PhD THESIS

Research on obtaining and characterizing extracts of *Prunus spinosa* for use as a food additive

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

Medicinal plants offer a number of natural remedies (SHAKERI, 2015), which can be used to prevent or treat diseases (SPONCHIADO, 2016; FOUDA, 2015). Apart from the fact that - they have such beneficial properties for human health, they can also be used in aromatic teas, cosmetics or food (URSO, 2016). A lot of medicinal plants are successfully cultivated on the territory of our country, most of them growing spontaneously, not needing special care. Romania has a diversified flora, with over 3600 plant species from which over 10% are used in phytomedicine.

In recent years, the use of phytotherapy in medicine is increasingly desired by limiting the excessive use of chemically synthesized drugs (AZIZ, 2016). Based on these considerations, new forms of use of medicinal plants were discovered: syrups, granules, teas, oils, extracts or balms (MORACZEWSKI, 2020; EL-GHAZOUANI, 2021). Regarding the use of these medicinal plants in the form of extracts, it should be noted that for therapeutic and pharmaceutical recovery (TOFANĂ, 2006) a thorough knowledge of active principles is required in order to correctly choose the extraction technique (BECZE, 2020).

Blackthorn, *Prunus spinosa*, is a shrub whose components are used in natural therapy in various forms (BĂBĂLĂU-FUSS, 2018). There are many uses for this plant, the main benefits are especially on the digestive system, the flowers have a sedative and laxative action, while the fruits have antidiarrheal and diuretic effects. This plant is slight studied in terms of pharmacodynamics, the study of its properties being the subject of this paper. The chemical composition of the plant is varied, in the flowers, flavonoids, quercitin, organic acids, kamferol, potassium and magnesium salts and glycosides have been found (BĂBĂLĂU-FUSS, 2020), while the fruits contain high amounts of sugars, anthocyanins, polyphenols, organic acids, prunicianine, tannins, gum-resins, vitamin C and calcium and magnesium salts (BHUYAN, 2013).

STRUCTURE OF THE THESIS

The present paper aims to obtain *Prunus spinosa* fruit extracts by using the accelerated solvent extraction (ASE) and supercritical fluids (CO_2) extraction techniques; characterization of these extracts and their introduction into food in the form of food additives. The samples of *Prunus*

spinosa fruits were taken from the neighboring area of Cluj-Napoca. The paper "Research on obtaining and characterizing extracts of *Prunus spinosa* for use as a food additive" contains a number of 142 pages, being written according to USAMV Cluj-Napoca regulations in force and structured in two parts. The first part "Current state of knowledge" consists of 2 chapters and extends over 38 pages, this part summarizing the general aspects of medicinal plants, plant extracts and food additives. Their main characteristics, classification, legislation on food additives and their use in food are described.

The second part of the thesis, "Personal Contributions" extends to 89 pages and is structured in 10 chapters. This part presents the objectives, the purpose of the paper, the materials and methods used, the results obtained from the determinations and the discussions based on them. The results were compared with data from the literature, which were summarized in 78 figures and 38 tables.

Chapter 3 presents the objectives, purpose and experimental design of this study. **Chapter 4** is dedicated to the study of the particularities of the natural environment in which the experimentation took place, a chapter in which the natural setting of the sampling area, the climate and the relief were described. **Chapter 5** described the biological material, sampling and analytical methodologies used for the matrices under investigation. **Chapter 6**, entitled "Characterization of the raw material used to obtain extracts of *Prunus spinosa*" aims at the comparative evaluation of the characteristics of the raw material and the evaluation of the impact of environmental factors on it. **Chapter 7** is intended entirely to obtaining and characterizing extracts from the fruits of *Prunus spinosa*. To obtain the extracts, two distinct techniques were used: the solvent-accelerated extraction technique and the extraction method with supercritical fluids. In **Chapter 8**, the results of the research on the comparative evaluation of *Prunus spinosa* extracts were interpreted. The concentration of total polyphenols and the antioxidant capacity for all samples of extracts obtained from the fruits of each sampling location were determined. **Chapter 9** was devoted to research on the use of *Prunus spinosa* fruit extracts as a food additive. The evaluation of the effects of the extract was performed on food of plant and animal origin. **Chapter 10** highlights the general conclusions and recommendations derived from this research, **Chapter 11** is dedicated to the aspects of originality and innovative contributions of the thesis, and **Chapter 12** highlights future research perspectives.

