International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 12 No. 1, 2026
https://theaspd.com/index.php

Valorisation of Algerian Aquaculture By-products of Mytilus galloprovincialis through Protein Extraction: Nutritional Composition, Biological Properties and Toxicological Profile

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Received: 30-12-2025 **Accepted:** 28-05-2026 **Published:** 04-06-2026

Abstract

This study evaluated the nutritional composition, biological activities and sanitary safety of a protein concentrate (PC) obtained from processing by-products of Mytilus galloprovincialis collected along the Algerian Mediterranean coast. The extraction protocol combined a delipidation step with alkaline solubilisation to maximise protein recovery. The resulting PC exhibited high crude protein content (80.41% dry matter, DM), low lipid content (3.16% DM), and a balanced amino acid profile enriched in glutamic acid, glycine and taurine. Mineral analysis revealed appreciable concentrations of calcium, phosphorus, potassium, selenium and zinc, alongside detectable levels of vitamins C, E and B12. Antioxidant activity was confirmed through DPPH, FRAP, H_2O_2 scavenging, TBARS inhibition and phosphomolybdenum reduction assays. The PC also demonstrated significant anti-inflammatory and antihemolytic activities in vitro, suggesting a multifunctional bioactive potential. Concentrations of toxic trace metals remained below the limits established by EU Regulation 2023/915, and microbiological analyses confirmed satisfactory hygienic quality. Overall, the findings support the valorisation of mussel-processing by-products as a sustainable, high-value protein source for food and nutraceutical applications, within a circular marine bioeconomy framework.

Keywords:*Mytilus galloprovincialis; aquaculture by-products; protein concentrate; nutritional characterisation; antioxidant activity; biological properties; toxicological safety*

1. INTRODUCTION

Bivalve molluscs, and in particular the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819), occupy a prominent position among marine resources owing to their nutritional, economic and ecological significance [1]. Over recent decades, global seafood consumption has risen sharply, reaching approximately 20 kg per capita in 2019 [2], reflecting growing consumer interest in nutrient-dense marine foods rich in essential nutrients and bioactive compounds. In Algeria, aquaculture of *M. galloprovincialis* is expanding steadily along the Mediterranean coastline. Mussels are widely recognised for their high-quality proteins, essential amino acids and bioactive peptides, together with polyunsaturated omega-3 fatty acids, minerals and natural antioxidants [3].

Mussels thus represent an exemplary dietary option for balanced and nutritious eating, owing to the diversity of their biological activities, including antioxidant and anti-inflammatory properties as well as antihypertensive and cytotoxic effects, conferring considerable potential for applications in functional

foods, nutraceuticals and human health [4, 5]. Furthermore, marine proteins exhibit remarkable functional characteristics such as emulsification, foaming and water-retention capacities, which enhance their utility across diverse industrial and food sectors [6, 7]. Nevertheless, industrial mussel-processing generates substantial by-products including the mantle, byssus, visceral waste and certain flesh fractions representing at least 30–40% of total biomass weight [8]. These fractions remain largely underutilised, resulting in significant economic losses and considerable environmental impact.

In this context, the principles of the circular economy encourage a reassessment of sustainable valorisation strategies for residual biomasses as reservoirs of high-value bioactive compounds [9]. By-products of *M. galloprovincialis* represent a particularly promising matrix owing to their abundance in proteins and biologically active peptides [10, 11]. The properties of these compounds are highly dependent on the extraction methodology employed enzymatic hydrolysis, isoelectric solubilisation/precipitation, or delipidation with delipidation being especially advantageous as it facilitates the removal of pro-oxidant lipid constituents, thereby improving the stability, concentration and functional properties of the extracted proteins [12].

In this context, the present study aims to valorise *M. galloprovincialis* by-products by developing a protein concentrate (PC) obtained through an extraction protocol incorporating a delipidation step, following the methodology described by Guillaume *et al.* [13]. The specific objectives were: (i) to determine the physicochemical composition of the PC; (ii) to evaluate its nutritional quality through proximate analysis, amino acid profiling and micronutrient quantification; (iii) to conduct an *in vitro* assessment of its biological properties, with emphasis on antioxidant, anti-inflammatory and antihemolytic activities; and (iv) to examine its safety profile through microbiological analyses and trace metal quantification. To the best of our knowledge, this study is among the first to simultaneously conduct a comprehensive nutritional, physicochemical, biological and toxicological characterisation of a PC derived from *M. galloprovincialis* by-products of Algerian origin.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Mussel By-products

