

Semicontinuous Lixiviation Process for Compound Extraction from *Cannabis sativa* grown in Colombia

Proceso semicontinuo de lixiviación para la extracción de compuestos a partir de *Cannabis sativa* cultivado en Colombia

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ABSTRACT

The extraction of compounds present in *Cannabis sativa* biomass from Colombian crops was studied using a semi-continuous lixiviation process. To this effect, three extraction stages were implemented, in which successive transfers were carried out at fixed times, seeking to emulate a continuous countercurrent process. In this way, the intention is to provide the Colombian agroindustry with an extraction method that is efficient and requires a lower initial investment than other techniques such as supercritical fluid extraction. Absolute ethanol was used as solvent, and constant temperature and stirring speed were applied. The obtained results indicate that, once the process is stabilized, extracted mass percentages (with respect to the total mass on a dry basis) of 10,5% at 40 °C and 9,5% at 19 °C are achieved, which are competitive compared to the 11,07% achieved through Soxhlet extraction. Besides, the proposed process is clearly a better alternative than a single-stage extraction method, through which it was possible to extract 5% of the mass. Five cannabinoids were identified in the obtained extract, and, with the proposed process, it was possible to extract 66% of cannabidiol present in the original biomass.

Keywords: *Cannabis sativa*, lixiviation, agroindustry, semicontinuous process, solvent extraction

RESUMEN

Se estudió la extracción de compuestos presentes en la biomasa de *Cannabis sativa* de cultivos colombianos mediante un proceso de lixiviación semicontinuo. Para tal fin se implementaron tres etapas de extracción, en las cuales se realizaron transvases sucesivos en tiempos determinados, buscando emular un proceso continuo a contracorriente. De esta forma se pretende proporcionar a la agroindustria colombiana un método de extracción que sea eficiente y requiera una inversión inicial más baja que otras alternativas tales como la extracción con fluidos supercríticos. Se empleó etanol absoluto como solvente y se aplicaron temperaturas y velocidades de agitación constantes. Los resultados obtenidos muestran que, una vez estabilizado el proceso, se logran porcentajes de masa extraída (con respecto a la masa total en base seca) del 10,5 % a 40 °C y de 9,5% a 19 °C, los cuales son competitivos frente al 11,07% alcanzado con extracción Soxhlet. Además, el proceso propuesto es una alternativa mejor que el método de extracción de una sola etapa, mediante el cual se logró extraer el 5% de la masa. Se identificaron cinco cannabinoides en el extracto obtenido y, con el proceso propuesto, se logró extraer el 66% del cannabidiol presente en la biomasa original.

Palabras clave: *Cannabis sativa*, lixiviación, agroindustria, proceso semicontinuo, extracción con solventes

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Introduction

Although the *Cannabis sativa* plant comes from Central Asia and has been cultivated for more than 5 000 years (Booth and Bohlmann, 2019), it has been stigmatized for decades due to its psychoactive compounds. However, due to the use of these compounds for medicinal purposes and the presence of others, including phytocannabinoids, terpenes, and flavonoids, (Koltai and Namdar, 2020), that are of interest in other sectors such as the pharmaceutical, nutraceutical, cosmetic, and pesticide industries (Fiorini et al., 2019), many changes have begun worldwide in the legislation for the regulation for the growth and manufacture of its derivatives.

Particularly in Colombia, Decree 2467 of 2015 (which regulates growth, processes, possession of seeds, and manufacture of derivatives, among others) and Decree 613

of April 2017 (access to medical and scientific cannabis) have generated an attractive alternative that can contribute to the agro-industrial, technical, scientific, and economic development of the country.

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The spectrum of compounds that can be extracted from the cannabis plant that are of interest in terms of their scientific, medicinal, and industrial use is very diverse. In fact, more than 500 compounds have been identified (Elkins *et al.*, 2019), which are distributed in different families. For example, Delgado-Povedano *et al.* (2019) analyzed 17 cultivars and identified 169 compounds in them (22 cannabinoids, 70 terpenoids, 31 lipids, 16 flavonoids, 6 amino acids, 5 organic acids, 4 benzenoids, 4 organic oxygen compounds, 3 hydrocarbons, 3 carbohydrates, 2 organoheterocyclic compounds, 2 organonitrogen compounds, and 1 alkaloid).

