# A STATISTICAL STUDY OF THE EFFECT OF MATURITY, STORAGE, AND PROCESSING ON THE ASCORBIC ACID CONTENT OF CABBAGE

By

Elizabeth Bernice Smith

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# INTRODUCTION

The results of a dietary survey (11) in four cities of Canada published in 1941 revealed that the level of the Canadian dietary is not satisfactory. Particularly in the low-income families there is a marked shortage of protective foods. In regard to ascorbic acid intake, the percentage receiving less than 70% of the Canadian Dietary Standard ranged from 6% in Toronto to 68% in Edmonton with a dangerously narrow safety margin for Halifax. A similar survey (11) in Winnipeg in 1942 showed that of all the nutritive elements, vitamin C was the most markedly deficient.

To what extent this serious deficiency of a vitamin intake is reflected in individual health and working capacity is not easily defined even in the light of present day knowledge. The gross symptoms of scurvy occur only with almost total deprivation of the vitamin over a long period of time. It must be appreciated, however, that there is a definite advantage in using larger amounts of the anticorbutic factor than are needed for mere prevention of frank scurvy. Plimmer (72) wrote

that in people on scorbutic diets there is a period of poor health before definite symptoms of scurvy appear, and that the same sort of poor health may also occur (and sometimes become chronic) in those who habitually take suboptimal amounts of vitamin C even though they get enough to prevent acute scurvy. Among the symptoms common in such cases are loss of energy and fleeting pains in the joints and limbs, often mistaken for rheumatism.

Hess (72) emphasized the fact that children, without showing any distinct scurvy symptoms, are frequently irritable, lacking in stamina, and more or less retarded in growth; and can be restored to better growth, higher stamina and better general health by the feeding of orange juice.

Recently, the development by Kruse (34) of more delicate diagnostic methods has made it possible to obtain objective criteria of such borderline states as have been recorded by Hess and by Plimmer.

A daily intake of 25 milligrams of vitamin C by normal adults (other than pregnant and lactating women who need decidedly more) or 1.0 milligrams per 100 calories of food in family dietaries might be regarded as a minimum, sufficient

for prevention of the gross signs of scurvy, according to Sherman (72).

In view of the facts now known, it would seem that a satisfactory allowance should provide a wide margin above the bare minimum as a safeguard for the body against injuries from infections and physical strains and for its general well-being.

The National Research Council (American) (57) has recommended the following amounts of vitamin C as being sufficient to protect the body:

Recommended Dietary Ascorbic Acid Intake

| | mgm (daily) |
|---|---|
| Men | 75 |
| Women | 70 |
| " (pregnancy-latter half) | 100 |
| " (lactation) | 150 |
| Children 10-12 yrs. | 75 |

The Canadian Council on Nutrition (10) in formulating the Canadian Dietary Standard in 1939 and revising it in 1942 and 1945 consider that for the purpose of evaluating group dietary studies 50 mgm for adults and 30 mgm for children is a satisfactory intake.

In considering what foods or class of foods

to emphasize as good sources of vitamin C with a view to relieving the condition of subvitaminosis C which has been shown to exist in Canada, thought naturally turns to vegetables which can be grown locally in all parts of the country; and preferably to types which are not necessarily seasonal, but are capable of storage for long periods.

Ever since Lind's recommendation (50) that all ships carry lemons for the health of the seamen, the use of citrus fruits, and more recently tomatoes, have come to be regarded in the popular mind as the only antiscorbutics. So highly overemphasized have they become, that other very good sources of vitamin C have been neglected.

For the low income group citrus fruits are often prohibitive in cost and, indeed, tomatoes also, when out of season, are in the same price category. In addition Canada must import her citrus fruits, and a constant, high level of supply is not necessarily guaranteed. For remote or isolated areas, both citrus fruits and tomatoes are an almost unobtainable luxury.

The vegetable which seems to meet completely fulfill the requirements of ease of production in all parts of the country, good storage properties, low

cost and high ascorbic acid potency in the cabbage (Brassica oleraceae). To express the ascorbic acid content of the Danish Ballhead cabbage, for instance, comparatively with that of one medium (5½ oz.) orange, this being the daily amount of orange juice as recommended by the Canadian Council on Nutrition, results already published suggest that 3 1/2 oz. of fresh, or 4 1/3 oz. of the stored cabbage (raw) is an equivalent substitute.

The need for a readily available source of vitamin C in the Canadian diet has been shown to exist; a completely satisfactory fulfillment is found in the cabbage. However, this statement may hold true for typical varieties of raw cabbage only. In practice, most vegetables, including the cabbage, are consumed in the cooked state, as a result of which there is bound to be a greater or less depreciation of vitamin value.

To determine just what proportion of the total dietary intake of ascorbic acid was supplied by cooked vegetables, a group of nine British laboratory workers under the direction of Olliver (59) kept a record of their daily intake of

vitamin C for one week of the year 1941. Cooked vegetables were found to supply about 70% of the total vitamin C. The mean daily intake was about 12 mgm per day, and it was only when cooked greens were included that the probable requirement of 30 mgm per day according to British standards was reached.

As served in the Canadian dietary also, cabbage frequently appears in the cooked state. Therefore, in a study of the adequacy of cabbage as a reliable source of ascorbic acid, it is important to know what percentage of loss can be expected to occur in cooking by the methods most commonly used, and also to ascertain what cooking practices are most favorable to optimum retention of the vitamin.

The problem involved in this investigation was to determine the average ascorbic acid content, together with the variance which might be expected to occur, under typical conditions of cultivation, harvest and storage, in varieties of cabbage commonly grown in Canada, with a view to demonstrating the extent to which this vegetable might be relied upon as a major dietary source of vitamin C. The greater part of the data obtained were subjected to statistical analysis, in order that it could be determined to what extent the values obtained were representative of the whole population of cabbages.

## REVIEW OF LITERATURE

It was the necessity to seek the cause of, or at least a cure for, the dreaded scourge of scurvy, that led to the discovery of antiscorbutic substances and began the unfolding of our present knowledge of the substance known as ascorbic acid, and its role in human nutrition.

Hippocrates and Pliny both wrote of scurvy and Straby described cases of the disease in the armies of Caesar Germanicus and Aelius Gallus. In the 13th century the French crusaders are reported to have suffered greatly from it. In Elizabeth's reign the disease was rampant, not only on chartered ships such as those sent to India, but also on the many pirate vessels and treasure ships. "The Plague of the Sea and Spoyle of Mariners" was estimated by Sir Richard Hawkins to have killed 10,000 men during his twenty years of life at sea.

It is difficult to ascertain just when or by whom various cures for scurvy were found. Jacques Cartier's men on their voyage to the Bay of Gaspé in 1535 were extremely fortunate in being cured of scurvy, but of the substance which actually effected the cure very little was, or is, known.

Sherman (71) refers to the records of an early mariner of Elizabethan times, Captain Lancaster, who, by practising prophylaxis, was the only commander of four able to bring his ship successfully to anchor in the East Indies. The crews of the other three consorts were too weakened by scurvy to make the effort. His secret of scurvy prevention was a supply of lemon juice which he had taken along. Each sailor was given three spoonfuls every morning.

By 1734 it was generally recognized that scurvy was a disease due to dietetic errors, occurring after deprivation of fresh foodstuffs for long periods. In the book compiled by the Medical Research Council (51) through the translation given by Lind, we find this statement: "Where persons do refrain from eating the fresh fruits of the earth, and greens, for a considerable time, no age, no climate or soil are exempted from its attack".

James Lind, a Scottish naval surgeon, might well be called the first naval hygienist, and indeed his contribution toward the control of scurvy alone is outstanding enough to place his name high in the annals of medical fame. In Karl Vogel's paper, "The Plague of the Sea and Spoyle of Mariners" (85)

frequent reference is made to James Lind's classical "Treatise of the Scurvy". As a result of many observations Lind convinced himself that oranges and lemons are the best antiscorbutics, the former slightly preferable.

He based his conclusions on the results of a practical experiment made on "H.M.S. Salisbury" in 1774. This experiment is of especial interest because it was the first rather fumbling attempt to study nutrition by means of biological assay, and also because it marks the beginning of the era which was to realize the virtual eradication of scurvy.

Lind tried six different treatments then in vogue on twelve scurvy patients. The "cures" with their respective dosages were: a quart of cider daily; 25 drops of elixir of vitriol three times per day; half a pint of sea water daily; two oranges and one lemon daily; and, lastly, a compound of garlic, mustard seed, radish, balsam of Peru and gum myrrh. In six days the lucky ones who received the oranges were well enough so that they could return to duty, while the others, except those who had received the cider, showed little or no improvement.

Lind's recommendation that thereafter

the syrup of lemon juice be carried on all ships for the safeguarding of the health of the sailors eventually aroused the apathetic Admiralty to action, but not for several years, and not before very insistent representations had been made by Sir Gilbert Blane, also a naval surgeon, who had published several treatises on the health of and sanitation for seamen. In 1804 the British Navy prescribed the administration of lemon juice, which was popularly called lime juice.

From the beginning of the 19th century the situation in regard to scurvy improved vastly both in Europe and America. The practice of growing vegetables became more widespread, and, later, methods of storing them for winter use were undertaken. It is interesting to note that the early expeditions to America were not only the cause of hundreds of cases of the disease but also led to a greater degree of control since these same mariners introduced potatoes into Northern Europe.

Any outbreaks which occurred as recently as the latter part of the nineteenth century were regarded as enigmatical in medical circles and became the subject of inquiry with a view to explaining the

cause. The crews of both of Scott's Antarctic expeditions and of the Nares expedition in 1875 suffered from scurvy. A commission of inquiry held by the Admiralty on the return of the latter expedition failed to elucidate the cause. It was known that the crew was provided with every recognized means to protect the health of its members, including the regular issue of lime juice. Yet a sledge party consisting of fifty-three officers and men in eleven weeks had three-quarters of its members badly crippled by the disease, one man dying and the others remaining in serious danger for weeks.

An explanation was not forthcoming until fifty years later when the British Privy Council published a report. This publication points out that originally it was not lime juice at all, but Mediterranean lemon juice that was being used. Because of the greater acidity of lime juice, it was popularly supposed to have more antiscorbutic power, and was gradually substituted for lemon juice so that by 1870 but little of the latter was used officially. It was not appreciated that lemon juice retains its potency on preservation, while lime juice becomes almost totally deficient in antiscorbutic power.

Mary Schwartz Rose (69) in her book, "Foundations of Nutrition" throws further light on the appearance of scurvy in this instance. The men no doubt had subclinical scurvy, before starting out in the spring on the sledge party. The manual labor entailed hastened the onset of the disease, the first case appearing within ten days of departure. In due course scurvy also broke out among the crews left behind on the ships during the spring and summer of 1876 but its onset was distinctly later. The findings of many later workers now confirm the belief that the human requirement of ascorbic acid is in relation to energy expenditure.

It is known that scurvy was endemic among the prospectors in the Alaska Gold Rush of 1898. War continued to take its toll. It was reported that 76,000 cases of scurvy occurred in the Russian Army during one year, 1916, of the First World War. German troops had twenty-three epidemics on the Eastern Front and one on the Western.

World War II did not experience a sufficiently large number of cases of scurvy to even approach epidemic proportions; and probably no cases occurred among the armed forces in any country. We do have evidence, however, of isolated clinical and

hospitalised cases among the civilian population in the very low income group. Macmillan and Inglis (49) have cited experimental work done on fifty-three cases of scurvy in an Edinburgh Hospital in 1943.

The incidence of these last mentioned and recent cases indicates clearly that even florid scurvy, and much less subclinical scurvy, have not yet been eradicated. This fact remains in spite of the progress in the study of scurvy in the medical field and the increasing awareness on the part of the public of the means of controlling the disease.

## THE CHEMICAL NATURE OF VITAMIN C

The first person to entertain the idea of antiscorbutic substances having a definite chemical entity was the American physician Budd (69), who predicted in 1841 that it would be identified in the "not too distant future". Ninety years later, Budd's prediction was fulfilled by C. G. King employing the methods of organic chemistry and experimental physiology in his researches at the University of Pittsburgh.

During the interval, such workers as Holst and Frolich (29) and Cohen and Mendel (12) paved the way for the identification and study of the chemical nature of the "chemical entity" by their observations of its effects on the guinea pig. All early experimenters attempting to isolate the vitamin from natural products encountered great difficulty because of its extreme sensitiveness to destruction by oxidation.

The confusion as to whether the antiscorbutic properties of foods depended on their physical character, their acidity, or on some specific chemical substance was cleared by Hess and Unger in 1918, when according to Rose (69) they showed that the white inner part of the orange peel was anti-scorbutic as well as the acid juice; and also that the antiscorbutic substance could be extracted from orange juice with alcohol and could cure scurvy when given by injection directly into the blood stream.

In 1921, at the Lister Institute in London, Dr. S. S. Zilva (93) began a long series of experiments to obtain a highly active concentrate from lemon juice by removal of the acid, sugar and most of the water and salts, and succeeded in making a preparation of the vitamin which would keep for

several months if protected from air and light. He and his associates showed that antiscorbutic substances have the power to bleach phenol-indophenol.

Other researchers for information regarding the chemical identity and properties of the anti-scorbutic principle were Tillmans and Hirsch (22) working at Frankfurt, Germany. The Tillmans School disagreed with Zilva regarding the active principle of antiscorbutic substances. Tillmans advanced the view that the vitamin might be hexuronic acid. Another worker, Szent-Gyorgyi (15) at approximately the same time (1928) and working independently, had already demonstrated the reversible oxidation of hexuronic acid. Later he satisfied himself of the resemblance of vitamin C to hexuronic acid.

In 1927 Professor C. G. King (69) of the University of Pittsburgh prepared a concentrate according to Zilva's method, but was able to remove more of the contaminating material than Zilva had been able to do. By August, 1931, he was able to report (77) a concentrate of the active substance of lemon juice over 20,000 times as potent as the original juice. He and his collaborators (75) in announcing their final crystalline vitamin preparation

claimed that it reached a constant antiscorbutic activity (minimal daily dose 0.5 mgm.) and had all the chemical and physical properties of Szent-Gyorgyi's hexuronic acid. King and Waugh share the credit for the crucial work that identified vitamin C as hexuronic acid.

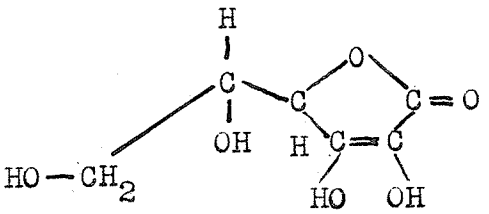
Waugh and King (37) in showing that vitamin C and hexuronic acid were probably identical set forth the steps followed in their procedure. Starting with lemon juice as the raw material, there followed decitration, solution in n-propyl alcohol, precipitation of impurities by petroleum ether, extraction with ethyl acetate, and, finally, precipitation at a low temperature by means of petroleum ether, followed by recrystallisation. The final product was claimed to have a minimal dose effect of not less than 0.8 mgm.

A paper published by Herbert, Hirst, Percival, Reynolds and Smith (25) in 1933 gave the structural formula which at that time found almost universal acceptance, viz., CHOH. CHOH. CH. C(OH):C(OH). CO. This superseded the original formula of Hirst and Zilva (26); CHOH. CHOH. CH. CO. CO. COOH, and various other formulae previously put forth. The new formula accommodated all the various observations made by

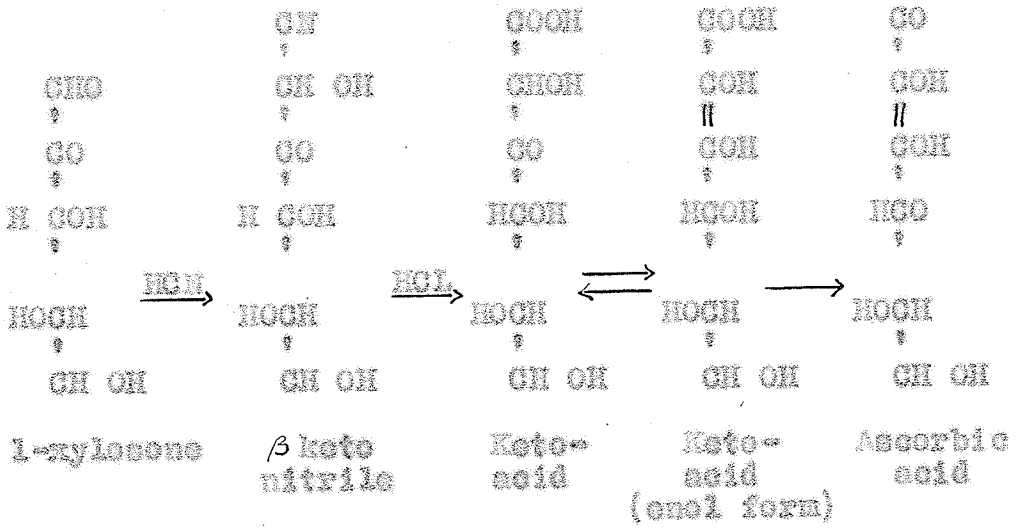
contemporary workers, and was accepted by them all. It also adequately accounted for the actual synthesis of ascorbic acid which was accomplished by Reichstein, Grussner and Oppenhauer (64).

The main insight into the structure of the molecule was gained by studying its behaviour with oxidising agents. By this means Herbert and his associates (25) showed ascorbic acid to be a derivative of l-gulose. The first oxidation product was identified as 2,3-diketo-l-gulonic acid. Hence ascorbic acid must be the corresponding reduced derivative of this lactone or 3-keto-l-gulonolactone.

The nature of the ring system was determined from a study of the methylated derivatives of the acid. This led to the conclusion that, in solution, ascorbic acid is the enolic form of 3 keto-l-gulo-furano-lactone. This formula accounts satisfactorily for the crystallographic properties, a consideration which had led to the rejection of various other alternatives. Data showed that the molecule must be extraordinarily flat, and this is borne out by a diagrammatic representation of the model.



Regarding synthesis of ascorbic acid, Reichstein and co-workers (64) described how, when osones are treated with hydrogen cyanide and then hydrolysed, products resulted which had properties similar to those of ascorbic acid. Starting with d-xylosone, the product was found to be identical with the d-form (optical antipode of natural or l-ascorbic acid). Its identity was proved by separating its acetone derivative, and determining its melting point, combustion value and iodine titre. The synthesis was represented as follows:




Modifications in King's original isolation method have been described (66) by which a yield of 100 to 150 mgm. per liter may be obtained from lemon juice. It was the discovery of the large

concentrations present in paprika (hungarian red pepper) which made it possible for Szent-Gyorgyi (76) to prepare no less than a pound of ascorbic acid for the purpose of the international co-operative study of its chemistry.

Although the oxidation-reduction behaviour of ascorbic acid in vitro was established early in its history, its nutritional chemistry is still largely unsolved. At the present time the nutritional functions of ascorbic acid which have been most clearly established (72) are (a) its relationship to the formation and maintenance of intercellular ground substance, (b) its significance in the safe-guarding of the body through the blood and (c) its role in regulating the rate of cellular respiration.

The appraisal of the extent of malfunction of any of these major roles of ascorbic acid in the animal organism is the basis of any type of animal assay. At present the most generally used methods of bioassay are (1) the Preventive Method developed by Sherman (73); (2) the Curative Method introduced by Harris (24) has the advantage of requiring a much shorter testing period than the Sherman method; the Mojer (46) Method based on the observation that

the degree of scurvy can be estimated by histological examination of the teeth of the guinea pig.

However, for food analyses, and, indeed, for clinical determinations also, the most widely used type of assay at present is the chemical one. In order to furnish absolute and final proof of actual biological effects, it is sometimes considered necessary to check by means of animal experimentation. The relative ease, simplicity and accuracy of the chemical method of assay makes it preferable for the routine analyses of most laboratories.

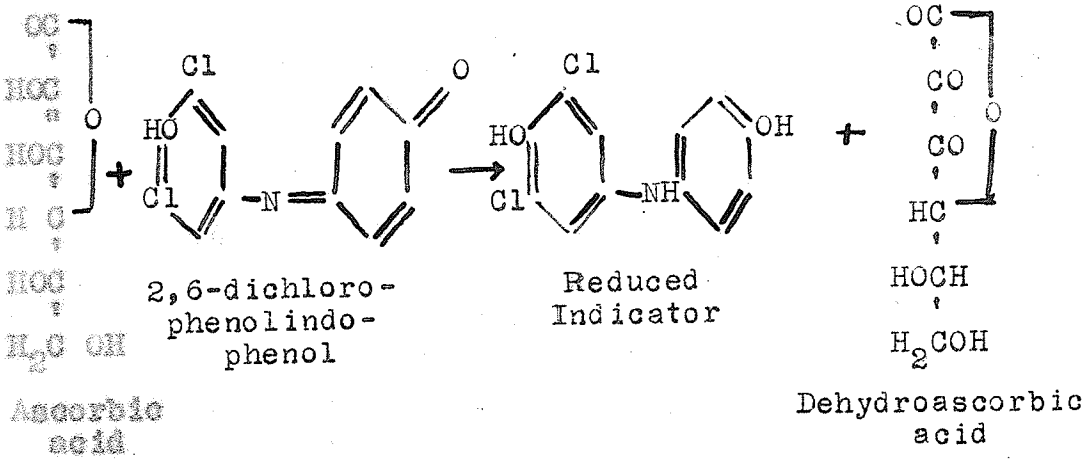
## CHEMICAL ASSAY FOR VARIOUS FORMS OF ASCORBIC ACID

The establishment of the identity of vitamin C by Waugh and King (67) stimulated experiments by Tillmans and his associates (22), Harris and Ray (23) and Bessey and King (6), which showed the close correlation between the amounts of vitamin C as determined by biological assay and by titration with 2,6-dichlorophenol-indophenol in many plant and animal tissues.

The essential features of the titration test are an extraction of the material under examination

with an acid-precipitating agent to remove certain interfering substances and to stabilise and bring into solution the vitamin C and subsequent titration of the vitamin with a standardised indophenol solution (13).

