International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM July 2024, 15(7): 01-10 **Article** AR5422

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2024.5422

Evaluation of Quality and Stability of Fish-vegetable Composite Bio-silage based Fish Feed Stored at Room Temperature

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ABSTRACT

The present experiment was conducted during March–May 2022 at ICAR–CIFE, Mumbai, Maharashtra, India to develop a unique fish feed from locally available vegetable, and fish processing waste. These two sources of the waste were used in composite bio-silage (CBS) production by combining fish (80%)–vegetable (20%) waste with probiotic proteolytic strain (*E. faecalis*+*L. acidophilus*) and jaggery (15% Jaggery) as a carbon source. The final CBS materials were used in experimental fish feed preparation by replacing fish meal (FM) protein. Changes in physicochemical, and microbiological quality characteristics of fish feed were measured every 15th day at room temperature (25–30°C). There was no change in color, the appearance of a moderately bad odour, a little bit of soft texture, and broken pellets were found in the later stages of the storage. Crude protein (38.50–36.10%) and fat (8.60–4.82%) content were decreased whereas, moisture content increased (9.15–11.10%) in all samples during the storage period. There were no notable changes in ash or crude fiber. Lipid oxidation product TBARs (8.0–13.205 MDA; nmol mg^{-1}), Total Plate Count (2.75–6.70 log cfu g^{-1}), and Fungal Count (1–2.50 log cfu g^{-1}) were within acceptable range. The study's findings indicated that composite bio-silage incorporated fish feed has a shelf life of up to 60th days and can be fed during this period. The study also suggested that manufactured fish feed should not be stored for more than two months, either in the place of production or at the farmer's store.

KEYWORDS: Composite bio-silage, fish feed, storage, quality, shelf life

***Citation* (VANCOUVER):** Ghosh et al., Evaluation of Quality and Stability of Fish-vegetable Composite Bio-silage based Fish Feed Stored at Room Temperature. *International Journal of Bio-resource and Stress Management,* 2024; 15(7), 01-10. HTTPS://DOI.ORG/10.23910/1.2024.5422.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 01st May 2024 RECEIVED in revised form on 25th June 2024 ACCEPTED in final form on 03rd July 2024 PUBLISHED on 06th July 2024

1. INTRODUCTION

India's aquaculture is shifting towards culture fisheries intensification because of rising demand for food fish and a fall in capture fisheries productivity (Anonymous, 2022). This intensification of the aquaculture system increases the demand for fish feed (Hossen et al., 2013). Fish feed is crucial to achieving optimum quality fish production (Sørensen et al., 2009; Wong et al., 2016). Feed costs are typically the biggest single operation expense in semi-intensive or intense grow-out aquaculture operations (Henriksson et al., 2021) Formulated fish feeds are expensive since ingredients are imported, and prices are steadily growing (Ragasa et al., 2022). To prevent excessive feed costs, it becomes vital to identify cost-effective replacements for feeding protein from locally available materials (Kop et al., 2019). Fish feed can be prepared from locally accessible plant protein sources (cereal seeds, bran, rapeseed, soybean meal, legume seeds, reddish, maize, vegetable by-products) and animal protein (meat bone and fish meal, poultry byproducts, powdered milk, animal fats, fish waste) supplemented with vitamins and minerals (Hossen et al., 2013; Ghosh et al., 2023a). In India various feed mills enterprises are producing commercial fish feeds using these plant and animal protein sources, but unfortunately, farmers are constantly confronted with fish feed scarcity, price disparity, and poor quality (Hundare et al., 2008; Kaushik and Hemre, 2008; Sørensen, 2012; Hasan and New, 2013).

Storage conditions, particularly temperature and humidity, have a significant impact on the microbiological and biochemical quality of fish feed (van der Meeren et al., 2008; Kong et al., 2020). The prevailing climatic conditions in the tropics are affected by an increase in temperature and relative humidity of over 25°C and 70%, respectively (Hossen et al., 2013). Such type of climatic conditions favour mold development and lipid oxidation in stored fish feed. The use of oxidized fish feeds in aquaculture influences fish body physiological functions, immune systems, growth, and overall fish health (HernAndez et al., 2014). Another type of large alteration that significantly impacts fish feed quality is occurrence of fungal toxins and microorganisms (Binder et al., 2007; Rodrigues and Naehrer, 2012). The risk of microbial contamination of fish feeds is reduced by using high-quality feed materials and maintaining hygienic storage conditions (Kubiriza et al., 2024). Improper storage temperature may extend the survival of microorganisms in the feed and produce potentially toxic substances (Kong et al., 2020). Furthermore, fish feed naturally undergoes different chemical and enzymatic processes during storage, which further affect their acceptability, shelf life, and safety (Draganovic et al., 2011). The researcher suggested that storing fish feed for a maximum of six months should be done with great care and attention (Hossen et al., 2013). Therefore, it is necessary to assess the nutritional value of fish feed and ingredients before and after preparation of feed.

