THESIS OF THE DOCTORAL DISSERTATION

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OYSTER MUSHROOM AS A MEAT SUBSTITUTE IN MEAT PRODUCTS

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INTRODUCTION AND OBJECTIVES

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

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

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

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

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

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

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

Microwave, HHP and UV Light) and fermentation on physicochemical and textural properties of oyster mushrooms. Are there differences in the effects of conventional and alternative pretreatment methods?

- To investigate the effect of different pretreatments (Blanching, Steaming, Oven, Microwave, HHP and UV Light) and fermentation on nutritional quality (total amino acid, essential amino acid, free amino acid and biogenic amine contents) of oyster mushrooms
- To compare the sausage quality under the partial substitution of meat with fermented oyster mushrooms. How does the use of different pretreatment technologies before the fermentation of the mushrooms affect the quality characteristics of the sausages? Is the effect of pretreatment methods significant?
- To assess the sausage quality under the partial substitution of meat with fermented oyster mushrooms. How does the mushroom ratio affect the quality characteristics of the sausages, when the mushrooms were identically pretreated. Is mushroom ratio replacing meat in the sausage formulation decisive?
- To examine the storage stability of sausages under the partial substitution of meat with fermented oyster mushrooms. Are the alternative pretreatment methods result in similar or better quality parameters of the sausages in comparison to the conventionally pretreated ones?

MATERIAL AND METHODS

Sausage production with fresh oyster mushrooms (Preliminary study)

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

Table 1: Sausage formulations with fresh oyster mushroom replacing meat

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

Mushroom pretreatments and fermentation

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

- Blanching in water

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

- Steaming

  Steaming was performed at 100°C for 3 minutes in a multifunctional oven (Lainox VE051P, Lainox, Vittorio Veneto, Italy) using only the steam function. Pretreated mushrooms were let to cool down to room temperature (21-22 °C) on stainless

steel trays until further processing.

- Oven pretreatment

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

- Microwave pretreatment

  For microwave pretreatment, mushroom samples were divided into 300 g portions and placed on porcelain plates. The mushrooms were then subjected to 900 W, 2.45 GHz at 85°C for 3 minutes using the A3 (vegetable) setting in a microwave oven (SHARP R722STWE, Sharp Electronics Europe Ltd., Middlesex, UK).

- HHP pretreatment

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

- UV Light pretreatment

  During UV light pretreatment, mushrooms were irradiated with a 30 W UV light at 312 nm (VL-115.M, Vilber Lourmat, Marne La Vallee, France) for 15 minutes at 20°C. To ensure even irradiation, mushrooms were placed on an open shelf, 20 cm below the light source.

- Mushroom Fermentation

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

Sausage production with pretreated fermented oyster mushrooms

Sausage mixtures were obtained by blending meat (70/30), fermented oyster

mushrooms, sodium nitrate, sodium ascorbate, phosphate, and ice in a cutter (Robot-Coupe R202, Robot-Coupe, Burgundy, France). A total of fifteen formulations were produced, involving six different pretreatment and three replacement ratios, as 0%, 25%, and 50% of the meat content. The sausage mixtures were manually stuffed into Ø30 mm synthetic casings (NaloShape, ViskoTeepak, Lommel, Germany) and subsequently subjected to heat treatment at 80 °C for 30 minutes using the steam function of a Lainox VE051P oven (Lainox, Vittorio Veneto, Italy). The sausages were then quickly chilled to below 5 °C. Evaluations of moisture content, pH, color, texture properties, and lipid oxidation were performed on the production day and at storage intervals of 7, 14, 21, and 28 days.

Applied measurements

- Moisture content

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

- Water activity (a_w)

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

- pH measurement

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

- Color measurement

  The color properties of the mushroom and sausage samples were assessed using the CIELAB scoring system (CIE, 1986). Lightness (L^*), redness (a^*), and yellowness (b^*) were measured with an 8 mm aperture CR-410 colorimeter (Konika Minolta Sensing Inc., Osaka, Japan), utilizing a 2° observer and calibrated with illuminant C against a standard white reflectance calibration plate (CRA43). Measurements were conducted at room temperature, with each sample undergoing

nine parallel assessments. For the mushroom samples, The Browning Index, Yellowness Index, and Total Color Change (ΔE) were also computed using the obtained L^*, a^*, and b^* values.

