Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology
Open Access

A Procedure for the Preparation of Some Natural Dietary Supplements Based on Coenzyme Q10 from Chicken Hearts and Oil Press Cakes

Cristina Anamaria SEMENIUC[1*], Andersina Simina PODAR[2], Floricuța RANGA[2], Simona Raluca IONESCU[1], Maria-Ioana SOCACIU[1], Melinda FOGARASI[1], Anca Corina FĂRCAȘ[2], Dan Cristian VODNAR[2], Sonia Ancuța SOCACI[2*]

[1] Department of Food Engineering; University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Mănăștur St., 400372 Cluj-Napoca, Romania
[2] Department of Food Science; University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Mănăștur St., 400372 Cluj-Napoca, Romania
**Corresponding authors: C.A. Semeniuc cristina.semeniuc@usamvcluj.ro; S.A. Socaci sonia.socaci@usamvcluj.ro*

RESEARCH ARTICLE

Abstract

This study proposes a procedure for preparing some natural dietary supplements based on CoQ10 (coenzyme Q10) from lyophilised chicken hearts, respectively, oil press cakes of sunflower, rapeseed, walnut, and pumpkin. It consists of homogenising the powders obtained from these matrices with 2-propanol, succeeded by sonication, overnight maceration, collecting the liquid phase and filtering it under vacuum, removing the solvent by rotary evaporation, eliminating the 2-propanol traces under a nitrogen stream, and, finally, packaging in 10-mL amber glass bottles. Five dietary supplements were prepared using the above-described procedure and kept at room temperature for 9 months to evaluate their storage stability. To this end, CoQ10, TEAC (Trolox equivalent antioxidant capacity), and PV (peroxide value) levels were determined initially and every 3 months of storage. The highest levels of CoQ10 were found in the dietary supplements from lyophilised chicken hearts (18.12 mg/10 mL) and pumpkin press cakes (5.48 mg/10 mL). However, those from rapeseed and walnut press cakes had the highest levels of TEAC (19.23 µmol TE/g and 15.31 µmol TE/g, respectively). Exceeding the maximum permissible level for PV revealed a shelf-life of 3 months for the dietary supplements from pumpkin and walnut press cakes and less than that for the others.

Keywords: Natural dietary supplement; coenzyme Q10; preparation procedure; food matrices.

Received: 27 May 2024
Accepted: 7 July 2024
Published: 15 November 2024

DOI: 10.15835/buasvmcn-fst:2024.0019

INTRODUCTION

Over the years, interest in coenzyme Q10 (CoQ10), the third most consumed dietary supplement, has grown as a possible candidate for treating several non-communicable diseases that rank among the top 10 causes of mortality worldwide (Arenas-Jal et al., 2020). Dietary supplements are covered in the European Union by Directive 2002/46/EC, which defines them as foodstuffs intended to supplement a normal diet. They are sources of concentrated nutrients or other substances with some physiological or nutritional effects, alone or in combination. The dietary supplements are marketed in dose form and designed to be taken in measured small unit quantities; they can be delivered only in a pre-packaged form to the ultimate consumer. CoQ10's effectiveness is compromised by its low oral bioavailability; therefore, several formulations have been created

to address this inconvenience. Initially, they were designed as lipid nanoparticles to encapsulate CoQ10 and distribute it through biological membranes, but later, formulations evolved towards chemical alterations of this molecule to reduce its hydrophobicity (Pastor-Maldonado et al., 2020). 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.

CoQ10, a.k.a. ubiquinone or ubidecarenone, is a lipid-soluble, vitamin-like compound present in the inner mitochondrial membrane of each cell in the body (Kommuru et al., 2001; Turkowicz and Karpinska, 2013). In chemical terms, it is 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone (Ercan and El, 2011), which has a benzoquinone ring and a side chain of 10 isoprenoid units (see Figure 1) (Souchet and Laplante, 2007; Zhang et al., 2018). While the benzoquinone ring predisposes CoQ10 to oxidation, the polyprenyl side chain makes it difficult to dissolve in polar solvents and water but easy to solubilise in nonpolar solvents and lipids. CoQ10 is susceptible to physicochemical changes; it becomes unstable and trans-forms in alkaline conditions and under light (Turkowicz and Karpinska, 2013).

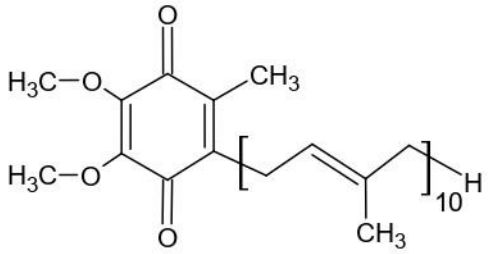


Figure 1. Chemical structure of CoQ10.

CoQ10 has two main functions in the body. It is a crucial molecule in the electron transport chain of mitochondria, where it is used to synthesise ATP, the primary energy source for cells; those with high energy needs — heart, brain, liver, and kidney cells — have the highest CoQ10 levels (Bank et al., 2011). The second function of CoQ10 is that of an antioxidant, especially in preventing lipid peroxidation (Mandrioli et al., 2018). The CoQ10 level in organs gradually declines with age, especially in the brain and heart, probably due to decreased synthesis, increased utilisation, or both (Zhang et al., 2018). Weber et al.'s study (1997) shows that CoQ10 is naturally present in certain food items and is absorbed into the human body by their consumption; according to a recent literature review by Podar et al. (2023), the richest food sources of CoQ10 have been found to include vegetable oils, organs, and meat. Dietary sources alone — which often supply < 3 mg per serving — are insufficient to raise CoQ10 levels to the population average of 1 μg/mL (1.16 μmol/L). Therefore, CoQ10 supplementation represents a convenient and feasible way to elevate CoQ10 status and, thus, promote health and prevent diseases (Zhang et al., 2018; Manasyan and Xia, 2020). Generally, liquid CoQ10 formulations are better absorbed in the body than solid ones (Martinefski et al., 2017); since CoQ10 is soluble in lipids, it is best absorbed when consumed with a meal containing fat or oil (Saini, 2011).

Ethanol (alone or mixed with *n*-hexane at different ratios) is among the most used solvents for CoQ10 extraction (Semeniuc et al., 2023). Another solvent that can withdraw CoQ10 from the food matrix is 2-propanol (isopropanol or propan-2-ol), CoQ10 being more soluble in it than in ethanol (Kubo et al., 2008; Stiff et al., 2011). Among the benefits of using the latter solvent are that triglycerides, which may be interference compounds in the food matrix, are not very soluble in 2-propanol (Souchet and Laplante, 2007), and the recovered solvent can be used to repeat the extraction (Semeniuc et al., 2023). According to industrial solvent selection guidelines, 2-propanol is the most environmentally safe chemical and ranks first on the list of green solvents (Yilmaz and Soylak, 2020). It was already approved as an extraction solvent for processing raw materials, foodstuffs, food components, or ingredients, with a maximum residue limit per kg food of 10 mg in the extracted foodstuff or food ingredient (Directive 2009/32/EC). 2-Propanol also can be used as a carrier solvent for flavourings, at a maximum level of 600 mg/L, in soft drinks (Anton et al., 2005).

