EFFECT OF TIME, TEMPERATURE, AND LEVEL OF ASCORBIC ACID FORTIFICATION ON THE QUALITY OF CANNED APPLE JUICE

DISSERTATION

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By

Mohamed Ibrahim Mahmoud, B.Sc., M.Sc.

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The Ohio State University

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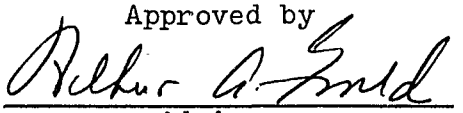
Reading Committee:

Wilbur A. Gould
Jean R. Geisman
Andrew C. Peng

Approved by



Adviser
Department of Horticulture

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INTRODUCTION

The fortification of apple juice with ascorbic acid is of considerable interest to both the manufacturer and consumer. This is especially of interest to the consumer because apple juice is naturally low in this vitamin and it is used interchangeably with juices high in ascorbic acid in the diet. It is the contention of some groups that it would be desirable to fortify juices low in ascorbic acid to make them more comparable with those rich in this vitamin to benefit the consumer by providing a significant vitamin C intake regardless of the choice of single fruit juice.

In recent years, new information and new trends in food product development and consumer eating habits have been causing a marked increase in consumer interest in nutrition. This consumer interest has already resulted in an increased demand for control of nutrient levels in the food manufacturing and marketing chain and culminated in the publishing of nutritional labeling law.

Nutritional labeling is continuing to gain momentum; and it is mandatory when a nutrient, such as ascorbic acid, is added to the food. If a processor is to make a claim for

the nutrient content of his product, he should know the rates of loss of, for example, a particular vitamin during processing and storage.

The loss of nutritional value during storage, however, has received very little study from a kinetic standpoint. Numerous papers reported losses of vitamins during storage, nevertheless, the most common method of reporting the effect of storage on vitamins has been simply to express the nutrient content as a percentage of the initial amount present. These data do not allow the determination of kinetic parameters necessary to describe the time-temperature effects on the nutrients.

Today, however, a knowledge of the reaction rate and kinetics of storage induced losses are needed desparately in order to predict the extent of deterioration of important nutritive factors during storage. A very recent survey of research and development needs in food processing revealed that the need for kinetics of nutrient degradation in the thermal processing and storage of foods ranked the 27th among 81 needs (71).

It was the purpose of this study to investigate the effect of addition of ascorbic acid on the retention of this vitamin in apple juice after specified storage conditions and duration and to extend the findings to the prediction of ascorbic acid retention in the fortified juice. Few studies

(19, 38, 62, 80, 106) have determined the retention of ascorbic acid in fortified apple juice mostly after storage at room temperature and employing only one fortification level. No conclusive studies, however, have been done to adequately predict the retention of ascorbic acid in fortified apple juice from a kinetic standpoint.

The objectives of this study were to determine:

1. the effect of the factors time, temperature and fortification level on the retention of ascorbic acid in apple juice,
2. the interaction of these factors during storage of the juice,
3. the effect of fortification on pH, titratable acidity, soluble solids, ^{o}Brix: acid ratio and vacuum during storage; and
4. kinetics degradation of ascorbic acid in apple juice in order to predict the retention of the vitamin in the fortified juice.

REVIEW OF LITERATURE

Since early colonial days in North America, large but unknown quantities of barreled apple juice have been made by small farms. It was about 1939 when commercial packs of canned and bottled apple juice were produced in the United States and Canada. Since that time apple juice has gradually increased in importance until it now constitutes a substantial portion of the fruit juice pack (101).

Product Description:

The definition of canned apple juice was promulgated on June 26, 1950 by U.S. Department of Agriculture in the U.S. Standards for Grades of canned apple juice, paragraph 52.301 as follows:

> Canned apple juice is the unfermented liquid prepared from the first pressing juice of properly sound, fresh apples, excluding the liquid obtained from any additional residual apple material. Such apple juice is prepared without any concentration, without dilution, or without the addition of sweetening ingredients; may be processed with or without the addition of antioxidant; and is sufficiently processed by heat to assure preservation of the product in hermetically sealed containers.

Packs produced under this description in 1973 totaled 15,623,000 cases of different container sizes (7) or enough

juice to provide 816,519,000 eight-ounce servings.

The natural ascorbic acid content of canned apple juice ranges from 0.2 to 3.6 mg. per 100 ml. of the juice (14, 15, 21, 52, 62, 106). Since fruit juices are the primary source of ascorbic acid in the diet, the enrichment of processed fruit juices naturally low in ascorbic acid has a sound nutritional basis and therefore has become accepted as common practice.

Apple juice as a medium for ascorbic acid enrichment:

Apple juice is an excellent choice as a carrier of ascorbic acid into the diet. Ascorbic acid is completely soluble in the juice and is more stable in an acid medium (14). Bauernfeind (14), Bunnel (21) and Siemers (103) recommended the standarization of fruit juices naturally low in ascorbic acid to provide an adequate intake of ascorbic acid regardless of consumer preference since these fruit juices are used interchangeably with those of high ascorbic acid content. The Canadian government specifications for vitamin C-enriched apple juice require a level of 35 mg. of ascorbic acid per 100 ml. of the juice (2, 6, 62).

Several investigators (2, 3, 4, 6, 19, 38, 56, 62, 80, 106) have proposed the fortification of apple juice with ascorbic acid. Andreae (6) indicated that ascorbic acid decomposition is not extensive in apple juice canning as

neither extreme heat is applied nor is the juice exposed to strong light. He also pointed out that apples do not contain ascorbic acid oxidase, which is specific for ascorbic acid oxidation; but, an enzyme system of similar nature and this system is partially inactivated by pasteurization and, fortunately, it is bound to the suspended tissue particles of the juice.

In addition to its nutritional value, ascorbic acid has been demonstrated to be effective in protecting apple juice from oxidative changes during the milling and pressing operations. Holgate et al. (56) reported that the addition of ascorbic acid during or immediately after crushing of the apples but before pressing retarded darkening and cloudiness of the juice. Similarly, Atkinson and Strachan (64) observed increased lightening of apple juice when apples were milled with ascorbic acid. In addition, Esselen et al. (38) indicated that the addition of ascorbic acid to bottled apple juice had a marked effect in lightening the color of the juice and retarding darkening during storage, thus, improving its appearance. Marshall (82) has also observed some protective action of ascorbic acid in apple juice; and Lee et al. (80) reported that ascorbic acid added to apple juice retarded development of brown color and slightly improved the flavor. Similarly, the addition of ascorbic acid to conventional unpasteurized apple juice to be frozen

prevented the development of a musty odor and helped to retain more of the original flavor (79).

Ascorbic acid, an essential nutrient:

Ascorbic acid is essential to man and other species including the guinea pig, who are unable to synthesize or store any significant quantity within the body, and when deprived of a dietary source for a sufficient length of time, develop scurvy (5, 14, 49, 68).

The therapeutic properties of ascorbic acid had been recognized since 1545. In 1753 Captain James Lind, physician to the British Fleet, published his paper on the successful treatment of scurvy by supplementation of the diet with oranges and lemon (49). This led ultimately to the issue of lime juice to men of the British navy.

At present, well-defined scurvy is uncommon in the United States (5, 68), and most of the population maintains a satisfactory tissue saturation (68), however, occasional cases of scurvy occur among infants fed exclusively on cow's milk (5), and small children and elderly people as a result of consuming severely restricted diets (68).

Since ascorbic acid is not stored in the body, it is necessary that the diet contains a regular, adequate supply of this vitamin. A daily intake of 10 mg. ascorbic acid is sufficient to alleviate and cure clinical signs of scurvy

in humans (54). This level, however, is not satisfactory for the preservation of health over long periods (5, 68). Actual utilization in the average adult human male shows that an intake of 30 mg. per day is more than sufficient to replenish the quantity of ascorbic acid metabolized daily (11, 12), and 40 mg. per day would satisfy the requirements of the largest healthy man (12).

Most studies suggest that 60 to 100 mg. ascorbic acid per day are needed in normal persons to maintain tissue saturation (99) or to provide a tissue concentration characteristic of good diets (68). The current recommended allowance for adult men is 60 mg. per day and 55 mg. per day for adult women. The RDA for children is 40 mg. per day and for infants under one year is 35 mg. per day; about the same amount supplied daily from 850 ml. of mother's milk in the United States (100), however, under certain conditions of continued stress and drug therapy there may be an increased need for ascorbic acid (11). Large amounts of ascorbic acid (0.5 to 1.5 mg. daily) have also been associated with undesirable side effects in certain situations (77, 91).

In addition to its antiscorbutic properties, ascorbic acid is necessary for wound healing (99), and neuromuscular regeneration (53). Ascorbic acid is also a known cofactor in hydroxylation of proline (112) and lysine (94), in collagen synthesis, hydroxylation of tryptophan to 5-hydroxytryptophan,

conversion of 3,4-dihydroxyphenylethylamine to norepinephrine, hydroxylation of p-hydroxyphenyl pyruvate to homogenistic acid in the metabolic pathway of tyrosine, hydroxylation of certain steroids synthesized in the adrenal gland (11), and stimulation of cholesterol oxidation (47). Recent studies have also indicated that ascorbic acid has an effect, although minor, on the duration and severity of the common cold (23, 35, 65).

In relation to other nutrients, ascorbic acid has been demonstrated to assist in the utilization of iron (37, 48, 96) and to afford a significant sparing or protective effect on several vitamins in the B-complex, and on vitamins A and E (68).

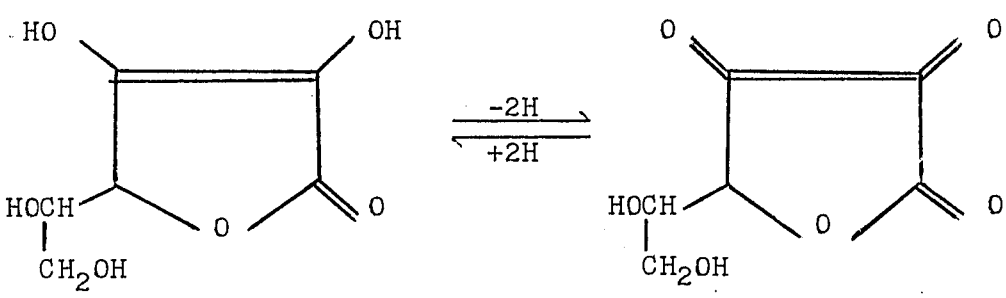
Although the need for ascorbic acid is well known, deficiencies among adults and children are reported in relatively large numbers. In 1971, the U.S. Department of Health, Education, and Welfare reported a major deficiency of ascorbic acid among poor Whites and Negroes in Kentucky, West Verginia, Texas, and Louisiana (114). Among all individuals living below poverty levels who were included in the study, 7.2 percent were listed as deficient or low. In the above poverty group, 4.3 percent were deficient or low.

Physical and chemical properties of ascorbic acid:

L-ascorbic acid, a 6-carbon compound, is an optically active crystalline solid melting at 192°C. It is freely soluble in water, but it is almost insoluble in oil (14). L-ascorbic acid is the generic name for L-threo-2,3,4,5,6-penta-hydroxy-2-hexenoic acid-4-lactone, also known as vitamin C, cevitamic acid and hexuronic acid (51). In structure it strongly resembles a simple sugar but is modified to contain an enediol and acid lactose group (14).

The unusual properties of the molecule are due to the enediol grouping (15). Normally it reacts as a monobasic acid with pK_a 4.25 in water (51). It is moderately strong reducing compound, is acidic in nature, and forms neutral salts with bases (15).

Ascorbic acid is reversibly oxidized to dehydroascorbic acid in the presence of oxygen by both enzymic and non-enzymic catalysts.



(reduced form)
ascorbic acid

(oxidized form)
dehydroascorbic acid

Dehydroascorbic acid has full vitamin C activity, but is more thermo-labile than ascorbic acid (52). It is further broken down irreversibly to 2,3 diketogulonic acid which has no biological activity (18, 51, 52, 81).

Aerobic oxidation of ascorbic acid is the catalytic effect of copper which is enhanced by iron (109, 110), resulting in the formation of dehydroascorbic acid and hydrogen peroxide (51, 52). The hydrogen peroxide produced in this reaction further reacts with ascorbic acid and the copper catalyst to give directly or indirectly oxygen and water (18, 52). Ferrous and ferric ions are less effective catalysts than cupric ion in aqueous solution (51), however, ferrous ions play an important role in the oxidation of ascorbic acid by hydrogen peroxide (25, 93).

The oxidation is inhibited by chelating or complex compounds such as EDTA, flavonoids, polybasic and poly-hydroxy acids by virtue of their ability to complex with metal ions (18, 51). The rate of aerobic oxidation is pH dependent exhibiting maxima at pH 5.0 and pH 11.5 (51).

Anaerobic destruction of ascorbic acid follows a period of relatively rapid oxidative degradation (15, 52). The rate of this reaction, in contrast to the aerobic degradation, hardly varies from pH 1-11 (42). However, a slight but definite maximum occurs at the pH range 3-4 (57), coincident with the pK_a value, and suggests the existence of a complex

between ascorbic acid and its monohydrogen anion (42). The reaction is accelerated by fructose, fructose-6-phosphate and fructose-1,6-diphosphate. Sucrose also increases the rate of the reaction, probably following its hydrolysis to fructose (57).

During high acidic anaerobic degradation, ascorbic acid yields approximately equimolar amounts of carbon dioxide and furfural (44, 45). The carbon dioxide evolved originates mainly from the lactonized carboxyl group of the ascorbic acid molecule (24, 43). The reactions occurring in the pH range of foods, with an optimum pH near the pK_a of ascorbic acid and the fructose-promoted reaction, may give 2,5-dihydro-2 furoic acid as the major product (29, 58), in addition to carbon dioxide.

Although copper does not appear to catalyze the anaerobic degradation of ascorbic acid (1), lead and aluminum are the most powerful inorganic catalysts of anaerobic degradation (45), but were effective only at concentrations far in excess of these likely to be found in fruit juices.

Aerobic and anaerobic degradation of ascorbic acid have also been shown to produce brown pigments. In the presence of sugars (31), amino acids (63, 116), and organic acids (27, 78) complex oxidation and decarboxylation of ascorbic acid may occur with subsequent polymerization and formation of colored compounds. The rate of the browning is increased

when available ascorbic acid is converted to dehydroascorbic acid (61).

Loss of ascorbic acid from canned foods:

Losses of ascorbic acid commence as soon as the fruit is harvested (52). These losses are mainly due to enzymatic reactions of which ascorbic acid oxidase, peroxidase, cytochrome oxidase and phenolase are the most significant (18, 81). In the tree-borne fruit, these enzymes are balanced by reductase systems. However, when this enzyme balance is disturbed by cellular disruption, as occurs during the extraction of fruit juices, the oxidases become free to react with ascorbic acid (18, 52, 81).

In the early stages of storage of canned foods, the degree of ascorbic acid destruction appears to be associated with aerobic oxidation (39, 40), the extent of which is largely determined by the amount of oxygen present in the food and in the headspace of the can (17, 18, 66). Bender (17) indicated that one ml. of oxygen in the headspace or included in solution destroys 15.7 mg. of ascorbic acid.

It is believed that free oxygen combines with the components of the product, including ascorbic acid, or the tin and steel of the container and the free oxygen is used up producing a highly reduced system within a few days after packing in plain tin cans (39, 40). In the case of

non-metallic containers the product combines with the oxygen (39, 40). Consumption of free oxygen by tin of the plain tin can is favorable to ascorbic acid retention (85).

After the disappearance of free oxygen from the stored canned foods, usually within a month of sealing, subsequent loss of ascorbic acid is due to anaerobic decomposition (57, 58).

Several workers (16, 17, 20, 42, 43, 44, 57, 58, 64, 66) have investigated the rate of aerobic and anaerobic destruction of ascorbic acid in model systems and in natural food products. All have indicated that ascorbic acid degradation follows a first-order reaction with respect to ascorbic acid concentration. While Huelin (57) indicated that the velocity constant of the anaerobic decomposition of ascorbic acid is not more than one thousandth that of the oxidation under optimum conditions, Kefford et al. (66) pointed out that a period of rapid aerobic loss of ascorbic acid in pasteurized orange juice is followed by a period of slow anaerobic loss at a rate of about one tenth of that in the first period, however, the total loss of ascorbic acid in anaerobic reactions soon exceeds the total loss from oxidative reactions.

The rate of aerobic and anaerobic destruction of ascorbic acid is influenced by time and temperature of storage and by the nature of the product (18, 19, 28, 40, 52). Data obtained

by several investigators indicate that for optimum ascorbic acid retention, storage at 10°C (50°F) or below is preferred.

Effect of refrigerated temperatures:

Ross (92) observed excellent retention of ascorbic acid in canned orange juice kept under refrigeration (44.6 to 51.8°F). At this temperature, he found 98 percent retained after 3 months, 95 percent after 6 months and 94 percent after 12 months. Guerrant et al. (50) held tomato juice at 30° and 42°F and reported 92 percent retention of ascorbic acid after one year of storage at both temperatures. When Moschette et al. (86) stored orange juice at 50°F, they observed 97 percent retention of ascorbic acid after one year of storage while pineapple juice retained essentially all the ascorbic acid content. Similarly, Sheft et al. (102) held citrus juices at 50°F for 18 and 24 months. They found that orange juice retained 96 percent and 95 percent of its ascorbic acid content after 18 and 24 months respectively, while pineapple juice retained 108 percent of ascorbic acid after 18 and 24 months and grapefruit juice retained 93 percent and 94 percent respectively, however, grapefruit juice stored for 2 years was unpalatable. Feaster et al. (41) also reported that canned orange juice stored for one year at 30°, 40° and 50°F retained 100 percent, 99 percent and 96 percent respectively of its ascorbic acid

content. He further indicated that storage of the juice at temperatures lower than 50°F would not improve the ascorbic acid retention to an extent greater enough to warrant the additional cost involved.

Recently, Pope (90) held unfortified and fortified tomato juice with different levels of ascrobic acid concentrations for 9 months. He observed that retention of ascorbic acid was from 86 percent to 98 percent at 35°F and from 85 percent to 95 percent at 50°F depending on the initial levels of ascorbic acid concentration.

Several workers have well established that small sacrifices of ascorbic acid may accompany 70°F or room temperature storage of canned fruit and vegetable juices (16, 20, 22, 28, 30, 40, 41, 50, 60, 74, 76, 83, 86, 90, 92, 102). Although actual losses of ascorbic acid varied from 10 to 20 percent upon one year's storage at 70° or at room temperature, the product was still considered to be an excellent source of ascorbic acid. Lamb (74) indicated that the average rate of ascorbic acid loss at 70°F was approximately one percent a month. Ross (92) pointed out that between 10° and 27°C (50° and 80.6°F) storage temperature the rate of ascorbic acid loss in orange juice doubled for each 10-degree rise in temperature.

Effect of elevated temperatures:

As the temperature of storage was increased above 70°F, retention of ascorbic acid decreased very rapidly, the rate of decrease being influenced by the product. Ross (92) stored canned orange juice up to 12 months at 99°F. At the end of 6 months, the juice retained only 31 percent of the initial ascorbic acid content, whereas the retention of ascorbic acid decreased to 16 percent after one year of storage. He further indicated that increasing the temperature from 27° to 37°C (80° to 99°F) nearly quadrupled the rate of ascorbic acid loss. Similarly, Bender (17) concluded that the rate of ascorbic acid loss in fruit squash was three times as rapid at 37°C as at room temperature.

Guerrant et al. (50) held tomato juice at 85° and 110°F for one year and reported that only 20 percent of ascorbic acid was present in juices stored at 110°F for one year. He further observed that, at 85°F the loss of ascorbic acid was about half of that occurring while the product was maintained at 110°F for the same length of time. Similarly, Moschette et al. (86) pointed out that grapefruit juice kept at 98°F for one year retained only 31 percent of its ascorbic acid content whereas those juices stored at 80°F for the same period of time retained 75 percent of ascorbic acid.

The effect of time on ascorbic acid retention at elevated temperatures can be readily seen from the data obtained by

Brenner at al. (20). They reported that the retention of ascorbic acid in orange juice stored at 90° and 100°F after 12 months was 54 and 26 percent respectively, and at the end of 18 months, it was 38 and 11 percent respectively. When Sheft et al. (102) held citrus juices at 80°F, they found that orange and grapefruit juices each retained 62 percent of ascorbic acid after 18 months of storage while orange juice retained 50 percent and grapefruit juice retained 57 percent after 24 months. Pineapple juice, however, showed better retention at this temperature, with 90 and 79 percent retention after 18 and 24 months respectively.

Pope (90) reviewed the literature concerning retention of ascorbic acid in canned tomato juice and indicated that storage at elevated temperatures resulted in lower stability of ascorbic acid. In addition, Pope (90) also held tomato juice, unfortified and fortified with varying concentrations of ascorbic acid, at 88° and 108°F. Depending on the initial levels of ascorbic acid concentration, he reported that ascorbic acid retention was from 56 to 77 percent after 9 months of storage at 88°F while the product retained from 17 to 35 percent of ascorbic acid when maintained at 108°F for 6 months.

It has been noted that an average storage temperature of 99°F is an abnormal average temperature so far as the

commercial warehousing of canned foods is concerned (28, 40, 84), except for some tropical regions and central continental areas during the summer months (70, 84). Monroe et al. (84) made a comprehensive temperature study for commercial warehouses at which detailed temperature observations, maximum and minimum temperatures were recorded. They indicated that the yearly average temperatures of cans in storage, even in areas of high prevailing temperatures, did not reach 80°F. However, in a survey of New Jersey food processors (95), it has been found that processors used the following procedure to determine shelf life: twelve weeks at 120°F provides acceleration 10:1 over 70°F storage and eighteen months at 100°F provides acceleration of 2 to 3:1 over 70°F storage. For adequate determination of shelf life, it has been suggested that 150-200 cans from each sample lot be stored at temperatures ranging from 80° to 120°F.

Fluctuating temperatures have been evaluated by Monroe et al. (84). They pointed out that retention of vitamins at the fluctuating temperatures encountered in commercial warehouses were of the same order as the samples stored at constant temperatures when the yearly average temperatures of the warehouses were comparable with the constant temperatures.

Factors affecting ascorbic acid content of apple juice:

The ascorbic acid content of apple juice is expected to be affected by the concentration of ascorbic acid in the fresh fruit and losses due to processing and storage.

The natural ascorbic acid content of apples depends on cultivar, stage of ripeness, growing environment, season and storage conditions.

A very marked variation in the ascorbic acid content of apples, not only between cultivars but also within the cultivar, has been reported by many investigators. Hulme (59) tabulated values of a trace to 34 mg. ascorbic acid per 100 grams of the fresh fruit of various cultivars taken from various sources. Smock and Neubert (104) reported 10 to 15 mg. ascorbic acid per 100 grams of the fresh fruit of Grimes Golden and Jonathan apples (both cultivars being used in this study) grown in West Virginia; while Jonathan apples grown in New York contained 11 to 17 mg. ascorbic acid per 100 grams of the fruit. Murneek et al. (88) observed 7.0 to 8.2 mg. ascorbic acid per 100 grams of Jonathan apples grown in Missouri.

Light intensity has been reported by several authors to greatly influence the ascorbic acid content of the fresh fruit. Highly colored fruit and fruit exposed to greater light intensity possessed higher values of ascorbic acid as compared with less colored fruit or fruit grown in the shade

(36, 88, 89, 111). Murneek et al. (87) and Murphy (89) also found considerable difference in ascorbic acid content between the shaded and exposed (to direct light) halves of the apple. The distribution of ascorbic acid varies markedly in different portions of the apple. Kidd and West (67) showed that the peel contains three to five times as much ascorbic acid as the pulp. Eheart (36) and Murneek et al. (87) found that smaller apples are higher in ascorbic acid than larger ones of the same cultivar.

As the apples mature and then ripen on the tree, the ascorbic acid content increases and reaches a maximum at physiological maturity then diminishes in the overripe fruit (59, 87, 88). Seasonal differences are also considerable. In general, summer cultivars have a higher ascorbic acid content than fall and winter cultivars (87, 88, 111).

Plant nutrition has also been associated with observed differences. It has been noted that high soil nitrogen supply resulted in a decrease in ascorbic acid content of the fruit (87, 88, 104), whereas spraying or brushing the fruit while still on the tree with calcium chloride solution resulted in a significant increase in ascorbic acid (13).

Length of storage and temperature employed also markedly affect ascorbic acid content of the fruit (59, 87, 88, 104). Smock and Neubert (104) found that apples stored at 32°F showed little or no loss of ascorbic acid during a six months'

period but those stored at 45°F lost one fourth of their original amount. Hulme (59) indicated that loss of ascorbic acid was somewhat higher in the gas-stored than in the ordinary cold-stored fruits. Thus, fresh apples can be expected to vary in ascorbic acid content depending on cultivar, maturity, season, growing environment and storage conditions.

Processing effects on ascorbic acid content of apple juice have been extensively studied by Strachan (106). During the manufacture of apple juice ascorbic acid may be oxidized to dehydroascorbic acid which is further broken down to degradation products with no vitamin C activity. The oxidation is enzymatic and non-enzymatic and is catalyzed by copper ions. The rate of oxidation is a function of dissolved oxygen, enzyme content, dissolved copper and temperature of the juice. Andreae (6) and Strachan (106) indicated that there is sufficient oxygen in apple tissue to completely oxidize all the ascorbic acid normally present in the fruit upon crushing of the cellular tissue.

Extraction of the juice involves both grinding of the apples and pressing the pulp (4). Both operations incorporate air in the juice. The destruction of ascorbic acid in apple juice seems to be due to the presence of peroxides which are formed very rapidly after the pulp is exposed to the atmosphere (4). Polarographic analysis showed that there was

practically no oxygen in apple juice shortly after pressing but there was a definite quantity of peroxides which were a definite factor in the destruction of ascorbic acid in apple juice (4). These peroxides are the product of enzyme action.

Strachan (106) determined the effect of the different unit operations employed in the manufacture of apple juice on the natural ascorbic acid content of the juice. The freshly expressed juice contained 7.6 mg. ascorbic acid per 100 ml. He indicated that deaeration had little effect on the ascorbic acid content. Both deaerated and non-deaerated juices lost 10 percent of ascorbic acid after 3 hours of juice extraction. Filtering with a commercial filter aid caused a slightly greater loss. Clarification of the juice with enzyme (pectinol) and filtering with filter aid caused 32 percent loss of ascorbic acid, while clarification with tannin-gelatin and filtering with filter aid resulted in 19 percent loss of ascorbic acid. Flash pasteurization at 85°C produced about 10 percent loss, whereas double pasteurization at 87°C (188.6°F) and 85°C (185.0°F) resulted in 24 percent loss of ascorbic acid. He further indicated that regardless of the deaeration, clarification, filtration and pasteurization processes employed, the ascorbic acid content of the juice diminished rapidly after canning and 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. Strachan (106) concluded that the loss of ascorbic acid in canned apple juice was due to factors present in the juice itself other than oxygen or enzyme since the ascorbic acid oxidizing enzyme was inactivated at a temperature of 76.7°C (170°F) during pasteurization.

Johnston (62) observed similar results with unfortified apple juice. The ascorbic acid content of the juice was 0.8 mg. per 100 ml. of the juice after 3 hours of processing and it was reduced to 0.3 mg. per 100 ml. after 2 weeks of storage at room temperature. Esselen et al. (38) also reported 0.88 mg. ascorbic acid per 100 ml. of unfortified apple juice before processing. When the processed juice was stored at room temperature (70-80°F) the ascorbic acid content was 0.95 and 0.10 mg. per 100 ml. after 6 and 12 months of storage respectively. It must be remembered that in estimating such minute amounts of ascorbic acid the accuracy of the determination is reduced.

Retention of ascorbic acid in fortified apple juice:

Since canned apple juice is low in ascorbic acid, the fortification of apple juice with ascorbic acid has been the subject of considerable interest.

