# MSc. Thesis

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**2024**

Hungarian University of Agriculture and Life Sciences

Institute of Food Science and Technology

# Oyster mushroom- a possible meat replacement in sausage and burger patties

**MSc. THESIS**

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**Budapest, 2024**

**Hungarian university of agriculture and life sciences**

**Specialization name:** Food technology and product development

**Place of thesis preparation:** Department of Livestock and Food Preservation Technology

**Student:** Batoul Khalil

**Thesis title:** Oyster mushroom- a possible meat replacement in sausage and burger patties

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**Date of issuing the thesis:** 29.04.2024



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Year of publication: 2024

Name of the Department: Department of Livestock and Food Preservation Technology

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## Table of Contents

# 1. Introduction

In several countries, the concept of plant-based meat was inspired by the rising health consciousness among individuals and sustainable food sources. The amount of vegetable proteins in food items has increased over time because of illnesses associated with animals, better eating habits, a growing need for healthful and religious food, as well as economic factors. More environmental resources are needed for a diet high in meat than for a diet rich in plant-based proteins. It can be quite difficult to produce and innovate a new food product that meets market demand, though. Meat analog, commonly referred to as imitation meat, has long been used in the food industry; it was first introduced in the beginning of the 1960s (Ismail et al., 2020). If more vital fatty acids, minerals, vitamins, and protein could be obtained from both plant- and animal-based sources, the well-being of a large number of the world's poorest people who live in developing nations would improve (Machovina et al., 2015). The mushroom industry is a growing global sector that produces more than two million tons of mushrooms annually worldwide. The three main types of mushrooms that are grown are Pleurotus ostreatus, Lentinus edodes, and Agaricus bisporus. Of all the edible mushrooms that are grown worldwide, oyster mushrooms are in second place (Gogavekar et al., 2014). Because of their high biological value and high nutritional content, edible grown mushrooms have been shown to have functional qualities that make them suitable for inclusion in cooked smoked sausages. This helps to improve consumer attributes, lower calorie content, and create valuable products (Stepanova et al., 2019). While the use of different protein texturizing processes has been thoroughly studied over time in an effort to create meat substitutes or analogs (Zahari et al., 2022), there is still a crucial need for comprehensive scientific investigation to fully grasp the implications of incorporating oyster mushrooms into meat products on product quality and consumer acceptance. This study seeks to address this gap by examining the effects of replacing pork meat in sausages with fresh oyster mushrooms and exploring the influence of different pre-treatments and mushroom percentage on key quality attributes. The disposition of this research within the scientific landscape lies at the intersection of food science, nutrition, and sustainability studies. This study adds significant knowledge to the continuing debate about alternative protein sources and their function in sustainable food systems by clarifying the impact of substituting meat for mushroom on the color, texture, and weight loss of sausages and by examining the effect of storage conditions.

## 2. Aim of study

The primary objective of this study is to explore how substituting pork meat with fresh oyster mushrooms in sausages, along with varying pre-treatments and mushroom percentage (ranging from 10% to 50%), affects the color, weight loss, and texture of the sausages. Additionally, it seeks to examine how storage conditions influence the quality attributes of mushroom-infused sausages. The thermal treatments applied in this investigation comprise blanching in water and steaming of oyster mushrooms.

# 3. Literature Review

## 3.1. Oyster Mushroom: Possible Meat Substitute

Fungi known as mushrooms exhibit rapid growth, producing edible fruit bodies rich in essential nutrients, embraced as both a culinary delight and a dietary necessity worldwide. Their cultivation and consumption have surged steadily across numerous nations due to their nutritional benefits and medicinal value. Particularly in developing countries, small and medium-scale enterprises play a pivotal role in mushroom production. Among the myriad species cultivated worldwide, button/white, shiitake, and oyster mushrooms are the most dominant (Nketia et al., 2020). Mushrooms are the meat of the plant world and can be prepared in a wide range of tasty ways. They are frequently used as a flavoring substitute for meat in recipes like stews (Nongthombam et al., 2021). One of the foods which is regarded as important due to their prominent contributions to health and nutrition is mushrooms. Oyster mushrooms are the most prevalent species within the Pleurotus genus. The tropical and temperate regions contain over 200 saprophytic species from the genus Pleurotus. These mushrooms are widely considered to be the most popular globally and have the third tank in the production of edible mushrooms, following the genera Agaricus and Lentinula. Oyster mushrooms are rich in vitamin B complex, vitamin C and mineral salts which serve as a valuable source of essential nutrients. Pleurotus mushrooms are nutritionally dense containing various bioactive compounds such as steroids, terpenoids, alkaloids, phenols, nucleotides, and lectins. Oyster mushrooms are gaining popularity as promoters of health and environmental scavenger in comparison to other medicinal mushrooms. This drives to increased research and development activities which focus on oyster mushrooms (Kumar, 2018). Pleurotus ostreatus bears an aroma that is somewhat similar to that of both anise and almonds which have a bitter and sweet scent due to benzaldehyde. It's considered a luxury treat in many regions, particularly in Asia. However, it is used in Slovakia and the Czech Republic as an alternative to beef (Piska et al., 2016).

## 3.2. Pleurotus Ostreatus Morphology

The structure of the mushroom body is the purpose behind its scientific and typical name. Pleurotus comes from the Latin name to sideways which signifies the horizontal extension of the stem in relation to the cap, whereas the word ostreatus, Latin for oyster denotes the structure of the cap where it is similar in appearance to the bivalve. Pleurotus ostreatus exhibits a fan-shaped cap

measuring from 5 to 25 cm in diameter and differing from white to grey or tan to dark brown in color. As for the margin, it is smooth at the very beginning then starts to get wrinkly and curvy. Figure 2 shows the body structure of P. ostreatus. Due to the stem configuration, the flesh's thickness differs while maintaining its white and sturdy appearance. Falling down from the stalk when found, the gills of the mushroom tend to be white to creamy in color. The wood is connected laterally with the stem off from the center. To have a good overlook of the spore print which seems to be white to lilac-grey in color, a dark background is required as shown in Figure 1. (Deepalakshmi and Mirunalini, 2014).


**Figure 2.** Taxonomic description of Pleurotus ostreatus mushrooms *(Lesa et al.,2022).*

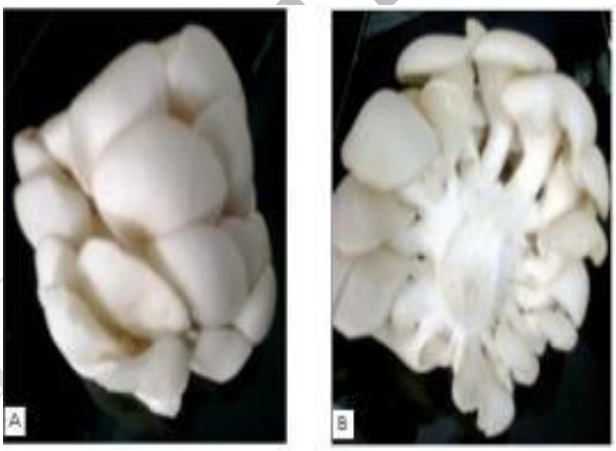



**Figure 1.** A,B shows fruit bodies of Pleurotus ostreatus *(Deepalakshmi and Mirunalini,2014).*

### 3.3. Nutritional Key Components

Mushrooms belong to a different group called fungus, differentiating them from plants and animals. Certain types of mushrooms are considered nutritious foods because they include a variety of nutrients, including fiber, vitamins (such as vitamin D and B vitamins), minerals (such as calcium, magnesium, iron, manganese, zinc, and copper), and vital amino acids (Antonelli and Donelli, 2023). Mushrooms are generally low in fat and high in vitamins, minerals, and dietary fiber. In addition to its flavor and texture, the fruit body of mushrooms is prized for its chemical and nutritional properties (Jayakumar et al., 2011). Oyster mushrooms' protein content surpasses many other foods since it contains all nine essential amino acids which makes it a great and potential substitute for a meat-based diet (Kumar, 2018). Pleurotus species' chitin-rich composition serves as a valuable source of dietary fiber, along with other vitamins (B1, B2, B12, C, D, and E),

essential micro and macro-nutrients, carbohydrates, minimal fat content, and negligible cholesterol content. Regarding its chemical constitution involving secondary metabolites like betalains and alkaloids, alongside glycoproteins and polysaccharides, the Pleurotus genus stands out as one of the most varied among edible and medicinal mushrooms (Torres-Martínez et al., 2022). Pleurotus mushrooms are well-known for having all the qualities one might want in a food: they are tasty, nourishing, and healthful. People enjoy them for their flavor and nutritional value. Mushrooms have unique odors and flavors that are attributed to their terpenes, lactones, amino acids, and carbohydrates. The most common variety of these mushrooms, Pleurotus ostreatus, is frequently used in soups, stir-fries with soy sauce, or even stuffed and consumed (Corrêa et al., 2016).

### 3.4. The rationale for substituting meat with alternative options

The escalating number of humans and exhaustion of natural resources have become among the most pressing issues faced globally in the recent few years. Due to the expected increase in the number of populations in the upcoming years, there should be an increase in the need for sustainable, healthy, and nutritious foods. Food systems are significant contributors to environmental issues such as global greenhouse gas (GHG) emissions, deforestation, and water usage. Meat production was identified to play a huge factor on GHG emissions and water consumption. This leads to a critical demand for alternative sources of valuable proteins (Quintieri et al., 2023). Human health problems have been linked to the growing production and consumption of animal products, including a higher risk of zoonotic diseases, long-term illnesses, and health problems brought on by air pollution A growing number of individuals are becoming vegetarians or flexitarians which are trying to consume as little meat as possible, as a result of the negative effects of meat on environmental sustainability (Mazumder et al., 2023). The influence of climate change such as droughts, flooding, and heat waves, have been demonstrated to adversely affect food security in every aspect. A previous study showed that livestock production contributed to climate change dramatically representing up to 14.5% of all human induced GHG emissions. Among all livestock categories, beef production was found mostly to pose noteworthy hurdles since it demands considerable resources and land-use in comparison to other meat sources. Moreover, it has been proposed that incorporating innovative plant-based alternatives, enhancing waste management and policy reforms could generate synergetic effects that address both environmental and food security concerns. CO2 emissions of major livestock and meat-free

products were studied. It showed that Ovine (lamb) meat production produces (39-52 kg of CO2 per kg of product) which is the highest among others. It was followed by bovine meat (14-39 kg of CO2 per kg of product), poultry (turkey) produced (4-11 kg of CO2 per kg of product) and porcine meat (4-9 kg of CO2 per kg of product). As for meat-free products, they produced about 2-7 kg of CO2 per kg of product (Jiang et al., 2020).

