EFFECT OF BLACK CUMIN (*NIGELLA SATIVA*) SEED AS A NATURAL PRESERVATIVE AND SODIUM ACETATE AS A SYNTHETIC PRESERVATIVE ON PROTEIN AND FAT CONTENTS OF ROHU (*LABEO ROHITA*) AND MORI (*CIRRHINUS MRIGALA*)

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Abstract

The advantages of fish meals for human nutrition and health have recently attracted considerable attention. Throughout the past few decades, their consumption has significantly increased due to their excellent nutritional content. Both the rohu (*Labeo rohita*) and mori (*Cirrhinus mrigala*), which are Pakistan commercial fish species, are significant aquatic food sources. The purpose of this study was to determine how sodium acetate and a particular natural preservative, black cumin (*Nigella sativa*), affected the protein and fat content of *L. rohita* and *C. mrigala*. We bought 96 species samples, cleaned them, and removed the guts. Six of the 96 samples were freshly examined, with the remaining samples serving as the experimental and control groups. The control group was without preservatives, and both species were preserved in the experimental group with black cumin and sodium acetate at concentrations of (3g/l, 5g/l, and 7g/l). Samples of both species were frozen at 4±1ºC for 15, 30 and 45 days. In the fresh sample *of L. rohita*, the highest % value of protein 30.382±1.835 was observed, while black cumin seed oil showed values from 28.437±1.546 to 24.791±1.263 and sodium acetate quantified the highest % value at 26.916±2.162 and the lowest % value at 19.687±2.177. In *C. mrigala* on the first day, the peak of protein was 28.437±1.786, black cumin seed oil indicated values from 27.708±1.262 to 21.729±2.409 and sodium acetate showed the maximum value at 26.979±1.262 and the least value at 21.145±1.263. While the % value of fat in the fresh sample of *L. rohita* on the first day was 11.67±0.235 with black cumin seed oil it ranged from 10.83±0.288 to 8.33±0.577 and sodium acetate indicated values from 10.66±0.288 to 7.66±0.763. In *C. mrigala*, the fresh sample had 12.67±0.288% fat content. Black cumin seed oil represented % values from 12.83±0.288 to 10.17±0.763 and sodium acetate ranged from 12±0.866 to 8.83±0.763. The results explained that when compared with the experimental group, the maximum % value was observed on the 15th day and the minimum on the 45th day.

Keywords: Nutrition, Consumption, *Labeo rohita, Cirrhinus mrigala,* Protein, Fat Content,

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1. Introduction

Fish are the largest group of animals used for animal-based food production (Hussain *et al*., 2015). Fish and other seafood are considered one of the most valuable resources for the human diet because of their high nutrient content. Therefore, their dietary intake has increased significantly over the last few decades. Overall, rates of fish consumption are growing rapidly due to increasing population growth, rising incomes, recognition of health benefits related to fish consumption, and increasing urbanization. In addition to directly supplying high-quality food, fisheries and aquacultures by raising, trading, and selling wild and farmed fish, create economic value. Therefore, fish is widely recognized as "the superfood of nature" (Obiero *et al*., 2019).

