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Altin Berisha

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

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Supervisor: Dr. Kenesei György

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Head of department	Supervisor
Dr. Friedrich László	Dr. Kenesei György

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1.Introduction

The global food landscape is witnessing a paradigm shift towards sustainable and healthier dietary choices, driven by concerns over environmental impact, animal welfare, and human health (Farr-Wharton, Foth, & Choi, 2014; Willett et al., 2019). In this context, the exploration of alternative protein sources has garnered significant attention to address these pressing challenges (Lynch, Johnston, & Wharton, 2018). One such promising candidate is the oyster mushroom (Pleurotus spp.), renowned for its nutritional profile, culinary versatility, and potential as a meat substitute (Cheung, Cheung, & Ooi, 2003; Dubey, Upadhyay, & Gidwani, 2019).

The quest for viable meat replacements in processed foods like sausages and burger patties has intensified, propelled by consumer demand for plant-based options without compromising taste, texture, or nutritional value (Asioli et al., 2017; Hartmann & Siegrist, 2017). Oyster mushrooms emerge as a compelling solution, offering a rich source of protein, fiber, vitamins, and minerals, coupled with a meat-like texture and umami flavor profile (Cheung et al., 2003; Dubey et al., 2019).

However, the successful integration of oyster mushrooms into meat-based products necessitates careful consideration of various factors, including processing techniques to enhance their functional properties and sensory attributes. Among these techniques, pretreatment methods such as ultraviolet (UV) irradiation and high hydrostatic pressure (HHP) have emerged as promising strategies to optimize the suitability of mushrooms for incorporation into processed foods.

UV irradiation and HHP represent innovative pretreatment methods that hold immense potential for modifying the physicochemical and structural characteristics of oyster mushrooms. UV irradiation, known for its ability to induce biochemical changes through the activation of secondary metabolites, can enhance the nutritional profile and flavor profile of mushrooms while potentially mitigating microbial contamination (Cheung et al., 2003; Gharibzahedi & Smith, 2018). Similarly, HHP, operating at elevated pressures, can induce structural modifications within the mushroom tissue, resulting in improved texture, shelf stability, and overall product quality (Gharibzahedi & Smith, 2018; Torres et al., 2019).

Against this backdrop, this thesis aims to investigate the efficacy of UV irradiation and HHP pretreatment techniques in enhancing the suitability of oyster mushrooms as meat replacements in

sausage and burger patty formulations. Through a comprehensive analysis encompassing color, weight loss, and texture parameters, this research endeavors to elucidate the impact of pretreatment on the physicochemical properties and sensory characteristics of the final products.

The findings of this research hold substantial implications for the food industry, offering valuable insights into novel strategies for formulating healthier and more sustainable processed foods. By elucidating the potential of oyster mushrooms as meat substitutes and the efficacy of pretreatment methods in optimizing their functionality, this study contributes to the ongoing discourse on alternative protein sources and sustainable food production practices.

2. Aim of the study

The main aim of this study is to comprehensively examine the impact of replacing pork meat with fresh oyster mushroom on several product characteristics of sausages. The characteristics encompass color, weight, and texture. Furthermore, the objective of this study is to evaluate the effects of several pretreatment techniques, including UV (Ultraviolet) pretreatment and HHP (High Hydrostatic Pressure), on the characteristics of oyster mushrooms and their subsequent implications for sausage production.

3.Literature review

3.1 Oyster Mushroom

Mushrooms are types of macro fungus, have unique fruiting bodies that can be epigeous or hypogenous. They lack chlorophyll, making them reliant on dead and decaying saprophytes (Chang., 1992; Oie, 2005). Mushrooms can help address global issues like food demand, unemployment, and environmental pollution. They produce high-quality, high-value food suitable for various age groups. Intensive cultivation may result in significant loss of product due to increased pest and disease incidents. To avoid or reduce risks associated with mushroom cultivation, it is crucial to understand common diseases and control them promptly and appropriately. With the growing global interest in organic food, proper precautions should be taken to reduce or avoid the use of chemical fertilizers (Nongthomban et al., 2021). Edible mushrooms are a crucial food source due to their easy digestibility and nutritional value. They are superior to vegetables and fruits but may be inferior to dairy products and meats (Aemu et al., 2009; Koyyalamud et al., 2009; Sharma et al., 2013). Mushrooms are also high in protein and have lower calorific value, making them suitable for heart patients. They have high mineral content, including phosphorus, potassium, calcium, iron, and copper. They are also rich in vitamin B and vitamin D. Edible mushrooms can be used as a substitute for meat in various dishes and can be used as a substitute in making stews (Nongthomban et al., 2021; Randive, 2012; Sivrikaya et al., 2002). Mushroom cultivation began in France around 1630, and since then, numerous experiments have been conducted to monitor growth parameters (Bernart, 2005). The optimal temperature for oyster mushroom growth is 20° to 30°C and humidity between 55-70% for 6-8 months per year (Abulude and Muhammed, 2013; Chang and Wasser, 2017). Mushroom cultivation during summer months requires extra humidity for growth and development. Mushroom production involves multiple steps and is typically grown in natural caves or well-controlled growth chambers. Substrate preparation is crucial for reducing disease occurrence and improving yield. Organic materials like sawdust, rice bran, rice straw, wheat bran, and wheat straw are used for this purpose. Sugarcane bagasse is pasteurized to eliminate potential competitors like Trichoderma spp. and Pleurotus spp., and benomyl-treated water is used to suppress Trichoderma spp. These treatments can be applied to both commercial and small-scale production of oyster mushrooms. Spawn, a substrate used for

mushroom propagation, is used as a seed for mycelium development. It can be prepared from wheat, sorghum, barley, and rice, with sorghum being a better mycelium carrier. The adoption of spawn can increase yield and biological efficiency, while reducing spawn running time. These treatments can be applied to both commercial and small-scale production of oyster mushrooms. The quality of carrier and moisture significantly impacts the growth and colonization of mycelium on substrates. The spawned substrate needs a temperature between 25-30°C and a dark room for proper growth and colonization. Incubation and fruiting require optimal temperatures between 20-250C and a minimum of 8-12 hours of sunlight. After harvesting, bags are kept in a growing chamber to allow other mycelium to grow and produce more fruiting bodies. Harvested mushrooms are packed in perforated polythene bags for marketing. In a period of one and a half months to two months, 500-700 kg of fresh mushrooms can be harvested from 1 ton of paddy straw.

Despite over 300 mushroom genera, only a few species are commercially cultivated. Understanding the causes of diseases is essential for controlling them and reducing the use of chemical fertilizers in mushroom cultivation. Oyster mushroom (Pleurotus ostreatus) belongs to the family Agaricaceae and class Basidiomycetes (also known as 'Dhingri' in India) (Randive, 2012). It is also known for its medicinal value in fighting diabetes and cancer, as well as its high potassium to sodium ratio, making it ideal for heart disease and hypertension. Oyster mushroom has no cholesterol content and can cure anemia due to its folic acid content. Mushroom species have a variety of metabolites, including antitumor, antioxidant, antigen toxic, antiplatelet aggregating, antihyperglycaemic, antimicrobial, and antiviral activities. Oyster mushroom species, such as Pleurotus ostreatus, have antitumor activity, while Pleurotus cystidiosus has strong antioxidant properties (Nongthomban et al., 2021; Oie, 2005; Randive, 2012; Sivrikaya et al., 2002).

The production of Pleurotus mushroom has significantly increased in recent years, reaching 6,288 tons (618%) from 876 tons in 1997 to 2010 (Royes 2014). This growth is attributed to its higher biological efficiency, low-cost production methods, and the ability to cultivate them on various substrates. Pleurotus is a significant commercially cultivated mushroom due to its nutritional properties and medicinal properties, which are used for various health applications (Mane et al.,2007; Rosado et al., 2002). Traditional medicinal properties of mushrooms, particularly in East Asian countries, have been well documented. The genus Pleurotus has a unique flavor and

aromatic properties, rich in carbohydrates, protein, vitamins, minerals, and fiber. Several species of *Pleurotus*, including *P. citrinopileatus, P. cornucopiae, P. cystidiosus, P. djamour, P. eryngii, P. euosmus, P. ostreatus, P. pulmonarius, and P. rhodophyllus*, are cultivated in markets. China is the major producer *of P. ostreatus*, which was first cultivated in the USA in 1900. Other species like P. sajor-caju were initially cultivated in India after the late 1940s.

3.2 Nutrition Value of Oyster mushroom

Oyster mushroom cultivation has gained popularity due to its medicinal properties and adaptability to various agro-climatic conditions on agricultural waste. Mushrooms can be used as biofertilizer, animal feed, and biogas production, making them eco-friendly. However, cultivation depends on factors like temperature, humidity, and substrate sterility. Infections on mushrooms are facilitated by cultivation conditions and pest presence, with bacterial and fungal-originating diseases and fungal viruses like mycoviruses being common challenges.

Pleurotus mushrooms are a profitable cash crop, with carbohydrates constituting between 50% and 60% of the dry matter (Kalac 2012; Vaz et al., 2011). These mushrooms contain both high and low molecular weight carbohydrates, with high molecular weight carbohydrates being polysaccharides like chitin and glucan, and low molecular weight carbohydrates being monosaccharides, disaccharides, and sugar alcohols (polyols) (Zou et al., 2016). According to Tolera and Abera (2017) the carbohydrate content of *P. ostreatus* is significantly affected by the drying method, with oven-dried mushrooms having a higher carbohydrate value (43.64%) than open sun-dried mushrooms (39.99%). Mushrooms also contain higher protein content than many vegetables and essential amino acids, making them an alternative to animal meat (Gonzalez et al., 2020; Wani et al., 2010). The protein content of mushrooms depends on the species, substrate composition, harvesting time, and pileus size. Tolera and Abera (2017) reported that oven-dried mushrooms have a lower protein content (24.99%) than open sun-dried mushrooms (27.14%). Mushrooms are rich in essential amino acids, particularly inessential amino acids like lysine and leucine, which are lacking in most cereal foods (Chang and Buswell, 1996). Pleurotus mushrooms have lower fat concentrations compared to their carbohydrate and protein contents (Deepalakshim and Mirunalini, 2014), with the main fatty acid being linoleic acid (Naraian and Baharti 2017). This

high concentration makes mushrooms a nutritionally healthy food, as they have a low risk of plaque formation in blood vessels.

Mushrooms also contain dietary fibers, such as polysaccharides and chitin, which are indigestible food components with various nutritional and physiological benefits (Deepalakshim and Mirunalin, 2014). Mineral levels in wild edible mushrooms are affected by the interval between the formation and age of mycelium, and mineral elements are unevenly distributed within the fruitbody. Some elements are toxic, and mushrooms absorb heavy metals from soil. Trace elements such as arsenic, barium, cobalt, copper, rubidium, silver, thallium, and vanadium are observed in edible mushrooms (Svoboda and Charstny, 2007). Pleurotus also contains several vitamins, such as Thiamine, Riboflavin, Niacin, Folic acid, and Ascorbic acid.

