

ORIGINAL RESEARCH ARTICLE

Molecular Characterization of Enterotoxin Genes from Food-borne Pathogen, *Bacillus cereus*

Ogori Akama F*¹, Utim Susan M²

1. Federal University of Agriculture, Department of Food Science and Technology, PMB 274, Benue State, Nigeria
2. Federal College of Education Kontagora, Department of Home Economics, PMB 39, Niger State, Nigeria

ABSTRACT

The research work is on physical, photochemical and chemical quality of traditionally processed cassava ball and chunks, lending credence to home use, small scale or medium enterprise/factories on the potential of the chunk and balls in the market. The quality characteristics of cassava flour from the meal of retted dried balls and chunks from randomized sampling had their physical, Physio-chemical and chemical characteristics analyzed using standard methods. Physical properties of flour had (2.50-11.25) g/g water binding capacity, (2.15-18.0) w/v gelation capacity and (25.75-45.35) viscosity. Physio-chemical properties of flour had titratable acidity (0.011-0.125), (4.07-4.99) pH and (1.85-8.75) mg/100g hydrocyanic acid content of the meal of retted balls flour which agreed with the standard. Chemical properties had (1.05-1.53) % crude protein, (3.75-7.0) % ash and (2.87-3.78) % crude fiber. The (79.38-83.1) carbohydrate and (324.49-345.0) energy value from dried chunks flour were high and compared favorably with the standard.

Keywords: Physiochemical, Chemical, Hydrocyanic Acid, Dried Cassava Ball, Dried Cassava Chunks.

INTRODUCTION

Tropical tuber crops constitute one of the most important staple food commodities in the world.

The major tuber crops include sweet potatoes, yam,

Irish potato, cassava and cocoyam. These are usually high in moisture content which affects the storage under ambient conditions.

Cassava (*Manihot esculenta* crantz) is a crop belonging to the Euphorbiaceae family, classified into bitter and sweet cassava type.¹ About 34 million tonnes of the world cassava produce are from Nigeria². Cassava potential uses cut across human consumption, animal feed stock, industrial

Address for correspondence:

Ogori Akama F*

Federal University of Agriculture, Department of Food Science and Technology, PMB 274, Benue State, Nigeria

E-mail: faraday1232002@yahoo.com

or medicinal products and valued market profit.^{3, 4} Traditional meals of retted dried cassava ball and chunks are processed by large segments of the Nigeria population by peeling washing slicing, fermenting, draining or molding and drying. Cassava chips flour has been reported to be a better quality food and of longer shelf life than a potato.⁵ The fleshy portion of cassava contains 62% moisture, 35% starch, 1% protein, 0.3% fat, 2% fibre and 1% ash. The fresh roots contain 35mg/100g of vitamin C, trace amount of niacin and fat soluble vitamins.¹ Cassava chips have a wide application for dough and paste, for composite flour making and starch as a source of fermentable sugar required in the production of alcoholic beverages.⁶ Processed cassava flour has been reported to be good weaning food, feed ingredient and bakery substitute.⁷

The most economical method of processing cassava is by drying. The traditional drying process is carried out by the local women who normally target the period of scarcity as the purpose of preservation. The drying is carried out under unhygienic environment resulting in products of low hygienic quality.⁸ It is important for food inspectors, processors and handlers to keep dried cassava balls and dried chunks and its food safe from pathogens. Several microorganisms have been known to affect the quality of food outside the natural flora, thereby constituting a health hazard when contaminated food is consumed, and posing

questions on food safety measures. Meal of retted dried Cassava balls are low acid foods during processing with limited and slow rate of water loss on sun drying which generally favours spoilage food intoxication, infection and quality reduction resulting from the chemical interaction.⁹ Food quality is that whole characteristics of food that make food important chemically, physically and economically, hence the quality of traditional processed dried cassava ball and dried chunks are of much importance. The Nigerian government has approved the inclusion of 10% cassava flour in wheat flour for economic purposes. Also the oil company had indicated interest in admixture of alcohol and petroleum as a substitute for crude oil, which may require varied source other than modern method of processing cassava root.

