

Supercritical CO₂ extraction: Methodological approaches and practical applications for vaporizing agro-industrial secondary products

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ABSTRACT

Secondary products from the agro-industrial complex in the Republic of Moldova are potential resources for biorefineries, that is, which process secondary bioproducts into raw materials for the production of value-added products. Rich in multifunctional biologically active compounds—including fatty and essential oils, phenolic substances, dietary fibers and other metabolites with demonstrated health benefits—they offer significant potential for the development of value-added ingredients. Supercritical CO₂ extraction (SFE-CO₂) provides a sustainable, selective and solvent-free method for isolating such compounds, particularly from plant- and animal-derived residues. This research presents a methodological overview and practical case applications of SFE-CO₂ for the valorization of agro-industrial by-products. Emphasis is placed on extraction principles, selective programming strategies and the technological advantages of supercritical processing. Examples from grape pomace, *Ginkgo biloba* leaves, insect biomass and other secondary materials illustrate the capacity of SFE-CO₂ to obtain high-quality extracts suitable for use in dietary supplements, food formulations, cosmetic products and pharmaceutical applications. The analysis demonstrates that SFE-CO₂ is a key technology for implementing innovative, waste-free biorefinery processes.

Keywords: agro-industrial complex, bioactive additives, dietary supplements, raw materials, secondary bio products, supercritical fluid extraction, SFE technologies, waste-free production.

Principles of Supercritical CO₂ Extraction

Supercritical fluid extraction (SFE) of natural materials, both of plant and animal origin, using solvents such as carbon dioxide is attracting growing scientific and industrial interest, as it represents a promising direction for advanced processing technologies and waste-free production. Carbon dioxide (CO₂) is the most commonly used solvent in SFE due to its safety, non-toxicity, non-flammability, affordability and complete removability from the final product. Moreover, CO₂ possesses low critical parameters and behaves as a hydrophobic, non-polar solvent, which facilitates the solubilization of small and low-polarity molecules (Reverchon, 2006; Agostini *et al.*, 2012).

The critical temperature of CO₂, close to ambient temperature, makes it particularly suitable for extracting thermolabile compounds without inducing chemical degradation. As a result, SFE allows processing of plant materials at relatively low temperatures, which minimizes microbiological contamination and prevents thermal decomposition of native substances,

while simultaneously eliminating the use of toxic organic solvents. For these reasons, SFE technologies are now firmly positioned within the family of green chemistry methods. Beyond its environmental advantages, SFE is fundamentally a **separation technology**, capable of selectively isolating a broad range of compounds present in natural substrates. Consequently, SFE has been increasingly applied in the food, cosmetic and pharmaceutical industries for the extraction and purification of natural active ingredients at both laboratory and industrial scales. Applications include the isolation of bioactive and herbal preparations, vitamins, essential and fatty oils, phytoncides, waxes, lipids and phytosterols from plant and animal raw materials—making it highly suitable for the processing of secondary products originating from numerous industrial sectors.

Key qualitative features of SFE driving its adoption include: complete absence of solvent residues in the final products; higher extraction efficiency compared to traditional liquid-liquid extraction; the ability to fine-tune the solvent power of supercritical CO₂ over a wide range of pressures and temperatures; compatibility with co-solvents when processing moderately polar or polar compounds (Reverchon *et al.*, 2006; Agostini *et al.*, 2012).

From a scientific standpoint, the most distinctive feature of supercritical fluids is their **programmable dissolving ability**, a universal property absent in traditional solvents and liquefied subcritical gases. This characteristic has stimulated extensive global research on the applied use of controlled solubility and other programmable properties of supercritical fluids in modern separation processes (Sovová, 1994; McHugh *et al.*, 1994). the laboratory-scale CO₂ SFE extractor (1-liter capacity) presented in this study, together with a schematic representation of the extraction workflow (Figure 1).

