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The effectiveness of the use of antioxidant formulations in the storage of fat from the Pacific sardines *Sardinops melanostictus*

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ABSTRACT

The potential of both natural and synthetic antioxidants, alone and in combination, to prevent the degradation of highly unsaturated fat isolated from the Pacific sardine, *Sardinops melanostictus*, during storage has been examined in this study. Accelerated aging methods on fish oil samples, enhanced with individual and combinations of antioxidants, were used. The fish oil was evaluated for quality by analyzing its physicochemical properties, fatty acid composition, and amounts of fat-soluble vitamins. The results showed a notable rise in peroxide value, acid value, and para-anisidine value in fat samples lacking antioxidants. This was accompanied by a decrease of 3.4–5 times in omega-3 polyunsaturated fatty acids (PUFAs) and 40–80% in fat-soluble vitamins. These findings suggest the presence of ongoing lipid oxidation processes and degradation. The use of specific antioxidants, such as α-tocopherol acetate at a concentration of 2%, betulin at a concentration of 0.3%, and ethoxyvine at a concentration of 0.14%, resulted in a slight decrease in the rate of fat autoxidation and the protection of PUFAs and vitamins. A synergistic effect was observed when α-tocopherol acetate and ethoxyvine, or betulin and ethoxyvine, were combined at half the single levels. This combination improved fish oil oxidation resistance. These antioxidant blends inhibited oxidative processes in unsaturated fish oil using salicylic acid amide. This slowed omega-3 PUFA and fat-soluble vitamin breakdown.

1. INTRODUCTION

Fish fat is important as it contains essential fatty acids and fat-soluble vitamins [1-4]. Polyunsaturated fatty acids (PUFAs) of the omega-3 family have biological activity and play a positive role in human metabolism, reducing the risk of developing noncommunicable diseases [5-7]. The fat obtained from the Pacific sardine *Sardinops melanostictus* has a high content of PUFAs [8]. The main composition of the PUFAs is omega 3 fatty acid (72.3–73.6%), among which docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) predominate.

It is known that during storage, under the influence of various factors (air, oxygen, temperature, light, and humidity), oxidative processes occur in highly unsaturated fish oil, which is carried out according to a free-radical mechanism [9,10]. As a result of the action of oxygen on fatty acids in the composition of triacylglycerols and free fatty acids (FFAs), hydroperoxides are formed, which in turn break down into aldehyde and free radicals. At the same time, hydrolytic reactions occur in fish oil, which leads to the breakdown of triacylglycerols into glycerol, accumulating FFAs and hydrolysis intermediates (mono- and diacylglycerols). The amount of FFA increases in oxidation and hydrolytic decomposition, peroxides and aldehydes toxic to the human body accumulate, and fat-soluble vitamins are destroyed, decreasing the amount of PUFAs. These changes lead to a decrease in fish oil's organoleptic properties and nutritional value, a deterioration in quality indicators, and a limitation of its shelf life and use [11,12]. Oxidized fish oil, when used in nutrition, poses a risk to human health in the form of oxidative stress or oxidative damage [13].

To slow down the processes of oxidation and hydrolytic cleavage of fish oils and increase their stability and shelf life, antioxidants are used that can interrupt the reactions of free radical oxidation and reduce the accumulation of lipid peroxidation products. The most effective are synthetic antioxidants such as butylatedhydroxyanisole, tert-butyl hydroquinone, and butylated hydroxytoluene [14,15]. Despite the relatively high efficiency of lipid stabilization, synthetic antioxidants can harm the human body.

Different mechanisms of action characterize antioxidant drugs; hence, using their combinations in fish oil is more effective than using one

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type of antioxidant [16,17]. Therefore, to increase the stability of fish oil with a high content of PUFAs during storage, it is relevant to develop synergistic compositions of antioxidants of both natural and artificial origin.

This study aims to evaluate the effects of antioxidants and their synergistic mixtures on the oxidative and hydrolytic processes of Pacific sardines' fat *Sardinops melanostictus* during storage.

2. MATERIALS AND METHODS

2.1. Examined Material

The object of the research was the fat of the Far Eastern sardines *S. melanostictus* obtained from a chilled crushed fish. The fat production technology from the fish included heat treatment at 90°C for 30 min, centrifugation, filtration, and drying [18].

