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Chemical Composition, Techno-Functional Properties, Lipid Oxidation, Microbiological, and Sensory Analyses of Quinoa -Fortified Common Carp Burgers

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ABSTRACT

In the present study quinoa seed powder (QSP) was added to common carp fish burgers at different weight percentages (0%, 5%, 10 %, and 15%). For every treatment group, the physicochemical techno-functional properties, lipid oxidation, microbiological, and sensory analyses were evaluated. According to the findings, the QSP has high levels of total antioxidants (91.4%), total phenolic content (308.6 mg/100g), and total flavonoid content (150.2 mg/100g). The nutritional composition of fish burgers is enhanced by the addition of QSP. The functional and sensory qualities of the fish burger treatments with 10 % QSP were superior to the other treatments' outcomes. In addition, these treatments shown increased capacity to swell as well as hold water and oil. The total volatile base nitrogen values for the treatments compared to the control showed strong stability, reaching at 18.40 (mg/100g) at month six. At the end of the storage period, it was noted that the microbial load of the treatments had reduced in comparison to the control. In conclusion, extending the freshness of fish burgers by using QSP as a natural preservative is a creative and sustainable method of food preservation.

INTRODUCTION

Globally, fish consumption has been promoted as a solution to both food security and economic development challenges, with the fish market experiencing notable growth in 2018 **(FAO, 2018)**. In response, developed nations are implementing policies aimed at ensuring environmentally sustainable and socioeconomically viable growth in the fisheries sector, in preparation for the projected global population of 9.2 billion by 2050 **(Vázquez *et al.*, 2019)**.

Fish is the most consumed source of animal protein worldwide, surpassing pork, chicken, and beef combined **(Sonoda & Shirota, 2012)**. Despite this, efforts are still needed to further increase fish consumption. The development of diverse fish-based products can help familiarize consumers with the taste and nutritional value of fish, thereby expanding the market for such products.

The common carp (*Cyprinus carpio*) is a freshwater fish species from the family

Cyprinidae **(Maiditsch & Ladich, 2014)**. According to **Ljubojevic *et al.* (2013)**, its flesh is nutritionally beneficial, containing essential amino acids, high levels of omega-3 and -6 polyunsaturated fatty acids, fat-soluble vitamins, key minerals, and relatively low levels of saturated fats and cholesterol **(Gökoglu & Yerlikaya, 2015)**. However, its consumption in Egypt remains limited due to the high abundance of intramuscular bones. Therefore, in the field of fish processing, it is crucial to explore transformation techniques to convert underutilized fish like carp into palatable, functional food products for human consumption **(Mohammed *et al.*, 2024)**.

Fish products are prone to oxidation, a complex biochemical process that begins shortly after slaughter. This reaction involves unsaturated fatty acids interacting with molecular oxygen to produce peroxides and initiate a chain of free radical formation. Oxidation affects the product's flavor, texture, color, and nutritional value by leading to lipid degradation and the accumulation of rancid compounds **(Min *et al.*, 2008)**. To mitigate this, synthetic antioxidants are often used. However, natural antioxidants derived from fruit extracts, residues, or seeds offer functional properties while also preserving product quality **(Ribeiro *et al.*, 2019)**.

Fibers are highly desirable in meat and fish products due to their water retention capacity (WRC) and potential to reduce production costs **(Lima *et al.*, 2022)**. Quinoa (*Chenopodium quinoa* Willd.), classified as a pseudocereal, offers a superior nutritional profile compared to traditional cereals like wheat. It is rich in lipids, proteins, carbohydrates, and fiber **(Gewehr *et al.*, 2012; Amaya-Farfan *et al.*, 2015; Ayala-Niño *et al.*, 2020)**. Pseudocereals have gained interest in the food industry due to their functional and nutritional properties.

Quinoa seeds contain bioactive compounds such as proteins, saponins, and polysaccharides, which are known for their antioxidant, anti-inflammatory, immunomodulatory, and anticarcinogenic effects **(Fuentes & Paredes-Gónzalez, 2013)**. Their polysaccharides resemble those found in fruits and vegetables and function as prebiotics, promoting the growth of beneficial gut bacteria **(Zhu, 2020)**. Additionally, quinoa has been linked to reductions in obesity risk **(Wang *et al.*, 2022)**. Although quinoa shares several nutritional traits with cereals, its botanical classification distinguishes it as a pseudocereal **(Mu *et al.*, 2023)**.

Despite these benefits, limited studies have investigated the application of quinoa seeds in improving the quality of fish-based products. Therefore, the objective of this study was to evaluate the effect of quinoa seed incorporation on the chemical composition, techno-functional properties, lipid oxidation (as measured by the thiobarbituric acid index—TBA), microbiological quality, and sensory attributes of common carp (*Cyprinus carpio*) burgers during frozen storage at –18 °C.

MATERIALS AND METHODS

1. Materials and reagents

Quinoa seeds (*Chenopodium quinoa* Willd.) were purchased from a local market (Harraz) in Cairo, Egypt. Seeds were cleaned to remove broken seeds, dust, and other foreign matter. Whole seeds were washed 4–5 times with cold water, or until the washing water showed no foam, to eliminate surface saponins. The washed seeds were then oven-dried at 45 ± 1°C for 24 hours or until completely dry. Dried quinoa seeds were ground into flour using a stainless-steel electric grinder (laboratory disc mill), then passed through a 60-mesh sieve. The resulting flour was packed in polyethylene bags and stored at –18 ± 1°C until use **(Osheba *et al.*, 2024)**.