- Texture measurement

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

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

- Yield

  The weights of the mushrooms were recorded both before and after the application of pretreatments and fermentation. Subsequently, the yield for each group was calculated using the formula below.

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

- Differential scanning calorimetry

  Differential scanning calorimetry (DSC) is utilized to monitor temperature and heat flow over time within a controlled environment. The thermal denaturation and alterations in the state of the sample proteins were observed using a Micro DSC III (SETARAM, France). As reference substance, bi-distilled water (776.6 mg) was employed alongside each sample. Sausage samples were weighed (776.6 ± 5 mg)

and placed in the stainless steel cylinder sample containers. The analysis began with a temperature stabilization phase at 20 °C for 2 minutes and followed by heating to 95 °C at a rate of 1 °C/min. Throughout this procedure, heat flow curves were captured. The DSC curves were then analyzed within the 35 to 90 °C range using the device's Calisto 7.6 software. The total denaturation enthalpy (ΔH, [J/g]) for each sample was determined by calculating the area between the DSC curve and a linear baseline. The peak temperatures were also recorded. Each sample underwent two parallel measurements.

- Lipid oxidation

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

- Sensory analysis

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

- Essential amino acids and proteinogenic (total) amino acids

  The mushroom samples were freeze-dried using a Christ Alpha 2-4 lyophilizer at

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

- Free amino acids and biogenic amines

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

- Near-Infrared Spectroscopy – NIR

  The measurements were conducted using a Hungarian-developed METRIKA NIR device (METRINIR 10-17 PR), which operates within the near-infrared frequency range of 700–1700 nm. Spectra were recorded within this range in transflexion mode, at 2 nm intervals, using the device's own MetriNIR® software. The measurements were performed on the samples in grinded form. Each sample was examined with two independent loadings, with three rotations of the sample holder per loading. The sample holder was rotated 120° in the same direction after each

measurement, resulting in six spectra recordings per sample for data analysis. The previously prepared samples were loaded into a 55 mm diameter sample holder in an even layer. Calibration of the device was performed before each sample series. The recorded datasets were exported, followed by spectrum smoothing (using a Savitzky-Golay filter: 2–2 left-right) and generation of the second derivative spectrum using the Unscrambler software. The resulting spectral data were then evaluated with discriminant analysis (CDA), using IBM SPSS (Version 29, SPSS Inc., Chicago, IL, USA).

- Statistical analysis

The experimental data were analysed with IBM SPSS (Version 29, SPSS Inc., Chicago, IL, USA) using multivariate analysis of variance (MANOVA) and principal component analysis (PCA). Levene's test was employed to verify the homogeneity of variances. Tukey's post hoc tests were conducted to identify significant differences between the samples if the homogeneity of variances was satisfied, otherwise Games-Howell's method was used. Statistical significance was determined at $p<0.05$. The obtained results were reported as mean values ± standard deviation. Discriminant Canonical Analysis with cross-validation was performed to evaluate the effects of different pretreatments and mushroom percentages on the classification of sausage samples.

RESULTS AND DISCUSSION

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

The moisture content of the sausages notably increased with higher levels of mushroom substitution ($p < 0.05$), where the 0P samples, containing no mushroom, exhibited the lowest moisture content (75.01 ± 1.20%), and the 10P samples, with complete mushroom replacement, showed the highest moisture content (88.35 ± 0.69%). Substituting pork meat with fresh oyster mushrooms in the sausage formulations did not lead to significant changes in water activity of the samples. The water activity values for all sample groups ranged between 0.94 and 0.95. The fresh oyster mushrooms used in this research had an average pH of 6.35. The pH of the sausage samples did not significantly differ across different formulations ($p > 0.05$), indicating that the fresh oyster mushroom had no discernible effect on the samples' pH. As the amount of oyster mushroom in the sausage formulations increased, the L^* values declined, resulting in a darker product. The a^* values also significantly decreased as the substitution level

increased, leading to a less red appearance in the sausages ($p < 0.05$). A significant increase in b^* values was noted ($p < 0.05$). Increasing the ratio of oyster mushroom up to 60% resulted in a reduction in both shear force and work values, enhancing tenderness and producing softer sausages. Based on the DSC curves, the control sample (0P) shows three distinct peaks representing the primary meat proteins, as it consists solely of pork meat. As the proportion of meat replacement increased, the denaturation peaks became smaller and less distinct in the diagrams. The calculated enthalpy values and peak temperatures aligned with the visual trends observed.

