

INFLUENCE OF VACUUM-PULSE DRYING ON THE CONTENT OF FREE AMINO ACIDS, TRYPSINE INHIBITOR ACTIVITY AND COMPOSITION OF VOLATILE COMPONENTS OF MUSHROOMS

I. V. Shcheglova, A. L. Vereshchagin*

Biysk Technological Institute (branch),
Altai State Technical University named after I. I. Polzunova,
ul. Trofimova 27, Biysk, 659305 Russia,
* e-mail: val@bti.secna.ru

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Abstract: Wild mushrooms traditionally are considered one of the sources of food fibers, vegetable proteins, macro- and - micronutrients, and also flavor components. However, the composition of mushrooms includes antinutritional substances capable to selectively reduce the absorption of certain nutrients. These are primarily antienzymes or proteinase inhibitors, which reduce the absorption of proteins. Previous studies have indicated applicability of vacuum-pulse drying to improve the nutritional value in the edible mushrooms (*Cantharellus cibarius Fr.*) autohydrolysis of bodies biopolymers of the mushrooms and increase of the rate of swelling in hot water. The possibility of applying a vacuum-pulse drying for increasing the content of free amino acids and reduction of the activity of trypsin inhibitors in edible mushrooms: chanterelles and autumn agarics (*Cantharellus cibarius Fr.*) is shown in this study. In addition, it is established, that the vacuum-pulse method of drying leads to reduction of flavor components content in the edible mushrooms. To study human body digestibility of vacuum-dried product further research is required. The effect of vacuum-pulse drying on flavor properties of mushrooms continues to be a controversial question.

Keywords: Mushrooms, vacuum-pulse drying, proteins, amino acids, trypsin inhibitors, volatile components

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INTRODUCTION

Nowadays there is a shortage of protein intake in different groups of population, which leads to a decrease in efficiency, metabolic disorders, and the emergence of a number of diseases. The proteins of mushrooms may play an important role in meeting the needs of food protein.

Mushrooms belong to vegetable products with a relatively high content of protein, which takes up to 40% solids, an average of $24.9 \pm 1.75\%$ [1]. However, up to date there is no consensus about the usefulness and comprehensibility of fungal proteins. Recognizing the great protein content in mushrooms, we cannot ignore the high content of dietary fiber and chitin. Definite proof of complexity of fungal protein digestibility by a human body can also be considered by the fact that the degree of extractability of the protein by various solvents, depending on the species of fungi is on the level 35–60% [2].

Biological value of food is determined by indication of protein quality, what reflects the extent to which its amino acid composition corresponds to the body needs in amino acids for protein synthesis. In fungal protein hydrolyzates up to 22 amino acids are revealed [3]. Essential amino acids are contained in mushrooms up to 33–44% of amino acids total sum. And their numbers are growing in direct proportion to an increase in protein content [4]. Alongside with the implementation of their biological function certain

amino acids make great contribution into flavor properties of mushrooms [5].

It is also known that fungi include antinutritional substances capable to selectively reduce the assimilation of certain nutrients. They are primarily antienzymes or proteinase inhibitors, which block the activity of enzymes of the gastrointestinal tract, and reduce the absorption of protein substances [6, 7]. These are trypsin inhibitors capable to form inactive complexes with enzymes that break down proteins in a human body; wherein the enzymes lose their catalytic activity. Therefore, prolonged use of such food leads to hypertrophy of a pancreas and hence a slower growth. Thus, high levels of proteinase inhibitors content significantly lowers the nutritional value of proteins and has negative effects on the body.

According to studies [8] of the content of trypsin inhibitors, in 55 kinds of edible mushrooms a trypsin inhibitor activity is observed to be within 0.36–10.42 mg/g of dry weight.

