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NEW STARCHES FROM IMPROVED YELLOW COLORED CASSAVA: ENZYMATIC AND ACID STURDINESS AND POTENTIAL OF INDUSTRIAL UTILIZATION

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Abstract: *Native and gelatinized starches from eight improved cassava varieties were extracted and subjected to enzymatic and acid hydrolysis in order to highlight their potential of industrial application. The enzymatic hydrolysis showed that native starches were less hydrolyzed (8.40–15.80 10^{-2} UI/mg of proteins) than the gelatinized ones with values ranged from 1.14.10^{2} UI (V_{23}) to 6.93 10^{2} UI/mg of proteins (V_{54}). Thin layer chromatography analysis showed glucose and a high molecular mass maltodextrin as the common products released from both starches. Maltose and another low molecular mass maltodextrin were in addition observed when gelatinized starches were used. The hydrochloric acid solution had showed predominant conversion of V_4, V_{54} and V_{55} with maximum values of reducing sugar products of about 70, 62, and 82 µmol, respectively. The starch variety V_{52} recorded the lowest value (36.23 µmol of reducing sugar). Due to the relatively high degrees of hydrolysis and the wide range of industrial application of modified starches products (glucose, maltose and maltodextrins), V_4, V_{54}, and V_{55} could constitute new exploring sources of starch for the food and pharmaceutical industry while V_{52} could be suggested as a raw material for the paper industry and for biofuel production.*

Keywords: *acid conversion - enzymatic hydrolysis - industry - modified cassava starch*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important food crops in the humid tropics, being particularly suited to low nutrient availability and able to survive drought [1]. Initially grown as a substantial food crop, cassava root derivatives especially flour and starch are now used for various industrial purposes [2]. This utilization has been taking place in the so-called "newly opened economies" of China and Vietnam, and to a lesser extent in Latin America. Starches can be classified as unmodified or modified. Unmodified or native starches are produced through the separation of naturally occurring starch from grain or

root crops. Irrespective of their source, native starches are undesirable for many industrial applications because of their inability to withstand processing conditions such as extreme temperatures. They yield pastes of poor stability which decreases products shelf life. Native starches also showed high ability to retrograde, loss of viscosity, syneresis tendency and thickening power upon cooking [3]. In order to improve their desirable functional properties and overcome their limitation uses, native starches are often modified. Modification of starches mainly consist in the alteration of physical and chemical characteristics. These processes enable to improve inherent poor structural and physicochemical properties of native starch thus tailor it to specific industrial applications [4]. Nowadays, practically every industry in existence uses starch or its derivatives in one form or another [5]. In foods and pharmaceuticals, starch truly serves as a multifunctional ingredient [4]. Indeed, it is used to influence or control such characteristics as texture, moisture, consistency and shelf stability. It can be used to bind, to densify, to opacify and to attract moisture in various products. As for the food industry, non-food industrial uses of cassava starch are also associated with larger scale firms. The textile industry, for example, uses cassava starch for sizing while the paper industry uses it for coating high quality paper. As all these industrial sectors develop, demand for cassava starch is likely to increase. Despite their importance, a large proportion of these agro-resources are lost yearly due to non-availability of appropriate technology and industry to harness these into various useful products [6]. Nowadays, most of African countries have taken in earnest their abundantly produced cassava and have just started to process more expended food and non-food end-products [7]. In this context, plant breeders, agronomists and recently molecular biologists have made substantial improvements in cassava varieties and yields during the last two decades [8]. In Côte d'Ivoire, the national agricultural center has implemented several improved varieties for rural dissemination. Recently, flours and starches of the new yellow colored cassava fleshes were extracted and extensively characterized through physicochemical and biochemical parameters [9,10]. In the present work, eight (8) of these starches were subjected to enzymatic and mild acid hydrolysis and their resulted products analyzed in order to explore the potential domains of industrial applications.

