretreatments ($p < 0.05$)

In the pretreated fermented sample group (Figure 31), the biogenic amine content ranged from 0.14 to 5.05 mg/g. The UV pretreated fermented oyster mushrooms exhibited the highest biogenic amine

content (5.05 mg/g), while the lowest was observed in blanched fermented samples (0.14 mg/g). Fermented samples generally displayed higher biogenic amine levels (overall 5.72 times more than their pretreated counterparts) except for the blanched fermented samples. This result is not surprising, as the microbes involved in fermentation produce biogenic amines from free amino acids through decarboxylase activity. However, the disproportionately high contribution of UV fermented samples to this overall increase should not be neglected. Among fermented samples, only the UV pretreated sample had a higher biogenic amine concentration (2.7 times) compared to the fresh fermented samples. In all other cases, a reduction in biogenic amine content was observed within the fermented group: blanched samples decreased by 92.8%, microwave pretreated by 76.0%, steamed by 57.3%, oven-treated by 52.1%, and HHP pretreated by 17.6%. These reductions highlight the effectiveness of pretreatments in mitigating biogenic amine production during fermentation.

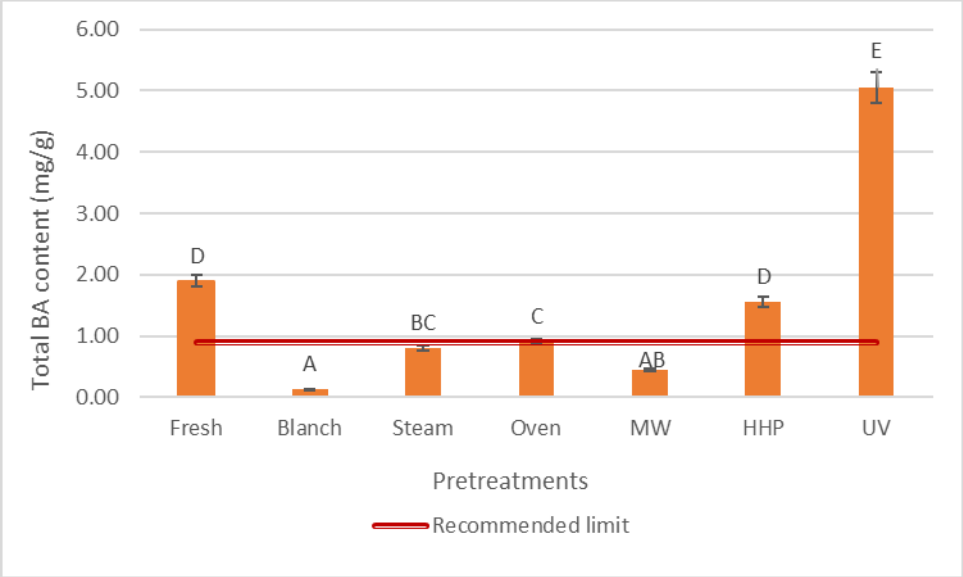


Figure 31. Total biogenic amine content of the pretreated mushroom samples after fermentation. A-D, Mean values with different letters differ significantly among Total BA values after fermentation ($p < 0.05$)

The biogenic amine profile of mushroom samples is presented in annex 5 and 6. In fresh oyster mushrooms, a small amount of spermidine (0.15 mg/g) and cadaverine (0.02 mg/g) were detected. In pretreated samples, 3 biogenic amines were identified: spermidine, cadaverine and tyramine. Spermidine (0.24-0.31 mg/g) was the dominant biogenic amine in pretreated samples, accounting for 94% of the total biogenic amine content. Spermidin and putrescine are reported as typical fungal polyamines, often occurring in the highest concentrations compared to other biogenic amines in mushrooms (Jabłońska-Ryś et al., 2022). Additionally, cadaverine was present in small amounts (0.01-0.04 mg/g) in fresh, steamed, and HHP pretreated samples, contributing 4% to the total biogenic

amine content. Tyramine was detected only in UV pretreated oyster mushrooms (0.04 mg/g), making up 2% of the total biogenic amine content. In fermented samples, seven biogenic amines were identified: histamine, tyramine, putrescine, cadaverine, spermidine, agmatine and spermine. Histamine was the most abundant, ranging between 0.15-1.23 mg/g, constituting 28% of the total biogenic amine content. It was present in all fermented samples except the blanched ones, with notably high levels (1.23 mg/g) observed in HHP fermented sample, exceeding the recommended limits (0.05–0.1 mg/g for food). Tyramine was the second most abundant biogenic amine (0.08-4.1 mg/g) representing 48% of the total biogenic amine content. It was detected in all fermented samples except the blanched and microwave pretreated ones, with an exceptionally high amount (4.1 mg/g) in the UV fermented sample, significantly surpassing the recommended limits (0.1–0.8 mg/g for food). Spermidine ranged between 0.12-0.21 mg/g across six pretreatments but was undetectable in the HHP pretreated samples. Putrescine (0.01-0.27 mg/g) was present in all samples except the blanched ones, contributing an average of 9.1% to the total biogenic amine content. Cadaverine accounted for 3.0% of the total biogenic amine content, with the highest concentration observed in the HHP fermented sample (0.15 mg/g). Spermine was only detected in the fresh fermented sample (0.48 mg/g), while agmatine (0.08 mg/g) was only found in the HHP fermented sample. In a study on button mushrooms, three biogenic amines: spermidine, putrescine, and tyramine were identified. Putrescine (ranging from 0.58 to10.11 mg/kg) and tyramine (ranging from 1.44 to 69.04 mg/kg) were only present in fermented mushroom samples, while histamine was not detected in their study (Jabłońska-Ryś et al., 2022). The biogenic amine profile of mushroom samples was also evaluated with principal component analysis, as shown in Figure 32. The first two principal components accounted for 75.7% of the variance, representing the majority of the dataset's variation.

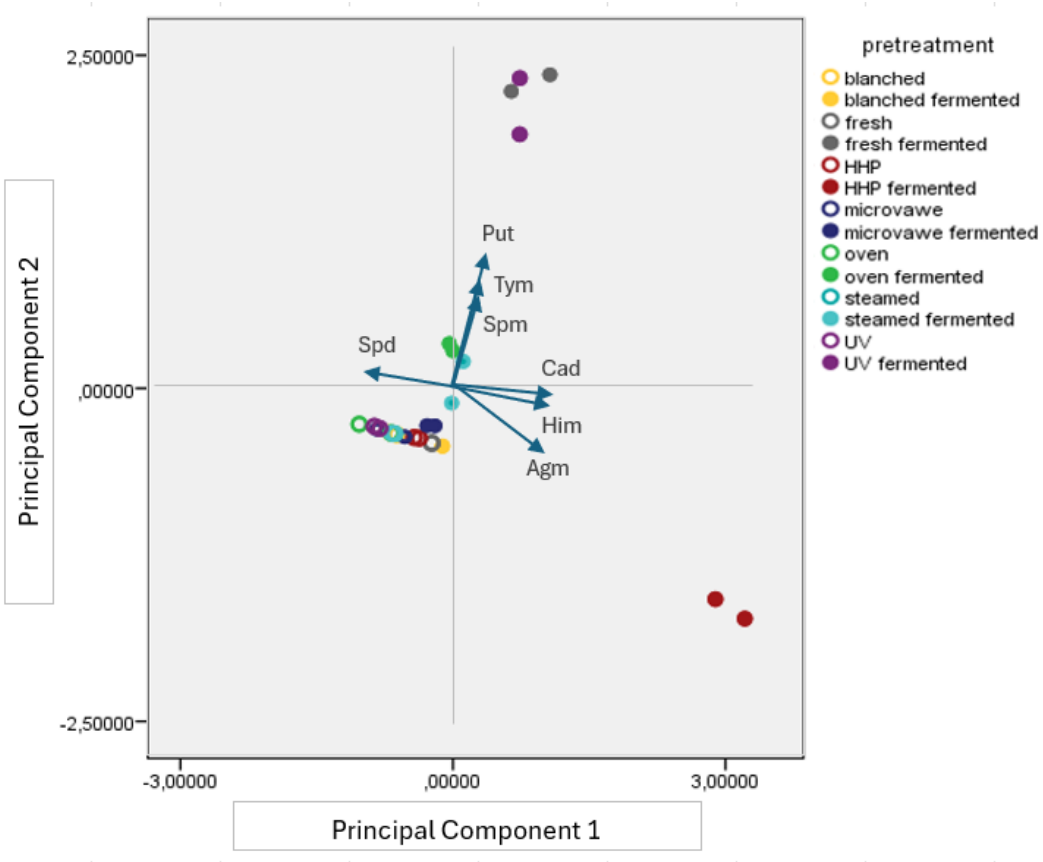


Figure 32. Principal Component Analysis plot (Component 1 vs. Component 2) of biogenic amine profile of mushroom samples

In Figure 32, the upper right quadrant contains markers corresponding to fresh fermented and UV fermented mushrooms, forming a distinct cluster from other sample groups, indicating similarities in their biogenic amine profiles. The lower right quadrant features markers only for HHP fermented samples, located far from all the other sample groups, suggesting a unique biogenic amin composition. Markers for oven fermented and steamed fermented samples are located near the center of the plot, indicating that their biogenic amine profiles closely resemble the overall dataset average. The lower left quadrant includes all pretreated samples along with blanched fermented and microwave fermented samples. This distinct clustering of pretreated samples highlights the minimal impact of pretreatments on their biogenic amine profiles, which are predominantly characterized by spermidine and show an absence of other biogenic amines. Notably, the blanched fermented and microwave fermented samples exhibit similar profiles, suggesting that fermentation had a limited effect on altering their biogenic amine composition, in contrast to the fresh fermented, UV fermented, and HHP fermented samples, which showed more pronounced changes. The arrows represent the biogenic amines' contribution to the principal components. Fresh fermented and UV fermented samples are positioned further along the positive PC2 axis, indicating elevated levels of biogenic amines such as putrescine, spermine, and

notably tyramine in UV fermented samples. In addition to agmatine being exclusively detected in HHP fermented samples, histamine was also most abundant in these samples, a factor that influenced their distinct positioning on the plot.

5.2.9. NIR evaluation of the mushroom samples

The obtained NIR spectral data were evaluated with canonical discriminant analysis (CDA) to assess the impact of various pretreatments and fermentation on the classification of mushroom samples, aiming to maximize the separation between groups based on their characteristics.

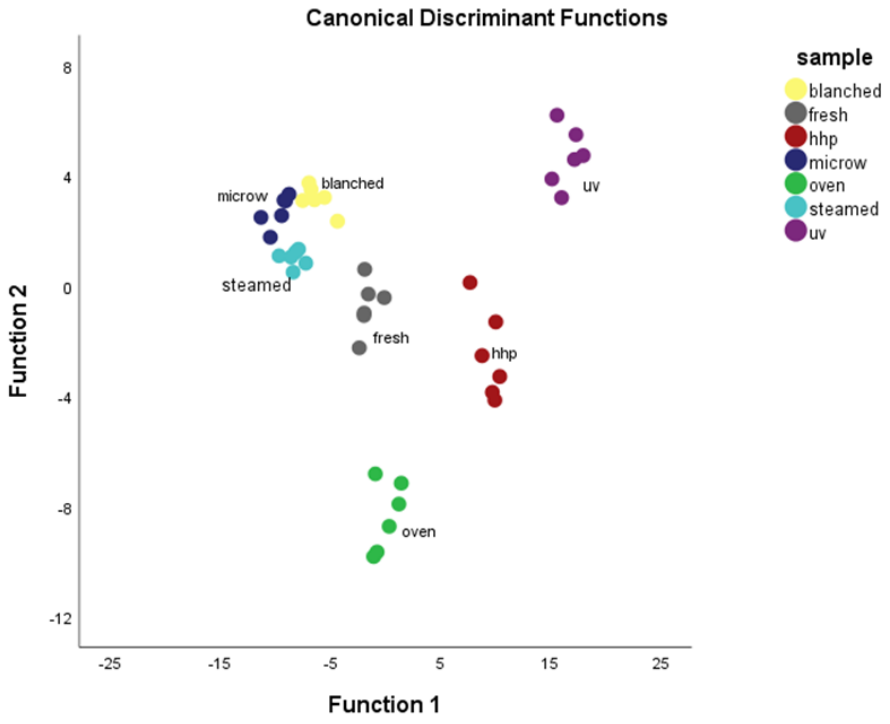


Figure 33. Canonical Discriminant Analysis plot (Function 1 vs. Function 2) illustrating the classification of pretreated mushroom samples based on NIR spectra

Figure 33 illustrates the NIR dataset, providing a visual comparison of mushroom pretreatments – fresh, blanched, steamed, oven, microwave, HHP and UV. This CDA plot represents the initially grouped cases, reflecting the dataset prior to any cross-validation or adjustments to the model. The original classification achieved an accuracy of 100%, demonstrating that the model correctly assigned all of the cases to their respective groups based on the discriminant functions derived from the dataset. Following cross-validation, the accuracy dropped to 42.9%, indicating cases where the model misclassified microwaved samples as either blanched or steamed, and vice versa. This outcome indicates a greater spectral similarity between the effects of these pretreatments on the mushrooms

compared to advanced methods such as HHP and UV pretreatments. Notably, HHP, UV and oven pretreatments formed distinct clusters indicating that their NIR spectra contain unique characteristics differentiating them.

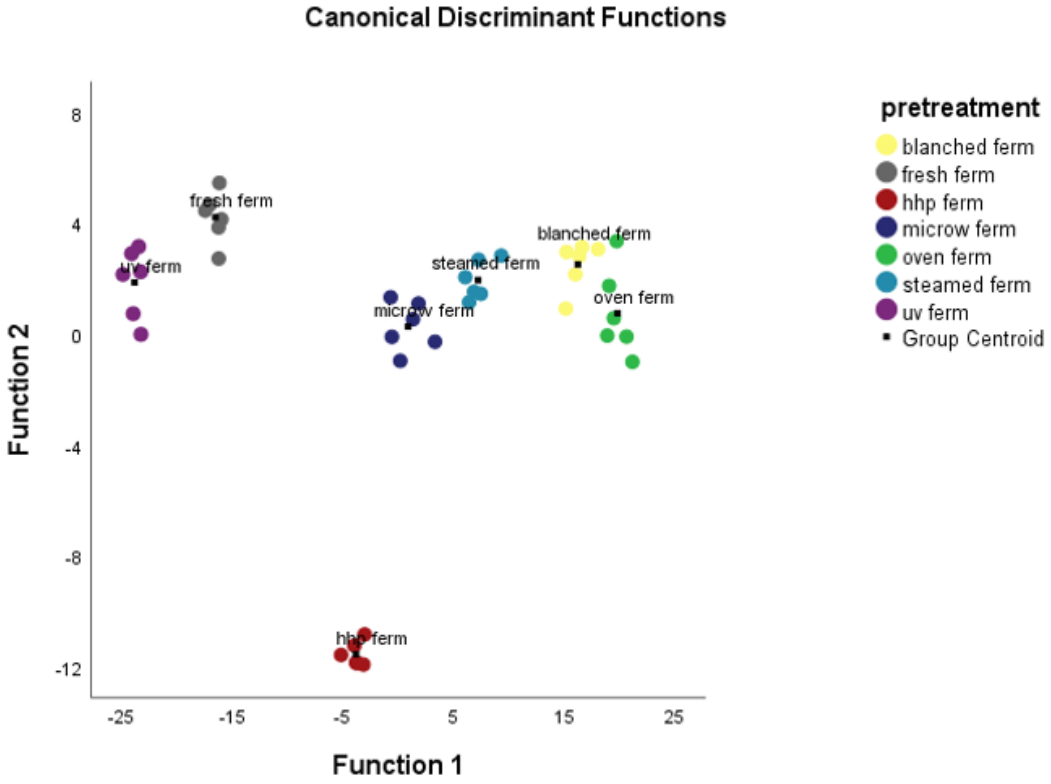


Figure 34. Canonical Discriminant Analysis plot (Function 1 vs. Function 2) illustrating the classification of pretreated fermented mushroom samples based on NIR spectra

Figure 34 illustrates the NIR dataset, providing a visual comparison of mushroom samples after their fermentation. The original classification achieved an accuracy of 100% based on the discriminant functions derived from the dataset. After cross-validation, the accuracy decreased to 61.9%, highlighting instances where the model misclassified microwave-fermented, steam-fermented, blanched-fermented, and oven-fermented samples among one another. This result suggests that the spectral similarities among these sample groups persisted even after their fermentation, with oven-fermented samples also aligning closely with this group. On the other hand, fresh-fermented and UV-fermented samples clustered closely together after fermentation, suggesting that fermentation produced similar effects on these sample groups. HHP-fermented samples formed distinctly separate clusters from all other groups, suggesting that fermentation induced unique characteristics in their NIR spectra, distinguishing them from the other samples.

