5-1960

Effects of Beta Rays, Gamma Rays, and Hydrochloric Acid on Tubers of Jerusalem Artichoke

Subhi Al-Sammarai
Utah State University

EFFECTS OF BETA RAYS, GAMMA RAYS, AND HYDROCHLORIC ACID ON TUBERS OF JERUSALEM ARTICHOKE

by

Subhi Al-Sammarai

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

The Inter-departmental Curriculum in

Nutrition and Biochemistry

UTAH STATE UNIVERSITY
Logan, Utah

1960

ACKNOWLEDGMENTS

The writer wishes to express his deepest gratitude to his major professor, Dr. E. B. Wilcox, for the generous encouragement and guidance throughout the periods of study. He is also indebted to Dr. D. K. Salunkhe, thesis director, for the suggestion of the problem and for his direction and guidance during the study.

Sincere appreciation is extended to Dr. R. L. Berger, committee member of this study, for valuable suggestions; to Dr. E. L. Olsen for suggestions and contributions to this study; to Professor D. V. Sissons for his help in the statistical analysis; to Mr. William Gallagher and Professor R. B. Neal of Stanford University and to Dr. E. S. Munan of Varian Associates, Palo Alto, California, for their work and help during the use of beta radiation.

Subhi Al-Sammarai

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

INTRODUCTION

Levulose (D-fructose, fruit sugar), $C_6H_{12}O_6$ is a white crystalline ketohexose. This sugar has distinctive properties which make it of commercial interest. It is more costly than ordinary table sugar (sucrose). Levulose is characterized by a high degree of sweetness (6), great solubility (20), and medical usefulness when administered intravenously. Joslin (21) and Root and Baker (32) found levulose to be of great value in the treatment of diabetes. According to Daniel (8), it is assimilated and oxidized more quickly than sucrose, supplying the necessary energy requirements for the human system. Additional uses of levulose have been reported by McGlumphy and Eichinger (27), which include its application to improve the quality and the flavor of jams, jellies, marmalades, canned fruits, carbonated beverages, and corn sugar.

Plants of the family compositae contain large amounts of levulose polymers, but the dahlia, chicory, and Jerusalem artichoke are the most promising sources because of the high levulose content and the ease of production (13). The tubers of Jerusalem artichoke were used in this study because they offer an inexpensive and prolific source from which levulose can be extracted.

Since most of the levulose units in the artichoke tubers are linked together to form inulin or carbohydrate intermediate compounds between inulin and levulose, it is necessary to hydrolyze the material in order to free the levulose. Several investigators have reported the use of acid compounds. Anderson and Greaves (1) used H_2SO_4 for

hydrolysis, while Yamasaki (41) used HCl for the same purpose. Kleiderer and Englis (23) obtained complete hydrolysis of inulin by use of CO_2 and SO_2 at a pressure of 1000 pounds per square inch at 150° C. for 60 minutes.

As far as can be determined from the literature, little work was done prior to 1953 using radioactive material to study peaceful uses of atomic energy. Since that time, a law was passed by the United States Congress authorizing the use of radioactive material for peaceful purposes, thereby making it possible to use atomic energy for this study. Since previous work has shown that gamma rays can convert starch to simple sugar (33), it was, therefore, assumed that the inulin and other carbohydrate intermediates of Jerusalem artichoke upon hydrolysis by beta or gamma rays might yield fructose (35).

The primary purpose of this study was to determine the effects of beta and gamma rays on inulin and other carbohydrate intermediate compounds present in the Jerusalem artichoke tubers and to compare the results with those obtained by hydrolysis with radiation plus hydrochloric acid.

REVIEW OF LITERATURE

Historical Background

The Jerusalem artichoke, Helianthus tuberosus L., grows prolifically as a weed even on marginal land.

It was seen by Champlain, according to Shoemaker (37), in the gardens of the Indians at Mallebrre July 21, 1605; now called Nauset Harbor, Cape Cod, Massachusetts. The range of the plant in the United States is usually given from New York to Minnesota, southward to Georgia and Arkansas or, in general, they can be grown in the same region as potato cultivation. Shoemaker (37) considered the Jerusalem artichoke a crop plant native to America but taken to some parts of Europe for cultivation. Probably it was introduced into France by Les Carbot, a companion of Champlain. It reached England in 1616 or 1617. Although it is American in origin, it has retained its importance only in a few localities where it is used as fodder for cattle. However, it has been used and studied more in Europe, especially in France where it has been used as a forage crop for livestock, food for humans, and as a source of alcohol. Finally it has been re-introduced into this country in a number of improved varieties. Shoemaker (37) stated that the wild types could be listed under thousands of different varieties.

It is well known that the Jerusalem artichoke has inulin as a reserve carbohydrate. This carbohydrate is useless as a food because it cannot be hydrolyzed by any of the enzymes of the human gastrointestinal tract. Further study on the composition of the Jerusalem artichoke tubers showed the fructans (inulin and levulans) (30) and

other intermediate carbohydrate compounds (4) can be easily hydrolyzed by acids into fructose. Therefore, an interest in the plant has developed in this country within the last 30 years because of its possible value as a source of raw material for the production of levulose, "the future sugar."

The Carbohydrate of the Jerusalem Artichoke

In spite of repeated investigations, divergences still exist as to natural and relative proportions of the carbohydrates found in the tubers of Jerusalem artichoke.

One of the well known carbohydrates in the Jerusalem artichoke and other compositae is inulin which was first found in the tubers by Connet according to Shoemaker (37). During investigations by Tanret as noted by Thaysen et al. (38), with tubers gathered in September and October eight different carbohydrate compounds were separated from the artichoke juice such as inulin, pseudo-inulin, inulenin, sucrose, levulose, dextrose, helianthenin, and synanthrin. Out of these, the last two levorotatory carbohydrates were named by him. Pseudo-inulin and inulenin are closely related to the inulin proper but are stated to differ from it in their specific rotations which are -32.2°, -29.6° for pseudo-inulin and inulenin, respectively. Of all the carbohydrates isolated by Tanret from the Jerusalem artichoke tubers, only sucrose and dextrose give positive specific rotation. It is to be expected, therefore, that the juice obtained by extracting the fresh tubers with water would show a negative rotation.

Colin, according to Thaysen et al. (38), found that an extract of tubers was strongly levorotatory when prepared from an autumn crop during September, while the extract of early spring tubers had a dextrorotatory rotation. This implies that considerable changes had

occurred in the carbohydrate content.

Other investigators have studied the change in the composition of the tubers with respect to their ages and maturity. They came to the conclusion that inulin may constitute only a small fraction of the total carbohydrate present in the artichoke tuber. The remainder, which resembles inulin, is composed essentially of fructofuranose units, having a great solubility in water and in aqueous ethanol.

Another factor of considerable importance has been the variety which has a great effect on the composition of the carbohydrates. Shoemaker (37) reported that the carbohydrate content ranges from 8.64 to 19.47 percent in different varieties. Since levulose is the only interesting sugar of the Jerusalem artichoke tubers, its percentage is more often reported than other sugars. McGlumphy et al. (27) stated that levulose can reach 24 percent and he believed it was possible to increase it to 30 percent by plant breeding. Yamazaki (41) in Japan, has given 11.56 and 14.80 as average values of percentage of levulose and total reducing sugar, respectively, obtained from the family of white Cortex Jerusalem artichoke.

In 1939, nine artichoke varieties cultivated at Iowa Agricultural Experiment Station (13) were analyzed on a wet basis and showed that the levulose content varied from 3.70 to 6.55 percent. However, in 1940, analysis on the same nine varieties showed higher levulose content varying from 8.40 to 17.64 percent. Bacon and Edelman (4) used a paper partition chromatographic technique to identify the carbohydrate content of the extract of the Jerusalem artichoke tubers. They showed the presence of five different compounds, which were levulose, sucrose, dextrose, inulin, and inulides.

Conversion of Inulin into Levulose

McGlumphy et al. (27) state that Crookewitt was apparently the first to hydrolyze inulin to an "uncrystallizable" sugar by heating for 15 hours in a water solution. Since that time, all attempts to crystallize the sugar failed, until the year 1880 when Jungfleisch found a method of crystallization and obtained crystallizable sugar, known today as levulose. Heat hydrolysis of inulin was repeated by Kilini who claimed a 96.7 percent conversion of inulin into levulose.

Weizsaeker according to Harding (15) converted sucrose to monosaccharides by taking 100 grams of pure sucrose, adding it to a liter of distilled water acidified with one gram of concentrated H_2SO_4 and boiling for five minutes.

In 1905, the Levulose Company of England (24) secured a patent on a process for preparing levulose from inulin by acid hydrolysis. Bourgulot and Bridel according to McGlumphy et al. (27) showed that the fructose molecules were the only molecules formed by acid hydrolysis of pure inulin. Willaman (40) published a method for the manufacture of levulose, of which the acid hydrolysis of the inulin components is one step. Jackson, Silsbee, and Proffitt (19) obtained white crystalline fructose by acidifying the artichoke juice with H_2SO_4 or HCl immediately after extraction and then heating to 70° to 80° C. for 30 to 40 minutes. The same investigators (20) reported a detailed method for the preparation of levulose from the Jerusalem artichoke and dahlia tubers and conducted an experiment to measure the velocity constant of conversion of inulin and cane sugar. They found that the velocity constant of conversion of ash-free inulin was 0.02, while under the same conditions cane sugar gave a velocity constant of about 0.27. In other words, the velocity conversion of cane sugar is more than

thirteen times as fast as that of inulin. By measuring a number of velocity constants, they found that the resultant of the various reactions occurring during the conversion process follows substantially the course of a unimolecular reaction. The same investigators also studied the effect of pH, temperature, and the heating time upon the decomposition of levulose by H_2SO_4. Their conclusion was that as the pH decreased, the amount of decomposed levulose increased. Also, the higher temperature and the prolonged heating caused more destruction.

