PUBLICATION 732 ISSUED MARCH, 1942
TECHNICAL BULLETIN 40
DOMINION OF CANADA—DEPARTMENT OF AGRICULTURE

FACTORS INFLUENCING ASCORBIC ACID RETENTION IN APPLE JUICE

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Published by authority of the Hon. JAMES G. GARDINER, Minister of Agriculture,
Ottawa, 1942

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INTRODUCTION

Meagre information is available on the ascorbic acid content of fresh and processed apple juice in spite of the increased consumption and economic importance of this product. While extensive investigations have been reported on similar products, particularly tomato and citrus fruit juices, a study of the literature reveals little published data of this nature concerning apple juice. Furthermore, under certain climatic and economic conditions it may become advantageous to fortify apple juice with ascorbic acid. So far as the writer has been able to discover there are no published data on this subject. In view of this lack of information it was considered advisable to investigate the problem. Data have been secured on (*a*) the ascorbic acid content of commercial apple juices now on the market, (*b*) the effect of the various factors involved in preparation and processing on the ascorbic acid content, and (*c*) the feasibility of ascorbic acid fortification at a suitable stage in processing of the product.

REVIEW OF LITERATURE

Ascorbic Acid Content of Apple Juice

The limited data available indicate that freshly expressed apple juice contains almost the same quantity of ascorbic acid as is present in the fresh fruit from which it is extracted. Fellers, Cleveland, and Clague (1933) found freshly expressed Baldwin apple juice to be nearly as rich in ascorbic acid as fresh apple and that little loss occurred during the first twenty-four hours after extraction. They employed the bio-assay method of Sherman, LaMer, and Campbell (1922). Results of such a test are difficult of interpretation in terms of milligrams of ascorbic acid but they nevertheless give an accurate comparison of the two substances under test.

Fawns and Martin (1938) investigated the ascorbic acid content of juices of a number of individual English apple varieties and of blended juices. They reported an average value for apple juice of 2·38 mg. ascorbic acid per 100 ml. with a maximum of 5·3 and minimum of 1·2. The indophenol dye chemical method which is now the most generally accepted method for ascorbic acid analysis was used. The technique employed was that of Birch, Harris, and Ray (1933) in which the juice or extract is titrated into a known volume of indicator. These investigators determined only the reduced ascorbic acid and did not treat with hydrogen sulphide to determine the reversibly oxidized ascorbic acid (dehydroascorbic acid). When one considers the rapidity with which apple juice is oxidized and the fact that many of these juices analysed had been clarified and some apparently held for a time prior to testing, the figures estimated as reduced ascorbic acid appear to be rather high.

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[1]Data presented to the Graduate School of Massachusetts State College, U.S.A., June, 1941, as a thesis in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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Charley (1938) gives the average ascorbic acid content of apple juice as 4 to 8 mg. per 100 ml. as estimated with indophenol dye, but makes no statement as to what varieties of apples were used or whether hydrogen sulphide reduction was employed. Presumably reduction was resorted to. Cruess (1939) reported that apple juice lost practically all its directly titratable ascorbic acid within a very few minutes after pressing. Tressler, Joslyn, and Marsh (1939) indicated that apple juice is lower in ascorbic acid content than the fresh fruit from which it is expressed, there being a marked loss in extraction. King apples containing 8 mg. of ascorbic acid per 100 grams on pressing in a large hydraulic press yielded juice containing 3 mg. of ascorbic acid per 100 grams. Bessey (1939) indicated that the ascorbic acid value of any apple juice is similar to that of the fresh apple from which it is produced.

The meagre data to be found in the literature on the ascorbic acid content of canned apple juice indicate that it contains none or only a trace of ascorbic acid. Fellers, Cleveland, and Clague (1933) found that pasteurized apple juice over 48 hours old failed to retain an appreciable quantity of vitamin C. When fed at the 8-gram level in the guinea pig assay it showed practically no protective value and was little better than the negative controls. Cruess (1939) reported that commercially bottled apple juice was practically useless as a source of ascorbic acid in guinea pig feeding tests. Krauss (1934) investigated the antiscorbutic value of commercial grade apple juice finding it to be lacking in measurable quantities of the vitamin whereas fresh apple juice was a fair source of the vitamin. The results of a limited survey of the ascorbic acid content of commercial fruit juices being marketed in Canada was recently given in a report compiled by McFarlane (1940). This report showed the ascorbic acid content to vary from 0·7 mg. to 3·3 mg. per 100 grams. The indophenol dye titration method was used to estimate the ascorbic acid after reduction with hydrogen sulphide to recover the dehydroascorbic acid. No further details were given as to methods employed. Several of these results are higher than might be expected. In certain instances in preparation of the juice the tannin-gelatin method of clarification in all probability was employed. An excess of tannin present in the juice might contribute towards a high result. If the titration, however, is carried out rapidly and in a very acid solution, interference from tannin is usually avoided.

Factors Affecting Ascorbic Acid Content

The ascorbic acid content of apple juice may be expected to be affected by the concentration of ascorbic acid in the fresh fruit. A number of studies have been made on the occurrence of ascorbic acid in apples. A few of the recent reviews or studies which indicate the wide range of ascorbic acid values in apples are: Smith and Fellers (1934), Daniel and Munsell (1937), Todhunter (1937 and 1939), and Fixsen and Roscoe (1940). A very marked variation in the ascorbic acid content of different varieties of apples has been reported by many investigators. This is well illustrated by Smith and Fellers (1934) who determined the potency of 21 varieties of Massachusetts grown apples using the biological assay method. They found that the daily protective level for guinea pigs varied from 4 grams for Baldwin to over 25 grams for McIntosh.

Length of storage and temperature employed also markedly affect the ascorbic acid content of the fruit, as shown by Todhunter (1936). The studies of Batchelder (1934), and Zilva, Kidd, and West (1938) showed that a storage temperature of 0° C. preserved the ascorbic acid potency of apples. Fruit kept at this temperature for a period of six months showed little or no loss of ascorbic acid.

The distribution of ascorbic acid varies markedly in different portions of the apple, the peel being the richest. According to Fellers, Isham, and Smith

(1932), Todhunter (1937 and 1939), and others, the peel of the apple is from four to six times as rich as the cortex of the fruit. Peel, however, only makes up a comparatively small portion of the total fruit. Since apple juice is produced by thorough grinding or milling and pressing of the whole fruit, some of the ascorbic acid present in the peel should theoretically pass into the juice. This is expected since ascorbic acid is quite water soluble and is not held in the combined state.

Zilva, Kidd, and West (1938) have shown that immature apples contain a large proportion of their ascorbic acid in dehydroascorbic acid form. At harvest time, however, it is over 90 per cent in the reduced state. This condition is maintained throughout the storage life of the fruit. Curran, Tressler, and King (1937) have also indicated that there is a small proportion of dehydroascorbic acid present in apples as shown by a consistently slight increase in titration value after reduction with hydrogen sulphide. In most plant tissues dehydroascorbic acid represents an insignificant portion of the total biologic value of fresh or storage food products.

Reedman and McHenry (1938) and a few other previous investigators have suggested that there may exist in certain plant tissues significant quantities of physiologically available combined ascorbic acid which is not extracted by ordinary methods being only liberated by acid hydrolysis or heating. Hence if ascorbic acid of this nature occurred in apple tissue it would fail to appear in the particle-free expressed juice. Stone (1937) showed fairly convincingly that the apparent increase in ascorbic acid in certain plant tissues after heating is attributable to the inactivation of the ascorbic acid oxidizing enzyme. Mack and Tressler (1937) also supported this view. Bessey (1938) using the accurate photoelectric indophenol method was unable to confirm the existence of any combined ascorbic acid in plant tissues.

The stability of ascorbic acid in a product appears to centre entirely on one type of reaction, oxidation. Ascorbic acid is very sensitive to destruction by oxidation as shown by numerous investigations reported in the literature both in studies on pure ascorbic acid in aqueous solution and in studies on the storage and processing of various food products. Barron, DeMeio, and Klemperer (1936) have shown that in aqueous solution below pH 7·6 the vitamin is not oxidized on exposure to air unless the reaction is catalysed by copper or some other activating agency. This is of special interest in showing that the vitamin is not auto-oxidizable within the normal pH range of plant tissues. Among the metallic salts tested (Mn, Ni, Fe, Co, Ca, and Cu) copper is the only catalyst they found for the oxidation of ascorbic acid, its action being detectable in concentrations as small as 46 micrograms of copper per liter. These results with copper have been substantiated by Mack and Kertesz (1936) who also found that in the presence of iron, copper exerts an increased catalytic activity.

The presence in certain plants of an oxidizing enzyme termed ascorbic acid oxidase has been shown by Tauber, Kleiner, and Mishkind (1935), and by Kertesz, Dearborn, and Mack (1936) to cause rapid oxidation of ascorbic acid. As indicated by Kertesz *et al* (1936) the activity of the enzyme in different plants varies markedly. These investigators also have shown that the ascorbic acid oxidase is completely inactivated in vegetables or in their extracts by heating to 100° C. for one minute. Inactivation of the enzyme while preventing rapid loss of ascorbic acid in vegetable extracts did not entirely eliminate it. They found a variable loss in different vegetables due to non-enzymic catalysis.

The investigations of Zilva (1934), Johnson and Zilva (1937), Barron, Barron, and Klemperer (1936), have shown apple juice to contain an ascorbic acid oxidizing enzyme. The activity of the enzyme as reported by Barron *et al* (1936) is less than that in certain vegetables.

The first oxidation of ascorbic acid is to dehydroascorbic acid which may be reversibly oxidized to ascorbic acid. Dehydroascorbic acid has approximately the same physiological activity as ascorbic acid as shown by Fox and Levy (1936), and King (1941). If oxidation continues further an irreversible arrangement occurs yielding substances having no physiological activity. While King (1939) has indicated that dehydroascorbic acid is fairly stable in aqueous solutions below pH 4, Kertesz, Dearborn, and Mack (1936) reported that dehydroascorbic acid is more readily decomposed by a non-enzymic reaction into a compound having no antiscorbutic activity.

