ANATOMICAL CHANGES IN THE FRUIT OF THE RUBEL BLUEBERRY DURING STORAGE IN CONTROLLED ATMOSPHERE

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Gerhard Bünemann

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A THESIS

Submitted to the College of Agriculture of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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AN ABSTRACT

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Approved by ______________________

The production of blueberries in Michigan amounts to over one million crates (12 pints per crate), of which about 50 percent are sold fresh and the rest are processed by canning or freezing. Since texture changes had been observed to occur in storage that seemed desirable for improving the firmness characteristics of frozen blueberries, the present study was made to determine the nature of these changes in the flesh and skin of the fruit, and to ascertain their practical application to the freezing industry.

Controlled atmospheres containing CO_2:O_2 percentages of 0.03:21 (air), 5:2, 11:10, 16:15 and 25:15 at 40° and 50°F, 11:10 at 32° and 25:15 at 72°F were employed for storing the fruit. Selected berries of defined maturities (blue for less than 6 days, 6 to 13 days, and over 13 days) were stored for anatomical examination; whereas, commercially harvested fruit of the "Great Lakes" grade was stored for determination of freezing quality.

The anatomical examinations were made with fruit sections prepared at 10 microns thickness, stained either with Safranin + Fast Green, or with Crystal Violet + Erythrosin. Photomicrographs are presented showing typical cell structures as determined by thorough study of the material.

Fruit which had been blue for 6 to 13 days before harvest were of the most favorable maturity condition for storage purposes. Younger berries were too tart in flavor;

older berries tended to disintegrate. Texture changes of the fruit which yielded a more firm condition occurred upon storage for 4 weeks in a controlled atmosphere of 16% CO_2 and 5% O_2 at 50°F. These berries, however, were unsuitable for consumption because of the development of off-flavors by fermentation.

While flesh firming was desirable, other changes were adverse. In some cases, the skin of the fruit became tough and caused a disagreeable texture of the fresh berry as well as of the frozen one. Breakdown of the walls of the cells of the epidermal and hypodermal layers in fruit stored in air was observed by microscopic examination and was evidenced by the release of anthocyanin into the syrup upon freezing.

A correlation of the tough skin characteristic with a "corrugation" condition of the cell walls was established, particularly for the fruit stored at 50°F in 5% CO_2:2% O_2. Flesh firmness observations could not be correlated to changes in the anatomical structure of the parenchyma tissue.

Storage treatment prior to freezing to improve the texture is of no practical value, since flavor deterioration and off-flavor formation occur in both fresh and processed berries.

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INTRODUCTION

The commercial cultivation of blueberries in Michigan started about 30 years ago on the muck soils around South Haven and Grand Junction.

In 1955, the area of cultivated blueberries amounted to 4200 acres, yielding over 1,000,000 crates of 12 pints capacity. About 50 percent of the crop is sold on the fresh produce market, 30 percent is canned, and 20 percent is frozen.

Frozen berries, although widely used, are unsuitable for use like fresh berries with cereals or as sauce for dessert because of the lack of a desirable texture that is characteristic of fresh fruit. BEDFORD and ROBERTSON (1954) compared several treatments to overcome this undesirable quality, which seems to be related to the development of a tough skin as a result of freezing. They recommend packing the berries in 40-50% sucrose syrup and suggest further work is necessary to improve a nicking procedure for slitting the skins, with which they obtained partial success in improving the texture of the fruit.

It is not definitely known if the changes in fruit texture are due to a softening of the flesh upon freezing so that the skins seem tough, or if the skins actually become tough. Preliminary studies (DEWEY) indicated that certain storage treatments tend to cause a more firm condition of the fresh fruit rather than a softening during storage as on most other crops.

The present study was made to determine the nature of the changes occurring in the flesh and skin of fresh fruit responsible for this firming characteristic, and to ascertain whether or not the changes could be brought about and employed to the advantage of the freezing industry.

REVIEW OF LITERATURE

Previous research on blueberries is limited and few reports are available in the literature on the behavior of the fruit under different storage conditions.

CHANDLER (1942, 1944) investigated the applicability of increasing the CO_2 and decreasing the O_2 content of the atmosphere for long-term storage of blueberries. He kept berries successfully at 41° in 13-15% CO_2 and 5% O_2 for 2 weeks, and he suggested that increased nitrogen concentrations in the storage atmosphere favor mold formation.

Preliminary studies were made in 1953 and 1954 by DEWEY (unpublished), whereby temperatures of 32-35° and 42°F and carbon dioxide concentrations of 5%, 10%, 15% and 25% were employed with sealed and aerated samples as controls. After 3 months, the fruit stored at 32° and 10% CO_2 had the best appearance, but was unsuitable for consumption because of undesirable interior changes. These changes were more pronounced, however, at somewhat higher temperature (42°). Preliminary anatomical studies of this material by WATSON (unpublished) indicated that changes had occurred in the tissue during the storage period and that the stage of maturity of the berries at harvest-time was a contributing factor.

Contributions on the development of the blueberry were made by BAILEY (1947) and YOUNG (1952). BAILEY found that the lapsed time between bloom and picking is too variable to be used for estimation of the proper picking time for optimal holding quality. YOUNG stated that upon changing color from green to dark blue, the berry passes through a very short period of red coloration. The position of the berry on the panicle is not related to the degree of maturity.

Anatomical studies on Vaccinium sp. were made by YARBROUGH and MORROW (1947) with particular emphasis on the presence of stone cells. The varieties Rubel and Dixi contained the smallest amount of stone cells. The number of stone cells could be directly correlated with the degree of "grittiness" and palatability.

Reports of changes during storage of other fruit than blueberries were given by BOYES (1955) on peaches, and by CHRIST and BATJER (1931) on pears. The development of sclereids on pears was observed throughout a vegetation period by STERLING (1954), showing some similarities to sclereids in blueberries.