Additionally, for medicinal purposes, the extraction procedure is critical because it influences the profile of the preparations that can be obtained; in particular, hard decarboxylation conditions lead to not having terpenes, while milder conditions allow obtaining terpene-rich preparations (Ternelli *et al.*, 2020). Among the medicinal uses of cannabis derivatives, it is worth highlighting pain relief in patients with cancer, chronic pain, multiple sclerosis-related pain, inflammation reduction, and treatment of symptoms related to neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and epilepsy (Koltai and Namdar, 2020).

It should also be considered that not only are the process conditions or the difference in cultivated varieties important, but the different growing conditions and the regions in which the sowing is done also influence the characteristics of the plants obtained (Amaducci *et al.*, 2008). In fact, even the position of the flower on the plant affects the composition of the extracts (Namdar *et al.*, 2018). Therefore, it is not correct to generalize the content of compounds in different varieties of cannabis that have been cultivated and characterized in different countries (or in different regions, even if they belong to the same variety or cultivar).

Regarding the procedures for the extraction of compounds, steam distillation and hydrodistillation are known alternatives, but there are other commonly available processes as well, such as mechanic techniques (cold-pressing), solvent extraction, ultrasonic-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and supercritical fluid extraction (SFE) (Baldino *et al.*, 2020). However, in comparison with conventional extraction methods, SFE has many advantages, such as a selective extraction, short processing times, a solvent-free product, low running costs, and low impact on the environment (Qamar *et al.*, 2021). Therefore, SFE is one of the most used techniques in commercial practice, due to the selectivity it allows, as well as the use of green solvents such as CO₂ (Moreno *et al.*, 2020; Da Porto *et al.*, 2012).

Specifically, SFE with supercritical CO₂ (SC-CO₂) is considered to be a green extraction technique, and it has been successfully employed in the extraction of different compounds from various vegetal matrices such as *Artemisia annua L* (to obtain antimalarial compounds), as well as in other fields such as micronization, nanonization, composite

microparticle formation, membrane production, and biomedical applications (Baldino *et al.*, 2017).

As for *Cannabis sativa L*, SFE with CO₂ has been used for compound extraction due to its characteristics. However, research still needs to be done in order to improve this extraction process. In particular, the different solubilities of the various compound families must be taken into account, since essential oil compounds show larger solubilities in SC-CO₂ than cannabinoids, and they are more soluble than other compounds with higher molecular weights. Therefore, it has been proposed to carry out extractions with sequential pressure increases (Baldino *et al.*, 2020).

For this research, the outlined aspects and the Colombian context are taken into account. The country has suffered the rigor of the war on drugs, and the regulation of cannabis crops is a great opportunity for farmers and local stakeholders to benefit from. Therefore, work is being done on finding alternatives that are not exclusive in terms of initial capital investment and technical understanding of the process. In particular, this research worked with vegetal material (biomass) from a plantation located in the department of Cauca in Colombia, and a solvent extraction process in several stages was carried out, emulating a continuous countercurrent process, which is efficient while relatively simple to understand and apply.

Solvent extraction

A simple process used in practice to extract compounds present in vegetal matrices is solvent extraction. The process of putting the solvent in direct contact with solids (in this case, flowers, leaves, stems, or other parts of the plant that have been previously dried and crushed) is also synonymous with lixiviation.

One way to evaluate the quantity of soluble compounds that are present in the solid and that can be extracted by means of the lixiviation is Soxhlet extraction. This procedure uses a device that allows the vapors of the boiling solvent in a container to ascend in a lateral arm and condense in such a way that they fall on a quantity of solid contained in a permeable cartridge, which is usually made of cellulose. When a sufficient volume of condensate is reached while in contact with the solid, and due to the siphoning effect, the lixivate returns to the solvent container (which is now the lixivate container) through another lateral detachment, and a new cycle begins (Figure 1).

