Total quality index of mushrooms packed in modified atmosphere

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Abstract:

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BACKGROUND: The aim of this study was to develop a total quality index and examine the effects of modified atmosphere packaging (MAP) on the quality of *Agaricus bisporus* mushrooms, stored for 22 days at 4°C. Mushrooms were packaged under three MAPs: high nitrogen packaging (HNP); low carbon dioxide packaging (LCP); and low oxygen packaging (LOP). Passive MAP with air inside initially was used as the atmosphere treatment (AIR).

RESULTS: This research revealed two phases in quality deterioration of *A. bisporus* mushrooms. During the first week, most of the quality parameters were not statistically different. As of the second period, the odor intensities were stronger for all four packaging. Color difference and browning index showed that significantly lower color changes were on AIR and LOP compared to HNP and LCP mushrooms.

CONCLUSION: Total quality index showed that the best total quality index was calculated for LOP, followed by LCP and AIR. The findings of this study are worthy, in respect to examining two component MAPs, separating the limiting factors (O_2 and CO_2) and evaluating quality deterioration effects and total quality index of *A. bisporus* mushroom.

Key words: *Agaricus bisporus*; modified atmosphere packaging; quality characteristics; total quality index

INTRODUCTION

Agaricus bisporus is the number one mushroom species on the world market because of its taste and pronounced nutritional value. [1] Large part of the mushroom production is marketed in fresh condition (45%) with a constantly growing trend. [2] On the other side this commodity is highly perishable due to high water content and an intensive transpiration. The consequences of the fast postharvest changes are weight loss and shrinkage as well as the browning of the fruiting bodies surface. Listed characteristic mainly attribute to the consumers non-acceptance of the commodity. Depending on the wholesale and retail conditions, mushrooms can stay fresh from 1-5 days. [3] Since *A. bisporus* is the main industrially cultivated and most consumed mushroom species, the problem of a short shelf life, seen through the loss of its nutritive and physico-chemical characteristics, causes economic losses. [4, 5]

Different approaches for the prolonging of the white button mushroom shelf life has been proposed by different authors; from freezing, salting, canning, washing with preservatives, cooling to active and modified atmosphere packaging (MAP). [2, 4, 6] The last one appeared to be the most promising bearing in mind that it does not include direct chemical reactions or the use of preservatives, which are strictly controlled by the EU regulative. [7] Moreover MAP has been economically stimulating, especially for mushrooms with a high price and a short shelf life. [2]

O_2 and CO_2 are marked as the main factors affecting the quality of mushrooms stored under MAP conditions. [2] When choosing gas composition in MAP, researches show two main streams. First approach is having N_2 of around 80% and varying O_2 and CO_2 as presented in the works of Jafri et al. with different gas compositions: O_2 10% and CO_2 5% and O_2 5% and CO_2 10% [8] and Oz et al. [9] with 12%O_2 and 2.5% CO_2 and 12% O_2 and 5% CO_2. On the contrary, Li et al. introduce a different approach with higher oxygen mixtures such as high oxygen packaging, 100% oxygen initially; medium oxygen packaging, 50% O_2 and balanced with N_2 initially; and low oxygen packaging, 3% O_2 + 5% CO_2, balanced with N_2 initially. [10] Both approaches appeared to express the positive effect when applied in high concentrations, separately. That led to the conclusions

which are contradictory, but might be connected to other factors such as temperature, relative humidity, maturity stage and porosity of the packaging material.

Literature review revealed many manuscripts analyzing different quality parameters of *Agaricus bisporus* mushrooms in MAP where mushroom quality is defined by a combination of various parameters. [11] Gormley determined whiteness, texture, development stage and microbial counts as the most important quality characteristics. [12] Color and color changes are identified as important quality parameters. [1, 13, 14] Sensory analysis is often used to evaluate mushroom quality. [15, 16] This led to defining and analyzing quality deterioration parameters such as off-odor, gill color, gill uniformity, cap surface uniformity, and presence of dark zones on the cap [17]. Back in 1998, Jolivet *et al.* [18] presented a review paper on browning *A. bisporus*, while Ares *et al.* focused their work on dehydration, texture and browning effects. [11]

In order to evaluate the effects of different modified atmospheres on the quality of *Agaricus bisporus* mushrooms during shelf-life it is necessary to evaluate these quality parameters. However, there is no single scoring system capable of explaining the total quality. Two main constraints in obtaining a single score able to describe in a concise way the total quality are: (i) the fact that quality parameters are evaluated using different units, and (ii) it is hard to define which parameter, can be considered more important than the others. [19] Introduction of a mathematical approach can be useful, in terms of defining a unique quality index parameter that will interpret all quality parameters. To the best of our knowledge, there were no attempts, in the previous literature, to determine a unique total quality index (TQI), for mushrooms in MAP.

Led by the perspective of MAP usage, easy way to be obtained and a relatively low economic price, the aim of this study was to examine the effect of the two component MAP systems, separating the limiting factors (O_2 and CO_2) in order to isolate and evaluate their effect on several quality characteristics of *A. bisporus* mushroom, stored for 22 days at 4°C. For the purpose of this study based on 11 quality parameters, a mathematical model for calculating a

single total quality index of *Agaricus bisporus* mushrooms packed in modified atmosphere during shelf-life has been introduced.

MATERIALS AND METHODS

Types of mushrooms

Agaricus bisporus mushrooms (Italspawn F599 strain) were commercially produced by a local producer in Belgrade, Serbia. Freshly harvested mushrooms at the closed cap stage (cap diameter of 3-6 cm) were selected from the first flush. Within one hour after picking the mushrooms were transferred to the laboratory in a refrigerated container (8-10^{o}C), where they were stored in a refrigerator at 4^{o}C prior to packaging. Pre-cooling is desirable to reduce the respiration rate.

Mushroom preparation

Fresh mushrooms, free from scars and blemishes, were sorted and cleaned to remove any extraneous material. [8] Samples used as a control every testing day, were grown in the same growing place under exactly the same conditions. The spawn and compost were always procured from the same manufacturer. Mushrooms were selected by uniform size and color for each experiment. Potential variation in quality was not observed. Only completely healthy and good-looking specimens were chosen for further work, while every defected mushroom was discarded from the study. Specific humidity is the standard parameter, applied in industrial conditions, and depends on the specific requirements of the strain. In our growing place specific humidity varied between 82 and 85% in the period of incubation and was maintaining on 95% in the period of fruit bodies formation.

