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Evaluations of the Sensory Quality Indices and Freshness Assessment of Nile Tilapia *Oreochromis niloticus* Fillets Fed Recycled Food Waste Materials

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Abstract: This study was designed to evaluate the sensory quality indices, freshness assessment and safety of eating Nile tilapia fed recycled food waste materials [food industry waste (FIW) and soy sauce waste (SSW)] for 32 weeks using K values, IMP content and microbial viable cell count. Five experimental diets were formulated at 0% and 20%-22% inclusion level of recycled food wastes. The diets were designated as D1: 0% of recycled food waste, D2: 20% inclusion of FIW, D3: 20% inclusion of FIW and SSW, D4: 20% inclusion of FIW and tryptophan, and D5: 22% inclusion of SSW. The result from the body composition shows that D1 had higher carcass protein, while D3 had the highest lipid content and there was no significant difference in the carcass moisture and ash contents among all treatments. The results of microbial viable cell counts showed that no significant differences were observed among the dietary treatments and all the fish fed experimental diets still remained fresh four days after refrigerated storage at 5 °C. In addition, no significant differences were noted among the K value concentrations of all the fish fed the experimental diets. From the result of this study, we concluded that using 20% inclusion of recycled food waste materials (FIW and SSW) in the diet of tilapia had no negative effect on the flesh of the fish; hence, recycled food waste could be a good alternative ingredient to aquaculture.

Key words: Recycled food waste, food industry waste, soy sauce waste, K value, Nile tilapia, *Oreochromis niloticus*, sensory quality.

1. Introduction

The aquaculture production of tilapia worldwide doubled between the years 1986 and 1992, and was only lower than that for Chinese carps total farm productions [1], such that the World Aquaculture Society labeled tilapia as the fish of the decade [2]. Tilapia is regarded as an alternative to catfish and flounder [3] because of its mild, non-fishy taste and white flesh, which account for its relatively high demand. To meet the increasing demand and emerging competitive market, many fish farmers have expanded their annual production. Many efforts to increase production of these cichlids have been based on control of reproduction [4, 5], hybridization [6] and improved nutrition and feeding [7-10].

Freshness of perishables is the most important determining factor for food quality assessment. Although a variety of chemical and physical methods have been used to assess the freshness of fish during storage, the main quality parameters for fresh fish are aroma, flavor, texture and sensory response [11, 12].

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Therefore, for decades, fish production has mainly focused on increasing yield and optimizing product quality in order to enhance consumer's acceptance and boost the price of cultivated organisms [13]. This is because the sensory features of fish are clearly visible to the consumer and are essential for consumer satisfaction [14]. Fish, among other perishables foods, are commonly processed by heating and adding preservatives such as salt, sugar or supplementation with various additives acids and alcohols [12]. However, these food-processing techniques cannot maintain most of the unique characteristics of the food because the techniques often have negative effects on the quality of foods, causing a change in taste, aroma, color and texture of the fish [12]. Studies have shown that endogenous and exogenous factors affect the chemical composition of the whole body of fish [13-16]. Among the exogenous factors are the type and the level of feed ingredients for the fish. Unlike marine species, little research has been performed on the factors, which modify flesh quality and market value of tilapia, to the effect, that the quality of farmed fish has occasionally been reported to be lower than that of wild fish [17]. It is a great source of worry that tilapia fed on recycled food waste materials may acquire off-flavor or other negative fillet characteristics, since research studies have shown for example, that channel catfish grown in ponds acquire off-flavor [18].

Therefore, the main objective of this study was to investigate, among other things, the detectable differences in the physical sensory characteristics of the fillets, the storage life and quality changes in Nile tilapia fed recycled food waste materials.