An important characteristic of the Soxhlet process is that it requires a heat supply to bring the solvent to its boiling temperature in each of the cycles, and that the solvent/solid ratio is usually high, so the required energy consumption is quite high.

Even though solvent extraction usually has a higher yield than SFE with supercritical CO₂, (Marzorati *et al.*, 2020), it

does not mean that it is the best choice, since extraction with SFE is usually more selective, green, and it allows obtaining higher-purity extracts. On the other hand, single-stage solvent extraction processes are often time consuming and produce diluted extracts (da Silva *et al.*, 2016).

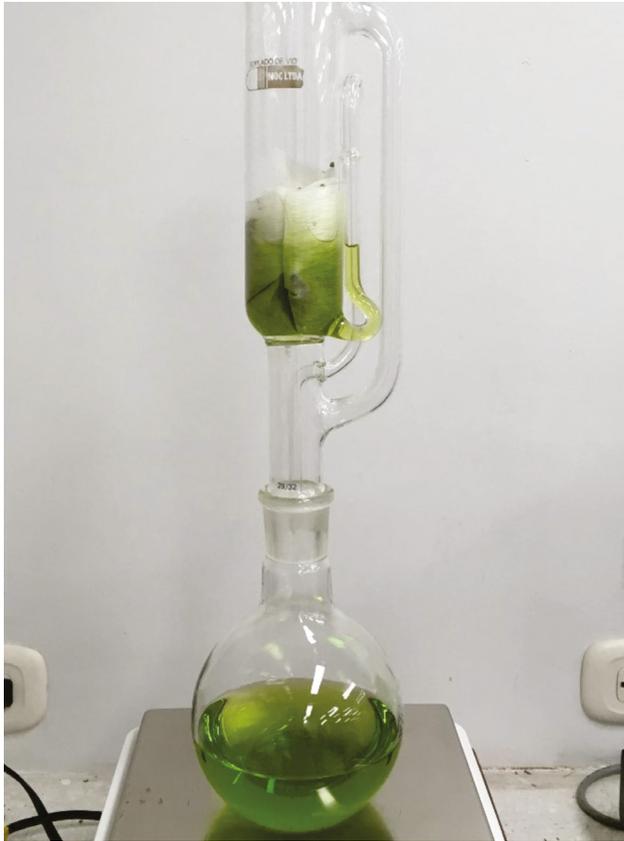


Figure 1. Soxhlet process to obtain compounds from cannabis biomass
Source: Authors

Although there are studies in which solvent extractions from cannabis material are analyzed with the purpose of calibrating analytical methods (Mudge *et al.*, 2017), they are only carried out in a single stage.

Despite the fact that a lixiviation process cannot be carried out continuously in countercurrent, it is possible to arrange a series of extraction tanks and adequately transfer the lixivate to emulate a continuous process, which is the foundation of the proposal presented in this work. Since there is no research that studies extraction from cannabis biomass with solvents in more than one stage, a three-stage process was designed, imitating a continuous countercurrent extraction process that seeks to increase the operation performance and decrease operating time. This three-stage process represents the main contribution of this work.

Selected solvent

Absolute ethanol was used as solvent because it is easy to obtain in Colombia, since, in Valle del Cauca, there are large sugar cane crops and sugar mills that produce ethanol.

This type of alcohol has also been used as a co-solvent in the extraction of compounds from cannabis using the SFE technique (Ribeiro Grijó *et al.*, 2019; Rovetto and Aieta, 2017; Gallo-Molina *et al.*, 2019). Furthermore, ethanol obtained through biotechnological processes from sugar cane is considered to be a green solvent.

Methodology

To extract compounds from the biomass of *Cannabis sativa* in Colombian crops, a ground mixture of stems, leaves, seeds, and some flower fragments was obtained, which was provided by the Finca Interactiva company, with potency of 3,24%. This biomass comes from the Colombian department of Cauca, and it was chosen for the ease of obtaining it and because there are many cannabis crops in the region (Figure 2a).