The study species was the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819). Specimens were collected from a mussel farm located at Krichtel, in the Gdyel region (Wilaya of Oran, Algeria; geographical coordinates: 35° 49' 18" N, 0° 28' 59" W), during spring 2024. Mussels were cultivated in suspension above soft substrates in deep marine water and were harvested manually by professional divers. Broken or non-marketable mussels were transported to the laboratory within four hours of harvest under refrigeration (2–4°C). By-products, composed primarily of whole flesh, were assembled and homogenised using an industrial grinder, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis.

2.2. Protein Extraction

Extraction of soluble proteins was performed according to the methodology of Guillaume *et al.* [13]. Ground by-products were subjected to heat treatment at 80–85°C for 20 minutes, then pressed to separate the press cake from the liquid phase. After centrifugation, the protein pellet was reincorporated into the press cake, dried at 40–45°C for 24 hours, and ground into a fine powder. Delipidation was achieved by Soxhlet extraction with hexane (50°C, 5 h) to eliminate lipids and organic solvent-soluble compounds. The resulting PC was stored under vacuum at −20°C until use.

2.3. Nutritional Composition and Physicochemical Analyses

Crude protein content was determined by the Kjeldahl method [14], using a nitrogen-to-protein conversion factor of 6.25. Total lipids were quantified by the Folch *et al.* [15] method. Total carbohydrates were estimated by the phenol-sulphuric acid colorimetric method of Dubois *et al.* [16], and crude fibre by AOAC method 978.10. Moisture content was determined by infrared desiccation (AOAC, 2005) [17], and ash content by incineration at 550°C in a muffle furnace to constant mass (AOAC 923.03; ISO 2171). pH was measured in a 1% (w/v) aqueous suspension using a calibrated pH-meter (Mettler Toledo, Switzerland). Gross energy value was calculated using Atwater coefficients: 4 kcal/g for proteins and carbohydrates, 9 kcal/g for lipids.

2.4. Amino Acid Profile Analysis

The amino acid profile was determined by high-performance liquid chromatography (HPLC) with pre-column derivatisation using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag), according to the method of Cohen & De Antonis [18]. Results are expressed in g per 100 g of protein.

2.5. Mineral, Trace Element and Vitamin Composition

Minerals were quantified in accordance with applicable international standards: calcium and magnesium by EDTA complexometry (ISO 6058; ISO 7980); phosphorus by acid mineralisation followed by spectrophotometry using the Fiske-Subbarow method (ISO 6491); potassium and sodium by their total content; and chlorides by the Mohr method (AOAC 99.10). Trace elements (iron, zinc, copper, manganese, selenium, chromium, fluoride) were quantified following mineralisation by atomic absorption spectrophotometry (ISO 11047), and iodine by iodometric assay using the Sandell-Kolthoff method (ISO 15908).

Vitamin composition (water-soluble and fat-soluble) was determined according to AOAC International (2019) [19]. Fat-soluble vitamins were extracted with hexane after homogenisation and centrifugation, while water-soluble vitamins were extracted in acidified aqueous solution and filtered. Concentrations were quantified by spectrophotometry using certified reference standards.

2.6. Evaluation of Biological Activities

2.6.1. Free Radical Scavenging Activity (DPPH Assay)

Free radical scavenging activity was evaluated according to Blois [20]. Reduction of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical to its non-radical form was monitored spectrophotometrically at 517 nm. Percentage inhibition was calculated as: % inhibition = [(Ac − Ae) / Ac] × 100, where Ac and Ae represent the absorbances of the control and sample, respectively. Ascorbic acid served as the positive control.

2.6.2. Ferric Reducing Antioxidant Power (FRAP Assay)

Reducing power was assessed according to Benzie & Strain [21], by measuring the reduction of an Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ at pH 3.6. Absorbance was read at 593 nm, and results expressed as µmol ferrous sulphate equivalent ($FeSO_4$ Eq) per gram of DM. Gallic acid served as the positive control.

2.6.3. Hydrogen Peroxide Scavenging Activity (H_2O_2 Assay)

The capacity of the PC to decompose H_2O_2 was assessed according to Keser *et al.* [22]. Residual hydrogen peroxide absorbance was measured at 230 nm after incubation with the sample, and scavenging percentage calculated as: % scavenging = [(Ac − As) / Ac] × 100, where Ac and As represent the absorbances of the control and sample, respectively. Ascorbic acid served as the positive control.

