

SUMMARY

Key words: *Capsicum* sp., extraction, isolation, capsaicinoids, carotenoids

The PhD thesis entitled „**Extraction and isolation of biological active compounds from *Capsicum* genus**” aimed the selection of the best variety of hot peppers for capsaicinoids extraction and the development of a laboratory technology for the isolation of a capsaicinoids concentrate.

Biological active substances existing in the genus *Capsicum*, particularly capsaicinoids, provide many therapeutical uses due to its anti-inflammatory properties, to treat chronic pain, such as rheumatoid arthritis and neuralgia, and due to its anticancer, antimicrobial and antioxidant activity. Also, the presence of carotenoids in *Capsicum* fruits, and in particular, capsanthin and capsorubin, determined their intensive use in the food industry, both because of their coloring and antioxidant characteristics.

The amount of these compounds varies depending on the cultivars used, the technological elements applied (culture density, period of planting in the field, fertilization regime) and the stage of ripening fruit and climatic conditions.

This work comprises two parts: the documentation part (chapter 1) and an experimental research part (4 chapters), and a list of references totaling with 387 book titles.

Documentary study (chapter I) based on the literature in this area reveals that to obtain the active ingredients of fruit *Capsicum* species, in industry are using various organic solvents, for cold or hot extraction, most used for this purpose, being methanol, ethanol, acetonitrile, acetone and petroleum ether. It should be noted that the literature data are not unanimous in choosing the best solvent for the extraction of oleoresins.

The experimental part (chapter II-IV) start with presenting the main objectives of the work:

- ✓ Identification of *Capsicum* varieties with high potential for obtaining biologically active compounds and testing them in the field, in the south of Romania, of some varieties of foreign origin, with a high Scoville score, for obtaining a biological material with reproducible characteristics
- ✓ Conducting experiments in the laboratory for obtaining biomass through specific plant biotechnology methods (cell culture in solid and liquid medium).
- ✓ Developing, verifying and implementing an optimal extraction methodology for biologically active product

✓ Optimization methods for isolation and concentration of capsaicinoids from *Capsicum* varieties

✓ Capsaicin valorification by designing and analysis of primary biopharmaceutical evolution of some topical products for pain therapy

Thus, the work plan of the thesis consisted of several steps, including obtaining biological material, establishing optimum conditions for grinding the biological material, the selection of solvent and the method of extraction, isolation of a capsaicinoids concentrate and the use of capsaicin to obtain the product of topical use.

The experimental part (chapter 2) starts with the presentation of the methods used to obtain the biological material (as in field crops as well as *in vitro* culture technique). Also, there are provided the steps of conditioning the plant material, the principles and equipment used for the extraction and analysis of compounds of interest, as well as the characterization and investigation of the anti-inflammatory action of the topical formulation.

Results and discussion chapter (Chapter III) is divided into eight subchapters.

The first chapter entitled „Obtaining biological material for oleoresin extraction” presents the experimental results obtained in order to identify the variety with great potential for capsaicinoids. Seven varieties were critically analyzed (*Congo Trinidad*, *Cayenne*, *Fresno*, *Guindilla Larga Roja*, *Fiesta*, *Hot Cherry*, *Pintea*), in two different locations (Drăgănești, Prahova county and Măgurele, Ilfov county). During the vegetation it was registered the behavior of varieties of foreign origin and the one homologate in Romania from the point of view of plant growth and productivity. Also, there was conducted several tests to determine the best conditions for obtaining of vegetable biomass with improved characteristics.

The second subchapter are showed the results of the influence of grinding conditions on the particle size distribution of the powder pepper obtained for extraction of compounds of interest. The data obtained have shown that the system of grinding influences both the average particle diameter and particle size dispersion.

In the three subchapter entitled „Oleoresin extraction from *Capsicum sp.* with organic solvents” are presented the results regarding the capsaicinoids extraction by Soxhlet method. Four solvents were tested: ethanol, methanol, acetonitrile and petroleum ether. The use of these solvents for the capsaicinoids extraction follows the same trend for all varieties, the best values are obtained in the extraction with ethanol, followed by methanol, acetonitrile and petroleum ether.