Fish and other aquatic foods contain high levels of free amino acids, and high-water content and many species of fish contain trimethylamine oxide (TMAO). Both Gram-positive and Gram-negative bacteria grow more and can survive a wide range of temperatures due to such features. Therefore, the quality deterioration of fish and other seafood is mainly affected with up to 25 to 30% attributed to microbial growth (Hassoun and Coban, 2017).
Major carp are commercial species, while *Labeo rohita* and *Cirrhinus mrigala* are valuable aquatic foods in Pakistan (Sheikh *et al*., 2017). The carp is the oldest domesticated species for food. According to the FAO, in 2004 common carp production made up 13% (3,387,918 tonnes) of all global freshwater aquaculture production. Asia yields more production of carp species and consumption rates are higher there as well (Basnet *et al*., 2013).
Several carp, especially the Morakhi (*C. mirrigala*), Rohu (*Labeo rohita*), and Thaila (*Catla catla*), are regarded as an important source of protein from Pakistani waterways. These carp have more commercial value and these species are preferred for cultivation. *L. rohita* is a member of the Cyprinidae family in the Cypriniformes order. It is native to the river systems of Bangladesh, India, Pakistan, and Myanmar. These fish species are more valuable due to their many attributes such as a faster growth rate, high market prices, greater food utilization, and the ability to inhabit all three columns of water bodies (Yeasmin *et al*., 2010). The most important species for India's freshwater fisheries are the major carp. *L. rohita* is highly recommended for cultivation among the major Indian carp and contributes to over 80 percent of India's major carp population. Its delicious taste, high commercial value, and white color enhance the carp's desirability (Dhanapal *et al*., 2013).
Southeast Asian countries have a higher rate of *C. mrigala* cultivation. This species traditionally plays a significant role in polyculture with other native species, particularly in India. Additionally, in Nepal, Bangladesh, Pakistan, Burma, the Lao People's Democratic Republic, and Thailand, *C. mrigala* has gained importance in fish culture. The *C. mrigala* is preferred for pisciculture due to its fast growth rate. When cultured in artificial environments, *C. mrigala* increases in size and it contains high protein content (Basnet *et al*., 2013). The annual loss of one-third of the world's food production is primarily due to microbial spoilage. Food preservatives have garnered significant attention as a means to extend the shelf life of food and enhance the quality of seafood (Toson *et al*., 2017). With the increase in species growth, the use of preservatives and additives has also risen over the past few years (Hassoun and Coban, 2017).
For ensuring food safety, natural antimicrobials including organic acids, essential oils, plant extracts, and bacteriocins, could provide viable alternatives (Ozpolat and Duman, 2007). Recently, the use of medicinal plants has gained popularity in comparison to chemical drugs due to reasons such as affordability, accessibility, and reduced harm. According to the WHO, around 80% of people get benefits from herbal remedies and further research is needed on many medicinal plants for their protective, therapeutic properties and mechanisms of action (Kooti *et al*., 2016).

This plant is known by various names including black cumin in English, habba-tu sawda in Arabic, Krishnajirika in Sanskrit, shonaiz in Persian, black caraway seeds in the U.S.A., Kalonji in English and Urdu, and kalajira in Bengali. Black cumin is the common name for the *Nigella sativa* species of the Ranunculaceae family. Black cumin seeds exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, making them biologically active (Eskandari *et al*., 2014). Due to its many beneficial properties, *N. sativa* holds great importance in Islamic countries. It is the black seed referenced as having healing powers by the Prophet Mohammad (S.A.W.W). The healing powers of black cumin are referenced in religious books, such as the Holy Bible (Salem and Hossain, 2000). Egypt is known for exporting high-quality black cumin seeds, which are rich in nearly 100 different chemical elements and essential fatty acids (Ramadan and Moersel, 2002). Black cumin seeds have a fat content of 35.5%, protein content of 22.7%, fixed oil content ranging

from 35.6% to 41.6%, and volatile oil content ranging from 0.5% to 1.6%, which can enhance broiler productivity and foster feed development (Azeem *et al.*, 2014).

The most commonly used additives in the food and industry sectors are salt, due to its economical nature and its unique and diverse properties, such as serving as a food preservative and antimicrobial agent. Salt in the form of sodium chloride (NaCl), possesses a significant capacity to reduce water activity while maintaining the shelf life and quality of food products. Salt is extensively employed as a preservative to inhibit microbial growth. An excessive concentration of NaCl serves to minimize the action of proteases, thereby preventing meat from spoiling (Oranusi *et al*., 2017).

The use of sodium salts or certain organic acids, such as sodium acetate and sodium lactate, can assist in reducing the factors contributing to reduced shelf life. These two preservatives are safe for human consumption and effectively prevent the proliferation of infections and spoilage microorganisms. Common preservatives like sodium acetate, sodium chloride, and sodium lactate do not pose a threat to human health. Moreover, research has demonstrated that the addition of sodium lactate and sodium acetate as preservatives extends the shelf life of meat by inhibiting microbial growth (Tangkham *et al*., 2012). In the past, acetates were used to prevent the growth of gram-negative spoilage bacteria in catfish fillets stored at 4°C (Kim *et al*., 1995).