The choice of pretreatment method impacted all examined quality attributes of oyster mushrooms. This part of the research explored the feasibility of integrating advanced technologies such as HHP, microwave, and UV light alongside traditional methods like steaming, oven cooking, and water blanching prior to mushroom fermentation. Among these, microwave pretreatment demonstrated favorable outcomes suggesting it could serve as viable alternative to water blanching. Conversely, UV and HHP pretreatments were deemed unsuitable due to significant quality degradation (Boylu et al., 2023a). A summary of the most beneficial pretreatment effects on oyster mushroom quality is provided in Table 9.

Table 9. Summary of the Most Beneficial Pretreatment Effects on Oyster Mushroom Quality

Quality Parameter	Most Beneficial Effect	
	After pretreatments	After Fermentation
Moisture (%)	Blanch, Oven, HHP, UV	HHP, UV
pH	HHP, UV	UV
Yield (%)	HHP, UV	Blanch, MW
Color (*L*, a*, b*,* BI, YI*)*	Blanch, UV	Blanch, Steam, MW
Texture (Fmax, N)	MW, UV	Blanch, Steam, MW
Essential/Total amino acid content (mg/g)	MW, HHP	Blanch, MW
Free amino acid content (mg/g)	Blanch, MW	Blanch, Steam
Biogenic amine content (mg/g)	Blanch, MW	Blanch, MW

5.3. QUALITY OF SAUSAGES DURING STORAGE WITH PARTIAL MEAT REPLACEMENT BY FERMENTED OYSTER MUSHROOMS

5.3.1. Moisture content of the sausage samples

Table 10 presents the moisture contents of sausage samples in which meat was partially replaced by 25% and 50% fermented oyster mushrooms. The results showed significant differences in moisture content across the samples ($p < 0.05$). Specifically, all samples with mushroom substitution exhibited higher moisture levels than the control sample, and the 50% substitution samples contained more moisture than both the control and 25% substitution samples. This increased moisture content is likely due to the high water content (85–90%) of the fermented mushrooms used as a meat substitute (Boylu et al., 2023a). Comparable findings were noted in another study, where replacing meat with oyster mushrooms in sausages resulted in the control sample having the lowest moisture content (67.62 ± 0.33%), while sausages with 60% fresh oyster mushrooms recorded the highest moisture level (75.29 ± 1.44%) (Yahya & Ting, 2020).

In the 25% meat replacement group, the UV25 samples exhibited the lowest initial moisture content on day 0 (62.04 ± 0.10%), comparable to the control samples (62.35 ± 0.11%). Between day 0 and day 7, however, the moisture content in the control samples significantly declined to 56.32 ± 0.56% and stayed the lowest for the remainder of storage, whereas the other samples generally showed a rising trend. The Blanch25 samples had the highest moisture level initially (67.82 ± 0.12%) and maintained this position throughout storage, reaching 70.36 ± 0.26% at the end. Significant differences were also observed in the moisture content of the sausage samples with a 50% meat replacement. Consistent with the 25% replacement group, the UV50 samples recorded the lowest moisture content on day 0 (71.10 ± 0.38%) and on day 28 (70.00 ± 0.11%). Apart from the Steam50 sample group, moisture content for all samples fluctuated throughout the storage duration. By the end of the storage period, the Blanch50 samples had the highest moisture level (75.55 ± 0.16%), followed closely by the Steam50 samples (75.37 ± 0.24%).

Table 10. The mean ± standard deviation values for the moisture content of sausage samples with meat replacements throughout the storage period

		Moisture Content (%)				
		Storage (day)				
	Samples	0	7	14	21	28
25% meat replacement	Fresh25	67.38 ± 0.06^{Ea}	67.60 ± 0.13^{Da}	68.44 ± 0.24^{Eb}	69.22 ± 0.09^{Fc}	68.15 ± 0.08^{Eb}
	Blanch25	67.82 ± 0.12^{Fa}	68.96 ± 0.03^{Eb}	69.92 ± 0.13^{Fc}	69.26 ± 0.19^{Fb}	70.36 ± 0.26^{Fc}
	Steam25	67.09 ± 0.04^{Eb}	67.42 ± 0.02^{Dbc}	67.80 ± 0.10^{Dc}	67.49 ± 0.11^{Ebc}	66.46 ± 0.31^{CDa}
	Oven25	64.79 ± 0.09^{Cb}	64.45 ± 0.09^{Cab}	64.42 ± 0.12^{Ba}	66.44 ± 0.06^{Dc}	67.06 ± 0.04^{Dd}
	MW25	66.10 ± 0.04^{Dd}	64.96 ± 0.13^{Cb}	65.66 ± 0.17^{Cc}	64.74 ± 0.03^{Cb}	66.01 ± 0.09^{Ccd}
	HHP25	64.15 ± 0.18^{Ba}	65.16 ± 0.47^{Cab}	65.33 ± 0.13^{Cbc}	66.31 ± 0.24^{Dcd}	66.51 ± 0.02^{CDd}
	UV25	62.04 ± 0.10^{Aa}	61.80 ± 0.08^{Ba}	63.92 ± 0.01^{Bb}	62.29 ± 0.01^{Bab}	64.30 ± 0.20^{Bb}
	Control	62.35 ± 0.11^{Ab}	56.32 ± 0.56^{Aa}	55.94 ± 0.29^{Aa}	56.11 ± 0.20^{Aa}	56.44 ± 0.09^{Aa}
50% meat replacement	Fresh50	72.92 ± 0.16^{Cc}	71.77 ± 0.20^{Bb}	70.75 ± 0.15^{Ba}	71.92 ± 0.08^{Bb}	70.37 ± 0.06^{Ba}
	Blanch50	75.19 ± 0.21^{Da}	75.95 ± 0.11^{Fb}	76.96 ± 0.19^{Fc}	76.91 ± 0.04^{Gc}	75.55 ± 0.16^{Eab}
	Steam50	75.48 ± 0.24^{D}	75.81 ± 0.08^{F}	75.56 ± 0.11^{E}	75.57 ± 0.24^{E}	75.37 ± 0.24^{E}
	Oven50	76.85 ± 0.28^{Ed}	74.94 ± 0.01^{Ec}	72.63 ± 0.09^{Ca}	74.15 ± 0.14^{Db}	73.76 ± 0.06^{Db}
	MW50	76.12 ± 0.13^{Db}	75.86 ± 0.01^{Fb}	76.39 ± 0.06^{Fb}	76.34 ± 0.06^{Fb}	72.62 ± 0.37^{Ca}
	HHP50	71.16 ± 0.11^{Ba}	72.33 ± 0.05^{Cb}	71.04 ± 0.29^{Ba}	72.67 ± 0.08^{Cb}	72.45 ± 0.06^{Cb}
	UV50	71.10 ± 0.38^{Bb}	73.32 ± 0.04^{Dc}	73.58 ± 0.05^{Dc}	73.80 ± 0.03^{Dc}	70.00 ± 0.11^{Ba}

$^{A–G}$: Means in the same column with distinct superscripts indicate significant differences among samples. $^{a–d}$: Means in the same row with distinct superscripts denote significant differences among storage days ($p < 0.05$). Control, 100% pork sausage; Fresh25–50: sausage containing 25–50% fresh fermented mushrooms; Blanch25–50: sausage containing 25–50% blanched fermented mushrooms; Steam25–50: sausage containing 25–50% steamed fermented mushrooms; Oven25–50: sausage containing 25–50% oven pretreated fermented mushrooms: MW25–50: sausage containing 25–50% microwave fermented mushrooms; HHP25–50: sausage containing 25–50% HHP fermented mushrooms; UV25–50: sausage containing 25–50% UV fermented mushrooms.

The elevated moisture levels in the Blanch25 and Blanch50 samples compared to the others likely result from the moisture-retention characteristics of the blanching pretreatment, which helps lock moisture within mushroom tissues, preserving water content prior to fermentation (Walde et al., 2006). As a result, when these mushrooms are incorporated into sausage formulations, they may add more moisture than those treated with other methods. Conversely, the UV25 and UV50 samples exhibited the lowest moisture content among the groups, possibly due to surface dehydration effects linked to UV exposure, as noted by other researchers (Forouzanfar et al., 2020); however, this effect was not found in our previous study. Other studies have similarly reported moisture variations in pork sausages with *P. eryngii* subjected to diverse treatments (Wang et al., 2019b). Additionally, differences in microbial activity during fermentation, influenced by various pretreatments and subsequent incorporation, may further explain the moisture content variations in the sausage samples.

5.3.2. pH of the sausage samples

Figure 35 (A, B) displays the pH values of sausage samples. Replacing meat with pretreated fermented mushrooms had a significant impact on sausage pH ($p < 0.05$). Beginning on day 0, the control samples showed higher pH values (pH = 5.87 ± 0.03) than the replacement samples, a trend that continued through the end of storage (pH = 6.04 ± 0.02). This difference is likely due to the inherently lower pH of fermented mushrooms relative to the meat they replaced. Among the samples with 25% meat replacement, the Blanch25 group demonstrated the highest pH values, starting from day 0 (pH = 5.63 ± 0.01) and continuing through day 28 (pH = 5.81 ± 0.01). In contrast, the UV25 samples had the lowest pH values from day 0 (pH = 5.23 ± 0.03) until the conclusion of the storage period (pH = 5.44 ± 0.01). All sample groups exhibited an increase in pH until day 7, followed by a decline leading up to the end of the storage period, with the exception of the control samples.

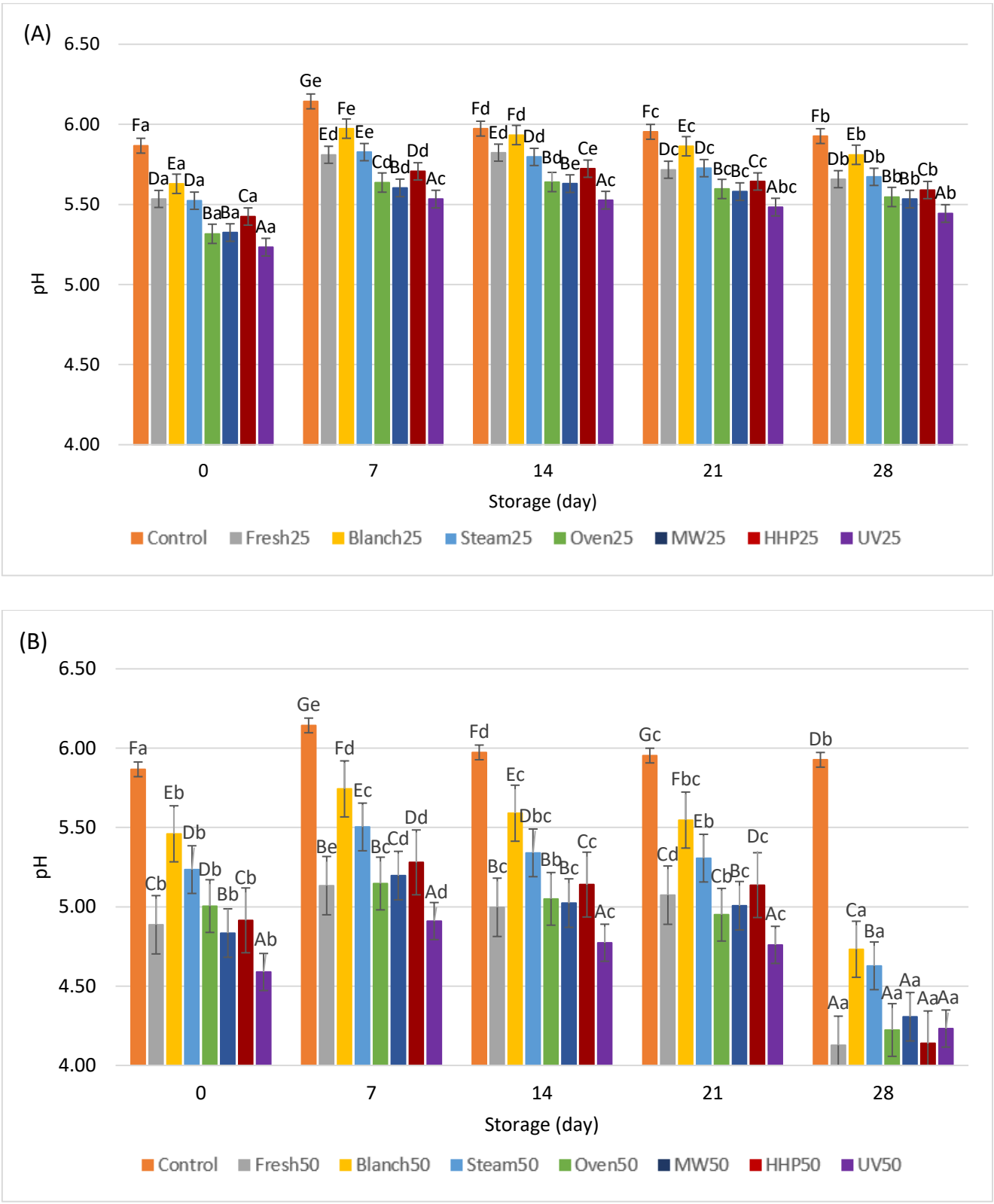


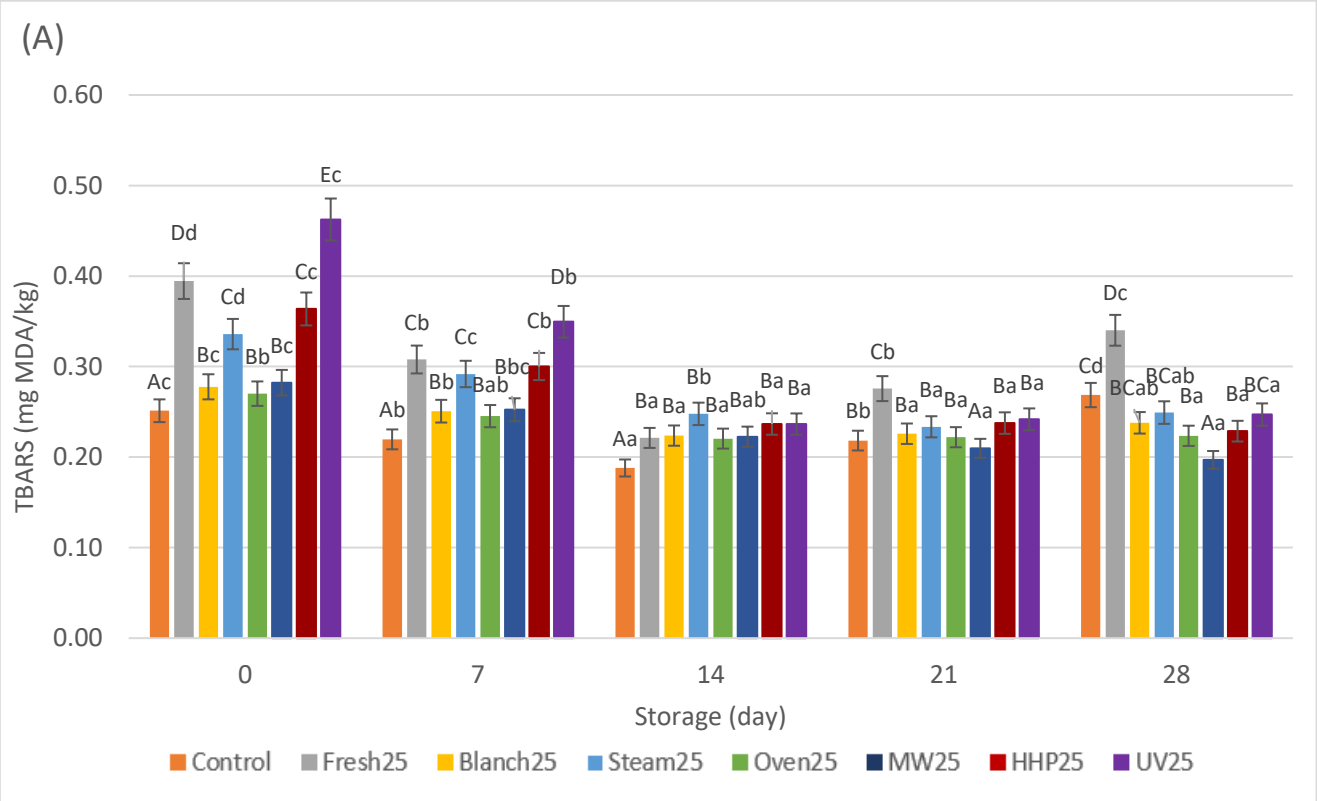
Figure 35. Mean ± standard deviation values of pH levels for sausage samples during storage with 25% meat replacement (A) and with 50% meat replacement (B). A–G: Distinct letters indicate significant differences among the samples. a–e: Different letters indicate significant differences among storage days (Tukey's post hoc test, $p < 0.05$).