METHODS AND MATERIAL

The storage conditions employed were selected as the ones most likely to bring about marked anatomical changes in the fruit. These were 40° and 50°F with 5 different atmosphere combinations as listed in Table I in the Appendix, and one lot at 70° with 25% CO_2, as well as the one lot held at 32° in 11% CO_2 and 10% O_2 as a check for the other storage conditions. The latter condition had proved desirable for the storage of blueberries in previous studies by DEWEY (1953, 1954). The desired atmospheric conditions were difficult to obtain, and the average concentrations of CO_2 and O_2 during the four periods of two weeks are shown in Table I. As can be seen, some difficulties arose in obtaining the desired levels of CO_2 and O_2. It was particularly difficult to maintain the relation of 5% CO_2 + 2% O_2. However, the values in this lot were maintained at a fairly uniform level near 7% CO_2 at both 40° and 50° by adding air each time the atmosphere was analyzed.

The berries were obtained from the "Triangle" plantation near South Haven, Michigan, from bushes approximately 15 years of age. They were harvested on July 28, which is in the early part of the Michigan blueberry season. On the same day the berries were transported to East Lansing, and were kept at 40° overnight. The next morning they were sorted for removal of injured or otherwise defective fruit, and filled into one pint paraffin-treated paper containers without lids, then placed into 5-gallon glass jars with tightly closing covers. Through two glass tubes, fitted tightly through the cover, the $CO_2:O_2$ relation was checked at least every other day with a Hayes-Orsat gas analyzer, and the atmosphere combination was corrected accordingly by flushing with nitrogen, carbon dioxide, or air as required. After adjustment, the composition of the atmosphere was not re-checked until the next day at which time further correction was made as necessary. When the desired level was obtained it was checked only every other day.

For the freezing after storage, a "commercial sample" of berries picked by commercial pickers on the same day (July 28) from the same field as the berries used for the anatomical studies was obtained. The fruit, picked between 1 and 3 p. m., was still slightly wet because of rain which occurred between 6:30 and 7:30 a.m. The fruit was of the "Great Lakes" grade (less than 145 berries in a 1/2 pint cup) as used by the Blueberry Growers' Association, Grand Junction, Michigan.

For the study of anatomical changes and for an evaluation of storage effects on fruit for fresh consumption, berries of well-defined maturity stages were utilized as follows:

Group I, "Ripe": Berries of blue skin color for more than 13 days.

To be certain of obtaining fruit that had been blue on the plant for more than two weeks, all "turning" fruit were picked off during the two weeks prior to harvest. To avoid inaccuracies, due to the rapid turning from green to blue color, only the most easily separated and deeply blue-colored berries were included when this sample was harvested.

Group II, "Firm Ripe": Berries blue for 6 to 13 days.

At the first picking all blue fruit were removed, 7 days later all "turning" and green berries were picked, so that fruit on the bush at harvest-time had been blue in color for 6 to 13 days.

Group III, "Hard Ripe": Berries blue for less than 6 days.

At the first two pickings all mature and "turning" berries were removed, at harvest only the ones which appeared fully mature were collected.

The soluble solids content of the harvested blueberries was immediately determined in the field with a Zeiss-Opton hand refractometer. The juice from 5 samples of 8-10 berries of each maturity group was expressed and examined, and the average values determined.

A sample of 10-12 berries of each maturity stage was placed into FAA Killing Solution* immediately, to be used as a control in comparison with the samples stored under different temperature and controlled atmosphere conditions in later anatomical studies.

Some of the berries were cut into quarters, the others were killed as whole berries, removing a part of the epidermis to allow the FAA solution to enter the flesh as quickly as possible.

Due to a leaching effect of the FAA, the solution became deeply colored. For this reason the solution was renewed once in the first few lots. Later on, when it seemed desirable to remove some pigment for easier microscopic examination, berries were placed either into fresh FAA or 70% alcohol. However, no further leaching of pigment was obtained.

Further attempts to bleach the blue color were made without positive results. The berries were treated unsuccessfully with hydrochloric acid and with acetic acid to take advantage of the possible indicator properties of anthocyanin. Ammonium hydroxide was undesirable because it induced cell wall enlargement. Basic lead acetate also was unsatisfactory as a bleaching agent. "Clorox", a commercial bleaching agent containing sodium hypochlorite, produced bleaching, but changed the structure of the tissue. Mounted

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*9 parts ~~formaldehyde~~ alcohol (70%)
0.5 " acetic acid
0.5 " ~~alcohol~~ (70%) formalin

tissue sections as well as quarters of a berry embedded in a paraffin block from which about 1/3 of the slides had been sectioned, were irradiated with the Electron Accelerator. The dosage was 5 million rep.* in 50 passes at 100,000 rep. per pass. This had a slight effect on the structure of the tissue, but no effect on the anthocyanin pigment. Bleaching with ultraviolet light was also tried without success.

The dehydration, infiltration, and embedding of the tissue was carried out according to the method of JOHANSEN (1940) using chloroform as a clearing agent to which "Parowax" was added, until the tissues were saturated. The material was embedded in paraffin of a melting point of 56-58°C. Paraffin of a lower melting point was too soft for sectioning purposes.

The sections were cut with a Spencer Rotary Microtome at 10 mu thickness. Most of the fruit samples were individual quarters of a berry cut in such a manner than the microtome knife cut the flesh part first, so as to prevent a compression or crushing of the epidermis.

Two staining procedures, one utilizing Safranin + Fast Green, the other Crystal Violet + Erythrosin, were developed for blueberries by modification of methods recommended by

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*Roentgen equivalent physical scale

SASS (1951) and JOHANSEN (1940). The safranin stain solution was prepared by dissolving 1 gm of safranin in 100 cc of a 50% alcohol; the fast green stock solution was prepared of 1.5 gm fast green in 100 cc absolute alcohol, 100 cc methyl cellosolve, and 100 cc clove oil. About 7 cc of stock solution were mixed with 100 cc clove oil and used for staining. Leaving the slides in safranin for 5 minutes and in fast green for 3 minutes was sufficient to give a good stain differentiation. Later, the stain combination of 1% aqueous crystal violet (15 minutes) and erythrosin B saturated in clove oil (3 minutes) was tried and gave more satisfactory results because of a better clearing and less danger of overstaining.

The slides were mounted in Canada balsam and were allowed to dry thoroughly at 60°C before examination.

Photomicrographs were made of parts which showed a typical situation, determined after thorough examination of all the slides available of the respective lot.

