



Replacement of Yellow Maize with Citrus Peels Fermented with *Saccharomyces Cerevisiae* on the Growth Performance and Biochemical Profile of Nile Tilapia

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ABSTRACT: The objective of this study was to investigate the effect of replacement of yellow maize with citrus peels fermented with *Saccharomyces cerevisiae* on the growth performance and biochemical profile of Nile tilapia using appropriate standardized procedures. Results showed that growth performance was the same ($P > 0.05$) in all the treatment groups. Fish weight gain, specific growth rate and feed conversion ratio followed the same trend. Fish weight gain was 10.8, 10.0, 9.86, 9.76 and 8.80g respectively in fish fed diets 1 -5. The peels significantly increased the carcass iron, and with marginal increments in other minerals. The effect of the peels reduced marginally the total cholesterol, malondialdehyde, glucose, triglyceride, low density lipoprotein; while the high-density lipoprotein increased marginally. In conclusion, *Saccharomyces cerevisiae* fermented citrus peels can replace 100% of yellow maize in the diets of Nile tilapia; and improve the nutritional qualities of the food fish.

DOI: <https://dx.doi.org/10.4314/jasem.v29i2.32>

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Cite this Article as: NWANNA, L. C, OBOH, G; AGBEDE, G; ATUFE, V (2025) Replacement of Yellow Maize with Citrus Peels Fermented with *Saccharomyces Cerevisiae* on the Growth Performance and Biochemical Profile of Nile Tilapia. *J. Appl. Sci. Environ. Manage.* 29 (2): 611-617

Dates: Received: 23 December 2024; Revised: 27 January 2025; Accepted: 09 February 2025; Published: 28 February 2025

Keywords: Nile tilapia; yellow maize; citrus peels; fermentation; *Saccharomyces cerevisiae*

Maize meal is the major carbohydrate/energy source in livestock and fish nutrition. But in the recent time, the cost of maize has tripled and this has consequently resulted in high costs of fish production and astronomical increase in the market price of the product. For instance, 1kg of live Catfish that used to cost N600, is now about N3,500, a kilogram of large size Tilapia that used to cost N900 now sells at N4, 500 or more depending on locations and cities. The skyrocketing price of maize is because of competition for use in human, animals and fish nutrition. Hence the need for research into non-conventional alternative cheaper feed ingredient that can replace maize, and without compromising growth, food fish quality and physiological functions. Nwanne *et al.* (2003; 2004) described the beneficial use of under-

utilized forest seed Acha (*Digitaria exilis*) and Tamarind (*Tamarindus indica*) meals as a replacement for maize in the diets of Nile tilapia. Citrus fruit peels, a by-product of the citrus industry, represent about 65% of the total weight of the processed fruits (Nwanne *et al.*, 2014). Therefore, citrus processing produces a considerable quantity of citrus peels that constitute problems since the plant material is usually prone to microbial spoilage, decay and contribute to environmental pollution (Nwanne *et al.* 2014).

The use of citrus peels as poultry / fish feed may be a practical and economic way of disposal of such materials. Dried citrus peels have similar protein level with maize (10.6%), but with more minerals

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and antioxidants, and therefore can effectively replace maize in animal feeds. The objective of this study was to investigate the effect of replacement of yellow maize with citrus peels fermented with *Saccharomyces cerevisiae* on the growth performance and biochemical profile of Nile tilapia.