In an earlier work (Semeniuc et al., 2023), we investigated the amount of CoQ10 in some food waste (chicken hearts and fish meat) and by-products (sunflower, pumpkin, walnut, linseed, and hempseed oil press cakes) to see if they constitute a substantial source of this compound; cold-pressed cakes contain significant amounts of oil, and the meat, poultry, and fish sector generates a lot of waste. CoQ10 was not found in fish meat or hempseed press cakes, although it varied from 36.56 to 84.80 μg/g in oil press cakes and from 114.39 to 383.25 μg/g in chicken hearts (raw and lyophilised, respectively). To recover higher concentrations of CoQ10 and get out the solvent, we decided to extract the fats from these matrices to be used as such. Therefore, this study aimed to provide a procedure for the preparation of five natural dietary supplements based on CoQ10, which consists of the lipid

fraction extraction from lyophilised chicken hearts and oil press cakes of sunflower, rapeseed, walnut, and pumpkin with a green solvent, 2-propanol, followed by solvent removal through vacuum evaporation. The formulae thus obtained are liquid, natural, and solvent-free. Hence, they meet the abovementioned limitations regarding synthetic dietary supplements; those prepared from oil press cakes can also be used by people who adopt a vegetarian diet or are fasting. No dietary supplement based on CoQ10 with these characteristics is available on the market. As far as we know, this is the first study reporting a preparation procedure for such a supplement by extraction with 2-propanol. The experimental design included (1) the preparation of four natural dietary supplements based on CoQ10 from oil press cakes and one from chicken hearts and optimisation 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].

MATERIALS AND METHODS

Reagents and Standards

HPLC grade methanol, HPLC grade 2-propanol, HPLC grade ethanol absolute, coenzyme Q10 standard, and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) while 2-propanol pure p.a. and hydrochloric acid 35-38% pure p.a. from Chempur (Piekary Śląskie, Poland); chloroform stabilised with about 0.6% ethanol and ammonium thiocyanate p.a. from VWR International bvba (Leuven, Belgium), methanol p.a. and hydrochloric acid 0.2 M from VWR Chemicals (Fontenay-sous-Boi, France), iron(II) chloride tetrahydrate p.a., iron(III) chloride hexa-hydrate p.a., and Trolox standard from Acros Organics (Geel, Belgium).

Food Materials

The chicken hearts were acquired from S.C. Puiul Regal S.R.L. (Gilău, Romania) in an amount of 2.1 kg and maintained at 4 °C (in a refrigerator) until expiration when they were considered waste. They were minced the following day with a meat grinder (N12; Lancom Distribution S.R.L., Bucharest, Romania), homogenised using a silicone spatula, divided into 100 g portions weighted with a precision balance (440-35N; Kern & Sohn GmbH Ziegelei, Balingen, Germany), and then lyophilised in a laboratory freeze-dryer (LyoQuest-55; Azbil Telstar Technologies S.L., Barcelona, Spain) operated under the next conditions: -80 °C for 24 hours at freezing, 0.01 mbar vacuum pressure, and -55 °C for 3 days at sublimation (until constant weight (±0.005 g) was achieved). The lyophilisation yield was 22.1%. The lyophilisate thus obtained was introduced into an amber glass jar, hermetically closed with the lid, and maintained at 4 °C (in a refrigerator) until use. Before use, it was milled to a fine powder with an electric grinder (Titan Mil 300 DuoClean; Grupo Cecotec Innovaciones S.L., Valencia, Spain). The oil press cake pellets of sunflower, rapeseed, walnut, and pumpkin (1 kg of each type) were obtained by a donation from Taf Presoil S.R.L. (Luncani, Romania); they were maintained at 4 °C (in a refrigerator) until use when were milled to fine powders with an electric grinder (Titan Mil 300 DuoClean; Grupo Cecotec Innovaciones S.L., Valencia, Spain).

Preparation of Dietary Supplements Based on CoQ10

Into an amber glass reagent bottle with a screw cap (of 500 mL), 40 g of lyophilised chicken hearts powder (or 100 g oil press cakes powder) was weighed using a precision balance (440-35N; Kern & Sohn GmbH Ziegelei, Balingen, Germany), to the nearest 0.01 g, over which 250 mL of 2-propanol (or 200 mL in case of oil press cakes) was added and homogenised by shaking and inverting the closed container until complete wetting of powder; a second 500 mL-bottle was prepared in the same manner. The two laboratory bottles were placed in a USC 300 THD ultrasonic water bath (VWR International, Singapore, Malaysia) and sonicated to an intensity of 200 W with a frequency of 45 kHz for 15 min, then left for 24 h at room temperature on a 3005-orbital shaker (GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany) set to 150 rpm to macerate. The next day, the liquid phase of each bottle was collected and filtered under a vacuum. The combined filtrate of the two bottles was brought to dryness by evaporation at 40 °C in a Hei-VAP Expert laboratory rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany); the 2-propanol traces were eliminated by passing a stream of nitrogen gas through the dried residue (the resulted lipid fraction) until a constant mass (change of no more than 1 mg). Ten millilitres of this fraction were transferred to a 10 mL-amber glass bottle, to which the dropper was then attached and closed by screwing on the cap fitted with a sealing ring. The dietary supplement (DS) thus obtained was stored at room temperature in the dark for 9 months.

All dietary supplements were prepared in triplicate and analysed every 3 months for CoQ10 content, TEAC, and PV.

Determination of CoQ10 Content in Dietary Supplements

As Rodriguez-Estrada et al. (2006) recommended in their investigation, 30–50 mg of dietary supplement was dissolved in 2 mL of *n*-hexane/2-propanol mixture (3:1, v/v), depending on the sample. CoQ10 was separated using a 1200 high-performance liquid chromatography (HPLC) system (Agilent Technologies Inc., Palo Alto, USA) fitted with a DAD (diode-array detector) set to 275 nm on a Kinetex XB-C18 column (150 mm L × 4.6 mm ID × 5 µm particle size; Phenomenex, Torrance, USA). The elution was performed in an isocratic mode with a methanol/2-propanol/ethanol mixture (70:15:15, v/v/v), as Semeniuc et al. (2023) described. The system additionally included an autosampler, a thermostated column compartment, a degasser, and a quaternary pump. For analysis, 20 µL of extract was injected into the HPLC apparatus. The column's oven temperature was kept at 25 °C, the PDA at 275 nm, and the mobile phase flow rate at 1.2 mL/min. The Rev B.04.02 SP1 of ChemStation software (Agilent Technologies Inc., Palo Alto, USA) was employed to acquire and process the chromatograms; data acquisition took place for 15 min. A 1000 µg/mL stock solution of CoQ10 in 2-propanol was made, and five working solutions were prepared from it at 1, 50, 100, 150, and 200 µg/mL by diluting with the same solvent. The working standards and stock solution were kept in the dark, at -18 °C, for later usage. The working standards and extracts were filtered using polyamide syringe filters (0.45 µm pore size, 25 mm diameter) before being injected into HPLC. The analyte of interest (CoQ10) was identified by comparing the retention times of peaks from the chromatogram to that of the standard analysed under the same conditions, which the UV absorption spectrum confirmed. The results were given as mg/10 mL sample.

