

ORIGINAL ARTICLE

How do *sous-vide* treatment and herb spices addition improve sensory acceptance and antimicrobial attributes of organic oyster mushrooms (*Pleurotus ostreatus*)?

Ana Doroški¹  | Anita Klaus¹ | Biljana Nikolić² | Igor Tomasevic¹ | Vesna Lazić¹ | Jovana Vunduk^{1,3} | Ilija Djekic¹

¹Faculty of Agriculture, Institute for Food Technology and Biochemistry, University of Belgrade, Belgrade, Serbia

²Faculty of Biology, University of Belgrade, Belgrade, Serbia

³Institute of General and Physical Chemistry, Belgrade, Serbia

Correspondence

Ana Doroški, Institute for Food Technology and Biochemistry, University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade 11080, Serbia.
Email: ana.doroski@gmail.com

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Abstract

Sensory and physical properties of organic oyster mushrooms (*Pleurotus ostreatus*) prepared by the *sous-vide* culinary method (50–90°C, 10') were investigated and compared with the cooking method (80°C, 30–60') to analyze the effects of different temperature and time regimes. To promote organic oyster mushroom's nutritional benefits and improve its taste, oregano and thyme were added in different percentages, and mushrooms were exposed to *sous-vide* treatments at 60°C for 20' and 30'. Sensory evaluation was focused on testing spices intensity, likeability, and recognizability with principal component analysis employed. While the likeability scores and color changes were higher for those with higher thyme content in the mixture, the antibacterial results showed the highest effect of oregano, proving certain positive effects and emphasizing the research's importance. As an added value of the research, the mixture concentrations of *sous-vide* vacuumed pouches containing spices and mushrooms, that is, *sous-vide* pure juice extracts were analyzed for the first time for antibacterial activity. The research highlights the sensory improvement of mushrooms using the convenient treatment and adding selected spices, altogether multiplying their health benefits by preserving beneficial compounds.

Novelty impact statement: *Sous-vide* culinary method, as an innovative choice in the world of gastronomy, gives the possibility to adjust treatment conditions and enables control of changes associated with the physical properties of mushrooms. Concerning the importance of living a healthy lifestyle, the fact that this method provides the ability to preserve bioactive compounds in well-known healthy foods, such as mushrooms, widens the additional importance of its application. The research highlights the sensory improvement of mushrooms using convenient heat treatment and adding selected spices generally used in traditional and world-known cuisine and altogether multiply their health benefits by preserving beneficial compounds.

1 | INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus*) (Jacq. ex Fr.), P. Kummer (1871), was recognized as one of the most consumed species, for its mild

and unique taste, which enriches world cuisine. Besides being commercially important, *P. ostreatus* is embedded with a health benefit potential, such as immunomodulatory, antitumor, anti-inflammatory, antioxidant, and antimicrobial effects (Corrêa et al., 2016).

According to Aisala et al. (2018), the reduced volume of research regarding the sensory properties of mushrooms is surprising considering the large interest in mushroom consumption. Considering that consumers' preferences toward mushrooms are diverse, and the ones who dislike their taste could not benefit from their health effects with existing food preparation methods, additional investigation, which potentially reveals alternatives to attract new consumers and expand a healthy diet practice, is encouraged.

Sous-vide is a new-age culinary method widely used in the food industry and haute cuisine. Its advantage over other widely used culinary methods, which attract attention in the world of gastronomy, includes a decrease in heat treatment variation and avoidance of evaporation and leaching of flavor compounds to the air or cooking medium (Aisala et al., 2018). Namely, the main key mushroom-like odor is a combination of 1-octen-3-ol, 3-octenone, and methional, found the highest in button mushroom samples. Additionally, any processing method (cooking, canning, drying) creates new volatile substances as a result of various chemical reactions of a considerable amount of reactive compounds already present in raw mushrooms (Misharina et al., 2009). Many researchers focused on descriptive sensory analysis of mushrooms and the connection of mushroom descriptors with their volatile aroma compounds (Aisala et al., 2018; Politowicz et al., 2018; Rotola-Pukkila et al., 2019). Research by Li et al. (2011) has shown the influence of cooking methods on the flavor profile of mushroom soup, regarding levels of free amino acids, presence of Maillard reaction, and aroma-active compounds. Namely, the heating treatment affected the loss of non-volatile components in chemical reactions and the volatile compounds' evaporation during the cooking process.

Sous-vide culinary method enables the preparation of different foodstuffs, including mushrooms, together with selected spices which noticeably contributes to a unique taste of processed food. Based on the fact that many people do not like the taste of mushrooms (Aisala et al., 2018), spices may potentially improve the taste and increase likability, besides the beneficial health and antimicrobial effect which they contribute (Boskovic et al., 2015; Radünz et al., 2021; Salehi et al., 2019). Black pepper (*Piper nigrum*, L.) is a widely used culinary spice and its secondary metabolites show a wide range of human health benefits (Salehi et al., 2019). On the contrary, spices such as thyme (*Thymus vulgaris*, L.) and oregano (*Origanum vulgare*, L.) exhibit antioxidant and antimicrobial effects through their major constituents (thymol and carvacrol), and also offer potential health benefits, such as application as a traditional medicine for treating diseases: type II diabetes mellitus and control of chronic non-communicable diseases (Radünz et al., 2021) by reducing the level of reactive oxygen species. Antimicrobial activity of oregano and thyme essential oil against several pathogens was widely investigated, such as *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, and *MRSA* (Methicillin-resistant *Staphylococcus aureus*) (Boskovic et al., 2015).

The main objective of this study was to analyze how cooking and *sous-vide* impact the physical properties and sensory perception of oyster mushroom prepared with selected spices, and what are the

relations among them. For obtaining a better insight, different temperatures and treatment times were investigated for the two culinary methods. Oyster mushroom was chosen due to its favorable sensory characteristics among mushrooms and richness in healthy components, while the used spices were black pepper, oregano, and thyme, selected in accordance with their flavor characteristics and biological activities. Finally, two widely used spices (thyme and oregano) were added during the preparation of *P. ostreatus* by *sous-vide* technique because of their antimicrobial effects as well as the impact on mushrooms' sensory attributes. The aim was to identify the sensory potential of mushrooms with spices, selected for two main factors: (i) proven antimicrobial effects (Boskovic et al., 2015); (ii) oregano and thyme are traditionally used and grown in some Serbian regions, while the black pepper is widely represented among the world market (Salehi et al., 2019).

2 | MATERIALS AND METHODS

2.1 | Types of mushroom

Organic oyster mushrooms (*Pleurotus ostreatus*) of commercial strain HK35 were cultivated by a local producer Ekofungi, Belgrade, Serbia. Fruiting bodies were freshly harvested and transferred to the laboratory 1 hour after picking, in plastic containers under refrigerated conditions (8–10°C). Mushrooms were prepared for the experiment right after the reception.

2.2 | Mushroom preparation

To prepare mushrooms for analysis, all damaged fruiting bodies or extraneous material were removed and fruiting bodies were sorted and cleansed. 100g of mushrooms were individually placed into plastic vacuum 85µm thick Polyamide/Polyethylene/Polyethylene bags (200mm × 300mm), and then vacuumed in the packaging machine HVC-510T/2A, applying a high vacuum level with a vacuum pump capacity of 20 m³/h.