2. Material and Methods

2.1 Fish selection and collection

Fish species *Labeo rohita* and *Cirrhinus mrigala* were purchased from the Baigowala fish farm in District Sialkot. Fresh samples of fish were transported in ice boxes. Within an hour, the fresh samples were taken to the lab for further experiments and analysis.

2.2 Preservative and preparation

As a natural preservative black cumin seed (*Nigella sativa*) oil was employed and sodium acetate, a synthetic preservative was used in varying amounts. Samples were washed with tap water and degutted. Ninety-six fillets of each species, with approximately the same weight (125-130g each) were prepared. These fillets were then divided into two groups.

2.3 Processing

Initially, 96 fillets of each species were obtained. Six fillets were used freshly in triplicates for both parameters to estimate protein and lipid contents in each fish species. The remaining 90 fillets were divided into control and experimental groups, respectively. In the case of the relative preservative, *L. rohita* fillets were immersed for half an hour. After proper use, the samples were frozen in plastic bags to prevent deterioration. After 15, 30, and 45 days, samples from the control and experimental groups were analyzed in triplicates for the estimation of protein, fat, dry matter, and moisture contents. The entire experiment was repeated for *C. mrigala* as well.

Table 1: The table shows control and experimental groups along with preservatives.

No. of Days of Examination	Control Group		Experimental Group								Total No. of Specimens
	No. of Specimen		No. of Specimen								
			Cumin Natural Preservative		Sodium acetate 3g/l Synthetic Preservative		Sodium acetate 5g/l Synthetic Preservative		Sodium acetate 7g/ l Synthetic Preservative		
	Fats Test	Protein Test	Fats Test	Protein Test	Fats Test	Protein Test	Fats Test	Protein Test	Fats Test	Protein Test	
0	3	3	0	0	0	0	0	0	0	0	6
15	3	3	3	3	3	3	3	3	3	3	30
30	3	3	3	3	3	3	3	3	3	3	30
45	3	3	3	3	3	3	3	3	3	3	30
Total	12	12	9	9	9	9	9	9	9	9	96

2.4 Oven drying

Samples in triplicate of both species were frozen, defrosted and excess water was drained. For analysis, the first step was oven drying. Triplicates of both species were placed on aluminum foils and dried at a temperature of 96-100 degrees Celsius to remove all the moisture. After drying, all the samples were removed from the oven and weighed. The dried samples were then ground in a mortar and pestle until a powder was formed. The powdered samples were saved in plastic bags for further analysis.

2.5 Estimation of protein

Powder for the estimation of protein was obtained from dried fish samples. A Kjeldahl apparatus was used for protein estimation, which calculated the amount of nitrogen for protein estimation following the method (Bano and Afzal, 2017). In a round-bottomed flask, 5 g of the digesting mixture (potassium sulfate, copper sulfate, and ferrous sulfate) was placed, along with 1 g of the sample and 25-30 ml of concentrated sulfuric acid (98%) was added. Afterward, the digestion flask was placed on a flame and when the dark color turned into light yellow or green, it indicated the completion of digestion. At room temperature, the flask contents were allowed to cool and 30 ml of water was added to prevent crystallization. The digestion material was filtered with filter paper.

In the Kjeldahl method, initially, a 250 ml round-bottom flask was taken and distilled water was added to it. It was then placed on a burner for heating. In the Kjeldahl apparatus, the processed sample was distilled by adding 10 ml of 40% NaOH and 10 ml of the sample. Steam was produced in the round-bottom flask and moved towards the mixture of the sample and NaOH, heating it. Subsequently, nitrogen gas began to be produced. Using steam distillation, the nitrogen traveled into a receiver vessel containing 10 ml of a 4% methyl red solution of boric acid, which was placed in a beaker as an indicator (1-2 drops). The color of the indicator turned yellow due to the collected nitrogen.

The final step was titration, which involved an acid-base titration using a solution of boric acid as the absorbing solution and sulfuric acid with a concentration of 0.01N as the standard solution. The reading indicated the nitrogen content as the protein content present in the sample.

