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A Comprehensive Evaluation of Curcumin and Oregano Extracts as Natural Preservatives: antioxidant and antibacterial effects on Quality of (*Cyprinus carpio*)Fish Meat During cold Storage

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Abstract: In response to customer demand for natural alternatives to synthetic preservatives, researchers delved into the antioxidant and antibacterial properties of these bioactive substances in order to keep fish fresh and safe. This study investigated at how oregano and curcumin preserved carp (*Cyprinus carpio*)fish meat. The trial design consisted of four treatment groups: 6% curcumin and oregano extract (G1), 3% oregano extract (G3), 3% curcumin extract (G2), and a control group that received no treatments. 18 carp fish fillets (45±2 gram each) were randomly allocated to each treatment group. The total phenolic content of plant extracts t-extracted with 70% ethanol is determined using the Folin-Ciocalteu technique. The objective of this study was to evaluate the efficacy of combined 6\% curcumin and oregano extracts (G1) as a natural preservative for fish samples stored under refrigeration 4 c over a two-week period.Quality attributes were assessed at defined intervals (24-hour, 4-day, and 2-week), focusing on pH markers of lipid oxidation ,TBARS, peroxide value), protein degradation TVB-N, and the total bacterial count.One-way ANOVA demonstrated significant differences ($p < 0.05$) between the control and G1 treatments. According to this study, oregano and curcumin extracts work well as natural seafood preservation. Positive correlations between TBARS, TVB-N, and peroxide levels are found using Pearson correlation analysis, indicating that protein decomposition and lipid oxidation occur during the preservation of fish meat. As a result, fish meat quality may be preserved from oxidative and degradative processes by antioxidants like curcumin and oregano.

Key words :- Fish Preservation, Curcumin , Oregano , Antioxidant, Microbial

Introduction A plant known for its culinary and medicinal uses, oregano is becoming more and more well-liked in the food industry due to its antibacterial, antioxidant, and preservation properties. Its primary active ingredients, carvacrol, thymol, and rosmarinic acid, are what give it its preservation properties. The primary phenolic molecule that gives oregano oil its antimicrobial properties is carvacrol. It is a powerful natural preservative due to its effective antibacterial, antifungal, and antioxidant qualities. Synthetic preservatives are becoming less popular due to the rising demand for natural ones .Fish are prone to microbial degradation and are perishable. Pseudomonas, Vibrio, and Lactobacillus bacteria can spoil fish and produce toxic compounds and off-flavors that affect the hygiene and overall quality of products. Oregano may improve the shelf life of fish by inhibiting specific spoiling microbes because of its antibacterial properties. Research has shown that adding oregano essential oil to water or ethanol inhibits the growth of fish bacteria. Pseudomonas putida is a major fish-rotting microbe that was found to be inhibited by oregano essential oil (1). Fish that have their bacterial growth monitored live longer and are safe for human consumption. The main ingredient in turmeric (Curcuma longa), curcumin, is attracting interest because of its numerous biological characteristics. It is well known for its anti-inflammatory, anti-bacterial, anti-cancer, and antioxidant properties. Curcumin has been investigated recently as a natural preservative for perishable foods like fish. Curcumin is a safe and effective alternative to synthetic preservatives for increasing the shelf life of fish products, especially carb fish. Fish's high polyunsaturated fatty acid content renders it susceptible to lipid oxidation, which results in rancidity, nutritional loss, and off-flavors. Antioxidants are required to maintain seafood quality during preservation. Curcumin's antioxidant effect scavenges free radicals and suppresses lipid peroxides, therefore protecting fish from oxidative damage. (2). Oregano, curcumin, and other plant-based chemicals have

