THE RETENTION OF VITAMIN C AND DEVELOPMENT OF COLOR IN SYNTHETIC JELLIES AND MARMALADE

by

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CHAPTER I

INTRODUCTION

A good retention of vitamin C in jams and jellies is of importance insofar as it will contribute to the daily vitamin C requirement of the diet.

The disease known as scurvy, resulting from a vitamin C deficiency in the body is under normal conditions comparatively rare. Hawk, et. al. (37), however, reported that widespread studies of ascorbic acid nutrition of large groups of the population revealed subclinical vitamin C deficiency.

The importance of a good retention of vitamin C in jellies and marmalades to serve as a supplement to the daily vitamin C requirements can therefore not be overlooked. This is especially important for the lower income groups where jellies and marmalades are commonly relied on to add to the palatability of the diet.

The objectives in these studies were to investigate the effect of different ingredients usually employed in jam and jelly manufacture and other factors likely to have a bearing upon the vitamin C retention.

The importance of copper as a catalyst in the

oxidation of vitamin C has always been realized, but in previous reported experiments on jams and jellies no attempt has been made to keep this factor constant. In these experiments copper was previously determined in all the ingredients used in the boilings. By adding copper as copper sulfate, the final level was brought up to 2 p.p.m. so as to eliminate copper as a variable factor.

Reports on the retention of vitamin C as influenced by the different sugars and sugar sirups in replacing sucrose -- which today is a common practice in the food processing field -- have been very contradictory. With this in mind, low conversion corn sirup, high conversion corn sirup, invert sirup (prepared in the laboratory) and crystalline dextrose were used to partially replace sucrose in the different jelly and orange marmalade boilings.

The effect of different containers (glass and plain tin), amount of vacuum in the head space, storage conditions (temperature and sunlight) and anti-oxidants (sodium chloride and d-Iso ascorbic acid) were also investigated. The reason for including both tin and glass containers in these investigations is that jellies and marmalades are marketed in countries like England and South Africa mainly in tin and relatively small quantities are put up in glass.

Ascorbic acid is readily oxidized to dehydro-ascorbic acid, a substance which is similar to ascorbic acid in biological activity. The formation of dehydro-ascorbic acid is of importance however, in that it is an indication of oxidation processes being underway, which may finally lead to complete destruction of the vitamin. The formation of dehydro-ascorbic acid was included in these studies. The development of reductones and reductone-like substances has been reported by various workers. These substances reduce the dye indophenol rapidly and although they are structurally somewhat similar to vitamin C, possess no antiscorbutic activity. It is of importance therefore that these substances should not be mistaken for vitamin C. The probable development of these substances in the jellies and orange marmalade was also investigated.

The formation of a brown color in many food products is usually accompanied by a series of other changes which lead to a product of low nutritional value and poor flavor, which again in general is concomitant with a low quality. Studies on the browning reaction were included in these experiments with the idea of finding a probable correlation between the latter reaction and losses of ascorbic acid. The formation of a complex compound involving the oxidation

products of ascorbic acid is one of the suggested theories of the browning reaction.

All these studies were carried out with synthetic jellies boiled from citrus pectin, citric acid and sugars; and orange marmalade. The complex system which exists between the natural substances present in orange marmalade and the other ingredients used for the boilings are eliminated in the synthetic jellies, the latter representing a relatively simple system as compared to the orange marmalade.

The limitations in these investigations can be found in the relatively short period of storage (6 months) and also the elevated temperatures used to speed up the reactions in some cases. To this can be added the need for improved and more specific methods for reductone and dehydro-ascorbic acid determinations. Several workers have pointed out that satisfactory methods for the determination of these substances as they occur in foods are not available.

In reviewing the literature it was found that very little work has been done in investigating the nutritional values of jams and jellies. In countries like South Africa and England where these products are consumed in large quantities their value in supplementing the diet

with the necessary nutritive ingredients cannot be over-emphasized.

CHAPTER II

LITERATURE REVIEW

A. The Oxidation of Ascorbic Acid as Influenced by

1. Sugars and sugar sirups. Several investigations in the food field have shown that the non-enzymatic oxidation of l-ascorbic acid is inhibited to varying degrees by sugars. Data indicating the relative effectiveness of these sugars as protective agents of oxidation by the different investigators are very contradictory and the results so far presented are very confusing.

Richardson and Mayfield (88) in experiments with sugar solutions prepared with special precautions as to the oxygen content of the solutions etc., found that sucrose and white corn sirup exerted a protective influence on the oxidation of ascorbic acid, but that dextrose had a more destructive influence during boiling. They, however, also point out the importance of the presence of heavy metals such as copper in catalysing the oxidation of vitamin C. They state in their final discussions that a possible explanation of these findings might be due to the copper content of the crystalline dextrose, which was higher than the copper content of the sucrose and corn sirup used. Japanese

investigators Sumiki, et. al. (104) to the contrary state that fructose, sucrose and citric acid promote oxidation while glucose inhibits it.

Munilla and Vogelsinger (75) report that sugar solutions stored at 20°C reduced the rate of ascorbic acid oxidation; lactose having the least, and dextrose and sucrose the most effect.

Riester, et. al. (89) working with canned citrus juice state that levulose either added directly, or due to the inversion of sucrose, helps to maintain the desirable flavor of the fresh juice but has no effect on the ascorbic acid content.

Chamrai (13) also reports a protective effect of sucrose and states that at a low pH the stabilization is due to its effect on reducing the rate of diffusion of oxygen into the solution; and at higher pH values to the formation of copper-saccharates.

Isaac (42) found a greater loss of ascorbic acid in solutions of caramelized fructose than in solutions of pure fructose. He also points out that at 98° F and higher temperatures pure fructose caramelizes spontaneously.

Joslyn (50) found in peach freezing experiments that in Kim nectarines ascorbic acid retention was much better in sirups containing 70 per cent or less invert

sugar than in 90 per cent invert sirups.

Studying the oxidation of ascorbic acid in sugar solutions allowed to stand exposed to air at 22° C, Joslyn (50) found that maltose, levulose and lactose were the most effective in retarding oxidation of ascorbic acid, and dextrose to be the least effective. Under conditions of vigorous oxidation he found the order of decreasing protection to be maltose, dextrose, sucrose and lactose. With sugar sirups tested under these conditions a high conversion corn sirup gave the best protection followed by sucrose, invert sirup and corn sirup (low conversion).

In a paper presented recently to a meeting of the American Chemical Society at San Francisco, Joslyn and Miller (51) state that the rate at which ascorbic acid is oxidized when the oxygen supply is limited was reduced by the addition of sugars and found the protective effect of lactose less than that of levulose. Under conditions of an ample oxygen supply the extent of protection by sugars increased with the increase of pH. The greatest protection was exerted by levulose followed by maltose, sucrose and dextrose in an order of decreasing protection. Curl (18) working with synthetic mixtures of sugar and other substances analogous to orange juice -- using 120° F storage temperatures -- found that the addition

of levulose resulted in considerably greater losses of ascorbic acid than did the addition of dextrose. Sucrose after one month storage had the same effect as added dextrose but after two months the loss of ascorbic acid was greater in the pack containing the sucrose. The greater loss after two months was attributed to the inversion of sucrose to levulose and dextrose.

The general trend of the work done by these investigators makes it appear plausible that levulose is the most reactive sugar for ascorbic acid destruction, especially under prolonged storage conditions and at elevated temperatures.

2. The Copper Ion. Copper acts as a catalyst in the oxidation of ascorbic acid. For this reason the copper content of food products in which the retention of vitamin C is concerned should be kept at the lowest possible level.

Szent-Guorgyi (105) first recorded the catalytic action of copper in the oxidation of ascorbic acid. He found Fe^{+++} and Mn^{++} had no effect upon the rate of oxidation.

Kellie and Zilva (53) on the other hand regarded both iron and copper as catalysts.

Copper in a concentration of 1 part per 20 million

parts at 25° C was reported by Barron, et. al. (5) sufficient to catalyze the oxidation of vitamin C at pH 3.17 in a citrate buffer. They also state that iron alone does not catalyze the oxidation of ascorbic acid but in the presence of iron, copper exerts an increased catalytic effect.

Mack and Kertesz (63) found that the catalytic activity of copper is considerably increased by the addition of small amounts of iron. They suggested that iron exerts a promoter action on copper since iron itself does not catalyze the oxidation of ascorbic acid.

Eddy (20) reports that 20 p.p.m. cupric ions catalyze the oxidation of ascorbic acid whereas stannous and stannic ions have a slight inhibiting effect.

Oxidation of ascorbic acid was found to be hastened by the presence of phosphates Cavalini (12). The speed of this reaction at a fixed pH was proportional to the concentration of phosphates and was favored by the presence of copper ions.

Peterson and Walton (82) in discussing the possible mechanism of ascorbic acid oxidation inhibitors, claim that the inhibitor ties up with the catalytic copper ion and forms a coordinate complex. The oxidation of ascorbic acid was found to take place in both alkaline and acid solutions if copper is present.

Sonovski (101) and Van der Laan, et. al. (110) warn against the selection of inappropriate metals for cooking utensils. Copper and copper containing alloys are unsuitable for utensils used in the preparation of foods containing vitamin C. Even if plated with tin and other harmless metals they cannot be recommended because of surface irregularities.

The following statement was made by Joslyn and Miller (51) regarding the oxidation of ascorbic acid by copper and iron: "The oxidation of ascorbic acid by oxygen in the presence of copper and iron was found to be first order with respect to ascorbic acid concentration. Its rate was directly proportional to the square root of the copper concentration and inversely as the square root of the H^{+} concentration in acid solutions. Ferric ions catalyze the oxidation of ascorbic acid in the more acid solutions but very slightly in comparison with copper".

From the work done by the various investigators there is no doubt about the importance of copper in catalyzing the oxidation of vitamin C. Many contradictory reports in the literature as to factors influencing the oxidation of ascorbic acid may be due to the fact that the importance of copper and other heavy metals in catalyzing these reactions have been overlooked.

3. Storage Conditions

(a) Storage temperature. Vitamin C losses are very closely associated with storage temperatures. Also, the most important single factor influencing the rate of browning has been found to be temperature. For this reason it is believed that the breakdown products of ascorbic acid may be involved in the formation of the brown color complexes.

Ross (93) experimenting with orange juice found that between 50° F and 80.6° F the rate of ascorbic acid losses in orange juice doubles for each 10 degrees in temperature rise.

Wokes and Organ (120) report losses of vitamin C in black currant sirup at 96.5° F nearly three times that at 78.5° F, and the rate of loss at 78.5° F was nearly three times that at 62.5° F.

Chaves (14), and later also Curl, et. al. (17) working with Brazilian orange juice concentrates (62 per cent solids) found the following vitamin C losses:

	Per cent Vitamin C Losses		
Storage Time	50° F	80.6° F	100.4° F
30 days	18	28	82
58 days	19	36	94

Curl, et. al. (17) found the rate of CO_2 production, ascorbic acid losses, and darkening (initial rate) of these orange concentrates, to increase approximately 4 times for an 18° F rise in temperature.

Guerrant, et. al. (31) recommended 70° F as a storage temperature for commercially canned foods. They found prolonged storage (12 months and more) at 110° F to reduce the ascorbic acid content of canned foods to a great extent, while almost complete retention was obtained after 12 months' storage at temperatures ranging from 45° to 85° F. Similar results were obtained by Brenner, et. al. (10).

(b) Effect of Sunlight. Usually where light, as an influencing factor on the vitamin retention in foods has been studied the samples were exposed to severe sunlight or conditions which are not common in commercial storage.

In this respect Esselen and Barnby (23) state that experimental results usually do not interpret changes which will occur under commercial storage conditions. According to them, the light that reaches the shelves in grocery stores is only 0.5 per cent of the intensity of the light outside the store.

Light intensity, length of exposure and degree of transmission are factors which will influence the product

exposed to it.

Visible light according to Hebo (38) will not destroy pure ascorbic acid. Ascorbic acid however is sensitive to ultra violet light but ordinary flint glass transmits little or no ultra violet light.

Wokes and Organ (120) found that black currant sirup exposed to sunlight will lose three to four times as much vitamin C as those wrapped in light-proof paper. They also state that amber colored bottles may not afford complete protection to the destructive properties of light.

Von Loesecke, Mottern and Pulley (114) believe darkening in orange juice to be due to oxidation. They found light to have little effect on darkening since orange juice stored away from the light darkened as much as that left in the light.

Newman, et. al. (78) state that in general light increases the oxidation of ascorbic acid but also stresses at the same time the importance of the oxygen content in the headspace of the container.

Fellers and Buck (27) found that when glass packed foods are stored in light, l-ascorbic acid losses are effected but it did not affect total losses much.

Fawns (26) experimenting with black currant sirups reports considerable losses in vitamin C when exposed to light. The destruction of the vitamin by light is

accompanied by simultaneous fading of color and a possible relation between the two is suggested.

On exposure of orange juice to sunlamps, Moore, Esselen and Fellers (73) report that no additional browning was formed. In connection with the stability of the brown color of honey to light Milum (70) found that honey stored in light does not darken as does honey stored in dark at the same temperature. Discoloration is considered to be chiefly due to the unstability of the levulose. The apparent bleaching effect of the light on the colored compounds derived from levulose is also pointed out by the latter investigator.

It is well known that all chemical reactions are greatly accelerated by heat and sometimes by light and the destruction of vitamin C accompanied by the formation of brown colored pigments may be no exception to this rule.

4. Amount of Oxygen in the Headspace. The amount of vacuum which will determine the amount of oxygen left in the headspace of a container is a factor of great importance as far as the oxidation of vitamin C in the container is concerned.

According to Strohecker, et. al. (103), the stability of ascorbic acid is more affected by the

oxygen content of the air and pH than by the temperature.

Beattie, et. al. (6) found a great loss of ascorbic acid and a deterioration of color in samples of raspberry, strawberry and currant juices when the bottles were partially filled with air.

Moore, Esselen and Fellers (73) point out that the presence of oxygen is directly associated with the browning of orange juice and that 50 ml. of headspace give much more browning of the juice than 10 ml.

Bottled grapefruit juice was reported by Pederson, et. al. (80) to deteriorate less rapidly without a headspace than with one.

Bennett (7) reported that the exclusion of air in containers does not entirely prevent the loss of ascorbic acid in processed citrus juice held at room temperature.

Clark (16) in discussing the effect of an excess of oxygen in sealed containers of fruit juices points out its effect on color, flavor and the reduction of ascorbic acid with possible darkening of the juice.

Tressler, et. al. (109) found darkening of orange juice to be more rapid in the presence of oxygen. It also proceeds rapidly even when the juice was deaerated to remove dissolved oxygen and stored in vacuum sealed containers.

5. Type of Container. It is of great importance to know the influence of the type of container used on the nutritive value and quality of the canned product in storage.

Several reports (some contradictory in nature) as to the influence of glass and plain tin containers on certain food products have been published.

Newman and Fellers (78) for example studied the vitamin C content of food products in glass and tin containers purchased from retail markets. They found that twelve products contained more vitamin C when packed in glass than the same foods in tin. In nine of the products packed in glass the vitamin C contents exceeded those packed in tin.

Riester, et. al. (89) found that distinctly different flavors developed in orange juice packed in plain and enamel cans but the type of can had no effect on the vitamin C content. To the contrary, Boyd and Peterson (9) found that vitamin C in orange juice is retained better in plain tin than in enameled cans. Lueck and Pilcher (61) made the same conclusions from their experiments with tomato juice.

Daniel and Rutherford (19) and also Hauck (36) made the observation that tomato juice canned in tin retained much more ascorbic acid than similar juice

packed in glass.

Reynolds (87) reported that metalic tin added to glass packed orange juice would yield a product higher in ascorbic acid than the juice packed without added tin.

On the other hand, Tressler and Curran (108) offer data to support their findings that ascorbic acid is not lost more rapidly from glass containers than from tin cans provided both types of containers are completely filled.

6. Antioxidants

(a) Sodiumchloride. The catalytic action of copper in the oxidation of ascorbic acid was reported by Mapson (65) to be greatly reduced by the addition of halides. The protective effect only sets in if the halides are present in sufficient amounts.

Armentano (2) found that the rate at which copper catalyses the oxidation of vitamin C is directly proportional to the copper concentration. He further found that calcium chloride, potassium chloride and sodium-chloride accelerates the oxidation in dilute solutions (1-2 molar) but in stronger concentrations (5-10 molar) they have an inhibiting effect even to the extent of completely suppressing the spontaneous oxidation of the ascorbic acid. This effect is attributed to the chloride

ion. The greater the concentration of copper present the greater the concentration of halide necessary to obtain the same protection.

According to Mapson (65) the maximum effect of halides were obtained with a pH range of 2.3 to 4.0. This effect diminishes rapidly between a pH of 4 to 6 and also below 2.0. Of the halides tested iodides were found to be the most effective and chlorides the least.

Pendleton (81) and Høygaard and Rasmussen (39) experimenting with sodiumchloride in cooked vegetables found a 16 to 19 per cent saving of ascorbic acid when the vegetables were cooked in salt water. Summarizing the literature it is found that the factors that will influence the degree of protection of ascorbic acid as secured by the adding of halide salts to foodstuffs may be the following:

1. The concentration of halide added.
2. The temperature.
3. The pH of the solution.
4. The copper content.
5. The nature and concentration of substances such as hydroxyacids and sugars.

Mapson (65) adds to this that in complex systems such as in the case of foodstuffs other factors not taken into consideration in the above summary may have an

influence on the action of the salt.

For practical purposes it should be remembered that the addition of such salts will effect the flavor and very high concentrations to protect vitamin C oxidation will then be out of the question.

Mapson (65) states that 1 to 2 per cent NaCl can be added to vegetables and 0.5 per cent may be added to fruits without affecting palatability.

(b) d-Iso Ascorbic Acid. Yourga, Esselen and Fellers (122) found that in a mixture of l-ascorbic acid and d-iso ascorbic acid the latter is preferably oxidized and thus protects the l-ascorbic acid from oxidation. It is on this principle that the properties of d-iso ascorbic acid as an antioxidant is based.

Esselen, Powers and Woodward (25) in their reports on citrus juice claim the same properties for d-iso ascorbic acid.

Moore (71) after using d-iso ascorbic acid in orange juice concluded that it rather increases than prevents or retards darkening in orange juice.

Beattie, et. al. (6) claim a protection of the color in tomato juice when 30 and 95 mg d-iso ascorbic acid were added per pint. The natural good flavor was also preserved.

B. The Role of Ascorbic Acid and Sugars in the Browning Reaction

The development of "browning" or dark colored pigments in food products is usually accompanied by undesirable changes in flavor, odor and nutritive values which mean a deterioration in quality.

Color deteriorations in food products involve a series of complex reactions in a complex system in which very many variables are involved. This makes the study of the browning mechanism so difficult. The brown pigments, huminlike in nature are also very complex in composition and this further complicates the study of these reactions.

Stadtman (102) lists the theories of the browning reaction as: 1. The Maillard or melanoidin condensation theory, which involves a condensation of reducing sugars and amino acids resulting in the formation of dark colored substances; 2. The ascorbic acid theory in which ascorbic acid and related compounds are said to be precursors. Upon oxidation of these compounds reactive products are supposedly formed, which upon polymerization and reaction with nitrogenous compounds form brown pigments. (Koppanyi (55)); 3. The active aldehyde theory. Sugars on decomposition form sugar acids and furfuraldehyde or

related compounds, which are characterized by having an active carbonyl group. These compounds may condense with nitrogenous compounds which may give rise to brown resinous materials.

Nelson, Mottern and Eddy (77) point out that the conditions favorable for the Maillard reaction are high sugar concentrations in a slightly alkaline media. The synthetic jellies in these investigations had a pH of 3.1 and for this reason and also for the fact that practically no nitrogen is present in these jellies, the discussion of the browning reaction as related to this reaction will be discontinued.

In orange marmalade we have a much more complex system and catalytic factors might be able to bring about the reaction with small amounts of soluble nitrogen present in the oranges.

The browning of juices especially citrus juice has been investigated quite extensively. Joslyn and Marsh (44), (46), (48) and Joslyn, Marsh and Morgan (47) were of the opinion that the browning of orange juice involves oxidation. It was also stated that the loss of ascorbic acid was regulated by the amount of available oxygen. The process of browning also paralleled the loss of ascorbic acid. They found, and so did Hamburger

and Joslyn (35) that darkening did not occur immediately in processed orange juice but only after the initial ascorbic acid has reached a very low level. They suggested that certain substances which prevent darkening may have to be oxidized before darkening can begin.

Loeffler (60) however to the contrary found that darkening in orange juice can be determined when the reducing value as measured by the indophenol dye has diminished only 10 to 15 per cent. This will suggest that the darkening starts coincidentally with the oxidation of vitamin C and not after part has first been lost. Further observations of Loeffler (60) show that vitamin C was lost in frozen samples without being accompanied by a change in color. This will indicate that the color change is not due to oxidation and is apparently not correlated with vitamin C losses.

Hamburger and Joslyn (35) advance the following theories for the auto oxidation of filtered orange juice: (a) The ascorbic acid is oxidized to the dehydro form and this in turn is decomposed to further oxidation products. When all the ascorbic acid in the reduced form has been depleted the darkening occurs as a result of polymerization of the oxidation products of ascorbic acid. However not all of the vitamin C participates in

the darkening.

"(b) After the reduced ascorbic acid has been depleted and only then, can an unknown substance X be oxidized and this in turn is quickly polymerized and darkening occurs".

Szent-Gyorgyi (106) reports that ascorbic acid can reduce all quinones and phenols and in this way prevent pigment formation. Polyphenols on the other hand form intensely dark compounds in the presence of ferric ions Zerban (123).

Moore, et. al. (72 and (73), experimenting with orange juice found that ascorbic acid is an important factor in browning and a marked increase in the browning of the juice is reported when stored in the presence of oxygen.

Certain chemical reactions were found to accompany the browning reaction of which the production of CO_2 is one. This CO_2 development has been reported by Hall (34), Curl, et. al. (17), Greer (29) and several other workers. Loeffler (60) demonstrates in his experiments that the amount of CO_2 produced is increased by raising the temperature of storage. The total quantity of CO_2 produced was reported to be ten times as great as the oxygen which had disappeared during the five months storage at 35° C.

As a result of certain chemical changes which sugars undergo in storage of food products, several investigators have tried to correlate browning with changes in reducing sugars.

Wilson (117) and Hall (34) state that reducing sugars are necessary for the darkening of certain concentrates. A slight decrease of reducing sugars during storage has been reported. Curl, et. al. (17) also report losses in reducing sugars in orange concentrates during storage. These reducing sugar losses were shown to be roughly paralleled by changes in color.

Curl (18) found the combination of sugars and amino acids the principal factor in the darkening of sugar solutions. When ascorbic acid was added to this mixture the darkening was increased to a great extent.

The decomposition of carbohydrates as carbohydrate derivatives which may yield colored bodies, or intermediates which on polymerization become colored, was pointed out by Joslyn (49). Uronic acid derivatives of simple sugars decompose in acid solutions, first into pentoses and CO_2 and subsequently into furfural. When the latter polymerizes various brown resins are formed. Joslyn continues in saying that ascorbic acid also yields furfural in the presence of acid and that CO_2

development from sterile juice concentrates has been ascribed to the decomposition of ascorbic acid. Yellow and reddish brown pigments are formed when furfural derivatives polymerize with other substituents. Stadtman (102) states that very small amounts of furfuraldehyde added to apricot sirups cause a great increase in the rate of browning.

Invert sugar solutions such as honey or other sirups containing levulose are particularly susceptible to discoloration on heating (Joslyn (49). Isaac (42) points out that levulose will caramelize spontaneously at 98° F or higher temperatures.

According to Kruisheer (56 and 57) hydroxymethylfurfural may be formed as a dehydration product of levulose particularly in acid solutions. This substance again will readily polymerize with other substances to form dark colored compounds.

In acid solutions both glucose and fructose are converted into anhydrides so-called "reversion products" (Zerban (123)). These anhydrides undergo further condensation and polymerization and will yield fructose-caramel. Glucose is found to be more stable under acid conditions than fructose. Eikelberg (22) found that the decomposition of levulose on heating is greatly affected by pH.

Stadtman (102) stresses the fact that relatively small chemical changes are required to produce brown pigments, which in small amounts can be the cause of excessive discoloration.

That compounds, other than reducing sugars, are also involved in the browning reactions is evident from the fact that Stadtman (102) in his experiments found that removal of sugars from apricot sirups by fermentation decreases the rate of browning to only half that of the original rate. Joslyn and Marsh (48) report the same thing on orange juice.

Uronic acids and ascorbic acid which can form furfuraldehydes were not removed in the above experiments and are still there to form polymerized complexes.

Haas and Stadtman (33) using ion exchange resins to identify types of compounds involved in the browning came to the conclusion that the overall browning is the result of at least four different types of reactions:

1. Reactions between nitrogenous constituents and sugar.

2. Reactions between nitrogenous constituents and organic acids.

3. Reactions between sugars and organic acids.

4. Reactions involving only organic acids.

In their separated neutral fraction it was found that glucose was one of the most important constituents with respect to browning.

From the above literature review it is evident that browning is produced by a series of complex reactions which make the study and characterization of an individual reaction very difficult. Many of the fundamental factors involved in the browning reaction are not known and work reported on these reactions are still contradictory in nature.

C. Formation of Reductones and Reductic Acids

The presence of substances known as reductones and reductic acids in various processed foods has been reported by several investigators.

These substances are of significance in that they also reduce the indophenol dye which is usually employed for ascorbic acid determinations. These substances differ from ascorbic acid however in that they have no antiscorbutic value and by reporting values of reduced indophenol as ascorbic acid may, when these reductions and reductic acids are present, be erroneous.

Hawk, Oser and Summerson (37) point out that the terms reductone and reductic acid are used rather loosely for reducing compounds which are formed during heat

processing and storage of certain foods. They report reductones to be hydroxypyruvic aldehydes and that reductic acids are formed when certain pentoses are treated with acids. Both these substances are according to them similar to vitamin C in structure, stability and chemical reaction. These same substances are referred to by Wokes, et. al. (119) as "apparent vitamin C".

Roe and Oesterling (91) found in their experiments that these reductones do not only rapidly decolorize the indophenol dye but also interfere with the dinitrophenyl hydrozine method of vitamin C determination.