The cannabis biomass was dried at 70° C for 24 h before being used in the extractions, since the water content forms a barrier during the process (Baldino *et al.*, 2020). Particle size is another factor to consider because, if particles are too small, the caking and channeling phenomena are possible during extraction, thus affecting extraction performance (Baldino *et al.*, 2020).

A test was performed by grinding vegetal material 1 680 µm in size, detecting the inconvenience of the formation of a paste (Figure 2b), which hinders the precipitation of the biomass and makes it impossible to separate the lixivate from the solid to make the transfers. For this reason, it was decided to work with the crushed material as provided by the supplier (Figure 2a).

Regarding the extraction solvent, 99,5% ACS reagent-grade absolute ethanol was used, with a 0,789 g/mL density and 57,3 hPa vapor pressure at 20°C. It was manufactured by PanReac AppliChem S.L.U. and provided by Físicoquímica Integral S.A.S.



Figure 2. a) Cannabis biomass from the department of Cauca, Colombia, and b) formed cake with ground material
Source: Authors

Three jacketed reactors mounted on heating plates with magnetic stirring were connected to perform the extractions (Figure 3). A water tank with a resistance in its interior, coupled with an automatic control, was used to keep the process temperature constant. Once the water reached the process temperature, it was pumped into the jacket of the

first reactor. From there, it went to the second, and then to the third, before returning to the tank. Additionally, the temperature of the stove was also set at a value equal to temperature of the tank, and the ethanol was preheated to that temperature before being used.

The amount of fresh and dry vegetal material that entered the reactors at each stage of the process was 2 g. Each time that fresh solvent was added, it was done in amounts of 40 g. The tests were carried out at room temperature in the city of Bogotá (19 °C) and at 40 °C. The latter was established in order not to move too far from room temperature (it can be 30 °C in the department of Cauca) and stay distant from the normal boiling point of ethanol.

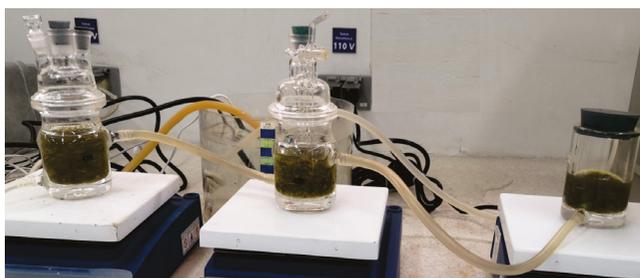


Figure 3. Assembly for the semicontinuous lixiviation of cannabis biomass

Source: Authors

The tests at room temperature were carried out with the purpose of determining if a suitable performance could be achieved, which allows omitting the heating of the solvent. A stirring value of 250 rpm was set. Each stage lasted five minutes and, once that time was completed, the transfers were made according to the scheme shown in Figure 4.

The number on the left in Figure 4 was called 'period' and corresponds to a five-minute interval for extraction plus the time taken by the respective transfers. T1, T2, and T3 refer to the three agitated tanks, FV is the fresh vegetal, FS is the fresh solvent, and the accompanying number refers to the order in which the vegetable and solvent batches entered the system. Each batch of vegetable remains in the system for three periods and does not change tanks. Each solvent batch also remains in the system for three periods but is transferred as indicated by the arrows in Figure 4.

In the same period, the smallest number identifies the exhausted vegetal (which leaves the system at the end of the period), and the largest refers to batch vegetal that has just entered the process. Something similar happens with the solvent (or lixivate): the smallest number identifies the concentrated liquid that leaves the process, and the largest one refers to the fresh solvent that enters to the process.

The three-stage extraction system is stabilized by repeated extraction periods. Eleven lixiviation periods were carried out according to the scheme shown in Figure 4. It is considered that, with this number of periods, the transition state is exceeded and there is a stable process. For comparison

purposes, Soxhlet extraction (for 2 h with a solvent/biomass ratio of 6:1) and single-stage extractions with absolute ethanol (40 g of solvent and 2 g of biomass) were performed.