Samples of all three maturity stages were removed from storage at two-week intervals for immediate subjective examination. About one dozen berries of each lot was killed in FAA killing solution for later microscopic examination. Berries seriously affected by decay organisms were discarded so that only berries of good condition were preserved in the FAA solution. Thus the results should not be interpreted as fully representative for the particular lot, but representative for berries which would have gone through a careful sorting process after storage.

At the final inspection, after 8 weeks of storage, the remaining berries of the individual lots of the maturity groups were used for pressure tests. The pressure resistance of the berries was measured with the BOUYOUCOS-MARSHALL (1951) small fruit pressure tester on position 6, i.e., 550 grams of pressure. The readings were taken after 10 seconds.

Simultaneously, samples of 10 oz. of commercial berries were selected and prepared for freezing in home freezer containers of one pint size. 4 oz. of 40% sucrose syrup were added to each 10 oz. lot of fruit; the packed fruit was then frozen and stored at 0°F.

About 3 months later, the frozen packs were thawed in a 68°F water bath. The drained weights were determined, and thereafter the berries were examined visually, tasted by a panel of five men, and tested for firmness with the Tenderometer.

RESULTS

Sugar Content

The berries at harvest were of similar size and color for the three maturity stages. Definite differences were evident in the soluble solids content of the expressed juices as tabulated in Table 1.

TABLE 1

SOLUBLE SOLIDS CONTENT OF THE EXPRESSED JUICE OF BLUEBERRY FRUIT AT HARVEST

Group	Maturity	% Solids (Av. of 5 samples)
I	"Ripe"	13.5
II	"Firm Ripe"	12.0
III	"Hard Ripe"	11.1

Little difference in taste was noted between Groups I and II, whereas Group III berries were markedly tart or sour in flavor.

Subjective Judgment of Stored Berries

Tables II, III and IV in the Appendix show the results of the judgment of the berries after the storage periods of 4, 6 and 8 weeks. The inspection after two weeks showed no great differences between the storage treatments. The observations are summarized as follows:

<u>After 2 weeks</u>: The 11% samples at 32°F and the 5% and the 11% CO_2 samples at 40° were practically like fresh berries. The 16% and 25% samples at 40° were still of fresh appearance, but had a slightly fermented flavor. Initial mold development was observed at 40° in 5% CO_2.

At 50° some detrimental effects of the two weeks storage period could be noticed in all lots. In the air-stored sample considerable mold had formed, and many berries showed shrinking and softening. The 11% CO_2 sample showed the least change, except for the development of a slight toughness.

At 72° (25% CO_2) a strongly alcoholic taste had developed and the berries which showed no mold infection were very woody in flesh texture.

<u>After 4 weeks</u>: The berries stored at 32° in 11% CO_2 were fairly fresh in appearance and taste, but a few soft or deteriorated berries were found. In the commercial berries stored in the same conditions, a nest of mold had developed covering a group of 5-8 berries.

At 40° mold infection was prevalent in the air sample, independent of the maturity group. At 5% CO_2, however, the flesh texture resembled that of fresh berries. Some of the original flavor was evident, but all of the berries had a very slightly fermented flavor. In 11% CO_2 and in 16% CO_2, the berries appeared to be slightly more firm than at harvest. The berries from 25%, however, seemed softer in texture than the other lots.

At 50°, considerable differences in mold infection were found between the "ripe" and "firm ripe" berries (I and II), and the "hard ripe" ones (III) of the aerated sample. In group I and II the mold growth was vigorous and in group III only slight mold development had initiated. All samples stored in controlled atmosphere showed a certain degree of toughness and were practically free of visible mold. The 72° sample (25% CO_2) contained some berries with a cracked skin, many others were still hard. The number of cracked berries was higher in the "hard ripe" group (III) than in the "ripe" sample (I). As previously noted, the berries in controlled atmospheres at 72° and 50°, tasted fermented.

<u>After 6 weeks</u>: The 32° sample (11% CO_2) was still fairly fresh in appearance, but had lost some of its original flavor. The flesh had softened in a few of the berries.

Samples from 40° and 5%, 11% and 16% CO_2 were quite similar in texture to fresh berries, or firmer; in the air sample a noticeable firmness had developed. The flavor, however, of all these berries was slightly alcoholic, or fermented. The 25% CO_2 samples showed interior disintegration as evidenced by general softening and distribution of the blue skin pigment throughout the flesh. Mold infections in the air sample caused a great amount of decay.

Samples from 50° had developed a degree of toughness and an alcoholic or fermented flavor. The off-flavor was strongest in the 25% CO_2 sample. Since there was no mold growth in 16% and 25% CO_2, the appearance of the fruit was still quite fair. At 72° (25%) the berries were completely disintegrated.

After 8 weeks: The 32° sample (11% CO_2) was still of fair appearance, but slightly alcoholic in flavor; the texture, though still more similar to fresh berries than in most of the other lots, tended to be mushy or mealy and soft. The "hard ripe" lot was of better texture than those of other maturities.

In 40°, a tendency toward a mealy texture was noted in some of the CO_2 lots (5%, 11%); in the higher CO_2 concentrations (16%, 25%) considerable interior disintegration had taken place; exudations were observed at the stem scar. The aerated sample at this temperature was mostly decayed or deteriorated by mold infection.

In 50° the aerated berries were completely broken down, and covered with mycelium. The 5% and the 16% sample showed the most pronounced woodiness in flesh texture; the 11% sample was seriously infected with mold, and general decay had occurred. In general, the higher CO_2 concentrations seemed to have retarded the mold growth. At 25%, strong fermentation had developed.

Pressure Tests

Due to differences in mold infection and general decay, the quantity of berries per treatment available for pressure tests varied from 13 to 50. The average pressures with their standard deviations are shown in Table V of the Appendix. These data show that the differences in flesh firmness were of measurable quantity only between the "hard ripe" and the other two maturities. Slight, but inconsistent, differences occurred between the "firm ripe" and "ripe" groups.