2. Material and Methods

2.1 Experimental Diets

Based on the nutritional requirements of tilapia, five iso-nitrogenous (40% crude protein) and iso-lipidic (8.5% lipid) diets were formulated [19], containing 20%-22% of different proportions of recycled food waste (Factory industrial waste, FIW and Soy sauce waste, SSW) as shown in Table 1. The experimental diets were designated as D1 (with 0% inclusion of recycled food waste, containing fishmeal only) as the control, D2 (with 20% inclusion of FIW, at 9.5% fishmeal protein being replaced by FIW), D3 (with 20% inclusion of recycled food waste, at 10% FIW and 10% SSW, 11% of fishmeal protein being replaced by FIW and SSW), D4 (with 20% inclusion of FIW and tryptophan, at 13% of fishmeal protein being replaced by FIW and tryptophan) and D5 (with 22% inclusion of SSW, at 14% of fishmeal protein being replaced by SSW). D4 was formulated to evaluate the effect of tryptophan that was very low in the food industry waste when amino acids analysis was carried out, hence the additional inclusion of tryptophan in D4. During the mixing small quantity of distilled water was added to enhance its pelletability. The diets were pelleted using a laboratory pelletizer (AFZ12M, Hiraga-Seikakusho, Kobe, Japan) and dried using a freeze drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan). The pelleted diets were then crumbled using a pestle and mortal. The diet was then screened into two using a 500 μ and 250 μ mesh and stored at 4 °C until use.

2.2 Experimental Culture System and Fish

Newly hatched tilapia larvae were obtained from a pure-bred stock and maintained in the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology. The feeding experiment was conducted using 13-day old tilapia fry, with initial standard length (SL) of 8.0 ± 0.01 mm and wet body weight of 0.01 g at the onset of exogenous feeding (10). Fresh, filtered dechlorinated tap water was supplied to a flow through system, consisting of 10 aquariums of 30-L approximate capacity, at a flow rate of 250-300 mL/min. Water temperature was maintained at 28 ± 0.5 °C using electric heaters placed in each tank. The aquariums were illuminated by overhead fluorescent lights, to maintain a constant photoperiod

Table 1 Composition and proximate analysis of the experimental diets for Nile tilapia (g kg^{-1}).

Diet code	D1	D2	D3	D4	D5
Fishmeal	630	570	560	545	540
Soy sauce waste	0	0	100	0	220
Food industry waste	0	200	100	200	0
α-starch	150	150	150	150	150
Vitamin premix*[1]	20	20	20	20	20
P-free mineral mixture*[2]	30	30	30	30	30
$Ca(H_2PO_4)_2 \cdot H_2O$	30	30	30	30	30
Soybean oil	15	0	0	0	0
L-Tryptophan	0	0	0	15	0
Cellulose	125	0	10	10	10
Moisture (%)	2.69	3.16	3.35	3.51	4.71
Crude protien (% d.b.*[1])	41.13	40.02	40.38	40.79	40.68
Crude lipid (% d.b.*[1])	8.25	8.25	8.20	8.15	8.51
Ash (% d.b.*[1])	15.87	17.08	16.88	16.28	15.51

*[1] Composition (mg/100 g): Thiamin HCl 6, riboflavin 10, pyridoxine HCl 4, cynocobalamin 0.01, ascrobic acid 500, niacin 40, Ca-pantothenate 10, inositol 200, biotin 0.6, folic acid 1.5, p-aminobenzoic acid 5, vitamin K3 5, vitamin A acetate 4000 IU, vitamin D3 4,000 IU,

*[2] Composition (g/100 g): NaCl 5.0, $MgSO_4 \cdot 7H_2O$ 74.5, $FeC_6H_5O_7 \cdot nH_2O$ 12.5; trace element mixture*3 5.0, cellulose 3.0,

*[3] Composition (mg/g): $ZnSO_4 \cdot 7H_2O$ 353, $MnSO_4 \cdot 5H_2O$ 162, $CuSO_4 \cdot 5H_2O$ 31, $AlCl_3 \cdot 6H_2O$ 10, $CoCl_2 \cdot 6H_2O$, KlO_3 3, cellulose 440.

of 12 h light and 12 h dark schedule (8:00-20:00) throughout the study. The aquariums were provided with continuous aeration through an air compressor.

