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RESEARCH ARTICLE

Technological solutions for enhancing the efficiency of supercritical fluid extraction of biologically active substances from plant raw materials

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Abstract

Objectives. The development of technological solutions aimed at increasing the efficiency of supercritical fluid extraction of polar biologically active substances from plant materials is a priority direction due to the demand for such substances in the food and pharmaceutical industries. The aim of this study is to develop an approach for stabilizing extractant composition by continuously feeding a polar cosolvent into a supercritical carbon dioxide flow.

Methods. The study was carried out on blackcurrant (*Ribes nigrum* L.) fruits, which contain polyphenols, one of the most difficult-to-extract compounds. Two types of prepared raw materials were used: heat-dried at 50°C for 48 h; freeze-dried at a pressure of 68 Pa and a temperature of up to 25°C for 48 h. Extracts were obtained by supercritical fluid extraction in a 250-mL high-pressure extractor. The process was carried out at a temperature of 50°C and a pressure of 200 bar in different modes: using pure CO_2; with the addition of ethanol; with the addition of acetone; and employing different methods for feeding the extractant into the high-pressure extractor.

Results. The highest efficiency of polyphenol extraction was achieved using heat drying of the raw materials in combination with a continuous supply of ethanol as a cosolvent, which enabled the highest yields of extract (22.47 wt %) and polyphenols (4.95 wt %). The experiments confirmed that the modes using acetone provide a high yield of extractive substances; however, their use is limited by the toxicity of the solvent, whereas a continuous supply of ethanol is a more promising and safer method for process intensification. Peat-dried samples consistently demonstrated higher yields of both extract and target polyphenols in comparison with freeze-dried raw materials in all extraction modes studied.

Conclusions. The choice of drying method at the stage of raw material preparation is shown to have a significant impact on extraction efficiency: heat drying provides a higher extract yield than freeze drying. The experiments confirmed that the addition of polar cosolvents increased the solubility of polyphenols. While the highest efficiency among single-addition modes was achieved using acetone, ethanol is recommended due to its non-toxicity. By using a continuous flow of a cosolvent instead of its a single addition, the total extract yield is increased by 6.4 times, and the polyphenol content by 17 times. The proposed universal approach can be applied to the extraction of a wide range of polar compounds; even higher efficiency is expected for less polar substances.

Keywords

supercritical fluid extraction, polar compounds, polyphenols, cosolvent, equipment modernization

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НАУЧНАЯ СТАТЬЯ

Технологические решения для повышения эффективности сверхкритической флюидной экстракции биологически активных веществ из растительного сырья

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Аннотация

Цели. Разработка технологических решений, направленных на повышение эффективности сверхкритической флюидной экстракции полярных биологически активных веществ из растительного сырья, является актуальной задачей. Целью данной работы является стабилизация состава экстрагента за счет непрерывной подачи полярного сорастворителя в поток сверхкритического диоксида углерода.

Методы. В качестве модельного объекта были использованы плоды черной смородины (*Ribes nigrum* L.), содержащие полифенолы, которые относятся к группе наиболее трудноизвлекаемых соединений. В работе были использованы два типа подготовленного сырья, полученные с помощью тепловой сушки при температуре 50°C в течении 48 ч и лиофильной сушки при давлении 68 Па и температуре до 25°C в течении 48 ч. Для получения экстрактов применялся метод сверхкритической флюидной экстракции в экстракторе высокого давления с объемом 250 мл. Процесс проводился при температуре 50°C и давлении 200 бар при пяти различных режимах: чистым CO_2, с добавлением этанола, с добавлением ацетона и с различным способом подачи экстрагента в экстрактор высокого давления.

