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Quality Evaluation of Mono-Sex Nile Tilapia (*Oreochromis niloticus*) Fed Supplemented Diets with Some Natural Pigments sources

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ABSTRACT

The quality evaluation of Mono-sex Nile tilapia (*Oreochromis niloticus*) fed diets containing 2% different red natural pigments sources (shrimp by-product meal (SH), red pepper meal (RP), and tomato meal (TM)) and stored at 5±0.5ºC was the main aim of this study. Fish samples were fed a diet meal (25% crude protein, CP), two times per day for 90 days, in a concrete tanks (5×2×1 m), at a density of 10 fish/tank. Concerning growth performance of studied trials; the findings indicated that, as compared to the control group, fish fed diets contained various natural red pigments had considerably higher biomass, weight gain, and specific growth rate, however, the differences were statistically insignificant (*P>0.05*). The chemical composition of fish flesh showed that the best treatment was in particular fish fed RP-diet compared to Ctr-diet. With regard to effect of red pigments on quality indices of refrigerated fish treatments, the data showed that TBARS value of Ctr-sample recorded the highest value (2.38±0.12 mg MDA/kg sample) while fish fed SH-diet recorded 0.35mg MDA/kg sample at 0 day and augmented to 0.7 mg MDA/kg after 4th day of refrigerated storage. The highest acceptable scores of sensory parameters were given for fish fed RP-diet, and the lowest was for Ctr-fish. In conclusion, the RP or SH as a source of natural pigments promoted the development and feed utilization of *O. niloticus* diet, which reflected on quality improvement of fish skin and flesh colors beside delaying in lipid oxidation.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) ranks as one of the finest fish for farming due to its incredible rate of growth, the capacity to sustain growth during elevated stocking densities, to resistance aquatic illness, staggering rates of food conversion, and motivation to ingest artificial feed (**Ng and Romano, 2013)**. On the other side, fish pigmentation constitutes a single of the key quality features which affects the acceptability of consumers, and its level affects marketing value and may also serve as an obvious indication of the fish's quality and health (**Chien and Jeng, 1992**). Aquatic organisms can transform these pigments into other substances, but they cannot synthesis them. Higher plants, algae, and specific microorganisms carry out this function (**Goodwin, 1984; Rekha *et al*., 2022**). Thus, all animals have to count on organisms that produce pigments to fitful their needs (**Gupta *et al*., 2007**; **Torrissen, 1989**). In addition, natural and artificial colors are added to fish diets to improve the fish skin and flesh colors. Nevertheless, natural pigments may be employed as cheaper substitutes for synthetic pigments, which can cost up to 15% of the overall cost of feed (**Latscha, 1989**; **Buttle *et al*., 2001**).

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Natural astaxanthin is commercially produced using crustacean byproducts such as crab meal, prawn meal, crayfish meal, Antarctic krill, etc. These serve as excellent suppliers of the carotenoid astaxanthin and serve a purpose in aqua feed (**Gupta *et al*., 2007; Nakano and Wiegertjes, 2020; Shahidi and Brown, 1998**). Red peppers (*Capsicum annum*), are aboundant plant supplier of vitamins A and C and powerful antioxidants, also include capsanthin, which strengthens immunity and assists in fighting infectious diseases (**Judan Cruz *et al*., 2021; Mobli and Piraste, 1994**). Capsanthin and capsorubin are the active substances that account for 80% of pepper's red coloring (**Tepić *et al*., 2009**). Tomatoes are an amazing source of nutrients, particularly lycopene along with carotenoids, ascorbic acid, folate, flavonoids, vitamin E, and potassium, that provides nutrients and several kinds of anti-infectious disease substances. Carotene and lycopene are two significant sources of carotenoids, with lycopene constituting roughly between 80 and 90% of the total carotenoids in a tomato fruit (**Shi and Maguer, 2000**). Three coloring substances; canthaxanthin, astaxanthin, and gammarus have been used successfully in the diets of rainbow trout (*Oncorhynchus mykiss*) (**Yanar, 1997**; **Diler *et al*., 2005**), electric yellow cichlids (*Labidochromis caeruleus*) (**Yılmaz and Ergün, 2011**), goldfish (*Carassius auratus*) (**Yeşilayer *et al*., 2011**) and snow trout (*Schizothorax richardsonii*) (**Jha *et al*., 2012**). Furthermore, marigold and beetroot have been effectively utilised with rainbow trout (**Yeşilayer *et al*., 2011**). Additionally, paprika oleoresin (**Scabini *et al*., 2011**; **Yesilayer and Erdem, 2011**) and red pepper (*Capsicum oleoresin)* boosted fish coloring (**Harpaz and Padowicz, 2007**).