The use of 2,6-dichlorophenol-indophenol (6) to determine vitamin C is based on the fact that the colored indicator solution is quantitatively and rapidly reduced by vitamin C in acid solution to a colorless compound as represented by the equation:




There are several limitations and hazards connected with the titration procedure of the indophenol method of assay. The process of titration is not precise for the determination of small quantities of ascorbic acid; and highly colored and turbid solutions present added difficulties (13). The method which overcomes most of the objections and limitations of the

titration technic, is the photoelectric indophenol method, introduced by Mindlin and Butler (32). This eliminates the error of subjective detection of the end-point. Its precision is superior to that of other methods (15).

Alternative methods to the titration method with indophenol have been worked out, but none have achieved the same success. For removing interfering substances which may reduce the indophenol, a process involving precipitation with mercuric acetate and subsequent reduction with hydrogen sulphide has been described (17). The interfering action of glutathione in the silver nitrate test was discussed by Svirbely (70).

Mellonry and Graham (48) did further work and reported their observations on the titration method using dichlorophenol-indophenol. These authors advocated multiple extractions when grinding material in the mortar in order to obtain complete extraction of the entire liquid phase.

Bessey published a review of the literature (5) and an experimental paper (4) in which each step in the analytical procedure for the colorimetric method was carefully examined. Emphasis was placed upon the importance of buffering within the pH range 3.5 to

3.7 and upon the failure to find evidence for incomplete extraction with the acid used. Points of technique in regard to the photoelectric measurement of ascorbic acid were described by Evelyn, Malloy and Rosen (16). The Evelyn method of ascorbic acid determination using the Evelyn photometer has been adopted widely. Kirkpatrick (33) also modified the indophenol method to overcome the difficulties of highly colored and turbid extracts. Morell (53) employed a standard curve prepared with regularly increasing increments of pure ascorbic acid in calculating the amount of ascorbic acid in a sample.

Hochberg, Melnick and Oser (27) made modifications of established methods in an attempt to discover something of the nature of possible interfering substances.

The effectiveness of various acids in stabilizing ascorbic acid during extraction from plant materials was tested (65). From these studies it appeared that oxalic acid and metaphosphoric acid are equally suitable. In the same group of investigations Roe (65) reported a method for the determination of ascorbic acid based on the observation that the reduced form gives furfural on boiling with hydrochloric acid,

whereas the reversibly oxidised form does not.

The problem of ascorbic acid oxidase activity of many vegetable tissues has been studied by Kertesz, Dearborn and Nack (32) from the viewpoint of preservation of ascorbic acid values in stored foods. Musulin and King (36) found that the use of metaphosphoric acid with acetic or trichloracetic acid in the extraction and titration procedures is very valuable in inhibiting the oxidase reaction.

The catalytic effect of extremely low concentrations of copper (46 ugm per liter) on the oxidation of ascorbic acid by atmospheric oxygen as studied by Barron, Barron and Klemperer (2) may be of great importance both in the handling of foods and in chemical assays of foods.

In order to obtain accurate estimates in ascorbic acid assay, the presence of interfering substances must be considered as well as dehydroascorbic acid.

A chemical assay method developed by Pepkowitz (60) was reported to be applicable to all plant materials assayed, whether fresh, frozen or dehydrated, and to be especially useful for highly colored or turbid extracts. It is dependent on the

quantitative extraction of the unreduced 2,6-dichlorophenol-indophenol dye from an acid aqueous solution by xylene.

Lugue (40) developed a method involving titration with a rather concentrated solution of 2,6-dichlorophenol-indophenol-indophenol using a microburet.

A micro adaptation of the indophenol method has also been described by Birch, Harris and Ray (7) by which titrations may be carried out with ease with an error of less than 3 per cent on amounts of tissue equivalent to no more than 0.03 cc. of orange juice. The test is reasonably specific for vitamin C, but when dealing with unusual types of material it is still necessary to exercise judgment and check results by biological assay.

Loeffler and Ponting (39) have combined Morell's (53) technique of preparing plant extracts with a modification of the Evelyn et al (16) colorimetric method for determining ascorbic acid. By using a large amount of 1% metaphosphoric acid as the extractant, the necessity for buffering is avoided, since the pH obtained is sufficiently low to prevent losses during blending, yet sufficiently high to prevent fading of the dye reagent. Rapid

ascorbic acid determinations can be made easily on highly pigmented berries and tough dehydrated vegetables.

Karrer, Salomon and Schopp (31) found that the reduction of ascorbic acid with the loss of two hydroxyl groups led to dehydroascorbic acid, considered to be an inner peroxide.

The reversibility of the first oxidation of ascorbic acid was confirmed by Johnson (30).

A careful study of oxidation-reduction potentials has been made by Borsook, Davenport, Jeffrey and Warner (8). They recognize at least three stages in vitro corresponding to the previously recognized stages of degradation (20). The first stage A, can be studied at a pH lower than 5.0, and corresponds to the reversible transformation occurring with increasing rapidity as the pH becomes greater than 6.0. The changes correspond to the transformation from dehydroascorbic acid to diketogulonic acid (B), and to the further degradation (C) to decomposition products, as l-threonic acid and oxalic acid. Only stage (A) is normally important biologically, and the importance of keeping ascorbic reduced in the tissues is clearly shown by the extreme lability of the dehydro form

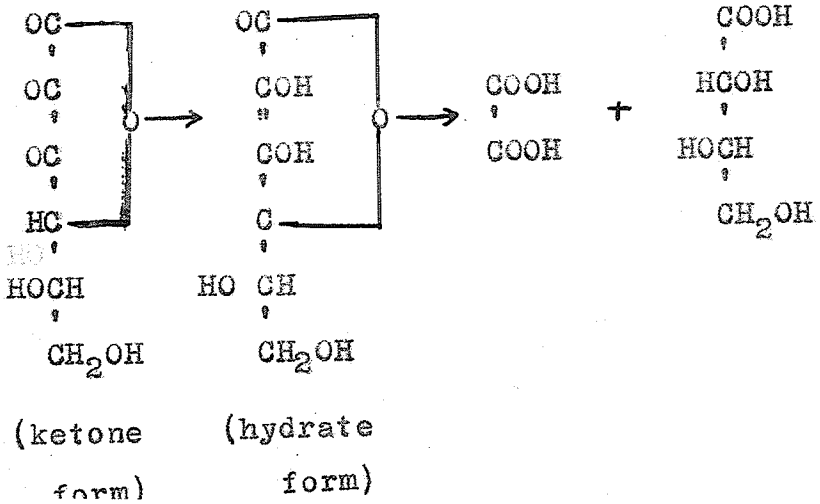
at body pH.

According to the reports of the work by Mellanby and Graham (47) on the "reversibly oxidized vitamin C" (dehydroascorbic acid) this substance is present in certain vegetables (e.g. lettuce, string beans, cabbage) in greater amounts than the reduced form, being especially evident in the cooked state. Tomatoes, lemon juice, peppers and adrenal glands, on the other hand, were said to contain all of the vitamin C in the reduced form.

That reversibly oxidized vitamin C has some antiscorbutic activity was demonstrated by Hirst and Zilva (26), and confirmed by Fox and Levy (18) and by Roe and Barnum (57). On the basis of a quantitative comparison of the effectiveness of the two forms of vitamin C in promoting growth of guinea pigs on a scorbutic diet, Roe and Barnum (66) concluded that reversibly oxidized vitamin C is about one-fourth as active as the reduced form. It has been shown that it is reduced in the body, Hirst and Zilva (26) having reported that individuals saturated with vitamin C excrete ingested dehydroascorbic acid in the reduced form.

Roe and Hall (67) developed a method for determining dehydroascorbic acid by preparing its

2,4-dinitrophenylhydrazine derivative and converting it to furfural, which could be measured colorimetrically by the aniline acetate method. Roe and Oesterling (68) have adapted the original 2,4-dinitrophenylhydrazine method for the determination of the total ascorbic acid in blood and urine to the direct determination of dehydroascorbic acid in the presence of ascorbic acid in plant tissue. The method in brief is as follows: the dehydroascorbic acid of a metaphosphoric acid extract, in which the ascorbic acid is stabilized with thiourea, is coupled with 2,4-dinitrophenylhydrazine and the resulting derivative is treated with sulphuric acid to produce a red color which is measured photometrically. The oxidative breakdown which dehydroascorbic acid is thought to undergo is shown; the change from the ketone to the hydrate form occurring first; followed by an irreversible arrangement giving rise to oxalic acid, and lastly to l-threonic acid:

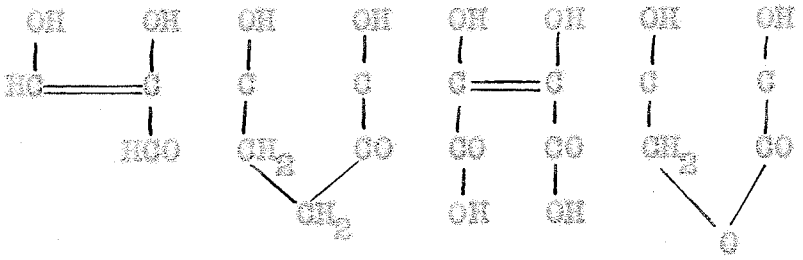


"Isovitamin C", a substance differing from vitamin C only in the steric arrangement of the groups on the fifth carbon atom has been found by Nemole (14) to possess one-twentieth to one-fifth the antiscorbutic activity of ascorbic acid. d-Ascorbic acid is inactive at forty times the dose of the active or l-form.

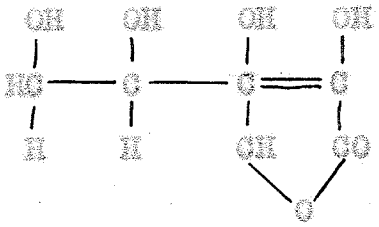
Foods contain a variety of substances resembling ascorbic acid in physical and chemical properties, which are not distinguished by the usual dye titration from true ascorbic acid. The term "apparent vitamin C" has been proposed to include all such substances, which according to Wokes, Organ, Duncan and Jacoby (91) probably contain the enediol group (reductones, reductic, dihydroxymaleic and hydroxytetronic acids). They are produced by the action of heat on pectins, glucides, etc. They are found in germinated grains, malt extract, cocoa, dehydrated fruits, vegetables and beer. The "apparent vitamin C" content of processed and dehydrated food can increase during storage and obscure the decrease in true vitamin C.

A suggested explanation of the similar chemical behaviour of "apparent vitamin C" and ascorbic acid is provided by Wokes et al (90).

Since the group of substances termed "apparent vitamin C" probably all contain the dienol group, the members undergo enol-keto tautomerism, which causes their rate of reaction with the dye, and other properties, to be affected by the pH, the range 4-6 appearing to be critical. An examination of their steric formulae shows clearly this similarity of structure:



Reductone | Reductic acid | Dihydroxymaleic acid | Hydroxytetronic acid



Ascorbic acid

The method of determination of "apparent vitamin C" most commonly used by early investigators of the substance and originated by Lugg (41) depended on the fact that it differs from true vitamin C in reaction with HCHO, only the latter

reacting rapidly at pH 3.5 and slowly at pH 1.5. Mapson (43) working chiefly with dehydrated foods slightly modified the method of Lugg, as he found a pH of 2 for the estimation of the apparent form of the vitamin more satisfactory than the less acidic range used by Lugg. Wokes et al. (90) considered that the rate of destruction of "apparent vitamin C" was least rapid at a pH of 4.5. They made the further modification of allowing several minutes to elapse after measuring the reduced acid and before measuring the apparent vitamin C, to allow for the slower rate of action of non-vitamin C reductants.

Levy (37) has developed a method based on a different principle, viz: When concentrated hydrochloric acid is added to ascorbic acid solutions, reversible oxidation becomes progressively slower as the hydrochloric concentration increases toward a point at which there is no titration of ascorbic acid. On dilution with water, indophenol is again reduced to the same extent as in non-acidified solution.

## THE ASCORBIC ACID CONTENT OF CABBAGES

As has already been mentioned, the ascorbic acid content of fruit and vegetables shows extreme variability. Since so many factors have been found to exert an influence in some degree it is difficult to study the relative importance of any particular one because of the practical impossibility of controlling all others. However, much experimental work has been directed toward the determination of ascorbic acid values for particular plants under specific conditions.

Beckurnch and Coates (1) have this to say in regard to this type of work:

> "Investigations into the differences between members of different plant families and genera, between different species of the same genus, between different varieties of the same species, between different parts of the same plant (leaves, flowers, stems, roots), between the different parts at different stages of development (cotelydons before and after sprouting) are typical of one kind of enquiry; in addition attention is being turned to the effects of different types of fertilizer and general conditions of cultivation on the nutrient make-up of the crop. Not merely is all this work to be encouraged, but it should be widely extended, particularly if the tendencies evident at the Hot Springs Conference are to be given qualitative as well as quantitative expression."

Tressler and Associates (80) conducted a comprehensive survey of the ascorbic acid content of American vegetables, with special attention given to the effect of soil condition, varietal differences, harvesting practices, maturity and size, storage conditions, freezing methods and cooking losses. For peas, snap beans, tomatoes, cabbage and spinach, they found that variety is a factor of considerable importance in determining the ascorbic acid content.

Thirty varieties and strains of cabbage, grown in the Ohio State University Gardens were studied by Burrel et al (9). The average value for the ascorbic acid content of the ten highest varieties was 1.53 mgm per gm and of the ten lowest, 0.59 mgm per gm. The extreme range was 0.48 to 1.81 mgm. This furnishes a striking demonstration of the extremely wide ranges found in different varieties of cabbages. The authors concluded that the accumulation of ascorbic acid in plants is a characteristic of species and variety and that this genetic influence may overwhelm any differences due to climate, soil or fertilization. Of climatic factors light seems to have the preponderating influence. The effect of fertilizers is relatively small and varies with the plant, they believed.

Lempitt, Baker and Parkinson (30) studied a large number of varieties of raw cabbages and chose four main classes of these varieties, arranging their mean ascorbic acid values and their ranges in the form of a table, as shown on page 35.

TABLE I

Ascorbic Acid Content of Four Varieties of Cabbage

| Variety | Range (mgm/gm) | Mean (mgm/gm) |
|---|---|---|
| Spring | 0.60-1.76 | 1.04 |
| Winter | 0.30-1.35 | 0.65 |
| Savoy | 0.32-0.99 | 0.62 |
| Red Pickling | ----- | 0.60 |

Truscott, Johnstone, Drake, Van Haarlem and Thomson (81) made a survey of the ascorbic acid content of a large number of fruits and vegetables and native plants of Ontario. In regard to genetic factors their data indicated that on a broad basis plants possess an inherent capacity to produce low, medium or high amounts of ascorbic acid in their edible parts. However, the results of their data also showed that usually the ascorbic acid content of most of the varieties of any one kind of fruit or vegetable are so much influenced by environment that differences controlled by heredity are masked.

Further confirmation of the relative influence of heredity was made by Poole, Grimball and Kanapaux (61) who stated that regression data showed that hereditary differences are more important than seasonal or positional differences in producing ascorbic acid differences between breeding lines.

Varieties of summer cabbage are much higher in vitamin C than winter varieties according to Gould, Fressler and King (20).

Maynard and Beeson (45) in studying the

effect of fertilisers stated that many investigators, as Todhunter and Sperling, for example, have reported that they found little or no effect of fertilization in the ascorbic acid content of a number of vegetables, particularly cabbages, spinach, carrots and potatoes. Some investigators found a difference in this respect, however, according to species. The authors report that Pfaff and Pfutzer found that fertilization with nitrogen and magnesium in plot or field tests resulted in increases in the ascorbic acid content of grass, spinach and cabbages but not of kohlrabi, tomatoes, potatoes or sugar beet.

Observations on the ascorbic acid content of cabbages and potatoes grown in several localities have been made by Mack, Tapley and King (42) but only in some cases were differences found associated with locality.

In regard to the influences of locality and season, Murphy (55) concluded that, "Environmental agencies markedly influenced the synthesis of vitamin C in tomatoes and cabbages, and geographical situation was not a contributing factor except insofar as environmental conditions were consistently

characteristic of that situation." An analysis of available weather data provided a certain basis for the assumption that sunlight, rainfall and probably temperature may all be causal agents in the variations in ascorbic acid.

Truscott and co-workers (81) in discussing environmental factors other than light and their effect on the ascorbic acid content of a fruit or vegetable, state that the data indicated that probably the meteorological environment has more effect than have ordinary variations in the chemical or physical condition of the soil.

In addition to studying the influences of locality and season on the ascorbic acid value of tomatoes and cabbages, Murphy (55) also made observations on the stage of maturity. She made the general statement that "paralleling maturation of the tissue, a definite rise in ascorbic acid concentration in the tomato and a decline in the cabbage was observed.". A study of the graphs accompanying the article shows that the high values found in the very immature cabbages in July drop rapidly to a minimum about September, and then for most varieties there is a slight but definite upward

swing as the cabbages approach maturity. This is particularly noticeable in the Penn State Ballhead variety.

The possibility of a relationship existing between potency of vitamin C, and head weight, both within varieties and from strain to strain has been investigated in several instances. There is very little conclusive evidence presented, but the results of two large-scale studies may be mentioned here. Serbon (92) described a study of the size and weight of vegetables in relation to their quality and to the amount of labor required for their processing on a large scale. It was found that small heads of cabbage require 70% more labor in processing, but at the same time contain an average of 37% more vitamin C. Poole and co-workers (61) found a significant negative correlation between ascorbic acid concentration and head weight among twenty-five breeding lines of cabbage.

In addition to the many external factors which are considered to cause variance in vegetables in general, and in the cabbage in particular, and which must be taken into account when assaying for vitamin C, there is also a considerable lack of

uniformity of vitamin concentration within the plant itself. This is associated with the selective distribution of the vitamin in different structural parts of the plant and seems to be species specific. For instance, Gallot (19) found the outer leaves of Milan cabbage to be an important source of vitamin C while the inner leaves contained less. Sheets, Leonard and Gilgor (70) found a higher concentration of vitamin C in the green outer leaves of a variety of summer cabbage and also a higher concentration in the leaf blades than in the mid-ribs.

On the other hand, Grollman (3) reviewed an article which reported that the leaves of white cabbage contain the least vitamin C, the ribs more and the stalk the highest amount. Ranganathan (62) found a similar situation, obtaining values of 132.6 mgm % for fresh inner leaves and 125.8 mgm % for fresh outer leaves.

Because ascorbic acid is so readily oxidisable, it is reasonable to expect that there would be an appreciable loss during storage. That the rate of loss can be reduced to a minimum under optimum conditions of storage has been demonstrated by several groups of workers.

Lengitt, Baker and Parkinson (35) studied the effect of storage on raw vegetables. They reported that owing to the change in weight of vegetables during storage, steps must be taken to calculate the amount of ascorbic acid to a common standard before and after storage. They found no evidence of a sufficient amount of development of interfering substances during storage to cause interference with the determination of the vitamin C content. During the storage of lettuce, broccoli, cabbage and spinach at room temperature, ascorbic acid was lost at different rates. Storage at 3°C. reduced the rate of loss. In fact at this temperature an increase in vitamin content was observed in lettuce, broccoli and cabbage during the first two days of storage. Gould, Tressler and King (20) also found that the rate of loss of ascorbic acid is much higher at ordinary room temperatures than when the cabbage is stored under refrigeration.

Van Duyne and associates (34) found that the ascorbic acid content of cabbages did not decrease appreciably when stored in closed containers for two months or when kept in a refrigerator for a week.

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losses occurred during the third month of storage and when held at room temperature for three days.

Mayfield and Richardson (44) studied fresh and stored Danish Ballhead cabbages. Representative portions of a number of heads of cabbage were tested when raw and after being cooked for ten minutes, both in the fall and spring. Their results are recorded in Table II, page 43.

TABLE II

Vitamin C content of Fresh and Stored Danish Ballhead Cabbage

| Condition of Cabbage | Number of tests | Mean vit.C (mgm/gm) |
|---|---|---|
| Fall | | |
| Raw | 15 | 0.63 |
| Cooked | 15 | 0.35 |
| Spring - Stored 6 months | | |
| Raw | 7 | 0.47 |
| Cooked | 2 | 0.27 |

Smith and Walker (74) in studying the relation of environmental and hereditary factors to the ascorbic acid content of cabbage considered also the dehydroascorbic acid and bound ascorbic acid found therein. They found no significant amounts of the latter forms of vitamin C in the cabbage studied.

McMillan and Todhunter (50) studied the development of dehydroascorbic acid in cabbages. Fresh cabbage, cut with a knife, after two hours' standing showed 10.6% loss of reduced ascorbic acid and 9% increase of dehydroascorbic acid; thus, there was destruction of only 1.6% of the total vitamin. Shredded cabbage after standing for two hours showed 27.2% loss of reduced acid and 22.2% increase of dehydroascorbic acid, or 5% total ascorbic acid loss.

To investigate the effect of dehydration on the ascorbic acid content of cabbage, Morgan and associates (54) obtained lots of fresh, blanched and dehydrated potatoes, cabbages, carrots and onions from thirteen plants. The fresh and dehydrated samples were compared. Ascorbic acid disappeared to the extent of 16% to 63% in all but five cases.

Titus, Brown, Werthern and Skofield (79) found that retention of vitamin C in vegetables was improved by dehydration in natural gas or $CH_4$ rather than air. Values in excess of 100% retention were observed sufficiently frequently to suggest that under certain conditions of blanching or dehydration an increase occurs.

Rao and Haugaker (63) confirmed Mapson's (43) observation that dehydrated vegetables contain reducing substances which interfere with the estimation of ascorbic acid by titration with 2,6-dichlorophenolindophenol.

Since such a large proportion of the fruit and vegetables of the Canadian diet for the greater part of the year is consumed in the cooked state, a consideration of the percentage loss of ascorbic acid by the more commonly used methods of cookery is necessary in order to estimate the biologic value of the food as served; and to discover what cooking practices will best conserve the vitamin.

Lampitt and associates (35) have made one of the most detailed investigations of losses of vitamin content during the cooking of vegetables. The study referred to shows the loss of ascorbic acid during each stage of the cooking period,

according to several different methods of cooking.

