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BIOCHEMICAL CHANGES INDUCED BY FIVE PATHOGENIC FUNGI ON SEEDS OF *Hibiscus sabdariffa* (YAKWA)

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ABSTRACT

Different biochemical analysis were carried out to determine the changes induced by some fungi inoculated on Hibiscus sabdariffa linn seed for 14days. The inoculated fungi are Aspergillus niger Van Tieghem, Aspergillus flavus Link Ex fr, Fusarium oxysporum Schlecht, Penicillium chrysogenum Thom and Penicillium roqueforti Thom. The moisture content of fungi– inoculated seeds increased (p=0.05) when compared with the uninoculated control. Fusarium oxysporum (64.7%) caused the highest increase in moisture followed by Penicillium chrysogenum (61.0%), Aspergillus flavus (58.9%) and Aspergillus niger (58.2%). There was a significant decrease (P=0.05) in Dry Matter from the control seeds when compared to the fungi inoculated seeds. There was also a decrease in carbohydrate of fungi inoculated seeds as when compared with the uninoculated control. The protein content of the fungi inoculated seeds, increased significantly when compared to the uninocualted control (25.5%). Penicillium chrysogenum (29.9%), Fusarium oxysporum (28.7%), Aspergillus flavus (28.4%), Aspergillus niger (27.8%) increased in that order. The increase could be due to the presence of proteinaceous mycelium in the fungi. There was a significant decrease (P=0.05) of lipid from the uninoculated control (10.38%) when compared to the fungi inoculated seeds. The decrease in oil content could be due to the hydrolysis of oil to free fatty acid. There was a drastic and significant decrease in fibre caused by utilization during fermentation. There was significant increase (P=0.05) in ash, when compared to the uninoculated control. This is due to the presence of minerals like potassium and phosphorous in the mycelia of the fungi.

Key words: Hibiscus sabdariffa Linn Seed, Biochemical changes and fungi.

INTRODUCTION

Hibiscus sabdariffa Linn (Yakwa) is an angiosperm of the family Malvaceae. It is an annual herb that grows to 180 cm. It is an important crop grown for its numerous uses. The seeds, calyxes and leaves are of economic importance to man (Babalola, 2000).

Roselle seeds are a commercial source of a vegetable oil that is low in-cholesterol and rich in other phytosterols and tocopherols, particularly *β*-sistosterol and *γ*-tocopherol. The global characteristics of roselle seed oil allow important industrial applications for the oil, which represent an added value for the culture of this plant (Mohamed *et al*., 2007). The brownish-yellow

seed oil is claimed to heal sores on camels (Adegunloye *et al*., 2000). In India, a decoction of the seeds is given to relieve dysuria, strangury and mild cases of dyspepsia and debility (Jain and Bal, 1997). The residue remaining after extraction of oil by parching, soaking in water containing ashes for 3 or 4 days, and then pounding the seeds, or by crushing and boiling them, is eaten in soup or blended with bean meal in patties. It is high in protein.

In Nigeria, a decoction of the seeds is given to augment or induce lactation in poor let down and maternal mortality (Okasha *et al.,* 2008). The seeds are considered excellent feed for chickens and have been used as aphrodisiac coffee substitute. The residue after oil extraction is valued as cattle feed when available in quantity. Roselle (*Hibiscus sabdariffa* var. *Sabdariffa)* seed meal and kenaf (*Hibiscus sabdariffa* var. *altissima*) seed meal are used as replacement for soybean meal in practical diets for fingerlings of nile Tilaapia, *Oreochromis niloticus* (Fagbenro, 2005).

Seeds of *Hibiscus cannabinus*, *Hibiscus asper*, *Hibiscus esculenta*, *Adansonia digitata* and *Ceiba pentandra* are also sometimes used along with *H. sabdariffa* seeds to produce Bikalga in certain areas of Burkina Faso. (Parkouda *et al.*, 2008). *Bikalga* is a food condiment obtained by a traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds in African countries, including Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others. It is also known as dawadawa botso (Niger), datou (Mali), Furundu (Sudan), Mbuja (Cameroon). Bikalga is mainly produced by women and constitute an economical source for the producers. It is mainly produced in areas of Burkina Faso where *Parkia biglobosa* seeds for production of Soumbala (alkaline food condiment) are not commonly found. Soumbala and Bikalga are the most popular food condiments in Burkina Faso; they are used as meat replacement mainly by low-income population. Bikalga is also used by some ethnic tribes to cure high blood pressure, diarrhoea, and rubella or is used as an antiseptic. (Parkouda *et al*., 2008).

