The resource-saving technology of anthocyanins extraction by the method of low-frequency vibration impact

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Abstract. The development and use of a practical method for producing antioxidants (less energy-consuming and more economically viable) is relevant and contributes to the expansion of the market of “functional” products and the receipt of new dietary supplements. An experimental model of a laboratory low-frequency vibration extractor was developed to intensify the technological process of extraction from plant material containing ALS. Some technological parameters of the anthocyanins extraction process have been determined: raw material / extractant ratio, % extractant concentration, as well as some design parameters of a pilot plant (vibration extractor). The possibility of obtaining anthocyanins from the waste of the fruit and berry industry with a high percentage yield of biologically active substances has been experimentally shown.

1. Introduction

One of the priority areas of economic development, which assumes an unprecedentedly important status, is a new direction in which the roadmap for 2025 is being developed. “Foodnet”, where the new paradigm of a healthy diet is, the main emphasis is on the positive aspects of the diet. Functional food products of a selective orientation help reduce the risk of the occurrence and prevention of acute and chronic diseases [1, 2]. Secondary metabolites of fruits and vegetables reduce the processes resulting from oxidative stress. “... Oxidative stress, which releases oxygen free radicals in the body, is the cause of many diseases: cardiovascular, cancer, rheumatism, cataracts and many other autoimmune diseases besides aging. Phytochemicals act as antioxidants, remove free radicals, and act as “cell savers” ...” [1, 2, 3, 4].

Numerous large-scale studies have proven the protective role of fruits and vegetables against cancer of the pancreas, bladder, and breast cancer [5]. Synthetic antioxidants: butyloxytoluene and butoxyanisole, along with the antioxidant effect, exhibit toxic and teratogenic properties. Biologically active compounds of various groups of compounds of fruits and vegetables exhibit the properties of snowing effects of singlet oxygen, these include compounds such as dietary fiber, catechins, polyphenols, chlorophyllin, sesaminol, soy proteins, glutathione, galate, epigalatecatechol, flavonoids, isoflavanones, vitamins A, B, C, E, conjugated isomers of linoleic acid, D-limonene, calcium, selenium, uric acid, indoles, thiocyanates and protease inhibitors. These substances can act independently or in combination as anticancer or cardioprotective components with a variety of mechanisms [5].
Anti-nutritional compounds, which include tannins, belong to the group of polyphenols and exhibit high antioxidant activity in comparison with ascorbic acid in fruits. In this case, polyphenols and phenolic substances are present in low concentrations relative to the substrate, while the final radicals formed during the neutralization process are quite stable, which allows them to exhibit antioxidant activity, acting as reducing components, hydrogen donors and singlet oxygen quenchers.

The vast majority of works devoted to the antioxidant potential of fruits are limited to grapes, berries and juices. Grapes and wine contain a large amount of polyphenols, including catechin, anthocyanins, flavan-3-ol, malvidin-3-glucoside, tartaric acid and caffeic acid esters. Grape juice showed the highest antioxidant activity, somewhat lower - samples of grapefruit juice, orange and apple [1–7].

The development and use of a practical method for producing antioxidants (less energy-consuming and more cost-effective) is relevant. This will lead to the expansion of the market of “functional” products and the receipt of new dietary supplements [3, 4, 8, 9, 10].

2. Main part

2.1. Materials and methods

The widespread use of biologically active additives (BAA) currently prompts the study of both new sources of biologically active substances (BAS) and high-tech methods for their preparation. It is well known that the most environmentally friendly, bioavailable, demanded by consumers and a relatively cheap source of biologically active substances that could be used for the production of dietary supplements is plant material. At the same time, the methods known in Russia and abroad for producing such substances are cumbersome in the performance of the plants, as well as a high degree of cost. In addition, the high-temperature regime used, as a rule, reduces the content of biologically active substances in the extract [1, 2, 3, 4, 7].

In recent years, a number of developments have appeared to intensify the extraction method by applying some physical and mechanical methods of exposure that optimize the extraction process.

Of greatest relevance at present is the production of antioxidants (antioxidants), which include flavonoids, the group of which includes anthocyanins (table 1).

| Flavonoid class | Representatives                                      |
|-----------------|------------------------------------------------------|
| Flavonols       | Quercetin, campferol, myricetin, fisetin             |
| Flavones        | Luteolin, apigenin                                    |
| Flavanols (flavan-3-ol) | Catechins, proanthocyanidins (catechin polymers) |
| Flavanones      | Hesperidin, Naringin, Eriodiktio                      |
| Anthocyanins    | Pelargonidin, cyanidin, peonidine, delphinidin, petunidin, malvidin |
| Flavononols     | Taxifolin                                             |

Anthocyanins are pigments dissolved in the vacuolar juice of the epidermal tissues of flowers and fruits, to which they give a variety of colors – pink, red, blue or purple, in addition, they, in accordance with the acid-base factor, can have not only a colored form, but and colorless.

Anthocyanins are found in red wine, some cereals, as well as in leafy vegetables and root vegetables (eggplant, cabbage, beans, onions, radish), but most accumulate in fruits and berries.