Many factors which may enter into the cooking process of cabbage to influence its vitamin content have been examined by one or more groups of workers with varying results. The various aspects studied, and a summary of the conclusions drawn by several experimenters is shown in Table III

TABLE III

Cooking Practices and Their Relation to Ascorbic Acid Retention

| Name(s) | Ratio of $H_2O$ to cabbage | Size of pieces | Rate of boiling or length of cooking period | Method of cooking | Covered Uncovered | Reheating | Advance preparation |
|---|---|---|---|---|---|---|---|
| Lampitt, Baker & Parkinson (36) | better in small volumes of $H_2O$ | | better when pieces added gradually so $H_2O$ does not go off boil | | | | |
| Noble and Waddell (58) | | | slightly better for short periods | equally good: steamer covered pan pressure cooker | covered better | | |
| Van Duyne, Chase and Simpson (83,84) | small volumes of $H_2O$ better | | little change for 7,15, 25 min., but more in $H_2O$ for longer periods | | covered better | Refrig. 1-3 days 28-33% loss | Shredded, stand 1 hr. in air or 3 hr. in $H_2O$ 6% loss |
| Hollyman, Brodie & Willard (28) | small volumes of $H_2O$ better | | | pressure cooker | | Refrig. 24 hr. 66% loss | Shredded, stand 30 min. in air 6% loss |
| Wellington and Tressler (88) | small volumes of $H_2O$ better | 1/6 loss large 2/3 loss shredded but much leaching in cooking $H_2O$ | | steaming | | | |
| Gould, Tressler & King (20) | | | rapid boiling (little destruction but greater loss in cooking $H_2O$ with longer cooking) | | | Refrig. 48 hr. 50% loss | |
| Olliver (59) | | | same as (20) simmering 78% loss boiling 65% loss | steaming 35% loss. Added to steamer 2 min. before $H_2O$ boils 48% loss | | Kept hot 2 hr. 80% loss | |

## EXPERIMENTAL AND DISCUSSION

The measurement of reduced ascorbic acid according to the method suggested by Loeffler and Ponting (30) is presented fully in Appendix A.

A number of moisture tests were made at different times on the cabbages from all three farms, by drying 5-gram samples in the vacuum oven for 16 hours. As seen from the table on page 49 there never was a greater variation than 6% at any time.

As a variation of 7% would cause less than 1% variation in the ascorbic acid calculation (30), it was considered that an approximate value from food analysis tables would be sufficiently accurate. As suggested by Winton and Winton (69) the value of 91.5% was used. This figure was used in calculating the values for all forms of vitamin C.

The method of Roe and Oesterling (48) was used to measure dehydroascorbic acid and is given in detail in Appendix B.

Appendix C contains a description of the assay for "apparent vitamin C" as suggested by Levy (37).

The cabbages were winter varieties, commonly

TABLE IV

Moisture Content of Some Cabbage Samples

| Date | Percentage Moisture Farm I | Farm II | Farm III |
|---|---|---|---|
| Oct. 9 | | | 90.1 |
| Oct.12 | | | 88.9 |
| Oct.19 | | | 89.3 |
| Nov. 5 | 89.2 | 91.5 | 88.1 |
| Dec. 3 | 87.1 | 88.0 | 87.8 |
| Jan.22 | 85.9 | 88.2 | 87.5 |

grown in Canada, obtained from three farms within the area of Greater Winnipeg. The first lot was obtained on September 4th, 1946, from Mr. Ashton in St. Vital and was of the Penn. State Ballhead variety. The second lot was grown on a plot owned by Mr. Kuyper of Headingly, harvested September 24th and was of the Danish Ballhead variety. The third lot was also of this variety but was obtained from the farm of Mr. Anema of St. Vital, on October 8th.

All cabbages were random samples of large plots. On the same day as harvested they were placed in a refrigerator at a temperature of 8-10°C. Ten cabbages were assayed over a ten-day period.

The cabbages used in the storage tests and several which were used for the cooking tests were obtained from the same plots as the fresh lots of cabbages, and stored in a root cellar. During the period of storage, the temperature varied from 32°F. to 40°F. and the relative humidity from 80% to 92%. The heads were placed core down, with spaces between them on wooden shelves. When received, all of the cabbages had been prepared as if for

market, that is, with a few outer leaves only, removed. Those assayed after the second week in January had any wilted or off-color leaves removed.

The storage tests were begun on October 22, 1946, and except for a three-week interval at Christmas were assayed weekly until January 14, 1947. Reduced ascorbic acid was determined throughout the series with the object of finding the rate of loss to be expected from the storage of winter cabbages, under conditions which are usual in Canada. Determinations for dehydro-ascorbic acid were begun later and continued to the end of the experiment. The results are presented in Table VIII.

Cooking tests were made to investigate the influence of various common cooking practices on the reduced vitamin C content and to observe changes, if any, on such amounts of other forms of the vitamin as might be present. These tests followed the storage tests. The assay material used until the last of March was from the same sources with one exception. An imported cabbage was used on March 3, presumably a spring variety. All cabbages used during April were imported and

nothing is known of their history,as they were bought on the open market.

Much of the experimental procedure for the cooking tests was as suggested by Noble and Waddell (58) in their excellent study of the effect of various cooking practices on the ascorbic acid content of cabbages. It was felt that an inquiry into the most commonly used methods for cooking cabbage,as carried out in the average home without special equipment,and only such variations of method as might be introduced easily and readily by the homemaker,was sufficient for the purpose of this study.

One series of assays, only, was run on two lots of dehydrated cabbages,prepared by Bulmans Ltd., of Vernon, B.C. One of these varieties was labelled "Ballhead". The other was unknown.

The first series of assays was made on three lots of ten cabbages each. The cabbages were assayed in the order: Farm No. 1; Farm No. 2 and Farm No. 3 respectively. The object was to determine by chemical assay the actual values of a large number of samples from each cabbage under the conditions already described and from a statistical analysis

of the mass of data to predict the values to be expected from similar cabbages under similar conditions.

The special cutting device was designed primarily to overcome the variations in ascorbic acid potency in different parts of the plant. As already noted this variation has been reported in the literature (3,84) and was confirmed in this laboratory by preliminary experiments. These results are shown in Table V, page 54.

TABLE V.

Variation in Ascorbic Acid Content of Winter Cabbage According to Distance from Center

| Part of Plant | Ascorbic acid mgm/100 gm |
|---|---|
| Outer quarter | 31.3 |
| 2nd quarter (toward center) | 32.0 |
| 3rd quarter (toward center) | 37.2 |
| Inner quarter | 42.4 |

Thus, by using the borer, any 25-gram sample from a cabbage was a cross-section, representative of all layers from the outer leaves toward the center ones.

In order to have the mean of all samples from one plant reflecting influences of each level, that is, the upper, middle and lower (or root) one-third, as well as all sections of the whole fruit, it was planned to take fifteen samples in all, five from each level. These five were taken in a clockwise direction in relation to the first and each sample roughly represented one-fifth of the total level. Samples No.6 to 10 were taken from the upper level in the same manner as samples 1 to 5 from the middle level, but care was taken to have sample No.6 approximately in a direct line with sample No.1. Samples 11 to 15 of the root level were directly under samples 1 to 5.

It can thus be seen from the diagram, page 57, (Figure 1) that the three levels according to the arrangement: Nos.1 to 5, 6 to 10 and 11 to 15 represent cross-sectioning along horizontal lines. A rearrangement of samples into five groups to read vertically, as: Nos. 1, 6 and 11, Nos. 2, 7 and

12, etc., represents even distribution of samples from vertical divisions or sectors of the cabbage. The mean of the fifteen samples obtained in this way would represent an average of the values from all parts of the cabbage.

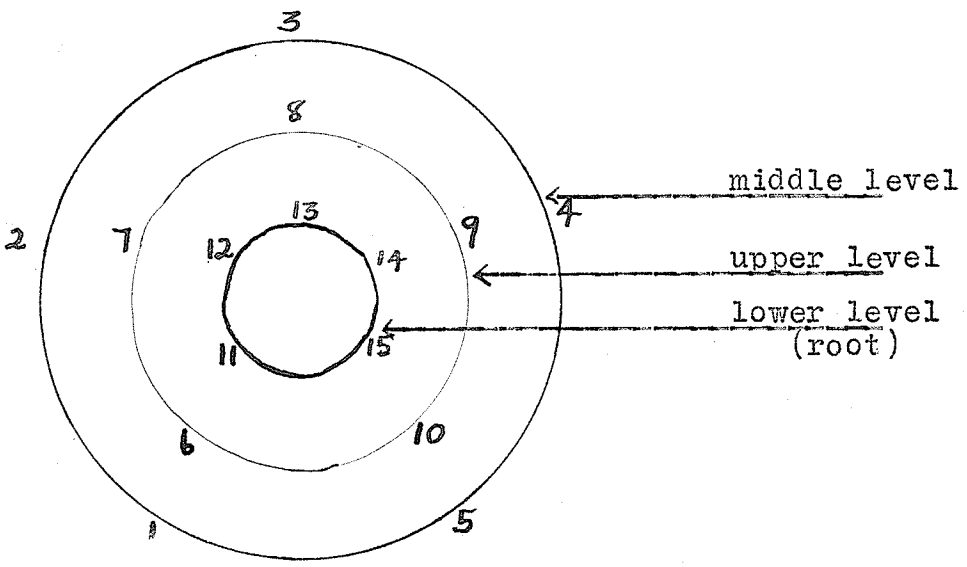
With the variations within each cabbage thus controlled by means of the special borer and by means of sampling from all parts, it would be logical to assume that any differences from plant to plant would be inherent differences and not attributable to laboratory sampling.

The results of the first series of determinations on fresh cabbages from Farms No. 1, 2 and 3 are presented in Table VI showing the mean value for each cabbage of each plot, also their ranges and standard deviations. Ascorbic acid values are arranged so that levels are read vertically and the sectors horizontally.

In order to show graphically the extent of variation in the means and standard deviations being shown by these cabbages, the statistical device of the control chart was selected as being the most suitable. The nature of the "quality control chart" and its application to modern

FIGURE 1

Pattern of Sampling From Cabbage for First Series of Tests



## TABLE VI

Ascorbic Acid Content of Fresh Cabbages from Farms 1, 2 and 3.

### Farm No.1

| Date | Ascorbic Acid (mgm/100 gm) | | | Date | Ascorbic Acid (mgm/100 gm) | | |
|---|---|---|---|---|---|---|---|
| Sept. 5 | 42.5 | 45.4 | 41.7# | Sept.12(A.M.) | 63.9 | 65.8 | 66.0 |
| | 44.4 | 36.5 | 42.9 | | 64.8 | 66.3 | 64.4 |
| | 41.7 | 44.4 | 42.5 | | 63.3 | 61.5 | 63.3 |
| | 41.7 | 41.7 | 42.5 | | 63.3 | 63.7 | 63.3 |
| | 42.5 | 44.4 | 43.9 | | 66.3 | 61.5 | 61.3 |
| | Mean = 42.6 ± 1.99 | | | | Mean = 64.2 ± 2.20 | | |
| | Range = 36.5 - 45.4 | | | | Range = 61.5 - 66.3 | | |
| | Weight = 1089 gm | | | | Weight = 1951 gm | | |
| Sept. 6 | 43.9 | 41.7 | 41.7 | Sept.12(P.M.) | 63.9 | 64.4 | 63.3 |
| | 43.9 | 42.5 | 45.0 | | 64.8 | 64.1 | 63.7 |
| | 42.9 | 42.9 | 42.9 | | 66.3 | 64.8 | 63.7 |
| | 44.4 | 43.9 | 42.9 | | 61.8 | 63.7 | 64.8 |
| | 42.5 | 44.4 | 41.7 | | 63.3 | 61.8 | 64.1 |
| | Mean = 43.1 ± 2.17 | | | | Mean = 63.9 ± 1.87 | | |
| | Range = 41.7 - 45.0 | | | | Range = 61.8 - 66.3 | | |
| | Weight = 1175 gm | | | | Weight = 957 gm | | |
| Sept.10(A.M.) | 66.3 | 62.6 | 62.6 | Sept.13(A.M.) | 68.5 | 68.5 | 65.2 |
| | 63.3 | 62.6 | 61.8 | | 67.3 | 69.3 | 64.8 |
| | 61.8 | 63.3 | 61.1 | | 69.3 | 67.0 | 69.3 |
| | 62.6 | 64.8 | 63.3 | | 67.3 | 67.0 | 68.5 |
| | 63.3 | 62.6 | 62.6 | | 67.0 | 66.3 | 68.5 |
| | Mean = 62.9 ± 2.05 | | | | Mean = 67.5 ± 2.90 | | |
| | Range = 61.1 - 66.3 | | | | Range = 65.2 - 69.3 | | |
| | Weight = 1010 gm | | | | Weight = 1155 gm | | |
| Sept.10(P.M.) | 64.1 | 63.3 | 63.3 | Sept.13(P.M.) | 66.3 | 65.5 | 65.8 |
| | 62.6 | 64.8 | 63.3 | | 66.3 | 65.8 | 65.2 |
| | 61.1 | 61.8 | 63.3 | | 65.5 | 67.8 | 65.5 |
| | 64.1 | 62.6 | 61.8 | | 64.8 | 66.3 | 66.3 |
| | 63.3 | 61.8 | 62.6 | | 66.3 | 66.3 | 66.3 |
| | Mean = 62.9 ± 1.10 | | | | Mean = 66.0 ± 3.66 | | |
| | Range = 61.1 - 64.8 | | | | Range = 64.8 - 67.8 | | |
| | Weight = 1740 gm | | | | Weight = 840 gm | | |
| Sept.11 | 64.8 | 64.1 | 63.3 | Sept.14 | 69.3 | 68.8 | 71.5 |
| | 63.3 | 64.1 | 61.1 | | 70.8 | 69.3 | 69.3 |
| | 61.8 | 64.8 | 64.1 | | 69.3 | 69.3 | 71.5 |
| | 63.3 | 63.3 | 61.5 | | 71.5 | 69.3 | 69.3 |
| | 64.8 | 62.6 | 63.3 | | 68.8 | 69.3 | 68.5 |
| | Mean = 63.4 ± 0.67 | | | | Mean = 69.7 ± 1.95 | | |
| | Range = 61.1 - 64.8 | | | | Range = 68.5 - 71.5 | | |
| | Weight = 1540 gm | | | | Weight = 1075 gm | | |

#The arrangement in three columns (for all farms) is according to levels, the middle level being in the first column, the upper in the second, and the lower (root) in the third.

## TABLE VI

Ascorbic Acid Content of Fresh Cabbages from Farms 1, 2 and 3.

### Farm No.2

| Date | Ascorbic Acid (mgm/100 gm) | | | Date | Ascorbic Acid (mgm/100 gm) | | |
|---|---|---|---|---|---|---|---|
| Sept.25 | 50.7 | 49.5 | 55.9 | Oct.1 | 50.4 | 49.2 | 50.7 |
| | 50.7 | 49.9 | 54.8 | | 50.7 | 52.4 | 50.7 |
| | 50.7 | 55.4 | 52.1 | | 48.4 | 49.5 | 51.4 |
| | 52.1 | 48.4 | 62.6 | | 53.3 | 52.9 | 52.1 |
| | 51.8 | 49.0 | 62.9 | | 50.4 | 52.1 | 50.4 |
| | Mean = 53.2 ± 4.3 | | | | Mean = 51.0 ± 1.5 | | |
| | Range= 49.5 - 62.9 | | | | Range= 48.4 - 53.3 | | |
| | Weight = 2303 gm | | | | Weight = 1678 gm | | |
| Sept.26 | 52.1 | 47.7 | 54.8 | Oct.2 | 47.7 | 49.5 | 52.9 |
| | 52.1 | 49.5 | 52.1 | | 52.1 | 47.7 | 52.9 |
| | 49.5 | 48.9 | 53.3 | | 55.4 | 47.7 | 53.9 |
| | 46.9 | 49.9 | 53.9 | | 46.9 | 45.9 | 50.4 |
| | 52.1 | 50.7 | 50.7 | | 49.5 | 47.7 | 50.4 |
| | Mean = 50.9 ± 3.1 | | | | Mean = 50.0 ± 2.8 | | |
| | Range= 46.9 - 54.8 | | | | Range= 45.9 - 55.4 | | |
| | Weight = 2353 gm | | | | Weight = 2102 gm | | |
| Sept.27 | 49.5 | 49.9 | 51.4 | Oct.3 | 46.4 | 54.8 | 48.9 |
| | 51.4 | 48.4 | 52.1 | | 48.4 | 52.1 | 47.4 |
| | 56.6 | 49.5 | 57.8 | | 51.4 | 54.8 | 47.4 |
| | 52.1 | 48.0 | 56.8 | | 49.5 | 53.9 | 47.4 |
| | 50.1 | 52.9 | 62.9 | | 49.5 | 51.4 | 47.4 |
| | Mean = 53.3 ± 4.4 | | | | Mean = 50.2 ± 2.2 | | |
| | Range= 48.0 - 62.9 | | | | Range= 47.4 - 54.8 | | |
| | Weight = 2807 gm | | | | Weight = 2123 gm | | |
| Sept.28 | 68.5 | 68.5 | 72.7 | Oct.4 | 49.5 | 54.4 | 52.1 |
| | 72.7 | 69.3 | 71.5 | | 49.2 | 53.9 | 52.1 |
| | 68.8 | 68.8 | 73.7 | | 49.5 | 49.5 | 50.7 |
| | 73.0 | 68.5 | 68.5 | | 49.5 | 49.5 | 52.9 |
| | 67.8 | 67.8 | 67.8 | | 56.6 | 49.5 | 52.1 |
| | Mean = 69.9 ± 2.1 | | | | Mean = 51.4 ± 2.2 | | |
| | Range= 67.8 - 73.7 | | | | Range= 49.2 - 56.6 | | |
| | Weight = 2493 gm | | | | Weight = 2668 gm | | |
| Sept.30 | 59.6 | 56.6 | 61.8 | Oct.5 | 67.0 | 66.3 | 61.1 |
| | 53.6 | 60.3 | 55.9 | | 67.0 | 63.7 | 61.8 |
| | 59.6 | 61.1 | 58.2 | | 64.8 | 63.7 | 64.1 |
| | 64.8 | 64.8 | 61.8 | | 67.8 | 61.1 | 60.3 |
| | 62.9 | 59.6 | 55.9 | | 61.4 | 61.4 | 65.7 |
| | Mean = 59.1 ± 3.5 | | | | Mean = 63.7 ± 0.6 | | |
| | Range= 53.9 - 64.8 | | | | Range= 60.3 - 67.8 | | |
| | Weight = 2048 gm | | | | Weight = 1290 gm | | |

TABLE V I

Ascorbic Acid Content of Fresh Cabbages from Farms 1, 2 and 3.

Farm No.3

| Date | Ascorbic Acid (mgm/100 gm) | | | Date | Ascorbic Acid (mgm/100 gm) | | |
|---|---|---|---|---|---|---|---|
| Oct. 9 | 55.4 | 55.4 | 57.8 | Oct.15 | 70.0 | 69.3 | 72.3 |
| | 55.9 | 56.6 | 55.4 | | 69.7 | 71.2 | 67.3 |
| | 56.4 | 54.4 | 56.6 | | 70.8 | 70.0 | 69.3 |
| | 55.4 | 57.8 | 54.4 | | 73.0 | 71.5 | 69.3 |
| | 53.6 | 50.6 | 53.6 | | 73.0 | 70.0 | 69.3 |
| | Mean = 55.4 ± 1.8 | | | | Mean = 70.4 ± 1.5 | | |
| | Range= 50.6 - 58.4 | | | | Range= 67.3 - 73.0 | | |
| | Weight = 1610 gm | | | | Weight = 1735 gm | | |
| Oct.10 | 66.7 | 67.8 | 68.5 | Oct.16 | 62.9 | 61.8 | 64.1 |
| | 68.5 | 67.0 | 67.0 | | 62.9 | 61.8 | 59.6 |
| | 66.3 | 65.6 | 67.0 | | 58.8 | 63.7 | 59.6 |
| | 65.6 | 66.7 | 66.7 | | 63.7 | 62.3 | 60.3 |
| | 66.3 | 65.6 | 66.3 | | 62.9 | 62.9 | 61.8 |
| | Mean = 66.7 ± 3.3 | | | | Mean = 61.9 ± 2.7 | | |
| | Range= 65.6 - 68.5 | | | | Range= 58.8 - 64.1 | | |
| | Weight = 1315 gm | | | | Weight = 1788 gm | | |
| Oct.11 | 63.7 | 64.1 | 65.3 | Oct.17 | 67.0 | 64.1 | 64.1 |
| | 63.7 | 64.1 | 62.6 | | 66.7 | 64.1 | 66.3 |
| | 61.1 | 59.3 | 62.8 | | 71.6 | 64.8 | 65.3 |
| | 61.8 | 61.1 | 61.4 | | 66.7 | 66.3 | 66.7 |
| | 62.6 | 59.6 | 62.9 | | 66.7 | 65.3 | 66.3 |
| | Mean = 62.4 ± 2.1 | | | | Mean = 66.2 ± 1.7 | | |
| | Range= 59.3 - 65.3 | | | | Range= 64.1 - 71.6 | | |
| | Weight = 1502 gm | | | | Weight = 1386 gm | | |
| Oct.12 | 58.6 | 60.3 | 59.3 | Oct.18 | 61.1 | 58.4 | 57.8 |
| | 57.8 | 64.1 | 64.1 | | 56.6 | 58.8 | 57.4 |
| | 61.1 | 60.3 | 64.8 | | 55.9 | 60.3 | 56.6 |
| | 64.8 | 61.8 | 64.8 | | 56.9 | 59.6 | 59.6 |
| | 59.9 | 60.3 | 59.9 | | 57.8 | 57.4 | 58.1 |
| | Mean = 61.5 ± 1.5 | | | | Mean = 58.2 ± 2.6 | | |
| | Range= 57.8 - 64.8 | | | | Range= 55.9 - 61.1 | | |
| | Weight = 1836 gm | | | | Weight = 1142 gm | | |
| Oct.14 | 63.7 | 64.8 | 64.8 | Oct.19 | 64.8 | 62.3 | 61.4 |
| | 67.0 | 69.3 | 62.6 | | 62.6 | 61.4 | 62.3 |
| | 67.8 | 66.3 | 67.0 | | 63.7 | 61.4 | 63.7 |
| | 66.3 | 66.7 | 67.8 | | 64.1 | 62.3 | 63.7 |
| | 66.3 | 63.7 | 64.8 | | 61.8 | 62.9 | 62.6 |
| | Mean = 65.9 ± 2.1 | | | | Mean = 62.7 ± 2.0 | | |
| | Range= 62.6 - 69.3 | | | | Range= 61.4 - 64.8 | | |
| | Weight = 1330 gm | | | | Weight = 1112 gm | | |

industry is fully discussed by E. L. Grant (21). Such charts make use of $\pm$ 3 sigma bands as control limits for the means, and in a normal distribution 99.3% of the mean values should fall within these limits. Thus 0.7% might be expected to fall outside and still be considered to be attributable to some assignable cause.