The technological application of flour from dried cassava balls and dried chunks will depend on some of their functional and nutritive properties. However, limited information are available on traditional processing, preservation or safety and quality of meal of retted dried cassava balls, cassava chunks and products, hence there is need to carry out in-depth study on flour from meal of retted dried cassava balls and dried chunks that are locally dried to furnish intending users with information, encourage production and make possible technical assistance on processing and marketing as raw materials for intending factories, consumers and health inspectors.

Processors are concerned with ways of processing cassava into utilizable states. Meal of retted dried cassava ball and dried chunks are common in Benue State with less concern for its quality, therefore there is an urgent need for quick physico-chemical and microbiological analysis of this dried cassava balls and cassava chunks to evaluate its potential quality, ascertain process divisibility for both intending users at industrial and international markets. Cassava roots base technology is raising rapidly and the need to appreciate a long time usage of traditionally processed cassava roots by local consumers or villages in the middle belt region of Nigeria are important. The technological application of meal of retted dried cassava balls and dried chunks will depend on some of their functional and nutritive properties. However, limited information is available on traditional processing, preservation or safety and quality of flour from dried cassava balls and chunks.

The research work seeks to establish relationship between traditional processed meal of retted dried cassava balls and dried chunks and its properties, by reasons of local processing approach, not all of their properties may deviate from standard restricting guide for specific processing or production of cassava based traditional products or use as raw materials. The study may add value to limited utilization of traditionally processed meal of retted cassava ball and dried chunks, careful target for health consumer and guide for food

safety measures. The knowledge of physical, chemical, quality of food eaten in a place is valuable in identified and solving nutritional and health problems of the population.

MATERIALS AND METHODS

Raw Material

The understudy site within the metropolis includes Wurukum, Wadata, North Bank, High-level and Fiidi, were ten respondent processors from each site are interviewed on how the meal of retted dried cassava balls and dried cassava, chunks were traditional processed, based on variety of cassava used, the fermentation equipments, the source of water, sanitary conditions and methods of packing from drying flour. Sample of meal of retted dried cassava balls or simply cassava ball and the dried cassava chunks for the study were obtained from different locations.

Random sampling from ten points within a site were done using the America microbiological specification of foods, and the three class manual methods of mixing, which is adopted by the Association of American Feed Control officials.¹⁰
¹¹ Samples were sealed in polyethylene bags stored in cooler and then conveyed to the laboratory.