Several methods have been suggested for fortifying apple juice (4): 1) adding crystalline ascorbic acid to the juice before pasteurizing, 2) adding tablets of ascorbic acid to

the containers previous to filling, 3) injecting measured quantities of ascorbic acid solution into the containers previous to filling, 4) injecting measured quantities of ascorbic acid solution into the juice just before pasteurizing and 5) adding ascorbic acid to apples at the grinder.

Strachan (106) was first to report on the feasibility of fortification of processed apple juice with ascorbic acid. He observed no difference in ascorbic acid retention when crystalline ascorbic acid was added to the juice before pasteurization or when it was placed in the container prior to filling with the hot pasteurized juice. Both treatments resulted in about 30 percent loss of ascorbic acid after one day as well as after 4 weeks of canning. However, Johnston (62) presented data indicating that the addition of ascorbic acid to apple juice after pasteurization, which inactivates ascorbic acid oxidizing enzymes, resulted in better retention after 3 months of storage at room temperature. This treatment resulted in 83 percent retention of ascorbic acid compared to 73 percent when crystalline ascorbic acid was added before pasteurization. Nevertheless, both treatments produced the same retention of ascorbic acid (90 percent) after 3 hours of canning. Similarly, Esselen et al. (38) fortified apple juice by placing special ascorbic acid tablets in the cans previous to filling and reported 90 percent retention after canning.

Oxygen has been found to be the most important factor that brings about the destruction of ascorbic acid in fortified apple juice. Attempts were made to lessen or eliminate its effect by deaeration of the juice or filling the headspace of the containers with an inert gas such as nitrogen or purified steam. Strachan (106) indicated that deaeration of the fortified apple juice tended to slightly increase the retention of ascorbic acid. He reported that the retention of ascorbic acid in non-deaerated juice with same headspace was about 50 percent after 2 months of storage at 68°F while it was from 92 to 96 percent in the deaerated, nitrogen-filled headspace product after 3 months of storage at the same temperature. Aitken (2) also used nitrogen gas for mixing a concentrated solution of ascorbic acid in apple juice. He pointed out that bubbling nitrogen gas into the juice tank during addition of ascorbic acid tended to remove the dissolved oxygen and prevented further incorporation of air. However, this treatment resulted in about 10 percent loss of ascorbic acid at the end of the run six hours later. Similarly, Johnston (62) presented data indicating that even vacuumization of apple juice, either before or after addition of ascorbic acid, resulted in about 10 percent loss of ascorbic acid after 3 hours of canning. He concluded that slack-filling with one-quarter of an inch before sealing markedly reduced the retention of ascorbic acid.

Effect of time and temperature of storage:

Few studies have been conducted to evaluate the effects of time and temperature of storage on the retention of ascorbic acid in fortified apple juice. Most studies reported the effect of room temperature storage.

Strachan (106) stored fortified apple juice containing 20 mg. ascorbic acid per 100 ml. of the juice and reported 55-70 percent of ascorbic acid can be retained under ordinary conditions of manufacturing after 3 months of storage at 68°F. Johnston (62) also held apple juice fortified with ascorbic acid at the rate of 35 mg. per 100 ml. of the juice and observed 77-82 percent retention of ascorbic acid after 4 months and 58-64 percent retention after 10 months of storage at room temperature. Similarly, Andreae (6) reported 75-80 percent retention of ascorbic acid in fortified apple juice after 4 months of storage without specifying storage temperature. In addition, Esselen et al. (38) obtained similar results with apple juice fortified with 50 mg. ascorbic acid per 100 ml. of the juice. They reported 73, 65 and 67 percent retention of ascorbic acid after 6, 9, and 12 months of storage respectively, at room temperature (70-80°F). When Lee et al. (80) held fortified apple juice containing 40 mg. ascorbic acid per 100 ml. of the juice at 18°, 34°, and 70°F, they observed 85, 80, and 75 percent retention respectively after 11 months of storage.

Bauernfeind and Pinkert (15) reviewed trials of apple juice fortification and reported 76-81 percent retention after 6 months of storage at 70-75°F. Higher results were observed by Brenner et al. (19) who reported 95 percent retention after both 3 and 6 months storage at room temperature. They also held fortified apple juice at 100°F and reported 75 and 50 percent retention after 3 and 6 months storage respectively.

Prediction of ascorbic acid stability:

Few studies (6, 62, 106) have been conducted, by Canadian investigators, for the purpose of predicting the retention of ascorbic acid in fortified apple juice. Strachan (106) indicated that a loss of at least 8 to 10 mg. of added ascorbic acid is expected per 100 ml. of the juice processed under commercial methods and this loss occurs irrespective of the quantity of ascorbic acid added; without presenting actual trials. Andreae (6) pointed out that 20-25 percent loss of added ascorbic acid in apple juice is expected after 4 months of storage and predicted no further loss will take place during further storage. Johnston (62) also predicted a loss of 15 mg. of ascorbic acid added per 100 ml. of the juice in processing and storage at room temperature during one year of storage. On the basis of the data obtained by these investigators, Andreae (6) and Johnston (58)

recommended that ascorbic acid be added to apple juice at the rate of 50 mg. per 100 ml. of the juice to meet the Canadian Government standards of 35 mg. per 100 ml. of the juice at any time during one year storage.

Other workers have developed models for the prediction of ascorbic acid stability during storage from time-temperature data. Freed et al. (46) prepared nomographs for estimating ascorbic acid retention in canned vegetables, fruits and fruit juices. However, they indicated that these nomographs cannot be expected to give fully accurate results in the extremities of the temperature range as browning increases rapidly at the higher temperatures.

Kwolek and Bookwalter (72) developed plots showing contours of equal product quality as related to temperature and storage time. They showed that product quality at any time in storage could be given by:

$$Y = a + b_i t + u$$

where Y equals a measure of product quality (ascorbic acid concentration), a is the response at zero time, t is the time of storage, b_i is the slope or rate of change in Y per unit change in t associated with the i temperature level and u is a random error associated with the deviation of observed Y from the model. However, they suggested that maximum temperatures could cause deviation from the

prediction equation.

Wanninger (115) presented a mathematical model postulated for rate of degradation of ascorbic acid in food products. He stated that the Arrhenius equation is the most acceptable expression of the degradation of ascorbic acid.

Pope (90) derived a prediction formula for estimating ascorbic acid retention in fortified tomato juice based on the equation of first-order reaction. However, he stated that his data did not satisfactorily fit the Arrhenius expression for ascorbic acid degradation.

MATERIALS AND METHODS

Two cultivars of apples, Grimes Golden and Jonathan, were grown and harvested at the Horticulture Farm of the Ohio State University and transported to the Food Processing Pilot Plant of the Department of Horticulture.

Processing and Ascorbic Acid Fortification

The two apple cultivars were blended at the ratio of 1:1 by weight. The apples were sorted, washed and ground to a pulp suitable for juice extraction. The juice was then extracted in a hydraulic press by the rack-and-cloth procedure. It was immediately flash pasteurized at 205°F for 30 seconds and filled hot from an eight-gallon filler bowl. To each eight gallons of the juice was added 0, 50, 75, 100, or 150 ml. of a solution of 90 grams ascorbic acid (Merck U.S.P. ascorbic acid) in 500 ml. of the previously pasteurized and cooled apple juice drawn from the filler bowl. The added solution was calculated to increase the ascorbic acid concentration by 0, 30, 45, 60, or 90 mg. per 100 ml. of the juice. The juice was then mixed and filled in No. 303 fruit enamel cans. The cans were sealed and

coded so as to identify the fortification level referred to hereafter as level 0, 30, 45, 60, or 90. The cans were inverted and then held for 3 minutes prior to spin cooling in water to 100°F. The cans within each fortification level were then divided into five groups and stored at 32, 50, 68, 86, and 104°F.

Sampling and Measurements

Two random samples from each fortification level were taken after cooling prior to storage. In addition, two other random samples from each fortification level were drawn at 3, 6, 9, and 12 months storage at each storage temperature.

The following measurements were made on each sample: vacuum, pH, titratable acidity, soluble solids, and ascorbic acid. All measurements were made at room temperature after the juice was removed from storage and allowed to equilibrate with room temperature.

Vacuum was measured with a vacuum gauge and recorded to the nearest half inch of mercury.

The pH was measured with a glass electrode Beckman pH meter using 10 ml. of the juice diluted to 100 ml. with distilled water. The pH was recorded to the nearest hundredth.

Titratable acidity was determined by titrating 10 ml. of the juice diluted to 100 ml. with distilled water with

a one-tenth normal solution of sodium hydroxide to a pH of 8.2 according to the official method of analysis of AOAC (8) and calculated as malic acid according to the formula:

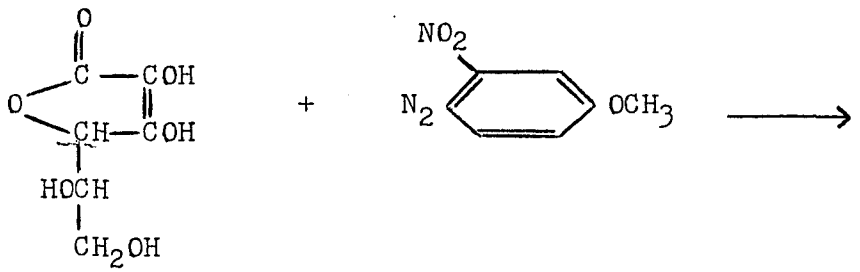
Percent malic acid =

$$\frac{(\text{No. ml. of NaOH})(\text{normality of NaOH})(0.067)}{10 \text{ ml. sample}} \times 100$$

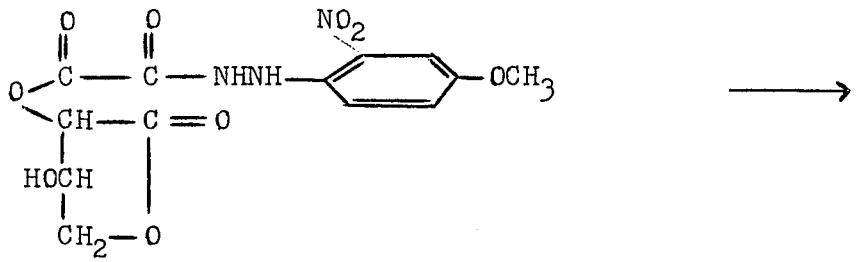
Percent soluble solids was determined by Abbé Refractometer which can be used to measure either refractive index or percent soluble solids of the sample. The refractive index of the sample was recorded and converted to percent soluble solids from an appropriate table after making corrections for the temperature. In addition, the ^{o}Brix:acid ratio was calculated by dividing the percent soluble solids by percent malic acid.

Ascorbic acid concentration was determined colorimetrically according to the method of Strohecker and Henning (107). The method was shown by Schmall et al. (97) to possess a high degree of specificity, rapid, simple and suitable for the assay of ascorbic acid in fruit juices fortified with ascorbic acid. The assay method involves the reaction of diazotized 4-methoxy-2-nitroaniline with ascorbic acid in acid medium (oxalic acid) followed by the addition of sodium hydroxide to yield an intensely blue color. In this reaction (98), ascorbic acid (I) and 4-methoxy-2-nitroaniline (II) undergo an oxidation-reduction reaction while the substituted benzene diazonium moiety forms a hydrazide. The ascorbic

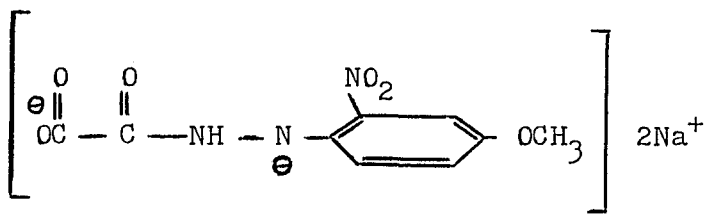
acid part suffers, in addition to oxidation, an opening of the furan ring. The resultant product is the 4-methoxy-2-nitrophenylhydrazide of the α-oxalate of D-threonic acid lactone (III). Further action of alkali on (III) yields finally the blue disodium oxalate derivative (IV).



(I) (II)



(III)



(IV)

This blue color, with a maximum absorbance at 570 nm, is compared with standards in a suitable colorimeter.

The 4-methoxy-2-nitroaniline reagent, referred to hereafter as amino reagent, was prepared by dissolving 500 mg. of the reagent in 125 ml. of glacial acetic acid and diluted to 250 ml. with sulfuric acid 10 percent W/V. This reagent is stable at room temperature for at least 2 months.

For the determination of ascorbic acid in the sample, 2 ml. of amino reagent were pipetted into 200 ml. volumetric flask, followed by 2 ml. of 0.2 percent sodium nitrite reagent. The solution was swirled to the disappearance of the orange color of the amino reagent; 75 ml. of alcohol were added, and the contents of the flask were mixed. Five ml. of 0.5 percent oxalic acid were added and the contents were mixed. A 5 ml. aliquot of the sample was added; the solution was mixed and rendered alkaline by the addition of 25 ml. of 10 percent soldium hydroxide. The volume was brought to mark with water and the contents again were well mixed. The blue color developed may be read after 1 minute without necessity of accurate timing, as it reaches its peak within less than a minute. This color is quite stable.

At the same time, reference solutions of ascorbic acid dissolved in 0.5 percent oxalic acid were made up and treated exactly in the same manner as the sample to establish

a standard curve. A sample blank also was prepared, containing the same amount of sample aliquot and all reagents with the exception of the amino reagent.

The absorbance of the sample, sample blank and ascorbic acid standard solutions was measured at 570 nm on a Bausch and Lamb Spectronic 20. The absorbance of the sample blank was subtracted from the sample absorbance to obtain a corrected sample absorbance. Concentration of ascorbic acid in the sample was calculated from a standard curve as shown in Figure 1. Ascorbic acid per 100 ml. of apple juice was recorded to the nearest 0.5 mg.

Only ascorbic acid was measured in this study, as it was found by Strachan (106), who studied the retention of ascorbic acid in fortified apple juice, that dehydroascorbic acid formed upon oxidation entirely disappears by the end of 42 days of storage and the reduced form represents all the ascorbic acid present in the fortified apple juice. Similarly, Brenner et al. (19) obtained convincing evidence that the reduction in ascorbic acid content in fortified foods which occurs on storage is due to a destructive loss of ascorbic acid rather than a conversion to the dehydro form and the breakdown process in the ascorbic acid molecule does not stop at the dehydro stage long enough to be detected. In addition, Deutsch and Weeks (32) who developed the micro-fluorometric assay of vitamin C, which is an official method

of analysis of AOAC, reported that their procedure, which measures both reduced and oxidized forms of ascorbic acid, gave results in fortified canned fruit juices and drinks statistically equivalent to those obtained by titration with 2,6-dichlorophenol-indophenol which measures only the reduced form of ascorbic acid.

Treatment of the Data

The effect of time, temperature and fortification level on each of the variables, ascorbic acid concentration as mg. per 100 ml. of the juice, percent ascorbic acid retention, pH, titratable acidity, soluble solids, ^{o}Brix:acid ratio and vacuum were determined. Percent retention of ascorbic acid was calculated as:

$$\text{Percent} = \frac{\text{Ascorbic acid measured during storage}}{\text{Initial concentration before storage}} \times 100$$

Percent retention was reported to the nearest one percent.

A factorial analysis of variance (105) was conducted at the Computer Science Department of the Ohio State University to evaluate the effect of the factors of time, temperature and fortification level and the effect of their interaction on each of the variables.

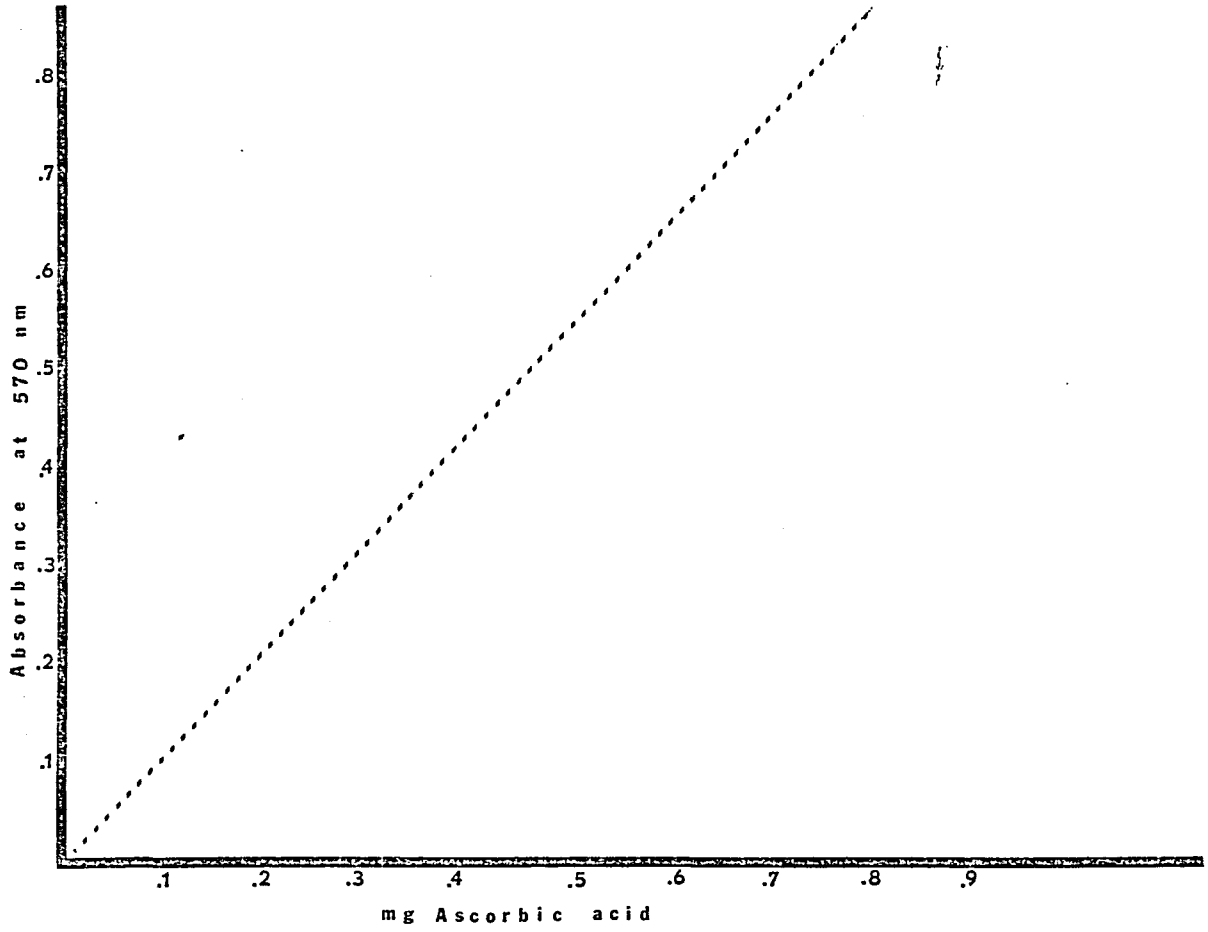


FIGURE 1: Standard curve of ascorbic acid

PRESENTATION OF RESULTS

Data Analysis

The analysis of data collected at 0, 3, 6, 9 and 12 months of storage for all storage temperatures is shown in Tables 1, 2 and 3. Factors significantly affecting the concentration of ascorbic acid (mg/100 ml. of the juice) were fortification level, temperature and storage time. Interaction of these factors significantly altered ascorbic acid concentration.

Percent retention was also significantly altered by time, temperature of storage and fortification level. Again interaction of these factors significantly affected the retention of ascorbic acid. The factors and their interactions shown in Tables 1 and 2 are listed in the order of their effect on ascorbic acid concentration (Table 1) and retention (Table 2).

Those factors which altered titratable acidity, pH, soluble solids content, ^{o}Brix: acid ratio and vacuum are shown in Table 3. Complete analysis of variance tables are presented in Appendix A.

TABLE 1: Analysis of variance - concentration of ascorbic acid in apple juice (mg/100 ml)

Source	Sum of squares	DF	Mean square	F value	Significance
Fortification level	122803.246	4	30700.811	11266.354	.01
Temperature	19725.584	4	4931.396	1809.686	.01
Time	17040.224	4	4260.056	1563.323	.01
Fortification x temperature interaction	8876.416	16	554.776	203.587	.01
Temperature x time interaction	8483.056	16	530.191	194.565	.01
Fortification x time interaction	7676.076	16	479.754	176.057	.01
Fortification x time x temperature interaction	3869.944	64	60.467	22.190	.01
Error	340.625	125	2.725		

TABLE 2: Analysis of variance - percent retention of ascorbic acid in apple juice

Source	Sum of squares	DF	Mean square	F value	Significance
Time	114602.188	4	28650.547	3041.503	.01
Temperature	75212.017	4	18803.004	1996.102	.01
Fortification level	48146.336	4	12036.584	1277.787	.01
Temperature x time interaction	23903.421	16	1493.964	158.597	.01
Fortification x time interaction	20664.680	16	1291.542	137.108	.01
Fortification x temperature interaction	3323.052	16	207.690	22.048	.01
Fortification x temperature x time interaction	23507.098	64	367.298	38.991	.01
Error	1177.483	125	9.419		

TABLE 3: Significance of the effects of the factors fortification level, time and temperature and their interactions on the variables pH, titratable acidity, soluble solids, °Brix: acid ratio and vacuum

Factor	pH	Titratable acidity	Soluble solids	°Brix: acid ratio	Vacuum
Fortification level	.01	.01	.01	.01	.01
Time	.01	.01	.01	.01	.01
Temperature	.01	.01	.01	.01	.01
Fortification x time interaction	.01	.01	.01	.01	.01
Fortification x temperature interaction	.01	.01	.01	.01	NS*
Time x temperature interaction	.01	.01	.01	.01	.01
Fortification x temperature x time interaction	.01	.01	.01	.01	.01

*NS = Not significant

Retention of Ascorbic Acid in Unfortified Apple Juice

Data on the ascorbic acid content and retention in the unfortified apple juice after processing and during storage are presented in Tables 4 and 5.

It may be seen from data in Tables 4 and 5 that the unfortified juice contained 1.5 mg. of ascorbic acid per 100 ml. of the juice after processing and cooling. At the end of 3 months storage retention was not different between juice stored at either 32°F or 50°F; both juices retained 67 percent or 1.0 mg. ascorbic acid per 100 ml. of the juice. Juice stored at 68, 86 or 104°F lost its ascorbic acid content after 3 months of storage.

After 6 months of storage no ascorbic acid was found, by the procedure employed in this study, in any unfortified juice stored at any temperature.

Retention of Ascorbic Acid in Fortified Apple Juice

The addition of given amounts of a solution of ascorbic acid in apple juice was carried out for the purpose of increasing the concentration in the final product by 0, 30, 45, 60 or 90 mg. per 100 ml. of apple juice, thus, producing 5 distinct fortification levels. Initial concentration of ascorbic acid, calculated as the average value of duplicate samples taken after cooling and before storage, were: level 0 (unfortified juice), 1.5 mg. per 100 ml.; level 30, 28.5 mg. per

100 ml.; level 45, 47.5 mg. per 100 ml., level 60, 66.5 mg. per 100 ml.; and level 90, 93.0 mg. per 100 ml. of the juice.

Effect of storage time

The data in Tables 1 and 2 show that the ascorbic acid concentration (mg/100 ml) and retention were significantly affected by storage time over all levels of fortification and temperatures of storage at the one percent level of significance. Interaction of time with either storage temperatures or fortification level or with both were also significant in altering ascorbic acid concentration and retention at the one percent level of significance.

The ascorbic acid concentration found in the samples of fortified apple juice is shown in Figures 2 through 6. The data show conclusively that ascorbic acid content of the juice decreased with time at all fortification levels.

At 32°F (Figure 2), level 30 maintained 24.0 mg. per 100 ml. of the juice at the end of 12 months of storage. Also juices fortified at higher levels lost ascorbic acid when stored at 32°F. After 12 months level 45 kept 39.5 mg. per 100 ml.; level 60 held 54.0 mg. per 100 ml., and level 90 maintained 73.5 mg. per 100 ml. of the juice. When expressed as percent retention of initial ascorbic acid concentration level 30 retained 98, 96, 93 and 84 percent after 3, 6, 9 and 12 months respectively. Level 45 retained 97, 92, 88 and 83

TABLE 4: Effect of fortification level, storage time and temperature on concentration of ascorbic acid in apple juice

Fortification level	Temperature °F	Storage time in months 0	3	6	9	12
		mg. ascorbic acid/100 ml. juice				
0	32	1.5	1.0	0.0	0.0	0.0
	50	1.5	1.0	0.0	0.0	0.0
	68	1.5	0.0	0.0	0.0	0.0
	86	1.5	0.0	0.0	0.0	0.0
	104	1.5	0.0	0.0	0.0	0.0
30	32	28.5	28.0	27.5	26.5	24.0
	50	28.5	26.5	25.0	24.5	22.0
	68	28.5	24.0	22.5	22.0	19.5
	86	28.5	23.5	21.0	16.0	12.5
	104	28.5	22.0	9.5	0.0	0.0
45	32	47.5	46.0	43.5	42.0	39.5
	50	47.5	43.0	40.5	38.0	35.0
	68	47.5	38.5	37.5	35.5	31.0
	86	47.5	38.0	33.5	28.0	19.5
	104	47.5	32.0	14.0	0.0	0.0
60	32	66.5	65.0	59.0	60.5	54.0
	50	66.5	62.0	58.0	53.0	48.5
	68	66.5	54.5	54.0	48.5	42.5
	86	66.5	55.0	46.5	38.0	25.0
	104	66.5	44.0	15.0	0.0	0.0
90	32	93.0	84.5	82.0	78.0	73.5
	50	93.0	80.0	75.5	74.5	66.0
	68	93.0	71.5	70.5	67.0	58.5
	86	93.0	71.5	58.5	46.5	30.5
	104	93.0	57.0	18.0	0.0	0.0

TABLE 5: Effect of fortification level, storage time and temperature on percent retention of ascorbic acid in apple juice

Fortification level	Temperature °F	Storage time in months			
		3	6	9	12
		percent retention			
0	32	67	0	0	0
	50	67	0	0	0
	68	0	0	0	0
	86	0	0	0	0
	104	0	0	0	0
30	32	98	96	93	84
	50	93	88	86	77
	68	84	79	77	68
	86	82	74	56	44
	104	77	33	0	0
45	32	97	92	88	83
	50	91	85	80	74
	68	81	79	75	65
	86	80	71	59	41
	104	67	29	0	0
60	32	98	89	91	81
	50	93	87	80	73
	68	82	81	73	64
	86	83	70	57	38
	104	66	23	0	0
90	32	91	88	84	79
	50	86	81	80	71
	68	77	76	72	63
	86	77	63	50	33
	104	61	19	0	0

percent after 3, 6, 9 and 12 months respectively. Level 60 retained 98, 89, 91 and 81 percent after 3, 6, 9 and 12 months respectively. Level 90 retained 91, 88, 84 and 79 percent after 3, 6, 9 and 12 months respectively.

Storage of the juice at 50°F (Figure 3) produced slightly lower retentions when compared to juice stored at 32°F with decrease in ascorbic acid content over time. At the end of 12 months storage at 50°F, level 30 maintained 22.0 mg. per 100 ml.; level 45 retained 35.0 mg. per 100 ml.; level 60 retained 48.5 mg. per 100 ml.; and level 90 retained 66.0 mg. per 100 ml. of the juice. Expressed as percent retention, level 30 retained 93, 88, 86 and 77 percent after 3, 6, 9 and 12 months respectively. Level 45 retained 93, 87, 80 and 73 percent after 3, 6, 9 and 12 months respectively. Level 90 retained 86, 81, 80 and 71 percent after 3, 6, 9 and 12 months respectively. It will be noted from the data in Figures 2 and 3 that there was a uniform drop in ascorbic acid values over time in juices stored at 32 and 50°F.

