ACETALDEHYDE AND ALCOHOL PRODUCTION IN FROZEN SNAP BEANS AND THEIR RELATION TO OFF-FLAVOUR FORMATION.

by

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INTRODUCTION

Off-flavour development in raw or underblanched vegetables occurring as a result of an imbalance in the enzyme reactions was recognized early in the development of the frozen vegetable industry by Joslyn (1930), Tressler (1932) and Diehl and Berry (1933). Since then, numerous investigations have been carried out on various aspects of this problem but the reactions involved are still largely unknown. The investigators seem to agree that off-flavours are due to an enzymatic process because they do not develop in properly blanched vegetables. However, on prolonged storage non-enzymatic changes resulting in disagreeable flavours are probably involved also.

The changes in flavour occurring in frozen, raw or underblanched vegetables have been attributed to autolysis, proteolysis, Tressler (1932) and Mergentime and Wiegand (1946), glycolysis, Joslyn (1949), and more recently to oxidative rancidity of lipids, Lee (1958). The present investigation was undertaken to study the correlation which may exist between off-flavour development and the accumulation of alcoholic fermentation ("anaerobiosis") products.

It is well established that plant tissues, stored in atmospheres of low oxygen content, will undergo fermentation with the formation of alcohol and acetaldehyde and the development of off-flavours. Joslyn (1930) and Kohman and Sanborn (1934) showed that bruised vegetables and vegetables held under anaerobic or semi-aerobic conditions accumulate large amounts of acetaldehyde and ethanol and develop off-flavours similar to those found in raw frozen vegetables. They suggested that off-flavours develop as a result of alcoholic fermentation, and that the acetaldehyde and ethanol content could be used as an objective measure of off-flavour development in frozen vegetables. Their theory was further strengthened by the work of Gutterman et. al. (1951), and Lovejoy (1952) who found a good correlation between off-flavour and acetaldehyde content in green peas, lima beans and asparagus. However, other investigators, Moore (1951) and Kramer (1954), found the correlation unsatisfactory. In spite of the fact that the Association of Official Agricultural Chemists adopted the determination of acetaldehyde as an official method for the objective measurement of off-flavour development in frozen peas, it has rarely been used, if at all.

The present investigation was carried out on snap beans for which relatively little information is

available. The experiments covered a shorter storage period than that used by other workers, which lasted for several years in some cases, but it is closer to the period used by the industry. Raw, underblanched and adequately blanched beans were stored under oxygen and nitrogen in addition to the usual air atmosphere to obtain information on the mechanism of off-flavour formation. Samples were stored at three different temperatures and acetaldehyde and ethanol content were determined and organoleptic tests were carried out periodically.

During this investigation it was found that some unidentified compounds, giving reactions similar to that of acetaldehyde and ethanol, occurred as heat degradation products from some unknown precursor during steam distillation. To clarify how much they interfered with our determinations, further work was carried out on this problem. This observation was further investigated to determine its effect on the experimental data obtained. In addition, it is possible that these compounds contribute to the flavour of cooked beans.

REVIEW OF LITERATURE

Acetaldehyde in plant tissues: It has been well established that plant tissues undergo fermentation (anaerobic respiration) in the absence of oxygen, and to a certain extent, even in its presence. Plant respiration, including fermentation, had been reviewed last by Stiles (1960). In the course of fermentation, carbohydrates are metabolized to pyruvic acid which is then decarboxylated, giving acetaldehyde and carbon dioxide as follows:

$$CH_3.CO.COOH \xrightarrow{\text{carboxylase}} CH_3.CHO + CO_2$$

The reaction is catalyzed by the enzyme carboxylase (pyruvic decarboxylase) which requires the presence of a co-factor named co-carboxylase. Klein and Pirschle (1926) demonstrated that acetaldehyde occupies an intermediate position in the respiration of higher plants. They identified it by means of dimedon (dimethylhydroresorcinol) in all strongly respiring plant organs such as flowers and embryos, and also in leaves and roots of a variety of plants under both aerobic and anaerobic conditions. That acetaldehyde is an intermediate product of "anaerobic respiration" of higher plants was also shown by Griebel (1925) and Neuberg and Gottschalk (1925). Pirschle (1926) demonstrated that acetaldehyde is an intermediary product in germinating seeds which contain fats and that this product may be formed in the

conversion of fat to carbohydrate.

In the presence of oxygen, part of the acetaldehyde condenses to acetoin (acetylmethylcarbinol) when the enzyme carboligase is present, James (1953).

$$2CH_3.CHO \xrightarrow{\text{carboligase}} CH_3.CHOH.CO.CH_3$$

The reaction was shown to proceed in peas by David and Joslyn (1953) and in wheat germ by Singer and Pensky (1951).

Many workers have demonstrated the occurrence of acetaldehyde in plant tissues. The products investigated include apples, Müller-Thurgau and Osterwalder (1915), Power and Chestnut (1920), Thomas (1925, 1931), Fidler (1934), Miller (1936), White (1950), Huelin (1952) and Henze et.al. (1954); strawberry, Dimick and Makower (1956), and Winter et.al. (1958); citrus fruits, Biale and Weiss (1939), Biale and Shepherd (1939), and Peyron (1959); tropical fruits, Steinmann (1935); banana, Griebel (1924); pears, Müller-Thurgau and Osterwalder (1915), Harley and Fisher (1927), Thomas (1931) and Gerhardt and Ezell (1938); Concord grape, Holley et. al. (1955); tomato, Gustafson (1934); onion, Niegisch and Stahl (1956); corn, Silberstein (1954); barley and rice, Phillips (1947); and various other plants, Kostychev et. al. (1913), Niethammer (1928), and Peyron (1959). Kirchner (1949), in his review article, described acetaldehyde as an important flavour constituent in a large

number of fruits and vegetables.

Peas were used as a model system by several investigators because of their economic importance. Acetaldehyde formation in peas was first demonstrated by Neuberg and Gottschalk (1924,1925). They showed that coarsely-ground young pea seedlings can produce acetaldehyde from a glucose solution under anaerobic conditions. Bodnar et. al. (1925) showed acetaldehyde production with whole pea seeds in absence of O_2.

Kohman (1932), and Kohman and Sanborn (1932, 1934) showed that bruised peas, lima beans and corn, under anaerobic conditions, developed abnormal flavours very rapidly. They also reported that unbruised peas and lima beans submitted to anaerobiosis, and raw frozen vegetables also developed off-flavours. Alcohol and acetaldehyde were formed in larger quantities in the bruised vegetables than in the unbruised, and their production was associated with the formation of off-flavours.

Arighi, Joslyn and Marsh (1936) were the first to report the presence and accumulation of volatile aldehydes in the tissues of frozen peas. Aldehyde accumulation was also found in artichoke hearts, Joslyn, Bedford and Marsh (1938); asparagus, Joslyn and Bedford (1940), and Lovejoy (1952); lima beans, Gutterman (1956);

and in Brussel sprouts and squash, Joslyn and Bedford (1938-1940). Lovejoy (1952) and Gutterman (1956) confirmed Joslyn's original observation with green peas. Acetaldehyde was found in spinach, Arighi, Joslyn and Marsh (1936; broccoli, Buck and Joslyn (1953), and peas, Kramer (1954), but little or no correlation was found with off-flavour development.

The volatile aldehydes which have been reported as acetaldehyde were examined by David (1949). He identified acetaldehyde as the chief aldehyde constituent in fresh and frozen peas by means of 2,4-dinitrophenylhydrazine. He found that accumulation of acetaldehyde increased under anaerobic conditions, and its content paralleled activity of pyruvic carboxylase. Blanching at temperatures and times sufficient to inactivate carboxylase resulted in reduction in acetaldehyde formation. He also reported that raw peas and those blanched at 60°C. accumulated aldehydes during freezing storage since their content was much higher than that of the peas subjected to other treatments.

Acetaldehyde accumulation and its relation to off-flavour development was investigated by Moore (1951) in raw and underblanched peas throughout the storage period. The peas were stored at -6.7°, -17.8° and -28.9°C. under oxygen and air in the container. He found differences

in acetaldehyde content between different series and the initial content was greatly reduced by blanching at the higher temperatures. Acetaldehyde accumulated in raw or lightly blanched peas only at the highest storage temperature where oxygen caused a definite increase compared to air treatment. Off-flavour development was observed in samples which did not show any increase in acetaldehyde.

Silberstein (1954) studied the 2,4-dinitrophenylhydrazones of the steam volatile carbonyl compounds of green peas and identified acetaldehyde as the major component. In freshly harvested peas, steam volatile carbonyl compounds or their precursors accumulated very rapidly in the immediate post-harvest period even if the peas were stored at 0°F.

Recently, Wager (1958) and Ralls (1960) demonstrated the presence of acetaldehyde in peas. Ralls identified it by means of flash exchange gas chromatography and found differences in the amounts found in samples from two successive years.

Relatively little information exists concerning the acetaldehyde content and accumulation in green beans. Bedford and Joslyn (1939) first reported its accumulation in string beans. They detected acetaldehyde in raw and blanched samples stored at -17°C. for over four years

and at -23°C. for seven months. They found that when the length or temperature of blanching increased, the acetaldehyde content decreased and the quality of the product improved. However, the acetaldehyde concentration was not a reliable index of flavour retention. It was quite significant that the difference between the two series was small although there was more than three years difference in storage time. This might indicate that if acetaldehyde accumulated, it took place in the early part of storage.

Studying the connection between the thermal destruction rates and the regeneration of green bean peroxidase, Zoneil and Esselen (1959) determined acetaldehyde accumulation and off-flavour formation in sterilized samples containing added peroxidase, stored at room temperature. They found that the increase in acetaldehyde and off-flavour were due to the presence of peroxidase. Rapid increases in acetaldehyde content were found in the first two weeks of storage which were followed by decrease.

Alcohol in plant tissues. It has long been known that plants, like yeasts, form ethanol as final product in a series of enzyme catalyzed reactions called alcoholic fermentation or incorrectly, "anaerobic respiration". Ethanol formation follows the same pathway as acetaldehyde, this compound is then reduced to ethanol as follows:

$$CH_3.CHO + DPNH_2 \underset{\longleftarrow}{\xrightarrow{\text{dehydrogenase}}} CH_3.CH_2.OH + DPN$$

The reaction is catalyzed by the enzyme alcohol dehydrogenase and requires the presence of coenzyme I (diphosphopyridine nucleotide or DPN) as donor of hydrogen ions. During the respiration of carbohydrates, as 3-phosphoglyceric acid is formed from 3-phosphoglyceraldehyde, DPN is reduced to $DPNH_2$. In the reduction of acetaldehyde to alcohol, DPN is regenerated.

Numerous investigators confirmed the occurrence of alcohol in plants since Lechartier and Bellamy (1869, 1872, 1874) and Pasteur (1872) first demonstrated that fruits deprived of oxygen could produce alcohol. A number of plant tissues have been found to contain ethanol: apple, Power and Chestnut (1920), Thomas (1925 , 1931), Fidler (1933, 1934, 1951), Miller (1936), Thomas and Fidler (1941), Hackney (1943), White (1950), Thompson (1951), Thewlis (1952), and Henze (1953); strawberry, Winter et. al. (1958); orange, Fidler (1936); Concord grape, Holley et. al. (1955; tomato, Gustafson (1934),

and Link et. al. (1952); carrot, Wetzel (1933), and Thewlis (1952); potato, Gustafson (1934), Barron et. al. (1950), and Barker and ElSaifi (1952); maize, Neal and Girton (1955); barley, Phillips (1947), Nance (1949), and Thewlis (1952); rice, Phillips (1947); and in the various other plants, Kostychev et. al. (1913), Ruhland and Ramshorn (1938), Laing (1940), and Thewlis (1952). Kirchner (1949) indicated that ethanol was an important flavour constituent of many fruits and vegetables.

Several investigators showed the presence of ethanol in pea tissues, Bodnar et. al. (1925), Neuberg and Gottschalk (1925), Gustafson (1934), and Wager (1958), but very little data is available on beans.

The bruising of vegetables was reported by Kohman (1932) and Kohman and Sanborn (1932, 1934) to result in an increase in alcohol production which they assumed to be ethanol without actually identifying it. Peas, lima beans, and corn were found to contain more alcohol after bruising than before.

David (1949) found that, in peas , whether fresh or frozen, ethyl alcohol was the only alcohol produced and its formation and accumulation paralleled that of aldehyde, but occurred at faster rates so that the alcohol: aldehyde ratio increased. On anaerobiosis, the alcohol content increased by five-to eight-fold in

comparison with a two-to three-fold increase in acetaldehyde content. Using a similar technique, Buck and Joslyn (1953) found accumulation of alcohol in frozen raw and underblanched broccoli.

Moore (1951) reported differences in alcohol content between different lots of peas. Its content was greatly reduced on blanching. Ethanol accumulation was found only with those raw or lightly blanched peas which were stored at the highest temperature. Increase in alcohol content was about six- and seven-fold, whereas increase in aldehyde content was only two-fold. Samples stored at the lower temperatures showed no increase and sometimes a decrease in their ethanol content. He found no direct relationship between alcohol accumulation and off-flavour development.

Occurrence of acetaldehyde and ethanol as heat breakdown products in plant material. It is rather difficult to estimate the original concentrations of such rapidly produced and degraded intermediates as α-keto acids or acetaldehyde in plant tissues. Maceration or slurrying frees the enzymes which are normally confined to small subcellular units such as mitochondria and microsomes. This probably accelerates the reactions because it makes possible for the enzymes to react at a higher substrate concentration. These compounds can also occur at an accelerated rate during the early part of heat inactivation. Furthermore, they can very often be produced in the course of non-enzymatic reactions occuring during heating or upon addition of chemicals, such as strong acids or bases.

Isherwood and Niavis (1956), calculated that, during treatment with boiling alcohol, the rate of enzyme catalyzed reactions will be about eight times that at room temperature. They found that among other α-keto acids, pyruvic acid, the immediate precursor of acetaldehyde, occurred in potato and pea tissues during inactivation with boiling methanol or with the use of hot acid or strongly alkaline media. Breslow (1958), using model systems, demonstrated the thiamine catalyzed non-enzymatic formation of acetoin from pyruvic acid and acetaldehyde together, and from acetaldehyde alone. Ralls

(1959) showed that the reaction appears in peas.

From our viewpoint, the most interesting results were those obtained by Wager (1958). When he compared his low-temperature diffusion method with steam distillation, he always found considerably higher values for acetaldehyde when steam distillation was used with frozen peas. To investigate the problem further, he determined the acetaldehyde and ethanol content of successive aliquots obtained on continuous steam distillation of the same sample. Acetaldehyde was detected after as long as 2½ hours of steam distillation. When the sample was further heated under reflux at 100°C. for 2 hours, a rather large amount of acetaldehyde was obtained. He concluded that the excess acetaldehyde arose as a result of heating and is probably not due to an enzymic reaction. Wager used the method of Barker and Summerson (1941), to determine acetaldehyde which employs p-hydroxydiphenyl, which is not specific for this compound. He reported the decreasing production of a volatile material determined as ethanol after as long as 2 hours continuous steam distillation. The experiment with ethanol was not as detailed as that with acetaldehyde.

Barker (1951), working with potato tubers, noted that a compound reacting with alkaline permanganate, such as ethanol, occurred as a degradation product due to the

strongly acidic conditions (pH 1.2) existing during extraction.

The occurrence of acetaldehyde on heating various foods of plant origin had been reported more often. It usually accompanies some form of the non-enzymic browning reaction. Acetaldehyde occurring on the course of heating of jellies and preserves had been identified in model systems by Steinberg et. al. (1956). Mattick and Robinson (1960) showed its presence among the volatiles produced during the baking of sherry wine by the Tressler process. In fresh bread, both acetaldehyde and ethanol occurred, Wiseblatt and Kohn (1960). In bread however, we can assume that at least some of these compounds were produced by yeasts and other microorganisms. Acetaldehyde formation was found to occur in maple syrup during the concentration of the maple sap, Underwood et. al. (1956).

Off-flavour development in frozen beans. It is well recognized that frozen raw or underblanched vegetables develop off-flavours as a result of enzyme activity. Joslyn and Marsh (1933) reported that raw and underblanched string beans stored at 0°F. developed hay-like flavours within two months after freezing. The off-flavours increased in intensity during storage. Lightly blanched beans developed a less intense off-flavour compared to that of the raw ones. Oxidases were suggested as responsible for off-flavour formation although underblanched samples gave negative reactions for peroxidase, protease, and tyrosinase. Diehl et. al. (1933) also reported the occurrence of undesirable flavours and odours which are accentuated by cooking in frozen snap beans after 4 to 6 weeks of storage. They suggested scalding as a means to prevent it.

Extensive investigations on the various aspects of freezing preservation of green snap beans were carried out by Bedford and Joslyn (1939). They blanched the beans for various lengths of time at various temperatures and stored them at -17°C. and -23°C. for over four years and for seven months, respectively. The catalase, peroxidase and ascorbic acid oxidase activity, and the acetaldehyde content were determined and compared to off-flavour development. Samples which were not sufficiently blanched to inactivate guaiacum peroxidase developed off-flavours. The odour and flavour developed in raw and

underblanched samples were alfalfa-like and disagreeable.

The development of off-flavours in frozen beans was attacked from a different point of view by Lee (1954) and Lee, Wagenknecht and Hening (1955). They proposed that enzymatic oxidation of the lipid matter is the actual cause of off-flavour since acid number began to increase after one week, and peroxides appeared after one month of storage at -17.8°C. in the lipid fraction of raw or underblanched beans. Off-flavours could be detected after as little as two weeks storage. The acid and peroxide numbers and the intensity of off-flavour continued to increase with longer periods of storage. The enzyme lipase was held responsible for the higher acid number, while the occurrence of peroxides in the lipid fraction was attributed to lipoxidase.

Dietrich et. al. (1959) ran a large scale investigation on commercial samples blanched for various lengths of time. The effect of a wide range of constant and fluctuating storage temperatures on the retention of colour and flavour, and on ascorbic acid and chlorophyll content, was determined. High-temperature-short-time blanching was found to give the best results. The flavour and colour deterioration rate doubled with every 5°F. increase in temperature between 0° to 25°F.

No case of reversal of deterioration was found.

Other workers who were mainly concerned with the evaluation of various enzyme tests and blanching procedures also detected off-flavour formation in underblanched beans, Woodroof et. al. (1946), Cruess (1947), and Fisher and VanDuyne (1952).

MATERIALS AND METHODS

The beans used in this investigation were obtained from David Lord Limited, St. Jean, Quebec. Freshly harvested beans were brought to Macdonald College, by car, and kept under refrigeration (4°C.) until processed. The variety Slendergreen was used in every series. They were more mature than those generally used for freezing. Blanching was carried out in a cabinet-type steam blancher. This was followed by spray cooling with tap water.

Series I: Whole beans were used in this series to avoid the possible accumulation of acetaldehyde and ethanol resulting from the tissue injury caused by cutting. The beans were harvested and transported on September 10, 1959. After overnight storage they were sorted, washed, and divided into four lots. One lot was left unblanched and the three others were steam-blanched for 1, 2 and 4 minutes. The beans were then placed in 20 oz. sanitary cans. Each of the four lots was sub-divided into three groups to study the effect of the composition of the atmosphere. Oxygen, nitrogen, and air were used. Oxygen was obtained from Canadian Liquid Air Company Limited and the nitrogen from Linde Air Products Company, Limited. The treatments were carried out as follows. A hole was punctured in each can. The cans were placed in a vacuum desiccator and evacuation was carried out

with a Cenco Pressovac 4 vacuum pump. The pressure was reduced to about 0.8 cm. The evacuation was continued for 5 minutes after which the vacuum was released slowly with gas from the cylinders or, in the case of the air treatment, from the surrounding air. When oxygen or nitrogen were used, a slight positive pressure was allowed to build up in the desiccator before lifting the lid to prevent air getting into the cans. The hole on each can was immediately sealed with solder.

The containers were placed on quick-freezing plates at -28.9°C. and kept there until the beans were completely frozen. Four days later, each of the twelve treatments were further sub-divided into three lots, which were stored at -12.2°, -17.8°, and -21.1°C. ±1° respectively. Initial determinations for acetaldehyde and ethanol were begun on the next day and subsequent determinations were carried out throughout the storage period.