Результаты. Установлено, что наибольшая эффективность извлечения полифенолов достигается при использовании тепловой сушки сырья в сочетании с непрерывной подачей этанола в качестве сорастворителя, что позволило получить максимальные значения выхода экстракта (22.47 мас. %) и полифенолов (4.95 мас. %). Экспериментально подтверждено, что режимы с применением ацетона обеспечивают высокий выход экстрактивных веществ, однако их использование ограничено токсичностью растворителя, тогда как непрерывная подача этанола является более перспективным и безопасным методом интенсификации процесса. Выявлено, что образцы, подвергнутые тепловой сушке, систематически демонстрируют более высокие показатели выхода как экстракта, так и целевых полифенолов по сравнению с лиофильно высушенным сырьем во всех исследованных режимах экстракции.

Выводы. Установлено, что выбор метода сушки на этапе подготовки сырья оказывает значимое влияние на эффективность экстракции, причем тепловая сушка обеспечивает более высокий выход экстракта по сравнению с лиофильной. Экспериментально подтверждено, что добавление полярных сорастворителей повышает растворимость полифенолов, при этом наибольшая эффективность среди режимов с однократным внесением достигается при использовании ацетона, однако рекомендуется использовать нетоксичный этанол. Переход от разового внесения сорастворителя к непрерывной подаче позволяет увеличить суммарный выход экстракта в 6.4 раза, содержание полифенолов в 17 раз. Предложенный подход универсален и может быть применен для экстракции широкого спектра полярных соединений; для менее полярных веществ ожидается еще более высокая эффективность.

Ключевые слова

сверхкритическая флюидная экстракция, полярные соединения, полифенолы, сорастворитель, модернизация оборудования

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INTRODUCTION

Polar compounds of plant origin are of significant interest to the food and pharmaceutical industries. This class of substances is in high demand for the production of functional foods and pharmaceutical products due to its pronounced antioxidant and anti-inflammatory activity, as well as its ability to perform certain prebiotic functions [1]. However, the efficient extraction of these substances from plant materials is challenging due to their high polarity and instability at elevated temperatures [2, 3]. The application of conventional extraction methods—in particular thermal and ultrasonic—frequently leads to partial destruction of biologically active substances or require large volumes of organic solvents to carry out the process [3]. In this context, supercritical fluid extraction (SFE) using carbon dioxide CO_2 is a promising alternative, since it combines such process advantages as the use of nontoxic solvents, the possibility of selective extraction of biologically active substances, and mild process conditions [4].

Despite the obvious advantages of SFE, its application for the extraction of polar substances, particularly polyphenols, from plant materials is limited by a number of process requirements. Pure CO_2, because of its low polarity, is characterized by limited solubility of polyphenolic compounds, which reduces the efficiency of extraction of polar biologically active substances. To increase the solvent capacity of the extractant, polar cosolvents, particularly ethanol and acetone, are commonly added [5]. It is worth noting that the conventional approach to introducing a cosolvent into the SFE process—namely, a single injection into the apparatus before extraction—does not maintain a stable concentration of the polar cosolvent throughout the process. As well as, limiting the total efficiency of the method, this may reduce the reproducibility of the results.

Thus, in order to improve the efficiency of the process, it is necessary not only to reconsider its operating modes, but also to change the principle of cosolvent introduction. As already mentioned, a single addition of a polar cosolvent before the start of extraction leads to its rapid washout and a further decrease in the solvent capacity of the extractant. A more effective approach presents itself in terms of providing a gradual introduction of the cosolvent throughout the process. To implement such an approach, it becomes necessary to modernize the equipment by introducing an additional pump into the process flowsheet for ensuring the continuous supply of the polar cosolvent into the supercritical CO_2 flow. This design permits the maintenance of a constant concentration of the cosolvent in the extractant, increases selectivity, and ensures more efficient extraction of polar biologically active substances. At the Kazan National Research Technological University, studies are being carried out on the yields of phenolic compounds and triterpenes from tinder fungus mown (*chaga*) in an experimental extraction pilot plant using ethyl alcohol as a cosolvent to predict and evaluate the efficiency of the SFE process [6].