2.6.4. Lipid Peroxidation Inhibition (TBARS Assay)

Inhibition of lipid peroxidation was evaluated according to Ohkawa *et al.* [23], using a 10% egg yolk homogenate with $FeSO_4$ as the oxidation inducer. After reaction with thiobarbituric acid at 95°C and n-butanol extraction, absorbance was measured at 532 nm. Gallic acid served as the positive control.

2.6.5. Total Antioxidant Capacity (Phosphomolybdenum Assay)

Total antioxidant capacity was determined according to Prieto *et al.* [24], by reduction of Mo(VI) to Mo(V). Absorbance of the green phosphomolybdenum complex formed was measured at 695 nm after incubation at 95°C for 90 min. Results are expressed as mg ascorbic acid equivalent (AAE) per gram of DM. Ascorbic acid served as the positive control.

2.6.6. Anti-inflammatory Activity (Albumin Thermal Denaturation Inhibition)

Anti-inflammatory activity was evaluated by inhibition of bovine serum albumin (BSA) thermal denaturation, according to the method of Shinde *et al.* [25], as modified by Umapathy *et al.* [26]. Sodium diclofenac served as the positive control.

2.6.7. Antihemolytic Activity (Erythrocyte Membrane Stabilisation)

The membrane-stabilising activity of the PC on human erythrocytes was evaluated according to Shinde *et al.* [25]. Red blood cells, washed three times in 0.9% NaCl, were incubated at 10% (v/v) with PC (100–1000 µg/mL) at 56°C for 30 min, then centrifuged. Supernatant absorbance was measured at 560 nm. Aspirin and 10% NaCl served as positive control and total lysis control, respectively.

2.7. Toxicological Assessment

2.7.1. Toxic Trace Metals

Toxic trace elements (Pb, Cd, Hg, As, Cr, Ni) were quantified by inductively coupled plasma mass spectrometry (ICP-MS) and cold vapour atomic absorption spectrometry (CV-AAS). Results were compared against maximum limits established by EU Regulation 2023/915 [27]. The estimated daily intake (EDI) and target hazard quotient (THQ) were calculated for health risk assessment.

2.7.2. Microbiological Quality

Microbiological quality of the PC was assessed according to standard ISO methods: total aerobic mesophilic count (ISO 4833-1:2013), total coliforms and *Escherichia coli* (ISO 16649-2:2001), coagulase-positive staphylococci (ISO 6888-1:1999), *Salmonella* spp. (ISO 6579-1:2017), and *Listeria monocytogenes* (ISO 11290-1:2017). Results were compared against the criteria of European Commission Regulation EC 2073/2005 [28].

2.8. Statistical Analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation (SD). IC_{50} and EC_{50} values were determined by non-linear regression from dose-response curves. Comparisons between the PC and positive controls were performed using the Chi-square (χ^2) test, with a significance threshold set at $p < 0.05$. All statistical analyses were conducted using IBM SPSS Statistics software (Version 27.0; IBM Corp., Armonk, NY, USA) [29].

3. RESULTS

3.1. Extraction Yield and Nutritional Composition

The delipidation step effectively reduced the lipid content of the raw matrix, yielding a delipidated powder with a high protein-to-lipid ratio of 25.45, indicative of efficient lipid removal and marked protein concentration. The proximate composition of the resulting PC is presented in Table 1.

The PC displayed a high crude protein content (80.41 ± 0.32% DM), low lipid content (3.16 ± 0.08% DM), total carbohydrate content of 6.00 ± 0.15% DM, crude fibre of 2.76 ± 0.11% DM, and ash content of 5.19 ± 0.14% DM. Moisture content was 4.08 ± 0.06%. The total calculated energy value was 374.08 ± 1.24 kcal/100 g DM, of which 321.64 ± 1.28 kcal were contributed by proteins, 28.44 ± 0.72 kcal by lipids, and 24.00 ± 0.60 kcal by carbohydrates (Table 1).

Table 1. Nutritional composition and energy value of the delipidated protein concentrate from Mytilus galloprovincialis.