3.3 Healthy Benefits of Oyster Mushrooms

Osyster Mushrooms offer numerous health benefits, including anti-tumor, anti-cancer, antibacterial, antifungus, anti-viral, anti-inflammatory, genoprotective, anti-oxidant, immuno-modulatory, anti-diabetic, anti-allergic, anti-mitogenic, anti-hypertensive, and anti-hypercholesterolemic properties. Mushrooms have shown antitumor and anti-cancer activities, with hot water extracts from the fruiting bodies of the family polyporaceae showing host-mediated antitumor activity against Sarcoma (S-180) (Choi et al., 2004; Choi et al., 2013). Ethanol extracts show antitumor activity towards lung cancer cells (A549). Bioactive compounds like ergosterol, glucans, aminoacids (arginine and glutamine), and proteoglucans have been correlated with antitumor activities. Examples include lactin isolated from *P. citrinopileatus* that exhibits antitumor activity in mice Sarcoma (S-180) and protein fractions extracted from P. ostreatus that show antitumor activity against different tumors of mice (Li et al., 2008). Pleurotus mushrooms also have antibacterial activity depending on the solvent used to extract the compounds. Ethanol extracts of *P. florida* are more effective against Streptococcus sp., Escherichia coli, Klebsiella pneumonia, Salmonellatyphi, Klebsiella pneumonia, Vibriocholera sp., Klebsiella oxytoca, and Proteus murabilus. Petroleum ether extracts (PE) and acetone extracts of P. ostreatus are effective antimicrobial agents for Bacillus subtillis and Escherichia coli (Aykuz and Kirbag, 2009; Iwaloku et al., 2007; Thillaimharani et al., 2013). Pleurotus mushrooms also exhibit bioprotective and nephroprotective activities, such as reducing DNA damage in lung cells of Chinese hamsters and

suppressing DNA damage in artificially mutated Drosophila. P. ostreatus extract has been found to reduce cadmium levels in renal tissues and restore DNA fragmentation in rats (El-Bohi et al., 2005; Taira et al., 2005). Antioxidants, such as ethanol extracts of *P. florida, P. cystidiosus*, and *P. ostreatus*, protect cells from damage by free radicals. Immuno-modulatory activities of Pleurotus mushrooms are modulated by various factors, including cytotoxic T cells, activated macrophages, natural killer (NK) cells, oxygen intermediates, reactive nitrogen, tumor necrosis factors, and interleukins (Elkhateeb and Daba, 2021; Thillaimharani et al., 2013; Yang et al., 2002). Consistent consumption of P. ostreatus may also improve kidney functions (Ravi et al., 2013).

3.4 Oyster Mushrooms -Based Meat Alternatives

In contemporary society, there's a growing emphasis on reducing meat consumption and developing new meat alternatives that prioritize health and sustainability. Mushrooms are increasingly recognized as a promising source of bioactive compounds for creating healthier meat products. With their natural antimicrobial and antioxidant properties, mushrooms can extend the shelf life of meat products while also enhancing their nutritional profile. By incorporating various types of mushrooms, meat products can see significant improvements in protein, dietary fiber, and mineral content without compromising their physical and chemical characteristics. Additionally, mushrooms, with their abundance of dietary fiber, easily digestible protein, and meat-like texture, offer a compelling option for replacing traditional additives like salt, phosphates, protein, and fat in meat formulations. Furthermore, the high levels of free amino acids in mushrooms contribute to the overall sensory appeal of meat products. (Perez-Montes et al., 2021)

Incorporating mushrooms as blends in meat products presents a promising strategy to reduce meat content while enhancing nutritional value. Mushrooms offer high protein and dietary fiber content, along with a meat-like texture and umami flavor, making them suitable meat substitutes. Various studies have successfully replaced meat with different mushroom species, such as Pleurotus ostreatus and Agaricus bisporus, in patty formulations and dishes like taco filling and fish patties. Although mushrooms soften products and increase moisture, they are well-accepted, especially when moderate meat percentages are substituted, providing additional dietary fiber. However, the perishable nature of fresh mushrooms necessitates drying procedures to extend shelf life, though

this may alter nutritional value and taste. Incorporating dried mushrooms in meat products, such as chicken frankfurters and pork sausages, has shown promising results, with slight texture modifications and increased dietary fiber content. Additionally, replacing meat with dried mushroom powder can improve water holding capacity and reduce cooking losses, resulting in softer nuggets. However, careful consideration of mushroom concentration is crucial to avoid undesirable texture and taste changes. (Rangel-Vargas et al., 2021)

3.5 Meat replacement trends

The intake of meat is generally seen unfavorably due to its adverse impacts on the environment, agriculture, slaughter, blood, and some religious practices. A growing number of customers are seeking sustainable food options and ecologically conscious methods of food production, which is inspiring others to embrace a vegetarian or vegan diet or decrease their weekly consumption of meat (Dagevos & Voordouw, 2017).

The meat analogues, which are plant-based foods that possess the organoleptic and chemical attributes of conventional meat products, show significant potential (Joshi & Kumar, 2015).

While not all meat analogs fall under the category of ultra-processed meals, a significant number of contemporary meat analog items available in the present food industry can be classified as that. According to Bohrer (2019) and Monteiro et al. (2013), ultra-processed foods can be characterized as food products that contain minimal or no whole foods, and instead rely on processed ingredients or substances that have been extracted or refined from whole foods. These processed ingredients may include protein isolates, oils, hydrogenated oils and fats, flours and starches, sugar variants, refined carbohydrates, and other value-added ingredients.

Consumers have valued modern meat analogues for their ability to meet consumer expectations by providing the appearance, quality, taste of meat, while alleviating the reluctance some consumers have with traditional meat production such as environmental concerns and animal welfare issues (Bohrer, 2019). Although there are objective arguments in favor of more environmentally sustainable consumption, a large proportion of consumers are still not really concerned about ethical considerations. The conditions under which animals are transported and slaughtered are rarely considered by buyers when purchasing food. Lower meat consumption

would significantly reduce the need to use natural resources (water, food, etc.) as well as the emissions associated with meat production (Wilkinson, 2011).

Another reason is that the ecological pressure on arable land is great due to lack or abundance of water supply, crops are becoming more and more doubtful. According to the forecast of the International Institute for Sustainable Development, the number of people on our planet will reach 9.9 billion by 2050 (International Institute for Sustainable Development, 2020). If such a mass were to be supplied with meat, it would lead, among other things, to the complete destruction of forests, a further increase in the amount of dead zones in the ocean and the amount of greenhouse gases emitted into the atmosphere, and catastrophic climate change. Thus, the benefits of reducing meat consumption are manifold in terms of nutritional and sensory characteristics. Numerous environmental studies have been conducted with protein-rich products, including plant-based meat analogs (soybean, green pea, lupine, rice, etc.), animal proteins (milk, meat, insects, lab-produced) and mycoproteins. Most of them have shown that plant-based meat analogues have less environmental impact than meat (Kyriakopoulou et al., 2019). For example, the production of soy and gluten-based meat analogues has been shown to be more environmentally friendly than chicken and even laboratory- and mycoprotein-based meat substitutes (Smetana et al., 2015), while pea-based products greens have been shown to be more suitable than pork (Zhu & Ierland, 2010).

3.7 High Hydrostatic Pressure (HHP)

High Hydrostatic Pressure (HHP) treatment is a non-thermal processing technology that is widely used in the food processing field (Tottes Bello et al., 2014). The food processing industry has been converting industrial equipment for food use since the 1990s, with two compression types available: direct compression and indirect compression. Direct compression involves intruding a piston into a vessel to reduce volume and raise pressure, while indirect compression uses an exterior compression system to pressurize pressure medium (Yamamoto., 2017). Vertical and horizontal types are commercialized for industrial applications (Figure 1). In the early days of HHP food processing, the vertical type was dominant, but recent installments are of the horizontal type. Large vessels are required for HHP food processing, which requires increasing either the diameter or length. Larger diameters require thicker vessel walls, while longer vessels ensure larger volume and higher height. Hybrid horizontal equipment requires water as pressure medium to be drained from the vessel after each treatment, but a high-speed pump recycles most of the drained water, minimizing water loss (Yamamoto., 2017).

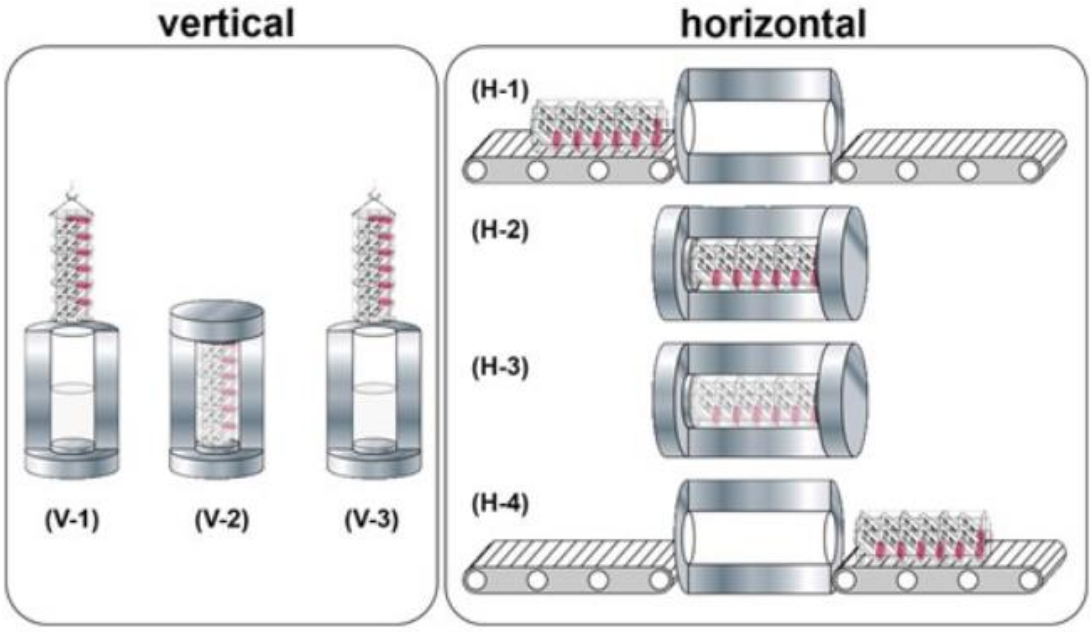

Figure 1. Vertical and horizontal installation of HHP in food processing: V-1, Pouched food in the basket is dipped in water (pressure medium) by crane; V-2, the lid is closed and HHP is applied; V-3, the lid is opened and the food is taken out by crane; H-1, the food in the basket is conveyed into the vessel; H-2, the lid is closed and the vessel is filled with water by water pump; H-3, HHP is applied by HHP pump; H-4, pressure is released, water in the vessel is recovered, the lid is opened, and the food is conveyed out (Source: Yamamoto et al., 2017)

High hydrostatic pressure is characterized by a minimal impact on food characteristics such as sensory, nutritional, and functional properties (Barba et al., 2012). Typically, food is subjected to an HHP level of 100 MPa or higher. Unlike conventional heat processes, which can damage food components associated with color, flavor, and nutrition through intensified chemical reactions, HHP minimizes damage and renders bacteria inactive, facilitating the production of high-quality and safe meals (Martin et al., 2002; Torres Bello et al., 2014; Yamamoto., 2017). The commercialization of HHP-processed foods began in 1990, initially with fruit products such as jams. Subsequently, various other products were introduced to the market, including retort rice products with enhanced water impregnation, cooked hams and sausages with extended shelf life, soy sauce with reduced salt content due to enhanced enzymatic reactions, and beverages with extended shelf life (Yamamoto et al., 2017).