Roselle seed is composed of 28.69 ± 1.15% of crude proteins, 21.93 ± 0.74% of crude lipids, 26.39 ± 1.03 % of carbohydrates as compared with the fermented seed with the following composition: 26.47 ± 1.5 for proteins 23.19 ± 1.25 for crude lipids and 13.7 ± 0.62 for carbohydrates. (Parkouda *et al*., 2008).

The world attitude is now oriented to develop low cost protein foods of plant origin to combat nutritional problems projected from protein/calorie deficiency. Traditional food processing techniques are believed to improve nutritive value of plant foods. Roselle "Karkade" (yakwa) seed was reported to be a prospective cheap protein source (Yagoub *et al*., 2007)

This study was carried out to know the effect of *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Penicillium chrysogenum* and *Penicillium roqueforti,* on the proximate component of *Hibiscus sabdariffa* seed when compared to the uninoculated control.

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MATERIALS AND METHODS

Isolation and identification of fungi

The fungi used in this study (*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Penicillium chrysogenum* and *Penicillium roqueforti)* were isolated from diseased seeds (Nwaukwu and Ataga, 2012) using the standard Blotter method recommended by the International Seed Testing Association (ISTA, 1976) and Agar method (Klement and Voros, 1974). The fungi were identified under a Stereobinocular microscope based on their habit characteristics. Slides were made to confirm identification following descriptions by International Mycological Institute (IMI fungi Descriptions). Pure, single spore cultures of each fungi were obtained by growing them on potato dextrose agar (PDA). The cultures were grown in complete darkness in an incubator for 7days at 21 ± 2^0C they were used as inoculum.

Inoculation of Seeds with Fungi

One hundred grams of healthy *Hibiscus sabdariffa* seeds were weighed out into 250ml conical flasks, plugged with non –absorbent cotton wool and covered with foil and then autoclaved at 121^{o}C for 15 minutes to eliminate any internal and external seed borne micro- organism. After autoclaving the flasks were allowed to cool and 100ml of sterile distilled water was added to each flask and shaken gently to wet all the seeds and to create a humid and conducive environment for the fungi to be inoculated to have an even distribution. Each flask containing seeds was inoculated with a disc of 7 day old mycelium spores of each fungus obtained from the pure culture of isolated fungi from infected seeds. This was done with a 1.5cm diameter sterile cork borer.

The flasks were gently shaken for about 15 minutes to obtain uniform distribution of the mycelium among the seeds. The control flask, received the same treatment, but there was no fungi added to it. The entire flasks which include the fungi inoculated and uninoculated seeds were incubated at room temperature (28^0C) in complete darkness for 14 days.

A total of 18 flasks were used, 3 flasks replicate for each set of fungi inoculated seeds and uninoculated seeds. At the end of the incubated period, the flasks of each fungal treatment and flasks for control were harvested for biochemical analysis. The seeds in each flask were transferred into a pre-weighed watch glass, dried at 45^{o}C for 24 hours and the spores and mycelia of the fungi removed by sieving.

Biochemical analysis of various nutrient component (dry matter, moisture, Extracted oil, crude protein, fibre, ash content and carbohydrates) in both fungus-inoculated and uninoculated seeds at the incubation period of 14 days were determined following procedures recommended by the Association of Official Analytical Chemists (AOAC, 1995). The results of each component were subjected to statistical analysis using the Analysis of Variance (ANOVA).

RESULTS

The results of the biochemical analysis of the various nutrient components of fungus- inoculated and uninoculated seeds of *Hibiscus sabdariffa* Linn for 14 days are represented in Table 1.

