

# Antioxidative Compounds from Fermented Olive mixed in Fish Feedstuffs

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## Abstract

Bioactive compounds like enzymes and antioxidants have been added to the feed to ensure its long-term viability and increase its nutritional value. However, feed additives may lose their efficacy and activity during the pelleting and storage processes. The stability of functional activity in feeds enriched with antioxidants, cellulases, and other bioactive extracts was the primary focus of this study. This bioactive extract (FBE) was produced by *Aspergillus ibericus* through solid-state fermentation of byproducts from olive mills and wineries. With or without lyophilized FBE (0.26 percent w/w) as a supplement, two isolipidic and isoproteic diets were developed. Both diets were kept at room temperature (RT), or 4 °C, for four months. The results indicated that feed stored at 4 °C was more stable for cellulase than for xylanase. The half-lives of cellulase and xylanase were approximately 60 and 14% longer when stored at 4 °C compared to RT, respectively. Antioxidant activity lost in the diet without supplements was the same at both storage temperatures, but antioxidant activity lost less with dietary FBE supplementation when stored at 4 °C. The addition of FBE reduced lipid peroxidation by 17.5% and 19.5%, respectively, when stored at 4 °C or RT. The current study is a first step toward enhancing bioactive compound-based diet storage conditions.

**Keywords:** Functional Aquafeeds; Solid-State Fermentation; Antioxidants

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## Introduction

Global fish production is supported by aquaculture and fisheries, two important industries that ensure food supply. While fisheries production has reached its highest level since the 1990s, aquaculture production has been steadily rising. Due to the use of fish meal and fish oil in the production of aquafeeds, there have been concerns raised regarding the expansion of aquaculture [1]. The majority of these ingredients were made from whole fish. There is one major drawback to using plant feedstuffs in aquafeed: Nutrients like non-starch polysaccharides (NSP) are in them. NSP may reduce the availability of nutrients by making digesta more viscous, preventing digestive enzymes from reaching the substrate, and slowing down gastrointestinal transit. Enzymes, organic acids, yeast products, probiotics, phytogenics, and mycotoxin binders are just a few examples of the many compositions and applications for feed additives. Additionally, fish growth performance may be compromised by

dietary NSP, as may unfavorable gut morphology and physiology changes that affect nutrient absorption and gut health [2]. Some feed additives have the ability to improve the feed's nutritional properties, reduce the negative effects of anti-nutritional factors, and increase fish consumption of the feed. Plant by-products, which are more cost-effective and friendly to the environment than other feed additives, are a possibility. In addition, there is a possibility that some will enhance the immune system and health of the fish, thereby reducing the amount of antibiotics required for aquaculture [3].

Enzymes like xylanases and cellulases are the primary functional feed additives that are utilized to enhance the digestibility of NSP. Fish, laying hens, minor poultry species, weaned piglets, pigs, and turkeys can all utilize some of these enzymes as feed additives [4]. Adding xylanase and cellulase to the diets of yellow river carp (*Cyprinus carpio*) was found to benefit their growth, feed digestibility, and digestive enzyme activities. affecting

the *Clarias gariepinus*, an African catfish, its gut microbiota; improving the digestibility of Nile tilapia (*Oreochromis niloticus*) dry matter, energy, total carbohydrates, and NSP; and making it easier for European seabass (*Dicentrarchus labrax*), meagre (*Argyrosomus regius*), white seabream (*Diplodus sargus*), and turbot (*Scophthalmus maximus*) to digest dry matter, energy, and starch [5].

Cost and stability during feed manufacturing and storage are the main obstacles to using enzymes as feed additives because denaturation processes may reduce their effectiveness. Utilizing inexpensive agro-industrial by-products to produce enzymes through solid-state fermentation (SSF) is one way to cut costs. For instance, the cost of producing cellulase using SSF is approximately 2.6 times lower than the cost of producing it using conventional submerged fermentation. SSF is an environmentally friendly bioprocess that makes use of water that doesn't flow freely or even close to it. Because of their hyphal growth, filamentous fungi are better suited for SSF because they can penetrate and disrupt the substrate and are tolerant of conditions with low water activity. Exoenzymes from fungi contain lignocellulolytic enzymes like cellulases, xylanases, and  $\alpha$ -glucosidases [6].

For consistent and high-quality feed production, quality management is essential from ingredient production to feed manufacturing and processing. Additionally, it is crucial to keep the feed's nutritional profile unchanged during storage. However, feed quality may be compromised by a number of processes. Oxidation is one of these cycles that could alter the feed's nature during production and capacity [7]. Autoxidation, photosensitized oxidation, thermal oxidation, and enzymatic oxidation are all examples of oxidation processes. Autoxidation occurs when feed radicals react with oxygen in the air [8]. A variety of fish diseases, including liver degeneration, skeletal anomalies, anemia, hemolysis, and jaundice, may be brought on by a diet high in oxidized lipids. Fish feeds that can be stored for an extended period of time either at room temperature or at low temperatures may experience protein depletion and degradation. Polyunsaturated fatty acids (PUFA) are abundant in fish diets, making them particularly vulnerable to lipid peroxidation. To prevent the oxidation of lipids and thus extend the shelf life of fish feeds, antioxidants have been added to fish diets. Additionally, during fish metabolism, dietary antioxidants may reduce reactive oxygen species, preventing fish health decline. As an antioxidant, ethoxyquin (EQ) has been utilized in fish feed [9]. However, due to concerns regarding the product's mutagenic and carcinogenic properties, the European Union recently prohibited its use. As a consequence of this, researchers have been looking for additional antioxidant compounds that are able to slow the oxidation of lipids.

