Scientific paper

The Stability of Coenzyme Q_{10} in Fortified Foods

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Abstract

Coenzyme Q_{10} (CoQ_{10}), also known as Ubiquinone, is a natural antioxidant with a fundamental role in cellular bioenergetics. Endogenous tissue levels drop progressively with increasing age and a deficiency has also been observed in various medical conditions and lifestyles. The limited supply to the organism by foods has been further reduced by food processing as it is known that processed products and foods with a lower amount of fat usually have smaller amounts of CoQ_{10}. This and the numerous health benefits of its supplementation are the main reason triggering the interest of the food industry which has started to use this compound to fortify food products. Due to its lipophilicity, until recently this goal was not easily achievable with most products. Forms of CoQ_{10} with increased water-solubility or dispersibility have been developed for this purpose, allowing the fortification of aqueous products. We studied the stability of Coenzyme Q_{10} in some fortified products that were enriched by water-soluble inclusion complex of CoQ_{10} and β-cyclodextrin (Q10Vital), with the use of different technological processes; fruit-based products, milk, yoghurt and some other dairy products have been investigated. The level of CoQ_{10} in form of Q10Vital in studied products was determined to be stable. The enrichment of some types of products (i.e. curd) should be performed at the end, especially if fermentation is a step in the technological process.

Keywords: CoQ_{10}, Ubiquinone, Q10Vital, Stability, Fortification, Functional Food

1. Introduction

Coenzymes Q are naturally occurring fat-soluble compounds present in every living cell; due to their ubiquitous occurrence in nature they are also called Ubiquinones.[1] They are essential for the functioning of an organism. The predominant form in humans and most animals is Coenzyme Q_{10}, containing 10 isoprenoid units attached to the quinone moiety. It was first isolated in 1957[2] and in 1978 a Nobel Prize was awarded for establishing its role in biological energy transfers at the cellular level.[3] It is now well established that Coenzyme Q_{10} (CoQ_{10}) is an essential component of the mitochondrial energy metabolism. It is responsible for energy conversion from carbohydrates, proteins and fatty acids into high-energy adenosine triphosphate (ATP), which plays an integral role in supplying energy to chemical reactions in the body and therefore driving cellular machinery and synthesis.[4] Further, CoQ_{10} has been known to be a very effective antioxidant for over 40 years,[5,6] protecting against lipid peroxidation, DNA and protein oxidation and being capable of functioning synergistically with other antioxidants.[7] A key advantage of CoQ_{10} lies in its presence in the mitochondria,[8] where free radicals are mainly formed. For this reason, it can be more effective than other antioxidants which are more evenly distributed throughout a cell. CoQ_{10} is present in the human body in both an oxidised and a reduced form. It is present in all cellular membranes, not only in mitochondria.[1] The beneficial role of CoQ_{10} for human health is reported in various clinical aspects,[9,10] particularly in cardiovascular,[11–14] neurodegenerative and mitochondrial diseases.[15–17]

The human body biosynthesises CoQ_{10}, but its endogenous tissue levels drop progressively with increasing age.[18] A deficiency is also observed in various medical conditions,[19] in people with inappropriate nutrition and in smokers.[20] Besides biosynthesis, small amounts of CoQ_{10} are supplied to the organism by various foods (3–6 mg/day), mostly from meat and fish.[21] Ubiquinone is reduced to ubiquinol during or following absorption in the intestine,[22] consequently its function is not affected by the form in which it is consumed. A connection has been found between the technological processing of food, its fat content and concentration of CoQ_{10}. Processed products and foods with a lower amount of fat usually have lower amounts of CoQ_{10}.[23]