Evaluation of the Frozen Blueberries

From the total number of 50 frozen samples a taste panel of 5 persons selected the 7 treatments listed below as the ones of best texture and flavor. Then the panel members were asked to pick out the three most desirable samples and their decision resulted in the following:

Storage Treatmt.	Control w/o stor.	32°-11% 4 wks.	40°-11% 2 wks.	Control w/o stor.	32°-11% 2 wks.	50°-5% 4 wks.	50°-25% 2 wks.
No. of people in favor	5	4	3	3	1	0	0

It should be noted that one of the controls was rated favorably by all 5 persons, and the other one only by 3 persons, although both samples were frozen right after harvest without any storage treatment.

As to the general characteristics, the controls were best, primarily because of the bland, mild flavor, which is considered to be desirable for frozen blueberries. For firmness of the flesh the sample from 50° and 5% CO_2 (4 weeks) was most agreeable, but undesirable because of a strongly fermented flavor. 50° with 25% CO_2 for 2 weeks contained some firm fleshed fruit. 40° with 11% CO_2 for 2 weeks was similar to the controls in flesh characteristics, but had some off-flavor due to fermentation prior to freezing.

The color of the syrup differed considerably between the individual samples (see Table 2). The syrups of the frozen products from the air-stored samples were more red in color than the syrup of the others. The syrup from fruit stored at 72° in 25% CO_2 for 2 weeks was intermediate in color; the rest were practically free of red coloration. No relation could be established with the drained weight and the tenderometer readings.

The drained weights and the tenderometer readings, shown on Table VI in the Appendix, do not permit any conclusions except of the fact that 25% CO_2 has given the frozen blueberries a largely increased resistance after a storage

TABLE 2

RED COLORATION OF THE 40% SUCROSE SYRUP USED IN THE FREEZING TEST OF STORED BLUEBERRIES AFTER THAWING

Time of Storage Prior to Freezing	Relative Red Coloration of Syrup		
	Deep	Medium	Slight
2 weeks	--	--	40° air
	50° air	--	50° 25%
	--	72° 25%	
4 weeks	--	40° air	
	50° air	--	
	--	--*	
6 weeks	40° air	40° 5%	
	--**	50° 11%	
8 weeks	40° air	40° 11%	
	50° 25%	50° 11%	

*The 72° 25% sample was not frozen because of decay in storage.

**50°-air sample was not frozen because of decay in storage.

period of 6 weeks at 50° and after 8 weeks at 40°F. The skin of these berries seemed leathery or rubbery, and the flesh soft and mushy, when they were organoleptically sampled after storage and prior to freezing (See Appendix Table IV).

Anatomical Examinations

Microscopical preparations of 20 blueberries were examined anatomically. In the epidermal and subepidermal cell layers conspicuous differences occurred as a result of the different storage treatments. Only the two extreme maturity groups were selected for examination. Within these groups the aerated samples were compared with the freshly harvested berries and with those CO_2-treated berries which showed the most pronounced woodiness and toughness, and little or no decay.

There was generally less anthocyanin deposit in "hard ripe" berries than in the "ripe" ones.

In all berries conspicuous sclereids were found scattered throughout the tissue, but their number differed widely even within the fresh fruit.

A good correlation was found between the appearance of "corrugation" of the epidermal and hypodermal cell walls on berries the skin of which was rated "tough" in the general judgment of texture and flavor. This "corrugation" on the sample stored at 50° in 5% CO_2 for 8 weeks is shown in Fig. 1