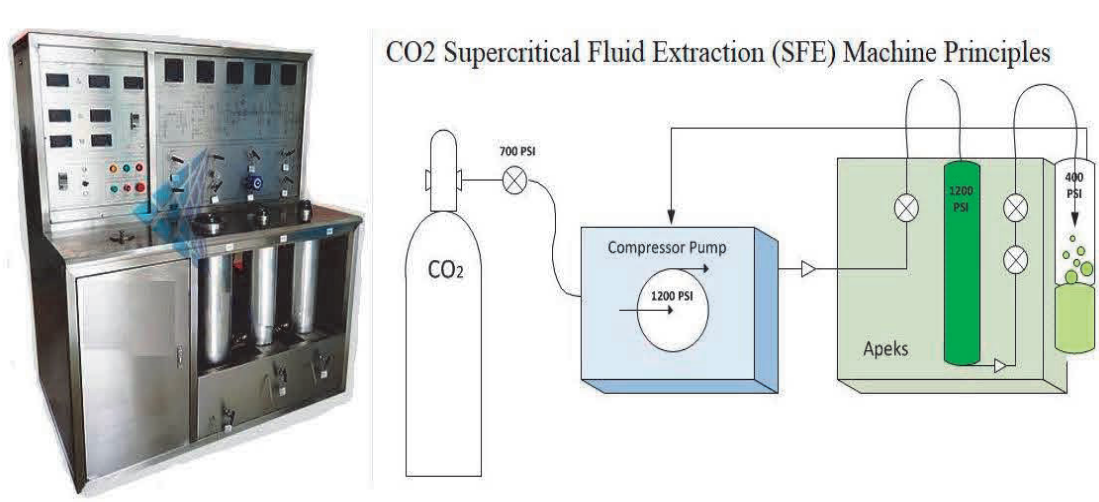


Figure 1. Photo of a laboratory SCF CO₂ extractor machine with a volume of 1 liter of loading source and schematic diagram of the technological process of supercritical fluid extraction.

Methodological Approaches for Selective SFE

A distinctive advantage of supercritical fluid extraction (SFE) is the ability to program the dissolving capacity of the supercritical solvent, enabling selective removal of undesired compounds while isolating target molecules in concentrated fractions (Pourmortazavi *et al.*, 2007; Díaz-Reinoso *et al.*, 2006). Through precise adjustment of pressure and temperature, it is possible to suppress the solubility of heavy, non-volatile constituents and simultaneously enhance the solubilization of desired low-molecular-weight compounds.



Figure 2a. Representatives of modern equipment for separating SCF extractor machines.



Figure 2b. Final products of SCF separation of various components of plant raw materials.

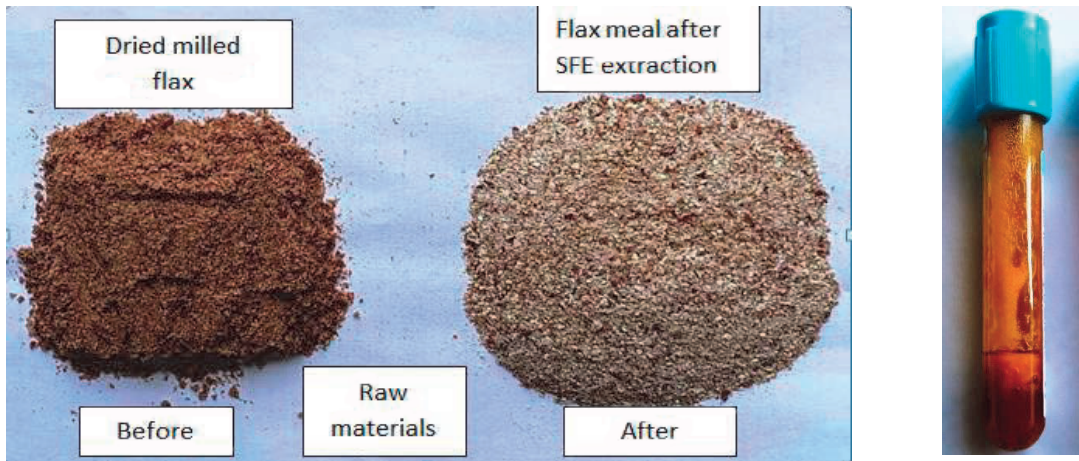


Figure 3. Vegetable raw materials before and after CO₂-SFE extraction and the final product

For example, during the extraction of essential-oil-bearing plants, SFE parameters can be programmed so that volatile terpenoids, responsible for the aromatic and functional profile of the extracts—are selectively dissolved in SCF-CO₂. Meanwhile, high-molecular-weight lipids, waxes, carotenoids and other heavy components remain insoluble and are excluded from the final product. This selective behavior is illustrated by the modern SCF extraction equipment (Figure 2a), which enables precise control of extraction parameters, and by the fractionated plant-derived products obtained through SCF separation (Figure 2b).

An important methodological development in SFE technology is the stepwise or fractionated separation of molecules with different physicochemical characteristics. Using multi-stage separators, substances contained in a single raw material can be separated into multiple distinct fractions during one extraction cycle (Arumugham *et al.*, 2021; Jokić *et al.*, 2016). This strategy greatly enhances the efficiency and economic value of SFE in biorefinery applications.