Schering (36) secured a patent on the hydrolysis of inulin by using volatile organic acid, such as HCOOH, CH_3COOH, or H_2CO_3.

Arsem (2) obtained many patents covering: the purification and hydrolysis of inulin. He used 0.01 N HCl at a temperature of 100° C. for conversion into fructose. He also used a purified inulin in a 70 percent water solution acidified with 0.015 N $C_4H_6O_6$ (tartaric acid). Arsem (3) in 1930 secured another patent for the hydrolysis of inulin. He mixed inulin with a required quantity of water and hydrolyzed it by adding a soluble acid anhydride, such as SO_2 or CO_2. The mixture was enclosed in a container and heated to about 100° C. under pressure.

Golovin, Bryukhanova, and Fridman (12) hydrolyzed chicory and artichoke juice with 0.2 N and 0.5 N H_2SO_4 at 70° C. for 30 to 45 minutes.

McGlumphy (25) attempted to hydrolyze inulin by using CO_2 under pressure following the method of Schering (36) at a temperature of 85° to 90° C. and CO_2 pressure of 200 pounds per square inch. The rotation measurement indicated only slight hydrolysis with CO_2. However, the same results were obtained without the use of CO_2. This indicated that hydrolysis probably was due chiefly to the temperature. In another

experiment, McGlumphy applied HCl to convert inulin. He added 465 cc. of concentrated HCl to 28 liters of artichoke juice, brought the normality of the mixture to 0.1923, left the mixture for nine hours at room temperature, and then measured the rotation which showed -4.5 at 24° C. Heating for 45 minutes at 80° C. changed the rotation to -6.4 at 24° C. Additional heating for 30 minutes at the same temperature showed the same rotation, indicating that the maximum hydrolysis was reached at the end of the first 45 minutes of heating.

In 1931, McGlumphy, Eichinger, Hixon, and Buchanan (26) selected conditions of 60 minutes interval, a temperature of 80° C. and a pH of 1.50 with H_2SO_4 or pH of 1.75 with HCl to hydrolyze the levulosans in the Jerusalem artichoke tubers to fructose. Kleiderer and Englis (23) have obtained nearly complete hydrolysis of inulin by the use of CO_2 and SO_2 at a pressure of 1000 pounds per square inch at 150° C. for 60 minutes. This hydrolysis might be due to the presence of SO_2 and the more drastic conditions of temperature and pressure than those used by McGlumphy (25) where he obtained only slight hydrolysis.

Eichinger, McGlumphy, Buchanan, and Hixon (11) determined some factors controlling the conversion reaction of Jerusalem artichoke juice. One part of their experiment dealt with the effect of pH on hydrolysis of Jerusalem artichoke juice. By measuring the rotation of the hydrolyzed material at intervals and applying the equation for unimolecular reaction, they calculated the velocity constants for the conversion of artichoke juice by H_2SO_4 and HCl. Their results showed that the velocity constant depended upon the pH of the mixture of juice and acid. The second factor was to determine the decomposition of levulose during conversion. Their data showed practically no decomposition in the levulose solution when heated for one hour at 80° C. and

pH values down to 1.12. In addition to the factors mentioned above, two other major factors studied which affect hydrolysis were temperature and percent total solids in the extract.

Dykins and Englis (10) reported other optimum conditions for the hydrolysis of the extract of the Jerusalem artichoke tubers. The conditions selected were pH 4.2 for 20 minutes at 130° C. Under these conditions, the salts resulting from neutralization of HCl were less than one percent and the loss of fructose as indicated by polariscopic and reduction tests probably not significant.

Anderson and Greaves (1) used the Jerusalem artichoke as a source for the preparation of d-lactic acid. They hydrolyzed the tuber material prior to fermentation by heating at 95° C. for one hour at a pH of 2.0 with H_2SO_4.

From the above reviews, heat, pressure, and acids were the only factors used to hydrolyze the artichoke extract. Another method used to purify and hydrolyze artichoke extract was the utilization of electricity. A report on this method has been made by Heubaum (18) and more complete information has been reported by Hardy (16). This method has the advantage of acidifying the extract without the addition of acid, thus reducing the ash content. Treatment with the electrodialytic apparatus also reduced the colloid content of the extract.

Thies, Souci, and Kallinich (39) showed that the acid hydrolysis of inulin was only a partly first order reaction. The hydrolysis during the first third of the reaction was highly curved, the velocity constant increased progressively. Later, the curve straightened out and the hydrolysis became a first order reaction.

According to the reviewed literatures, the hydrolysis rate is

dependent upon the following factors: pH, heating time, pressure, temperature, and the concentration of solids in the extract.

METHODS AND MATERIALS

General Outline of Experiment

Jerusalem artichoke tubers were analyzed for inulin, reducing sugar, and fructose in four different experiments: (a) non-irradiated tubers which were harvested in June and October; (b) June gathered tubers which were irradiated by beta rays obtained from the Mark IV Linear Electron Accelerator at Stanford University, Palo Alto, California; (c) tubers harvested in March which were irradiated by gamma rays obtained at the National Reactor Testing Station near Arco, Idaho; (d) non-irradiated, beta irradiated, and gamma irradiated tubers which were hydrolyzed with heat and hydrochloric acid to obtain additional fructose. Total solids were also determined in experiments (a) and (b).

Beta Radiation Technique

Locally grown Jerusalem artichoke of unknown variety (Figure 1) were harvested June 13, 1959. The tubers were transferred to the laboratory the same day, and the soil was removed under running water. As far as possible uniform sized tubers were selected for the experiment. They were put in polyethylene bags, then sealed in No. 10 tin cans. Each can was labeled indicating the dose and rate of beta rays to be given. The cans were transported to the High Energy Linear Electron Accelerator at Stanford University, Palo Alto, California, for irradiation. Electron linear accelerators are suitable for a wide range of research applications. The electron beam can be used directly or a portion of its energy can be converted by suitable targets for the production of X-rays and neutrons. These machines produce bursts of

Figure 1. The Jerusalem artichoke plant

electrons, X-rays, or neutrons in pulses of 0.1 to several micro-seconds long. Intense beams of high energy electrons produce high density ionization tracks. High energy output permits penetration of thick targets and the use of electrons permits focusing by strong magnetic fields. Several factors are interrelated and associated with the amount of energy passing through the sample. Such factors are the original electron energy (Mev.) to be used and the radius of the cylindrical beam. With regard to the second factor, Mr. William Gallagher, Professor R. B. Neal of Stanford Research Center, and E. S. Munan, Varian Associates, Palo Alto, California, suggested the use of smaller volumes than No. 10 cans in order to get the specified energy through the whole sample. The electrons beam, which was about 1 centimeter in diameter, was spread out by letting it scatter through a one-fourth inch aluminum plate 18 inches in front of the jar which produced a sufficient cone to cover the 3-inch diameter Mason jar containing the tubers. For this reason, two Mason jars were filled with Jerusalem artichoke tubers. The samples were irradiated June 15, 1959, with the Mark IV accelerator using a 1 microamper electron beam at 70 Mev. The beam current collected directly behind the jar measured three-fourths microamper.

The first jar containing Jerusalem artichoke was irradiated for 45 minutes to an estimated dose of 8×10^6 rads.[1] The second jar was

[1]A rad is equal to the amount of energy absorbed in tissue and is equivalent to 100 ergs per gram of tissue. In comparing the relative biological effectiveness of the radiation, it is now considered best to do this on the basis of the total energy absorbed in the tissue rather than the amount of ionization taking place. As recommended by the Sixth International Congress of Radiology in 1950, the dose is expressed as follows: "For the correlation of the dose of an ionizing radiation with its biological or related effects the International Commission on Radiological units recommends that the dose be expressed in terms of quantity of energy absorbed per unit mass (ergs per gram) of irradiated

irradiated for 2 hours to an estimated dose of 20 x 10^6 rads.

The electron energy of 70 Mev. used in our experiment allowed an estimated penetration distance of about 37 centimeters through the sample as calculated from the equation below (22):

$$R = 530\ E - 106$$

where

R = range in mg/cm^2

E = maximum energy (Mev.)

therefore

$$R = 530 \times 70 - 106 = 36994\ mg/cm^2$$

If we assumed that the density of artichoke tubers is 1 gram/ml., the estimated distance of penetration would be 36.994 cm.

When radiation was completed the jars were tested at intervals for radiation by means of a Geiger counter until no rays were emitted from the jars. They then were opened for the ethanol slurry preparation. At that time it was noticed that the irradiated tubers had turned yellow-white in color. The intensity of the color increased as the dose was advanced. A breakdown of the tubers into tissue and separated juicy material was noticed with 20 Mega rads treatment.

Slurry preparation

A representative sample of 100 grams of sliced tubers was weighed in a Mason jar and the sample then transferred to a quart Waring blender jar. About 100 ml. of 95 percent ethanol were added and the mixture blended for 4 minutes. The blender was stopped and the sides of the container were washed down with a small amount of 95 percent ethanol from a wash bottle. The blending was then continued for 1

---

material." For this reason, the radiation dose is expressed in rads rather than reps.

minute. The mixture was transferred quantitatively to a tared Mason jar with the aid of 95 percent ethanol, making the total slurry weight 400 grams. The jar was sealed and the slurry saved for the various chemical determinations.

Total solids

Total solids were determined by drying the aliquot of the ethanol slurry. The slurry was vigorously agitated by means of a motor driven stirrer. During the agitation a 12.5 to 13.5 gram aliquot was removed and transferred to a tared moisture dish which was immediately capped and weighed. The sample was dried in an air oven for 24 hours at 70° C and in a vacuum oven at 70° C for 48 hours. It was then cooled in a desiccator, weighed, and calculated as percent total solids in the Jerusalem artichoke.