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

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

After pretreatment, samples Blanch, HHP, Oven, and UV exhibited comparable moisture contents, ranging from 88.90% to 90.47%, which is similar to that of fresh oyster mushrooms (89.66%). Further significant differences in moisture content were noted after the fermentation of mushrooms. Samples Fresh and UV post-fermentation displayed the highest moisture content, both at 90%, while MW samples recorded the lowest at 85.56%. The control sample (Fresh) exhibited the lowest pH, while the Blanch samples showed the highest pH value of 7.60 ± 0.02, compared to the mean pH of 6.35 ± 0.04 for fresh mushrooms. All pretreated samples recorded a higher pH than fresh oyster mushrooms As anticipated, all sample groups experienced a decrease in pH after fermentation. After pretreatment, the highest final weights were observed in the UV (99.71%) and HHP (98.65%) samples. In

contrast, the lowest yield (80%) was recorded for mushrooms subjected to microwave treatment (MW). Following fermentation, the highest yield was observed in MW samples (95.46%), followed by Blanch (92.32%) and Steam (89.59%) samples.

All pretreated samples exhibited significantly lower L^* values than untreated samples (Fresh), except for UV samples ($P<0.05$). This trend aligned with the Browning Index, suggesting that browning was most pronounced in Steam, MW, and Oven samples and least observed in UV samples. The Yellowness Index showed a consistent trend with the Browning Index, matching the b^* values. The lowest a^* values were observed in HHP and Oven samples. Following fermentation, the highest L^* values were recorded for Blanch and MW samples, suggesting that water blanching and microwave treatments may help reduce browning in mushrooms, as reflected in the BI results. Additionally, fermentation led to a reduction in lightness and redness (except for Blanch samples) and significantly increased yellowness across all samples.

Pretreatment type caused significant differences in the mushrooms' texture ($p < 0.05$). Fresh (54.79 ± 4.86), UV (54.41 ± 3.10), and MW (52.41 ± 3.39) samples required notably higher shear force than other samples, while HHP (35.30 ± 4.39) samples required the least. Significant textural differences were found between samples post-fermentation (Fig 22), with Blanch (46.85 ± 4.29), MW (46.37 ± 4.85), and Steam (44.88 ± 5.35) samples requiring greater shear force compared to others, whereas UV (8.53 ± 1.12) samples needed the least force.

The oyster mushroom samples contained 17 protein-building amino acids, with their total amounts ranging from 144.34 to 248.91 mg/g for the pretreated samples. All pretreatment methods caused a significant increase of essential amino acid and total amino acid content of the mushroom samples ($p < 0.05$). Among these, microwave pretreatment increased the amino acid concentration the most, (72.4%) reaching 248.91 mg/g. In general, fermentation reduced the total amino acid content of the pretreated samples, except in the case of fresh fermented ones. Of the nine essential amino acids, eight were identified in the oyster mushroom samples, with tryptophan being the exception.

The oyster mushroom samples contained 22 free amino acids, with their total amounts ranging from 25.95 to 55.68 mg/g for the pretreated samples. The total free amino acid content of the fresh oyster mushroom was 34.07 mg/g. Blanching (23.8%) and microwave (14.9%) pretreatments caused a significant decrease on

free amino content of the mushroom samples, while HHP (63.4%), oven (61.9%), UV (60.1%) and steam (8.0%) pretreatments caused an increase ($P < 0.05$). In the fermented sample group (Figure 28), the fresh fermented samples exhibited the highest free amino acid content at 53.64 mg/g, while blanched fermented resulted in the lowest content at 26.23 mg/g.

In the pretreated sample group, the total biogenic amine content ranged from 0.17 to 0.34 mg/g, with an average of 0.27 mg/g. The lowest biogenic amine content was observed in fresh samples (0.17 mg/g), while the highest was in UV pretreated (0.34 mg/g) samples. The UV pretreated fermented oyster mushrooms exhibited the highest biogenic amine content (5.05 mg/g), while the lowest was observed in blanched fermented samples (0.14 mg/g). Fermented samples generally displayed higher biogenic amine levels except for the blanched fermented samples. Histamine was the most abundant and present in all fermented samples except the blanched ones, with notably high levels (1.23 mg/g) observed in HHP fermented sample. Tyramine was the second most with an exceptionally high amount (4.1 mg/g) in the UV fermented sample. HHP, UV and oven pretreatments formed distinct clusters indicating that their NIR spectra contain unique characteristics differentiating them.

