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COMPARATIVE ANALYSIS OF THE NICOTINE EXTRACTION PROCESS FROM *NICOTIANA TABACUM* L. IN SUPERCRITICAL CONDITIONS UNDER VARIOUS EXTRACTANT FLOWS

Abstract. In this paper, we present a comparative analysis of various methods and conditions for extraction from the plant *Nicotiana tabacum* L., harvested in the Almaty region of the Republic of Kazakhstan in 2019. Extraction was carried out at various temperatures at during the extraction process while maintaining pressure in the system 120 bar and a flow of 100 ml/min and 4000 ml/min with the subsequent development of the technology for producing nicotinic acid by oxidation in an aqueous medium.

The supercritical extraction method was carried out on a CO_2 extraction unit under the following conditions: the temperature range in the reactor 40-80 °C, the pressure 120 bar, a gas flow of 100 ml / min. Extraction was also carried out on the installation which was developed jointly with LLC «Superhydrophobic Coatings» (Nizhny Tagil, Russia) at similar temperatures and pressure but with a 40-fold increase in flow (4000 ml/min).

The obtained extracts were studied on a gas chromatograph with a mass selective detector Agilent Technologies 7890N / 5973N GC / MS. Data processing included determining retention times, peak areas and processing of spectral information obtained by using a mass spectrometric detector. The libraries Wiley 7th edition and NIST'02 were used for evaluation of mass spectra.

As a result, an almost two-fold increase in the efficiency of the extraction process was achieved while maintaining the previous parameters.

In addition, the maximum selectivity of the process is achieved at 120 bar and a temperature 70 ºC. The nicotine content in the final extract increased significantly with reducing the quantitative and qualitative content of impurities which are represented in most higher hydrocarbons and alcohols, which do not have special biological activity and, as a consequence, do not affect the quality of the final product.

Our comparative analysis with previously published data shows that a temperature change significantly increases the selectivity of the extraction process, while the highest nicotine content was noted in the extract obtained at 70 ºC. Also under these conditions the smallest impurity content was noted. In addition, only 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (11.31%) and Tetratetracontane (4.70%) were identified in the extract with nicotine.

Natural nicotine is in demand in the production of alternative methods of tobacco consumption, such as e-cigarette. Nicotine can also be potentially used in medicine as an anesthetic and in the treatment of Alzheimer's disease. In addition, nicotine is used as an insecticide to protect plants. For these purposes, nicotine was used in the form of a pure substance, its sulfate, tobacco dust, and the pure substance was the most active.

Keywords: Nicotiana tabacum L, SFE extraction, nicotine, high flows.

Introduction. Extraction is the main technological process that allows extracting biologically active substances from plant materials

Classical extraction of plant materials is a process of extraction plant raw material with a solvent (extractant).

Moreover, the solvent used often cannot be completely removed from the obtained extract, in addition, the feedstock undergoes a number of changes due to the use of chemical solvents, which casts doubt on the "nativeness" of such extracts. In addition, solvents are not able to provide extraction of a full range of biologically active substances [1].

The prevailing actual environmental and social conditions in the world urgently require new approaches to the extraction of biological components.

The use of a number of extractants with a toxic or mutagenic effect in the pharmaceutical industry is prohibited. One of the solutions to this problem is the use of supercritical carbon dioxide as an extractant. And the technology itself is called supercritical fluid carbon dioxide extraction of plant raw material [2].

Most organic solvents are highly toxic compounds and exhibit an accumulation effect which requires the introduction of additional purification stages for the drug substance and additional quality control methods for such preparations. These measures lead to the inevitable rise in price of the final product. In addition, a significant portion of organic solvents are one way or another petrochemical products and their price will inevitably increase in the future [4].

In this regard, in recent decades, new methods for the extraction of biologically active substances complexes from plant materials have been actively studied and developed.

Supercritical extraction methods have several effective advantages.

Supercritical fluids have been investigated since the last century and at first the greatest commercial interest was the use of supercritical toluene in the processes of shale oil processing in the 1970s. Supercritical water is also being investigated as a means of destroying toxic waste and as an exotic synthesis medium [5].

Supercritical carbon dioxide, the critical temperature 31 °C. and a pressure of 71 bar has great interest in the field of chemistry of natural compounds. Biological materials can be processed at 35 ° C which contributes to their preservation from thermal degradation. The density of supercritical CO2 at a pressure of about 200 bar is close in efficiency to hexane and the solvation characteristics during extraction are also similar to hexane [6].

