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ORAL PRESENTATION

The physical-chemical characteristics of the Albanian *Capsicum annuum*, a complete SC-CO₂ extract for the aromatic medicinal plant.

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Abstract

Known by the plant's botanical name: *Capsicum annuum*, the chili pepper is considered an excellent source of antioxidants, including capsaicin, alkaloids, fatty acids, flavonoids, carbohydrates, carotene, essential oil, vitamins A, B1, B2, B3, B5, B6, B9, and C, fibers, folic acid, manganese, lutein, zeaxanthin, b-carotene, and lycopene. Capsaicin is used against atherosclerosis, diabetic vascular disease, stroke, hypertension, metabolic syndrome, etc.

After collecting the plant, its integrity and health are examined under a microscope, and the humidity is determined. Before the supercritical CO₂ extractions, the plant was under investigation for its physical properties. The extraction method used is supercritical CO₂ extraction, which requires a maximum humidity of 6% for production. The result provided by the GC-FID analysis of the essential oil extract of *Capsicum annuum*, with the SC-CO₂ has shown that the content of capsaicin is 36.82 % followed by nonivamide at 7.98%, which is used as a topical analgesic, as a flavoring ingredient, and exhibits anti-inflammatory properties in peripheral blood mononuclear cells and U937 macrophages. The GC-FID method was used for the identification of the components of the *Capsicum annuum*. The essential oil of *Capsicum annuum*, was under investigation for the presence of heavy metals, and ISO 11212 Spectrophotometry was used for the identification. Lead was the heavy metal that resulted in higher concentration in comparison with the other metals, but still, its presence was according to FAO / WHO levels for plants. All the analyses were performed according to the ISO standards.

Keywords: *Capsicum annuum*, Supercritical CO₂ extraction, GC -FID analysis,

INTRODUCTION

The antioxidant effects of peppers, also known as capsicum, are well studied. Peppers come in a variety of colors (green, yellow, orange, and red), and it has been heavily studied for their positive effects in treating a variety of illnesses, including gastrointestinal problems and antipyretics, particularly in problems related to mental health. Along with substantial levels of beta-carotene (pro-vitamin A) and other active compounds, pepper also contains several bioactive substances. Red peppers had the highest concentration of beta-carotene and yellow peppers had the lowest when comparing pepper color and levels of the active substance. It has been discovered that the antioxidant activity in all peppers is similar. Pepper has been proven to stop the oxidation of important fatty acids when used in cooking. (Sun T et. al 2007). Capsaicin, alkaloids, fatty acids, flavonoids, carbohydrates, carotene, essential oils, vitamins A, B1, B2, B3, B5, B6, B9, and C, fiber, folic acid, manganese, lutein, zeaxanthin, b-carotene, and lycopene are just a few of the antioxidants that pepper is a great source of. The pharmacological effects of *Capsicum annuum*, sometimes known as chili paper, include anti-diarrheal, antibacterial, anti-rheumatic, and antispasmodic properties. Recent research has demonstrated that capsicum can stimulate the circulatory system, improve poor circulation, and treat excessive blood clotting. It is advised to take capsicum to avoid cardiovascular disorders because it can even lower high blood cholesterol levels. Of course, it has additional advantages beyond those mentioned; it can also be used to treat toothaches, nausea, drunkenness, malaria, and swallowing difficulties. Externally applied, it can help against osteoarthritis, and

rheumatoid arthritis (Gyula Mozsik, 2018). Fresh pepper is one of the vegetables with such a higher vitamin C concentration (Vanderslice et al., 1990).

The major active substance of the *Capsicum annuum* is Capsaicin and Nonivamide. Capsaicinoids show antioxidant properties and potent antimutagenic and anticarcinogenic possessions. (Young – Joon Surh, 2002). Improved endothelial function, including eNOS activation/stimulation; induction of LXRalpha in foam cells, which promotes cholesterol export; Capsaicin is used to treat *atherosclerosis*.

Improved endothelial function, including eNOS activation/induction, in the treatment of *stroke and diabetic vascular disease*

Improved endothelium-dependent coronary artery vasodilation in angina

- *Hypertension*: reduced renal sodium retention and activation/induction of eNOS
- *Metabolic syndrome*: PPARgamma induction and decreased adipose inflammation
- *Cardiac hypertrophy* – PPARdelta induction
- Hepatocyte UCP2 induction in *fatty liver*; reduced adipose inflammation increased secretion of GLP-1.