Preparation of Flour from Rettened Dried Cassava Balls

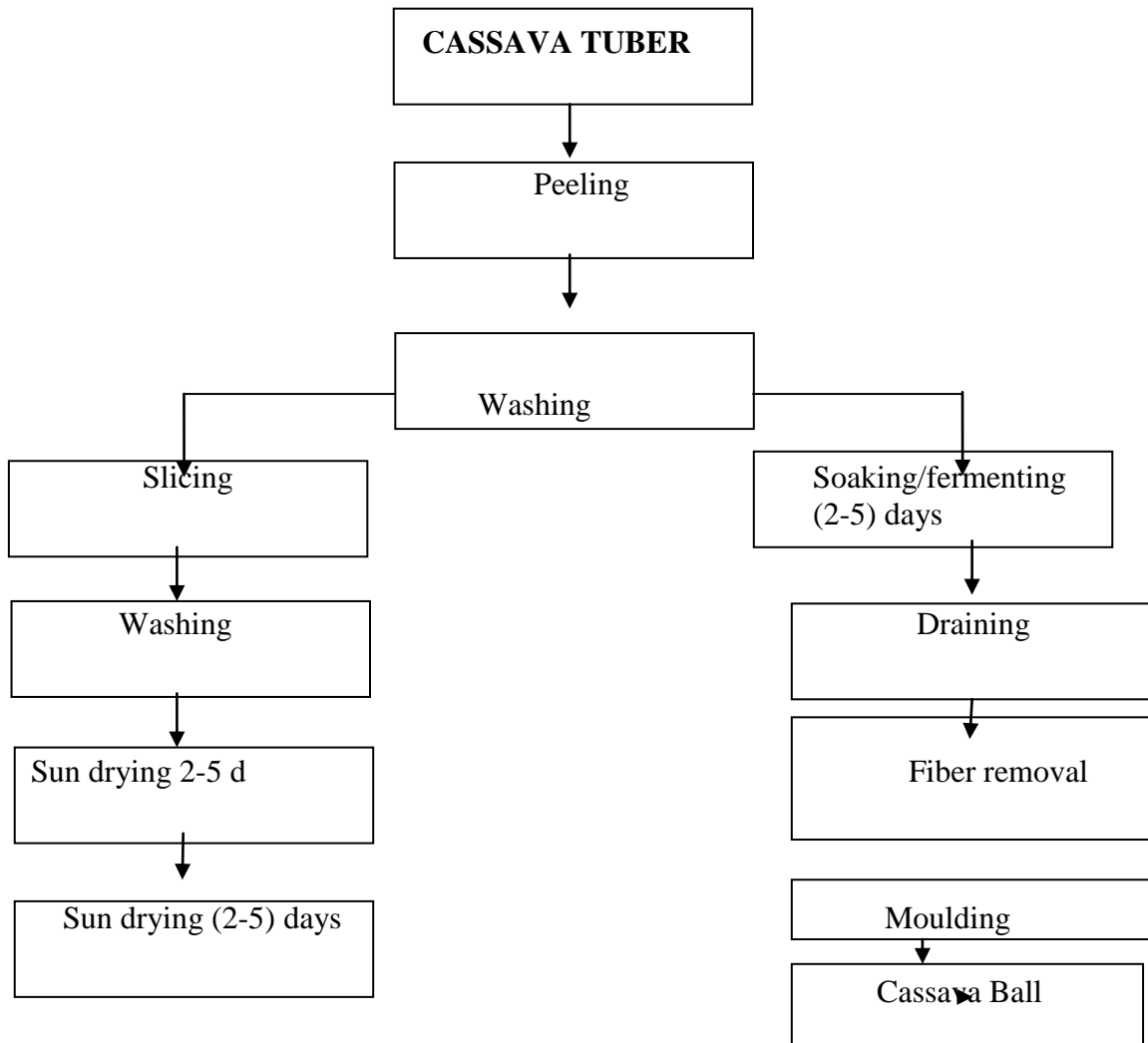
Two kilogram weight of dried cassava balls from each represented sample was thoroughly hand mixed in an aluminium bowl, about 10kg weighed of representative dry sampled cassava balls, were milled with a hammer mill (type 8' lab mill). The resulting flour was saved using 100 um aperture size.

Preparation of Flour from Dried Cassava Chunks

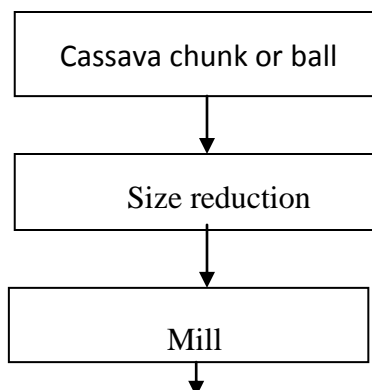
Two kilogram weight of dried cassava chunks from each sample was thoroughly hand mixed in an aluminium bowl, about 10kg weighted of preventative dry sampled cassava chips, were milled with the hammer mill (type 8' lab mill). The resulting flour was sieved using 100 um aperture size

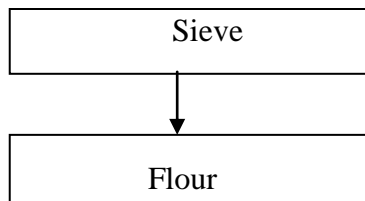
Referral sample or standard: National agency for food drugs administration and control with standard organization of Nigeria, flour characteristics properties were used.

