Research in the field of processing plant materials, and obtaining new materials, biologically active substances and medicines

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Abstract. Complex processing of renewable plant materials is becoming the most relevant in modern socio-economic conditions. In this regard, it seems appropriate to single out the problem of the development and production of biologically active food products of rehabilitation and medical and preventive purposes from renewable plant materials and waste from their industrial processing as a priority area of innovation. The growing interest in wastes from processing plant materials is due to the fact that potential volumes, for example, sawdust, grape, apple, beetroot, and other squeezes allow them to be classified as industrial raw materials. The complete extraction of all valuable components from it is one of the ways to increase the profitability of the agricultural industry by expanding the range and volume of products. The environmental aspect is also important. Waste volumes pose a serious threat to the environment and require the development of effective ways of their disposal. This paper presents an original non-waste, energy-saving technology for the integrated processing of plant materials, in particular waste of larch wood, obtaining new structural materials and biologically active substances on its basis. The work considers the latest data, mainly by the authors, on the structure and chemical properties of dihydroquercetin (taxifolin) as one of the representatives of plant flavonoids (FL). The latter are excellent means not only for the prevention and maintenance of health, but also for the treatment of many diseases.

1. Introduction

The solution of the environmental problems of the agro-industrial complex is the creation of waste-free, resource- and energy-saving technologies for processing waste from renewable biological raw materials, the isolation and identification of extraction products, and the determination of their physical and chemical properties. Conducting basic research on the study of their biological activity, obtaining on their basis new modified derivatives with higher rates, for use as biological active food additives and drugs.

The proposed original technology using a universal mechanoacoustic rotary pulsation apparatus (RPA) of Russian production (Fig. 1) involves reducing energy consumption and metal consumption of equipment, significantly reducing the time of technological processes of grinding and extraction by convergent combination.
RPA is of industrial importance [1]. Linear productivity on water of 20 m³/hour; heating capacity 27 Mcal/hour; instant power when dispersing wood with a particle size <3 mm, 29 kW; particle size of dispersed wood <0.5 μm. For several years, the device has established itself in the production of various products and, especially, food. The device can simultaneously perform the functions of a homogenizer, dispersant, wet colloidal mill, heater, centrifugal pump and pasteurizer. Due to the simultaneous partial pasteurization of the product, it is possible to save up to 30 % of thermal energy. In processed juices with pulp, the sedimentation coefficient of suspended particles is several times lower than when using other grades of industrial homogenizers, which simultaneously with improved organoleptics dramatically improves the consumer properties of the products.

The mechanoacoustic method allows a high-quality and whole processing of virtually any fruit that does not contain hard bones: apples, pears, tomatoes, tangerines, grapes, etc.; while there is no production waste. There is no need for additional equipment to remove or grind the peel, membranous chamber and seeds.

![Figure 1. Rotary pulsation apparatus: And – a general view, – a rotor](image)

2. Creation of a new generation of complex green fertilizer based on humate and waste from the wine industry

Currently, using the technology of alkaline extraction, many types of liquid humic preparations are produced. A common disadvantage of this method of obtaining humic preparations is that in the finished product the content of physiologically active substances is in low concentrations, the preparations are characterized by high alkalinity and are less active in relation to plants in comparison with native humates. Of interest is the creation of a new generation of complex green fertilizer based on humate and waste from the wine industry by improving the process of obtaining a liquid humic preparation and enhancing its physiological activity by introducing a complex of biologically active substances of grapes.

It is known that grape squeeze contains a number of vitamins, macro- and microelements, proteins, fatty acids, biologically active substances, for example, anthocyanins. However, the isolation and transfer into the liquid phase of this valuable complex of substances from this type of raw material and its practical use is complicated by the very strong fibrous structure of the seed shells of grape seed, which make up 60–62 % of the mass of dry squeezed. At the same time, the temperature regime for processing grape marc must be gentle to avoid the loss of biologically active substances [2–5].