Fish were kept at a density of 60 larvae per aquarium per treatment during the first 12 weeks in 30-L capacity aquariums and experimental feed was manually administered 6 times daily at 30 % of body weight (dry weight basis), at 0900, 1100, 1300, 1500, 1700 and 1900 h. Eight juvenile fish were randomly selected from each treatment and reared separately for 20 weeks in 30-L aquariums, fed twice daily at 1000 and 1500 h. Feeding rates were subsequently adjusted according to the growth rates recorded per aquarium. Fish were denied feed 24 h prior to sampling.

2.3 Preparation of Samples and Storage Condition

Feeding was stopped 48 hours prior to harvesting of the experimental fish. Fish were sacrificed by immersion in crushed ice; the fish were individually weighted using a digital electronic weighing balance (AW 220; Shimadzu Corporation, Kyoto, Japan), whereas total length and standard length were measured using digimatic calipers (CD-20CP; Mitutoyo Corporation, Tokyo, Japan). Each treatment was divided into three lots; one lot contained 4 fish, which were individually placed in nylon-polyethylene bag (35 × 50 cm) and afterward, in 5 °C regulated refrigerator (Fukushima multi-solution) for four days and subsequently used for raw sensory evaluation and variable cell count of the fish meat. The second lot contained 3 fish from each treatment and were appropriately labeled and placed in 20 °C regulated refrigerator (Fujistu Er-F428 ME) and subsequently used for K value and IMP contents analysis. The third lot contained 1 fish from each treatment which were used for biochemical analyses.

2.4 Sensory Test Conditions

Sensory evaluation of whole fish and fillet was carried out on physical sensory indices of the meat of fish fed experimental diets for 32 weeks and stored refrigerated at 5 °C for 4 days. After this time period, a test panel consisting of 15 under graduate and graduate students from fish culture laboratory, Fish

Nutrition laboratory and Laboratory of Safety Management in Food Supply Chain Tokyo university of Marine Science and Technology were used for the sensory evaluation. The panel was trained in fish quality assessment according to methods of Stone and Sidle [20], before the experiment. The panel performed the sensory evaluation without the foreknowledge of the experimental design, whereas the samples were also coded. Only the physical attributes of the raw, whole fish was assessed, the fillet of which were scored according to a modified scheme from Ruiz-Capillas and Moral [21]. A 5-point scoring scale was modified and used to quantitatively evaluate the general appearance, the eye quality, fillet color and the quality of gills of the fish.

2.5 Biochemical Analyses

About 20 g of the initial samples and 20 g of final samples from each aquarium were pooled separately and homogenized, using a mincing machine (ZM200, Retsch Inc., Haan, Germany). The experimental diets and fish body samples were subjected to chemical analysis. Proximate analysis and lipid analysis were done according to the methods detailed by Takeuchi [22] and Folch et al. [23].

2.6 Viable Cell Count Analysis

Two fish, from each treatment inside a nylon bag stored at 5 °C in the refrigerator for four days, were taken out on the fourth day and the fish were analyzed for viable cell count. Blends of 25 g of fish meat (including skin) were collected aseptically and 225 mL of diluent (0.8% NaCl, Physiological saline water) was used to homogenize the sample using a stomacher (Stomacher 400 LAB Blender, Seward, England). After serially diluting the sample solutions, 0.1 mL of each dilution was spread on agar plates and the plates were incubated (Sanyo incubator MIR-153) at 37 °C for 2 days. The numbers of colony forming units (CFU) were then counted and the viable cell count of each sample was calculated.