Separation of soluble sugars from inulin

Each sample of the irradiated Jerusalem artichoke and the control in ethanol slurry was weighed into a stoppered 50 ml. centrifuge tube. The samples were centrifuged and the supernatant liquid was transferred into 100 ml. volumetric flasks together with two washings of 15 ml. of cold 80 percent ethanol. This solution was made up to the mark with distilled water and used for fructose and total reducing sugar analyses, while the residue was used for inulin analysis.

Fructose

The colorimetric determination of this natural sugar in biological systems has been difficult because of interference from aldose sugar. The use of the aromatic amines, such as *p*-anisidine or 3.3 dimethoxybenzidine, made the determination of fructose possible in the presence of glucose and other aldoses.

Fructose was determined by the method developed by Hessler (17)

with the following modifications.

1. Two ml. of each sugar solution and the standard fructose solutions were pipetted into similar test tubes together with 4 ml. of 0.5 percent *p*-anisidine in 85 percent phosphoric acid.
2. All tubes were covered with glass globes, placed in a water bath, maintained at 90° C for 10 minutes, and cooled to room temperature.
3. After 30 minutes of cooling, the color intensity reached its maximum and the optical density was read with Bausch and Lomb Spectronic 20 colorimeter.
4. The optical density of the standard fructose solutions was plotted against concentrations and the fructose concentration of the samples were estimated from the graph so obtained (Figure 2; Appendix, table 7).

Inulin

After the ethanol soluble materials containing the sugars were extracted from the samples, the residue was used for inulin analysis. This was carried out in the following manner:

The residue was transferred to a 250 ml. beaker with the aid of 60 ml. of distilled water. With constant stirring a controlled temperature brought each sample to the boiling point in 5 minutes and the residue was then filtered through a No. 2 filter paper. The filtrate with 2 washings of 50 ml. boiling distilled water was collected and made up to the volume in a 250 ml. volumetric flask. A further dilution (5 ml. of the filtrate to a 50 ml.) was made, except for the samples harvested in October which were made up to a volume of 200 ml. in order to measure the percent transmission.

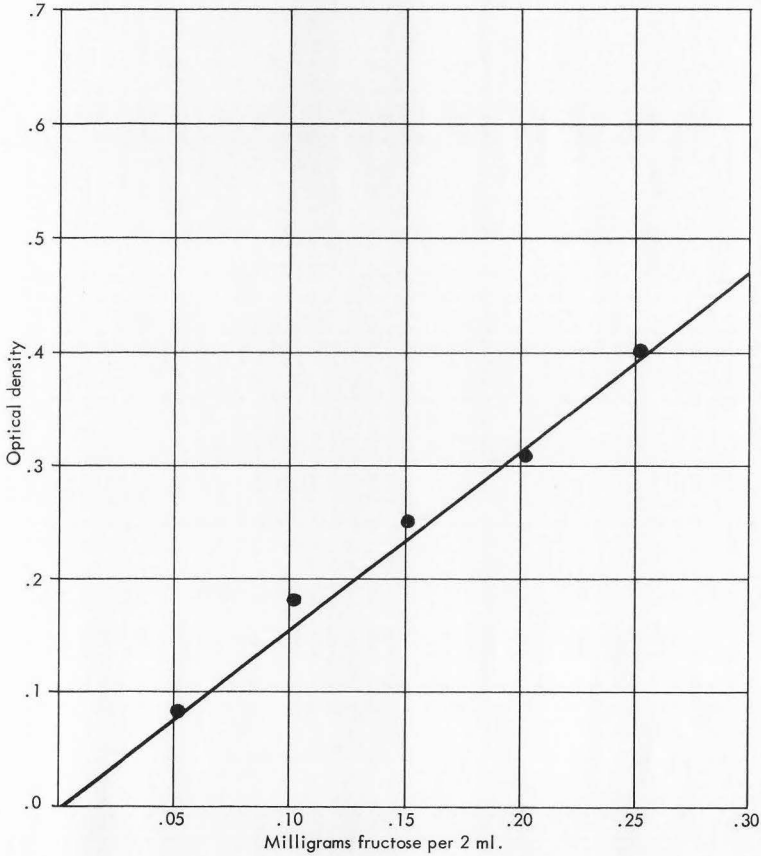


Figure 2. Fructose standard curve

Inulin was estimated by the method used by Preedy (31) with the following modifications.

1. Two ml. of each diluted sample and the standard inulin solutions were pipetted into similar test tubes together with 1 ml. of resorcinol-thiourea solution and 7 ml. of HCl solution.
2. All tubes were covered with glass globes and placed in a water bath, maintained at 90° C for 10 minutes. The tubes were removed and cooled immediately by placing them in tap water.
3. A Bausch and Lomb Spectronic 20 colorimeter was employed to determine the percent transmission.
4. The percentage transmissions of the standard inulin solutions were plotted against concentration on semilogarithmic paper and the inulin concentration of the unknown solution was estimated from the graph so obtained (Figure 3; Appendix, table 8).

Reducing sugar

Reducing sugar was estimated by the Munson and Walker method (28) as modified by Shaffer and Hartmann.

This method has employed the alkaline copper reagent and heating conditions specified by Munson and Walker so that the milligrams reducing sugar expressed as fructose could be read from the table worked out by Hammond (14).

Gamma Radiation Technique

The means of gamma radiation were obtained from an area on the station grounds called the Materials Testing Reactor Area. In that area there was a building referred to as the Gamma Building which houses a "swimming pool" type of canal for experiments.

In this canal there was an upright 18-foot length of 8-inch (inside

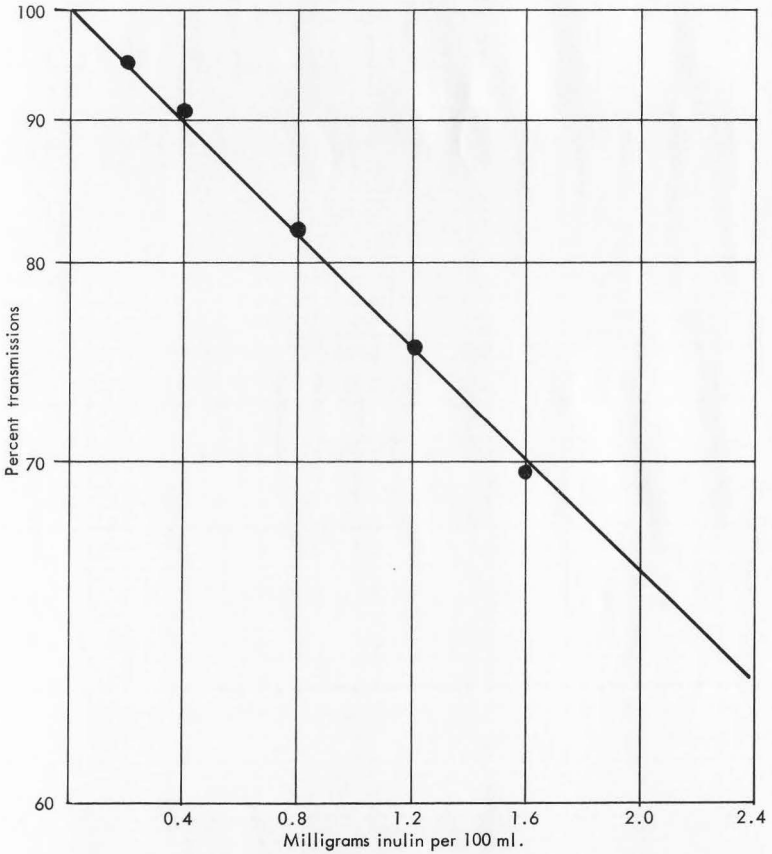


Figure 3. Inulin standard curve

diameter) aluminum pipe (35). It was lead weighted and sealed at the bottom so that no radioactively contaminated canal water may enter. This column had a separate source of uncontaminated "flushing" water flowing through it continually. This meant that at no time did the canal water or the gamma radiation source touch the container holding the samples for irradiation. The above described column is referred to as the U. I. A. Column since the branch of the University of Idaho at Aberdeen provided the facility (Figure 4). The source of gamma radiation came from spent fuel rods. These fuel elements, which are 6 x 6 x 24 inches, were placed around the U. I. A. Column to facilitate desired doses and rates.

On the bottom of a fuel rod insert was a piece of metal narrowed down on four sides. This narrow piece was fitted into a hole in what was called the Fuel Element Support Grid at the bottom of the canal. In this manner it was possible to stand a fuel element upright (Figure 4). At the gamma facilities building the useful life of this source was considered to be near 30 days. After this time it was sent to the Chemical Processing Plant. Part of the material was usually reclaimed and the rest discarded in a safe place.

