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The Quality Control Assessment of Commercially Available Coenzyme Q_{10}-Containing Dietary and Health Supplements in Japan

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Summary **Coenzyme Q_{10} (CoQ_{10}) has been widely commercially available in Japan as a dietary and health supplement since 2001 and is used for the prevention of lifestyle-related diseases induced by free radicals and aging. We evaluated CoQ_{10} supplements to ensure that these supplements can be used effectively and safely. Commercially available products were selected and assessed by the quality control tests specified in the Japanese Pharmacopoeia XV. When the disintegration time of CoQ_{10} supplements was measured, a few tested supplements did not completely disintegrate even after incubation in water for an hour at 37°C. In the content test, many samples were well controlled. However, a few supplements showed low recovery rates of CoQ_{10} as compared to manufacturer's indicated contents. Among soft capsule and liquid supplements, the reduced form of CoQ_{10} (H_2CoQ_{10}), as well as the oxidized form, was detected by HPLC with electrochemical detector. The results for experimental formulated CoQ_{10} supplements demonstrated that H_2CoQ_{10} was produced by the interaction of CoQ_{10} with vitamins E and/or C. From these results, we concluded that quality varied considerably among the many supplement brands containing CoQ_{10}. Additionally, we also demonstrated that H_2CoQ_{10} can be detected in some foods as well as in CoQ_{10} supplements.**

Key Words**: coenzyme Q_{10}, ubiqinol-10, quality control, dietary and health supplement, food**

Introduction

It is well known that coenzyme Q (CoQ) serves as an essential carrier for electron transport and proton translocation in the mitochondrial respiratory chain [*1*]. Besides its role in electron-transfer reactions, CoQ_{10} serves as a free radical scavenger, thereby preventing oxidative damage in the human body. Several researchers have pointed out that the reduced form of CoQ_{10} (H_2CoQ_{10}) is an efficient scavenger of lipid radicals and inhibitors of lipid peroxidation in low-density lipoprotein [*2*, *3*], biomembrane [*4*], and liposomes [*5*, *6*]. CoQ in the bodies of human beings is thought to be provided by both dietary intake, from foods or dietary supplements [*7*, *8*], and *de novo* biosynthesis [*9*, *10*]. Therefore, decreases in the biosynthesis or intake of CoQ_{10} might affect

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the physiological action of CoQ_{10}. In fact, decreased serum levels of CoQ_{10} were observed in patients undergoing total parenteral nutrition therapy without dietary intake [*11*], and in patients [*12*, *13*] and animals [*14*, *15*] receiving HMG-CoA reductase inhibitor (statin) administration.

CoQ_{10} was introduced into clinical therapy in 1974 in Japan. Its generic name is ubidecarenone. Many clinical trials showed that oral administration to patients was effective for mild congestive heart failure symptoms such as edema, lung congestion, and swollen liver. On the basis of these clinical findings, CoQ_{10} was classified in the group of cardiovascular drugs for metabolic disturbances. In 1991, CoQ_{10}, whose generic name is ubiquinone-10, was also made available as an over-the-counter drug in pharmacies.

While sales of dietary and health supplement products have been rapidly increasing in Japan, it is vital to supply quality-controlled products for consumers. In 2001, the Ministry of Health, Labour and Welfare in Japan permitted the use of CoQ_{10} as a food additive as long as no claims were made about its pharmacological effectiveness and application. Currently, it is estimated that more than 200 kinds of CoQ_{10}-containing dietary and health supplements (CoQ_{10} supplements), produced by more than 100 different manufacturers, are available. In particular, CoQ_{10} supplements are used for the prevention of lifestyle-related diseases induced by free radicals and aging. However, the products may vary in quality. Because CoQ_{10} has a rather complicated chemical structure and possesses several physical properties, such as low melting point, hydrophobic nature, and light sensitivity, that do not favor large-scale commercial production, highly sophisticated techniques should be employed at all production stages to obtain a satisfactory product [*16*]. Moreover, many Japanese CoQ_{10} supplements also contain other components, e.g., vitamins, minerals, amino acids, antioxidative compounds, and enzymes, in the same tablet, soft capsule, or other product. Therefore, the chemical stability of CoQ_{10} supplements is also an important point to be considered.