2.7 K Value and IMP Analysis

Three fish from each tank were killed (as earlier described), tagged and coded. 2-2.5 g of the fresh, raw fish muscle was taken and the rest were kept in a refrigerator (Fujistu Er- F428 ME) at 5 °C. Samples of the muscle kept at 5 °C were taken every day at 10 am for a total of 1 week. About 2-2.5 g of muscle taken from each fish was homogenized with 15 mL of 10% perchloric acid at 0 °C. The homogenate was centrifuged (Hitachi himac CF 15R) at 3,000 *g* for 10 min and the supernatant was made up to 20 mL. An aliquot (1 mL) of supernatant was neutralized with potassium hydroxide. After standing at 0 °C for 10 min, potassium perchlorate was removed by centrifuging at 4 °C. The supernatant was filtered for subsequent HPLC analysis. Separations were achieved on Asahipak GS-320 HQ column (7.6 × 300 mm) with guard column (GS-2G), equilibrated at 25 °C. The mobile phase of 0.02 M $Na_2H_2PO_4$ (disodium dihydrogen orthophosphate, pH 2.9) dissolved in MilliQ purified distilled water (Millepore, Bedford, MA) was used at a flow rate of 1 mL/min. The eluent was monitored at 260 nm. The K-value, a freshness index, was defined as follows [24]: The process of ATP breakdown was used as an indicator of fish freshness [12]. By measuring the concentrations of the six components, a ratio of concentration of the hypoxanthine (Hx) and inosine (HxR) to total concentration (ATP, ADP, AMP, IMP, HxR and Hx) gives a quantity (K-value, %), which increases from 0% towards 100% with time. During the initial storage, reduction in ATP and increase in hypoxanthine by endogenous enzymes allows this measurement to be used for determining freshness Hx in formula (1);

$$\text{K} - \text{Value}\ (\%) = \frac{[\text{HxR}] + [\text{Hx}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{HxR}] + [\text{Hx}]} \times 100 \quad (1)$$

2.8 Statistical Analyses

Data were analyzed using one-way analysis of variance (ANOVA) using Statistica 8.0 (Stat-Soft, Inc.,

USA). Differences between treatments were compared by Tukey's Test. Level of significance was tested at $P < 0.05$.

3. Results

3.1 Sensory Evaluation of the Raw Fish Meat

Figure 1 shows the results of the physical sensory indices of the raw meat of Nile tilapia fed experimental diets for 32 weeks. Changes in the sensory attributes of the raw fish stored refrigerated at 5 °C for 4 days were recorded using descriptions given by the panel of judges. The panel were unable to point out any physical differences among all the fish fed different experimental diets, hence there was no significant ($P > 0.005$) difference between fish fed the control diet and those fed the recycled food waste based diets (containing FIW and/or SSW).

3.2 Chemical Compositions

Whole body proximate compositions of fish, sampled at the beginning and end of the feeding trial is given in Table 2. The highest moisture content was observed in the fish fed D5 and the lowest value in D3; these values were however, not significantly different among all the dietary treatments ($P > 0.05$). Fish fed D1 had the highest protein content, which was significantly different when compared with the other treatments ($P < 0.05$). D3 contained significantly the highest lipid content, than D1 but which did not differ significantly from D2, D4 and D5. There were no significant differences among all the treatments in the ash content.