METHODS AND MATERIALS

Collection and fermentation of citrus peels: Citrus peels (green cover) were collected from the Fruit Market Vendors at the school of Agriculture and Agricultural Technology of the Federal University of Technology Akure. The peels were rinsed in clean water and dried at room temperature before blending them into fine powder. Then 2 kg of the blended citrus peels meal were fermented with yeast, *Saccharomyces cerevisiae* for 2 weeks and stored at room temperature before use. Briefly, Nutrient solution for fermentation was prepared by mixing 58g $Mg_2SO_4 \cdot 7H_2O$, 50g urea, 8.1g KH_3PO_4 , 12.5 g Citric acid in 5 litre of distilled water. These reagents were shaken thoroughly until complete dissolution. Then 2 kg of the blended peels were placed in the fermenter and compacted leaving a well in the middle. Then the prepared nutrient solution was gradually added until it reached a moist consistency. The solution was mixed thoroughly with the peels before the yeast was introduced and thoroughly mixed again. To maintain good fermentation environment, the fermenter was covered immediately with a hydrophilic absorbent cotton wool material, weighing 500g. During fermentation, little water was added to the citrus peels to remain moist, and fermentation lasted for 14 days

Experimental diets and setup: A completely randomized design was used in this study where fish were randomly distributed in tanks and administered five dietary treatments in triplicates. Five isonitrogenous diets (30% crude protein) were formulated. Coarse ingredients such as fishmeal, soyabean, groundnut cake, and yellow maize were finely blended, weighed on a top load balance (Mettler Toledo, Pb 8001, London), and mixed thoroughly with distilled water to obtain a homogenous mass. Then, methionine and lysine and vitamin-mineral premix were weighed accordingly, and thoroughly mixed with other ingredients, before fish oil was added and mixed again with other ingredients. Carboxyl methylcellulose (CMC) was added as a binder and mixed with the other ingredients. The resulting dough was pelleted using a pelleting machine with a 2 mm diameter die opening using a Hobart pelletizer (A-2007 Model, UK) to form pellets and then dried at room temperature for 2 days, and stored at 4°C before use. Proximate

compositions of diets were analyzed in triplicates according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2006).

Feeding trials: A total of 225 Nile tilapia fingerlings were purchased from a reputable hatchery. The fish were acclimated for 14 days in rectangular concrete tanks (1 m x 1 m x 1 m), filled with 300 L de-chlorinated water. The fish were fed a 2 mm commercial diet (Proximate Composition: Crude protein = 30%, Lipid = 12%, Ash = 9.5%, Crude fibre = 1.9%, NFE (Carbohydrates) = 10%, and Moisture = 8.1%) during this period. The fish were fed two times daily between 08:00 - 10:00 and 14.00 -16.00hours, at 5% biomass throughout the acclimation period. At the end of acclimatization, fish of approximately the same size were sorted at 15 fingerlings/tank, weighed (average body weight 2.01 ± 0.03 g), and randomly distributed into 15 glass tanks (70 cm x 50 cm x 50 cm) containing 70 litres of water. The fish were fed using the point feeding method to apparent satiation twice daily between 08:00 - 10:00 and 16:00 – 18:00 hours for 56 days.

Growth indices: All fish from each tank were measured bi-weekly and the values were used in calculating the growth parameters. Before each measurement, fish were anaesthetized using clove oil at 95 mg/L (Adeshina *et al.*, 2016) to reduce handling stress. At the end of the feeding trial, (fish were not fed for the last 24 hours), all fish from each tank were counted and weighed using a Camry digital 10 kg scale. The survival rate, weight gain, feed intake, specific growth rate, feed conversion ratio, and protein efficiency ratio were calculated as described in Nwanna and Helen-Tope-Jegede (2017).

Mineral composition of the experimental fish: Three Nile tilapia were randomly collected from each tank at the end of the experiment and the whole body was analyzed for proximate and mineral composition using standard methods of (AOAC, 2006).

Serum biochemical analyses: At the end of the experiment, 24 hours after the last feeding, 9 randomly selected fish from each treatment group were anaesthetized using clove oil at 95 mg/L before the blood was taken to reduce handling stress. Blood samples were collected from the caudal vein with a 2-ml sterile hypodermic syringe and carefully transferred into 1.5 ml sterile ethylene diamine tetra-acetic acid (EDTA) heparinized tubes and after standing for 12 hours at 4 °C, centrifuged at $850 \times g$ for 15 minutes at 4 °C. The supernatant, i.e., serum, was collected and stored at -8 °C until further use. Serum glucose and cholesterol were evaluated using

enzymatic-colorimetric methods (Naito, 1984) with the aid of specific kits (BT29 4QY: RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom). Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red - violet quinoneimine dye as indicator.