A container known as an aeration chamber (Figures 5 and 6) was used for lowering the cans containing the artichoke tubers into the U. I. A. This chamber was constructed from a 2-foot section of 7-inch diameter aluminum irrigation pipe. An aluminum bottom was welded on one end and the cylindrical tank was weighted with 35 pounds of lead. A maneuverable aluminum lid was designed to fit the other end and was made water tight with a rubber gasket. A 6-inch piece of three-fourths inch iron pipe was fastened with a gasket into the lid to which was clamped a 25-foot length of 1-inch inner diameter Tygon tubing. A 25-foot length

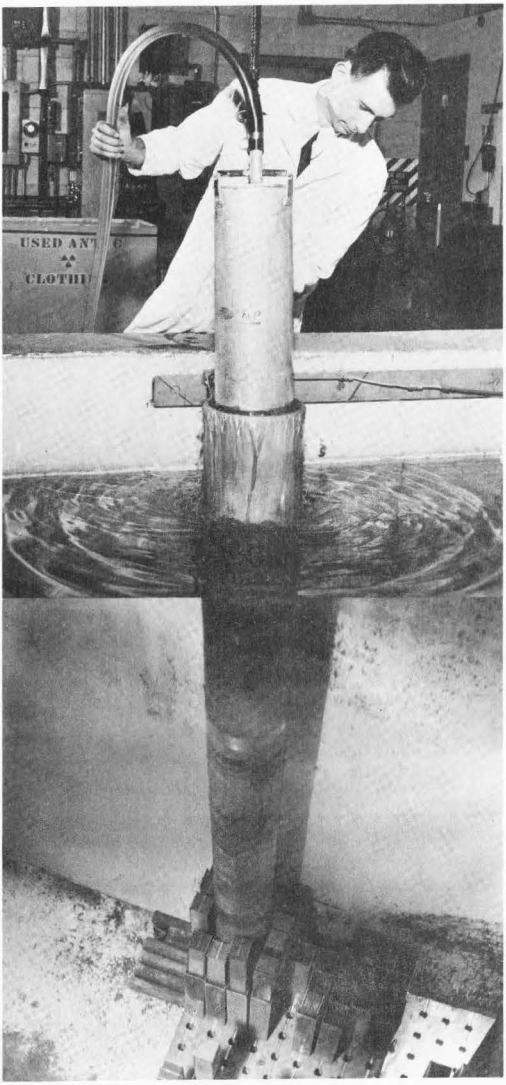


Figure 4. Entire radiation apparatus showing U. I. A. Column, aeration device, and fuel element

Figure 5. Parts of aeration chamber

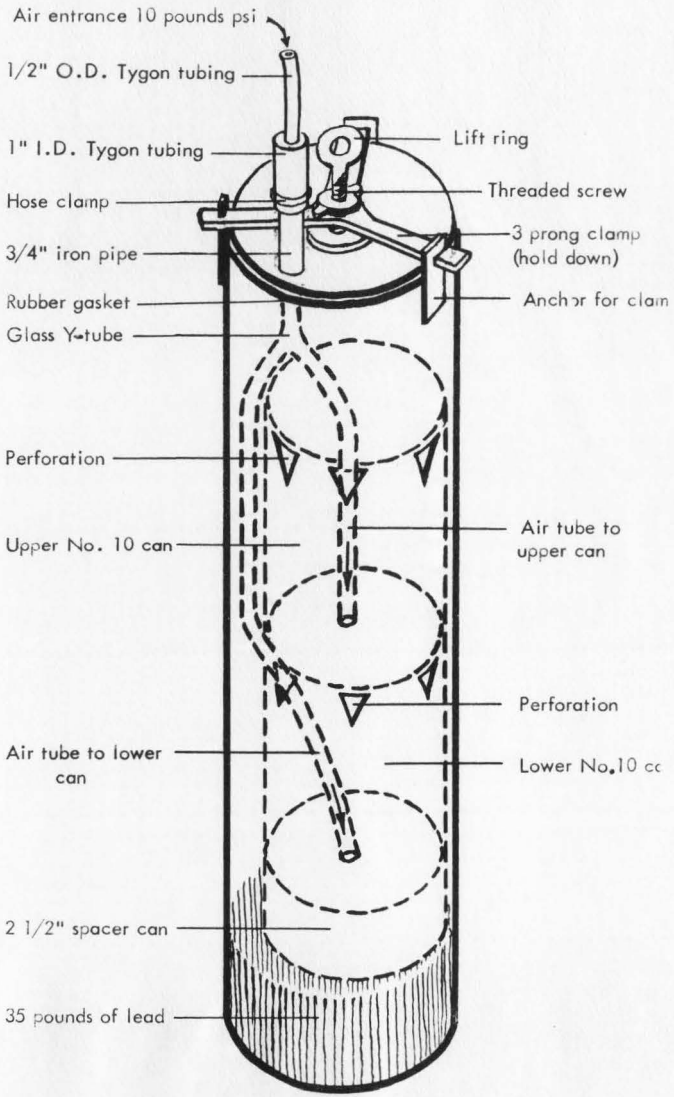


Figure 6. Aeration chamber used during the process of radiation under water (cross section)

of one-half inch outer diameter Tygon tubing was threaded through the larger piece of Tygon tubing.

For irradiation, two No. 10 cans of artichoke tubers were placed in the chamber and air under 10 pounds pressure per square inch was forced down the one small tube. Within the chamber, the air or gas was divided with a glass Y-tube and taken by auxiliary tubes to each punctured can. The exhaust air escaped from the chamber through the space between the small tube and the wall of the large tube.

The chamber was then lowered to the bottom of the water column. It was allowed to remain for a calculated length of time.

Dosimeter measurements were used to calculate the dose (amount of radiation) and rate (length of exposure to gamma rays). Details of this procedure are given by Cotton and Siu (7).

To insure equal dosage for both cans in the aeration chamber, they were irradiated for half the calculated time required for the desired dose. The cans were then raised to the surface and the top can placed on the bottom. The cans were rearranged because the fuel rods do not emit rays at the same rate along their length. By changing the cans in this manner, both of them received the same amount of radiation.

The doses of the gamma radiation used were estimated to be 1, 2, 4, 8, 10, 20, 40, and 60 x 10^6 rads. Out of these doses, the tubers irradiated with 8 and 20 x 10^6 rads were used for the chemical anlysis. After irradiation, each can was inspected by health officials for the emission of radiation. Then they were stored at 40° F until the time of the preparation of ethanol slurry, which was saved up to the time of the chemical analysis.

Acid Hydrolysis Technique

Samples of 21 to 22 grams of ethanol slurry containing irradiated or non-irradiated Jerusalem artichoke tubers were taken for hydrolysis.

The ethanol portion of each sample was evaporated by setting the samples overnight in an oven at 60° C. Fifty milliliters of hydrochloric acid, pH 2, was added to each sample. By the dropwise addition of 1 N HCl, each solution was brought to pH 2. All samples were placed at the same time in a water bath maintained at 95° C for one hour, then cooled to room temperature before centrifuging. The supernatant liquid which was separated from the residue by decantation was assumed to contain the sugar part of the hydrolyzed extract.

The excess acid in the supernatant liquid was neutralized by adding a 15 percent NaOH solution, and the volume was made up to 250 ml. with distilled water. For the reducing sugar determination, 25 ml. of each solution were used. To carry out the fructose determination, a further dilution was made in order to obtain a colormetric reading within the measurable limits.

The unhydrolyzable inulin remaining in the residue was extracted by the method previously mentioned and then made up to 100 ml. A further dilution was found to be necessary to obtain a colormetric reading within the measurable limits.

RESULTS AND DISCUSSION

Experiment 1. Comparison in the Composition between Jerusalem Artichoke Tubers Harvested in June and October 1959

Analyses of the non-irradiated control tubers of Jerusalem artichoke harvested in June were made to determine the total solids, inulin, total reducing sugar, and fructose. The average value of reserve inulin was 1.29 percent for the tubers harvested in June (table 1). This yield was too low to be an economical source for the production of fructose after the conversion of inulin by radiation or HCl. Because of this fact, other tubers were harvested in October for analyses. Results given in table 1 showed that the percent total solid of Jerusalem artichoke tubers harvested in October was approximately twice that of tubers harvested in June during the growing season, when the plant tops measured about 4 feet.

The inulin content of the tubers was shown to be approximately 9 times greater in October than in June. This may be due to the fact that more translocated simple sugar synthesized in the leaves was stored in the tubers as reserve inulin or inulides during the period between June and October. Free fructose which averaged 3.13 percent in June was found to be 2.76 in October; during the same season, total reducing sugar decreased from an average value of 3.51 percent to 3.05 percent. This decreased value in free fructose and total reducing sugar in tubers harvested in October may be a result of a higher rate of conversion of free sugars to inulin and inulides during October. Earlier in the season during the growing period, the reverse process takes place, supplying the nutrient to the young plant.

Table 1. Effect of harvesting time (June and October) on total solids, inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke[a]

No. of sample	Time of harvesting (1959)	Total solids (percent)	Inulin (percent)	Total reducing sugar (percent)	Fructose (percent)
1	June	11.23	1.22	3.59	3.10
2	June	11.09	1.49	3.42	3.28
3	June	11.02	1.17	3.51	3.02
Mean		11.11	1.29	3.51	3.13
1	October	20.00	9.43	3.19	2.83
2	October	19.71	9.65	3.05	2.60
3	October	20.77	9.20	2.92	2.67
Mean		20.16	9.43	3.05	2.70
S. e. of difference[b]		0.23	0.09	0.21	0.28

[a]The detailed analysis of variance is presented in Appendix tables 9 and 10.

[b]S. e. of differences were obtained from data presented in tables 1 and 4.

The total reducing sugar which had a greater value than that of fructose might prove the presence of other reducing sugar molecules such as glucose.

According to statistical analysis (29) presented in Appendix tables 9 and 10, a significant difference in the percent total solids, inulin, total reducing sugar, and fructose at the 1 percent level was due to the effect of harvesting time.

Experiment 2. Effect of Beta Radiation Dose on Total Solids, Inulin, Total Reducing Sugar, and Fructose Contents of Jerusalem Artichoke Tubers Harvested in June 1959

Tubers of Jerusalem artichoke harvested in June were irradiated to estimated doses of 8 and 20 x 10^6 rads with Mark IV accelerator at Stanford University, Palo Alto, California, to study the effect of beta radiation. Observation following the radiation process showed that the epidermal layer of the tubers turned from a light tan color to yellow-white color. The color became more intensified with the 20 x 10^6 rads treatment, while the control tubers showed the normal light brown color which was due to the pigments in the epidermal layer. This color change in the irradiated tubers probably was due to the enzymatic activity change during irradiation or due to the browning reactions. The different color intensities in the supernatant and the residue of the ethanol slurry of the control and the irradiated samples are shown in Figure 7.