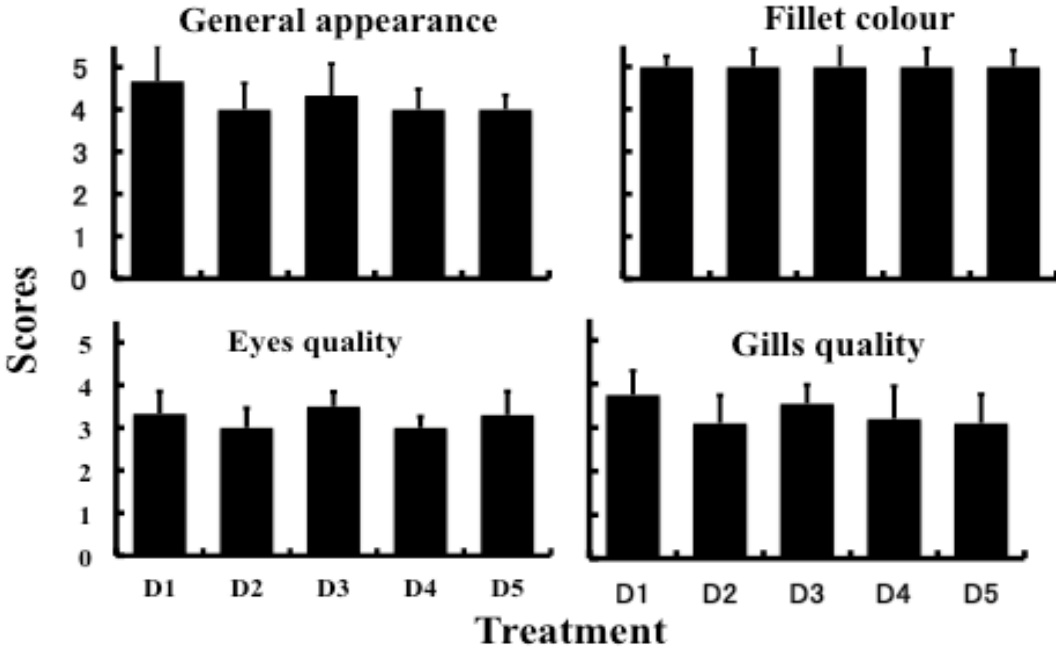

Fig. 1 Physical sensory indices of raw meat of *Oreochromis niloticus* fed experimental diets for 32 weeks.

Points represent mean value of fifteen determinations ± standard deviation ($n = 15$).

Table 2 Body composition (dry basis) of *O. niloticus* fed experimental diet for 32 weeks.

Component (%)	Initial	Final				
		D1	D2	D3	D4	D5
Moisture	75.4	74.1 ± 1.2	74.3 ± 1.3	73.5 ± 0.5	74.2 ± 1.2	74.5 ± 1.4
Protein	56.7	60.2 ± 1.1^{a}	56.4 ± 1.1^{b}	58.1 ± 1.1^{b}	57.0 ± 1.2^{b}	57.2 ± 1.1^{b}
Lipid	20.3	21.2 ± 0.9^{b}	22.6 ± 0.7^{ab}	23.4 ± 0.6^{a}	22.9 ± 0.7^{ab}	22.7 ± 0.8^{ab}
Ash	12.8	12.7 ± 0.2	13.2 ± 0.3	132. ± 0.1	13.2 ± 0.3	12.9 ± 0.2

Values in the same row with different superscript letters are significantly different. ($P < 0.05$) from each other ($n = 3$).

3.3 Viable Cell Count

Fig. 2 shows the result of the viable cell count of Nile tilapia fed experimental diets for 32 weeks. The viable cell count was generally good, as indicated by the low number of bacterial colony growth (10^3 CFU/g) among all the fish fed the experimental diets. However, fish fed D4 diet recorded the highest viable cells count, while those fed D2 and D5 diets were noted with the lowest viable cell count, even though these values did not significantly differ among the dietary treatments.

3.4 K Value and IMP Content

Figs. 3 and 4 show the K value and IMP concentration of Nile tilapia fed experimental diets for 32 weeks. From the K value result experimental fish fed D5 had the highest K value (%) index while those fed D2 had the lowest K value index however there was no significant differences among all the treatments. The result of IMP concentration shows that experimental fish fed D2 had the highest concentration at the end of the 7 days analysis while fish fed D5 had the lowest IMP concentration at the

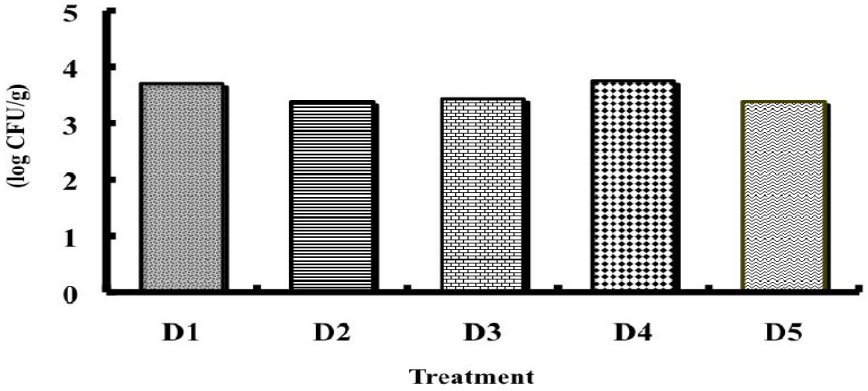

Fig. 2 Viable cell count of *O. niloticus* fed experimental diets for 32 weeks.
Points represent mean value of six determinations ± standard deviation ($n = 2 \times 3$).