Determination of TEAC in Dietary Supplements

The DPPH assay detailed by Semeniuc et al. (2024) was used for this. Initially, a stock solution was done by dissolving 24 mg of DPPH in 100 mL of methanol; the working solution was prepared by combining 24 mL of this solution with 90 mL of methanol to achieve an absorbance at 515 nm of 1.1 ± 0.02 units. Subsequently, using an ABJ-220-4NM analytical balance (Kern & Sohn GmbH, Balingen, Germany), 20-50 mg of the dietary supplement was weighed into a 2-mL screw-top vial to the nearest 0.01 mg. Ultimately, this was dissolved in a 150-750 µL chloroform-methanol mixture (2:1, v/v). A 16-mL glass container with a rubber stopper was filled with 150 µL of this solution, to which 2850 µL of DPPH working solution was added and vortexed (6776; Corning Life Sciences, Monterrey, Mexico). This mixture was kept for 1 hour at room temperature in the dark. Its absorbance value was measured at 515 nm using a UV-1900i double-beam UV-VIS spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) against a chloroform-methanol mixture (2:1, v/v). Instead of the dietary supplement solution, a chloroform-methanol mixture (2:1, v/v) was used to prepare the blank, which was treated similarly. The blank's absorbance value was subtracted from that of the sample. Each sample was tested in triplicate. Trolox was employed as standard at 25 to 800 µmol/L concentrations to create the calibration curve. The results were given in µmol Trolox equivalent (TE)/g sample.

Determination of PV in Dietary Supplements

The PV test was carried out as Semeniuc et al. (2016) have described, with a few adjustments. It is based on the spectrophotometric method published in the ISO 3976:2006 | IDF 74:2006 standard. An ABJ-220-4NM analytical balance (Kern & Sohn GmbH, Balingen, Germany) has been used to weigh approximately 0.040 g of dietary supplement to the nearest 0.1 mg in a 16-mL glass vial with a rubber stopper. Initially, 9.0 mL of a chloroform/methanol mixture (2:1, v/v) (CM) was added to the test sample and gently mixed to dissolve it. Subsequently, 50 µL of a 30% (w/v) ammonium thiocyanate solution (NH_4SCN) was introduced and vortexed (6776; Corning Life Sciences, Monterrey, Mexico) and then 50 µL of an iron(II) chloride solution ($FeCl_2$) [c(Fe)=1 mg/mL]. The resulting mixture was poured into a 10-mL volumetric flask, completed with CM to the mark, vortexed, and left to stand in the dark for 10 min at room temperature. Using a UV-1900i double-beam UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), its absorbance value was read at 500 nm against the blank. The blank sample (made with 9.9 mL of CM, 50 µL of NH_4SCN, and 50 µL of $FeCl_2$) was subjected to the same treatment as the test sample. Each sample was tested in triplicate. An iron(III) chloride solution ($FeCl_3$) [c(Fe)=10 µg/mL] was used to prepare the standards, in concentrations ranging from 5 to 50 µg Fe^{3+}, for the calibration curve. The results were given in milliequivalents (meq) O_2/g sample.

Statistical Analysis

Using the 19.1.1 version of Minitab statistical software (LEAD Technologies, Inc., Charlotte, NC, USA), a one-way ANOVA with a post-hoc Tukey's test at a 95% confidence level ($p < 0.05$) was applied to assess whether differences between the mean levels of CoQ10, TEAC, and PV in oil press cakes are statistically significant. By calculating Pearson's correlation coefficient (r), the strength of the relationship between CoQ10 and TEAC, CoQ10 and PV, and TEAC and PV, respectively, was determined.

RESULTS AND DISCUSSIONS

Figures 2, 4, and 5 show the CoQ10 content in the dietary supplements obtained and analysed as described above and their stability at room temperature. These represent graphically the changes in CoQ10 content, respectively, in levels of TEAC and PV during 9 months of storage at three-month intervals.

CoQ10 content

The highest CoQ10 content was found in the dietary supplement prepared from lyophilised chicken hearts (DS_LCH) [18.12 mg/10 mL (1435 μg/g)], followed by the dietary supplement prepared from pumpkin press cakes (DS_PPC) [5.48 mg/10 mL (582 μg/g)], the dietary supplement prepared from walnut press cakes (DS_WPC) [4.17 mg/10 mL (406 μg/g)], the dietary supplement from prepared rapeseed press cakes (DS_RPC) [3.23 mg/10 mL (343 μg/g)], and the dietary supplement prepared from sunflower press cakes (DS_SPC) [2.88 mg/10 mL (298 μg/g)], with non-significant differences between levels of the last three ones (see Figure 2); however, the CoQ10 content decreased with storage time in all dietary supplements.

As the preparation procedure of the natural dietary supplements based on CoQ10 proposed in this study involves the lipid fraction extraction from some food matrices, the CoQ10 levels found in them are between 3.7 and 11.1 times higher than in the extracts tested by us in a previous study (Semeniuc et al. 2023), in which the method for chromatographic analysis of CoQ10 was validated. An in-depth characterisation of the fats extracted from chicken hearts and oil press cakes using the Folch method modified by Boselli showed that they also contain triglycerides, diglycerides, monoglycerides, free fatty acids, and tocopherols (Semeniuc et al. 2024).

The amount of active ingredient per total volume of dietary supplement will vary from batch to batch depending on the CoQ10 content in the raw material (oil press cakes or lyophilised chicken hearts) at the time of extraction. However, according to European Union regulations (Directive 2002/46/EC), this does not represent a problem if the amount of CoQ10 from each container and the recommended daily dose is written on the label.

The least stable at ambient storage were DS_LCH, DS_RPC, and DS_SPC; in the 3rd month of storage, they showed levels of 15.76 mg/10 mL, 2.57 mg/10 mL, and 2.55 mg/10 mL, respectively, significantly lower than initial ones (18.12 mg/10 mL for DS_LCH, 3.23 mg/10 mL for DS_RPC, and 2.88 mg/10 mL for DS_SPC). DS_PPC and DS_WPC reached a 3-month shelf-life, their CoQ10 content (of 5.06 mg/10 mL and 4.17 mg/10 mL, respectively) being non-significant compared to the initial ones (5.48 mg/10 mL for DS_PPC and 4.17 mg/10 mL for 4.17 mg/10 mL) from this storage time onwards, but significantly higher than those in the 6th month of storage (3.84 mg/10 mL for DS_PPC and 3.57 mg/10 mL for 4.17 mg/10 mL). Previous reports of CoQ10 content have been made for rapeseed oil (63.5 μg/g) by Mattila and Kumpulainen (2001), sunflower oil (8.7 μg/g) by Rodríguez-Acuña et al. (2008), and chicken hearts fat (2310 μg/g) by Villanueva-Bermejo and Temelli (2021).