All experiments have been conducted with whole carpophores. Control samples were fresh mushrooms, harvested early in the morning of every testing day, and delivered to the laboratory under conditions that have already been explained above.

The mushrooms were individually weighed and packaged in 85μm thick (PA/PE/PE) bags with the transmission rates of 60ml O_2, 12ml N_2, 180mlCO_2/m^2/24h/1atm and the size of 200mm×300mm. The bags were packed and sealed with a HVC-510T/2A packaging machine.

Modified atmosphere

Mushrooms selected for uniform size and color were packaged under three active modified atmosphere packaging conditions: (1) high nitrogen packaging (HNP), 100% N_2; (2) low carbon dioxide packaging (LCP) 30% CO_2:70% N_2; (3) low oxygen packaging (LOP), 30% O_2:70% N_2. Passive modified atmosphere packaging (4) with air inside initially was used as the atmosphere treatment (AIR).

Six packages of each MAP (three packages for sensory and texture properties and three for chemical properties) were used in all analyses. All the packaged samples were stored in a constant temperature (4°C) and relative humidity of approximately 95% room for up to 22 days pointing the following testing days ("5", "8", "12", "15", "19", "22" days).

Weight loss

Weight loss was calculated according to the weights of each sample and expressed as a percentage of the initial weights of mushrooms (equation 1). Results were expressed as an average of six replicates. [8, 20]

$$Weight\ loss\ (\%) = \frac{W_o - W_d}{W_o} x100 \quad (1)$$

W_o refers to the weight on day "1" and W_d refers to the weight on the day of observation.

Texture profile analysis (TPA)

Texture profile analyses of the mushroom caps were done using a texture analyzer (TA.XT Plus, Stable Micro Systems, Ltd., UK). A 50kg load cell and 75mm diameter compression platen probe were used for the purpose. Mushroom caps 30 – 40mm in diameter were compressed by 20% of the sample height. The speed of the probe was 2.0mm/s during the penetration. Force and time data were recorded with Texture Expert from Stable Micro Systems. From the force vs.

time curve, hardness and chewiness of the samples were calculated. Measurements were performed at 25°C on six mushroom caps for each treatment.

Chemical analysis

Mushroom extracts preparation

Mushroom samples were lyophilized (Telstar LioAlpha 15-85, Terrassa, Spain), powdered and stored at 4°C prior to extraction. 50g of the sample was extracted with 1 L Milli-Q (MQ) water by autoclaving (45 min, 121°C). The extract was cooled and filtered through Whatman No.1 paper. The liquid part was concentrated to 10% of its initial volume. Two volumes of 96% ethanol were added and the sample was left over night in the fridge, at 4°C. After centrifugation (9,000g, 10 min) the pellets were collected, dried at 40°C and powdered in the mortar. The fine powder mushroom extracts were kept at 4°C prior to further analysis.

Total phenolic compounds (TPC)

Method of Folin-Ciocalteu reaction adapted for 96-well microplate reader (Hanna Instruments EC 215 Conductivity Meter, Chelmsford, England, UK) was used in order to determine the total phenolic compounds in crude water extracts of mushrooms [21]. Gallic acid was used as standard (0.015-0.25 mg/ml; $y = 6.588x - 0.0534$; $R^2 = 0.9991$) and the results were expressed as mg of gallic acid (GAE) per g of extract. The assay was performed in 96-well microtiter plates (Sarstedt, Germany). The solution of mushroom extract (25 µL, 1 mg/mL) was mixed with 125 µL 0.1M Folin-Ciocalteu solution. After 10 minutes of incubation 100 µL of sodium carbonate (6%) was added, and the reaction was kept in the dark for two hours. The absorbance was measured at 630 nm.

Membrane permeability changes

Tissue electrolytic leakage was examined as an indicator of the membrane permeability changes according to the modified method given by Liu et al. [22] 1 g of fresh mushroom tissue was prepared in form of discs (8-mm diameter, 3-mm thick) and submerged in deionized water at 25°C. After 30 min the conductivity of the surrounding solution was measured (Absorbance

Microplate Reader ELx808 BioTek). The samples were then boiled for 30 min and cooled to 25°C prior to final conductivity measurement. Electrolyte leakage was expressed as the percentage of total electrolytes in the tested tissue.

Color changes

Visual color of sliced mushroom was measured based on Hunter color parameters (L*, a* and b*) using Computer vision system (CVS). A Sony Alpha DSLR-A200 digital camera featuring a 10.2 Megapixel CCD sensor was used for image acquisition. The Colorchecker was photographed using the implemented CVS to obtain the input device RGB signals in the theoretical range of 0–255 (the RGB values are expressed as sRGB D65 and CIELab D50 2° observer). The L*, a*, and b* values were measured on the digital image of the sample visualized on the monitor by pointing the cursor at the center of the area (11x11 pixels) to be evaluated by clicking on it. The L*, a* and b* values from RGB images were measured from RAW photographs. The i1Profiler 1.5.6 software was used to create the ICC monitor profile while Adobe Photoshop CC (64 bit) software was used for image analysis. L_o*; a_o* and b^o* and L*, a*, b* are experimental data obtained at the beginning of storage and at a given time, respectively. A total of 15 measures from three replicate packages were used for data analysis.

Total color difference (ΔE) was determined using the equation (2), as follow:

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

Browning index (BI) was calculated using Equation (3) [13, 14].

$$BI = \frac{100(x-0.31)}{0.17} \qquad \text{where} \qquad x = \frac{a^*+1.75L^*}{5.645L^*+a^*-3.012b^*} \quad (3)$$

Sensory analysis

Sensory shelf-life can be estimated by measuring the degree of difference between stored samples and a product, considered as “control”, using intensity scales. [23] A trained eight-member panel consisting of researchers from the departments that participated in the research was used to evaluate the mushrooms. Over a period of one week, three 1-hour training sessions

were performed using mushrooms of different levels of freshness to help in both the training of panelists and use of intensity scales. This procedure was repeated three times until a level of consistency in scoring was obtained, [24] meaning that at least four panelists scored the same. When the panel members had become familiar with the test facilities and scoring regime, they were invited to score samples.