The main advantages of supercritical carbon dioxide are:

- universal dissolving ability of organic compounds. It does not cause concern physiologically because is the final product of the metabolism of a number of living organisms, including humans;
- carbon dioxide is chemically inert and does not with with recoverable substances;
- CO_2 is relatively safe for the environment which suggests the possibility of creating an environmentally friendly type of production;
- carbon dioxide is one of the most accessible and widely used gases in the food industry.

A large number of industrial products are obtained using supercritical technologies (decaffeinated coffee, cholesterol-free oil, lean meat, rose oil, etc.).

The solvation characteristics of supercritical CO_2 can be modified by the addition of a co-solvent such as ethanol which can significantly increase the extraction efficiency. However, the solvent residue in the product somewhat eliminates the main advantage of the process which consists in the complete absence of impurities in the final extract [7, 8].

In connection with the foregoing, the development of the scientific foundations and the development of extraction processes and techniques that will allow the operation of extraction plants using carbon dioxide in the liquefied and compressed state to be switched on to a high-performance and energy-saving mode are necessary, while it is necessary to reduce the pressure level in the apparatuses, create conditions of steady and safe work.

The improvement of the technique and technology for the extraction of plant materials with carbon dioxide is possible on the basis of deepening research on both the extraction process itself and the operation of the entire complex of apparatuses of the extraction plant.

In earlier articles, we determined the optimal pressure for the process of extraction of nicotine from the plant *Nicotiana tabacum* L. [9-17].

In this article we show transfer factor and its effect on the efficiency of the extraction process in more detail the mass.

Materials and methods. Plant raw material (*Nicotiana tabacum* L.) for the extraction was harvested in the Almaty region in 2019. The tobacco was dried and crushed in a cutting mill to an average particle size 3 mm for increasing the specific area and the efficiency of the extraction process accordingly.

Prepared plant raw material was divided into 6 samples weighing 500 g to determine the completeness of extraction based on the loss in mass [18-20].

Then, samples numbered 1–3 were extracted on a extraction unit Thar SFE-1000 under the following conditions: temperature 40–70 ° C; CO_2 pressure 120 bar; a gas flow of 100 ml/min.

Samples 4-6 were extracted with the installation developed jointly with LLC "Superhydrophobic Coatings" (Nizhny Tagil, Russia) under the Target Financing Program BR05236420 "Angry Technologies Based on Supercritical Media" at similar temperatures (40-80ºC) and a CO_2 pressure 120 bar but with a flow of carbon dioxide of 4000 ml / min) [21-22].

The obtained extracts were studied by gas chromatography with a mass selective detector. The analysis was performed on a gas chromatograph with a mass spectrometric detector 6890N/5973C (Agilent, USA) equipped with a autosampler Combi-PAL (CTC Analytics, Switzerland). 1.00 µl of the sample was injected into the gas chromatograph injector using an autosampler at an injector temperature 250 °C for GC-MS analysis. Chromatography was performed using an HP-5ms capillary column (Agilent, USA): a length 30 m, an inner diameter 0.25 mm, a film thickness 0.25 µm at a constant carrier gas velocity 1.0 ml/min (helium> 99.995%, Orenburg-Tekhgaz, Russia). The program for heating the chromatographic column: holding 5 min at 40 ° C, heating at a speed 10 °C / min to 280 ° C, holding for 5 min. The total chromatographic time was 34 minutes. The temperatures of the quadrupole and the detector ion source were 150 and 230 °C, respectively.

Mass spectrometric detection was carried out in the ion scanning mode in the m/*z* range 40 to 550 with a solvent delay of 5 min. The peaks found in the chromatograms were identified using the NIST'11 and Wiley 10 mass spectral libraries [23–25].

Results and discussion. We found that increasing the flow almost 2 times increases the efficiency of the extraction process, in addition, the duration of the process is significantly reduced from 2 hours to 15 minutes, with a relatively small increase in energy consumption.

The results are presented in table 1.

Table 1 – Results of the effectiveness of the process of extraction of nicotine from the plant *Nicotiana tabacum* L.

№	Flow ml/min	T, ºC	Duration extraction, min	Mass, g	Loss in mass relative to plant raw material, %	P, bar
1	100	40	120	500	0,43	120
2	100	50	120	500	0,5	120
3	100	70	120	500	1,05	120
4	4000	40	15	500	1,54	120
5	4000	50	15	500	1,8	120
6	4000	70	15	500	2,1	120

It is possible to achieve an extremely high yield of nicotine, which is 46.40% at a flow of 100 ml/ min and 83.9% at a flow of 4000 ml/min, and this is a very high rate for alkaloids under the conditions of supercritical fluid CO_2 extraction at a pressure 120 bar and the temperature 70 °C. At lower temperatures, and specifically 40 ºC, the yield is 18.2% with a smaller and 68.2% with a larger flow, and at 50 ºC 25.9% with a lower and 71.1%, respectively.