Table 1: Changes in levels of nutrients in *Hibiscus sabdariffa* Linn (Yakwa) Seeds inoculated with Fungi and incubated at 28 ± 2^0C for 14 days

	Nutritional Composition (%W/W)/100g						
Fungi	**Dry Matter**	**Moisture**	**Carbohydrate**	**Protien**	**Lipid**	**Ash**	**Fibre**
Uninoculated Control	47.3*	52.7	7.1	25.5	10.38	5.2	8.30
Aspergillus Flavus	41.1	58.9	5.1	28.4	9.90	7.9	2.40
Aspergillus niger	41.8	58.2	4.6	27.9	9.04	7.6	1.81
Fusarium oxysporum	35.3	64.7	3.6	28.7	8.00	9.5	1.20
Penicillium chrysogenum	39.0	61.0	4.8	29.9	8.72	9.1	0.50
Penicillium roqueforti	39.9	60.1	4.9	28.5	8.62	8.5	0.48
LSD 0.05	**6.2**	**6.2**	**0.01**	**0.3**	**0.09**	**0.2**	**0.02**

**Key ***Means of two determinations with three replicates.

L.S.D. Least significant difference for comparison of treatments mean

DISCUSSIONS

The inoculation of Yakwa seed with the following fungi; *F. oxysporum, P. chrysogenum A. niger, P.roqueforti, and A. flavus* at room temperature for 14 days resulted in various degrees of deterioration. The moisture content of the fungi–inoculated seeds increased (p=0.05) when compared with the uninoculated control. *Fusarium oxysporum* (64.7%) caused the highest increase in moisture followed by *Penicillium chrysogenum* (61.0%), *Aspergillus flavus* (58.9%) and *Aspergillus niger* (58.2%). The increase caused by the fungi is due to their utilization of the components of the seeds as food nutrient thereby producing water in the process. Similar results were recorded by Ataga and Akueshi (1986) in sunflower seeds inoculated with fungi. Ataga and Umechuruba (1997) also reported increase in moisture content of African Yam bean inoculated with storage fungi.

The reverse is the case for Dry Matter. There was a significant decrease (P=0.05) from the control seeds when compared to inoculated seeds. *Fusarium oxysporum* had the highest decrease when compared with the other fungi. This fungus produces extracellular cellulolytic and pectic enzyme and secondary metabolites which may be responsible for the drastic depletion of dry matter (Okonkwo *et al*., 1990).

There was also a decrease in carbohydrate of fungi inoculated seeds as when compared with the uninoculated control. The decrease in the inoculated seeds could be due to the utilization of storage starch and sugar as a carbon source by the fungi during respiration and also a source of energy for microbial growth (Monday and Ataga,

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2005). Nwaukwu and Ikechi-Nwogu, (2012) obtained similar result in their research on effect of six pathogenic fungi on *Dialium guineense.*

The protein content of the fungi inoculated seeds, increased significantly when compared to the uninocualted control (25.5%). For the fungi, Penicillium chrysogenum(29.9%), *Fusarium oxysporum* (28.7%), *Aspergillus flavus* (28.4%), *Aspergillus niger* (27.8%) increased in that order. The increase caused by the above fungi could be due to the presence of proteinaceous mycelium in the fungi. Cherry and Beuchat, (1975) obtained a similar result in their study of protein changes in groundnut seeds infected with *Neurospora sitophila and Rhizopus oligosporu* which he said resulted from slight protein synthesis by the proliferation of the fungal hyphae and the synthesis of enzyme protein or other constituents. Ataga and Akueshi, (1986) also recorded similar report for sunflower seeds.

There was a significant decrease of lipid from the uninoculated control (10.38%) when compared to the fungi inoculated seeds. *Fusarium oxysporum* (8.00%) caused the most decrease when compared to the other fungi. This agrees with the findings of Ogundero, (1992) who explained that the decrease in oil content could be due to the hydrolysis of oil to free fatty acid (FFA). This occurred at different rates for the individual fungi.

There was a drastic and significant decrease in fibre of all the seeds inoculated with the individual fungi. This experiment agrees with the report of (Onifade et al, 2004), about a decrease in crude fibre content of sweet potato flour enriched with *A. niger*. He also explained that the crude fibre tends to decrease during fermentation. This he concluded was a result of being utilized by the fermentation microbes.

There was significant increase in ash of the individual fungi when compared to the uninoculated control. Ataga and Umechuruba (1997) resolved that the increase could be attributed to the presence of minerals like potassium and phosphorous in the mycelia of the fungi.

In conclusion, the result of this study indicates that all the five seedborne fungi caused deterioration in the alteration of the nutritional value of Yakwa seeds.

REFERENCES

Adegunloye B.J., Mojiminiyi F.B.O., Egbeniyi, Y. A., Okolo, R.U. (2000). An investigation of the diuretic effect of an aqueous extract of the petals of *Hibiscus sabdariffa. J Medicine Medical Sci.*; 2:77–80.

AOAC (1995). Official Methods of Analysis (16th edition) Association of Official Analytical Chemists. Virginia, USA. 1018p.