Mushrooms were served in open, odorless plastic containers at room temperature. Each sample was coded with a randomly selected 3-digit number. The sensory evaluations were performed in two replicates on each sample by a sensory panel. Fresh mushrooms were used as 'control' (score = 5). During sensory evaluation, each panelist was asked to evaluate samples during the storage time (testing days “5”, “8”, “12”, “15”, “19”, “22”).

Intensities of attributes of the four packaging atmospheres were compared with the 'control' mushroom. An intensity scale test of 9-points was used for each attribute: cap surface color (1=too light, 5=control, 9=too dark); gill color (1=too light, 5=control, 9=too dark); mushroom odor (1=not enough odor, 5=control, 9=too much odor). In addition, defects, if any, were evaluated using a 5-point intensity scale (5=control; 9=very intense defects). The following defects were considered: cap surface color defects; gill color defects; and odor defects. [17,20] Semantic differential chart were used to visualize the sensory profile of mushrooms in various packaging atmospheres.

Total quality index

The quality parameters have been divided in two groups, in line with work of Finotti et al. [19] Parameters of the first kind for which there is a target value, i.e. the measured valued of quality parameter at Day#01. The following rule applies - the nearer to the target values the parameter is, the better the quality is, equation 4:

$$QI = \left|\frac{2*(x_i - T)}{x_{max} - x_{min}}\right| \quad (4)$$

Where: QI – quality index for a parameter; x_i – measured value in the subset of values; T - target value; x_{max} – maximal value in the subset of values; x_{min} – minimal value in the subset of values. The following quality parameters were included in this group (hardness with target value = 26.22; chewiness with target value = 1,693.00; cohesiveness with target value = 0.71; total phenolic compounds with target value = 2.70; electrolyte leakage with target value = 21.24; odor with the target value = 5).

Parameters of the second kind have the following rule: the smaller the value is, the better the quality is. For this type of parameters, QI is calculated based on equation 5:

$$QI = \frac{x_i}{x_{max}} \quad (5)$$

Where:

QI – quality index for a specific quality parameter; x_i – measured value in the subset of values; x_{max} – maximal value in the subset of values. The following quality parameters were included in this group (weight loss; total color difference; browning index; gill color and cap color).

Upon calculation of all QIs, we can assume that in the new Euclidean space R^N (N is the number of quality parameters) quality indexes are considered as vectors QI = (QI_1, QI_2, . . . , QI_N) ϵR^N [25]. The Euclidean norm of the vector, whose components are the indexes QI_N, will represent the overall quality index (TQI) equation 6: [19]

$$TQI = \sqrt{\sum_{j=1}^{N} (QI_j)^2} \quad (6)$$

As a conclusion, the “rule of thumb” is that the farther from the origin the vector, the worse is its “TQI” and the nearer the origin the vector, the better is its “TQI”. [19]

Statistical analysis

In order to analyze data in respect to type of packaging atmosphere and storage time two-way ANOVA was used. To distinguish statistical differences between the experimental data, LSD and Tukey's post hoc tests were performed. Data obtained from the difference from control test

were processed using one-way ANOVA and Tukey's HSD post hoc test to distinguish statistical differences between the packaging atmospheres during the same storage period. The level of statistical significance was set at 0.05. Statistical processing was performed using Microsoft Excel 2010 and SPSS Statistics 17.0.

RESULTS AND DISCUSSION

Weight loss

The highest retention of weight loss was observed in the treatment with AIR while the lowest was observed in HNP and LCP (Table 1). Treatment with high CO_2 in MAP reduces the weight loss of mushroom, probably due to the reduction in the respiration rate. [26] Weight loss is mostly caused by the loss of water from the package to the surrounding atmosphere. [1] Comparison of results from different MAPs during the same storage period showed no statistical difference ($p>0.05$). However, comparison within the same packaging during the shelf-life showed statistical differences after the first week (starting from day "8") for LCP, LOP and AIR while HNP expressed statistical differences after two weeks (from day "15"). Liu et al. [22] found that mushrooms kept in air atmosphere exhibited significantly less weight loss than those that were stored under high oxygen atmosphere, after 12 days storage at 2°C. Ares et al. reported weight loss of whole (Shiitake) mushroom results up to 6% [17]. The results are within the range of acceptable market values of up to 10% as proposed by Mahajan et al. [3]

Texture

Firmness of mushrooms is a very important quality parameter in respect to consumer preference. [9] Hardness of mushrooms showed a gradual decrease in all MAPs from the fifth day onwards with HNP and AIR showing the highest loss (Table 2). The same results were observed by Liu et al. [22] for the mushroom stored in air. This trend could be attributed to protein and polysaccharide degradation, hyphae shrinkage, central vacuole disruption and expansion of the intercellular space at the pileal surface. [27]

Analysis of shelf-life of each MAP shows that statistically significant loss of hardness occurs in HNP (starting from day "12") and AIR (starting from day "19"). For other two MAPs hardness changes were not statistically significant ($p>0.05$). Comparing of MAPs showed that statistically significant differences occur after day "19", whereas HNP hardness is statistically significant compared to LCP and LOP. At day "22" HNP hardness is statistically significant compared to LCP ($p<0.05$).

Chewiness results showed statistically significant differences only for HNP. Changes of chewiness in other three types of MAP was not statistically significant during the observed shelf-life ($p>0.05$). Comparison of packaging atmosphere during the same storage period showed no statistical difference ($p>0.05$). Postharvest loss of firmness in mushroom is attributed to the cell growth, high water content and water migration, protein and polysaccharide degradation, and loss of cell turgency due to changes in cell membrane permeability. [9, 20, 26, 28]

Cohesiveness results showed statistically significant differences for all types of mushrooms ($p<0.05$). On the other side, comparison of packaging atmosphere during the same storage period showed no statistical difference ($p>0.05$). As presented in Table 2, cohesiveness increases with storage time for all treatments. This trend was been explained by Zivanovic *et al.* [27] stating that due to an increase in chitin content and formation of covalent bonds between chitin and R-glucan, rigidity of the hyphal wall increases.

