OFF-FLAVOUR DEVELOPMENT IN FROZEN CAULIFLOWER

by

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I. INTRODUCTION

The early attempts to preserve vegetables by freezing were not very successful, even under laboratory conditions, because of the development of undesirable flavours and odours during storage. Joslyn and Cruess (1929) were the first to report the successful retention of colour and flavour during storage at -18° C by blanching the vegetables prior to freezing. Joslyn (1930), Tressler (1932) and Diehl and Berry (1933) recognized early in the development of the frozen vegetable industry that the off-flavour development in raw and underblanched vegetables occurred as a result of an imbalance in the normal enzyme reactions. Even though numerous investigations have been carried out on various aspects of this problem, the reactions involved are still not completely elucidated. The investigators agree that an enzymatic process is involved since off-flavours do not develop in properly blanched vegetables. Prolonged storage may result in non-enzymatic changes giving disagreeable flavours.

The four enzymes which are now thought to be associated with quality changes during frozen storage are

lipoxidase, lipase, catalase and peroxidase, Lee and Wagenknecht (1958). There is reasonable evidence that lipid oxidation is the major factor responsible for off-flavour production in frozen vegetables as was suggested by Lee and Wagenknecht (1951). The enzymes actually involved in off-flavour formation, the substrates acted upon, the factors influencing rate and extent of chemical change and the chemical components responsible for off-flavour formation are not fully elucidated. Flavour changes occurring in frozen raw or underblanched vegetables have also been attributed to autolysis and proteolysis (Tressler, 1932; Mergentime and Wiegand, 1946) and glycolysis (Joslyn, 1949).

It is well established that when raw vegetables are stored in an atmosphere of low oxygen content, they will undergo fermentation with the formation of acetaldehyde and alcohol. It has been shown by Joslyn (1930) and Kohman and Sanborn (1934) that bruised vegetables and vegetables held under anaerobic or semi-anaerobic conditions accumulate large amounts of acetaldehyde and ethanol and develop off-flavours similar to those found in raw frozen vegetables. They suggest that as a result of alcoholic fermentation, off-flavours develop and that the acetaldehyde and ethanol content could be used as an objective measure of off-flavour development in frozen

vegetables. Their theory was 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. The correlation was not found satisfactory by Moore (1951), Kramer (1954), Füleki (1961) and Bengtsson and Bosund (1964).

This investigation was undertaken to study the correlation, if any, between aldehyde and ethanol content and off-flavour development which was judged organoleptically.

Lee and Wagenknecht (1951), Wagenknecht (1952), Wagenknecht and Lee (1956), Lee and Wagenknecht (1958) and Wagenknecht and Lee (1958) have presented data which suggest that the products of lipid oxidation are partially, if not completely, responsible for off-flavour development. Experiments were carried out with 2-thiobarbituric acid (TBA), a test used for detecting fat oxidation in many substances such as dairy products, pork, bakery products, oysters, fishery products and other biological material because of its sensitivity. It must be pointed out that this test is open to criticism since, according to Tarladgis et al. (1962) the reagent itself is not stable and various side reactions may occur under the experimental conditions frequently employed. A procedure was developed

which seemed to give satisfactory results. Cauliflower samples were tested periodically throughout a 60-day storage period. Since the results were found to agree with flavour scores, the test was used in all further studies on the development of off-flavour in cauliflower.

During this investigation, malonaldehyde (MA), which shows a maximum absorption at 245 mμ, when the pH of the solution is below 3, could not be detected in any of the cauliflower samples. This concurs with the results of Kwon and Watts (1963), who reported that the ultra-violet spectra of distillates from peas were complex and very different in appearance from those of MA. A large number of carbonyl compounds are present in cauliflower as evidenced by the many 2,4-dinitrophenylhydrazine (2,4-DNPHine) derivatives obtained. The condensation product between TBA and one or more of these carbonyl compounds could be responsible for the reddish colour formation which showed an absorption maximum at 535 mμ since, according to Patton (1960), the TBA reagent is not specific for MA. Saslaw and Waravdekar (1965) have presented evidence from thin-layer chromatography studies of irradiated fatty acids which showed that none of the thiobarbituric acid reactive substances (TBRS) was MA.

II. REVIEW OF LITERATURE

Acetaldehyde in Plant Tissues

In the absence of oxygen and to a certain extent even in its presence, plant tissues undergo anaerobic respiration or fermentation. In the course of respiration and fermentation, carbohydrates are metabolized to pyruvic acid which is then decarboxylated, giving acetaldehyde and carbon dioxide as follows:

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

The reaction is catalyzed by pyruvic decarboxylase. 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. It was shown by Griebel (1925) and Neuberg and Gottschalk (1925) that acetaldehyde is an intermediate product of anaerobic respiration in higher plants. Pirschle (1926) was able to demonstrate that acetaldehyde was an intermediary product in germinating

seeds which contain fats and that it may be formed during the conversion of fat to carbohydrate.

James (1953) reported that in the presence of oxygen, part of the acetaldehyde condenses to form acetoin (acetylmethylcarbinol) when the enzyme carboligase is present:

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

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

Because of their economic importance, peas have often been investigated. Neuberg and Gottschalk (1924, 1925) first demonstrated the presence of acetaldehyde in this vegetable. They showed that under anaerobic conditions, coarsely-ground young pea seedlings can produce acetaldehyde from a glucose solution. It was shown by Bodnar *et al.* (1925) that whole peas in the absence of oxygen produced acetaldehyde.

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 lima beans and unbruised peas submitted to anaerobiosis and raw frozen vegetables developed off-flavours. Larger quantities of acetaldehyde

and alcohol were found in vegetables which had been bruised as compared with unbruised vegetables. Off-flavour formation was associated with these compounds.

The presence and accumulation of volatile aldehydes in the tissues of frozen peas were first reported by Arighi et al. (1936). Joslyn et al. (1938) found that aldehydes accumulated in artichoke hearts. They have also been found in asparagus, Joslyn and Bedford (1940) and Lovejoy (1952), in lima beans, Gutterman (1956), and in Brussels sprouts and squash, Joslyn and Bedford (1938-1940). The findings of Arighi et al. (1936) were confirmed by Lovejoy (1952), Gutterman (1956) and Bengtsson and Bosund (1964). Acetaldehyde was found in spinach, Arighi et al. (1936), broccoli, Buck and Joslyn (1953) and peas, Kramer (1954), but little or no correlation was found with off-flavour development. Füleki (1961) found that aldehydes, determined as acetaldehyde but not identified as such, accumulated in green snap beans but that its accumulation could not be suggested as an objective measure of off-flavour development.

David (1949) examined the volatile aldehydes of peas. He identified acetaldehyde as the chief aldehyde constituent in fresh and frozen peas by means of 2,4-DNPHine. He found that acetaldehyde content increased under anaerobic conditions and parallelled the activity of pyruvic

decarboxylase. A reduction in acetaldehyde content was obtained by blanching at times and temperatures sufficient to inactivate decarboxylase. He also found that raw peas and those blanched at 60° C accumulated aldehydes during freezing storage since their content was much higher than that found in peas blanched at higher temperatures.

The 2,4-dinitrophenylhydrazones (2,4-DNPHs) of steam volatile carbonyl compounds of green peas were studied by Silberstein (1954). He identified acetaldehyde as the major component. In freshly harvested peas, he found that steam volatile carbonyl compounds or their precursors accumulated very rapidly in the immediate post-harvest period even if the peas were stored at -17.8° C.

The relationship between acetaldehyde accumulation and off-flavour development was investigated by Moore (1951). In his investigation in raw and underblanched peas stored at different freezing temperatures, he found that differences existed in acetaldehyde content between series. He also found that the initial content could be greatly reduced by blanching at higher temperatures. Acetaldehyde accumulated in raw or lightly blanched peas only at the highest storage temperature where oxygen caused a definite increase compared with air treatment. Off-flavour development was observed in samples which did not show an increase in acetaldehyde.

Wager (1958), Ralls (1960), Matthews (1961), Pendlington (1962) and Self et al. (1963) identified acetaldehyde in green peas. Ralls, using flash exchange gas chromatography, found differences in the amounts from samples in two successive years. He also reported that volatile carbonyl compounds obtained by flash exchange gas chromatography do not have the characteristic pleasant odour of cooked peas. Gutterman et al. (1951) demonstrated that whenever the acetaldehyde level in frozen peas and asparagus exceeded a certain range, organoleptic taste panels have invariably classed the product as being of inferior quality or inedible. According to Pendlington (1962) the concentration of acetaldehyde is not high enough to be responsible for the development of off-flavour.

Acetaldehyde was described by Kirchner (1949) as being an important flavour constituent in a large number of fruits and vegetables. It can be rather disconcerting, as was suggested by Lea (1963), to find volatile carbonyl content being used as a measure of desirable flavour production in one product and of off-flavour development in another.

Bengtsson and Bosund (1964), using gas chromatographic techniques, identified acetaldehyde in raw green peas. They indicated that the same compounds which are

formed slowly at freezing temperatures in storage are also characteristic of post-harvest changes at room temperature. Hexanal was also found in the stored material. They suggested that the level of hexanal may be used as an indication of off-flavour development.

Acetaldehyde content and its accumulation in green beans were also investigated. It was first reported to accumulate in string beans by Bedford and Joslyn (1939). 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 the acetaldehyde content decreased and the quality of the product improved when the length or the temperature of blanching increased. They found that acetaldehyde concentration was not a reliable index of flavour retention since the accumulation of acetaldehyde was about the same in the two series even though there was more than three years difference in storage time. This might indicate that acetaldehyde accumulation took place in the early part of storage.

Zoneil and Esselen (1959) studied the connection between the thermal destruction rates and the regeneration of green bean peroxidase, in material stored at room temperature. They studied the accumulation of acetaldehyde and the formation of off-flavour in sterilized samples containing added peroxidase. They attributed the

increase in acetaldehyde content and the off-flavour formation to the presence of peroxidase. The acetaldehyde content increased rapidly during the first two weeks of storage, after which there was a decrease.

Fűleki (1961) found that the initial acetaldehyde content depended mainly on handling methods and the treatments given before the beans were frozen. Blanching resulted in decrease in the initial content of acetaldehyde but raw and lightly blanched frozen beans retained their ability to produce acetaldehyde. He found that the amount which accumulated over the initial content depended on three main factors: extent of blanching, length of storage and temperature of storage. Storage in oxygen, nitrogen and air had no significant effect. Fűleki also found that the highest rate of acetaldehyde accumulation was found to occur early in the storage period. The content levelled off or decreased later.

Bailey et al. (1961) studied the volatile sulfur components of cabbage, using gas chromatographic techniques. Among a number of other volatiles, they reported the presence of various aldehydes. However, they did not report the identity of any of the aldehydes.

Ethanol in Plant Tissues

During alcoholic fermentation plants form ethanol as a final product. The production of ethanol follows the

same pathway as acetaldehyde which is reduced to ethanol as follows:

$$CH_3.CHO + DPNH_2 \xrightleftharpoons{\text{dehydrogenase}} CH_3.CH_2.OH + DPN$$

The reaction is catalyzed by the enzyme alcohol dehydrogenase, and requires the presence of $DPNH_2$ or coenzyme I (reduced diphosphopyridine nucleotide) as donor of hydrogen ions.

Numerous investigators have 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. Kirchner (1949) was of the opinion that ethanol was an important flavour constituent of many fruits and vegetables. The bruising of vegetables results in an increase in alcohol production, as was reported by Kohman (1932) and Kohman and Sanborn (1932, 1934). They assumed it was ethanol without actually identifying it. Peas, lima beans and corn were found to contain more alcohol after bruising than before.

Several investigators have shown the presence of ethanol in pea tissues, Bodnar et al. (1925), Neuberg and Gottschalk (1925), Gustafson (1934), Joslyn and David (1952), Wager (1958), Ralls (1960), Matthews (1961), Pendlington (1962) and Bengtsson and Bosund (1964).

It was found by David (1949) that, in peas, both fresh and frozen, ethanol was the only alcohol produced. Its formation and accumulation parallelled 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. Buck and Joslyn (1953), using a similar technique, found accumulation of alcohol in frozen raw and underblanched broccoli.

Moore (1951) reported differences in the alcohol content between different lots of peas. Ethanol content was greatly reduced on blanching and its accumulation was found to occur in raw or lightly blanched peas stored at the highest temperature. Under these conditions, a six- to seven-fold increase in alcohol content was obtained as compared with a two-fold increase in aldehyde content. At lower temperatures, the samples did not show an increase in ethanol. A decrease was sometimes observed. No direct relationship between alcohol accumulation and off-flavour development was detected.

Using gas chromatography, Bengtsson and Bosund (1964) identified ethanol in peas. They found that, whereas acetaldehyde and hexanal increased rapidly with increasing storage temperature, the ethanol content

remained fairly constant. They advanced two possible explanations for these results: either the maximum concentration of ethanol had been reached in the raw material used or the formation of ethanol was completely stopped at temperatures of -8° C and lower. They also found that the same types of reactions occur, only much more rapidly, at temperatures above 0° C.

Amla and Francis (1960) found that when the oxygen content reached 2 to 3% in prepeeled potatoes, alcohol production began and its content increased rapidly. The alcohol was identified as ethanol. The experiment was conducted at 7.8° to 8.9° C. They also found that at lower temperatures, 3.3° to 4.4° C, the same trends were evident, but at slower rates. They reported that the changeover from aerobic to anaerobic respiration occurred at approximately 3 to 4% oxygen.

Füleki (1961), working with green snap beans, found that variations in the initial ethanol content depended mainly on handling methods and the treatments given before the beans were frozen. Blanching resulted in a decrease in the initial ethanol content. He also found that raw and lightly blanched beans retained their ability to produce alcohol. The amount present 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 accumulation was found to occur early in the storage period. Later the content levelled off or decreased.

Bailey et al. (1961), in their studies on the volatile sulfur components of cabbage, reported the presence of various alcohols without identifying them.

Occurrence of Acetaldehyde and Ethanol as Heat Breakdown Products in Plant Material

The original concentrations of rapidly produced and degraded intermediates such as ∝-keto acids and acetaldehyde in plant tissues are rather difficult to estimate. The enzymes are normally confined to small subcellular units such as mitochondria and microsomes. Maceration or slurrying frees these enzymes. This could accelerate the reactions because the enzymes can react at a higher substrate concentration. These compounds can also be produced at an accelerated rate during the early part of heat inactivation. They can be formed by non-enzymatic reactions occurring during heating or upon addition of chemicals, such as strong acids or bases.

Isherwood and Niavis (1956) found that, during treatment with boiling alcohol, the rate of enzyme-catalyzed

reactions was about eight times that at room temperature. They also found that, during inactivation with boiling methanol or hot acid and strongly alkali media, pyruvic acid, the immediate precursor of acetaldehyde was produced in potato and pea tissues. Breslow (1958) demonstrated the formation of acetoin from pyruvic acid and acetaldehyde together and from acetaldehyde alone in a thiamine-catalyzed non-enzymatic system. The reaction was shown to appear in peas by Ralls (1959).

Wager (1958) compared a low temperature diffusion method with steam distillation. He always found considerably higher values for aldehydes with steam distillation methods from frozen peas. He was able to detect acetaldehyde in successive aliquots even after two and one-half hours of steam distillation. He concluded that the additional acetaldehyde arose as a result of heating and was probably not due to an enzymatic reaction. He also reported the production of other volatile materials, which were determined as ethanol, for as long as two hours of continuous steam distillation.

Füleki (1961), working with green beans, reported that part of the acetaldehyde appeared to occur from some unknown precursor or precursors, as a heat degradation product during steam distillation. Besides the carbonyls he also found that some other volatile organic reducing

compound or compounds, determined as ethanol, appeared, presumably, as heat breakdown products during steam distillation.

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 strong acidic conditions during extraction (pH 1.2).

Role of Enzymes in Off-flavour Development

Four enzymes have often been studied in association with quality changes during frozen storage, Lee and Wagenknecht (1958). They are lipoxidase, lipase, catalase and peroxidase. Catalase and peroxidase are both used to determine the adequacy of blanching. Much of the recent research suggests that successful freezing preservation of vegetables requires a negative peroxidase test.

Diehl and Berry (1933) were of the opinion that catalase activity served as an index of adequacy of blanching for peas. Arighi *et al.* (1936) suggested that enzymes concerned in the production of off-flavours in peas were more heat stable than catalase. Balls (1942) suggested that peroxidase activity might serve as an index of adequacy of blanching. From earlier work, Joslyn (1946), in a review paper, found that peroxidase activity more closely parallelled the formation of

off-flavours in frozen vegetables than did catalase activity. Masure and Campbell (1944) did a quantitative study relating degree of peroxidase inactivation to quality retention in frozen vegetables. They showed that samples heated for a time sufficient for quality retention during freezing storage, contained 1 to 2% residual peroxidase.

Lindquist et al. (1951) presented data which indicated that blanching long enough to inactivate catalase is not sufficient for quality retention in frozen Brussels sprouts. They found that peroxidase activity served as a satisfactory index of the adequacy of the blanch in this commodity. Upon comparing samples blanched enough to inactivate catalase but not peroxidase and samples in which peroxidase was destroyed, it was found that the former showed the following changes: marked development of off-flavour, decrease in natural flavour and development of pink colouration at centres.

In his review, Joslyn (1949) reported that aldehyde accumulates in raw or underscalded artichoke hearts, asparagus, Brussels sprouts, green snap beans, peas, lima beans and squash progressively during freezing storage. The extent of accumulation and the rate of production of acetaldehyde were reduced as the temperature and time of blanching increased and did not occur in tissue blanched

sufficiently to completely inactivate peroxidase.

In their studies, Dietrich et al. (1955) showed that heating to a point sufficient to inactivate catalase was sufficient for chlorophyll and ascorbic acid retention in samples stored under conditions considered good for commercial practice. However, it was not sufficient for flavour retention. Samples which were blanched for fifty seconds showed no active catalase but contained 40 to 50% residual peroxidase. Their findings agreed with the report of Joslyn (1946).

Boetcher (1962), working with green peas, beans, cauliflower, Brussels sprouts, kale, spinach, sliced carrots and asparagus showed that the higher the peroxidase activity after blanching, the poorer the quality of the vegetable after freezing preservation.

The most likely cause of off-flavour formation in underscalded frozen vegetables is the enzyme-catalyzed oxidative deterioration of lipids. According to Joslyn (1961) the lipid content of vegetables is very small. On a dry weight basis, the crude lipid content varies from 1.25 to 3.33% by weight depending on the vegetable. The enzymatic oxidation of lipids could be very important considering the activation energy for lipoxidase as compared with the activation energy for autoxidation. Siddiqi and Tappel (1956) reported in their work that the

activation energy for lipoxidase was 4.3 K cal/g mole as compared with an activation energy of 15.2 K cal/g mole for autoxidation. Therefore the damage caused by autoxidation of the lipid material will be negligible in comparison with that caused by enzymic oxidation at low temperatures of storage.

Lee and Wagenknecht (1951) lyophilized unblanched Thomas Laxton peas which had been stored for five years at -17.8° C. The lipid material was then extracted with peroxide-free anhydrous ethyl ether. They also prepared similar extracts from blanched and unblanched fresh peas of the same variety immediately after harvesting. Tests involving taste, odour, peroxide number, iodine number and acid number were carried out. These results showed that rancidity of the lipid material was one of the primary causes, if not the main cause, of off-flavour development in unblanched frozen peas.

These research workers found that there was a considerable loss of chlorophyll and carotene during the protracted storage of frozen unblanched peas. However, no significant changes were observed in the reducing and non-reducing sugar fractions and in the starch fraction. Thus it appeared that these components of the carbohydrate fraction did not contribute to the development of off-flavour during the storage of frozen unblanched peas. No

changes were observed in total nitrogen content, such as would have occurred had ammonia or volatile amines or other volatile nitrogenous compounds been evolved during storage.

Work done by Wagenknecht (1952) showed that marked changes had occurred in the lipid fractions of unblanched peas which had been frozen for several years. These changes were considered to be mostly responsible for the off-odour and flavour. In the raw peas frozen and stored for five years, more than 50% of the chlorophyll and carotene had disappeared. Very little change occurred in the blanched samples held for five years. No change could be noticed in the protein, sugars and starch.

The chemical changes taking place in the crude lipids during the storage of frozen asparagus, corn, snap beans, lima beans, spinach and peas were reported by Lee (1954). The results show that corn, snap beans and spinach were similar to peas in that high peroxide numbers were found in their crude lipid extracts after storage. Crude lipid extracts from asparagus gave a high acid number, but a negative peroxide test, whereas the extract from raw lima beans gave low peroxide and acid numbers. However, these two vegetables are low in lipoxidase which could account for these results.

Lee *et al.* (1955) reported results on a chemical

study of the progressive development of off-flavour in frozen raw peas, corn and snap beans. They found that off-flavour could be detected by a taste panel in from two to four weeks of storage. The crude lipids extracted from frozen unblanched vegetables showed a definite increase in acid numbers after storage for one week. This rise was found to continue during long storage, but the main increase took place during the early months of storage. They also reported that the crude lipids extracted from frozen unblanched vegetables showed a positive test for peroxides after the vegetables had been held in frozen storage for periods of time as follows: peas, 3 weeks; snap beans, 1 month; and sweet corn, 2 months.

Lee (1954) found peroxides in the lipid extracted from blanched sweet corn which had been held in frozen storage at -17.8° C for one and one-half years. These results were confirmed by Lee et al. (1955). They found that this was in accord with taste tests on blanched corn which begins to develop off-flavours after a year in frozen storage.

The presence of a lipoxidase in pea extracts was demonstrated by Siddiqi and Tappel (1956). They based their findings on the fact that pea extracts contained an enzyme which did not possess fatty acid oxidase or

fatty acid dehydrogenase activity, and which oxidized linoleate but not oleate. Therefore, the enzyme behaved like a true lipoxidase.

These research workers were of the opinion that the two lines of investigation on the development of off-flavour in frozen raw peas could be explained. They proposed that the lipoxidase of the intact pea could react with the unsaturated fatty acids present to form hydroperoxides which, in turn, decompose to give aldehydes. This would explain the oxidation of lipid material to give off-flavour. It would also explain the findings which show that aldehyde accumulation parallels off-flavour development.

The action of lipoxidase in frozen raw peas was reported by Wagenknecht and Lee (1956). They were able to demonstrate the presence of lipoxidase in frozen peas. They were also able to demonstrate two major actions of lipoxidase, using partially purified pea lipoxidase: chlorophyll destruction and peroxidation of lipid material. Strain (1941) was of the opinion that unsaturated fat oxidases were involved in the oxidation of certain unsaturated plant pigments such as chlorophyll or carotene.

Lee et al. (1956) reported that peas stored in the pod developed off-flavour at a slower rate than vined peas. They showed a greater loss of chlorophyll and the

crude lipids had higher peroxide values. Lee (1964) also indicated that peas held in the pod or vined and then stored at -17.8° C showed the same increase in acid number in the lipid material. Peroxides were found to increase at a more rapid rate in the lipids of peas held in the pods than in those which had been mechanically vined. He suggested that this increase might account for the fact that the colour deterioration in the peas held in the pods was greater than those which had been mechanically vined.

The importance of peroxidase in the development of off-flavour in frozen peas was discussed by Wagenknecht and Lee (1958). They found that peas blanched for sixty seconds, upon prolonged storage, had regenerated 24% of the original peroxidase activity. The fact that this amount of peroxidase produced no off-flavour was taken as further evidence that peroxidase is not of prime importance in off-flavour production in frozen raw peas since they had also found that peroxidase added to blanched peas produced only mild changes in flavour. Catalase also produced only mild off-flavour. Lipoxidase-treated samples were moderately off-flavoured. The lipase-treated samples had disagreeable off-flavours which were attributed in part to the flavour imparted by the lipase preparation itself. Lipase did cause the greatest losses in chlorophyll.

Changes in the chlorophyll were produced by all enzymes. Deterioration in lipids was caused by lipase and lipoxidase.

Wagenknecht and Lee (1958) gave a possible explanation of how lipase could function in off-flavour production. During the determination of residual lipoxidase activity it was observed that samples containing both lipoxidase and lipase showed considerable amounts of oxygen uptake in the absence of added linoleic acid substrate. This effect was not apparent when lipoxidase only was present, nor when catalase and/or peroxidase were present along with lipoxidase. Under the above conditions the sole source of lipoxidase substrate was the endogenous supply of lipids in the blanched peas. From the acid number determinations on these samples it was evident that considerable amounts of triglyceride had been split by the added lipase. Apparently the free fatty acids were more readily utilizable as lipoxidase substrates than the intact triglycerides. During the progressive development of off-flavour in stored frozen raw peas, the increase in acid number invariably preceded the development of peroxides detectable in the lipids.

Lee and Wagenknecht (1958) reported the use of enzymes prepared from garden peas, to study enzyme action and off-flavour in frozen peas. They were able to

obtain lipoxidase, lipase, catalase and peroxidase in partially purified form from lyophilized raw peas. The addition of these partially purified preparations of native pea enzymes to blanched peas resulted in the production of off-flavour following prolonged frozen storage. The greatest change in flavour score was associated with added catalase, but lipoxidase and lipase produced pronounced off-flavour. Peroxidase produced only a mild change in flavour which was not objectionable. Changes in green colour were brought about by each enzyme. The greatest lowering of visual colour scores was produced by lipoxidase and lipase.

Pinsent (1961) found that there was a regeneration of peroxidase at -18° C. In this respect pea peroxidase was reported to be similar to cauliflower peroxidase. He reported that the regenerated peroxidase did not affect the quality of the peas. However, peas which contained residual peroxidase immediately after blanching developed off-flavours.

Boettcher (1962) reported that heating for two minutes at 60° C destroyed more than 50% of the peroxidase activity in several vegetables but not in cauliflower, kale and Brussels sprouts.

Crude lipids were prepared from peas stored for five and one-half years by Lee (1958). Both raw and

blanched stored samples were used plus fresh peas raw and blanched. The peas which had been held in storage unblanched, yielded crude lipids which contained appreciable amounts of unsaturated carbonyl compounds. Unsaturated carbonyl compounds were not found in the blanched samples nor in either of the fresh blanched and unblanched samples.

The crude lipids of spinach were estimated by Mattick and Lee (1960). They showed that the total free fatty acids increased in the unblanched samples during storage. Palmitic acid increased during storage, whereas the longer-chain fatty acids, particularly linolenic acid, decreased. Lee and Mattick (1960) studied the changes taking place in the fatty acids of lipid material from peas. They found that large losses occurred in all the fatty acids in the phospholipid fraction of the raw samples. The neutral fats in the same samples showed decreases in all the unsaturated fatty acids in the raw material as compared with the blanched. The fatty acids in the neutral fat and the free fatty acids of the raw material showed a net gain in the total quantity of palmitic acid.

