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Research Article

Effects of Turmeric, Cinnamon, and Lemon Extracts on Shelf Life, Nutrients, and Preservation of Carp Fish in Cold Storage

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The present study aimed to investigate the effect of extracts of turmeric, cinnamon, and lemon on shelf life, macronutrients, and oxidative spoilage of the carp fish in cold storage at 4°C. Fishes were divided into five groups: control (NT); immersed fish in extracts of cinnamon, turmeric, and lemon (CTL); immersed fish in extracts of lemon and turmeric (LT); immersed fish in extracts of cinnamon and turmeric (CT); and immersed fish in extracts of lemon and cinnamon (LC). Results showed immersion of the carp fillet in extracts of turmeric, cinnamon, and lemon improved spoilage indices such as thiobarbituric acid, volatile nitrogen bases, and pH. The used extracts maintained the nutritional value and increased the fish's shelf life. The compounds existed in the studied extracts decreased the values of the spoilage indices during cold storage. The highest spoilage indices were found for CL and CT treatments, and the lowest was for CTL treatment. The total bacterial load as well as the number of psychrophilic bacteria in the fish fillet found a significant decrease compared to the NT. Therefore, the best treatment was CTL, and inappropriate treatments were CT and CL. The fish shelf life for nine days was extended with the used extracts because the total count of cryophilic bacteria was $0.41 \times 10^4 \pm 0.50$, which was the lowest compared to other treatments.

1. Introduction

Because of the high effects of industrial preservatives on consumer health, today, natural preservatives are used, which not only do not have harmful side effects but also improve the smell, taste, shelf life, and flavor of seafood products. Fresh fish can be easily spoiled due to internal enzymes and fast bacterial growth in the fish or through postcatch spoilage [1]. In the fish spoilage process, material degradation and the formation of new compounds occur, which lead to the destruction of protein and oxidation of lipids, as well as changes in the smell, taste, and texture of fish. Therefore, the development of effective processing methods to increase the shelf life of fish is inevitable [2]. The soft tissue of the fish limits its shelf life, thus hindering its marketing. During postmortem transport, storage temperature, oxygen, endogenous or microbial proteases, and moisture can lead to destructive changes in fish color, odor, texture, and flavor [3, 4]. Therefore, fish are traditionally preserved in crushed ice, seawater, or by exposure to natural chemicals. At the same time, the fisheries industry is always looking for new preservation methods to increase fish shelf life and provide the best quality in terms of sensory and nutritional to consumers [5].

The activity of spoilage bacteria and endogenous enzymes in fish leads to the production of volatile nitrogen bases resulting in the production of various compounds including ammonia, methylamine, dimethylamine, and trimethylamine [6]. According to various researchers, the permissible TVB-N limit for fresh fish is 30 mg nitrogen per 100 g of fish samples [7].

Additives have been approved for their antimicrobial and antioxidant properties. Antioxidants are compounds that increase shelf life by delaying rancidity and preventing bad taste and odor in fish. The use of antioxidants in the food industry is to prevent or reduce spoilage, maintain the desired quality, and increase the shelf life [8].

Turmeric extract contains phenolic compounds such as folic acid and protocatechuic acid. Among polyphenols, cinnamon contains bioactive compounds, including vanillic, caffeic, gallic, protocatechuic, p-coumaric, and ferulic acids [9]. The most important group of secondary metabolites in the lemon includes flavonoids and other compounds, such as phenolic acids, coumarins, carboxylic acids, amino acids, and vitamins. The main compounds of essential oil are monoterpenoids, especially D-limonene [10, 11]. The mechanism of action of phenolic compounds is that they stimulate the phospholipid bilayer membrane of the cell and increase the permeability and release of essential intracellular components such as Fe, ATP, nucleic acids, and amino acids. They may also damage the bacterial enzyme system, resulting in bacterial death [12]. The essential oils can inhibit enzymes responsible for energy regulation and synthesis of structural components including several enzyme systems [13]. The effects of essential oils against lipid oxidation and microbial growth as fish preservatives have been extensively demonstrated by many authors. These different effects depend on the plant's essential oils, its concentration, as well as the characteristics of the raw material [14]. The present study aimed to investigate the effect of turmeric, cinnamon, and lemon extracts on oxidative spoilage and shelf life in carp fish in cold storage.

2. Materials and Methods

2.1. Preparing Fish and Samples. This research was conducted from 2015 to 2016 at the Behbahan Khatam Alanbia University of Technology in Iran. The carp fishes were purchased from a fish market and placed in a dish containing ice and then transferred to the laboratory. The purchased samples were washed with distilled water to remove contaminants and viscous materials from the fish skin, and then the scales and abdominal wastes were removed. Each of the carp fish had an average weight of 200 g. The weight of the fillet of each fish was 50 ± 2 g.

2.1.1. Extraction of Turmeric Extract. The purchased turmeric rhizome was thoroughly grounded and powdered. Alcoholic extract was prepared from turmeric powder using the Soxhlet method, which is one of the common extraction methods. In this method, the first 100 g of turmeric powder was weighed. Turmeric powder was placed in the filter thimble of the Soxhlet extractor. Then, 600 ml of 70% alcohol was poured into the balloon. Simultaneously, with the heating of the heat bag, the alcohol was heated slowly, and the turmeric extract was mixed with alcohol returned to the balloon. Thus, extraction was performed with balloon heat to collect a thick liquid at the bottom of the balloon [15]. Finally, the solvent was removed from the alcoholic extract, and the obtained extracts were poured into autoclaved jars and covered with aluminum foil around the jar and kept until use.

2.1.2. Extraction of Cinnamon Extract. First, the cinnamon peel was grounded and passed through a 40 mesh in 1 cm^2 sieve. The methanol solvent was used for the extraction of the cinnamon peel extract by a cold solvent method. In this way, a mixture of cinnamon peel and the solvent was mixed on a shaker (Parmis Azma, Iran) in a ratio of 1:10 (w/v) for 24 h at room temperature. Then the filtration was done in the first stage using filter paper and using a vacuum pump. In the next step, a centrifuge (Parmis Azma, Iran) was used at a speed of 4,000 rpm (448 g) for 15 min [16]. In order to remove the solvent, the liquid obtained in the rotary apparatus was distilled to remove the alcohol from it. After distillation, the cinnamon extract was poured into dark-colored autoclaved jars, which were wrapped in aluminum foil around the jar and kept until use.

2.1.3. Preparation of Lemon Extract. The lemon extract was purchased from Adonis Gol Daroo Company (Tehran-Iran). The sour lemon is a kind of lemon that has a cold and dry nature with the best quality and is available in Iran. Iranian lemon with the scientific name *Citrus latifolia* is one of the large acidic lemons. All chemicals were purchased from the company Parmis Azma, Iran.