Currently, many experiments are being performed using foreign-made equipment from *Rexo Engineering* (South Korea) and *Waters* (USA) [7–9]. For supercritical CO_2 extraction, pilot plants have been developed by the Russian companies *Karavan*[1], *NITs ER GORO*[2], and *Yaventa*[3]. However, the ability to obtain target polar compounds from plant materials using the implemented technological solution is hampered by the impossibility of adding a cosolvent during the supercritical extraction process. Moreover, despite the construction of supercritical extraction plants, serial production of high-tech high-pressure equipment on an industrial scale in Russia is virtually nonexistent [6, 10, 11].

The aim of the present work is therefore to improve the efficiency of polyphenol extraction from plant materials using SFE by optimizing process conditions and upgrading extraction equipment. Solutions for stabilizing the extractant composition by providing a continuous supply of cosolvent are proposed. The presented experimental data allow us to establish the influence of technological and design factors on the SFE process, thus opening up opportunities for the creation of more efficient and selective technologies for obtaining extracts with the required biological activity from plant raw materials.

As a model plant material in this work, blackcurrant (*Ribes nigrum* L.) fruits were chosen due to their high content of biologically active substances, including anthocyanins, quercetin, and other polyphenols offering pronounced antioxidant properties [12]. Meanwhile, polyphenols are among compounds that are the most difficult to extract by SFE, making them a preferred object for testing the effectiveness of the developed approach. In addition, blackcurrant fruits are an accessible and widespread berry raw material in Russia. Among berries commercially grown in Russia, blackcurrants make up 33% of the valuable total (in the world, on

[1] Karavan. URL: https://kuban-co2-karawan.ru/. Accessed October 27, 2025 (in Russ.).

[2] NITs ER GORO. URL: https://extract.ru/. Accessed October 27, 2025 (in Russ.).

[3] Yaventa. URL: https://javenta.ru/. Accessed October 27, 2025 (in Russ.).

average 11%) [13]. Currently, a significant quantity of blackcurrant fruit is processed annually; the resulting processed products, such as cake, can be reused for the extraction of biologically active substances.

EXPERIMENTAL

The SFE process was studied using a setup with a 250-mL extractor. Figure 1 depicts the flow diagram of the modernized setup for the SFE process.

During SFE, liquid CO_2 is fed from cylinder *1* into condenser *5* and is cooled there to 5°C. Then pneumatic pump *7* powered by compressor *8* pumps liquid CO_2 into the system to a pressure of 200 bar. Before being fed to extractor *15*, CO_2 is heated in thermostat *12*. Upon reaching a temperature above 31°C, CO_2 becomes a supercritical fluid, expands sharply, and is fed to high-pressure extractor *15* with preloaded dried plant material (40 g). After passing through extractor *15*, CO_2 with dissolved biologically active substances passes through fine adjustment valve *18*, which controls the flow rate of the mixture leaving the extractor. The mixture then enters separator *19*, where CO_2 and extract are separated: with the transition of CO_2 from a supercritical to a gaseous phase, the extract is to precipitated. This process causes a sharp drop in pressure and temperature. Following extraction, the pressure in extractor *15* is reduced to atmospheric pressure, the temperature equalizes with the ambient temperature (20–25°C), the extractor is depressurized, and the cake is removed.

To overcome the limitations associated with the single introduction of a cosolvent, the extraction setup was upgraded. Additional high-pressure membrane electric pump *23* was added to the process

