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Formulation and evaluation of a natural dietary supplement from quail egg and arugula leaves

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ABSTRACT

This study focused on formulating a natural dietary supplement based on a combination of freeze-dried quail egg powder and dried arugula (Eruca sativa) leaves. The integration of animal- and plant-derived components produced a nutritionally dense product enriched with essential nutrients and bioactive substances. Compositional analysis revealed that the supplement is a substantial source of high-quality protein (30 g/100 g), lipids (19 g/100 g), and carbohydrates (16 g/100 g). Furthermore, it provides appreciable levels of key minerals, including calcium (210 mg), magnesium (54 mg), and iron (5.7 mg). The presence of bioactive compounds was confirmed by the high contents of total phenolics (1500 mg GAE), flavonoids (500 mg QE), and vitamin E (160 mg/100 g), supporting its functional and antioxidant potential.

A short-term human intervention was conducted in which participants consumed three capsules (1.5 g) of the supplement daily. Biochemical assessments demonstrated that serum uric acid (4.41–4.58 mg/dL) and blood glucose levels (82.59–85.59 mg/dL) remained within normal ranges throughout the study. A modest enhancement in total antioxidant capacity (1.02–1.15 µmol TE/g) was observed, whereas malondialdehyde concentrations showed minimal variation (3.21–3.27 nmol/mL). These limited physiological changes are likely attributable to the low intake level, brief supplementation period, and inter-individual variability. In addition, chemical stability evaluation indicated favorable storage properties, as evidenced by low moisture content (3.40%), near-neutral pH (6.42), and a very low peroxide value (1.18 meq O_2/kg fat), reflecting minimal lipid oxidation. Collectively, these results suggest that the developed supplement is chemically stable, safe for consumption, and may provide moderate nutritional and antioxidant benefits in humans.

1. Introduction

Nutrition is a key determinant of public health and plays a major role in lowering the risk of chronic diseases. With the continuous global increase in the consumption of dietary supplements, scientific attention has shifted toward natural alternatives that combine safety with meaningful functional benefits [1]. Within this framework, there is a growing demand to investigate local and unconventional food resources that can be developed into dietary supplements capable of supporting health without reliance on synthetic compounds. Recent research has emphasized the importance of plant-based proteins and phytochemicals as sources of bioactive peptides and molecules with antioxidant and anti-inflammatory activities. This perspective is further supported by recent reviews highlighting egg-derived peptides and their promising applications in functional food systems [1,2,3].

Quail eggs are characterized by a concentrated nutritional profile rich in high-quality proteins, lipids, and extractable bioactive peptides. Experimental and analytical studies have demonstrated that oils and extracts obtained from quail egg yolk exhibit significant antioxidant and anti-inflammatory properties in laboratory and animal models. At the molecular level, additional investigations have reported their ability to modulate the expression of genes involved in antioxidant defense mechanisms [4,5]. Studies focused on egg composition further indicate that variations in feeding regimes and processing conditions can enhance the nutritional and functional properties of quail eggs, supporting their potential use in natural dietary supplement formulations.

From a botanical standpoint, *Eruca sativa* leaves contain substantial levels of glucosinolates, phenolic compounds, flavonoids, and essential vitamins, all of which have shown antioxidant and anti-inflammatory effects across chemical, physiological, and biomimetic systems. Recent studies have explored the practical applications and protective biological effects of *Eruca sativa*, along with comparative analyses revealing differences in phenolic content and antioxidant capacity under diverse environmental and varietal conditions [6-8]. Moreover, applied research has indicated that suitable processing and preservation techniques can effectively maintain the stability and antioxidant activity of *Eruca sativa* extracts after formulation [9,10].

A careful review of the available scientific literature reveals a growing body of evidence supporting the functional and bioactive properties of both animal- and plant-derived ingredients used in dietary

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supplementation. However, despite extensive individual investigations into quail eggs and *Eruca sativa*, limited attention has been given to their combined application within a single functional formulation. Given the complementary nature of these ingredients where quail eggs provide bioactive proteins, peptides, and lipids, and *Eruca sativa* contributes a diverse range of antioxidants and phytochemicals, this synergy represents a promising opportunity to translate existing scientific knowledge into practical product development. Accordingly, the present study aims to formulate and evaluate a natural functional supplement derived from these resources, examining its chemical composition, functional characteristics, antioxidant potential, and overall safety as a dietary supplement.