An excellent safety record is demonstrated for the oral application of CoQ_{10} in many clinical trials[24] and the decrease of its level in foods due to processing along with the many health benefits of this compound are the main reason triggering the interest of the food industry which has started to use this compound to fortify food products. The fortification of foods with Coenzyme Q_{10} entered the market in Japan as part of the country's FOSHU (Foods for Specified Health Use)[25] concept. In many other countries, the regulation of functional-food remains unsettled, especially regarding claims about the health benefits of such foods in terms of their nutrient content and function, and disease risk,[26] although CoQ_{10}-fortified products are already available in many countries across the world. Functional foods must be fortified with a sufficient amount of an active component to provide evidence-based health benefits for consumers. As CoQ_{10} is lipid-soluble crystalline powder with a relatively high molecular weight (M_r = 863) its insolubility in water represents the main limitation on the fortification of foods, particularly those with a low fat content. A very small increase in the CoQ_{10} level can usually be achieved with the use of a crystalline compound. New forms of CoQ_{10} have been developed to solve this problem.[27–30]

The stability of CoQ_{10} is a continuous issue, especially in processes performed at increased temperatures or when products are stored in the light (UV irradiation).[31,32] Despite this, to our knowledge no stability studies of Co-Q_{10}-fortified products have been published in the scientific literature even though such products are already available in many markets. We investigated the stability of some food products fortified with a water-soluble CoQ_{10}/β-cyclodextrin complex (Q10Vital). The reported results will also help the industry to choose a proper fortification strategy. It should be mentioned that the stability of the final food product is, of course, the responsibility of the producer and greatly depends on the production procedure, the form of CoQ_{10} used in this process and on the packaging. Therefore, the stability of every final product has to be verified.

2. Experimental

Chemical determinations of CoQ_{10} concentrations were performed using High Pressure Liquid Chromatography (HPLC) at the National Institute of Chemistry (NIC), Ljubljana, Slovenia or at Chelab s.r.l. (Chelab), Resana, Italy. A modified reported analytical procedure was employed,[23] depending on the nature of the sample. A validated analytical procedure was applied to determine CoQ_{10} levels in milk. Analyses at the NIC were carried out in accordance with the standard procedures of the QASKI quality assurance system, whereas Chelab is accredited by the Italian Laboratory Certification System (SINAL) according to UNI CEI EN ISO/IEC 17025. Results of determinations of CoQ_{10} concentrations in both laboratories were comparable.

2. 1. Typical Analytical Procedure (NIC)

Reagents: Liquid chromatography solvents (Merck, Darmstadt, Germany), CoQ_{10} standard (Sigma-Aldrich, Steinheim, Germany), Q10Vital (CoQ10/β-cyclodextrine complex; 7.5% CoQ_{10} content, Valens Int., Celje, Slovenia)

Apparatus: Vortex mixer Vibromix 204 EV (Tehtnica, Železniki, Slovenia), centrifuge J-21C (Beckman Instruments, Palo Alto, CA), 10 mL polypropylene tubes with plug and screw caps (Beckman Instruments), a rotary evaporator Rotavapor R-144, equipped with a water bath B-480 (Büchi, Flawil, Switzerland), Surveyor LC system (Thermo Finnigan, Riviera Beach, CA, USA) equipped with a UV detector, LC column Gemini C18, 150 mm x 4.6 mm i.d. and a 5 mm particle diameter (Phenomenex, Torrance, CA, USA).

Standard solutions and calibration curve: A stock solution of 500 mg/L was prepared by dissolving 10 mg of Coenzyme Q_{10} in 6 mL of 1.4-dioxane in a 20 mL flask; the flask was then filled with 2-propanol. The stock solution was used for the preparation of six calibration standards from 0.2 to 25 mg/kg (ppm) by dilution with 2-propanol. Values of slope (b), intercept (a), correlation coefficient (R) and the standard deviation of slope V(b) were calculated using a weighted calibration curve. Weight factors were calculated from the relationship $fw_i = 1/(100 + 5*X_i)^2$. The limit of detection (LOD) and the limit of quantisation (LOQ) were calculated from the confidence interval.