The spectrum of their action on humans is extremely wide. Especially it should be noted antiradiation, anti-inflammatory, antimicrobial, capillary-strengthening, antihypertensive, anti-allergic and general strengthening effect.

Of greatest relevance is currently the methods for producing antioxidants of antioxidants, which include flavonoids, to the group of which anthocyanins belong. Moreover, the level of consumption of
flavonoids is 23 mg / day. Anthocyanins are widely represented in red berries and fruits - such as cherries, strawberries, raspberries, black currants, blueberries. It is this class of flavonoids that gives fruits, berries, leaves and flowers the color of a wide variety of shades - from pink to black-violet. Blackcurrant berries, due to their rich chemical composition, are widely used both in food (jam, jams, juice) and in the pharmaceutical industry (gels, dietary supplements). They contain 5-6% soluble sugars; 2-4% organic acids; 0.6% of essential oils. The vitamin composition of blackcurrant is also unique: up to 400 mg% of vitamin C; 0.1 mg% β-carotene; vitamin P - 0.72 mg%. In addition, they contain B vitamins, biotin.

Blackcurrant is rich in macro- and microelements, such as potassium, calcium, magnesium, sodium, sulfur, phosphorus, boron, iodine, manganese, molybdenum, zinc.

In currant berries, the content of anthocyanins is 250-500 mcg / kg.

The quantitative content of anthocyanins is presented in table 2.

| R1 | R2 | aglycon |
|----|----|---------|
| H  | H  | pelargonidine |
| OH | H  | cyanidin |
| OMe| H  | peonanidin |
| OH | OH | dolphidin |
| OMe| OMe| petunidine |
| OMe| OMe| malvidin |

Table 2. The quantitative content of anthocyanins.

![General anthocyanin formula](image1)

**Figure 1.** General anthocyanin formula (Note: R3, R4 - H or glycoside).

The following anthocyanin glycosides were isolated from blackcurrant fruits: dolphidin-3-rutinoside, cyanidin-3-rutinoside, dolphidin-3-glucoside, cyanidin-3-glucoside, peonidine-3-rutinoside, malvidin-3-rutinoside, malvidin-3, myricetin-3-rutinoside, myricetin-3-glucoside, quercetin-3-rutinoside (figure 2).
**Figure 2.** Structural formulas of some anthocyanins.

**Table 3.** The content of some representatives of the classes of anthocyanins in blackcurrant berries (mg / 100 g fresh weight).

| Food Raw               | Anthocyanins         |
|------------------------|----------------------|
|                        | cyanidin             |
| Black currant (Ribes nigrum L.) | dolphinidin         |
|                        | pelargonidine        |
|                        | peonidine            |
|                        | petunidine           |
|                        | 53.36 –              |
|                        | 69.77 –              |
|                        | 0.79 –               |
|                        | 0.26 –               |
|                        | 0.07 –               |
|                        | 149.40               |
|                        | 272.81               |
|                        | 1.39                 |
|                        | 1.09                 |
|                        | 12.30                |

2.2. **Results and discussions**

An experimental model of a laboratory low-frequency vibration extractor was developed to intensify the technological process of extraction from plant materials containing ALS [8, 9, 10].

The goal is to reduce the cost of the obtained BAS extracts by intensifying the extraction process and increasing the yield of substances, i.e. reducing raw material costs, increasing productivity.
In recent years, a number of developments have appeared to intensify the extraction method by applying some physical and mechanical methods of exposure that optimize the hydrolysis-extraction process.

We conducted a patent search, found 2 patents with similar, but different in design extractors.

The developed experimental model of a laboratory low-frequency vibration extractor for intensification of the extraction technological process differs in the configuration of the mixing blade (a truncated prism with perforated holes - 1 cm in diameter) with the possibility of controlling the frequencies of vibration exposure (1 - 20 Hz; 2 - 50 Hz) (figure 3, figure 4).

The laboratory setup consists of 1 - electromagnetic vibration drive; 2 - extraction chamber; 3 - acceleration sensors in 2 modes; Thermostat working blades.

Figure 3. Experimental plant for extracting fruit and berry raw materials.

Figure 4. Extractor’s blade.

The scheme of the experiment and its comparative analysis of the output with the traditional:

The experiment was carried out using waste from the canning industry.

We offer an alternative to the extraction method described by Belgorod State University, according to which anthocyanins are obtained from pulp of black currant according to the following scheme.

A portion of pulp (50 g) was infused in 100 ml of extractant in 70% ethanol solution with 1% HCl solution, the mixture was kept for 3 days at room temperature with periodic stirring. The disadvantage is that with an increase in the extraction time, there is a release of ballast substances from which it is difficult to be released during pericrystallization.

Currently, dietary supplements containing alcohol extract from blueberry and blackcurrant waste cost from 90 - 125 rubles and more for a volume of 25 ml, which despite high demand is not a cheap dietary supplement, given the volume.

The main processes of the production technology that determine the production efficiency and quality of the target product is the hydrolysis and extraction of flavonoids.

