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Fortification of Dark Chocolate with Coenzyme Q10: A Novel Approach to Improve Its Nutritional and Health Benefits

Alireza Mohammadi[1], Mohsen Barzegar[1], Mohammad Ali Sahari[1], and Leila Kamali Rousta[2]

ABSTRACT

Coenzyme Q10 (CoQ10), a bioactive, fat-soluble compound with potent antioxidant and therapeutic properties, plays an essential role in cellular energy production and protection against oxidative stress. In this study, dark chocolate was selected as a promising vehicle for CoQ10 delivery. Standard dark chocolate manufacturing processes were employed, and different concentrations of CoQ10 (0.0, 0.2, 0.4, 0.6, and 0.8%) were added to the chocolate formulation. The products were stored at 25°C for 90 days, and the physicochemical properties and stability of formulated samples were evaluated during storage. The peroxide value (PV) increased with higher CoQ10 concentrations during storage, peaking at 6.74 meq peroxide/kg of oil (in the sample containing 0.8% of CoQ10) after 60 days, which was influenced by the dark chocolate's matrix ingredients. HPLC analysis indicated that CoQ10 content was 27.0% and 82.7% in samples containing 0.4% and 0.6% CoQ10, respectively. Colorimetric assessments showed visual changes, while sensory evaluation revealed no significant differences in texture, flavor, or overall acceptability between fortified and control samples, suggesting consumer acceptance at all CoQ10 levels. This study confirms the feasibility of utilizing dark chocolate as a delivery system for CoQ10, despite certain processing and storage limitations. Future research should prioritize enhancing CoQ10 stability, examining its long-term health benefits, and evaluating its effectiveness in promoting consumer health.

Key words: CoQ10 stability, Functional food, Health, HPLC, Oxidative stress.

INTRODUCTION

Non-communicable diseases (NCDs) such as cardiovascular diseases, diabetes mellitus, cancer, and neurological disorders rank among the top ten causes of mortality worldwide (WHO, 2024). Emphasizing a balanced diet and appropriate nutritional supplementation plays a crucial role in

[1] Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
[2] Zar Industrial and Research Group, Alborz, Islamic Republic of Iran.
*Corresponding author; e-mail: mbb@modares.ac.ir

reducing healthcare costs and minimizing adverse effects associated with more invasive treatments. This highlighting the need for continued research in food science and nutrition to address these emerging public health challenges (Arenas-Jal *et al.*, 2020). Micronutrients- vitamins and minerals- are vital for maintaining optimal physiological function and preventing disease. Since the human body has a limited capacity for the endogenous synthesis of many essential micronutrients, these nutrients must be obtained through diet or supplementation. Effective strategies to combat micronutrient deficiencies include oral supplementation, public health initiatives, and food-based interventions such as food fortification (Mannar & Wesley, 2025).

Coenzyme Q10, also known as ubiquinone or ubidecarenone, is the third most widely consumed dietary supplement after fish oil and multivitamins (Kapoor & Kapoor, 2013). Its biological activity is largely attributed to its antioxidant properties, with its reduced form, ubiquinol, acting as a hydrophobic antioxidant that protects lipids within cellular membranes and helps preserve mitochondrial proteins and DNA from oxidative damage (Aaseth *et al.*, 2021).

Numerous strategies have been developed to improve CoQ10 bioavailability, including the use of nanoemulsions, cyclodextrin complexes, and encapsulation with suitable coating materials (Barakat *et al.*, 2013; Xia *et al.*, 2006). *In vitro* studies have corroborated CoQ10's neuroprotective properties, demonstrating its ability to mitigate dysfunction and cell death associated with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's (Somayajulu *et al.*, 2005).

Several studies have demonstrated successful fortification approaches, such as encapsulating CoQ10 using the complex coacervation method in functional yogurt (Ahmadi *et al.*, 2015) and assessing the effects of thermal processing and simulated gastrointestinal digestion on its retention in processed meat products (Tobin *et al.*, 2014). The suggested daily intake of CoQ10 for healthy individuals ranges from 30 to 100 mg, with potential increases to 1200 mg in specific medical conditions (Pravst *et al.*, 2010). Dietary sources typically provide only 3- 6 mg daily (Kubo *et al.*, 2008).

Cocoa and chocolate products are widely consumed globally, with increasing per capita intake across diverse populations (Arunkumar & Jegadeeswari, 2019; Zugravu & Otelea, 2019). Given their widespread consumption, chocolate products presents an attractive matrix for bioactive compounds delivery. Previous research has successfully fortified chocolate with phytosterols, fish oil, peanut oil, cinnamon, and bulk sweeteners, while maintaining products stability and consumer

acceptance (Kiumarsi et al., 2017; Agibert & Lannes, 2018; Botelho et al., 2014; Dwijatmoko et al., 2016; Hadnađev et al., 2023).

Dark chocolate`s high lipid content offers an advantageous matrix for CoQ10 fortification, as it enhances the solubility of this lipophilic compound. The relatively mild thermal processing involved in chocolate production may better preserve CoQ10`s structural integrity compared to other food matrices. Additionally, the presence of dietary lipids facilitates CoQ10 absorption via lipid-mediated pathways in the gastrointestinal tract, potentially improving its bioavailability (Pravst *et al.*, 2010). The widespread consumer acceptance of chocolate makes it a promising vehicle for delivering CoQ10, provided that stability and sensory qualities are maintained throughout processing and storage.

The study aimed to evaluate the feasibility of fortifying dark chocolate with CoQ10 to develop a scalable, consumer-acceptable functional food. The objectives included assessing product quality and stability through physicochemical analyses- such as peroxide value (PV), acidity, and colorimetry- and evaluating consumer acceptance via sensory evaluation. Additionally, HPLC was employed to quantify CoQ10 content during a 90-day storage period at 25°C, thereby determining its stability within the chocolate matrix.

