To the Graduate Council:

I am submitting herewith a thesis written by Fanny B. Larez entitled "Nutritional Value of Sweet Potato Residue Following Fermentation for Ethanol." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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We have read this thesis
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NUTRITIONAL VALUE OF SWEET POTATO RESIDUE FOLLOWING FERMENTATION FOR ETHANOL

A Thesis

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Degree

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This thesis is dedicated to my Father, Luis B. Larez and my Mother, Luisa E. de Larez.

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ABSTRACT

Two sweet potato cultivars, Centennial, an orange-flesh type, and TG-75-96-1, an industrial white cultivar, were comminuted and allowed to ferment to produce ethanol. An enzyme alpha-amylase, was used to hydrolyze starch to increase the concentration of fermentable sugars. Saccharomyces cerevisiae was used to ferment the sugars to ethanol. Fermentation was completed in two weeks at 21.1-23.9°C. Ethanol production was 2.02% for the orange and 1.73% for the white sweet potatoes. Analysis of the fermented residue indicated that the percentage protein was increased for Centennial, from 4.7% to 7.0%, and for the white-fleshed cultivar, from 4.3% to 5.1%. The amount of fat, crude fiber, caloric content, calcium and iron increased also. The essential amino acid (EAA) analysis showed no significant change in amino acid content and that isoleucine was the limiting amino acid for both cultivars.

Centennial was found to be significantly more nutritious than the white cultivar in terms of the B-vitamins and vitamin C. The white cultivar, however, contained a significantly greater solids content indicating a potentially higher yield of ethanol.

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CHAPTER I

INTRODUCTION

Concern for a solution to the protein deficiency problem in the world has caused investigators to look at different methods by which higher protein foods may be produced economically. One possibility is the use of carbohydrate waste materials to produce a product of increased protein content through controlled fermentation. This process is of great interest not only to flour millers, but also to refiners, and processors of sugar, potatoes, yams, cassava, and other starchy crops (1).

This study considers sweet potato as a potential raw material. Sweet potato (Ipomoea batatas (L) Lam) is a crop which contains nutritionally significant amounts of high quality protein, but usually does not have enough protein to provide an adequate protein-caloric ratio (39). Sweet potato is also rich in vitamins and such minerals as calcium and phosphorus (19).

This study investigated the nutritional value of the sweet potato residue that is a by-product of an alcohol fermentation. Such a product could be used as animal feed, fertilizer or possibly as a nutritional supplement in foods (53).

CHAPTER II

REVIEW OF LITERATURE

I. SWEET POTATO

History and Source

Sweet potatoes (Ipomoea batatas (L) Lam.) belong to the Convolvulaceae or morning-glory family (9, 13, 32, 48). The genus Ipomoea contains 400 or more species widely distributed over the world, chiefly in tropical countries (33, 44).

Sweet potatoes are thought to be native to South and Central America (9, 46, 48) from where they were disseminated to the Polynesian triangle, New Zeland and Guam (32). Records show that sweet potatoes were widely cultivated in tropical America and the warm islands of the Pacific Ocean by the latter part of the 15th Century (13, 32).

Christopher Columbus carried the plant to Spain and the sweet potato soon became a favorite treat throughout Spain and Europe (9, 27). In North America, it was cultivated in Virginia as early as 1648 after English colonists brought it to the area (13, 27, 46). In the United States, sweet potatoes are now extensively grown in all southern states and portions of New Jersey, Delaware, Maryland, Illinois, Missouri and California (13).

There are basically two types of sweet potatoes, the moist-fleshed type and the dry-fleshed type (13, 27, 46). The moist-fleshed cultivar is called "yam" (27, 48). The term yam is applied to the Porto Rico cultivar and its strains and many shippers used a brand

name which includes the word "yam." This term has buying appeal to the housewife, the principal buyer of sweet potatoes in retail stores (13).

The dry-fleshed cultivar, which grows best in the North, is preferred by consumers in that region (13, 27). According to Kane (27) the dry-fleshed cultivars possess the characteristics of becoming sweeter during long periods of storage. Furthermore, these cultivars have a higher starch content compared to the orange-colored cultivars as well as a higher resistance to black rot and fusarium wilt (13).

Production and Consumption

Sweet potatoes have a great potential for being used to feed people. More people can be fed from an acre of sweet potatoes than from an acre of cereal grains (32, 34). Sweet potatoes are of primary importance in Asia and Africa. In the Americas, the main producers are Brazil, Cuba, United States, Jamaica and Argentina (32).

Sweet potatoes produce high yields, about 14.435 metric ton per hectare. In 1980, total world wide production ranged between 133.9 and 135.9 million metric tons of which 538.12 metric tons are produced in the U.S. with minimum applications of fertilizer and pesticides (2, 34). Sweet potatoes are not affected much by drought since they require a very low amount of moisture during production (27). On the other hand, sweet potato production has decreased due to reduced popularity over the years. One of the reasons responsible for this decrease is the changes in food consumption. People in the U.S., Western Europe, U.S.S.R. and Japan have increased consumption

of animal protein such as beef, pork and poultry. This is due to the increase in affluence and improved standards of living of these countries (13, 34). Other reasons responsible for the decline in consumption are the price, and storage difficulties, and that sweet potatoes have been associated as a low status or "country food" for many people (7). Despite this decrease in popularity, sweet potatoes ranked ninth in acreage and eleventh in value of the 22 principal vegetable crops grown in the U.S. in 1971. Production decreased from 1260.04 metric tons to 629.67 metric tons during the period of 1949 to 1977, but farm value increased from $96,377,000. to $11,142,000. in 1977 (48).

The increasing interest in alcohol production for fuel purposes and possible utilization of the residue as an animal or human food product could bring about an increase in the production and utilization of the sweet potato.

Utilization

The sweet potato is a multipurpose root. It is a rich food containing carbohydrate, vitamins and minerals. Therefore, it is considered good for feeding dairy cattle, hogs and for pigmentation of poultry feed products.

