The concentration of melanin in powder materials obtained from white-rot fungi by the mechanochemical method

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Abstract. The effect of mechanochemical treatment of solid mixtures of white-rot fungi as chaga, ganoderma, tinder with alkalines powders on concentrations of biologically active substance melanin was studied. The rise of alkalinity of mechanochemical reagent in the range “sodium bicarbonate – carbonate – hydroxide” leads to the increase in yield of water soluble substances and melanin among them. The use of 5% solid sodium hydroxide changes the yield of water soluble substances from tinder from 4 to 24%.

1. Introduction

The class of higher basidiomycetes embraces well-known white-rot fungi or woody mushrooms. They break down the lignin and possibly cellulose in wood. Some of these materials are conjugated with polyphenols to yield melanin. Otherwise, melanin would be rather insoluble and has a low biological availability. The white-rot fungi are popular in folk medicine: Reishi - tinder conk mushroom (Fomes fomentarius), shiitake (Lentinus edodes), which has surpassed the traditional champignons and oyster mushrooms in production volumes, and chaga [1].

Chaga is a sterile form of true tinder mushroom, Inonotus obliquus (Fr.) Pil., has been investigated by biochemists most thoroughly. The main active ingredient that provides the biological activity of pharmacological drugs is a dark polyphenol carbon complex containing strong antioxidant melanins. Water extract of chaga is a system with complex composition, which additionally includes salts of organic and mineral acids, polysaccharides and low molecular weight phenols [2]. The concentration of the water-soluble polyphenolic complex is a qualitative indicator of the biological activity of chaga-based drugs.

Melanins are able to neutralize free radicals that occur in a living cell. The concentration of melanins is particularly high in tinder mushrooms. It has been found that melanins of chaga and other tinder fungi possess photo- and radioprotective, antioxidant and genoprotective properties [3] and have pronounced antitumor and antiviral effects [4, 5].

Mechanochemical interactions between solid polyphenols and alkalis can be used to obtain biologically active preparations and to increase the extraction from plant raw materials. Alkalines incorporated in plant raw materials interact with solid acids to form water-soluble salts [6]. The extraction process can be significantly simplified. Biologically active acids and alkalis can interact with auxiliary substances to form soluble mechanocomposites, dispersions, co-crystals [7].
Previously, we investigated the effect of mechanochemical treatment of chaga (mainly with alkaline reagents) on the increase in melanin yield [8]. The use of solid-phase mechanochemical methods opens up prospects for the production of powder preparations with increased yield and dissolution rate in aqueous media.

Powder materials obtained from mushrooms are used in food industry as components of functional food to improve the quality of life of patients with severe diseases after chemical and radiation therapy.

The aim of this work was to study the effect of mechanochemical treatment on the concentration of water-soluble components of three types of white-rot fungi (Inonotus obliquus - true tinder mushroom, chaga; Ganoderma lucidum – lacquered tinder, Reishi; Fomes fomentarius – tinder conk mushroom) and to determine the effectiveness of mechanochemical treatment employed to produce powder preparations of a number of fungi used in food industry and traditional medicine.

2. Experimental part
Powders of woody mushrooms are slightly acidic, and pH of solution in the extraction of 1 g of powder in 100 ml of water is 4.3-5.6. As polyphenolic compounds dissolve better in alkaline form, the following mixtures of mushroom powder and solid reagent with increasing alkalinity were used for mechanochemical treatment: sodium bicarbonate, 5% by weight, sodium carbonate, 5% by weight, sodium hydroxide, 5% by weight [9]. It is assumed that the formation of alkaline soluble forms of polyphenols occurs in joint mechanical treatment of powders as was shown previously for the case of diantronic compounds from St. John’s wort [10], or there occurs the formation of mechanocomposites that are easily transformed into soluble polyphenol salts when they get into water. The high reactivity of composites is predetermined by a larger contact area of reagents compared to the usual contact area of reagents and the reduction of diffusion difficulties [11].

The concentration of water-soluble substances in powder preparations obtained from chaga, ganoderma and tinder was determined as follows. A sample of powder material weighing 5.0 g was kept for 15-16 hours in water at the weight ratio of solid and liquid phases of 1:4 at room temperature with subsequent water extraction at 60±2°C in a Soxhlet extractor with a reverse fridge until soluble components are completely removed from the samples (8-10 hours). The solution (400 ml) was filtered, pH was determined and the solution was evaporated at 60±2°C to the dry residue of water-soluble substances. Dry product was weighed, and the yield was calculated relative to the weight of initial raw material.