The effect of canning on ascorbic acid content of numerous food products is thoroughly reviewed by Fellers (1936) and recently briefly reviewed by King and Tressler (1940). Bing, Bailey, and Fisher (1938) reported that canned orange juice is only slightly lower in ascorbic acid than the fresh juice. Bessey (1939) reported that canned grapefruit and orange juice are about 70 to 90 per cent as potent as the fresh juice and like most canned products, remain stable while canned but slowly lose their vitamin C when left open to the air. Maclinn and Fellers (1938) reported that after 400 days' storage of tomato juice in either tin or glass the loss of ascorbic acid was not greater than 25 per cent. Kohman, Eddy, and Gurin (1933) showed that deaeration and anaerobic handling in the manufacture of tomato juice largely prevented impairment of the ascorbic acid content. Loss in flavour, colour, and ascorbic acid content of juices has been shown by Joslyn (1941) and others to be correlated with the oxygen present.

Lueck and Pilcher (1941) report significant data to show that the type of container markedly affected the retention of ascorbic acid. They found the retention of ascorbic acid in tomato and grapefruit juice packed commercially in tin containers to be excellent and superior to that in glass containers. The rate of decline of ascorbic acid during storage was greater in glass than in tin. They ascribed this to the reduced state of the juice system due to the presence of stannous and ferrous iron when preserved in metal containers. This of course is particularly applicable to the plain type of can employed in the preservation of tomato and grapefruit juice.

With regard to the role of oxygen in the development of off flavours, loss of ascorbic acid and effect upon the tin containers, Lueck and Pilcher (1941) reported that Clark and Lachele of the American Can Company laboratories demonstrated the necessity of almost complete elimination of oxygen where inside enamelled cans are used for orange and lemon juice. These investigators found that the presence of oxygen in the can in amounts exceeding 0·10 per cent by volume promotes an attack on the container which disrupts the bond between the enamel film and the tin-plate. Disagreeable flavours were generated. Lueck and Pilcher further state that of necessity, all cans must be filled so as to leave some free space (head space) above the juice level. It was found that the amount of air trapped in the head space of a can in the usual filling and sealing operation was greater than that removed by the most effectual deaeration of the juice contained in it. To avoid this they found it was necessary to devise a can-sealing machine whereby the remaining oxygen can be eliminated from the can with a stream of inert gas forced into the head space during the assembly of can and cover. The necessity of leaving a head space in smaller sizes of cans as stated by the authors may be questioned in the packing of a product such as apple juice. Care must be exercised though in such highly evacuated cans, particularly in the larger sizes, to avoid damage to the container. By leaving a head space or by filling the head space with an inert gas (probably nitrogen) an excessive vacuum is avoided within the tin container.

Investigations on the effect of light on ascorbic acid in black currant juice by Fawns (1939) and on buffered ascorbic acid solutions under anaerobic conditions by Arcus and Zilva (1940) showed ascorbic acid to be subject to photochemical decomposition. However, since processed apple juice is largely canned this fact would have little practical significance with regard to this product.

Certain substances having an anti-browning or anti-oxidant effect on fruit tissues have in some instances been shown to exert a stabilizing effect on the ascorbic acid present. Contrary to some other findings, Joslyn (1941) has shown that sulphur dioxide in addition to being a good anti-oxidant is also a preservative of ascorbic acid in citrus juices. Sulphur dioxide apparently prevented the rapid oxidation of ascorbic acid to its dehydro form especially in concentrations of 500 p.p.m. of sulphur dioxide. Also the dehydroascorbic acid was found to decrease more slowly in the presence of sulphur dioxide than in its absence. It is suggested that sulphur dioxide prevents discoloration by protecting ascorbic acid and other reducing substances. The protective action of sulphur dioxide towards ascorbic acid has been substantiated by Sills (1939) working with strawberry and black currant juices.

The amino acid cysteine, the tripeptide glutathione and the "fixed-SH" groups associated with proteins are stated by King (1939) to have a reducing action on dehydroascorbic acid or to protect ascorbic acid. These substances have been proposed by Balls and Hale (1935) for inhibiting discoloration of fruits and vegetables. Another substance which is proposed by Denny (1935) for inhibiting browning of cut surfaces of fruits and vegetables or juices is thiourea. Other sulphur-amino-derivatives of carbonic acid had a similar action according to this investigator. No data were found in the literature as to the possible effect of these compounds on the retention of ascorbic acid.

Fortification

Fortification with vitamins or minerals has been practised in proprietary products. Up to within the past year or two, however, the only commonly used foods fortified with vitamins have been dairy products, chiefly milk with Vitamin D and substitutes for butter such as oleomargarine with vitamin A. Recently there has been a keen interest, intensified by war and economic conditions, to increase the vitamin content of other staple foods by the addition of synthetic or concentrated vitamins. The interest has been particularly in the fortification of cereal products with thiamin. This was first proposed in North America by Cowgill (1939).

The Council on Foods (1939) of the American Medical Association approves of the fortification of staple foods with the provision that such additions be limited to vitamins or minerals or other dietary essentials for which a distribution is considered to be in the interest of the public health. Mitchell (1941) indicates that the attitude of nutritionists on fortification is now one of limited recommendation under proper control, stating that it is realized that education in food values cannot alone solve all nutrition problems.

In warm countries where ample supplies of citrus fruits and juices are available at low prices they constitute the logical medium through which to include ascorbic acid in the diet. In countries with colder climates where citrus fruits are less plentiful and more expensive, it may be necessary for people to secure their vitamin C in some other form. Apples are readily produced in many of these cooler countries and the juice of this fruit may prove to be a suitable medium for the addition of ascorbic acid. So far as the writer is aware, there are no published data on the fortification of processed apple juice either with synthetic ascorbic acid or by blending with some other natural substance rich in ascorbic acid.

EXPERIMENTAL PROCEDURE

Material and Processing Equipment

In the survey of the ascorbic acid content of commercially processed juices several blends of apple varieties and different methods of processing were involved. In the studies of the ascorbic acid content of fresh apple juice and in the investigation of factors affecting ascorbic acid retention and fortification, apples of the Newtown variety were used.

The apples were harvested from heavy crop trees on October 11 and after four days placed in storage at 0° C. Quantities of fruit were removed from storage as required for preparation of experimental juice packs.

In general the processing equipment used was the same as that described by Atkinson and Strachan (1939) for apple juice processing plants. Because of the small quantities of juice being processed a glass tube flash pasteurizer was employed for much of the experimental work. This consisted simply of coiled glass tube of about 6 mm. bore immersed in a tank of vigorously boiling water.

Type L tin-plate cans with double enamel or special fruit-juice enamel coating were used. For sealing bottled juice an aluminum spotted crown cap was employed.

The deaeration of the juice was accomplished by the method of Mottern and von Loesecke (1933). The apparatus as set up for this study is illustrated in Fig. 1. In every instance the vacuum was released with nitrogen. The efficiency of this deaerator is shown in table 1.

TABLE 1.—EFFICIENCY OF DEAERATOR

	Temp. of juice	Gas per 100 ml. juice at 0° C. (760 mm.)				Approx. reduction in total gas
		Total gas	CO_2	O_2	N_2	
	°C	ml.	ml.	ml.	ml.	%
1. Raw juice	.....	2·16	0·82	0·06	1·28	.....
Deaerated	2°	0·47	0·21	0·00	0·26	78·2
2. Raw juice	.....	2·75	1·25	0·00	1·50	.....
Deaerated	10°	0·24	0·10	0·00	0·14	91·7
3. Raw juice	.....	1·72	0·54	0·00	1·18	.....
Deaerated	19°	0·26	0·06	0·00	0·20	84·9
4. Raw juice	.....	2·10	0·92	0·00	1·18	.....
Deaerated	21°	0·14	0·05	0·00	0·09	93·4

It will be noted that the temperature of the juice has some influence on the efficiency of the deaerator. However, at temperatures of 10° to 20° C. at which most deaerations were carried out, the removal of total gas amounted to at least 85 per cent and often to better than 90 per cent.

Treatment of Juice

Juice was analysed immediately after expression by a hydraulic press. It was then stored in an open container at 12° C. and analysed at definite intervals.

Several samples of commercially canned apple juice of different brands were analysed. These samples were selected as being typical of various types and blends.

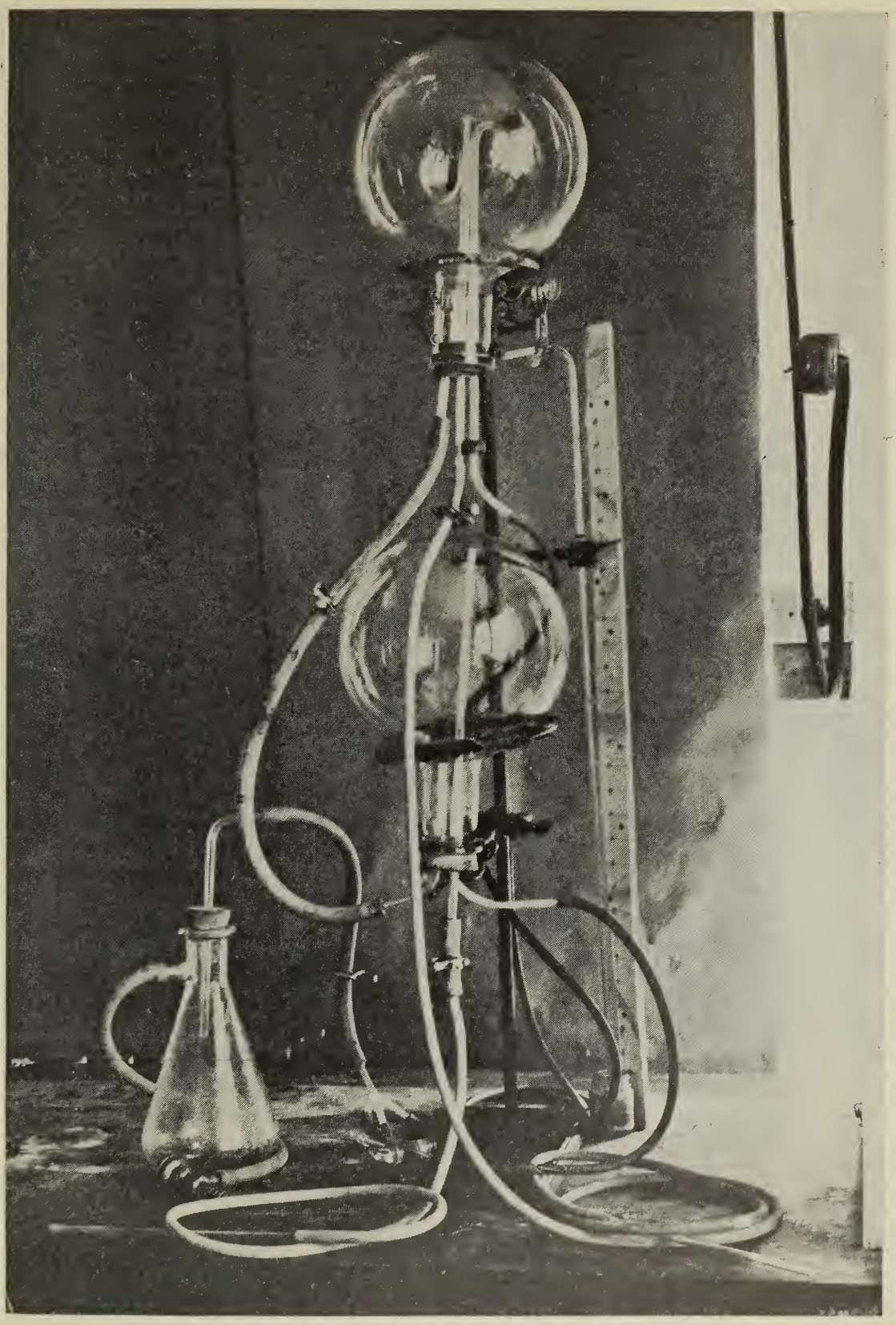

FIGURE 1.—Apparatus for deaerating juice.
(Photograph by J. E. Britton)