In this study, we evaluated the quality of commercially available CoQ_{10} supplements to ensure that these supplements can be used effectively and safely. In addition, to investigate the distribution of CoQ_{10} in foods from a nutritional point of view, we also measured both CoQ_{10} and H_2CoQ_{10} contents in selected foods.

Materials and Methods

Reagents

Authentic CoQ homologues from CoQ_7 to CoQ_{11} were kindly supplied by Nisshin Pharma Inc., Tokyo. High performance liquid chromatography (HPLC) solvents and ethanol were purchased (HPLC grade) from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All other chemicals used were of analytical grade, available from commercial suppliers.

CoQ_{10} supplements

CoQ_{10} supplements were commercially purchased from pharmacies, health food stores, department stores, convenience stores, and sport goods shops in the Kobe city area. Forms tested in this study included tablet (TB), hard capsule (HCP), soft capsule (SCP), granule (GN), liquid (LQ), jelly (JL), and inclusion complex with γ-dextrin (ICD). The quality control tests of CoQ_{10} supplements were completed within 6 months before the product's consume-by date. CoQ_{10} supplements obtained for testing were preserved in tight, light-resistant containers at room temperature until the quality control tests were conducted.

Experimental manufacturing preparations for SCPs, a LQ, and an HCP of "Formulas 1 to 5"

Experimental manufacturing products for 3 SCP forms, a LQ form, and an HCP form of formulas 1 to 5 were kindly provided by Shiseido Medical, Co., Ltd., Tokyo, Japan. The detailed formulas of their components are shown in Table 1.

Quality control tests

Disintegration test—This test was performed using the apparatus (Model TMB-81, Toyama Sangyo Co., Ltd., Osaka, Japan) and test conditions specified in the Japanese Pharmacopoeia XV (J.P.XV). Water and the first (pH 1.2) and the second (pH 6.8) fluids of the J.P.XV method were used as the immersion solution at 37 ± 2°C. The time required for each TB, HCP, and SCP to completely disintegrate was recorded, and the mean disintegration time was calculated. Complete disintegration is defined by the J.P.XV as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining

Table 1. Experimental formulas of CoQ_{10} supplement forms of soft capsule, liquid, and hard capsule

Contents	"Formula 1"	"Formula 2"	"Formula 3"	"Formula 4"	"Formula 5"
Intake form	Soft capsule	Soft capsule	Soft capsule	Liquid	Hard capsule
Based Oil	Safflower oil	Safflower oil	Olive oil	—	—
CoQ_{10}	30 mg	10 mg	30 mg	30 mg	30 mg
Vitamin E	10 mg	30 mg	10 mg	10 mg	10 mg
Vitamin C	30 mg	—	30 mg	30 mg	30 mg

on the screen (0.25–0.31 mm) of the test apparatus is a soft mass having no palpably firm core.

Content test—All procedures for the content test of CoQ_{10} supplements were carried out in the dark.

i) *Pre-preparation of CoQ_{10} supplements*

TB and ICD products were crushed carefully in a mortar to obtain a homogenous fine powder. This powder was transferred completely to a 50-ml brown volumetric flask, and about 30 ml of ethanol was added to the flask.

HCP and SCP products were carefully opened, the contents were transferred completely to a brown 50-ml volumetric flask, and about 30 ml of ethanol was added to the flask. If ethanol-insoluble contents and/or fragments were observed, these were sonicated on ice for 1 min with output 3 to 5 (Ultrasonic Distruptor, Model UR-200P, Tomy Seiko Co, Ltd., Tokyo, Japan).

In the case of LQ products, an aliquot of 5 ml of drink solution was pipetted into a 50-ml brown volumetric flask directly, and about 30 ml of ethanol was added to the flask. For JL and GN products, an equivalent of 5 mg was weighed accurately and dissolved in 10 ml of water and about 20 ml of ethanol in a mortar to obtain a homogenous solution. The solution was completely transferred to a brown 50-ml volumetric flask.

ii) *Preparation of CoQ_{10} supplements*

After the ethanol solution was prepared as described above, it was incubated for 10 min at 50°C and allowed to stand for 10 min at room temperature. Then, the ethanol solution was added to the flask again to scale up to 50 ml. The solution was filtered with filter paper (No. 3) and diluted with ethanol to adjust to a 10 μg/ml CoQ_{10}-containing ethanol solution. Finally, except for SCP products, an aliquot of 10 μl of the ethanol solution was injected into the column to determine H_2CoQ_{10}.