2.2. Experimental Treatments. For the present research, there were four treatments in addition to the control (T1). The first treatment (T2) was a mixture of extracts (CT); the second treatment (T3) was a mixture of extracts (TL); the third treatment (T4) was a mixture of extracts (CL); and the fourth treatment (T5) was a mixture of extracts (CTL), each with a concentration of 1.5% and with triplicate. The experimental treatment is shown in Table 1.

An extract of 1.5% (1.5 g in 100 ml distilled water) was prepared for each treatment with triplicates.

According to the method of Jeon et al. [17], the fish fillets were completely immersed in the prepared solution for each treatment separately. First, the carp fish fillet was immersed in the prepared solution for 30 s, and then samples were immersed in the solution again for 30 s separately. The fillets soaked in each solution were placed on sterilized wire nets under the hood for 1 h. All samples were placed in sterile plastic containers and transferred separately to the refrigerator at 4°C. Then, all samples were evaluated from point of chemically every 3 days for 12 days [17]. NT was also evaluated without immersion in extracts.

2.3. Proximate Analysis. At the end of the experiment, for measuring the chemical composition of the fish fillets, samples were put in the oven at 60°C for 24 h to remove interstitial moisture. The dried sample was then powdered and transferred to the laboratory for chemical analysis.

2.3.1. Proximate Composition. The analysis of proximate composition in the fish fillet was carried out with triplicate methods [18]. To measure the moisture content, 10 g of the sample was placed in the oven at 105°C, and after about 18 h, it was weighed again until the weight was fixed. To determine the amount of ash, 0.5 g of the dry sample was placed in a furnace at 550°C for 5 h. The amount of protein was measured by the Kjeldahl method [18] with a conversion factor

Table 1: Composition of the five different experimental treatments used.

Treatments	Ingredients	Concentration (%)
T1 (control)	Without extract	—
T2	Mixture of CT extracts	1.5
T3	Mixture of TL extracts	1.5
T4	Mixture of CL extracts	1.5
T5	Mixture of CTL extracts	1.5

of 6.25. Total fat measured by chloroform/methanol method in Soxhlet apparatus.

2.3.2. Measurement of Thiobarbituric Acid. This index was measured according to Siripatrawan and Noipha's [19] method. About 97.5 ml of distilled water and 2.5 ml of 4 M hydrochloric acid were added to 10 g of the homogenized sample. Five milliliter of distillation liquid of this mixture was added to 5 ml of thiobarbituric acid reagent (Parmis Azma, Iran) and then placed at 100°C for 35 min. The amount of pink liquid after cooling at 538 nm was measured in a spectrophotometer (Parmis Azma, Iran), and the absorption number was measured. The number measured was multiplied by a constant of 7.8 to obtain the thiobarbituric acid content of the sample.

2.3.3. Measurement of Free Fatty Acid Content (FFA). To measure the free fatty acids, 25 ml ethyl alcohol neutralized with normal NaOH was added to the oil sample (resulting from the solvent evaporation and remaining in the lower phase of the decanter). The amount of free fatty acids was determined by the amount of consumed normal NaOH during titration in the presence of phenolphthalein and the percentage of oleic acid [20].

2.3.4. Measurement of Volatile Nitrogen Bases (TVB-N). The volatile nitrogen compounds by the Kjeldahl method and titration of the obtained extract were measured. The 10 g of sample along with 2 g of magnesium oxide and 500 ml of distilled water were put into a Kjeldahl balloon (Parmis Azma, Iran) and obtained extract with a solution of 2% boric acid and 1–2 drops of methyl red as an indicator mixed. The resulting yellow solution was titrated with sulfuric acid until a purple color was obtained. The number of volatile nitrogen compounds (mg/100 g) in the fish sample was obtained [21].

2.3.5. Measurement of Peroxide Value. To measure the peroxide content of the samples, the fish fillets were transferred to the laboratory of Sari after freezing along with the dry ice. The 50 g of the sample was placed in an Erlenmeyer flask of 500 ml, and 200 ml of chloroform was added to a container. For extraction, the Erlenmeyer was shaken on the shaker for 2 h. Then, the contents of the Erlenmeyer were then filtered and the solution under the filter was transferred to the Erlenmeyer sanding door. For solvent evaporation, the samples were transferred to a rotating evaporator, and after the solvent evaporated, the weight of the remained oil was determined in Erlenmeyer. To measure the peroxide value according to the AOAC method [18], the extracted oil was dissolved in 30 ml of chloroform acetic acid mixture, and 0.5 ml of saturated potassium iodide was added to the obtained mixture, and the mixture was shaken vigorously for one min [18]. Then, 30 ml of distilled water was added to the mixture. After complete mixing, the mixture was titrated with sodium thiosulfate solution 0.01 normal until a light-yellow color appeared. Then, 0.5 ml of 0.01 starch reagent was added to the mixture, and the color of the mixture turned dark blue. The titration process continued until the blue color was removed and a light color appeared.

2.3.6. pH Value Measurement. The 5 g of the fish fillet was homogenized with 45 ml distilled water in a 250 ml dish with an electric mixer. The pH value of the samples was then measured using a 713 Metrohm digital pH meter [22]. It should be pointed out that each experiment was conducted three times.

2.3.7. Measuring the Rate of Acidity. To determine the acidity rate, 2.5 to 3 g of the extracted oil was weighed in a 250 ml Erlenmeyer flask. The 30 ml of neutral alcohol and 2 ml of phenolphthalein reagent were added to it and titrated with 0.01 N normal solution until a pink color appeared. The amount of acidity was calculated in terms of oleic using the following relation [23]:

$$\text{Acidity rate (free fatty acids according to oleic, } QQ) = \frac{W}{2.28 \times NN \times V}, \quad (1)$$

where V = volume of NaOH (ml), M = 0.01 M normal solution of NaOH, W = sample weight (g), and Q = free fatty acids according to oleic.

2.3.8. K Value. The K value is known as a chemical quality and freshness index for fish determined by nucleotide degradation [24]. This value is obtained by quantifying

ATP (adenosine triphosphate) and its degradation product series, including adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx) [25]. In this way, HxR and Hx are correlated with the loss of fish freshness, and their accumulation indicates the start of autolysis and bacterial spoilage [26].

