

QUALITY ASSURANCE, SAFETY, AND ACCEPTABILITY OF STORED YELLOW LACTIC LAFUN: AN INDEX OF INCREASING IT SHELF LIFE

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ABSTRACT

Storability and shelf life of lafun has been a major challenge, especially in Nigeria, being a developing country that has limited or no storage facilities. Hence, necessitates a continuous search for remedies. This study thus evaluates the quality, safety, and acceptability of stored lactic lafun using different packaging materials. A 120 kg of cassava “TMS – IBA 01137” was procured from International Institute of Tropical Agriculture, Oyo state, Nigeria, and was fermented using *Weissella koreensis* stock culture obtained from University of Readings, United Kingdom. The fermented product was allowed for further processing; to obtain the end product “Lactic lafun.” The samples were then introduced into four different packaging materials which include Ziploc transparent bag, Ziploc opaque bag, vacuum sealed transparent bag, and vacuum sealed opaque bag for 90 days. Physicochemical, pasting, sensory, and microbial analyses were carried out every 30 days. The results showed for microbial analysis, showed that the Ziploc materials contain high microbial count as it has the highest count of bacteria (6.8–7.5 LogCFU) and fungi (7.0–9.0 LogCFU) throughout the storage period. The proximate analysis also showed increase in moisture content (p<0.05) in transparent packaging materials when compared with their baseline sample at day 0 (3.5±0.09) whereas, there was no significant changes in the ash, fiber, CHO, and lipid across all the samples. The carotenoid content level decreased significantly (0.212±0.04) in the transparent packaging materials with increasing days of storage when compared to the baseline sample (1.977±0.012). There is a significant reduction (p<0.05) in the hydrogen cyanide of the lafun sample when compared to the baseline. The organoleptic property showed no significant difference in the acceptability of all the samples. It can, therefore, be concluded that lactic lafun produced from yellow cassava stored in opaque materials presents a viable and sustainable means of tackling carotenoid deficiencies and spoilage of lafun over a long period of time.

Keywords: Fermentation, Pasting properties, Lactic lafun, Sensory evaluation.

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop that is found in many tropical and subtropical countries, including Asia, Latin America, and especially found in West Africa (Sayre *et al.*, 2011). This root crop is identified in different names such as manioc, mandioca, tapioca, or yucca and it is referred to as a cyanogenic food crop (Abban *et al.*, 2013). Cassava is known to grow under harsh conditions and still do well than other food crops (Cach *et al.*, 2006). The cassava plant is resistance to most diseases and pests and can also withstand drought (Setter and Fregene, 2007). In addition, cassava is a form of food that is eaten routinely and constitutes a dominant diet for over 500 million people in more than 90 countries and also part of the third most important source of calories in the tropics after rice and corn (Murugan *et al.*, 2014). Nevertheless, toxicity of cassava happens due to presence of the cyanogenic glucosides or cyanogens (naturally occurring substrates), there are two common cyanogenic compounds found in cassava. They are linamarin (2-hydroxy-isobutyronitrile/~-D-glucopyranoside) and lotaustralin (2-hydroxyl 2-methylbutyronitrile/~-D-glucopyranoside) which have fatal consequences when consumed in unprocessed food (Montagnac *et al.*, 2009). Cyanogens are leading cause of cyanide poisoning which symptoms include of dizziness, stomach pain, headache, nausea, vomiting, and occasionally death (Lambri *et al.*, 2013). In addition, daily consumption of cassava food products that still retain residual levels of these cyanogenic glucosides (cyanogens) can cause several chronic diseases which are potentially harmful to human (Ahaotu *et al.*, 2013; Ogbonnaya, 2016). According to the World Health Organization, the recommended maximum safe intake of cyanide-containing food/feed for both humans and animals should be around 10 mg HCN/kg body weight (Tritscher *et al.*, 2013;

Chikezie and Ojiako, 2013). The cassava undergoes submerged fermentation that is a process in which the growth and anaerobic or partially anaerobic decomposition of the carbohydrates by the action of microorganisms in a liquid medium with very more than enough free water available (Ray *et al.*, 2007) takes place. The fermentation process usually takes 3–4 days and shows an increase in crude protein content of cassava peels. Freshly harvested cassava tubers and leaves are very high in cyanogenic glucosides. This glucosides content may vary between 137 and 1515 ppm. Traditional fermentation processing of these tubers ensures the significant reduction of cyanogenic glucosides contents by 70–75% (Dhellit *et al.*, 2015). A drying procedure with and without the fermentation process for elimination of cyanogens in cassava tubers (pressed pulp) was investigated (Lambri *et al.*, 2013). The process of fermentation was carried out in the presence of yeast (*Saccharomyces cerevisiae*) and detoxification was found effective. In drying conditions, a temperature of 60°C, even for a shorter duration (say 8 h), lowered the cyanide content (>90%). However, the process of dehydration followed by fermentation showed maximum removal of cyanide content. Fermented products of cassava constitute a major part of the daily diets of many homes in Nigeria and most parts of West Africa. The most popular of these products are gari, fufu, and lafun. Lafun is a fermented cassava food product (fine powdery) commonly consumed in West Africa, mainly in the western states of Nigeria and Benin (Falade and Akingbala, 2010). Many constraints have been identified in the processing and storage of lafun including the processing method used, the age and variety of cassava as another constraint, and another factor initiated by chance in inoculation by microorganisms from the environment (Achi and Akomas, 2006). Hence, this study now aimed by fermenting the fortified cassava with particular lactic

acid bacteria (*Weissella koreensis*) without allowing any other external microorganisms and also understudying the storability using different packaging materials.

MATERIALS AND METHODS

Samples collection

The cassavas used (fortified) were collected from International Institute of Tropical Agriculture (IITA), IITA Ibadan, Oyo state. The lactic acid bacteria used (*W. koreensis*) were obtained from University of Reading, United Kingdom, as starter for the fermentation process. The strain was preserved in 2 ml cryovials of 1860 µl cultured broth and 140 µl dimethyl sulfoxide. It was originally preserved at -80°C and brought to Nigeria in ice pack. It was quickly transferred to a freezer (-40°C) on arrival.

Collection of materials

Petri dishes, conical flask, measuring cylinder, weighing balances, spirit lamps, stirrer, spatula, McCartney bottles, autoclave, cotton wool, stopwatch, inoculating loop, potato dextrose agar (PDA), plate count agar (PCA), and eosin methylene blue (EMB) were all collected.