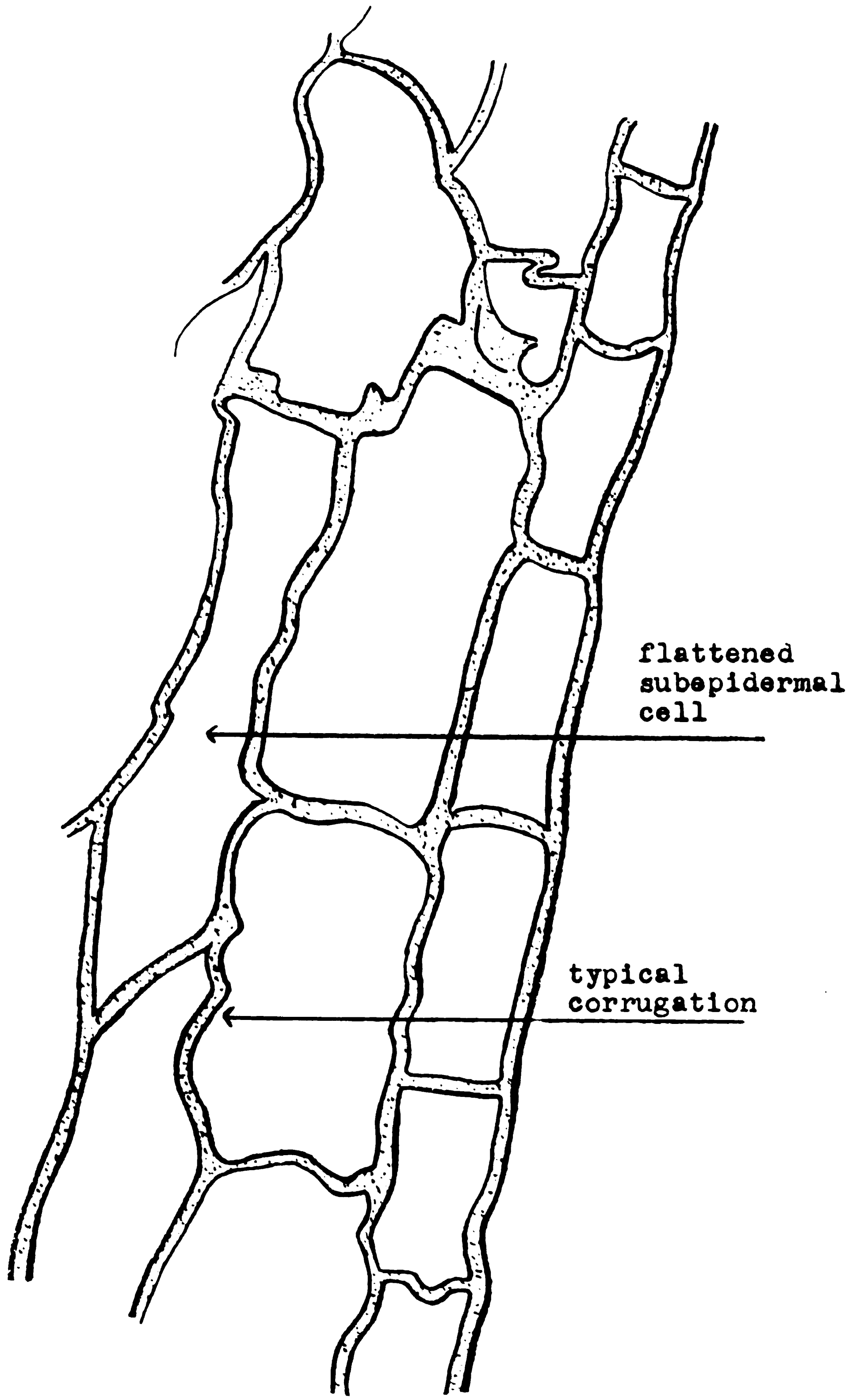


Fig. 1. Free-hand drawing (400x) showing epidermis and two subepidermal cell layers of a "ripe" blueberry stored at 50° in 5% CO_2 and 2% O_2 for 8 weeks; the "corrugation" of the subepidermal layers is prominent.

in comparison to the one from 50° in 16% CO_2 (8 weeks) where it did not occur.

In the photomicrographs (Figs. 3 to 10) some cross-sections of non-stored and of air-stored controls are compared with berries from the two treatments which produced the most marked changes of skin texture (50°F, 5% CO_2, 8 weeks) and flesh texture (50°F, 16% CO_2, 8 weeks). Descriptions of the cell structure are included in the captions accompanying the figures.