MATERIALS AND METHODS

Chemicals and Materials

Coenzyme Q10 (CoQ10, ≥99%) was purchased from Xi'an Natural Field Bio-Technique Co., Ltd. (CAS No: 303-98-0, China). Various chemicals and reagents were used, including n-hexane, ethanol, potassium iodide, potassium hydroxide, acetic acid glacial, chloroform, sodium thiosulfate, methanol, and ferric chloride ($FeCl_3$), all supplied by Merck Chemical Co. (Darmstadt, Germany). Acetonitrile, tetrahydrofuran (THF), and 2-propanol were obtained from Caledon Laboratories Ltd. (Canada). All chemicals were of analytical grade.

Dark Chocolates Preparation

Dark chocolate was industrially produced and fortified in a pilot at Zarkam Food Research and Industry Company (Alborz, Iran). The base formulation consisted of natural cocoa mass (46%), crystalline sugar (40%), cocoa butter (6% added initially, with an additional 6% incorporated during the final conching stage), cocoa powder (1.5%), and vanilla essential oil (0.03%). All

ingredients were mixed to obtain a uniform structure and refined to minimize particle size, achieving a smooth, grit-free texture. Five formulations were prepared: a control sample without CoQ10 (A) and four fortified variants containing CoQ10 at concentrations of 0.2% (B), 0.4% (C), 0.6% (D), and 0.8% (E). The refined chocolate was conched at 45°C for 3 h to enhance flavor and texture (Dand, 2011). During the final 30 min of conching, the remaining cocoa butter (6%)- in which CoQ10 was easily dissolved due to its lipophilicity- was added along with lecithin (0.43%) and polyglycerol polyricinoleate (PGPR) emulsifier (0.04%) to ensure a homogeneous mixture in 4 kg batches. Following conching, the chocolate was tempered to stabilize β_2 crystal formation, essential for achieving optimal texture, gloss, and resistance to fat bloom. Manual tempering was performed by rapidly cooling the chocolate from 44°C to 28°C (Castro-Alayo *et al.*, 2023). Subsequently, the chocolate was molded, cooled, and packaged in dark, air-impermeable aluminum foil to preserve stability and quality. Samples were stored at 25°C for 90 days. Tests were conducted each 30 days, with three replicates per analysis.

Acid Value Determination

The acid value- defined as the milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of sample oil- was determined according to the AOAC method (2023a) during 90 days storage. A 5.00 ± 0.05 g sample of extracted oil (using n-hexane) combined with 75 mL of neutralized ethanol in a 250 mL flask. The solution was titrated immediately with 0.1 M potassium hydroxide in the presence of 0.5 mL phenolphthalein indicator until a stable pink endpoint was observed.

This results were expressed as the acid value itself or as the percentage of free fatty acids (%FFA), expressed as percent of oleic acid.

Peroxide Value (PV) Determination

The PV quantifies all substances that react with potassium iodide (KI) under specified test conditions, expressed as milliequivalents of peroxide per kilogram of oil. These substances are typically recognized as peroxides or other oxidative by-products of fats. PV was determined using the AOAC method (2023b). A 5.00 ± 0.05 g of oil sample oil was mixed with 30 mL of an acetic acid-chloroform solution (3:2, v/v), followed by the addition of 0.5 mL of saturated potassium iodide (KI). After one minute, the peroxides in the sample reacted with KI in the acidic conditions.

The liberated iodine was titrated with 0.01M sodium thiosulfate solution in the presence of 0.5 mL of 1.0% starch indicator until the dark blue color disappearance. The peroxide value was calculated as follows:

$$\text{PV (milli equivalent peroxide/kg of oil)} = \frac{(S-B)\times M\times 1000}{W} \quad [1]$$

where B, S, M, and W are the volume of the used titrant (mL) for the blank, volume of the used titrant (mL) for the sample, molarity of the sodium thiosulfate solution, and sample weight (g), respectively.

Color Evaluation

The effect of CoQ10 fortification on chocolate color and appearance was evaluated using a HunterLab ColorFlex colorimeter (USA). Prior to measurement, the device was calibrated using standard black and white reference plate. Color parameters L^* (lightness), a^* (red-green coordinate), and b^* (yellow-blue coordinate), were recorded. The color differences (ΔE) among the samples was calculated as follows (Hadnađev et al., 2023):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad [2]$$

Interpretation of ΔE values: ΔE < 1, color difference not perceptible to the human eye; ΔE < 3, color difference barely perceptible; ΔE > 3, color difference clearly visible.

Determination of CoQ10 Content

The remaining CoQ10 content was quantified using a modified AOAC method (Lunetta & Roman, 2008). Extraction was performed using a solvent mixture of acetonitrile, tetrahydrofuran (THF), and water (55:40:5, v/v/v), which served as both the mobile phase and the extraction solvent. To oxidized the reduced form of CoQ10 (ubiquinol) into oxidize form (ubiquinone), 0.1% Fe(III) chloride solution in alcohol (90% ethanol, 5% methanol, and 5% 2-propanol) was added to the extract.

A CoQ10 standard (125.0 ± 0.1 mg, 99.2% purity) was accurately weighed and transferred to a 100 mL volumetric flask. Approximately 50 mL of the mobile phase was added, and the solution was sonicated for 30 min, then diluted to volume with the mobile phase to obtain 1.25 mg/mL stock solution.. A five-point calibration curve (linear range 0.025 to 0.125 mg/mL) was ploted.