Preparation of media

PDA and PCA were prepared according to the manufacturer specification weighed into conical flasks. The mixture was homogenized and corked. It was sterilized using autoclave at 121°C for 15 min. After sterilization, it was allowed to cool and was aseptically poured into sterile Petri dishes.

Preparation of samples

Preparation of cassava roots

After collecting the cassava (fortified) from IITA. It was washed thoroughly, sorted, and then peeled. It was cut into pieces and rewashed. After being washed, it was then packed into sterile bowl.

Preparation of lactic acid bacteria

The strains of lactic acid bacteria (*W. koreensis*) obtained were preserved in cultured broth. They were reactivated by first culturing plating on the media agar. Incubation was performed anaerobically.

Preparation of lactic lafun

W. koreensis were used in control fermentation of the prepared cassava roots. The prepared roots were steeped into prepared sterile water inside screwed drum. Stock cultured of *W. koreensis* was then inoculated into the steeped cassava roots (23 kg of fortified cassava was steeped into 2300 ml of sterile water inoculating 23 ml of stock cultured of *W. koreensis*). It was fermented in anaerobic condition for 3 days checking the temperature (thermometer EXTECH SD200 3-Channel) and pH in each day. After fermenting, it was oven dried. The dried fermented cassava was then milled, packed, and stored. The packaging materials used are transparent and opaque vacuum and transparent and opaque Ziploc.

Microbiological analysis

One gram of each samples was suspended into 9 ml of sterile and was homogenized for 1 min. Serial dilution (10^{-1} – 10^{-9}) was prepared and 0.5 ml from dilution factor 10^{-3} , 10^{-5} , and 10^{-7} was inoculated into already solidify PCA aseptically using sterile syringe and was spread over it using sterile spreader. This procedure was repeated for PDA and EMB aseptically. Plates were incubated using incubator (Gollenknop ONP 9052 YA) for 24 h at 37°C. It was then counted using colony counter (TT 201, Techmel and Techmel, USA).

Determinations of functional properties lafun samples

Loose bulk density and packed bulk density determination

The loose bulk density and packed bulk density were determined by the modified method of (Musa et al., 2008). About 10 g of the sample was weighted into a measuring cylinder (without tapping), noting the

volume occupied. Packed bulk density was determined after tapping the cylinder containing 10 g of flour samples on a table and noting the final volume. The loose and packed bulk density was calculated as mass by volume in grams per milliliter (g/ml).

$$\text{Loose bulk density (g/ml)} = \frac{\text{Weight of sample(g)}}{\text{Volume of the sample without tapping}} \quad (1)$$

$$\text{And packed bulk density (g/ml)} = \frac{\text{Weight of sample(g)}}{\text{Volume of the sample after tapping}} \quad (2)$$

Dispersibility

Percentage dispersibility was determined using the methods of Kulkarni et al. (1991). Ten grams of the flour sample were weighed into 100 ml measuring cylinder, distilled water was added to reach 100 ml mark. The set up stirred vigorously and allowed to stand for 3 h. The volume of settled particles was recorded and subtracted from 100. The dispersibility was then calculated by equation.

$$\% \text{ Dispersibility} = 100 \times \frac{\text{volume of settled particle}}{\text{total volume}}$$

Swelling power and starch solubility index

Swelling power and starch solubility index were carried out using the method of Leach et al. (1959). One gram (1.0 g) of the dried sample was weighed into a 125 ml conical flask. Then, 15 ml of distilled water was added and then shake on a shaker for 5 min at low speed. The sample was then transferred into a water bath (Grant Instrument (Cambridge) Limited Serial no. 22026, Type SB 2) and heated for 4 min at 80°C with constant stirring. The gel formed was then transferred into a pre-weighed centrifuge tube and 7.5 ml of distilled water was added. It was then centrifuged at 2200 rpm for 20 min. The supernatant was then carefully decanted into a pre-weighed drying can and dried at 100–105°C to constant weight. The weight of the gel in the centrifuge tube was also determined. The swelling power and starch solubility index were then calculated by equations.

Calculation:

$$\text{Swelling Power} = \frac{\text{Weight of the wet sediment (g)}}{\text{Weight of the sample (g)}} \times 100 \quad (3)$$

$$\text{Starch Solubility index} = \frac{\text{Weight of soluble(g)}}{\text{Sample weight(g)}} \times 100 \quad (4)$$

WAI

WAI was carried out using the modified method of Ruales et al. (1993). The sample (1 g) was suspended in 15 ml distilled water at 30°C in a centrifuge tube, stirred for 30 min intermittently, and then centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the weight of the sediment formed was recorded. The WAI was then calculated as sediment weight per gram dry sample. The WAI was then calculated by equation.

$$\text{Water Absorption Index (WAI)} = \frac{\text{Bound water(g)}}{\text{Sample weight(g)}} \times 100 \quad (5)$$

Determinations of proximate analysis of lafun samples

Moisture content (MC) determination

MC determination was carried out using the air oven method as described in AOAC (2010). Crucibles were washed and dried in an oven (BST/HAO-1123). They were allowed to cool in the desiccator and weight was noted. A known weight of samples was then transferred into the crucibles and dried at a temperature between 103° and 105°C. The dry samples were cooled in a desiccator and the weight noted. They were later returned to the oven and the process continued until constant weights were obtained.

$$\text{Calculation: Moisture content\%} = \frac{(\text{Weight Loss})}{(\text{Weight of Sample})} \times 100 \quad (6)$$

Determination of ash content

Ash percentage was determined using the method described in AOAC (2010). Briefly, a known weight of finely ground sample was weighed into clean, dried previously weighed crucible with lid (W1). The sample was ignited over a low flame to char the organic matter W1 removed. The crucible was then placed in muffle furnace (Guangzhou Medsinglong muffle furnace MSL/200B) at 600°C for 6 h until it turned to ashes completely. It was then transferred directly to desiccators, cooled, and weighed immediately (W2).

$$\text{Ash \%} = \frac{(W2-W1)}{(\text{Weight of Sample})} \times 100 \quad (7)$$