Ascorbic acid content decreased more rapidly in juice stored at room temperature, 68°F (Figure 4). After 12 months of storage at 68°F, level 30 retained 19.5 mg. per 100 ml.; level 45 retained 31.0 mg. per 100 ml.; level 60 retained 42.5 mg. per 100 ml.; and level 90 maintained 58.5 mg. per 100 ml. of the juice. Expressed as percent retention, level 30 retained 84, 79, 77 and 68 percent after 3, 6, 9 and 12

months respectively. Level 45 retained 81, 79, 75 and 65 percent after 3, 6, 9 and 12 months respectively. Level 60 retained 82, 81, 73 and 64 after 3, 6, 9 and 12 months respectively. Level 90 retained 77, 76, 72 and 63 percent after 3, 6, 9 and 12 months respectively. As it may be seen from the data in Figure 4, the first three months of storage at 68°F resulted in a greater loss of ascorbic acid than any subsequent three-month period of storage. Loss of ascorbic acid in the first three months was about half that occurred in the total 12 months storage at 68°F in all fortification levels.

Similarly, loss of ascorbic acid in juice stored at 86°F (Figure 5) was also rapid in the first three months of storage accounting to 17-23 percent or about one-third of the total loss occurred during the entire storage period. After 12 months, level 30 juice retained 12.5 mg. per 100 ml.; level 45 retained 19.5 mg. per 100 ml.; level 60 retained 25.0 mg. per 100 ml.; and level 90 retained 30.5 mg. per 100 ml. of the juice. Expressed as percent retention, level 30 retained 82, 74, 56 and 44 percent after 3, 6, 9 and 12 months respectively. Level 45 retained 80, 71, 59 and 41 percent after 3, 6, 9 and 12 months respectively. Level 60 retained 83, 70, 57 and 38 percent after 3, 6, 9 and 12 months respectively. Level 90 retained 77, 63, 50 and 33 percent after 3, 6, 9 and 12 months respectively.

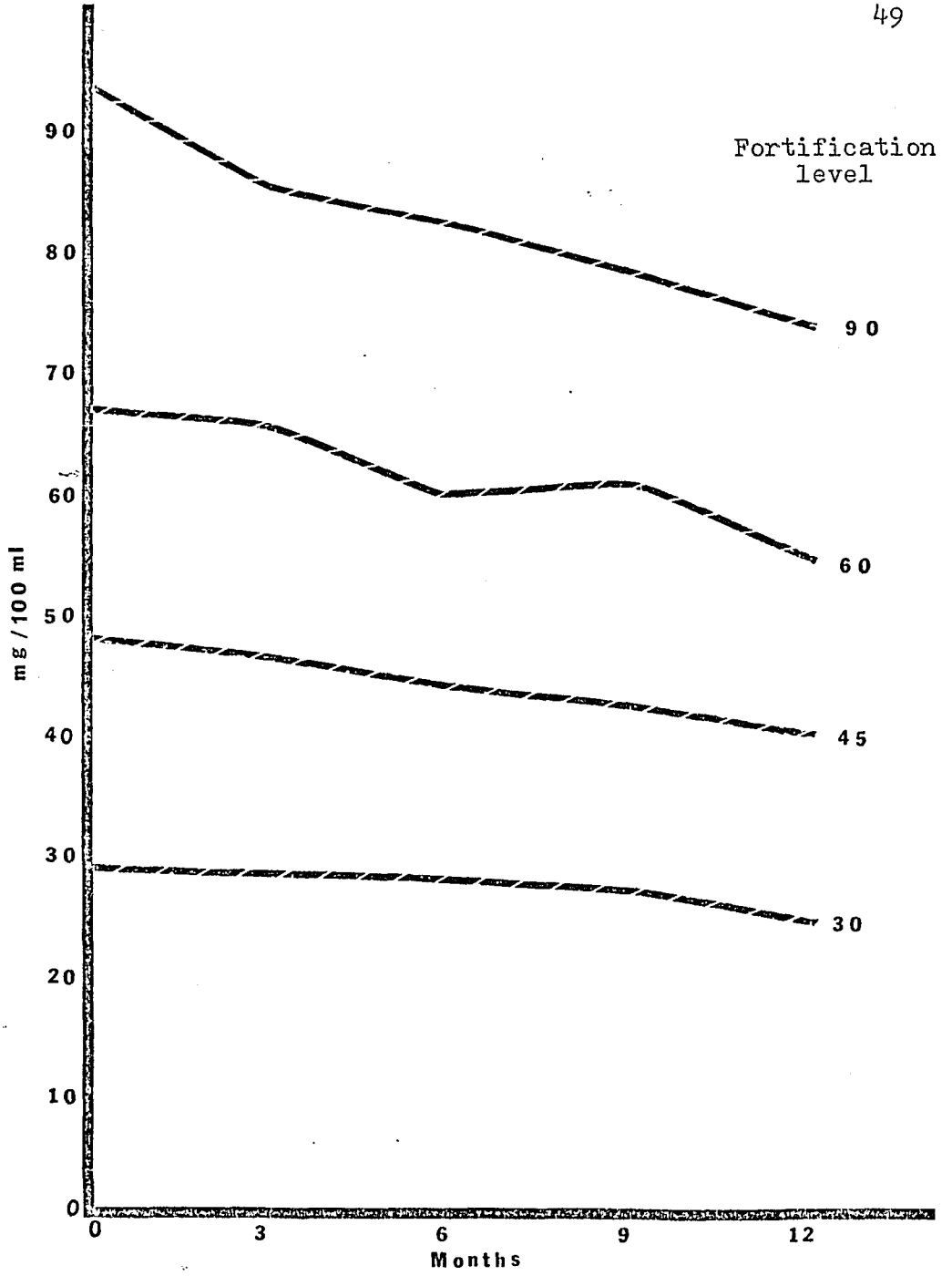


FIGURE 2: Relationship between ascorbic acid concentration in fortified apple juice and storage time at 32°F

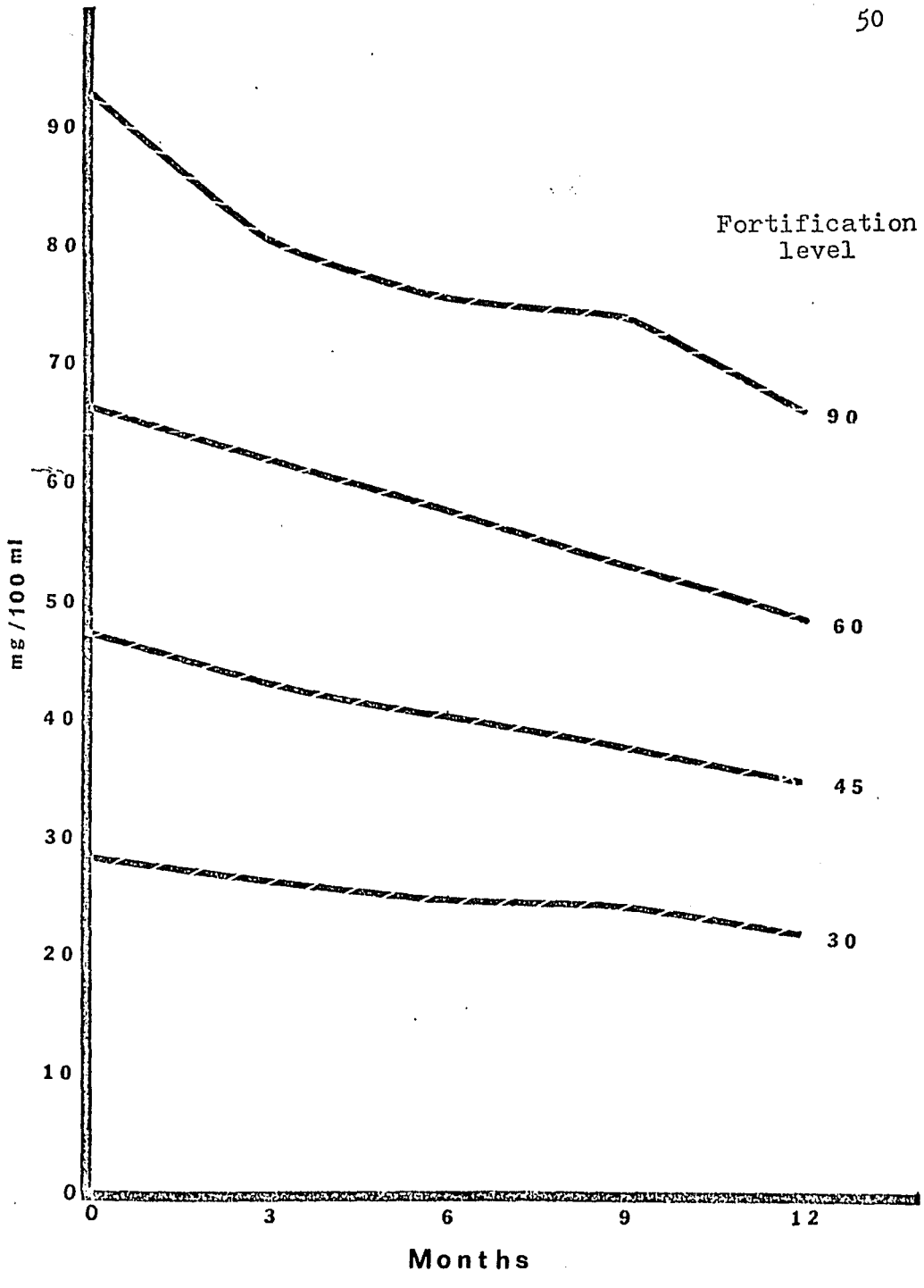


FIGURE 3: Relationship between ascorbic acid concentration in fortified apple juice and storage time at 50°F

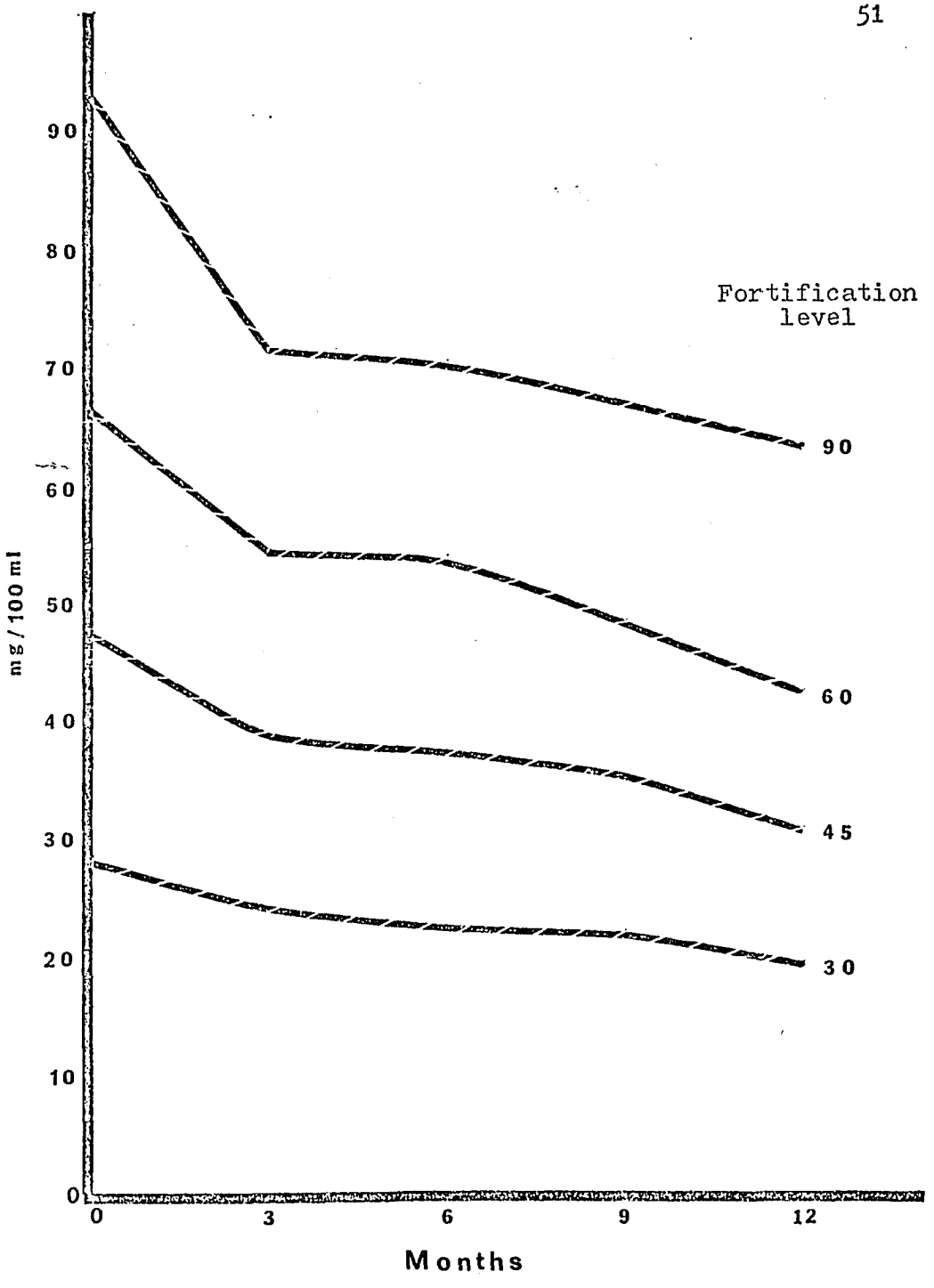


FIGURE 4: Relationship between ascorbic acid concentration in fortified apple juice and storage time at 68°F

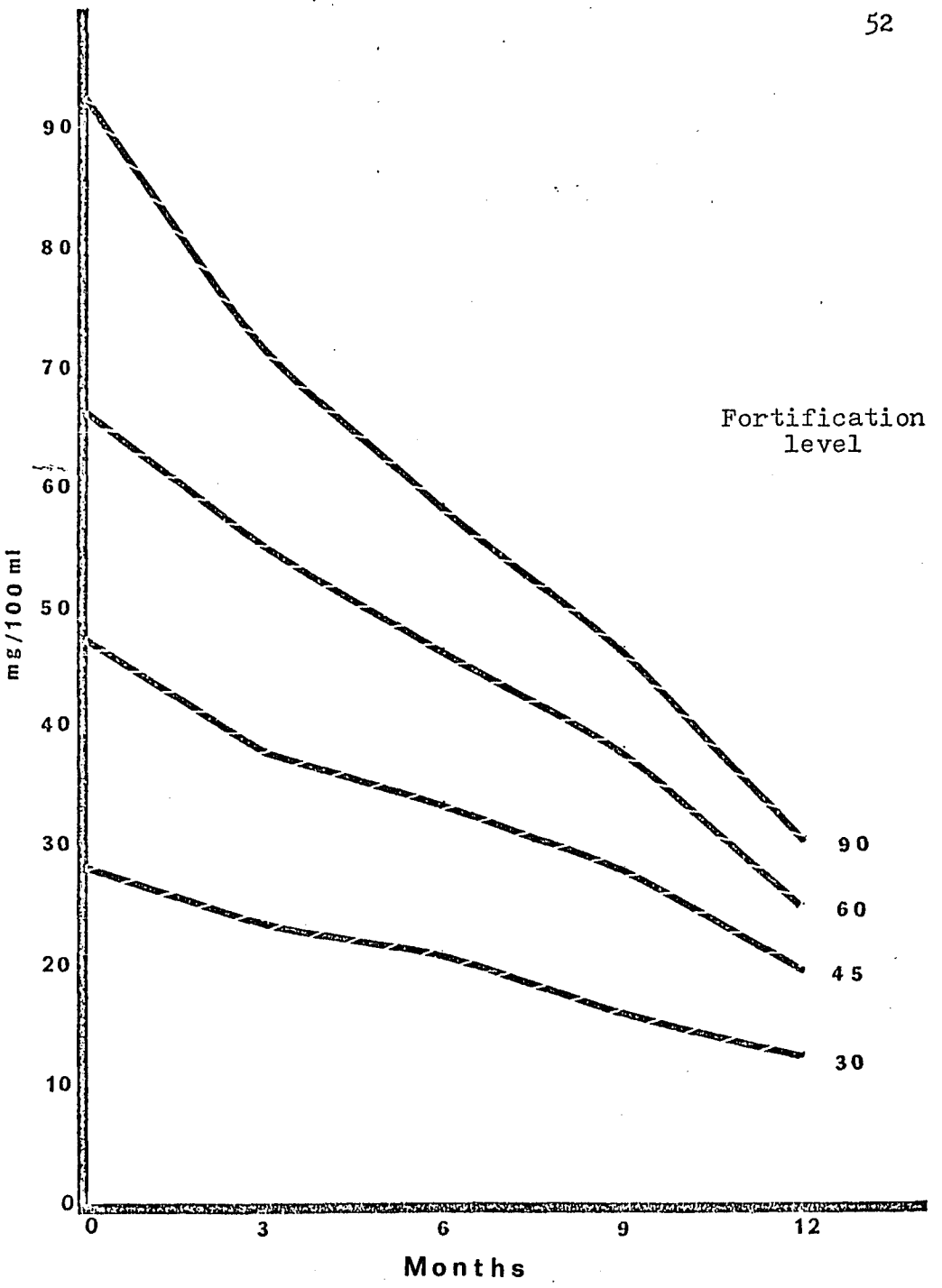


FIGURE 5: Relationship between ascorbic acid concentration in fortified apple juice and storage time at 86°F

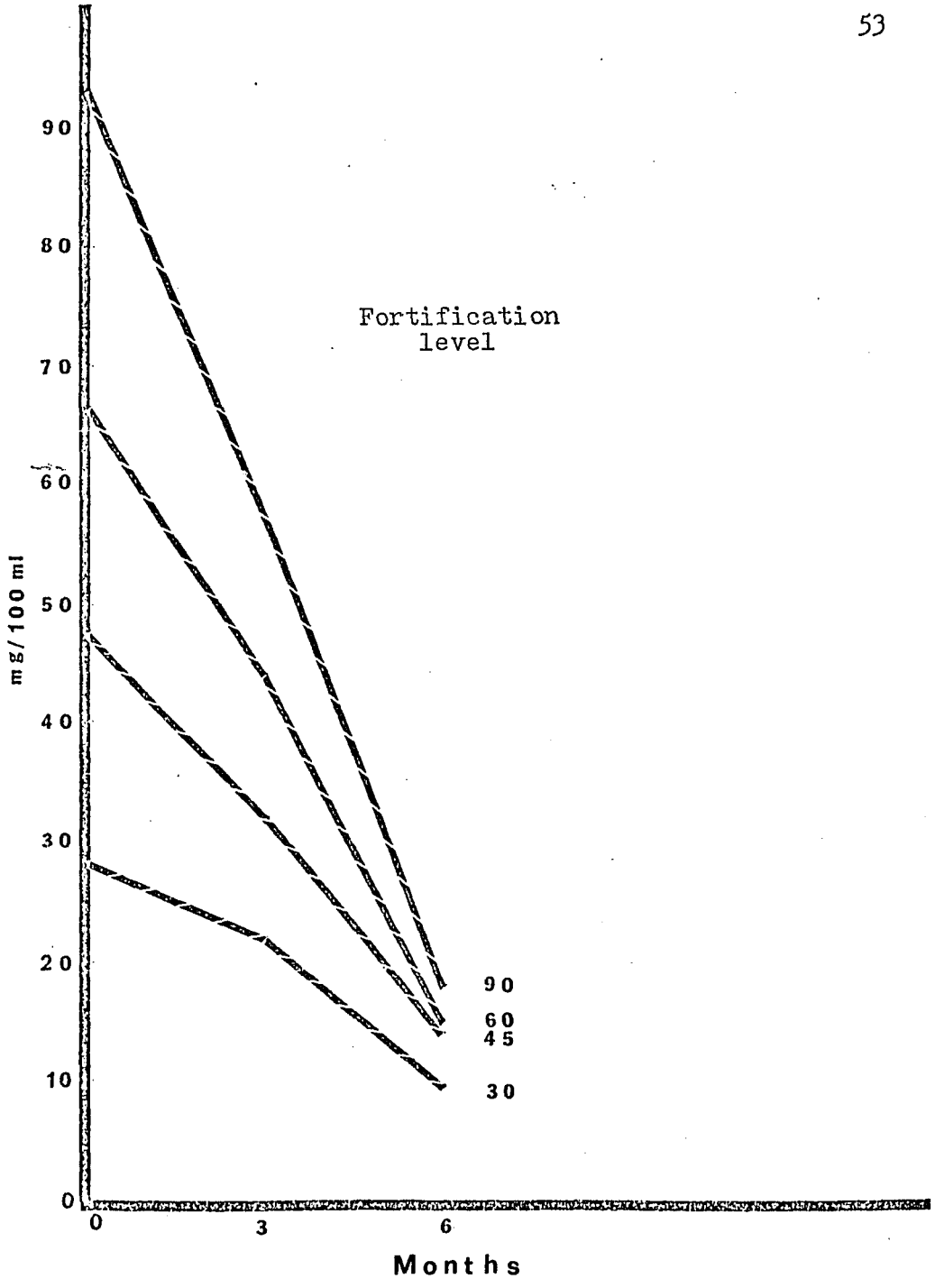


FIGURE 6: Relationship between ascorbic acid concentration in fortified apple juice and storage time at 104°F

Ascorbic acid content was decreased very rapidly in juice stored at 104°F. The data in Figure 6 represent storage after only 6 months. Level 30 kept 9.5 mg. per 100 ml.; level 45 retained 14.0 mg. per 100 ml.; level 60 retained 15.0 mg. per 100 ml.; and level 90 retained 18.0 mg. per 100 ml. of the juice. In all fortification levels, loss of ascorbic acid was more rapid from three to six months than for the first three months of storage. Level 30 lost 23 percent of initial ascorbic acid content in the first three months and 44 percent during the next three. Level 45 lost 33 percent during the first three months and 38 percent during the next three. Level 60 also lost 34 percent in the first three months and 43 percent for the following three months. Level 90 juice suffered the greatest loss of its ascorbic acid content with 39 percent in the first three months and 42 percent during the subsequent three months of storage.

Effect of storage temperature

The temperature of storage influenced the retention of ascorbic acid in all fortification levels as shown in Table 5. Results of the effect of storage temperature on the ascorbic acid content of fortified apple juice after 12 months of storage are shown in Figure 7. These results clearly indicate the dramatic effect of accelerated storage temperatures on the ascorbic acid content of the juice.

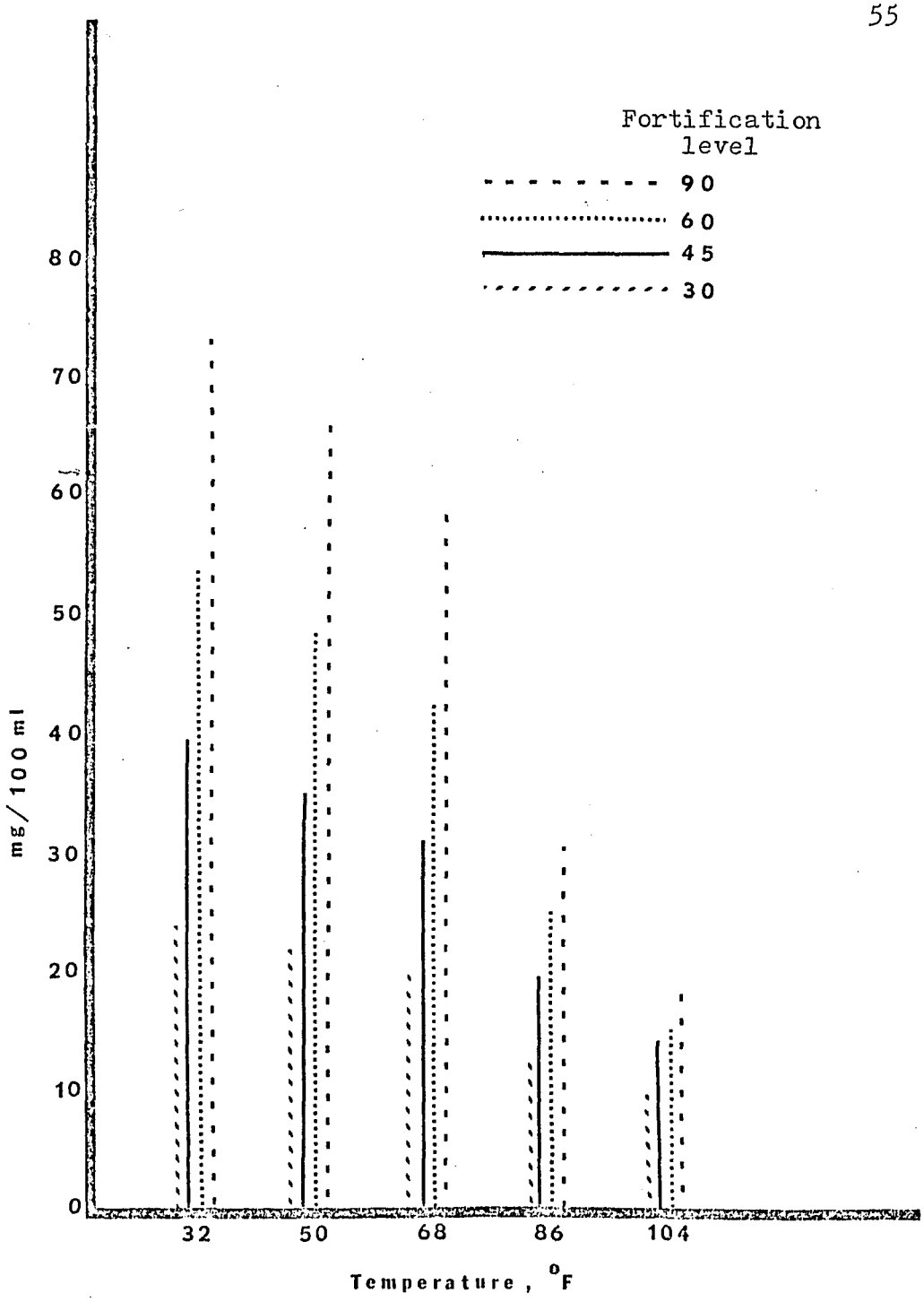


FIGURE 7: Relationship between concentration of ascorbic acid retained in fortified apple juice after 12 months (after 6 months for 104°F) and storage temperature

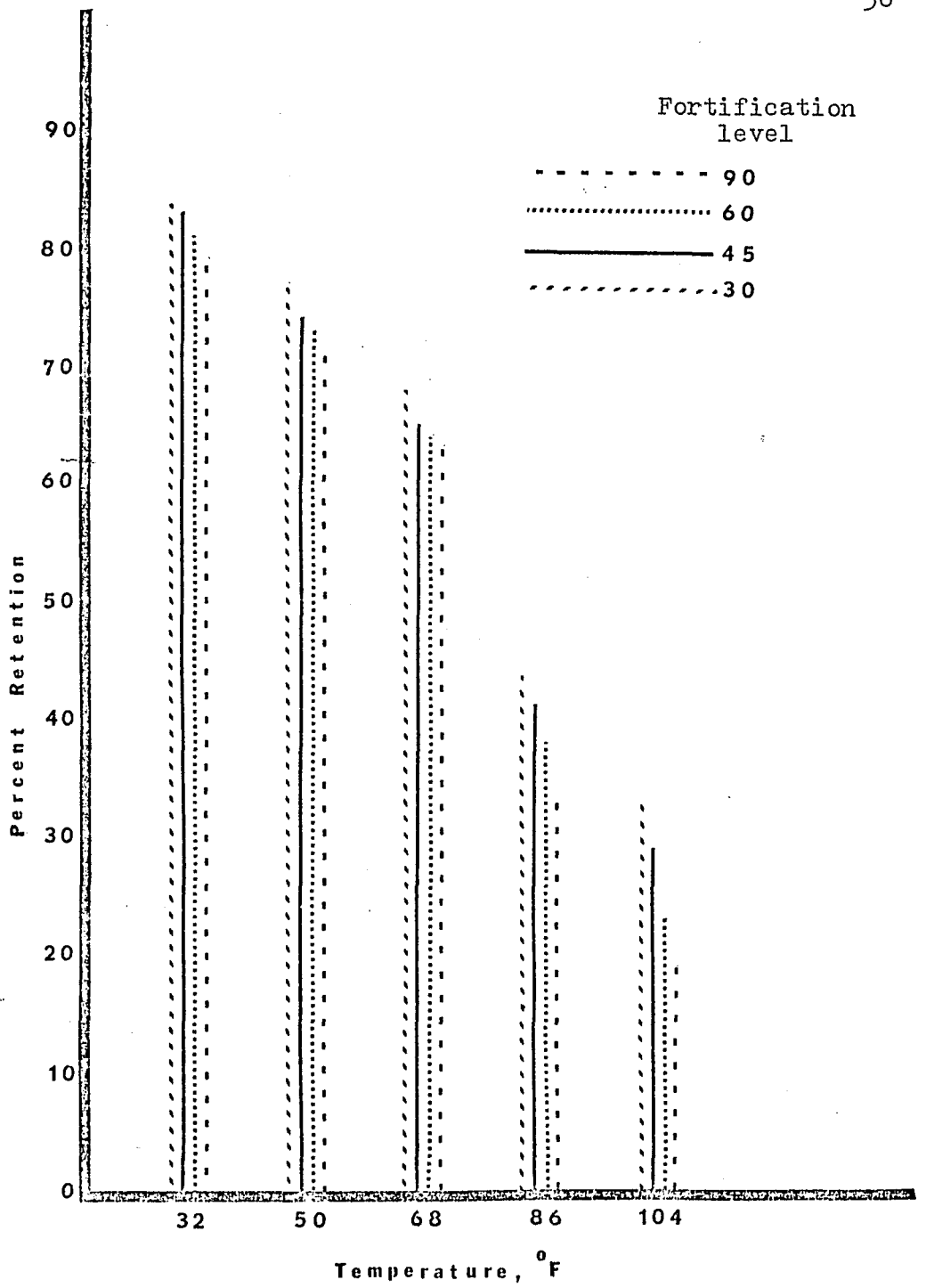


FIGURE 8: Relationship between percent ascorbic acid retained in fortified apple juice after 12 months (after 6 months for 104°F) and storage temperature

Storage at 104°F resulted in the greatest loss of ascorbic acid. At the end of 9 months of storage at 104°F, no ascorbic acid was detected in fortified apple juice by the method employed in this study. No juice, even when fortified to 93.0 mg. per 100 ml., retained over 18.0 mg. per 100 ml. of the juice after 6 months of storage at 104°F. Data in Figure 8 show these results expressed as percent retention of initial ascorbic acid concentration.

