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SOLID STATE TECHNOLOGY OF PRODUCTION OF ANTIOXIDANTS PREPARATIONS FROM PLANT MATERIAL

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Abstract

Antioxidants plays one of the main role in protection of normal vital functions of organisms. The main sources of natural antioxidant are plant extracts. Because of antioxidants are reducers, they have strongly activity with oxygen and intermediates of oxidizing of organic compounds. Decreasing of yield of antioxidants, nonreproductivity of concentrations, problems with storage are technical effect of high activity of antioxidants. It is known that stability of reducers in the solid phase in comparison with those in the liquid phase is strongly higher. We showed availability of using of solid phase mechanochemical reaction for producing of preparations of unstable antioxidant compounds from plant material. The ratio of oxidizing of active component – green tea catechins and naphtodiantrones from St. John's Wort in the solid mechanochemical preparation in comparison with those in liquid preparation was decreased not less than 500 times. The yield of active components increased in 2-10 times.

Key words: *green tea catechins, hypericin, antioxidant activity, mechanochemistry, extraction.*

1. INTRODUCTION

Common external factors such as unhealthy environmental conditions, radioactive and UV - radiation, smoking, alcohol abuse leads to increased levels of free radicals in the human body. There is the connection between elevated level of free radicals and the development of many diseases. For the prevention of disease to consuming of antioxidants in the body through food, beverages, food additives is proposed. It is believed that the safest way is to use substances - antioxidants of natural origin, which is an essential part is polyphenol compounds (Minina, 2009).

Numerous studies have shown that natural polyphenolic compounds are characterized by low toxicity combined with high efficiency. In connection with this trend the use of stabilizing additives and medicinal products derived from the plant material, is constantly increasing.

St. John's wort (*Hypericum perforatum* L.) contains a number of naphtodiantronoic compounds: hypericin (Hyp), pseudohypericinand their protonaited forms. Hypericin (Fig. 1) is considered one of the most active natural antioxidant. The content of hypericin in plant material 0.5 – 1 wt. %. Green tea (*Camellia sinensis* L.) is the most popular beverage in the world. Tea leaf contains from 6 to 16 wt. % polyphenols compounds green tea catechins (GTC). The main of them are epicatechin, epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). Structural formulas are shown in Fig. 2, 3. Apparently, the catechins - is one of the large natural antioxidant resources.

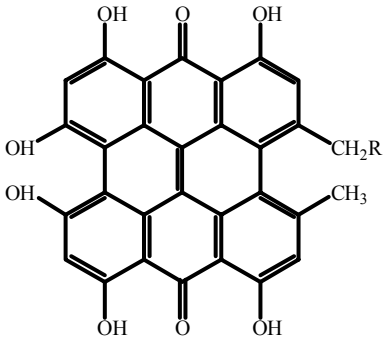


Fig. 1. Structure of hypericin.

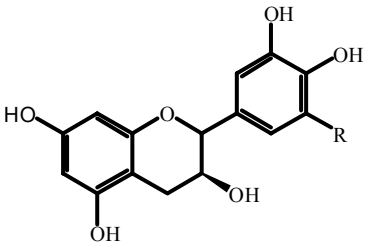


Fig.2 Structure: R = H Epicatechin
R = OH Epigallocatechin

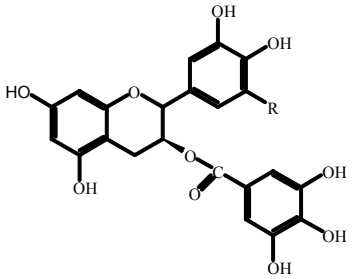


Fig.3 Structure R = H Epicatechin gallate
R = OH Epigallocatechin gallate

The tendency of recent years in mechanochemistry is the processing of renewable raw materials - crop and livestock production, industrial microorganisms grown, industry wastes, peat (Gorokhova, 1995, Efanov, 2001, Kashnskaya, 2003, Yudina, 2004). Mechanochemistry has been applied to processing plants and other biogenic raw materials to produce biologically active compounds - alkaloids, organic acids, glycosides, glycoproteins (Bychkov, 2008, Lebovka, 2011, Pankrushina, 2007, Korolev, 2003).