Fish samples

Sixty kilograms of fresh common carp fish (*Cyprinus carpio*), with an average weight of 3.95 kg and average length of 55.15 cm, were obtained from the Institute of Fisheries Research, Al-Abbasa, Sharkia Governorate, Egypt.

Other ingredients and reagents

Spices (black pepper, cumin powder, thyme powder, onion powder, and garlic powder), salt, and wheat flour were sourced from the local market in Cairo, Egypt. Analytical-grade solvents and chemicals were supplied by Sigma Chemical Co. (St. Louis, MI, USA).

Culture media

The BG-11 medium was prepared according to **Al-Rikabey and Al-Mayah (2018)**. LB broth, LB agar, total count agar, Baird-Parker agar, potato dextrose agar, and MacConkey agar were prepared following the protocols described by **Zimbro *et al.* (2009)**.

Microorganisms for antimicrobial testing

Three indicator bacterial strains were used to evaluate antimicrobial activity: two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus* ATCC 13565) and one Gram-negative strain (*Escherichia coli* O:157 ATCC 1659). All bacterial strains were obtained as live cultures from the Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

2. Preparation of Common carp fish burgers (CCFB)

Fresh common carp fish were delivered chilled to the Food Science Laboratory, Fisheries Research Department, Abbasa, Sharkia, Egypt. Upon arrival, the fish were thoroughly cleaned, gutted, and filleted by hand. The fillets were then minced using a mechanical meat grinder fitted with a 4 mm coarse blade. The minced fish was stored at 4°C until further use.

Common carp fish burgers were prepared based on the formulation provided in Table (1), following the procedure outlined by **Kenawi and Abdelaal (2021)**. All ingredients were thoroughly mixed, and the mixture was divided into 50 g portions. Each portion was hand-shaped into a patty approximately 10 cm in diameter and 0.5 cm in thickness. The formed burgers were placed in polystyrene foam trays with low-density polyethylene (LDPE) sheets between them to prevent sticking.

Table 1. Common carp fish burger formula

Ingredient	Control common carp fish burger (C)	Common carp fish burger fortified with quinoa seeds		
		5.0% (Q1)	10.0% (Q2)	15.0% (Q3)
Ground catfish Fillet	89.8	85.31	80.82	76.33
Quinoa seeds powder	0.00	4.49	8.98	13.47
Wheat flour	3.5	3.5	3.5	3.5
Salt	2.5	2.5	2.5	2.5
Black pepper powder	1.0	1.0	1.0	1.0
Onion powder	1.0	1.0	1.0	1.0
Garlic powder	1.0	1.0	1.0	1.0
Cumin powder	1.0	1.0	1.0	1.0
Thyme powder	0.2	0.2	0.2	0.2

3. Determination of total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity

The total phenolic content (TPC) of quinoa seed powder (QSP) and common carp fish burger (CCFB) samples was determined according to **Alonso-Riaño *et al.* (2020)**. A gallic acid (GA) standard curve was constructed, and results were expressed as mg gallic acid equivalents per gram (mg GAE/g) of sample.

The total flavonoid content (TFC) of QSP and CCFB samples was measured following the method of **Aminzare *et al.* (2022)**. A standard curve of quercetin in ethanol was used, and TFC was expressed as mg quercetin per gram (mg quercetin/g).

Antioxidant activity was assessed based on the 1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenylethylamino) (DPPH) free radical scavenging method, following **Hashemian and Nouri (2023)**. The radical scavenging percentage was calculated using the following equation:

DPPH radical scavenging (%) = A (blank) − A (sample)/A (blank) × 100

4. High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) analysis was carried out using an Agilent 1260 Series HPLC system, following the procedure described by **Khalil *et al.* (2020)**.

5. Physicochemical assessments

The physicochemical properties of the burger samples were assessed as follows:

- pH was measured using a digital pH meter (Zenit, Germany).
- Moisture (%), ash (%), protein (%), lipid (%), carbohydrate (%), and fiber (%) contents were determined following the standard procedures of the Association of Official Analytical Chemists **(AOAC, 2012)**.

- Carotenoid content was estimated according to **Kumar and Sharma (2019)**.
- Total volatile base nitrogen (TVB-N), expressed in mg N/100 g, was determined based on **Mahmoudzadeh *et al.* (2010)** using the equation:

$$\text{TVB−N (mg N/100 g)} = [1.4 \times \text{volume of used } H_2SO_4 \times 100] / \text{sample weight (mg)} \times 1000$$

6. Oxidative index measurement

The peroxide value (PV) was determined following **Amiri *et al.* (2019)** and reported in milliequivalents per kilogram (meq/kg).

Thiobarbituric acid (TBA) levels, representing lipid oxidation, were expressed as mg malondialdehyde per kg (mg MDA/kg) of sample, following **Maghami *et al.* (2019)**.

7. Functional properties of fish burgers

The functional properties of powdered fish burger samples were measured as follows:

- Swelling capacity (SWC) was assessed using the method described by **Kuniak and Marchessault (1972)**.
- Water holding capacity (WHC) was measured following **Okezie and Bello (1988)**.
- Oil holding capacity (OHC**)** was determined according to **Wong and Cheung (2000)**.