In the case of SCP products only, an aliquot of 0.5 ml of 10 μg/ml CoQ_{10}-containing ethanol solution was pipetted into a brown glass-stoppered centrifuged tube, and then 0.5 ml of distilled water, 1.5 ml of ethanol, and 5 ml of *n*-hexane were added in turn. The mixture was shaken vigorously reciprocally at a rate of 80 times per minute for 10 min and centrifuged at 500 *g* for 10 min. This extraction procedure was repeated three times. Subsequently, the combined *n*-hexane layer was concentrated *in vacuo* under a stream of nitrogen. The resulting residue was dissolved in 0.5 ml of ethanol, and an aliquot of 10 μl of the ethanol solution was injected into the column to determine H_2CoQ_{10}.

Separately, an aliquot of 10 μl of 0.25% sodium borohydride solution (0.25 mg/ml water) was added to 0.4 ml of the ethanol and the mixture allowed to stand for 10 min at room temperature. An aliquot of 10 μl of the solution was injected into the column to determine total CoQ_{10}, the sum of reduced and oxidized CoQ_{10}.

iii) *HPLC conditions for determination of CoQ_{10} and H_2CoQ_{10}*

The content of CoQ_{10} supplements was determined by an HPLC method with electrochemical detection (ECD), as previously described [*17*]. Authentic CoQ homologues from CoQ_7 to CoQ_{11} were prepared by dissolution in ethanol to yield concentrations of 10 μg/ml. Authentic CoQ homologues were freshly prepared from the corresponding CoQ, adding 25 μg of sodium borohydride (10 μl of a 0.25% solution of sodium borohydride in water) to give a concentration of 1 μg/ml prior to HPLC-ECD analysis.

Food samples

Food samples were obtained from local food stores or supermarkets in the Kobe city area. All food items were analyzed raw, without freezing. In some cases, samples of the same food were collected on separate days.

Measurement of CoQ homologue contents in foods

Food samples were homogenized with distilled water at 4°C using a Polytron homogenizer (Type PT 10/35; Kinematica, Lucerne, Switzerland) at a setting of 7 to 30 seconds. The final volume of the homogenate was adjusted so as to contain about 1 to 2 μg of H_2CoQ_{10}. An aliquot of 0.5 ml of the homogenate was pipetted into a brown glass-stoppered centrifuged tube, and then 2 ml of ethanol and 5 ml of n-hexane were added. The solution was extracted as described above for SCP products, and the contents of H_2CoQs and CoQs were determined by HPLC-ECD [*17*].

Results

Internal profile for SCP forms of CoQ_{10} supplements

Among CoQ_{10} supplements, SCP is the most common commercially available intake form in Japan. According to the United States Pharmacopoeia, SCPs (sometimes called soft gelatin capsules or softgels) are filled with liquid contents in most cases. Typically, CoQ_{10} is dissolved or suspended in a liquid vehicle such as vegetable oil or the lower-molecular-weight polyethylene glycols.

First, we assessed the internal profile of different SCP CoQ_{10} supplement products. Most samples showed the typical internal profile of SCPs. However, as shown in Fig. 1, in a few SCPs, CoQ_{10} crystallized itself inside and existed in the solid state but not the lipid-soluble form. Moreover, a certain SCP did not show the uniform distribution of CoQ_{10} in the capsule.

Disintegration tests for TB, HCP, SCP, and ICD forms of CoQ_{10} supplements

According to the disintegration test specified in the J.P.XV, TB supplements should disintegrate with water as the immersion fluid within 30 min after operating the apparatus. For both HCP and SCP supplements, the time limit is 20 min. Although ICD is the inclusion complex form of CoQ_{10} and γ-

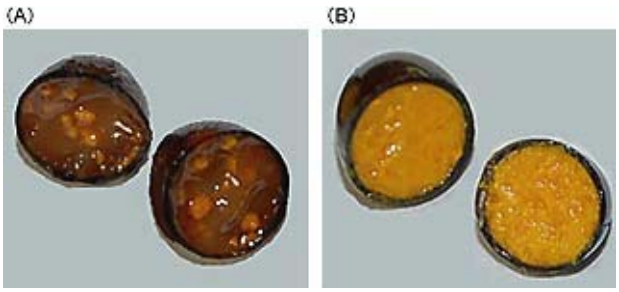

Fig. 1. Internal appearance of soft capsule forms of CoQ_{10}-supplements. (A) Crystallized CoQ_{10}; the yellowed compound is crystallized CoQ_{10}. (B) Typical soft capsule appearance; CoQ_{10} distributes uniformly in the soft capsule.

dextrin, the disintegration time is not specified by J.P.XV.

