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The coenzyme Q10 content of food supplements

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Summary

In the European Union, food supplements are regulated as food and their use is expanding rapidly. There is no enforcement to ensure good manufacturing practices (GMPs) are followed in the manufacturing process but producers are fully responsible for their products. Recently, the safety and quality of supplements available in the market has come into question. In our surveillance, we examined coenzyme Q10 content of 58 food supplements available in three EU member states or from Internet stores using High-Performance Liquid Chromatography methodology. While some of the tested supplements contained almost exactly the same quantity of active ingredient as labelled, one-third of the products contained less than 70% of the labelled content. In the food supplements obtained online the medium content was lower than in the products purchased in pharmacies. To protect the consumer and assure the safety and quality of products, the market authorities need to exert better control. In addition, it would make sense to enforce additional requirements to ensure GMPs are followed in the manufacturing process of food supplements.

Keywords: *Supplement, quality, CoQ10, ubiquinone, Belgium, Slovenia, Spain*

1 Introduction

Nutrition is considered one of the main factors affecting human health and well-being; a wise choice of a wide variety of foods is the best strategy for promoting optimal health and reducing the risk of chronic diseases (Marra et al. 2009). People who cannot meet their nutritional needs with foods can achieve this by using food supplements. These are concentrated sources of nutrients or other substances with a nutritional or physiological effect, marketed in a dose form, mostly as capsules, tablets, syrups and other similar forms designed to be taken in measured small unit quantities. The ingredients of food supplements are often connected with effects on human health and supplements are therefore very interesting for marketing not only to people with improper nutrition, but also to people who would like to stay healthy – meaning the general population. For this reason, food supplements are being used in extreme volumes (Gershwin et al. 2010; Skeie et al. 2009). Producers achieve this by employing aggressive marketing supported by health claims.

In the European Union (EU) food supplements are regulated as food and covered by general food legislation and EU Directive 2002/46/EC on food supplements. To ensure the protection of consumers and to facilitate their choices, products must be safe and appropriately labelled. The quality and safety of food supplements is the full responsibility of the producer but can be controlled by national authorities. In practice, such controls mainly focus on assuring adequate safety by controlling contaminants and additives (Bourgoin et al. 1993; Ross et al. 2000). Nutritional composition is usually not considered a health risk and is therefore less controlled. In fact, while labelling requirements exist in many countries for more than a decade, many nutrient analyses are still challenging. Connected with the lack of standards used in the production and labelling of food supplements (Betz et al. 2007; Jiang 2009; Sturtz et al. 2008), this situation has led to concerns about the poor quality of products on the market (Eberhardie 2007). While EU legislation currently concentrates on regulating the use of vitamins and minerals, the use of other substances with a nutritional or physiological effect is not regulated in detail (Eisenbrand 2008). The Commission and the majority of EU member states believe that the current legislation is sufficient to provide consumer protection for these other substances (FSA 2010) although products containing such ingredients are poorly controlled in many EU countries.

When discussing the safety and quality control assessment of food supplements, we must distinguish products on the basis of their active ingredients; i.e. chemically stable dietary minerals, less stable vitamins, chemical compounds other than vitamins and minerals, living microorganisms (i.e. probiotics) etc. The appropriate content of these ingredients in final

products must be achieved using suitable production standards (including quality control of both raw materials and the final product) and stability during the manufacturing process and shelf life. The low content of an ingredient in a final product is often connected with either improper manufacturing (inappropriate purity or insufficient ingredients used in the production, uncontrolled manufacturing conditions and inappropriate formulation) or its decomposition during shelf life. On the other hand, when not enough ingredients are used during manufacturing, the primary concern is about misleading consumers. Such scenarios can pose a risk to human health, i.e. in instances of adulteration or where such supplements are used by people who cannot meet their nutritional needs from other sources (Cole et al. 2003).