The purpose of this study was to investigate the influence of mechanochemical treatment on the extraction of hypericin from St. John's wort, catechins from green tea and the creation of the production technology of antioxidant preparations with a high content of active ingredients.

2. MATERIALS AND METHODS

2.1 Initial material and reagents

Initial green tea was provided by Ltd. DagomisChai (Russia) spec. No 9191-003-00570186-04. Initial St. John's wort was provided by Central Siberian Botanic Garden SB RAS in accordance with GOST 15161-93. All another reagents was not less than > 99,9 % purity.

2.2 Extraction and storage of material

Storage of material in solid phase was carried out on plastic jars with presence of air at room temperature.

Material was extracted by water with water/solid ratio 1/20 at 1-3 hours than filtered on membrane filter 0.45 μm at room temperature. Samples for analisys of kinetics of degradation of antioxidants was stored at presence of light and air. Samples for analysis of yield of extraction was analyzed immediately.

2.3 Analysis of extracts

Analysis of antioxidant activity was carried out by method, described in (Pakhomov, 2003).

Analysis of content of hypericin and GTC was carried out by HPLC methods on MiliChrom 02. For analysis of GTC was used method described in (Mizukami, 2007).

For analysis of hypericin was used 20 vv colum with sorbent ProntoSil C18. Elution was carried out in gradient mode water / acetonitrile. Detection was carried out by photometric detector on 230 nm. Ratio of elution 200 μl/min.

2.4 Mechanical activation

Material was treated in planetary ball mill AGO-2 with calculated intensity of treatment 5 - 15 W/g (Estimated acceleration of milling bodies 200-600 m/s^2) (Avvakumov, 2001) and attritor (Courtesy of Union Process, USA) with intensity 1 W/g. The ratio of material/milling bodies 1/20.

2.5 Analysis of crystallinity

Analysis of crystallinity was carried out by X-ray diffractometer Bruker D8 Advance with approximation by Segal method (Segal, 1962).

2.6 *Analysis of surface area and size of the particles*

Surface Area was measured by thermal desorption of nitrogen with approximation by BET on Sorbtometr (Russia).

Analysis of the size of the average weighted particles was made on Microsezer 201 (Russia) with approximation of the round particles.

2.7 *Microscopic studies*

Microscopic studies was made on SEM Hitachi TM-1000.

3. RESULTS AND DISCUSSION

3.1 Kinetics of degradation of GTC and Hyp in solution and solid phase

The extracts of green tea and St. John's wort ware stored and analyzed for the content of active components. The degradation of polyphenols is a multi-step process that involves several parallel reactions and diffusion processes, but the kinetic data are well described by pseudo-first order equation. The corresponding constants (1/s) are given in Table 1.

Table 1. The effective constant (1/s) of degradation of GTC and Hyp in water extract and solid phase (plant material).

.	ECG	EGCG	ECG	Hyp
Water extract	$8.7 \pm 0.7 * 10^{-5}$	$10 \pm 1.5 * 10^{-5}$	$8.0 \pm 0.9 * 10^{-5}$	$2.3 \pm 0.7\ 10^{-5}$
Solid phase	$3.5 \pm 0.3 * 10^{-8}$	$3.0 \pm 0.2 * 10^{-8}$	$4.3 \pm 0.6 * 10^{-8}$	$1.2 \pm 0.4 * 10^{-8}$

These data show that in the solid phase green tea catechins and hypericin is 500 times more stable than in solution. These experiment shows that it is desirable to process technological operation will be carried out in the solid phase.

3.2 Stability of antioxidants under mechanical action

Many organic compounds, especially with the complex structure, are unstable under the intence mechanical loading. The purpose of the experiment was to determine the treatment conditions in which degradation of polyphenolic compounds does not occur or is minimal.