When we measured the disintegration time of 2 kinds of CoQ_{10}-containing over-the-counter (OTC) drugs as controls, their disintegration times were 3.8 min for OTC-TB and 4.6 min for OTC-SCP, respectively. OTC drugs were well controlled and met the necessary requirements specified in the J.P.XV (Table 2).

For CoQ_{10} supplements, as shown in Table 2, the disintegration time was 3.2 min to >60 min for TBs, 3.2 min to 23.8 min for HCPs, 5.6 min to >60 min for SCPs, and 8.8 min to 32.5 min for ICDs; considerable differences were observed among the many supplements. In particular, the disintegration time of about half of the SCPs was more than 60 min. Among the different forms of CoQ_{10} supplements, the disintegration performance of ICDs was not always faster than that of other forms. Moreover, as tested supplements might be enteric-coated tablets, we also carried out the disintegration tests again for the first fluid, simulated gastric fluid (pH 1.2), and the second fluid, simulated intestinal fluid (pH 6.8). However, the results did not differ from those of water as the immersion fluid.

Content tests for CoQ_{10} supplements

Although the J.P.XV specifies the HPLC-UV method as the identification and content test for ubidecarenone, we applied the HPLC-ECD method as described in Materials and Methods instead because this method can measure both the reduced and oxidized forms of CoQ_{10} and shows higher sensitivity than the HPLC-UV method does.

Of course, the content of the over-the-counter drugs used as controls was well controlled within an extremely limited range (99–100%). Most CoQ_{10} supplements showed a content value of more than 80%. However, a few supplements exhibited low recovery rates of CoQ_{10} as compared to manufacturers' indicated contents. Moreover, in several SCP and LQ products, H_2CoQ_{10} as well as CoQ_{10} was detected. Interestingly, in LQ-6, as shown in Table 3, CoQ_{10} was

Table 2. The disintegration time of CoQ_{10} supplements, tablets, hard capsules, soft capsules, and inclusion complex with γ-dextrin

Samples*	Disintegration Time (min)		
	Water	First fluid (pH 1.2, J.P.XV)	Second fluid (pH 6.8, J.P.XV)
OTC-TB	3.8 ± 0.2	4.1 ± 0.4	6.0 ± 0.4
OTC-SCP	4.6 ± 0.1	6.8 ± 0.1	9.8 ± 0.5
TB-1	11.3 ± 0.9	16.5 ± 0.6	11.1 ± 0.7
TB-2	3.2 ± 0.4	3.6 ± 1.0	2.4 ± 0.1
TB-3	10.7 ± 0.4	12.8 ± 0.6	13.1 ± 0.3
TB-4	11.1 ± 0.1	12.1 ± 0.1	13.4 ± 0.1
TB-5	>60	>60	>60
TB-6	55.2 ± 0.9	56.4 ± 0.4	55.9 ± 0.8
TB-7	4.6 ± 0.6	3.3 ± 0.0	4.1 ± 0.8
TB-8	31.5 ± 2.6	32.1 ± 13.1	33.7 ± 3.9
TB-9	23.4 ± 0.7	32.8 ± 0.5	21.4 ± 0.4
TB-10	11.8 ± 3.2	11.8 ± 1.9	14.8 ± 4.2
TB-11	17.5 ± 2.8	19.6 ± 1.3	16.5 ± 2.0
TB-12	>60	>60	>60
TB-13	18.3 ± 0.2	18.6 ± 0.5	18.8 ± 0.6
TB-14	56.7 ± 1.3	53.4 ± 0.8	58.7 ± 1.6
TB-15	19.6 ± 1.4	18.9 ± 1.5	18.7 ± 1.6
TB-16	9.3 ± 1.0	11.3 ± 0.3	11.6 ± 0.5
TB-17	18.8 ± 1.0	18.6 ± 0.8	25.1 ± 1.9
HCP-1	6.9 ± 1.9	7.2 ± 1.5	17.6 ± 0.4
HCP-2	10.8 ± 0.6	9.9 ± 0.3	11.9 ± 0.7
HCP-3	3.2 ± 0.2	4.0 ± 0.4	3.6 ± 0.3
HCP-4	23.8 ± 3.0	22.5 ± 3.8	13.9 ± 0.9
HCP-5	4.9 ± 0.9	3.2 ± 0.1	5.3 ± 0.9
SCP-1	10.2 ± 1.0	12.9 ± 1.0	11.2 ± 1.0
SCP-2	5.6 ± 0.2	5.6 ± 0.5	10.7 ± 0.5
SCP-3	47.2 ± 1.5	54.9 ± 2.8	57.2 ± 0.9
SCP-4	>60	>60	>60
SCP-5	>60	44.0 ± 2.5	52.7 ± 2.0
SCP-6	>60	>60	>60
SCP-7	>60	>60	>60
SCP-8	41.0 ± 0.9	40.7 ± 2.5	32.6 ± 1.9
SCP-9	19.0 ± 1.5	21.5 ± 0.5	13.8 ± 0.9
SCP-10	>60	>60	>60
SCP-11	14.8 ± 0.7	16.9 ± 1.5	14.0 ± 1.6
SCP-12	>60	>60	>60
SCP-13	11.4 ± 0.7	7.8 ± 0.3	8.0 ± 0.7
ICD-1	32.5 ± 3.4	30.8 ± 2.7	41.6 ± 2.1
ICD-2	29.9 ± 2.1	41.3 ± 1.1	28.1 ± 1.5
ICD-3	12.1 ± 0.8	15.6 ± 1.9	11.6 ± 0.9
ICD-4	30.0 ± 2.2	29.8 ± 0.7	31.4 ± 2.4
ICD-5	26.4 ± 3.3	36.4 ± 2.7	25.1 ± 2.8
ICD-6	8.8 ± 0.7	12.1 ± 0.9	7.6 ± 0.6

