Potentials of Sodom apple *(Calotropis procera)* extract as a coagulant to substitute Alum in soy cheese production in Ghana

Chikpah S. K[1,*], Teye M[2], Annor J. A. F[1] and Teye G. A[3]

[1]Department of Biotechnology, Faculty of Agriculture, University for Development Studies, Tamale, Ghana.
[2]Department of Animal Science, School of Agriculture, University of Cape Coast, Cape Coast, Ghana.
[3]Department of Animal Science, Faculty of Agriculture, University for Development Studies, Tamale, Ghana.

ABSTRACT

This study was conducted to determine the potentials of Sodom apple (*Calotropis procera*) extract as a sole coagulant of soymilk during soy cheese production. Four alum concentrations were prepared and used as control treatments: A5 (0.5 % conc.), A10 (1.0 % conc.), A15 (1.5 % conc.) and A20 (2.0 % conc.). Four strengths of *Calotropis procera* extracts (CPE) (C15, C20, C25 and C30) were obtained from 15 g, 20 g, 25 g and 30 g respectively of the fresh plant. Each of the concentrations of alum and *CPE* was used as a sole coagulating agent of soy milk and the effects on clotting time, product yield, whey volume and organoleptic properties of the curd were assessed. The average milk clotting time, curd yield, and whey volume were significantly ($P < 0.05$) influenced by the type and concentration of coagulant used to achieve curdling. The average clotting time, curd yield, and whey volume were in the ranges of (6.0 - 31 minutes), (120.2 – 207.8 g/l), and (495.0 – 785.0 ml/l) respectively. Treatments A20 and C30 recorded the shortest clotting times of 6.0 and 9.0 minutes respectively whereas treatment A5 had the longest clotting time (31.0 min). The curd yields were high in treatments A10 (207.8 g/l), C30 (191.2 g/l) and C25 (183.9 g/l) whereas A20 recorded the least curd yield (120.2 g/l). In the alum treatments, positive correlation ($r = 0.585$) was observed between soy milk clotting time and soy curd yield. On the contrary, there was strong negative correlation ($r = - 0.803$) between clotting time and curd yield when *CPE* were used as coagulants. The different concentrations of alum and *C. procera* added to soy milk had significant ($P < 0.05$) effects on the organoleptic properties (taste, colour, texture and overall acceptability) of the soy cheese produced. The soy cheese flavour did not vary significantly ($P > 0.05$) among the treatments. Generally, it was observed that the 25g CPE can be used to substitute for alum in soy curd formulation.

Introduction

Cheese is a food obtained from milk and is produced in a wide range of flavours, textures and forms by coagulation of the milk protein. Cheese production serves as a means of preserving essential nutrients in milk, and is an excellent source of proteins, fat, minerals and vitamins (O'Conner, 1993). Generally, cheese making in Africa has not been properly documented and is largely dictated by tradition. However, the production of cheese in African countries has increased and about one-third of the total volume of milk obtained from lactating cows is used for this purpose (FAO, 2002).

For the production of high quality cheese, rennet enzyme is added for the effective curdling of the milk. Rennet is a general term used to describe a variety of enzymes from animals (usually calves), plants or microbial origin used for the coagulation of milk (Craw, 1983). The milk clotting properties are of much importance with regards to both yield and organoleptic quality of cheese. The shelf life, protein content, quality and quantity of cheese produced are consequently affected by the type of coagulants used (Awolola *et al*., 2010).

Milk obtained from a lactating cow is the most widely used milk in cheese formulation, but milk from goat and sheep are sometimes also used. However, recent consumer concerns regarding higher cholesterol levels in animal milk, and inadequate quantities of milk obtained from animals, have caused a tremendous increased in the use of milk from soybeans (*Glycine max*) to formulate soy cheese.

Soymilk is extracted from soybeans and could be described as a milk-like suspension of fine soy solids in water (Lal *et al*., 1987). Soymilk is of considerable interest to nutritionists due to its great potentials to substitute animal milk. It is reported to be suitable for use as infant formula for infants suffering from malnutrition and those who are allergic to pasteurized cow milk protein (Merritt and Jenks, 2004). Soy products are now spreading across the world as healthy foods and as a low-cost source of nutrients (Soya-Agrodok, 2005).

