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A Comparative Study on the Structural Properties and Lipid Profile of Mushroom (*Pleurotus ostreatus*) Powder Obtained by Different Drying Methods

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Abstract: Mushroom powders, as functional food ingredients, have attracted much attention in recent years. In the present study, four drying methods, i.e., freeze drying (FD), hot air drying (HAD), microwave drying (MWD), and sun drying (SD), were investigated to determine the effects on the structure and lipid profile of mushroom powder. The morphology of the mushroom powder was studied by using X-ray microtomography. The surface of the particles was studied by using scanning electron microscopy. The identification of lipophilic components was carried out by using gas chromatography in a powder extract obtained under in vitro conditions simulating digestion. The FD powder extract, with the widest range of particle size distribution (17.7–2270.3 μm), represented flake shapes with a porous structure. In addition, particles with minimal sizes (17.7–35.4 μm) were recorded only in the FD powder extract. Among the samples, the representation of large granules (1135.5–2270.3 μm) was ranked in the order: MWD < SD < FD < HAD, where the MWD sample was characterized by a narrow particle size composition (35.4–1135.1 μm), whereas the HAD granules were characerizedd by a lamellar structure with multiple deformations. The MWD particles were fused microaglomerates, whereas the SD powder consisted of amorphous particles with a strongly wrinkled surface. Sixty compounds were identified in the lipophilic powder extracts. Regarding the number of compounds identified, the powder extracts were ranked in the order MWD > FD > HAD > SD. Based on the content of linoleic acid, the samples were ranked in the order HAD < MWD < FD < SD, and, based on the stearic acid concentration, they were ranked in the order FD < HAD < MWD < SD. Oleic acid was identified in the HAD and MWD powder extracts, and palmitic acid was only identified in the SD powder extract. According to the number of fatty acid esters, the extracts were ranked in the order SD < FD < MWD < HAD. As per the concentration, alkanes were obtained from HAD and MWD samples and fatty alcohols were obtained from the FD samples. Lipophilic substances with a possible undesirable effect were identified only in the FD and HAD powder extracts. The results of this study expand the currently limited knowledge about the effect of various drying methods on the structural properties of mushroom (*Pleurotus ostreatus*) powder and its lipophilic component. The new information obtained will contribute to better management of mushroom raw materials in terms of optimization, taking into consideration the manufacturer's interest in the technological and functional properties of mushroom powders as a food ingredient or biologically active substance for the production of nutraceuticals.

Keywords: freeze drying; hot air drying; microwave drying; sun drying; *Pleurotus ostreatus*; mushroom powder; structural properties; lipophilic components

1. Introduction

Recently, increasing consumer demand has been directed toward functional nutrition products, whose ingredients, through direct or indirect influence, are capable of exerting a regulatory effect on the body as a whole or on its specific systems and organs [1]. This increased interest in these products is the result of the number of public health problems that can be solved by using drug therapy, and also by using food products [2]. This trend has motivated the food industry to develop products, with improved physical structures and chemical compositions, that require the inclusion of additional food ingredients [3], and regarding this, special attention is given to mushrooms today. As ingredients in products, mushrooms have been used to increase the nutritional value of the products. For example, replacing 5–10% of the flour used to produce noodles and cookies with mushroom powder can increase the contents of protein, dietary fiber, iron, calcium, and potassium in the products [4,5]. Crushed mushrooms have been added to the dough of bakery products [6,7], meat emulsions [8], and yogurt [9] to improve organoleptic, rheological, and textural qualities. In some products, mushrooms are used as new ingredients to improve their functional properties, thus, contributing to health promotion. For example, an increase in the antioxidant properties of pasta has been confirmed when mushrooms are added to their composition [10]. A decrease in the glycemic index of bread has been experimentally proven when a mushroom component is added to the recipe [11].

One of the potential mushrooms for the food industry is *Pleurotus ostreatus*. The fruit bodies of *Pleurotus ostreatus* have a high nutritional value, and, in terms of dry weight, they contain proteins (17–42%), carbohydrates (37–48%), and dietary fiber (24–31%). Mushroom proteins include 18 amino acids, eight of which are essential. In addition, mushrooms contain the following mineral elements: K, P, Na, Ca, Mg, Cu, Zn, Fe, Mo, and Cd. They are rich in B vitamins (thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, nicotinamide, folic acid, and cobalamin), vitamins C, and vitamin D2 [12,13]. The fruit bodies of *Pleurotus ostreatus* are characterized by a high content of phenolic substances, beta-glucans, ergothioneine, lectin, glycopeptides, proteoglycans, and lovastatin [12]. These biologically active metabolites have antioxidant [14,15], anti-inflammatory [16,17], antihyperlipidemic [18], and antidiabetic [19,20] effects.

Fresh fruiting bodies of *Pleurotus ostreatus* contain a large amount of water, have high enzymatic activity, and therefore, they are a perishable product. For example, after 7–12 days of storage at 4 °C, the sensory, nutritional, and medicinal properties of the mushroom are significantly reduced [21].

Mushrooms require effective post-harvest processing and canning methods. In this regard, mushroom powder represents a cost-effective approach for maintaining the desired product functionality and stability over an extended period of time, while minimizing mushroom processing, packaging, and transportation costs. Different methods of drying mushrooms, particularly freeze drying, hot air drying, microwave drying, and sun drying, are commercial options for producing mushroom powders. However, each drying method has its advantages and limitations. The final product obtained by these methods may differ in terms of physicochemical properties, nutritional value, and microstructure [22,23]. In addition, the content of biologically active substances of mushroom powder may depend on the conditions of pretreatment, in particular, the degree of grinding of mushrooms before drying (whole mushrooms, slices, and homogenate) [24]. Therefore, when studying the applicable properties of the final product, all processing conditions must be taken into consideration.

The structure of food powders has been studied in terms of their effects on product digestibility, stability, and yield rate of biologically active substances [25,26]. Regarding mushroom powder, a number of researchers have conducted studies to evaluate the effects of various drying

methods and conditions on the physicochemical properties of *Pleurotus ostreatus* powder [27,28]. However, currently, there is no comprehensive information on the effect of drying methods on the structural properties of ground *Pleurotus ostreatus* fruiting bodies.

Our previous work [24,29] and that of our colleagues [30,31] have shown the effects of different pretreatment and drying conditions on the chemical composition and bioactive properties of *Pleurotus ostreatus*. However, most of these studies have mainly focused on the assessment of the content of water-soluble bioactive components, i.e., phenols, flavonoids, glucans, and peptides, as well as their antioxidant activity. Moreover, information on the effect of drying methods on the lipophilic component of *Pleurotus ostreatus* is still limited. In this regard, lipids are one of the least studied components of *Pleurotus ostreatus* since their total content is in the range of 6–8% dry weight. Therefore, although the amount of lipophilic substances in mushrooms is relatively low, the study of these substances is especially important as they are mainly composed of essential fatty acids [32].

As compared with animal products, the lipid components of mushrooms contain mainly mono- and polyunsaturated fatty acids. Therefore, depending on the ratio of unsaturated to saturated fatty acids, mushrooms can be used especially for restrictive vegetarian and vegan diets [33].

Accordingly, the results of this study are important due to the growing interest in *Pleurotus ostreatus* powder as a functional food ingredient along with the lack of adequate information regarding the effect of various drying methods on its structural properties and lipid profile. Therefore, the aim of this study was to complement the currently limited knowledge regarding the effect of various drying methods on the structural properties of *Pleurotus ostreatus* powder and its lipophilic compounds. In addition, the findings of this study could contribute to better management of mushroom raw material in the context of its use as a functional food ingredient.

2. Materials and Methods

2.1. Raw Materials

For the experiments, we used fresh *Pleurotus ostreatus* mushrooms (culture NK35, SYLVAN, Hungary, Dunaharasti) grown in the Stavropol Region of the Russian Federation. To ensure the uniformity and reproducibility of measurements, we used only intact fruit bodies of the same size and maturity. The initial moisture content in the selected samples was 87.64 $\pm$ 0.3%.

2.2. Chemicals

In accordance with our previous work [29], we used the following chemicals in the experiments: pepsin from porcine gastric mucosa with activity 600–1800 U/mg, hydrochloric acid 37%, sodium hydroxide $\geq$98% (Sigma-Aldrich, St. Louis, MI, USA), pancreatin with activity: amylase 22,500 FIP E/g, lipase 22,500 FIP E/g, and protease 1050 FIP E/g (AppliChem, Darmstadt, Germany); bile extract (millipore); petroleum ether 40–60 °C, $\geq$90%; chloroform $\geq$ 99%; methanol $\geq$ 99%; hexane $\geq$ 95%; acetic acid $\geq$ 99%; diethyl ether $\geq$99%; and molybdophosphoric acid (LenReactive, Saint Petersburg, Russia).