At the end of 12 months storage, level 30 retained 84 percent of its ascorbic acid content when stored at 32°F while the retention was 44 percent when the juice was held at 86°F for the same length of time. Similar losses in ascorbic acid were also observed in higher levels of fortification. Level 45 decreased from 83 percent retention after 12 months of storage at 32°F to 41 percent at 86°F. Level 60 decreased from 81 percent retention at 32°F to 38 percent at 86°F after one year storage. Level 90 decreased from 79 percent at 32°F to 33 percent at 86°F after 12 months of storage.

In general, storage at 32°F for 12 months resulted in a favorable retention of ascorbic acid by all fortification levels. When stored for the same period at 32°F the loss in ascorbic acid appeared to be approximately one-third of that occurring while the product was maintained at 86°F.

Data in Table 5 conclusively illustrate that the retention of ascorbic acid was higher when the juice was stored at refrigerated temperatures. Storage at room temperature , 68°F, resulted in lower retention while storage at accelerated temperatures markedly caused a very rapid loss of ascorbic acid.

It was further noted that fortified apple juice stored at 32°F lost from 1.3 to 1.7 percent of ascorbic acid per month whereas juice maintained at 50°F lost approximately 2.0 to 2.5 percent per month. When storage was held at room temperature, loss of ascorbic acid increased over the refrigerated juice. Approximately 2.6 to 3.0 percent loss per month occurred when the juice was maintained at 68°F. A more rapid increase in ascorbic acid loss was noticed when the juice was stored at 86°F compared to room temperature and refrigerated storage. Loss was from 4.5 to 5.5 percent per month in juice stored at 86°F. A dramatic loss was observed in juice stored at 104°F with approximately 11.0 to 13.5 percent loss per month at all fortification levels.

Effect of fortification level

The initial concentration of ascorbic acid in fortified apple juice significantly influenced ascorbic acid content and retention within each storage temperature at the one percent level of significance as shown in Tables 1 and 2.

Data in Figures 9 through 13 show the effect of increasing the original concentration of ascorbic acid on percent retention in the fortified juice.

It may be noted that level 30 retained only 84 percent of its original ascorbic acid content after 12 months of storage at 32°F (Figure 9) while 79 percent appeared to be present in level 90 when stored for the same length of time at the same storage temperature. Levels 45 and 60 also showed a difference in percent retention of their ascorbic acid content. They retained 83 and 81 percent respectively at the end of 12 months storage at 32°F.

Storage at 50°F (Figure 10) also showed the same trend. A decrease in percent retention of ascorbic acid was observed as the ascorbic acid concentration was increased from level 30 to level 90. Level 30 retained 77 percent at 50°F after 12 months storage, while level 45 retained 74 percent. Level 60 retained 73 percent whereas level 90 maintained 71 percent at the end of 12 months storage at 50°F.

Storage at room temperature, 68°F, produced results after 12 months presented as shown in Figure 11. Retentions decreased with increasing fortification level from 68 percent in level 30 to 65, 64 and 63 percent in levels 45, 60 and 90 respectively.

The difference in percent retention due to fortification level in juice stored at 86°F (Figure 12) was more pronounced than that when the juice was maintained at either refrigerated

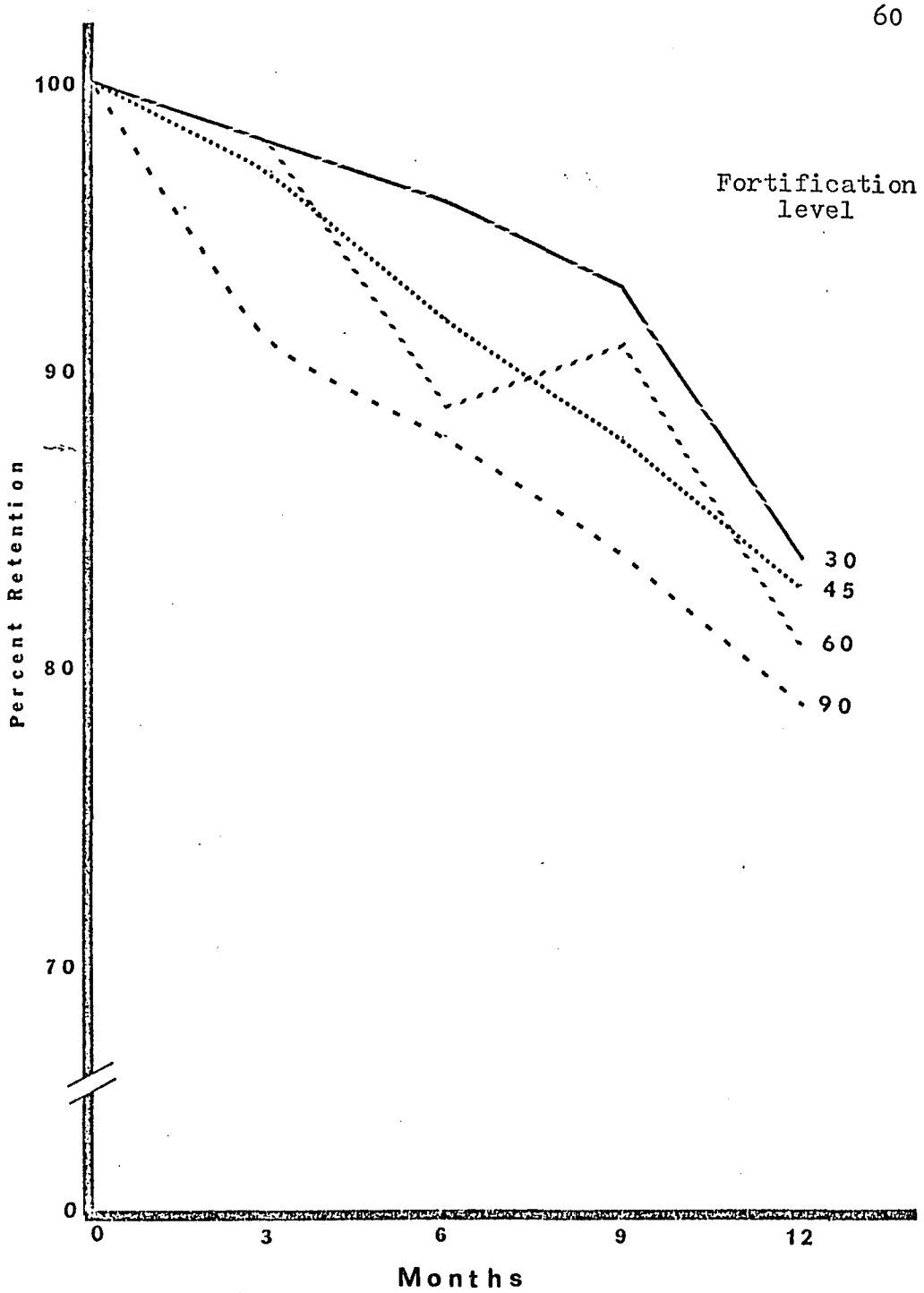


FIGURE 9: Relationship between percent retention of ascorbic acid in fortified apple juice and storage time at $32^{o}F$

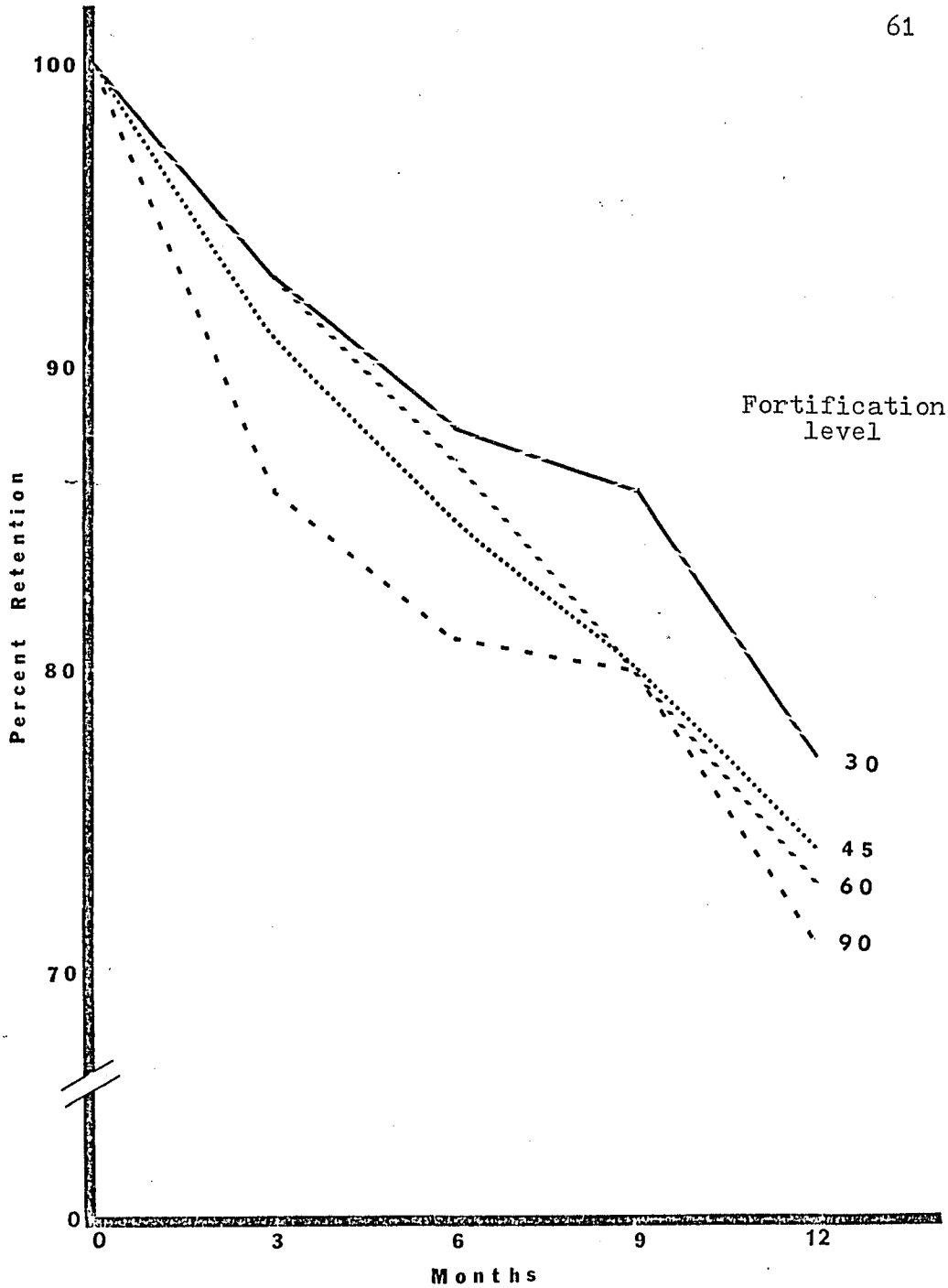


FIGURE 10: Relationship between percent retention of ascorbic acid in fortified apple juice and storage time at 50°F

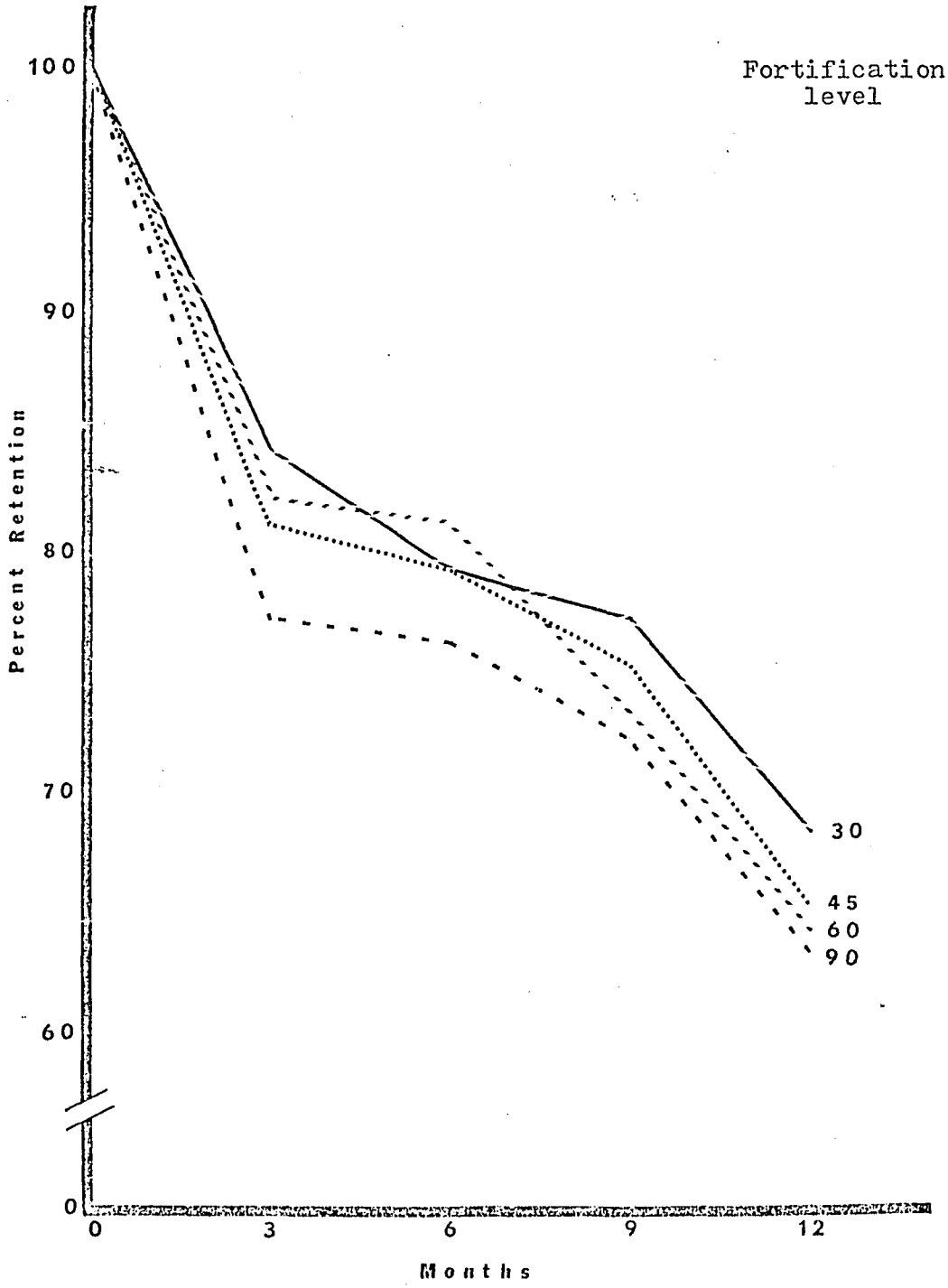


FIGURE 11: Relationship between percent retention of ascorbic acid in fortified apple juice and storage time at $68^{\circ}F$

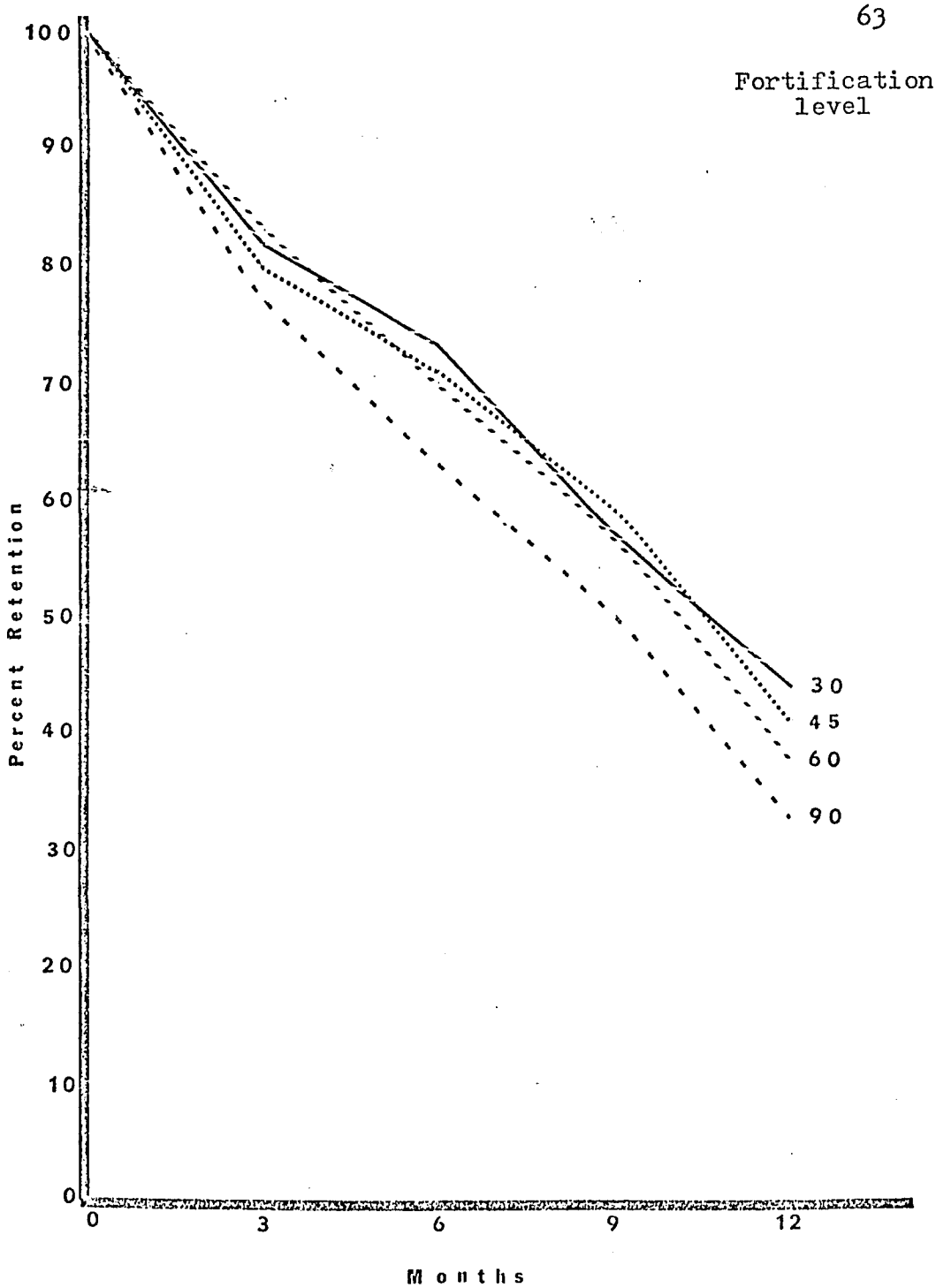


FIGURE 12: Relationship between percent retention of ascorbic acid in fortified apple juice and storage time at 86°F

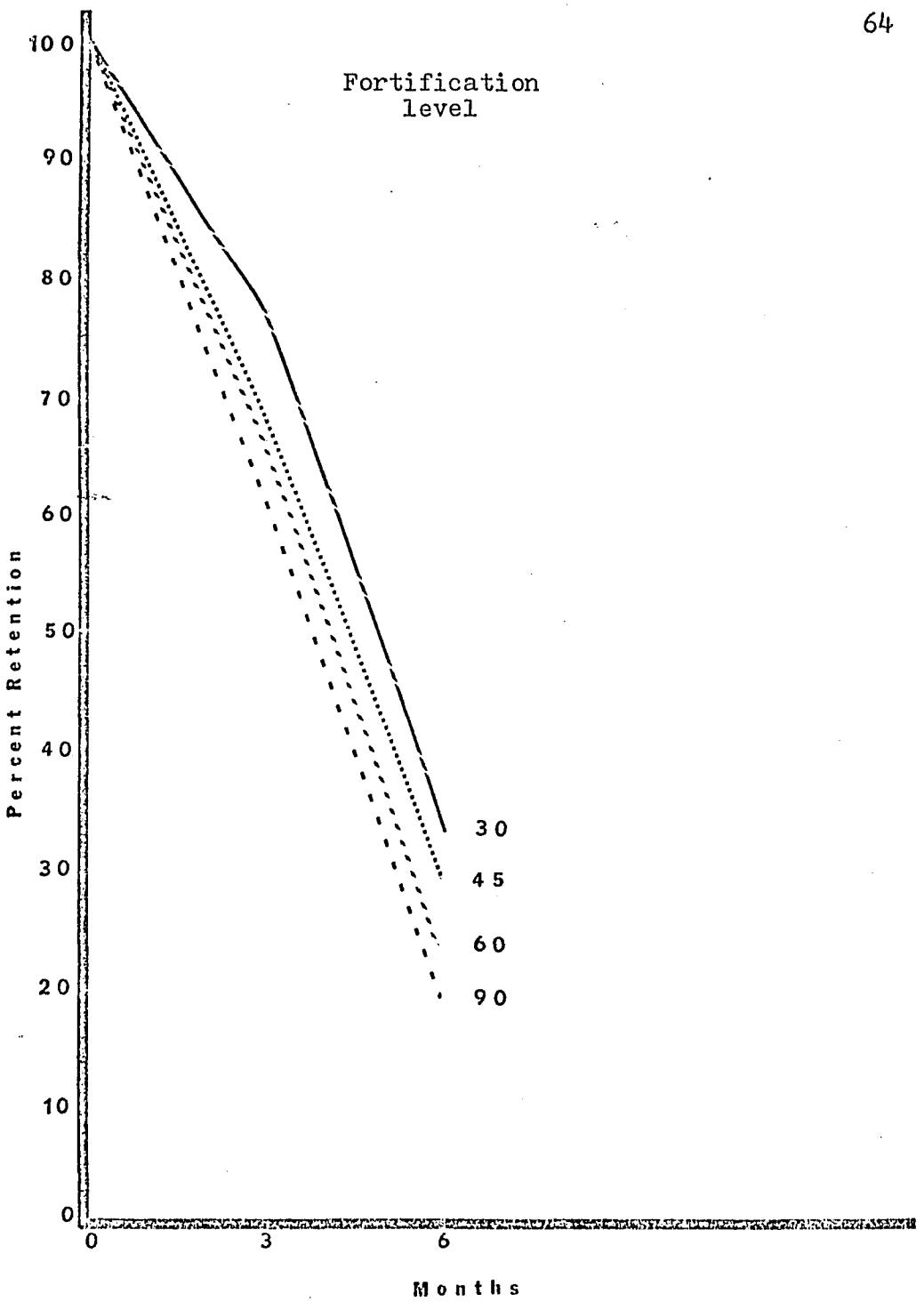


FIGURE 13: Relationship between percent retention of ascorbic acid in fortified apple juice and storage time at 104°F

or room temperatures. At the end of 12 months storage at 86°F, levels 30, 45, 60 and 90 retained 44, 41, 38 and 33 percent respectively.

Storage at 104°F produced similar results as shown in Figure 13, however, the decrease in percent retention due to fortification level effect was very pronounced at the end of 6 months storage. Level 30 retained 33 percent of its original ascorbic acid content while only 19 percent appeared to be present in level 90 stored for the same period of time. Levels 45 and 60 retained 29 and 23 percent respectively after 6 months storage at 104°F.