Apple juice was canned and bottled by the various procedures at present employed commercially. Each step in the process was investigated critically to ascertain its effect on retention of ascorbic acid.

Certain modifications were tried, the principal one being the use of the antioxidants thiourea and cysteine hydrochloride. These substances were used on the sliced fruit or added to the expressed juice. In the former procedure the apples were cut into slices of a thickness not greater than one-quarter inch, immersed in a weak water solution (0·05 or 0·1 per cent usually) of the compound for 4 to 5 minutes, thoroughly drained, milled and pressed. When thiourea or cysteine was added to the previously expressed juice an amount was used to give a concentration in the juice of 0·01 per cent.

All juice processed in these experiments was, unless otherwise noted, pasteurized at 85° C. directly into the container which was filled full and sealed. Cans were laid on their sides for a period of 2 minutes, then cooled as quickly as possible, about 3 to 6 minutes being required for cooling depending on the size of can. Bottles were placed on their sides and allowed to air cool.

Samples of canned and bottle juice were stored at 20° C. for further examination.

Samples of juice were fortified with pure crystalline ascorbic acid (Merck) by mixing in the juice prior to passing through the pasteurizer or by placing quantitative amounts in the container prior to filling with the hot pasteurized juice. Ascorbic acid was usually added at the rate of 20 to 25 mg. per 100 ml. of juice. Several methods of procedure in adding the ascorbic acid were used.

Samples of the various fortified processed juice were stored at 20° C. for further examination.

Determination of Ascorbic Acid

The visual titration method of determining ascorbic acid with the dye 2,6-dichlorophenolindophenol in a pH range of 1 to 3·5 gives a reasonably accurate and satisfactory measure of the ascorbic acid content of most tissues according to King (1941). Zilva, Kidd, and West (1938) carried out extensive biological tests on apples in conjunction with indophenol dye titrations. They reported that the dye titration figures were an accurate index of the ascorbic acid content of the apple tissue extracts after reduction with hydrogen sulphide. Curran, Tressler, and King (1937) also found that results obtained by the biological assay were in close agreement with the titration values for ascorbic acid in apples and apple products. Because of its accuracy and wide acceptance by investigators the indophenol dye titration method was used in this study to estimate ascorbic acid.

The technique used at the commencement of the investigation was that of Bessey and King (1933) as modified by Bessey (1939). This modification employs 3 per cent metaphosphoric acid as the sole extractant. The reduced ascorbic acid was determined by direct titration. To determine any reversibly oxidized ascorbic acid (dehydroascorbic acid) aliquots of the metaphosphoric acid extract were treated with hydrogen sulphide for a period of 15 minutes, allowed to stand 20 minutes in a corked glass cylinder and freed of hydrogen sulphide by bubbling nitrogen through the solution for two hours or until a negative test for hydrogen sulphide was obtained. This reduced extract was then titrated with the indophenol dye. According to Mack and Tressler (1937) this procedure should measure all the biologically active ascorbic acid.

In the course of analysing freshly expressed apple juice for dehydroascorbic acid inconsistent results were obtained where any variation in the period of hydrogen sulphide saturation of the extract had occurred. It appeared that possibly all the reversibly oxidized ascorbic acid was not being recovered under these conditions. Longer periods of hydrogen sulphide saturation yielded increased ascorbic acid values. Bessey (1938) reported that reduction of

dehydroascorbic acid in more acid solutions than pH 3·0 was slow and incomplete. Shamrai (1939) reported a similar finding. The metaphosphoric acid extract of apple juice under test had a pH of 1·47.

An investigation was therefore undertaken to determine the effect of pH of the extract and type of extractant on the recovery of reversibly oxidized ascorbic acid in apple juice. The following three acid media were tried: citrate buffered metaphosphoric acid (Bessey 1938), one normal sulphuric acid solution containing 2 per cent metaphosphoric acid (Mack and Tressler 1937), and 3 per cent metaphosphoric acid. These extracts of apple juice had respectively the following pH values as determined with the glass electrode: 3·5, 0·4, and 1·47. Each was saturated with hydrogen sulphide for varying intervals of time. The results of these tests are indicated in table 2.

TABLE 2.—EFFECT OF pH ON THE REDUCTION WITH H_2S OF DEHYDROASCORBIC ACID IN APPLE JUICE

H_2S Treatment	Ascorbic acid per 100 ml.		
	Extraction medium		
	Buffered HPO_3 pH 3·5	1/N H_2SO_4 plus 2% HPO_3 pH 0·4	3% HPO_3 pH 1·47
hrs.	mg.	mg.	mg.
½	4·91	0·70	0·52
1	5·61	1·00	.....
2	6·30	0·94	1·17
4	6·32	1·17	2·34
20	6·30	1·64	5·10

It will be noted that recovery of dehydroascorbic acid was rapid and complete in less than two hours at pH 3·5. The poorest recovery was obtained at pH 0·4.

Evidence that the increased titration value obtained with the citrate-buffered extract is entirely due to dehydroascorbic acid being reduced to ascorbic acid is presented by Bessey (1938). The possibilities of interfering substances being produced under conditions of the preceding tests were unlikely. However, further investigations were carried out to determine the accuracy of the assumption that it was truly reversibly oxidized ascorbic acid that was being estimated under these circumstances.

The same three acid media as previously employed were used to make extracts of juice which had been treated so as to contain little or no ascorbic acid either reduced or reversibly oxidized. The results are presented in table 3.

TABLE 3.—COMPARISON OF RESULTS OF REDUCTION FOR TWO HOURS WITH H_2S IN DIFFERENT EXTRACT MEDIA OF APPLE JUICES CONTAINING NEGLIGIBLE AMOUNTS OF DEHYDROASCORBIC ACID

Juice treatment	Ascorbic acid per 100 ml.		
	Extraction medium		
	Buffered HPO_3 pH 3·5	1/N H_2SO_4 plus 2% HPO_3 pH 0·4	3% HPO_3 pH 1·47
	mg.	mg.	mg.
Canned—1	0·46	0·41	0·41
2	0·41	0·41	0·46
3	0·20	0·20	0·20
Boiled fermented	*0·10 or less	*0·10 or less	.....
Fermented	0·50	0·25	.....
Oxidized	0·52	0·46	.....
Partially oxidized plus 5 p.p.m. each of Cu^{++} and Fe^{+++}	0·50	0·30	.....

* H_2S treated for 20 hours.

It is apparent that there is only slight variation in the titration values in the three media. Also when crystalline ascorbic acid was added to the three acid extracts of thoroughly boiled apple juice and saturated with hydrogen sulphide, quantitative recovery of ascorbic acid was obtained in each case. While the evidence secured was indirect it appears that almost all of the increased titration value after reduction with hydrogen sulphide in a buffered metaphosphoric acid medium of pH 3·5 was due to dehydroascorbic acid.

Bessey (1938) has shown that in the buffered medium both ascorbic and dehydroascorbic acid are stable for several hours. For direct visual titrations King (1941) prefers a solution closer to pH 1·0 because interference from other substances is decreased. In this investigation the writer has found that in a few instances the end point was not so definite at pH 3·5 as in the more acid solutions, but the agreement usually was excellent. Evelyn, Malloy, and Rosen (1938) have shown that the reaction of ascorbic acid with the indophenol dye is almost instantaneous (complete in 5 seconds) while that of all other interfering substances proceeds at a slower rate. With extracts very low in ascorbic acid it is difficult to get a high degree of accuracy for, as pointed out by Ahmad (1935), the end point tends to be indefinite. Maclinn and Fellers (1938) and Lueck and Pilcher (1941) have shown that stannous and ferrous ions cause no serious interference in estimating ascorbic acid in canned juices unless each is present in excess of 100 p.p.m.

Having regard to the information presented above, the following procedure was adopted for the determination of ascorbic acid in apple juice.

The 2,6-dichlorophenolindophenol dye was prepared by dissolving approximately 50 mg. of the dye with successive portions of hot water, filtering and making up to a volume of 200 ml. with water. This prepared a 0·025 per cent solution of the indicator. Under conditions where ascorbic acid had been added to the sample under test markedly increasing the ascorbic acid, a dye strength of 0·05 per cent was employed. Fresh dye solution was prepared every two days. The dye was standardized by the method of Buck and Ritchie (1938).

Metaphosphoric acid of 6 per cent strength was prepared by dissolving clear sticks of glacial metaphosphoric acid in glass-distilled water and filtering. This stock solution was kept in a cold storage room at 0° C. for periods not greater than two weeks. Bessey (1938) has shown that the solution thus prepared remains satisfactory for this length of time. A 3 per cent solution was prepared from this stock solution as required. The citrate buffer was prepared exactly as described by Bessey (1938).