In addition to solubility programming, the selectivity of SFE can be improved through the use of co-solvents, such as ethanol or diethyl ether. These enhance the extraction of moderately polar or nonionic compounds from hydrophobic matrices. In applications requiring highly clean analytical samples—such as chromatographic-mass spectrometric analysis of pharmaceutical creams—non-polar supercritical solvents (e.g., low-molecular-weight fluoroalkanes) may replace CO₂ to minimize co-extraction of fatty impurities (Taylor, 1996; Clifford, 1998).

Moreover, selective extraction can be further advanced by incorporating chemical modifying agents into the supercritical phase. A well-documented example is the extraction of dicarboxylic acids, which cannot dissolve in SCF-CO₂ due to their high polarity and strong hydrogen bonding. However, when tertiary alkylamines are introduced during the SFE process, the solubility of these acids increases significantly, allowing their selective isolation in pure form after removal of the modifier (Chiu *et al.*, 2002; Wrona *et al.*, 2017; Capuzzo *et al.*, 2013).

Together, these approaches, pressure-temperature programming, co-solvent integration and chemical modification—form a versatile methodological framework that enables precise, selective and clean extraction of a wide spectrum of bioactive compounds from plant and animal biomass.

RESULTS AND DISCUSSIONS

Supercritical CO₂ extraction was applied in the laboratory using a 1-L SFE installation to evaluate the technology's capacity to process diverse natural secondary materials.

The results obtained confirm that SFE-CO₂ provides efficient and selective extraction of valuable bioactive compounds from a wide spectrum of plant- and animal-derived residues. The extracts obtained under the applied operating conditions were clean, free of solvent residues, and characterized by a broad biochemical profile corresponding to the composition of the original raw materials.

A variety of secondary products were processed, including amaranth, *Ginkgo biloba* leaves, dried maggots (fly larvae), plankton/krill, grape seeds and grape pomace, cannabis seeds, flax, corn and other grains.

The chromatogram of the *Ginkgo biloba* extract (Figure 4) illustrates the presence of multiple bioactive components and demonstrates the ability of CO₂-SFE to recover thermolabile compounds without degradation.