Processing of Cassava Balls and Chunks from Cassava Tube¹²



Processing Cassava Ball and Chunks into Flour





Chemical analysis

Proximate analysis was by AOAC¹³ and carbohydrate was by differential according to AOAC. Phytochemical analysis for PH was by Persons¹⁴, Hydrocyanic acid was by Attire and See and titrable acidity was by AOAC.¹³

Physical analysis

Swelling index was by Fleming et al, Gelation capacity was by Coffman and Garcia, Water absorption capacity was by acacia and Bello and Energy value was by Osborne and Voogt.^{15, 16}

RESULT TABLES

Table 1: Physical properties of flour from dried cassava balls and chunks

Sample	Water (binding)	Gelation		Swelling index	Viscosity
	Capacity (g/)	Capacity (w/)	40°C	70°C	cpp)
RS	34.5
CBWU	0.25±0.20d	4.35±0.700a	0.0±0.00d	2.20±0.00a	42.9±0.10b
CBWA	7.75±0.25a	2.16±0.32b	25±0.02b	1.52.5±0.50b	35.75±0.3c
CBH	2.50±0.29c	4.15±0.50b	20±0.50b	80.20±0.50d	35.75±0.35c
CBFD	3.75±0.25b	4.25±0.50b	20±0.50b	81±0.20d	25.75±0.35d
LSD	0.00	0.83	0.00	6.19	0.76
CCWu	2.75±0.25c	10.35±0.70c	37.5±0.5b	196±0.04a	33.50±0.50b

CCWA	2.50±0.00d	2.15±0.31a	4.0±0.3c	183.5±0.11bc	44.90±0.10a
CCN/B	5.25±0.25b	18.0±0.35a	2.0±0.10c	190.5±0.12ba	27.75±0.25c
CCH/L	11.25±0.45a	4.22±0.45d	2.0c±0.20c	190.5±0.10ba	27.75±0.25c
CC FD5	5.25±0.25b	16.17±0.35b	50.0±0.13c	178.5±0.13c	45.05±0.019a
LSD	0.00	0.96	4.06	9.88	4.10

Means in the same column followed by the same superscript are not significantly different ($P \geq 0.05$).

Mean \pm standard deviation from duplicate determinations

ND = Non detected

RS = referral Standard

Cbwu=cassava ball flour from wurukum. CCwu=cassava chunksflour from wurukum

Cbwa=cassava ball flour from wadata. Ccwa=cassava chunk flour from wadata

CBnb=cassava ball from north bank. Ccwn/b=cassava chunk flour from north bank

CBh/l=cassava ball flour from high level. Ccwh/l=cassava chunk flour from high level

CBfd=cassava chunksflour from fiidi. CCfd=cassava chunk flour from fiidi

TABLE 2: Physio-chemical properties of flour from dried cassava balls and chunks

Sample	P^H, Titratable Acidity and Hydrocyanic Acid content		
	pH	TA	HCN (mg/100)
RS	5.00	0.1	10.0
CBWU	4.99±0.1ad	0.011±0.10a	1.85±0.10d
CBWA	4.89±0.1ab	0.013±0.10a	8.65±0.50a
CBH	4.07±0.2cb	0.030±0.20a	2.75±0.25a
CBFD	4.40±0.3ba	0.019±0.20a	4.25±0.10c
LSD	0.5	0.01
CCWu	5.0±0.10c	0.125±0.01a	4.95±0.1d
CCWA	5.32±0.30b	0.106±0.10b	18.5±0.65a
CCN/B	6.32±0.30b	0.019±0.10c	14.95±0.60b
CCH/L	6.03±0.30a	0.004±0.20d	9.5±0.35d

CC FD	5.44±0.25b	0.016±0.40c	13.0±0.510c
LSD	0.24	0.007

Means in the same column followed by the same superscript are not significantly different ($P \geq 0.05$).

Mean ± standard deviation from duplicate determinations

ND = Non detected

RS = referral Standard

Cbwu=cassava ball flour from wurukum. CCwu=cassava chunksflour from wurukum

Cbwa=cassava ball flour from wadata. Ccwa=cassava chunk flour from wadata

CBnb=cassava ball from northbank. Ccwn/b=cassava chunk flour from northbank

CBh/l=cassava ball flour from high level. Ccwh/l=cassava chunk flour from high level

