

## ABSTRACT

“Producing of biological active substances in supercritical media”

**Zair Tairovich Ibraimov**

Educational Program “8D07102 – Chemical Engineering”

**General Characteristics of the work.** The research is aimed at developing a waste-free technology for obtaining biologically active substances (BAS) from cultivated raw materials and winemaking industry waste by supercritical fluid CO<sub>2</sub> extraction (SC-CO<sub>2</sub>).

**Relevance of the study.** Currently, plant-derived products are becoming increasingly popular, including as food additives, functional ingredients, and sources of biologically active substances. In conditions of high information load, increased stress levels, climate change, and growing concern about the safety of consumed products, issues of maintaining human health become particularly relevant. In this regard, the number of studies devoted to obtaining natural products and studying their properties increases every year.

These products include essential and vegetable oils. Essential oils are rich in terpene compounds, while vegetable oils contain unsaturated fatty acids, polyphenols, phytosterols, vitamins, and other biologically active components. The essential oils of spearmint *Mentha spicata* L. and purple basil *Ocimum basilicum* L. possess high biological activity, including antioxidant, antimicrobial, antibacterial, antiviral, and chemoprotective properties, which makes them in demand in medicine, the food industry, perfumery and cosmetology, as well as in agrochemistry as natural insecticidal agents.

The high biological value of grape seed oil *Vitis vinifera* (GSO) and black cumin seed oil *Nigella sativa* (BCO), due to the content of saturated and unsaturated fatty acids, vitamins, minerals, and phenolic compounds, arouses significant interest among researchers. GSO can be used in the food industry as a preservative and a functional additive that improves the physicochemical properties of products. BCO is characterized by pronounced anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. However, at the same time, they are characterized by low oxidative stability.

The possibility of complex processing of winemaking industry waste adds additional relevance to the study. Globally, the volume of wine production waste reaches 8-9 million tons per year, in which the content of phenolic compounds is 70%, making it a promising raw material for obtaining valuable products. Moreover, the favorable climatic conditions of Kazakhstan allow for the cultivation of a wide range of spicy and medicinal plants, which are a potential source of essential oils and other biologically active substances.

Essential oils (EOs) are traditionally obtained by hydrodistillation, maceration, and liquid extraction, while vegetable oils are obtained by pressing and liquid extraction. However, these methods have a number of disadvantages (duration and multi-stage processes, use of toxic and flammable solvents, high temperatures, and relatively low yield). In this regard, the development of highly efficient, environmentally safe, and waste-

free technologies for obtaining products with specified properties is an urgent scientific and practical task.

One of the most promising technologies is SC-CO<sub>2</sub> extraction, which has several advantages (high diffusion coefficient, safety, availability, and economic efficiency).

**Degree of topic development.** One of the key parameters required for scaling SC-CO<sub>2</sub> extraction processes is the yield and solubility of extracted compounds in SC-CO<sub>2</sub>. Currently, studies are known on determining the yield and solubility of rosemary oil (A. Zermane *et al.*, 2010), lavender oil (E. Cruz-Sánchez *et al.*, 2024), clove oil (M.C. Wei *et al.*, 2016), red palm kernels (W.J. Lee *et al.*, 2018), and grape seeds (D.S. Kurabachew *et al.*, 2016) in SC-CO<sub>2</sub>. Also known is work on determining the solubility of carvone (K.H. Kim *et al.*, 1999), the main component of spearmint EO, in SC-CO<sub>2</sub>. However, the solubility of *Mentha spicata* L. and *Ocimum basilicum* L. EO in SC-CO<sub>2</sub> directly from plant raw material by the dynamic method remains a poorly studied area of research.

