

THE EFFECT OF CARBON DIOXIDE ON THE QUALITY OF THE MUSHROOMS

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ABSTRACT

Mushrooms' quality may be significantly changing depending on their type, strain, growing cycle, packing, cooling, postharvest handling (PHH), and conditions of storage. This work aimed to define the influence of the type and mushrooms' strain, the regime of the PHH by carbon dioxide on their preservation (marketability, loss of weight (LW)), changes in the chemical substances, and physiological activity (intensity respiration (IR) and heat release (HR)). Mushrooms *Agaricus bisporus* (AB) (strains IBK-25 and IBK-15) and *Pleurotus ostreatus* (PO) (strains HK-35 and Amycel 3000) were used for testing. Three regimes of treatment by CO₂ with a concentration of 20% were applied: 2 h; 12 h and 22 h. The control was the mushrooms without treatment by CO₂. Changes in the chemical substances such as dry matters (DM), protein nitrogen (PN), and ascorbic acid (AA) in the researched mushrooms were observed. The best result of mushroom preservation was provided by the regime of CO₂ treatment during 12 h. The yield of marketable AB was 94.9% (IBK-25) and 94.2% (IBK-15) comparison to control 93.5%, and 92.5%, respectively. The regime of PHH 2 h almost has no influence but 22 h harmed this indicator. PHH of mushrooms by carbon dioxide was promoted to preserve the DM and increasing concentration of CO₂ was supplied better results. Thus, DM at the end of storage in the AB of strain IBK-25 depend on the regime were 8.5, 8.6, and 8.4%, against – 8.3% in the control variant. Significant quantitative changes in the PN and AA as a result of treatment by CO₂ were not established. PHH also affected the IR and HR. The increased duration of treatment by CO₂ inhibited the intensity of physiological processes in the mushrooms. But, as in previous cases, the best result was provided PHH by 20% CO₂ during 12 h. Similar trends of treatments effect by carbon dioxide were observed in the mushrooms of PO.

Keywords: mushrooms; *Agaricus bisporus*; *Pleurotus ostreatus*; postharvest handling; carbon dioxide

INTRODUCTION

Mushroom growing is an ecologically clean and waste-free production (Varoquaux et al., 1999). There are whole mushroom industries were created in France, England, Holland, Germany, and the USA (Yoo et al., 2005). The world leaders in mushroom growing are China, the USA, and France (Zhang et al., 2014). The industrial production of cultivated mushrooms is one of the most perspective industries of agriculture in Ukraine. The main types of mushrooms that are officially allowed to grow in our country are AB and PO (Dubinina and Timofeeva, 2009; Nesterenko, 2011). Mushroom is a rich source of good quality proteins (Simahina, 2008), having most of the essential amino acids, fatty acids, minerals, and vitamins with low calories (Mattila et al., 2002; Lee et al., 2011). Consumption of mushrooms has a positive effect on the human body. Their components enhance immunity, have hepatoprotective, antitumor, antidiabetic, cardiac effects, help reduce the level of "harmful" cholesterol, improve the functional state of certain organs and systems of the body (including nervous, sexual) (Wasser, 2002; Lindequist,

Niedermeyer and Julich, 2005; Patel and Goyal, 2012; Kalač, 2013).

The shelf life of the fresh mushrooms is limited to 1 – 3 days at ambient temperature and it may be increased up to 4 – 7 days at the 4 °C. Browning and texture changes are the main processes that responsible for the loss of the sensory quality of mushrooms. The free water high level in the fruiting bodies of mushrooms promotes their high IR and causes dehydration and metabolic activity.

The IR of the harvested mushrooms is high in comparison to the other horticultural crops and this main cause their shorter postharvest life. Many short-term storage measures are followed to retard the deterioration in quality at the level of mushroom grower till it reaches the consumer. By following proper packing, cooling, PHH, and transportation, the shelf life of mushrooms can be extended (Kumar et al., 2014).

Good results in extending the shelf life of mushrooms have been obtained as a result application of the Controlled Atmosphere Storage (CAS), Modified Atmosphere Packaging (MAP), and Modified Humidity Packaging

(MHP) (Barron et al., 2002; Kim et al., 2006; Mahajan et al., 2008; Jiang et al., 2010).

In the case of CAS, the product is stored in cold storage chamber where created atmospheric composition with certain relative humidity (RH) and concentration of O₂ and CO₂ that is maintained constant throughout storage. CAS reduces IR of mushrooms, their texture changes, and brown discoloration (enzymatic browning) as a result shelf life is extended (Djekic et al., 2017; Park et al., 2020). A major disadvantage of CAS is the significant cost of equipment and its maintenance.

MAP is a method of storage when fresh product is in a sealed package and is creating a modified atmosphere by respiratory gas exchange, namely oxygen intake and carbon dioxide evolution (Zhang, Pu, and Sun, 2018; Zalewska et al., 2018). Equilibrium concentrations of O₂ and CO₂ are consequently established in the case the rate of gas permeation through the packaging material equals respiratory gas exchange (Gantner et al., 2017; Gholami, Ahmadi and Farris, 2017). The balance of the gas exchange depends on product weight, temperature, the respiration rate of a certain product, permeability O₂ and CO₂ through packaging material, free volume in the package, and film area. MAP helps to extend the shelf life and keep quality as a result creation of a corresponding atmosphere around the products that are packaged in plastic films (Ozturk, Havsut and Yildiz, 2021; Vunduk et al., 2021). There are two methods of creating a modified atmosphere: active and passive modifications. The product is only sealed in a polymeric package at the passive modification. The atmosphere is modified as a result of the fresh product breathing and permeation of gases into the package. It takes a long time to reach the steady-state conditions within the package in passive modification. In the case of the active modification, air initially compulsorily is pushed into the package. Therefore, a stable state of the atmosphere is reached after packaging quickly (Li et al., 2014; Han Lyn et al., 2020). MAP of mushrooms has been shown successfully to delay senescence and keep quality after harvest.

Modified Humidity Packaging

The many polymeric films used for fresh product packaging have lower water vapor transmission rates compared to rates of breathing of this product. As a result, the packages creating saturated conditions of water vapor. The high relative humidity in the package (HRHP) can be cause condensation of water vapor within a package and promote microbial growth. It is may be increasing or decreasing the spoilage depending on the products, their breathing coefficients, and water potentials. There are two possible ways for reaching the desired HRHP: perforation of the package (Dhalsamant et al., 2015) and use of in-package water-absorbing compounds like calcium chloride that can be keeping the required RH (Villaescusa and Gil, 2003). Combination MAP with MHP promotes improving the shelf-life of fresh mushrooms. The best result for keeping mushrooms obtained at the HRHP of 87 – 90% during the storage.

