EFFECTS OF FREEZE-DRIED AND OVEN-DRIED SOURSOP (*Annona muricata*) PULP POWDERS ON BLOOD GLUCOSE LEVELS OF NORMO-GLYCAEMIC ADULTS

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ABSTRACT
Background: Soursop is a tropical underutilized fruit of great nutritional importance.
Objective: This study determined the effects of freeze-dried and oven-dried soursop (Annona muricata) pulp powders on blood glucose levels of normo-glycaemic adults.

Materials and Methods: Mature soursop fruits were purchased at a local market in Nsukka, Enugu State, Nigeria and were allowed to ripen for 5 days at room temperature (37°C). The ripe fruits were washed, peeled, seeds handpicked and the pulp dried. Freeze-drying was done at -40°C for 72 hours and oven-drying at 70^0C for 48 hours. The amount of the freeze-dried and oven-dried soursop powders providing 50g available carbohydrate were liquefied with water to formulate unsweetened drinks. Effect of the unsweetened freeze-dried and oven-dried drinks on blood glucose levels of normo-glycaemic adults was determined by finger prick blood sample every 30 minutes at 30, 60, 90 and 120 minutes postprandial using sterile lancets and glucometer (Accu-Chek). Data obtained from the study were analyzed using Statistical Product for Service Solution (SPSS) for Windows version 21. Results were presented as means and standard deviations. Analysis of Variance (ANOVA) was used to separate means at $p < 0.05$.

Results: Mean values of blood glucose levels of subjects at 30, 60, 90 and 120 minutes from baseline showed that the drinks did not spike blood glucose but maintained it within the normal range.
Conclusion: Freeze-dried and oven-dried soursop pulp has potential in the prevention of diabetes due to their hypoglycaemic effect.

KEYWORDS: Soursop, oven-dried, freeze-dried, proximate composition, blood glucose.

Introduction
There has been an alarming increase in diet and lifestyle-related non-communicable diseases (NCDs) including cardiovascular diseases, obesity and cancer. The chronic disease of diabetes mellitus afflicts a large proportion of people all around the world. There is thus a need for an effective natural adjuvant therapy that will be beneficial to diminish diabetic complications and augment the quality of life for diabetic patients (1). This then implies a reassessment of our dietary choices. Diverse indigenous fruits could contribute to providing culturally-acceptable, sustainable and diversified nutritious diets (2). The health benefits of fruits' consumption have been associated with a significant impact on the prevention of diabetes, different kinds of cancer and cardiovascular diseases (3). The challenge is that availability of these fruits needed to constitute a diversified diet is limited. This is particularly the problem in the developing world where diets often consist of starchy staples with limited fruits (4). The problem of underutilizing fruits can increase the social problem of health and nutrition insecurity, poverty and unemployment (5).
In spite of extensive research on the composition of most fruits, little is known about the many tropical underutilized fruits in developing nations. One of such underutilized fruits is the soursop fruit, scientifically known as *Annona muricata*. It is known as 'graviola' in Brazil and as 'guanábana' in Mexico (6). It is known as 'Ebo' or 'Apekan' in Yoruba, 'Fasadarur' or 'Tuwon biri' in Hausa and 'Sawansop' in Igbo (7). The soursop tree is prevalent in the rainforests of Africa, South America and Southeast Asia. The fruits are consumed as food and the leaves and stems are used in traditional medicine to treat symptoms associated with inflammation and infection (8). Soursop is an evergreen tree native to Mexico, Cuba, the Caribbean, northern South America and especially Eastern Nigeria in Africa. The fruit is very delicate, dark green and firm when unripe, and covered with soft spines. It is relatively large, and has a thin, leathery skin, and can weigh about 2.5kg to more than 4kg (9). The pulp is white, creamy, meaty, juicy and slightly acidic having many seeds. The fruit skin becomes smooth, its green colour slightly pale, and yields to touch as the fruit matures (10). All parts of the soursop plant are used in natural medicine (9).
Fresh soursop pulp has been reported to have high fibre content which could be harnessed in the control of blood glucose levels in normal and diabetic individuals. It will also be useful in protecting man against excessive weight gain and its associated diseases (6). Due to the traditional application of soursop (*Annona muricata)* against diabetes, several

studies are investigating this potential *in vivo* (1). Availability of soursop fruits, however, is known to be short-lived due to seasonality and their perishable nature (11). Soursop is highly susceptible to quality deterioration after harvest if not well preserved (6).