2. Materials and methods

This study was approved by the Ethics Committee of the University of Basrah, Approval No. 73AP, in March 19, 2025.

2.1. Method of preparing freeze-dried quail egg powder

After obtaining fresh quail eggs from the production fields at the College of Agriculture, University of Basrah, the eggs were prepared for freeze-drying according to the method mentioned by [١١], which includes washing the eggs well, then breaking them and mixing the yolk and white until the mixture is homogeneous. Then, it is gently heated (pasteurized) at 60–63°C for 3–5 min to reduce the microbial load, followed by rapid cooling to below 10°C. After that, the mixture is poured onto flat trays and rapidly frozen at -40 to -80°C until excellent. Then, it is transferred to a freeze dryer, where the primary drying stage takes place under vacuum and at a low shelf temperature (about -20°C) to defrost by sublimation, followed by a secondary drying stage in which the temperature is gradually raised to 20–30°C to remove any remaining moisture. Once the drying process is finished, the material is milled into a fine powder, then sealed in airtight, moisture-resistant containers and kept in a cool, dry environment for storage.

2.2. Method of preparing watercress leaf powder

Fresh watercress leaves were sourced from the farms of the Field Crops Department at the College of Agriculture, University of Basrah. The leaves were carefully washed to eliminate surface impurities and then left to drain in order to reduce excess moisture. In accordance with the method described in [12], the leaves were converted into a finely powdered dried material. Briefly, the leaves were spread as a thin layer within an air-drying oven and subjected to temperatures ranging from 50 to 60 °C to eliminate moisture while preserving phenolic compounds and antioxidant activity. Drying was continued until a constant weight was achieved, confirming complete dehydration. After drying, the leaves were ground using a high-speed mill to obtain a homogeneous powder. This powder was then sifted through a fine sieve (60–80 mesh) to standardize particle size. Finally, the powder was stored in moisture-proof and light-proof containers to preserve the active compounds.

2.3. Preparation of the dietary supplement

The dietary supplement powder was prepared from dried quail egg and dried watercress leaf powder according to the methods described in [١١,12]. 250 g of each powder was weighed, and 100 g of microcrystalline cellulose (MCC) was added as a carrier, along with 5 g of silica as an anti-caking agent and 5 g of vitamin E powder as an antioxidant, for a total of 610 g. Table 1 shows the components of the dietary supplement made from natural materials, along with their weights and proportions. All ingredients were mixed in a dry blender for 10–15 min until homogeneous. Silica and vitamin E were added during the last 2–3 min. After measuring the powder density, it was packaged into volume 0 HPMC vegetable capsules with a fill weight of 500 mg per capsule, yielding 1,220 capsules. The capsules were stored in airtight containers with a desiccant bag in a cool, dry place, away from light, to maintain the stability of the active compounds. Fig. 1. shows the final packaging of the natural food supplement made from quail eggs and Arugula leaves.

Table 1. Ingredients of the dietary supplement formulation and their weights

Ingredient	Weight (g)	Total Weight (%)
Freeze-dried quail egg powder	250	41.00%
Dried arugula leaf powder	250	41.00%
Microcrystalline Cellulose (MCC)	100	16.40%
Food-grade silica	5	0.80%
Vitamin E powder	5	0.80%
Total	610	100%

Fig. 1. Final packaging of the natural food supplement, from quail eggs and Arugula leaves