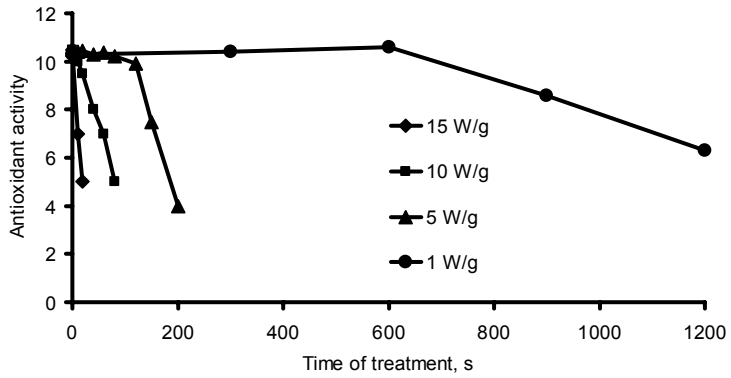


Fig. 4. Antioxidants activity of plant material depends on time and intensity of mechanical treatment.

The Fig. 4 show that the antioxidants in the matrix of plant materials are unstable at high intensities of mechanical treatment (15 – 10 W/g) and begins to decompose almost immediately. Practical interest is the behavior of the sample at an intensity of treatment 5 W/g and 1 W/g. Under these conditions, the content of polyphenols remains constant until the threshold time of 120 and 600 seconds respectively, then begins to decompose. The samples treated 150 s with intensity 5 W/g and 600 s with intensity 1 W/g has the equal energy supply. The differences in products described below.

3.3 Changes in cryctallynity structure and morphology of green tea

The reasons for the threshold behavior of the efficiency of chemical degradation under mechanical action could be associated with a change in the crystal structure of the processed materials (Boldyrev, 2006). The crystallinity of cellulose matrix was investigated by common Segal's method.

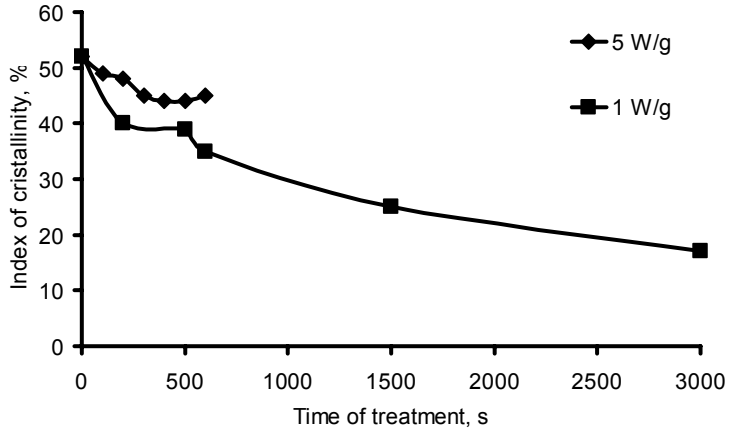


Fig. 5 Index of crystallinity of matrix of green tea depends of time and intensity of mechanical treatment.

In both types of treatment, first there is an intensive decrease in the crystallinity of the cellulose domain in matrix. This indicates that there is a significant plastic deformation. When the plastic deformation of polymers occurs there is an intense point heat generation. Therefore, if the deformation is sufficiently effective, then green tea catechins are destroyed. If the plastic deformation goes slowly, the heat generated during deformation of polymers has time to dissipate.

This phenomena was confirmed by microscopic studies. The original plant material consists of two types of particles: large porous particles (Fig. 6 a, b) and long flat hairs particles (Fig. 6 c). The product, in both cases are isotropic particles (Fig. 6 d, e). Morphological characteristics of initial material are not saved. There are visible cracks, resembling a particle.