Effect of Fortification on pH

The level of fortification of ascorbic acid significantly affected the pH of apple juice. Average pH values of duplicate samples taken at 0, 3, 6, 9 and 12 months of storage in each fortification level stored at each temperature are presented in Appendix B. It was noted that the addition of ascorbic acid to apple juice resulted in an immediate decrease in the pH of the juice and the decrease in pH was increased with increasing the fortification level. Level 0, unfortified juice, had a pH of 3.80 while level 90 maintained a pH of 3.63 after processing and cooling. Storage time and temperature also significantly altered the pH of the juice, however, the change in pH of the juice stored at refrigerated

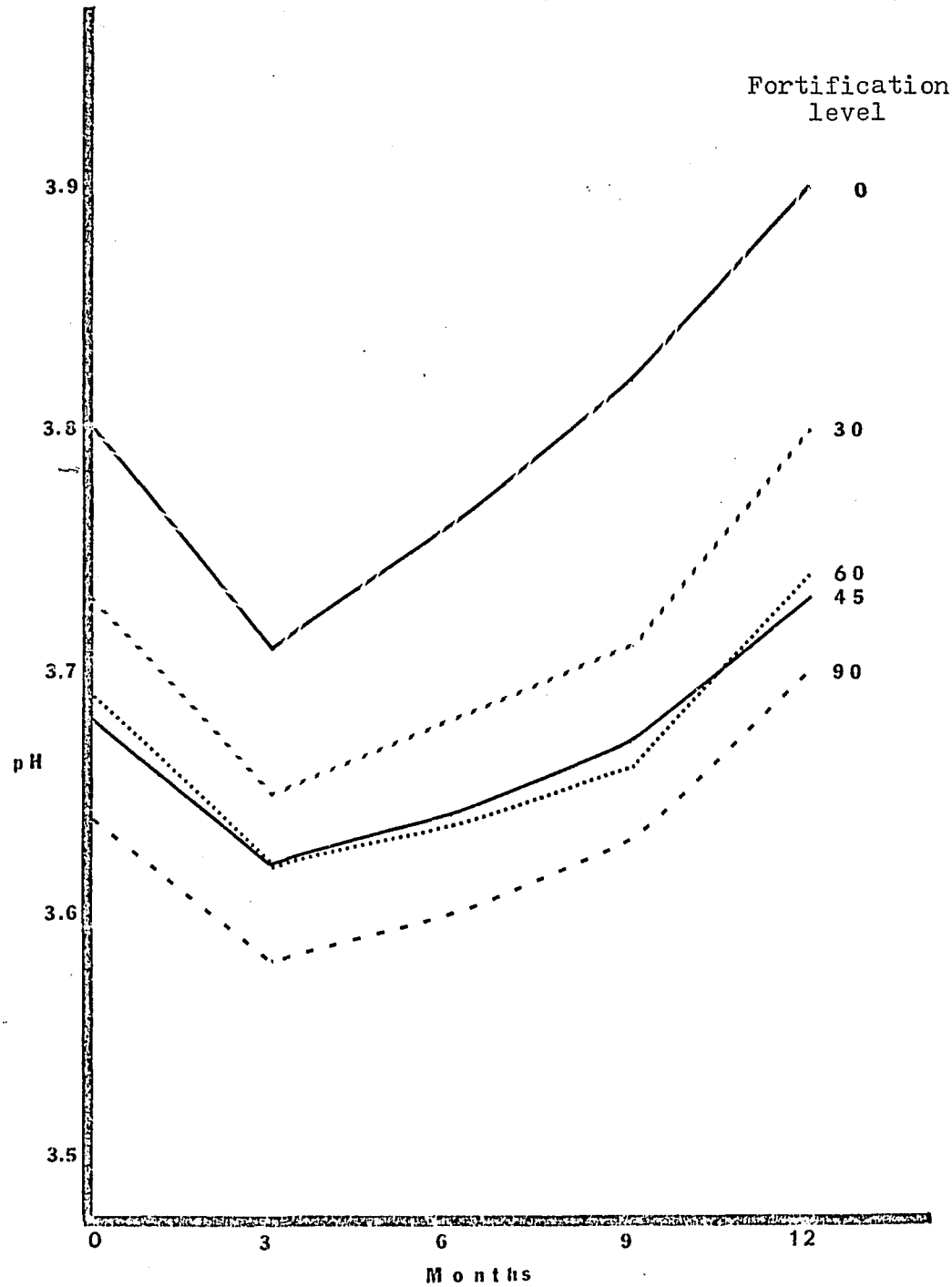


FIGURE 14: Relationship between pH of apple juice and storage time in each fortification level

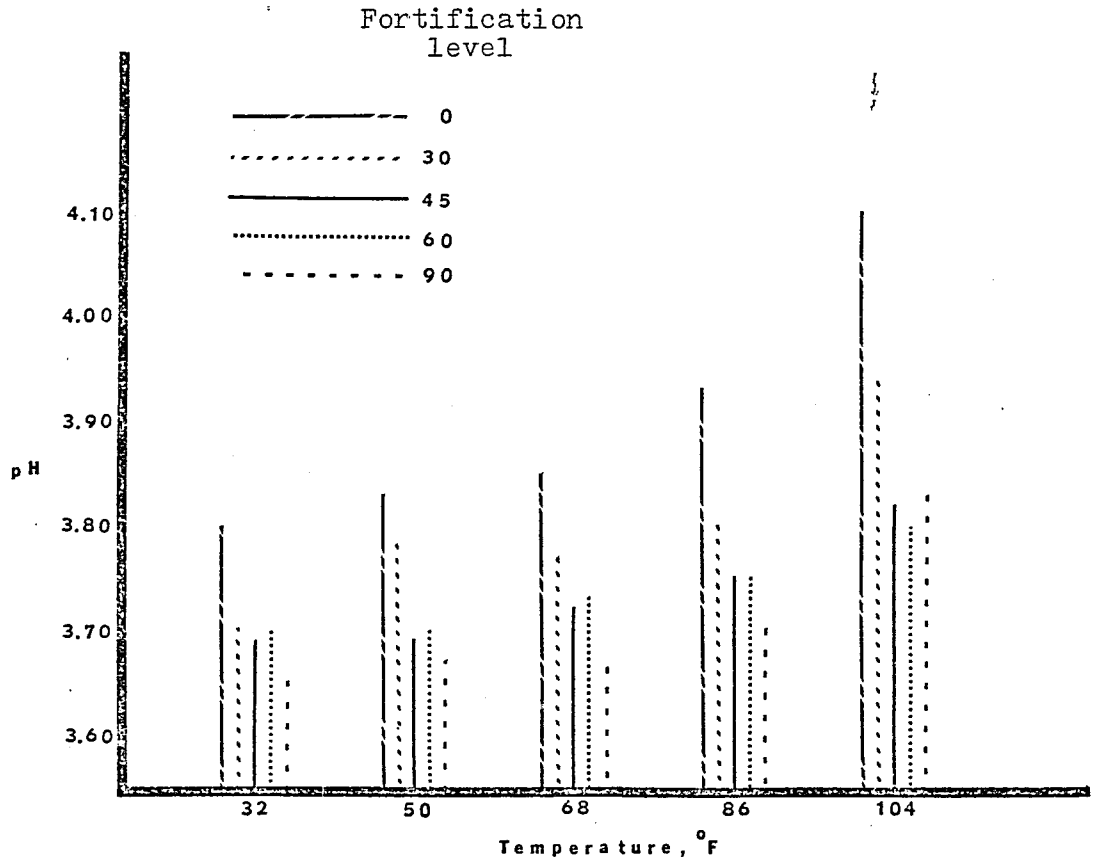


FIGURE 15: Relationship between pH of apple juice after 12 months of storage and storage temperature at each fortification level

and room temperatures was not pronounced over time as that when the juice was held at 86° and 104°F.

Average pH values for all storage temperatures measured at 0, 3, 6, 9 and 12 months in each fortification level is presented in Table 6. It was noticed that the pH of the juice decreased after 3 months of storage in all fortification levels, then it tended to increase with subsequent storage time until the end of 12 months where it reached a level higher than that before storage. At the end of 12 months storage, level 0 had a pH value of 3.90 while level 90 maintained a pH value of 3.70. It was further observed that the change in pH with storage time was parallel in all fortification levels (see Figure 14). The effect of storage temperature on the pH of apple juice can be also seen from the data in Figure 15. At the end of 12 months storage, the pH of the juice was increased with increasing storage temperature in all fortification levels.

Effect of Fortification on Titratable Acidity

The fortification of apple juice with ascorbic acid significantly affected the titratable acidity measured as malic acid. Average values of percent malic acid of duplicate samples taken at 0, 3, 6, 9 and 12 months of storage in each fortification level held at each temperature are shown in Appendix B.

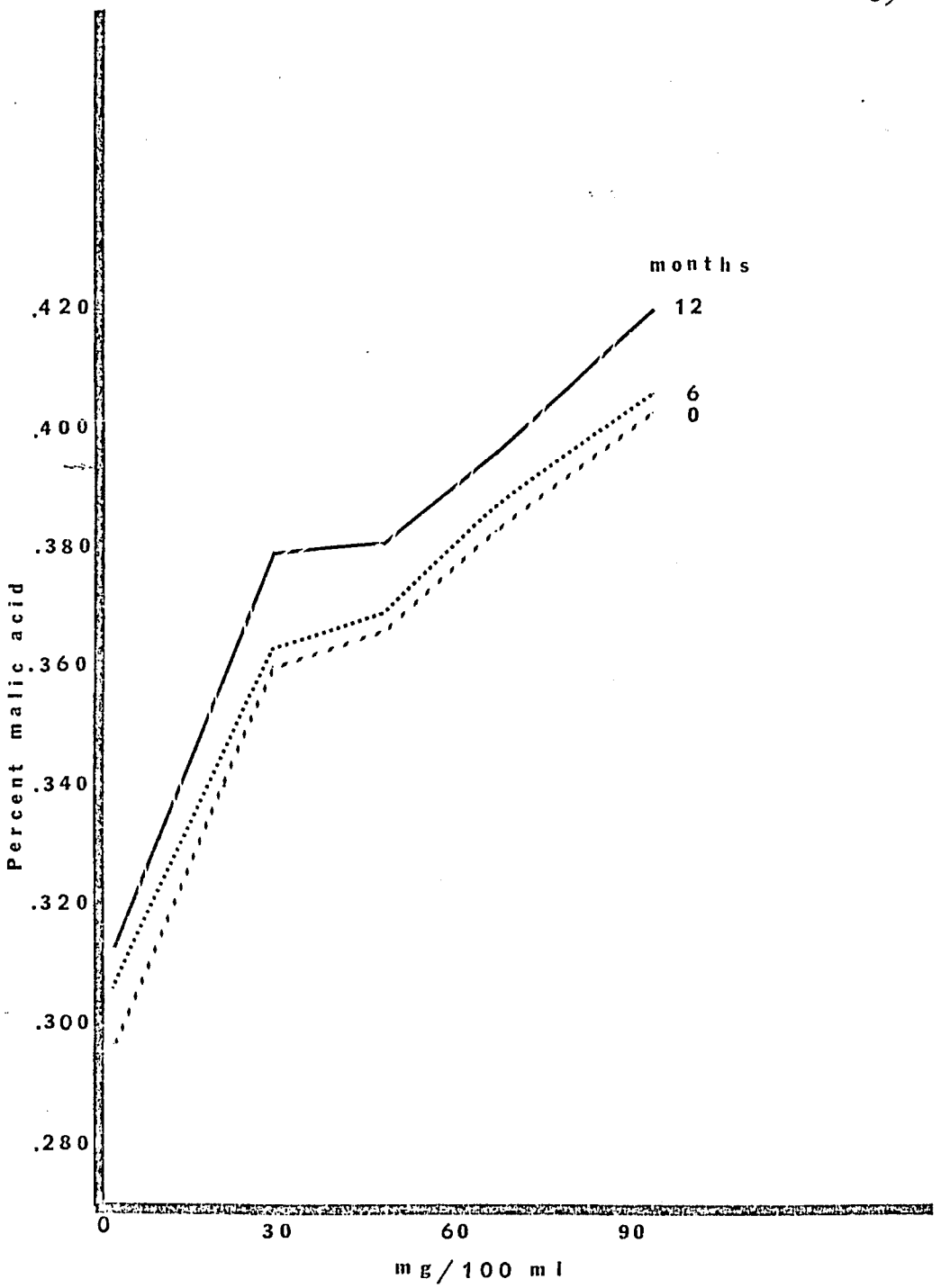


FIGURE 16: Relationship between percent malic acid of apple juice and initial ascorbic acid concentration at 0, 6 and 12 months of storage

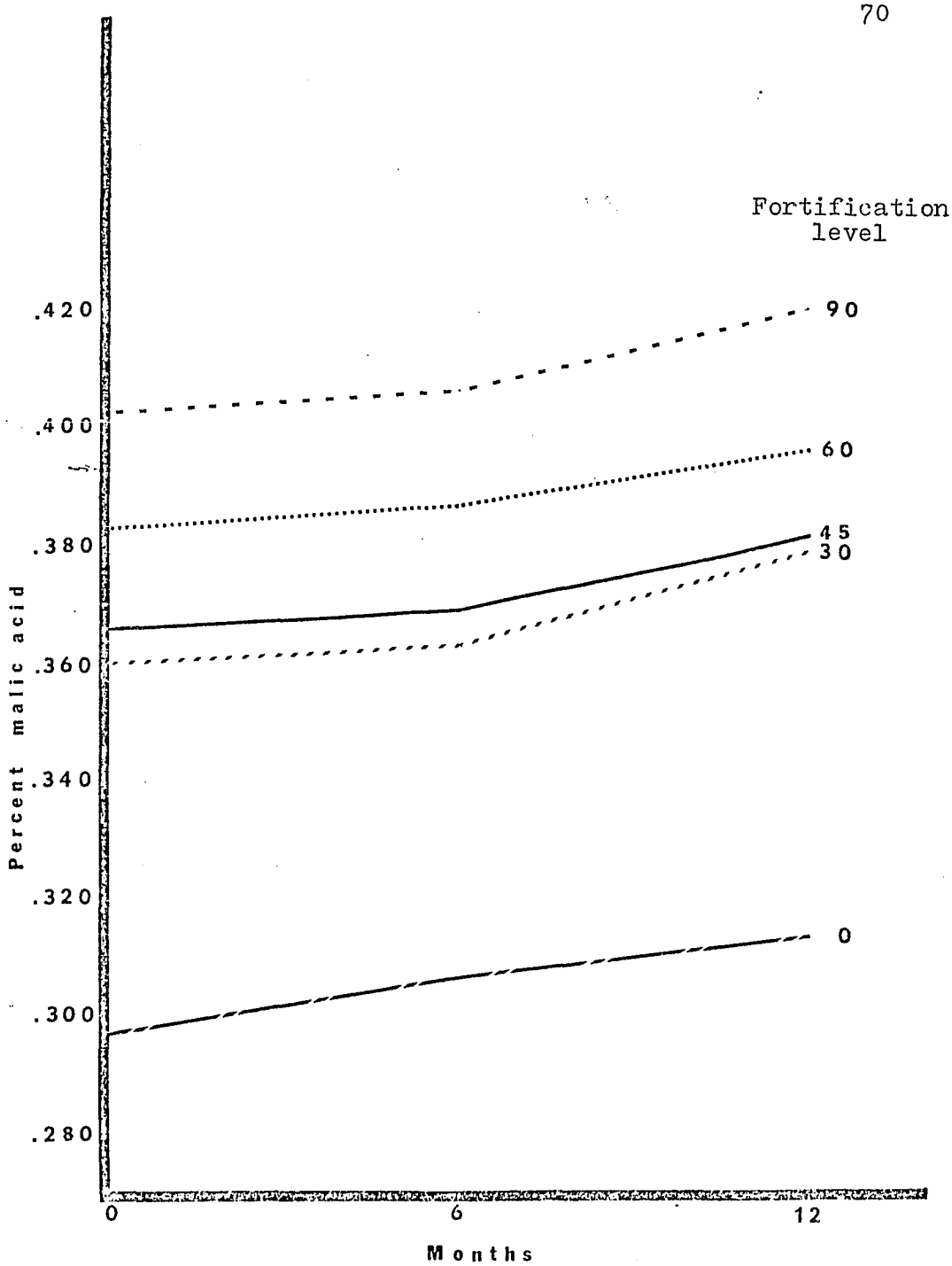


FIGURE 17: Relationship between percent malic acid of apple juice and storage time after 0, 6 and 12 months in each fortification level

An immediate increase in percent acidity was observed upon the addition of ascorbic acid as shown in Figure 16. Percent malic acid was increased from 0.297 in level 0, unfortified juice, to 0.366 in level 45 to 0.403 in level 90 after processing and cooling. Storage time and temperature also significantly influenced percent acidity, however, percent malic acid was less affected by temperature than by time of storage as indicated from the data of the analysis of variance (see Appendix A). Average percent malic acid for all storage temperatures measured at 0, 3, 6, 9 and 12 months of storage in each fortification level are presented in Table 7.

It was noticed that increasing storage time resulted in an increase in percent malic acid. The effect of 0, 6 and 12 months of storage on percent acidity of apple juice is shown in Figure 17. Percent malic acid was increased from 0.297 in level 0 after processing to an average of 0.313 at the end of 12 months storage and from 0.403 in level 90 before storage to an average of 0.420 after 12 months. As with pH, the increase in percent acidity over storage time was parallel in all fortification levels.

Effect of Fortification on Soluble Solids

Analysis of variance of apple juice soluble solids showed fortification level to affect significantly percent

soluble solids, however, level 30 juice had a percent soluble solids of 14.37 while level 90 had a value of 13.93 percent immediately after processing. Levels 0, 45 and 60 had percent soluble solids of 13.51, 13.61 and 13.74 respectively before storage. Range over the lots was from 13.51 to 14.37 percent with an average value of 13.81 percent in all fortification levels after processing. Average values of percent soluble solids of duplicate samples measured at 0, 3, 6, 9 and 12 months in each fortification level stored at each temperature are listed in Appendix B.

Storage time and temperature also significantly altered the soluble solids content of the juice. Accelerated storage temperatures at 86° and 104°F resulted in a decrease in percent soluble solids after 9 and 12 months of storage.

Average percent soluble solids for all storage temperatures at 0, 3, 6, 9 and 12 months are listed in Table 8. A slight increase in soluble solids content occurred after 3 months of storage followed by a decrease until the end of storage period as shown in Figure 18. Level 0 decreased from 13.51 percent to an average of 13.29 percent; level 30 decreased from 14.37 to an average of 14.30 percent; level 45 decreased from 13.61 to an average of 13.35 percent; level 60 decreased from 13.74 percent to an average of 13.54 percent and level 90 decreased from 13.93 percent to an average of 13.66 percent during 12 months storage. As may be seen

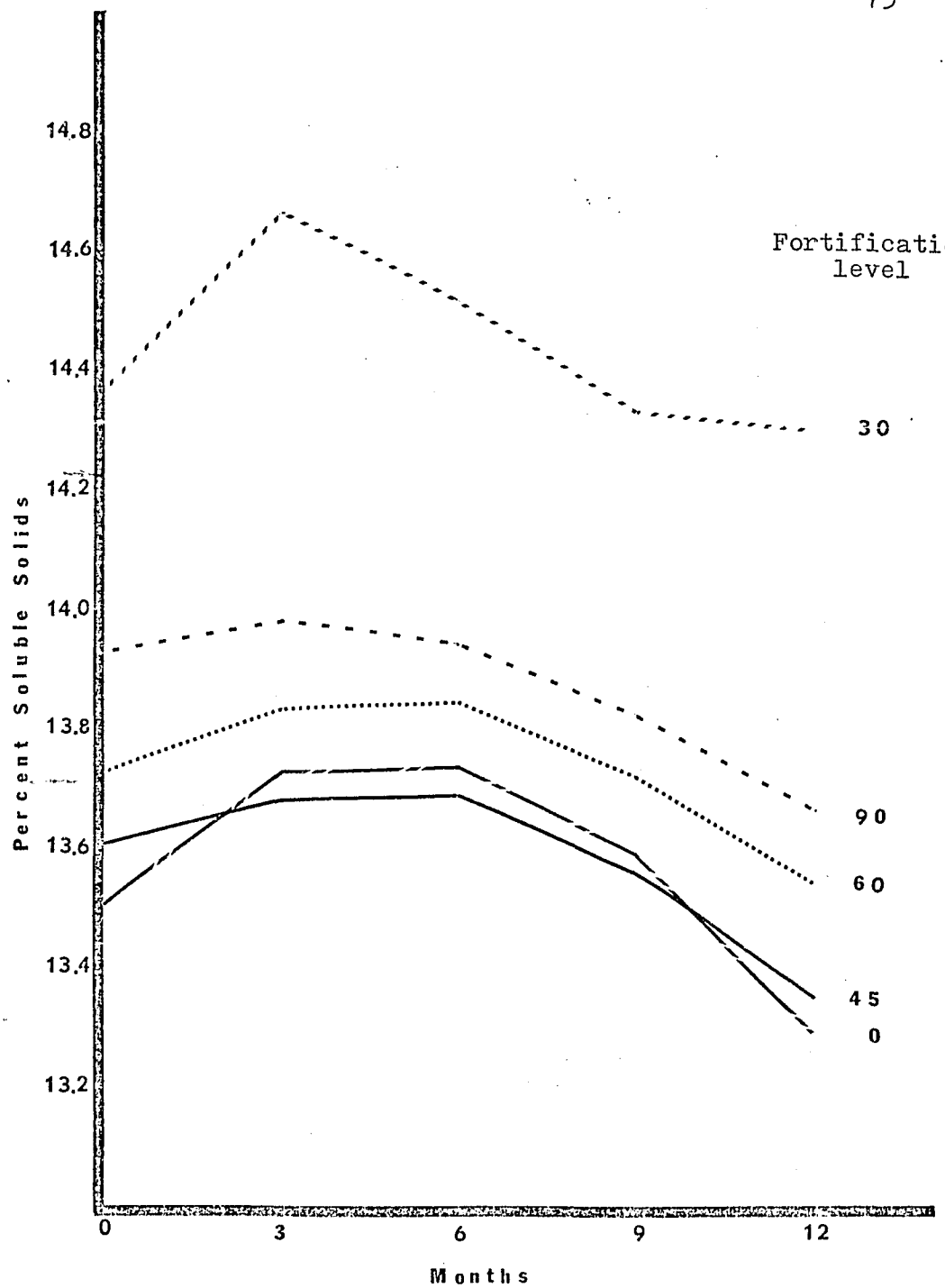


FIGURE 18: Relationship between percent soluble solids of apple juice and storage time in each fortification level

from Figure 18, the change in percent soluble solids of the juice over time was also parallel in all fortification levels.

Effect of Fortification on °Brix: Acid Ratio

The addition of ascorbic acid to apple juice significantly altered the °Brix: acid ratio. Average values of °Brix: acid ratio of duplicate samples taken at 0, 3, 6, 9 and 12 months of storage in each fortification level held at each storage temperature are shown in Appendix B. An immediate decrease in the ratio was observed upon fortification with ascorbic acid and the decrease was accentuated by increasing the initial concentration of ascorbic acid as shown in Figure 19. The ratio was decreased from 45.4:1 in level 0, unfortified juice, to 37.2:1 in level 45 and 34.6:1 in level 90.

Storage time and temperature also significantly altered the ratio, however, the effect of storage temperature on the ratio was less pronounced than that due to storage time (see Appendix A).

Average °Brix:acid ratio for all storage temperatures measured at 0, 3, 6, 9 and 12 months of storage in each fortification level is listed in Table 9. The data indicate that the ratio decreased with increasing storage time particularly in the last 6 months of storage. During 12 months of storage, the ratio decreased from 45.4:1 to 42.5:1

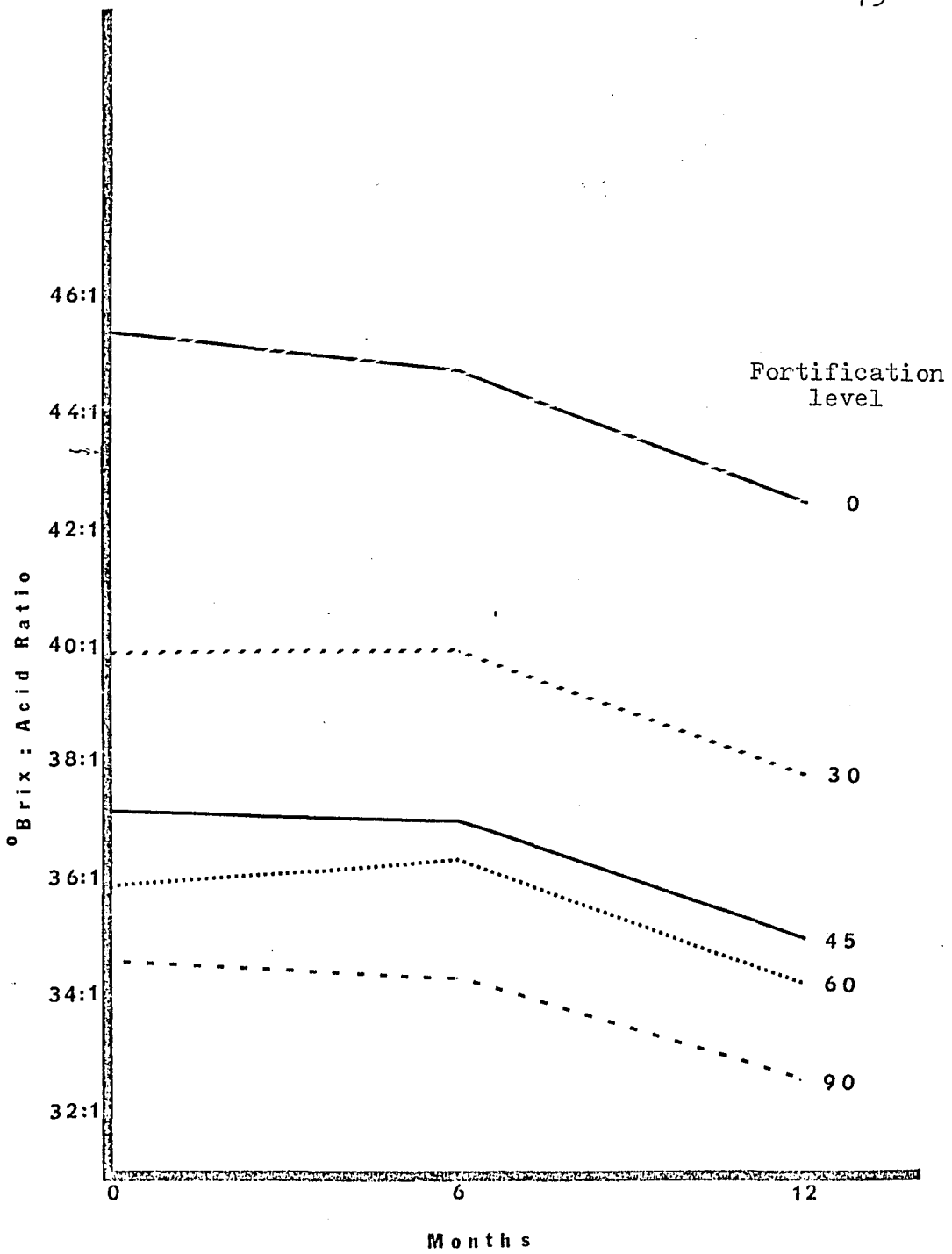


FIGURE 19: Relationship between °Brix:acid ratio of apple juice and storage time at 0, 6 and 12 months in each fortification level

in level 0; from 39.9:1 to 37.8:1 in level 30; from 37.2:1 to 35.0:1 in level 45; from 35.9:1 to 34.2:1 in level 60 and from 34.6:1 to 32.5:1 in level 90. Again the change in °Brix: acid ratio over time was parallel in all fortification levels.

Effect of Fortification on Vacuum

Analysis of variance of can vacuum showed fortification level to significantly affect the maintenance of the vacuum, however, the effect of fortification level was much less than that due to either storage temperature or time or temperature x time interaction as may be seen from the analysis of variance data in Appendix A. Average values of can vacuum of duplicate samples taken at 0, 3, 6, 9 and 12 months in each fortification level stored at each temperature are presented in Appendix B. Can vacuum measured after processing and cooling was 4.50, 7.75, 9.00, 4.50 and 6.50 in. mercury for levels 0, 30, 45, 60 and 90 respectively. Average can vacuum in all fortification levels before storage was 6.45 in. mercury. Range over the lots was from 4.50 to 9.00 in. mercury.

Storage temperature had the greatest significant effect on can vacuum followed by storage time and temperature x time interaction. Average can vacuum of all fortification levels at 0, 3, 6, 9 and 12 months storage held at each storage temperature is listed in Table 10.

Vacuum did decrease with time and with increasing storage temperature. Storage at 32°F resulted in a decrease in vacuum from an average of 6.45 in. mercury after processing and cooling to an average of 3.5 in. mercury after 12 months storage. Likewise, storage at 50°F caused a decrease from an average of 6.45 in. mercury to an average of 3.40 in. mercury during 12 months storage. Storage at room temperature, 68°F, also resulted in a vacuum loss to an average of 1.25 in. mercury after12 months storage.

Vacuum did decrease rapidly at 86°F storage temperature from an average of 6.45 in. mercury to an average of 0.90 in. mercury over 9 months storage and pressure developed at 12 months with an average of 0.90 in. mercury. When stored at 104°F, vacuum was lost rapidly and pressure developed after 3 months storage and it was increased from an average of 1.95 in. mercury after 3 months to an average of 9.00 in. mercury at the end of 12 months storage.