The computation for Charts I, II and III shown as Figures 2, 3 and 4, consisted in setting up the control limits on either side of the mean value of each of the ten mean ascorbic acid values, at distances of $\pm \frac{3\sigma}{\sqrt{N}}$ from the mean (N being the number of determinations, or 16).

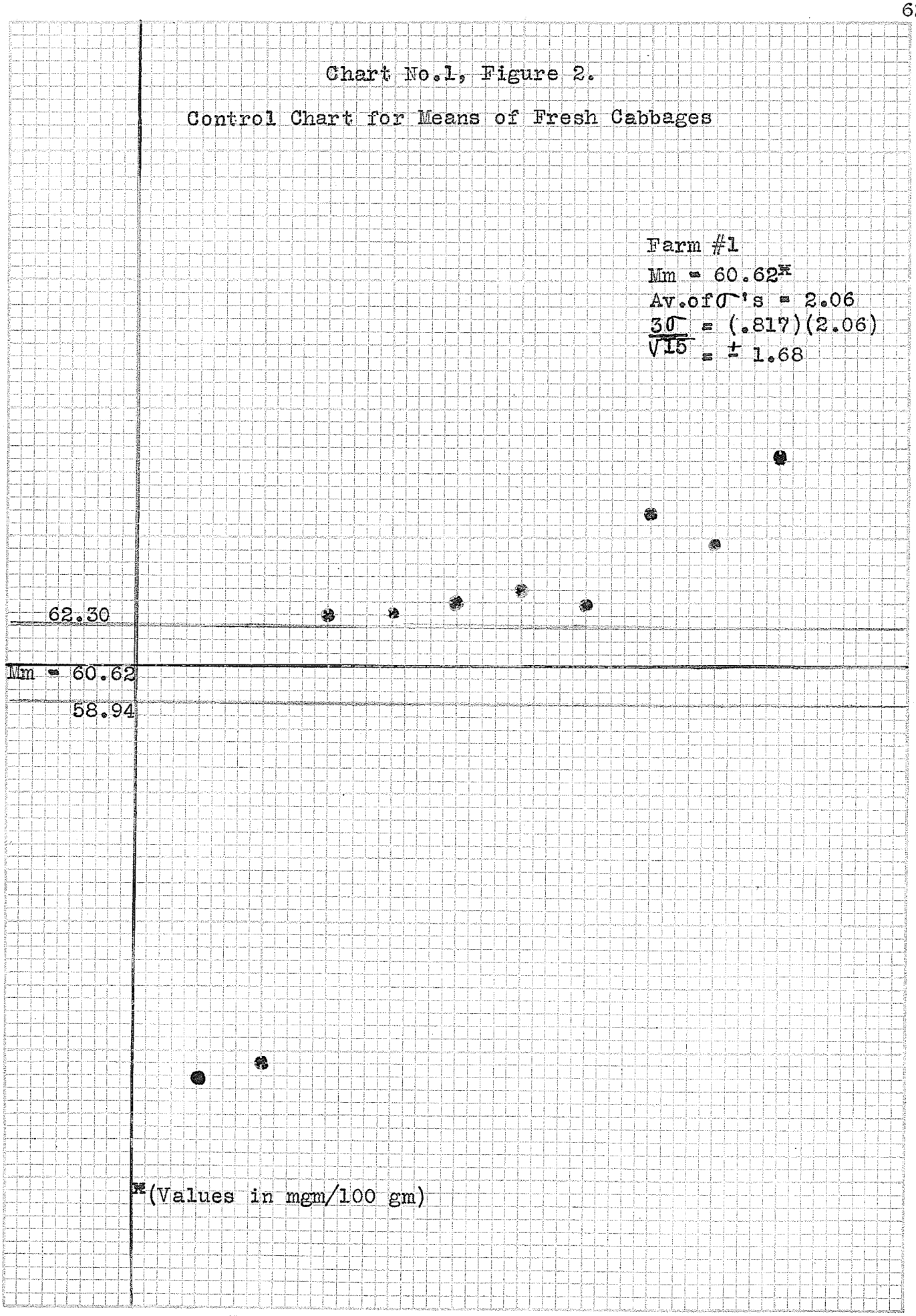
An examination of the values as plotted on these charts shows extreme lack of conformity to a normal distribution, the greatest variation being shown in the cabbages from Farm No.1.

Chart IV, Figure 5, shows the amount of control in the standard deviations of these same determinations by showing the extent to which they fall within their control limits. A lesser degree of variation is found in the standard deviations of Chart IV than in the means of Charts I, II and III.

A further statistical measure, actual values for which were derived for the control charts, was

Chart No.1, Figure 2.

Control Chart for Means of Fresh Cabbages

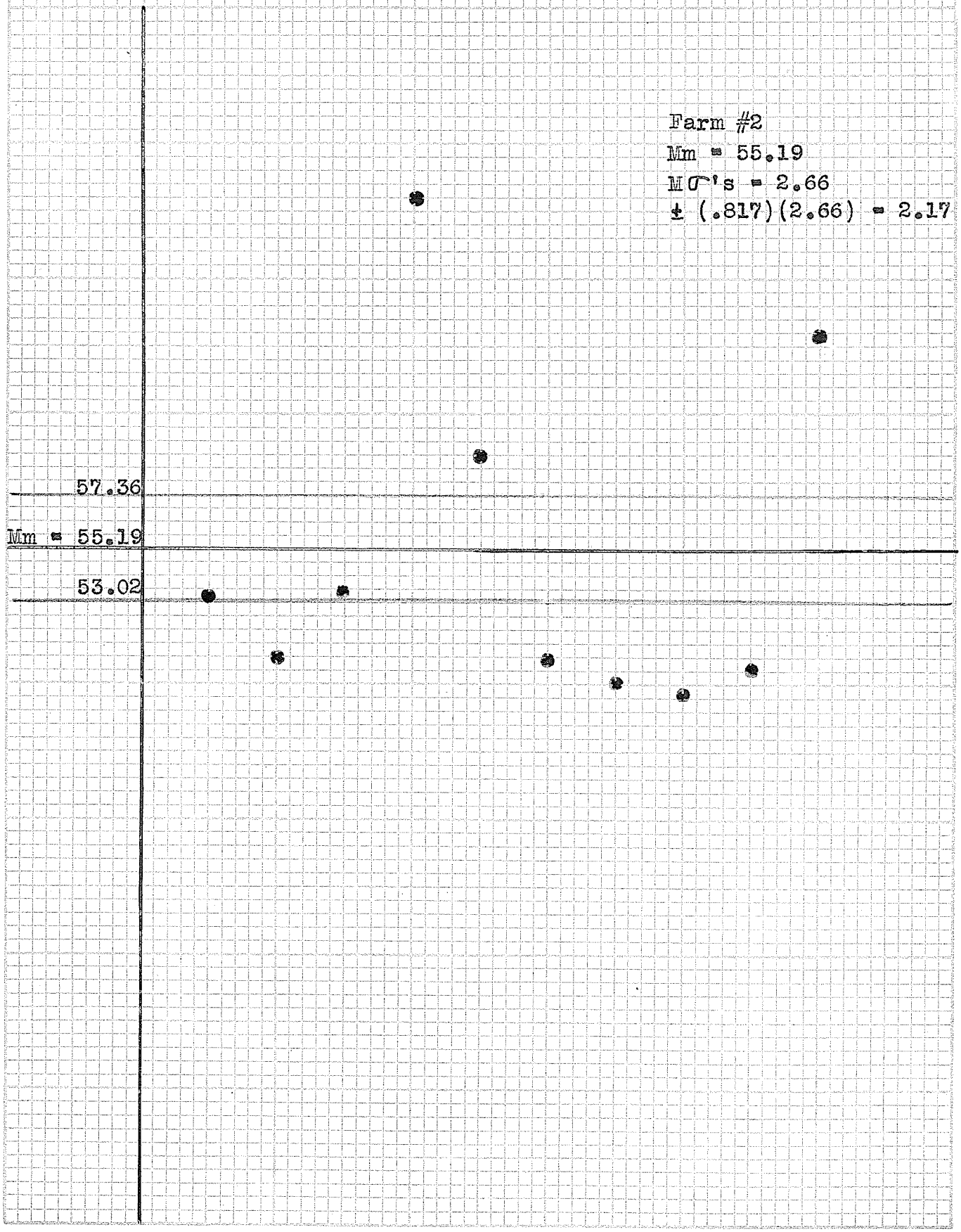



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Chart No.2, Figure 3.

Control Chart for Means of Fresh Cabbages

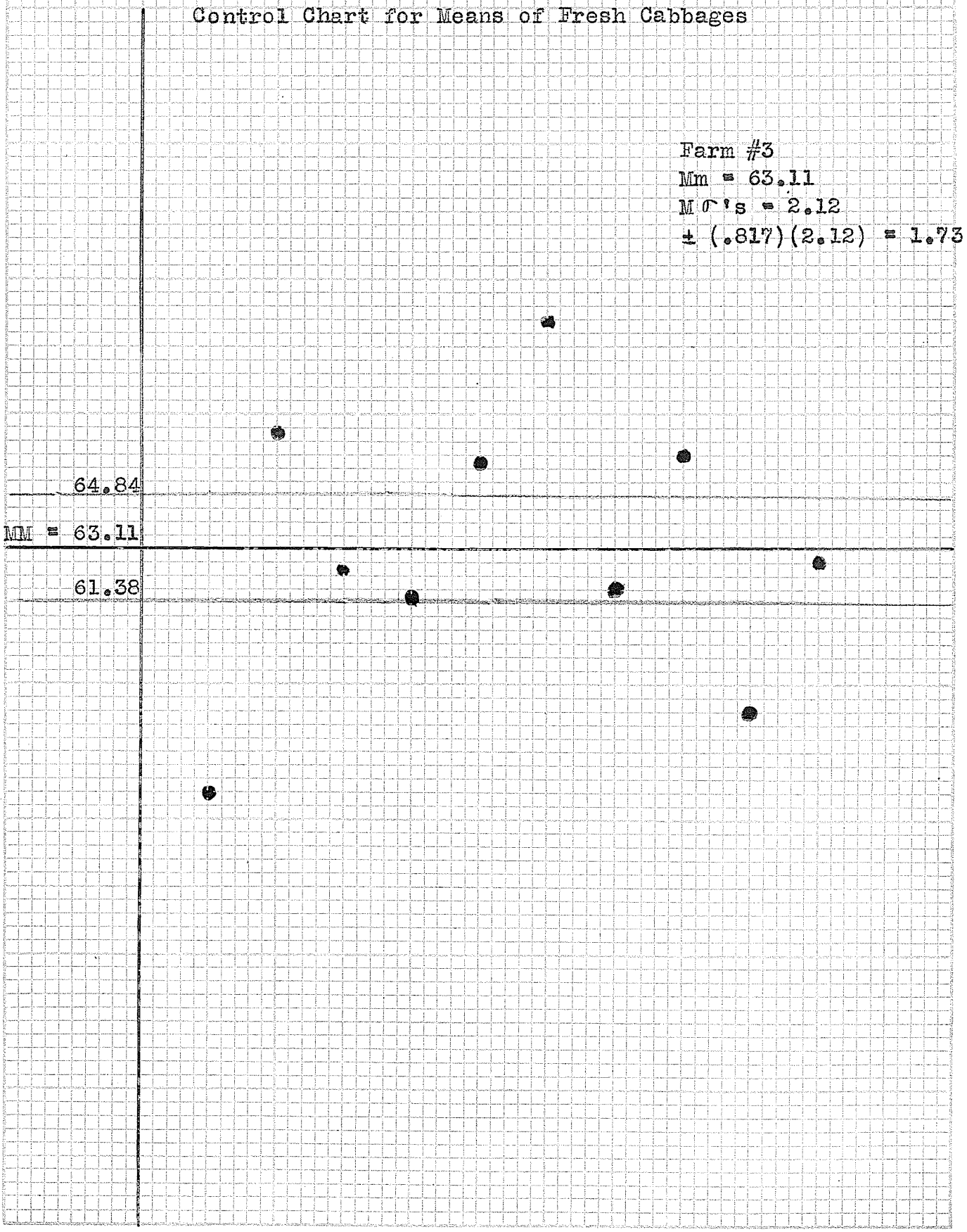



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

Chart No.3, Figure 4.

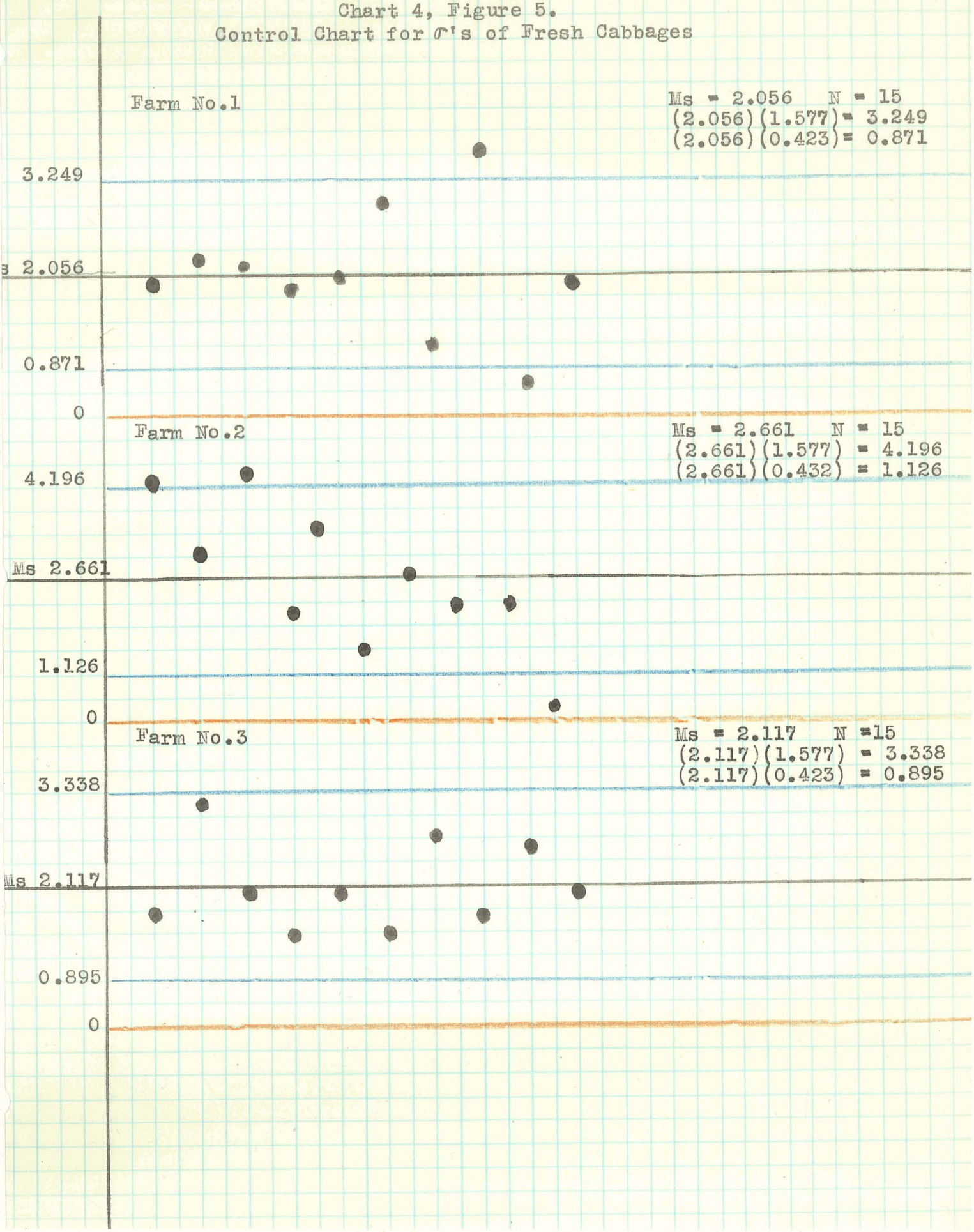
Control Chart for Means of Fresh Cabbages




This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Chart 4, Figure 5.
Control Chart for $\sigma$'s of Fresh Cabbages



the mean of the means of the vitamin C content of the cabbages from each plot, and also the mean of the standard deviations of the cabbages from each plot.

The most striking fact brought out in a study and comparison of the results of this series is the wide range shown within the mean values for Farm No.1. There is a progressive increase from a comparatively low concentration for the first few cabbages to a higher value which is comparable to those found in the lots assayed at later dates, and seems too marked a trend to be due to pure chance. As the plants were harvested at an earlier than customary date for a winter variety such as the Penn State Ballhead, it was thought that the relative immaturity of the plants might be responsible for the low level obtained during the first few days after harvest. Support for this hypothesis is found in Murphy's (55) study on the influence of the stage of maturity on the ascorbic acid content of vegetables. Accompanying graphs show the tendency for the Penn State Ballhead variety of cabbage to decrease sharply from a high concentration in the very immature stage to a low point, and then during the last two or three weeks of growth there is a

slight upward trend.

A study of the actual dates of Murphy's experiment shows that this low point was reached at the end of four weeks of assay on August 30 and the levelling off and upward swing extended throughout September until the end of the testing period on October 11. It is considered probable that the maturity dates for the Penn State Ballhead in Maine and in Manitoba are somewhat similar and that during the first week in September the tendency for the cabbages used in the tests, to increase in ascorbic acid concentration was operating, and at the time of harvest the full potency of the fully matured cabbage head had not been reached. This immaturity factor is no doubt also partly responsible for the wide range in the mean values from cabbage to cabbage in the case of Farm No.1.

There seemed to be no consistent relationship between head weight and ascorbic acid potency. Here, also, if the immaturity factor was operating, a wider range in the weights of the cabbages than would have obtained otherwise, would be found. However, as mentioned earlier, inconsistent reports regarding the relationship of head weight and

vitamin C potency have been found throughout in the literature.

An inspection of the values according to levels shows that in the majority of cases there is a tendency for the lower levels to contain a slightly higher concentration of the vitamin than either of the other two levels. Thus the precaution of sampling equally from all levels, based on the presumption that some such variability might exist, proved to be warranted.

When the ascorbic acid concentration for the Penn State and Danish varieties of the Ballhead cabbage are compared with those found in the literature there is relatively close agreement in mean values. For example, Noble and Waddell (58) found means of 46.9 and 44.9 mgm per 100 gm for two series of determinations on the Danish Ballhead. Hollyman and co-workers (28) found an average of 44.0 mgm per 100 gm for the same variety. For reported ranges in the literature, however, there is less agreement, the majority being wider. For instance, in the same experiment already reported by Hollyman et al (28) the range for twenty samples of a Ballhead having a mean ascorbic acid content of 44.0 mgm per 100 gm was 28 to 64. Treasler,

Mack and King (60) found that for four varieties of cabbages having a mean of 31 mgm per 100 gm the range was 22 to 38.

As already mentioned, a mean of means was determined, representing one value for each farm. From these a figure of 59.6 mgm per 100 gm was arrived at, which should express with reasonable accuracy the approximate value of native-grown Ballhead cabbage, for a period within a month or six weeks of harvest, and which should serve to evaluate the contribution of winter cabbages to the diet as a source of vitamin C. On the basis of the values determined by this series of assays, a total day's requirement, according to the Canadian Standard, could easily be met on an intake of 100 grams of raw cabbage.

The second series of determinations was made on the same varieties of cabbages obtained from the same farm plots. The purpose of the series was to follow the decrease in the retention of ascorbic acid during the course of several months of storage, under conditions similar to home or commercial storage, and to determine whether there is still any appreciable concentration as

long as the cabbage is considered fit for serving.

One cabbage from each farm was assayed on the same day at weekly intervals from October 22, 1946 until January 14, 1947.

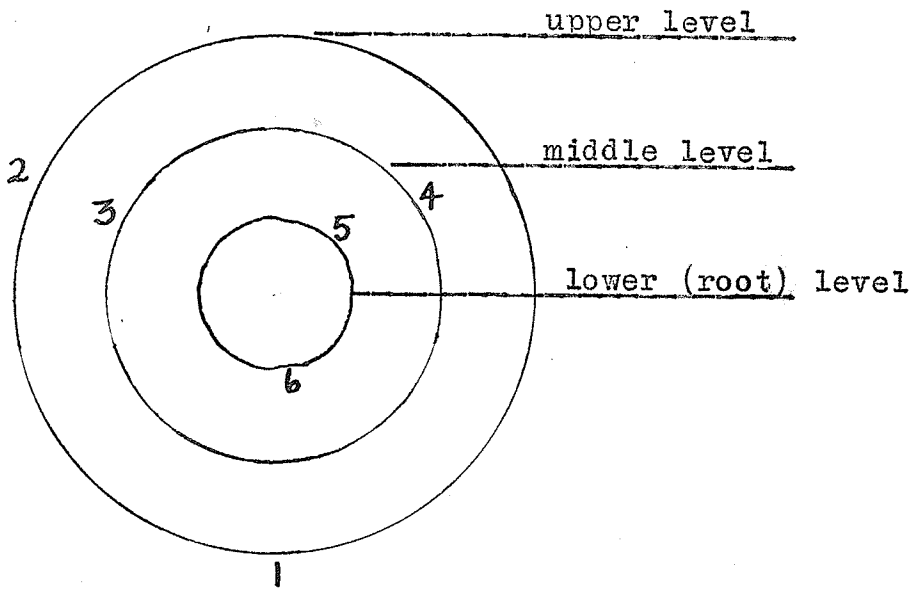
Six samples were taken from each cabbage, two being from each level. In order to represent each sector, a spiral pattern was followed, that is, the first two samples taken from the upper level included about one-third of the circumference; the third and fourth samples from the middle level included the next one-third of the circumference in a clockwise direction from the first two; and the fifth and sixth samples were taken from the remaining one-third of the cabbage. This is shown in Figure 6.