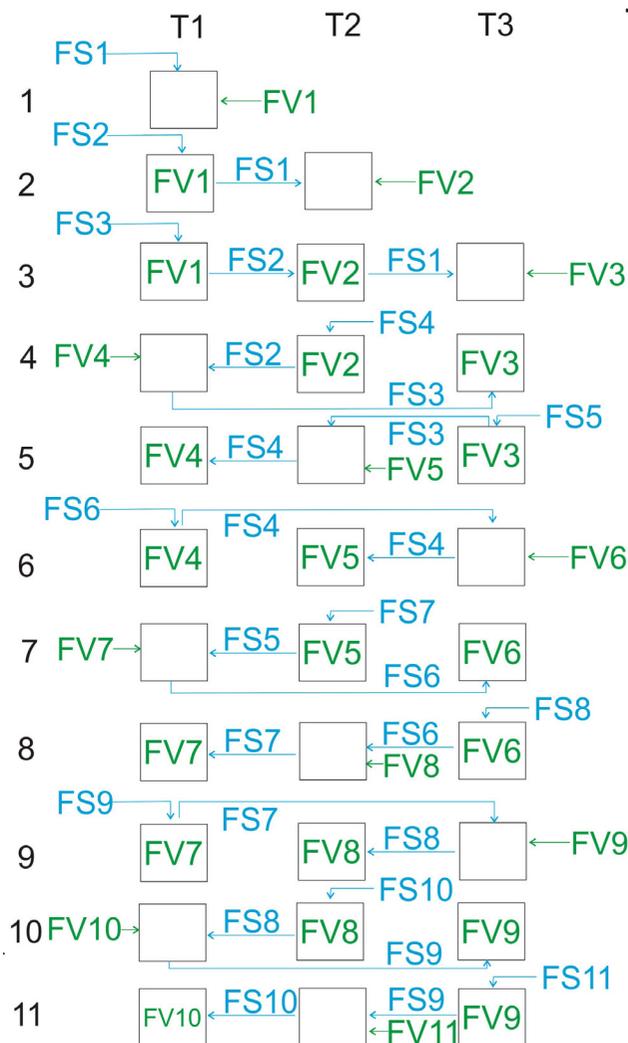


Figure 4. Implemented scheme for transfer between tanks; FS stands for Fresh Solvent and FV indicates Fresh Vegetal

Source: Authors

In the extract (FS9 in period 11) obtained through the proposed process, cannabinoids were identified and quantified, as they are compounds with high added value and with which comparisons can be established with other varieties of cannabis. High Performance Liquid Chromatography (HPLC), according to AOAC-SMPR, was used for this purpose.

Results and discussion

Although the product is described as dry by the supplier, drying tests were carried out on the biomass. The results shown in Table I indicate that the average moisture is 9,41%.

Initially, although it is a qualitative measure, and it is associated with the amount of chlorophyll extracted, the

difference in color can give an idea of the way in which the extraction process progresses. Figure 5 shows the result of a lixiviation carried out with a single stage at 19 °C for 20 min.

Table 1. Moisture of the biomass

Sample	Temperature	Time	Moisture
1	70 °C	48 h	9,38%
2	70 °C	48 h	9,27%
3	70 °C	48 h	9,27%
4	70 °C	48 h	9,73%

Source: Authors



Figure 5. Lixiviate obtained by extraction with ethanol in a single stage at 20 °C

Source: Authors

The extracts obtained in the three stages of period 11 for the proposed process at 40 °C are shown in Figure 6. The contrast in tonality between the lixiviates from the stabilized process is evident.



Figure 6. Color differences in each of the three stages of the stabilized process

Source: Authors

For all the extraction processes performed, the weight percentage of the total extracted compounds was calculated by using the weight difference between the initial dry biomass and the exhausted dry biomass, expressed as a percent of initial dry biomass.

The obtained percentage of extracted mass with the Soxhlet method was 11,07% (weight of extracted compounds/initial

dry biomass weight), whereas single-stage extraction (5 min) obtained a percentage of extracted mass of 2,41% at 19 °C and 3,47% at 40 °C. The performance was higher with increasing contact time; as for the test at 19 °C for 20 min, the percentage of extracted mass reached 5%.