Determination of crude fat

The Soxhlet extraction method as described in AOAC, 2010 was used to determine the crude fat percentage. A known weight of sample was weighed into a weighed filter paper and folded neatly. This was put inside pre-weighed thimble (W1). The thimble with the sample (W2) was inserted into the Soxhlet apparatus (Lab tech Grey LTSW-5) and extraction under reflux was carried out with petroleum ether (40°C–60°C boiling range) for 6 h. At the end of extraction, the thimble was dried in the oven for about 30 min at 100°C to evaporate off the solvent and thimble was cooled in a desiccator and later weighed (W3). The fat extracted from a given quantity of sample was then calculated:

$$\% \text{ Fat (w/w)} = \frac{(\text{Loss in Weight of sample (W2-W3)})}{(\text{Original Weight of sample (W2-W1)})} \times 100 \quad (8)$$

Crude protein determination

The crude protein content was determined using micro Kjeldahl method as described in AOAC (2010). Sample (1 g) was weighed into a long necked Kjeldahl flask. One tablet of Kjeldahl catalyst was added to the sample in the flask with 25 cm³ of concentrated H₂SO₄. The flask was swirled, gently clamped in an inclined position, and heated using electricity in a fume cupboard. The heating continue until a clear solution was obtained. The clear solution was cooled, poured into a 100 cm³ volumetric flask, and made up to mark with distilled water 10 ml of the resulting mixture which was measured into the distillation set through the funnel. Boric acid (5 cm³) was pipette into a 100 cm³ conical flask and placed at the receiving end of the distillatory. The conical flask was placed such that the delivery tube dipped completely into the boric acid inside the flask. NaOH (40%) was used to liberate ammonia out of the digest under alkaline condition during the distillation. Two drops of methyl orange were added to the round bottom flask containing the digested sample before 40% NaOH was added. As soon as the contents became alkaline, the red color changed to yellow showing NaOH to be in excess. Steam was then generated into the distillation set using a steam chest. The liberated ammonia was trapped in the boric acid solution and about 50 cm³ of the solution collected into a conical flask. The solution in the flask was titrated against 0.1 MHC1 until the first permanent color change was observed.

$$\% \text{ N} = \frac{(\text{Molarity of HCl} \times \text{Sample titre-Blank}) \times 0.014 \times \text{DF}}{(\text{Weight of sample used})} \times 100 \quad (9)$$

% was converted to the percentage crude protein by multiplying by 6.25.

Where, DF is dilution factor; % N is percentage nitrogen, and 6.25 is the nitrogen: protein conversion factor.

Crude fiber determination

Two hundred (200 ml) freshly prepared 1.25% (1 M) H₂SO₄ were added to a known weight of the residue obtained from fat extraction and this was brought to quick boil. Boiling was continued for 30 min. The mixture was filtered and residue washed until it was free from

acid. The residue was transferred quantitatively into a digestion flask, 1.25% (0.1 M) NaOH was added and brought to boiling point quickly. Boiling was continued for 30 min. The mixture was filtered and residue washed free of alkali. The residue was then washed with methylated spirit, thrice with petroleum ether using small quantities. It was allowed to properly drain and the residue was transferred to a silica dish (previously ignited at 600°C and cooled). The dish and its content were dried to constant weight at 105°C. The organic matter of the residue was burnt by igniting for 30 min in a muffle furnace at 600°C. The residue was cooled and weighed. The weight difference before and after ignition was reported as crude fiber (AOAC, 2010).

Estimation of carbohydrate

The carbohydrate content was estimated by difference.

% CHO = 100 - (Sum of the percentages of moisture, ash, fat, protein, and crude fiber).

Determination of cyanide content

Duplicate 100 g samples of lafun were added to a small plastic bottle, a buffer/enzyme paper was added, followed by 1 L of 1 M pH 6 phosphate buffers, a picrate paper, and a screw cap lid. The bottles were allowed to stand overnight at 30°C, the picrate papers were removed from the plastic support, and 5.0 L of water added to elute the color. The absorbance was measured in a spectrophotometer at 510 nm and the total cyanide content in mg HCN equivalents/100 g fresh weight was evaluated.

Determination of carotenoid content

The carotenoid content of the cassava flour sample was determined using spectrophotometric method (AOAC 2005). About 2.5 g of each sample was weighed into a conical flask, 30 ml n-Hexane, and 20 ml ethanol was added to the sample while 2 ml of 2% NaCl was also added and shaken together. After thorough mixing, the mixture was transferred into a separating funnel and allowed to stand for about 10 min for complete extraction of carotenoid. Thereafter, the lower layer was allowed to run off while the upper layer was collected and the absorbance measured using a spectrophotometer at 436 nm.

Total carotenoid was calculated as thus:

L= cell length=1 cm

W=weight of the sample=2.5

Sensory methodology

The quantitative descriptive analysis was used for appearance, odor, and texture. This descriptor was printed on a paper for scoring which was carried out in the laboratory at room temperature in the department of Biology (The Polytechnic, Ibadan). Four samples were given a code name. The samples were freshly prepared on the day of the profiling session and served hot for each analysis day (day 0, day 30, day 60, and day 90, respectively).

Consumer liking testing

Twenty participants were obtained with a varying ratio of about 7–9 males to 10–13 females for each analysis day. The participant was obtained in a way that encourages between familiar and unfamiliar consumers by tagging from state origin with familiarity to lafun. The participant consent was obtained before their participation in the study. The prepared samples were presented to five groups of consumers in a disposable serving plate with clean water for hand washing in a hand washing basin. The evaluation forms were thoroughly explained to the consumers, and they were asked to score liking (overall appearance odor and texture using 5-point hedonic anchored from dislike extremely to like extremely). To access consumption and purchased intents, 5-point scales anchored from definitely would not eat/buy to definitely would eat/buy. The questionnaire started with demographic question and familiarity with lafun question.

Statistical analysis

General linear model procedure of SPSS for the analysis of mean and SME or SD was used for all equipment.