2. Experimental

2.1. Cassava varieties

All cassava varieties used in this study were harvested at eleven months old and provided to us by the National Agricultural Research Centre (CNRA, Adiopodoumé, Côte d'Ivoire 5°19'40" latitude North and 4°23'00" longitude West). As starches which are extracted, these varieties were encoded V_{64}, V_{65}, V_{66}, V_{69}, V_{71} and V_{73}, and resulted from various crossbreeding's of two already disseminated varieties: V_4 (white coloured flesh) and V_{23} (yellow coloured flesh).

2.2. Enzymatic source and amylolytic enzymes extraction

Ten active snails (*Achatina achatina*) were denied water and food for three days to liquefy their initial bolus and then, their shells were cracked. The digestive fluid was collected with syringe, filtered through sterile cotton wool and centrifuged at 10,000 g for 15 min. The supernatant was

used as the crude extract source of amylolytic enzymes.

2.3. Proteins estimation

Proteins concentration in the crude extract was measured by using the Folin-Ciocalteu's phenolic reagent [11]. Bovine serum albumin was used as the standard protein.

2.4 Starch extraction

Fresh cassava roots were washed, peeled, chopped and then pulverized in a high speed blender. The cassava mash was suspended in ten times its volume of water, stirred for 5 min and filtered by using double fold cheesecloth. The filtrate was allowed to stand for 2 h and the top liquid was decanted and discarded. A 4% (w/v) NaCl solution was added to the sediment and the mixture stirred for 5 min. Filtration was carried out using sieves with reverse mesh sizes varying from 500 to 100 µm. After decanting the top liquid, starch paste was dried at 45 °C for 48 h, crushed to a fine powder and then stored for further treatments.

2.5. Native and gelatinized starch substrate preparation

Both starch substrates were prepared at 1% (w/v). Native starch substrates were obtained by dissolving 1 g of the above dried powder in 100 ml of distilled water. For gelatinized starches, the same protocol was initially followed in 50 ml of distilled water. Then, the mixture was heated at 70 °C for 10 min and the residual solution adjusted to 100 ml with distilled water.

2.6. Enzymatic and mild-acid hydrolysis

The enzymatic hydrolysis were performed on both starch substrates. Reactions were carried out at 37 °C with 2.5 µg of proteins in a 100 mM sodium acetate buffer (pH 5.6) for 24 hours. Concerning the mild acid hydrolysis, only native starches were used. A 2.2 N hydrochloric acid solution was used at 25 °C to catalyse reactions for three (3) months. All the experiments were performed in triplicate under sporadic stirring with a total mixture volume of 200 µl by using a screw-cap glass cells. Amylolytic enzymes activities were expressed as the amount of catalysts which release 1 µmol of reducing sugar from 1% (w/v) starch in a minute under the experimental conditions. For the acid conversion, activities were expressed in percent (mg of dextrose equivalent per 100 mg of starch). The initial velocities were graphically determined from the beginning rising side (linear part) of each hydrolysis profile by taking into account the earlier upward trend of reducing sugar production.

2.7. Analysis of hydrolysed products

At regular time intervals, 100 µl volume samples were withdrawn to quantify total reducing sugars [12], while 5 µl of the same solution were spotted onto thin layer chromatograph plates (Model 60 F_{254}, Merck) to monitor the hydrolysis of native and gelatinized starches. TLC plates were run with butanol-acetic acid-water mixed at 9:3.75:2.25 (v/v/v), and sprayed with a naphto-resorcinol in ethanol and H_2SO_4 20% (v/v) solution. Then, sugar spots were visualized by heating plates at 110 °C for 5 min.

3. Results and Discussion

3.1. Enzymatic hydrolysis

The kinetics of the enzymatic hydrolysis of native and gelatinized starches are depicted in figure 1. These profiles obeyed the Michaelis-Menten kinetics which are

characterized by logarithmic curves. So, they enabled us to determine the different initial velocities topped in table 1. These values reflect the strength of each starch overlooked amylolytic enzymes. The results indicated that, at the earlier stage of the reactions, and irrespective of the starch variety, native starches were less susceptible to enzymes attack than the gelatinized ones. Concerning native starches, the best hydrolysis rate (15.80×10^{-2} UI/mg of proteins) was observed with V_{55}.