Figure 3 shows the preparation yields of natural dietary supplements based on CoQ10. Since these were low, it was decided to optimise their preparation procedure by replacing the orbital shaking operation when macerating the sample with magnetic stirring (50 rpm; digital stirrer w/heating RSLAB-11C; RSLab, Heraklion, Crete) and reusing the recovered solvent (with its completion at the initial volume) for two more successive extractions.

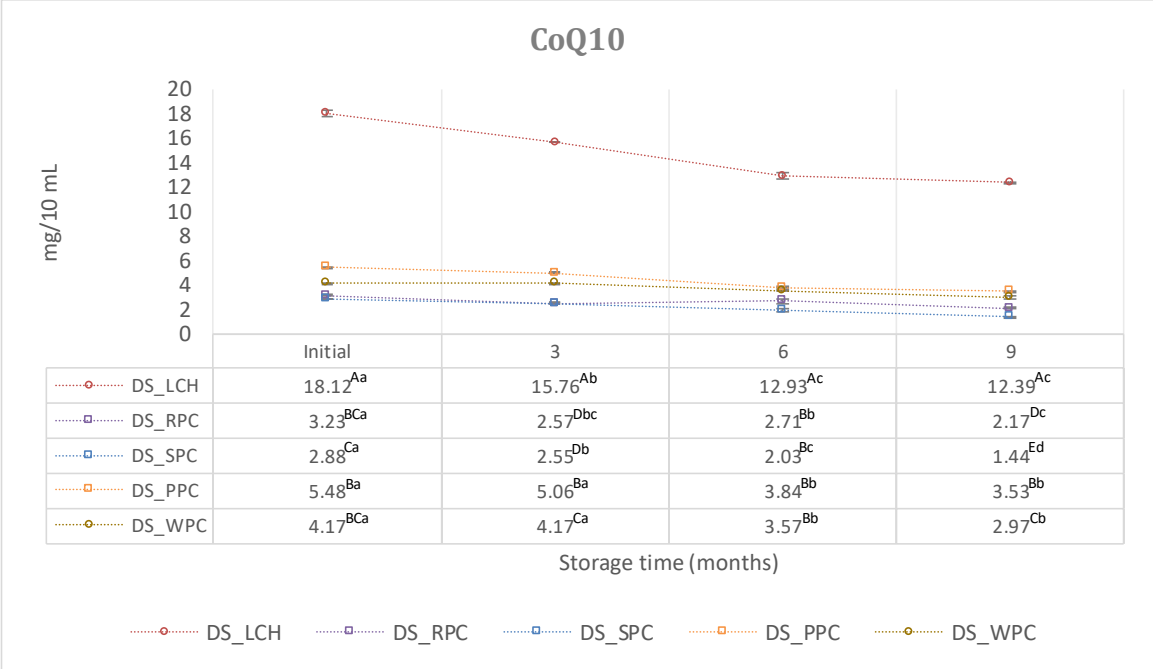


Figure 2. Variation of CoQ10 content in dietary supplements during the stability test. Data are presented as mean ± standard deviation of triplicate data (n=3). Different uppercase letters in a row indicate significant differences between dietary supplements ($p < 0.05$, Tukey's test), and different lowercase letters show significant differences between storage times ($p < 0.05$).

The highest extraction yield obtained by the initial procedure was for DS_LCH (12.5 mL/100 g) because the matrix from which it was prepared was concentrated (96.8% dry matter) due to lyophilisation. From 100 g of rapeseed and pumpkin oil press cakes, 7.3 mL of dietary supplement resulted, 6.6 mL from those of sunflower, and 4.0 mL from the walnut press cakes; optimisation of the extraction process increased the preparation yield by 2.13 times for DS_LCH and DS_RPC, 2.56 for DS_SPC, 1.89 for DS_PPC, and 1.45 for DS_WPC.

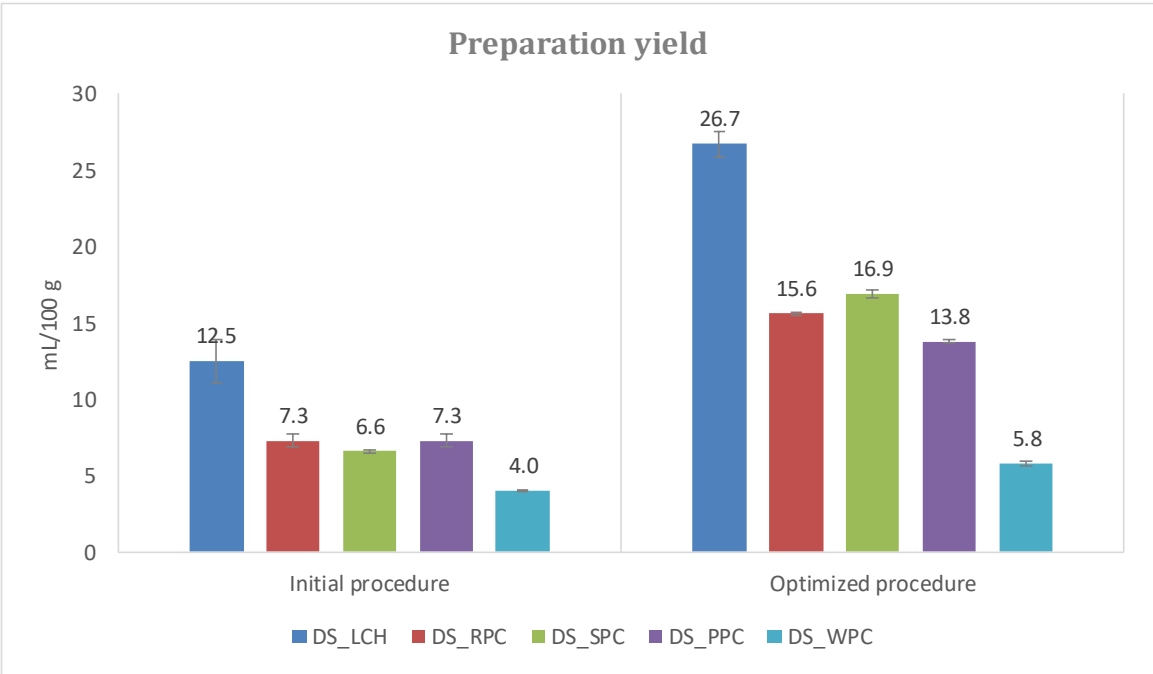


Figure 3. Preparation yields for the dietary supplements obtained by initial procedure vs optimised procedure.

To determine which food matrix is more economical for producing a natural dietary supplement based on CoQ10, inputs like raw material (oil press cakes and chicken hearts), the solvent, lyophilisation, and packaging bottle were evaluated. Table 1 shows the production cost per 10 millilitres dietary supplement prepared using the optimised procedure. Sunflower press cakes generate the lowest production cost (3.59 €), followed by rapeseed (3.87 €) and pumpkin press cakes (4.31 €). Walnut press cakes are not cost-effective for preparing such a dietary supplement (9.85 €), as large amounts of raw material and solvent are needed. As expected, lyophilisation considerably increased the production cost of DS_LCH, being the highest (11.26 €). The other costs (with inputs like energy, labour or capital investment) involved in the large-scale production of a natural dietary supplement based on CoQ10 could not be estimated in this study.