2.3. Drying Process

The drying process was carried out according to the protocol described in our previous work [29]. Before drying, the mushrooms were washed with running water at room temperature for 30 s. The remaining water was removed with a paper towel. The whole mushrooms were dried using four different methods: freeze drying (FD), hot air drying (HAD), microwave drying (MWD), and sun drying (SD).

In each experiment, 200 g of oyster mushrooms were dried. The drying process was carried out until a constant moisture content was achieved in the samples. The reference point for the target moisture content in the dried mushrooms was the data of Keomixay et al. (2019) and Ofodile et al. (2020) [34,35]. The initial and final moisture contents of the samples were measured with a moisture analyzer (MB 25, Ohaus Corporation, Parsippany, NJ, USA) with

an accuracy of 0.001%. The values were considered when selecting the automatic measuring option with a heating temperature of 100 °C and a measuring time of 5 min.

The final moisture contents of the mushrooms dried using FD, HAD, MWD, and SD were 5.72 ± 0.1%, 6.94 ± 0.1%, 6.11 ± 0.1%, and 7.48 ± 0.2%, respectively. The finished dried oyster mushroom samples were stored in hermetically sealed containers and stored in a dark place at a temperature no higher than 25 °C for further analysis.

2.3.1. Freeze Drying (FD)

The first batch of mushrooms was frozen in the freezer (TEFCOLD SE-45, Viborg, Denmark) at −40 °C for 72 h [29]. Then, they were dried in a LS-500 freeze dryer (Prointech, St. Petersburg, Russia) with a sublimator and a vacuum station. The average operating pressure in the drying chamber reached 80.0–90.0 Pa, the condenser temperature was −48.0–49.0 °C, and the mushroom heating during the entire drying process did not exceed 30 °C. The average drying time was 26–27 h.

2.3.2. Hot Air Drying (HAD)

The second batch of mushrooms was dried at atmospheric pressure and 55 °C in a drying cabinet model FD 115 (Binder, Tuttlingen, Germany) with forced ventilation. The mushrooms in the dryer were placed in single layers on trays. Temperature and air velocity were kept constant at 50 °C and 1.3 m/s, respectively. The drying time was 10 h [29].

2.3.3. Microwave Drying (MWD)

The third batch of mushrooms was dried in a household microwave (model WD 900 EL 23-2III, Erisson, Kaliningrad, Russia). The single-layer sample arrangement on the turntable ensured uniform heating. Microwave drying was performed at a power of 200 W for 40 min [29]. The sample surface temperature was 75 °C and was measured using an electronic thermometer with an ETS-D6 steel probe (IKA, Staufen, Germany), immediately after the drying process.

2.3.4. Sun Drying (SD)

The fourth batch of mushrooms was placed in stainless steel dishes and dried in the open sun at an ambient temperature of 25 ± 5 °C and a relative humidity of 40 ± 5% for 3 days (average 9 h per day) [29].

2.4. Preparation of Dried Mushroom Powder

The samples of dried mushrooms prepared by various drying methods were ground into powder using a VT-1541 BK grinder (Vitek, Wien, Austria) using a nozzle for grinding dry products. The grinding time was the same for all samples and was 60 s. Samples of the obtained mushroom powder for further research were stored in tightly sealed plastic containers at a temperature of minus 20 °C.

2.5. Powder Morphology

The external structure of the mushroom powder was studied by image analysis [36]. A laboratory microscope of the research class Axio ZOOM.V16 (Carl Zeiss Microscopy, Oberkochen, Germany) was used at a magnification of ×25 with image fixation using a specialized AxioCam MRc5 camera (Carl Zeiss Microscopy, Oberkochen, Germany) and the Zen 2 software (Carl Zeiss Microscopy, Oberkochen, Germany). To obtain a representative sample, powders were taken from different locations of the powder container. For the analysis of individual particles, powders were placed on slides in the form of an array of non-overlapping particles.

2.5.1. X-ray Microtomography Analysis

The volumetric structural properties of mushroom powder samples were investigated by X-ray microtomography (μCT) with an X-ray microcomputer tomography system

Skyscan 1176 (Bruker, Kontich, Belgium), according to our own method of microtomographic scanning of food products [37]. The powder samples were placed and lightly tamped with the same force into Eppendorf tubes. The samples were scanned with the following parameters: X-ray voltage and current of 40 KV and 600 uA, respectively; without filter; image pixel size of 8.87 um; scanning time of 55 min. The scan protocol included a 180° rotation at 0.3° rotation. The exposure time was 535 ms per image, and the image averaging was 4. Two-dimensional reconstruction and orientation in space (x, y, z) of powders were carried out using DataViewer (version: 1.5.6.2, Bruker, Kontich, Belgium). The following settings were used for the microtomographic reconstruction: without smoothing, correction of ring artefacts = 10%, and correction of beam hardening = 10–30% [29].

The μCT analysis of particle size and particle size distribution was performed using the CTAn software (version: 1.18.4.0, Bruker, Kontich, Belgium). The major diameter of the powder particles was taken into consideration.

2.5.2. Scanning Electron Microscopy

The surfaces of the powder particle structures were observed using a scanning electron microscopy (SEM) (MIRA-LMU, Tescan, BrnoKohoutovice, Czech). Thoroughly mixed samples of mushroom powders were applied to conductive carbon tape and covered with a thin layer of carbon (10 nm) using a QR 150 spraying system. Images with different magnification were taken for each sample.

2.6. Thin Layer Chromatography

Samples of mushroom powders, in the amount of 20 mg, were extracted in 1 mL of a mixture of chloroform and methanol (2:1). The extract was centrifuged for 5 min at 1300 rpm. The supernatant, in the amount of 2 micrograms, was applied to a chromatographic plate with an aluminum substrate with the sorbent silica gel STX-1A "Sorbfil" (IMID, Krasnodar, Russia). Extracted lipids of mushroom powders were separated in a chromatographic chamber consisting of hexane, diethyl ether, and acetic acid (73:25:2). Thin layer chromatography (TLC) was manifested with a 10% alcohol solution of molybdenum phosphoric acid.

2.7. In Vitro Digestion Methods

The study of the lipid profile of mushroom powder was carried out in lipophilic fractions of the extract obtained under in vitro conditions simulating the process of gastrointestinal digestion. The digestive model was reproduced according to the method of McDougall et al. [38], with some modification.

To 5 g of mushroom powder, 100 mL of distilled water was added, then 33% HCl solution, up to 0.5%, was added and the mixture was incubated with 0.1% pepsin in a thermoshaker ES 20/60 (Biosan, Riga, Latvia) for 120 min at 37 °C, at 120 rpm shaking mode. The mixture was neutralized with 1 M NaOH to pH 7.0 (pH meter S400-B, Mettler Toledo, Barcelona, Spain), then a mixture of pancreatin 2 mg/mL and 4 mL bile extract was added, and incubated in a thermoshaker for 120 min at 37 °C, at 120 rpm shaking mode. The extraction was stopped by boiling for 10 min. The extracts were centrifuged for 60 min at 8000 rpm (Universal 320 centrifuge table, Hettich, Germany). After centrifugation, the sludge was separated, and petroleum ether was added to the supernatant (1:3). Then, the solution was mixed using a magnetic stirrer for 4 h. The water and lipid fractions were separated in the separating funnel. All subsequent analyses were performed with the lipophilic fraction of the extract [29].

2.8. Gas Chromatographic Analysis

The composition of the lipophilic substances was investigated using a gas chromatograph Agilent 6850 (Agilent Technologies, Moscow, Russia) with a mass detector Agilent 5975V. The percentage composition of the lipophilic compounds was calculated using peak ranges without the use of correction factors. The qualitative analysis was based on the

comparison of mass spectra of substances with the corresponding data of the NIST0.5a mass spectra library [29].

2.9. Statistical Analysis

All statistical analyses were performed with GraphPad Prism for Windows, Version 6.01 (GraphPad Software, San Diego, CA, USA). The statistical analysis was conducted by performing a one-way variance analysis (ANOVA); $p < 0.05$ was considered to be statistically significant. The results are expressed as mean ± SD ($n = 3$) [29]. Additionally, heatmaps were created using ClustVis [39].

3. Results and Discussion

3.1. Particle Morphology and Microstructural Properties

Since grinding characteristics depend on the drying process, and the morphology of food powders affects their final properties [40,41], the external and volumetric structures of the mushroom powders were evaluated, and the microstructural properties of the surface of their constituent particles were studied. The dependence of these characteristics on four different drying methods was determined.

The visual appearance and optical microscopy results of the studied powders are shown in Figure 1. The FD, MWD, and SD samples had a floury structure. At the same time, the MWD powder was characterized externally by its maximum smoothness and uniformity. The HAD sample was somewhat out of the range of powders. It is known that HAD is characterized by uneven removal of moisture over time, i.e., fast at the initial stage and slow during subsequent drying, and, as a consequence, the formation of a dense layered structure of food products. Observing the texture of mushrooms (*Pleurotus* spp.) dehydrated by hot air drying, Kotwaliwale, N. et al. [42] recorded a high density of dried mushrooms. We confirmed this for fruiting body pieces of mushrooms in a previous study [43]. Dense dry product was more difficult to grind into small particles, therefore, the consistency of the HAD powder was characterized as granular.