The disadvantages of MAP and MHP are the need for special packaging materials, water-absorbing compounds, and packaging equipment that increases the cost of products. Besides, as a result, MAP storage takes place excessive accumulation of CO₂ that may be damage the cell

membrane and physiological injuries to the mushrooms, such as severe enzymatic browning and loss of firmness (Varoquaux et al., 1999).

The perspective method of PHH of mushrooms and extended their shelf life is the treatment by carbon dioxide with high concentration during a short time.

The effect of this influence depends on the stage developmental of mushrooms, the concentration of CO₂, and the time of exposure (Fonseca, Oliveira and Brecht, 2002; Li et al., 2013).

This work aimed to investigate the effect of the short-term treatment by high concentrations of carbon dioxide on the mushrooms' quality and their physiological activity.

Scientific hypothesis

Mushrooms are products with a high level of water in the fruiting bodies (more than 90%). That explains high IR and metabolic activity to comparison other horticultural crops and causer loss of the sensory quality of mushrooms (browning and texture changes). Mushrooms have a large loss of moisture as a result of evaporation, especially at the storage in the reduced RH. The shelf life of mushrooms depends on their type, packing, regimes, and ways of cooling, technologies of PHH, and conditions of transportation.

MATERIAL AND METHODOLOGY

Samples

Materials of the study were mushrooms of AB (strains IBK-25 and IBK-15) and PO (strains HK-35 and Amycel 3000) from the collection of Institute of Botany after name M.G. Xolodnogo of National Academy Science of Ukraine. These strains are widespread, suitable to grow all year, and have universal purposes. Mushrooms AB and PO were supplied by the Trynchuk Mushroom Farm (Kyiv region, Fastiv district, Borova village) and transported to the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) at 2 ±2 °C and 90% ±1 RH.

Chemicals

Barium Hydroxide (brand LLC "Khimlaborreaktiv", producer NVP LLC Alfarus, Ukrain, clean for analysis), Hydrochloric Acid (brand LLC "Khimlaborreaktiv", producer NVP LLC Alfarus, Ukrain, chemically pure), and Tillman's reagent (2,6-Dichlorophenolindophenolate Sodium, brand Himtest Ukraine, Ukrain) were used in the analysis.

Animals and Biological Material:

No animals and biological materials were used for the studies

Instruments

The four cold chambers KH-6U with volume 6 m³ were used for the mushrooms treatment by CO₂ and their following storage. The required concentration of CO₂ was created by the rotameter of the brand RM-2.5 GUZ (GK PriborMarket, Russia). The gaseous medium in the chamber was stirred by fans.

Concentration CO₂ in the chambers was monitoring by a VTI-2 gas analyzer (Thermal Engineering Institute, Russia). The temperature and RH in the chamber were controlled daily. The air temperature was measured by alcohol thermometer TLC-5 (PJSC "Glass Device", Ukraine, I accuracy class) with the division price of 0.5 °C

but RH – by August psychrometers (Lab Time, Ukraine, I accuracy class), daily.

Samples were weighted by laboratory scales ADG2200C (AXIS) from the company "Scales of AXIS Ukraine" with the 2nd class of accuracy.

Laboratory Methods

For chemical analyses, only the first wave mushrooms were used. In mushrooms, before storage and after 6 days were determined DM, PN, and AA.

Content of DM was performed by the weighted method (DSTU 7804, 2015; Skaletska, Podpryatov and Zavadska, 2014).

Content of protein nitrogen (PN) was performed by the state standard of Ukraine DSTU 4923 (2008).

Content of AA – restoring the Tillman's reagent, by extraction acid solution of mushrooms sample followed by filtration of the resulting substrate by the titrimetric method according to the state standard of Ukraine DSTU 7803 (2015).

Description of the Experiment

The scheme of researches is presented in Figure 1.

Sample preparation: Mushrooms that were used in the investigations were harvested at the peak of fruiting of the first, second, and third waves.

Mushrooms were harvested manually, immediately placed in plastic boxes with the volume of 5 kg where they were stored. Every sample before storage was weighed,

numbered, and added a label with indication weight, temperature, time of the start storage, and repeatability.

PHH of mushrooms by CO₂

Regimes PHH of mushrooms of AB and PO: 20% CO₂ during 2 h; 20% CO₂ during 12 h and 20% CO₂ during 22 h. The control was the mushrooms without treatment by carbon dioxide. The temperature of the product storage was 1 °C. Time after mushrooms' harvesting to the PHH by CO₂ did not exceed 3 h.

Density of carpophore

The density of carpophore (DC) of fruiting bodies of AB mushrooms before and after storage was determined by the formula:

$$DC = \frac{m_c}{d_h} \quad (1)$$

Where:

DC – density of carpophore (g.cm⁻¹); m_c – weight of carpophore (g); d_h – diameter of mushroom hat (cm).