The sweet potato can be classifed according to its use into two types: (1) for human consumption; and (2) forage for animal feed (8, 32, 46). Sweet potato vines can also be used for animal feed. Its yield is greater than the yield of green corn silage. Also, it has been proven that sweet potato leaves, when dried, provide

a feeding value comparable to that of alfalfa hay when utilized as forage (8, 32). However, these vines are usually not used due to the preference in the growing areas to harvest and to store the roots (13, 34, 46).

The sweet potato is used for the production of industrial products which include starch, syrup, alcohol and related compounds, flour, carotene, protein-enriched pulp and feed, yeast and silage. In addition, sweet potato is utilized fresh, frozen, canned and as dehydrated food products (13, 32).

Composition

Sweet potato is considered to be a very nutritious vegetable (13, 27, 29, 34). Carbohydrates are a major component of sweet potato roots and usually average approximately 28% of the root. Since the carbohydrate material consists primarily of glucose, sucrose, dextrin and starch (approximately 98%) and is easily digested, sweet potato roots are a good source of energy. Furthermore, sweet potatoes have the highest content of non-sugar carbohydrate (starch) of any vegetable (10, 13). Sweet potato, because of its lower water content (70.6%), compared to most vegetables, is a highly concentrated source of food, and provides other types of minor chemical constituents (10).

B-carotene is important in this crop not only as a nutritional component, as a precursor of vitamin A, but also to contribute to a pleasant orange color. Carotene content is 5,280 mg/100g for raw sweet potatoes and 4,680 for canned sweet potatoes (49). In most cultivars, beta-carotene comprises 80-90% of the total carotenoid

pigment. On the contrary, some cultivars may have as little as 22% beta-carotene (17).

This vegetable is also an excellent source of ascorbic acid (21 mg/100g) (49). It is also fairly rich in B-vitamins, especially thiamin (0.10 mg/100g), niacin (0.6 mg/100g) and riboflavin (0.06 mg/100g) (49). In addition, it has appreciable quantities of phosphorus (47.0 mg/100g), calcium (32.0 mg/100g), and iron (0.7 mg/100g) (43, 49).

Although sweet potato is considered a low protein food because it provides a high caloric-low protein ratio (38) its protein is of good quality and its nutritive value is complemented by vitamins and minerals (19, 29, 37). The major portion of the sweet potato protein is composed of a globulin, ipomoein (10, 25). It is isolated from newly harvested sweet potatoes (25). There is another protein which is an intermediate product from the degradation of ipomoein as the root ages. This other protein forms by action of proteolytic enzymes on ipomoein (10, 25).

Protein content ranges from 2-11% (7, 10) on a dry weight basis, but most cultivars contain between 4.5-7% (37). Sweet potato contains most of the essential amino acids (34). However, it is low in sulfur amino acids and tryptophan (38, 43) when compared to the FAO reference, (51) but other amino acids are in excess (43).

Importance of Fermentation

Interest in alcohol production by fermentation from agricultural crops or waste is now under consideration as an alternative due to

the continued increase in prices of fossil fuel petroleum (47). To transform biomass into fermentable sugars which can be utilized by yeast to yield ethanol, a pretreatment to remove lignin is necessary. Cellulose, a crystalline structure, is protected by lignin which is a polysaccharide that prevents hydrolysis of cellulose from occurring. The hydrolysis of cellulose makes it available for fermentation (22, 52). Use of enzyme is also necessary to break down starch prior to fermentation.

New biological systems have been developed to overcome situations such as toxicity of metabolites which limits fermentation. Production of liquid fuels from cellulose carbohydrates has occurred and will continue. The discovery of new microorganisms with genetic versatility and altered membrane compositions capable of producing useful fuels from a variety of renewable carbohydrates are one of the challenges which scientists will have to find solutions for in the future.

At the same time, due to the great concern for the protein deficiency problem in the world, scientists are looking for solutions (19, 20, 40). One solution may be the new developments in the utilization of carbohydrate waste materials as substrates for high protein microorganisms (1).

Products from fermentation could be one of the answers for the protein deficiency problem. The primary goal is the synthesis of protein from carbohydrate in a short period of time (19, 33). Studies indicate that carbohydrate containing materials are excellent raw materials for this process. Furthermore, carbohydrate materials are available throughout the year and in surplus (1, 5, 19, 20).

Fermentation technology advances in ethanol production from cellulose and starch have been slow for the past 40 years (15). Nevertheless, significant advances have been made which provide ways for cellulose and starch conversion to ethanol (15, 21, 22, 47, 50, 52).

Process

Fermentation of foods is a process that has been developed for centuries by accidental observation of a natural phenomenon. In addition, for thousands of years, man has used fermentation as a method of preserving foods (35). It occurs as a process with different and complex chemical transformations of the organic substrate by the catalytic action of enzymes which can be either native to that substrate or can be produced by microorganisms. Man has chosen and developed methods conducive to more desirable changes (35).

Enzyme Function

Enzymes are distinguished one from another by the specific property of the chemical reaction catalysed. All of the known enzymes can be classified in one of the following classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases or synthetases. Oxidizing enzymes, which catalyse hydrogen transfer, are treated as a separate group (12). Glycosidases are a subgroup included in the hydrolases class. This subgroup includes not only enzymes acting on simple glycosides, but also enzymes like amylases which hydrolyse glycosidic links in polysaccharides (12).

Alpha-amylase is termed an endoamylase and beta-amylase an exoamylase because alpha-amylase splits glycosidic links in the middle of polysaccharide chains whereas beta-amylase splits glycosidic links from the nonreducing end of the chains splitting off maltose residues one at a time. The mechanism of this class of enzymes is not very well known. It is known that alpha-amylases require Cu^{++} for activity. However, it is not known if it is involved in the catalytic reaction or in maintaining the conformation of the enzyme. The reaction catalysed by the alpha-amylases produces no inversion of configuration (12).

