S U M M A R Y

EXTRACTION AND ISOLATION OF ACTIVE PRINCIPLES FROM VEGETABLE RAW MATERIALS

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This work, through the research studies included, aims to valorize the pigmented maize bran of the Bloody Butcher (red maize) variety, a by-product of cereal residues, by obtaining extracts rich in phenolic species and testing them as an antioxidant additive for dermato-cosmetic emulsions, a topic of interest in modern society. In carrying out the proposed studies, aspects related to the improvement of the environmental impact have been taken into account, through the use of low-energy processes and low-toxicity materials, and most importantly, through the development of a strategy for the valorization of a by-product used exclusively as animal feed (red maize bran, with a hard, glassy structure).

In view of the current state of research in the field, the originality of this doctoral thesis consists in the following:

- *the valorization of a by-product derived from red maize kernels, cultivated in Romania, for obtaining polyphenolic extracts.* There is a low volume of literature reports exclusively for red maize compared to other pigmented maize variants (such as purple or blue maize). Furthermore, there is a low volume of publications on the valorization of some varieties of pigmented maize cultivated on the territory of our country.
- *novel elements deriving from the investigation of the bran of red maize with a glassy structure, used exclusively as animal feed.* This highlights the usefulness of this cereal raw material in a research strategy that may lead to value-added products - polyphenolic extracts - adaptable to industries with societal impact (pharmaceutical, cosmetic, biomedical, food).
- *the fact that the testing was conducted exclusively with environmentally friendly extraction media and using low impact and low energy consumption processes.* The commitment to responsible processes has led to a work plan harmonized with

sustainability strategies. Moreover, the choice of raw materials that constitute a by-product is harmonized with the concept of sustainability.

- *use of polyphenolic extracts from Bloody Butcher red maize bran in an application on the borderline between the pharmaceutical and cosmetic fields - active ingredient as antioxidant for dermato-cosmetic emulsions.* The specialized literature does not present such an approach.

Against this background, in order to achieve the proposed aim, the PhD thesis comprises three main objectives, which constitute three experimental research chapters:

○ ***Objective 1****: Valorization of a by-product (bran) derived from colored maize for the isolation of polyphenolic extracts, establishing the optimal extraction process and optimizing working parameters, by environmentally-responsible processes.*

○ ***Objective 2:*** *Fractionation of polyphenolic extract by environmentally friendly membranous processes in order to isolate valuable fractions with phytochemically differentiated content and biochemical evaluation of the resulting fractions.*

○ ***Objective 3:*** *Valorization of a selection of polyphenolic fractions obtained from red maize bran as antioxidant active ingredient for dermato-cosmetic emulsions.*

Objective 1, achieved through the activities detailed in the Chapter III of this thesis, aimed at optimizing the extraction parameters in order to obtain a fraction rich in phenolic compounds, starting from Bloody Butcher red maize bran. The selection of this raw material was carried out in a rational manner, taking into account, on the one hand, the strategy of valorization of a by-product and, on the other hand, the high phenolic content associated with the aleuronic layer and the pericarp of pigmented maize kernels.

The research was carried out from both raw bran, a raw material referenced as milled maize (MM), and sifted bran, a raw material referenced as sifted milled maize (SMM). For comparison, experiments were carried out on the extraction of polyphenols from the bran of yellow maize, the KWS Kashmir variety. The raw materials were obtained by milling maize kernels grown in 2021 and 2022, in the region of Brăila, Romania.

The two types of maize differ in structure. Yellow maize has a floury/semi-glassy structure, the endosperm adhering to the bran being in small amount, while red maize has a glassy structure (an elevated hardness), and in this case, the bran shows a significant starch fraction attached to the pericarp; also, MM and MMC are much harder compared to yellow maize bran.

For MM and SMM, a sequence of experimental phases was carried out and the extracts obtained were further investigated by modern characterization techniques, the optimal extraction method as well as the optimal working parameters (medium and extraction time) were established.

Extraction of polyphenols from MM and SMM was performed using distilled water (DW), water-ethanol mixture (1/1, v/v, DW-EtOH) and DW-EtOH acidified with

hydrochloric acid (1%, sol HCl 1N) as extraction media. The mass ratio of plant material/solvent used was 1:10.

In a first working sequence, MM and SMM were extracted for 60 min by two methods: magnetic stirring and ultrasonic treatment, respectively, at room temperature, shielded from light.

The second phase of the experiment aimed to identify the optimal extraction time for MM and SMM between 1 min and 60 min.

The third experimental phase utilized an unconventional extraction technique for the extraction of phenolic compounds from MM and SMM, namely microwave-assisted extraction. Extraction times between 5 and 20 seconds and two different power levels, 350 and 700 W, were tested.

All the extracts resulting from the 3 experimental sequences were investigated by determining the total phenolic content, and on the basis of the obtained results, the optimal solvent variant capable of isolating a fraction rich in phenolic species, the optimal extraction time, in accordance with the strategy of using responsible processes, and the most efficient method were determined.