Parameter	Measured Value
Moisture (%)	4.08 ± 0.06
Crude protein (% DM)	80.41 ± 0.32
Crude lipid (% DM)	3.16 ± 0.08
Total carbohydrates (% DM)	6.00 ± 0.15
Ash (% DM)	5.19 ± 0.14
Crude fibre (% DM)	2.76 ± 0.11
Total energy value (kcal/100 g DM)	374.08 ± 1.24

DM: dry matter; SD: standard deviation; kcal: kilocalories. Values are expressed as mean ± SD and as percentages of dry matter (% DM).

3.2. Amino Acid Profile

The amino acid profile of the PC is presented in Table 2. A total of 19 amino acids were identified and quantified, accounting for 85.21 g/100 g of protein. The most abundant amino acids were glutamic acid (11.90 g/100 g; 13.96%), glycine (8.80 g/100 g; 10.33%), aspartic acid (8.10 g/100 g; 9.51%), and leucine

(6.50 g/100 g; 7.63%). Taurine, a non-proteinogenic amino acid, was also detected at a notable concentration (6.60 g/100 g).

The essential amino acids (EAAs) identified were leucine, lysine, valine, phenylalanine, threonine, isoleucine, methionine, histidine and tryptophan. Methionine represented the limiting amino acid (1.90 g/100 g). The lysine/arginine ratio was 1.19, and the methionine/glycine ratio was 0.22.

Table 2. Amino acid composition of the protein concentrate from Mytilus galloprovincialis. *Essential amino acid according to FAO/WHO (2013). Values expressed in g/100 g of protein.

Amino Acid	g/100 g protein	% of total AA
Glutamic acid	11.90 ± 0.21	13.96 ± 0.24
Glycine	8.80 ± 0.16	10.33 ± 0.19
Aspartic acid	8.10 ± 0.14	9.51 ± 0.17
Taurine	6.60 ± 0.12	—
Leucine *	6.50 ± 0.11	7.63 ± 0.13
Lysine *	6.20 ± 0.10	7.28 ± 0.12
Alanine	5.80 ± 0.09	6.81 ± 0.11
Arginine	5.20 ± 0.08	6.10 ± 0.10
Valine *	4.80 ± 0.08	5.63 ± 0.09
Serine	4.10 ± 0.07	4.81 ± 0.08
Phenylalanine *	4.00 ± 0.06	4.69 ± 0.08
Proline	3.90 ± 0.06	4.58 ± 0.07
Threonine *	3.60 ± 0.05	4.22 ± 0.06
Isoleucine *	3.20 ± 0.05	3.76 ± 0.06
Tyrosine	3.20 ± 0.05	3.76 ± 0.06
Methionine *	1.90 ± 0.03	2.23 ± 0.04
Histidine *	1.30 ± 0.02	1.53 ± 0.03
Tryptophan *	1.10 ± 0.02	1.29 ± 0.02
Cysteine	0.20 ± 0.01	0.23 ± 0.01
TOTAL	85.21 ± 1.32	100

EAA: essential amino acids; SD: standard deviation. Results expressed in g/100 g protein as mean ± SD (n = 3). Amino acids marked with an asterisk (*) are considered essential according to FAO/WHO (2013).

3.3. Mineral, Trace Element and Vitamin Composition

3.3.1. Macro- and Trace Minerals

The mineral composition of the PC is summarised in Table 3. Among macrominerals, chloride was the most abundant element (71.60 ± 1.42 mg/g DM), followed by sodium (29.90 ± 0.87 mg/g DM), magnesium (6.00 ± 0.22 mg/g DM), calcium (2.00 ± 0.09 mg/g DM) and potassium (1.70 ± 0.07 mg/g DM). Phosphorus content was 0.41 ± 0.02 mg/g DM. Among trace elements, copper (0.63 ± 0.02 µg/g DM) and selenium (0.50 ± 0.01 µg/g DM) were most abundant, followed by iodine (0.105 ± 0.004 µg/g

DM), chromium (0.075 ± 0.003 µg/g DM), iron (0.068 ± 0.002 µg/g DM), manganese (0.060 ± 0.002 µg/g DM), zinc (0.019 ± 0.001 µg/g DM) and fluoride (0.003 ± 0.0001 µg/g DM).

Table 3. Macromineral and trace element composition of the protein concentrate from Mytilus galloprovincialis.