Therefore, the primary goal of this investigation was to explore the effect of diets supplemented with 2% of various natural pigments; tomatoes meal (TM), red pepper meal (RP), and shrimp by-product meal (SH) on growth rate performance, body chemical composition, and also quality indices of Mono-sex Nile tilapia (*O. niloticus*) stored under refrigerated temperature (5 ± 0.5°C).

MATERIALS AND METHODS

Natural pigments sources: They were purchased from local market, Alexandria, Egypt, during spring, 2021. Wastes of shrimp **(***Metapenaeopsis Stridulans)*, red pepper (*Capsicum annuum L*.), and tomatoes (*Solanum lycopersicum L*.) were thoroughly cleaned with drinking water, dried in the oven at 50°C for 48 hrs, grinded using an electrical mixer, and then packed in polyethylene bags until using.

Experimental diets: Three experimental diets were developed via the addition of three red natural pigments at level 2% (shrimp by-product meal (SP), tomatoes meal (TM), and red pepper meal (RP)) in comparison to control diet (Ctr) (0%). According to **NRC (1993)**, all experimental diets included 25% crude protein and designed to provide all nutrients required for adult tilapia. **Table 1** displays the components and proximate contents of each trial diet. All finely powdered components in each experimental diet were homogenized for 30 min and formed into pellets using a meat mincer, then dried for 48 hrs at 50°C. Each dried diet packed into labeled airtight plastic bag and stored in a cool and dry place until the feeding trial commenced.

Table 1. Ingredients and proximate composition (% on dry weight basis) of the experimental diets containing 2% natural red pigments for the Nile tilapia (*O. niloticus*)

Ingredient	Composition (g kg^{-1}) of experimental diets			
	Ctr	**Shrimp shells meal (SH)**	**Red pepper meal (RP)**	**Tomatos meal (TM)**
Fish meal (65%)	70	70	70	70
Soybean meal	250	250	250	250
Corn gluten	80	80	80	80
Yellow corn	100	80	80	80
Shrimp by-product meal (SH)	0	20	0	0
Red pepper meal (RP)	0	0	20	0
Tomatoes meal (TM)	0	0	0	20
Wheat bran	150	150	150	150
Rice brine	300	300	300	300
Fish oil	20	20	20	20
Premix[1]	30	30	30	30
Total	1000	1000	1000	1000
	Chemical composition (%)			
Dry matter (DM)	91.4	91.7	91.2	91.1
Crud protein (CP)	25.3	25.4	25.1	25.4
Ether extract (Lipid)	10.4	10.2	10.6	10.4
Crude fibre	5.5	5.4	5.3	5.8
Ash	8.1	8.7	8.4	8.2
Nitrogen free extract (carbohydrate)	50.7	50.3	50.6	50.2
G.E.(kcal/100g DM)[2]	423.95	420.88	424.49	422.35

[1]Premix composition: each 1 kg contains Vit. A (400000 i.u.), Vit. D3 (100000 i.u.), Vit. E (230 mg) Vit. K3 (165 mg) Vit. B1 (300 mg), Vit. B2 (80 mg), Vit. B6 (200 mg), Vit. B12 (1 mg), Vit. C (650 mg), Niacin (1000 mg), Methionine (3000 mg), Choline chloride (10000 mg), Folic acid (100 mg), Biotin (2 mg), Pantothenic acid (220 mg), Magnesium sulphate (1000 mg), Copper sulphate (1000 mg), Iron sulphate (330 mg), Zinc sulphate (600 mg), Cobalt sulphate (100 mg), Calcium carbonate (up to 1000 g); [2]Gross energy (GE)

Experimental design: Just 120 adult Mono-sex Nile tilapia (Oreochromis niloticus) having a typical body weight of 100.8 ± 5.5g were obtained in May 2021 from a commercial fish pond, Kafr El-Sheikh Governorate, Egypt. Fish were acclimated in concrete tanks (5×10×1.2 m), fed diet that included 25% crude protein for two weeks. Twelve concrete tanks with ten fish each were selected at random to represent four treatments, each treatment was three replicated. Each tank supplied with underground freshwater and the experimental tank's aeration using a 0.5 hp air blower (Vortex MODEL; XGB-370). The water flow-through system with a daily water exchange of 20% was employed. For 12 weeks, fish received experimental diets till satiation (3-5 g) two times per day at 09.00 and 14.00 h. The Fish Nutrition Lab at the Baltim Research Station for Aquatic Resources of Egypt's National Institute of Oceanography and Fisheries (NIOF) was where the experimental investigation was conducted. Fish samples were harvested at the conclusion of feeding trail, packed whole in polyethylene bags and kept chilled (5 ± 0.5oC) through four days. Manual fish filleting carried out to examine the flesh quality.