Series II: Beans in this series were harvested on July 26th, 1960. They were washed and cut in one inch lengths at the canning plant on the following day, and then taken to Macdonald College where they were processed immediately. They were divided into three lots. One lot was left unblanched and the other two lots were steam-blanched for 1 and 2½ minutes. The beans were then placed

in 15 oz. sanitary cans. Each group was again sub-divided into three lots for treatment with oxygen, nitrogen, and air, respectively. The gas treatments were carried out as described previously. The beans were quickly frozen and each group was divided into three lots and stored at -12.2°, -17.8°, and -21.1° C. ±1° respectively. Three days later, initial acetaldehyde and ethanol determinations were carried out in triplicate on the raw samples for the three gas treatments, and on the 1 and 2½ minute-blanched samples, stored in air.

Series III: The washed and cut beans were transported to Macdonald College and processed on August 30th, 1960. They had been harvested on the previous afternoon and were somewhat older than the beans used in the second series. The treatments given differed from those used in the other series. The beans were divided into three lots, one lot was left unblanched and the other lots were blanched for ½ and 2½ minutes. They were then frozen on trays and placed in the containers on the following day, avoiding warming up as much as possible. The cans were placed back on the freezing shelves and a few days later each group was subdivided into three lots and stored at -12.2°, -17.8°, and -21.1° C. ±1° respectively. Gas greatments were omitted in this series. The acetaldehyde and alcohol determinations were run in duplicate

immediately after blanching and, as in the first two series, after the samples had been placed at their respective storage temperatures.

The volatile aldehydes and alcohol were obtained by steam distillation of the finely ground material. Grinding was carried out in a cooled Waring Blendor, using 100 g. of beans and 200ml. of cold water. The distillate was collected in a 500 ml. receiving flask containing 50 ml. distilled water into which dipped the tip of the condenser. The receiving flask was kept in an ice bath to prevent losses by volatilization. The distillation was discontinued after about 400 ml. had been collected. The distillate was then transferred to a 500 ml. volumetric flask and made up to volume with the washings from the condenser and the receiving flask. The distillation time was standardized to 30 minutes.

In some cases,distillation was continued further to determine the extent to which aldehydes occurred as degradation products during steam distillation. Successive aliquots up to 2000 ml. were obtained from 100 g. of beans for aldehyde determination. The first 500 ml. were collected in 100 ml. aliquots in 100 ml. volumetric flasks containing 30 ml. distilled water into which dipped the tip of the condenser. Further aliquots were distilled into a 500 ml. volumetric flask in the

usual manner. The receiving flask was changed as fast as possible to avoid loss of aldehydes.

Quantitative determination of aldehydes. Aldehydes were determined by the iodometric bisulfite method of Jaulmes and Espezel (1935) omitting the addition of water after making the solution alkaline and titrating the liberated bisulfite with 0.01 N iodine solution instead of 0.1 N because of the small amount present, Joslyn and David (1952).

A 100 ml. aliquot of the steam distillate was placed in a 500 ml. Erlenmeyer flask containing 50 ml. of a neutral buffer solution (3.35 grams KH_2PO_4 and 15 grams $Na_2HPO_4.12H_2O$) per litre and 10 ml. of bisulfite solution (18.9 grams anhydrous Na_2SO_3 and 150 ml. of N H_2SO_4 per litre). The flask was stoppered and shaken. After standing for 20 minutes, 1 ml. of freshly prepared 1% starch solution and 10 ml. of acid solution (250 ml. conc. HCl per litre) were added. The excess bisulfite was then titrated with 0.1 N iodine solution until blue end point was reached. The solution was then made alkaline by adding 100 ml. of an alkaline buffer (8.75 grams boric acid and 400 ml. of N NaOH per litre). This liberated the bound bisulfite and the blue colour disappeared. One ml. freshly prepared 1% starch solution was added again and the liberated bisulfite was then

titrated with 0.01 N iodine solution until the blue end point returned. A blank was run on the reagents using 100 ml. of water in place of the distillate. 1 ml. of 0.01 N iodine = .22 mg. acetaldehyde.

Quatitative determination of ethanol. Before proceeding to the quantitative determination of ethanol, it was necessary to remove any interfering carbonyl compounds present in the steam distillate. This was accomplished by the method proposed by Friedmann and Klaas (1936) and Friedmann (1938), which consisted of a distillation of the sample from acid Na_2WO_4-$HgSO_4$ and a redistillation from $Ca(OH)_2$-HgO. The procedure used by Joslyn and David (1952) was followed throughout.

A 100 ml. aliquot of the steam distillate was introduced in a 500 ml. round bottom flask fitted with a glass connection, and 10 ml. of $HgSO_4$ solution (100 grams $HgSO_4$ dissolved in 1 litre of 2 N H_2SO_4) were added, followed by 15 ml. of 10% Na_2WO_4 solution. After adding enough water to bring the volume to about 150 ml., the flask was connected to an all glass apparatus, the connections wetted and the distillation started. After having collected about 100 ml. in another round-bottom flask, the distillation was stopped. Five ml. of $HgSO_4$ and an excess of $Ca(OH)_2$ (about 10 ml. of a suspension containing 132 grams $Ca(OH)_2$ per litre) were now added

to this new distillate and, after vigorous shaking, the volume was brought to about 150 ml. and the sample was redistilled into a 100 ml. glass-stoppered volumetric flask.

The quatitative determination was carried out by a modification of the dichromate-sulfuric acid procedure of Semichon and Flanzy (1929) as follows: an aliquot containing not more than 3 mg. alcohol was added to 5ml. of 0.1 N $K_2Cr_2O_7$ in 25% H_2SO_4 in a 25 ml. Erlenmeyer flask, which was then tightly stoppered. After heating in a boiling water bath for 15 minutes, the flask was cooled and 3 ml. of a 15% KI solution were added. The iodine liberated was titrated with 0.1 N thiosulfate. Whenever an aliquot larger than 5 ml. was thought necessary, a 50 ml. Erlenmeyer was used and 1 ml. of conc. H_2SO_4 was added for every 5 ml. of aliquot used to maintain the proper acidity. The time of heating was increased to 30 minutes. A blank was run simultaneously and the difference, expressed as ml. of 0.1 N thiosulfate was recorded. 1 ml. of 0.1 N thiosulfate = 1.15 mg. ethyl alcohol.

Preparation of 2,4-dinitrophenylhydrazones (DNPHs).

Among the reagents suitable for the identification and characterization of carbonyl compounds, 2,4 -dinitrophenylhydrazine (2,4-DNPHine) has often been recommended

and used, David (1949). It has many advantages. Its derivatives are very insoluble in water and can therefore be isolated from dilute solutions. They are easily crystallized from alcohol. The relatively high molecular weight of 2,4-DNPHine permits to obtain a reasonable amount of the derivative from small quantities of carbonyl compounds.

The steam distillate was redistilled in an all glass apparatus using a Vigreaux distilling column. The distillate was collected into a receiving flask containing 75 ml. of a saturated 2,4-DNPHine solution in 2 N HCl, the tip of the condenser dipping into the solution to prevent any losses by volatilization. The distillation was stopped after about 75 ml. had distilled over. Twenty-four hours were allowed for the formation of the DNPHs which were then filtered off, washed with 2 N HCl, water and dried in a desiccator. Melting points were determined using a Fisher-Jones melting point apparatus. The separation of the DNPHs was attempted by adsorption chromatography using the technique described by David (1949). The fractions obtained were tested for purity by ascending paper chromatography, using the method of Ellis, Gaddis and Currie (1958).

EXPERIMENTAL RESULTS

ALDEHYDE PRODUCTION

Since acetaldehyde production is affected by a number of factors, the effect of blanching, storage at various temperatures, and composition of the atmosphere were studied. The presence of aldehydes in the steam distillate of raw and blanched samples was demonstrated by preparing the 2,4-dinitrophenylhydrazine derivatives from them. The melting point determinations indicated that a mixture of DNPHs were present. This was confirmed when adsorption chromatography was used. The fractions separated by this technique were not pure as evidenced by ascending paper chromatography, since each of them yielded more than one distinctly different spot. During this study it appeared that aldehydes occured as degradation products from some unknown precursors during steam distillation. Investigations were then carried out to determine the nature of this reaction and the extent to which it contributed to the aldehyde values obtained in our experiments.

Effect of blanching and processing on aldehyde content. The results on the effect of blanching and processing on acetaldehyde content are reported in Table 1 and plotted in Figure 1*. They show that the initial aldehyde content is appreciably effected by such a mild treatment as a ½ - minute steam blanch.

* All the Figures appear in the Appendix.

TABLE 1

Effect of blanching and processing on the aldehyde content of beans expressed in mg. %

Blanching time min.	Series I		Series II		Series III	
	Air	Oxygen	Air	Nitrogen	Before Freezing	After Freezing
Raw	1.87	0.99	1.30	1.64	0.87	1.36
½	-	-	-	-	0.16	0.26
1	0.25	0.26	0.32	0.24	-	-
2	0.21	-	-	-	-	-
2½	-	0.26	0.26	-	-	0.22
4	0.20	-	-	-	-	-

Differences in the initial acetaldehyde content were found between the three series. Besides the variations in the raw material, these are likely due to differences in handling during the preparation of the samples. Raw samples accumulated large amounts of aldehyde during handling and freezing in the first two series in which the unfrozen material was packaged before freezing. The greatest amount accumulated in nitrogen-treated samples, less in air-treated ones, and least in samples stored under oxygen.

In series III, aldehyde determinations carried out before and after freezing. The results indicate that there was considerable aldehyde product in the course of freezing, since the beans were not frozen in cans as in the previous two series. Freezing is gradual and relatively

slow in still air. It may then be presumed that, in the course of freezing, the frozen outer layer blocks the normal gas exchange, creating anaerobic conditions in the still unfrozen inner tissues of the beans. This might be responsible for the accumulation of acetaldehyde in raw and slightly blanched beans during freezing, in addition to the freezing injury discussed by Kohman and Sanborn (1934).

Effect of blanching on aldehyde production during storage. The results of analyses after various storage conditions are presented in Tables 5 to 11 and in Figures 2 to 22. In every series, raw beans were able to accumulate acetaldehyde during storage when stored at the higher temperatures (-12.2°C., and -17.8°C.). Blanching for 1 minute and ½ minute in series I and III was not sufficient to completely inactivate the enzymes responsible for aldehyde production. Some destruction occurred as can be seen from the greatly reduced acetaldehyde production. The 1 minute blanch in series I was less effective than in series II. This can be attributed to the fact that cut beans were used, allowing better heat penetration. In samples blanched for 2 minutes or longer, no significant increase of aldehyde content was observed.

Effect of storage temperature on aldehyde accumulation.

Bedford and Joslyn (1939) reported that an increase in aldehyde content results from prolonged storage of raw and underblanched frozen green beans. Various storage temperatures (-12.2°, -17.8°, and -21.1°C.) were used to determine the effect they would have on accumulation of acetaldehyde. The results of these experiments are given in Tables 5 to 11 and in Figures 2 to 22. The highest storage temperature resulted in the greatest accumulation of aldehyde. A smaller accumulation was observed in samples stored at -17.8°C. and at the lowest storage temperature, very little increase occurred, if at all.

Acetaldehyde accumulation usually took place in the early stages of storage. The rate of accumulation in raw samples was highest during the first two or five weeks. The lightly blanched samples required some time to regain their enzyme activity and the highest rate of accumulation was delayed accordingly. The highest aldehyde concentrations were reached fastest with raw samples stored at the highest temperature than with those stored at the lower temperatures, or blanched. After the peak was reached, a slow decrease often took place.

Effect of composition of atmosphere in the container on aldehyde accumulation. These experiments were carried out to study whether completely aerobic and anaerobic conditions would decrease or increase aldehyde production;

and to test the two controversial hypotheses that off-flavour development results from an anaerobic or an oxidative process. Samples of the first two series were stored under oxygen and nitrogen along with the standard air treatment. The effect of the gas treatments on acetaldehyde content are recorded in Tables 5 to 10 and in Figures 2 to 19. The amount of aldehyde found was, in most cases, in the following order: nitrogen> air> oxygen. The results obtained indicate that this order occurs more as a result of fermentation during handling of packaged material prior to freezing rather than from the actual aldehyde accumulation during storage.

TABLE 2

Effect of composition of atmosphere on the highest acetaldehyde accumulation over the initial content during storage, expressed in mg. %.

Storage Temp. °C.	Blanching time min.	Series I			Series II		
		Oxygen	Air	Nitrogen	Oxygen	Air	Nitrogen
-12.2	Raw	0.85	0.95	0.69	1.22	1.51	1.28
	1	0.36	0.25	0.20	-	-	-
-17.8	Raw	0.11	0.38	0.53	0.64	1.08	0.68
	1	0.19	0.20	0.15	-	-	-
-21.1	Raw	-	0.00	-	0.21	0.28	0.25
	1	-	0.00	-	-	-	-

Table 2 shows the extent of acetaldehyde accumulation over the initial content during storage at different treatments. Initial determinations on series I were not complete since they were carried out on the air treated samples only. In this series, the results of the first determinations on the same treatment stored at the lowest temperature were therefore used as the initial content for the calculations. Examining the maximum aldehyde accumulation during storage, the following order generally was found: air> nitrogen> oxygen. Completely aerobic conditions did not result in considerably lower aldehyde production. These results support the theory that anaerobic conditions prevail regardless of the atmosphere of the container.

Occurrence of carbonyls as heat breakdown products on steam distillation. Finding almost identical amounts of acetaldehyde with samples blanched for different lenths of time arose suspicion that all or a great part of the aldehyde determined occurred as degradation product on distillation. Continuous distillation was carried out until 2000 ml. of distillate were obtained. In the first 500 ml., every 100 ml. aliquot was collected separately. Then 500 ml. aliquots were collected. The average results of triplicate samples, expressed as acetaldehyde, are reported in Table 3.

TABLE 3

Occurrence of carbonyls in subsequent aliquots during continuous distillation, expressed as acetaldehyde.

No. of 100 ml. aliquots	Time of distillation min.	Sample	
		Raw	Blanched
		Acetaldehyde, mg. per 100 ml. distillate	
1	10*	1.31	0.11
2	5½	0.09	0.03
3	4½	0.03	0.03
4	5	0.03	0.02
5	5	0.03	0.02
6 to 10	26	0.03	0.02
11 to 15	27	0.03	0.02
16 to 20	25	0.03	0.02

* the first drop of distillate appeared after 5 minutes.

These experiments indicate that all the aldehyde present in the tissues was distilled off in the first 100 - 200 ml. After that, acetaldehyde production fell off to a steady level, presumably occurring as heat degradation product from some unknown precursors. From these results, it can be assumed that approximately 15 mg. % aldehyde occurred as heat degradation product during steam distillation in the 500 ml. distillate collected regularly. Our experimental data was not corrected for this value because it was assumed this error appeared in every determination in about the same extent. Additional proof

of the occurrence of carbonyls was obtained by preparing 2,4-dinitrophenylhydrazine derivatives from the 500 to 2000 ml. fractions.

Further evidence on the production of aldehyde as a degradation product occurring on heating and on the nature of the reaction was furnished by keeping the slurry of beans which had been previously distilled at 90°C. under air and nitrogen for 150 minutes. A second distillation then was carried out and 250 ml. of distillate were collected. The aldehyde content found in different samples is reported in Table 4.

TABLE 4

Carbonyl content of bean slurry held at 90°C. for 150 minutes after the first distillation, expressed as acetaldehyde mg. %.

Atmosphere in flask	Sample			
	Raw		Blanched	
	First distillate	Second distillate	First distillate	Second distillate
Air	2.38	0.56	0.28	0.60
	1.86	0.71	-	-
Nitrogen	1.45	0.17	0.28	0.18
	-	-	0.52	0.17

The data in Table 4 indicate that extended periods of heating resulted in large aldehyde production in

samples kept under air. The reaction seems to be accelerated by oxygen since greater aldehyde production was observed in air as compared to nitrogen. Stronger browning of the slurry was observed on heating under an air atmosphere than nitrogen. Other experiments showed that, in addition to the factors already mentioned, the extent of aldehyde production depends on the length and temperature of heating. The above results indicate that possibly non-enzymatic browning is responsible for the formation of aldehyde during steam distillation.

TABLE 5

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans stored under oxygen, series I.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		44		140		275		500	
-12.2	Raw	1.87	A	1.97	C	2.56	D	2.16	D	___	
	1	0.25	A	0.24	B	0.55	B	0.21	C	___	
	2	0.21	A	___		0.20	A	0.28	A	___	
	4	0.20	A	___		0.19	A	0.26	A	___	
-17.8	Raw	1.87	A	1.81	A	1.73	B	1.82	D	___	
	1	0.25	A	___		0.38	B	0.21	B	___	
	2	0.21	A	___		0.28	A	0.22	A	___	
	4	0.20	A	___		0.23	A	0.24	A	___	
-21.1	Raw	1.87	A	1.71	A	1.69	B	1.47	C	1.45	D
	1	0.25	A	0.19	A	0.22	B	0.19	B	___	
	2	0.21	A	___		___		0.20	A	___	
	4	0.20	A	___		___		0.17	A	___	

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 6

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans stored under air, series I.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		44		140		275		500	
-12.2	Raw	1.87	A	2.82	B	2.67	C	2.25	D	____	
	1	0.25	A	0.33	A	0.50	C	0.22	C	____	
	2	0.21	A	0.20	A	0.22	A	0.21	A	____	
	4	0.20	A	0.17	A	____		0.20	A	____	
-17.8	Raw	1.87	A	2.25	A	2.21	C	2.24	C	____	
	1	0.25	A	0.45	A	0.38	C	0.23	B	____	
	2	0.21	A	0.23	A	0.28	A	0.21	A	____	
	4	0.20	A	0.20	A	____		0.21	A	____	
-21.1	Raw	1.87	A	1.76	B	1.78	C	1.80	C	____	
	1	0.25	A	0.19	A	0.21	B	0.19	B	____	
	2	0.21	A	____		0.19	A	0.22	A	____	
	4	0.20	A	____		0.18	A	0.23	A	____	

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 7

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans stored under nitrogen, series I.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		44		140		275		500	
-12.2	Raw	1.87	A	2.88	C	2.76	D	2.75	D	2.21	D
	1	0.25	A	0.26	B	0.38	B	0.26	B	____	
	2	0.21	A	____		0.22	A	0.26	A	____	
	4	0.20	A	____		0.22	A	0.23	A	____	
-17.8	Raw	1.87	A	2.65	B	2.72	D	2.33	D	____	
	1	0.25	A	____		0.33	B	0.28	B	0.24	C
	2	0.21	A	____		0.30	A	0.23	A	____	
	4	0.20	A	____		0.27	A	0.20	A	____	
-21.1	Raw	1.87	A	2.19	A	2.14	B	2.09	C	2.19	D
	1	0.25	A	0.18	A	____		0.19	B	____	
	2	0.21	A	____		____		0.20	A	____	
	4	0.20	A	____		____		0.21	A	____	

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 8

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans stored under oxygen, series II.

Storage temperature °C.	Blanching time mins.	Storage time in days					
		Initial	12	32	48	77	184
-12.2	Raw	0.99 A	1.64 A	1.61 C	2.21 C	1.78 D	1.85 D
	1	0.26 A	____	____	0.23 A	____	0.20 D
	2½	0.26 A	____	____	____	____	0.18 C
-17.8	Raw	0.99 A	1.15 A	1.37 A	1.47 C	1.63 D	1.52 D
	1	0.26 A	____	____	____	____	0.22 C
	2½	0.26 A	____	____	____	____	0.20 A
-21.1	Raw	0.99 A	1.13 A	1.07 A	1.07 B	1.06 B	1.20 D
	1	0.26 A	____	____	0.19 A	____	0.22 B
	2½	0.26 A	____	____	____	____	0.21 A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 9

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans stored under air, series II.