Various substances used in the technology of food and feed production were used as antioxidant preparations: α-tocopherol acetate and food additive – "betulin," 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyvine), and salicylic acid amide. The selected antioxidant compounds were injected into the resulting fat from the sardine according to the manufacturer's recommendations in the following quantities per weight of fat: ethoxyvine 0.14%, α-tocopherol acetate 2.0%, betulin 0.3%, and salicylic acid 0.0015%. The synergistic mixtures included antioxidants in the following ratios: ethoxyvine:α-tocopherol acetate (50:50), ethoxyvine:betulin (50:50), ethoxyvin:betulin:salicylic acid amide (69.9:30:0.1), and ethoxyvin:α-tocopherol acetate:salicylic acid amide (69.9:30.0:0.1).

2.2. Determine the Effects of Antioxidants on Lipid Stability

The effects of antioxidants on fat stability were determined using the accelerated method. Experimental fat samples (with antioxidants) were stored in glass containers in a thermostat for 45 days at 45°C. For comparison, a control variant, antioxidant-free fat, was stored and investigated at the same time [19].

2.3. Assessment of the Physicochemical Parameters for Evaluating the Quality of Fish Oil

The evaluation of physicochemical parameters to ascertain the quality of fish oil adhered to the prescribed techniques of the American Oil Chemists' Society (AOCS) [20]. The fat density (g/L) was measured using a glass pycnometer. The refractive index was measured using the refractometric method at 20°C. The moisture and volatile chemical content were measured by vacuum drying using the AOCS Ca 2d-25 standard.

The peroxide content and primary oxidation levels were assessed using AOAC Official Method 965.33 [21], and the results were documented based on peroxide values (PVs). The degree of hydrolytic alterations in the oil was assessed using the AOCS Official Method Cd 3d-63 [22] and reported according to acid values (AVs). The oil oxidation degree was evaluated using the AOCS Official Method Cd 18-90 [23], which measures the presence of secondary oxidation products, specifically aldehydes, expressed as para-anisidine values (p-AVs). The overall rate of oil oxidation was determined by calculating the TOTOX values, which include measurements of PV and p-AV. The relationship between p-AV and PV was used to make these calculations.

2.4. Determination of the Composition of Fatty Acids

The oil fatty acid composition was analyzed using the AOCS Official Method Ce 1h-05 with modifications [24], and the fatty acid methyl esters were prepared following the AOCS Official Method Ce 2-66 [25]. Gas chromatography analysis was done using a Hewlett–Packard model 5890 II gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with a flame ionization detector and a Supelcowax 10 column (30 m × 0.25 mm × 0.25 mm). The temperature of the injector was held constant at 240°C while running in split mode at a ratio of 1:25. The hydrogen flow rate was adjusted to 1 mL/min while maintaining an oven temperature of 220°C. The temperature of the detector was adjusted to 240°C. The peaks were identified by comparing their retention durations with those of a FAME reference standard.

The concentration of FFAs was measured by acidometric titration using a 0.1 M solution of NaOH, following the AOCS Official Method Ca 5a-40. The results were quantified as a percentage of oleic acid.

2.5. Determination of Vitamin Content

High-performance liquid chromatography (HPLC) analysis of vitamin A was performed according to the method described by Nadeeshani et al. [26]. Vitamin A was analyzed in triplicate by injecting 20 µL of samples and standards to HPLC (Thermo Scientific™ Dionex™ UltiMate™ 3000 standard system, UK). At the same time, a C18 column (4.6 × 250 mm, 5 µm) (Agilent ZORBAX Eclipse Plus™, USA, 5 µm, 4.6 × 250 mm) was used with a linear gradient of methanol at a constant flow rate of 1 mL/min and UV detection at 325 nm. All procedures were carried out under subdued light conditions.

Vitamin D3 content was analyzed using a published stability-indicating method and an HPLC Agilent 1100/1200 Series instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV detector and ChemStation data acquisition system. In brief, a reversed-phase column Gemini C18 100 × 3.0 mm, 3 µm particle size column (Phenomenex, Torrance, CA, USA) at 40°C was used with a mobile phase of acetonitrile–water (99:1, v/v) at a flow rate of 1 mL/min. The detection was carried out at 265 nm [27].

2.6. Statistics

All data are presented as mean ± standard deviation for parametric variables or the median (interquartile range) for nonparametric variables and as number (%) for categorical variables. P-values were derived from a paired Student's *t*-test. The results were analyzed using the computer programs "Microsoft Excel" 2014 and Statistica 7.0.