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demonstrated potential as fish preservatives. Rosemary, thyme, and clove essential oils contain phenolic chemicals similar to oregano. These compounds possess potent antibacterial and antioxidant activities (3). Rosemary extract improves fish taste and shelf life by inhibiting lipid oxidation and microbiological development. Phenolic acids found in botanicals, such as ferulic and caffeic acids, aid in antioxidant defense by neutralizing free radicals. These organic preservatives harm microbial cells by disrupting their membranes and cell walls. They also reduce oxidizing reactive oxygen species (5). Herbal extracts are efficient food preservatives because of their dual characteristics. Consumer demand for healthful, chemical-free food has fueled research into natural replacements (6). Integrating these plant extracts into edible coatings or packaging materials is another unique way to preserve seafood Combining essential oils or extracts can boost antibacterial activity while lowering concentrations and sensory changes (7). (8).It has been determined that curcumin prolongs the shelf life of fish fillets by preventing lipid oxidation. Compared to untreated samples, curcumin decreased lipid breakdown in fish storage solutions, extending the time that fish remain fresh. Because it slows lipid oxidation while preserving fish's flavor, texture, and nutrients, as well as a natural preservative. Fish are susceptible to microbial contamination, especially from bacteria that cause spoiling, like Lactobacillus, Vibrio, and Pseudomonas.In 2006, Yanishlieva-Maslarova et al. Fish preservation benefits greatly from curcumin's antimicrobial qualities.The bacteria put consumers at risk by contaminating seafood. Curcumin has a potent antibacterial effect on fish diseases. itreduced Staphylococcus aureus and Escherichia coli in fish fillets, according to (9). In conclusion, oregano, curcumin, and other phytochemicals offer sustainable and viable fish preservation methods.

Material and Method

Ethical approval:

The present study was conducted according to the standards for animal care and use and was approved by the Ethical Committee at college of veterinary medicine/ University of Al-Qadisiyah (No. 4420 in 23/11/2023).

Prepare Curcumin and Oregano extract

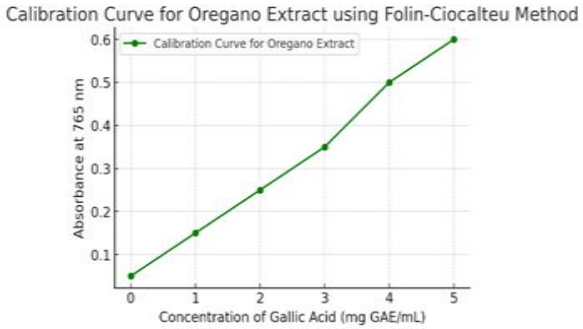
The active compounds were extracted from turmeric (Curcuma longa L.) and oregano leaves which bought from commercial market using the Soxhlet extraction procedure. The plant materials were initially desiccated and finely ground using a mortar and pestle or grinder. Each Curcumin and Oregano material were weighed at approximately 50 g, and 500 mL of 70% ethanol was added to a boiling vessel. In order to guarantee the comprehensive extraction of bioactive compounds, the extraction was conducted for a duration of 6-8 hours. Subsequently, the solution was filtered to eliminate any solid detritus. The bioactive compounds were preserved by evaporating the solvent (ethanol) at low temperatures (below 60°C) under reduced pressure using a rotary evaporator. The concentrated extract was diluted with ethanol or water to achieve 3 g of extract per 100 mL for the 3% concentration and 6 g per 100 mL for the 6% concentration in order to produce extracts of 3% and 6% concentration (10).

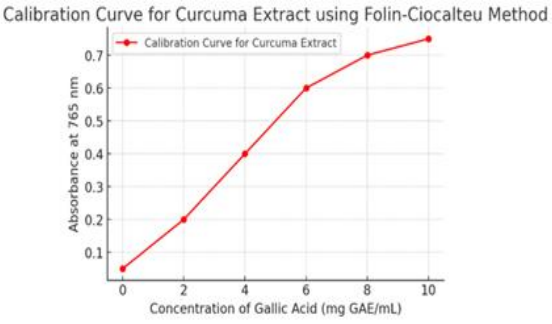
The total phenolic content (TPC)

of the plant extracts was quantified using the Folin-Ciocalteu method. This technique involved the mixture of the extract with Folin-Ciocalteu reagent and sodium carbonate, and the absorbance was measured at 765 nm using a spectrophotometer. The phenolic concentration was determined by referencing a gallic acid standard curve and expressed as mg GAE per gram of dried extract (11).