Sources in which alpha-amylases are found in high concentrations include: Bacillus stearothermophilus, Bacillus subtilis, Aspergillus oryzae, porcine pancreas and malt (12).

Important consideration should be given to the specificity of enzymes such as amylases which hydrolyse starches. The difference among them is in the particular link attacked at a specific stage in the reaction. Alpha-amylase produces a mixture of dextrins due to random attack on the chains (12).

Yeast Function

Further breakdown of sugars is carried on by yeast with the species, Saccharomyces cerevisiae, being the major one utilized. Its role is very important in bakeries, wineries and breweries. Few other species play roles in alcohol fermentation. The role of yeasts in the food industry is highly prized because their enzymes catalyze several chemical reactions producing alcohol and carbon

dioxide. These products are important in synthesizing vitamins like the B-group (11). Yeasts get their energy by breaking down sugars to simpler compounds which in the presence of oxygen are carbon dioxide and water and in the absence of oxygen are alcohol and carbon dioxide. Yeasts can also be used for synthesizing proteins from industrial waste (11).

Concern for the Production of Alcohol

Significant interest is being shown today in the production of ethanol from grain or other carbohydrate sources for use as a gasoline additive (45) which can extend the quantity of fossil fuel used. American agriculture is dependent on petroleum fuel sources (28). Therefore, researchers are determined to produce fuel (ethanol) from crop residues in order to reduce the requirement for the petroleum fuel. However, to increase production of ethanol, crop yields will also need to be increased. Furthermore, it is very important to consider the production of higher quality crops (28). There are processes now available that are technically and economically feasible (41) if the carbohydrate substrate is available.

Brazil is one of the countries in which the concern over energy has led the government to promote and to stimulate increased production of ethanol from agricultural residues (30, 44). The effort is now to explore various raw materials which can be economically justifiable. Alcohol distilleries produce alcohol from sugar cane, via molasses, and also from crops rich in starch such as cassava

or mandioc, babassu nuts and sweet potatoes, and from sorghum grass, which is rich in both sugar and starch (30).

Food Development

Food value of starchy crops can be increased by increasing protein content (5). Cassava contains about 70% starch and 3% protein which is less protein than corn (5, 20). Increased protein content can be achieved through fermentation processes which use yeast. The end protein increase is the result of the presence of the synthesized yeast biomass (5). Results indicate that not only the amount of protein is large but the quality in terms of amino acid also is well balanced. Corn increased its protein content 18-20% parallel to that of lysine and cystine (5).

Furthermore, positive results have been found utilizing grain fermentation by-products, from corn and wheat. The development of DPC, distillers protein concentrate, to produce food blends and testing these blends in model food systems has been satisfactory, showing that DPC can be a human consumable protein (40).

Fermentation also appeared to improve the nutritional quality of the proteins in the black gram and rice blend, referred to as idli. Amino acid contents of the batter were increased with the increase in fermentation time with the exception of isoleucine and cysteine (33).

In a recent study on fermentation of pumpkin flesh to produce ethanol (24) it was found that there was a relative increase in protein content of 7.56% after fermentation. The dry matter left from the fermentation can be used as an animal feed supplement.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE AND TYPES OF SWEET POTATO

Two cultivars of sweet potato, Centennial, an orange-colored flesh, and a red skinned white-fleshed cultivar (industrial type), TG-75-96-1, were grown in 1981 on the Plant and Soil Science farm near Knoxville, Tennessee. They were grown under irrigation as needed, and 505.43 kg of 6:12:12/hectare were applied. The sweet potatoes were harvested after 120 days in the field.

Preparation of the Sweet Potato

Freshly harvested roots were washed under running tap water and brushed until all soil was removed. The unpeeled sweet potatoes were chopped into small pieces less than 0.2 cm using a Hobart Food silent knife Model 84142. The chopped sweet potato was blended at a ratio of 1:2 w/w with distilled water for one minute in a Waring Blender to give a pourable mixture.

Preparation of the Sample

Samples of unfermented and fermented sweet potatoes were prepared as follows for each cultivar. A total of 14,000 g of the sweet potato/water slurry was prepared. Samples of 2,000g were removed and freeze dried in a Virtis freeze dryer for further analysis. Four fermentation vats were filled with 3,000g of the slurry for each of the two sweet potato cultivars.

The fermentation vats were heated to approximately 80°C and held for 25 minutes. They were cooled to 75°C and alpha-amylase, 1g per vat, was added. Bacillus subtilus was the source of alpha-amylase which was supplied by Sigma Chemical Company. The slurries were held at 75°C for 45 minutes, and then cooled to 25°C. A 1% inoculum of yeast, Saccharomyces cerevisiae, which had been inoculated into yeast dextrose broth 24 hours before, was added to each of the sweet potato mashes. This procedure was developed from Satterle (46) which reported on a method for use in grains. The procedure was modified by adding the enzyme to the sweet potato slurries without adjusting the pH to 6.8-7.0. The slurries were initially heated only to 80°C rather than 90°C utilized in Satterle's (43) procedures. Fermentation was allowed to proceed at room temperature, 21.1-23.9°C until it ceased (two weeks). Each day, each of the fermentation vats were shaken to mix the solid and liquid portions.

Each batch of the mash was centrifuged at 3,000 rpm for five minutes and the supernatant decanted. The supernantant was later analysed for alcohol content by the GLC.

The remaining four solid portions from each of the two cultivars were combined into two combinations. they were thoroughly mixed, frozen and then freeze-dried. The freeze-dried portions were stored in the freezer for further analysis.

II. CHEMICAL ANALYSIS OF THE SWEET POTATO

Freeze-dried samples of fermented and unfermented sweet potato were analyzed for the components described below.