To obtain a liquid preparation that stimulates the growth and development of crops, we used vermicompost and grape marc. The initial vermicompost with a particle size not exceeding 3 mm and a moisture content of 55–57 % was first treated in RPA with water buffered with ammonia or potassium hydroxide to a pH in the range of 9.5-10.9, with a mass ratio of vermicompost: water equal to 1: 3–4, and a temperature of 55–60 °C, for 2–3 minutes. Then, to the resulting pulp, grape squeezes with a particle size not exceeding 20 mm, a moisture content of 6–9 % were loaded into the rotary pulsation apparatus with a mass ratio “grape squeezes: vermicompost” equal to 1: 6–9, and they were jointly disintegrated at a temperature 55–60 °C until the particles reach a solid phase of size 5–10 microns and a pH in the range of 7.2–7.5. The resulting dispersion was cooled and poured into the
container as the target product. Joint disintegration is carried out for 2–5 minutes. The technical result is to obtain a more uniform dispersion in the mode of self-heating of the pulp with its simultaneous pasteurization, as well as expanding the spectrum of biogenic components that make up the target product. The proposed method allows to obtain a dispersion of high-quality vermicompost due to the hard grape seed contained in the squeezes, which plays the role of a micronizer in the process, is accompanied by the simultaneous release of grape oil from the seed, which leads to a decrease in the surface tension of the dispersion medium and their more complete extraction. [6, 7].

3. Recycling of logging waste

One of the most important types of renewable natural resources characteristic of our country is forest. The products, logging, primarily include the stem parts of the tree, the use of which is well known. In addition, there are: needles, bark, branches, comli and other components. All of them are included in the processing only occasionally, using semi-artisanal methods. Mostly they go to the dump, creating environmental problems for the environment. Meanwhile, the listed substances are “stores” of valuable compounds of various nature, often having a complex structure: mono-, oligo- and polysaccharides, various phenols and flavonoids, terpenes and terpenoids, alkaloids, porphyrins, wax, etc. It should be noted that the isolation of such products in their pure form is a non-trivial task.

Among conifers, larch is of particular interest. At the end of the 50s of the last century, it was found that the butt of the larch contains the most valuable flavonoid – dihydroquercetin [8] and other useful products (resins, arabinogalactan, cellulose), which are currently finding wider application in the national economy [9].

An important scientific and practical task was the development of technological principles and methods for the complete processing of larch wood waste into practically important products [10].

To accomplish this task, the solid structure of larch wood was disintegrated using RPA, the separation of the obtained cavitated mass, the extraction of biologically active substances [11], the implementation of their chemical modifications in the original drugs.

4. Natural Flavonoid – Dihydroquercetin

One of the current trends in the development of the pharmaceutical industry is the intensification of the use of a unique biologically active complex of substances contained in green plants.

In the 60s, it was found that Daurian larch contains up to 4 % of the strongest biologically active antioxidant dihydroquercetin (DHQ) [12]. The chemical and technological solutions developed and proposed by us made it possible to reduce energy consumption by several times, increase the yield percentage and the purity of DHQ [13, 14]. The molecular structure of DHQ is shown in Fig. 2.

![Figure 2: a – The structure of the DHQ molecule, b – packing of structural units of DHQ in the crystal](image-url)
The heterocyclic ring of dihydroquercetin has the conformation of an asymmetric half-chair with a flat system of four atoms O-1, C-9, C-4, C-3. In an independent molecule, an intramolecular hydrogen bond is realized between the oxygen atom of the carbonyl group and the hydrogen atom of the hydroxyl group in the fifth position. The dihydroquercetin molecules in the crystal are combined with each other and with hydrated water molecules by intermolecular hydrogen bonds into a complex structural motif (Fig. 2). In dihydroquercetin, the torsion angle around the C (2) -C (3) bond is 68º.

In addition, we determined the activity of hydroxyl groups for dihydroquercetin. They change in the following sequence: 7> 4 3> 5> 3. These data are of great importance for the chemical modification of DHQ, including the preparation of its water-soluble form.

4.1. Water soluble dihydroquercetin

Having unique biological properties, DHQ has one drawback – poor solubility in water at normal temperature, which prevents its absorption by the body. We have developed a method for producing previously unknown DHQ adducts and synthesized complexes for incorporating DHQ into α- and β-cyclodextrins. Scheme for preparing a complex of DHQ with β-cyclodextrin:

\[ \text{DHQ} + 2 \beta\text{-cyclodextrin} \rightarrow \text{DHQ-β-cyclodextrin complex} \]

It was shown that the solubility in water at 20 °C of the isolated supramolecular compound DHQ: β-cyclodextrin (DHQ -CD) of 1: 1 composition compared to free DHQ (0.3 g/l) and free β-cyclodextrin (18.5 g/l) significantly increases and amounts 53.2 g/l [15].