Malondialdehyde (MDA) in the tissue was determined according to the methods of (Varshney and Kale 1990) and expressed as micromolar of malondialdehyde (MDA) g/ tissue.

Triglycerides (TG) concentration was determined according to methods of Tietz (1990), while, the High-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentrations were determined according to methods of Friedewald *et al.*, (1972).

Statistical analyses: All data were expressed in terms of mean \pm standard deviation. Treatment effects on different parameters were subjected to one-way

analysis of variance (ANOVA) as described by Steel and Torrie (1980). Duncan's New Multiple Range Test (Duncan, 1955) was used to separate differences among the means. All statistical analyses were performed with the aid of the computer software SPSS (Statistical Package for Social Science Version 20) and (Microsoft office Excel programme 2010).

The study evaluated the effects of fermenting citrus peels with yeast (*Saccharomyces cerevisiae*) on the chemical composition of the peels and the consequential effects of the fermented peels in replacing maize in the diets of Nile tilapia, *Oreochromis niloticus*. The gross and proximate composition of the experimental diets are presented in table 1. The table shows feed formulation where the fermented peels replaced up to 100% of maize in the diets. The table also posited that fermentation improved marginally the ash, fat, and protein contents of the diets, but significantly improved the fibre content. However, the values of moisture and NFE are similar across the treatments, but declined with increasing levels of the peels in the diets.

Table 1. Gross composition of experimental diets (g/100g DM)

Feed Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0% peels	25% peels	50% peels	75% peels	100% peels
Fish meal (65% CP)	20.4	20.4	20.4	20.4	20.4
Soybean meal (45 CP)	17.0	17.0	17.0	17.0	17.0
Ground nut cake (48% CP)	19.0	19.0	19.0	19.0	19.0
Yellow maize	34.6	25.95	17.3	8.65	0.00
Fermented Citrus peels	0.00	8.65	17.3	25.95	34.6
Fish oil	5.00	5.00	5.00	5.00	5.00
Vitamin	1.80	1.80	1.80	1.80	1.80
Methionine	0.60	0.60	0.60	0.60	0.60
Lysine	0.40	0.40	0.40	0.40	0.40
Calcium	0.20	0.20	0.20	0.20	0.20
Starch	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Proximate composition of experimental diets					
	8.96	8.64	8.16	8.40	
Moisture	$\pm 0.03^a$	$\pm 0.00^a$	$\pm 0.00^a$	$\pm 0.00^a$	8.16 $\pm 0.08^a$
	10.0	10.01	11.0	11.0	
Ash	$\pm 0.00^a$	$\pm 0.02^a$	$\pm 0.00^b$	$\pm 0.00^b$	11.5 $\pm 0.00^b$
	10.2	10.2	10.7	10.9	
Fat	$\pm 0.00^a$	$\pm 0.00^a$	$\pm 0.00^b$	$\pm 0.00^b$	11.9 $\pm 0.00^c$
	10.1	10.2	11.0	11.2	
Fibre	$\pm 0.00^a$	$\pm 0.00^a$	$\pm 0.00^b$	$\pm 0.00^b$	11.7 $\pm 0.00^b$
	30.0	30.1	30.2	30.4	
Protein	$\pm 0.01^a$	$\pm 0.15^a$	$\pm 0.00^a$	$\pm 0.00^a$	30.5 $\pm 0.03^a$
	30.8	30.7	28.9	28.1	
NFE	$\pm 0.06^a$	$\pm 0.09^a$	$\pm 0.04^a$	$\pm 0.06^a$	26.2 $\pm 0.07^a$

There was over 100% increment in the protein (28.5%) content of the fermented peels in comparison to the unfermented peels with 10.5% protein. Similarly, increases were observed in the ash, fibre and lipid contents of the peels. Increasing values of protein due to fermentation of citrus peels has been

reported by Gökhan and Baytal (2023). Also, increasing fat levels in the diets with fermented citrus peels supports the work of Orzuna-Orzuna and Granados-Rivera (2024). Similarly, the increment in ash values obtained from the present study is in agreement with the findings of Abo El-Ward *et*

al. (2019), who recorded similar results from citrus peels.

