Development of a technology for obtaining hesperidin from citrus fruit processing waste

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Abstract. When processing citrus fruits into concentrated juice, enterprises generate waste in the form of peel, pit, and pulp. These wastes, especially the peel, are rich in useful biologically active substances. The most representative in terms of content and valuable from the point of view of use in medicine and the food industry, groups of biologically active substances of citrus fruits are flavonoids and carbohydrates. Flavanones are known for their multifunctional and antioxidant action. The dominant flavanone of citrus fruits, hesperidin, can be used as an active substance for the preparation in medicine and in medicinal food products. Research in the ways of using various valuable products from citrus fruit processing waste are presented in foreign scientific sources, while Russian scientists have paid insufficient attention to this urgent problem. This article substantiates and experimentally confirms the optimal method for obtaining hesperidin from citrus fruit processing waste.

1 Introduction

Natural products have always been used for the prevention and treatment of diseases. In the face of complex diseases, such as the COVID-19 pandemic, for which there were no effective treatments, nature could offer new therapeutic options. Review [1] experimentally found that natural metabolites from the flavonoid and terpenoid classes are promising active compounds in the maintenance treatment of COVID-19. Attention has been drawn to flavonoid-rich citrus peel waste and apple pomace as sources of natural metabolites against COVID-19. Since such wastes are mass produced all over the world, this makes them available for obtaining useful products from them and attractive for future researches.

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It has been established that citrus fruit presses contain (% of dry weight) up to 40 % mono- and disaccharides, 10.5 % pectin, 20 % fiber, about 10 % flavonoid compounds, 56 mg% vitamins [1-7]. In the food industry, only pectin concentrates from the peel of citrus fruits are widely used [3, 4, 5].

The flavanone glycoside, hesperidin, is found in all types of citrus fruits [2-6, 9-15]. Individually isolated, it has a wide range of pharmacological activity: choleretic, hepatoprotective, venotonic, antitumor, antiviral, antibacterial, and others [2, 5, 10, 11, 12]. Due to its multifunctionality, high safety, low cost, and wide availability, hesperidin is of great interest as an active component of useful products. Waste processing of fruits of citrus plants is a significant industrial source of its production [1, 3, 15].

The aim of this work was to find an optimal method for obtaining hesperidin flavanone from agro-industrial waste of citrus fruits in order to create therapeutic and prophylactic products on its basis.

2 Materials and methods

In experimental studies, dried industrial waste from the processing of citrus fruits (mandarin, orange and lemon) was used.

Raw materials were treated with chemically pure calcium oxide. Raw materials were extracted by the classical remaceration method. Purified water was used as the extractant.

Infrared spectra of hesperidin were recorded on a Specord 71 UR spectrophotometer in the range 600-3600 cm^{-1} in thin vaseline oil.

Measurements of proton magnetic resonance were carried out on the "TESLA BS-487" 100 (MHz), in a deuteropyridine solution.

3 Results and discussion

The most common is the method of obtaining hesperidin from dried and ground orange peel by sequential extraction with petroleum ether and methanol. The resulting methanol extract is evaporated until the consistency of syrup is reached, the residue is mixed with 50 ml of 6 % acetic acid. The resulting precipitate is crude hesperidin. It is filtered off, washed with 6 % acetic acid, and dried at 60 °C to constant weight. Recrystallization of crude hesperidin is carried out from a 5 % dimethyl sulfoxide solution by heating it to 60-80 °C [14].

The second known method for obtaining hesperidin involves the treatment of raw materials with lime water (calcium hydroxide) followed by precipitation of flavanone [15]. This makes it possible to selectively extract flavanones, since the latter are characterized by decyclization in this medium with the formation of an easily soluble chalcone-calcium complex. The resulting anion easily interacts with divalent metal cations, for example, Ca^{2+}, with the formation of chelate complexes. The described method is more environmentally friendly and eliminates the use of organic solvents.