When a fish dies, the adenosine triphosphate (ATP) is broken down by enzymes into other compounds as follows:

$$\begin{aligned} ATP + Tpase &\longrightarrow A\ DP + myokinase \longrightarrow AMP + \text{dea min ase} \longrightarrow IMP + \text{alkaline phosphatase} \\ &\longrightarrow HxR + \text{nucleoside phospharylase} \longrightarrow Hx + \text{anthine oxidase} \longrightarrow UA, \end{aligned} \tag{2}$$

The shelf life limit of fresh tropical food fish, based on sensory evaluation, was when handled well, between 12 and 28 days of ice storage depending on species, with the K value ranging from 24 to 37%.

$$\text{The } K \text{ value, expressed as } K \text{ value\%} = \frac{(HxR + Hx)}{(ATP + A\ DP + AMP + IMP + HxR + Hx)} \times 100. \tag{3}$$

2.3.9. Microbial Analysis. The amount of 10 g of sample in 90 ml of 0.9% sodium chloride solution was mixed under sterile conditions, and then it was used for a total count of bacteria in a special culture medium. One milliliter of each dilution was cultured in the plate count agar (PCA) medium by pour plate method. The cultured samples were incubated for 48 h at 37°C to identify the total count of bacterial load at 7°C and for 7 days to identify psychrophilic bacteria. After the incubation period, the colony was counted. The total count was based on cfu/g [27].

2.4. Statistical Analysis. Data analysis was performed using SPSS software and to draw graphs used Excel software. One-way analysis of variance (ANOVA) was used to evaluate the significant difference between the values obtained from each sample at time 0, day 3, day 6, day 9, and day 12 for refrigerated samples. The least significant difference (LSD) test was used to compare the mean in cases where significant differences were observed, and Duncan's test was used to compare multiple treatments. All data were analyzed in triplicate.

3. Results and Discussion

The results of the analysis of the proximate composition and spoilage indices in the carp fillet immersed in extracts of turmeric, cinnamon, andlemonwerepresentedinFigures 1–9. According to the obtained results, immersion of the carp fish in turmeric, cinnamon, and lemon extracts led to significant changes in the number of proximate compounds in the fish tissue.

Control was T1 is without extract. T2 is a mixture of CT extracts; T3 is a mixture of TL extracts; T4 is a mixture of CL extracts; and T5 is a mixture of CTL extracts.

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3.1. Microbial Analysis Results. Counting the bacteria present in the carp tissue immersed in the extracts of turmeric, cinnamon, and lemon changed the number of bacteria total count and psychrophile bacteria present in the carp tissue. These changes were significantly different from each other. The results of microbial analysis of the carp tissue showed in Table 2.

Note. The same letters in each column indicate that there is no significant difference ($p < 0.05$). "±number" was a standard deviation.

Control (NT) was without extract. T2 is a mixture of CT extracts; T3 is a mixture of TL extracts; T4 is a mixture of CL extracts; and T5 is a mixture of CTL extracts.

Results shown in Table 2 showed that extracts of turmeric, cinnamon, and lemon changed the total count of bacterial colonies. Except for the 0th day and 3rd day, the

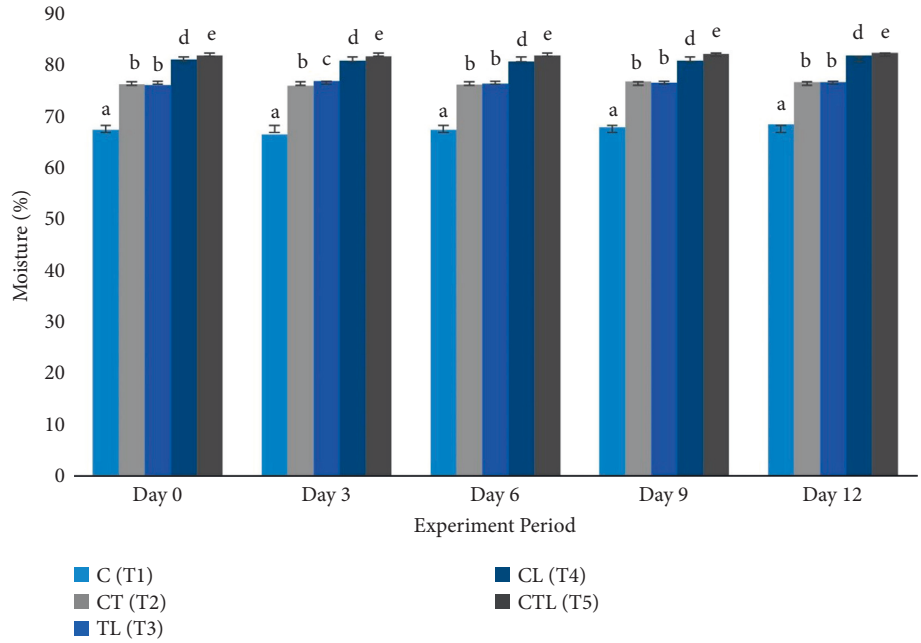

Figure 1: Percentage of moisture content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

number of bacteria in CL treatment was higher than in other treatments. In other experimental periods, the number of bacteria in the control treatment the on 0th day and 3rd day was higher than in other samples, which were significantly different in all treatments ($p < 0.05$). According to obtained results, all the experiments differed significantly compared to the NT, which was affected by the used extracts, and the values differed significantly in different samples ($p < 0.05$). On the 0th day, 6th day, and 12th day, the number of psychrophile bacteria in all treatments was significantly different with control and with each other ($p < 0.05$), but on the 3rd day, except for CL and CT treatments, the other treatments had significantly different ($p < 0.05$). On the 9th day, the treatments CT, TL, and CTL were not found significantly different ($p < 0.05$). Either 10^6 or 10^7 cfu/g indicated respective upper acceptable limits for psychrotrophic and mesophilic bacteria [28].

Immersion of the fish in turmeric, cinnamon, and sour lemon extracts in all samples had a positive effect on their bacterial load so that in experimental treatments, the total count in the carp tissue compared to the NT was significantly less. The inhibitory effects of turmeric, cinnamon, and lemon extracts on the total bacterial load in the carp were clear. Either 10^6 or 10^7 cfu/g indicated respective upper acceptable limits for psychrotrophic and mesophilic bacteria [28].

The antimicrobial effects of plant extracts have been attributed to their ability to disrupt cell walls/membranes, inhibit adenosine triphosphate (ATP) production, and disrupt protein synthesis and intracellular pH imbalances. These phenolic compounds are in extracts that have mainly antimicrobial effects [29].

Essential oils, such as thyme oil and cinnamon oil, have promising antibacterial properties against foodborne pathogens (such as *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, and *Listeria monocytogenes* in different food matrices. The food industry should be more cautious about using essential oils because the deadly stress of EOs can lead to resistance [30].

Studies have shown that grape seed extract has excellent antibacterial and antioxidant properties and shows good inhibitory effects on *Pseudomonas*, *Staphylococcus aureus*, and *Salmonella* [31].