In addition to the color change, a softening in the flesh of the tubers was observed with the 8 x 10^6 rads treatment. More drastic changes resulting in the breakdown of the flesh similar to cooked tubers was observed with 20 x 10^6 rads treatment. The chemical analysis of irradiated and non-irradiated tubers harvested in June are presented in table 2. A decreased value was shown in total solids and inulin as the

Figure 7. Color of ethanol slurries made of the control and the beta irradiated Jerusalem artichoke tubers harvested in June 1959. (No. 1, treatment with 8 x 10^6 rads; No. 2, treatment with 20 x 10^6 rads; No. 3, control, unirradiated.)

Table 2. Effect of beta radiation on total solids, inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in June 1959[a]

No. of sample	Dose x 10^6 rads	Total solids (percent)	Inulin (percent)	Total reducing sugar (percent)	Fructose (percent)
1	Control	11.23	1.22	3.59	3.10
2	Control	11.09	1.49	3.42	3.28
3	Control	11.02	1.17	3.51	3.02
Mean		11.11	1.29	3.51	3.13
1	8	10.70	0.61	3.98	3.44
2	8	10.88	0.66	3.89	3.27
3	8	10.48	0.59	3.69	3.50
Mean		10.69	0.62	3.85	3.40
1	20	10.26	0.59	4.14	3.92
2	20	10.63	0.63	4.04	3.78
3	20	10.40	0.50	4.14	3.12
Mean		10.43	0.57	4.11	3.61
S. e. of difference[b]		0.10	0.05	0.12	0.15

[a]The detailed analysis of variance is presented in Appendix tables 11 and 12.

[b]S. e. of differences were obtained from data presented in tables 2 and 5.

dose increased, while the amount of total reducing sugar and fructose increased as the dose of radiation was increased. These increased values in total reducing and fructose content of the irradiated tubers was due to the conversion of inulin and other carbohydrate intermediate compounds into simple sugar during the radiation process.

The statistical analysis (29) presented in Appendix tables 11 and 12 showed a significant difference due to the radiation doses in the percent of total solids, inulin, total reducing sugar, and fructose at the 1 percent level.

Experiment 3. Effect of Gamma Radiation Dose on Inulin, Total Reducing Sugar, and Fructose Contents in Tubers of Jerusalem Artichoke Harvested in March 1957

Tubers of Jerusalem artichokes were irradiated to estimated doses of 1, 2, 4, 8, 10, 20, 40, and 60 x 10^6 rads with gamma rays from the material Testing Reactor facility near Arco, Idaho.

In a previous study on these irradiated samples in our laboratories (34), a progressive darkening showed in the epidermal layer of the Jerusalem artichoke tubers from the dose of 1 to 4 x 10^6 rads and then a successively yellowish-white color as the dose increased from 8 to 60 x 10^6 rads. A breakdown in the tubers had been observed only with 40 and 60 x 10^6 rads treatments as shown in Figure 8.

The catechol test for the oxidase enzyme had showed that the radiation had accelerated the enzymatic activity of the tubers as the dose increased from 1 to 4 x 10^6 rads and then the activity had decreased as the dose increased from 8 to 10 x 10^6 rads.

At doses of 20 to 60 x 10^6 rads the catechol test was negative, which may be the result of the enzymatic inactivations.

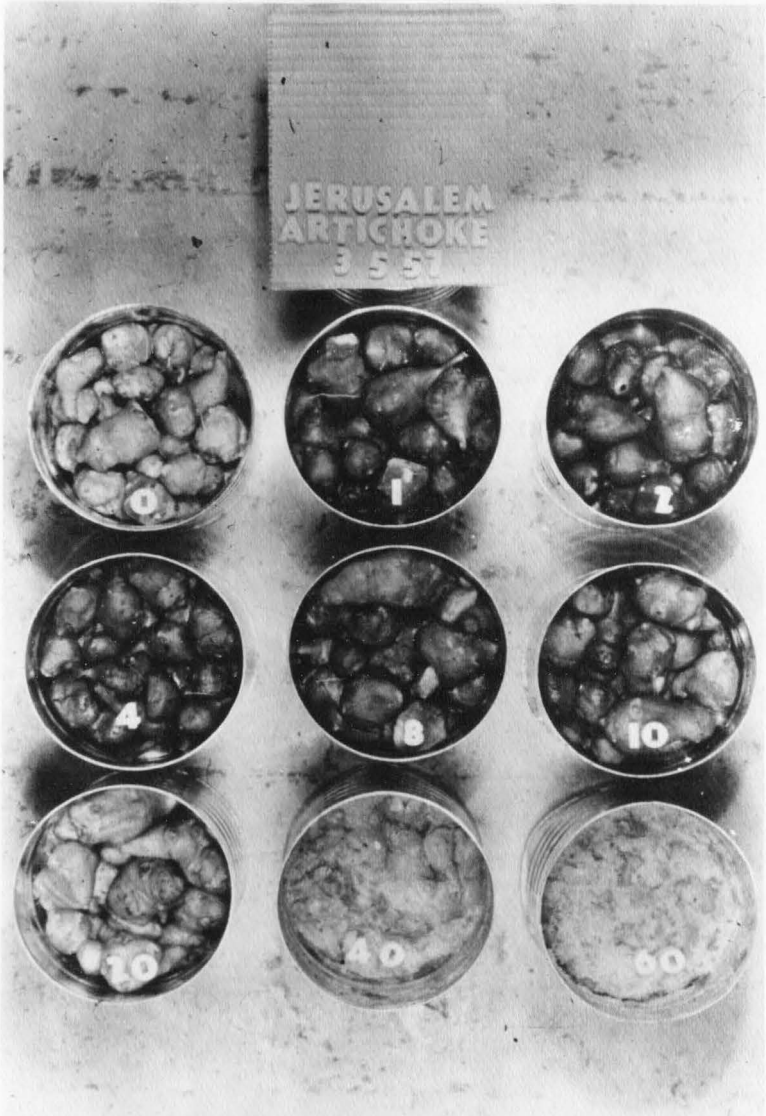


Figure 8. Effect of gamma radiation dose on the darkening of epidermis of tubers of Jerusalem artichoke harvested in March 1957 (each number represents the dose given in mega rads)

The chemical analysis on the dry weight basis which was made on the irradiated and the control tubers presented in table 3 showed that the control had an average value of 30.65 percent inulin while the radiation with 8 x 10^6 rads had dropped the amount of inulin approximately in half. The higher dosage of 20 x 10^6 rads showed more inulin conversion and only an average of 9.82 percent inulin was left as unhydrolyzable form. Correspondingly, the fructose content increased from 1.22 percent at the control level to 13.48 and 20.47 percent with 8 and 20 x 10^6 rads, respectively. The total reducing sugar had also increased as the radiation doses advanced. The statistical analysis, presented in the Appendix table 13, showed radiation doses had a significant effect on all the determined constituents at the 1 percent level.

Experiment 4. Effect of Hot HCl on Inulin, Total Reducing Sugar, and Fructose Content in Control Tubers and Beta and Gamma Irradiated Tubers of Jerusalem artichoke

In order to obtain additional fructose, samples of controls and irradiated tubers were hydrolyzed under our selected conditions of heating time, temperature and pH.

Effect of hot HCl on inulin, total reducing sugar, and fructose contents in control tubers harvested in June and in October in 1959

In table 4 are shown the percent of inulin, total reducing sugar, and fructose subsequent to acid hydrolysis. An average value of 0.68 percent of unhydrolyzable inulin remained in June harvested tubers. This means that there was approximately 47 percent conversion to simple sugars taking place during the hydrolysis process.

Acid hydrolysis had increased the percent total reducing sugar and

Table 3. Effect of gamma radiation on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in March 1957[a]

No. of sample	Dose x 10^6 rads	Inulin (percent)[b]	Total reducing sugar (percent)[b]	Fructose (percent)[b]
1	Control	30.53	1.09	1.01
2	Control	30.19	1.60	1.49
3	Control	31.23	1.19	1.17
Mean		30.65	1.29	1.22
1	8	16.20	12.76	12.23
2	8	16.80	11.68	10.55
3	8	16.22	12.72	17.66
Mean		16.41	12.39	13.48
1	20	9.99	21.24	20.51
2	20	9.65	21.65	20.97
3	20	9.82	20.64	19.92
Mean		9.82	21.18	20.47
S. e. of difference		0.22	0.28	1.25

[a]The detailed analysis of variance is presented in Appendix table 13.

[b]Determinations were calculated on the dry weight basis.

Table 4. Effect of hydrochloric acid on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in June and October[a]

No. of sample	Time of harvesting (1959)	Inulin (percent)	Total reducing sugar (percent)	Fructose (percent)
1	June	0.59	4.70	4.53
2	June	0.71	3.93	3.88
3	June	0.75	4.50	4.31
Mean		0.68	4.38	4.24
1	October	0.85	12.72	12.17
2	October	0.87	14.11	13.88
3	October	0.77	13.48	13.43
Mean		0.83	13.56	13.16
S. e. of difference[b]		0.09	0.21	0.28

[a]The detailed analysis of variance is presented in Appendix table 19.

[b]S. e. of differences were obtained from data presented in tables 1 and 4.

fructose in June harvested tubers from average values of 3.51 and 3.13 to 4.38 and 4.24, respectively. This small change in the percent of reducing sugar and fructose in June harvested tubers was because of the low amount of reserve inulin present in the tubers.