HHP treatment can effectively inactivate bacterial cells and many enzymes, making it very attractive to consumers who value the organoleptic characteristics of products processed by this non-thermal food preservation technology. A study by Ma et al. (2021) investigated the effects of HHP treatment on the physicochemical properties, texture parameters, and volatile flavor compounds of oysters. The results showed that HHP treatment increased the water content while reducing the crude protein and ash content of the oyster. Texture parameters showed that HHP treatment improved the hardness, springiness, chewiness, and cohesiveness of oysters, compared with the control group. Moreover, Braspaiboon and Laokuldilok. (2024) consolidated relevant research findings elucidating the effects of HHP on protein structure, allergenicity, bioactivities, and functional properties across diverse protein sources. They encompass cereals, legumes, nuts, meat, poultry products, milk, eggs, seafood, algae, insects, seeds, and vegetables. The research findings presented by Rodrigues, (2016), which emphasize the usage of HHP treatment to obtain bioactive compounds from natural sources like mushrooms, we can infer that using HHP treatment on oyster mushrooms may enhance the extraction of beneficial compounds. Moreover, high hydrostatic pressure can contribute to the prevention of enzymatic browning in fruits and vegetables, implying that HHP treatment may also aid in preserving the quality and extending the shelf life of oyster mushrooms. Therefore, exploring the impact of HHP treatment on oyster mushrooms could unveil valuable insights into their nutritional content, potential health benefits, and overall quality, thus warranting further investigation in the domain of functional food development.

3.8 Ultraviolet light-emitting diode (UV-LED)

Ultraviolet light-emitting diode (UV-LED) technology has revolutionized the food industry by preserving liquid fruit and vegetable foods at different wavelengths. This non-thermal and non-chemical treatment addresses product stability, quality, and safety during storage. UV-LED treatment affects microbe and enzyme inactivation and improves the retention of bioactive compounds, ensuring better quality (Salazar et al., 2022). Ultraviolet (UV) treatment has also been applied to food packaging, with UV blocking films being used to protect food from photooxidation and maintain its quality attributes (Tripathi et al., 2023). These films absorb, reflect, or scatter UV light, reducing its transmittance through packaging film. Recent advances in UV-C light science and engineering have made it a viable choice for food manufacturers, as it can improve food safety without significant loss in quality or nutrient content (Koutchma, 2009). The historical background of UV treatment in the food industry has evolved significantly, from water disinfection in the late 19th century to its current applications in food preservation and packaging. The integration of historical perspectives with modern technological advancements provides a comprehensive understanding of UV treatment's significance in food processing, shedding light on its transformative potential for food preservation, including oyster mushroom preservation.

The application of UV treatment on mushrooms has been shown to significantly increase their nutritional value, particularly in terms of vitamin D2 content. A study conducted in Thailand by Judparasong et al. (2023) examined the effect and stability of ultraviolet B (UV-B) irradiation on the vitamin D content in commonly consumed mushrooms. The results showed that vitamin D2 in all varieties of mushrooms significantly increased after UV-B irradiation according to the exposure time[1]. The highest level of vitamin D2 was found in enokitake mushrooms. In addition, 25-OH D2 and vitamin D4 contents increased after UV-B irradiation in enokitake mushrooms. The vitamin D2 true retention in all cooked mushrooms ranged from 53 to 89% and was highest in stir-fried mushrooms (Judprasong et al., 2023). Moreover, UV treatment has been found to enhance the phytochemical content of mushrooms, including phenolics, flavonoids, and folic acid (Banlangsawan and Sanoamuang, 2015; Tidke et al., 2024; Zhong et al., 2022) . After 120 minutes of UV treatment, there was a 0.6-fold increase in phenolic content for Agaricus bisporus and a 0.7-

fold increase for Pleurotus ostreatus (Tidke et al., 2024). This enhancement of phytochemical content further contributes to the nutritional value of the mushrooms, making them a more beneficial dietary choice. In addition to enhancing the nutritional value of mushrooms, UV treatment also has implications for the commercial viability of mushroom cultivation. One study suggested that UV-B irradiation for 15 minutes with low energy was the optimum treatment for the production of vitamin D2 recommended per day and also vitamin D2 concentration remained relatively stable in oyster mushrooms during storage (Banlangsawan and Sanoamuang, 2015; Szabo and Gyorfi, 2012). This indicates that UV treatment can be integrated into the cultivation process in a cost-effective manner, paving the way for sustainable and nutritionally enriched mushroom production.

4.Material and Methods

Fresh oyster mushrooms and ground pork were sourced from a local market in Budapest, Hungary. The sausage production took place at the Department of Livestock Products and Food Preservation Technology, Hungarian University of Agriculture and Life Sciences.

To prepare the sausage emulsions, a mixture of meat, fresh oyster mushrooms, sodium nitrate, phosphate, and ice (Table 1) was processed in a cutter (Robot-Coupe R201). The oyster mushrooms underwent initial inspection where damaged parts were removed, and the remaining mushrooms were cleaned, longitudinally sliced, and then subjected to two different pretreatment methods: High Hydrostatic Pressure (H) for 3 min at 20 ºC, 300 MPa (Resato B2441) and Ultraviolet Light treatment (U) for 15 min at 20 ºC, Power: 30 W, 312 nm (VL-115.M) fig.2&3.

Evaluation of the fresh and pretreated mushrooms included assessments of color, texture, and weight after cooking and after 10-day storage periods. Four sausage formulations were prepared, each with increasing proportions of oyster mushroom substituting meat. The sample groups included a control with 0% mushroom substitution, as well as formulations with 10%, 20%, 30%, 40%, and 50% mushroom substitution, labeled as F10-F50. Similarly, formulations with UV pretreated mushrooms were labeled as U10-U50, and those with HHP pretreated mushrooms were labeled as H10-H50.

Prior to heat treatment, the sausage batters were weighed and placed into petri dishes. Heat treatment involved baking the sausages at 80°C for 25 minutes in an oven with steam function (Lainox VE051P). The color, texture, and weight after cooking properties of the sausage samples were assessed on the same day as production. Each treatment was repeated independently twice, with six parallel measurements performed for each sausage sample.

Table 1 Ingredients Composition for Sausage Preparation

Mushroom %	Ice(g)	Meat(g)	Mushroom(g)	Phosphate(g)	Sodium nitrate(g)

10%	160	286.2	38.16	2	9.6
20%	160	254.4	76.32	2	9.6
30%	160	222.6	114.48	2	9.6
40%	160	190.8	152.64	2	9.6
50%	160	159	190.8	2	9.6

Figure 2 UV pretreated mushrooms

Figure 3 HHP pretreated mushrooms

Figure 4 Sample preparation

4.1 Color measurement

The color attributes of the sausage samples were assessed utilizing the CIELAB scoring system, which provides quantitative measures of lightness (L*), redness (a*), and yellowness (b*). These measurements were conducted employing a CR-410-type colorimeter manufactured by Konika Minolta Sensing Inc., Japan. Before each measurement session, the colorimeter underwent calibration procedures using a white standard plate (CRA43) to ensure accuracy and consistency in the color readings.

4.2 Weight measurement

The sausage batter was meticulously prepared and subsequently dispensed into individual petri dishes for uniformity. Prior to any cooking processes, the weight of each sample was meticulously measured using a digital scale, with an approximate weight of 80 grams per sample. This initial weight measurement served as a baseline for assessing weight loss throughout subsequent stages. Following the cooking procedure, the samples were once again subjected to weight measurement to ascertain any alterations incurred during the cooking process, thereby facilitating a comprehensive evaluation of weight loss. Furthermore, to investigate potential weight variation over time, the samples underwent additional weight measurements after a period of 10 days of storage. These sequential weight assessments aimed to provide insights into the dynamics of weight fluctuation from preparation through to storage, thereby enriching the understanding of sausage product stability and quality attributes.

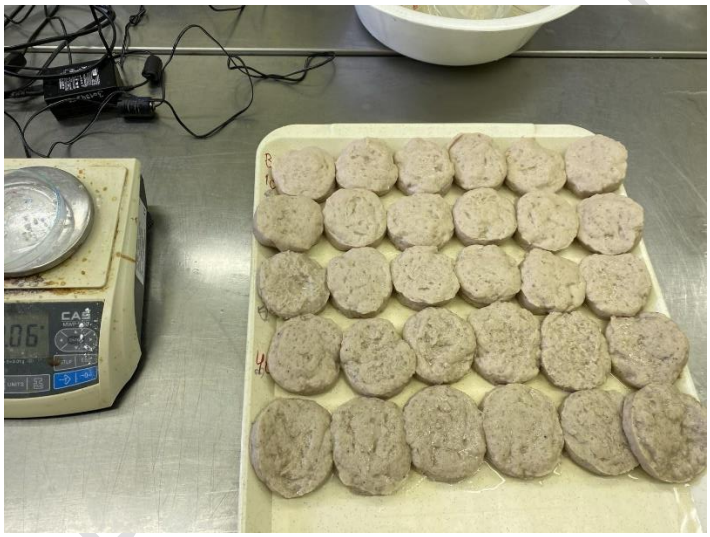

Figure 5 Weight measurement of each sample

4.2 Texture measurement

The textural properties of sausage samples were evaluated utilizing a TA. XT Plus texture analyzer manufactured by Stable Micro Systems, Surrey, United Kingdom. Shear force analysis was conducted by slicing samples with a Warner-Bratzler with 5 mm Czl stainless rod, at a consistent speed of 2 mm/s both prior to and during measurement, with a set distance of 20 mm. Force (N)

was recorded over time or distance. The maximum peak force observed on the resultant graph was identified as the shear force value, indicative of the tenderness or firmness of the meat. Additionally, the area under the force-distance curve from the onset of the test to the designated deformation distance was calculated, representing the work (Nmm) exerted during each test.