Pollard, Kieser and Steedman (84) ascribe the apparent synthesis of ascorbic acid in their sirups and juices, to the development of these reductones; and concluded that heat treatment, previous history of the product, oxidation and adverse storage conditions will influence the development of these substances.

According to Mapson (66) and to Miller (69) the presence of reductone-like substances is not to be expected in many fresh fruit and vegetables and the usual indophenol titration method can be used for vitamin C determinations without any modifications.

Several workers like Mapson (66), Lugg (62), Snow and Silva (100), Robinson and Stotz (90) have

devised formaldehyde modification methods for the determination of these interfering substances. Of all these methods the one by Robinson and Stotz (90) is relatively simple and more adapted for control work on foods. The latter workers however point out that due to the unknown nature of these reducing substances their formaldehyde modification method cannot be claimed to offer an exact differentiation between these reductone substances and vitamin C.

Miller (69) making reductone determinations on orange marmalade found a reductone interference amounting to 8 per cent of the dye reducing substances after five months storage at 40° F. It was further stated that the reductone development appears to be inversely proportional to the amount of vitamin C initially present. The high initial vitamin C content of rose hip jam (337 mg. per 100 grams) for example, seems to stabilize the product. Orange marmalade with 27.3 mg. vitamin C initially showed slight reductone formation while cranberry sauce with only 1.8 mg. per 100 grams shows a development of large percentages of interfering substances.

Lincoln and McCay (59) report the development of non-specific substances of the reductone type in orange marmalade to be insignificant when storage was carried out at cool temperatures. Higher temperatures seem to

decrease the vitamin C content and correspondingly increase reductones.

Miller (69) reporting similar findings, adds that an increase in reductones in commercial orange concentrates corresponds to a progressive change in color from light to dark.

Previous reported vitamin C content of processed foods may, due to the formation of these reductones be only "apparent vitamin C", whereas actually very little "true vitamin C" may be present. The importance of introducing methods which will correct for these interfering substances is thus very evident.

D. Formation of Dehydro-Ascorbic Acid

On oxidation l-ascorbic acid is converted to dehydro-ascorbic acid, a process which, according to Borsook, et. al. (8) is reversible. They further state that dehydro-ascorbic acid undergoes a spontaneous irreversible change and gives rise to a compound which is a stranger acid than dehydro-ascorbic acid itself; namely, 2:3 diketo l-gulonic acid. This change is brought about by the irreversible hydrolysis of the lactone ring of dehydro-ascorbic acid. On subsequent oxidation the reaction products formed are l-threonic acid and oxalic acid.

According to Rosenberg (92) and Borsook (8) the biological activity of dehydro-ascorbic acid is the same as that of l-ascorbic acid. The significance of the presence of dehydro-ascorbic acid in food products is the indication that oxidation processes are underway. The further transformation of dehydro-ascorbic acid to other non-biologically active substances is very likely to ensue (Joslyn (50). Hawk, et. al. (37) state that once the lactone ring of dehydro-ascorbic acid has opened the molecule readily undergoes further oxidation and may degrade rapidly to oxalic acid.

Reporting on the oxidation of filtered orange juice, Hamburger and Joslyn (35) concluded that dehydro-ascorbic acid at first decreases at a rate which is proportional to that at which free vitamin C was reduced but then later reaches a minimum value which stays constant.

Lincoln and McCay (59) in experiments with the retention of ascorbic acid in marmalades report the dehydro-ascorbic acid content to be rather constant and they did not find it correlated with the darkening of the marmalade.

CHAPTER III

EXPERIMENTAL PROCEDURES

A. Preparation of Synthetic Jellies

Ingredients: 1950 gram sugar

1125 ml. of 0.1 per cent citric acid soln.

12.5 gram pectin (0.4 per cent) 197 grade.

1.5 gram crystalline l-ascorbic acid.

(50 mg. per 100 grams final jelly)

Procedure: 600 grams of the sugar (sucrose) were weighed out separately and with this the powdered pectin was thoroughly mixed. 600 ml. of the citric acid was now added, stirred and brought to a boil. After the mixture reached the boiling point it was allowed to boil for exactly one minute so as to dissolve the pectin. The remaining 1350 grams of sugar was now added and the temperature again raised to boiling point. The batch was now removed from the flame and the ascorbic acid and copper sulfate, where necessary, were washed in with the remaining citric acid solution. Again the mixture was brought to a boil and boiled down to a final weight of 3000 gram. The final total soluble solids of the

batch were 66 per cent as determined by refractometer.

Where sirups were used adjustments were made in the final weights of the batch to allow for the water content of these sirups.

The top foam of the batch was skimmed off and the jellies filled immediately into the glass or tin containers. Six-ounce jelly glasses and 8-ounce plain tin containers filled with 170 gram and 250 gr. jelly, respectively, were used. The headspace left between the jelly and the lid of containers was 22 ml. for glass and 22.5 for tin.

The lids of both containers were boiled in water for a few minutes and placed loosely on the containers immediately after they were filled and weighed. The containers were allowed to cool for at least 3 hours, after which they were vacuum sealed. A mercury marometer was used to measure the vacuum in the chambers of the sealing machines. Ten inch and 25 inch vacuums were used.

The different boilings were stored at 32° F, room temperature (avg. 72° F), in sunlight of a south window (avg. 51° F) and at 100° F. The first analyses were made the day after boiling and thereafter again at 1, 3 and 6 month intervals.

B. Preparation of Orange Marmalade

The oranges were hand-peeled and the peels and centers minced separately. Fifteen hundred grams minced peel, 4000 ml. distilled water, and 50 grams citric acid were weighed out and boiled for about 20 minutes in a steam jacketed kettle until the peel was soft and tender. Minced centers (7550 g.) were separately boiled for about 15 minutes and to this the boiled peel was added. After thorough mixing the mixed batch was boiled together to a final weight of 6330 grams.

A preliminary marmalade boiling from the above prepared pulp, to find the vitamin C retention was made the same day. Analysis showed a 6.66 mgm. per 100 gram marmalade retention. Lincoln and McCay (59) found a retention of 2 to 14 mgm./100 gram marmalade in commercial marmalades.

Copper analysis of the pulp showed it to contain 0.41 ppm. copper.

The orange pulp was kept at 32° F overnight and the next day the marmalade boilings were made using the following ratios:

185 grams pulp
100 ml. glass distilled water
400 grams sugar

This mixture was boiled to a final weight of 630 grams

which resulted in a marmalade containing 66 per cent total soluble solids.

Additional crystalline l-ascorbic acid was added to bring the level of the final boiling up to about 50 mgm./ 100 gram marmalade.

The citric acid added to the minced peel was enough to adjust the pH of the marmalade batch to the desired 3.1 so that no additional citric acid was required.

No additional pectin was used in these boilings.

The containers, vacuum sealing and storage temperatures for the marmalade were the same as for the synthetic jellies.

C. Materials Used

1. Sugars and Sugar Sirups

(a) Sucrose - commercial granulated cane sugar

Copper content = 1.25 ppm.

(b) Commercial crystalline dextrose

Copper content = 0.63 ppm.

(c) High conversion corn sirup, abbreviated hereafter as H.C. corn sirup

(acid inverted)

Copper content = 4.5 ppm.

Total soluble solids = 82.5%

Manufacturers analysis:

Baume' = 43° Baume'

Dextrose equivalent = 58-62 D.E.

pH = 4.7-5.0

Ash = 0.03%

Sugar analysis, dry basis:

Dextrose = 40.5%

Maltose = 28.5%

Dextrins = 23.0%

Higher Sugars = 8.0%

Fe = 0.0001%

(d) Low conversion corn sirup, abbreviated as L.C. corn sirup

Copper content = 1.84 ppm.

Total soluble solids = 83.5%

Manufacturers analysis:

Baume' = 43°

Dextrose equivalent = 43 avg.

pH = 4.7-5.0

Ash = 0.3

Fe = 0.0004%

Sugar analysis, dry basis:

Dextrose = 22%

Maltose = 21%

Dextrins = 37%

Higher Sugars = 20%

(e) Invert sirup made in the laboratory as follows:

453 gram of above mentioned sucrose

0.563 gram chemically pure citric acid

120 ml. distilled water

The above mixture was heated on a water bath at 212°F for one hour. The analysis of this sirup was as follows:

Reducing sugars = 66.8%

Total soluble solids (Refr.) = 81.8%

Copper content = 0.14 p.p.m.

2. Sugar and Sugar-Sirup Ratios

For both the synthetic jellies and orange marmalade the following combination of sugar and sugar-sirups were used for the boilings:

(a) 100% sucrose

(b) 50% (wt) H.C. corn sirup (60.4 D.E.) + 50% sucrose

(c) 50% (wt) L.C. corn sirup (43.9 D.E.) + 50% sucrose

(d) 50% (wt) Invert sirup + 50% sucrose

(e) 25% dextrose + 75% sucrose

The reason for using 25% dextrose in the last case was to simulate commercial procedures where a 25 to 30 per cent inversion in jams is considered optimum to prevent crystallization of the sucrose.

The consequence of using these corn sirups with

their high dextrin contents is that the boilings made from them contain less sugar than the others.

3. Oranges

The oranges used for the marmalade were the first Navels of the winter crop of 1948. They were not very ripe and a large percentage still had some green spots.

D. Methods of Analysis

1. Copper Determinations. Copper was determined by using a micro-method of Eden and Green (21) which is a modification of the Callan and Henderson (11) method. A "Lumetron" photo-electric colorimeter was used for measuring color density.

For copper concentrations of 5 gammas and higher a 530 millimicron wavelength filter was used and for concentrations below 5 gammas per tube a 420 millimicron filter was employed, as suggested by Eden and Green (21). According to them a better extinction coefficient was obtained for the very low copper concentrations with the 420 millimicron filter. Two separate standard curves, one for each filter employed were used for calculations.

2. l-Ascorbic Acid Determinations. l-Ascorbic acid was determined by the indophenol-xylene extraction

method of Robinson and Stotz (90) using a "Lumetron" photoelectric colorimeter for the color measurements. A 530 millimicron filter was used and a standard curve was prepared.

3. Reductone and Reductic Acids Determinations. The formaldehyde modification method of Robinson and Stotz (90) which is assumed to correct for the interfering action of reductones, was used. Color measurements were made with the same instrument as described under l-ascorbic acid using a 530 millimicron wavelength filter. Formaldehyde forms a complex with ascorbic acid and prevents it from reducing the dye.

4. Total Ascorbic Acid Determinations. The method used here was the same as the one described in "Methods of Vitamin Assay" (4). This method is an adaptation of the method of Roe and Oesterling (91) which is based on the osazone formation of 2,4-dinitrophenylhydrazine with dehydro-ascorbic acid after oxidizing the ascorbic acid with bromine.

5. Dehydro-ascorbic Acid Determinations. Direct dehydro-ascorbic acid determinations were made by the method of Roe and Oesterling (91) as outlined in "Methods of Vitamin Assay" (4).

6. Reducing Sugar Determinations. Reducing sugars were determined by the Lane-Eynon general volumetric method as described in the 6th edition of Methods of Analysis of the Association of Official Agricultural Chemists (3).

7. Color Determinations. The color changes taking place in the jellies and marmalades are marked by the formation of yellow- to dark brown shades. The following method was used for the color determination.

100 grams of sample were dissolved in 100 ml of 5 per cent metaphosphoric acid. The slurry was allowed to filter slowly, first through a linen cloth and thereafter with suction through a No. 41 Watman's filter paper. Resulting air bubbles in the clear filtrate were centrifuged out.

The color index of the filtrate was determined in a "Lumetron" photo-electric colormeter using a 420 milli micron wavelength filter because a better spread was obtained with this filter. Five per cent metaphosphoric acid was used as a blank.

The galvanometer reading was adjusted to 100 per cent transmission with the blank, after which the sample tube was introduced and the per cent transmission noted. A high reading indicates a light colored product

whereas a low reading, indicates a brown colored product.

8. Spectrophotometric Analysis. A Beckman Quartz Spectrophotometer was employed for these determinations. In the visible range the 5 per cent clear metaphosphoric acid filtrate as prepared for the color measurements described previously was used.

For the ultra violet range the samples were taken up in distilled water since the ultra violet light was not transmitted through the metaphosphoric acid. Quartz tubes with the appropriate ultra violet lamp were used for the latter readings.

9. Other Determinations. A Beckman glass electrode model M pH meter was used for determining pH values.

Soluble solids were determined by means of a Zeiss refractometer.

Vacuum was determined by using an ordinary laboratory vacuum gauge (Puncture type) which was previously calebrated with a mercury manometer type vacuum gauge.

CHAPTER IV

EXPERIMENTAL RESULTS

A. Effect of Sugars and Sugar Sirups in Synthetic Jellies And Orange Marmalade on

1. Losses of l-ascorbic Acid and Total Ascorbic Acid. The influence of sugars and sugar sirups, as ingredients usually employed in the manufacturing of jams and jellies, on the retention of vitamin C was investigated first, by employing them in a relatively simple system such as synthetic jellies (made with citrus pectin and citric acid) and secondly, in boilings of orange marmalade.

The experimental data of losses of l-ascorbic acid as influenced by the different sugar and sugar-sirup combinations used in synthetic jellies are presented in Table I and the losses in the glass and tin containers are graphically illustrated in Figure 1.

Referring to the data in Table I, but especially to that in Figure 1, it is noted that if the losses of l-ascorbic acid after three as well as six months storage at 100° F are taken into consideration, the order of decreasing retention of ascorbic acid in both glass and tin containers was L. C. corn sirup, H. C. corn sirup,

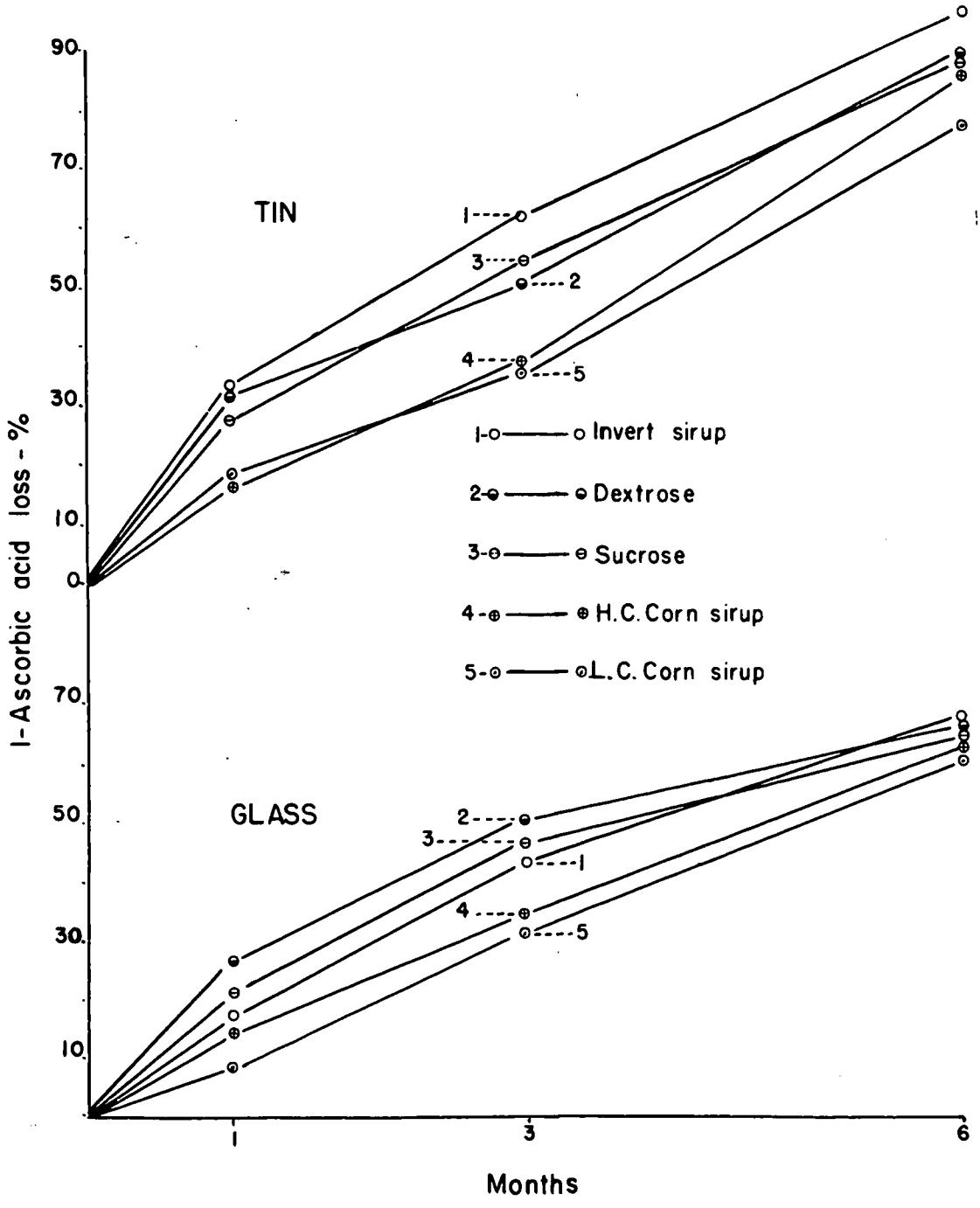
TABLE I

Losses of l-ascorbic Acid in Synthetic Jellies and Orange Marmalade at 100°F. as influenced by Sugars and Sugar Sirups.
(10" vacuum. Copper in all cases adjusted to 2 p.p.m.)

Storage Time	L.C. Corn Sirup*		H.C. Corn Sirup		Sucrose		Dextrose		Invert Sirup	
	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
Months	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss
				Synthetic Jellies						
1	8.7	18.8	14.3	16.7	21.7	28.3	26.5	32.3	17.4	32.5
3	31.4	36.0	34.5	36.9	46.7	54.5	50.3	45.7	43.4	61.9
6	61.4	77.1	62.0	88.1	62.4	87.0	63.8	88.7	65.2	95.6
				Orange Marmalade						
1	26.1	29.3	26.7	28.5	26.7	26.7	28.3	33.7	33.3	35.0
3	45.6	41.5	50.4	50.4	50.0	46.7	55.5	59.9	65.6	67.4
6	74.0	81.6	71.5	79.2	77.8	83.3	78.3	89.2	86.6	93.4

* The ratios of sucrose replacements were outlined in the experimental procedures.

FIG. 1 Losses of l-ascorbic acid in synthetic jellies stored at 100°F as influenced by sugars and sugar sirups



sucrose, dextrose and invert sirup.

Another outstanding feature of the data in Table I is the excessively high losses of vitamin C in the tin containers as compared to the losses in the glass containers under the same conditions.

Comparing the losses of l-ascorbic acid in the tin and glass containers it was found that the average losses for the above mentioned synthetic jellies were the following:

	Per cent Losses After 3 Months. Stored at 100°F.	Per cent Losses After 6 Months. Stored at 100°F.
Glass	41.2	62.2
Tin	47.0	87.3

Comparing the percentage losses of total ascorbic acid of the different synthetic jelly boilings stored in tin containers (Table II) it is evident that the order of decreasing retention was the same as in the case of l-ascorbic acid losses.

Data for the total ascorbic acid losses in glass do not follow the above mentioned sequence of retention very distinctly. From the analysis of the jellies in the glass containers it is however clear that there was a better retention of ascorbic acid in the corn sirup samples than in any of the other sugar jellies tested.

The average losses for total ascorbic acid of these jelly boilings were as follows:

	Percent Total Ascorbic Acid Losses (average) after 3 Months at 100°F	Percent Total Ascorbic Acid Losses (average) after 6 Months at 100°F
Glass	35.9	58.0
Tin	39.0	74.7

Again the relatively large losses of total ascorbic acid in the tin containers are very evident.

The level of total ascorbic acid lost is much lower than that of l-ascorbic acid. The conversion of l-ascorbic acid to dehydro-ascorbic acid which is included in the total ascorbic acid determinations may account for this.

The influence of the different sugar and sugar-sirup mixtures on the l-ascorbic acid losses in orange marmalade is presented in Table I and graphically illustrated in Figure 2.

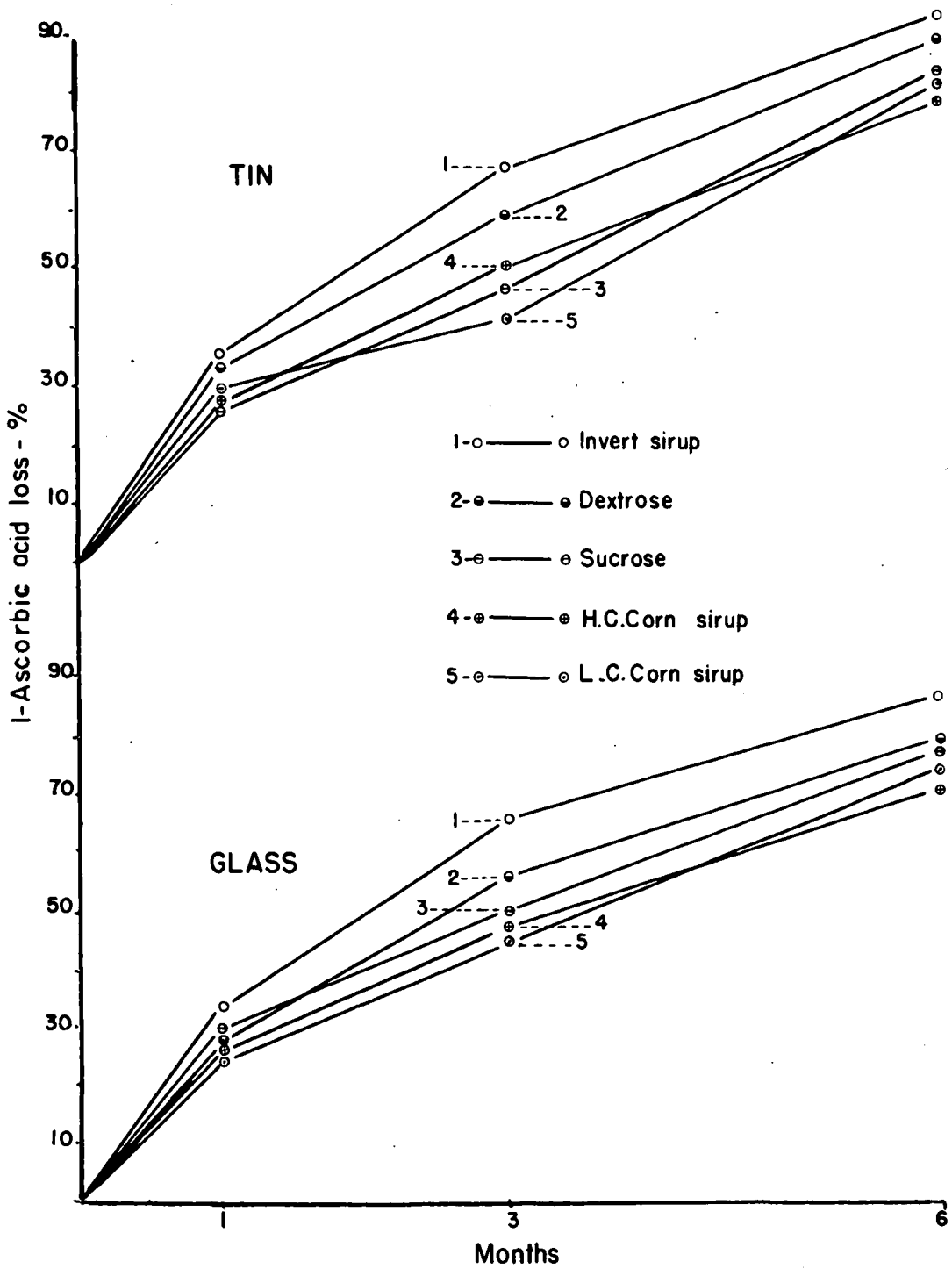
From the graphs illustrating the l-ascorbic acid losses in the tin containers, it is found that the order of decreasing retention of l-ascorbic acid is: L.C. corn sirup, H.C. corn sirup, sucrose, dextrose and invert sirup; which is the same order as for synthetic jellies.

TABLE II

Losses of Total Ascorbic Acid in Synthetic Jellies and Orange Marmalade at 100°F. as influenced by Sugars and Sugar Sirups.
(10" vacuum. Copper in all Cases adjusted to 2 p.p.m.)

	L.C. Corn Sirup		H.C. Corn Sirup		Sucrose		Dextrose		Invert Sirup	
Storage Time	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
Months	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss	% Loss
					Synthetic Jellies					
1	3.3	15.7	9.4	11.1	15.7	24.8	22.1	26.5	11.8	30.9
3	26.7	31.0	26.8	25.8	37.5	43.1	48.6	48.0	29.9	47.3
6	51.4	56.6	51.2	75.1	75.8	75.0	53.6	76.3	60.3	90.8
					Orange Marmalade					
1	19.9	22.3	19.7	22.7	25.1	19.9	21.5	26.4	26.9	28.6
3	40.0	40.1	45.4	51.0	48.8	43.7	48.4	48.0	58.8	58.2
6	55.0	73.5	58.0	68.7	61.4	68.6	68.3	76.0	74.7	76.7

FIG. 2 Losses of l-ascorbic acid in orange marmalade stored at 100°F. as influenced by sugars and sugar sirups.



From the data in Table I under orange marmalade, higher losses of l-ascorbic acid in the tin than in the glass is very clear. This was also found with the synthetic jellies.

Average losses of l-ascorbic acid in orange marmalade as compiled from Table I are as follows:

	Percent l-ascorbic Acid Losses after 3 Months at 100°F	Percent l-ascorbic Acid Losses after 6 Months at 100°F
Glass	53.4	77.7
Tin	53.2	85.3

Comparing the percentage losses of <u>total</u> ascorbic acid of the different orange marmalades in Figure 3, it is found that the sequence of decreasing retention exerted by the different sugars is the same as that for the l-ascorbic acid losses. This was also found with the control samples of orange marmalade boiled without adjusting the copper to the 2 p.p.m. level.