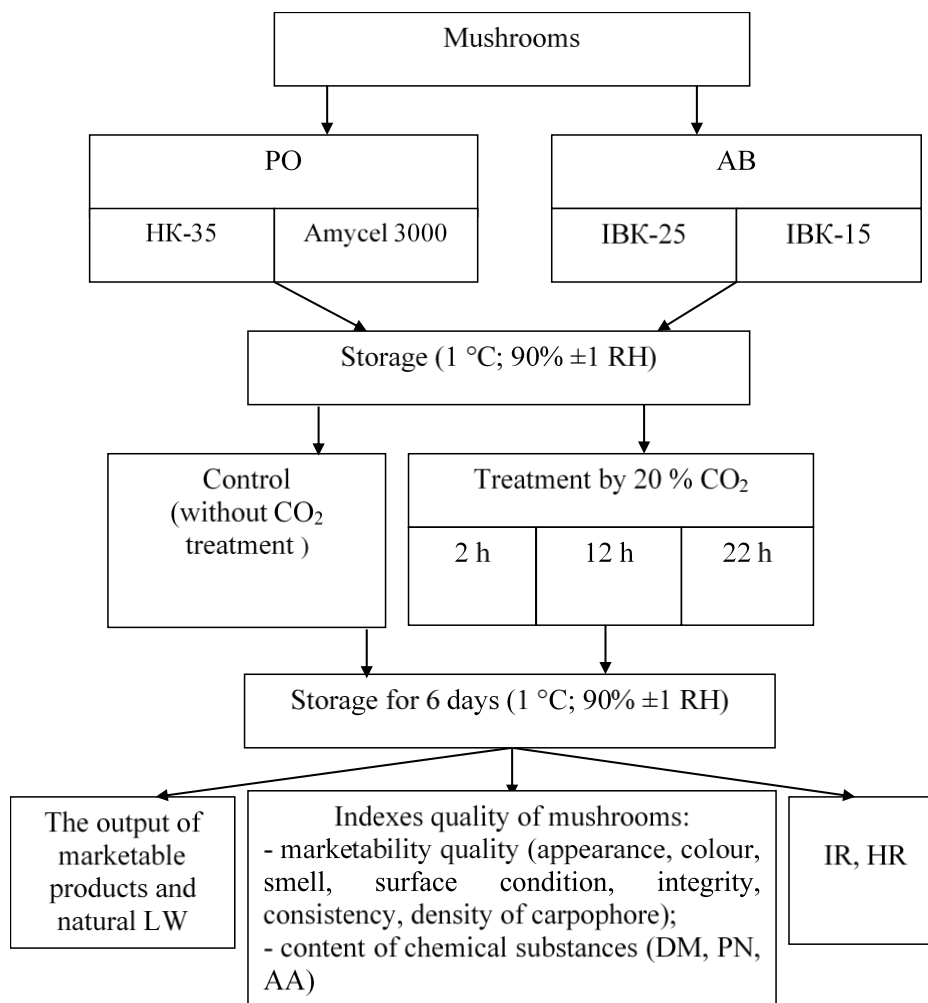


Figure 1 Scheme of researches the influence of treatment by CO₂ on the quality and physiological activity of mushrooms.

Loss of weight

LW was determined by the method of fixed samples. Samples were weighed before storage and every day. The calculation LW was performed by the formula:

$$LW = \frac{W_{bs} - W_{eds}}{W_{eds}} \cdot 100\% \quad (2)$$

Where:

LW – loss of weight (g); W_{bs} – the weight of the sample before storage (g); W_{eds} – the weight of a sample of every day of storage (g).

Intensity of respiration

The IR of mushrooms was determined experimentally in desiccators every day during the storage. This method is based on the absorption of CO_2 by solutions of alkalis ($Ba(OH)_2$) with known concentration and followed by the determination of the amount of alkali that did not react with acid for titration (HCl). Simultaneously, the alkali was titrated from a desiccator without mushrooms (control). IR was determined by the formula:

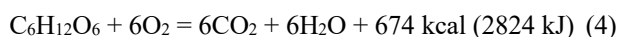
$$IR = \frac{(Q_c - Q_e) \cdot a \cdot 480000 \cdot T}{w_m \cdot b \cdot t} \quad (3)$$

Where:

IR – the intensity of respiration ($mL \cdot kg^{-1} \cdot h^{-1}$ for CO_2); Q_c – quantity of acid which was used for control titration (mL); Q_e – quantity of acid which was used for titration in the experiment (mL); w_m – the weight of the mushrooms (g); t – time duration of the product in the desiccator (min); T – correction to a titter of the 0.1 N alkali; a – average between atmospheric pressure at the start and end of the experiment; b – average between the temperature at the start and end of the experiment.

Heat release

The amount of HR by mushrooms was determined by the amount of CO_2 released during aerobic respiration:



For each g of CO_2 that was released during respiration spent 2,553 kcal or 10.69 kJ of heat. The amount of carbon dioxide expressed by $mL \cdot kg^{-1} \cdot h^{-1}$ but heat – $kJ \cdot kg^{-1} \cdot h^{-1}$. Thus the final formula has the following form:

$$HR = IR \cdot 10.69 / 1000 \cdot 24, \quad (5)$$

Where:

HR – heat released from the product ($kJ \cdot kg^{-1} \cdot d^{-1}$); IR – intensity respiration ($mL \cdot kg^{-1} \cdot h^{-1}$ for CO_2).

Number of samples analyzed: The weight of samples for investigations was up to 4 kg. The average sample for chemical analysis was 20 mushrooms fruit bodies with average weight.

Number of repeated analyses: 3

Number of experiment replication: Every variant was replied three times. Mushrooms were stored for 6 days. The marketable quality, the chemical composition of mushrooms, the yield of marketable products, and natural

LW were determined before and after the storage. The intensity of physiological processes was monitored daily.

Statistical Analysis

The experiment was established as completely randomized designs with three repetitions in 2017 – 2019 years. The data are reported as mean values \pm standard deviation (*SD*). As the statistical analysis software was used Microsoft Excel version 2016.

RESULTS AND DISCUSSION

Many publications report that reduced O_2 and elevated CO_2 concentration have beneficial effects on the shelf life of mushrooms (Lin et al., 2017; Iqbal et al., 2009; Singh et al., 2010; Simón, González-Fandos and Tobar, 2005; Hosseini and Moradinezhad, 2018).

The atmospheres with a high concentration of CO_2 can potentially reduce respiration rate, production of ethylene, sensitivity, decay, and physiological changes in the mushrooms (Farber et al., 2003).

The IR depends on the product, its developmental stage, CO_2 concentration, and time of exposure (Fonseca, Oliveira and Brecht, 2002). CO_2 has significant and direct antimicrobial activity. Aerobic bacteria, such as *Pseudomonas*, are inhibited by moderate to high levels of CO_2 (10-20%) (Farber et al., 2003; Lee et al., 1995). A combination of 3% O_2 and 10% CO_2 is reported to extend the storage life of mushrooms up to 12 to 15 days at 0 °C (Cantwell and Suslow, 2002). Another report says that 10 to 15% CO_2 concentration reduces cap opening, browning, and stripe elongation (Zhang, Pu and Sun, 2018).

The low level of O_2 and very high CO_2 in the atmosphere may cause the development of off-flavors, veil opening, and stripe elongation (Farber et al., 2003; Lee et al., 1995).

Besides, the excessive level of CO_2 can cause cell membrane damage and physiological damages to the product, such as strong enzymatic browning and loss of mushroom density (Briones et al., 1992).

So, low concentrations of CO_2 did not allow obtaining the desired effect but high has negatively affected the quality and shelf life of the mushrooms.