In this context, the present study selects fish and vegetable wastes that are generated in significant amounts in the Indian market. Fish waste serves as a nutritious medium with a high nitrogen content but a low quantity of biodegradable carbon. Whereas vegetable waste such as cabbage and cauliflower are high in carbohydrates (C) and low in nitrogen. By combining these two waste mixtures, the carbon/nitrogen ratio will be improved and the permitting bacteria will break down the materials more quickly (Alvarez et al., 2010; Rughoonundun et al., 2012). It produces composite bio-silage, which can potentially serve as a source of protein for the aquaculture and animal feed industries (Ghosh et al., 2023a; Ghosh et al., 2023b). Therefore, the current study objective was to utilize the composite bio-ensilage as a fish feed and study its quality and shelf life at room temperature.

2. MATERIALS AND METHODS

2.1. Controlled bio-silage preparation using selected microbes

The present experimental setup was conducted in Post Harvest Technology laboratory during March–May, 2022 at ICAR-CIFE, Mumbai. Fresh vegetable waste, including cabbage and cauliflower leaves, and fish waste were collected from Versova vegetable and 4-Bungalows fish market respectively. Vegetable waste was cleaned and partially sun-dried before being mashed with fish waste in a mixer grinder for uniform mixing. To make composite bio-silage, native, isolated starter cultures of *Enterococcus faecalis* (ON 843903) and non-native starter cultures of *Lactobacillus acidophilus* (MTCC 10307) were used. The starter culture cell suspension count was simultaneously adjusted to the standard (10^8 cfu ml^{-1}), and jaggery powder (15%) was added as a source of carbohydrates (Bhaskar et al., 2008). A total of four treatments of control (without starter culture), T_1 (with *E. faecalis*), T_2 (with *L. acidophilus*), and T_3 (with a mixture of *L. acidophilus* and *E. faecalis*) were prepared in triplicate using a fish waste-to-vegetable waste (FW: VW) ratio of 80:20, and the total weight of the material was built up to 2 kg in each reactor. All treatments were undertaken at room temperature (30±2°C), and variations in physical, biochemical, and microbiological parameters were assessed throughout the treatment period of 15 days. Based on the data obtained from the fish-vegetable composite bio-ensilage physicochemical and microbiological quality assessment T_3 sample materials were chosen for the final fish feed preparation (Ghosh et al., 2023b).

2.2. Proximate composition of fish feed and ingredients

The proximate value of feed ingredients and experimental fish feed was checked by standard methods (Anonymous,

2023). The Kjeldahl Method was used to quantify the amount of total nitrogen (TN), and the true protein content was obtained by multiplying the protein nitrogen (PN) value with the conversion factor (PN×6.25). The moisture amount of feeds was determined by taking the sample in a petri dish of known weight and kept for drying in a hot air oven (100±5°C) still a stable weight was reached. The moisture value was computed using the difference in sample weight with the formula as follows:

Moisture(%)=(The initial weight of the sample-the dried weight of the sample)/The initial weight of the sample.....(1)

The micro-Kjeldahl technique was used to determine the crude protein concentration (Kelplus, PELICAN, and India). The ether treated extract of dry feed was measured using the soxhlet instrument with petroleum ether solvent (Boiling point: 62°C). The following formula was used to calculate the ether extract.

EE(%)={(The initial weight of sample-Final Weight of sample)/The initial weight of the sample)}×100.....(2)

To evaluate the ash value of feed, a known weight of dry samples was burned for 5–6 hours at 600°C in a silica crucible. The following formula was used to perform the calculation.

