EFFECT OF PROCESSING ON AMYLOSE, RESISTANT-STARCH, RAPIDLY-DIGESTIBLE-STARCH, AND SLOWLY-DIGESTIBLE-STARCH FOR *GARI*, A PROCESSED CASSAVA PRODUCT.

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ABSTRACT
The objective was to investigate the effect of domestic processing on the digestion attributes of gari, a low-moisture food product from cassava commonly consumed in tropical developing nations, and important ethnic food amongst population groups in Europe and elsewhere.
During the production of gari, cassava-mash samples were analysed for amylose whilst pH of enzyme extracts from all samples were measured. Resistant-starch types 2 and 3 (RS_2, RS_3), rapidly-digestible-starch (RDS), and slowly-digestible-starch (SDS) in gari were determined.
The pH was almost stable at 5.3. SDS, RDS, RS_2 and RS_3 variations were 27.4-39.5, 18.2-24.7, 41.7-51.6, and 8.2-15.3 mg/100mg, respectively. Increase in RS_3, lower RDS, higher SDS and RS_2 suggested that gari has better potential as functional food compared to potato-chips (3.5gRS/100g), rice-cake (0.2gRS/100g), raw-banana (4.0gRS/100g), and cooked-white-rice (1.3gRS/100g). Therefore, gari may be considered for its benefits in the diets of wider population groups.

Introduction

Cassava is a key crop with underground edible tuber which is an important source of dietary calories for a large population in tropical countries in Asia, Africa and Latin America (Aryee *et al*, 2006., Cock, 1985). It can tolerate drought (Spencer & Associates, 1997), attracts high market values, able to replace other food crops and useful as starch-based industrial material (Nweke, 1992., Cardoso *et al*, 2005). There is, however, a storage problem for cassava tubers with rotting readily occurring within the first few days of harvest. In addition, the tubers are bulky which makes transport both difficult and expensive. Consequently, the crop is processed for better upkeep and to enhance its food and industrial utilisations.

One previous constraint to utilisation of some cassava genotypes as food products is the occurrence of high cyanogenic compounds (linamarin, lotaustralin (methyl linamarin)). Cassava naturally uses these compounds as defence mechanisms against predators during propagation. High or prolonged intake of the cyanogens could be deleterious to human because this has been suggested to result in food poisoning with symptoms like vomiting, nausea, dizziness, stomach pains, and weakness. In particular, adverse consumption had been associated although without any epidemiological data (Oluwole *et al*, 2002., Oluwole *et al*, 2003) with prominent *konzo* disease in Eastern, Southern & Central Africa. Tropical ataxic neuropathy (TAN) disease due to

intolerable level of cyanide is also associated with loss of vision and unsteady walking in older people in Nigeria (Oluwole, 2008., Bradbury, 2009).
Cyanogenicity in cassava genotypes varies widely, and its residuals in human foods depend on the method of processing used to produce the foods (Wilson, 2003). The initial concentration of cyanide in cassava including the genotype that is processed to foods however determines residual cyanide in foods. Several genotypes of cassava with very low level of cyanide below the concentration that the WHO recommended for human consumption (10ppm) have been developed, and these are early maturing, resistance to diseases, and of improved yield (Oboh & Akindahunsi, 2003; Ashaye et al, 2009). Processing of cassava into foods such as *fufu*, *lafun* and *gari* plays significant role in reducing the toxicity in cassava due to cyanide level which is predominantly present in the peels to a tolerable level in human. The peels are normally removed during the processing of the tuber crop for foods including *gari*.
Gari is a food product usually creamy-white, granular flour with a mild sour taste made from fermented, partially-gelatinized cassava tubers. It is very popular in many developing countries including Nigeria (Westby & Twidy, 1992), Sierra Leone (Blanshard *et al*, 1994), Ghana (Oduro *et al*, 2000), and Mozambique (Bradbury, 2009), and frequently consumed as ethnic food amongst population groups in Europe. Various cyanide levels have been found in the *gari* samples in most of the

geographical locations where the food is predominantly consumed, and in a recent study in Nigeria comparable mean residual values of cyanide to the WHO recommended value (10ppm) have been found in the endemic, south-eastern, south-western and northern areas of Nigeria where *gari* is major staple food as 9, 4, 7 and 13ppm, respectively (Oluwole, 2008).