CBfd=cassava chunksflour from fiidi. CCfd=cassava chunk flour from fiidi

Table 3: The proximate composition of flour from dried cassava Ball and Chunks

Samples	protein (kca)	Crude Fat (%)	Ash (%)	Moisture (%)	Crud fibre (%)	Carbohydrate (%)	Energy Value
RS -	0.60	10.00	2.00	67.50	-----
CB W	1.42±0.00a	1.05±0.23d	4.0±0.50	12.50±0.5a	3.67±0.43a	77.36±0.36a	324.49±0.15a
CB WA	1.20±0.00bc	1.20±0.02c	2.75±0.35a	11.25±0.75b	3.55±0.01a	80.05±0.40a	80±0.16a
CB N/B	1.14±0.01bc	1.60±0.02a	3.75±0.25a	12.62±0.38a	3.48b±0.00ba	77.41±0.10a	328.60±0.6a
CB H/L	1.31±0.01ba	1.31±0.01b	2.75±0.39a	10.0±0.00c	3.12±0.30bc	81.51±0.28a	343.05±0.11a
CB FD	1.09±0.00c	1.05±0.31d	7.0±0.40a	9.0±0.10d	3.07±0.13	79.79±0.39a	329.05±0.10a
LSD	0.19	0.00	5.84	7.53	0.37	11.24	45.30
CC WU	1.31±0.10b	1.25±0.75a	3.65±0.75a	10.0±0.00a	3.78±0.03a	79.82±0.10b	
	3.36.35±0.4ab						
CC WA	1.05±0.01c	1.0±0.00d	4.35±0.00a	7.5±0.75b	3.00±0.21a	83.10±0.00a	345.0±0.00a
CCN/B	153±0.50a	1.20±0	5.0±0.25a	10.0±0.00a	3.67±0.03a	78.60±0.50b	345.0±0.20b

CCH/L	1.31±0.32b	1.0±0.25d	5.0±0.39a	9.75±0.25a	2.87±0.03a	80.10±0.28b	332.65±0.40b
LSD	0.06	0.00	1.49	0.40	0.94	2.54	10.02

Means in the same column followed by the same superscript are not significantly different ($P \geq 0.05$).

Mean \pm standard deviation from duplicate determinations

Cbwu=cassava ball flour from wurukum. CCwu=cassava chunksflour from wurukum
 Cbwa=cassava ball flour from wadata. Ccwa=cassava chunk flour from wadata
 CBnb=cassava ball from northbank. Ccwn/b=cassava chunk flour from northbank
 CBh/l=cassava ball flour from high level. Ccwh/l=cassava chunk flour from high level
 CBfd=cassava chunksflour from fiidi. CCfd=cassava chunk flour from fiidi

DISCUSSION

Physical properties of Flour from Dried Cassava Ball and Chunks

The significant high water binding capacity exhibited by cassava ball flour may be due to the presence of more hydrophilic carbohydrate exposed sights may be due to cell disarrangement of the cassava ball during rationing.¹⁷ The higher water binding capacity from high level cassava chunks indicate that it is more hygroscopic than the other samples. The low gelatin capacity in cassava balls flour showed that fermentation change carbohydrate quality and composition of the product failure. This may be due to activity of hydrolytic enzymes, changing gelling properties to other metabolite resulting in a decrease in gelatin concentration obtained. This is an advantage to wheat miller and bread maker using cassava ball

flour as an admixture since little bond may have to be broken by their mixers. The high gelatin capacity of cassava chunks flour showed that when war is no fermentation of cassava there is little or no change in carbohydrate composition. A Little endogenous enzyme activity may have resulted in the breakdown of starch with no further conversions, making more flour available for cell formation hence the increase in dilation concentration obtained. This is an advantage to animal feed and ethanol producer. The observed high swelling power of the flour obtained from cassava chunks at 70oC, indicate that steaming may increase the solubility of cassava chunks flour. This may be due to leaching of dextrin because of glucosidal bond breakage. Due to little or no complexity resulting from fermentation of cassava chunks flour, this may encourage sedimentation and flocculation ability for pharmaceutical

purposes. The higher swelling power of flour obtained from cassava chunks indicate that this may give higher yield of fufu past compared to the flour from cassava balls flour.

