

Impact of an Herb-Based Nano-Emulsion on the Sensory, Chemical, and Microbiological Characteristics of Rainbow Trout Fillets under Ice Preservation

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Abstract

This article's goal was to evaluate the effects of Nano-emulsions made with different vacuum packing and laurel essential oil (LEO) (1%, 2%) concentrations on the span of time of rainbow trout (RT) preserved in ice preservations. Trout fillets were sprayed with LEO. Changes were noted in the microbiological, chemical, and sensory quality features after 14 days of storage. After performing microbiological, chemical, and sensory tests, it was determined that 2% LEO prevented microbial deterioration in vacuum-packed RT, lengthening the RT span of time by around 4 days and improving its sensory qualities. Using Nano-emulsions made from all essential oil (EO) lower the values of the biochemical metrics and inhibited bacterial growth. Moreover, using LEO instead of synthetic additives may be advised to provide microbiological protection and so lengthen the span of time of various meat and meat products.

Keywords: Nano-emulsions, Rainbow trout (RT), Span of time, Laurel Essential oil (LEO),

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1. Introduction

Food processing, packaging, safety, nutrition, and nutraceuticals are just a few of the areas of food science where nanotechnology may be used. Due to their simplicity in production, tiny particle size, improved bioavailability, bio-efficacy, and kinetic stability, nano-emulsions are gaining recognition as the best vehicle for the delivery of lipophilic drugs. The water content in their scale models leaves no accessible water for microbes, nano-and emulsions are also considered self-storing antimicrobials [1]. Fish and fish products are loved by people all over the globe because of their great nutritious value and delectable flavor. Hence, ensuring the safety of edible fish is crucial in the global fishing sector. Fish and other seafood are among the foods that spoil quickly owing to lipid oxidation and microbial development. It might be challenging to provide safe and high-quality seafood when the need for natural goods devoid of chemical preservatives is taken into account. As a result, it has been a surge in involvement in the herbal extract and antibacterial properties of organic additives including EOs as a replacement for synthetic

additives to improve food's oxidative and microbiological stability and lengthen the span of time [2]. Secondary metabolites from plants known as EOs have been used in the culinary, cosmetic, and pharmaceutical industries. Due to rising consumer need for organic products and the substitution of artificial chemicals in the food sector, the usage of EO as an aroma and flavoring element has expanded lately. In sustaining the death standard and increasing the span of time of RT, many EOs have been tested [3]. Due to fish's high level of unsaturated fatty acids and the fact that it spoils more rapidly than other fresh foods, one of the most significant problems facing the food industry and consumers is the oxidative rotting of aquaculture products [3]. The product's span of time is shortened as a consequence of oxidative deterioration, and undesirable modifications occur to the taste, fragrance, and color of the product. Synthetic antioxidants and antimicrobials are often employed to decrease the incidence of rancid flavor and odor [4]. The majority of customers want food free of dangerous artificial flavors, such as those

added as conditioners and antimicrobials. As a substitute to antimicrobial compounds often employed to lengthen the span of time of food and combat foodborne diseases, it has thus been an increase in involvement in more natural and non-artificial antimicrobial substances [5].