No work has been published on the effect of processing on amylose content and the various starch compositions in *gari*, despite its being a low-moisture food product which continues to increase as ethnic diet in the West. There are literatures suggesting that ethnic groups may become vulnerable to diet-related diseases compared to obesity, cardiovascular disease and diabetes affecting some mainstream populations due to indiscriminate diet consumption (Gilbert & Khokhar, 2008., Stunkard, 1996). Incomplete and fragmented information on the composition of ethnic foods in Europe may exacerbate the poor state of diet-related diseases amongst ethnic groups. Therefore, it is necessary that food composition databases are extended to include important ethnic foods that are consumed by both mainstream and ethnic populations. This study has been undertaken to determine effect of processing on amylose content in cassava-mash whilst enzyme extracts of the fermenting mash were analysed for pH and amylase activity. The particle size, digestion profile in relation to resistant starch contents (RS_2, RS_3), rapidly-digestible-starch (RDS) and slowly-digestible-starch (SDS) of *gari* from five cassava genotypes were also determined. The parameters are suitable to determine the potential health benefits of *gari* as a food for wider population groups.

Materials & methods
A total of 5 cassava genotypes (12 months old) were obtained at the International Institute of Tropical Agriculture, Ibadan (IITA, Ibadan) Nigeria and used in this study. All reagents that were used were of analytical grade.

Preparation of *gari*
A solid fermentation procedure reported by Oguntoyinbo (2007) was used to prepare *gari* from cassava tubers. The tubers were harvested and manually peeled with knife, the peeled cassava tubers were washed with water and grated with a locally fabricated grater at IITA (Ibadan). The grated mash was packaged in coarsely knitted sacks for *gari* production. The sack containing grated cassava-mash was placed under hydraulic jack to simultaneously allow for spontaneous fermentation and dewatering process for up to 72 h. After this time, the solid cassava-mash was crumbled by hand and sieved on a locally woven flat basket. After sieving, the cassava-mash was dry roasted (fried) in a wide aluminium pot over naked fire from firewood to produce *gari*. Samples for analyses were taken from grated cassava and from the cassava-mash after 24, 48 & 72 h of simultaneous dewatering and fermentation. Samples were also taken from the sieved cassava-mash and roasted cassava-mash (*gari*). All samples except the roasted mash were dried in the cabinet drier at 60°C for 10h and stored in a dessicator placed at room temperature until required for analyses.

Determination of amylose content and pH in cassava-mash samples and *gari*
One hundred mg of samples were weighed into test tubes, five ml of 85 % methanol was added, the mixture vortexed and placed in a shaking water-bath that was maintained at 60°C for 30 min. Samples were centrifuged at 3000 x g for 15 min, supernatant liquors discarded and the centrifugation process repeated twice to obtain lipid-free samples. Two ml of 2M NaOH and 4 ml of H_2O were then added to the lipid-free samples (in order to release amylose into the solution), test tubes were capped and heated at 85°C for 15 min in the shaking water-bath to obtain solution 1.

Exactly 0.1 ml of the solution 1 was added to 5 ml of 0.5 % trichloroacetic (TCA) in a separate test-tube. The solution was mixed and 0.05 ml of 0.01 N I_2-KI solution (1.27 g of I_2 per L + 3 g of KI per L) was added and the mixture vortexed immediately. The blue colour was read after 30 min on a spectrophotometer at 620 nm whilst H_2O was used as the blank (Chrastil, 1987). A regression equation from a prepared amylose standard curve was used to convert the absorbance value to amylose content.

The pH was measured with a pH 210 Microprocessor pH Meter (HANNA Instruments, Japan). Determinations were in triplicates.