In both methods, the production of hesperidin involves the use of citrus fruit waste immediately after juice production, which means that such raw materials are characterized by a high moisture content [15]. This method is not always justified, since in large-scale production, raw materials must be preserved in order to avoid spoilage. Therefore, in our opinion, a technologically more convenient way to obtain hesperidin is the use of calcium oxide instead of lime-water and the use of dried waste of citrus fruits. Dry citrus fruits waste is thoroughly mixed with dry powder of calcium oxide. It is very important that waste can be stored in this form for a long time (up to 12 months), since calcium oxide in this case is a good preservative.

To determine the optimal raw material/calcium oxide ratio, a series of experiments was carried out, in which the amount of dry calcium oxide was varied and the “output” of hesperidin (the amount of the obtained substance expressed as 100 % of the mass of the raw material) was evaluated. The results are presented in Table 1.

Table 1. Dependence of the “output” of hesperidin at the ratio of raw materials: calcium oxide

Waste source	“Output” of technical hesperidin, % at the ratio of raw materials : calcium oxide				
	Ratio 10:0.1	Ratio 10:0.5	Ratio 10:1.0	Ratio 10:1.5	Ratio 10:2.0
tangerines	10,11±0,26	10,42±0,22	11,70±0,28	11,92±0,26	12,01±0,28
oranges	8,52±0,35	8,81±0,35	9,54±0,36	9,24±0,31	9,33±0,38
lemons	4,60±0,47	4,52±0,45	5,55±0,49	5,60±0,48	5,63±0,46

As can be seen from the data obtained, the most optimal ratio of raw materials/calcium oxide is 10:1. Based on this, all further studies were carried out with this ratio.

The next stage of the research was the study of the dependence of the “output” of technical hesperidin on the extraction time. Experimental data are presented in Table 2 (note: average values from 5 determinations and deviations from the arithmetic mean are given).

Table 2. The dependence of the "output" of hesperidin on the duration of extraction at a ratio of raw material-extractant 1:10

Waste source	Extraction duration, min				
	30	40	50	120	180
tangerines	10,16±0,21	11,71±0,24	11,70±0,25	11,63±0,26	11,64±0,22
oranges	8,32±0,32	9,52±0,33	9,47±0,34	9,52±0,35	9,44±0,35
lemons	4,81±0,41	5,63±0,42	5,62±0,46	5,57±0,49	5,65±0,42

An analysis of the experimental data obtained allows us to state that the maximum “output” of hesperidin is observed during extraction for 40 minutes. When studying the multiplicity of extraction, it was found that the highest "output" of technical hesperidin is observed with a three-fold extraction with water of the raw material treated with calcium oxide, in a ratio of 1:180. The “output” of hesperidin from citrus fruit processing waste can reach about 11.7%, provided that the extraction efficiency in this technology is 85 %. According to the proposed method, crude hesperidin (technical product) is obtained, which is then purified by recrystallization from a 6 % acetic acid solution. The obtained purified hesperidin was subjected to physical and physico-chemical research methods in order to confirm the authenticity, the absence of impurities and the preservation of the structure.

Purified hesperidin is a white crystalline precipitate with a melting point of 261-264 °C. The R_f value of the isolated substance is 0.48 (thin-layer chromatography in the system butanol-acetic acid-water (4:1:5).

In the infrared spectrum (Figure 1) there are absorption bands corresponding to stretching vibrations: aromatic system – 1650, 1600, 1560 cm^{-1}; carbonyl group – 1350, 1260 -1175, 3370 cm^{-1}; phenol and hydroxyl groups – 1035, 910, 835 cm^{-1} and 3400-3300 cm^{-1} and γ-pyrone of the cycle 1640 cm^{-1}.