2.4. Analysis of nutritional composition of the dietary supplement

a. Protein: The protein content was determined using the Kjeldahl method, which measures total nitrogen and converts it to protein using a conversion factor, following the procedures recommended by [13].
b. Total Fat: Total fat was measured using the Soxhlet extraction method with petroleum ether as the solvent.
c. Carbohydrates: Carbohydrate content was calculated by difference, subtracting the sum of protein, fat, ash, and moisture from 100 [14].
d. Moisture: Moisture content was determined by oven-drying at 105°C until a constant weight was reached.
e. Ash and minerals: The ash content was quantified by incinerating the samples at 550 °C until complete mineralization was achieved. Major mineral elements, including calcium, magnesium, potassium, sodium, iron, and zinc, were subsequently determined following acid digestion using atomic absorption spectroscopy [13,15].
f. Bioactive compounds: Total phenolic content was evaluated using the Folin–Chiocalteu assay and expressed as gallic acid equivalents (GAE). Total flavonoid content was assessed by the aluminum chloride colorimetric method and reported as quercetin equivalents (QE), providing an estimation of the antioxidant capacity of the plant-derived components [16,17].
g. Final nutritional profile: The combined results of these analyses enabled the establishment of a comprehensive nutritional profile of the formulated food supplement powder, allowing for the estimation of nutrient composition per 100 g of product as well as per individual capsule.

2.3. Chemical testing

The chemical characteristics of the formulated food supplement were assessed using internationally recognized analytical parameters to evaluate its quality and stability as a dried food product, as outlined below:

a. Moisture content measurement: Moisture content was determined as a critical parameter for controlling water activity within the product, thereby reducing the risk of oxidative reactions and chemical deterioration during storage [18,19].
b. pH measurement: The pH value was measured to characterize the acidity of the powder, as this factor plays a key role in the stability of bioactive compounds throughout storage [20].
c. Peroxide value (PV): Peroxide value was analyzed to estimate the extent of primary lipid oxidation in the product, following established and standardized methods for the evaluation of fats and dried food materials [21,22].

2.6. Microbiological testing

The microbiological quality of the dietary supplement was evaluated as a critical indicator of product safety and

overall quality in dried natural formulations. Standard microbiological analyses were performed, including the determination of the total aerobic microbial count (TAMC) According to the procedures mentioned [23], as well as the total yeast and mold count (TYMC) conducted according to the method reported by [24].

2.7. Some biochemical blood tests

The study was carried out on fifty male students enrolled in the College of Physical Education and Sports Sciences at the University of Basrah. Prior to sample collection, all participants were instructed to fast for at least 8 h and to abstain from any physical activity. At 10:00 AM, an initial blood sample (5 mL) was collected from the right arm of each participant. Immediately thereafter, participants ingested three capsules of the natural dietary supplement with 250 mL of water in order to evaluate the acute physiological response.

All participants were clinically healthy, with heights ranging from 170 to 175 cm and body weights between 65 and 68 kg, resulting in body mass index (BMI) values within the normal range. None of the participants had a history of chronic illness or metabolic disorders, nor were they consuming dietary supplements or medications that could influence the assessed biochemical parameters. To ensure consistency, all participants avoided physical exertion prior to blood sampling, and all experimental procedures were performed under controlled laboratory conditions.

Three hours following supplement consumption, a second blood sample (5 mL) was collected from the left arm of each participant to allow comparison between pre- and post-supplementation values. This protocol was repeated once weekly every Thursday for four consecutive weeks. Serum samples were separated by centrifugation, and biochemical analyses, including total antioxidant capacity (TAC), malondialdehyde (MDA), uric acid, and glucose levels, were determined using modern spectroscopic analytical techniques as described by [25,2٦].

2.8. Statistical analysis

Statistical analyses were performed using SPSS software [27]. Data are presented as Mean ± standard error (SE). Pre- and post-supplementation measurements obtained from repeated weekly assessments were analyzed using repeated-measures analysis to evaluate within-subject changes over time. When appropriate, Duncan's multiple range test was applied for pairwise comparisons. Statistical significance was established at $p \leq 0.05$.

3. Results and discussion

3.1. Nutritional evaluation results of the natural supplement

As presented in Table 2, the dietary supplement formulated from dried quail egg powder and watercress leaves exhibits a well-balanced nutritional profile, supporting its suitability as a multifunctional natural supplement. The calculated energy value of the product was 362.5 kcal/100 g, which reflects the balanced contribution of its macronutrient components. Notably, the supplement contained a high protein level (30 g/100 g), highlighting the substantial biological value of proteins derived from quail eggs.

