SUMMARY OF PhD THESIS

Recovery of coenzyme Q10 from agri-food by-products in order to develop food supplements

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SUMMARY

The coenzyme Q term refers to a class of homologous quinones found in various living organisms, including microorganisms, plants, animals, and humans, that share a benzoquinone ring structure with an isoprenoid side chain of varying lengths [SIEMIENIUK and SKRZYDLEWSKA, 2005; BHAGAVAN et al., 2007]. Over the last decades, CoQ10 has gotten much attention, given that it is the only endogenously synthesized lipid-soluble antioxidant in cells [WEBER et al., 1997; SOUCHET and LAPLANTE, 2007; STIFF et al., 2011]. It is involved in mitochondrial processes, such as respiration, cellular ATP (adenosine triphosphate) synthesis, maintaining heart muscle strength, neutralizing free radicals in the fight against aging, and stimulating the immune system [ATLA et al., 2014].

Food loss and food waste are a global challenge; one third of all food produced around the globe is lost or wasted at some point in the food supply chain [SMEU et al., 2022]. Smeu et al. (2022), state that, traditionally, food waste is usually incinerated or dumped in landfills, which subsequently results in air, water, and soil pollution; food waste from several agrifood industries (vegetables, fruits, beverages, sugar, meat, aquaculture and marine products, seafood, etc.) is an interesting and cheaper source of potentially functional or bioactive compounds so food by-products can be used in nutraceutical and pharmaceutical products, not just to get animal feed and different fertilizers.

Several methods are available in the literature for CoQ10 determination in food matrices by using high-performance liquid chromatography with diode array detection (HPLC-DAD), which first consists of analyte extraction with different solvents, followed by the instrumental analysis of the obtained extracts [PODAR et al., 2023].

We have to consider the advantages and disadvantages of available techniques as well as several factors to choose the appropriate analytical method for a given problem. In addition, revalidation or verification is necessary whenever a method is changed or applied to a new circumstance (such as a different sample matrix), depending on the change's degree and the unique circumstance's nature [AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY]. Also, typical characteristics such as accuracy, precision, specificity, detection limit, quantification limit, linearity, and range should be considered when validating an analytical method [EUROPEAN MEDICINES AGENCY].

From a sustainable point of view, isopropanol, a polar solvent, was used in this work for the extraction of CoQ10 from food matrices, which is at the top of the list of green chemicals and which is considered environmentally safe in industrial solvent selection guidelines [YILMAZ and SOYLAK, 2020]. According to Directive 2009/32/EC of the European Parliament and the Council, isopropanol is already approved as an extraction solvent for processing raw materials, foodstuffs, food components, or ingredients, with a maximum residue limit of 10 mg/kg food in the extracted foodstuff or food ingredient.

Embracing the concept of "good for me, good for the earth", many consumers are interested in products with ”clean label” and sustainability credentials; 33% of supplement users prefer products derived from natural sources [ADM, 2024]. Therefore, the dietary supplement market has a high demand for such products.

The aim of the research was to assess the possibility of exploiting food by-products and waste as a source of coenzyme Q10 and identify the most suitable method to recover this molecule for further use in preparing natural dietary supplements. To achieve this, we proposed the following objectives:

O1. The development of an analytical procedure for recovering and quantifying CoQ10 from food by-products and waste through ultrasonic extraction with 2-propanol, preceded by HPLC-DAD analysis

O2. To characterise the fats extracted from the selected food by-products/waste (oil press cakes of rapeseed, sunflower, pumpkin, linseed, walnut, and hempseed; whole fish; chicken hearts) in terms of their content in CoQ10, tocopherols and tocotrienols, or cannabinoids (only for HPC fat).

O3. The preparation of different natural dietary supplements based on CoQ10 from food by-products/waste and the assessment of their stability at storage in ambient conditions.

The results of this thesis were published in one review article (ISI-indexed journal with IF 4.1 – *Metabolites*) and three original articles (one ISI-indexed journal with IF 5.2 – *Foods Journal*; the second ISI-indexed journal with IF 3.2 – *Waste and Biomass Valorization* and the third is still In Press in the *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology*.