Ash(%)=(Weight of ash/Weight of sample)×100.....(3)

Fibertech (Tulin equipment, India) equipment was used to estimate the fibre, and ash value was measured in a muffle oven at 550°C for 5 hours. Nitrogen-free extract (NFE) was computed by deducting other nutrients percent from 100 as follows:

NFE (%)=100–[Crude protein (%)+Ether extract (%)+Ash (%)+fiber (%)].....(4)

The total carbohydrate (TC) content of feed ingredients as well as tested feeds was calculated as follows. TC (%)=100–[Moisture (%)+Crude protein (%)+Ether extract (%)+Ash (%)].....(5)

The following formula was used to determine the digestible energy value of experimental feed under normal physiological parameters (Halver, 1976). Digestible energy (Kcal 100 g^{-1})=[Crude Protein (%)×4+Ether extract (%)×9+Nitrogen free extract (%)×4].....(6)

Gross energy=[5.7×g protein+9.4×g fat+4.×(g NFE+g fibre)].....(7)

2.3. Experimental fish feed preparation

The experimental fish feed were prepared in four different group with one control group, and intended to meet the nutrition recommendations of Pangasius catfish (Phumee et al., 2011; Asdari et al., 2011). A total of five iso-nitrogenous (38.0±0.06% crude protein kg^{-1}) and isocaloric (273.54±0.80 digestible energy Kcal 100 g^{-1}) experimental fish feed with Control (C): Composite bio-silage 0%; T_1 (CBS 25%): Composite bio-silage 25%; T_2 (CBS 50%): Composite bio-silage 50%; T_3 (CBS 75%): Composite bio-silage 75%; T_4 (CBS 100%): Composite bio-silage 100% were formulated. To prepare the experimental feed, all the ingredients along with composite bio-silage were weighed separately as per the requirement. The required amount of water was added to the mixture of feed components to create dough. After around 30 minutes of steaming in a pressure cooker, the dough was slowly cooled. The measured concentration of the oils, vitamins, and minerals mixture were added to the steamed dough and well combined once it had cooled. Following a thorough mixing process, the dough was fed through a pelletizer to produce uniform-sized pellets, which were then spread out on a piece of paper to dry naturally. The pellets were labelled with the different treatments, sealed in airtight polythene bags, and kept at 4°C until use.

2.4. Storage conditions and Sampling procedure of fish feed

The storage experiment was carried out under standard room temperature conditions (25–30°C). Dry feed (1 kg each) were stored in bags similar to those used by feed companies, closed and protected from light. These bags were stored in two batches at room temperature, in a room with a temperature that varied between 25 and 30°C throughout the storage period. Samples of dry feed were taken for quality analysis at intervals of the 3rd, 15th, 30th, 45th, and 60th days of the storage period. All samples were placed in vacuum bags and stored at 4°C until the time of analysis.

2.5. Determination of biochemical and microbiological quality of fish feed

Analysis of the secondary lipid oxidation product of storage fish feed was conducted using the Grigorakis et al. (2010) methodology. Thiobarbituric acid (TBA) value was used to assess the oxidation condition of all prepared fish feeds. Fish feed weighing 1 g was examined for TBA reactive chemicals (TBARs). Microbial quality parameters like Aerobic Plate Count (APC) and Total Fungal Count (TFC) were analyzed by following the standard method. Experimentally tested fish feed weighing about 10g was aseptically collected, ground, and mixed for three minutes in 90 ml of sterile physiological saline solution (0.85% NaCl). Additional decimal dilutions were prepared, and 0.1 ml of each dilution was pipetted in triplicate onto the surface of Red Bengal agar (Hi Media, Mumbai) plates for total fungal colony count. The total aerobic plate count was estimated (log cfu g^{-1} of the sample) using plate count agar followed by incubating the plate at 37°C for 24 hours.

2.6. Statistic interpretation

The significant difference in all the biochemical parameters was determined using the one way analysis of variance (ANOVA). To compare the means, Duncan multiple

range tests were used for Post hoc analysis. The statistical package for social science (SPSS for Windows Inc. Version 22. Chicago, Illinois) was used for all the statistical interpretations. The research data were represented as Mean±SD with a significant (p<0.05) difference.