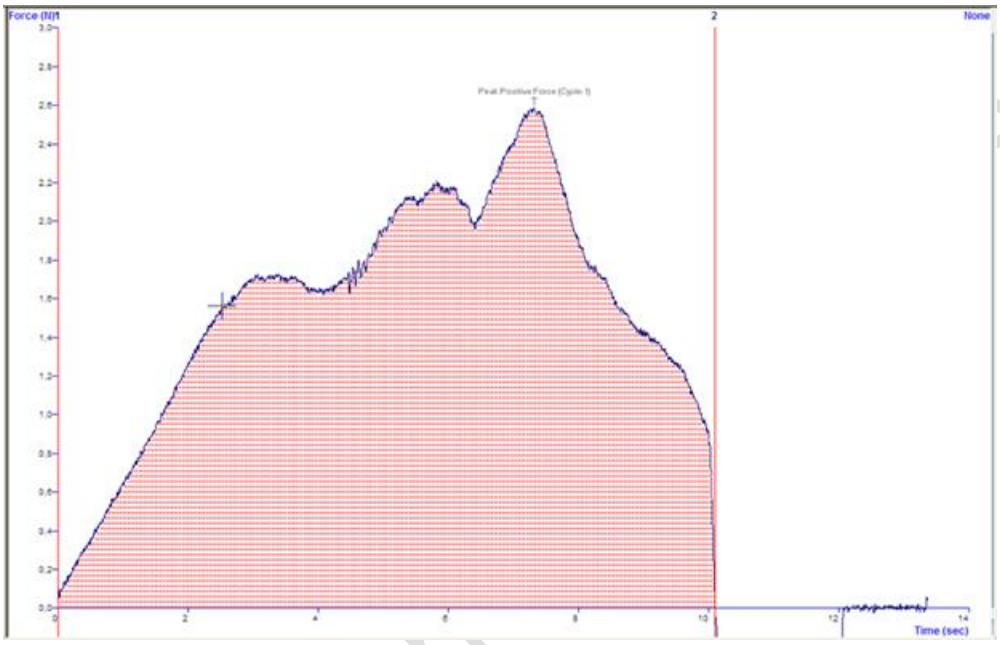

Figure 6 Texture measurement using a TA. XT Plus texture analyzer

4.3 Statistical analysis

One-way multivariate analysis of variance (MANOVA) models was conducted using SPSS version 23 software (SPSS Inc., an IBM Company, USA) to analyze color, weight loss, and texture parameters. Significant differences between different groups were determined by one-way ANOVA method, Tukey's post-hoc test and Canonical discriminant analysis. The T -test was performed to identify significant differences between different groups based on storage days.
The differences were regarded to be statistically significant at $P < 0.05$.

5 Results

5.1 Canonical Discriminant Analysis (CDA)

Canonical Discriminant Analysis (CDA) is a statistical technique used to identify and characterize the underlying linear relationships between multiple variables and groups, aiming to maximize the separation between groups based on their measured characteristics.

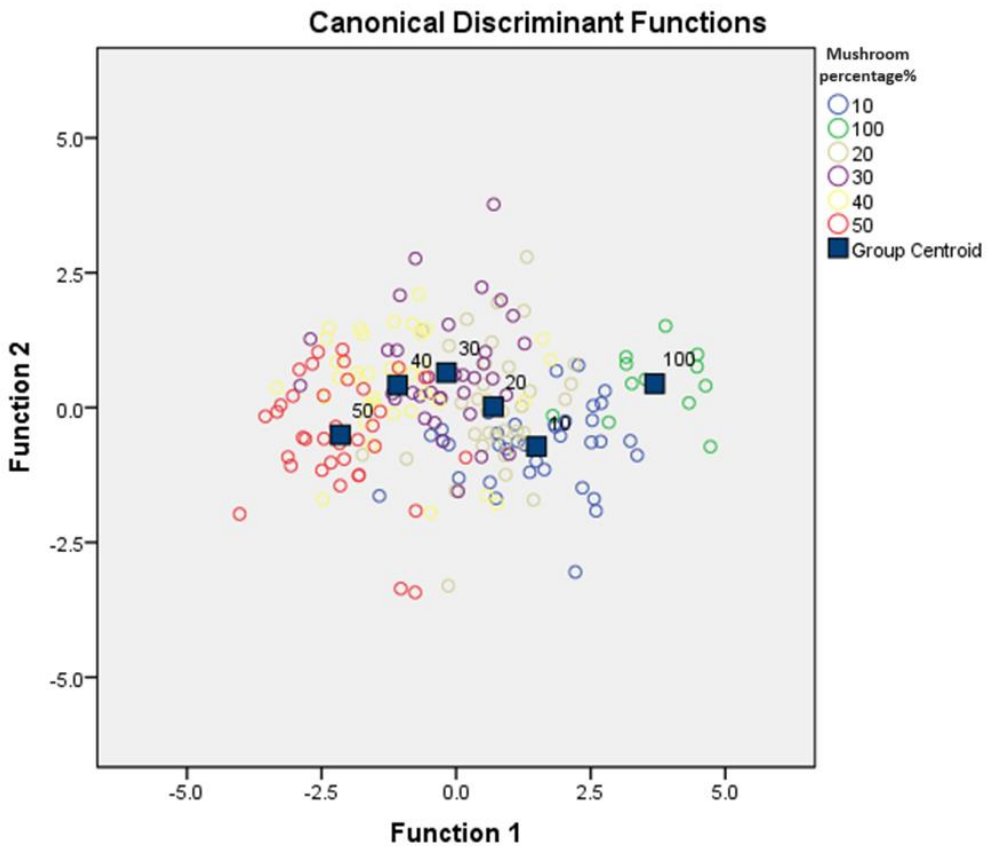

Figure 7 CDA score plot (Function 1 versus Function 2) Mushroom Percentage classification on sausage samples.

The diagram visually represents the entire data set, focusing on the impact of mushroom substitution percentages. Specifically, it showcases the following mushroom inclusion levels: 10%, 20%, 30%, 40%, and 50%. These percentages correspond to the proportion of mushrooms that were substituted in the sausage samples. Canonical Discriminant Analysis (CDA) was applied to this data set. Initially, the model was trained on the original data set. It successfully classified 58.9% of the grouped cases correctly. This accuracy rate indicates how well the discriminant functions derived from the data can predict the group membership of each sample.

The cross-validated accuracy rate was 53.6%, which is slightly lower than the original classification rate. This discrepancy highlights the importance of cross-validation in evaluating the model's performance.

This diagram provides valuable insights into the relationship between mushroom substitution percentages and the discriminant functions. While the original classification accuracy was moderate, cross-validation allowed us to gauge the model's predictive capability more rigorously.

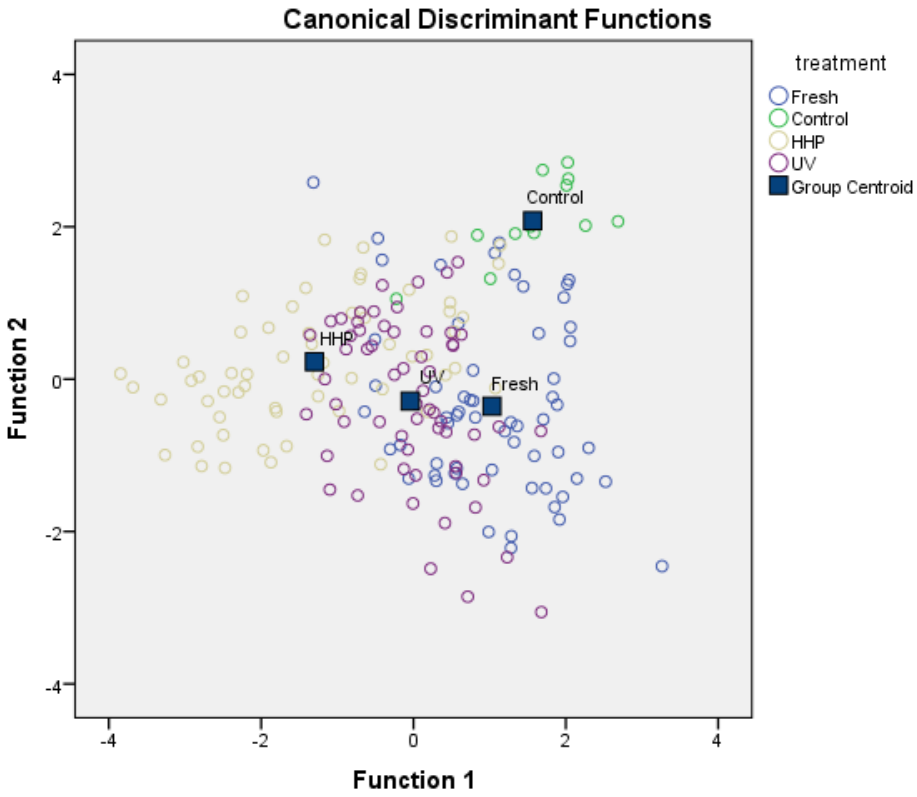

Figure 8 CDA score plot (Function 1 versus Function 2) Treatment Comparison: HHP, UV, and Fresh on sausage samples.

When applying the classification functions derived from all cases to the original dataset, 59.4% of the cases were accurately identified as belonging to their respective treatment groups. This accuracy rate reflects how well the discriminant functions, based on the features of the samples, can predict the group membership. For each case, the model predicted its group membership using functions derived from all other cases except the one being classified. The cross-validated accuracy rate was 56.3%, which slightly differs from the original classification rate. The CDA results reveal the effectiveness of the discriminant functions in distinguishing between the treatments (HHP, UV, fresh)

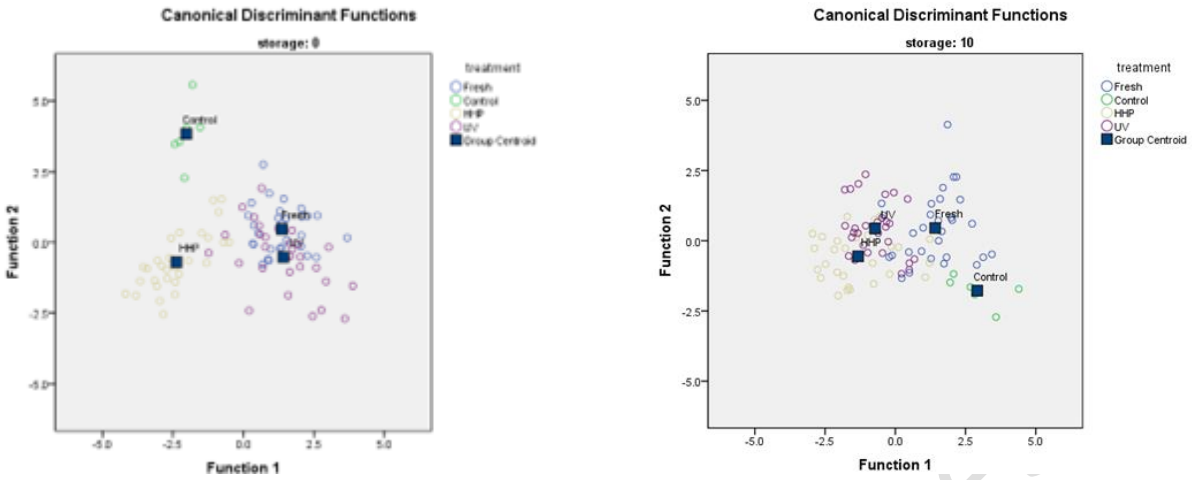

Figure 9 CDA score plot (Function 1 versus Function 2) Treatment Effect Plot based on Storage Days

The canonical discriminant analysis revealed that both storage days and pretreatments impacted the chemical composition of the sausage samples. There was a clear separation between day 0 and day 10 samples, indicating compositional changes during storage. Additionally, the distinct clustering of fresh, control, and HHP pretreatments suggests these methods have a measurable effect. The classification accuracy achieved acceptable levels, with 82.3% of fresh samples correctly classified at day 0 (dropping slightly to 75.0% with cross-validation). However, a small decrease in accuracy was observed for samples stored for 10 days (68.4% and 63.2% for original and cross-validated data).