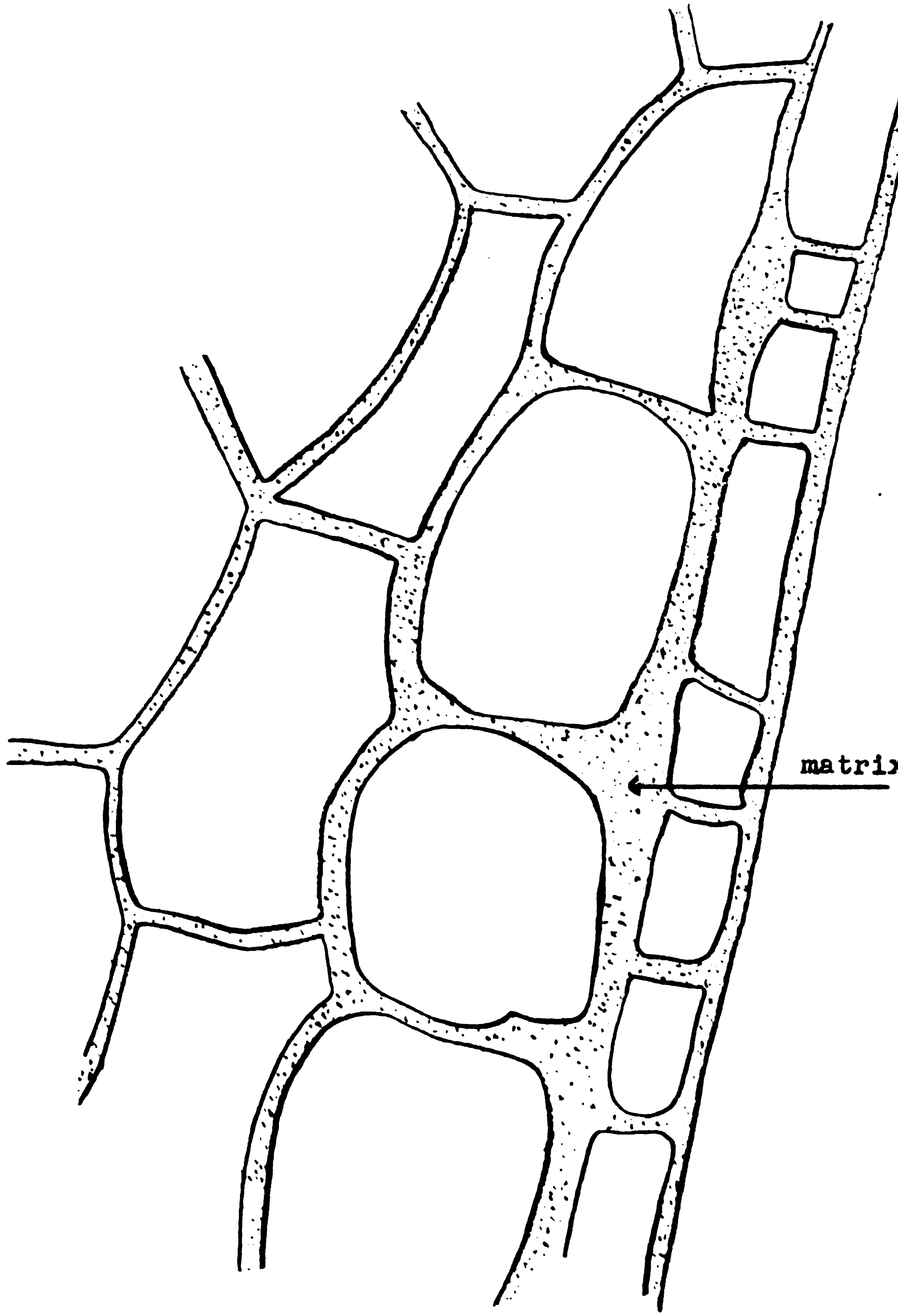


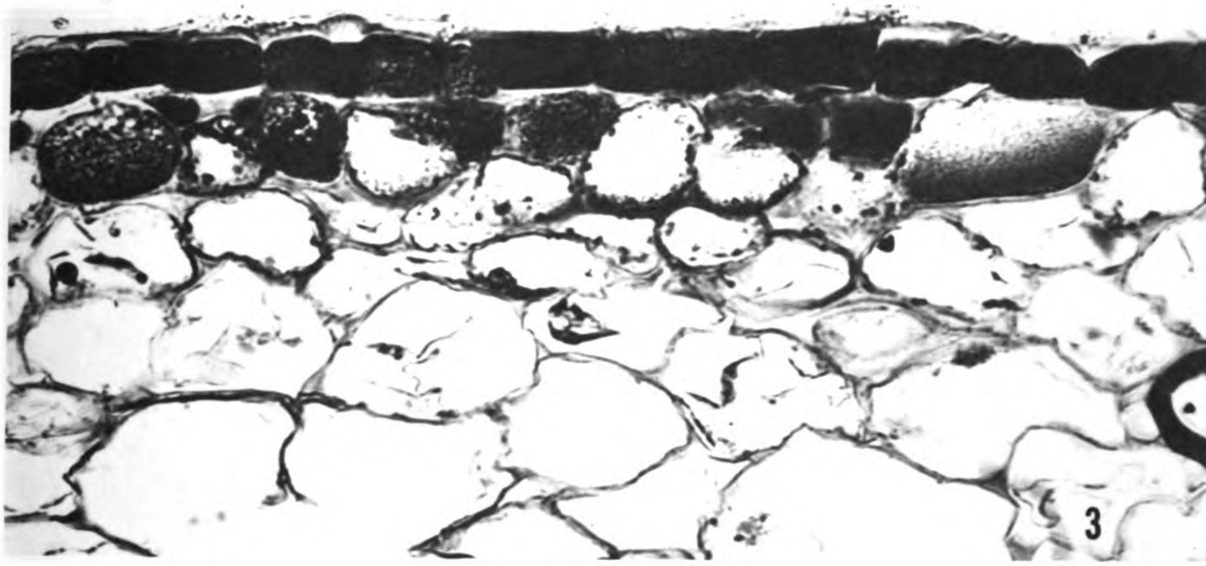
Fig. 2. Free-hand drawing (400x) showing epidermis and two subepidermal cell layers of a "ripe" blueberry stored at 50° in 16% CO_2 and 5% O_2 for 8 weeks; the cells maintained the original shape as in a freshly harvested berry; the wide matrix is typical.

Fig. 3. Group I "Ripe" fruit at time of harvest, control.

The cells of the epidermal layer are conspicuously defined and heavily filled with pigment. Two more or less organized subepidermal layers form a hypodermis. Beneath these layers, parenchyma forms the flesh of the berry. In the epidermal cells a heavy, solid-appearing pigment prevails, in some of which a granular structure can be detected. The quantity of this content diminishes from the epidermal to the subepidermal cells. No anthocyanin is found in the parenchyma except for occasional secondary penetration possibly because of cellular breakdown of the fruit. Among the cells of the three outermost layers is a matrix which readily takes up the erythrosin component of the stain. Occasionally the cell content contracts somewhat.

Fig. 4. "Ripe", 50°, aerated, 4 weeks of storage.

Many cells of the first subepidermal layers have disintegrated walls. The cells of the second subepidermal layer are elongated in the plane parallel to the epidermal layer. The first subepidermal layer as well as the epidermis appears somewhat more flat. Occasionally a slight "corrugation" of the cell wall can be observed. This is more severe in some samples described later (Fig. 5). The matrix between the cells is slightly enlarged. The granular appearance of the pigment in the cells of the outer layers is conspicuous.



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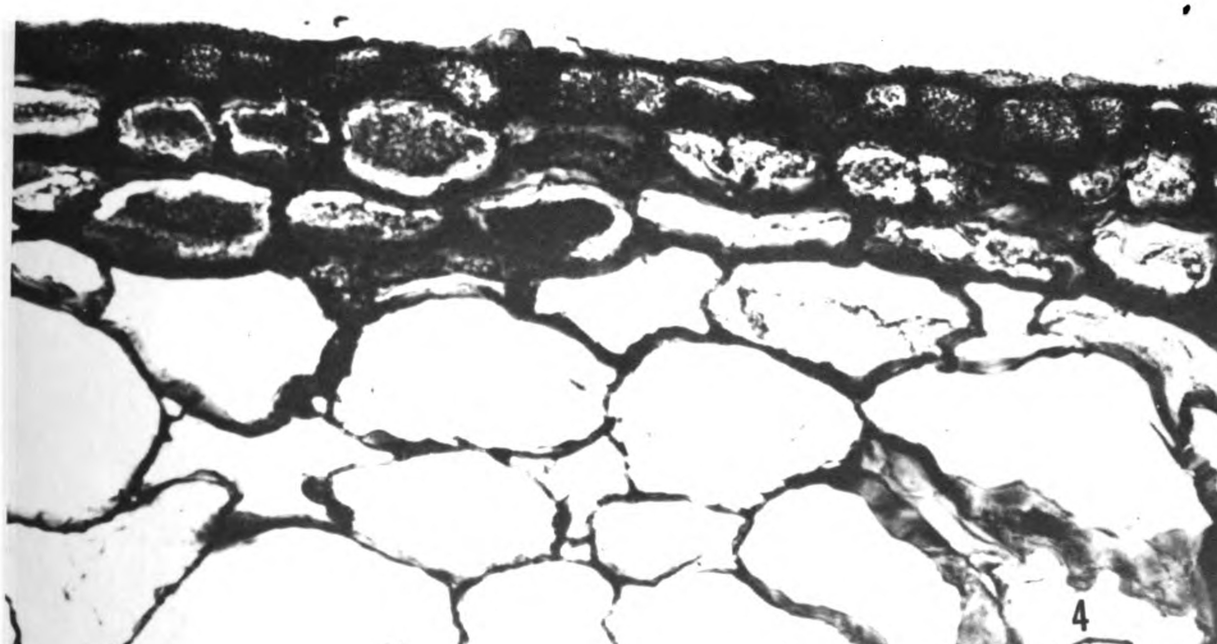