X-ray diffraction analysis of a single-crystal inclusion complex confirms its composition DHQ: β-cyclodextrin in a ratio of 1: 1:11 (Fig. 3).

It is known that cyclodextrin is able to incorporate different molecules into a hydrophobic cavity with the formation of nanoparticles transporting the active molecule (Fig. 3). However, we have shown that in aqueous solutions at different concentrations and temperatures more complex states of the DHQ-CD complex arise. So, at 25 °C, two independent groups of nanoparticles are characteristic in a mass ratio of about 1: 1. The bimodal nature of the volume distribution of particle sizes changes to monomodal with increasing solution temperature to 40 °C (Fig. 4). It seems possible to control the composition and structure of such nanoscale entities, which, according to [16], apparently also react with biological objects. The DHQ-CD complex was studied at a diffusion rate comparable to that of free DHQ through a bilayer of standard bilayer egg lecithin liposomes, which is a good model of cell membranes. It has been established that “unincorporated” DHQ strongly binds to membranes and practically does not enter the cells. At the same time, the “on” taxifolin for several hours is dosed into the cells, that is, it is a drug of prolonged action.
The permeability of DHQ through biomembranes is determined by its content in the blood after oral administration to the stomach as a dietary supplement. It was proved that only the introduction of the DHQ-CD complex ensured the effective administration of DHQ into the bloodstream of rats. Therefore, the use of the complex DHQ-CD is more promising in the medical aspect than pure DHQ.

Figure 4. Volumetric distribution of particle sizes in an aqueous solution of the complex DHQ-CD

Figure 5. Change in the melting temperature of liposome lipids in the presence of DHQ and DHQ-CD: 1 – initial liposomes, 2 – liposomes + DHQ, 3- liposomes + DHQ-CD

Figure 5 shows curves characterizing the change in the melting temperature of liposome lipids in the presence of DHQ and the complex DHQ-CD. It is clearly seen that the complex is less prone to incorporation into the lipid membrane. This observation was confirmed by a comparative test of DHQ and the DHQ-CD complex in the direction of recording the degree of accumulation of these substances in rat liver (studies were carried out at the Institute of Cell Biophysics, RAS).

4.2. Water-soluble pharmaceutical composition L-arginine-dihydroquercetin (Arg-DHQ)

We obtained a molecular composition of dihydroquercetin with L-arginine, which has increased biological activity and higher water solubility [17]. The technical result is achieved by mechanical activation of a mixture of L-arginine and dihydroquercetin in a molar ratio of 1: 1–5 in an argon atmosphere to a dispersion of 1–3 microns.

It was found that the solubility of the molecular composition of Arg-DHQ in water compared with individual DHQ (0.03 % at 20 °C) and free arginine (15.0 % at 20 °C) significantly increases and reaches 64.5 % at 20 °C, that is, 11.3 % higher than the previously described complex DHQ-CD (53.2 %). It was found that the solubility of the molecular composition of Arg-DHQ in water compared with individual DHQ (0.03 % at 20 °C) and free arginine (15.0 % at 20 °C) significantly increases and reaches 64.5 % at 20 °C, that is, 11.3 % higher than the previously described complex DHQ-CD (53.2 %). The pronounced chemical shift of the signal from the seventh carbon atom of the resorcinol part of the molecule of the starting dihydroquercetin, amounting to 166.6 ppm, to a value of 177.8 ppm, observed on the 13C NMR spectrum (in D20), confirms the formation of the ionic system upon dissolution of the composition Arg-DHQ. (Fig. 6).

Figure 6. The structure of ARG-DHQ in the aquatic environment
During testing of Ap-DHQ, the results of its interaction with collagen and membrane lipids were analyzed. It is known that with age, due to the glycation process, a systematic decrease in the melting temperature of collagen occurs, which leads, in particular, to a decrease in the efficiency of receptors. At the same time, altered collagen fibers retain water worse and deform [18]. It was established that Ap-DHQ increases the melting temperature of collagen to a greater extent than free DHQ, and also interacts weaker with lipids of cell membranes than pure DHQ, therefore it penetrates the blood faster and effectively enters metabolic processes.