A similar pattern was noted among the 50% replacement samples, which exhibited lower pH values compared to the 25% replacement samples, with values ranging from 4.58 ± 0.01 to 5.46 ± 0.01 immediately after production. A gradual decline in pH values was observed from day 7 to day 21, followed by a more significant decrease between day 21 and day 28. By the end of the storage period, there were no significant differences in pH among the Fresh50, Oven50, MW50, HHP50, and UV50 samples, all of which displayed low pH levels near 4.3. This result may be linked to previous research indicating that fresh, oven-cooked, HHP pretreated, and UV pretreated fermented mushroom samples had lower pH values than those that were blanched and steamed (Boylu et al., 2023a). Comparable findings were observed with *Agaricus bisporus* mushrooms, where boiled mushrooms exhibited higher pH values than those that were sous-vide cooked, pan-fried, oven-cooked, or barbecued. This phenomenon is believed to result from the prolonged exposure of the samples to hot water during pretreatments like blanching and steaming, which can cause thermal degradation of organic acids, their leaching into the water, and a subsequent decrease in acidity (Erhan et al., 2023). In contrast, UV and HHP pretreatments do not lead to the degradation of organic acids due to heat, while microwave pretreatment heats the samples unevenly and internally, which may help retain more of the natural acidity compared to traditional thermal methods. In a study examining a vegan boiled sausage analog, the pH of samples containing *Pleurotus sapidus* was recorded as 4.7 ± 0.01 on the production day and decreased to 4.6 ± 0.02 after four weeks of storage. The authors observed that the pH decreased over time not only in meat products but also in vegetarian and vegan systems containing fungal mycelia, as various microorganisms metabolize sugars and proteins, resulting in higher concentrations of organic acids (Stephan et al., 2018). Another study focusing on fermented sausages made from soybean protein and *Coprinus comatus* mushrooms reported a pH value of 5.09 within 18 hours (Yuan et al., 2022b). Also, when dry *Lentinula edodes* was utilized as a substitute for pork in sausages, higher pH values were recorded compared to this study, with values of 6.65 ± 0.03 and 6.69 ± 0.03 for the 25% and 50% replacement samples, respectively (Wang et al., 2019a).

5.3.3. Lipid oxidation of the sausage samples

The malondialdehyde (MDA) levels in the sausage samples during storage are presented in Figure 36 (A, B). Replacing meat with pretreated fermented mushrooms significantly influenced the TBARS values of the sausage samples ($p < 0.05$). For the samples with 25% meat replacement, the TBARS values on day 0 varied between 0.27 ± 0.01 and 0.46 ± 0.01 mg MDA/kg. The control samples consistently showed lower TBARS values than the 25% replacement samples, measuring 0.25 ± 0.00 mg MDA/kg on day 0 and decreasing to 0.19 ± 0.01 mg MDA/kg by day 14. On the other hand, the

TBARS values for both Control and Fresh25 samples increased following day 14, while the other sample groups remained fairly consistent, fluctuating between 0.20 and 0.25 mg MDA/kg. At the conclusion of the storage period, both the Control and Fresh25 samples reverted to TBARS values similar to those recorded on day 0. The other sample groups (B25, Steam25, Oven25, MW25, HHP25, UV25) displayed a notable decline in TBARS values by the end of the storage period compared to their initial readings ($p < 0.05$). On day 28, the Fresh25 samples recorded the highest TBARS value at 0.37 ± 0.03 mg MDA/kg, while the MW25 samples had the lowest value at 0.20 ± 0.02 mg MDA/kg.



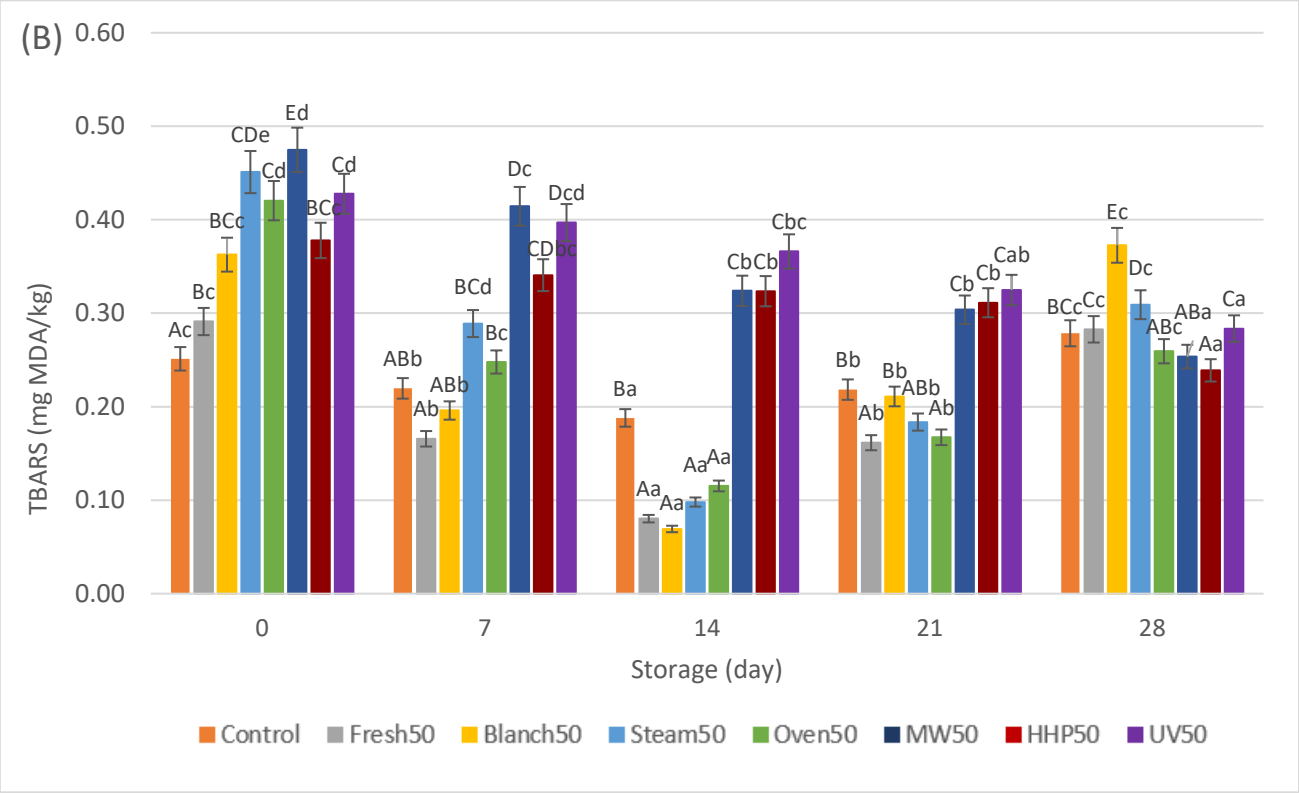


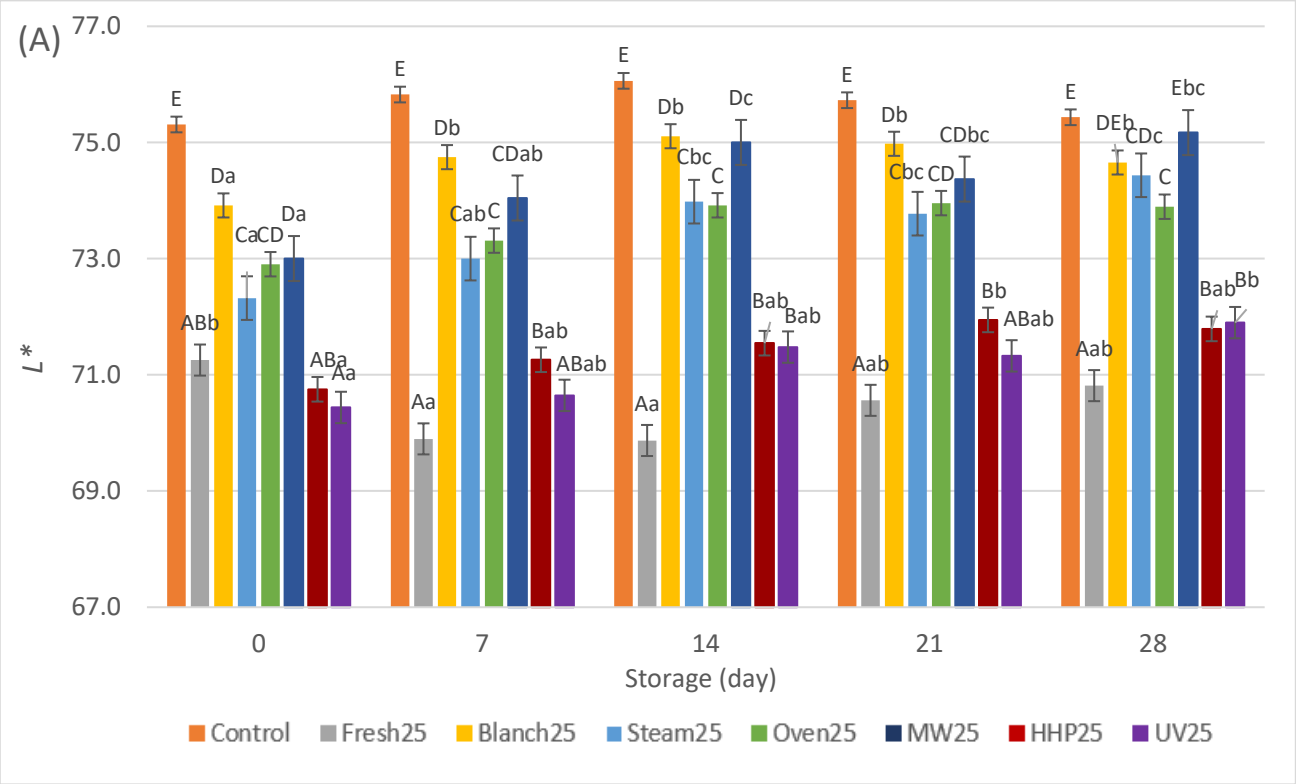
Figure 36. Mean ± standard deviation values of TBARS levels for sausage samples during storage with 25% meat replacement (A) and with 50% meat replacement (B). A–E: Distinct letters denote significant differences across sample groups. a–e: Distinct letters denote significant differences across storage days (Tukey's post hoc test, $p < 0.05$).

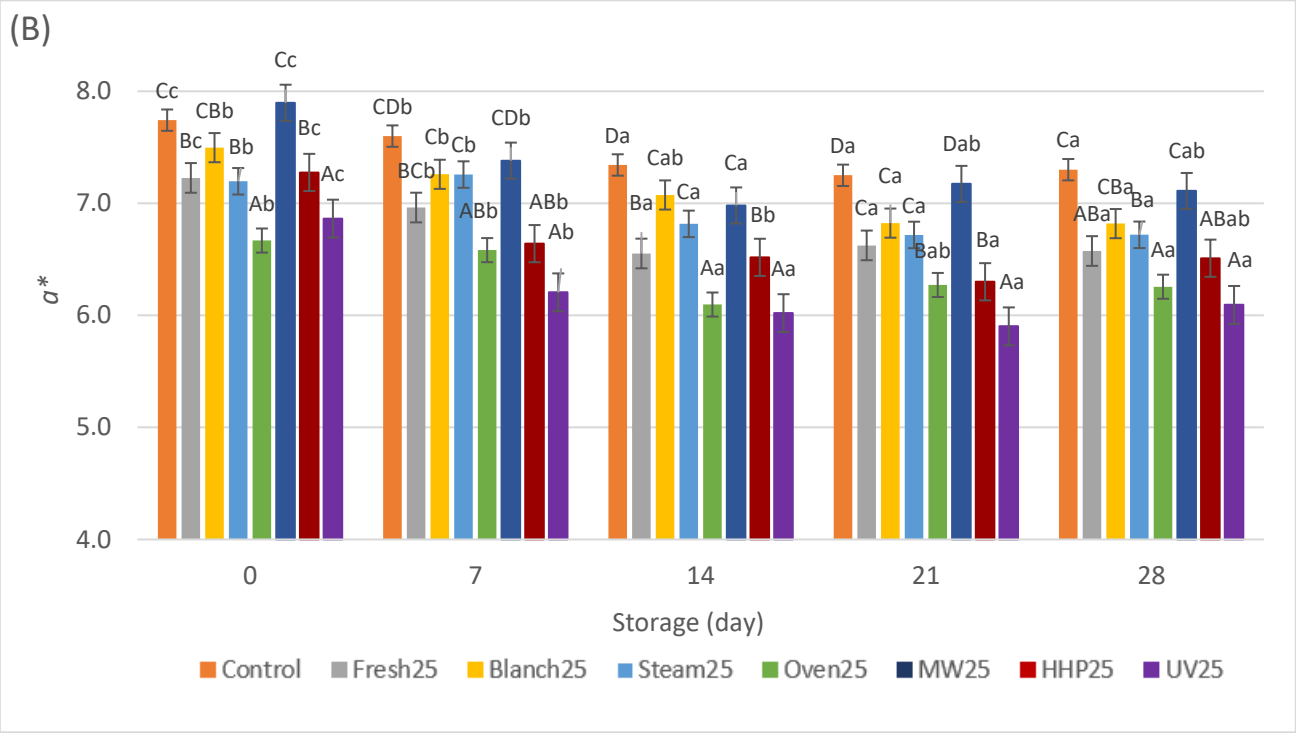
Among the samples with 50% meat replacement, TBARS values on day 0 varied from 0.29 ± 0.01 to 0.50 ± 0.01 mg MDA/kg. While all sample groups showed a decrease in TBARS values by day 14, a clear division into three distinct categories was noted at that point. The TBARS values for the MW50, HHP50, and UV50 samples (I) were significantly elevated compared to the values of the Control (II), Fresh50, Blanch50, Steam50, and Oven50 (III) samples. This classification can be explained by the unique characteristics of the different pretreatment techniques employed. The antioxidant properties of mushrooms, and consequently the lipid oxidation in sausages, may respond differently to innovative methods such as microwave, HHP, and UV light pretreatments in contrast to conventional thermal treatments. This difference could be partly due to the presence of different free amino acids in the mushroom samples, which may act as scavengers of reactive oxygen species, thereby reducing lipid peroxidation. A notable decrease in total antioxidant activity was reported with microwave blanching for 3 minutes at 85 °C. Improved results related to temperature distribution, inactivation of polyphenol oxidase, weight loss, shrinkage, total antioxidant activity, and browning of samples were attained using a combined approach (1 minute in the microwave at 85 °C followed by a 20-second immersion in water at 92 °C) (Jabłońska-Ryś et al., 2019). For example, UV exposure is known to damage plant

tissues by inducing oxidative stress, which leads to lipid peroxidation, denaturation of proteins, oxidation of carbohydrates, and damage to DNA (Jiang et al., 2010). Researchers agree that HHP treatments frequently trigger oxidation and have proposed various hypotheses. These include the release of iron from heme and conformational changes in hemoproteins due to pressure, which enhance the accessibility of unsaturated fatty acids to catalytic heme groups (Cava et al., 2020; Guyon et al., 2016). A study involving pork samples found that an increase in the pressure levels of HHP treatments resulted in a greater presence of secondary oxidation products and elevated TBARS values (Kenesei et al., 2024). In contrast, conventional thermal pretreatments like blanching, steaming, and oven cooking can lead to the creation of new compounds with antioxidant properties, including those generated by the Maillard reaction (Roncero-Ramos et al., 2017). One study indicated that the phenolic content of *Pleurotus ostreatus* decreased due to the blanching and fermentation, likely because soluble phenolics diffused into the fermentation brine (Radzki et al., 2016). In this study, by the end of the storage period, the variations in TBARS values among the samples had mostly diminished. The marked reduction in TBARS values observed on day 14 can be attributed to an initial phase of oxidative stress that occurred after processing, resulting in elevated TBARS values at the beginning of storage. As this oxidative stress lessens and the product reaches a more stable state over time, TBARS values typically decrease, as demonstrated on day 14 (Domínguez et al., 2019). Also, a study revealed that the antioxidant activity of Porcini mushrooms (*Boletus edulis*) incorporated into frankfurters peaked around day 20, during cold storage. However, this activity subsequently declined due to the exhaustion of the mushrooms' inherent antioxidant reserves, a trend also noted in this research after day 14 (Novakovic et al., 2020). Concerning the TBARS data, it is important to highlight that all sample groups maintained lipid oxidation levels below 0.50 mg MDA/kg throughout the entire storage period, which is considered acceptable for processed meat products (Patinho et al., 2019). This finding suggests that the antioxidant properties of fermented oyster mushrooms, as previously reported, can retain a degree of effectiveness when added to sausage products (Jabłońska-Ryś et al., 2016). The antioxidant properties are influenced by the pretreatment methods applied to the mushrooms. Moreover, a steady decline in TBARS values was noted for the MW, HHP, and UV samples during the storage period, even though they began with higher initial TBARS values. Another study reported that the use of dried *Lentinula edodes* to replace 25%, 50%, 75%, and 100% of pork meat in sausages resulted in enhanced total phenolic content and antioxidant activity compared to the control samples (Wang et al., 2019a).