Using the formula:

$$\text{Phenol percentage} = \left(\frac{5\text{mg/mL} \times 1\text{ mL}}{0.1\text{g}} \right) \times 100 = 5\%$$





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Samples preparation

The raw carb (*Cyprinus carpio*) fish were obtained from the local market, and arrived to our laboratory (in 1 hour) in an incubator with ice crystals (0°C–4°C). The samples were cleaned with distilled water to remove the contaminants and sticky materials attached to the fish skin, and the scales and abdominal wastes were collected. The average weight of a carp fish was 200 g. Fish fillets were weighed with the same displacement of 45±2 grams. The fish in each treatment, 72 g on average, were divided randomly into four groups of 18 portions each. the preservation therapies, including 3% curcumin, 3% oregano, 6% curcumin + oregano. (12)

PH Measurement

As mention in Leroy, F., & Opara 2020 claim Fish Meat pH Calculation Method Sample fresh fish then Standardize fish meat. And process fish flesh in a blender. Ten to twenty grams of homogenized fish meat. Mix the sample with 50–100 mL distilled water. Sample and water should be mixed. Fish juice can be separated with filter paper or a centrifuge. Calibrate the pH meter with pH 4.00, 7.00, and 9.00 buffers. add pH meter electrode to filtered fish juice.

thiobarbituric acid reactive substances test

Consume five to ten grams of fish meat. Add chloroform-methanol (2:1, v/v) to extract lipids from homogenized fish. Concentrate the lipid extract by evaporating the solvent with a rotary evaporator or nitrogen gas. Dissolve TBA in acetic acid to generate TBA reagent (14). Furnish TBA with a specified amount of lipid extract. A pink chromogen is produced by MDA and TBA during a 45-minute immersion in water at 95°C subsequent to lipid oxidation. Allow the reaction mixture to cool to room temperature following heating. To eradicate chromogen, introduce butanol into the aqueous solution. The spectrophotometer detects absorption at 532 nm. The standard TBA formula is as follows:

$$\text{TBA value} = \frac{\text{Absorbance} \times \text{Dilution Factor}}{\text{Molar Extinction Coefficient} \times \text{Volume of the sample}}$$

*The molar extinction coefficient is specific to MDA-TBA complex and is generally used as 1-56× 10^5 M^-1cm^-1

Determination of total volatile base nitrogen

Total volatile base nitrogen (TVB-N) content was determined using a Kjeldahl automated distillation device (Kjeltec™ 8400, FOSS, Denmark) and the procedure described by (15) is used. TVB-N is measured in milligrams per 100 grams of fish sample and is based on the use of hydrochloric acid (0.1 mol/L). Three duplicates of each experiment were carried out.

Determining the Peroxide Value

Fish fillets were frozen on dry ice and brought to Sari for peroxide testing. In a 500ml Erlenmeyer flask containing 50g sample, add 200ml chloroform. The solution was shaken for 2 hours, filtered, and placed on Erlenmeyer's sanding door. Samples were placed in a rotating evaporator to imitate solvent evaporation to estimate Erlenmeyer oil weight. Materials and procedures the peroxide value The AOAC technique (16) tested peroxide value by soaking 2g of extracted oil in 30ml chloroform acetic acid, adding 0.5ml saturated potassium iodide, and stirring for one minute. The mixture was mixed with 30ml distilled water. After vigorous stirring, sodium thiosulfate solution 0.01 normal titrated the mixture to light-yellow. With 0.5ml of 0.01 starch reagent, the liquid became dark blue. Titration continued until the blue disappeared and the bright tone developed.

Bacterial load assessment

During Storage Period 24 hr day 4 day and 2weeks storage 10 g of the fillet portion were homogenised and mixed with 90 mL of peptone-physiological saline solution (0.1% peptone +0.85% NaCl). Thereafter, different dilutions were made. Microbial analysis was performed. Into 1 mL of each diluted sample, 1 mL of plate count agar culture was used to culture bacteria. For determination of culturable counts, the inoculated dishes were incubated 24 h at 37 °C. the microbial count relative to the surface area of the sample. All the results were performed in triplicate and expressed as log 10 CFU g−2 (17).

Statistical analysis

The study compares different treatments (3% and 6% curcumin, 3% and 6% oregano) within storage periods All samples were conducted at least in triplicate. To investigate the difference among different variables, an ANOVA (one-way analysis of variance) were performed by SAS 9.1(Statistical Analysis System, version 9.1) with results showing a p-value > 0.05. Pearson correlation coefficient was used to assess pH-bacterial counts connections. We accepted significance at $p < 0.05$.