8. Microbiological analyses

Microbiological assessments included counts of:

- Aerobic plate count
- *Escherichia coli*
- Yeasts and molds
- *Staphylococcus aureus*

All microbiological tests were conducted in accordance with ISO 7218:2007 standards.

9. Sensory evaluation

Sensory analysis of cooked burger samples was performed by a semi-trained panel using a nine-point hedonic scale, where 1 indicated strong dislike and 9 indicated strong preference. Evaluated attributes included color, taste, odor, and texture **(Watts *et al.*, 1989)**.

10. Statistical analysis

All tests were conducted in duplicate. Data were statistically analyzed using SPSS version 22.0. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to assess significant differences among means at a 95% confidence level ($P < 0.05$).

RESULTS AND DISCUSSION

1. Nutritional composition of quinoa seeds powder

Table (2) displays the proximate composition of quinoa seeds (QSP). The seeds contained 6.2% moisture, 17.70% protein, 6.20% fat, 15.70% crude fiber, and 2.6% ash. These values are in agreement with those previously reported by **Pellegrinia *et al.* (2018)**.

In terms of bioactive content, Table (2) also shows that the total phenolic content

(TPC), total flavonoid content (TFC), and DPPH radical scavenging activity of QSP were 308.6 mg/100 g, 150.2 mg/100 g, and 91.4%, respectively. These findings are consistent with those reported by **Al-Anazi *et al.* (2022)**, who recorded TPC and TFC values of 488.32 mg/100 g and 367.11 mg/100 g, respectively.

The total carotenoid content of quinoa seed powder was 20.90 mg/100 g, while phycocyanins and chlorophyll were not detected, which aligns with results presented by **Multari *et al.* (2018)**.

2. HPLC analysis of quinoa seed extract

Table (3) summarizes the results of the high-performance liquid chromatography (HPLC) analysis of quinoa seed extract. A total of 21 phenolic compounds were identified, with concentrations ranging from 0.33 to 108.5 ppm. The phenolic compounds detected included:

Gallic acid, Pyrogallol, 4-Aminobenzoic acid, Protocatechuic acid, Catechin, Chlorogenic acid, Catechol, Caffeine, *p*-Hydroxybenzoic acid, Caffeic acid, Vanillic acid, *p*-Coumaric acid, Ferulic acid, Isoferulic acid, α-Coumaric acid, Ellagic acid, Benzoic acid, Coumarin, 3,4,5-Trimethoxycinnamic acid, Salicylic acid, and Cinnamic acid.

Among these, pyrogallol was the predominant compound, with a concentration of 108.8 ppm.

These results are consistent with findings by **Farajzadeh *et al.* (2020)**, who reported that similar phenolic compounds—including protocatechuic acid, catechin, chlorogenic acid, catechol, caffeine, and various cinnamic acid derivatives—were the main constituents in chia seed extracts.

Table 2. Comparison of the nutritional composition of quinoa seeds powder

Components	QSP
Proximate composition (on dry matter basis)	
Moisture	6.2± 0.4
Protein	17.70 ± 0.9
Fat	6.2 ± 0.4
Ash	2.6 ± 0.2
Crude Fiber	15.7 ± 0.5
Phytochemical and phytopigments properties	
TPC mg/100g	308.6 ± 3.2
TFC mg/100 g	150.2 ± 2.3
DPPH inhibition (%)	91.4±1.6
Carotenoids (mg/100g)	20.90

* Values (means ±SD)

Table 3. The phenolic compounds determined in the quinoa seeds extract

Phenolic Compounds	(mg/100g)
Gallic	1.38
Pyrogallol	108.5
4-amino-benzoic	1.62
Protocatchein	18.70
Catectein	10.80
Chlorogenic	3.50
Catechol	1.20
Caffeine	4.60
p-oh-benzoic	12.20
Caffeic	1.44
Vanillie	1.05
p-coumaric	11.78
Ferulic	5.65
Ios-ferulic	0.54
Alpha – coumaric	0.42
Ellagic	1.94
Benzoic	3.57
Coumrin	1.42
3.4.5-methoxy-cinnamic	1.55
Salyeilic	6.84
Cinnamic	0.33

3. Effect of QSP and frozen storage at - 18±1°C for 6 months on proximate composition of fish burger

Table (4) presents the results of the proximate composition analysis, including moisture, protein, ether extract (fat), ash, and crude fiber contents of the common carp fish burger (CCFB) treatments.

Moisture content in the CCFB samples ranged from 65.92 to 70.02%. Protein content ranged from 14.98 to 18.20%, with the highest level recorded at the beginning of storage (18.20%) in the control group. Over the storage period, protein content decreased by 18.20, 17.75, 17.20, and 16.70% for the control, and by 1, 2, and 3% for the 1, 2, and 3% QSP-added treatments, respectively. This trend indicates that quinoa seed powder (QSP) helped to mitigate protein degradation during storage.

Ether extract (fat content) ranged from 2.60 to 4.60%. The highest fat percentages were observed in the fish burger samples containing QSP, with fat content increasing proportionally to the QSP addition level. This trend can be attributed to the naturally high fat content of quinoa seeds.

Ash content varied from 3.02 to 3.96%, with the lowest values recorded in samples containing QSP. The addition of QSP was associated with a decreasing trend in ash content, likely due to the lower mineral content of quinoa compared to fish muscle. Interestingly, all QSP treatments had lower ash percentages than the control, supporting findings reported by **Bhinder *et al.* (2021)**.