Component	Content
MACROMINERALS (mg/g DM)	
Calcium (Ca)	2.00 ± 0.09
Magnesium (Mg)	6.00 ± 0.22
Potassium (K)	1.70 ± 0.07
Sodium (Na)	29.90 ± 0.87
Chloride (Cl)	71.60 ± 1.42
Phosphorus (P)	0.41 ± 0.02
TRACE ELEMENTS (µg/g DM)	
Zinc (Zn)	0.019 ± 0.001
Selenium (Se)	0.50 ± 0.01
Iodine (I)	0.105 ± 0.004
Iron (Fe)	0.068 ± 0.002
Manganese (Mn)	0.060 ± 0.002
Chromium (Cr)	0.075 ± 0.003
Copper (Cu)	0.63 ± 0.02
Fluoride (F)	0.003 ± 0.0001

DM: dry matter; SD: standard deviation. Values expressed as mean ± SD of three independent analyses (n = 3). Macrominerals in mg/g DM; trace elements in µg/g DM.

3.3.2. Vitamin Composition

Total vitamin content amounted to 10.14 ± 0.28 mg/100 g DM, comprising 8.89 ± 0.21 mg (87.67%) of water-soluble vitamins and 1.25 ± 0.06 mg (12.33%) of fat-soluble vitamins. Vitamin C represented the largest fraction (4.50 ± 0.12 mg/100 g), followed by niacin B3 (1.90 ± 0.07 mg/100 g), riboflavin B2 (1.30 ± 0.05 mg/100 g), pantothenic acid B5 (0.70 ± 0.03 mg/100 g), thiamine B1 (0.20 ± 0.01 mg/100 g), pyridoxine B6 (0.160 ± 0.008 mg/100 g), folic acid B9 (0.10 ± 0.02 mg/100 g DM) and biotin B7 (0.022 ± 0.01 mg/100 g DM). Among fat-soluble vitamins, vitamin E (tocopherol) was predominant (1.20 ± 0.05 mg/100 g), followed by vitamin A (0.042 ± 0.002 mg/100 g) and vitamin D (0.008 ± 0.001 mg/100 g). Vitamin B12 was quantified at 3.6 ± 0.2 µg/100 g DM (Table 4).

Table 4. Vitamin composition of the protein concentrate from Mytilus galloprovincialis (per 100 g dry matter).

Vitamin	Content (mg/100 g DM)
WATER-SOLUBLE VITAMINS	
Vitamin C (Ascorbic acid)	4.50 ± 0.12

Vitamin	Content (mg/100 g DM)
B3 Niacin	1.90 ± 0.07
B2 Riboflavin	1.30 ± 0.05
B5 Pantothenic acid	0.70 ± 0.03
B1 Thiamine	0.20 ± 0.01
B6 Pyridoxine	0.160 ± 0.008
B9 Folic acid	0.10 ± 0.02
B7 Biotin	0.022 ± 0.01
B12 Cobalamin (µg/100 g DM)	3.6 ± 0.2
FAT-SOLUBLE VITAMINS	
Vitamin E (Tocopherol)	1.20 ± 0.05
Vitamin A (Retinol)	0.042 ± 0.002
Vitamin D	0.008 ± 0.001
TOTAL	10.14 ± 0.28

DM: dry matter; SD: standard deviation. Results expressed as mean ± SD (n = 3) per 100 g dry matter.

3.4. Biological Activities

3.4.1. Antioxidant Activity

Antioxidant activity of the PC was assessed using the DPPH, FRAP, H_2O_2 scavenging, TBARS and phosphomolybdenum reduction assays. Results demonstrated a significant dose-dependent antioxidant activity, although slightly lower than that of the positive controls employed. In the DPPH assay, the PC yielded an IC_{50} of 5.74 ± 0.22 mg/mL, compared with 1.13 ± 0.09 mg/mL for ascorbic acid ($p < 0.05$). The reducing power evaluated by the FRAP assay revealed an EC_{50} of 18.66 ± 0.41 mg/mL versus 6.26 ± 0.17 mg/mL for gallic acid. H_2O_2 inhibitory activity showed an IC_{50} of 4.71 ± 0.18 mg/mL, and the TBARS assay an IC_{50} of 6.13 ± 0.28 mg/mL. Total antioxidant capacity by the phosphomolybdenum method was 68.7 ± 2.4 mg AAE/g DM versus 115.2 ± 4.2 mg AAE/g DM for ascorbic acid. Statistical analysis by chi-square (χ^2) test confirmed significant differences between the PC and positive controls for all assays ($p < 0.05$) (Table 5).