An aliquot of apple juice (usually 20 ml.) was placed in a 100 ml. volumetric flask containing 3 per cent metaphosphoric acid. Sufficient citrate buffer (approximatey 21·3 ml.) was added to bring the pH of the extract to 3·5 when made up to volume. Aliquots of this extract for the estimation of ascorbic acid were made up to the same volume with 3 per cent metaphosphoric acid and titrated with the indophenol dye. The reversibly oxidized ascorbic acid (dehydroascorbic acid) was determined by bubbling hydrogen sulphide slowly for 15 minutes through 25 to 30 ml. of the buffered extract in a glass cylinder, allowing to stand at room temperature for two hours, and then freeing of hydrogen sulphide by bubbling with cylinder nitrogen for two hours or until a negative test for hydrogen sulphide was obtained. Aliquots of this extract were made up to the same volume with metaphosphoric acid and titrated with the indicator. The difference between this latter titration which gives the total ascorbic acid and the direct titration represents the dehydroascorbic acid. The final titre was in every case corrected for the blank titration.

Titrations were always made in triplicate. The end point of the titration was taken where the faint pink colour of the extract persisted for 10 to 15 seconds.

Ascorbic acid analysis of apple tissues was carried out as follows:—

The tissue extract was prepared essentially according to the volumetric method of Thornton (1938). A 10-gram radial sector of apple was extracted with 3 per cent metaphosphoric acid and buffered to a pH of 3·5 when made up to 100 ml. volume. This preparation was filtered in a Buchner funnel instead of separation by centrifuging. The ascorbic acid was then estimated as described for apple juice. In order to obtain a representative analysis duplicate radial samples were taken from each of twelve representative apples.

Determination of Gas

The method and apparatus used for gas analysis was essentially that developed by Lachele (1939). This apparatus permits of analysis of the gas in cold or hot juice or the canned or bottled product. A further advantage is that an analysis may be carried out at any point in the manufacture of a processed juice. The accuracy of the method and equipment was determined and found to be reasonably good. An oxygen analysis of a water sample by the chemical method (A.O.A.C. 1940) showed the water to contain 2·79 ml. of oxygen per liter of water. With the Lachele type of equipment, 2·89 ml. of oxygen per liter of water were indicated. These figures are corrected to 0° C. and 760 mm. pressure. Blank determinations on thoroughly boiled water yielded on an average less than 0·03 ml. of oxygen per 100 ml. of water at 0° C. (760 mm.). The method is rapid, requiring only 10 to 15 minutes for each determination. It is very suitable for comparative determinations of gas content but requires a standardized technique for the most accurate results.

The modified apparatus as designed and set up for the present investigation is illustrated in Fig. 2. It consists of a 50 ml. Lunge nitrometer gas burette with 500 ml. levelling bottle; a water jacket around the gas burette so as to control the gas temperature; a 1,000 ml. gas collecting bulb; a 2,000 ml. aspirator bottle with tubulature at bottom and supplied with a two-hole rubber stopper in the mouth of the bottle (one hole for tube leading to the vacuum pump, the other for release); a puncturing device with the piercing point completely covered by a one-hole rubber stopper; a vacuum pump or water tap aspirator; and heavy rubber tubing for connections together with several wire spring clamps.

Briefly the equipment is operated as follows: The system is filled with thoroughly boiled water, being careful to release all air in the apparatus by upward flow of water. About 500 ml. of water should remain in the large aspirator bottle and about 250 ml. in the levelling bottle. The system may readily be tested for leaks by drawing a vacuum.

An operating vacuum of 18 to 20 inches was found quite suitable. A vacuum in excess of 25 inches tends to produce leaks. To maintain a consistent vacuum, a vacuum gauge is useful. A simple mercury column was employed in this investigation.

The sample under test should be at a temperature of at least 80° C. so that the sample will boil vigorously at the reduced pressure used. A temperature of 82° to 88° C. is desirable. The canned or bottled sample if cold may be placed in boiling water for a definite period of time to raise it to the desired temperature.

The can or bottle is pierced, a vacuum is drawn and the gas is collected in the collecting bulb. The tube leading to the piercing clamp is then closed with a clamp before gas completely fills the bulb, and the gas is transferred to the nitrometer tube by releasing the vacuum and lowering the levelling bottle

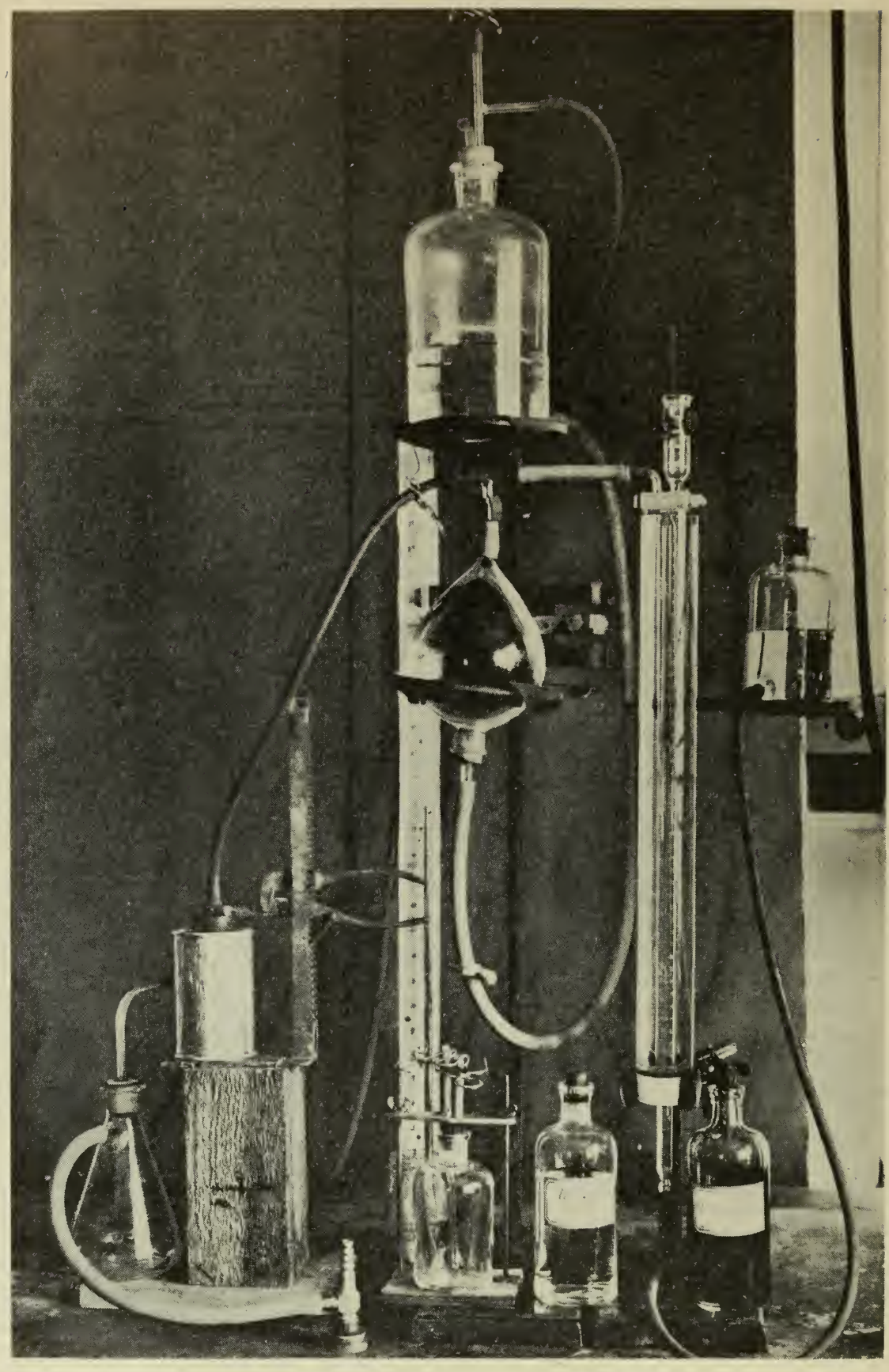

FIGURE 2.—Gas analysis apparatus.
(Photograph by J. E. Britton)

attached to the nitrometer tube. Three vacuumizations usually are sufficient, removing well over 90 per cent of the gases. The time for each vacuumization is about 30 to 60 seconds.

After the gas in the tube has assumed a constant temperature the total gas content is noted. Then 10 to 15 ml. of 10 per cent sodium hydroxide solution is allowed to trickle slowly down the side of the burette from the funnel. The carbon dioxide is rapidly absorbed. If large quantities of carbon dioxide are present the operation should be repeated. Oxygen is absorbed similarly with 10 to 15 ml. of 5 to 10 per cent sodium hydroxide solution containing 5 to 10 grams of pyrogallic acid crystals per 100 ml. of solution.

For the determination of gases in fresh juice or juice not bottled or canned, a 300 ml. pyrex bottle with a ground glass neck was fitted with a two-hole rubber stopper. A glass tube was inserted in each hole, one of the tubes extending to near the bottom of the bottle, the other tube being made flush with the bottom of the stopper. The exterior tips of the tubes are each fitted with a short piece of rubber tubing. The bottle is then placed in a metal vice arrangement which was designed to hold the stopper securely in place, thus reducing the possibility of leakage. The sample for analysis is then siphoned slowly from below the surface of the liquid under test into the bottle through the longer tube until the bottle is filled and all the air forced out. The juice is allowed to flow for a time to make certain of a representative sample. The two short pieces of rubber tubing are clamped close to their ends. The entire assembly is placed in a boiling water bath which is close to the gas analysis equipment. The rubber tube connected to the shorter piece of glass tubing is then connected to the gas analysis equipment by joining with the tube formerly connected to the puncturing device. This is accomplished by inserting a small piece of glass tubing between the two rubber tubes. Water is allowed to flow from the equipment during assembly. The sample is allowed to heat in the bath for 6 to 8 minutes or until it has reached a temperature of at least 80° C. This temperature is determined by previous trials. The gas analysis is then carried out exactly as described for canned or bottled juices.

PRESENTATION OF RESULTS

Ascorbic Acid Content of Fresh and Commercially Canned Apple Juice

Data on the ascorbic acid content of apple juice as determined after reduction with hydrogen sulphide to recover dehydroascorbic acid are presented in table 4.