One might expect that the quality of food supplements containing inexpensive essential elements is less problematic due to chemical stability but studies in which the quality of such ingredients was controlled show that this is not always the case (Cuderman et al. 2010; Osterc et al. 2006; Restani et al. 2008; Stibilj et al. 2005; Vale et al. 2010). Supplements containing living organisms have also been found to be in question (Aureli et al. 2010). The monitoring of supplements containing bioactive chemical compounds other than vitamins and minerals is very rare in Europe. The quality of food supplements containing coenzyme Q10 was addressed in Japan; 80-130% of the labelled CoQ10 content was found in 86% of tested samples (Kettawan et al. 2007). A recent study performed by the National Institute of Chemistry on over 30 food supplements showed that 40% of tested products contained less than 80% of the labelled lutein content; no active ingredient was found in some samples (Jaksic 2010).

Stimulated by these observations, we decided to study the quality of food supplements containing coenzyme Q10 (CoQ10) in EU countries. CoQ10 is a naturally occurring molecule with a role in cellular bioenergetics. It has a range of potential health benefits (Littarru et al. 2010; Pravst et al. 2010) and is widely used in supplements. The study's main objective was to assess the quality of food supplements by comparing the content of the active ingredient with the labelled value. Supplements from four different markets were included in our study: three EU countries (Belgium, Slovenia and Spain), and internet stores.

2 Material and methods

2.1 Samples sources

Altogether, 58 CoQ10-containing food supplements were purchased from pharmacies in Ljubljana/Slovenia (18), Seville/Spain (18) and Brussels/Belgium (7) or ordered on-line (15).

Most supplements were in the form of either softgel or hard capsules (47 (81%)), while some were also tablets or powders (16%) and syrups (3%). In each country, three pharmacies were visited and all available CoQ10-containing food supplements were purchased. In cases where more than one product of the same brand was available, only the one with the lowest labelled CoQ10 content was purchased. Specific products which were already included in our survey were not purchased from other sources; i.e. if a product had already been purchased in one pharmacy, we did not purchase the same product in other pharmacies. The online purchases were performed through many different websites; products were found by using internet search engines (i.e. Google). The producers or sellers of food supplements were not informed about the inclusion of their product in the study. All samples were purchased and no gifts were accepted. The packagings of the supplements were examined for product composition and the use by date. To avoid an increase in oxidation processes and product decomposition, the samples were delivered to the laboratory unopened. They were stored at room temperature until the analyses were performed. All samples were analysed well before the products' use by dates.

2.2 Chemical analyses

Chemical determinations of CoQ_{10} in food supplements were performed using High-Performance Liquid Chromatography-UV (HPLC) as described by Lunetta (2008) with modifications. Analyses were conducted at the Cell Physiopathology and Bioenergetics Laboratory at Pablo de Olavide University, Seville, Spain. They were performed in line with the UPO quality assurance programme and reasonably transferred current guidelines of the European regulatory authorities; the laboratory is accredited to conform to ISO 15189:2007. The samples were prepared in the same facility shortly before the analyses.

2.2.1 Reagents and Equipment

HPLC-grade solvents (methanol, propanol, water) were purchased from Scharlab (Spain). The CoQ10 standard was purchased from Sigma-Aldrich. All other chemicals were of analytical grade and available from commercial suppliers. Propanol:water (4:1 v/v) was used as extraction solvent.

The HPLC System Gold (Beckman Coulter Ltd.) equipped with a double head pump plus a diode array UV-Vis detector (ESA, Dionex Corporation) was used.

2.2.2 Preparing the samples

Solid formulations were carefully ground in a mortar. The homogenous fine powder was weighed and transferred to a volumetric flask. Hard and soft capsules were opened and their contents were weighed and transferred to a volumetric flask. For liquid formulations, an aliquot quantity of product was pipetted to a volumetric flask. The samples were then dissolved in fresh degassed propanol:water (4:1), mixed and injected. The solubility was optimised by adjusting the dilution in additional propanol solvent. Typically 10 to 20 mg of a sample was dissolved in 5 to 15 ml of fresh degassed propanol:water (4:1) and carefully mixed to the total dissolution using polycarbonate plastic tubes.