When the averages of the total ascorbic acid losses of orange marmalade in the glass and the tin containers are compared, (Table II) the following

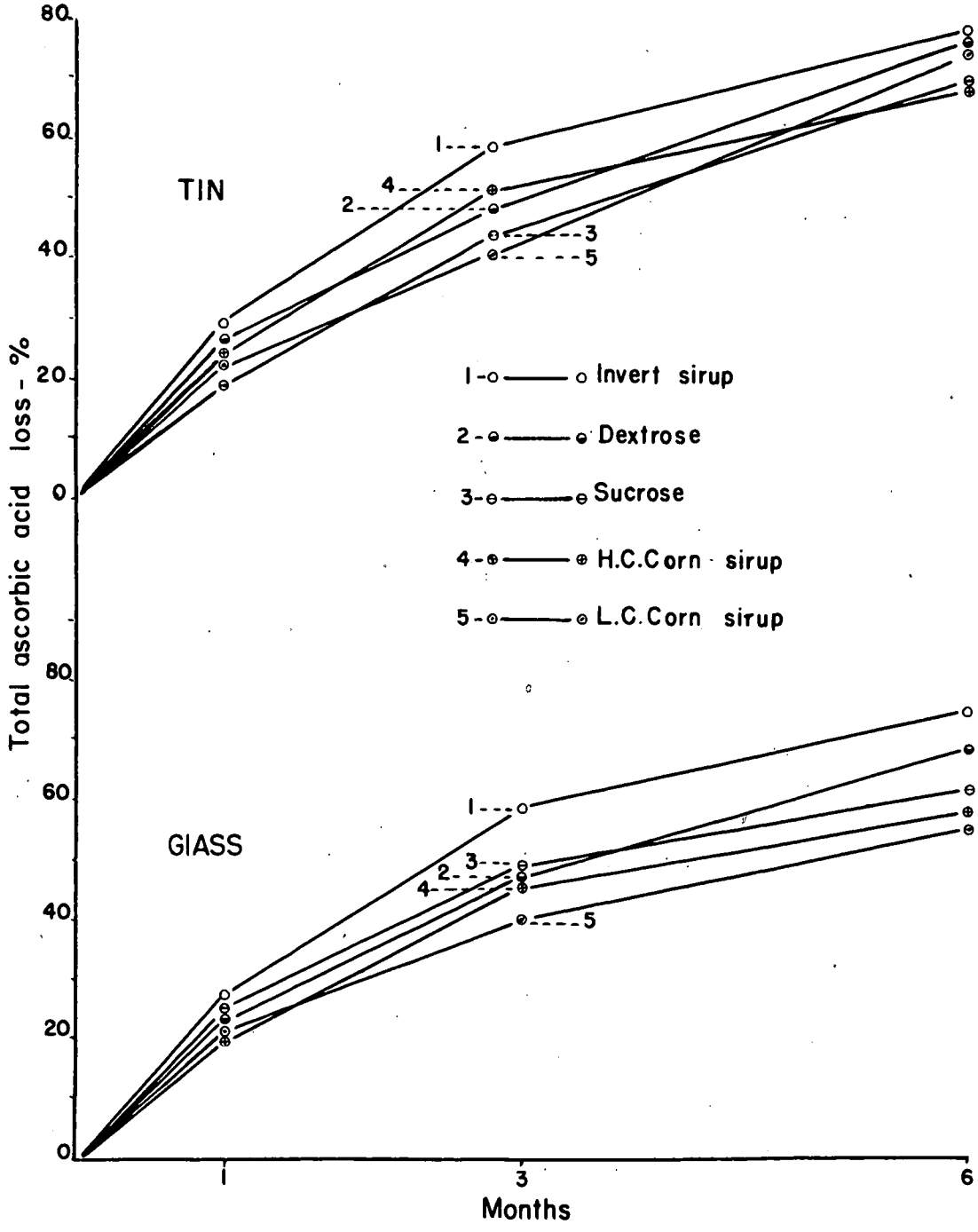
differences are found:

	Losses of Total Ascorbic Acid after 3 Months at 100°F	Losses of Total Ascorbic Acid after 6 Months at 100°F
Glass	48.3 %	63.5 %
Tin	56.2 %	72.7 %

Orange marmalades therefore appear to lose l-ascorbic acid and total ascorbic acid in both glass and tin containers at a much faster rate than the synthetic jellies.

In considering the better retention of vitamin C in the corn sirup samples, their high dextrin content should be borne in mind, which results in a slightly lower sugar content.

FIG. 3 Losses of total ascorbic acid in orange marmalade stored at 100°F. as influenced by sugars and sugar sirups.



2. Color Changes. A distinct brown color developed in the synthetic jellies first only at the higher storage temperature (100° F) but later also became noticeable at room temperature.

These color developments were first only slight but the figures in Table III indicate that as the storage time progressed the intensity of the color also increased.

The graphical illustrations of these data (Figure 4) show very distinctly how much more pronounced the dark color development was in the tin than in the glass container.

The sequence of increased color development as formed in the different sugars and sugar sirup boilings was invert sirup, dextrose, sucrose, H.C. corn sirup and L.C. corn sirup. In other words, the invert sirup was responsible for the darkest color developed whereas the corn sirup boilings showed the least discoloration.

There was very little difference between the color development as caused by sucrose and the sucrose-dextrose mixture.

The degree of intensity of the brown color as developed in the synthetic jellies after 6 months storage at 100° F is demonstrated by a series of photographs in Figure 5. The excessively dark color as developed in the invert sugar boilings

TABLE III

Effect of Sugars on Color Changes in Synthetic Jelly and Orange Marmalade at 100°F. (10" Vacuum. Copper in all Cases adjusted to 2 p.p.m.)

Storage Time	L.C. Corn Sirup		H.C. Corn Sirup		Sucrose		Dextrose		Invert Sirup	
	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
Months	% Trans.	% Trans.	% Trans.	% Trans.	% Trans.	% Trans.	% Trans.	% Trans.	% Trans.	% Trans.
				Synthetic Jellies						
0	96.0	96.0	96.0	96.0	96.0	96.0	96.0	96.0	96.0	96.0
1	97.0	94.5	94.5	92.5	94.0	95.0	88.0	89.0	91.0	84.0
3	88.5	89.0	88.0	87.0	89.0	87.0	88.0	97.0	81.0	74.0
6	83.5	73.5	79.5	64.5	78.5	40.5	73.0	59.5	71.5	28.0
				Orange Marmalade						
0	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.0	71.0
1	-	-	-	-	-	-	-	-	-	-
3	42.0	46.5	40.0	40.0	47.0	51.0	44.0	50.0	25.0	24.0
6	28.0	26.0	26.5	17.5	31.0	28.0	30.5	29.0	16.0	17.0

FIG. 4 Effect of sugars and sugar sirups on color changes in synthetic jellies stored at 100°F.

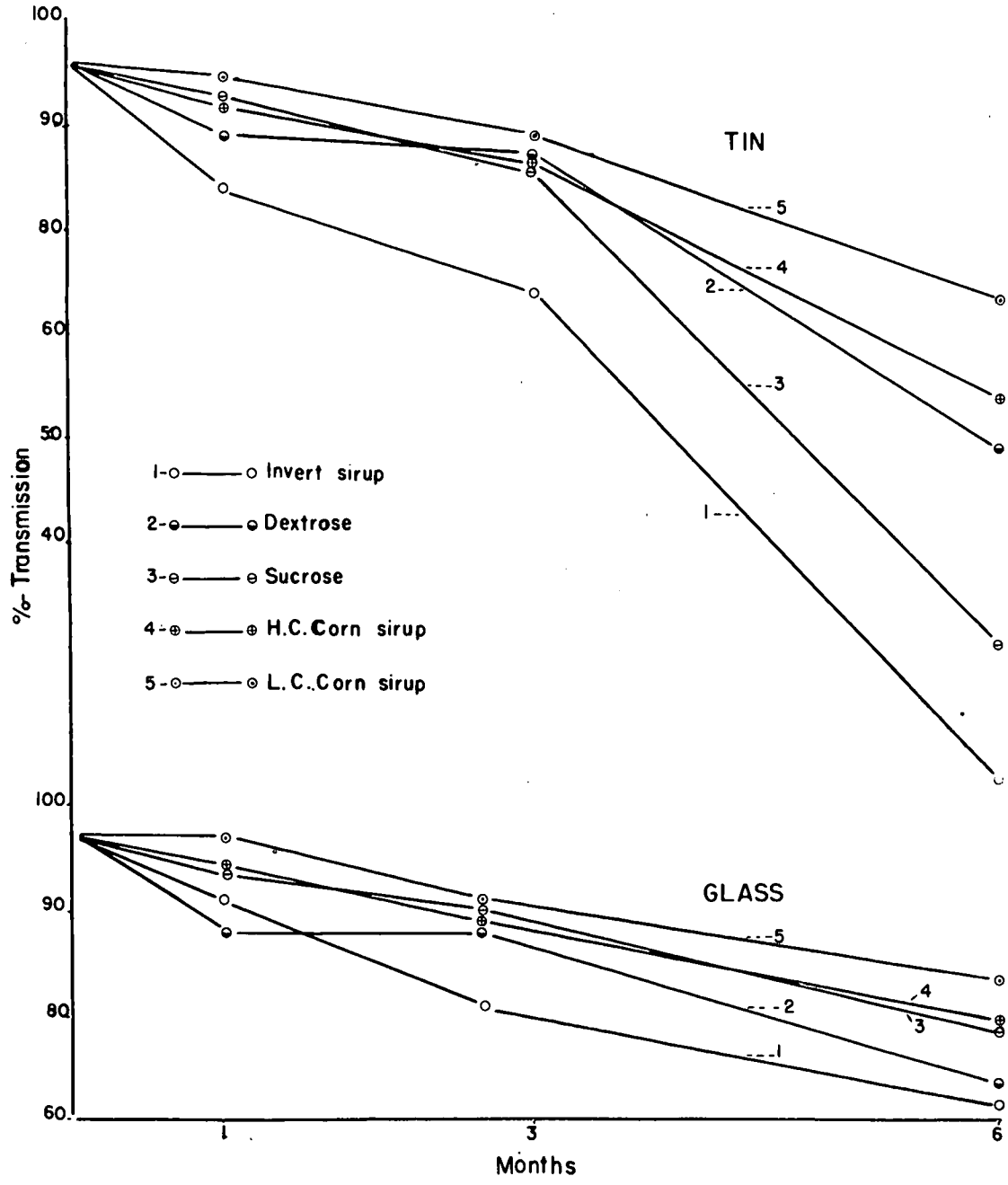


Fig. 5. Illustrations of Color Development in Synthetic Jellies After 6 Months Storage (10" vacuum in all containers)

A. Sucrose, 100°F no d-iso ascorbic acid (83%)*
B. Sucrose, 100°F + 20 mg. d-iso ascorbic acid/100 g. (86%)
C. Sucrose, Sunlight (51°F) no d-iso ascorbic acid (94%)
D. Sucrose, Sunlight (51°F) + 20 mg. d-iso ascorbic acid/100 g.(94%)
E. L.C. Corn sirup, 100°F (86%)
F. L.C. Corn sirup - Copper, 100°F (83%)
G. Sucrose, 100°F (82%)
H. Sucrose + Copper, 100°F (79.5%)
I. Invert Sirup, 100°F (75%)
J. Invert Sirup + Copper, 100°F (71.5)
K. H.C. Corn sirup + Copper, 100°F (78%)
L. H.C. Corn sirup, 100°F (82%)

* % Transmission

(containers I and J) as compared with containers E and F, containing the corn sirup boilings, is very clear. The per cent transmission given for each sample may help in distinguishing the color intensity differences of these illustrations.

The same dark color development which was observed in the synthetic jellies also develops in the orange marmalade. These color developments were especially severe at the higher storage temperatures and became noticeable first also in the top layers of the containers. Containers 2, 3, and 12 in Figure 6 illustrate this very clearly.

From the data in Table III and from the graphs in Figure 7 it is shown that invert sirup is responsible for excessive discoloration in the orange marmalade. Compare containers 1 and 2 (invert sirup marmalade) with the containers in the bottom row of Figure 6. This is in agreement with the synthetic jelly results.

The least color development was found in the sucrose and sucrose-dextrose boilings. The L.C. corn sirup and H.C. corn sirup boilings fit in intermediately between the sucrose and invert sirup samples.

Fig. 6. Illustration of Color Development in Orange Marmalade due to Type of Sugar, Added Copper and Storage Temperatures.

TOP ROW: (left to right)

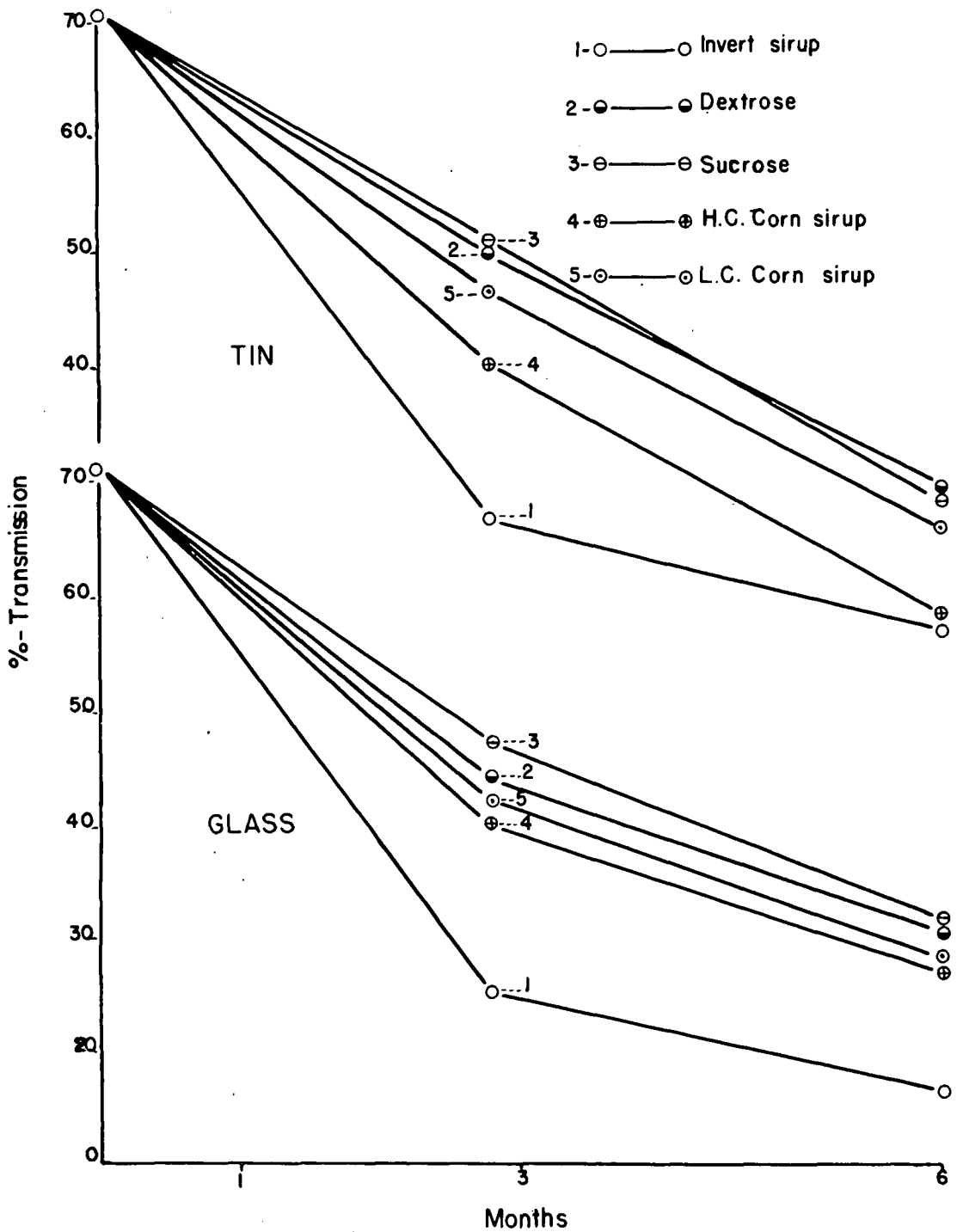
1. Invert sirup 100°F, copper added. (25%)*
2. Invert sirup 100°F, (32%)
3. Sucrose - room temperature (66%)
4. Sucrose - room temperature, copper added (64%)
5. Sucrose Sunlight (51°F) (72%)
6. Sucrose Sunlight (51°F), copper added (67%)

BOTTOM: (left to right)

7. Sucrose 100°F (50%)
8. Sucrose 100°F, copper added (47%)
9. H.C. Corn Sirup 100°F (47%)
10. H.C. Corn Sirup 100°F, copper added (40%)
11. L.C. Corn Sirup 100°F (42.0%)
12. L.C. Corn Sirup 100°F, copper added (38%)

* Color - Per cent Transmission.

FIG. 7 The effect of sugars and sugar sirups on color changes in orange marmalade stored at 100°F.



3. Degree of Inversion in Storage. By using the different sugars and sugar sirup combinations a large variation in the per cent of reducing sugars of the different boilings was obtained.

The per cent inversion of the different synthetic jelly boilings and the progress of inversion during the six months storage at 100°F is graphically illustrated in Figure 8. After six months storage the invert sirup jellies showed the largest per cent of inversion followed in decreasing order by dextrose, sucrose, H.C. corn sirup and L.C. corn sirup.

As pointed out in the literature review on this subject previously, several workers, Hall (34), Wilson (117) and Curl (17) tried to correlate invert sugars with losses of ascorbic acid. By doing this with the experimental results the data presented in Table IV show a very definite correlation between the per cent reducing sugars present, browning, and the losses of vitamin C.

FIG. 8 The degree of inversion in synthetic jellies prepared from different sugars and sugar sirups stored in glass at 100°F.

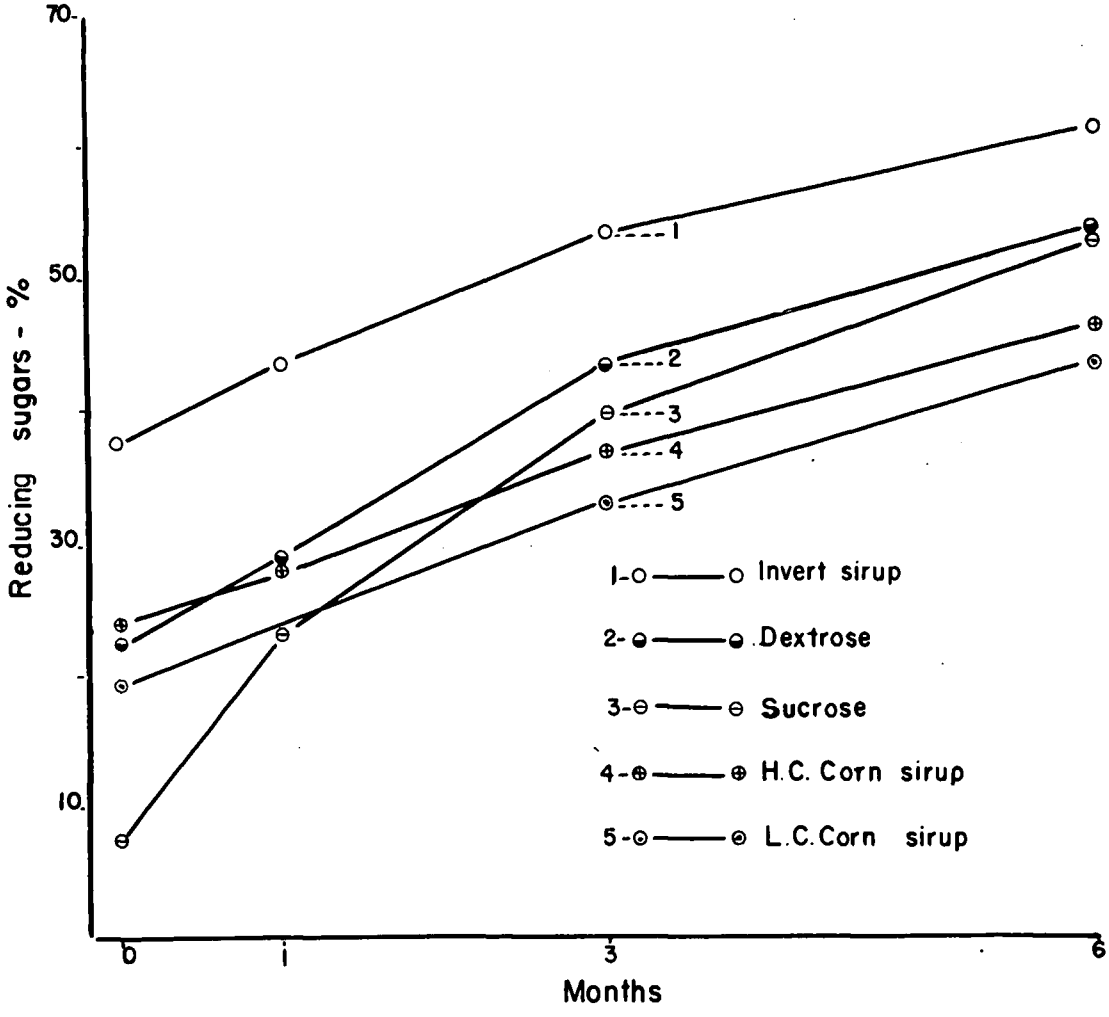


TABLE IV

Comparison between the Degree of Inversion, Color and Loss of l-ascorbic Acid as Related to the Type of Sugar of Synthetic Jellies at 100°F.

Months	L.C. Corn Sirup			H.C. Corn Sirup			Sucrose			Dextrose			Invert Sirup		
	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss
							Glass Containers								
0	19.1	96.0	-	23.7	96.0	-	7.6	96.0	-	22.6	96.0	-	37.0	96.0	-
1	-	-	-	28.4	95.5	9.5	23.3	94.0	15.7	28.4	91.0	12.8	43.7	90.5	9.9
3	33.2	90.0	17.7	37.0	87.6	25.0	40.0	89.0	37.5	43.4	84.0	27.3	53.6	84.5	22.2
6	43.7	86.0	43.9	46.6	82.0	49.7	58.0	79.0	-	58.8	76.0	51.1	61.4	75.0	57.7
							Tin Containers								
0	19.1	96.0	-	23.7	96.0	-	7.6	96.0	-	-	96.0	-	37.9	96.0	-
1	28.4	97.0	8.6	30.8	95.0	7.9	25.8	95.0	24.8	-	89.0	25.0	48.6	88.0	16.6
3	34.8	91.0	15.7	38.8	86.0	32.8	45.0	87.0	43.0	-	85.0	21.0	55.0	76.0	32.7
6	46.4	72.0	56.0	47.6	64.0	68.3	58.5	40.5	74.9	-	57.0	73.3	63.8	28.0	76.7

The per cent loss of ascorbic acid in the presence of the various sugars in increasing order is L.C. corn sirup, H.C. corn sirup, sucrose, dextrose and invert sirup. Comparing the per cent inversion with this it will be seen that L.C. corn sirup had the lowest per cent invert sugars after 3 and 6 months, followed by the other sugars in the same sequence as listed above. The figures for color changes follow exactly the same pattern, that is, corn sirup jellies developed much less color than invert sirup jellies under the same conditions.

It is also interesting to note that the per cent invert sugars were in most cases always slightly greater in the tin than in the glass containers. The strikingly higher losses of l-ascorbic acid and excessive discoloration in the tin containers has already been pointed out.

The per cent reducing sugars formed may be tied up to a certain extent with the losses of ascorbic acid and the browning reaction, but this is by far not the only factor involved in these reactions.

The excessively higher losses of ascorbic acid in the tin containers cannot be blamed on the copper since the copper content of the jellies in both types of containers was the same.

Another possibility may be the tin of the plain

tin container. Eddy (20) however found that the cupric ion increased the rate of oxidation of ascorbic acid greatly; zinc ions increased it slightly and stannic and stannous ions had a slight inhibiting effect. This also is in agreement with the findings of Joslyn and Marsh (47).

Several workers pointed out the importance of iron as a factor which influences the oxidation rates of ascorbic acid (50), (51), and (115).

Barron, et. al. (5) state that iron alone does not catalyze the oxidation of ascorbic acid but found that in the presence of iron, copper exerts an increased catalytic effect.

Mack and Kertesz (63) also found that the catalytic activity of copper is markedly increased by the addition of small amounts of ion. They suggest that the iron exerts a promoter action on the copper since it does not itself catalyze the oxidation of ascorbic acid.

The amount of iron in the different boilings is not known but it seems reasonable to predict that due to irregularities in the surface of the tin coating of the container some of the iron of the base plate may be exposed and probably play a part in the oxidation of vitamin C as suggested by the latter investigators.

Whether the excessive browning in the tin over

that in glass is due to the oxidation of ascorbic acid alone or whether the invert sugars also play a part is not shown by these data. Other data to be presented later show that the major portion of the browning in tin is caused by high sugar concentrations with the ascorbic acid playing only a minor part.

While the storage experiments of the synthetic jellies were in progress it was noted that the brown discoloration always shows up first at the top of the container and gradually migrates to the bottom. To see if there is any correlation between this color formation, vitamin C losses and the per cent invert sugars of the top and bottom parts of the same container, separate analyses of these layers were made. The sides and middle portions of the tin containers were used for separate analysis.

Typical analytical results of the two separate layers for the sucrose jellies stored at 100° F for 6 months are given in Table V.

TABLE V

Losses of Ascorbic Acid, Color Changes and Per cent Invert Sugars in Different Parts of Synthetic Jellies in the same Container

		Ascorbic Acid Loss Per cent	Color Per cent Transm.	Per cent Invert Sugar
Glass	Top	61.2	80.0	50.0
	Bottom	45.2	89.0	54.0
Tin	Sides	83.0	49.0	60.2
	Middle	81.8	61.5	59.6

These data show the marked increase of color which is paralleled with the heavy loss of vitamin C in the top layer of the glass and the side layers of the tin containers.

The color formation in the top layer of the glass container may be explained by the rapid development of oxidation products of ascorbic acid since these layers are directly exposed to the oxygen of the headspace. These oxidation products are definitely the cause of browning as proved by experiments on the browning reaction of which the data are to be presented later. It has been suggested by other investigators that these substances may include furfuraldehyde. Haas and Stadtman (32), for example, point out that substances that may form furfural are sugar, ascorbic acid, uronic acid, etc. and

in experiments with apricot pulp they found that when sugars are removed by fermentation with yeast the rate of browning is also reduced to about half of the normal rate. Very little furfural accumulated in this fermented material during storage. This will show the important part played by sugars in the browning reaction which also in some cases may be a major source of furfural formation.