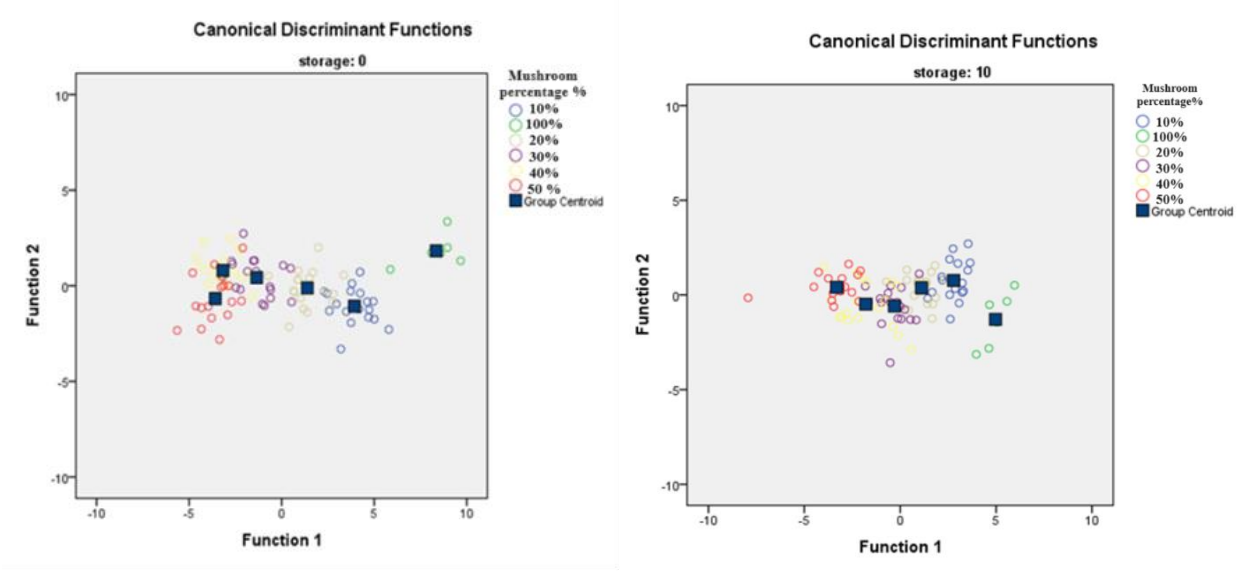

Figure 10 CDA score plot (Function 1 versus Function 2) Mushroom percentage effect Plot based on Storage Days

The canonical discriminant analysis revealed that both storage days and mushroom percentage impacted the chemical composition of the mushroom-based sausage samples. There was a clear separation between day 0 and day 10 samples, indicating compositional changes during storage. Additionally, the clustering of samples by mushroom content (10%, 20%, 30%, 40%, and 50%) suggests a measurable effect of this factor as well. The classification accuracy achieved acceptable levels, with 77.1% of original samples correctly classified at day 0 (dropping slightly to 75.0% with cross-validation). Interestingly, the accuracy for samples stored for 10 days showed a different trend, with a slight improvement over day 0 (82.1% and 71.6% for original and cross-validated data

5.2 Effect of mushroom percentage and pretreatment

5.2.1 Storage effect

The influence of storage on various parameters (color, texture, weight loss) within consistent pretreatment and mushroom percentage conditions was statistically significant, as evidenced by the results of t-tests.

Figure 11 Samples after 10 days storage

5.2.2 Color -Effect of mushroom content

L*: It's evident that the effect of mushroom percentage on L* values varied depending on the pretreatment method HHP and UV and the duration of storage (Day 0 and Day 10). Under HHP

treatment at Day 0, an increase in mushroom percentage from 30% to 40% resulted in an increase in the mean L* value (from 71.75±3.19 to 75.13±1.27), indicating a tighter distribution of data around the mean. Conversely, at Day 10 under HHP treatment, an increase in mushroom percentage from 10% to 40% led to a decrease in the mean L* value (from 75.15±0.45 to 72.95±0.69). For the UV treatment on Day 0, there was a slight increase in mean L* value when going from 10% to 20% mushroom percentage, but a decrease in mean L* value from 20% to 50% mushroom percentage (from 74.05±2.12 to 68.34±5.03). On Day 10 under UV treatment, there was a consistent decrease in mean L* value with increasing mushroom percentage from 10% to 50% (from 76.35±1.37 to 70.15±0.70). In the fresh condition at Day 10, the mean L* value increased from 74.89±0.67 at 10% mushroom percentage to 68.49±3.79 at 50% mushroom percentage. These findings highlight the complex interactions between mushroom percentage, treatment method, and storage duration on L* values. (Figure 12).

The a* values across different mushroom percentages, treatment methods, and storage durations reveals distinct trends. In the fresh condition on Day 0, a decrease in mean a* value is observed from 10% to 40% mushroom percentage (from 3.07±0.11 to 1.97±0.32), indicating a shift towards lower a* values and possibly a change towards a greener color. Under HHP treatment at Day 0, there is a substantial decrease in mean a* value from 10% to 50% mushroom percentage (from 3.29±0.48 to 0.58±0.30), suggesting a significant shift towards lower a* values and possibly a greener coloration. Similarly, under UV treatment on Day 0, there is a noticeable decrease in mean a* value from 10% to 50% mushroom percentage (from 3.14±0.38 to 1.59±0.12), indicating a shift towards lower a* values and possibly a greener color. Moving to Day 10, in the fresh condition, a decrease in mean a* value is observed from 10% to 40% mushroom percentage (from 4.54±0.19 to 3.74±0.20), suggesting a shift towards lower a* values and potentially a greener color. Under HHP treatment on Day 10, there is a significant decrease in mean a* value from 10% to 50% mushroom percentage (from 3.70±0.28 to 0.86±0.32), indicating a shift towards lower a* values and possibly a greener color. Similarly, under UV treatment on Day 10, there is a slight decrease in mean a* value from 10% to 50% mushroom percentage (from 3.61±0.25 to 2.00±0.24), suggesting a potential shift towards lower a* values and a change in color. These findings underscore the influence of mushroom percentage, treatment method, and storage duration on a* values, particularly indicating decreases in a* values with higher mushroom percentages, suggesting a shift towards greener colors (Figure 13).

b*: At Day 0, under fresh conditions, there seems to be a slight increase in mean b* value from 10% (12.60±0.71) to 50% (14.48±0.59) mushroom percentage. Under HHP treatment, there's an decrease in mean b* value from 20% (15.41±0.77) to 50% (13.90±0.96) mushroom percentage. However, under UV treatment, there appears to be a increase in mean b* value from 10% (13.71±0.73) to 40% (15.29±0.62) mushroom percentage.

Moving to Day 10, in the fresh condition, there is a slight decrease in mean b* value from 30% (12.63±0.29) to 40% (11.84±0.29) mushroom percentage, while there's a slight increase observed at 50% (12.78±0.50). Under HHP treatment, there's a decrease in mean b* value from 30% (12.33±0.70) to 40% (12.14±0.55) mushroom percentage. Under UV treatment, there seems to be an increase in mean b* value from 10% (12.44±0.70) to 40% (13.48±0.30) mushroom percentage.It appears that the trend varies across different treatment methods and storage durations, with some instances of increase and decrease in b* values (Figure 14)

When comparing with the literature this thesis's findings align with previous data reported in mushrooms. A study by Borges et al., (2023) in white button mushrooms found that coated mushrooms had less color change after 14 days showing that treatment methods can significantly impact the color stability of mushrooms, similar to observations with HHP and UV treatments. In another study, different substrates used for mushroom cultivation were found to affect the yield and mineral content of the mushrooms (Siwulski et al., 2018). This could explain the color changes observed with varying mushroom percentages, as the nutritional composition of the mushrooms might have been altered. Furthermore, a study on 5D food printing demonstrated that the growth of probiotics could induce a controllable color change in food products (Chen et al., 2023). This study showed that biological factors can also contribute to color changes in food products, leading that the color changes observed may be influenced by other biological factors. Moreover, a review of the industrial development and applications of eco-friendly colorants highlighted the direct association between the color of food products and their flavor, safety, and nutritional value (Renita et al., 2023). This highlights the importance results in understanding how mushroom percentage, treatment method, and storage period can influence the color, and potentially other quality attributes, of food products.

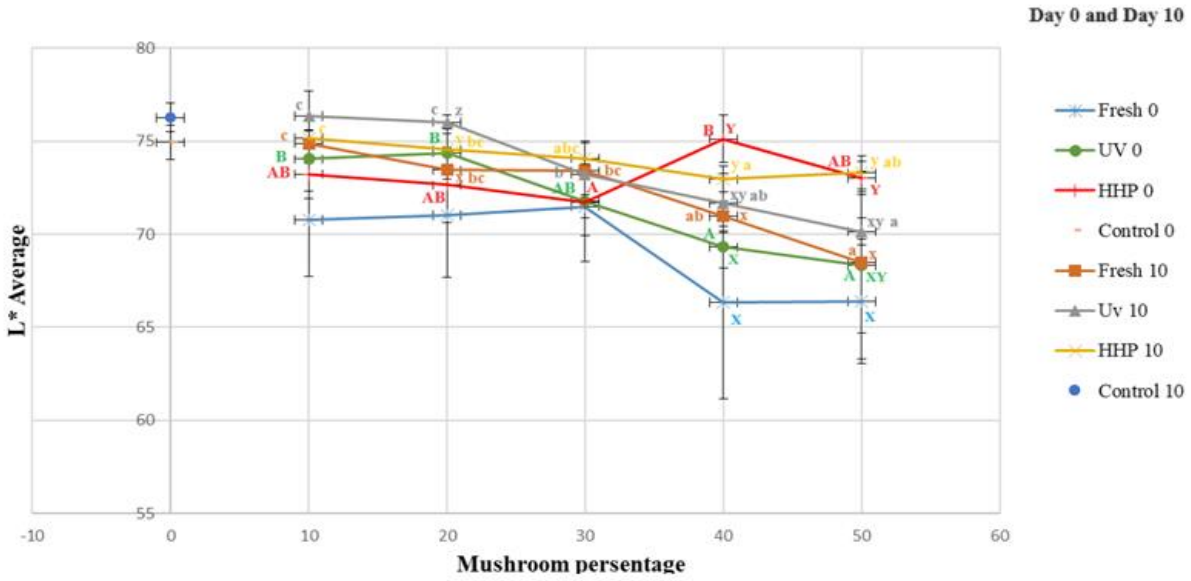

Figure 12 Variations in L Values: Mushroom Percentage and Pretreatment Effects (Samples with significant differences are marked with different letters.1)*

[1] When comparing different pretreatments within the same mushroom percentage on Day 0, we denote the comparison with capital letters X,Y,Z. Similarly, when comparing pretreatments within the same mushroom percentage on Day 10, lowercase letters x,y,z are used. When comparing different mushroom percentages within the same pretreatment on Day 0, uppercase letters A, B,C are employed. Conversely, when comparing mushroom percentages within the same pretreatment on Day 10, lowercase letters a,b,c are utilized.