The acid hydrolysis on the October harvested tubers had increased the percent total reducing sugar and fructose from average values of 3.05 and 2.70 to 13.56 and 13.16, respectively. Thus October harvested tubers produced approximately 4 times as much total reducing sugar and fructose as that produced in June harvested tubers. Therefore, October is a wise time to harvest the Jerusalem artichoke tubers in order to get the maximum amount of fructose.

The statistical test for significant at the 1 percent level as it is given in Appendix table 10 showed a significant difference in the determined constituents due to the effect of acid.

Effect of hot HCl on inulin, total reducing sugar, and fructose contents in control and beta irradiated tubers of Jerusalem artichoke harvested in June 1959

The effect of acid on the percent of inulin, total reducing sugar, and fructose is shown by a comparison of values in tables 2 and 5. The percent of unhydrolyzable inulin remained in the control and the irradiated tubers (8 and 20 x 10^6 rads) had decreased from averaged values of 1.29, 0.62, and 0.57 percent to average values of 0.68, 0.51, and 0.40 percent, respectively. This decreased value in the percent inulin was caused by the hydrolysis brought by acid.

Calculation made on data of table 2 showed that the percent hydrolysis caused by beta radiation alone had a value of 52 and 55.8 percent as the dose advanced from 8 to 20 x 10^6 rads, while the same

Table 5. Effect of beta radiation and hydrochloric acid on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in June 1959[a]

No. of sample	Dose x 10^6 rads	Inulin (percent)	Total reducing sugar (percent)	Fructose (percent)
1	Control	0.59	4.70	4.53
2	Control	0.71	3.93	3.88
3	Control	0.75	4.50	4.31
Mean		0.68	4.38	4.24
1	8	0.56	5.26	4.99
2	8	0.39	4.67	4.44
3	8	0.57	4.87	4.73
Mean		0.51	4.93	4.72
1	20	0.44	5.93	5.83
2	20	0.41	5.95	5.91
3	20	0.36	5.97	5.93
Mean		0.40	5.95	5.89
S. e. of difference[b]		0.50	0.12	0.15

[a]The detailed analysis of variance is presented in Appendix table 12.

[b]S. e. of differences were obtained from data presented in tables 5 and 8.

type of calculation made on data presented in table 5 showed that the total percent hydrolysis has increased to 60 and 69 with the same doses. The effect of acid on the control samples caused approximately 47 percent conversions of inulin to simple sugars. This increased value in the percent hydrolysis of inulin in the irradiated samples was due to the further hydrolysis by the acid. The total reducing sugar had increased from average values of 3.51, 3.85, and 4.11 percent in the absence of acid to values of 4.38, 4.93, and 5.95 in the presence of acid as the radiation dose advanced from the control to 20 x 10^6 rads.

As can be seen from tables 2 and 5 this increased value in the total reducing sugar was due to the conversion of inulin to simple sugars during the hydrolysis process and also probably due to the incomplete separation of the soluble carbohydrates units such as fructofuranose units passing in the solution which was used for sugar analysis.

These soluble carbohydrate compounds might be hydrolyzed by the reagent added or the thermal condition applied during the fructose and total reducing sugar determination.

A similar change had occurred with the percent of fructose. It had increased from average values of 3.13, 3.40, and 3.16 in the absence of acid to values of 4.24, 4.72, and 5.89 percent in the presence of acid. The statistical analysis, presented in Appendix table 12, showed that there was a significant difference in the percent of determined constituents due to the effect of the use of acid for additional hydrolysis.

Effect of hot HCl on inulin, total reducing sugar, and fructose contents in control and gamma irradiated tubers of Jerusalem artichokes harvested in March 1957

In order to measure the effect of HCl on the percent inulin, total reducing sugar, and fructose, a comparison was made on data presented in tables 3 and 6. Gamma radiation dose of 8 x 10^6 rads had caused a 47 percent hydrolysis of the reserve inulin as calculated from table 3. The increment of 12 x 10^6 rads had increased the percent hydrolysis to 68 as calculated from the same table.

The total hydrolysis produced by gamma radiation plus HCl as calculated from tables 3 and 6 showed an increased hydrolysis of 91.4 and 92.6 percent for the same two doses, respectively. The hydrolysis produced by the HCl on the control tubers showed approximately 90 percent conversion. Thus, such a small difference in the percent hydrolysis between the treatment with the radiation plus acid and the treatment with acid only points to the fact that the expense of the radiation is not necessary.

Total reducing sugars in the control and irradiated tubers had average values of 1.29, 12.39, and 21.18 percent as the dose increased from the control to 20 x 10^6 rads as shown in table 3.

Because of the combined treatments of radiation and acid, the percent of total reducing sugars increased to average values of 53.69, 55.85, and 60.78 percent, respectively.

A similar change had occurred in the percent of fructose. In the absence of acid the average values were 1.22, 13.48, and 20.47 percent, while the present of the acid had increased to 52.94, 55.42, and 60.47 percent as the dose advanced from the control to 20 x 10^6 rads. These high values of total reducing sugar and fructose as shown in table 6

Table 6. Effect of gamma radiation and hydrochloric acid on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in March 1957[a]

No. of sample	Dose x 10^6 rads	Inulin (percent)[b]	Total reducing sugar (percent)[b]	Fructose (percent)[b]
1	Control	3.13	50.37	49.67
2	Control	3.02	57.02	56.21
Mean		3.07	53.69	52.94
1	8	2.84	51.74	51.28
2	8	2.55	59.97	59.57
Mean		2.64	55.85	55.42
1	20	2.64	60.99	60.68
2	20	1.92	60.57	60.27
Mean		2.28	60.78	60.47
S. e. of difference		0.23	3.00	3.10

[a]The detailed analysis of variance is presented in Appendix table 14.

[b]Determinations were calculated on the dry weight basis.

would not be necessarily only from the conversion of the reserve inulin to a simple sugar during the hydrolysis process, but it might be due to the presence of other carbohydrate intermediate compounds which were hydrolyzed by the acid.

The statistical analysis, presented in Appendix table 14, showed that there is no significant difference in the percent inulin, total reducing sugar, and fructose of the irradiated and non-irradiated tubers subsequent to acid hydrolysis.

The treatment with hydrochloric acid to the tubers of Jerusalem artichoke indicates that the chloride ion must be left in the solution, or neutralization with soda would form sodium chloride. If the goal sought is a palatable syrup, the amount of sodium chloride which would form after our hydrolysis conditions would not be objectionable in the finished syrup. However, if the product sought is the crystalline sugar, this sodium chloride and the natural impurities will not interfere since they remain in the mother liquor after the separation of fructose as calcium fructosate. It, therefore, could be concluded that the selected conditions of the acid hydrolysis used in our experiment can be applied for the production of palatable syrup or crystalline fructose.

GENERAL DISCUSSION

Chemical and Biological Effects of Ionizing Radiations

The chemical and biological effects of any ionizing radiation are believed to depend upon: (a) the energy absorbed per unit mass of the materials and (b) the spatial distribution of the ions produced by the radiation. The latter depends upon the energy and the type of radiation used, but for a particular kind of radiation, the biological effects should be closely correlated with the energy absorbed by the material at the place of interest. A result of the absorption of beta rays or gamma rays by matter is the ionization and excitation of the atoms of the absorber. In the ionization process, electrons are ejected from the absorber atoms leaving positive ions. Each free electron is eventually either captured by another atom or molecule to produce a negative ion or is recaptured by the same or another positive ion. The fate of the positive ions and the free electrons depends upon their spatial distribution and upon both the physical state and the chemical nature of the absorber. These primary ionizations, caused by the absorption of the energy, initiate further processes leading to chemical changes in the irradiated materials.

Gamma rays interact with matter mainly by three processes. The first process is called the photo-electric effect in which the gamma ray knocks out an orbital electron. The energy distribution is such that the following equation holds:

$$h\frac{c}{\lambda} = W + \frac{1}{2}mv^2$$

where h = planck's constant

c = velocity of light

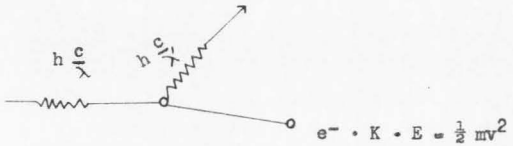
λ = wave length of the gamma ray

W = work function of the material, i. e., work required to remove electron from stable atomic or molecular orbit

$\frac{1}{2} mv^2$ = kinetic energy of ejected electron.

This electron then acts as a free electron with very high energy.

The second process is called Compton scattering. In this process the incoming gamma ray is scattered off an electron as indicated in the diagram below.



This electron again acts as a free electron of high energy.

In the third process, if the incoming gamma ray has energy greater than 1.05 Mev., pair production can occur, that is, the gamma ray is annihilated and an electron and positron pair are produced. Any energy over 1.05 Mev. goes into the kinetic energy of the electron and positron pair. Therefore, it should be expected that similar results can be obtained by direct irradiation with electrons (beta rays) or gamma rays. This agrees with our experiments and the experiments of others which showed a similar chemical change in beta and gamma irradiated materials (5).

Since tissues contain a large proportion of water, the chemical effects in pure water and in the water containing simple solutes is a logical starting point in the study of the effect of radiation on biological materials. These effects have been widely studied, but the nature of the reaction which occurs is still not fully understood. Chemical changes during the radiation process could be explained by the

following theories. These theories are tentative and may require modification in the future. Young (42) has outlined the present status as follows.