2.3 | Applied culinary methods and experimental stages

2.3.1 | The preliminary experimental stage

The preliminary experimental stage was the sensory screening of organic oyster mushrooms prepared with three cooking methods: (i) blanching in hot water at 88°C for 2' (control), (ii) cooking at 80°C for 60', and (iii) *sous-vide* (60°C for 22'), both methods performed with three selected spices (oregano, thyme, and black pepper). The sensory panel consisted of nine researchers that participated in the preliminary research. Prepared combinations were evaluated on a five-point scale (1—totally unacceptable to 5—totally acceptable)

TABLE 1 Preliminary sensory evaluation of oyster mushroom prepared with three selected spices (thyme, black pepper, and oregano)

Culinary method	Taste	Odor	Overall quality	Defect
Cooking, 80°C 60'				
Oyster mushroom (control)	4.50 ± 0.55 ^a	4.17 ± 1.17 ^a	4.33 ± 0.82 ^a	0.00 ± 0.00 ^a
Oyster mushroom with thyme	4.50 ± 0.55 ^a	4.50 ± 0.55 ^a	4.33 ± 0.52 ^a	0.00 ± 0.00 ^a
Oyster mushroom with black pepper	1.83 ± 0.75 ^b	4.17 ± 0.41 ^a	2.33 ± 0.82 ^b	1.83 ± 0.41 ^b
Oyster mushroom with oregano	4.33 ± 0.82 ^a	4.33 ± 0.82 ^a	4.17 ± 0.75 ^a	0.17 ± 0.41 ^a
Blanching, 88°C 2'				
Oyster mushroom (control)	2.67 ± 1.51 ^b	2.33 ± 0.82 ^b	2.50 ± 1.38 ^b	1.33 ± 0.82 ^b

Note: Means of six replications ± standard deviation. Means in the same column with different small letters are significantly different ($p < .05$).

using three sensory attributes: taste, odor, and overall quality, leaving the panelist the possibility to detect potential defects. This experimental stage was prepared in line with the methods employed by Rotola-Pukkila et al. (2019) and presented in Table 1.

The additional evaluation included minimum inhibitory concentrations of spices in crude hot water extracts and mixture concentrations of *sous-vide* pure juice extracts against certain strains of pathogenic bacteria, as well as their interaction effect, which together with preliminary study influenced the decision of spice type and concentrations in the main experiment.

2.3.2 | The main experimental stages

The main experiment consisted of two stages. In the first part, culinary methods, treatment temperatures, and times were applied with mushrooms only. The details are presented in Table 2. These two culinary methods with the pre-defined temperature vs. time treatments were chosen based on the research of Rotola-Pukkila et al. (2019) and Aisala et al. (2018).

The second part included the preparation of oyster mushrooms using the *sous-vide* culinary method, with different combinations of spices added in the mushroom vacuum bag, presented in Table 3. Based on preliminary microbial and sensory analysis, only thyme and oregano were chosen as an addition to the mushrooms, while the previous stage influenced the decision of the main culinary method selection used with spices addition. The spices were finally added in a concentration of 40 mg g⁻¹ in total (4% on 100g of oyster mushroom), and the concentration of each spice was dependent on the percent in the mixture. The concentration percentages are presented in Table 3.

2.4 | Sensory evaluation procedure

Sensory analysis was performed using a trained panel consisting of nine researchers from the Faculties of Agriculture and Biology (University of Belgrade). Two training sessions of 90' each were conducted to familiarize the subjects with the sensory methods used.

Mushrooms were served at constant room temperature in plastic containers, within 2 h from sample preparation, to avoid

TABLE 2 Culinary methods with the corresponding temperature and time modes applied with mushrooms only in the first part of the main experimental stage

Sample no.	Culinary method	Temperature (°C)	Time (')
1	Cooking	80	30
2			40
3			45—control*
4			50
5			60
6	<i>Sous-vide</i>	50	10
7			60
8			70—control*
9			80
10			90

Note: (*) denotes control samples used during sensory analysis.

loss of off-odors. The testing was performed in a sensory laboratory designed in accordance with ISO 8589:2007 at the Faculty of Agriculture.

The preliminary stage and the first part of the main investigation consisted of two tests. The first test was based on a 9-point intensity scale with seven main sensory attributes, related to visual, odor, textural, and taste attributes, as well as observation of atypical tastes and estimation of product acceptability. In the second sensory test, panelists compared samples to the control one, applying a degree of difference testing to measure the variability in key sensory attributes from the target (control) sample. The panelists used a bi-directional 9-point category-type scale, with anchors set at "1" and "9" and with "Target" indicated as a score "5"; scores from 1–4 indicated "less intense" and 6–9 "more intense." The values were as follows: 1/9—unacceptably different, 2/8—significantly different, 3/7—marginally different, 4/6—slightly different, 5—as control, similar as described in the work of Djekic et al. (2017). For the main experimental results, semantic differential charts were used to visualize the sensory profile of the mushrooms.

The second part of the main evaluation was performed by assessing the intensity of selected sensory attributes (odor—oyster mushroom, thyme, and oregano; crispness; hardness; taste—oyster mushroom, thyme, and oregano) using 15 cm line scales with verbal

TABLE 3 Culinary methods with the corresponding temperature, time modes and added spices applied in the second part of the main experimental stage

Sample no.	Culinary method	Temperature and time (°C, ')	Combination and proportion of added spices	Percentage of added spices	Code
1	<i>Sous-vide</i>	60°C, 20'	100% Thyme	4%	100TH
2			75% Thyme 25% Oregano	3%: 1%	75TH25OR
3			50% Thyme 50% Oregano	2%: 2%	50TH50OR
4			25% Thyme 75% Oregano	1%: 3%	25TH75OR
5			100% Oregano	4%	100OR
6			/	0%	CONTROL20*
7		60°C, 30'	100% Thyme	4%	100TH
8			75% Thyme 25% Oregano	3%: 1%	75TH25OR
9			50% Thyme 50% Oregano	2%: 2%	50TH50OR
10			25% Thyme 75% Oregano	1%: 3%	25TH75OR
11			100% Oregano	4%	100OR
12			/	0%	CONTROL30*

Note: (*) denotes control samples used during sensory analysis.

anchors at both ends (none—intense for odor, taste, and crispiness; soft-hard for hardness), as proposed by ASTM (1992). Panelists used the scales in their way by comparing the samples to each other since there were no reference standards (Romano et al., 2008). For odor evaluation, spices were kept in closed glass jars. Finally, using 15 cm line scales with anchors at both ends panelists ranked: (i) whether the intensity of spices is lower/higher than in their preferred cousins (lower intensity and higher intensity; mid-point was defined as “exactly the same”) and (ii) overall likeliness of the mushroom with spices (dislike extremely and like extremely; mid-point was defined as “neither like nor dislike”).

All sensory testing was performed in two replicates. Every panelist was instructed to drink still water and chew a piece of cracker between the samples testing, to neutralize residual flavors (Aisala et al., 2018). No specific instructions were provided to panelists on whether to swallow or expectorate individual bites.

2.5 | Color change

Visual color for each mushroom sample was measured by hunter color parameters (L^* , a^* , and b^*) and L_0^* , a_0^* , and b_0^* for control samples, using Computer Vision System (CVS) method (Tomasevic et al., 2018). A total of 10 measurements of each sample were analyzed and attached to the data calculation. Due to the visible differences in the color of different segments of mushroom fruiting body, the color values were measured from three different segments of mushroom fruiting bodies with similar shades, in 10 replicates.