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Result

Table 1: Effect of Curcumin and Oregano Additives on the pH Stability of Fish Meat During Different Storage Conditions

Groups Time	Control	G1	G2	G3	$LSD_{0.05}$
After 24 hr storage at 4 c quality of fish meat	6.6 ± 0.14 Ac	6.05 ± 0.05 Ba	6.3 ± 0.00 Aa	6.35 ± 0.07 Ab	0.37 (S)
Quality of fish meat after 4 days storage in -8 c	6.97 ± 0.11 Ab	5.15 ± 0.07 Cb	5.95 ± 0.21 Bb	5.8 ± 0.14 Bc	0.43 (S)
Quality of fish meat After 2 weeks in -8 freeze	7.45 ± 0.49 Aa	5.02 ± 0.01 Cc	5.6 ± 0.14 Bc	7.45 ± 0.49 Aa	0.51 (S)
$LSD_{0.05}$	0.53 (S)	0.45 (S)	0.38 (S)	0.44 (S)	—

G1: Curcumin & oregano 6%, G2: Curcumin 3%, G3: Oregano 3%
Capital litters represent horizontal comparison of data, and small litters represent vertical comparison of data.
S: significant differences between groups (p value ≤ 0.05)
NS: non-significant difference between groups (p value > 0.05)

Figure 1: Effect of Curcumin and Oregano Additives on the pH Stability of Fish Meat During Different Storage Conditions

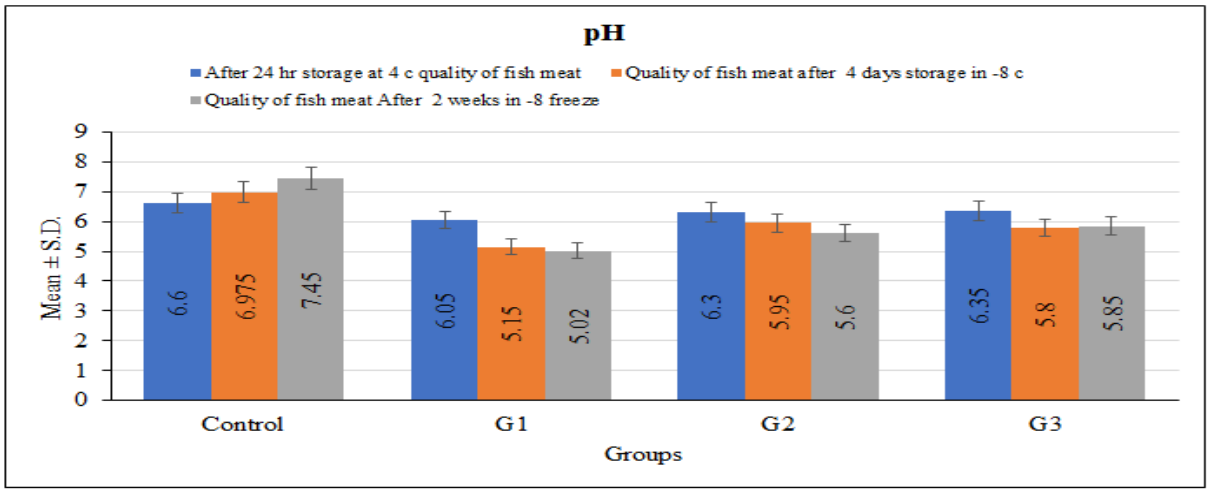

Table 2: Effect of Curcumin and Oregano Additives on the Thiobarbyturic Acid Stability of Fish Meat During Different Storage Conditions

Groups Time	Control	G1	G2	G3	$LSD_{0.05}$
After 24 hr storage at 4 c quality of fish meat	26.65 ± 0.21 Ab	24.07 ± 0.05 Aa	24.73 ± 0.46 Aa	25.45 ± 0.21 Aa	2.55 (NS)
Quality of fish meat after 4 days storage in -8 c	28.17 ± 0.18 Aa	22.5 ± 0.57 Cb	24.03 ± 0.04 Ba	25.35 ± 0.36 Ba	2.08 (S)
Quality of fish meat After 2 weeks in -8 freeze	28.96 ± 0.08 Aa	21.6 ± 0.14 Cc	23.8 ± 0.42 Ba	24.65 ± 0.07 Ba	1.84 (S)
$LSD_{0.05}$	1.35 (S)	1.15 (S)	0.94 (NS)	1.27 (NS)	—