L.A. Gzogyan showed that the fertile bodies of 18 different species of basidiomycetes in Krasnodar region contain these enzymes, with the exception of polypore (*Coriolus versicolor (Fr.) Karst*) and blackberries (*Hericium erinaceus (Fr.) Quel*). The highest level of activity of trypsin-like proteinases was found in fruit bodies of brown cap boletus (*Leccinum melanum (Fr.) Karst*) (5.3 mg/g), white mushrooms (*Boletus edulis*) (3.7 mg/g) and the chanterelles natural

(*Cantharellus cibarius Fr.*) (3.6 mg/g), autumn honey fungus (*Armillariella mellea (Fr.) Karst.*) (2 mg/d) [6].

Studies of V. I. Bakaytis and S. N. Basalaeva of seven species of wild mushrooms, grown in Novosibirsk Region and Altai territory, showed lower activity of trypsin inhibitors than in those of Krasnodar territory.

Thus, the highest activity of trypsin inhibitors is presented in white mushrooms (*Boletus edulis*) (0.97–1.20 mg/g), the average level of activity is stated in autumn honey agarics (*Armillariella mellea (Fr.) Karst.*), mokhoviki (*Boletus variegates*) and presented in natural chanterelles (*Cantharellus cibarius Fr.*) (0.67–0.44 mg/g), the minimum level of activity of trypsin inhibitors is in white podgruzdky (*Russula delicata Fr.*) and real milk mushrooms (*Lactarius resimus Fr.*) (0.35–0.39 mg/g) [9].

It should be noted that trypsin inhibitors have sufficiently high resistance to inactivation. From some literature data we find trypsin [7, 10, 11], for example, after treatment of aqueous soy extract, containing trypsin inhibitors nearly for an hour, at 120°C their activity is reduced up to 30–35% [12]. The effect of heat treatment is increased by presoaking [13] and microionization [14].

Studies conducted earlier have shown the applicability of the method of vacuum-pulse drying to improve the nutritional value of chanterelles (*Cantharellus cibarius Fr.*) due to autohydrolysis of biopolymers of mushroom bodies and increase the rate of swelling in hot water [15].

Drying of mushrooms is an effective and popular way of their preservation and conservation. Dried mushrooms have unusually pleasant and strong aroma and flavor, thus they are considered delicacies.

During the drying process the composition of products significantly changes. Together with the removal of moisture, losses of volatile organic substances take place, concentration of low molecular weight compounds (peptides, amino acids, sugars, organic acids) significantly increases, and enzyme activity is also changed. All this leads to the change in aroma and taste of foods. Under high-temperature drying the reaction occurs between amino acids and sugars, leading to the synthesis of new organic compounds, including volatile products. That generates an aroma of fragrance-dried products. In dried products changes occur during storage, particularly in composition of volatile substances stipulated by their loss through volatilization or oxidation [16, 17].

As a result of research, in various kinds of mushrooms nearly 150 volatile substances have been found, belonging to different classes of organic compounds, liable to significant changes. Basic compounds forming raw mushroom flavor, are aliphatic alcohols and ketones with carbon numbers 8: 1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octanol, 1-octen-3-one and 3-octanone [18-20].

The purpose of this study is to investigate the influence of vacuum-pulse drying on protein quality, trypsin inhibitor activity and composition of the volatile compounds of edible mushrooms.

OBJECTS AND METHODS OF STUDY

As research objects, we chose one of the most common precious wild mushrooms of the third category: chanterelles natural (*Cantharellus cibarius Fr.*) and autumn honey fungus (*Armillariella mellea (Fr.) Karst.*), collected in pine forest tracts "Soshnikovo" Priobsk forest massive, Altai territory. It is also known that these types of mushrooms contain the least amount of protein among the major harvested species [1].

Being cut into cubes of 5–10 mm in size lateral mushroom bodies were placed into the working chamber of a dryer and subjected to vacuum-pulse drying under temperature of 55°C. Processing was performed under certain pressure, lowering it from atmospheric pressure up to 100 Pa for the period of 30 seconds, and then again it was raised to atmospheric pressure and mushrooms were kept for 100 seconds. The process of successive vacuuming and holding mushrooms in contact with atmosphere was carried out periodically 2–5 times to constant weight, depending on the consistency of mushrooms with a definable age.