Determination of particle size of starch granules in *gari*
The particle size of the starch granules in *gari* was determined using a light microscope (Nikon Optiphot Model, Japan). Slides for determining particle size were prepared with 100 μl of deionised water. The light microscope 50X lens was used to view the particles immediately the water was added within 45 seconds. Granules were simultaneously viewed on a monitor coupled to the microscope whilst the image was captured with the camera attached to the light microscope. The actual size of granules was determined by interpolation with a standard scale. Readings were made in triplicates.

Determination of RDS, SDS and RS_2 in *gari* samples
The *in vitro* starch digestion method of Sang & Seib (2006) was used with slight modifications to determine the starch digestion profile for cassava samples: ~ 0.2g sample (dry basis) was weighed into test tube. Pepsin solution (5ml) prepared as 25mg pepsin in 0.05 M hydrochloric acid was added to the sample in the test tube and the mixture vortexed to remove protein. The vortexed mixture was placed in a shaking water bath for 30 min at 37°C. After the incubation, 0.25 M sodium acetate solution (5ml) was added, and the mixture was heated for 30min in boiling water in the water bath at 99°C. The heated mixture was cooled for 10 min under running tap water, and then digested with

pancreatin/amyloglucosidase. The enzyme mixture solution was prepared by adding 20 ml of distilled water to pancreatin (3.0g) and stirred at room temperature for 20min to obtain enzyme slurry. The slurry was centrifuged at 1500 g for 10 min. Supernatant was collected and 15.0 ml of this was mixed with amyloglucosidase solution (60.0 mg of amyloglucosidase in 1.7 ml of water).

The freshly prepared enzyme mixture solution (1.67 ml) was added to the sample for digestion after the heated sample which contained sodium acetate solution had been cooled for 10 min under running tap water. After 20 min and 120 min digestion of the mixture in a controlled shaking water bath at 37°C, ~0.5ml of the aliquots were taken for analyses of Rapidly-Digestible-Starch (RDS) and the Slowly-Digestible-Starch (RDS+SDS), respectively. Each of the samples was mixed with 20 ml of 66% ethanol before centrifugation in a centrifuge @ 3000 X g for 15 min each. The supernatants (precipitates were discarded) were collected and diluted 10 times (1ml supernatant + 9ml distilled water) before the glucose assays were carried out. RS_2 was determined by difference between the total starch content and the resistant starch value after 120 min digestion of the samples.

Determination of thermally stable RS (RS_3) in cassava-mash samples and *gari*

Thermally stable resistant starch was determined using the method of Goni *et al* (1996); 100 mg of cassava sample was weighed into 50 ml centrifuge tube and 10 ml of KCl - HCl buffer (pH 1.5) was added. 0.2 ml of Pepsin (Sigma P7000) solution was added to the volume and shaken in water-bath which was maintained at 40°C for 60 min in order to isolate protein. The sample was cooled at ambient temperature; trismaleate buffer (9 ml) was added to it and vortexed immediately. Also, 1 ml of σ- amylase (Sigma A– 3176) solution was added to the volume and the mixture was incubated at 37°C for 16 h in a shaking water bath to hydrolyse the starch. The hydrolysed sample was centrifuged at 3000 x g for 15 min, and the supernatant was discarded. The precipitate was washed with 10 ml distilled water, centrifugation repeated and supernatant was discarded again. The on the amylose content in cassava-mash samples and *gari*.

residue was dissolved in 3ml distilled water and 3 ml 4M KOH, content was mixed and kept for 30 min at room temperature under constant shaking using orbital shaker.

Thereafter, 5.5 ml of 2M HCl and 3ml of 0.4 M sodium acetate buffer (pH 4.75) were added to the dissolved residue. Amyloglucosidase (sigma A– 9913) (80µl) was then added to the volume and the sample kept in shaking water-bath at 60°C for 45 min. After centrifugation at 3000 x g for 15 min, supernatant was collected into a volumetric flask. Residue was washed with 10 ml distilled water, centrifuged again and the 2nd supernatant was added to the 1st supernatant. A ratio of 1:1:2 of Carrez solutions 1 and 2, and 0.1 M NaOH was added to ensure the precipitation of proteins before glucose contents were subsequently determined. Glucose hexokinase kit (Sigma GAHK-20) was used to determine the glucose concentration. Measurements were carried out in triplicates.