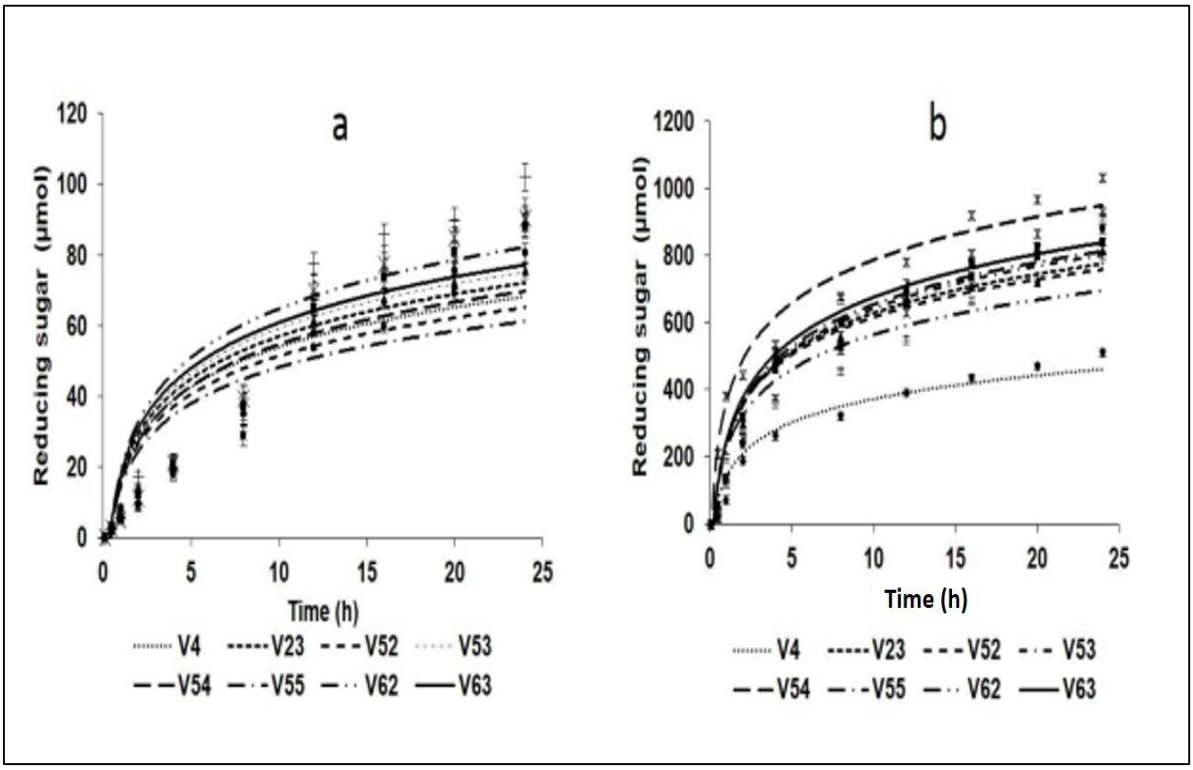

Figure 1. Kinetics of enzymatic hydrolysis at 37°C of native (a) and gelatinized (b) cassava starches by the digestive fluid of the giant snail *Achatina achatina.* (V_4, V_{23}, V_{52}, V_{53}, V_{54}, V_{55}, V_{62} and V_{63}, Cassava starches)

The other varieties are hydrolyzed to a lesser extent and in almost the same proportion with rates of between 8.40 and 10.37×10^{-2} UI/mg of proteins (Table 1). Unlike native starches, gelatinized starches had better tendency to hydrolysis of at least 1,000 times higher. V_{54} and V_{62} showed the highest values of respectively 6.93 and 3.26×10^{2} UI/mg of proteins while V_{23} recorded the lower rate of conversion ($1.14\pm1.03\times10^{2}$ UI/mg of proteins). The other gelatinized starches showed intermediate values of between 2.01 and 2.24×10^{2} UI/mg of proteins (Table 1).