As a control sample the authors used the fruit body dried at atmospheric pressure, under temperature of 55°C bringing up to its constant weight (conventional convection drying).

Determination of mass fraction of the total protein in mushrooms sample was carried out by Dumas method on the express-analyzer Rapid N cube, the concentration of amino acids - by the method of ion exchange chromatography on an amino acid analyzer Aracus. Trypsin inhibitor activity (AIT) was determined by the method described in "Methods of biochemical research ..." (1987) [21]. Reagents of the firm ISN-Biomedical (US) were used, in particular a substrate - BAPA (Na-benzoyl-DL-arginine-p-nitroanilide) in accordance with the method of Iu.Ia. Gofmana and I.M. Vaysblaya (1975) [22]. The method used is based on the spectrophotometric measurement of proteinaceous substrate decay products optical density (BAPA) by an action of trypsin at a wavelength of 405 nm. The amount of inhibitors, extracted by distilled water from the air-dried mushroom flour (moisture content 6%) at the ratio of 1 : 50, was kept in a refrigerator for the whole night. The flour was obtained by grinding of dried mushrooms and their sieving through a sieve with a cell diameter of 0.1 mm. 0.05 M Tris-HCl-0.02 M CaCl₂ was used as a buffer. Determination of AIT was carried out at pH = 7.7; before operating all the solutions were incubated for an hour at 25°C.

Indices of the qualitative and quantitative composition of volatile aromatic mushrooms substances were determined by a gas chromatography. For this purpose, 5 g of crushed dried mushrooms were added in 100 ml of distilled water and 250 mg (5000 mg per 100 g of mushroom) of n-dodecane as an internal standard. The volatile components were removed within 45 min with 20 ml of diethyl ether, freshly distilled by continuous distillation-extraction. The extracts were dried with 2 g of anhydrous sodium sulfate and concentrated to a volume of 0.1 ml using ether distillation at 40°C with a Vigreux column on the length of 35 cm. The ether

extracts obtained were analyzed by gas-liquid chromatography.

For gas chromatography studies a capillary gas chromatograph with a HP 5730A with a flame-ionization detector, a quartz capillary column FFAP (50 m×0.32 mm, 0.5 micron layer of phase) were used. Analysis of the ethereal extracts was conducted at a column temperature programming mode as follows: isotherm at 77°C for 6.5 minutes, then the at temperature programming up to 210°C at a rate of 10°C/min.

RESULTS AND DISCUSSION

Experimental data on the determination of protein and amino acid composition are shown in Tables 1 and 2.

Table 1. Mass fraction of total protein in mushroom samples

Mushrooms	Mass fraction of total protein in fungi samples, %	
	VIS	Control
Chanterelles	21.6	18.6
Honey fungus (autumn)	31.0	29.2

Table 2. Results of determining concentration of amino acids in samples

Name of determined amino acid	Amino acid concentration, mg/100 g dry substance			
	Chanterelles		Honey fungus	
	VIS	control	VIS	control
Aspartic acid	2890	2547	4575	4380
Threonine	495	466	1130	838
Serine	894	704	190	165
Glutamine acid	3585	3240	3679	3501
Proline + Glycine	605	570	2715	2556
Alanine	285	283	2778	2634
Umteine	1707	1238	2736	2566
Methionine	311	293	260	231
Isoleucine	966	916	1037	956
Leucine	1848	1515	1223	1159
Tyrosine	614	597	839	740
Phenilalanyl	3273	2988	2335	2206
Gistadin	2213	1860	2427	2307
Lysine	399	307	2715	2365
Arginine	1621	1230	2526	2334
Total content of amino acids	21706	18754	31165	28938

Note. Values are valid at P = 95%.