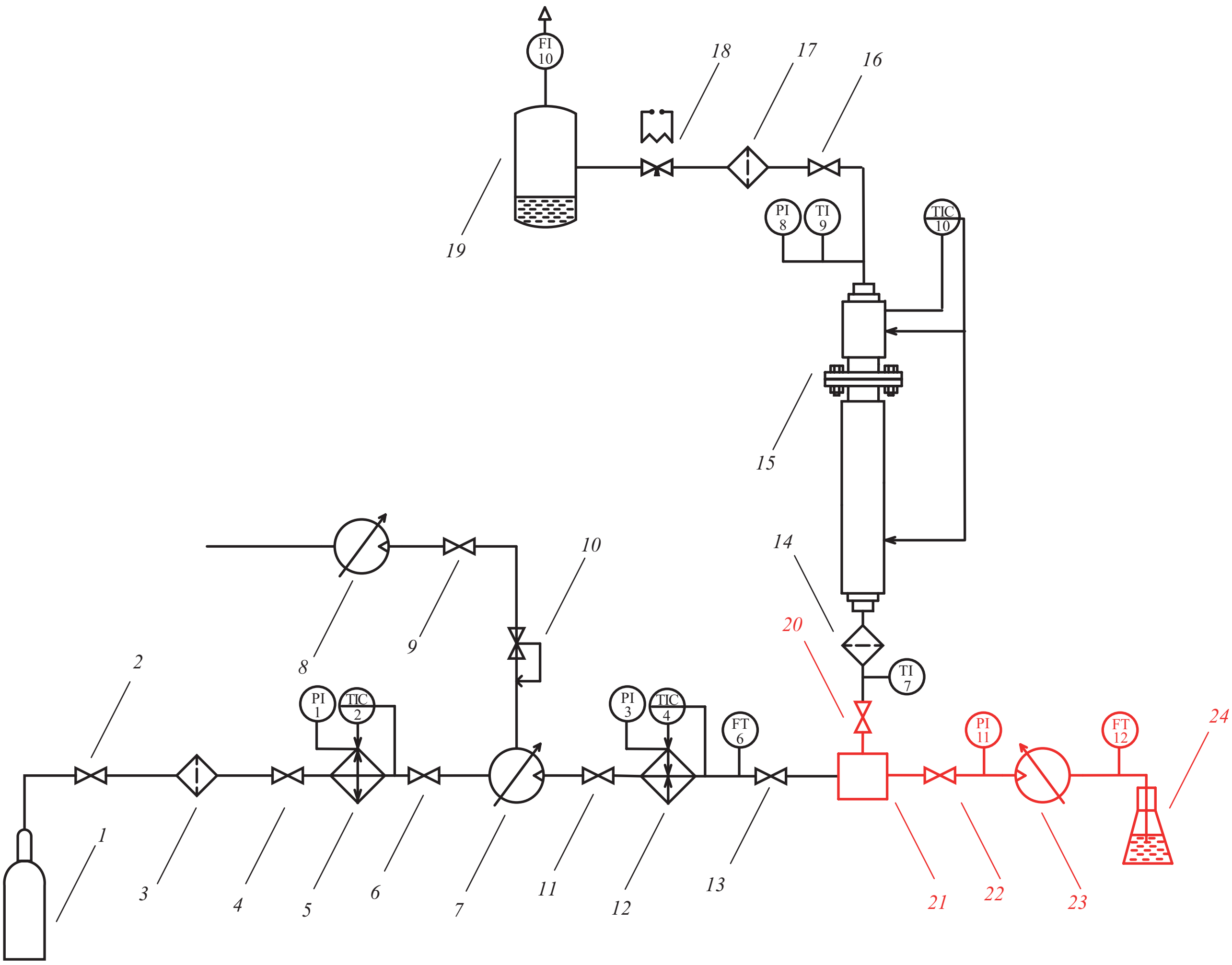

Fig. 1. Process flow diagram of the supercritical fluid extraction setup:
(*1*) CO_2 cylinder; (*2*, *4*, *6*, *9*, *11*, *13*, *16*, *20*, *22*) shut-off valves; (*3*, *14*, *17*) micron filters;
(*5*) condenser; (*7*, *23*) pumps; (*8*) compressor; (*10*) pressure reducer; (*12*) thermostat;
(*15*) 250-mL high-pressure extractor; (*18*) fine adjustment valve; (*19*) separator; (*21*) mixer; (*24*) container with cosolvent;
(TIC2, TIC4, TIC9) temperature controllers; (FT5, FT12) mass flow meters; (FI10) rotameter;
(PI1, PI3, PI7, PI11) pressure gauges; (TI6, TI8) thermometers

flow, ensuring continuous delivery of a cosolvent directly into the supercritical CO_2 flow upstream of the extractor. Figure 2 presents a photograph of the modified setup.