The growth performance and nutrient utilization indices of the experimental fish is shown in Table 2. From the table, fish fed diet 1(100% maize) and those fed diets 2, 3, 4 and 5 (25%, 50%, 75% and 100% of fermented peels) respectively, have the same ($P>0.05$) final weight, mean weight gain and specific

growth rate (SGR). Meanwhile, fish fed diet containing 100% fermented peels have lower final weight, mean weight gain and SGR than others. This means that fermented citrus peels can replace up to 100% of maize in the diets of Nile tilapia. The protein efficiency ratio improved marginally with increasing levels of the peels in the diets, while food conversion ratio is the same in fish fed all the different diets.

Table 2. Growth performance and nutrient utilization of Nile tilapia fed citrus peels fermented with *Saccharomyces cerevisiae*

Treatments	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0% peels	25% peels	50% peels	75% peels	100% peels
Initial Wt (g)	2.18 ±0.02 ^a	2.15 ±0.02 ^a	2.04 ±0.05 ^a	2.12 ±0.03 ^a	2.00 ±0.03 ^a
Final Wt (g)	12.98 ±0.05 ^a	12.15 ±0.04 ^a	11.9 ±0.15 ^a	11.88 ±0.12 ^a	11.8 ±0.60 ^a
Wt gain (g)	10.8 ±0.02 ^a	10.0 ±0.00 ^a	9.86.7 ±0.00 ^a	9.76 ±0.03 ^a	8.80 ±0.00 ^a
SGR	1.93 ±0.04 ^a	1.79 ±0.03 ^a	1.76 ±0.03 ^a	1.74 ±0.03 ^a	1.58 ±0.02 ^a
FCR	2.00 ±0.03 ^a	2.20 ±0.47 ^a	2.32 ±0.07 ^a	2.56 ±1.03 ^a	2.82 ±2.03 ^a
PER	0.64 ±0.02 ^a	0.65 ±0.03 ^a	0.68 ±0.04 ^a	0.74 ±0.03 ^a	0.76 ±0.03 ^a

In the growth and nutrient utilization indices, similar values obtained from the present study is contrary to the reports of Guimaries *et al.* (2010); Siyal *et al.* (2016); Mohammed *et al.* (2018) and Afaf *et al.* (2019) which stated that citrus peels significantly improved growth performance in Nile tilapia. Mohammed *et al.* (2020) similarly submitted that citrus peels improved growth and health of tilapia. The poorest FCR recorded in the fish fed 100% fermented citrus peels is in disagreement with the report of El-Boushy and Van der Poel (2013) which revealed that orange fruits by-products enhanced the FCR of animals in poultry sector when compared with traditional feed. However, protein efficiency

ratio (PER) increased with increasing levels of the peels in the diets which supports the work of Nwanna *et al.* (2024) which demonstrated that phytogetic aloe vera leaf iron oxide nanoparticles improved the PER in African catfish.

Table 3 shows the mineral contents of the fish fed fermented citrus peels, which indicated good minerals deposition in the fish. The fermented peels did not cause significant changes in the values of Na, K, Ca, Ma, P and Z. However, feeding the fish with the fermented peels improved Fe deposition in the fish significantly in relation to the fish fed 100% of maize.