To establish the optimal regime of AB and PO mushrooms treatment by carbon dioxide, we were kept them in sealed chambers with 20% CO_2 in the environment for 2, 12, and 22 h, and then stored with temperature 1 °C for 6 days in the normal atmosphere.

The influence of parameters and regimes of the PHH on the quality of mushrooms was determined by organoleptic and chemical indexes. Organoleptic evaluation of AB and PO showed that PHH by 20% CO_2 for 2 h had almost no effect on changes in color, smell, and consistency of fruiting bodies. Prolonged treatment by carbon dioxide during 22 h significantly deteriorated the color of mushrooms (Figure 2). In our opinion, prolonged contact of mushroom tissues with high concentrations of CO_2 has caused respiratory disorders and inhibited the biochemical processes associated with oxygen deficiency. This caused the death of individual cells, enzymatic browning, and a decrease in the fruit density of mushrooms, which corresponding with the results obtained by other researchers (Briones et al., 1992). Besides, products had a slight characteristic smell of carbon dioxide.

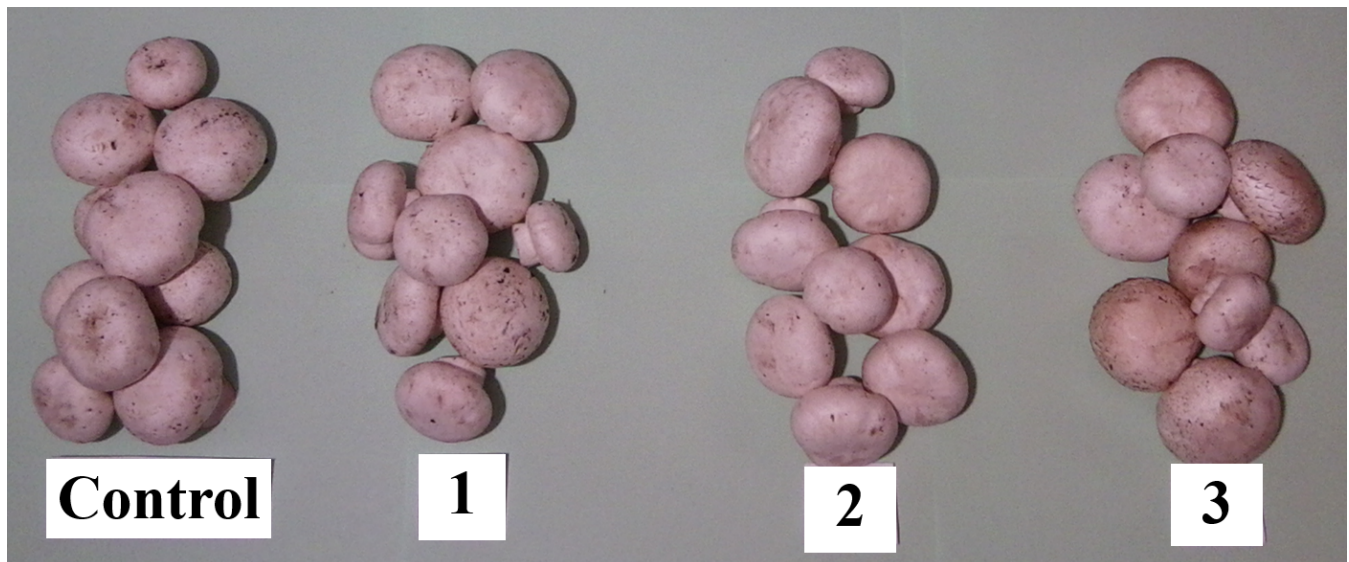


Figure 2 Influence of the treatment duration by 20% carbon dioxide on the color of the mushroom: 1 – 2 h; 2 – 12 h; 3 – 22 h.

Table 1 Preservation of mushrooms AB depending on the regimes of PHH by CO₂.

The regime of treatment, % CO ₂ – h	Output non-commodity mushrooms, %				Output of the commodity mushrooms, %				DC, g.cm ⁻¹
	FGC*	SGC**	TGC***	average	FGC*	SGC**	TGC***	Average	
Strain IBK-25									
control	2.0 ± 0.08	2.0 ± 0.02	1.5 ± 0.03	1.8 ± 0.05	93.0 ± 0.51	93.1 ± 0.61	93.9 ± 0.55	93.5 ± 0.36	5.58 ± 0.21
20% – 2 h	1.8 ± 0.05	2.0 ± 0.04	1.7 ± 0.02	1.8 ± 0.05	93.3 ± 0.47	93.3 ± 0.47	93.7 ± 0.43	93.5 ± 0.41	5.60 ± 0.18
20% – 12 h	1.2 ± 0.05	1.0 ± 0.02	1.0 ± 0.03	1.0 ± 0.02	94.5 ± 0.6	94.9 ± 0.52	95.0 ± 0.61	94.9 ± 0.47	5.65 ± 0.22
20% – 22 h	4.0 ± 0.18	4.8 ± 0.22	4.5 ± 0.18	4.4 ± 0.24	91.9 ± 0.38	91.3 ± 0.47	91.5 ± 0.37	91.6 ± 0.31	5.42 ± 0.21
Strain IBK-15									
control	2.5 ± 0.06	2.9 ± 0.01	2.2 ± 0.03	2.5 ± 0.12	92.3 ± 0.52	92.0 ± 0.69	93.0 ± 0.61	92.5 ± 0.51	5.48 ± 0.24
20% – 2 h	2.5 ± 0.09	3.1 ± 0.04	2.0 ± 0.04	2.5 ± 0.18	92.4 ± 0.68	92.0 ± 0.8	93.3 ± 0.44	92.6 ± 0.47	5.51 ± 0.27
20% – 12 h	1.9 ± 0.1	1.5 ± 0.06	1.0 ± 0.01	1.5 ± 0.03	93.7 ± 0.6	94.2 ± 0.51	94.9 ± 0.52	94.2 ± 0.64	5.56 ± 0.19
20% – 22 h	5.1 ± 0.04	5.4 ± 0.18	4.9 ± 0.16	5.1 ± 0.11	90.6 ± 0.47	90.5 ± 0.44	91.0 ± 0.71	90.7 ± 0.41	5.37 ± 0.16

Note: * – The first growing cycle; ** – The second growing cycle; *** – The third growing cycle. Values are means ± standard deviation, $p \leq 0.05$.

