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Obtaining Nettle Extracts (*Urtica dioica*) by Means of Hydro-cavitation

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Stinging nettle (*Urtica dioica L.)* is considered a great source of secondary metabolites of commercial interest and the extraction of this kind of metabolites is important for the process viability and scale-up. Different alternatives have been evaluated, including Soxhlet extraction and the use of supercritical fluids, mainly on laboratory scale. As an innovative approach, hydro-cavitation has emerged as an efficient alternative for extracting principles from plants at low temperatures and short operational times. This work presents the experimental extraction of ß-carotene from Stinging nettle employing hydro-cavitation. Ethanol was used as solvent at different concentrations (100%, 90%, 80% and 60%) and two w/v solute/solvent ratio (1:30 and 2:30). The concentration of chlorophyll A, B, total chlorophylls (A+B), carotenes, and ß-carotenes were determined. The best concentration of ß-carotenes was obtained at 2:30 solute/solvent ratio with ethanol at 100% and an operational time of 2 min, corresponding to 153.975 mg ß-carotenes/kg, with a power consumption of 0.7698 kJ/mg ß-carotene extracted. The results support the viability of the use of hydro-cavitation as fast and efficient process for obtaining of Stinging nettle extracts on a pilot scale and open the possibility for the design of process on an industrial scale..

1. Introduction

Stinging nettle (*Urtica dioica L.*) is a plant considered as a weed in Colombia, which grows in different thermal floors and under wild conditions. However, nettle has shown high contents of metabolites, minerals, and vitamins that have begun to awaken research and commercial interest. In particular, nettle has been reported as a source of polyphenols(Orcic et al., 2014), fatty acids (Dhouibi et al., 2020), carotenes(Otles & Yalcin, 2012), vitamin B2, vitamin B5(Upton, 2013), calcium, potassium and magnesium (Salih, 2015). Consequently, this vegetable matrix has been used for therapeutic purposes in the treatment of breast cancer(Mansoori et al., 2017); as well as antioxidant, antimicrobial, and analgesic (Di Virgilio et al., 2015). Also, it has been used in treatments for patients with chronic cardiovascular deficiency (Xu et al., 2017), and for the control of allergies (Bourgeois et al., 2016).

Of the wide variety of metabolites of interest that have been identified in nettle, carotenes have received particular attention due to their potential for the manufacture of products for therapeutic purposes(Shonte et al., 2020). In particular, ß-carotenes are known as pro-vitamin A. Concentrations of up to 720 mg of carotenes/kg of fresh nettle have been reported(Otles & Yalcin, 2012) and associated with anti-inflammatory, antioxidant, and anti-carcinogenic effects(Ba et al., 2020); as well as anti-allergens and skin protectors (Freitas et al., 2015).

ß-carotenes are sensitive to changes in temperature and exposure to light, due to the high risk of degradation. It has been reported that the maximum temperature to which they can be exposed is 80°C(Baek et al., 2020). Consequently, the extraction of ß-carotenes must be carried out under conditions that guarantee their stability, at low temperatures and with pressure control (Sovová et al., 2004), which considerably increases the cost of the process. Concurrently, the effect of the drying process has been studied, taking into account that the reduction of the water content is a fundamental preliminary stage to achieve the successful extraction of the metabolites. For example, it has been reported that while fresh nettle shows ß-carotene concentrations of

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580.58 mg/kg, in lyophilized nettle the value decreased to 563.41 mg/kg and the material dried in an oven at 70°C for 15 h reached 525.04 mg/kg (Shonte et al., 2020). Subsequently, drying is an important stage for the degradation of ß-carotenes and that must be taken into account when designing a process for their extraction.
The extraction of active ingredients of phytochemical origin generally employs solvents such as methanol, ethanol, hexane, and water. During the extraction, the preferential solubilization of the metabolites of interest is sought (Akalin et al., 2013). With this aim, different operations have been evaluated, such as maceration, Soxhlet extraction (Köszegi et al., 2015), ultrasound-assisted extraction (UAE) (Xu et al., 2017), microwave-assisted extraction (MAE) (Ince et al., 2014), and extraction under supercritical conditions (Sajfrtová et al., 2005).The high energy costs, the use of toxic solvents in some cases (Chen et al., 2020), and the need for equipment that is difficult to scale-up are especially complex. That is why it is necessary to design new extraction alternatives, where hydrodynamic cavitation has begun to be evaluated by some researchers (Lee et al., 2019). Cavitation is a physical phenomenon that refers to the formation of bubbles in a liquid when it is subjected to a pressure low enough to change from liquid to gaseous state and then from gaseous state to liquid again (Choi et al., 2019). This phenomenon generates shock waves and microjets that release energy in very large proportions (Talebian et al., 2020)(Alexander Ladino et al., 2016). The application of hydro-cavitation has been studied and is considered an extraction method that can be applied on an industrial scale due to its low cost and high efficiency (Cravotto et al., 2021).
The aim of this paper is to propose the use of a hydrodynamic cavitation reactor for the extraction of ß-carotenes from nettle using ethanol as solvent, to promote the development of industrial processes that start from the use of vegetable matrices available.