Sample preparation: melted chocolate samples were weighed (15 g for samples B and C, 10 g for samples D and E) and transferred to 100 mL volumetric flasks. About 50 mL of the mobile

phase was added, and the mixtures were sonicated for 30 min. The solutions were then diluted to volume, filtered through DP 595090 filter paper (Hahnemühle Dassel, Germany), and 8 mL of each solution was transferred to another 100 mL volumetric flask. To each, 10 mL of Fe(III) chloride in alcohol was added and the volum adjusted with mobile phase. The mixtures were filtered through 0.45 µm PTFE syringe and injected into the HPLC system under the following conditions: HPLC System (Waters liquid chromatograph with 600E pump and 2487 Waters dual wavelength UV detector (USA)); column (Develosil ODS-UG-5 (4.6 mm × 250 mm, 5 µm, Japan)); mobile phase flow rate= 1.0 mL/min (isocratic); injection volume (20 µL); detection wavelength (λ= 275 nm); column temperature (25°C).

To validate analytical accuracy, known quantity of CoQ10 standard was spiked into the samples, and all procedures were repeated.

Sensory Evaluation

A total of 40 panelists (21 males and 19 females, aged 12-70 years) participated in the sensory evaluation. All participants confirmed that they were not on special diets and had no allergies to chocolate ingredients. The molded chocolate samples, wrapped in aluminum foil, were labeled with five-letter random codes. Before evaluating each sample, participants rinsed their mouths with warm water to remove residual flavors. A 5-point hedonic scale was used, where 5 represented "extremely good" and 1 represented "extremely poor". Participants rated each attribute- color, taste, texture, melting properties, oil extrusion, and overall acceptance- caaording to their preference (Dwijatmoko et al., 2016).

Statistical Analysis

Data were analyzed using a completely randomized design and one-way analysis of variance (ANOVA). Statistical analysis was performed using IBM SPSS Statistics 27 software, and graphs were generated in Microsoft Excel 2019. Tukey's test was applied to determine significant differences at a 95% confidence level ($p < 0.05$). Results are reported as mean ± standard deviation (n=3).

RESULTS AND DISCUSSION

Acidity Results

As presented in Table 1, formulations with higher concentrations of CoQ10 exhibited significantly lower acid values. Notably, formulation E (containing 0.8% CoQ10) recorded the lowest acidity at 1.76 ± 0.09 % (FFA) after 90 days, demonstrating a statistically significant difference ($p< 0.05$) compared to the control formulation.

According to international quality standards, the permissible limit for free fatty acid (FFA) content in chocolate and cocoa butter is generally below 1.75 % (expressed as oleic acid) (Codex Alimentarius Commission, 2022). FFA values exceeding this threshold typically indicate hydrolytic rancidity and lipid degradation, which can adversely affect both the sensory attributes and storage stability of chocolate products. As presented in Table 1, all CoQ10-fortified formulations, including the control, exhibited FFA levels within or close to the acceptable range throughout the 90-day storage period. Although the control sample (A) showed a significant increase in acidity, reaching 2.72 ± 0.13 %, slightly above the recommended limit, formulations containing CoQ10- particularly samples D and E- maintained considerably lower FFA values (1.91 ± 0.04% and 1.76 ± 0.09%, respectively) after 90 days of storage. These results suggest that CoQ10 incorporation effectively mitigates lipid hydrolysis and oxidation deterioration, thereby preserving the chemical integrity of the lipid fraction during storage. Moreover, the lower FFA levels observed in CoQ10-fortified samples suggest enhanced resistance to rancidity, likely duo to the antioxidant properties of CoQ10. Overall, CoQ10 fortification appears to be a promising strategy for improving both the nutritional value and physicochemical stability of chocolate products, extending their shelf life while maintaining compliance with international quality standards.

The observed reduction in acidity is likely due to the potent antioxidant properties of CoQ10, which suppress lipid oxidation and consequently limit the formation of free fatty acids. In lipid-rich matrices such as chocolate, lipid oxidation results in the formation of acidic by-products, including free fatty acids. CoQ10 seems to mitigate these reactions by neutralizing free radicals and interacting with fatty acids through its functional groups. Supporting this, a meta-analysis by Zhai *et al.* (2017) indicated that CoQ10 significantly decreases malondialdehyde levels- a biomarker of lipid peroxidation associated with increased acidity- in various food systems. Table 1 illustrates the trends in acidity over the storage period, revealing that formulations E and D, which contain higher concentrations of CoQ10, exhibited a decreasing trend in acidity. This trend

is consistent with previous studies demonstrating that antioxidant-rich matrices can delay the onset of lipid oxidation (Littarru & Tiano, 2007). Conversely, a study by Rossini *et al.* (2011) on white chocolate reported a significant increase in acidity over a 10-month storage period across three treatments, including two antioxidant agents (casein hydrolysate and Grindox 562) and a control, indicating variability depending on formation and antioxidant type.

Table1. Acidity of CoQ10-fortified chocolates during 90 days storage (% free fatty acid).

Sample	Storage time (day)		
	30	60	90
A*	2.21±0.05^{aB}	2.21± 0.02^{aB}	2.72±0.13^{aA}
B	2.29±0.03^{aA}	2.02± 0.02^{cB}	1.88±0.02^{bB}
C	2.09±0.05^{bA}	2.05± 0.01^{cA}	1.78±0.08^{bB}
D	2.23±0.02^{aA}	1.96± 0.01^{dB}	1.91±0.04^{bB}
E	2.27±0.01^{aA}	2.11± 0.01^{bA}	1.76±0.09^{bB}

*A: control without CoQ10; B: sample containing 0.20% CoQ10; C: sample containing 0.40% CoQ10; D: sample containing 0.60% CoQ10; E: sample containing 0.80% CoQ10. Values are means ± standard deviation of three replicates. Different higher case letters within the same row and different lowercase letters within the same column are significantly different ($p< 0.05$).