Table 3. Mineral contents of Nile tilapia fed citrus peels fermented with *S. cerevisiae*

Treatments	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0% peels	25% peels	50% peels	75% peels	100% peels
Na	55.9 ±0.05 ^a	55.8 ±0.04 ^a	56.0±0.11 ^a	55.6 ±0.01 ^a	55.6 ±0.03 ^a
K	99.4x10 ³ ±06 ^a	99.4x10 ³ ±01 ^a	99.5x10 ³ ±02 ^a	99.4x10 ³ ±02 ^a	99.4 ±01 ^a
Fe	0.85 ±0.01 ^a	0.95 ±0.01 ^b	0.96 ±0.00 ^b	0.98 ±0.00 ^b	0.97 ±0.00 ^b
Ca	30.1 ±0.02 ^a	30.2 ±0.0 ^a	30.4 ±0.5 ^a	30.3 ±0.5 ^a	30.5 ±0.5 ^a
Mg	41.0 ±0.72 ^a	41.3 ±0.01 ^a	42.5 ±1.66 ^a	41.0 ±0.02 ^a	41.1 ±0.01 ^a
P	16.0 ±0.02 ^a	16.0 ±0.02 ^a	16.0 ±0.5 ^a	16.3 ±0.05 ^a	16.5 ±0.05 ^a
Zn	0.03 ±0.001 ^a	0.03 ±0.01 ^a	0.03 ±0.00 ^a	0.03 ±0.00 ^a	0.04 ±0.00 ^a

Table 4. Serum biochemical profile of the experimental fish

Treatments	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0% peels	25% peels	50% peels	75% peels	100% peels
MDA	2.56 ±0.88 ^{ab}	2.16 ±0.88 ^a	1.82 ±0.60 ^a	1.25 ±0.42 ^a	1.17 ±0.41 ^a
TC (mg/dl)	383.2 ±0.26 ^a	341.1 ±0.27 ^a	313.8 ±0.45 ^a	280.1 ±2.26 ^a	273.2 ±5.26 ^a
TG (µ/ml)	207.0 ±6.25 ^a	185.3 ±9.25 ^a	170.7 ±9.75 ^a	154.7 ±10.25 ^a	128.0 ±12.25 ^a
HDL (mg/dl)	47. ±12.69 ^a	52.5 ±7.89 ^a	53.0 ±12.64 ^a	55.4 ±4.43 ^a	61.2 ±9.75 ^a
LDL (mg/dl)	284.3 ±4.04 ^a	284.3 ±9.05 ^a	283.3 ±21.05 ^a	282.1 ±20.5 ^a	280.3 ±9.05 ^a

MDA-Malondialdehyde; TC-Total cholesterol; TG- Triglycerides concentration; HDL-High density lipoprotein concentration; LDL-Low density lipoprotein concentration

The improvement in the carcass iron deposition recorded in the present study corroborates the work of Nwanna *et al.* (2014) which showed that unripe plantain peels increased the carcass iron deposition in

African catfish. This increment in iron deposition is also in agreement with the work of Nwanna and Ikuesan (2024) which established that dietary aloe vera leaf iron oxide nanoparticles significantly

improved iron deposition in African catfish, *Clarias gariepinus*. In another development, Nwanna *et al.* (2014), reported that feeding African catfish with unripe plantain peels resulted in increase in carcass ash and consequently increase in the minerals deposition in the fish.

Table 4 above presents the serum biochemical profile of the experimental fish, which shows no significant differences in the values of malondialdehyde (MDA), total cholesterol (TC), total triglycerides (TG) and low-density lipoprotein (LDL) concentrations. However, the values decreased marginally with increasing levels of the fermented peels in the diets. While, the high-density lipoprotein (HDL) concentrations increased marginally with increasing levels of the peels in the diets. Lipid peroxidation and free radicals are abnormal situation and should be controlled. Free radicals react with lipids causing peroxidation, resulting in the release MDA. An increase in lipid peroxides indicate damage to cell membrane, inhibition of several important enzymes, reduced cellular function and death (Pompella, *et al.*, 2003). Cholesterol is measured in animal studies because elevated levels increase the risk of occurrence of heart diseases. Triglycerides are the most common type of fat in animal, and they store excess energy from diets, elevated levels are risk of heart diseases. Malondialdehyde is a marker of lipid peroxidation and oxidative stress. Lower MDA levels suggest a potential antioxidant effect of the diets,