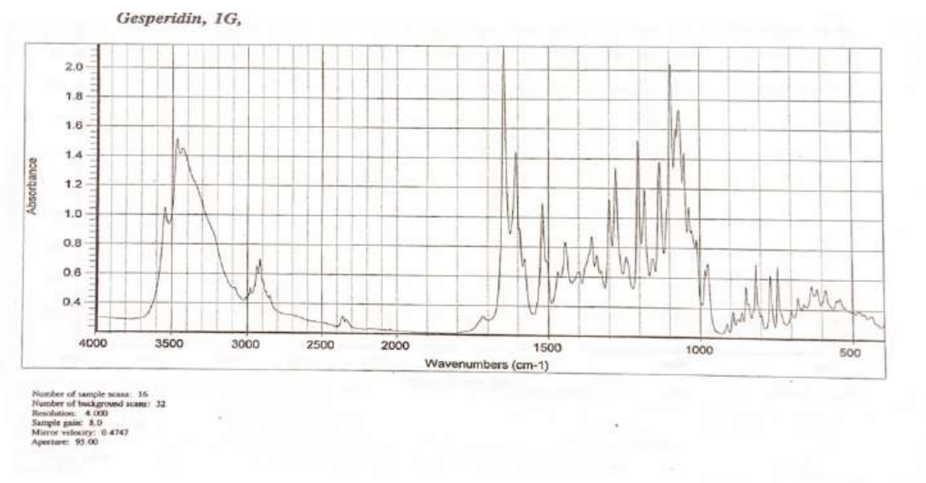

Fig.1. Infrared spectrum of hesperidin

In proton magnetic resonance spectrum of hesperidin (DMSO,δ, m.d.) we observed signals: 6,74 (d. H-2′, 5′, 6′; J=5 Hz); 5,99 (c. H-8); 5,92 (c.H-6); 5,18 (d.d.H-2); 2,83 (d.d. 2H-3); 3,72 (c. OCH_3-4′); 4,84 (d. H-1», J=4 Hz); 4,28 (c. H-1′′′) и 0,93 (d.3H-6′′′, J=5 Hz). The given data made it possible to conclude that free OH groups are located in positions 5 and 7 and 3^1, and the methoxy group is in position 4' of the flavan core (Fig. 2).

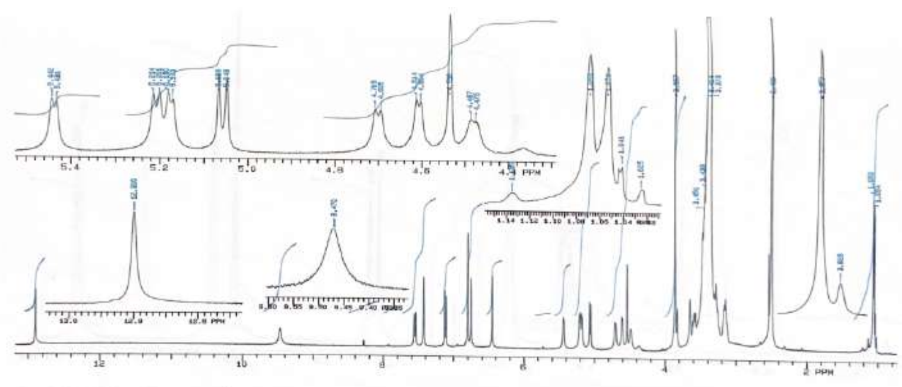

Fig.2. Proton magnetic resonance spectrum of hesperidin

As a result of physical and physicochemical studies, the substance was identified as 5,7,3^1-trihydroxy-4^1-methoxyflavane [9, 14], authenticity and the absence of impurities in hesperidin were established.