3. RESULTS

This study utilized five samples of fat from the sardine *S. melanostictus*, harvested on various production dates.

3.1. Characteristics of the Initial Indicators Regarding Fat Quality From Sardine *S. melanostictus*

The fat obtained from *S. melanostictus* sardine for this study displayed a translucent, pale yellow hue without any noticeable foreign scent or flavor. Table 1 shows the physicochemical characteristics of the fat from the sardine species *S. melanostictus*. The parameters consist of the refractive index at 20°C and density, which is determined by comparing the mass of fat at 20°C to the mass of the same volume of water at 4°C. The values were similar to those of vegetable oils. Furthermore, the PV, AV, ranitidine value (p-AV), and moisture and volatile content were determined to be at a low level, suggesting excellent fat quality and the lack of secondary oxidation products.

Table 1: Physicochemical indicators of the quality of fat from the Pacific sardines *Sardinops melanostictus.*

Index	Meaning
Density, g/L	0.89 + 0.12
Refractive index	1.38 + 0.23
Peroxide value (PV), meq/kg	1.89 + 0.41
p-Anisidine value (*p*-AV), mmol/kg	1.28 + 0.14
Acid value (AV), mg KOH/g	0.89 + 0.06
Free fatty acids, % as oleic acid	0.19 + 0.08
Soap, mg/kg of sodium oleate	0.18 + 0.04
Moisture and volatiles, %	0.48 + 0.07

Table 2: Fatty acid composition of the fat obtained from Pacific sardines *Sardinops melanostictus*, % of the total amount of fatty acids.

Saturated		Monounsaturated		PUFAs	
Fatty Acid	Content	Fatty Acid	Content	Fatty Acid	Content
14:0	7.79 ± 0.63	16:1 n-7	6.70 ± 0.84	18:2 n-6	1.45 ± 0.02
15:0-i	0.30 ± 0.63	16:1 n-5	0.30 ± 0.02	18:2 n-4	0.31 ± 0.03
15:0-ai	0.14 ± 0.02	17:1	0.72 ± 0.05	18:3 n-6	0.23 ± 0.4
15:0	0.51 ± 0.04	18:1 n-9	9.00 ± 0.91	18:3 n-3	0.94 ± 0.06
i-16:0	0.13 ± 0.01	18:1 n-7	2.62 ± 0.43	18:4 n-3	3.38 ± 0.52
16:0	17.96 ± 1.13	18:1 n-5	0.51 ± 0.03	20:2 n-6	0.20 ± 0.01
17:0-a	0.20 ± 0.01	20:1 n-11	4.29 ± 0.72	20:3 n-3	0.13 ± 0.02
17:0	0.42 ± 0.03	20:1 n-9	2.16 ± 0.41	20:4 n-3	1.05 ± 0.31
i-18:0	0.28 ± 0.01	20:1 n-7	0.17 ± 0.01	20:5 n-3	13.85 ± 0.92
ai-18:0	0.17 ± 0.01	22:1 n-11	4.73 ± 0.75	21:4 n-6	0.57 ± 0.04
18:0	2.41 ± 0.43	22:1 n-9	0.11 ± 0.01	21:5 n-3	0.53 ± 0.06
20:0	0.18 ± 0.02	Sum	31.31 ± 0.64	22:4 n-6	0.14 ± 0.02
Sum	30.93 ± 0.48			22:5 n-6	0.19 ± 0.01
				22:5 n-3	2.11 ± 0.36
				22:6 n-3	11.10 ± 0.75
				Sum	36.93 ± 0.78

It is important to note that the fresh fat from *S. melanostictus* sardine has a significant feature: a high concentration of FFAs, as previously found [8]. Although the high content does not affect its sensory qualities, it can be seen as a sign of possible instability during storage.

3.2. Fatty Acid Composition of the Sardine Fat *S. melanostictus*

The fat sample obtained from *S. melanostictus* sardines showed a saturated fatty acid composition of 30.93%. Among the members of this group, a total of 13 fatty acids and their isomers were detected. The most abundant fatty acid present was palmitic acid (16:0), followed by myristic acid (14:0) and stearic acid (18:0). However, the levels of myristic and stearic acids were considerably lower compared to palmitic acid, as shown in Table 2.