Crude fiber content ranged from 0.30 to 1.50%. The fish burgers fortified with QSP exhibited significantly higher crude fiber levels, with values increasing in direct proportion to the amount of QSP added. These results confirm that quinoa contributes substantial dietary fiber to the fish burger matrix.

Overall, the addition of QSP to fish burgers resulted in increased fat and crude fiber contents, while ash content showed a decline. The observed increase in fat and fiber is consistent with the nutritional properties of quinoa, which is known to be rich in both **(Bhinder *et al.*, 2021)**.

5. Effect of freezing storage on proximate composition

Frozen storage at –18 ± 1 °C for six months had a significant effect ($P < 0.05$) on the proximate composition of the fish burgers. Specifically, extended frozen storage resulted in a significant decrease in moisture and protein contents.

As noted by **Osheba *et al.* (2024)**, the reduction in moisture content may be due to evaporation through the polyethylene packaging and drip loss during thawing. The decrease in protein content could be attributed to enzymatic hydrolysis by endogenous fish enzymes and microbial enzymes, along with the loss of water-soluble proteins in the drip **(Siddik *et al.*, 2021)**.

Conversely, fat, ash, and fiber contents significantly increased ($P \leq 0.05$) over the storage period. These increases can be explained by the relative concentration effect caused by moisture and protein loss during frozen storage.

These findings are consistent with those reported by **Zapata and Pava (2022)** and **Felyes *et al.* (2021)**, who observed similar increases in fat and fiber contents in fish and beef burgers supplemented with QSP compared to control samples.

Table 4. Effect of quinoa seed powder and frozen storage at -18±1°C for 6 months on proximate composition of common carp fish burger

Components (%)	Storage period (months)	Treatments			
		C	Q1	Q2	Q3
Moisture	0	70.02±0.45^{a}	69.58±0.50^{a}	69.26±0.38^{ab}	68.80±0.54^{b}
	3	68.20±0.64^{c}	67.58±0.52^{d}	67.30±0.45^{d}	66.90±0.45^{de}
	6	67.50±0.75^{d}	66.45±0.48^{e}	66.32±0.50^{e}	65.92±0.54^{f}
Crude protein	0	18.20±0.40^{d}	17.75±0.44^{c}	17.20±0.36^{b}	16.70±0.40^{a}
	3	17.35±0.52^{a}	16.80±0.64^{b}	16.25±0.48^{b}	15.76±0.45^{b}
	6	16.40±0.55^{a}	15.90±0.65^{b}	15.42±0.56^{b}	14.98±0.55^{b}
Crude either extract	0	2.60±0.14^{d}	2.90±0.17^{d}	3.20±0.09^{e}	3.50±0.08^{e}
	3	3.40±0.14^{a}	3.70±0.15^{b}	4.00±0.15^{b}	4. 40±0.16^{c}
	6	3.50±0.12^{a}	4.00±0.12^{b}	4.30±0.14^{b}	4. 60±0.14^{c}
Ash	0	3.02±0.04^{g}	2.96±0.04^{f}	2.82±0.06^{e}	2.65±0.06^{d}
	3	4.14±0.11^{c}	4.00±0.05^{bc}	3.86±0.08^{b}	3.80±0.05^{ab}

	6	4.25±0.14^{bc}	4.15±0.06^{b}	4.02±0.05^{ab}	3.96±0.07^{a}
Crude Fiber	0	0.30±0.04^{i}	0.65±0.03^{gh}	0.90±0.05^{e}	1.30±0.04^{c}
	3	0.40±0.05^{h}	0.80±0.05^{f}	1.02±0.04^{d}	1.42±0.05^{b}
	6	0.46±0.03^{g}	0.92±0.04^{e}	1.24±0.06^{c}	1.50±0.04^{a}

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$). C: common carp fish burger, Q_1: common carp fish burger fortified with 5% quinoa seed powder. , Q_2: common carp fish burger fortified with 10% quinoa seed powder. , Q_3: common carp fish burger fortified with 15% quinoa seed powder**.**

4. Effect of quinoa seed powder as well as frozen storage at-18±1°C up to 6 months on chemical quality characteristics and pH value fish burgers

4.1. The total volatile base nitrogen (TVB-N) levels of burgers

The TVB-N levels of CCFB samples at the beginning of storage showed no significant differences among treatments, with values recorded at 13.04 mg N/100 g for the control and approximately 13.00 mg N/100 g for QSP-treated samples (Table 5). However, after six months of frozen storage at –18 ± 1°C, the TVB-N level in the control group significantly increased to 18.40 mg N/100g. In contrast, the QSP-fortified samples exhibited lower TVB-N values of 15.35, 14.20, and 13.90 mg N/100 g for the 1%, 2%, and 3% QSP treatments, respectively.

These reductions were statistically significant ($P < 0.05$) and correlated with increasing QSP concentrations. This trend supports the hypothesis that QSP possesses antibacterial properties, helping to suppress microbial activity and delay protein degradation **(Osman & Zidan, 2014; Dong *et al.*, 2020; Osheba *et al.*, 2024)**.

4.2. Thiobarbituric acid (TBA) index

Table (5) illustrates the changes in TBA values of fish burgers during frozen storage. At the beginning of storage, all CCFB treatments showed similar values (~0.42 mg MDA/kg). Over time, TBA values significantly increased ($P < 0.05$), indicating progressive lipid oxidation.