Effect of MAP on TPC amounts

TPC are known as secondary metabolites which play an important role in absorbing and neutralizing free radicals formed under abiotic stress conditions, like those in MAPs. [29] The amount of the total phenolic compounds appeared to change in a wave-like manner (Table 3). Sudden decrease followed by a moderate increase is present in all tested MAPs in the first 10 days. During storage, phenolic compounds breakdown due to enzymatic activity resulting in the decline of TPC concentrations. [30] The highest value is reached at the end of the second week

after which TPC levels decrease. The most pronounced increase was observed in LOP and AIR which is in agreement with the findings of Liu and Wang. [31] They noticed that the amount of TPC increases in mushroom samples stored under high oxygen conditions. In case of fruit additional phenolic compounds are formed as a defense response. [32] According to the statistical analysis AIR atmosphere in the package appeared as the least stressful for the fruiting bodies since there is no significant difference between TPC levels measured on day 1, 5, 8, 12 and 15. The peak for the TPC levels occurred again in the AIR indicating that at the end of third week the conditions in the package are drastically changed and the mushroom tissue is severely stressed. Such results can be expected since it has been proved that the activity of phenylpropanoid pathway increases under stressful conditions and phenolic compounds are synthesized and accumulated. [33] Evidently, high nitrogen, low carbon-dioxide and low oxygen atmospheres act as a stress factors from the beginning of the storage which trigger changes, probably oxidation of phenolic compounds, at the very first week. [2, 34] In case of HNP a sudden decrease in TPC amount appears after 5 days of storage followed by a drastic change of color (ΔE), which implies that TPC were involved in the enzymatic oxidation and browning process [32]. According to Coria-Cayupan et al., Nitrogen availability effects the reduce of TPCs amounts. [35]

Electrolyte leakage

Electrolyte leakage (EL) of plant and mushroom tissue has been associated with its cell membrane integrity. [10, 22, 36, 37] In previous studies it was found that different storage conditions may affect this parameter in various degrees.

There was no difference in EL in the samples at the end of the 4th day of storage (Table 3). After one week, EL increased significantly only in AIR, compared to the control to LCP), but not comparing to LOP and HNP. The difference between the samples was seen after 11 days of storage; EL increased in absolute terms in all samples, but the increase was significant only in AIR and HNP. EL did not increase significantly in LOP and LCP even after 2 weeks of storage. After this period, all samples except those kept in LOP, showed relatively high rate of EL

increase. At the end of the storage period, EL was the lowest in LOP (34.3%), in both absolute and relative terms; EL of the samples kept in AIR and LCP was higher (44.5% and 41.9%, respectively) and HNP was shown to have the highest effect on membrane integrity of the *A. bisporus* mushrooms, with the highest value of EL (55.1%).

The EL analysis showed that all tested atmospheres are suitable for packaging of the white button mushroom at the short term period (<7 days), with AIR being the preferred one, as being the least expensive. However, neither AIR nor HNP are suitable for the long term storage of the *A. bisporus* (>7 days). LCP and LOP, but particularly the latter are found to be better choice as these atmospheres showed lower negative impact on the membrane integrity of the tested samples. Liu and Wang [31] noticed that the atmosphere enriched with oxygen protects the membrane integrity through the preservation of lipids. They also stated that mushrooms kept in high oxygen atmosphere exhibit lower browning index, which also proves to be the same in our study (Table 4). The same membrane preservation effect of oxygen enriched package has been described by Li et al. [10]; they found that low oxygen atmosphere (3%) was not suitable due to inducement of anaerobic respiration and increased ethanol accumulation in the sample tissue, leading to lysis.

However, LCP also showed a protective effect on cell membrane integrity during first 2 weeks of storage, compared to AIR and HNP; CO_2 enriched atmospheres were shown to extend shelf-life of various products. [38, 39] Ye et al. [37] investigated the effect of the atmospheres with different $O_2/CO_2/N_2$ ratios on tissue integrity of shiitake mushrooms and found that CO_2 enriched MAPs have protective effect on the samples' membrane integrity, but under the concentration of 10%. Higher CO_2 concentration may have different effect on different kinds of mushrooms.

Color changes

Browning after harvest is a common phenomenon in mushroom crops, which decreases the commercial value of the products and enzyme oxidation, senescence and microbial growth are the main triggers that cause color changes from white to brown. [17, 18]

As presented in Table 4, it can be concluded that in most cases, AIR and LOP showed significantly lower total color difference and overall lower browning compared to HNP and LCP mushrooms. Higher CO_2 concentration can cause severe browning. [28] Also, for HNP and LCP mushrooms, after half of the period, statistically significant ΔE and BI occurred. For ΔE > 5, it is considered as such a color difference that average observer notices two different colors. [40] It appeared that the effect of active MAP with oxygen over 10% (oxygen (LOP and AIR) significantly retards the BI of button mushroom, compared to higher concentration of Nitrogen (HNP) or carbon-dioxide (LCP). After harvest, the mushroom color gradually changes from white to brown. [17 18] Increase in BI value was observed in all treatment groups during shelf-life, which is in concurrence with similar previous research. [9, 18] Liu et al. [34] stated that TPC affects the BI since the phenolic compounds are the key elements in the enzymatic browning. Indeed, TPC levels in HNP and LCP were significantly lower than in LOP and AIR.

Rajarathnam et al., [41] concludes that there is a direct relationship between browning and weight loss for the mushrooms stored in air conditions. In our study this was not the case. Although LOP and AIR samples showed the highest weight loss (Table 1) it was still less than 5% and does not affect the material severely. [3]

Sensory analysis

Sensory evaluation of the mushrooms packed in four different atmospheres during the shelf life showed that HNP was the mushroom with the majority of worst scores depending on the sensory attributes compared to “control” (Figure 1).