The studies and experiments described in this thesis were carried on in the Faculty of Food Science and Technology, and in the Institute of Life Sciences from the same University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, under the coordination of Prof. Dr. habil. Sonia Ancuţa Socaci and Prof. Dr. habil. Cristina Anamaria Semeniuc and the supervision and guidance of the co-authors listed in the publications.

The PhD thesis is structured in two main parts state-of-the-art containing a literature review article (Chapter 1) and personal contributions containing the research objectives (Chapter 2), followed by the own research articles (Chapters 3-5), the own patent application entitled "Process for the preparation of some natural dietary supplements based on coenzyme Q10" (Chapter 6), and respectively, general conclusions and recommendations (Chapter 7).

In what concerns the **first part** (literature review article), it was found that analytical methods available in the literature for determining CoQ10 contents in foods consist of a combination of extraction and quantification techniques, each with strengths and limitations, so: generally, a direct extraction method is used with 2-propanol or a mixture of ethanol/*n*-hexane. High-performance liquid chromatography, spectrofluorimetry, and differential pulse voltammetry are used to analyse CoQ10

quantitatively in the obtained extracts. Also, the richest sources of CoQ10 are oils, organs, and meat.

In what concern the **second part**, the experiments included: i)determination of CoQ10 content in food by-products and waste by HPLC-DAD; ii) chacaterisation of fats extracted from oil press cakes, fish meat, and chicken hearts as potential CoQ10 supplements; iii) a procedure for the preparation of some natural dietary supplements based on CoQ10 from chicken hearts and oil press cakes. And a differentiated chapter is represented by the patent application with the title "Process for the preparation of some natural dietary supplements based on Coenzyme Q10"

Chapter 3 shows how the CoQ10 level is determined in some food by-products (oil press cakes) and waste (fish meat and chicken hearts) to recover this compound for further use as a dietary supplement. An analytical method was used: ultrasonic extraction using 2-propanol, followed by HPLC-DAD. The method was validated in terms of linearity and measuring range, limits of detection (LOD) and quantification (LOQ), trueness, and precision.

Chapter 4 is about the characterisation of fats extracted from rapeseed press cakes (RPC), sunflower press cakes (SPC), pumpkin press cakes (PPC), linseed press cakes (LPC), walnut press cakes (WPC), hempseed press cakes (HPC), whole fish (WF), and chicken hearts (CH) in terms of their content in CoQ10, tocopherols and tocotrienols, or cannabinoids (only for HPC fat); the main lipid classes and fatty acids composition were also determined. The antioxidant capacity of each type of fat was assessed by the DPPH method, while the oxidative status was evaluated by determining the PV and TBARS. Several lipid quality indices were calculated to estimate the benefit-harm balance of each potential dietary supplement.

Chapter 5 presents (1) the preparation of four natural dietary supplements based on CoQ10 from oil press cakes and one from chicken hearts and the optimization of this procedure, as well as (2) the assessment of their stability at storage in ambient conditions [by regular determination (every three months) of CoQ10 content, TEAC, and PV for nine months].

Chapter 6 includes the patent application with number A/00138 from 24.03.2023, entitled "*Process for the preparation of some natural dietary supplements based on Coenzyme Q10*". Inventors: Cristina-Anamaria Semeniuc, Andersina-Simina Podar, Sonia-Ancuța Socaci, Floricuța Ranga, Simona-Raluca Ionescu, Maria-Ioana Socaciu, Melinda Fogarasi, Dan-Cristian Vodnar, Anca-Corina Fărcaș.

Future perspective:

- Our new line of research will focus on conducting *in vitro* tests on skin cancer and normal cell lines. We have already applied the MTT assay to assess cell viability and cytotoxicity (data not shown here). We also intend to evaluate the antiproliferative effects of these dietary supplements and investigate other molecular markers to understand their potential therapeutic effects and mechanisms. These tests' findings will provide crucial insights into the efficacy and safety of our

dietary supplements, advancing the understanding of their potential in clinical applications.

Our future work will focus on the bioavailability assessment of dietary supplements with the highest CoQ10 content, namely DS_LCH (animal origin) and DS_PPC (vegan).