Storage temperature °C.	Blanching time mins.	Storage time in days											
		Initial		12		32		48		77		184	
-12.2	Raw	1.30	A	2.01	A	2.20	B	2.81	C	2.67	D	2.78	D
	1	0.32	A	____		____		0.26	A	____		0.28	D
	2½	0.26	A	____		____		____		____		0.24	B
-17.8	Raw	1.30	A	1.53	A	1.84	B	1.82	B	1.98	D	2.38	D
	1	0.32	A	____		____		0.19	A	____		0.21	C
	2½	0.26	A	____		____		____		____		0.19	B
-21.1	Raw	1.30	A	1.32	A	1.42	A	1.33	B	1.48	C	1.58	D
	1	0.32	A	____		____		0.18	A	____		0.20	C
	2½	0.26	A	____		____		____		____		0.19	A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 10

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans stored under nitrogen, series II.

Storage temperature °C.	Blanching time mins.	Storage time in days											
		Initial		12		32		48		77		184	
-12.2	Raw	1.64	A	2.00	A	2.25	A	2.92	C	2.60	D	2.86	D
	1	0.24	A	0.25	A	0.22	A	0.24	A	____		0.23	D
	2½	0.26	A	____		____		____		____		0.21	B
-17.8	Raw	1.64	A	1.62	A	1.96	B	2.15	B	2.32	D	2.13	D
	1	0.24	A	____		____		0.17	A	____		0.24	C
	2½	0.26	A	____		____		____		____		0.24	A
-21.1	Raw	1.64	A	1.46	A	1.56	A	1.59	B	1.60	B	1.89	D
	1	0.24	A	____		____		0.19	A	____		0.21	B
	2½	0.26	A	____		____		____		____		0.22	A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 11

Effect of blanching and storage temperature on acetaldehyde content, expressed in mg.%, and flavour of beans, series III.

Storage temperature °C	Blanching time mins.	Storage time in days									
		Initial		14		31		59		145	
-12.2	Raw	1.36	A	2.22	B	2.02	C	2.12	D	2.00	D
	½	0.26	A	0.17	A	0.29	A	0.31	B	0.60	D
	2½	0.22	A	____		____		____		0.28	B
-17.8	Raw	1.36	A	1.53	A	1.50	B	1.59	C	1.86	D
	½	0.26	A	0.19	A	0.26	A	0.23	A	0.29	C
	2½	0.22	A	____		____		____		0.21	A
-21.1	Raw	1.36	A	1.37	A	1.29	A	1.38	C	1.39	D
	½	o.26	A	0.25	A	0.29	A	0.24	A	0.23	D
	2½	0.22	A	____		____		____		0.20	A

Flavour scores: A = good, B = slightly off, C= off, D = very off.

ALCOHOL PRODUCTION

The presence of alcohol in plant tissues, particularly under anaerobic conditions, has often been reported together with acetaldehyde, its precursor. The results of our experiments presented in Tables 12 to 20, show that ethanol, like acetaldehyde, occurred in various amounts, depending on the treatment given to the sample. Ethanol was always found in greater amounts than acetaldehyde.

Effect of blanching and processing on alcohol content. The results in Table 19, and Figure 1, show that the effect of blanching and subsequent processing on ethanol content was similar to that found with acetaldehyde. The initial alcohol content decreased as the time of blanching increased, but this was more gradual than that of acetaldehyde probably due to the higher boiling point of ethanol (78.5°C.) as compared with that of acetaldehyde (21.0°C.)

As with aldehyde, the results presented in Table 19 show that there is a difference in the initial ethanol content between different series. Differences in the raw material and in handling during the preparation of the samples are likely responsible for a large part of it. Raw beans accumulated large quantities of ethanol during the processing in the first two series. The greatest amount was produced by nitrogen-treated samples, less in air-treated ones, and least in beans stored

TABLE 12

Effect of blanching and storage temperature on ethanol content, expressed in mg.%, and flavour of beans stored under oxygen, series I.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		44		140		275		500	
-12.2	Raw	33.53	A	50.34	C	46.38	D	42.83	D	____	
	1	19.92	A	28.28	B	20.61	B	17.02	C	____	
	2	17.66	A	____		18.35	A	13.10	A	____	
	4	11.96	A	____		14.17	A	10.36	A	____	
-17.8	Raw	33.53	A	40.87	A	41.86	B	27.05	D	____	
	1	19.92	A	____		22.86	B	19.73	B	____	
	2	17.66	A	____		16.45	A	12.19	A	____	
	4	11.96	A	____		12.88	A	14.63	A	____	
-21.1	Raw	33.53	A	37.31	A	36.06	B	37.75	C	30.91	D
	1	19.92	A	19.84	A	lost	B	14.93	B	____	
	2	17.66	A	____		____		17.98	A	____	
	4	11.96	A	____		____		14.02	A	____	

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 13

Effect of blanching and storage temperature on ethanol, expressed in mg.%, and flavour of beans stored under air, series I.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		44		140		275		500	
-12.2	Raw	33.53	A	46.79	B	45.72	C	38.88	D	____	
	1	19.92	A	22.50	A	22.22	C	13.81	C	____	
	2	17.66	A	18.59	A	12.24	A	11.89	A	____	
	4	11.96	A	17.68	A	____		10.06	A	____	
-17.8	Raw	33.53	A	47.97	A	52.16	C	32.68	C	____	
	1	19.92	A	24.38	A	22.22	C	15.78	B	____	
	2	17.66	A	18.59	A	12.24	A	10.67	A	____	
	4	11.96	A	17.30	A	____		12.19	A	____	
-21.1	Raw	33.53	A	40.87	B	32.20	C	34.37	C	____	
	1	19.92	A	18.51	A	lost	B	12.11	B	____	
	2	17.66	A	____		13.85	A	14.32	A	____	
	4	11.96	A	____		15.78	A	11.28	A	____	

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 14

Effect of blanching and storage temperature on ethanol content, expressed in mg.%, and flavour of beans stored under nitrogen, series I.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		44		140		275		500	
-12.2	Raw	33.53	A	55.08	C	48.94	D	45.08	D	45.10	D
	1	19.92	A	26.95	B	22.86	B	15.50	B	_____	
	2	17.66	A	_____		12.24	A	14.93	A	_____	
	4	11.96	A	_____		16.74	A	11.28	A	_____	
-17.8	Raw	33.53	A	47.97	B	50.23	D	29.59	D	_____	
	1	19.92	A	_____		19.32	B	14.09	B	12.80	C
	2	17.66	A	_____		11.91	A	11.58	A	_____	
	4	11.96	A	_____		13.52	A	10.36	A	_____	
-21.1	Raw	33.53	A	35.54	A	31.88	B	34.37	C	28.01	D
	1	19.92	A	22.21	A	_____		16.06	B	_____	
	2	17.66	A	_____		_____		11.28	A	_____	
	4	11.96	A	_____		_____		12.19	A	_____	

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 15

Effect of blanching and storage temperature on ethanol content, expressed in mg.%, and flavour of beans stored under oxygen, series II.

Storage temperature °C.	Blanching time mins.	Storage time in days											
		Initial		12		32		48		77		184	
-12.2	Raw	30.70	A	39.07	A	39.85	C	45.88	C	41.23	D	52.86	D
	1	15.66	A	____		____		12.41	A	____		16.60	D
	2½	12.90	A	____		____		____		____		12.45	C
-17.8	Raw	30.70	A	38.00	A	28.00	A	27.99	C	37.52	D	32.52	D
	1	15.66	A	____		____		____		____		14.30	C
	2½	12.90	A	____		____		____		____		10.48	A
-21.1	Raw	30.70	A	33.93	A	39.30	A	38.14	B	38.21	B	37.03	D
	1	15.66	A	____		____		15.58	A	____		14.95	B
	2½	12.90	A	____		____		____		____		12.87	A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 16

Effect of blanching and storage temperature on ethanol content, expressed in mg.%, and flavour of beans stored under air, series II.

Storage temperature °C	Blanching time mins.	Storage time in days											
		Initial		12		32		48		77		184	
-12.2	Raw	34.72	A	40.37	A	38.59	B	40.12	C	52.81	D	60.74	D
	1	17.76	A	____		____		17.32	A	____		15.24	D
	2½	12.90	A	____		____		____		____		14.23	B
-17.8	Raw	34.72	A	40.37	A	39.08	B	40.22	B	42.60	D	45.10	D
	1	17.76	A	____		____		13.85	A	____		13.52	C
	2½	12.90	A	____		____		____		____		12.30	B
-21.1	Raw	34.72	A	42.07	A	36.98	A	34.92	B	41.85	C	40.10	D
	1	17.76	A	____		____		14.43	A	____		16.10	C
	2½	12.90	A	____		____		____		____		11.56	A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 17

Effect of blanching and storage temperature on ethanol content, expressed in mg.%, and flavour of beans stored under nitrogen, series II.

Storage temperature °C.	Blanching time mins.	Storage time in days					
		Initial	12	32	48	77	184
-12.2	Raw	59.88 A	70.56 A	66.90 A	78.54 C	85.43 D	85.34 D
	1	14.08 A	11.87 A	15.94 A	15.30 A	____	14.08 D
	2½	12.90 A	____	____	____	____	11.81 B
-17.8	Raw	59.88 A	55.30 A	lost B	61.47 B	60.36 D	58.47 D
	1	14.08 A	____	____	14.14 A	____	17.41 C
	2½	12.90 A	____	____	____	____	11.75 A
-21.1	Raw	59.88 A	65.48 A	65.48 A	60.03 B	59.13 B	61.82 D
	1	14.08 A	____	____	17.32 A	____	15.46 B
	2½	12.90 A	____	____	____	____	13.50 A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

TABLE 18

Effect of blanching and storage temperature on ethanol content, expressed in mg.%, and flavour of beans, series III.

Storage temperature °C.	Blanching time mins.	Storage time in days									
		Initial		14		31		59		145	
-12.2	Raw	24.82	A	32.32	B	32.50	C	33.12	D	40.49	D
	½	16.74	A	22.51	A	26.84	A	25.12	B	33.23	D
	2½	9.09	A	____		____		____		10.24	B
-17.8	Raw	24.82	A	29.44	A	26.26	B	28.66	C	32.20	D
	½	16.74	A	20.20	A	19.05	A	19.26	A	19.19	C
	2½	9.09	A	____		____		____		9.61	A
-21.1	Raw	24.82	A	28.57	A	30.59	A	26.40	C	26.50	D
	½	16.74	A	19.62	A	22.22	A	18.68	A	18.03	D
	2½	9.09	A	____		____		____		10.30	A

Flavour scores: A = good, B = slightly off, C = off, D = very off.

under oxygen. Anaerobic fermentation before freezing is undoubtedly responsible for most of the large increase noted in the nitrogen treated samples. The results of determinations on series III before and after freezing showed a similar, although relatively smaller increase in ethanol content in the course of freezing, as was noted with acetaldehyde. This observation supports the theory advanced in a previous chapter that, during freezing, anaerobic conditions prevail in the still unfrozen inner part of the tissues regardless of the composition of the atmosphere in the container.

TABLE 19

Effect of blanching and processing on the alcohol content of beans, expressed in mg. %.

Blanching time min.	Series I	Series II			Series III	
					Before Freezing	After Freezing
	Air	Oxygen	Air	Nitrogen		
Raw	33.53	30.70	34.72	59.88	20.74	24.82
½	-	-	-	-	18.64	16.74
1	19.92	15.66	17.76	14.08	-	-
2	17.66	-	-	-	-	-
2½	-	12.90	12.90	12.90	-	9.09
4	11.96	-	-	-	-	-

Effect of blanching on alcohol production during storage. The data of ethanol analyses on beans stored under different conditons for various lengths of time are presented in Tables 12 to 18 and in Figures 2 to 22. They show that ethanol accumulated to a greater extent than aldehyde but the effect of blanching on ethanol production was much the same. Raw beans were able to accumulate alcohol in every series, even at the lowest storage temperature. Samples blanched for 1 minute and ½-a-minute in series I and III retained their ability to produce ethyl alcohol as well as acetaldehyde. A 1-minute steam blanch was sufficient to inactivate the enzymes responsible for alcohol production in series II where cut beans were used. Blanching for 2 minutes or more was enough to prevent considerable accumulation of ethanol in every case.

Effect of storage temperature on alcohol accumulation. The results presented in Tables 12 to 18 and in Figures 2 to 22 show that ethanol production, like aldehyde accumulation, largely depends on the temperature of storage. In most of the cases, the greatest amounts were obtained when the beans were stored at -12.2°C. Lower temperatures usually resulted in smaller accumulation, but this pattern was not as clearcut as that with acetaldehyde.

The highest rate of accumulation was always found

in the very early part of storage. The peak was often reached in the first 50 days of storage and was followed by an unexplained decrease. Condensation of ice crystals was observed on the internal walls of the can. It was thought that this condensation might contain ethanol in an amount equal to the decrease observed. Determinations were carried out on samples of series I stored for 275 days, but the amount found could account for only part of the decrease. Moore (1951) noticed decrease of ethanol content with blanched peas.

Effect of composition of atmosphere in the container on alcohol accumulation. The results reported in Tables 12 to 17, and 20, and Figures 2 to 19 show that oxygen did not prevent the formation of ethanol. Anaerobic conditions seemed to prevail in the frozen tissues, regardless of the atmosphere of the container.

Table 20 was prepared in the same way as that for acetaldehyde. In series I, results of the first determination on the same treatment stored under the lowest temperature were used as the initial content in the calculations. The data in Table 20 show no definite influence of any of the gas treatments used. When these results are compared with the highest amounts of alcohol reported in Tables 12 to 17, it can be seen that ethanol, like acetaldehyde, occurred during the handling of the canned unfrozen material.

TABLE 20

Effect of composition of atmosphere on the highest ethanol accumulation over the initial content during storage, expressed in mg. %

Storage Temp. °C.	Blanching time min.	Series I			Series II		
		Oxygen	Air	Nitrogen	Oxygen	Air	Nitrogen
-12.2	Raw	13.03	13.26	19.54	22.16	26.02	25.55
	1	8.44	2.58	4.74	0.94	-	1.86
-17.8	Raw	4.55	18.63	14.69	7.30	10.38	1.59
	1	3.02	4.46	-	-	-	3.33
-21.1	Raw	-	7.34	-	8.60	7.35	5.60
	1	-	-	-	-	-	3.24

Occurrence of non-carbonyl volatile reducing compounds as heat breakdown products on steam distillation. During the investigation on the production of aldehydes as heat degradation products during steam distillation, a limited number of experiments were undertaken to find out if non-carbonyl compounds occurred as well. While specific tests were not carried out to identify the compound or compounds present, the results are reported as ethanol. The determinations were carried out on aliquots obtained during continuous distillation. Interfering carbonyl compounds were removed in the usual manner.

The data, presented in Table 21, indicate that the ethanol present in the beans is distilled over in the first 500 ml. It may be suggested that the compound obtained on further distillation is part of the alcohol originally present in the tissues, its high boiling point and affinity for water being responsible for the slow distillation. However, distillation of blanks containing 200 p.p.m. ethanol gave an average recovery of 98.7%, indicating an almost complete removal of alcohol during the first 500 ml. of distillation. It appears, therefore, that some non-carbonyl volatile compounds or compounds, perhaps ethanol, occur as heat degradation products during steam distillation.

TABLE 21

Production of non-carbonyl volatile compounds during prolonged distillation, expressed in mg. ethanol %.

	Number of the subsequent 100 ml. aliquot							
Sample	1	2	3	4	5	10	15	19
1	25.60	19.81	9.15	7.93	-	3.66	3.96	1.53

	Number of the subsequent 100 ml. aliquot				
	1 - 5	5 - 7.5	5 - 10	10 - 15	15 - 20
2	15.58	-	6.64	7.50	5.19
3	22.50	6.51	-	-	-
4	22.22	5.48	-	-	-
5	17.68	6.25	-	-	-
6	52.16	3.38	-	-	-

Description of the samples in Table 21:

Number	Series	Blanch min.	Atmosphere of container	Storage Temp. °C.	Storage time (days)
1	I	Raw	Air	-17.8	251
2	II	1	Oxygen	-21.1	76
3	I	1	Air	-12.2	67
4	I	1	Air	-12.2	132
5	I	4	Air	-17.8	73
6	I	Raw	Air	-17.8	131

OFF-FLAVOUR DEVELOPMENT

Several investigators reported that there is a good correlation between off-flavour development and accumulation of acetaldehyde and ethanol in frozen vegetables. The main purpose of this investigation was to determine the relationship between off-flavour development and the accumulation of these fermentation products in snap beans.

Each sample was tested by two trained persons and their unanimous observations are recorded in Tables 5 to 18 and in Figures 2 to 22. Organoleptic tests were carried out simultaneously with the ethanol and aldehyde determinations on the same samples used for the chemical analyses. The off-flavour development of beans can be described as the formation and accumulation of malodorous bitter-tasting compound or compounds. In this experiment, four flavour scores were used: good, slightly off, off, very off. The last term was used for a very wide range of flavours, ranging from a strong and definitely unpleasant bitterness to an unbearable disgusting bitter flavour. The term "off" was used in cases where the off-flavour was definte but still not very strong. "Slightly off" was used in cases where a very mild off-flavour was noted.

Effect of blanching on off-flavour development during storage. The strongest off-flavour always developed in

raw samples. It could be detected earlier in these samples. The ½, 1, and 2½-minute blanched samples retained their ability to produce off-flavours. However, in Series I, a 2-minute blanch was sufficient, to completely inactivate the enzymes responsible for off-flavour formation. This indicates that there was an initial difference in the ability to develop off-flavour between the three series. From data presented, it can also be seen that off-flavour could be detected earlier in samples of series III. At the last tasting, the samples of series III showed the highest off-flavour development, when compared to those of the other series stored for approximately the same length of time. Samples of series II gave fairly close results to those of series III, but series I was definitely slower in producing those compounds responsible for off-flavour.

Comparing the results of the organoleptic tests with those of ethanol and acetaldehyde determinations, it may be concluded that a longer blanching was required to inactivate the enzymes responsible for off-flavour formation than to inactivate the fermentative enzymes. The above conclusion is particularly well demonstrated in samples of Series II, blanched for 1 and 2½ minutes and those of Series III blanched for 2½ minutes.

Effect of storage temperature on off-flavour development. The inhibitory effect of low temperatures on the enzyme system taking part in the formation of acetaldehyde and ethanol was quite different to that on those unknown enzymes producing off-flavour. Upon studying the experimental results from the blanching treatments which retained their ability to produce ethanol and acetaldehyde, it becomes obvious that fermentation was completely inhibited by storage temperatures which did not prevent off-flavour formation. Nevertheless, lowering the temperature slowed down the rate of off-flavour development. Some 2½-minute blanched samples stored at -21.1°C. did not show any detectable off-flavour at the last determination. This can be explained by the combined effect of low temperature inhibition and heat inactivation on the enzyme system taking part in off-flavour formation.

It is very difficult to draw a graph showing the accumulation of off-flavour throughout the storage period because of the lack of an objective test. Nevetheless, studying the data presented in Tables 5 to 18 and in Figures 2 to 22, and considering the definition given of the "very off" term, it can be visualized that this accumulation would be linear rather than levelling off as found with acetaldehyde and ethanol. The slope of

the line would depend mainly on the blanching treatment and the temperature of storage.

The effect of storage temperature on colour was quite different. Every sample stored at -12.2°C. developed a pronounced brownish colour whether it was blanched or not. Beans stored at -17.8°C. developed a slightly brownish discolouration, but all samples stored at -21.1°C. retained their original colour even when they were stored for as long as 500 days. From the above observations, it can be concluded that discolouration depended entirely on the storage temperature and that there is a critical temperature somewhere between -17.8°C. and -21.1°C. at which no detectable discolouration would occur. Since a 4-minute blanching treatment had very little effect on this discolouration, it would appear that it is not the result of an enzymatic process, or that the enzymes involved are extremely heat-resistant.

Effect of composition of atmosphere in the container on off-flavour development. The results of the organoleptic tests show that the gas treatments had no significant effect on the off-flavour development. This is in agreement with the results of acetaldehyde and ethanol determinations. It gives further support to the theory discussed in the previous chapters that the conditions inside of the material were not effected by the outside atmosphere because of the frozen state of the tissues.