Table 1.
Initial velocities of enzymatic hydrolysis of native and gelatinized cassava starches by the digestive fluid of the giant snail *Achatina achatina*

Starch varieties	Initial velocities of amylolytic enzymes on native starches (10^{-2} UI/mg of proteins)	Initial velocities of amylolytic enzymes on gelatinized starches (10^{2} UI/mg of proteins)
V_4	8.90±0.08	1.57±1.03
V_{23}	10.37±0.08	1.14±1.03
V_{52}	8.89±0.09	2.24±1.45
V_{53}	8.65±0.07	2.03±2.06
V_{54}	8.40±0.08	6.93±2.07
V_{55}	15.80±1.03	2.21±2.44
V_{62}	8.67±1.04	3.26±2.22
V_{63}	11.37±1.06	2.01±1.03

The observed variability at the hydrolysis rate of native starches may be related to the degree of damage in isolated starch granules. Indeed, damage starch granules have larger surface area for enzymes hydrolysis than intact native starch granules with smooth surface structure lacking pinholes and channels, thus rendering them highly resistant to amylolytic enzymes attack [13]. The strong susceptibility of a native starch to enzymes attack could be also explained by both their relatively high amylopectin content and granules size [14]. Indeed, the small granules would be more resistant to external influences and less inclined to the transformations therefore less subjected to enzymes attack. Because most foods are consumed after cooking, the digestibility of ungelatinized starch granules is only applicable mostly for animal feed. So, the native starch V_{55} which is greatly hydrolysed by digestive enzymes could constitute an inexpensive and commodity feed material for livestock as substitute of maize starch usually used [15]. Elsewhere, in the renewable energy industry, this starch variety could serve as an alternative substrate for selected yeasts in the production of bioethanol [16].

The difference at the hydrolysis behavior between native and gelatinized starch results in the modification of the native starch granules structure during gelatinization [17]. Indeed, when native starches granules are gelatinized during a hydro thermal processing, the hydrogen bonds between starch chains are disrupted, allowing the granules to swell and then disintegrate. As a result, the availability of amylose and amylopectin chains to the digestive enzymes increases [18]. The great tendency to hydrolysis of the gelatinized starch V_{54} could also be linked to its higher solubility attribute partly to the high swelling it undergoes during gelatinization. Also, this property could be due to its low proportion of amylose of about 14%, as already reported [10], which reduces the presence of inhibitors such as phosphate and promote gelatinization [19]. The potential gelling property of this starches lead to its use in the food industry as thickener and ambient products stabilizer. The other starches which have shown weak digestibility may be less gelatinized due to high proportion of small granules and amylose, and the large number of associative forces [20]. Therefore, V_{54} could be more explore in infant diet preparations because of its high

degree of digestion. Also, it could be incorporated into food ratio of sportsmen such as an ingredient of choice to substitute lipid foods in order to reduce prevalence of obesity and cardiovascular diseases [21]. As for the other gelatinized starches which have low digestibility rates, they could considered to be beneficial for dietary management of metabolic disorders, including diabetes and hyperlipidemia [22]. Moreover, the low digestibility of these starches showed close resemblance with dietary fiber which is healthy and has previously been reported to reduce postprandial glycaemia [23]. The weaker associative bonds between the starch molecules in the granules also favours its hydrolysis. Beyond influencing hydrolysis, cassava starch with weaker associative forces enables it to have better clarity when gelatinized, and such property has much relevant in food and textile applications. Therefore, the great hydrolysed starches reported in this study for gelatinized starches V_{54} and V_{62} could promote their use as suitable materials in foods as pie fillings and in textile industry for sizing. The opposed hydrolysis behavior of V_{55} face to amylolytic enzymes when gelatinized, in comparison to its native form, remind us of two new qualities of starches namely resistant starch type 3 or 4 (RS3 or RS4). These kind of starches are formed through retrogradation such they are no longer well recognised by alpha-amylases, thus rendering them lower hydrolysed. In the same context, native starches such as V_{53}, V_{54} and V_{62} that resist to enzymatic hydrolysis could be categorized as type 2 resistant starch (RS2) which escape digestion in the stomach and the small intestine so that they can be labelled as "dietary fibre". Resistant starches are considered as ideal ingredients for the fibre fortification of low-calorie products. In nutrition, they have potential physiological benefits in relation to the biochemistry of the colon and glucose/insulin metabolism with healthy benefit to diabetics [24].