G1: Curcumin & oregano 6%, G2: Curcumin 3%, G3: Oregano 3%
Capital litters represent horizontal comparison of data, and small litters represent vertical comparison of data.
S: significant differences between groups (p value ≤ 0.05)
NS: non-significant difference between groups (p value > 0.05)

Figure 2: Effect of Curcumin and Oregano Additives on the Thiobarbyturic Acid Stability of Fish Meat During Different Storage Conditions

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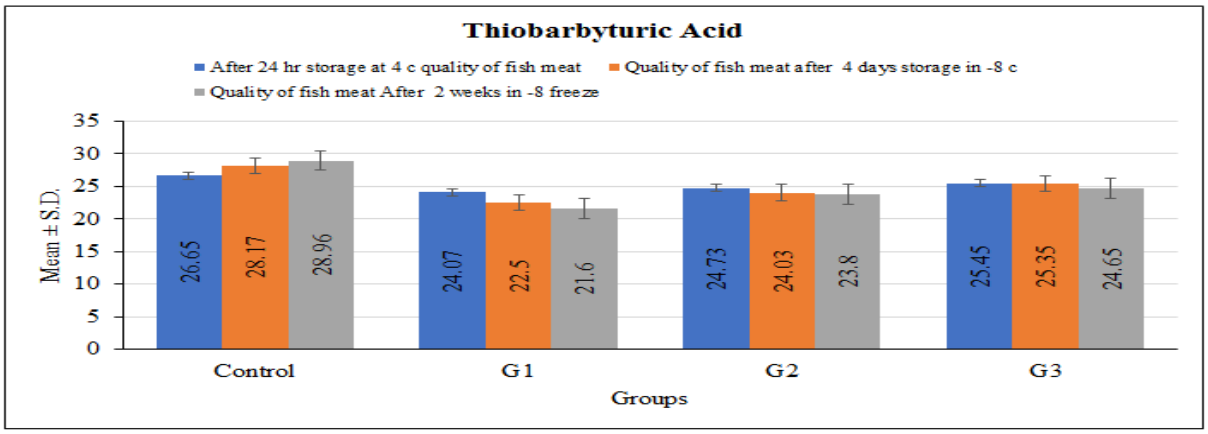

Table 3: Effect of Curcumin and Oregano Additives on the Total Volatile Nitrogen Stability of Fish Meat During Different Storage Conditions

Groups Time	Control	G1	G2	G3	$LSD_{0.05}$
After 24 hr storage at 4 c quality of fish meat	36.5 ± 0.42 Ac	30.29 ± 0.3 Ba	31.65 ± 0.78 Bb	36.35 ± 4.25 Aa	3.25 (NS)
Quality of fish meat after 4 days storage in -8 c	37 ± 0.28 Ab	31 ± 0.85 Ba	33.6 ± 0.14 Ba	35 ± 0.28 Ab	2.28 (S)
Quality of fish meat After 2 weeks in -8 freeze	38.23 ± 0.25 Aa	30.7 ± 0.42 Ba	33.05 ± 0.07 Ba	35.6 ± 0.42 Ab	2.07 (S)
$LSD_{0.05}$	1.47 (S)	0.84 (NS)	1.07 (S)	1.54 (S)	

G1: Curcumin & oregano 6%, G2: Curcumin 3%, G3: Oregano 3%
Capital litters represent horizontal comparison of data, and small litters represent vertical comparison of data.
S: significant differences between groups (p value ≤ 0.05)
NS: non-significant difference between groups (p value > 0.05)

Figure 3: Effect of Curcumin and Oregano Additives on the Total Volatile Nitrogen Stability of Fish Meat During Different Storage Conditions