Peroxide Value (PV) Results

In this study, significant differences in oxidative stability among various formulations were observed (Table 2). The control treatment (A) showed the lowest PV throughout the storage period, reaching 1.79 ± 0.06 meq peroxide/kg of oil after 90 days. In contrast, samples fortified with CoQ10 displayed progressively higher PVs correlating with increased CoQ10 content. Specifically, formulation E, containing 0.8% CoQ10, recorded the highest PV of 6.74 meq peroxide/kg of oil; however, all values remained below the Codex Alimentarius Commission`s recommended limit of 10 meq peroxide/kg of oil (Codex Alimentarius Commission, 2023)

The elevated PVs observed in the CoQ10-fortified samples suggest a potential pro-oxidant effect of CoQ10. The isoprenoid side chain of CoQ10, which is rich in double bonds, inherently predisposes it to oxidation. This characteristic may facilitate its role as a scavenger antioxidant, potentially protecting the lipid matrix of chocolate from oxidative degradation (Valgimigli, 2023). While multiple double bonds in CoQ10 might theoretically compete with lipids for oxidation, current evidence indicates that CoQ10 primarily acts as an antioxidant within the chocolate matrix. Its ability to react with free radicals may mitigate extensive lipid peroxidation, thereby preserving the integrity of the fat structure in chocolate.

Table 2. Peroxide value of CoQ10-fortified chocolates during 90 days of storage (meq peroxide/kg oil).

	Storage time (day)		
Sample	**30**	**60**	**90**
A*	0.70 ± 0.01^{eB}	0.89 ± 0.01^{eB}	1.79 ± 0.06^{eA}
B	1.84 ± 0.06^{dB}	1.52 ± 0.01^{dC}	2.37 ± 0.02^{dA}
C	3.47 ± 0.08^{cA}	2.25 ± 0.09^{cC}	2.56 ± 0.01^{cB}
D	4.59 ± 0.09^{bA}	3.13 ± 0.16^{bC}	3.43 ± 0.02^{bB}
E	6.31 ± 0.03^{aB}	6.74 ± 0.14^{aA}	5.80 ± 0.05^{aC}

*For abbreviations, see Table 1 footnote. Values are means ± standard deviation of three replicates. Different higher case letters within the same row and different lowercase letters within the same column are significantly different ($p<0.05$).

Supporting this hypothesis, the thiobarbituric acid-reactive substances (TBARS) assay conducted on sample E at day 90 revealed a malondialdehyde (MDA) content of 0.026 mg MDA/kg of oil, accompanied by a PV of 5.80 ± 0.05 meq peroxide/kg of oil. Low MDA levels, reflected by minimal TBARS values, are widely recognized in the literature as indicative of limited secondary lipid oxidation (Aguilar Diaz De Leon & Borges, 2020). In food systems, the efficacy of antioxidant treatments is often validated by their capacity to inhibit increases TBARS value (Li *et al.*, 2023). Therefore, the observed MDA concentration of 0.026 mg/kg of oil suggests that CoQ10 effectively mitigated secondary lipid oxidation under the storage conditions employed, despite the concurrent increase in PV, which primarily reflects primary oxidation products. This indicates that CoQ10 may exert a protective effect against the progression of lipid oxidation beyond the initial peroxidation stage.

Comparable studies have also investigated the effect of various fortifying agents on the oxidative stability of chocolate. For instance, Rossini *et al.* (2011) examined the impact of two different antioxidants on the physicochemical properties of white chocolate over its shelf life. Their findings align with our results, showing increased PVs in antioxidant-treated samples. Specifically, they reported a maximum PV of approximately 8.1 meq peroxide/kg of oil in samples stored at 28°C with Grindox antioxidant after 10 months. Similarly, Vercet (2003) reported a PV of 9.5 meq peroxide/kg of oil after 15 months of storage, further emphasizing the influence of antioxidant type and storage conditions on oxidative stability.

Other comparable findings have been reported in chocolate fortification studies. Quispe-Sánchez *et al.* (2023) observed that fortifying dark chocolate with essential oils (*Cymbopogon citratus*, *Pimpinella anisum*, and *Minthostachys mollis*) resulted in a concentration-dependent increase in peroxide value during storage, particularly at higher inclusion levels.

In summary, while the incorporation of CoQ10 into dark chocolate formulations resulted in higher PVs, its overall antioxidant capacity appears to play a crucial role in protecting the lipid fraction from oxidative degradation. These findings highlight the complex role of CoQ10, which may exhibit pro-oxidant tendencies under certain conditions but predominantly functions as an antioxidant within the product matrix. Further research should focus on elucidating the mechanisms underlying CoQ10`s dual role in oxidative processes, aiming to optimize formulations for enhanced shelf life and quality preservation in chocolate and other lipid-based foods.

Colorimetric Results

Appearance and color are crucial parameters influencing consumer acceptability of food products. In this study, colorimetric tests were conducted to investigate the impact of CoQ10 fortification on the color parameters and total color difference (ΔE) of formulated dark chocolates. The coloration of chocolate is influenced by multiple factors, including production processes, formulation composition, and the crystalline structure of the fat used (Saputro et al., 2017). Measurements were taken over a 90-day storage period, and ΔE was calculated to quantify perceptible color deviations induced by fortification and storage conditions (see Table. 3).