Soy cheese, popularly known as "Tofu" in most Asian countries, is an unfermented soy product made by curdling fresh hot soymilk (Oboh, 2006). In recent years, the consumption of soymilk and soy curd had increased, perhaps due to the perceived health problems associated with the high consumption of animal products. For this reason, soy cheese production has become a great business for quite a number of women in developing countries like Ghana.

In Ghana, the soy curd is generally prepared by curdling soy milk with concentrated alum solution. However, research had indicated that the use of alum in treating water for humans could induce Alzheimer's disease and other carcinogenic problems in consumers (Miller *et al*., 1984; McLachlan, 1995). Therefore, it is necessary to find alternative sources of coagulating agents that

Tele: +233-246927641
E-mail addresses: braveboy.solo@gmail.com

are readily available, affordable and with minimal or no health risks for use in soy cheese formulation. Proteolytic enzymes from certain plants have been reported to serve as coagulants of soy milk (O'Connor, 1993). One of such plants is the Sodom apple (*Calotropis procera*).

Extracts from the succulent leaves and stems of *C. procera* has been reported to contain rennet enzymes called calotropin that coagulates cow milk at temperatures less than 100 ^{0}C (Belewu and Aina, 2000). The extracts from the Calotropis plant has been used traditionally as the sole coagulant of cow milk in a number of African countries for the production of a soft cheese called '*Wakashi*' (Ibiama and Griffiths, 1987). However, the ability of this extract to coagulate soy milk for the formulation of soy curd is not known. This study therefore sought to investigate the potential of *Calotropis procera* extract (CPE) as a coagulant during soy curd preparation.

Materials and methods
Study location
The study was conducted at the Food Technology Unit of the Faculty of Agriculture, University for Development Studies (UDS), Tamale, Ghana.
Soybeans and Soymilk Extraction
Soy beans used in this study were obtained from the Tamale central market in the Northern region of Ghana. The milk was extracted from the soybeans according to the method described by Oboh (2006) with some modifications. The soybeans were washed and then soaked in clean water at a temperature of about 30°C for 9 hours. The water was then drained off and the beans were washed again with fresh water. The testa (seed coat) was removed manually by rubbing the soaked beans in between the palms. The dehulled soybeans were milled using a domestic electric blender. Three kilograms of the milled soy bean was manually mixed with 12 litres of distilled water and stirred thoroughly for uniform mixing. The slurry was filtered with the use of an autoclaved-linen filter cloth. The milk was collected in a glass jar for later use.

Coagulants used
Alum solution and *Calotropis procera* extracts (CPE) of varying concentrations were used as clotting agents of the soy milk.
Preparation of Alum Solutions
Alum crystals were obtained from the Tamale market. Alum stock solutions of concentrations; 0.5 %, 1.0 %, 1.5 % and 2.0 % were prepared by dissolving 5 g, 10 g, 15 g and 20 g of alum respectively in 1 litre each of distilled water, then agitated with a mechanic shaker at 150 rpm for 10 minutes.
Processing of *Calotropis procera* leaves and stems
Equal quantities of succulent leaves and stems of *C. procera* plants were harvested in Tamale. Four different strengths of extracts were prepared with approximately 15 g, 20 g, 25 g and 30 g of the succulent leaves and stems of the plant were measured and ground separately using a laboratory mortar and pestle. Each of the homogenized plant materials was transferred into a 100 ml volumetric flask and 50 ml of soy milk each was added to each flask and agitated with a mechanical shaker at 150 rpm for 5 minutes. The mixture in each of the flasks was filtered and the filtrate was then used as coagulant of soymilk. The quantities of plant extracts used was based on preliminary studies conducted by the authors, where it was realized that extracts obtained from less than 15 g plant parts resulted in no coagulation of milk whiles plant parts greater than 30 g resulted in an undesirably bitter curd with an extremely green colour.

Soy curd preparation
Soy curds were prepared according to the method used by the traditional milk processors. The soymilk was pasteurized at a temperature of 70° C for 20 minutes using an electric plate. Approximately 200 ml of each of the alum solutions (0.5 %, 1.0 %, 1.5 % and 2.0 %) was measured and added to 1000 ml of the pasteurized soymilk and heated at a temperature of about 50 ^{0}C and carefully observed until coagulation occurred.