Use of TBA in the Study of Oxidative Rancidity

It was noted by Dox and Plaisance (1916) that a reaction occurred between TBA and a number of aromatic aldehydes to form highly coloured derivatives. Kohn and

Liversedge (1944) observed that various animal tissues, after incubation under aerobic conditions, produced compound(s) which gave a colour reaction when heated with TBA. Their research established the absorption spectrum of the colour complex and certain chemical characteristics of the colour reaction. Their work indicated that the responsible compound(s) were carbonyl in nature since semi-carbazide or phenylhydrazine blocked the reaction. They did not demonstrate the nature of the colour producing compound(s). Bernheim et al. (1948) found that the colour obtained by Kohn and Liversedge (1944), upon addition of TBA to incubated tissues, was due to a product of the oxidation of unsaturated fatty acids, particularly linolenic acid. They also found that egg lecithin, fatty acids from linseed oil and brain protein-lecithin-complex gave colours with TBA after aerobic incubation with ascorbic acid. Their data indicated that a three-carbon compound reacted with the reagent to produce the colour.

The TBA colour reaction with regard to certain sugars and aldehydes, as well as the oxidation products of linolenic and some other unsaturated fatty acids was studied by Wilbur et al. (1949). These studies revealed that no specific compound gave a colour spectrum identical to those obtained from oxidized lipid materials or

aerobically incubated animal tissues which give a single absorption peak at 532 mμ. They also found that after incubation with ascorbic acid, oleic and stearic acid were inactive.

Patton and Kurtz (1951) subjected a large number of compounds, in addition to those studied by Wilbur et al. (1949), to the TBA reagent and they also developed a test which could be applied to oxidized milk fat. Evidence indicated that the colours produced with malonic dialdehyde and oxidized milk fat were identical. The absorption maxima at 532 mμ and the shape of the transmission curves for the two colour systems were essentially the same as those obtained for oxidized methyl linolenate by Wilbur et al. (1949) and for aerobically incubated animal tissues by Kohn and Liversedge (1944) and Wilbur et al. (1949). Subsequent investigations have indicated that the reaction can serve as a measure of oxidation in a wide variety of food products.

After the work of Patton and Kurtz (1951), several attempts were made to further characterize the TBA pigment. Patton et al. (1951) pointed out the similarity between epihydrin aldehyde and MA. They reported that the TBA reactive material from oxidized milk fat was a water soluble, low molecular weight, Kreis positive carbonyl compound, similar to MA. In addition, they postulated

that MA would be strongly acidic, enolic, and relatively stable on heating with dilute mineral acids. It was suggested by Kurtz et al. (1951) that the oxidation product of milk fat and MA were the same and theorized that the reaction with TBA occurs by attack of the mono-enolic form of MA on the active methylene groups of TBA, followed by ring closure. Using a spectrophotometric technique, Jennings et al. (1955) suggested that the MA-TBA pigment was formed by an equi-molar reaction without loss of water. They also reported that the pigments prepared from oxidized milk fat, MA and sulfadiazine exhibited the same spectral characteristics in the visible range. However, this did not justify the conclusion that the compounds were identical.

Work was done by Sinnhuber et al. (1958) on the characterization of the red pigment formed in the TBA determination of oxidative rancidity. They prepared crystalline TBA pigment from rancid salmon oil, sulfadiazine and MA. Results obtained by elemental analyses, absorption spectrophotometry and paper chromatography all suggested that the pigments were identical. Their data indicated that the crystalline pigment was a condensation product between 1 molecule of MA and 2 molecules of TBA with the probable elimination of 2 molecules of water.

Several research workers have applied the test to

milk products, Patton et al. (1951), Patton and Kurtz (1951), Biggs and Bryant (1953) and Sidwell et al. (1955). Turner et al. (1954) and Zipser et al. (1964) used the test to detect rancidity in frozen pork while Caldwell and Grogg (1955) applied it to cereal and baked products. TBA was used as a quantitative measure of determination in cooked oysters by Schwartz and Watts (1957). Yu and Sinnhuber (1957) applied the test to fishery products. Chang et al. (1961) applied the TBA test to study tissue lipids in precooked beef. Zipser and Watts (1961) used it to study oxidative rancidity in cooked mullet. Kwon (1965) used TBA to study the reactivity of MA with food constituents. Wilbur et al. (1949) followed the effects of ultraviolet radiation and different catalysts on the TBA-chromogen produced from unsaturated fatty acids and their esters. The TBA test was used by Tarladgis and Watts (1960) to study MA production during the oxidation of pure unsaturated fatty acids under controlled conditions. It has also been used to study ultraviolet photolysis of unsaturated fatty acid by Saslaw et al. (1963).

Various TBA methods have been developed for food products. Each research worker has modified the test to suit his own needs. Most of the methods are similar in that they employ heating the food at a low pH which is

claimed to be essential for the liberation of MA from some precursor as well as for the condensation of MA with TBA.

The methods for applying the TBA test can be classified under two main categories. (a) A solution of TBA in a strong acid is added to the food product and the whole mixture is heated from 10 to 35 minutes in a water bath to obtain maximum colour development. The coloured complex is then extracted with a suitable solvent and measured in a spectrophotometer. (b) The reactive material is first distilled off from a sample adjusted to a low pH. A portion of the distillate is added to the TBA-acid reagent and this is heated to develop the colour. The red pigment is then read directly on a spectrophotometer.

Those who have used the outlined procedures are: Bernheim et al. (1948), Wilbur et al. (1949), Dunkley (1951), Dunkley and Jennings (1951), Patton et al. (1951), Patton and Kurtz (1951), Sidwell et al. (1954), Turner et al. (1954), Sidwell et al. (1955), Caldwell and Grogg (1955), Yu and Sinnhuber (1957), Schwartz and Watts (1957), Sinnhuber and Yu (1958), Sinnhuber et al. (1958), Patton (1960), Tarladgis et al. (1960), Tarladgis and Watts (1960), Zipser and Watts (1961), Chang et al. (1961) and Zipser et al. (1964). All the TBA methods invariably employ the addition of TBA dissolved in acid.

Tarladgis et al. (1960) were of the opinion that the distillation method appeared to have advantages in that prolonged heating of the food product with the TBA reagent was avoided. This keeps to a minimum any further oxidative or decomposition changes during the test, as the acid-heat treatment necessary to effect the liberation and distillation of MA from the sample is less drastic than that required for maximum colour development with the TBA reagent. Only the volatile constituents of the food are distilled over, thus avoiding any reactions of the TBA with non-volatiles of the food which may react with it. Also the acid of the TBA reagent is diluted as the reagent is added to the distillate in equal amounts before the heating begins. When a distillation method is used, the MA is obtained in a clear aqueous solution and the reaction product with TBA does not need to be extracted with solvents.

Tarladgis et al. (1962) published data which indicated that some important side reactions could occur during acid-heat treatment of TBA. They presented evidence by ultraviolet, visible and infrared spectra as well as by paper and column chromatography of variously treated TBA-acid reagents which showed that the structure of TBA is altered upon acid-heat treatment. A more pronounced but similar effect resulted from the treatment of

TBA with hydrogen peroxide. They found that some of the degradation products of TBA absorb at the same wavelengths as the TBA-MA complex, as do many compounds which are reported in the literature to react with TBA.

Further research by Tarladgis et al. (1964) revealed that acid-heat treatment is not necessary for the condensation of TBA with MA nor for maximum colour development. They found that heating without acid accelerated the condensation of TBA with MA without affecting the E_m^{530}.

Sinnhuber and Yu (1958) proposed using a standard curve as a means of reporting results. 1,1,3,3-tetraethoxypropane (1,1,3,3-TEP), on hydrolysis, will yield 1 mole of MA which reacts quantitatively with TBA. They suggested that the term TBA number or mg of MA per 1000 g of material be used to express results. Standard curves have been used for reporting results, by Tarladgis et al. (1960), Chang et al. (1961), Zipser and Watts (1961), Zipser et al. (1964) and Tarladgis et al. (1964).

It was reported by Kwon and Watts (1963) that MA occurred mainly as the enol form (CHOH=CHCHO) in aqueous solution. The ultraviolet absorption spectrum was found to be pH dependent. Below pH 3 the compound is s-cis, planar, having an intramolecular H bond, with absorption maximum at 245 mμ and molar absorptivity (ϵ) = 1.34×10^4.

Above pH 7, the compound is completely dissociated and the maximum absorption of the enolate anion was found to occur at 267 mμ with $\epsilon = 3.18 \times 10^4$. They suggested that the absorbance difference between acidic and basic MA solutions at 267 mμ could be used as a measure of MA even in the presence of other compounds that absorb in this spectral region, provided their absorption is not pH-dependent. The difference was found to be directly proportional to MA concentrations from 5×10^{-6} M to 3×10^{-5} M. The method was successfully applied to the assay of MA in distillates from rancid foods. Its sensitivity was only about 40% of the TBA test, but was sufficient to detect threshold levels of rancidity. The test was simpler, much more rapid and more specific than the TBA test.

Kwon and Watts (1964) reported that when distillation is employed to separate MA from other food constituents, maximum volatilization even of preformed MA would not be expected at pH values above 3, since the volatile hydrogen-bonded ring form undergoes progressive ionization with increasing pH from 3 to 6.5 (Kwon, 1963). They found that recovery of known amounts of MA below pH 3 was constant at about 65%, while above pH 6.5 the recovery was negligible. Between pH 3 and 6.5, the recoveries differed, and were dependent upon the proportions of the volatile

chelated form and the nonvolatile enolate anion.

Kwon and Watts (1964) were of the opinion that where extraction rather than distillation is used as the initial separation step, water could be used as solvent for free preformed MA or its metal complexes. They found that higher yields of MA could be obtained from fish, using an acid extraction or distillation. They were of the opinion that this was evidence that the acid treatment either produces more MA from a precursor or that preformed MA is freed from a secondary combination with some other food constituents by the acid treatment. They believed that the high correlations between MA content and rancid odours appeared to be limited to moist foods, especially animal tissues. In the pH range of such tissues, MA produced from lipid oxidation was mainly dissociated into the enolate anion, which is nonvolatile and might conceivably be stabilized against further irreversible reactions by the formation of metal chelates from which MA could be recovered by heat and acid.

Kwon et al. (1965) found that free MA was readily converted into its volatile form by acidification only, whereas both acidification and heating were necessary to volatilize bound MA from its reaction products with proteins. Tarladgis et al. (1964) prepared simple aqueous extracts from foods for the TBA test. It was the opinion

of Kwon et al. (1965) that such a procedure would be useful only in the initial stages of lipid oxidation when insoluble TBRS-protein products are unlikely to occur. From their experiments, they found that water extracts could not be considered satisfactory, since aqueous extracts contained only the free TBRS and water-soluble products, but would not include TBRS bound to water-insoluble proteins and other food constituents.

Kwon et al. (1965) suggested that in the pH range of moist foods, especially animal tissues, free TBRS produced from lipid oxidation would not volatilize. It is probable that the hydration of the TBRS alone, as with the MA anion, could lead to the accumulation of the compound at low concentration. When the TBRS concentration in foods increases with advanced lipid oxidation, the anion might react further with amino acids, peptides, proteins, glycogen and other food constituents. If the TBRS concentration is further increased, binding sites would be saturated by the compound. Eventually cross-linking with other protein molecules could occur with resultant loss of solubility and recoverable TBRS. The ability of the compound to combine with proteins at -18° C, as indicated by the in situ formation of TBRS-protein products in frozen tuna, accounts for the fate of some of the compound in the moist food systems. The accumulation

of the compound through its interaction with food constituents makes the TBA test more desirable than others in assessing rancidity in foods.

The above discussion by Kwon et al. (1965) was based on the supposition that the reactions of TBRS were similar to those of MA. However, several lines of evidence suggest that the two are not the same. Their observations suggested strongly that the prevailing concept that MA is the sole end product of lipid oxidation needs careful re-evaluation. Evidence presented by Saslaw and Waravdekar (1965) from thin-layer chromatography studies of extracts of irradiated fatty acids showed that none of the TBRS was MA. Nevertheless, MA and TBRS are alike in being water-soluble, dialyzable, TBA-reactive, able to react with proteins and having a pH-dependent volatility, Kwon et al. (1965).

The Separation of 2,4-DNPHs by Thin-layer Chromatography

The chromatographic separation of 2,4-DNPHs of carbonyl compounds is becoming very important in the identification of volatile food constituents. Thin-layer chromatography of 2,4-DNPHs has been reported by Dhont and De Rooy (1961), Rosmus and Deyl (1961), Anet (1962), Urbach (1962), Nano and Sancin (1963), Denti and Luboz (1965) and Byrne (1965).

The adsorbents employed, their preparation, the thickness of the adsorbent, their activation, the solvent systems used, and the method of preparation of the 2,4-DNPHine derivatives vary from one worker to another.

Anet (1962) used aluminum oxide G and silica gel G in a ratio of 1 g of adsorbent to 2 ml of water. The slurries were mixed for 1 1/2 minutes and applied at a thickness of .25 mm. Some plates were air dried, others were activated at 100° C for 2 hours and stored in a dessicator and some were deactivated by holding for a few hours in an atmosphere of 70% relative humidity. Anet found mixtures of toluene and ethyl acetate the most useful solvent systems. The hydrazones were prepared by the general method of Neuberg, Grauer and Pisha (1952) and dissolved in acetone for application. He used both normal and two-dimensional separations. He sprayed his plates with a 2% solution of NaOH in 90% ethanol to give intense characteristic colours.

Denti and Luboz (1965) used alumina G, silica gel G, alumina G + 25% $AgNO_3$ and silica gel G + 25% $AgNO_3$ as adsorbents. The slurry was applied at a thickness of .30 mm, the plates air dried for five minutes and then activated for thirty minutes at 110° C. The plates which contained $AgNO_3$ were stored in the dark. In some cases the plates were deactivated for twelve hours in an

atmosphere of 60% relative humidity, others were dried and stored over $CaCl_2$. They used several solvent systems: benzene-petroleum ether, chloroform-petroleum ether, benzene-n-hexane, and cyclohexane-nitrobenzene-petroleum ether. The hydrazones were prepared according to the method described by Brady (1931). The plates were sprayed with a 2% NaOH solution in 90% ethanol.

In his work, Bryne (1965) used silica gel G and aluminum oxide G in a ratio of 1 g of adsorbent to 2 ml of water. The slurries were applied at a thickness of .25 mm. The plates were air dried and then activated for two hours at 110° C or further dried without heating. Bryne employed the following solvent systems: 80 to 100° C light petroleum-diethyl ether, benzene-tetrahydrofuran, and benzene-tetrahydrofuran-glacial acetic acid. Most of the 2,4-DNPHs were prepared according to the procedure of Neuberg et al. (1952). Complex mixtures were separated using normal, multiple, two-dimensional and continuous horizontal development. The hydrazones were dissolved in benzene, or in ethyl acetate, or in tetrahydrofuran to which benzene was added later. The plates were sprayed with ethanolamine which gives characteristic colours for different hydrazones.

Anet (1962) reported that, to obtain reproducible rf values, the water content of the adsorbents must be

constant since water lowers their activity. He found that in hot humid weather, loss of activity took place during handling. Geiss and Schlitt (1960), working with mixtures of polyphenols, indicated that the results obtained depended markedly on the relative humidity in the laboratory or in the developing chamber. They found that most of the difficulties caused by relative humidity variations could be overcome by introducing the coated plates into the developing chamber after they had been heated to 40° C. Badings (1961) also found that relative humidity played an important part in the separation of hydrazones.

III. MATERIALS AND METHODS

Respiration

Bruising and cutting plant tissue will greatly increase the rate of respiration, Joslyn (1930), Kohman and Sanborn (1934) and Stiles and Leach (1952). In these experiments, the cauliflower had to be cut into florets. Thus, the rate of respiration was affected. Since it takes a few hours to process the material for an experiment, the effect of cutting the tissue on the rate of respiration was studied.

The apparatus consisted of 2 gas washers, a flow meter, a water vacuum pump and a 5000 ml desiccator. The gas washers were Pyrex brand fritted ware, 29 cm high and had a capacity of 250 ml. The air passed through the fritted dispersion unit, giving very fine bubbles, providing a large surface for carbon dioxide absorption. Since it was important to know the respiration rate of the intact material, a 5000 ml desiccator equipped with a 2-hole rubber stopper was used as the respiration vessel.

The air was first drawn through a gas washer containing KOH to remove any carbon dioxide present. It

then circulated about the cauliflower placed on a perforated disc about one inch from the bottom of the vessel, after which it entered a second gas washer where the carbon dioxide from the respiring cauliflower was absorbed in 0.1 N KOH. The flow meter was inserted into the system between the first gas washer and the dessicator. An air flow of 600 cc per minute was used.

Each gas washer contained 200 ml of 0.1 N KOH. Titration with 0.1 N HCl, using phenolphthalein as indicator, was carried out every hour. At the beginning and at the end of the experiment, a blank was run on the reagents. The difference between the blank and sample was recorded as ml of 0.1 N HCl. The rate of respiration was calculated in cc CO_2/Kg/hr.

A large head of cauliflower was obtained locally. The leaves were removed and the sample was stored at 5° C for 24 hours. The sample was weighed and the whole head was transferred into the respiration vessel. The cover was fitted and sealed.

Two initial one-hour determinations were made on the whole head. The sample was then removed, cut into florets and excess stem removed. The weight was recorded again, after which the material was transferred back into the respiration vessel. Readings were taken every hour for 13 hours, after which the apparatus was left operating

overnight. The overnight average was not used since most of the KOH had been neutralized and some of the carbon dioxide was lost. Readings were taken at 22 and 23 hours to see if the respiration rate were still constant.

Preparation of Material

Cauliflower, obtained from various sources, was cut into florets to simulate commercial practice. At first, the cut material was used immediately but later on, it was held for 18 hours before using, after it was found that cutting had a profound influence on the rate of respiration.

The cauliflower was blanched in a cabinet-type steam blancher and cooled by spraying with tap water. After cooling, the blanched material was spread out on cheesecloth and allowed to drain for 30 minutes.

For the storage experiments, 125 g of material were placed in 20 oz metal cans. After closing the containers, the covers were perforated with a single hole to permit evacuation and replacement of the atmosphere.

The cans were placed in a vacuum dessicator and evacuated to a pressure of about .5 cm in one minute and maintained at this level for another three minutes. The

vacuum was then released slowly with nitrogen, oxygen or air to provide different atmospheres. When nitrogen or oxygen were used, a slight positive pressure was allowed to build up in the dessicator before lifting the lid to prevent air from getting into the can. The hole in each can was immediately sealed with solder.

In the experiments on aldehyde and ethanol content, the cans were stored at the desired temperatures without prior freezing after the required treatments were given. In the other series, the cans were taken to the freezer after each hour of operation, placed on plates held at -25° C and subdivided into 3 temperature groups, 24 hours later.

Aldehydes and Ethanol

Steam Distillation of Aldehydes and Ethanol

The volatile aldehydes and ethanol were obtained by steam distillation of the ground material. The cauliflower was put through the fine cutting blade on a number 2 Universal food chopper. One hundred g were weighed into a tared round bottom distilling flask. One hundred cc of water were then added to the flask.

The distillate was collected in a 500 ml receiving flask containing 50 ml of distilled water. The condenser was set up so that the tip dipped into the water in the

receiver which 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 45 minutes because it was shown by Wager (1958) and Füleki (1961) that volatiles occur as heat degradation products upon prolonged heating.

In some cases it was decided to let the material stand for a while to see if there were any further production of aldehydes and ethanol. After the initial steam distillation the flask was closed off while still hot so as to keep the contents free of microbial contamination. Forty-eight hours later another distillation was carried out.

Vacuum Distillation of
Aldehydes and Ethanol

Vacuum distillation was used in an attempt to remove the volatile aldehydes and ethanol from cauliflower. The apparatus was set up so that the volatiles were recovered in two liquid nitrogen traps in series. A Virtis Mcleod Gauge was used to determine the vacuum obtained with a Welch Duo-Seal vacuum pump.

Cauliflower was ground in a manner similar to the

one described for aldehyde and ethanol determinations. Two hundred g of material were divided evenly between two Virtis freeze-drying flasks. The samples were spread out evenly around the side of the flasks and quick frozen in liquid air. A vacuum of 5 μ was maintained during the distillation.

Every four hours, the loss in weight was calculated. At the same time, the condensed material in the liquid air traps was removed and made up to a final volume of 500 ml. The aldehydes and ethanol recovered were determined. In the first test, vacuum distillation was carried out for 4 hours. The second one lasted for 8 hours. The third test was carried out on material blanched for 3 minutes. The distillation lasted for 12 hours.

While each sample was being vacuum distilled, a duplicate sample was steam distilled so that the results could be compared.

Known amounts of acetaldehyde and ethanol were vacuum distilled and steam distilled so that the two methods could be compared.

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 due to the small amount present, Joslyn and David (1952) and Füleki (1961).

A 100 ml aliquot of steam distillate was placed in a 500 ml Erlenmeyer flask containing 50 ml of neutral buffer solution (3.35 g of KH_2PO_4 and 15 g of $Na_2HPO_4.12H_2O$ per litre) and 10 ml of bisulfite solution (18.9 g of 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 of concentrated HCl per litre) were added. The excess bisulfite was then titrated with 0.1 N iodine solution until a light blue end point was reached. The solution was then made alkaline by adding 100 ml of an alkaline buffer (8.75 g of boric acid and 400 ml of N NaOH per litre). This liberated the bound bisulfite and the blue colour disappeared. The solution was then titrated with 0.01 N iodine until the blue end point returned. A blank was run on the reagents, using 100 ml of distilled water in place of the distillate. One ml of 0.01 N iodine = .22 mg of aldehyde.

Identification and Quantitative Determination of Ethanol

It was necessary to remove any interfering carbonyl compounds present in the steam distillate before proceeding to identification and quantitative determination of ethanol. 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) and Füleki (1961) was followed throughout.

A 100 ml aliquot of steam distillate was introduced in a 500 ml round bottom flask, fitted with a ground glass connection. Ten ml of $HgSO_4$ solution (10 g of $HgSO_4$ dissolved in 1 litre of 2N 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. The distillation was stopped after having collected about 100 ml in another round bottom flask. Five ml of H_2SO_4 solution and an excess of $Ca(OH)_2$ (about 10 ml of a suspension containing 132 g of $Ca(OH)_2$ per litre) were added to the new distillate. After vigorous shaking, the volume was brought to about 150 ml and the sample was redistilled into a 100 ml glass-stoppered flask.

Identification of ethanol was carried out according to the procedure of Shriner and Fuson (1948). The presence of iodoform was detected by its odour which was compared with iodoform made from pure ethanol.

The quantitative determination of ethanol was carried out by a modification of the dichromate-sulfuric acid procedure of Semichon and Flanzy (1929) and used by David (1949), Moore (1951) and Füleki (1961) as follows: an aliquot containing not more than 3 mg of ethanol was added to 5 ml of 0.1 N $K_2Cr_2O_7$ in 25% H_2SO_4 in a 25 ml Erlenmeyer flask. The flask was tightly stoppered and put in a boiling water bath for 15 minutes. After cooling, 3 ml of 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. One ml of concentrated H_2SO_4 was added for the extra 5 ml aliquot used, thus maintaining the proper acidity. The time of heating was increased to 30 minutes. Duplicate determinations were made on the final distillate. A blank was run simultaneously and the difference, expressed as ml of 0.1 N thiosulfate, was recorded. One ml of 0.1 N thiosulfate = 1.15 mg of ethanol.

Organoleptic Tests

Organoleptic tests were conducted on the samples throughout the storage period. These tests were conducted at the same time as other determinations were carried out. Flavour scores were assigned as follows: "A" good, "B" slightly off, "C" off, "D" very off. In series 1 the samples were also cooked for 4 minutes before tasting. After cooking, the water was drained off and the sample was allowed to cool for a few minutes before tasting. Flavour scores similar to those for the raw material were assigned.

Extraction of TBRS

Several methods were investigated for the extraction of TBRS from cauliflower. Chloroform and carbon tetrachloride were tried but were abandoned. These organic solvents, being immiscible with water, could not be filtered easily.

A steam distillation procedure was tried, first without acid and then with acid. When acid was not used, very little reactive material could be obtained. With acid, a very low pH was required to obtain a significant amount of TBRS. This acidity brought about a considerable amount of browning in the sample during distillation and was thought to cause severe degradation. Therefore

this procedure was discarded.

A water extraction procedure was tried. This seemed to give a good recovery of TBRS. However, because the extract was very cloudy and a thick precipitate occurred upon heating with the TBA reagent, this method was abandoned.

Extractions with butanol, propanol and ethanol were tried. The butanol and propanol extracts were extremely TBA reactive. However, the reactivity was presumably due to aldehydes present in these alcohols. Since vigorous purification would be necessary before these alcohols could be used, no further work was done with them. The ethanol extraction procedure seemed to give the best results of any method tried. The blanks were very clear and comparable to distilled water so the ethanol was not purified any further. However, there was still some cloudiness in the samples after heating and cooling. Upon further investigations, it was found the addition of water would cause cloudiness, especially if it was used to bring the solutions to volume after heating on a hot water bath. When 95% ethanol was used instead, the solutions remained fairly clear. Some material precipitated out when the extracts were left standing overnight at 5^{o} C. These samples, when heated with TBA, were much clearer. When ethanol was used to

bring the samples to volume after heating, the extracts from the fresh samples were the only ones to remain cloudy. This cloudiness disappeared when the volume was increased one-fold with 95% ethanol.

The final procedure developed for the extraction of TBRS was as follows: 100 g of material were blended with 100 ml of 95% ethanol in a Waring blendor at high speed for 3 minutes. The slurry was filtered through Whatman filter paper Number 3 in a Buchner funnel, using a vacuum of 3 to 4 pounds. This low vacuum was employed to minimize any losses which may occur through volatilization. The blendor was washed twice with 25 ml of 95% ethanol. These washings were added successively to the Buchner funnel to rinse the residue. When filtration was complete, the filtrate was transferred to a 250 ml volumetric flask. The sample was then made up to volume with washings of 95% ethanol from the suction flask.

The filtrates were stored for 18 hours at 5^{o} C. They were then removed from the storage and shaken. An aliquot was diluted 1:1 (v/v) with 95% ethanol and centrifuged to remove any precipitate. If the extract contained too much reactive substance, further dilutions were made.

The test was conducted using 5 ml of TBA reagent

and 5 ml of extract. The samples were heated in boiling tubes, 22 mm in diameter and 175 mm long. They were brought to a final volume of 20 ml with 95% ethanol after heating. The absorbance was then read at 520 mμ. A reagent blank was used to adjust the instrument to 100 per cent transmittance. In place of the ethanol extract, 5 ml of 80% ethanol were used. A sample blank was also used. The sample blank contained 5 ml of extract and 5 ml of reagent which did not contain any TBA reagent. This sample blank was used to eliminate any natural colour in the system or colour production during heating which might absorb at 520 mμ, but which was not due to the TBA reaction.