Moisture

Moisture content of the sweet potato samples were determined according to the AOAC method 7.003 (3). Five grams of sample were placed in the vacuum oven at 70°C for 19 hours at 25.0 psi to obtain constant weight. Results were expressed as percentage of moisture. Three replications of triplicate samples of each of the two fermented and unfermented sweet potato samples were prepared.

Crude Protein

Crude protein was determined by the Kjeldahl procedure according to the AOAC method 7.015 (3). Results were expressed as percentage of crude protein (%N x 6.25) on a dry weight basis. Six replications of triplicate samples of each of the two fermented and unfermented sweet potato samples were performed.

Crude Fiber

Crude fiber was determined by a modified AOAC method 7.061 (3). The modification was the use of glass wool to replace asbestos as a filter mat. Two replications were prepared on each of the two fermented and unfermented sweet potato samples. Results were reported as percentage of crude fiber on a dry weight basis.

Ash

Ash was determined according to the AOAC method 7.009 (3). Two grams of sweet potato sample were ashed in a muffle furnace at 525°C for eight hours. Results were reported as a percentage of ash to first decimal place on a dry weight basis. Six replications

of triplicate samples for each of the two fermented and unfermented sweet potato samples were prepared.

Gross Energy Value

Gross energy value was determined with an adiabatic calorimeter, Parr Cat. No. 1241. A 0.8g sample of freeze-dried sweet potato was placed in a bomb and 35 psi of oxygen was added to it. The change in temperature was measured and used to determine the amount of kilocalories which were calculated on a dry weight basis.

Crude Lipid

Crude lipid was determined by the lipid extraction procedure using a Soxhlet-type extractor according to the AOAC method 7.056 (3). Two grams of freeze-dried samples were used. Results were expressed as percentage of fat on a dry weight basis. Six replications of triplicate samples for each of the two fermented and unfermented sweet potato samples were measured.

Nitrogen-Free Extract (NFE)

The percentage of NFE was determined by subtracting the percentage of protein, lipid, ash and fiber from 100% and was reported on a dry weight basis.

Calcium and Iron

Calcium and iron were determined using atomic absorption spectrophotometry according to AOAC method 2.122 (3). Results were expressed as percentages of calcium and iron on a dry weight basis.

One analysis was performed on each of the two fermented and unfermented sweet potatoes. One gram of each sample was used.

Phosphorus

Phosphorus was determined according to the AOAC method 22.045 (3). Results were expressed as mg P_2O_5/100g on a dry weight basis. Two replications for each sample were analysed using two gram samples.

Vitamins

Niacin, pyridoxal, pyridoxine and thiamin (vitamin B_1) were extracted following the method by Augustine et al., (4). The vitamins were determined using high performance liquid chromatography (HPLC).

A Lambda-Max Model 480 LC Spectrophotometer 6,000 pump and a 254nm detector was used (Waters Associates). A Partisil-10 ODS-2 column was used (Waters Associates). The mobil phase consisted of 75/25 v/v phosphate buffer pH 6.86/methanol. Standards for each of the vitamins were prepared to a final concentration of 10 µg/ml in 6% HPO_3.

One gram of each of the samples was ground to pass a 30 mesh, and was allowed to macerate in 50 ml 6% HPO_3 for 15 hours at room temperature. Then, samples were heated in a water bath at 90°C for 30 minutes. The slurries were filtered through Whatman No. 1 filter paper. The filtrates were filtered through a Millipore filter (average size 0.45 millimicrons) prior to injection into the HPLC. A flow rate of 1.5 ml/min and an attenuation of 0.5 were used for the standards and samples.

Vitamin C

Vitamin C was determined following the AOAC method 43.060 (3). Results were expressed as mg Vit C/100g sample. Because of the development of a pink color during preparation of the samples for the white sweet potato cultivar, samples were filtered through carbon decolorizing Nuchar C-190N to eliminate color prior to the determination of the vitamin. A standard solution of ascorbic acid was also filtered through the carbon decolorizing material for comparison.

Color

Color was measured with the Hunter Color/Difference Meter. Hunter L, "a" and "b" values were recorded. The instrument was standardized against a standard plate No. C2-138 L = 79.40, a = -2.40 and b = 23.60. Measurements were taken from a measuring cup filled with raw unfermented and centrifuged fermented sweet potatoes.

Alcohol

Ethanol was determined by gas chromatography following the procedure of Bouthilet et al. (6). Acetone was used as an internal standard. A Shimadzu Mini-2 gas chromatograph equipped with a flame ionization detector, Dorhaur recorder, and a Shimsdzu Chromatopac E/A electronic calculating integrator. Conditions for the gas chromatography were as follows:

Column temperature - 150°C

Injection/Detector temperature - 165°C

Attenuation 64

Carrier gas (nitrogen) flow rate - 60 ml/minute

Hydrogen flow rate - 34 ml/minute

Air flow rate - 345 ml/minute

Ethanol concentrations were reported on a weight/volume basis.

Amino Acid Analysis

Amino acids were determined following the method of Kaldy et al. (26). Freeze-dried samples of sweet potato were ground to pass a 60 mesh. Lipids were extracted from the samples by the crude lipid procedure using a Soxhlet-type extractor according to AOAC method (3).

Five mg of each freeze-dried, lipid free sample were weighed into 1 ml test tubes. One ml of mercaptoethanosulfonic acid (3N) and 0.032 ml of norleucine as an internal standard indicating transfer losses were added to each tube. Samples were degassed for 15 minutes and frozen at -60°C for 15 minutes.

The tubes were connected to a high vacuum line and heat-sealed while still connected to the vacuum. The acid hydrolysis was allowed to proceed in an oil bath at 110°C for 22 hours in one analysis and 72 hours in another. To remove any suspended particles, samples were filtered. Then, the pH was adjusted to 2.20 $\pm$ 0.01 using small amounts of NaOH (11N) and HCl (0.01N). A few drops of sodium citrate buffer were added to complete neutralization. One ml of the prepared sample was injected into the machine. The column operates on the basis of a cation exchange resin. The column eluant was mixed with

ninhydrin and passed through a reaction bath where the ninhydrin reaction occurs. The eluant then proceeded through a colorimeter. The colorimeter was connected to a recorder which recorded the color changes as peaks. By the location and size of the peak, the amino acid and its concentration were identified. The machine used was a Beckman 116 amino acid analyzer modified to a single column system.