3. Results and discussion
Mechanochemically produced powders consist mainly of particles with sizes 0.07 – 0.08 mm, every composite particle contains fungi material and micron and submicron solid alkaline.

Table 1 presents some properties of powders obtained by mechanochemical method from different types of white-rot fungi mushrooms.

Table 1 shows that mechanochemical treatment with reagents of increased alkalinity increases the concentration of water-soluble substances.

Generally, pH rises under the use of more alkaline mechanochemical reagent in the range: sodium bicarbonate – carbonate – hydroxide. The decrease of pH in the case of chaga and ganoderma from 6.9 to 6.7 and from 8.8 to 8.5 can be result of higher reactivity of sodium hydroxide in relation to sodium carbonate and possibility for more stable plant polymer substances to take part in reaction of hydrolysis with melanin formation.

The use of powders with alkalinity providing pH of extracts of more than 8 when adding 1 g of powder in 100 ml of water should be taken into account in the development of food formulations.

The water soluble substances considered in Table 1 are not really pure fungal melanin. Purification of water soluble substance and production of pure melanin was made by redeposition as in [8].

As it has been shown previously, melanin yield from chaga increases after mechanochemical treatment (Table 2) [8].
Table 1. The concentration of water-soluble substances in powders obtained by mechanochemical method from chaga (*Inonotus obliquus*), ganoderma (*Ganoderma lucidum*) and tinder (*Fomes fomentarius*) mushrooms.

| Variant | Concentration of water-soluble substances, % of the mushroom weight / pH of solution - 1 g of sample in 100 ml of water |
|---------|---------------------------------------------------------------------------------------------------------------|
|         | Chaga                                                                                                        | Ganoderma                             | Tinder                                                                 |
| Grinding, fraction less than 2 mm (initial raw material) | 26.0 ± 1.2 / 6.1                                                                                             | 19.4 ± 1.0 / 4.3                       | 3.8 ± 0.2 / 5.6 |
| Mechanochemical treatment with 5 % NaHCO₃ | 30.0 ± 1.4 /6.9                                                                                             | 19.1 ± 1.2 / 6.9                       | 7.0 ± 0.3 / 7.2 |
| Mechanochemical treatment with 5 % Na₂CO₃ | 33.0 ± 1.4 /6.9                                                                                             | 21.2 ± 1.4 / 8.8                       | 8.7 ± 0.5 / 8.3 |
| Mechanochemical treatment with 5 % NaOH | 35.2 ± 1.9 / 6.7                                                                                             | 27.6 ± 1.6 / 8.5                       | 23.7 ± 1.7 /9.6 |

Table 2. Melanin concentration with different methods of preparation of raw materials and extraction conditions (average data).

| Particle sizes in chaga powder obtained with different methods | Variant # | Extraction conditions | Melanin yield, % |
|-----------------------------------------------------------------|-----------|----------------------|------------------|
| Particle sizes less than 7 mm (pharmaceutical raw materials)   | 1         | 2 days at 50°C       | 11.0 ± 1.0       |
| Particle sizes less than 2 mm (IKA MF 10 grinder)              | 2         | 2 days at 50°C       | 21.3 ± 1.1       |
|                                                                 | 3         | 30 minutes at 100°C  | 18.2 ± 1.5       |
|                                                                 | 4         | 30 minutes at 112°C  | 24.4 ± 1.8       |
| Particle sizes of 0.07-0.08 mm, mechanochemical treatment (PM-1 grinder) | 5         | 2 days at 50°C       | 28.2 ± 2.3       |
|                                                                 | 6         | 30 minutes at 112°C  | 30.1 ± 2.0       |

Comparing the results of Table 1 and Table 2 for chaga we can see that an increase in the concentration of water-soluble substances from 26 to 33% enhances melanin concentration from 21 to 30%. The temperatures used are as follows: 112°C - temperature recommended in [8] as optimal, 100°C and 50°C – temperatures recommended by Pharmacopeia cited in.

The concentration of water-soluble substances can be used for rapid analysis of the quality of mushroom powders obtained by mechanochemical method.

4. Conclusions
Mechanochemical treatment of white-rot fungi materials with solid alkalis leads to formation of powders with composite particle 0.07 – 0.08 mm size containing enhanced concentration of soluble substances. The highest concentration of soluble substances of polyphenolic nature including melanin in powders obtained from white-rot fungi is achieved by solid-phase mechanochemical treatment using solid sodium hydroxide. The use of 5% solid sodium hydroxide changes the yield of water soluble substances from tinder from 4 to 24%.

Concentration of water soluble substances and melanin rises under the use of more alkaline mechanochemical reagent in the range: sodium bicarbonate – carbonate - hydroxide.

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