There are known studies on the evaluation of the oxidative stability of various vegetable oils, including corn oil (M.F. Ramadan *et al.*, 2012) and BCO (M.S. Choudhury *et al.*, 2023). It has been shown that the addition of GSO to corn oil (M. Cisneros-Yupanqui *et al.*, 2021), the introduction of saffron stigmas into linseed oil (M.N. Ahmed *et al.*, 2024), as well as the addition of BCO to rapeseed oil (M. Rudzińska *et al.*, 2016) contribute to increasing their resistance to oxidation. Obtaining functional oil (FO) by SC-CO<sub>2</sub> extraction from black cumin seeds and grape seeds, as well as studying its oxidative stability, remain insufficiently studied.

**Objective of the study:** Determination of optimal conditions for the extraction of essential oils from spearmint (*Mentha spicata* L.) and basil (*Ocimum basilicum* L.), as well as obtaining a functional oil with increased oxidative stability based on vegetable oils of black cumin (*Nigella sativa*) and grape seeds (*Vitis vinifera*) by supercritical CO<sub>2</sub> extraction.

In accordance with the objective, the following **tasks** were set:

1. To determine the conditions for the extraction of essential oils from spearmint *Mentha spicata* L. and basil *Ocimum basilicum* L. by supercritical CO<sub>2</sub> extraction;
2. To calculate the kinetic parameters of mass transfer of essential oils from the cellular structure of plant raw materials into the volume of supercritical CO<sub>2</sub> using mathematical models;
3. To evaluate the oxidative stability of functional oil obtained by supercritical CO<sub>2</sub> extraction from black cumin seeds (*Nigella sativa*) and grape seeds (*Vitis vinifera*);
4. To develop a basic technological scheme for processing plant raw materials and winemaking industry waste.

**Objects of study.** Spearmint (*Mentha spicata* L.), basil (*Ocimum basilicum* L.), black cumin (*Nigella sativa*), grape seeds (*Vitis vinifera*).

**Subject of study.** Investigation of the vacuum drying process with a desiccant. Optimization of conditions for saturating supercritical CO<sub>2</sub> with spearmint and basil essential oils, optimization of supercritical CO<sub>2</sub> extraction parameters for functional oil, study of the oxidative stability of black cumin seed oil, grape seed oil, and functional oil.

**Research methods.** Gravimetric determination of yield, atomic absorption spectroscopy, Soxhlet and Clevenger extraction, supercritical CO<sub>2</sub> extraction, dynamic method for determining solubility, gas chromatography with mass spectrometric detection, oxidative stability (peroxide value, anisidine value, total oxidation value).

**Scientific novelty of the research.**

1. It was established for the first time that the effective extraction of EO from dried leaves of spearmint *Mentha spicata* L. and basil *Ocimum basilicum* L. by SC-CO<sub>2</sub> extraction is possible only under conditions that ensure the capture of components in the separator at a pressure of 10-20 bar and the achievement of phase equilibrium in the extractor due to the SC-CO<sub>2</sub> flow rate of 3 ml/min.

2. The kinetics model of SC-CO<sub>2</sub> extraction (Broken and Intact Cells) was adapted and experimentally verified to describe the extraction of EO from dried leaves of spearmint *Mentha spicata* L., which showed the adequacy of describing the process with a coefficient of determination  $R^2 = 0.9002-0.9789$  and an average absolute relative deviation (AARD) equal to 5.84-28.65 %.

3. For the first time, FO was obtained by co-SC-CO<sub>2</sub> extraction from black cumin seeds and grape seeds, which possesses increased oxidative stability when stored in the dark, manifested in a decrease in the peroxide value by 20-60 % and the anisidine value by 2.5-4 times compared to individual oils.

**Validity and reliability of the obtained results.** All experiments were carried out in triplicate, and a statistical evaluation of the obtained results was performed. The adequacy of the mathematical models used was confirmed by determining the following statistical indicators: coefficient of determination ( $R^2$ ), sum of squares of errors (SSE), average absolute relative deviation (AARD), and root mean square error (RMSE).