3.2. Thin layer chromatography products

Products spots on thin layer chromatograms of the enzymatic reaction mixtures are showed by figure 2. Glucose and Maltodextrin 1 were distinctly observed as the common products from both substrates (Figures 2a and 2b). In addition to the latter products, maltose and another maltodextrin with relatively lower molecular mass (Maltodextrin 2) were obtained for gelatinized starches (Figure 2b).

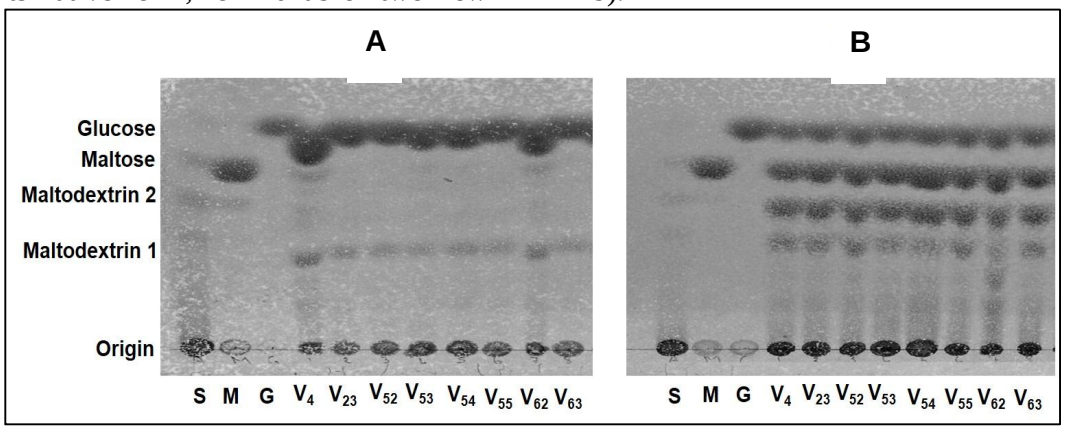

Figure 2. Thin layer chromatography of products from the reaction mixture of native (a) and gelatinized (b) cassava starches catalyzed by the digestive fluid of the giant snail *Achatina achatina*. (V_4, V_{23}, V_{52}, V_{53}, V_{54}, V_{55}, V_{62} and V_{63}, Cassava starches; S, standard of starch; M, standard of maltose; G, standard of glucose).

Though starch truly serves as a multifunctional ingredient in the food industry, its released product such as glucose, maltose and maltodextrins also are essential and useful products for food confectionary industries [5,6] and widely applied in sugar, spirits, and textile as well as in brewing. For example, glucose syrup could serve as substrates for the production of a wide range of fermentation products such as monosodium glutamate [25], lysine for the animal feed industry [26] and ethanol for biofuel industry [16]. Dextrins' are used in backed goods and in confectionary where their main functions are viscosity control, softening, texturing and coating [6].

3.3. Thinned-acid starches

The acid hydrolysis profiles of the studied cassava starches were shown in figure 3. The two-stage of hydrolysis pattern was quite obvious in all cases. A relatively fast hydrolysis rate during the first two weeks linked to the depolymerisation of the amorphous regions of starch granules. While the crystalline material is slowly degraded during the second stage [27].

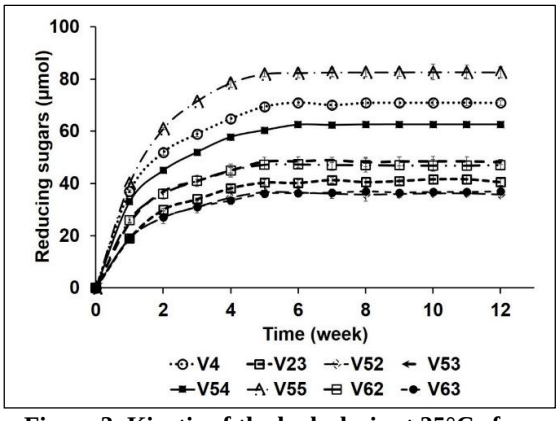

Figure 3. Kinetic of the hydrolysis at 25°C of native cassava starches by a mild hydrochloric acid (2.2 N) solution.