The major portion of these activities has been linked to EOs and other secondary plant metabolites. Fragrant plants and their constituent parts have been studied as possible inhibitors of germ growth. Due to their possible antibacterial characteristics, EOs from various sources are often advised. Associated with several adverse impacts of chemical additives on human health, natural additives have proliferated. Vegetable oils are the most used natural addition. Innovative nano-technological conservation techniques are being developed by researchers for the use of natural additives in aquaculture products [6]. One of these novel techniques is the need for nano-emulsions to develop the span of time of RT goods without compromising the flavor of the products. As nano-food may increase product output, span of time, performance, and security, they have sparked policy discussions in several nations [7]. Labeling of nano-food items is required by the European Commission. Several nations have different views on the regulation, labeling, and approval of food nanotechnology products. Regulations are also required concerning the prospective use of nanotechnology in the field of food science [8]. Vacuum packing entails sealing off the package with a hermetic seal after sealing the product within a low-permeability film. Vacuum packaging has been found to increase the food span of time by six days or more [9]. On the other side, while rancidity does not form as a result of prolonged storage, unfavorable odor, and flavor caused by bacterial activity may happen [10]. To enhance the span of time of vacuum-packed RT, this research explores the possible application of LEO in varied concentrations (1%, 2%). Even at cold temperatures and under various packing circumstances, the microbial load is a key spoiling factor in chilled preserved seafood. In chilled-storage circumstances, EOs provide a helpful natural tool for suppressing microbial load and deterioration. The use of bulk EO is constrained by several drawbacks [11]. The development of Total Mesophilic Aerobic Bacteria (TMAB) in skinless RT fillets kept at 41°C was inhibited using nano-mats. The skinless fish fillets treated with AuZ-Nm also showed dielectric characteristics and then control samples. The samples examined with AuZ-Nm underwent sensory alterations while the course of the 8-day storage period. By utilizing AuZ-Nm, it is possible to maintain the dielectric characteristics, restrict microbiological spoilage, and postpone sensory degradation of the fish fillets [12]. The average width of electro spun nanofibers and the zeta potential value was determined to be 3.38 mV and 150.88 nm, correspondingly. Fibrous, smooth, and ultrafine nanofibers were effectively produced, according to SEM pictures. The RT fillets coated with nisin-loaded PVA-based nanofibers were kept from outside contamination by being packaged in PE [13]. Worldwide, user's intake a lot of fish as a primary source of omega-3 fatty acids and protein. Nevertheless, putrefaction may occur in fish muscles while preservation. This reduced beginning load could have had an impact on the product's microbial load up to the conclusion of the fermentation time [14]. The span of time fish was

increased by 6 days with the application of nano-emulsions, and throughout the storage time, it was found that the chemical and microbiological parameters were lower than they were with the control. Nano-emulsions have a suppressive impact on bacteria and viruses because they prevent germs from accessing water due to their water-binding ability [15].

The remaining portion is structured as follows: the technique of the nano-emulsion based on herb EO was presented in section II, the performance analysis was shown in section III, and the study was concluded in section IV.

2 Material and Methods

2.1 Nano-emulsion preparation

Laurel oil, 2% ethanol, and 2% surfactant-Tween 80 (GRAS = deemed safe-harmless in general) were utilized to form an oil phase in the oil nano-emulsion in water. These oil phase components were combined and maintained at 86 °C for one hour in the oven. Once the mixture was taken out of the oven and allowed to cool to room temperature, sterilized clean water was added. The liquid was then put into a beaker filled with ice and homogenized for 15 minutes at 72 AMPL using an ultrasonic homogenizer (Optic Ivymen System CY-500, Barcelona, Spain). The nano-emulsions viscosity, surface tension, droplet size, and thermodynamic stability were all measured. Using a Mastersizer 2000 (Malvern, UK) that relies on laser diffraction, the average particle size of the droplets in the emulsions was calculated. A rheometer was used to test the viscosity (TA Instruments ARES Rheometer).

2.2 Plant Material

Fresh laurel leaves were gathered and dried. To separate the EO, the dried laurel leaves were mechanically pulverized. Using a Clevenger device, water steam distillation was used to create the laurel oil from crushed leaves (Wisd-Wise Therm). 500 mL of purified water was poured into a round-bottom flask that contained 50 g of the ground material for this purpose, and the sample was then injected into the Clevenger contraption. The EO produced by a three-hour purification operation was stored at 4°C in a tightly packed, dark container until use in the testing.