In addition, the fat content was moderate (19 g/100 g), providing an appropriate source of energy, while carbohydrates accounted for 16 g/100 g of the formulation. The ash content reached 6.1 g/100 g, indicating a considerable mineral contribution. This mineral fraction included nutritionally important elements such as calcium, iron, zinc, potassium, and magnesium, thereby enhancing the overall nutritional quality of the supplement.

Table 2. Nutritional profile of the formulated dietary supplement (per 100 g of product and per capsule, 0.5 g)

Nutrient	Per 100 g	Per Capsule (0.5 g)
Energy (kcal)	362.5 kcal	1.81 kcal
Protein (g)	30.0 g	0.15 g (150 mg)
Total Fat (g)	19.0 g	0.095 g (95 mg)
Carbohydrates (g)	16.0 g	0.08 g (80 mg)
Dietary Fiber (g)	3.75 g	0.0188 g (18.8 mg)
Ash (g)	6.1 g	0.0305 g (30.5 mg)
Calcium (mg)	210 mg	1.05 mg
Iron (mg)	5.7 mg	0.0285 mg (28.5 µg)
Zinc (mg)	1.8 mg	0.009 mg (9 µg)
Magnesium (mg)	54 mg	0.27 mg
Potassium (mg)	423 mg	2.115 mg
Sodium (mg)	57 mg	0.285 mg
Total Phenolics (mg GAE)	1,500 mg	7.5 mg GAE
Total Flavonoids (mg QE)	500 mg	2.5 mg QE
Vitamin E (mg)	160 mg	0.8 mg

With respect to bioactive constituents, elevated levels of phenolic and flavonoid compounds were observed, underscoring the significant contribution of watercress leaves to the antioxidant potential of the formulation. The presence of vitamin E further supported product stability by limiting oxidative processes during storage.

When expressed on a per-serving basis (0.5 g capsule), the supplement delivered concentrated amounts of essential nutrients while remaining within recommended daily intake levels. This profile supports its suitability for regular consumption and confirms its acceptability from a nutritional safety standpoint.

3.2. Results of the chemical stability parameters of the natural dietary supplement

Table 3 summarizes the chemical stability parameters of the formulated natural dietary supplement, including moisture content, pH, and peroxide value (PV), which together reflect its physicochemical quality and storage stability. The moisture content of the supplement was 3.4%, indicating effective dehydration and low water activity. Such a low moisture level is known to limit microbial proliferation, reduce enzymatic activity, and preserve the structural stability of both egg-derived proteins and plant-based phenolic compounds. This value is consistent with international quality criteria for dried powdered supplements, where moisture contents below 5% are considered optimal for prolonged shelf life.

The measured pH of the reconstituted powder was 6.42, corresponding to a slightly acidic to near-neutral environment. This pH range is chemically advantageous for maintaining the stability of bioactive constituents, particularly phenolic compounds and flavonoids originating from arugula leaves, while also supporting the structural integrity of egg-derived peptides. Maintaining a pH within this range minimizes undesirable chemical reactions, such as non-enzymatic browning or protein denaturation, during storage.

The peroxide value of the supplement was 1.18 meq O_2/kg fat, indicating a very low degree of lipid oxidation. This low PV reflects the effectiveness of the freeze-drying process, appropriate storage conditions, and the protective role of vitamin E as a natural antioxidant. Peroxide values below 2 meq O_2/kg fat are generally associated with high lipid stability and the absence of rancidity, ensuring both product safety and acceptable sensory properties. Collectively, the results presented in Table 3 confirm that the applied drying procedures, antioxidant fortification, and packaging strategies were successful in producing a chemically stable dietary supplement with favorable shelf-life characteristics.