The results of the second series of assays are tabulated in Table VII, showing also the mean value for each farm, the ranges and standard deviations.

The percentage loss for each plot during the entire period was 25.1%; 30.7%; and 22.9% respectively for Farms No.1, 2 and 3.

Control charts were plotted similar to

FIGURE 6




Pattern of sampling for Storage Tests

## TABLE VII

Ascorbic Acid Content of Stored Cabbages from Farms No.1, 2 and 3.

| Date | Ascorbic Acid in mgm/100 gm Farm No.1 | 2 | 3 | Date | Ascorbic Acid in mgm/100 gm Farm No.1 | 2 | 3 |
|---|---|---|---|---|---|---|---|
| 22/10/46 | 61.8 | 56.3 | 59.9 | 29/10/46 | 55.3 | 41.0 | 52.1 |
| | 61.1 | 55.4 | 61.1 | | 53.3 | 41.7 | 52.9 |
| | 61.4 | 55.6 | 59.9 | | 55.9 | 40.0 | 52.9 |
| | 61.1 | 58.8 | 59.6 | | 52.9 | 43.9 | 52.9 |
| | 65.3 | 58.6 | 62.6 | | 49.5 | 44.9 | 54.4 |
| | 63.7 | 58.8 | 61.6 | | 53.7 | 42.9 | 54.4 |
| Range | 61.1-63.7 | 55.4-58.8 | 59.6-62.6 | Range | 49.5-55.9 | 41.0-49.9 | 5.21-54.4 |
| Mean | 62.1±.26 | 57.4±1.4 | 60.7±1.04 | Mean | 53.1±1.9 | 44.6±3.4 | 53.2±0.6 |
| Wt(gm) | 1512 | 2003 | 1410 | Wt(gm) | 2455.5 | 2478.8 | 1395 |
| 5/11/46 | 56.4 | 56.6 | 60.3 | 12/11/46 | 57.8 | 52.4 | 58.4 |
| | 56.6 | 57.8 | 59.3 | | 57.8 | 55.1 | 58.4 |
| | 57.8 | 57.8 | 57.8 | | 59.3 | 54.8 | 57.4 |
| | 56.9 | 57.8 | 56.9 | | 57.8 | 57.4 | 57.8 |
| | 57.8 | 56.9 | 59.3 | | 59.6 | 53.9 | 58.1 |
| | 58.4 | 56.9 | 57.8 | | 58.1 | 52.9 | 57.8 |
| Range | 56.6-58.4 | 56.6-57.8 | 56.9-60.3 | Range | 57.8-59.3 | 52.4-57.4 | 57.4-58.4 |
| Mean | 57.6±.66 | 57.3±.51 | 58.6±.98 | Mean | 58.4±.76 | 54.4±1.52 | 57.9±.23 |
| Wt(gm) | 1808 | 1868 | 1658.5 | Wt(gm) | 2173 | 2043 | 1260 |
| 19/11/46 | 45.0 | 39.9 | 46.4 | 26/11/46 | 38.0 | 40.5 | 41.7 |
| | 45.4 | 45.0 | 43.9 | | 43.2 | 41.0 | 40.5 |
| | 44.4 | 43.2 | 43.2 | | 39.9 | 40.5 | 43.9 |
| | 45.0 | 43.2 | 42.5 | | 42.0 | 40.5 | 42.0 |
| | 43.2 | 41.7 | 47.4 | | 42.5 | 39.9 | 41.7 |
| | 44.7 | 39.5 | 47.4 | | 41.7 | 39.5 | 42.0 |
| Range | 43.2-45.4 | 39.5-45.0 | 42.5-47.4 | Range | 38.0-43.2 | 39.5-41.0 | 40.5-43.9 |
| Mean | 44.6±0.14 | 42.1±2.0 | 45.0±1.86 | Mean | 41.2±1.66 | 40.3±0.11 | 42.0±0.65 |
| Wt(gm) | 1275 | 1983.5 | 1518 | Wt(gm) | 2063 | 1706 | 1433 |
| 3/12/46 | 40.5 | 40.5 | 40.2 | 10/12/46 | 38.0 | 33.5 | 32.0 |
| | 41.0 | 40.2 | 45.2 | | 34.3 | 33.5 | 33.2 |
| | 42.5 | 38.0 | 41.0 | | 33.5 | 31.5 | 35.0 |
| | 42.5 | 38.7 | 42.5 | | 35.7 | 34.3 | 36.9 |
| | 42.5 | 41.7 | 42.5 | | 34.3 | 33.2 | 33.2 |
| | 41.0 | 41.7 | 43.2 | | 34.3 | 32.8 | 34.3 |
| Range | 40.5-42.5 | 38.0-41.7 | 40.2-45.2 | Range | 33.5-38.0 | 31.5-34.3 | 32.0-36.9 |
| Mean | 41.7±0.67 | 40.1±1.5 | 42.1±1.1 | Mean | 35.1±1.4 | 33.1±0.98 | 34.1±1.56 |
| Wt(gm) | 2803 | 2185 | 1703.5 | Wt(gm) | 1877 | 2055 | 1812 |

TABLE VII (Continued)

Ascorbic Acid Content of Stored Cabbages from Farms No.1, 2 and 3.

| Date | Ascorbic Acid in mgm/100 gm Farm 1 | 2 | 3 | Date | Ascorbic Acid in mgm/100 gm Farm 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|
| 17/12/46 | 35.0 | 32.8 | 50.1 | 7/1/47 | 50.4 | 36.1 | 39.0 |
| | 36.5 | 30.1 | 35.0 | | 53.6 | 43.9 | 41.7 |
| | 35.0 | 31.6 | 35.0 | | 50.4 | 41.7 | 38.0 |
| | 35.7 | 32.8 | 35.0 | | 43.9 | 36.1 | 34.3 |
| | 34.6 | 31.6 | 35.0 | | 53.6 | 37.9 | 35.0 |
| | 33.5 | 33.5 | 35.0 | | 43.9 | 39.6 | 34.3 |
| Range | 33.5-36.5 | 30.1-33.5 | 35.0-36.1 | Range | 43.9-53.6 | 36.1-43.9 | 34.3-41.7 |
| Mean | 35.1±0.93 | 32.1±1.01 | 35.2±0.64 | Mean | 49.3±4.0 | 39.1±2.9 | 37.0±2.76 |
| Wt(gm) | 1788 | 1925 | 1810 | Wt(gm) | 1648 | 2040 | 2042 |
| 14/1/47 | 48.9 | 40.5 | 49.2 | | | | |
| | 50.7 | 36.0 | 44.4 | | | | |
| | 40.5 | 30.1 | 49.5 | | | | |
| | 41.0 | 32.0 | 49.4 | | | | |
| | 50.7 | 40.5 | 48.9 | | | | |
| | 44.7 | 39.9 | 44.4 | | | | |
| Range | 40.5-50.7 | 30.1-40.5 | 44.4-49.5 | | | | |
| Mean | 46.5±4.4 | 36.8±4.2 | 46.6±3.3 | | | | |
| Wt(gm) | 2137.5 | 2315 | 1370 | | | | |

those made for the fresh samples. Charts V, VI and VII, as Figures 7, 8 and 9, show the amount of control in the means of the ascorbic acid values for Farms 1, 2 and 3 respectively. Although slightly less variation appeared in the stored samples than in the fresh, there was but little tendency for the means of each cabbage to fall within the limits which were charted at distances of $\pm \frac{3\sigma}{\sqrt{6}}$ on either side of the mean of means. Chart VIII, Figure 10, shows a much greater degree of control in the standard deviations of these same cabbages.

The determination of the mean of the means of the individual values and the means of the standard deviations was carried out in the same manner as for the fresh cabbages. The values are shown on the charts.

The graphs in Figure 11 show the course of the decrease in the ascorbic acid potency during the period of storage.

A review of the literature regarding experimental work on the effect of storage for a relatively long period at comparable conditions of temperature and humidity shows the rate of loss of

Chart No.5, Figure 7.

Control Chart for Means of Stored Cabbages

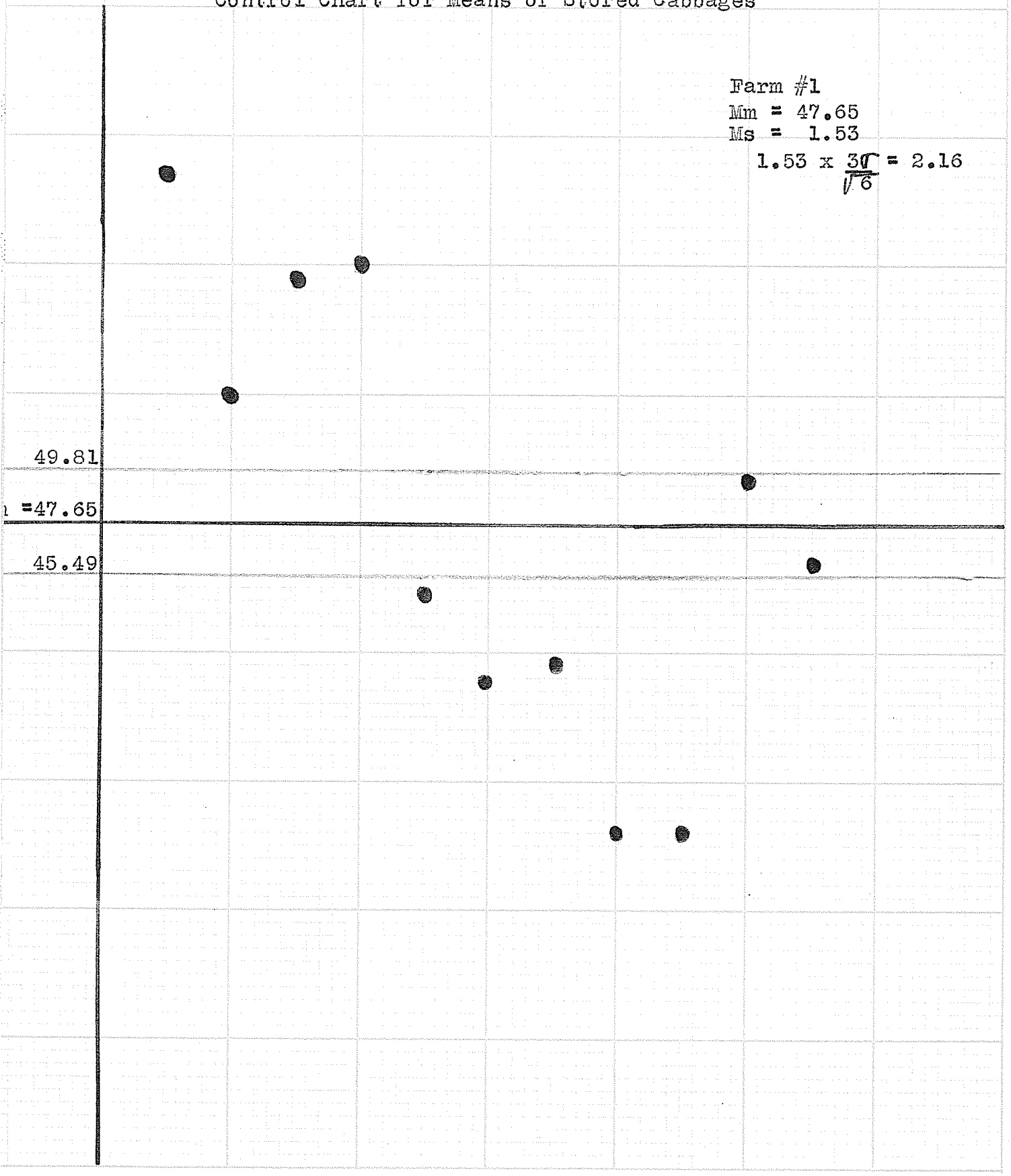


Chart No.6, Figure 8.

Control Chart for Means of Stored Cabbages

Farm #2
Mm = 43.40
Ms = 1.78
$1.78 \times \frac{3\sigma}{\sqrt{6}} = \pm 2.51$

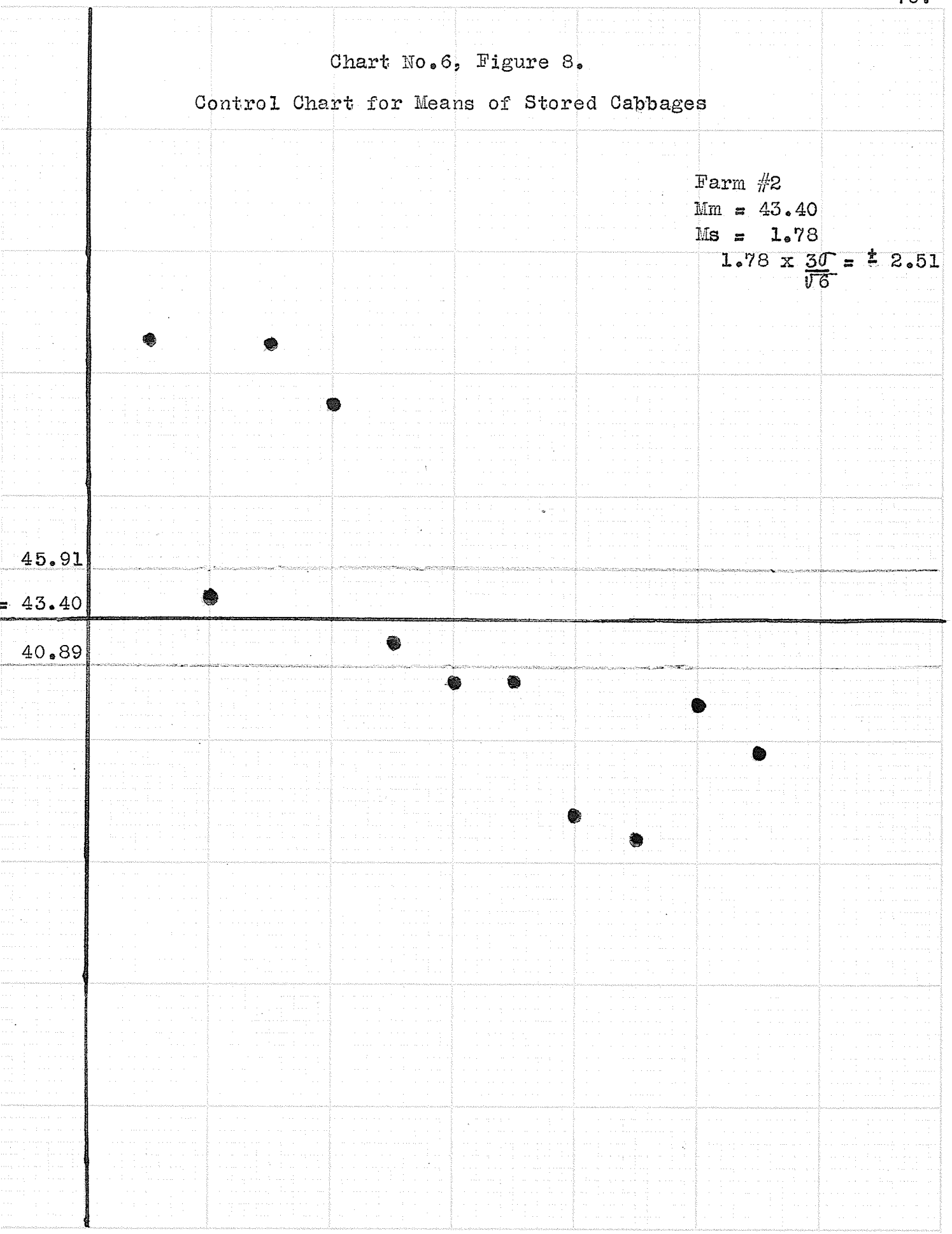


Chart No.7, Figure 9.

Control Chart for Means of Stored Cabbages

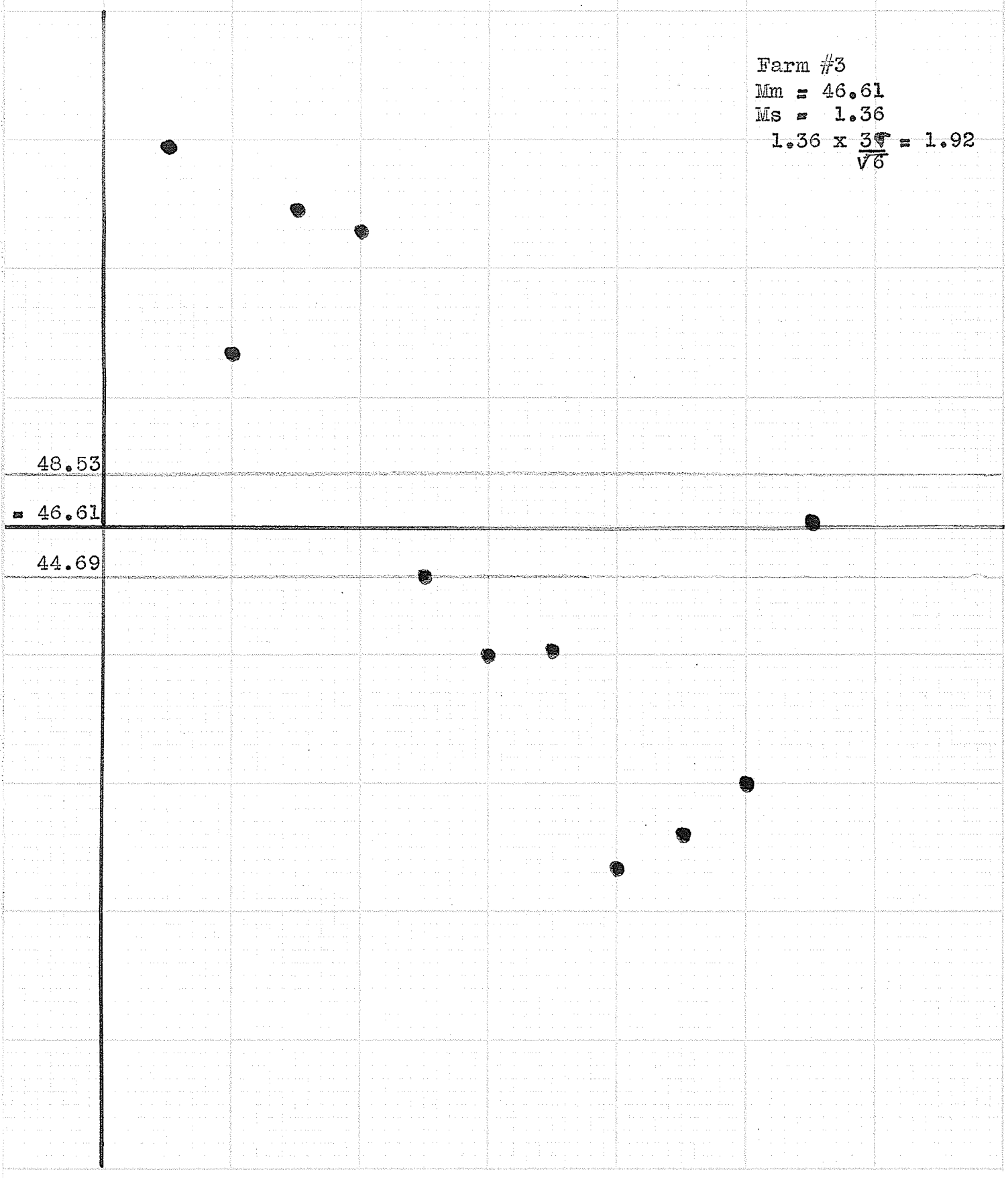


Chart No.8, Figure 10.