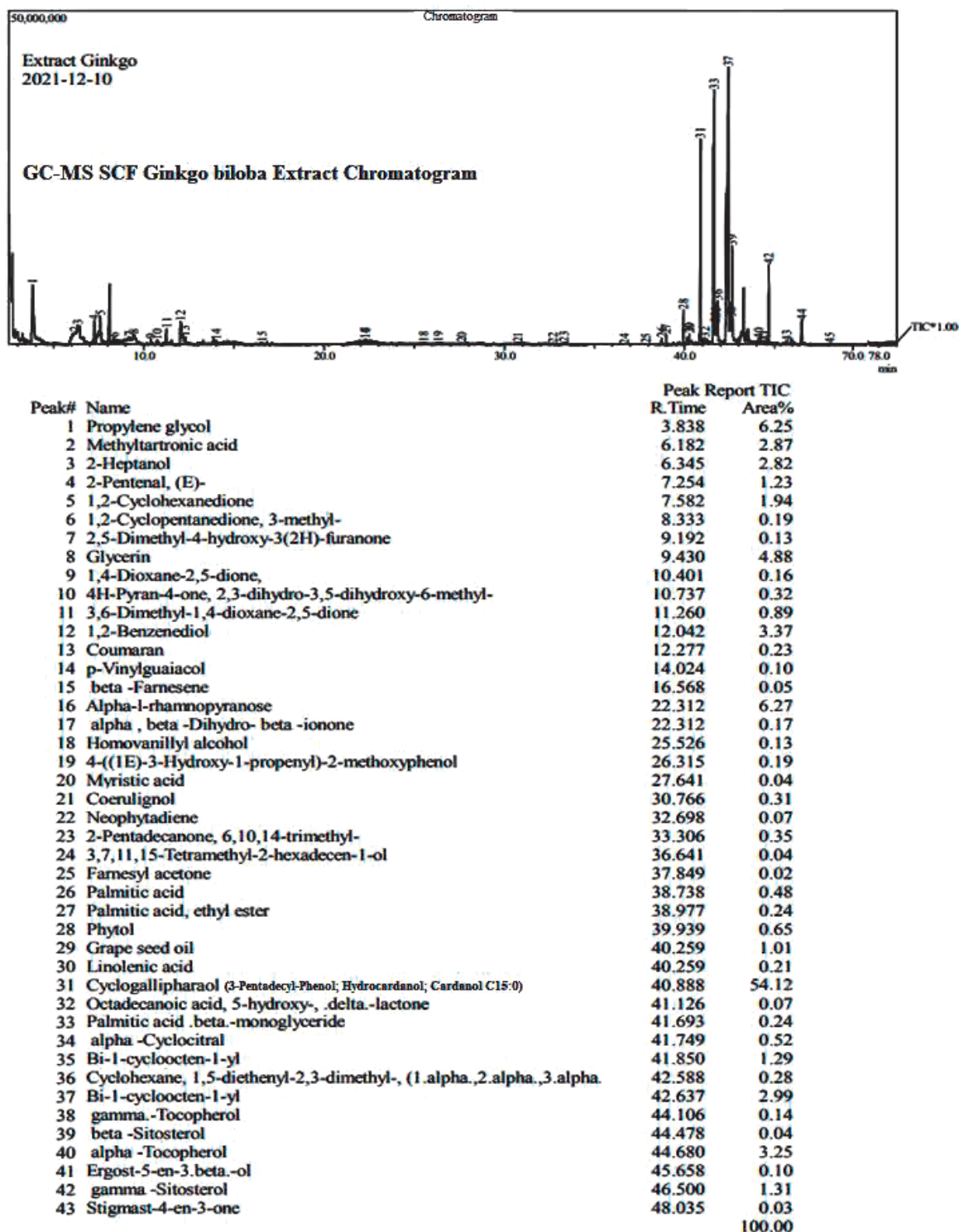


Figure 4. Ginkgo biloba leaves SCF CO₂-extract Chromatogram

Extraction conducted at 35 MPa and 50 °C for 2 hours yielded >15% fatty-wax fraction from the grape pomace of 'Cabernet Sauvignon', 'Saperavi', 'Fetească albă' and 'Fetească neagră' cultivars. The resulting extracts contained a wide range of biologically active compounds, including fatty acids, fatty alcohols and esters, vitamins, phytosterols and other lipidic constituents. Representative chromatograms of grape-derived extracts confirming the extraction selectivity and chemical diversity of these fractions (Figures 5a and 5b).