When the first samples were tested, it was found that maximum absorption was occurring at 520 mμ on a Coleman Junior Spectrophotometer Model 6A. However, Patton and Kurtz (1951) reported an absorption maximum at 532 mμ for MA and for oxidized milk fat which was the same as that found by Wilbur et al. (1949) for oxidized methyl linolenate. Turner et al. (1954) found an absorption maximum at 535-538 mμ for the pigment produced from rancid pork while Sinnhuber and Yu (1958) reported an absorption maximum at 532-535 mμ for a sample of rancid salmon oil. An oxidized oil sample was then used to check the readings obtained on the Coleman Junior

Spectrophotometer. This sample also showed a maximum absorption at 520 mμ. The same material was then tested on a Bausch and Lomb Spectronic 505 and a Bechman Model DU. Both instruments showed an absorption peak for the sample between 530 and 535 mμ. Since the grating on the Coleman Junior Spectrophotometer is not as fine as that of the Bausch and Lomb Spectronic 505 and the Bechman Model DU, this difference could be attributed to the difference in gratings. The absorption curve obtained on the Spectronic 505 showed a shoulder at 500 mμ, exhibited by the normal curve in this vicinity. This could move the absorption maximum on the Coleman Junior Spectrophotometer to the right.

In each series a different procedure was used for making the TBA reagent. In series 1, 100 ml of water were used to dissolve .67 g of TBA. The flask was heated in hot water to facilitate solution. The reagent was then diluted with an equal volume of glacial acetic acid. Thus 2.5 ml of glacial acetic acid were being used in each test. It was thought that this was a rather severe treatment, considering the fact that the sample contained approximately 80% ethanol before it was mixed with the TBA reagent. Most, if not all, of the ethanol is volatilized off during heating.

In series II, the samples were heated both with

and without acid. When acid was used, .67 g of TBA were dissolved in 160 ml of water. Enough glacial acetic acid was then added to bring the reagent to 200 ml. A 5 ml aliquot of the reagent was equivalent to using 1 ml of glacial acetic acid in the test. When acid was not used, .67 g of TBA were dissolved in 200 ml of water.

In series I, a heating time of 35 minutes was chosen. This time interval gave good colour development and there was sufficient time to prepare another sample and take readings on previous samples. A 35-minute heating time was used by Tarladgis et al. (1960). When the samples were removed from the hot water bath, they were cooled in cold tap water for 10 minutes, Tarladgis et al. (1960).

Before starting series II, it was decided to compare the absorption values between cauliflower stored for 11 years and fresh cauliflower, to see if the differences between the samples became constant after heating with TBA for a certain period. The comparisons were made without heating the samples with acid. The absorption values were determined after heating for 20, 25, 30, 35, 40, 45, 50 and 55 minutes. From these results, a heating time of 40 minutes was chosen.

In the first series, the samples were placed in a steam-heated hot water bath. However, it was difficult

to maintain a uniform temperature throughout the bath. In the second series a glycerine-water bath was used. The temperature of the bath was set at 100° C. An electric stirrer was used to maintain the temperature of the bath constant at all points.

Most of the results in the literature are reported as TBA numbers. The TBA number is defined as the number of mg of MA per 1000 g of material. Since MA could not be detected in the cauliflower samples, this definition does not really apply. In this investigation, the TBA number might better be described as the number of mg of TBA reactive substances per 1000 g of sample, calculated as MA.

The use of a standard curve was first proposed by Sinnhuber and Yu (1958). Standard curves were prepared, with and without acid, using known amounts of 1,1,3,3-TEP. One mole of 1,1,3,3-TEP, on hydrolysis, produces 1 mole of MA.

Purification of TBA-TBRS Complex

An attempt was made to purify the TBA-TBRS complex formed and to determine its melting point. An acid distillation was carried out from a mixed nitrogen and oxygen ethanol extraction. The ethanol extract contained a small amount of TBRS and only a

part of this was recoverable by distillation. About 8 liters of extract were distilled, which represented 3200 g of cauliflower.

The pH for the distillation was adjusted to 3.0 for maximum recovery of TBRS. The distillate was collected in 50 ml of water containing 1 g of TBA. The tip of the condenser dipped into the TBA solution. About 800 ml of distillate were collected from each liter. The distillate was refluxed for 30 minutes, after which the ethanol was distilled off. All the samples were collected together and the TBA complex was allowed to precipitate out overnight. The crystals were filtered and washed with 100 ml of ethyl ether and then dried. They were then refluxed with 400 ml of water for 30 minutes. After precipitation the crystals were filtered and again washed with ethyl ether. The refluxing and washing were repeated 3 times.

MA from the acid hydrolysis of 1,1,3,3-TEP was also reacted with TBA. The crystals were purified in a manner similar to that used for purification of crystals from cauliflower extract.

After the crystals had been purified and dried, their colour was noted and a melting point determination made. Their absorption spectrum was determined on a Bausch and Lomb Spectronic 505. The samples were spotted

on thin-layer chromatography plates, which were eluted with 95% ethanol.

Recovery of Volatiles for Ultraviolet Absorption Studies

A distillation procedure was used to obtain the volatiles of cauliflower in order to study their characteristics. A 200 g sample of material was ground at high speed in a Waring blendor for 3 minutes, with 200 ml of distilled water. The slurry was transferred to a distilling flask, using 100 ml of water. The suspension was acidified with 5 ml of 3 N HCl. A pH of 2 was obtained which was satisfactory for the recovery of MA. Kwon and Watts (1964) found that maximum volatilization of free preformed MA would not be expected at pH values above 3. Kwon et al. (1965) stated that free MA is readily converted into its volatile form by acidification only, whereas both acidification and heating are necessary to free the protein-bound MA.

Tarladgis et al. (1960) found that maximum recovery of TBRS from oxidized food by steam distillation was obtained only under acid conditions.

A small amount of Dow Corning Antifoam A was used to stop any foaming. The distillate was collected in a 100 ml graduated cylinder. The tip of the condenser dipped into 25 ml of water. The receiver was kept in an

ice bath to minimize any losses by volatilization.

Fifty ml were collected in 10 minutes. The distillate was transferred to a 100 ml volumetric flask and the solution brought to volume with washings from the receiving flask. The pH of the sample was adjusted to 2 so that any MA present could be detected because it showed a maximum absorption at 245 mμ as reported by Kwon and Watts (1963).

The absorption curve was determined from 200 mμ to 400 mμ on a Bausch and Lomb Spectronic 505. A water blank adjusted to pH 2 was used.

Preparation and Separation of 2,4-DNPHs

A reagent which is often used for the separation, identification and characterization of carbonyl compounds is 2,4-DNPHine. Thin-layer chromatography was used for separating the 2,4-DNPHs of carbonyl compounds by Dhont and DeRooy (1961), Rosmus and Deyl (1961), Anet (1962), Denti and Luboz (1965) and Byrne (1965).

The use of 2,4-DNPHine has many advantages. Its derivatives can be easily crystallized from water or alcohol. Because 2,4-DNPHine has a relatively high molecular weight, a reasonable amount of derivative can be obtained from small quantities of carbonyl compounds. The 2,4-DNPHine reagent was prepared according to the

method of Neuberg, Grauer and Pisha (1952). One and one-fifth g of 2,4-DNPHine was dissolved in 50 ml of 30% $HClO_4$.

Most of the hydrazones examined during this investigation were prepared from an ethanol extract of the cauliflower. The DNPHs were prepared from the same extract used for determining the TBA numbers. Two hundred ml of extract and 40 ml of the 2,4-DNPHine solution were placed in a round bottom flask and refluxed for 30 minutes, using a Glas-Col heating mantle. After cooling, the 2,4-DNPH solution was placed in a 5^{o} C storage for 18 hours. The crystals were filtered off on a Whatman filter paper Number 42 and washed with a solution of $HClO_4$ (30 ml of 60% $HClO_4$ in 220 ml of water). The crystals were then dissolved in 50 ml of acetone.

In some cases the 2,4-DNPHs were prepared from a steam distillate. Two hundred g of cauliflower were blended with 200 ml of water in a Waring blendor at high speed for 3 minutes. The slurry was transferred to the distilling flask, using 100 ml of water. Dow Corning Anti-Foam A was used to stop any foaming.

The distillate was collected in a 500 ml round bottom flask, containing 25 ml of 2,4-DNPHine solution, into which dipped the tip of the condenser. The receiver was heated with a Glas-Col heating mantle during the

distillation. About 400 ml of distillate were collected in 45 minutes. The sample was then refluxed for 15 minutes. The 2,4-DNPHs were treated similarly to those obtained from the ethanol extract.

Derivatives were also prepared from distillates obtained from an acid distillation. The procedure outlined above was used except that the pH of the slurry was adjusted to 1.5.

For purposes of comparison, derivatives were prepared from MA, diacetyl, acetaldehyde, acrolein, propionaldehyde, butyraldehyde, valeraldehyde and glyoxal. Each compound was refluxed with an excess of 2.4-DNPHine. The derivatives were recovered from the solution, after cooling and crystallization had occurred. The crystals were washed with an $HClO_4$ solution and dissolved in acetone.

The plates for thin-layer chromatography were prepared according to the method used by Bryne (1965). Thirty g of silica gel G (E. Merck, A. G. Darmstadt) were mixed with 60 g of water. After shaking vigorously for 90 seconds, the slurry was applied at a thickness of .25 mm, using a Shandon spreader. Plates were also prepared in a similar manner with aluminum oxide G, but they did not give results as good as those obtained with silica gel G.

The plates were air dried for 40 minutes, after which they were placed in a drying oven. They were dried at 110° C for 1 hour and then stored over anhydrous $CaSO_4$ until used.

Each spot contained 15 λ of 2,4-DNPH solution. For photography, only one spot was applied. In all cases, the plates were spotted 2 cm from the bottom. The spot was developed, using two dimensional chromatography and two different solvent systems. The solvents were allowed to travel the full length of the plate. When known derivatives were run with the unknown, several spots were applied to the same plate for comparison purposes. The spots were placed 1.5 cm apart. These plates were developed only in one solvent system. The solvent was allowed to move to the top of the plate.

The chromatograms were developed in Desaga developing tanks. The ascending technique was used. The plates were tilted 15 to 20° from the vertical. To achieve saturation in the developing chamber, it was necessary to line the tank on 3 sides with Whatman filter paper Number 1. The solvent was placed in the chamber at least 1 hour before developing the chromatograms. The cover of the tank was made tight by using several 2-pound lead weights. The temperature in the chromatography room was kept at 20° C.

Several solvent systems were investigated. Benzene-petroleum ether (3:1) (v/v), and benzene-ethyl acetate (20:1) (v/v) and (10:1) (v/v) were unsatisfactory. Chloroform-ethanol (5:1) (v/v), (10:1) (v/v) and (15:1) (v/v), chloroform-methanol (10:1) (v/v) and (15:1) (v/v) and toluene-ethyl acetate (1:1) (v/v) resulted in very fast movement of spots but the separation was not complete. Chloroform-ethanol (20:1) (v/v) and toluene-ethyl acetate (2:1) (v/v) gave a fairly good separation. Chloroform-ethanol (25:1) (v/v) and toluene-ethyl acetate (4:1) (v/v) resulted in movement a little slower than that required for good separation. Chloroform-methanol (20:1) (v/v) gave a good separation but still too fast, and a ratio of (25:1) (v/v) was found to elute too slowly.

The best developments of the 2,4-DNPHs from the ethanol extract were obtained with chloroform-methanol (22:1) (v/v) and toluene-ethyl acetate (3:1) (v/v). The latter solvent system was used by Anet (1962). The plates were developed first in chloroform-methanol. After drying, they were turned 90° and developed in toluene-ethyl acetate.

The plates made from the 2,4-DNPHs of the steam distillate were not developed two-dimensionally. The solvent was composed of 80 to 100° C light petroleum-diethyl ether (7:3) (v/v). The plates were developed for identification of the 2,4-DNPH derivative of

acetaldehyde. The rest of the plates were developed with either chloroform-methanol or toluene-ethyl acetate.

An attempt was made to trace the development of off-flavour by photographing the developed chromatograms of the 2,4-DNPHs from the ethanol extract and examining the films to see if new spots appeared or if any, or all, the original spots increased in intensity. High Speed Ektachrome Type B film was used. The plates were illuminated from underneath. Photographs were taken before and after spraying. A 2% solution of NaOH in 90% ethanol was used in spraying, as suggested by Anet (1962).

IV. EXPERIMENTAL RESULTS

Respiration

Preliminary research on cauliflower revealed that there was a wide variation in the metabolic activity of different samples as indicated by differences in aldehyde and ethanol production. Since the bruising of plant tissue changes the rate of respiration, an experiment was performed to find what changes take place when cauliflower is cut into florets. The material for this experiment was obtained locally.

The results of the effect of cutting cauliflower into florets on the rate of respiration are reported in Table 1 and plotted in Figure 1. They show that there is a sharp increase in the rate of respiration due to the bruising of the tissue. The maximum rate of respiration was reached 2 hours after the cauliflower had been cut into florets, after which the rate decreased. Eleven hours after cutting, the rate of respiration had reached a fairly constant level. The variations which were noticed afterwards could be due to experimental error.

The time required to process the material for any

TABLE 1.--Effect of cutting cauliflower in florets on the rate of respiration

Time in hours	cc of CO_2/kg/hour
Whole head, first hour	24.1
Whole head, second hour	25.5
Hours after cutting	
1	51.7
2	57.0
3	53.8
4	51.7
5	50.8
6	48.7
7	46.7
8	44.1
9	42.1
10	39.4
11	38.3
12	38.3
13	38.3
14	39.0
15-21 (overnight)	
22	38.0
23	38.0

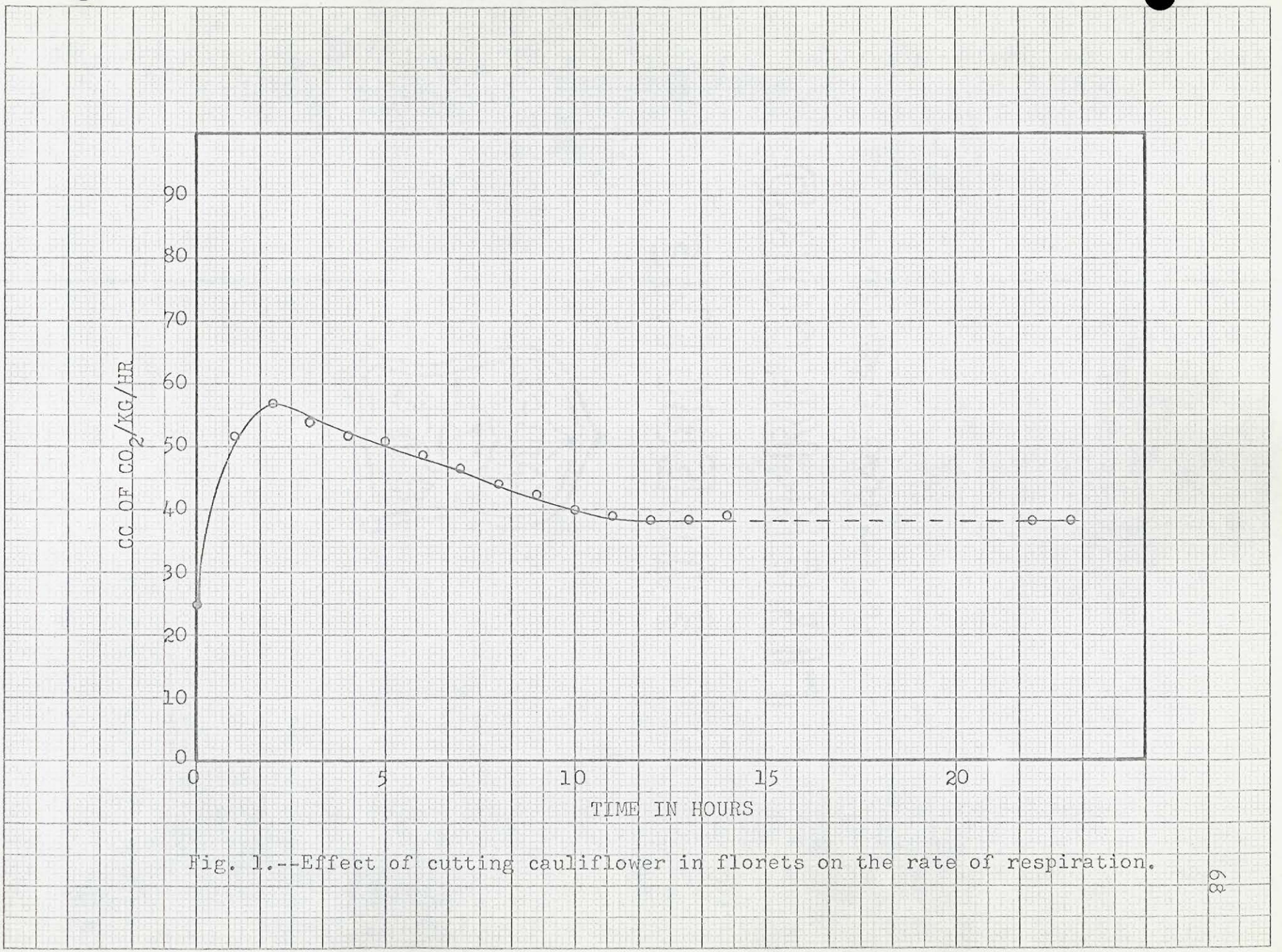


Fig. 1.--Effect of cutting cauliflower in florets on the rate of respiration.

one experiment varies, depending on the amount of cauliflower to be processed. If the material is processed immediately after cutting, a new variable is introduced into the results which would not be similar in all segments of the experiment. It was decided to hold the material at 5° C for 18 hours after cutting it to insure that the rate of respiration was constant.

Aldehydes

Recovery of Aldehydes

Steam distillation is the accepted procedure to remove volatiles from plant tissues, Moore (1951), Wager (1958) and Füleki (1961). However, since Wager (1958) and Füleki (1961) have reported that aldehydes appear to form as heat breakdown products in peas and green beans during this process, an experiment was carried out to find if this phenomenon occurs in cauliflower. Samples were held for 48 hours after the initial distillation and then distilled again. In the first distillation, 1.53 mg of aldehydes were recovered. In the second distillation 48 hours later, 1.08 mg were recovered. These results indicate that a non-enzymatic production of aldehyde occurs also in cauliflower probably as heat breakdown products.

Vacuum distillation which does not have the undesirable characteristics of steam distillation was

attempted for the recovery of the aldehydes. The results obtained were quite variable and the quantities recovered depended upon the length of the distillation to a considerable extent. The first distillation lasted 4 hours using unblanched cauliflower. A recovery of 1.18 mg of aldehydes was obtained as compared with 2.00 mg by steam distillation. In the second experiment on unblanched material, two consecutive distillations of four hours resulted in a total recovery of 1.14 mg of aldehydes, or .57 mg during each 4 hours of operation. Using the steam distillation method, 1.86 mg were recovered. A third vacuum distillation on cauliflower blanched for 3 minutes was continued for 12 hours with a recovery of .38, .50 and .35 mg respectively for each 4-hour period. The total recovery was 1.23 mg as compared with 1.91 mg by steam distillation.

A known amount of acetaldehyde was vacuum distilled for 2 hours and steam distilled. A recovery of .62 mg was reported by vacuum distillation and .61 mg by steam distillation indicating that while it is possible to obtain complete recovery when pure acetaldehyde is used, it is quite difficult to obtain the same results with a complex system such as ground cauliflower. Since vacuum distillation is also very time-consuming, it was not adopted and steam distillation was retained.

Preliminary Experiments on Cauliflower Stored in Air

Three preliminary experiments of short duration were performed with cauliflower stored in air at different temperatures. The material for the experiments was obtained locally. About 2 hours were required to process the cauliflower, after cutting it into florets. Steam distillation was used to recover the aldehydes. The initial determinations were made after processing the material for storage. Determinations were then made at intervals throughout the experiments. The results for the aldehyde determinations are given in Tables 2, 3 and 4.

In all cases, the aldehyde content decreased at the beginning of storage. After several hours, depending on the storage temperature, there was an increase in the aldehyde content, the greatest changes taking place at the highest temperatures.

These results can possibly be explained by the fact that cutting affects the rate of respiration, the highest rate being reached after 2 hours, followed by a decrease, as shown previously. In the present experiments, the initial aldehyde determinations were made about 2 hours after the material had been cut into florets, favouring aldehyde production as is the case with bruised peas and lima beans, Kohman (1932) and Kohman and Sanborn (1934). Therefore, it would appear that the first

TABLE 2.--Aldehyde production, expressed in mg%, at the beginning of storage of cauliflower stored in air

Storage temperature °C	Time in hours	Aldehyde
20.6	Initial	1.07
	1	0.98
	2	0.83
	3	0.83
	4	0.86
	5	0.87
	7	0.83
	20	1.83
	30	2.30

TABLE 3.--Aldehyde production, expressed in mg%, at the beginning of storage of cauliflower stored in air

Storage temperature °C	Time in hours	Aldehyde
20.6	Initial	1.45
	4	1.25
	8	1.21
	12	1.43
	28	2.34
	50	3.01
10.6	Initial	1.45
	4	1.01
	8	1.44
	12	2.08
	28	3.36
	48	3.56
	72	4.62
0.6	Initial	1.45
	6	1.05
	10	1.38
	30	1.61
	50	1.67
-9.4	Initial	1.45
	6	1.42
	10	1.40
	30	1.30
	48	1.41
	72	1.38

TABLE 4.--Aldehyde production, expressed in mg%, at the beginning of storage of cauliflower stored in air

Storage temperature °C	Time in hours	Aldehyde
20.6	Initial	1.86
	4	0.98
	8	1.39
	12	1.98
	24	2.75
	48	3.87
10.6	Initial	1.86
	4	0.85
	8	0.88
	12	0.88
	24	1.98
	28	4.71
0.6	Initial	1.86
	4	0.97
	8	0.88
	12	0.77
	24	0.88
	28	2.55
-9.4	Initial	1.86
	4	1.76
	8	2.12
	24	2.49
	48	2.52

determinations were made when the cauliflower was showing its greatest metabolic activity. Thus, the rate of aldehyde production would be higher than that expected in the succeeding determinations while the material was still respiring aerobically. During storage the oxygen content would be expected to decrease to a point where the material would undergo anaerobic respiration. Under these conditions, aldehydes accumulate. It was observed that, after several hours, there was an increase in the aldehyde content of the cauliflower, which can be attributed to the development of anaerobic conditions in the container.

The cauliflower stored at -9.4° C showed little change in the aldehyde content. This could be due to a decrease in enzyme activity at low temperatures. Since there was only a very small decrease in carbonyl content, which in some cases was followed by an increase, it is possible that when plant cells are frozen, anaerobic conditions develop. If this were so, then anaerobic conditions within the frozen cauliflower would exist sooner than in similar material stored at higher temperatures. It was also suggested by Kohman and Sanborn (1934) that frozen material may accumulate aldehydes due to freezing injury.

The higher the temperature of storage above 0°C,

the sooner the aldehydes started to accumulate. This can be explained by the fact that the rate of respiration is influenced by temperature. Therefore, at higher temperatures, the oxygen in the container will disappear faster, resulting in earlier anaerobic conditions.

Preliminary Experiments on Cauliflower Stored in Nitrogen

Three experiments of short duration were also performed with cauliflower stored in nitrogen at different temperatures. The material was obtained locally. It was cut into florets and then kept at 5° C for 18 hours.

The initial aldehyde determinations were made on the material stored for 18 hours at 5° C. Determinations were also made at intervals throughout the experiments. The results are reported in Tables 5, 6 and 7.

The material which was stored in nitrogen underwent anaerobic respiration immediately at all temperatures. This was evidenced by the fact that in all cases there was an increase in the aldehyde content within a short period after the material had been processed. The fastest rate of aldehyde accumulation occurred in the cauliflower stored at the highest temperature.

The rate of accumulation of aldehydes in cauliflower stored in air and nitrogen cannot be readily compared in these preliminary experiments because of the

TABLE 5.--Aldehyde production, expressed in mg%, at the beginning of storage of cauliflower stored in N_2

Storage temperature °C	Time in hours	Aldehyde
20.6	Initial	0.77
	2.5	1.36
	4.5	2.07
	6.5	3.21
	8.5	3.61
	9.5	3.44
	11.5	5.16
	24	8.38
	28	8.73
10.6	Initial	0.77
	2.5	2.84
	4.5	2.66
	6.5	2.79
	9.5	2.99
	24	5.61
	28	5.98

TABLE 6.--Aldehyde production, expressed in mg%, at the beginning of storage of cauliflower stored in N_2

Storage temperature oC	Time in hours	Aldehyde
20.6	Initial	1.32
	4	2.59
	8	2.64
	24	3.15
	52	4.57
	76	5.24
10.6	Initial	1.32
	4	2.56
	8	2.56
	24	2.77
	52	4.07
	76	4.44
0.6	Initial	1.32
	4	2.22
	8	2.02
	24	2.23
	52	2.26
	76	3.22
-9.4	Initial	1.32
	4	1.93
	8	2.01
	24	2.45
	52	2.32
	76	3.40

TABLE 7.--Aldehyde production, expressed in mg%, at the beginning of storage of cauliflower stored in N_2

Storage temperature °C	Time in hours	Aldehyde
20.6	Initial	1.10
	4	2.95
	8	3.45
	30	6.09
	48	6.88
	97	8.73
10.6	Initial	1.10
	4	3.63
	8	3.19
	30	3.22
	48	4.87
	97	5.98
0.6	Initial	1.10
	4	2.60
	8	3.18
	30	3.74
	48	3.92
	97	4.40
-9.4	Initial	1.10
	4	2.31
	8	2.52
	30	2.70
	48	2.07
	97	2.48

different treatments given the material at the beginning. However, it was noted that the initial aldehyde content of cauliflower showed a wide variation for different lots. This was probably due to different rates of metabolic activity which might be related to varietal differences, the age of the cauliflower and/or the time between harvest and analysis. The metabolic activity of immature cauliflower would be higher than that of mature cauliflower, while that of freshly harvested samples would be higher than that found in samples which had been harvested and stored.

Since there is a wide variation in the rate of respiration of cauliflower, care must be taken when preparing samples for experimentation. The cauliflower should be cut into florets as quickly as possible and then the prepared material should be well mixed to insure uniformity.

Those results obtained from the cauliflower stored at -9.4° C are of particular interest. They show a rapid rate of accumulation of aldehydes in the early part of storage. Füleki (1961) found that raw green beans accumulated large amounts of aldehyde during handling and freezing. He also found that variations observed in samples stored under the same conditions were due to differences in the preparation and handling of the material.