Control Chart for 's of Storage Tests

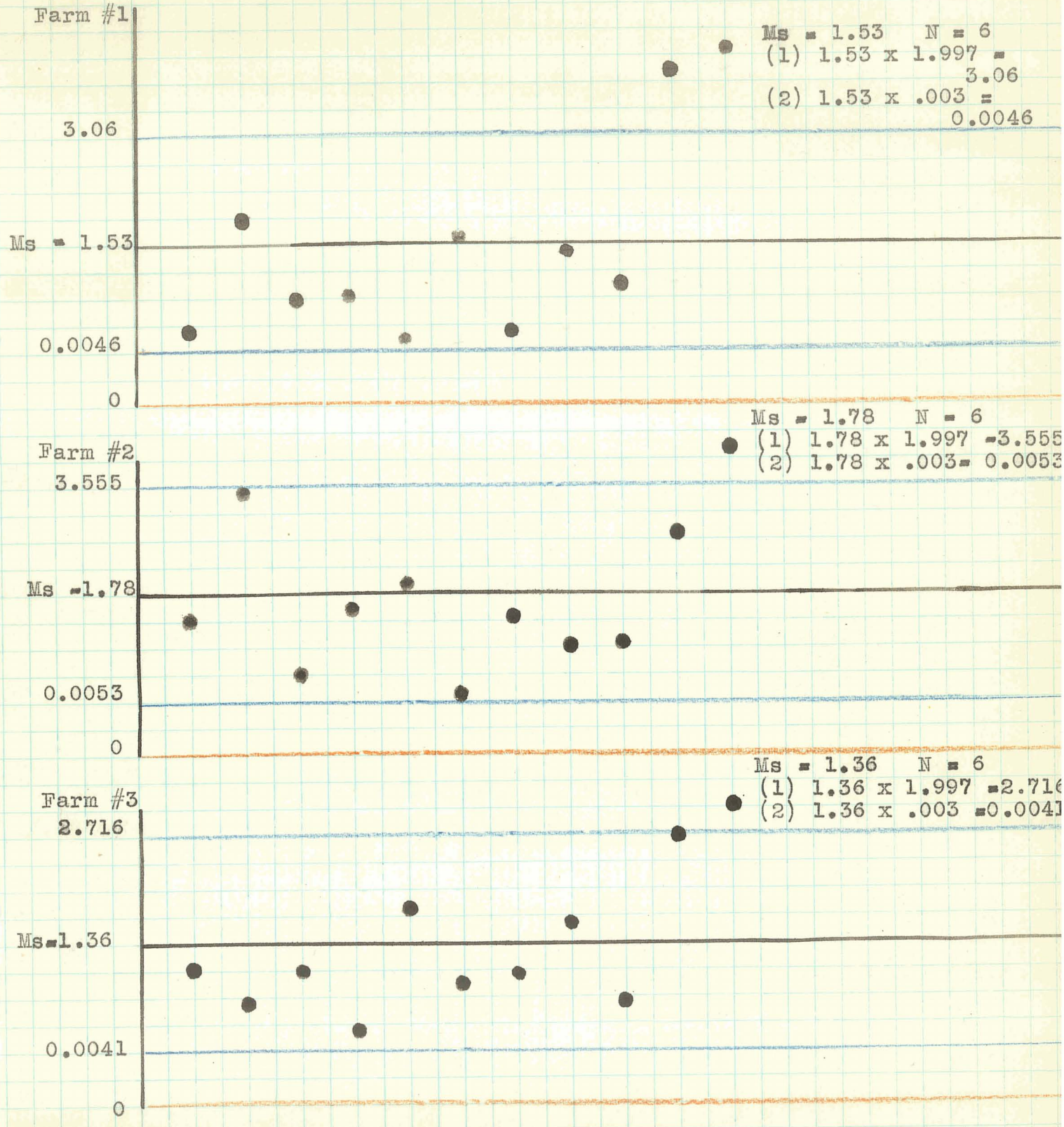
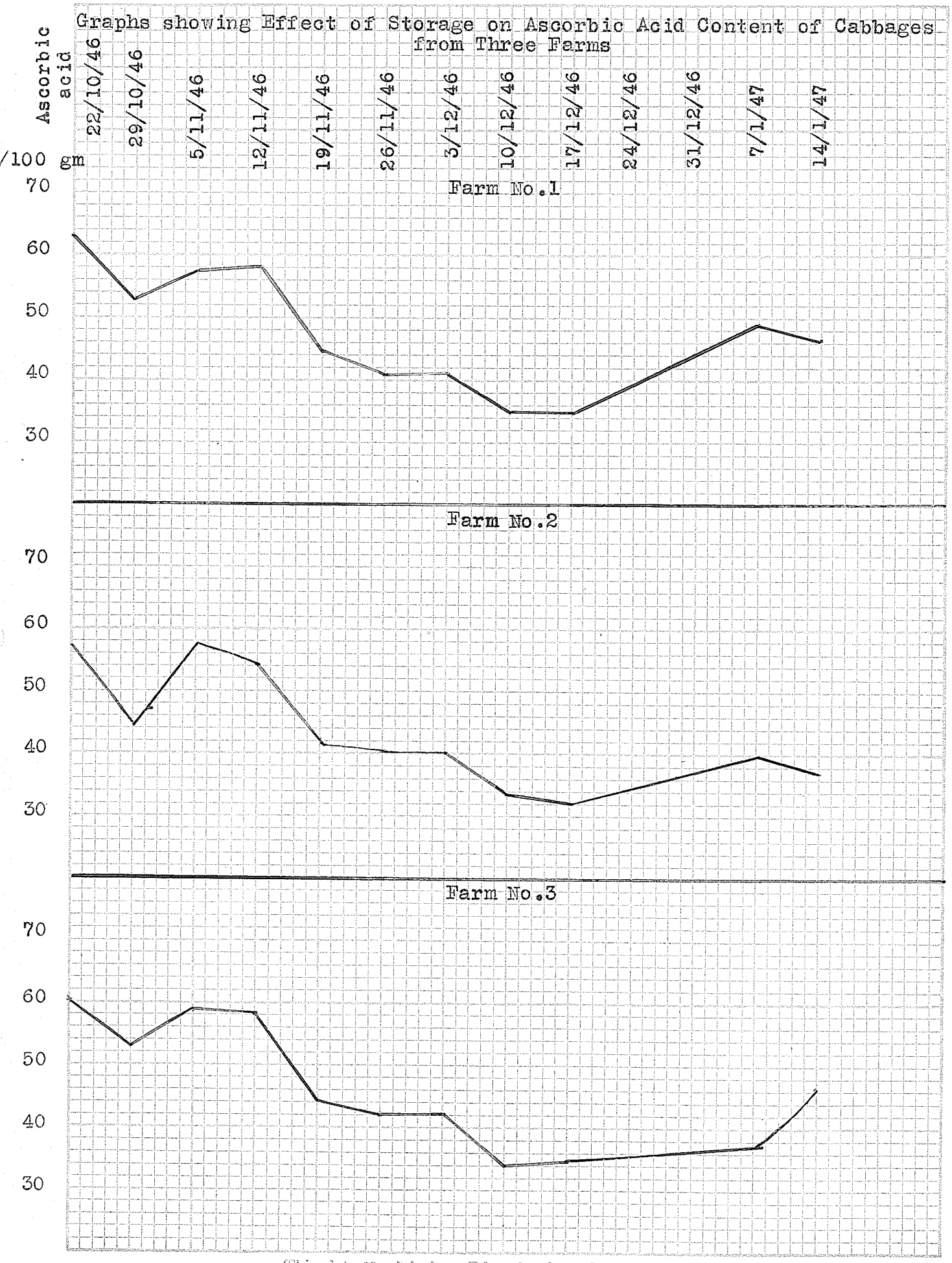


Figure 11




This plate 10 x 7 inches. Edge of each small square 1/10 inch.

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vitamin C to be in reasonably close agreement to that found in this series of assays. For instance, Mayfield and Richardson (44) found a 25% loss in Danish Ballheads stored for six months. Gould and co-workers (20) found that Glory cabbages stored at 5 to 9°C for eighty-four days lost 37% of their original vitamin C content.

That temperature is vitally important in obtaining good storage facilities has been evidenced by the results of many groups of workers, the study by Gould et al (20) already mentioned being typical. This fact was also brought out in the present study. Lots of cabbages from the same plots as were used throughout in the experimental work were stored separately in a vegetable room belonging to the Food Service Department of the University. These cabbages deteriorated much more quickly than those stored in the root cellar, it being necessary to discard them after the beginning of January. The rapid spoilage was no doubt due to the higher temperature of the room, which, as it was not intended for storage purposes for long periods, did not have a uniformly low temperature.

The cabbages used in the tests were all in good condition when assayed. A very small number

showed some spoilage by the middle of January. These were discarded and the remainder had any withered or yellowed leaves removed. In all cases the remainder of the plant was crisp and firm and a good color. There seemed to be no toughening of the fibers and no signs of sprouting during this series of assay.

The moisture tests which were made periodically showed that the percentage never went below 89% (Table IV), which was not sufficient to alter the calculation as used for the fresh samples.

That it would be a simple matter to duplicate these conditions for storage of a winter's supply of cabbage in the ordinary home has also been brought out in this study. The ideal conditions appear to be a temperature of 40°F. or under and a moderately humid atmosphere.

A study of the graphs of Figure 11 shows a fairly steady rate of decrease from the initial values until the middle of December, and then a slight upward trend by the time the analyses were resumed in January. Although dehydroascorbic acid was not measured until late in the series, and apparent vitamin C assays were not carried out on these groups

of cabbages until a few weeks later, the discovery of appreciable amounts of non-vitamin C reductants leads to the assumption that their development may have begun in the cabbages during the latter part of December. As very small amounts of dehydroascorbic acid were found in the cabbages at any time, it is not considered that the reversible form of the vitamin played any significant part in altering the total vitamin value. Such concentrations as did appear are shown in Table VIII. As already noted, Smith and Walker (74) were unable to find appreciable amounts of dehydroascorbic acid in the cabbages studied. Thus, any estimate of the vitamin C content of the cabbages studied in this series is an evaluation of their total reducing power, and not necessarily, as will be shown in the following series of assays, an expression of reduced ascorbic acid only.

It was also observed by making determinations on samples of raw cabbages, representative of the same three farms, at weekly intervals during the series of cooking tests which followed the storage tests, that the slow rate of loss of ascorbic acid concentration continued, but with considerable retention of the original amount. These results,

TABLE VIII

Dehydroascorbic Acid in Stored Cabbage

(Vitamin values expressed as mgm/100 gm)

| Date | Origin | No. of detns. | Dehydro-asc. acid | % of total vitamin |
|---|---|---|---|---|
| Nov. 26 | from 3 farms | 3 each | neg* | --- |
| Dec. 3 | Farm 1 | 3 | 2.1 | 5.0 |
| | Farm 2 | 3 | 2.1 | 5.2 |
| | Farm 3 | 3 | 2.1 | 5.0 |
| Dec. 10 | from 3 farms | 3 each | neg. | --- |
| Dec. 17 | Farm 1 | 3 | neg. | --- |
| | Farm 2 | 3 | 2.1 | 6.5 |
| | Farm 3 | 3 | 2.1 | 6.0 |
| Jan. 7 | from 3 farms | 3 each | neg. | --- |
| Jan. 14 | from 3 farms | 3 each | neg. | --- |
| Jan. 21 | from 3 farms | 3 each | neg. | --- |

*Amounts less than 2.0 mgm/100 gm. but sufficient to cause detectible color change, were considered "negligible".

which are shown in Tables IX(a) and IX(b), indicate that these varieties have the tendency to preserve an appreciable amount of ascorbic acid for as long as their keeping qualities remain good.

A figure which might serve to express the vitamin C potency of stored Ballhead cabbages under normally encountered conditions of storage during the winter months is obtained by averaging the means of the means of the values found for each farm, viz., 47.7; 43.4 and 46.0 mgm per 100 gm which gives a value of 45.0 mgm per 100 gm. As already explained, it is granted that some portion of the total ascorbic acid found in these cabbages by chemical assay was undoubtedly in the form of non-vitamin C reductants; but even with the deduction of a 20% portion of the value as an allowance for the apparent vitamin C content, there remains well over 30 mgm per 100 gm of reduced ascorbic acid.

Since winter cabbages are possibly, and in the case of lower income groups probably, the sole important source of dietary vitamin C for the greater part of the year, it is highly desirable

TABLE IX(c)

Retention of Reduced Ascorbic Acid as Measured by the Spectrophotometer

in Cooked Cabbages and Cooking Water

(All vitamin values in mgm/100 gm)

| Date | Variety | No. of detns. | Raw Cabbages Asc. acid Range | Raw Cabbages Asc. acid Mean | Cooked Cabbages Asc. acid Range | Cooked Cabbages Asc. acid Mean | Cooking $H_2O$ Asc. acid Mean | Total Retent. cabb. + $H_2O$ | % retent. in cabb. | % retent. cabb. + $H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 28/1/47 | Danish Ball-head | 6 (1 raw) | - | 44.7 | 9.0-22.5 | 16.2 | 7.4 | 23.6 | 36.2 | 52.8 |
| 4/2/47 | " | 6 (1 raw) | - | 67.8 | - | - | - | - | - | - |
| 11/2/47 | Ball[a]-head | 3 | 52.9-66.7 | 60.2 | 33.1-49.3 | 42.8 | 10.7 | 53.5 | 71.1 | 88.8 |
| 18/2/47 | " | 3 | 40.2-61.1 | 52.6 | 26.6-53.6 | 37.4 | 9.0 | 46.4 | 61.1 | 88.2 |
| 24/2/47 | " | 3 | 40.2-55.9 | 51.7 | 21.7-24.7 | 23.4 | 9.8 | 33.1 | 45.2 | 64.0 |
| 3/3/47 | Imp-orted | 3 | 35.7-43.9 | 38.9 | 16.3-34.7 | 21.3 | 10.5 | 31.8 | 54.8 | 81.7 |

[#]The method of treatment used in cooking was similar in all cases, viz., boiling for 8 minutes in 4 volumes of water in an uncovered dish.

[a]From this date the cabbages were treated without regard to the particular farm from which they came.

## TABLE IX(b)

### Retention of Reduced Ascorbic Acid as Measured by Titration and Occurrence of Apparent Vitamin C in Cooked Cabbages and Cooking Water

(All vitamin values in mgm/100 gm)

| Date | Variety | No. of detns. | Raw (1) Asc. acid Range | Raw (1) Asc. acid Mean | Raw (2) App. Vit. C Range | Raw (2) App. Vit. C Mean | Raw (3) Total Asc. acid Range | Raw (3) Total Asc. acid Mean | Cooked: Cabbage (4) Asc. acid Range | Cooked: Cabbage (4) Asc. acid Mean | Cooked: Cabbage (5) App. Vit. C Range | Cooked: Cabbage (5) App. Vit. C Mean | Cooked: Cabbage (6) Total Asc. acid Range | Cooked: Cabbage (6) Total Asc. acid Mean | Cooking Water (7) Asc. acid Mean | Cooking Water (8) App. Vit. C Mean | Cooking Water (9) Total Asc. acid | (10) Total asc acid (6)+(9) | % retent. of (4) in cabb. | % retent. of (4)+(7) in cabbage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 28/1/47 | Danish Ball-head | 6 (no raw) | -- | -- | -- | -- | -- | -- | 12.4-13.4 | 13.1 | 5.2-5.6 | 5.4 | 17.9-19.2 | 18.5 | -- | -- | -- | -- | -- | -- |
| 4/2/47 | " | 6 (no raw) | -- | -- | -- | -- | -- | -- | 27.0-33.7 | 29.0 | 9.7-12.0 | 10.9 | 34.0-45.3 | 39.9 | 14.5 | 5.7 | 20.2 | -- | -- | -- |
| 11/2/47 | " | 3 (no raw) | -- | -- | -- | -- | -- | -- | 22.7-28.4 | 24.9 | 9.1-10.3 | 9.7 | 31.8-35.7 | 34.6 | 10.3 | 3.2 | 13.5 | | | |
| 18/2/47 | " | 3 | 33.9-43.3 | 37.8 | 8.1-9.4 | 8.9 | 42.0-52.7 | 46.7 | 13.9-27.2 | 20.5 | 8.7-9.7 | 9.4 | 23.6-35.0 | 29.8 | 9.3 | 3.4 | 12.7 | 42.5 | 54.2 | 79.0 |
| 24/2/47 | " | 3 | 37.6-40.3 | 39.0 | 8.3-9.8 | 9.6 | 47.0-50.1 | 48.6 | 20.1-20.6 | 20.2 | 8.4-9.3 | 9.0 | 28.5-29.6 | 29.2 | 8.8 | 4.2 | 13.0 | 42.2 | 51.8 | 74.3 |
| 3/3/47 | Imp-orted | 3 | 31.6-40.8 | 38.4 | 7.9-8.8 | 8.3 | 44.5-49.6 | 46.7 | 20.7-22.0 | 21.2 | 4.4-5.4 | 4.7 | 25.2-27.4 | 25.9 | 7.8 | 2.1 | 9.9 | 36.8 | 55.2 | 75.5 |

* See Table IX (a)

that this source should rate well as compared to other foods high in antiscorbutic activity. That the Penn State and Danish varieties of the Hollhead cabbage can be relied upon to furnish well over the minimum daily requirement (30 mgm) without overemphasizing them in the diet, and even after they have undergone a long period of storage, has been proved by chemical assay.

For the cooking tests, as for the other series, care was taken to have samples evenly distributed over the entire cabbage, so that mean values and ranges would be representative of all areas. Due to the larger amount of tissue required for each test (in most cases 240 grams were cooked at a time) the minimum number of determinations possible to make from an average sized cabbage was three raw and from six to nine cooked.

Except for the first three or four assays the practice of making determinations by both the photometric and titration method for reduced ascorbic acid was followed. This procedure had a double checking value, for while the spectro-photometer values should be expected to agree reasonably closely with the reduced ascorbic acid

found by difference in the titration method, any wide discrepancies in these values would be presumably accounted for by higher "apparent vitamin C".

Changes in the moisture content due to cooking, never greater than 4% as shown by periodic moisture tests, were not sufficiently large to make a 1% change in the calculation used for the first two series of assays.

Tables IX(a) and IX(b) show the results of the first six cooking tests. This set is grouped for presentation because the method of treatment in cooking the samples was the same throughout. That is, the cabbage was boiled for 8 minutes in an uncovered beaker in 4 volumes of water, the size of the pieces being just as cut by the borer. The water went off the boil when the cabbage was added for approximately two minutes. The column "No. of detns." refers to the cooked vegetable only. In most cases, not more than three assays, one from each level, were made for the raw vegetable. In cases where the three levels of cabbage were cooked simultaneously in the same water, the cheesecloth bags being marked according to the levels, the one sample of cooking water

served for all three levels.

A consideration of the percentage retentions of ascorbic acid in the cooked vegetable and in the vegetable plus the cooking water as shown in Tables IX (a) and (b) shows rather wide ranges. Computation of the mean for the values found photometrically gives an average of 53.7% retention for the cabbage alone and when the cooking water is included it becomes 75.1%. It is noteworthy that whereas the cabbage alone has little more than 50% retention, the addition of the cooking water enhances its value to 3/4 of the original amount.

As "apparent vitamin C" was not determined in the raw vegetables for the first three assays, it was not possible to calculate percentage retentions. The figures shown are valuable only as an indication of the occurrence of apparent vitamin C in these cooked vegetables and cooking water. An illustration of the checking value of the two methods of assay is shown when the figures just mentioned for Table IX(a) are computed for Table X(a). Mean ascorbic acid retentions for cabbage alone and for cabbage plus cooking water are 53.7 and 76.3% respectively.

These values not only agree within themselves but correspond closely with those found by Van duyne et al (84). They found that uncovered cabbage cooked in 4 volumes of water retained 51% of its ascorbic acid.

"Apparent vitamin C" occurred in the cabbages tested at a mean rate which varied from 8.3 to 9.6 mgm/100 gm in the raw vegetable and from 4.7 to 10.9 mgm/100 gm in the cooked vegetable. The percentage changes based on the ratio of "apparent" to total ascorbic acid in the raw vegetable compared with their ratio in the cooked are shown in Table XII.

Some variations of the method of cooking used for the first six assays were tested, the percentage retention of ascorbic acid measured both photometrically and by titration, the occurrence of "apparent vitamin C" noted, and the results tabulated in Tables X(a) and X(b).

An examination of each day's results and a comparison of the values found by the two methods bring out several points regarding cooking practices and their effect on the original vitamin C value of the cabbages.

TABLE X(a)

Comparison of Amounts of Retention of Reduced Ascorbic Acid in Cabbages,

According to Various Cooking Practices, as Measured by the Spectrophotometer

(All vitamin values in mgm/100 gm)

| Date | Variety | No. of* Detns. | Raw Asc. acid Range | Raw Asc. acid Mean | Cooked Method of treatment | Cooked Cabbages Asc. acid Range | Cooked Cabbages Asc. acid Mean | Cooking $H_2O$ Asc. acid Mean | Total Retent. Cabb. + $H_2O$ | % retent. in cabb. | % retent. cabb. + $H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11/3/47 | Ballhead | 3 | 33.1-41.7 | 37.0 | Boiled 8 min. | 15.9-22.4 | 18.8 | 10.5 | 29.3 | 50.8 | 79.2 |
| | " | 3 | | | " 12 " | 14.7-22.8 | 18.4 | 10.8 | 29.2 | 50.0 | 79.0 |
| 17/3/47 | Ballhead | 3 | | | Boiled 8 min. | 16.2-22.7 | 19.4 | 6.1 | 25.5 | 45.5 | 60.0 |
| | | 3 | 40.5-44.7 | 42.6 | " 12 " | 13.4-21.0 | 17.9 | 7.7 | 25.6 | 42.0 | 60.0 |
| | | 3 | | | " 8 min.cov | 21.0-22.7 | 19.1 | 9.4 | 28.5 | 44.8 | 66.9 |
| 24/3/47 | Ballhead | 3 | | | " 8 min) un- | 14.6-22.4 | 19.8 | 8.4 | 28.2 | 55.8 | 79.4 |
| | | 3 | 34.7-37.0 | 35.5 | " 10 " ) cov | 14.6-21.6 | 17.0 | 12.0 | 29.0 | 47.9 | 81.7 |
| | | 3 | | | " 12.5 ) | 14.9-24.6 | 23.1 | 10.1 | 33.2 | 65.1 | 93.5 |
| 31/3/47 | Ballhead | 3 | | | " 8 min) | 20.6-33.5 | 26.3 | 9.1 | 35.4 | 66.4 | 87.1 |
| | | 3 | 35.0-43.2 | 39.6 | " 10 " ) cov | 22.1-26.1 | 25.0 | 9.5 | 34.5 | 63.1 | 89.4 |
| | | 3 | | | " 12.5 ) | 31.3-38.7 | 24.6 | 12.6 | 37.2 | 62.1 | 93.9 |
| 4/4/47 | Imported | 3 | 43.2-46.2 | 44.4 | " 8 min un- | 23.7-32.0 | 27.4 | 10.9 | 38.3 | 61.7 | 86.3 |
| | | 3 | | | " cov. | | | | | | |
| | | | | | " 8 min cov | 23.4-32.8 | 27.7 | 11.1 | 38.8 | 62.4 | 87.4 |
| 7/4/47 | " | 3 | 33.5-36.1 | 34.9 | " 8 min un- | 14.6-20.9 | 18.7 | 7.7 | 26.4 | 53.6 | 75.6 |
| | | 3 | | | cov. | | | | | | |
| | | | | | " 8 min cov | 21.2-23.1 | 22.2 | 6.7 | 28.9 | 63.6 | 82.8 |
| 11/4/47 | " | 3 | | | 2 vol. $H_2O$ | 19.4-25.3 | 22.4 | 9.5 | 31.9 | 65.7 | 93.5 |
| | | 3 | 33.5-34.6 | 34.1 | 10 " $H_2O$ | 14.0-19.4 | 16.0 | 7.0 | 23.0 | 46.9 | 67.4 |
| | | 3 | | | Boil. $H_2O$ | 19.4-34.3 | 26.1 | 8.7 | 34.8 | 76.5 | 102.0 |
| 14/4/47 | " | 3 | 31.0-38.0 | 33.7 | Shredded | 15.7-19.2 | 17.5 | 12.6 | 30.1 | 51.9 | 89.3 |
| | | 3 | | | whole | 12.6-22.3 | 16.2 | 8.4 | 24.6 | 48.1 | 73.0 |

*Three determinations only were made on the raw cabbage, one from each level. The number in the column refers to the cooked cabbages. Usually the cooking water from each level was measured, but not in every case.