Ataga, A. E. and Akueshi, C.O. (1986). Fungi associated with sunflower seeds in Nigeria. *Seed Research* 1(24): 64 -65.

Ataga, A. E. and Umechuruba, C.I. (1997). Biochemical changes in Africam Yam bean seeds caused by *Botryodiplodia theobromae, Fusarium palidoreseum* and *Penicillium cxaliam. Global Journal of Pure and Applied Science* 4(4): 381-384.

Babalola, S. O. (2000). Chemical analysis of Roselle leaf (*Hibiscus sabdariffa*) in proceeding of 24th annual conference of NIFST, pp. 228-229.

Cherry, J. O. P. and Beuchat, L.R. (1975). Comparative studies of protein and amino acid changes in peanuts, infected with *Neurospora sitophila and Rhixzopus oligosporus. Cereal Chemistry* 53:750-761.

Fagbenro, O. A. (2005) Soybean meal substitution with roselle (*Hibiscus sabdariffa* L.) seed meal in dry practical diets for fingerlings of the African Catfish, *Clarias gariepinus*.

Journal of Animal and Vetwrinary Advances 4, 473-477.

ISTA (1976). International Seed Testing Association. International Rules for Seed Testing. *Seed Science and Technology* 4: 51-77.

Jain, R. K. and Bal, S. (1997). Properties of pearl millet. *Journal of Agriculture Engineering Researc*h 66, 85 - 91

Klement, Z. K. and Voros, I. C. (1974) Methods in Pathology. Elsevier Scientific Publishing Co., Amsterdam, London. Pp. 220-288.

Mohamed R, Fernadez J, Pineda M, Aguilar M. (2007). roselle (*Hibiscus sabdariffa*) seed oil is a rich source of γ-tocopherol. *Journal of Food Science. 72*: 207-211.

Monday, N.A.G. and Ataga A.E. (2005). The Role of *Aspergillus niger, Aspergillus flavus, and Fusarium oxysporum* on the nutritional composition and contents of *Cyperus esculentus L. (B.Sc Thesis)* University of Port Harcourt

Nwaukwu, I. A. and Ataga, A. E. (2012). Fungi Associated with *Hibiscus sabdariffa* Linn (Yakwa) seed from Plateau State. *Scientia Africana,* Vol.11 (No. 1), June 2012. Pp 125-129.

Nwaukwu, I. A. and Ikechi – Nwogu, C. (2012) Biochemical Changes Induced By the Effect of Six Pathogenic Fungi on *Dialium guineense:* Black Velvet Edible Fruit. *IOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS) ISSN: 2278-3008 Volume 2, Issue 4 (July-August), PP 20-24. www.iosrjournals.org*

Ogundero, V. W. (1992). Hydrolysis of vegetable oils and triglycerides by thermotolerant and zoopathogenic species of *Aspergillus* from Nigeria Palm Produce. *Mycopathogia*, 77: 43-46.

Okasha, M.A.M., Abubakar, M.S. and Bako, I.G. (2008). Study of the effect aqueous *Hibiscus sabdariffa l.* seed extract on serum prolactin level in lactating albino rats. *Eur. J. Sci. Res*., 22(4): 575-583.

Okonkwo, S.N., Amund, O.O and Ogunsanya, C.O. (1990). Microbial rotting and preservation of banana fruit (*Musa sapientium L*) in Nigeria. *Microbes.* Letters 44:175-176

Onifade, A. K., Atum, H. N. And Adebolu, T. T. (2004). Nutrient enrichment of Sweet potato (*Ipomoea batatas L.*). *Global Journal of Pure and Applied Sciences* 10(1):31-36

Parkouda, C., Diawara, B., Labia, I. and Ouoba, I. (2008) Technology and physico-chemical characteristics of Bikalga, alkaline fermented seeds of *Hibiscus sabdariffa. African Journal of Biotechnology* Vol. 7 (7), pp. 916-922.

Yagoub, A., Mohammed, A. and Abu, E. G. (2007). Fururndu, from Fermented/Sprouted Roselle (*Hibiscus sabdariffa* L.) Seed: Investigation on Chemical Composition, Antinutritional Factors, HCl-extractability of Minerals, Amino Acids Composition, *In vitro* Protein Digestibility and Microbial Growth. *Research Journal of Agriculture and Biological Sciences*, 3(6): 876-885.