Sample preparation: 1 g of homogenized sample was weighed into a 10 mL plastic centrifuge tube together with 500 μL of 10% perchloric acid and 2 mL of *n*-hexane. The sample was mixed vigorously for 1 min with the help of a vortex mixer and centrifuged at 2,500 rpm for 3 min. Hexane extract was removed. The extraction was repeated with 2 mL of chloroform; the sample was again mixed vigorously for 1 min with the help of the vortex mixer and centrifuged at 2,500 rpm for 3 min. Extraction with chloroform was repeated twice. The combined organic extracts were concentrated at reduced pressure at 40 °C. The residue was redissolved in 1 mL of 2-propanol and left for 1 h. Prior to the HPLC analyses, the sample was filtered through a membrane filter 0.45 μm (millipore Millex-HV, Hydrophilic PVDF).

HPLC analyses: the separation and quantitative determinations of CoQ_{10} were performed with the Surveyor LC system equipped with a UV detector. A Gemini C18 column, 150 × 4.6, 5μ was used, the flow rate was 1.0 mL/min, the injection volume was 20 μL, and the temperature of the column was 30 °C. The mobile phase was a mixture of 1,4-dioxane : methanol : ethanol (5 : 30 : 65, v/v/v); the detection was performed at 280 nm. Separated peaks were quantified with ChromQuest 4.0 (TSP) software.

2. 2. Samples and their Fortification

Food products from Central and Eastern Europe were used (milk with 1.6% fat, kefir with 3.5% fat, curd, apple nectar and fruit syrup). The products were fortified with water-soluble CoQ_{10}/β-cyclodextrin complex (Q10Vital, Valens Int., Celje, Slovenia).[30]

Milk (1.6% fat) was fortified before steam sterilisation (140 °C, 4 s) and homogenisation (150 bar, 75–80 °C) with 40 mg CoQ_{10} per L. Samples of packaged fortified UHT milk (TetraPak packages) were stored at a controlled temperature (25 °C) and analysed monthly.

Kefir (3.5% fat) was fortified after the fermentation process with 50 mg CoQ_{10} per L. Packed samples (250 mL) were stored in a refrigerator at 4 °C and analysed every few days until day 29.

Two samples of curd were prepared. The first sample (180 g of curd) was prepared using 400 mL of fresh milk, which was previously fortified with 30 mg CoQ_{10} in the form of Q10Vital. The second sample was prepared by the direct fortification of curd with 30 mg CoQ_{10} per 180 g of curd. Both samples were put in a refrigerator at 4 °C and reanalysed after 2 weeks of storage.

Apple nectar that contained 70% of apple juice (not from a concentrate) and L-ascorbic acid as an antioxidant was fortified with 22.5 mg CoQ_{10} (Q10Vital) per L by a local food producer and packed in sterile conditions (TetraPak packages). The sample was stored at room temperature for one year, when it was analysed for its CoQ_{10} content.

Commercially available syrup (ingredients: sugar, strawberry, blueberry, raspberry and elder juice from concentrated juice (30%), citric acid (0.06%); with 60% dry solids) was fortified with 15 g CoQ_{10} per L. The samples were stored in dark glass bottles (200 mL) and protected from light at either room temperature (sample series A) or in a refrigerator at 4 °C (sample series B). Both series were analysed immediately after production as well as after 6, 9 and 12 months of storage.

3. Results and Discussion

We performed a stability study of CoQ_{10} in fortified dairy and fruit-based products. The products were enriched with a water-soluble inclusion complex of CoQ_{10} and β-cyclodextrin,[30] commercially available as Q10Vital. This form of CoQ_{10} is already widely used in the food industry for fortifying various food products such as diary products (e.g. milk, yoghurt, kefir etc.), fruit juice or nectar, syrup and other beverages, honey, tea, as well as a food supplement in a variety of formulations like effervescent tablets, capsules, syrups etc. Further, highly increased bioavailability has recently been shown for Q10Vital by *in vivo* pharmacokinetic studies,[33–35] making it even more attractive to the food industry.

