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Impact of Glazing and Freezing Preservation on the Chemical Composition and Caloric Value of Common Carp, *Cyprinus Carpio* L.1758.

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Abstract

The study focused on the preservation of economically important common carp *Cyprinus carpio* L. by glazing and evaluated the characteristics and properties changes in nutritional composition during fresh and frozen flesh at (–18± 2) °C compared to fresh fish (before treatment in glazing solutions). Fish samples were subjected to four treatments, including treatment control (non-treatment), immersion in drinking water, 5% table salt solution, and 5% white vinegar solution T1, T2, T3, and T4, respectively, measurement of their quality as well as suitability for human consumption, for three months 30, 60, and 90 days. The statistical analysis results showed that at a probability level of $p<0.05$, moisture, protein, and fat contents in T1 and T2 decreased, while T3 and T4 showed preservation benefits over 90 days. In all treatments, the ash contents increased with extended freezing. Caloric content from protein and fat exceeded that of the fresh sample, suggesting potential nutritional losses during frozen preservation. The reason for maintaining nutritional (moisture, protein, fat, ash, and Caloric content) value may be due to the synergistic effect of the glazing materials. However, the study identified a 5% table salt solution and white vinegar as ideal glazing materials for preserving the nutritional value of fish. In conclusion, storage of these fish species for up to 90 days did not adversely affect their nutritional value.

Keywords: Impact of Glazing, *Cyprinus Carpio*, Chemical Composition, Caloric Value.

Introduction

Fish resources worldwide are essential for economic development and a vital natural resource supporting millions of people (1). With the significant growth in water farming and the increasing global demand for fish meat due to its high nutritional value, distinctive flavor, and ease of preparation, carp has become a significant economic fish in water bodies. Due to its adaptability to the environment and ease of cultivation, carp is considered one of the most important types of fish that reproduce in Iraqi fish farms (2).

Due to its high protein and fat content, which are believed to support the maintenance of good health through the prevention and treatment of cardiovascular, inflammatory, and neurological conditions, fish is now the ideal food and an essential part of the human diet(3,4). In addition to a diverse range of soluble vitamins such as A, E, D, and K, fish oils are rich in unsaturated fatty acids, particularly omega-3 fatty acids (5). Due to its elevated moisture content and near-neutral pH, which create favorable conditions for microbial growth in the cardiovascular system, fish is considered highly perishable and susceptible to contamination by harmful microbes on its surface and internal organs. To mitigate spoilage, various preservation methods are employed(6,7). Glazing, a method that involves immersing the fish in cold water to prevent lipid oxidation during freezing, creates a thin layer of ice crystals. The function of this layer resulting from glazing is to prevent the penetration of moisture and oxygen and improve the quality of fishery products in freezing conditions (8). These layers effectively reduce protein texture alterations and water loss in frozen fish meat (9).

The fish industry, which encompasses both fish production and its byproducts, has evolved into one of the most thriving sectors, extending beyond the traditional practice of raw consumption (after boiling).Many types of fish undergo repeated freezing for transportation to distant nations and markets, allowing for prolonged preservation as a sustainable source of sustenance for humans (10). Therefore, the current study aims to increase the shelf life of frozen fish and evaluate its nutritional value using safe preservation techniques, especially glazing with effective materials that improve the quality of fish under freezing conditions, such as carp, due to the increased demand for it.

Materials and methods

Fish Sample : The common carp *Cyprinus carpio* used in this study was obtained from local markets in Basra city in September 2022. The samples were transported using an insulated container cooled with ice to the Chemical Analysis Laboratory of the Vertebrate Department at the Marine Science Center. Upon arrival at the laboratory, the fish were washed well to remove any contaminants. The weights of the fish ranged from (199.78 - 257.5) gm and the total length was from (20.85 - 25.6) cm. Then, a random sample was taken to conduct a chemical composition analysis on a fresh fish sample. These samples were divided into four treatments, each with three

replicates, and the glazing solutions were prepared, including treatment (T1) as a control group without additives, treatment (T2) was immersed in drinking water only, treatment (T3) using table salt with a purity of 98.5%, which is available in local markets under the name of iodized salt and prepared by American Garden Company - United States of America, by the wet salting method with a concentration of 5% sodium chloride, i.e. a ratio of (1:1) ml/gm of fish, and treatment (T4) used white vinegar with a concentration of 5%, which is available in local markets under the name Natural Distilled white and prepared by the same company above, where all treatments were completely immersed in the glazing solutions prepared for use by placing them in containers suitable for immersion.