Analytical methods

Water quality parameters: Each week, water temperature, dissolved oxygen (Jenway- Ltd., Model 970, dissolved oxygen-meter) and pH (Jenway- Ltd., Model 350, pH-meter) were monitored using standard **APHAAWWA (1998).**

Growth performance and Feed utilization parameters: Parameters of weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated (**Soltan *et al*., 2017)** as follows:

WG (g) = [(FW) – (IW)] (g/fish)

SGR (% day^{-1}) = 100 × [(FW) - (IW)] / experimental days

FCR = total feed intake (g) / WG (g)

PER = (WG /total protein intake (g))

Where; initial and final body weights (g) are denoted by IW and FW, respectively.

Proximate composition: The components of moisture, crude protein, crude lipid and ash of experimental fish diets and fish flesh have been defined using established procedures (**AOAC, 2007**). According to the procedure of **ISO (2000)**, the intermediate filtration method was followed to calculate the crude fibre content. By removing the total percentage of protein, fat, and ash in the dry matter from 100%, the carbohydrate or nitrogen free extract content was computed. Gross energy (G.E.) was computed using the following protein, fat and nitrogen-free extract values; 5.64, 9.44, and 4.11 kcal/100g; respectively **(NRC, 1993).**

pH value: The pH of tilapia flesh samples (**Wiklund *et al*., 2008**) was evaluated using an Orion Research digital Lonalyzer 1501 pH-meter.

Thiobarbituric acid reactive substances (TBARS) value: According to **Shi *et al*. (2014)**, the distillation method was used to determine the TBARS value. As shown below, TBARS were computed as mg *Malonaldehyde* (MDA) per kg *sample*:

TBARS (mg MDA/ kg *sample*) = ($OD_{at\ 538\ nm}$ of sample – blank) × 7.8

Total volatile bases nitrogen (TVB-N): The distillation method provided by **Hu *et al*. (2013)** was followed to ascertain the TVB-N content. The data were represented as mg TVB-N/100 g sample.

Sensory evaluation

Organoleptic attributes of whole raw tilapia and cooked skinless fillets after steaming in a covered pan for 10 min, were performed at the completion of the experiment. Ten trained panelists were chosen from the staff members of fish processing and technology laboratory and fish nutrition laboratory, (NIOF), Alexandria, Egypt to evaluate each treatment using the scale (0-10) described by **Kirk and Sawyer (1991)**.

Statistical analysis

The acquired findings were subjected to one-way ANOVA analysis to examine the influence of treatment inclusion on fish growth performance and biochemical composition. Also, tow-way analysis was performed to assess the impact of treatment addition on fish refrigerated storage. The results were analysed using the SPSS program, Version 16 (**SPSS, 1997**). Mean differences were also examined at the *P<0.05* level using Duncan's multiple range tests. The data were presented as Mean ± SD.

RESULTS AND DISCUSSION

Water quality parameters

Water temperature, dissolved oxygen level, and pH value were all kept constant throughout the experiment at 23.0 ± 3.0°C, 6.8 ± 3.0 mgL^{-1}, and 7.8 ± 0.2; respectively. These values were all within the acceptable limits for tilapia culture.

Growth performance and Feed utilization parameters

Table 2 displays the impact of different red natural pigments sources on growth performance parameters and feed-utilization. The changes in final weight (FW), weight gain (WG), and specific growth rate (SGR) among the control sample (Ctr) and the rest-treated samples (SH, RP, and TM) were recorded but no statistically significant difference (P>*0.05*). The results gathered demonstrated that adding 2% natural pigments sources to the diets had a negligible impact on growth performance. However, fish fed at RP had greater FW (261.67 g), WG (130.83 g), and SGR (0.77% day^{-1}) than other fish treatments.

Table 2. Growth performances and feed utilization indices of monosex Nile tilapia (*O. niloticus*) as affected by different natural pigments at the end of feeding trail for 90 days.