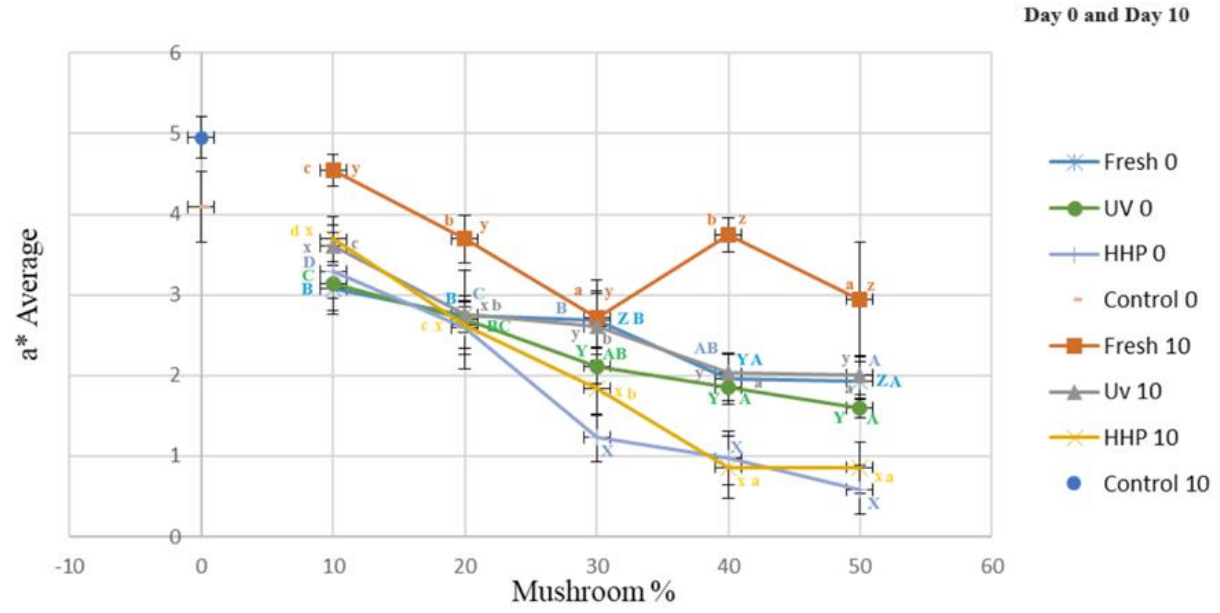

Figure 13 Variations in a Values: Mushroom Percentage and Pretreatment Effects (Samples with significant differences are marked with different letters[1].)*

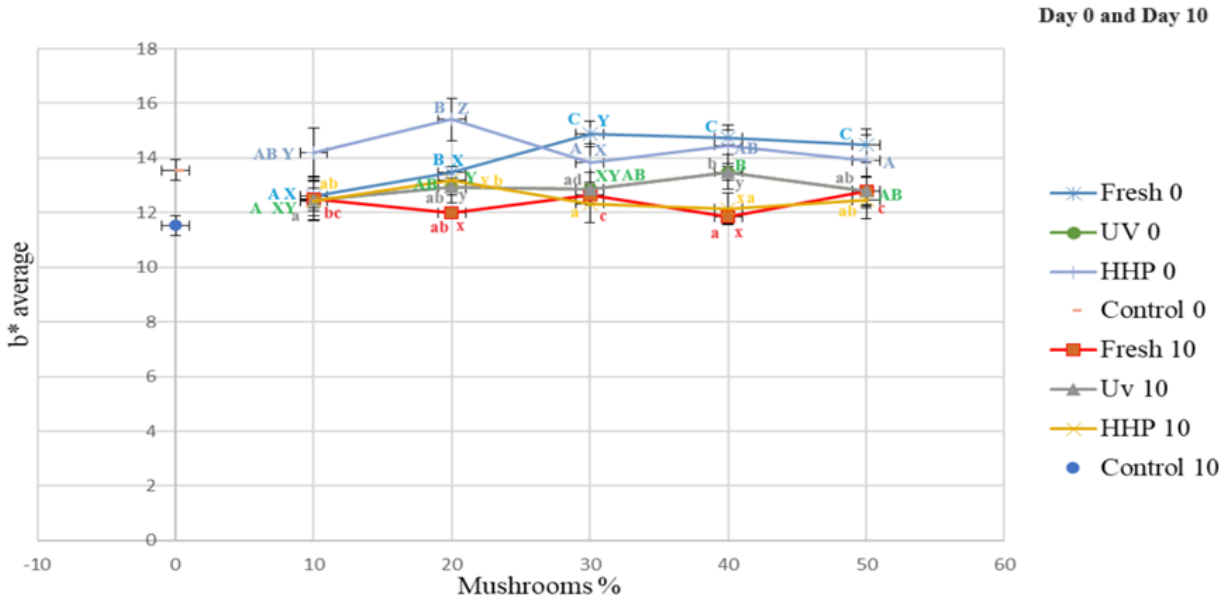

Figure 14 Variations in b Values: Mushroom Percentage and Pretreatment Effects (Samples with significant differences are marked with different letters.)*

5.2.3 Color -Effect of pretreatment

The results indicate notable variations in the L* values across different treatments and storage durations. On Day 0, at a mushroom percentage of 40%, the mean L* values for Fresh, UV, and HHP treatments were (66.34±5.15), (69.32±1.12), and (75.13±1.27), respectively. Comparing these, it's evident that the HHP treatment exhibited the highest mean L* value, followed by UV and Fresh treatments, indicating an increase in lightness with HHP treatment. At a mushroom percentage of 50%, only Fresh and HHP treatments were observed, with mean L* values of (66.40±3.32) and (73.02±0.88), respectively. The HHP treatment again showed a higher mean L* value, suggesting an increase in lightness compared to Fresh treatment. Moving to Day 10, at 20% mushroom percentage, the mean L* values for Fresh, HHP, and UV treatments were (73.46±0.77), (74.54±0.85), and (76.02±0.37). Here, UV treatment exhibited the highest mean L* value, followed by HHP and Fresh treatments, indicating an increase in lightness with UV treatment. At 40% mushroom percentage, Fresh and HHP treatments had mean L* values of (70.98±0.82) and (72.95±0.69). The HHP treatment showed a slightly higher mean L* value, suggesting a slight increase in lightness compared to Fresh treatment. Lastly, at 50% mushroom percentage, Fresh and HHP treatments had mean L* values of (68.49±3.79) and (73.30±0.88), respectively. Again, the HHP treatment exhibited a higher mean L* value, indicating an increase in lightness compared to Fresh treatment. Throughout the experiment, it's shows that HHP treatment consistently resulted in higher mean L* values, indicating an overall increase in lightness compared to Fresh and UV treatments (Figure 12).

The analysis reveals distinct variations in the a* values across different pretreatments within the same mushroom percentage and storage durations. On Day 0, at a mushroom percentage of 30%, the mean a* values for Fresh, HHP, and UV treatments were (2.68±0.33), (1.23±0.30), and (2.11±0.22). Here, the Fresh treatment exhibited the highest mean a* value, followed by UV and HHP treatments, indicating an increase in redness with Fresh treatment. At 40% mushroom percentage, the mean a* values for Fresh, UV, and HHP treatments were (1.97±0.32), (1.85±0.16), and (0.97±0.33), . Again, the Fresh treatment showed the highest mean a* value, followed by UV and HHP treatments, suggesting an increase in redness with Fresh treatment. Similarly, at 50% mushroom percentage, Fresh treatment had the highest mean a* value of (1.94±0.23), followed by

UV with (1.59±0.11), and HHP with (0.58±0.30), indicating an increase in redness with Fresh treatment compared to UV and HHP treatments. Transitioning to Day 10, at 10% mushroom percentage, UV treatment exhibited shows the mean a* value of (3.61±0.25), HHP with (3.69±0.28), and Fresh with (4.54±0.19), suggesting an increase in redness with Fresh treatment compared to UV and HHP treatments. At higher mushroom percentages, similar trends were observed, with Fresh treatments consistently showing higher mean a* values compared to UV and HHP treatments. Throughout the experiment, Fresh treatments consistently displayed the highest mean a* values, indicating an overall increase in redness compared to UV and HHP treatments (Figure 13).

The examination of b* values within the same mushroom percentage and storage duration highlights discernible trends. On Day 0, at a 10% mushroom percentage, Fresh and HHP treatments displayed mean b* values of (12.60±0.72) and (14.19±0.90), indicating a decrease in yellowness with Fresh treatment compared to HHP treatment. At 20% mushroom percentage, Fresh treatment showed a mean b* value of (13.48±0.22), while UV and HHP treatments exhibited values of (14.43±0.60) and (15.41±0.78). Here, Fresh treatment showcased a decrease in yellowness compared to UV and HHP treatments. Similarly, at 30% mushroom percentage, Fresh treatment had a mean b* value of 14.87±0.46, higer than that of HHP treatment, which had a value of (13.83±0.70), suggesting a decrease in yellowness with HHP treatment. Transitioning to Day 10, at 20% mushroom percentage, Fresh treatment exhibited a mean b* value of (11.99±0.23), lower than both UV and HHP treatments, which displayed values of (12.92±0.27) and (13.17±0.32), indicating a decrease in yellowness with Fresh treatment. At 40% mushroom percentage, Fresh treatment showcased a mean b* value of (11.85±0.29), again lower than that of UV treatment (13.48±0.30) and HHP treatment (12.14±0.56), suggesting a decrease in yellowness with Fresh treatment compared to UV treatment. Throughout the experiment, Fresh treatments consistently exhibited lower mean b* values, indicating an overall decrease in yellowness compared to UV and HHP treatments. (Figure 14).

The findings of this study demonstrate that pretreatment methods significantly impact the color characteristics of the final product containing mushroom inclusions. Notably, HHP treatment consistently resulted in higher L* values, indicating a tendency towards increased lightness compared to Fresh and UV treatments. This aligns with observations made by (Sun et al., 2018)

who reported that HHP pretreatment of oyster mushrooms led to a significant increase in lightness (L*) compared to the control group. The authors attributed this effect to the ability of HHP to minimize enzymatic browning reactions, thereby preserving the lighter color of the mushrooms.

In contrast, the impact of pretreatments on the a* value (redness) displayed a more complex pattern. Fresh treatments generally exhibited higher a* values, suggesting a greater degree of redness compared to UV and HHP treatments. This observation is consistent with the findings of (Singh et al., 2017) who reported that UV pretreatment of button mushrooms resulted in a significant decrease in redness (a*) compared to the control group. The authors suggested that UV treatment might inactivate enzymes responsible for anthocyanin biosynthesis, leading to a reduction in red pigments.