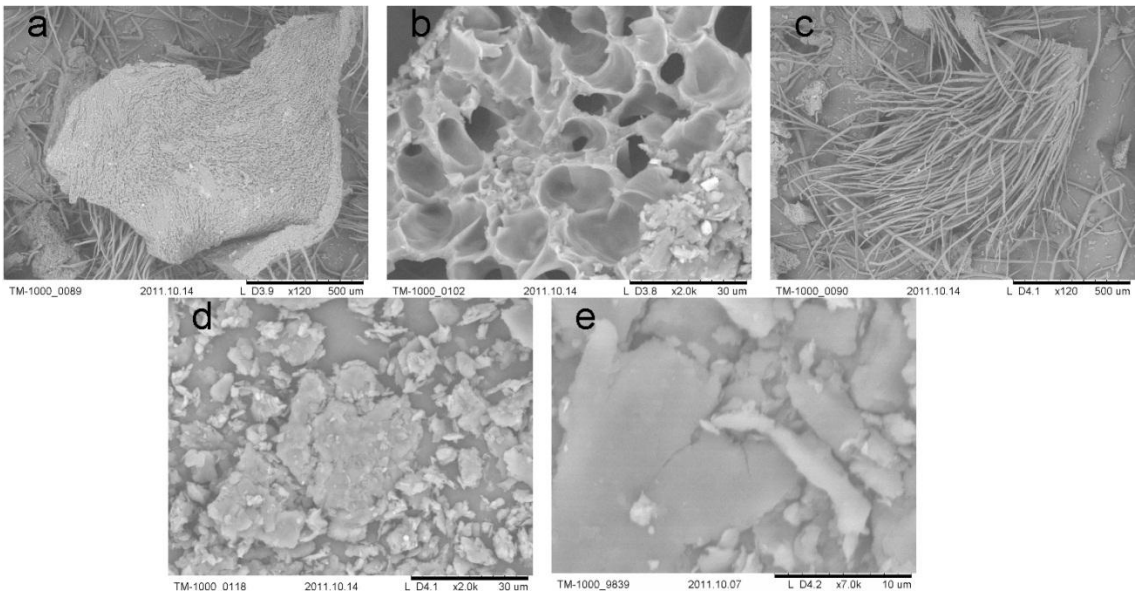


Fig. 6. Microscopic photo of green tea. a, b, c – initial material; d, e – product of mechanochemical treatment.

The initial material has a highly porous surface. The studies of the changing of size of average weighted particles and surface area showed that The size of average weighted particle reduced from 500 to 50 μm. After the mechanical treatment with intensity of 5 W/g there is increasing of surface area from 1.8 to 2.4 m^2/g. However, when material was activated with an intensity of 1 W/g surface area remains unchanged. This suggests that in addition to reducing the size of particles there is collapsing of pores of plant material occurs.

3.4 Changes in crystallinity structure and morphology of St. John' wort

There is no significant change is in the crystallinity index of raw materials under mechanochemical treatment with an intensity of 5 W/g. But when the intensity of treatment was 1 W/g there is the fall of index of crystallinity (Fig. 9).

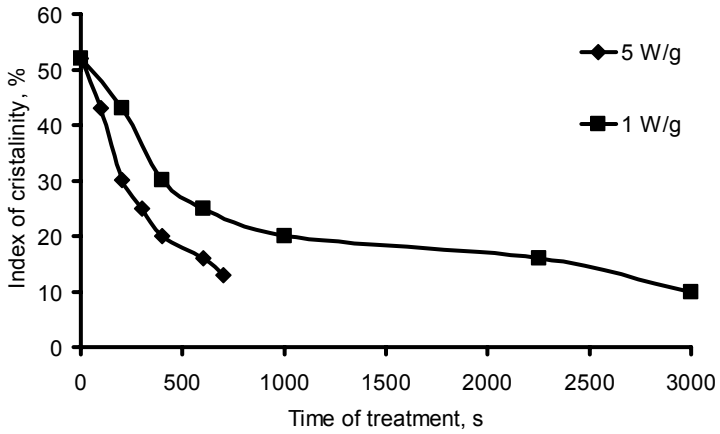


Fig. 7. Index of crystallinity of matrix of green tea depends of time and intensity of mechanical treatment.

The size of the average weighted particle in both cases is very close 150 μm. But the main difference that when you make high energy treatment the crystallinity of the material does not change. It is mean, that there is only brittle failure of material accrue. If you make low energy treatment, the index of crystallinity decrease in 3 times. It is mean, that there is the plastic deformation of matrix and ducktail failure occur.