3. RESULTS AND DISCUSSION

3.1. Changes in proximate composition of raw feed ingredients and fish feed during the storage period

The proximate chemical makeup of the various raw feed ingredients, including composite silage, defatted soybean meal, fish meal, deoiled rice bran, a cake made from ground nut oil, and gross energy were given in Table 1.. The proximate value of the prepared experimental feed is represented in Table 2. The initial value of the crude protein content in various experimental fish feeds ranged from 38.0 to 38.50. However, after 60 days, the value decreased and remained at 36.10 to 36.20 at room temperature. During the two-month storage period, the lipid contents of the various test feeds varied significantly. Initial lipid contents of the test feed ranged from 8.20 to 8.60 and decreased from 5.10 to 4.82 correspondingly. As the value of lipids decreased over the storage period, moisture content likewise changed in an upward trend. The initial moisture content ranged from 9.15 to 9.68, and during storage, it increased to range from 11.10 to 10.90. The initial ash concentration ranged from 8.04 to 8.55, and it dropped to a range from 7.10 to 6.90 after 60 days of storage, respectively. Ash and fibre contents of the prepared fish feed did not differ significantly. During the 60-day storage period, smaller variations in fibre content occurred, falling a range from 6.27–6.81 to 5.15–5.27 (Table 3).

In the current experimental study isonitrogenous (38.0%) and isolipidic (8.20%) feeds were prepared from locally available fish and vegetable waste. The proximate composition such as crude protein (13.40%) and ether

Table 1: Proximate composition of various feed ingredients (on % dry matter basis) used in the preparation of fish feeds

Variables	Ingredients					
	CBS[1]	FM[2]	DORB[3]	DSBM[4]	GNOC[5]	Wheat flour
Moisture	6.81±0.13	6.50±0.12	8.30±0.15	5.28±0.05	3.67±0.20	7.70±0.05
Crude protein	69.42±0.06	51.33±0.05	13.40±0.20	50.0±0.10	41.80±0.08	10.05±0.08
Ether extract	2.36±0.26	5.56±0.10	1.75±0.05	1.32±0.05	4.55±0.05	2.05±0.10
Crude fibre	1.99±0.01	3.44±0.12	14.40±0.20	5.52±0.10	6.12±0.06	3.65±0.06
Nitrogen free extract (NFE)	12.35±0.05	15.34±0.08	47.25±0.25	32.30±0.30	38.65±0.25	74.90±0.20
Total ash	1.78±0.00	24.33±0.10	14.75±0.26	5.50±0.10	4.64±0.15	1.47±0.12
GE (Kcal 100 g^{-1})	476.67±10.34	421.84±0.87	345.59±8.75	452.47±7.90	464.58±7.85	398.61±10.30

Data expressed as mean±SE, n=3; Mean values in the same column with different superscripts differ significantly (p<0.05); NFE: Nitrogen free extract=100%- (% Crude protein+% Ether extract+% Ash+% crude fibre); GE: Gross energy=(5.7×g protein+9.4×g fat+4.1×(g NFE+g fibre)

Table 2: Proximate content of different fish feeds (on % dry weight basis)

Variables	Diet/Treatment group				
	Control (C)	T_1 (CBS 25%)	T_2 (CBS 50%)	T_3 (CBS 75%)	T_4 (CBS 100%)
Dry matter content	90.80±0.27	90.77±0.71	90.70±0.25	90.85±0.40	91.32±0.26
Crude protein	38.0±0.06	38.10±0.97	38.16±0.83	38.06±0.11	38.50±1.2
Ether extract	8.40±0.10	8.60±0.35	8.30±0.1	8.20±0.16	8.52±0.03
Crude fibre	6.77±0.19	6.71±0.33	6.81±0.28	6.27±0.19	6.32±0.59
Nitrogen free extract	11.46±0.53	11.88±0.95	11.74±0.45	11.10±0.56	11.28±0.55
Ash value	8.10±0.10	8.34±0.06	8.04±0.86	8.55±0.39	8.50±0.02
GE (Kcal 100 g^{-1})	461.123±0.80	461.54±1.17	460.97±2.21	459.86±2.22	462.31±2.32

Data expressed as mean±SE, n=3; Mean values in the same column with different superscripts differ significantly (p<0.05). Control (C): Composite bio silage 0%; T_1 (CBS 25%): Composite bio silage 25%; T_2 (CBS 50%): Composite bio silage 50%; T_3 (CBS 75%): Composite bio silage 75%; T_4 (CBS 100%): Composite silage 100%; NFE: Nitrogen free extract=100%- (% Crude protein+% Ether extract+% Ash+% crude fibre); GE: Gross energy=(5.7×g protein+9.4×g fat+4.1×(g NFE+g fibre)