RESULTS

Total viable count in lafun sample with zero packaging and lafun samples stored in different packaging materials

The total viable count of bacteria, fungi count, and coliform count in lafun samples at different storage duration is shown in Table 1. It was observed that there was no significant increase in microbial count of the packaged samples for the first 30 days of storage (6.8–6.9 LogCFU for Ziploc materials but decreased in vacuum materials which was 5.2–5.3 LogCFU) when compared to lafun of day 0 (6.8 LogCFU). However, there was a significant increase in microbial count of all the samples at both 60 (7.3, 7.0 LogCFU for Ziploc materials and 6.3, 6.5 LogCFU for vacuum sealed polyethylene bag) and 90 days (7.3, 7.5 LogCFU for Ziploc materials and 6.2, 6.7 LogCFU for vacuum sealed polyethylene bag). The microbial loads of the samples vary considerably between packaging materials. There was a significant difference in the microbial count of lafun samples packaged in Ziploc materials (both transparent (6.9–7.5 LogCFU) and opaque (6.8–7.3 LogCFU)) and the vacuum sealed polyethylene bag (transparent (5.2–6.7 LogCFU) and opaque (5.5–6.2 LogCFU)). The same trend occurs in fungi count as there was no growth found on coliform plates.

The proximate composition of raw cassava, freshly processed lafun with zero packaging, and lafun samples packed in different packaging materials

The percentage of proximate composition of the raw cassava and lafun samples with zero storage and lafun samples stored in different packaging materials in Table 2 shows that MC value 73.00 ± 0.32 was observed in raw cassava sample which reduced drastically after processing into lafun with a value of 3.50 ± 0.09 . As the MC reduced in raw sample when compared to lafun sample with zero packaging, other proximate characteristics such as ash, lipid, fiber, protein, and carbohydrate increased. It was observed that the MC in all samples was gradually increased as the storage duration increased. MC of lafun in transparent Ziploc material ranged 3.56 ± 0.38 – 9.70 ± 0.43 , 2.62 ± 0.37 – 6.80 ± 0.66 in opaque Ziploc material, 4.13 ± 0.62 – 10.85 ± 0.14 in vacuum sealed transparent material, and 4.07 ± 0.005 – 6.30 ± 0.42 in vacuum sealed opaque material. The increase recorded in the MC was in the order vacuum transparent > Ziploc transparent > Ziploc opaque > vacuum opaque at the end of the storage period. After 90 days of storage, vacuum sealed material had the highest ash content with 5.69 ± 0.82 and 4.06 ± 0.35 for vacuum transparent and vacuum opaque material, respectively. No significant difference was observed in the lipid and crude fiber content of the stored lafun samples even as the storage duration increased. The protein content value of the stored lafun samples reduced after 90 days of storage in all the packaging materials. Ziploc opaque material had the highest protein value of 2.05 ± 0.09 .

Table 1: Total viable count in lafun sample with zero packaging and lafun samples stored in different packaging materials

Days	Packaging material	Bacteria		Fungi		Coliform	
		CFU g/ml	LogCFU	CFU g/ml	LogCFU	CFU g/ml	LogCFU
Day 0	None	7.0×10^6	6.8	2.0×10^6	6.3	NG	NG
Day 30	Ziploc transparent	7.0×10^6	6.9	1.0×10^7	7.0	NG	NG
	Ziploc opaque	7.0×10^6	6.8	1.0×10^7	7.0	NG	NG
Day 60	Vacuum transparent	1.5×10^5	5.2	1.7×10^5	5.2	NG	NG
	Vacuum opaque	3.5×10^5	5.5	1.0×10^5	5.0	NG	NG
	Ziploc transparent	2.0×10^7	7.3	2.0×10^7	7.3	NG	NG
	Ziploc opaque	1.0×10^7	7.0	3.0×10^7	7.5	NG	NG
Day 90	Vacuum transparent	2.0×10^6	6.3	1.0×10^7	7.0	NG	NG
	Vacuum opaque	3.0×10^6	6.5	1.7×10^5	5.2	NG	NG
	Ziploc transparent	3.0×10^7	7.5	1.0×10^9	9.0	NG	NG
	Ziploc opaque	2.0×10^7	7.3	3.0×10^8	8.5	NG	NG
	Vacuum transparent	6.0×10^6	6.7	1.8×10^7	7.3	NG	NG
	Vacuum opaque	1.6×10^6	6.2	3.0×10^5	5.5	NG	NG

Table 2: The proximate composition of raw cassava, freshly processed lafun with zero packaging, and lafun samples packed in different packaging materials

Storage duration (days)	Packaging materials	Proximate (%)					
		Moisture	Crude lipid	Ash	Crude fiber	Crude protein	Cho
30	RAW	73.00 ± 0.32^g	1.49 ± 0.01^a	1.16 ± 0.01^a	1.95 ± 0.01^a	1.55 ± 0.01^a	20.67 ± 0.26^a
	LAFUN	3.50 ± 0.09^{ab}	3.47 ± 0.12^{abc}	1.83 ± 0.03^a	2.60 ± 0.13^a	1.95 ± 0.09^{abc}	86.65 ± 0.05^c
	ZT	3.56 ± 0.38^{ab}	2.83 ± 0.64^{abc}	2.95 ± 0.07^a	2.18 ± 0.49^a	2.54 ± 0.11^{cde}	85.95 ± 0.42^c
	ZO	2.62 ± 0.37^a	1.93 ± 0.35^{ab}	2.95 ± 0.07^a	2.06 ± 0.21^a	3.32 ± 0.15^{ef}	87.13 ± 0.03^c
60	VT	4.13 ± 0.62^{abc}	4.30 ± 2.16^{bc}	2.70 ± 0.28^a	1.94 ± 0.24^a	2.04 ± 0.08^{abc}	84.91 ± 3.37^c
	VO	4.07 ± 0.05^{abc}	2.67 ± 0.71^{abc}	2.80 ± 0.42^a	2.10 ± 0.01^a	1.88 ± 0.16^{abc}	86.49 ± 0.51^c
	ZT	5.75 ± 1.20^{cde}	1.99 ± 0.12^{ab}	2.06 ± 0.08^a	2.06 ± 0.19^a	2.32 ± 0.30^f	85.84 ± 0.74^c
	ZO	4.40 ± 0.71^{abcd}	2.25 ± 1.63^{ab}	1.85 ± 0.13^a	1.97 ± 0.06^a	3.65 ± 0.32^f	85.89 ± 2.59^c
90	VT	4.95 ± 0.07^{bcde}	2.42 ± 0.71^{ab}	2.02 ± 0.03^a	2.15 ± 0.06^a	1.77 ± 0.11^{abc}	86.70 ± 0.62^c
	VO	6.45 ± 0.78^e	1.80 ± 0.37^{ab}	2.01 ± 0.02^a	2.15 ± 0.08^a	3.02 ± 0.14^{def}	84.59 ± 0.32^c
	ZT	9.70 ± 0.43^f	3.71 ± 0.26^{abc}	6.59 ± 1.46^b	2.82 ± 0.54^a	1.66 ± 0.47^{ab}	75.54 ± 1.22^b
	ZO	6.80 ± 0.66^e	2.05 ± 0.08^{ab}	1.81 ± 1.58^a	2.88 ± 0.78^a	2.05 ± 0.09^{abc}	84.43 ± 0.32^c
	VT	10.85 ± 0.14^f	5.69 ± 0.82^c	3.58 ± 2.24^{ab}	3.06 ± 0.22^a	1.95 ± 0.11^{abc}	74.89 ± 3.32^b
	VO	6.30 ± 0.42^{de}	4.06 ± 0.35^{abc}	1.38 ± 0.45^a	2.04 ± 0.06^a	1.87 ± 0.31^{abc}	84.37 ± 0.90^c