RESULTS OF THE RESEARCH

Chapter 6 - The characterization of the raw material used to obtain *Prunus spinosa* extracts was performed by comparative evaluation of the characteristics of the biological material. The determination of the essential minerals in blackthorn fruit showed the impressive amount of potassium and calcium in these fruits. Comparison of the obtained concentrations of the essential minerals with the concentration of the recommended daily dose revealed that the fruits contain a significantly increased amount of these essential minerals. By analyzing the concentrations of polyphenols and antioxidant capacity, a directly proportional increase of these two parameters was noticed. The determination of the content of polycyclic aromatic hydrocarbons in fruit showed that phenanthrene, fluorene, naphthalene, pyrene and fluoranthene are the most present contaminants in the samples.

The assessment of the impact of environmental factors on the raw material (*Prunus spinosa* fruit) was performed by calculating transfer factors of metals from soil to fruit and polycyclic aromatic hydrocarbons from soil to fruit. In the case of calculating the transfer factors of essential metals from soil to fruit, the highest ratio was recorded for potassium at Borhanci (2.6459). The transfer of polycyclic aromatic hydrocarbons from soil to fruit indicated significantly increased values for most of the indicators analyzed in Floresti location. Pollution of this area due to heavy traffic is also found in the vegetation of the area.

The results obtained, in their entirety, showed that *Prunus spinosa* fruits are a valuable source of vitamins and minerals, being recommended in the treatment of certain diseases, but their location is a particularly important factor in choosing fruits, because soil pollution and of air can influence the quality of their healing properties.

Chapter 7 - *Prunus spinosa* extracts were made by two techniques: the solvent-accelerated extraction technique and the supercritical fluid extraction method, using carbon dioxide and a small amount of solvent, ethanol. The extracts obtained by accelerated solvent extraction were analyzed in terms of total polyphenol concentration and antioxidant capacity to demonstrate the effectiveness of the extraction. The results obtained were not satisfactory, the extracts showing significant concentrations of polyphenols (154 mg / ml), and the antioxidant capacity showed extremely low values. The correlation matrix of the two

parameters indicated a correlation of only 0.42, which is why these extracts were not used in future analyzes, and implicitly this extraction technique was discarded.

Fig. 1. Extracts obtained by CO_2 extraction

Fig. 2. Extracts obtained by ASE extraction

The second extraction technique, that of supercritical fluids, was developed using carbon dioxide as a supercritical fluid and a small amount of solvent. The extracts were analyzed in order to determine the characteristics of interest for this paper. Total polyphenol concentrations (1212 mg / ml) and antioxidant capacity (2407 µg / ml) were determined and it was observed that these parameters show maximum values in the last sampling campaign, November 2019. Significance of these values increased after the first frost, confirms that these fruits reach full maturity only after contact with low temperatures. The results obtained with this type of extraction were significantly positive, which is why the concentrations of essential and toxic heavy metals were determined.

Fig.3. Comparative situation of the extractor evaluation parameters obtained by ASE technique

Fig.4. Comparative situation of the evaluation parameters of the extracts obtained by the CO_2 technique

Considering the data obtained regarding characterization of the extracts, it can be stated that the favorable extraction of this type of fruit is the extraction with carbon dioxide used as supercritical fluid. The

parameters of the extraction made with carbon dioxide used as supercritical liquid help to transfer minerals and beneficial properties of fruits in extracts, making them a valuable product for human consumption, and due to antioxidant properties, the extract obtained by research underlying this thesis can be consumed as such or can be introduced into food.