Glazing Procedure

Before the treatment, the samples were frozen for four days at -18±2°C. After that, the fish were kept alive in the glazing solutions (T1, T2, T3, and T4) that were made using the immersion method to create an ice layer that would protect them from air exposure and slow down oxidative processes. Then they were frozen again for different periods of 30, 60, and 90 days, three replicates for each treatment. Then the changes in the chemical composition and calorific value of all treatments were monitored and compared with fresh fish samples before treatment and freezing.

Chemical Composition Evaluation

The fat percentage was calculated using the soxhlet apparatus and the organic solvent hexane according to the method (12).

Caloric Value

According to the procedure described in (13), the caloric value was calculated as digestible energy in kcal/100 g = Total protein × 4 + Total fat × 9, where the numbers 4 and 9 represent the amount of energy provided to the body in calories per 1 gram of protein and fat, respectively.

Statistical analysis

Experimental data was statistically analyzed using SPSS Statistics v.20.0, and the results are reported as the mean ± standard deviation. Univariate Analysis of Variance (ANOVA) was analyzed to determine differences, and $p < 0.05$ was considered statistically significant.

Results and Discussion

Table (1) shows the analysis of the chemical composition and calorific value of fresh common carp fish, where the percentage of moisture, protein, fat, ash, and calories reached 74.37, 17.58, 4.45, 1.49%, and 110.39 kcal, respectively. The moisture percentage was lower than that obtained by (14), which reached about 78.20%, and higher in each of (protein, fat, and ash), which reached 16.89, 3.43, and 1.44%, respectively. The calorific value was 98.5 kcal/100 g. When studying the nutritional value of common carp *Cyprinus carpio* L. from different sources, the results were consistent with the results (15) when studying the health status of imported carp and the effect of freezing on the nutritional value (protein, fat, and ash) compared to fresh, which amounted to about 17.62%, 4.54%, and 1.17% respectively, due to many

factors, including food composition, as diet, feeding habits, feeding rate, age, size, gender, habitat, genetic characteristics, and season/migration play an important role in determining the chemical composition of different fish species (16,17)

Table 1: Chemical composition and caloric value of fresh fish samples.

Chemical Composition	Moisture %	Protein %	Fat %	Ash %	caloric value kcal / 100 g
	74.36	17.58	4.45	1.49	110.37

Effect of glazing and freezing on fish

The ice glazing application rate was maintained at 5%, within the acceptable range of 2 to 20%. It is crucial to note that excessive glazing, particularly in specific fish products, can significantly impact the economic value and consumer acceptance of frozen fish (18). Freezing storage presents challenges for maintaining the quality of high-water activity and neutral pH products, such as common carp. However, this preservation method may have adverse effects on nutritional value, freshness, and overall quality. Freezing induces various chemical reactions, including fat and protein oxidation, protein breakdown, and ice crystal crystallization, leading to undesirable characteristics such as unpleasant tastes, dryness, weight loss, juice loss, and susceptibility to microbial spoilage (19,20,21).

Moisture

From the statistical analysis shown in Table (2), it was found that the effects of freezing fish at 18±2°C for different amounts of time and treating them with 5% white vinegar for 60 days were significantly different at the $p<0.05$ level. The T4 therapy had the greatest overall average (74.19%). Extending the storage period to 90 days revealed a slight decrease in the moisture content across all treatments (T4, T3, T2, T1), reaching 72.92, 73.82, 73.63, and 73.93% compared to the fresh fish sample, which had a moisture content of 74.37%. However, the general average in the T1 treatments was the lowest moisture percentage at 73.68%. The results of (22)'s investigation, which evaluated the impact of frozen storage on the chemical composition of five freshwater commercial fish species in the Nile River, aligned with the decreased moisture content of the tested samples. Comparatively, the study conducted by (23) investigated the effects of lactic acid bacteria metabolites and brine solutions on the qualitative traits of grouper fillets, specifically *Epinephelus coioidis* and *Scomberoides commersonianus*, that were undergoing freeze preservation.The disruption of stromal cell tissues, which allowed water to seep into the glazing material from the fish tissues, explained the observed variations. This phenomenon became very apparent when table salt-treated drinking water was added to increase the glazing material's osmotic pressure (24). Contributors to the creation of ice crystals include the freezing process itself, the significant loss of liquid after thawing, particularly during the evaporation of free water, and other factors (25).

Table 2: Impact of Glazing and Freezing at (18 ± 2) °C on moisture (%) of fish samples.