Values are given as means of triplicate determinations \pm standard deviation. Similar letters in a column are not significantly different ($p > 0.05$). ZT: Ziploc transparent bag, ZO: Ziploc opaque bag, VT: Vacuum sealed transparent bag, VO: Vacuum sealed opaque bag, Raw: Raw cassava sample, lafun: Cassava flour with zero packaging

but no significant difference was observed in its reading compared to that of the vacuum opaque material and vacuum transparent material. Carbohydrate content reduced in all the packaging materials has the storage period increased. Ziploc opaque had a value of 84.43 ± 0.32 while vacuum opaque material had 84.37 ± 0.90 both values were the highest carbohydrate value recorded after 90 days of storage and are no significantly difference from each other.

Functional properties of lafun samples before packaging and in different packaging materials

The functional properties of lafun sample after processing and stored in different packaging material are shown in Table 3. Lafun sample with zero packaging had a packed bulk density value of (0.49 ± 0.02) . It was observed that the packed bulk density level increased in all the stored lafun samples. Opaque Ziploc material had the highest value of 0.68 ± 0.01 after 90 days of storage. The vacuum sealed packaging material had values that were not significantly different from each other. The loose bulk density value ranged from 0.38 ± 0.00 to 0.60 ± 0.02 . Transparent vacuum sealed packaging material had the highest value (0.60 ± 0.02) after 90 days of storage. Dispersibility, water absorption, and starch solubility index increased as the storage period increased in all packaging materials. It was observed that swelling power value 6.47 ± 4.32 from lafun samples with zero packaging decreased after 60 days of storage and later increased in values after 90 days of storage. Ziploc materials had the highest value in swelling power.

The total carotenoid content of samples used in this study is shown in Table 4. The fortified raw cassava used in this study contained a total carotenoid value of 1.98 ± 0.01 which reduced after processing. The lafun sample with zero packaging had a total carotenoid level of 0.85 ± 0.01 . Total carotenoid level continued to decrease under different packaging materials as the storage duration increased from days 30 to 90. After 90 days storage, the opaque and transparent vacuum sealed bag had a better carotenoid retention with a value of 0.385 ± 0.072 and 0.320 ± 0.63 while the opaque Ziploc material also had a reading of

0.284 ± 0.08 but there was no significant difference observed between them.

The result of the total carotenoid content of raw cassava, lafun processed with lactic acid bacteria and lafun samples in the different packaging materials in Table 5 showed that concentration of carotenoid decreases with increase in storage days. The raw cassava had the highest carotenoid content ($1.977 \text{ mg}/100 \text{ g}$) when compared to the processed cassava ($0.848 \text{ mg}/100 \text{ g}$). Whereas, a reduction in this carotenoid was observed with transparent containers and days of storage.

Pasting characteristic of lafun processed with lactic acid bacteria and lafun samples in the different packaging materials

The pasting characteristics such as peak 1, trough 1, breakdown, final viscosity (FV), set back, peak time, and pasting temperature were observed and recorded (Table 4). Lafun samples with zero packaging had a peak velocity of 550.33 which significantly reduced across all the packaging material which has the storage duration increased. Opaque Ziploc material had the highest value of 255.08 after 60 days of storage but transparent Ziploc material had the highest peak velocity after 90 days of storage. It was observed that other pasting characteristics also reduced as the storage duration with exception to the peak time of the lafun samples which were consistent. There was no significant difference in the interaction of the packaging material and days on the peak time of the lafun samples.

Sensory evaluation of lafun samples consumer attribute and intent for lafun samples in different packaging materials

Different sensory attribute was tested among consumer (Table 6). The overall liking of the lafun sample without packaging was at 3.68 ± 1.25 . There was no significant difference in the overall liking of consumers on all the lafun samples stored in different packaging materials for day 30 and day 60. The opaque vacuum sealed material had the highest value for overall liking after 90 days and was significant different from other samples. The appearance liking ranged from 3.20 ± 0.77 to

Table 3: Functional properties of lafun samples before packaging and in different packaging materials