The viscosity values are in confirmation by, that granules rupture so easily on stirring. This may indicate the weak type of bonding force to maintain granules structures in cassava balls and chunks flours.¹⁸ The cassava balls flour had higher viscosity value compared to cassava chunks flour, this indicate that cassava balls flour will have high resistance to rupture due to high concentrated metabolites and easy to leaching due to its weak bound forces. The increase in viscosity of the ball flour also observed in Wurukm samples may also be due to activity of hydrolytic enzymes and natural floral during fermentation, producing metabolites, starch, sugar which may have re-concentrated during sun drying. The low viscosity of the cassava chunks flour may be as a release of bound water and unbound water.¹⁹ This indicates that it can be used as weaning formula since it will easily digest (in the absence of cyanide toxicity), flour concentration would be required to form gels.

pH Titratable Acidity and Hydrocyanic Acid of flour from dried cassava balls and chunks

From table 2 above, Cassava chunks PH of flour were significantly high. The slight increase may be due to little or no fermentation of the slice chunks

of sun drying after washing. These were however below PH values reported by for bitter and sweet cassava processed flour. The titratable acidity of both cassava balls and chunks flours were in agreement with the work of who reported that periodic fermentation of Gari from dried cassava chunks increased titratable acidity of the flour.²⁰

The hydrocyanic acid content of cassava balls flour agreed with (WHO) and the Indonesian standard, there were significantly lower than cassava chunks. The observed lower value in the hydrocyanic acid content of cassava balls flour could be due to retained dried balls and more hydrophilic sites, maybe humidity variation during drying which may have changed the chemical makeup of the drying balls. According to cassava cyanide disease network wetting of the flour reduces hydrocyanic acid content to third size of the original quantity.

Proximate Composition of Flour from Dried Balls and Chunks

From table 3 above, the moisture, crude fiber, carbohydrates and energy values of the flour from cassava balls and chunks flour were similar to the values reported by.^{16, 21} The value in crude protein, Ash, carbohydrate content and energy value from cassava ball flour may be due to enzymatic actions and micro floral activities which might have used soluble substrate during tuber retting.²²

Fat content were above the value reported by Iwuoha et al. The similarity values in fat content from cassava balls and cassava chunks may be due to reduction in lipolytic enzyme. It may also be due to little or no alteration in protein, ash, crude fiber content of the flour as reflect in table three. The values were consistent will reports by Iwuoha et al. The high ash content from cassava ball of Fiidi sample may be due to tuber returning. Since fermentation liberate mineral ions like sodium, iron and soluble zinc. The high values in moisture observed in cassava ball flour from Wurukum, Wadata and North Bank may be due to starch modification during fermentation which could have allowed more hydrophilic regions; this could affect storage of cassava balls flour. Lower values observed in moisture from cassava chunks flour may be due to chunks sizing and its small surface area.¹⁸

The high crude fiber values were significantly above referral standard this may be resulting from poor peeling and sizing before washing and fermenting .The significant difference in cassava ball flour over cassava chunks flour could be due to micro fungi surface contaminations during fermentation. This could promote peristaltic movement of the gastrointestinal tracks of fufu made from cassava balls. The significant high carbohydrate content of the cassava ball and the chunks were consistent with reports by Njoku and Aluyah. Cassava ball flour had lower carbohydrate

content due to hydrolytic enzyme activity producing other macromolecules and metabolites.²²

The energy value of cassava ball flour samples were significantly below that of cassava chunks flour samples this was obvious in the High-level sample. This may be due to rationing resulted in breakdown of starch. Fermentation allows less interaction of protein, fat, crude fiber and ash. This bound loss may have resulted in interaction with leaching processes. The energy value obtained from cassava chunks flour from North Bank samples were significantly higher which may be due to less activity of micro flora and hydrolytic enzymes on the slice dried chunks.¹²

CONCLUSION

Proximate properties of cassava chunks flour were higher than cassava balls, hence an attraction for feed and ethanol productions. Cassava ball flour can keep longer than the chunk flour because of its moisture content. The water binding capacity, viscosity, pH titratable acidity and hydrocyanic acid content in cassava ball flour were high, compared to cassava chunks flour which may be potentially good for composite flour technology. Proper hygiene and sanitary practice by processors of dried balls and chunks during hand drying , packing from dried flour should be improved and wire quash , plat-form and drying using tarpaulins should be adopted on drying instead of drying balls and chunks by the roadsides.