The sardine fat contained 31.31% monounsaturated fatty acids out of the total fatty acids. Twelve fatty acids were detected, with oleic acid (18:1 n-9) being the most abundant, followed by palmitoleic acid (16:1 n-7). The *cis*-isomer of erucic acid (22:1 n-11) accounted for 4.73% of the overall fatty acids. Erucic acid (22:1 n-9) was detected in lesser amounts, comprising 0.11% of the overall fatty acid composition. Erucic acid has been found to reduce the quality of fats and oils because it negatively affects growth and reproductive maturity and can harm the heart muscle [28]. On the other hand, the *cis*-isomer of erucic acid (22:1 n-11) helps regulate the metabolism in the human body, improving the nutritional quality of fat obtained from *S. melanostictus* sardines.

The sardine lipids contained 36.93% PUFAs in their total fatty acid composition. Sixteen fatty acids were detected, with omega-3 fatty acids being the most abundant, including EPA (20:5 n-3) and DHA (22:6 n-3), which together comprised over 25.0% of all the fatty acids.

3.3. Changes in Quality Parameters of Sardine *S. melanostictus* Fat Throughout Storage

To assess the influence of antioxidants on fat samples' long-term stability, lipid oxidation, and hydrolysis markers were measured at 5-day intervals.

PV is a key measure of fat oxidation, indicating the speed at which peroxides and hydroperoxides are formed. Then, peroxides decompose into aldehyde and ketone substances, measured by the anisidine number (p-AV), which indicates the presence of secondary oxidation products that cause rancidity. Figure 1 illustrates the variations in PV in sardine fat samples of *S. melanostictus* when antioxidants are present.

The control group, which did not have antioxidants, showed a faster oxidation process than the experimental groups, as indicated by the rapid rise in PV. Following 45 days, the control group's peroxidizability value (PV) increased to 25.3 mmol O_2/kg of fat, indicating significant oxidative alterations. Including antioxidant compounds significantly reduced the increase in PV. The antioxidant formulations consisting of ethoxyvine + α-tocopherol acetate + salicylic acid amide and ethoxyvine + betulin + salicylic acid amide showed the highest effectiveness in preventing the accumulation of peroxide and hydroperoxide. After 45 days, the PV values of these formulations were 2.14–2.21 times lower than the control, indicating a significant slowdown in oxidation.

The statistical analysis produced regression equations, as shown in Table 3, that accurately describe the lipid oxidation of *S. melanostictus* sardine fat. These equations were validated using approximation coefficients that approach 1, confirming the precision of the equations.

Similarly, the path metric measures the buildup of secondary oxidation products, which closely reflects the trends observed in PV. The fat samples enriched with antioxidants showed decreased p-AV values compared to the control, especially in three-component antioxidant formulations, indicating effective protection against oxidation.

The AV measures the extent of fat hydrolysis during storage. The control sample's antioxidant activity (AV) increased by a factor of 2.12 after 15 days and 4.51 after 45 days. In contrast, the samples stabilized with antioxidants exhibited less significant increases in AV. The antioxidant combinations consisting of ethoxyvine + α-tocopherol acetate + salicylic acid amide and ethoxyvine + betulin + salicylic acid amide showed the lowest AV values, which suggests that they are highly effective in inhibiting hydrolysis.

The regression equations for AV changes closely mirrored the trends observed in p-AV during statistical analysis, strengthening the lipid degradation evaluation's reliability.

