SOME ASPECTS OF PHYSIOCHEMICAL PROPERTIES AND EVALUATION OF PROBIOTIC ISOLATED FROM AFRICAN STAR APPLE (*Chrysophyllum albidum*)

Lawal, R. T. & Ishola, A. D.
Science Laboratory Technology Department, Osun State Polytechnic, Iree, **NIGERIA**
Sulaiman, W. K.
Chemistry Department, Oduduwa University, Ile-Ife, Osun State, **NIGERIA**
Ajao, F. D.
Food Technology Department, Osun State Polytechnic, Iree, **NIGERIA**
&
Adetuberu, I. A.
Applied Sciences Department, Osun State Polytechnic, Iree, **NIGERIA**

ABSTRACT

Two LAB bacteria were isolated from African star apple (*Chrysophyllum abidum*), characterized and identified as *Lactobacillus lactis*, *Lactobacillus plantarum*. The *Lactobacillus lactis* and *Lactobacillus plantarum* survived at different temperatures, pH and tolerance to bile salt.The proximate, pH and minerals analysis of African star apple were determined using standard methods which varied in contents. The fruit had crude fibre of 18.18%, protein of 1.71% and moisture content of 64.32%. The potassium content was 112.00ppm followed by calcium, sodium and iron with 78.00ppm, 55.50ppm and 0.69ppm respectively. On the basis of the findings, nutritional content of the *C. albidum* gave an indication of its usefulness in jelly or jam manufacturing and the ability of LAB, especially *Lactobacillus* sp isolated from *C. albidum.*

Keywords: *C. albidum*, Probiotic, Temperature, pH, Tolerance, Bile-Salt.

INTRODUCTION

African star apple *(Chrysophyllum albidum*), a wildly grown fruit in the Southwestern part of Nigeria, it belongs to the family of trees known as *Sapotaceae*. Its fruit is commonly known as "*Agbalumo*" or "*Osan*" (Yoruba) or "*Udala*" (Igbo) in the local languages. It is pale yellow with pink coloured endocarp and relished by children and adults when in season. Its fully ripe fruit becomes available from December through April. The pink-coloured pulp and the whitish cover of the brown-coloured seeds of the fruit are consumed, while the empty pale yellow pericarp is often discarded by some people (Adepoju and Adeniji, 2012). The plant often grows to a height of 36.5m though it may be smaller. It has in recent times become a crop of commercial value in Nigeria. The fruit has been found to have high content of ascorbic acid with 1000 to 3,330 mg of ascorbic acid per 100gm of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew (Bada, 1997).

The fruits are not usually harvested from the trees; they are left to drop naturally to the forest floor, from where they are picked. The fruit pulp is used in table jelly manufacture and also useful in the formation of soft drink in beverage industry. Although, research work had been carried out on the nutritive value of pulp, storage of the fruits and physical properties of the seeds of African star apple but there is dearth of information on the physicochemical properties of the components of the fruit (Adindu*et al*., 2003; Amusa*et al*., 2003; Oyelade*et al*., 2005). *Bifidobacterium bifidum*, also a probiotic culture inhabit within the mucus lining of the large intestine and/or vaginal tract. *B. bifidum* creates favorable changes in pH levels by producing lactic and acetic acids thereby preventing pathogenic bacteria and yeast from invading. In addition, this species increase absorption of iron, calcium, magnesium and zinc.

B. infantis simulates the production of cytokines that affect the immune system, and can kill off such pathogens as *Clostridia*, *Salmonella* and *Shigella*. *B. longum* colonizes the large intestine and prevents the growth of harmful bacteria and yeast which decrease the frequency of gastrointestinal problems, such as diarrhea, and nausea during antibiotic use (Andrew, 2013). This organism eliminates nitrate, promoting nitric oxide levels and decreases pathogens. *L. rhamnosus* has a high tolerance to bile salts, surviving in less than favorable environments. This species has shown benefit to the elderly and infants alike. Ithelps with lactose intolerance, protects the small intestine, and produces lactic acid in the large intestine (Andrew, 2013). The objectives of this work was to assess proximate analysis, minerals compositions and survival of LAB isolate at varying pH, temperatures and tolerance to bile salt concentrations.

MATERIALS AND METHODS

Collection of Samples

Africa star apple (*Chrysophyllum albidum*) fruits were purchased from *Oja Oba* market at Ikirun, Ifelodun Local Government area of Osun State, transported to the laboratory and washed with clean water to remove extraneous matter and surface sterilized with 70% ethanol.