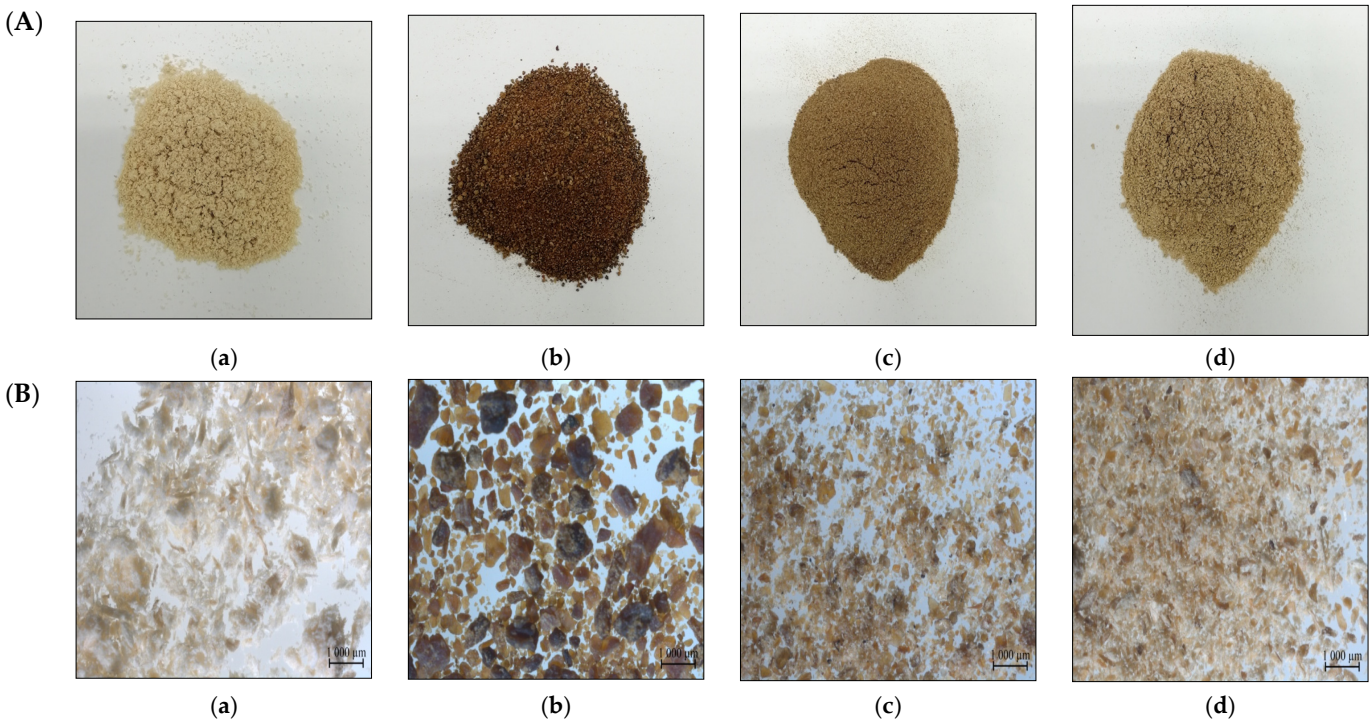


Figure 1. (**A**) Photographs of mushroom powders obtained by different drying methods; (**B**) the light microscope of mushroom powders (×25): (**a**) FD; (**b**) HAD; (**c**) MWD; (**d**) SD.

3.1.1. Particle Size Analysis

The granulometric composition is one of the important structural properties affecting the flowability and functionality of food powders [44]. The μCT analysis of the particle size distribution of the mushroom powders is shown in Figure 2.

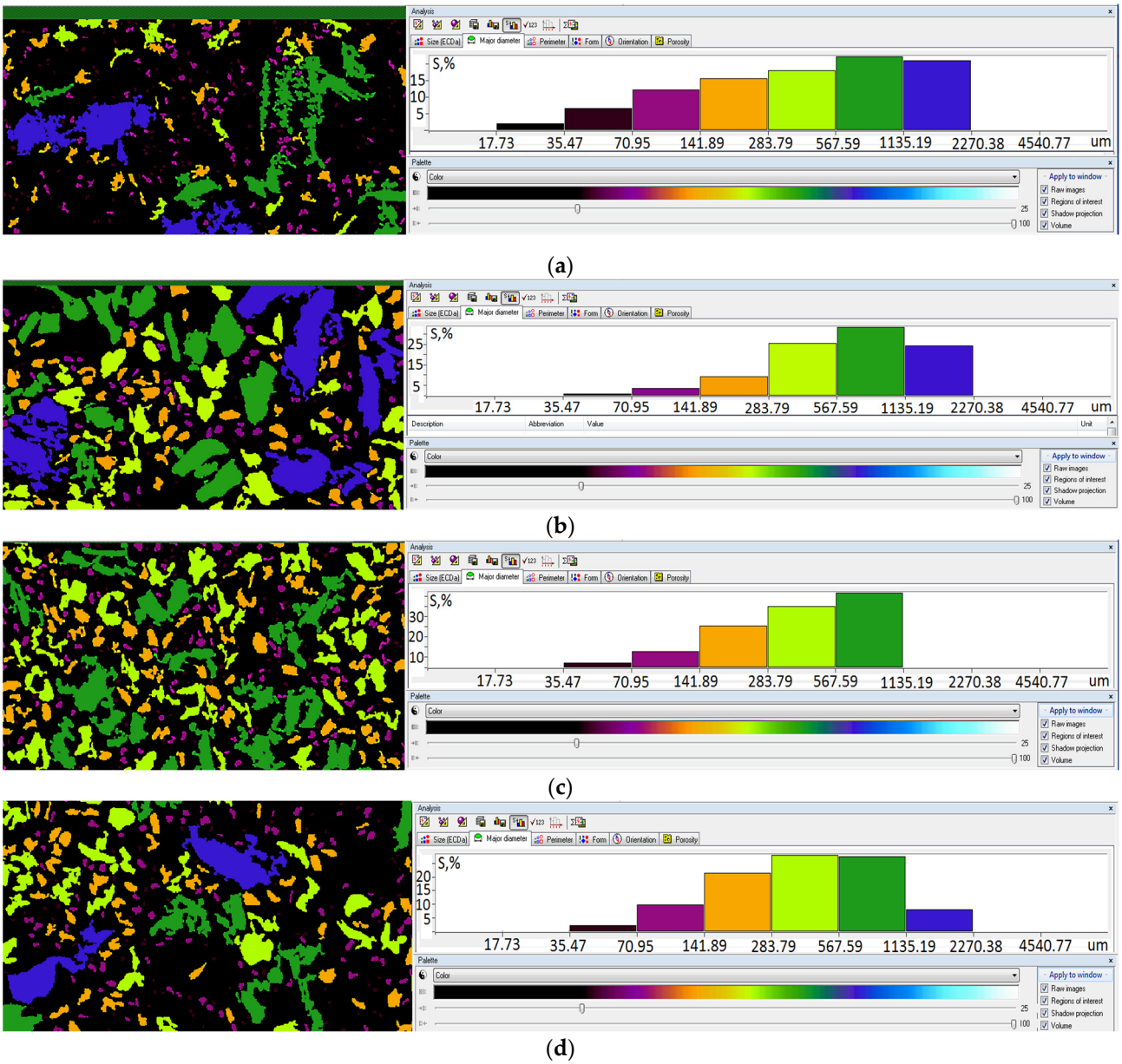


Figure 2. A diagram of the granulometric composition of the mushroom powders based on microtomographic data of the particle size in the program CTAn: (**a**) FD; (**b**) HAD; (**c**) MWD; (**d**) SD.

It can be noted that among the four samples, the MWD powder was characterized by a relatively narrow granulometric composition. The FD samples, on the contrary, demonstrated the widest range of particle size distribution. Moreover, particles with a minimum dimension of 17.73–35.47 μm were registered only in FD powder. The HAD and SD samples occupied an intermediate position between the MWD and FD samples and had relatively similar granulometric compositions.

The presence of large particles (1135.59–2270.38 μm) in the samples in the order of their increase was as follows: SD < FD < HAD. The registered increase in the average particle size in the HAD powder, according to Majid, I. and Nando, V. [36], may provide an increase in the flow rate of the powder. The MWD sample did not have any large particles

of dimension 1135.59–2270.38 µm. This logically confirmed the results of Nejatdarabi, S. and Mohebbi, M. [45], according to which the particle sizes of *Agaricus bisporus* mushroom powder decreased with increasing drying temperature. Among the drying methods we used, MWD involved the highest temperature. The revealed relatively smaller average particle size of MWD powder may be its advantage for solubilization in food matrices, as was also noted by Forero, D.P. [46]. According to Hitayezu, E. and Kang, Y.H. [47], finely ground mushroom (*Hypsizygus marmoreus*) powder had higher values of water absorption index and water solubility index than powder consisting of large particles.