Thus we can see from Tables 1 and 2, as a result of pulsed-vacuum treatment, the total amount of protein and the content of individual amino acids increased. However, the degree of increase in a number of free amino acids is not the same. For example, in chanterelles the qualitative composition of real free amino acids varies with the destruction of glutamic acid, tyrosine and phenylalanine and with increasing proportion of cysteine and serine. In autumn honey fungus the relative content of aspartic and glutamic acids decreased, but lysine and threonine fraction increased.

Increase in the total amino acid content was 15.7% and 7.7% for chanterelles and honey fungus respectively. Changes in a proportion of essential amino acids are stated in the range of measurement error (less than 1%).

Table 3 shows the results of calculating the balance in composition of fungal amino acid protein according to the standard FAO (the calculations of tryptophan and valine were not involved, as their content was not determined).

Table 3. Amino-acid score of fungi protein

Amino acid	Amino-acid score of fungal protein, %			
	Chanterelles natural		Honey fungus	
	VIS	control	VIS	Control
Threonine	57	62	91	72
Methionine + cysteine	266	233	275	286
Isoleucine	111	122	83	83
Leucine	122	115	56	57
Phenilalanyl + tyrosine	298	319	180	170
Lysine	33	30	158	149
Total	887	881	843	816

These data suggest that limiting amino acids in chanterelles are lysine and threonine, and phenylalanine + tyrosine, while methionine + cysteine being predominant. In autumn honey fungus limiting amino acids are leucine, isoleucine and threonine, and methionine + cysteine are predominant. It should be noted, that no significant amino acid changes came soon after vacuum- pulse processing.

Thus, as a result of vacuum-pulse treatment, the qualitative and quantitative composition of the fungal protein varies. The data obtained can be attributed to the fact that by increasing water activity due to the vacuum treatment the pulse partial hydrolysis of chitin-glucan protein complexes and hard-digestive mushroom proteins occur. In this case free amino acids are generated.

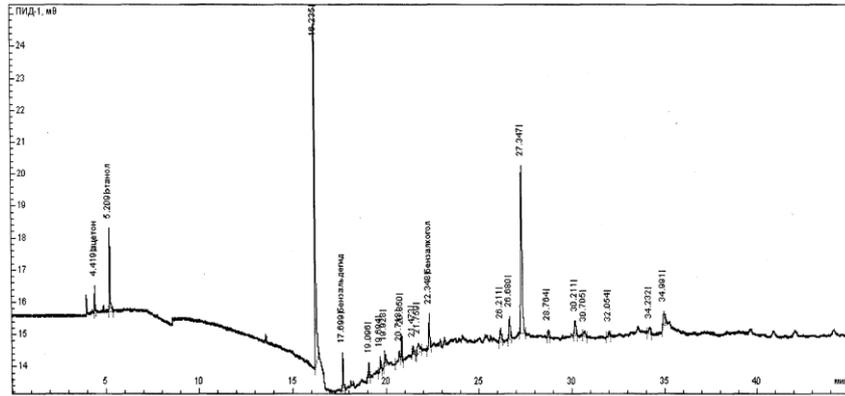
Experimental data on the determination of trypsin-inhibitor activity are shown in Table 4.

Table 4. The activity of trypsin inhibitors in mushrooms

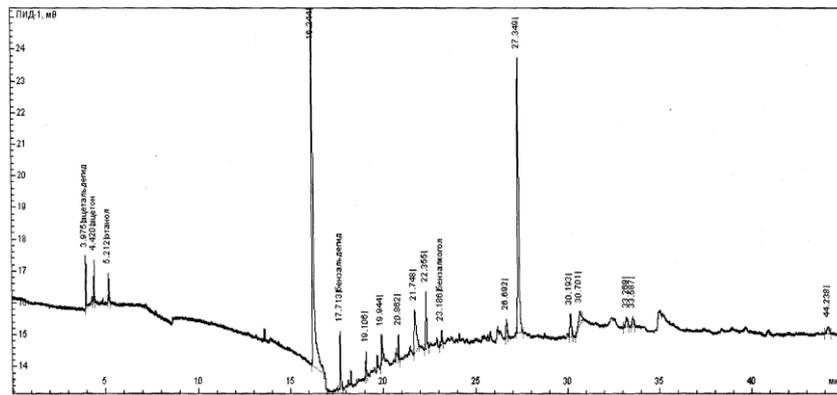
Mushrooms	AIT, mg/g dry weight		Trypsin inhibitor destruction degree versus control samples, %
	VIS	control	
Chanterelles real	0.45±0.03	0.77±0.05	41.6
Agarics autumn	0.57±0.04	0.77±0.05	26.0