3.2. Proximate Composition Analysis Results. The percentage of moisture in the treatments and control is shown in Figure 1. In all periods, the percentage of moisture in the fish immersed in CL extracts was higher than in the other samples. All samples had significant differences from each other in each treatment ($p < 0.05$). The percentage of ash in the NT was higher than in other samples (Figure 2). Except for the examples of CTL (0.65%) and CT (0.67%), there was a significant difference between other treatments on day 0 ($p < 0.05$). The sample CTL (0.67%) on the 3rd day had not significantly different from the sample CT (0.68%). The samples CTL (0.65%) and CL (0.63%) on the 6th day had significant differences ($p < 0.05$), but other samples found significant differences. The percentage of ash on the 9th day in samples CTL (0.65%) and CT (0.67%) also on the 12th day in treatments CT (0.65%) and CL (0.65%) were found significantly different ($p < 0.05$). According to obtained results from the present study, cinnamon, turmeric, and lemon extracts affected the fat percentage of the carp fillet. The fat percentage in the NT was higher than in other treatments on the 1st, 3rd, and 6th day but, for treatment CT on the 9th day and for treatment CTL on the 12th day, was found higher than in other samples (Figure 3). The percentage of fat from 6% to 5% was decreased for treatment CL on day 12 of storage.

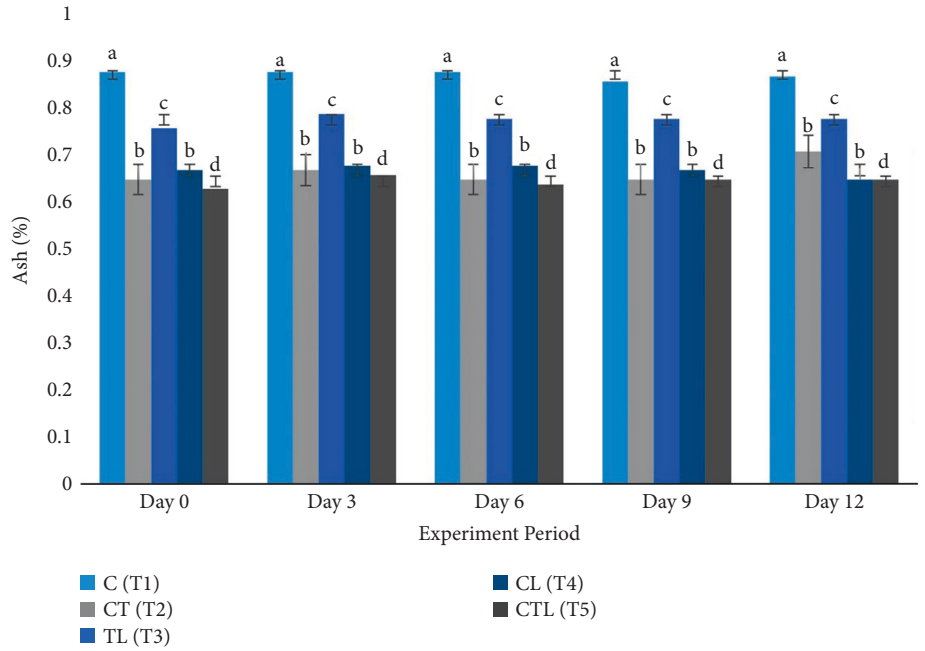

Figure 2: Percentage of ash content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

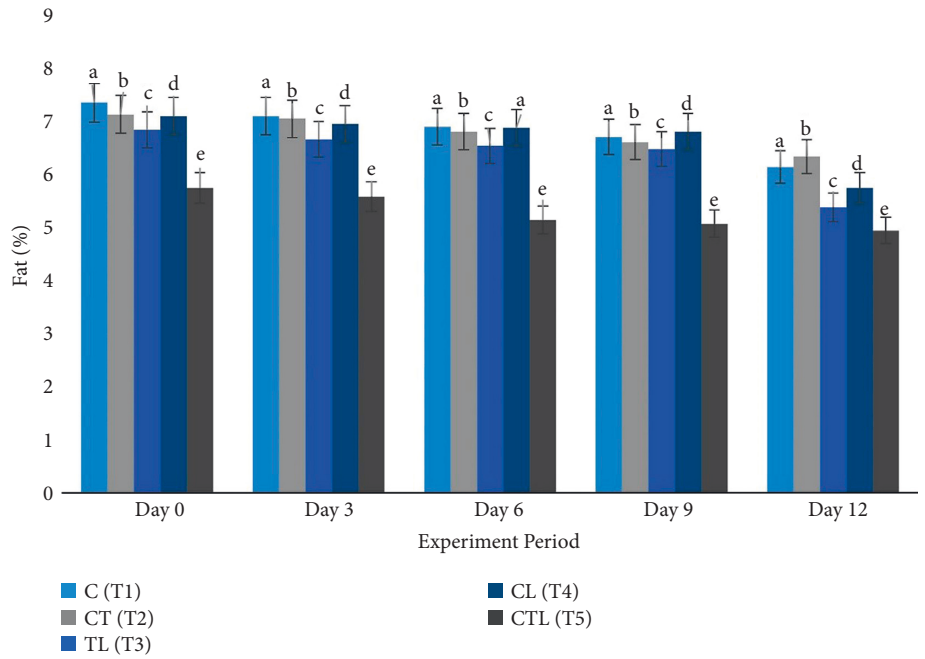

Figure 3: Percentage of fat content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

The fat content in treatment CL was lower than the other treatments in all storage periods that may be due to the breakdown of the fish fat by chemical compounds that exist in the extracts, while the protein content in treatments CT and CL decreased during storage periods, which was due to high volatile nitrogen compounds in these treatments.

The percentage of protein in all samples had a significant difference with some treatments except on the 0th day ($p < 0.05$). The effects of turmeric, cinnamon, and lemon extracts decreased the spoilage indices that had significant differences from each other ($p < 0.05$). The effects of turmeric, cinnamon, and lemon extracts decreased the spoilage indices that had significant differences from each other. There was a significant difference between pH levels in the samples ($p < 0.05$; Figure 5). The value of pH for the control from 6.2 to 4 decreased for CTL on the 12th day. There was not a significant difference in peroxide index on the 0th day between control and other treatments ($p < 0.05$; Figure 6), but on other days, there was a significant difference between the treatments. The peroxide value for the control from