In the tin container the darkening was the most severe where the jelly was in contact with the sides of the container. In the interior or center parts much less browning occurred. This may explain the additional effect exerted by the iron of the side walls of the tin container which, as suggested by Mack and Kertesz (63) have a promotive action on the primary catalyst copper, which is known to be present. The copper then in turn catalyzes the oxidation of ascorbic acid and starts a series of reactions which all contribute to the excessively dark complexes which are formed in the tin.

The evidence from a series of papers (38), (33) and (64) by the Food Technology Division of the University of California is that the browning is not due to a simple process but it is the result of a series of unrelated reactions of various kinds each giving rise to a dark pigment.

In the orange marmalade boilings the degree of

inversion for the different sugars and sugar-sirup mixtures that took place during the six months storage at 100° F show the same trends as the previously described synthetic jellies. The invert sirup boilings showed the highest degree of inversion followed by dextrose, sucrose and lastly by corn sirup which showed the lowest per cent of reducing sugars. (See Figure 9).

When the per cent invert sugar, color and the per cent l-ascorbic acid loss (Table VI) of the orange marmalade boilings are compared it is found that a high per cent invert sugar also here is associated with a high percentage loss of vitamin C.

As in the synthetic jellies it is also found that the invert sirup samples developed the darkest color. The color development of the rest of the marmalade samples was not so distinctly correlated with the degree of inversion and l-ascorbic acid losses as in the case of the synthetic jellies. The L.C. corn sirup and H.C. corn sirup marmalades with a lower per cent inversion showed slightly more color development than the sucrose and dextrose boilings which had a higher per cent inversion.

FIG.9 The degree of inversion in orange marmalade prepared from different sugars and sugar sirups stored in glass at 100°F.

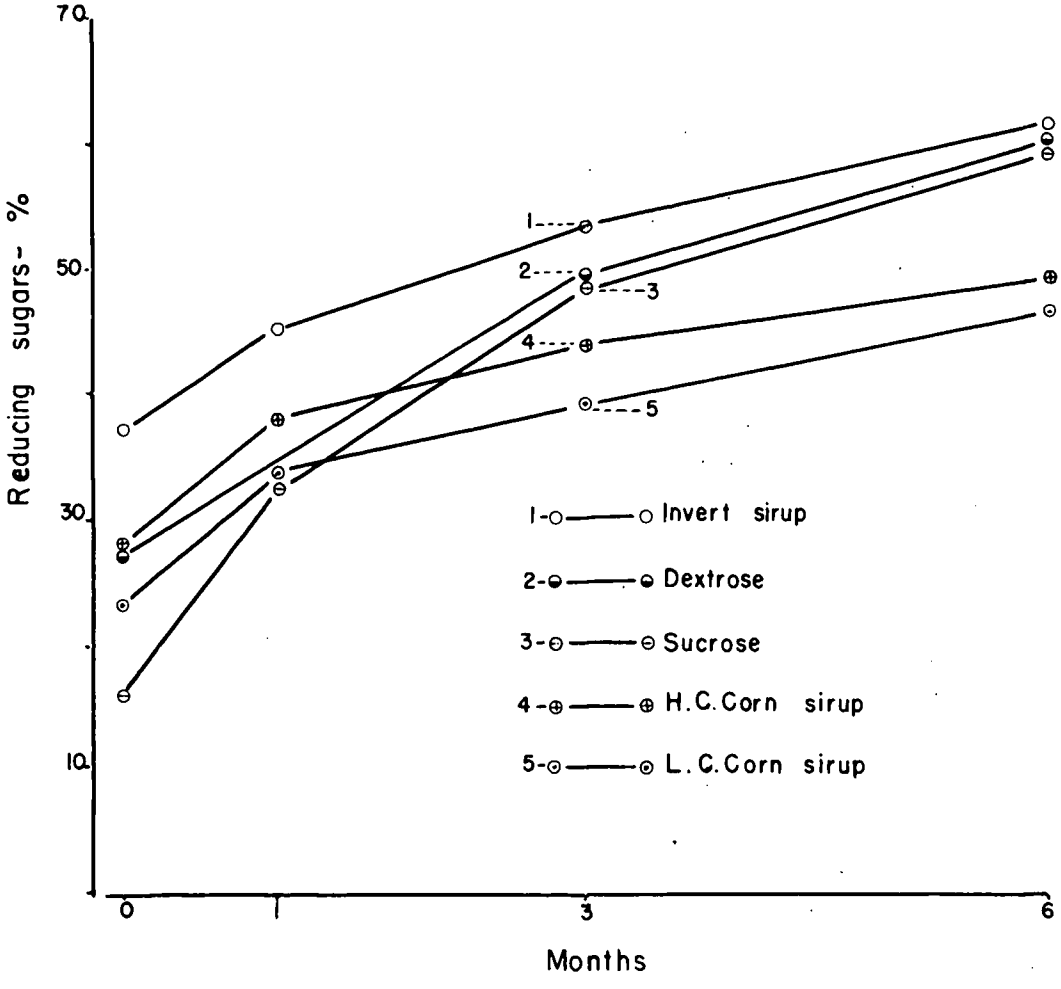


TABLE VI

Comparison between the Degree of Inversion, Color and Loss of l-ascorbic Acid in Orange Marmalade as Related to Type of Sugar at 100°F.

Storage Time	L.C. Corn Sirup			H.C. Corn Sirup			Sucrose			Dextrose			Invert Sirup		
Months	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss	% Inv.	% Trs.	% A.a. loss
							Glass Containers								
0	23.6	71.0	-	28.0	71.0	-	16.0	71.0	-	27.4	71.0	-	37.4	71.0	-
1	33.8	-	18.7	38.0	-	32.4	32.3	-	20.0	-	-	22.7	45.0	-	26.9
3	39.0	42.0	42.2	44.0	47.0	48.3	48.7	50.0	49.5	49.6	50.0	47.1	53.6	32.0	52.8
6	46.6	28.0	68.9	49.0	28.0	73.7	59.2	32.0	78.8	59.8	32.5	75.0	61.0	19.0	84.2
							Tin Containers								
0	23.6	71.0	-	28.0	71.0	-	16.0	71.0	-	27.4	71.0	-	37.4	71.0	-
1	34.4	-	31.5	37.2	-	32.4	30.7	-	27.7	-	-	20.5	48.0	-	33.3
3	41.0	45.0	47.3	44.0	49.0	47.3	47.8	51.0	42.3	50.4	53.5	60.2	53.2	34.0	58.9
6	46.8	27.0	77.3	52.0	26.0	81.3	58.0	29.0	48.5	60.2	31.0	80.7	58.4	26.0	81.1

These same trends are followed by the marmalade in the tin containers and there is slight evidence that the degree of inversion in the tin containers is a little higher than that of the marmalade in the glass jars. With this higher per cent inversion in the tin, the higher losses of l-ascorbic acid and the development of a darker color are closely associated.

B. Effect of Copper Added

1. Losses of l-ascorbic and Total Ascorbic Acid. The boilings made using the different sugars were run in duplicate. To the first series the sugars and other ingredients were used without copper supplementation but to the second series additional copper in the form of copper sulfate was added so as to bring the copper level in the final batch up to 2 p.p.m.

The copper analysis of the ingredients used was as follows:

Ingredient	Copper (p.p.m.)
Sucrose	1.25
Dextrose	0.63
H.C. Corn Sirup	4.5
L.C. Corn Sirup	1.84
0.1% Citric Acid Solution	0.0069
Citrus Pectin	25.8

The effects of the addition of copper to the different boilings on the losses of l-ascorbic and total ascorbic acid are presented in Tables VII and VIII, respectively.

TABLE VII

Losses of l-ascorbic Acid due to the Addition of Copper to a 2 p.p.m. Level in Synthetic Jellies at 100°F. (10" Vacuum)

Storage Time Months	L.C.Corn Sirup				H.C.Corn Sirup				Sucrose				Dextrose				Invert Sirup			
	Glass		Tin		Glass		Tin		Glass		Tin		Glass		Tin		Glass		Tin	
	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %
1	-	8.7	10.8	18.8	12.2	14.2	13.3	16.6	17.3	21.7	23.8	28.3	13.3	26.5	19.9	32.2	15.5	17.4	20.0	32.5
3	22.3	31.4	25.7	36.0	35.4	34.5	35.4	36.9	32.5	46.7	41.1	54.5	41.1	50.3	33.3	45.7	37.7	43.4	48.9	61.9
6	60.0	61.1	71.4	77.4	61.9	65.5	80.5	88.1	58.6	55.4	89.1	86.9	62.2	63.8	81.1	88.7	61.1	65.2	90.1	95.6

TABLE VIII

Losses of Total Ascorbic Acid due to the Addition of Copper to a 2 p.p.m. Level in Synthetic Jellies at 100°F. (10" Vacuum)

Storage Time Months	L.C.Corn Sirup				H.C.Corn Sirup				Sucrose				Dextrose				Invert Sirup			
	Glass		Tin		Glass		Tin		Glass		Tin		Glass		Tin		Glass		Tin	
	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %	%	+Cu %
1	-	3.3	8.6	15.7	9.5	7.9	9.4	11.1	13.6	15.7	20.4	24.8	12.8	22.1	24.9	26.5	9.9	11.8	16.6	30.9
3	17.7	26.7	15.7	31.0	25.0	23.8	26.8	25.8	26.9	37.5	31.8	43.1	27.3	48.6	21.1	48.1	22.2	30.0	32.7	47.3
6	49.7	51.4	56.0	56.6	44.0	68.3	51.2	75.1	47.2	76.0	73.6	75.0	51.1	53.6	73.4	76.3	57.7	60.3	73.4	90.8

In both the glass and tin containers the losses of total and l-ascorbic acid were markedly increased by the addition of copper. It is also very evident from these data that the retention of vitamin C was much better in the boilings where the two corn sirups were used.

A comparison of the total ascorbic acid losses in the corn sirup and invert sirup jellies is graphically illustrated in Figure 10.

Jellies in the glass containers with and without additional copper retained ascorbic acid much better than the jellies in the tin. This is especially evident in the case of the invert sirup jelly boilings (See Figure 10).

Computations made from the data of the different sugar boilings (copper not adjusted) in Tables VII and VIII show that after a six months storage period at 100° F, the average losses of l-ascorbic acid were 19.1 per cent and for total ascorbic acid were 19.8 per cent higher in the tin than in the glass container. When the copper level was adjusted to 2 p.p.m. these losses increased to 25.1 per cent and 25.4 per cent for l-ascorbic and total ascorbic acid, respectively.

If copper were the only major factor responsible for the losses of ascorbic acid then according to the

amounts of copper present in these jellies, one would predict that the order in which they should retain vitamin C should be:

Invert sirup jelly (0.61 p.p.m. Cu)* > Dextrose (0.75 p.p.m. Cu) > Sucrose (0.92 p.p.m. Cu) > L.C. Corn Sirup (1.22 p.p.m. Cu) > H.C. Corn Sirup (1.55 p.p.m. Cu).

From the analytical data in Tables VII and VIII however the sequence in which the different jellies retain vitamin C is not in the above mentioned order. To the contrary the two corn sirups with the highest copper content showed the best vitamin retention.

The orange marmalade boilings were also made in duplicate in the same manner as described for the synthetic jellies. Copper determinations were made of the orange pulp before it was boiled into marmalade. The first series were boiled with the ingredients as found but to the second series copper was added to bring the level to 2 p.p.m.

The analytical data show that in the majority of cases the addition of the minute amounts of copper was responsible for increased losses in both l-ascorbic

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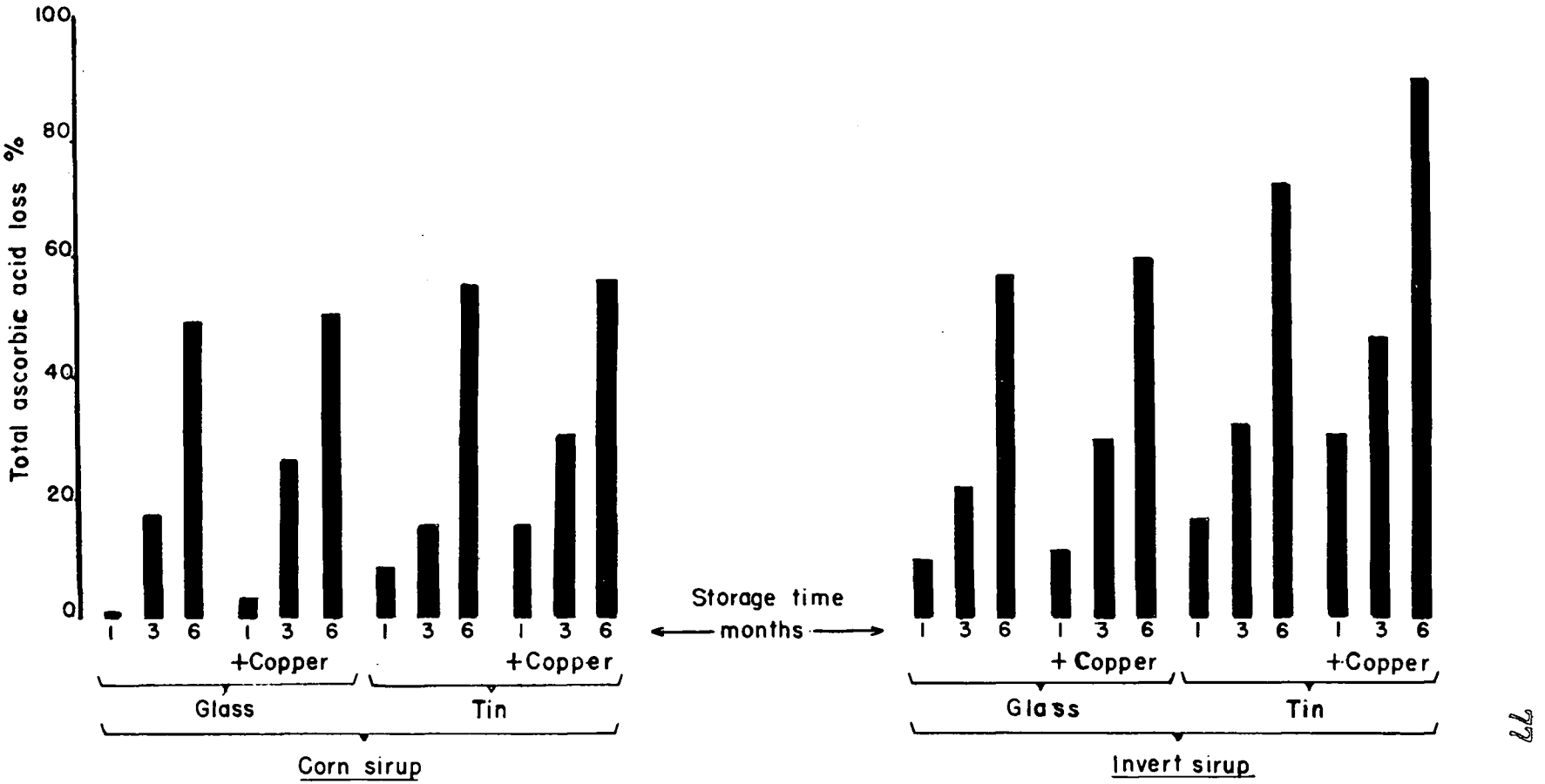
*p.p.m. copper per final jelly batch.

FIG. 10 The influence of copper on total ascorbic acid losses in corn and invert sirup jellies stored at 100°F.



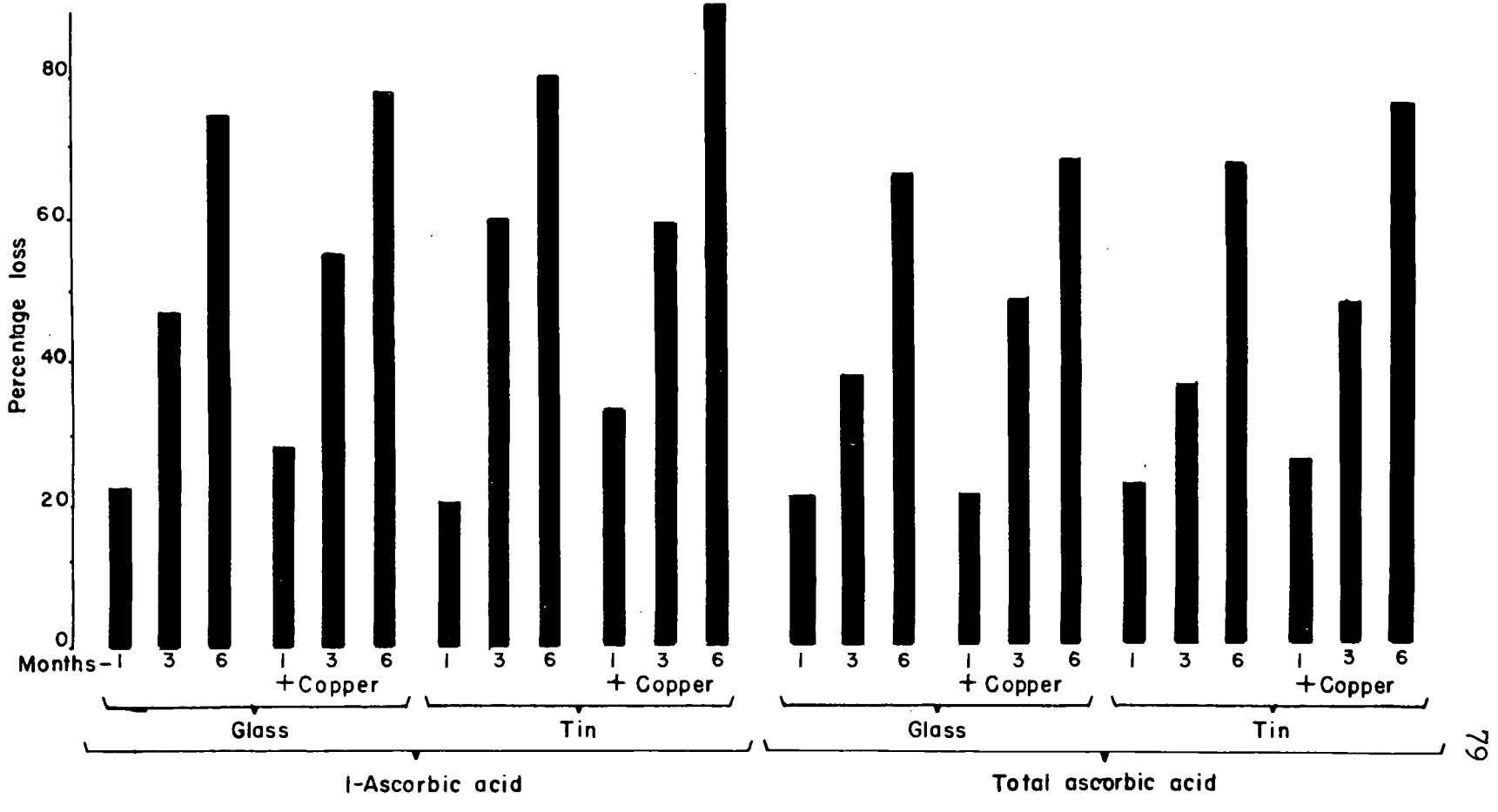
and total ascorbic acid, which is in agreement with the findings of synthetic jellies.

Typical data to illustrate the influence of added copper on vitamin C losses in orange marmalade is illustrated by graphs in Figure 11.

The additional destructive effect exerted by the plain tin container and also by the tin container plus the copper on l-ascorbic acid losses is well illustrated in these graphs.

Computations made from the analytical data show averages of 7.1 per cent and 7.2 per cent higher losses in the tin than in the glass container for l-ascorbic and total ascorbic acid, respectively. When the copper level was adjusted to 2 p.p.m. these losses also increased to 14.7 per cent and 8.3 per cent for l-ascorbic and total ascorbic acid, respectively.

FIG. 11 The effect of copper on l-ascorbic acid and total ascorbic acid losses in sucrose - dextrose orange marmalades at 100°F.



2. Color Changes. From the data compiled in Table IX it will be seen that in the case of all five different sugar mixture boilings the addition of copper had a distinct effect in increasing the darkening of the jelly as the storage progressed.

For comparison purposes the per cent losses of l-ascorbic acid is included in this table and again the direct correlation between the loss of vitamin C and the increase of the dark color is very evident.

The tin container plus the additional copper is responsible for much more excessive darkening and simultaneously high losses in ascorbic acid. In the glass container the addition of copper increased the formation of the dark color to a much lesser degree.

The influence of the type of container and the addition of copper upon the color of sucrose jellies is graphed in a typical set of curves in Figure 12. The browning of the other sugar jellies follows the same general trend.

These color formations due to the added copper are also illustrated in Figure 13. A comparison of container B (with added copper) with container A stored at room temperature, with a 10" vacuum shows a distinct brown discoloration especially noticeable at the top of container B.

Containers E and F, containing additional copper and sealed with 10" and 25" vacuum, respectively, developed a distinctly darker color at 100° F than containers I and J under the same conditions but with no additional copper.

The actual color developed in containers I and E is illustrated in color by the containers 2 and 3 in Figure 14.

Container 12 in Figure 14 (Invert Sirup + Copper) in comparison with container 11, which contain no added copper, may serve as another example of the increase in the dark color due to copper.

TABLE IX

The Effect of Copper (Added to a 2 p.p.m. Level) on l-ascorbic Acid Losses and Color Changes in Synthetic Jellies at 100°F.

Mo.	Type of Cont.	L.C.Corn Sirup		H.C.Corn Sirup		Sucrose		Dextrose		Invert Sirup	
		% A.a. loss	% Trs.	% A.a. loss	% Trs.	% A.a. loss	% Trs.	% A.a. loss	% Trs.	% A.a. loss	% Trs.
					NO COPPER ADDED						
0	Glass	-	96.0	-	96.0	-	96.0	-	96.0	-	96.0
	Tin	-	96.0	-	96.0	-	96.0	-	96.0	-	96.0
1	Glass	-	-	12.2	95.5	17.3	95.5	13.3	91.0	15.5	90.5
	Tin	10.8	97.0	13.4	95.0	23.8	95.0	20.0	89.6	20.0	88.0
3	Glass	23.3	90.0	35.4	87.0	32.5	89.5	41.1	84.0	37.8	84.5
	Tin	25.7	91.0	35.4	86.0	41.1	87.0	33.3	85.0	49.0	76.0
6	Glass	60.0	86.0	61.9	82.0	58.6	82.0	62.3	76.0	61.1	75.0
	Tin	77.1	72.0	80.5	64.0	89.1	44.5	81.1	57.0	90.1	28.0
					COPPER ADDED						
0	Glass	-	96.0	-	96.0	-	96.0	-	96.0	-	96.0
	Tin	-	96.0	-	96.0	-	96.0	-	96.0	-	96.0
1	Glass	8.7	97.0	14.3	94.5	21.7	94.0	26.5	88.0	17.4	91.0
	Tin	18.8	94.5	16.7	92.5	28.3	95.0	32.2	89.0	32.8	84.0
3	Glass	31.4	88.5	34.5	88.0	46.8	89.0	50.3	88.0	43.4	81.0
	Tin	36.0	89.0	37.0	87.0	54.8	86.0	45.7	87.0	62.0	74.0
6	Glass	61.1	83.5	65.5	74.0	55.4	79.5	63.8	77.0	65.2	71.5
	Tin	77.1	73.5	88.1	64.5	86.9	40.0	88.7	59.5	95.6	28.0

FIG. 12 The influence of type of container and the addition of copper on the color changes in sucrose jellies stored at 100°F.