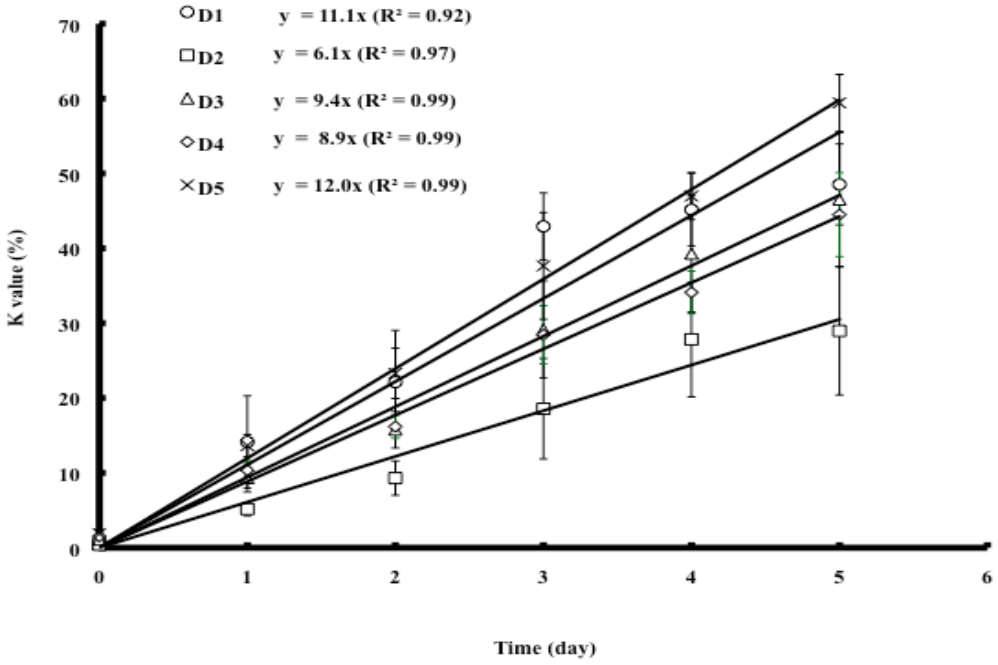

Fig. 3 K value of *Oreochromis niloticus* fed experimental diets for 32 weeks (%).
Points represent mean value of six determinations ± standard deviation ($n = 2 \times 3$).