Milk and dairy products are very suitable for CoQ_{10} fortification[23] as they have a low CoQ_{10} content and their consumption by the average population is quite high; unfortified milk (1.6% fat) contains about 1.2 mg CoQ_{10}/kg.[30] While the CoQ_{10} level in milk can only be doubled with the use of crystalline CoQ_{10}, as much as a 5000-times increase in the initial CoQ_{10} concentration can be accomplished with Q10Vital.[30]

Milk with 1.6% fat was fortified with 40 mg CoQ_{10}/L in the form of Q10Vital. After short steam sterilisation and homogenisation, fortified UHT milk was packed and stored at room temperature. The samples were analysed monthly for their CoQ_{10} concentration and sensorial properties. First analyses of the produced sample confirmed the desired CoQ_{10} content (39.7 mg/L ± 0.3%). In addition, no change in appearance, taste or odour was observed. These results confirmed that short high-temperature sterilisation does not cause the decomposition of CoQ_{10} in the form of Q10Vital. Analyses were repeated monthly; after three months no changes in milk properties were observed and the CoQ_{10} level was determined to be stable and only slightly lower than in the fresh sample (38.6 mg/L ± 0.3%; Figure 1). Very similar results were obtained with those milk samples stored in a refrigerator.

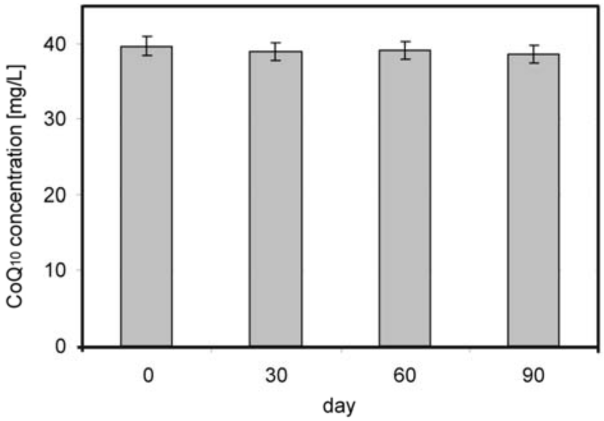

Figure 1. Three-month stability of the CoQ_{10} level in UHT milk (1.6% fat)

Further, we tested the stability of the CoQ_{10} level in kefir, an enriched fermented milk drink. The content of CoQ_{10} in the kefir (3.5% fat) was determined to be 1.8 mg/kg. The sample was fortified with 50 mg CoQ_{10}/kg in the form of Q10Vital after the fermentation process, packed and stored at about 4 °C. An analytical determination of CoQ_{10} was performed every few days for a month (10 analyses); a selection of representative data is shown on Figure 2. The fortification of the kefir with CoQ_{10} did not result in any change in the product properties, including its taste or odour. No decrease in the CoQ_{10} content was observed.

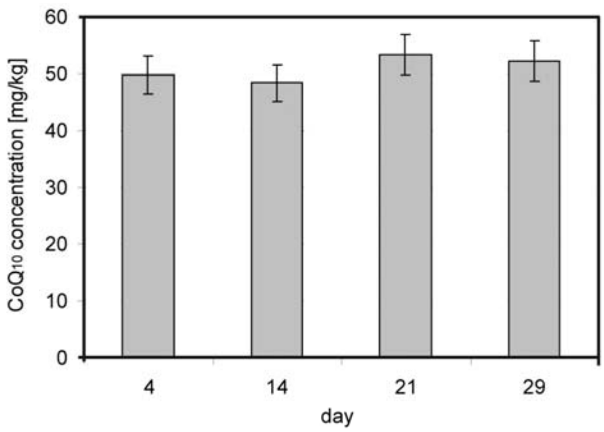

Figure 2. One-month stability of the CoQ_{10} level in a fermented milk product (kefir, 3.5% fat)