The MWD powder particles were characterized by a uniform structure to a greater extent than those of the FD and SD samples. Probably, the comparative uniformity of the MWD powder particles is caused by the microwave heating of mushrooms, which, due to high-frequency electromagnetic energy, is accompanied by the destruction of fibrous and cellular tissue structures. As a consequence, with MWD powder, the fragility of the dry material increases and its shrinkage decreases. A similar fact was noted in the results of Luo, Z. et al. [48] and Zhou, W. et al. [49], who studied the effect of drying conditions on the structure of fruit, vegetables, and herbal powders.

3.1.2. X-ray Microtomography Diffraction Analysis

The comparative analysis of the µCT volume structures of powders is clearly presented on the reconstructed 2D images (Figure 3).

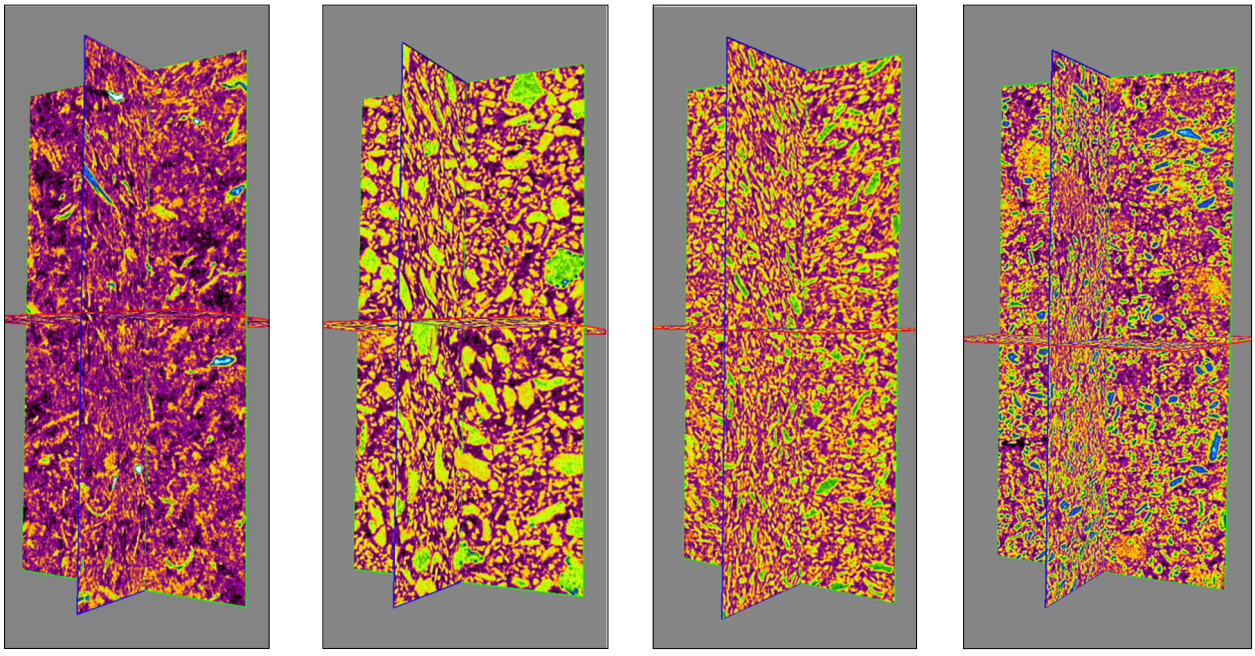


Figure 3. Reconstructed 2D images of X-ray diffraction analysis of the mushroom powders: (**a**) FD; (**b**) HAD; (**c**) MWD; (**d**) SD.

The FD powder exhibited the minimum volumetric density. The remaining samples were characterized as denser: SD < HD < MWD. Similar results have been observed in experiments with fruit powders [50] and, according to the researchers, the volume density depended on the porosity of the structure, and also on the agglomeration of powder particles. We identified partial aggregation of mushroom powder in the FD sample, probably caused by freezing processes, which was also noted by Ouaabou, R. et al. [51] in a study of the microstructure of freeze-dried cherry powder. In addition, according to Zhao, X. et al. [52], the agglomeration could be explained by higher specific surface area and electrostatic interaction between ultrafine powder particles. It is logically consistent with the granulometric indicators given above, which distinguish the FD sample as the

only one containing a fraction of ultrafine particles. The MWD powder had the highest volume density. This correlated with its relatively smaller average particle size, which according to the data of Lv, G. et al. [53], were characterized by a larger contact surface with the environment and a more uniform shape, resulting in a decrease in pore space and, consequently, an increase in bulk density.

3.1.3. SEM of Mushroom Powder

The surface of the particles is also of considerable importance in the production and use of food powders. The surface morphology of powder particles is the result of complex interactions between production processes and is associated with the destruction of the structure of the raw material. The overall properties of the powder may depend on the surface properties of the powder particles [54].

The SEM of powder samples confirmed the results of light microscopy and X-ray μCT and provided additional and detailed information on the structural properties of dried oyster mushroom fruit bodies after grinding.

Figure 4 shows the SEM images of mushroom powder particles obtained by using the FD, HAD, MWD, and SD methods. The surfaces of all the powders presented were irregularly shaped, which was probably due to the nature of the raw materials and the grinding process. However, the SEM results showed clear differences in the surface morphology of the mushroom powders' particles.

The surface microstructure of the FD sample particles differed significantly from the structure of other powders. Ice sublimation of cells in the FD method occurs slowly, which significantly affects the structural properties of the cells, even when the lyophilized material is crushed into powder [55]. The FD sample exhibited irregular morphology, with flake-like particles and a highly porous structure, probably formed during the formation of ice crystals at the freezing stage and characterized by Vardanega, R. et al. [3] as a typical structure for sublimates. In some places, the ice formation bound the cells together and made it difficult to distinguish them. Similar porous structures were observed by Taskin, O. et al. [56] and Ouaabou, R. et al. [51] when freeze-dried fruits were powdered.

The particles of the HAD sample were densely arranged and had flattened cellular structures, probably associated with a decrease in cell pressure and collapse of cell walls due to faster moisture removal and high dehydration rate. Morphologically, the surface of the HAD powder mainly had a lamellar structure (scaly shape) with multiple cracks and other deformations. The mechanical impact of grinding led to the formation of particles with sharp edges on their surface. Similar images were observed for carrot powders prepared using the HAD method in an experiment by Özbek, H.N. et al. [57].

The MWD powder particle samples appeared more compact and were characterized by an uneven surface with significant dents, potholes, and dotted with remnants of destroyed cell membranes. The surface of the MWD particles resembled the appearance of fused microaglomerates, probably formed due to cell fusion caused by cell wall damage under microwaves treatment. Similar destruction of the cellular structure was recorded by Isic, N.I.E. and Islam, N. [58] in an experimental search for the optimal model of microwave dehydration of mushroom (*Agaricus bisporus*).

Grinding of the SD samples into a powder form led to the formation of amorphous particles with a strongly wrinkled surface, but in contrast to the HAD and MWD samples, with a large preservation of the morphology of the mushroom tissue. Similar structures were observed by Kong, D. et. al. [59] for vegetable and fruit powders obtained using SD. The observed features were probably related to the unavoidable influence of ultraviolet radiation during sun drying, which according to Forouzanfar A. et. al. [60], could reduce the destructive force of temperature and other drying conditions.