Proximate Analysis of African Star Apple

Moisture content was determined at 105^0C using air oven, ash content was determined at 550^0C with muffle furnace while crude protein, fat and fibre were determined according to the procedures of AOAC (1990).

pH Determination

The pH of the sample was determined using pH meter (Model Jenway 3051) standardized with buffer solutions of pH_4 and $_7$.

Minerals Analysis of African Star Apple

Various elements like Na, Ca, Mg, Fe and P were analysed from African star apple. The ash of the samples were analysed for their mineral contents. The sodium (Na) and Potassium (K) content determined by Flame Emission Spectrophometer (FES) while others calcium (Ca) Magnesium (Mg), Iron (Fe) and Phosporus (P) were determined using Atomic Absorption Spectrophotometer method (AAS) (AOAC 1995).

Isolation of Bacteria from African Star Apple

The fruits were expressed manually and the juice produce was serially diluted in test tube containing 9.0ml sterile distilled water and it was labeled as 10^{-1}. The dilution process was carried out serially until it got to the test tube labeled as $10^{4.}$ Nutrient agar and De-Mann Rogosa Sharpe (MRS) agar were used for the isolation of bacteria. The 0.5ml of diluted sample was spread plated onto nutrient agar and De-Mann Rogosa Sharpe (MRS) agar and incubated aerobically and anaerobically at 37^0C for 24 hours respectively for isolation. The selected colonies were purified by streak plate techniques and preserved on agar slants at 4^0C for further analysis.

Survival of LAB Isolates at Varying pH and Temperature

Lactic acid bacteria (LAB) isolates obtained from overnight culture were harvested by centrifugation for 15 minutes at 5000rpm and 4^oC washed twice with phosphate buffer solution (PBS) ($pH_{7.2}$) and adjusted to different temperature range (37^0C and 45^0C) and pH range (pH $_{3.0, 4.8, 6.5}$ and $_{9.6}$). Survival was assessed in duplicates in terms of viable colony counts and enumeration on MRS agar after incubation at 37^0C for 24 hours.

Tolerance to Bile Salt at different Concentration

MRS medium containing 0.3% and 0.5% (w/v) bile concentration was inoculated with overnight culture of lactic acid bacteria. Viable colonies were counted after incubation period 24 hours on MRS agar.

RESULTS

Proximate Compositions of African Star Apple

The moisture, crude fibre, carbohydrate, ash, fat, protein content and pH value were presented in table 1.

Minerals Analysis of African Star Apple

The potassium, calcium and sodium content were higher while iron had the lowest value.

Survival of LAB isolates at varying pH, Temperatures and Tolerance to Bile Salt Concentration

The growth and survival of the lactic acid bacteria at different temperatures, pH and bile salt concentration varied as shown in Table 3. *Lactobacillus lactis* and *Lactobacillus plantarum* grew at 37^0C and at 45^0C for *Lactobacillus lactis* while weak growth was noticed for *Lactobacillus plantarum*. They both survived at $pH_{3.0}$, $pH_{4.8}$, $pH_{6.5}$, $pH_{9.6}$ and at 0.3% and 0.5% bile salt.

Table 1: Proximate Compositions of African Star Apple (%)

Parameter	Value
Ash	1.72±0.03
Moisture	64.32±0.12
Fat	2.66±0.02
Crude fibre	18.18±0.02
Protein	1.71±0.09
Carbohydrate	11.41±0.23
pH	3.32±0.07

Table 2: Minerals Analysis of African Star Apple (ppm)

Parameter	Value
Sodium(Na)	55.50+0.13
Potassium(k)	112.00+0.08
Calcium(Ca)	78.00+0.23
Magnesium(Mg)	27.60+0.16
Iron(Fe)	0.69+0.03

Table 3: Growth Characteristics of Isolates at different Temperatures, pH and Bile Salt at different Concentration

Characteristics	A	B
Growth at 37^0C	+	+
Growth at 45^0C	+	±
Growth at pH 3.0	+	+
Growth at pH 4.8	+	+
Growth at pH 6.5	+	+
Growth at pH 9.6	+	+
Growth at 0.3% Bile salt	+	+
Growth at 0.5% Bile salt	+	+

Key:

+ good growth

±very weak

A: *Lactobacillus lactis*

B: *Lactobacillus plantarum*

DISCUSSION

Food items with high moisture content readily deteriorate due to activities of microorganisms. The moisture content of *Chrysophyllum albidum* was 64.32%, which was considered low compared to 86.30% reported for sample by Abiodun and Oladapo, (2011). This may be due to some factors, such as location, species, maturity and point at which harvesting was done. Ash content (1.72%) was low compared to 2.20% reported by Adepoju and Adeniji (2012). Fat content of 2.66% was very low to *Vitexdoniana* fruit (30.0%) and mango seed (14.80%) as reported by Agbede and Ibitoye (2007) and Fowomola, (2010) respectively. Low fat diets have been promoted for the prevention of heart disease and would be useful for obese patients that require low fat in their diets. The crude fibre had higher value of 18.18% compared to 2.40% obtained for the mango seed (Fowomola, 2010). The amount of crude fibre may influence the digestibility of the fruit and may also help to maintain normal internal distention of the intestinal tract and thus aid peristaltic movement (Abiodun and Oladapo, 2011). The pulp may be a good source of dietary fibre, meeting part of the recommended dietary fibre requirement of between 15 and 20g for prevention of nutritionally related diseases such as obesity, lowering of blood sugar and serum cholesterol, as well as cell growth and differentiation resulting into cancer (Sakata, 1993; Adepoju and Adeniji, 2012). Crude protein content of (1.71%) was lower compared to 5.89% obtained by Ureigho and Ekeke, (2010) and lower than the value reported for *Butyrospermum paradoxum*, by Adepoju and Ketiku (2003). Carbohydrate content had higher content of 11.41% than 10.38% reported by Ureigho and Ekeke (2010). ThepH of *C. albidum*(pH 3.32) was similar to orange juice of (pH3.5) reported by Egbekun and Akubor (2006). Low pH had been reported to inhibit the growth of undesirable microorganisms. The potassium content of the sample (112.00ppm) is low compared to the value (198.700ppm) obtained for *Carica papaya* by Oyeleke and Odedeji (2011). The *C. albidum* varieties can be good sources of potassium and calcium which are needed for electrolyte balance, neurotransmission, development of strong bones and teeth (Roth and Townsend, 2003).The low sodium content of the fruit makes it suitable for consumption by hypertensive patients (Adepoju and Adeniji, 2012). *C. albidum* was high in magnesium than that of other fruit pulps

such as *Spondiasmombin*, *Mordiiwhytii* and *Dialiumguineense* (Adepoju, 2009) but comparably lower in iron. The two isolates *Lactobacillus lactis* and *Lactobacillus plantarum* showed tolerability to bile salt at 0.3% and 0.5% concentrations. This is similar to the bile tolerance of different *Lactobacillus* sp isolated from conventional yogurt samples and traditional fermented food in Thaiup by Klayraung*et al*., (2008) and Ashraf*et al*., (2009). The tolerance of these LAB isolates to 0.3% and 0.5% bile salt is indicative of its potential as good probiotic bacteria adapted to adhere to intestinal, tolerate the gastrointestinal tract acid and bile conditions (Dunne *et al*., 1999; Pereira *et al*., 2003; Schillinger*et al*., 2005). The LAB isolates from *Chrysophyllumalbidum* survived at different pH3.0, 4.8, 6.5, 9.6 and temperature 37^0C and 45^0C which is in line with the LAB isolated from fermented milk ergo at different temperature of 20, 30, 37 and 45^0C as reported by Jermen*et al*., (2015).

CONCLUSION

This research showed African star apple as an excellent source of crude fibre, carbohydrates and minerals such as iron, potassium and calcium needed for digestibility, electrolyte balance, neurotransmission, development of strong bones and teeth. The lactic acid bacteria isolated from the fruit showed a great potential for probiotic thereby enhancing its health benefits.

REFERENCES

Abiodun, O.A. and Oladapo, A.S. (2011).Physico-chemical Properties of African Star Apple (*Chrysophyllumalbidum*) Components.*Nutr& Food Sci*. Vol. 41 (1): 8-11.

Adepoju, O.T. and Adeniji, P.O. (2012). Nutrient Composition and Micronutrient Potential of three Wildly Grown Varieties of African Star Apple (*Chrysophyllumalbidum*) from Nigeria. *African Journal of Food Science* Vol. 6(12): 344-351.

Adepoju, O.T. (2009). Nutrient Composition and Micronutrient Potentials of three Locally Available Wildly-grown Fruits in Nigeria.*Afr. J. Agric. Res*., 4(9): 887-892.

Adepoju, O.T. and Ketiku, A.O. (2003). Chemical Composition and Contribution of Shea Butter (*Butyrospermumparadoxum)* Fruit Pulp to Nutrient Intake of its Consumers. *J. Trop. For. Res*., 19(2): 20-28.

Adindu, M.N., Williams, J.O. and Adiele, E.C. (2003).Prelminary Storage Study on African Star Apple (*C.albidum*). *Plant Foods for Human Nutrition*, Vol. 58 No. 3, pp. 1-9.