TABLE 4.—ASCORBIC ACID CONTENT OF NEWTOWN APPLE JUICE

Sample	Treatment	Ascorbic acid per 100 ml.
		mg.
1	Fresh expressed juice	7·0
	After 24 hrs. in open container at 12° C	6·6
	After 48 hrs. " "	5·0
	After 72 hrs. " "	4·7
2	Fresh expressed juice	7·6
3	Fresh expressed juice	6·5

It may be seen from table 4 that freshly expressed apple juice contained about 7 mg. of ascorbic acid per 100 ml. of juice. Upon standing, the reversibly oxidized ascorbic acid became gradually less, but even after 72 hours the juice

still contained 4·7 mg. per 100 ml. It should be pointed out that during the entire progress of this study only mere traces of the reduced form of ascorbic acid were found in apple juice. This was true even when juice was sampled during the pressing operation.

The results of a survey to determine the ascorbic acid values of commercially canned apple juices are presented in table 5. These juices had been packed for three or four months. The values shown are those obtained after reduction with hydrogen sulphide. No naturally occurring reduced ascorbic acid was detected in any of these samples. It is evident from these data that the ascorbic acid content of processed apple juice is negligible, amounting to only 0·2 to 0·3 mg. ascorbic acid per 100 ml. of juice. It must be remembered that in

TABLE 5.—ASCORBIC ACID CONTENT OF COMMERCIALLY CANNED APPLE JUICE

Brand number	Type of Juice	Ascorbic acid per 100 ml.
		mg.
I	Crushed, deaerated, N_2 filled head space	0·2
		0·4
		0·4
II	Clarified, filtered	0·2
		0·2
III	Clarified	0·2
		0·2
IV	Clarified, filtered	0·3
		0·3

estimating such minute amounts of ascorbic acid the accuracy of the determination is reduced. It is interesting to note that even highly deaerated juices from which all except mere traces of air had been excluded during processing and in the final container failed to retain their ascorbic acid in a physiologically active form.

Factors Affecting Ascorbic Acid Content of Apple Juice

Data on the ascorbic acid content of the Newtown apples employed in this investigation are presented in table 6.

TABLE 6.—ASCORBIC ACID CONTENT OF NEWTOWN APPLES

Length of storage	Ascorbic acid per 100 g.	
	Fresh wt. basis	Dry wt. basis
	mg.	mg.
2 months at 0° C.—		
Maximum	10·2	66·5
Minimum	5·5	35·9
Average	7·3	47·6
4·5 months at 0° C.—		
Maximum	10·0	65·2
Minimum	5·0	32·6
Average	7·1	46·3
7 weeks at 15° C.—		
Maximum	2·5	15·8
Minimum	2·0	12·6
Average	2·3	14·6

It will be noted that during the 4½-month storage period at 0° C. the average ascorbic acid value of the apples remained practically constant at about 7 mg. of ascorbic acid per 100 grams of fresh apple tissue. As a matter of interest an analysis was made of some fruit which had become overripe but was still in reasonably good eating condition. This fruit had been held for 7 weeks at 15° C. The results presented in table 6 show that these apples had lost approximately two-thirds of their original ascorbic acid.

A number of experiments were conducted to secure information on the effect of processing on the ascorbic acid value of apple juice. The juice was analysed at each step in the various methods employed in the manufacture of canned or bottled juice. The results of these experiments are shown by the data presented in table 7.

TABLE 7.—EFFECT OF PROCESSING ON ASCORBIC ACID CONTENT OF APPLE JUICE

Treatment	Age of sample	Ascorbic acid per 100 ml.		
		Fresh	Canned	Bottled
		mg.	mg.	mg.
Fresh juice		7·6		
Deaerated		7·5		
Deaerated, pasteurized	3 hrs.		6·9	5·7
" "	2 wks.		0·2	
" "	4 wks.		0·2	0·3
Not deaerated, pasteurized	3 hrs.		6·9	5·5
" " "	2 wks.		0·2	
Fresh juice (stood 20 hrs. with pulp)		9·2		
Filtered with commercial filter aid		8·9		
Filtered juice, pasteurized	24 hrs.		4·6	3·1
" "	2 wks.		0·3	
" "	4 wks.		0·2	0·2
Juice clarified with Pectinol and filtered with filter aid		5·2		
Pasteurized	24 hrs.		4·6	
"	4 wks.		0·3	0·2
Juice clarified with Pectinol, deaerated		4·6		
Pasteurized	24 hrs.		4·6	
"	4 wks.		0·2	
Juice clarified with tannin and gelatin, filtered with filter aid		6·2		
Deaerated, pasteurized	24 hrs.		4·8	
" "	4 wks.		0·3	
Fresh juice double pasteurized at 87° and 85° C.	24 hrs.		5·5	
	2 wks.		0·4	

The figures in table 7 indicate that deaeration had little effect on the ascorbic acid content. Filtering with a commercial filter aid such as "Super-cell" or "Celite" caused a slightly greater loss. The usual clarification employing "Pectinol" or tannin-gelatin and filtering with a commercial-type diatomaceous filter aid tended to cause a reduction in original ascorbic acid of 1 to 2 mg. per 100 ml. of juice. In general it may be noted that flash pasteurization produced less than a 10 per cent loss in ascorbic acid. Deaeration tended to be beneficial to retention during pasteurization but the results are not entirely consistent, possibly due to quantities of air being trapped in the container at time of sealing. Greater loss of ascorbic acid occurred in the glass packed juice. As indicated in table 7 the difference between the canned and bottled product after pasteurizing was approximately 1·3 mg. of ascorbic acid per 100 ml. of juice. The slow cooling of the bottled juice is probably a factor in this regard.

It is significant that regardless of the deaeration, pasteurization, filtering, and clarification processes employed, the ascorbic acid content of the juice

diminished rapidly after canning. In every instance the ascorbic acid value was reduced to a fairly constant level of 0·2 mg. per 100 ml. in two weeks or less.

The use of antioxidants was investigated to determine if they would assist in retention in the processed product of ascorbic acid originally present in the fresh juice. Thiourea and cysteine hydrochloride were used. The data obtained with these two antioxidants are given in table 8. Evidence presented in table 8 shows that the antioxidants used were definitely ineffective. No ascorbic acid was found naturally present in the reduced state.

TABLE 8.—EFFECT OF ANTIOXIDANT ON RETENTION OF ASCORBIC ACID

Treatment	Age of sample	Ascorbic acid per 100 ml.		
		Fresh	Canned	Bottled
		mg.	mg.	mg.
0·05% thiourea treated slices, macerated 20 hrs., juice deaerated	.....	8·0	.....	.....
Pasteurized	3 days	.....	4·2	.....
"	2 wks.	.....	0·4	.....
0·05% thiourea treated slices, juice deaerated, pasteurized	24 hrs.	.....	4·0	.....
	2 wks.	.....	0·6	.....
Deaerated juice plus 0·01% cysteine, pasteurized	24 hrs.	.....	.....	3·6
	4 wks.	.....	.....	0·2
Deaerated juice plus 0·01% thiourea, pasteurized	25 hrs.	.....	.....	3·6
	4 wks.	.....	.....	0·2

In order to determine if it were possible to obtain an increased concentration of ascorbic acid from the fruit in the juice, an experiment was carried out in which the macerated fruit was allowed to stand about 20 hours before pressing. This produced a dark coloured juice. Analyses were made of the expressed juice and resulting pomace. The juice was found to have an ascorbic acid content of 7·2 mg. per 100 grams on the fresh-weight basis or 54·5 mg. per 100 grams on the dry-weight basis. The pomace contained 13·0 mg. of ascorbic acid per 100 grams on the fresh-weight basis or 65·4 mg. per 100 grams on the dry-weight basis. This treatment did not noticeably increase the ascorbic acid content of the juice. The pomace apparently retains an appreciable amount of ascorbic acid which may possibly be accounted for by the skins in the pomace. The experiment was repeated with thiourea treated slices. Only a slight increase in ascorbic acid was noted in the expressed juice over that normally obtained as previously shown.

While the usually recommended temperatures for flash pasteurization of apple juice are in the range of 82° to 88°C., temperatures as low as 74° C. have been employed to some extent. Furthermore, the actual flash pasteurization is only a matter of a few seconds. The final sterilization is accomplished by holding the sealed container for varying times, usually 1 to 5 minutes before rapid cooling. Thus, the product is at an elevated temperature for a very short time. In view of this, experiments were undertaken to determine the point of inactivation of the ascorbic acid oxidizing enzyme in the flash pasteurization technique. Raw juice was pasteurized directly into cans at temperatures ranging from 74° C. to 93° C. The cans (16-oz.) were filled full and sealed. This operation was carried out as rapidly as possible. The cans were immediately cooled. Thus, the juice was at an elevated temperature for only about 60 seconds altogether. It required approximately 5 minutes to cool the canned juice. To samples of this canned juice were added quantitative amounts of pure ascorbic acid in the form of an aqueous solution. In Test A each sample contained 0·1 mg. of ascorbic acid per ml. of liquid while in Test B the amount of ascorbic

acid added was increased to 0·4 mg. of ascorbic acid per ml. The samples were then incubated for 7 hours at 30° C. at the end of which time aliquots were taken for the estimation of reduced ascorbic acid. Samples of raw and thoroughly boiled juice were similarly treated for control purposes. This juice had a pH value of 3·4. The results are presented in table 9.

TABLE 9.—INACTIVATION OF ASCORBIC ACID OXIDIZING ENZYME BY FLASH PASTEURIZATION

Treatment	Temperature	Loss of added ascorbic acid	
		Test A	Test B
	°C.	%	%
Pasteurized	73·9	94·4	52·5
"	76·7	83·4	.....
"	79·4	83·4	38·6
"	82·2	83·4	40·0
"	87·8	83·8	.....
"	90·5	83·6	.....
"	93·3	83·8	.....
Boiled	100·0	83·8	38·6
Unheated	.....	100·0	100·0

It may be readily seen that under the conditions of the experiment the enzyme was inactivated at a temperature of 76·7° C. The enzyme was still active after pasteurization at 73·9° C.

To assist in interpretation of data on the ascorbic acid retention in apple juice, numerous gas analyses were carried out on both commercial and experimental processed juice. In table 10 are presented the results of analyses of a number of commercial canned apple juices.