2.3 Analyzing Volatile Components

Analyses using a gas chromatography-mass spectrometer (GC-MS) may be performed on solid, liquid, or gaseous materials. The gas chromatograph is the initial stage of the analysis process. At this stage, the data is vaporized into the gas phase and then passed down a capillary column that is covered with a stationary (liquid or solid) phase. This phase then separates the data into its parts. In addition to an SGE-BPX5 MS glass capillary from Science Instrumentation Solutions Incorporated of Ringoes, New Jersey, Thermally Finnigan's Gas chromatographic DSQ/A1300 (EI quadrupole) were utilised to analyses the EOA 70 eV electron ionisation device was employed for the

GC-MS identification process. One millilitre per minute (mL/min) of helium was pumped as the carrier gas. The MS power line and injectors were tuned to 220 and 290°C, respectively.

The temperature range for the temperature range was 50–150°C with a 3°C/min rate, kept isothermal for 10 min, and then enlarged to 250°C at a rate of 10°C/min. In the splitless mode, diluted samples of 1.0 microliter (1/100, v/v, in methylene chloride) were injected. The components were identified by contrasting the mass spectra and relative retention durations of the samples based on value, Wiley7N, the TRILIB database information from the GC-MS equipment, and data from other studies. The quantitative data was presented using Area%.

2.4 Sampling and Packing

We provided RT fillets, every measuring around 100 g. The samples were delivered to the lab in an icing chain, and suddenly placed in ice preservation in a cooled incubator, and the packaging procedure was started right away. In this investigation, three testing groups in all were created. The fillets were vacuum-packed in polyamide film bags using an untreated Henkelman small Jumbo machine to create the vacuum-packed. The fillets in the laurel group were sprayed with 5 mL LEO at concentrations (1% and 2%) in purified water with 0.2% Tween 80 before being vacuum-packed similarly to the vacuum-packed. The full sample set was kept at 4°C, and on days 0, 2, 4, 6, 8, 10, and 12 of ice preservation, microbiological, chemical, and sensory evaluations were carried out.

2.5 Microbiological Analysis

Regarding this investigation, 10 grams of every data was taken, weighed, and put into stomacher bags under aseptic circumstances. After that, the stomacher was homogenized in the stomacher for 2 minutes with 90 mL of sterile physiological saline solution added. The samples were decimally diluted before being inoculated using the pour plate and streak plate procedures to a microorganism-specific medium.

Oxoid CM 325 Standard Methods Agar was fermented for 48 hours at 30°C to determine the total viable counts; at 7°C for 10 days to determine the total psychrotrophic bacteria; at 30°C for 48 hours to calculate the activation of *Pseudomonas* species; at 30°C for 48 hours to calculate the activation of lactic acid bacteria (LAB); and at 30°C for 3 to 5 days to determine the presence of "Enterobacteriaceae, Violet red bile glucose agar (Oxoid CM 485) was incubated at 35°C for 48 hours, violet red bile lactose agar (Oxoid CM 107) was incubated at 37°C for 24 hours, and violet red bile lactose agar (Oxoid CM 107) was incubated at 44.5°C for 24-48 hours" to incubate fecal coliform.

2.6 Chemical Analysis

2.6.1 Total Volatile Basic Nitrogen (TVB-N) calculation:

The Antonocopoulos technique was followed in the evaluation of TVB-N. For this purpose, ten grams of the homogenised sample were measured in a 0.1 mg weighing scale and then added to the tube of the Kjeldahl instrument together with 100 mL of distilled water and 1 g of mgo. 10 mL of 3% boric acid, 8 drops of methyl-red, and an average of 100 mL of purified water were added to an Erlenmeyer flask before it was set on the distillate collecting part of the distillation machine. A total of 200 mL of liquid was produced after further distillation was performed. After that, 0.1N hydrochloric acid was titrated into the distillate until the color changed.

2.6.2 pH measurement

One hundred millilitres of purified water was used to homogenize a 10 g fish from each group's sample after it had been weighed. A digital pH meter was used to test the homogenizer's pH level (Thermo-Orion 3 Star).