**Theoretical and practical significance of the research.** The theoretical significance lies in obtaining new data on the vacuum drying of spearmint (*Mentha spicata* L.) and basil (*Ocimum basilicum* L.) leaves using CaCl<sub>2</sub> as a desiccant, as well as in establishing the parameters of SC-CO<sub>2</sub> extraction (flow rate and pressure in the separator) that affect the yield and solubility of EO. It was established that co-SC-CO<sub>2</sub> extraction of BCO and GSO makes it possible to obtain FO with improved oxidative stability.

The practical significance of the work consists of developing a waste-free technology for obtaining EO and FO. The proposed method of vacuum drying with a desiccant can be used to prepare plant raw materials before extraction. The obtained extraction conditions, solubility values, and mass transfer coefficients can be useful in scaling SC-CO<sub>2</sub> extraction processes, and the proposed technology allows obtaining high-value-added products that are competitive in the market.

**Main provisions submitted for defense.**

1. Efficient extraction of essential oil (spearmint EO yield  $1.70 \pm 0.04\%$ ) from dried leaves of spearmint *Mentha spicata* L. and its saturation in SC-CO<sub>2</sub> (equilibrium concentration of spearmint EO  $0.185 \pm 0.009$  mg/g) is achieved at a flow rate of 3 ml/min and a pressure of 10 bar in the separator.

2. An increase in the density of supercritical CO<sub>2</sub> from 602.58 to 900.30 kg/m<sup>3</sup> leads to an increase in mass transfer coefficients: internal mass transfer from  $0.23 \times 10^{-5}$  to  $0.54 \times 10^{-5}$  m/s, external mass transfer from  $1.62 \times 10^{-5}$  to  $2.31 \times 10^{-5}$  m/s.

3. Co-supercritical CO<sub>2</sub> extraction of oils from black cumin seeds and grape seeds at a pressure of 300 bar in the extractor, a temperature of 50 °C, a pressure of 10 bar in the separator, a CO<sub>2</sub> flow rate of 12 ml/min, and a black cumin seed to grape seed ratio of 1:1 leads to a synergistic increase in the oxidative stability of FO, which is expressed in a decrease in the peroxide value by 10-20 % and the anisidine value by 3-6 times compared to individual oils.

### **Main results of the study.**

1. Vacuum drying with a CaCl<sub>2</sub> desiccant proceeds 21.1% faster than without it. The cost of drying 1 kg of fresh spearmint *Mentha spicata* L. leaves is 26.5% cheaper than without a desiccant. The EO content in the extract after vacuum drying at T=40 °C: with desiccant –  $0.90 \pm 0.22\%$ , without desiccant –  $0.83 \pm 0.12\%$ . The main compound of the spearmint *Mentha spicata* L. EO is carvone, the content of which is 51.7%.

2. The EO yield increased from  $1.5 \pm 0.05$  to  $1.6 \pm 0.09\%$  when increasing the pressure in the separator from 10 to 30 bar. With a further increase in pressure, the yield rapidly drops to  $0.1 \pm 0.06\%$ . As the pressure in the separator increases, the yield of volatile compounds decreases, while the yield of high-molecular compounds increases. At a CO<sub>2</sub> flow rate of 3 ml/min, equilibrium is achieved between the spearmint *Mentha spicata* L. EO and SC-CO<sub>2</sub>, which is confirmed by the yield and equilibrium concentration reaching maximum values.

3. It was established that the linear driving force model describing the mass transfer of EO components does not provide an adequate description of the kinetics of SC-CO<sub>2</sub> extraction of spearmint *Mentha spicata* L. EO ( $R^2 = 0.7138-0.9058$ , AARD = 30.5-42.1 %). It was shown that the broken and intact cells model, which takes into account the different availability of components localized in broken and intact cells, adequately describes the extraction process ( $R^2 = 0.9002-0.9789$ , AARD = 5.84-28.65 %).