SUMMARY AND CONCLUSIONS

Raw, underblanched, and sufficiently blanched beans were stored under air, oxygen and nitrogen atmosphere at -12.2°C., -17.8°C. and -21.1°C., ±1°C. Three different lots were tested for 145, 184 and 500 days. Acetaldehyde and ethanol content were determined throughout the storage period. Organoleptic tests were carried out concurrently.

Variations were found in the initial acetaldehyde and ethanol content. They depended mainly on handling methods and the treatments given before the beans were frozen. Blanching resulted in a decrease in the initial content of both compounds.

Raw and lightly blanched beans retained their ability to produce acetaldehyde and alcohol. The amount accumulated over the initial content depended on three main factors: extent of blanching, length of storage, and temperature of storage. Oxygen, nitrogen and air treatments had no significant effect. The initial alcohol content and the quantity accumulated were always much higher than the corresponding acetaldehyde values. The highest rate of acetaldehyde and alcohol accumulation was found to occur early in the storage period. Later, their content levelled off or decreased.

Part of the acetaldehyde found appears to occur

from some unknown precursor or precursors, as a heat degradation product during steam distillation. This may occur as the result of a non-enzymatic browning reaction. The presence of carbonyl compounds in the breakdown products as well as in the beans was demonstrated by preparing their 2,4-dinitrophenylhydrazine derivatives. Besides the carbonyls, some other volatile organic reducing compound or compounds, determined as ethanol, appeared presumably as a heat breakdown products on steam distillation.

Off-flavour development was found to be influenced by the same external factors as acetaldehyde and alcohol accumulation. However, a different enzyme system seems to be involved since it is more heat resistant. It can also function at lower temperatures. The compounds responsible for the off-flavour continues to accumulate throughout the whole storage period.

Beans which accumulated acetaldehyde or ethanol developed off-flavour, but as the result of the differences between the two enzyme systems, off-flavour development could occur without any increase in acetaldehyde or alcohol content.

The experimental results showed that a straight relationship does not exist between off-flavour formation and acetaldehyde or alcohol production. It appears that acetaldehyde or alcohol accumulation cannot be suggested as an objective measure of off-flavour development.

LITERATURE CITED

Arighi, A.L., M.A. Joslyn and G.L. Marsh. 1936.
Enzyme activity in frozen vegetables.
Ind. Eng. Chem. 28: 595-598.

Barker, J. 1951.
A note on the determination of alcohol in potato tubers. J. Exp. Botany. 2: 238-241.

_____ and A.F. El Saifi. 1952.
Studies in the respiratory and carbohydrate metabolism of plant tissue. II. Interrelation between the rates of production of carbon dioxide, of lactic acid and of alcohol in potato tubers under anaerobic conditions.
Proc. Roy. Soc. (London). B140: 385-403. cf.Chem.Abs. 47: 1243a.

Barker, S.B. and W.H. Summerson. 1941.
The colorimetric determination of lactic acid in biological material. J. Biol Chem. 138: 535-554.

Barron, E.S.G., G.K.K. Link, R.M. Klein, and B.E. Michel. 1950.
The metabolism of potato slices.
Arch. Biochem. Biophys. 28: 377-398.

Bedford, C.L. and M.A. Joslyn. 1939.
Enzyme activity in frozen vegetables. Stringbeans.
Ind. Eng. Chem. 31: 751-758.

Biale, J.B. and A.D. Shepherd. 1939.
Identifaction of acetaldehyde among the volatile products of citrus fruits.
Proc. Am. Soc. Hort. Sci. 37: 543-547.

_____ and F.T. Weiss. 1939.
Identification of acetaldehyde in the steam distillate of the peel of citrus fruits.
J. Am. Chem. Soc. 61: 635-636.

Bodnar, J., C. Szepessy and J. Ferenczy. 1925.
Use of the Neuberg acetaldehyde method with the alcoholic fermentation of higher plants.
Biochem. Z. 165: 16-22. cf. Chem. Abs. 20: 2518.

Breslow, R. 1958.
On the mechanism of thiamine action. IV. Evidence from studies on model systems.
J. Am. Chem. Soc. 80: 3719-3726.

Buck, P.A. and M.A. Joslyn. 1953.
Accumulation of alcohol in underscalded frozen broccoli. J. Agr. Food Chem. 1: 309-312.

Cruess, W.V. 1947.
Blanching - its frozen pack importance.
Canner. 104 (2): 62-64.

David, J.J. 1949.
Acetaldehyde and related carbonyl compounds in frozen peas.
Thesis for degree of Ph.D., University of California.

_____ and M.A. Joslyn. 1953.
Acetaldehyde and related compounds in frozen green peas. Food Research. 18: 390-398.

Diehl, H.C. and J.A. Berry. 1933.
Relation of scalding practice and storage temperature to quality retention in frozen pack peas.
Proc. Am. Soc. Hort. Sci. 30: 496-500.

_____ J.H. Dingle and J.A. Berry. 1933.
Enzymes can cause off-flavour even when foods are frozen. Food Inds. 5: 300-301.

_____ H. Campbell and J.A. Berry. 1936.
Some observations on the freezing preservation of Alderman peas. Food Research. 1: 61-71.

Dietrich, W.C., M.D. Nutting, R.L. Olson, F.E. Lindquist, M.M. Boggs, G.S. Bohart, H.J. Neumann and H.J. Morris. 1959.
Time-temperature tolerance of frozen foods. XVI. Quality retention of frozen green snap beans in retail packages. Food Technol. 13: 136-145.

Dimick, K.P. and B. Makower. 1956.
Volatile flavour of strawberry essence. I. Identification of the carbonyls and certain low-boiling substances. Food Technol. 10: 73-75.

Ellis, R., A.M. Gaddis and G.T. Currie. 1958.
Paper chromatography of 2,4-dinitrophenylhydrazones of saturated aliphatic aldehydes.
Anal. Chem. 30: 475-479.

Fidler, J.C. 1933.
Studies in zymasis. IV. The accumulation of zymasic products in apples during senescence.
Biochem. J. 27: 1614-1621.

_____ 1934.
Studies in zymasis. VII. The estimation of the ethyl alcohol and acetaldehyde content of apples.
Biochem. J. 28: 1107-1120.

_____ 1936.
The aerobic and anaerobic breakdown of carbohydrates and acids by apples and oranges. Gt. Brit. Dep. Sci. Ind. Research. Food Invest. 1935: 114-115. cf. Chem. Abs. 32: 2237.

_____ 1951.
A comparison of aerobic and anaerobic respiration of apples. J. Exp. Botany. 2: 41-64.

Fisher, W.B. and F.O. Van Duyne, 1952.
Effect of variations in blanching on quality of frozen broccoli, snap beans and spinach.
Food Research. 17: 315-325.

Friedmann, T.E. 1938.

The identifaction and quantitative determination of volatile alcohols and acids.
J. Biol. Chem. 123: 161-184.

_____and R.Klaas. 1936.
The determination of ethyl alcohol.
J. Biol. Chem. 115: 47-61.

Gerhardt, F. and B.D. Ezell. 1938.
Respiration and emanation of volatiles from Bartlett pears as influenced by ripening and storage.
Proc. Am. Soc. Hort. Sci. 36: 423-426.

Gortner, W.A., F. Fenton, F.E. Voltz and E. Gleim. 1948.
Effect of fluctuating storage temperature on quality of frozen foods. Ind. Eng. Chem. 40: 1423-1426

Griebel, C. 1924.
Some observations on the ripening process in bananas. Z. Untersuch. Nahr. u. Genussm. 49: 221-227.
cf. Chem. Abs. 19: 666.

_____ 1925.
The occurrence of acetaldehyde in fruits and other plant tissues.
Z. Untersuch. Nahr. u. Genussm. 49: 105-110.
cf. Chem. Abs. 19: 2225.

Gustafson, F.G. 1934.
Production of alcohol and acetaldehyde by tomatoes.
Plant. Physiol. 9: 359-367.

Gutterman, B.M. 1956.
Determination of acetaldehyde in frozen vegetables.
J. Assoc. Offic. Agr. Chemists. 39: 282-285.

_____ R.D. Lovejoy and L.M. Beacham. 1951.
Report on quality factors in processed vegetables. Preliminary report on correlation of acetaldehyde with off-flavour in frozen vegetables.
J. Assoc. Offic. Agr. Chemists. 34: 231-232.

Hackney, F.M.V. 1943.
Metabolism of apples. II. Respiratory metabolism of Granny Smith apples of commercial maturity after various periods of cool storage.
Proc. Linnean Soc. N.S. Wales. 68: 33-47.
cf. Chem. Abs. 37: 5761[7].

Harley, C.P. and D.F. Fisher. 1927.
The occurrence of acetaldehyde in Bartlett pears and its relation to pear scald and breakdown.
J. Agr. Research. 35: 983-993.

Henze, R.E., C.E. Baker and F.W. Quackenbush. 1953.
The chemical composition of apple storage volatiles. I. Acids, alcohols, and esters.
Proc. Am. Soc. Hort. Sci. 61: 237-245.

_____ 1954.
Carbonyl compounds in apple storage volatiles.
J. Agr. Food Chem. 2: 1118-1120.

Holley, R.W., B. Stoyla and A.D. Holley. 1955.
The identifiaction of some volatile constituents of Concord grape juice. Food Research. 20: 326-331.

Huelin, F.E. 1952.
Volatile products of apples. III. Identification of aldehydes and ketones.
Australian J. Sci. Research. B5: 328-334.

Isherwood, F.A. and C.A. Niavis. 1956.
Estimation of α-keto acids in plant tissue: a critical study of various methods of extraction as applied to strawberry leaves, washed potato slices and peas. Biochem. J. 64: 549-558.

Jaulmes, P. and R. Espezel. 1935.
Determination of acetaldehyde in wines and spirituous liquors. Ann. fals. et fraudes. 28: 325-335. cf. Chem. Abs. 29: 7008.

Joslyn, M.A. 1930.
Preservation of fruits and vegetables by freezing storage. Calif. Dept. Agr.Exp. Sta. Circ. 320: 1-35.

_____ 1949.
Enzyme activity in frozen vegetables tissue. Advances in Enzymol. 9: 613-652.

_____ and C.L. Bedford. 1938-1940.
Unpublished data.

_____ 1940.
Enzyme activity in frozen vegetables. Asparagus. Ind. Eng. Chem. 32: 702-706.

_____ and G.L. Marsh. 1938.
Enzyme activity in frozen vegetables. Artichoke hearts. Ind. Eng. Chem. 30: 1068-1073.

_____ and J.J. David. 1952.
Acetaldehyde and alcohol in raw or underblanched peas. Quick Frozen Foods. 15:(4), 51-53, 151-153.

_____and G.L Marsh. 1933.
The role of peroxidase in the deterioration of frozen fruits and vegetables. Science. 78: 174-175.

_____ 1933.
Observations on certain changes occurring during freezing and subsequent thawing of fruits and vegetables. Fruit Prod. J. 12: 330-332. Chem.Abs. 27: 4319.

Kirchner, J.G. 1949.
The chemistry of fruit and vegetable flavors. Advances in Food Research. 2: 259-296.

Klein, S. and K. Pirschle. 1926.
Acetaldehyde as an intermediate product in plant respiration.
Biochem. Z. 168: 340-360. cf. Chem. Abs. 20: 2519.

Kohman E.F. 1932.
New facts relating to quality in canned peas. Canner. 74: No. 11, 64-66.

_____ and N.H. Sanborn. 1932.
Special chemical studies.
Canner. 74: No. 11, 132-134.

Kohman E.F. and N.H. Sanborn. 1934.
Effect of respiration on vegetable flavor.
Ind. Eng. Chem. 26: 773-776.

Kostychev, S., E. Hubbenet and A. Sheloumov. 1913.
Formation of acetaldehyde through anaerobic respiration of poplar blossoms.
Z. physiol.Chem. 83: 105-111. cf. Chem. Abs. 7: 1531.

Kramer, A. 1954.
Mandatory standards program on quality factors for frozen asparagus and peas - an industry approach.
III. Evaluation of results. Food Technol. 8: 468-470

Laing, H.E. 1940.
Respiration of the rhizomes of Nuphar advenum and other water plants. Am. J. Botany. 7: 574-581.

Lechartier, C. and F. Bellamy. 1869.
De la fermentation des fruits.
Compt. rend. 69: 466-469.

_____ 1872.
De la fermentation des fruits.
Compt. rend. 75: 1203-1206.

_____ 1874.
De la fermentation des fruits.
Compt. rend. 79: 1006-1009.

Lee, F.A. 1954.
Chemical chages taking place in the crude lipids during the storage of frozen raw vegetables.
Food Research. 19: 515-520.

_____ 1958.
The blanching process.
Advances in Food Research. 8: 63-109.

_____ A.C. Wagenknecht and J.C. Hening. 1955.
A chemical study of the progressive development of off-flavor in frozen raw vegetables.
Food Research. 20: 289-297.

Link, G.K.K., R.M. Klein and E.S.G. Barron. 1952.
Metabolism of slices of the tomato stem.
J. Exp. Botany. 3: 216-236.

Lovejoy, R.D. 1952.
Report on quality factors in processed vegetable products. Acetaldehyde content as a measure of flavor in frozen peas and frozen asparagus.
J. Assoc. Offic. Agr. Chemists. 35: 179-181.

Mattick, L.R. and W.B. Robinson. 1960.
Changes in volatile constituents during the baking of sherry wine by the Tressler process.
Food Technol. 14: 30-33.

Mergentime, M. and E.H. Wiegand. 1946.
Low-temperature characteristics of pea proteinase.
Fruit Prod. J. 26: 72-80, 89, 91-92.

Miller, E.V. 1936.
Distribution of acetaldehyde and alcohol in the apple fruit. J. Agr. Research. 53: 49-55.

Moore, R.E. 1951.
Formation of acetaldehyde and alcohol in frozen peas and their relation to off-flavour development.
Thesis for degree of M.Sc., McGill University.

Müller-Thurgau, H. and A. Osterwalder. 1915.
Formation of acetaldehyde in fruit .
Landwirtsch. Jahrb. Schweiz. 1915: 400-407.
cf. Chem. Abs. 10: 2474.

Nance, J.P. 1949.
A comparison of carbohydrate loss and carbon dioxide production during fermentation by barley roots.
Am. J. Botany. 36: 274-276.

Neal, M.J. and R.E. Girton. 1955.
The Pasteur effect in maize.
Am. J. Botany. 42: 733-737.

Neuberg, C. and A. Gottschalk. 1924.
Observation on the course of anaerobic respiration of plants.
Biochem. Z. 151: 167-168. cf. Chem. Abs. 19: 1584.

_____ 1925.
The proof that acetaldehyde is an intermediary product in the anaerobic respiration of higher plants.
Biochem. Z. 160: 256-260. cf. Chem. Abs. 20: 1095.

Niegish, W.D. and W.H. Stahl. 1956.
The onion: gaseous emanation products.
Food Research. 21: 657-665.

Niethammer, A. 1928.
Continued studies on the chemistry of Augiosperm seeds and the natural and artificial external germination factors. II. Acetaldehyde.
Biochem. Z. 197: 245-256. cf. Chem. Abs. 23: 183.

Pasteur, L. 1872.
Note sur la production de l'alcool par les fruits.
Compt. rend. 75: 1054-1056.

Peyron, L. 1959.
Micro-recherche qualitative des composes carbonyles par chromatographic radiale de leurs hydrazones.
La France et ses parfums. 12: 41-48.

Phillips, J.W. 1947.
Studies on fermentation in rice and barley.
Am. J. Botany. 34: 62-72.

Pirschle, K. 1926.
Acetaldehyde is an intermediary product in the germination of seeds which contain fats.
Biochem. Z. 169: 482-489. cf. Chem. Abs. 20: 3715.

Power, F.B. and V.K. Chestnut. 1920.
The odorous constituents of apples. Emanation of acetaldehyde from the ripe fruit.
J. Am. Chem. Soc. 42: 1509-1526. cf. Chem.Abs. 14: 2829.

Ralls, J.W. 1959.
Non-enzymatic formation of acetoin in canned vegetables. J. Agr. Food Chem. 7: 505-507.

_____ 1960.
Flash exchange gas chromatography for the analysis of potential flavor components of peas.
J. Agr. Food Chem. 8: 141-143.

Ruhland, W. and K. Ramshorn. 1938.
Aerobe Garung in Activen Pflanzlichen Meristemen.
Planta. 28: 471-514.

Semichon, L. and M. Flanzy. 1929.
Determination of alcohol in wines and liquors by means of potassium dichromate and sulfuric acid.
Ann. fals. et fraudes. 22: 139-152. cf. Chem.Abs. 23:3537.

Silberstein, O. 1954.
Volatile carbonyl compounds of vegetables and their possible role in flavor.
Proc. Am. Soc. Hort. Sci. 63: 359-370.

Singer, T.P. and J. Pensky. 1951.
Acetoin synthesis by highly purified α - carboxylase. Arch. Biochem. Biophys. 31: 457-459.

Steinberg, M.A., G.E. Livingston and C.R. Fellers. 1956.
Chemical aspects of pectin gel darkening.
Food Technol. 10: 470-476.

Steinmann, A. 1935.
The presence of acetaldehyde in tropical fruits.
Z. Untersuch. Lebensm. 69: 479-481.
cf. Chem. Abs. 29: 7517.

Stiles, W. 1960.
Respiration III. Botan. Rev. 26: 209-260.

Thewlis, B.H. 1952.
The identification of ethyl alcohol in plant tissues.
New Phytologist. 51: 263-265.

Thomas, M. 1925.
The controlling influence of CO_2. V. A quantitative study of the production of ethyl alcohol and acetaldehyde by cells of the higher plants in relation to concentration of oxygen and carbon dioxide. Biochem. J. 19: 927-947.

_____ 1931.
The production of ethyl alcohol and acetaldehyde by fruits in relation to the injuries occurring in storage. Part II. Injuries to apples and pears occurring in the presence of oxygen and in the absence of accumulation of carbon dioxide in the storage atmosphere. Ann. Appl. Biol. 18: 60-74.

_____ and J.C. Fidler. 1941.
Studies in zymasis. VIII. The discovery and investigation of aerobic HCN zymasis in apples treated with hydrogen cyanide; and comparison with other forms of zymasis. New Phytologist. 40: 217-239.

Thompson, A.R. 1951.
Volatile products of apples. I. Identification of acids and alcohols.
Australian J. Sci. Research. B.4: 283-290.
cf. Chem. Abs. 46: 193g.

Tressler, D.K. 1932.
Chemical problems of the quick-freezing industry.
Ind. Eng. Chem. 24: 682-686.

Underwood, J.C., H.G. Lento, Jr. and C.O. Willits. 1956.
Triose compounds in maple syrup.
Food research. 21: 589-597.

Wager, H.G. 1958.
Determination of ethanol and acetaldehyde in plant tissue by low temperature diffusion.
Analyst. 83: 291-295.

Wetzel, K. 1933.
Zur Physiologie der Anaeroben Atmung Hoherer Pflanzen.
Ber. deut. botan. Ges. 51: 46-51.

White, Jr. J.W. 1950.
Composition of volatile fraction of apples.
Food Research. 15: 68-78.

Winter, M., B. Willhalm, M. Hinder, E. Palluy and E. Sundt.1958.
Chromatography on columns and paper in the study of the volatile fruit flavour of strawberries.
Perfumery and Essential Oil Record. 49: 250-255.

Wiseblatt, L., and F.E. Kohn. 1960.
Some volatile aromatic compounds in fresh bread.
Cereal Chem. 37: 55-66.

Woodroof, J.G., I.S. Atkinson, S.R. Cecil and E. Shelor. 1946.
Studies of methods of scalding (blanching) vegetables for freezing.
Georgia Agr. Exp. Sta. Bull. 248: 1-44.

Zoneil, M.E. and W.B. Esselen. 1959.
Thermal destruction rates and regeneration of peroxidase in green beans and turnips.
Food Research. 24: 119-133.

APPENDIX

F I G U R E 1.

EFFECT OF STEAM BLANCHING AND PROCESSING ON ACETALDEHYDE AND ETHANOL CONTENT OF BEANS.