5.3.4. Color attributes of the sausage samples

The color attributes (*L**, *a**, *b**) of the sausage samples were measured instrumentally throughout the storage period, as given in Figures 37 (A, B, C) and 38 (A, B, C). Notably, significant variations ($p < 0.05$) were found across the samples in terms of color properties. The *L** values for the Control samples consistently remained higher than those for the 25% and 50% replacement samples on each storage day. Moreover, the *L** values of the Control samples showed minimal change, from an initial 75.31 ± 1.45 on day 0 to 75.43 ± 0.7 by day 28.





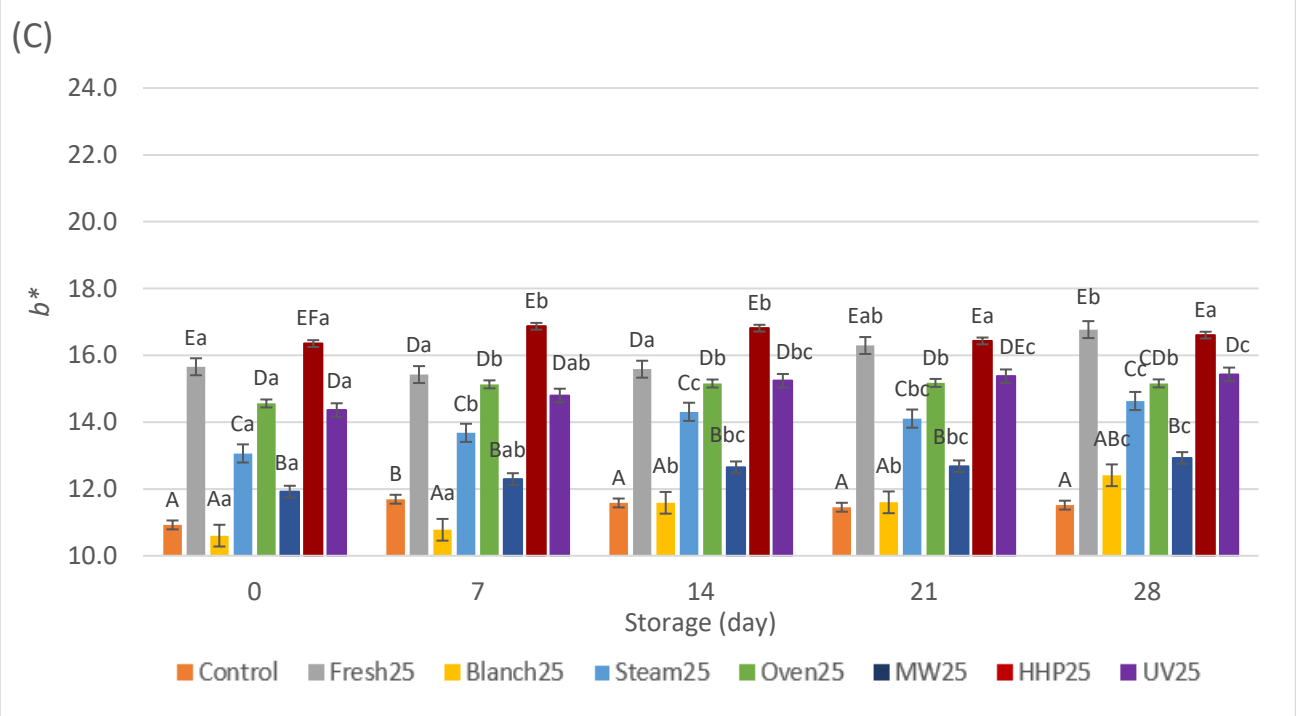
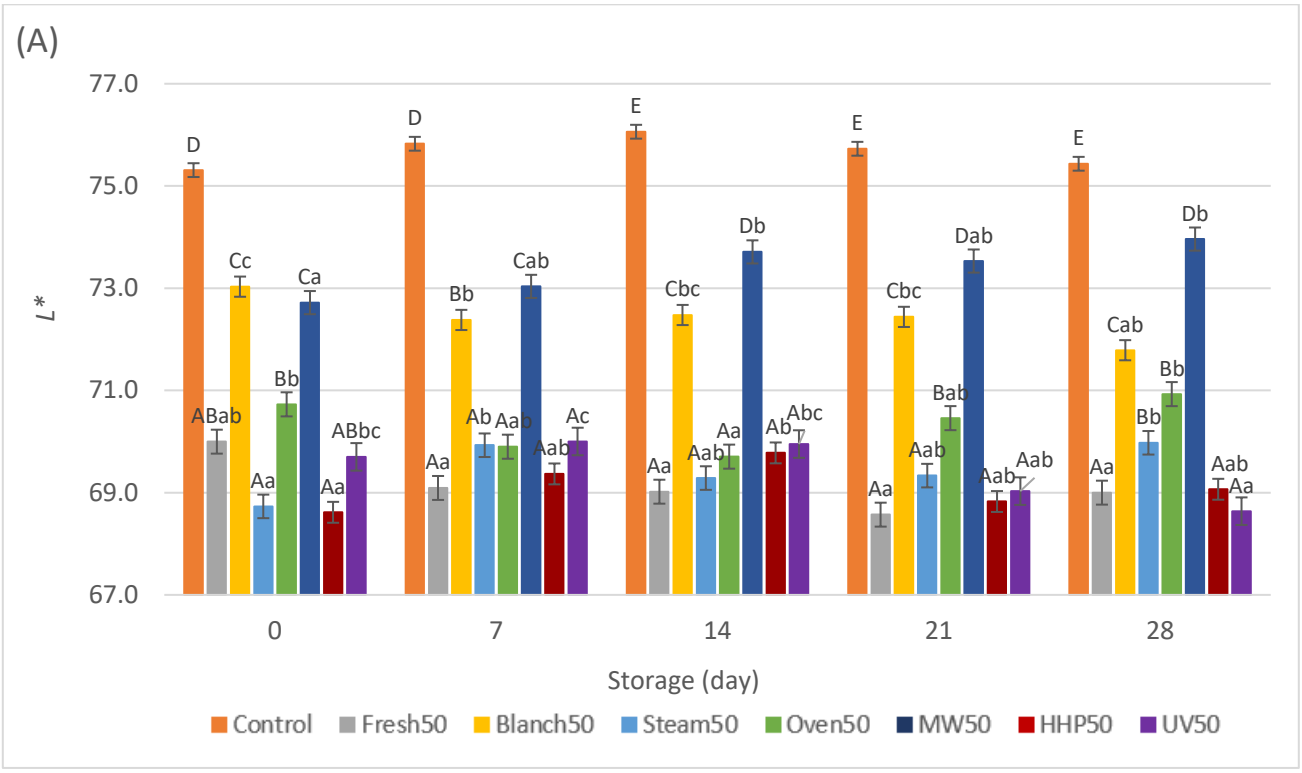


Figure 37. The mean ± standard deviation values of color parameters (A) *L**, (B) *a**, and (C) *b** of the sausage samples containing 25% meat replacement across the storage period. A–F: Distinct letters denote significant differences across sample groups. a–c: Distinct letters denote significant differences across storage days (Tukey's post hoc test, $p < 0.05$).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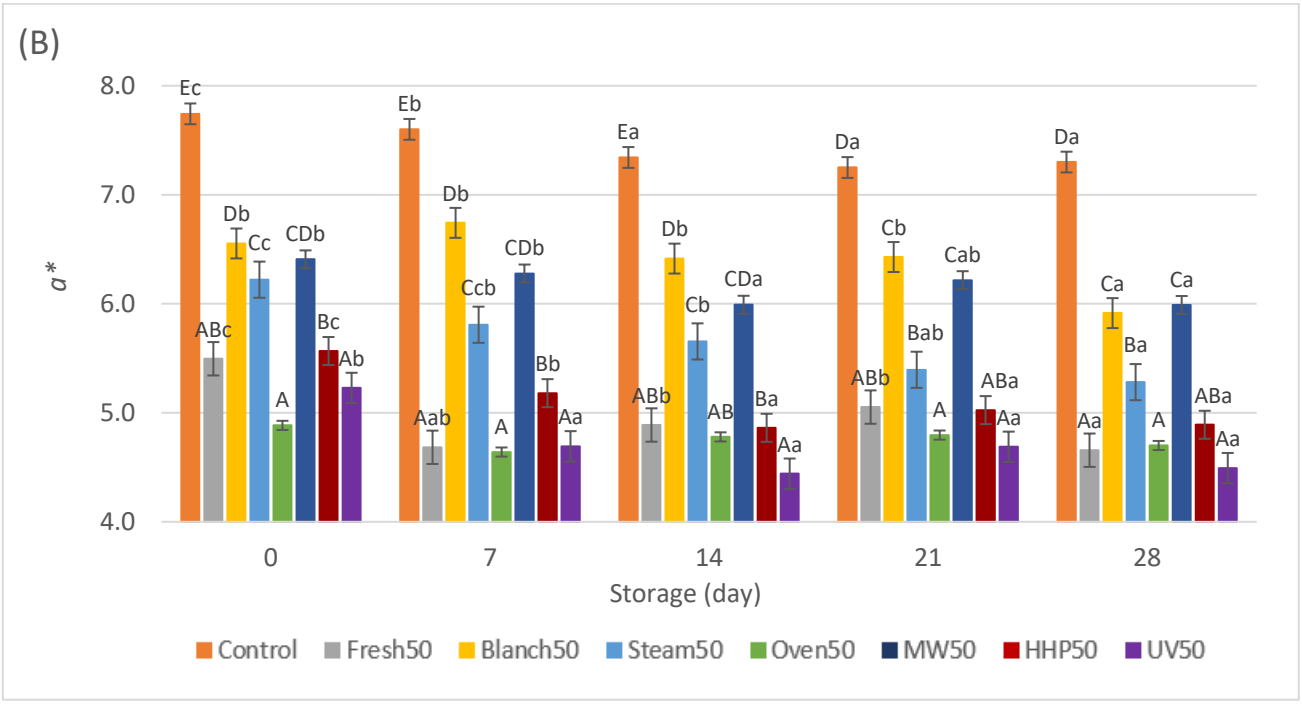
Oven50
MW50
HHP50
UV50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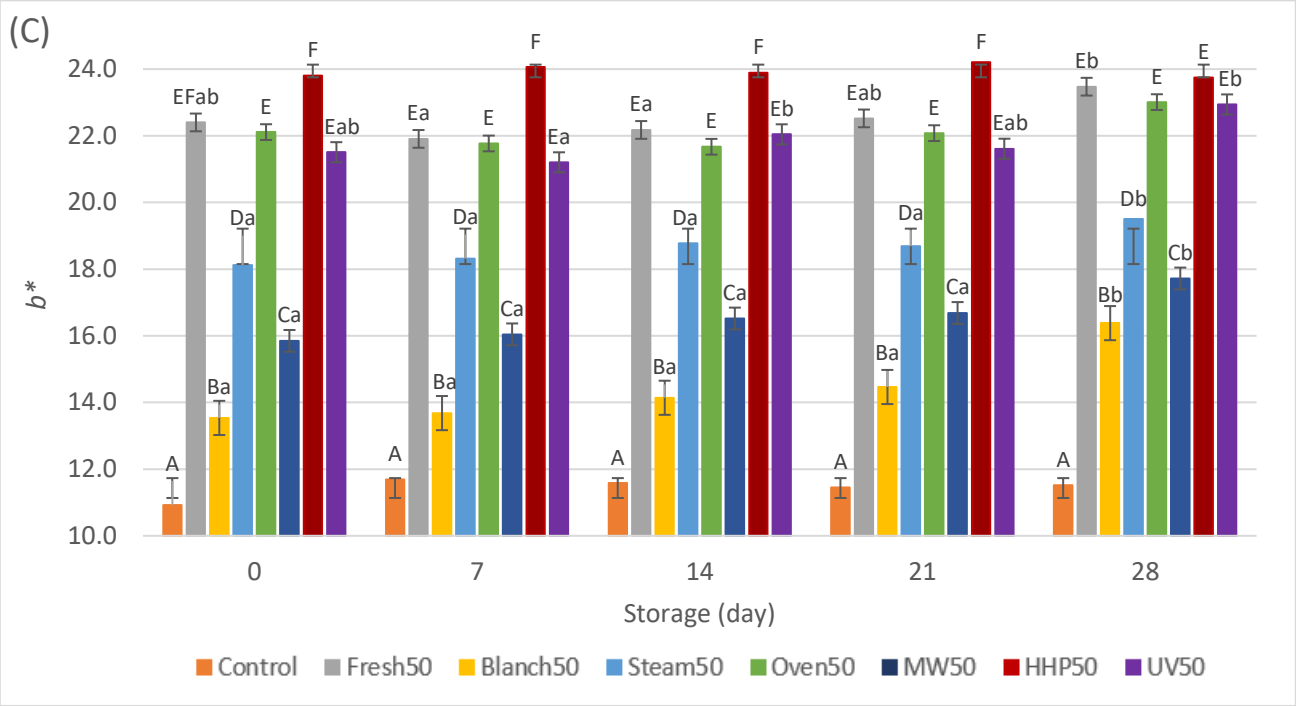


Figure 38. The mean ± standard deviation values of color parameters (A) *L**, (B) *a**, and (C) *b** of the sausage samples containing 50% meat replacement across the storage period. A–F: Distinct letters denote significant differences across sample groups· a–c: Distinct letters denote significant differences across storage days (Tukey's post hoc test, $p < 0.05$).

During the storage period, the Fresh25 samples consistently showed the lowest *L** values, spanning from 69.87 ± 1.08 to 71.25 ± 0.5, with the HHP25 and UV25 samples following in order. In contrast, the Steam25, Oven25, MW25, and Blanch25 samples exhibited significantly higher *L** values. Additionally, lightness increased in all 25% replacement samples, except for Fresh25, indicating a slight, progressive brightening over time, likely attributable to fat oxidation. A comparable order was noted in the 50% replacement samples, where MW50 and Blanch50 demonstrated higher *L** values relative to the other samples. In contrast, the Fresh50, Steam50, HHP50, and UV50 samples exhibited the lowest *L** values, with minor variations ranging from 68.63 ± 0.45 to 69.97 ± 0.68 during the storage period. It can be inferred that substituting meat with fermented oyster mushrooms led to a slight darkening of the sausage color, with the extent of this effect varying based on the pretreatment method used. The impact was less pronounced in sausage samples subjected to blanching or microwave pretreatments. Previous research has reported similar influences of blanching and microwave treatments on the color attributes of canned mushrooms, resulting in comparable enzymatic inactivation and structural modifications within the cells, which have minimal effects on the pigment composition and retention of the mushrooms, thereby producing similar color results (Baldwin et al., 1986). Also, the darkening observed with the HHP treatment in this research can be

linked to the activity of polyphenol oxidase at a pressure of 300 MPa. Among the polyphenol oxidase family, tyrosinase is a key enzyme that primarily catalyzes the browning process in *A. bisporus* and *P. ostreatus* due to its high concentration (Lyn et al., 2020). When polyphenol oxidase inactivation does not occur, the increased permeabilization of cell membranes resulting from phospholipid crystallization enables extracellular polyphenol oxidase to interact with phenolic compounds, resulting in enhanced browning with pressure treatment (Matser et al., 2000). An increasing trend in L^* and b^* values was observed in carambola puree as pressure levels rose from 200 MPa to 600 MPa and 800 MPa. This behavior is attributed to the enzymatic inactivation of polyphenol oxidase occurring under elevated pressure conditions (Yi et al., 2012; Zhang et al., 2021a). In a study on oyster mushrooms, increasing the UV-C exposure time beyond 30 minutes led to browning. This darkening effect was attributed to the activation of polyphenol oxidase by UV treatment, which oxidizes phenolic compounds into quinones. Subsequently, these quinones undergo polymerization, resulting in the formation of brown pigments and contributing to a darker coloration of the mushrooms (Desmukh et al., 2014). This same effect was noted despite the exposure time in this research being only 15 minutes. These results are consistent with findings from other researchers, who reported a reduction in sausage lightness from 75.59 ± 0.84 to 72.16 ± 0.63 when substituting chicken meat with fresh oyster mushrooms at levels of up to 45% in their samples (Yahya & Ting, 2020). Comparable results have also been reported when using *L. edodes* as a meat substitute in sausages, as well as with the incorporation of *A. bisporus* and *P. ostreatus* flour in frankfurter sausages (Cerón-Guevara et al., 2020; Wang et al., 2019a).

