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CAPSAICINOIDS EXTRACTION FROM SEVERAL *CAPSICUM* SPECIES CULTIVATED IN ROMANIA

Roxana-Mădălina STOICA, Ovidiu POPA, Liliana-Claudia BLASS, Narcisa BĂBEANU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67, Faculty of Biotechnologies, Email: roxym_stoica@yahoo.com

Corresponding author email: roxym_stoica@yahoo.com

Abstract

The latest world trends in scientific research are directed towards the production and application of secondary metabolites. The plant of the genus Capsicum produces a fruit (chilli pepper) with unique bioactive compounds. Pepper fruits are a rich source of metabolites with potential health-promoting properties, for example carotenoids (provitamin A), ascorbic acid (vitamin C), tocopherols (vitamin E), capsaicinoids and flavonoids.
Capsaicinoids are the compounds responsible for the hot, spicy flavour presented by many varieties of peppers. There were identified over 20 compounds, capsaicin analogues, of which most important (>95%, w/w) are: capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin. Capsaicinoid extraction from peppers is typically performed using organic solvents, however, the extraction efficiencies can vary with peppers, their parts and pre-extraction processing. A major component of this group is capsaicin.
This study involves extraction of capsacinoids from three varieties cultivated in Romania. Capsaicinoids were identified in all extracts with concentration ranging from 0.5 to 0.8% (dry weight) and the best results were obtained with 96% ethanol as solvent.

Key words*: capsaicinoids, extraction, Capsicum sp., ethanol.*

INTRODUCTION

Secondary plant metabolites represent a significant economic group used in different areas such as production of food additives, pigments, pharmaceuticals and biopesticides. Around the world is known five varieties of *Capsicum sp.*, which are *C. annuum, C. frutescens, C. chinense, C. baccatum* and *C. pubescens* (Dhaliwal et al, 2014).
The most important components in the group of secondary metabolites are derived from the biologically active components of the species *Capsicum annum L.*. (Tilahun et al., 2013). The pungent metabolites in the fruits of *Capsicum* species are called capsaicinoids, and among the most abundant of these components are capsaicin (CAP) and dihydrocapsaicin (DHC), which are responsible for about 90% of total pungency (Islam et al., 2015; Amruthraj et al., 2013; Anwar et al., 2014).
In addition to capsaicin and dihydrocapsaicin, many less abundant capsaicinoids have been detected in *Capsicum* extracts, including nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin (Mozsiksi et al., 2009).
Capsaicinoids are alkaloids produced by a condensation reaction between an aromatic moiety and a C9-C11 branched-chain fatty acid and are synthesized exclusively in the epidermal cells of the placenta of *Capsicum* fruits (Zaki et al., 2013).
Capsicum is the only genus known to produce capsaicinoids (Cisneros-Pineda et al., 2007), and capsaicin is the major pungent lipophilic alkaloid of the *Capsicum* fruits (Chen et Kang, 2013).
The amount of capsaicinoids in peppers is dependent on the genetic makeup of the plant, developmental stage, and the environment where it is grown, such as geographical origin and temperature, nutrients soil, light, water stress (Zewdie and Bosland, 2000; Bosland and Baral, 2007; González-Zamora et al., 2013; Sganzerla et al., 2014).
Jurenitsch et al. (1979) found considerable differences in total capsaicinoids within cultivars grown in greenhouses, laboratory field

studies, and regular plantations, highlighting the effect of environmental conditions on capsaicinoid content.

Capsaicin is currently used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia, and psoriasis, as well as there are many patents on insecticides, insect or animal repellents, and pesticides containing capsaicinoids (Dang et al., 2014).

Therefore, the present study was undertaken to determine the capsaicinoids content of three varieties of Capsicum cultivated in Romania during 2014, using different organic solvents for extraction (ethanol, methanol, acetone and acetonitrile).

MATERIALS AND METHODS

1. Standards and Chemical Reagents

The solvents, such as methanol, acetonitrile, acetone and ethanol used for extraction of capsaicinoids were purchased from Merck (Germany) and pure capsaicin obtained from Sigma Aldrich was used as reference standards. HPLC grade chemicals were used for the mobile phase and all the other reagents used in the analysis were analytical grade.

1.1. Plant material

Dried fruits of three varieties of hot peppers, such as *Pintea* (SP1), *Habanero rouge* (SP2) and *De Cayenne* genotypes (SP3) were used for extraction of capsaicinoids. Peppers used in this study were harvested from a local farm in September 2014 at maturity stage and at full fruit size.

1.2. Capsaicinoids extraction

Capsaicinoids are soluble in various solvents such as chloroform, acetone, ethyl ether, ethyl acetate, methyl chloride, ethanol, and 2-propanol, methanol, acetonitrile, among others (Santamaria R.I. et al., 2000).