Total color difference (ΔE), Browning index (BI), Yellowness index (YI), and Whiteness index (WI) as key color parameters, previously described by Pathare et al. (2013), were determined based on Equations (1)–(4):

$$\Delta E = \sqrt{(a^* - a_0^*)^2 + (b^* - b_0^*)^2 + (L^* - L_0^*)^2} \quad (1)$$

$$BI = \frac{100(x - 0.31)}{0.17}, \text{ where } x = \frac{(a^* + 1.75 L^*)}{(5.645 L^* + a^* - 3.012 b^*)} \quad (2)$$

$$YI = 142.86 \cdot \frac{b^*}{L^*} \quad (3)$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad (4)$$

2.6 | Texture profile analysis

All culinary-treated mushrooms were exposed to a single compression cycle by using Texture CT3 (Brookfield Engineering Labs, Inc., USA). Testing parameters were defined as follows: speed—3.3 mm s⁻¹ and deformation depth—1 mm using a needle probe of 2 mm diameter (Djekic et al., 2017). Ten mushroom samples of each mushroom treatment were horizontally placed and compressed at ambient conditions, measuring the force of the first bite along with adhesion parameters.

2.7 | Extracts preparation

2.7.1 | Crude hot water extracts preparation

The crude hot water extracts were prepared according to Sudha et al. (2012) and Rotola-Pukkila et al. (2019), respectively. The organic *P. ostreatus* fruiting bodies were powdered and prepared as crude hot water extracts at 85°C for 1 h after they were air-dried at 55°C to constant weight. Extracts were prepared in a concentration of 160 mg ml⁻¹ distilled water. Thyme, black pepper, and oregano were purchased dry in the local market from the well-known manufacturer (Premia—private label of the biggest retailer in Serbia), and prepared by the same method in different concentrations: 40 mg ml⁻¹ for thyme and 20 mg ml⁻¹ for oregano and black pepper crude hot water extract. After

thermal treatment, extracts were centrifuged for 30' at 11,000rpm and the supernatant was stored at 4°C for further analysis.

2.7.2 | *Sous-vide* pure juice extract preparation

Sous-vide pure juice extracts were prepared according to the "Mushroom preparation section of this study," with the addition of spices, in the same percentages as listed in Table 3. Six vacuumed bags were exposed to *sous-vide* treatment for 30' at 60°C. Juice segregated in each bag was collected in sterile conditions and stored at 4°C for further analysis.

2.8 | MIC assay

MIC assay was performed in 96-well microplates, as previously described by Nikolić et al. (2019). The Gram-positive bacterial species *Enterococcus faecalis* (ATCC 29219) and *Staphylococcus aureus* (ATCC 25923), as well as Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Proteus mirabilis* (ATCC 12453) were used for antimicrobial activity assay analysis performed with crude hot water extracts of spices and organic oyster mushroom. Crude hot water extracts were tested in concentration ranges from 0.625 to 10 mg ml⁻¹ (oregano and black pepper), from 1.25 to 20 mg ml⁻¹ (thyme) and 2.5 to 80 mg ml⁻¹ (oyster mushroom).

Selected species of bacteria originate from ATCC (American Type Culture Collection, Rockville, Maryland). The concentration of *sous-vide* pure juice extracts was 80 mg g⁻¹. The bacterial suspension was adjusted to approximately 5–6 log CFU ml⁻¹ and incubated at 37°C for 24 h. The lowest concentrations of added extracts without visible bacterial growth were defined as minimum inhibitory concentrations (MIC). Testing was performed twice in three replicates.

2.9 | Checkerboard assay

Checkerboard assay was performed in 96-well microplates, according to Mulyaningsih et al. (2010). *MRSA*–Methicillin-resistant *Staphylococcus aureus* (human isolate), *Enterococcus faecalis* (human isolate), *Escherichia coli* (human isolate), and *Salmonella enteritidis* (ATCC 13078) were used for antimicrobial activity assay of *sous-vide* pure juice extracts analysis. Concentrations of prepared extracts ranged from 4MIC to 1/32MIC. Bacterial inoculum in each well was adjusted to 5–6 log CFU ml⁻¹ concentration. Plates were incubated at 37°C for 24 h. Testing was performed twice in three replicates. Fractional inhibitory concentration index (FICI) was calculated according to Equation (5), for two combinations of antimicrobials, which was used to differentiate the effect as follows: synergetic effect (FICI ≤ 0.5); additive effect (0.5 < FICI ≤ 1); indifferent effect (1 < FICI ≤ 4) and antagonistic effect (FICI > 4).

$$FICI = \frac{MIC_{A \text{ in comb.}}}{MIC_{A \text{ alone}}} + \frac{MIC_{B \text{ in comb.}}}{MIC_{B \text{ alone}}} \quad (5)$$

2.10 | Statistical analysis

Statistical analysis was obtained using a *t*-test and/or one-way analysis of variance (ANOVA). *Tukey's* post hoc test was used to differentiate statistical differences between the data, with statistical significance set at the level $p < 0.05$. A general overview of the sensory profile was done using Principal Component Analysis (PCA), including eight descriptors from the raw data. SPSS Statistics 23 and Microsoft Excel 2013 were used for statistical data analysis.

3 | RESULTS

3.1 | Preliminary sensory evaluation results

According to the preliminary evaluation (Table 1), the most acceptable taste and overall quality, with insignificant noticed defects, were recorded for the control sample and oyster mushroom with thyme and oregano, which influenced the decision not to consider black pepper for further experiments. As for other culinary methods, overall quality showed *sous-vide* (T = 60°C) as a promising culinary method, so this was further explored according to the sensory and textural properties evaluation behind.

3.2 | The main evaluation stage: Cooked and *sous-vide*-treated mushrooms

3.2.1 | Sensory profiling of mushrooms prepared by different culinary methods

The central line on each chart of Figure 1 represents the control sample result: treatment 80°C 45' for the cooking method (a) and treatment 70°C 10' for *sous-vide* (b). Each sensory attribute was evaluated in comparison to the control sample. According to Figure 1a, obvious differences were observed in the hardness, slipperiness, juiciness, and taste attribute of the treatment at 80°C 30' with regard to the control and sample at 80°C 60'. Namely, shorter time treatment affected the stronger juiciness and taste intensity, increased slipperiness mouthfeel, as well as the softening of the mushroom bite.

Figure 1b points out the conclusion that *sous-vide* treatment temperature significantly affected the impression of the sample differences. First, the color of sample 60° 10' was notably lighter than the control and sample 80°C 10', respectively. This can be related to the color properties data which originate from the instrumental CVS method, presented in Table S1 (Supporting material), which indicates the brown and yellow shades enhancement with higher temperature treatment. The same relation was also presented for the following attributes: odor, crispness, and juiciness. The sample softness grew with a higher temperature, confirmed by the texture analysis (Table 4). Additionally, the juiciness intensity of sample 80° 10' was outstanding, which is expected with higher temperature treatment.