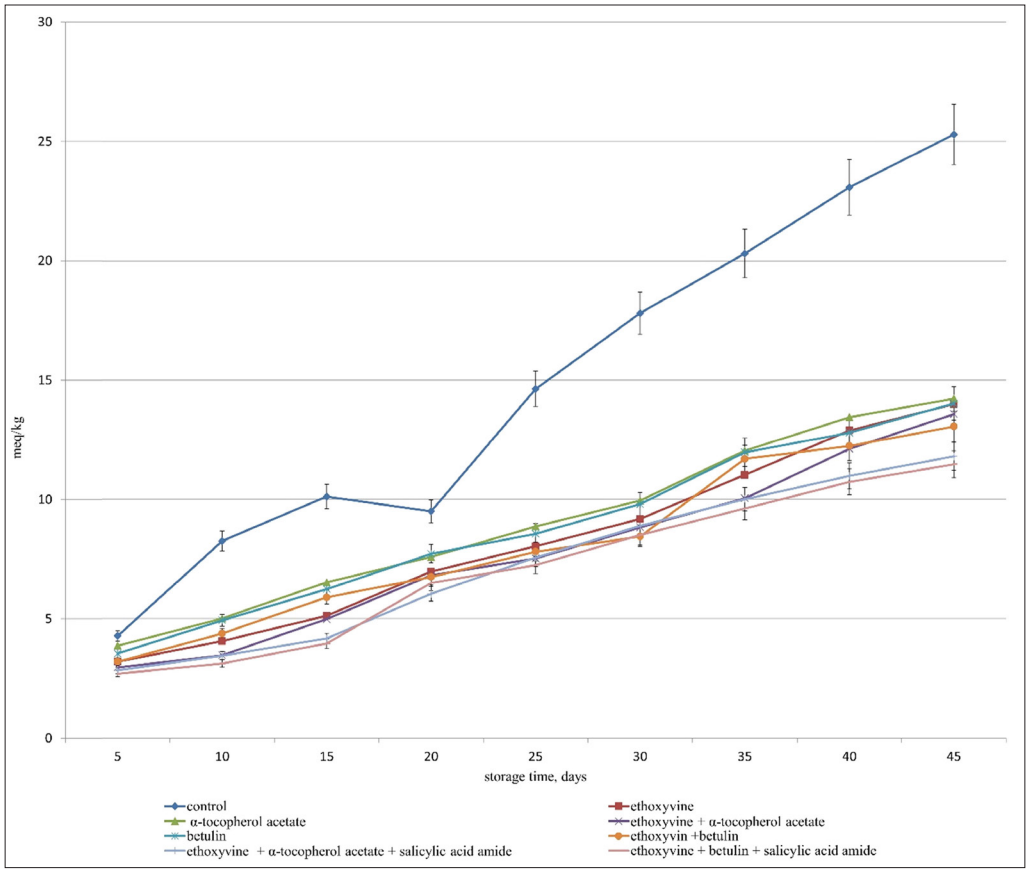

Figure 1: Dynamics of changes in PV in samples of fat of Pacific sardines *Sardinops melanostictus* with antioxidants during storage.

Table 3: Regression equations of PV changes in Pacific sardine fat samples.

Name of Samples	Regression Equation	R^2
Fat (control)	$y^* = 0.0514x2 + 2.1065x^{**} + 2.6493$	0.979
Fat + α-tocopherol acetate	y = 0.0129x2 + 1.2066x + 2.6162	0.9952
Fat + botulin	y = 0.0012x2 + 1.3063x + 2.2743	0.9952
Fat + ethoxyvine	y = 0.0385x2 + 1.0106x + 2.0069	0.9962
Fat + ethoxyvine + α-tocopherol acetate	y = 0.0383x2 + 0.9603x + 1.8021	0.9938
Fat + ethoxyvine + botulin	y = 0.0176x2 + 1.0959x + 2.1262	0.9779
Fat + ethoxyvine + betulin + salicylic acid	y = –0.0081x2 + 1.2974x + 1.0752	0.9879
Fat + ethoxyvine + α-tocopherol acetate + salicylic acid	y = –0.0163x2 + 1.3508x + 0.8593	0.9839

Designation: y^* – PV value, x^{**} – storage duration, days.

3.4. Modifications in Fatty Acid Composition During Fat Storage in the Presence of Antioxidants

Table 4 displays the data on the fatty acid composition of fat samples obtained from *S. melanostictus* sardines after 45 days of experimental storage.

Throughout the trial duration, the control sample, which did not receive any antioxidant treatment, showed noteworthy elevations in FFAs (15.8-fold rise) and saturated fatty acids (specifically myristic acid 2-fold increase, and palmitic acid 17% increase) compared to the

Table 4: Fatty acid content in fat samples of Pacific sardines *Sardinops melanostictus* after experimental storage for 45 days.