indicating a reduction in oxidative stress in fish subjected to certain dietary treatments. Amer *et al.* (2018) reported that essential oils improved the antioxidant capacities in Nile tilapia, improving catalase activities in the blood and reduction of malondialdehyde (MDA) in the tissues. This observation is in consonance with the findings from the present study which shows reduction in MDA levels with increasing dietary fermented citrus peels. Orzuna-Orzuna and Granados-Rivera (2024) and Ngugi *et al.* (2017) asserted that Nile tilapia fed fermented citrus peels exhibited significant reduction in serum cholesterol, bilirubin, triglycerides, low density lipoprotein, and these findings are in line with the results of the present study. In addition, Aanyu *et al.* (2018) indicated that lipid metabolism was regulated by dietary citrus peels which increased expression of lipoprotein lipase and alkaline phosphatase genes. Lipoprotein lipase is a crucial enzyme responsible for the breaking down of plasma lipids releasing fatty acids which are then transported from the blood circulation to tissues for production of energy (Georgiadi and Kersten, 2012). Improved expression of lipoprotein lipase proposes that citrus peels increased energy availability from dietary lipids and used protein for somatic growth (Li *et al.*, 2014).

Glucose levels in the fish (Figure 1) explains that dietary peels reduced marginally the glucose concentrations with increasing levels of the peels in the diets.

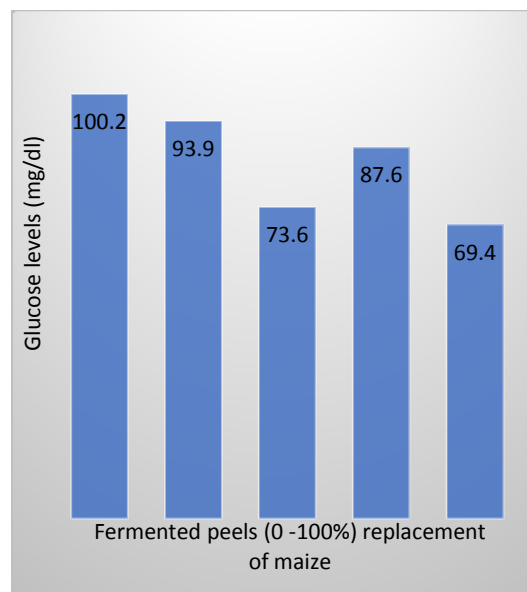


Fig 1. Blood glucose levels of Nile tilapia fed fermented citrus peels

This observation on reduction in glucose levels in the fish is in consonance with the report of Nwanna *et al.* (2014) which stated that unripe plantain peels

lowered the blood glucose and cholesterol in African catfish. This is also supported by the works of Nwanna and Helen Tope-Jegede (2017) and Nwanna

et al. (2024), which explained that dietary *Lactobacillus plantarum* and Thymol essential oil reduced the blood glucose and cholesterol levels in African catfish, *Clarias gariepinus*. In Nwana and Ikuesan (2024), aloe vera leaf oxide nanoparticles also reduced the carcass glucose and cholesterol of *Clarias gariepinus*.

Conclusion: Fermentation improved the nutritional qualities of the citrus peels. Fermented citrus peels can replace maize in the diets of Nile tilapia, without compromising the physiological functions in the fish. Also, fermented peels caused increase in carcass minerals and reduction in glucose levels in the fish. Dietary citrus peels similarly increased the white blood cells in the fish. However, more studies are required to validate the results from the present study.

Acknowledgement: The authors extend their appreciation to Tertiary Education Trust Fund (TETFUND) of Nigeria for funding this research with a Grant Number: (TETF/ES/DR & D-CE/NRF 2021/CC/EWC/00053/VOL.1)

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability: Data are available upon request from the first author or corresponding author or any of the other authors

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