Table 3. Chemical stability parameters of the natural dietary supplement

Test	Result	Interpretation
Moisture Content	3.40%	Excellent stability; low water activity; reduced microbial and oxidation risk
pH	6.42	Chemically stable matrix; compatible with proteins and phenolic
Peroxide Value (PV)	1.18 meq O_2/kg	Indicates very low oxidation; high lipid stability

3.3. Results of the microbiological count of the natural food supplement

The microbiological evaluation of the natural food supplement formulated from dried quail egg powder and dried watercress leaves is presented in Table 4. The obtained results demonstrate that the product exhibits a high level of microbiological quality and complies with the acceptable limits established for dried dietary supplements. The total aerobic microbial count (TAMC) was low, confirming the effectiveness of the applied drying and packaging procedures in limiting bacterial contamination. Similarly, the total yeast and mold count (TYMC) remained within minimal levels, which can be attributed to the low water activity of the powder and its favorable storage stability. Collectively, these findings indicate that the formulated supplement is microbiologically safe and suitable for consumption under the applied processing and storage conditions.

Table 4. Microbial count results of the manufactured dietary supplement

Test	Result (CFU/g)	Result (log_{10} CFU/g)	Standard Limit	Interpretation
Total Aerobic Microbial Count (TAMC)	1.2×10^2	2.08	$< 10^4$ CFU/g	Within acceptable limits
Total Yeast and Mold Count (TYMC)	4.8×10^1	1.68	$< 10^3$ CFU/g	Within acceptable limits

3.4. Results of antioxidant capacity (TAC) and MDA levels

As presented in Table 5, a modest increase in total antioxidant capacity (TAC) was observed three hours following supplementation across all study weeks; however, these changes did not reach statistical significance. This trend may be attributed to the high content of bioactive plant compounds and vitamin E in the formulation, both of which are recognized for their role in supporting the body's antioxidant defense system. In contrast, malondialdehyde (MDA) concentrations remained relatively stable after supplementation and were comparable to fasting values, indicating that the intake of the supplement did not promote lipid peroxidation or induce oxidative stress. Collectively, these findings suggest that the product is chemically stable and capable of providing a mild, short-term enhancement of antioxidant status without adverse effects.

Table 5. Total antioxidant capacity and MDA levels before (fasting) and 3 hours after natural dietary supplement (Mean ± SE)

Week	Total Antioxidant Capacity (TAC) µmol TE/g		MDA (TBARS) nmol/mL	
	Fasting (Pre-dose)	Post-dose (3 h)	Fasting (Pre-dose)	Post-dose (3 h)
1	1.02±0.03	1.14±0.02	3.21±0.06	3.22±0.03
2	1.04±0.02	1.11±0.01	3.31±0.04	3.31±0.05
3	1.05±0.02	1.13±0.03	3.26±0.05	3.27±0.02
4	1.06±0.04	1.15±0.05	3.21±0.04	3.23±0.03
Sig.	N.S	N.S	N.S	N.S

N.S: Indicates no significant differences between the Means.

3.5. Results of uric acid and glucose measurement

As shown in Table 6, serum uric acid concentrations did not differ significantly between fasting and post-supplementation measurements throughout the four-week study period and remained within normal physiological ranges. These findings indicate that the administered supplement does not elevate purine metabolism or disrupt uric acid homeostasis. With respect to blood glucose levels, a slight reduction was observed three hours after supplementation; however, this change was not statistically significant. This minor decrease may be associated with the action of plant-derived bioactive compounds that contribute to improved metabolic regulation. Nevertheless, the administered dosage and the relatively short observation period may have been insufficient to elicit a measurable effect. Overall, these results suggest that the acute intake of the supplement is safe and does not induce abrupt or adverse alterations in key metabolic parameters.

Table 6. Uric acid and glucose levels before (fasting) and 3 hours after natural dietary supplement (Mean ± SE)

Week	Uric Acid (mg/dL)		Glucose (mg/dL)	
	Fasting (Pre-dose)	Post-dose (3 h)	Fasting (Pre-dose)	Post-dose (3 h)
1	4.50±0.06	4.46±0.04	87.59±1.27	85.59±1.18
2	4.56±0.05	4.52±0.02	85.20±1.29	83.20±1.13
3	4.62±0.03	4.58±0.01	83.59±1.53	82.59±1.33
4	4.45±0.04	4.41±0.02	85.32±1.42	83.32±1.14
Sig.	N.S	N.S	N.S	N.S

N.S: Indicates no significant differences between the Means.