Cap surface color showed darkening range from 5.88 – 6.88 (day “5”) to 6.50 – 8.13 (day “22”). HNP mushrooms were with the darkest cap surfaces (days “8”, “12”, “15”, “22”) while AIR were with the lightest (days “5”, “8”, “12”, “22”). The results correspond to CVS results presented in Table 4. This is in concurrence with Antmann *et al.* [20] stressing that the color of mushrooms gradually becomes browner over time.

Cap surface defects were in the range 5.81 – 6.44 (day 5) until 6.31 – 7.88 (day 22). In four out of six evaluations, HNP mushrooms had the highest intensity of defects, mostly classified as "dark zones" and "marbleness or unequal color". Our results confirm research of Antmann *et al.* [20] stating that color of mushrooms becomes less uniform during shelf-life.

Gill color had darkening range from 5.63 – 6.25 (day 5) to 6.44 – 7.69 (day 22). The majority of mushrooms with the darkest gill were HNP mushroom. Similar to cap surface color defects, the highest intensity of defects were identified as "dark zones" and "marbleness or unequal color". HNP mushrooms prevailed with the highest level of defects in most of the observed days (days 8, 12, 15, 22).

Odor evaluation showed two moments. During the first 10 days, odor intensities were less intense compared to control (day 5, range 4.25 – 4.88; day 8, range 4.69 – 5.31). At the end of the research, odor intensity was between 6.56 and 7.00 (day 22). Intensity of odor defects reached 6.88 – 7.56 at day 22. These defects were recognized as "pungent odor", "rotten odor" and "artificial odor". HNP mushrooms had the highest intensity of odor defect at the end of the research (days 19 and 22). Off-odor development is attributed to fermentative metabolism under anaerobic conditions [20].

Total quality index

Figure 2 shows result of TQI for 11 selected parameters. As it can be seen, HNP showed the worst TQI starting from day 5 to end of the observed shelf life. Starting from day 12, a clear distinction of the results may be observed. AIR is the second worst MAP while LOP has been observed as the best scoring MAP. Related to quality deterioration, this figure confirms two periods during shelf life dividing the period in half.

This method of calculating a unique TQI shows the ability of evaluating and comparing mushrooms packed in different MAPs in a quantitative way. This mathematical formulation is sensitive to the displacement of QI from their optimal values. [19]

This approach can enable a large-scale comparison of mushrooms packed in MAPs. The model presented in this study was found to be reliable, precise, and simple tool for monitoring TQI during shelf-life. Outcomes are understandable in relation to various types of mushrooms and throughout the shelf-life.

Consequently this model enables detection of critical to quality characteristics during shelf-life. A critical (food) quality characteristic is defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. [42] Based on experimental data related to the evolution of quality indexes during shelf life, TQI is capable to identify which QI values should be modified in order to increase TQI or optimize the technological processes [19].

CONCLUSION

Authors established a mathematical index of TQI in order to evaluate total quality of mushrooms packed in different MAPs during shelf life. It has been confirmed that this model enables evaluation and comparison of different MAPs during the shelf-life.

This research enables examining the correlation between initial gas composition and quality characteristics of *A. bisporus* mushroom for the postharvest extension of whole mushrooms. The three week shelf-life research revealed two phases in quality deterioration of *A. bisporus* mushrooms. TQI showed that measurable changes occur during the second half of the shelf-life where it is possible to clearly distinguish differences in the overall TQI.

Regarding individual quality parameters, during the first week, most of the quality parameters were not statistically affected. The EL analysis during the first week also confirmed that all tested atmospheres are suitable for packaging. Statistical differences in weight loss occurred after the first week. Statistically significant loss of hardness occurred on HNP during the second week. The same MAP was the only packaging with statistically significant differences in

chewiness ($p<0.05$). During the first 10 days, odor intensities were less intense compared to control.

As of the second period, the odor intensities were stronger compared to control for all four MAPs. TPC highest value was reached at the end of the second week after which TPC levels decrease. High nitrogen, low carbon-dioxide and low oxygen act as a stress factors and trigger changes (oxidation) of phenolic compounds. LCP and LOP showed lower negative impact on the membrane integrity of the tested samples as EL result indicate.

Color difference and overall browning showed that significantly lower color changes were on AIR and LOP compared to HNP and LCP mushrooms. Sensory evaluation confirmed that HNP mushrooms had the highest intensity of cap surface defects, mostly classified as “dark zones” and “marbleness or unequal color” and the darkest cap surfaces and gills.

This research can provide incentives for a more complex measure of large scale of different quality parameters. Its practical application is its simplicity without constraints in the number of selected quality parameters. This tool can be a part of the postharvest quality assurance system.

Further research should deploy investigation of different critical values during shelf-life related to the evolution of TQI and to show directions of possible modifications in order to increase the TQI. Development of specific TQI models for various types of mushrooms and packaging technologies could be a research challenge in the future.

The limitation of this research is the fact that possible correlation between quality parameters has not been included.