First fully ripe fruits samples were allowed to dry at 50°C for 24-48 hours, with a moisture content ranging between 3-4%, and crushed into powder before the extraction. So, 25 g of powder was dissolved in 300 mL organic solvents, using Soxhlet extraction, for 5 hours, and the temperature was adjusted to obtain at least 40 cycles (Figure 1). Then, the solvent was removed after extraction using a rotary evaporator, and the vacuum level was adjusted so the temperature to be less than 40 degrees Celsius.

Figure 1: Soxhlet extraction

The capsaicinoids contents, such as capsaicin, dihydrocapsaicin and nordihydrocapsaicin were determined using High performance liquid chromatography. It was used a standard solution of capsaicin with a concentration of 0.05%, 0.025% nordihydrocapsaicin, and 0.025% dihydrocapsaicin in methanol. Also, a phenylsilicagel column (5 µm), l = 0.250 m, Ø = 4.6 mm, temperature = 30 °C it was used. Mobile phase was acetonitrile R, 1g/L solution of phosphoric acid R (40:62 V/V), flow rate: 1.0 mL/min, detection: spectrophotometer at 225 nm, injection:10µL and the elution order: nordihydrocaspsaicin, nonivamide, capsaicin, dihydrocapsaicin.

RESULTS AND DISCUSSIONS

From all three *Capsicum* varieties, the best results were obtained with Habanero rouge genotype (SP2), while the lowest results were obtained with Pintea genotype (SP1) (Table 1)

Table 1. The percentage of capsaicinoids content from ethanolic extracts of *Capsicum sp.*/ dry weight

Genotype / Capsaicinoids	Pintea	Habanero rouge	De Cayenne
Nordihydrocapsaicin (%)	0.086	0.201	0.102
Capsaicin (%)	0.241	0.417	0.359
Dihydrocapsaicin (%)	0.173	0.182	0.194
Total (%)	0.500	0.800	0.655

As we can see from the data presented in Table 1 the amount of capsaicin and dihydrocapsaicin concentrations ranges between 75%-85%. (Figure 2).

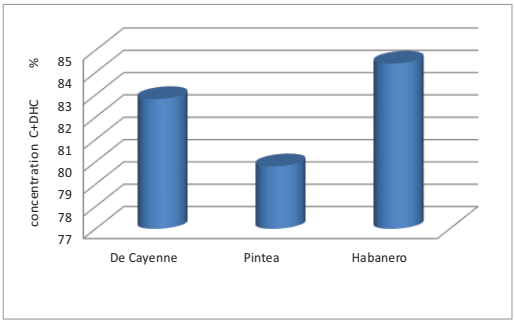

Figure 2: Comparative chart on the capsaicinoids concentrations of *Capsicum sp.*

Comparing the efficiency extraction of capsaicinoids from three varieties of *Capsicum* sp. with the four solvents tested, it can be observe that ethanol showed the higher content of capsaicinoids (0.8%), and methanol have the lowest content of capsaicinoids (0.599%) (Table 2).

Table 2. The percentage of capsaicinoids content obtained with organic solvents from *Capsicum sp.*/dry weight

Solvents / Capsaicinoids	EtOH	MeOH	Me_2Co	Acetonitrile
Nordihydrocapsaicin (%)	0.201	0.155	0.199	0.194
Capsaicin (%)	0.417	0.272	0.405	0.366
Dihydrocapsaicin (%)	0.182	0.172	0.177	0.199
Total (%)	0.800	0.599	0.776	0.759

So, the organic solvents influence the total amount of capsaicinoids, but not their ratio in extract (Table 3, Figure 3).

Table 3: The influence of solvents on total amount of capsaicinoids

Solvents / Capsaicinoids	EtOH	MeOH	Acetone	Acetonitrile
Capsaicin (%)	52.12	45.41	52.19	48.22
CAP (%) + DHC(%)	74.88	74.12	75.0	74.44

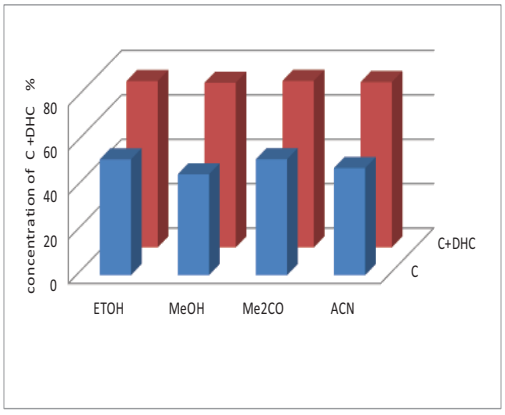

Figure 3 : Comparative chart on the influence of solvents on concentrations of capsaicinoids

CONCLUSIONS

This study showed the influence of genotype on capsaicinoids extraction from three varieties of peppers. Among four solvents tested, ethanol showed the higher content of capsaicinoids comparing with methanol, which showed the smallest amount of capsaicinoids.

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