Item	Ctr	SH	RP	TM
Initial wt.	130.33±2.74	130.0±0.76	130.83±2.20	131.67±2.74
Final wt.	250.83±7.18	251.00±0.25	261.67±1.93	248.17±10.15
Weight gain	120.50±5.29	121.00±2.92	130.83±2.21	116.50±7.00
SGR	0.72±0.01	0.73±0.01	0.77±0.03	0.72±0.03
Feed intake	223.67±4.06	220.33±6.35	214.67±4.81	211.67±2.72
FCR	1.76±0.06	1.83±0.08	1.64±0.08	1.94±0.15
PER	2.25±0.05	2.17±0.11	2.41±0.01	2.06±0.15

SGR: Specific growth rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio; SH: shrimp by-product meal; RP: red pepper meal and TM: tomatoes meal.

On the other hand, the feed utilization parameters showed that RP-treatment had the lowest (best) FCR (1.64), followed by Ctr (1.76), while TM-treatment had the highest FCR (1.94). Additionally, the RP dietary group had the highest PER (2.41), whereas TM-treatment had the lowest value (2.06).

The findings in this investigation were comparable to **Yilmaz *et al*. (2013)'** study on the impact of adding paprika, astaxanthin, and capsicum to the diets of tilapia (*Oreochromis mossambicus*) where the paprika groups only showed significantly better growth performance, recording the highest FW, WG, SGR, and FCR. Also, **Wassef *et al*. (2010)** found that gilthead sea-bream fed red bell-pepper meal as an all-natural dietary source compared with control showed no significant variations (P>*0.05*) in the FW and FCR. High dose of *C. annum* and its extracts caused a bad impact on rainbow trout and fresh-water sprat (*Zacco platypus*) development (**Ingle de la Mora *et al*., 2006; Lee, *et al*., 2010**). The growth performance may be affected by anti-nutritional substances like strong Pungency (**Lee *et al*., 2010**), Tannin (**Esayas *et al*., 2011)**, or Saponins (**Rao and Gurfinkel, 2000)** beside abiotic factors like salinity and temperature (**Leng and Li, 2006**).

Biochemical properties

The chemical profile (moisture, crude protein, crude lipid, and ash) of fish flesh before starting feeding trails were 83.83±0.26, 75.45±0.36, 16.34±0.68, and 8.10±0.43; respectively. By the completion of the feeding trail, fish samples fed diets with natural pigments revealed significant variations (P<0.05) in fish flesh chemical composition in comparing with Ctr group

(**Table 3**). Fish flesh in RP group had significantly (*P<0.05*) the lowest protein content (72.17±0.41) and the highest lipid and ash contents (20.35±0.20 and 7.39±0.69, respectively) in comparing with Ctr-group and other groups. The chemical composition of TM fish flesh was close to Ctr-group except in ash content. Feeding on natural pigments from animal and plant sources under this study led to a slight reduction in protein content especially in RP treatment whereas lipid content increased especially in RP and SH treatments.

The chemical composition of tilapia flesh of SH treatment in current study was contradict with **Arous *et al*. (2014)** who stated that crustacean meals are considered an alternative protein sources in diets of several fish species. Also, they found that squilla and crayfish meals have positive impact on crude protein percentage in fish composition. In the current study, marketing size tilapia had superior nutritious components when contrasted with the outcomes of **Valente *et al*. (2016)**, who found that Nile tilapia (*O. niloticus*) at commercial size (300g) had protein and fat contents ranging from 52.24 to 54.43% and 19.83 to 25.75%, respectively.

Table 3. Biochemical composition of different fish flesh treatments pre-fed diets containing 2% different natural pigments sources.

Constituent (%)	Treatments				
	Initial	Ctr	SH	**RP**	**TM**
Moisture	83.83±0.26	81.18±0.08[a]	80.23±0.06[b]	79.65±0.20[c]	81.17±0.02[a]
			on dry weight basis		
Crude protein	75.45±0.36	74.41±0.78[a]	73.25±0.36[a]	72.17±0.41[b]	73.90±0.24[a]
Crude lipid	16.34±0.68	18.81±0.08[c]	19.77±0.11[b]	20.35±0.20[a]	18.83±0.03[c]
Ash	8.10±0.43	6.68±0.13[c]	6.9±0.36[b]	7.39±0.69[a]	7.22±0.66[a]

SH: shrimp by-product meal; RP: red pepper meal and TM: tomatoes meal.
Means in the same row with different superscript letters are significantly different at (*P < 0.05*).