A positive effect on the organoleptic characteristics of the mushrooms was established in the variant of treatment by 20% CO₂ for 12 h. There was no foreign smell; the fruiting bodies remained whole and dense. As can be seen in Figure 2, the mushrooms of AB had advantages over control in appearance at this regime.

However, it should be noted that photos do not fully demonstrate changes in the appearance of mushrooms. The difference is more clearly visible in the organoleptic evaluation products in the boxes. The better appearance of mushrooms at this regime, compared with the control, can be explained by inhibition of the activity of the enzyme tyrosinase as a result of CO₂ treatment. As a consequence, the formation of melanins slows down, which causes enzymatic browning of AB. A similar result in terms of inhibition of the enzyme tyrosinase activity as a result of treatment by high concentrations of CO₂ was obtained in the investigations of straw mushrooms (*Volvariella volvacea*) (Jamjumroon et al., 2013; Jamjumroon et al., 2012).

PO in contrast to the AB did not change in appearance at different regimes of CO₂ treatment.

Preservation of AB and PO depending on the regime of PHH was determined by the results of studies of three cycles

of cultivation (repetitions). The highest yield of marketable products was observed at the regime of treatment by 20% CO₂ within 12 h (Table 1).

For mushrooms AB of the IBK-25 strain, on average, was 94.9% that is 1.4% more than the control variant (93.5%). For strain IBK-15, this index was 94.2% (in the control variant – 92.5%). The duration of treatment during 2 h did not significantly affect the output of marketable fruit bodies. Depending on the growing cycle, they were 93.3 – 93.7%, while in the control variant – 93.0 – 93.9%.

Negatively affected a variant of treatment by CO₂ for 22 h on the yield of marketable products. On average, during three growing cycles, their quantity decreased relative to control by 1.8-1.9%, depending on the strain of the AB. The regime of treatment by 20% CO₂ during 2 h had little effect on the natural LW (Table 2) and quantity of non-marketable fruiting bodies of mushrooms. Such a short-term treatment cannot significantly change the vital processes that take place in the mushrooms after harvest. The treatment by carbon dioxide also affected the

Table 2 Natural LW of mushrooms AB depending on the regimes of PHH by CO₂.

The regime of treatment, % CO ₂ – h	Natural LW, %			average	
	FGC*	SGC**	TGC***		
Strain IBK-25					
control	5.0 ±0.2	4.9 ±0.14	4.6 ±0.2	4.8 ±0.15	2.0 ±0.08
20% – 2 h	4.9 ±0.1	4.7 ±0.18	4.6 ±0.14	4.7 ±0.21	1.8 ±0.05
20% – 12 h	4.3 ±0.11	4.1 ±0.1	4.0 ±0.17	4.1 ±0.17	1.2 ±0.05
20% – 22 h	4.1 ±0.12	3.9 ±0.15	4.0 ±0.12	4.0 ±0.12	4.0 ±0.18
Strain IBK-15					
control	5.2 ±0.14	5.1 ±0.19	4.8 ±0.1	5.0 ±0.17	2.5 ±0.06
20% – 2 h	5.1 ±0.11	4.9 ±0.12	4.7 ±0.15	4.9 ±0.12	2.5 ±0.09
20% – 12 h	4.4 ±0.18	4.3 ±0.16	4.1 ±0.09	4.3 ±0.1	1.9 ±0.1
20% – 22 h	4.3 ±0.1	4.1 ±0.11	4.1 ±0.12	4.2 ±0.19	5.1 ±0.04

Note: * – The first growing cycle; ** – The second growing cycle; *** – The third growing cycle. Values are means ± standard deviation, *p* ≤ 0.05.

Table 3 Natural LW of mushrooms PO depending on the regimes of PHH by CO₂.

The regime of treatment, % CO ₂ – h	Natural LW, %			average
	FGC*	SGC**	TGC***	
Strain HK-35				
control	6.1 ±0.15	6.0 ±0.11	5.7 ±0.12	5.9 ±0.17
20% – 2 h	5.9 ±0.11	5.8 ±0.17	5.7 ±0.1	5.8 ±0.1
20% – 12 h	5.4 ±0.12	5.2 ±0.14	5.1 ±0.1	5.2 ±0.14
20% – 22 h	5.2 ±0.15	5.0 ±0.14	5.1 ±0.14	5.1 ±0.12
Strain Amycel 3000				
control	5.5 ±0.15	5.4 ±0.18	5.1 ±0.21	5.3 ±0.11
20% – 2 h	5.3 ±0.1	5.3 ±0.14	5.2 ±0.17	5.3 ±0.15
20% – 12 h	4.9 ±0.1	4.7 ±0.12	4.6 ±0.14	4.7 ±0.2
20% – 22 h	4.7 ±0.2	4.5 ±0.11	4.6 ±0.17	4.6 ±0.13

Note: * – The first growing cycle; ** – The second growing cycle; *** – The third growing cycle. Values are means ± standard deviation, *p* ≤ 0.05.

DC of mushrooms (Table 1). There best values were observed for treatment during 12 h (5.56 and 5.65 g.cm⁻¹), and in the control variant – 5.48 and 5.58 g.cm⁻¹, respectively.

The regime of the mushrooms treatment during 22 h had positive effects on the natural LW (Table 2). In this regime, a natural loss is slightly lower (4.0 – 4.2% depending on the strain), compared with the control (4.8 – 5.0%), but at the same time in the experimental version significantly increases the quantity of non-marketable fruiting bodies (from 1.8 – 2.5 to 4.4 – 5.1%, respectively).

The fruiting bodies of AB of the strain IBK-15 have larger fruiting bodies compared to the strain IBK-25, respectively, and a larger area of moisture evaporation. This, in turn, affected the natural LW during storage (4.2 – 5.0% for strain IBK-15) against 4.0 – 4.8% for strain IBK-25, depending on the regime of PHH.

The tendencies concerning the influence of PHH of 20% CO₂ on the natural LW, quantity of a commodity, and non-commodity fruit bodies in the AB were characteristic and for PO (Table 3). There was a slight increase in the yield of marketable products – by 0.6 – 0.9% relative to the control for the twelve-hour treatment by carbon dioxide. This is due to the breaking of vital processes in mushrooms as a result of CO₂ treatment. The total LW during storage of PO mushrooms in these conditions was formed mainly due to natural losses. For strain NK-35 their value was 5.2%, for Amycel 3000 – 4.7%. The quantity of non-commodity fruit bodies was 0.5 and 1.5%, respectively (Table 4). PO of

strain Amycel 3000 had a higher number of non-marketable fruiting bodies that explain the botanical features of the formation of their clusters. The clusters of this strain had a large number of small fruit bodies, which after storage for 6 days dried up or became watery.