Before carrying out SFE, a mandatory step involves the removal of moisture from plant materials by drying, since water is virtually insoluble in supercritical CO_2 [14]. Moreover, the choice of drying method has a significant impact on both the preservation of the cellular structures of the raw material and the preservation of biologically active substances.

Two types of prepared raw materials were obtained during the study depending on the drying method (freeze or heat drying). Raw materials were prepared using an LM-1000 laboratory mill (*Stegler*, China). For heat drying, plant materials were loaded into an MT-FD188A fruit and vegetable dryer (*MARTA*, Russia). The process was carried out at a set temperature of 50°C for 48 h. The dried fruits were ground using the Stegler LM-1000 laboratory mill. After grinding, the powder was sieved through a 0.5 mm sieve until the achievement of a uniform particle size. This approach ensured the production of a powder with particles smaller than 0.5 mm.

In the case of freeze drying, the whole raw material was initially ground to a homogeneous mass, frozen, and then placed in a freeze dryer. The freeze drying was carried out using a CoolSafe Touch 100-9 system (*LaboGene*, Denmark).

A series of experimental studies was conducted for each type of raw material. Extraction was carried out in five modes, which differed in terms of the type of cosolvent used and the method of its application in the process. The process was performed at a pressure of 200 bar and a temperature of 50°C. Figure 3 presents a detailed diagram of the experimental stages.

In mode I, samples were treated in a pure CO_2 medium for 30 min, after which the extraction was continued with pure CO_2 at a flow rate of 1 kg/h for 60 min. In mode II, a 30-min treatment in a CO_2 medium with the addition of ethanol was followed by extraction with pure CO_2 at a flow rate of 1 kg/h for 20 min. In mode III, the raw material was treated in a CO_2 medium with ethanol for 30 min, after which the extraction was terminated by an immediate pressure release. In mode IV, a 30-min treatment in a CO_2 medium with the addition of acetone was followed by extraction with pure CO_2 at a flow rate of 1 kg/h for 20 min. In mode V, samples were also treated in a CO_2 medium with acetone for 30 min, then the extraction was terminated by an immediate pressure release.

Each sample was assigned a code. The first letter denoted the method of raw material preparation: "H" for heat drying and "F" for freeze drying. The second part

Fig. 2. Supercritical fluid extraction setup

of the code was a number corresponding to the specific extraction mode (I–V). For each sample, a series of three experiments was conducted.

To overcome the limitations associated with the single introduction of the cosolvent, the extraction setup was modernized. The dissolving power of supercritical CO_2 was increased by adding ethanol, which has polar properties [15]. Its addition alters the dielectric properties of the extractant, promotes increased solubility of polyphenolic compounds, and intensifies mass transfer [16].

Extraction with continuous ethanol feed was carried out by means of supercritical CO_2 constantly pumped through the extractor for 1 h at a rate of 1 kg/h. Ethanol was simultaneously introduced into the flow in an amount ensuring its content in the extractant at a level of 10 wt %. For samples obtained in this mode, the