Treatment	Freezing Duration (Day)			Overall average
	30	60	90	
T1	74.17^{aA} ±0.19	73.96^{aA} ±0.06	72.92^{bA} ±0 .10	73.68
T2	74.18^{aA} ±0.19	74.10^{aA} ±0.27	73.82^{bB} ±0.16	74.03
T3	74.31^{aA} ±0.00	74.26aA ±0.03	73.63^{bC} ±0.02	74.06
T4	74.34^{A} ±0.05	74.32^{aB} ±0.05	73.93^{bD} ±0.09	74.19

***Small different letters denote significant differences between groups ($p \leq 0.05$).**
Note: (T1) as a control group without additives, (T2) Glazed with water only, (T3) Glazed with salt 5%, (T4) Glazed with white vinegar 5%.

Protein

The results indicated a decrease in the protein content in the frozen glazed fish and across all treatments used in the study over a 90-day storage period, as presented in Table 3. Treatment T3 demonstrated the highest protein content throughout the storage periods of 30, 60, and 90 days (17.56, 17.49, and 17.40%), respectively. Treatment T4 followed closely with an overall average of 17.42%, while treatment T1 experienced the most significant impact from the freezing process, with its protein content reaching 16.52% during the freezing period.The drop in protein levels was significant ($P < 0.05$) compared to the fresh fish sample (17.58%), and the protein percentages kept going down during the freezing periods because of oxidation and direct contact with air. This is because sulfhydryl oxidation forms a disulfide bond that lowers the amount of protein that can dissolve in salt (26). The results of this study agree with (27, 28, 29), who all looked at how glazing and freezing affect different properties and composition. Chemically, their results showed that with increasing preservation times, the protein content decreased significantly, and that microbiological or enzymatic autolytic mechanisms may be responsible for the observed decrease in the quality of fish species throughout the glazing and freezing processes These processes may degrade frozen fish proteins individually or in combination. Another possible cause is the release of nitrogenous bases during protein degradation, which may lead to the loss of the separated liquid during the thawing process. Fish proteins are rapidly degraded by bacterial and endogenous enzymes after death. The loss of volatile amines (NH3) and the conversion of nitrogen to various non-protein nitrogen molecules are the result of this enzymatic activity, and as a result of these events, amino acids are damaged and agglomerated, reducing the amount of protein in the body. The interaction between protein molecules and lipids intensifies the decrease in protein levels (30).

Table 3: Impact of Glazing and Freezing at (18 ± 2) °C on protein (%) percentage

Treatment	Freezing Duration (Day)			Overall average
	30	60	90	
T1	17.06A ±0.08	16.56^{aA} ±0.37	15.94^{bA} ±0.87	16.52
T2	17.33A ±0.05	17.38^{aB} ±0.02	16.94^{bB} ±0.07	17.22
T3	17.56A ±0.07	17.49^{aC} ±0.03	17.40^{bC} ±0.03	17.48
T4	17.48A ±0.01	17.45^{aD} ±0.01	17.32^{bD} ±0.04	17.42

***Small different letters denote significant differences between groups ($p \leq 0.05$).**
Note: (T1) as a control group without additives, (T2) Glazed with water only, (T3) Glazed with salt 5%, (T4) Glazed with white vinegar 5%.

Fat

Table 4 shows the results of the statistical analysis. A significance level of 0.05 (p 0.05) means that there are important differences in how the glazing treatments affect the average fat percentage of common carp fish, especially when they are frozen for a longer time. In contrast to a sample of fresh fish, where the fat percentage was 4.45% of the dry weight, fat percentage values for the glazing treatments consistently decreased as the duration of freezing preservation increased. Over the 90-day preservation period, the fat percentage values for treatments T1, T2, T3, and T4 (4.13%, 4.26%, 4.36%, and 4.32) %, respectively showed a gradual and consistent decline. The T3 treatment, involving freezing with 5% salt and resulting in a fat percentage of 4.36%, exhibited the highest average. The addition of 5% salt led to a decrease in moisture percentage, which was identified as the cause of the observed increase (31). The results align with several studies investigating the relationship between the increased duration of freezing fish preservation and a subsequent decrease in fat percentage, impacting the chemical composition and qualitative characteristics. Noteworthy studies supporting this trend include those conducted by (23,32,29). The hydrolysis process, catalyzed by lipase and phospholipase enzymes, plays a pivotal role in converting free fatty acids, contributing to variations in fish fat proportion during freeze-preservation (33). Myofibrillar proteins' susceptibility to oxidative rancidification processes leads to their insolubility with an extended freezing duration, attributed to substances like malonaldehyde, byproducts of lipid oxidation (34). Furthermore, fish spoilage results from changes caused by three major mechanisms: (i) the breakdown of tissue by the fish's enzymes (autolysis of cells), (ii) the growth of microorganisms, and (iii) oxidative reactions. To reduce the loss in freshness, different preservative methods, such as glazing (35). The current study's findings regarding the decrease in fat percentage during the preservation period are consistent with these results.