TABLE 10.—GAS CONTENT OF COMMERCIAL CANNED APPLE JUICE

Brand number	Sample number	Can volume	Gas per 100 ml. juice including head space at 0° C. (760 mm.)			
			Total gas	CO_2	O_2	N_2
		oz.	ml.	ml.	ml.	ml.
I	1	12	3·84	0·44	0·00	3·40
	2	12	1·70	0·59	0·00	1·11
	3	12	1·01	0·37	0·00	0·64
II	1	20	2·46	1·22	0·00	1·24
	2	20	2·17	1·07	0·00	1·10
	3	48	*5·00	*2·94	0·00	2·06
	4	48	1·96	0·79	0·00	1·17
III	1	10	1·14	0·57	0·00	0·56
	2	10	1·87	0·67	0·00	1·20
	3	20	1·79	0·71	0·00	1·08
	4	20	2·32	1·06	0·00	1·26
	5	20	3·38	0·76	0·00	2·62
	6	48	2·11	1·20	0·00	0·91
	7	48	2·27	1·21	0·00	1·06
IV	1	10	5·61	3·04	0·00	2·57
	2	26	*10·55	*6·83	0·00	3·72
	3	48	*3·71	*1·19	0·00	2·52

*Estimate only, as volume too great to measure accurately.

The data in table 10 show a variation in gas content from can to can, and between cans of different sizes. Also certain brands contain markedly more gas than others indicating quite variable fills with quantities of entrapped

air. No oxygen was found as it rapidly reacts with the juice constituents or the tin container. An approximate estimate of oxygen may be obtained by multiplying the figure given for nitrogen by the factor 0·265. This is assuming that the gases are present in the proportion of air which is only true of the entrapped gas. It will be noted that carbon dioxide constitutes a large proportion of the gas present in canned apple juice. It is indicated that a properly filled can contains close to 1·0 ml. of nitrogen per 100 ml. juice. It should be pointed out that Brand No. I is a crushed type deaerated product with a nitrogen-filled head space. This will account for the lower carbon dioxide content and porportionately high nitrogen content. The other brands are clear juices not deaerated.

In table 11, data are given on the gas content of processed apple juice prepared under experimental conditions. Care was taken to fill the cans or bottles full.

It is again shown that a well-filled can of non-deaerated juice contains approximately 1·0 ml. of nitrogen gas per 100 ml. of juice. The bottled product shows a trifle less gas than the canned product. Deaeration resulted in much lower gas content. The thiourea-prepared deaerated processed juice tended to have a slightly lower gas content than a comparable sample of juice which did not contain thiourea. Some variation in gas content may also be noted with the other treatments. Carbon dioxide gas is again shown to make up half or more of the gas present in the preserved product.

Table 11.—GAS CONTENT OF PROCESSED APPLE JUICE UNDER EXPERIMENTAL CONDITIONS

Treatment	Can or bottle volume	Gas per 100 ml. juice including head space at 0° C. (760 mm.)			
		Total gas	CO_2	O_2	N_2
		ml.	ml.	ml.	ml.
Pectinol, not filtered or deaerated	10-oz. can	2·20	0·94	0·00	1·25
	"	1·81	0·97	0·00	0·84
	12-oz. bottle	1·46	0·76	0·00	0·70
Tannin-gelatin, not filtered or deaerated	10-oz can	2·10	1·02	0·00	1·08
	"	2·09	1·02	0·00	1·07
	12-oz. bottle	2·00	1·02	0·00	0·97
Pulp filtered, deaerated	10-oz. can	1·36	0·88	0·00	0·48
	"	1·53	0·88	0·00	0·65
	12-oz. bottle	1·31	0·68	0·00	0·64
Pectinol Celite filtered, deaerated	10-oz. can	1·16	0·75	0·00	0·41
	12-oz. bottle	1·04	0·54	0·00	0·50
Thiourea (0·05%) treated slices, deaerated	10-oz. can	0·82	0·49	0·00	0·33
	"	0·72	0·50	0·00	0·22
	12-oz. bottle	0·61	0·49	0·00	0·12
Strained only, double pasteurized	16-oz. can	2·39	1·86	0·00	0·53
	12-oz. bottle	1·42	0·96	0·00	0·46
Strained only, pasteurized	16-oz. can	2·67	1·85	0·00	0·83
	12-oz. bottle	1·94	1·35	0·00	0·60

Gas analyses were made under commercial processing conditions in two factories. Plant 1 employed a patented deaerated process for crushed type juice with nitrogen filled head space in the container. Plant 2 produced the usual highly clarified non-deaerated canned juice. The results of this investigation are shown in table 12.

TABLE 12.—GASES IN APPLE JUICE AT VARIOUS STEPS IN COMMERCIAL PROCESSING

Steps in juice processing	Gas per 100 ml. juice at 0° C. (760 mm.)			
	Total gas	CO_2	O_2	N_2
	ml.	ml.	ml.	ml.
PLANT 1—				
After pasteurizing and prior to filling into can	0·34	0·13	0·02	0·19
After pasteurizing but juice filled into can at 8-inch height, then poured from one can to another prior to analysing	0·38	0·09	0·01	0·28
After pasteurizing, canned (12-oz.) nitrogen-filled head space	2·18	0·47	0·00	1·71
PLANT 2—				
Fresh expressed juice	1·89	0·65	0·08	1·16
After filtering	3·54	2·07	0·37	1·09
After pasteurizing	1·89	1·08	0·06	0·75
After pasteurizing, canned (48-oz.) including head space (duplicate samples)	*3·84	*1·39	0·48	1·97
	1·92	0·39	0·32	1·28

* Estimate only as volume too great to measure accurately.

It will be noted from table 12 that deaeration of the product in Plant 1 was very efficient. Figures given for oxygen of a magnitude of 0·03 or less are insignificant as has been previously shown in discussion of the methods of gas analysis. Air is not very readily picked up by the juice while at the pasteurizing temperature as is indicated by comparison of results secured with rough and regular methods of filling cans.

In Plant 2 considerable air is indicated to have been absorbed during filtering. This is probably greater than is normal due to failure to maintain a continuous flow at this particular time resulting in some air being pumped into the juice. Pasteurizing reduced the gas content but several samples of the canned juice contained large quantities of gas due to excessive head space. Some carbon dioxide gas is possibly produced in the juice during clarification with Pectinol.

A study was made of the gases present in apple juice at each step in processing under experimental conditions. Care was exercised to have all cans filled full. A summary of the data secured is presented in table 13.

As shown in table 13, fresh expressed apple juice prepared by grating the fruit and expressing in a hydraulic press contained 2·18 ml. of total gas of which carbon dioxide made up about one-third and nitrogen the remainder.

TABLE 13.—GASES IN APPLE JUICE AT VARIOUS STEPS OF PROCESSING UNDER EXPERIMENTAL CONDITIONS

Steps in juice processing	Gas per 100 ml. juice at 0° C. (760 mm.)			
	Total gas	CO_2	O_2	N_2
	ml.	ml.	ml.	ml.
Fresh juice	2·18	0·73	0·03	1·41
After pasteurizing, not deaerated, canned, or cooled	1·10	0·61	0·03	0·45
After pasteurizing and sealing in 16-oz. cans	1·39	0·80	0·05	0·54
Fresh juice expressed from thiourea treated slices	1·96	0·50	0·06	1·39
After pasteurizing deaerated juice, not canned	0·25	0·09	0·02	0·13
After pasteurizing deaerated juice and sealing in 16-oz. cans	0·60	0·36	0·03	0·21
Juice kept in open container for 3 hours at 0° C.	2·16	0·82	0·06	1·28
Juice kept in open container for 10 hours at 0° C.	2·08	0·68	0·18	1·22

Only traces of oxygen were found in fresh apple juice, due apparently to extremely rapid reaction of oxygen with the juice. However, when apple juice was allowed to stand in an open container for a few hours, particularly at cold temperatures, oxygen was found in the juice although there was little actual change in total gas content of the juice. It will be noted that flash pasteurization expelled a quantity of the gases present, reducing the content in the juice by at least 50 per cent.

Fortification

Extensive investigations were conducted on the fortification of apple juice with pure crystalline ascorbic acid.

To secure evidence on the stability of the crystalline substance when mixed into the juice prior to pasteurizing the following experiment was undertaken. Juice was clarified by the enzyme method using the preparation Pectinol A and also by the tannin and gelatin method. These clarified juices were employed for the tests. Ascorbic acid crystals (Merck) were added at the rate of 25 mg. per 100 ml. to 2,000 ml. volumes of the juice, dissolved and thoroughly mixed by gently submerged agitation. A portion of this mixture was allowed to stand open to the air at 20° C. for a definite period. This was repeated with previously deaerated juice. Another portion was agitated vigorously for a minute or two, then allowed to stand for nearly two hours when it was again violently agitated for 15 minutes. Analyses were made for both reduced ascorbic and dehydroascorbic acid. The reduced ascorbic acid determined was also calculated as percentage of the crystalline acid originally added. The results of this experiment are shown in table 14.

From the data given in table 14, it is clear that ascorbic acid (reduced form) is fairly rapidly lost, being changed to the reversibly oxidized form. After 3 hours about 68 per cent was retained in non-deaerated juice; at the end of 24 hours only 21 to 27 per cent remained. Agitation under conditions of this experiment was without noticeable effect. Deaeration tended to increase the retention of ascorbic acid. However, there was still present after 24 hours at least 90 per cent of the total physiologically active ascorbic acid.

Table 14.—STABILITY OF CRYSTALLINE ASCORBIC ACID IN APPLE JUICE

Juice treatment	Analysis per 100 ml.			Retention of reduced ascorbic acid
	Ascorbic acid	Dehydro-ascorbic acid	Total	
	mg.	mg.	mg.	%
Pectinol Clarified—				
1. Held without agitation, 3 hrs.	17·0	9·5	26·5	68·0
Held without agitation, 24 hrs.	6·9	15·3	22·2	27·6
2. Agitated, held for 2 hrs.	16·9	9·3	26·2	67·6
3. Deaerated, held without agitation for 3 hrs.	17·5	8·4	25·9	70·0
Tannin-gelatin Clarified—				
1. Held without agitation, 3 hrs.	19·6	5·3	24·9	78·4
Held without agitation, 24 hrs.	5·4	19·9	25·3	21·6
2. Agitated, held for 2 hrs.	17·3	7·2	24·5	69·2
3. Deaerated, held without agitation for 3 hrs.	18·7	5·2	23·9	74·8

Information was secured on the effect of canning on the retention of added ascorbic acid. The concentration of ascorbic acid employed was 25 mg. per 100

ml. of juice. In most instances the crystals were mixed into the juice by gentle stirring immediately prior to pasteurizing, the delay being not greater than 15 minutes. In a few instances the crystals were added to each can. Analyses were made at short intervals. The results are presented in table 15.