Table 3: Changes in proximate composition (%) of composite silage added fish feed stored at room temperature (25–30°C)

Test feeds	Characteristics	Storage period (Days)				
		2-3rd days	15th days	30th days	45th days	60th days
Control	Protein	38.0±0.06^{c}	37.50±0.02^{b}	37.05±0.05^{b}	36.25±0.04^{a}	36.10±0.08^{a}
	Lipid	8.40±0.10^{d}	7.35±0.02^{c}	6.25±0.06^{b}	5.18±0.12^{a}	5.10±0.10^{a}
	Moisture	9.20±0.27^{a}	9.95±0.12^{ab}	10.15±0.20^{c}	10.75±0.06^{c}	10.90±0.05^{cd}
	Ash	8.10±0.10^{d}	7.90±0.06^{bc}	7.55±0.12^{b}	7.10±0.10^{b}	6.90±0.02^{a}
	Crude fibre	6.77±0.19^{cd}	6.10±0.10^{c}	5.85±0.05^{ab}	5.27±0.06^{a}	5.15±0.19^{a}
T_1 (CBS 25%)	Protein	38.10±0.97^{c}	37.54±0.05^{b}	37.08±0.06^{b}	36.28±0.02^{a}	36.12±0.04^{a}
	Lipid	8.60±0.35^{d}	7.32±0.04^{c}	6.22±0.08^{b}	5.15±0.10^{a}	5.05±0.12^{a}
	Moisture	9.23±0.71^{a}	9.97±0.10^{ab}	10.19±0.10^{c}	10.77±0.05^{c}	10.95±0.06^{cd}
	Ash	8.34±0.06^{d}	7.92±0.05^{c}	7.58±0.10^{b}	7.15±0.12^{b}	6.96±0.04^{a}
	Crude fibre	6.71±0.33^{cd}	6.15±0.05^{c}	5.87±0.06^{ab}	5.30±0.07^{a}	5.19±0.10^{a}
T_2 (CBS 50%)	Protein	38.16±0.83^{c}	37.57±0.06^{b}	37.10±0.05^{b}	36.30±0.10^{a}	36.15±0.02^{a}
	Lipid	8.30±0.1^{d}	7.27±0.05^{c}	6.15±0.06^{b}	5.10±0.05^{a}	5.00±0.12^{a}
	Moisture	9.30±0.25^{a}	9.98±0.06^{ab}	10.25±0.12^{c}	10.80±0.02^{c}	10.97±0.05^{cd}
	Ash	8.04±0.86^{d}	7.95±0.06^{bc}	7.60±0.08^{b}	7.20±0.10^{b}	6.98±0.05^{a}
	Crude fibre	6.81±0.28^{cd}	6.17±0.02^{c}	5.90±0.04^{ab}	5.35±0.06^{a}	5.20±0.08^{a}
T_3 (CBS 75%)	Protein	38.06±0.11^{c}	37.60±0.04^{b}	37.15±0.10^{b}	36.37±0.12^{a}	36.17±0.04^{a}
	Lipid	8.20±0.16^{d}	7.22±0.05^{c}	6.12±0.06^{b}	5.05±0.04^{a}	4.95±0.05^{a}
	Moisture	9.15±0.40^{a}	10.05±0.04^{b}	10.27±0.10^{c}	10.85±0.05^{c}	11.05±0.06^{cd}
	Ash	8.55±0.39^{d}	7.97±0.05^{bc}	7.62±0.06^{b}	7.24±0.12^{b}	7.05±0.06^{a}
	Crude fibre	6.27±0.19^{bc}	6.19±0.06^{b}	5.92±0.05^{ab}	5.37±0.08^{a}	5.25±0.10^{a}
T_4 (CBS 100%)	Protein	38.50±1.2^{c}	37.64±0.05^{b}	37.17±0.12^{b}	36.38±0.15^{a}	36.20±0.05^{a}
	Lipid	8.52±0.03^{d}	7.15±0.06^{c}	6.10±0.05^{b}	4.90±0.10^{a}	4.82±0.12^{a}
	Moisture	9.68±0.26^{a}	10.10±0.06^{b}	10.25±0.12^{bc}	10.90±0.08^{cd}	11.10±0.02^{cd}
	Ash	8.50±0.02^{d}	8.02±0.04^{cd}	7.65±0.05^{bc}	7.25±0.06^{b}	7.10±0.05^{a}
	Crude fibre	6.32±0.59^{d}	6.20±0.05^{c}	5.95±0.06^{ab}	5.40±0.10^{a}	5.27±0.12^{a}