The *C. procera*-soymilk filtrates prepared earlier were used to coagulate equivalent volumes (1000 ml) of soymilk at a temperature range of 40-50 ^{o} C and observed carefully until clotting begun. Coagulation was allowed to continue for about 20 minutes when adequate coagulation of the mixture was observed. The mixture of coagulum and water (whey) was allowed to cool and then filtered using an autoclaved linen cloth to separate the whey from the coagulum or raw curd. The soy curd was then pressed to drain excess whey and to make the curd firm (Plate 1). Each product preparation was repeated three times.

Plate 1: A - curd made from soymilk coagulated with alum solution; B - curd produced from soymilk coagulated with *C. procera* extract

Measurement of certain parameters of the curds
The parameters measured include milk clotting time (minutes), the product yields (g/l) and sensory evaluations.
Measurement of Soy Milk Clotting Time
Clotting time is the time taken for the first clot to form. The set up for soy curd preparation was carefully observed and the time (minutes) of first clotting after the coagulant was added was recorded for each treatment. The average clotting time of the three replicates of each treatment was calculated and used as the mean clotting time.
Determination of curd yield and whey volume
The yield or weight (g/ litre of milk) of the final soy curd produced was weighed using an electronic weighing scale (Sartorius, TE 612). The average of the three replicates was computed and used as the mean curd yield of each treatment. Similarly, the volume of whey that was discharged at the end of the cheese making process was measured with a volumetric flask and the average of the three replicates was calculated for each treatment.
Sensory evaluation
A five-point hedonic scale (Table 1) described by Sugri and Johnson (2009) with some modifications was used to assess the sensory characteristics of the soy curd samples. The sensory qualities considered include: taste, flavour, colour, texture and overall acceptability of the products. Raw soy cheese samples were used for colour and texture evaluations whiles cooked (fried) soy curds were used for the evaluation of the taste, flavour and overall acceptability of the products. Fifteen panelists were randomly selected and trained for the sensory evaluation of the products.

Data Analysis

The data generated was subjected to analysis of variance (ANOVA) using GenStat statistical software (fourth edition) and where significant differences occurred, the means were compared at 5% level of significance.

Results and Discussion

Effects of coagulant type and concentration on soy milk clotting time, curd yield and whey volume

The clotting times, curd yields and whey volumes of alum and fresh PCE treated soy milk are shown in Table 2. The average milk clotting time, curd yield, and whey volume were significantly (p < 0.05) influenced by the type and amount of coagulant used for milk clotting (Table 2). The mean soymilk clotting time ranged between 6.0 - 31 minutes. Treatment A20 (2.0 % of alum) recorded the shortest clotting time (6.0 minutes) followed by treatment C30 (9.0 minutes) whereas treatment A5 (0.5 % alum) had the longest clotting time (31.0 minutes). The average clotting time for treatments A10 and C20 were similar (p > 0.05). Again, treatments A15, C25 and C30 were also not significantly (p > 0.05) different, but these were significantly different (p< 0.05) from the other treatments (Table 2). The differences between the clotting times of the various treatments confirms the earlier findings of Shih *et al.* (1997) who reported that coagulant characteristics such as coagulant types and amount of coagulant added to soy milk affect the rate of coagulation. Generally, the average milk clotting time decreased when concentrations of the coagulants were increased. This agrees with findings of Lopez *et al.* (1998) and Risso *et al.* (2008) which stated that clotting time decreases when enzyme concentration is increased because of a higher level of proteolysis of k- casein. It can therefore be suggested that to achieve effective coagulation in one (1) litre soy milk during soy curd formulation, 1.5 - 2.0 % alum concentration and extracts from 25 – 30 g of *C. procera* leaves and stems can be used for quick coagulation of soy milk.

The average values of soy curd yields ranged between 120.2 – 207.8 g/l. Treatment A10 had the highest curd yield (207.8 g/l) while treatment A20 recorded the least curd yield (120.2 g/l). The curd yields for treatments A5 (178.1 g/l), C25 (183.9 g/l) and C30 (191.2 g/l) were not significantly (p > 0.05) different from each other. Similarly, the curd yields of treatments A15 (123.5 g/l) and C15 (133.7 g/l) did not vary significantly (p > 0.05). The soy curd yield reduced when alum concentrations decreased below or increased above 1.0 % concentration. On the contrary, increase in soy curd yields were observed with increasing strengths of fresh CPE (Table 2).