The rapid accumulation of aldehydes in the early part of storage, which was in evidence at all temperatures in material stored in nitrogen, emphasizes the importance of freezing the samples as soon as possible after they have received the desired treatment.

Series I

In series I, experiments were conducted on unblanched and blanched cauliflower. However, the 3-minute steam-blanch given was not sufficient since off-flavours developed in the material during freezing storage. The samples were stored in nitrogen and oxygen at -9.4° , -15° and -20.6° C.

Effect of Storage Temperature on Aldehyde Content in Unblanched and Blanched Cauliflower

The results of the aldehyde determinations on unblanched and blanched cauliflower stored in nitrogen and oxygen are reported in Tables 8, 9, 10 and 11. The results for unblanched cauliflower stored in nitrogen and oxygen are plotted in Figures 2 and 3.

In the unblanched samples stored in nitrogen, it was found that there was an increase in aldehyde content at all temperatures. After 66 days of storage, the aldehyde content was still increasing in all samples. The biggest increase occurred at the highest temperature.

TABLE 8.--Effect of storage temperature on aldehyde content, expressed in mg%, of unblanched cauliflower stored in N_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days							
	Initial	2	9	18	25	32	40	66
- 9.4	0.43 A	0.88 A	2.04 C	2.49 D	3.19 D	3.08 D	3.19 D	3.37 D
-15	0.43 A	-	1.17 B	1.63 D	1.40 D	1.96 D	1.90 D	2.18 D
-20.6	0.43 A	-	0.97 A	1.32 D	1.67 D	1.67 D	1.53 D	1.69 D

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

TABLE 9.--Effect of storage temperature on aldehyde content, expressed in mg%, of blanched cauliflower stored in N_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days			
	Initial	16	42	66
- 9.4	0.24 A	0.54 B	0.64 D	0.62 D
-15	0.24 A	0.48 A	0.53 D	0.53 D
-20.6	0.24 A	0.47 A	0.47 D	0.47 D

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

Fig. 2.--Effect of storage temperature on aldehyde content, expressed in mg%, of unblanched cauliflower stored in N_2, series I.

TABLE 10.--Effect of storage temperature on aldehyde content, expressed in mg%, of unblanched cauliflower stored in O_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days							
	Initial	2	9	18	25	32	40	66
- 9.4	0.43 A	1.25 A	3.72 D	3.47 D	3.73 D	3.54 D	3.36 D	3.34 D
-15	0.43 A	-	2.24 C	2.48 D	2.67 D	2.92 D	2.62 D	2.67 D
-20.6	0.43 A	-	1.61 B	2.16 D	2.23 D	2.26 D	2.07 D	2.29 D

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

TABLE 11.--Effect of storage temperature on aldehyde content, expressed in mg%, of blanched cauliflower stored in O_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days			
	Initial	16	42	66
- 9.4	0.24 A	0.50 B	0.61 D	0.65 D
-15	0.24 A	0.50 A	0.53 D	0.62 D
-20.6	0.24 A	0.44 A	0.43 D	0.53 D

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

Fig. 3.--Effect of storage temperature on aldehyde content, expressed in mg%, of unblanched cauliflower stored in O_2, series I.

It was found that a fairly large increase occurred in the first few days in storage. This might be due to a delay in freezing. It was found in the preliminary experiments on cauliflower stored in nitrogen at -9.4° C that a large increase in aldehyde occurred during the first 4 hours of storage before the material had a chance to freeze, and that at temperatures above 0° C, large increases in aldehyde occurred rapidly due to anaerobic conditions. A 7.8-, 5.1- and 3.9-fold increase in aldehyde content occurred at -9.4°, -15° and -20.6° C, respectively.

The blanched material stored in nitrogen showed an increase in aldehyde content at all temperatures. At the end of the first 16 days in storage, the greatest change in aldehyde content had occurred. Small changes were later observed at the higher temperatures of storage. The greatest increase occurred at the highest temperature. The final aldehyde content was only slightly higher than that found in the initial unblanched samples. A 2.7-, 2.2- and 2.0-fold increase in aldehyde content occurred at -9.4°, -15° and -20.6° C, respectively.

The samples stored in oxygen showed an increase in aldehyde content at all temperatures with the largest increase occurring at the higher temperatures. After 66 days in storage, the aldehyde content was showing signs of decreasing at -9.4° C. At -15° C the aldehyde content

seemed to have reached a constant level, while at -20.6° C it seemed to be still increasing slowly. An 8.7-, 6.8- and 5.3-fold increase in aldehyde content occurred at -9.4°, -15° and-20.6° C, respectively.

The blanched material stored in oxygen showed an increase in aldehyde content at all temperatures. After the first 16 days in storage, the rate of aldehyde accumulation was rather slow. Again, the greatest accumulation was associated with the highest storage temperature. The final aldehyde content was only slightly higher than that found in the initial unblanched samples. A 2.7-, 2.6- and 2.2-fold increase in aldehyde content occurred at -9.4°, -15° and -20.6° C, respectively.

Effect of Atmosphere on Aldehyde Content in Unblanched and Blanched Cauliflower

The results showing the effect of the atmosphere on the highest accumulation of aldehydes at each temperature are found in Table 12. The highest accumulation of aldehydes at each temperature occurred in the unblanched samples stored in oxygen. Unblanched samples stored in nitrogen accumulated more aldehydes than the blanched samples. The blanched samples stored in nitrogen accumulated slightly less than those stored in oxygen.

TABLE 12.--Effect of temperature and composition of atmosphere on the highest aldehyde accumulation over the initial content during freezing storage, expressed in mg%

Storage temperature °C	Treatment of material			
	N_2 unblanched	N_2 blanched	O_2 unblanched	O_2 blanched
- 9.4	2.94	.40	3.30	.41
-15.0	1.75	.29	2.49	.38
-20.6	1.26	.23	1.86	.29

Effect of Blanching on Aldehyde Content of Cauliflower

The results of the effect of blanching on aldehyde accumulation are reported in Table 12. All samples showed an increase in aldehyde content, the greatest increase occurring in the oxygen blanched samples. The highest accumulation was found to occur at the highest temperature in both the nitrogen and oxygen atmospheres. These results are in accord with the unblanched material where it was found that samples stored in oxygen accumulated the greatest amount of aldehydes. The blanched samples did not accumulate an appreciable quantity of aldehydes. This indicates that the enzyme systems involved in the production of aldehydes were partially if not completely inactivated by blanching for 3 minutes.

Relationship Between Aldehyde Content and Off-flavour Scores

It was apparent that the unknown compound or compounds responsible for off-flavour were different organoleptically in the oxygen and nitrogen samples. The material stored in nitrogen and oxygen had a pungent odour and taste. The samples stored in oxygen were the most disagreeable because of the strong oxidized flavour. The compound or compounds producing off-flavour in the samples stored in nitrogen seemed to be more volatile than those causing off-flavour in cauliflower stored in oxygen, since cooking for 4 minutes resulted in a marked improvement in the flavour of the material stored in nitrogen but not in the product stored in oxygen. A comparison of flavour scores between cooked and uncooked cauliflower is found in Tables 13 and 14.

There seems to be no relationship between the development of off-flavour and aldehyde content in unblanched and blanched samples stored in the same atmospheres, even though the blanched samples were not heated long enough and off-flavour did develop in them. Blanching for 3 minutes in a steam blancher delayed the development of off-flavour but it did not prevent it.

It was noted that in some cases samples which were judged as "off" had a higher aldehyde content than samples which were judged as "very off." The differences in

TABLE 13.--Comparison of flavour scores of stored unblanched cauliflower before and after cooking, series I

Storage atmosphere	Storage temperature °C	Storage time in days											
		Initial		9		18		25		32		40	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Nitrogen	- 9.4	A	A	C	A	D	B	D	B	D	D	D	D
Nitrogen	-15.0	A	A	B	A	D	A	D	A	D	B	D	C
Nitrogen	-20.6	A	A	A	A	D	A	D	A	D	A	D	B
Oxygen	- 9.4	A	A	D	D	D	D	D	D	D	D	D	D
Oxygen	-15.0	A	A	C	D	D	D	D	D	D	D	D	D
Oxygen	-20.6	A	A	B	B	D	D	D	D	D	D	D	D

(a) columns = Uncooked; (b) columns = Cooked
Taste scores: A = good; B = slightly off; C = off; D = very off

TABLE 14.--Comparison of flavour scores of stored blanched cauliflower before and after cooking, series I

Storage atmosphere	Storage temperature °C	Storage time in days							
		Initial		16		42		66	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Nitrogen	- 9.4	B	A	B	A	D	B	D	D
Nitrogen	-15.0	A	A	A	A	D	B	D	D
Nitrogen	-20.6	A	A	A	A	D	A	D	C
Oxygen	- 9.4	A	A	B	A	D	D	D	D
Oxygen	-15.0	A	A	A	A	D	D	D	D
Oxygen	-20.6	A	A	A	A	D	D	D	D

(a) columns = Uncooked; (b) columns = Cooked
Taste scores: A = good; B = slightly off; C = off; D = very off

aldehyde content were small between samples which were judged as "good" and samples which were judged as "very off."

Off-flavour developed very rapidly in samples stored in oxygen. These samples had a higher aldehyde content than samples stored in nitrogen with the same flavour score.

In the blanched material, there was little difference in aldehyde content between samples judged as "off" and "good" when stored in nitrogen or oxygen. Temperature differences did not affect aldehyde content to a great extent. It was found that samples which were judged as "very off" at one temperature contained less aldehyde than samples which were judged as "good" at another temperature. Blanched cauliflower stored in nitrogen at -20.6° C had the same aldehyde content in samples with flavour scores of "good" and "very off."

It would seem from these results that a poor relationship exists between aldehyde content as determined by this method and the development of off-flavour.

Ethanol

Recovery of Ethanol

Ethanol determinations are usually made on the steam distillate from plant tissues. Since it was found that aldehydes are formed as heat breakdown products, the

second steam distillate obtained was also analyzed for ethanol. The results were negative, indicating that ethanol is not formed under these conditions.

The vacuum distillates used for aldehyde determinations were also analyzed for ethanol. These results were also quite variable, and the quantity recovered depended upon the length of the vacuum distillation. The first distillation lasted 4 hours using unblanched cauliflower. A recovery of 40.3 mg of ethanol was obtained as compared with 48.9 mg by steam distillation. In the second experiment on unblanched material, two consecutive distillations of 4 hours each, resulted in a total recovery of 25.9 mg of ethanol, 17.3 mg during the first 4 hours operation and 8.6 mg during the second 4 hours. Using steam distillation, 41.7 mg were recovered. A third vacuum distillation on cauliflower blanched for 3 minutes was continued for 12 hours with a recovery of 23.0, 17.3 and 14.4 mg, respectively, during each 4 hours operation. The total recovery was 54.7 mg as compared with 43.1 mg by steam distillation.

A known quantity of ethanol was vacuum distilled and steam distilled. A recovery of 102.1 mg was obtained by vacuum distillation as compared with 104.9 mg by steam distillation.

Preliminary Experiments on Cauliflower Stored in Air

The steam distillates of the cauliflower used in the preliminary experiments on aldehyde production were also analyzed for ethanol content. The initial determinations were made about 2 hours after the cauliflower had been cut into florets, followed by determinations at intervals throughout the experiments. The results are given in Tables 15, 16 and 17.

In the early part of storage, the ethanol content either stayed at the same level or decreased. In some instances, the decrease was quite pronounced. After several hours of storage, there was generally a sharp increase in the ethanol content. Wide variations in the final content were noted specially at the higher temperatures.

The same factors which caused a decrease in the aldehyde content of cauliflower stored in air were probably responsible for the decrease in ethanol content as well. As anaerobic conditions developed within the container, there was a sharp increase in the ethanol content.

Preliminary Experiments on Cauliflower Stored in Nitrogen

The steam distillates obtained in the preliminary experiments on aldehyde production in cauliflower stored

TABLE 15.--Ethanol production, expressed in mg%, at the beginning of storage of cauliflower stored in air

Storage temperature °C	Time in hours	Ethanol
20.6	Initial	20.1
	1	14.3
	2	10.8
	3	12.2
	4	13.0
	5	14.4
	7	21.5
	20	56.2
	30	103.7

TABLE 16.--Ethanol production, expressed in mg%, at the beginning of storage of cauliflower stored in air

Storage temperature °C	Time in hours	Ethanol
20.6	Initial	34.2
	4	34.5
	8	34.9
	12	38.2
	28	78.8
	50	205.6
10.6	Initial	34.2
	4	32.6
	8	37.6
	12	37.4
	28	45.6
	48	48.1
	72	151.1
0.6	Initial	34.2
	6	28.8
	10	31.6
	30	41.7
	50	42.4
-9.4	Initial	34.2
	6	34.5
	10	29.6
	30	29.6
	48	33.8
	72	41.2

TABLE 17.--Ethanol production, expressed in mg%, at the beginning of storage of cauliflower stored in air

Storage temperature °C	Time in hours	Ethanol
20.6	Initial	18.8
	4	12.1
	8	30.3
	12	71.8
	24	148.4
	48	352.6
10.6	Initial	18.8
	4	14.3
	8	18.8
	12	23.0
	24	64.8
	28	144.5
0.6	Initial	18.8
	4	13.0
	8	21.6
	12	24.5
	24	26.5
	28	30.7
-9.4	Initial	18.8
	4	23.0
	8	27.4
	24	31.6
	28	34.5

in nitrogen were also analyzed for ethanol content. The initial determinations were made after the material had been cut into florets and stored for 18 hours at 5° C. Determinations were also made at intervals throughout the experiments. The results for the ethanol determinations are found in Tables 18, 19 and 20.

In these experiments, the cauliflower underwent anaerobic respiration immediately at all temperatures. This was evidenced by the fact that in all cases there was an increase in ethanol content within a short period after the beginning of storage. The fastest rate of ethanol accumulation occurred in the cauliflower stored at the highest temperatures.

The rate of accumulation of ethanol in cauliflower stored in air and in nitrogen cannot be readily compared in these preliminary experiments because of the different treatments given the material at the beginning. The initial ethanol content of the material showed considerable variation as did the rate of accumulation during storage. The factors which might have affected aldehyde accumulation could also contribute to ethanol accumulation. Those factors which might affect the metabolic activity, and thus the ethanol content, are varietal differences, the age of the cauliflower and/or the time between harvest and analysis.

TABLE 18.--Ethanol production, expressed in mg%, at the beginning of storage of cauliflower stored in N_2

Storage temperature °C	Time in hours	Ethanol
20.6	Initial	30.3
	2.5	24.4
	4.5	40.3
	6.5	92.0
	8.5	145.3
	9.5	122.3
	11.5	140.9
	24	333.4
	28	370.9
10.6	Initial	30.3
	2.5	40.3
	4.5	71.0
	6.5	63.2
	9.5	94.8
	24	178.2
	28	201.2

TABLE 19.--Ethanol production, expressed in mg%, at the beginning of storage of cauliflower stored in N_2

Storage temperature °C	Time in hours	Ethanol
20.6	Initial	30.2
	4	53.1
	8	92.0
	24	175.4
	52	388.1
	76	595.1
10.6	Initial	30.2
	4	56.1
	8	76.3
	24	148.1
	52	280.3
	76	376.6
0.6	Initial	30.2
	4	40.3
	8	41.7
	24	84.8
	52	143.8
	76	202.7
-9.4	Initial	30.2
	4	47.5
	8	44.7
	24	44.7
	52	48.9
	76	37.4

TABLE 20.--Ethanol production, expressed in mg%, at the beginning of storage of cauliflower stored in N_2

Storage temperature °C	Time in hours	Ethanol
20.6	Initial	20.9
	4	74.7
	8	123.6
	30	393.9
	48	635.4
	97	1028.0
10.6	Initial	20.9
	4	48.9
	8	67.6
	30	179.8
	48	296.2
	97	540.5
0.6	Initial	20.9
	4	30.3
	8	48.9
	30	103.4
	48	152.4
	97	267.4
-9.4	Initial	20.9
	4	31.6
	8	40.3
	30	43.1
	48	40.3
	97	41.7

Series I

The material used to determine the aldehyde content of cauliflower stored in nitrogen and oxygen was also analyzed for ethanol content.

Effect of Storage Temperature on Ethanol Content in Unblanched and Blanched Cauliflower

The results of the ethanol determinations on unblanched and blanched cauliflower stored in nitrogen and oxygen are reported in Tables 21, 22, 23 and 24. The results for the unblanched material are plotted in Figures 4 and 5.

In the unblanched samples stored in nitrogen, an increase in ethanol content was found at all the storage temperatures used. This increase appeared to have taken place by the time the first determinations were made. The fluctuations occurring during further storage could be due to experimental error and biological variation.

Since most of the ethanol in the cauliflower seems to have accumulated in the early part of storage, there is a possibility that the ethanol accumulated before or during freezing. In the preliminary experiments on samples stored in nitrogen at -9.4° C, it was found that most of the ethanol accumulated during the first 8 hours of storage, with the greatest production occurring during the first 4 hours of storage before the samples were

TABLE 21.--Effect of storage temperature on ethanol content, expressed in mg %, of unblanched cauliflower stored in N_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days							
	Initial	2	9	18	25	32	40	66
- 9.4	33.1 A	139.5 A	123.6 C	122.3 D	120.8 D	123.6 D	139.5 D	135.1 D
-15	33.1 A	-	89.1 B	86.2 D	84.8 D	93.5 D	99.2 D	94.8 D
-20.6	33.1 A	-	110.8 A	97.7 D	99.2 D	106.2 D	96.3 D	94.8 D

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

TABLE 22.--Effect of storage temperature on ethanol content, expressed in mg %, of blanched cauliflower stored in N_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days			
	Initial	16	42	66
- 9.4	24.5 A	14.4 B	8.6 D	5.8 D
-15	24.5 A	17.3 A	8.6 D	7.3 D
-20.6	24.5 A	14.4 A	7.3 D	11.5 D

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

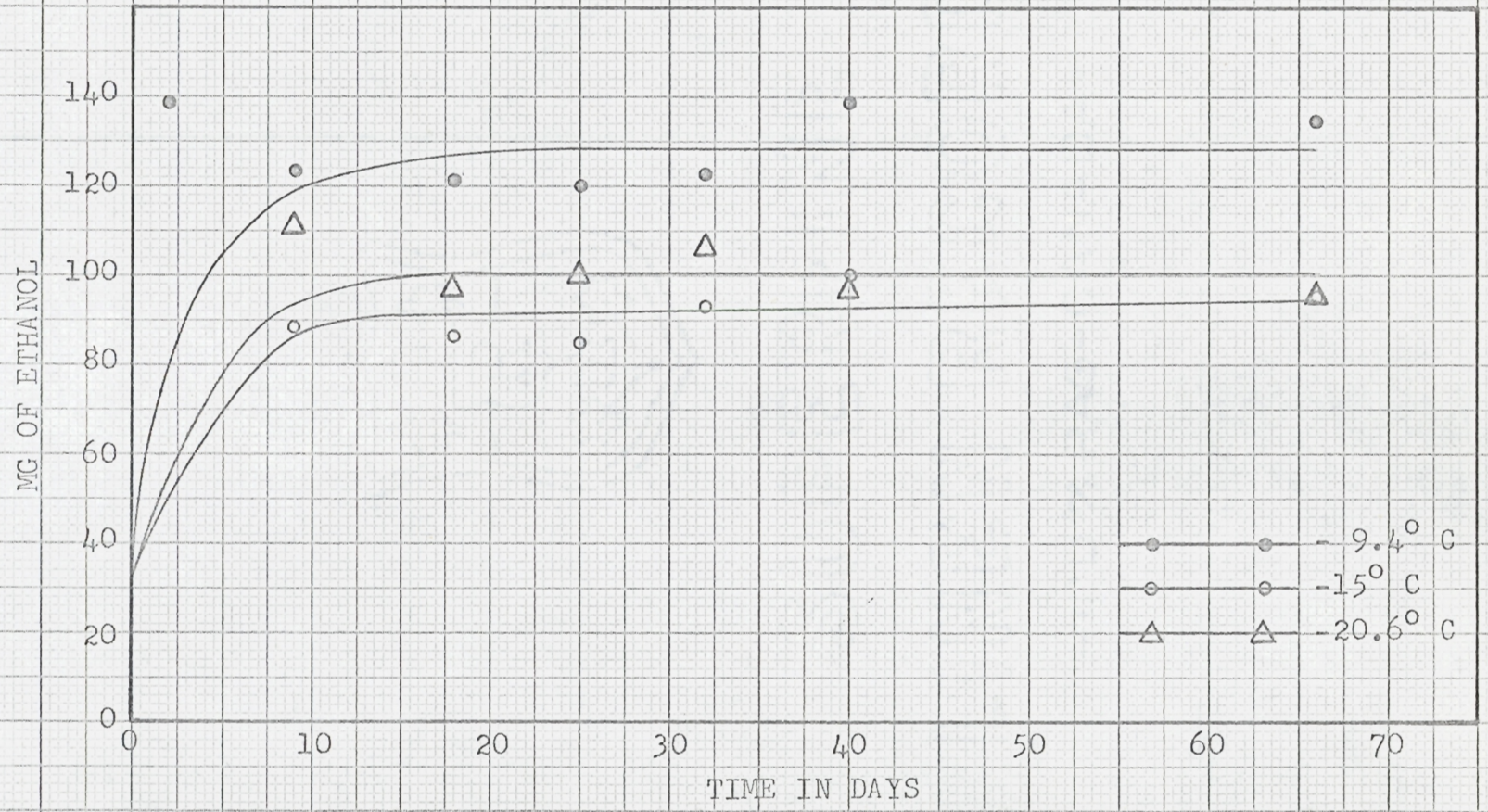


Fig. 4.--Effect of storage temperature on ethanol content, expressed in mg%, of unblanched cauliflower in N_2, series I.

TABLE 23.--Effect of storage temperature on ethanol content, expressed in mg %, of unblanched cauliflower stored in O_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days							
	Initial	2	9	18	25	32	40	66
- 9.4	33.1 A	50.4 A	37.4 D	30.3 D	28.8 D	17.3 D	14.4 D	17.3 D
-15	33.1 A	-	27.4 C	23.0 D	15.9 D	15.9 D	13.0 D	11.5 D
-20.6	33.1 A	-	25.9 B	18.8 D	17.3 D	18.8 D	15.9 D	17.3 D

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

TABLE 24.--Effect of storage temperature on ethanol content, expressed in mg %, of blanched cauliflower stored in O_2. Letters indicate the flavour scores, series I

Storage temperature °C	Storage time in days			
	Initial	16	42	66
- 9.4	24.5 A	14.4 B	10.1 D	7.3 D
-15	24.5 A	17.3 A	10.1 D	5.8 D
-20.6	24.5 A	14.4 A	10.1 D	7.3 D

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

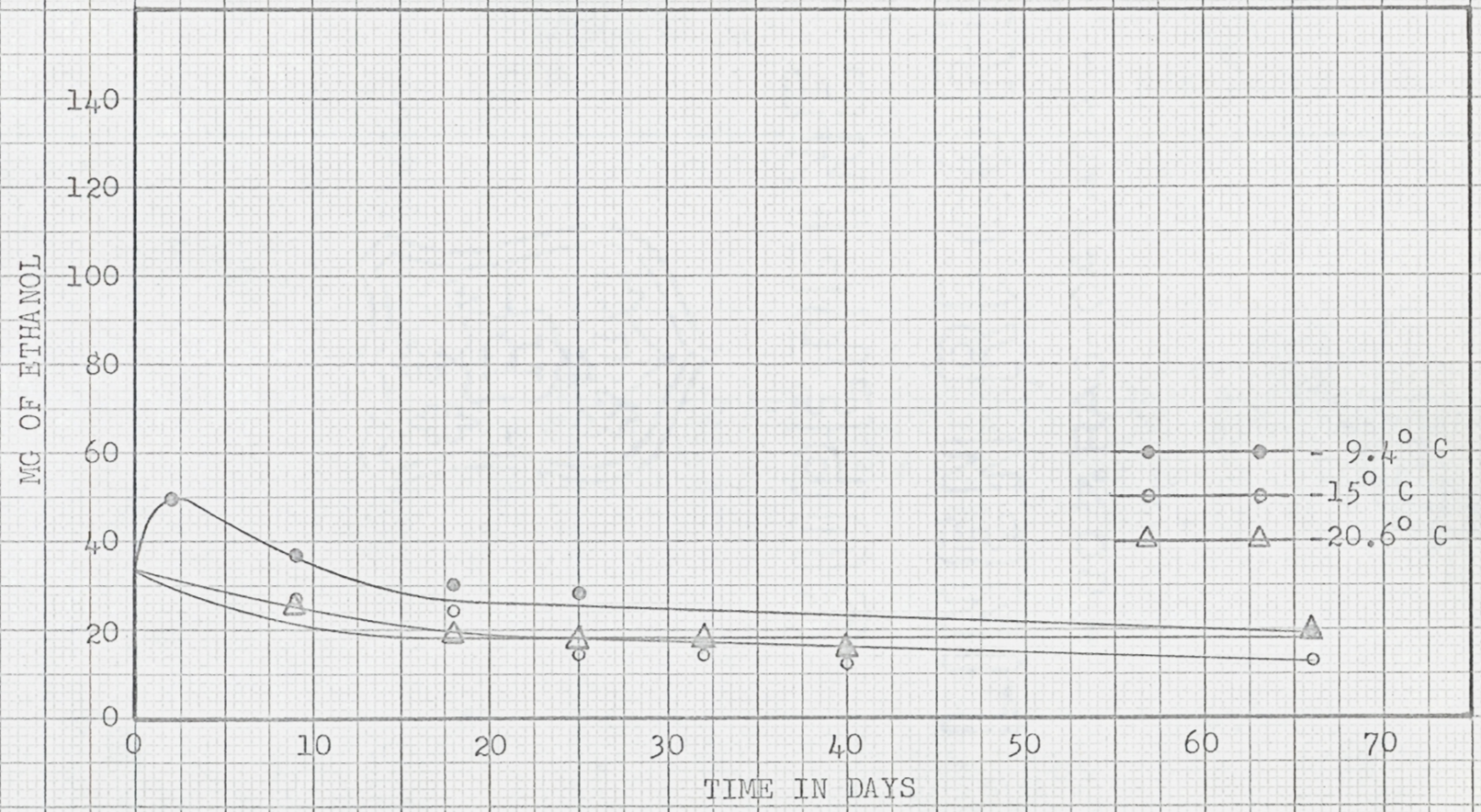


Fig. 5.--Effect of storage temperature on ethanol content, expressed in mg%, of unblanched cauliflower stored in O_2, series I.

completely frozen. Large increases were found to occur rapidly at temperatures above 0° C due to anaerobic conditions. Thus, it would seem that ethanol accumulation under anaerobic conditions was due to handling before freezing.