2.2.3 Calibration curves

A stock solution of CoQ10 standards in an extraction solvent was used for the preparation of five calibration standards for each compound studied by dilution with an extraction solvent (25 pM, 50 pM, 75 pM, 100 pM, 125 pM). Values of the slope (b), intercept (a), correlation coefficient (R) and standard deviation of the slope V(b) were calculated using the 32Karat™ software (Beckman Coulter Ltd.).

2.2.4 HPLC analyses

The separation and quantification of the studied compound was performed with the LC system equipped with a double head pump plus a diode array UV-Vis detector. The reported results are the average of at least three replicates (injections). The standard deviation of the *de novo* analyses was determined to be below 10%.

HPLC conditions: Ultrabase C18 column, 150 x 4.6, 5 µM (BC, Aplicaciones analíticas s.a., Spain) was used, the flow rate was 1 mL/min, the injection volume was 20 µL and the temperature of the column was 37°C. Analyses were performed using isocratic conditions; the mobile phase was a mixture of methanol:propanol:$AcNH_4$ 1M pH 4.4 (49:49:2, v/v/v). Detection was performed using a UV-Vis detector set at 275 nm.

2.3 Statistics

Statistical analyses were carried out in MS Excel 2003. Group comparisons were performed using unpaired t-test and differences of $P<0.05$ were considered significant.

3 Results and Discussion

3.1 Content of the active ingredient

All samples were analysed using HPLC technique to determine the CoQ10 content. The ratio between the analytically determined and labelled ingredient content per unit was calculated (CR, content ratio) (Table 1). One product was only labelled with information about the presence of CoQ10 (the exact quantity was not specified) and therefore the content ratios were calculated for 57 samples. On average, 81% ±32% of the labelled CoQ10 content was found in the tested supplements. The content ratio ranged from 0% (no CoQ10 found) to 165% (65% more than expected). A group comparison of food supplements purchased in pharmacies between countries did not show statistical significant differences (P>30).
In products obtained online, the medium content (71% ±9%) was lower than in the pharmacy products (85%±33%) but statistical difference between both groups was not significant (p=0.11). The active ingredient was found in all products, except for one purchased from an (non-EU) Internet store. The distribution of the tested food supplements in relation to the content ratio of Coenzyme Q10 is presented in Figure 1.

((Table 1 somewhere here))
((Figure 1 somewhere here))

Coenzyme Q10 is included in the European Pharmacopeia (Monograph 1578: Ubidecarenonum). Since food supplements are not considered to be drugs, we used food tolerances in our evaluation. Currently, there are no generally accepted tolerances for declaration of nutrients and other active ingredients in the EU (DG SANCO 2006), although guidelines on this issue have been accepted in some countries (Table 2). The task of setting tolerable margins was identified as a priority 10 years ago during the discussion that led to the adoption of Directive 2002/46/EC on food supplements but this goal has not yet been achieved. Nevertheless, there is a general agreement that such tolerances should be defined at the Community level in order to avoid trade barriers and ensure consumer protection (DG SANCO 2006).

((Table 2 somewhere here))

On the basis of tolerances currently practiced in the EU for oil-soluble vitamins we decided to check whether the CoQ10 content in the tested supplements is within the following limits:

(A) 80-130% of declared ingredient content; comparable to tolerances for oil-soluble vitamins practiced in Slovenia and Belgium, where the majority of the samples was

purchased, and in line with Italian regulation of the use of coenzyme Q10 in foods (CIAA 2007); and

(B) 70-130% of declared ingredient content; the tolerance practiced for oil-soluble vitamins in non-liquid foods in the UK.

((Table 3 somewhere here))

Altogether, 26 (46%) of the 57 tested products contained 80-130% of the declared CoQ10 content; 28 products contained less active ingredient (Table 3, section a). Insufficient CoQ10 contents were observed in 57% of products purchased online and in 47% of supplements purchased in pharmacies. When applying wider tolerances (70-130% of declared ingredient content), we observed that 36 (63%) of the tested supplements were compliant (Table 3, section b). The difference between pharmacy and online samples become more distinct as 28% and 43% of the supplements contained less CoQ10 than labelled, respectively.