Sympathetic stimulation of brown fat thermogenesis in *obesity*. Better ability to manage appetite thanks to improved adipocyte capacity for lipolysis and GLP-1, a vagal signal to appetite centers. Capsaicin exposure through the gastrointestinal tract lowers calorie and fat consumption.

Shengping Zhang MD, et. al. were able to look into the anticancer properties of capsaicin, according to a study that was published in 2019. They conclude that through stimulating capsaicin receptors or controlling other signaling pathways, capsaicin has potent anti-cancer effects on a variety of cancer types. Even the potential for clinical application in cancer therapy has been investigated, along with the impact of capsaicin on conventional chemotherapeutic medicines and radiotherapy in many cancer types. Capsaicin has broad clinical application prospects and may be used as a potential chemo-preventive or new auxiliary therapeutic drug for cancer, according to researchers who confirmed that when combined with conventional chemotherapy drugs or radiotherapy, it can improve patients' sensitivity to chemo-radiotherapy, reduce treatment dosages, and improve patient tolerance.

Table 1. Characteristics of *Capsicum annuum*.

Name	Sweet peppers
Scientific Name	Capsicum annuum
Native	Central and South America
Common/English Name	Capsicum Pepper , Cayenne, Cayenne Pepper, Cherry Pepper, Chili, Chilly Pepper , Green Capsicum, Paprika , etc.
Name in Other Languages	Albanian : Specë djegës
Plant Growth Habit	Small, branched, mostly erect, annual or perennial herbaceous shrub
Growing Climate	Warm-season crop, which performs well under an extended frost-free season
Origin of the plant:	Gjinar, Elbasan.
Cultivation	collected by the harvesters from August to October
Major Nutrition*	Vitamin C (Ascorbic acid), Vitamin B6 (Pyridoxine) Copper, Cu, Vitamin K (phylloquinone), Manganese, Mn Vitamin B1 (Thiamin), Total dietary Fiber Iron, Fe, Potassium, K, Carbohydrate
Health Benefits	It has anti-diarrheal actions. It can be used as an antibiotic, antirheumatic, antispasmodic, appetizing, antirheumatic, antiseptic, and sudorific. It has tonic and digestive actions. - It can stimulate the cardiovascular system. It can improve poor circulation and reduces high blood cholesterol levels. It can prevent patients from cardiovascular (Kim C-S, 2007). disease and treats excessive blood clotting.

The need for developing novel processing methods has increased because of the high cost of organic solvents, environmental considerations, and demands for ultrapure goods from, for example, the food and medical industries. In the manufacturing of pharmaceuticals, carbon dioxide is getting more attention. The "clean" synthesis of medicinal compounds utilizing carbon dioxide as a reaction medium holds tremendous potential for large-scale application in the pharmaceutical industry. These applications include particle creation with dense carbon dioxide. Since the end of the 1970s, supercritical fluid extraction has been used to separate natural products, but for a very long time, the applications were limited to a select few goods.

Supercritical extraction has the advantage of being able to extract much larger molecules like lipids (omega-3 and omega-6 fatty acids), chlorophyll, and waxes. Experiments and results have shown that these extracts are free of microorganisms, solvents, heavy metals, and preservatives. Since they are entire extractions of the plants, which comprise the essential oils as well as the fat- and water-soluble components, they are exceptionally concentrated. This means that while using CO₂ total extracts, we are utilizing both the plant's essential oil and other components that were also supercritically CO₂ extracted (T. A. Safapuri et.al 2019). After the essential oil is obtained, it goes through different analyses, from the physical-chemical, even the microbial ones. GC – FID provided information regarding the composition of the chili pepper, after that using the UV-VIS Spectrophotometer we were able to determine the concentration of the heavy metals.