The unblanched samples stored in oxygen showed a decrease in ethanol content. Initially, at -9.4° C there seemed to be an increase in ethanol which was later followed by a decrease. This change may also have taken place at other storage temperatures. However, analysis was not carried out early enough after freezing to detect any increase which may have occurred. The accumulation of ethanol under aerobic conditions may have been caused by freezing injury, as suggested by Kohman and Sanborn (1934), or due to anaerobic conditions developing inside the cells after partial freezing, Füleki (1961).

The blanched material stored in nitrogen and oxygen also showed a decrease in ethanol content at all temperatures.

Effect of Atmosphere on Ethanol Content in Unblanched and Blanched Cauliflower

The results showing the effect of the atmosphere on the maximum change in ethanol content at each temperature are given in Table 25. The unblanched samples stored in nitrogen were the only ones showing an increase

in ethanol content. All the other samples showed almost identical decreases.

TABLE 25.--Effect of temperature and composition of atmosphere on the maximum change in ethanol content over the initial content during freezing storage, expressed in mg%

Storage temperature °C	Treatment of material			
	N_2 unblanched	N_2 blanched	O_2 unblanched	O_2 blanched
- 9.4	106.4	-18.7	-18.7	-17.2
-15.0	66.1	-17.2	-22.6	-18.7
-20.6	77.7	-17.2	-17.2	-17.2

Effect of Blanching on Ethanol Content of Cauliflower

The results of the effect of blanching on ethanol content are reported in Table 25. The blanched samples stored in nitrogen and oxygen showed a decrease in ethanol content. The losses in both atmospheres were about equal. These results show that a 3-minute steam-blanch was sufficient to inactivate alcohol dehydrogenase. If dehydrogenase was not inactivated, there should have been an increase in ethanol in samples stored in nitrogen, since there was an accumulation of aldehyde in these samples.

Relationship Between Ethanol Content and Off-flavour Scores

From the results obtained, it is quite evident that there is no relationship between off-flavour formation and ethanol content. The unblanched samples stored in nitrogen were the only ones to show an increase in ethanol content. There is evidence that the accumulation of ethanol in samples stored in nitrogen was due to anaerobic conditions before freezing. Ethanol may have accumulated at the beginning of storage in the samples stored in oxygen, followed by a decrease throughout the storage period.

2-Thiobarbituric Acid

Tarladgis et al. (1960) used a 35-minute heating time in a boiling water bath to develop the colour of the TBA complex. A 35-minute heating time was tried on the cauliflower extract. It was found to give a good colour development and was used in series I. Before starting series II, a comparison of the colour development in extracts obtained from cauliflower stored for 11 years in oxygen and extracts from fresh cauliflower were made. The results are reported in Table 26. Acid was not used in making the determinations. From these results, it was decided to use a 40-minute heating time.

Standard curves were prepared from 1,1,3,3-TEP,

TABLE 26.--Determination of heating time for the TBA reaction. Acid not used in determinations

Heating time in minutes	(2-log G) Sample stored in oxygen for 11 years	(2-log G) Fresh raw sample	Difference (2-log G)
20	.198	.051	.147
25	.203	.058	.145
30	.258	.092	.166
35	.305	.117	.188
40	.357	.137	.220
45	.380	.163	.225
50	.419	.191	.228
55	.453	.217	.233

using both concentrations of acid employed in the preparation of the TBA reagent and without acid. The curves obtained when acid was used at either concentration were essentially the same. The slope of the curve, when acid was omitted, was slightly different from the slope of the curve when acid was used. Figure 6 shows the standard curve obtained when acid was used. Figure 7 shows the standard curve obtained when acid was omitted.

In series I, acid was used in the preparation of the TBA reagent. Since the acid content was quite high, it was decided to use less acid in series II. Because the use of acid in the TBA reagent was criticized by Tarladgis et al. (1962, 1964), it was decided to prepare the TBA reagent without acid in series II as well.

The reproducibility of the method for the extraction of the TBRS was studied, using unblanched cauliflower stored in oxygen for 11 years. The TBA numbers were determined without using acid. The results are reported in Table 27. They indicate that the ethanol extraction method developed gives satisfactory reproducible results.

TBA Experiments on Stored Cauliflower

In series I, analysis with the TBA reagent was carried out on cauliflower stored in nitrogen and oxygen

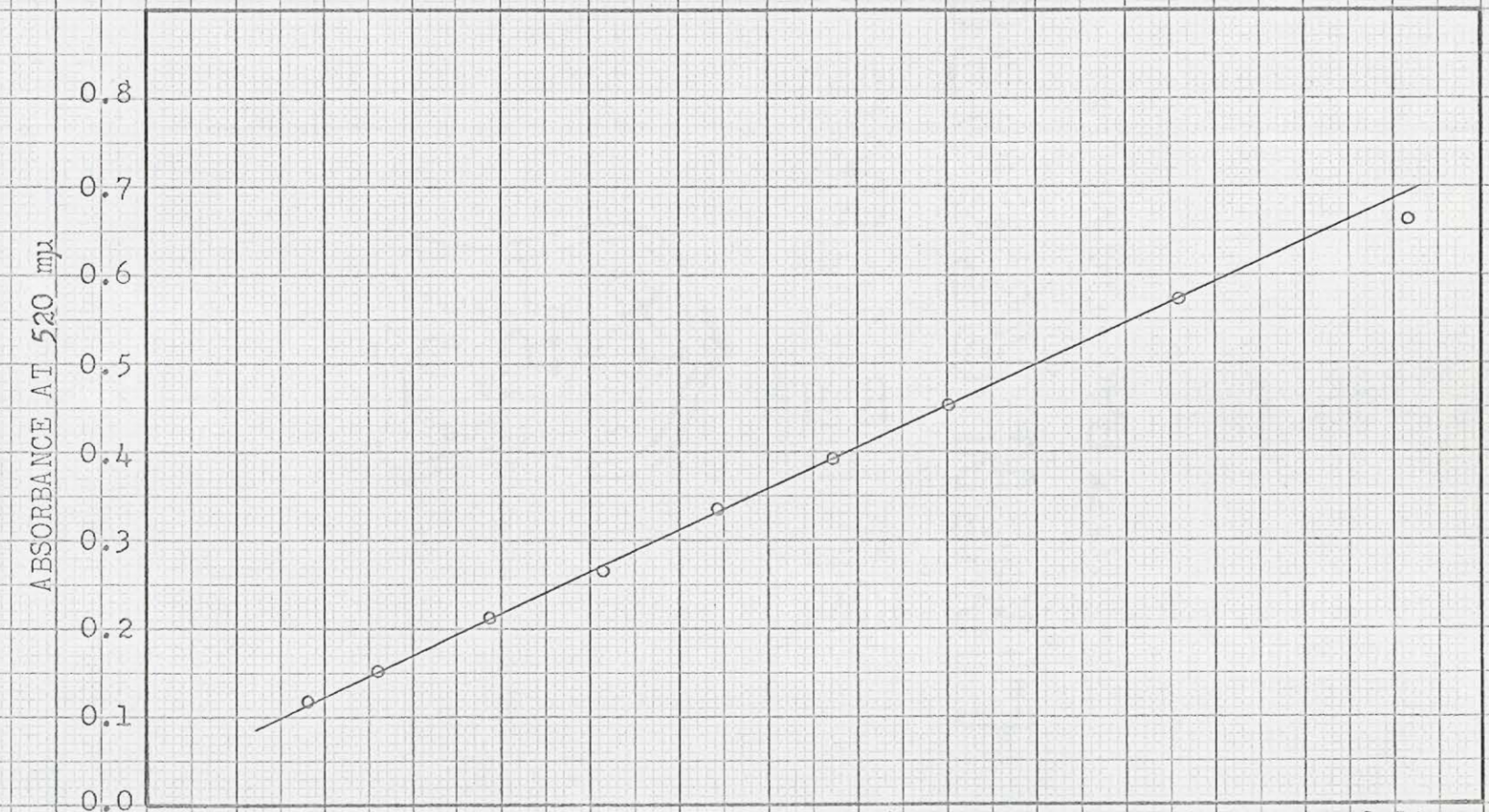


Fig. 6.--Standard curve for the determination of TBA reactive substances. Acid used in determinations.

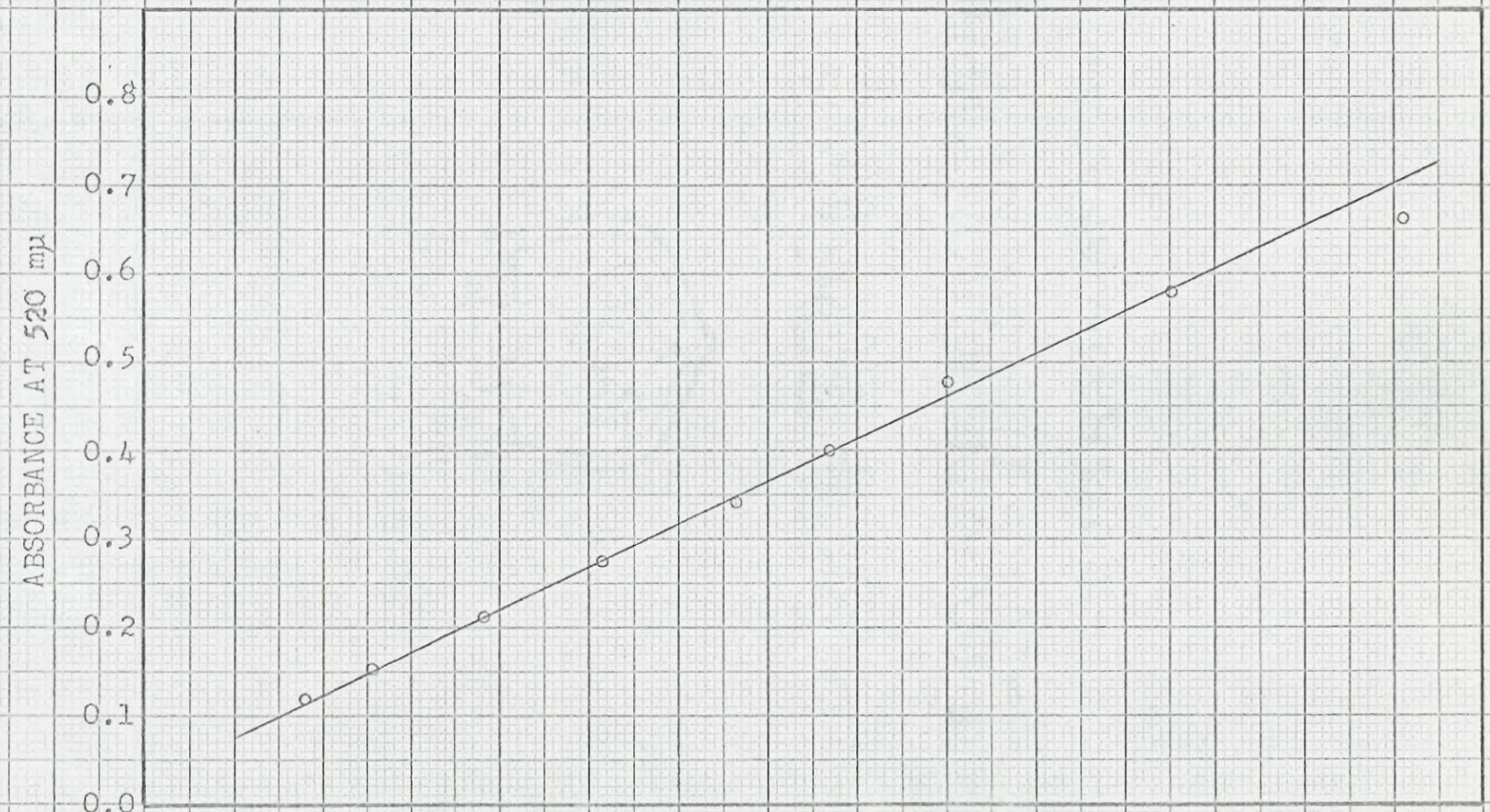


Fig. 7.--Standard curve for the determination of TBA reactive substances. Acid not used in determinations.

TABLE 27.--A comparison of results obtained with three different samples of unblanched cauliflower stored in oxygen for 11 years

Sample number	mg of TBRS
1	6.90
2	6.85
3	7.05

at -9.4, -15 and -20.6° C. Both unblanched and blanched cauliflower were used. However, the blanched cauliflower developed an off-flavour. The results are reported in Tables 28, 29, 30 and 31, and plotted in Figures 8, 9, 10 and 11.

In series II, samples were stored in air at -15° C, and in nitrogen and oxygen at the three storage temperatures used in series I. The results, using an acidified TBA reagent, are reported in Tables 32, 33, 34, 35 and 36. The results obtained for the unblanched material (Tables 32, 33 and 35) are plotted in Figures 12, 13 and 14. A comparison of air, nitrogen and oxygen at -15° C is found in Table 37 and plotted in Figure 15. The results, using a non-acidified TBA reagent, are found in Tables 38, 39, 40, 41 and 42. The results obtained for the unblanched material (Tables 38, 39 and 41) are plotted in Figures 16, 17 and 18. A comparison of air, nitrogen

TABLE 28.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in N_2. Letters indicate the flavour scores. Acid used in determinations, series I

Storage Temperature °C	Storage time in days							
	Initial	2	9	18	25	32	42	60
- 9.4	0.96 A	1.08 A	2.45 C	3.56 D	4.76 D	5.68 D	6.81 D	7.91 D
-15	0.96 A	-	2.32 B	3.45 D	4.10 D	4.81 D	5.91 D	7.21 D
-20.6	0.96 A	-	2.23 A	3.04 D	3.89 D	4.26 D	4.93 D	5.91 D

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

TABLE 29.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in N_2. Letters indicate the flavour scores. Acid used in determinations, series I

Storage Temperature °C	Storage time in days			
	Initial	16	42	60
- 9.4	1.05 A	2.63 B	4.78 D	5.87 D
-15	1.05 A	2.25 A	4.22 D	4.92 D
-20.6	1.05 A	2.05 A	3.98 D	4.64 D

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

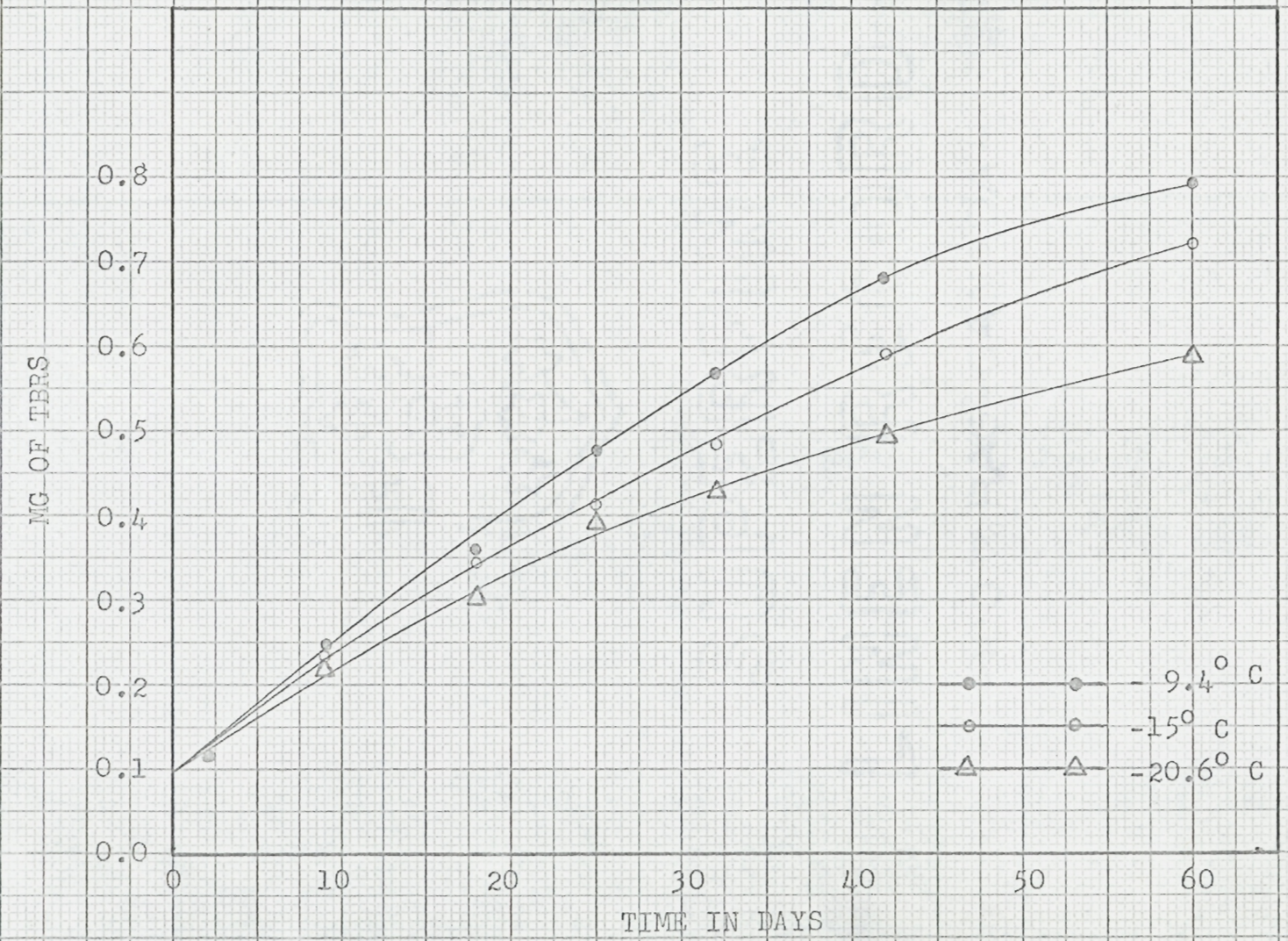


Fig. 8.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in N_2. Acid used in determinations, series I.

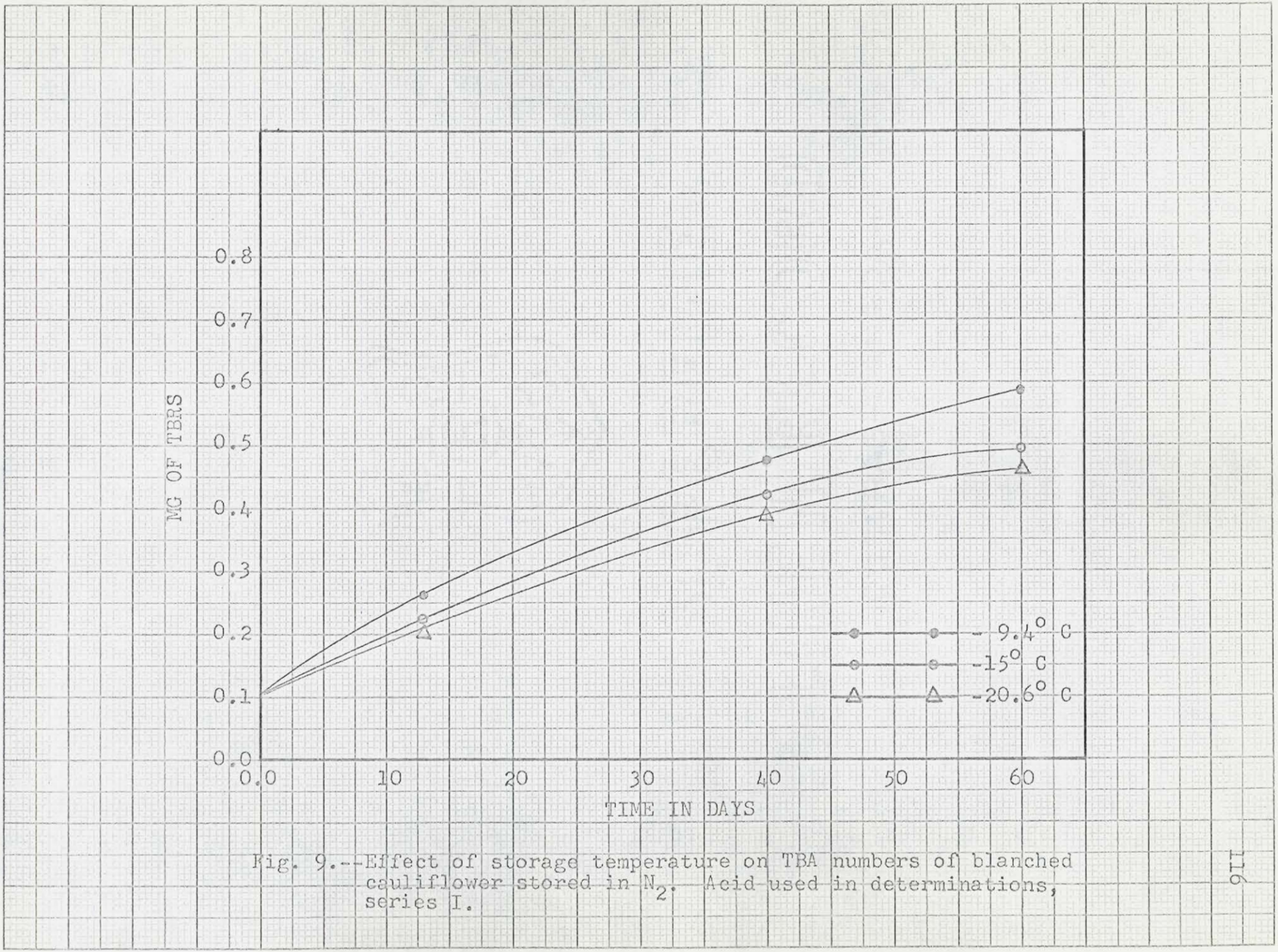


Fig. 9.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in N_2. Acid used in determinations, series I.

TABLE 30.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in O_2. Letters indicate the flavour scores. Acid used in determinations, series I

Storage temperature °C	Storage time in days							
	Initial	2	9	18	25	32	42	60
- 9.4	0.96 A	1.93 A	4.36 D	5.52 D	6.68 D	7.67 D	8.57 D	9.51 D
-15	0.96 A	-	3.79 C	4.88 D	5.93 D	7.00 D	7.98 D	9.07 D
-20.6	0.96 A	-	3.16 B	4.30 D	5.13 D	6.35 D	7.17 D	8.50 D

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

TABLE 31.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in O_2. Letters indicate the flavour scores. Acid used in determinations, series I

Storage temperature °C	Storage time in days			
	Initial	16	42	60
- 9.4	1.05 A	3.08 B	6.41 D	7.91 D
-15	1.05 A	2.61 A	5.51 D	7.17 D
-20.6	1.05 A	2.37 A	4.85 D	6.29 D

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

Fig. 10.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in O_2. Acid used in determinations, series I.

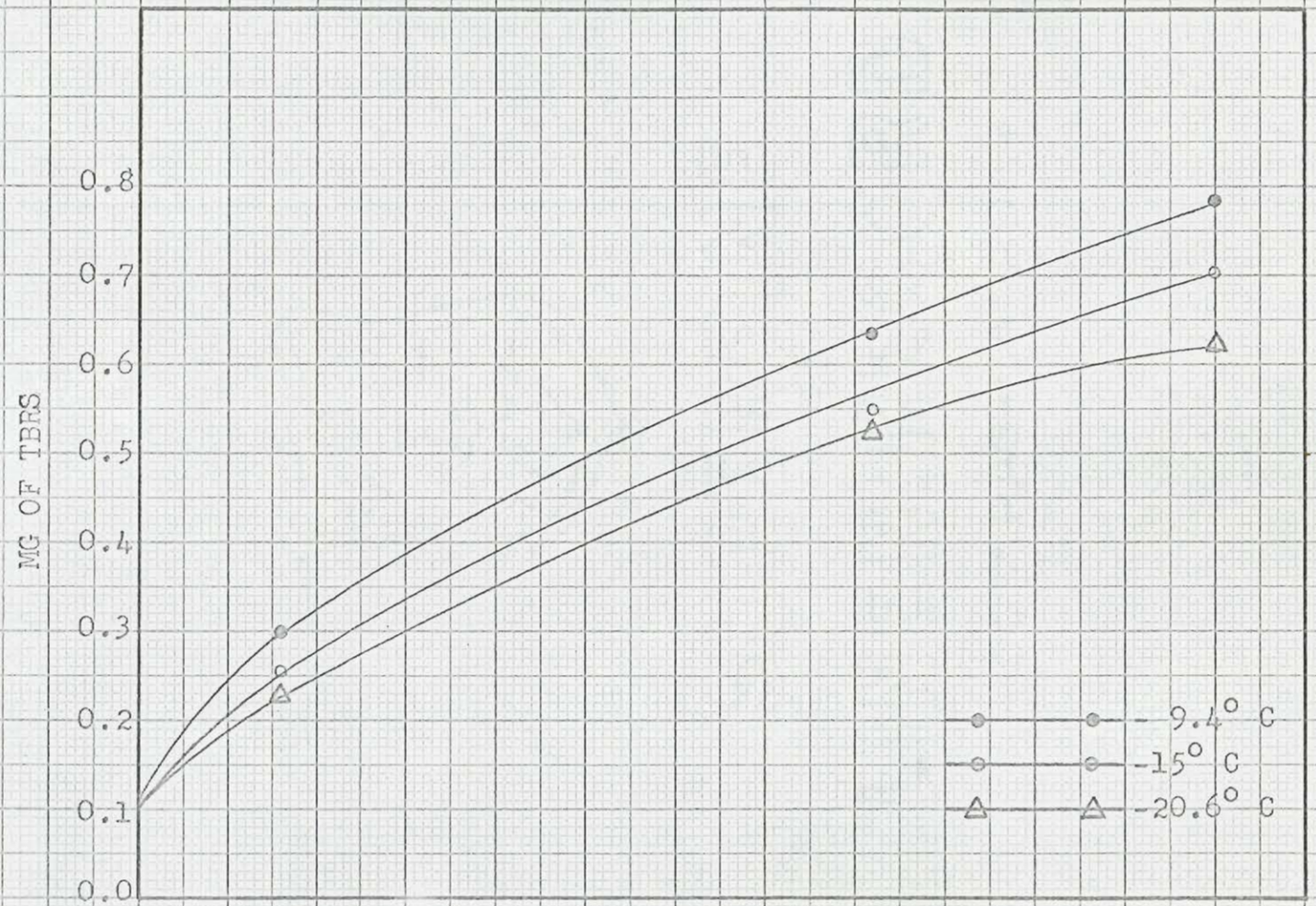


Fig. 11.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in O_2. Acid used in determinations, series I.