All values are represented as Mean±SE (n=3). Control (C): Composite bio silage 0%; T_1 (CBS 25%): Composite bio silage 25%; T_2 (CBS 50%): Composite bio silage 50%; T_3 (CBS 75%): Composite bio silage 75%; T_4 (CBS 100%): Composite silage 100%

extract (1.75%) values of DORB estimated in the present study was supported by Ranjan et al. (2019). Similarly, the crude protein level (41.80%) of GNOC, as observed in the present study was within the reported range of 32% to 46.4% (Hertrampf and Piedad-Pascual, 2012). Nitrogen-free extracts (74.90%) and crude protein (10.05%) contents of wheat flour used in the present study were similar to the reported values of Hertrampf and Piedad-Pascual (2012).

3.2. Changes in physical characteristics of fish feed during the storage period

The unique colour of composite silage-based fish feed was brown and light green because fish waste and vegetable waste were blended (Figure 1). After 60th days of storage at ambient temperature (25–30°C) no colour change was seen. The odour of fish feed had a pleasant aroma at first. The smell of the experimental feed was vaguely muddled with the flavour of dried fish and vegetables. On 60th day, the characteristic odour started to shift slightly, and on 75th day a fairly unpleasant odour was detected. The typical texture of a small number of pellets changed to a slightly softer texture after 60th days, and after 75th days some flies were discovered. It could happen as a result of inappropriate tropical climatic conditions, feed handling, and an increase in moisture content. This softening of the texture may be caused by an increase in moisture content and the natural deterioration of feeds kept at ambient room temperature. After the 75th day, the experimental feed's overall quality started to lose

Figure 1: Changes in colour of composite bio-silage added fish feed during the storage periods at ambient temperature (25–30°C)

some luster and produce an unpleasant odour, but the fish feed was still at an acceptable level (Table 4). The current investigation's physical attributes of fish feed revealed that the experimentally produced test feed had comparable color and texture quality. Hossen et al. (2013) experimented to ascertain how the nutritional value of various commercial fish feeds changed when kept at low temperatures (5–8°C) for two months and normal temperatures (25–30°C). They revealed that both storage regimes had lower crude protein and fat contents. While moisture content reduced when stored at low temperatures (5–8°C) and increased slightly when stored at room temperature. Other compositions like ash and crude fibre have not seen any notable changes. Although the quality of fish meals degraded more quickly at room temperature (25–30°C) than at low temperature (5–8°C), both settings were suitable for storage for up to

Table 4: Changes in physical characteristics of composite bio-silage added fish feed stored at room temperature (25–30°C)

Test feeds	Characteristics	Storage period (Days)					
		2-3rd days	15th days	30th days	45th days	60th days	75th days
Control	Colour	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/ Light green	Brown/Light green
	Odour	Characteristic odour	Characteristic odour	Characteristic odour	Characteristic odour	Moderately bad odour	Moderately bad odour
	Texture	Normal texture	Normal texture	Normal texture	Normal texture	A bit of soft texture	A bit of soft texture
	Infestation	No infestation	No infestation	No infestation	No infestation	No infestation	No infestation
	Broken pieces and fines	No fines	No fines	No fines	No fines	No fines	A little bit of fines
	Overall quality	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable but starting of spoilage symptom
T_1 (CBS 25%)	Colour	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/ Light green	Brown/Light green
	Odour	Characteristic odour	Characteristic odour	Characteristic odour	Characteristic odour	Moderately bad odour	Moderately bad odour
	Texture	Normal texture	Normal texture	Normal texture	Normal texture	A bit of soft texture	A bit of soft texture
	Infestation	No infestation	No infestation	No infestation	No infestation	No infestation	No infestation
	Broken pieces and fines	No fines	No fines	No fines	No fines	No fines	A little bit of fines
	Overall quality	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable but starting of spoilage symptom

Table 4: Continue...