The treatment OP by CO₂ during 22 h harmed the quantity of non-marketable products.

Their numbers increased up to 3.4 and 3.6%, compared with controls – 0.8 and 1.8%, depending on the strain. Some mushrooms had brown spots. In our opinion, the reason for this is the burns of the tissues of the mushrooms by carbonic acid that was formed by the interaction of condensate, which appeared as a result of respiration of products and carbon dioxide of high concentration.

Natural losses of mushrooms during storage were formed due to the evaporation of moisture and loss of chemical substances (Tables 5 and 6).

The postharvest handling by carbon dioxide during the storage of AB mushrooms contributes to the preservation of DM. It explains by the inhibition effects of CO₂ on the respiration processes and development of the mushroom's fruiting body that decreased sugar consumption. Thus, at the end of storage for the mushroom of strain IBK-25, the amount of DM in the fruiting bodies of the experimental variants were 8.5, 8.6, and 8.4%, while in the control variant – 8.3%.

Table 4 Preservation of mushrooms PO depending on the regimes of PHH by CO₂.

The regime of treatment, % CO ₂ -h	Output non-commodity mushrooms, %				Output of the commodity mushrooms, %			
	FGC*	SGC**	TGC***	average	FGC*	SGC**	TGC***	average
Strain HK-35								
control	0.9 ± 0.01	0.9 ± 0.01	0.5 ± 0.02	0.8 ± 0.03	93.0 ± 0.41	93.1 ± 0.36	93.8 ± 0.5	93.4 ± 0.47
20% – 2 h	0.7 ± 0.01	0.9 ± 0.01	0.7 ± 0.03	0.8 ± 0.02	93.4 ± 0.3	93.3 ± 0.32	93.6 ± 0.62	93.4 ± 0.35
20% – 12 h	0.5 ± 0.02	0.6 ± 0.02	0.4 ± 0.01	0.5 ± 0.02	94.1 ± 0.28	94.2 ± 0.21	94.5 ± 0.41	94.3 ± 0.4
20% – 22 h	3.0 ± 0.01	3.7 ± 0.03	3.4 ± 0.02	3.4 ± 0.04	91.8 ± 0.21	91.3 ± 0.37	91.5 ± 0.64	91.5 ± 0.62
Strain Amycel 3000								
control	1.3 ± 0.01	2.5 ± 0.01	1.6 ± 0.01	1.8 ± 0.03	93.2 ± 0.4	92.1 ± 0.5	93.3 ± 0.28	92.9 ± 0.24
20% – 2 h	1.6 ± 0.01	2.0 ± 0.02	1.6 ± 0.01	1.7 ± 0.02	93.1 ± 0.38	92.0 ± 0.2	93.2 ± 0.33	92.8 ± 0.3
20% – 12 h	1.2 ± 0.01	1.9 ± 0.01	1.5 ± 0.02	1.5 ± 0.02	93.9 ± 0.5	92.7 ± 0.3	93.9 ± 0.3	93.5 ± 0.2
20% – 22 h	3.5 ± 0.04	4.2 ± 0.03	3.1 ± 0.02	3.6 ± 0.02	91.8 ± 0.33	91.3 ± 0.3	92.3 ± 0.3	91.8 ± 0.2

Note: * – The first growing cycle; ** – The second growing cycle; *** – The third growing cycle. Values are means ± standard deviation, $p \leq 0.05$.

Table 5 The changes in the chemical substances of mushrooms AB depend on the regimes of PHH by CO₂.

The regime of treatment, % CO ₂ -h	DM, %		PN, %		AA, mg%	
	before storage	after storage	before storage	after storage	before storage	after storage
Strain IBK-25						
control	9.0 ± 0.11	8.3 ± 0.08	3.7 ± 0.02	3.7 ± 0.01	7.5 ± 0.05	6.4 ± 0.03
20% – 2 h	9.0 ± 0.11	8.5 ± 0.07	3.7 ± 0.02	3.7 ± 0.02	7.5 ± 0.05	6.5 ± 0.01
20% – 12 h	9.0 ± 0.11	8.6 ± 0.04	3.7 ± 0.02	3.7 ± 0.01	7.5 ± 0.05	6.4 ± 0.02
20% – 22 h	9.0 ± 0.11	8.4 ± 0.04	3.7 ± 0.02	3.5 ± 0.01	7.5 ± 0.05	6.4 ± 0.02
Strain IBK-15						
control	8.6 ± 0.14	7.8 ± 0.11	3.5 ± 0.00	3.5 ± 0.02	7.4 ± 0.03	6.3 ± 0.01
20% – 2 h	8.6 ± 0.14	7.9 ± 0.14	3.5 ± 0.00	3.5 ± 0.01	7.4 ± 0.03	6.4 ± 0.03
20% – 12 h	8.6 ± 0.14	8.0 ± 0.17	3.5 ± 0.00	3.5 ± 0.01	7.4 ± 0.03	6.3 ± 0.02
20% – 22 h	8.6 ± 0.14	7.8 ± 0.12	3.5 ± 0.00	3.4 ± 0.01	7.4 ± 0.03	6.2 ± 0.02

Note: Values are means ± standard deviation. $p \leq 0.05$.

Table 6 The changes in the chemical substances of mushrooms OP depend on the regimes of PHH by CO₂.

The regime of treatment, % CO ₂ -h	DM, %		PN, %		AA, mg%	
	before storage	after storage	before storage	after storage	before storage	after storage
Strain HK-35						
control	12.5 ± 0.21	11.4 ± 0.24	3.3 ± 0.01	3.3 ± 0.01	10.5 ± 0.24	9.2 ± 0.17
20% – 2 h	12.5 ± 0.21	11.5 ± 0.18	3.3 ± 0.01	3.2 ± 0.02	10.5 ± 0.24	9.3 ± 0.1
20% – 12 h	12.5 ± 0.21	11.7 ± 0.14	3.3 ± 0.01	3.3 ± 0.02	10.5 ± 0.24	9.5 ± 0.13
20% – 22 h	12.5 ± 0.21	11.3 ± 0.11	3.3 ± 0.01	3.1 ± 0.02	10.5 ± 0.24	9.5 ± 0.08
Strain Amycel 3000						
control	12.3 ± 0.18	11.2 ± 0.04	3.3 ± 0.02	3.3 ± 0.01	10.6 ± 0.2	9.2 ± 0.12
20% – 2 h	12.3 ± 0.18	11.3 ± 0.08	3.3 ± 0.02	3.3 ± 0.01	10.6 ± 0.2	9.2 ± 0.14
20% – 12 h	12.3 ± 0.18	11.5 ± 0.01	3.3 ± 0.02	3.3 ± 0.01	10.6 ± 0.2	9.6 ± 0.04
20% – 22 h	12.3 ± 0.18	11.1 ± 0.03	3.3 ± 0.02	3.2 ± 0.02	10.6 ± 0.2	9.6 ± 0.08

Note: Values are means ± standard deviation, $p \leq 0.05$.

