FTIR-Spectrophotometry of Differently Processed Cassava Roots and Effects of their Formulated Diets on the Weight Gains of *Oreochromis niloticus*

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Abstract

This study compared the Fourier Transform Infrared (FTIR) Spectrophotometry of differently processed cassava roots for partial replacement of maize and the effects on the percentage weight gain of Oreochromis niloticus fed on their composed diets. The processed cassava meals were prepared by subjecting peeled dewatered mashed cassava tubers to sun-drying and gelatinization after fermentation to obtain sundried cassava root meal (SD) and fermented gelatinized cassava root meal (FG), respectively. FTIR analysis of maize (MZ), SD and FG showed common but varying intensities of anti-nutritional factors such as cyanides (6.06%: 50.50% and 16.00%, respectively), nutrients such as amides (1.52%: 0% and 5.00%, respectively) and therapeutic such as phenolic functional groups (0.85%: 2.40% and 3.00%, respectively). FG had the highest intensities of hydroxyl, phenolic groups, aromatic rings, carboxylic acids and amides. But SD had highest intensities of cyanide, ketone and legnin functional groups than FG with essentially none in maize. FG 25% inclusion Group had the highest final mean weight (FMW) of 77.77±0.03g) and mean weight gain (MWG) of 57.77 ± 0.03g followed by MZ with FMW of 75.44 ±0.03g and MWG of 55.44 ±0.03g while SD 75% had the least FMW (64.07±0.16g) and MWG (44.07±0.17g). Consequently, FG had highest amides (usually found in proteins) than in SD and its 25% inclusion yielded the highest FMW and MWG followed by MZ but least in SD 75% inclusion. The higher MWG of FG could also be justified by the lower intensities of anti-nutritional factors in FG than in SD.

Key words: FTIR-Spectroscopy; Processing; Cassava; Fish; Growth

INTRODUCTION

Conventionally, maize has been the main source of energy in fish feed. The demand for maize has, however, exceeded its supply in Nigeria because it is also used as staple food for a large proportion of Nigerians, as well as industrial raw material (Udedibie and Asoluka, 2008). Efforts are being made to replace maize (Jimoh, Sodamola and Ayeola, 2014).

One of the energy sources that have great potential as alternative to maize is cassava root. Various studies have documented the replacement value of processed cassava root as an energy ingredient. Cassava is more digestible compared to maize due to its high content of amylopectin (Talthawan, Lie and Froyland, 2002) and it is underutilized than maize (Lukuyu et al., 2014). However, its anti-nutritional properties have been the major setback for their limited success (Salim, Nehvi, Mir, Tyagi, Ali and Bhat, 2023).
Some of the anti-nutritional factors (ANFs) or non-beneficial compounds that can affect human and animal growth as well as reduce their nutrient intake, absorption and utilization are phytic acid, saponins, alkaloids, certain oligosaccharides, protease inhibitors, glucosinolates, tannins, and cyanogenic glycosides (Ali, Devarajan, Manickavasagan and Ata, 2022 and Dey, Saxena, Kumar, Maity, Tarafdar, 2022).

This study assessed and compared the intensities of chemical functional groups in cassava roots processed differently with that of maize.

MATERIALS AND METHODS

Fresh cassava (*Manihot esculata*) roots were procured from a local farm in Obinze, Owerri West LGA of Imo State, Nigeria. The cassava roots were washed with water to remove any possible dirt and then peeled. The peeled cassava roots were mashed and divided into two portions, one portion was used to prepare fermented gelatinized cassava root meal while the other portion was used for sundried cassava root meal.

Processing of Fermented Gelatinized Cassava Meal

The peeled and mashed roots were fermented for 4 days in plastic vats under ambient temperature (15^0 to 25^0C). The fermented cassava roots were then put in sacs, pressed to reduce water content and then sundried. The dusty cassava products were then subjected to gelatinization.
Gelatinization involved mixing the meal in water in a pot seated on fire at the rate of 1kg of the meal to 1litre of water and the mixture stirred until it sufficiently gelatinized into fufu, the form of cassava meal which is usually prepared and eaten locally.
The products were taken bit by bit and flattened on polyethylene sheets and sun-dried for a given period. The flakes were considered adequately dried when they become crispy to the touch and when they snap at bending.
The flakes were milled in a hammer mill with 2mm mesh size sieve to produce peeled, fermented and gelatinized cassava root meal (Enyenyi et al., 2013).

Processing of Sundried Cassava Meal

The peeled and mashed cassava roots were de- watered and sun dried to form sundried cassava root meal.

Fourier Transform Infrared (FTIR) Spectrophotometry of the samples of differently processed cassava root meal compared with the maize meal.