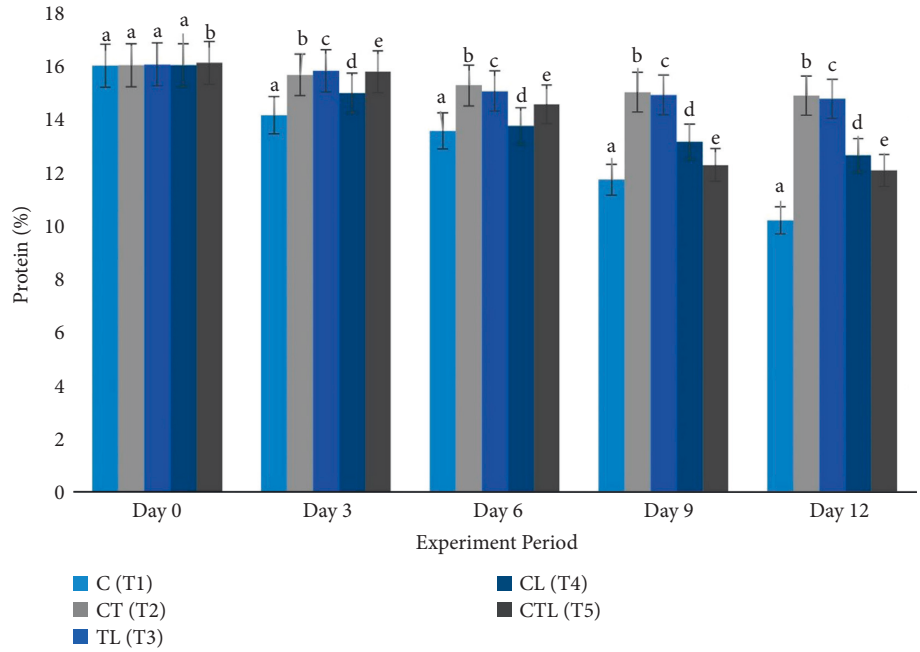

Figure 4: Percentage of protein content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

1 meq O2/100g on the first day to 4 O2/100g increased for CL on the 12th day. Thiobarbituric acid index in the control was higher than in other samples ($p < 0.05$; Figure 7). There was a significant difference between the control and other treatments except for CL treatment on day 0 and between the control and CL samples on the 3rd day ($p < 0.05$). In other periods, all samples had significant differences from each other. The results showed that the TBA index increased with time in the carp fillet in cold storage.

The pH value in treatment CT was highest, due to high volatile nitrogen compounds due to the breakdown of protein, which made the alkaline environment. The peroxide, TBA, volatile nitrogen, and acidity levels in all samples in all storage periods were found lower than the control, which was because of extracts in maintaining the fish's good quality, but the highest value of these indices belongs to treatments CL and CT and the lowest was for treatment CTL. Therefore, the best treatment was CTL and inappropriate treatments were CT and CL.

Foroughi et al. [32] investigated the effect of a combination of shallot extracts (*Allium ascalonicum*) and turmeric (*Curcuma longa*) on silver carp paste stored in freezing conditions. Their results showed that turmeric and shallot extract due to their antimicrobial and antioxidant properties were able to delay microbial spoilage, fat oxidation, and protein spoilage of carp paste. These extracts maintained the safety and nutritional value of fish paste by preventing fat changes and increasing the shelf life of the product.

Pezeshk and Hosseini [33] studied the antibacterial and antioxidant effects of turmeric extract (*Curcuma Longa*) in vitro on rainbow trout (*Oncorhynchus mykiss*). Their studies showed that turmeric extract significantly delayed lipid oxidation in treated fish and maintained nutritional value and improved spoilage in fish.

Ojagh et al. [34] investigated the effect of antimicrobial and antioxidant coatings of CT extract on increasing the shelf life of rainbow trout under laboratory conditions. Their results showed that cinnamon extract significantly reduced the number of volatile nitrogen bases in the samples. Texture, odor, color, and overall acceptance were significantly reduced only in NT.

The acidity levels in the experimental treatments were different, and this effect was shown for the 6th, 9th, and 12th day, while there was a significant difference between the other samples. The acidity values for all treatments on the first day were not significantly different ($p < 0.05$; Figure 9).

In the present study, the pH level in all experimental treatments was found significantly lower than the control, which was because of the extracts on carp tissue. A pH higher than 7.9 leads to spoilage and loss of tissue quality and reduces the acceptability of the product [35]. Low pH in samples can be treated with extracts of lemon; CT can be related to the acidic and antibacterial properties of lemon and CT extracts, which prevents microbial activity and ultimately prevents decomposition of protein and amine production [36]. Low pH prevents the activity of spoilage bacteria and endogenous enzymes and therefore leads to suitable preservation of fish. The reason for the decrease in pH at the beginning of the period is because of carbonic acid and the presence of ammonium compounds, which are produced due to spoilage due to bacterial activity [37].

Consistent with the results of Jedi et al. [38], the peroxide index showed a significant increase during the storage period, while the highest rate was observed on the 9th and 12th day of the experiment, but in all treatments, its amount was significantly lower than the control. The permissible limit of peroxide 7–8 M eq/kg of fat has been reported [39]. In the present study, except for the NT and the treatments of lemon

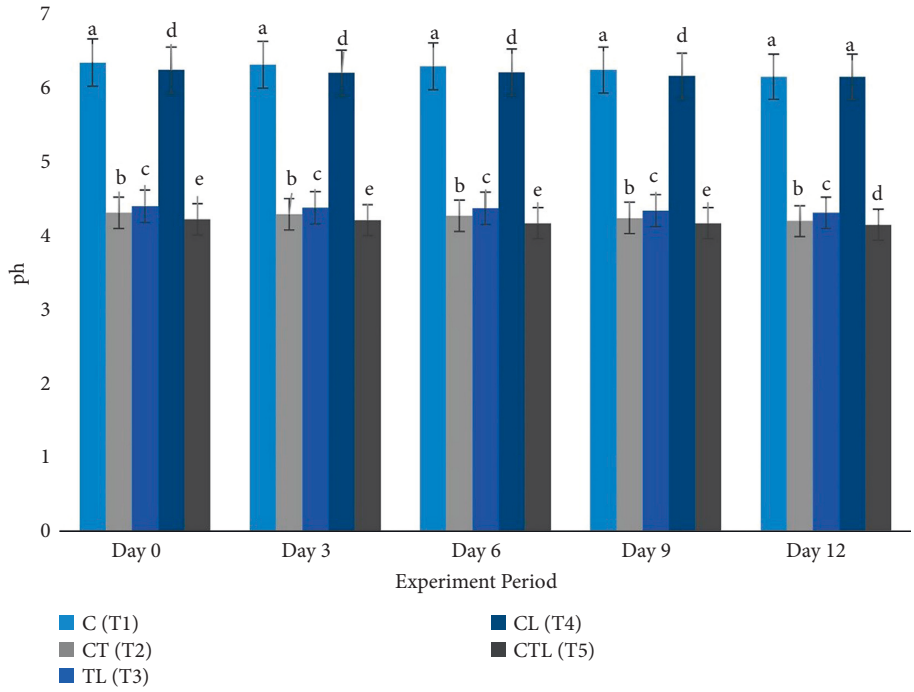

Figure 5: pH content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

and cinnamon on the 12th day of storage, the other samples had less than this allowable limit. Although the amount of volatile nitrogen in the carp fillets increased during the storage period on the 12th day, the amount of this index in fish immersed in cinnamon, turmeric, and lemon extracts showed a significant decrease compared to the control. The results of the present study agreed with the results of studies of Ojagh et al. [34] and Taghizadeh Andvari and Rezaei [40]. In the present study, this index in all samples was lower than in the control.