Table 4: Impact of Glazing and Freezing at (18 ± 2) °C on fat (%)

Treatment	Freezing Duration (Day)			Overall average
	30	60	90	
T1	4.30^{aA} ±0.03	4.10^{bA} ±0.01	3.99^{cA} ±0.05	4.13
T2	4.22aE ±0.03	4.17^{a} ±0.02	4.04aB ±0.08	4.26
T3	4.41aB ±0.04	4.37bB ±0.02	4.32cC ±0.02	4.36
T4	4.39E ±0.08	4.32aC ±0.11	4.25bD ±0.15	4.32

***Small different letters denote significant differences between groups ($p \leq 0.05$).**
Note: (T1) as a control group without additives, (T2) Glazed with water only, (T3) Glazed with salt 5%, (T4) Glazed with white vinegar 5%.

Ash

When analyzing the ash content in the freezing period and the effect of glazing and frozen fish treatments from T1 to T4 over three months, Table 5 did not reveal any statistically significant changes ($p>0.05$). In treatments T1, T2, T3, and T4, the overall average of common carp meat was 1.56, 1.59, 1.65, and 1.61%, respectively. As the storage duration increased to 30, 60, and 90 days, we observed a gradual increase among the glazing treatments. In contrast, the fresh fish sample contained 1.49% ash. The results showed that T3 with 5% salt addition was the best with an overall average of 1.65%, while T4 with 5% white vinegar addition came in second place with an average of 1.61%. These results are consistent with the results of the (23) study, which examined the effect of brine solutions and lactic acid bacteria metabolic products at different concentrations on the quality characteristics of frozen grouper *Epinephelus coioidis* and rib *Scomberoides commersonianus* fillets. Specifically, the ash content of the fish fillets was 3.06 and 2.71%, respectively, when 6 % salt was added, and 2.04 and 1.87 %, respectively, when 10 % lactic acid bacteria products were added during a 90-day preservation period. Additionally, the results of (36) support the idea that increasing the brine concentration by 8 or 20% significantly increased the ash content, which the researcher then used to salt horse mackerel *Trachurus trachurus*, yielding ash contents of 10.55 and 11.03%, respectively. On the other hand, this indicates that the increased salt concentration caused the increased ash.

The inverse relationship between moisture and ash may be the cause of the slight increase in the ash percentage. According to (37), this relationship led to an increase in the concentration of sodium chloride within the fleshy tissues and a decrease in the moisture percentage with the continuous extension of the storage period. Alternatively, ash is a content of mineral elements found in fish, such as calcium, sodium, zinc, iron, phosphorus, etc., and its percentage varies according to the quantity

and quality of fish feed and the amount of movement performed (38).

Table 5: Impact of Glazing and Freezing at (18 ± 2) °C on ash (%)

Treatment	Freezing Duration (Day)			Overall average
	30	60	90	
T1	1.46aA ±0.02	1.50bA ±0.03	1.72E ±0.02	1.56
T2	1.57aB ±0.02	1.58bB ±0.01	1.61cA ±0.01	1.59
T3	1.62aC ±0.02	1.63bC ±0.02	1.71E ±0.02	1.65
T4	1.57aD ±0.00	1.61bD ±0.01	1.66cB ±0.01	1.61

***Small different letters denote significant differences between groups ($p \leq 0.05$).**
Note: (T1) as a control group without additives, (T2) Glazed with water only, (T3) Glazed with salt 5%, (T4) Glazed with white vinegar 5%.

Caloric Value:

Table 6 shows the significant differences at the probability level ($p<0.05$) in the importance of the caloric value of common carp fish for the studied species. When the glazed fish were preserved by freezing, the general average in treatment T1 was 103.13 kcal/100 gm, then the average calorific value decreased in the glazing treatment (T2) with the advancement of the storage period to reach 106.16 kcal/100 g, then it increased again in the glazing treatments T3 and T4 to reach averages of (109.23, 108.53) kcal/100 g. Compared to the fresh fish sample, which had a general average of 110.37 kcal/100 g, which is a document expressing the fish content in terms of the amount of protein and fat consumed, which is a direct relationship, one gram of digested protein in the body gives energy estimated at 4 kcal, and one gram of fat give 9 kcal (39,40). The differences in these values can be attributed to the unique characteristics of the fish's body, as well as seasonal fluctuations that affect food intake and energy storage beyond the body's needs (41).