MATERIALS AND METHODS

From August to October, harvesters in Gjinari Village, which is a component of the Administrative Unit of Gjinari, Elbasan Municipality, cultivate the plant. The fruit is the plant element that is extracted with supercritical CO₂. A regulated, dry, and ventilated environment is used for both plant selection and milling. The integrity and health of the plant are evaluated using a humidity monitor and a microscope. Regarding the current state of production: The plant particle size used is 0.3 mm by SCFE (supercritical fluid extraction) technology criteria with a maximum humidity of 6%. The extraction of solids from ground materials (or pellets, granulates) is frequently carried out in batch mode utilizing food carbon dioxide as a solvent. The apparatus comes with two separators where the CO₂ is removed from the extract before being recycled and two or more extractors that work above the CO₂'s critical pressure. The temperature and pressure of the carbon dioxide gas are increased until they reach the supercritical state as the first step in the supercritical CO₂ extraction procedure. At a temperature of 31.1 °C and a pressure of 1071 psi (72.87723 atm), carbon dioxide reaches a supercritical state. To do this, a heater and a high-pressure pump are employed. There is a lot of organic raw material in the extractors that the supercritical CO₂ passes through.

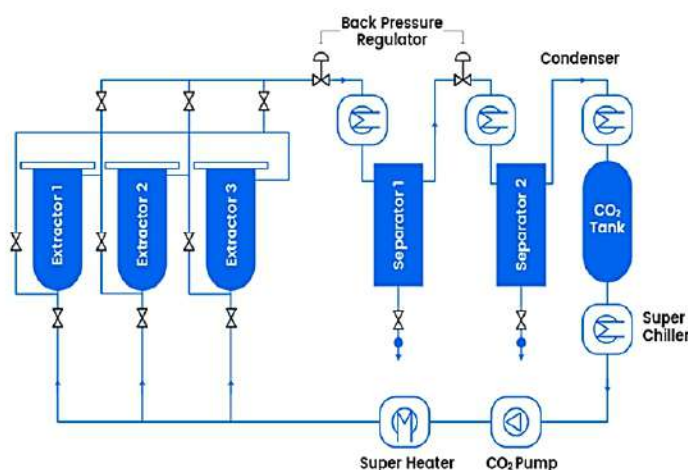


Figure 1. The Supercritical CO₂ extraction apparatus.

Depending on the elements present in the raw materials and the pressure-temperature conditions, the supercritical CO₂ removes the oils from the plant (*Capsicum annuum*) and passes through a series of pressure-regulating valves before entering the separators. The separator's two pressures are independently adjusted to separate the various extract components. The CO₂ is recycled by condensing and storing it as a liquid in the tank after the oil is removed and separated from the CO₂ that is released as gas. The oil is then collected in collection containers. The entire cycle is then repeated by utilizing this CO₂ throughout the batch. After obtaining the entire CO₂ extract, the extract is diluted with n-hexane in a ratio of 1:2 before being used for analysis.



Figure 2. The Supercritical CO₂ extraction apparatus while performing in the laboratory.

RESULTS AND DISCUSSION

After CO₂ extraction, the main components were found using the GC-FID method performed by Essential's in-house laboratory. These data are shown in Table 2. The GC - FID method is faster, more sensitive, reproducible, and precise. (Branca M. Silva, 2019).

Table 2. GC- FID analysis of Capsicum annum CO₂ Total Extract

	Compound	%
1	heptadecane	0.17
2	octadecane	0.12
3	palmitic acid (hexadecanoic)	0.22
4	methyl 11-(3-pentyl-2-oxiranyl) undecanoate	0.13
5	1-octadecanamine	3.92
6	7,11-hexadecadienal	0.2
7	oxacyclohexadecan-2-one	0.48
8	1-acetoxynonadecane	0.88
9	9,12-octadecadien-1-ol	0.2
10	tetradecylcyclohexane	0.32
11	oleic acid	0.1
12	heneicosane	0.7
13	nonivamide	7.98
14	nordihydrocapsaicin	0.65
15	capsaicin	36.82
16	dihydrocapsaicin	16.33
17	N-vanillyldecanamide	2.72
18	homocapsaicin	0.18
19	docosane	0.9
20	homocapsaicin II	1.12
21	homodihydrocapsaicin	3.49
22	pentacosane	1.4
23	β-tocopherol	0.1
24	γ-tocopherol	0.2
25	hexatriacontane	0.84
26	vitamin E	5.71
27	ergost-5-en-3-ol	0.39
28	β-sitosterol	0.6
29	α-amyrin	1.02
30	β-amyrin	1.5
31	Lupeol	0.12



Figure 3. Capsicum annum extract injection in GC – FID in the laboratory.