The results of the proximate analysis in the present study showed that the extracts had a positive effect on the proximate composition of the carp fillet, and these changes had significant differences from each other. The percentage of moisture in the treated samples was significantly higher than in the NT. The extracts preserved the moisture content of the carp fish fillet during cold storage.

Results showed that the ash content significantly decreased in all samples and all storage, periods compared to the control. All samples had significant differences from each other ($p < 0.05$), but the percentage of difference in ash values for CTL treatment on the first day from 0.65% to 0.7% increased on the 12th day. Shabanpour et al. [41] stated that, during the storage of salmon in the refrigerator, there was no significant change in moisture and protein contents between the experimental samples, while in the present study, the percentage of protein and moisture of samples immersed in the extracts of turmeric, cinnamon and lemon were higher than the NT, which indicated the positive effect of these materials on the nutritional value of the processed product. The results of Shabanpour et al. [41] showed that the fat content of carp and farmed fish samples stored in the refrigerator decreased during storage, which was consistent with the results of the present study.

Fat oxidation includes enzymatic and nonenzymatic oxidation, which leads to degradation and loss of fish quality. Fish is rich in unsaturated fatty acids that can be oxidized and eventually lead to dryness and poor quality. These compounds lead to inappropriate odors, colors, textures, and nutritional values. The products of fat oxidation also led to protein denaturation, food loss, and loss of internal antioxidant systems. Accordingly, fat oxidation leads to the unacceptability of fish by consumers [42].

Fish contain a high amount of free amino acids, high pH after rigor mortis, and high moisture, and many species of fish contain trimethylamine oxide (TMAO), which promotes bacterial growth in a wide range of temperatures [43]. Enzymatic spoilage is the main reason for the decrease in fish quality, which causes the loss of 30 to 25% of these products [44]. The plant-derived essential extracts have shown excellent antimicrobial and/or antioxidant activities in fish preservation [45].

Although fresh fish is highly perishable, and even if stored in cold storage to prolong its shelf life, these processes are not sufficient to prevent fat oxidation or bacterial growth. In most cases, improving the quality of fish is also necessary [46]. For this reason, it is necessary to add preservatives to the fish properly during storage. Natural preservatives in food have been used. Generally, people choose foods without preservatives, but if these substances are not available, the same consumer chooses foods containing natural preservatives instead of artificial foods [47].

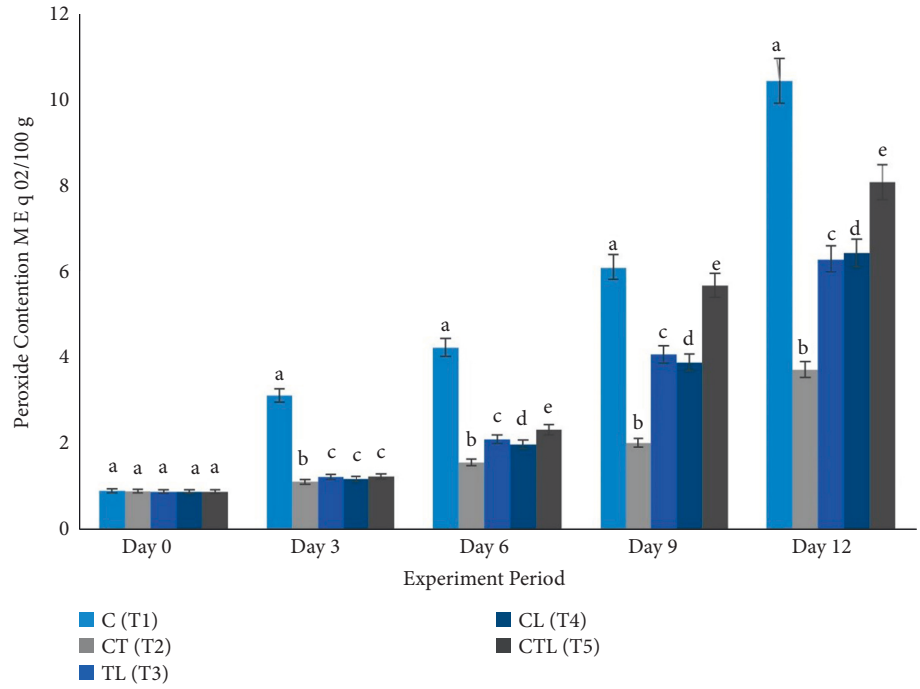

Figure 6: Percentage of peroxide content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

3.3. Changes in Total Volatile Nitrogen and Biogenic Amines. The initial volatile nitrogen of all treatments was in a low range of 20–40 mg N/100 g (Figure 8). The total volatile nitrogen value of the control treatment fluctuated during the earlier period of storage and increased significantly ($p < 0.05$) from day 3. A similar increasing trend was also observed in previous studies. The sharp increase of total volatile nitrogen usually occurs in the late period of storage, which may be because the generation of total volatile nitrogen requires a long degradation process to convert nitrogen-containing macromolecules to volatile small molecular compounds under the action of microbes. This significant difference in total volatile nitrogen content could be attributed to the antimicrobial property of the extracts. In addition, phenolic compounds in the extracts could prevent protein decomposition caused by bacteria and hinder their capacity for the oxidative deamination of nitrogenous compounds from nonprotein sources [48].

The volatile nitrogen value in the carp fillet in all treatments especially on the 9th and 12th day of the experiment was found significantly different from each other (Figure 8; $p < 0.05$). The highest value of this index was observed for the control, and the lowest was observed for the fillets immersed in cinnamon, turmeric, and lemon extracts. The volatile nitrogen value in the fresh fillet was lower than the permissible level (30 mg) on the 3rd day and found for treatment CTL on the 12th day lower than 30 mg. Total volatile basic amines are one of the most widely used indices of fisheries products quality and appear in marine fish spoilage [49]. The concentration of total volatile nitrogen in freshly caught fish is typically reported to vary between 5 and 20 mg/100 g [50]. The total volatile nitrogen values increased according to time of storage [51] proposed that the quality classification of fish and fish products regarding total volatile nitrogen values would be "high quality" up to 25 mg/100 g, "good quality" up to 30 mg/100 g, "limit of acceptability" up to 35 mg/100 g, and "spoilt" above 35 mg/100 g [11]. At the beginning of the experiment (the same day of the catch), the TVB-N values of the untreated samples were very low and close to 9 mg $100\,g^{-1}$ fish muscle, indicating the high standard of the freshness of the samples of *Mullus surmuletus* [52].