The quantification of the total polyphenol content (TPC) with the Folin-Ciocâlteu spectrophotometric method showed that the DW-EtOH mixture leads to the most polyphenol-rich extractions when ultrasonic treatment is used, for both tested raw materials, MM and SMM. Also, a longer extraction time (60 minutes) indicates a better yield, but in accordance with sustainability principles, aiming for low energy consumption, the optimal extraction time of 20 minutes was set. The increase in the concentration of phenolic compounds in the extracts obtained was taken into account by proportionally determining the TPC value at the different extraction times tested, by comparison with the TPC value for the extract resulting from 1 minute of processing (either by magnetic stirring or ultrasonic treatment).

Lower TPC values were obtained for extracts from yellow maize bran (KWS Kashmir), validating the selection strategy of red maize bran for the extraction of polyphenolic species.

Concerning microwave-assisted extraction, as a modern and environmentally friendly technique, particularly useful in the isolation of bioactive molecules from plant sources, performing the process at higher power (700 W) as well as longer exposure (20 seconds), leads to higher total polyphenol content.

The assessment of the antioxidant capacity was carried out by the TEAC (Trolox Equivalent Antioxidant Capacity) method. The results showed better results for SMM in both magnetic stirring and ultrasonic extraction. The highest antioxidant activity (451.71 mM TE/100 g DM) was observed in the extract after 60 minutes of processing by magnetic stirring. A similar result was also recorded for the SMM extract after 60 minutes of ultrasonic treatment (430.71 mM TE/100 g DM).

The fractions obtained by microwave-assisted extraction (20 seconds at 350W) showed moderate antioxidant capacity while the MM and SMM extracts obtained after 20 seconds of microwave irradiation at 700 W had very good antioxidant activity, 385.34 mM TE/100 g DM and 396.67 mM TE/100 g DM, respectively.

Thus, based on the experimental results obtained, it was concluded that Bloody Butcher red maize can be considered in future experiments for the isolation of fractions rich in phenolic compounds and isolation of fractions with differentiated content. Also, the extracts obtained show potential for future studies and their testing in different application fields (cosmetics, pharmaceuticals, nutraceuticals, biomedical, and foodstuffs) as additives with antioxidant activity. This is also encouraged by the processes selected to obtain fractions rich in antioxidant phenolic species, using only solvents compatible with cells (water and ethanol). These aspects of the experimental protocols established in this study are also harmonized with sustainability strategies.

Objective 2 was achieved by the results presented and discussed in Chapter IV of this work, which focused on the optimal extract of red maize bran of the Bloody Butcher variety, as detailed for Chapter III, for the separation of fractions with differentiated phenolic compound content by membrane processes.

Separations of the optimal extract (P0, as determined in Chapter III) were carried out by separation experiments using different membrane types and also different experimental parameters:

- Regenerated cellulose (RC) membranes with a mean pore diameter (cut-off) of 5KDa at a working pressure of 5 bar;

- Polyethersulfone (PES) membranes with a mean pore diameter (cut-off) of 5KDa at a working pressure of 5 bar;

- Polyethersulfone (PES) membranes with a mean pore diameter (cut-off) of 10KDa, using two working pressures, 2 bar (first pass) and 3 bar (second pass), respectively.

- The obtained fractions, named *concentrate fraction* (C) and *permeate fraction* (P) were characterized in terms of total phenolic compounds content, total flavone content, antioxidant activity. Also, phenolic acids, rutin and quercetin, molecules with recognized biological effect according to literature data, were quantified for the resulting extract fractions.

The extract fractions evaluated for total polyphenol content (TPC) showed a dependence of these contents on the separation experiment used (membrane type, pore size, working pressure). The differences obtained can be attributed, on the one hand, to the pore size of the membranes, which are capable of separating the polyphenols present in the P0 extract in relation to their average molecular weight, but on the other hand, they can be attributed to a lower or higher affinity of the extract for the membrane used.

A comparative analysis of the obtained data shows that the 5 KDa RC membrane helps to a higher retention of polyphenols, promoting the enrichment of the resulting extract fraction (P1-C) in valuable phenolic molecules, compared to the 5 KDa PES membrane. It was also determined that the PES 10 KDa membrane (at a working pressure of 3 bar) favors the polyphenol content for the concentrated fraction (P3-C2), probably allowing the suspension of higher molecular weight compounds in the hydroalcoholic medium, avoiding their loss by deposition/capture on the membrane surface/pore.

For the RC 5 KDa (5 bar) and PES 10 KDa (2 bar) membranes, concentrate fractions with high content of phenolic species were obtained, while PES 5 KDa (5 bar) and PES 10 KDa (3 bar) membranes lead to concentrate and permeate fractions with differentiated content of phenolic species, being considered the most efficient separation processes according to the proposed strategy.

The total polyphenol content and antioxidant activity of red maize extract fractions show very good results especially for the concentrated parts, which showed levels comparable with other reports in the literature.