### 3.5. Plant-Based Alternatives to Meat

The market for plant-based meat substitutes is expanding rapidly, moving from a small market to a broader segment. Since 2015, over 6485 new items have been introduced globally. Known by various names such as faux meat, imitation meat, or meat substitutes, analogous meats are plant-based products that resemble the texture, taste, and visual characteristics of animal meat (Boukid, 2020). Soybeans are a common meat substitute that have been utilized historically in numerous Buddhist meal dishes. Soy meal flour, soy protein isolates, and soy protein concentrate were used to create artificial soy goods. About 70% of the protein in the soy concentrate is derived from soybeans, which are widely accessible for eating (Singh et al., 2021). It is possible to improve the physiological and nutritional quality of meat by investigating novel sources of protein and substituting traditional sources either entirely or partially. One such component that works well for this is jackfruit. Customers seeking vegan products prefer jackfruits because they have a feel and taste that's equivalent to that of meat (Mishal et al., 2022). When manufacturing meat analogs, soy and wheat gluten are most commonly utilized as possible sources of protein. However, sources of protein from legumes including peas, faba beans, kidney beans, and mushrooms, as well as fungi like mycoprotein are also employed for the production of meat alternatives (Mazumder et al., 2023). Among the most widely grown vegetables in the world, potatoes offer an abundance of protein .It has been found that potato proteins extracted from potato juice have great solubility as well as foaming and emulsifying qualities. These characteristics make them an excellent texturizer, along with their high gelling capability. The majority of plant-based proteins, especially those derived from potatoes, have the best ratio of essential amino acids, matching or surpassing that of animal proteins and soybean proteins (Nowacka et al., 2023). Edible mushrooms are being employed extensively as a substitute for meat from livestock in modern times. As food 3D printing technology advances, it might be possible to create protein analogues made of edible mushrooms

that precisely mimic the flavor and taste of animal meat, which would increase the nutritional value of edible mushroom food (Wang and Zhao, 2023).

### 3.6. Mini-livestock meat

The world is currently experiencing a severe scarcity of animal protein, but mini livestock, which has been a staple of human diets for millions of years, has an opportunity to greatly reduce this (Tabassum-Abbasi et al., 2016). Mini-livestock meat comes from small animals bred in captivity. Capybaras, a type of rodent, has been found to have its meat lower in cholesterol, leaner and abundant in omega 3 fatty acids, making it a suitable choice for human consumption. In different regions of the world such as in Asia, Africa and Latin America, insects have been widely recognized for their nutritional richness, taste, and wholesomeness that serve to deliver high-quality proteins. Insects are abundant in iron, copper, minerals, and few vitamins (Jiang et al., 2020). The recognition of health, social, and environmental issues linked to excessive meat consumption has prompted demands to push for a reduction in meat intake, leading to an ongoing worldwide debate among policymakers, professionals, and scholars (Apostolidis and McLeay, 2016).

### 3.7. Market of Meat alternatives

Despite some growth in the adoption of meat substitutes within the UK and Europe, the market remains relatively small, comprising only 3.6% of the total meat market value. Challenges in popularizing meat substitutes stem from factors such as food neophobia, perceived lower product quality and healthiness, as well as higher prices compared to traditional meat, hindering efforts to transition towards diets with reduced meat consumption levels through substitution. However, meat substitutes are gaining popularity among vegetarians and meat reducers actively aiming to decrease their meat intake due to concerns about religion, animal welfare, health, and the environment. This suggests potential for promoting less meat-based lifestyles, offering opportunities to reduce the social, environmental, and economic impacts of diets. (Apostolidis and McLeay, 2016). Pleurotus cultivation has been going on in Europe for about thirty years; from 1980 to 1996, it increased steadily, with the exception of a little decline in 1997. Due in part to the method created by two Hungarian expert groups (the HTTV procedure, patented by Balázs, Kovácsné Gyenes, Tóth), Italy has maintained its top place for ten years. With a 230% boost in yield, Spain made a huge breakthrough and moved up to the second rank, with France falling to

third (Győrfi, 2001). In 2010, more than a million tons of mushrooms entered the European Union market; among other fruits and vegetables, Hungary is notable for its capacity to grow mushrooms that exceed export criteria. Hungary currently grows 10–20 tons of shiitake, 2300 tons of oyster mushrooms, and 25,000 tons of white button mushrooms annually. Merely 2% of mushrooms grown in Europe are exported to Hungary, but 40–45% of this production is meant for export sales (Bio-fungi Kft. Bio., 2017). From 18,000 to over 39,000 tons, Hungary's mushroom production has increased dramatically in the past ten years. Ninety percent of the mushrooms are champignons, and the remaining seven to eight percent are oyster mushrooms. These mushrooms are exported in nearly two thirds (About Hungary, 2018). As stated by (Győrfi, 2001), about thirty distinct species of mushrooms were grown in 1994, but only ten of them which accounted for 95.2% of the total production have had a significant commercial value:

- 37,6 % Agaricus bisporus (including Agaricus bitorquis),
- 16,8 % Shiitake, Lentinula edodes,
- 16,3 % Oyster mushroom, Pleurotus spp.,
- 8,5 % Auricularia spp.(mostly Auricularia judea),
- 6,1 % Volvariella volvacea,
- 4,7 % Flammulina velutipes,
- 10,0 % Tremella ficuformis, Hypsizygus marmoreus, Hericium erinaceus, Pholiota nameko and others.

### 3.8. Protein content in Mushrooms

Because it performs so many crucial physiological tasks for the body, including hormone regulation and enzyme activity facilitation, protein is essential to the growth and maintenance of the human body. While the nutritional quality of animal-derived proteins is great, the cost of manufacturing is significantly higher than that of vegetable-derived proteins. The food business and scientific community have recently become interested in proteins generated from fungi because of their remarkable nutritional value, especially when compared to vegetables because they contain more essential amino acids (Gonzalez et al., 2021). For those who avoid meat, protein from mushrooms is a fantastic substitute. It can give those who avoid animal products the nourishment they need and is a decent replacement for animal protein. Due to their concern for animals or their beliefs regarding religion, many people select non-animal products. When making

decisions, they also take the advantages for their health and the environment into account (Ayimbila and Keawsompong, 2023). Edible fungi proteins are attracting considerable attention for their beneficial nutritional content. Among the most commonly cultivated fungal species are Agaricus bisporus (including button mushroom varieties, white or brown, and portobello, comprising roughly 40% of global production), Lentinula edodes (representing approximately 25% of global production), Pleurotus spp. (primarily P.ostreatus), and Flammulina velutipes. Protein content in edible fungi varies from 19% to 45% of dry matter based on species, maturation stage, parts of fungi, substrate, and cultivation methods. Analysis of Pleurotus spp. shows all essential amino acids present, with leucine, aspartic acid, phenylalanine, and lysine most abundant, along with umami and non-essential amino acids like GABA and ornithine (Quintieri et al., 2023). Approximately 39.9% of the dry weight of mushroom fruiting bodies is made up of carbohydrates, 17.5% is protein, and 2.9% is fat. The remaining material is made up of minerals. Numerous papers have argued that mushrooms' amino acid contents are similar to those of animal proteins. The significance of this information lies in the fact that diseases linked to the use of animal meat have made human nutrition more complex. Comprehensive research is needed to determine the nutritional effects of gradually substituting meat with mushrooms, including in-depth chemical and biological studies (E.A. and J. K, 2017). There are thirteen amino acids in Pleurotus ostreatus, eight of which are non-essential and five of which are essential. A list of the amino acids discovered is as follows: proline, serine, asparagine, hydroxyproline, cysteine, glutamine, phenylalanine, lysine, alanine, leucine, threonine, methionine, and aspartic acid. A concentration of 492.12 mg of aspartic acid per 100 grams makes it the most abundant amino acid in Pleurotus ostreatus, while a concentration of 9.32 mg per 100 grams of cysteine is the least (Effiong et al., 2024). White oyster mushrooms have around 37.87 g carbs, 17.12 g protein, and 2.60 g fat per 100 g dry matter, with a total caloric content of 243.66 kcal. It also has 4.8 g of ash and around 30.25 g of fiber (Dündar et al.,2008). Pleurotus ostreatus fresh mushrooms have a moisture content of between 80% and 90%, which is comparable to other fungal species (Piska et al.,2016). Chemical compounds known as "biogenic amines" influence protein synthesis, DNA replication, and cellular membrane permeability, among other aspects of cellular activity. For healthy cells to form, remain in good condition, and function as intended, they are crucial (Jabłońska-Ryś et al., 2020)**.** White oyster mushrooms have a salty or Umami flavor which is due to the fact that it comprises about 41 mg/g of glutamic acid of dried mushrooms (Rahmah et al., 2020).

### 3.9. Application areas of mushrooms

The various application areas of mushrooms are illustrated in Figure 3. It is easy to differentiate mushrooms from other foods owing to their unique texture, flavor, and odor. In general, by enhancing the texture and juiciness of food items, the inclusion of mushroom powder boosted customer acceptance (Hamza et al., 2023).


**Figure 3.** Different application areas of mushrooms *(Guo et al.,2022)*

Due to the fact that Pleurotus powder and extracts are rich sources of prebiotics, the significant amount of fiber of this species has been used to create functional dairy products (Ritota and Manzi,2023). To improve the functions of the liquid goods, mushrooms were also added as powder or extracts. Due to Ganoderma lucidum's special ability to promote health, traditional Chinese medicine has utilized it instead of meals since it contains a variety of bioactive chemicals. Beer was supplemented with G. lucidum extract to boost its physiologically active components. It was suggested that it might be used as a brewing raw material to create a new kind of beer with better functioning and palatable sensory qualities (Moon et al., 2013). Treatment of various solid waste kinds can be accomplished effectively and economically with the help of mycoremediation. Fungi, including mushrooms, thrive in soil and aid in the breakdown of harmful substances. Growing in both hydrocarbon- and non-hydrocarbon-contaminated soils, mushrooms release the remediation-useful enzymes laccase, manganese-dependent peroxidase, and lignin peroxidase (Jebapriya et al., 2013). Studies on mushrooms belonging to the Pleurotus genus have shown a number of

potentially medicinal characteristics. Fruiting bodies, their extracts, and the mycelium itself all contain medicinal compounds. It is possible to obtain medicinal benefits by eating fresh oyster mushroom fruiting bodies, meals that contain dried oyster mushrooms, or supplements that comprise such mushrooms. A glucan-based product derived from P. ostreatus fruiting bodies is available in the market. In cases where an individual's immunity is low and infections and allergies are common, it is employed in treatment (Golak-Siwulska et al., 2018).

### 3.10. Consumers' Acceptance of Meat Substitutes

The most significant individual-related factor influencing adoption of meat substitutes is food neophobia, or the unwillingness to try new meals, especially when it involves fear of unpleasant sensory experiences. The adoption of these foreign foods has been found to be facilitated by information on appropriate use, favorable flavor or resemblance of recognized food ("tastes like food X"), and engagement over a period of time (Caparros Megido et al., 2016). Despite the modern emphasis on sustainability and well-being in consumer preferences, the sensory attributes, flavor, taste, and texture, remain paramount in influencing consumers' decisions to buy or repeatedly select food items. The tailored formulation of Mixed Mushroom Meat Substitutes (MMMS), consisting of 37.5% Pleurotus Sajor-caju (PSC) mushrooms, 12,5% chickpea flour, 0.2% beetroot extract, and 5% canola oil, aimed to meet consumer expectations and replicate the sensory qualities of traditional animal-based minced meat. Evaluation through sensory analysis indicated that Mixed Mushroom Meat Substitutes formulations obtained considerable acceptance among 120 untrained panelists. However, comparative analysis revealed that the sensory attributes, encompassing appearance, taste, color, texture, aroma, and overall acceptability of the cooked MMMS, were slightly inferior ($p < 0.05$) to those cooked animal-based minced meat (Mazumder et al., 2023). Moreover, price is a significant factor in determining consumer interest in alternative proteins, in addition to flavor and accessibility. Even though plant-based foods were frequently far cheaper to create because they used less expensive raw materials, they were nevertheless frequently more expensive than animal-based products. The costs of supply networks, manufacturing volume, and post-processing contributed to higher retail pricing (Szenderák et al., 2022).