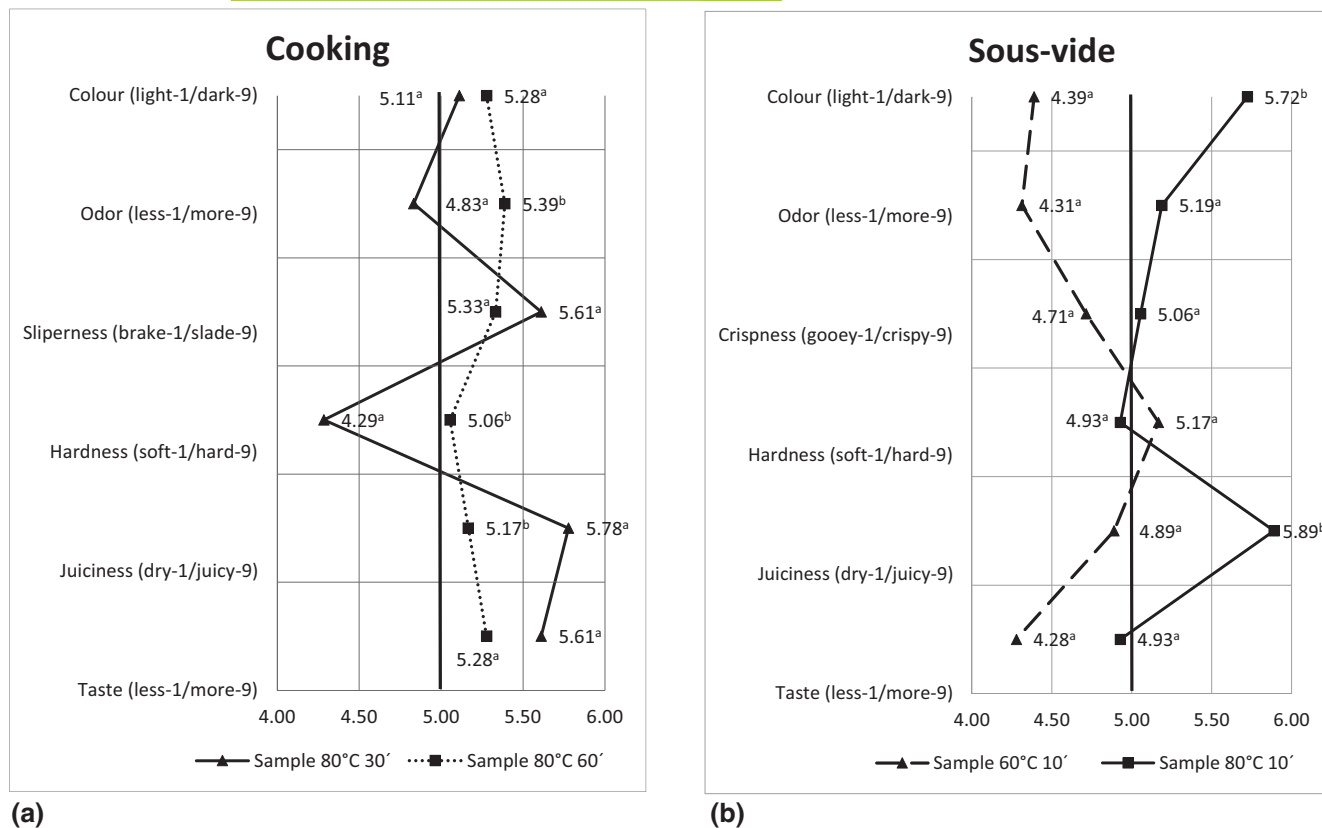


FIGURE 1 Semantic differential chart for mushrooms treated by different culinary methods, temperatures, and time: (a) cooking at 80°C for 30' and 60', compared to 45' for a control; (b) *sous-vide* for 10' at 60°C and 80°C, comparing to 70°C for a control.

TABLE 4 The effects of culinary method, temperature and time on the textural properties of mushrooms

<i>Sous-vide</i> for 10'	50°C	60°C	70°C (control)	80°C	90°C
Peak load (N)	0.60 ± 0.10 ^a	0.78 ± 0.30 ^{a,b}	0.86 ± 0.24 ^{a,b}	0.92 ± 0.40 ^b	0.92 ± 0.26 ^b
Adhesive force (N)	0.06 ± 0.10	0.05 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
Adhesion (mJ)	42.11 ± 62.28	61.87 ± 23.34	38.57 ± 50.13	40.77 ± 49.93	57.10 ± 58.98
Cooking at 80°C	30'	40'	45' (control)	50'	60'
Peak load (N)	1.06 ± 0.30 ^a	1.00 ± 0.28 ^a	1.02 ± 0.24 ^a	0.72 ± 0.31 ^b	0.71 ± 0.20 ^b
Adhesive force (N)	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
Adhesion (mJ)	58.39 ± 48.41	49.61 ± 36.62	63.14 ± 51.15	47.52 ± 50.11	35.45 ± 36.27

Note: Means of ten replications ± standard deviation. Means in the same row with different letters are significantly different ($p < 0.05$).

3.2.2 | Color properties of organic mushrooms

Regarding the total color difference of the central part of the mushroom cap, the treatments 80°40' and 80°50' had the most similar color to the control sample. A similar trend was noticed for the whiteness index, which was higher for the central part of mushroom caps of the treatments with similar cooking time as a control. The treatment with the shortest cooking time had the highest mushroom cap edge whiteness index, while the treatments 40' and 50' have shown this parameter being the highest for mushroom gills. Concerning browning and yellowing indices of mushroom caps, treatments for 40' and 50' showed lower values for central parts and higher values

for the edges. As for mushroom gills, browning and yellowing indices were highest for treatment 60'.

Sous-vide treatment showed a lower influence on color change generally, with lower indices values. The exception was noticed in a total color difference of mushroom gills, which showed a lower color difference of samples with higher temperature treatment. On the contrary, the whiteness of mushroom samples was lower with the increase in temperature, in all mushroom parts analyzed. Browning and yellowing of the central part of the mushroom cap were increasing with the higher temperature treatment. A similar trend was noticed for mushroom cap edge browning and yellowing indices, with a milder growth curve at higher temperatures. Mushroom gills indices

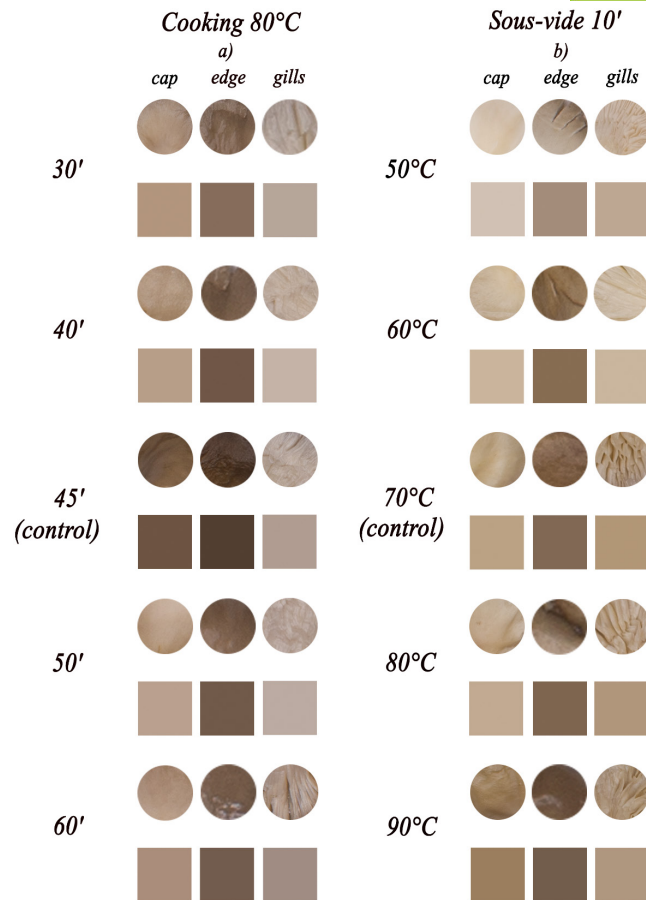


FIGURE 2 Images and color chips of mushrooms treated by different culinary methods, temperatures, and time (cap, edge, and gills). (a) cooking—at 80°C for 30'; 40'; 45' (control); 50' and 60' (b) *sous vide*—at 50°C; 60°C; 70°C (control); 80°C and 90°C for 10'.