There are two plausible explanations for these results: Decomposition could have occurred during production or shelf life, as CoQ10 is known to be sensitive to light and temperature (Matsuda et al. 1983; Pravst et al. 2009), or economisation on raw material during manufacturing. CoQ10 is a relatively expensive material and some manufacturers might have tried to save on production costs. Since official market controls are rare, especially for non-vitamins, and consumers are not able to check the actual CoQ10 content, this could result in consumer misleading practices.

It is interesting to compare our observations with the results of a similar study performed on products containing CoQ10 from the Japanese market (Kettawan et al. 2007). The authors concluded that the quality of tested supplements varied considerably among brands. The CoQ10 content of over-the-counter (OTC) drugs was found within 99-100% of the labelled value and most supplements also showed a content value of more than 80%. Only a few products exhibited insufficient quality performance. In relation to this, it should be mentioned that Japan has a very well-developed CoQ10 market with over 200 different food supplements available from over 100 different producers (Kettawan et al. 2007). This strong competition might also partially explain the overall better content ratio of CoQ10 containing supplements on the Japanese market.

3.2 Other aspects of quality and consumer protection

We did not observe any significant correlation between the price of a supplement and its quality in relation to the compliant content of the active ingredient. However, the lowest price was found for a product purchased online which did not contain any CoQ10. Several aspects of quality were not monitored in this study, including appropriate labelling and use of health claims, presence of contaminants, disintegration time and quality of ingredients etc. Pure coenzyme Q10 is a crystalline powder with a relatively high molecular weight that is insoluble in water due to its lipophilicity; its solubility in lipids is also limited. For these reasons, crystalline CoQ10 is poorly absorbed in the gastrointestinal tract and different formulations have been developed to improve bioavailability (Bhagavan et al. 2007; Žmitek et al. 2008b; Žmitek et al. 2008a). However, in this study, we did not focus on the quality of used CoQ10 source.

Possible misleading of consumers was observed with some multivitamin products with added CoQ10. Due to promotion campaigns, Coenzyme Q10 is well-known as a health-related ingredient. Some manufacturers take advantage of this situation and use "Q10" as part of their brand names, even though the content of this ingredient in such products is very low (a few milligrams). Five of the tested products with "Q10" as part of their brand name contained less than 5 mg of CoQ10 while competitive products usually contain at least 30 mg. Such a manipulation might be easily overlooked by consumers.

3.3. Limitations of the study

Chemical analyses of food supplements were performed in a laboratory well experienced with determinations of CoQ10 in different matrixes including food products. However, we must note the unavailability of suitable standard reference materials for CoQ10 containing food supplements.

4 Conclusions

The use of food supplements in the EU is rapidly increasing and concerns have been raised about the poor quality of products on the market. In our survey of 58 CoQ10-containing supplements, one-third of the products were found to contain less than 70% of the labelled content.

The study reveals that regulator cannot rely on manufacturers' good practices in the production of food supplements. To protect the consumer and assure the safety and quality of products, better controls are needed. Moreover, it would make sense to enforce additional requirements such as that good manufacturing practices (GMPs) are followed when food

supplements are produced. Such a regulation was already accepted in the USA in 2008 and requires that proper controls are in place so that supplements are processed in a consistent manner and meet quality standards including consistent identity, purity, strength and composition (Frankos et al., 2010).

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Table 1: *Content of CoQ10 in tested food supplements*