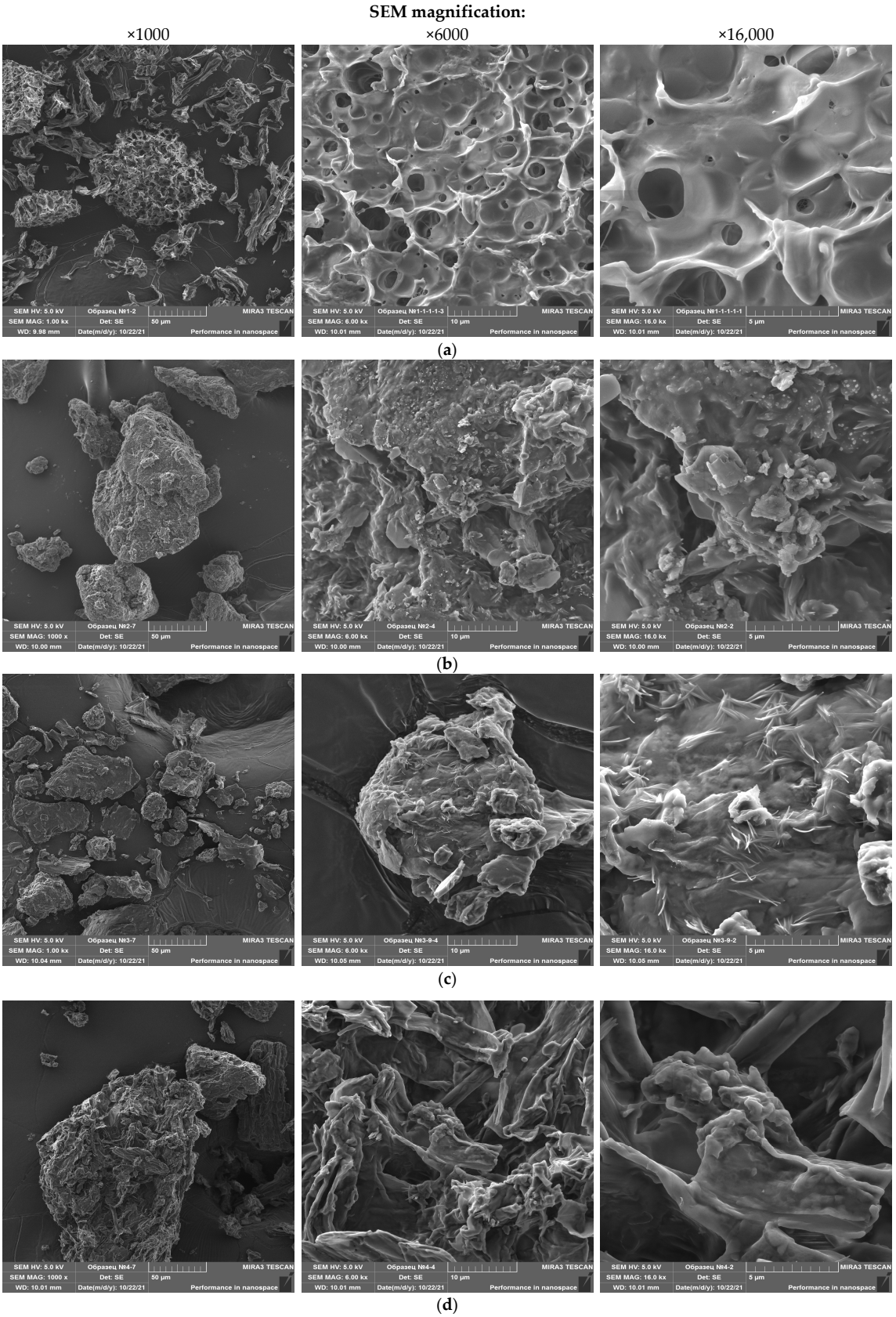


Figure 4. Scanning electron microscopy images of mushroom powders obtained by different drying methods: (**a**) FD; (**b**) HAD; (**c**) MWD; (**d**) SD.

The presented SEM images proved to be good evidence of structural changes resulting from drying and subsequent grinding. The maximum destruction of the initial porous structure of the mushroom was observed after the application of the HAD and MWD methods. The particle surfaces of the FD and SD samples were characterized by a lower degree of structural damage, which according to Sadowska A. [61], could provide a higher level of rehydration properties and moisture holding capacity of the powder.

Next, we summarize the results of a comparative analysis of the structural properties of mushroom seeds obtained by various drying methods and relying on data [61] showing the relationship of the microstructure with the properties of the powder. The degree of rehydration directly depends on the microstructure of the dried product, with important indicators including: structural disturbance, porosity, surface and capillary structure, amorphous structure, and surface density [62]. As a rule, the dehydrated product does not effectively restore its original properties after rehydration due to the destruction of the cellular structure, its compaction, shrinking of capillaries and, as a consequence, a decrease in the hydrophilic properties and water-absorbing capacity of the dry product. Therefore, it can be confidently stated that the observed microstructure features of the particles of the FD and SD samples, characterized by porosity, comparatively low volume density, and relative preservation of the cellular structure, fully agree and justify the results of Maray, A.R. et al. [63] and Lee, M.J. [22], according to which the mushroom (*Pleurotus ostreatus* and *Inonotus obliquus*) powders obtained by the FD and SD methods differed in high levels of rehydration and water retention as compared with powders obtained using HAD drying.

The property of rehydration is an important criterion for choosing the drying process, since some of the dry products are restored by soaking in water before cooking or eating. The structural feature characteristics of FD and SD powders give them an advantage for inclusion in such products. For example, the inclusion of mushroom (*Pleurotus ostreatus*) powder as an ingredient in powdered soups and instant porridges, is increasingly being considered and used by manufacturers of functional nutrition products [64,65].

The most distinctive feature of the mushroom powder obtained by HAD drying was the relatively large particle size. We believe that this structural characteristic will be especially useful for inclusion as an ingredient in products where particle sensitivity occurs, for example, in sauces. According to Keomixay, P. et al. [34], the best sensory quality of the *Pleurotus ostreatus* mushroom is provided by large particles.

Microwave drying and subsequent grinding proved to be effective in reducing the size of the mushroom powder, ensuring its granulometric uniformity and increasing the bulk density. These structural properties give it an advantage in solubilization in the food matrix and advantageous use in products where particle solubility is required, for example, beverages and instant soups. The search and development of new formulations of such products with the inclusion of mushroom (*Pleurotus ostreatus*) powder [66–68] is increasingly of interest today.

In general, the complex of morphological characteristics obtained in our study can be used to optimize the technological properties of mushroom powders.

3.2. Thin Layer Chromatography

Total lipids extracted from mushroom powders were fractionated by TLC (Figure 5).

The analysis of the TLC results showed that the powders contain free fatty acids, sterols, and phospholipids. At the same time, differences between some samples were clearly expressed, which increased interest in a detailed study of the composition of lipophilic substances of mushroom powders. This interest was reinforced by a lipidomic analysis recently conducted by Pellegrino, R.M. et al. [32] on dried fruiting bodies of *Pleurotus os-treatus*, with an emphasis on the study of the dependence of the lipid composition of mushroom on the nutrient substrates used for its cultivation. However, this experiment was carried out only with oyster mushroom sublimates.

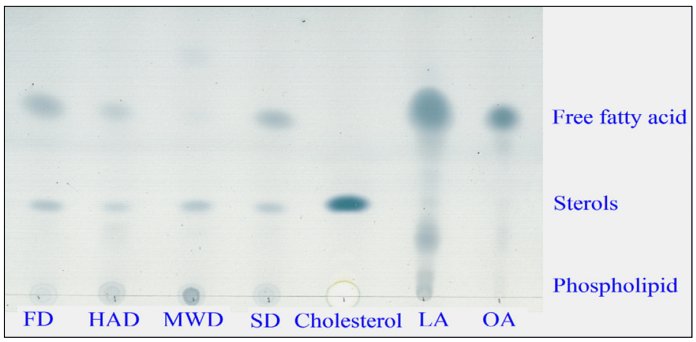
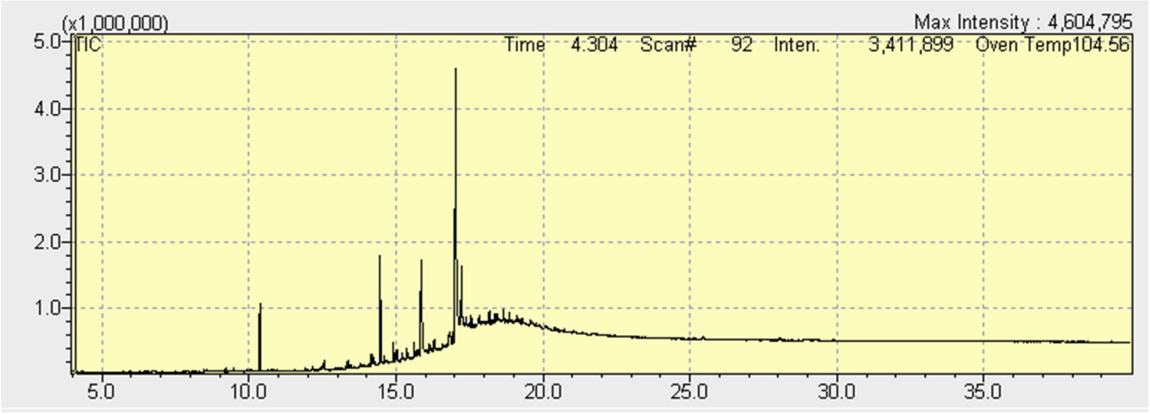


Figure 5. Chromatographic separation of the lipids from mushroom powders and lipid standards: FD—freeze drying; HAD—hot air drying; MWD—microwave drying; SD—sun drying; OA—oleic acid; LA—linoleic acid.

