Effects of Long-Term Frozen Storage on Fat-Soluble Vitamin Content in the Muscle of Common Carp (*Cyprinus carpio* L.)

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Abstract

In this study, the stability of fat-soluble vitamins (A, D, E, and K) in common carp (*Cyprinus carpio* L.) during frozen storage at –18°C for up to 12 months was investigated. It was found that HPLC analysis showed that there is a statistically significant decrease ($p \leq 0.05$) of the vitamins' concentration with time. The highest level of loss was shown by vitamin D (59.1%) and vitamin K (55.9%), while vitamin A and vitamin E loss were 50.7% and 49.8%, respectively. This progressive decline of the vitamin's concentration suggests that a considerable decrease in its nutritional value occurs with prolonged frozen storage. This work contributes to the knowledge about the chemical stability of vitamins in fish muscle and provides important information concerning their quality for both aquaculture and the food industry. The presented data show that nutrition value is considerably reduced after six months of frozen storage.

Keywords: *Cyprinus carpio*, Fat-soluble vitamins, High-performance liquid chromatography, Frozen storage, Vitamin degradation kinetics.

Introduction

Common carp (*C. carpio* L.) is one of the leading fish species used in aquaculture globally owing to their highly efficient production process as well as nutritional benefits (FAO, 2024). It is one of the fish species with high amounts of high-quality proteins and unsaturated fats (Ma *et al*., 2023). Common carp fish is one of the fish species with fat-soluble vitamins A, D, E, and K necessary for boosting the immune system, metabolism, vision, and bone structure (Andrès *et al*., 2024).

Fat-soluble vitamins are extremely important vitamin A is responsible for cell differentiation and vision (Xie *et al*., 2025), vitamin D controls calcium and phosphorous balance which is necessary for bone health; vitamin E acts as a powerful antioxidant

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protecting cell membranes and vitamin K plays an important role in blood clotting (Sadler *et al*., 2024). However, despite being extremely important, these vitamins demonstrate chemical instability, being highly sensitive to temperature, sunlight, and oxygen, making it difficult to preserve their stability (Herrera Ardila *et al*., 2022

Freezing is considered to be among the most popular and efficient techniques of preservation of fish due to the dramatic decrease in microbial and biochemical activity. Though the extremely low temperatures during the process of freezing dramatically decrease biochemical reactions, gradual changes may occur during long-term storage (Du *et al*., 2023). One of the most significant processes is called lipid oxidation. This is a process of gradual oxidation of lipids, causing the decrease in vitamin concentrations, especially in vitamin E, this results in a progressive decrease in the amount of vitamins, particularly vitamin E, which acts as the first line of protection against free radicals. The stability of vitamins D and K is highly variable, demonstrating the dynamism of these vitamins while stored frozen (Rakuša *et al*., 2021; You *et al*., 2024)

Although several studies have been performed on the influence of freezing on fish species like herring and mackerel, it appears that there are very few empirical results on the stability of fat-soluble vitamins in the common carp (*Cyprinus carpio* L.) species. As a result, the existing knowledge base concerning nutritional stability in the most cultivated fish in the world remains underdeveloped, thus requiring scientifically-based shelf-life guidelines to be set forth. Therefore, the purpose of the current research is to conduct an adequate quantitative and qualitative analysis of the loss of vitamins A, D, E, and K in carp muscle at freezing storage times of 0, 3, 6, 9, and 12 months using high-performance liquid chromatography (HPLC).

Materials and Methods

Sample Preparation

Fresh common carp (*Cyprinus carpio* L.) samples were obtained from local markets in Basra Governorate and then transported to the Chemical Analysis Laboratory of the Marine Science Center in cold storage. All samples were ensured to be fresh when purchased. The samples had weights between 500-1000g and total lengths of 28-35cm. A total number of 15 fishes were used in the experiment; 3 fish in the control (fresh), while other 3 fishes in each experimental group (kept at–18°C for 3, 6, 9 and 12 months). Fishes were used as experimental units and their muscles tissues were taken for vitamins determination. Samples in the control group were analyzed before storing, while the rest of the groups were analyzed after storing period.