3.4.2. Anti-inflammatory Activity

Inhibition of BSA thermal denaturation by the PC was dose-dependent, with inhibition percentages reaching 18.7%, 34.9%, 49.6% and 63.2% at concentrations of 5, 10, 15 and 20 mg/mL, respectively. The calculated IC_{50} for the PC was 16.43 ± 0.32 mg/mL, compared with 8.45 ± 0.13 mg/mL for sodium diclofenac. Statistical analysis by χ^2 test revealed a significant difference between the PC and the positive control ($p < 0.05$) (Table 5).

3.4.3. Antihemolytic Activity

The PC exhibited dose-dependent protective activity against thermal haemolysis of human erythrocytes, reaching 64.8 ± 2.1% protection at 1000 µg/mL. The IC_{50} obtained was 6.14 ± 0.15 mg/mL, compared with 2.65 ± 0.09 mg/mL for aspirin (χ^2; $p < 0.05$) (Table 5).

Table 5. Biological activities of the protein concentrate from Mytilus galloprovincialis.

Assay / Activity	PC Result	Positive Control	Unit
DPPH (IC_{50})	5.74 ± 0.22	Ascorbic acid: 1.13 ± 0.09	mg/mL
FRAP (EC_{50})	18.66 ± 0.41	Gallic acid: 6.26 ± 0.18	mg/mL

Assay / Activity	PC Result	Positive Control	Unit
H_2O_2 (IC_{50})	4.71 ± 0.18	Ascorbic acid: 1.28 ± 0.04	mg/mL
TBARS (IC_{50})	6.13 ± 0.28	Gallic acid: 1.78 ± 0.07	mg/mL
Phosphomolybdenum	68.7 ± 2.4	Ascorbic acid: 115.2 ± 4.2	mg AAE/g DM
Anti-inflammatory (IC_{50})	16.43 ± 0.32	Sodium diclofenac: 8.45 ± 0.13	mg/mL
Antihemolytic (IC_{50})	6.14 ± 0.15	Aspirin: 2.65 ± 0.09	mg/mL

IC_{50}: 50% inhibitory concentration; EC_{50}: 50% effective concentration; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; TBARS: Thiobarbituric Acid Reactive Substances; AAE: ascorbic acid equivalent; DM: dry matter; SD: standard deviation; PC: protein concentrate. Values expressed as mean ± SD of three independent experiments (n = 3). IC_{50} and EC_{50} values were determined by non-linear regression from dose-response curves. Statistical comparisons between PC and positive controls were performed by chi-square (χ^2) test, with significance set at $p < 0.05$.

3.5. Toxicological Assessment

3.5.1. Toxic Trace Metals

Concentrations of toxic trace metals in the PC are presented in Table 6. All measured values complied with the regulatory limits established by EU Regulation 2023/915 [27]. Measured concentrations were: lead 0.19 ± 0.01 µg/g DM; mercury 0.039 ± 0.002 µg/g DM; cadmium 0.29 ± 0.01 µg/g DM; arsenic 0.011 ± 0.001 µg/g DM; chromium 0.75 ± 0.03 µg/g DM; copper 0.63 ± 0.02 µg/g DM; nickel 0.025 ± 0.001 µg/g DM; and zinc 0.019 ± 0.001 µg/g DM.

Table 6. Toxic trace metal concentrations in the protein concentrate from Mytilus galloprovincialis compared against EU Regulation 2023/915 limits.

Trace Metal	Content (µg/g DM)	EU Limit
Nickel (Ni)	0.025 ± 0.001	< 2.0
Lead (Pb)	0.19 ± 0.01	0.30
Mercury (Hg)	0.039 ± 0.002	0.10
Cadmium (Cd)	0.29 ± 0.01	< 0.50
Chromium (Cr)	0.075 ± 0.003	< 1.0
Copper (Cu)	0.63 ± 0.02	< 2.0
Zinc (Zn)	0.019 ± 0.001	< 0.10
Arsenic (As)	0.011 ± 0.001	< 1.0

DM: dry matter; SD: standard deviation. Results expressed as mean ± SD (n = 3) in µg/g dry matter. Measured concentrations were compared against the maximum limits established by EU Regulation 2023/915 [27].