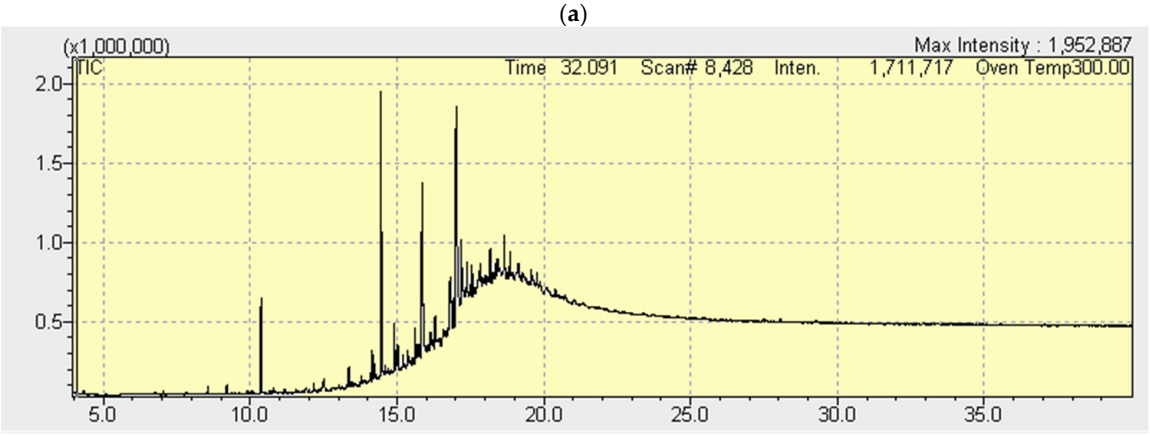
3.3. Gas Chromatographic Analysis

Lipophilic components were identified by gas chromatography with mass spectrometry (GC-MS). Considering that the net efficacy of food-associated bioactive substances depends on their actual level and activity in the digestive tract [69], we carried out a comparative analysis and identification of lipophilic components of mushroom powders in the lipophilic fraction of their extracts. The extracts were obtained under in vitro conditions simulating the process of gastrointestinal digestion.

The GC-MS chromatograms are shown in Figure 6. The chromatographic profile of the main identified lipophilic substances, whose presence and content reflect the differences between the studied samples of mushroom powder extracts, is presented in Table 1.

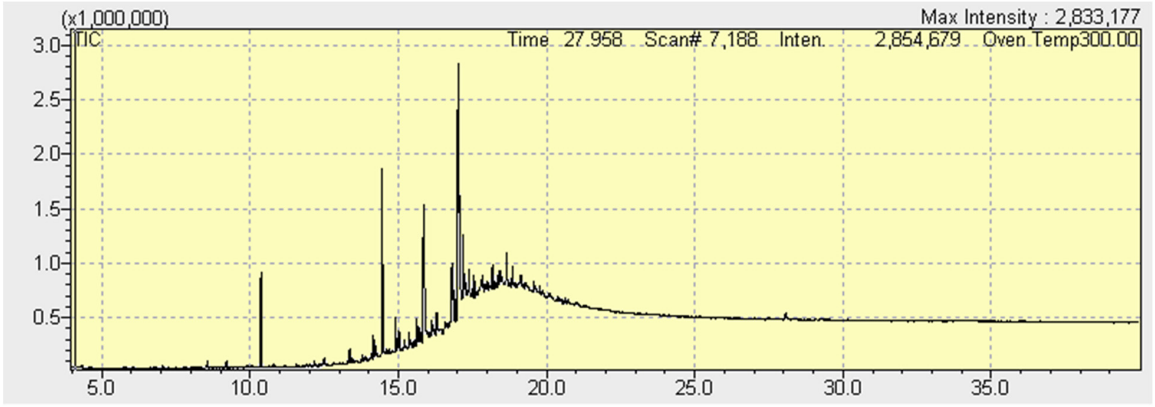


(**a**)

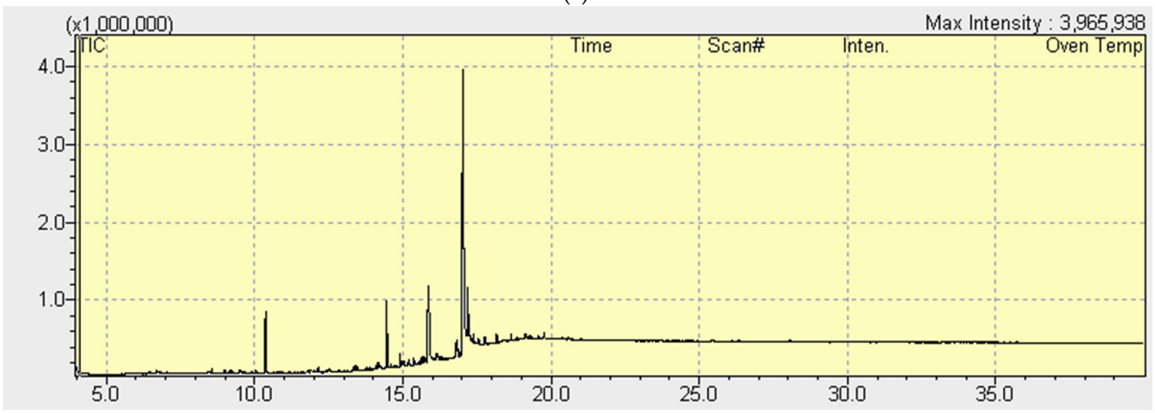


(**b**)

Figure 6. *Cont.*



(**c**)



(**d**)

Figure 6. GC-MS chromatograms of *Pleurotus ostreatus* powder lipophilic extract: (**a**) FD; (**b**) HAD; (**c**) MWD; (**d**) SD.

Table 1. Profile of substances of *Pleurotus ostreatus* powder lipophilic extract.

Name of the Compounds	Molecular Formulae	Relative Peak Area (%)			
		FD	HAD	MWD	SD
Isobornyl acetate	$C_{12}H_{20}O_2$	4.11	3.46	3.94	4.94
Butylated hydroxytoluene	$C_{15}H_{24}O$	0.58	nd	nd	nd
Hexadecane	$C_{16}H_{34}$	0.50	0.71	0.57	nd
Heptadecane	$C_{17}H_{36}$	0.88	1.16	0.97	1.21
Sulfurous acid, cyclohexylmethyl heptyl ester	$C_{14}H_{28}O_3S$	6.59	11.94	7.6	5.37
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.39	0.71	0.86	0.58
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	1.56	nd	nd	nd
1-Heptanal, 3,5,5-triethyl-	$C_{13}H_{26}O$	nd	1.03	0.91	0.52
Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	0.70	0.96	2.43	0.47
Tetratetracontane	$C_{44}H_{90}$	nd	5.0	5.03	nd
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	0.5	nd	0.34	0.47
Benzoic acid, 2-hydroxy-, phenylmethyl ester	$C_{14}H_{12}O_3$	0.71	0.57	0.58	0.94
Heneicosane	$C_{21}H_{44}$	5.03	5.76	4.98	0.59
7,7-Diethylheptadecane	$C_{21}H_{44}$	nd	nd	nd	0.54
Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	nd	0.92	0.89	nd
1-Eicosanol	$C_{20}H_{42}O$	0.91	nd	nd	nd
Dibutyl phthalate	$C_{16}H_{22}O_4$	10.15	10.61	10.53	6.55
Decane, 5,6-Bis(2,2-dimethylpropylidene)-, (E,Z)-	$C_{20}H_{38}$	nd	0.67	0.55	nd
Nonadecane, 4-methyl-	$C_{20}H_{42}$	0.8	nd	nd	nd
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	0.77	nd	nd	0.53