A notable decrease in a^* values was recorded for all sample groups throughout the storage period. The a^* values for the samples with 25% meat replacement varied between 5.90 and 7.89 throughout the storage period. The Blanch25 and MW25 samples demonstrated a^* values comparable to those of the Control sample (7.30–7.74), consistently higher than those of the other groups on all days assessed. The lowest a^* values were measured in the UV25 (6.09 ± 0.41) and Oven25 (6.25 ± 0.47) samples by the end of storage. A similar pattern emerged for the 50% replacement samples, where a^* values ranged from 4.49 to 6.74. In this instance, the Control sample was clearly distinct from the others, maintaining higher a^* values throughout the entire storage period ($p < 0.05$). Notably, substituting 25% of the meat with fermented oyster mushrooms did not lead to a reduction in the red color for the Blanch25 or MW25 samples. Moreover, studies investigating the effects of microwave blanching on frozen *A. bisporus* mushrooms showed that the color spectrum of microwave-treated mushrooms shifted more toward red and less toward yellow in comparison to those treated with sodium metabisulfite blanching (Bernaś & Jaworska, 2015). This phenomenon can be attributed to microwave

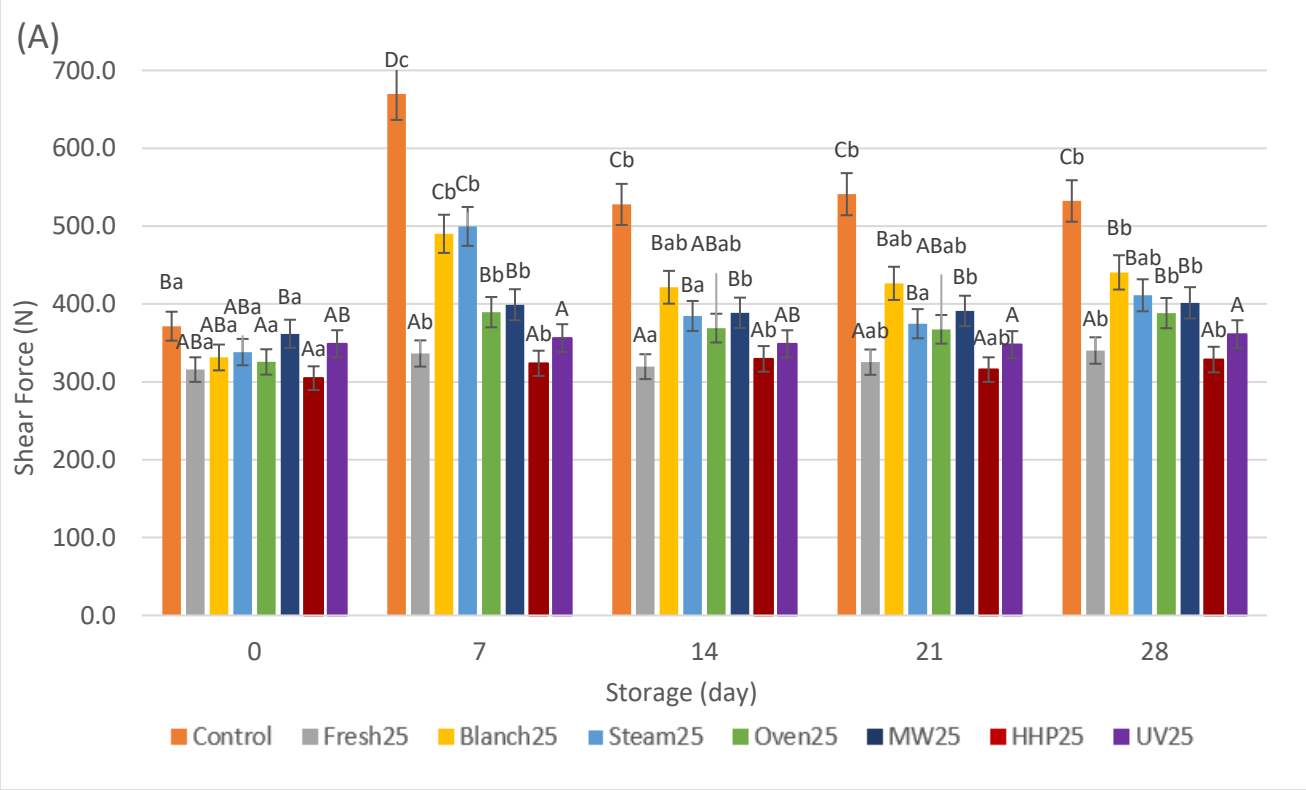
radiation penetrating the mushroom tissues, which influences the distribution and structure of pigments, potentially altering their absorption and reflection characteristics. Such changes can enhance the intensity of red hues while diminishing the yellow tones, leading to the observed color shift toward red. However, when the replacement ratio increased to 50%, all sample groups exhibited a decrease in red compared to the control sample, primarily due to the reduced meat content in the formulations. Similar reductions in a^* values were noted in frankfurter sausages with the addition of *A. bisporus* and *P. ostreatus* (Cerón-Guevara et al., 2020), as well as in goat meat nuggets containing *F. velutipes* mushrooms (Banerjee et al., 2020). In contrast, no significant differences were found in the a^* values of beef patties when button mushrooms were utilized as meat extenders, even at a substitution ratio of up to 50% (Wong et al., 2019).

During the storage period, samples with 25% meat replacement demonstrated a significant increase in b^* values ($p < 0.05$). The highest b^* values were observed in Fresh25 (16.77 ± 0.47) and HHP25 (16.60 ± 0.31) samples by the end of the storage period. In contrast, the lowest b^* values were found in Blanch25 (10.60 ± 0.53 to 12.40 ± 0.46) and Control (10.92 ± 0.60 to 11.51 ± 0.73) samples. A comparable pattern was noted in the 50% replacement samples, which exhibited higher b^* values (13.53 ± 0.44 to 24.21 ± 0.20) in relation to their 25% counterparts, while preserving a consistent order among the sample groups. Moreover, when the replacement ratio rose to 50%, all sample groups had a more yellow color relative to the control sample ($p < 0.05$) due to the natural coloration of the mushrooms. The highest b^* values were recorded in the HHP50 samples (23.75 ± 0.70 to 23.80 ± 0.39) across all assessment days. The elevated yellowness index noted with pressure treatment was linked to a rise in beta-carotene content in the carambola pure samples (Zhang et al., 2021a). The color findings align with those of another study, which indicated that incorporating *P. eryngii* after various treatments, as substitutes for pork back fat in sausages led to an increase in yellowness. However, the b^* values of the sausages did not show significant changes when the mushrooms were added in their raw state, but changed if boiled, deep fried, or fried (Wang et al., 2019b).

The resulting color characteristics are greatly influenced by the color of the ingredients in the formulation, the species involved, and the amounts used. Our research indicated that the pretreatments applied to mushrooms before fermentation led to notable differences in the color attributes of the sausage samples with meat replacements. The most negative impacts on sausage color were noted when the mushrooms were fresh, pretreated with HHP or UV light before fermentation. In contrast, the least detrimental effects were noted when the mushrooms underwent blanching or microwave pretreatment before fermentation (Boylu et al., 2024).

5.3.5. Texture evaluation of the sausage samples

The texture analysis of the sausage samples is presented in Figure 39 (A, B). Replacing meat with pretreated fermented mushrooms had a significant impact on sausage texture ($p < 0.05$). The control samples consistently exhibited the highest shear force values, from day 0 (371.50 ± 32.53 N) to the end of storage (532.33 ± 29.12 N), reflecting a greater hardness in the sausages. Within the 25% replacement samples, Blanch25, Steam25, Oven25, and MW25 demonstrated a firmer texture than Fresh25, HHP25, and UV25 ($p < 0.05$). Most samples, except for UV25, showed a significant increase in hardness values by day 7. By the end of storage, all these samples had shear force values that were significantly higher than their initial measurements ($p < 0.05$). A similar trend was observed in the 50% replacement samples, with Blanch50, Steam50, Oven50, and MW50 exhibiting a firmer texture than Fresh50, HHP50, and UV50. However, at the 50% replacement level, force values ranged from 18.56 ± 5.24 to 322.45 ± 37.36, while at the 25% replacement level, they spanned from 325.58 ± 14.37 to 499.67 ± 21.23 throughout storage. This indicates that increasing the proportion of fermented oyster mushrooms from 25% to 50% in the sausage formulations contributed to a softer texture. Additionally, all replacement samples, except UV, displayed a significant rise in force values by the end of the storage period compared to their initial measurements.



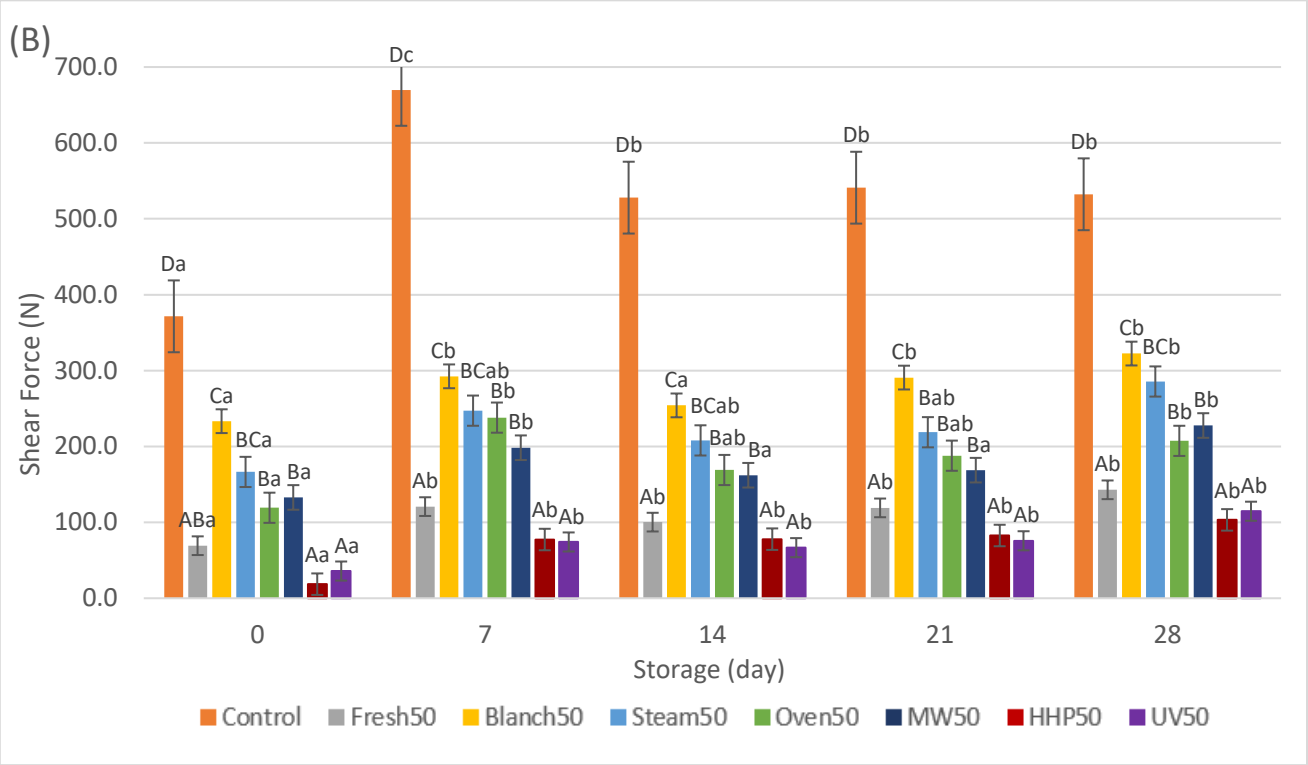


Figure 39. The mean ± standard deviation values of Shear force of the sausage samples during storage with 25% meat replacement (A) and 50% meat replacement (B). A–D; Distinct letters denote significant differences across sample groups. a–c: Distinct letters denote significant differences across storage days (Tukey's post hoc test, $p < 0.05$).

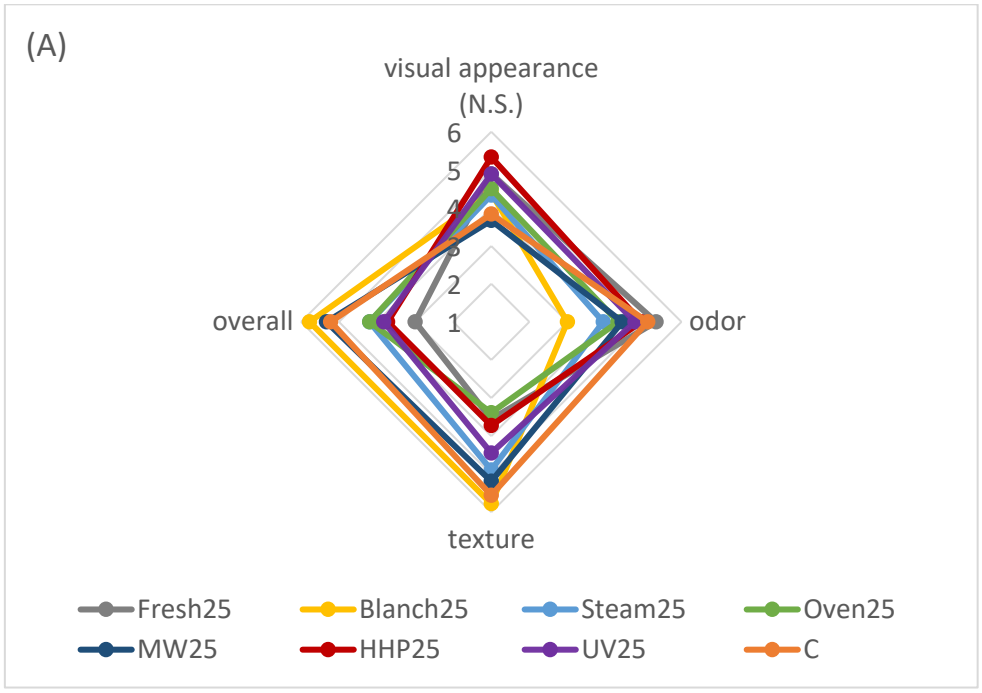
Comparable findings were observed in frankfurters containing *A. bisporus* and *P. ostreatus* mushrooms, where cold storage led to increased hardness due to purge loss and incorporating *P. ostreatus* contributed to softer sausage samples (Cerón-Guevara et al., 2020). In another study investigating emulsion-type sausages, substituting chicken breast meat with *A. bisporus* mushrooms at levels above 10% led to significantly reduced hardness, springiness, cohesiveness, and chewiness compared to control sausages. This outcome suggests that the softer texture of mushrooms, linked to their lower protein and higher moisture content relative to chicken breast, influences sausage hardness, which is closely associated with protein and water contents (Fu et al., 2023). Generally, incorporating various mushrooms into meat products tends to produce softer textures. This effect is attributed to dietary fibers in mushrooms that trap fluids and the lower concentration of solubilized muscle proteins, collectively resulting in a softer texture in the final muscle-based products (Das et al., 2021). A study on pork sausages incorporating *P. eryngii* with various treatments found that adding raw, boiled, and deep-fried mushrooms led to reduced sausage hardness, while sausages containing fried mushrooms displayed a firmer texture (Wang et al., 2019b).