Because the largest amount of capsaicin for all varieties studied was obtained using ethanol and because this solvent is not show toxicity comparing with other solvents, it was further tested the influence of ethanol concentration on capsaicinoids extraction, through Soxhlet method (subchapter 4). It was noted that in terms of the amount of capsaicin, dihydrocapsaicin and

nordihydrocapsaicin, this is influenced by the concentration of the solvent used, the best results being obtained with the maximum concentration of the solvent, by Soxhlet method. It was also, studied the influence of maceration time (24 hours, 36 hours, and 48 hours respectively) on the amount of capsaicinoids, at different concentrations of ethanol. In the case of maceration, the concentration of capsaicin increases with duration of extraction, the highest values recorded in maceration for 48 hours. The amount of capsaicin obtained by maceration is higher than that obtained by Soxhlet method. Regarding the influence of ethanol concentration on capsaicin extraction it can conclude that regardless the method and extraction time, the best results are obtained with 96% ethanol.

In subchapter 5 it was studied the influence of solvent/solid phase ratio on extraction and duration of capsaicinoids concentration. Increasing the volume solvent/ solid phase ratio leads to an increase in the amount of capsaicinoids extracted. The increase is higher in the case of maceration for 48 hours. The largest quantities of capsaicinoids extracted were recorded for *Congo Trinidad* variety, followed by the *Cayenne*, and the lowest were obtained for *Pintea* and *Hot Cherry* varieties. Energy consumption increases with increasing the solvent/solid phase ratio. Taking this into account, this increase extraction yield does not justify the additional energy costs. For this reason, it is preferred to use an extraction ratio of 10: 1 (v/w). If for *Congo Trinidad* and *Cayenne* varieties the capsaicinoids quantity extracted by maceration is higher than that extracted by Soxhlet method, the other varieties presents no significant differences between the methods.

In subchapter 6 it was studied the influence of low concentrations of ethanol (30-45%) on capsaicinoids and carotenoids extracted at 40°C, stirred Erlenmeyer flasks was observed that the best selectivity ($C_{\text{caps}}/C_{\text{car}} = 37$) is obtained from the concentration of ethanol of 30%. At this concentration of alcohol, capsaicinoids extraction yield was 66% of the value obtained by extraction with 95% ethanol for 48 hours at a ratio solvent/solid phase of 20: 1. Using an alcohol concentration of 45%, increased by 5-6% capsaicinoids quantity extracted, but significantly decreases selectivity. The optimum conditions for the selective capsaicinoids extraction with ethanol are: 40°C (30%), during 36 hours, ratio solvent/solid phase 20: 1. The solid residue remaining after the first extraction was re-extracted with ethanol 96% for extraction total carotenoid. This phase lasted 12 hours. The sequential extraction were recovered 85% of capsaicinoids, and 78-82% of carotenoids extracted with 95% ethanol.

Further, it was investigated the possibility of isolating a capsaicinoids concentrate by supercritical CO₂ extraction. The experimental results obtained have shown that the optimal procedure for the isolation of a capsaicinoids concentrate is the extraction with supercritical CO₂, carried out in two stages:

1. 140 barr, 30 minutes (30% and 85% of capsaicinoids oleoresins)

2. 400 barr, 2 hours (complete extraction of the remaining capsaicinoids and carotenoids).

The advantage of this method is the possibility of applying the different varieties of *Capsicum* (those with high content of capsaicinoids and carotenoids), because it is obtained two types of extracts: the first containing 85% of the capsaicinoids, and the second, which contains most of the carotenoids.

In subchapters 7-8 are presented the results that have been directed to obtain a topical formulation that combines the effects of capsaicin, known for its analgesic action and local vasodilator with those of ketoprofen, a non-steroidal anti-inflammatory, which fall into the category of minor anti-inflammatory. Semisolid pharmaceutical forms for topical application of a non-steroidal anti-inflammatory can be bought without a prescription and can be applied to a first measure of acute and even chronic pain. Considering these aspects, the realization of this topical formulation is a viable alternative in pain therapy.

Chapter 4 contains the conclusions of experimental research and final part presented at the end the bibliographic sources used in the original thesis.