The findings of the present study demonstrate that the dietary supplement developed from freeze-dried quail egg powder and dried arugula leaf powder exhibits favorable nutritional and functional characteristics. Chemical and nutritional evaluations confirmed that the formulation provides a diverse and concentrated profile of high-quality proteins, bioactive peptides, phenolic compounds, and beneficial lipid components.

1. Contribution of quail egg components to bioactivity

Recent evidence indicates that quail eggs are a rich source of bioactive constituents, particularly peptides exhibiting antioxidant and anti-inflammatory properties. Experimental studies have shown that these peptides can enhance the expression of key antioxidant enzymes, such as superoxide dismutase (SOD1) and catalase, while simultaneously suppressing inflammatory gene pathways [4]. In addition, recent reviews have emphasized the broad functional potential of egg-derived peptides and their diverse physiological roles in health promotion [1]. Together, these mechanisms provide a plausible biological explanation for the modest increase observed in total antioxidant capacity (TAC) following supplementation.

2. Functional role of arugula leaves in enhancing antioxidant capacity

Arugula (*Eruca sativa*) is widely recognized as a rich source of phenolic compounds and flavonoids, which are strongly associated with free radical scavenging and protection against oxidative damage. Previous studies have demonstrated that drying conditions comparable to those applied in the present work are effective in retaining a substantial proportion of these phytochemicals [12]. Moreover, existing research has confirmed that *Eruca sativa* possesses pronounced anti-inflammatory and cytoprotective properties [6,7]. Collectively, these findings provide a clear scientific basis for the modest and non-significant increase in total antioxidant capacity (TAC) observed following supplementation.

3. Stability of uric acid and glucose levels

The absence of significant changes in serum uric acid levels is anticipated, as the total protein intake provided by three capsules (0.45 g) is insufficient to exert a measurable effect on purine metabolism, a finding consistent with previous reports on the metabolic impact of animal-derived dietary proteins [5]. Likewise, the slight yet non-significant decrease in glucose concentrations may be attributed to the acute metabolic actions of phenolic compounds, which have been shown

to enhance insulin sensitivity and reduce oxidative stress [25].

4. Stability of MDA as an indicator of lipid oxidation control

The lack of significant variation in malondialdehyde (MDA, TBARS) levels indicates that the lipid fraction of the supplement remained oxidatively stable throughout the intervention. This stability can be primarily attributed to two key factors. First, the freeze-drying process is known to effectively preserve sensitive lipids and bioactive constituents by limiting oxidative degradation [11]. Second, the enrichment of the formulation with vitamin E provides strong protection against lipid peroxidation, thereby preventing the formation of secondary oxidation products [21,22].

5. Synergistic biological interaction between animal and plant components

The synergistic combination of animal-derived bioactive peptides and plant-based antioxidants forms a complementary functional matrix capable of enhancing physiological responses without inducing metabolic stress. The overall pattern of findings characterized by an increase in total antioxidant capacity, stable malondialdehyde levels, unchanged uric acid concentrations, and a modest reduction in glucose supports the conclusion that the formulated supplement is safe, exhibits mild bioactivity, and warrants further investigation in nutritional and clinical settings. As reported in previous studies, this also demonstrates the protection of sensitive compounds and their stability using encapsulation and stabilization techniques [28,29,30].

4. Conclusions

We can conclude that the natural dietary supplement formulated from freeze-dried quail egg powder and dried arugula leaves offers a nutritionally rich composition characterized by valuable proteins, essential minerals, and phenolic antioxidants. The evaluated chemical stability parameters moisture content (3.40%), pH (6.42), and peroxide value (1.18 meq O_2/kg fat) demonstrate excellent preservation, minimal lipid oxidation, and high overall product quality. Microbiological assessments confirmed a safe and contamination-free status, while the human intervention results indicated stable uric acid, glucose, and malondialdehyde levels, accompanied by a slight but non-significant improvement in total antioxidant capacity. Collectively, these findings confirm that the developed supplement is safe, well-formulated, and functionally promising. Further investigations involving longer intervention periods and higher dosage levels are recommended to fully elucidate its potential health benefits.

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