In order to meet the demand for soursop throughout the year in all areas, the fruits could be preserved using different techniques (10). Soursop pulp could be preserved dry by spray-drying, freeze-drying, vacuum-drying (12), or by oven-drying the pulp (13). Drying is an excellent way to preserve fruits which can add variety to meals and also provide delicious, nutritious snacks (14). Oven-drying with an ordinary kitchen oven is the simplest way to dry foods in most households. It is faster and safer than sun-drying and can be used on a small scale. This process does reduce the heat labile nutrients such as vitamin C (14). However, the significant reduction in moisture content results generally in higher nutrient content (13). Conversely, freeze-drying involves placing the food to be dried on large racks inside a vacuum chamber. The temperature is lowered below freezing and then slowly raised so that the water in the food moves from a solid state to a gaseous state, maintaining the structure of the food. Freeze-drying produces higher-quality products, with minimal changes in colour and nutrients (13). Apart from fewer nutrient losses, freeze-drying also results in a product that can be more successfully rehydrated in the future (14). The soursop fruit pulp concentrated into powder will increase its commercial value and demand in Nigeria, make it available during its off-season, encourage its inclusion in our diets, and its use in varied food products (nutritious snacks, complementary food formulations and confectioneries). It will also encourage dietary diversity and improve nutritional health both now and in future.

The aim of this work is to determine the effects of freeze-dried and oven-dried soursop (*Annona muricata*) pulp powder on blood glucose levels of normo-glycaemic adults.

Materials and Methods

Procurement of Materials

Fresh, mature soursop fruits (fruit skin smooth, its green colour slightly pale) were selected and purchased from a local market in Nsukka, Enugu State, Nigeria.

Preparation of samples

The soursop fruits were allowed to ripen (until they were yellowish green and yielding to touch) at room temperature (37°C) for about five days. Below is the flowchart showing the steps involved in the extraction of the soursop pulp for freeze-drying and oven-drying.

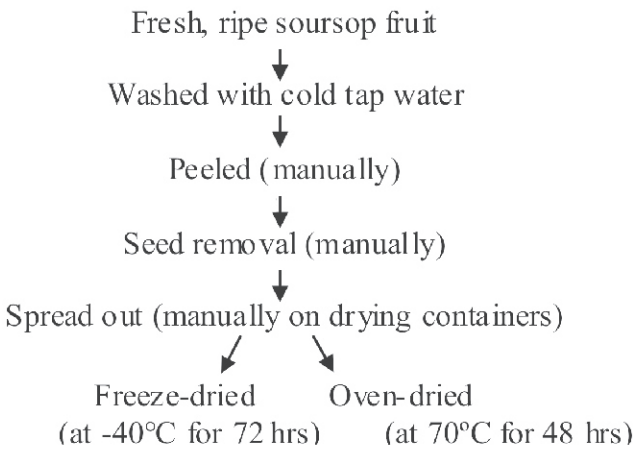

Figure 1: Flow chart for the preparation of soursop pulp for freeze-drying and oven-drying

Freeze-drying of the pulp was done at -40°C for 72 hours (13) using a freeze-dryer, Virtis (Genesis25, USA), with an average freezing speed of 2.04°C min-1, a chamber pressure of 1.3 ± 0.3 mmHg, a plate temperature between 10°C and 80°C, and a maximum product temperature of 60 ± 5 °C (12). The freeze-dried samples were homogenized by blending into powder using a kitchen blender (Sonik® Japan, SB-1212 model). The powder was very similar in colour to that of the fresh soursop pulp.

Oven-drying pulp was done at 70 □C for 48 hours in a single layer using a hot air oven. The dried fruit was then ground into flour using a kitchen blender (Sonik® Japan, SB-1212 model) (13). The powdered oven-dried flour was yellowish brown in colour. The freeze-dried and oven-dried samples were stored in air-tight containers prior to use for analysis.

Preparation of unsweetened soursop drinks

Soursop drinks were prepared from the freeze-dried and oven-dried powders using the following recipes adapted from Onyechi et al. (15).

Sample recipe for Freeze-dried soursop pulp drink (unsweetened) (FrSDp)
Table 1: Preparation of unsweetened freeze-dried soursop pulp drink

Ingredients	Quantity
Water	500mls
Freeze-dried soursop pulp	100g

Method of Preparation
a. One hundred grammes (100g) of the freeze-dried pulp were weighed out into an electric blender (Sonik® Japan, SB-1212 model).
b. Five hundred millimeters (500mls) of water was added into the blender, and then blended for 5 minutes to homogenize the drink.
c. The homogenized drink was poured into different clean bottles, cooled and it was ready for drinking.