The plant extract (cinnamon) was added to the food, which led to a significant reduction in the accumulation of biogenic amines. Biogenic amines are a nitrogenous organic and toxic compounds in foods when the biogenic amine content is higher than required. Many studies have shown that beneficial microorganisms or starter cultures can reduce biogenic amine accumulation and prevent the growth of harmful microorganisms. Tea polyphenols suppressed biogenic amines formation by inhibiting the growth of biogenic amines-producing bacteria. This study showed that plant extract (cinnamon) effectively inhibits the growth of biogenic amines-producing bacteria, thus reducing the content of biogenic amines in food. Cinnamon essential oil can be used to reduce biogenic amine accumulation and inhibit the growth of spoilage bacteria in food, thereby improving food safety [53].

The plant extracts have broad application prospects in fish preservation. The antimicrobial activities of plant extracts may be attributed to the combined effects of polyphenols adsorption to the bacterial membrane with membrane disruption and subsequent cellular contents leakage, and the generation of hydroperoxides from polyphenols. Plant extracts also show antifungal activities, antioxidant, and antimutagenic activities and inhibit lipid

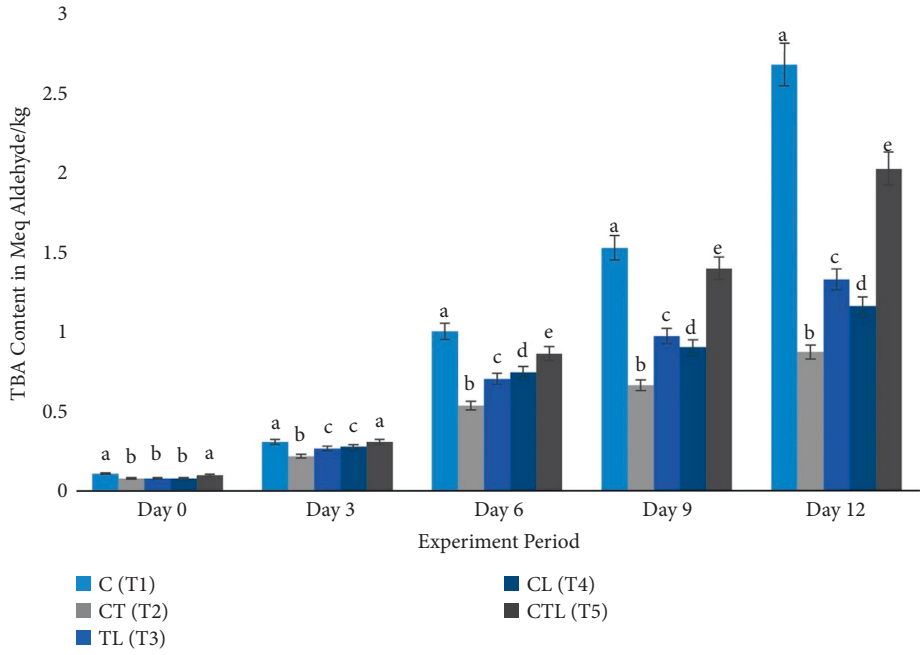

Figure 7: TBA content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

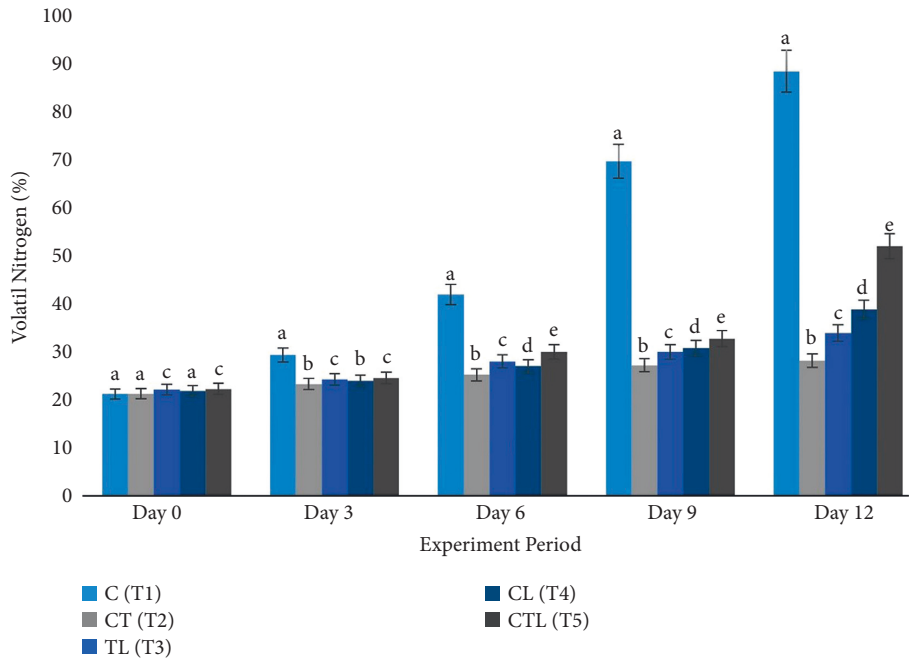

Figure 8: Percentage of volatile nitrogen content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

oxidation in food. The plant crude extracts generally contain flavonoids in the form of glycosides, in which the sugar in them decreases the effectiveness against some foodborne pathogens. The plant-derived compounds could extend fish shelf life by reducing the total aerobic plate count and retarding lipid oxidation and may also be used together with other natural preservatives or in different packaging ways. Rainbow trout (*Oncorhynchus mykiss*) using turmeric extract and shallot extract and their combination with vacuum packaging could reduce the growth of total viable count and extend the shelf life. The plant-derived compounds are also combined with nisin to extend the shelf life of fish and fish products [54].

The grape seed extract, as a functional substance, consists mainly of proanthocyanidins and a small number of monomeric polyphenols such as gallic acid and catechins.