showed a mild decline with increasing temperature, starting at 60°C *sous-vide* treatment. The color data is visualized by the images of cooked and *sous-vide* mushrooms (Figure 2a,b) and presented in Table S1. of Supporting material.

3.2.3 | Texture analysis of organic mushrooms

The difference in texture properties of *sous-vide* treated and cooked mushrooms are highlighted by the peak load parameter. It represents “the maximum force to break into the food in a combination of adhesive and cohesive effects” (Zorrilla et al., 2000). This parameter increased with the higher *sous-vide* treatment temperature and decreased with longer cooking treatment time. Namely, the higher the *sous-vide* treatment temperature was, the higher the maximal compression force was required. Longer *sous-vide* treatment did not influence the mushroom texture parameters (data not shown). For the cooking treatment, the rule was reversed. The adhesion parameter also showed an opposite trend for the two culinary methods. The control sample of *sous-vide* treatment had the lowest adhesion parameter and the highest for cooking treatment (Table 4).

3.3 | The main evaluation stage: *Sous-vide*-treated mushrooms with spices

3.3.1 | Sensory evaluation results of mushrooms with spices

In accordance with previously analyzed data, oregano and thyme spices, as well as *sous-vide* at 60°C as a culinary method, were selected for further analysis. Our previous experimentation pointed out that both of them could be used to improve the taste of oyster mushrooms processed in that way. However, since the treatment that lasted for 10' did not result in the expected intensity of spices' taste (data not shown), the *sous-vide* processing was prolonged to 20' and 30'. Intensity impressions related to added spices were positively correlated to the percent of present oregano. On the contrary, the likeliness of the mushrooms was negatively correlated to the oregano content. Comparing two *sous-vide* time treatments (Figure 3), total intensity scores for each sample after 20' (a) and 30' (b) were similar, with exception of 100OR, rated with much stronger intensity after 30' of treatment. Likewise, the likeliness of two negatively scored samples: 25TH75OR and 100OR were closer to control (less negative) after a shorter time of *sous-vide* treatment. Likeliness scores for samples with higher thyme content were slightly higher for the longer *sous-vide* treatment.

3.3.2 | Principal component analysis

In order to separate the impression of different added spices, interpretation of the data collected in this investigation was conducted by principal component analysis (PCA) (Figure 4). Loading plot shows that the sensory attributes could be visually grouped into three clusters and two separated independent attributes: (i) the first cluster contains sensory attributes of thyme: thyme taste and thyme odor, with positive loadings in the second principal component (PC 2) and high negative loadings in first principal component (PC 1) (ii) the second cluster consists of sensory attributes of mushroom: mushroom taste and mushroom odor, with high positive loadings in second principal component (PC 2) and (iii) the third cluster comprising of sensory attributes of oregano: oregano taste and oregano odor, with near zero loadings in PC 2. Hardness and crispiness are separated and visually independent attributes, with high positive loadings in PC 2 and negative loadings in PC 1, as well as high negative loadings in PC 1, respectively.

The scores plot depicts a model that separated the samples into the mushrooms with higher thyme content, with high positive scores, and the mushrooms with higher oregano content, with high negative scores. All the samples with combined spices are grouped within the PC 1 positive scores, positively correlated with the mushroom odor and taste attributes. Mushroom samples with 100% thyme had near-zero scores in PC 1 but were negatively correlated with the thyme odor and taste attributes. Mushroom samples with 100% oregano were, on the contrary, positively correlated with the oregano taste

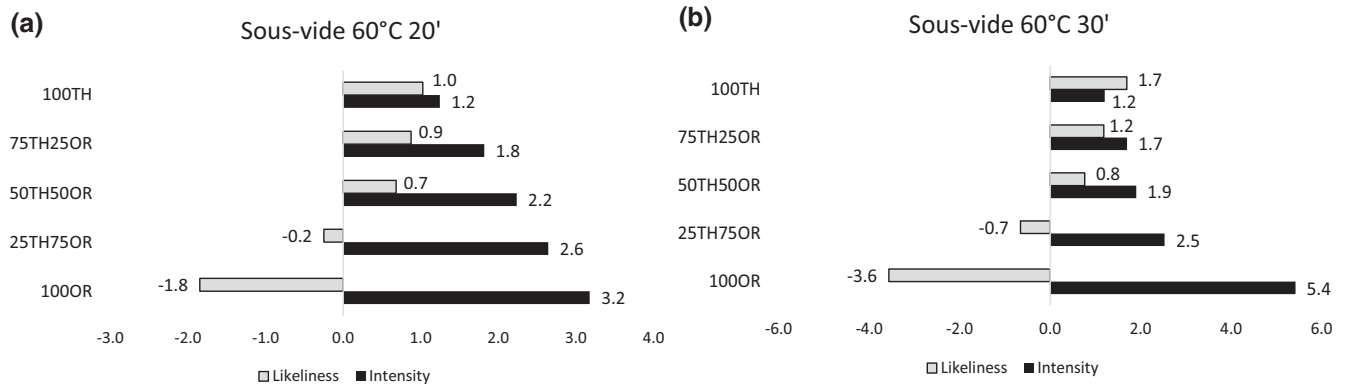


FIGURE 3 Intensity of spices and likeliness of mushrooms with spices (a) *sous-vide* 60°C 20'; (b) *sous-vide* 60°C 30'. Intensity scale: lower intensity (negative values)—“0” (exactly the same)—higher intensity (positive values). Likeliness scale: dislike extremely (negative values)—“0” (neither like nor dislike)—like extremely (positive results). Sample codes: 100TH—100% Thyme; 75TH25OR—75% Thyme: 25% Oregano; 50TH50OR—50% Thyme: 50% Oregano; 25TH75OR—25% Thyme: 75% Oregano; 100OR—100% Oregano.