For the proposed semicontinuous lixiviation process, the measurements carried out indicate that, from period six onwards, the process can be considered to be stabilized. However, for a greater certainty regarding a stable process, the reported results correspond to period eleven. With respect to the weight of the final lixiviate obtained in each period, this corresponds to 81% of the weight of fresh solvent initially added in each tank. In other words, about 19% of the solvent remains impregnated in the biomass or is difficult to transfer. For this reason, designing a mechanism that allows increasing the percentage of liquid that can be passed from one tank to another can be an interesting matter to resolve.

For the lixiviates of the three stages of period 11, the percentage of extracted mass was calculated for the processes at 19 °C and 40 °C. The results are shown in Table 2. The initial stage refers to the tank in which the fresh vegetal is in contact with the oldest lixiviate (this liquid leaves the process). In the intermediate stage, there are both vegetal and lixiviate that have already passed through a stage, and, in the final stage, the exhausted vegetal (which has remained longer in the process and will leave it at the final period) and fresh absolute ethanol come in contact.

Table 2. Percentage of extracted mass in each stage of the proposed process (dry basis)

Stage	Description	Biomass	Lixiviate	Yield 19°C	Yield 40°C
1	Initial	Fresh	Saturated	4,0%	4,0%
2	Intermediate	Intermediate	Intermediate	5,5%	6,0%
3	Final	exhausted	Fresh	9,5%	10,5%

Source: Authors

For both studied temperatures, the results indicate that, at the end of the process, a noticeably higher percentage of extracted mass is obtained by the three-stage extraction in comparison with a single-stage process. However, the difference between the process results at 40 °C and at 19 °C is one percentage point.

In comparison with the Soxhlet method, the proposed process (with any of the temperatures studied) exhibits a similar extraction performance, but it must be considered that it is more economic regarding energy consumption, since the vegetal/solvent ratio is lower, and it is not necessary to evaporate the solvent (referring only to the extraction operations). Additionally, the required equipment is simpler, and this process is carried out at lower temperature and pressure levels than the Soxhlet and SFE techniques. Additionally, for the 19 °C process, it seems convenient to run the process with three stages at room temperature and an increase in contact time.

The results of the quantification of identified cannabinoids present in the final extract are shown in Table 3. Since cannabinoids are normally found in the hemp flowers and not in the fiber, stem, or other parts of the plant, low amounts were expected in the extracts. However, the amounts found cannot be considered negligible. Also, surprisingly, a considerable amount (in comparison with non-psychoactive cannabinoids) of D-9 THC is present in the lixiviate.

Based on the results reported in Table 3 and the potency of the used biomass, the extraction yield can be calculated. For comparison purposes, the extraction yield is calculated as μg of compound/g of waste (or dry biomass). The obtained extraction yield is 1,77%, and, particularly for cannabidiol (CBD), it is 11 158 μg /g of waste, which corresponds to 66% of the one found in the original dry biomass.

On the other hand, since the biomass used is a hemp waste threshing, it is possible to compare our results with those obtained through SFE with CO₂ carried out on hemp waste, particularly the resulting dust in a processing plant by separating the fibers from the rest of the stem in hemp grown in North Yorkshire, UK (Attard *et al.*, 2018). In that case, the highest extraction crude yields are 1,57%, obtained with 65 °C and 400 bar.

Table 3. Identified cannabinoids and percentage in the final extract

Cannabinoid	%
Cannabidiol (CBD)	0,1284
Cannabidiolic Acid (CBDA)	0,0203
Cannabichromene (CBC)	0,0056
Cannabigerol (CBG)	0,0203
Delta-9-tetrahydrocannabinol (D9-THC)	0,0294

Source: Authors

The yield of 1,77% achieved with our semi-continuous extraction method does not seem to be significantly higher than the value of 1,57% reported by Attard *et al.* (2018), but it is indeed, since our yield only considers cannabinoids and theirs includes fatty acids, fatty aldehydes, hydrocarbons, sterols, wax esters, and cannabinoids. Considering cannabinoids specifically, the difference is more noticeable. In the case of North Yorkshire hemp dust, 5 832,5 \pm 118,9 μg CBD/g of dust with heptane as solvent and 1 138 μg CBD/g of dust with SFE-CO₂ at 50 °C and 350 bar were extracted (Attard *et al.*, 2018), compared to the 11 158 μg CBD/g of waste obtained with our method. Apart from the differences between the used methods, the characteristics of the vegetal material on which the extractions are made are a major cause of the gap in the results.