Sample recipe for Oven-dried soursop pulp drink (unsweetened) (OSDp)
Table 2: Preparation of unsweetened oven-dried soursop pulp drink

Ingredients	Quantity
Water	500mls
Oven-dried soursop pulp	100g

Method of Preparation
a. One hundred grammes (100g) of the oven-dried pulp were weighed out into an electric blender (Sonik® Japan, SB-1212 model).
b. Five hundred millimeters (500mls) of water was added into the blender, and then blended for 5 minutes to homogenize the drink.
c. The homogenized drink was poured into different clean bottles, cooled and it was ready for drinking.

Selection of subjects
A total of eight healthy non-diabetic subjects were screened and selected from the students of the Department of Human Nutrition and Dietetics, University of Nigeria, Nsukka. Inclusion and exclusion criteria were used to select subjects. Subjects were not obese and not diabetic (fasting blood glucose level of <100mg/dl), there were no pregnant or lactating subjects, none on any medication, suspected alcohol or drug use, or had severe allergic conditions. Written protocol of the study was explained to the subjects, thereafter they signed their consent. The study was approved by the University of Nigeria Teaching Hospital Ethical Committee, Ituku/Ozalla, Enugu State.

Study procedure
The test was conducted in the diet therapy laboratory of the Department of Human Nutrition and Dietetics, University of Nigeria, Nsukka. The selected subjects who had completed a three-day pre-screening and fasting blood glucose test (FBG<100mg/dl) on non-consecutive days (16) were divided into two groups for the freeze-dried (group 1) and oven-dried (group 2) test samples. The subjects came in after fasting of 12 hours. Fasting blood sample was obtained from finger prick using a sterile lancet and read using a glucometre. The freeze-dried and oven-dried samples were reconstituted to provide 50g available carbohydrate (17) using the recipe described above for unsweetened freeze-dried and oven-dried soursop drinks. Subjects took either the freeze-dried soursop drink or the oven-dried drink within 15 minutes on the same day but in their different groups. The blood glucose levels of the subjects were determined by finger prick every 30 minutes at 30, 60, 90 and 120 minutes postprandial using a sterile lancet and a glucometer (Accu-Chek).

Statistical analysis
Data obtained from this study were analysed using Statistical Product for Service Solution (SPSS) for windows version 21 for mean and standard deviations. Analysis of Variance (ANOVA) was used to separate at $p < 0.05$.

RESULTS
Table 4a shows the baseline characteristics of 8 healthy subjects who took the test drinks containing 50g available carbohydrate. Mean age of subjects ranged from 21.00±1.41 to 22.50±2.88 years, mean body mass index (BMI) ranged from 23.00±2.41 to 23.24±0.18 kg/m^2.

Table 4a: Baseline characteristics of subjects

Group	Sample	Amount of test food containing 50g available CHO (g)	Total weight of test drinks (g)	Age (years)	Body Mass Index(kg/m^2)
1	Freeze-dried	104.76	525	21.00±1.41	23.24±0.18
2	Oven-dried	91.66	500	22.50±2.88	23.00±2.41

Table 4b shows the mean values of postprandial blood glucose levels of selected subjects from baseline values. In subjects fed the FrDSp, the blood glucose levels ranged from the baseline of 81.00 ± 1.41 mg/dl to 86.00 ± 2.83 mg/dL after 120 minutes of intake. Also, in subjects fed ODSp, the blood glucose concentration was found to vary from baseline of 73.17 ± 13.66 mg/dl to 85.67 ± 3.93 mg/dL, 120 minutes after intake. There was a gradual rise in the means of the blood glucose levels of the two groups for the intervals of 30, 60, 90 and 120 minutes as shown in the table below.

Table 4b: Effect of freeze-dried and oven-dried soursop drinks on blood glucose levels of healthy subjects (mg/dl).

Test samples	At 0mins	After 30mins	After 60mins	After 90mins	After 120 mins
FrDSp	81.00±1.41	↑83.50±4.95	↑85.00±0.00	↑90.50±3.54	↑86.00±2.83
ODSp	73.17±13.66	↑80.00±5.40	↑83.67±3.88	↑89.17±6.88	↑85.67±3.93

Key: FrDSp=Freeze-dried soursop drink (unsweetened)
ODSp=Oven-dried soursop (unsweetened)

Figure 2 is the graph showing variations in blood glucose levels of the subjects at the various time intervals.