The data on the nicotine content in the extracts are presented in figure 1.

The indicated changes in the composition are explained by the fact that during supercritical processes, pressure and temperature are crucial not only for the completeness of extraction but also for the properties of the extractant [26–35].

Comparative analysis of the chemical composition with previously published data shows that a change in temperature significantly increases the selectivity of the extraction process while the highest nicotine content was observed in the extract obtained at 70 °C and under these conditions the smallest quantity of impurities was noted, in addition, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (11.31%) and Tetratetracontane (4.70%) were identified.

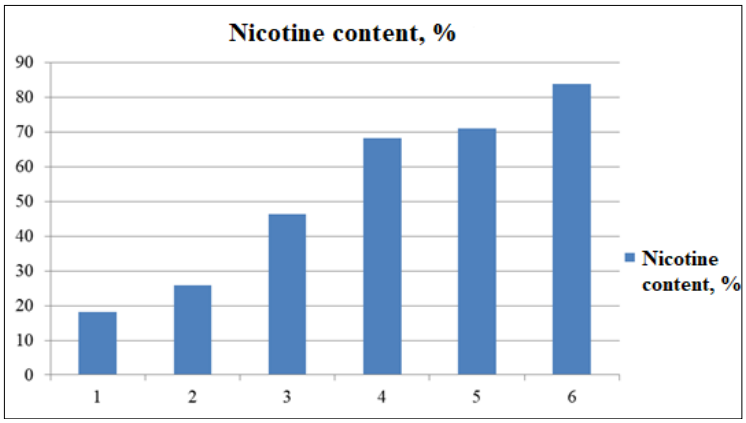

Figure 1 – Nicotine content in supercritical extracts obtained from spreading *Nicotiana tabacum* L. at different flows

Conclusion. As a result, an almost two-fold increase in the efficiency of the extraction process was achieved with maintaining the previous parameters.

That can significantly improve the environmental friendliness and energy efficiency of the extraction process.

In addition, the maximum selectivity of the process was achieved at 120 bar and a temperature 70 ºC. The nicotine content in the final extract increased significantly with reducing the quantitative and qualitative content of impurities. These impurities are represented in most higher hydrocarbons and alcohols which do not have special biological activity and, as a result, do not affect the quality of the final product.

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ЭКСТРАГЕНТТІҢ ТҮРЛІ АҒЫМДАРЫ КЕЗІНДЕ АСА СЫНИ ЖАҒДАЙЛАРДА NICOTIANA TABACUM L ӨСІМДІГІНЕН НИКОТИНДІ ЭКСТРАКЦИЯЛАУ ПРОЦЕСІН САЛЫСТЫРМАЛЫ ТАЛДАУ

Аннотация. Бұл мақалада біз 2019 жылы Қазақстан Республикасының Алматы облысында жиналған *Nicotiana tabacum L.* өсімдігінен алудың әртүрлі әдістері мен шарттарына салыстырмалы талдау ұсынамыз. Экстракция кезінде әр түрлі температурада жүйеде қысымды ұстап тұру кезінде 100 бар / мин және 4000 мл/мин. Сулы ортада тотығу арқылы никотин қышқылын алу технологиясын одан әрі дамыта отырып.

Экстракционды экстракция әдісі CO_2 алу қондырғысында келесі жағдайларда жүргізілді: реактордағы температура диапазоны - 40-80ºC, CO2 қысымы - 120 бар, газ шығыны 100 мл / мин. Superhydrophobic Coatings LLC-мен (Нижний Тагил, Ресей) бірлескен қондырғыда ұқсас температурада (40-80ºC) және CO_2 қысымы - 120 бар, бірақ ағынның 40 есе ұлғаюымен (4000 мл / мин).

Алынған сығындылар Agilent Technologies 7890N / 5973N GC / MS массалық іріктеу детекторымен газды хроматографта зерттелді. Мәліметтерді өңдеудің құрамына ұстап тұру мерзімдерін, шың аудандарын және масс-спектрометриялық детектордың көмегімен алынған спектрлік ақпаратты өңдеу кіреді. Алынған масс-спектрлердің шифрын ашу үшін Wiley 7 басылымы мен NIST'02 кітапханалары пайдаланылды.

Нәтижесінде, алдыңғы параметрлерді сақтай отырып, өндіру процесінің тиімділігі екі есеге артты.