Table 4 displays the changes in the TBARS values of tilapia flesh resulted from feeding trail with diets containing red natural pigments during refrigeration at 5 ± 0.5 ºC for 4 days. The TBARS values as indicator of secondary lipid oxidation of SH, RP and TM samples were low and increased slightly during the refrigerated storage but still <2 *mg* MDA/*Kg* sample. The TBARS results of SH in this investigation showed 0.35 *mg* MDA/ *kg* sample at day 0 of storage and steadily increased during extended periods of refrigeration, reaching a peak of 0.7 *mg* MDA/*kg* sample on the fourth day. Additionally, it was found that TBARS in the Ctr sample had a greater value on the fourth day (2.38 *mg* MDA/ *kg*) than in the other treatments. All fish flesh samples not exceeded 4.5 mg MDA/kg according to **EOS (2005)** as indicator of fat rancidity. The threshold range of TBARS was 1–2 *mg* MDA /*kg* sample according to **Lakshmanan (2000).** The current results are indicated that pigments deposited in SH, RP and TM tilapia flesh samples are powerful antioxidants where delaying in lipid oxidation led to Keep flesh quality during refrigerated storage period. These results were in line with those of **Arous *et al*. (2014)** who emphasis the role of natural carotenoid supplies in inhibiting the oxidation of lipids in tilapia during frozen storage and keep TBARS values less than 1.

Table 4. Effect of refrigerated storage at 5 ± 0.5ºC on TBARS value of raw whole Nile tilapia (*O. niloticus*) fed diets containing natural pigments for 90 days.

storage time (days)	TBARS value (mg MDA/kg)			
	Ctr	SH	RP	TM
0	0.28±0.03[Bc]	0.37±0.01[ABc]	0.35±0.04[ABbc]	0.48±0.17[Ab]
2	0.80±0.01[Ab]	0.48±0.04[BCb]	0.45±0.05[BCb]	0.55±0.04[Bb]
4	2.38±0.12[Aa]	0.70±0.04[Da]	0.94±0.05[Ca]	1.26±0.03[Ba]

SH: shrimp by-product meal; RP: red pepper meal and TM: tomatoes meal. Means in the same row with different superscript letters A-C and mean in the same column having different letters a-c are significantly different at (*P < 0.05*).

Table 5 indicates the influence of chilled storage times at 5 ± 0.5 ºC on the TVB-N value of tilapia flesh samples fed diets containing natural pigments sources. The TVB-N levels of RP and SH treatments increased significantly (P<*0.05*) slightly less than those of TM- and Ctr-groups that reached to 22.68±1.07 and 25.62±0.54 mg/ 100 g, respectively at the completion of refrigerated storage time. However, the TVB-N of Ctr- and TM-treatments recorded 13.55±0.93 and 13.93±0.53 mg/100g, respectively at zero time yet they were 33.66±1.07 and 32.72±0.86; respectively, more than acceptable limit (<30 mg/100 g) by the conclusion of the fourth day of refrigeration, according to **EOS (2005)** and **Directive, E.C.** (Commission of the European Community) **(1995)**. From the current study, pigments deposited in SH- and RP-flesh samples have good effects on delaying microbial and enzymatic activity during refrigerated storage time while TM- samples did not appeared that. The level of TVB-N between the current study and that reported in others, such **Khalafalla *et al*. (2015)**, may differ depending on the species, catch season, age, area, sex of the fish and feeding ingredients.

Table 6 exhibits the pH changes of raw whole tilapia fed diets containing natural pigments during refrigerated period at 5 ± 0.5 ºC. At zero time, pH values of Ctr and other treatment tilapia samples were exceeded 6.5 as the maximum acceptable limits that recommended by **EOS (2005)**. The initial higher pH values identified in this study could be related to the species, nutrition and season, as well as the degree of stress experienced throughout harvesting and kind of muscle. At the completion of fourth day of refrigeration period, the pH values of all flesh treatments were considerably higher (P<*0.05*) especially Ctr- and TM-treatments which reached to 7.25±0.01 and 7.22±0.03, respectively. Increasing pH levels during refrigeration of flesh samples might be attributable to a rise in volatile bases like ammonia generated through microbial or enzymes activity (**Li *et al*., 2012**). Despite pH values that above the permissible range, the samples were deemed acceptable by sensory examination. These findings are similar with **Özyurt *et al*. (2009)** who reported that pH levels exceeding 7.1 indicate the breakdown in fish. **Newton and Gell (1981)** revealed that the pH of fish fluctuates between 5.8 through 7.2 based on the level of struggle at the time of harvesting.