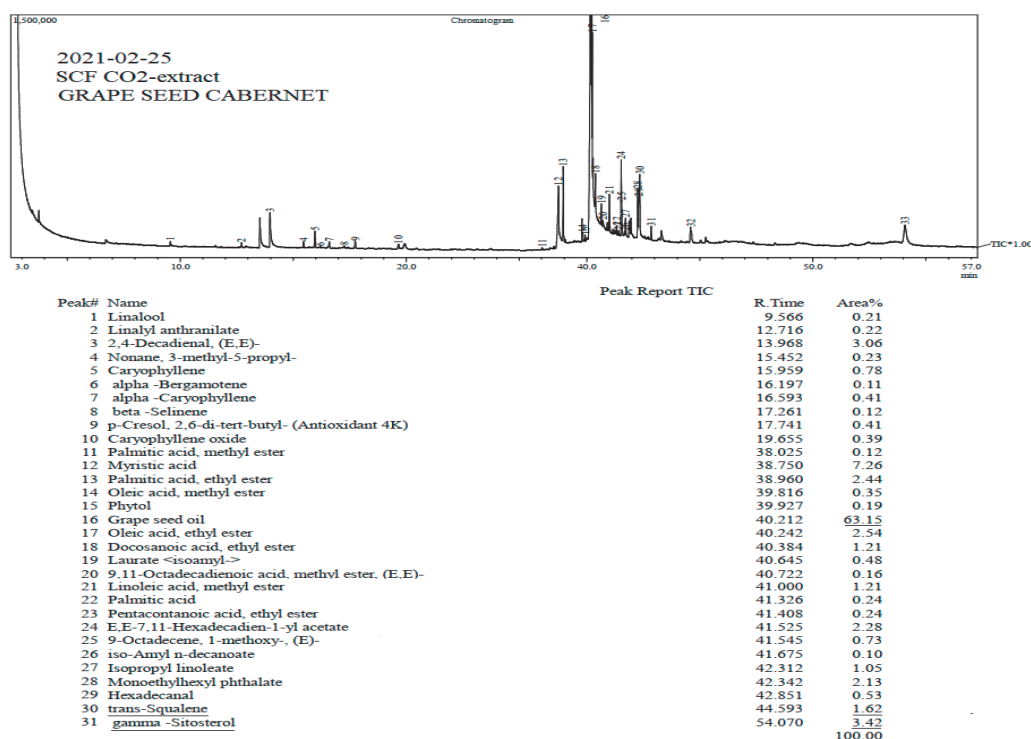


Figure 5a. SCF CO₂ extract chromatogram of Cabernet grape seeds

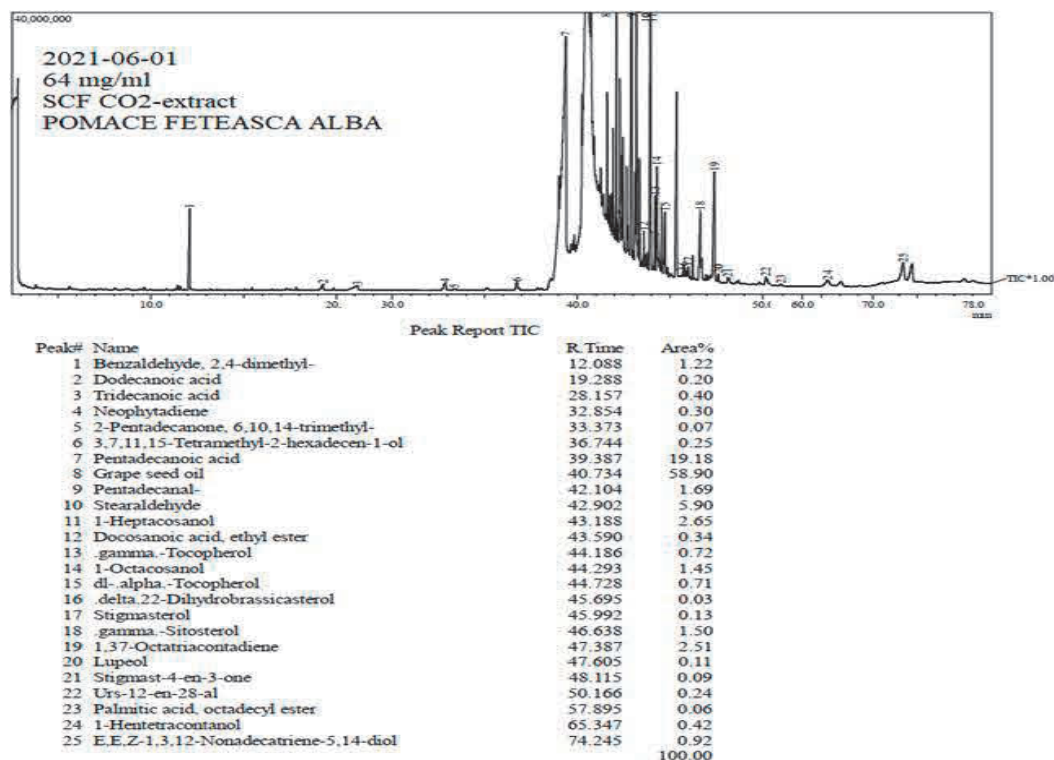


Figure 5b. SCF CO₂ extract chromatogram of 'Fetească albă' grape seeds

Across all processed materials, SFE-CO₂ produced fractions rich in bioactive molecules, primarily concentrated in the fatty-wax portion of the extracts.

Compared to conventional pressing, SFE-CO₂ generated significantly higher yields and a broader spectrum of extractable substances. The difference is illustrated by comparing the extract composition obtained by classical pressing of dried maggots with that achieved through CO₂-SFE, clearly demonstrating the superior extraction efficiency and selectivity of the SFE method (Figure 6).