The analysis showed that the best economic effect in this area is achieved using non-waste technologies, i.e. with a closed cycle, including the processing of waste that remains in the production process of canned fruit and berry raw materials.

We propose the use of polyharmonic low-frequency exposure, which allows to increase the efficiency of the process.
The main stages of the hydrolysis-extraction process are:
1) diffusion of the hydrolyzing agent (hydrogen ion) into the feed;
2) hydrolysis;
3) diffusion of anthocyanin molecules to the interface;
4) mass transfer at the interface;
5) diffusion of molecules of anthocyanins of substances in the extractant from the interface in the volume of the solution.

The limiting phase of the total process of hydrolysis-extraction are stages 3, 4, 5.

When the vibration effect is applied, an intensive renewal of the interphase surface is created due to the active hydrodynamic regime, while the concentration of extractable substances on the surface of the solid phase and in the liquid layers adjacent to the particles of the raw material decreases sharply, which accelerates the middle phase of the process (steps 3-5).

The amount of reagents used can be significantly reduced, since with the appropriate choice of hydromodules for each operation, the vibration effect practically does not affect the rate of hydrolysis, but due to the reciprocating movement of the nozzle blades, the extraction process is greatly accelerated, while in the mixture the phenomenon of vibro-jet effect occurs, which creates intensive mixing of the liquid, which significantly intensifies the extraction process. At the same time, the yield increased 1.5 times (figure 6, figure 7) and the amount of ballast substances decreased significantly.

Extraction was carried out at various ratios of raw materials / extractant.

Extraction process time reduced from 3 days to 1.5 hours; the output increased 1.5 times and the decrease in the amount of ballast substances.

We used ethanol 55 and 60%, at an extraction temperature of 60 ° C, used fresh raw materials to prevent losses during drying. All this helps to reduce the cost of the obtained extracts. The highest yield was achieved with a hydraulic module of 1: 10 (figure 5).

![Figure 5. The output of anthocyanins in% depending on the ratio (raw material / extractant).](image-url)
Figure 6. Comparative analysis of the yield of anthocyanins using the low-frequency vibration extractor and the traditional extraction process.

Table 4. Anthocyanin composition of squeezed blackcurrant R. nigrum obtained using the low-frequency vibration extractor and the traditional extraction process.

| Sort         | Total content of anthocyanins mg / 100g (production method - maceration, exposure – 3 days) | Total content of anthocyanins mg / 100g, the experimental method – (solution for extraction: 1% HCl in 55-60% ethanol, exposure –3 series for 30 min) with an additional low-frequency vibration exposure | Total content of anthocyanins mg / 100g in fresh peel | Total content of anthocyanins mg / 100g in dry peel | Percent age loss during drying |
|--------------|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|-------------------------------------------------|--------------------------------|
| Datchnitsa   | 43±5                                                                                     | 66.7                                                                                                                                                    | 1.59                                              | 5.16                                            | 16 %                                        |
| Black Pearl  | 55 ± 9                                                                                   | 74.8                                                                                                                                                    | 3.189                                             | 7.898                                           | 19.7 %                                      |

The degree of extraction (DE,%) of anthocyanins was calculated by the equation:

\[ DE = \left( \frac{A}{B} \right) \cdot 100, \]  

where A and B are the content of substances determined respectively in the extract and weighed portion of the plant material.

Qualitative determination of anthocyanins was carried out by the method of ascending paper and thin layer chromatography (TLC), in different solvent systems (25% H3PO4 - phosphoric acid - H2O - HCOOH; HCOOH - H2O - HCl in ratios 5: 2: 3; 45: 1: 3 ), the developer is pairs of ammonia + ultraviolet (figure 7) (Ermakov, Arasimovich, 1987).
Figure 7. TLC of anthocyanins (1 - cyanidin (attestor); 2 - dolphinidin (attestor); 3 - sum of anthocyanins).
3. Conclusion

Obtaining an extract using this equipment allows you to reduce the cost of both extracts (BAA) and functional foods, increase the turnover of dietary supplements and functional foods, and contribute to the health of the population.

The production technology of the antioxidant extract does not limit the scope of the designed model of a low-frequency extractor.

Our proposed method allows us to reduce the time of the extraction process from 3 days to 1.5 hours; increase the yield by 1.5 times and reduce the amount of ballast substances. All this helps to reduce the cost of the obtained anthocyanins.

Conclusions:

The possibility of obtaining anthocyanins from a waste of the fruit and berry industry with a high % yield of biologically active substances has been experimentally shown.

Some technological parameters of the anthocyanins extraction process have been determined: raw material / extractant ratio, % extractant concentration, as well as some design parameters of a pilot plant (vibration extractor).

The range of products produced from blackcurrant can be expanded by processing wastes from the canning industry (blackcurrant squeezes in the production process remain up to 40% of anthocyanin-rich squeezes).

The use of integrated processing of blackcurrant will reduce the amount of waste and get biologically active substances (anthocyanins) that can be used:

- as antioxidants; like dyes;
- as anti-allergic components for the production of functional foods.

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