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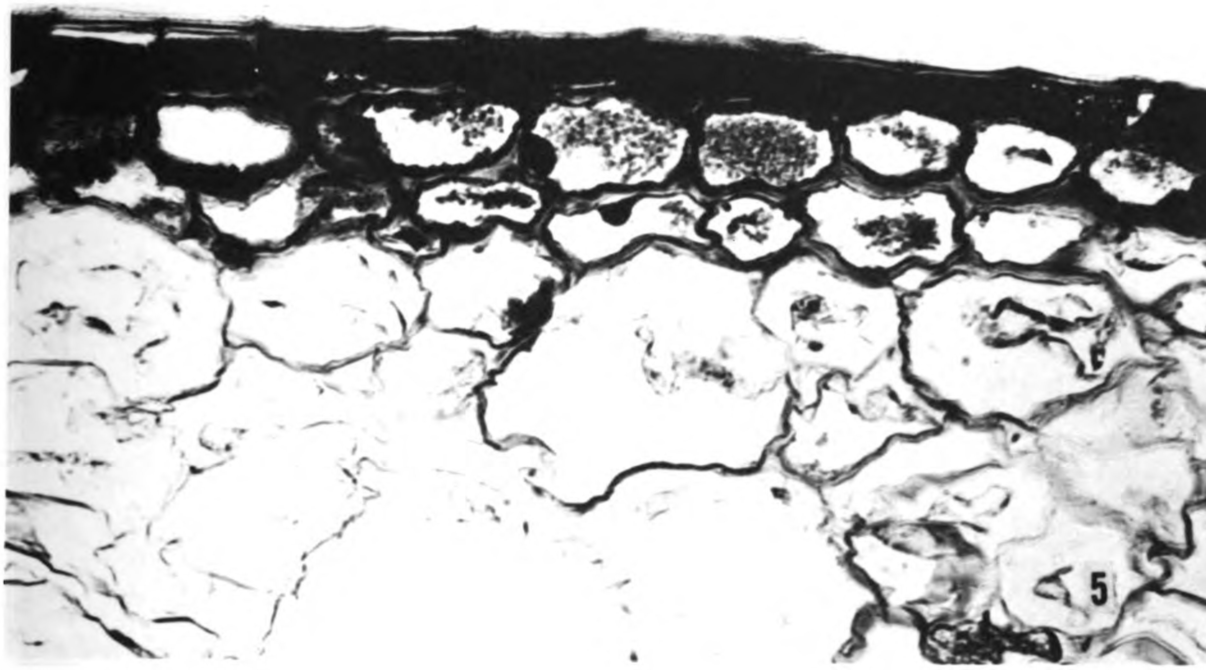
Fig. 5. "Ripe", 50°, 5% CO_2, 8 weeks storage.

Not as large a matrix is found between the cells as in the "ripe" fruit at time of harvest. Pronounced "corrugation" of the cell wall is apparent in the second subepidermal layer, adjacent to the parenchymatous tissue. In the first subepidermal layer "corrugation" is less severe but is even present in the epidermis. The cells of the epidermis seem to be slightly more flattened than those in "ripe" fruit at time of harvest. In the second subepidermal layer many of the cells appear crushed.

Fig. 6. "Ripe", 50°, 16% CO_2, 8 weeks storage.

A matrix, similar to the one described in the "ripe" fruit at time of harvest (control) lies between the cells. The content of this matrix is not uniform. While it appears homogeneous in the "ripe" fruit at time of harvest, it now appears to have a darker line running through, more clearly defining the middle-lamella. Occasionally slight "corrugation" can be noticed.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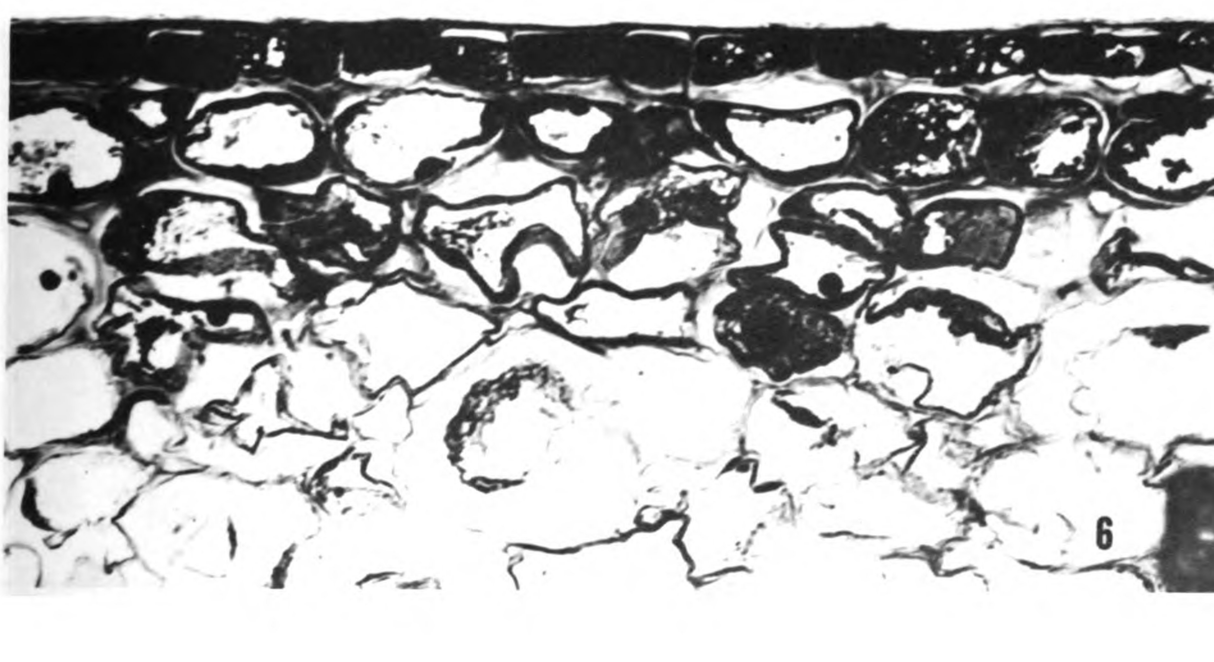
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Fig. 7. Group III, "Hard Ripe" fruit at time of harvest, control.

The epidermal cells are as heavily filled with pigment as in the "ripe" berry at harvest (group I). The first subepidermal cell layer contains only a slight amount of pigment of a granular nature. The second subepidermal layer contains very few scattered deposits of pigment. The cell walls are thin; occasionally a thin, rather transparent matrix can be observed. A very slight contraction of the cell content can be noticed, but not enough to change the smooth round appearance of the cells. The epidermal cells seem to be more flattened than the subepidermal cells. The second subepidermal layer is often more similar to the parenchymatous tissue than to any of the cells in the first subepidermal layer.