### 3.11. Therapeutic advantages of Pleurotus mushroom

Apart from plants, various fungal species exhibit medicinal properties, with several already employed for therapeutic applications. The different pharmacologocal effects are summarized in Table 1 from the study of (Waktola and Temesgen, 2020), where it is discussed that the various natural compounds have been identified for their potential therapeutic properties, spanning from antifungal to immune modulatory effects. Okamoto et al., (2002) highlighted the antifungal activity of hexane-dichloromethane compounds, while Vamanu (2012) discussed the antibacterial properties of β-D glucan (pleuran). El-Fakharany et al., (2010) shed light on the efficacy of laccase against hepatitis C virus and the antiviral activity of ubiquitin-like protein. De-Silva et al., (2012) explored the anticancer potential of water-soluble proteins or polysaccharides, whereas Bello et al., (2017) focused on α-amylase and α-glucosidase as anti-diabetic agents. Additionally, Bauerova et al., (2009) investigated the anti-arthritic effects of β-(1,3/1,6) D-glucan. Wang & Ng, (2000) introduced a novel ubiquitin protein with potential inhibition of HIV-1 reverse transcriptase. Eye health was associated with unspecified bioactive compounds by Isai et al., (2009), while Wang et al., (2000) suggested polysaccharides-peptides and polysaccharide-protein complexes for immune modulation. Furthermore, Cowan, (1999) discussed the inhibition of protein synthesis and proteolytic enzymes by phenolic and tannin compounds. Ethanol was explored for its anti-hyperlipidemic properties by Mohamad et al., (2017). Mushrooms, revered as "the ultimate health food", have been utilized in medicinal contexts across diverse cultures since ancient times (Patel et al., 2012). Mushroom-derived proteins and peptides have a number of health benefits, including the ability to reduce blood pressure, modulate the immune system, fight bacterial and fungal infections, and potentially fight cancer, viruses, and oxidative stress. They also inhibit the angiotensin-converting enzyme (Ayimbila, and Keawsompong, 2023). The β- and α-glucans, which are polysaccharides made of glucopyranose molecules joined by glycosidic linkages of the (1→3)-β, (1→6)-β-, or (1→3)-α type, are found in the cell wall of mushrooms. A subset of polysaccharides known as β-glucans has been the subject of substantial research. Their benefits for improving health are numerous and varied (Golak-Siwulska et al., 2018).

**Table 1**. Therapeutical benefits of Pleurotus ostreatus *(Waktola and Temesgen, 2020)*

| No | Pharmacological Effect | Extracted Substances | References |
|---|---|---|---|
| 1 | Antifungal | Hexane-dichloromethane | Okamoto et al., (2002) |
| 2 | Antibacterial | β-D Glucan (pleuran) | Vamanu, (2012) |
| 3 | Hepatitis C virus | Laccase | El-Fakharany et al., (2010) |
| 4 | Antiviral | Ubiquitin-like protein | El-Fakharany et al., (2010) |
| 5 | Anticancer | Water soluble protein (or) polysaccharides | De-Silva et al., (2012) |
| 6 | Anti-diabetic | α- amylase | Bello et al., (2017) |
| | | α-glucosidase | |
| 7 | Anti-tumor | β-D Glucan (pleuran) Glycopeptides | Devi et al., (2013) |
| | | Proteoglycans | |
| 8 | Anti-hypercholesterolic | Lovastatin | Weng et al., (2010) |
| 9 | Anti-arthritic | β-(1,3/1,6)Dglucan | Bauerova et al., (2009) |
| 10 | Inhibit HIV-1 reverse transcriptase | novel ubiquitin protein | Wang and Ng, (2000) |
| 11 | Eye health | Unspecified bioactive | Isai et al., (2009) |
| 12 | Immune modulatory | polysaccharides-peptides, and polysaccharide-protein complex | Wang et al., (2000) |
| 13 | Inhibition of protein synthesis,proteolytic enzymes | Phenolic and tannin | Cowan, (1999) |
| 14 | Anti-hyperlipidemic | Ethanol | Mohamad et al., (2017) |

### 3.11.1 Antiviral Properties

The compounds found in Pleurotus mushrooms have the ability to fight viruses either directly or by triggering their immune-stimulating systems. Amongst those, an antiviral protein named ubiquitin has been discovered and extracted from oyster mushroom fruiting bodies. Both water-soluble sulphated derivatives and water-insoluble β-glucans that were isolated from P. tuberculum sclerotia exhibited anti-herpes simplex virus types 1 and 2 properties. Due to sulphated β-glucans' potential to adhere to virus particles and prevent them from infecting host cells, this antiviral function is claimed. Furthermore, P. ostreatus extracellular extracts and intracellular proteins both comprise polysaccharides which possess immunomodulatory functions (Patel et al., 2012).

### 3.11.2 Antimicrobial Effects

Oyster mushrooms (OM) have been investigated for their efficacy against both simple and multiple drug-resistant strains of Escherichia coli, Staphylococcus epidermidis, and S. aureus, as well as various species of Candida, Streptococcus, and Enterococcus. Utilizing methanolic extracts from Pleurotus species, researchers have observed inhibitory effects on the proliferation of Bacillus megaterium, S. aureus, E. coli, Klebsiella pneumoniae, C. albicans, C. glabrata, as well as Trichophyton and Epidermophyton species, albeit with varying degrees of efficacy, which were comparatively lower than those of two antifungal agents: Streptomycin and Nystatin (Patel et al., 2012).

### 3.11.3 Antifungal Activity

The antifungal properties of oyster mushrooms originate from a peptide found in its fruiting body called Pleurostrin (Xia et al.,2005). The bioactive substances p-anisaldehyde, chitin (chitosan), and pleurostrin are frequently credited with the antifungal characteristics of P. ostreatus mushrooms isolated using various solvents (Törős et al., 2023). P. ostreatus was found to create p-anisaldehyde as a defense mechanism against other species in a different investigation. This aids in its defense against fungus and bacteria. The potent antifungal activity of P. ostreatus var. florida against Trichoderma sp. in water-based extracts may be attributed to the presence of tannins, saponins, and flavonoids in the mushroom (Owaid et al., 2017).

### 3.11.4 Anticancer Effect

Anticancer activity in mice diagnosed with sarcoma and hepatoma was illustrated from lectin, which was extracted from the oyster mushroom body (Wang et al., 2000). Apoptosis was stimulated in tumor cells by RNase Po1, a guanylic acid-specific ribonuclease originated from oyster mushrooms (Jesenak et al., 2014).

### 3.11.5 Lipid Metabolism

The impact on serum lipids was examined where triglycerides and total cholesterol, two indicators of infection, decreased following the initial P. ostreatus treatment, along with fasting plasma glucose (Khatun et al., 2007). Lovastatin, a drug licensed to treat dyslipidemia, is one of the main ingredients of P. ostreatus. It functions by blocking HMG-CoA reductase (Piska et al., 2016). The activity of the rate limiting enzyme responsible for biosynthesizing cholesterol, HMG CoA

reductase, is hindered by mevinolin (lovastatin) which therefore works on decreasing cholesterol biosynthesis (Hossain et al., 2003).

### 3.11.6 Antioxidant Activity

Oxidative stress is caused by free radicals which can lead to various illnesses like cancer, cardiovascular disease, and Alzheimer's. To protect the cells and stop free radicals, antioxidants are essential. They are abundant in oyster mushrooms. High content of vitamins and selenium is identified in Pleurotus mushrooms which in biological systems are considered as natural antioxidants (Jamir, 2023). β-glucans in fibers and high-quality proteins found in P. ostreatus can be potentially employed as a functional food component. β-glucans are used to lower the glycemic index and have an antioxidant role (Bulam et al., 2022)

### 3.11.7 Anti-diabetic

Impairment in insulin production, resistance or both are the leading causes of Diabetes mellitus. To regulate blood sugar and diabetic implications, mushrooms are used as a treatment in traditional medicine. When streptozotocin-induced diabetic rats were given dietary supplements containing oyster (P. ostreatus) and shiitake (L. subnudus), the rats' fasting blood glucose levels significantly decreased. Furthermore, relative to diabetic rats not receiving treatment, there were appreciable decreases in the activities of α-amylase, α-glucosidase, ACE, and arginase (Devi et al., 2024).

### 3.11.8 Uric acid

Gout symptoms like soreness and inflammation in the joints are worsened by high levels of uric acid. One of the compounds that influences and controls the uric acid level in the body is purines which are found in oyster mushrooms (Irfan et al., 2022).

## 3.12. Heat treatment as a food preservative method

One crucial procedure that is often used in the food sector is heat treatment. Protein, fat, and starch are among the nutrients that can be altered by a variety of heat processing methods. These alterations can affect the physical and chemical attributes of food, such as its color, texture, taste, and nutritive value. Food processing commonly uses traditional processing techniques such convection with hot air drying, vacuum drying, microwave heating, burning charcoal treatment, boiling, steaming, and frying (Fang et al.,2022). Heat generation and distribution, as well as the

interaction of temperature and time, are all important factors to consider when working on thermal treatment processes. The impact of heat on food functionality can be characterized by both favorable and unfavorable outcomes. By chemically modifying the nutrients and producing or destroying different exogenous or endogenous biologically active molecules, these methods influence the nutritional value of food (Hardy et al., 1999).

### 3.12.1 Blanching

Thermal methods like blanching are typically carried out before food is processed, including canning, freezing, frying, and drying. Enzymes and bacteria that could infect raw fruits and vegetables during manufacturing, harvesting, and transportation are inactivated and destroyed during long-term storage, making blanching crucial to maintain product quality. Blanching is the process of quickly reaching a predefined temperature and holding it there for a predetermined length of time, usually one to ten minutes or fewer. After blanching, the product is either quickly chilled or sent straight on to the following step (Xiao et al., 2017). In the food sector, hot water and steam are the most often utilized thermal techniques for blanching (Alenyorege et al., 2024).

#### 3.12.1.1 Hot water blanching

The most widely used and widely accepted blanching technique is hot water blanching since it is straightforward to set up and manage. Materials are submerged in boiling water (70 to 100 C) for a couple of minutes during a standard hot water blanching process. Prior to proceeding on to the following stage of processing, the blanched materials are then drained and cooled down (Xiao et al., 2017). It has been demonstrated that hot water blanching, an effective heat treatment technique, enhances the visual appeal and rehydration properties of dried mushrooms (Gothandapani et al., 1997). As a result of the short contact with heat, the activity of enzymes is rendered inactive, prohibiting additional nutritional loss, color change, and production of bizarre flavors (Mutukwa, 2014).

#### 3.12.1.2 Steam blanching

It is known worldwide that steaming is a traditional and nutritious culinary technique. Steam from continuously boiled water serves to prepare food in steam cooking. Steam heating is a method of making food using heat that dates back thousands of years. Distinct from boiling food, steamed food is cooked directly over hot steam on a tray which holds it apart from the boiling water. In

addition to being tasty, food prepared in steam retains most of its vitamins and minerals, color, texture, and taste (Shi et al.,2020). The high enthalpy levels of hot steam contribute to it being an effective heating medium for blanching. Because the product temperature remains cooler than that of the vapor during the initial stages of steam blanching, a significant quantity of latent heat passes to the product itself as the steam condenses on its top. Upon hitting the threshold temperature for enzymes or organisms' action, the products' temperature progressively rises, at which point they become inactive. Because steam blanching has minimal leaching impact compared to water blanching, it is thought to be more cost-effective while retaining the majority of minerals and aqueous-soluble compounds (Xiao et al.,2017).