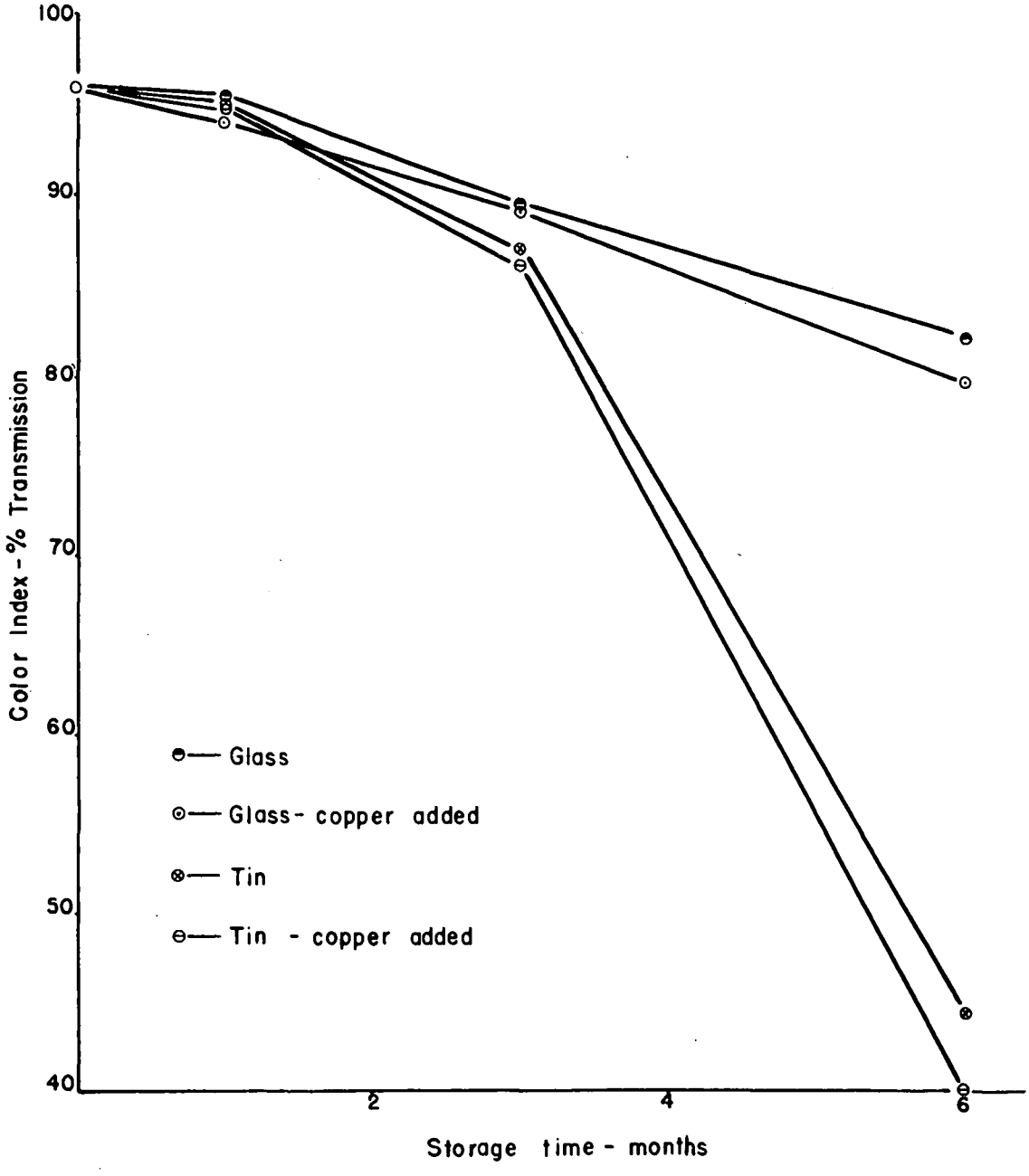
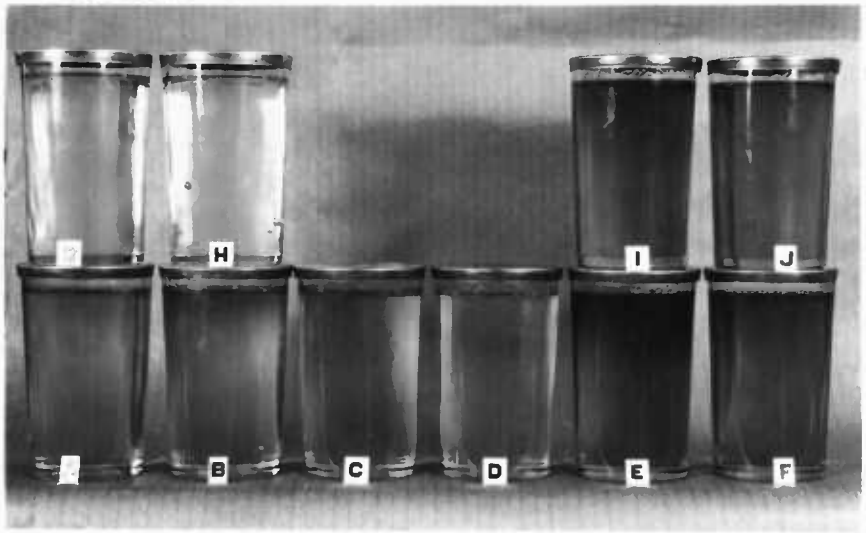


Fig. 13. Illustrations of Color Development in Synthetic Jellies due to Added Copper and the Amount of Oxygen in the Headspace



A. Sucrose, R. Temp. (72°F) 10" vacuum (95.5%)*
B. Sucrose + Copper, R. Temp. (72°F) 10" vacuum (92.5%)
C. Sucrose + Copper, R. Temp. (72°F) 25" vacuum (96%)
D. Sucrose, 32°F, 10" vacuum (94%)
E. Sucrose + Copper, 100°F, 10" vacuum (79%)
F. Sucrose + Copper, 100°F, 25" vacuum (82%)
G. Sucrose, Sunlight (51°F) 10" vacuum (98%)
H. Sucrose, Sunlight (51°F) 25" vacuum (96%)
I. Sucrose, 100°F, 10" vacuum (82%)
J. Sucrose, 100°F, 25" vacuum (88%)

* Per cent Transmission

Fig. 14. Color Photograph of Synthetic Jellies After 6 Months Storage, showing browning related to type of sugar, added Copper and Storage Temperature.

TOP ROW: (left to right)

1. Dextrose Room Temperature (72°F.) (88%)*
2. Sucrose 100°F (82%)
3. Sucrose 100°F., Copper added. (78.0%)
4. Sucrose in Sunlight (51°F) (98%)
5. Sucrose in Sunlight, Copper added (94%)
6. Sucrose 125°F, (6 weeks) 50 mg.Asc.acid (51.5%)

BOTTOM ROW: (left to right)

7. Dextrose 100°F, copper added (73.0%)
8. Dextrose 100°F (76%)
9. L.C. Corn Sirup 100°F 86%
10. L.C. Corn Sirup 100°F, copper added (83.5%)
11. Invert Sirup 100°F (75%)
12. Invert Sirup 100°F, copper added (71.5%)

* Per cent Transmission.

Where the increased darkening due to the adding of copper was very prominent in synthetic jellies it is found that in the case of orange marmalade the copper definitely increased the rate of darkening but the degree of intensity is not so marked. (See Table X).

In Figure 6, comparing containers 4 (room temp. + copper), and 6, (sunlight + copper) with containers 3 (room temp. no copper added) and 5 (sunlight, no copper added) a distinct discoloration can be seen, especially at the top of the containers. This is also true for the other orange marmalade samples containing additional copper (Figure 6).

Where the l-ascorbic acid losses are compared with the color changes (Table X) a correlation is noticeable although not so distinct as in the case of the synthetic jellies.

The conclusions that can be drawn from these results are that the acceleration of the oxidation of ascorbic acid by the catalytic effect of the added copper is responsible for large amounts of ascorbic acid breakdown products to be formed which again is tied up with the browning reactions.

TABLE X

The Effect of Copper (Added to a 2 p.p.m. Level) on l-ascorbic Acid Losses and Color Changes in Orange Marmalade at 100°F.

Storage Time Mo.	Type of Cont.	L.C.Corn Sirup % A.a. loss	L.C.Corn Sirup % Trs.	H.C.Corn Sirup % A.a. loss	H.C.Corn Sirup % Trs.	Sucrose % A.a. loss	Sucrose % Trs.	Dextrose % A.a. loss	Dextrose % Trs.	Invert Sirup % A.a. loss	Invert Sirup % Trs.
					NO COPPER ADDED						
0	Glass	-	71.0	-	71.0	-	71.0	-	71.0	-	71.0
	Tin	-	-	-	-	-	-	-	-	-	-
1	Glass	18.7	-	32.4	-	20.0	52.0	22.7	61.5	26.9	-
	Tin	31.5	-	32.4	-	27.7	53.5	20.5	63.0	33.3	-
3	Glass	42.2	42.0	48.3	47.0	49.5	50.0	47.1	50.0	52.8	32.0
	Tin	47.3	45.0	47.3	49.0	42.3	51.0	60.2	53.5	58.9	34.0
6	Glass	68.9	28.0	73.7	28.0	79.8	32.0	75.0	32.5	84.2	19.0
	Tin	77.3	27.0	81.3	26.0	84.5	29.0	80.7	31.0	81.1	26.0
					COPPER ADDED						
0	Glass	-	71.0	-	71.0	-	71.0	-	71.0	-	71.0
	Tin	-	-	-	-	-	-	-	-	-	-
1	Glass	26.1	-	26.2	-	26.7	54.5	33.7	58.5	33.3	-
	Tin	29.3	-	28.5	-	26.7	55.0	28.3	59.0	35.0	-
3	Glass	45.6	38.0	50.4	40.0	50.0	47.0	55.5	44.0	65.6	25.0
	Tin	41.5	40.0	50.4	40.0	46.7	51.0	59.9	50.0	67.0	24.0
6	Glass	74.0	28.0	71.5	26.5	77.8	31.0	78.3	30.5	86.6	16.0
	Tin	81.6	26.0	79.2	17.5	83.3	28.0	89.2	29.0	93.4	17.0

C. The Effect of Storage Temperature and Sunlight

1. Losses of l-ascorbic Acid and Total Ascorbic Acid. The influence of storage temperature on the losses of vitamin C was found to be a factor of great importance. As pointed out in the literature review, this point was also stressed by other investigators.

The ascorbic acid losses increased rapidly as the storage temperature was increased. (See Table XI.)

The additional influence exerted on l-ascorbic acid losses by the tin container and the copper is again very evident and shows up with all the different storage temperatures used for the synthetic jellies.

The samples stored in sunlight was exposed to the light by placing the glasses in a south window. However, the intensity of the winter sunlight to which these samples were subjected over the storage period of six months was not very great.

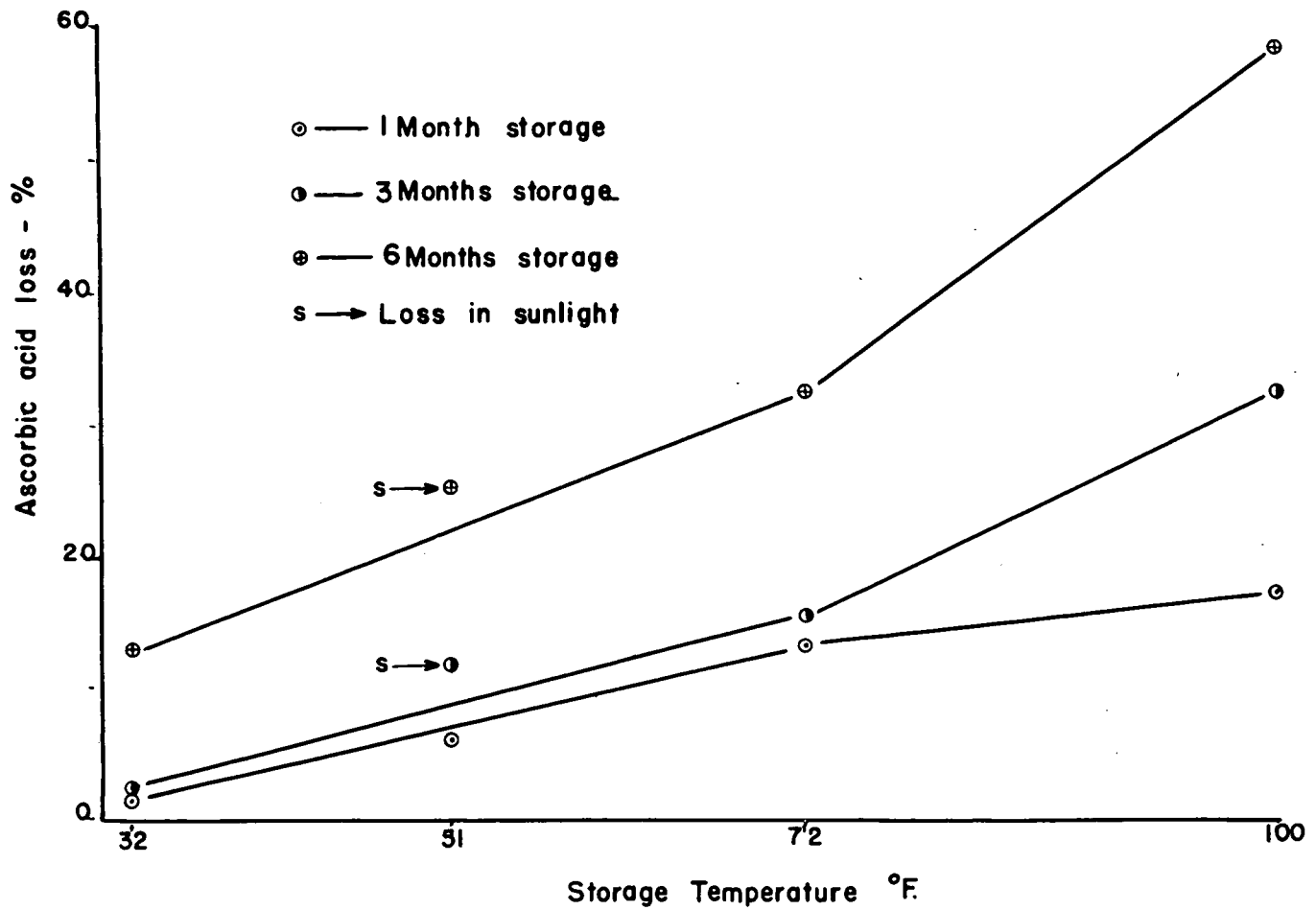
Although the storage temperatures of the samples in the sun were lower than that of the room temperature samples, it is found by computations from Figure 15, where storage temperatures are plotted against losses of ascorbic acid, that these losses were 3.0 and 3.6 per cent units higher than the expected vitamin C losses in the containers stored in the sunlight after three and six

TABLE XI

The Effect of Storage Temperature and Added Copper on l-ascorbic Acid Losses in Synthetic Jellies and Orange Marmalade (Sucrose)

Storage Time Months	Type of Cont.	32°F A.a. loss %	Sun (51°F) A.a. loss %	Sun (51°F) +Cu A.a. loss %	Room Temp. 72°F A.a. loss %	Room Temp. 72°F +Cu A.a. loss %	100°F A.a. loss %	100°F +Cu A.a. loss %
				Synthetic Jellies				
1	Glass	2.8	6.3	10.8	13.3	10.9	17.3	21.7
	Tin	1.7	-	-	13.3	-	23.8	28.3
3	Glass	2.9	12.0	23.1	15.6	17.4	32.5	46.7
	Tin	4.0	-	-	15.6	-	41.2	54.5
6	Glass	13.1	25.7	31.5	32.7	35.9	58.6	62.0
	Tin	16.2	-	-	35.0	-	89.1	87.0
				Orange Marmalade				
1	Glass	6.3	9.7	6.7	6.5	12.2	20.0	26.7
	Tin	9.7	-	-	8.7	-	27.7	26.7
3	Glass	6.9	16.2	15.6	9.7	16.7	49.5	50.0
	Tin	7.5	-	-	-	-	42.3	46.7
6	Glass	7.6	21.1	21.1	19.3	22.2	78.8	77.8
	Tin	8.6	-	-	19.3	-	84.5	83.3

FIG.15 The effect of storage temperature and sunlight on ascorbic acid losses in sucrose jellies



months, respectively. In the orange marmalade samples these losses were a little higher, i.e., losses higher by 3, 8 and 10.5 per cent units than expected by the curve, were obtained in the sunlight after 1, 3 and 6 months, respectively. (See Figure 16.) The additional losses must be attributed to the effect of the sunlight.

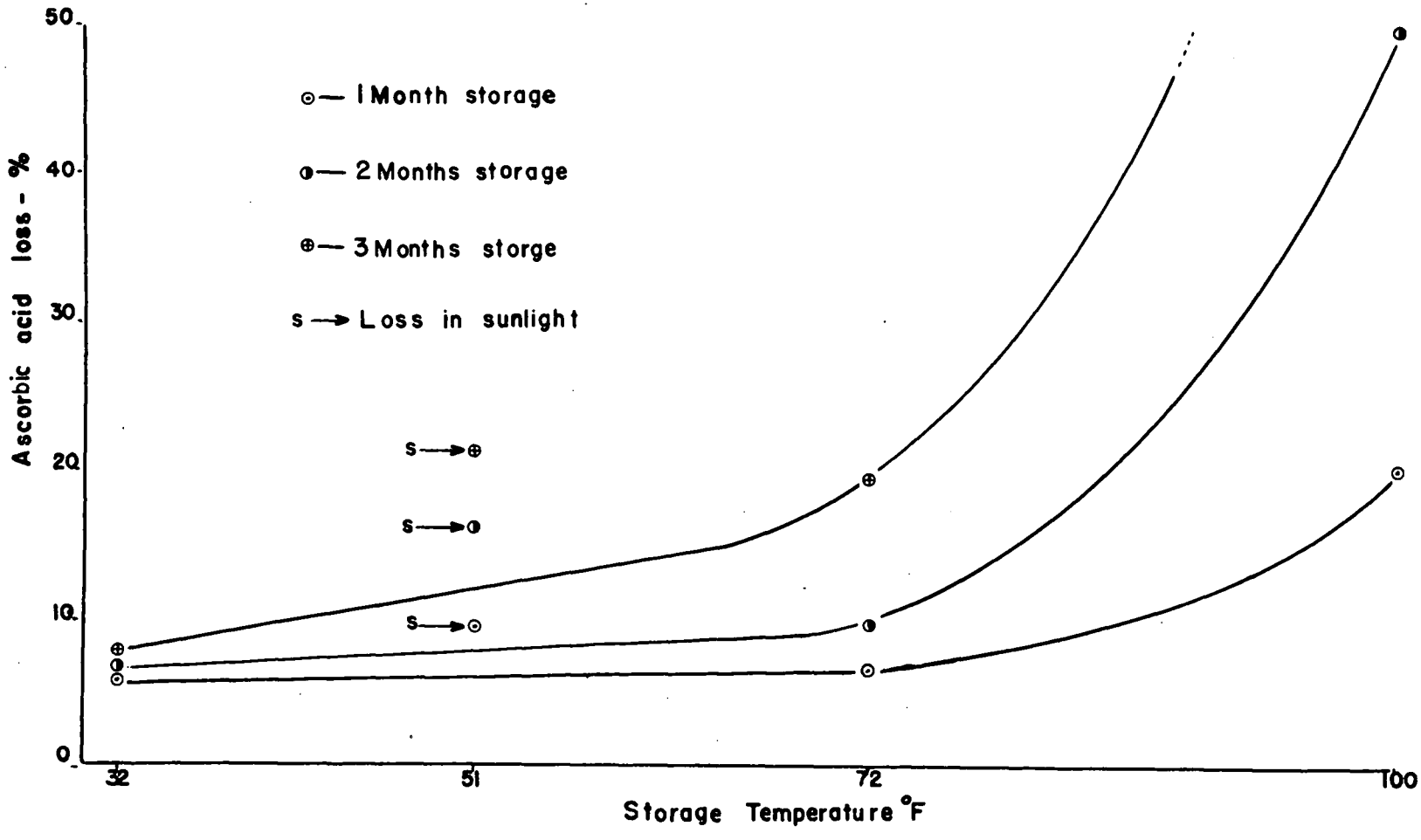
The relative influence of the different storage temperatures on l-ascorbic acid losses are summarized in Table XII.

TABLE XII

The Increased Losses of l-ascorbic Acid as caused by Different Storage Temperatures, in Synthetic Jellies (Sucrose) and Orange Marmalade (Sucrose) Stored in Glass and Tin Containers (After 6 Months).

Container	% Losses of Asc.Acid greater at Room Temp. than at 32°F.		% Losses of Asc.Acid greater at 100°F. than at Room Temp.		% Losses of Asc.Acid greater at 100°F. than at 32°F.	
	S.Jelly	O. Marm.	S.Jelly	O. Marm.	S.Jelly	O. Marm
Glass	19.6	11.7	25.9	59.5	45.5	71.2
Tin	18.3	10.6	54.1	65.3	72.4	75.9

FIG.16 The effect of storage temperature and sunlight on ascorbic acid losses in orange marmalade



Losses of total ascorbic acid as influenced by storage temperature follow the same trends as that of l-ascorbic acid. The experimental data are presented in Table XIII. At 32°F. the total ascorbic acid losses in synthetic jellies were found to be very small and the retention was practically 100 per cent over the study period of six months.

For the orange marmalade boilings the losses of total ascorbic acid at 32°F. is slightly more than for synthetic jellies. (See Table XIV.)

2. Color Changes. Sucrose jelly boilings stored at different temperatures show a distinct increase in the brown color development as the temperature increases and as the storage time progresses. (See Table XIV.)

At 32°F. hardly any color changes took place in both tin and glass containers. At room temperature a slight but distinct discoloration was observed, especially in the upper layers of the jellies.

The color of the jellies stored in the sunlight also showed very little changes and in some cases there was slight evidence of a bleaching effect.

Color changes at 100°F. as well as the influence of the tin container and the additional copper have been discussed previously.

TABLE XIII

The Effect of Storage Temperature and Added Copper on Total Ascorbic Acid Losses in Synthetic Jellies and Orange Marmalade (Sucrose).

Storage Time Months	Type of Cont.	32°F	Sun (51°F)		Room Temp. 72°F		100°F	
				+Cu		+Cu		+Cu
		A.a. loss %	A.a. loss %	A.a. loss %	A.a. loss %	A.a. loss %	A.a. loss %	A.a. loss %
				Synthetic Jellies				
1	Glass	2.6	-	10.6	1.2	6.1	13.6	15.7
	Tin	*	-	-	1.2	-	20.4	24.8
3	Glass	*	4.6	17.9	3.0	15.1	26.9	37.5
	Tin	*	-	-	4.8	-	31.8	43.1
6	Glass	*	16.0	21.6	9.2	21.6	47.2	75.9
	Tin	*	-	-	17.7	-	73.6	74.9
				Orange Marmalade				
1	Glass	0.9	1.8	7.6	5.1	7.8	18.1	25.1
	Tin	1.4	-	-	10.0	-	21.3	19.9
3	Glass	0.9	12.8	15.6	8.5	20.6	46.6	48.8
	Tin	1.8	-	-	-	-	40.6	43.7
6	Glass	8.0	18.1	21.8	15.4	21.8	64.6	61.4
	Tin	8.5	-	-	15.4	-	70.6	68.6

* Losses insignificant.

TABLE XIV

The Effect of Storage Temperature and Added Copper on Color Changes in Synthetic Jellies and Orange Marmalade (Sucrose)

Storage Time Months	Type of Cont.	32°F	Sun (51°F)		Room Temp. 72°F		100°F	
		Trs. %	Trs. %	+Cu Trs. %	Trs. %	+Cu Trs. %	Trs. %	+Cu Trs. %
Synthetic Jellies								
0	Glass	96.0	96.0	96.0	96.0	96.0	96.0	96.0
	Tin	96.0	-	-	96.0	-	96.0	96.0
1	Glass	95.0	94.5	97.0	95.5	96.0	95.5	94.0
	Tin	94.5	-	-	95.5	-	95.0	95.0
3	Glass	94.0	96.0	94.0	95.0	96.0	89.5	89.0
	Tin	96.0	-	-	93.0	-	86.0	87.0
6	Glass	94.0	98.0	94.0	92.5	92.5	82.0	79.5
	Tin	94.0	-	-	92.0	-	44.5	40.5
Orange Marmalade								
0	Glass	71.0	71.0	71.0	71.0	71.0	71.0	71.0
	Tin	71.0	-	-	71.0	71.0	71.0	71.0
1	Glass	-	-	67.0	-	65.5	52.0	54.5
	Tin	-	-	-	-	-	53.5	55.0
3	Glass	69.5	72.5	75.5	64.0	66.0	50.0	47.0
	Tin	70.0	-	-	66.5	-	51.0	51.0
6	Glass	71.0	72.0	71.0	65.0	62.0	32.0	31.0
	Tin	70.0	-	-	64.0	-	29.0	28.0

The color intensity at the different storage temperatures is illustrated in Figure 13. Container G stored in the sun is entirely clear, whereas containers A and D stored at room temperature and 32°F., respectively, are slightly darker but not readily distinguishable from each other. Container I at 100°F. shows the dark discoloration very clearly and the gradation of the color from the top to the bottom is also noticeable.

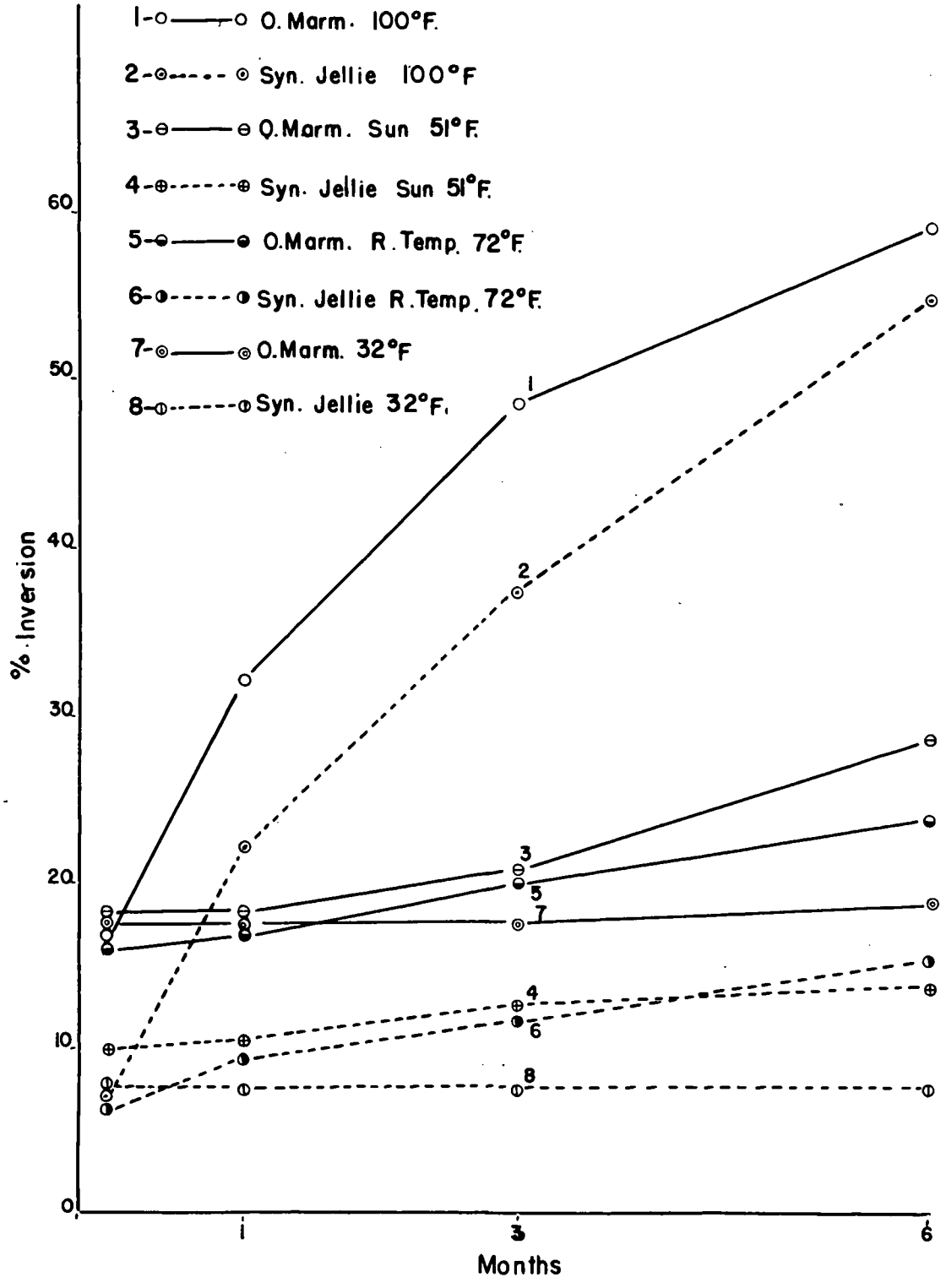
The orange marmalade stored at the different temperatures follow the same trends as that described for the synthetic jellies. Here again was slight evidence of a bleaching effect of sunlight. Compare containers 3 and 5 in Figure 6 for illustrations of this.