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

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

All samples with mushroom substitution exhibited higher moisture levels than the control sample, and the 50% substitution samples contained more moisture than both the control and 25% substitution samples. Replacing meat with pretreated fermented mushrooms had a significant impact on sausage pH ($p < 0.05$). Beginning on day 0, the control samples showed higher pH values ($pH = 5.87 \pm 0.03$) than the replacement samples, a trend that continued through the end of storage ($pH = 6.04 \pm 0.02$). Replacing meat with pretreated fermented mushrooms significantly influenced the TBARS values of the sausage samples ($p < 0.05$). The

control samples consistently showed lower TBARS values than the 25% replacement samples. Concerning the TBARS data, it is important to highlight that all sample groups maintained lipid oxidation levels below 0.50 mg MDA/kg throughout the entire storage period, which is considered acceptable for processed meat products (Patinho et al., 2019).

Pretreatments applied to mushrooms before fermentation led to notable differences in the color attributes of the sausage samples with meat replacements. The most negative impacts on sausage color were noted when the mushrooms were fresh, pretreated with HHP or UV light before fermentation. In contrast, the least detrimental effects were noted when the mushrooms underwent blanching or microwave pretreatment before fermentation. Notably, the greatest texture changes occurred in sausages containing fresh fermented, UV fermented, and HHP fermented mushrooms at both 25% and 50% replacement levels. The pronounced softness in fresh fermented samples underscores the importance of pretreatment processes to maintain mushroom quality before fermentation.

The overall sensory rankings revealed that blanched, microwave pretreated and steamed samples received similar rankings with the control samples at both replacement levels, with slight variations in ranking depending on the replacement ratio. NIR spectra of fresh fermented, UV fermented, and HHP fermented sausage samples clustered together, suggesting shared spectral characteristics.

CONCLUSIONS AND RECOMMENDATIONS

The complex challenges associated with animal meat production have prompted many consumers to reduce their intake of muscle-based foods. In response to these challenges, the FAO has highlighted the need for alternative protein sources to effectively feed the growing global population. This has spurred increased research into developing meat alternatives and enhancing the nutritional content of traditional meat products by incorporating sustainable, potentially health-promoting ingredients. In this context, mushrooms have emerged as a promising alternative, either as direct replacements or as innovative ingredients in various meat products. Mushrooms are typically used in meat products in the form of dried powder, fresh ground, or extracts. However, there is limited research on the application of emerging technologies—such as microwave, high hydrostatic pressure, and ultraviolet light treatments—and their comparative effects on mushroom quality in relation to traditional methods. Furthermore, the use of fermentation processes in the production of meat alternatives remains

underexplored, despite its potential to enhance functionality, improve nutritional content, and create appealing aromas. This study aimed to investigate: the replacement of meat with fresh oyster mushrooms in sausage formulations, the effects of different pretreatments and fermentation on the quality characteristics of oyster mushrooms, and the incorporation of pretreated fermented mushrooms as meat substitutes in sausage formulations.

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

The feasibility of integrating advanced technologies, such as HHP, microwave, and UV light, alongside traditional methods like steaming, oven cooking, and water blanching prior to mushroom fermentation, was investigated. Among these methods, microwave and steaming pretreatments showed the most promising results. They better preserved the color and texture of the samples, resulting in higher yield values after fermentation, suggesting they could be viable alternatives to water blanching. Fermentation led to a decrease in pH and redness, while increasing yellowness in the pretreated mushroom samples. The total and essential amino acid content of the pretreated samples were significantly higher than that of fresh oyster mushrooms, with the microwave-pretreated samples exhibiting the most substantial improvement. All essential amino acids, except tryptophan, were detected in the mushroom samples. Essential amino acids, including arginine, comprised 35–44% of the total amino acids in the pretreated mushroom samples, indicating that oyster mushrooms maintain high protein quality regardless of the pretreatment applied.