Name of Samples	Content, % of the Amount of Fatty Acids					
	Free fatty acids	14:0	16:0	18:1 n-9	20:5 n-3	22:6 n-3
Fat after receiving	0.19	7.19	16.96	9.00	13.85	11.10
After 45 days of storage						
Fat (control)	3.02	15.93	19.85	12.17	4.08	2.35
Fat + α-tocopherol acetate	1.41	11.24	17.84	10.07	6.05	4.81
Fat + botulin	1.35	11.80	17.67	10.79	6.92	5.09
Fat + ethoxyvine	1.29	10.06	16.26	10.97	8.56	7.12
Fat + ethoxyvin +α-tocopherol acetate	1.04	9.28	16.03	10.54	9.23	8.06
Fat + ethoxyvine + botulin	1.06	9.55	16.91	10.08	9.8	7.66
Fat + ethoxyvine + betulin + salicylic acid	0.94	8.99	16.64	9.92	10.09	8.47
Fat + ethoxyvine +α-tocopherol acetate + salicylic acid	0.88	8.83	17.08	9.70	10.58	8.95

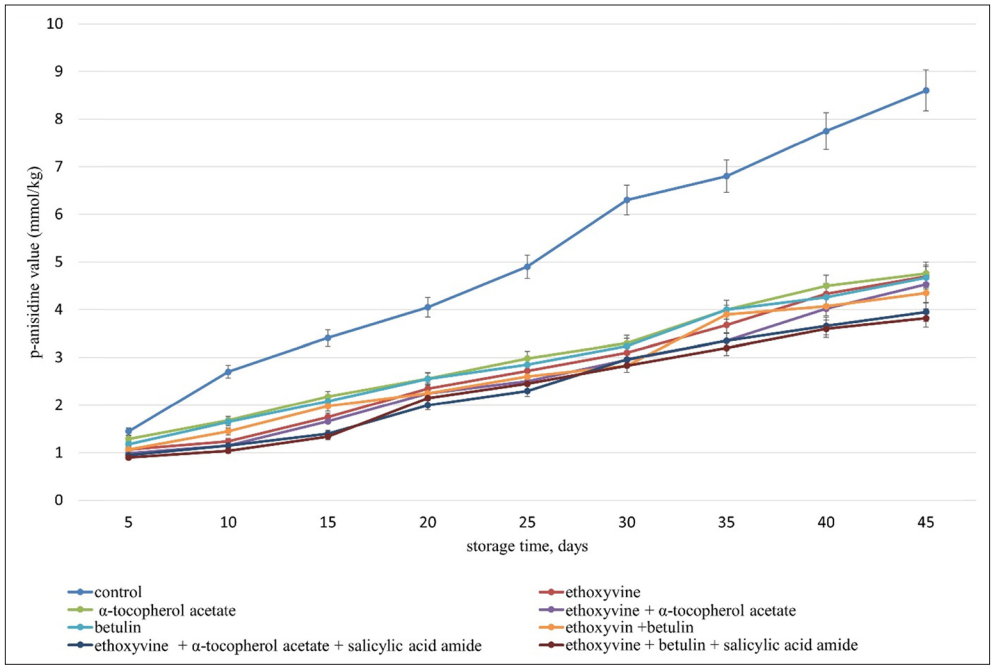

Figure 2: Dynamics of changes AVs in *Sardinops melanostictus* fat samples with antioxidants during storage.