The dark color which develops at 100°F. is illustrated by container 7 in Figure 7.

3. Per cent Inversion. For both orange marmalade and synthetic jellies the per cent invert sugar increases rapidly at the higher storage temperature (100°F.) At 32°F. practically no additional inversion took place, whereas at room temperature and at the temperature in the south window the percentages increase very gradually and more or less at the same rate.

These temperature influences on the rate of inversion are graphically illustrated in Figure 17.

FIG. 17 The effect of storage temperature on the degree of inversion in synthetic jellies and orange marmalade.



At 100°F. more than 50 per cent of the sucrose was in the inverted form after six months storage. There is slight evidence as is shown from the data in Table XV that the per cent invert sugars formed was greater when copper was added. In several cases for orange marmalade it was also found that the per cent invert sugar was always a few per cent higher in boilings where copper was added. A possible explanation for this is that the copper catalyzes the oxidation of ascorbic acid to dehydro-ascorbic acid and from there to such compounds as threonic and oxalic acids. Borsook, et. al. (8) pointed out that acids originating as oxidation products of ascorbic acid are stronger acids than ascorbic acid itself and would thus cause greater inversion in a given time.

When the l-ascorbic acid losses of the synthetic jellies are compared with the losses in orange marmalade, it is found that at 100°F. the losses in orange marmalade were always higher than in the jellies. (See Table XI.) At room temperature, however, just the opposite was found. At 32°F. and in sunlight (51°F.) higher losses were recorded in the synthetic jellies only after a six months storage.

The higher per cent reducing sugars in the orange marmalade at 100°F. may possibly be tied up with the higher losses of l-ascorbic acid. To the contrary it is

TABLE XV

The Effect of Storage Temperature on the Per cent Inversion in Synthetic Jellies (Sucrose) and Orange Marmalade (Sucrose) in Glass Containers.

Storage Time Months	32°F.	Sun (51°F.)		Room Temp. 72°F.		100°F.	
	% Inv.	% Inv.	+Cu % Inv.	% Inv.	+Cu % Inv.	% Inv.	+Cu % Inv.
Synthetic Jellies							
0	7.8	9.8	7.8	6.8	-	6.8	7.6
1	7.3	10.2	15.5	9.2	-	22.2	23.3
3	7.5	12.7	17.2	11.7	-	37.4	40.0
6	7.6	13.7	19.2	15.4	-	55.0	58.0
Orange Marmalade							
0	18.0	18.0	-	17.2	17.2	16.0	17.2
1	17.4	17.8	-	17.3	18.2	32.2	32.8
3	17.6	20.7	-	20.6	20.6	48.7	50.0
6	18.7	28.6		23.8	24.6	59.2	60.0

found that at the lower temperature (72°F.) the per cent reducing sugars in the orange marmalade is higher than that of the synthetic jellies and still the loss of ascorbic acid in the marmalade is less than that in the jellies.

D. Effect of Amount of Oxygen in the Headspace

1. Losses of l-ascorbic and Total Ascorbic Acid. In all cases where the influence of vacuum were studied, it was found that in both the glass and the tin containers stored at room temperature and at 100°F., the losses of l-ascorbic acid and of total ascorbic acid at 10" vacuum always exceeds the losses at 25" vacuum.

The data in Table XVI, typical of the findings in the synthetic jelly and orange marmalade experiments, will serve to illustrate these statements.

Although the amount of headspace in both types of containers were approximately the same (22 ml. headspace in glass and 22.5 ml. for tin) the higher losses of ascorbic acid in the tin is still very evident.

TABLE XVI

The Effect of Vacuum on Losses of Ascorbic Acid and the Color Changes in Synthetic Jellies and Orange Marmalade (Sucrose) at 100°F.

Storage Time Months	2 p.p.m. Copper				No Copper Added			
	Glass		Tin		Glass		Tin	
	10" Vac.	25" Vac.	10" Vac.	25" Vac.	10" Vac.	25" Vac.	10" Vac.	25" Vac.
	Losses of l-ascorbic Acid In Synthetic Jellies							
1	21.8	20.9	28.3	14.1	17.3	17.3	23.8	12.9
3	46.5	34.8	54.5	39.2	32.5	24.9	41.1	26.0
6	62.0	52.2	86.9	86.6	58.6	52.1	89.1	86.0
					In Orange Marmalade			
1	-	-	-	-	20.0	16.6	27.7	22.2
3	-	-	-	-	49.5	34.5	42.3	34.5
6	-	-	-	-	78.8	67.4	84.5	80.0
	Losses of Total Ascorbic Acid In Synthetic Jellies							
1	15.7	7.9	24.8	8.4	13.6	16.2	20.0	14.8
3	37.5	20.7	43.1	26.1	26.9	19.5	31.8	22.6
6	-	46.1	74.9	71.8	47.2	40.0	73.6	41.1
					In Orange Marmalade			
1	-	-	-	-	18.1	13.5	21.3	18.1
3	-	-	-	-	46.6	33.5	40.6	45.1
6	-	-	-	-	64.6	58.4	70.6	69.6
	Color Changes In Synthetic Jellies							
0	96.0	96.0	96.0	96.0	96.0	96.0	96.0	96.0
1	94.0	95.0	95.0	94.0	95.5	94.5	95.0	95.0
3	89.0	91.0	87.0	91.0	89.5	90.0	86.0	91.0
6	78.0	85.0	40.5	48.5	82.0	88.0	44.5	50.0

The difference in ascorbic acid losses between samples at 10" vacuum and those at 25" vacuum were greater for orange marmalade in the glass than for synthetic jellies in the same containers. In the tin container just the opposite is found and the losses were higher for synthetic jellies than they were for marmalade.

2. Color Changes. The formation of the dark pigments in jelly starting at the surface of the container and gradually penetrating towards the bottom seems to be directly correlated with the vacuum in the container (amount of oxygen present) see Table XVI.

The color development as caused by the difference in vacuum in the orange marmalade samples is only very slight and not so conclusive as in the case of the synthetic jellies. Less analytical data are available for orange marmalade to give conclusive evidence as to the effect of vacuums on color development.

In the case of the jellies it is very clear that the higher the vacuum the less intense is the formation of the brown color. (Compare containers I and J and containers E and F in Figure 13.) These findings are in agreement with those of Moore, et. al. (73). They state that if the volume of air in bottled orange juice

increases, the rate of browning also increases.

At both vacuums studied, the tin container again showed a marked influence on the development of color. During the first three months of storage at 100°F. there was not much difference between the discoloration in the glass and in the tin, but between the third and the sixth month the browning in the tin containers was excessively heavy. To illustrate this, the color changes expressed as per cent transmission for synthetic jellies is graphically presented in Figure 18.

After observing the distinct darkening formed at the top of the containers (part in contact with the oxygen of the headspace), analysis of the top and bottom parts of the samples were made separately. Data in Tables XVII and XVIII.

FIG. 18 The influence of the amount of vacuum in glass and tin containers on the color changes in sucrose jellies at 100°F.

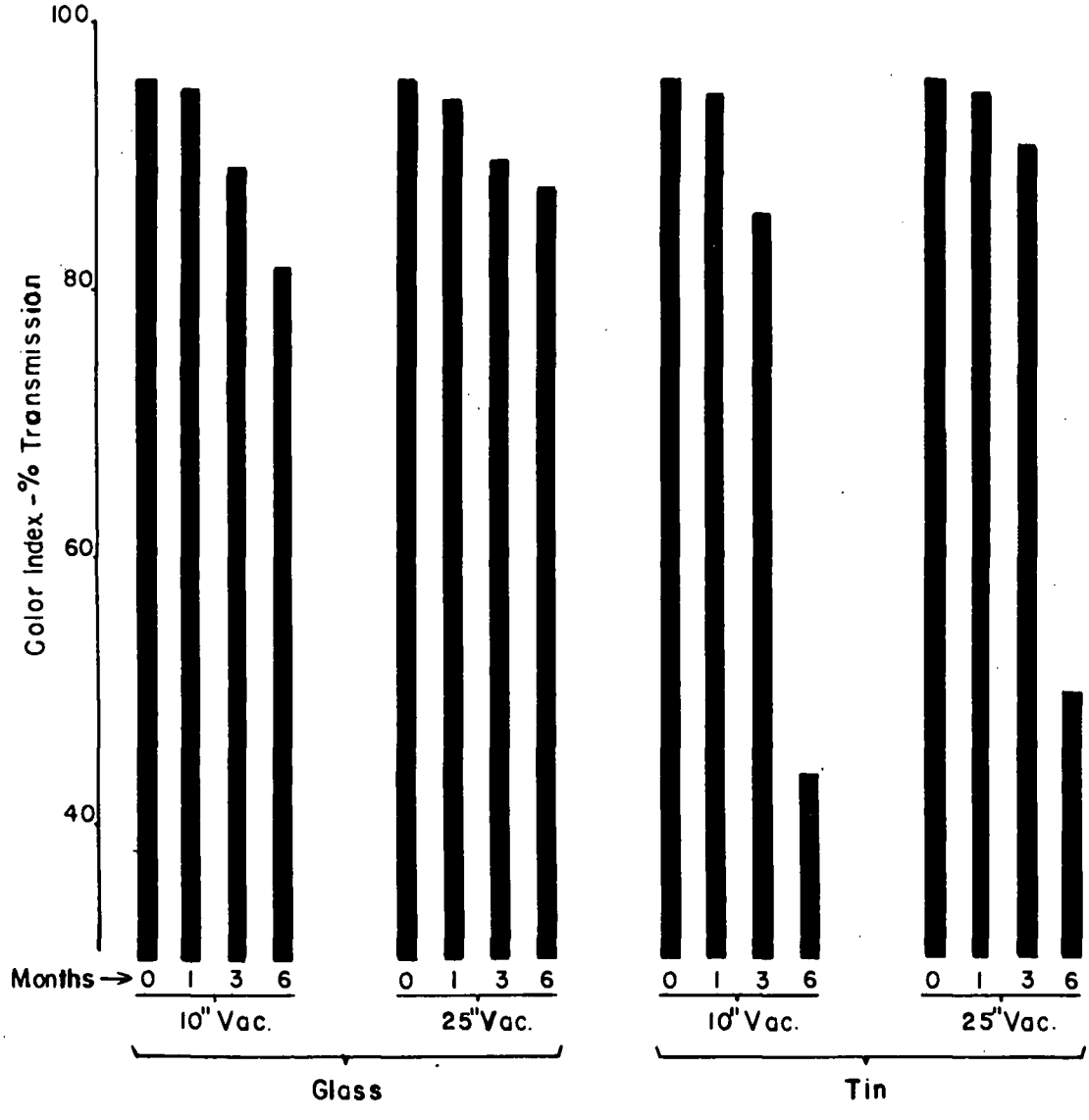


TABLE XVII

Ascorbic Acid Losses, Color Changes and Per cent Inversion in Different Parts of Synthetic Jellies in Glass and Tin Containers Stored at Different Temperatures (10" Vacuum).

GLASS					TIN			
Sample Description	Part of Container	% A.A. loss	% Trs.	% Inv.	Part of Container	% A.a. Loss	% Trs.	% Inv.
Control - Sucrose	Top	-	88.0	50.1	Sides	-	75.0	54.0
With no A.a. (100°F)	Bottom	-	90.0	48.6	Middle	-	81.0	53.6
Sucrose + 50 mg A.a.	Top	61.2	80.0	55.0	Sides	83.0	49.0	60.2
per 100 gm (100°F	Bottom	45.2	89.0	54.0	Middle	81.8	61.5	59.6
Sucrose + 50 mg A.a.	Top	29.0	91.0	-				
per 100 g.(Room Temp.)	Bottom	3.1	96.0	-				
Sucrose + 50 mg A.a.	Top	26.5	98.0	-				
per 100 g.(Sunlight)	Bottom	9.5	98.0	-				
Sucrose + 50 mg A.a.	Top	9.5	95.0	-				
per 100 g. (32°F)	Bottom	0.4	96.0	-				
L.C.Corn Sirup + 50mg	Top	60.0	84.0	45.0				
A.a. per 100g (100°F)	Bottom	38.0	92.5	44.6				

TABLE XVIII

Ascorbic Acid Losses and Color Changes in Different Parts of Orange Marmalade Samples Stored in Glass Containers at Different Temperatures (10" Vacuum).

Sample Description	Part of Container	Asc. Acid loss %	Color % Transmission
Sucrose Marmalade at 100°F.	Top	59.2	38.0
	Bottom	42.6	47.0
Sucrose Marmalade at Room Temp. (72°F.)	Top	17.2	64.0
	Bottom	9.7	68.0
Sucrose Marmalade in Sunlight (51°F.)	Top	21.8	70.5
	Bottom	10.6	74.5
Sucrose Marmalade at 32°F.	Top	10.6	68.5
	Bottom	6.9	72.0
Invert Sirup Marmalade at 100°F.	Top	80.5	22.0
	Bottom	60.1	27.0

These color developments also took place in the top parts of the samples stored at room temperature and at 32°F. but the intensity is much less.

In Tables XVII and XVIII the ascorbic acid losses in the different parts of the container are also given and a direct correlation between the color changes and ascorbic acid losses may be seen. An average of about 20 per cent units higher losses of ascorbic acid were found in the top half of the glass containers stored at 100°F.

Although the glass containers in the sun show a distinct loss of ascorbic acid in the top half (Tables XVII and XVIII), the per cent transmission is still higher than that of the same sample stored at room temperature. Thus is demonstrated the bleaching effect of the sunlight.

Analysis of synthetic jellies in the tin containers show an intense dark color development at the sides of the container where the jelly is in contact with the tin. This color development gradually migrates towards the center where the discoloration was much less.

Several investigators report the production of CO_2 as a result of ascorbic acid breakdown: Curl, et. al. (17), Hall (34) and Stadtman (102).

The evidence of CO_2 development in these synthetic jellies as could be detected by a decrease in vacuum measured by an ordinary vacuum gauge, were very slight. Even after six months storage where the ascorbic acid lost was over ninety per cent in some cases, only slight decreases in the vacuum could be detected.

In two cases where jellies containing 50 mg. ascorbic acid per 100 grams were stored at 130°F., gas bubbles could be seen entrapped in the jelly after six weeks storage. However some of the other containers stored under the same conditions did not show this phenomena.

The vacuum of the orange marmalade samples at 100°F. as measured by the vacuum gauge show a little more evidence of decreasing than could be observed with the synthetic jellies. This is also associated with the l-ascorbic acid losses which were in general, as pointed out previously, higher for orange marmalade, especially when stored in the glass containers.

From the above observations the importance of oxygen on both color formation and vitamin C losses is very evident. To this the effect of the tin container must be added.

The evidence that the per cent inversion (Table XVII) sugars had an influence on the color formation

and vitamin C losses is, due to insufficient analytical data and the very small percentage differences obtained in cases where it was analyzed, not conclusive.

E. The Influence of Added Ascorbic Acid on Color Changes and Per cent Inversion of Sucrose Jellies and Orange Marmalade

Color development in the control samples of sucrose jellies (100°F. storage) where no ascorbic acid was added show very little darkening in the glass containers but a definite discoloration sets in after three months storage in the tin containers.

When l-ascorbic acid, 50 mg./100 grams jelly, was added, a very distinct additional discoloration was noticeable in both types of containers, but more so in the tin containers. Figure 19 presents these data graphically.

The color data indicate that a certain amount of browning does take place in the ordinary sugar boilings when no ascorbic acid is present but when ascorbic acid is added the browning reaction is accelerated and additional brown color is formed.

By comparing container B (control no ascorbic acid) with container C (50mg. ascorbic acid) in Figure 20, the amount of additional browning due to the

FIG.19 The effect of ascorbic acid on color changes in sucrose jellies stored in glass and tin 100°F.

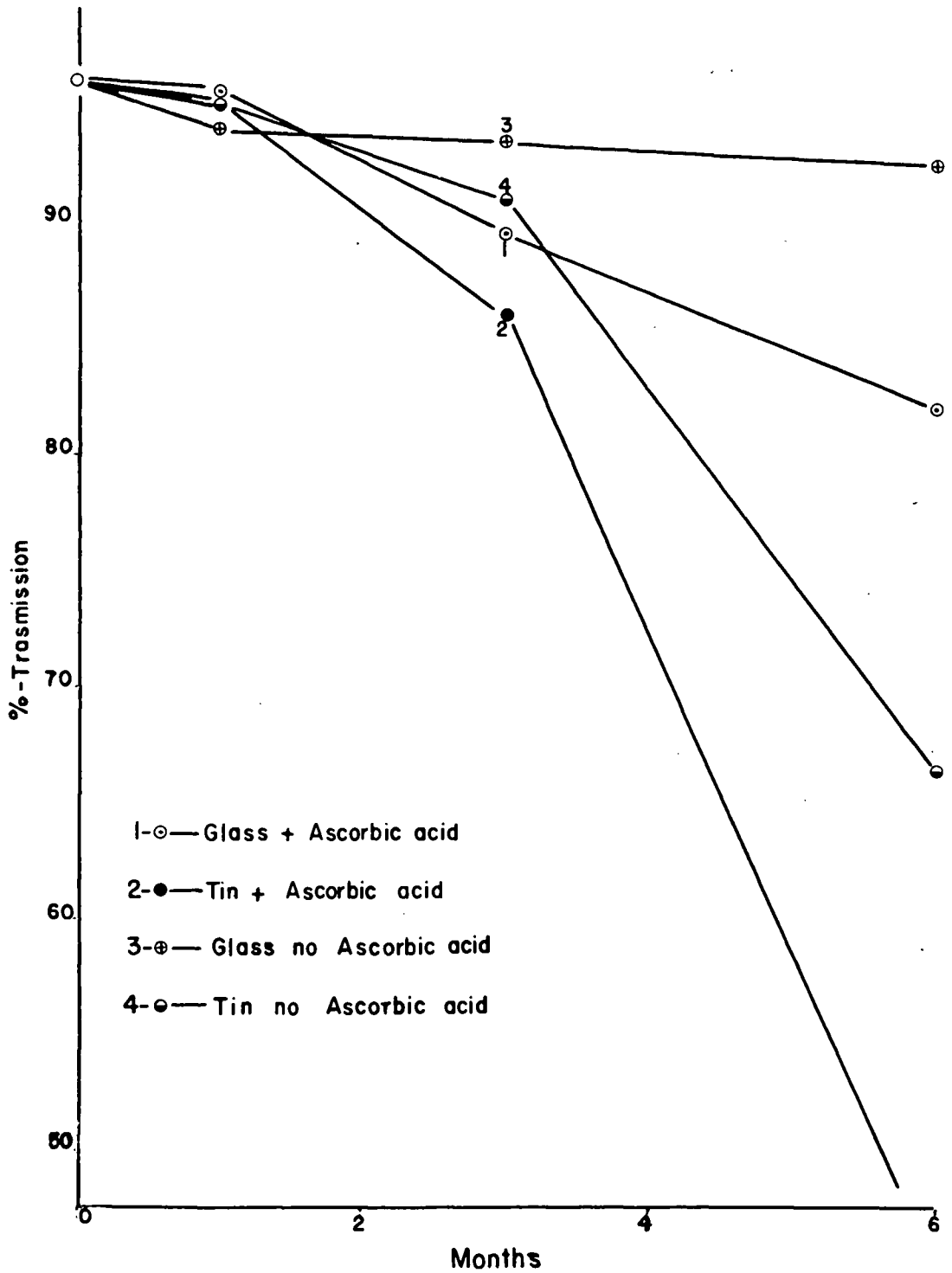
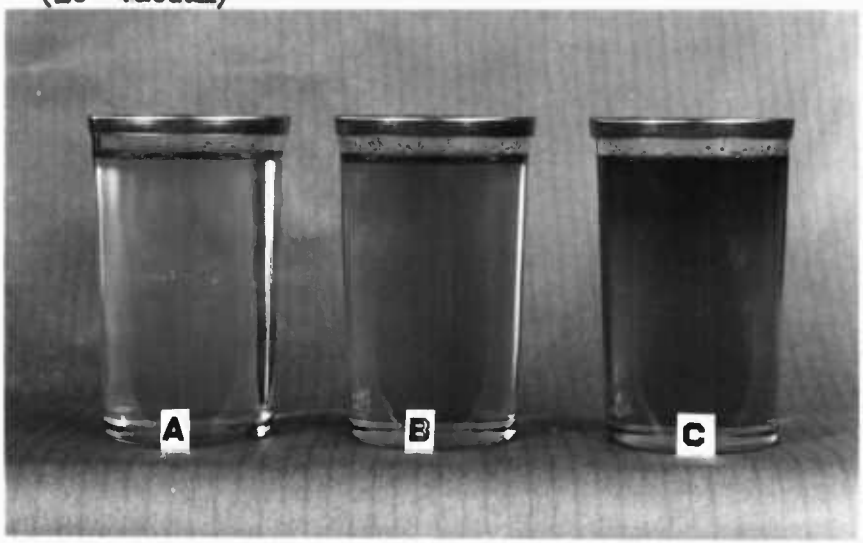


Fig. 20. Illustrations of Color Development in Sucrose Jellies as Influenced by the Addition of Ascorbic Acid (10" vacuum)



A. Sucrose, no ascorbic acid, Sunlight (51°F)

B. Sucrose, no ascorbic acid, 100°F

C. Sucrose, + 50mg. ascorbic acid per 100g., 100°F

ascorbic acid added is clearly seen. Container A stored in the sun is clear and does not show any discoloration.

Discoloration in the control samples (no ascorbic acid) stored in glass was much less than in the tin containers. If the per cent reducing sugars of these same samples is compared it is found (Table XVII) that the tin containers had 5.5 per cent units (average of top and bottom analysis) more reducing sugars than the glass.

Referring to the further data in Table XVII, it will be seen that the samples to which the ascorbic acid was added showed 5.2 per cent units higher reducing sugars for samples in glass and 6.1 per cent units higher reducing sugars for samples in tin, than the control samples which contain no vitamin C.

The more intense discoloration of samples containing the ascorbic acid may also be due to the higher per cent invert sugars which they contain (as previously explained) plus the degradation products of ascorbic acid which very likely may also be tied up in these color complexes.

The theories of Moore, et. al. (72) and Moore, et. al. (73) are that the oxidation products of ascorbic acid are involved in the browning reaction.

Control samples to which no l-ascorbic acid was added were also included in the orange marmalade experiments.

Analysis made directly after the marmalades were boiled show them to contain 8 mg. natural l-ascorbic acid per 100 grams of marmalade. The initial analysis of the samples to which synthetic ascorbic acid was added show it to contain ca. 51 mg. per 100 grams.

Lincoln and McCay (59) report the ascorbic acid content of commercial orange marmalade to be between 4 and 10 mg. per 100 grams.

As in the case of synthetic jellies it is also very evident from the analytical data of orange marmalade (Figure 21) that the added ascorbic acid increased darkening to a marked degree. From the curves in Figure 21 the influence of the tin container in accelerating the formation of the brown color and the bleaching effect exerted by the sunlight is clearly illustrated.

Color photos of the control marmalade boilings (containing no synthetic ascorbic acid) are shown in Figure 22. The influence of the higher storage temperature in accelerating the brown color development, (container 3) and the bleached appearance of container 1 stored in the sunlight, as compared to container 2 at

room temperature, is shown very clearly.

Reducing sugar determinations made on the orange marmalade were not enough to correlate the data with the color changes of the same samples.

FIG.21 The effect of added ascorbic acid on the color changes in orange marmalade

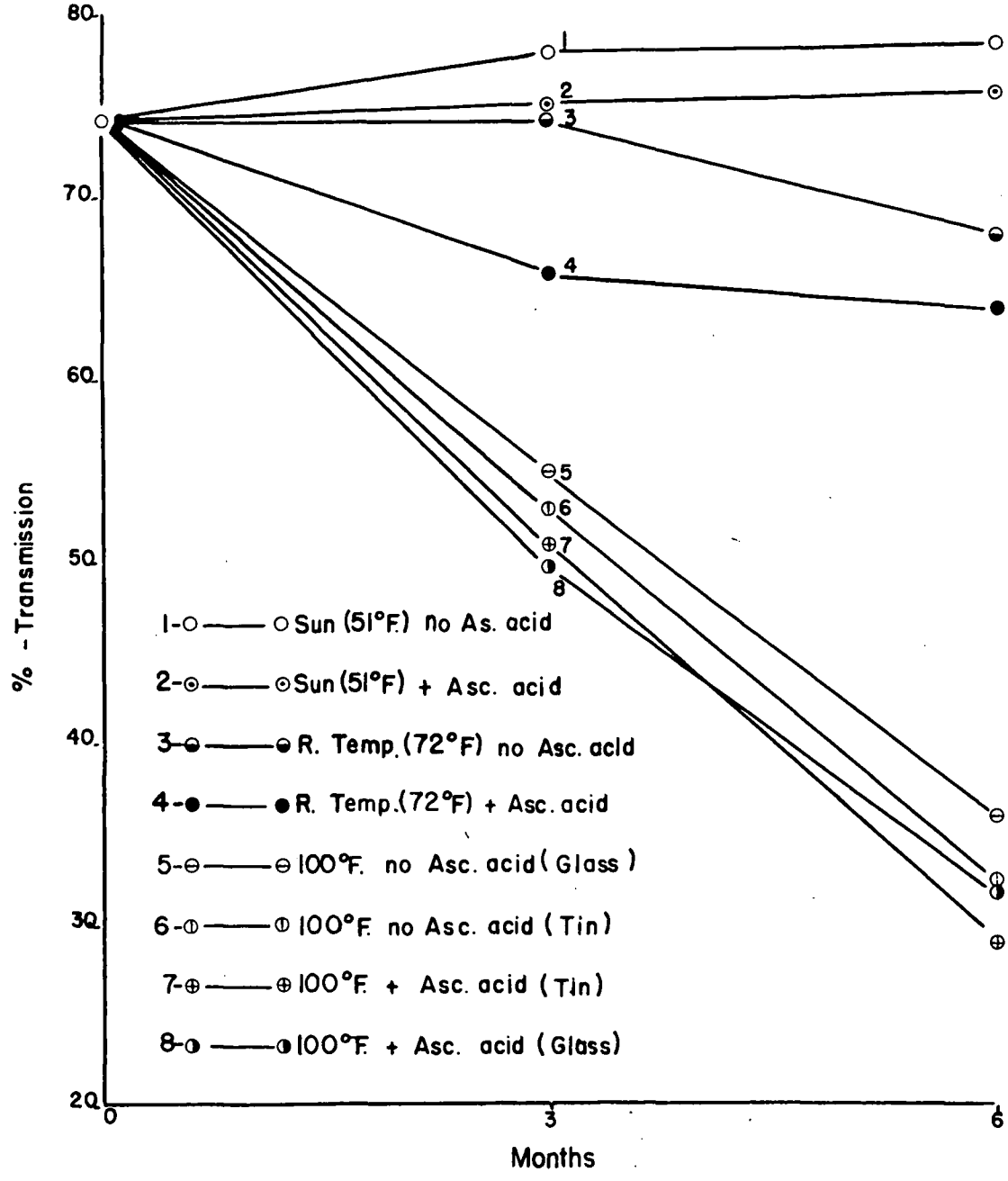


Fig. 22. Color Developments in Orange Marmalade due to Storage Temperatures and Sunlight.