# 4. Materials and Method

## 4.1 Materials

The oyster mushrooms and minced pork meat used in this research were provided from a Hungarian market in Budapest. The experiments were done at the Hungarian University of Agriculture and Life Sciences, Department of Livestock Products and Food Preservation Technology. The preparation of homogenous sausage batter with increasing percentage of oyster mushrooms as a meat replacement was carried out using the food processor (Robot Coupe, R 201 Ultra E). The recipe included cut oyster mushrooms, minced pork meat, ice, phosphate, and sodium nitrate salts. The preparation was divided according to the amount needed to obtain six samples for each pre-treated sausage percentage where one sample weighs around 80 g. Table 2 shows the recipe of 480 g sausage batter which produces six samples for each meat replacement percentage. Water loss by mushrooms was considered as 20% when calculating amount of mushrooms to be used.

**Table 2.** *Recipe of the mushroom sausages.*

| Percentage of mushroom in sausage batter | MEAT (g) | MUSHROOM (g) | ICE (g) | Phosphate (g) | Sodium nitrate (g) |
|---|---|---|---|---|---|
| **10%** | 288 | 38.4 | 160 | 2 | 9.6 |
| **20%** | 256 | 76.8 | 160 | 2 | 9.6 |
| **30%** | 224 | 115.2 | 160 | 2 | 9.6 |
| **40%** | 192 | 153.6 | 160 | 2 | 9.6 |
| **50%** | 160 | 192 | 160 | 2 | 9.6 |

Two thermal pre-treatments were performed on oyster mushrooms, namely steaming in oven (Lainox VE051P) for 3 minutes at 100 ˚C and blanching in water for 3 minutes at 100˚C. Six control samples from pork meat only (53 g meat and 27 g ice per sample) were prepared. Whereas six different samples for each percentage replacement were done using fresh oyster mushrooms as untreated control samples. Images of the pretreated mushroom samples are presented in Figure 4. and Figure 5.

**Figure 4.** Mushrooms pre-treated inside steaming oven.

**Figure 5.** Blanching pre- treatment of mushrooms.

**Figure 6.** Blanch pretreated mushroom sausages.

**Figure 7.** Steam pretreated mushroom sausages.

After the sausage batter preparation, around 80 g were weighed in a petri dish on a digital scale then placed on trays to perform the cooking process. Cooking took place in the steam oven (Lainox VE051P) for 25 minutes at 80 ˚C. The samples (Figures 6 and 7) were left out to cool at

room temperature before performing the different evaluations in terms of weight loss percentage, color, and texture. The evaluation took place on the same day of production denoted by (day 0) and after 10 days of storage denoted by (day 10).

### 4.2 Methods

#### 4.2.1 Measurement of Color

The CIELAB scoring system was used to measure the color aspects of the mushroom sausage samples. The mushroom sausage samples' lightness (L*), redness (a*), and yellowness (b*) values were measured with a CR-410-type colorimeter (Konika Minolta Sensing Inc., Japan). The colorimeter was calibrated using a white reference plate (CRA43) before every measurement. For every sample, six parallel readings were taken and averaged to ensure precision on day 0 and day 10.

#### 4.2.2 Texture Analysis

A TA.XT Plus texture analyzer (made by Stable Micro Systems, Surrey, UK) was used to examine the texture of the samples of mushroom sausage. The analysis of maximal force was conducted using a CyI. stainless rod with a diameter of 5 mm and a 20 mm positioning from sample. The pre-test and test speed were both set at 2 mm/s. The maximal force value (N), which defined the samples' hardness, was represented by the maximum peak force seen on the graph. Furthermore, the area under the curve was used to calculate the work (Nmm) performed during each test (Figure 8). For every sample, six replicate values were obtained and averaged on day 0 and day 10.

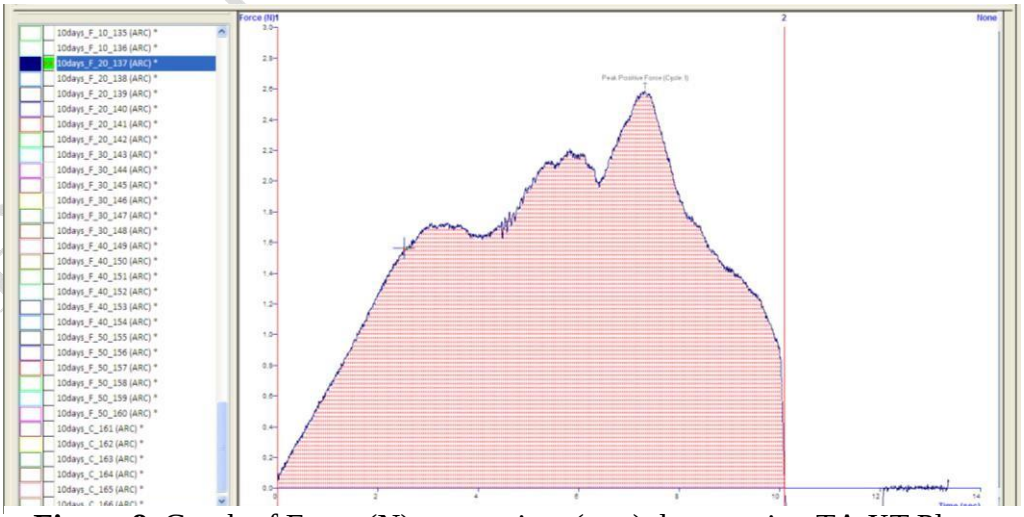


**Figure 8.** Graph of Force (N) versus time (sec.) drawn using TA.XT Plus texture analyzer.

### 4.2.3 Measurement of Weight Loss

The weight of the mushroom sausage samples was recorded before and after cooking took place on day 0 using a digital scale. The weight loss percentage was then calculated based on the method stated by Alves et al. (2016) with some modifications. The following equation was used to assess the weight loss percentage:

$$\text{Weight loss percentage} = \frac{Weight \text{ of sausage } before\ cooking - Weight\ of \text{ sausage } after\ cooking}{Weight\ of\ sausage\ after\ cooking} x100$$

The weight loss was evaluated again on day 10 of storage by measuring the mushroom sausage samples to examine any effect of storage on them. Six measurements were done and averaged for each sample.

### 4.2.4 Statistical Analysis

The statistical analysis of the collected data was performed using one-way multivariate ANOVA in SPSS-20 software (SPSS Inc., IBM Company, US). To find any significant differences between the samples, post hoc analysis and Tukey's HSD were used. The data were sorted first by storage and percentage of mushroom in sausage to evaluate the effect of pretreatment on the different studied parameters. Then the data was sorted by storage and pretreatment to evaluate the effect of increasing mushroom percentage in sausages on the different studied parameters. The samples were considered to be significantly different at a P value <0.05. Discriminant Canonical Analysis with cross validation was done to assess the impact of various pretreatments and increasing percentage of mushrooms on day 0 and day 10 on the classification of mushroom sausages samples. Additionally, one sample t-test was performed to evaluate the effect of storage on the different parameters within the same pretreatment and percentage of mushroom sausages.

# 5. Result and Discussion

## 5.1. Canonical Discriminant Analysis

Figure 9, displays the entire dataset used in the study, providing a visual comparison of sausage samples with increasing mushroom percentage across all parameters examined, including color, texture, and weight loss percentage. This Canonical Discriminant Analysis (CDA) plot illustrates the original grouped cases, which refers to the dataset before any cross-validation or model adjustments were made. The original grouped cases achieved a 58.3% accuracy which indicates that the classification model correctly predicted the group for approximately 58.3% of the cases. In simpler terms, this means that the model accurately classified the majority of cases into their respective groups based on the discriminant functions derived from the data. The cross-validation yielded an accuracy rate of 52.6%, marginally below the original classification rate. Cross-validation entails testing a model on various samples, a practice essential for evaluating the reliability and applicability of the results.

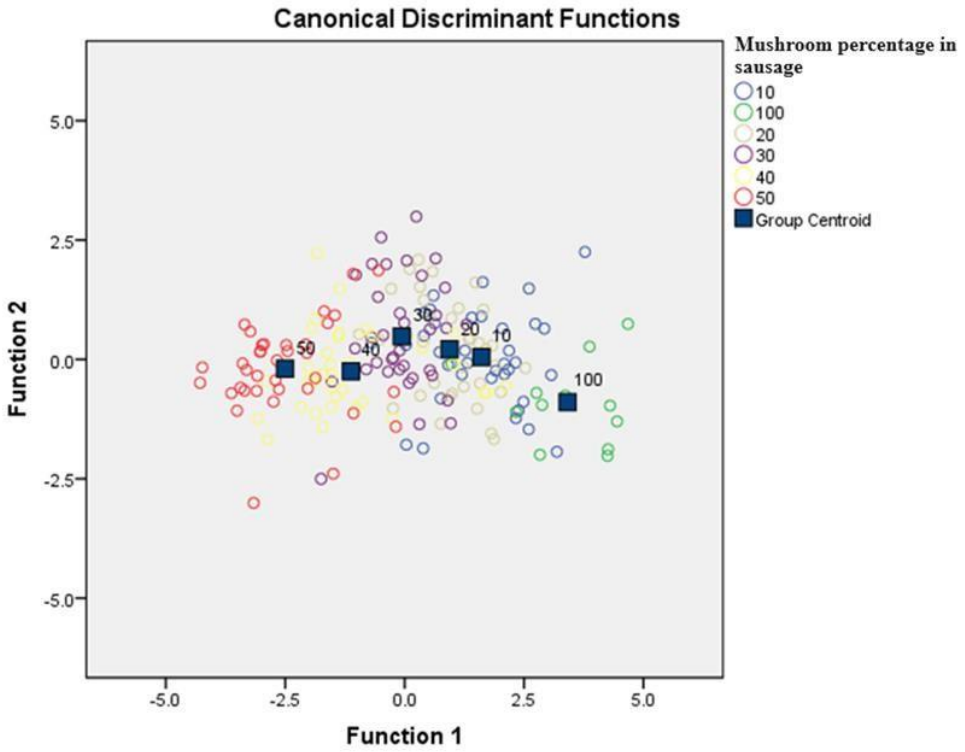



**Figure 9.** Classification of sausage samples by mushroom percentage using Canonical Discriminant Plot (Function 1 vs. Function 2)

Figure 10 presents the complete dataset utilized in the investigation, offering a visual comparison among mushroom sausages subjected to different pretreatments—Blanched, Steamed, and Fresh samples. This Canonical Discriminant Analysis (CDA) plot showcases the initial grouped cases, depicting the dataset before any cross-validation or model adjustments occurred. The original grouped cases attained a 69.3% accuracy, indicating that the classification model accurately predicted the group for approximately 69.3% of the cases. In simpler terms, this denotes that the model effectively classified the majority of cases into their respective groups based on the discriminant functions derived from the data. Subsequent cross-validation revealed an accuracy rate of 66.7%, slightly below the original classification rate. Table 3 shows the predicted group membership of the different samples. The classification model sometimes predicted the blanched samples as steamed and vice versa which means that these two pretreatments were more correlated and similar to each other than the fresh samples.