To employ the CO₂ extract for UV-VIS spectrophotometry analysis, the CO₂ extract must first be thoroughly extracted using CO₂ and then diluted with n-hexane in a ratio of 1: 2. The analyses were carried out using spectrophotometry by ISO-11212 Spectrophotometry.

The results regarding the presence of heavy metals are shown in the table below. These findings demonstrate that these metals' amounts for plants and vegetables are within WHO guidelines.

Table 3. The detection of heavy metals in Capsicum annum with UV -VIS Spectrophotometer.

Heavy Metals	Results	Specifications	Methods
Arsenic	0.03 ppm	≤1.5 ppm	ISO-11212 Spectrophotometry
Cadmium	0.02 ppm	≤1.0 ppm	
Mercury	< 0.05 ppm	≤0.1 ppm	
Lead	0.27 ppm	≤5.0 ppm	

The extract was obtained while it was still melting, applied without further processing, and then put in plates with the medium. The medium used for the identification of gram-positive bacteria was blood agar (Staphylococcus aureus), for gram-negative bacteria Endo Agar was used (i.e. Escherichia coli; Pseudomonas aeruginosa) and Sabouraud Dextrose Agar (SDA) for the identification of yeasts and molds. Since it contains waxes and esters, it is packaged at a temperature of 60 degrees Celsius so that the extract can be put into the container while it is still in a liquid state before freezing. The microbial analysis was performed according to the methods of ISO 21149, for the presence of Bacteria) and with ISO 16212 for Yeast and Mould.

0.1 gram of sample is mixed with 1 ml pure water, and centrifuged for 5 minutes / 3500 rpm at 20°C. Then 25 microliters are collected with a pipette and placed on the medium. These soils are then put in the thermostat for 48 hours at 36°C.

Table 4. The microbial analysis of Capsicum annum.

MICROBIAL ANALYSIS	RESULTS	SPECIFICATIONS	METHOD
Bacterial Count			
Staphylococcus aureus	CONFORMS	< 1000 CFU/g	ISO 21149
Escherichia coli	CONFORMS	< 1000 CFU/g	
Pseudomonas aeruginosa	CONFORMS	< 1000 CFU/g	
Yeast and Mould			
Candida Albicans	CONFORMS	< 100 CFU/g	ISO 16212
Aspergillus fumigatus	CONFORMS	< 100 CFU/g	

CONCLUSION

1. According to GC- FID analysis of Capsicum annum CO₂ Total Extract, the main active ingredient of Capsicum annum is capsaicin, which is present in 36.82% of the plant (together with its derivatives dihydrocapsaicin 16.33%) and nonivamide 7.98%. As a result of which chili pepper has a variety of beneficial effects.

2. By employing spectrophotometry, ISO-11212 Spectrophotometry was able to identify the presence of heavy metals. These results show that the levels of these metals in plants and vegetables are within WHO standards. Lead 0.27 ppm and mercury less than 0.05 ppm were the two most abundant heavy metals.
3. Microbial analyses are negative for the presence of various bacteria and yeast and molds. In some published regulations on standards and microbial analysis, the extracts obtained by extraction with carbon dioxide in the supercritical state are excluded from microbial analysis since the extract itself produced in this way does not allow microbial growth.
4. Supercritical CO₂ extraction has a variety of advantages, some of which we can list below:
 - a) Compared to oil products made using the steam distillation process, CO₂ extraction oil products are more pure and superior in quality.
 - b) CO₂ extracts have an advantage over steam distillation in that heating does not destroy the elements in the material while the essential oil is being extracted. Supercritical extraction processes need temperatures between 88 and 100 degrees Fahrenheit to operate, whereas steam distillation processes need temperatures between 140 and 212 degrees Fahrenheit. Temperature manipulation during steam distillation can change the molecules of the plant material and essential oil. However, CO₂ extract is more chemically similar to the plant from which it is generated since it has a larger variety of the plant's elements.
 - c) The CO₂ Extraction technique produces excellent lipids and aromatics (carrier oils). Many plant-based ingredients that are useful but difficult to extract using the cold pressing method are present in these products.
 - d) The aroma of the actual herb, spice, or plant is present in CO₂ extracts. It is thicker than the same essential oils because of the variety of elements it includes. In products extracted by steam distillation, this amount is lower.

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