The results demonstrated variations in the CIELAB color parameters- L^* (lightness), a^* (red-green), and b^* (yellow-blue)- corresponding to different CoQ10 concentrations. Both CoQ10 level and storage duration had statistically significant effects ($p< 0.05$) on these parameters, suggesting that CoQ10 addition can alter surface coloration, likely due to its inherent coloration and potential interactions with the cocoa matrix components.

Throughout the 90-day storage period, changes in color parameters were observed. The control sample maintained relative stability, whereas CoQ10-fortified chocolates exhibited incremental changes in L^*, a^* and b^* values. These findings imply that the color stability of fortified chocolates may be compromised over time, potentially due to oxidative processes or matrix interactions.

Measurement accuracy was carefully considered; potential sources of error include instrument calibration drift, surface irregularities of samples, and environmental factors such as ambient light and temperature fluctuations during storage. These variables can introduce minor variability in color measurements.

The observed increase in ΔE values over time, particularly at higher CoQ10concentrations, suggests that oxidation, degradation processes, and interactions between CoQ10 and other

306 chocolate ingredients may adversely affect color stability. Although most ΔE values remained
307 below the threshold of human perceptibility, sensory evaluations corroborated these findings,
308 indicating minimal perceptible color changes.

309 Comparative studies highlight varying impacts of fortification on chocolate color stability. For
310 instance, Botelho *et al.* (2014) reported that phytosterols-fortified chocolates maintained color
311 stability up to 90 days, after which surface bloom caused lightening. Similarly, Rajabi and
312 Sedaghati (2024) found that chocolates fortified with encapsulated herbal extracts exhibited a
313 significant ($p< 0.05$) increase in lightness shortly after production, which was deemed acceptable
314 by sensory panels.

315 In the present study, the dark coloration of chocolate likely masked the orange hue of CoQ10,
316 especially given the low concentration and small particle size of the CoQ10, minimizing visually
317 perceptible color alterations.

318

319 **Sensory Evaluation Results**

320 The sensory evaluation of CoQ10-fortified dark chocolates provided valuable insights into
321 consumer acceptability. Across CoQ10 concentrations ranging from 0.0 to 0.8%, no statistically
322 significant differences ($p< 0.05$) were observed in sensory attributes such as color, taste, texture,
323 melting properties, oil extrusion (surface oil separation), and overall acceptance. Tukey's post hoc
324 tests confirmed that even at the highest fortification level, sensory qualities remained comparable
325 to control samples (Fig. 1).

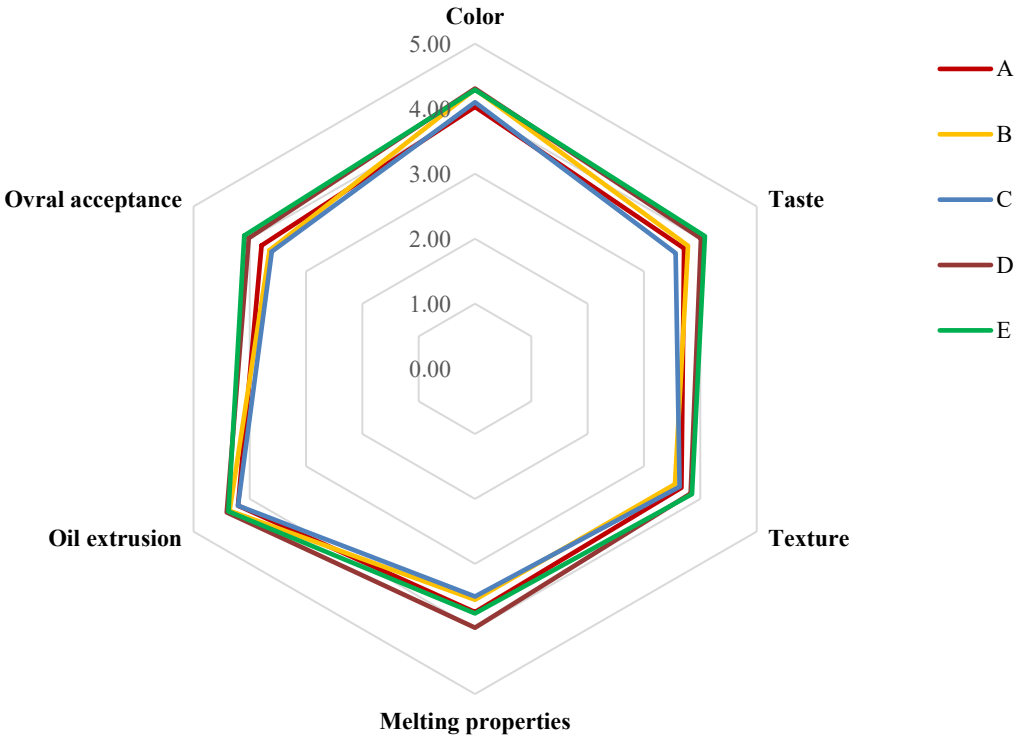


Figure 1. Sensory evaluation of CoQ10-fortified dark chocolates. A: control without CoQ10; B: sample containing 0.20% CoQ10; C: sample containing 0.40% CoQ10; D: sample containing 0.60% CoQ10; E: sample containing 0.80% CoQ10. Values are mean ± standard deviation.

Although CoQ10`s intrinsic orange coloration initially raised concerns about potential visual alterations, instrumental colorimetric data (Table 3) and consumer ratings indicated negligible differences. This likely results from the deep brown color of the chocolate matrix and effective dispersion of CoQ10 particles within the product. Similar findings were reported by Botelho *et al.* (2014), who observed that phytosterols fortification did not affect chocolate appearance.