Acid-thinned starches V_4, V_{54} and V_{55} showed the highest amounts of reducing sugars with values of about 70.07, 61.52 and 82.58 µmol, respectively. These results corroborate those obtained for yam and wheat starches [28]. The strong hydrolysis rate of V_4, V_{54} and V_{55} could suggest a predominant attack of their amorphous granule parts so that higher concentrations of these starches can be used without excessive thickening. Such starch property are preferentially sought in the manufacture of glues and gums, and in the paper and textile industry for coating and sizing. Also, their great conversion into lower molecular mass products could be used to improve printability and abrasion resistance on paper surfaces, as well as in calendar and size press applications [29].

4. Conclusion

The present study has revealed that starches from eight improved yellow colored fleshes of cassava have variable sturdiness towards enzymatic and acid hydrolysis. Gelatinized starches exhibited more enzymes attack than the native ones. Starch V_{54} which is easily hydrolyzed under it gelatinized form, could be proposed as food dietary of sportsmen and workers. The other starches which were less hydrolyzed, could serve as raw materials in food products formulation for diabetics and persons who suffer from cardiovascular diseases. By considering the acid hydrolysis, the native starch V_{55} could be recommended in the production of biofuel. It could be conclude that, as native starches have limited industrial utilization, enzymatic and chemical modification opens new prospects for their use in various food, textile, pharmaceutics and papers industrial processes.