Table 1. Production costs of natural dietary supplements based on CoQ10 prepared using the optimised procedure

Dietary supplement	Inputs used per production						Production costs (€/10 mL)*
	Oil press cakes/chicken hearts		2-Propanol		Lyophilisation	Glass bottle	
	Quantity (g)	Cost (€)*	Volume (mL)	Cost (€)*	Cost (€)*	Cost (€)*	
DS_LCH	167**	0.27	140	2.44	8.35	0.2	11.26
DS_RPC	64	0.03	209	3.64	-	0.2	3.87
DS_SPC	59	0.03	193	3.36	-	0.2	3.59
DS_PPC	72	0.04	234	4.07	-	0.2	4.31
DS_WPC	172	0.17	545	9.48	-	0.2	9.85

Note: *, based on costs available during autumn 2023; **, 37 g of lyophilisate.

TEAC

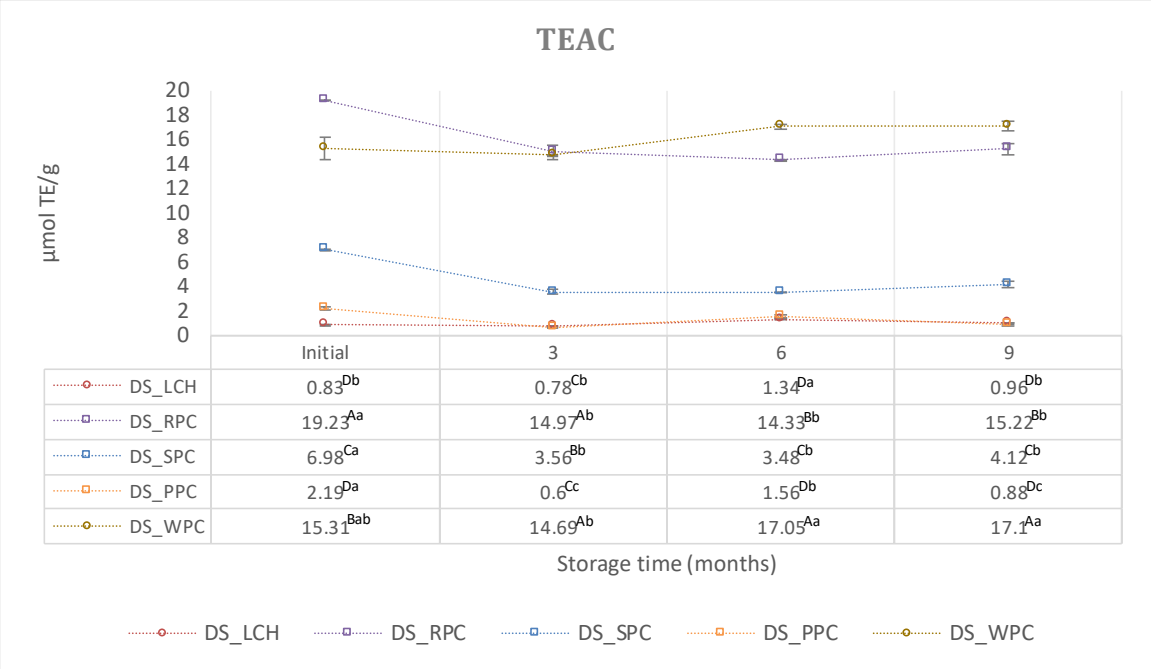
DS_RPC had the highest antioxidant capacity (19.23 μmol TE/g), followed by DS_WPC (15.31 μmol TE/g), DS_SPC (6.98 μmol TE/g), DS_PPC (2.19 μmol TE/g), and DS_LCH (0.83 μmol TE/g), with a statistically non-significant difference between DS_PPC and DS_LCH (see Figure 4); surprisingly, the dietary supplement with the highest CoQ10 content (DS_LCH) had the lowest TEAC. Simple linear regression revealed no significant correlation (r = – 0.604; p = 0.281^{NS}) between initial CoQ10 levels of dietary supplements and their TEACs. It can thus be deduced that the antioxidant capacity of DS_RPC, DS_WPC, and DS_SPC is not only due to the CoQ10 content but also to other compounds with such an effect present in dietary supplements, like fat-soluble vitamins; we have found tocopherols (Semeniuc et al., 2024) in the fats extracted from these matrices using the Folch method modified after Boselli et al. (2011). There are also no linear relationships between initial levels of PV and TEAC (r = 0.295; p = 0.630^{NS}), respectively, of CoQ10 and PV (r = – 0.830; p = 0.082^{NS}).

The TEAC in DS_LCH slightly varied during the 9 months of storage, significantly increasing from an initial level of 0.83 to 1.34 μmol TE/g in the 6th month and then decreasing to 0.96 μmol TE/g in the 9th month, a non-significant level compared to the initial one and that from the 3rd month (0.78 μmol TE/g). No data regarding the antioxidant capacity of chicken heart fat is available in the literature.

The antioxidant capacity of DS_RPC significantly decreased in the 3rd month of storage, from an initial value of 19.23 μmol TE/g to 14.97 μmol TE/g, after which it remained unchanged till the last month of storage (15.22 μmol TE/g) at significant levels compared to the initial one. The same trend was also noticed for the DS_SPC, having an initial level of 6.98 μmol TE/g, 3.56 μmol TE/g in the 3rd month of storage, and 4.12 μmol TE/g in the 9th month. Much lower antioxidant capacities were found by Deng et al. (2014; 1.32 μmol TE/g), Symoniuk et al. (2018; 1.81–1.96 μmol TE/g), Symoniuk et al. (2022; 3.12 μmol TE/g), Rabiej-Kozioł et al. (2022; 3.89 μmol TE/g), and Rabiej-Kozioł et al. (2023; 2.93 μmol TE/g) in cold-pressed rapeseed oil by using the DPPH assay. However, comparable levels were reported in cold-pressed oil of rapeseed by Symoniuk et al. (2018; 1.75–2.34 μmol TE/g) and Rabiej-Kozioł et al. (2023; 2.41 μmol TE/g).

For the DS_PPC instead, TEAC showed a decreasing (0.60 μmol TE/g; month 3)/increasing (1.56 μmol TE/g; month 6)/decreasing (0.88 μmol TE/g; month 9) behaviour at storage, starting from an initial level of 2.19 μmol TE/g; the difference between antioxidant capacities from the 3rd and 9th months of storage was non-significant but significant between those from the initial day and the 6th month. TEAC levels between 1.95 and 1.96 μmol TE/g were reported by Symoniuk et al. (2018), between 1.25 and 2.73 μmol TE/g by Grajzer et al. (2020), 2.54 μmol TE/g by Symoniuk et al. (2022), and 3.97 μmol TE/g by Rabiej-Kozioł et al. (2023) in cold-pressed oil of pumpkin.