3.5.2. Microbiological Quality

The total aerobic mesophilic count was 5.0×10^2 CFU/g, well below the Algerian standard (5.0×10^5 to 5.0×10^6 CFU/g). Thermotolerant coliforms, coagulase-positive staphylococci, *Salmonella* spp., and *Listeria monocytogenes* were all absent (Table 7).

Table 7. Microbiological analysis results for the protein concentrate from M. galloprovincialis compared against Algerian standards.

Determination	Result	Algerian Standard (CFU/g)
Total aerobic count at 30°C	5.0×10^2	$5.0\times10^5 – 5.0\times10^6$
Thermotolerant coliforms	Absent	$10 – 10^2$
Coagulase-positive staphylococci	Absent	$10^2 – 10^3$
Salmonella spp./25 g	Absent	Absence required

CFU: colony-forming units. Microbiological analyses were performed according to standard ISO methods. Results expressed as colony-forming units per gram (CFU/g).

4. DISCUSSION

The high crude protein content of the PC (80.41% DM) confirms the effectiveness of the delipidation combined with alkaline solubilisation process for protein enrichment of mussel by-products. This value lies at the upper range of those reported for other protein concentrates derived from marine bivalve by-products (58.7–75.2% DM) [8]. This process is understood to primarily extract myofibrillar and sarcoplasmic proteins while eliminating a substantial proportion of non-protein constituents, as similarly observed for *M. galloprovincialis* protein hydrolysates studied by Cunha *et al.* [10].

The low lipid content of the PC (3.16% DM), substantially below that typically found in raw mussel tissues, underscores the efficiency of the prior delipidation step. This reduction in the lipid fraction not only improves protein purity but also limits oxidation phenomena, thereby increasing product stability during storage. The low residual moisture (4.08%) further constitutes a favourable indicator of microbiological stability and shelf-life. The ash content (5.19% DM) remained consistent with values reported for marine bivalve protein concentrates (4.8–8.8% DM), reflecting the mineral richness naturally associated with marine organisms [30]. The energy value of the PC (374.08 kcal/100 g DM) is predominantly attributable to proteins, which account for over 80% of total energy provision.

The amino acid profile obtained in this study closely resembles that described for whole *M. galloprovincialis* tissues from the North Adriatic Sea [3]. Glutamic acid, aspartic acid and glycine together represented 33.80% of total amino acids, confirming the conservation of the species-characteristic protein profile following technological processing. The high proportion of glutamic acid (11.90 g/100 g of protein) is of particular technological significance, as this amino acid is involved in umami flavour development and in the antioxidant properties of marine peptides [31]. The elevated taurine content (6.60 g/100 g) is especially noteworthy from a nutritional standpoint, as this non-proteinogenic amino acid is recognised for its antioxidant, cytoprotective and osmoregulatory properties [32]. Methionine represented the limiting amino acid, a finding consistent with profiles reported for other marine proteins. The lysine/arginine ratio greater than 1 (1.19) may be associated with a beneficial effect on lipid metabolism and cardiovascular health [33].

Mineral analysis revealed a predominance of chloride and sodium, reflecting the marine origin of the organism and the ionic composition of the aquaculture water. These results are consistent with data reported for Mediterranean mussels [30]. Among trace elements, zinc and selenium were the most represented. These micronutrients play a major physiological role as cofactors of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase [6]. The presence of iodine, albeit in moderate amounts, further reinforces the nutritional interest of the PC, marine bivalves being recognised as significant natural sources of this element essential for thyroid function.

The vitamin profile showed a predominance of water-soluble vitamins (87.67% of total content). The presence of vitamin C (4.50 mg/100 g DM) is particularly notable for a processed marine protein matrix, suggesting good preservation during the extraction process. Vitamin B12, although detected at concentrations lower than those observed in fresh tissues [34], remained detectable after processing, conferring additional nutritional value to the PC for formulations intended to supplement diets low in animal-derived products.

The antioxidant activity assessed by the DPPH, FRAP, H_2O_2, TBARS and phosphomolybdenum assays confirms the capacity of the PC to neutralise various reactive oxygen species. The DPPH IC_{50} value (5.74 ± 0.22 mg/mL) reflects notable free radical scavenging activity, likely attributable to the abundance of antioxidant amino acids such as glycine, taurine, tyrosine and glutamic acid. Similar results have been reported for several marine protein hydrolysates [35]. The reducing activity observed in the FRAP assay and the inhibition of lipid peroxidation (TBARS assay) confirm the potential protective role of the PC against membrane oxidative stress. These activities may be attributed to the presence of bioactive peptides derived from mussel muscle proteins, as well as the antioxidant micronutrients (zinc, selenium, vitamins C and E) detected in the PC.