To evaluate the antioxidant activity of Arg-DHQ in comparison with DHQ, the ABTS test was used [19]. It has been established that the antioxidant activity of Arg-DHQ is 10–20 higher than that of pure DHQ. Therefore, the use of the Arg-DHQ molecular composition is more promising in the medical aspect than the use of DHQ alone.
4.3. The biological activity of phosphorylated derivatives of DHQ

It is known that phosphorylation of some natural compounds leads to an increase and expansion of the spectrum of their biological action, including the appearance of antiproliferative properties [20]. That is why, to obtain compounds with cytotoxic and antitumor activity, DHQ phosphorylation was used.

An analysis was made of the cytotoxic effect of a number of phosphorus-containing derivatives of DHA [21]: 7-diethylamidoethylthionphosphate, 7-tetraethyl diamidothionphosphate, 7-tetraethyl diamidoselenene phosphate, 7-dipiperidyl dithionphosphate, 7-dipiperidylselenose phosphate.

The object of the study was a line of human tumor cells – ovarian carcinoma CaO\textsubscript{V}. Cell cultures were grown in RPMI 1640 medium containing 10 % human fetal serum. The cytotoxic activity of the drugs was evaluated by the ability of the compounds to inhibit the growth of tumor cells. The effect on the growth of tumor cells was evaluated using a standard MTT assay. A compound was considered active if a concentration of 100 μM caused inhibition of cell growth of at least 50 %. The measurement error did not exceed 5 %. The antitumor activity was evaluated in vivo on transplanted tumors of first-generation BDF hybrid mice weighing 18–25 g. The antitumor effect was evaluated by inhibition of tumor growth (ITG) and by the increase in the life expectancy of treated animals (LET). The minimum criteria for ITG activity are \( \geq 50 \% \), LET \( \geq 25 \% \).

From the results presented in table. 1, it follows that dihydroquercetin 7-diethylamidoethylthionphosphate at a concentration of 100 μM inhibited the growth of CaO\textsubscript{V} cell lines by 83.9 %. In addition, the compound showed antimetastatic action.

| Compound | Inhibition of cell proliferation | \( I_{50}, \mu M \) |
|----------|---------------------------------|--------------------|
| DHQ      | 0                               |                    |
| DHQ 7-diethylamidoethylthionphosphate | 83.9 | 50 |
| DHQ 7-tetraethyl diamidothionphosphate | 85.3 | 50 |
| DHQ 7-tetraethyl diamidoselenene phosphate | 79.2 | 60 |
| DHQ 7 dipereditionfosfat | 76.3 | 65 |
| DHQ 7-Dipeperidyl Selenone Phosphate | 77.0 | 70 |

4.4. The group of peracil derivatives of dihydroquercetin

We have obtained and carried out biomedical studies of new aromatic peracil derivatives of dihydroquercetin. The pentaacetylsalicylate (I), penta-n-nitrobenzoate (II), and dihydroquercetin pentanicotinate (III) were synthesized and studied [22]. Tests conducted at the Russian Military Medical Academy prove that these drugs have anti-inflammatory effects.

5. Conclusion

In the framework of solving environmental problems for the disposal of industrial waste processing of biological raw materials, a rotary pulsation apparatus (RPA) was developed and introduced as a laboratory, pilot-industrial installation. The use of RPA can significantly intensify the technological processes associated with lowering the metal consumption of equipment, reducing the time of operations and the conversion of batch processes to continuous ones. Methods have been developed for the production of a new generation of complex green fertilizer based on humate and waste from the wine industry, the isolation of biologically active substances of plant origin, their identification and research by various physicochemical methods. Methods have been found for preparing water-soluble forms of dihydroquercetin by guest-host interaction, with the formation of a complex for the incorporation of DHA into β-cyclodextrin and L-arginine. As a result of the chemical modification of DHA and the preparation of derivatives based on it, the spectrum of the biological action of this unique flavonoid is significantly expanded. It is shown that the use of soluble forms of DHA and its derivatives is more promising in the medical aspect than pure DHA.
Acknowledgments
This work was supported by the Russian Foundation for Basic Research (RFBR), grant No. 18-03-00466

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