Test feeds	Characteristics	Storage period (Days)					
		2-3rd days	15th days	30th days	45th days	60th days	75th days
T_2 (CBS 50%)	Colour	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green
	Odour	Characteristic odour	Characteristic odour	Characteristic odour	Characteristic odour	Moderately bad odour	Moderately bad odour
	Texture	Normal texture	Normal texture	Normal texture	Normal texture	A bit of soft texture	A bit of soft texture
	Infestation	No infestation	No infestation	No infestation	No infestation	No infestation	No infestation
	Broken pieces and fines	No fines	No fines	No fines	No fines	No fines	A little bit of fines
	Overall quality	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable but starting of spoilage symptom
T_3 (CBS 75%)	Colour	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green
	Odour	Characteristic odour	Characteristic odour	Characteristic odour	Characteristic odour	Moderately bad odour	Moderately bad odour
	Texture	Normal texture	Normal texture	Normal texture	Normal texture	A bit of soft texture	A bit of soft texture
	Infestation	No infestation	No infestation	No infestation	No infestation	No infestation	No infestation
	Broken pieces and fines	No fines	No fines	No fines	No fines	No fines	A little bit of fines
	Overall quality	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable but starting of spoilage symptom
T_4 (CBS 100%)	Colour	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green	Brown/Light green
	Odour	Characteristic odour	Characteristic odour	Characteristic odour	Characteristic odour	Characteristic odour	Moderately bad odour
	Texture	Normal texture	Normal texture	Normal texture	Normal texture	Normal texture	A bit of soft texture
	Infestation	No infestation	No infestation	No infestation	No infestation	No infestation	No infestation
	Broken pieces and fines	No fines	No fines	No fines	No fines	No fines	A little bit of fines
	Overall quality	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable but starting of spoilage symptom

two months. De Silva and Anderson (1994), observed that feeds became lumpy and less appetizing when stored, and they noted significant flavour and visual alterations. Although adequate storage slows down the rate at which a feed degrades, storage never improves the quality of the fish feed.

3.3. Changes in biochemical and microbiological quality of fish feed during the storage period

The primary cause of the deterioration in fish feed quality is the influence that processing and subsequent storage have on the lipid oxidation of the feed. The feed and interior air chambers' porous surface structures encourage contact between fat and airborne oxygen, which supports the initiation of a lipid peroxidation process. The oxidative stability of the feed is crucial for the nutritional quality of the fish as well as their growth and health. On the other hand, microbiological infestation like fungal growth is one of the primary problems during the storage of fish feed in tropical countries. Secondary lipid oxidation products including TBARs were measured in the test feed for up to 60th days of storage at room temperature (25–30°C). It was found that the value of TBARs significantly increased from 8.0 to 13.205 throughout storage. The majority of the microbiological plates revealed fungal growth, with low colony-forming units (cfu) g^{-1} of fish feed sample (1 to 2.5 log cfu g^{-1}). The total aerobic plate count was also within the acceptable range for fish feed (2.80 to 6.70 log cfu gm^{-1}). The total fungal and bacterial colonization intensity of the plate count agar and the rose Bengal agar, which were inoculated with samples of fish feed during storage in a normal room environment, were summarised in Table 5. The feed's oxidative stability is crucial not only for fish growth and health but also for nutritional quality (Grigorakis et al., 2010). Supporting the above, Van den Bergh et al. (2008) and Ruiz et al. (2000) discovered that lipids are essentially unstable at temperatures above 30°C. Under such conditions, lipids hydrolyze to produce ketonic acids, which then undergo auto-oxidation, resulting in the degradation of free radical products (John and Hamilton, 1994; Hamre et al., 2010). Long-term storage has been shown to affect the biochemical makeup of fish feeds, but there is inadequate information on the effects of shorter storage durations on fish feed quality and stability (Hossen et al., 2013). Various synthetic antioxidants are also used to prevent all these undesirable effects, primarily butylhydroxytoluene and butylhydroxyanisole in fish oil and ethoxyquin in fish meal (Hamre et al., 2010). These chemicals have high antioxidant action. However, it has been demonstrated that they are transmitted to the muscle in unsettling proportions and have detrimental health effects (Lundebye et al., 2010). As a result, natural antioxidants are gaining popularity in the aquafeed business due to their anticipated safety and increased customer acceptance. Fish feeds retain good oxidative conditions throughout preparation and storage for 24 weeks at ambient temperature (20–28°C) or in a refrigerator (4–1°C). The difference in oxidation resistance during storage at both temperatures could be attributed to the loss of activity of aromatic substances. Thyme essential oils lose activity near the end