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6. References

[1]. BURRELL M.M, Starch: The need for improved quality or quantity – an overview, *J. Exp. Bot.*, 54. 451-456. (2003)
[2]. DEMIATE I.M., KOTOVICZ V., Cassava starch in the Brazilian food industry, *Food Sci. Technol. (Campinas)*, 31. 388-397, (2011)
[3]. SINGH J., KAUR L., MCCARTHY O.J., Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications-A review, *Food Hydrocoll.*, 21. 1-22, (2007)
[4]. MIYAZKI M.R., HUNG P.V., MAEDA T., MORITA N., Recent advances in application of modified starches for bread making, *Trends Food Sci. Technol.*, 17. 591-599, (2006)
[5]. AKPA G.J., DAGDE K.K., Modification of cassava starch for industrial uses, *Int. J. Eng. Technol.*, 2. 913-919, (2012)
[6]. ZAINAB A., MODU S., FALMATA A.S., MAISARATU T., Laboratory scale production of glucose syrup by the enzymatic hydrolysis of starch made from maize, millet and sorghum, *Biokemistri*, 23. 1-8, (2011)
[7]. GOLETTI F., RICH K., WHEATLEY C., The Cassava starch industry in Vietnam: Can small firms survive and prosper?, *Int. Food Agribus. Manage. Rev.*, 2. 345-357, (2001)
[8]. FREGENE M.A., SUAREZ M., MKUMBIRA J., KULEMBEKA H., NDEDYA E., KULAYA A., MITCHEL S., GULLBERG U., ROSLING H., DIXON A.G., DEAN R., KRESOVICH S., Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop, *Theor. Appl. Gen.*, 107. 1083-1093, (2003)
[9]. KOUA G.A-Y., MEGNANOU R-M., KRA K.S., N'ZUE B., TIAN-BI D.R., AKPA E.E., NIAMKE S.L., Biochemical characterization of new varieties of yellow colored pulp cassava flours from Côte d'Ivoire, *J. Appl. Biosci*., 53. 3760-3772, (2012)
[10]. DOUE G.G., MEGNANOU R-M., BEIKOU E.M., NIAMKE L.S., Physicochemical characterization of starches from seven improved cassava varieties: Potentiality of industrial utilization, *J. Appl. Biosci.*, 73. 6002-6011, (2014)
[11]. LOWRY H.O., ROSEBROUGH N.J., FARR L.A., RANDALL R.J., Protein measurement with the Folin Phenol reagent, *J. Biol. Chem.*, 193. 265-275, (1951)
[12]. BERNFELD P., Amylases α and β, *Method Enzym.*, 1. 149-158, (1955)
[13]. DHITAL S., SHRESTHA A.K., GIDLE M.J., Effect of cryomilling on starches: functionality and digestibility, *Food Hydrocoll.*, 24. 152-163, (2010)
[14]. SANDSTEDT R.M., SITRAHAN D., VEDA S., ABBOTT R.C., The digestibility of high amylose corn starches compared to that of other starches. The apparent effect of the *ae* gene and susceptibility to amylase action, *Cereal Chem.*, 39. 123-131, (1962)
[15]. COOPER R.J., MILTON C.T., KLOPFENSTEIN T.J., SCOTT T.L., WILSON C.B., MASS R.A. Effect of corn processing on starch digestion and bacterial crude protein flow in finishing cattle, *J. Anim. Sci.*, 80. 797-804, (2002)
[16]. DUHAN J.S., KUMAR A., TANWAR S.K., Bioethanol production from starchy part of tuberous plant (potato) using Saccharomyces cerevisiae MTCC-170, *Afr. J. Microbiol. Res.*, 7. 5253-5260, (2013)
[17]. AMANI N.G., TETCHI F.A., COULIBALY A., Propriétés physico-chimique de l'amidon de gingembre (*Zingiber officinale roscoe*) de Côte d'Ivoire, *Tropicultura*, 22. 77-83, (2004)
[18]. CHUNG H.J., LIM H.S., LIM S.T., Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch, *J. Cereal Sci.*, 43. 353-359, (2006)
[19]. NUWAMANYA E., BAGUMA Y., EMMAMBUX N., TAYLOR J., RUBAIHAYO P., Physicochemical and functional characteristics of cassava starch in Uganda varieties and their progenies, *J. Plant Breed. Crop Sci.*, 2. 001-011, (2010)
[20]. ADEJUMO A.L., ADERIBIGBE A.F., LAYOKUN S.K., Cassava Starch: Production, Physicochemical Properties and Hydrolysation - A Review, *Adv. Food Energy Sect.*, 2. 8-17, (2011)
[21]. MILLER J.C., MILLER, J.C., MCMILLAN P., STEINBECK K., CATERSON I., Carbohydrates: The good, the bad and the whole grain, *Asia Pac. J. Clin. Nutr.*, 17. 16-19, (2008)
[22]. WOLEVER T.M., MELHING C., High-carbohydrate-low-glycemic index dietary advice improves glucose disposition index in subjects with impaired glucose tolerance, *Br. J. Nutr.*, 87. 477-487, (2002)

[23]. NUTTALL F.Q., Dietary fiber in management of diabetes, *Diabetes*, 42. 503-508, (1993)
[24]. NUGENT A., Health properties of resistant starch, *Br. Nutr. Found Nutr. Bull.*, 30. 27-54, (2005)
[25]. NAMPOOTHIRI K.M., PANDEY A., Fermentation and recovery of L-glutamic acid from cassava starch hydrolysate by ion exchange resin column, *Rev. Microbiol.*, 30. 258-264, (1999)
[26]. MOOSAVI-NASAB M., ANSARI S., MONTAZER Z., Fermentative Production of Lysine by *Corynebacterium glutamicum* from Different Carbon Sources, *Iran Agric. Res.*, 25. 99-106, (2007)
[27]. ROBIN J.P., MERCIER C., CHARBONNIERE R., GUILBOT A., Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch, *Cereal Chem.*, 51. 389-406, (1974)
[28]. DALLY L.I., SAKO A., KOFFI A., SIDIBE D., LIA J.A., N'GUESSAN A.G., Influence des traitements acide et enzymatique sur la structure granulaire d'amidon d'igname «kponan» (*Dioscorea cayenensis rotundata*. Poir), *J. Sci. Pharm. Biol.*, 9. 17-26, (2008)
[29]. LIM W.J., LIANG Y.T., SEIB P.A., Cationic oat starch: preparation and effect on paper strength, *Cereal Chem.*, 69. 237-239, (1992)