The DS_WPC's antioxidant capacity did not vary significantly during the 9 months of storage, with an initial level of 15.31 µmol TE/g and a final one of 17.10 µmol TE/g. Much lower TEAC levels (0.88–1.37 µmol TE/g; 1.33 µmol TE/g) were found by Grajzer et al. (2020) and Gao et al. (2022), respectively, in cold-pressed walnut oil.



	Initial	3	6	9
DS_LCH	0.83^{Db}	0.78^{Cb}	1.34^{Da}	0.96^{Db}
DS_RPC	19.23^{Aa}	14.97^{Ab}	14.33^{Bb}	15.22^{Bb}
DS_SPC	6.98^{Ca}	3.56^{Bb}	3.48^{Cb}	4.12^{Cb}
DS_PPC	2.19^{Da}	0.6^{Cc}	1.56^{Db}	0.88^{Dc}
DS_WPC	15.31^{Bab}	14.69^{Ab}	17.05^{Aa}	17.1^{Aa}

Figure 4. Variation of TEAC in dietary supplements during the stability test. Data are presented as mean ± standard deviation of triplicate data (n=3). Different uppercase letters in a row indicate significant differences between dietary supplements ($p < 0.05$, Tukey's test), and different lowercase letters show significant differences between storage times ($p < 0.05$).

PV

Initial PV levels (see Figure 5) were significantly different between dietary supplements, the highest ones being in DS_PPC (18.12 meq O_2/kg), DS_WPC (17.77 meq O_2/kg) and DS_SPC (15.06 meq O_2/kg), followed by DS_RPC (13.59 meq O_2/kg) and DS_LCH (7.89 meq O_2/kg, but without a significant difference between those of DS_RPC and DS_PPC. The maximum permissible level for PV in cold-pressed oils, specified in the Codex CXS 210-1999 standard, is 15 meq O_2/kg oil; this was exceeded in DS_PPC, DS_WPC, and DS_SPC but not in DS_RPC. Therefore, it is plain to see that the initial oxidative status of vegan dietary supplements is due to the type and age of oil press cakes (8 months for RPC, 8 months for SPC, 9 months for PPC, and 8 months for WPC) used for their preparation. As regards the DS_LCH, its initial PV level (7.89 meq O_2/kg) was below the maximum permissible one (10 meq O_2/kg) stipulated in the Codex CXS 211-1999 standard, probably because the chicken hearts were sampled and lyophilised immediately after expiration.

Storage time has significantly affected the PV level in dietary supplements, except for DS_PPC and DS_WPC. In DS_LCH, PV increased considerably up to the 3rd month of storage (14.14 meq O_2/kg), from an initial level of 7.89 meq O_2/kg, after which it decreased in the 6th month to a non-significant level (9.35 meq O_2/kg) compared to the initial one; then, PV remained unchanged in DS_LCH until the 9th month of storage (9.23 meq O_2/kg). A similar trend of increase in month 3 (18.29 meq O_2/kg)/decrease in month 6 (7.44 meq O_2/kg)/maintenance until month 9 (5.47 meq O_2/kg) in PV level during storage was also noticed for DS_RPC, only that the levels in the 6th and 9th months were significantly lower than the initial one (13.59 meq O_2/kg). In the study carried out on cold-pressed rapeseed oil by Wroniak and Rękas (2016) during 12 months of storage at room temperature, PV increased from an initial level of 2.9 meq O_2/kg to 28.2 meq O_2/kg for the sample kept in the dark, respectively to 28.2 meq O_2/kg for that exposed to light.

The PV significantly increased with storage time in DS_SPC until the 6th month (19.57 meq O_2/kg), from an initial level of 15.06 meq O_2/kg, reaching a value of 21.53 meq O_2/kg in the 9th month of storage, non-significant compared to that in the 6th month; the PV level of 17.54 meq O_2/kg from the 3rd month of storage was non-significant compared to the initial level and that of the 6th month. As for the PV level in DS_PPC, this ranged non-significantly during storage, from 18.12 meq O_2/kg (initial) to 20.10 meq O_2/kg (in the 9th month). A non-significantly variation of PV with storage time was noticed in DS_WPC as well, the initial level being 17.77 meq O_2/kg and the final one

17.40 meq O_2/kg. All these findings indicate that DS_PPC and DS_WPC are the most stable during storage in terms of oxidation.

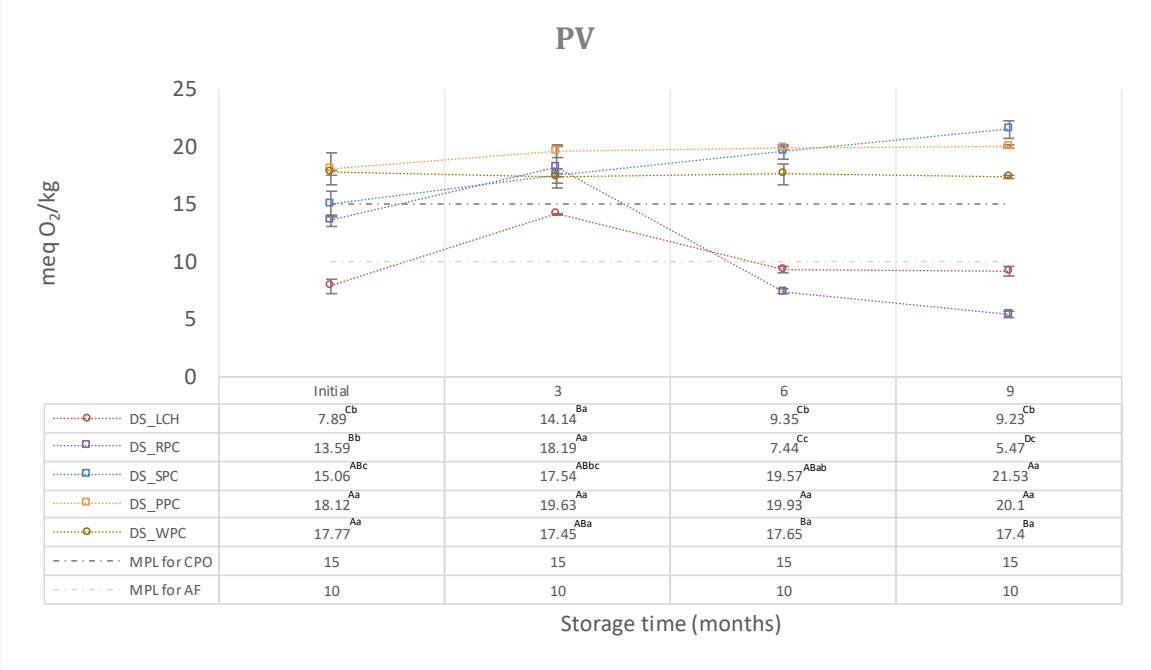


Figure 5. Variation of PV in dietary supplements during the stability test. MPL for CPO, maximum permissible level for cold-pressed oils; MPL for AF, maximum permissible level for animal fats. Data are presented as mean ± standard deviation of triplicate data (n=3). Different uppercase letters in a row indicate significant differences between dietary supplements (p < 0.05, Tukey's test), and different lowercase letters show significant differences between storage times (p < 0.05).