Results and discussions
Particle size and amylose content
The microscopic analysis of the particle size of the cassava cultivars used for *gari* in this study showed a range of 22.3 to 31.3µm (Table 1). This particle size is comparable to a range of 2.0 to 35.0µm that have been reported in the literatures when starch is extracted from freshly harvested cassava tubers (Defloor *et al*, 1998., Sanguanpong *et al.,* 2003). The microscopic investigation also showed lumps of starch granules in the *gari* samples, and this attribute which may be due to effect of pressing and roasting can inhibit the enzymatic hydrolysis of the food product because of the effect of surface area. Amylose contents in the cassava-mash and *gari* was found to increase in the grated raw cassava tuber from 9.2 to 35.0 mg/100mg in the roasted product for TMS 97/4763, from 10.0 to 34.0 mg/100mg for M98/0068, from 10.5 to 51.4 mg/100mg for TMS 98/0505, from 10.0 to 39.2 mg/100mg for TMS 95/0289 and from 9.0 to 56.8 mg/100mg for TMS 98/0510. The increase in amylose was much more pronounced in TMS 98/0510 as compared to other 4 cassava cultivars as Figure 1 shows the effect of genotype and processing

Table 1: Particle size, Rapidly-digestible-starch (RDS), slowly-digestible-starch (SDS), resistant starch type 2 (RS_2) of *gari*, and effect of processing on the pH of the enzyme extracts of fermenting cassava mash

Cassava Clones	Particle Size*	Rapidly Digestible Starch (RDS)*	Slowly Digestible Starch (SDS)*	Resistant Starch Type 2 (RS_2)*	pH (Fermentation/Unit Operations)**					
	µm	mg/100mg	mg/100mg	mg/100mg	0 h/ grating	24 h	48 h	72 h	Sieving	Roasting
TMS 97/4763	28.0±0.7	18.2±0.45	33.5±2.10	48.4±0.83	5.27	5.52	5.24	5.11	5.22	5.27
M98/0068	22.3±4.2	24.7±0.78	34.1±1.31	41.3±0.78	5.31	5.40	5.12	5.01	5.27	5.26
TMS 98/0505	31.3±1.1	22.7±0.46	32.8±1.48	44.5±0.78	5.25	5.46	5.10	5.01	5.24	5.30
TMS 95/0289	22.6±3.1	18.6±0.12	39.5±1.44	41.9±0.82	5.35	5.35	5.12	5.10	5.25	5.27
TMS 98/0510	27.2±3.3	21.0±0.45	27.4±1.44	51.6±0.82	5.19	5.38	5.12	4.94	5.21	5.24

*Values are means of 3 replicates readings **Values are means of duplicate readings

One of the reasons for the increasing interest in fermented foods and, hence, its consumption is its ability to promote the functions of human digestive system in a variety of positive ways. Amylose content has been implicated to have a direct influence on resistant starch (RS) (Brouns *et al.*, 2002., Haralampu, 2000., Peisong *et al.*, 2004., Sagum and Arcot, 2000). Investigation of the changes in amylose as affected by food unit processing is imperative because, boiling of starchy flours from food grains may produce higher glucose and insulin responses than the corresponding boiled kernels (Granfeldt *et al.,* 1994), large amounts of RS may be formed upon heating and cooling of pure high-amylose starches (Bjock, 1996), an increase in availability of starchy foods for enzymic digestion may be caused by milling (Granfeldt *et al.*, 1994., Fahey *et al.*, 2001), and generally increased amylose

content may be correlated with reduction in enzymatic food digestibility (Sagum & Arcot, 2000).

The production of *gari* requires solid fermentation (Oguntoyinbo, 2007), and this may have allowed a

retention of „amylose-like material" (Moothy & Mathew, 1998) in the cassava mash during *gari* production which caused an increase in the contents of amylose. Some studies have identified increase in apparent amylose content of cassava and some other crops such as lentil during fermentation, and have considered this to be due to the action of micro-organism during food processing because of its effects on some constituents of the fermented product (Numfor *et al.,* 1995., Sotomayor *et al.,*1999., Sobowale *et al*, 2007).