The microscopic studies confirm this. Initial material consist of two type of particles (Fig. 8 a, b).

After mechanochemical treatment with intensity 5 W/g all morphological features of particles remains the same (Fig. 8 c,d). The size of the average weighted particle reduced to 150 μm, the particles remains anisotropic.

After the treatment with intensity 1 W/g can be observed only one species (Fig. 8 e). These particles do not have the morphological characteristics of raw materials, are strongly deformed and isotropic. This indicates that the plastic deformation of the matrix of plant material occur only under conditions of low-intensity treatment.

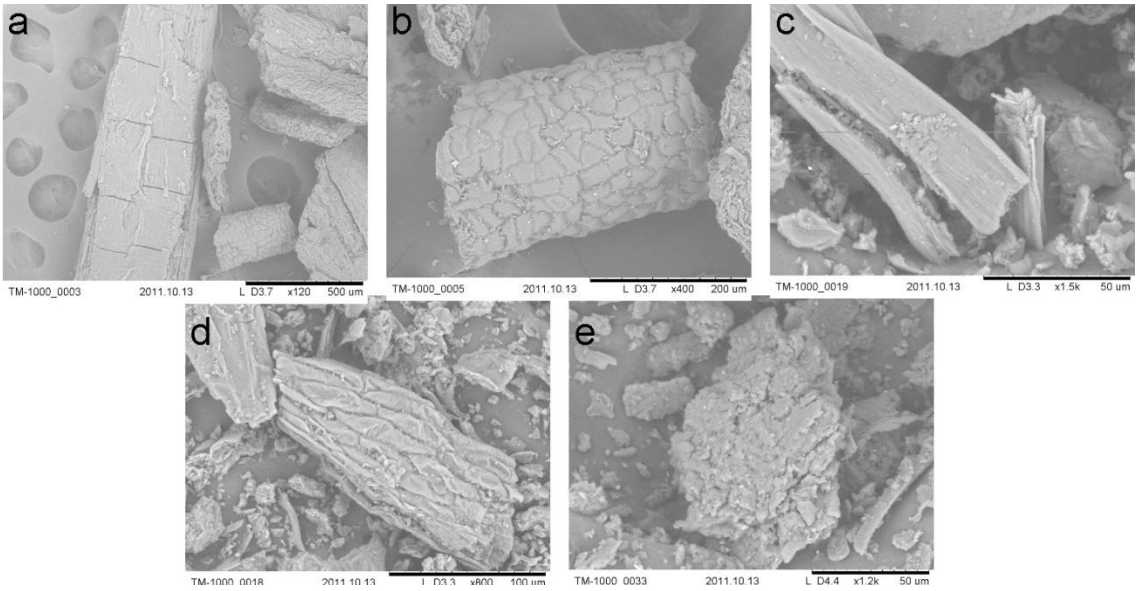


Fig. 8 Microscopic photo of St. John's wort. a, b – initial material; c, d, – product of mechanochemical treatment with intensity 5 W/g; e - . product of mechanochemical treatment with intensity 1 W/g.

3.5 Effect of mechanochemical treatment on the yield and stability of antioxidants

The mechanical activation of material with additives was made. The additives do not change mechanisms of activation but can strongly vary the yield of active components. Extraction of products of mehanochemicaly activated green tea showed that activation with alkaline additives leads to a increasing of catechins yield. Presumably because of their rapid degradation during extraction. Activation with ascorbic acid increase yield of catechins on 30% (Fig. 9).

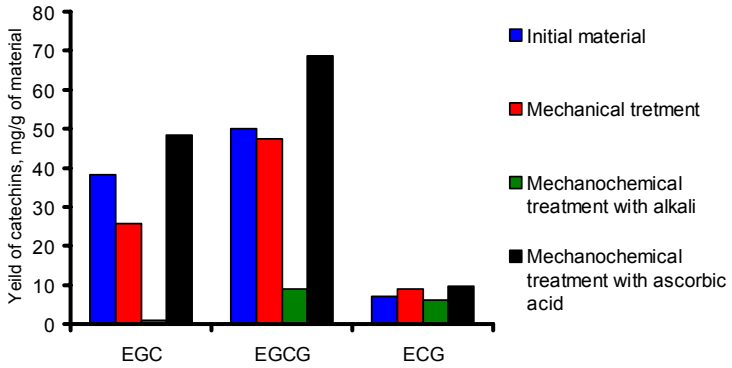


Fig. 9 The Yield of GTC depends of additives on mechanochemical treatment.