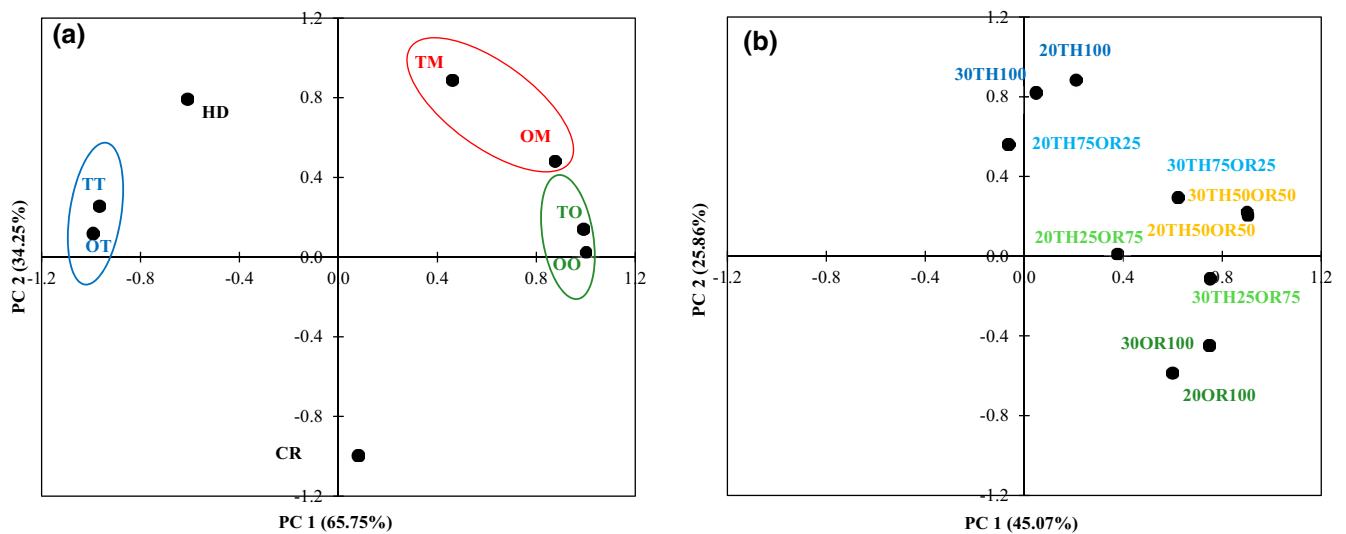


FIGURE 4 Principal component analysis loadings (a) and scores (b) for eight sensory attributes and 10 samples of oyster mushroom prepared by *sous-vide* culinary method with different combination and amount of added spices and two temperature regimes (a): *Sous-vide* 20°C, 100% thyme (20TH100); *sous-vide* 20°C, 75% thyme, 25% oregano (20TH75OR25); *sous-vide* 20°C, 50% thyme, 50% oregano (20TH50OR50); *sous-vide* 20°C, 25% thyme, 75% oregano (20TH25OR75); *sous-vide* 20°C, 100% oregano (20OR100); *sous-vide* 30°C, 100% thyme (30TH100); *sous-vide* 30°C, 75% thyme, 25% oregano (30TH75OR25); *sous-vide* 30°C, 50% thyme, 50% oregano (30TH50OR50); *sous-vide* 30°C, 25% thyme, 75% oregano (30TH25OR75); *sous-vide* 30°C, 100% oregano (30OR100). Descriptors were (b): Intensity of oyster mushroom odor (OM); intensity of thyme odor (OT); intensity of oregano odor (OO); intensity of crispness (CR); intensity of hardness (HD); intensity of oyster mushroom taste (TM); intensity of thyme taste (TT); intensity of oregano taste (TO).

and odor attributes, which indicated good recognizability of oregano attributes in those samples. *Sous-vide* treatment time had a slight and insignificant influence on each sample perception. The principal component analysis method was also applied in the research of Aisala et al. (2018) in the overview of the sensory attributes of Nordic edible mushrooms.

3.3.3 | Color properties of mushrooms with spices

Control samples used for the estimation were: (i) pure oyster mushroom treated by *sous-vide* at 60°C for 20' (ii) pure oyster mushroom

treated by *sous-vide* at 60°C for 30' (Tables 5 and S2 of Supporting information).

Color changes on the central part of the mushroom cap are noticeable under the influence of *sous-vide* treatment duration. The total color difference was generally higher for all samples after 30' of treatment. Otherwise, mushroom gills and mushroom cap edge color changed oppositely, regarding the treatment duration. It is noticeable that the central part of the mushroom cap and cap edge browning parameter decreased with longer time treatment in samples with 100% thyme, while this trend was the opposite with 100% oregano. Also, the browning and yellowing index of the samples with a higher percent of thyme was higher than those with a

TABLE 5 The effects of combination and amount of added spices, as well as of processing time on the color properties of mushroom cap

<i>Sous-vide</i> at 60°C	100TH	75TH25OR	50TH50OR	25TH75OR	100OR
<i>Color properties of the central part of mushroom cap</i>					
Total color difference (ΔE)					
20'	16.27 \pm 2.87 ^A	15.93 \pm 2.88 ^A	5.07 \pm 1.35 ^B	14.79 \pm 7.37 ^A	9.59 \pm 3.13 ^B
30'	21.73 \pm 3.22 ^B	32.66 \pm 2.74 ^D	26.35 \pm 4.68 ^C	16.03 \pm 3.36 ^A	23.86 \pm 3.32 ^{BC}
Browning index (BI)					
20'	84.17 \pm 10.57 ^A	82.21 \pm 8.07 ^A	42.83 \pm 10.21 ^B	70.03 \pm 20.92 ^A	69.28 \pm 12.93 ^A
30'	64.35 \pm 8.05 ^{AB}	92.99 \pm 16.73 ^C	78.01 \pm 11.20 ^{BC}	61.74 \pm 9.33 ^A	84.62 \pm 13.36 ^C
Yellowing index (YI)					
20'	70.32 \pm 6.92 ^A	69.70 \pm 5.24 ^A	41.31 \pm 8.86 ^B	61.10 \pm 14.45 ^A	61.17 \pm 9.79 ^A
30'	57.56 \pm 6.29 ^{AB}	74.86 \pm 12.02 ^C	67.27 \pm 7.34 ^{BC}	56.71 \pm 7.19 ^A	72.56 \pm 7.30 ^C
Whiteness index (WI)					
20'	41.87 \pm 2.72 ^A	42.08 \pm 2.81 ^A	59.88 \pm 4.03 ^C	44.44 \pm 8.65 ^{AB}	50.44 \pm 4.21 ^B
30'	48.46 \pm 3.10 ^B	37.86 \pm 2.82 ^D	43.89 \pm 4.61 ^C	54.41 \pm 3.22 ^A	46.49 \pm 3.28 ^{BC}

Note: Means of ten replications \pm standard deviation. Means in the same row with different capital letters are significantly different ($p < 0.05$). 100TH–100% Thyme; 75TH25OR–75% Thyme: 25% Oregano; 50TH50OR–50% Thyme: 50% Oregano; 25TH75OR–25% Thyme: 75% Oregano; 100OR–100% Oregano. Control samples: *Sous-vide* mode, no spices added, 60°20' and 60°30'.

higher oregano percent, pointing out the contribution of thyme to the stronger color shades.

The whiteness index followed the trend presented for the browning and yellowing of mushrooms, but in the opposite way: when the browning index grew, the whiteness index dropped. Mushroom gills color change generally dropped with longer time treatment, with the exception of whiteness index, which increased with longer *sous-vide* treatment. These data also contain some inconsistencies, regarding the samples coded 75TH25OR and 25TH75OR for mushroom cap color, as well as 50TH50OR for mushroom gills. Namely, the browning/yellowing parameter grows or drops with time without a logical connection with the previous statement regarding the influence of spices on the browning/yellowing trend connected to the treatment duration. Generally, a sample with an equal percentage of both spices always had the lowest browning index for the treatment duration of 20', which calls into question the certain influence of the spices mixing on the color neutralization process (Figure 5).