Conclusions

The glazing method emerges as the optimal approach for preserving the nutritional integrity of common carp fish, compared to freezing without glazing. Throughout the freezing and glazing processes, the chemical content underwent changes, with the percentages of key components (moisture, protein, and fat) decreasing as the preservation period extended. However, the reduction in these percentages was more moderate in glazed fish compared to unfrozen fish, with notable differences in caloric values. Significantly, when subjected to a 90-day freezing period, glazing with a 5% concentration of table salt and white vinegar improved the nutritional value and extended the fish's shelf life.This

makes it a suitable choice for human consumption, emphasizing the efficacy of glazing in preserving both the quality and longevity of common carp fish during freezing.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

Table 6: Impact of Glazing and Freezing Duration (18 ± 2) °C on Caloric Value kcal/100 gm.

Treatment	Freezing Duration (Day)			Overall average
	30	60	90	
T1	106.49^{aA} ±3.59	103.14^{bA} ±1.74	99.76^{cA} ±1.32	103.13
T2	107.30^{aB} ±0.80	107.05 ±0.28	104.12^{bB} ±0.28	106.16
T3	109.93^{aC} ±1.83	109.29^{bB} ±1.19	108.48^{cC} ±0.33	109.23
T4	109.43^{aD} ±0.45	108.64^{bC} ±0.40	107.53^{cD} ±0.37	108.53

***Small different letters denote significant differences between groups ($p \leq 0.05$).**
Note: (T1) as a control group without additives, (T2) Glazed with water only, (T3) Glazed with salt 5%, (T4) Glazed with white vinegar 5%.

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تأثير التزجيج والحفظ بالتجميد على التركيب الكيميائي والقيمة الحرارية لسمك الكارب الشائع، *Cyprinus Carpio* L.1758.

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الخلاصة

تضمنت الدراسة الحالية على حفظ أسماك الكارب الشائع المهمة اقتصاديًا *Cyprinus carpio* L. عن طريق التزجيج وتقييم الخصائص والتغيرات في التركيب الغذائي أثناء التجميد اعند درجة (-18 ± 2) °م ومقارنتها مع الأسماك الطازجة (قبل المعاملة في محاليل التزجيج). قسمت عينات الأسماك الى اربعة معاملات بواقع ثلاث مكررات لكل معاملة، تضمنت T1 معاملة السيطرة (بدون معاملة)، T2 الغمر بالماء الصالح للشرب، T3 الغمر بمحلول ملح الطعام بتركيز 5 % وبنقاوة 98.5 %، T4 الغمر بمحلول الخل الأبيض بتركيز 5 %، وقياس جودتها ومدى ملاءمتها للاستهلاك البشري، لمدة ثلاثة أشهر (30، 60 و90) يومًا. أظهرت نتائج التحليل الإحصائي عند مستوى احتمالية $p < 0.05$ انخفاض محتوى الرطوبة والبروتين والدهون في T1 وT2، بينما أظهرت T3 وT4 فوائد الحفظ على مدى 90 يومًا. في حين كان المتوسط العام لنسب الرماد متزايدة مع زيادة مدة التجميد في جميع المعاملات، قد يكون سبب الحفاظ على القيمة الغذائية (الرطوبة، البروتين، الدهون، الرماد) هو التأثير التآزري لمواد التزجيج، في حين كانت قيم السعرات الحرارية التي هي مجموع معدل المحتوى البروتيني والمحتوى الدهني كان اقل في جميع المعاملات مقارنةً مع العينة الطازجة، تعود الاختلافات الحاصلة في قيم السعرات الى الغذاء المتناول والتخزين الفائض من الطاقة عن حاجة الجسم بشكل عام يؤدي الحفظ المجمد إلى فقدان جودة القيمة الغذائية للأسماك وبالنظر إلى النتائج، فإن أفضل مادة لتزجيج الاسماك هي المعاملة بملح الطعام والخل الأبيض بتركيز 5 % وفي الختام، فإن تخزين هذه الأنواع من الأسماك لمدة تصل إلى 90 يومًا لم يؤثر سلبًا على قيمتها الغذائية.

الكلمات المفتاحية: تأثير التزجيج، *Cyprinus carpio* L.، التركيب الكيميائي، القيمة السعرية الحرارية