Table 5. Effect of refrigerated storage at 5 ± 0.5ºC on TVB-N content value of raw whole Nile tilapia (*O. niloticus*) fed diets containing natural pigments for 90 days.

Storage time (days)	TVBN (Mg /100 g flesh)			
	Ctr	SH	RP	TM
0	13.55±0.93^{ABc}	12.05±0.40^{BCc}	11.01±0.73^{Cc}	13.93±0.53^{Ac}
2	23.67±0.64^{Ab}	19.79±0.44^{BCb}	19.06±0.78^{Cb}	20.94±0.56^{Bb}
4	33.09±0.66^{Aa}	25.62±0.54^{Ba}	22.68±1.07^{Ca}	32.72±0.86^{Aa}

SH: shrimp by-product meal; RP: red pepper meal and TM: tomatoes meal. Means in the same row with different superscript letters A-C and mean in the same column having different letters a-c are significantly different at (*P* < *0.05*).

Table 6. Effect of refrigerated storage at 5 ± 0.5ºC on pH value of raw whole Nile tilapia (*O. niloticus*) fed diets containing natural pigments for 90 days.

Storage time (days)	PH value			
	Ctr	SH	RP	TM
0	6.69±0.02^{Ab}	6.73±0.03^{Ab}	6.72±0.03^{Ab}	6.57±0.03^{Bb}
2	7.18±0.03^{Aa}	7.06±0.08^{Ba}	7.02±0.03^{Ba}	7.11±0.01^{ABa}
4	7.25±0.01^{Aa}	7.09±0.11^{Ca}	7.13±0.04^{BCa}	7.22±0.03^{ABa}

Ctr: control, SH: shrimp by-product meal, RP: red pepper meal, and TM: tomato meal.
Means in the same row with different superscript letters A-C and mean in the same column having different letters a-c are significantly different at (*P* < *0.05*)

Sensory evaluation

Table 7 and **Fig. 1** describe the sensory changes during refrigerated storage in whole raw fish fed diets containing one of natural pigments (2%) compared to control sample. The sensory related to appearance, clarity of eye, gills color and odor, flexibility of body, flesh consistency, fish odor and overall acceptability of the samples were examined. At day zero, all samples had higher scores. The treatments especially RP-sample had significantly (*P<0.05*) the highest appearance and overall acceptability scores (9.6±0.52 and 9.4±0.52, respectively) while SH-sample had the lowest appearance score (8.8±0.42). The current results indicated that red pigments found in RP (Capsanthin and Capsorubin) and TM (Lycopene) were deposited and accumulated in tilapia skin leading to attractive appearance. In other side, most pigments (free astaxanthin and β-carotene) present in SH-source may be deposited and stored in flesh while mono and diesters of astaxanthin pigments accumulated in fish skin leading to more skin brightness (**Sindhu and Sherief, 2011; Nakano and Wiegertjes, 2020; Šimat *et al*., 2022**). According to **Maoka (2015)**, the level of carotenoids in the meal determines how much carotenoids are deposited in fish.

Table 7. Effect of refrigerated storage at 5 ± 0.5ºC on sensory evaluation (mean ± SD) of raw whole Nile tilapia (*O. niloticus)* fed diets containing natural pigments for 90 days.