Method:
The crushed powder sample (0.1g) was mixed with dry Potasium bromide (KBr) (0.4g) and transferred to sample compartment of the Thermoscientific NICOLET IS5 Infrared Spectrophotometer. The spectrophotometer was set at 100% transmittance with pure KBr

pellet. Crushed sample mixed with KBr cell was scanned at a wavenumber range of 600 – 4000 cm^{-1} with scanning period of 20 seconds and the transmittance reading was obtained and stored. In FTIR analyses, Infrared light from the light source passes through a Michelson interferometer along the optical path. The Michelson interferometer comprises a beam splitter, moving mirror, and fixed mirror. The light beam split into two by the beam splitter is reflected from the moving mirror and fixed mirror, before being recombined by the beam splitter.

As the moving mirror makes reciprocating movements, the optical path difference to the fixed mirror changes, such that the phase difference changes with time. The light beams are recombined in the Michelson interferometer to produce interference light.

The intensity of the interference light is recorded in an interferogram, with the optical path difference recorded along the horizontal axis.

Fish Feed Formulation

Eight isocaloric and isonitrogenous diets were formulated and designated as; Diets (FG (0%), FG (25%), FG (50%), FG (75%), SD (0%), SD (25%), SD (50%) and SD (75%)) table 3.4. Diets FG (0%) and SD (0%) are the control and had maize as the main source of energy. In Diets (FG (25%), FG (50%) and FG (75%)), maize was substituted with fermented gelatinized cassava and in Diet (SD (25%), SD (50%), and SD (75%)) maize was substituted with sundried cassava. Both were substituted at graded levels of 25%, 50% and 75%, respectively. Maize and the processed cassava were the major energy sources; fishmeal and soybeans in the ratio of 1:2 were the protein sources; the fixed ingredients which include wheat bran, fish premix, bone meal, cassava starch, cod liver oil, common salt, palm oil, methionine lysine and vitamin C made up the 11.5% by weight of respective experimental diets.

These ingredients were ground into fine texture. The ground ingredients were mixed in dry form before addition of water at 0.5 L kg-1 of mixture. Water used was at a temperature of 70^{o}C. This enabled dough formation before pelleting was carried out using a 2 mm dice. The pelleted diets were sundried for three days and packed in plastic air tight containers and kept in a refrigerator prior to use.

Experimental fish and feeding procedure:
A total of one thousand and fifty (1050) O. niloticus juveniles of average weight of 20g were sourced from a reputable fish farm in Imo State.
The experimental fish were acclimatized and fed with commercial feed for one week. All the fish were starved for 24 hours prior to the commencement of the feeding trial. This was necessary to enable the juveniles to prepare their gastrointestinal tract and adjust to the new diet (Okoye and Sule, 2001) and environment.

Fifty of the experimental juveniles of Nile tilapia were randomly distributed into 1m x 1m x 1m net cage culture. The net cage cultures were covered with mesh nylon screens and lowered into the river.
Fish were fed twice daily morning (08.00-09.00h) and evening (17.00-18.00h) at 5% of their body weight. The weighing was carried out an hour before feeding. Fish from each tank were

weighed to the nearest gram at the commencement and subsequently biweekly using weighing scale and corresponding adjustments made in the amount of feed fed.

The study period which lasted for 56 days, compared the proximate composition of diets formulated by partial replacement of maize with differently processed cassava meals and the carcass quality of O. *niloticus* fed with the diets

Determination of the Effects of Formulated Feeds with Maize, Sun dried Cassava and Gelatinized Fermented Cassava on the percentage mean weight gain of O. *niloticus* Cultured in Cages in Otamiri River.

The effect of the treatments on the growth performance was assessed using the following indices:

i. Final Mean Weight (FMW) (g) = Total final Weight of all fish (g) divided by the Number of Fish Eqt 1

ii. Mean Weight Gain (MWG) (g) = Final total Weight of fish – initial total weight of fish (g) / Number of fish Eqt 2

RESULTS AND DISCUSSION

The results of the FTIR analyses are shown in Table 1.

Table 1: The FTIR Spectroscopic Transmittance (Intensity) of the Functional Groups In Fermented Gelatinized (FG), Maize (MZ) And Sundried (SD) Cassava.

FUNCTIONAL GROUP	INTENSITY (%) IN FGC	INTENSITY (%) IN MAZ	INTENSITY (%) IN SDC
O–H stretching vibration of hydroxyl and phenolic groups.	3.00	0.85	2.40
Symmetric and asymmetric stretching vibrations of C–H group from aromatic rings and alkanes	6.00	1.25	9.29
Vibration of C≡N strength (cyanides)	16.00	6.06	50.50
Vibration of C=O strength from carboxylic acid	9.00	4.78	11.26
Vibration of C=O strength of carboxylic acid contains of ketone	8.00	2.80	17.29

Vibration of -OH group strength from carboxylic acid	6.00	3.42	1.03
Vibration of C – H group strength from alkynes	9.00	3.45	10.43
C-H bending vibration	11.00	3.78	15.29
O–H bending vibration from hydroxyl group	6.00	1.88	11.26
Vibration of C–N stretching of amide bond	5.00	1.52	-
C-OH secondary alcohol or C–O stretching vibration from carboxylic acid	3.00	3.42	-
Vibration of C=C strength of alkene	12.00	4.04	11.87
C – C from phenyl ring substitution bend from lignin	14.00	5.28	18.60
C-O-C bend	12.00	4.77	13.70
C – O – C band Vibration	11.50	3.66	7.29