Chapter 8 - The concentration of total polyphenols was determined from all the fruits and extracts obtained, the results being interpreted comparatively in order to demonstrate the transfer of these compounds in the new product obtained. For the Chinteni location, the values of polyphenol concentrations in fruits and extracts increase in direct proportion, the highest value being recorded in the last sampling campaign (fruit - 628 mg / kg, extract - 909 mg / ml). The same ascending slope is observed in the case of samples from the Pata sampling area (fruit - 782 mg / kg, extract - 1071 mg / ml). Borhanci location shows low concentrations of polyphenol concentrations in the sampling campaign October 2018. The same phenomenon is encountered at the Floresti sampling location in October 2018. For all sampling locations, the concentration of polyphenols in fruits and implicitly, extracts are increased in the last month of sampling, November 2019. The comparative situation of the averages of polyphenol concentrations, shows that the Borhanci location is the location with the highest values of both fruit concentrations and extracts. The correlation matrix of the two parameters indicates a positive correlation 0.81.

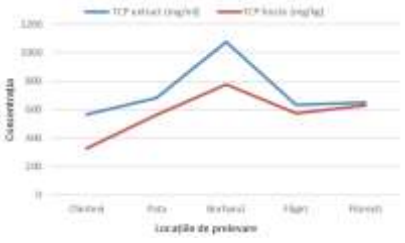

Fig. 5. Comparative situation of polyphenols in fruits from the sampling period 2018-2019 and extracts

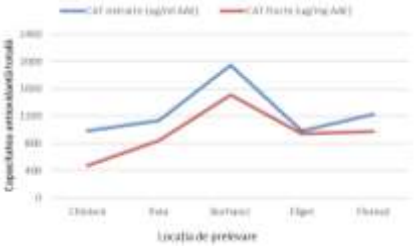

Fig. 6. Comparative situation of antioxidant capacity of fruits from the sampling period 2018-2019 and extracts

The antioxidant capacity of the extracts was determined compared to that of the fruits, in the case of Chinteni location the situation being similar to that of the concentration of total polyphenols. The increase of the antioxidant capacity is progressive, the maximum value being registered in

the last month of sampling. The Pata location shows a decrease in the antioxidant capacity of the extracts compared to that of the fruits, in the last month of sampling (October 2018 fruit - 645 μg/mg AAE, extract - 594 μg/ml AAE) the situation being totally opposite in the last sampling campaign (November 2019 fruit - 1125 μg/mg AAE, extract - 2098 μg/ml AAE). In the case of Borhanci location, a significant decrease of the antioxidant capacity is observed in the second sampling campaign, the maximum value being reached in the last sampling campaign (extract 2407 μg/ml AAE). The case of the Făget location is similar to the case of the Pata location, the recorded values being close. The values obtained for the Floresti location show a continuous and linear increase of the antioxidant capacity for both matrices. As with other locations, the maximum value is obtained in the last sampling campaign. The comparative situation of the averages of the antioxidant capacity of fruits and extracts grouped by location shows that after extraction, the antioxidant capacity of the extracts is higher than that determined in fruits, except for Făget location, where the values are approximately equal (fruit - 1509 μg / mg AAE, extract - 1947 μg/ml AAE). The Borhanci location presents the highest values for the antioxidant capacity of fruits and extracts.

Chapter 9 - The analysis performed on the fruit matrix of *Prunus spinosa* resulted in a valuable extract in terms of composition and properties. This extract contains a number of essential minerals for the human body (Ca–187mg/kg K–11458 mg/kg, Mg–753 mg/kg,) and significant concentrations of polyphenols (872 mg / kg). The antioxidant capacity of this extract (1947 μg/ml AAE) makes its use multiple and beneficial to several industry sectors.