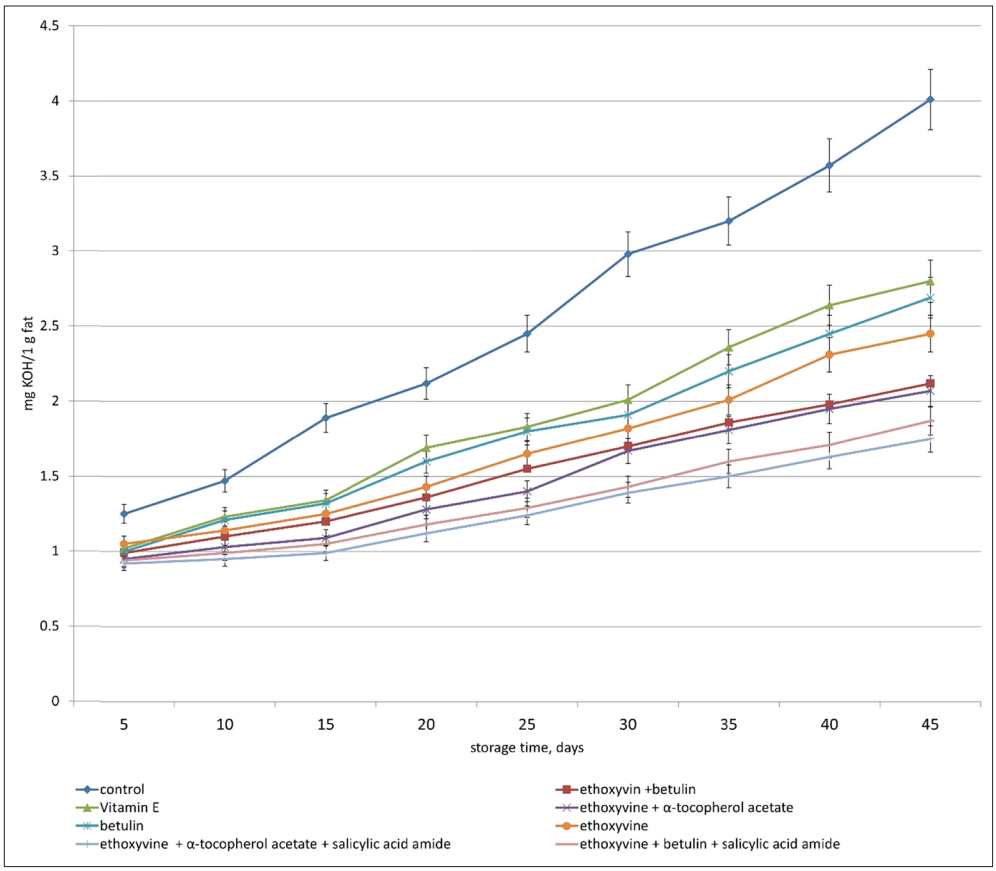

Figure 3: Dynamics of changes in the AV in samples of fat of Pacific sardines *Sardinops melanostictus* with antioxidants during storage.

original fat sample. The control fat sample had a significant decline in the levels of PUFAs, including a 3.4-fold reduction in EPA (22:6 n-3) and an approximately 5-fold decrease in DHA (20:5 n-3). This indicates that there was active degradation of PUFAs.

During storage, the presence of antioxidants in fat samples slowed down the degradation of PUFAs. When α-tocopherol acetate, betulin, and ethoxyvine were present separately, the levels of EPA and DHA fell by 54%, 50%, and 35–38%, respectively. Nevertheless, using a blend of

Table 5: Vitamin content in fat samples of Pacific sardines *Sardinops melanostictus* after experimental storage for 45 days.

Fat samples	Vitamin A (Retinole)		Vitamin D^3	
	IU/kg	Losses, %	IU/kg	Losses, %
Fat after receiving	29700		18400	
Fat (control)	17800	40.1	2200	88.0
Fat + α-tocopherol acetate	20100	32.3	5100	72.3
Fat + botulin	20600	30.6	5400	70.7
Fat + ethoxyvine	22800	23.2	7500	59.2
Fat + ethoxyvine + α-tocopherol acetate	23600	20.5	8900	51.6
Fat + ethoxyvine + botulin	24000	19.2	8700	52.7
Fat + ethoxyvine + betulin + salicylic acid	24700	16.8	9600	47.8
Fat + ethoxyvine + α-tocopherol acetate + salicylic acid	25300	14.8	10300	44.0

antioxidant compounds led to a reduction in the level of PUFA compared to those using individual antioxidants. For example, when a fat sample containing a mixture of ethoxyvin, betulin, and salicylic acid amide was tested, it showed a drop in EPA and DHA content of approximately 27–33%. However, when the fat sample had a mixture of ethoxyvin, α-tocopherol acetate, and salicylic acid amide, the decrease was 30%.

3.5. Variations in the Levels of Fat-Soluble Vitamins in Fat Samples Throughout Storage

The data in Table 5 demonstrate the changes in the concentrations of vitamins A and D3 in fat samples during the storage experiment. It was noted that the control fat sample saw a 40% decline in the initial vitamin A concentration and an 88% fall in vitamin D3. The presence of individual antioxidants led to a further decrease in the vitamin levels in fish oil samples. Significantly, including ethoxyvine in the fat led to the least vitamin loss. In addition, antioxidant combinations consisting of three components exhibited the greatest preservation of vitamins A and D3 in fish oil samples during the storage period.

4. DISCUSSION

This study investigates the effects of natural antioxidants (α-tocopherol acetate and betulin) and synthetic antioxidants (ethoxyvine and salicylic acid amide) on the breakdown of lipids in fish oil during storage. The antioxidant capacity was assessed by subjecting fat to accelerated aging by utilizing individual antioxidants and their mixtures. This work used sardine *S. melanostictus* fat, renowned for its elevated levels of omega-3 PUFA [8].