*OTC, over-the-counter drug; TB, tablet; HCP, hard capsule; SCP, soft capsule; ICD, inclusion complex with γ-dextrin. Data are expressed as means ± SD (n = 6).

Table 3. Content test of CoQ_{10} supplements, tablets, hard capsules, soft capsules, liquids, jellies, granules, and inclusion complex with γ-dextrin

CoQ_{10}-supplements*	Content (mg)		
	Labeled CoQ_{10}	H_2CoQ_{10} (%)**	Total CoQ_{10} (%)***
OTC-TB	10	ND****	100.5 ± 0.1 (100)
OTC-SCP	10	ND	99.4 ± 0.2 (99)
TB-1	10	ND	9.6 ± 0.3 (96)
TB-2	6.3	ND	3.4 ± 0.1 (54)
TB-3	11.1	ND	9.4 ± 0.5 (85)
TB-4	1.7	ND	1.6 ± 0.1 (94)
TB-5	5	ND	4.6 ± 0.2 (92)
TB-6	16.7	ND	17.2 ± 0.4 (103)
TB-7	10	ND	9.3 ± 0.4 (93)
TB-8	60	ND	55.6 ± 1.4 (93)
TB-9	20	ND	20.0 ± 0.5 (100)
TB-10	0.34	ND	0.4 ± 0.01 (118)
TB-11	3.75	ND	3.7 ± 0.3 (99)
TB-12	0.83	ND	0.9 ± 0.1 (108)
HCP-1	90	ND	92.3 ± 0.5 (103)
HCP-2	80	ND	4.2 ± 0.1 (5)
HCP-3	30	ND	28.7 ± 1.47 (96)
HCP-4	60	ND	59.3 ± 1.3 (99)
HCP-5	30	ND	30.8 ± 0.9 (103)
SCP-1	33.3	5.8 ± 0.5 (17)	33.8 ± 1.4 (101)
SCP-2	30	14.1 ± 1.0 (46)	30.6 ± 0.9 (102)
SCP-3	30	7.0 ± 0.8 (22)	31.0 ± 1.2 (103)
SCP-4	30	13.5 ± 0.4 (44)	31.1 ± 0.7 (104)
SCP-5	30	13.7 ± 0.7 (52)	26.4 ± 1.0 (88)
SCP-6	35	1.6 ± 0.5 (4)	35.8 ± 0.8 (102)
SCP-7	30	1.8 ± 0.03 (6)	28.0 ± 0.6 (94)
SCP-8	30	25.3 ± 0.7 (88)	28.8 ± 0.6 (96)
SCP-9	150	6.2 ± 0.4 (5)	120.8 ± 3.8 (80)
SCP-10	30	1.4 ± 0.1 (4)	30.7 ± 0.3 (102)
SCP-11	30	3.8 ± 0.7 (12)	30.7 ± 0.9 (102)
SCP-12	50	ND	49.6 ± 1.0 (99)
SCP-13	30	ND	31.1 ± 0.4 (104)
SCP-14	30	5.9 ± 0.7 (19)	31.0 ± 0.5 (103)
SCP-15	10	ND	10.1 ± 0.1 (101)
SCP-16	10	ND	10.0 ± 0.1 (100)
SCP-17	10	1.1 ± 0.2 (14)	8.0 ± 0.6 (80)
SCP-18	30	5.2 ± 0.4 (16)	31.4 ± 0.4 (105)
SCP-19	30	2.6 ± 0.7 (9)	30.4 ± 0.6 (101)
SCP-20	45	3.9 ± 0.2 (8)	46.5 ± 0.7 (103)
SCP-21	15	8.0 ± 0.5 (53)	14.9 ± 0.6 (99)
SCP-22	30	3.4 ± 0.2 (12)	27.2 ± 0.9 (91)
SCP-23	20	0.9 ± 0.1 (4.2)	20.2 ± 1.1 (101)
SCP-24	60	ND	58.0 ± 4.1 (97)
SCP-25	0.25	0.04 ± 0.1 (16)	0.2 ± 0.1 (96)