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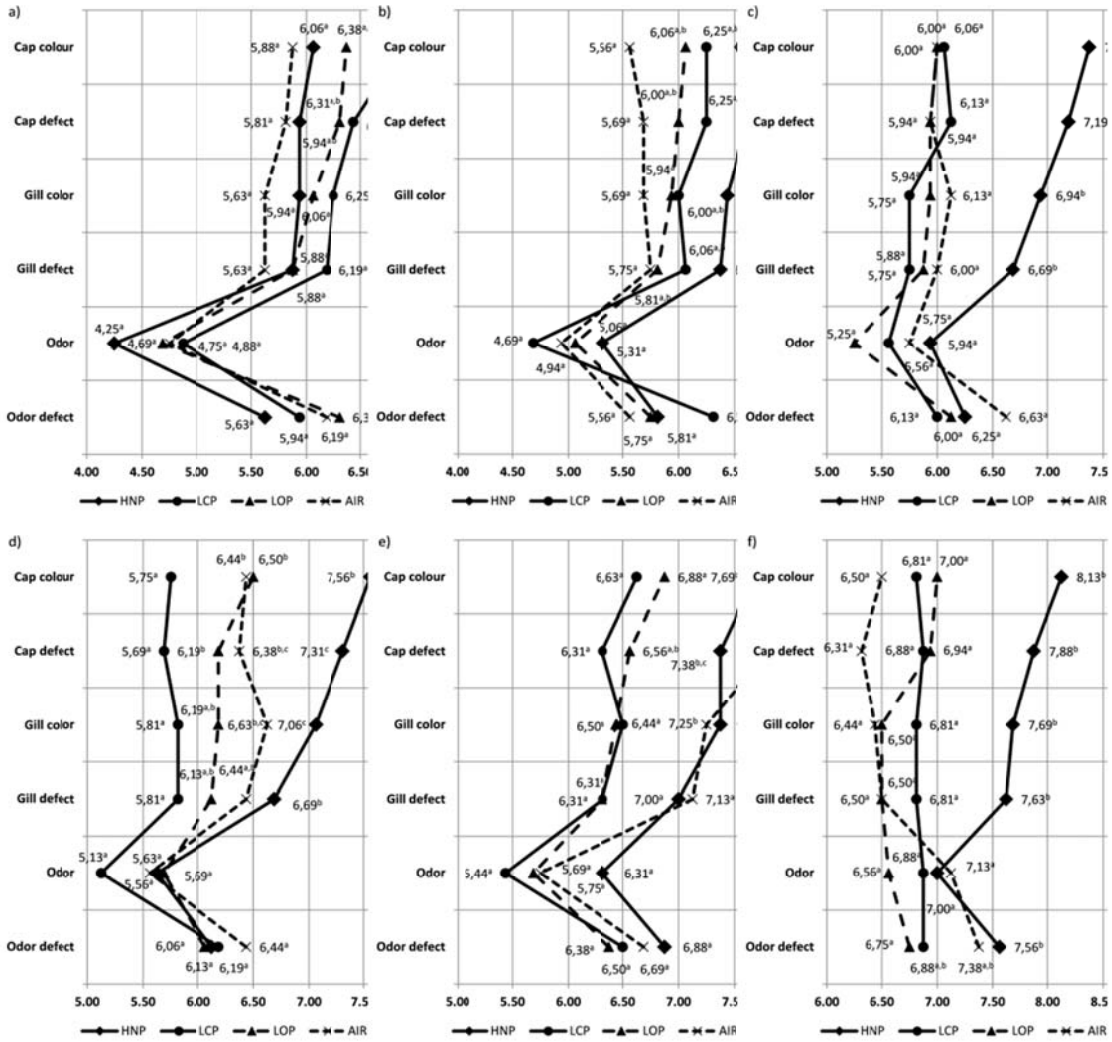

Figure 1. Semantic differential chart a) Day # 5; b) Day #8; c) Day #12; d) Day #15; e) Day #19; f) Day #22

[1] Mean values within the same row with the different superscripts differ significantly ($p < 0.05$)

Legend: Cap surface color (1=too light, 5=control, 9=too dark); gill color (1=too light, 5=control, 9=too dark); mushroom odor (1=not enough odor, 5=control, 9=too much odor). Defects of all three (5=control; 9=very intense defects).

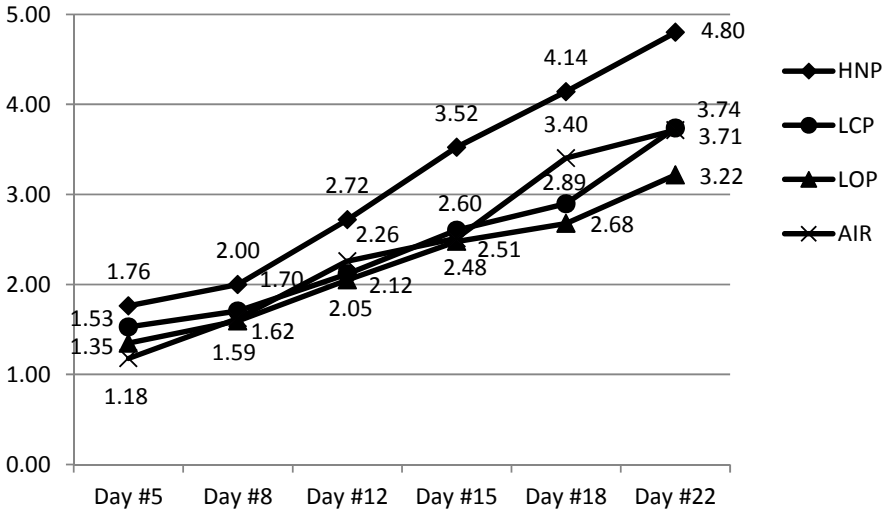

Figure 2 - Overall quality index of *Agaricus bisporus* mushrooms packed in modified atmosphere during shelf-life

Legend: High nitrogen packaging (HNP), 100% N_2; Low carbon dioxide packaging (LCP) 30% CO_2:70% N_2; Low oxygen packaging (LOP), 30% O_2:70% N_2; Passive modified atmosphere packaging with air inside initially was used as the atmosphere treatment (AIR).

Table 1. The effects of packaging atmosphere on the weight loss of mushrooms [%]

Days	HNP	LCP	LOP	AIR
5	$1.09 \pm 0.06^{a.A}$	$1.07 \pm 0.85^{a.A}$	$1.15 \pm 0.62^{a.A}$	$0.78 \pm 0.13^{a.A}$
8	$1.39 \pm 0.52^{a.A}$	$1.61 \pm 0.53^{a.b.A}$	$1.68 \pm 1.34^{a.b.A}$	$1.54 \pm 0.57^{a.b.A}$
12	$1.92 \pm 0.85^{a.b.A}$	$2.22 \pm 0.76^{b.c.A}$	$2.51 \pm 0.85^{a.b.A}$	$2.27 \pm 1.50^{a.b.c.A}$
15	$2.38 \pm 0.63^{b.A}$	$2.30 \pm 0.70^{b.c.A}$	$2.63 \pm 0.81^{a.b.A}$	$2.61 \pm 0.59^{b.c.A}$
18	$2.60 \pm 0.24^{b.A}$	$2.52 \pm 0.13^{b.c.A}$	$2.73 \pm 0.57^{a.b.A}$	$3.51 \pm 2.12^{b.c.A}$
22	$2.66 \pm 0.56^{b.A}$	$2.74 \pm 0.53^{c.A}$	$3.06 \pm 2.67^{b.A}$	$3.57 \pm 0.24^{b.c.A}$

Means of six replications ± standard deviation.