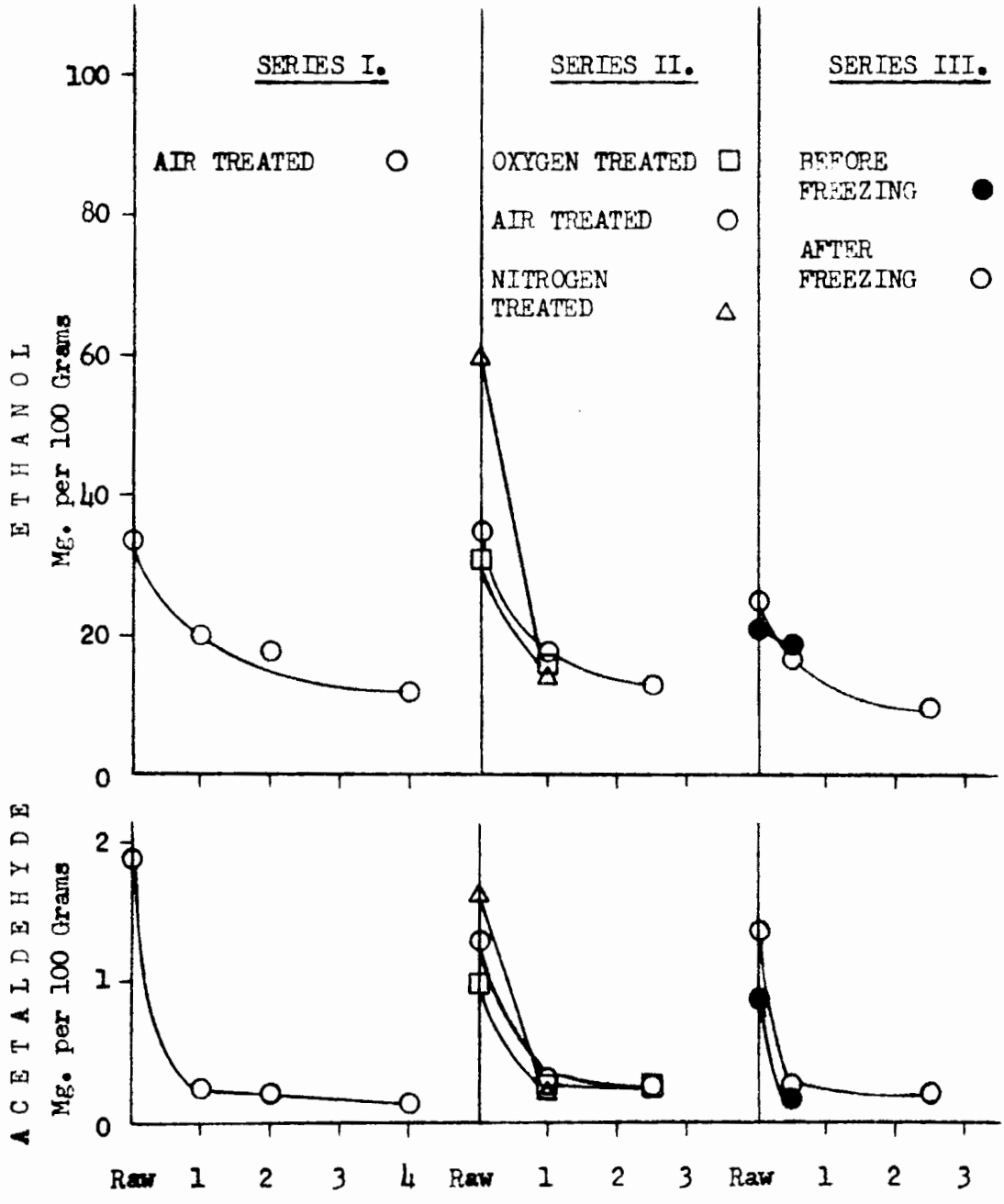


FIGURE 2.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER OXYGEN, SERIES I.

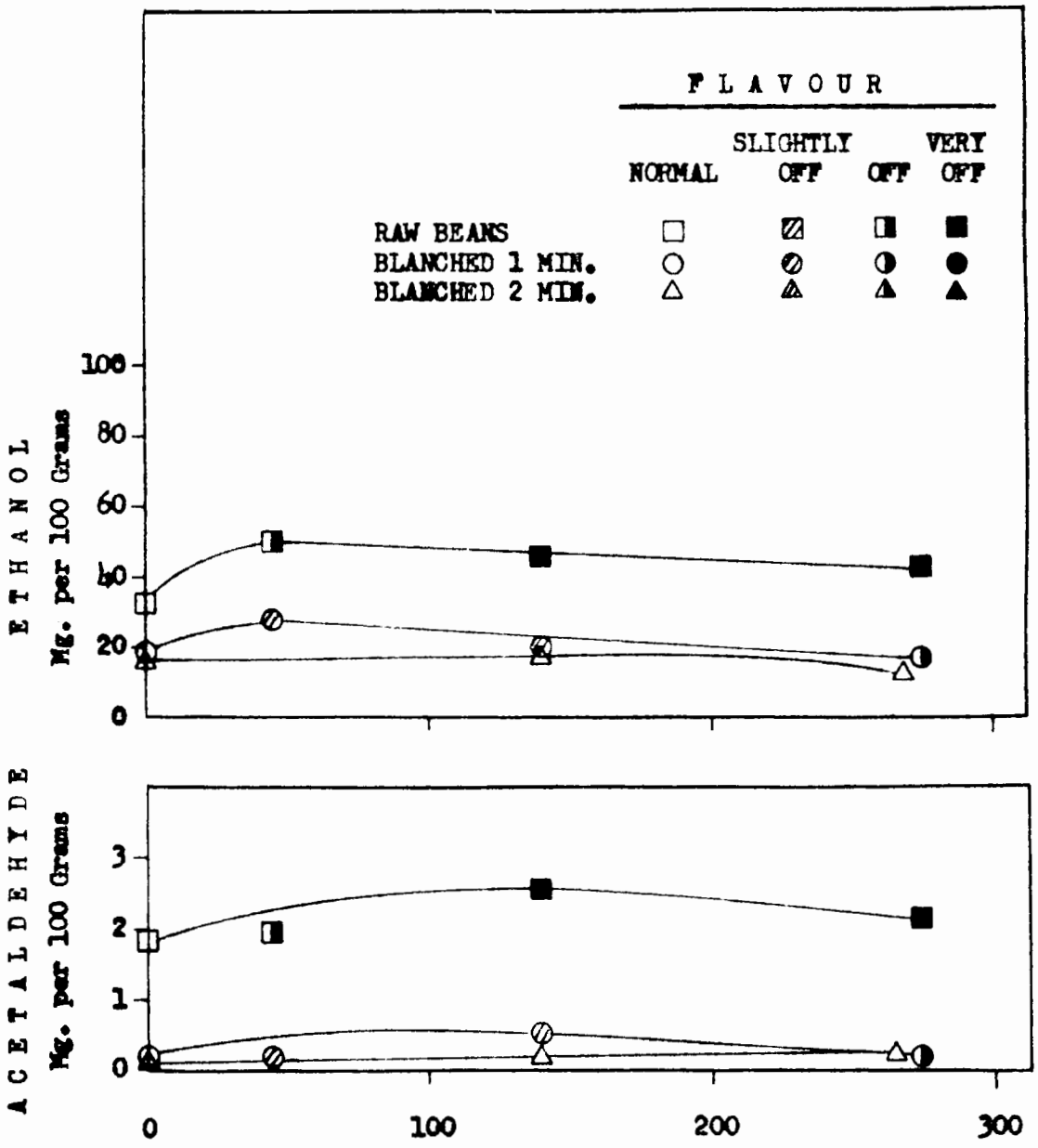
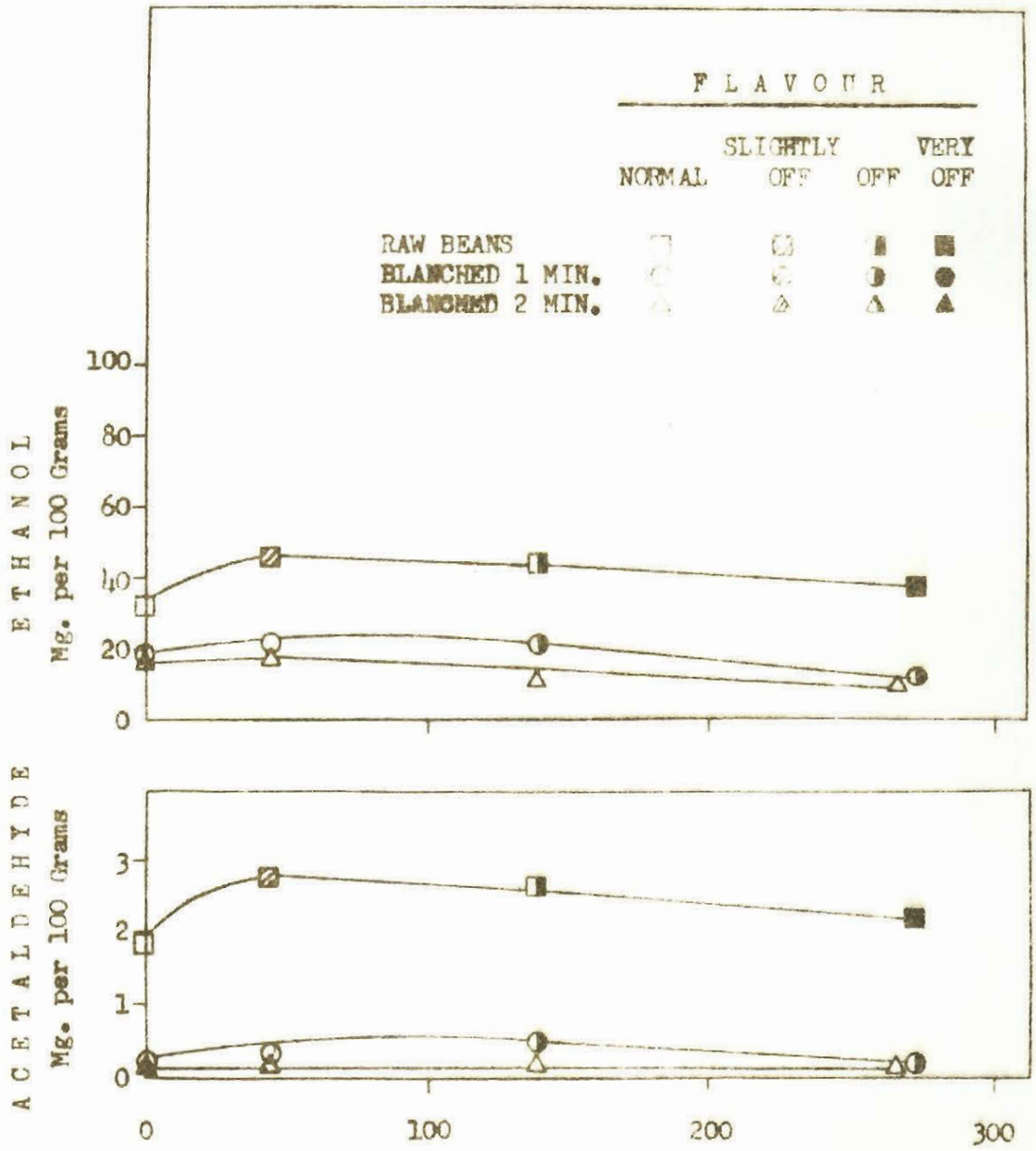


FIGURE 3.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER AIR, SERIES I.



F I G U R E 4.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER NITROGEN, SERIES I.

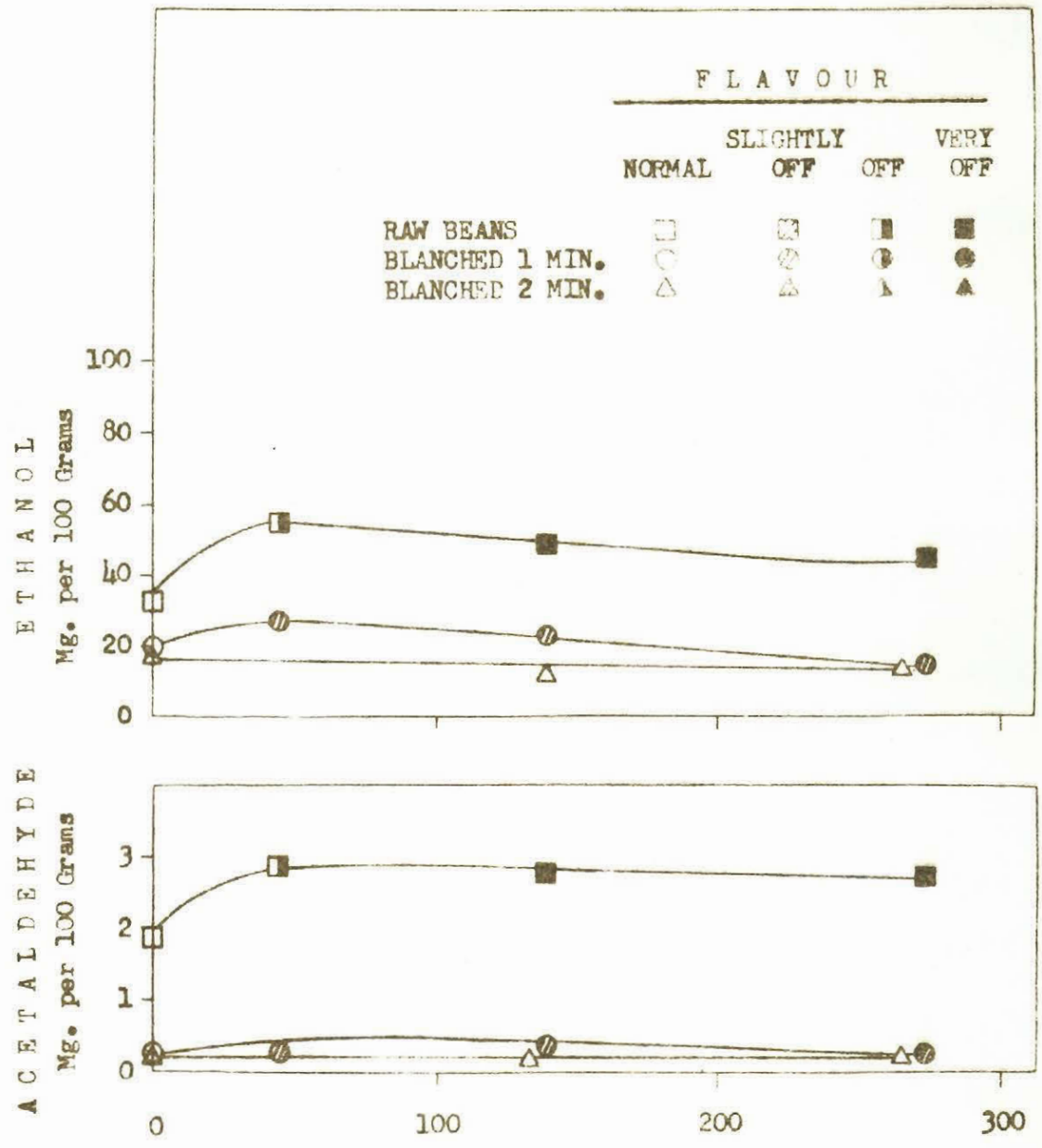
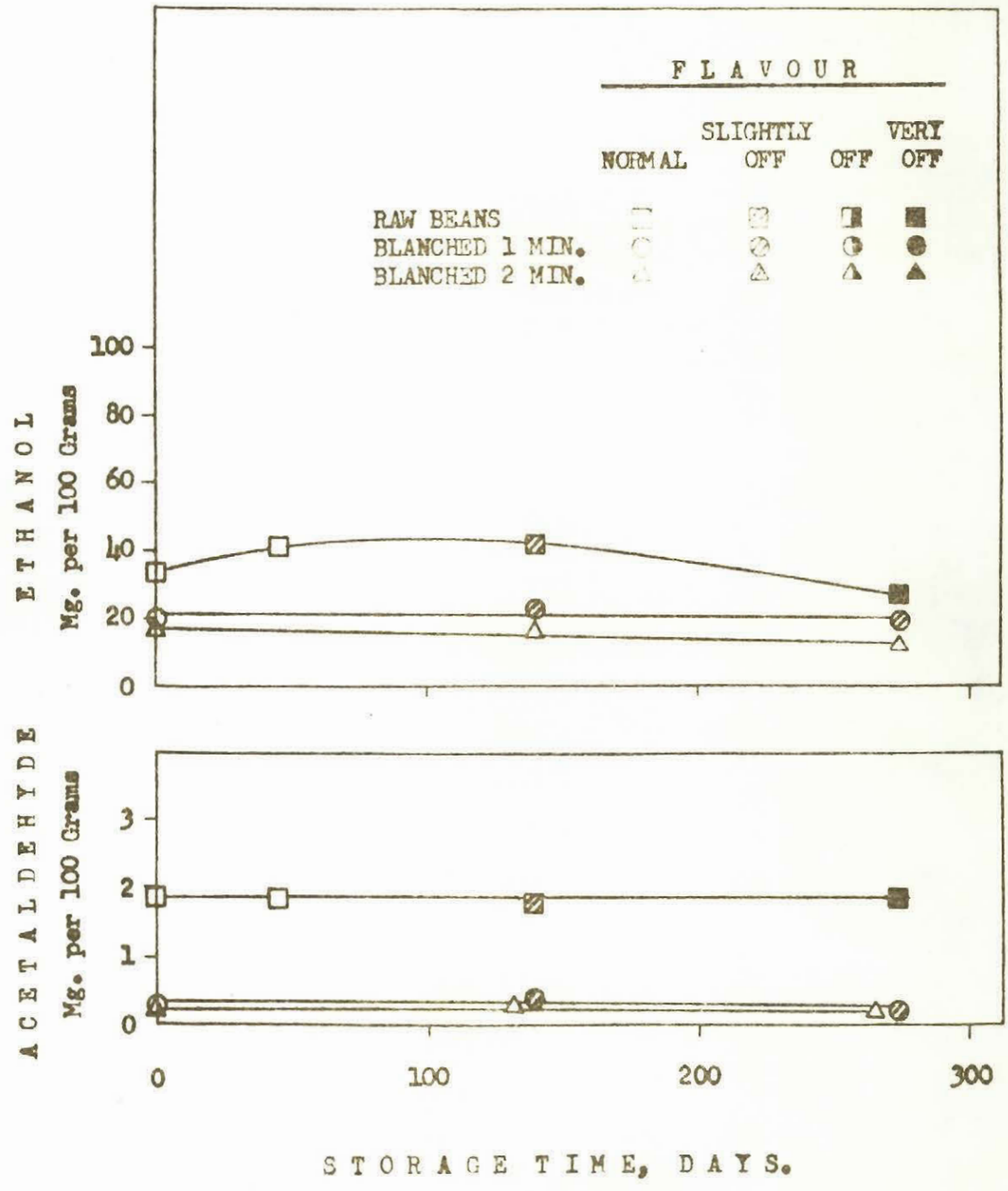


FIGURE 5.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER OXYGEN, SERIES I.



F I G U R E 6.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER AIR, SERIES I.