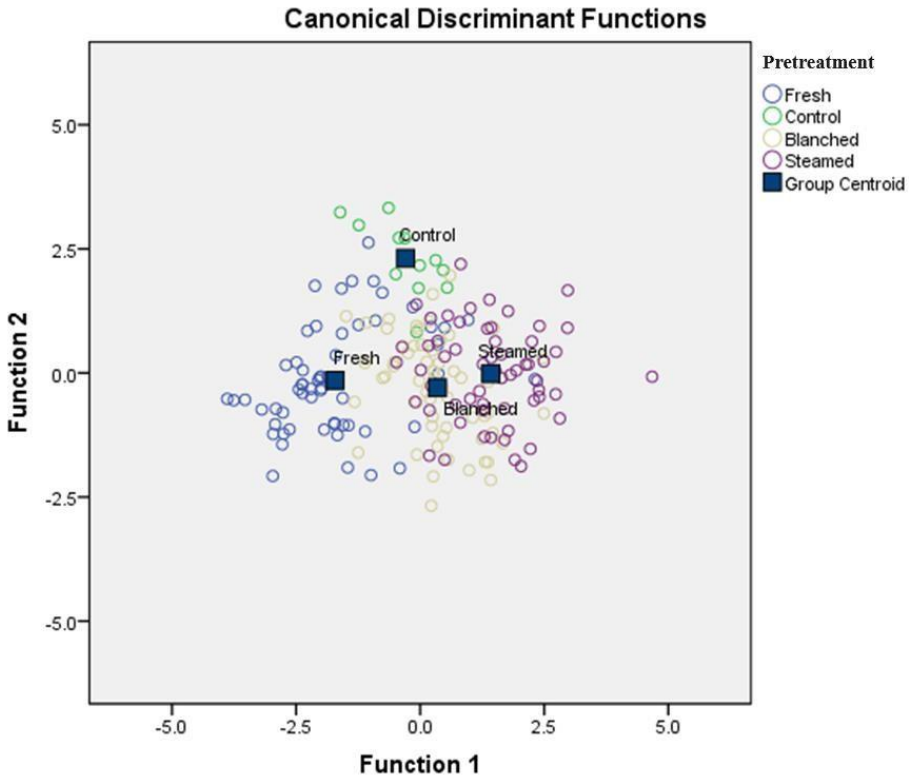



**Figure 10.** Classification of sausage samples by mushroom sample pretreatment using Canonical Discriminant Plot (Function 1 vs. Function 2).

**Table 3.** Predicted Group Membership of the different pretreatment samples with cross validation.

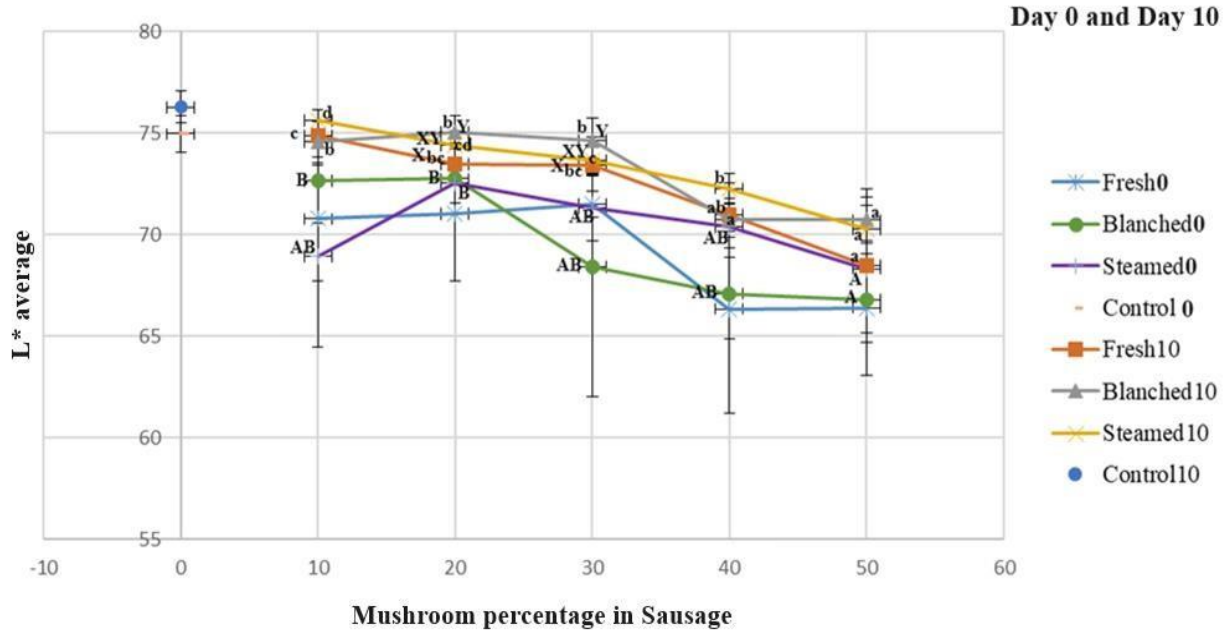
| | | treatment | Predicted Group Membership | | | | Total |
|---|---|---|---|---|---|---|---|
| | | | Fresh | Control | Blanched | Steamed | |
| Original | Count | Fresh | 42 | 9 | 8 | 1 | 60 |
| | | Control | 0 | 11 | 1 | 0 | 12 |
| | | Blanched | 4 | 5 | 39 | 12 | 60 |
| | | Steamed | 0 | 4 | 15 | 41 | 60 |
| | % | Fresh | 70.0 | 15.0 | 13.3 | 1.7 | 100.0 |
| | | Control | .0 | 91.7 | 8.3 | .0 | 100.0 |
| | | Blanched | 6.7 | 8.3 | 65.0 | 20.0 | 100.0 |
| | | Steamed | .0 | 6.7 | 25.0 | 68.3 | 100.0 |
| Cross-validated | Count | Fresh | 41 | 9 | 9 | 1 | 60 |
| | | Control | 0 | 11 | 1 | 0 | 12 |
| | | Blanched | 5 | 6 | 38 | 11 | 60 |
| | | Steamed | 0 | 4 | 18 | 38 | 60 |
| | % | Fresh | 68.3 | 15.0 | 15.0 | 1.7 | 100.0 |
| | | Control | .0 | 91.7 | 8.3 | .0 | 100.0 |
| | | Blanched | 8.3 | 10.0 | 63.3 | 18.3 | 100.0 |
| | | Steamed | .0 | 6.7 | 30.0 | 63.3 | 100.0 |

## 5.2. Color Analysis.

### 5.2.1. Effect of the different pretreatment within the same percentage of mushroom and storage day on L*, a* and b* values.

As Figure 11 represents for the L* parameter, no significant differences were observed across the pretreatment groups for all percentage from 10% to 50% of mushrooms in sausage, indicating that pretreatment did not have a significant impact on lightness value on day 0. Compared to Fresh samples, Blanched treatments showed significantly higher L* values at both the 20% and 30% of mushrooms in sausage on day 10 ($P < 0.05$). Specifically, at 20%, Fresh sausage samples had a mean L* value of 73.46 ± 0.77, while Blanched ones had a mean L* value of 75.04 ± 0.80. Similarly, at 30%, Fresh sausage samples had a mean L* value of 73.40 ± 0.38, while Blanched ones had a mean L* value of 74.63 ± 1.09. L* value increased with blanching on day 10. Even when stored in the fridge, browning processes and dehydration reduce the shelf life of fresh oyster mushrooms to a few days. Polyphenol oxidase (PPO) is a key enzyme responsible for browning.

It is possible to process oyster mushrooms to prolong their shelf life. Our results indicate no significant difference on day 0 across pretreatments, but on the long-term storage, blanching induced an increase in lightness value. This highlights how blanching effectively reduces enzymatic browning and prolongs the shelf life of the samples. The blanching technique results in an enhanced product for subsequent industrial activities, inhibits enzymatic browning, and causes size shrinkage and air leakage (Vullioud et al., 2011).




[1]

**Figure 11.** Lightness, L* value for the different pretreatments and increasing mushroom percentage in sausage samples.

The result of a* value is displayed in Figure 12. Compared to fresh samples (3.07 ± 0.12), both Blanched (3.56 ± 0.42) and Steamed (3.45 ± 0.13) pretreatments on day 0 resulted in significantly higher redness values (a*) for 10% of mushrooms in sausage samples. Compared to fresh samples (1.94 ± 0.23), both Blanched (1.29 ± 0.24) and Steamed (1.00 ± 0.49) pretreatments on day 0 resulted in significantly lower redness values (a*) for 50% of mushrooms in sausage samples. On

[1] A-B on day 0, a-d on day 10: Mean values with different letters differ significantly among the increasing percentage of mushroom in sausage within the same treatment ( $P < 0.05$).

X-Y on day 10: Mean values with different letters differ significantly among the pretreatments of mushroom sausage within the same mushroom percentage ( $P < 0.05$).

day 10, compared to Fresh untreated samples, at the 10% mushroom sausages, both pretreated samples (blanched and steamed) showed significantly lower a* values. Specifically, Fresh sausage samples had the highest mean a* value of 4.54 ± 0.20, followed by the Blanched samples with a mean a* value of 4.11 ± 0.18, and Steamed samples had the lowest mean a* value of 3.77 ± 0.12. Relative to the fresh samples, both the Blanched and Steamed pretreatment methods resulted in significant difference in a* values for sausages containing 40% and 50% mushrooms. Specifically, at the 40%, Blanched (1.86 ± 0.24) and Steamed sausage samples (1.89± 0.09), showed lower mean a* value than fresh samples (3.74 ± 0.21). At the 50% mushroom content, both Blanched and Steamed samples displayed identical mean a* values of 1.15 ± 0.23 which is lower than that of the fresh sausage samples (2.95 ± 0.71). Our results show that steaming and blanching caused a decrease in a* value which is in contrary to (Lespinard et al., 2009) and (Yao et al., 2023) where the a* values were increased after cooking the mushrooms indicating browning reactions.

[2]