These data show a decrease in the activity of trypsin inhibitors by 41.6 and 26.0% in natural chanterelles and honey agaric mushrooms (autumn) respectively.

Fig. 1 and 2 are chromatograms of volatile component samples.

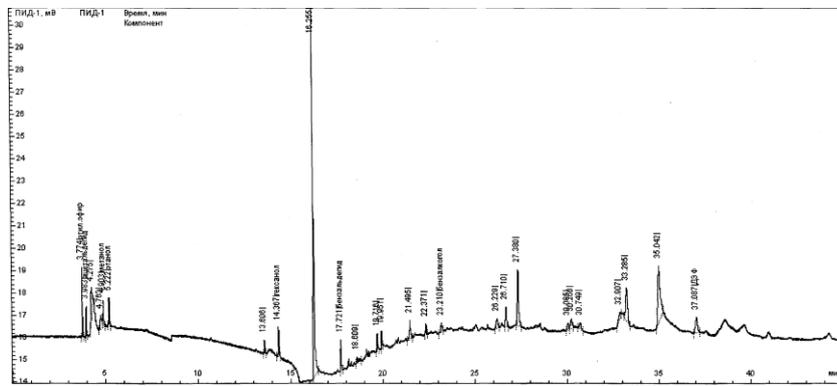


(a)

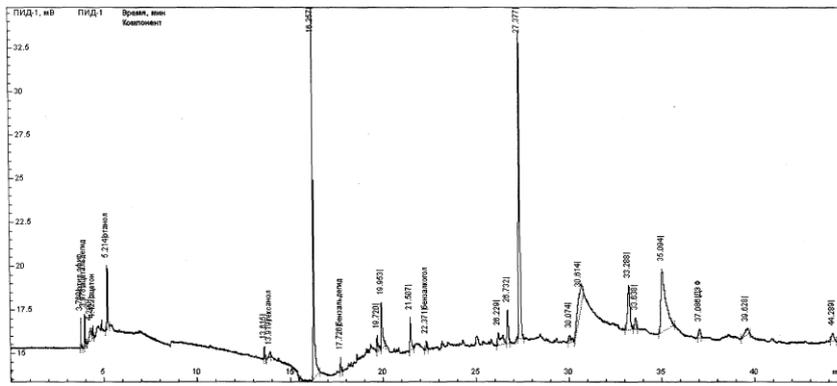


(b)

Fig. 1. Chromatograms of volatile samples of dried chanterelles: (a) vacuum impulse drying; (b) convective drying.



(a)



(b)

Fig. 2. Chromatograms of volatile samples of dried honey fungus: (a) vacuum-pulse drying; (b) convective drying.

In the study we found that treatment of mushrooms by vacuum-pulse method resulted in a decrease in comparison with the convective drying of the total volatile content of 45.7% and 55.6% in natural chanterelles and honey fungus respectively.

At the same time, the content of the treated samples increased in some volatile compounds: in agarics - hexanol benzalcohol, diethyl phthalate, benzaldehyde, ethyl ether, acetone; in chanterelles - ethanol and benzalcohol.

Despite the fact that these substances are not key odorants of mushroom flavor, they can give an important and even decisive contribution to mushroom smell, changing it and adding new hints to it.

Thus, the vacuum-pulse treatment leads to increasing of free amino acids content and reducing the activity of trypsin inhibitors in edible mushrooms. Perhaps, this will increase the nutritional value of mushrooms, but the additional study of the product digestibility by a human body is needed.

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