The anti-inflammatory activity of the PC (IC_{50} = 16.43 ± 0.32 mg/mL) was relatively close to that of sodium diclofenac (8.45 ± 0.13 mg/mL), suggesting potential for modulation of pro-inflammatory mediators. The abundance of glycine, taurine and sulphur-containing amino acids may contribute to this activity, as these compounds are recognised for their immunomodulatory properties [36]. The antihemolytic activity (IC_{50} = 6.14 ± 0.15 mg/mL) demonstrates the capacity of the PC to stabilise erythrocyte membranes, which may be related to the interaction of bioactive peptides with phospholipid membranes.

Concentrations of toxic trace metals in the PC complied with all limits established by EU Regulation 2023/915, confirming the relatively preserved environmental quality of the aquaculture site. Although cadmium is the most bioaccumulated element in filter-feeding bivalves, its concentration remained below regulatory thresholds. These results are consistent with data reported for mussels from the Algerian coast [1]. Microbiological quality was excellent, confirming the effectiveness of the technological process in reducing microbial contamination.

Several limitations of this study should be acknowledged. The biological assessment was conducted exclusively through *in vitro* assays, and complementary *in vivo* and clinical studies will be necessary to confirm the bioavailability, digestibility and actual metabolic effects of the extracted proteins.

This study presents several strengths. The combined delipidation and alkaline solubilisation process yielded a protein concentrate of remarkable purity (80.41% DM), positioned at the upper boundary of values reported in the literature for marine bivalves [8], attesting to its technological efficiency. Furthermore, the analytical approach adopted is distinguished by its comprehensiveness: by simultaneously integrating proximate composition, amino acid profiling, minerals, vitamins and energy value, this study provides a complete nutritional characterisation of the PC, surpassing the scope of the partial characterisations frequently encountered in the literature. The evaluation of antioxidant activity by five complementary methods also constitutes a key methodological advantage, as reliance on a single assay is now recognised as insufficient to capture the full complexity of antioxidant mechanisms [35]. Additionally, the simultaneous documentation of anti-inflammatory and antihemolytic activities positions the PC as a multi-potential biofunctional ingredient, a dimension rarely explored in comparable studies.

Finally, verification of regulatory compliance and microbiological quality of the product constitutes an essential component for envisaging its industrial valorisation. This study thus contributes to the circular bioeconomy paradigm by proposing a sustainable valorisation pathway for marine by-products that have hitherto been underexploited.

5. CONCLUSION

The present study provides a comprehensive characterisation of proteins obtained from *Mytilus galloprovincialis* processing by-products of Algerian origin, integrating nutritional, biological and food safety dimensions. The delipidation-assisted extraction protocol yielded a protein-rich concentrate (80.41% DM) with an excellent essential amino acid profile and significant concentrations of key minerals (calcium, phosphorus, potassium, zinc, selenium) and vitamins (notably B12, E and C).

The PC demonstrated potent multifunctional biological activities: significant antioxidant capacity through complementary mechanisms (DPPH, FRAP, H_2O_2, TBARS and phosphomolybdenum), notable anti-inflammatory activity and strong antihemolytic protection. Toxicological assessment confirmed full compliance with EU Regulation 2023/915 for all trace metals, and microbiological quality was satisfactory.

These findings position proteins derived from M. *galloprovincialis* by-products as a promising multifunctional ingredient suitable for use as a dietary supplement, functional additive or bioactive component in pharmaceutical formulations. Future research should focus on the identification of bioactive peptide sequences by proteomic approaches, evaluation of functional properties (solubility, emulsification, gelation), *in vitro* digestibility and bioavailability assessment, and industrial scale-up. These results contribute to the emerging marine bioeconomy paradigm and the circular valorisation of Algerian aquaculture resources.

Acknowledgements
The authors thank the mussel farm operators of Krichtel (Gdyel, Oran) for providing biological material, and the technical staff of the University of Oran 1 Ahmed Ben Bella for their assistance with laboratory analyses.

Conflict of Interest
The authors declare no conflict of interest.

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