Storage duration (days)	packaging materials	Packed bulk density	Loose bulk density	Dispersibility	Water absorption index	Swelling power	Starch solubility index
30	LAFUN	0.49 ± 0.02^a	0.39 ± 0.00^{ab}	59 ± 1.41^a	218 ± 4.72^{ab}	6.47 ± 4.62^a	4.25 ± 0.49^a
	ZT	0.58 ± 0.03^{bc}	0.39 ± 0.03^{ab}	62 ± 1.41^{ab}	215.5 ± 6.36^{ab}	4.93 ± 0.08^a	4.6 ± 0.14^{ab}
	ZO	0.55 ± 0.03^{bc}	0.41 ± 0.01^{ab}	61 ± 1.41^{ab}	220.5 ± 6.36^{abcd}	3.49 ± 0.01^a	6.6 ± 0.42^{abc}
	VT	0.57 ± 0.01^{bc}	0.40 ± 0.00^{ab}	61 ± 1.41^{ab}	210.2 ± 9.62^a	3.26 ± 0.06^a	3.95 ± 0.35^a
	VO	0.55 ± 0.01^{bc}	0.38 ± 0.01^{ab}	61 ± 0.00^{ab}	217 ± 4.24^{ab}	4.75 ± 0.08^a	5.3 ± 0.28^{ab}
60	ZT	0.56 ± 0.00^{bc}	0.39 ± 0.02^{ab}	61.5 ± 0.71^{ab}	236.5 ± 3.54^{abcd}	3.95 ± 0.78^a	7 ± 2.83^{abc}
	ZO	0.55 ± 0.02^{abc}	0.41 ± 0.01^{ab}	59 ± 0.00^a	242.5 ± 6.36^{abcd}	4.1 ± 0.01^a	10 ± 1.41^{bc}
	VT	0.53 ± 0.00^{ab}	0.38 ± 0.01^a	62 ± 0.00^{ab}	218.5 ± 19.09^{abc}	4.26 ± 0.08^a	12 ± 2.83^c
	VO	0.56 ± 0.00^{bc}	0.39 ± 0.02^{ab}	60.5 ± 0.71^{ab}	245 ± 15.56^{bcd}	4.41 ± 0.27^a	8 ± 2.83^{abc}
	ZT	0.61 ± 0.02^c	0.45 ± 0.03^b	66.5 ± 2.12^b	254.29 ± 6.56^d	5.9 ± 0.28^a	4.88 ± 0.54^{ab}
90	ZO	0.68 ± 0.01^d	0.44 ± 0.01^{ab}	62 ± 1.41^{ab}	252.2 ± 8.49^{cd}	9.22 ± 0.26^a	7.25 ± 0.36^{abc}
	VT	0.56 ± 0.00^{bc}	0.45 ± 0.03^b	61.5 ± 4.95^{ab}	251.83 ± 6.52^{cd}	4.94 ± 0.14^a	6.02 ± 1.02^{ab}
	VO	0.59 ± 0.00^{bc}	0.60 ± 0.02^c	64 ± 2.83^{ab}	252.18 ± 6.52^{cd}	3.21 ± 0.14^a	6.13 ± 1.59^{ab}

Values are given as means of triplicate determinations \pm standard deviation. Similar letters in a column are not significantly different ($p > 0.05$). ZT: Ziploc transparent bag, ZO: Ziploc opaque bag, VT: Vacuum sealed transparent bag, VO: Vacuum sealed opaque bag, Raw: Raw cassava sample, lafun: Cassava flour with zero packaging

Table 4: Pasting profile of lafun samples

Packaging material	Duration (day)	Peak 1	Trough1	Breakdown	Final viscosity	Set back	Peak time	Pasting temperature
LAFUN	0	550.33	256.08	294.25	336.25	80.17	4.47	76.65
ZT	60	250.83	136.25	114.58	183.75	47.50	4.40	75.10
	90	223.92	120.58	103.33	168.92	48.33	4.20	75.05
ZO	60	255.08	141.58	113.50	188.83	47.25	4.47	75.90
	90	165.58	103.42	61.08	148.75	45.00	4.60	75.00
VT	60	219.58	119.83	99.75	168.58	48.75	4.40	75.85
	90	177.08	105.67	71.42	150.67	45.00	4.47	75.10
VO	60	252.92	135.75	117.17	183.42	47.67	4.40	75.85
	90	194.17	110.00	84.17	156.58	46.58	4.27	75.10

ZT: Ziploc transparent bag, ZO: Ziploc opaque bag, VT: Vacuum sealed transparent bag, VO: Vacuum sealed opaque bag, lafun: Cassava flour with zero packaging

4.05±0.95 vacuum transparent material had the highest in appearance liking. There was no significant difference between samples. Result for consumption intent and purchase intent is shown in Tables 4.6. The vacuum transparent sample was the highest after 90 days of storage.

DISCUSSION

Effect of storage duration (30, 60, and 90 days) on microbial stability of cassava flour was monitored in all samples. The result indicates an increase in microbial count of all samples stored in different packaging materials as the days of storage increased. Microbial load of lafun samples in different packaging materials was influenced by the MC in the samples. According to Ogugbue and Gloria, 2011, most products from cassava are hygroscopic in nature and can absorb moisture which in turn encourages the growth of microbes.

Table 5: Total carotenoid content of raw cassava, lafun processed with lactic acid bacteria, and lafun samples in the different packaging materials

Duration (days)	Packaging material	Carotenoid
Baseline	Raw	1.977±0.012 ^e
	Lafun	0.848±0.011 ^d
30	ZT	0.351±0.020 ^{abc}
	ZO	0.539±0.048 ^c
	VT	0.382±0.104 ^{abc}
	VO	0.538±0.055 ^c
60	ZT	0.312±0.004 ^{ab}
	ZO	0.45±0.31 ^{bc}
	VT	0.351±0.064 ^{abc}
	VO	0.417±0.019 ^{bc}
90	ZT	0.212±0.04 ^a
	ZO	0.284±0.082 ^{ab}
	VT	0.320±0.63 ^{ab}
	VO	0.385±0.072 ^{abc}

Values are given as means of triplicate determinations±standard deviation. Similar letters in a column are not significantly different (p>0.05). ZT: Ziploc transparent bag, ZO: Ziploc opaque bag, VT: Vacuum sealed transparent bag, VO: Vacuum sealed opaque bag, Raw: Raw cassava sample, lafun: Cassava flour with zero packaging

The variation in water activity had significant effect on the microbial load of the stored lafun samples which is shown in Table 1. Vacuum packaging material had better microbial count for bacteria at day 90 (6.2 log cfu/g and 6.7 log cfu/g for vacuum opaque and vacuum transparent material, respectively). This result was better than that of the Ziploc packaging material which has a high microbial count compared to the vacuum packaging material at day 90. This trend occurs in the fungi count of all the samples in storage durations. The highest fungi count was also observed in Ziploc transparent material which has 9.0 logCFU at day 90. The total viable bacteria count and fungi count varied between the packaging as it was observed that Ziploc material has higher bacteria and fungi count than vacuum material as the days of storage increased and this was suggested to be due to different relative permeabilities of packaging materials either to atmospheric conditions, gases, and environmental changes (Butt *et al.*, 2004). Coliform growth was not detected in any of the packaging materials which indicates that the lafun samples produced was safe for consumption due to controlled fermentation used in processing the lafun samples such as blanching to reduce microbial load and also, lactic acid bacteria used during fermentation have been known to inhibit the growth of several spoilage and pathogenic bacteria through their ability to initiate rapid decrease in pH of a fermenting raw material (Rault *et al.*, 2009).