Significant levels of flavonoids and phenolic acids were also observed for the concentrated fractions derived from Bloody Butcher maize extract. From the phenolic acids category, syringic acid was the predominant representative in all the extract variants analyzed. No less significant are the concentrations of chlorogenic acid and 4-coumaric acid, phenolic acids known for their therapeutic effects. Rutin and quercetin, particularly important flavonols with potential applications in the pharmaceutical and food supplement industries, are found in rather low concentrations, but the overall phytochemical profile may contribute to the whole biological activity of the extract fractions isolated from red maize bran.

Thus, it was concluded that the concentrated P2-C and P3-C2 extract fractions resulting from separation using PES membrane (5 KDa, 5 bar and 10 KDa, 3 bar) represent the best variants with notable antioxidant activity and therefore deserve further study and investigation.

Objective 3, which entailed the valorization of the most promising Bloody Butcher red maize bran extract variants in the development of a dermato-cosmetic preparation, was achieved through the activities detailed in Chapter V of this work. Thus, the concentrated extract fraction resulting from the separation using PES membrane 5 KDa (5 bar) - *P2-C* AA (antioxidant capacity ~ 40 mg TE/ g DM) and the concentrate fraction resulting from the separation using the PES 10 KDa membrane (3 bar, second pass) - *P2-C2* (antioxidant capacity above 36 mg TE/ g DM) were used as an antioxidant ingredient for an oil-in-water (O/W) dermato-cosmetic emulsion with a concentration of 0.5%.

A basic formulation for an emulsion-type dermato-cosmetic preparation was established, keeping the list of ingredients as simple as possible, in order to be able to

determine whether the proposed antioxidant extracts exert a protective role for these emulsions.

The resulting emulsions (1 control emulsion, M, and 2 preparations with added extract, P_2C and P_3C_2) were tested by different methods, specific for the cosmetic/ dermato-cosmetic industry, and the stability of the resulting samples (creams) was estimated in correlation with the addition of maize polyphenolic extract (antioxidant ingredient, 0.5%).

The cold stability test (4°C, refrigerator) indicates the stability of all the studied preparations after 6 weeks of storage under these conditions, with no changes in organoleptic characteristics (appearance, color, odor).

Another stability test for the prepared emulsions was carried out by direct exposure to solar radiation. During the first 2 weeks of exposure no organoleptic changes were observed, but after 6 weeks of exposure a rancid odor was perceived for emulsion M, not antioxidant-activated.

Significant differences in behavior were observed between the control emulsion (M) and the two emulsions with added antioxidant extract (P_2C and P_3C_2, 0.5% polyphenolic extract added) during the stability test involving temperature input (accelerated ageing test/swing test). In this case, for the M emulsion, changes in organoleptic characteristics were perceived at the beginning of the 2nd test cycle (out of a total of 4 cycles, under pre-set conditions). The emulsion exhibits destabilization, by separation into component phases and thus dramatic changes in appearance, as well as changes in color and the appearance of rancid smell.

In contrast, the emulsions additivized with antioxidant extract (both extract variants, added at 0.5%) maintained their characteristics throughout the stability tests, including the Swing test (4 cycles, equivalent to 4 weeks of exposure to thermal stress), and the antioxidant effect of red maize extracts was evidenced.

The shelf life of the cosmetic preparations was estimated using a calculation protocol adapted from the Arrhenius equation, according to which the accelerated aging process is based on the relationship between temperature and reaction rate, where an increase of the test temperature by 10°C raises the reaction rate by approximately 2-3 times. The data obtained from the Swing test were used for this calculation, each developed and studied preparation being given a certain 'shelf life'. For the polyphenolic extract-additivized products, P_2C and P_3C_2, the estimated shelf life was more than 2.5 times higher than that estimated for the M emulsion, according to the results obtained (35 days for M and 76 days for P_2C and P_3C_2 emulsions).

The pH evaluation of the initial emulsions indicated an optimal level of use with a pH compatible with the skin (5-5.5) and dramatic changes were observed in the stability tests for the emulsion not activated with antioxidant extract, especially after the Swing test (M, pH 2-3). It was established that exposure to heat stress favors oxidative degradation processes of vegetable oils in the cream composition, forming acidic species (most likely free fatty acids) responsible for the decrease in pH values.

In all the tests carried out in this chapter, the protective role against oxidation processes exerted by the extracts from red maize bran P_2C and P_3C_2, selected in the study as antioxidant ingredients, was highlighted.

Thus, the results contained in this PhD thesis, validate the proposed research strategy on the superior valorization of red maize bran (by-product) of the Bloody Butcher variety.

In the context of sustainable processes towards value-added products in a circular economy approach, for this research strategy, possibilities for further valorization by valorization of the bran down to 0 level of process waste were also identified. In this sense, the bran waste resulting after extraction processes could be transformed into activated carbon with wide applications in different industrial segments. The polyphenolic extracts from red maize bran could also be tested in other applications in different industrial sectors (such as food industry, pharmaceutical/biomedical industry, veterinary, as feed additive).

Thus, by integrating these by-products into sustainable strategies linked to different production areas, the range of use of cereal by-products/waste can be broadened and processes adapted to the concept of circular economy can be implemented, whereby a plant product, having gone through efficient processing steps, can be revalorized to minimum/zero waste levels.