The amount of protein was further calculated by using the formula:

$$\text{Protein percentage} = \frac{V \times Nx14 \times 250x6.25}{1000 \times Wt.} \times 100$$

V= H_2SO_4 volume used
N= normality of NaOH
Estimated Protein =Nitrogen contents (N) x 6.25
14= conversion factor
Wt. = sample weight
250 =Volume of dilution after organic compound digestion

2.6 Estimation of extract of ether

The Soxhlet apparatus was used to extract fat. First, the samples were pre-weighed, and 2 grams of each sample were then wrapped in filter paper properly and stapled. All wrapped samples for each concentration and duration of the trial were placed in the Soxhlet apparatus. After that, the Soxhlet tube was set on the round-bottom flask containing petroleum ether. An electric mantle was used to heat the solvent, and vapors from the flask traveled up to the Soxhlet chamber. All the solvent moved down toward the flask from the Soxhlet chamber automatically. Petroleum ether was used to extract all the lipid contents present in the sample. The entire process was repeated three times for both species and every sample for every concentration. Finally, the packets of the samples were manually removed, dried, and weighed.

2.7 Evaluation of dry matter

For the control and experimental groups difference was measured by comparing the weight of the fresh sample and the sample after drying by following (Bano and Afzal, 2017). The amount of moisture contents was calculated by the given formula.

$$\text{Dry Matter \%} = \frac{\text{Original Wt. of S.} - \text{Wt. of dried S.}}{\text{The original Wt. of the Sample}} \times 100$$

2.8 Evaluation of moisture contents

The moisture content was measured by subtracting the dry matter content from 100. The percentage of the moisture content was determined using the following formula.

Moisture Content % = 100 - %age Dry Matter

All the data was tabulated and statistically analyzed for protein contents, ether extracted, moisture contents, and dry matter.

3. Results and Discussion

In *Labeo rohita*, the impact of black cumin seed oil was observed with maximum protein content of 28.437% (±1.546) on day 15 and 24.791% (±1.263) on day 45, showing a decreasing trend in protein content. The control group also exhibited a decreasing trend in protein content with 24.791% (±1.263) on day 15 and 20.125% (±1.515) on day 45. The results were highly significant when black cumin seed oil was used. This trend aligns with the findings of Gandotra *et al*. (2012), who reported that at 4±1°C, the muscle of *Mystus seenghala* contained 18.01% (±0.06) protein initially, with the lowest value of 8.22% (±0.2) noted on day 21. A similar experiment conducted by Siddique *et al*. (2011) on *L. rohita* revealed that at -50ºC, the protein content in the muscle of frozen fish decreased over 20 days.

The *L. rohita* treated with sodium acetate at concentrations of 3g/l, 5g/l, and 7g/l exhibited

varying protein contents. When preserved in sodium acetate at a concentration of 3g/l, it showed the highest protein content of 26.916% (±2.162) on day 15, while the lowest protein content of 23.333% (±1.262) was recorded on day 45. Tangkham *et al*. (2012) conducted research that demonstrated the effectiveness of sodium acetate as an inhibitor against microorganisms. Sodium acetate at 3.48% concentration showed a reduction in the production of aerobic microorganisms.

The *Cirrhinus mrigala* when preserved in black cumin seed oil, exhibited a decreasing trend in protein content, with values of 27.708% (±1.262), 26.979% (±1.262), and 21.729% (±2.409) on days 15, 30 and 45, respectively. The results of Ahmed and Beg (2013) noted a gradual decline in protein content from 13.02% (±0.09%) to 10.13% (±0.06%) in sparidae fish with an increasing number of days, attributing the difference in values to temperature, fish species, and storage duration. Another author observed that protein levels decreased due to denaturation, the passage of time, and proteolysis induced by the activities of psychotropic microorganism enzymes.