Carbon dioxide in most cases did not significantly affect the changes in the amount of PN and AA compared to the control. Only after treatment duration by 22 hours, the amount of PN partially changed.

For strain IBK-25 its amount was 3.5% for the experimental variant and 3.7% for the control; for strain IBK-15 – 3.4 and 3.5%, respectively. This may be due to oxygen deficiency and protein breakdown. Similar trends in the effect of postharvest handling by carbon dioxide on the content of chemical substances were observed for OP mushrooms. The amount of DM in mushrooms of strain

NK-35 during the storage period decreased from 12.5 to 11.3 – 11.7% depending on the exposure and in the control variant – up to 11.4%.

Most DM was preserved in fruiting bodies after twelve hours of treatment: in strain NK-35 – 11.7% and in the strain Amycel 3000 – 11.5%, that it is significantly more than the control variant.

There are similar to the AB in PO mushrooms wasn't observed significant changes in the quantity AA during the treatment by CO₂.

Table 7 IR of mushrooms AB depends on the regime of PHH by carbon dioxide, mL·kg⁻¹·h⁻¹ for CO₂.

Regime PHH, %CO ₂ -h	Before storage	Duration of storage, days						average
		1	2	3	4	5	6	
Strain IBK-25								
control	4.5 ±0.2	9.2 ±0.34	11.3 ±0.33	5.3 ±0.19	3.4 ±0.03	3.0 ±0.04	2.5 ±0.02	5.6 ±0.08
20% – 2 h	4.3 ±0.17	8.7 ±0.27	10.5 ±0.21	5.0 ±0.12	3.2 ±0.01	2.7 ±0.01	2.3 ±0.01	5.2 ±0.04
20% – 12 h	4.3 ±0.21	8.0 ±0.21	9.2 ±0.21	4.5 ±0.12	3.0 ±0.01	2.5 ±0.02	2.2 ±0.03	4.8 ±0.05
20% – 22 h	4.4 ±0.08	7.5 ±0.21	8.1 ±0.28	4.0 ±0.08	2.7 ±0.02	2.5 ±0.01	2.1 ±0.01	4.5 ±0.05
Strain IBK-15								
control	4.2 ±0.2	9.2 ±0.22	11.0 ±0.28	5.3 ±0.12	3.5 ±0.01	3.3 ±0.02	2.6 ±0.01	5.6 ±0.05
20% – 2 h	4.3 ±0.11	8.6 ±0.15	10.5 ±0.21	4.9 ±0.11	3.4 ±0.01	2.8 ±0.01	2.3 ±0.02	5.3 ±0.12
20% – 12 h	4.0 ±0.14	7.8 ±0.1	8.9 ±0.14	4.1 ±0.05	3.0 ±0.02	2.6 ±0.02	2.4 ±0.01	4.7 ±0.09
20% – 22 h	4.4 ±0.11	7.2 ±0.17	7.9 ±0.11	3.8 ±0.05	2.6 ±0.01	2.5 ±0.02	2.0 ±0.01	4.3 ±0.05

Note: Values are means ± standard deviation, *p* ≤0.05.

Table 8 IR of mushrooms OP depend on the regime of PHH by carbon dioxide, mL·kg⁻¹·h⁻¹ for CO₂.

Regime PHH, %CO ₂ -h	Before storage	Duration of storage, days						average
		1	2	3	4	5	6	
Strain HK-35								
control	6.0 ±0.24	11.1 ±0.21	12.5 ±0.15	7.1 ±0.04	4.0 ±0.01	3.4 ±0.02	2.9 ±0.01	6.7 ±0.03
20% – 2 h	6.3 ±0.28	9.2 ±0.17	11.1 ±0.11	6.5 ±0.02	3.7 ±0.01	3.0 ±0.01	2.8 ±0.00	6.1 ±0.03
20% – 12 h	6.2 ±0.22	8.1 ±0.11	10.4 ±0.12	5.8 ±0.03	3.6 ±0.01	3.0 ±0.02	2.6 ±0.01	5.7 ±0.04
20% – 22 h	6.5 ±0.21	7.4 ±0.04	9.9 ±0.11	5.6 ±0.02	3.1 ±0.02	2.4 ±0.02	2.3 ±0.01	5.3 ±0.04
Strain Amycel 3000								
control	5.7 ±0.17	9.8 ±0.2	13.7 ±0.05	8.4 ±0.03	4.5 ±0.01	3.5 ±0.00	3.2 ±0.01	7.0 ±0.21
20% – 2 h	6.0 ±0.13	8.8 ±0.18	10.7 ±0.02	8.0 ±0.03	4.0 ±0.01	3.1 ±0.00	3.0 ±0.01	6.2 ±0.14
20% – 12 h	6.0 ±0.12	7.5 ±0.12	9.6 ±0.04	7.2 ±0.01	4.0 ±0.02	2.7 ±0.00	2.4 ±0.01	5.6 ±0.11
20% – 22 h	5.6 ±0.12	6.8 ±0.08	8.3 ±0.03	6.8 ±0.02	3.2 ±0.01	2.7 ±0.02	2.2 ±0.01	5.1 ±0.12

Note: Values are means ± standard deviation, *p* ≤0.05.

Table 9 HR of mushrooms of AB after PHH by CO₂, kJ·kg⁻¹·day⁻¹.