TABLE 32.--TBA numbers of cauliflower stored in air at -15° C. Letters indicate the flavour scores. Acid used in determinations, series II

Treatment	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
Unblanched	1.72 A	3.23 B	3.46 C	3.58 D	3.64 D	3.66 D	3.96 D	3.97 D	-	4.39 D
Blanched	2.25 A	1.98 A	-	1.93 A	-	2.07 A	1.97 A	2.14 A	-	-

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

Fig. 12.--TBA numbers of cauliflower stored in air at -15° C. Acid used in determinations, series II.

TABLE 33.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in N_2. Letters indicate the flavour scores. Acid used in the determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	1.72 A	1.85 B	2.12 C	2.03 D	2.04 D	2.03 D	2.17 D	2.10 D	2.26 D	2.70 D
-15	1.72 A	1.59 A	1.62 B	1.76 B	1.80 B	1.79 C	1.82 D	1.96 D	2.09 D	2.36 D
-20.6	1.72 A	1.54 A	1.59 A	1.73 A	1.77 B	1.79 B	1.81 B	1.80 C	1.86 C	1.90 C

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

TABLE 34.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in N_2. Letters indicate the flavour scores. Acid used in determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	2.25 A	1.85 A	-	2.01 A	-	1.93 A	2.04 A	2.04 A	1.96 A	2.38 A
-15	2.25 A	1.79 A	-	1.76 A	-	1.78 A	2.12 A	2.07 A	1.82 A	2.23 A
-20.6	2.25 A	2.00 A	-	1.99 A	-	1.79 A	1.84 A	2.05 A	1.92 A	2.21 A

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

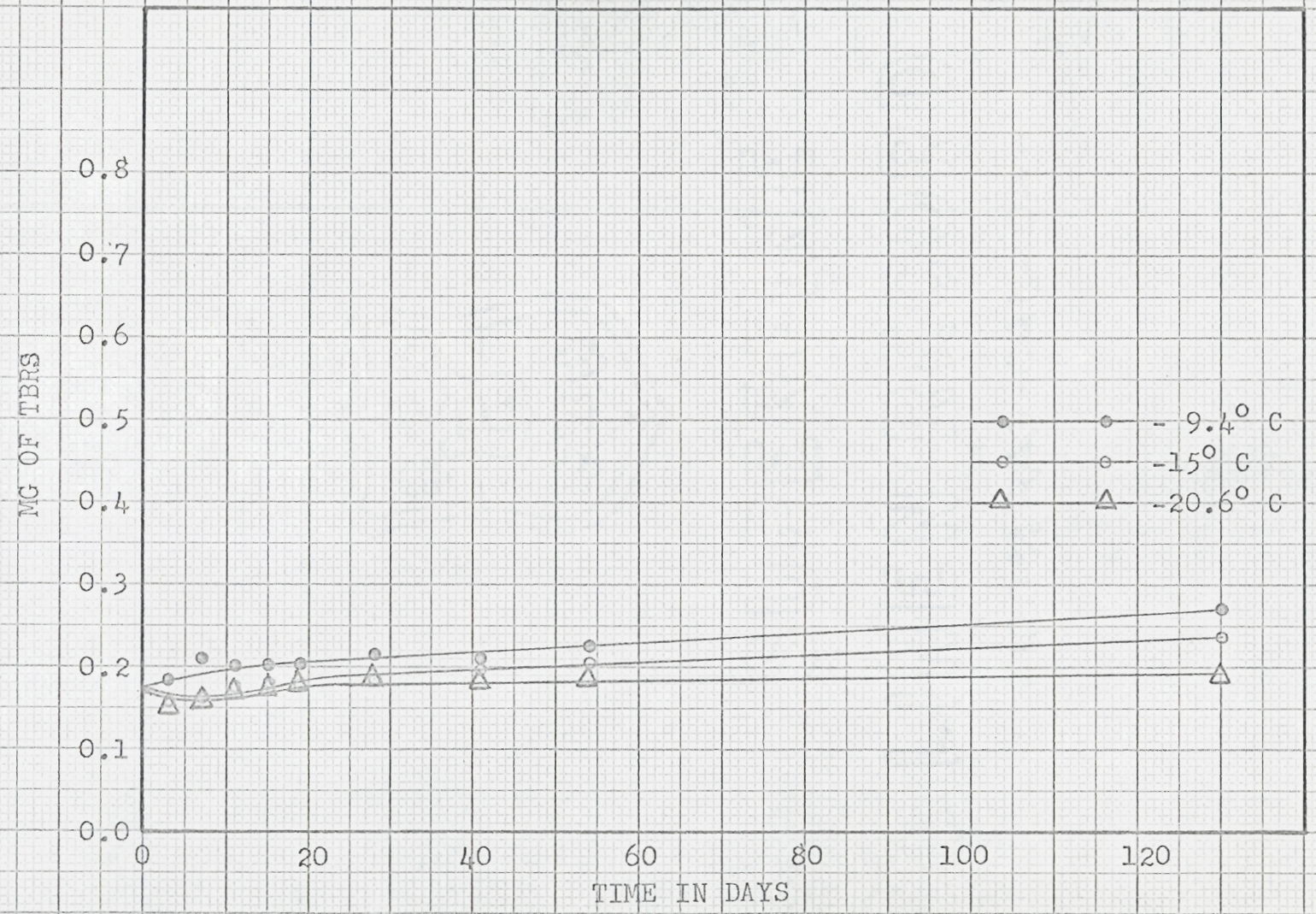


Fig. 13.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in N_2. Acid used in determinations, series II.

TABLE 35.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in O_2. Letters indicate the flavour scores. Acid used in the determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	1.72 A	4.19 D	4.65 D	5.13 D	5.21 D	5.54 D	5.56 D	5.81 D	6.04 D	6.38 D
-15	1.72 A	3.11 B	4.07 C	4.11 D	4.31 D	4.37 D	4.93 D	5.08 D	5.16 D	5.37 D
-20.6	1.72 A	2.96 A	3.19 A	3.29 A	3.25 A	3.35 B	3.37 B	3.56 C	3.65 C	3.83 C

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

TABLE 36.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in O_2. Letters indicate the flavour scores. Acid used in determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	2.25 A	1.94 A	-	2.00 A	-	1.74 A	1.95 A	1.92 A	2.14 A	2.26 A
-15	2.25 A	2.01 A	-	1.89 A	-	2.00 A	1.71 A	1.78 A	1.97 A	2.01 A
-20.6	2.25 A	1.84 A	-	2.00 A	-	1.86 A	1.83 A	1.86 A	1.78 A	1.78 A

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

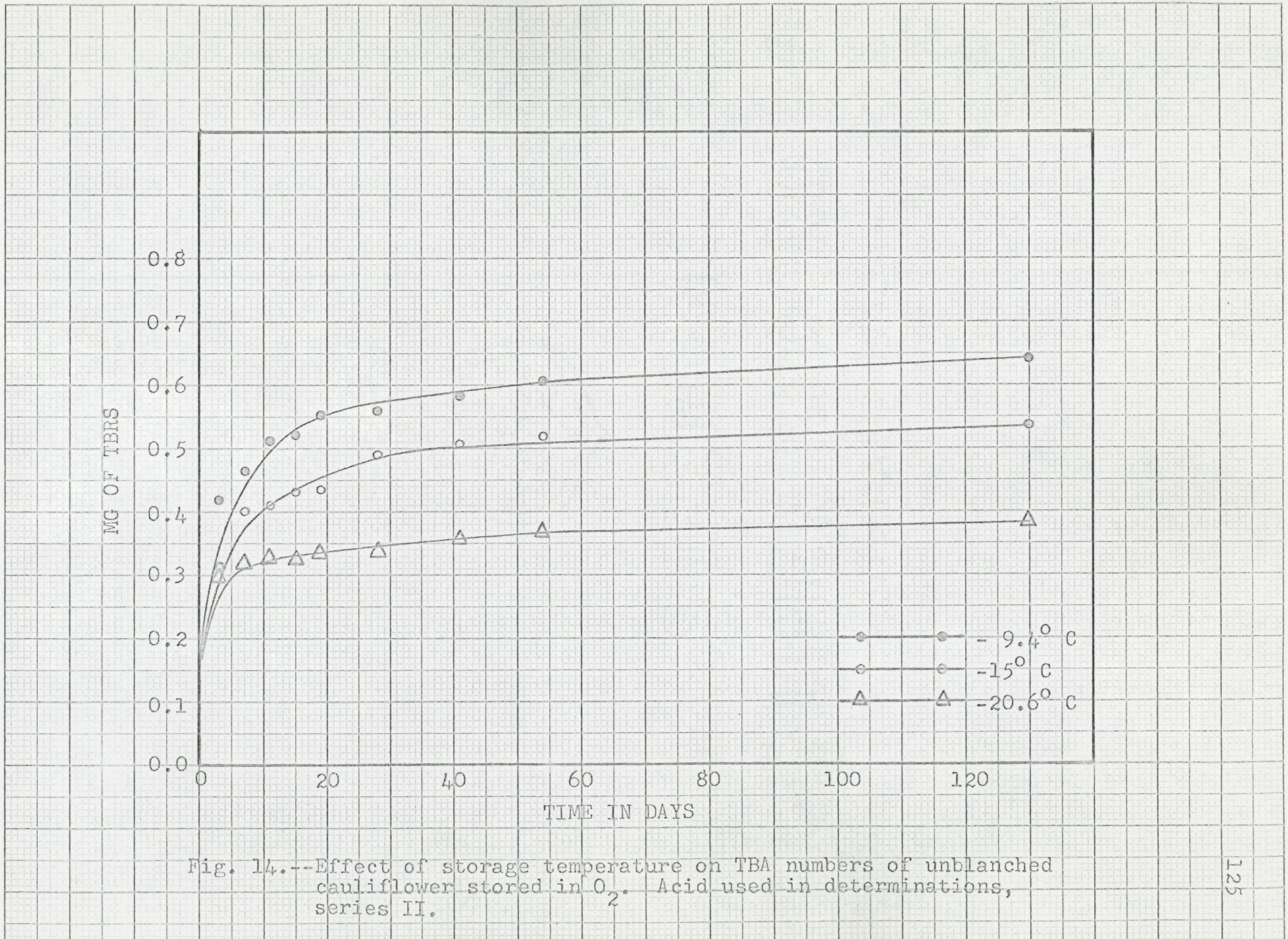


Fig. 14.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in O_2. Acid used in determinations, series II.

TABLE 37.--Comparison of TBA numbers of unblanched cauliflower stored in air, N_2 and O_2 at 15° C. Letters indicate the flavour scores. Acid used in determinations, series II

Atmosphere	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
Air	1.72 A	3.23 B	3.46 C	3.58 D	3.64 D	3.66 D	3.96 D	3.97 D	-	4.39 D
N_2	1.72 A	1.59 A	1.62 B	1.76 B	1.80 B	1.79 C	1.82 D	1.96 D	2.09 D	2.36 D
O_2	1.72 A	3.11 B	4.07 C	4.11 D	4.31 D	4.37 D	4.93 D	5.08 D	5.16 D	5.37 D

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

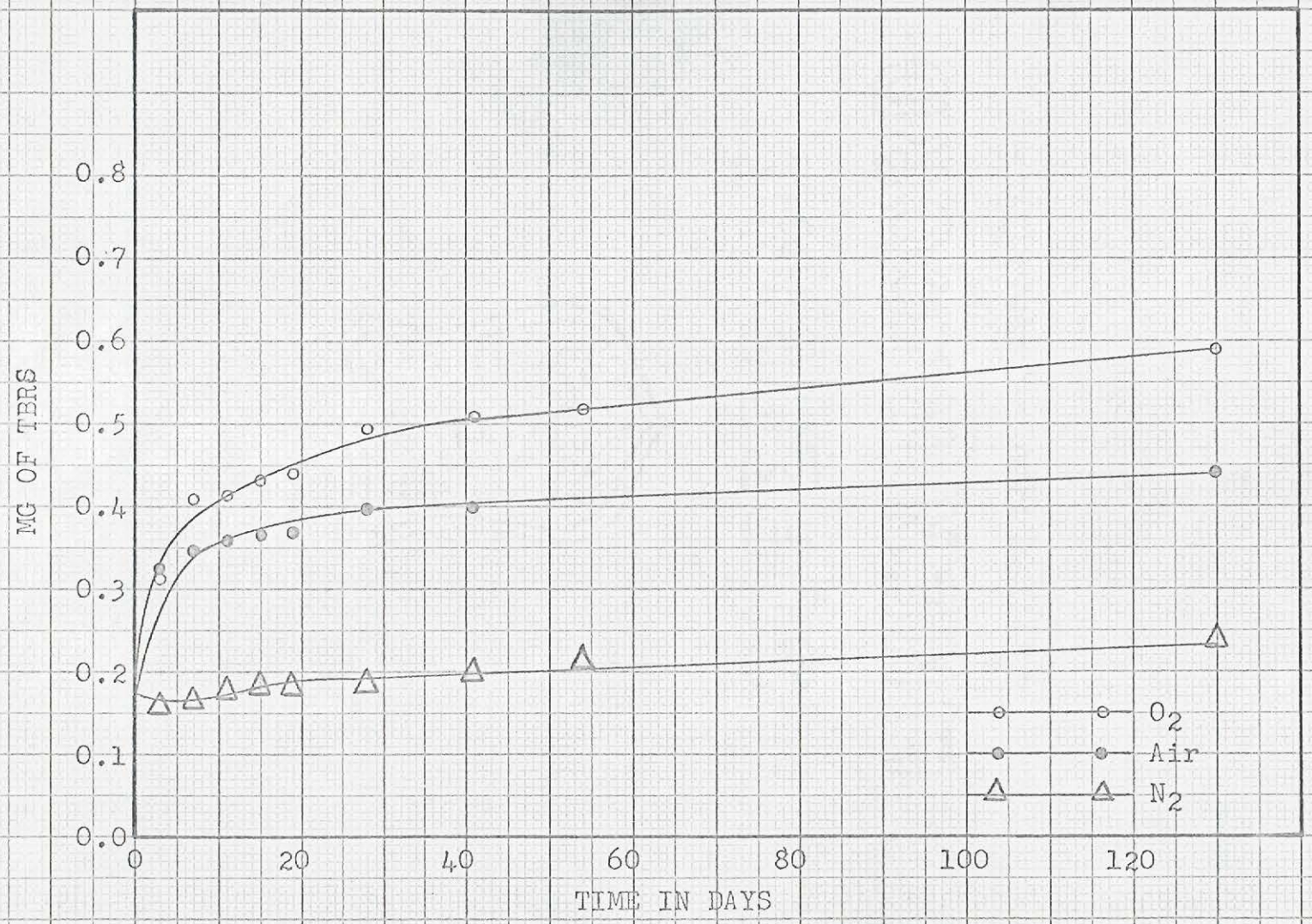


Fig. 15.--Comparison of TBA numbers of unblanched cauliflower stored in air, N_2 and O_2 at -15° C. Acid used in determinations, series II.

TABLE 38.--TBA numbers of cauliflower stored in air at -15° C. Letters indicate the flavour scores. Acid not used in determinations, series II

Treatment	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
Unblanched	1.09 A	2.44 B	2.46 C	2.56 D	2.57 D	2.77 D	2.84 D	3.20 D	-	3.33 D
Blanched	1.53 A	1.50 A	-	1.49 A	-	1.32 A	1.41 A	1.49 A	-	-

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

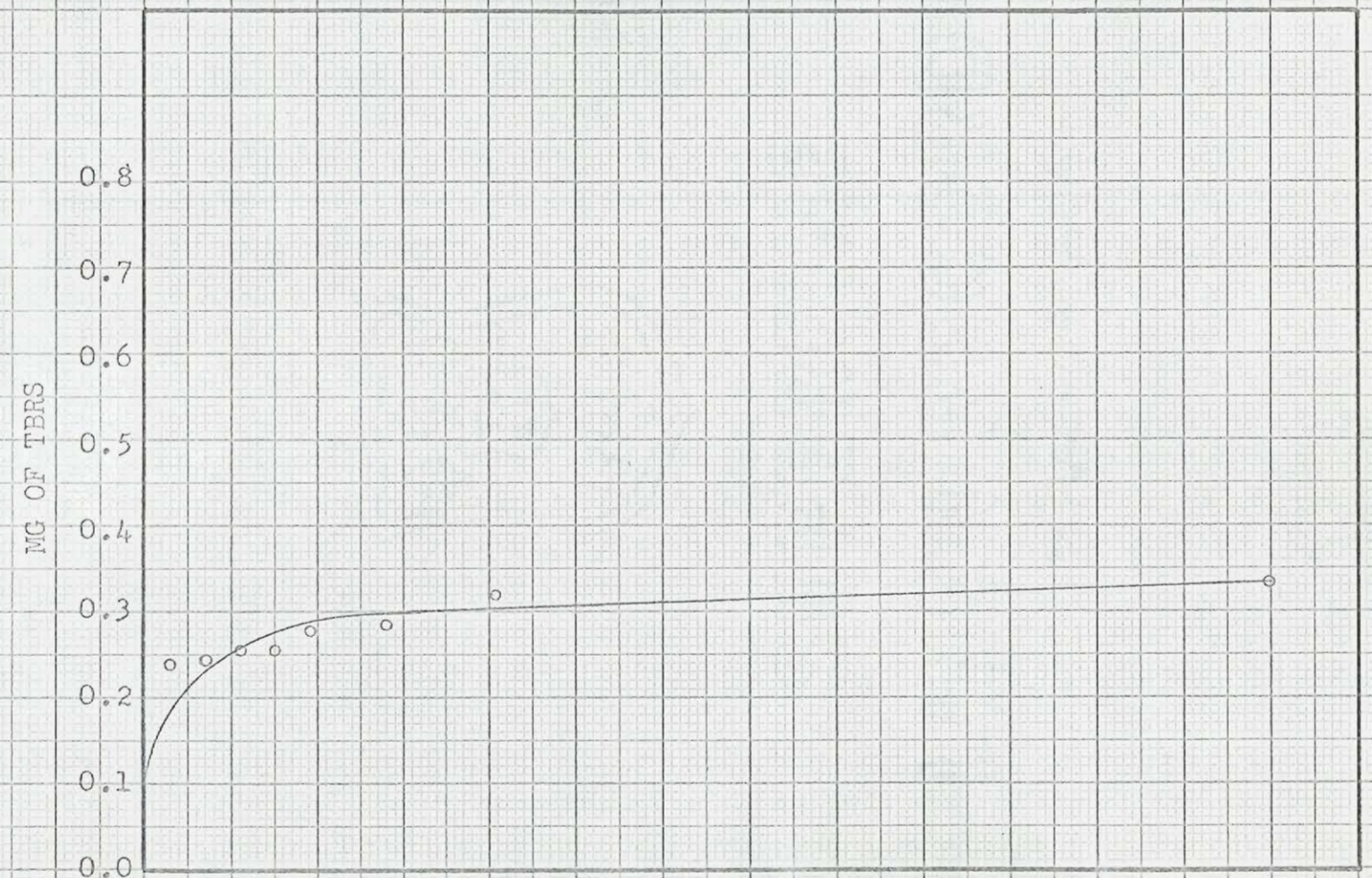


Fig. 16.--TBA numbers of cauliflower stored in air at -15° C. Acid not used in determinations, series II.

TABLE 39.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in N_2. Letters indicate the flavour scores. Acid not used in determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	1.09 A	1.22 B	1.41 C	1.43 D	1.43 D	1.49 D	1.47 D	1.51 D	1.65 D	2.10 D
-15	1.09 A	1.22 A	1.26 B	1.24 B	1.38 B	1.37 C	1.38 D	1.39 D	1.41 D	1.63 D
- 20.6	1.09 A	1.08 A	1.16 A	1.14 A	1.21 B	1.25 B	1.26 B	1.36 C	1.33 C	1.43 C

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

TABLE 40.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in N_2. Letters indicate the flavour scores. Acid not used in determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	1.53 A	1.27 A	-	1.37 A	-	1.42 A	1.30 A	1.22 A	1.22 A	1.61 A
-15	1.53 A	1.48 A	-	1.31 A	-	1.39 A	1.25 A	1.29 A	1.32 A	1.34 A
-20.6	1.53 A	1.26 A	-	1.42 A	-	1.45 A	1.21 A	1.38 A	1.26 A	1.20 A

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

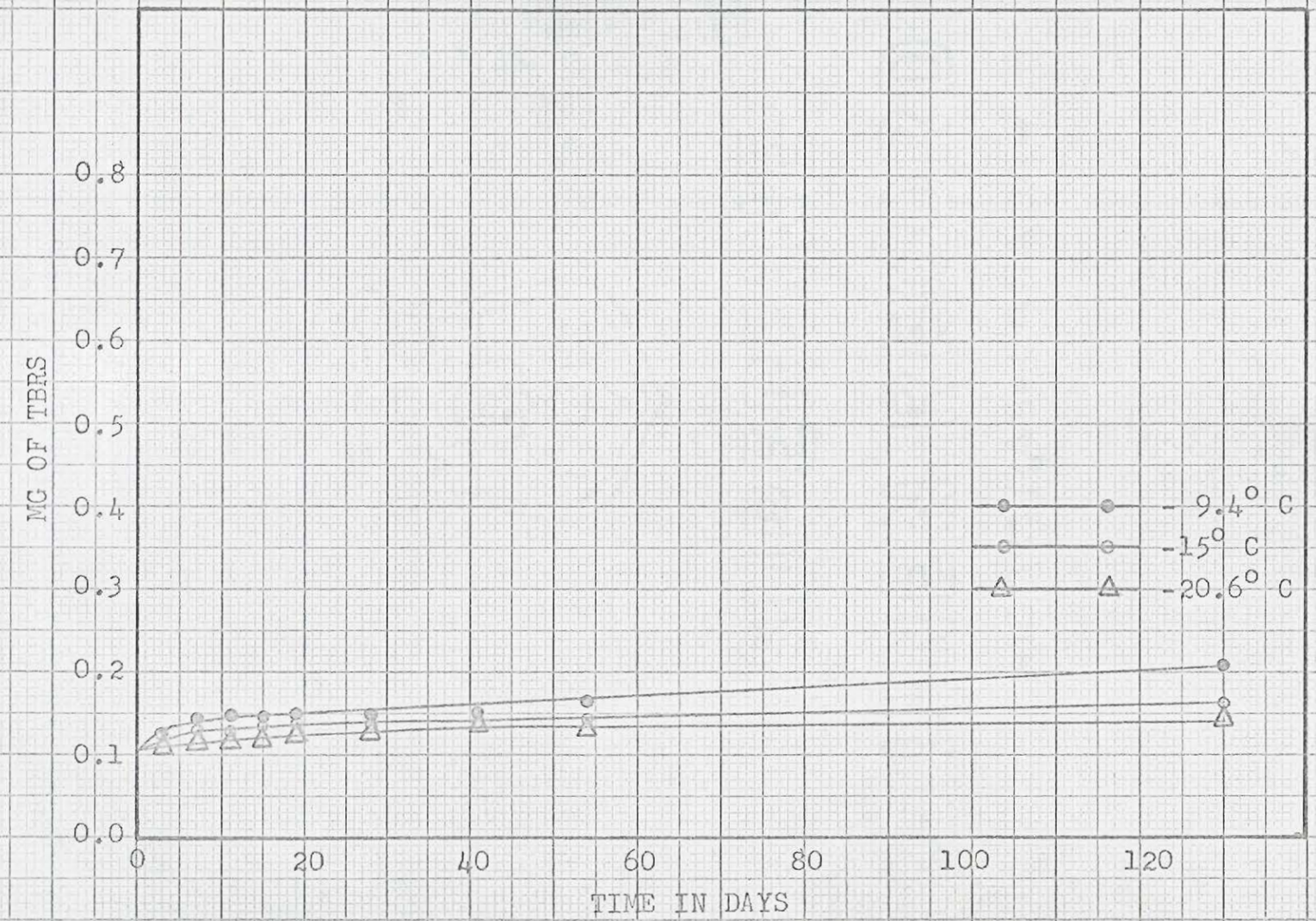


Fig. 17.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in N_2. Acid not used in determinations, series II.

TABLE 41.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in O_2. Letters indicate the flavour scores. Acid not used in determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	1.09 A	3.29 D	3.73 D	3.75 D	3.98 D	4.50 D	4.80 D	5.34 D	5.95 D	6.69 D
-15	1.09 A	2.77 B	3.28 C	3.35 D	3.56 D	4.03 D	4.33 D	4.60 D	4.95 D	5.47 D
-20.6	1.09 A	2.09 A	2.37 A	2.36 A	2.38 A	2.44 A	2.59 B	2.83 B	2.85 B	3.31 C

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

TABLE 42.--Effect of storage temperature on TBA numbers of blanched cauliflower stored in O_2. Letters indicate the flavour scores. Acid not used in determinations, series II

Storage temp. °C	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
- 9.4	1.53 A	1.42 A	-	1.28 A	-	1.22 A	1.28 A	1.23 A	1.54 A	1.70 A
- 15	1.53 A	1.44 A	-	1.50 A	-	1.26 A	1.40 A	1.24 A	1.31 A	1.35 A
-20.6	1.53 A	1.19 A	-	1.14 A	-	1.22 A	1.09 A	1.17 A	1.05 A	1.14 A

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

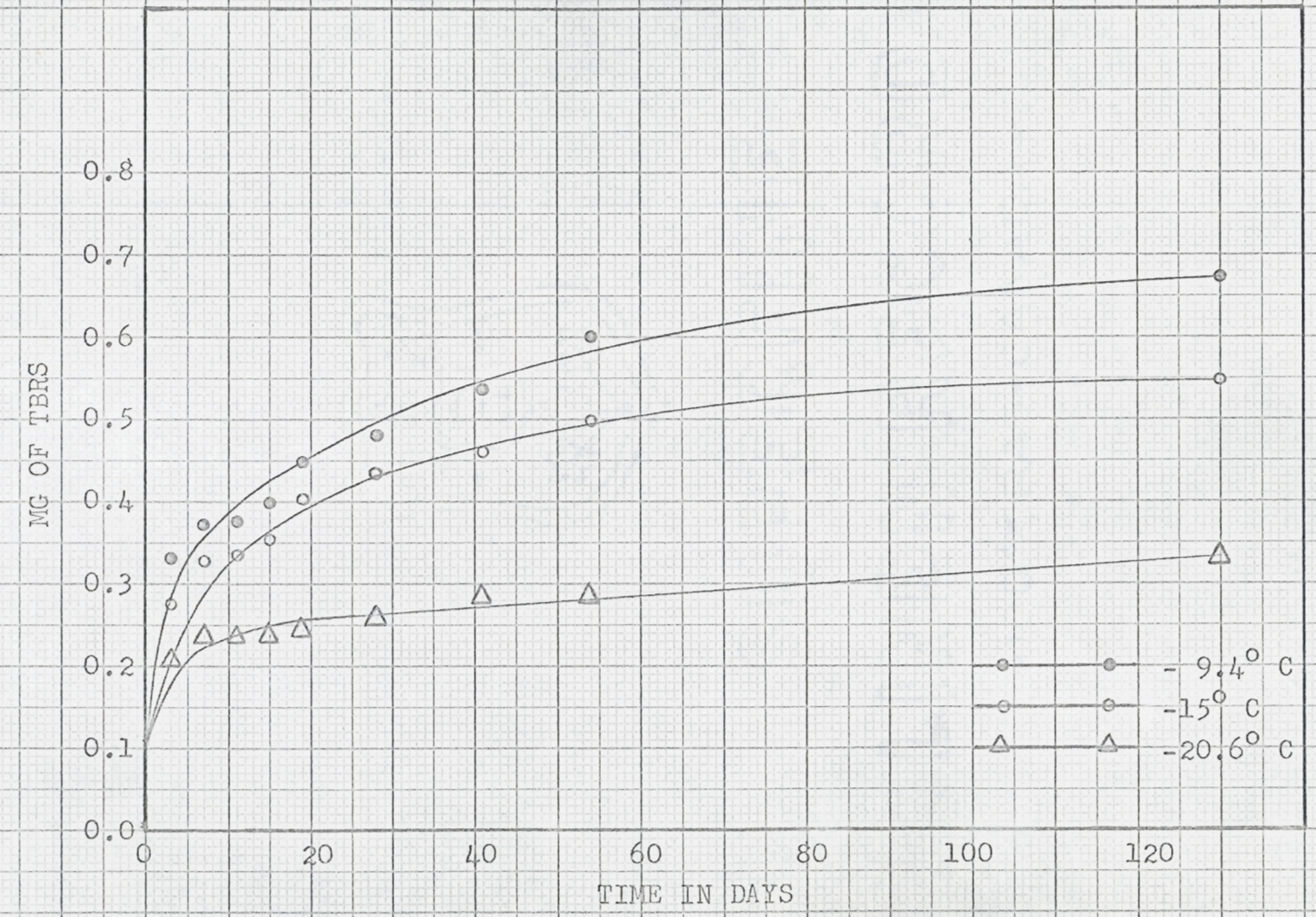


Fig. 18.--Effect of storage temperature on TBA numbers of unblanched cauliflower stored in O_2. Acid not used in determinations, series II.