2.7 Sensory Analysis

The Quality Index Method (QIM) was modified to conduct the sensory analysis on days zero, two, four, six, eight, ten, twelve, and fourteen of storage. In the sensory analysis, a score of zero means very fresh fish, while values that steadily increase suggest deterioration with time. The QIM system is shown in table 1.

3. Results and Discussions

LEO GC-MS analysis identified 92.98% of the components. According to analysis, 1,8-cineole (68.82%), α -terpinyl acetate (6.94%), and sabinene (12.2%) make up the majority of LEO. Figures 1 and 2 display the results of the microbiological tests conducted on vacuum-packed (control group), 1%- and 2%-LEO-examined vacuum-packed materials on different days of ice preservation. The control, 1%, and 2% LEO groups' starting stage (day zero) Enterobacteriaceae counts were found to be 1.44, 1.11, and 0.78 cfu/g log, correspondingly (P greater than 0.05). The figure was 7.60 cfu/g log in the vacuum-packed on day fourteen of ice preservation, whereas 6.37 cfu/g log was found in the 2% LEO group. As a result, there were no significant changes between the 1% LEO group and the vacuum-packed (P-value is greater than 0.05), however, there was a difference with the 2% LEO group. The control, 1%, and 2% LEO groups' starting stage (day zero) coliform counts were found to be 2.52, 1.90, and 0.77 cfu/g log, correspondingly (P-value is greater than 0.05). This value was 7.35 cfu/g log in the vacuum-packed on day fourteen of ice preservation, whereas it was 6.30 cfu/g log in the 2% LEO group.

Table 1: QIM scheme

Quality Parameter			
Meat	Breakdown status	Intensely broken down	3
		Broken down yet still intact	2
		Not entirely intact, somewhat damaged	1
	Color	Yellow, completely pink	2
		Slightly yellowish, slightly pinkish	1
	Brightness	Milky	2
		Transparent	0
		Dull	1
		Bright, shining	0
	Blood	Clouded, brown	2
		Bright red, none	0
		Clouded, brown	2
		Pale red, dull	1
	Smell	Laurel oil smell	Yes/No
		Acetic /Ammonia	3
		Sour milk	2
		Fresh	0
		Seaweed	1
	Texture	Very soft	2
		Slightly soft	0
Firm		1	
Skin	Mucus	Thick, yellowish	2
		Transparent, Thin	0
	Brightness	Dull, Slightly thick	1
		Slightly dull	1