In the case of sodium acetate at concentrations of 3g/l for *C. mrigala*, a decreasing trend in protein levels was observed. The highest protein content of 26.779% (±1.262) was measured at 7g/l, while the lowest protein content of 21.145% (±1.263) was recorded. This study's findings align with Tangkham *et al*. (2012), which demonstrated that 3.48% sodium acetate or a combination of 1.74% sodium lactate and 1.74% sodium acetate had a significant impact against microorganisms at 3ºC, extending the shelf life of raw chicken breasts for 39 days.

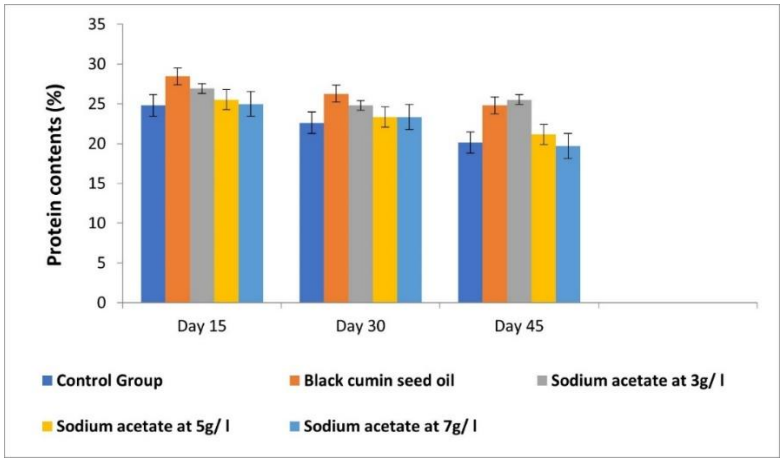

Figure 1: Protein contents (%) comparison between the control and experimental group in *L. rohita* on days 15, 30, and 45**.**

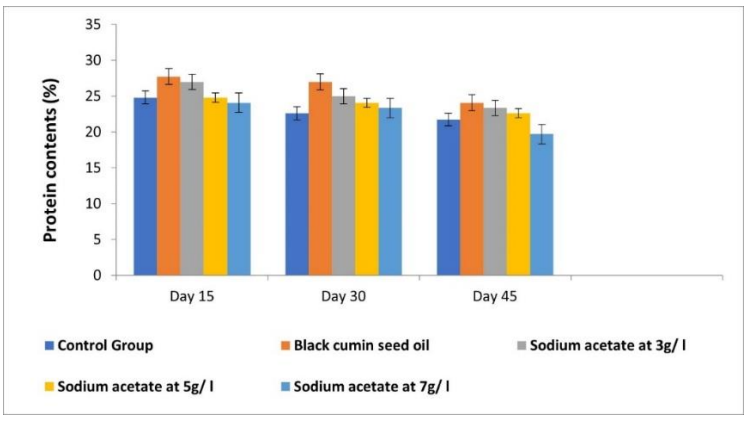

Figure 2: Protein contents (%) comparison between the control and experimental group in *C.mrigala* on the days 15, 30, and 45.

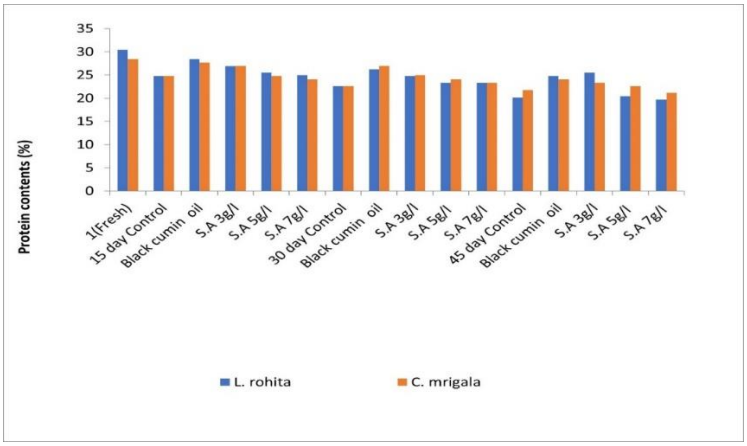

Figure 3: In Experimental And Control Group Comparison Of Protein Contents (%) In *L. Rohita* And *C. Mrigala*

The *L. rohita* showed a maximum fat content of 12.83% ± 0.288 on the 15th day and a minimum value of 10.17% ± 0.763 on the 45th day. On the 15th day, the control group exhibited a peak fat content of 11.5% ± 0.5 and a minimum fat content of 9.17% ± 0.763 on the 45th day. In the control group, a decreasing trend in protein content was observed from 24.791% ± 1.263 on the 15th day to 20.125% ± 1.515 on the 45th day. These results are in agreement with Ismail *et al*. (2019), who explained that a fine amount of essential fatty acids present in shrimp can lead to deterioration in samples with an increasing number of days.