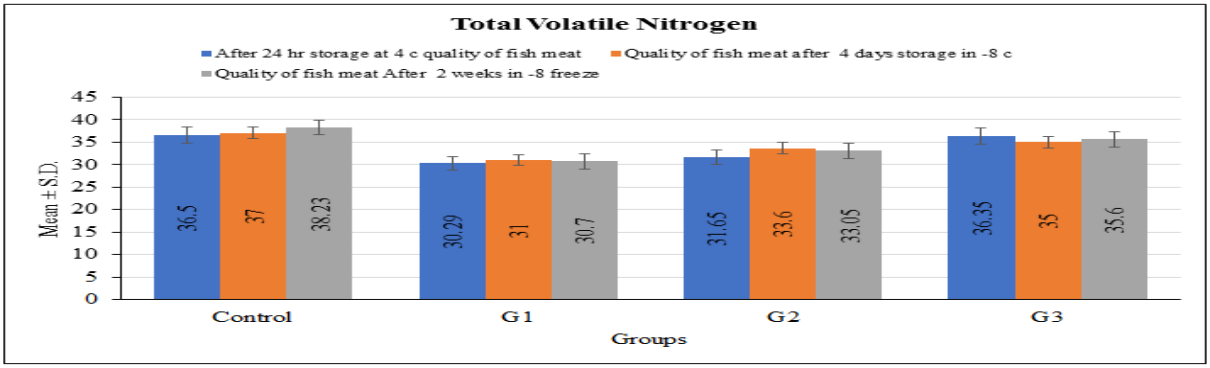

Table 4: Effect of Curcumin and Oregano Additives on the Peroxide value Stability of Fish Meat During Different Storage Conditions

Groups Time	Control	G1	G2	G3	$LSD_{0.05}$

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After 24 hr storage at 4 c quality of fish meat	2.71 ± 0.06 Ab	2.22 ± 0.01 Ba	2.22 ± 0.02 Bb	2.28 ± 0.01 Bb	0.38 (S)
Quality of fish meat after 4 days storage in -8 c	2.95 ± 0.08 Ab	2.08 ± 0.11 Bb	2.38 ± 0.08 Ba	2.6 ± 0.03 Ba	0.49 (S)
Quality of fish meat After 2 weeks in -8 freeze	3.01 ± 0.1 Aa	2.08 ± 0.04 Bb	2.22 ± 0.04 Bb	2.41 ± 0.08 Bb	0.46 (S)
$LSD_{0.05}$	0.28 (S)	0.18 (S)	0.21 (S)	0.24 (S)	

G1: Curcumin & oregano 6%, G2: Curcumin 3%, G3: Oregano 3%
Capital litters represent horizontal comparison of data, and small litters represent vertical comparison of data.
S: significant differences between groups (p value ≤ 0.05)
NS: non-significant difference between groups (p value > 0.05)

Figure 4: Effect of Curcumin and Oregano Additives on the Peroxide value Stability of Fish Meat During Different Storage Conditions

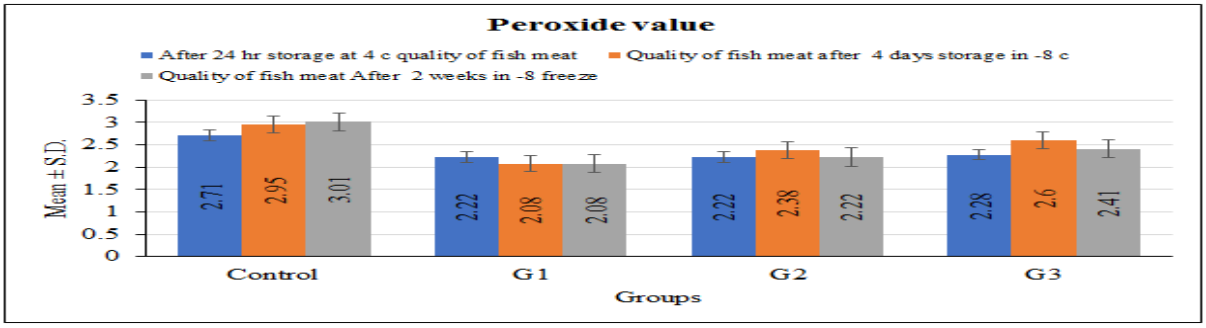

Table 5: Effect of Curcumin and Oregano Additives on the Total Bacterial Count ($\times 10^2$) (cfu/gm) Stability of Fish Meat During Different Storage Conditions