TABLE 43.--Comparison of TBA numbers of unblanched cauliflower stored in air, N_2 and O_2 at -15 C. Letters indicate the flavour scores. Acid not used in determinations, series II

Atmosphere	Storage time in days									
	Initial	3	7	11	15	19	28	41	54	130
Air	1.09 A	2.44 B	2.46 C	2.56 D	2.57 D	2.77 D	2.84 D	3.20 D	-	3.33 D
N_2	1.09 A	1.22 A	1.26 B	1.24 B	1.38 B	1.37 C	1.38 D	1.39 D	1.41 D	1.63 D
O_2	1.09 A	2.77 B	3.28 C	3.35 D	3.56 D	4.03 D	4.33 D	4.60 D	4.95 D	5.47 D

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

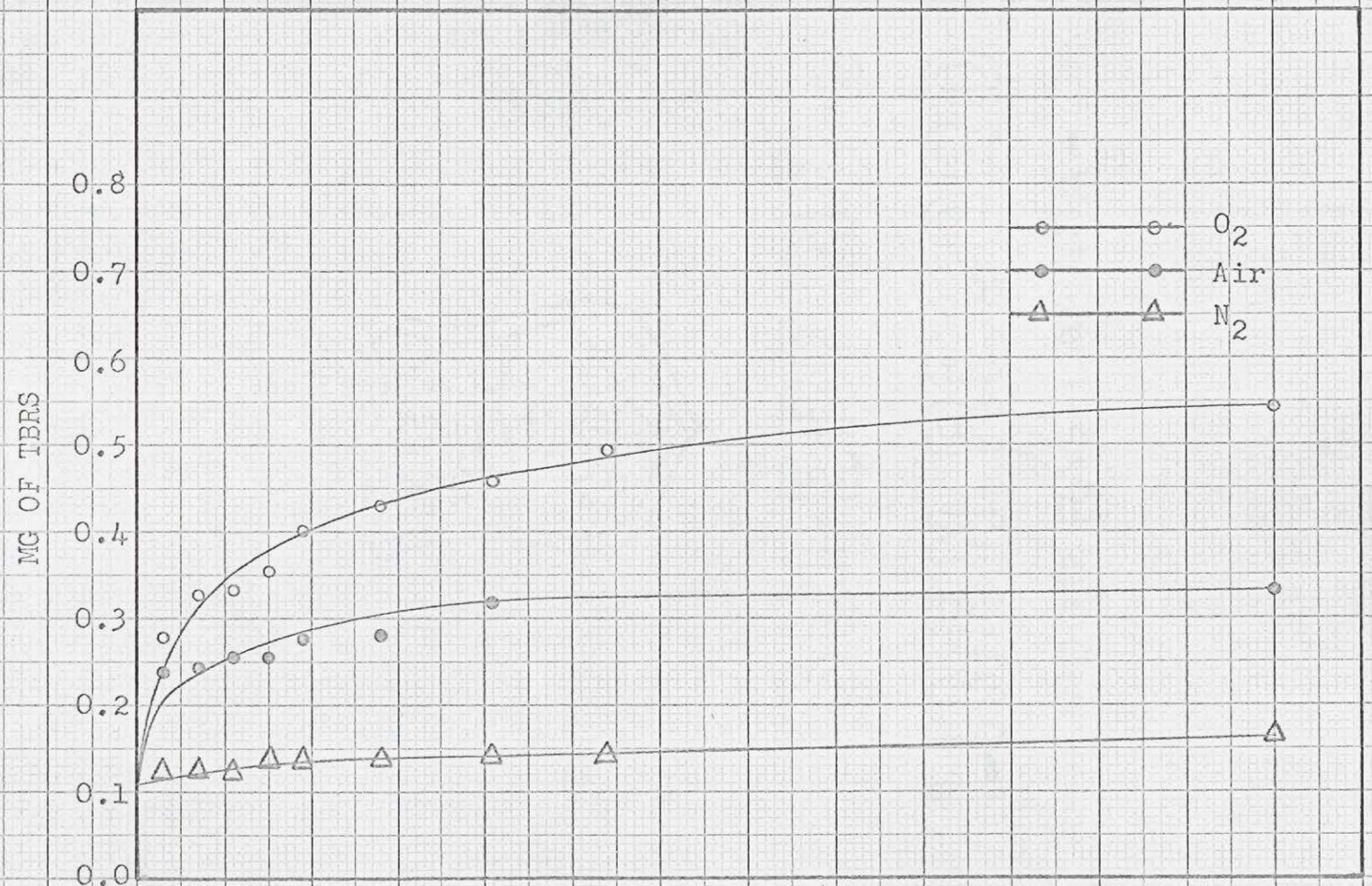


Fig. 19.--Comparison of TBA numbers of unblanched cauliflower stored in air, N_2 and O_2 at -15° C. Acid not used in determinations, series II.

and oxygen at -15° C is found in Table 43. The results are plotted in Figure 19.

Effect of Temperature on the Production of TBRS

In all cases, the higher the storage temperature, the greater the amount of TBRS produced in cauliflower. In the first series more TBRS was produced than in the second, in samples given the same storage treatment. The differences found between series I and II were believed to be due, in part at least, to the higher acidity of the TBA reagent in series I and also to differences in cauliflower, obtained from different sources.

Effect of Atmosphere on the Production of TBRS

The atmospheres used in storage had a pronounced effect on the production of TBRS. This effect was not as great in series I, in which the TBA reagent was very acid. In series II, in which the reagent was not as acid or not acidified at all, the effect of the atmospheres was more evident. In series I, more TBRS was produced in the unblanched samples stored in oxygen than in the samples stored in nitrogen. Off-flavour developed in the blanched samples in series I, and, as in the case of the unblanched material, more TBRS was produced in samples stored in oxygen. The amount produced in the blanched material stored in oxygen was comparable to the amount

produced in unblanched samples stored in nitrogen. In series II, the unblanched samples stored in oxygen showed the highest accumulation of TBRS, while the smallest amount of TBRS was formed in the unblanched samples stored in nitrogen. The material stored in air at -15° C produced an amount that was intermediate between samples stored in nitrogen and oxygen at that temperature probably because of the lower oxygen content of air. In this series, the blanched material, stored in either nitrogen, oxygen, or in air at -15° C, showed only slight fluctuations in TBA numbers which could be attributed to biological variations.

Effect of Blanching on the Production of TBRS

In series I, the material was steam-blanched for 3 minutes. The blanch was not sufficiently long to inactivate the enzymes responsible for off-flavour development. In series II, samples which were steam-blanched for 4 minutes did not develop off-flavour.

Because the material was underblanched in series I, the TBA number of the samples increased throughout the storage period. The greatest increase occurred in the samples stored under oxygen. In series II, it was found that very little change occurred in the TBA numbers of the blanched samples throughout the storage period. In

the initial determinations, blanched samples gave higher TBA numbers than the unblanched. This is possibly due to the fact that the heat treatment destroys the plant tissues and that some TBRS is more readily extracted.

Effect of the Use of Acid in the Preparation of the TBA Reagent

Tarladgis et al. (1962) found that acid caused a degradation of TBA and that some of the products absorbed at 530 mμ. When an acid reagent was used in experiments on cauliflower, any absorbance due to the degradation of TBA should have been cancelled out because the reagent blank and samples were treated similarly. Therefore, the differences observed in the TBA numbers of identical samples analyzed with acid and non-acid reagents should not be due to an acid degradation of TBA.

The differences in the TBA numbers between identical samples, determined with acid and non-acid reagents, showed a similarity under all storage conditions. They were consistently higher for the acid reagent and ranged from .4 to 1. This range in values could be due to experimental error since small differences in the spectrophotometer readings may become much larger because of the multiplication factor involved in changing these readings to TBA numbers.

The differences in TBA numbers observed in similar

samples, when different reagents were used, may have been caused by the condensation of a compound or compounds with TBA in the presence of acid, a reaction which would not take place in its absence. Their concentration does not appear to be affected by freezing storage or blanching since the difference does not seem to change except in samples where further dilutions were necessary to obtain readings on the spectrophotometer. In these samples, the extra dilutions may have affected the readings. This hypothesis would be in agreement with the findings of Tarladgis et al. (1960) that acid was necessary for the liberation of TBRS from food products during distillation, the maximum recovery being obtained at pH 1.5. The difference in TBA numbers obtained may have resulted from the liberation of TBRS from some complex in the extract in the same way, but the quantities liberated were constant. It would therefore appear that the use of acid in the preparation of the TBA reagent is not essential.

Tarladgis et al. (1964) stated further, that heating without acid accelerates the condensation of TBA with MA without affecting the maximum colour development to any extent. They also indicated, on the other hand, that the acid-heat treatment affected the absorbance value without accelerating the condensation reaction any more than heat alone. The results obtained with cauliflower

extracts would appear to indicate that acid favoured the condensation of TBRS with TBA. The standard curves prepared with 1,1,3,3-TEP (Figures 6 and 7) indicate there is a higher condensation of MA with TBA in the absence of acid, the effect being greater at higher concentrations of MA. This is in agreement with the findings of Tarladgis et al. (1964), but contrary to the results obtained with cauliflower extracts where the use of acid gave consistently higher TBA numbers.

Relationship Between Flavour Scores and TBA Numbers

As mentioned previously, the compound or compounds responsible for off-flavour development in cauliflower stored in nitrogen and oxygen seemed to be quite different organoleptically. The material stored in air seems to develop the same taste characteristics as the material stored in oxygen.

In the unblanched and blanched samples stored in nitrogen in series I, the following relationships were found between organoleptic scores and TBA numbers: flavour scores of "good" were given to samples having TBA numbers up to 2.25; "slightly off" to two samples, 2.32 and 2.63; "off" to one sample, 2.45; and "very off" to samples with TBA numbers of 2.45 and higher. In series II, very little change occurred in samples stored

in nitrogen. The following relationship between flavour scores and TBA numbers, obtained with acid TBA reagent was found: "good" up to 1.73; "slightly off" from 1.62 to 1.85; "off" from 1.79 to 2.12; and "very off" from 1.82 and up. These results indicate that if the compound or compounds responsible for the development of off-flavour in samples stored in nitrogen are TBA-reactive, then they must be extremely strong tasting. No off-flavour developed in the blanched samples in series II.

When acid was not used in the reagent, the following relationship between off-flavour development and TBA numbers was established in cauliflower stored in nitrogen: "good" up to 1.22; "slightly off" from 1.21 to 1.38; "off" from 1.36 to 1.43; and "very off" from 1.38 and up. The blanched samples showed only slight variations in TBA numbers with no off-flavour development.

In series I and II, a relationship seemed to exist between flavour scores and TBA numbers in samples stored in oxygen. In the unblanched and blanched samples of both series, the following relationships were noted in the flavour scores: when an acid reagent was used, "good" ranged up to 3.29, "slightly off" from 3.08 to 3.37; "off" from 3.56 to 4.07; and "very off" from 4.11 and up. The difference in TBA numbers between samples which are "good" and samples which are "very off" is small. This indicates

that the compound or compounds responsible for off-flavour, if they are TBA-reactive, are probably quite strong tasting and are readily detected organoleptically above a certain taste threshold. The underblanched samples stored in oxygen in series I were also within this range of values. The blanched samples in series II did not develop off-flavours and the TBA numbers varied only slightly.

In series II, when the non-acid reagent was used, cauliflower stored in oxygen gave the following results: "good" up to 2.44; "slightly off" from 2.59 to 2.83; "off" from 3.28 to 3.31; and "very off" from 3.29 and up. The blanched samples showed only slight variations in TBA numbers. Off-flavours did not develop.

The samples stored in air in series II were closely related to the samples stored in oxygen with small variations. When acid TBA was used, the results were within the limit of those found under oxygen storage. When acid was not used, the results were slightly different. The difference could be due to experimental error.

A different mechanism seems to be involved in the development of off-flavour in cauliflower stored in nitrogen and oxygen as indicated by flavour scores and TBA numbers. In both cases, however, enzymic activity appears to be involved, since adequate blanching prevents

off-flavour development and the formation of TBRS. In the samples stored in nitrogen, off-flavour formation likely results from the action of hydrolytic enzymes and anaerobic respiration. In the samples stored in oxygen or air, oxidative enzymes such as lipoxidase probably contribute a great deal more to off-flavour development.

It should be noted that in the unblanched material of series I, the results obtained with TBA are in agreement with the aldehyde content found in the steam distillates. The TBA tests indicated that samples stored in oxygen accumulated a larger quantity of aldehydes than samples stored in nitrogen. However, aldehyde content in the blanched samples which developed off-flavours did not increase materially while the TBA numbers showed appreciable increases and were in agreement with the organoleptic scores and TBA numbers of unblanched cauliflower.

A commercial sample of frozen cauliflower was tested with the TBA reagent. TBA numbers of .85 with the non-acid reagent and 1.50 with the acid reagent were obtained. These values are lower than those obtained for adequately blanched cauliflower in this investigation and are well within the range of TBA numbers associated with good quality.

The results indicate that a relationship appears

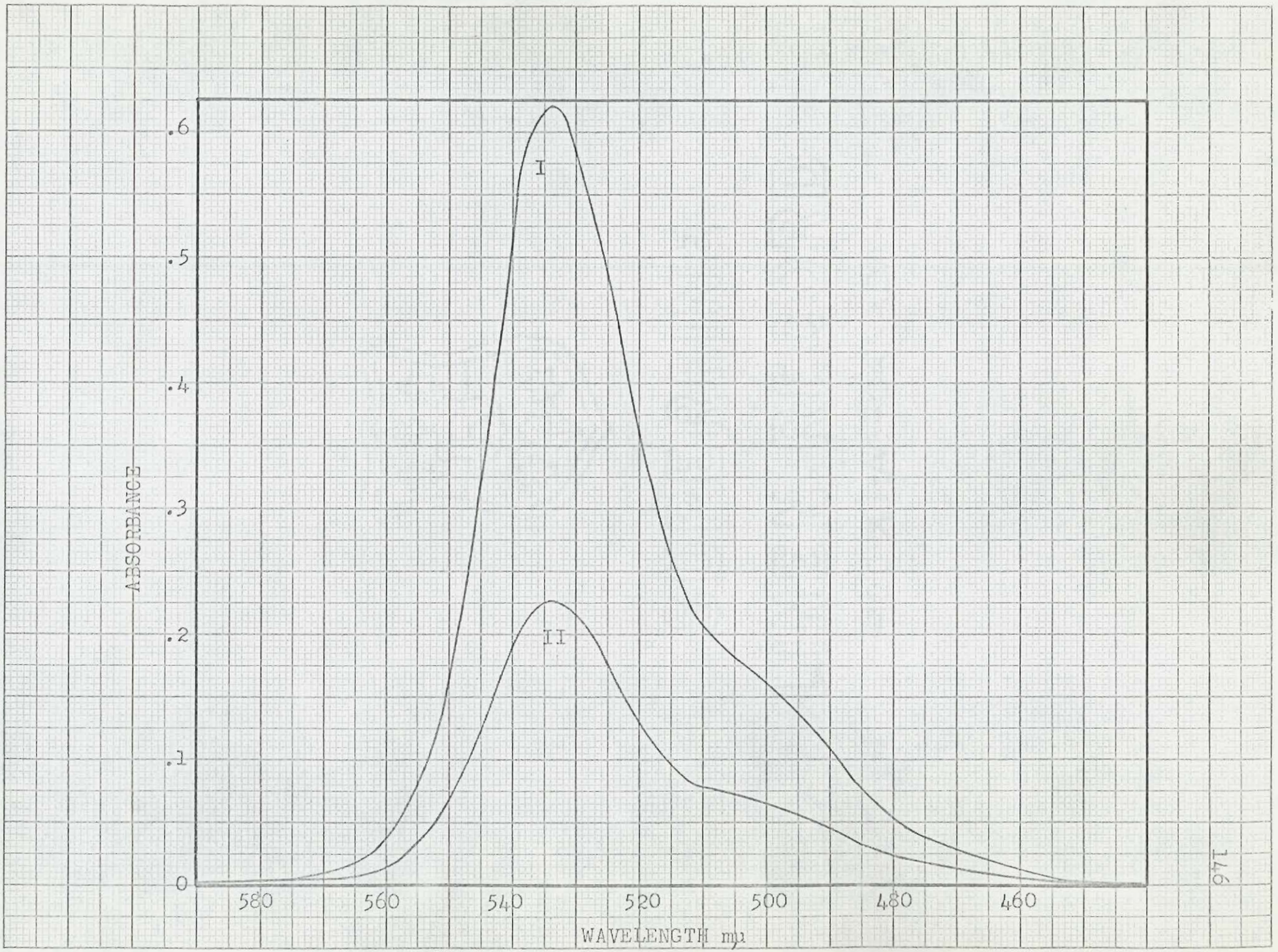
to exist between off-flavour development and TBA numbers and that the TBA reagent could be used to detect the development of off-flavours in frozen cauliflower.

Characteristics of TBRS

Several attempts were made to identify TBRS. A melting point determination was made on the purified TBA-TBRS pigment from cauliflower. No definite melting point could be observed up to 280° C. Pigment prepared from hydrolyzed 1,1,3,3-TEP did not exhibit a melting point up to 280° C either. The crystals from the TBA complex from 1,1,3,3-TEP were purple-black in colour, while those prepared from cauliflower were a reddish purple-black. The compounds were spotted on thin-layer chromatography plates and the spots eluted with 95% ethanol. Both compounds gave an rf value of .80.

Using a Spectronic 505, the absorption spectrum of the substance prepared from 1,1,3,3-TEP showed a single peak at 534 mμ. The compound or compounds obtained from cauliflower showed a peak at 535 mμ with 3 minor peaks at 346, 362 and 380 mμ. These peaks may have been due to other TBA reactive material not removed during purification. The main absorption peaks at 534 and 535 mμ are shown in Figure 20. The curves appear to be quite similar.

Fig. 20.--(I) Absorption spectrum for purified MA-TBA complex.
(II) Absorption spectrum for purified TBRS-TBA complex. TBRS obtained by distillation from a mixture of acidified cauliflower extracts.


.6
.5
.4
.3
.2
.1
0
ABSORBANCE
I
II
580
560
540
520
500
480
460
WAVELENGTH mμ
1746

The ultraviolet absorption spectra of steam distillates from the unblanched and blanched cauliflower were studied and are shown in Figures 21 and 22. The steam distillate from the oxygen-stored samples had to be diluted 1:1 with water adjusted to pH 2. Absorption maxima from cauliflower distillates were found to occur between 210 and 225 mμ and between 280 and 288 mμ, depending on the sample. Kwon and Watts (1963) reported that saturated aldehydes such as formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde and hexaldehyde absorbed at 278 to 288 mμ. They also reported that α,β-unsaturated aldehydes, acrolein and crotonaldehyde, absorb in the region of 210 to 225 mμ. The absorption curves obtained from unblanched samples stored in nitrogen are different from those from unblanched samples stored in oxygen. A comparison between the unblanched and the blanched samples shows that considerable changes had occurred after 175 days at -15° C.

Thin-layer chromatography and visible absorption spectrum of the TBA-TBRS complex indicate that the reactive material obtained from cauliflower appears to be similar to the MA-TBA complex formed from MA.

Fig. 21.--(I) Ultraviolet absorption spectrum of a steam distillate from acidified unblanched cauliflower stored in nitrogen for 175 days at -15° C. The pH of the distillate adjusted to 2.
(II) Ultraviolet absorption spectrum of a steam distillate from acidified blanched cauliflower stored in nitrogen for 175 days at -15° C. The pH of the distillate adjusted to 2.

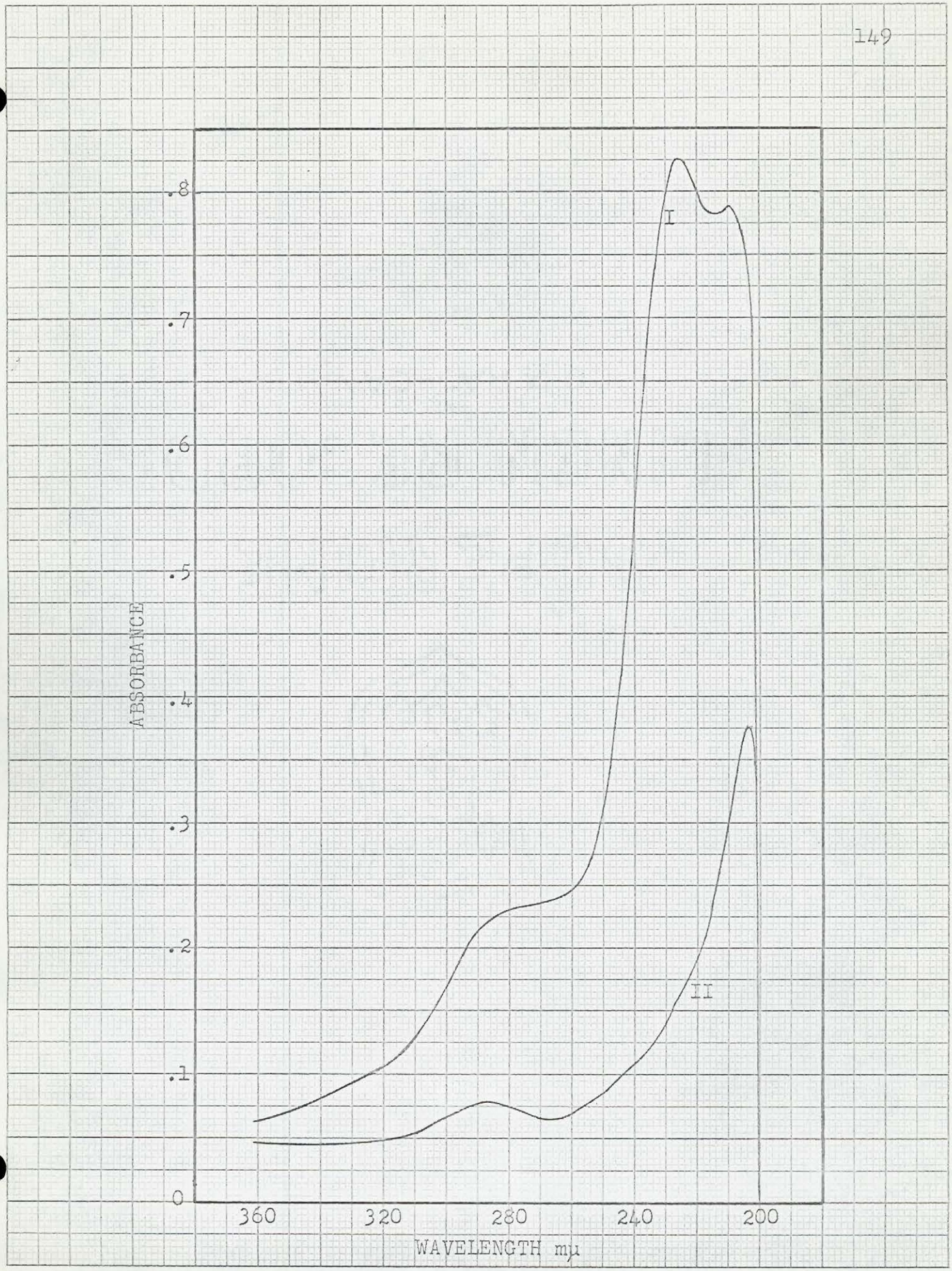
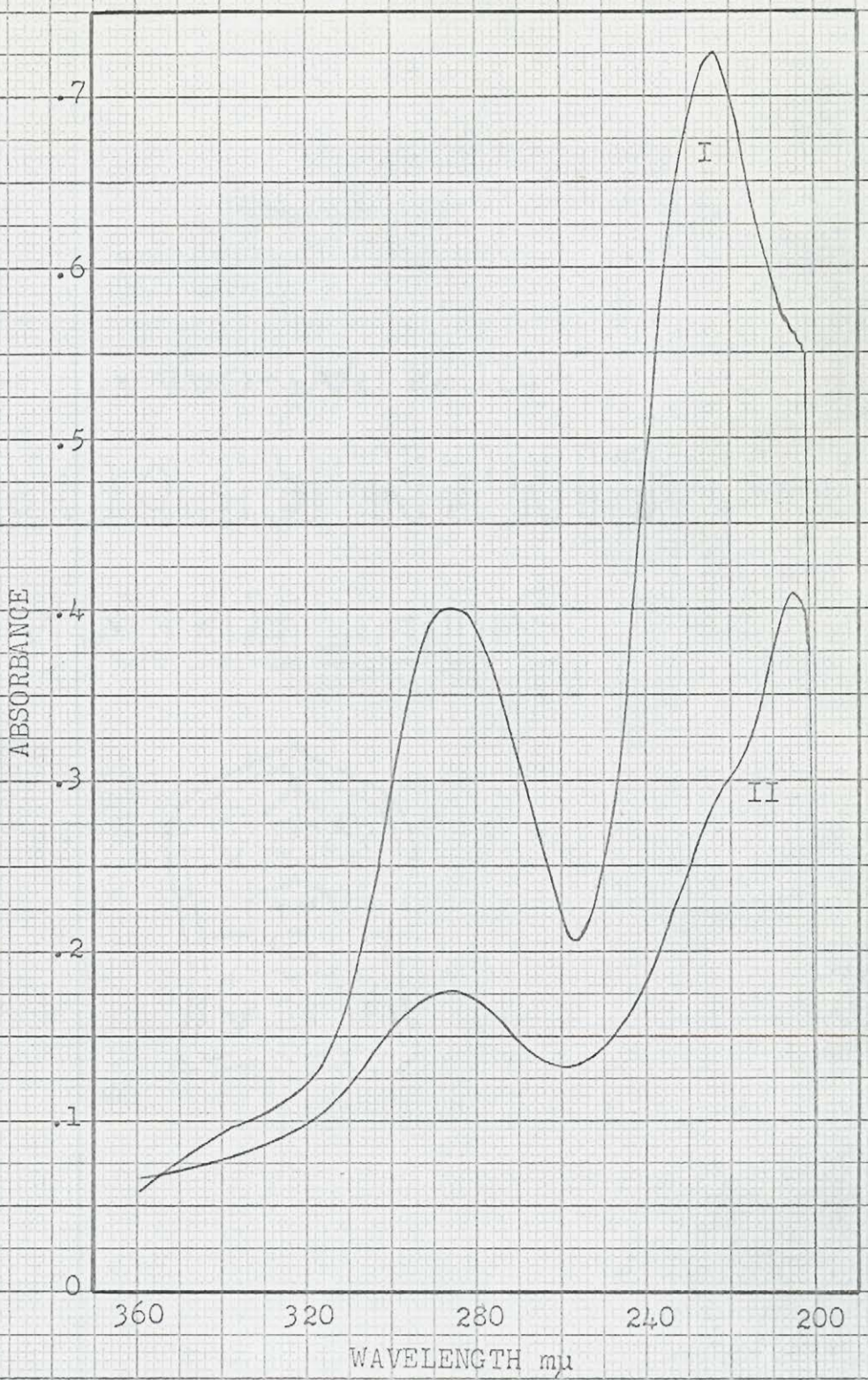


Fig. 22.--(I) Ultraviolet absorption spectrum of a steam distillate obtained from a sample of acidified unblanched cauliflower stored in oxygen for 175 days at -15° C. The pH of the distillate adjusted to 2.
(II) Ultraviolet absorption spectrum of a steam distillate obtained from a sample of acidified blanched cauliflower stored in oxygen for 175 days at -15° C. The pH of the distillate adjusted to 2.