3.4 | Antimicrobial activity of spices and mushroom extracts

Results of the MIC assay applied are presented in Table 6. Black pepper had the weakest antimicrobial effect, with a MIC of 10 mg ml⁻¹ detected only for *S. aureus*, which also influenced its exclusion from the main experiment. On the contrary, *E. coli* was the less susceptible and no spice had an effect on it in applied concentrations, but oyster mushroom induced an inhibitory effect at the highest tested concentration (80 mg ml⁻¹). MIC concentrations were initial values for checkerboard startup extract concentrations.

Interactions between spices mutually, and spices and oyster mushroom, were displayed through checkerboard assay, using MIC values as initial for extracts concentration ranges determination.

Calculated FICI values mostly pointed out the indifferent interaction between two components. For some combinations of oregano-thyme and thyme-pepper, antagonism was also determined, but with no synergism and additivism (Table 7).

Finally, MIC assay was applied to the *sous-vide* pure juice extracts, obtained with samples of mushrooms spiced with thyme and/or oregano, applied in different ratios. Higher temperature affected the water bounding by a mushroom. After cooling, mushrooms released juice and it was segregated, enabling easily volatile spices' compounds to be dissolved. Bearing in mind that volatile compounds are actually the ones responsible for their antimicrobial potential, screening the *sous-vide* juice for its antibacterial effect seemed reasonable. Results obtained (Table 8) clearly confirmed this. Comparing the effects of 100% oregano and 100% thyme *sous-vide* pure juice extracts it could be noted that oregano extract induced higher effects, especially against *S. aureus* and MRSA. The only exception was *S. enteritidis*, which was inhibited by 100% thyme extract in lower concentrations.

4 | DISCUSSION

Textural and sensory properties of Dried King Oyster (*Pleurotus eryngii*) mushroom samples that were raw, cooked fresh, and cooked dry were investigated by Boin et al. (2016), with similar properties examined regarding the mushroom attributes. The difference in color was noticed for raw samples. Cooked samples were different in the case of the hardness attribute, where the dried sample got a higher score. Aroma intensity was equal, while chewiness was easier for freshly cooked samples.

Color is a very important food quality parameter that determines product value and influences the attractiveness of products to potential consumers (Selli et al., 2021). A comparison of a CVS and

traditional colorimeter for color evaluation was done by Tomasevic et al. (2018), and then confirmed by Milovanovic et al. (2021) for milk products. The results of both investigations indicated the CVS system contributed to better results. The occurrence of browning compounds in mushrooms after the thermal process was explained by the presence of the Maillard reaction. Many researchers mentioned this phenomenon, whether the cause of this reaction was

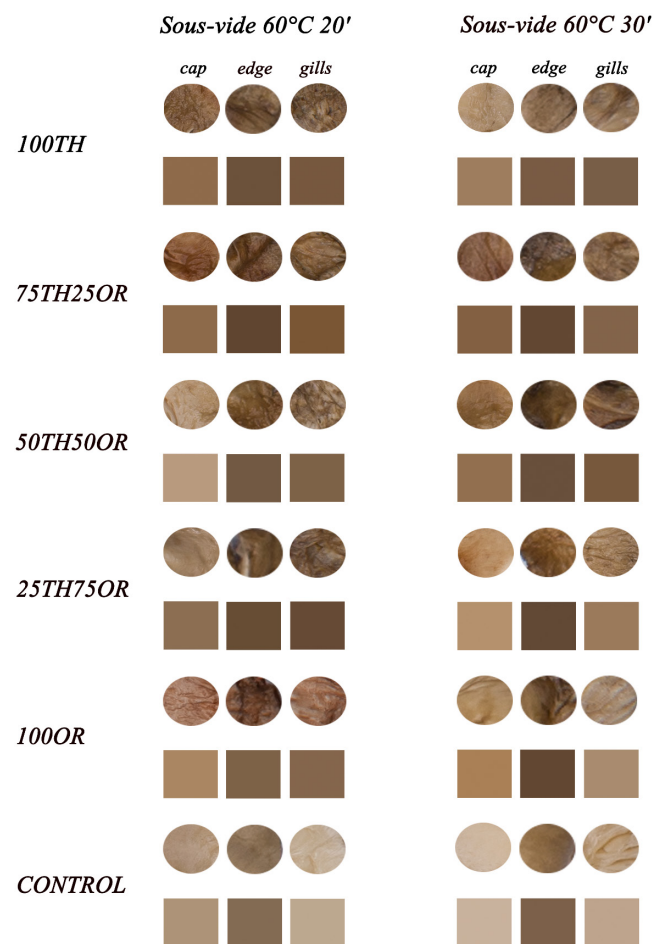


FIGURE 5 Images and color chips of mushrooms with aromatic spices in regard to different time *sous-vide* treatments (cap, edge, and gill). 100TH–100% thyme; 75TH25OR–75% thyme: 25% oregano; 50TH50OR–50% thyme: 50% oregano; 25TH75OR–25% thyme: 75% oregano; 100OR–100% oregano. control samples: *Sous-vide* mode, no spices added, 60°20' and 60°30'.

TABLE 6 MIC values of oregano, black pepper, thyme, and oyster mushroom crude hot water extracts determined in microdilution assay

MIC (mg ml ⁻¹)	<i>Enterococcus faecalis</i> (ATCC 29219)	<i>Proteus mirabilis</i> (ATCC 12453)	<i>Staphylococcus aureus</i> (ATCC 25923)	<i>Escherichia coli</i> (ATCC 25922)
Oregano (<i>Oregano vulgare</i> L.)	10	10	10	n.d.
Black pepper (<i>Piper nigrum</i> L.)	n.d.	n.d.	10	n.d.
Thyme (<i>Thymus vulgare</i> L.)	20	20	20	n.d.
Oyster mushroom (<i>Pleurotus ostreatus</i>)	80	n.d.	80	80

Note: n.d.—Not determined in applied concentration ranges (up to 10 mg ml⁻¹ for oregano and black pepper, 20 mg ml⁻¹ for thyme, 80 mg ml⁻¹ for oyster mushroom).

culinary treatments of mushrooms such as boiling or oven-cooking (Selli et al., 2021) or drying method (Politowicz et al., 2018). An increase in the browning index may be also caused by the duration of storage, which can be induced by enzymatic oxidation or microbial growth. This was observed in previous studies (Djekic et al., 2017; Doroški et al., 2020).

Ko et al. (2007) revealed that the thermal treatments (boiling at 70–100°C) significantly lower the hardness and adhesiveness, and in contrast increase the springiness and cohesiveness of winter mushrooms.

The solubilization ability of oregano essential oil, among the three phenolic-rich essential oils including thyme, proved to be the lowest, which may explain weaker color properties (Edris & Malone, 2012). Similarly, the color intensity of the bologna sausages with the addition of thyme essential oil showed higher scores with regard to oregano essential oil samples in the study of Viuda-Martos et al. (2009).