The average whey volume also differed significantly (p< 0.05) among some treatments. The average whey volume expulsed at the end of the curd making process ranged between 495.0 – 785.0 ml/l representing 49.5 % - 78.5 % of initial milk volume. It was generally observed that less whey volumes were discharged when soymilk were coagulated with fresh CPE as compared to the alum coagulated milk treatments.

The significant differences in yield of soy curd and whey volume recorded among the various treatments suggest that these curd parameters were significantly affected by the type and amount of the coagulant added to the milk. This observation agrees with the findings of Akinloye and Adewumi (2014), who reported that the type of coagulant used in cheese production results in variations in yields.

It was generally observed that the average soy curd yields were higher when CPEs were used as coagulants, compared with those produced by using the chemical coagulant, alum. This

result agreed to the earlier findings of Awolola *et al.* (2010) that in the production of 'Tofu'-soy curd, natural coagulants produced higher yields than those produced with chemical coagulants. It is conclusive that Treatments A10, C25 and C30 had outstanding performances in terms of curd yields.

Correlation between soy milk clotting time and soy curd yield

Figure 1 shows the relationship between clotting time and soy curd yield. There was a positive correlation (r = 0.585) between coagulation time and curd yield when alum solution was used. This indicates that a decrease in clotting time of soy milk will result in a decrease in curd yield and vice versa (Figure 1). On the contrary, there was a strong negative correlation (r = -0.803) between soy milk clotting time and curd yield when coagulation was done using *CPE* From Figure 1, it can be observed that the use of CPEs increased yield of curd when clotting time decreases. It is therefore conclusive that Treatments C25 and C30 could be used to effectively substitute alum solution in soy curd production.
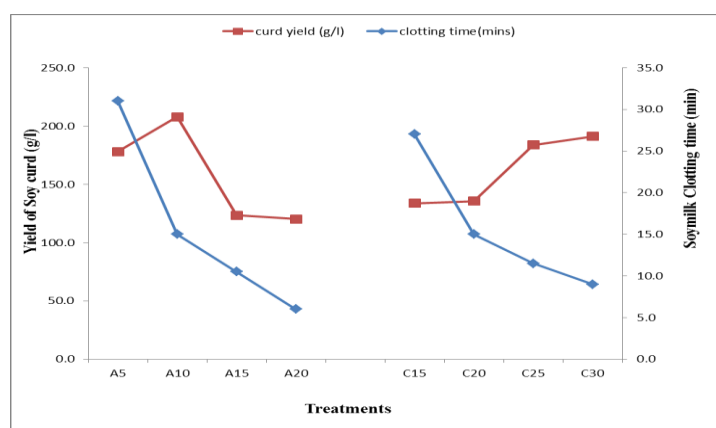
Figure 1: Relationship between soy milk clotting time and curd yield. (Treatments A5, A10, A15, and A20 represent 0.5 %, 1.0 %, 1.5 % and 2.0 % concentrations of alum solution while C15, C20, C25, and C30 represent extracts from 15 g, 20 g, 25 g and 30 g of *C. procera* leaves and stems).

Effects of different concentrations of alum and *Calotropis procera* extracts as coagulants on the organoleptic properties of soy cheese

The mean scores of the organoleptic properties (taste, flavour, colour, texture, and overall acceptability) of soy cheese prepared with different concentrations of alum and CPE as milk coagulants are shown in Table 3. The type and amount of coagulants that were added to the soymilk for curdling had significant (p < 0.05) effect on the taste, colour, texture, and overall acceptability of soy cheese. The taste of soy cheese were scored between the range of 1.77- 2.43. The result indicates that all the soy cheese samples were slightly sweet. The colour of raw soy curd samples ranged between almost white to slightly green. All the curd samples produced from the curdling of soy milk with different concentrations of alum solutions were almost white in colour (scored 1.18 – 1.23) whereas the different concentrations of CPEs impacted a slightly green colour (scored 1.63- 2.40) on the curd samples. The colour of soy curd samples of treatment C25 and C30 were significantly (p < 0.05) different from the soy curd of treatment C15 and C20. The degree of greenness increased with an increasing concentration of CPE.

Table 1: Sensory parameters used to evaluate the products.