The control group had the highest TBA value (0.80 mg MDA/kg) at the end of storage, while the burger sample treated with 3% QSP had the lowest (0.50 mg MDA/kg). This inverse relationship between QSP concentration and TBA levels highlights the antioxidant efficacy of QSP’s bioactive compounds in neutralizing free radicals **(Campos-Rodriguez *et al.*, 2022)**. These findings align with previous reports demonstrating QSP’s ability to delay oxidative degradation in stored meat products **(Osheba *et al.*, 2024)**.

4.3. Peroxide value (PV)

Peroxide values (PV) of CCFB samples are presented in Table (5). At the start of storage, all samples showed a baseline PV of 0.50 meq/kg. By the end of the storage period, the control group exhibited the highest PV at 2.50 meq/kg, whereas the sample treated with 3% QSP had the lowest (1.07 meq/kg).

The reduced peroxide formation in QSP-treated samples confirms the antioxidant capacity of quinoa seed components, particularly in slowing hydroperoxide formation during frozen storage **(Osman & Zidan, 2014; Choque-Quispe *et al.*, 2021)**.

4.4. pH values

The pH values of burger samples stored at –18 °C for 0, 3, and 6 months are shown in Table (5). Across all treatments, pH increased over time. However, the control sample displayed a decrease in pH during the sixth month, whereas all QSP-treated samples exhibited a consistent upward trend, especially at higher concentrations.

This elevation in pH in the presence of QSP may reflect its antimicrobial action, which reduces microbial activity and subsequent acid production, thus slowing pH decline **(Abdel-Moatamed *et al.*, 2024)**. Additionally, the slightly alkaline nature of QSP—attributable to its polyphenols, sugars, minerals, and flavonoids—may contribute to this effect **(Osheba *et al.*, 2024)**.

The results emphasize QSP's dual role in enhancing the oxidative stability and modulating the pH environment, likely contributing to microbial suppression and improved shelf-life of stored burgers.

Table 5. Chemical quality characteristics and pH value of quinoa seed-fortified common carp fish burgers during frozen storage at -18±1°C for 6 months

Components (%)	Storage period (months)	Treatments			
		C	Q1	Q2	Q3
TVB-N (mg/100g)	0	13.02±0.35[f]	13.00±0.15[f]	13.04±0.16[f]	13.00±0.10[f]
	3	15.64±0.80[c]	13.50±0.22[d]	13.30±0.15[e]	13.20±0.16[e]
	6	18.40±0.68[a]	15.35±0.16[b]	14.20±0.14[c]	13.90±0.22[d]
TBA(mg malondialdehyde /kg)	0	0.42±0.05[f]	0.42±0.02[f]	0.40±0.03[f]	0.42±0.03[f]
	3	0.72±0.03[b]	0.56±0.04[c]	0.47±0.03[d]	0.42±0.04[e]
	6	0.80±0.04[a]	0.65±0.02[bc]	0.60±0.04[c]	0.50±0.03[d]
Peroxide value meq/kg	0	0.50±0.04[g]	0.51±0.03[g]	0.52±0.02[g]	0.50±0.03[g]
	3	1.85±0.07[b]	1.36±0.04[d]	1.03±0.03[e]	0. 90±0.05[f]
	6	2.50±0.12[a]	1.71±0.03[b]	1.45±0.04[c]	1.07±0.03[e]
pH	0	6.38±0. 03[f]	6.50±0.05[e]	6.58±0.03[d]	6.64±0.02[c]
	3	6.22±0. 04[g]	6.60±0. 04[d]	6.62±0. 05[c]	6.73±0. 03[b]
	6	6.07±0. 02[h]	6.66±0. 03[c]	6.71±0. 04[b]	6.84±0. 02[a]

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$). C: common carp fish burger, Q_1: common carp fish burger fortified with 5% quinoa seed powder. , Q_2: common carp fish burger fortified with 10% quinoa seed powder. , Q_3: common carp fish burger fortified with 15% quinoa seed powder.

5. Functional properties of fish burgers

Table (6) presents the functional properties of common carp fish burgers (CCFB) with and without quinoa seed powder (QSP) supplementation, including water holding capacity (WHC), oil holding capacity (OHC), and swelling capacity (SWC).

At the beginning of storage, WHC values increased significantly ($P< 0.001$) in quinoa-fortified samples compared to the control. This enhancement is likely due to the fiber content of quinoa, which has high water-retention capability. The ability of QSP to hold water suggests its potential as a functional food ingredient, helping to improve product texture, reduce dehydration during storage, and lower energy density by retaining moisture.

Similarly, OHC values were significantly higher (P < 0.001) in QSP-supplemented burgers than in the control. This increase in fat-binding capacity is particularly beneficial in industrial food processing and storage, where fat retention contributes to product quality and stability. These effects may be attributed to the excellent fat-binding ability of quinoa fibers and possibly microalgal-like cell wall structures **(Roohinejad *et al.*, 2016)**.

As for SWC, quinoa-fortified burgers, especially those containing 3% QSP, exhibited significantly higher swelling capacity than the control at the start of storage. This suggests that quinoa's fiber-rich composition enhances the matrix's ability to absorb water and expand, improving texture and moisture retention.