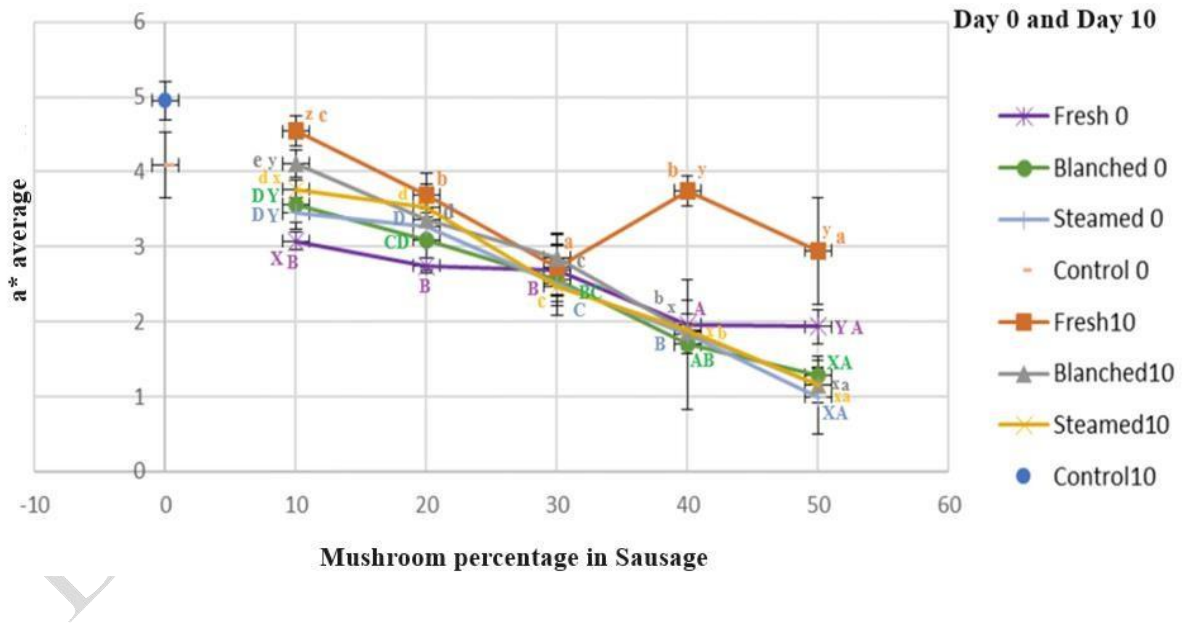
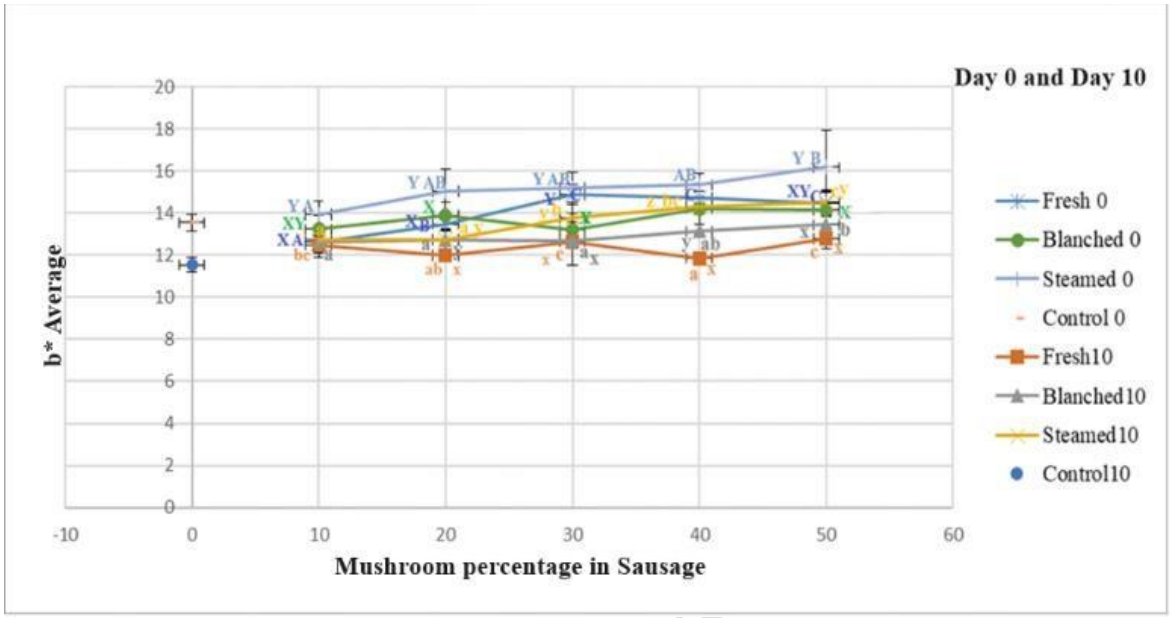



**Figure 12.** Redness, a* value for the different pretreatments and increasing mushroom percentage in sausage samples.

[2] A-D on day 0, a-e on day 10: Mean values with different letters differ significantly among the increasing percentage of mushroom in sausage within the same pretreatment ( P< 0.05).
X-Y on day 0, x-z on day 10: Mean values with different letters differ significantly among the pretreatments of mushroom sausage within the same mushroom percentage ( P< 0.05).




[3]

**Figure 13.** Yellowness, b* value for the different pretreatments in function of mushroom percentage in sausage samples.

The result of b* value is represented in Figure 13. Significant variations in the b* values at day 0 were observed across the different pretreatment samples within the same percentage of mushrooms in sausage. At the 10% and 20% mushroom sausage, significant differences were observed between Fresh (12.60 ± 0.72 and 13.48 ± 0.22, respectively) and Steamed (13.93 ± 0.64 and 15.04 ± 1.04, respectively) sausage samples, indicating a higher b* value associated with Steamed samples. Conversely, at the 30% mushroom sausage, significant results were observed between both Fresh (14.87 ± 0.46) and Steamed (15.22±0.69) with the Blanched (13.19 ± 1.68) samples , suggestive of a lower b* value for Blanched sausages. Furthermore, at the 50% mushroom sausage, marked differences were shown between Blanched (14.14 ± 0.84) and Steamed (16.18 ± 1.74) sausages, with the b* value at day 0 being higher for Steamed samples. On day 10, compared to untreated samples (Fresh), at the 20% mushroom sausages, significant differences were observed between Fresh and both Blanched and Steamed pretreatments. Specifically, blanched (12.73 ±

[3] A-C on day 0, a-c on day 10: Mean values with different letters differ significantly among the increasing percentage of mushroom in sausage within the same pretreatment ( P< 0.05).

X-Y on day 0, x-z on day 10: Mean values with different letters differ significantly among the pretreatments of mushroom sausage within the same mushroom percentage ( P< 0.05).

0.41) and steamed sausages(12.72 ± 0.50) showed a higher mean b* value than the Fresh untreated samples (11.99 ± 0.23). Compared to steam pretreated samples, at the 30% and 50% mushroom sausage on day 10, significant differences were observed between Steam pretreated samples and both Fresh and Blanched samples. Particularly, steamed sausages at 30% and 50% (13.76 ± 0.22 and 14.49 ± 0.56 respectively) showed a higher mean b* value than Fresh untreated sausages (12.63 ± 0.29 and 12.78 ± 0.50 respectively) and blanched samples (12.68 ± 0.26 and 13.44 ± 0.36 respectively). For the 40% mushroom sausages , Fresh untreated samples (11.85 ± 0.30) showed a significantly lower mean b* value compared to Blanched (13.13 ± 0.32), which, in turn, indicated a significantly lower mean b* value than Steamed sausages (14.31 ± 0.37). Our results showed that steaming caused the b* values to increase in mostly all percentage when compared with Fresh and Blanched samples. Blanching didn't have any significant effect on the b* value compared with fresh. Our results were in align with those reported by (Yao et al., 2023) where the b* value was increased with steaming pretreatment. Also, (Eissa et al., 2008) had similar results where steaming of mushrooms had higher b* values than both water-blanched and fresh samples.

### 5.2.2. Effect of mushroom percentage within the same pretreatment and storage day on L*, a* and b* values.

The percentage of mushroom replacement in sausage had a significant impact on color parameters at day 0, within the same pretreatment samples. For the L* parameter (Figure 11), the fresh samples showed no significant differences among different mushroom percentages (10% to 50%). Compared to both the 10% (72.65 ± 2.09) and 20% (72.76 ± 1.75) mushroom sausages in Blanched-pretreated samples, the 50% mushroom sausage had a significantly lower L* mean value (66.79 ± 1.65). Similarly, in steam-pretreated samples, the 50% mushroom sausages had a significantly lower mean value (68.31±-1.95) than the 20% (72.55±1.01). The results show a consistent decrease in L* values with increasing mushroom content (a darkening effect) within blanched and steamed samples on day 0. For the L* parameter on day 10, in fresh untreated samples, in blanched, and in steamed, significantly lower L* values were observed as the percentage of mushrooms in sausage increased from 10% to 50%. Specifically, for Fresh samples, the mean L* value was 74.89 ± 0.67 at 10% and decreased to 68.49 ± 3.79 at 50%. For Blanched samples, the mean L* value was 74.56 ± 1.05 at 10% and decreased to 70.72 ± 1.13 at 50%. For Steamed samples, the mean L* value was 75.61 ± 0.54 at 10% and decreased to 70.26 ± 1.19 at

50%. Our results showed that as the percentage of mushrooms increased, the lightness L* value decreased within the 3 samples (Fresh, Blanched and Steamed) on day 10 indicating a darkening effect of mushrooms. The changes in L* value in the sausage with increasing mushroom percentage in our study were in align with those reported by Fu et al. (2023) where the result showed that L* values decreased significantly as the amount of Agaricus Bisporus mushrooms increased in the sausage. This can be attributed to factors such as enzymatic browning, cellular changes, and suboptimal storage conditions (Kaur et al., 2014).

For the a* redness value (Figure 12), the results on day 0 showed that in fresh samples, the 10%, 20%, and 30% mushroom sausages had no significant differences between each other but were significantly different from the 40% and 50%, indicating a significant decrease in a* value as the percentage increased from 10% to 50%. The mean values for the 10% mushroom content were 3.07 ± 0.12, while for the 50% mushroom content, they were 1.94 ± 0.23. In blanched samples, similarly, the 50% showed a significantly lower a* value (1.29 ± 0.24) than the 10% (3.56 ± 0.33). Also, for steam-pretreated samples, the 10% and 20% showed similar values but were significantly different from all other percentages. As the percentage of mushrooms increased in the sausage, the a* value decreased. Specifically, for the 10% mushroom content, the mean value was 3.45 ± 0.13, while for the 50% mushroom content, it was 1.00 ± 0.50. The results show a consistent decrease in a* values with increasing mushroom content for all samples on day 0. Similarly, for the a* value, it decreased significantly as the percentage of mushrooms in sausage increased from 10% to 50% on day 10. In fresh untreated samples, at 10% mushroom content, the mean value was 4.54 ± 0.20, which decreased to 2.95 ± 0.71 at 50%. In blanched samples, at 10% mushroom content, the mean value was 4.11 ± 0.18, which decreased to 1.15 ± 0.23 at 50%. For steamed samples, at 10% mushroom content, the mean value was 3.77 ± 0.12, which decreased to 1.15 ± 0.23 at 50%. Our results illustrate a lower a* value on both days 0 and 10 for all samples (fresh, blanched and steamed) as the percentage of mushrooms increased in the sausage. Our study's variations in the a* value of the sausage with increasing mushroom percentage were consistent with findings published by Fu et al. (2023), which demonstrated that a* values dramatically decreased as the proportion of Agaricus Bisporus mushrooms in the sausage increased.

On day 0, in fresh untreated samples, there was a significant increase in b* value (Figure 13) between the 10% and 50% mushroom sausages, with the 10% mushroom content showing a mean b* value of 12.60 ± 0.72, while the 50% mushroom content showed a mean b* value of 14.48 ±

0.59. However, the 30%,40% and 50% mushroom content showed no significant difference. While blanch pre-treated samples showed no significant differences between the different percentage, steam pre-treated samples displayed significant differences in b* values between 10% and 50% mushroom content, with the mean value of b* increasing from 13.93 ± 0.64 at 10% to 16.18 ± 1.74 at 50%. On day 10, in fresh, blanched, and steamed pretreated samples, the b* value significantly increased as the percentage of mushroom in sausage increased (Figure 13). For instance, in Fresh samples, at 20% mushroom content, the mean value was 11.99 ± 0.23, significantly increasing to 12.78 ± 0.50 at 50% mushroom content. Similarly, for Blanched samples, at 10% mushroom content, the mean value was 12.63 ± 0.14, significantly increasing to 13.44 ± 0.36 at 50% mushroom content. For Steamed samples, at 10% mushroom content, the mean value was 12.71 ± 0.20, significantly increasing to 14.49 ± 0.56 at 50% mushroom content. Our results demonstrated that the b* values increased with the increase in mushroom percentage in sausage in all samples on both days 0 and 10 except for blanching on day 0 where no significant effect was observed. Our results are consistent with Wang et al. (2018) which observed that adding increasingly straw mushrooms affected the color of the sausages, making them more yellow (b*) and less red (a*). Also, Qing et al. (2020) had similar results where the addition of edible mushroom to beef paste increased the yellowness (b* value). But our results were in contrary to Fu et al. (2023) for the b* value.