TABLE 6: Effect of fortification level and storage time on pH of apple juice

Fortification level	Storage time in months				
	0	3	6	9	12
	pH				
0	3.80	3.71	3.76	3.82	3.90
30	3.73	3.65	3.68	3.71	3.80
45	3.69	3.62	3.64	3.67	3.73
60	3.68	3.62	3.63	3.66	3.74
90	3.64	3.58	3.60	3.63	3.70

TABLE 7: Effect of fortification level and storage time on titratable acidity in apple juice

Fortification level	Storage time in months				
	0	3	6	9	12
	Percent acidity (malic)				
0	0.297	0.299	0.306	0.299	0.313
30	0.360	0.358	0.363	0.355	0.379
45	0.366	0.363	0.369	0.361	0.381
60	0.383	0.378	0.387	0.375	0.396
90	0.403	0.402	0.406	0.398	0.420

TABLE 8: Effect of fortification level and storage time on percent soluble solids in apple juice

Fortification level	Storage time in months				
	0	3	6	9	12
	Percent soluble solids as sucrose				
0	13.51	13.72	13.73	13.59	13.29
30	14.37	14.66	14.52	14.33	14.30
45	13.61	13.68	13.68	13.56	13.35
60	13.74	13.83	13.84	13.72	13.54
90	13.93	13.98	13.94	13.82	13.66

TABLE 9: Effect of fortification level and storage time on °Brix:acid ratio in apple juice

Fortification level	Storage time in months				
	0	3	6	9	12
	°Brix:acid ratio				
0	45.4:1	45.9:1	44.8:1	45.4:1	42.5:1
30	39.9:1	40.9:1	39.9:1	40.4:1	37.8:1
45	37.2:1	37.7:1	37.0:1	37.6:1	35.0:1
60	35.9:1	36.5:1	36.3:1	36.6:1	34.2:1
90	34.6:1	34.8:1	34.3:1	34.7:1	32.5:1

TABLE 10: Effect of storage temperature and time on vacuum of apple juice

Temperature °F	Storage time in months				
	0	3	6	9	12
	Vacuum in inch of mercury				
32	6.45	5.50	6.10	5.10	3.50
50	6.45	7.20	4.65	4.60	3.40
68	6.45	5.50	3.05	3.50	1.25
86	6.45	4.20	3.60	0.90	+0.90
104	6.45	+1.95	+3.50	+7.80	+9.00

Figures with + indicating pressure

DISCUSSION

The factorial analysis of variance described by Snedecor and Cochran (105) allowed the singular effect of each factor: time, temperature and level of ascorbic acid fortification, as well as any interaction among these factors to be shown.

The factors fortification level, temperature and time, as well as their interactions, were shown to significantly alter the concentration of ascorbic acid. Fortification level, of course, affected the ascorbic acid concentration as the range of concentration among the fortified lots was directly attributed to the added ascorbic acid.

Temperature was a significant alterant of ascorbic acid concentration which was clearly portrayed by the spectacular effect of accelerated storage temperatures at 86° and 104°F. Even as fortification forced the concentration range to greater than 90 mg. per 100 ml., the dramatic loss of ascorbic acid from all fortification levels held at 86° and 104°F was sufficient to make temperature a significant factor. Juices stored at refrigerated and room temperatures also lost ascorbic acid although not as rapid as those stored at 86° and 104°F.

Time did significantly alter the concentration of ascorbic acid. Data in Figures 2 through 6 provided evidence that ascorbic acid was consistently lost over storage time. This was in contrast to observation of Andreae (6) who expected no further loss of ascorbic acid in fortified apple juice will take place after 4 months of storage. These data also show that loss of ascorbic acid in the first three months of storage, in most cases, appears to be more rapid than in subsequent three-month storage periods. This confirms observations of other workers (17, 39, 40) that destruction of ascorbic acid is more rapid during the first month of storage after packing, during which period any free oxygen within the container is being consumed.

The results of this study are more clearly illustrated by examining the effect of each factor on percent ascorbic acid retention. Here, again, time, temperature and fortification level each individually and their interactions affected percent retention. This was in agreement with other studies (15, 19, 38, 80) that time and temperature are operative in altering retention of ascorbic acid in fortified apple juice, but in contrast to other studies which stated that percent loss of ascorbic acid in fortified apple juice during processing and storage occurs irrespective of the quantity of ascorbic acid added (106).

The following discussion examining the results from fortified and unfortified apple juice will be based on the analysis of the factors affecting percent retention.

Retention of Ascorbic Acid in Unfortified Apple Juice

The results of the investigation of ascorbic acid content of processed apple juice were in close agreement with other workers. Bunnel (21) reported a value of 1.3 mg. per 100 ml. of the juice and Esselen et al. (38) reported 1.10 mg. per 100 ml. after processing. However, a value of 1.5 mg. per 100 ml. of the juice found in this study was slightly higher than that obtained by Johnston (62) who reported 0.8 mg. per 100 ml. after processing. A possible explanation is that the juice for this study was made directly from freshly harvested apples.

The unfortified juice maintained at refrigerated temperatures (32° and 50°F) retained 67 percent of its ascorbic acid content after 3 months of storage, after which time no ascorbic acid was detected by the method employed in this study. Previous reports did not evaluate the effect of refrigerated temperatures on the retention of the naturally occurring ascorbic acid of processed apple juice.

This loss of ascorbic acid (33 percent) appears to be very high with regard to the low temperature and short time of storage. The loss, however, was calculated on the basis

of initial concentration of ascorbic acid which was originally very low. When expressed as mg. per 100 ml. of the juice, the loss was 0.5 mg. per 100 ml. which was similar to that observed in level 30 juice maintained at refrigerated temperatures (see Table 4).

No ascorbic acid was detected after 3 months of storage in the unfortified juice held at either room or accelerated temperatures. This was in agreement with results obtained by Strachan (106) who reported a constant value of 0.2 mg. per 100 ml. of the processed juice after 2 weeks of storage at room temperature even when the juice was highly deaerated and the headspace of the cans were nitrogen-filled. Johnston (62) also reported 0.3 mg. per 100 ml. of processed apple juice stored at room temperature for 2 weeks. It must be remembered that in estimating such minute amounts of ascorbic acid the accuracy of the determination is reduced.

It is evident from the data of this study and other workers that the ascorbic acid content of processed apple juice is negligible and ordinary canned apple juice is thus apparently valueless as an antiscorbutic product.

Factors Affecting Retention in Fortified Apple Juice

Findings of this study indicated that the retention of ascorbic acid was adversely affected by storage at higher temperatures for relatively short periods. The results also

indicate that in addition to the significant singular effects of time and temperature, the effect of time x temperature interaction also significantly contributed to the loss of ascorbic acid in fortified apple juice. The drastic effect of storage at 104°F was seen after 9 months of storage at which time no ascorbic acid was found in any juice even when fortified to 93.0 mg. per 100 ml. This was in agreement with data reported by Pope (90). When stored at 104°F; level 30, for example, retained only 33 percent of its original ascorbic acid content after 6 months. Some reports show a higher stability of the vitamin in fortified apple juice than that reported here, for example, Brenner et al. (19) found 50 percent retention in apple juice fortified to 35 mg. per 100 ml. and stored at 100°F for 6 months. However, results of this study agree with those obtained by Pope (90) who reported 31 percent retention in tomato juice fortified to the same level of ascorbic acid and held at 108°F for the same period.

At 86°F for one year, which is probably more severe than commercial storage conditions for canned foods, fortified apple juice retained 33 to 44 percent of its ascorbic acid content. This was in contrast to the findings of Guerrant et al. (50) and Moschette et al. (86) who reported 75 to 85 percent retention in citrus juices held at 80° to 85°F for one year.

Ascorbic acid retentions were decidedly better when the product was stored at room temperature. This was in agreement with the many studies (6, 15, 38, 62, 80, 106) reported for fortified apple juice held at room temperature. Data reported in the literature showed retentions of 55 to 96 percent after 3 months, 76 to 81 percent after 6 months and 67 percent after 12 months at room temperature. The values found in this study were 77 to 84 percent after 3 months, 76 to 79 percent after 6 months and 63 to 68 percent after 12 months. Brenner et al. (19) always found higher ascorbic acid stability in fortified apple juice. They reported 95 percent retention after both 3 and 6 months at room temperature.

The excellent retention of ascorbic acid in fortified apple juice kept under refrigeration is evident from the data in Table 5 and Figure 8. Juices stored at 50°F for 12 months retained 71 to 77 percent of their ascorbic acid content while those held at 32°F for the same period retained 79 to 84 percent. These findings agree with those of Lee et al. (80) for fortified apple juice. The results suggest that storage temperature of 50°F or below may be used for maximum retention of ascorbic acid.

From data in Table 5, one can observe readily the seriousness of high temperature storage conditions and conversely the nondetrimental effect of the time element when low temperatures of storage are employed. Although

some decline of ascorbic acid may be associated with long storage, the extent of decline appears to be associated with the storage temperature.

Factors affecting retention can be readily seen from analyzing for the rate of ascorbic acid loss (mg. per 100 ml. per month) in fortified apple juice. According to Kwoleck and Bookwalter (72), the rate of ascorbic acid loss, b, may be used to evaluate the effect of time and temperature on retention.

Linear regressions seemed appropriate and were used to determine the relationship between ascorbic acid loss and storage time at each temperature using a linear function of $y = a + bx$. The results of the linear regression fit and the correlation coefficients are presented in Table 11.

As may be seen from Table 11, the high correlation coefficients indicated that a linear relationship between loss of ascorbic acid and time exists. Using the F value test, the relationships were all significant at the one percent level.

Data in Table 11 also indicate that the rate of ascorbic acid loss (mg. per 100 ml. per month) is a function of temperature. Increasing the storage temperature resulted in an increase in the rate of ascorbic acid loss, however, the increase in the rate of ascorbic acid loss with increased temperature was less dramatic at refrigerated and room

TABLE 11: Coefficients describing relationship between rate of ascorbic acid loss and temperature of storage in fortified apple juice

Fortification level	Temperature °F	a	b	r	S.E.E.	F value
30	32	29.0	-0.350	-.85	.07615	21.12*
	50	28.3	-0.500	-.88	.09354	28.57*
	68	27.3	-0.667	-.93	.09538	48.85*
	86	28.2	-1.317	-.97	.10721	150.84*
	104	27.8	-2.633	-.97	.24552	115.04*
45	32	47.7	-0.667	-.89	.12388	28.95*
	50	46.8	-1.000	-.94	.13202	57.37*
	68	45.2	-1.200	-.92	.18690	41.22*
	86	46.5	-2.200	-.98	.14884	218.48*
	104	44.1	-4.233	-.96	.41020	106.50*
60	32	66.9	-0.983	-.92	.15241	41.62*
	50	66.6	-1.500	-.98	.10992	186.20*
	68	64.0	-1.800	-.95	.20983	73.59*
	86	66.3	-3.342	-.99	.11818	799.50*
	104	60.5	-5.900	-.95	.65490	81.16*
90	32	91.3	-1.517	-.94	.19284	61.85*
	50	89.7	-1.983	-.93	.28019	50.10*
	68	86.8	-2.450	-.90	.42894	32.62*
	86	90.0	-5.000	-.99	.19983	626.08*
	104	82.2	-8.100	-.95	.97365	69.21*

a = intercept
b = rate of ascorbic acid loss, mg. per 100 ml. per month
r = correlation coefficient
S.E.E. = standard error of estimate
* = significant at the one percent level.

temperatures than at accelerated temperatures. Previous studies have not evaluated the effect of storage temperatures on fortified apple juice except to state that retention of ascorbic acid decreased with increasing temperature (15, 19, 38, 62, 80).

By plotting the rate of ascorbic acid loss (b) within each fortification level on logarithmic scale versus temperature on linear scale (Figure 20), the results indicate that the rate of ascorbic acid loss is a logarithmic function of temperature. In addition, it was also noted that the exponential increase in the rate of ascorbic acid loss was parallel in all fortification levels.

Data in Table 5 indicate as the level of ascorbic acid concentration was increased, the stability of the vitamin was decreased. Previous studies have not evaluated the effect of initial ascorbic acid concentration in fortified apple juice on percent retention. Again, data in Table 11 show that the rate of ascorbic acid loss increased with increasing the fortification level. This was in contrast to the observation of Strachan (106) who stated that loss of ascorbic acid in fortified apple juice upon processing and storage is irrespective to the quantity of ascorbic acid added; although he did not present actual trials.

By plotting the rate of ascorbic acid loss at each temperature on logarithmic scale versus initial ascorbic acid

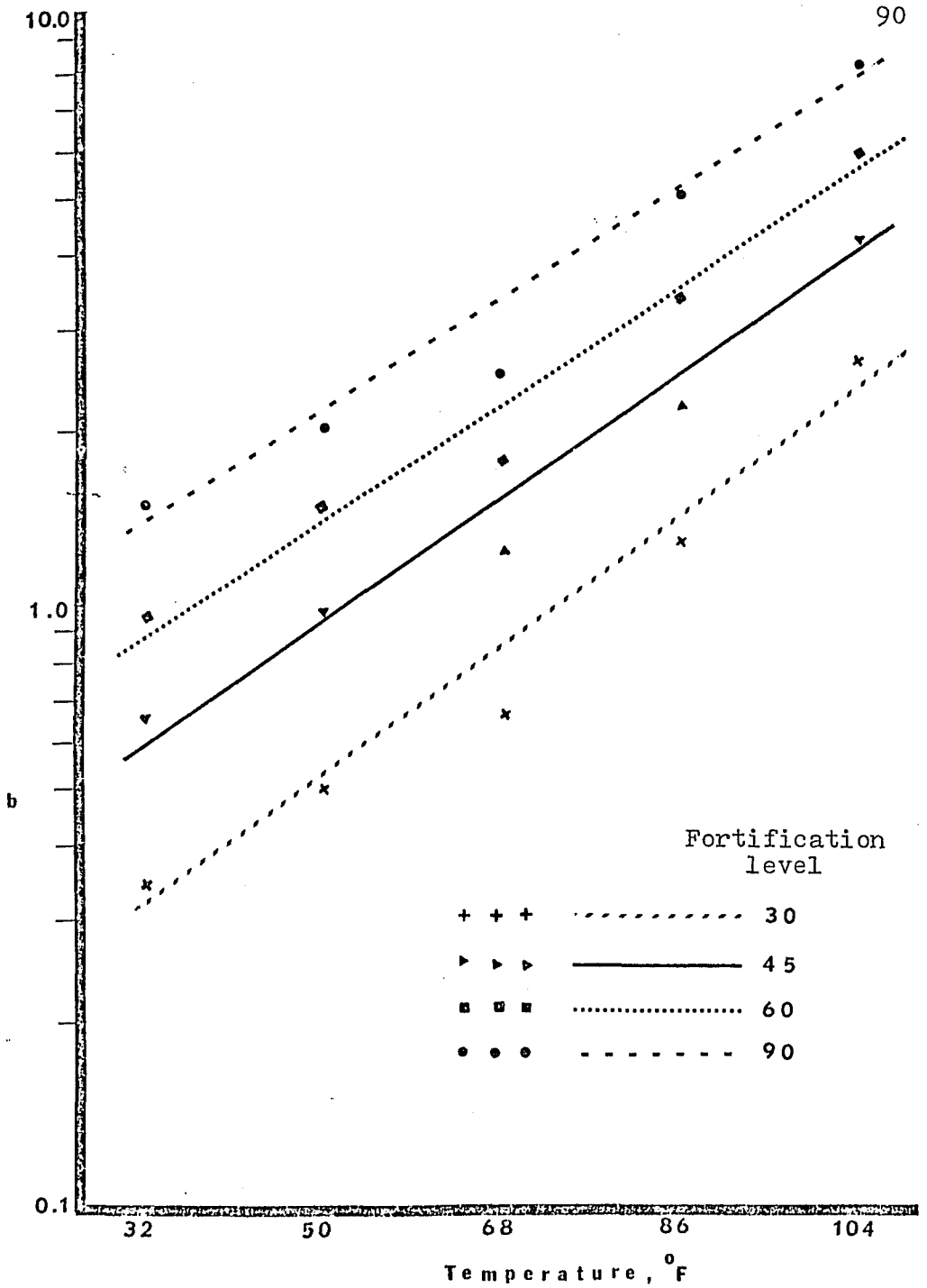


FIGURE 20: Relationship between rate of ascorbic acid loss (mg/100 ml/month) in fortified apple juice and storage temperature

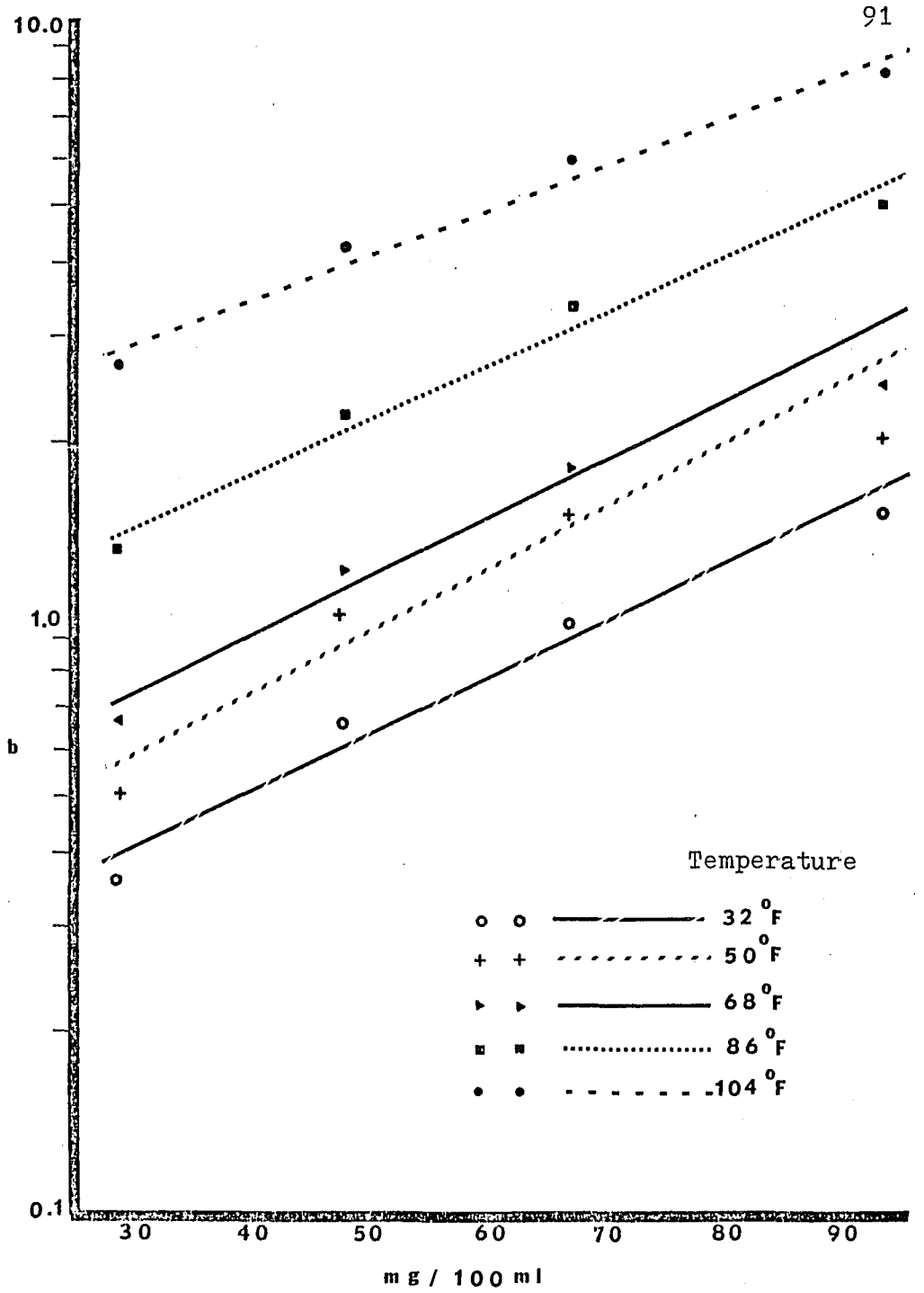


FIGURE 21: Relationship between rate of ascorbic acid loss (mg/100 ml/month) in fortified apple juice and initial ascorbic acid concentration

concentration on linear scale (Figure 21), the results indicate that the rate of ascorbic acid loss is also a logarithmic function of initial ascorbic acid concentration. The direct proportionality between rate of ascorbic acid loss and original ascorbic acid concentration supports the results of Pope (90) and Beattie et al. (16). These reports indicated that rate of ascorbic acid loss was increased with increasing initial ascorbic acid concentration.

Effect of Fortification on pH

Data in Table 6 have shown the pH of the fortified juice decreased upon the addition of ascorbic acid and this decrease was increased with increasing the initial ascorbic acid concentration. Since ascorbic acid was the only acid added to apple juice, the decrease in pH upon fortification is attributed to release of the hydrogen ions from ascorbic acid. This is clearly illustrated by investigating the hydrogen ion concentration of the juice after processing. Level 0 (unfortified juice) had a pH value of 3.80 or a hydrogen ion concentration (H^+) of 1.58×10^{-4} mole per liter. Upon the addition of 27.0 mg. ascorbic acid per 100 ml. of the juice (level 30) the hydrogen ion concentration increased to 1.86×10^{-4} mole per liter or an increase of 2.8×10^{-5} mole per liter. Similarly the addition of 91.5 mg. ascorbic acid per 100 ml. of the juice (level 90) resulted in an increase

of (H^+) by $7.1x10^{-5}$ mole per liter. Statistical analysis of data reported by Pope (90) indicated that the fortification of tomato juice with ascorbic acid significantly affected the pH of the juice, however, he did not present pH values of the juice upon fortification and before storage for which comparisons can be made.

Findings of this study have also shown the pH of the juice increased with storage time and temperature, however, data in Appendix B clearly illustrate the influence of storage time and temperature on the pH of the juice. It is only with temperatures higher than 68°F and storage time greater than 9 months that force the pH to increase by up to 0.3 unit as shown in Figure 15.

Storage at refrigerated and room temperatures up to 9 months, however, resulted in a slight decrease in the pH usually by .05 unit. Even when the juice was stored at these temperatures for 12 months the pH remained either essentially constant or increased by no greater than .05 unit. Pope (90) also presented data showing a decrease in the pH of tomato juice fortified with ascorbic acid and stored for 9 months. It is apparent that the increase in the pH of the juice occurred in the last three months of storage at accelerated temperatures.

Huelin (57) studying anaerobic degradation of ascorbic acid in model system reported an increase in the pH from

3.0 to 7.2 when a solution of ascorbic acid was heated in vacuum at 100°C for 10 days. It must be remembered that meaningful comparisons cannot be made with such model systems since a temperature of 100°C should never be encountered in storage of canned foods.

Effect of Fortification on Titratable Acidity

Percent titratable acidity was calculated as malic acid from the formula on page 33. The milliequivalent weight of malic acid is 0.067 or $\frac{\text{formula weight}}{1000}$ divided by 2, the number of acidic (H^+) ions per mole. Since ascorbic acid is a monobasic acid (51), its milliequivalent weight is 0.176 and the formula for percent acidity as ascorbic acid is:

Percent ascorbic acid =

$$\frac{(\text{ml. of NaOH})(\text{Normality of NaOH})(0.176)\ 100}{10 \text{ ml. sample}}$$

Findings of this study have shown that percent acidity of apple juice increased with increasing fortification level and storage time. Since ascorbic acid was the only added acid, the increase due to fortification may be related to percent ascorbic acid as measured in mg. per 100 ml. of the juice.

Calculating from percent malic acid, the increase due to fortification presented as increase in acidity as malic acid is:

Percent malic increase =

$$\frac{\frac{(\text{Percent ascorbic})(10 \text{ ml. sample})}{(100 \text{ percent})(0.176)} \cdot (0.067)}{10 \text{ ml. sample}} \times (100)$$

Simplifying:

Percent malic increase = percent ascorbic acid (0.381).

The data on percent acidity as malic during the entire storage period (Appendix B) were analyzed by regression equation, containing linear function of initial ascorbic acid concentration, of the form:

$$Y = a + bx$$

where Y is percent malic acid; a is the intercept; b is the regression coefficient and x is the initial ascorbic acid concentration. The regression coefficient (b), correlation coefficient (r); intercept (a) and standard error of estimate (S.E.E.) are shown along with the corresponding curve in Figure 22.

It is noted from Figure 22 that percent malic acid was increased by 0.00129 per 1 mg. ascorbic acid added. Translating the ascorbic acid into percent malic acid equivalent yields:

1 mg. ascorbic acid/100 ml. = 0.001 percent

(0.001)(0.381) = .000381 percent malic/0.00129 percent malic increase or, 0.295 percent malic/one percent malic increase.

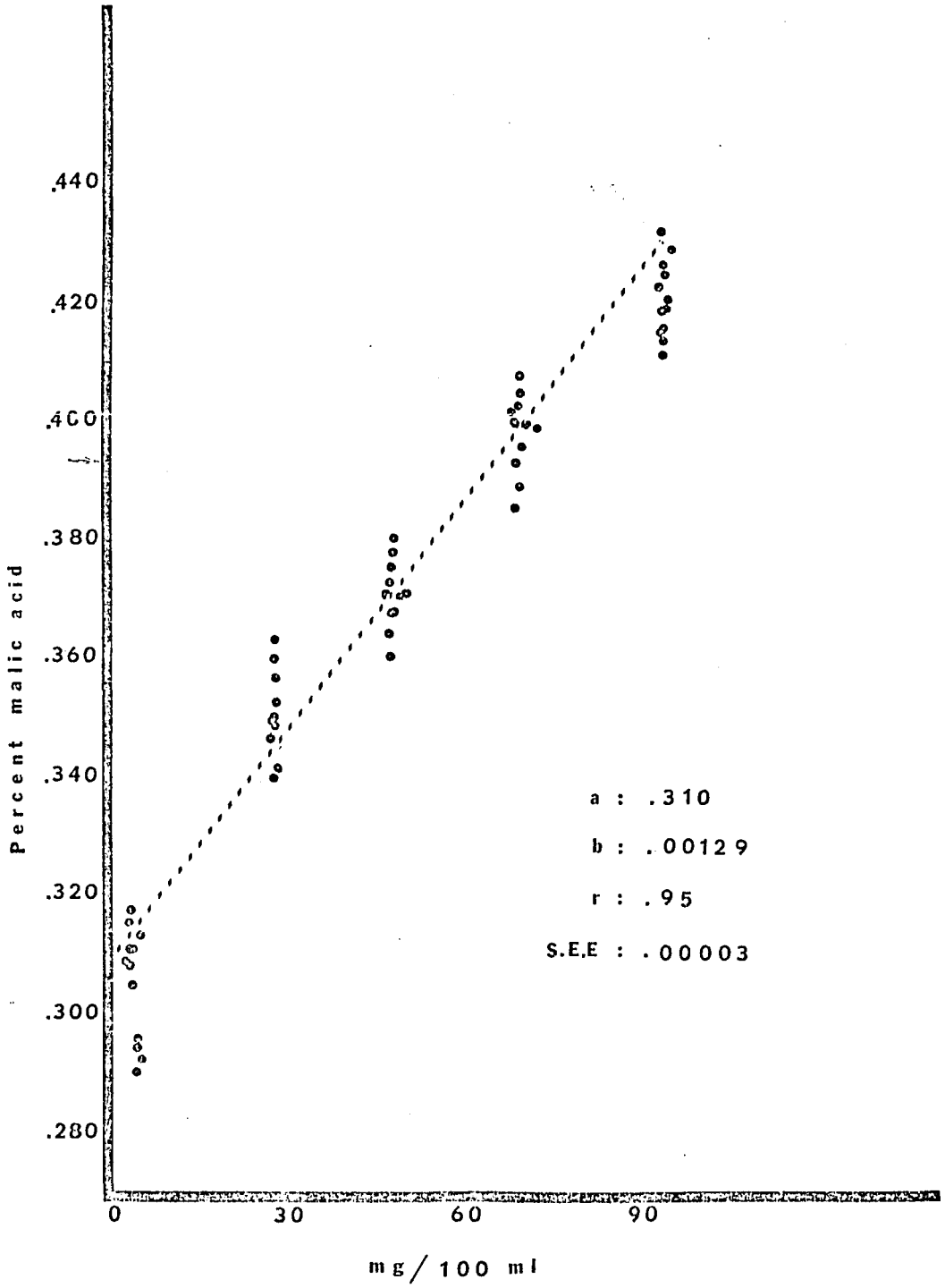


FIGURE 22: Regression plot of percent malic acid increase in apple juice due to fortification with ascorbic acid

Thus, 29.5 percent of the increase in percent acidity calculated as malic may be related directly to ascorbic acid in the juice.