4. Increasing the density of supercritical CO<sub>2</sub> from 602.58 to 900.30 kg/m<sup>3</sup> leads to intensification of mass transfer processes. In this case, the internal mass transfer coefficient increases from  $0.23 \times 10^{-5}$  to  $0.54 \times 10^{-5}$  m/s (by 2.3 times), and the external mass transfer coefficient increases from  $1.62 \times 10^{-5}$  to  $2.31 \times 10^{-5}$  m/s (by 1.4 times).

5. It was found that BCO is characterized by a higher rate of primary oxidation compared to GSO, which is expressed in an increase in the peroxide value by 62.5% over 5 days. At the same time, FO demonstrates increased oxidative stability: the increase in the peroxide value is 33% and is observed over 12 days, indicating a slowdown of the primary oxidation processes. At a later storage stage (after 19 days), an increase in secondary oxidation was noted in BCO (increase in anisidine value by 51%), whereas no significant changes were detected in GSO and FO, indicating the suppression of the formation of secondary oxidation products.

**Connection of the dissertation with research and state programs.** This research was carried out within the framework of: grant funding for young scientists under the project "Zhas Galym" for 2025-2027 on the topic "Study of the stability of black cumin

and grape seed oil due to the synergistic effect observed during combined supercritical CO<sub>2</sub> extraction" IRN AP25796043.

**Author's contribution to the research work.** The author conducted a detailed review of the scientific literature on raw material drying methods, extraction methods and evaluation of EO solubility, vegetable oil extraction methods, and the effect of combining vegetable oils on oxidative stability. The author performed work on the study of vacuum drying of spearmint (*Mentha spicata* L.) and basil (*Ocimum basilicum* L.) leaves with/without desiccant, and on the study of the influence of extraction parameters on the yield and solubility of spearmint (*Mentha spicata* L.) and basil (*Ocimum basilicum* L.) essential oils. The author took direct part in the preparation of scientific publications, abstracts, and the dissertation.

**Publications.** The results of the completed work are reflected in the following scientific works:

1. **Rustam Tokpayev, Zair Ibraimov\***, Khavaza Tamina, Bauyrzhan Bukenov, Bagashar Zhaksybay, Amina Abdullanova, Yekaterina Chshendrygina, Kanagat Kishibayev and **Luca Fiori**. Supercritical fluid CO<sub>2</sub> extraction of essential oil from spearmint leaves dried by vacuum drying with a desiccant // Foods, No. 15 (2), 2026. Scopus percentile 99%, WoS Q1, IF = 5.1. <https://10.3390/foods15020213>. (methodology, investigation, formal analysis, software, writing-original draft, writing-review and editing, conceptualization).

#### Conference Presentations

1. **Ibraimov Z.T., Tokpayev R.R.**, Zhaksybay B.B., Khavaza T.N., Nauryzbayev M.K., **Luca Fiori**. Study of the solubility of spearmint *M. spicata* L. essential oil in supercritical CO<sub>2</sub> // Proceedings of the IV International Symposium dedicated to the 91st anniversary of Professor Lev A. Ostroumov, September 18–19, 2025, pp. 166–169. (methodology, investigation, conceptualization).

2. **Ibraimov Z.T.**, Abdullanova A.M., Zhaksybay B.B., Dyusenkulova B.Zh. Supercritical CO<sub>2</sub> extraction of basil essential oil // Proceedings of the International Conference of Students and Young Scientists “Farabi Alemi”, Almaty, April 4–6, 2024, pp. 26–27. (methodology, investigation, conceptualization).

3. **Ibraimov Z.T.**, Khavaza T.N., Zhaksybay B.B., Abdullanova A.M., **Tokpayev R.R.** Investigation of supercritical CO<sub>2</sub> extraction processes of spearmint *M. spicata* L. essential oil // Abstracts of the XII International Beremizhanov Congress on Chemistry and Chemical Technology, Almaty, December 4–6, 2024, p. 205. (methodology, investigation, conceptualization).