A reduced level of amylose could cause improvement in starch digestibility as exemplified for rice (Sagum & Arcot, 2000). An increase in the particle size due to a lower surface area may however inhibit the rate of enzymatic digestibility of foods. Variation in the particle size of the food product due to genetic modification of cassava may have impact on the extent of its digestibility, and hence useful in its characterisation. Slowly digested and absorbed carbohydrates have been reported to be favourable for dietary management of diabetes and hyperlipidemia (Peisong *et al.*, 2004). Amylose content also affect the glycemic index (GI), a physiological concept that is used to classify the elevation of postprandial blood glucose levels in the body (Adjei-Seffah & Khokhar, 2005). Therefore, it may be plausible to suggest that, the higher the amylose content in the fermented cassava-based diet, the lower will be the GI of the food.

The implied effect of amylose on the GI suggests that amylose variation due to the effect of processing in the preparation of the cassava-based low moisture food product may be of particular interest in the treatment of non-insulin-dependent diabetes (Urooj & Puttaraj, 2002). Therefore, the monitoring of changes in amylose content of the cassava-mash during the production of *gari* a commonly consumed product in tropical countries such as Nigeria, Ghana, Benin, Cote d"Ivoire,

Mozambique and Sierra Leone, and as ethnic diets in the West may provide a means for the characterisation of the foods in different localities where the food is consumed (Oyewole & Afolami, 2001., Oyewole & Ogundele, 2001., Kostinek *et al.*, 2005., Oyelade & Khokhar, 2008). In the West the number of people familiar with the consumption of cassava-based food products from the developing world is significantly on the increase. Gilbert & Khokhar (2008) has reported that ethnic groups in the UK constitute between 19 and 21.7 % of the total population.

Effect of processing on pH
The pH of the enzyme extracts from the unmodified GM cassava starch varied between 5.40 and 5.48 whilst it was 5.27 and 5.30 in the extracts for *gari* (Table 1).
The pH was initially at 5.6 and may not be able to provoke optimal extent of enzymatic activity which is known to have optimal value at pH of 5.4 for amylolytic process. The pH nearly stayed at this value because there was no significant change in its value.
Similar seemingly low change in pH values in this study had also been observed by Moothy & Mathew (1998). Oyewole & Odunfa (1992) attributed pH decline during fermentation to be due to disappearance of amylolytic micro-organisms from the fermenting spectrum or due to other inhibitions. Westby & Twiddy (1992) also noticed a change in the pH of the root during fermentation from 7.0 to 6.1, after the first 24 h. The trend of the pH in the production of *gari* supports these findings and suggests that due to the disappearance of the amylolytic organisms in the fermenting cassava mash, it may be that the rate of hydrolysis will reduce. Although, the results of the pH and amylase activity in this study are in line with those obtained for cassava-mash by previous workers (Amund & Ogunsina, 1987., Westby & Twiddy 1992., Oyewole & Odunfa 1997), the experimental conditions were not the same as in the present study. The process of converting the freshly harvested cassava tubers to *gari* involved heat process before enzyme was extracted from the food samples, and could have produced significant influence on the pH and amylase activity values that were obtained in this study, regardless of the agreement in trend.

RDS, SDS and RS_2 in *gari* samples
The effect of cassava genotype on the digestion profile of *gari* is presented in Table 1. Variation in values of the RDS (mg/100mg), SDS (mg/100mg) and RS_2 (mg/100mg) exist for the food. The RDS ranged between 18.2 and 24.7 whilst the SDS changed from 27.4 to 39.5 with RS_2 varying between 41.3 and 51.6, respectively for *gari*.
The variations in the values of RDS and SDS due to the unit processing in this study confirms the fact that, digestibility characteristics of starch-based foods mostly depends on the processing conditions adopted and the resulting retrogradation steps (Siljestrom *et al.*, 1986), because during processing the starch molecule

undergoes several physical modifications depending on the type of starch and severity of the conditions employed (Goni *et al.*, 1996), leading to the formation of RS (Namratha *et al.*, 2003).