III. STATISTICAL ANALYSIS

Proximate analysis, caloric content, nutrients and essential amino acids were analyzed by analysis of variance as a split-split-plot design. Duncan's Multiple Range Test was used to determine significance among the means. The 1979 Statistical Analysis Program was used to compute the data.

CHAPTER IV

RESULTS AND DISCUSSION

I. PROXIMATE ANALYSIS OF UNFERMENTED AND FERMENTED, ORANGE AND WHITE SWEET POTATOES

The mean squares from the analysis of variance for the proximate composition of unfermented and fermented orange (Centennial) and white (TG-75-96-1) sweet potatoes are presented in Table 1. The moisture and ash contents were different at the 0.01 level, while protein, fat, crude fiber and nitrogen free extract (NFE) were different at the 0.05 level. The average moisture contents were 69.15% before and 79.58% after fermentation for the Centennial cultivar, and 63.46% before and 64.80% after fermentation for the TG-75-96-1 cultivar. The difference in the moisture content between the orange and white cultivars resulted in a greater solid content for the white-flesh sweet potato compared to the orange-flesh sweet potato. This would result in an increase of approximately 735.68 kg per hectare in solids material if both cultivars yielded the same amount per hectare of raw sweet potato. The significant increase in percent moisture in the fermented orange-colored cultivar (Table 2) might be explained by changes in the physical properties which increased the water holding capacity. This would have allowed more water to be retained by the fermented Centennial sweet potato during the centrifugation process.

There was no significant difference ($P<0.05$) between the protein content of the unfermented Centennial sweet potatoes, 4.7%,

TABLE 1. Mean Squares from the Analysis of Variance for the Proximate Composition of Unfermented and Fermented, Orange and White Sweet Potatoes

Source	D.F.	Moisture	Protein	Fat	Ash	Crude Fiber	NFE[a]
Model	3	106.696**	2.937*	0.633*	1.127**	0.488*	4.027*
Error	4	3.110	0.283	0.041	0.015	0.048	0.447

*Significant at 0.05 level.

**Significant at 0.01 level.

[a]Nitrogen-Free extract = (100 - (% protein + % ash + % fat + % fiber)).

TABLE 2. Means for the Proximate Composition of Unfermented and Fermented, Orange and White Sweet Potatoes

Variable[a]	Orange colored		White colored	
	Unfermented	Fermented	Unfermented	Fermented
	---------- % ----------		---------- % ----------	
Moisture[b]	69.15±0.01^{x}	79.58±2.10^{w}	63.46±0.01^{y}	64.80±2.82^{y}
Protein[c]	4.75±0.07^{y}	7.05±1.06^{w}	4.30±0.01^{y}	5.10±0.01^{x}
Fat[c]	1.49±0.28^{y}	2.51±0.09^{w}	1.31±0.08^{z}	2.16±0.25^{x}
Ash[c]	3.00±0.14^{w}	1.50±0.14^{y}	2.30±0.01^{x}	1.40±0.14^{y}
Crude Fiber[b]	1.55±0.21^{z}	2.05±0.21^{x}	1.80±0.14^{y}	2.70±0.28^{w}
NFE[d]	89.22±0.28^{x}	86.90±1.22^{z}	90.29±0.22^{w}	88.64±0.39^{y}

[a]Moisture is for the raw sweet potatoes and centrifuged fermented sweet potatoes and all other values are for the freeze dried samples on a dry weight basis (dwb).

[b]Each value is the average of 6 samples.

[c]Each value is the average of 12 samples.

[d]Nitrogen-free extract (100 - (% protein + % ash + % fat + % fiber)).

$^{w-z}$Pairs of means within a row followed by different letters are different at the level of probability in Table 1.

and the TG-75-96-1 sweet potatoes, 4.3% (Table 2). There was, however, significant increases in protein content in the sweet potato cultivars following fermentation. The Centennial sweet potatoes increased from 4.7% to 7.0% protein (dwb) and the TG-75-96-1 increased from 4.3% to 5.1% protein (dwb). This increase can be explained by the presence of the synthesized yeast biomass. These results were expected since they agree with results reported by Johnson (24) and Azoulay et al. (5). This increase in protein content shows promise for improving the nutritional value of the sweet potatoes.

The orange and the white cultivars of sweet potatoes increased ($P<0.05$) in fat content during fermentation from 1.5% to 2.5% and from 1.3% to 2.2%, respectively (Table 2). This increase in both cultivars could be accounted for by the decrease in carbohydrate and ash contents following fermentation as shown in Table 2. The difference in fat content between the two types of cultivar was also significant.

There was a significant decrease ($P<0.01$) in the ash content of the sweet cultivars following the fermentation process (Table 2). The percentage of ash content was greater for the unfermented Centennial sweet potatoes, 3.0%, compared to 2.3% for the TG-75-96-1 sweet potatoes, however, they were not different following fermentation, 1.5% and 1.4%, respectively. In both cases, the decrease in ash content could be based mainly on the solubility of minerals in water. Since two parts water were added, for each part of sweet potato, this was an ideal medium for mineral loss.

The crude fiber contents of the sweet potato cultivars increased during the fermentation process (Table 2). The orange-fleshed cultivar increased from 1.5% to 2.0% and the white-fleshed cultivar increased from 1.8% to 2.7% in crude fiber content. These increases in the crude fiber percentage can be explained by the presence of high molecular weight carbohydrates, such as cellulose, which were not broken down by the enzyme system during fermentation while other carbohydrates and the ash contents were reduced.