> The main experimental observations which any theory must explain are as follows:
>
> 1. In water vapor, the ions produced by radiation are H_2O^+ (the most abundant), HO^+, H^+, and HO_3^+ with very small quantities of O^+ and H_2^+ as identified by using a mass spectrometer.
>
> 2. In pure air-free liquid water which is exposed to beta radiation, hydrogen peroxide (and sometimes some oxygen) is produced together with an equivalent amount of hydrogen. On the other hand, gamma radiation produces very little if any of these substances.
>
> 3. In aerated or impure water, exposure to gamma radiation, as well as beta radiation, results in the production of hydrogen peroxide.
>
> 4. If solutes are dissolved in air-free water which is exposed to radiation, the solutes may be either oxidized or reduced. Powerful oxidizing agents are reduced but oxidative reactions preponderate. (Only systems having a redux potential greater than about 0.9 V. are reduced).

Most of the above cases can be explained by postulating the formation of hydrogen atoms and hydroxyl radicals in irradiated water. There are two principal theories of the mechanism by which the free radicals are produced. The theory which is more accepted is that the hydroxyl radicals are produced by the decomposition on hydration of the positive ions, $H_2O^+ \longrightarrow H^+ + OH$. Then a free electron combines with a neutral molecule of water to form a negative ion which also dissociates on hydration.

$$e + H_2O \longrightarrow H_2O^-$$

$$H_2O^- \longrightarrow OH^- + H.$$

(Some free electrons may also react with the products formed in "1." above: $e + H^+ \longrightarrow H.$ and $e + OH. \longrightarrow OH^-$.) The above theory assumes that the free electron escapes from the field of the positive ion.

The other theory which was suggested by some radiation chemists is that the electron recaptured by the positive ion to form a strongly excited water molecule which dissociates according to the equation below:

$$e + H_2O^+ \longrightarrow H_2O \text{ (strongly excited)} \longrightarrow H\cdot + OH.$$

Some free radicals may also be produced by the dissociation of molecules excited directly by the radiation but the number formed in this way is uncertain. In the tracks of densely ionizing radiation such as beta rays, similar radicals will be able to recombine producing both hydrogen peroxide and hydrogen.

$$2\ OH. \longrightarrow H_2O_2$$

$$2\ H. \longrightarrow H_2$$

Whereas in the tracks of high-energy electrons and gamma radiation, the separation of successive ion pairs is too great for similar radicals to have a finite probability of meeting and the only recombination process likely to occur will be between dissimilar radicals. $H. + OH. \longrightarrow H_2O$. In the presence of dissolved oxygen the formation of hydroxyl radicals is unaltered but the free electrons or the hydrogen atoms are believed to react with the dissolved oxygen to form an HO_2 radical.

$$H + O_2 \longrightarrow HO_2\cdot$$

$$\text{or} \quad e + O_2 \longrightarrow O_2^-$$

$$\text{followed by} \quad O_2^- + H_2O \longrightarrow HO_2\cdot + OH^-$$

The formation of hydrogen peroxide by gamma rays in water containing oxygen is probably due to the secondary reactions of the HO_2 radical, e. g., $2HO_2\cdot \longrightarrow H_2O_2 + O_2$ but the exact processes are not clear. As the concentration of the primary products of any of these reactions increases, the products will be liable to interact with newly formed

radicals, for example in air-free water exposed to beta radiation the following processes are all possible.

1. $OH. + H_2 \longrightarrow H_2O + H.$
2. $OH. + H_2O_2 \longrightarrow HO_2. + H_2O$
3. $H. + H_2O_2 \longrightarrow H_2O + OH.$
4. $HO_2 + H_2O_2 \longrightarrow H_2O + OH. + O_2$
5. $H. + O_2 \longrightarrow HO_2.$, giving a net back reaction $H_2 + H_2O_2 \longrightarrow 2H_2O$.

The great majority of the chemical reactions produced when water containing solutes is exposed to ionizing radiations can be attributed to reactions of the solute with either hydroxyl radicals, hydrogen atoms, HO_2 radicals (mainly in oxygenated water) or hydrogen peroxide, although details of some reactions are not yet satisfactorily explained.

From the preceding discussion it is clear that the solute under study receives energy indirectly by transfer from the molecules of the suspending medium and the action of the radiation. This action is said to be indirect, whereas, if the molecules under study are themselves ionized or excited by the radiation, the action is said to be direct. Thus, when a substance is irradiated dry, then only the direct action of the radiation is possible; but in biological material, which is irradiated in aqueous solution, both direct and indirect effects may occur. In the indirect action, the number of molecules affected by the radiation is independent of their initial concentration. Therefore, the observed effect is only due to the affected molecules by the radiation. If the effect is direct, the number of molecules altered by the radiation will increase as the number present increases. The magnitude of the effect produced by gamma radiation upon a great variety of materials varies in a characteristic manner with the oxygen tension in

the organism or solution during the irradiation. In general, the dose of radiation required to produce a given effect is two to three times as great in the absence of oxygen as under normal oxygen tension, whereas, an increase in ambient oxygen tension above the level in air has a relatively small effect. The diversity of the radiation effects which are modified similarly by the presence of oxygen, although there are some exceptions, provides very strong evidence for the view that in the cases which respond in this way the radiation effect is indirect and the effect of oxygen is due to the modification it causes in the nature of the free radicals which are produced.

Young (42) further remarked that

> In synthetic polymers which are of comparable molecular weight, it has been established that the major direct effects are either cross-linking or main-chain degradation leading to a decrease in molecular weight. It has also been shown that the energy required to produce a single break or link varies from less than 20 eV. to more than 2000 eV. in different polymers. Both cross-linking and degradation can also be produced by indirect action, if aqueous solutions of synthetic polymers are irradiated and it is possible that similar reactions occur in biological systems.

Therefore, in our experiments the conversion of inulin to fructose can be attributed to the breakdown of the cross-linking chain (glycoside linkage) by gamma rays or beta rays, and probably due to the recombining of separated main-chains with the ions or radicals formed by irradiating the Jerusalem artichoke tubers which contain about 80 to 90 percent water. It would then be interesting to see if irradiation in the dry state of the tubers would produce a similar yield, since this would greatly reduce storage problems.

Effects of Acid with Temperature on the Carbohydrate System with Special Reference to Inulin

In general complex carbohydrates are hydrolyzed into their simple constituents--monosaccharides--by boiling with dilute (0.5 to 1.0 N) acid such as hydrochloric or sulfuric acid.

In general monosaccharides are relatively stable to hot dilute acid, although the ketoses are appreciably decomposed by prolonged action. When the concentration of an acid is increased to several normalities, the monosaccharide molecules decompose producing changes which involve dehydration and subsequent formation of furfural derivatives.

Inulin, which consists of long chains of D-fructose units, like other carbohydrates can be hydrolyzed by a hot dilute acid, resulting in a breakdown at glycosidic bonds to free D-fructose units. In general chemical reactions, both catalytic as well as non-catalytic, occur at a faster rate as the temperature increases. The rate of hydrolysis, therefore, increases with increase in temperature.

SUMMARY AND CONCLUSIONS

Experiments were conducted to study the effect of harvesting time, beta radiation, gamma radiation, and combination of radiation with hydrochloric acid and heat (pH 2.0, temperature 95° C. and heating duration of one hour) on the tubers of Jerusalem artichoke.

From studies conducted in 1959 on June and October harvested tubers of artichoke, it could be concluded that to obtain high yield of tubers per acre and also high yield of D-fructose the crop should be harvested when it matures. The results obtained from October harvested tubers showed that October was a wise time to harvest the Jerusalem artichoke tubers.

June harvested Jerusalem artichoke tubers treated with beta rays (8 and 20 x 10^6 rads) showed a decrease in total solids, a decrease in inulin, and subsequent increase in D-fructose. The amount of original reserve inulin present in these tubers was too low to be an economical source for the production of fructose after the radiation treatment.

In order to get high yield of D-fructose, tubers of high amount of reserve inulin such as October harvested tubers should be treated with beta rays.

March (1957) harvested tubers treated with gamma rays (8 and 20 x 10^6 rads) showed a similar chemical change to those treated with beta rays.

For measuring the difference in the effect of beta rays and gamma rays on the artichoke tubers, representative samples from the same lot should be used in order to eliminate the time harvesting factor.

In all cases the combination of radiation with hydrochloric acid of pH 2.0 at 95° C. for one hour showed higher percent hydrolysis of inulin to D-fructose than the hydrolysis brought by radiation alone.

LITERATURE CITED

(1) Andersen, A. A., and Greaves, J. E. d-Lactic acid fermentation of Jerusalem artichoke. Ind. Eng. Chem. 34:1522-1526. 1942.

(2) Arsem, W. C. U. S. Patent No. 1,616,165. Fructose from inulin; Patent No. 1,616,172. Fructose; Patent No. 1,616,170. Fructose; and Patent No. 1,616,166. Levulose. 1927.

(3) _____ U. S. Patent No. 1,763,080. Levulose from inulin. 1930.

(4) Bacon, J. S., and Edelman, J. The carbohydrates of the Jerusalem artichoke and other compositae. Biochem. Jour. 48:114-125. 1951.

(5) Bacq, Z. M., and Alexander, P. Fundamentals of Radiobiology. Academic Press Inc., Publishers. New York, N. Y. 1955.

(6) Biestor, A., Wood, M. W., and Wahlim, C. S. Carbohydrate studies. 1. The relative sweetness of pure sugars. Amer. Jour. Physio. 73:387-396. 1925.

(7) Cotton, E. S., and Siu, R. G. H. Detection and dosimetric measurement of ionizing radiation, Chapter 8, Radiation Preservation of Food. Washington, D. C. Government Printing Office. 1957.

(8) Daniel, A. Fructose. Chem. Ztg. 45:4-5. 1921. Cited in reference (27), Original article not examined.

(9) Dean, A. L. On inulin. Amer. Chem. Jour. 32:69-84. 1904.