Means in the same column with different small letters and means in the same row with different capital letters are significantly different ($P<0.05$)

Legend: High nitrogen packaging (HNP), 100% N2; Low carbon dioxide packaging (LCP) 30% CO2:70% N2; Low oxygen packaging (LOP), 30% O2:70% N2; Passive modified atmosphere packaging with air inside initially was used as the atmosphere treatment (AIR).

Table 2. The effects of different atmospheres and storage time on the textural properties of mushrooms

Days	HNP	LCP	LOP	AIR
Hardness (N)				
1	26.22 ± 5.95 $^{a.A}$	26.22 ± 5.95 $^{a.A}$	26.22 ± 5.95 $^{a.A}$	26.22 ± 5.95 $^{a.A}$
5	24.91 ± 8.28 $^{a.A}$	25.61 ± 1.58 $^{a.A}$	25.38 ± 5.96 $^{a.A}$	23.44 ± 4.73 $^{a.b.A}$
8	24.37 ± 4.88 $^{a.A}$	23.84 ± 5.50 $^{a.A}$	22.83 ± 4.61 $^{a.A}$	22.15 ± 2.64 $^{a.b.A}$
12	19.89 ± 4.38 $^{a.b.A}$	23.47 ± 4.79 $^{a.A}$	21.86 ± 5.58 $^{a.A}$	19.05 ± 7.06 $^{a.b.A}$
15	15.41 ± 3.05 $^{b.A}$	20.77 ± 5.28 $^{a.A}$	18.88 ± 5.59 $^{a.A}$	17.87 ± 6.82 $^{a.b.A}$
19	13.47 ± 3.92 $^{b.A}$	19.89 ± 4.20 $^{a.B}$	18.84 ± 3.38 $^{a.B}$	16.05 ± 1.65 $^{b.A.B}$
22	12.58 ± 3.93 $^{b.A}$	19.12 ± 4.66 $^{a.B}$	18.46 ± 4.78 $^{a.A.B}$	15.80 ± 4.43 $^{b.A.B}$
Chewiness				
1	1,693.82 ± 477.46 $^{a.A}$	1,693.82 ± 477.46 $^{a.A}$	1,693.82 ± 477.46 $^{a.A}$	1,693.82 ± 477.46 $^{a.A}$
5	1,526.46 ± 436.58 $^{a.b.A}$	1,691.9 ± 137.67 $^{a.A}$	1,666.69 ± 318.22 $^{a.A}$	1,614.82 ± 336.93 $^{a.A}$
8	1,439.9 ± 390.15 $^{a.b.c.A}$	1,618.96 ± 401.08 $^{a.A}$	1,642.97 ± 398.36 $^{a.A}$	1,567.75 ± 145.47 $^{a.A}$
12	1,311.02 ± 332.14 $^{b.c.A}$	1,585.69 ± 239.86 $^{a.A}$	1,559.74 ± 402.34 $^{a.A}$	1,556.46 ± 490.56 $^{a.A}$
15	1,184.49 ± 276.91 $^{b.c.A}$	1,413.1 ± 499.56 $^{a.A}$	1,384.75 ± 386.31 $^{a.A}$	1,441.83 ± 614.30 $^{a.A}$
19	1,078.9 ± 270.21 $^{b.c.A}$	1,349.66 ± 550.15 $^{a.A}$	1,298.35 ± 489.54 $^{a.A}$	1,203.41 ± 157.50 $^{a.A}$
22	926.58 ± 310.16 $^{c.A}$	1,200.63 ± 479.4 $^{a.A}$	1,141.68 ± 391.25 $^{a.A}$	1,192.44 ± 297.23 $^{a.A}$
Cohesiveness				
1	0.71 ± 0.06^{a,A}	0.71 ± 0.06^{a,A}	0.71 ± 0.06^{a,A}	0.71 ± 0.06^{a,A}
5	0.75 ± 0.07^{a,b,A}	0.75 ± 0.07^{a,b,A}	0.73 ± 0.07^{a,b,A}	0.71 ± 0.06^{a,b,A}
8	0.77 ± 0.05^{a,b,c,A}	0.77 ± 0.07^{a,b,A}	0.76 ± 0.05^{a,b,A}	0.76 ± 0.04^{a,b,c,A}
12	0.79 ± 0.04^{a,b,c,A}	0.80 ± 0.04^{a,b,A}	0.77 ± 0.02^{a,b,A}	0.78 ± 0.02^{a,b,c,A}
15	0.81 ± 0.04^{b,c,A}	0.81 ± 0.09^{a,b,A}	0.79 ± 0.04^{a,b,A}	0.79 ± 0.03^{a,b,c,A}
19	0.83 ± 0.02^{b,c,A}	0.81 ± 0.05^{a,b,A}	0.79 ± 0.03^{a,b,A}	0.80 ± 0.03^{b,c,A}
22	0.84 ± 0.02^{c,A}	0.84 ± 0.01^{b,A}	0.81 ± 0.01^{b,A}	0.82 ± 0.02^{c,A}

Means of six replications ± standard deviation.

Means in the same column with different small letters and means in the same row with different capital letters are significantly different ($p<0.05$)

Legend: High nitrogen packaging (HNP), 100% N_2; Low carbon dioxide packaging (LCP) 30% CO_2:70% N_2; Low oxygen packaging (LOP), 30% O_2:70% N_2; Passive modified atmosphere packaging with air inside initially was used as the atmosphere treatment (AIR).