5.2.4 Texture-Effect of mushroom content

The force values across different mushroom percentages within the same pretreatment and storage conditions provides insights into the impact of mushroom percentage on force properties. At Day 0, under HHP treatment, there's a clear decrease in mean force values as mushroom percentage increases, with a decrease from 20% (2.16±0.52) to 50% (0.59±0.19). Similarly, under UV treatment, there's a decrease in mean force values from 10% (1.36±0.31) to 50% (0.80±0.25) mushroom percentage. These trends indicate a consistent decrease in force with an increasing mushroom percentage for both HHP and UV treatments at Day 0 (Figure 15).

Moving to Day 10, under fresh conditions, there's a decrease in mean force values from 30% (2.33±0.47) to 50% (1.1±0.36) mushroom percentage. Under HHP treatment, there's a decrease in mean force values from 10% (2.05±0.34) to 50% (0.83±0.23) mushroom percentage. Similarly, under UV treatment, there's a decrease in mean force values from 10% (1.80±0.34) to 50% (0.90±0.25) mushroom percentage. These observations suggest a consistent decrease in force values with an increasing mushroom percentage across all treatment methods at Day 10 (Figure 16).

Examining the work values across various mushroom percentages within same pretreatment and storage reveals discernible trends. On Day 0, within HHP treatment, a conspicuous decline in mean work values accompanies the rise in mushroom percentage, decreasing from 20% (11.81±2.39) to 50% (3.95±1.33). Similarly, under UV treatment, there's a decrease in mean work values from

10% (8.66±0.96) to 50% (4.71±1.45) mushroom percentage. These trends underscore a consistent reduction in work as mushroom percentage increases for both HHP and UV treatments on Day 0 (Figure 15).

Transitioning to Day 10, within HHP treatment, a decline in mean work values is evident from 10% (13.88±1.74) to 50% (4.96±1.62) mushroom percentage. Likewise, under UV treatment, there's a decrease in mean work values from 10% (11.97±1.44) to 50% (5.92±1.40) mushroom percentage. These observations underscore a consistent reduction in work values across all treatment methods on Day 10 as mushroom percentage increases (Figure 16).

Comparisons with relevant literature provide valuable insights into the observed texture changes. (Boylu et al.,) investigated the use of fresh oyster mushroom as a partial meat substitute in sausages, reporting a softer texture with increased mushroom content. This aligns with our findings and suggests that the moisture-retaining properties of mushrooms contribute to the observed texture modifications. While (Mantihal et al.,) did not specifically mention force values in their study on shiitake mushroom powder (SMP) in chicken sausages, the overall texture alterations they observed correspond to our findings. Additionally, (Mazumer et al.2023) work on mushroom-based meat extenders (MBMEs) emphasized texture modifications resulting from mushroom substitution, further supporting our observed reduction in force values.

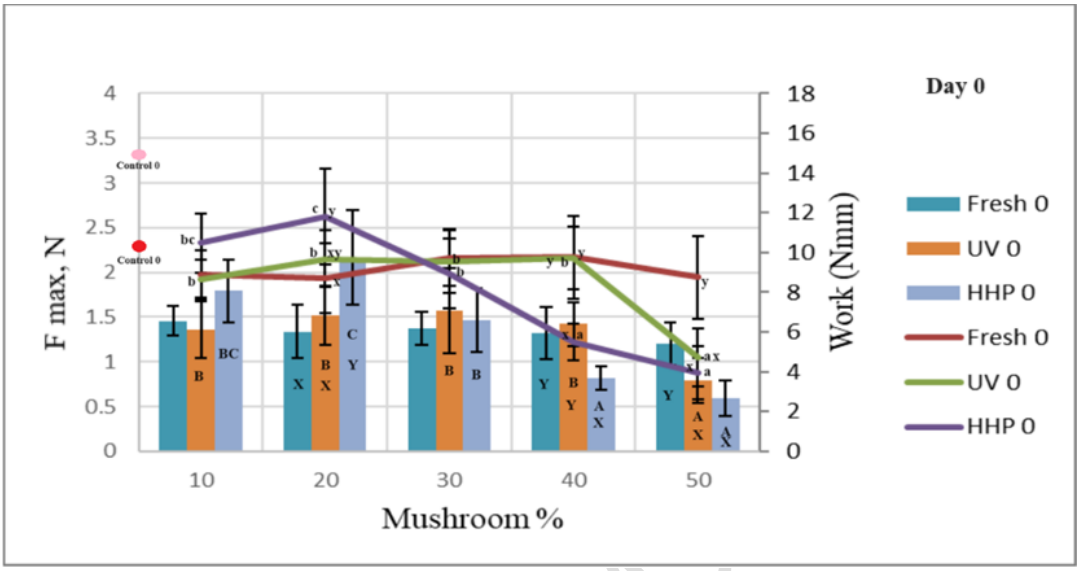

Figure 15 Comparison of Force and Work in Sausages on Day 0: Mushroom Percentage vs. Pretreatment effects (Samples with significant differences are marked with different letters.2)

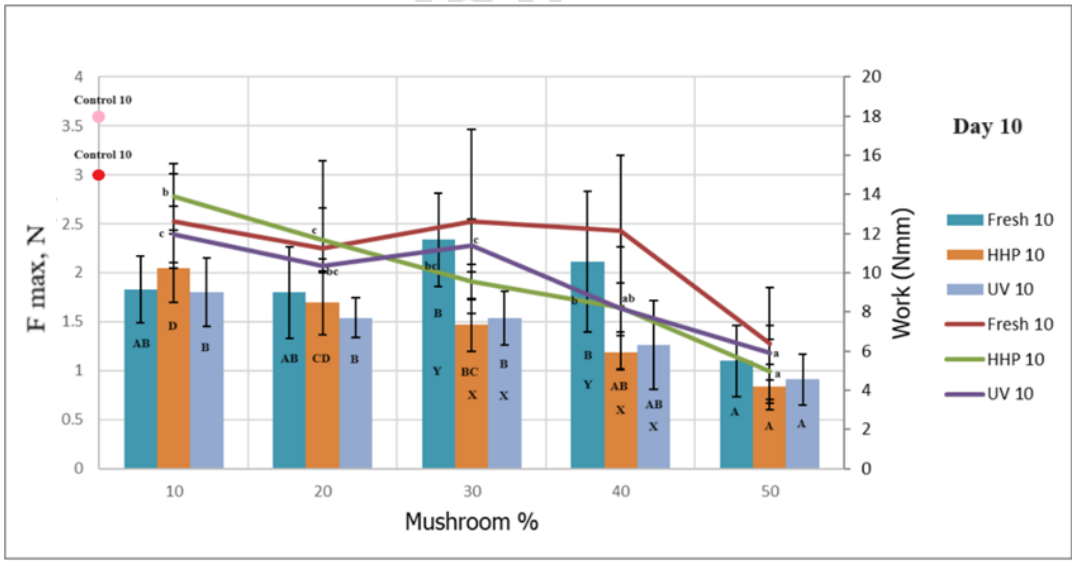

Figure 16 Comparison of Force and Work in Sausages on Day 10: Mushroom Percentage vs. Pretreatment effects (Samples with significant differences are marked with different letters)

[2] When comparing different pretreatments within the same mushroom percentage on Day 0, we denote the comparison with capital letters for force X, Y, Z and lowercase letters for work x,y,z.

When comparing different mushroom percentages within the same pretreatment on Day 0, uppercase letters are employed for force A, B, C and lowercase letters for work a,b,c.

This applies same for day 10.

5.2.5 Texture-Effect of pretreatment

The investigation into force values within the same mushroom percentage and storage duration unveils distinct patterns. On Day 0, at a 20% mushroom percentage, Fresh, UV, and HHP treatments displayed mean force values of (1.34±0.30), (1.52±0.32), and (2.16±0.53). Here, the HHP treatment exhibited the highest mean force value, followed by UV and Fresh treatments, indicating an increase in force with HHP treatment compared to UV and Fresh treatments. At 40% mushroom percentage, Fresh, UV, and HHP treatments showcased mean force values of (1.32±0.30), (1.42±0.25), and (0.82±0.13). In this case, the HHP treatment displayed the lowest mean force value, suggesting a decrease in force with HHP treatment compared to Fresh and UV treatments. Similarly, at 50% mushroom percentage, HHP, UV, and Fresh treatments had mean force values of (0.60±0.20), (0.80±0.25), and (1.20±0.23). Here, the HHP treatment exhibited the lowest mean force value again, indicating a decrease in force with HHP treatment compared to UV and Fresh treatments (Figure 15). Transitioning to Day 10, at 30% mushroom percentage, Fresh, HHP, and UV treatments displayed mean force values of (2.34±0.48), (1.47±0.27), and (1.53±0.27). Fresh treatment exhibited the highest mean force value, followed by UV and HHP treatments, suggesting an increase in force with Fresh treatment compared to UV and HHP treatments. At 40% mushroom percentage, Fresh, HHP, and UV treatments showcased mean force values of (2.11±0.72), (1.19±0.17), and (1.26±0.45). Here, Fresh treatment displayed the highest mean force value again, indicating an increase in force compared to UV and HHP treatments. Throughout the experiment, Fresh treatments consistently displayed higher mean force values, suggesting an overall increase in force compared to UV and HHP treatments (Figure 16).

Work values within the same mushroom percentage and storage duration reveals notable trends. On Day 0, at a 20% mushroom percentage, Fresh and HHP treatments displayed mean work values of (8.70±1.74) and (11.82±2.39). Here, the HHP treatment exhibited the highest mean work value, indicating an increase in work with HHP treatment compared to Fresh treatment. At 40% mushroom percentage, Fresh, UV, and HHP treatments showcased mean work values of (9.76±2.07), (9.71±1.60), and (5.48±0.93). In this case, the HHP treatment displayed the lowest mean work value, suggesting a decrease in work with HHP treatment compared to Fresh and UV treatments. Similarly, at 50% mushroom percentage, Fresh, UV, and HHP treatments had mean work values of (8.75±2.05), (3.96±1.33), and (4.71±1.45). Here, both UV and HHP treatments

exhibited lower mean work values compared to Fresh treatment, indicating a decrease in work with UV and HHP treatments (Figure 15). Transitioning to Day 10, no significant differences were observed between pretreatments, suggesting that the effect of pretreatment on work values diminished by Day 10. Throughout the experiment, HHP treatments consistently exhibited either the highest or lowest mean work values, indicating variable effects on work compared to Fresh and UV treatments (Figure 16)

The textural properties of the sausages, as measured by force and work values, revealed a complex interplay between pretreatment type, mushroom percentage, and storage duration. Our observation of HPP treatment increasing firmness (force) at lower mushroom content on Day 0, but this effect reversing at higher inclusion levels, aligns with concepts presented in (Donato et al., 2020). Their work on high-pressure processing of meat products highlights the potential for pressure to disrupt muscle protein networks, impacting textural properties. While not directly applicable to sausages with mushrooms, it provides a foundation for understanding how HPP might influence texture in our system. Conversely, Fresh treatments generally displayed the highest force and work values across storage durations at specific mushroom percentages. This is in contrast to (Gallego et al., 2019) who found that addition of dehydrated mushrooms to beef patties resulted in a decrease in hardness compared to the control group. These contrasting results highlight the potential influence of mushroom processing methods and their impact on the final texture of the meat product.