The percentage of nitrogen free extract (NFE) (Table 2) is mainly the carbohydrate material such as starch which could be broken down by the enzyme noticeably occurring in the sweet potato as well as the alpha-amylase that was added to the mixture. The NFE also include the fermentable sweet potato sugars. The white-fleshed unfermented sweet potato contained more NFE, 90.29% dwb than the orange-fleshed, 89.22% dwb. This was due to the greater amounts of protein, fat and ash in the orange-fleshed sweet potato. Since the white-fleshed cultivar also had a higher solid content, the total amount of fermentable content, if the NFE was completely digested by the enzymes to fermentable sugars, would be higher. The potential fermentable content was 32.99% in the white cultivar and 27.61% in the orange cultivar.

The average ethanol percentages for the liquid portion of the fermented Centennial sweet potatoes was 2.02% (w/v) and the TG-75-96-1 sweet potatoes was 1.73% (w/v). This was equal to 6.0% and 5.2% yields of ethanol from the raw sweet potatoes. Analysis of

carbohydrate material in the centrifuged, fermented sweet potatoes indicate a total absence of glucose and fructose. Therefore, the absence of these sugars indicate their utilization by the yeast Saccharomyces cerevisiae for ethanol production. The cause of this difference might be based on an increased presence of natural enzymes in the Centennial sweet potatoes which would contribute to more fermentable carbohydrates available or there could have been more fermentable carbohydrates initially present in the Centennial cultivar.

II. COLOR ANALYSIS OF FERMENTED SWEET POTATOES

The mean squares from the analysis of variance of the color analysis are presented in Table 3. The L values for the fermented sweet potatoes were not significantly different ($P<0.05$) while the "a" and "b" values were both significantly different ($P<0.01$). The average L, "a" and "b" values for the centrifuged fermented sweet potatoes are presented in Table 4. The average lightness (L value) for the fermented white-colored sweet potato was 49.55 and for the fermented orange-colored sweet potato was 46.8. The L values are based on the lightness or luminous reflectant which is only slightly higher for the white sweet potato than for the orange.

The "a" average value for the orange sweet potato was 14.7 and for the white sweet potato was 1.55. These results indicate the association of the orange sweet potato towards the red color and of the white sweet potato towards a more neutral color. The white cultivar had a slight red color due to the red skin which remained when it was prepared for fermentation.

TABLE 3. Mean Squares from the Analysis of Variance for the Effect of Fermentation on Hunter L, "a", and "b" Color Values for Orange and White Sweet Potatoes

Source	D.F.	L	a	b
Model	1	7.563^{ns}	172.923**	31.923**
Error	2	6.043	0.113	0.293

^{ns}Not significant.

**Significant at 0.01 level.

TABLE 4. Mean Hunter L, "a", and "b" Color Values for Fermented Sweet Potatoes

Variable	Orange-colored fermented	White-colored fermented
L[a]	46.80±0.2[x]	49.55±3.4[x]
a[a]	14.70±0.4[x]	1.55±0.2[y]
b[a]	20.10±0.4[x]	14.45±0.6[y]

[a]Each value is the average of two samples with ± one standard deviation.

[x-y]Means followed by different letters are different at the 0.01 level of probability.

The "b" average value for the orange was 20.10 and for the white sweet potato was 14.45. These results indicate the association of the orange-fleshed sweet potato "b" value to an intense yellow color in contrast to the white "b" value which associates to a less yellow color.

III. CALORIC CONTENT AND NUTRIENT ANALYSIS OF SWEET POTATOES

The mean squares from the analysis of variance for the caloric and nutrient contents of unfermented and fermented, Centennial and TG-75-96-1 cultivars are presented in Table 5. There were no significant differences in the caloric, niacin and thiamin contents following fermentation. Vitamin C, pyridoxine, calcium, iron and phosphorus were significantly different among the sweet potato samples ($P<.05$).

The average caloric and nutrient contents are shown in Table 6. The slight increase exhibited by the orange cultivar and white cultivar (Table 6) in caloric content following fermentation was due to the increase in crude fat content in both cultivars. The caloric values for fermented orange, 422.1 kcal/100g and for fermented white, 416.3 kcal/100 sweet potatoes on dry weight basis (dwb) were higher than the average published value, 360 kcal/100g (dwb) (42) for canned Centennial cultivar. The fermented caloric values were also higher compared to the U.S.D.A. value, 342 kcal/100g (dwb) (49). The higher caloric values were again due to the increase in fat content in the fermented sweet potatoes compared to the canned sweet potatoes (42) or raw sweet potatoes (49).

TABLE 5. Mean Squares from the Analysis of Variance for Caloric Content and Nutrients of Unfermented and Fermented, Orange and White Colored Sweet Potatoes

Source	Isoleucine[a]	Leucine[a]	Lysine[a]	TSAA[ac]	TAAA[ad]	Threonine[b]	Tryptophan[a]	Valine[b]
Model	3.002[ns]	5.900[ns]	10.581[ns]	33.262[ns]	59.700[ns]	553.800*	23.500[ns]	25.322[ns]
Error	6.500	63.030	18.743	53.754	111.481	46.200	11.620	118.195

[a]Isoleucine, leucine, lysine, TSAA, TAAA and tryptophan had three degrees of freedom for the model statement and four degrees of freedom for the error statement.

[b]Threonine and valine had three degrees of freedom for the model statement and three degrees of freedom for the error statement.

[c]Total sulfur amino acids.

[d]Total aromatic amino acids.

[ns]Not significant.

*Significant at 0.05 level.