In this research, all replacement samples consistently showed a softer texture than the control samples

across all examination days. Notably, the greatest texture changes occurred in sausages containing fresh fermented, UV-fermented, and HHP-fermented mushrooms at both 25% and 50% replacement levels. The pronounced softness in fresh fermented samples underscores the importance of pretreatment processes to maintain mushroom quality before fermentation (Jabłońska-Ryś et al., 2019). The softer texture of the HHP-pretreated samples is consistent with findings from a study on fresh *P. eryngii* treated at pressures of 100–300 MPa for 3–18 minutes. The study demonstrated that as pressure and holding time increased, PPO enzyme activity and elasticity initially dropped before rising again. In contrast, the *L** values and hardness of the samples steadily declined, while *b** values showed an increase (Castellanos-Reyes et al., 2021). In contrast to fruits and vegetables, that have sturdy cell walls for structural integrity, mushrooms mainly consist of glucan and chitin. Applying high pressure can disrupt their cell membranes, significantly redistributing water microscopically and gelatinizing cell biopolymers. Under pressure, cell proteins in mushrooms undergo denaturation, increasing membrane permeability. This increased permeability allows cellular solutes and water to escape, which consequently softens the cells (Kadam et al., 2015). The softer texture seen in UV-pretreated samples is consistent with findings from a study on UV-B pretreatment effects on mushrooms. The study noted that UV-B pretreatment lowers the fracture force in treated samples relative to controls. This decrease in fracture force is likely attributed to pretreatment, which accelerates drying and damages cell walls, thereby softening the samples' texture (Forouzanfar et al., 2020). Most research on UV light treatment in mushrooms emphasizes its effects on vitamin D levels, with few studies examining textural changes. On the other hand, pulsed light treatment was found to be significantly altering the texture of fresh-cut *Agaricus bisporus* mushrooms due to thermal damage from high energy doses (Oms-Oliu et al., 2010). White LED irradiation was observed to more effectively preserve textural qualities like firmness, chewiness, and resilience in shiitake mushrooms (Li et al., 2021). The differences in results may stem from variations in light type, light dosage, and mushroom species used in the studies.

5.3.6. Sensory evaluation of the sausage samples

For the interpretation of sensory evaluation results, a spiderweb diagram is provided in Figure 40 (A, B), illustrating the rankings for visual appearance, odor, texture, and overall acceptability across all formulations.



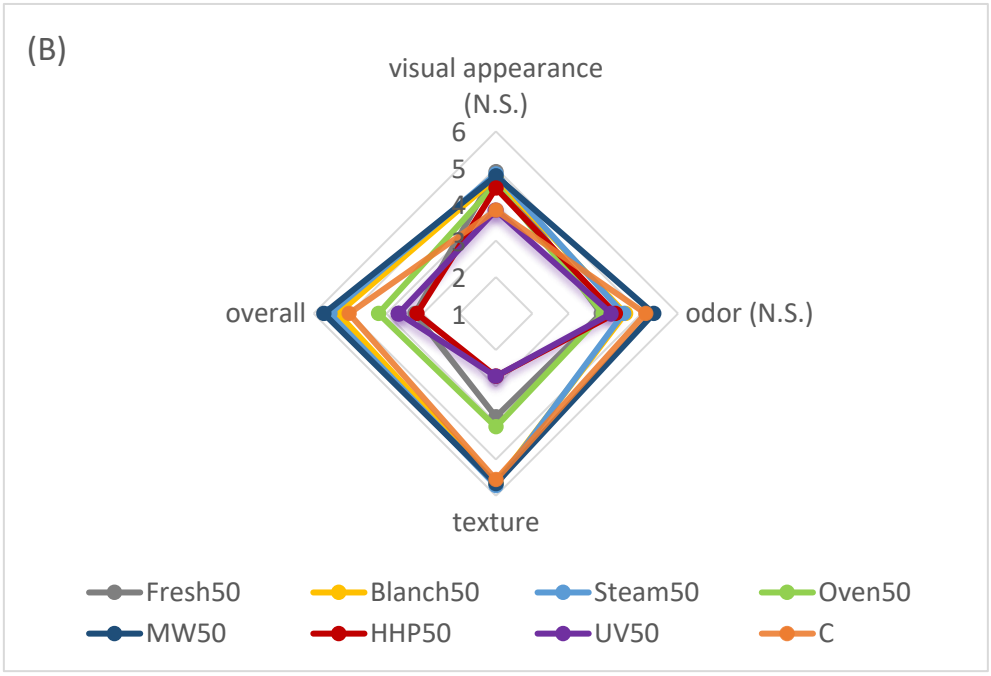


Figure 40. Spider web chart of the sensory evaluation of the sausage samples containing 25% meat replacement (A) and 50% meat replacement (B)

The panelists found no significant differences in the visual appearance of the samples for both the 50% and 25% replacement levels ($p > 0.05$), suggesting that the color changes identified through instrumental analysis were not noticeable to the panelists. In terms of texture, the blanched, microwave pretreated, and steamed samples achieved higher rankings, comparable to those of the control samples at both the 25% and 50% replacement ratios. Minor variations in the ranking order were observed

depending on the replacement ratio. This aligns with the texture analysis, which indicated that the fresh, HHP pretreated, and UV pretreated samples exhibited a softer texture compared to the other groups at both replacement levels. This soft characteristic was detected and ranked unfavorably by the panelists. Similar findings were reported in a study on sausages where *L. edodes* was used as a pork meat replacer. Authors indicated that the replacement did not cause significant changes in the appearance of the samples, however, increasing the mushroom ratio beyond 25% led to sausages with a softer texture, that panelists found unappealing (Wang et al., 2019a). Regarding odor, no significant differences were detected among samples with a 50% replacement. In contrast, for the 25% replacement samples, the fresh, HHP pretreated, and UV light pretreated samples received significantly higher rankings than the other sample groups ($p < 0.05$). This can be attributed to HHP treatment, which retains volatile aromatic compounds while minimizing degradation, and UV light, which promotes the formation of desirable aromas through photochemical reactions. In contrast, thermal methods may alter the volatile profile, potentially resulting in the loss of aromatic compounds or the development of off-flavors, adversely affecting the overall odor (Xie et al., 2023). The overall rankings revealed that blanched, microwave pretreated and steamed samples received similar rankings with the control samples at both replacement levels, with slight variations in ranking depending on the replacement ratio. Studies on frankfurters with porcini mushrooms (Novakovic et al., 2020) and on fermented sausages with shiitake mushrooms (Van Ba et al., 2016) reported no significant differences in acceptability scores among the treatments. Specifically, the blanched samples were ranked as the overall best for the 25% replacement level, despite having the least favorable odor ranking, while the microwave pretreated samples attained the best ranking for the 50% replacement. In both cases, the fresh and HHP pretreated samples were identified as the least liked samples. It is important to highlight that these samples were also ranked unfavorably for their texture, underscoring the significance of texture as a key characteristic influencing overall product acceptance. In another study on pork sausages with *P. eryngii*, the raw, boiled, and fried *P. eryngii* containing samples scored lower than the control in terms of overall acceptability, while the sausage containing deep-fried *P. eryngii* received the highest overall rating (Wang et al., 2019b). This study demonstrated that the inclusion of fermented oyster mushrooms in place of meat influenced the odor, texture, and overall sensory characteristics of the reformulated products, varying according to the pretreatment method applied.

5.3.7. NIR evaluation of the sausage samples

Figure 41 displays the NIR dataset, offering a visual comparison between sausage samples with meat replacements in relation to control sample.

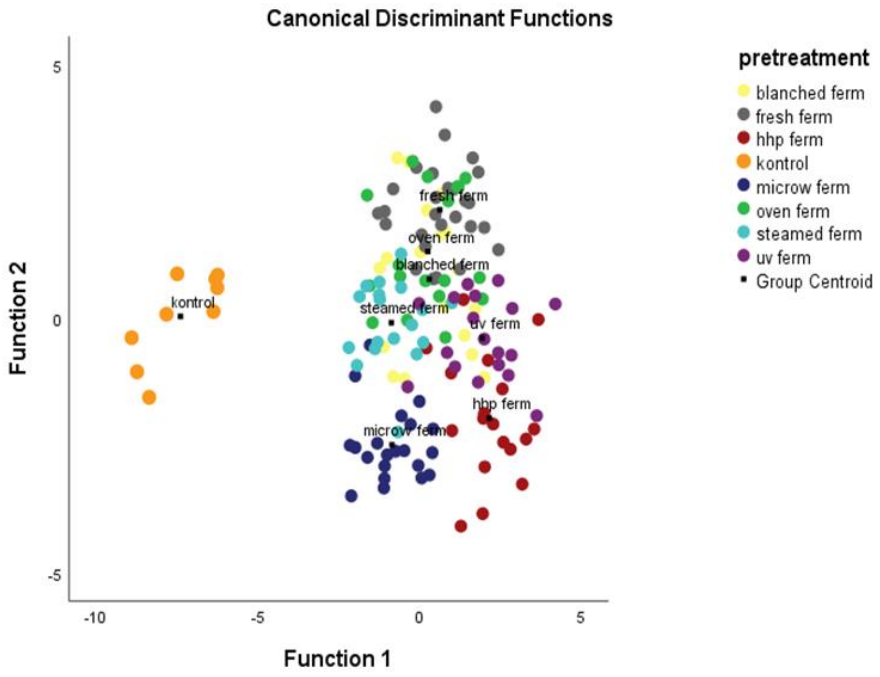


Figure 41. Canonical Discriminant Analysis plot (Function 1 vs. Function 2) illustrating the classification of sausage samples with meat replacements in relation to control sample based on NIR spectra

The original classification achieved an accuracy of 91.2%, demonstrating that the model correctly assigned most of the cases to their respective groups based on the discriminant functions derived from the dataset. After cross-validation, the accuracy decreased to 35.4%, revealing often instances where the model misclassified sausage samples with different pretreatments among one another, indicating a notable spectral similarity between the meat replaced sausage samples. The control sample remained clearly distinct, indicating its distinct characteristics compared to the mushroom-containing sausages. HHP-fermented and UV-fermented sausages are located farthest from the control along both Function 1 and Function 2. This suggests these pretreatments result in sausages that deviate the most from the characteristics of the control group.

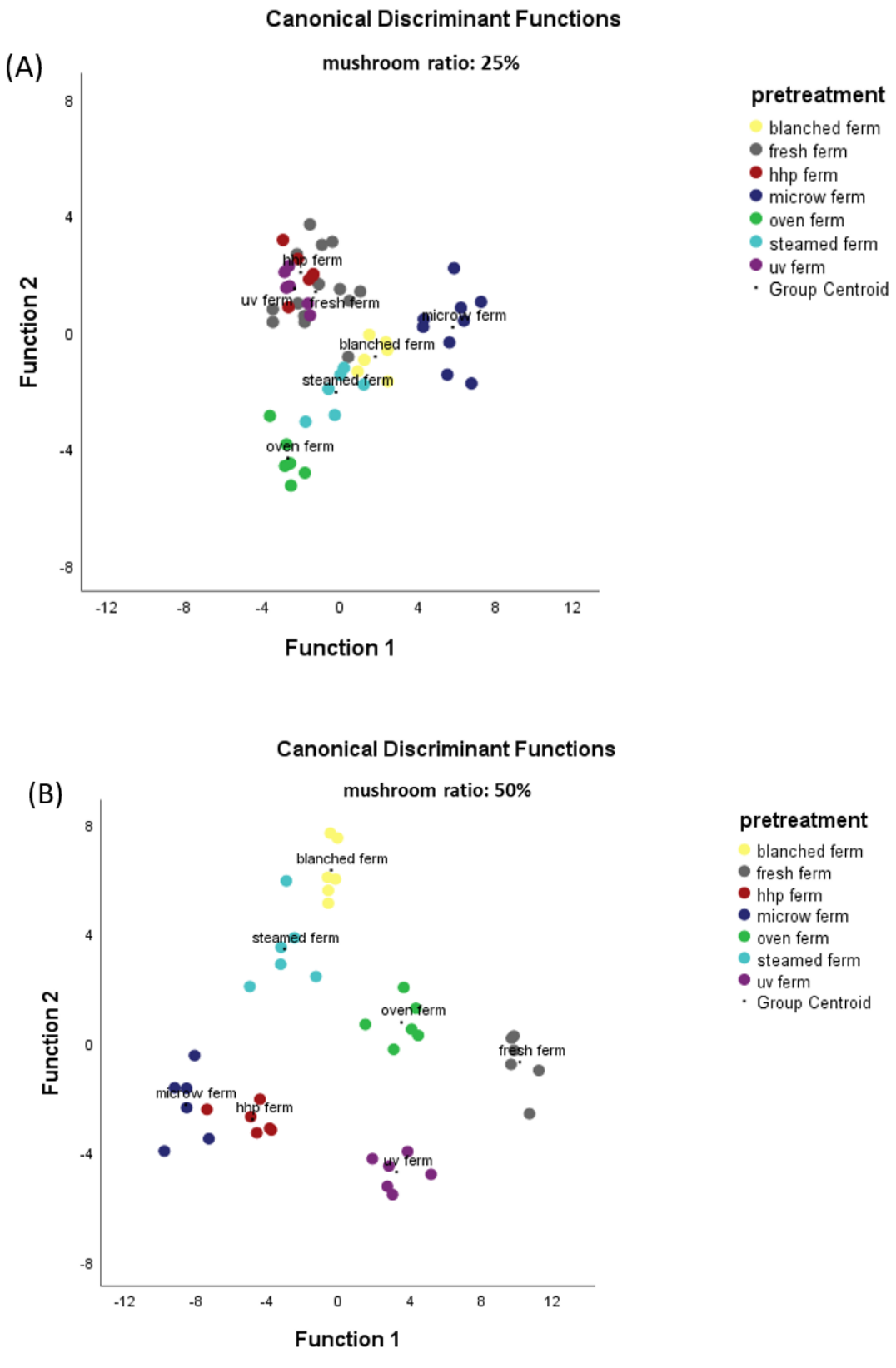


Figure 42. Canonical Discriminant Analysis plot (Function 1 vs. Function 2) illustrating the classification of sausage samples containing 25% meat replacement (A) and 50% meat replacement (B) based on NIR spectra

Figure 42 (A, B) displays the NIR dataset, illustrating the classification of sausage samples containing 25% meat replacement (A) and 50% meat replacement (B) based on NIR spectra. For the 25% replacement samples, the original classification achieved an accuracy of 96.3%, demonstrating that the model correctly assigned most of the cases to their respective groups based on the discriminant functions derived from the dataset. After cross-validation, the classification accuracy dropped to 38.9%, reflecting instances of misclassification among sausage samples with different pretreatments. This decrease indicates significant spectral overlap between certain pretreatment groups. Specifically, fresh-fermented, UV-fermented, and HHP-fermented sausage samples clustered together, suggesting shared characteristics. Blanched-fermented and steamed-fermented sausage samples were closely grouped, while microwave-fermented samples also showed some proximity to the blanched group, indicating partial spectral similarities. In contrast, oven-fermented sausage samples remained distinct, highlighting their unique spectral properties compared to the other groups.

For the 50% replacement samples, the original classification achieved an accuracy of 100%, demonstrating that the model correctly assigned all the cases to their respective groups based on the discriminant functions derived from the dataset. After cross-validation, the classification accuracy decreased to 42.9%, suggesting some spectral overlap between certain pretreatment groups. Specifically, blanched-fermented and steamed-fermented samples clustered closely together, as did microwave-fermented and HHP-fermented samples, pointing to shared spectral characteristics among these groups. Despite this, the increased separation of sample groups in the 50% replacement plot in comparison to the 25% replacement plot suggests that as the mushroom ratio increases, the effects of different pretreatments on the mushrooms become more pronounced, leading to greater spectral differentiation. This supports the idea that higher mushroom content makes the pretreatment-specific differences more visible in the NIR spectra. Since different pretreatments affect mushrooms in unique ways (e.g., structural, chemical, or compositional changes), these differences are likely amplified with a higher mushroom ratio, leading to more distinct clusters in the CDA plot.

6. CONCLUSIONS AND RECOMMENDATIONS

The complex challenges associated with animal meat production have prompted many consumers to reduce their intake of muscle-based foods. In response to these challenges, the Food and Agriculture Organization has highlighted the need for alternative protein sources to effectively feed the growing global population. This has spurred increased research into developing meat alternatives and enhancing the nutritional content of traditional meat products by incorporating sustainable, potentially health-promoting ingredients. In this context, mushrooms have emerged as a promising alternative, either as direct replacements or as innovative ingredients in various meat products. Mushrooms are typically used in meat products in the form of dried powder, fresh ground, or extracts. However, there is limited research on the application of emerging technologies—such as microwave, high hydrostatic pressure, and ultraviolet light treatments—and their comparative effects on mushroom quality in relation to traditional methods. Furthermore, the use of fermentation processes in the production of meat alternatives remains underexplored, despite its potential to enhance functionality, improve nutritional content, and create appealing aromas. This study aimed to investigate: the replacement of meat with fresh oyster mushrooms in sausage formulations, the effects of different pretreatments and fermentation on the quality characteristics of oyster mushrooms, and the incorporation of pretreated fermented mushrooms as meat substitutes in sausage formulations.