## 5.3. Texture Analysis

### 5.3.1. Effect of the different pretreatment within the same percentage of mushroom and storage day on texture parameters

Results of texture analysis on day 0 are displayed on Figure 14, where the texture parameters, force (N) and work (Nmm), showed significantly different mean values after applying the pretreatments (Blanching and Steaming) within the same mushroom percentage in sausages ($P < 0.05$). Compared to untreated samples (Fresh), at the 10%, 20%, 30%, and 40% mushroom sausages, significant differences were observed for the force value between Fresh and both Blanched and Steamed pretreatments. Specifically, the force for Fresh mushroom sausages at 10%, 20%, 30%, and 40% had lower mean values (1.46 ± 0.17, 1.34 ± 0.30, 1.38 ± 0.18, and 1.32 ± 0.29 respectively) than the Blanched (2.31 ± 0.51, 2.19 ± 0.60, 2.18 ± 0.31, and 2.20 ± 0.50 respectively) and Steamed samples (2.65 ± 0.35, 2.65 ± 0.36, 1.96 ± 0.26, and 1.99 ± 0.19 respectively). The

results showed that there was no significant difference between the force value for Blanched and Steamed sausage samples at any of the mushroom content levels.

Compared to Fresh untreated samples, at the 10% mushroom sausages, significant differences were observed for the work value between Fresh and both Blanched and Steamed pretreatments. Specifically, the work value for Fresh samples had a lower mean value (8.91 ± 1.22) compared to Blanched (13.06 ± 2.23) and Steamed samples (14.21 ± 1.33). Additionally, there was no significant difference between the work value of Blanched and Steamed sausages at the 10% mushroom content level. At the 20% mushroom sausages, significant differences were observed among all samples in terms of work value. Steam-pretreated samples showed the highest mean value for work (15.34 ± 1.34), followed by Blanched (11.84 ± 1.53), and then Fresh untreated samples (8.70 ± 1.74). At the 30% and 40% mushroom sausages, significant differences were observed only between Fresh and Blanched-pretreated samples in terms of work value.

Specifically, at 30%, Fresh samples had a mean work value of 9.72 ± 1.39, while Blanched ones had a higher mean work value of 12.38 ± 0.92. Similarly, at 40%, Fresh samples had a mean work value of 9.76 ± 2.08, whereas Blanched ones had a higher mean work value of 13.26 ± 1.85. On day 10 of storage (Figure 15), at the 10% mushroom sausages, the force value for Fresh and Blanched-pretreated samples were not significantly different from each other. However, Steamed-pretreated samples had the significantly highest force value through the sausage samples, with a mean value of 3.37 ± 0.75, followed by Blanched (2.26 ± 0.53), and then Fresh (1.83 ± 0.34). Similarly, the work value for Fresh and Blanched-pretreated samples at 10% mushroom content were not significantly different from each other, with Fresh having a mean value of 12.64 ± 2.42 and blanched having a mean value of 11.94 ± 1.42. However, both Fresh and Blanched samples were significantly different from Steamed-pretreated samples, which had the significantly highest Work value of 18.30 ± 2.16. At the 20% mushroom sausages, significant differences were observed only between Fresh and Steamed-pretreated samples in terms of the force. The mean force value of the sausages was higher for the Steamed pretreatment (2.65 ± 0.29) compared to Fresh untreated samples (1.80 ± 0.47). Our results demonstrated that the force and work increased with steaming and blanching pretreatments. In some percentages, steamed samples had higher values than blanched ones. This means that the hardness of mushroom sausage samples increased with the pretreatments. Our results partially agree with a study done by (Jaworska et al., 2010), where a higher force and lower work values were observed in the blanch pretreated mushroom samples

compared to the fresh in Agaricus bisporus species. In contrary to our results, (Yao et al., 2023) observed that the hardness was reduced after steaming of raw shiitake mushrooms was done.

[4]

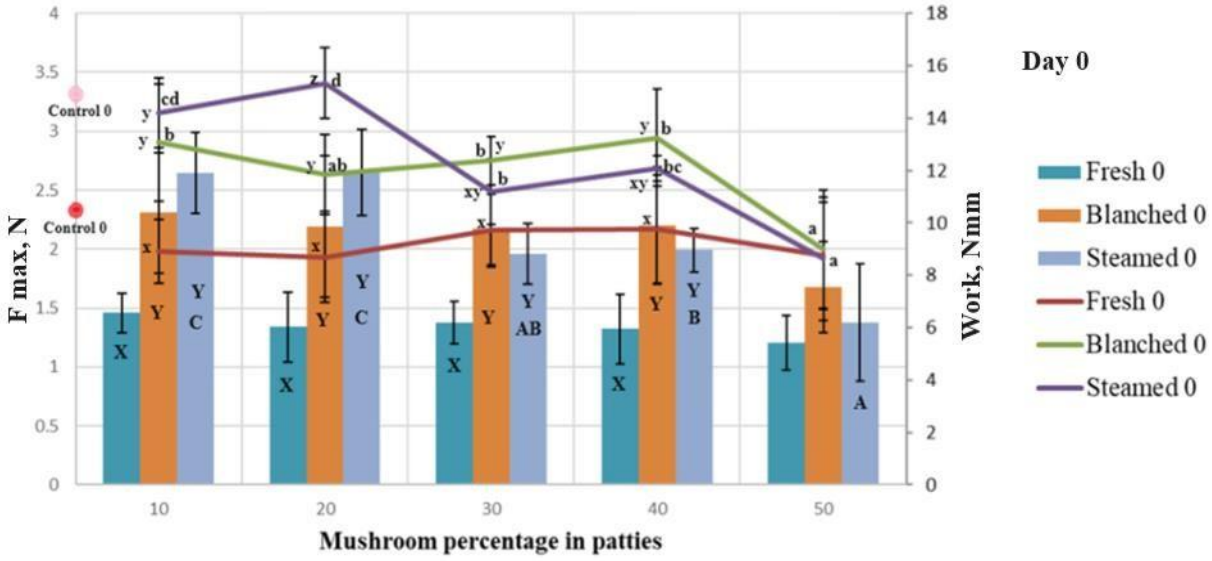



**Figure 14.** Hardness (Fmax, N) of the mushroom sausage samples for the different pretreatments and increasing mushroom percentage on day 0.

[4] A-C for Force, a-d for work : Mean values with different letters differ significantly among the increasing percentage of mushroom in sausage within the same pretreatment ( P< 0.05) on day 0.

X-Y For Force, x-z for work: Mean values with different letters differ significantly among the pretreatments of mushroom sausages within the same mushroom percentage ( P< 0.05) on day 0.

5

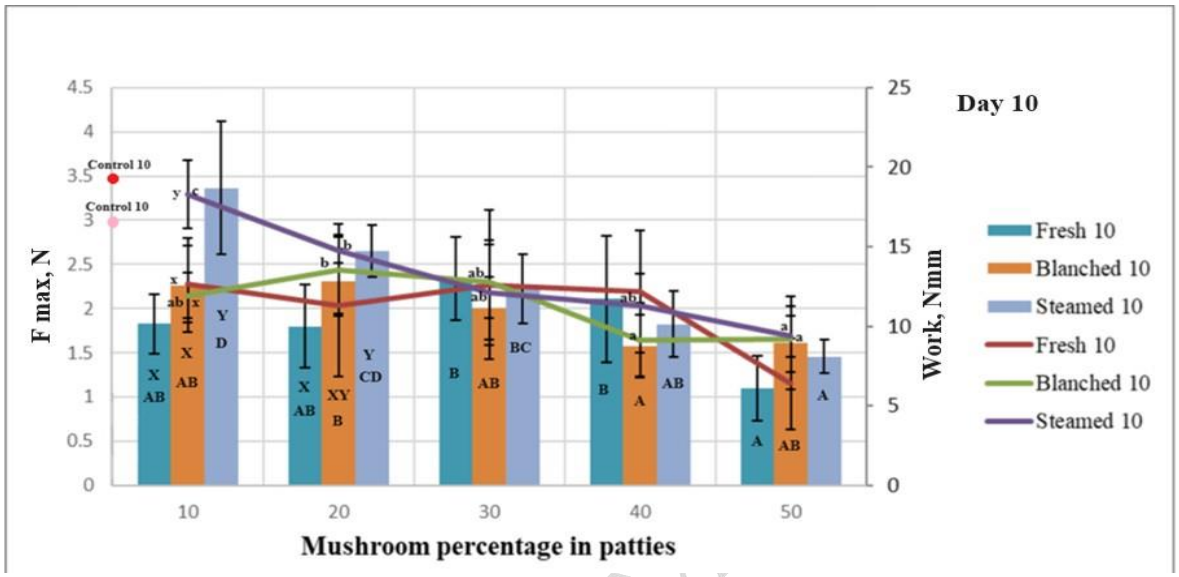



**Figure 15.** Texture analysis of the mushroom sausage samples for the different pretreatments and increasing mushroom percentage on day 10.

### 5.3.2. Effect of mushroom percentage within the same pretreatment and storage day on texture parameters

On day 0 (Figure 14), the force value in steamed samples showed a significant decrease, as the percentage of mushrooms increased in sausages from 10% to 50%. At 10% mushroom content, the mean force value was 2.65 ± 0.35, which decreased significantly to 1.37 ± 0.50 at 50% mushroom content in sausages. However, no significant differences were observed for both fresh and blanched samples among the different percentages of mushrooms. Similarly, for the work value in steamed and blanched samples, as the percentage of mushroom increased in sausages, the work value significantly decreased. For blanched samples, at 10% mushroom content, the mean work value was 13.06 ± 2.23, which decreased to 8.99 ± 2.25 at 50% mushroom content. For

[5] A-D for Force, a-c for work : Mean values with different letters differ significantly among the increasing percentage of mushroom in sausage within the same pretreatment ( P< 0.05) on day 10.

X-Y For Force, x,y for work: Mean values with different letters differ significantly among the pretreatments of mushroom sausages within the same mushroom percentage ( P< 0.05) on day 10.

steamed samples, at 10% mushroom content, the mean work value was 14.21 ± 1.33, which decreased to 8.63 ± 2.36 at 50% mushroom content. However, no significant differences were observed for the fresh untreated samples among the different percentages of mushrooms. On storage day 10 (Figure 15), the force value for fresh samples showed a significant decrease only between the 30% and 50% mushroom sausages, where at 30% mushroom content, the mean value was 2.34 ± 0.48, which decreased to 1.10 ± 0.37 at 50% mushroom content. For blanched samples, a significant decrease was observed between 20% (2.31 ± 0.37) and 40% (1.57 ± 0.35) mushroom content. For steamed samples, a significant decrease was observed between all percentage values from 10% (3.37 ± 0.75) to 50% (1.46 ± 0.19). This indicates that the force value was significantly decreasing and varying with the increase of mushroom percentage in sausages. For the work value, no significant differences were observed for the fresh samples among the different percentages of mushrooms. However, for blanched and steamed pretreated samples, the work value decreased with the increase of mushroom content in sausages. For blanched samples, a significant decrease was observed when comparing the work value at 20% mushroom content (13.51 ± 2.89) to that at 50% (9.17 ± 2.08). Similarly, for steamed samples, at 10% mushroom content, the mean work value was 18.30 ± 2.16, which significantly decreased to 9.37 ± 1.28 at 50% mushroom content. Our results depicted that as the percentage of mushrooms increased in sausages, the texture parameters decreased for both steamed and blanched samples. The fresh samples only showed a decrease in force on day 10 between 30 and 50% mushrooms. This result is in line with the results reported by Fu et al. (2023), which indicated that the inclusion of Agaricus bisporus decreased the TPA parameters of sausages with the increase in mushroom percentage .