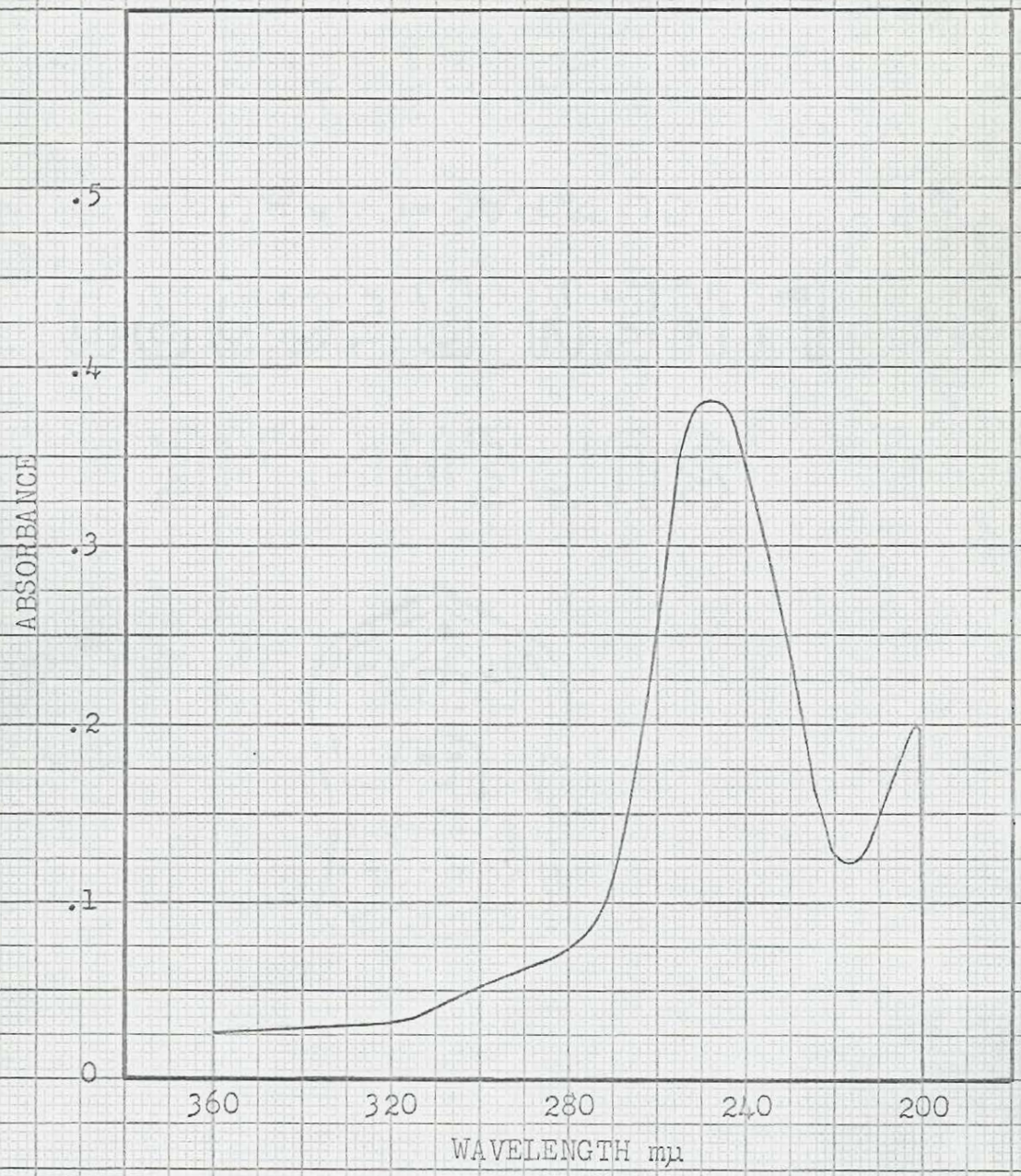


Fig.23--Ultraviolet absorption spectrum of sample of MA obtained from the steam distillation of an acidified solution of 1,1,3,3-TEP. The pH of the distillate adjusted to 2.5.

However, a comparison of the ultraviolet absorption spectrum of MA, Figure 23, and the spectra of steam distillates, Figures 21 and 22 does not support the hypothesis that MA may be present in stored cauliflower.

Thin-layer Chromatography of the 2,4-DNPHs

In series I, part of the ethanol extract was refluxed with 2,4-DNPHine. The 2,4-DNPHs were collected and analyzed, using thin-layer chromatography. Photographs were taken before and after spraying the plates with a solution of NaOH in ethanol.

Several plates were spotted with 2,4-DNPHs from extracts of unblanched and blanched cauliflower. It was found that there was a greater quantity of reactive material in the blanched cauliflower than in the unblanched, initially. This observation is substantiated by Figures 24 and 25. These results indicate that blanching increases the ease with which the carbonyl compounds are extracted from cauliflower. These findings parallel those obtained during the TBA determinations where the TBA numbers of unblanched material were lower than the TBA numbers of blanched material. The aldehyde content determined by steam distillation did not show this increase in the blanched material.

Other observations were made throughout the storage

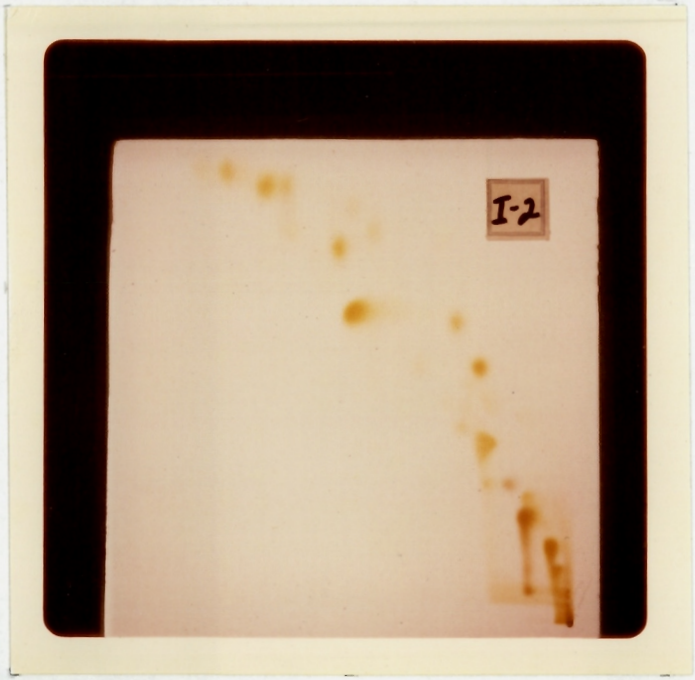


Fig. 24--Chromatogram of 2,4-DNPHs from an ethanol extract of fresh unblanched cauliflower. Chromatogram not sprayed, series II.

Fig. 25--Chromatogram of 2,4-DNPHs from an ethanol extract of fresh blanched cauliflower. Chromatogram not sprayed, series II.

period. After 19 days of storage at -9.4° C, photographs of the 2,4-DNPHs were again taken, Figures 26, 27, 28 and 29. They indicate that there was more reactive material in unblanched samples stored in nitrogen than in blanched samples in the same atmosphere. Those spots located at the top left and bottom right sides of the plate showed the greatest increase in intensity. Many of the spots decreased in intensity in the blanched samples stored in nitrogen for 19 days, when compared with the original. The unblanched samples stored in oxygen showed the greatest change in concentration of the 2,4-DNPHs. The blanched material stored in oxygen also showed considerable change when compared with the original. A comparison of the 2,4-DNPHs of unblanched and blanched cauliflower, after 19 days storage in oxygen, shows that the greatest changes in hydrazones occurred at the top left and bottom right sides of the plate. At the end of 19 days of storage, the unblanched samples stored in nitrogen did not seem to have any compounds showing more intense colour than those found on the blanched material stored in oxygen. Some of the spots at the bottom right may have increased in intensity in the material stored in nitrogen for 19 days. However, because of incomplete separation in this area, it was hard to detect any change unless it was quite apparent.

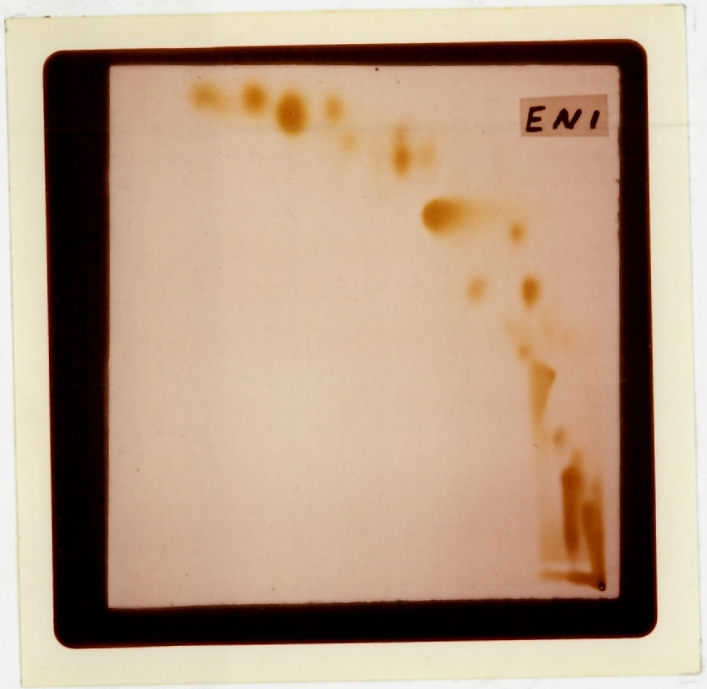


Fig. 26.--Chromatogram of 2,4-DNPHs from an ethanol extract of unblanched cauliflower stored in N_2 for 19 days at -9.4° C. Chromatogram not sprayed, series II.

Fig. 27.--Chromatogram of 2,4-DNPHs from an ethanol extract of blanched cauliflower stored in N_2 for 19 days at -9.4° C. Chromatogram not sprayed, series II.

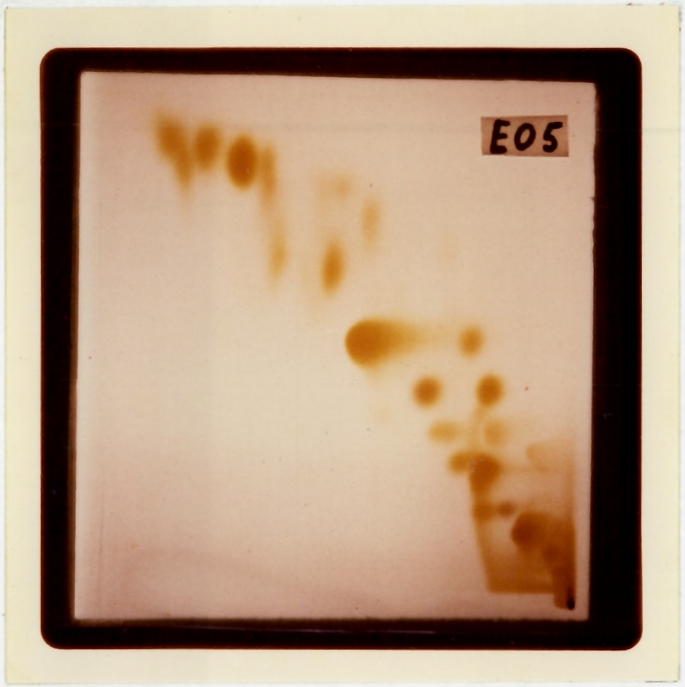


Fig. 28.--Chromatogram of 2,4-DNPHs from an ethanol extract of unblanched cauliflower stored in O_2 for 19 days at -9.4° C. Chromatogram not sprayed, series II.

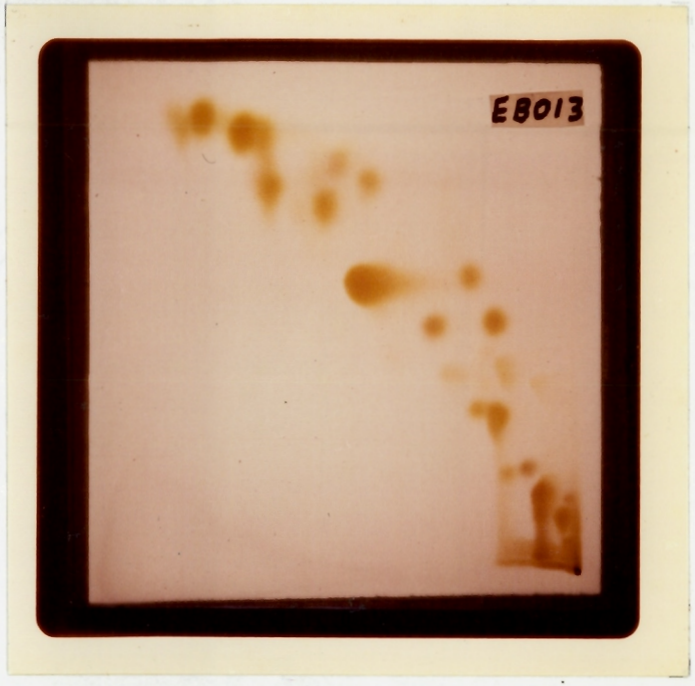


Fig. 29.--Chromatogram of 2,4-DNPHs from an ethanol extract of blanched cauliflower stored in O_2 for 19 days at -9.4° C. Chromatogram not sprayed, series II.

Figures 30, 31, 32, 33, 34 and 35 show the 2,4-DNPHs prepared from fresh cauliflower and from unblanched and blanched samples stored in nitrogen and oxygen for 19 days. The photographs were taken after spraying the plates with an alcoholic NaOH solution. Because of the unevenness of the spraying, the plates cannot be readily compared. They do show the presence of a large number of bis-aldehydes which turn blue or purple in the presence of NaOH.

Several attempts were made at identifying some of the carbonyl compounds from ethanol extracts and from steam distillates of cauliflower. The 2,4-DNPHs were prepared and the samples spotted as outlined previously. Standards were prepared and spotted on the same plates. The presence of acetaldehyde, propionaldehyde, acrolein, crotonaldehyde, butyraldehyde, methyl ethyl ketone, malonaldehyde and valeraldehyde could not be demonstrated.

Two carbonyl compounds were of interest in that they had the same rf value as 2,4-DNPHs of unknown spots from cauliflower extracts and distillates. These compounds were glyoxal and diacetyl. Both the known and the unknown compounds turned purple when the chromatography plates were sprayed with ethanol amine. When petroleum ether-ethyl ether (7:3) (v/v) was used as a solvent, the 2,4-DNPHs from cauliflower did not separate well enough

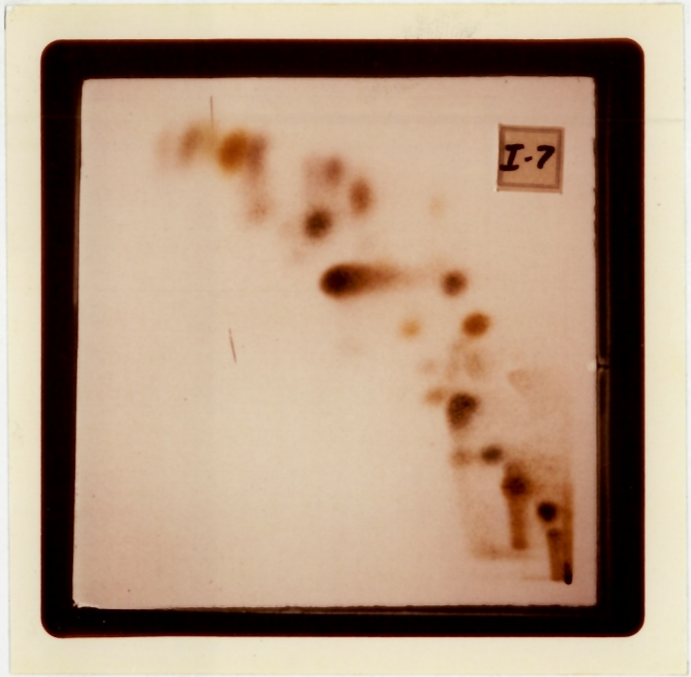


Fig. 30.--Chromatogram of 2,4-DNPHs from an ethanol extract of fresh unblanched cauliflower. Chromatogram sprayed with a 2% NaOH solution in 90% ethanol, series II.

Fig. 31.--Chromatogram of 2,4-DNPHs from an ethanol extract of fresh blanched cauliflower. Chromatogram sprayed with a 2% NaOH solution in 90% ethanol, series II.

Fig. 32.--Chromatogram of 2,4-DNPHs from an ethanol extract of unblanched cauliflower stored in N_2 for 19 days at -9.4° C. Chromatogram sprayed with a 2% NaOH solution in 90% ethanol, series II.

Fig. 33.--Chromatogram of 2,4-DNPHs from an ethanol extract of blanched cauliflower stored in N_2 for 19 days at -9.4° C. Chromatogram sprayed with a 2% NaOH solution in 90% ethanol, series II.

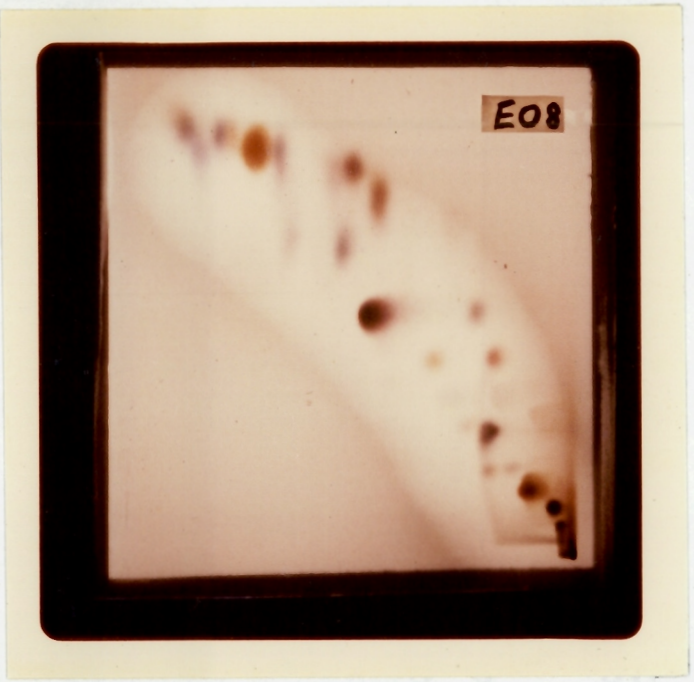


Fig. 34.--Chromatogram of 2,4-DNPHs from an ethanol extract of unblanched cauliflower stored in O_2 for 19 days at -9.4° C. Chromatogram sprayed with a 2% NaOH solution in 90% ethanol, series II.

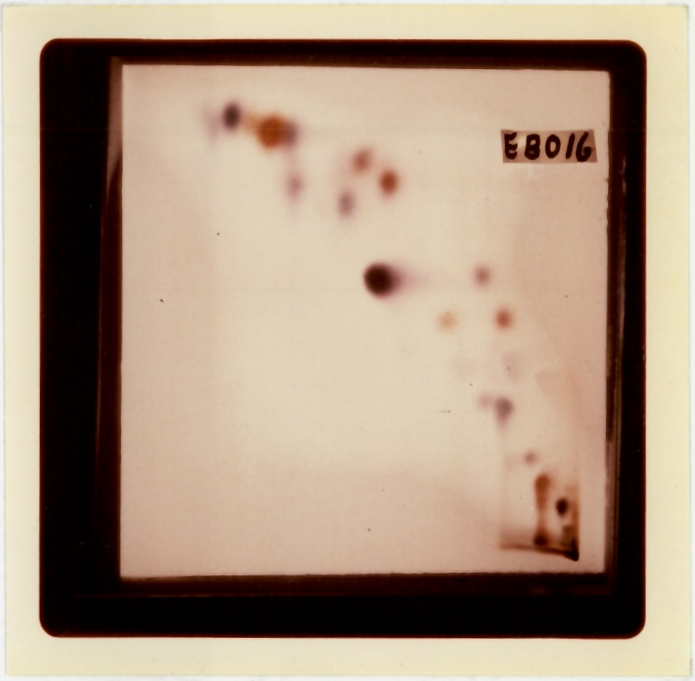


Fig. 35.--Chromatogram of 2,4-DNPHs from an ethanol extract of blanched cauliflower stored in O_2 for 19 days at -9.4° C. Chromatogram sprayed with a 2% NaOH solution in 90% ethanol, series II.

for good identification. However, one of the unknown 2,4-DNPHs and the 2,4-DNPH of glyoxal seemed to have about the same rf value of .17. With toluene-ethyl acetate (3:1) (v/v) as the solvent system, the compounds had an rf value of .64. When chloroform-ethanol (20:1) (v/v) was used as a solvent, the rf value was found to be .61. The unknown appeared to be present in greater quantities in unblanched and blanched cauliflower stored in oxygen than in nitrogen.

Small amounts of diacetyl seemed to be present in all four samples at about the same concentration. In petroleum ether-ethyl ether, an rf value of .19 was obtained, but the separation of the 2,4-DNPHs was incomplete. In toluene-ethyl acetate, a better separation was obtained and an rf value of .22 was obtained. When chloroform-ethanol was used, the rf value was found to be .61.

While glyoxal and diacetyl have been tentatively identified, their flavour does not resemble the off-flavour observed in cauliflower.

V. SUMMARY AND CONCLUSIONS

Aldehyde and ethanol determinations were carried out on cauliflower stored under various conditions of temperature and atmosphere, using steam distillation. The development of oxidative rancidity was investigated, using 2-thiobarbituric acid (TBA) as the reagent. Organoleptic tests were conducted concurrently.

Variations were found in the initial aldehyde and ethanol content. These were thought to be due to biological variations as well as handling methods. In addition, these variations could be attributed to different rates of respiration affected by variety, maturity of the cauliflower and time elapsed between harvest and processing.

Raw and underblanched cauliflower retained their ability to produce aldehydes in frozen storage. However, the accumulation of aldehydes in the underblanched samples had no relationship with the development of off-flavours. Large amounts of ethanol accumulated in unblanched cauliflower stored in nitrogen. This was thought to be due to handling methods since very little

change in ethanol content occurred after the first few days of storage. Unblanched cauliflower, stored in oxygen, also showed an increase early in storage, followed by a decrease. This decrease was also observed in the blanched material stored in nitrogen and oxygen. The initial increase in ethanol content in unblanched cauliflower stored in oxygen was probably due to anaerobic conditions developing in the cell during freezing. Ethanol production appears to cease at temperatures of -9.4° C and lower.

No relationship could be established between aldehyde and ethanol production and off-flavour development. The enzyme systems involved are probably different since a heat treatment sufficient to stop production of aldehydes did not prevent off-flavour formation. It would appear that the aldehyde and/or ethanol content, as determined by steam distillation, is not a satisfactory objective method for the determination of off-flavour development.

A satisfactory method for the extraction of 2-thiobarbituric acid reactive substances (TBRS) from cauliflower was developed, using 95% ethanol. Ethanol extracts of raw, underblanched and blanched cauliflower, stored in air, nitrogen and oxygen, were analyzed periodically with TBA reagents, prepared with and without

acid. The results indicate that this method is sensitive enough to detect changes in raw and underblanched cauliflower. These changes parallelled the development of off-flavours. While acid in the TBA reagent enhanced colour development, it does not appear to be necessary since the difference in results between acid and non-acid reagents was fairly constant throughout the experiments.

In series I, large changes were observed in the TBA numbers of cauliflower under all storage conditions. In series II, the changes were not quite as pronounced, specially in samples stored in nitrogen. These differences may have been due to biological variation between the two lots of cauliflower used. The results indicate that a relationship appears to exist between off-flavour development and TBA numbers and that the TBA reagent could be used to detect the development of off-flavours in frozen cauliflower.

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