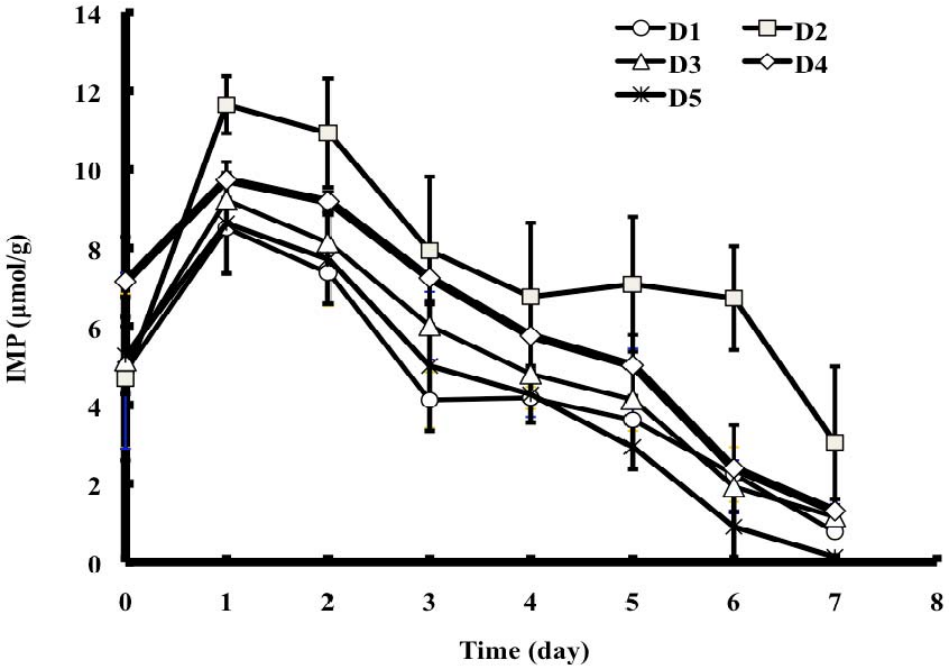

Fig. 4 IMP content of *Oreochromis niloticus* fed experimental diets for 32 weeks (mg/g).
Points represent mean value of six determinations ± standard deviation ($n = 2 \times 3$).

end of day 7 analyses. D2 was significantly higher and different ($P < 0.005$) from D5, however was not significantly different from D1, D3 and D4.

4. Discussions

From the result, it can be observed that the fish fed diets containing recycled food waste materials were still fresh, bright, of firm texture and with no off-odor, after refrigerated storage for 4 days at 5 °C. This observation agrees with that of LIU et al. [25], where tilapia was reported to still maintain good quality within 9 days of storage. Interestingly, the inclusion of the recycled food waste materials did not affect the morphological characteristics of the fish fed the experimental diets.

The higher carcass protein recorded in D1 may be due to the ability of easily digestible and utilizable proteins in fishmeal, the principal source of proteins. Anderson et al., Wee and Wang [26, 27] reported that the replacement of fishmeal by plant protein sources in diets for fish may likely lead to a decline in the final fish carcass protein in fish fed diets with high plant protein level. Muscle lipid and protein may be the primary factors determining carcass quality of raw fish [28], whereas according to Shearer [29], Rasmussen [13] and Grigorakis et al. [30], high lipid content in the flesh of fish is closely related to its flavor and the time taken to spoilage. Higher lipid content produce stronger fish flavor and is likely quicker to spoil and vice versa [13, 30]. Salmonids are considered to be oily fish with 12%-16% lipid in their flesh and consequently have stronger fish flavors [15]. Tilapia in contrast, contain relatively lower lipid content of about 8% [31], which could even decline to below 3% [32]. In the present study, the lipid composition ranged between 5%-6% and did not tend to have any negative effect on the fillet quality of the fish, judging by the results of the sensory assessment indices.

The viable cell count of all the fish fed experimental diets did not exceed 3-log CFU/g, which is in the range expected for many fresh water fish, according to Huss [33]. Generally when the viable cell count of fresh meat is recorded at between 10^2-10^4 cells/g or lower, they are considered to be fresh, whereas fish starts to rotten when the viable cell count is between 10^6-10^7 cell/g and fish meat is considered

completely rotten when the viable cell count exceeds 10^8. Since the number of viable cells count gives an indicates of the degree of freshness from the microbiological point of view, all the fish fed experimental diets still remained fresh four days after refrigerated storage at 5 °C. This result is similar to the initial microbial count recorded by Liu et al. [25], at the onset of their experiment. The result from the current study shows that recycled food waste materials (FIW and SSW) fed to the experimental fish does not make the body of the fish easily susceptible to bacterial growth under storage conditions.