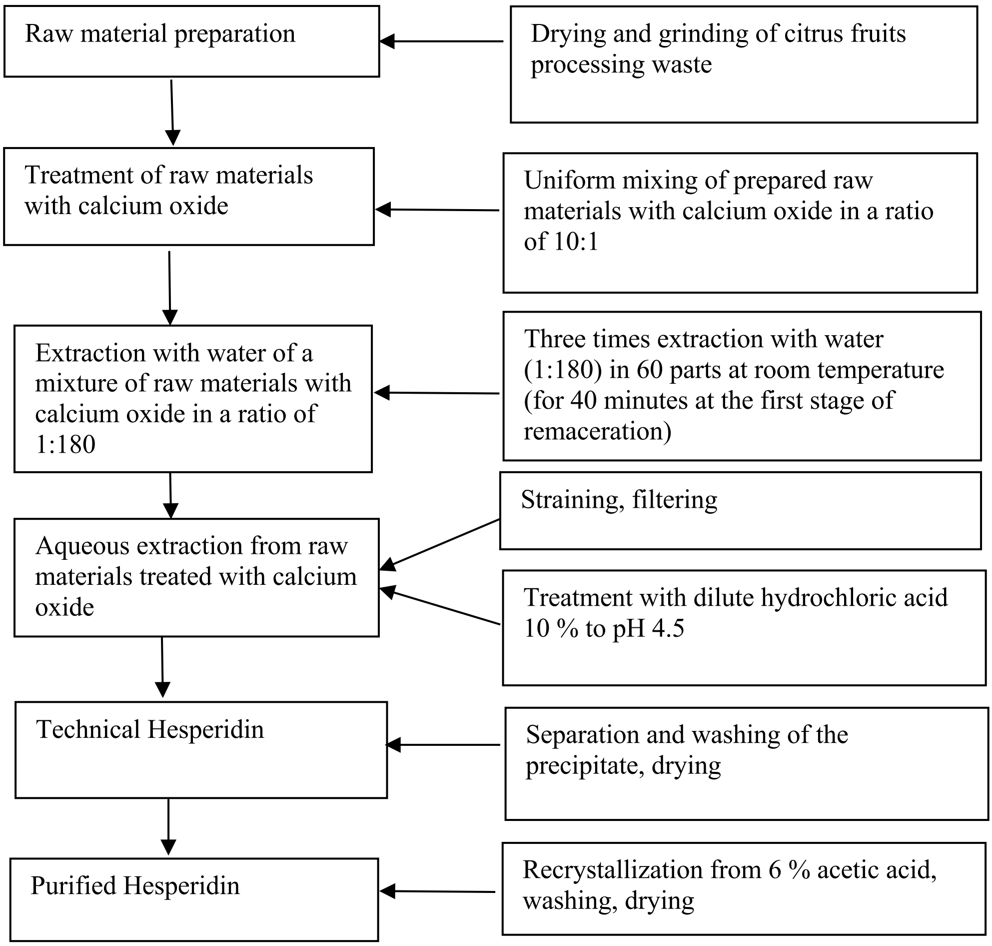

Fig.3. Scheme for obtaining hesperidin from citrus fruit processing waste

The proposed method for obtaining hesperidin (Figure 3) is as follows: citrus fruit processing waste, after obtaining juice from them, is crushed and dried, mixed with calcium oxide in a ratio of 10:1, a mixture of 60 parts of water is then poured and mixed again. After 40 minutes, the mass is filtered through a medium density cotton fabric, and the mixture is again poured with 60 parts of water and squeezed through the same fabric. This operation is repeated one more time. The filtrates are combined, acidified with 10 % hydrochloric acid until the pH of the medium reaches 4.5 and left for 18-20 hours at a temperature not higher than 10 °C. The resulting crude hesperidin is separated and washed with water until neutral and dried at 60 °C. Hesperidin is then purified by recrystallization three times from a 6 % acetic acid solution. The "output" of hesperidin from various types of citrus fruits according to the proposed scheme for obtaining is from 4.5 to 10.1 %: in lemons – 4.5 %; in oranges – 8.8 %; the maximum amount of hesperidin is found in mandarin waste – 10.1%.

4 Conclusion

To create new polyfunctional biologically active food supplements based on flavanones, an ecologically and economically acceptable modified method for obtaining hesperidin from agro-industrial waste from processing citrus fruits has been developed. This method provides for the treatment of the prepared raw material with calcium oxide, followed by aqueous extraction, isolation and recrystallization of the target product.

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