REFERENCES

1. Purselove, J. W. Tropical Crops. Dicotyledons Longman Scientific and Technical Co. Pub. In USA John Wiley and Sons. New York, 1991.
2. F.A.O. Global cassava production food outlook. 2001, 4 Oct, vol 22.
3. Asonye C.C. Fortification of common Nigeria food cassava meals food and nutrition bulletin. 2001; 22(40): 67-86.
4. Spencer D.S AND Lyman, J.K. The cassava transformation. Michigan state university press, East Lansing, 2002, page no. 273-290.
5. Bokanga, M. Mechanism of the elimination of cyanogens from cassava during traditional processing In: Tropical African Foods. Quality and nutrition ed. West by A. and Reilly P.J.A Proc. Of the Regional Workshops held by the international foundation for science (IFS) Dar as Salaam Tanzania. 1991, page no. 157-160.
6. Amutha, R. Gunusekaram, P. Production of ethanol from lignified cassava starch using Co immobilized cell of zymomonas Mobilis and Sac diastiticus J Biosci Bio Eng. 2001; 92, 6: 560-564.
7. Lekule, F.P. AND Sarwatt, S.V. Processing and Utilazation of cassava as livestock feed in Tanzania in review from cassava as livestock feed I Africa Ed Ravidran S and Kenkpan D. 1992; 135-138.
8. Kwaisa, J.T. Cassava processin Ghana, in: In praise of cassava in: proc of the inter regional experts group meeting, 1998.
9. Cheftel, J. L lug, J.L and Lorient, D. Amino Acid, Peptide and protein. In: Fennema O.R ed. Food Chemistry. 2nd ed. New York. 1985 245-369.
10. America Microbiological Specification of Food (AMSF) In: Bethy Hobbs and Diane Roberts (ed) food poisoning and food hygiene. Publisher Edward Arnold in Great Britian. 1980; page. 281-285.
11. Ameriaca Association of Feed Control Officials, (AAFCO). In: John Richard (ed), sampling and sample preparation for mycotxin analysis. Publisher Roner labs guid Inco. USA. 2002; Page 19-24.
12. Akpapunam, M. A, Inyang, C.U and TSAV-WUA, J. A. Studies on traditional processing and preservation of fermented cassava flour kpor-u-million mash. (M.Sc.) Thesis, 2000.
13. AOAC. Official Method of analysis 15th Ed. Association of official analytical chemist Washington D.C. 1995.
14. Pearson, D. The Chemical Analysis of Foods, 6th ed. Churchill London. 1976, 173.
15. Fleming, S.F., Salsulkin, K. W. Kulana, D. and Humbert, E.S. Viscosity and water absorption slurries of some flower and soy bean flour

- concentrate and isolate J. Food Science. 1974; 53:188-19.
16. Osborne, D. R and Voogt, P. The analysis of nutrient in food, 1978.
17. Ogbo, F.C. Improvement of organoleptic quality of retted cassava products by alkali pretreatment of roots and addition of sodium nitrate during retting Int. J. Food Micro. 2007; 15, 89-9
18. Njoku, B. A and Banigo, E.O.I. Physico-chemical properties of precooked cassava manihot esculenta crantz flour prepared by adaption of a traditional process. Nig food J. 2006; Vol. 24. 100-105.
19. Gallat, S. Preliminary study of the effect of Lactic fermentation on the rheological and pH of sorghum porridge abridon oxon; UK over sea development. Natural resource institute (ODNRI), 1989.
20. Oluwole, O.B. olatunji, O. and Odunta, S.A. A process technology for conversion of dried cassava chips into gari Nig. Food Journal. 2004; Vol. 22. 67-76.
21. Oyenuga V.A. Nigeria food and feeding stuffs, their chemistry nutritive value 3rd Edi Ibandon University press, 1980; page no. 99.
22. Oboh, G. Akudahunsin, A.A. Oshodi, A.A. Dynamics of phytate zine balance of funji fermented cassava product (flour and gari) plant food human nutrition. 2003; 58: 1-7.
23. Agboola, S. O Akingbola, J. O. and Oguntimein, G. B. processing of cassava starch for adhesive product starch. 1990; 42 (1) 12-15.