Regime PHH, %CO ₂ -h	Before storage	Duration of storage, days						average
		1	2	3	4	5	6	
Strain IBK-25								
control	1.2 ±0.2	2.4 ±0.34	2.9 ±0.33	1.4 ±0.19	0.9 ±0.03	0.8 ±0.04	0.6 ±0.02	1.4 ±0.08
20% – 2 h	1.1 ±0.17	2.2 ±0.27	2.7 ±0.21	1.3 ±0.12	0.8 ±0.01	0.7 ±0.01	0.6 ±0.01	1.3 ±0.04
20% – 12 h	1.1 ±0.21	2.1 ±0.21	2.4 ±0.21	1.2 ±0.12	0.8 ±0.01	0.6 ±0.02	0.6 ±0.03	1.2 ±0.05
20% – 22 h	1.1 ±0.08	1.9 ±0.21	2.1 ±0.28	1.0 ±0.08	0.7 ±0.02	0.6 ±0.01	0.5 ±0.01	1.1 ±0.05
Strain IBK-15								
control	1.1 ±0.2	2.4 ±0.22	2.8 ±0.28	1.4 ±0.12	0.9 ±0.01	0.8 ±0.02	0.7 ±0.01	1.4 ±0.05
20% – 2 h	1.1 ±0.11	2.2 ±0.15	2.7 ±0.21	1.3 ±0.11	0.9 ±0.01	0.7 ±0.01	0.6 ±0.02	1.3 ±0.12
20% – 12 h	1.0 ±0.14	2.0 ±0.1	2.3 ±0.14	1.1 ±0.05	0.8 ±0.02	0.7 ±0.02	0.6 ±0.01	1.2 ±0.09
20% – 22 h	1.1 ±0.11	1.8 ±0.17	2.0 ±0.11	1.0 ±0.05	0.7 ±0.01	0.6 ±0.02	0.5 ±0.01	1.1 ±0.05

Note: Values are means ± standard deviation, *p* ≤0.05.

Table 10 HR of mushrooms of PO after PHH by CO₂, kJ · kg⁻¹ · day⁻¹.

Regime PHH, %CO ₂ -h	Before storage	Duration of storage, days						average
		1	2	3	4	5	6	
Strain HK-35								
control	1.5 ±0.24	2.8 ±0.21	3.2 ±0.15	1.8 ±0.04	1.0 ±0.01	0.9 ±0.02	0.7 ±0.01	1.7 ±0.03
20% – 2 h	1.6 ±0.28	2.4 ±0.17	2.8 ±0.11	1.7 ±0.02	0.9 ±0.01	0.8 ±0.01	0.7 ±0.00	1.6 ±0.03
20% – 12 h	1.6 ±0.22	2.1 ±0.11	2.7 ±0.12	1.5 ±0.03	0.9 ±0.01	0.8 ±0.02	0.7 ±0.01	1.5 ±0.04
20% – 22 h	1.7 ±0.21	1.9 ±0.04	2.5 ±0.11	1.4 ±0.02	0.8 ±0.02	0.6 ±0.02	0.6 ±0.01	1.4 ±0.04
Strain Amycel 3000								
control	1.5 ±0.17	2.5 ±0.2	3.5 ±0.05	2.2 ±0.03	1.2 ±0.01	0.9 ±0.00	0.8 ±0.01	1.8 ±0.21
20% – 2 h	1.5 ±0.13	2.3 ±0.18	2.7 ±0.02	2.1 ±0.03	1.0 ±0.01	0.8 ±0.00	0.8 ±0.01	1.6 ±0.14
20% – 12 h	1.5 ±0.12	1.9 ±0.12	2.5 ±0.04	1.8 ±0.01	1.0 ±0.02	0.7 ±0.00	0.6 ±0.01	1.4 ±0.11
20% – 22 h	1.4 ±0.12	1.7 ±0.08	2.1 ±0.03	1.7 ±0.02	0.8 ±0.01	0.7 ±0.02	0.6 ±0.01	1.3 ±0.12

Note: Values are means (n = 3) ± standard deviation, *p* ≤0.05.

The amount of PN changed only after treatment during 22 h (3.1 and 3.2% against 3.3% in the control variant).

Analysis of the IR of mushrooms indicated that after harvesting quantity of carbon dioxide released during respiration begins to increase sharply, and then decreases during storage due to low temperatures (Table 7 and 8).

Significantly affected the amount of CO₂ released had postharvest treatment by carbon dioxide, especially in the first 3 days.

As can be seen from Table 7 and Table 8, there is a tendency that with increasing duration of treatment by CO₂, the process of respiration is suppressed. It is the least intense respiration was as a result of treatment CO₂ during 22 h. It was typical for both AB mushrooms and OP.

Thus, for AP of strain IBK-25 after a day of storage, the amount of CO₂ released in the experimental variant was 7.5 but in the control – 9.2 mL.kg⁻¹.h⁻¹ for CO₂; after two days – 8.1 and 11.3 mL.kg⁻¹.h⁻¹ for CO₂; after three days – 4.0 and 5.3 mL.kg⁻¹.h⁻¹ for CO₂, respectively. In the future, the IR of the control and experimental variants was gradually equalized and at the end of storage did not differ significantly (2.1 – 2.3 mL.kg⁻¹.h⁻¹ for CO₂ in the experimental variants and 2.5 in the control) (Table 7 and 8).

Based on the data on the intensity of respiration, calculations of the HR of mushrooms were performed (Tables 9 and 10).

Postharvest short-term treatment from 2 till 22 h by carbon dioxide at a concentration of 20% affected the average HR of mushrooms during storage.

Since the intensity of HR directly depends on the IR, so the trends of its change are similar.

Thus, for the OP of the strain Amycel 3000, these indexes were: after two hours of treatment – 1.6 kJ.kg⁻¹.day⁻¹; after 12 h – 1.4; after 22 hours – 1.3; control – 1.8 kJ.kg⁻¹.day⁻¹.

CONCLUSION

Postharvest short-term handling mushrooms of AB and PO by carbon dioxide at a concentration of 20% is an effective method that reduces the level of natural losses, increases the yield of marketable products, helps preserve their chemical substances, and inhibits the intensity of physiological processes in the product (IR and HR). According to all the obtained data, the most effective is the application of the regime of mushrooms treatment by CO₂ during 12 h. Increasing the time of treatment by carbon dioxide up to 22 h most effectively inhibits the processes of respiration (on the 0.4 – 3.2% for AB and the 0.6 – 5.4% for PO) and HR (on the 0.1 – 0.8% for AB and the 0.1 – 1.4% for PO), compared with the control and depending on the mushrooms' strain and duration of their storage. However, this regime promoted sharply increases the quantity of non-marketable products (on the 2.6% for AB and the 1.8 – 2.6% for PO) compared to the control and depending on the strain and wave of fruiting.

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Funds:

This research received no external funding.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

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