Raw cassava had high MC (73.00±0.32) which significantly reduced after processing into lafun (3.50±0.09) due to one of the processes involved in processing cassava to lafun which is drying (oven drying). The MC in all our packaging material increased significantly as the duration of storage increased from 30 to 90 days. The lowest MC was found in Ziploc packaging materials (ZO 3.56±0.38 and ZO 2.62±0.37) while the highest MC was found in transparent packaging materials (ZT 9.70±0.43 and VT 10.85±0.14).

Protein content increased in all the packaging material comparing to lafun and raw with exception to vacuum opaque material after 90 days of storage. The previous studies (Fawole, 2019) showed due to fermentation process and that protein content in fortified cassava increases. The protein value later decreased after 90 days duration in all the packaging material. This was observed by Çabuk *et al.* (2018), they noted the reduction in protein concentration after fermentation could indicate that the samples impacted contain more sulfur amino acids,

Table 6: Sensory evaluation of lafun samples consumer attribute and intent for lafun samples in different packaging materials

Days	Packaging material	Attribute				Intent	
		Overall liking	Appearance liking	Odor liking	Texture liking	Consumption	Purchase
Day 0	None	3.68±1.25 ^a	3.76±1.05 ^a	3.08±1.12 ^a	3.52±1.39 ^a	2.76±1.73 ^a	3.12±1.45 ^a
Day 30	Ziploc transparent	3.65±1.09 ^a	3.60±1.19 ^a	3.45±1.09 ^a	3.7±1.17 ^a	3.35±1.14 ^a	3.45±1.23 ^a
	Ziploc opaque	3.75±1.21 ^a	3.55±1.32 ^a	3.5±0.76 ^a	3.8±0.69 ^a	3.3±1.53 ^a	3.25±1.48 ^a
	Vacuum transparent	3.7±1.17 ^a	3.65±1.04 ^a	3.75±0.91 ^a	3.6±1.91 ^a	3.3±1.34 ^a	3.3±1.34 ^a
	Vacuum opaque	3.35±1.09 ^a	3.30±1.0 ^a	3.35±0.93 ^a	3.55±0.99 ^a	3.35±1.08 ^a	3.30±1.08 ^a
Day 60	Ziploc transparent	3.6±1.09 ^a	3.35±1.09 ^a	3.65±1.18 ^a	3.8±0.89 ^a	3.25±1.25 ^a	3.25±1.25 ^a
	Ziploc opaque	3.8±0.95 ^a	3.35±0.93 ^a	3.75±0.79 ^a	3.95±0.76 ^a	3.75±0.97 ^a	3.8±1.28 ^a
	Vacuum transparent	3.65±1.31 ^a	3.65±1.14 ^a	4.1±0.97 ^a	3.8±0.95 ^a	3.75±1.59 ^a	3.75±1.52 ^a
	Vacuum opaque	4.05±1.05 ^a	3.75±1.02 ^a	4.15±1.04 ^a	3.95±0.99 ^a	3.95±1.23 ^a	4.1±1.17 ^a
Day 90	Ziploc transparent	3.85±0.75 ^a	3.80±1.00 ^a	3.70±0.80 ^a	3.90±0.85 ^a	3.65±0.99 ^a	3.70±0.98 ^a
	Ziploc opaque	3.70±0.98 ^a	3.50±0.95 ^a	3.45±1.00 ^a	3.55±1.23 ^a	3.45±1.23 ^a	3.45±1.09 ^a
	Vacuum transparent	4.20±0.77 ^a	4.05±0.95 ^a	4.05±0.95 ^a	4.15±0.93 ^a	4.15±0.93 ^a	4.30±0.92 ^a
	Vacuum opaque	4.20±0.86 ^b	3.20±0.77 ^a	3.50±0.89 ^{ab}	4.00±0.92 ^b	4.05±0.73 ^b	4.00±0.73 ^b

Result was determined using the 5-point hedonic scale. Similar letters in a column are not significantly different (p>0.05)

which the LAB starters may have metabolized. Lafun sample packaged in Ziploc opaque material contained the highest protein content at the end of 90 days of storage. The observed increase in protein content could be attributed to increase in MC as there will be increase in the activities of microbes (Butt *et al.*, 2006), although there was no significant difference in all the packaging materials during this period.

The lipid contents of lafun samples slightly decreased during the storage period. The fat content value between all the packaging materials was not significantly different in day 30 and day 60 of storage but significantly different in day 90. This decrease could be attributed to the possible high proteolytic and lipolytic activities of the corresponding enzymes which, in turn, led to the loss in the nutrients (Agrahar-Murugkar and Jha, 2011). The highest lipid content was found in VT day 90 (5.69 ± 0.82) while the lowest was found in VO day 60 (1.80 ± 0.37).

Ash content decreased significantly in all packaging material as the storage time increased. The crude lipid, ash, crude fiber, and CHO show no significant difference when observed down the column. The samples were statically the same with lafun. The highest CHO was observed in lafun packed in opaque Ziploc bag (84.5 ± 0.26) while the lafun in the vacuum transparent material had the lowest CHO value (75.67 ± 2.71). This finding suggests that at lower MC, percentage CHO could increase depending on the percentage of all other components (protein, fat, and ash). This corresponds with the literature on the effect of different packaging materials on garri quality (Ogiehor and Ikenebomeh, 2006). The authors observed that package with the highest MC gave a corresponding decrease in percentage CHO and vice versa.

The bulk density of the lafun varied from 0.49 to 0.68 g/ml for the packed bulk density and 0.39 to 0.60 g/ml for loose bulk density which was desirable. This value falls within the value 0.52–0.62 g/ml reported by Nwancho *et al.* (2014) which shows that lafun samples in all the packaging material still had a good bulk density even after a long storage duration. The storage and packaging materials had significant ($p < 0.05$) effect on the packed bulk density and loose bulk density of lafun. Dispersibility is a measure of the reconstitution of flour starch in water, the higher the dispersibility, the better samples reconstitute in water as reported by Adebowale *et al.* (2008) and Awoyale *et al.* (2020). Dispersibility varied from 61.00 to 62.33% after 30 days, 59–62% after 60 days, and 61–67% after 90 days of storage. Lafun samples in Ziploc transparent material had the highest dispersibility which will allow it to be easily reconstituted in water without lump formation. For the dispersibility in this study, no significant difference was observed between the packaging material in storage day 30 and day 90.