TABLE 6. Means for the Caloric Content and Nutrients of Unfermented and Fermented, Orange and White Colored Sweet Potatoes

Variable	Orange colored		White colored	
	Unfermented	Fermented	Unfermented	Fermented
Caloric Content[a]	401.4±0.04[W]	422.1±0.06[W]	401.2±0.05[W]	416.3±0.09[W]
Vitamin C[b]	40.10±1.41[W]	34.20±0.01[W]	21.50±2.1[X]	20.60±0.01[X]
Pyridoxine[b]	45.84±0.0005[W]	None detected	3.4±0.00014[X]	None detected
Thiamin[c]	0.34±0.002[W]	0.29±0.0002[W]	0.19±0.0002[W]	0.23±0.0006[W]
Niacin[b]	1.20±0.01[W]	1.03±0.01[W]	0.19±0.0003[W]	None detected
Calcium[b]	18.0±0.001[X]	22.0±0.005[W]	15.7±0.002[Z]	16.3±0.002[Y]
Iron[b]	0.66±0.0004[X]	0.84±0.0004[W]	0.55±0.0002[Z]	0.61±0.0005[Y]
Phosphorus[b]	195.92±2.1[W]	99.95±3.0[X]	190.40±1.09[W]	101.86±0.1[X]

[a]Caloric content in kilocalories per gram on dry weight basis (dwb).

[b]Vitamin C, Pyridoxine, thiamin, niacin, calcium, iron in mg/100/g and phosphorus in P_2O_5/100g (dwb).

[w-z]Means in the same row followed by the different letters are different at the level of probability in Table 5.

Comparing thiamin content of fermented orange 0.29 mg/100g and fermented white, 0.23 mg/100g (dwb) (Table 6) to the values of canned Centennial sweet potatoes, (42) and of an average amount of raw sweet potatoes, 0.9 mg/100g (dwb), there was a noticeable decrease in thiamin content in the fermented sweet potato cultivars. Niacin content (Table 6) was a higher value in the Centennial fermented cultivars, 1.03 mg/100g (dwb) compared to the canned Centennial (42) value, 0.9 mg/100g and was lower than the U.S.D.A. (49) value, 1.8 mg/100g (dwb). Niacin was not detected in the fermented white-colored cultivar.

The mean values for vitamin C, pyriodoxine (vitamin B_6), calcium, iron and phosphorus are also presented in Table 6. The decrease in vitamin C content in the fermented centrifuged orange and white sweet potato cultivars was probably caused by a loss of the vitamin in water due to its solubility. Other reasons which accelerated the decrease are exposure to oxygen and light which would increase oxidation of the vitamin. The vitamin C contents for the fermented Centennial sweet potato cultivar (dwb) (Table 6) were higher when compared to the value for canned Centennial cultivar, 33 mg/100g (dwb) (42) and lower to the U.S.D.A. (49) value, 63 mg/100g (dwb).

Pyridoxal, an active form of vitamin B_6, was difficult to detect in the sweet potato in this experiment since it was at a low concentration. The decrease of this vitamin after fermentation can be explained due to its destruction when exposed to light, air and heat. Also, it is very soluble in water. Even though samples were kept in the freezer throughout this experiment to minimize losses,

storage should also be considered as a factor for decreasing activity of pyriodoxal.

The majority of vitamin B_6 is in the pyridoxal form. It was only found in the unfermented Centennial and unfermented TG-75-96-1 cultivars (Table 6). Solubility in water is probably the main reason for its decrease in the fermented centrifuged sweet potato.

There was a significant increase of calcium and iron contents in both fermented white and orange sweet potato cultivars (Table 6). The cause of this increase has not been determined. Calcium values for orange fermented, 22.0 mg/100g and for white fermented, 16.3 mg/100g (dwb) sweet potatoes were lower compared to the values for canned (42) Centennial sweet potatoes, 45 mg/100g (dwb) and for the U.S.D.A. value, 96 mg/100g (dwb) (49). Iron content for the Centennial fermented sweet potato was 0.84 mg/100 g and for the white-colored cultivar was 0.61 mg/100g (dwb) which were lower compared to 3.3 mg/100g dwb value for canned Centennial sweet potato (42) and 2.1 mg/100g dwb from the U.S.D.A. (49). These nutrients are also soluble in water and may have been lost from the sweet potato slurries when the water was removed.

The phosphorus content decreased significantly in both the fermented Centennial cultivar and TG-75-96-1 cultivars which can be accounted for since phosphorus is very soluble in water. The mean values (Table 6) for both fermented cultivars were lower compared to the U.S.D.A. value, 323.21 mg P_2O_5/100g (49).

IV. ESSENTIAL AMINO ACID ANALYSIS OF UNFERMENTED AND FERMENTED, ORANGE AND WHITE COLORED SWEET POTATOES

An essential amino acid analysis was performed on the sweet potato samples before and after fermentation. The analysis was determined in duplicate for the two sweet potato varieties, fermented and unfermented and two replications. The concentrations were calculated in mg of the amino acid per gram of protein. The mean squares from the analysis of variance of the essential amino acids in the sweet potatoes are presented in Table 7. The amino acid, threonine, was the only one which was significantly different among the sweet potato samples. It decreased following fermentation (Table 8).

Comparing the mg of amino acid per g of protein for the fermented sweet potato cultivars to the recommended FAO amino acid pattern (51) for an "ideal" protein (Table 8) shows that isoleucine is the limiting amino acid for both the Centennial and the TG-75-96-1 cultivars. The total aromatic amino acids, phenylalanime and tyrosine, are present at the best level in the protein but they only provide 77.1% to 60.8% of the FAO pattern (51).

It is important to indicate that there were difficulties in determining the amino acids, because of the presence of unknown modified amino acids which caused elution problems, and the presence of an unknown cation possibly Na^+ or K^+ which interfered also in the elution of the peaks. There were other amino acids also present in the sweet potatoes other than the essential amino acids. These amino acids were histidine, arginine, aspartic acid, serine, glutamic acid, proline,

TABLE 7. Mean Squares from the Analysis of Variance for the Essential Amino Acid Content of Unfermented and Fermented, Orange and White Colored Sweet Potatoes

Source	Caloric[a] Content	Vitamin C[a]	Pyridoxine[b]	Thiamin[a]	Niacin[c]	Calcium[a]	Iron[a]	Phosphorus[a]
Model	0.0224[ns]	184.680**	0.000074**	0.00000082[ns]	0.000055[ns]	0.00000016**	0.0000031*	5685.677**
Error	0.0041	1.625	0.00000017	0.0000018	0.000057	0.000000	0.00000020	3.678

[a]Caloric content, vitamin C, thiamin, calcium, iron and phosphorus had three degrees of freedom for the model statement and four degrees of freedom for the error statement.