However, WHC, OHC, and SWC values in all burger samples declined during frozen storage. This reduction is likely due to structural changes in the protein and fiber matrix under prolonged freezing conditions, affecting their capacity to retain water and oil.

The differences observed between control and QSP-treated formulations may also result from the specific structural characteristics of quinoa fibers, which influence hydration and fat retention behaviors. These findings are consistent with previous studies reporting improved functional properties in meat and fish products fortified with plant-based fibers **(Özer & Secen, 2018; Zapata & Pava, 2018; Cristofel *et al.*, 2021)**.

Table 6. Functional parameters of quinoa seed-fortified common carp fish burgers during frozen storage at -18±1°C for 6 months

Components (%)	Storage period (month)	Treatments			
		C	Q1	Q2	Q3
SWC (mL/g DW)	0	2.92±0.11^{f}	3.45±0.07^{c}	3.74±0.16^{b}	4.12±0.18^{a}
	3	2.55±0.14^{g}	3.05±0.12^{f}	3.50±0.14^{c}	3.70±0.16^{bc}
	6	2.30±0.16^{h}	2.80±0.20^{g}	3.15±0.22^{e}	3.45±0.12^{d}
OHC (g/g DW)	0	0.82±0.03^{e}	1.12±0.05^{c}	1.28±0.06^{b}	1.35±0.04^{a}
	3	0.70±0.04^{f}	1.04±0.05^{d}	1.16±0.04^{b}	1.26±0.06^{b}
	6	0.57±0.02^{g}	0.94±0.03^{e}	1.05±0.06^{d}	1.14±0.04^{b}
WHC (g/g DW)	0	2.02±0.12^{g}	2.580.06^{d}	2.75±0.12^{c}	3.12±0.06^{a}
	3	1.94±0.11^{h}	2.44±0.15^{e}	2.70±0.14^{cd}	2.94±0.12^{b}
	6	1.70±0.14^{i}	2.32±0.08^{f}	2.60±0.05^{d}	2.77±0.16^{c}

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$). C: common carp fish burger, Q_1: common carp fish burger fortified with 5% quinoa seed powder. , Q_2: common carp fish burger fortified with 10% quinoa seed powder. , Q_3: common carp fish burger fortified with 15% quinoa seed powder.

6. Antioxidant properties of fish burgers:

Table (7) shows that fish burgers fortified with quinoa seed powder (QSP) exhibited significantly higher ($P < 0.05$) antioxidant activity and carotenoid content at the beginning of storage compared to the control. The DPPH radical scavenging activity of CCFB samples fortified with 1%, 2%, and 3% QSP was 66.00%, 81.20%, and 89.70%, respectively—markedly higher than the 47.50% observed in the control group ($P < 0.05$). A similar trend was observed in total phenolic content (TPC). Specifically, TPC values for unfortified and QSP-fortified CCFB samples were 10.70, 24.50, 35.40, and 48.20 mg/100 g for the control, 1%, 2%, and 3% QSP treatments, respectively ($P < 0.05$). These results confirm that increasing the concentration of QSP significantly boosts the antioxidant capacity of fish burgers.

Although antioxidant activity and carotenoid content declined in all treatments during frozen storage, the QSP-fortified burgers consistently maintained higher levels than the control. The reduction may be attributed to oxidative degradation during prolonged storage.

The enhancement in antioxidant activity may be linked to the presence of polysaccharides and polyphenols in quinoa, which have been shown to support enzymatic activity and DNA repair mechanisms, in addition to providing immune system benefits **(Zhu, 2020)**. These components, particularly phenols, flavonoids **(Wang *et al.*, 2022)**, and polysaccharides **(Tan *et al.*, 2021)**, contribute to the increased free radical scavenging activity observed in the QSP-fortified burgers.

Importantly, the results indicate that fish burger processing did not negatively affect the antioxidant components of QSP. The findings in this study are consistent with those reported by **Haiam and Elsawy (2021)**, who observed similar enhancements in antioxidant potential in fish burgers formulated with quinoa seed powder.

Table 7. Antioxidant activities and pigments content of quinoa seed-fortified common carp fish burgers during frozen storage at -18±1°C for 6 months

Components	Storage period (months)	Treatments			
		C	Q1	Q2	Q3
Carotenoids (mg/100g DW)	0	0.00	1.04±0.05^{f}	1.72±0.09^{c}	2.55±0.12^{a}
	3	0.00	0.88±0.06^{g}	1.44±0.08^{d}	2.32±0.07^{b}
	6	0.00	0.79±0.08^{h}	1.30±0.09^{e}	1.98±0.06
TPC mg/100g	0	10.70±1.12^{dh}	24.50±1.1^{e}	35.40±1.4^{c}	48.20±1.2^{a}
	3	7.20±0.88^{i}	19.70±1.5^{f}	31.20±1.6^{d}	36.80±1.5^{b}
	6	3.30±0.85^{j}	15.20±1. 3^{g}	26.40±1. 2^{e}	32.50±1.4^{d}
Scavenging activity (%) *	0	47.50±2.2^{i}	66.00±1.4^{g}	81.20±1.6^{c}	89.70±1.5^{a}
	3	41.80±1.6^{k}	60.80±1.2^{h}	74.90±1.5^{e}	84.20±1.8^{b}
	6	35.20±2.3^{l}	47.30±1.5^{j}	71.20±1.9^{f}	77.30±1.4^{d}

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$). C: common carp fish burger, Q_1: common carp fish burger fortified with 5% quinoa seed powder. , Q_2: common carp fish burger fortified with 10% quinoa seed powder. , Q_3: common carp fish burger fortified with 15% quinoa seed powder.