At sodium acetate concentrations of 3g/l, 5g/l, and 7g/l, *L. rohita* was preserved for 15, 30, and 45 days. The protein content on the 15th day at a concentration of 3g/l was 10.66% ± 0.288, showing the highest amount, while the least amount was observed at a concentration of 7g/l (7.66% ± 0.763) on the 45th day. This result is aligned with the Siddique *et al*. (2011), who found that the level of lipid contents decreased in three species of Puntius during chill storage. Another author, Arannilewa *et al*. (2005), calculated a reduction in fat content in Tilapia stored in a freezer for 60 days due to lipid oxidation.

When *C. mrigala* was preserved in black cumin seed oil, the fat content values declined to 12.83% ± 0.288, 11.83% ± 0.577 and 10.17% ± 0.763 on the 15, 30 and 45 days, respectively. The control group exhibited a decreasing trend. The Ozpolat and Duman (2017), reported that the level of Enterobacteriaceae in the experimental group was less if compared to the control group. Black cumin oil was used for the preservation of fish meat, mainly consisting of thymoquinone and p-cymene, which showed antioxidant properties.

At a concentration of 3g/l of sodium acetate, the highest fat content value of 12% ± 0.866 was recorded on the 15th day, with the minimum fat content value of 9.5% ± 0.5 on the 45th day.

Nerbrink *et al*. (1999) viewed that the production of *Listeria monocytogenes* is affected by sodium acetate, and a mixture of lactate and acetate was indicated to have an antimicrobial effect.

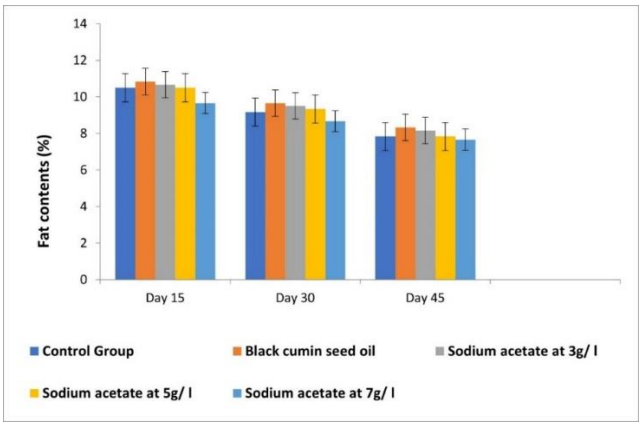

Figure 4: Fat contents (%) comparison between the control and experimental group in L. rohita on the days 15, 30, and 45.

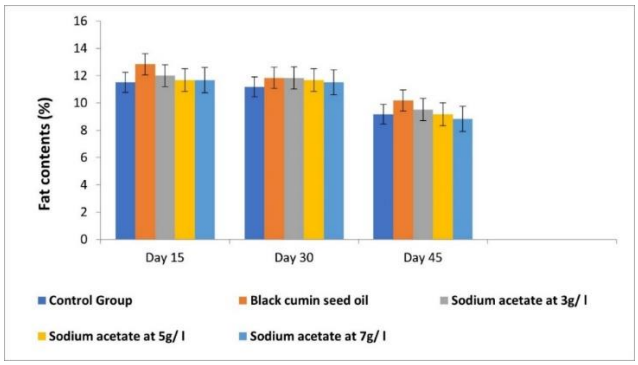

Figure 5: Fat contents (%) comparison in between the control and experimental group in *C.mrigala* on the days 15, 30, and 45.