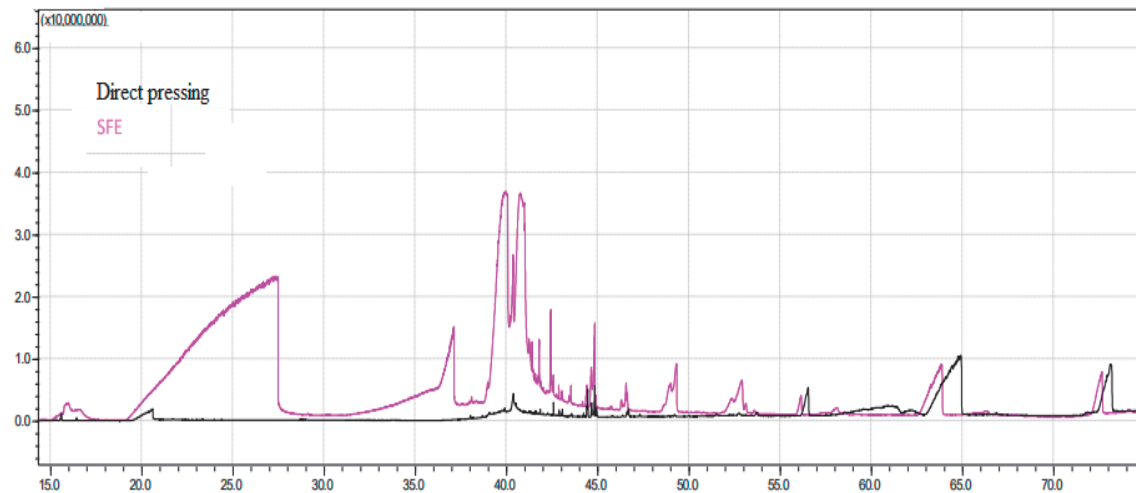
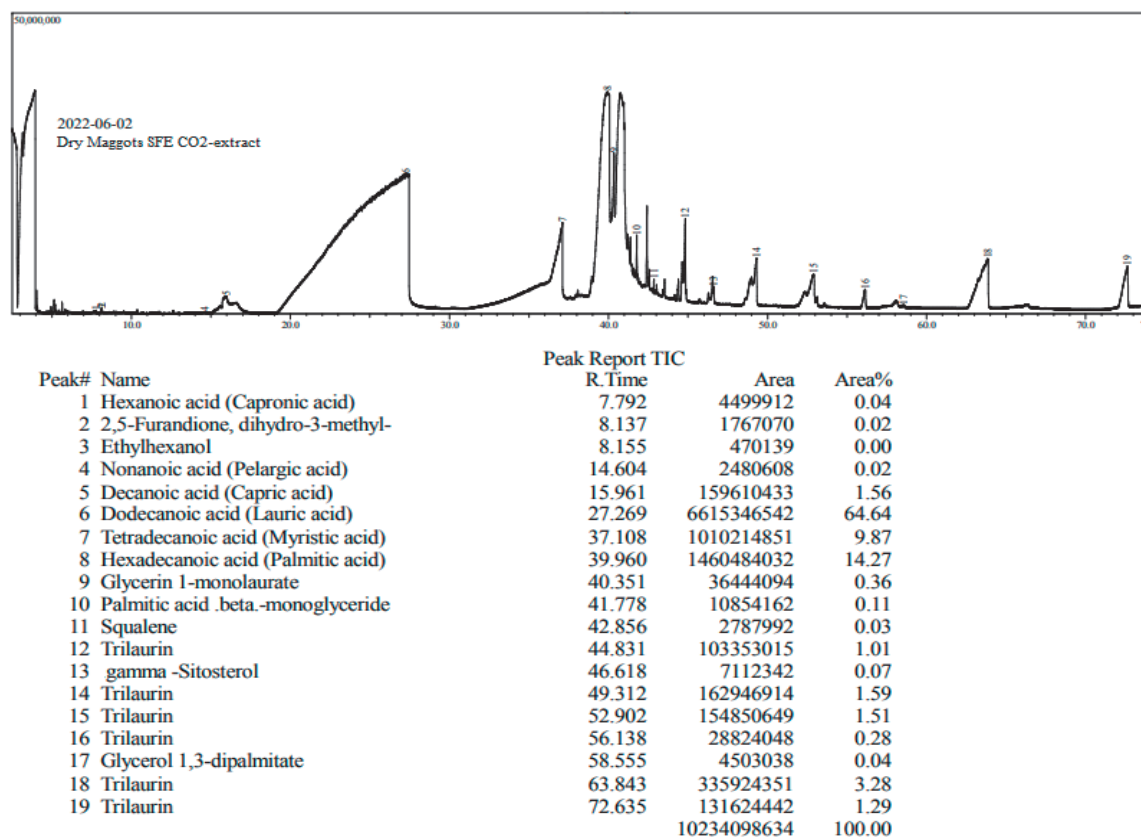


Figure 6. Comparison of extract yield and composition obtained from dried maggots by classical pressing and SFE-CO₂.

To summarize the practical applications demonstrated in this study, provides an overview of the raw materials processed, extract types, representative compounds identified (figures 4–6), extraction outcomes, and application fields of interest (Table 1).