Extraction of Fat-Soluble Vitamins

The fat-soluble vitamins (A, D, E, and K) were extracted using the method by Dahl-Lassen *et al*. (2018), slightly modified. Around 0.05-0.1 g of the muscle tissue sample was weighed, and then 5 mL of methanol with 0.1% butylated hydroxytoluene (BHT) was added. The purpose of using BHT was an antioxidant which helped avoid vitamin destruction during the process of extraction. To regulate pH of the reaction medium, a 0.4

M lithium perchlorate (pH 2.4) solution was used. The extract was kept in Opaque containers for 2 hours under constant stirring for 20 minutes at room temperature. The next step was isolating the extract by centrifuging at 10,000 rpm for 10 minutes. A volume of 100 µL was injected into the HPLC system for analysis.

HPLC Chromatographic Conditions

The analysis was performed using an HPLC system (SYKAM, Germany) under the following operating conditions:

- **Column:** C18–ODS (25 cm × 4.6 mm)
- **Mobile phase:** Acetonitrile/distilled water mixture (75:25, v/v) in isocratic mode
- **Flow rate:** 0.7 mL/min
- **Detector:** Ultraviolet (UV) detector at 280 nm

Statistical analysis

Statistical analysis was performed using SPSS software (Version 26). A one-way analysis of variance (ANOVA) was applied to determine differences among storage periods, followed by Duncan's multiple range test for post-hoc comparisons at a significance level of **(**$p \leq 0.05$**)**. Results were expressed as means ± standard deviation (Mean ± SD).

Results and Discussion

Degradation of Vitamins and Its Relationship to Lipid Stability

Vitamin A (Retinol)

Vitamin A recorded the highest loss among the studied vitamins, declining significantly ($p \leq 0.05$) from 12.81 ppm in fresh samples to 6.31 ppm after 12 months of frozen storage, representing a total loss of 50.7% (Table 2). The degradation curve (Fig. 1) shows a marked decline after six months. Similar findings have been reported in fatty fish species during frozen storage (Li *et al.*, 2025; Du *et al.*, 2023), and recent reviews confirm that retinol is chemically unstable under long-term storage conditions, leading to significant nutritional losses (Abeywardana *et al.*, 2026).

Vitamin E (α-tocopherol)

The concentration of vitamin E declined significantly ($p \leq 0.05$) from 33.56 ppm in fresh samples to 16.86 ppm after 12 months of frozen storage, representing a loss of more than 49.8 % (Table 1). This progressive reduction is clearly illustrated in the percentage-loss curve (Fig. 1), where vitamin E shows a sharp decline after six months. Tocopherol depletion has been widely recognized in previous studies on fatty fish species (Wang *et al.*, 2016; López-Puebla *et al.*, 2025). Comparable decreases have also been reported in herring and mackerel (Gimmel *et al.*, 2022), underscoring the universal role of vitamin E in maintaining muscle quality in aquaculture species (El Sayed and Izquierdo, 2021).

Vitamin K

A statistically significant reduction was observed ($p \leq 0.05$) in vitamin K content from 20.58 ppm in fresh samples to 9.06 ppm following 12 months of frozen storage, representing a reduction of about 55.9%. The decline trend (Fig. 1) suggests gradual reduction with marked reduction occurring after 6 months of storage. Previous studies have established that vitamin K is sensitive to light and oxygen, which makes it unstable under storage conditions (Silva *et al.*, 2024; Hassan *et al.*, 2025). Considering the few numbers of studies available on vitamin K stability in fish, the current results offer new information regarding significant loss in vitamin K content during frozen storage.

Vitamin D

The vitamin D content showed a considerable reduction ($p \leq 0.05$) from 11.09 ppm in fresh samples to 4.54 ppm upon storing for 12 months under frozen conditions, amounting to an overall loss of 59.1% (Table 2). An accelerated reduction was noted from the sixth month onwards (Fig. 1). The consistent retention time (8.16-8.22 minutes) similar to that of the reference standard peak indicated the reduction to be quantifiable and due to the vitamin D compound, itself. Such patterns of loss have been noted in other fatty fish species stored under frozen conditions (Cheng *et al.*, 2023; Hu and Xie, 2021; Knuth *et al.*, 2021; Ladole *et al.*, 2018).

Table 1: Levels of vitamins (Mean ± SD) in the muscle of fish during the frozen storage at -18°C for 12 months.