7. Microbiological quality standards for quinoa seed -fortified catfish burgers

Table (8) presents the effects of different concentrations of quinoa seed powder (QSP) (0%, 1%, 2%, and 3%) on microbiological parameters—including total bacterial count, total yeast and mould count, total coliform group, and total *Staphylococcus aureus* count—in common carp fish burgers (CCFB) during frozen storage at −18 °C for 0, 3, and 6 months.

Total bacterial count

The logarithmic values of total bacterial counts ($\log_{10}$ cfu/g) for the 1% QSP CCFB treatment were 5.02, 3.70, and 2.60 over 0, 3, and 6 months, respectively. The 2% QSP treatment recorded values of 5.04, 3.05, and 2.46, while the 3% QSP treatment showed the lowest values: 5.03, 2.60, and 2.32. In contrast, the control group showed significantly higher bacterial loads: 5.06, 4.87, and 3.38.

These results demonstrate that QSP significantly reduced the total bacterial count throughout the storage period, with the effect becoming more pronounced at higher concentrations.

Total yeast and mold count

For yeast and mold, the logarithmic counts at 0, 3, and 6 months were as follows:

- 1% QSP: 3.82, 2.58, 1.07
- 2% QSP: 3.80, 2.49, 1.00
- 3% QSP: 3.82, 2.42, 0.93

The progressive reduction in yeast and mould counts over time in QSP treatments indicates its potential antifungal activity compared to the control.

Total coliform group

The total coliform group counts ($\log_{10}$ cfu/g) were:

- 1% QSP: 3.94, 3.28, 2.70
- 2% QSP: 3.90, 3.16, 2.37
- 3% QSP: 3.91, 3.08, 2.22
- Control: 3.96, 3.66, 3.14

As with bacterial counts, coliform numbers declined more significantly in QSP-treated burgers, particularly with 3% QSP, suggesting an inhibitory effect on coliform growth during frozen storage.

***Staphylococcus aureus* count**

The *Staphylococcus aureus* counts were:

- 1% QSP: 4.22, 3.47, 1.36
- 2% QSP: 4.23, 3.26, 1.24
- 3% QSP: 4.24, 3.03, 1.28
- Control: 4.25, 4.00, 3.26

These values again show a significant reduction ($P < 0.05$) in microbial counts with increasing QSP concentrations, emphasizing the antimicrobial effectiveness of QSP against pathogenic bacteria.

Overall, the results indicate that increasing levels of QSP supplementation effectively reduced microbial load across all parameters during six months of frozen storage. The antimicrobial properties of quinoa may be attributed to its phenolic compounds, saponins, and flavonoids, which are known to disrupt microbial cell membranes and inhibit microbial metabolism.

The observed trends support the conclusion that QSP enhances microbiological stability and extends the shelf life of frozen fish burgers. These findings are consistent with earlier reports by **Cristofel *et al.* (2021)** and **El-Sohaimy *et al.* (2022)**, who highlighted the antimicrobial potential of plant-based additives in meat and fish preservation.

Table 8. Change in microbial counts (log10 cfu/g) of quinoa seed-fortified common carp fish burgers during frozen storage for 6 months

Components (%)	Storage period (month)	Treatments			
		C	Q1	Q2	Q3
Total bacterial count (log10 cfu/g)	0	5.06±0.02^{a}	5.02±0.04^{a}	5.04 ±0.03^{a}	5.03±0.05^{a}
	3	4.87±0.04^{b}	3.70±0.05^{c}	3.05±0.04^{e}	2.60±0.03^{f}
	6	3.38±0.03^{d}	2.60±0.04^{f}	2.46±0.05^{fg}	2.32±0.03^{g}
Total yeast and mold count (log10 cfu/g)	0	3.80±0.06^{a}	3.82±0.03^{a}	3.80±0.02^{a}	3.82±0.05^{a}
	3	3.05±0.02^{b}	2.58±0.04^{cd}	2.49±0.05^{cd}	2.42±0.04^{e}
	6	2.80±0.05^{d}	1.07±0.06^{f}	1.00±0.04^{f}	0.92±0.03^{g}
Total Coliform group count (log10 cfu/g)	0	3.96±0.04^{a}	3.94±0.04^{a}	3.90±0.08^{a}	3.91±0.05^{a}
	3	3.66±0.03^{b}	3.28±0.05^{c}	3.16±0.03^{d}	3.08±0.04
	6	3.14±0.08^{d}	2.70±0.04^{f}	2.37±0.05^{g}	2.22±0.05^{h}
Total Staphylococcus count (log10 cfu/g)	0	4.25±0.07^{a}	4.22±0.05^{a}	4.23±0.02^{a}	4.24±0.04^{a}
	3	4.00±0.05^{b}	3.47±0.03^{c}	3.26±0.05^{cd}	3.04±0.03^{d}
	6	3.26±0.07^{cd}	1.36±0.04^{e}	1.24±0.06^{ef}	1.15±0.06^{f}

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$). C: common carp fish burger, Q_1: common carp fish burger fortified with 5% quinoa seed powder. , Q_2: common carp fish burger fortified with 10% quinoa seed powder. , Q_3: common carp fish burger fortified with 15% quinoa seed powder.