It has been suggested that starchy foods that are digested slowly and result in low blood glucose are more beneficial to health and in the management of diabetes and hyperlipidemia (Jenkins *et al.*, 1985., Panlasigui *et al.*, 1991) than are starchy foods that are digested rapidly and result in high blood pressure. The inference is that degradability of ingested starch is important, especially among diabetics and hyperlipidemic individuals (Riley *et al.*, 2004). Jenkins *et al.*, (1982) have observed that starchy foods which are easily degraded will tend to have a higher insulin demand than the slower degrading starches. This can affect the sensitivity to insulin (Lehmann & Robin, 2007), and lead to or reduce the risk of developing type II diabetes (Salmeron *et al.*, 1997), myocardial infarction in women (Liu *et al.*, 1999., Riley *et al.*, 2004), and HDL cholesterol levels (Riley *et al.*, 2004).

Previous studies have shown that physicochemical properties affect rate of starch hydrolysis, where it has been proven that digestibility of starch is generally inversely proportional to amylose content (Rooney & Pflugfelder, 1986., Hoover, 2001., Lehman & Robin, 2007). It is possible that unit processing operations which affected amylose contents in the *gari* has produced synergistic impact on the digestion profile of the food. The RS_2 fraction of starch was determined by difference in the food. The values of RDS, SDS and RS_2 obtained in this study are within the range of values that have been reported in the literature. Sajilata *et al.*, (2006) reported that RDS is found in high amounts in starchy foods that are cooked by moist heat, such as bread and potatoes, and reported a range of values that varied between 25 and 94mg/100mg for some beans, peas, biscuits, spaghetti, bread and flour. The SDS for these categories of foods was between 4 and 59mg/100mg. This study suggests that the cassava-based food product have comparable rapidly digestibility rates with most cereal grains (barley, corn, white rice, brewer''s rice, brown rice, wheat, millet, oats and sorghum) that have been reported with RDS between 24.9 and 68.4mg/100mg , less RDS rates than most flours (corn, wheat, rice, potato, barley and sorghum) with RDS between 37.7 and 75.5mg/100mg, grain-based food products (macaroni, corn meal, rolled oats and hominy grits) with RDS of between 36.8 and 60mg/100mg, corn starch (71.8mg/100mg) (Bednar *et al.*, 2001).

Effect of genotype and unit processing on thermally stable resistant starch (RS_3) in cassava-mash and *gari*.

Figure 1 show that processing has remarkable effect on the quantities of RS starch that were obtained from the cassava-based low-moisture food product. The RS_3 obtained when five cassava cultivars were converted to

gari varied from 8.2 to 15.3 mg/100mg across the unit processing operations. RS_3 is of particular interest as an ingredient in the food industry because of its physical and nutritional functionality and processing stability (Haralampu, 2000., Thompson, 2000., Onyago *et al.*, 2006).
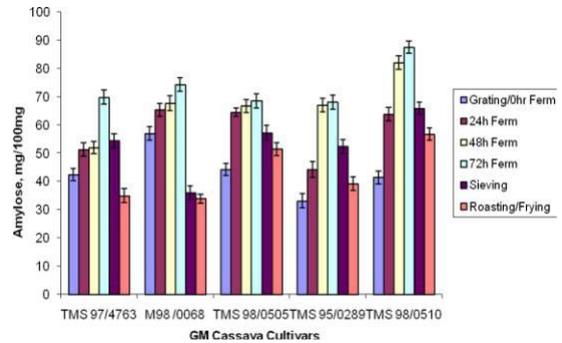

Figure 1: **Effect of genotype and unit processing on amylose during the production of *gari*.**

The trend of RS_3 levels being thermally stable resistant starch in this study suggests that GM cassava cultivars such as TMS97/4763, M98/0068, TMS98/0505, TMS95/0289 and TMS98/0510 will be good starting cultivars for development of cassava-based functional foods in relation to digestibility. In particular, the effect of unit processing on RS starches in the food product supports the findings of Englyst & Englyst (2005) that, the value of RS is dependent on the degree of processing and, hence, the need to determine RS in foods *as they would normally be eaten* becomes imperative.