Groups Time	Control	G1	G2	G3	$LSD_{0.05}$
After 24 hr storage at 4 c quality of fish meat	255 ± 14.14 Ab	140.5 ± 21.92 Ca	181.5 ± 9.19 Bb	208 ± 15.56 Bb	35.48 (S)
Quality of fish meat after 5 days storage in -4 c	290.5 ± 3.54 Aa	109 ± 4.24 Cb	229 ± 12.73 Ba	255.5 ± 7.78 Ba	40.73 (S)
Quality of fish meat After 2 weeks in -8 freeze	282 ± 4.24 Aa	105.5 ± 0.21 Cc	128 ± 4.24 Cc	181.5 ± 3.54 Bc	38.67 (S)
$LSD_{0.05}$	15.08 (NS)	20.37 (S)	18.19 (S)	16.04 (S)	

G1: Curcumin & oregano 6%, G2: Curcumin 3%, G3: Oregano 3%
Capital litters represent horizontal comparison of data, and small litters represent vertical comparison of data.
S: significant differences between groups (p value ≤ 0.05)
NS: non-significant difference between groups (p value > 0.05)

Figure 5: Effect of Curcumin and Oregano Additives on the Total Bacterial Count (cfu/gm) of Fish Meat During Different Storage Conditions

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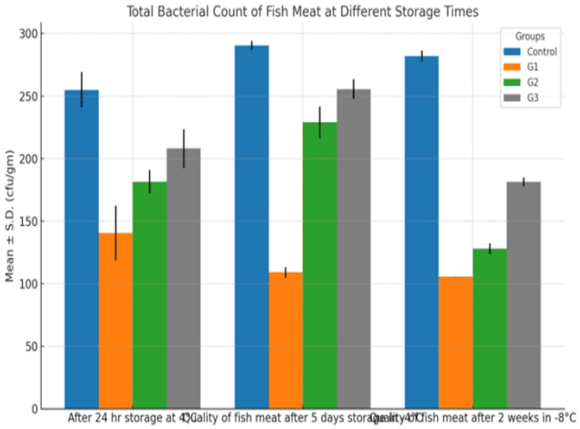

G1 (6% Curcumin + Oregano)	0.987	0.013
G2 (3% Curcumin)	0.945	0.055
G3 (3% Oregano)	0.980	0.020

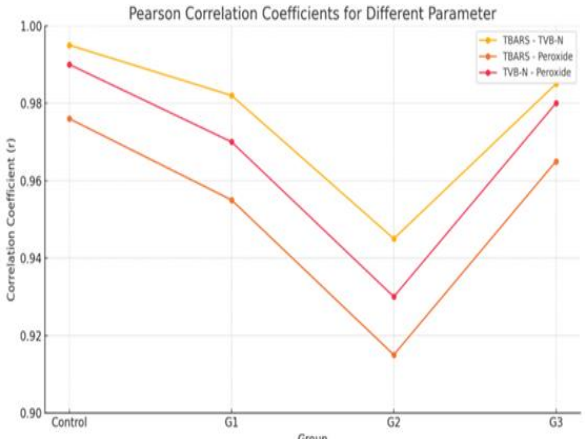

Table (6) Pearson Correlation Analysis between pH and Bacterial Count

Treatment Group	Pearson Correlation (r)	p-value

Table (7) The Pearson correlation coefficients and p-values between TBARS, TVB-N, and Peroxide Value for different treatment groups

Group	Parameter	Pearson Correlation (r)	p-value
Control	TBARS - TVB-N	0.995	0.005
Control	TBARS - Peroxide	0.976	0.024
Control	TVB-N - Peroxide	0.990	0.010
G1	TBARS - TVB-N	0.982	0.018
G1	TBARS - Peroxide	0.955	0.045
G1	TVB-N - Peroxide	0.970	0.030
G2	TBARS - TVB-N	0.945	0.055
G2	TBARS - Peroxide	0.915	0.085
G2	TVB-N - Peroxide	0.930	0.070
G3	TBARS - TVB-N	0.985	0.015
G3	TBARS - Peroxide	0.965	0.035
G3	TVB-N - Peroxide	0.980	0.020

Discussion

According to the study's outcomes, carp fish meat may be considerably preserved by oregano and curcumin extracts under a range of storage circumstances. pH, total volatile base nitrogen (TVB-N), thiobarbituric acid reactive compounds (TBARS), peroxide value, and total bacterial count were all higher in the treated samples than in the control group. The pH levels in the treated groups