The product treated with an intensity of 1 W/g has high storage stability in the solid phase (Table 2). Most likely this is due to the formation mechanocomposites during plastic deformation of the matrix of plant material and ascorbic acid. First, a reduction of the porosity of the product complicates

diffusion of air oxygen in the matrix of plant material. Second, a uniformly distributed ascorbic acid enters into a competing reaction with oxygen, preventing oxidized catechins.

Table 2. Constant of degradation of catechins (1/s) in solid product of mechanochemical treatment.

	EGC	EGCG	ECG
Usual mixture	$3.4 \pm 0.4 * 10^{-8}$	$3.1 \pm 0.3 * 10^{-8}$	$4.7 \pm 0.5 * 10^{-8}$
Treatment 5 W/g	$3.7 \pm 0.4 * 10^{-8}$	$3.0 \pm 0.3 * 10^{-8}$	$5.1 \pm 0.5 * 10^{-8}$
Treatment 1 W/g	$1.3 \pm 0.2 * 10^{-8}$	$1.4 \pm 0.1 * 10^{-8}$	$4.2 \pm 0.5 * 10^{-8}$

Extraction of mechanochemicaly activated St. John's wort showed significant increase in the yield of hypericin (Fig. 10). The product of mechanochemical activation at intensity of 5 W/g shows the increase in the yield of 6. As seen in columns for initial material and mixture of material with alkali this effect is not easily explained in the introduction of alkali. In this instance, the mechanocomposite is formed. The particles of alkali in this mechanocomposite are distributed in a matrix of plant material. When water is added, a rapid dissolution of the alkali in the immediate vicinity of Hyp, fast neutralization reaction and the subsequent diffusion of the product matrix occur.

In the case of mechanical activation with the intensity of 1 W/g yield increased in 12 times. In addition to formation of mechanocomposite in this case there is also a disorder of the matrix of plant material that reveals the diffusion difficulties. Since the mass transfer, secured by plastic deformation, is an imperative condition for the occurrence of solid-phase reaction, it is possible neutralization of hypericin in the solid phase during the mechanical activation.

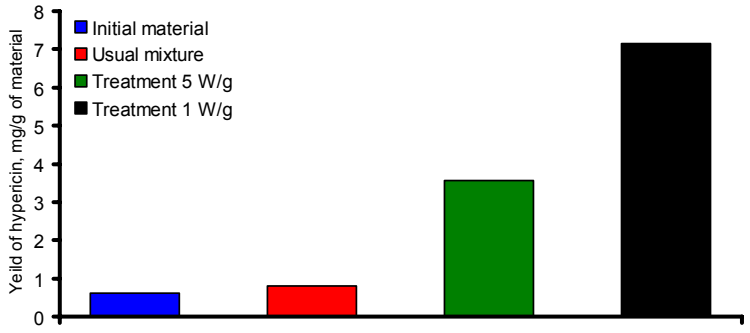


Fig. 10. The Yield of hypericin depends of additives on mechanochemical treatment.

4. CONCLUSIONS

Mechanical activation of plant material is a very affective method for enhancing of extraction processes. This method has particularly importance in case of very reactive antioxidant polyphenols because the GTC and Hyp in solid phase in more stability in 2.7 orders than in water. Mechanical

activation results in several effects. The first is the reduction of the size of the particles, increasing of the surface area and collapsing of the pores. The second of plastic deformation of the cellulose matrix of the material, disordering of the cell wall and decreasing of the diffusion complicates. The third is the formation of mechanocomposite and solid state reaction that leads to more stable product with increased yield of antioxidants.

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