Table 1. *Cont.*

Name of the Compounds	Molecular Formulae	Relative Peak Area (%)			
		FD	HAD	MWD	SD
1-Eicosanol, 2-hexadecyl-	$C_{36}H_{74}O$	0.62	nd	nd	nd
Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	1.33	2.88	3.29	1.48
Tridecane, 7-cyclohexyl-	$C_{19}H_{38}$	0.73	nd	nd	nd
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	28.85	9.39	12.52	41.42
17-Pentatriacontene	$C_{35}H_{70}$	nd	1.61	nd	nd
Cyclohexadecane, 1,2-diethyl-	$C_{20}H_{40}$	nd	1.16	nd	nd
Octadecanoic acid	$C_{18}H_{36}O_2$	3.22	4.1	4.75	6.68
Cyclopentane, heneicosyl-	$C_{26}H_{52}$	nd	0.78	nd	nd
Heneicosane, 11-decyl-	$C_{31}H_{64}$	nd	1.38	nd	nd
Oleic acid	$C_{18}H_{34}O_2$	nd	5.86	9.01	nd
Pentatriacontane	$C_{35}H_{72}$	nd	0.95	0.34	nd
Eicosane	$C_{20}H_{42}$	nd	4.46	1.25	0.35
Octadecane	$C_{18}H_{38}$	nd	0.54	1.58	0.57
trans,trans-9,12-Octadecadienoic acid, propyl ester	$C_{21}H_{38}O_2$	6.99	nd	nd	nd
Tetracosane	$C_{24}H_{50}$	nd	nd	nd	0.56
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	nd	3.44	3.26	5.48
2-Methyltetracosane	$C_{25}H_{52}$	1.88	0.92	nd	nd
Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	2.48	4.08	2.88	1.36
Cyclopentane, decyl-	$C_{15}H_{30}$	nd	nd	0.71	0.89
n-Heptadecylcyclohexane	$C_{23}H_{46}$	nd	nd	nd	1.53
Erythro-9,10-dibromopentacosane	$C_{25}H_{50}Br_2$	nd	nd	nd	1.06
n-Pentadecylcyclohexane	$C_{21}H_{42}$	2.57	1.67	nd	nd
2-Methyloctacosane	$C_{29}H_{60}$	1.48	nd	nd	nd
Tridecane, 3-cyclohexyl-	$C_{19}H_{38}$	2.81	nd	nd	nd
Pentadecane, 2,6,10,14-tetramethyl-	$C_{19}H_{40}$	1.42	0.6	0.57	0.45
Heptadecane, 3-methyl-	$C_{18}H_{38}$	1.62	nd	nd	nd
2-Methylhexacosane	$C_{27}H_{56}$	4.62	2.88	5.81	nd
Methacrylic acid, nonadecyl ester	$C_{23}H_{44}O_2$	nd	nd	nd	0.78
Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	nd	1.38	nd	nd
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	nd	0.6	nd	nd
Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	1.74	nd	nd	nd
Octadecanoic acid, butyl ester	$C_{22}H_{44}O_2$	0.88	1.85	1.55	0.65
1-Decanol, 2-octyl-	$C_{18}H_{38}O$	nd	nd	nd	0.88
Diisooctyl phthalate	$C_{24}H_{38}O_4$	nd	nd	nd	0.54
N,N-dimethyldodecanamide	$C_{14}H_{29}NO$	nd	nd	nd	0.71
9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	nd	nd	1.83	0.8
4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone	$C_{13}H_{23}NO_3$	nd	nd	nd	0.52
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	nd	nd	nd	7.58
Anthracene	$C_{14}H_{10}$	nd	nd	nd	1.19
Isopropyl myristate	$C_{17}H_{34}O_2$	0.38	nd	0.35	0.44

nd, not detected. Identified substances with a relative peak area less than 0.3% were not listed in the table. FD—freeze drying; HAD—hot air drying; MWD—microwave drying; SD—sun drying.

Following comparison with the databases of the NIST 05a library, we identified 60 compounds in *Pleurotus ostreatus* powder lipophilic extracts obtained by modeling gastrointestinal digestion in the peak area from 0.34% to 41.42%. The identified compounds included fatty acids, fatty acid esters, alkanes, fatty alcohols, fatty amides, terpenoids, and fatty aldehydes. According to the total content of the identified lipophilic substances, the experimental samples were distributed as follows: MWD > FD > HAD > SD.

To better consider the differences in the lipid profile of *Pleurotus ostreatus* powders obtained by various drying methods, a heat map was compiled showing how the mushroom powder samples differ in fatty acids and fatty acid ester composition (Figure 7). The Euclidean distance was used as a measure of similarity. The red color in the figure reflects the maximum increase in the concentration of the metabolite, the blue color is the minimum, and the intensity of the color indicates the degree of changes in each sampling interval.

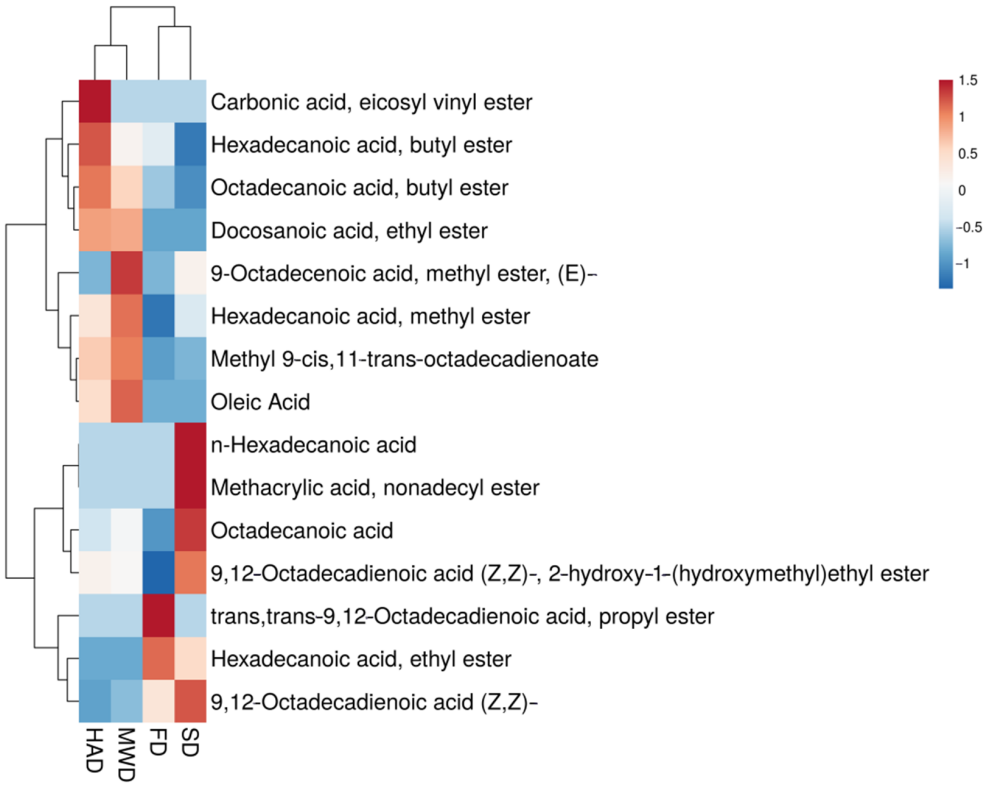


Figure 7. Heat map of fatty acids and fatty acid esters of *Pleurotus ostreatus* powder lipophilic extracts. FD—freeze drying; HAD—hot air drying; MWD—microwave drying; SD—sun drying. The increase and decrease in the content of substances are shown in red and blue, respectively.

According to the approximation of the composition and content of identified fatty acids and fatty acid esters, the samples were grouped as HAD–MWD and FD–SD.

We found four fatty acids in the composition of lipophilic extracts of mushroom powders: linoleic, oleic, palmitic, and stearic. The obvious difference between the extract samples was the content of linoleic acid (9,12-octadecadienoic acid). This is a polyunsaturated fatty acid with a number of biologically active properties: anti-inflammatory, hypocholesterolemic, oncoprotective, hepatoprotective, antihistamine, antiandrogenic, antiarthritis, anticoronary, and antimicrobial [70]. The content of linoleic acid was dominant in all samples. Other researchers have also reported a similar result [32,71].

Analysis of Figure 7 showed that the content of linoleic acid in the samples was distributed as follows: HAD (9.39%) < MWD (12.52%) < FD (28.85%) < SD (41.42%). The obtained data overlapped, to some extent, with the results of Dong, W. et al. [72] that characterized FD drying as an effective method of preserving fatty acids. The revealed fact of a high content of linoleic acid in FD samples as compared with HAD and MWD samples was consistent with the conclusions of Tu, X. et al. [73], who studied the chemical composition of lipophilic extracts of sun-dried and lyophilically dried black morel (*Morchella importuna*) and may be caused by an increase in the degree of oxidation of fatty acids in the product with an increase in the drying temperature, as noted by He, Z. et al. [74].

In our experiment, in terms of linoleic acid level, the SD sample registered the best result, probably due to the cellular mechanisms of lipid metabolism in mushroom under the influence of ultraviolet radiation during SD drying. Bhasin, A. et al. [75] showed that the postharvest effect of ultraviolet radiation on champignons was accompanied by an increase in the concentration of linoleic acid in the mushroom powder.

According to the content of stearic acid (octadecanoic acid), powder extracts were ranked in the next order: FD (3.22%) < HAD(4.1%) < MWD(4.75%) < SD(6.68%). The revealed certain superiority of the SD extract could logically be explained by less aggressive SD conditions. During sun drying, the integrity of the cells is preserved for a long time, and

enzymatic processes, biosynthesis, and transformation of substances continue. Probably for the same reason, the presence of palmitic acid (n-hexadecanoic acid) was registered only in the SD extract (7.58%).

The presence of oleic acid in its composition was characterized by extracts of mushroom powders obtained at relatively higher temperatures: HAD (5.86%) and MWD (9.01%). Similar facts were described by Uribe, E. et al. [76] and may be related to the peculiarities of the oxidative stability of individual unsaturated fatty acids.

According to the total content of fatty acid esters, the extracts were distributed in the next order: SD < FD < MWD < HAD. The FD sample was characterized by the lowest content of methyl ester of heptadecanoic acid, which according to Shaaban, M.T. et al. [77] had a high antimicrobial effect against clinical pathogenic bacteria, and also carried antioxidant, hypocholesterolemic activity [78], and anticancer effect [79].

Ethyl ether of hexadecanoic acid is an antimicrobial compound [80], and also acts as a pronounced antioxidant and inhibitor of 5-α reductase [70], and it was identified only in theFD and SD powder extracts. The presence of docosanoic acid, ethyl ester, known for its antimicrobial and antioxidant effect [79] characterized the HAD and MWD samples.