[b]Pyridoxine had one degree of freedom for the model statement and two degrees of freedom for the error statement.

[c]Niacin had two degrees of freedom for the model statement and two degrees of freedom for the error statement.

[ns]Not significant.

*Significant at the 0.05 level.

**Significant at the 0.01 level.

TABLE 8. Means for the Essential Amino Acid Content of Unfermented and Fermented, Orange and White Colored Sweet Potatoes

Variable	Orange Colored		White Colored		Amino Acid Pattern FAO (51)	% of FAO (51) Fermented	
	Unfermented	Fermented	Unfermented	Fermented	Mg/g protein	Orange	White
	------mg/g protein----		------mg/g protein------				
Isoluecine	12.011±0.3[W]	9.610±3.2[W]	9.710±1.6[W]	9.402±3.5[W]	40	24.0	23.5
Leucine	38.462±4.1[W]	36.766±3.8[W]	37.616±8.4[W]	34.841±12.2[W]	70	52.6	49.7
Lysine	22.832±2.0[W]	23.269±6.5[W]	18.338±3.8[W]	20.343±7.3[W]	55	42.4	36.9
TSAA[a]	18.242±4.4[W]	19.469±12.9[W]	13.256±1.7[W]	10.853±4.8[W]	35	55.7	31.1
TAAA[b]	49.274±0.9[W]	46.235±7.8[W]	44.245±14.6[W]	36.471±12.9[W]	60	77.1	60.8
Threonine	58.295±0.01[W]	24.234±6.4[X]	16.872±9.1[X]	10.010±3.4[Z]	40	60.5	25.0
Tryptophan	11.790±6.1[W]	6.610±1.8[W]	3.803±2.1[W]	5.628±0.4[W]	10	66.0	56.3
Valine	14.390±13.7[W]	22.400±3.7[W]	15.710±12.2[W]	21.784±0.01[W]	50	44.8	43.6

[a]Total sulfur amino acids.

[b]Total aromatic amino acids.

[W-Z]Means in the same row followed by the different letters are different at the level of probability in Table 7.

glycine, and alanine. Other nitrogen containing compounds included phosphorine, taurine, phosphoethanoamine, urea, ornithine, pre-aspartic and pre-glutamic.

Pre-aspartic was present in significantly large amounts which can be added as aspartic and be reported as such. This fact could also explain the lower percentage of the essential amino acids compared to the ideal amino acid pattern recommended by the FAO.

The amino acids were analysed at 22 hr hydrolysis and 72 hr hydrolysis. For the purposes of this study, the EAA 72 hr hydrolysis determination was chosen because of clearer resolution of the peaks.

CHAPTER V

CONCLUSION

The objective of this investigation was to evaluate the nutritional value of sweet potato residues after producing ethanol by yeast fermentation combined with enzymatic hydrolysis of starch.

Results from the proximate composition indicated that there was an increase in protein, fat and fiber content for both orange-colored (Centennial) and white-colored (TG-75-96-1) sweet potato cultivars. Ash content decreased as a result of the process.

Nitrogen free extract results indicated that there was a decrease in starch and various sugars in both sweet potato cultivars. The orange cultivar produced more alcohol than the white and had a greater decrease in %NFE. This is a contradiction with their initial carbohydrate contents. Because of greater yield and higher initial NFE content, white sweet potato would probably give greater ethanol production than orange-fleshed sweet potatoes, if increased enzyme was used to break down the carbohydrate material making available more fermentable sugars. Both sweet potatoes yielded less ethanol than expected due to poor break-down of starch to sugar.

High pressure liquid chromatographic analysis of thiamin and niacin indicated that there was no significant changes in their contents in the fermented sweet potato residues. Pyridoxine or vitamin B_6 was not detected in the fermented sweet potato residues and, therefore, must have been lost during fermentation.

Results of the colorimetric analysis of vitamin C or ascorbic acid content indicated that it decreased in both the orange and the white colored fermented sweet potato residues. The decrease of this vitamin as well as the expected amount of B-vitamins is mainly due to the loss of the mentioned vitamins in water which was the solvent system used for the process.

Calcium and iron contents increased for both orange-flesh and white-flesh sweet potato cultivars. Because the cause is unknown, further research would be needed to determine why.

Results from the phosphorus analysis indicated that there was a decrease in phosphorus content possibly because of the fermentation but more likely due to its solubility in the solvent used for the process.

Statistical analysis of essential amino acids indicated that fermentation did not significantly affect isoleucine, leucine, lysine, total sulfur amino acids, total aromatic amino acids, tryptophan and valine contents of the sweet potato residues but significantly affected threonine.

It is concluded that fermentation increased the nutritional value of sweet potatoes, particularly the percent protein. Improved fermentation of the carbohydrate material should increase the amount of protein even more. The orange, Centennial cultivar possessed more nutrients than the white, TG-75-96-1 cultivar prior to, and, therefore, after fermentation. Because of the increase in the protein content and nutritional value, fermented sweet potatoes could be a good source to supplement other vegetables low in this protein content.

Because of the interest in alcohol production, sweet potato can be considered a feasible substrate for alcohol providing the development of a way to increase carbohydrate breakdown for more fermentable sugars. The utilization of the residue or waste is an alternative for protein supplement for animal feed, and for human consumable protein. More research would be necessary in food applications taking in consideration the sensory attributes of the sweet potato residue.

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VITA

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