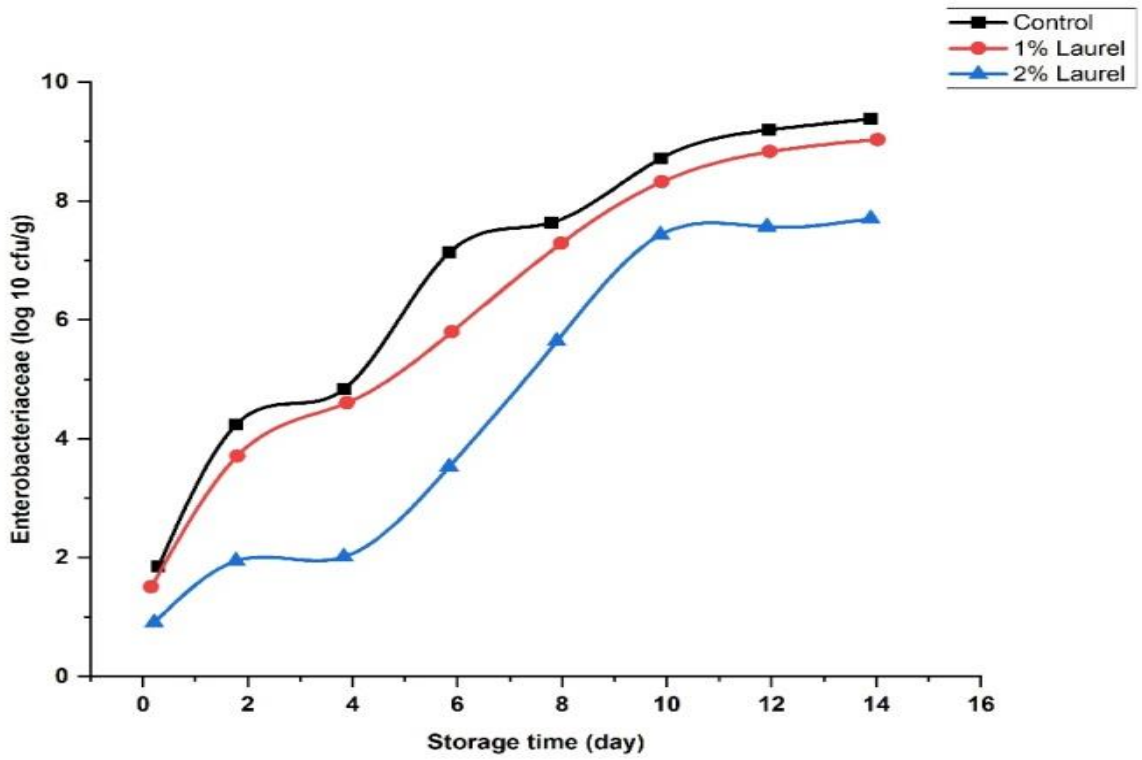


Figure 1: Enterobacteriaceae LEO during ice preservation

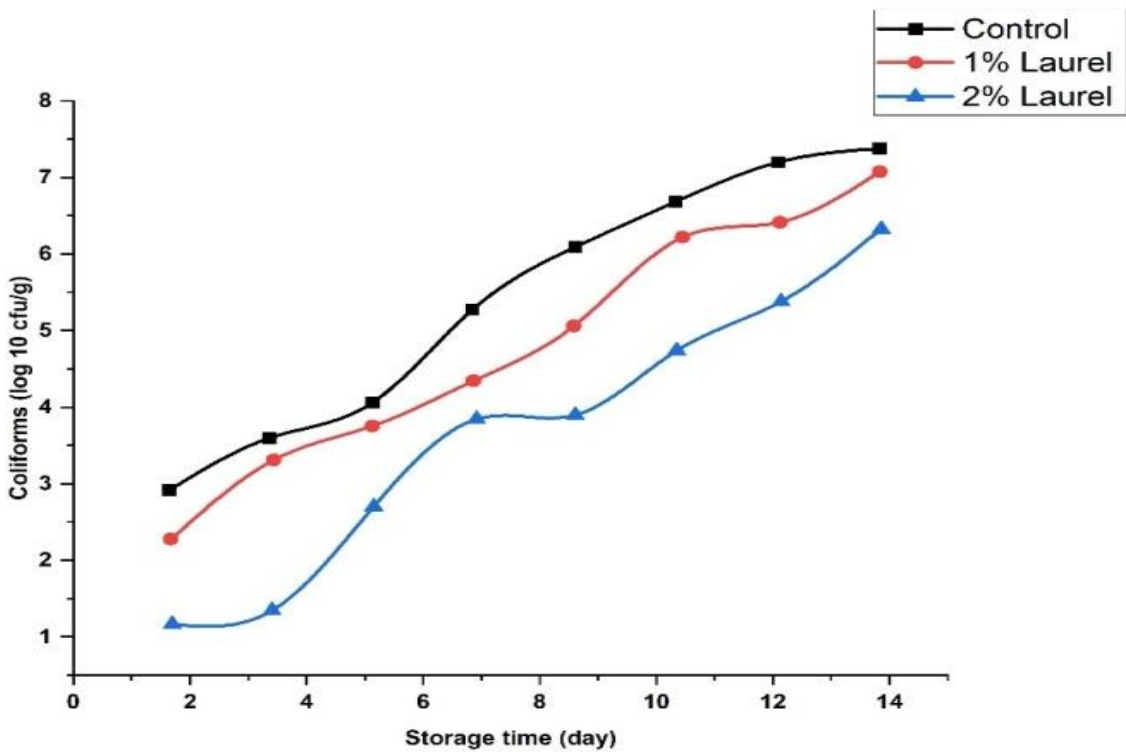


Figure 2: Coliform LEO during ice preservation

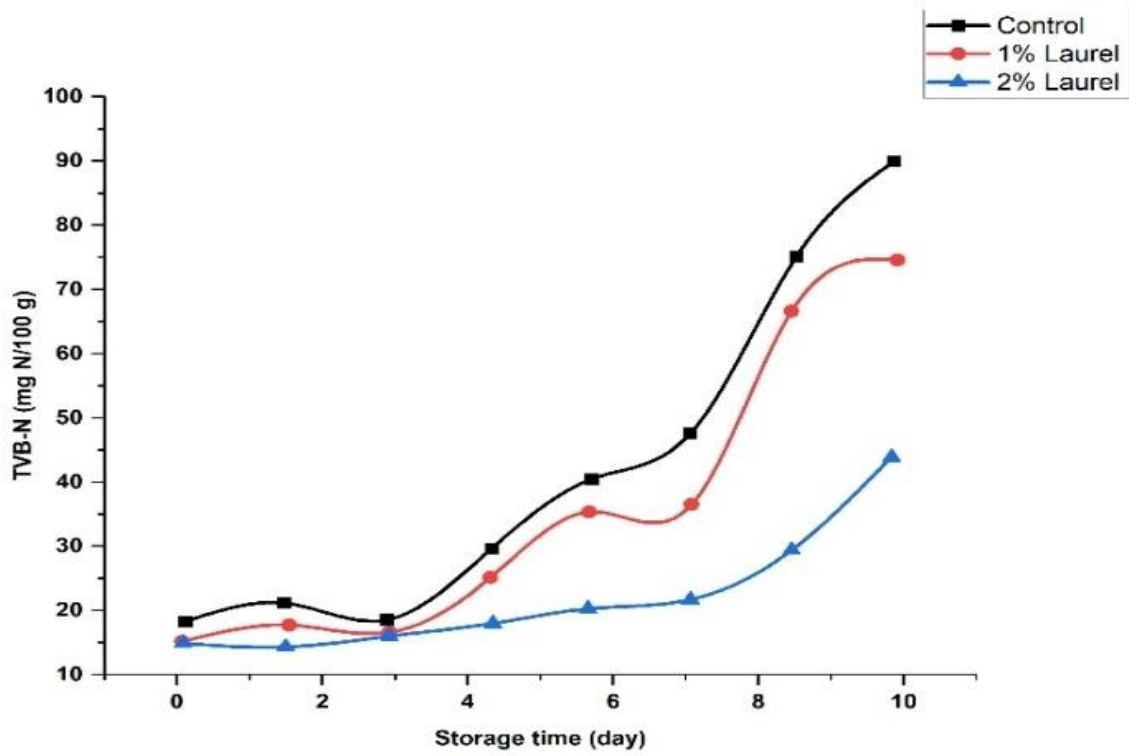


Figure 3: Total Volatile Basic NitrogenLEO during ice preservation

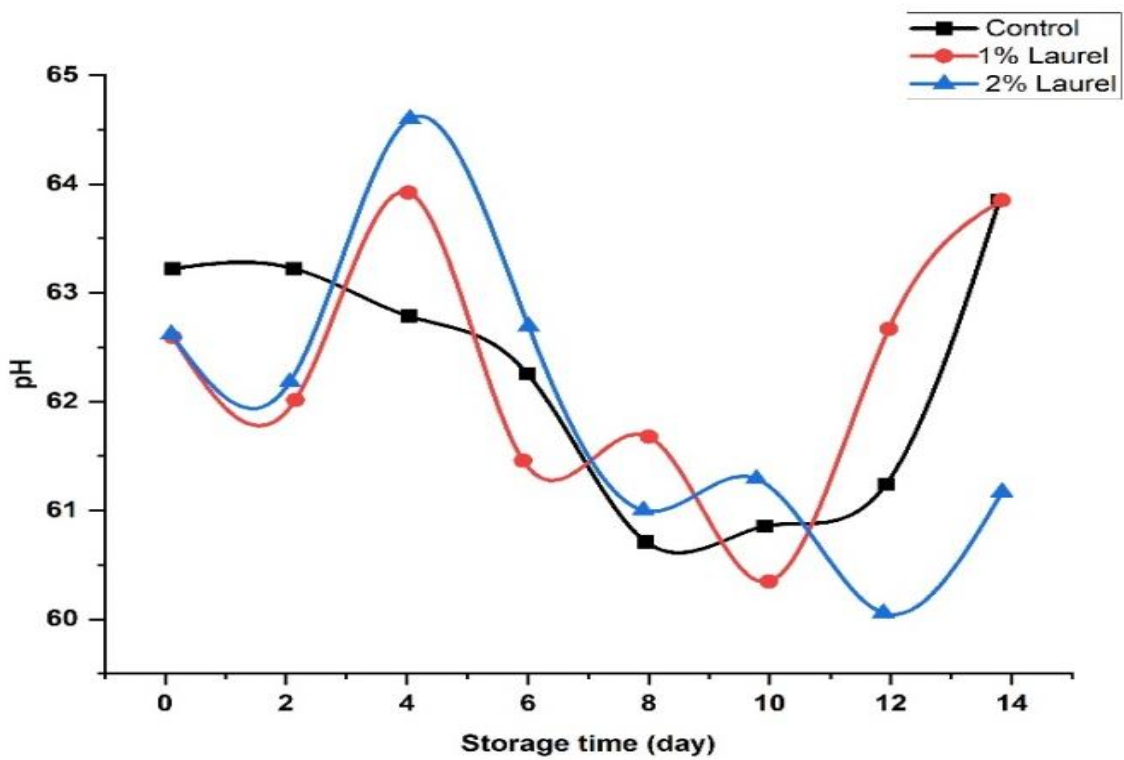


Figure 4: pH LEO during ice preservation

There were found to change with a high probability between the groups (P less than 0.05). Fecal coliform group bacteria were not identified in any of the 1% or 2% LEO groups, controls, or comparison groups. In the control, 1%, and 2% LEO groups, it was shown that the TVB-N level rose over the course of storage. There was no statistically significant difference (P-value is greater than 0.05) at the starting (day zero) TVB-N values among the control, 1%, and 2% LEO groups (16.6, 16.3, and 14.9 mg/100 g, correspondingly). On day fourteen of ice preservation, 92.2 mg/100 g of this level was found in the vacuum-packed but it was found to be 45.6 mg/100 g in the 2% LEO group. A statistically significant change between these values was found between these values (P less than 0.05). Figure 3 displays the TVB-N measurements made for the samples during the course of ice preservation.

The control, 1%, and 2% pH levels LEO categories did not change statistically significant at the beginning (day zero), but on day fourteen of ice preservation, there were statistically significant changes between the groups (P-value is less than 0.05). Figure 3 displays the samples' observed pH values during the course of ice preservation.

4 Conclusions

EOs have been studied as potential natural antibacterial food preservation agents. It was discovered that nano-emulsions reduced fishy odor during storage from a sensory standpoint. The usage of nano-emulsions based on all of the EOs, however, resulted in lower values for the biochemical parameters and was successful in reducing the rate of bacterial growth. In vacuum-packed RT, it was discovered that 2% LEO prevented microbiological deterioration, prolonging the span of time by around 4 days and improving sensory qualities. So, it has been determined that the combination of LEO and vacuum packaging is likely to increase the seafood's span of time and be utilized in the food industry.

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