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maintained constant, which is in line with the findings of (18). The 6% curcumin and oregano treatment (G1) had the highest pH stability; measurements remained within freshness levels even after storage. But the control group's pH increased the most, indicating that breakdown was occurring quickly. The significantly lower pH shifts in the treated groups ($p < 0.05$) further confirm the greater inhibitory effect of the combined extract, indicating suppression of microbial metabolism responsible for increased pH during spoilage. The Oregano and curcumin exhibit antibacterial properties that inhibit the growth of spoilage microorganisms, including Lactobacillus, Vibrio, and Pseudomonas (19). The pH stability of the 6% curcumin and oregano therapy (G1) was excellent; even after storage, the measurements remained within the freshness limits. Conversely, the control group's pH increased the most, suggesting that the degradation was occurring rapidly. The combined extract appears to have a more potent inhibitory effect, as the pH shifts in the treated groups were significantly lower ($p < 0.05$).This suggests that it obstructs microbial metabolism, which results in an elevation of pH levels during the decomposition process. This is corroborated by the antibacterial properties of curcumin and oregano, which prevent the growth of hazardous bacteria such as Lactobacillus, Vibrio, and Pseudomonas (19).The lipids in G1 (6% curcumin + oregano) were the least affected by oxidation, as evidenced by their low TBARS score. In contrast, the control group exhibited the maximum TBARS, suggesting that it was particularly susceptible to oxidative corrosion. Reduced TBARS levels in treated samples were associated with reduced lipid oxidation, as evidenced by statistically significant variance between groups ($p < 0.05$).The antioxidant activity of phenolic substances detected in both extracts, which prevent the oxidation of fish polyunsaturated fatty acids, is consistent with this decrease. In addition, the concentration of total volatile base nitrogen (TVB-N) decreased significantly in the treated samples. This is a critical indicator that proteins are being degraded. The findings suggest that the rate of enzymatic and microbiological degradation has been reduced (17). During storage, the fish's nutritional value remained constant, and the extracts' antioxidant effect was corroborated by the lower levels of peroxide (20). The overall number of microorganisms was significantly decreased by the most effective combination of curcumin and oregano (G1) at 6%, as demonstrated by microbiological studies. This advantageous interaction is in accordance with prior research that indicates that phytochemical combinations may enhance antibacterial efficacy (21). The results were consistent, as indicated by a significant difference between groups ($p < 0.05$) in an ANOVA study. The G1 therapy's efficacy in inhibiting bacterial growth and maintaining pH stability was demonstrated by a statistically significant correlation ($r = 0.987$, $p = 0.013$). The oregano-only group (G3) also exhibited a significant association, as demonstrated in (22; $r = 0.980$, $p = 0.020$). The levels of TBARS, TVB-N, and peroxide were all significantly positively related in all treatment groups, as demonstrated by Pearson correlation analysis (Table 7). This corroborates the results of 23) the evaluation of oxidative rancidity in seafood by demonstrating a robust correlation between lipid oxidation and protein degradation during the preservation of fish tissue. The significant positive correlation between TBARS and peroxide level is evidence of the accumulation of secondary oxidation products as lipid peroxidation progresses. In light of the consistent beneficial correlation between TVB-N and TBARS (24), oxidative stress causes fish to break down into smaller pieces, which releases more volatile nitrogenous chemical compounds. Examining several biochemical indicators at once to evaluate the freshness and quality of fish has become easier due to several significant links. The findings imply that the antioxidants in the curcumin and oregano extracts successfully protected the fish flesh from oxidative and degradative processes (25; 26).In conclusion, our findings indicate the efficacy of oregano and curcumin extracts as natural preservatives and are in line with the increased preference for plant-based ingredients over synthetic alternatives. Finding appropriate doses and lucrative potential inside commercial food systems should be the main objectives of future studies.

Conclusion

Researchers discovered that extracts of oregano and curcumin can considerably improve the quality and shelf life of carp meat. In both refrigerated and frozen storage conditions, these natural extracts exhibited outstanding antibacterial and antioxidant capabilities, significantly lowering lipid oxidation, breakdown of protein, and microbial growth when compared to untreated controls. Curcumin and oregano had the highest preservation benefits at 6% concentration, showing an effective synergistic interaction.

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Conflict of interest

There is no conflict of interest in this study as stated by the authors.

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