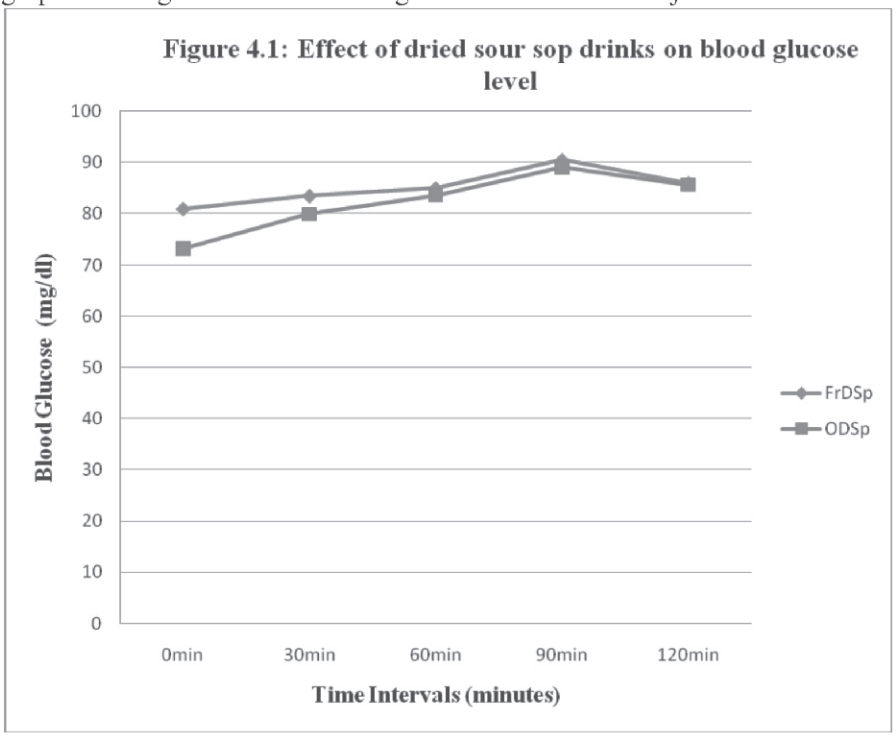

Figure 2: Effect of the dried soursop drinks on blood glucose levels.
Key: FrDSp = Freeze-dried soursop
ODSp = Oven-dried soursop

The graph showed that for both samples blood glucose levels rose slightly initially and peaked after 90 minutes before they began to drop but not quickly to the baseline after 120 minutes.

DISCUSSION

The results from the table showed that at =0.05 level of significance, the freeze-dried and oven-dried sour-sop pulp drinks had no statistically significant ($p < 0.05$) effect on blood glucose levels from baseline at the varying time intervals of 30, 60, 90 and 120 minutes of consumption of the test drinks. The p-values obtained (0.912, 0.115, 0.673, 0.836) for the various time intervals respectively, were all greater than 0.05. This means that the available carbohydrate of the samples did not spike blood glucose and so does not pose any threat to blood glucose levels of the subjects which remained within the normal range of <100mg/dl. The observed slight increase in the mean blood glucose levels of the subjects which remained fairly constant over the various time intervals showed sustained energy and satiety from the test drinks. This is what is desired from any food as observed by Bogs (18) in her study using apples. Also, the postprandial blood glucose (PPG) level is an important risk factor of diabetes. The human body requires blood sugar to be maintained in a very narrow range (19) which is what the test drinks have achieved. This shows that high consumption of soursop drinks can have a protective effect in the management of chronic diseases such as diabetes (20). Therefore, dried sour-sop drink could be recommended to dietitian nutritionists and diabetic patients for optimum blood glucose control. The establishment of a normal range of fasting blood glucose, FBG, for the subjects (<100mg/dl) as well as body mass index, BMI, (<25.0kg/m^2) provided some control.

CONCLUSION

Dried soursop pulp and drinks from it which were used in this study will help improve the dwindling consumption of the fruit all year round. It will also help enhance the nutritional status of consumers. The study also shows that high consumption of soursop drinks can have a protective effect in the management of chronic diseases such as diabetes. Therefore, dried sour-sop drink could be recommended to dietitian nutritionists and diabetic patients for optimum blood glucose control. There is need for further investigations on the nutritional, biochemical and physiological functions of the active compounds of dried soursop in vitro and in vivo. These will be completely pivotal for the development of nutritional, pharmaceutical and agricultural products.

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