The preliminary study demonstrated the potential of fresh oyster mushrooms as a meat substitute in sausages. However, challenges emerged with higher substitution ratios, resulting in undesirable changes in texture and color, such as increased softness and a darker, less red appearance. Based on these findings, the research was continued with the 25% and 50% substitution ratios, as they maintained acceptable product quality. Additionally, the limitations observed and the short shelf life of fresh oyster mushrooms emphasized the need to optimize mushroom processing techniques. To build upon this foundation, the main study focused on replacing meat with pretreated fermented oyster mushrooms at 25% and 50% substitution ratios. Prior to their incorporation into sausage formulations, a comprehensive quality analysis was conducted on the oyster mushrooms following their pretreatments and fermentation.

The feasibility of integrating advanced technologies, such as HHP, microwave, and UV light, alongside traditional methods like steaming, oven cooking, and water blanching prior to mushroom fermentation, was investigated. Among these methods, microwave and steaming pretreatments showed the most promising results. They better preserved the color and texture of the samples, resulting in higher yield values after fermentation, suggesting they could be viable alternatives to

water blanching. Fermentation led to a decrease in pH and redness, while increasing yellowness in the pretreated mushroom samples. The total and essential amino acid content of the pretreated samples were significantly higher than that of fresh oyster mushrooms, with the microwave-pretreated samples exhibiting the most substantial improvement. All essential amino acids, except tryptophan, were detected in the mushroom samples. Essential amino acids, including arginine, comprised 35–44% of the total amino acids in the pretreated mushroom samples, indicating that oyster mushrooms maintain high protein quality regardless of the pretreatment applied.

Blanching and microwave pretreatments reduced the free amino acid content, whereas steaming, oven, HHP, and UV pretreatments increased it compared to fresh oyster mushrooms (34.07 mg/g). When pretreatments were combined with fermentation, a decrease in free amino acid content was observed only in the blanched samples. A total of 22 free amino acids were identified in the oyster mushroom samples. The PCA plot revealed distinct clustering: blanching and microwave pretreatments, which caused a decrease in free amino acid content, were clearly separated from oven, HHP, and UV pretreatments, which caused an increase. Fresh mushrooms displayed the most pronounced shift, indicating that fermentation alone (without pretreatments) significantly alters the free amino acid profile of oyster mushrooms, with levels increasing by 1.5 times.

Three types of biogenic amines—spermidine, cadaverine, and tyramine—were detected in the pretreated control samples, while fermentation additionally produced histamine, putrescine, spermine, and agmatine. The total biogenic amine content in fresh (0.17 mg/g) and pretreated mushroom samples (0.17–0.34 mg/g) remained below the overall recommended limits (0.75–0.9 mg/g food). However, due to the enzymatic activity of microorganisms during fermentation, significant quantities of biogenic amines were generated from free amino acids. Fresh fermented (1.8 mg/g), HHP fermented (1.5 mg/g), and UV fermented (5.05 mg/g) samples exceeded these limits, with UV pretreatment causing the highest increase in BA content (95.7%). Blanching (0.14 mg/g) and microwave (0.45 mg/g) pretreatments effectively minimized biogenic amine formation during fermentation. Among the most concerning biogenic amines, histamine was absent in all pretreated samples but present in all fermented samples except the blanched ones. Notably, HHP fermented samples contained histamine at levels of 1.23 mg/g, far exceeding the recommended limits (0.05–0.1 mg/g for food). Tyramine was absent in fermented samples pretreated with blanching or microwaving but was found in others, with the UV fermented sample exhibiting the highest tyramine level (4.1 mg/g), significantly surpassing the recommended limits (0.1–0.8 mg/g for food). Overall, both pretreatment methods and fermentation, as well as their combination, significantly influenced the amino acid, free amino acid,

and biogenic amine profiles of oyster mushrooms, highlighting considerable differences in the quality-enhancing efficiency of the applied pretreatments.

The incorporation of fermented oyster mushrooms as a meat substitute in sausage formulations affected all examined quality attributes of the samples during storage. Samples with mushroom substitution had higher moisture levels than the control (Control), with 50% substitution samples retaining more moisture than both the control and 25% substitution samples. Among the groups, sausages substituted with 25% UV pretreated fermented mushrooms and 50% UV pretreated fermented mushrooms had the lowest moisture content, while sausages substituted with 25% blanched fermented mushrooms and 50% blanched fermented mushrooms recorded the highest. Sausages with 50% mushroom replacement exhibited lower pH values than those with 25%, with a consistent decline from day 7 to day 28, stabilizing around pH 4.5, indicating that higher substitution ratios and specific pretreatments (oven, microwave, HHP, UV) foster an environment conducive to ongoing acid production during storage. The antioxidant properties of fermented oyster mushrooms varied depending on the pretreatment method applied. Despite higher initial TBARS values in microwave, HHP, and UV samples, a consistent decline was observed throughout storage. Notably, all sample groups maintained lipid oxidation levels below 0.50 mg MDA/kg, a threshold acceptable for processed meat products. In terms of color and texture, incorporating blanched, steamed, or microwave-pretreated fermented mushrooms resulted in reduced darkening and firmer textures compared to fresh, UV, or HHP-fermented mushrooms. Sensory tests revealed no significant visual differences between sausages with 50% and 25% mushroom substitution. Texture emerged as a key factor in panelists' evaluations, with blanched, microwave, and steamed samples receiving higher rankings, comparable to control samples at both substitution levels. For the 25% substitution level, fresh, HHP, and UV-pretreated samples received notably higher odor rankings, likely due to their elevated free amino acid content. NIR spectroscopy effectively differentiated pretreatments based on pH, moisture, texture, amino acid, free amino acid, and biogenic amine profiles. HHP and UV pretreatments, especially when followed by fermentation, produced distinct chemical and spectral profiles. In contrast, blanching, steaming, and microwave pretreatments caused minimal spectral changes, clustering together due to similar chemical characteristics. Overall, the 25% substitution level yielded more favorable results compared to the 50% level.

These findings indicate that fermented oyster mushrooms can serve as a feasible meat substitute in sausage production, though their effectiveness is constrained by the proportion of mushroom used and the pretreatment method applied. Fermented oyster mushrooms offer the potential to create meat-

substituted products with improved health benefits and sustainability, providing valuable insights for the food industry. Blanching and microwave pretreatments were identified as the most suitable methods, considering their positive influence on the mushrooms' physical, chemical, and nutritional properties, as well as their effects when incorporated into sausage formulations. Conversely, HHP and UV pretreatments were deemed unsuitable for mushroom pretreatment prior to fermentation, due to significant increases in biogenic amine content, which exceeded recommended safety limits, along with their undesirable impacts on color and texture.

Although pH measurements confirmed the successful fermentation of mushroom samples, further research is recommended to assess the microbiological safety of the sausage products incorporating these mushrooms. The combination of pretreatments and fermentation influenced the biogenic amine composition across sample groups in varied ways, highlighting the need for deeper analysis to establish underlying mechanisms and correlations. Additionally, investigating nitrosamine formation in the sausage products is worthwhile, as the presence of nitrites and acidic conditions could pose a risk of nitrosamine development.

7. NEW SCIENTIFIC RESULTS

1. I observed that microwave pretreatment of fresh oyster mushrooms (900 W, 2.45 GHz, 85 ºC for 3 minutes) resulted in the highest fermentation yield (95.46%) when compared to other thermal (Blanching, Steaming, Oven) and non-thermal (HHP, UV) pretreatment methods.
2. I observed that the best results for preserving mushroom color and texture stability were achieved with blanching (100 ºC for 3 minutes) and microwave pretreatment (900 W, 2.45 GHz, 85 ºC for 3 minutes) prior to fermentation.
3. I found that the total amino acid content and the essential amino acid content of oyster mushrooms significantly increased with the application of pretreatments ($p < 0.05$). Among all the samples, only the steamed fermented samples (100 ºC for 3 minutes, at 21-22 ºC for 8 days) exhibited a lower essential amino acid content (52.50 mg/g) compared to fresh oyster mushrooms (58.05 mg/g).
4. I found that the total biogenic amine content of fresh (0.17 mg/g) and pretreated mushroom samples (0.17-0.34 mg/g) were under the overall BA content limits (0.75-0.9 mg/g food). The HHP fermented (300 MPa, at 20 ºC for 3 min, at 21-22 ºC for 8 days) samples exceeded the recommended Histamine limits (0.05–0.1 mg/g for food) with 1.23 mg/g, while UV fermented (30 W, 312 nm at 20 ºC for 15 min, at 21-22 ºC for 8 days) samples exceeded the recommended Tyramine limits (0.1–0.8 mg/g for food) with 4.1 mg/g.
5. I observed that NIR spectroscopy successfully detected (100% original classification accuracy) and reflected chemical and spectral differences between thermal and non- thermal pretreatments. A lower cross-validation accuracy (42.9%) was observed due to the model misclassifying thermal pretreatments among each other, indicating spectral similarities.
6. I observed that during the sensory analysis, panelists did not detect significant differences in the visual appearance of the sausage samples between the 50% and 25% mushroom substitution levels. Sausage samples containing blanched (at 100 ºC for 3 min), microwave pretreated (at 900 W, 2.45 GHz, 85 ºC for 3 min) and steamed (at 100 ºC for 3 min) fermented mushrooms received higher overall rankings, comparable to the control samples at both replacement levels.

8. APPENDIXES

8.1. References

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Annex 1. The proteinogenic amino acid profile of pretreated mushroom samples

Amino acid (mg/g)	Fresh		Blanched		Steamed		Oven		Microwave		HHP		UV	
	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.
Asp	24.37	0.80	36.27	0.01	51.90	0.00	26.63	0.00	37.50	1.06	30.91	0.00	34.17	0.00
Thr*	6.70	0.12	8.34	0.00	8.91	0.00	6.04	0.01	11.07	0.48	9.27	0.01	7.94	0.00
Ser	1.47	0.03	2.13	0.01	3.16	0.00	1.93	0.00	3.15	0.10	2.31	0.00	2.55	0.01
Glu	29.16	0.00	43.48	0.00	41.12	0.00	39.44	0.00	52.45	0.39	45.76	0.00	51.48	0.00
Pro	5.19	0.07	4.84	0.02	7.47	0.01	6.46	0.00	8.38	0.06	7.65	0.00	5.90	0.00
Gly	8.36	0.21	10.43	0.00	11.21	0.00	13.66	0.01	14.46	0.10	15.70	0.00	11.41	0.00
Ala	12.53	0.22	12.26	0.00	12.35	0.00	14.67	0.00	22.36	0.24	12.45	0.00	10.51	0.01
Val*	7.22	0.34	11.94	0.00	9.71	0.00	8.53	0.01	13.47	0.36	14.80	0.01	13.82	0.00
Cys	1.43	0.03	1.95	0.01	3.17	0.00	2.13	0.00	2.33	0.02	2.32	0.00	2.49	0.00
Met*	1.98	0.09	3.68	0.00	2.83	0.01	3.68	0.00	3.29	0.11	3.83	0.00	2.30	0.00
Ile*	4.22	0.04	4.96	0.00	8.48	0.00	8.94	0.00	8.10	0.25	6.36	0.00	4.46	0.00
Leu*	11.83	0.15	18.58	0.00	15.26	0.00	10.41	0.01	21.15	0.13	11.22	0.00	18.04	0.00
Tyr	3.78	0.20	4.50	0.00	5.55	0.00	6.78	0.00	6.08	0.05	5.65	0.00	6.79	0.01
Phe*	5.81	0.00	7.20	0.00	7.94	0.00	7.47	0.01	10.43	0.24	7.69	0.00	8.82	0.00
Lys*	8.38	0.01	9.16	0.00	7.36	0.00	7.65	0.00	14.80	0.22	8.17	0.00	8.94	0.00
His*	3.66	0.19	4.28	0.00	5.56	0.00	4.50	0.00	6.57	0.06	31.50	0.01	4.74	0.01
Arg*	8.25	0.05	9.32	0.00	8.30	0.00	8.34	0.00	13.34	0.13	7.31	0.00	7.21	0.00
Total AA	144.34	1.82	193.32	0.00	210.29	0.02	177.26	0.2	248.91	1.55	222.90	0.00	201.57	0.02
Total EAA*	58.05	0.86	77.46	0.01	74.35	0.00	65.56	0.00	102.22	0.79	100.15	0.00	76.27	0.00

Annex 2. The proteinogenic amino acid profile of pretreated fermented mushroom samples

Amino acid (mg/g)	Fresh fermented		Blanched fermented		Steamed fermented		Oven fermented		Microwave fermented		HHP fermented		UV fermented	
	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.
Asp	53.79	0.00	28.45	0.00	22.22	0.48	27.72	0.00	27.33	0.01	36.27	0.00	32.89	0.74
Thr*	10.36	0.01	7.30	0.01	5.04	0.11	7.99	0.00	8.03	0.00	7.65	0.00	6.37	0.21
Ser	1.07	0.00	1.92	0.00	1.30	0.11	1.89	0.01	1.90	0.00	3.17	0.00	1.33	0.03
Glu	20.40	0.00	27.59	0.00	27.15	0.23	32.46	0.00	38.02	0.00	22.30	0.02	35.61	0.26
Pro	5.18	0.00	6.81	0.00	4.53	0.35	7.01	0.00	7.67	0.00	4.38	0.00	6.53	0.08
Gly	16.82	0.00	9.69	0.01	7.57	0.13	13.11	0.01	8.87	0.00	7.02	0.00	9.75	0.16
Ala	11.24	0.01	19.29	0.00	11.32	0.08	12.71	0.00	15.34	0.01	18.78	0.00	14.88	0.04
Val*	11.95	0.00	14.52	0.01	7.15	0.27	15.64	0.00	11.61	0.00	8.76	0.00	9.44	0.00
Cys	1.43	0.00	1.67	0.00	1.16	0.01	1.71	0.00	2.38	0.00	2.82	0.00	1.47	0.00
Met*	3.87	0.00	2.37	0.00	2.00	0.09	3.79	0.00	2.05	0.00	3.81	0.00	2.58	0.04
Ile*	6.20	0.00	9.12	0.02	4.26	0.22	7.21	0.00	5.26	0.00	8.01	0.01	5.58	0.10
Leu*	10.56	0.00	17.78	0.00	11.05	0.00	14.62	0.00	13.07	0.02	17.18	0.00	13.68	0.06
Tyr	5.90	0.00	6.13	0.00	3.09	0.10	4.08	0.00	5.33	0.00	5.42	0.00	3.70	0.16
Phe*	9.74	0.00	8.93	0.00	5.53	0.07	8.60	0.00	8.53	0.00	8.68	0.00	7.27	0.01
Lys*	7.54	0.00	8.83	0.00	7.83	0.13	8.56	0.02	8.76	0.01	7.67	0.01	9.72	0.07
His*	3.94	0.00	4.80	0.00	2.89	0.02	3.74	0.00	6.76	0.00	4.81	0.00	4.32	0.02
Arg*	6.97	0.01	9.16	0.00	6.75	0.10	8.37	0.00	9.16	0.00	6.77	0.00	8.89	0.11
Total AA	186.96	0.01	184.36	0.02	130.86	0.85	179.21	0.01	180.07	0.00	173.50	0.00	174.02	0.35
Total EAA*	71.13	0.00	82.81	0.01	52.50	0.31	78.52	0.00	73.23	0.00	73.34	0.00	67.87	0.40