Table 3. The amounts of total phenolic compounds (TPC) and electrolyte leakage (EL)

Days	HNP	LCP	LOP	AIR
Total phenolic compounds				
1	$2.70 \pm 0.01^{a, A}$	$2.70 \pm 0.01^{a,A}$	$2.70 \pm 0.01^{a,A}$	$2.70 \pm 0.01^{a,A}$
5	$2.04 \pm 0.00^{b,A}$	$2.27 \pm 0.03^{b,B}$	$2.35 \pm 0.04^{b,B}$	$2.77 \pm 0.02^{a,b,C}$
8	$2.15 \pm 0.04^{c,A}$	$2.37 \pm 0.02^{b,B}$	$2.97 \pm 0.12^{c,C}$	$2.86 \pm 0.03^{b,c,C}$
12	$2.58 \pm 0.04^{d,A}$	$2.94 \pm 0.03^{c,B}$	$3.35 \pm 0.02^{d,C}$	$2.87 \pm 0.14^{b,c,B}$
15	$2.27 \pm 0.03^{e,A}$	$2.68 \pm 0.1^{a,B}$	$2.55 \pm 0.02^{e,g,C}$	$2.94 \pm 0.14^{c, D}$
19	$2.95 \pm 0.03^{f,A}$	$2.66 \pm 0.03^{a,B}$	$3.12 \pm 0.09^{f,C}$	$3.5 \pm 0.15^{d,D}$
22	$2.27 \pm 0.02^{e,A}$	$2.3 \pm 0.05^{b,A}$	$2.66 \pm 0.12^{a,g,B}$	$2.71 \pm 0.08^{a,e,B}$
Electrolyte leakage				
1	$21.24 \pm 1.82^{a,A}$	$21.24 \pm 1.82^{a,A}$	$21.24 \pm 1.82^{a,A}$	$21.24 \pm 1.82^{a,A}$
5	$21.97 \pm 1.78^{a,b,A}$	$20.06 \pm 1.91^{a,A}$	$21.31 \pm 2.81^{a,A}$	$21.09 \pm 1.92^{a,A}$
8	$24.92 \pm 3.15^{a,b,A,B}$	$20.28 \pm 0.63^{a,A}$	$23.25 \pm 1.25^{a,b,A,B}$	$27.95 \pm 2.06^{a,b,B}$
12	$30.29 \pm 2.03^{b,c,A}$	$22.22 \pm 2.00^{a,B}$	$24.64 \pm 1.22^{a,b,B}$	$32.07 \pm 2.48^{b,c,A}$
15	$34.00 \pm 1.62^{c,A}$	$25.04 \pm 2.44^{a,B}$	$26.24 \pm 0.98^{a,b,B}$	$32.5 \pm 2.63^{b,c,A}$
19	$44.73 \pm 3.81^{d,A}$	$34.33 \pm 2.04^{b,B,C}$	$28.93 \pm 0.52^{b,C}$	$38.32 \pm 5.6^{c,d,A,B}$
22	$55.15 \pm 4.14^{e,A}$	$41.85 \pm 2.21^{c,B}$	$34.31 \pm 1.49^{b,C}$	$44.5 \pm 2.73^{d,B}$

The results are expressed as means of three replications ± standard deviation

Means in the same column with different small letters and means in the same row with different capital letters are significantly different ($P<0.05$)

Legend: High nitrogen packaging (HNP), 100% N_2; Low carbon dioxide packaging (LCP) 30% CO_2:70% N_2; Low oxygen packaging (LOP), 30% O_2:70% N_2; Passive modified atmosphere packaging with air inside initially was used as the atmosphere treatment (AIR).

Table 4. The effects of different atmospheres and storage time on the color properties of mushrooms

Days	HNP	LCP	LOP	AIR
Total color difference (ΔE)				
5	9.25 ± 2.78 ^{a,b,A}	7.39 ± 1.81 ^{a,A}	7.08 ± 3.96 ^{a,A}	2.69 ± 0.90 ^{a,B}
8	13.11 ± 5.01 ^{a,b,A}	8.44 ± 2.03 ^{a,B}	7.66 ± 3.48 ^{a,b,B}	6.82 ± 3.43 ^{b,B}
12	19.72 ± 7.30 ^{b,c,A}	10.70 ± 4.23 ^{a,B}	9.57 ± 2.16 ^{a,b,c,B}	9.16 ± 3.28 ^{b,B}
15	26.67 ± 8.11 ^{d,A}	24.97 ± 7.75 ^{b,A}	12.88 ± 4.69 ^{c,B}	9.54 ± 2.76 ^{b,B}
19	26.49 ± 7.60 ^{d,A}	23.68 ± 7.57 ^{b,A}	12.43 ± 4.03 ^{c,B}	9.22 ± 3.15 ^{b,B}
22	26.15 ± 4.84 ^{c,d,A}	23.98 ± 6.10 ^{b,A}	11.40 ± 2.73 ^{b,c,B}	13.30 ± 3.79 ^{c,B}
Browning index (BI)				
5	12.47 ± 3.74 ^{a,A}	9.56 ± 2.38 ^{a,A,B}	8.98 ± 5.23 ^{a,B}	3.74 ± 0.72 ^{a,C}
8	18.19 ± 7.27 ^{a,b,A}	11.14 ± 2.47 ^{a,B}	10.21 ± 4.03 ^{a,b,B}	8.30 ± 3.66 ^{b,B}
12	28.27 ± 12.55 ^{b,c,A}	13.37 ± 4.63 ^{a,B}	13.11 ± 2.64 ^{a,b,c,B}	11.75 ± 3.96 ^{b,c,B}
15	40.98 ± 17.04 ^{c,A}	36.18 ± 12.89 ^{b,A}	15.85 ± 5.93 ^{c,B}	12.50 ± 3.58 ^{c,B}
19	40.67 ± 15.71 ^{c,A}	33.6 ± 11.72 ^{b,A}	15.31 ± 4.84 ^{c,B}	11.99 ± 4.39 ^{b,c,B}
22	39.13 ± 9.70 ^{c,A}	34.77 ± 11.65 ^{b,A}	14.95 ± 3.59 ^{b,c,B}	16.36 ± 3.41 ^{d,B}

Means of 15 replications ± standard deviation.

Means in the same column with different small letters and means in the same row with different capital letters are significantly different ($p<0.05$)

Legend: High nitrogen packaging (HNP), 100% N_2; Low carbon dioxide packaging (LCP) 30% CO_2:70% N_2; Low oxygen packaging (LOP), 30% O_2:70% N_2; Passive modified atmosphere packaging with air inside initially was used as the atmosphere treatment (AIR).