Of particular note is the identification, only in the FD powder extract, of the formed trans isomer (propyl ester of 9 trans,12 trans-octadecadienoic acid), which, according to Kumari, R. et al. [81], disrupts the metabolism of essential fatty acids and the synthesis of prostaglandins and other cellular regulators.

Attention should also be focused on the bis(2-ethylhexyl) phthalate compound registered only in the HAD powder extract, a lipophilic substance that is a common food contaminant and can have a toxic effect on the endocrine system [82]. The presence of this substance did not exceed the permissible reference concentrations established for food products and their daily consumption [83].

In terms of the content of conjugated linoleic acid (2-hydroxy-1-(hydroxymethyl) ethyl ester of 9Z,12Z-octadecadienoic acid), known as an antioxidant capable of protecting membranes from damage [70], the greatest content was found in the SD sample.

Butyl esters of palmitic and stearic acids in extracts were quantitatively distributed in the order of increase as follows: SD < FD < MWD < HAD.

A heat map was also used for comparative visualization of the content of alkanes, fatty alcohols, and aldehydes (Figure 8).

In terms of alkane concentration, the highest concentrations were found in the HAD and MWD samples, and fatty alcohols were identified in the FD powder extract. The FD extract was in a separate cluster from the other extracts, which suggests its significant differences. For example, fatty alcohols 1-eicosanol and 2-hexadecyl were only identified in FD extract, which have antibacterial effects and are considered by some researchers to be natural food preservatives [84]. In addition, 3,5-di-tert-butyl-4-hydroxybenzaldehyde with antiangiogenic activity was identified only in the FD extract [85] and tetrapentacontane alkane, 1,54-dibromo- bearing a pronounced antioxidant effect [86].

The content of 2-methylhexacosane, with hypolipidemic and antibacterial effects [87], prevailed in the MWD samples. At the same time, 2-methylhexacosane was not detected at all in the SD samples. The content of the secondary metabolite dibutyl phthalate, known from the results of Khatiwora, E. et al. [88] to have antimicrobial and antifungal properties, was about 10% in the FD, HAD, and MWD samples. In the SD sample, dibutyl phthalate was significantly lost (6.55%).

Alkanes, tetratetracontane, and pentatriacontane, known for their antibacterial properties [89,90], as well as decane, 5E,6Z-bis(2,2-dimethylpropylidene) were registered only in the HAD and MWD samples. 2-Methyltetracosane, exhibiting antiradical activity [91], was identified in the FD and HAD samples. Tetracosane alkane, known for anti-inflammatory, antioxidant, anti-ulcer, cardiotonic, and anticancer properties [92], as well as the substances n-heptadecylcyclohexane, erythro-9,10-dibromopentacosane, nonadecyl ester of methacrylic acid, 1-decanol, 2-octyl-, diisooctyl phthalate, N,N-dimethyldodecanamide, 4-t-butyl-2-(1-methyl-2-nitroethyl)cyclohexanone, and anthracene were only found in the SD samples.

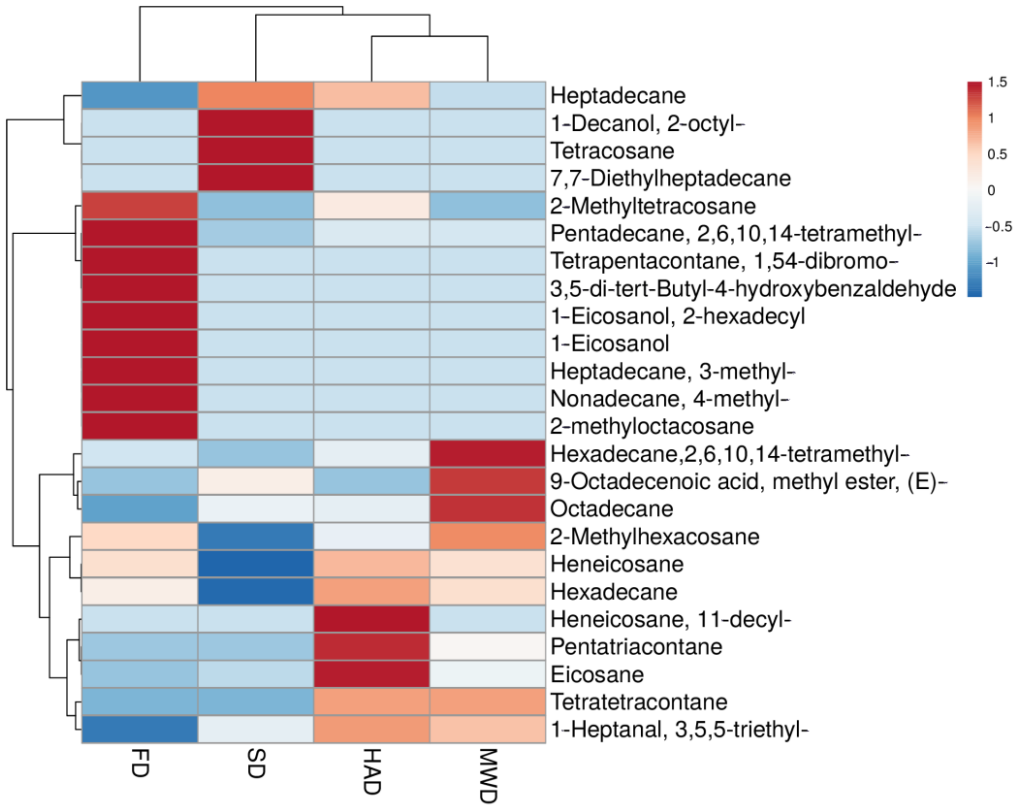


Figure 8. Heat map alkanes, fatty alcohols, and aldehydes of lipophilic extract of *Pleurotus ostreatus* powder lipophilic extracts. FD—freeze drying; HAD—hot air drying; MWD—microwave drying; SD—sun drying. The increase and decrease in the content of substances are shown in red and blue, respectively.

The highest number of biologically active alkanes were identified in the HAD sample. In particular, the HAD samples differed in the content of 17-pentatriacontene, which, according to Albratty, M. et al. [93] and Sargunam, J.H. and Thilakavathy, S. [94], has antibacterial, anti-inflammatory, anti-arthritis and anticancer properties. In addition, only in the HAD samples, we identified: cyclohexadiene, 1,2-diethyl-; cyclopentane, heneicosyl-; heneicosane, 11-decyl-; eicosyl vinyl ester of carbonic acid.

Thus, it can be seen that different drying methods can change the composition of the lipophilic components of mushroom (*Pleurotus ostreatus*) powder. Based on the data obtained, we are confident that in the future, when using mushroom powders in the food industry, standards for their preparation will be introduced, taking into consideration the effect of the drying method on the resulting properties, structure, and nutritional value of mushrooms.

4. Conclusions

In this work, we studied the influence of four drying methods on the morphology and lipid profile of mushroom (*Pleurotus ostreatus*) powder. The data obtained proved to be strong evidence of the structure and composition of biologically active lipophilic components of *Pleurotus ostreatus* as a result of drying and subsequent grinding. Lyophilic drying and sun drying proved to be the most effective in terms of obtaining mushroom powder with relatively high porosity, partial preservation of cellular structures, and low volume density, which was caused by pores and by the agglomeration of particles. These structural features give an advantage in the properties of rehydration and inclusion in products requiring water reduction before use. Dewatering with hot air produced coarse-grained mushroom powder, with the advantage of flow properties optimal for ensuring the sensory qualities of the particles. Microwave drying proved to be more effective in reducing particle size, ensuring uniformity of the structure, and increasing the volumetric density

of the mushroom powder. MWD improves the solubilization property of the mushroom powder for its use in types of products with a rapidly soluble matrix.

The drying process and subsequent grinding of *Pleurotus ostreatus* mushrooms were reflected in the composition of their lipophilic components. According to the composition of fatty acids and fatty acid esters under extraction conditions in simulated digestive fluids, the extrcts were ranked in the order SD < FD < MWD < HAD. Drying in the sun turned out to be the most effective drying method to obtain mushroom powder with a high content of linoleic and stearic fatty acids. The preservation of oleic acid in powders was ensured by drying with hot air and in a microwave oven. In terms of lipophilic metabolites with proven beneficial biological activity, the best result was identified for the powder obtained using the microwave method. Lipophilic compounds formed during drying with a possible undesirable effect were identified in sublimates and powders dried with hot air.

In general, this study expanded the currently limited knowledge about the effect of various drying methods on the structural properties of mushroom (*Pleurotus ostreatus*) powder and its lipophilic component. The resulting new information will contribute to better management of mushroom raw materials in terms of optimization, taking into consideration the manufacturer's interest in the technological and functional properties of mushroom powders as a food ingredient or biologically active substance for the production of nutraceuticals.

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Conflicts of Interest: The authors declare no conflict of interest.

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