Conclusions

A proposal was made for the extraction of compounds from *Cannabis sativa* biomass using a multistage lixiviation process (with absolute ethanol as solvent). The proposed process seeks to imitate a continuous countercurrent

process through the successive transfer of lixiviates. Three extraction tanks with devices for temperature control were used, and the extractions were carried out at 40 °C and at room temperature in the city of Bogotá D.C (19 °C).

The results show that the proposed method is better than conventional single-stage extraction because the yield is more than double. In comparison with the Soxhlet process, our proposal achieves a slightly lower percentage of extracted mass (11,07% and 10,5%, respectively). However, it requires less energy consumption and shorter operation times. Finally, the results of the quantification carried out by HPLC indicate that the content of cannabinoids in the final extract of the stabilized process is 0,204%, with an extraction yield of 11 158 μg CBD/g of waste. This allows us to conclude that the proposed process extracts compounds of high added value that are present in the studied biomass.

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References

- Amaducci, S., Zatta, A., Pelatti, F., and Venturi, G. (2008). Influence of agronomic factors on yield and quality of hemp (*Cannabis sativa* L.) fibre and implication for an innovative production system. *Field Crops Research*, 107(2), 161-169. <https://doi.org/10.1016/j.fcr.2008.02.002>
- Attard, T. M., Bainier, C., Reinaud, M., Lanot, A., McQueen-Mason, S. J., and Hunt, A. J. (2018). Utilisation of supercritical fluids for the effective extraction of waxes and Cannabidiol (CBD) from hemp wastes. *Industrial Crops and Products*, 112, 38-46. <https://doi.org/10.1016/j.indcrop.2017.10.045>
- Baldino, L., Reverchon, E., and Della, G. (2017). An optimized process for SC-CO₂ extraction of antimalarial compounds from *Artemisia annua* L. *The Journal of Supercritical Fluids*, 128, 89-93. <https://doi.org/10.1016/j.supflu.2017.05.018>
- Baldino, L., Scognamiglio, M., and Reverchon, E. (2020). Supercritical fluid technologies applied to the extraction of compounds of industrial interest from *Cannabis sativa* L. and to their pharmaceutical formulations: A review. *Journal of Supercritical Fluids*, 165, 104960. <https://doi.org/10.1016/j.supflu.2020.104960>
- Booth, J. K. and Bohlmann, J. (2019). Terpenes in *Cannabis sativa* – From plant genome to humans. *Plant Science*, 284, 67-72. <https://doi.org/10.1016/j.plantsci.2019.03.022>
- da Porto, C., Voinovich, D., Decorti, D., and Natolino, A. (2012). Response surface optimization of hemp seed (*Cannabis sativa* L.) oil yield and oxidation stability by supercritical carbon dioxide extraction. *Journal of Supercritical Fluids*, 68, 45-51. <https://doi.org/10.1016/j.supflu.2012.04.008>
- da Silva, R. P. F. F., Rocha-Santos, T. A. P., and Duarte, A. C. (2016). Supercritical fluid extraction of bioactive compounds. *TrAC - Trends in Analytical Chemistry*, 76, 40-51. <https://doi.org/10.1016/j.trac.2015.11.013>