2. Materials and Methods

2.1 Materials

70 kg of nettle were collected in the Savannah of Bogotá, Colombia. As the solvent for the extraction of metabolites, absolute ethanol (99%, Panreac) was used. Diethyl ether (Chemi) was employed for the quantification of chlorophylls and β-carotene in the extracts. The nettle was dried naturally at an average temperature of 17 °C for 21 days. The dried nettle was then processed to reduce the particle size, making use of a food grinder (Nex with a capacity of 1 L, CH3600). Once the nettle was pulverized, it was sieved on a standard mesh A.S.T.M. E-11, selecting the material with a size smaller than 600 microns. The larger material was reprocessed until meeting the established parameter.

2.2 Hydrocavitator

The hydro-cavitation instrument used was designed and built by the Universidad Santo Tomás (Bogotá, Colombia). In this equipment, hydrocavitation is produced by forcing a fluid through a hole at high pressure. The equipment consists of (Figure 1): A. Tank, B. Centrifugal pump, C. Flowmeter, D. Globe valve, E. Manometer, F. Reactor, G. Manometer, H. Globe valve. The total capacity of the tank is 8 liters, and the fluid is driven by a centrifugal pump (JET1100G1, 1.5 HP, IHM®). The thermocouple flow meter (SM6004, IFM) records temperature and flow. The inlet pipe to the reactor is SCH 40 and has a diameter of 1 inch. The reactor has an orifice plate with 5 holes of 3mm diameter. The pressure at the inlet and outlet of the reactor is regulated by globe valves. At the inlet of the reactor a pressure of 345 kPa is used, and 103.4 kPa at the outlet.

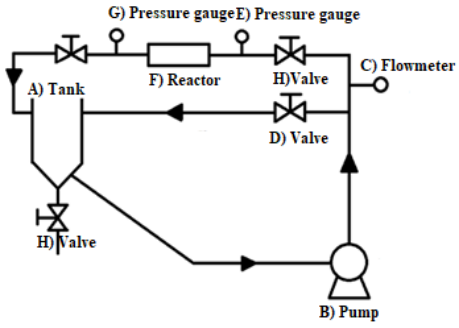

Figure 1. Hydrocavitator built by Universidad Santo Tomás

2.3 Experimental design

Ethanol was used as a solvent for the extraction. Four concentrations of ethanol were evaluated, to study the effect of the addition of water and the consequent change in polarity: 100%, 90%, 80% and 60%. From the bibliographic review, the solute/solvent ratio were determined as 1:30 and 2:30 of weight to volume ratio, w/v (Ince et al., 2014) (Sajfrtová et al., 2005). All tests were run for 10 min and samples were taken every minute for 10 min. All tests were done in duplicate, and the flow rate was fixed as 19.3 L/min. The Soxhlet method was used as a control for the extraction of chlorophylls and carotenes. 350mL of ethanol was put into the Soxhlet equipment, then the nettle was added in a 1:30 or 2:30 ratio, according to the experimental design. The temperature was kept at 80° C by an electric heater (CORNING PC-420D) for 8 h. At the end of the test, the solvent was recovered with the extract. Power used for extraction was calculated from voltage, electric current and time used in each experiment.

2.4 Characterization of nettle extract

High-performance liquid chromatography (HPLC) was used to quantify the ß-carotenes obtained. The solvent present in the nettle extracts was evaporated using a rotary evaporator (RV10 DS1, IKA) at 40°C and 60 rpm. The concentrated extract was injected into the chromatograph (1100 series, Agilent), employing methanol: isopropanol 35:65 as mobile phase at a flow of 1mL/min in isocratic mode. A Hypersil ODS column (1100, Agilent) and a UV/VIS detector were used with an injection volume of 10 µL at 25ºC and a run time of 5 min.
Additionally, the amount of chlorophyll A, chlorophyll B, and total carotenoids were quantified according to the methodology proposed by Đurović et al. (Đurović et al., 2017). According to this methodology, the samples obtained in the hydro-cavitation equipment were centrifuged at 1000 g and diluted in diethyl ether with a dilution factor of 1:50. As a blank, a solution consisting of 1 mL of ethanol + 49 mL of diethyl ether was used. The absorbance at 660 nm, 642 nm, and 480 nm was measured, using a UV-Vis spectrophotometer (NANOCOLOR UV/VIS, Macherey-Nagel). The concentrations of the metabolites were established according to the equations proposed in the literature (Đurović et al., 2017):