(10) Dykins, F. A., and Englis, D. T. Production of palatable artichoke syrup. Ind. Eng. Chem. 25:1165-1168. 1933.

(11) Eichinger, J. W., McGlumphy, H., Buchanan, J. H., and Hixon, R. M. Commercial production of levulose. II. Conversion of Jerusalem artichoke juice. Ind. Eng. Chem. 24:41. 1932.

(12) Golovin, P. V., Bryukhanova, N. A., and Fridman, A. I. Extraction of crystallized levulose. Jour. Sugar Ind. (U.S.S.R.) 3:140-142. 1929.

(13) Haber, E. S., Gaessher, W. G., and Hixon, R. M. Levulose from chicory, dahlias, and artichoke. Iowa State College Journal of Science 16:291-297. 1942.

(14) Hammond, L. D. Revised Hammond table for calculation dextrose, levulose, lactose, invert sugar alone, and invert sugar in presence of sucrose. Official Methods of Analysis of the Association of Official Agricultural Chemists (8th ed.), Table 42-12, 896-900. 1955.

(15) Harding, T. S. The sources of the rare sugars. VIII. History of levulose its discovery and methods of preparation. Sugar 25:406-408. 1923.

(16) Hardy, V. R. Purification and acidification of polysaccharide solution by electricity. Illinois State Academic Science 26:80-81. 1934.

(17) Hessler, L. E. Sensitive colormetric method for determination of fructose. Anal. Chem 31:1234-1236. 1959.

(18) Heubaum, U. The use of electricity in the manufacture of sugar syrups. Facts About Sugar 28:284-287. 1933.

(19) **Jackson**, R. F., Silsbee, C. G., and Profitte, M. J. A method for the manufacture of levulose. Ind. Eng. Chem. 16:1250-1251. 1924.

(20) _____, _____, and _____. The preparation of levulose. U. S. Bur. Stand. Sci. 519:587-617. 1926.

(21) Joslin, E. P. Diabetic metabolism with high and low diets. Carnegie Inst., Pub. 323:211-279. 1923. Cited in reference (27). Original article not examined.

(22) Kinsman, S. Radiological Health Handbook. U. S. Department of Health, Education, and Welfare. P. B. 121784:53. 1957.

(23) Kleiderer, E. C., and Englis, D. T. Hydrolysis of inulin under pressure. Ind. Eng. Chem. 23:332-334. 1931.

(24) Levulose Co. of England. British Patent 353,670. 1905.

(25) McGlumphy, J. H. Problems related to the commercial production of levulose. Ph. D. thesis. Iowa State College. Ames, Iowa. 1930.

(26) McGlumphy, J. H., Eichinger, J. W., Hixon, R. M., and Buchanan, J. H. Commercial production of levulose. Ind. Eng. Chem. 23: 1202-1204. 1931.

(27) _____, _____, _____, and _____. Levulose the sugar of the future. Jour. Chem. Educ. 10:453-462. 1933.

(28) Munson, L. S., and Walker, P. H. Modified method. U.S.D.A. Agric. Res. Admin. Bur. of Agric. and Ind. Chem. Bull. AIC - 333:8-11. 1951.

(29) Ostle, B. Statistics in Research. The Iowa State College Press, Ames, Iowa (2nd ed.) 1956.

(30) Pigman, W. The Carbohydrates--Chemistry, Biochemistry, Physiology. Academic Press, Inc., Publishers. New York, N. Y. pp. 683. 1957.

(31) Preedy, J. R. A method for the estimation of inulin in plasma and urine containing dextran. Jour. Biol. Chem. 210:651-655. 1954.

(32) Root, H. F., and Baker, M. L. Inulin and artichoke in the treatment of diabetes. Arch. Inter. Med. 36:126-145. 1925.

(33) Salunkhe, D. K. Histological and histochemical changes in gamma-irradiated lima beans, Phaseolus lunatus. L. Nature (England) 179:585-586. 1957.

(34) _____. Physiological and biochemical effects of gamma radiation on tubers of Jerusalem artichoke. The Botanical Gazette 120(3):180-183. 1959.

(35) Salunkhe, D. K., Pollard, L. H., Gerber, R. K., Wilcox, E. B., and Simon, M. Packaging effects on the flavor and shelf life of gamma-irradiated fresh fruits and vegetables. Package Engineering 4:40-46. 1959.

(36) Schering, British patent 272,876. 1926.

(37) Shoemaker, D. N. The Jerusalem artichoke as a crop plant. U.S.D.A. Tech. Bull. 33:1-23. 1927.

(38) Thaysen, A. E., Bakes, W. E., and Green, B. M. On the nature of the carbohydrates found in the Jerusalem artichoke. Biochem. Jour. 23:444-455. 1929.

(39) Thies, H., Souei, S. W., and Kallinich, G. The course of acid hydrolysis of inulin (Univ. Munich, Germany). Z. Lebensen. Unlersueh U. Forsch. 96:41-46. 1953. Cited in Chem. Abs. 46:7775 h. Original article not examined.

(40) Willaman, J. J. The preparation of inulin with special reference to artichoke tubers as a source. Jour. Biol. Chem. 51:275-283. 1922.

(41) Yamasaki, J. Manufacture of levulose from Jerusalem artichoke. Chem. Soc. of Japan Bull. of 1954.

(42) Young, M. E. J. Radiological physics. H. K. Lewis and Co., Ltd., London, England. 1957.

APPENDIX

Table 7. Optical density obtained from fructose standard solutions

Fructose standard	Optical density		
mg. per 2 ml.	First estimation	Second estimation	Mean
0.05	0.084	0.082	0.083
0.10	0.181	0.181	0.181
0.15	0.240	0.260	0.250
0.20	0.310	0.306	0.308
0.25	0.400	0.395	0.398

Table 8. Transmissions obtained from inulin standard solutions

Inulin standards	Percent transmission		
mg. per 100 ml.	First estimation	Second estimation	Mean
0.2	95.0	95.0	95.0
0.4	91.0	90.0	90.5
0.8	82.2	82.6	82.4
1.2	75.4	75.2	75.3
1.6	69.0	70.0	69.5

Table 9. Analysis of variance for the effect of harvesting time on total solids content in tuber of Jerusalem artichokes

Constituent analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Total solids	Dates	1	122.7632	122.7632	787.954**
	Within dates	4	0.6231	0.1558	
	Total	5	123.3863		

**Highly significant at 1 percent level.

Table 10. Analysis of variance for the effect of harvesting time and hydrochloric acid on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke

Constituent analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Inulin	Dates	1	51.4188	51.4188	2285.28**
	Acid	1	63.5721	63.5721	2825.43**
	DXA	1	47.8401	47.8401	2126.23**
	Error	8	0.1800	0.0225	
	Total	11	163.0110		
Total reducing sugar	Dates	1	55.5561	55.5561	409.71**
	Acid	1	94.9781	94.9781	700.43**
	DXA	1	67.8776	67.8776	500.57**
	Error	8	1.0848	0.1356	
	Total	11	219.4966		
Fructose	Dates	1	54.0177	54.0177	233.14**
	Acid	1	100.3409	100.3409	433.06**
	DXA	1	65.6135	65.6135	283.18**
	Error	8	1.8533	0.2317	
	Total	11	221.8254		

**Highly significant at 1 percent level.

Table 11. Analysis of variance of the effect of beta radiation on total solids content in tubers of Jerusalem artichoke harvested in June 1959

Constituent analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Total solids	Dose	2	0.7149	0.3575	12.413**
	Error	6	0.1729	0.0288	
	Total	8	0.8878		

**Highly significant at 1 percent level.

Table 12. Analysis of variance of the effect of beta radiation and beta radiation plus hydrochloric acid on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in June 1959

Constituent analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Inulin	Dose	2	0.8725	0.4363	48.478**
	Acid	1	0.3990	0.3990	47.333**
	DXA	2	0.2218	0.1109	12.322**
	Error	12	0.1083	0.0090	
	Total	17	1.6016		
Total reducing sugar	Dose	2	3.5761	1.7881	37.964**
	Acid	1	7.1946	7.1946	152.752**
	DXA	2	0.7872	0.3936	8.357**
	Error	12	0.5653	0.0471	
	Total	17	12.1232		
Fructose	Dose	2	3.4785	1.7393	25.921**
	Acid	1	11.0763	11.0763	165.072**
	DXA	2	1.1816	0.5908	8.805**
	Error	12	0.8046	0.0671	
	Total	17	16.5410		

**Highly significant at 1 percent level.

Table 13. Analysis of variance for the effect of gamma radiation on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in March 1957

Constituent analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Inulin	Treatment	2	680.1456	340.0728	2393.2**
	Error	6	0.8525	0.1421	
	Total	8	680.9981		
Total reducing sugar	Treatment	2	595.6731	297.8366	1265.77**
	Error	6	1.4120	0.2353	
	Total	8	597.0851		
Fructose	Treatment	2	569.3453	284.6727	60.368**
	Error	6	28.2933	4.7156	
	Total	8	597.6386		

**Highly significant at 1 percent level.

Table 14. Analysis of variance for the effect of gamma radiation and hydrochloric acid on inulin, total reducing sugar, and fructose contents in tubers of Jerusalem artichoke harvested in March 1957

Constituents analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F value
Inulin	Treatment	2	0.6324	0.3162	3.088 (N.S.)[a]
	Error	3	0.3073	0.1024	
	Total	5	0.9397		
Total reducing sugar	Treatment	2	52.7457	26.3729	1.411 (N.S.)
	Error	3	56.0659	18.6886	
	Total	5	108.8116		
Fructose	Treatment	2	58.9693	26.4847	1.398 (N.S.)
	Error	3	55.8319	18.9440	
	Total	5	114.8012		

[a]N.S. = Not significant at 1 percent level.