5.2.6 Weight Loss-Effect of mushroom content

Analyzing the weight loss across various mushroom percentages within identical pretreatment and storage conditions reveals distinct trends. At Day 0, within the fresh condition, there's an apparent increase in weight loss as mushroom percentage rises, with values of 17.65% at 10%, 22.86% at 20%, 33.90% at 30%, and 26.33% at 50%. Under HHP treatment, weight loss shows a fluctuating pattern, decreasing from 13.63% at 10% to 5.58% at 40%, then increasing to 7.17% at 50%. Conversely, under UV treatment, weight loss exhibits a more consistent increase with mushroom percentage, rising from 9.87% at 10% to 34.14% at 50% (Figure 17). Transitioning to Day 10, under HHP treatment, there's a noticeable increase in weight loss from 1.95% at 10% to 10.03% at 50% mushroom percentage. This suggests an escalating trend in weight loss with increasing mushroom percentage. Similarly, under UV treatment, weight loss increases steadily from 1.64%

at 20% to 10.03% at 50% mushroom percentage. These observations underscore a consistent rise in weight loss across all treatment methods on Day 10 as mushroom percentage increases (Figure 18).

The observed positive correlation between mushroom content and weight loss at Day 0, likely due to the high moisture content of mushrooms disrupting the sausages' moisture balance, aligns with the data on shiitake mushrooms (Sun et al., 2017). Moreover, HHP displayed a potential time-dependent effect, with initial moisture retention at Day 0 potentially followed by increased dehydration at Day 10 due to complex interactions with mushroom components or disruption of the sausage structure (Alpas et al., 2015). Conversely, UV treatment showed a consistent rise in weight loss with mushroom content across storage days, suggesting interactions with specific mushroom compounds. The significant increase in weight loss with mushroom content at Day 10 for both HHP and UV treatments highlights the need for further investigation into the underlying mechanisms and the impact of different mushroom varieties. Overall, this findings demonstrate the multifaceted influence of mushroom content, pretreatment method, and storage duration on weight loss in sausages, paving the way for optimizing formulations and exploring the potential of pretreatments for managing moisture content (Shahidi et al., 2015).

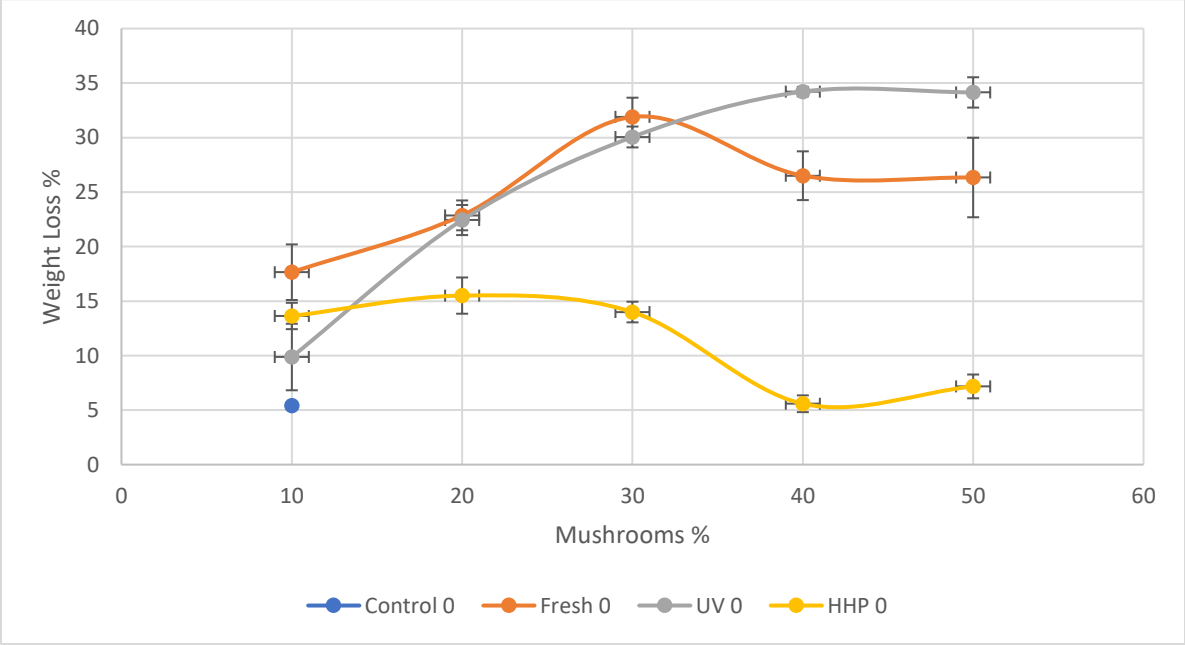

Figure 17 Weight Loss % Day 0 (after cooking)

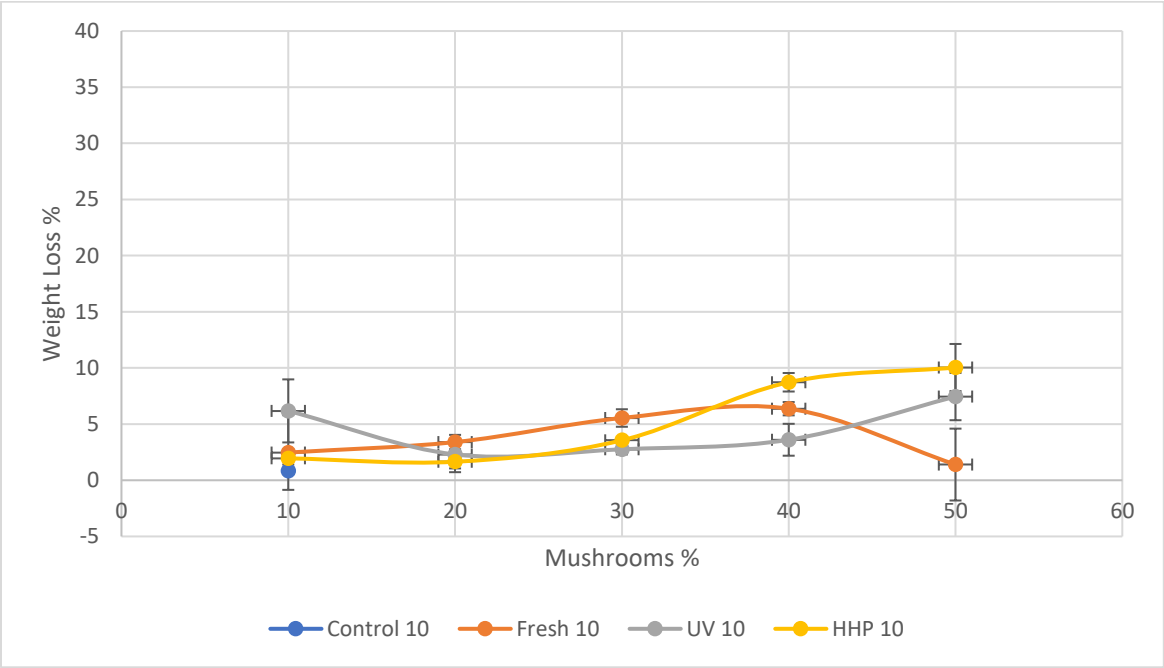

Figure 18 Weight Loss % (after 10 days storage)

5.2.7 Weight Loss-Effect of Pretreatment

The analysis of weight loss across various mushroom percentages and storage durations reveals clear trends. On Day 0, at a 20% mushroom percentage, UV treatment showed the highest mean weight loss at 22.44, closely followed by Fresh treatment at 22.86, whereas HHP treatment had the lowest mean weight loss at 15.50. This indicates that both UV and Fresh treatments led to higher weight loss compared to HHP treatment. At 30% mushroom percentage, Fresh exhibited the highest mean weight loss at 31.90, followed by UV treatment at 30.05, while HHP treatment had the lowest mean weight loss at 14.00. Likewise, at 40% and 50% mushroom percentages, UV treatments consistently resulted in the highest mean weight loss values of 34.21 and 34.14, respectively, with Fresh treatments following closely at 26.50 and 26.33, respectively, and HHP treatments consistently displaying the lowest mean weight loss values of 5.59 and 7.17, respectively (Figure 17). Transitioning to Day 10, at 40% mushroom percentage, UV treatment maintained the highest mean weight loss at 3.60, while HHP treatment exhibited a higher mean weight loss at 8.72. Throughout the experiment, UV treatments consistently led to the highest mean weight loss values, indicating an overall increase in weight loss compared to Fresh and HHP

treatments, while HHP treatments consistently showed the lowest mean weight loss values, suggesting a decrease in weight loss compared to UV and Fresh treatments (Figure 18).

The analysis of weight loss revealed distinct patterns influenced by pretreatment type (Fresh, HHP, UV) within the same mushroom percentage and storage duration. UV treatments consistently exhibited the highest weight loss, potentially due to localized heating within mushroom tissue by UV irradiation, facilitating moisture release similar to findings of (Wang et al., 2021). Conversely, HHP treatments consistently minimized weight loss throughout the experiment, potentially by enhancing moisture retention within pretreated mushrooms aligning with HPP's effects on meat products in (Sheen et al., 2015).

6. Summary

This diploma thesis investigates the potential of oyster mushrooms as a sustainable meat replacement in sausage and burger patties, addressing the growing demand for healthier dietary options. By examining the nutritional composition and culinary versatility of oyster mushrooms, this study highlights their suitability as a plant-based protein source with a meat-like texture and umami flavor profile, aligning with global trends towards more ethical and environmentally friendly food choices.

The research explores the application of advanced food processing techniques such as High Hydrostatic Pressure (HHP) and Ultraviolet light-emitting diode (UV-LED) irradiation to enhance the functional properties and sensory attributes of oyster mushrooms. Through comprehensive analyses of color, weight, and texture parameters, valuable insights are provided into optimizing the incorporation of oyster mushrooms into processed foods.

The findings of this thesis contribute significantly to the discourse on alternative protein sources, sustainable food production practices, and the development of healthier and more environmentally conscious food products. Key findings include the influence of mushroom percentage, treatment

method, and storage duration on color values, with trends indicating shifts towards greener colors and decreases in redness and yellowness with higher mushroom percentages.

Consistent trends were observed in texture parameters, with decreases in force values and increases in weight loss as mushroom percentage increased, particularly identified across different treatment methods and storage durations. HHP treatments consistently resulted in higher lightness values, while Fresh treatments exhibited higher redness and lower yellowness values. UV treatments consistently led to higher weight loss values, while HHP treatments showed the lowest weight loss values.

Overall, these findings offer valuable insights into the potential of oyster mushrooms as a sustainable meat alternative and provide guidance for optimizing their incorporation into processed foods, thereby contributing to the advancement of sustainable food production and the promotion of healthier dietary choices.

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Author's declaration

I, as the undersigned Altin Berisha (Neptun Code: X4DE56) declare that the MSc thesis* titled:

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

Those parts, which were taken from other authors are clearly specified and the references are listed.

If my declaration is false, I understand that the Final Exam Committee will exclude me from the final exam, and I must prepare a new thesis work.

Budapest, 26.04.2024