$$ChlA = 9.993A_{660} - 0.78A_{642} \quad (1)$$

$$ChlB = 17.60A_{642} - 0.78A_{660} \quad (2)$$

$$ChlA + ChlB = 7.12A_{660} + 16.80A_{642} \quad (3)$$

$$TC = \frac{1000A_{480} - 0.52ChlA - 7.25ChlB}{226} \quad (4)$$

Where ChlA, ChlB, ChlA + ChlB, TC represent the contents of chlorophyll A, chlorophyll B, chlorophyll A + B and total carotenoids, respectively, in mg/L; A_{660}, A_{642}, and A_{480} represent the absorbances at 660 nm, 642 nm, and 490 nm, respectively. To calculate the total content of each metabolite in the dry leaves, expressed in mg/g, the dilution factor and the amount of plant material used for each extraction were taken into account.

3. Results and discussion

The humidity content of the fresh nettle was 82% and after the natural drying stage, it decreased to 9%. The final moisture content is within the range reported avoiding the decay of the vegetable material until its use (Otles & Yalcin, 2012) (Đurović et al., 2017). Drying is also a fundamental stage in the process of extracting metabolites, since the presence of water interferes with the extracting agent (ethanol), diluting it and affecting the extraction efficiency. Drying must be carried out under conditions that assure the conservation of β-carotenes, since when the temperature is higher than 80ºC, their degradation occurs (Baek et al., 2020)(Phan-thi et al., 2016). Accordingly, in this study, a natural drying strategy was employed under average environmental conditions of 17°C and relative humidity of 67.5%. Additionally, during the extraction the temperature was prevented from exceeding 60ºC; the highest temperature reached in the assays was 37.6°C, which ensured that there was no degradation of ß-carotene neither during drying nor during extraction. Regarding the particle size of the plant material for extraction, it was less than 600 µm as recommended by literature (Cravotto et al., 2021), to avoid clogging the reactor holes.
The extraction in the hydro-cavitation and in the Soxhlet equipment was carried out with the relations of nettle mass to volume of solvent recommended in the literature (1:30 and 2:30) (Ince et al., 2014). Table 1 presents the results obtained for the extraction using ethanol in different concentrations (100%, 90%, 80% and 60%) for the two previous ratios, in terms of the metabolite concentrations obtained. As depicted in Table 1, results show the presence of chlorophylls and carotenoids, which are typical plant pigments and have been previously reported in nettle (Paulauskienė et al., 2021a). Chlorophylls are responsible for the green color in vegetables

and fruits and are the main photoreceptors required for the photosynthesis process (Hojnik et al., 2007). There are two types of chlorophyll, A and B, according to the radicals present at C-3 of the molecule.

Table 1. Content of chlorophyll A, B, total chlorophyll (A+B), carotenes, and ß -carotenes.

R 1:30	**ChlA**	**Chl B**	**Chl A+B**	**TC**	**HPLC (mg ß-carotene/kg of SN)**
1	3256,04	3525,36	6776,28	887,49	93,42
0,9	3898,53	2986,76	6880,59	858,98	27,80
0,8	2577,01	2229,46	4803,06	576,14	16,00
0,6	1792,62	1981,52	3771,27	554,88	0,00
Soxh 1:30	2621,45	1486,77	4105,66	504,77	161,60
R2:30	**ChlA**	**Chl B**	**Chl A+B**	**TC**	**HPLC (mg ß-carotene/kg of SN)**
1	4781,51	5773,92	10547,23	1246,10	153,98
0,9	4130,30	5401,27	9524,01	1146,56	45,00
0,8	2011,25	2476,58	4484,33	569,96	22,50
0,6	1220,85	1699,51	2918,01	348,12	0,00
Soxh 2:30	3709,35	2270,16	5975,70	717,35	248,55

In all the experiments the amount of total chlorophylls is higher than the one of carotenes, which is similar to previous works where the Soxhlet extraction was carried out with 96% ethanol and methylene chloride (Đurović et al., 2017). The extraction of chlorophylls decreased as the concentration of ethanol was reduced, which is similar to that reported in the literature, since it is stated that chlorophylls have high solubility in ethanol(Đurović et al., 2017). The concentration of chlorophylls in ethanol and carotenoids is lower than previously reported for fresh nettle in laboratory tests using ethanol as extraction solvent (2510 mg of chlorophylls/kg fresh nettle and 630 mg of carotenoids/kg fresh nettle) (Zeipiņa et al., 2014). The content of these components depends on the degree of maturity of the plant used, an effect that was not evaluated here (Paulauskienė et al., 2021b).