Hedonic scale	Taste	Flavour	Colour	Texture	Overall acceptability
5	Extremely sweet	Extremely strong	Very green	Very hard	Excellent
4	Very sweet	Very strong	Green	Hard	Very good
3	Sweet	Strong	Pale green	Slightly hard	Good
2	Slightly sweet	Slightly strong	Slightly green	Soft	Fair
1	No sweetness/Bitter	Off-flavours	Almost white	Very soft	Dislike

Sugri and Johnson (2009)

Table 2: Effects of different concentrations of alum and *Calotropis procera* extracts on soy milk clotting time, soy curd yield and whey volume.

Treatments	Clotting time (minutes)	Curd yield (g/l)	Volume of whey(ml/l)
A5	31.0^a	178.1^b	705.0^b
A10	15.0^c	207.8^a	715.0^b
A15	10.5^d	123.5^{cd}	785.0^a
A20	6.0^e	120.2^d	765.0^{ab}
C15	27.0^b	133.7^{cd}	545.0^{cd}
C20	15.0^c	135.7^c	565.0^c
C25	11.5^d	183.9^b	540.0^{cd}
C30	9.0^d	191.2^b	495.0^d
P- value	0.001	0.001	0.001

Values represent means of three replicates. Means in the same column with similar superscripts are significantly different ($p < 0.05$). Treatments A5, A10, A15, and A20 represent 0.5 %, 1.0 %, 1.5 % and 2.0 % concentrations of alum solution while C15, C20, C25, and C30 represent extracts from 15 g, 20 g, 25 g and 30 g of *C. procera* leaves and stems

Table 3: Effects of the type and concentration of milk coagulant on the organoleptic characteristics of the soy cheese

Treatment	Taste	Flavour	Colour	Texture	Overall acceptability
A5	1.87^{bc}	2.30	1.23^c	2.43^a	2.87^{ab}
A10	2.20^{abc}	2.07	1.20^c	1.90^b	3.00^{ab}
A15	2.43^a	2.30	1.20^c	2.70^a	3.23^a
A20	2.33^a	2.60	1.18^c	2.33^a	2.97^{ab}
C15	1.77^c	2.03	1.73^b	1.40^c	2.30^c
C20	2.17^{abc}	2.30	1.63^b	1.80^b	2.60^{bc}
C25	2.27^{ab}	2.40	2.13^a	1.87^b	2.50^{bc}
C30	1.87^{bc}	2.27	2.40^a	2.40^a	2.23^c
P-value	0.030	0.201	0.001	0.001	0.001

Values represent means of three replicates. Means within the same column having no superscript in common are significantly different ($p < 0.05$). A5, A10, A15, and A20 represent 0.5 %, 1.0 %, 1.5 % and 2.0 % concentrations of alum while C15, C20, C25, and C30 represent extracts from 15 g, 20 g, 25 g and 30 g of *C. procera* leaves and stems.

The green colour of soy curd samples emanated from the chlorophyll present in the leaves and stems of *C. procera*.

For the texture, the soy curd samples of all the alum treatments (A5, A10, A15 and A20) were slightly harder than the CPE curds (C15, C20, C25, and C30) which were generally soft in texture. The hardness of the soy curds increased progressively with increasing concentrations of coagulants. deMan *et al*. (1986) reported that the texture and microstructure of the soybean curd were greatly influenced by the type of coagulant used. The generally soft nature of the curd produced with the CPEs confirms earlier reports that extracts from this plant are not suitable for increasing hardness in cheese (O'Connor and Tripathi, 1992).

The type of coagulant and the amount added to soy milk did not significantly ($p > 0.05$) influence the product flavour. The flavours of the products were slightly strong. The different concentrations of alum and *C. procera* had significant ($p < 0.05$) effect on the overall acceptability of the soy curd samples. The overall acceptability of the soy curd products were scored between fair and good. Generally all the soy products of the alum treatments and that of C20 and C25 had good preference whereas soy curd samples of treatment C15 and C30 were fairly preferred and scored between (2.23-2.30) as compared to the rest of the soy cheese samples.

Values represent means of three replicates. Means within the same column having no superscript in common are significantly different ($p < 0.05$). A5, A10, A15, and A20 represent 0.5 %, 1.0 %, 1.5 % and 2.0 % concentrations of alum while C15, C20, C25, and C30 represent extracts from 15 g, 20 g, 25 g and 30 g of *C. procera* leaves and stems.