ID	formul.	CoQ10 content (mg)[1] label.	anal.	%[2]
Samples purchased in pharmacies:				
M063	capsule[4]	1	0.10 ± 0.01	6%
M065	tablet[4]	2	0.40 ± 0.02	22%
M014	efferv.[3]	3	3.3 ± 0.2	111%
M018	syrup[3]	4	6.2 ± 0.3	154%
M015	tablet[3]	5	2.6 ± 0.1	52%
M069	capsule[4]	5	7.1 ± 0.4	142%
M009	efferv.[3]	10	7.0 ± 0.4	70%
M012	tablet[3]	10	7.5 ± 0.4	75%
M062	capsule[4]	10	8.8 ± 0.4	88%
M013	tablet[3]	10	10.9 ± 0.5	109%
M070	capsule[4]	10	16.5 ± 0.8	165%
M008	syrup[3]	15	16.5 ± 0.8	110%
M073	tablet[4]	20	17.4 ± 0.9	87%
M067	capsule[4]	30	19.4 ± 1.0	65%
M076	capsule[4]	30	20.8 ± 1.0	70%
M079	capsule[4]	30	10.1 ± 0.5	34%
M068	capsule[4]	30	14.4 ± 0.7	48%
M003	capsule[3]	30	18.9 ± 0.9	63%
M075	capsule[4]	30	17.2 ± 0.9	57%
M019	capsule[5]	30	17.0 ± 0.9	57%
M006	capsule[3]	30	22.9 ± 1.1	76%
M077	capsule[4]	30	29.5 ± 2.1	98%
M017	capsule[3]	30	23.8 ± 1.2	79%
M004	capsule[3]	30	26.7 ± 1.3	89%
M001	capsule[3]	30	28.3 ± 1.4	94%
M071	capsule[4]	30	34.1 ± 2.4	114%
M005	capsule[3]	30	30.3 ± 1.5	101%
M002	capsule[3]	30	30.9 ± 1.5	103%
M072	capsule[4]	30	32.3 ± 1.6	108%
M026	capsule[3]	30	33.4 ± 1.7	111%
M078	capsule[4]	30	24.5 ± 1.7	82%
M024	capsule[5]	30	35.2 ± 1.8	117%
M020	capsule[5]	30	35.6 ± 1.8	119%
M074	capsule[4]	40	27.5 ± 1.9	70%
M010	capsule[3]	50	40.8 ± 2.0	82%
M016	capsule[3]	50	43.3 ± 2.2	87%
M021	capsule[5]	50	23.4 ±1.6	47%
M011	powder[3]	50	51.6 ± 2.6	103%
M025	capsule[5]	50	36.1 ± 1.8	72%
M023	capsule[5]	50	38.7 ± 1.9	77%
M064	capsule[4]	100	122.6 ± 11.0	123%
M022	capsule[5]	100	49.5 ± 2.5	50%
M066	capsule[4]	120	74.9 ± 6.7	62%
Samples purchased from Internet sources				
M039	capsule	NA [6]	29.6 ± 2.1	
M034	capsule	10	7.4 ± 0.4	74%
M061	capsule	15	10.1 ± 0.6	67%
M031	capsule	20	12.9 ± 0.6	65%
M028	capsule	20	13.9 ± 0.7	70%
M032	capsule	30	12.9 ± 0.6	43%
M037	capsule	30	16.5 ± 0.8	55%
M030	capsule	30	18.7 ± 0.9	62%
M036	capsule	30	24.2 ± 1.2	81%
M033	tablet	30	25.2 ± 1.3	84%
M027	capsule	30	28.1 ± 1.4	94%
M029	capsule	30	28.2 ± 1.4	94%
M035	capsule	30	29.9 ± 1.5	100%
M038	capsule	30	30.4 ± 1.5	101%
M058	capsule	100	0.1 ± 0.01	0%

Notes: [1] CoQ10 content in mg per unit (i.e. capsule, tablet, effervescent tablet [efferv.], mL of syrup); [2]the ratio between the analytically determined and labelled CoQ10 content (in %); [3]origin: Slovenian pharmacy; [4]origin: Spanish pharmacy; [5]origin: Belgian pharmacy; [6]no CoQ10 quantity indicated on label

***Table 2**: Tolerance values accepted or practiced in some EU member states* (CIAA 2007; DG SANCO 2006; IVZ 2009).

	Tolerances for added nutrients [1]		
	Minerals	Water-soluble vitamins	Fat-soluble vitamins
Country			
Belgium	90% - 120%	90% - 120%	90% - 120%
Denmark	80% - 150%	80% - 150%	80% - 150%
France	80% - 200%	80% - 200%[2]	80% - 200%[2]
Italy	75% - 100%	80% - 130%[2]	80% - 130%[2]
Slovenia	80% - 150%	80% - 150%[2]	80% - 130%
The Netherlands	80% - 150%	80% - 200%	80% - 200%
United Kingdom	50% - 200%	50% - 200%	70% - 130%