Sensory characteristics	**Storage time (days)**	Treatments			
		Ctr	**SH**	**RP**	**TM**
Appearance	0	9.0 ± 0.47^{ABa}	8.8 ± 0.42^{Ba}	9.6 ± 0.52^{Aa}	9.2 ± 0.42^{ABa}
	2	7.4 ± 0.52^{Ab}	7.4 ± 0.52^{Ab}	7.9 ± 0.32^{Ab}	7.6 ± 0.52^{Ab}
	4	5.3 ± 0.48^{Bc}	6.1 ± 0.32^{Ac}	6.4 ± 0.52^{Ac}	6.0 ± 0.74^{Ac}
Eye	0	8.5 ± 0.53^{Ba}	8.3 ± 0.48^{BCa}	9.2 ± 0.52^{Aa}	8.5 ± 0.48^{Ba}
	2	7.4 ± 0.69^{ABb}	7.6 ± 0.52^{Aab}	7.5 ± 0.52^{Ab}	7.6 ± 0.52^{Ab}
	4	5.5 ± 0.53^{Bc}	5.8 ± 0.79^{ABc}	6.5 ± 0.52^{Ac}	5.8 ± 0.79^{ABc}
Gills	0	8.4 ± 0.52^{Ba}	8.2 ± 0.42^{BCa}	9.3 ± 0.48^{Aa}	8.4 ± 0.52^{Ba}
	2	7.3 ± 0.82^{Ab}	7.4 ± 0.52^{Ab}	7.7 ± 0.48^{ABb}	7.4 ± 0.52^{Ab}
	4	5.2 ± 0.42^{Cc}	5.9 ± 0.32^{ABc}	6.4 ± 0.52^{Ac}	5.5 ± 0.52^{BCc}
Odour	0	8.4 ± 0.52^{Ba}	8.9 ± 0.57^{ABa}	9.2 ± 0.42^{Aa}	8.8 ± 0.42^{ABa}
	2	7.1 ± 0.74^{ABb}	7.6 ± 0.52^{Ab}	7.7 ± 0.48^{Ab}	7.5 ± 0.53^{Ab}
	4	4.6 ± 0.32^{Cc}	6.2 ± 0.63^{ABc}	6.6 ± 0.52^{Ac}	5.7 ± 0.48^{Bc}
Texture	0	8.5 ± 0.53^{Aa}	8.8 ± 0.42^{Aa}	9.0 ± 0.47^{Aa}	8.9 ± 0.32^{Aa}
	2	7.4 ± 0.7^{Ab}	7.3 ± 0.48^{Ab}	7.8 ± 0.42^{Ab}	7.3 ± 0.682^{Ab}
	4	5.4 ± 0.52^{Bc}	5.9 ± 0.32^{ABc}	6.5 ± 0.53^{Ac}	6.1 ± 0.74^{ABc}
Flesh conditions	0	8.2 ± 0.42^{Aa}	8.5 ± 0.53^{Aa}	8.6 ± 0.52^{Aa}	8.5 ± 0.53^{Aa}
	2	7.2 ± 0.42^{Ab}	7.2 ± 0.63^{Ab}	7.6 ± 0.52^{Ab}	7.4 ± 0.7^{Ab}
	4	5.9 ± 0.32^{Ac}	5.9 ± 0.74^{Ac}	6.2 ± 0.63^{Ac}	5.8 ± 0.42^{Ac}
Overall acceptability	0	8.4 ± 0.52^{Ba}	8.6 ± 0.52^{Ba}	9.4 ± 0.52^{Aa}	9.3 ± 0.48^{Aa}
	2	6.9 ± 0.57^{BCb}	7.1 ± 0.32^{BCb}	7.7 ± 0.48^{Ab}	7.3 ± 0.48^{Bb}
	4	5.1 ± 0.32^{Bc}	6.0 ± 0.47^{Abc}	6.2 ± 0.42^{Ac}	5.7 ± 0.82^{ABc}

Ctr: control, SH: shrimp by-product meal, RP: red pepper meal, and TM: tomato meal.
Means in the same row with different superscript letters A-C and mean in the same column having different letters a-c are significantly different at ($P < 0.05$).

As the storage time increased to 4 days, the sensory scores declined significantly ($P<0.05$) but RP-sample still had the best overall acceptable scores (6.2±0.42) while Ctr-sample had the lowest scores, due to bad appearance (5.3±0.48), rancidity odor (4.6±0.32) and soft flesh (5.9±0.32). Evaluation scores of SH- and TM-samples are close to but less than sensory scores of RP-treatment. The findings from the current study are well in line with several workers (**Abraham-Olukayode and Oramadike, 2015**; **Adoga *et al*., 2010**; **Farag, 2012**; **Goliat *et al*., 2016**; **Kapute *et al*., 2013**; **Liu *et al*., 2010**; **Reshika *et al*., 2013**; **Rodrigues *et al*., 2016**). Natural pigments that found in whole fish treatments under this research showed significant gradual reduction especially in appearance and odor, due to their roles in pigmentation and antioxidant properties compared to control fish. **Aracati *et al*. (2022)** stated that the development of microorganisms during storage are induced an elevation in pH which has a significant impact on fish quality, particularly sensory attributes like color, odor and texture.