Table 2: **Results of the effects of feeding graded levels of sun-dried and fermented gelatinized cassava roots on the Weight gains of *O. niloticus*.**

PROC_M X INCL	IMW (g)	FMW(g)	MWG(g)
MZ 0%	20	75.44±0.03	55.44±0.03
FG 25%	20	77.77±0.03	57.77±0.03
FG 50%	20	74.88±0.09	54.88±0.44
FG 75%	20	67.58±0.04	47.58±0.04
SD 25%	20	73.49±0.05	53.50±0.05
SD 50%	20	67.30±0.03	47.30±0.02
SD 75%	20	64.07±0.16	44.07±0.17
LSD (0.05)	0.002	0.15	0.15

Keys: PROC_M =Processing method; INC=Inclusion; MZ= Maize; FG =Fermented gelatinized cassava root diet; SD= Sundried cassava root diet; IMW =initial mean weight; FMW=final mean weight; MWG=mean weight gain. (S.D when p< 0.05 < LSD)

The results from the transmittance of FTIR Spectrophotometer showed that the low or high transmittance indicated the intensity (quantity) of the chemical identified in the samples. The hydroxyl and phenolic groups, aromatic rings and alkanes, carboxylic acid of ketone, alkene groups and phenyl ring substitution band from lignin were present in FG, maize and SD.

The results from the weight gains among the experimental diets fed *O. niloticus* for 56 days showed that there were significant difference ($p<0.05$).
The result revealed that final mean weight (FMW) of the fish varied significantly ($P<0.05$) in all the experimental diets with highest value (77.77±0.03g) being observed in FG25% followed by MZ0% with the value FMW (75.44±0.03g) and the lowest value (64.07±0.16g) in SD75%. Similar result was observed in mean weight gain (MWG) where all the diets at different processing and inclusion methods varied significantly (0.05 <LSD) from each other.FG25% had the highest value (57.77±0.03g), followed by MZ0% with the value MWG (55.44±0.03g) and the lowest value (44.07±0.17g) being observed in SD75%.

Based on the FTIR spectroscopy results reported in the study, there were similar functional groups commonly associated with the chemical composition of cassava and maize-based materials across the fermented gelatinized cassava (FGC), maize meal (MAM), and sun-dried cassava (SDC) samples, including, hydroxyl and phenolic groups, aromatic rings and alkanes, ketone groups. Similar components have been reported in other studies (Torrenegra, Solano, Herrera, León and Pajaro, 2018; Monroy, Rivero and García, 2018). For example, Rahaman et al. (2021) examined FTIR spectra of cassava flour and found similar functional groups, including hydroxyl, carbonyl, and aromatic compounds. The presence of similar functional groups provides insight into the chemical properties and potential applications of these feedstuffs. However, as expected, the spectra showed lowest intensity of cyanide, ketones and lignin compounds in the maize meal but the cyanide, ketone and lignin concentrations (50.50%, 17.29% and 18.60%, respectively) were comparatively highest in sundried cassava more than in fermented gelatinized cassava (16.06%, 8.00% and 14.00%, respectively).This disparity is attributed to the possible destructive effect of heat on cyanides, lignins and other possible anti-nutritional constituents (Midau, et al., 2011). Processing methods have been shown to reduce cyanogen in cassava and could enhance its utilization by fish and such processing and modifications of substrate meals have been reported by Isiyaku, Mohammed, Laurat and Lawee, (2021). Moreover, fish is known to be sensitive to antinutritional factors ANF (Krogdahl, Lea and Olli, 1994). Thus, the differences in transmittance/absorbance intensities in the present study suggest variations in the modifications, quantities or concentrations of these functional groups, and the operating processes of the three fish meals (Abdulah et al., 2021). This also support the lower mean weight gain MWG in fish fed sundried cassava root diet particularly at higher inclusion levels SD75% which is4 44.07g.
However, the C-O-C bond intensity which may be related to carbohydrate structure was higher in SDC. This implies that more polysaccharide formations may reduce digestibility.
More so, the higher presence of hydroxyl (OH) and amide (C-N) bond intensities in FGC may enhance the availability of protein which supports more weight gain in the fish feed the diet formulated with FG.

CONCLUSION

The results of FTIR spectroscopy depicted that the cardinal chemical functional differences between fermented gelatinized cassava and sundried cassava directly influenced fish growth. The lower antinutritional factors in fermented gelatinized diets supported better final and mean weight gain in *O. niloticus*. While the higher lignin and oxidation levels in sundried cassava diets, negatively impacted growth. These findings suggest that fermentation and gelatinization improved the nutritional value of cassava, making it a better alternative to maize in *O. niloticus* diets, particularly at 25-50% inclusion levels.

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