TABLE X(b)

Comparison of Amounts of Retention of Reduced Ascorbic Acid in Cabbages, as Measured by Titration According to Method of Cooking, and Occurrence of Apparent Vitamin C.

(All vitamin values in mgm/100 gm)

| | | | Raw | | | | | | Cooked | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Cabbage | | | | | | Cooking Water | | | | | | |
| Date | Variety | No. of* Detns. | (1) Asc. acid Range | (1) Asc. acid Mean | (2) App. Vit. C Range | (2) App. Vit. C Mean | (3) Total Asc. acid Range | (3) Total Asc. acid Mean | (4) Asc. acid Range | (4) Asc. acid Mean | (5) App. Vit. C Range | (5) App. Vit. C Mean | (6) Total Asc. acid Range | (6) Total Asc. acid Mean | (7) Asc. acid Mean | (8) App. vit. C Mean | (9) Total Asc. acid | (1) Total asc. acid - (6) + (9) | % retent. of (4) in cabb. | % retent. of (4) + (7) in cabb. |
| 11/3/47 | Ballhead | 3 | 33.5-37.0 | 35.0 | 5.4-6.1 | 5.6 | 38.9-43.1 | 40.6 | 17.3-19.3 | 18.5 | 3.4-4.0 | 3.7 | 20.7-23.3 | 22.2 | 8.0 | 1.3 | 9.3 | 31.5 | 52.9 | 75.7 |
| | | 3 | | | | | | | 11.6-15.0 | 13.3 | 4.4-5.4 | 4.7 | 17.0-19.4 | 18.0 | 8.0 | 1.3 | 9.3 | 27.3 | 38.0 | 60.9 |
| 17/3/47 | Ballhead | 3 | 35.7-39.6 | 37.2 | 5.8-6.2 | 6.0 | 41.5-45.8 | 43.2 | 17.0-21.3 | 18.7 | 4.0-4.5 | 4.2 | 21.0-25.3 | 22.9 | 8.7 | 2.6 | 11.3 | 34.2 | 50.3 | 73.6 |
| | | 3 | | | | | | | 16.1-20.4 | 17.9 | 3.7-4.0 | 4.1 | 19.8-24.1 | 22.0 | 8.2 | 2.7 | 10.9 | 32.9 | 48.1 | 70.2 |
| | | 3 | | | | | | | 18.1-20.4 | 19.6 | 3.7-4.0 | 4.2 | 22.6-24.4 | 23.8 | 8.1 | 2.9 | 11.0 | 34.8 | 52.7 | 74.5 |
| 24/3/47 | Ballhead | 3 | 32.4-36.2 | 34.3 | 5.6-6.4 | 6.1 | 38.8-42.6 | 40.3 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | | 3 | | | | | | | | | | | | | | | | | | |
| 31/3/47 | Ballhead | 3 | 35.0-43.2 | 35.0 | 4.3-4.6 | 4.5 | 37.3-40.6 | 39.5 | 16.7-18.1 | 17.5 | 4.8-6.1 | 5.2 | 21.5-24.2 | 22.7 | 8.7 | 2.4 | 11.1 | 33.8 | 50. | 74.9 |
| | | 3 | | | | | | | 14.4-16.0 | 15.4 | 4.3-5.8 | 5.0 | 20.2-23.7 | 20.4 | 9.0 | 2.4 | 11.4 | 31.8 | 44. | 69.9 |
| | | 3 | | | | | | | 16.5-17.8 | 17.3 | 4.5-5.1 | 4.7 | 21.1-22.9 | 22.0 | 9.2 | 3.1 | 12.3 | 34.3 | 49.4 | 75.7 |
| 4/4/47 | Imported | 3 | 36.2-37.5 | 36.8 | 4.3-4.3 | 4.3 | 40.5-41.8 | 41.1 | 22.4-27.3 | 24.6 | 3.1-3.3 | 3.2 | 25.7-30.6 | 27.8 | 7.8 | 2.1 | 9.9 | 37.7 | 66.8 | 88.0 |
| | | 3 | | | | | | | 24.0-30.8 | 26.3 | 3.1-3.8 | 3.5 | 27.1-34.6 | 29.8 | 8.9 | 2.1 | 11.0 | 40.8 | 71.5 | 95.7 |
| 7/4/47 | Imported | 3 | 28.9-30.4 | 29.7 | 5.0-5.0 | 5.0 | 33.9-35.4 | 34.7 | 13.2-15.8 | 14.6 | 4.2-5.0 | 4.6 | 17.4-20.2 | 19.2 | 6.7 | 2.1 | 8.8 | 28.0 | 49.9 | 71.7 |
| | | 3 | | | | | | | 16.2-17.2 | 16.9 | 4.2-5.0 | 4.7 | 20.4-22.2 | 21.6 | 6.7 | 2.1 | 8.8 | 30.4 | 56.9 | 79.4 |
| 11/4/47 | Imported | 3 | 25.9-27.7 | 27.0 | 6.5-7.1 | 6.7 | 32.4-34.8 | 33.7 | 16.8-20.1 | 18.5 | 5.8-5.8 | 5.8 | 22.6-25.9 | 24.3 | 9.6 | 1.9 | 11.5 | 35.8 | 68.5 | 104.1 |
| | | 3 | | | | | | | 12.9-17.8 | 14.9 | 4.4-4.8 | 4.5 | 17.3-22.6 | 19.4 | 9.3 | 1.5 | 10.8 | 30.2 | 55.2 | 95.2 |
| | | 3 | | | | | | | 16.2-27.4 | 21.4 | 5.8-6.5 | 6.0 | 22.0-33.9 | 27.4 | 8.3 | 2.5 | 10.8 | 38.2 | 79.3 | 119.3 |
| 14/4/47 | Imported | 3 | 25.3-29.1 | 27.1 | 4.5-5.2 | 4.7 | 29.8-34.3 | 31.8 | 15.5-15.5 | 15.5 | 3.0-3.7 | 3.3 | 18.5-19.2 | 18.8 | 11.0 | 3.0 | 14.0 | 32.8 | 57.2 | 97.8 |
| | | 3 | | | | | | | 13.3-17.8 | 15.0 | 3.0-3.0 | 3.0 | 16.3-20.8 | 18.0 | 9.5 | 2.2 | 11.7 | 29.7 | 55.3 | 90.4 |

* See Table X(a)

1. When a Ballhead cabbage was boiled for 6 minutes, the percentage retention of ascorbic acid as measured by both methods was in close agreement with the mean value found in the previous six assays. The 12 minute cooking period did not cause a destruction due to longer cooking according to Table X(a), but Table X(b) does show what we should expect to see according to the literature. The absolute values of "apparent vitamin C" of Table X do not convey as much meaning as their relative increases found in Table XII. As a greater development of "apparent vitamin C" took place during the four minutes of extra cooking, it is this factor which is suspected as the cause of the higher value of Table X(a).

2. The cabbage assayed March 17 was treated in the same way as that done the week before, with an additional test of covering the beaker for the 6 minute boiling period. There was a slightly favorable effect shown by both means of measurement, for the 6 minute cooking over the 12 minute, both in the cabbage alone, and in the cabbage plus cooking water. There should be greater retention than is shown in the third trial if the results of several

investigators, as Noble and Waddell (58) are correct. Further tests were made later in which the covering was the only variable factor. The results are discussed in the paragraph concerning tests No.5 and 6.

3. The length of the cooking period using an uncovered dish for cabbages cooked in four volumes of water was tested. The spectrophotometer showed results which conflicted with similar tests discussed in paragraph No.4.

4. The following assay also tested for relative retention of ascorbic acid in 8, 10 and 12.5 minute cooking periods and this time the vegetable was covered. Table X(a) showed best retention for the shorter cooking period for the cabbage alone and increasing leaching into the cooking water according to the length of time for cooking. Table X(b) shows approximately the same situation but the final column of both tables suggests a different deduction because of the opposite trends within the two values of which it is composed.

It may be added here that any conclusions regarding length of cooking period are not very definite or clear cut, the majority of investigators

finding only slight advantages in the shorter time for cooking. For instance, Noble and Waddell (58) found that one variety of cabbage showed very little decrease in ascorbic acid retention when cooked for 12 as against 4 minutes and the Danish Ballhead variety showed little difference when cooked for 10 as against 12.5 minutes. Van duyne, Chase and Simpson (84) report similarly on cooking cabbages for periods of different lengths. When cabbage was boiled in twice its weight of water for 7, 15 and 25 minute periods 55, 52 and 52% respectively of the original ascorbic acid remained.

When the retention of the covered cabbage of March 31 is compared with that for the uncovered lots of the previous week, a considerable advantage due to covering, according to both methods of measurement of ascorbic acid appeared.

5 and 6. The same conclusions in regard to covering the vegetable while cooking for greater conservation of the vitamin are made from an observation of the two assays done on the 4th and 7th of April. The differences are a little more marked in Table X(b) where the "apparent vitamin C" does not influence the values.

7. The samples were cooked for 8 minutes, covered in variable amounts of water in the assays of April 11. The last three samples were added gradually to four volumes of water so that it never went off the boil, before covering the vegetable. The relative rates of retention are in accordance with the results of similar trials found in the literature, but the too-high value for the set of samples added to continuously boiling water first leads to the assumption that "apparent vitamin C" may be to blame. Examining Table X(b) merely shows "apparent vitamin C" concentration to be in line with that found for other samples of cooked vegetable studied. This table also shows impossibly high retentions for reduced ascorbic acid.

Consulting Table XII for percentages of apparent vitamin C throws no light on the matter except to show that the amount of non-vitamin C reductants was very high in the raw vegetable.

Allowing for a certain amount of experimental error in this assay it is considered that they confirm the findings of Van duyne et al (84), which showed that there is a definite advantage in

cooking in as small a volume of water as possible; also that adding the vegetable gradually to the boiling water, so that the high temperature inactivates the enzymes as quickly as possible, makes for better conservation of vitamin C value.

This last conclusion is also brought out convincingly by the data of Gould, Tressler and King (20). By means of tables and graphs they showed that approximately one-quarter of the total ascorbic acid is destroyed before the cooking water begins to boil and that very little is lost thereafter, although a considerable portion is dissolved in the cooking water.

8. Whether large pieces or finely shredded cabbage is preferable from the viewpoint of ascorbic acid retention was tested on April 14th. A sharp, stainless steel knife was used to cut 100-gram sections, and to finely shred the cabbage, care being taken not to bruise the tissue. The cooking was done in two volumes of water for 8 minutes in a covered beaker. Tables X(a) and X(b) both show very little difference in the amount of ascorbic acid retained by the cabbage tissue, but a much greater amount in the water from the

shredded cabbage. Wellington and Tressler (88) encountered the same results with their cabbage cooking tests and concluded that finely shredded cabbage with the cooking water retained more ascorbic acid than if it were boiled in strips or quarters. This is explained by the fact that the heat does not penetrate the larger pieces as quickly as the shredded or finely chopped cabbage, so that the ascorbic acid oxidases continue their destruction for a longer time before they are inactivated.

Dehydroascorbic acid determinations, as well as those for reduced and apparent forms of vitamin C, were made on each cabbage used for the cooking tests. With the exception of the first four assays, both raw and cooked samples were measured for the purpose of demonstrating any change due to the cooking process.

An increase would be a confirmation of the findings of Wokes et al (90) and Tuba, Hunter and Steele (82) with the important difference that their studies were made with foodstuffs subjected to comparatively high temperatures for a longer period of time.

As already noted, assays made on raw

vegetables during and after the storage tests revealed small or insignificant amounts of dehydroascorbic acid. Table VIII, page 83, showed the percentage of the total vitamin content in the form of dehydroascorbic acid.

Because the proportion of dehydroascorbic acid to the total vitamin C content of these cabbages was so small and any change in amount of dehydroascorbic acid due to cooking practices so difficult to measure, no attempt was made to find percentage retentions. Net amounts as they appeared are recorded. Amounts which were present in sufficient concentration to make a slightly visible color change after incubation of samples, but less than 2.0 mgm/100 gm were recorded as "negligible".

Table XI presents the results of the analyses for dehydroascorbic acid. By matching the dates shown in this table with those of Tables IX(a) and IX(b) the corresponding concentrations for the other forms of the vitamin are seen.

In most cases only one determination was made on the cooking water.

Comparison of the dehydroascorbic acid concentrations before and after cooking shows no

TABLE XI

Dehydroascorbic Acid in Fresh* and Stored Cabbages Before and After Cooking

(Concentrations in mgm/100 gm)

| Date | Variety | No. of detns. | Dehydro-asc.acid raw | Dehydro-asc.acid cooked | Dehydro-asc.acid cooking $H_2O$ | Date | Variety | No. of detns. | Dehydro-asc.acid raw | Dehydro-asc.acid cooked | Dehydro-asc.acid cooking $H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 28/1/47 | Danish Ballhead | 6 | --- | 2.0 | nil | 17/3/47 | Ballhead | 3 | 2.1 | neg. | nil |
| 4/2/47 | " | 6 | --- | 2.7 | nil | 24/3/47 | " | 3 | 2.2 | neg. | nil |
| 11/2/47 | " | 3 | --- | 2.4 | nil | 31/3/47 | " | 3 | 2.1 | neg. | nil |
| 18/2/47 | " | 3 | --- | 2.9 | nil | 4/4/47 | Imported | 3 | 2.1 | neg. | nil |
| 25/2/47 | " | 3 | neg. | neg. | nil | 7/4/47 | " | 3 | neg. | nil | nil |
| 3/3/47 | Imported | 3 | neg. | nil | nil | 11/4/47 | " | 3 | neg. | nil | nil |
| 11/3/47 | Ballhead | 3 | neg. | nil | nil | 14/4/47 | " | 3 | neg. | nil | nil |

*The imported cabbages were fresh

significant effect due to the cooking process. Because such amount of dehydroascorbic acid which may be present in fresh or stored cabbages is not noticeably altered during the cooking process, it is concluded that the presence of the reversible form of vitamin C does not appreciably influence the antiscorbutic property of cabbages.

That the development of non-vitamin C reductants might be favored during cooking in accordance with the findings of Wokes and associates (91) was considered possible and so a comparison of the relative amounts of apparent vitamin C before and after cooking was made. As the first three determinations did not include the measurement of "apparent vitamin C in the raw vegetable, per cent changes could not be calculated. The following sets of determinations showed increases in every case, however. These values are shown in Table XII. They were computed by comparing the proportion of "apparent vitamin C" in the raw cabbages, expressed as a percentage of the total, and that found by adding the amounts in the cooked cabbage and cooking water, (column 5 column 8 of Tables IX(b) and X(b), and expressed

## TABLE XII

Percentage change in "Apparent Vitamin C" Content of Cabbages Before and After Cooking

("apparent vitamin C" values in percentages)

| Date | Variety | No.of detns. | Method of treatment | App.vit.C raw* cabb. | App.vit.C in cooked cabb.+ cooking $H_2O$ | % change after cooking |
|---|---|---|---|---|---|---|
| 18/2/47 | Ballhead | 3 | Boiled 8 min. uncovered in 4 vol. $H_2O$ | 19 | 23.1 | 4.1 |
| 24/2/47 | " | 3 | " | 19.9 | 31.3 | 11.4 |
| 3/3/47 | Imported | 3 | " | 17.7 | 19.0 | 1.3 |
| 11/3/47 | Ballhead | 6 | Boiled 8 min<br>" 12 min | 13.0 | 15.9<br>19.0 | 1.4<br>6.0 |
| 17/3/47 | " | 9 | " 8 min<br>" 12 min<br>cov. 8 min | 13.2 | 20.0<br>20.7<br>20.4 | 6.8<br>7.5<br>7.2 |
| 24/3/47 | " | 9 | Boiled(8 min<br>uncov(10.0 "<br>(12.5 " | 14.9 | -- | --- |
| 31/3/47 | " | 9 | Boiled(8 min<br>(10.0 "<br>cov.(12.5 " | 11.4 | 22.5<br>20.8<br>22.9 | 11.1<br>9.4<br>11.5 |
| 4/4/47 | Imported | 6 | uncov. 8 min<br>cov. 8 min | 10.5 | 14.1<br>13.7 | 3.6<br>3.2 |
| 7/4/47 | " | 6 | uncov. 8 min<br>cov. 8min. | 14.4 | 23.9<br>22.4 | 9.5<br>8.0 |
| 11/4/47 | " | 9 | 2 vol. $H_2O$<br>10 vol. $H_2O$<br>Boiling $H_2O$ (4 vol) | 19.9 | 21.5<br>19.9<br>22.2 | 1.6<br>0.0<br>2.3 |
| 14/4/47 | " | 6 | Shredded<br>whole | 14.9 | 19.1<br>17.5 | 4.2<br>2.6 |

*3 detns. each for raw cabbage.

as a percentage of the total ascorbic acid content (column 9).

Although the increases are consistent in that they appeared to some degree in every test, yet they are inconsistent when an attempt is made to correlate the amount of increase with the length of cooking period, or other variable factor.

It is considered possible that definite trends would be evident if this part of the study had been carried out on a much larger number of samples. The observations made by several groups of workers, for example Levy (38) and Tuba, Hunter and Steele (82) show general agreement in that oxidation and heat are considered largely responsible for the development of reductones and other forms of vitamin C having no biological value.

Table XII also provides figures which may serve as a basis for estimating the proportion of "apparent vitamin C" to be expected in stored winter cabbages, from the values which are shown for the raw Ballhead varieties. It was from the computation of the values for Table XII that the range of 11.4 to 19.9% "apparent vitamin C" was

found, the upper limit of which was used to express the mean value on page 84.

Very little information was obtainable in regard to the dehydrated cabbage on hand. As any conclusions regarding ascorbic acid content would be of little practical value when such details as variety, method of dehydration and length of storage after processing were lacking. only one series of determinations was made on each tin.

The unknown variety gave a mean concentration of ascorbic acid of 36.1 mgm/100 gm and the Ballhead had a mean value of 14.6 mgm/100 gm as determined by the spectrophotometer, and as shown in Table XIII. Respective dehydroascorbic acid and "apparent vitamin C" concentrations were 7.3; 8.3 and 8.6; 7.1 mgm/100 gm. It was noted that the unknown variety was a good color, much like fresh cabbage, while the Ballhead was very brown.

These assays proved of value in that they showed the tendency which is most noticeable in reports of similar analyses of dehydrated cabbage, such as those of Mapson (43) and Morgan et al (54), viz., a greater proportion of dehydroascorbic acid

## TABLE XIII

Reduced Ascorbic Acid, Dehydroascorbic Acid and "Apparent Vitamin C" Content of Two Lots of Dehydrated Cabbage

(vitamin values in mgm/100 gm)

| Variety | No.of detns. | Asc.acid (spectro) Range | Asc.acid (spectro) Mean | Dehydro asc.acid Range | Dehydro asc.acid Mean | Asc.acid (titration) Range | Asc.acid (titration) Mean | Apparent vitamin C Range | Apparent vitamin C Mean |
|---|---|---|---|---|---|---|---|---|---|
| Unknown | 2 | 35.7-36.5 | 36.1 | 7.2-7.4 | 7.3 | 25.5-26.2 | 25.9 | 8.3-8.3 | 8.3 |
| Ballhead | 2 | 14.2-14.9 | 14.6 | 7.8-9.5 | 8.6 | 8.6-8.6 | 8.6 | 6.8-7.5 | 7.1 |

and of "apparent vitamin C" than in the untreated vegetable. That dehydrated cabbage is a very undependable source of reduced ascorbic acid is inferred from this assay.

## SUMMARY

1. A method of sampling has been devised, which, by the use of a specially designed borer, cuts cross-sections of a cabbage head so that when the data from a large number of these, taken over all parts of the head, are analyzed, a mean value is obtained which is a reliable estimate of the ascorbic acid content of that cabbage.

2. Three lots of fresh winter cabbages when analyzed showed mean ascorbic acid values of 60.6 ± 2.1; 55.2 ± 2.7; and 63.1 ± 2.1 mgm/100 gm.

3. Cabbages from the same three market gardens as the fresh lots, when stored under conditions adequate for maintaining them in a satisfactory condition for 4 to 5 months, and analyzed weekly, showed an overall loss in ascorbic acid potency of 25.1; 30.7; and 22.9% by the end of 11 weeks. The mean ascorbic acid values for the three lots for this period were 47.7±2.2;

43.4±2.5; and 46.6±1.9 mgm/100 gm respectively. Dehydroascorbic acid was not present in sufficient concentration to alter the antiscorbutic properties of the vegetables. "Apparent vitamin C" assays made on similar lots of cabbages after the storage tests, showed that this form of the vitamin never exceeded the concentration of 20% of the total ascorbic acid content.

4. The effect of various cooking practices on the total ascorbic acid retention of cabbages was investigated, the reduced ascorbic acid being measured by both photometry and titration. Cooking in an uncovered dish for 8 minutes in 4 volumes of water was repeated 6 times giving data from 24 determinations. The mean reduced ascorbic acid for the cabbage tissue was 53.7% of the original and that found in the cooking water increased the value to 76.3%. The "apparent vitamin C" content of these cooked cabbages varied from 4.7 mgm/100 gm to 10.9 mgm/100 gm. No appreciable amount of dehydroascorbic

acid was encountered.

Results of variations of the method of cooking showed general agreement with those of similar cooking trials published in the literature which establish the following factors as being conducive to optimum retention of vitamin C in cabbage; small volumes of water; exclusion of air by covering the cooking vessel, and reducing the amount of exposed surface by preparing cabbage in large pieces; short cooking period; and adding the vegetable to continuously boiling water.

# APPENDIX A.

## Ascorbic Acid Determination

It was necessary to make slight modifications in the Loeffler and Ponting (39) method because of the substitution of a Coleman spectrophotometer for the Evelyn colorimeter.

The apparatus used included:

A Waring Blendor, for the macerating of the vegetable tissue. This device quickly accomplishes the reduction of the plant tissues to a pulp without causing loss of ascorbic acid.