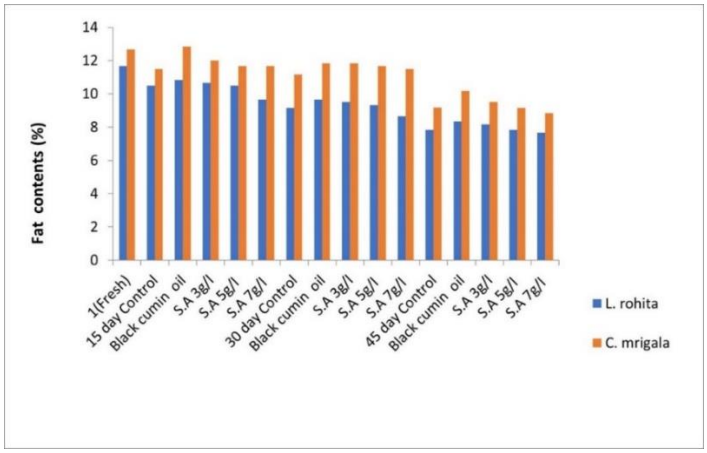

Figure 6: In experimental and control group comparison of fat contents (%) in *L. rohita* and *C. mrigala*

For the evaluation of moisture and dry matter contents with black cumin seed oil, *L. rohita* showed highly significant results. In the experimental group, moisture contents were lower, and dry matter was higher compared to the control group. Our findings were similar to Abbas *et al*. (2009), it was indicated that a decrease in moisture contents in food, along with maintaining a certain water activity, can block mold growth and prolong the shelf life of fish.

When *C. mrigala* was preserved with different concentrations of sodium acetate (3g/l, 5g/l, and 7g/l) for all days ranging from 15 to 45, the maximum moisture level was observed with a concentration of 3g/l. In a study, Rani *et al*. (2017) demonstrated that moisture content in *C. mrigala* decreased with an increasing number of days.

In *C. mrigala*, the moisture content of black cumin seed oil decreased with an increasing number of days. These results are consistent with the findings of Rani *et al*. (2017), who observed a declining trend in moisture content when *C. mrigala* was preserved for 3 months at -18ºC. This decrease in moisture content was associated with an increase in the number of days of preservation.

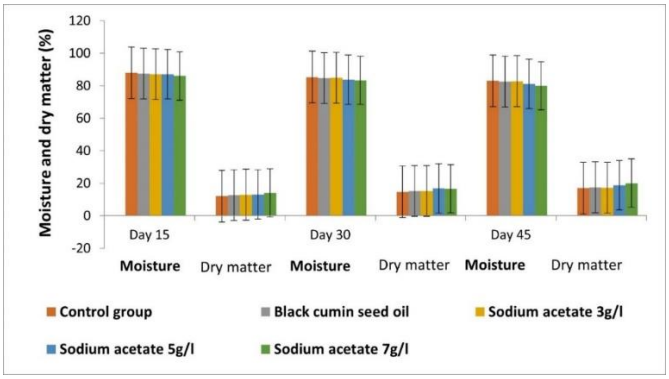

Figure 7: Moisture and dry matter (%) comparison between the control and experimental group in *L. rohita* on the days of 15, 30, and 45.

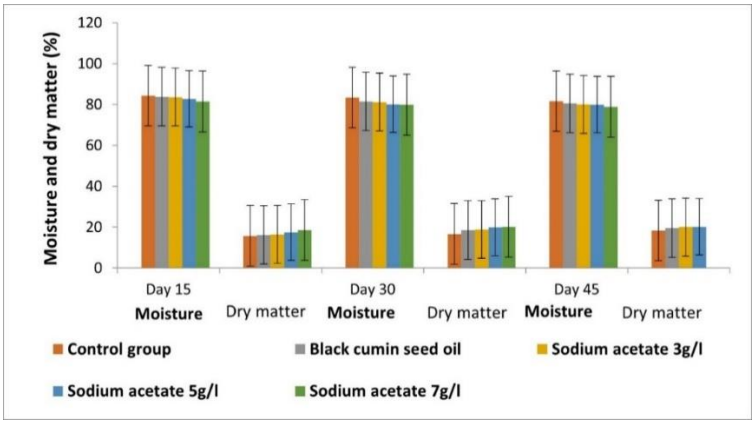

Figure 8: Moisture and dry matter (%) comparison between the control and experimental group in *C. mrigala* on the days of 15, 30, and 45.