Table 1. Practical applications of SFE-CO₂ on various secondary raw materials

Raw material	Type of extract obtained	Key compounds identified	Extraction outcome mentioned in text	Application potential	Reference
<i>Ginkgo biloba</i> leaves	CO ₂ -SFE extract	Terpenoids, bioactive plant metabolites	Chromatogram shown in Figure 4	Nutraceuticals, phytotherapeutics	Figure 4
Grape pomace ('Cabernet', 'Saperavi', 'Fetească')	Fatty-wax fraction	Fatty acids, fatty alcohols, esters, vitamins, phytosterols	>15% yield at 35 MPa, 50°C, 2 h; Figures 5a–5b	Cosmetics, supplements, food formulations	Figures 5a–5b
Grape seeds	Lipid-based fraction	Fatty acids, vitamin E, sterols	Wide biochemical profile	Cosmetic oils, nutraceuticals	Figures 5a–5b
Dried maggots (fly larvae)	Lipid extract	Fatty acids, nutrients	SFE yield much higher vs. pressing (Figure 6)	Pet nutrition, protein-lipid concentrates	Figure 6
Plankton / krill	Marine lipid fraction	Omega fatty acids, wax esters	Obtained by SFE-CO ₂ in laboratory tests	Marine nutraceuticals	Author's data
Amaranth, cannabis, flax, corn seeds	Seed oil fractions	Fatty acids, sterols, vitamins	Extracts obtained successfully by SFE-CO ₂	Functional food, cosmetic oils	Author's data

Technological considerations and practical outcomes

The practical applications presented in this study demonstrate that supercritical CO₂ extraction (SFE-CO₂) is not only a selective and clean technology, but also one that provides significant technological advantages over conventional extraction methods. The ability to adjust pressure and temperature enables highly controlled extraction of bioactive compounds from a wide variety of secondary raw materials, including *Ginkgo biloba* leaves, grape pomace, grape seeds, dried insect biomass (maggots), plankton/krill and numerous seed materials.

One of the primary technological benefits of SFE-CO₂ is its high extraction efficiency, illustrated by the >15% yield of fatty-wax fractions obtained from grape pomace under optimized laboratory conditions (35 MPa, 50 °C, 2 hours). These extracts contain a wide spectrum of biologically active compounds, including fatty acids, fatty alcohols, their esters, vitamins and phytosterols. The chromatographic profiles shown in Figures 4, 5a and 5b confirm the selectivity of the process and the chemical diversity of the extracted fractions.

Another important technological outcome is the superior quality of extracts, which are free of solvent residues and require minimal or no post-processing. Compared to classical pressing, SFE-CO₂ produced both a higher yield and a broader range of extractable substances, as illustrated in Figure 6 for dried maggots. This highlights the technological advantage of SFE-CO₂ for processing lipid-rich insect biomass and other unconventional protein-fat sources.

The method also enables comprehensive valorization of raw materials, as the programmable solubility of supercritical CO₂ allows recovery of multiple fractions from a single substrate

through stepwise separation. This contributes to waste minimization and increases the economic value of secondary products, aligning with modern biorefinery principles.

In practical terms, the extracts obtained by SFE-CO₂ in this study exhibit potential applications in the nutraceutical, food, cosmetic and pharmaceutical sectors. The ability to process thermolabile materials without degradation, combined with the absence of residual solvents, positions SFE-CO₂ as a technologically sound and environmentally compatible extraction method.

Overall, the laboratory results confirm that SFE-CO₂ is a robust, efficient and scalable technology capable of transforming diverse secondary materials into high-value bioactive products suitable for multiple industrial uses.

CONCLUSIONS

The methods of fine programming and customization of selective supercritical CO₂ extraction described in this work represent only a portion of the strategies currently implemented in the rapidly developing field of SFE technology. Owing to its highly tunable physicochemical properties, SFE-CO₂ offers virtually unlimited potential for the efficient isolation of valuable components from both plant and animal raw materials, as well as from secondary products and accumulated biowaste generated across various industrial sectors.

The expanding commercial applicability of SFE-CO₂ confirms its technological relevance. It is increasingly employed in the production of dietary supplements, nutraceuticals, specialized cosmetic ingredients, pharmaceutical preparations, flavoring substances and food-grade extracts, including highly purified individual compounds. The technology places no inherent limitations on the type of raw material that can be processed, and current industrial applications already include residues from fruit and vegetable processing, berry seeds, and other agro-industrial by-products.

Overall, CO₂-based supercritical extraction has emerged as a modern, environmentally compatible and highly efficient technology. Its selectivity, solvent-free processing, and capacity to valorize secondary materials position SFE-CO₂ as a key enabling technology for sustainable biorefinery models and for the development of high-value bioactive products.