Storage Duration	Vitamin A (ppm)	Vitamin E (ppm)	Vitamin K (ppm)	Vitamin D (ppm)
Control (Zero)	12.81 ± 0.26^{a}	33.56 ± 0.37^{a}	20.58 ± 0.29^{a}	11.09 ± 0.23^{a}
3 Months	11.95 ± 0.24^{b}	32.48 ± 0.36^{b}	19.00 ± 0.32^{b}	10.42 ± 0.22^{b}
6 Months	8.88 ± 0.34^{c}	25.78 ± 1.20^{c}	14.56 ± 0.88^{c}	8.68 ± 1.20^{c}
9 Months	6.69 ± 0.57^{d}	19.83 ± 0.84^{d}	10.92 ± 0.67^{d}	6.01 ± 0.57^{d}
12 Months	6.31 ± 0.54^{d}	16.86 ± 0.58^{e}	9.06 ± 0.61^{e}	4.54 ± 0.66^{e}

Note: Data are presented as Mean ± SD ($n = 3$). Values within the same column followed by different lowercase superscript letters are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Table 2: Percentage loss of fat-soluble vitamins in common carp (*Cyprinus carpio*) muscles during frozen storage at -18°C for up to 12 months ($n = 3$).

Storage Period (Months)	Vitamin A Loss %	Vitamin E Loss %	Vitamin K Loss %	Vitamin D Loss %
3	6.7	3.2	7.7	6.0
6	30.7	23.2	29.2	21.7
9	47.8	40.9	46.9	45.8
12	50.7	49.8	55.9	59.1

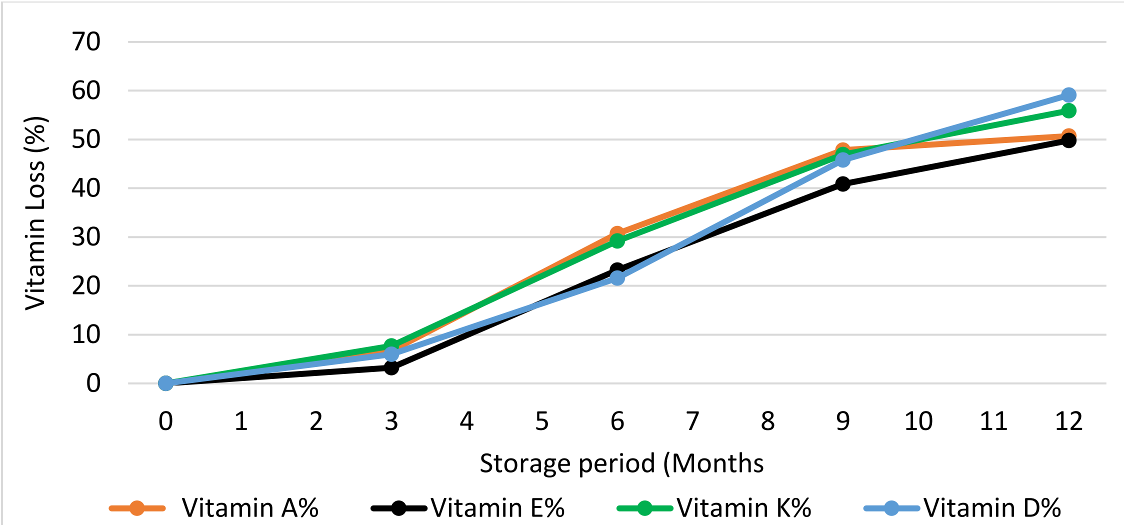

Fig. 1. Percentage loss curves of fat-soluble vitamins (A, D, E, and K) in common carp (*Cyprinus carpio*) muscles during a 12-month frozen storage period at -18°C.

Method Selectivity and analytical reliability

These results are supported by chromatographic analysis, which demonstrated baseline stability and clear peak resolution without any interference. This is considered a strong indicator of method selectivity according to ICH Q2(R2) guidelines (2023). Such evidence confirms that the degradation products resulting from lipid rancidity and cellular disruption did not generate chemical impurities affecting the original peaks. Recent studies have validated similar stability-indicating HPLC methods for vitamins, proving that the loss of vitamins was exclusively due to frozen storage conditions rather than analytical errors or unwanted interferences (Dobreva *et al*., 2013; Coad and Bui, 2020; Żandarek *et al*., 2023).