Table 5: Changes in the biochemical and microbiological quality of composite silage added fish feed stored at room temperature (25–30°C)

Test Feeds	Characteristics	Storage period (Days)				
		2-3rd days	15th days	30th days	45th days	60th days
Control	Bengal red (log cfu g^{-1})	ND	ND	1.0	1.50	2.50
	PCA (log cfu g^{-1})	2.80	3.50	4.25	5.60	6.45
	TBARs (MDA; nmol•mg^{-1})	8.106	8.550	10.450	11.605	13.205
T_1 (CBS 25%)	Bengal red (log cfu g^{-1})	ND	ND	1.0	1.30	2.30
	PCA (log cfu g^{-1})	2.85	3.45	4.20	5.50	6.50
	TBARs (MDA; nmol•mg^{-1})	8.045	8.250	10.320	11.502	13.105
T_2 (CBS 50%)	Bengal red (log cfu g^{-1})	ND	ND	1.0	1.20	2.20
	PCA (log cfu g^{-1})	2.90	3.40	4.15	5.30	6.70
	TBARs (MDA; nmol•mg^{-1})	8.100	8.105	10.120	11.320	13.085
T_3 (CBS 75%)	Bengal red (log cfu g^{-1})	ND	ND	1.0	1.40	2.35
	PCA (log cfu g^{-1})	2.75	3.30	4.20	5.40	6.60
	TBARs (MDA; nmol•mg^{-1})	8.008	8.210	10.062	11.120	13.005
T_4 (CBS 100%)	Bengal red (log cfu g^{-1})	ND	ND	1.0	1.30	2.40
	PCA (log cfu g^{-1})	2.84	3.35	4.00	5.45	6.55
	TBARs (MDA; nmol•mg^{-1})	8.0	8.005	10.150	11.210	13.002

The Bengal red and plate count agar plates were kept at 25°C for 96 hours before being scored as follows: Not detected (ND) colony; Log colony forming unit (log cfu g^{-1}); Malonaldehyde (MDA) levels in fish feed (nmol mg^{-1})

of the storage period, whereas butyl hydroxytoluene and rosemary extract provide the best oxidation resistance at room temperature. The nature and extent of these changes may differ based on the feed's initial state, particularly its moisture content, and the storage environment (HernAndez et al., 2014). Dethlefsen et al. (2016) discovered that astaxanthin has antioxidative and self-protective capabilities, which prevent oxidation and extend the shelf life of fish feed pellets. According to Filipe et al. (2023), the antioxidant activity of the bioactive extract (FBE) from Aspergillus ibericus during solid-state fermentation of olive mill and winery by-product-supplemented feed was more stable over time and helped to reduce lipid peroxidation in the later stages of storage.

A considerable amount of mycotoxin, such as ochratoxin A (OTA), was found in a fishmeal-based fish feed after being stored for a month under warm (above 25°C) and humid conditions (above 60% relative humidity), resulting in the feed inappropriate for feeding to farmed fish. This highlights how quickly the mycotoxin can increase in fish feed during storage and underlines its significance as a prevalent fish feed contaminant (Pietsch et al., 2020). Pietsch et al. (2020), recommended that future studies should focus on plant-based fish feed storage stability and quality, as aquaculture feeds increasingly contain plant components. The current experiment maintained the proper storage environments, however, it was found that feed quality reduced at normal room temperature near the end of the two-month storage period. In order to extend the shelf life of fish feed, carefully controlled storage conditions must be maintained. According to the study, the feed should not be stored at room temperature for more than two months. This type of fish feed must be used within two months of production.

4. CONCLUSION

Shelf life of composite bio-silage incorporated fish feed was 60 days. Furthermore, storage conditions such as temperature, relative humidity, and hygienic storage facilities possessed an important impact to ensured higher fish feed storage quality. The composite silage-added fish feed should not be stored for more than two months, either in the production center or at the farmer's store.

5. ACKNOWLEDGMENT

The authors thankfully acknowledge the ICAR-Central Institute of Fisheries Education for student's fellowship and National Agricultural Higher Education Project (NAHEP) for the financial and infrastructure support to carry out the research work.

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