LQ-1	40	ND	41.4 ± 1.9 (104)
LQ-2	30	ND	31.7 ± 1.4 (106)
LQ-3	30	ND	32.5 ± 0.3 (108)
LQ-4	1	ND	1.0 ± 0.03 (100)
LQ-5	50	12.3 ± 2.4 (24)	52.1 ± 0.8 (104)
LQ-6	—	5.2 ± 0.1 (—)	5.2 ± 0.1 (—)
JL-1	30	ND	29.3 ± 0.1 (98)
JL-2	50	ND	61.5 ± 4.6 (123)
GN-1	30	ND	28.5 ± 0.3 (95)
GN-2	30	ND	30.4 ± 0.2 (101)
GN-3	30	ND	30.2 ± 0.2 (101)
GN-4	30	ND	30.1 ± 0.1 (100)
ICD-1	3.75 as ICD	ND	1.0 ± 0.01 (27)
ICD-2	84 as ICD	ND	10.4 ± 0.3 (12)
ICD-3	18 as ICD	2.0 ± 0.6 (50)	3.9 ± 0.72 (22)
ICD-4	100 as ICD	ND	21.8 ± 1.4 (22)
ICD-5	12.5 as ICD	ND	0.9 ± 0.1 (7)
ICD-6	100 as ICD	ND	22.8 ± 0.1 (23)

*OTC, over-the-counter drug; TB, tablet; HCP, hard capsule; SCP, soft capsule; LQ, liquid; JL, jelly; GN, granule; ICD, inclusion complex with γ-dextrin. Data are expressed as means ± SD ($n = 6$).

** [H_2CoQ_{10}/total CoQ_{10} (sum of CoQ_{10} and H_2CoQ_{10})] × 100.

*** [Sum of CoQ_{10} and H_2CoQ_{10}] × 100.

****Not detected.

recovered entirely as the reduced form, H_2CoQ_{10}.

ICD is the inclusion complex form of CoQ_{10} and γ-dextrin. The purposes of developing this inclusion complex with CoQ_{10} seem to be reduction of instability to light and enhancement of bioavailability. However, the manufacturer's indicated contents were mentioned and listed on the package only as "CoQ_{10} inclusion complexes with γ-dextrin." Thus, the CoQ_{10} content of the product was uncertain. As shown in Table 3, we estimated that the inclusion ratio of CoQ_{10} to γ-dextrin in the tested ICD will be in the range from 5% to 30%.

H_2CoQ_{10} production in the SCP and LQ forms of CoQ_{10} supplements

We recognized that all CoQ_{10} supplements in which H_2CoQ_{10} was detected contained vitamin E (α-tocopherol) and/or vitamin C (ascorbic acid). Therefore, we presumed that H_2CoQ_{10} might be produced by the interaction of CoQ_{10} with vitamin E and/or vitamin C. To confirm this possibility, we prepared the experimental formulas listed in Table 1.