Conclusion and Recommendations

It is conclusive that the milk clotting time, yield and quality of soy curds depend on the type and concentration of coagulant added to the milk during curdling. The highest concentrations of the two coagulant types: A20 (2.0 % of alum) and C30 (extracts from 30 g of *C. procera*) recorded the shortest milk clotting time. Generally, soy milk clotting time decreases with increasing concentration of the milk coagulant. Treatment A10, C25 and C30 gave the best curd yield whereas treatment A20 had the least yield. Soy curds produced from treatments A10 and A15 had the best overall acceptability whiles curds of treatment C30 were less preferred by the consumers due to the green coloration. Treatment C25 had a general good effects on curds, thus is a good substitute for alum in soy curd formulation.

It is recommended that further work should be done on the effects of CPE on the nutritional composition and shelf life of soy curds. Efforts should be made to minimize the green - coloration of CPE in order to reduce the colouration effects on its products.

References

Akinloye, A. M. and Adewumi, O. O. (2014). "Effects of local coagulants on the yield of cheese using cow and sheep milk", *International Journal of Development and Sustainability*, Vol. 3 No. 1, pp. 150-161.

Awolola, G.V., Oluwaniyi, O.O., Solanke, A., Dosumu, O.O., and Shuiab, A.O. (2010). Toxicity assessment of natural and chemical coagulants using brine shrimp (Artemia salina) bioassay, Int. J. Biol. Chem. Sci. 4(3): 633-641.

Belewu, M.A and A.B.J Aina, (2000). Microbial evaluation of indigenous milk products with special reference to microbial flora of public health importance in Nigeria. Afr. J. Exp. Microbial., 1: 13-19.

Craw, R. J. (1983). Future development in rennet and is uses in the cheese factory, International Diary Federation Bulletin, (14), pp.194

deMan, J. M., deMan, L., and Gupta, S. (1986). Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *Food Microstructure* 5, 83-89.

FAO (2002). Production yearbook, volume 56. Rome: Food and Agriculture Organization of United Nations (FAO).

Ibiama, E. and Griffiths, M. W. (1987). Studies on milk coagulating enzymes calotropin, obtained from Sodom apple (Calotropis procera). Journal of food and agriculture, pp. 157-167.

Lal, R.R., Siripurapu, S.C., and Singh, B.P. (1987). Physical Properties of Sweetened and Unsweetened Soymilk. Journal of Food Science and Technology. 24: 60-62.

Lopez MB, Lomholt SB, Qvist KB, 1998. Rheological properties and cutting time of rennet gels. Effect of pH and enzyme concentration. Inter. Dairy J 8: 289-293.

Merritt, R.J. and B.H. Jenks (2004). "Safety of soy-based infant's formulas containing isoflavones: the clinical evidence". Journal of Nutr. 134 (5): 1220s-1224s.

McLachlan D.R.C. (1995). Aluminium and the risk for Alzheimer's disease. *Environmentrics*, 6: 233.

Miller R.G., Kopfler F.C., Kelty K.C., Strober J.A., Ulmer N.S. (1984). The occurrence of aluminum in drinking water. J. Am Water Work Assoc., 76(1):84-91.

Oboh Ganiyu. (2006). African Journal of Biotechnology Vol. 5 (3), pp. 290-294.

O'Connor C. B. (1993). Traditional cheese making manual. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia.

O'Connor C.B and Tripathi B.R. (1992). Milk processing techniques- sour milk. Audiotutorial module 2. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia. 20 pp.

Risso P, Borraccetti M, Araujo C, Hidalgo ME, Gatti C, 2008. Effect of temperature and pH on the aggregation and the surface hydrophobicity of bovine κ-casein. Colloid Poly Sci. 286: 1369-1378.

Shih, M. C., Hou, H. J., and Chang, K. C. (1997). Process optimization for soft tofu. Journal of Food Science 62, 833–837.

Soya-Agrodok. (2005). Cultivation of soya and other legumes. In: Extrusion cooking, Mercier, C.P. Linko and J.M Harper (Eds). Agromisa foundation, USA, pp: 321-341.

Sugri I. and P.N.T Johnson (2009). Effect of two storage methods on the keeping and sensory qualities of four plantain varieties. African Journal of Food, Agriculture, Nutrition and Development, vol. 9, No. 4, June, 2009, pp. 1091-1109.