Correlations

No significant linear relationships (correlations) were found between the CoQ10 and TEAC, CoQ10 and PV, respectively TEAC and PV levels of dietary supplements during storage (see Table 2), except for that between CoQ10 and PV for DS_SPC; as the CoQ10 content in DS_SPC decreased with storage time, the PV increased.

Table 2. Correlations between CoQ10 content, TEAC, and PV in dietary supplements during storage

Dietary supplement	Parameter	CoQ10 content	TEAC	PV
DS_LCH	CoQ10 content TEAC PV	- r = -0.350; p = 0.650^{NS} r = 0.002; p = 0.998^{NS}	r = -0.350; p = 0.650^{NS} - r = -0.650; p = 0.350^{NS}	r = 0.002; p = 0.998^{NS} r = -0.650; p = 0.350^{NS} -
DS_RPC	CoQ10 content TEAC PV	- r = 0.764; p = 0.236^{NS} r = 0.439; p = 0.561^{NS}	r = 0.764; p = 0.236^{NS} - r = 0.290; p = 0.710^{NS}	r = 0.439; p = 0.561^{NS} r = 0.290; p = 0.710^{NS} -
DS_SPC	CoQ10 content TEAC PV	- r = 0.581; p = 0.419^{NS} r = -0.984; p = 0.016*	r = 0.581; p = 0.419^{NS} - r = -0.717; p = 0.283^{NS}	r = -0.984; p = 0.016* r = -0.717; p = 0.283^{NS} -
DS_PPC	CoQ10 content TEAC PV	- r = -0.081; p = 0.919^{NS} r = -0.574; p = 0.426^{NS}	r = -0.081; p = 0.919^{NS} - r = -0.753; p = 0.247^{NS}	r = -0.574; p = 0.426^{NS} r = -0.753; p = 0.247^{NS} -
DS_WPC	CoQ10 content TEAC PV	- r = -0.892; p = 0.108^{NS} r = 0.508; p = 0.492^{NS}	r = -0.892; p = 0.108^{NS} - r = -0.131; p = 0.869^{NS}	r = 0.508; p = 0.492^{NS} r = -0.131; p = 0.869^{NS} -

Note: r, Pearson correlation coefficient; p-value, calculated probability; NS, non-significant correlation ($p \geq 0.05$); *, significant correlation (p < 0.05).

To summarise, the benefits of the optimised procedure proposed in this study to prepare a dietary supplement based on CoQ10 include that it is a green extraction technique, involves a few steps and a minimum of equipment, uses a single extraction solvent, reduces the amount of solvent needed due to its reuse after recovery, and is easy to adapt at the industrial level; in addition, formulations obtained using it are liquid, natural, and ultimately free of solvent, among the drawbacks. Among the drawbacks of the procedure is that other fat-soluble compounds are extracted with CoQ10 from the matrix, and the dietary supplement thus obtained cannot be standardised.

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.

CONCLUSIONS

The chicken heart and oil press cake matrices used in this study are suitable for preparing natural dietary supplements based on CoQ10, provided they are subjected to the extraction process immediately after expiration (in the case of chicken hearts) or pressing (for oil press cakes). The preparation procedure proposed here for a natural dietary supplement based on CoQ10 is simple and, therefore, easily adapted for large-scale production; it uses only one extraction solvent (2-propanol), which is green. Among the disadvantages of a natural dietary supplement is that it cannot be standardised (because the amount of active ingredient depends on the CoQ10 content in the matrix) and has low storage stability. However, Directive 2002/46/EC allows the commercialisation of a non-standardised dietary supplement if the name and amount of the substance that characterises it and the portion recommended for daily consumption are mentioned on the label. Our future work will focus on the safety and bioavailability assessment of dietary supplements with the highest CoQ10 amount, namely DS_LCH (animal origin) and DS_PPC (vegan).

Abbreviations

CoQ10, coenzyme Q10
TEAC, Trolox equivalent antioxidant capacity
PV, peroxide value
DS_LCH, the dietary supplement prepared from lyophilised chicken hearts
DS_PPC, the dietary supplement prepared from pumpkin press cakes
DS_WPC, the dietary supplement prepared from walnut press cakes
DS_RPC, the dietary supplement from prepared rapeseed press cakes
DS_SPC, the dietary supplement prepared from sunflower press cakes
MPL for CPO, maximum permissible level for cold-pressed oils
MPL for AF, maximum permissible level for animal fats

Patent

Patent Application A/00138 from 24.03.2023: "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

Author Contributions: Conceptualisation, C.A.S. and S.A.S.; methodology, C.A.S.; formal analysis, F.R., A.S.P., S.R.I., M.-I.S., M.F., and A.C.F.; resources, C.A.S., S.A.S., and D.C.V.; data curation, C.A.S.; writing—original draft preparation, C.A.S. and A.S.P.; writing—review and editing, S.A.S.; visualisation, D.C.V.; supervision, S.A.S.; project administration, S.A.S.; funding acquisition, S.A.S. and D.C.V. All authors have read and agreed to the published version of the manuscript. Cristina Anamaria Semeniuc and Andersina Simina Podar have contributed equally to this work as co-first authors.

Funding Source: This work was supported by a grant from the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN-III-P4-ID-PCE-2020-1847, within PNCDI III.

Acknowledgements

We are grateful for the administrative and financial support from the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Data Availability Statement

Data sharing does not apply to this article as no new data were created or analysed in this study.

REFERENCES

1. Anton R, Barlow S, Boskou D, Castle L, Crebelli R, Dekant W, Engel K-H, Forsythe S, Grunow W, Larsen JC, Leclercq C, Mennes W, Milana M-R, Pratt I, Rietjens I, Svensson K, Tobback P, Toldrá F. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Propan-2-ol as a carrier solvent for Flavourings. EFSA J. 2005; 202:1–10.
2. Arenas-Jal M., Suñé-Negre JM, García-Montoya E. Coenzyme Q10 supplementation: Efficacy, safety, and formulation challenges. Compr. Rev. Food Sci. Food Saf. 2020; 9(2):574–594.
3. Bank G, Kagan D, Madhavi D. Coenzyme Q10: clinical update and bioavailability. J. Evid. Based Complementary Altern. Med. 2011; 16(2):129–137.
4. Boselli E, Velazco V, Caboni MF, Lercker G. Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. J. Chromatogr. A 2011; 917(1-2):239–244.
5. Codex Alimentarius Commission. Standard for named animal fats CXS 211-1999. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B211-1999%252FCXS_211e.pdf (accessed on 14 March 2024).
6. Codex Alimentarius Commission. Standard for named vegetable oils CXS 210-1999. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B210-1999%252FCXS_210e.pdf (accessed on 14 March 2024).
7. Deng Q, Yu X, Xu J, Wang L, Huang F, Huang Q, Liu C, Ma F. Effects of endogenous and exogenous micronutrients in rapeseed oils on the antioxidant status and lipid profile in high-fat fed rats. Lipids Health Dis. 2014; 13(December):198.
8. Ercan P, El SN. Changes in content of coenzyme Q10 in beef muscle, beef liver and beef heart with cooking and in vitro digestion. J. Food Compos. Anal. 2011; 24(8):1136–1140.
9. European Union. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Off. J. Eur. Union 2002; L 183:51–57.
10. European Union. Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. Off. J. Eur. Union 2009; L 141:3–11.
11. Gao P, Ding Y, Chen Z, Zhou Z, Zhong W, Hu C, He D, Wang X. Characteristics and antioxidant activity of walnut oil using various pretreatment and processing technologies. Foods 2022; 11(12):1698.
12. Grajzer M, Szmalcel K, Kuźmiński Ł, Witkowski M, Kulma A, Prescha A. Characteristics and antioxidant potential of cold-pressed oils—Possible strategies to improve oil stability. Foods 2020; 9(11):1630.
13. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int. J. Pharm. 2001; 212(2):233–246.
14. Kubo H, Fujii K, Kawabe T, Matsumoto S, Kishida H, Hosoe K. Food content of ubiquinol-10 and ubiquinone-10 in the Japanese diet. J. Food Compos. Anal. 2008; 21(3):199–210.
15. Manasyan L, Xia F. Determination and validation of a single laboratory method for coenzyme Q10 (ubiquinone) by UHPLC. LC GC N. Am. 2020; 38(5):299–304.
16. Mandrioli M, Semeniuc CA, Boselli E, Rodriguez-Estrada MT. Ubiquinone in Italian high-quality raw cow milk. Ital. J. Food Sci. 2018; 30(1):144–155.
17. Martinefski M, Samassa P, Buontempo F, Höcht C, Lucangioli S, Tripodi V. Relative bioavailability of coenzyme Q10 formulation for paediatric individualized therapy. J. Pharm. Pharmacol. 2017; 69(5):567–573.