TABLE 15.—EFFECT OF CANNING ON RETENTION OF ADDED ASCORBIC ACID

Juice treatment	Storage	Analysis per 100 ml.			Retention of	
		Ascorbic acid	Dehydro-ascorbic acid	Total	Reduced ascorbic acid	Total ascorbic acid
	days	mg.	mg.	mg.	%	%
ASCORBIC ACID MIXED IN JUICE—						
Pectinol clarified	1	18·0	9·7	27·7	72·0	.....
	14	16·5	2·7	19·2	66·0	76·8
	28	17·2	1·6	18·8	68·8	75·2
Pectinol clarified, deaerated	1	18·7	8·9	27·6	74·8	.....
	14	17·4	3·1	20·5	69·6	82·0
	28	17·7	2·4	20·1	70·8	80·4
	42	18·8	0·3	19·1	75·2	76·4
Tannin-gelatin clarified	1	17·6	11·5	29·1	70·4	.....
	14	17·8	3·3	21·1	71·2	84·4
	28	17·8	2·6	20·4	71·2	81·6
	42	19·1	0·3	19·4	74·6	77·6
Tannin-gelatin clarified, deaerated	1	18·7	12·1	30·8	74·8	.....
	42	18·9	0·2	19·1	75·6	76·4
ASCORBIC ACID ADDED TO EACH CAN—						
Pectinol clarified	1	16·8	8·0	24·8	68·3	.....
	14	18·1	1·8	19·9	73·6	80·8
	28	16·6	1·6	18·2	67·4	74·0

It may be seen from table 15 that approximately 30 per cent loss in ascorbic acid occurred on canning. There was little further decrease in ascorbic acid during the next 42 days. It is indicated that the ascorbic acid lost on pasteurization was oxidized to dehydroascorbic acid. The dehydroascorbic acid it will be noted almost entirely disappears by the end of 42 days so that the reduced form represents all the ascorbic acid present in the fortified apple juice. Deaeration had slight beneficial results. Samples of bottled juice treated similarly to the canned juice retained on pasteurization approximately 2 per cent less ascorbic acid than comparable canned samples. However, as mentioned later in this bulletin, the bottled juice retained more ascorbic acid after a few months' storage than the similarly canned product. It may also be noted that there was as much loss of ascorbic acid when the hot pasteurized juice was filled into the containers in which crystalline ascorbic acid had just previously been placed.

In table 16 are presented data concerning the effect of thiourea and cysteine on the retention of added ascorbic acid in canning. The crystals were added at the rate of 25 mg. per 100 ml. of juice. It may be seen from comparison with data given in table 15 that these antioxidants were not very effective in increasing the retention of ascorbic acid.

TABLE 16.—EFFECT OF ANTIOXIDANTS ON RETENTION OF ADDED ASCORBIC ACID IN CANNING

Juice treatment	Storage	Analysis per 100 ml.			Retention of	
		Ascorbic acid	Dehydro-ascorbic acid	Total	Reduced ascorbic acid	Total ascorbic acid
	days	mg.	mg.	mg.	%	%
Clarified plus ·01% cysteine	1	18·5	11·0	29·5	74·0	.....
	28	15·7	0·8	16·5	62·8	66·0
Clarified plus ·01% thiourea	1	18·5	11·3	29·8	74·0	.....
	14	16·9	3·3	20·2	67·6	80·8
	28	15·3	1·2	16·5	61·2	66·0
Unclarified plus ·01% thiourea	1	16·3	14·4	30·7	65·2	.....
	14	16·1	3·2	19·3	64·4	77·2
	28	14·9	1·0	15·9	59·6	63·6
Unclarified, deaerated plus ·01% thiourea	1	16·5	13·3	29·8	66·0	.....
	28	15·9	1·5	17·4	63·6	69·6
·05% thiourea treated slices, juice deaerated	1	20·6	9·4	30·0	82·4	.....
	14	21·0	2·0	23·0	84·0	92·0
	28	19·4	3·3	22·7	77·6	90·8

In table 17 are shown the results of analyses of fortified canned apple juice after three to four months' storage at 20° C. The ascorbic acid was added to each container at the rate of 20 mg. per 100 ml. of juice unless otherwise noted. The data show that under ordinary conditions at least 55 per cent of the ascorbic acid is retained. Deaeration and thiourea treatment appeared to increase retention to some extent. Thiourea treated juice on the average contained 1 to 2 mg. more of dehydroascorbic acid per 100 ml. of juice than did juice not so treated. It may be mentioned that bottled samples of fortified juice showed in most instances 10 to 20 per cent more retention of ascorbic acid after storage than did comparable canned samples. Occasionally there was no difference in degree of retention.

TABLE 17.—EFFECT OF THREE TO FOUR MONTHS' STORAGE AT 20° C. ON RETENTION OF ADDED ASCORBIC ACID IN CANNED APPLE JUICE

Juice Treatment	Analysis per 100 ml.			Retention of	
	Ascorbic acid	Dehydro-ascorbic acid	Total	Reduced ascorbic acid	Total ascorbic acid
	mg.	mg.	mg.	%	%
Rough filtered	10·8	0·1	10·9	54·8	55·3
Rough filtered, double pasteurized	11·3	0·2	11·5	57·3	58·3
Pectinol clarified, deaerated once	10·9	0·6	11·5	55·3	58·3
Tannin-gelatin clarified, deaerated once	11·8	.....	.....	59·9	.....
Rough filtered, deaerated twice	13·8	.....	.....	70·0	.....
Pectinol clarified, deaerated twice	12·8	1·2	14·0	65·0	71·1
	*27·8	0·6	28·4	70·7	72·2
·05% thiourea treated slices, deaerated once	†12·4	0·9	13·3	62·0	66·4
	*29·6	1·9	31·5	75·3	80·1
·05% thiourea treated slices, deaerated twice	12·7	1·3	14·0	64·5	71·0
	*28·3	2·4	30·7	72·0	78·1

* Ascorbic acid added at rate of 40 mg. per 100 ml.

† Ascorbic acid mixed in juice at rate of 20 mg. per 100 ml.

A few experiments were carried out to obtain information on the retention of added crystalline ascorbic acid to canned apple juice under commercial conditions. Two commercial plants were selected. Plant 1 produces a patented crushed type of thoroughly deaerated juice employing plain type L tin-plate cans and filling the head space of the container with nitrogen. A gas analysis indicated this product was free of oxygen. Plant 2 is typical of those plants producing the usual highly clarified non-deaerated canned juice. Type L tin-plate double enamel lined cans were used. In each case ascorbic acid crystals were added to the container at the rate of 20 to 25 mg. of ascorbic acid per 100 ml. of juice just prior to filling with flash pasteurized juice. Temperature of pasteurization was 82° to 85° C. The results of this experiment are presented in table 18.

TABLE 18.—FORTIFICATION OF COMMERCIAL APPLE JUICE WITH CRYSTALLINE ASCORBIC ACID

Juice treatment	Ascorbic acid added per 100 ml.	Analysis per 100 ml.			Retention of	
		Ascorbic acid	Dehydro-ascorbic acid	Total	Reduced ascorbic acid	Total ascorbic acid
	mg.	mg.	mg.	mg.	%	%
Plant 1—						
Patented crushed type juice, deaerated, N_2 filled head space	24·4	22·6	0·2	22·8	92·6	93·4
After 3 months (12-oz. can)	24·4	23·5	0·5	24·0	96·3	98·3
Plant 2—						
Clarified, filtered juice, not deaerated	24·6	13·7	1·8	15·5	55·7	63·0
After 2 months (26-oz. can)	24·6	12·1	2·1	14·2	49·3	57·7

It will be noted that after three months' storage the product entirely free of oxygen retained at least 92 per cent of the added ascorbic acid in the reduced state. Only traces of dehydroascorbic acid were present in this product. Retention of reduced ascorbic acid in the non-deaerated product with some head space was markedly poorer being only about 50 per cent after two months. The variation between the duplicate samples may in all probability be attributed to differences in head space with varying quantities of entrapped air. Again the total ascorbic acid present was almost entirely in the reduced form.

DISCUSSION

Ascorbic Acid Content of Fresh and Commercially Canned Apple Juice

The results of the investigation of ascorbic acid content of processed apple juice substantiate the early work of Fellers, Cleveland, and Clague (1933). Fresh apple juice has been shown to be as rich in ascorbic acid content as the fresh apple from which it was expressed. There is a continual loss in ascorbic acid but even after 48 hours' storage in a cool place (12° C.) the juice still retained 71 per cent. Only the reversibly oxidized (dehydro) ascorbic acid was found to be present in apple juice. It is apparent that the reduced ascorbic acid is oxidized to this form within a matter of a few seconds of crushing of the cellular fruit tissue. The scientific literature indicates that there is generally more than sufficient oxygen present in apple tissue to completely oxidize all the ascorbic acid normally present in the fruit. However, the dehydroascorbic acid is reasonably stable in the unpreserved juice for a day or two.

In every instance irrespective of method of processing or type of apple juice packed, only negligible quantities of ascorbic acid (0·4 mg. or less per 100 ml.) were found after a short storage period. These findings are in general agreement with the literature. Ordinary canned apple juice is thus apparently valueless as an antiscorbutic product.

Factors Affecting Ascorbic Acid Content of Apple Juice

An interesting point about the ascorbic acid in apple juice is that a large proportion of it is still retained after the various clarification processes and pasteurization but that it is almost entirely lost on storage of two weeks or less in the can or bottle. It would appear that the dehydroascorbic acid is very unstable, being subject to fairly rapid destruction by non-enzymic catalysis. This is also indicated in the studies on fortification. Kertesz, Dearborn, and Mack (1936) have indicated that non-enzymic catalysis occurs to varying degrees in heated vegetable extracts.

Antioxidant preparations of juice employing cysteine and thiourea failed to improve the retention of ascorbic acid. This possibly further indicated that the loss of ascorbic acid was due to factors present in the juice itself other than oxygen or enzyme since evidence was also secured indicating that under commercial pasteurizing conditions the ascorbic acid oxidizing enzyme was inactivated at temperatures below 76·7° C., whereas these experiments were carried out at 82° to 85° C. Thiourea and cysteine had the disadvantage that they resulted in a very poor product, cysteine producing an especially disagreeably flavoured juice. Fellers (1941) in a private communication (unpublished data) stated that they had found that several other antioxidants failed to conserve ascorbic acid in grapefruit juice.