Nutritional Standards and reliability of results

When the data from the twelfth month is compared with the nutritional standards, it clearly indicates that the nutritive value of fish deteriorates due to significant depletion of fat-soluble vitamins. This finding agrees with Liu (2025) because she found significant depletion of vitamins in the case of frozen fish stored for a long time. The relatively low standard deviation among replicates in the analysis shows the high precision and reproducibility of the method, but it does not give an insight into the chemical processes behind the degradation of the vitamin. The statistical methods used for comparison are current: Gimmel *et al*. (2022) proved reproducible patterns of vitamin stability by means of HPLC; Azeem *et al*. (2025) predicted vitamin C degradation with the help of Bayesian inverse Weibull models; and Hu *et al*. (2025) showed that the vitamin A decrease follows predictable global trends with the use of ARIMA forecasting. Overall, these results confirm the accuracy of measurements, and vitamin loss during the frozen storage is systematic (Ali *et al*., 2022; Khan *et al*., 2024). Statistical analysis confirmed significant differences among storage durations ($p \leq 0.05$), supporting the recommendation to limit frozen storage of fish to six months to preserve nutritional quality (You *et al*., 2024; Li *et al*., 2025).

General Interpretation

Collectively, the progressive decline of vitamins A, D, E, and K (Tables 1,2; Fig. 1) confirms that the nutritional value of carp muscle decreases significantly **(**$p \leq 0.05$**)** after six months, supporting the recommendation to limit frozen storage to this period for optimal quality. According to previous literature, this systematic and reproducible degradation pattern across all studied vitamins is typically attributed to the secondary effects of frozen storage, such as lipid oxidation and cryoconcentration phenomena, which accelerate the chemical breakdown of fat-soluble nutrients over time.

Conclusion

This study demonstrates that fat-soluble vitamins (A, D, E, and K) in common carp undergo systematic and progressive losses during frozen storage at −18°C. Vitamin D showed the highest reduction (59.1%), followed by vitamins K (55.9%), A (50.7%), and E (49.8%). The data (Tables 1–2; Fig. 1) indicate that the nutritional value declines significantly ($p \leq 0.05$) after six months, even though physical quality may remain acceptable. However, the mechanisms underlying this deterioration could not be confirmed in the present study, since oxidative markers (TBARS, peroxide value), cryoconcentration indicators (ice crystal formation, water migration), and other quality parameters (microbial load, sensory attributes, texture, nitrogenous bases, pH) were not measured. Consequently, the noted decrease ought to be regarded as a measurable diminishment in vitamin levels, while additional research is necessary to ascertain the exact reasons and to formulate thorough shelf-life guidelines for frozen carp.

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تأثير الحفظ بالتجميد طويل الأمد على محتوى الفيتامينات الذائبة في الدهون في عضلات أسماك الكارب الشائع (*Cyprinus carpio* L)

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المستخلص

تهدف هذه الدراسة إلى تقييم استقرار الفيتامينات الذائبة في الدهون (A ،D ،E ،K) في أسماك الكارب الشائع (*Cyprinus carpio* L.) خلال فترة التخزين المجمد عند –18°م لمدة تصل إلى 12 شهرًا. أظهرت نتائج التحليل باستخدام كروماتوغرافيا السائل عالية الأداء (HPLC) انخفاضًا معنويًا (P < 0.05) في تراكيز الفيتامينات بمرور الوقت. كان فيتامين D الأكثر انخفاضًا بنسبة 59.1%، يليه فيتامين K بنسبة 55.9%، ثم فيتامين A بنسبة 50.7%، وأخيرًا فيتامين E بنسبة 49.8%. يعكس هذا التراجع التدريجي انخفاضًا ملحوظًا في القيمة الغذائية خلال التخزين الطويل الأمد. وتوفر هذه النتائج رؤى مهمة حول الثبات الكيميائي للفيتامينات في عضلات اسماك الكارب، وتبرز انعكاساتها على جودة المنتج في قطاع الاستزراع المائي والصناعات الغذائية. وتشير النتائج إلى أن القيمة الغذائية تنخفض بشكل كبير بعد ستة أشهر من التخزين المجمد، مما يستدعي إجراء المزيد من الدراسات لوضع توصيات دقيقة لعمر التخزين المسموح به.

الكلمات المفتاحية: *Cyprinus carpio*، الفيتامينات الذائبة في الدهون، كروماتوغرافيا السائل عالية الأداء (HPLC)، الحفظ بالتجميد، تحلل الفيتامينات.