Agbede, J.O. and Ibitoye, A. A. (2007). Chemical Composition of Black Plum (Vitexdoniana): an Under-utilized Fruit. *J.food, Agric. & Environ*. Vol.5 (2) : 95-96.

Amusa, N.A., Ashaye, O.A. and Oladapo, M.O. (2003).Biodeterioration of the African Star Apple (*Chrysophylumalbidum*) in Storage and the Effect on its Food Value.*Afr. J. Biotechnol*., Vol. 2(3): 56-59.

Andrew, B. (2013). Probiotic Manufacturing. www.nutraceutix.com.

AOAC, (1990).*Official Methods of Analysis*, 15th Edn., Association of Official Analytical Chemists. Washington, DC.

AOAC, (1995).*Official Methods of Analysis*, 15th Edn., Association of Official Analytical Chemists. Washington, DC.

Ashraf, M., Siddique, A.M. and Muhammad, G. (2009).*In-vitro* Screening of Locally Isolated Lactobacillus species for Probiotic Properties. *PakistanVet. J*. 29(4): 186-190.

Bada, S.O. (1997). Preliminary Information on the Ecology of *Chrysophillumalbidum* G. Don, in West and Central Africa In: Proceedings of a National Workshop on the Potentials of the Star Apple in Nigeria (eds) Denton OA, Ladipo DO, Adetoro MA, Sarumi MB, 16-25.

Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., Morrissey, D., Thornton, G., Fitzgerald, G., Daly, C., Kiely, B., Quigley EMM, Gerald, C., O'Sullivan, G.C., Shanahan, .F and Collins, J.K. (1999). Probiotics: from Myth to Reality: Demonstration of Functionality in Animal Models of Disease and in Human Clinical trials. AntonievanLeeuwenhoek. 76:279-292.

Egbekun, M.K. and Akubor, P.I. (2006).Chemical Composition and Sensory Properties of Melon Seed Milk-Orange Juice Beverage.*Nig. Food J*., Vol. 24 (1): 42-9.

Fowomola, M.A. (2010). Some Nutrients and Antinutrients Contents of Mango (*Magniferaindica*) seed. *Afr. J. Food Sci*. Vol. 4(8): 472-476.

Jermen, M. N., Fassil, A. T. and Anteneh, T. T. (2015). Evaluation of the Antagonistic Effect of Six Mixed Cultures of Lactic Acid Bacteria, Isolated from the Ethiopian Fermented Milk *ergo*, Against Some Foodborne Pathogens Inoculated into the Ethiopian Cottage Cheese *ayib*. *Afri.J.Microbio. Res*. Vol. 9(29): 1789-1797.

Klayraung, S., Viernstein, H., Sirithunyalug, J. and Okonogi, S. (2008). Probiotic Properties of *Lactobacilli* Isolated from thai Traditional Food. *Sci. Pharm*. 76:485-503.

Oyelade, O.J., Odugbenro, P.O., Abioye, A.O. and Raji, N.L. (2005). Some Physical Properties of African Star Apple (*Chrysophyllumalbidum*) seeds. *Journal of Food Engineering*, Vol. 67: 435-440.

Oyeleke, W.A. and Odedeji, J.O. (2011). Effect of Different Waxing Material on Some Chemical Properties, Minerals and Antinutrients Compositions of Pawpaw (*Carica papaya*). *Pakistan Journal of Nutrition* 10 (11): 1008-1012.

Pereira, D.I., McCartney, A.L. and Gibson, G.R. (2003). An in Vitro Study of Theprobiotic Potential of a Bile Salt Hydrolyzing *Lactobacillus fermentum*Strain, and Determination of its Cholesterol-lowering Properties. *Appl. Environ. Microbiol*. 69: 4743-4752.

Roth, A.R. and Townsend, C.E. (2003).Nutrition and Diet Therapy 8th edn. Delmar Learning, Thomson Learning Inc. Canada.pp. 132.

Sakata, T. (1993).Effects of Short-chain Fatty Acids on Gastrointestinal Epithelial Cells. In: Dietary fibre. Mechanisms of Action in Human Physiology and Metabolism, ed. Cherbut C, Barry JL, Lairon D & Durand M. *Paris, France*, pp. 61-68.

Schillinger, U., Guigas, C. and Holzapfel, W.H. (2005). In Vitro Adherence and other Properties Of *Lactobacilli* Used in Probiotic Yogurt-like Products. *Int. Dairy J*. 15: 1289-1297.

Ureigho, U. N. and Ekeke, B.A. (2010). Nutrient Values of *Chrysophyllum albidum* Linn African Star Apple as a Domestic Income Plantation Species.pp.50-56.