The WAI represents the ability of a product to associate with water under conditions where water is limited. The WAI across different packaging material in day 30 and day 90 had no significant difference but there was significant difference between the packaging materials in day 60. The lafun sample ranged from 217 to 253% in Ziploc transparent, 222–255% in opaque Ziploc, 225.3–253% in vacuum transparent, and 218–253% in vacuum opaque swelling power (SP) of starchy foods reveals the extent of associative forces within the granules; thus, the higher the SP, the lower the associative forces (Sanni, 2005). The swelling power of the freshly processed lafun samples with zero packaging decreased as the storage day increased in day 30 and day 60 but had a slight increase in day 90 with exception to the vacuum opaque material which continued to decrease in this study, swelling power showed significant difference between the packaging materials in all the storage duration ($p < 0.05$). The solubility index (SI) is related to the extent of leaching of amylose out of starch granules during swelling and affected by intermolecular forces, and the presence of surfactants and other associated substances (Awoyale *et al.*, 2020) of the stored lafun ranged from 4.03 ± 0.29 to 6.70 ± 0.35 in day 30, 6.33 ± 2.31 to 11.33 ± 2.21 in day 60, and 4.75 ± 0.44 to 77.33 ± 0.29 in day 90. A significant difference in all the packaging materials ($p < 0.05$) existed in the solubility index during the storage periods.

The carotenoid decreased significantly in raw cassava (1.977 ± 0.012 – 0.848 ± 0.011) in lafun sample. Decreased was observed in all samples when compared to lafun. It was also observed that a decrease in the carotenoid content was much in transparent packaging materials when compared to lafun. At the last day of storage (90 days), Ziploc transparent has the least value of carotenoid content of 0.212 ± 0.04^a and this is due to the fact that samples in transparent packages are more exposed to light than those in opaque thus, leading to decreased in carotenoid content as carotenoid is heat and light sensitive (Chavez *et al.*, 2007).

Pasting properties are functional properties relating to the ability of an item to act in paste-like manner. According to Wang and Copeland (2013), starch granules when heated become hydrated, swell, and are transformed into a paste. The peak viscosity is the maximum viscosity developed during or soon after the heating of the floury product (Adebowale *et al.*, 2008), and in this study, it ranged from here it ranged from 165.08 RVU to 550.33 RVU, the highest value was found in freshly processed the lafun samples before it was packaged into different packaging materials. Nwancho *et al.* (2014) reported similar value (322.67 RVU) for gari produced from dried cassava chips. The peak velocity reduced significantly across the packaging materials as storage day increased. After 90 days of storage, the Ziploc opaque material had the lowest peak velocity value (165.58 RVU) followed by the vacuum opaque material (194.17 RVU). Trough viscosity (TV) is the minimum viscosity that occurs after the initiation of product cooling; thus, it measures the ability of the paste to withstand breakdown during cooling. TV value for the lafun sample after processing was 256.08. It was observed that as the storage day increased the TV value decreased across all the packaging materials. This value ranged from 103.42 to 141.58 RVU. The Ziploc opaque material had the lowest TV value. Breakdown viscosity (BDV) reflected the ability of the sample to withstand shear stress and heating during cooking for this study, after 90 days of storage, the BDV value of the lafun samples stored in all the packaging material has a significant decrease from the lafun sample after processing. The highest value BDV was recorded for zero storage, and the value was noticed to decrease with storage periods the value ranged from. This was also noticed by Awoyale *et al.* (2020) in their storage study on gari in different packaging material. FV is the ability of the flour to form starch and viscous paste or gel after cooking and cooling (Maziya-Dixon *et al.*, 2007). The FV value ranged from 148.75 to 336.25 in lafun samples stored in different packaging materials with the highest value recorded for freshly processed lafun with zero storage. The FV value too also decreased with storage duration in all the packaging materials. Setback viscosity (SBV) gives an idea about the retrogradation tendency of starch in the flour sample after 50°C. However, the highest value was seen in the lafun samples with zero storage day: High SBV values have been reported to affect dough digestibility (Shittu *et al.*, 2001), while lower values, which were recorded for the 3rd month of storage, are beneficial as they indicate a lower tendency for retrogradation (Sandhu *et al.*, 2007). The vacuum transparent had the lowest SBV value after 30 days of storage. Peak time is reported by Adebowale *et al.* (2005) to be a measure of the cooking time of the flour. The peak times obtained for this study ranged 4.20–4.47 for lafun stored in Ziploc materials while for the vacuum packaging material, the value also ranged from 4.27 to 4.47. There was no significant difference in the peak time value between the packaging materials and storage period. Pasting temperature (PT) is an index of the minimum energy required to initiate rapid water ingestion, swelling, and eventual gelatinization of starch granules (Awoyale *et al.*, 2016). Thus, the PT of the lafun samples was observed to fall below 100°C. The highest PT value was in the lafun samples with zero storage and the lafun samples in all four packaging materials ranged from 75.00° to 75.90°C which is between the value in Awoyale *et al.* (2016) (78.36–80.40°C), and values reported by Sanni *et al.* (2008) (63.40–64.65°C).

However, there was decreased in hydrogen cyanide of the samples illustrated in Fig. 1. Before processing of cassava, the hydrogen cyanide gives 0.6 while after processing (day 0), and the hydrogen

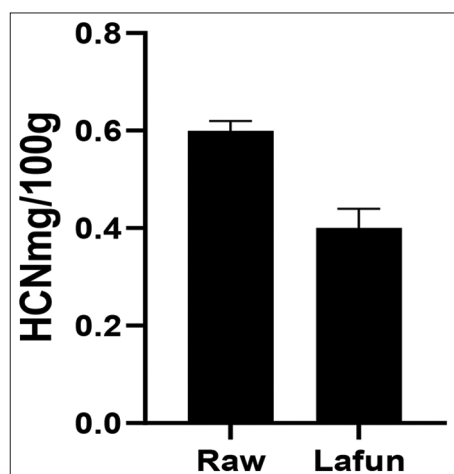


Fig. 1: Hydrogen cyanide in raw and cassava flour

cyanide decreased to 0.4. The reduction in HCN could be credited to the processing method of the cassava, for example, grating and drying as both methods have been the rate of linamarin breakdown and cyanogens reduction (Eleazu and Eleazu, 2012).

The sensory evaluations carried out on the sample are shown in Table 4.7. The table review that the odor, texture attribute, overall liking, appearance, and intent were all rated at the same level as there was no significant difference in the samples.

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