As shown in Fig. 2, co-existence of CoQ_{10} and vitamin E and/or vitamin C produced reduction of CoQ_{10} to H_2CoQ_{10} in SCPs and a LQ. The content ratio of vitamin E to CoQ_{10} was also important. The existence of safflower oil in SCPs was also an essential condition for production of H_2CoQ_{10} from CoQ_{10} in SCPs. In particular, a LQ form of CoQ_{10} that co-existed with vitamin E and vitamin C exhibited a high ratio of the reduced form to total CoQ_{10} in a time-dependent manner. In an HCP, however, the reduction of CoQ_{10} to H_2CoQ_{10} was not observed even if CoQ_{10} co-existed with vitamin E and C.

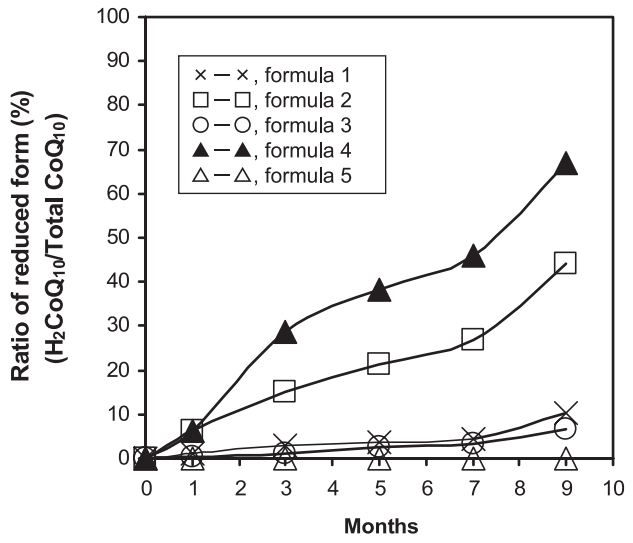

Fig. 2. H_2CoQ_{10} produced by interaction of CoQ_{10} with vitamins C and/or E in soft capsule and liquid. Each formula is shown in Table 1.

H_2CoQ_{10} and CoQ_{10} contents in foods

To determine the dietary intake of H_2CoQ_{10} as well as CoQ_{10} in human beings, we analyzed the CoQ_{10} contents of raw foods. Table 4 shows both the CoQ_{10} and H_2CoQ_{10} contents of meats and vegetables. The greater part of the CoQ homologues in meats and vegetables was CoQ_{10}. Moreover, H_2CoQ_{10} as well as CoQ_{10} was detected in all examined samples. In particular, squid and eel meats showed high ratios of the reduced form, H_2CoQ_{10}, as compared to other animal products. H_2CoQ_9 and CoQ_9 were also detected in small amounts in beef, pork, and chicken.

Discussion

Although CoQ_{10} supplements have not been required to meet the same rigorous product quality performance standards as medicines do, impaired product performance, such as failure to disintegrate in the gastrointestinal tract, might limit the absorption of CoQ_{10}. However, standardized guidelines and methods for assessing the quality control of dietary and health supplements have not been established in Japan. So, we applied the Japanese Pharmacopoeial Convention General Tests to evaluate the quality of CoQ_{10} supplements in this study.

Externally administered CoQ_{10} appears to be converted to H_2CoQ_{10} in the human body and then to act as an antioxidant against lipid peroxidation. In fact, a CoQ_{10} supplement given to a human increases the serum level of H_2CoQ_{10} as well as CoQ_{10} [*18*]. The enzymes responsible for the reduction of CoQ_{10} to H_2CoQ_{10} have been reported to be not only mitochondrial respiratory enzymes but also NADPH-CoQ reductase [*19–21*], NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) [*22*, *23*], thioredoxin reductase [*24*], and lipoamide dehydrogenase [*25*, *26*]. CoQ in the human body is thought to be provided by both dietary intake from foods and/or dietary supplements and biosynthesis *de novo.*