Regarding the content of carotenoids, it can be seen that the maximum concentration of ß -carotenes obtained from nettle extract is obtained when 100% ethanol is used as solvent. The highest concentration is obtained by using a ratio of 2:30 (153,975 mg of ß-carotene/ kg of dried nettle) compared to that obtained with a ratio of 1:30 (93,42 mg of ß-carotene/kg of dried nettle). This reflects that the presence of water decreases the amount of solubilized ß-carotenes. Previous studies (Mihaylova et al., 2018), show that sometimes the presence of carotenoids in the extract does not necessarily lead to the presence of ß-carotenes, which depends on the solvent used. This study confirms that the use of ethanol assures the extraction of ß-carotenes.

As shown in Table 1, in the hydro-cavitation equipment, it is possible to extract in the same order of magnitude ß-carotenes than the Soxhlet assay. For the test with a 2:30 ratio in the hydrocavitator 153.98 mg of ß-carotene/ kg of dried nettle were extracted, while for the similar ratio were extracted 248 mg of ß-carotene/ kg of dried nettle in the Soxhlet equipment. However, the Soxhlet method required heating to 70ºC for 8 h, compared to the hydro-cavitation assays that started at room temperature (17ºC) without heating for 10 min. These conditions are suitable for the scaling up of the process since it is possible to place a greater amount of dried material in the hydro-cavitation equipment and thus achieving a higher net content of ß-carotenes. Since no previous work was found where ß-carotene extraction was carried out from nettle using hydro-cavitation, this study is a pioneer in the application of this type of technology.

On a laboratory scale, Đurović et al. employed the Soxhlet method to extract total carotenoids and chlorophylls, evaluating the effect of three different solvents and a weight-to-volume ratio of 1:30. With ethanol, they obtained 5.47 mg/g of carotenoids, 16.55 mg/g of chlorophyll A, 7.58 mg/g of chlorophyll B, 24.13 mg/g of total chlorophylls on a dry basis from nettle extract, while using methylene chloride, they reported 6.48mg/g of carotenoids, 15.55 mg/g of chlorophyll A, 5.6 mg/g chlorophyll B, 21.25 mg/g of total chlorophylls on a dry basis from nettle extract and 12.71 mg/g of carotenoids, 4.12 mg/g of chlorophyll A, 0 mg/g of chlorophyll B and 4.12 mg/g of total chlorophylls with n-hexane as solvent, on a dry basis from nettle extract (Đurović et al., 2017).

These results have shown that hydro-cavitation could be implemented on an industrial scale for extracting active vegetable principles (Cravotto et al., 2021). One advantage of this method is the elimination of toxic solvents, which were changed for ethanol. For all the experiments, the initial temperature was room temperature (17ºC), and the final one was less than 37.6ºC. In this way, it is not only possible to reduce energy consumption but also to protect the integrity and stability of the metabolites of interest. The time required for extracting ß-carotenes and chlorophylls by hydro-cavitation is about 2 min, compared with 8 h required for the Soxhlet extraction method, corresponding to a reduction of two orders of magnitude and a promising alternative for industrial-scale process design. Finally, the power used for the extraction was 0.7698 kJ/mg ß-carotene extracted; however, this value could not be compared with other authors since no paper evaluating nettle extraction with hydro-cavitation was found.

4. Conclusions

Results of this study confirm that hydrodynamic cavitation is a successful extraction method with low energy consumption (0.7698 kJ/mg ß-carotene extracted) and that it could be implemented for obtaining extracts in pharmaceutical and chemical industry. Hydro-cavitation is a green and efficient extraction method, as it allows the use of nontoxic solvents producing yields comparable to other industrial approaches such as supercritical extraction. In this work, hydro-cavitation was used for the extraction of active principles from Stinging nettle, taking advantage of the low operational temperatures 37.6°C for the maximum temperature and short times 10 min that offered protection to the highly sensitive metabolites of interest. The presence of ß-carotene, chlorophyll A and chlorophyll B in the extract was quantified 153.98 mg/ kg of dried nettle, 4781.51 98 mg/ kg of dried nettle, 5773.92 98 mg/ kg of dried nettle for the maximum yield extraction with 100% ethanol and R2:30 w/v supporting the idea that nettle is an important source of secondary metabolites that are not currently being employed industrially and therefore open the possibility to develop a pharmaceutical and consequently constitutes an important development platform in the pharmaceutical and food field.

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