An influence of unit operations and genotypic interference on the changes in RS which occurred during the time course of spontaneous fermentation of the cassava mash during conversion to *gari* is shown in Figure 2. The quantity of RS_3 obtained from the five GM cassavas when converted to *gari* varied from 8.2 to 15.3 mg/100mg across the processing stages and are comparable with the reported 11 % RS in corn flour (Bednar *et al.*, 2001). However, literature has cautioned on drawing parallel comparison in numerical values of RS of food products. This is because such determination could be method of estimation dependent (Thompson, 2000).
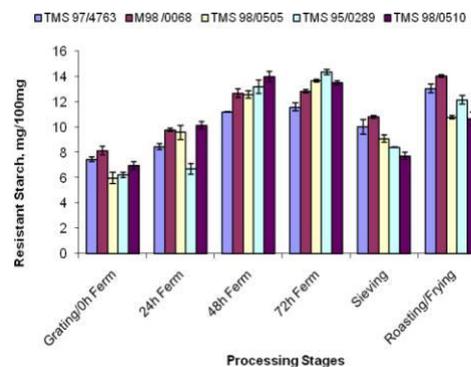
Figure 2: **Effect of unit processing and genotype on**
Literatures have shown a general correlation in amylose level with RS levels of some starchy food products (Berry, 1986; Knutson *et al.*, 1982). This emanated from the observation that high-amylose starch granules with increasing granule size have higher amylose content and lower digestibility. According to McNaught *et al.*, (1999), there is usually an optimal granule size for RS, and that this size is not necessarily associated with the fraction with the highest amylose content (Thompson, 2000). The observation in this study showed that the GM cassava cultivar with the highest amylose content did not produce highest RS_3. In general, RS_3 levels increased as the amylose content increased in the time course of fermentation. It is possible that during the production of the food product the starch granules swell reversibly (Rooney & Pflugfelder, 1986), and consequently affect the internal structure of the starch which invariably has implication on the associated RS_3 content. The molecular composition and physical structure of RS have also been reported to affect its prebiotic and butyrogenic properties. A comprehensive potential actions of RS in regards to its placement in the functional food drive as derived from *in vitro*, animal and human studies have been reviewed elsewhere (Brouns *et al.*, 2002).

Conclusions
The physicochemical and functional properties which are pH, particle size, amylose, and RS_3 obtained during the processing of cassava to *gari* are affected by genotype and unit operations. The increase in the amylose content during the production of the food product may be due to production of „amylose-like" material associated with spontaneous fermentation of cassava. However, since changes in amylose and RS_3 are minimal between 48 and 72 h of fermentation for all the GM cassava cultivars, it can be considered that 72 h is the optimum period in the time course of fermentation of cassava mash to *gari* in relation to RS_3. This form of RS is the most thermally stable, and hence significantly useful in industrial production of functional foods. Also, the microscopic investigation on the particle size of starch granules in *gari* shows lumps of granules which probably can produce significant effect on the digestibility of the food, and can be linked to the effect of pressing and roasting unit operations.
Over all, the digestion profile of the food product in relation to RDS, SDS, RS_2 and RS_3 shows that the food product has the potential of being used as functional food than most cereal grains (barley, corn, white rice, brewer"s rice, brown rice, wheat, millet, oats and sorghum), flours (corn, wheat, rice, potato, barley and sorghum) and grain-based food products (macaroni, corn meal, rolled oats and hominy grits). The food product is also comparable with corn starch, potato starch, and amylomaize. Therefore, *gari* could be appreciably suggested as valuable international foods amongst wider population groups. However, the pressing and roasting unit operations merit optimal

RS_3 in cassava-mash and *gari*
investigations in relation to the production of *gari* with better RS_3 values.

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