- Delgado-Povedano, M. M., Sánchez-Carnerero Callado, C., Priego-Capote, F., and Ferreiro-Vera, C. (2019). Untargeted characterization of extracts from *Cannabis sativa* L. cultivars by gas and liquid chromatography coupled to mass spectrometry in high resolution mode. *Talanta*, 208, 120384. <https://doi.org/10.1016/j.talanta.2019.120384>
- Elkins, A. C., Deseo, M. A., Rochfort, S., Ezernieks, V., and Spangenberg, G. (2019). Development of a validated method for the qualitative and quantitative analysis of cannabinoids in plant biomass and medicinal cannabis resin extracts obtained by super-critical fluid extraction. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1109, 76-83. <https://doi.org/10.1016/j.jchromb.2019.01.027>
- Fiorini, D., Molle, A., Nabissi, M., Santini, G., Benelli, G., and Maggi, F. (2019). Valorizing industrial hemp (*Cannabis sativa* L.) by-products: Cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation. *Industrial Crops and Products*, 128, 581-589. <https://doi.org/10.1016/j.indcrop.2018.10.045>
- Gallo-Molina, A. C., Castro-Vargas, H. I., Garzón-Méndez, W. F., Martínez-Ramírez, J. A., Rivera-Monroy, Z. J., King, J. W., and Parada-Alfonso, F. (2019). Extraction, isolation and purification of tetrahydrocannabinol from the *Cannabis sativa* L. plant using supercritical fluid extraction and solid phase extraction. *Journal of Supercritical Fluids*, 146, 208-216. <https://doi.org/10.1016/j.supflu.2019.01.020>
- Koltai, H. and Namdar, D. (2020). Cannabis phytomolecule "entourage": From domestication to medical use. *Trends in Plant Science*, 25(10), 976-984. <https://doi.org/10.1016/j.tplants.2020.04.007>
- Marzorati, S., Friscione, D., Picchi, E., and Verotta, L. (2020). Cannabidiol from inflorescences of *Cannabis sativa* L.: Green extraction and purification processes. *Industrial Crops and Products*, 155, 112816. <https://doi.org/10.1016/j.indcrop.2020.112816>
- Moreno, T., Montanes, F., Tallon, S. J., Fenton, T., and King, J. W. (2020). Extraction of cannabinoids from hemp (*Cannabis sativa* L.) using high pressure solvents: An overview of different processing options. *Journal of Supercritical Fluids*, 161, 104850. <https://doi.org/10.1016/j.supflu.2020.104850>
- Mudge, E. M., Murch, S. J., and Brown, P. N. (2017). Leaner and greener analysis of cannabinoids. *Analytical and Bioanalytical Chemistry*, 409(12), 3153-3163. <https://doi.org/10.1007/s00216-017-0256-3>
- Namdar, D., Mazuz, M., Ion, A., and Koltai, H. (2018). Variation in the compositions of cannabinoid and terpenoids in *Cannabis sativa* derived from inflorescence position along the stem and extraction methods. *Industrial Crops and Products*, 113, 376-382. <https://doi.org/10.1016/j.indcrop.2018.01.060>
- Qamar, S., Torres, Y. J. M., Parekh, H. S., and Falconer, J. R. (2021). Extraction of medicinal cannabinoids through supercritical carbon dioxide technologies: A review. *Journal of Chromatography B*, 1167, 122581. <https://doi.org/10.1016/j.jchromb.2021.122581>
- Ribeiro Grijó, D., Vieitez Osorio, I. A., and Cardozo-Filho, L. (2019). Supercritical extraction strategies using CO₂ and ethanol to obtain cannabinoid compounds from cannabis hybrid flowers. *Journal of CO₂ Utilization*, 30, 241-248. <https://doi.org/10.1016/j.jcou.2018.12.014>
- Rovetto, L. J. and Aieta, N. V. (2017). Supercritical carbon dioxide extraction of cannabinoids from *Cannabis sativa* L. *Journal of Supercritical Fluids*, 129, 16-27. <https://doi.org/10.1016/j.supflu.2017.03.014>
- Ternelli, M., Brighenti, V., Anceschi, L., Poto, M., Bertelli, D., Licata, M., and Pellati, F. (2020). Innovative methods for the preparation of medical Cannabis oils with a high content of both cannabinoids and terpenes. *Journal of Pharmaceutical and Biomedical Analysis*, 186, 113296. <https://doi.org/10.1016/j.jpba.2020.113296>