A Coleman Universal Spectrophotometer Model 11 with filter PC-4, of a special glass which levels out the response of the photocell in the visible region. The general principles of the single cell photoelectric colorimeter are: A beam of light falls on a photocell which produces a deflection in a galvanometer to which it is connected. If an absorption cell containing a colored solution is placed between lamp and photocell, the percentage of light transmitted through the solution is proportional to the ratio of the final to the initial galvanometer deflection. The concentration of the colored

substance in the sample can then be read from a chart showing the variation of light transmission with concentration. Increased accuracy is obtained by using color filters which transmit only that portion of the spectrum in which the solution has the correct degree of absorption. In the Coleman spectrophotometer a movable diffraction grating obviates the necessity of using color filters.

Two 8-volt lead storage batteries. A battery charger was used for recharging. By alternating their use, it was possible to recharge one battery while the other was in use.

Cuvettes 112 mm. long and 19 mm. in external diameter. To calibrate these tubes, they were washed, filled to the half-way mark with distilled water, the wave band selector set at 440 mμ. By taking the transmission readings on all tubes, they were matched in sets of three, and marked.

Whatman No. 12 24.0 cms. fluted filter paper.

Gram scales for the measurement of samples. Fine balances for weighings for moisture tests,

and in making up all solutions.

A special cutting device, designed to make a complete cross-section of the cabbage head, which would be representative of outer, middle and center leaves. Based on the idea of an apple corer, it had a sharp, saw-toothed cutting edge, with a diameter and cylindrical length calculated to cut approximately 25 grams of cabbage. The material was steel and in order to render it absolutely resistant to any action by the vegetable juices, it was electroplated with chromium. A wooden plunger aided in expelling the sample from the borer.

Reagents used were:

Sodium 2,6-dichlorobenzenone indophenol (Eastman Kodak Co.). A stock solution of the concentration 800 mgm per litre was prepared. This was stable for at least a month. A working solution of the concentration 16 mgm of the stock solution per litre of distilled water was prepared and standardized daily.

1 per cent metaphosphoric acid.

Synthetic ascorbic acid crystals for use in calibrating the standard curve. To calibrate the standard curve, a fresh solution of ascorbic

acid was prepared by dissolving 125 mgm. in 250 ml. of 1% metaphosphoric acid. One to ten ml. amounts by 1 ml. increments, were added to 100 ml. volumetric flasks, which were then made to volume with the 1% metaphosphoric acid. These contained from 5 to 50 mgm. ascorbic acid per ml. 9 ml. portions of the dye solution were added to each of a series of matched cuvettes. The instrument was set at 100 with distilled water. A blank reading was obtained using 9 ml. of the standardized dye solution and 1 ml. of 1% metaphosphoric acid. Readings of the ten samples were taken until checks differing by not more than 0.5 drum dial units were obtained. The logarithm of the readings (Gs) minus the logarithm of the standardized dye solution ($G_b$) were plotted against the ascorbic acid content in micrograms per ml.

In Table XIV, page 114, the data obtained for the calibration are presented. By applying the method of least squares, the equation for the line best satisfying the experimental points was found to be Y = 0.0066X+0.0050, where X = micrograms ascorbic acid per milliliter and

TABLE XIV

Calibration data for Photometric Determination of Ascorbic Acid

| Micrograms per ml. | Log $G_s$ - Log $G_b$ | Calculated Log $G_s$ - Log $G_b$ | (Calculated - Observed)$^2$ |
|---|---|---|---|
| 5 | 0.0208 | 0.0380 | 0.000196 |
| 10 | 0.0637 | 0.0710 | 0.000053 |
| 15 | 0.1063 | 0.1040 | 0.000006 |
| 20 | 0.1461 | 0.1370 | 0.000083 |
| 25 | 0.1669 | 0.1700 | 0.000010 |
| 30 | 0.2149 | 0.2030 | 0.000142 |
| 35 | 0.2441 | 0.2360 | 0.000066 |
| 40 | 0.2711 | 0.2690 | 0.000004 |
| 45 | 0.2957 | 0.3020 | 0.000040 |
| 50 | 0.3347 | 0.3350 | 0.000000 |

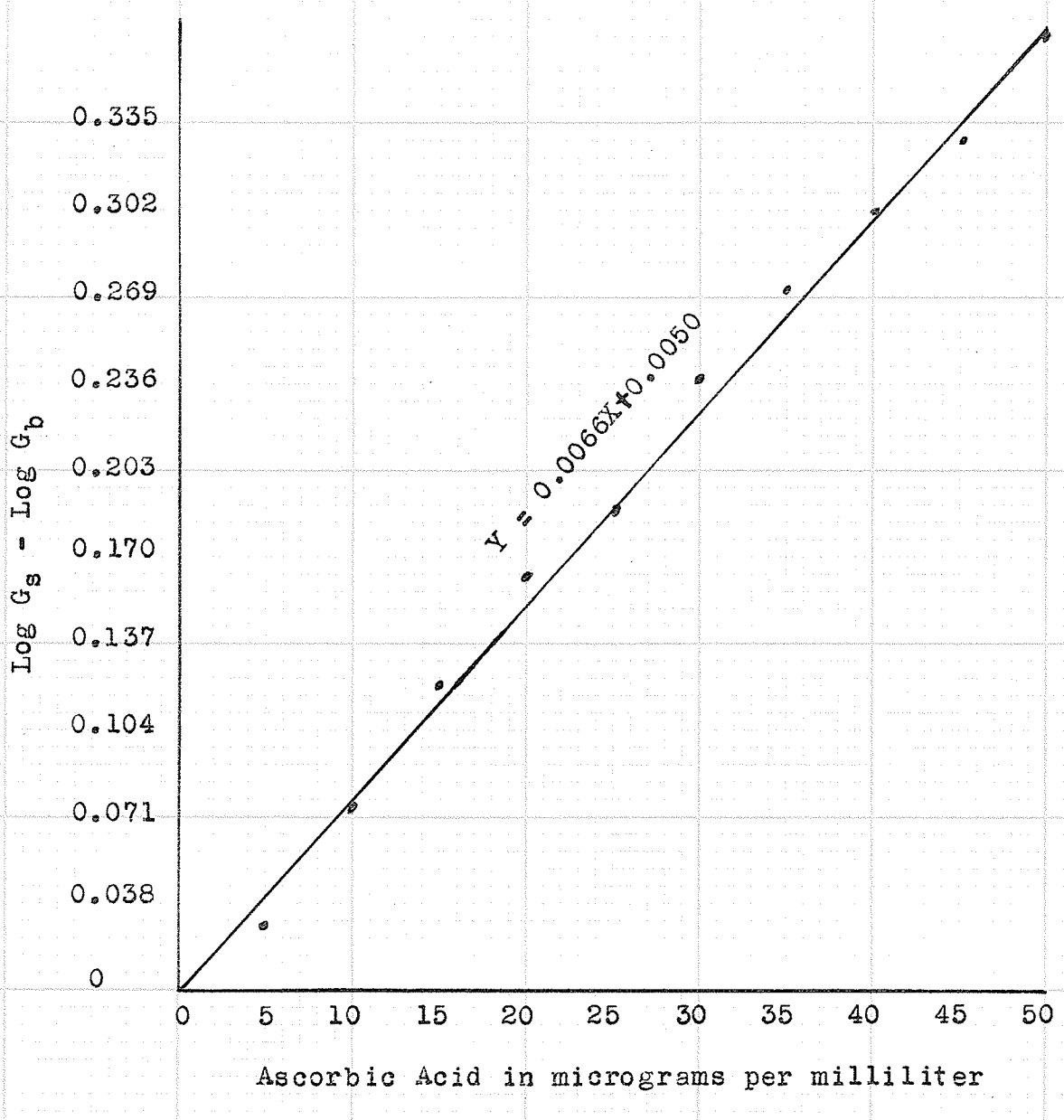
Y = logarithm of drum scale readings for samples minus that for the blank (Figure 12).

The dye was standardized by noting the 15-second reading with light at 520 mμ wave length given by a tube containing 1 ml. of 1% metaphosphoric acid and 9 ml. of the dye solution, and the instrument calibrated to 100 with distilled water. This was done in duplicate. Tubes which differed by more than 0.5% transmittance were rejected.

The procedure for the assay of a sample of cabbage as practised in the laboratory is presented in full: Using the specially designed borer, a sample is removed and 25 grams weighed to the nearest 0.1 gms. When assaying vegetable tissue of low ascorbic acid content a 50 gm sample is needed. The sample is blended with 350 ml. of 1% metaphosphoric acid in the Waring Blendor operated for 5 minutes at high speed. The extract is filtered through fluted Whatman filter paper. Moderate turbidity does not interfere, since the instrument is calibrated with proper blanks. When approximately 100 ml. has filtered through, 1 ml. aliquots are

Figure 12.

Standard Curve for Photometric Determination of Vitamin C using Light with a 520 mμ Wave Length



pipetted into three matched cuvettes. To one tube 9 ml. of distilled water is added as a blank. To each of the other tubes 9 ml. of the previously standardized indophenol dye solution is added from a rapid delivery pipette, and quickly stirred before placing in the colorimeter.

In making readings from the spectrophotometer, the null method, by which readings are made directly from the drum scale, was used. This eliminates possible galvanometer errors and has a precision of about $\pm 0.5\%$, which is considerably greater than that obtainable by direct readings from the galvanometer scale.

The first few steps in the operation of the colorimeter are completed before placing the reference sample in the holder, viz., the wave length dial is set at 520 and the galvanometer dial is made to register zero by means of the coarse knob. The "OFF-GAL-ON" switch is turned to "ON" and the instrument is allowed to warm up for about 5 minutes before making readings.

The reference sample is placed in the beam for transmittance measurement. Then the drum dial is quickly set at 100% by means of the "BAL" knob,

and the galvanometer is brought to zero by the fine "GALV" knob. The sample to be measured is moved into the beam. The drum dial is rotated until the galvanometer again reads zero. The % transmittance is then read from the scale of the drum dial.

The actual reading operation should not require more than a total of 15 seconds after the start of the dye addition. The duplicate tubes must agree to within 0.5 units, otherwise they are rejected. If the values are not identical, their mean value is used. This reading is $G_2$ from which the corresponding $L_2$ value is obtained by subtracting the logarithm of the dye reading from the logarithm of the sample reading. This $L_2$ value is referred to the standard curve and the concentration of ascorbic acid per milliliter of filtrate is obtained.

In order to calculate the amount of ascorbic acid per 100 grams of sample, it is necessary only to determine the ascorbic acid concentration of the filtrate (per milliliter of liquid phase) and to know the total volume of liquid.

A single extraction and only a few millilitres of liquid are necessary. The total amount of liquid is the sum of the volume of the $HPO_3$ solution added plus the liquid originally present in the sample. The calculation required to determine the amount of ascorbic acid per 100 grams of vegetable tissue is as follows:

$$\frac{100\ (\text{micrograms ascorbic acid})\left[350+(\%\text{moisture})(\text{wt. of sample})\right]}{1000\ (\text{wt. of sample})}$$

When calculating from the data of cooking water or dehydrated cabbage 100% moisture in the first case and no moisture in the second made any adjustment for moisture unnecessary.

## APPENDIX B

### Dehydroascorbic Acid Determination

Dehydroascorbic acid assays were made according to the method of Roe and Oesterling (68) which, as has been mentioned, is a colorimetric method based on the quantitative reduction of the reversibly oxidized form of ascorbic acid by means of the dye 2,4-dinitrophenylhydrazine.

The working solution concentration of the dye was 2% 2,4-dinitrophenylhydrazine in approximately 9N $H_2SO_4$ and was made up weekly in volumes of 100 ml. at a time.

5% metaphosphoric acid containing 1% thiourea was used for stabilizing the ascorbic acid.

A calibration curve and chart were made with standard solutions of dehydroascorbic acid in concentrations ranging from 0.004 to 0.032 micrograms per milliliter. To prepare the dehydroascorbic acid standard a solution of 25 milligrams of ascorbic acid in 25 ml. of 5% metaphosphoric acid was treated with 1 or 2

drops of bromine, shaken until yellow, decanted from the excess of bromine into a large test-tube, and aerated until colorless. Standards of appropriate concentrations were made by diluting with 5% metaphosphoric acid containing 1% thiourea. After preparing the standards in the same manner as will be described below for the preparation of the samples, they were read in the instrument which was calibrated with a blank in the same way as for ascorbic acid, but with the wave-length knob set at 540 mu units.

Table XV shows the data obtained for the calibration of the reference curve. By the method of least squares it was found that the type of curve which was a best fit for the data was the hyperbolic, and the equation best satisfying the curve was $Y = 0.0056 + \frac{0.0051}{X}$, where X = micrograms dehydroascorbic acid per milliliter and Y = antilog. of (log. of drum drum scale - log. of blank), as shown in Figure 13.

The procedure for assaying samples of cabbage for dehydroascorbic acid according to the method of Roe and Oesterling (68) is as

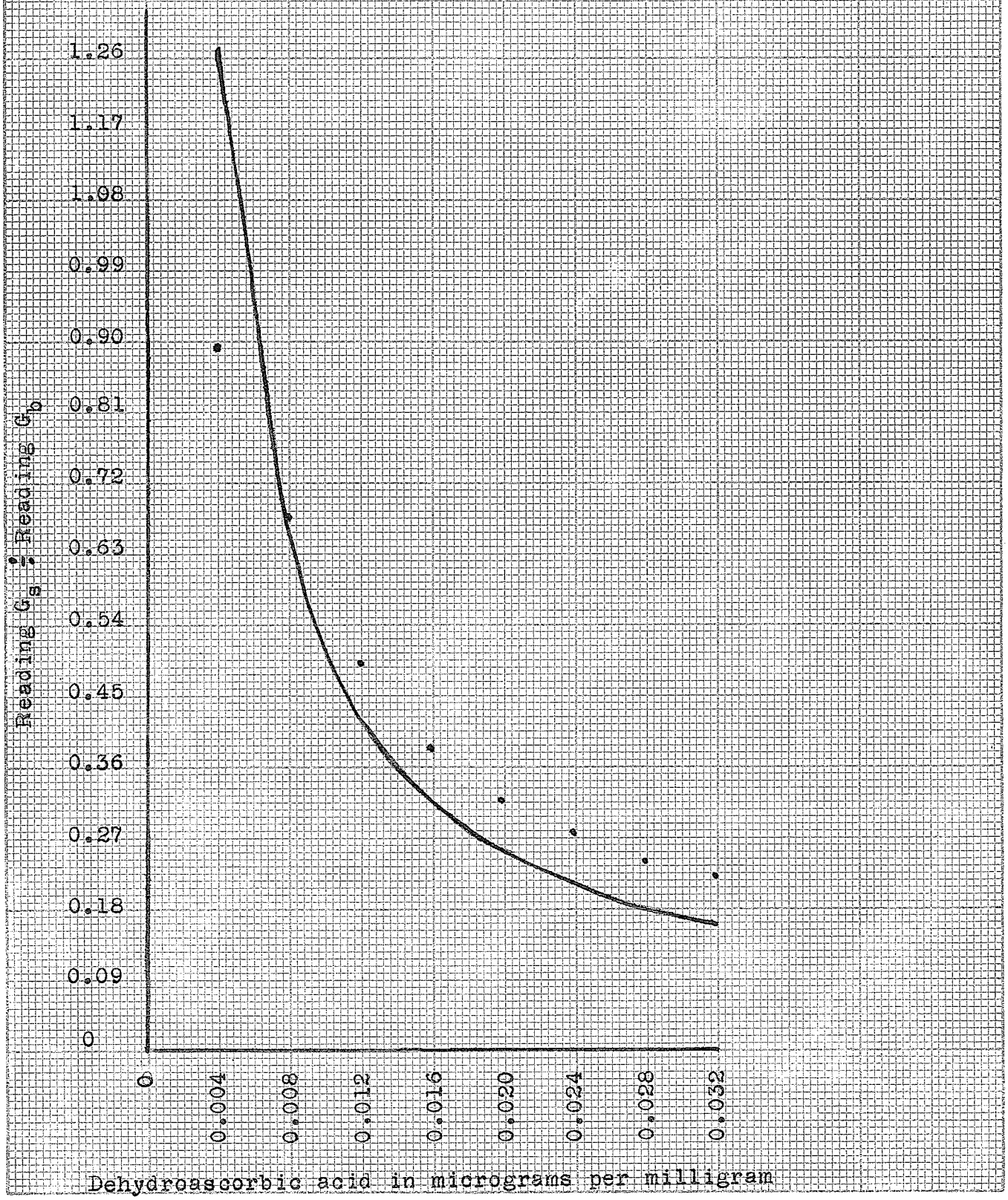
TABLE XV

Calibration data for Photometric Determination of Dehydroascorbic Acid

| Micrograms per ml. | Reading $G_s$ / Reading $G_b$ | Calculated $\frac{G_s}{G_b}$ | $(\text{Calculated } \frac{G_s}{G_b} - \text{Observ.} \frac{G_s}{G_b})^2$ |
|---|---|---|---|
| 0.004 | 0.89 | 1.2806 | 0.1526 |
| 0.008 | 0.66 | 0.6431 | 0.0003 |
| 0.012 | 0.49 | 0.4306 | 0.0035 |
| 0.016 | 0.39 | 0.3244 | 0.0043 |
| 0.020 | 0.32 | 0.2606 | 0.0035 |
| 0.024 | 0.28 | 0.2181 | 0.0039 |
| 0.028 | 0.25 | 0.1878 | 0.0039 |
| 0.032 | 0.23 | 0.1650 | 0.0043 |

Figure 13.

Standard Curve for Photometric Determination of Dehydroascorbic Acid using a Light of Wave Length 540 mμ




No. 5780-20
20 SQUARES TO THE INCH
Made in U. S. A.

follows: The tissue is ground under usually 50 and never less than 20 parts of a solution containing 5% metaphosphoric acid and 1% thiourea. After filtration 4 ml. of extract are placed in each of three matched cuvettes. One tube is kept for a blank. To each of the others is added 1 ml. of 2% 2,4-dinitrophenylhydrazine in approximately 9N $H_2SO_4$. These are held at 37°C. for three hours, then cooled, together with the blank in ice water. To each of the three tubes, while in the ice water bath, 5 ml. of 85% $H_2SO_4$ are added from a burette, a drop at a time, during not less than 1 minute. (Sulphuric acid, not grease, should be used for lubricating the stop-cock of the burette). Finally 1 ml. of the 2% 2,4-dinitrophenylhydrazine solution is placed in the blank tube. The tubes are shaken thoroughly under the ice water, removed to a rack, wiped dry after 30 minutes, and transmittance measured in the spectrophotometer with light of 540 mμ wave length.

It is important that colorimeter readings should not be made before 30-45 minutes after removal from the ice bath. In some plants a slight yellowish coloration appears when the

85% $H_2SO_4$ is added. This is compensated by the blank, to which the colorimeter is adjusted before the unknown is read. As the center setting does not remain constant in all cases, a greater increase in color sometimes takes place in the blank tube than in the unknown, the colorimeter readings should be made as directed above.

The instrument is operated and the readings are made in the same manner as for ascorbic acid.

In order to calculate the amount of dehydroascorbic acid contained in 100 grams of vegetable tissue, the value obtained from the reference standard is substituted in the following:

$$\frac{100(\text{micrograms dehydroasc.acid})[500+(\%\text{moisture})(\text{wt.of sample})]}{1000\ (\text{ml.of aliquot})(\text{wt.of sample})}$$

# APPENDIX C

## "Apparent Vitamin C" Determination

The principle of the titration method of Levy (37) has already been presented, (page 31).

20% hydrochloric acid and 2,6-dichlorophenolindophenol were used. The dye was standardized daily against ascorbic acid in concentrations of 0.05, 0.10, and 0.15 micrograms per milliliter in order to determine the indophenol equivalent.

An example is presented showing in detail the procedure followed in assaying for, and calculating the amount of "apparent vitamin C" present in a sample of cabbage.

A 25-gram sample is blended and filtered in the usual way. A 2-ml. aliquot is titrated against the standardized indophenol dye by means of a microburet. The amount [I] required to produce a pink color with 1 drop of indophenol and remain visible for 2 minutes is the amount of dye required to titrate the total ascorbic acid present. This amount is used later as a check.

To another 2-ml. aliquot, from 3-4 ml. of

36% HCl is added. 2.5 ml. 36% HCl suffices for an initial concentration of 20% HCl, but an additional 0.5-0.6 ml. of dye is necessary in order to compensate for dilution of the acid by the aqueous indophenol. The end-point [2] is taken when the pink color persists for 20 seconds ("20 sec. end-point"). On dilution with 5 volumes, or 30 ml. in this example, of water the titration is continued and the amount recorded [3].

Because of the difficulty in detecting a sharp end-point at least two and frequently several aliquots must be titrated and their mean value used in order to obtain an accurate titration.

In the example cited, previously standardized dye gave the following titrations:

0.05 mcg/ml. ascorbic acid ≡ 10 ml.dye (1.26% equiv.)
0.10 mcg/ml. " " ≡ 20 ml.dye "
0.30 mcg/ml. " " ≡ 30 ml.dye "

2 ml.sample HCl ≡ 1.4 [2] ml.dye or .0070 mcg.app.vit.C.
" HCl 5 vol.$H_2O$ 9.2 [3] ml.dye or.0460 mcg.
Total ascorbic acid

100 grams contain $0.0035(350+\frac{91.5 \text{ of } 25)\ 100}{25}$ or 5.22 mgm "apparent vitamin C"

and 0.0195 x 1491.2 or 29.07 mgm ascorbic acid
or a total of 34.3 grams Total ascorbic acid

From [1] the total ascorbic acid = 8.8 ml.dye or
0.0440 mcgm Total ascorbic acid.

and 100 grams contain 0.0220 x 1491.2 = 32.8 mgm

When the value from [1] is checked against the value obtained from [2] and [3] there is a variation of 1.5 mgm per 100 gm, which is probably due to the slower rate of reaction of the reducing substances on the indophenol dye. A reliable check which has been found to give values in close agreement to the method just mentioned is by using the spectrophotometer to give a reading for the reduced ascorbic acid, which may then be checked against the value found by difference in the titration procedure.

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