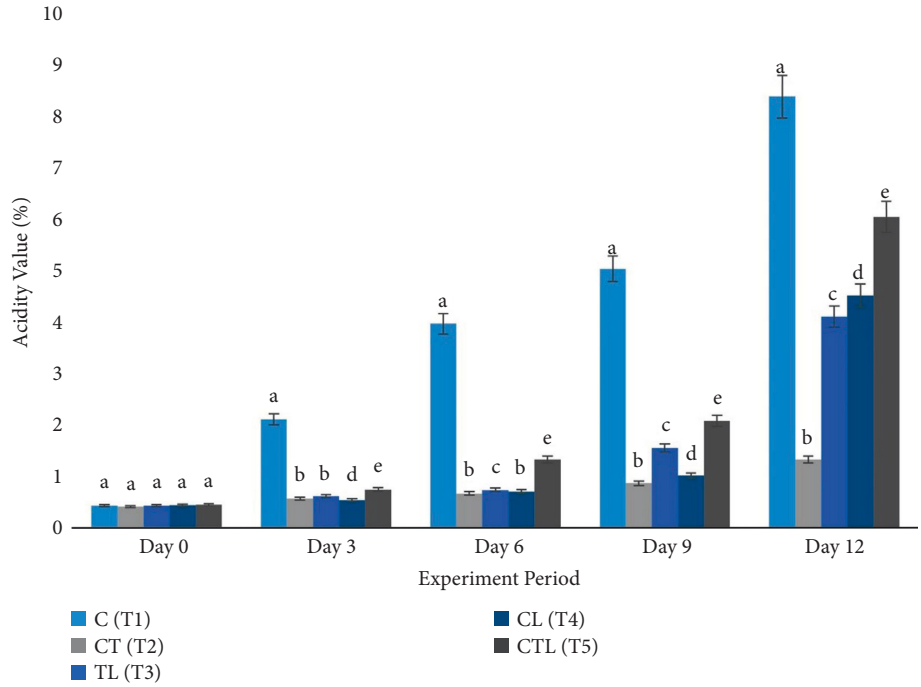

Figure 9: Percentage of acidity content in the carp fillet immersed in mixed different extracts of turmeric, cinnamon, and lemon at 4°C.

Table 2: Bacterial analysis (CFU/gr) of common carp tissue immersed in mixed different extracts of turmeric, cinnamon, and lemon before cold storage.

Storage time (Days)	Treatments	Total count of bacteria	Psychrophile bacteria
0th day	NT (T1)	$1.9\times10^{4}\pm0.24^{a}$	$1\times10^{2}\pm0.4^{a}$
	CT (T2)	$1.3\times10^{4}\pm0.43^{b}$	$0.93\times10^{2}\pm0.66^{d}$
	TL (T3)	$1.5\times10^{4}\pm0.73^{c}$	$0.84\times10^{3}\pm0.22^{c}$
	CL(T4)	$2.1\times10^{4}\pm0.21^{d}$	$5.2\times10^{2}\pm0.23^{d}$
	CTL (T5)	$1.3\times10^{4}\pm0.56^{b}$	$0.83\times10^{2}\pm0.78^{b}$
3rd day	NT (T1)	$4.7\times10^{5}\pm0.86^{a}$	$13\times10^{2}\pm0.34^{a}$
	CT (T2)	$0.75\times10^{5}\pm0.54^{c}$	$5.2\times10^{2}\pm0.23^{d}$
	TL (T3)	$0.77\times10^{5}\pm0.28^{c}$	$6.7\times10^{2}\pm0.74^{c}$
	CL(T4)	$1.10\times10^{5}\pm0.60^{e}$	$5.2\times10^{2}\pm0.23^{d}$
	CTL (T5)	$0.58\times10^{5}\pm0.33^{b}$	$3.1\times10^{2}\pm0.22^{b}$
6th day	NT (T1)	$73\times10^{5}\pm0.50^{a}$	$56\times10^{3}\pm0.65^{a}$
	CT (T2)	$2.3\times10^{5}\pm0.76^{d}$	$11\times10^{3}\pm0.4^{d}$
	TL (T3)	$4.2\times10^{5}\pm0.81^{c}$	$1.7\times10^{3}\pm0.45^{c}$
	CL(T4)	$7.50\times10^{5}\pm0.21^{e}$	$2.3\times10^{3}\pm0.76^{e}$
	CTL (T5)	$3.1\times10^{5}\pm0.56^{b}$	$1.4\times10^{3}\pm0.23^{b}$
9th day	NT (T1)	$7.7\times10^{8}\pm0.42^{a}$	$6.20\times10^{4}\pm0.34^{a}$
	CT (T2)	$7.2\times10^{5}\pm0.87^{d}$	$0.52\times10^{4}\pm0.56^{b}$
	TL (T3)	$8.7\times10^{5}\pm0.32^{c}$	$0.65\times10^{4}\pm0.36^{b}$
	CL(T4)	$9.9\times10^{5}\pm0.56^{e}$	$6.2\times10^{4}\pm0.40^{c}$
	CTL (T5)	$6.8\times10^{5}\pm0.86^{b}$	$0.41\times10^{4}\pm0.50^{b}$
12th day	NT (T1)	$9.4\times10^{10}\pm0.30^{a}$	$7.5\times10^{8}\pm0.24^{a}$
	CT (T2)	$2.3\times10^{7}\pm0.35^{d}$	$1.1\times10^{5}\pm0.54^{d}$
	TL (T3)	$2.8\times10^{7}\pm0.56^{c}$	$9.1\times10^{4}\pm0.23^{c}$
	CL(T4)	$1.9\times10^{7}\pm0.44^{e}$	$5.1\times10^{6}\pm0.78^{e}$
	CTL (T5)	$1.9\times10^{7}\pm0.71^{b}$	$9.9\times10^{4}\pm0.89^{b}$

4. Conclusions

It can be concluded that the use of extracts of turmeric, cinnamon, and lemon led to the improvement and reduction of bacterial load in the carp fillets. Results showed that the compounds used in the extracts in the present research led to a significant reduction in microbial load during cold storage. Indeed, while the importance of the use of plant extracts in enhancing antioxidant and antimicrobial stability of seafood is widely recognized, their combination with another

preservation method such as cooling is resulting in further superior results. The present study showed the changes in volatile nitrogen compounds in the carp fish. Based on the results of the present study, it can be concluded that the use of extracts of turmeric, cinnamon, and lemon led to improve proximate composition and reduce the rate of spoilage in the carp fish fillet. The extracts led to moisture retention, increase protein content, and decrease fat and ash content in experimental treatments. Results showed that the compounds that exist in the extracts led to a significant decrease in the spoilage indices during the cold storage. The highest spoilage indices belong to treatments LD and DZ, and the lowest was found for treatment LDZ. Therefore, the best treatment was LDZ, and inappropriate treatments were DZ and LD.

Data Availability

All data included in this study can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Ali Aberoumand conceptualized and designed the study; Semira Maleki Mugahi contributed to bacteriology testing; Ali aberoumand reviewed the manuscript; Ali Aberoumand and Saeed Ziaeie nejad approved the final manuscript; and Ali Aberoumand contributed to the submission of the paper.

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