18. Mattila P, Kumpulainen,J. Coenzymes Q9 and Q10: contents in foods and dietary intake. J. Food Compos. Anal. 2001; 14(4):409–417.
19. Munuera-Cabeza M, Suárez-Carrillo A, Talaverón-Rey M, Sánchez-Alcázar JA. Coenzyme Q10: Novel formulations and medical trends. Int. J. Mol. Sci. 2020; 21(22):8432.
20. Podar AS, Semeniuc CA, Ionescu SR, Socaciu M-I, Fogarasi M, Fărcaş AC, Vodnar DC, Socaci SA. An overview of analytical methods for quantitative determination of coenzyme Q10 in foods. Metabolites 2023; 13(2):272.
21. Rabiej-Kozioł D, Momot-Ruppert M, Stawicka B, Szydłowska-Czerniak A. Health benefits, antioxidant activity, and sensory attributes of selected cold-pressed oils. Molecules 2023; 28(14):5484.
22. Rabiej-Kozioł D, Tymczewska A, Szydłowska-Czerniak A. Changes in quality of cold-pressed rapeseed oil with sinapic acid ester-gelatin films during storage. Foods 2022; 11(21):3341.
23. Rodríguez-Acuña R, Brenne E, Lacoste F. Determination of coenzyme Q10 and Q9 in vegetable oils. J. Agric. Food Chem. 2008; 56(15):6241–6245.
24. Rodriguez-Estrada MT, Poerio A, Mandrioli M, Lercker G, Trinchero A, Tosi MR, Tugnoli V. Determination of coenzyme Q10 in functional and neoplastic human renal tissues. Anal. Biochem. 2006; 357(1):150–152.
25. Saini R. Coenzyme Q10: The essential nutrient. J. Pharm. Bioallied Sci. 2011; 3(3):466–467.
26. Semeniuc CA, Cardenia V, Mandrioli M, Muste S, Borsari A, Rodriguez-Estrada MT. Stability of flavoured phytosterol-enriched drinking yogurts during storage as affected by different packaging materials. J. Sci. Food Agric. 2016; 96(8):2782–2787.
27. Semeniuc CA, Mandrioli M, Podar AS, Ranga F, Socaciu M-I, Ionescu SR, Fogarasi M, Fărcaş AC, Gallina Toschi T, Vodnar DC, Socaci SA. Fats extracted from oil press cakes, fish meat, and chicken hearts as potential CoQ10 supplements. Waste Biomass Valori. 2024.
28. Semeniuc CA, Ranga F, Podar AS, Ionescu SR, Socaciu M-I, Fogarasi M, Fărcaş AC, Vodnar DC, Socaci SA. Determination of coenzyme Q10 content in food by-products and waste by high-performance liquid chromatography coupled with diode array detection. Foods 2023; 12(12):2296.
29. ISO 3976:2006 | IDF 74:2006. Milk fat-determination of peroxide value; ISO: Geneva, Switzerland, 2006.
30. Souchet N, Laplante S. Seasonal variation of Co-enzyme Q10 content in pelagic fish tissues from Eastern Quebec. J. Food Compo. Anal. 2007; 20(5):403–410.
31. Stiff MR, Weissinger AK, Danehower DA. Analysis of CoQ10in cultivated tobacco by a high-performance liquid chromatography–ultraviolet method. J. Agric. Food Chem. 2011; 59(17):9054–9058.
32. Symoniuk E, Ratusz K, Ostrowska-Ligęza E, Krygier K. Impact of selected chemical characteristics of cold-pressed oils on their oxidative stability determined using the Rancimat and pressure differential scanning calorimetry method. Food Anal. Methods 2018; 11(November):1095–1104.
33. Symoniuk E, Wroniak M, Napiórkowska K, Brzezińska R, Ratusz K. Oxidative stability and antioxidant activity of selected cold-pressed oils and oils mixtures. Foods 2022; 11(11):1597.
34. ADM [Internet]. The Dietary Supplement Consumer of Tomorrow. Chicago: Archer Daniels Midland; 2024. 18 p. https://www.adm.com/globalassets/news/adm-stories/the-dietary-supplement-consumer-of-tomorrow-report-min.pdf/
35. Turkowicz MJ, Karpińska J. Analytical problems with the determination of coenzyme Q10 in biological samples. BioFactors 2013; 39(2):176–185.
36. Villanueva-Bermejo D, Temelli F. Extraction of oil rich in coenzyme Q10 from chicken by-products using supercritical CO2. J. Supercrit. Fluids 2021; 174(August):105242.
37. Weber C, Bysted A, Hølmer G. Coenzyme Q10 in the diet-daily intake and relative bioavailability. Mol. Aspects Med. 1997; 18(Supplement 1):251–254.
38. Wroniak M, Rękas A. Nutritional value of cold-pressed rapeseed oil during long term storage as influenced by the type of packaging material, exposure to light & oxygen and storage temperature. J. Food Sci. Tech. 2016; 53(2):1338–1347.
39. Yilmaz E, Soylak M. Type of green solvents used in separation and preconcentration methods. In: Soylak M, Yilmaz E, editors. New generation green solvents for separation and preconcentration of organic and inorganic species. 1st ed. Amsterdam: Elsevier; 2020. p. 207–266.
40. Zhang Y, Liu J, Chen X-Q, Chen C-YO. Ubiquinol is superior to ubiquinone to enhance Coenzyme Q10 status in older men. Food Funct. 2018; 9(11):5653–5659.