Left to Right

1. Sucrose - no ascorbic acid added - in Sunlight ($51^{o}F$)
2. Sucrose - no ascorbic acid added - Room Temp. ($72^{o}F$)
3. Sucrose - no ascorbic acid added - $100^{o}F$

F. The Effect of Antioxidants on Ascorbic Acid Oxidation

1. The Influence of Sodium Chloride. Several investigators like Mapson (56), Mystovski (76), Armentano (2) and Vonesch and Remezzano (112) found that the presence of sodium chloride reduces the catalytic action of the copper ion in the oxidation of vitamin C. The greater the concentration of copper present the greater was the concentration of halide found necessary to obtain the same protection.

As a result of these findings, 2 per cent sodium chloride was added to a series of sucrose jellies to investigate its influence on the vitamin C retention.

The experimental data for synthetic jellies is presented in Table XIX. The results for jellies in the glass containers show a definite better retention of the vitamin where the sodium chloride was added. A still better retention was obtained in the samples where the copper concentrations were low (where no copper was added). This is in agreement with the findings of Armentano (2) and Mapson (56). These retentions were not found for the synthetic jellies in the tin containers. To the contrary, there is some evidence that the addition of NaCl in the tin containers accelerates l-ascorbic acid losses rather than reduces them. This may indicate

TABLE XIX

THE EFFECT OF SODIUM CHLORIDE (2%) ON ASCORBIC ACID LOSSES, COLOR CHANGES AND PER CENT INVERSION OF SUCROSE JELLIES AT 100°F

Storage Time Months	No Copper Added				Copper Added to 2 p.p.m.			
		Glass Plus NaCl		Tin Plus NaCl		Glass Plus NaCl		Tin Plus NaCl
l-Ascorbic Acid Loss - Per cent								
1	17.3	13.3	23.8	22.2	21.7	19.1	28.3	19.2
3	32.5	24.3	41.1	51.1	46.8	26.6	54.5	47.8
6	58.7	49.7	89.1	92.2	55.4	51.1	86.9	92.5
Color - Per cent Transmission								
0	96.0	96.0	96.0	96.0	96.0	96.0	96.0	96.0
1	93.5	94.0	95.0	92.0	94.0	95.0	95.0	96.0
3	89.5	92.0	86.0	80.0	89.0	95.0	87.0	86.0
6	82.0	82.0	44.5	27.5	75.5	84.0	40.5	30.0
Invert Sugars - Per cent								
0	6.8	28.0	7.6	28.0	-	-	-	-
1	22.2	51.0	25.8	54.0	-	-	-	-
3	37.4	58.5	45.0	61.0	-	-	-	-
6	55.0	64.2	58.0	64.2	-	-	-	-

that the influence of the tin containers in accelerating the oxidation losses is greater than the protective action which may be exerted by the 2 per cent NaCl.

Comparing the color changes and ascorbic acid losses of the jellies in the glass containers (Table XIX), the direct correlation between the vitamin C losses and color formation is again very evident. The higher the losses of ascorbic acid the more intense is the discoloration.

In the tin containers the added sodium chloride was responsible for intense discoloration which, as already said previously, is paralleled with much higher l-ascorbic acid losses.

From the results of l-ascorbic acid losses and color changes in the orange marmalade samples as presented in Table XX, the antioxidative effect of NaCl is not clear and as positive as it was for the synthetic jelly in the glass containers. Orange marmalade being a more complex system may introduce more complex reactions which might interfere with the antioxidative action of sodium chloride as experienced in the synthetic jellies which, comparatively speaking, is a more simple system.

At the 100°F storage temperature the sodium chloride added to sucrose jellies in the glass containers was responsible for a 8.2 and 9.0 per cent

TABLE XX

THE EFFECT OF SODIUM CHLORIDE (2%) ON ASCORBIC ACID LOSSES, COLOR CHANGES AND PER CENT INVERSION IN SUCROSE MARMALADE AT 100°F

Storage Time Months	No Copper Added				Copper Added to 2 p.p.m.			
		Glass	Glass Plus NaCl	Tin	Tin Plus NaCl		Glass	Glass Plus NaCl	Tin	Tin Plus NaCl
l-Ascorbic Acid Loss - Per cent										
1	20.0	25.1	27.7	24.0		26.7	22.2	26.7	22.2	
3	49.5	50.0	42.3	54.4		50.0	51.2	46.7	51.2	
6	78.8	68.5	84.5	66.5		77.8	68.9	83.3	75.3	
Color - Per cent Transmission										
0	71.0	71.0	71.0	71.0		71.0	71.0	71.0	71.0	
1	-	57.0	-	55.5		-	55.5	-	55.0	
3	50.0	39.0	51.0	37.0		47.0	40.0	51.0	41.0	
6	29.0	28.0	29.0	32.0		31.0	26.0	28.0	25.5	
Invert Sugars - Per cent										
0	16.0	26.7	16.0	26.7						
1	32.0	45.0	30.7	48.4						
3	48.7	60.2	47.8	59.4						
6	59.2	63.0	58.0	63.0						

units better retention of ascorbic acid after 3 and 6 months storage, respectively. (Table XIX). In the sunlight (51°F), however, just the reverse was found. The samples to which sodium chloride was added lost in the sun 12.5 and 21 per cent units more vitamin C after 3 and 6 months storage, respectively. (Table XXI). Therefore it seems that sodium chloride under the influence of sunlight is much more destructive to vitamin C than sunlight alone.

TABLE XXI

THE EFFECT OF SODIUM CHLORIDE (2%) ON ASCORBIC ACID LOSSES, COLOR CHANGES AND PER CENT INVERSION OF SUCROSE JELLIES IN GLASS CONTAINERS STORED IN SUNLIGHT (51°F)

Storage Time Months	l-ascorbic a. loss %		Color % Transmission		% Inversion	
		plus NaCl		plus NaCl		plus NaCl
0			96.0	96.0	9.8	28.0
1	6.3	17.8	94.5	95.0	10.2	36.9
3	12.0	24.5	96.0	95.0	12.8	37.8
6	25.7	46.7	98.0	93.5	13.7	47.4

The color of the jelly samples containing the sodium chloride in the sunlight turn slightly darker and is therefore correlated with the heavy vitamin C losses. However, when the color figures of the two containers, one at 100°F (Table XIX) and one in the sunlight (Table XXI) showing about the same ascorbic acid losses, are compared, it is found that the intensity of the color developed in the sun is much less than at 100°F. This may also serve as a proof of the bleaching effect of the sun on this particular color complex.

The percentage invert sugars of the synthetic jelly boilings to which NaCl was added was about 20 per cent units higher at zero time the next day after preparation. During the storage in the sun the per cent reducing sugars in samples containing the NaCl increased with 9.8 and 19.4 per cent units after 3 and 6 months, respectively. In comparison with this, only 3 and 4 per cent units more invert sugars were formed in the control samples where no sodium chloride was added.

The higher degree of invert sugar formation may also here, as explained previously, be a factor responsible for the higher losses of ascorbic acid and the increase in the color development.

At 100°F, however, the better retention of ascorbic acid in the glass containers with the added

sodium chloride takes place in spite of higher reducing sugars content. The reduction of the catalytic effect of copper by sodium chloride as suggested by Mapson (56) and others may serve as an explanation for the function of sodium chloride under these conditions.

2. d-Iso Ascorbic Acid. d-Iso ascorbic acid differs from l-ascorbic acid only in the arrangement of the -oH group on the asymmetric 5th carbon of the ascorbic acid molecule. d-Iso ascorbic acid as applied to food products is used mostly for its "antioxidation" properties and not for its antiscorbutic value. The antiscorbutic value of d-iso ascorbic acid is, according to Yourga, et. al. (122), only 1/20th that of l-ascorbic acid.

The so-called "antioxidation" properties of d-iso ascorbic acid are explained to be due to the fact that d-iso ascorbic acid is preferentially oxidized and thus will leave the l-ascorbic acid in the reduced form.

In this investigation d-iso ascorbic acid was applied to sucrose jellies in four different levels of 10, 20, 30 and 50 mg. per 100 grams of jelly. The l-ascorbic acid was kept constant at 50 mg. per 100 grams. The different boilings were filled in glass containers only, sealed with 10" of vacuum and stored at 100°F and in the sunlight (51°F).

From the experimental data in Table XXII it will be seen that 10 mg. d-iso ascorbic acid per 100 g. added to jellies containing 50 mg. l-ascorbic acid/100 g. did not prevent the oxidation of the vitamin in the samples at 100°F for even one month. At the latter temperature 20 mg. d-iso ascorbic acid however prevented vitamin C losses for one month, whereas 30 and 50 mg. protect it for at least three months.

In the sunlight (51°F), 10 mg. d-iso ascorbic acid prevented ascorbic acid oxidation for three months, whereas 20 mg. and the larger amounts tried showed an antioxidative effect even after six months storage, so that all the original content of vitamin C remained in the jelly.

Therefore at ordinary room storage temperatures a quantity of 20 to 30 mg. d-iso ascorbic acid per 100 g. of jelly seems an advisable amount to use as an antioxidant for ascorbic acid if the product is put up in glass containers.

From the figures indicating color changes (Table XXII) it would appear that all the samples darkened at nearly the same rate. No appreciable differences were noticeable between the d-iso ascorbic acid containing samples and the controls. The same is true for the sunlight stored samples.

TABLE XXII

THE EFFECT OF d-Iso ASCORBIC ACID ON l-ASCORBIC ACID LOSSES AND COLOR CHANGES OF SUCROSE JELLIES IN GLASS CONTAINERS

d-Iso Ascorbic Acid (mg.)	Losses of l-asc.acid mg./100 gr. at 100°F.					Color Changes - % Transmission at 100°F.				
	0	10	20	30	50	0	10	20	30	50
Months										
0*	0.9	-	0.0	0.0	0.0	96	96	96	96	96
1	6.6	8.7	0.0	0.0	0.0	92	95	92	94	95
3	16.3	20.0	6.0	0.0	0.0	88	90	88	89	88
6	28.3	28.0	21.2	26.0	23.4	83	83	86	79.5	82
	Losses of l-asc.acid mg./100 gr. in Sunlight (51°F.)					Color Changes - % Transmission in Sunlight (51°F.)				
0	0.9	0.0	0.0	0.0	0.0	96	96	96	96	96
1	2.0	-	0.0	0.0	0.0	95	95	95	96	97
3	6.0	0.0	0.0	0.0	0.0	94	95	94	94	95
6	12.9	8.0	0.0	0.0	0.0	94	96	94	93	93

* Zero time equals 24 hours.

G. The Development of Reductones and Reductic Acids

The Robinson and Stotz (90) formaldehyde method for differentiating between vitamin C and reductones was carried out on all synthetic jelly and orange marmalade samples.

The amounts of interfering substances that could be detected by this method in synthetic jellies was never higher than 2 mg. per 100 grams of jelly. This figure, being rather constant for all the analyses of the samples stored at the different temperatures, leads to the conclusion that this amount may be an experimental error rather than indicating actual reductones formed.

The analysis of the control samples to which no vitamin C was added did not differ from the other samples containing 50 mg. ascorbic acid per 100 grams. None of the treatments and storage temperatures (not even 135°F) used showed any significant development of reductones.

No appreciable amounts of reductones could be detected in orange marmalade either. No evidence to support the statement of Miller (69) that the extent of reductone development is inversely proportional, roughly to the amount of vitamin C initially present could be found.

In different samples of orange marmalades con-

taining initially 50 mg. and 8 mg. ascorbic acid per 100 grams the same insignificant quantity of reductones was shown.

The highest amount of reductones that were detected was found to be present in the orange marmalade sample boiled with invert sirup to which copper was added. After 6 months storage at 100°F, 5.7 mg. per 100 g. of an interfering substance was found present in both glass and tin containers. This amount appeared to be wholly reductones since the control sample containing no formaldehyde and the formaldehyde treated sample showed the same analysis.

As in the case of synthetic jellies the amount of interfering substances very seldom exceeds 2 mg. per 100 grams. Again here this was a general figure for most samples analysed so that hardly any significance can be attached to it.

H. Formation of Dehydro Ascorbic Acid

Direct dehydro ascorbic acid determinations were made in all the different synthetic jelly and orange marmalade samples using the dinitrophenylhydrazine method.

Typical analytical data are presented in Table XXIII.

The actual amounts of dehydroascorbic acid present in both the jellies and the marmalade were found to be very small and only slight changes occur during the entire storage period of 6 months. A slight increase in dehydro-ascorbic acid in some cases may, as the storage time progresses, be observed but the amounts were so small that hardly any significance can be attached to these figures.

Hamburger and Joslyn (35) working with orange juice and Lincoln and McCay (59) in experiments with marmalades also find dehydro-ascorbic acid values to remain rather constant.

There is some doubt as to the specificity of this phenylhydrazine method for dehydro-ascorbic acid determinations since diketogulonic acid or other reductones are probably included. Hawk, Oser and Summerson (37) state that diketogulonic acid biologically inactive oxidation product of vitamin C reacts like the vitamin with phenylhidrazine. Joslyn (50) also points

out that as yet no satisfactory method for dehydro-ascorbic acid as it occurs in food products is available.

Dehydro-ascorbic acid in these jellies and marmalades is present in very small amounts and for this reason the original samples could not be diluted very much. The result was that the samples used for analysis contain high percentages of sugars which probably interfere with the reaction of the phenylhydrazine reagent and dehydro-ascorbic acid.

TABLE XXIII

DEHYDRO-ASCORBIC ACID CONTENT OF SYNTHETIC JELLIES AND ORANGE MARMALADE IN GLASS AND TIN CONTAINERS STORED AT DIFFERENT TEMPERATURES - (mg. per 100 grams.)

Synthetic Jellies

Storage Months	Sucrose					Invert Sirup	
	32°F	Sun (51°F)	Room Temp. (72°F)	100°F	100°F	100°F	100°F
	Glass	Glass	Glass	Glass	Tin	Glass	Tin
0	2.25	-	2.5	-	-	2.1	2.1
1	2.0	3.5	3.6	1.7	1.5	2.4	1.9
3	2.9	3.8	3.4	1.5	1.9	2.5	2.7
6	3.0	3.0	3.0	1.9	3.5	2.4	2.8

Orange Marmalade

0	1.1	1.1	1.4	1.4	1.4	1.6	1.6
1	1.5	1.6	2.0	1.0	1.3	2.1	2.1
3	2.8	2.7	2.1	2.5	3.0	2.3	2.2
6	2.2	1.4	2.5	3.0	3.1	2.6	2.9

I. Studies on the Browning Reaction

With an attempt to throw more light on the various factors involved in the browning reaction of synthetic jellies, a new series of jellies were made and stored at 135°F. Sucrose was used as the sugar and after the batch was boiled down to a specified weight it was poured in glass and tin containers and sealed at 10" vacuum after proper cooling.

1. Influencing Factors

(a) The hydrogen ion concentration (citric acid). By using citric acid and distilled water, synthetic jellies were boiled with three different pH levels. Analytical data in Table XXIV.

The trends of the brown color development as influenced by pH are hard to explain. This may be due to the rapid reaction rate at 135°F and also to the insufficient number of samples available.

A comparison of data in columns 4 and 5 where ascorbic acid was present (Table XXIV) shows very clearly that the citric acid (pH 3.3) exerts a slight but consistent protective effect, and less browning was formed in comparison with the distilled water samples. This phenomena was observed in both the glass and tin containers.

Vitamin C losses of these boilings are also strikingly lower in the samples containing the citric acid. Again the correlation between l-ascorbic acid losses and color development is very evident.

In a publication by Pfizer and Co. (124) it is claimed that citric acid decreases the atmospheric oxidation of ascorbic acid under the more acid conditions. They also claim that citric acid forms complexes with traces of iron and copper and in this manner retards the destructive effect of these metals on ascorbic acid.

TABLE XXIV

COLOR CHANGES AND l-ASCORBIC ACID LOSSES IN SUCROSE SYNTHETIC JELLIES STORED AT 135°F*

Column	1		2		3		4		5		6		7		8	
	pH 3.6		pH 3.3		pH 2.8		pH 3.5		pH 3.3		pH 3.2		pH 3.3		pH 3.3	
Months	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
							Color Changes - Per cent Transmission									
0	95.5	95.5	93.0	93.0	96.0	96.0	92.5	92.5	94.0	94.0	93.0	93.0	99.0	99.0	96.0	96.0
1	86.0	61.0	86.0	46.0	85.0	44.5	75.0	40.0	79.0	44.5	72.0	31.0	83.0	26.0	85.0	96.0
2	59.0	4.5 (40.0)**	67.0	6.5 (48.0)	57.0	7.0 (52.0)	36.0	4.0 (39.5)	44.0	5.5 (47.0)	50.5	8.0 (55.0)	56.5	3.5 (37.0)	79.0	89.0
							l-Ascorbic Acid Losses - per cent									
1	-	-	-	-	-	-	63.6	87.4	58.6	75.7	61.3	81.4	29.6	56.0	69.4	14.0
2	-	-	-	-	-	-	92.8	91.4	82.1	89.2	84.0	90.7	59.9	78.5	73.9	29.3

Identification of Columns

1 - Distilled water no ascorbic acid
2 - Citric acid no ascorbic acid
3 - Citric acid no ascorbic acid
4 - Distilled water plus ascorbic acid
5 - Citric acid plus ascorbic acid
6 - Citric acid plus ascorbic acid plus copper 10 p.p.m.
7 - Citric acid plus ascorbic acid no pectin
8 - Citric acid plus ascorbic acid no sucrose

* Sealed at 10" vacuum
**Per cent Transmission with 530 m.micron wave length filter

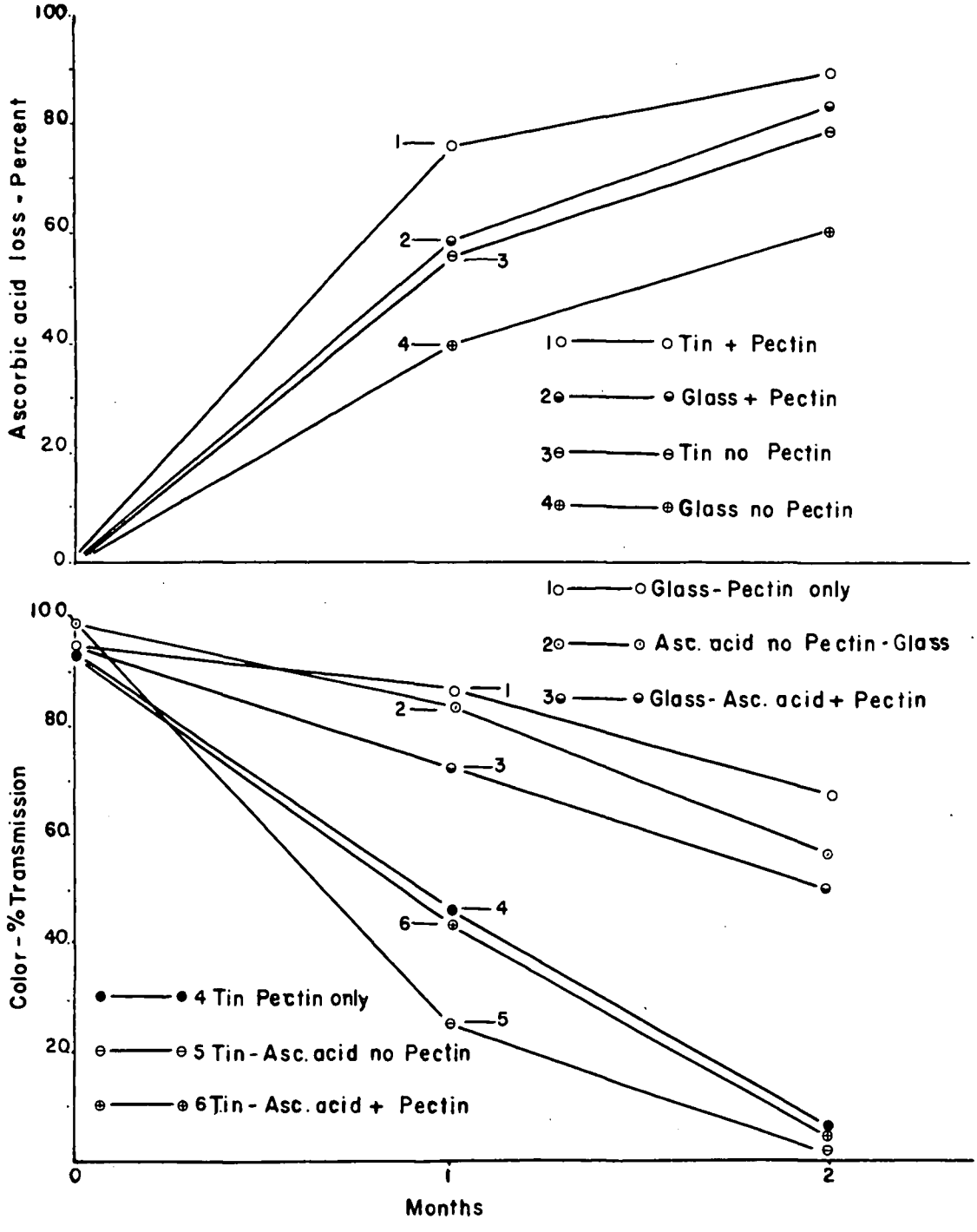
(b) Ascorbic acid. Synthetic l-ascorbic acid added to the sucrose jellies (50 mg. per 100 grams) was found to be responsible for the development of a much more intense brown color. (Compare data in columns 4 and 5 with columns 1 and 2 in Table XXIV).

(c) Copper. To sucrose jellies boiled with 50 mg. ascorbic acid per 100 grams, copper was added to bring the final level up to 10 p.p.m.

When jelly samples containing ascorbic acid but no added copper (column 5) is compared with samples containing 10 p.p.m. copper (column 6) it is found that after 1 month storage at 135°F the added copper plus the ascorbic acid was responsible for a much more intense discoloration than the ascorbic acid alone. These observations were made in both the tin and the glass containers. Analyses after two months storage however show that the discoloration in samples to which the copper was added was less than the ones containing ascorbic acid only.

(d) Pectin. Comparing the sucrose jelly samples in glass containers containing pectin (column 5 in Table XXIV and curve 3 in Figure 23) with the samples containing no pectin (column 7 in Table XXIV and curve 2 in Figure 23) it is found that the pectin containing jellies developed more color. Copper analysis of the

FIG. 23 The effect of pectin on color changes and ascorbic acid losses in sucrose jellies stored at 135°F



pectin show it to contain 25 p.p.m. of copper. This may show the influence of the copper on ascorbic acid losses, the oxidation products of which are involved in the excessive dark color development.

In the tin containers exactly the opposite was found true. The samples containing ascorbic acid but no pectin (column 7, Table XXIV and curve 5 in Figure 23) showed severe browning (much more than the pectin plus ascorbic acid samples -- curve 6, Figure 23), which may be due to the solid pectin-sugargel, causing only a relatively small portion of the jelly to be actually in contact with the tin walls. In the samples boiled without pectin no gel is formed and more of the viscous sugar liquid may, by means of convection currents or other means come in contact with the tin container which causes a discoloration not produced in glass.

In both tin and glass containers the vitamin C retention was much better where no pectin was added (column 7, Table XXIV, and curves 3 and 4 in Figure 23). The high copper content of the pectin may account for the rapid oxidation of ascorbic acid in this case.

In general throughout these studies it was found that high ascorbic acid losses are usually accompanied by the dark color development. In the case of the synthetic jellies in tin containers boiled without

pectin however, this was not experienced. In spite of the fact that less vitamin C was lost in the tin containers where no pectin was present, still the color formation was more intense than where the larger amount of ascorbic acid was oxidized (tin containers plus pectin). This might show that in the tin container the oxidation products of ascorbic acid only play a minor part in color development and the main reaction responsible for the color compound formation is between the sugars and the tin container itself.

(e) Invert sugar. The high storage temperature (135°F) was responsible for a very rapid inversion of the sucrose so that it was hard to make correlations between the degree of inversion and color formation in these jellies.

Data of the per cent reducing sugar formation in the jellies are given in Table XXV from which the following observations can be made:

1. Added ascorbic acid (50 mg. per 100 grams) causes a slight decrease in pH and this simultaneously increases the per cent invert sugars (columns 1 and 4).
2. Where copper was added to the jellies containing the ascorbic acid a slight decrease of pH (column 6) again was observed which simultaneously increases the per cent invert sugar. (More rapid oxidation of ascorbic acid to form acids which are more readily ionized.)
3. Pectin seems to prevent inversion slightly (columns 5 and 7).

TABLE XXV

PER CENT INVERT SUGARS IN SUCROSE JELLIES STORED AT 135°F*

Column	1 **		2 **		3 **		4 **		5 **		6 **		7 **	
	pH 3.56		pH 3.3		pH 2.8		pH 3.49		pH 3.26		pH 3.2		pH 3.3	
Months	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
0	0.0	0.0	6.3	6.3	58.8	58.8	3.0	3.0	7.1	7.1	8.5	8.5	18.6	18.6
1	52.0	54.6	62.8	63.1	63.2	63.6	60.2	60.2	62.6	65.6	63.8	65.8	65.0	65.0
2	63.4	63.6	63.1	65.6	65.0	65.0	66.0	66.0	65.6	65.6	65.8	65.8	66.0	65.8

Identification of Columns

1 - Distilled water no ascorbic acid
2 - Citric acid no ascorbic acid
3 - Citric acid no ascorbic acid
4 - Distilled water plus ascorbic acid
5 - Citric acid plus ascorbic acid
6 - Citric acid plus ascorbic acid plus 10 p.p.m. copper
7 - Citric acid plus ascorbic acid no pectin

* Sealed at 10" vacuums
**Mean pH of 5 determinations

4. Inversion in the tin containers was always a little higher than the inversion of the sugars in glass containers. Evidence of a slightly higher pH in the tin than in the glass was also observed. Data of pH of jellies in glass and tin containers however are not enough to be conclusive.