Mushroom sensory analysis was usually done with no additives. The only study which covers the sensory evaluation of mushroom with slight taste modification was done by Khaskheli et al. (2017) using the Chinese traditional anaerobic method of shiitake mushrooms pickles preparation with mustard oil or soft water, plus vinegar, and salt. The color, flavor, and overall acceptability of samples with mustard oil were found to be the best as compared to the ones with soft water, which indicated an increased likeability of the mushroom sensory characteristics with taste additives.

Oregano and thyme were mostly added in the form of essential oils and the products that were represented as the research subjects were different kinds of meat products. As opposed to these results, in the sensory evaluation of bologna sausages with the addition of citrus wastewater, oregano, and thyme essential oil (Viuda-Martos et al., 2009), the sample with the addition of oregano oil only had the obvious advantage regarding the odor likeliness and general quality, while the sample with the addition of thyme oil was only scored as better in case of color intensity, juiciness, and hardness. Moreover, sensory taste and aroma profile analysis of pork meatballs and hamburgers with the addition of oregano and thyme essential oils, investigated by (Szymandera-Buszkowska et al., 2020), indicated the consumer preference to the oregano aroma and taste. On the contrary, thyme essential oil in these products contributed to meat and bitter aroma

TABLE 7 Interaction between spices and mushroom mutually in checkerboard assay

Oregano (MIC)	Thyme (MIC)	FICI	Interaction
<i>P. mirabilis</i> (ATCC 12453)			
4	0.03125	2.0625	Indifferent
2	2	5	Antagonistic
1	4	8.5	Antagonistic
<i>E. faecalis</i> (ATCC 29219)			
4	0.03125	4.03125	Antagonistic
2	0.5	2.5	Indifferent
1	2	3	Indifferent
0.5	4	4.5	Antagonistic
<i>S. aureus</i> (ATCC 25923)			
2	0.03125	2.03125	Indifferent
1	1	2	Indifferent
0.03125	2	2.03125	Indifferent
Oregano (MIC)	Pepper (MIC)	FICI	Interaction
<i>S. aureus</i> (ATCC 25923)			
2	0.015625	2.015625	Indifferent
1	1	2	Indifferent
0.5	2	2.5	Indifferent
Thyme (MIC)	Pepper (MIC)	FICI	Interaction
<i>S. aureus</i> (ATCC 25923)			
4	0.5	4.5	Antagonistic
2	0.015625	2.015625	Indifferent
1	2	3	Indifferent
Oregano (MIC)	Oyster mushroom (MIC)	FICI	Interaction
<i>E. faecalis</i> (ATCC 29219)			
2	0.0078125	2.0078125	Indifferent
<i>S. aureus</i> (ATCC 25923)			
2	0.0078125	2.0078125	Indifferent

Note: Tested concentrations presented in Table are expressed in MIC units (tested range of each substance 4MIC–1/32MIC). Only combinations with the lowest concentrations tested and indicating a certain type of interaction are presented. FICI values indicated the type of interaction in the following manner: synergism (FICI ≤ 0.5); additivism (0.5 < FICI ≤ 1); indifferent effect (1 < FICI ≤ 4) and antagonism (FICI > 4).

attribute, while the oregano meatballs and hamburgers were spicier and more peppery related to aroma impression.

Bearing in mind the problem of microbial food contamination, as well as the fact that natural preservation methods that could prolong the shelf life are emphasized, we have also screened for antibacterial activities of crude hot water extracts of the spices and oyster mushrooms. Research articles so far have mostly covered the antimicrobial effect of spices in form of essential oils and extracts, as well as their interaction effect. Boskovic et al. (2015) reported MIC values (μgml^{-1}) for both thyme and oregano essential oils, including the following bacteria: *Salmonella Enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, and MRSA.

TABLE 8 MIC values of sous-vide pure juice extracts of mushrooms with spices determined in microdilution assay

Mixture MIC (mg g^{-1})	100TH (80 mg g^{-1} thyme)	75TH25OR (60 mg g^{-1} thyme +20 mg g^{-1} oregano)	50TH50OR (40 mg g^{-1} thyme +40 mg g^{-1} oregano)	25TH75OR (20 mg g^{-1} thyme +60 mg g^{-1} oregano)	100OR (80 mg g^{-1} oregano)	Pure oyster mushroom juice (control)
<i>P. mirabilis</i> (ATCC 12453)	1/2	1/2	1/2	1/2	1/4	n.d.
<i>S. enteritidis</i> (ATCC 13078)	1/8	1/8	1/8	1/4	1/4	1/2
<i>Escherichia coli</i> (ATCC 25922)	1/4	1/4	1/4	1/4	1/4	n.d.
<i>E. coli</i> (human isolate)	n.d.	1/2	1/2	1/4	1/4	n.d.
<i>S. aureus</i> (ATCC 25923)	n.d.	1/2	1/2	1/4	1/16	n.d.
MRSA (human isolate)	1/4	1/8	1/16	1/16	1/32	n.d.
<i>E. faecalis</i> (ATCC 29219)	1/2	1/2	1/2	1/2	1/2	n.d.
<i>E. faecalis</i> (human isolate)	1/2	1/2	1/2	1/4	1/4	n.d.

Note: n.d.—Not determined in applied concentration ranges. 100TH–100% Thyme; 75TH25OR–75% Thyme: 25% Oregano; 50TH50OR–50% Thyme: 50% Oregano; 25TH75OR–25% Thyme: 75% Oregano; 100OR–100% Oregano.

5 | CONCLUSIONS

This research confirmed that the *sous-vide* culinary method as an innovative option in the gastronomy world maintains the quality properties and sensory characteristics of thermally treated food. The results of the *sous-vide* method showed the possibility to control changes in mushrooms' physical properties by the adjustment of treatment conditions and in that way to provide predictability of the regime effect. With an idea to potentially improve mushrooms' taste and multiply health benefits, the addition of selected spices indicated increased likeability, with the exception of mushrooms with a higher amount of oregano that showed certain defects. For the first time, the antibacterial effect of *sous-vide* pure juice extracts was screened in microdilution assay for the antibacterial potential and obtained results pointed out that spices added in vacuum bags in certain concentrations and processed together with oyster mushroom preserve notable antimicrobial effect, which gave the research additional significance. Further research may include the antifungal effect of spices being common in mushroom processing, as well as the influence of spices' bioactive compounds on the slight changes in color intensity.

AUTHOR CONTRIBUTIONS

Ana Doroški: Conceptualization; Investigation; Funding acquisition; Writing—original draft; Writing—review & editing; Visualization; Validation; Methodology; Software; Formal analysis; Data curation; Project administration. **Anita Klaus:** Funding acquisition; Conceptualization; Methodology; Validation; Investigation; Resources; Supervision. **Biljana Nikolić:** Conceptualization; Methodology; Software; Validation; Investigation; Resources; Writing—review & editing; Supervision. **Igor Tomasević:** Methodology; Software; Validation; Formal analysis; Investigation; Resources. **Vesna Lazić:** Investigation. **Jovana Vunduk:** Investigation; Resources. **Ilija Djekic:** Conceptualization; Methodology; Software; Validation; Investigation; Formal analysis; Supervision; Resources; Writing—review & editing; Funding acquisition; Project administration.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

ORCID

Ana Doroški  <https://orcid.org/0000-0002-1940-4713>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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