Blanching and microwave pretreatments reduced the free amino acid content, whereas steaming, oven, HHP, and UV pretreatments increased it compared to fresh oyster mushrooms (34.07 mg/g). When pretreatments were combined with

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

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

The incorporation of fermented oyster mushrooms as a meat substitute in sausage formulations affected all examined quality attributes of the samples during storage. Samples with mushroom substitution had higher moisture levels than the control, with 50% substitution samples retaining more moisture than both the control and 25% substitution samples. Among the groups, UV25 and UV50 had the lowest moisture content, while Blanch50 and Blanch25 recorded the highest. Sausages with 50% mushroom replacement exhibited lower pH values than those with 25%,

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

These findings indicate that fermented oyster mushrooms can serve as a feasible meat substitute in sausage production, though their effectiveness is constrained by the proportion of mushroom used and the pretreatment method applied. Fermented oyster mushrooms offer the potential to create meat-substituted products with improved health benefits and sustainability, providing valuable insights for the food industry. Blanching and microwave pretreatments were identified as the most suitable methods, considering their positive influence on the mushrooms' physical, chemical, and nutritional properties, as well as their effects when incorporated into sausage formulations. Conversely, HHP and UV pretreatments were deemed unsuitable for mushroom pretreatment prior to fermentation, due to significant increases in biogenic amine content, which exceeded recommended safety limits, along with their undesirable impacts on color and texture.

Although pH measurements confirmed the successful fermentation of mushroom

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

NEW SCIENTIFIC RESULTS

1- I observed that microwave pretreatment of fresh oyster mushrooms (900 W, 2.45 GHz, 85 ºC for 3 minutes) resulted in the highest fermentation yield (95.46%) when compared to other thermal (Blanching, Steaming, Oven) and non-thermal (HHP, UV) pretreatment methods.

2- I observed that the best results for preserving mushroom color and texture stability were achieved with blanching (100 ºC for 3 minutes) and microwave pretreatment (900 W, 2.45 GHz, 85 ºC for 3 minutes) prior to fermentation.

3- I found that the total amino acid content and the essential amino acid content of oyster mushrooms significantly increased with the application of pretreatments ($p < 0.05$). Among all the samples, only the steamed fermented samples (100 ºC for 3 minutes, at 21-22 ºC for 8 days) exhibited a lower essential amino acid content (52.50 mg/g) compared to fresh oyster mushrooms (58.05 mg/g).

4- I found that the total biogenic amine content of fresh (0.17 mg/g) and pretreated mushroom samples (0.17-0.34 mg/g) were under the overall BA content limits (0.75-0.9 mg/g food). The HHP fermented (300 MPa, at 20 ºC for 3 min, at 21-22 ºC for 8 days) samples exceeded the recommended Histamine limits (0.05–0.1 mg/g for food) with 1.23 mg/g, while UV fermented (30 W, 312 nm at 20 ºC for 15 min, at 21-22 ºC for 8 days) samples exceeded the recommended Tyramine limits (0.1–0.8 mg/g for food) with 4.1 mg/g.

5- I observed that NIR spectroscopy successfully detected (100% original classification accuracy) and reflected chemical and spectral differences between thermal and non- thermal pretreatments. A lower cross-validation accuracy (42.9%) was observed due to the model misclassifying thermal pretreatments among each other, indicating spectral similarities.

6- I observed that during the sensory analysis, panelists did not detect significant differences in the visual appearance of the sausage samples between the 50% and 25% mushroom substitution levels. Sausage samples containing blanched (at 100 ºC for 3 min), microwave pretreated (at 900 W, 2.45 GHz, 85 ºC for 3 min) and steamed (at 100 ºC for 3 min) fermented mushrooms received higher overall rankings, comparable to the control samples at both replacement levels.

LIST OF PUBLICATIONS

Journal article publications

Boylu M, Hitka G, Kenesei G. (2024). Sausage Quality during Storage under the Partial Substitution of Meat with Fermented Oyster Mushrooms. *Foods*; 13(13):2115. DOI:10.3390/foods13132115

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Yıldız Turp G., **Boylu M**. (2018). Medicinal and Edible Mushrooms and Usage in Meat Products. *Yüzüncü Yıl University Journal of Agricultural Sciences*, 28 (1); 144-153. DOI: 10.29133/yyutbd.397683

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