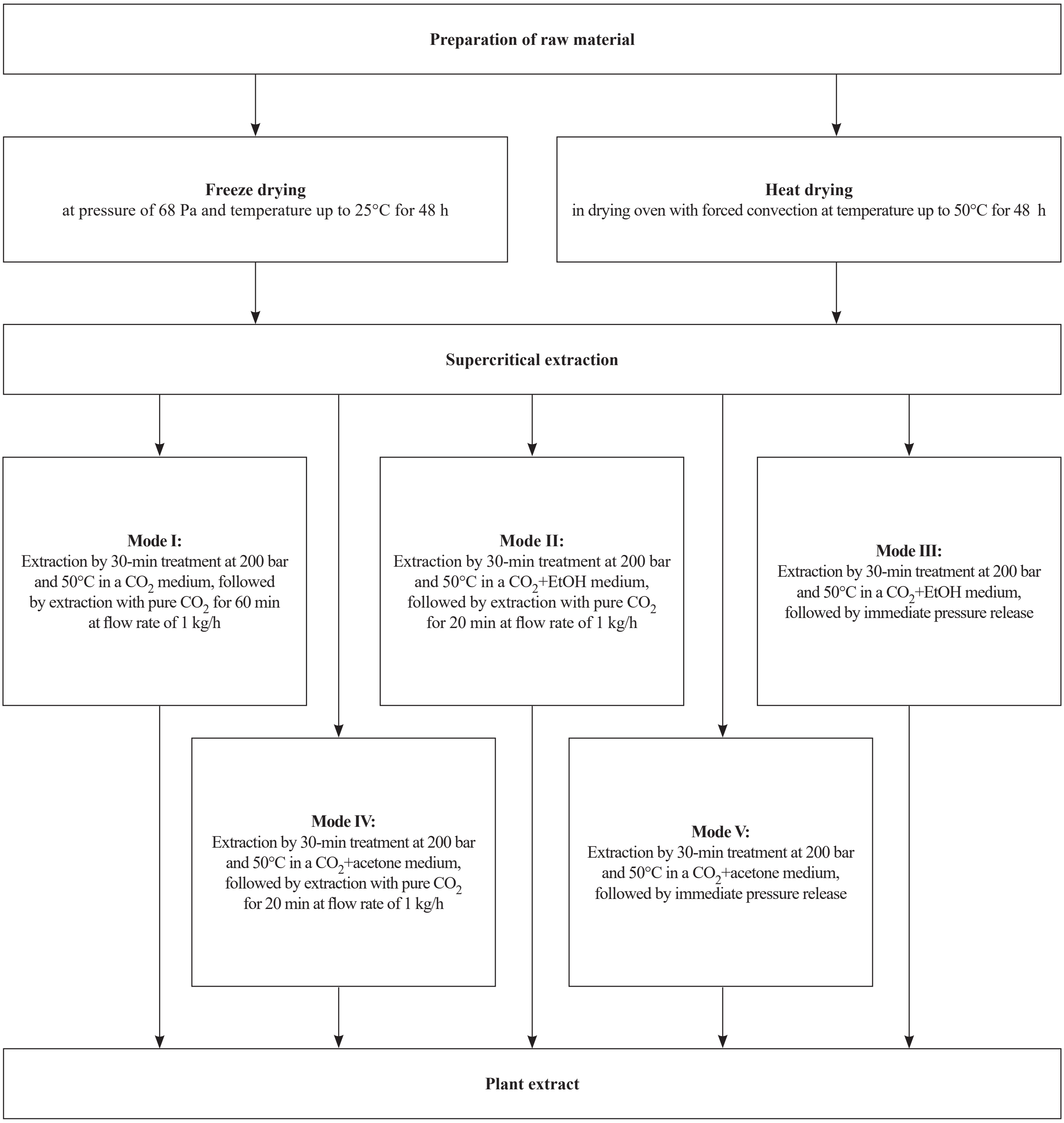

Fig. 3. Block diagram of the processes for obtaining plant extracts

conventional designations "H-E" are given for raw materials prepared by heat drying, and "L-E"—for raw materials prepared by freeze drying.

It should be noted that the proposed approach requires additional investment in pumping equipment and complicates the system design. However, the successful implementation of this technical solution ensures a stable extractant composition and reproducible process conditions, which in turn increases process reliability and simplifies scaling to industrial scale.

The total content of polyphenols in each extract obtained was determined by a colorimetric method using the Folin–Ciocâlteu reagent in accordance with a published procedure [17].

Hot water extraction and ethanol extraction using a reflux condenser were chosen as comparative methods for evaluating the efficiency of polyphenol extraction. Each sample was assigned a code. The second part of the code was a letter corresponding to the specific extraction mode: "W" for water extraction and “A” for ethanol (alcohol) extraction.

RESULTS AND DISCUSSION

Table presents the results of the experiments. The mass yields of extract and polyphenols were calculated based on the amount of dry raw material loaded into the extractor. For each sample, a series of three experiments was conducted.