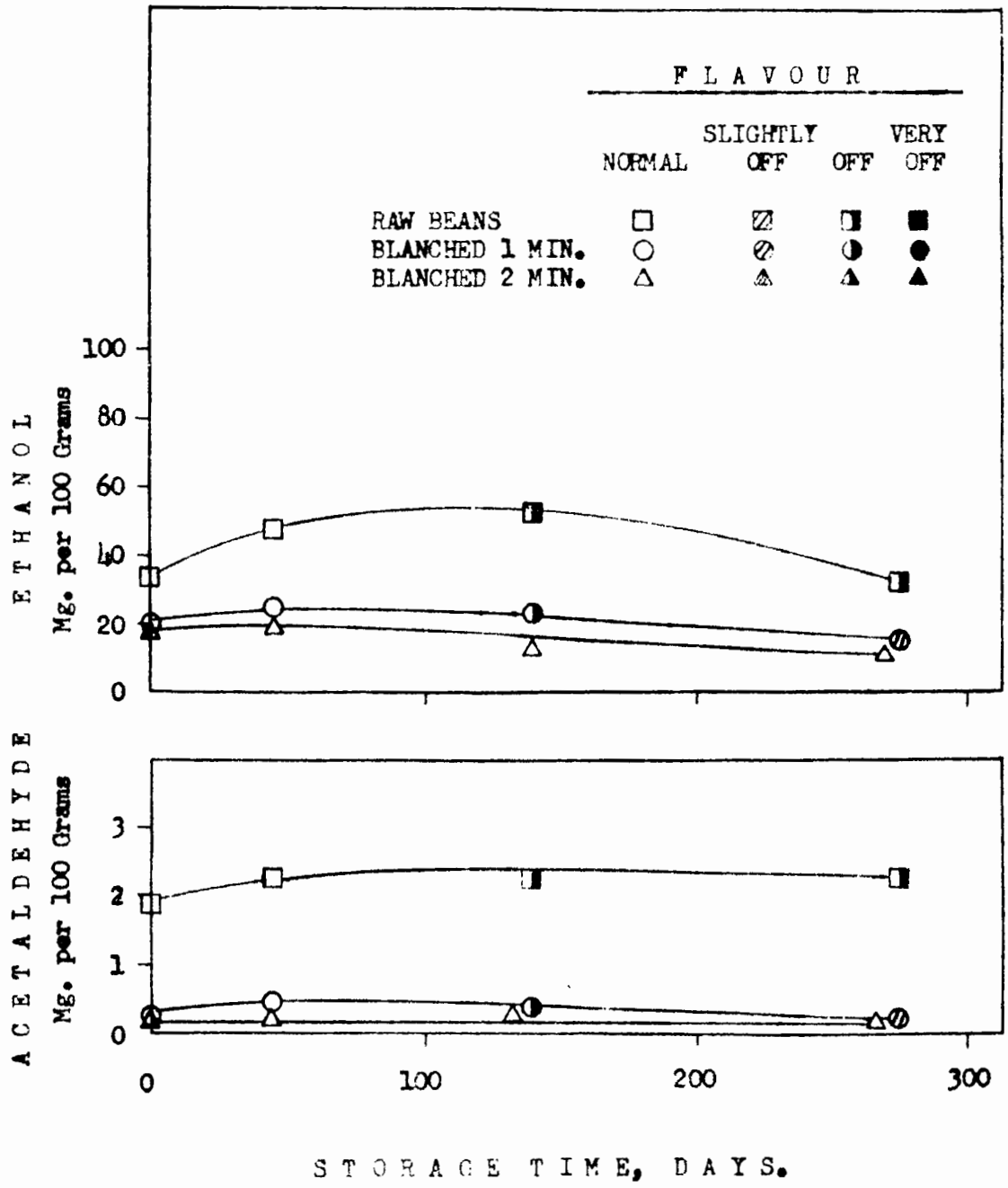


FIGURE 7.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER NITROGEN, SERIES I.

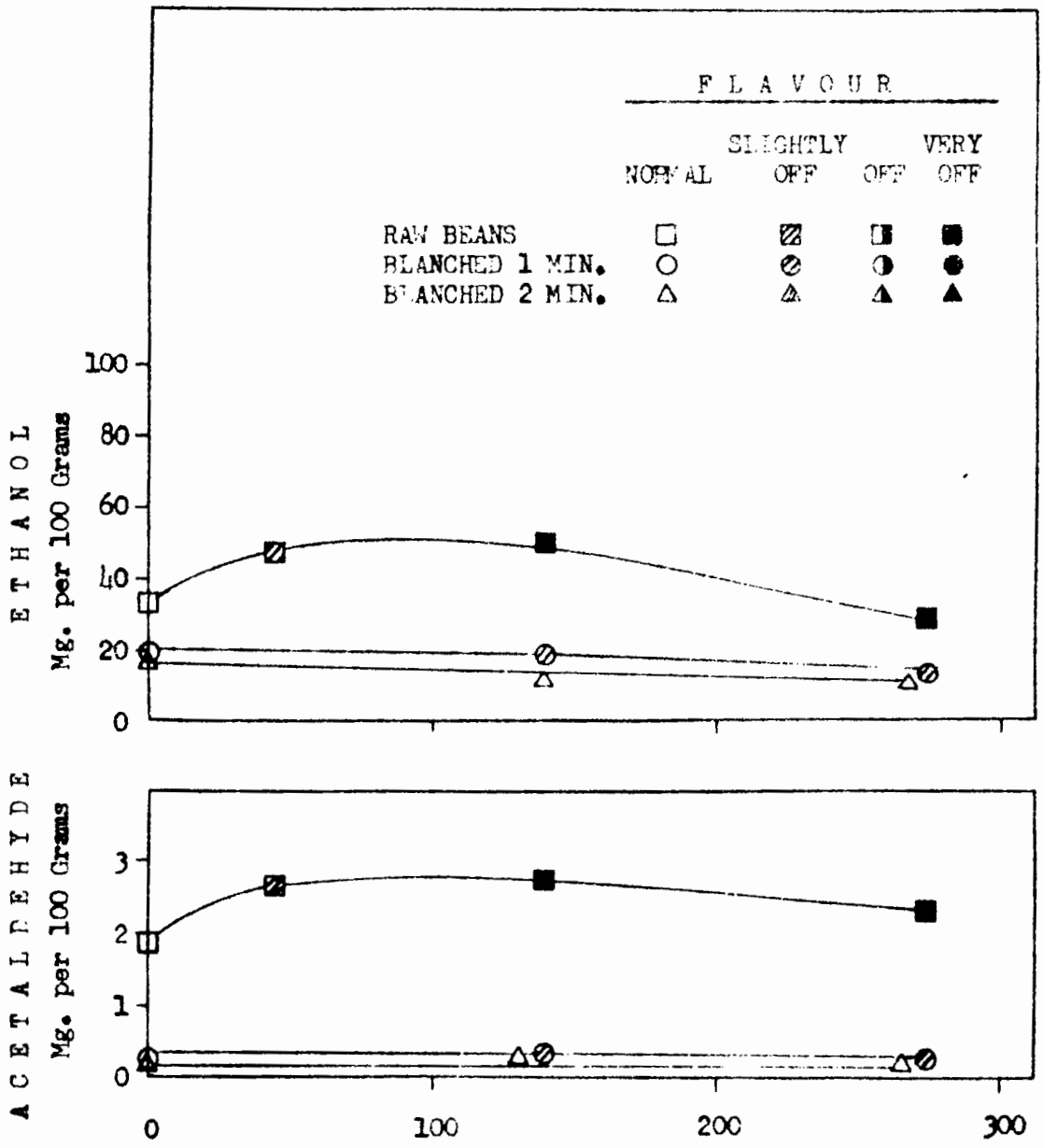
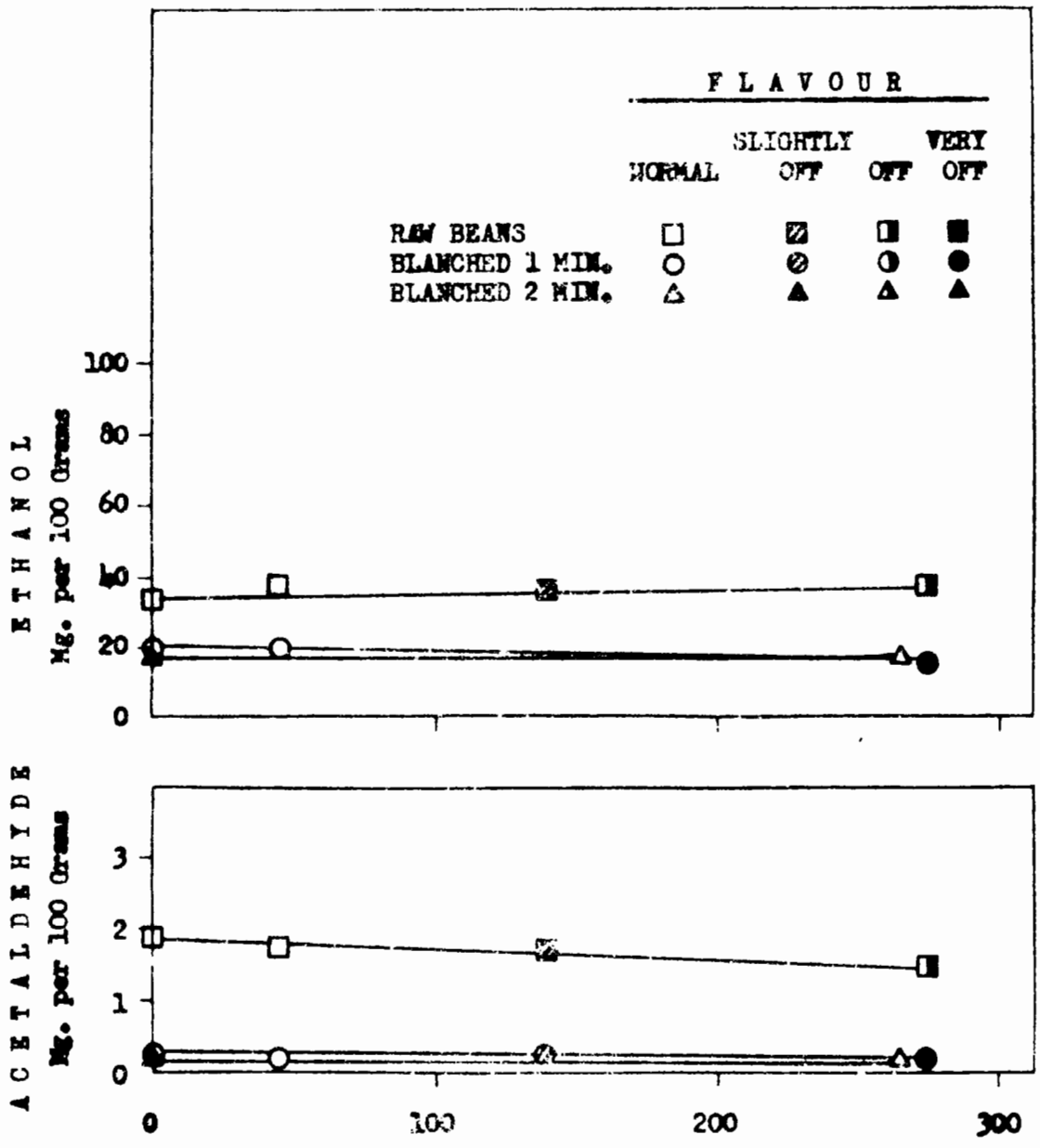


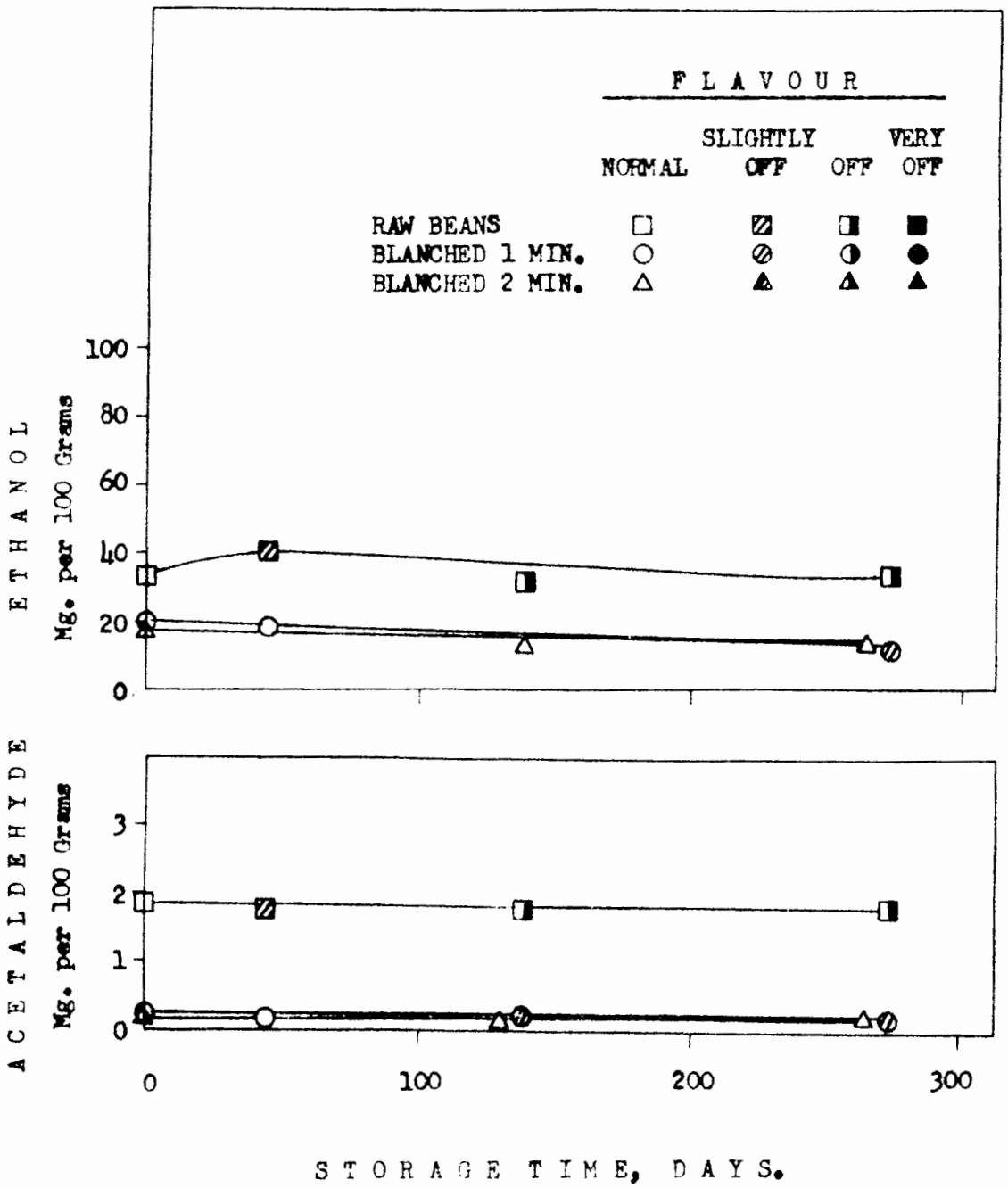
FIGURE 8.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER OXYGEN, SERIES I.



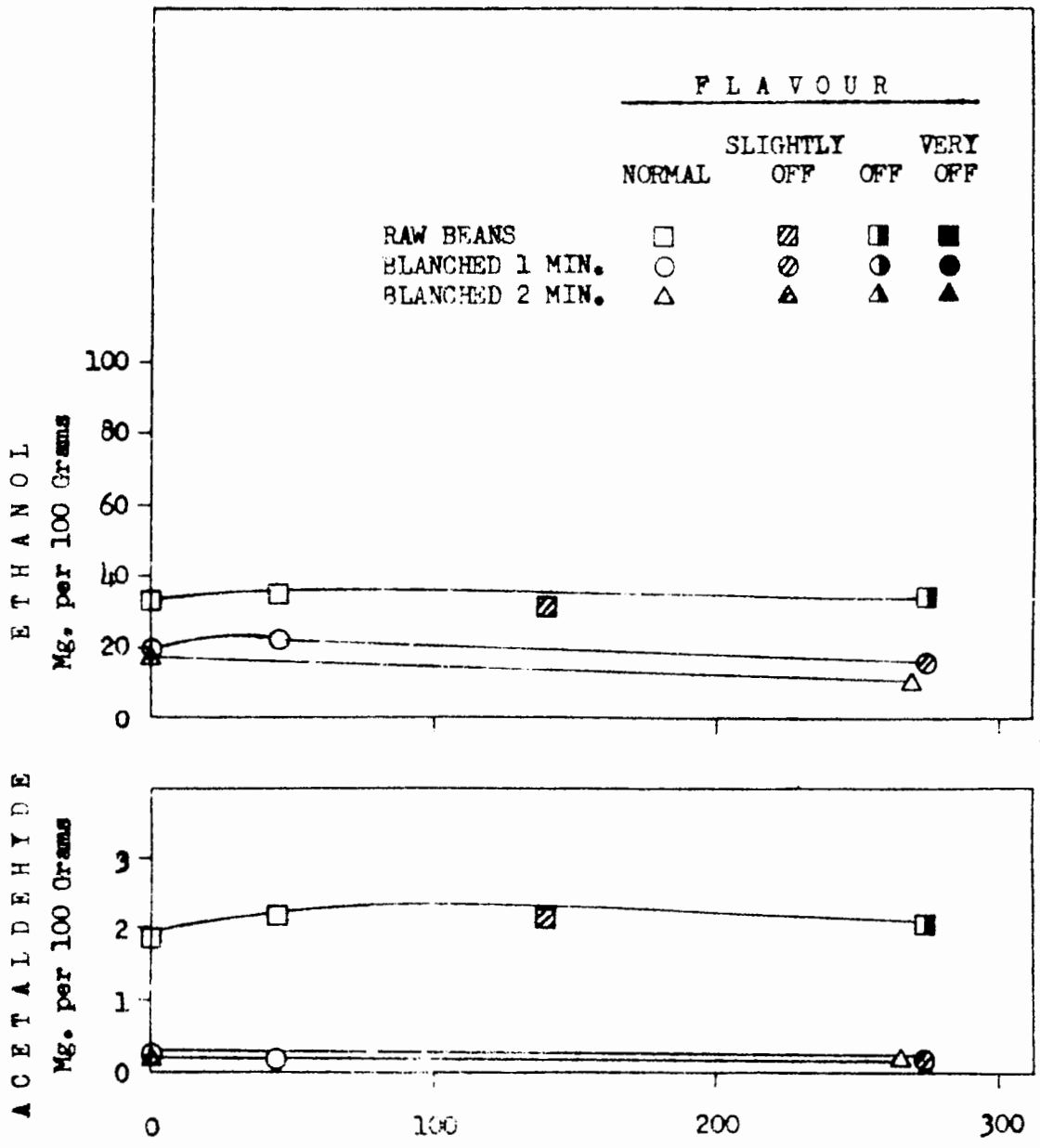
F I G U R E 9.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER AIR, SERIES I.



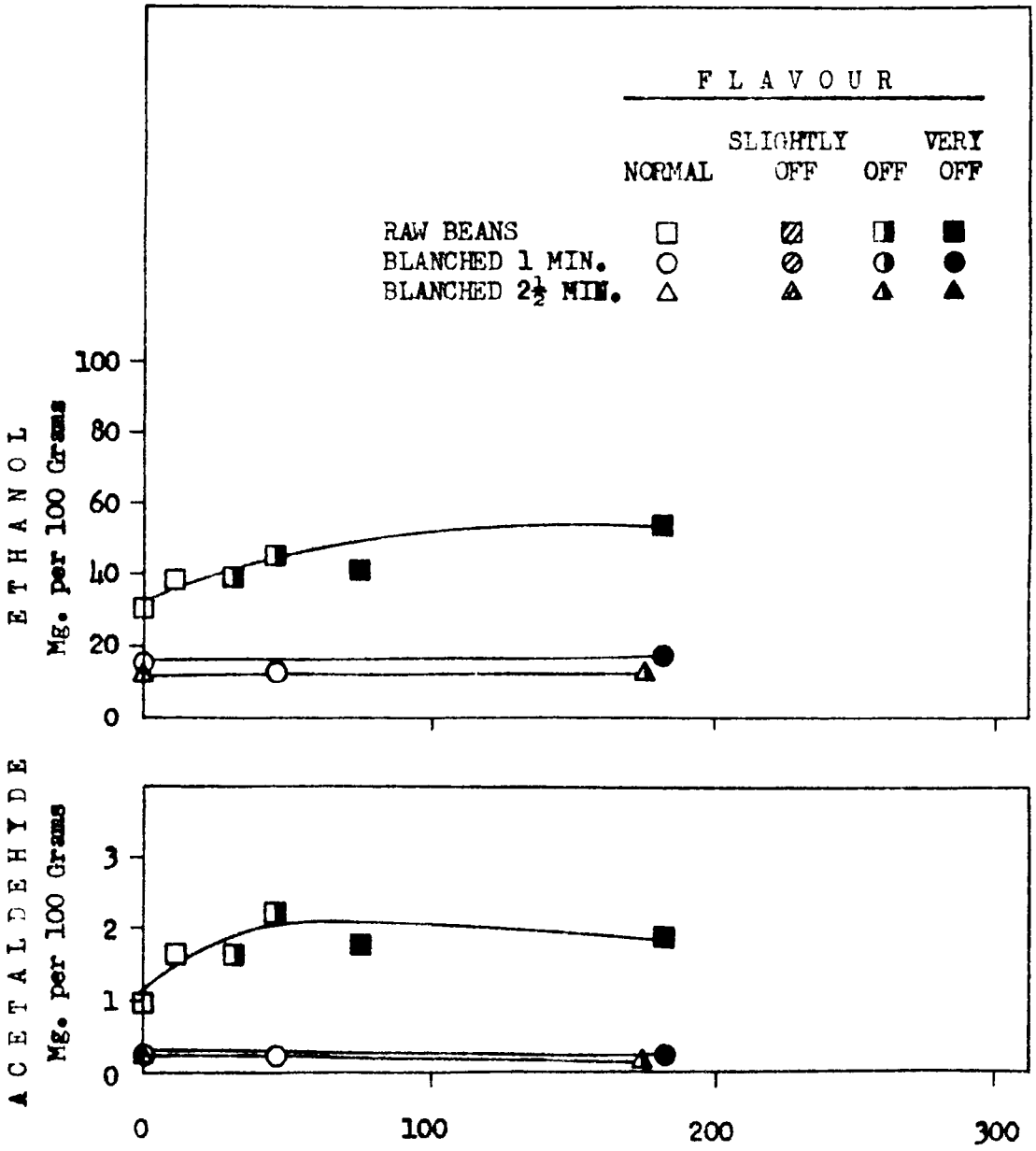
F I G U R E 10.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER NITROGEN, SERIES I.