Despite its relatively high fat content, curd naturally contains quite low levels of CoQ_{10} (below 1 mg/kg).[23] The stability of CoQ_{10} in enriched curd was verified with two production strategies (Figure 3). The curd samples were prepared at the NIC. The first sample was prepared from cow milk that was previously fortified with 75 mg CoQ_{10}/L; 180 g of curd was produced from 400 mL of fortified milk. While we expected to get about 30 mg CoQ_{10} in curd sample (theoretical value; 167 mg/kg), analyses of the produced sample did not confirm these expectations. The fortification strategy used was unsuccessful as only a third of the expected CoQ_{10} was established after production (10.8 ± 0.9 mg; 60 ± 5 mg/kg). The CoQ_{10} level in whey was below the detection limit, meaning that the compound was probably consumed as food for lactic acid bacteria. Nevertheless, the level of CoQ_{10} remained relatively stable and dropped to 57 ± 5 mg/kg after 14 days. The second enrichment strategy, in which 30 mg of CoQ_{10} in the form of Q10Vital was added to 180 g of curd prior to homogenisation, was more successful. The first analyses of the curd confirmed that the CoQ_{10} concentration in the enriched curd (159 ± 11 mg/kg) was just 1.8% lower than expected. The level of CoQ_{10} in the curd was confirmed to be stable; after two weeks at about 4 °C no decrease in the CoQ_{10} content was observed.

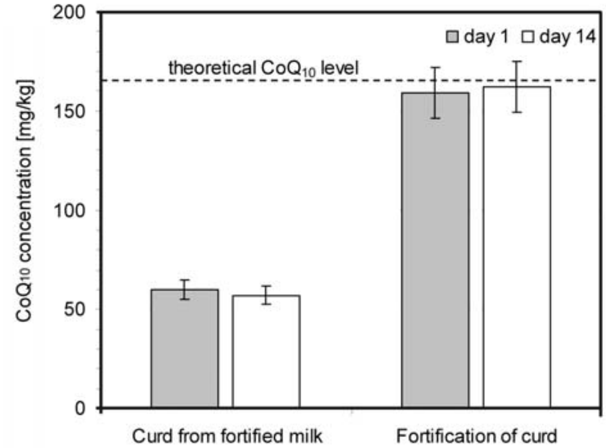

Figure 3. Two-week stability of the CoQ_{10} level in curd

Apart from dairy products, fruit-based drinks are also very suitable for enrichment with CoQ_{10}. With the exception of avocado,[36] most fruit contains very low levels of CoQ_{10}.[21] It has to be noted that fruit juices and nectars represent some of the most important nutritional sources of some water-soluble vitamins, but they contain only negligible levels of lipophilic nutrients, such as CoQ_{10} (i.e. 0.3 mg CoQ_{10}/kg in orange juice).[37] It is known than orange juice can be successfully enriched with CoQ_{10}; very high acceptability in the testing of organoleptic properties on individual consumers was shown with orange juice fortified with 75 mg CoQ_{10}/L (appearance, smell, taste).[30] We studied the stability of CoQ_{10} in apple nectar (70% apple juice) fortified with 22.5 mg CoQ_{10}/L in the form of Q10Vital and stored at room temperature. One year after production the level of CoQ_{10} was determined to be stable (23 mg/L).

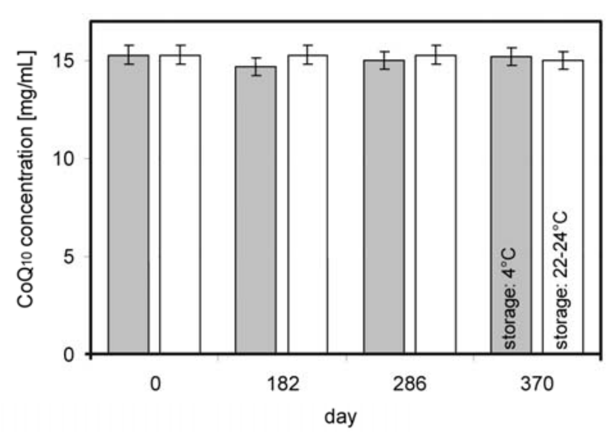

Figure 4. One-year stability of the CoQ_{10} level in fruit syrup (65% sugar)