8. Sensory evaluation of quinoa seed-fortified fish burgers

Table (9) presents the results of the sensory evaluation for common carp fish burgers (CCFB), assessing key attributes such as flavor, aroma, color, and texture. Overall, the hedonic scores indicated that burgers fortified with 10% (w/w) quinoa seed powder (QSP) received the highest panel ratings for most organoleptic attributes.

Fish burgers containing 3% QSP generally showed lower sensory acceptance scores compared to those with lower concentrations and the control group ($P < 0.05$). Specifically, sensory attributes such as colour and taste were negatively affected at this higher concentration. In contrast, burgers with 1% and 2% QSP maintained comparable sensory characteristics to the control, with minimal differences noted in flavor and aroma. Interestingly, taste scores for burgers containing 10% QSP were statistically similar to the control group ($P > 0.05$), indicating acceptable palatability at this level of fortification.

However, as the concentration of QSP increased beyond 10%, particularly at 15%, a noticeable decline in taste and colour acceptance was observed, suggesting sensory saturation or undesired textural changes at higher inclusion levels.

Regarding color, a gradual decline in hedonic ratings was observed with increasing QSP concentration. At lower inclusion levels, however, color remained comparable to the control. The darker hue associated with higher quinoa concentrations likely contributed to reduced visual appeal.

In terms of texture, some panellists noted improvements with moderate QSP addition. However, statistically significant improvements ($P < 0.05$) were only observed in comparisons between the QSP treatments and the control, particularly for burgers containing up to 10% QSP.

Overall, treatments containing 15% QSP were the least preferred in terms of flavour and appearance, although panellists still found them acceptable. These results suggest that 10% QSP is the optimal concentration at which quinoa flour can be added to fish burgers without negatively affecting sensory quality. This level maintains consumer acceptance while also providing the nutritional benefits of quinoa.

As expected, sensory scores for all treatments declined as the frozen storage period increased, reflecting changes in texture, flavor, and appearance over time. These findings align with those of **Haiam and Elsawy (2021)**, who reported that the substitution of meat with up to 10% quinoa flour in burger formulations maintained favorable sensory acceptance.

Table 9. Sensory evaluation of supplemented common carp fish burgers during frozen storage (at -18 °C for 6 months)

Components (%)	Storage period (months)	Treatments			
		C	Q1	Q2	Q3
Colour	0	$8.30±0.12^{a}$	$8.30±0.22^{a}$	$8.20±0.14^{ab}$	$8.00±0.16^{b}$
	3	$7.30±0.16^{c}$	$7.30±0.15^{c}$	$7.10±0.20^{d}$	$7.00±0.13^{d}$
	6	$6.80±0.18^{de}$	$6.70±0.18^{de}$	$6.50±0.15^{e}$	$6.40±0.16^{e}$
Taste	0	$7.30±0.14^{a}$	$7.20±0.16^{ab}$	$7.10±0.18^{b}$	$7.00±0.14^{c}$
	3	$7.10±0.15^{b}$	$7.20±0.15^{ab}$	$6.90±0.22^{bc}$	$6.70±0.16^{c}$
	6	$7.00±0.12^{b}$	$6.80±0.14^{bc}$	$6.70±0.18^{c}$	$6.50±0.15^{d}$
Odor	0	$7.80±0.20^{b}$	$7.90±0.12^{b}$	$8.00±0.16^{ab}$	$8.20±0.17^{a}$
	3	$7.50±0.14^{c}$	$7.60±0.18^{bc}$	$7.80±0.21^{b}$	$8.00±0.13^{ab}$
	6	$7.20±0.15^{d}$	$7.30±0.17^{cd}$	$7.50±0.15^{c}$	$7.70±0.16^{bc}$
Texture	0	$7.40±0.12^{bc}$	$7.60±0.15^{b}$	$7.80±0.18^{ab}$	$8.00±0.22^{a}$
	3	$7.00±0.13^{cd}$	$7.30±0.14^{c}$	$7.50±0.17^{b}$	$7.80±0.15^{ab}$
	6	$6.80±0.15^{d}$	$7.10±0.12^{cd}$	$7.30±0.16^{c}$	$7.50±0.16^{b}$

* Values (means ±SD) with different superscript letters are statistically significantly different ($P \leq 0.05$). C: common carp fish burger, Q_1: common carp fish burger fortified with 5% quinoa seed powder. , Q_2: common carp fish burger fortified with 10% quinoa seed powder. , Q_3: common carp fish burger fortified with 15% quinoa seed powder.

CONCLUSIONS

Quinoa seed, being rich in dietary fiber and bioactive compounds, significantly enhances the functional properties of fish-based products—particularly water and oil holding capacities, which are essential for maintaining product texture and quality. The results of this study support the recommendation to incorporate 10% quinoa seed powder into fish burger formulations. At this level, quinoa not only improves the nutritional value but also maintains acceptable sensory characteristics, offering a natural and health-promoting alternative to synthetic additives. Given its favorable nutritional profile, technological functionality, and consumer acceptability, quinoa seed presents itself as a viable natural fortifying agent in fish-based products. It contributes to the development of healthier, more satisfying food options while eliminating the need for artificial chemical fortifiers, which may have adverse health effects. Thus, quinoa seed can be considered an effective and sustainable ingredient for improving both the quality and health appeal of processed fish products.

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