F I G U R E 11.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER OXYGEN, SERIES II.



F I G U R E 12.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER AIR, SERIES II.

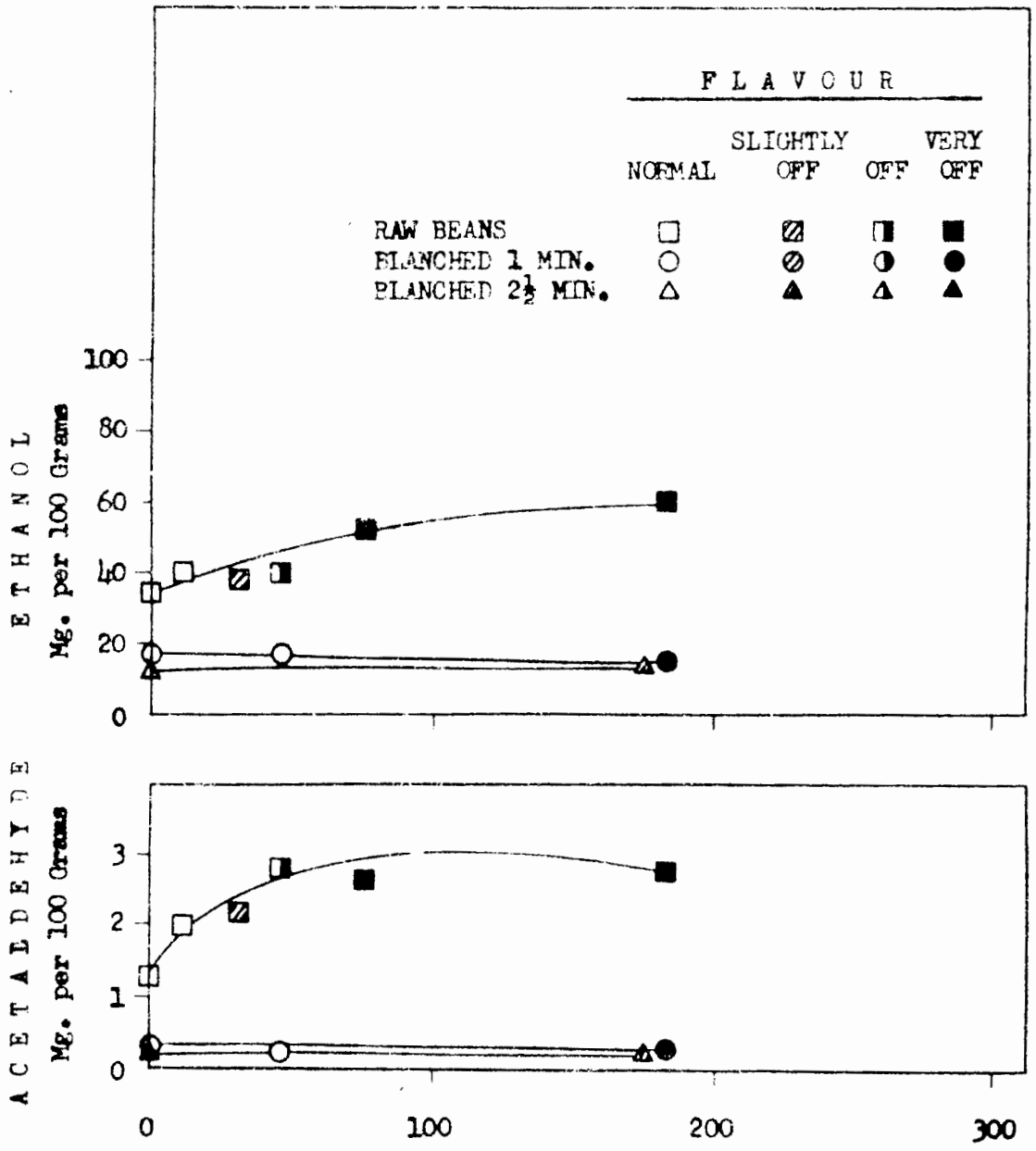


FIGURE 13.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER NITROGEN, SERIES II.

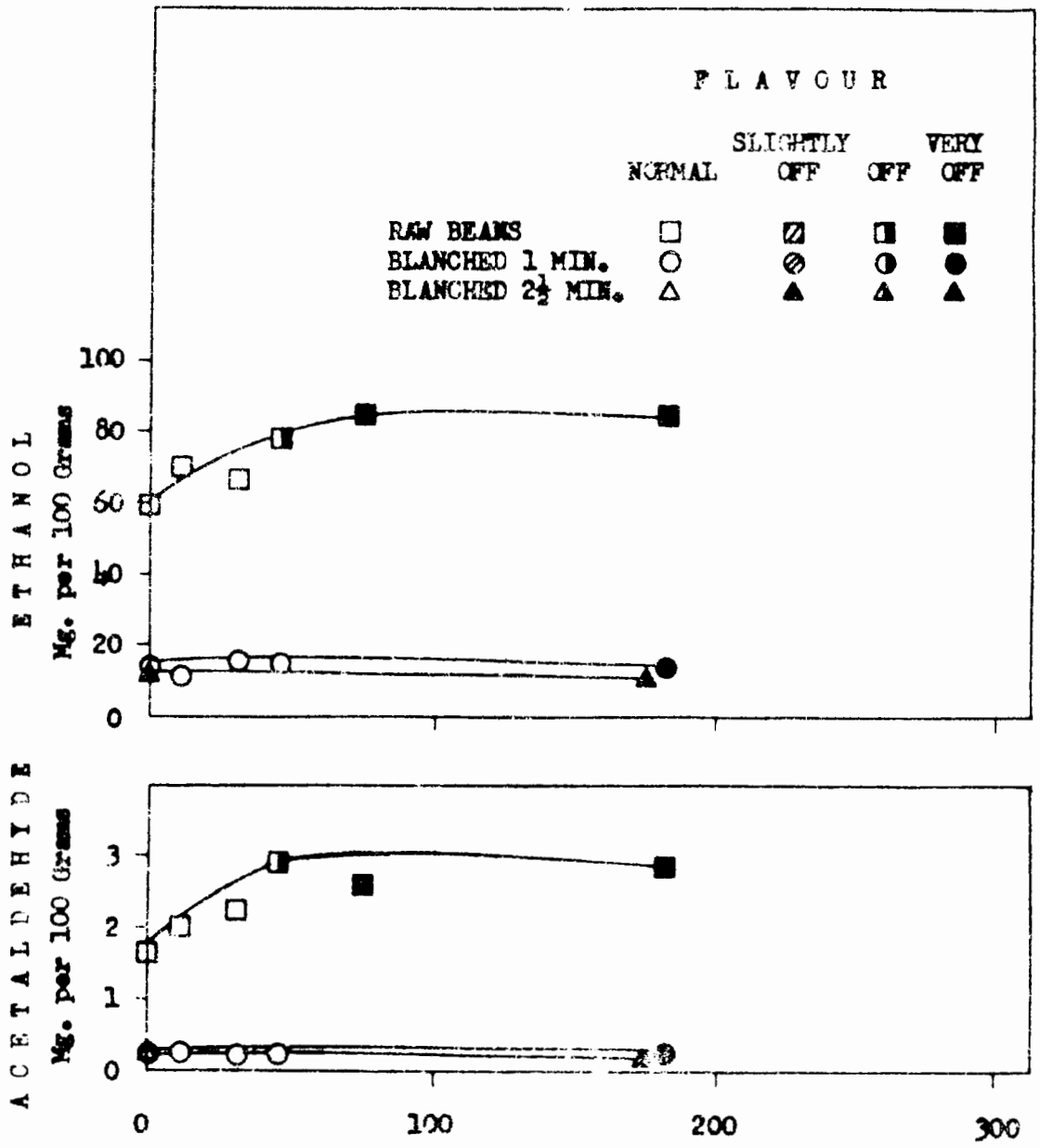
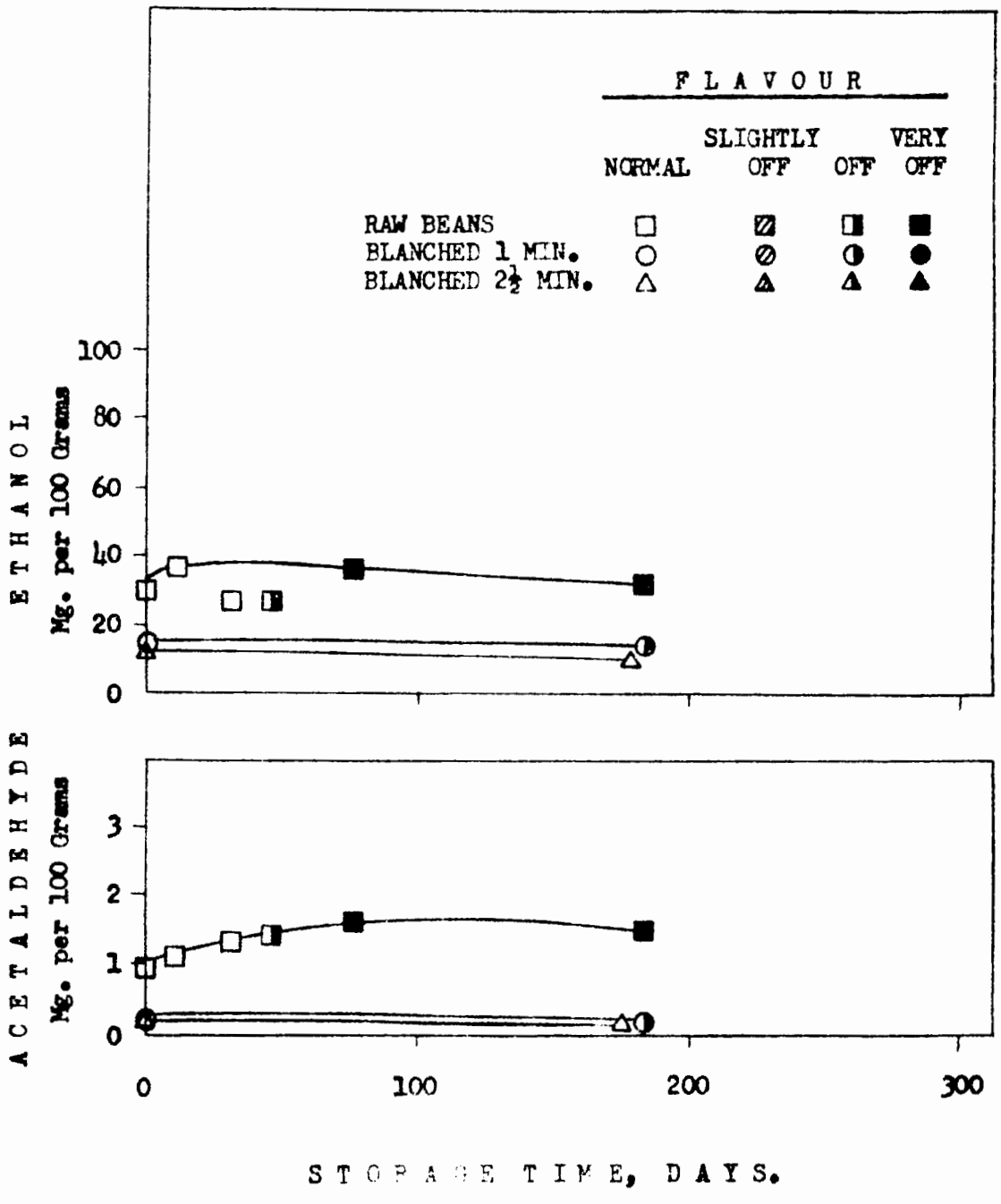


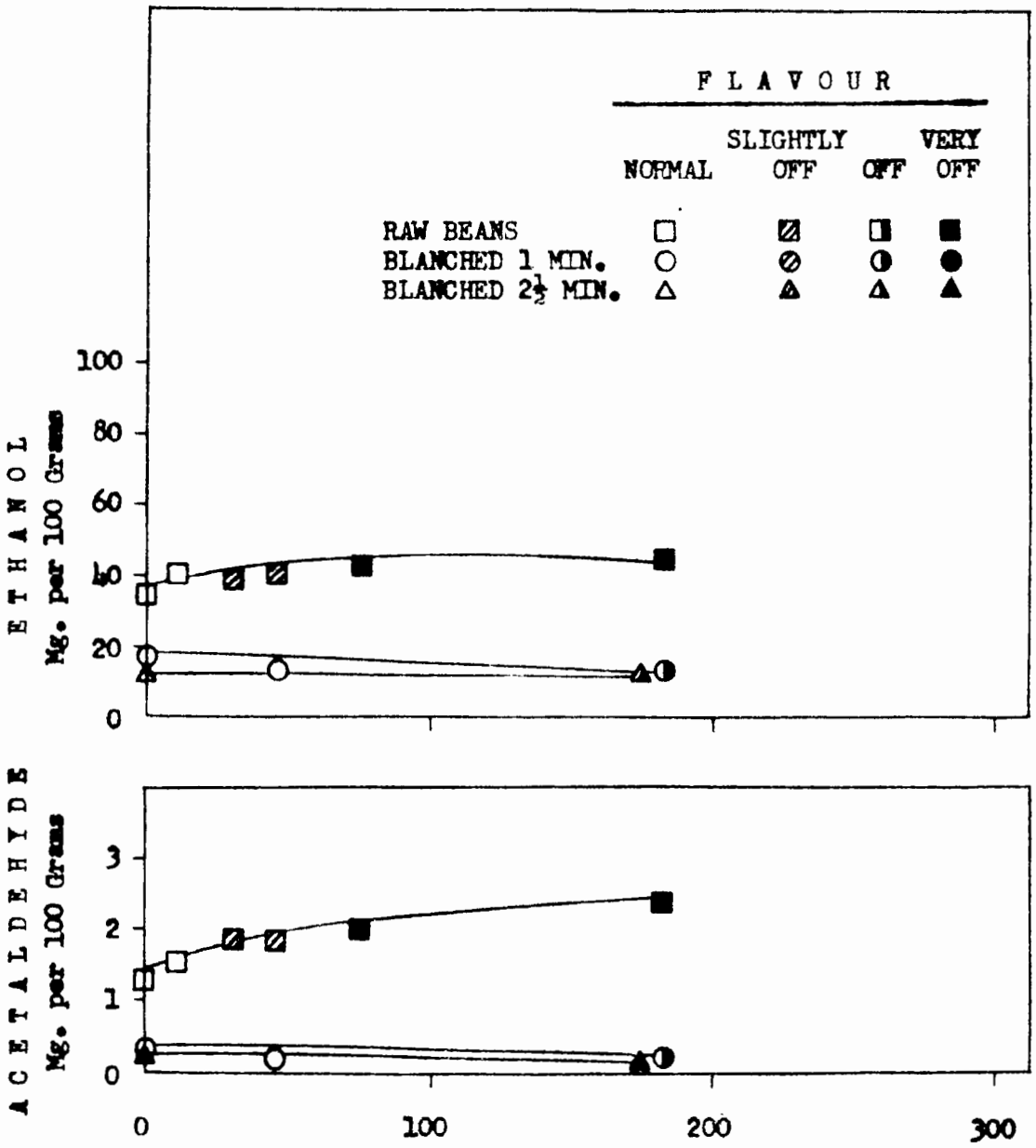
FIGURE 14.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER OXYGEN, SERIES II.



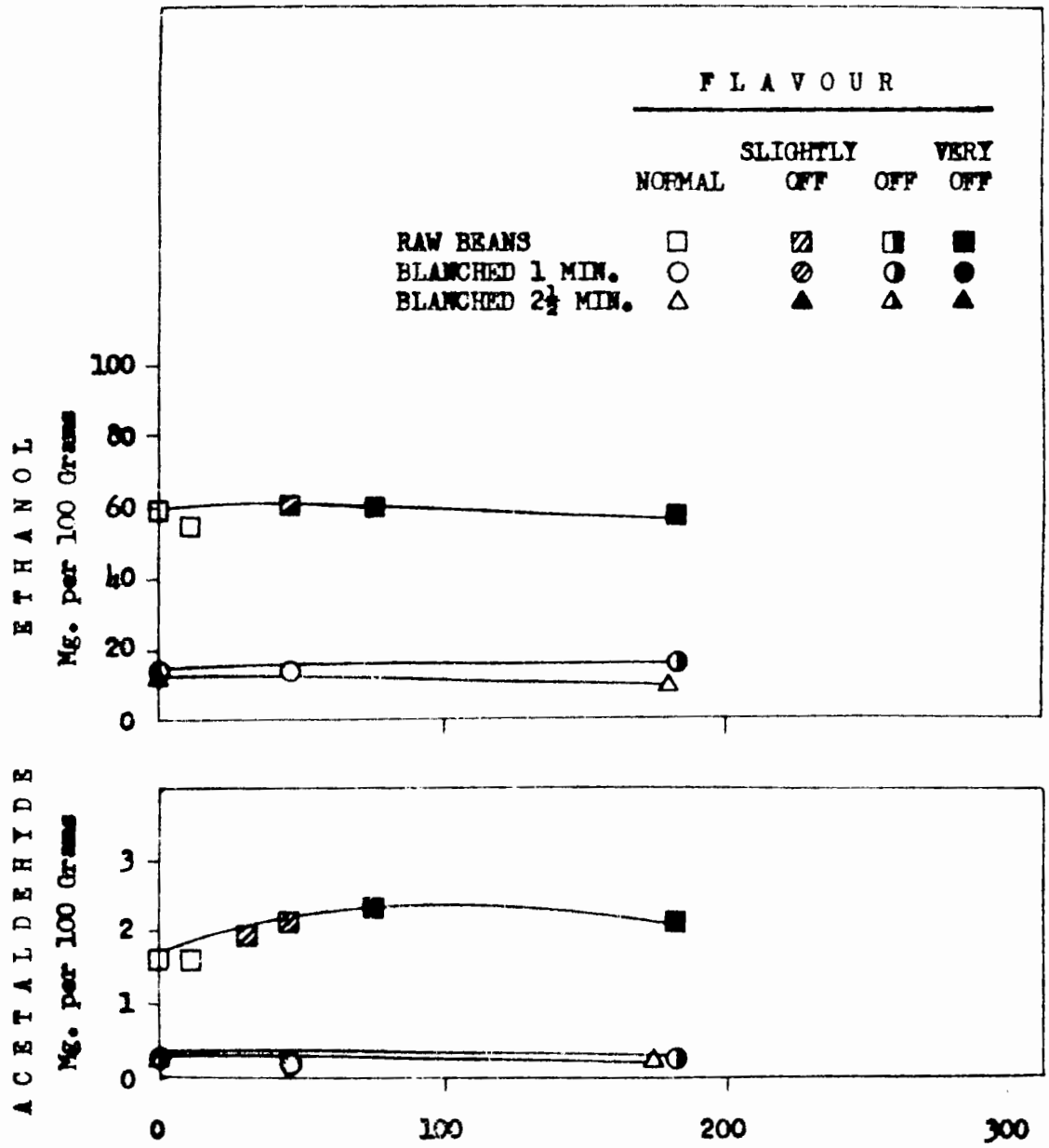
F I G U R E 15.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER AIR, SERIES II.