Taste scores remained high, with no significant differences ($p < 0.05$). Contrasting findings from Rajabi and Sedaghati (2024) indicated that consumers could detect differences between control and fortified samples, highlighting variability based on formulation and detection thresholds. Other research (Hadnađev *et al.*, 2023) noted consumer preference for control chocolates over those fortified with fish oil, which can impart off-flavors.

Overall, the sensory data suggest that dark chocolate is an effective delivery matrix for CoQ10, maintaining sensory quality and consumer acceptability. These findings support further exploration of bioactive compounds incorporating into food products, with potential for functional food development.

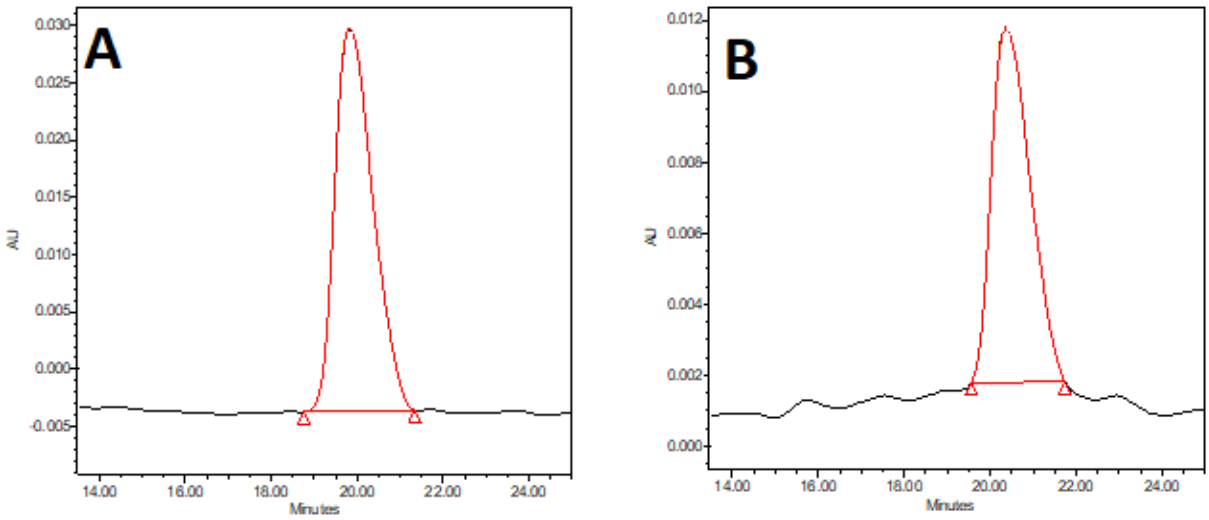
Table 3 Color parameters (L^*, a^*, b^*, and ΔE) of CoQ10-fortified chocolates during 90 days storage.

	Storage time (day)											
	30				60				90			
Samples	**L^***	**a^***	**b^***	**ΔE**	**L^***	**a^***	**b^***	**ΔE**	**L^***	**a^***	**b^***	**ΔE**
A*	20.01±0.12^{aB}	14.80±0.09 aA	19.98±0.34 aA	0.00	21.69 ± 0.01^{bA}	13.54±0.23^{b} B	16.51± 0.10^{cB}	0.00	18.92±0.43^{bC}	13.74±0.09 cB	17.13±1.41^{bB}	0
B	20.14±0.04^{aB}	14.54±0.06 abA	20.59±0.06 aA	0.68	20.57 ± 0.06^{dA}	14.25±0.11^{a} B	19.70± 0.14^{aB}	3.19	20.15±0.04^{aB}	14.55±0.07 bA	18.88±0.19^{abC}	2.28
C	20.20±0.11^{aB}	14.60±0.09 abA	20.07±0.15 aA	0.30	20.48 ± 0.04^{dA}	14.06±0.08^{a} B	17.42± 0.53^{bB}	1.6	19.85±0.05^{aC}	14.65±0.07 abA	19.19±0.31^{aA}	2.43
D	20.16±0.08^{aB}	14.35±0.29 bA	19.83±0.71 aA	0.51	21.80 ± 0.02^{aA}	13.41±0.04^{b} B	17.23± 0.29^{bcB}	0.73	19.72±0.04^{aC}	14.70±0.05 abA	20.21±0.10^{aA}	3.32
E	20.08±0.05^{aB}	14.76±0.03 aA	20.69±0.17 aA	0.71	21.33 ± 0.04^{cA}	13.54±0.07^{b} B	17.50± 0.11^{bC}	1.05	20.05±0.04^{aB}	14.74±0.06 aA	20.06±0.30^{aB}	3.27

*For abbreviations see Table 1 footnote. Values are mean ± standard deviation of three replicates. Different higher case letters within the same row and different lowercase letters within the same column are significantly different ($p< 0.05$).

363 **CoQ10 Content of Produced Samples**

364 The residual CoQ10 content was quantified using RP-HPLC-UV. Representative
365 chromatograms are shown in Fig. 2A for the standard solution and Fig. 2B for CoQ10 extracted
366 from the fortified chocolates. The calibration curve exhibited excellent linearity within the tested
367 concentration range, with an R^2 value of 0.9997 and the equation Y= 19,960X + 42,252, indicating
368 reliable quantification. Initial analyses indicated that residual CoQ10 content in the samples ranged
369 from approximately 54.86% to 82.66% of the initially added amount, suggesting variable retention
370 efficiency across samples. Over the storage period, as depicted in Fig. 3, there was a gradual
371 decrease in CoQ10 levels, which could be attributed to several factors. Potential mechanisms
372 include interactions between CoQ10 and components of the chocolate matrix- such as lipids,
373 proteins, and polyphenols- that may influence its stability and bioavailability. Additionally,
374 incomplete extraction of CoQ10 from the matrix and possible oxidative degradation may
375 contribute to the observed decrease.