The majority of the world's olive oil and wine are produced by European wineries and olive mills, which also produce a lot of byproducts. Due to their phytotoxic effects when used as fertilizers, these byproducts are typically burned as fuel. Antioxidant compounds become less bioavailable because they are bound to plant cell walls by these byproducts. Reusing these byproducts is essential to the environmental sustainability of these agricultural industries under a circular economy strategy.

Fungi may benefit from combining these byproducts [10].

## Result and Discussion

D-values, which indicate the amount of time it takes for the enzyme activity to decrease by 90% from its initial value, were also estimated using the first-order model. The diet that was kept at 4 °C had a D-value that was 1.6 times higher than the diet that was kept at room temperature. To increase the nutritional bioavailability of plant-based diets, supplements containing exogenous enzymes are currently being used more frequently. However, very few studies have examined the stability of dietary enzymes during storage. Cellulases were added to a barley-based diet for poultry for 32 weeks at room temperature [11]. After three and six weeks, the enzyme activity dropped to 42% and 33%, respectively. After 19 weeks at RT and 31 weeks at 4 °C, cellulase activity only decreased by 50% in this study [12].

The shelf life of enzymes that are not included in a diet is the subject of additional research. In addition, cellulase outperformed xylanase in terms of stability. Cellulase activity was 100 and 75% of its initial value after 7.5 months of storage at 4 °C and 30 °C in commercial liquid feed enzymes that were not incorporated into a diet, respectively. At 4 °C and 30 °C, xylanase residual activity decreased to 20% and 50%, respectively.

After nine days of storage at 4 °C, *Paenibacillus chitinolyticus*'s cellulase activity decreased by 33%. After 39 weeks of storage, *Aspergillus terreus* cellulase retained 75% of its initial activity at 5 °C, whereas only 40% of its initial activity was retained when stored at RT. After 19 weeks at RT and 31 weeks at 4 °C, cellulase activity decreased by 50%.

At 4 °C, this study discovered that xylanase was more stable than RT. On the other hand, the  $t_{1/2}$  was only nine days behind schedule. The shelf life of the *Pseudomonas* sp.-produced xylanase XPB-6 at 4 °C and RT was previously examined. After six weeks and remaining stable for 25 days at 4 °C, the xylanase activity of *Anoxybacillus kamchatkensis* NASTPD13 decreased by 70% from its initial level, according to. When stored at 25 °C, its activity was reduced to 16 days, and then gradually decreased thereafter. This enzyme was also stable for 15 days at RT, but after six weeks, its activity dropped by 40%. Alkalophilic *Bacillus subtilis* ASH produced a purified extracellular xylanase that was kept at 4 °C for six weeks before decreasing. However, after 10 weeks of storage at 4 °C, the enzyme maintained 80 percent of its initial activity. In this study, xylanase activity decreased by 50% after nine weeks at RT and ten weeks at 4 °C [13].

One of the factors that render xylanases and cellulases inactive is the activity of proteases. The diets' lack of protease activity was the focus of this study. As a consequence of this, enzyme inactivity during storage was primarily brought on by temperature.

## Inhibition of Lipid

Their oxidation susceptibility is influenced by their diet. Fish oil, which is particularly prone to oxidation and has a high content of unsaturated fatty acids, is present in 12.3% of the experimental diets in this study. Fish fed oxidized lipids are more likely to suffer damage to their digestive system and other organs as well as

serious conditions like muscular dystrophy. The susceptibility to and mortality from fish diseases, as well as performance, feed consumption, animal weight, and feed conversion ratio, will all be affected. Adding antioxidants to the diet to prevent lipid peroxidation is the only way to maintain the quality of fish feed. In addition, it has been demonstrated that including antioxidant supplements in a fish's diet is a novel method for maintaining cell metabolism and preventing excessive free radical production in oxidatively stressed fish [14].

Lipid peroxidation levels in FBE-unsupplemented and supplemented diets remained constant 28 days after storage, according to this study. However, the FBE-supplemented diet lead's lipid peroxidation was lower than the control diet after 42 days at 4 °C. From the 41st to the 91st day of storage, the lipid peroxidation of the FBE-supplemented diet was lower than that of the control diet in diets stored at RT. After 119 days of storage at 4 °C, the control diet's lipid peroxidation was 17% higher than the FBE-supplemented diet's, indicating the greater difference

between the two diets. After 119 days of storage, this result is well correlated with the higher antioxidant activity of the FBE diet compared to the control [15].

## Conclusions

The results of this study made it possible to investigate how bioactive compounds added to aquaculture diets store. During storage, the activity of cellulosease was more stable than that of xylanase. The storage temperature, which could be as low as 4 °C or as high as room temperature, had a significant impact on the enzymes' rate of deactivation. Cellulase and xylanase saw reductions of up to 42% and 79%, respectively, in both temperatures at the conclusion of the experiment.

The antioxidant activity of the FBE-supplemented diet was more stable over time than that of the control diet. The diet supplemented with FBE maintained its higher antioxidant activity even after 119 days of storage. Lipid peroxidation was lessened by the FBE in later stages of storage.

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