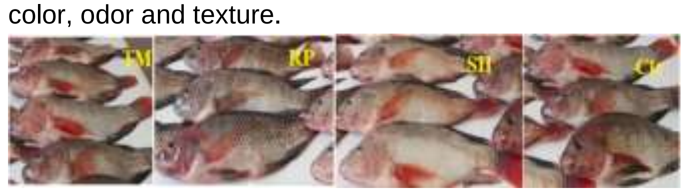

Fig.1. Colour of (marketable size) external features *Oreochromis niloticus* fed control (Ctr) or experimental diets containing 2% natural pigments of (red pepper meal (RP), shrimp by-product meal (SP), tomatoes meal (TM)) for 90 days.

Table 8 illustrates changes in sensory parameters scores of steamed flesh tilapia fed diets with and without natural pigments during refrigerated storage.

There was non-significant variance (P> *0.05*) appeared among Ctr and other treated samples at day 0. SH-flesh sample had got the highest scores for taste, odor, color, texture and overall acceptability followed by RP-sample. By extending the refrigerated storage time, all sensory scores decreased significantly (*P*< *0.05*) especially in Ctr- sample but SH-sample kept the best sensory scores among the rest treated samples after 4 days of storage. These findings related to astaxanthin deposited in SH flesh treatment and its ability to withstand the high temperature during steaming more than Capsanthin and Capsorubin in RP flesh treatment and Lycopene in TM flesh treatment (**Martínez-Delgado *et al*., 2017**).

Table 8. Effect of refrigerated storage at 5 ± 0.5ºC on sensory evaluation of steamed Nile tilapia flesh fed diets containing natural pigments for 90 days.

Sensory characters	Storage time (days)	Treatments			
		Ctr	**SH**	**RP**	**TM**
Odour	0	$8.67±0.50^{Ba}$	$9.22±0.67^{ABa}$	$9.0±0.71^{ABa}$	$8.78±0.67^{Aba}$
	3	$7.11±0.33^{Ab}$	$7.44±0.53^{Ab}$	$7.33±0.50^{Ab}$	$7.25±0.46^{Ab}$
	6	$5.56±0.53^{Cc}$	$7.22±0.44^{Ab}$	$6.89±0.33^{Bc}$	$6.44±0.53^{Bc}$
Taste	0	$8.67±0.71^{Aa}$	$9.11±0.78^{Aa}$	$8.89±0.78^{Aa}$	$8.67±0.71^{Aa}$
	3	$7.44±0.53^{Ab}$	$7.67±0.50^{Ab}$	$7.56±0.53^{Ab}$	$7.33±0.50^{Ab}$
	6	$5.77±0.44^{Cc}$	$7.11±0.33^{Abc}$	$7.0±0.50^{ABbc}$	$6.56±0.53^{Bc}$
Texture	0	$8.67±0.71^{Ba}$	$9.33±0.71^{Aa}$	$9.0±0.71^{ABa}$	$8.78±0.67^{Ba}$
	3	$7.33±0.50^{Ab}$	$7.56±0.53^{Ab}$	$7.22±0.44^{Ab}$	$7.25±0.46^{Ab}$
	6	$5.88±0.33^{Bc}$	$6.67±0.50^{Ac}$	$6.89±0.33^{Ab}$	$6.33±0.50^{ABc}$
Overall acceptability	0	$8.78±0.83^{Aa}$	$9.33±0.87^{Aa}$	$9.11±0.78^{Aa}$	$8.89±0.93^{Aa}$
	3	$6.89±0.33^{Bb}$	$7.56±0.33^{Ab}$	$7.44±0.53^{ABb}$	$7.0±0.00^{ABb}$
	6	$5.33±0.5^{Cc}$	$7.0±0.5^{Ac}$	$6.56±0.53^{ABc}$	$6.22±0.44^{Bb}$

Ctr: control, SH: shrimp by-product meal, RP: red pepper meal, and TM: tomato meal.
Means in the same row with different superscript letters A-C and mean in the same column having different letters a-c are significantly different at (*P* < *0.05*).

CONCLUSION

In accordance with the data acquired, the fish fed diets containing 2% natural pigments sources demonstrated the best growth performance and feed utilization indices. The best PER and maximum energy utilization confirmed this approach. Additionally, the improvement in the flesh quality of Monosex (*O. niloticus*) in particular those fed 2% RP-diet. Also, adding natural pigments sources especially RP and SH to the diets of Monosex (*O. niloticus*) fish improved their body composition, feed utilization, and fish quality indices too.

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