It was also noticed that percent titratable acidity increased upon storage. This is in agreement with results obtained by Pope (86) who reported increased acidity with storage time in ascorbic acid fortified tomato juice. This increase in percent titratable acidity may be due to degradation products of ascorbic acid. Tatum et al. (108) isolated 15 compounds as degradation products from ascorbic acid among which 3 acids were reported. The increase in percent titratable acidity probably did not result in loss of malic acid of the juice as indicated by Lamden and Harris (78) who reported no loss or breakdown of citric or malic acid during browning of an ascorbic acid-citric or malic mixture in a model system.

Effect of Fortification on Soluble Solids

Percent soluble solids was determined by measuring the refractive index of the juice by Abbé refractometer. The refractive index was converted to percent soluble solids as sucrose by use of a conversion table with temperature correction.

Analysis of variance have shown that fortification level significantly affected percent soluble solids. Data in

Table 8 indicated that level 0 had a soluble solids content of 13.51 percent whereas level 30 had a value of 14.37 percent. If the addition of ascorbic acid to apple juice had significantly resulted in an increase in percent soluble solids, level 30, which contains 27 mg. ascorbic acid per 100 ml. more than level 0 or 0.027 percent ascorbic acid, would be expected to have a soluble solids content of 13.54 percent which was not the case. Similarly, level 90 would have a soluble solids of 13.60 percent instead of 13.93 percent after processing. It must be noted that the refractive index as measured by the refractometer is graduated on the basis of pure sucrose solutions and soluble solids other than sucrose do not affect the refractive index to the same extent as sucrose (75). For these reasons, ascorbic acid additions did not alter percent soluble solids.

The variation observed in the soluble solids content of the juice upon the addition of ascorbic acid, however, may probably be due to variation in blending the two cultivars employed in this study and/or to variation in the quantity of the free-run juice in each fortification lot. The free-run juice expressed in the early stage of pressing apple pulp contains a higher soluble solids content. In this study, each fortification lot was processed as soon as more than 8 gallons of the juice were collected.

Findings of this study have also shown the percent soluble solids decreased with storage time and temperature, however, the effect of temperature was much less pronounced than time effect. The decrease in the soluble solids content occurred mostly in the last three months of storage (see Figure 18) particularly at accelerated storage temperatures of 86° and 104°F. In any event, the decrease was no greater than 0.40 percent soluble solids in any fortification level.

The decrease in percent soluble solids upon storage may be due to inversion of sucrose of apple juice. The sugars of apple juice consist predominantly of fructose followed by sucrose then glucose (104). Aitken (4) presented data indicating that stored apple juice usually shows only a very low percentage of sucrose or none at all due to inversion of sucrose in the acid medium of apple juice. Furthermore, the inversion increases the amount of glucose and disturbs the glucose-fructose ratio. This process mainly depends on the pH, temperature of the juice and duration of storage. On the basis of these findings it could be concluded that a storage period longer than 9 months and a temperature greater than room temperature was necessary to significantly alter the percent soluble solids of apple juice.

Effect of Fortification on °Brix:Acid Ratio

The °Brix:acid ratio was determined by dividing the percent soluble solids by percent acidity as malic acid.

Findings of this study have indicated that the addition of ascorbic acid to apple juice significantly decreased the ratio upon fortification and the decrease was increased with increasing the initial ascorbic acid concentration as may be seen from Figure 19. The decrease in °Brix:acid ratio was directly related to the increase in percent malic acid upon fortification. It has also been shown that the ratio further decreased with increasing storage time which was evidently due to increase in percent malic acid and decrease in percent soluble solids upon storage.

The °Brix:acid ratio, rather than Brix degrees or percent soluble solids alone, has been employed to indicate the flavor and palatability of fruit juices. It is also required for grading apple juice. U.S. Standards for Grades of canned apple juice specify a Brix value of not less than 11.5 and 10.5 degrees for Grades A and C respectively; and percent malic acid not less than 0.35 nor more than 0.70 for Grade A and not less than 0.30 nor more than 0.80 for Grade C (113).

It is apparent that percent soluble solids of the juice in all fortification levels has met the requirement for Grade A canned apple juice; however, level 0 with a percent malic acid of 0.297 was considered Grade C at the most. The

addition of ascorbic acid to apple juice has increased percent malic acid from 0.297 in level 0 to 0.360 in level 30 and the acidity was further increased with increasing fortification level. Thus, fortification of apple juice with ascorbic acid, even at level 30, upgraded the juice to Grade A.

Although fortified apple juice held at 86° and 104°F for 6 months of storage or longer maintained a °Brix:acid ratio characteristic of Grade A, these juices were brown in color and much darker than juices stored at refrigerated or room temperatures for the same period of time and developed objectionable flavor. Therefore, the °Brix:acid ratio of juices stored at 86° and 104°F cannot be considered the only quality index for the flavor of these juices.

Effect of Fortification on Vacuum

Data of the analysis of variance have shown that the can vacuum was significantly influenced by fortification level. However, the results of the investigation of can vacuum after processing and cooling indicate no relationship between can vacuum and initial ascorbic acid concentration of apple juice. Can vacuum measured after processing and cooling was 4.50, 7.75, 9.00, 4.50 and 6.50 in. of mercury for levels 0, 30, 45, 60 and 90 respectively. It is apparent that variation in vacuum existed among fortification lots but this variation did not follow a specific trend in relation to ascorbic acid

concentration of the juice.

It is well established that the vacuum of a can will vary according to the temperature of the can contents at time of sealing. As the closing temperature is increased, the vacuum in the can increases upon cooling. Thus, the variation in can vacuum observed among fortification lots after processing and cooling was mainly due to variation in the product temperature upon sealing.

Storage temperature, time and temperature x time interaction were found to have the greatest significant effect on can vacuum. Data in Table 10 illustrated the dramatic effect of high storage temperature on can vacuum. Storage at 86°F resulted in not only a rapid decrease in vacuum but also produced pressure in the cans at the end of 12 months storage, whereas storage at 104°F produced pressure in the cans after only 3 months of storage and the pressure was increased with increasing storage time. It is also noteworthy that cans stored at 104°F developed a hard swell after 6 months of storage due to increased pressure inside the cans.

The rapid loss of vacuum and development of pressure in cans stored at elevated temperatures is probably due to the formation of carbon dioxide resulting from ascorbic acid decomposition. Several investigators (17, 20, 24, 42, 43, 44, 57, 58, 64, 66) have indicated that anaerobic degradation of ascorbic acid in model systems and fruit juices leads to the

formation of furfural and carbon dioxide especially at high temperatures and acidities. Gresswell (49) also pointed out that carbon dioxide produced during anaerobic destruction of ascorbic acid results in an increased pressure in the container. This was also in agreement with results obtained by Pope (90) who reported rapid decrease in vacuum and development of pressure in cans of fortified tomato juice stored at 108°F for 6 months.

Prediction of Ascorbic Acid Retention in Fortified Apple Juice

Many workers (16, 17, 20, 26, 43, 44, 57, 58, 64, 66, 73, 115) have assumed that loss of ascorbic acid in fruit juices and related aqueous systems is a first-order reaction in which the reaction rate is proportional to ascorbic acid concentration and may be expressed as a rate constant, k.

$$\text{Ascorbic acid} \xrightarrow{k} \text{degradation products.}$$

The following differential equation may be written to describe the reaction at constant temperature (115);

$$dC/dt = -kC \qquad [1]$$

where C = ascorbic acid concentration

t = time in months

k = first-order rate constant.

If $C = C_o$ at $t = 0$, then integration of equation [1] yields:

$$\ln \frac{C}{C_o} = -kt \qquad [2]$$

Changing to log 10

$$2.303 \log \frac{C}{C_o} = -kt \qquad [3]$$

or,

$$k = \frac{2.303}{t} \log \frac{Co}{C} \qquad [4]$$

Therefore, there is a linear relation between log ascorbic acid concentration and time and a plot of log ascorbic acid concentration versus time yields a straight line for a first-order reaction, the slope of the resulting line is k/2.303.

This aspect was specifically examined using the data in Table 4 and the regression lines from Table 11. The results of plotting log ascorbic acid concentrations in fortified apple juice versus time of storage at each storage temperature are sown in Figures 23 through 27. As may be seen from these graphs, the plots yielded straight lines indicating that destruction of ascorbic acid in the fortified juice follows a first-order reaction. In addition, the regression lines were nearly parallel for all fortification levels stored at the same temperature and had almost the same slopes. The reaction rate constant, k, was calculated by multiplying the slopes of the regression lines by 2.303 according to equation [4]. The values of k (Table 12) indicate that the rate constants for ascorbic acid degradation in fortified apple juice are relatively small at the refrigerated and room

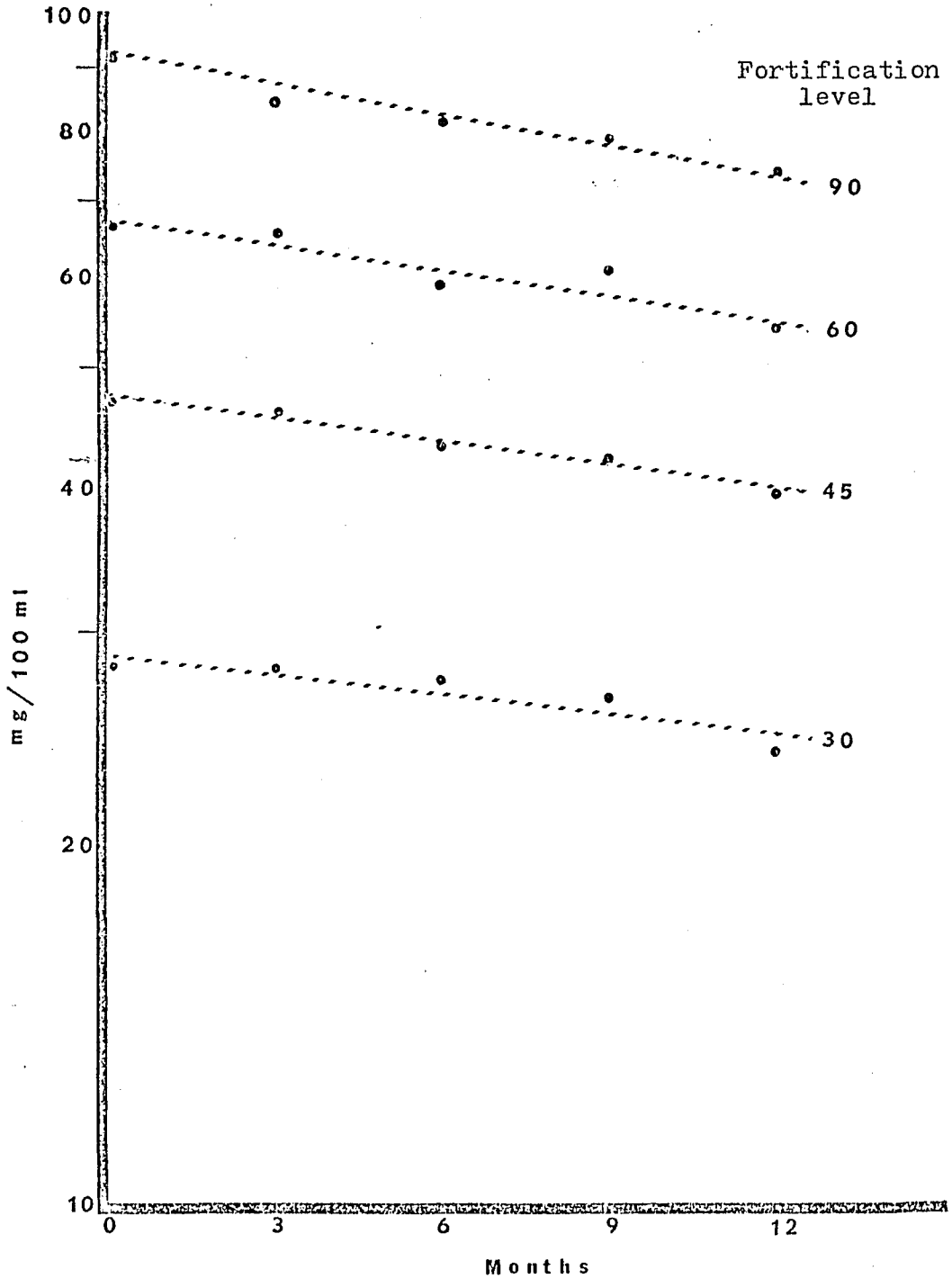


FIGURE 23: Regression lines of log ascorbic acid concentration in fortified apple juice vs. storage time at 32°F

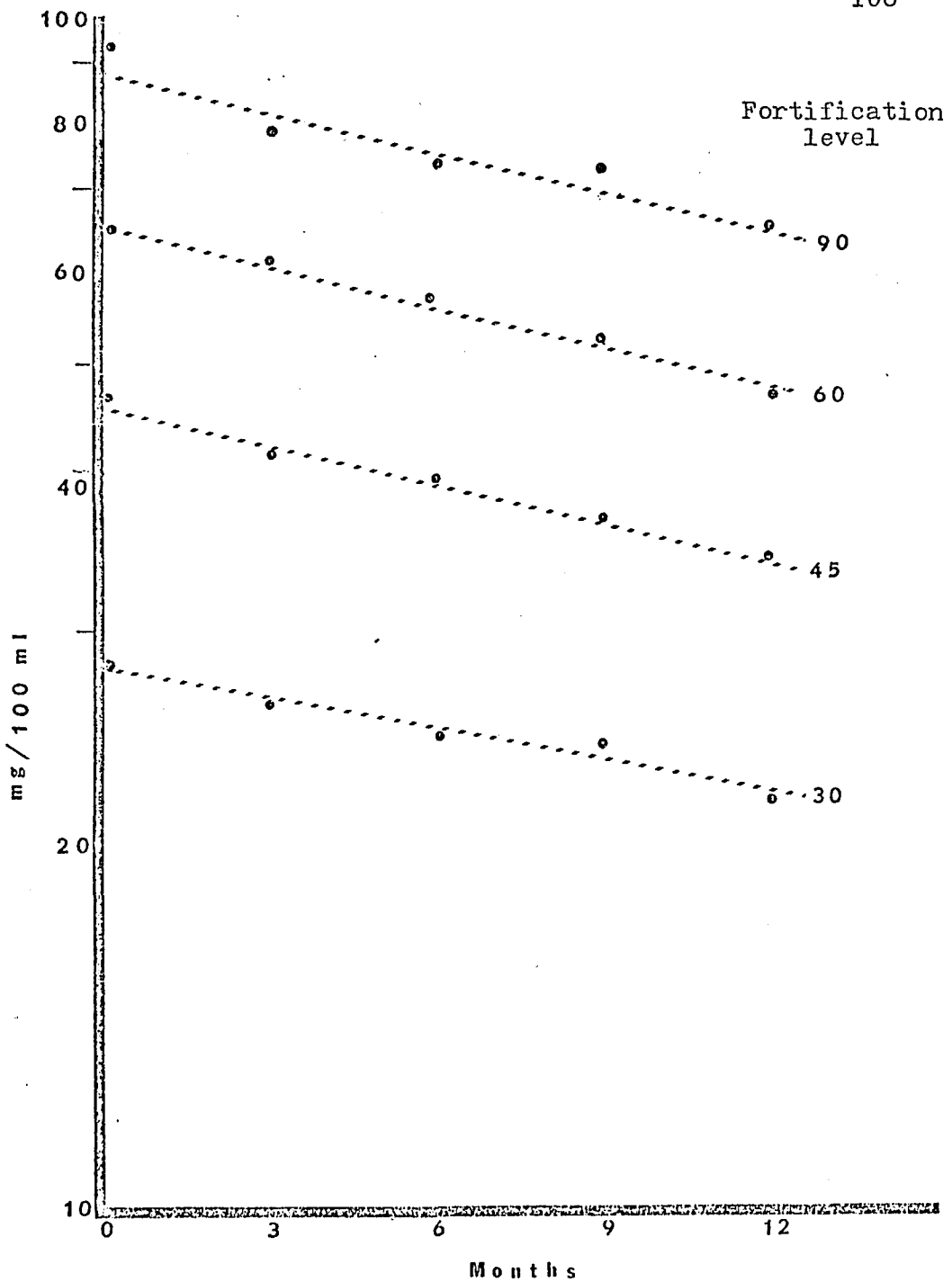


FIGURE 24: Regression lines of log ascorbic acid concentration in fortified apple juice vs. storage time at 50°F

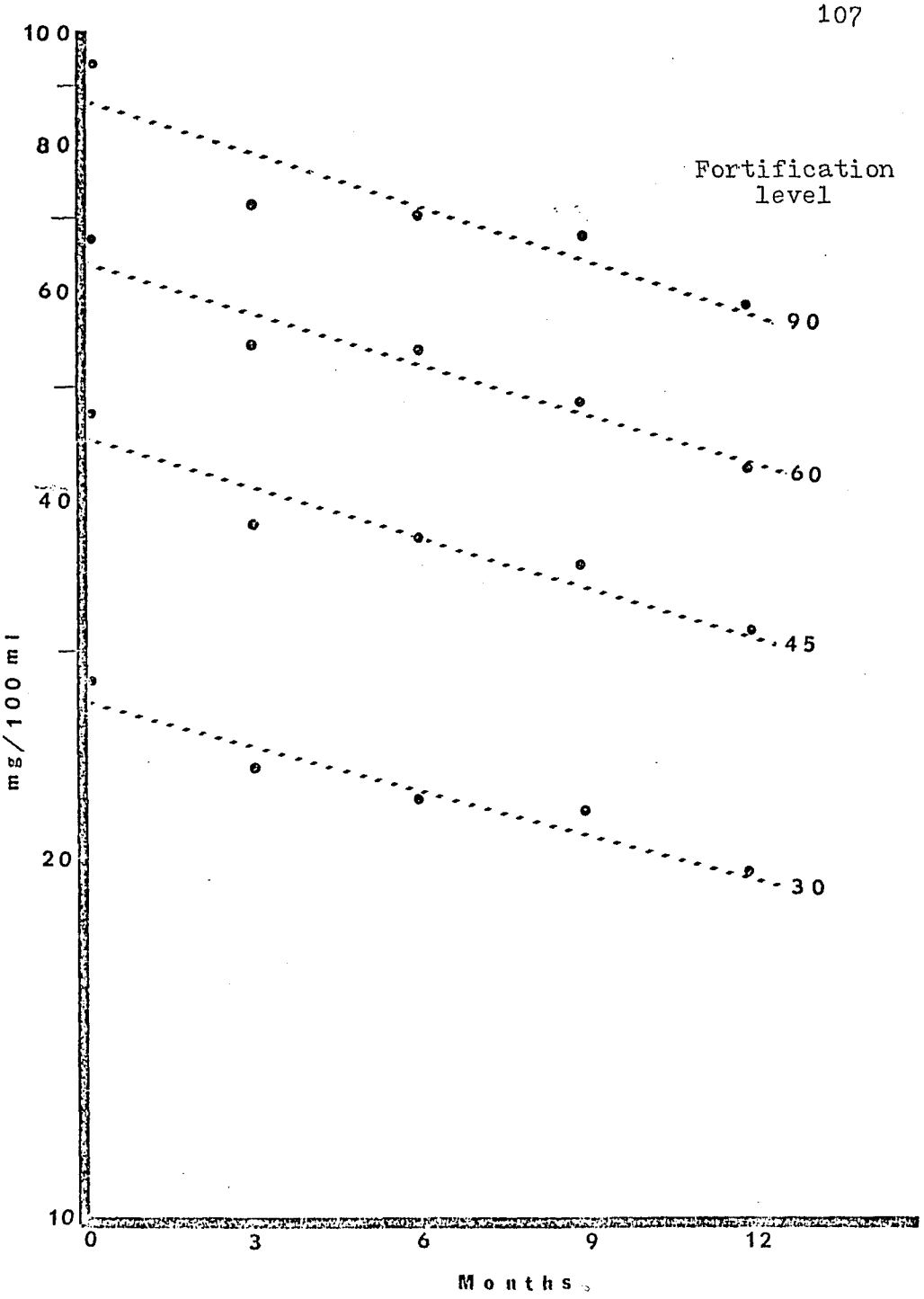


FIGURE 25: Regression lines of log ascorbic acid concentration in fortified apple juice vs. storage time at 68°F

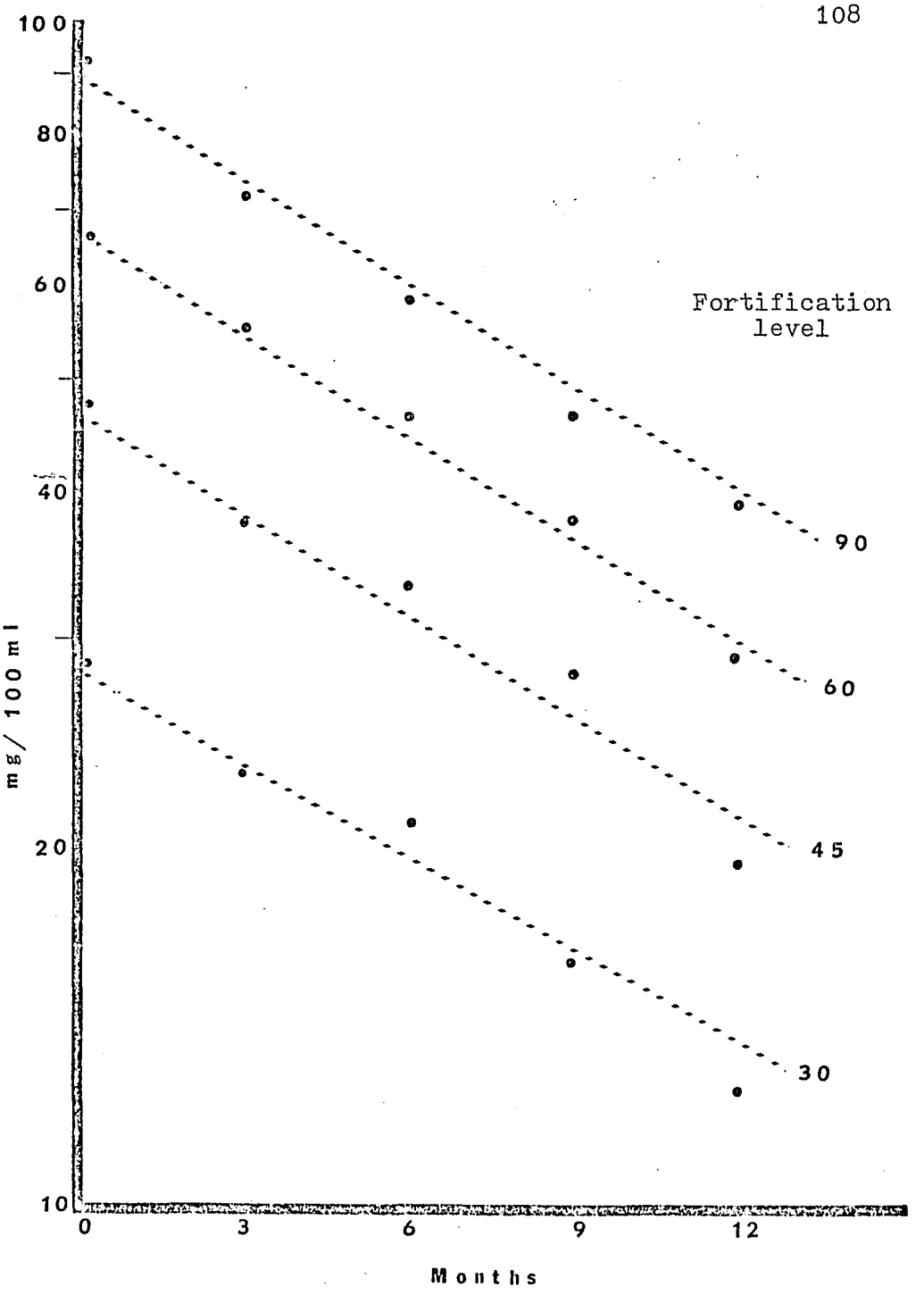


FIGURE 26: Regression lines of log ascorbic acid concentration in fortified apple juice vs. storage time at 86°F

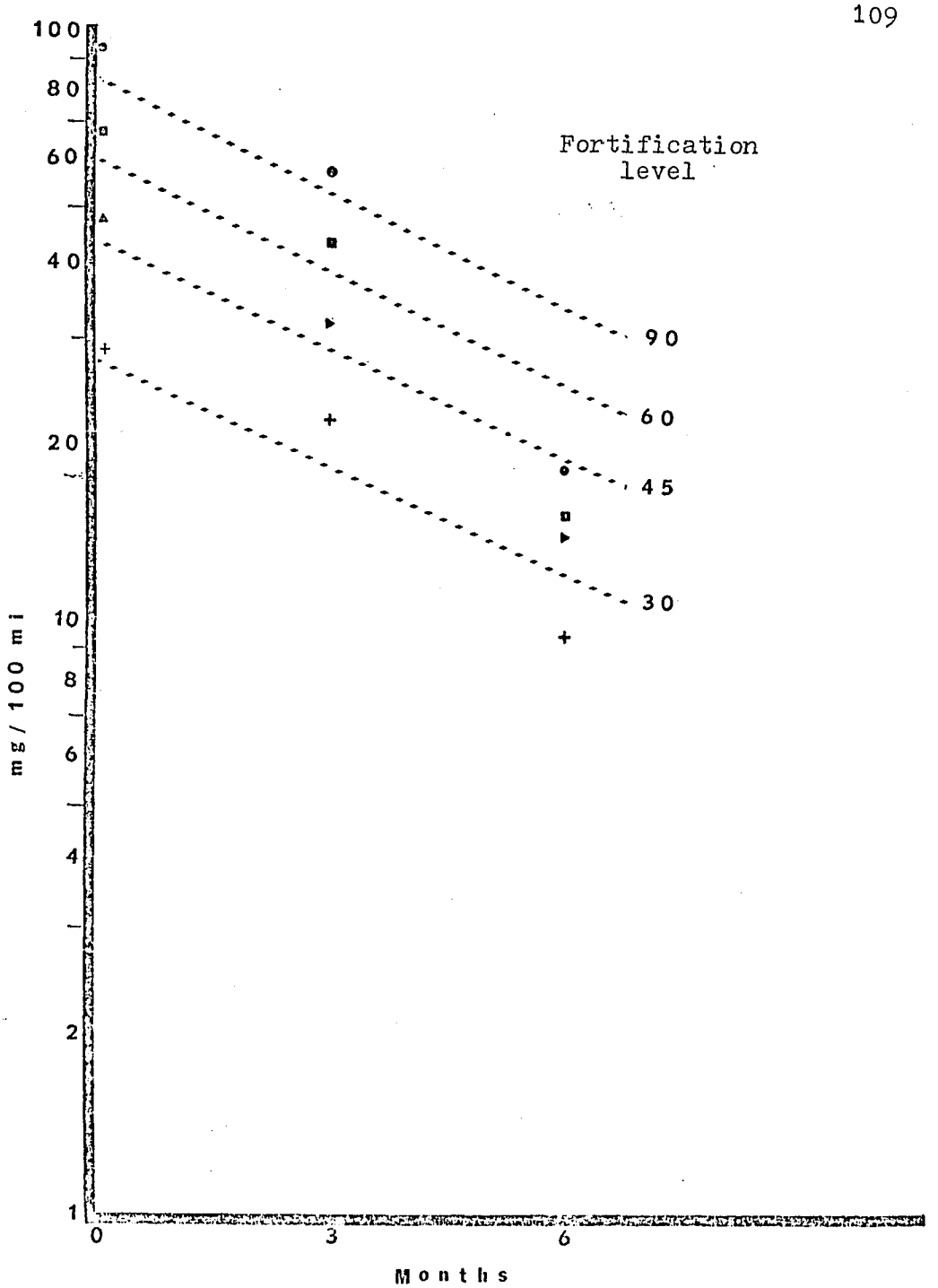


FIGURE 27: Regression lines of log ascorbic acid concentration in fortified apple juice vs. storage time at 104°F

temperatures while the reverse is true at accelerated storage temperatures. This suggests negligible loss of the vitamin at lower temperatures of storage.