Сонымен қатар, 120 барда және 70 ºC температурада процестің максималды селективтілігіне қол жеткізіледі. Соңғы сығындыда никотиннің мөлшері айтарлықтай жоғарылады, бұл көмірсутектер мен спирттердің көпшілігінде кездеседі, олар арнайы биологиялық белсенділікке ие емес және нәтижесінде өнімнің сапасына әсер етпейді.

Бұрын жарияланған мәліметтермен салыстырмалы талдау көрсеткендей, температураның өзгеруі экстракция процесінің селективтілігін едәуір арттырады, ал 70 никельден алынған сығындыда никотиннің ең жоғары мөлшері байқалды, сонымен қатар бұл жағдайда никотиннен басқа экстракцияда тек 3 анықталды. 7,11,15-тетраметил-2-он алтазид-1-ол (11,31%) және тетра-тетраконтан (4,70%).

Табиғи шикізаттан алынған никотин темекіні тұтынудың балама әдістерін, мысалы, электронды темекі өнімдерін өндіруде сұранысқа ие, никотин сонымен қатар медицинада анестезия ретінде және Альцгеймер ауруын емдеуде қолдануға болады. Сонымен қатар, никотин инсектицид ретінде өсімдіктерді қорғау үшін қолданылады. Бұл мақсаттарда никотин таза зат түрінде қолданылды, оның сульфаты, темекі шаңы, ал таза зат ең белсенді болды.

Түйін сөздер:. *Nicotiana tabacum* L, СКФ-экстракция, никотин, жоғары ағындар.

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ ПРОЦЕССА ЭКСТРАКЦИИ НИКОТИНА ИЗ РАСТЕНИЯ *NICOTIANA TABACUM* L В СВЕРХКРИТИЧЕСКИХ УСЛОВИЯХ ПРИ РАЗЛИЧНЫХ ПОТОКАХ ЭКСТРАГЕНТА

Аннотация. В данной статье нами представлен сравнительный анализ различных методов и условий экстракции из растения *Nicotiana tabacum* L., заготовленного в Алматинской области Республики Казахстан в 2019 году. При различных температурах проведения процесса экстракции с сохранением давления в системе 120бар и потока 100 мл/мин и 4000 мл/мин, с последующей отработкой технологии получения никотиновой кислоты путём окисления в водной среде.

Метод сверхкритической экстракции был проведён на установке CO_2-экстракции при следующих условиях: температурный диапазон в реакторе – 40-80ºС, давление CO_2 – 120 бар, при потоке газа 100 мл/мин. И на установке, разработанной совместно с ООО “Супергидрофобные покрытия” (Нижний Тагил, Россия), при аналогичных температурах (40-80ºС) и давления CO_2 – 120 бар, но с увеличенным в 40 раз потоком (4000 мл/мин).

Полученные экстракты исследовали методом газовой хроматографии на газовом хроматографе с масс-селективным детектором Agilent Technologies 7890N/5973N GC/MS. Обработка данных включала в себя определение времен удерживания, площадей пиков, а также обработку, спектральной информации, полученной с помощью масс-спектрометрического детектора. Для расшифровки полученных масс-спектров использовали библиотеки Wiley 7th edition и NIST’02.

В результате достигнуто практически двукратное повышение эффективности процесса экстракций при сохранении прежних параметров.

Кроме того, при 120 бар и температуре 70 ºС достигается максимальная селективность процесса. Содержание никотина в конечном экстракте значительно возросло, одновременно с уменьшение количественного и качественного содержания примесей, которые представлены в большинстве высшими углеводородами и спиртами, не имеющими особой биологической активности и, как следствие, не влияющими на качество конечного продукта.

Проведённый нами сравнительный анализ с ранее опубликованными данными показывает, что изменение температуры значительно повышает селективность процесса экстракции, при этом наибольшее содержание никотина отмечено в экстракте, полученном при 70 ºС, также при данных условиях отмечено наименьшее содержание примесей, в экстракте помимо никотина идентифицированы только 3,7,11,15-тетраметил-2-гексадецен-1-ол (11,31%) и тетратетраконтан (4,70%).

Никотин, полученный из натурального сырья, востребован в производстве альтернативных способов потребления табака, таких как e-cigarette, также никотин потенциально может быть использован в медицине в качестве анестетика и в терапии болезни Альцгеймера. Кроме того, никотин применяется в качестве инсектицида для защиты растений. Для этих целей использовали никотин в виде чистого вещества, его сульфат, табачную пыль, причём наибольшей активностью обладало чистое вещество.

Ключевые слова: *Nicotiana tabacum* L, СКФ-экстракция, никотин, высокие потоки.

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