Gas analysis of commercial samples indicated wide variation in fill of containers with resultant entrapping of considerable quantities of air which could be expected to materially increase the possibility of loss of ascorbic acid. If excessive air were entrapped it might cause deterioration in flavour and undesirable changes in the juice with even probable deleterious effects upon the container such as pin-holing or separation of the enamel lining from the tin-plate. With regard to this latter point there is apparent variation in the stability of any one enamel. Separation has been noted in certain packs of apple juice. This would be accentuated by the presence of excessive quantities of oxygen as shown by Clark and Lachele (Lueck and Pilcher, 1941) in the case of lemon and orange juice. According to these authors, the oxygen content should not exceed 0·10 per cent by volume which is a very small amount. In terms of nitrogen this is equivalent to 0·377 ml. of nitrogen per 100 ml. of juice, assuming the gases are present in the same proportion as they are in air. This can only be attained in canning apple juice under extremely exacting processing conditions where the can is filled full and little or no spilling occurs as is indicated in table 13. If allowance is made for the nitrogen present in the juice prior to sealing the can, it is readily seen that only about 0·10 per cent by volume of nitrogen is entrapped in the can during closing which is equivalent to 0·026 per cent of oxygen. However, by referring to the tables of gas analyses under commercial conditions of ordinarily packed apple juice, it is noted that even under the very best possible conditions at least 0·50 per cent by volume of nitrogen is entrapped. Usually the amount is considerably greater than this.

It appears, however, that a nitrogen content of 1·0 to 1·25 ml. per 100 ml. of canned juice which may be obtained under commercial conditions where a little care is exercised, or even a quantity slightly in excess of this, causes no deleterious effects either on flavour or appearance of the juice, or upon the container itself. That it does allow loss of ascorbic acid is convincingly shown in tables 17 and 18. The exact amount of air in canned apple juice that may

cause a decrease in quality of the juice or materially affect the can was not determined. If a plain can is being used for a crushed type of apple juice, oxygen must be almost entirely eliminated to prevent action on the container or discoloration of the product.

Fortification

The results of this investigation show definitely the feasibility of the fortification of processed apple juice with crystalline ascorbic acid. With certain precautions and depending upon the exact method employed, retention after several months' storage may be expected to be at least 50 per cent and possibly as much as 96 per cent. This is true when ascorbic acid is added at the rate of 20 mg. per 100 ml. or more. The maximum retention was attained with the highly deaerated crushed type of juice with nitrogen-filled head space. Deaeration of clear juice and filling the containers full was only slightly more effective in retaining the ascorbic acid than employing non-deaerated juice. These latter two procedures retained only 55 to 70 per cent of the ascorbic acid, however, compared with the 92 to 96 per cent retention in the deaerated nitrogen-treated product. It is possible that the plain tin type of can exerts some effect in retention of the ascorbic acid due to the stannous ions which may consequently be present in the juice. Mixing the ascorbic acid crystals into the juice (preferably deaerated juice) by submerged agitation not more than 15 to 20 minutes prior to pasteurization resulted in only a small loss of ascorbic acid. No increase in retention of ascorbic acid in the canned product was obtained by the difficult procedure of adding quantitative amounts of the crystals to the container prior to filling with pasteurized juice. This, together with the fact that bottled juice had slightly better retention of ascorbic acid than deaerated carefully canned juice and that deaerated juice canned with nitrogen-filled head space had by far the best retention, strongly indicates that the loss of ascorbic acid is directly associated with the oxygen entrapped in the head space at time of closing the container.

In fortifying canned apple juice with crystalline ascorbic acid, a loss of at least 8 to 10 mg. of added ascorbic acid may be expected per 100 ml. of juice when processed under the commercial methods at present generally employed. This loss occurs irrespective of the quantity of ascorbic acid added. Hence the percentage loss is less when greater amounts of ascorbic acid are used. Fortification of deaerated nitrogen packed juice results in a loss of only 2 to 3 mg. of ascorbic acid per 100 ml. of juice.

The greatest loss of ascorbic acid occurs at time of sealing in the can or within a few hours following canning. It appears to be largely due to enclosing some air in the sealing operation.* This results in partial oxidizing of a portion of the ascorbic acid to the reversibly oxidized (dehydro) ascorbic acid which as previously pointed out, rapidly disappears in canned apple juice. Therefore, to produce a fortified canned apple juice retaining most of the added ascorbic acid it would be necessary to employ deaerated apple juice and fill the head space with an inert gas such as nitrogen or purified steam. Leuck and Pilcher (1941) have indicated that vacuum closing of the container did not entirely remove the oxygen. They state that the last traces of oxygen must be eliminated with a stream of inert gas forced into the head space during assembly of can and

* Since the preparation of this bulletin, W. H. Fitzpatrick, J. A. Powers, and C. R. Fellers presented a paper on the "Ascorbic Acid-Oxygen Relationships in Glass Packed Foods" to the American Chemical Society meeting at Atlantic City, N.J., on September 8, 1941, which supports this contention. These investigators showed that the total decrease in ascorbic acid is approximately proportional to the enclosed oxygen. Their results indicate that high storage temperature and light exposure merely accelerate the ascorbic acid-oxygen reaction in glass packed foods, the final total loss of ascorbic acid being unaffected by these factors.

cover. Fellers and Buck (1941) have recently shown that storing canned food products at lowered temperatures (2·2° C.) retains ascorbic acid to a greater extent as well as resulting in superior flavour retention.

The addition of crystalline ascorbic acid to apple juice had no detectable effect on the flavour of the juice but it did produce a lighter colour due to the reducing action of the vitamin. It tended to make cloudy juices more attractive. With tannin-gelatin clarified juices it tended to produce too pale a colour.

The feasibility of fortification of apple juice by blending with a natural substance rich in ascorbic acid such as the juice of black currants is worthy of investigation. Black currant juice has been shown by Sills (1939), Charley, Curtis, and Sills (1937) to contain 135 to 223 mg. of ascorbic acid per 100 ml. of juice with a probable mean value of 190 mg. Furthermore, these investigators as well as Fawns (1939) have shown the ascorbic acid in black currant juice to be comparatively stable. Black currants are grown fairly extensively in certain portions of Canada. Experiments carried out at the Summerland Experimental Station a few years ago and reported by Strachan and Atkinson (1939) showed that a blend of two or three of apple juice to one of a sweetened (35° Balling) black currant juice made a very acceptable beverage. By calculation it would appear possible to produce a blended product containing approximately 30 to 40 mg. of ascorbic acid. If this was largely retained on processing it would be a relatively good source of the vitamin. Black currants at present are comparatively expensive. Under certain conditions, however, the manufacture of this product might become economically feasible.

SUMMARY

A study has been made of (*a*) the ascorbic acid content of fresh and commercially canned apple juice, (*b*) factors affecting the ascorbic acid content during preparation, processing and storage of the canned or bottled apple juice, and (*c*) the feasibility of ascorbic acid fortification of processed apple juice.

Fresh Newtown apple juice was found to be practically as rich in ascorbic acid as the fresh fruit, containing about 7 mg. of ascorbic acid per 100 ml. of juice. After 48 hours the juice still retained 70 per cent of its ascorbic acid. Commercially canned apple juice contained about 0·2 mg. of ascorbic acid. Only the reversibly oxidized form of ascorbic acid (dehydroascorbic acid) was found in apple juice.

While an appreciable amount of ascorbic acid was retained on canning it rapidly disappeared so that in two weeks or less the ascorbic acid value was only 0·2 mg. per 100 ml. Apparently the reversibly oxidized (dehydro) ascorbic acid is unstable in apple juice. Deaeration and the use of antioxidants failed to have any beneficial effect.

Evidence was secured indicating that the ascorbic acid oxidizing enzyme in apple juice was inactivated under commercial conditions at a flash pasteurizing temperature of 76·7° C. but was still active at 73·9° C.

Gas analysis of commercially canned apple juices showed them to vary markedly in their gas content indicating quite variable fills.

Freshly expressed apple juice obtained from a hydraulic press contained about 2·0 ml. of total gas, 0·7 ml. of carbon dioxide, 1·4 ml. of nitrogen, and only a trace of oxygen per 100 ml. of juice. Flash pasteurizing alone reduced this by 50 per cent or more. Under very exacting conditions canning increased the nitrogen content of the juice by about 0·10 ml. per 100 ml. of juice. Under best commercial conditions however the nitrogen increased on canning by 0·5 ml. per 100 ml. of juice. Properly filled cans contained 1·0 to 1·25 ml. of nitrogen per 100 ml. of juice.

The greatest loss of ascorbic acid in fortified apple juice appears to be directly due to the enclosing of some air (oxygen) in the head space of the container in the closing and sealing operation.

The feasibility of fortifying processed apple juice with crystalline ascorbic acid at the rate of 20 mg. or more per 100 ml. of juice is shown. The conditions under which this may be best accomplished are indicated. Briefly, the ascorbic acid crystals are dissolved and carefully mixed into the deaerated juice which is then flash pasteurized and canned without delay; or the ascorbic acid is dispensed directly into each can immediately prior to filling with flash pasteurized juice. All but possibly minute traces of oxygen are removed from the container when sealing by displacement with an inert gas. Juice fortified commercially under these specified conditions retained after three months' storage over 90 per cent of the added ascorbic acid in the reduced state.

ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation to Dr. C. R. Fellers, Head of the Department of Horticultural Manufactures, Massachusetts State College, Amherst, U.S.A., for his encouragement and many helpful suggestions offered throughout the course of this investigation. Grateful acknowledgment is made to Mr. R. C. Palmer, Superintendent, Dominion Experimental Station, Summerland, B.C., for his co-operation in carrying out the work at this station and for his advice and criticism in preparation of the manuscript. The writer is indebted to Mr. F. E. Atkinson of the Summerland Station for suggestions and assistance in certain phases of the study. Acknowledgment is also made to Mr. R. W. Arengo-Jones of the Central Experimental Farm, and Mr. F. Johnston of the Division of Chemistry, Science Service, for their helpful criticism of the manuscript.

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