In this study, we tested two quality control tests, disintegration and content tests. The disintegration profile and content test are thought to be essential parameters relative to absorption and intake efficacy. Many commercially available CoQ_{10} supplements in Japan were well controlled and met the requirements specified by J.P.XV. However, a few products exhibited insufficient quality performance. In particular, the disintegration of many SCPs required more than 60 min. The gelatin shell of an SCP is somewhat thicker than that of an HCP shell and is plasticized by the addition of a polyol such as sorbitol or glycerol. The ratio of dry plasticizer to dry gelatin determines the hardness of the shell and may be varied to accommodate environmental conditions as well as the nature of the contents. Therefore, the disintegration time of SCPs may have a higher score than that of HCPs. In this study, we demonstrated that the quality

Table 4. H_2CoQs and CoQs contents in foods

Samples	CoQ_9	(μg/g wet weight)	CoQ_{10}	(μg/g wet weight)
	H_2CoQ_9 (%)*	Total CoQ_9**	H_2CoQ_{10} (%)*	Total CoQ_{10}**
Meats:				
Beef				
sirloin	0.1 ± 0.1 (17)	0.6 ± 0.1	1.8 ± 0.2 (6)	30.6 ± 1.4
tenderloin	ND***	ND	1.8 ± 0.2 (7)	26.5 ± 1.2
Pork				
sirloin	0.1 ± 0.1 (14)	0.7 ± 0.2	0.6 ± 0.2 (4)	14.0 ± 0.6
heart	ND	ND	6.7 ± 1.9 (6)	118.1 ± 12.2
liver	0.3 ± 0.1 (17)	1.8 ± 0.3	11.1 ± 1.6 (21)	54.0 ± 5.7
Chicken				
chest	ND	ND	0.9 ± 0.1 (5)	16.6 ± 1.6
heart	ND	ND	5.0 ± 1.2 (4)	123.2 ± 7.2
liver	0.2 ± 0.1 (12)	1.7 ± 0.4	18.9 ± 3.0 (16)	116.2 ± 6.2
Salmon	ND	ND	1.2 ± 0.3 (16)	7.6 ± 0.9
Eel	ND	ND	4.6 ± 1.6 (62)	7.4 ± 1.8
Squid	ND	ND	2.3 ± 0.2 (60)	3.8 ± 0.8
Vegetables:				
Chinese cabbage	ND	ND	0.4 ± 0.1 (15)	2.7 ± 0.4
Eggplant	ND	ND	0.2 ± 0.1 (9)	2.2 ± 0.6
Parsley	ND	ND	7.5 ± 0.8 (28)	26.4 ± 1.9

Data are expressed as means ± SD ($n = 5$).

* [H_2CoQn/total CoQn (sum of CoQn and H_2CoQn)] × 100 (%).

**Sum of CoQn and H_2CoQn.

***Not detected.

of the many supplement products containing CoQ_{10} in Japan varies considerably, and thus, we recommend introducing simple quality control tests for CoQ_{10} supplements.

Among CoQ_{10} supplements, in SCPs and LQs, H_2CoQ_{10} as well as CoQ_{10} was detected by the HPLC-ECD method. The results for experimentally formulated CoQ_{10} supplements suggested that H_2CoQ_{10} was produced by the interaction of CoQ_{10} with vitamins E and/or C. Moreover, although H_2CoQ_{10} is unstable when exposed to air, and can easily be oxidized into oxidized CoQ, chemically unstable H_2CoQ_{10} is thought to exist in the body to serve as an antioxidant. Recently, Yan *et al.* have reported [*27*] that dietary supplementation with H_2CoQ_{10} decreased the degree of senescence in middle-aged SAMP1 mice. H_2CoQ_{10} produced by the interaction of CoQ_{10} with vitamins E and/or C, therefore, may exert beneficial effects in the human body.

Many investigators have reported that exogenous CoQ_{10} exhibits useful health effects. If humans consume H_2CoQ_{10} as well as CoQ_{10} in meals every day, foods containing H_2CoQ_{10} and CoQ_{10} might have some physiological activities. Therefore, it is important to investigate the distribution of these substances in foods. Some investigators have reported the CoQ homologue contents in foods. However, they could not analyze reduced forms of CoQ homologues because of the HPLC-UV method and insufficient sensitivity. In our study, we measured the distribution of both CoQ_{10} and H_2CoQ_{10} in food items. The results clarify that humans consume not only CoQ_{10} but also H_2CoQ_{10} from foods. In other words, the present study confirms the human intake of H_2CoQ_{10} in daily foods and dietary CoQ_{10} supplements.

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