It is known empirically that a rise of $10^{o}C$ ($18^{o}F$) in temperature increases the rate of most chemical and biological reactions from two to four times.

Thus, the temperature coefficient, Q_{10}, of the rate of ascorbic acid destruction was calculated from the relationship:

$$Q_{10} = \frac{k_{(t)}}{k_{(t-18)}} \qquad [5]$$

in which $k_{(t)}$ is the reaction rate constant at temperature T_1, and $k_{(t-18)}$ is the reaction rate constant at a temperature $18^{o}F$ ($10^{o}C$) below T_1. The values of Q_{10} are tabulated in Table 13 and indicate that between 32^{o} and $68^{o}F$, the rate of ascorbic acid destruction was increased by approximately one and half times for each $18^{o}F$ rise in temperature. Calculated Q_{10} value from 68^{o} to $86^{o}F$ shows the rate slightly more than doubled while increasing the temperature from $86^{o}F$ to $104^{o}F$ tripled the rate of ascorbic acid destruction. Here again, the dramatic effect of accelerated storage temperatures on the rate of ascorbic acid degradation can be readily seen.

To further illustrate the effect of storage temperature on the rate of ascorbic acid degradation, the half-life, $t_{\frac{1}{2}}$, of ascorbic acid at each storage temperature was calculated

TABLE 12: The reaction rate constants, k(months^{-1}), at constant temperature

Temperature ^{o}F	k(months^{-1})
32	.01588
50	.02410
68	.03158
86	.07606
104	.22877

TABLE 13: Temperature coefficient, Q_{10}, of rate of ascorbic acid destruction

Temperature range ^{o}F	Q_{10}
32-50	1.5
50-68	1.3
68-86	2.4
86-104	3.0

from the following relationship (73) for a first-order reaction:

$$t_{\frac{1}{2}} = \frac{0.693}{k} \qquad [6]$$

This value represents the time required to destroy 50 percent of ascorbic acid originally present in the juice, thus, yielding useful information as to the shelf life of fortified apple juice with respect to ascorbic acid.

The calculated values of the half-life of ascorbic acid (Table 14) clearly demonstrate the excellent expected shelf life of ascorbic acid in fortified apple juice stored at refrigerated temperatures. Favorable shelf life would also be anticipated if the juice is held at room temperature; slightly less than 2 years storage will be required to reduce the original ascorbic acid content by half. On the other hand, storage at accelerated temperatures would result in a drastic decrease in the shelf life of ascorbic acid with only 50 percent of the vitamin remaining after 9 and 3 months storage if the juice is stored at 86° and 104°F respectively.

Reaction rate constant as a function of temperature:

The most satisfactory method for expressing the influence of temperature on the reaction rate constant, k, is the Arrhenius equation which gives a linear relation between log k and reciprocal of absolute temperature (26):

TABLE 14: The half-life, $t_{\frac{1}{2}}$, of ascorbic acid in apple juice at constant temperature

$$t_{\frac{1}{2}} = \frac{0.693}{k}$$

Temperature ^{o}F	$t_{\frac{1}{2}}$ (months)
32	43.6
50	28.8
68	21.9
86	9.1
104	3.0

$$\log k = \log A - \frac{E_a}{2.303\ RT} \qquad [7]$$

where k = reaction rate constant;

A = pre-exponential frequency factor;

E_a = activation energy (calories/mole);

R = gas constant (1.987 calories/mole K); and

T = absolute temperature (Kelvin).

Data in Table 12 were utilized to plot log rate constant, k, versus the reciprocal of absolute temperature in an Arrhenius plot. From Arrhenius plot (Figure 28), the corresponding activation energy, E_a, was computed. The activation energy, which is a measure of the dependence of the reaction rate temperature, is equal to the slope of Arrhenius plot times the gas constant [(R) x 2.303].

As is evident from Figure 28, two rather distinct curves are obtained when the experimental data are plotted. It appears that a change in the kinetics of the reaction apparently occurs between 68° and 86°F. This is evident from the break in the curve in this region which occurs at 75°F. Below this temperature, the activation energy, E_a, is much lower than the value of the activation energy at temperatures above 75°F as may be seen from data in Figure 28. This indicates that changes in temperature above 75°F have a more significant influence on the rate at which ascorbic acid is lost from the product. The change in kinetics between

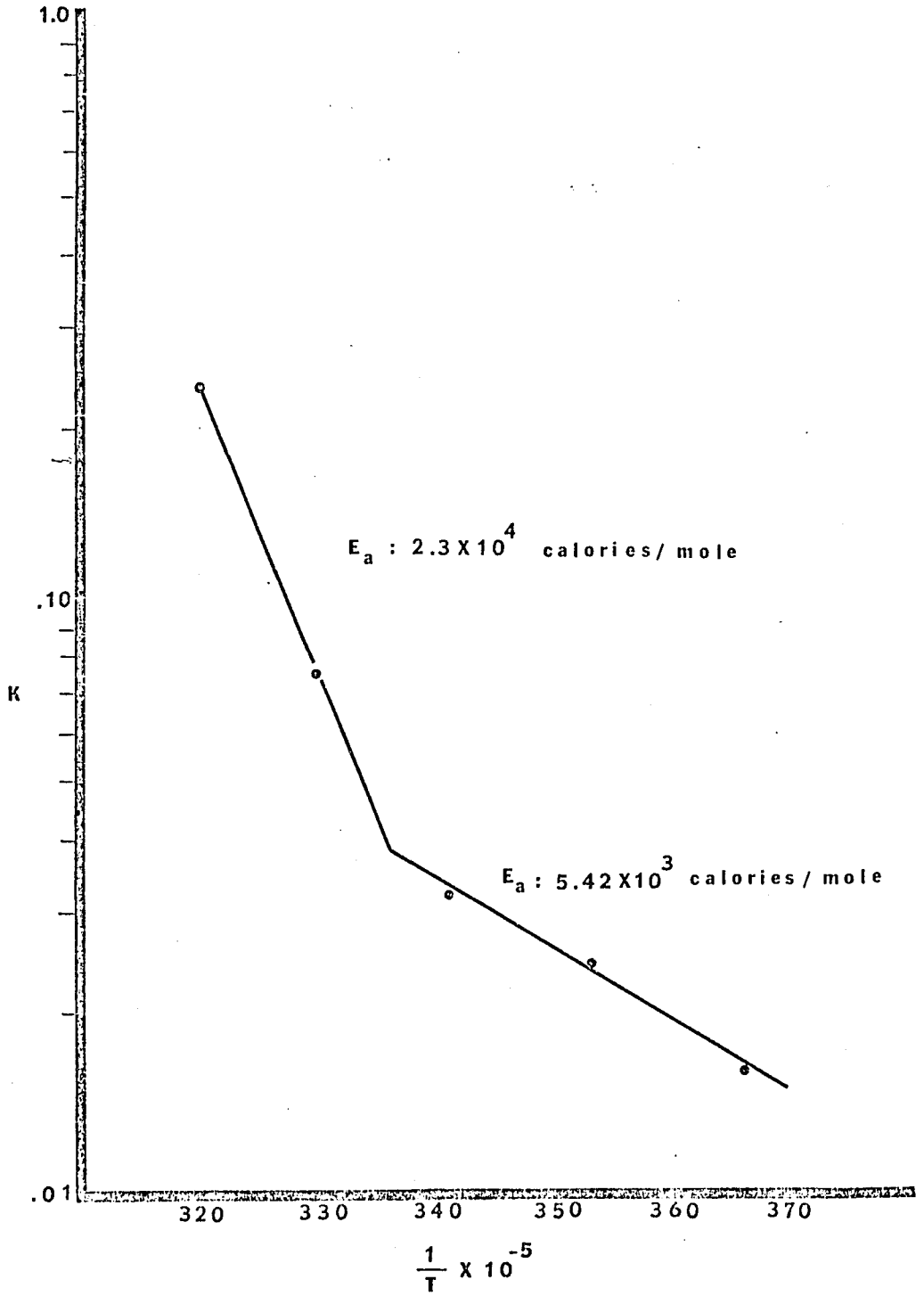


FIGURE 28: Arrhenius plot. Effect of storage temperature on rate of ascorbic acid destruction in fortified apple juice

68° and 86°F suggests that another mechanism may be governing the rate of change here. It must be noted that a break in Arrhenius plot is not unusual and has been observed by Charm (26) when analyzing data collected by Dietrich et al. (33) concerning ascorbic acid degradation in frozen spinach. Charm (26) observed a change in kinetics of ascorbic acid destruction in frozen spinach between 15° and 20°C, and Pope (90) indicated that rates of ascorbic acid degradation in fortified tomato juice did not satisfactorily fit Arrhenius equation. Pope's data (90) for rates of ascorbic acid destruction were not determined by regression analysis.

It should be noted that fortified apple juice held at 104°F developed a dark brown color and a caramelized flavor. Therefore, values of k above 86°F, while valid, do not give any information about the acceptability of apple juice.

From data in Figure 28, values of k for temperature intermediate to the temperatures employed in this study could be estimated.

The above discussion illustrates that the various kinetic parameters can be used to compare degradation of ascorbic acid under different storage conditions. The kinetic analysis is particularly useful in predicting the shelf life of the product with respect to a particular nutrient and optimizing the nutrient retention. By measuring the concentrations of ascorbic acid over a period of time,

the parameters, such as reaction rate constant and activation energies can be calculated. These parameters are in turn useful in selecting the optimum storage conditions for the product.

More specific prediction formula for ascorbic acid in fortified apple juice would be useful.

Rearranging equation [4]:

$$k = \frac{2.303}{t} \log \frac{C_o}{C} \qquad [4]$$

yields:

$$2.303 \log \frac{C_o}{C} = kt \qquad [8]$$

$$\frac{C_o}{C} = 10^{\frac{kt}{2.303}} \qquad [9]$$

$$C_o = C \cdot 10^{\frac{kt}{2.303}} \qquad [10]$$

from equation [3]:

$$2.303 \log \frac{C}{C_o} = -kt \qquad [3]$$

$$\frac{C}{C_o} = 10^{\frac{-kt}{2.303}} \qquad [11]$$

$$C = C_o \cdot 10^{\frac{-kt}{2.303}} \qquad [12]$$

The initial concentration C_o, after processing and cooling and before storage, required to give a final concentration C can be predicted from equation [10] when the storage

temperature and time are known. Similarly, the final concentration C can be also predicted from equation [12].

In case of fluctuating storage temperatures as may usually occur in commercial warehouses, it is possible to determine the change for a first-order reaction by using the temperature-time record of the warehouse. In this situation, the temperature of the warehouse is plotted graphically versus time of storage. For each time interval (t_1), the average warehouse temperature (T_1) is obtained from the graph. The rate constant (k_1) at temperature T_1 is obtained from Arrhenius plot in Figure 28. This process is repeated for a second and third time interval and so on during the entire storage period. By this process, the rate constant k for each time interval is obtained and the sum of the exponent in equations [10] and [12] is calculated to give the initial or the final concentration desired at fluctuating temperatures. For example:

$$\frac{k_1 t_1}{2.303} + \frac{k_2 t_2}{2.303} + \frac{k_3 t_3}{2.303} \quad \cdots\cdots\cdots\cdots + \frac{k_n t_n}{2.303}$$

and equation [10] becomes[1]:

$$C_o = C \cdot 10^{\Sigma(\frac{k\Delta t}{2.303})} \qquad [13]$$

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[1]Blaisdell, J.L. 1976. Personal communication. Department of Food Science and Nutrition. The Ohio State University, Columbus, Ohio.

Equation [12] becomes:

$$C = C_o \cdot 10^{\Sigma\left(\frac{-k\Delta t}{2.303}\right)} \qquad [14]$$

The initial concentrations, C_o, required to give 100 percent of RDA of ascorbic acid per 6-fluid ounce serving of apple juice under given storage conditions and durations have been calculated from the data obtained in this study and appear in Table 15. Similarly, an apple juice processor can determine the necessary initial concentration of ascorbic acid required to meet his label claim after a specific storage time and temperature.

TABLE 15: Initial concentration, C_o, required to produce 60 mg. ascorbic acid (100 percent RDA) per 6-fluid ounce serving of apple juice

Time (months)	Temperature (°F)							
	50	55	60	65	70	75	80	85
1	61.4	61.5	61.7	61.9	62.1	62.3	63.3	64.7
2	63.0	63.1	63.5	63.8	64.2	64.6	66.7	69.7
3	64.5	64.8	65.4	65.8	66.4	67.0	70.3	75.1
4	66.1	66.4	67.2	67.9	68.7	69.6	74.2	81.0
5	67.7	68.2	69.2	70.1	71.1	72.2	78.2	87.3
6	69.3	69.9	71.2	72.3	73.6	74.9	82.5	94.1
7	71.0	71.7	73.2	74.5	76.1	77.7	86.9	101.4
8	72.8	73.6	75.4	76.9	78.8	80.7	91.7	109.3
9	74.5	75.5	77.5	79.3	81.5	83.7	96.7	117.8
10	76.3	77.4	79.8	81.8	84.3	86.9	101.9	127.0
11	78.2	79.4	82.1	84.4	87.2	90.1	107.5	136.9
12	80.1	81.5	84.5	87.0	90.2	93.5	113.3	147.6

SUMMARY AND CONCLUSIONS

Apple juice fortified with ascorbic acid at levels of 0, 30, 45, 60 and 90 mg. ascorbic acid per 100 ml. of the juice was used in this study. Samples were stored at 32, 50, 68, 86 and 104°F for periods of 3, 6, 9 and 12 months.

Factors significantly affecting the retention of ascorbic acid were time, temperature, fortification level and their interactions. The pH, titratable acidity and °Brix:acid ratio were also significantly affected by the same factors. Vacuum and soluble solids were not altered by the addition of ascorbic acid, however, time, temperature and their interaction significantly changed vacuum and soluble solids.

The following conclusions can be drawn from this study:

1. The naturally occurring ascorbic acid in ordinary processed apple juice is negligible. Unfortified canned apple juice is valueless as an antiscorbutic product.
2. In fortified apple juice:
   a) Ascorbic acid was consistently lost over time of storage. A linear relationship between ascorbic acid and time existed.

b) The percent retention of ascorbic acid decreased with increasing storage temperature and initial ascorbic acid concentration.

c) The rate of ascorbic acid loss expressed as mg. per 100 ml. per month increased logarithmically with an increase in storage temperature and initial ascorbic acid concentration.

3. Fortification of apple juice with ascorbic acid affected pH, titratable acidity and ^{o}Brix:acid ratio; that is,

a) The pH was decreased upon fortification due to release of hydrogen ions from ascorbic acid. A storage time greater than 9 months and a temperature higher than 68^{o}F were necessary to increase the pH of the juice.

b) Percent malic acid increased upon fortification and with time; with 29.5 percent of the increase in titratable acidity was directly attributed to ascorbic acid.

c) The ^{o}Brix:acid ratio decreased with fortification and time mainly due to increase in percent malic acid. Fortification of apple juice with ascorbic acid resulted in a product that is Grade A.

4. Fortification of apple juice with ascorbic acid did not alter the soluble solids nor the vacuum; however,

a) Variation in the soluble solids content observed upon fortification was partially due to the ratio of blending the two cultivars of apples and partially to the amount of free-run juice in each fortification lot. Soluble solids were slightly decreased with time and temperature.

b) Vacuum decreased with time and increasing storage temperature. Pressure developed at accelerated storage temperatures.

5. The kinetic analysis indicated that ascorbic acid degradation in apple juice can be best described by assuming a first-order reaction, that is,

a) From 86° to 104°F, the rate of ascorbic acid destruction tripled and from 68° to 86°F the rate slightly more than doubled while between 32° and 68°F the rate increased by approximately one and half times for each 18°F rise in temperature.

b) The half-life values expressed the expected shelf life of fortified apple juice with respect to ascorbic acid. At 86° and 104°F storage temperatures the shelf life of ascorbic acid is drastically reduced.

c) A break in Arrhenius plot at 75°F suggested a change in the mechanism governing the rate of ascorbic acid destruction at this temperature.

d) Results obtained from Arrhenius plot indicated that the magnitude of the activation energy is an indicator of the temperature influence on the rate of ascorbic acid degradation.

6. The initial concentration, C_o, required to produce 100 percent RDA of ascorbic acid per 6-fluid ounce serving of fortified apple juice under given storage conditions and durations have been predicted from k values.

7. Values of k under fluctuating storage temperatures can also be determined.

8. An apple juice processor can determine the necessary initial concentration of ascorbic acid required to meet his label claim after specified storage time and temperature from the equations developed in this study.

APPENDIX A

Analysis of Variance - pH

Source	Sum of squares	DF	Mean square	F value	Significance
Fortification level	0.813388	4	0.203347	1083.939	.01
Time	0.569472	4	0.142368	758.891	.01
Temperature	0.212192	4	0.053048	282.771	.01
Temperature x time interaction	0.136136	16	0.008508	45.354	.01
Fortification x time interaction	0.027640	16	0.0017275	9.208	.01
Fortification x temperature interaction	0.014620	16	0.000913	4.870	.01
Fortification x temperature x time interaction	0.041192	64	0.000644	3.430	.01
Error	0.023450	125	0.0001876		

Analysis of Variance - Titratable Acidity in Apple Juice

Source	Sum of squares	DF	Mean square	F value	Significance
Fortification level	0.29211404	4	0.07302851	11276.815	.01
Time	0.01268664	4	0.00317166	489.756	.01
Fortification x temperature interaction	0.00142396	16	0.00008899	13.742	.01
Temperature	0.00034360	4	0.0000859	13.264	.01
Fortification x time interaction	0.00063252	16	0.00003953	6.104	.01
Temperature x time interaction	0.00045616	16	0.00002853	4.402	.01
Fortification x temperature x time interaction	0.00188388	64	0.00002943	4.545	.01
Error	0.00080950	125	0.00000648		

Analysis of Variance - Soluble Solids Content in Apple Juice

Source	Sum of squares	DF	Mean square	F value	Significance
Fortification level	25.595072	4	6.398768	803.179	.01
Time	3.747232	4	0.936808	117.589	.01
Temperature	0.723700	4	0.180925	22.709	.01
Fortification x temperature interaction	1.343568	16	0.083973	10.540	.01
Temperature x time interaction	0.588428	16	0.036777	4.616	.01
Fortification x time interaction	0.500596	16	0.031287	3.927	.01
Fortification x temperature x time interaction	1.848844	64	0.028888	3.626	.01
Error	0.995850	125	0.007967		

Analysis of Variance - °Brix:Acid Ratio in Apple Juice

Source	Sum of squares	DF	Mean square	F value	Significance
Fortification level	3445.34096	4	861.33524	9918.646	.01
Time	244.87936	4	61.21984	704.972	.01
Fortification x time interaction	8.85424	16	0.55339	6.372	.01
Temperature	1.46296	4	0.36574	4.211	.01
Temperature x time interaction	6.13424	16	0.38339	4.414	.01
Fortification x temperature interaction	6.07864	16	0.379915	4.374	.01
Fortification x temperature x time interaction	23.09016	64	0.36078	4.154	.01
Error	10.85500	125	0.08684		

Analysis of Variance - Vacuum

Source	Sum of squares	DF	Mean square	F value	Significance
Temperature	2315.726	4	578.9315	218.547	.01
Time	1367.626	4	341.9065	129.070	.01
Temperature x time interaction	783.664	16	48.9790	18.489	.01
Fortification level	81.616	4	20.4040	7.702	.01
Fortification x time interaction	294.474	16	18.4046	6.947	.01
Fortification x temperature interaction	49.774	16	3.1108	1.174	NS*
Fortification x temperature x time interaction	309.936	64	4.8427	1.828	.01
Error	331.125	125	2.6490		

*NS = Not significant

APPENDIX B

Effect of Fortification Level, Storage Time and Temperature on pH of Apple Juice

Fortification level	Temperature °F	Storage time in months				
		0	3	6	9	12
		pH				
0	32	3.80	3.74	3.73	3.77	3.80
	50	3.80	3.69	3.74	3.77	3.83
	68	3.80	3.69	3.75	3.80	3.85
	86	3.80	3.69	3.74	3.81	3.93
	104	3.80	3.74	3.82	3.93	4.10
30	32	3.73	3.64	3.67	3.70	3.70
	50	3.73	3.63	3.67	3.69	3.78
	68	3.73	3.66	3.67	3.70	3.77
	86	3.73	3.63	3.65	3.69	3.80
	104	3.73	3.67	3.73	3.77	3.94
45	32	3.69	3.59	3.63	3.67	3.69
	50	3.69	3.60	3.65	3.66	3.69
	68	3.69	3.62	3.63	3.65	3.72
	86	3.69	3.63	3.63	3.65	3.75
	104	3.69	3.65	3.67	3.75	3.82
60	32	3.68	3.61	3.61	3.64	3.70
	50	3.68	3.60	3.61	3.64	3.70
	68	3.68	3.62	3.62	3.63	3.73
	86	3.68	3.61	3.63	3.64	3.75
	104	3.68	3.65	3.68	3.74	3.80
90	32	3.64	3.57	3.58	3.61	3.65
	50	3.64	3.55	3.59	3.61	3.67
	68	3.64	3.59	3.59	3.62	3.66
	86	3.64	3.57	3.59	3.62	3.70
	104	3.64	3.62	3.67	3.71	3.83

Effect of Fortification Level, Storage Time and Temperature on Titratable Acidity of Apple Juice

Fortification level	Temperature °F	Storage time in months: 0	3	6	9	12
		Percent titratable acidity as malic				
0	32	.297	.294	.300	.295	.310
	50	.297	.286	.302	.290	.307
	68	.297	.296	.306	.295	.317
	86	.297	.310	.310	.312	.317
	104	.297	.308	.314	.303	.313
30	32	.360	.357	.366	.355	.374
	50	.360	.360	.361	.355	.378
	68	.360	.357	.364	.355	.378
	86	.360	.358	.364	.355	.385
	104	.360	.357	.361	.355	.378
45	32	.366	.358	.371	.359	.378
	50	.366	.364	.366	.362	.385
	68	.366	.360	.366	.362	.381
	86	.366	.370	.376	.362	.385
	104	.366	.362	.368	.358	.378
60	32	.383	.378	.385	.376	.390
	50	.383	.384	.380	.377	.395
	68	.383	.375	.378	.374	.392
	86	.383	.378	.380	.376	.392
	104	.383	.375	.383	.372	.410
90	32	.403	.409	.414	.400	.422
	50	.403	.404	.407	.405	.419
	68	.403	.399	.403	.396	.412
	86	.403	.401	.407	.396	.419
	104	.403	.397	.397	.392	.430

Effect of Fortification Level, Storage Time and Temperature on Soluble Solids of Apple Juice

Fortification level	Temperature °F	Storage time in months 0	3	6	9	12
		Percent soluble solids as sucrose				
0	32	13.51	13.57	13.42	13.38	13.29
	50	13.51	13.40	13.36	13.34	13.09
	68	13.51	13.62	13.85	13.18	13.52
	86	13.51	14.00	14.01	14.08	13.16
	104	13.51	14.00	14.01	13.96	13.36
30	32	14.37	14.59	14.48	14.40	14.35
	50	14.37	14.69	14.48	14.40	14.17
	68	14.37	14.69	14.46	14.48	14.44
	86	14.37	14.65	14.53	14.38	14.23
	104	14.37	14.69	14.64	14.00	14.32
45	32	13.61	13.66	13.52	13.60	13.29
	50	13.61	13.44	13.52	13.44	13.26
	68	13.61	13.57	13.77	13.52	13.36
	86	13.61	13.97	13.93	13.73	13.56
	104	13.61	13.75	13.69	13.52	13.29
60	32	13.74	13.88	13.85	13.77	13.52
	50	13.74	13.75	13.85	13.77	13.49
	68	13.74	13.75	13.77	13.75	13.49
	86	13.74	13.87	13.90	13.73	13.69
	104	13.74	13.88	13.82	13.59	13.49
90	32	13.43	14.07	14.11	13.77	13.69
	50	13.93	13.94	13.85	13.77	13.69
	68	13.93	13.94	13.93	13.85	13.69
	86	13.93	13.87	13.90	13.93	13.69
	104	13.93	14.07	13.91	13.77	13.52

Effect of Fortification Level, Storage Time and Temperature on °Brix:Acid Ratio of Apple Juice

Fortification level	Temperature °F	Storage time in months 0	3	6	9	12
		°Brix:Acid Ratio				
0	32	45.4:1	46.0:1	44.6:1	45.3:1	42.9:1
	50	45.4:1	46.7:1	44.2:1	46.0:1	42.6:1
	68	45.4:1	45.9:1	45.2:1	44.6:1	42.6:1
	86	45.4:1	45.2:1	45.1:1	45.1:1	41.5:1
	104	45.4:1	45.4:1	44.6:1	46.0:1	42.6:1
30	32	39.8:1	40.8:1	39.6:1	40.6:1	38.3:1
	50	39.8:1	40.7:1	40.0:1	40.6:1	37.5:1
	68	39.8:1	41.1:1	39.7:1	40.8:1	38.2:1
	86	39.8:1	40.8:1	39.8:1	40.5:1	36.9:1
	104	39.8:1	41.1:1	40.5:1	39.4:1	37.9:1
45	32	37.2:1	38.1:1	36.3:1	37.8:1	35.1:1
	50	37.2:1	36.9:1	36.9:1	37.1:1	34.4:1
	68	37.2:1	37.7:1	37.6:1	37.3:1	35.0:1
	86	37.2:1	37.7:1	37.0:1	37.9:1	35.4:1
	104	37.2:1	38.0:1	37.2:1	37.7:1	35.1:1
60	32	35.9:1	36.6:1	36.0:1	36.6:1	34.6:1
	50	35.9:1	35.8:1	36.4:1	36.4:1	34.1:1
	68	35.9:1	36.6:1	36.3:1	36.7:1	34.4:1
	86	35.9:1	36.6:1	36.6:1	36.5:1	34.9:1
	104	35.9:1	36.9:1	36.0:1	36.5:1	32.9:1
90	32	34.6:1	34.4:1	34.0:1	34.3:1	32.4:1
	50	34.6:1	34.5:1	34.0:1	33.9:1	32.7:1
	68	34.6:1	34.9:1	34.5:1	34.5:1	33.2:1
	86	34.6:1	34.6:1	34.1:1	35.2:1	32.7:1
	104	34.6:1	35.4:1	35.0:1	35.1:1	31.4:1

Effect of Fortification Level, Storage Time and Temperature on Vacuum of Apple Juice

Fortification level	Temperature °F	Storage time in months: 0	3	6	9	12
		Vacuum in inches of mercury				
0	32	4.50	4.00	9.00	6.00	5.00
	50	4.50	8.00	6.25	4.00	1.75
	68	4.50	9.00	4.50	3.50	0.00
	86	4.50	5.00	5.00	+0.50	+4.50
	104	4.50	+2.75	+2.50	+8.50	+9.50
30	32	7.75	7.00	6.50	6.50	2.00
	50	7.75	6.00	5.50	6.00	5.50
	68	7.75	7.00	3.25	6.00	1.75
	86	7.75	4.00	3.50	3.00	0.00
	104	7.75	+2.00	+5.00	+8.00	+8.00
45	32	9.00	6.00	5.00	2.50	5.50
	50	9.00	9.00	1.50	4.50	5.00
	68	9.00	2.50	1.50	2.50	2.50
	86	9.00	4.00	2.00	2.50	0.00
	104	9.00	0.00	+1.00	+8.50	+9.00
60	32	4.50	5.50	6.00	6.00	4.50
	50	4.50	9.00	6.00	4.50	2.50
	68	4.50	5.50	3.50	4.50	1.50
	86	4.50	6.50	6.50	0.00	0.00
	104	4.50	+2.00	+3.00	+10.00	+10.00
90	32	6.50	5.00	4.00	4.50	0.50
	50	6.50	4.00	4.00	4.00	2.25
	68	6.50	3.50	2.50	1.00	0.50
	86	6.50	1.50	1.00	+0.50	0.00
	104	6.50	+3.00	+6.00	+4.00	+8.50

Figures with + indicating pressure.

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