Annex 3. The free amino acid profile of pretreated mushroom samples

Free amino acid (mg/g)	Fresh		Blanched		Steamed		Oven		Microwave		HHP		UV	
	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.
Asp	1,36	0,02	1,43	0,02	2,77	0,00	1,60	0,01	1,52	0,19	1,79	0,09	1,48	0,03
Thr	0,71	0,00	0,60	0,01	0,77	0,03	0,70	0,02	0,53	0,04	0,87	0,01	1,00	0,05
Ser	1,73	0,27	1,39	0,02	1,50	0,22	2,66	0,04	1,13	0,31	2,46	0,36	2,82	0,07
Asn	3,64	0,04	2,10	0,08	2,69	0,41	8,06	0,27	2,60	0,09	6,56	0,14	6,04	0,16
Glu	3,62	0,02	3,11	0,11	5,14	0,12	4,22	0,17	4,64	0,10	4,44	0,11	7,68	0,22
Gln	2,53	0,09	2,16	0,08	5,89	0,00	6,59	0,08	3,59	0,12	4,39	0,14	3,46	0,12
Pro	0,62	0,03	0,57	0,05	0,54	0,05	0,54	0,01	0,61	0,03	0,71	0,00	0,59	0,04
Gly	0,94	0,05	0,50	0,02	0,71	0,01	0,97	0,05	0,56	0,07	1,20	0,24	1,60	0,06
Ala	3,80	0,15	1,95	0,13	2,63	0,05	5,43	0,40	3,21	0,31	6,84	0,22	6,67	0,11
Val	1,35	0,24	1,14	0,20	1,28	0,01	2,29	0,02	0,95	0,21	2,51	0,19	2,26	0,08
Cys	0,34	0,07	0,30	0,02	0,44	0,00	0,48	0,04	0,58	0,05	0,50	0,05	0,73	0,05
Met	0,74	0,03	0,23	0,02	0,34	0,00	0,89	0,03	0,19	0,05	1,29	0,16	1,06	0,08
Cysta	0,62	0,07	0,41	0,01	1,08	0,00	1,19	0,05	0,84	0,19	1,04	0,10	1,36	0,06
Ile	1,03	0,17	1,01	0,02	0,67	0,00	1,30	0,01	0,55	0,09	1,59	0,11	1,31	0,02
Leu	2,62	0,03	1,48	0,08	1,67	0,00	3,58	0,06	1,42	0,21	5,23	0,45	3,70	0,02
Tyr	1,52	0,09	0,89	0,02	1,24	0,07	2,37	0,02	0,92	0,13	2,79	0,28	2,48	0,05
Phe	1,30	0,04	1,32	0,05	1,09	0,08	1,98	0,04	0,89	0,11	2,83	0,21	2,14	0,04
Gaba	1,62	0,04	1,33	0,03	1,23	0,02	1,73	0,03	1,27	0,04	1,49	0,01	1,31	0,02
Orn	0,51	0,12	0,39	0,01	0,85	0,00	1,17	0,09	0,83	0,12	0,67	0,06	1,20	0,08
Lys	1,33	0,02	1,24	0,04	1,93	0,00	3,16	0,08	0,81	0,11	2,72	0,09	1,98	0,04
His	0,77	0,10	0,80	0,01	0,71	0,00	1,66	0,01	0,47	0,09	1,27	0,10	1,61	0,06
Arg	1,38	0,30	1,61	0,22	1,65	0,03	2,63	0,03	0,89	0,18	2,46	0,26	2,07	0,08
Total FAA	34,07	0,82	25,95	0,22	36,80	0,48	55,17	0,39	28,98	2,58	55,68	3,16	54,54	0,58

Annex 4. The free amino acid profile of pretreated fermented mushroom samples

Free amino acid (mg/g)	Fresh fermented		Blanched fermented		Steamed fermented		Oven fermented		Microwave fermented		HHP fermented		UV fermented	
	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.
Asp	2,12	0,20	2,19	0,04	2,25	0,05	3,23	0,15	1,79	0,04	1,56	0,10	3,19	0,06
Thr	1,71	0,08	1,10	0,11	1,27	0,02	2,00	0,02	0,48	0,07	2,00	0,02	1,52	0,03
Ser	3,74	0,06	1,08	0,11	1,09	0,04	2,05	0,07	1,00	0,09	2,63	0,22	2,61	0,05
Asn	2,14	0,04	1,69	0,01	1,72	0,02	2,34	0,16	1,16	0,10	4,32	0,19	3,04	0,06
Glu	6,40	0,07	1,20	0,01	1,27	0,06	7,01	0,03	4,35	0,37	7,00	0,02	2,18	0,12
Gln	2,37	0,05	1,41	0,01	1,27	0,08	1,38	0,03	1,40	0,12	1,68	0,02	1,55	0,04
Pro	0,98	0,05	0,85	0,04	0,68	0,12	0,75	0,01	0,82	0,07	0,80	0,07	0,86	0,06
Gly	2,28	0,02	1,02	0,05	0,83	0,05	2,00	0,02	0,99	0,08	2,01	0,01	2,11	0,06
Ala	6,57	0,04	1,24	0,06	2,75	0,01	5,13	0,23	3,49	0,23	5,16	0,18	6,13	0,19
Val	3,20	0,03	1,69	0,04	1,42	0,01	3,31	0,05	1,59	0,07	3,22	0,11	3,17	0,06
Cys	0,47	0,03	0,40	0,01	0,33	0,01	0,29	0,02	0,53	0,01	0,26	0,05	0,39	0,02
Met	1,11	0,03	0,33	0,02	0,34	0,01	0,80	0,00	0,45	0,01	1,01	0,04	1,11	0,05
Cysta	0,80	0,00	0,79	0,02	0,76	0,07	0,45	0,01	0,72	0,03	0,48	0,02	0,79	0,02
Ile	2,10	0,02	1,73	0,04	0,68	0,03	2,20	0,07	0,72	0,17	2,20	0,01	2,12	0,10
Leu	5,36	0,33	1,87	0,04	1,69	0,06	5,35	0,08	2,32	0,10	5,01	0,04	5,57	0,16
Tyr	1,43	0,09	0,80	0,02	0,85	0,00	1,29	0,14	0,66	0,06	2,63	0,05	0,87	0,05
Phe	2,96	0,06	1,00	0,02	1,00	0,04	2,06	0,10	1,26	0,04	2,46	0,08	2,57	0,14
Gaba	1,11	0,17	1,12	0,06	4,85	0,09	1,68	0,04	2,29	0,18	1,24	0,03	4,26	0,14
Orn	0,59	0,02	0,70	0,00	1,42	0,16	1,50	0,03	1,86	0,11	0,89	0,02	0,65	0,05
Lys	3,52	0,08	1,34	0,27	2,09	0,13	1,30	0,04	2,60	0,20	2,15	0,06	2,74	0,14
His	1,59	0,03	1,73	0,04	1,10	0,08	0,78	0,09	0,99	0,07	1,39	0,10	1,39	0,01
Arg	1,11	0,03	0,96	0,09	0,98	0,06	1,10	0,00	1,19	0,07	1,04	0,01	0,84	0,17
Total FAA	53,65	0,69	26,24	0,56	30,64	0,93	48,00	0,41	32,68	1,67	51,17	0,53	49,67	0,09

Annex 5. The biogenic amine profile of pretreated mushroom samples

Biogenic amine (mg/g)	Fresh		Blanched		Steamed		Oven		Microwave		HHP		UV	
	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.
Him	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tym	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,04	0,01
Put	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cad	0,02	0,00	n.d.	n.d.	0,01	0,00	n.d.	n.d.	n.d.	n.d.	0,04	0,01	n.d.	n.d.
Spd	0,15	0,00	0,24	0,02	0,26	0,01	0,31	0,08	0,25	0,07	0,26	0,01	0,30	0,01
Agm	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Spm	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total BA	0,17	0,01	0,24	0,02	0,26	0,00	0,31	0,08	0,25	0,07	0,30	0,02	0,34	0,02

Annex 6. The biogenic amine profile of pretreated fermented mushroom samples

Biogenic amine (mg/g)	Fresh fermented		Blanched fermented		Steamed fermented		Oven fermented		Microwave fermented		HHP fermented		UV fermented	
	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.	Mean	±St.dv.
Him	0,15	0,02	n.d.	n.d.	0,46	0,07	0,44	0,00	0,24	0,03	1,23	0,12	0,50	0,07
Tym	0,76	0,19	n.d.	n.d.	0,08	0,01	0,11	0,00	n.d.	n.d.	0,10	0,02	4,10	0,44
Put	0,27	0,01	n.d.	n.d.	0,10	0,05	0,15	0,01	0,03	0,00	0,01	0,00	0,26	0,03
Cad	0,08	0,03	0,02	0,00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,15	0,00	0,03	0,00
Spd	0,16	0,01	0,12	0,01	0,17	0,03	0,21	0,01	0,18	0,01	n.d.	n.d.	0,17	0,03
Agm	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,08	0,01	n.d.	n.d.
Spm	0,48	0,10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total BA	1,90	0,06	0,14	0,01	0,81	0,05	0,91	0,02	0,45	0,02	1,56	0,15	5,05	0,57

Annex 7. Correlations

Correlations

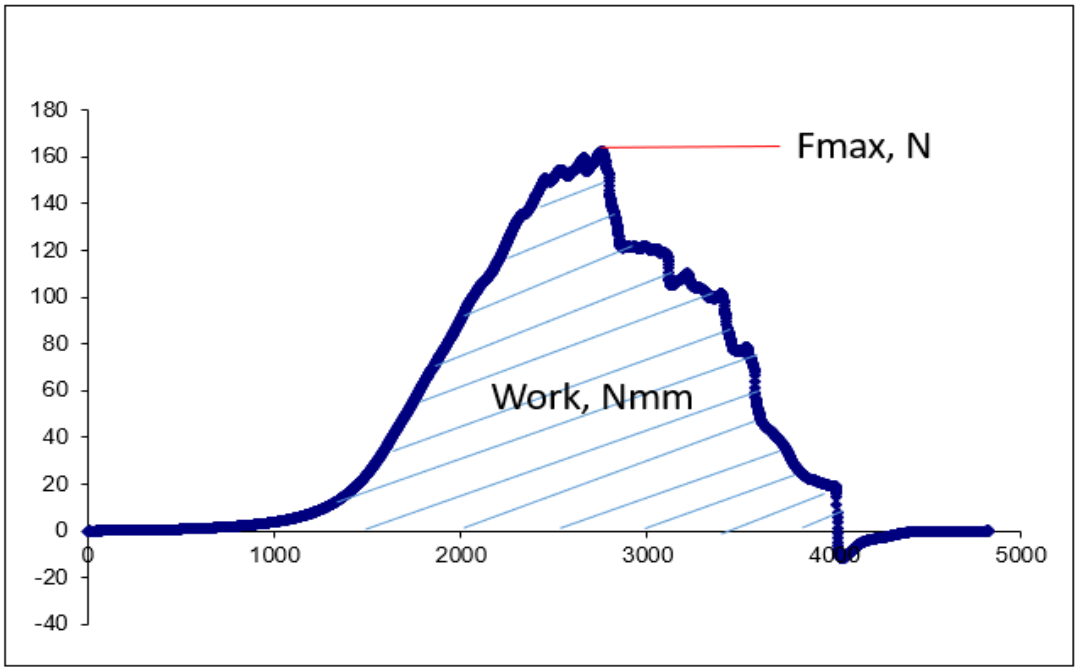
fermentation			L	a	b	Force	work	dm	Ph
Not fermented	L	Pearson Correlation	1	-,195**	-,185*	,286**	,449**	-,131	-,288**
		Sig. (2-tailed)		,007	,011	,000	,000	,073	,000
		N	189	189	189	189	189	189	189
	a	Pearson Correlation	-,195**	1	-,087	,223**	,280**	-,151*	,209**
		Sig. (2-tailed)	,007		,235	,002	,000	,038	,004
		N	189	189	189	189	189	189	189
	b	Pearson Correlation	-,185*	-,087	1	,079	-,273**	,365**	-,010
		Sig. (2-tailed)	,011	,235		,279	,000	,000	,889
		N	189	189	189	189	189	189	189
	Force	Pearson Correlation	,286**	,223**	,079	1	,813**	,307**	-,328**
		Sig. (2-tailed)	,000	,002	,279		,000	,000	,000
		N	189	189	189	189	189	189	189
	work	Pearson Correlation	,449**	,280**	-,273**	,813**	1	,040	-,437**
		Sig. (2-tailed)	,000	,000	,000	,000		,587	,000
		N	189	189	189	189	189	189	189
	dm	Pearson Correlation	-,131	-,151*	,365**	,307**	,040	1	-,042
		Sig. (2-tailed)	,073	,038	,000	,000	,587		,569
		N	189	189	189	189	189	189	189
	Ph	Pearson Correlation	-,288**	,209**	-,010	-,328**	-,437**	-,042	1
		Sig. (2-tailed)	,000	,004	,889	,000	,000	,569	
		N	189	189	189	189	189	189	189
Fermented	L	Pearson Correlation	1	-,371**	,031	,344**	,367**	,212**	,147*
		Sig. (2-tailed)		,000	,667	,000	,000	,003	,044
		N	189	189	189	189	189	189	189
	a	Pearson Correlation	-,371**	1	-,008	,087	,039	-,054	,082
		Sig. (2-tailed)	,000		,916	,236	,594	,462	,263
		N	189	189	189	189	189	189	189
	b	Pearson Correlation	,031	-,008	1	-,534**	-,467**	-,277**	-,169*
		Sig. (2-tailed)	,667	,916		,000	,000	,000	,020
		N	189	189	189	189	189	189	189

	Force	Pearson Correlation	,344**	,087	-,534**	1	,969**	,799**	,617**
		Sig. (2-tailed)	,000	,236	,000		,000	,000	,000
		N	189	189	189	189	189	189	189
	work	Pearson Correlation	,367**	,039	-,467**	,969**	1	,827**	,643**
		Sig. (2-tailed)	,000	,594	,000	,000		,000	,000
		N	189	189	189	189	189	189	189
	dm	Pearson Correlation	,212**	-,054	-,277**	,799**	,827**	1	,686**
		Sig. (2-tailed)	,003	,462	,000	,000	,000		,000
		N	189	189	189	189	189	189	189
	Ph	Pearson Correlation	,147*	,082	-,169*	,617**	,643**	,686**	1
		Sig. (2-tailed)	,044	,263	,020	,000	,000	,000	
		N	189	189	189	189	189	189	189

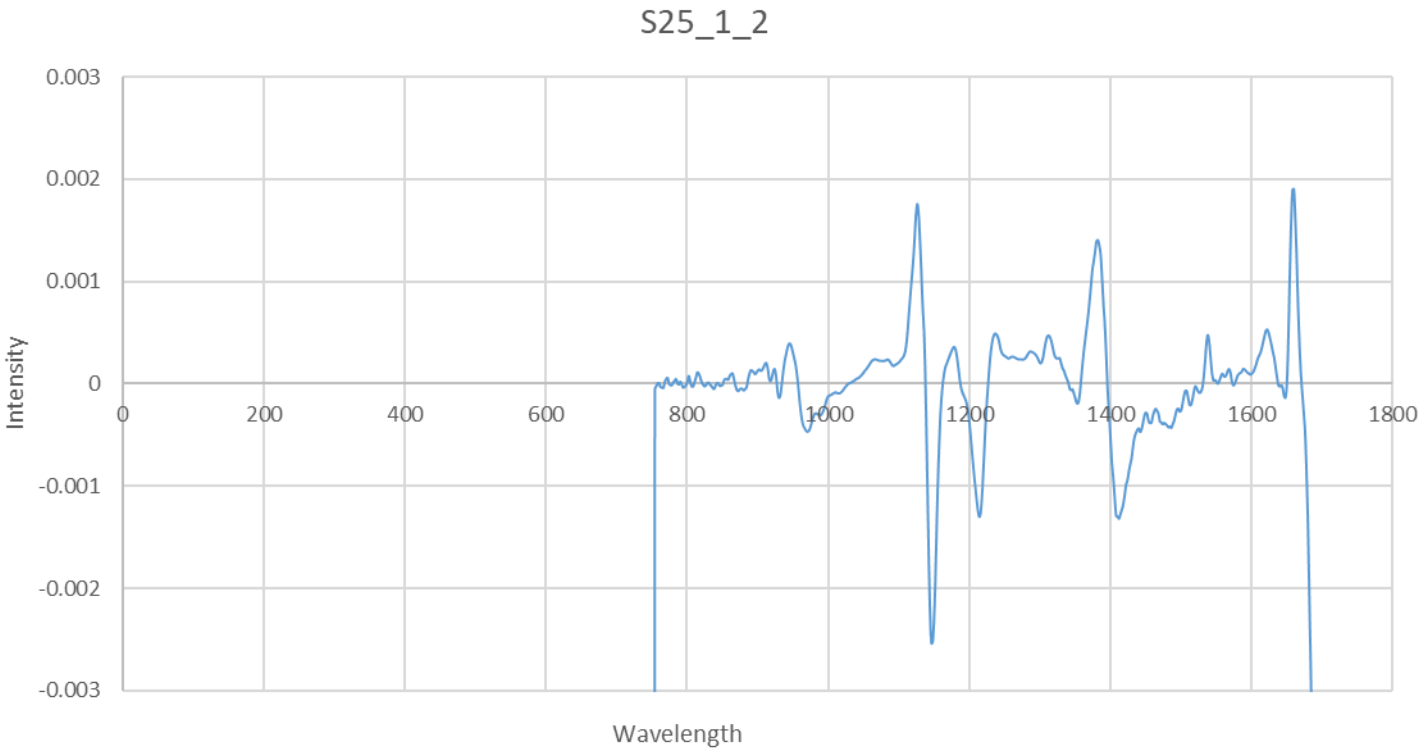
**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Annex 8. Texture measurement curve of Steam50 sample



Annex 9. NIR spectra (secondary weight) of Steam25 sample



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