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REFERENCES

1. Agostini, F., Bertussi, R.A., Agostini, G., Atti dos Santos, A.C., Rossato, M., Vanderlinde, R. (2012) Supercritical extraction from vinification residues: fatty acids, α -tocopherol, and phenolic compounds in the oil seeds from different varieties of grape. *Scientific World Journal*, <https://doi.org/10.1100/2012/790486>
2. Aizpurua-Olaizola, O., Ormazabal, M., Vallejo, A., Olivares, M., Navarro, P., Etxebarria, N., Usobiaga, A. (2015) Optimization of supercritical fluid consecutive extractions of fatty acids and polyphenols from *Vitis vinifera* grape wastes. *Journal of Food Science*, 80 (1), pp. E101–E107. doi:10.1111/1750-3841.12715
3. Arumugham, Th., Rambabu, K., Hasan, Sh. W., Show, P. L. (2021) Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications – A review. *Chemosphere*, 271, 129525. <https://doi.org/10.1016/j.chemosphere.2020.129525>
4. Capuzzo, A., Maffei, M.E., Occhipinti, A. (2013) Supercritical fluid extraction of plant flavors and fragrances. *Molecules*, 18, pp. 7194–7238. <https://doi.org/10.3390/molecules18067194>
5. Chiu, K.-L., Cheng, Y.-C., Chen, J.-H., Chang, C. J., Yang, P.-W. (2002) Supercritical fluids extraction of *Ginkgo* ginkgolides and flavonoids. *The Journal of Supercritical Fluids*, 24 (1), pp. 77–87. [https://doi.org/10.1016/S0896-8446\(02\)00014-1](https://doi.org/10.1016/S0896-8446(02)00014-1)
6. Clifford, T. (1999) *Fundamentals of supercritical fluids*. Oxford: Oxford Science Publications. ISBN 978-0-19-850137-4.

7. Díaz-Reinoso, B., Moure, A., Domínguez, H., Parajó, J. C. (2006) Supercritical CO₂ extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*, 54 (7), pp. 2441–2469. <https://doi.org/10.1021/jf052858j>
8. Duba, K.S., Fiori, L. (2015) Supercritical CO₂ extraction of grape seed oil: effect of process parameters on the extraction kinetics. *The journal of supercritical fluids*, 98, pp. 33-43. <https://doi.org/10.1016/j.supflu.2014.12.021>
9. Fiori, L., Lavelli V., Duba K. S. *et al.* (2014) Supercritical CO₂ extraction of oil from seeds of six grape cultivars: modeling of mass transfer kinetics and evaluation of lipid profiles and tocol contents. *The journal of supercritical fluids*, 94, pp. 71-80. <https://doi.org/10.1016/j.supflu.2014.06.021>
10. Jokić, S., Bijuk, M., Aladić, K., Bilić, M., Molnar, M. (2016) Optimisation of supercritical CO₂ extraction of grape seed oil using response surface methodology. *International Journal of Food Science and Technology*, 51, p. 403. <https://doi.org/10.1111/ijfs.12986>
11. McHugh, M.A., Krukonis, V.J. (1994) *Supercritical fluid extraction – principles and practice*. Butterworth-Heinemann Series In Chemical Engineering (2nd Edition). ISBN 0-7506-9244-8
12. Pourmortazavi, S.M., Hajimirsadeghi, S.S. (2007) Supercritical fluid extraction in plant essential and volatile oil analysis. *Journal of Chromatography A*, 1163 (1-2), pp. 2-24. <https://doi.org/10.1016/j.chroma.2007.06.021>
13. Reverchon, E., De Marco, I. (2006) Supercritical fluid extraction and fractionation of natural matter. *The Journal of Supercritical Fluids*, 38 (2), pp. 146-166. <https://doi.org/10.1016/j.supflu.2006.03.020>
14. Sovová, H. (1994) Rate of the vegetable oil extraction with supercritical CO₂—I. Modelling of extraction curves. *Chemical Engineering Science*, 49 (3), pp. 409-414. [https://doi.org/10.1016/0009-2509\(94\)87012-8](https://doi.org/10.1016/0009-2509(94)87012-8)
15. Taylor, L. T. (1996) *Supercritical fluid extraction*. John Wiley and Sons, Inc. ISBN 978-0-471-11990-6. <https://www.wiley.com/en-us/Supercritical+Fluid+Extraction-p-9780471119906#aboutauthors-section>
16. Turner Ch., Eskilsson C. S., Björklund E. (2002) Collection in analytical-scale supercritical fluid extraction. *Journal of chromatography A*, 947 (1), pp. 1-22. [https://doi.org/10.1016/s0021-9673\(01\)01592-8](https://doi.org/10.1016/s0021-9673(01)01592-8)
17. Wrona, O., Rafińska, K., Możejński, C., Buszewski, B. (2017) Supercritical fluid extraction of bioactive compounds from plant materials. *Journal of AOAC International*, 100 (6). <https://doi.org/10.5740/jaoacint.17-0232>