Note: [1] if legislation prescribes minimum and maximum values for the addition of nutrients, the analysed amount must not exceed these limits; [2] with exceptions

***Table 3**: Distribution of tested food supplements in relation to the content ratio of CoQ10*

	Food supplement origin		
Content ratio of CoQ10 [1]	EU pharmacies	Internet	All sources
a) Distribution using a tolerance interval of 80% - 130%:			
below 80%	20 (46.5%)	8 (57%)	28 (49%)
80% - 130%	20 (46.5%)	6 (43%)	26 (46%)
over 80%	3 (7%)		3 (5%)
b) Distribution using a tolerance interval of 70% - 130%:			
below 70%	12 (28%)	6 (43%)	18 (32%)
70% - 130%	28 (65%)	8 (57%)	36 (63%)
over 130%	3 (7%)		3 (5%)

Note: [1] the ratio between the CoQ10 contents obtained by the analyses and those declared

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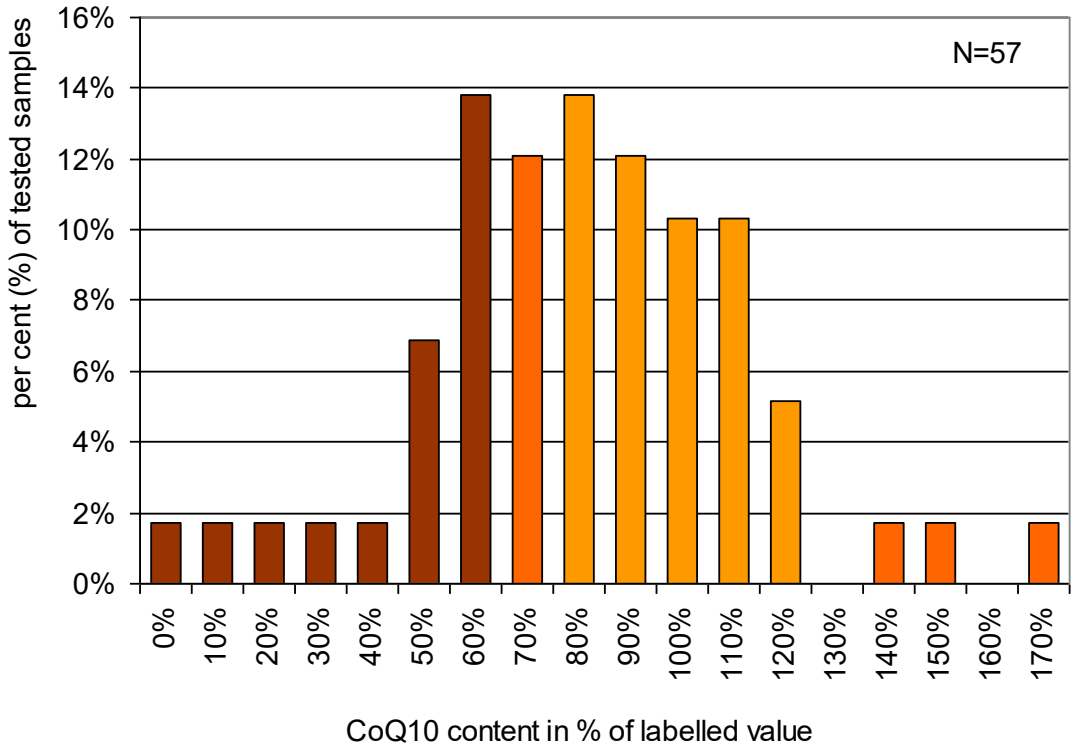

***Figure 1**: Distribution of tested food supplements in relation to the content ratio of CoQ10*