### 5.4. Weight loss percentage analysis

#### 5.4.1. Effect of the different pretreatment within the same percentage of mushroom and storage day on weight loss

As displayed on Figure 16, on day 0, significant differences in weight loss were observed for the pretreated samples compared to Fresh samples within the same percentage of mushrooms in sausages. For all mushroom percentages (10% to 50%), Fresh samples showed significantly higher weight loss % compared to both Blanched and Steamed samples. However, no significant difference was found between Blanched and Steamed sausages at any mushroom percentage. At 10% mushroom content, Fresh sausages had a higher weight loss percentage (17.65±3.07)

compared to Blanched (7.96±0.70) and Steamed (5.83±0.79) samples. Similarly, at 20% mushroom content, Fresh sausages had a higher weight loss percentage (22.86±1.34) compared to Blanched (8.60±1.08) and Steamed (6.99±1.40) samples. At 30% mushroom content, Fresh sausages had a higher weight loss percentage (31.90±1.78) compared to Blanched (10.11±1.05) and Steamed (10.22±3.85) samples. At 40% mushroom content, Fresh sausages had a higher weight loss percentage (26.50±2.14) compared to Blanched (10.04±2.14) and Steamed (8.05±2.25) samples. Finally, at 50% mushroom content, Fresh sausages had a higher weight loss percentage (26.34±4.07) compared to Blanched (8.69±1.49) and Steamed (7.74±1.99) samples. Results of weight loss on day 10 are represented in Figure 17. On storage day 10, no significant differences were observed between the pretreatments for the different percentages of mushroom. Our results indicated that steaming and blanching were similar in terms of weight loss but were significantly lower than fresh. So, these two thermal pretreatments decreased weight loss in sausage samples. This can be explained by water absorption by the mushrooms during the heat pretreatment where (Vullioud et al.,2011) explains that weight gain is observed during blanching process of mushrooms and causes a decrease in weight loss.

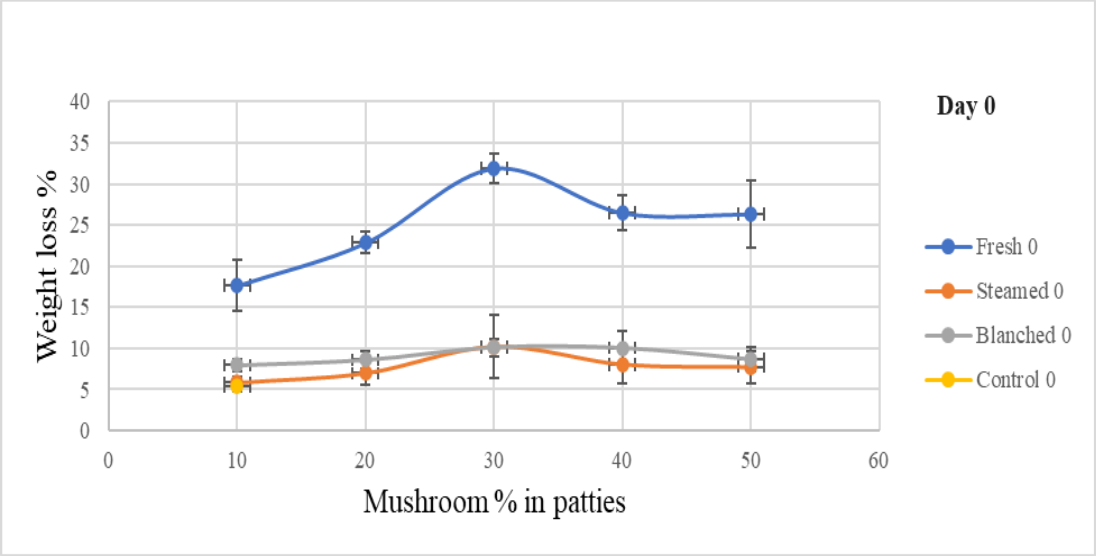



**Figure 16.** Weight loss percentage mean value for the different pretreatments and increasing mushroom percentage in sausage samples on day 0.

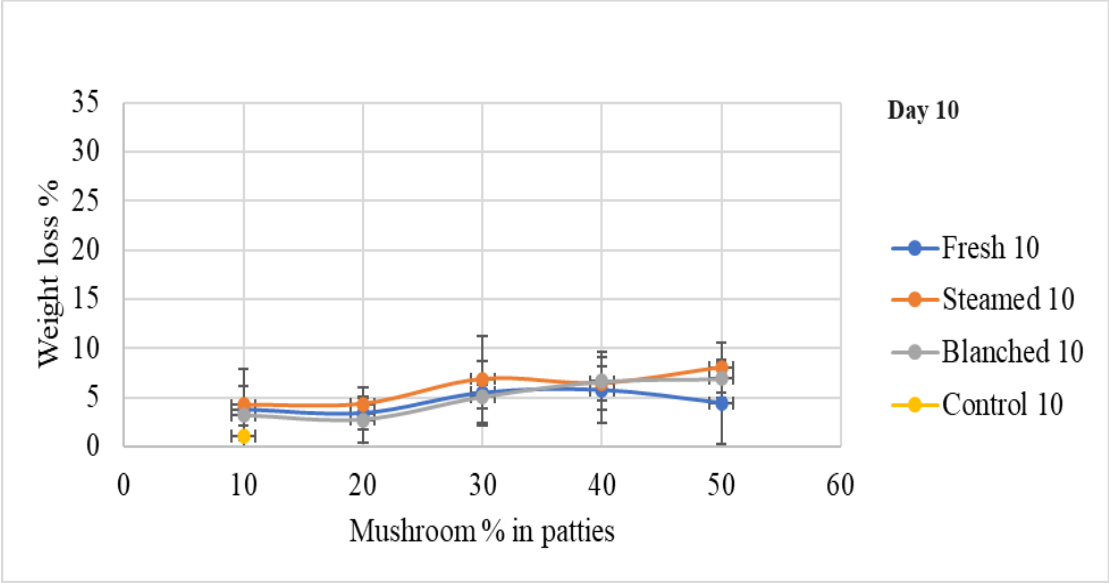



**Figure 17.** Weight loss percentage mean value for the different pretreatments and increasing mushroom percentage in sausage samples on day 10.

### 5.4.2. Effect of mushroom percentage within the same pretreatment and storage day on weight loss

Based on Figure 16, on day 0, for blanch pre-treated samples, no significant differences were observed in weight loss (%) among the different mushroom percentages in sausage samples. For weight loss %, in fresh samples, as the percentage of mushroom increased in the sausage, the weight loss % increased significantly. At 10% mushroom content, the weight loss mean value was (17.65±3.07), which increased to (26.34±4.07) at 50%. Similarly, for the steamed samples, significant differences in weight loss mean values were observed between the 10% and 30% mushroom content. At 10% mushroom content, the mean value was (5.83±0.79), which increased to (10.22±3.85) at 30% mushroom content. All other mushroom sausage percentages had mean values that lay in between the 10% and 30% mushroom sausages. On storage day 10 (Figure 17), for blanch pre-treated samples, an increase in the percentage of mushrooms from 10% to 50% resulted in a significant increase in the weight loss %. Specifically, sausages with 10% had a mean value of (3.17±1.10) and those with 20% had a mean value of (2.73±2.28), both showing significantly lower weight loss % compared to those with 40% (6.63±2.94) and 50% (6.90±1.95) mushroom content. Conversely, no significant differences were observed for weight loss (%) in fresh and steam pre-treated samples. In our study, weight loss increased with the increase of

mushroom percentage in both fresh and steamed samples on day 0 while on day 10 only blanched samples showed significantly higher differences among the increasing mushroom percentage. Our results were consistent with those of Fu et al. (2023), who reported that the cooking loss of the substitution groups increased dramatically in parallel with increases in Agaricus bisporus mushroom inclusion rates. The significant cooking loss in substitution groups may be due to the higher water content of Agaricus bisporus mushrooms than chicken breast meat.

### 5.5. Effect of Storage

The findings of one sample t-test indicated that the storage impact was statistically significant for the different parameters studied (Color, weight loss, and texture parameters) among the different pretreatments and mushroom percentage in sausage samples.

## 6. Conclusions and Suggestions

The results of this study suggest a viable direction for innovation in the meat processing industry by emphasizing the possibility of replacing pork meat in sausages with fresh oyster mushrooms. The impacts of several pre-treatments and mushroom percentage have been carefully examined, and significant impacts on parameters related to color, texture, and weight loss have been identified. Both pretreatments (steaming and blanching), the increasing mushroom percentage, and storage days played a significant role on the quality of sausages.

The observed increase in lightness (L*) value following blanching, particularly on day 10, suggests the efficacy of enzymatic browning inhibition. Conversely, the consistent lightness value with steaming pretreatment indicates a less impact on color. The decrease in lightness (L*) and redness (a*) values with increasing mushroom percentage underscores the mushrooms' darker color and higher moisture content relative to meat, influencing overall color attributes. Both blanching and steaming resulted in decreased redness (a*) values, due to pigment breakdown and surface texture alterations. The yellowness b* value of the sausage samples increased with the increase in mushroom percentage and with steaming. However, no significant effect was observed with blanching on day 0 even with increasing mushroom percentage. This shows that blanching has less impact on color compared to steaming.

The texture analysis showed that maximal force and work values were increased by pretreatments but decreased with higher mushroom percentages, reflective of mushrooms' softer texture. Moreover, the increase in weight loss with higher mushroom content, despite decreased losses with steaming and blanching, underscores the complex dynamics of moisture retention and structural changes in mushrooms during cooking. The mushroom sausages were influenced by storage days where day 10 had less impact on the different parameters especially for texture and weight loss.

Overall, our study contributes to a deeper understanding of sausage formulation and quality enhancement through mushroom substitution, paving the way for continued advancements in the field of meat alternative products. Still, further research is necessary to determine the ideal mushroom percentage for a high-quality sausage, especially in the form of sensory analysis that considers flavor, aroma, visual appeal, and customer acceptability as a whole.

## 7. Summary

This study investigates substituting pork meat in sausages with fresh oyster mushrooms, suggesting potential innovation in the meat processing industry. It examines various pre-treatments and mushroom proportions, finding significant impacts on color, texture, and weight loss. Blanching inhibits enzymatic browning, increasing lightness values, while steaming shows consistent color impact. Higher mushroom content softens texture but increases weight loss. Storage duration also influences sausage quality. Overall, this research contributes to understanding sausage formulation and quality enhancement through mushroom substitution, with further sensory analysis recommended for optimal mushroom percentage determination.

## 8. Acknowledgments

I am deeply grateful to Professor Kenesei György for his steadfast support and invaluable guidance throughout the writing of this thesis. Without his feedback and assistance, this work would not have been possible. I also extend my heartfelt thanks to the Hungarian University of Agriculture and Life Sciences for providing the necessary resources for the experimental phase of this research.

To my colleagues and friends, your encouragement has been a constant source of motivation. Thank you for standing by me through this journey.

Lastly, I owe an immense debt of gratitude to my family. Their love, prayers, and support have been the bedrock of my academic endeavors. Mom and Dad, your belief in me has been my greatest strength.

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## List of Figures

## List of Tables