With an invert sirup prepared in the laboratory, jellies were boiled containing 56 per cent total soluble solids as compared with total soluble solids of 67 per cent of the sucrose jellies. When losses of ascorbic acid in these boilings are compared it is found that the samples containing the highest percentage of reducing sugar also show the highest losses in vitamin C. See Table XXVI.

TABLE XXVI

THE INFLUENCE OF REDUCING SUGARS ON ASCORBIC ACID LOSSES IN SYNTHETIC JELLIES AT 135°F

Months Storage	Sucrose Jellies				Invert Sirup Jellies			
	Reducing Sugar %		A-Acid Loss %		Reducing Sugar %		A-Acid Loss %	
	Glass	Tin	Glass	Tin	Glass	Tin	Glass	Tin
0	7.1	7.1			48.0	48.0		
1	62.6	65.6	58.6	75.7	53.0	53.6	45.7	71.1
2	65.6	65.6	82.1	89.2	55.2	55.2	65.7	79.7

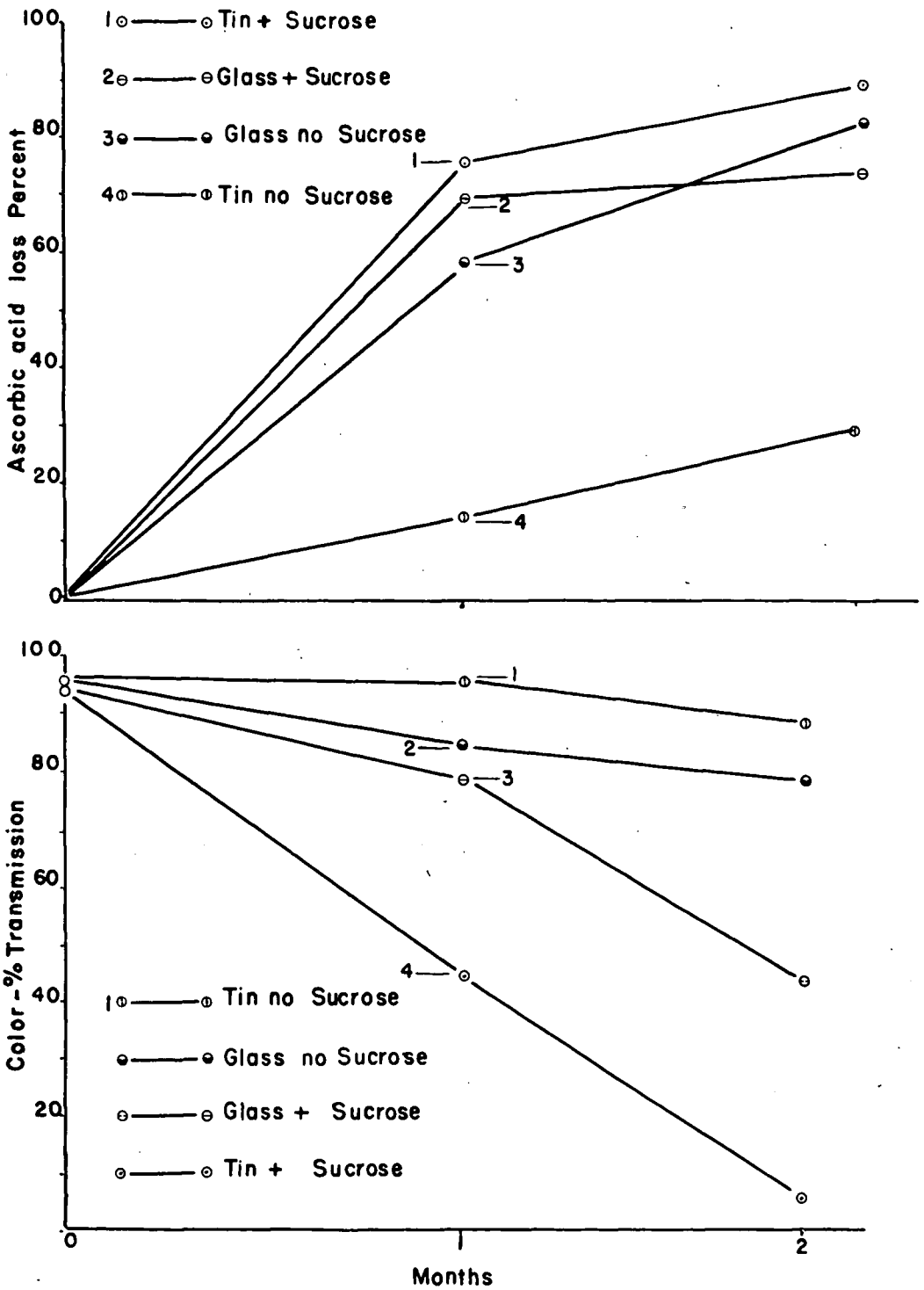
It should be noted that the rate of inversion at this high temperature (135°F) was so rapid that sugars were practically totally inverted after the first month of storage. For rates of inversion at lower temperatures the reader is referred to Figure 17.

(f) Effect of the presence of sugar. To eliminate sugar as a factor in the browning reactions a control was included in these experiments containing only pectin, citric acid (pH 3.3) and the 50 mg. per 100 g. ascorbic acid. Sucrose was left out.

Referring to Figure 24 it will be seen that there was a slight color development in the control samples to which no sugar was added. A very striking and interesting observation was that there was more color development in the glass than in the tin containers. This is exactly opposite to the findings of jellies which were boiled with sucrose.

The ascorbic acid losses illustrated in the same figure also were much higher in the glass than in the tin.

FIG. 24 The effect of sucrose on color changes and ascorbic acid losses of syntetic jellies stored at 135° F.



These observations lead to the following conclusions:

(a) The plain tin container reduces vitamin C losses when sucrose is absent in high concentrations. This is in agreement with the findings of Daniel and Rutherford (19) and Hauck (36) who made the observations that in juices such as orange and tomato with a relatively low sugar content as compared with the jellies, the retention of vitamin C is better in plain tin containers than in glass.

(b) The high losses of ascorbic acid in the glass containers are paralleled by the development of color. The oxidation products are probably responsible for this color formation.

(c) The plain tin container plus sugars is responsible for development of excessive dark compounds. This reaction is responsible also for large vitamin C losses and the presence of ascorbic acid adds to additional discoloration.

2. Spectrophotometric analysis of the brown color. The clear 5 per cent metaphosphoric acid filtrate used for the color development studies was also used for the first spectrophotometric studies. Wave lengths ranging from 320 to 500 millimicron were used.

Figure 25 is representative of typical data obtained for the color analysis of synthetic jellies stored at 135°F. The colored compounds developed in synthetic jellies where ascorbic acid was added are represented by straight lines, whereas curved lines are obtained when no ascorbic acid was present. These differences are only slight however.

After only minor differences in the colored compounds could be detected by the visual range, the spectrophotometric studies were carried out in the ultra violet range. Instead of the 5 per cent metaphosphoric acid as a solvent, distilled water was used because it was found that ultra violet light is not transmitted by the 5 per cent metaphosphoric acid. These analyses were made with wave lengths ranging from 230 to 310 millimicron. Data typical for these analyses are graphically presented in Figure 26.

FIG. 25 Spectrophotometric analysis (visible range) of the brown color developed in synthetic jellies in glass containers at 135°F. (5% Metaphosphoric acid as solvent)

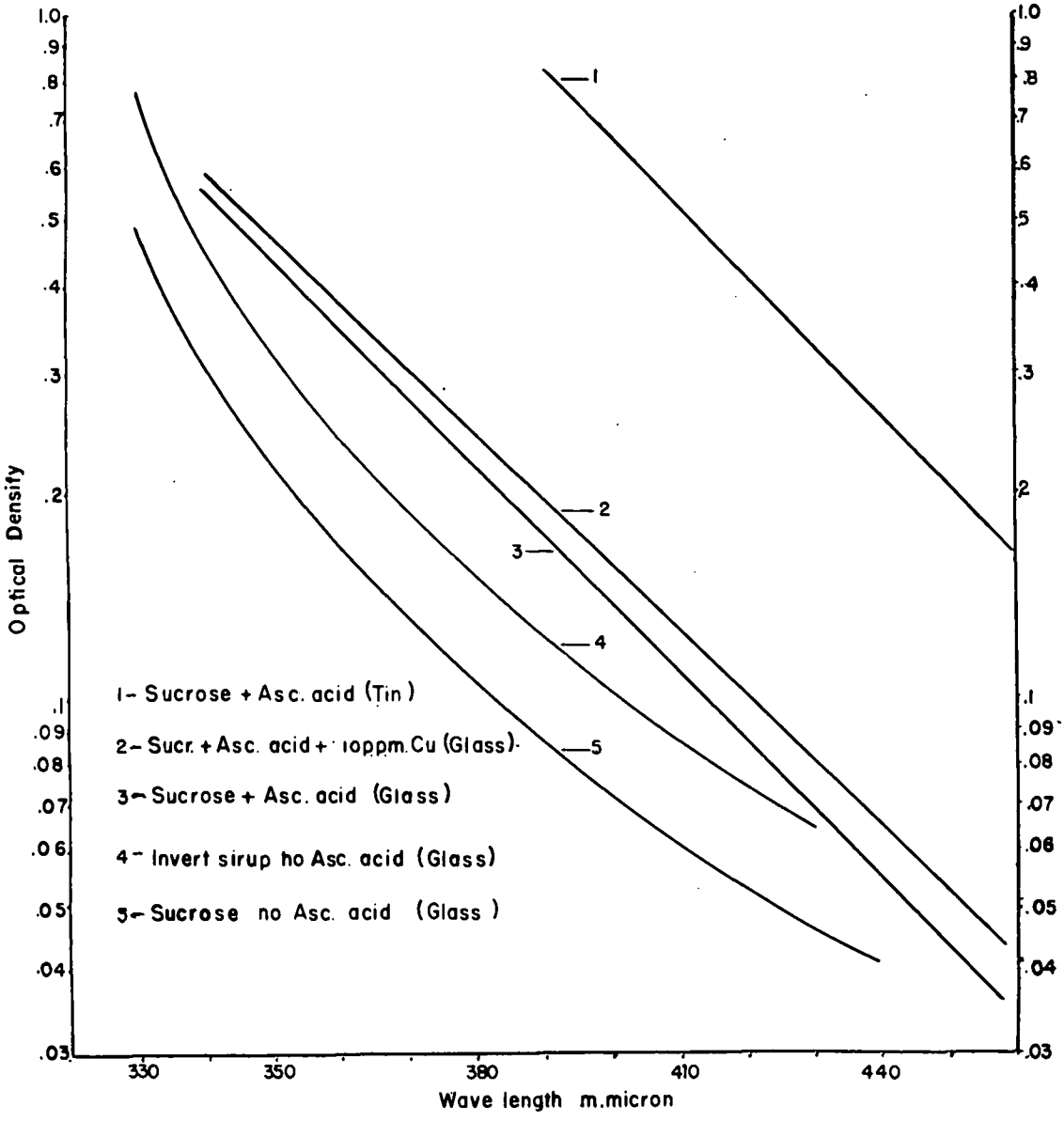
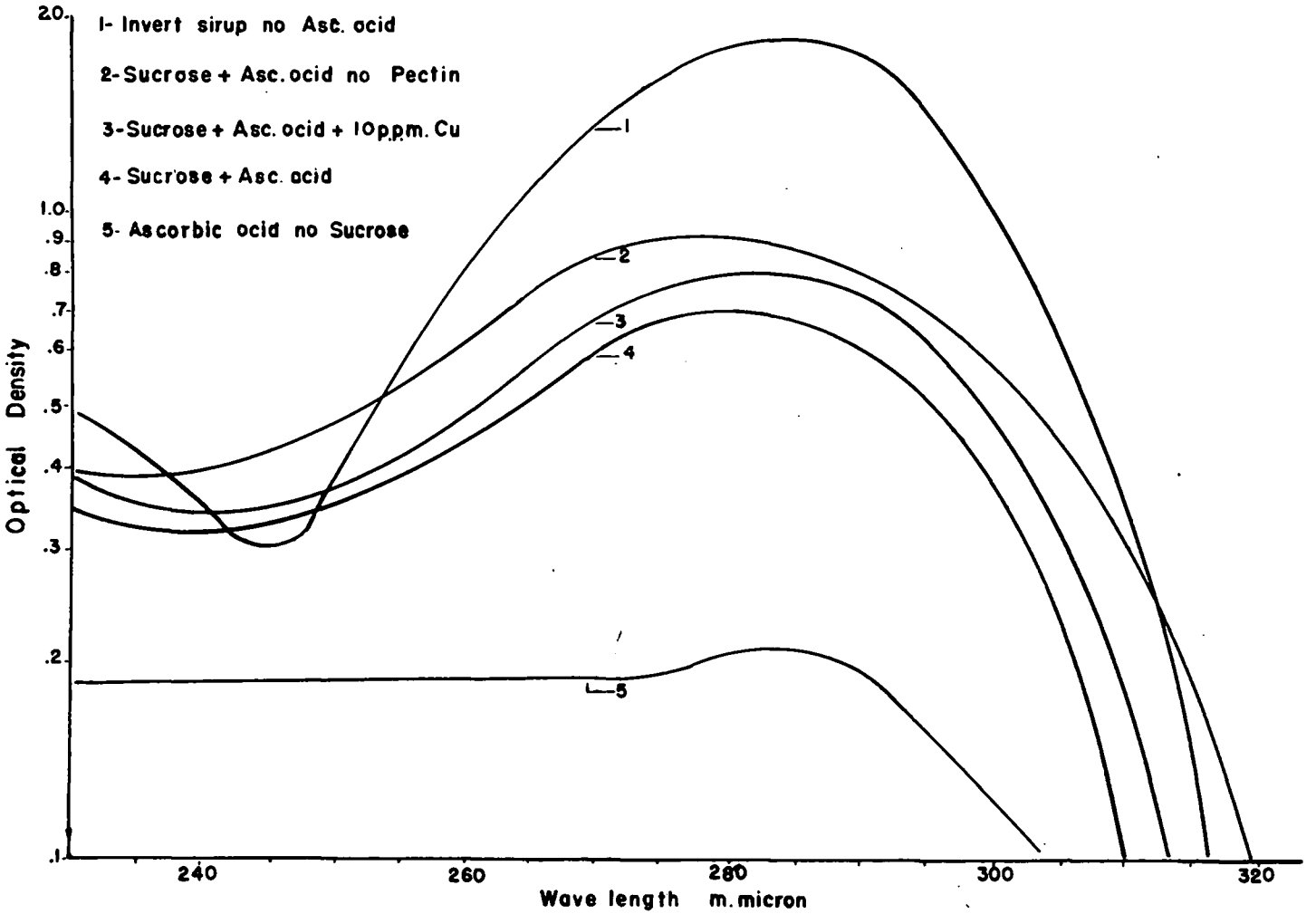


FIG. 26 Spectrophotometric analysis (ultra violet range) of synthetic jellies stored at 135° F. (Distilled water as solvent)



The curves for the color analysis of the different sucrose jellies all show the same trends with an absorption maximum at 285 millimicrons which would indicate that the brown compound is the same in the different samples.

Curve No. 1 in Figure 26 of the invert sirup jellies boiled with no ascorbic acid also shows a maximum absorption at ca. 285 millimicrons, but the point of minimum absorption which is formed at ca. 245 millimicrons is more acute than with the other curves. This may indicate that the brown compound formed when ascorbic acid is not present is somewhat different from the compound formed with ascorbic acid.

The sucrose sample plus ascorbic acid and 10 p.p.m. copper also show the same trends as the other curves representing jelly samples to which no copper was added.

Singh, et. al. (99) and Wolfrom, et. al. (121) both found that d-glucose can be converted to 5-(hydroxy methyl) furfural in acid solutions. Spetrophotometric analyses done by the latter investigators, by MacKinney and Tammer (64) and also by Proctor, et. al. (85) showed the absorption maximum of 5-(hydroxy methyl) furfural, indeed all furfurals to be ca. 285 millimicrons.

Singh, et. al. (99) and Haas, et. al. (32) both state that the major portion of coloring matter arises

from the decomposition of sugars and that these brown compounds are closely associated with the furfurals. The absorption curves of the color formed in the samples containing ascorbic acid, citric acid and pectin but without the sugars also show an absorption maximum at ca. 285 millimicrons (Figure 26, curve 5). This may indicate that the breakdown products of ascorbic acid, pectin and citric acid are also related to 5-(hydroxy methyl) furfural. It will be noted that in this curve without the presence of sugars there is no minimum at 245 millimicrons which would indicate that the brown color developed is caused by or accompanied by furfurals, unless the concentration was too low to give an appreciable figure.

Haas and Stadtman (33) however list sugars, ascorbic acid, uronic acid and reductic acid as substances that may give rise to furfural. They also found the brown compounds in lemon concentrates, dried cabbage and caramels to have an absorption maximum at 285 millimicrons in an aqueous medium.

CHAPTER V

SUMMARY AND CONCLUSIONS

The retention of vitamin C and a study of brown color development in synthetic jellies and orange marmalade, as influenced by storage conditions and ingredients usually employed for the manufacture of these products, were the main objectives of this investigation.

In the preparation of these jellies and marmalades synthetic ascorbic acid was added to a 50 mg. per 100 g. level. Boilings were made with different sugars and sugar sirup replacements of 50 per cent for the most part, poured in glass and plain tin containers, cooled and sealed at 10" and 25" vacuum.

The storage conditions investigated were: 32°F., sunlight (51°F.), room temperature (72°F.), 100°F., and 135°F.

Copper as an influencing factor was eliminated by predetermining the copper in all the ingredients used, and adjusting the final copper level of the boilings to 2 p.p.m. Controls without added copper were also included.

The effects of sodium chloride and d-iso ascorbic acid as antioxidants were also investigated.

Analyses including l-ascorbic acid, total ascorbic

acid, dehydro-ascorbic acid, reductones, color measurements and reducing sugar determinations were made the day following the preparation and thereafter at 1, 3 and 6 months intervals.

Spectrophotometric analyses of the brown color developed in synthetic jellies were carried out in a separate study.

The principal conclusions were as follows:

A. Factors Associated with or Causing Losses of Vitamin C

1. Type of sugar. The retention of l-ascorbic acid as affected by the different sugars and sugar sirup combinations used was found in a decreasing order to be: low conversion corn sirup, high conversion corn sirup, sucrose, dextrose and invert sirup, the losses of ascorbic acid being the highest (of the order of 85 per cent at 100°F. after 6 months) in the invert sirup jellies. Retention of total ascorbic acid followed the same sequence. These findings apply for both synthetic jelly and orange marmalade.

2. Type of container. l-Ascorbic acid and total ascorbic acid losses in the tin containers were found in all cases to exceed these losses in the glass containers, eg. by about 10 per cent units after 6 months at 100°F.

Again this was true for both commodities tested. In control samples where sucrose was omitted the opposite result was experienced and higher vitamin C losses were found in the glass than in the tin containers.

3. Type of preserve. Higher losses for l-ascorbic and total ascorbic acid were obtained in orange marmalade than in synthetic jellies stored at 100° F., but not enough data were obtained at other temperatures to be conclusive.

4. Storage time. Losses of ascorbic acid in synthetic jellies (sucrose) after 6 months storage at 100° F. were 26 and 54 per cent units higher for tin and glass, respectively, than at room temperature (72° F.). For orange marmalade stored for 6 months these losses were 59.5 and 65.3 per cent units higher in glass and tin, respectively, than at room temperature.

5. Reducing sugars. High vitamin C losses were found to be concomitant with high percentages of invert sugars present so that conditions producing high inversion also produced high vitamin C losses.

6. Copper. Copper added to these samples up to a 2 p.p.m. level caused a marked increase in ascorbic acid losses over an average original copper content of

ca. 1 p.p.m. The tin container plus the added copper was responsible for much higher losses of vitamin C as compared with the same losses in the glass containers.

7. Storage temperature. Vitamin C losses rapidly increase as storage temperature is increased, eg. after 6 months storage the l-ascorbic acid losses for synthetic jellies (sucrose) in glass containers were: 13.1 per cent at 32°F., 32.7 per cent at 72°F. and 58.6 per cent at 100°F. These same losses for orange marmalade were: 7.6 per cent at 32°F., 19.2 per cent at 72°F. and 78.8 per cent at 100°F.

8. Sunlight. Sunlight caused slightly increased vitamin C losses in jellies and orange marmalade; more so in the latter case than in the former.

9. Oxygen content in the headspace. The amount of oxygen in the headspace was found of great importance in the retention of vitamin C even though the product was a solid gel. Losses at 10" vacuum always exceeded the losses at the 25" vacuum. Analysis of the top and bottom parts of the samples in glass (10" vacuum) showed a 20 per cent unit higher destruction in the top half after 6 months at 100°F.

10. Use of antioxidants.

(a) Sodium chloride. Two per cent sodium chloride as an antioxidant added to synthetic jellies in glass containers showed a definite antioxidative effect. In the tin containers the added sodium chloride had an accelerating rather than an inhibiting effect on vitamin C losses. In the orange marmalade conflicting results were obtained and further investigation is necessary.

(b) d-Iso ascorbic acid. In glass containers at 100°F. sucrose jellies were completely protected from any loss of vitamin C for at least 3 months by the addition of 30 mg. d-iso ascorbic acid per 100 g. At 51°F. 20 mg. d-iso ascorbic acid per 100 g. gave complete protection for 6 months. Other additions produced proportionate results.

11. Low pH (citric acid). Comparing citric acid sucrose jellies (pH 3.3) with distilled water boilings (pH 3.6) it was noticed that the citric acid definitely exerts a protective effect against vitamin C losses. This phenomenon was observed in both glass and tin containers.

12. Pectin. It was observed that a better vitamin C retention was obtained in ungelled samples containing no pectin. The high copper content of the pectin may be responsible for the high losses of ascorbic acid where pectin was present.

13. The presence of sucrose. Where sucrose was omitted higher retentions of ascorbic acid were found, especially after two months at 135°F. These results were not obtained under atmospheric conditions and is not to be construed as conflicting with reported results under the latter conditions.

B. The Same Factors Causing Losses of Ascorbic Acid Correlate with Color Development with the Following Comments and Modifications:

1. Color development in control samples containing no ascorbic acid was only slight as compared to the ascorbic acid containing samples. Analyses of orange marmalade showed it to contain 8 mg. natural l-ascorbic acid per 100 g. By increasing the ascorbic acid content to 50 mg. per 100 g. (adding synthetic ascorbic acid), the brown color formation was increased to a marked extent. Thus ascorbic acid is responsible for color formation but the data indicate that sugars contribute more to discoloration than vitamin C.

2. No protection against color formation was obtained by the 2 per cent NaCl or the d-iso ascorbic acid that were employed as antioxidants.

3. Sunlight did not permit as much discoloration as the accompanying destruction of ascorbic acid might indicate. That is, evidence was obtained that the brown color developed is bleached by the sunlight.

4. An apparent exception to the general trend of discoloration data, was the fact that sucrose jellies in tin containers containing pectin, (135°F. storage) developed less browning than the samples without pectin which is probably due to their lack of fluidity. In the glass containers exactly the opposite was experienced and more browning resulted where pectin was present, although all glass samples were less discolored than those in tin. It was observed that the ascorbic acid losses of samples in the tin where no pectin was present were much less and still the browning was much more excessive than in the samples containing the pectin. These findings may demonstrate that in the tin container the oxidation products of ascorbic acid only play a minor part in color development and the major reaction responsible for the colored compounds is that between the sugars and the tin container itself.

C. Ascorbic Acid Degradation Products

1. No appreciable amounts of reductones or other interfering substances in the jellies and marmalades could be detected by means of the formaldehyde method used. Even at the 135°F. storage temperature there was no indication of these reducing substances to be formed in synthetic jellies even with high discoloration after two months storage.

2. The actual amounts of dehydro-ascorbic acid present in both synthetic jellies and marmalade were found to range from 1 to 5 mg. per 100 g., and only slight changes occurred during the entire storage period of 6 months.

D. Spectrophotometric Study

1. From the spectrophotometric analysis of the brown color in synthetic jellies, slight evidence was obtained to indicate that the compounds formed in the presence of both ascorbic acid and sugars are different from those formed when no ascorbic acid is present.

2. The absorption maximum of the brown compound as developed in synthetic jellies was found to be ca. 285 millimicrons with a minimum absorption at ca. 245 milli-

microns. When sucrose was excluded only slight discoloration set in. Analyses of the compounds formed in the latter case show them to have an absorption maximum at ca. 285 millimicrons but no minimum at 245 millimicrons. This may indicate that the color developed from ascorbic acid, pectin and citric acid without sugar, gave rise to a different compound than that from the sugars, unless the concentrations were too low in the latter case to give an appreciable figure.

E. Minor Observations

1. Inversion

(a) Rapid inversion of sucrose samples took place at the 100°F. storage temperature. At room temperature (72°F.) the rate of inversion was much slower but still very distinct. No changes in the invert sugar content could be detected at 32°F.

(b) When copper was added to samples containing ascorbic acid the per cent reducing sugars formed was increased.

(c) A comparison between ascorbic acid containing samples of sucrose jellies and samples containing no ascorbic acid show the former to contain 10

per cent units more reducing sugars than the latter at room temperature (72°F.).

(d) Samples in tin containers always showed slightly higher invert sugars than samples in glass containers.

(e) Pectin seems to prevent the inversion of sucrose slightly.

(f) The per cent invert sugars in samples to which sodium chloride was added was always much higher than the samples boiled without added NaCl. The results were consistent but further work needs to be done to assign a cause for this phenomenon.

2. Synergistic combinations. From the practical standpoint about the worst combination for vitamin C retention and development of color appears to be tin containers, high invert sugars, high oxygen content of headspace and 2 p.p.m. or more of copper.

3. CO_2 production. Little evidence of gas production in the synthetic jellies as indicated by a decrease in the vacuum could be found. There was however a slight detectible reduction in the vacuum of the orange marmalade samples after 6 months storage at 100°F. which may be indicative of gas formation.

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