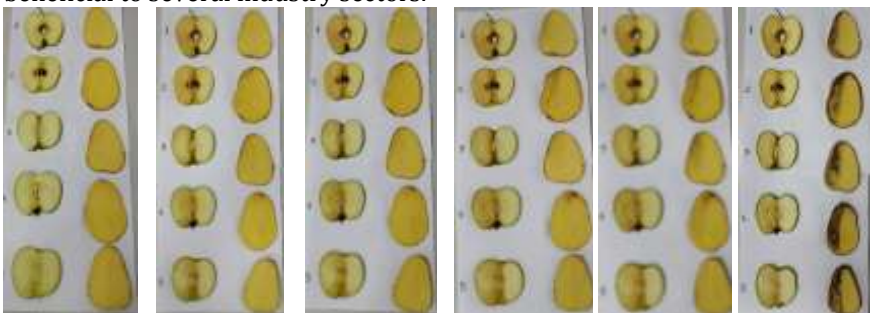

Fig.7. Testing the antioxidant capacity of the extract on products of vegetable origin

Extracts obtained by the extraction technique using carbon dioxide

as a supercritical fluid were introduced into vegetable and animal products. The ability of the extract to stop the oxidation produced by polyphenoxidase enzymes has been demonstrated in samples of vegetable origin. This product stopped the oxidation of the apple samples throughout the experiment. The part of the untreated apple samples completely oxidized after 6 hours, whereas the complete oxidation of the potato samples was performed after 24 hours. As far as animal products are concerned, the extract has completely stopped the formation of hydrogen sulphide and ammonia compounds, these two substances being responsible for the putrefaction process of meat products. By determining these parameters, it can be stated that the addition of extract to products of plant or animal origin leads to the extension of the shelf life of the products, so this new product can be used as a food additive.

GENERAL CONCLUSIONS

The analysis of *Prunus spinosa* fruits revealed the special character of the fruits and the wide range of possible fields of their use:

1. Both fruit and fruit extracts possess an impressive amount of essential mineral elements, present in concentrations well above the recommended daily dose.
2. Chemical analyzes have shown that the fruit contains, in addition to vitamins and minerals, significant amounts of polyphenols, saturated fatty acids, chlorophyll.
3. The data obtained show that the transfer of contaminants determined in the analyzed soil and air samples is performed only in polluted areas with heavy traffic, this being demonstrated by the calculated correlations.
4. The extraction technique is a determining factor for an optimal transfer and increase of all beneficial parameters, from fruit to extracts. In the case of the present work the optimal method proved to be the one with the help of supercritical fluids.
5. The extraction carried out by means of carbon dioxide used as supercritical liquid results in obtaining an extract in which the vitamins and minerals contained in the fruits are concentrated, contributing to the obtaining of a new product - the extract with multiple effects and benefits.
6. Studies and experiments have shown that low temperature has the most important role in the full ripening of fruits. These increased values are found in both fruits and extracts.

7. Borhanci has proven to be the ideal location for picking and eating *Prunus spinosa* fruit.
8. The product obtained, the extract, has been introduced into products of vegetable and animal origin in order to demonstrate its ability to improve the quality of the food in which it is introduced. Thus, following the analyzes performed on samples of apple, potato and meat, the results obtained demonstrated its property to extend the shelf life of the product in which it was introduced. This new extract, obtained from the fruits of *Prunus spinosa* can be used as such, as it has a number of benefits for human consumption, but the most important aspect is that it can bring improvements to the food in which it is introduced.
9. Given the high concentrations of essential minerals, vitamins, organic acids, calcium and magnesium salts found in the *Prunus spinosa* fruit studied and their total transfer in the extracts obtained, it is recommended to use these fruits, but especially the extracts, especially in food industry as a food additive.
10. Given that higher concentrations of PAHs were determined in the Făget location than in the other locations studied, it is recommended to avoid the use of plants for curative purposes if they were harvested from areas where there is information on the existence of a high degree of pollution.
11. In order to ensure that the fruit or plant products used are not contaminated and do not present a risk to the consumer, it is necessary to implement sets of analyzes and measures including the appropriate choice of sampling site and monitoring of environmental pollution from which they come.

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