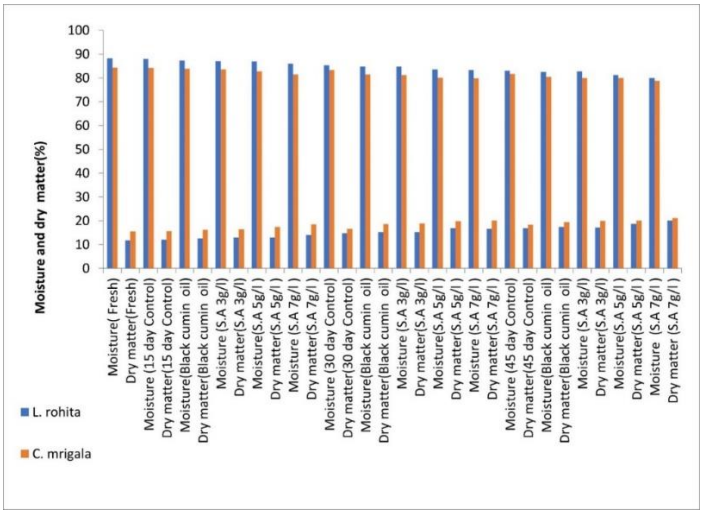

Figure 9: In Experimental And Control Group Comparison Of Moisture Contents And Dry Matter (%) In *L. Rohita* And *C. Mrigala*

Regression analysis

In Microsoft Office Excel 2007, regression analysis was applied to determine the correlation between protein, fat, moisture contents, dry matter, and selected days.

Table 2: Regression analysis for experimental and control groups in *L. rohita* and *C. mrigala*

Conditions	**Protein contents**		**Fat contents**		**Moisture and dry matter**	
Fishes	***L. rohita***	***C.mrigala***	***L. rohita***	***C.mrigala***	***L. rohita***	***C.mrigala***
Control	0.9382	0.9771	0.9998	0.9252	0.9316	0.9316
Black cumin seed oil	0.9868	0.8928	0.9986	0.9799	0.9971	0.9416
Sodium acetate (3g/l)	0.9886	0.9966	0.9983	0.8007	0.999	0.9613
Sodium acetate (5g/l)	0.9931	0.9949	0.9949	0.753	0.9908	0.8062
Sodium acetate (7g/l)	0.9541	0.9541	0.8133	0.7947	0.9946	0.9881

4. Conclusion and Recommendations

The shelf life of *Labeo rohita* and *Cirrhinus mrigala* can be prolonged by using sodium acetate and black cumin seed preservatives at concentrations of 3g/l, 5g/l, and 7g/l to reduce lipid and protein oxidation. Additionally, the current study provides greater insight into the potential of black cumin seed oil and sodium acetate as natural and synthetic preservatives. These preservatives are considered a sufficient source of antioxidants and antibacterial agents for fish preservation. The

fat, protein, and moisture contents for both species showed ($p < 0.05$), indicating high significance.

The use of sodium acetate and black cumin seed preservatives could have significant commercial applications in the fish preservation industry. Consider exploring opportunities for partnerships or collaborations with food processing companies or fisheries to develop and market these preservatives. Evaluate the safety and regulatory compliance of these preservatives in fish preservation. Ensure that the concentrations used are within safe limits for human consumption and meet relevant food safety standards. Investigate the impact of various storage conditions (temperature, humidity, and packaging) on the effectiveness of these preservatives. This will help identify the best practices for fish storage to maximize their shelf life. Explore potential applications of sodium acetate and black cumin seed preservatives in other food preservation contexts beyond fish. Their efficacy as natural and synthetic preservatives might extend to various food products.

5. Acknowledgment

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6. Conflict of Interest

All authors have declared that there is no conflict of interest regarding the publication of this article.

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