In addition to fruit juices and nectars, syrups are also interesting products for CoQ_{10}-fortification but much higher CoQ_{10} levels are necessary to achieve a suitable

CoQ_{10} content in diluted drinks. As syrups are also a very attractive formulation of food supplements, we decided to determine the stability of such a product with a very high content of CoQ_{10}. Commercially available syrup from a forest-fruit concentrate (65% sugar) was fortified with 15 g CoQ_{10} per L, homogenised and packed in black glass bottles. The samples were divided into two series; one was stored at room temperature, while the other was kept in a refrigerator. After it was produced the syrup was analysed for its CoQ_{10} content (15.3 mg/mL ± 1.5%). The stability of the syrup was confirmed after one year following production for both series (room temperature, 4 °C; Figure 4). The level of CoQ_{10} was determined to be stable; only a 0.7% decrease in the CoQ_{10} concentration was found in the samples stored at room temperature (15.2 mg/mL ± 1.5%). Further, no changes in organoleptic and physical properties were observed (no layering or change of colour or odour). The samples were also negative for mould.

4. Conclusions

Coenzyme Q_{10} is a natural antioxidant present in all human cells with a fundamental role in cellular bioenergetics. Besides endogenous synthesis it is also supplied to the organism by food, but its average dietary intake is low. Numerous health benefits of CoQ_{10} supplementation have been reported, reflected in growing demand for its use in functional foods, especially after the development of Q10Vital, a water-soluble form of CoQ_{10}, which enables the fortification of low-fat aqueous-based products and exhibits improved bioavailability. We tested the stability of CoQ_{10} levels in some products enriched with Q10Vital, such as milk, fermented milk drink – kefir, curd and fruit syrup. The CoQ_{10} level in studied products was determined to be stable. In some cases, the enrichment was more successful if performed at the end of the technological process (curd). Only a slight decrease in CoQ_{10} was determined in UHT milk after three months and in fruit syrup after one year, while no decrease was observed in apple nectar (one year), fermented milk product drink (one month) and curd (two weeks) samples. In addition, no changes in sensorial, microbiological, physical and chemical properties were observed.

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Povzetek

Koencim Q_{10} (CoQ_{10}), poznan tudi kot ubikinon, je naravni antioksidant z zelo pomembno vlogo v celičnih energetskih procesih. Endogena koncentracija CoQ_{10} v telesnih tkivih pada s staranjem, zmanjšane koncentracije pa so opazili tudi pri različnih bolezenskih stanjih in življenjskih slogih. Že sicer dokaj omejen vnos CoQ_{10} v telo s hrano se je še dodatno zmanjšal zaradi povečanega uživanja obdelane hrane, v kateri je vsebnost tega koencima pogosto zelo nizka. To in številni opaženi ugodni učinki dodajanja CoQ_{10} na zdravje ljudi so spodbudili živilsko industrijo, da je začela izbrana živila bogatiti s koencimom Q_{10}. Bogatenje večine živil zaradi lipofilnosti CoQ_{10} ni bilo enostavno izvedljivo. Zato so bile razvite nove oblike CoQ_{10} s povečano vodotopnostjo ali disperzibilnostjo, s katerimi je izvedljivo tudi bogatenje živil na vodni osnovi. Študirali smo stabilnost koencima Q_{10} v izbranih živilih, obogatenih z vodotopnim inkluzijskim kompleksom CoQ_{10} in β-ciklodekstrina (Q10Vital). Stabilnost smo opazovali v sadnih napitkih, mleku, jogurtu in nekaterih drugih mlečnih izdelkih. Koncentracija CoQ_{10} v obliki Q10Vital je bila v vseh študiranih proizvodih stabilna. Ugotovili smo tudi, da je izkoristek bogatenja nekaterih živil (npr. skute) lahko večji, če se CoQ_{10} dodaja v zaključku proizvodnega procesa, še posebej, če se tekom proizvodnje proizvod fermentira.