The K value index, calculated from ATP degradation products, has been reported as a good indicator of freshness in various species of fish [34-36]. Many factors affect the K value concentration of fish, including fish species [37] type of muscle [38], stress of fish during capture [39] and storage temperature [40]. There was good time-independent relationships between the K value concentrations in fish fed the experimental diets, with storage time (as shown in Fig. 3). The K value concentration in fish fed the different experimental diets and stored at 20 °C increased linearly and were varied among the treatments (D1: $r^2 = 0.98$; D2: $r^2 = 0.97$; D3: $r^2 = 0.99$; D4: $r^2 = 0.99$ and D5: $r^2 = 0.99$ over the storage time). At day 0 of storage, the K value concentration was negligible among the fish from all treatments, showing that the fish were still very fresh. Subsequently, the K value began to gradually increase over the storage period. Finally during the storage period, the K value of fish from different treatments ranged between 29.0% and 59.4%. From the result, fish from D5 treatment recorded the highest K value concentration, while D2 had the lowest K value. However, no significant differences were noted among the K value concentrations of all the fish fed the experimental diets. The gradual increase in the concentration of the K value of the fish fed all experimental diets in the current experiment followed a linear pattern, although K value concentration varies among fish species [41-44].

The result of the K value concentration also showed that the fish fed the experimental diets are fresh from day 0 to day 2 and that the fillets were of good quality when their K values was below 20%, since it is generally regarded that fish with evaluated K-value of less than 20% could be eaten raw (Sashimi). At higher K-values than 20%, but less than 65% could be cooked before being eaten, but with a K-value of more than 65%, fish is considered to be at rejection point [45]. Therefore, judging by the K value result, fish fed recycled food waste materials and stored refrigerated at 5 °C were still fresh and could be eaten raw from day zero to day 3 of the storage period, and could be and eaten when stored for a week at 5 °C. The increase in the K value may likely be the result of the gradual reduction of IMP content in the fish flesh. This speculation is in line with those of Özogul et al. [46] and Guizani et al. [40] who showed that the K value was strongly affected by the gradual degradation of IMP. Fig. 4 shows the result of the IMP concentration of tilapia fed experimental diets for 32 weeks. The IMP concentration of all the experimental fish was lower at day zero compared to day 1 and 2 of the storage period. The IMP then gradually increased with increase in the storage period, such that at the end of the storage period, fish fed D2 had significantly the highest IMP concentration ($P < 0.005$) than those fed D5 but was however, not significantly different from D1, D3 and D4. IMP is a relative compound produced as a result of ATP degradation. ATP degradation is associated with stress and struggle either during capture or death of the fish [47]. This may likely be the reason for the lower IMP at day 0. The fish were captured stresses free and killed by submerging them inside crushed ice; hence struggling of the experimental fish was reduced to barest minimal. Jones [48] and Hiltz et al. [49] reported that ATP is usually converted to IMP on the first day of the fish death. This observation agrees with the results of the current study, since the first samples were taken

immediately the fish died and as such the increase in IMP at days 1 and 2.

The IMP plays an important role in the umami-flavour, especially for L-glutamate in meaty foods [50-52], hence it contributes to the pleasant flesh flavour of the meat. Kuda et al. [53] stated that higher concentration of IMP in fish meat may lead to higher umami taste. The significant differences between D2 and D5 may likely be the result of ingredients make up of the diets fed [54]. Although soy sauce usually contains high IMP content, the low concentration of IMP in the fish fed SSW could be due to the production process of the ingredient. During SSW production, the liquid soy sauce is pressed out, leaving the solid waste and since IMP is very soluble, it is likely that it was completely pressed out. Most of the foods eaten in Japan are usually seasoned with soy sauce, it is thus likely that IMP may be absorbed by FIW before being recycled. This may therefore, account for fish fed on FIW to have more IMP in the fillet compared to those fed SSW.

5. Conclusion

There was no feed rejection during the experiment and all the experimental fish responded very well in terms of growth and feed utilization to the experimental diets. Judging from the data obtained from the sensory assessment indices, proximate composition, the viable cell count, the K value and the IMP concentration analysis, we could conclude that using 20% inclusion of recycled food waste materials (FIW and SSW) in the diet of tilapia had no negative effect on the flesh of the fish hence, recycled food waste could be a good source of alternative ingredients in aquaculture. Furthermore, there is need for further evaluation of the sensory indices of Nile tilapia fed at higher inclusion levels of these recycled food waste materials.

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