376 **Figure 2.** Chromatogram of CoQ10 standard (0.10 mg/ml); B: Chromatogram of extracted CoQ10
377 from produced dark chocolate (0.8% CoQ10).
378
379 Over a 90-day storage period, CoQ10 levels exhibited a gradually decline, as illustrated in Fig.
380 3. For example, treatment E retained approximately 49% CoQ10 after 30 days, decreasing to 37%
381 at 90 days. The observed trend aligns with previous studies indicating that CoQ10 stability is
382 influenced by formulation composition, the presence of antioxidants, and storage conditions.

Temova Rakuša et al. (2021) demonstrated that the reduced (ubiquinol) and oxidized (ubiquinone) forms of CoQ10 exhibit different stability profiles, with degradation rates varying depending on the carrier systems- being more pronounced in capsule formulations. Similarly, Tobin *et al.* (2014) reported a 21% reduction in CoQ10 content following cooking processes in meat products, highlighting the influence of thermal and processing conditions on CoQ10 retention. Pravst *et al.* (2009) found stable CoQ10 levels in fortified dairy products during storage, although the hydrophobicity nature of CoQ10 poses challenges for water-base formulations. These findings underscore the critical importance of formulation strategies aimed at enhancing CoQ10 stability. Incorporating antioxidants into the formulation or employing encapsulation techniques can mitigate oxidative degradation and improve shelf life (Domínguez *et al.*, 2021; Sereti *et al.*, 2025). For instance, microencapsulation using lipid-based carriers or antioxidant coatings has been shown to significantly increase CoQ10 stability during storage.

In addition, the general observations showed that when compounds are added under controlled condition, phenolic compounds, vitamins, or other bioactive fortificants remain stable. It has been also proven that the addition of heat-sensitive compounds such as phytosterol esters during tempering substantially increases total phenolics and antioxidant capacity, while high temperatures can degrate such compounds and accelerate oxidation in unsaturated bioactive compounds (Topka *et al.*, 2024). Similarly, adding CoQ10 at the final stage of conching, likely minimized thermal stress, preserving its integrity.

Despite the observed degradation, the residual CoQ10 levels in the fortified dark chocolate remained within the effective intake range of 30-100 mg/day (Pravst *et al.*, 2010). This suggests that fortified dark chocolate can serve as a viable functional carrier for CoQ10, providing a convenient and palatable matrix. Future research should focus on the impact of varying storage conditions, including temperature and light exposure, to enhance CoQ10 retention and bioavailability.