During the experimental storage, there was a noticeable breakdown of lipids in fish oil without antioxidants (control sample) due to oxidation. This was demonstrated by the large increases in PV, AV, and p-AV, as well as a decrease in PUFA levels. The decline in quality is ascribed to the active processes of oxidation and hydrolysis [9-12], with higher temperatures speeding up the peroxidation of lipids [29].

The inhibitory effect of synthetic ethoxyvine on fish oil oxidation was greater than that of individual natural antioxidants (α-tocopherol acetate and betulin). This is consistent with previous studies that have shown that synthetic antioxidants have a greater ability to prevent oxidation [30-32]. Tocopherols operate as antioxidants by inhibiting the formation of lipid radicals and slowing down the chain events of autoxidation [29,33]. However, their efficiency decreases once the process of oxidation begins. Therefore, it is possible that α-tocopherol acetate alone, when taken at the prescribed levels, may not be sufficient for achieving optimum inhibition. Betulin, a naturally occurring molecule, exhibits antioxidant effects in fatty meals [4,34]. However, its mechanism of action is still not fully understood. Ethoxyvine's antioxidant mechanism involves the interaction with peroxide radicals, forming molecular products and inactive antioxidant radicals. This process stops the oxidation chains [32].

The combination of two antioxidants (ethoxyvine + α-tocopherol acetate or ethoxyvine + betulin) exhibited synergistic effects due to various antioxidant interactions, surpassing the solo contributions of each antioxidant [29,31]. It is worth mentioning that mixes of three antioxidants, which include salicylic acid amide, showed the strongest combined inhibition of autoxidation. This resulted in improved stability of fats and preservation of fat-soluble vitamins during storage. This information is supported by Figures 1–3 and Table 4. When salicylic acid amide is coupled with other antioxidants, it synergizes, enhancing the overall antioxidative action [35]. The results of this study align with prior research [32], which emphasizes the capacity of salicylic acid amide to eliminate hydroperoxides by as much as 70–75% without producing any free radicals.

The initial fish oil samples exhibited elevated concentrations of vitamins A and D3 (Table 5). The stability of vitamins in fats and oils decreases as secondary lipid oxidation products accumulate [18,36]. A significant decrease in vitamin content was seen in samples without antioxidants, resulting in increased PV, AV, and ranitidine value (p-AV). Although the individual antioxidants had a small positive impact on preserving vitamins, the synthetic ethoxyvine had the most significant effect. Prior research [32] has shown that bicomponent antioxidant combinations improve the stability of fat-soluble vitamins during storage. When salicylic acid amide was used with other antioxidants, it effectively prevented fat oxidation and lipid peroxidation accumulation. This resulted in improved stability of vitamins in fish oil samples during storage experiments.

5. CONCLUSIONS

During the experimental aging of Pacific sardine *S. melanostictus* fat without antioxidants, there were significant increases in PV, AV, and p-AV. These increases were accompanied by a notable decline in omega-3 PUFAs by 3.4–5 times and fat-soluble vitamins by 40–80%. These findings indicate the presence of vigorous oxidative processes and lipid degradation.

A slight reduction in the oxidation of fats, degradation of PUFAs, and loss of vitamins was seen when specific antioxidants (α-tocopherol acetate 2%, betulin 0.3%, ethoxyvine 0.14%) were present. The synthetic antioxidant ethoxyvine had superior antioxidant effectiveness. The combination of two antioxidants, α-tocopherol acetate and ethoxyvine, along with betulin and ethoxyvine, at a concentration of half the usual amount, resulted in a synergistic effect. This impact improved the stability of fish oil during storage. The addition of salicylic acid amide as a synergist showed strong antioxidant activity in mixtures of antioxidants (α-tocopherol acetate + ethoxyvine + salicylic acid amide, betulin + ethoxyvine + salicylic acid amide), resulting in improved preservation of fats during storage.

Following sardine *S. melanostictus* fat preservation, the omega-3 PUFA content remained at 70–73% of its initial level. Similarly, the retention

rates for vitamin A and D3 were 83.2–85.2% and 51.2–56%, respectively. Salicylic acid amide in a synergistic antioxidant composition for preserving highly unsaturated fish oils is an efficient method for preventing oxidative reactions and minimizing the degradation of omega-3 PUFAs and fat-soluble vitamins during storage.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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8. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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