Analysis of the experimental data showed that heat drying ensures higher extract and polyphenol yields than freeze-drying. These results can be explained by the thermal destruction of cellular structures, which facilitates access of supercritical CO_2 and cosolvent to the intracellular contents.

Although the addition of ethanol contributed to an increase in the yield of polyphenols, a decrease in the total yield was observed upon completion by releasing the pressure (H-3, F-3) in comparison with the mode where the 30-min treatment was followed by a further extraction with pure CO_2 (H-2, F-2).

The use of acetone led to the highest yields among the modes with a single addition of a cosolvent. In

Table. Results of experimental studies of supercritical fluid extraction of polyphenols from blackcurrant fruits

No.	Sample	Extract yield, wt %	Polyphenol yield, wt %
1	H-1	2.80	0.06
2	H-2	2.11	0.18
3	H-3	1.37	0.28
4	H-4	3.51	0.29
5	H-5	0.58	0.03
6	H-E	22.47	4.95
7	F-1	1.90	0.02
8	F-2	1.68	0.08
9	F-3	1.14	0.02
10	F-4	2.84	0.06
11	F-5	0.11	0.01
12	F-E	4.45	3.48
13	H-W	5.45	3.91
14	H-A	25.94	6:40
15	F-W	15:44	3.56
16	F-A	22.57	5.62

mode IV, the extract yield reached 3.51 wt % (H-4) at a polyphenol content of 0.29 wt %. However, in mode V (with the process terminated by pressure release), the yields decreased sharply in the case of both heat and freeze drying.

The best results were achieved using the modernized setup with the continuous ethanol feed. For samples after heat drying, the extract yield was 22.47 wt %, while the polyphenol content was 4.95 wt % (H-E), significantly exceeding the results obtained for all other modes. For freeze-dried samples, the values were somewhat lower (16.45 and 3.48 wt %, respectively); however, even these results significantly exceeded the results of series I–V. The obtained results confirm that stabilization of the extractant composition by continuous cosolvent feed is the most promising technological solution for increasing the efficiency of SFE of polar compounds.

The use of acetone as a cosolvent is limited by its toxicity and possible negative impact on the subsequent use of extracts in food and biotechnological processes. For this reason, it was decided to exclude from further studies and leave only ethanol as a cosolvent.

A wide range of components in extracts can be used to positively influence the growth of bifidobacteria [18]. Therefore, despite the lower yield of polyphenols, extracts obtained using supercritical technologies will be further investigated as an additive to culture media to influence the growth of probiotics such as *Bifidobacterium breve* L. and *Bifidobacterium bifidum* L.

CONCLUSIONS

The conducted studies confirm that the efficiency of supercritical fluid extraction of polyphenols from plant materials depends on both the pretreatment method and the method of cosolvent introduction. The higher yields of extract and polyphenols obtained from heat-dried feedstocks than freeze-dried ones are likely due to factors including the destruction of cellular structures and facilitated mass transfer. The addition of polar cosolvents increased the solubility of polyphenols, with acetone achieving the highest efficiency among the modes involving the addition of a single cosolvent. However, the use of acetone is limited by its toxicity and requires additional product purification steps, complicating its use in food and biotechnological processes.

The best results in supercritical fluid extraction were achieved with a continuous supply of ethanol. This approach allowed us to maintain a stable extractant composition and ensure maximum extract yields (up to 22.47 wt % at a polyphenol content of 4.95 wt % after heat drying and up to 16.45 wt % at a polyphenol content of 3.48 wt % after freeze drying). The resulting extracts will be tested for biological activity and compared with extracts obtained by hot water extraction and ethanol extraction using a reflux condenser.

Thus, the proposed method represents not only a viable technique for intensifying the supercritical fluid extraction of polyphenols, which are among the most difficult-to-recover compounds, but also a new process design concept aimed at increasing the efficiency of isolating a wide range of polar components. Preliminary experiments with less polar compounds, including carotenoids, demonstrated even higher yields, confirming the versatility and potential of the developed approach.

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Authors' contribution

All authors contributed equally to the research work.

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