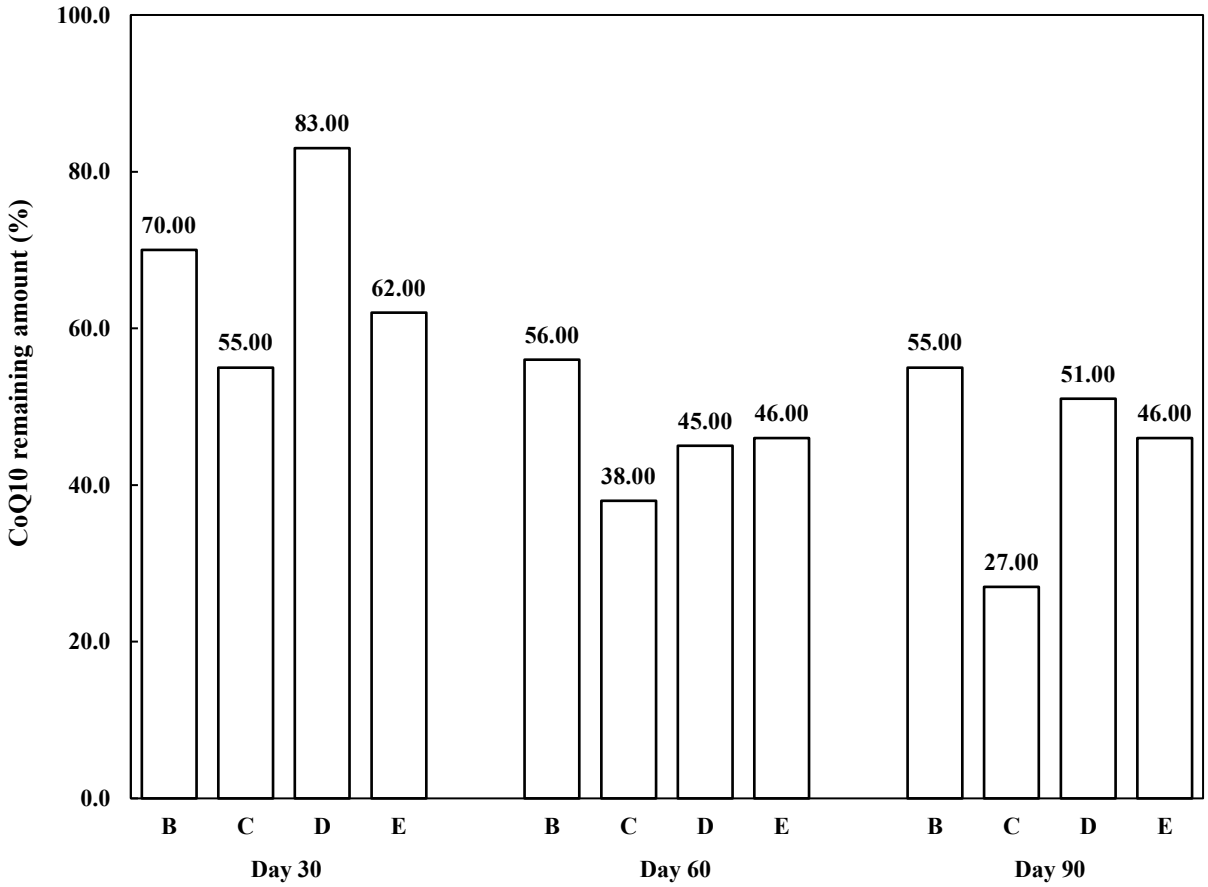


Figure 3. 90-day stability study of CoQ10-fortified dark chocolates. A: control without CoQ10; B: sample containing 0.20% CoQ10; C: sample containing 0.40% CoQ10; D: sample containing 0.60% CoQ10; E: sample containing 0.80% CoQ10. Values are mean ± standard deviation of three replicates.

CONCLUSIONS

This study demonstrated that CoQ10 fortification and storage duration markedly influence the physicochemical and sensory properties of dark chocolate. Fortified chocolates showed a reduction in acidity and an increase in peroxide values over time, indicative of oxidative processes potentially catalyzed by CoQ10. Nonetheless, CoQ10 retention remained acceptable after 90 days, confirming the feasibility of using dark chocolate as a delivery matrix for this bioactive compound. Sensory evaluation revealed no significant differences in appearance, aroma, texture, or overall acceptability between control and fortified samples, indicating that CoQ10 incorporation does not compromise consumer perception. Based on the findings, CoQ10 concentrations of 0.6% and 0.8% were identified as optimal for dark chocolate fortification. The 0.6% formulation exhibited better

CoQ10 recovery and good oxidative stability, while the 0.8% formulation showed the lowest acidity (1.76% FFA) and maintained excellent sensory acceptability. Therefore, fortification within this range effectively balances CoQ10 stability, retention, and overall product quality, confirming its suitability for functional chocolate development. To improve stability and preserve functional benefits, future research should explore strategies such as natural antioxidants addition or advanced encapsulation techniques. Overall, the results support the potential of CoQ10-fortified dark chocolate as a functional food, offering a convenient and palatable means of delivering nutraceutical benefits. Further studies assessing long-term health impacts and optimizing formulation stability are recommended to maximize the efficacy and consumer acceptance of such products.

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غنی‌سازی شکلات با کوآنزیم کیوتن، رویکردی نوین برای بهبود مزایا و خواص تغذیه‌ای و سلامتی آن

علیرضا محمدی، محسن برزگر، محمدعلی سحری، و لیلا کمالی روستا

چکیده

کوآنزیم کیوتن (CoQ10) ترکیبی زیست‌فعال و محلول در چربی با خواص ضداکسایشی و درمانی موثر است که نقش اساسی در تولید انرژی سلولی و محافظت در برابر تنش اکسایشی ایفا می‌کند. در این پژوهش، شکلات تلخ به‌عنوان یک حامل برای انتقال CoQ10 انتخاب شد. برای تولید شکلات، از فرآیندهای استاندارد صنعتی استفاده گردید و نمونه‌ها به‌مدت ۹۰ روز در دمای ۲۵ درجه سانتی‌گراد نگهداری شدند. طی این مدت ویژگی‌های فیزیکوشیمیایی و پایداری کوآنزیم کیوتن مورد ارزیابی قرار گرفت. نتایج نشان داد مقدار اندیس پراکسید (PV) با افزایش غلظت CoQ10 در طول نگهداری افزایش یافته و در روز شصتم در نمونه دارای بیشترین میزان CoQ10 به مقدار 6/74 میلی‌اکی‌والان پراکسید بر کیلوگرم روغن رسید که به نظر می‌رسد این امر تحت تأثیر برهم‌کنش‌های ترکیبات شکلات بوده است. تعیین مقدار CoQ10 با HPLC نشان داد که درصد بازیابی آن در نمونه‌های غنی‌شده با 0/4 و 0/6 درصد کوآنزیم کیوتن به‌ترتیب 27/0 و 82/7 درصد بوده است. برای بررسی تغییرات ظاهری احتمالی، آزمون‌های رنگ سنجی انجام شد. همچنین، ارزیابی حسی با استفاده از مقیاس پنج امتیازی هدرونیک نشان داد تفاوت معنی‌داری از نظر بافت، طعم یا پذیرش کلی بین نمونه‌های غنی‌شده و شاهد وجود ندارد که نشان‌دهنده پذیرش مصرف‌کننده در تمامی سطوح CoQ10 است. به‌عنوان مثال، نمونه حاوی 0/8 درصد CoQ10 امتیاز بالاتری در پذیرش کلی (4/10)، طعم (4/08) و بافت (3/85) نسبت به نمونه شاهد به‌دست آورد که این تفاوت‌ها از لحاظ آماری معنادار نبودند. این مطالعه قابلیت استفاده از شکلات تلخ به‌عنوان یک سامانه موثر جهت انتقال CoQ10 را با در نظر گرفتن محدودیت‌های تولید و نگهداری تأیید می‌کند. نتایج این پژوهش همسو با روند رو به افزایش تولید غذاهای فراسودمند بوده و رویکرد ارزشمندی برای بهینه‌سازی فرمولاسیون در اختیار توسعه‌دهندگان محصولات غذایی قرار می‌دهد. پیشنهاد می‌شود تحقیقات آینده بر بهبود پایداری CoQ10، بررسی مزایای سلامت‌بخش بلندمدت آن، و ارزیابی نقش آن در ارتقای سلامت مصرف‌کنندگان متمرکز شوند.