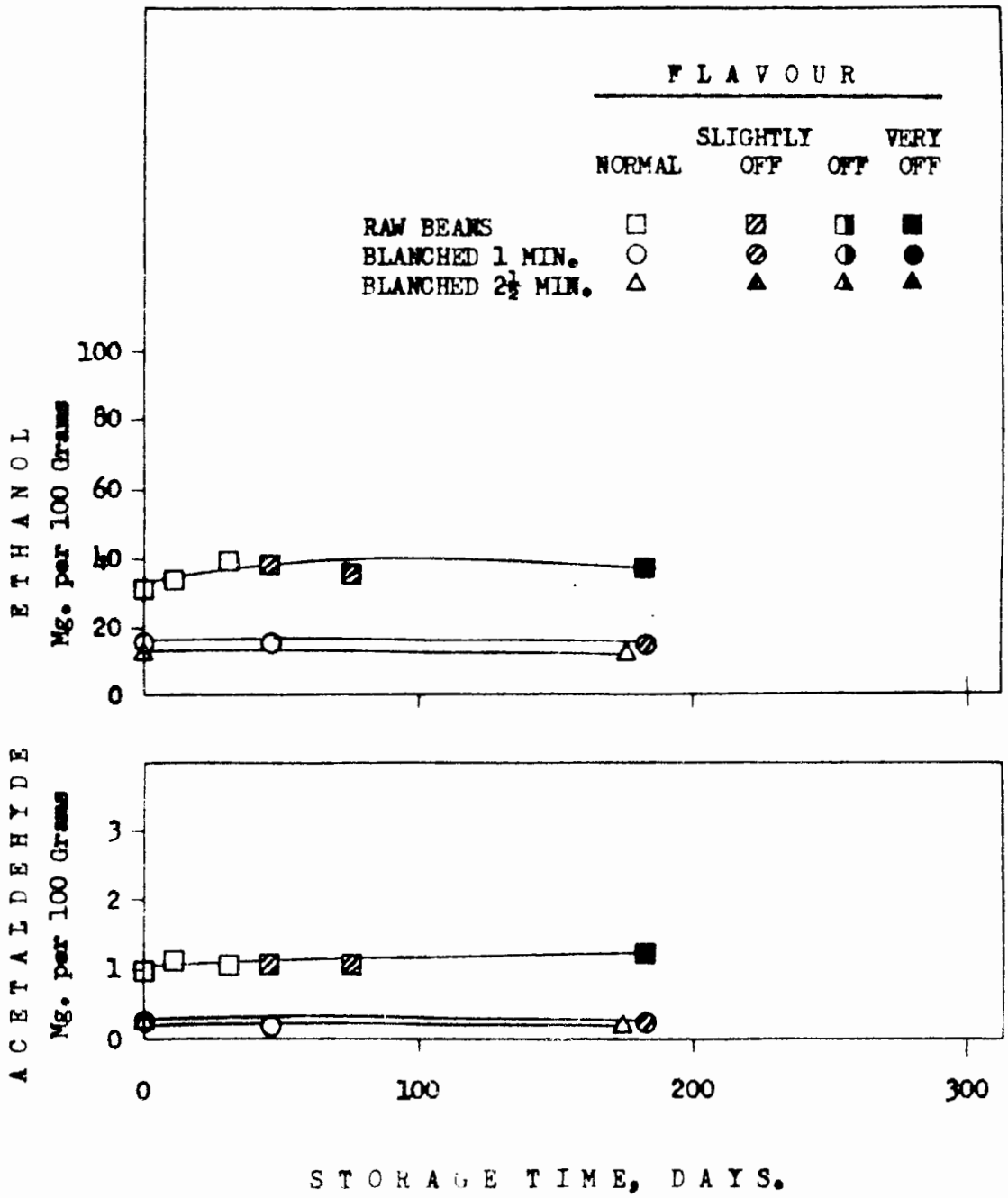
F I G U R E 16.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER NITROGEN, SERIES II.



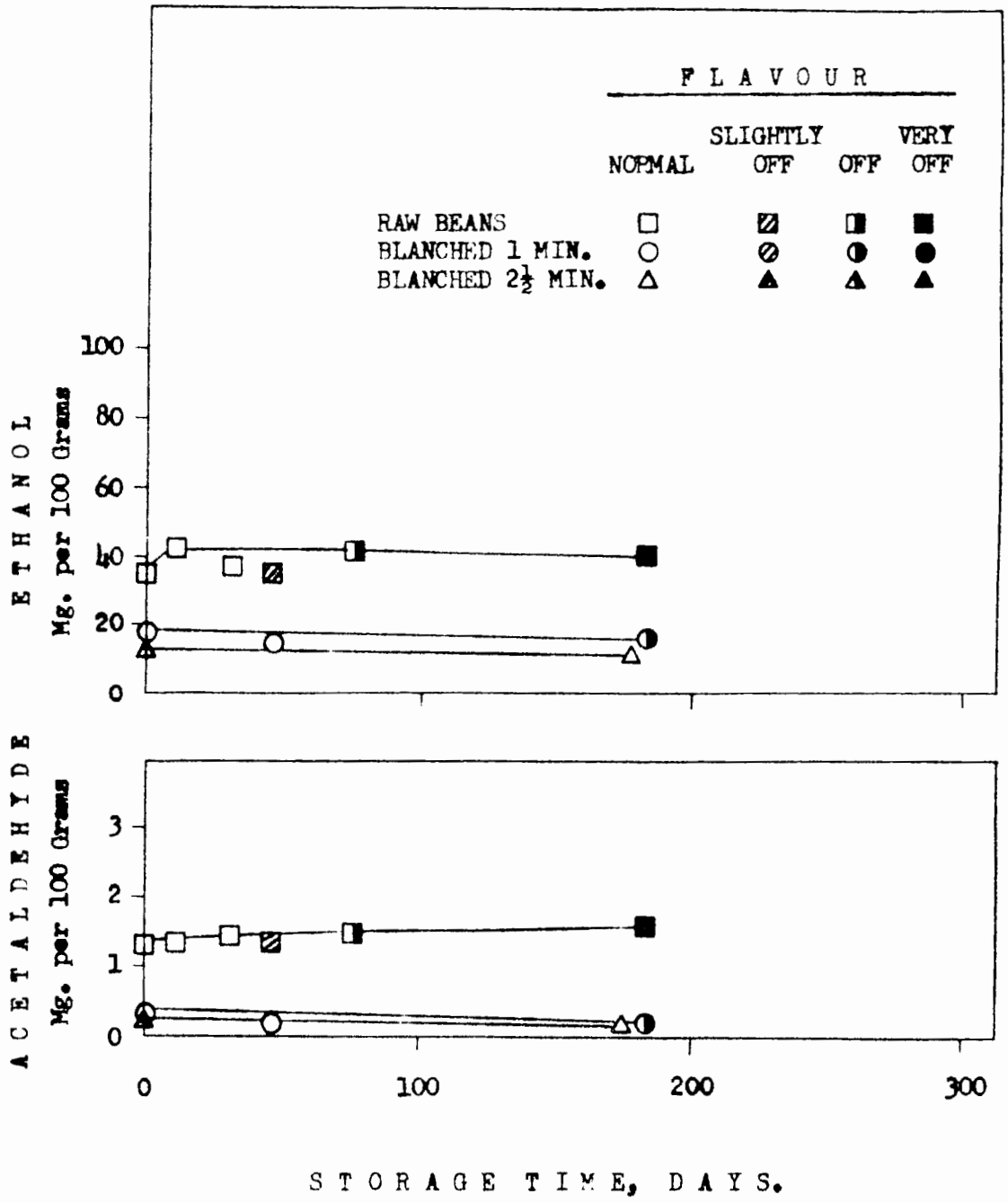
F I G U R E 17.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER OXYGEN, SERIES II.



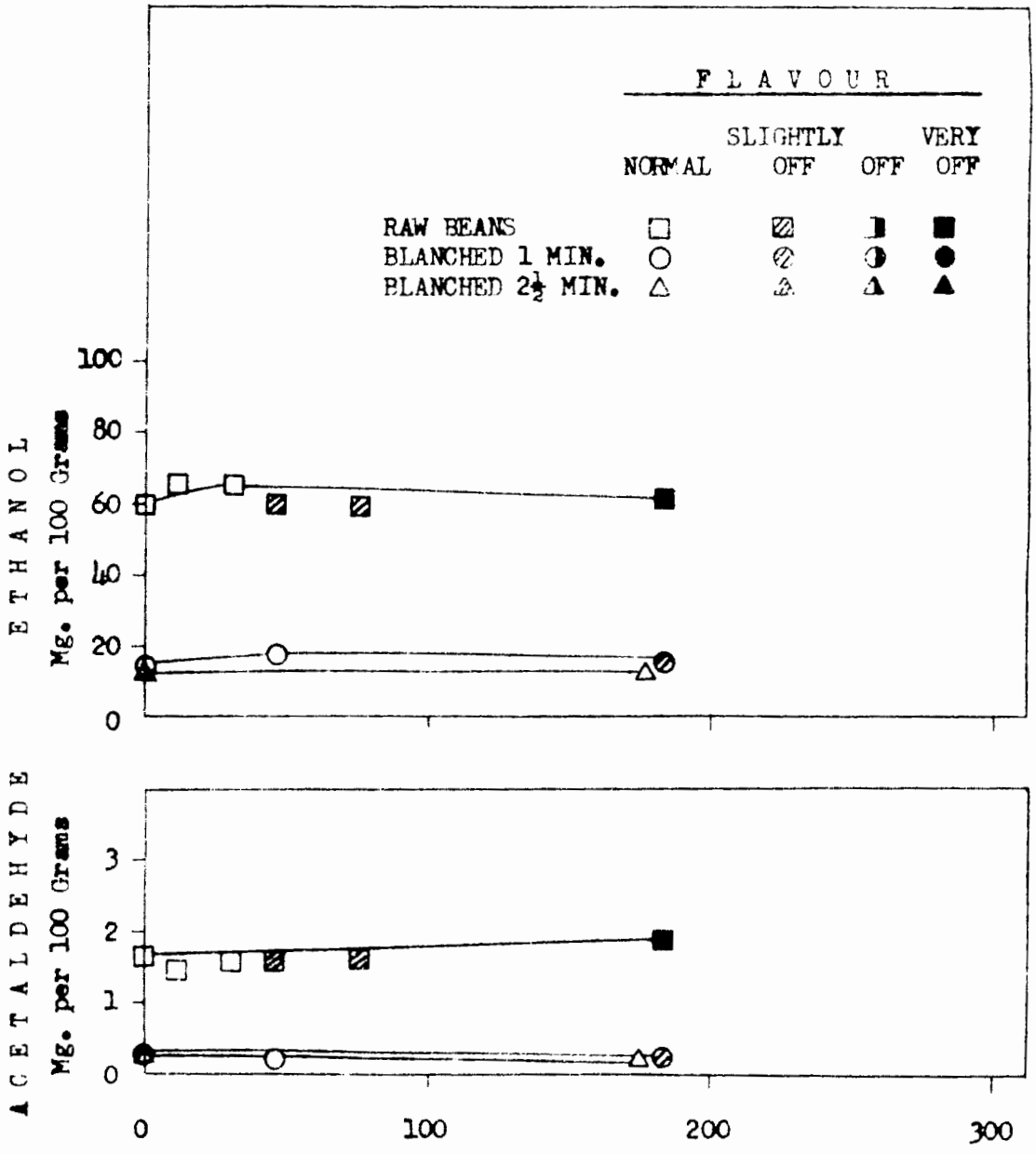
F I G U R E 18.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER AIR, SERIES II.



F I G U R E 19.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS STORED UNDER NITROGEN, SERIES II.



F I G U R E 20.

EFFECT OF STORAGE AT -12.2° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS, SERIES III.

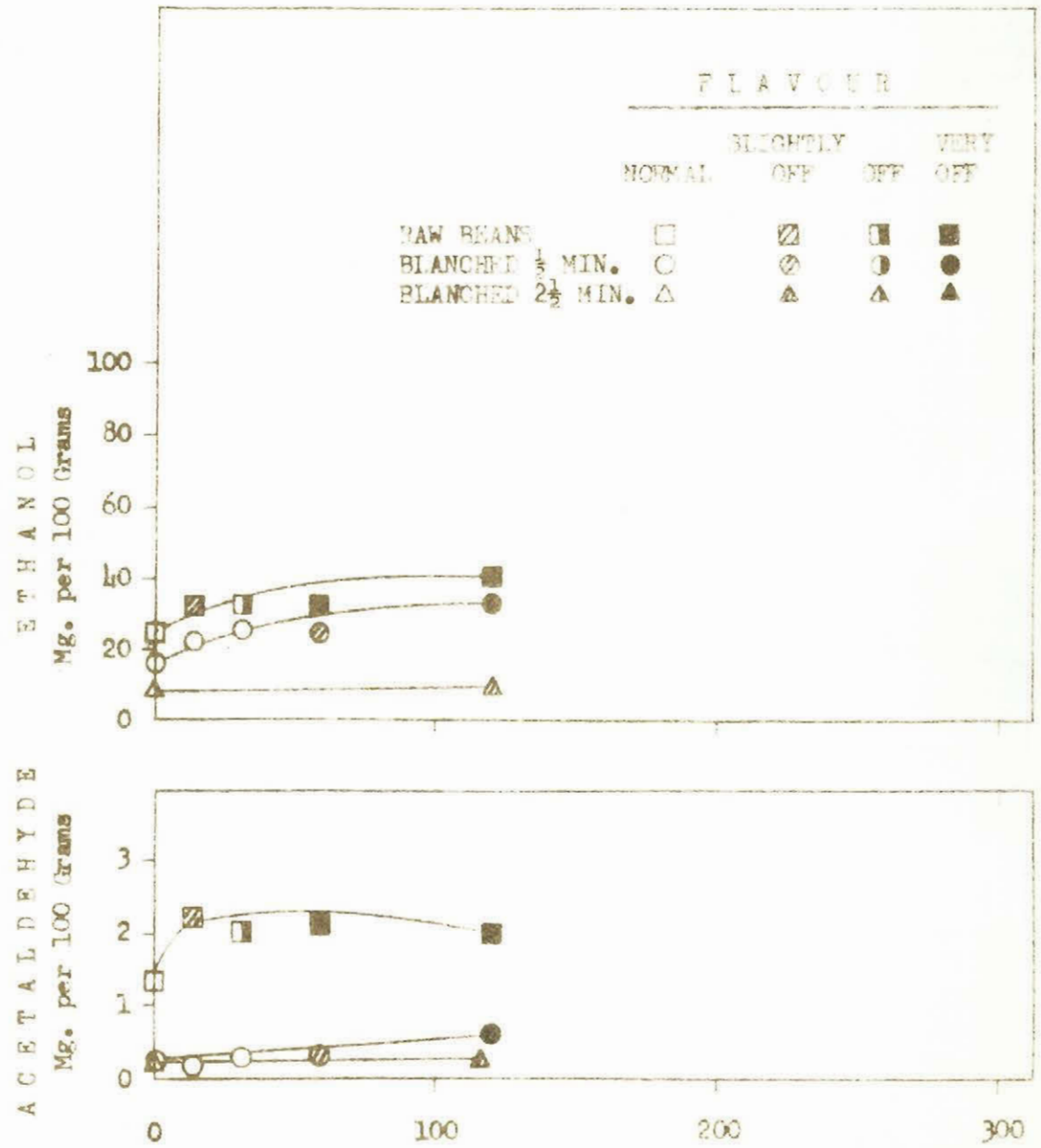


FIGURE 21.

EFFECT OF STORAGE AT -17.8° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS, SERIES III.

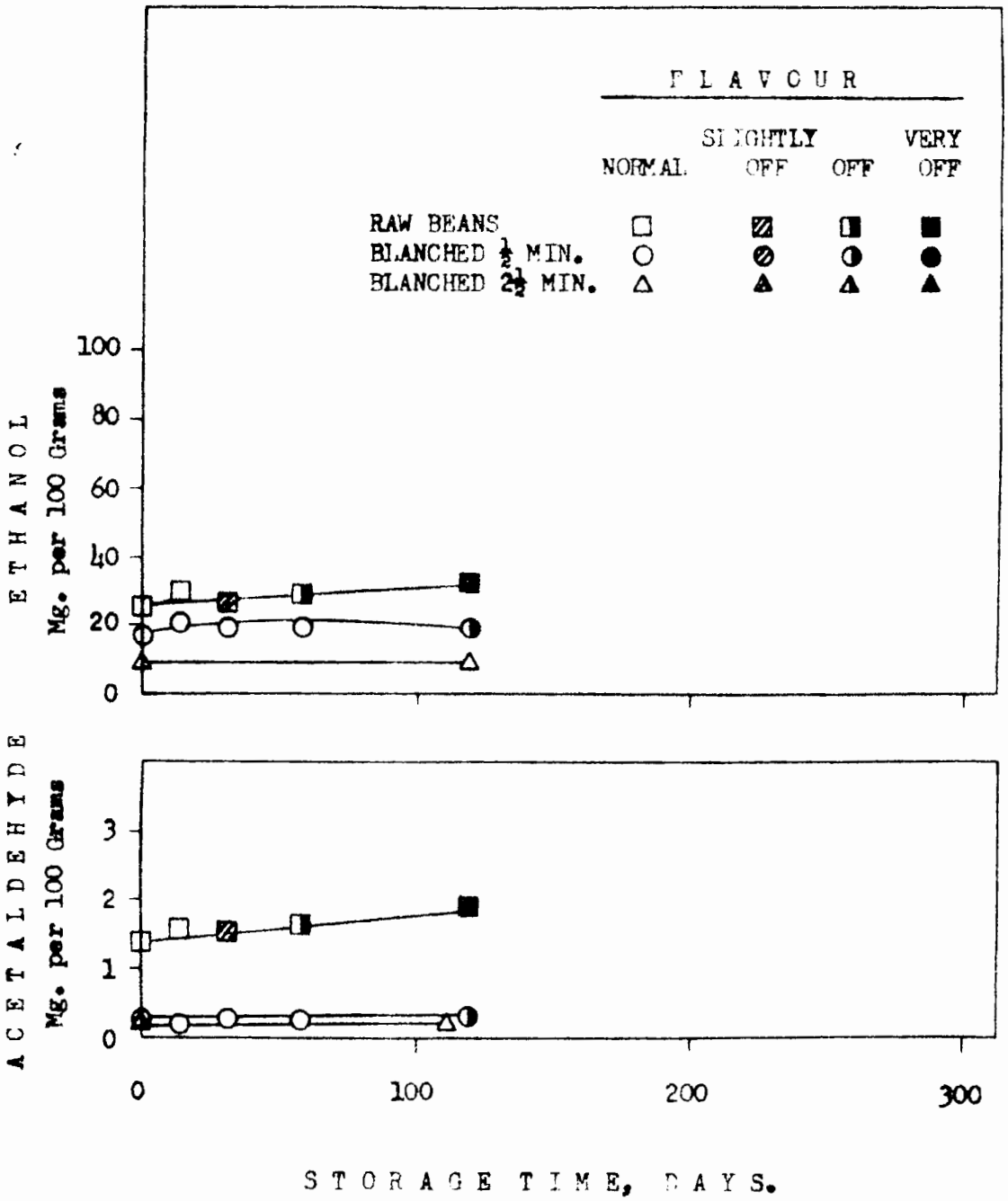


FIGURE 22.

EFFECT OF STORAGE AT -21.1° C. ON ACETALDEHYDE AND ETHANOL CONTENT AND FLAVOUR OF BEANS, SERIES III.