Fig. 8. "Hard Ripe", 50°, aerated, 6 weeks of storage.

The epidermis shows a heavy anthocyanin pigment, the first subepidermal layer contains only a slight, granular deposit; the second subepidermal layer is almost free of pigment. Very little "matrix" exists between the rigid cell walls which show some corrugation, mainly in the subepidermal layers. Particularly the second, and to a certain extent also the first, subepidermal layer appear compressed and elongated.

7

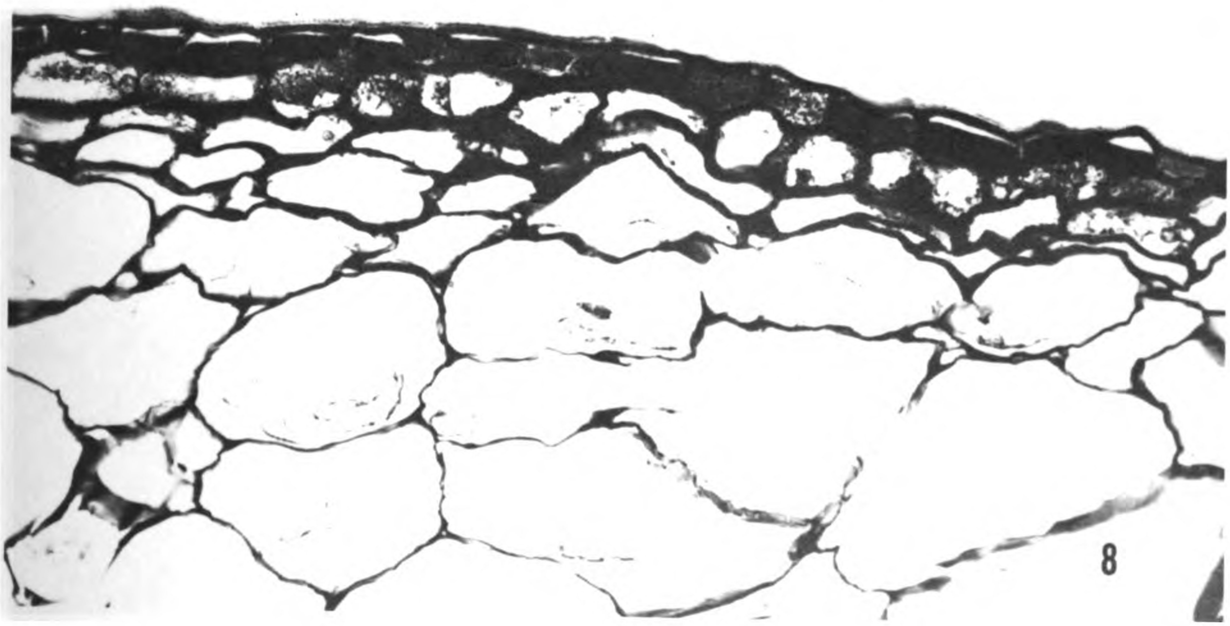

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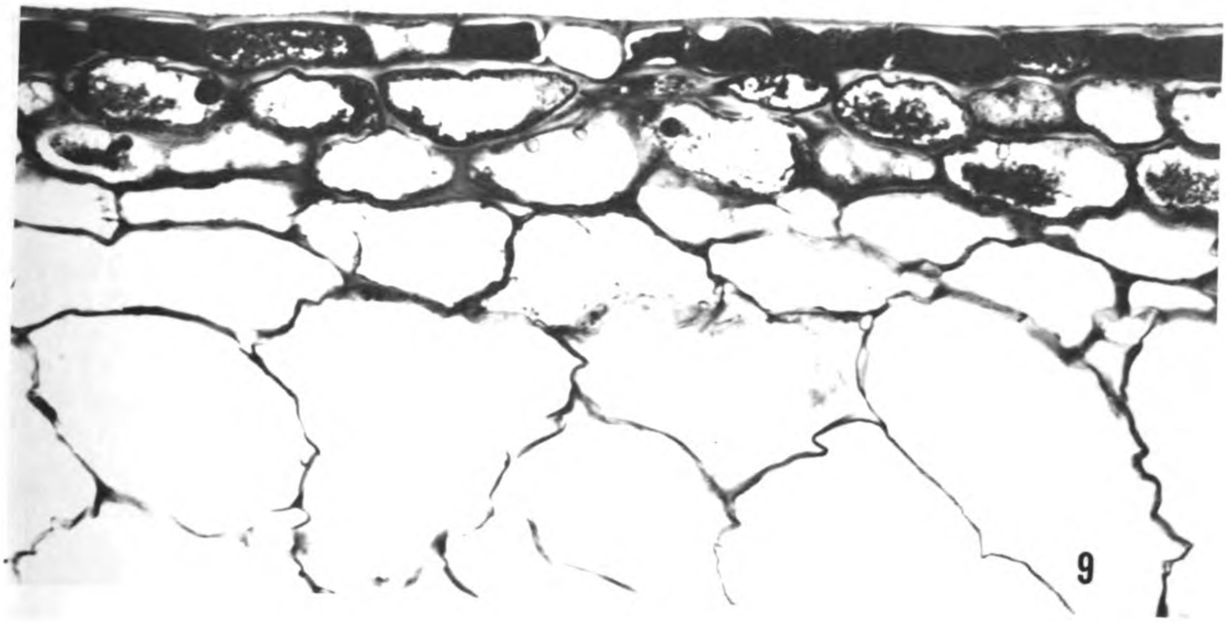
Fig. 9. "Hard Ripe", 50°, 5% CO_2, 8 weeks of storage.

A similar "corrugation" effect as in the "ripe" berry of the same storage lot is apparent. The epidermal cells are more flat than in the more mature sample, and there is no matrix. The cell walls are rigid.

Fig. 10. "Hard Ripe", 50°, 16% CO_2, 8 weeks of storage.

The structure of the epidermis closely resembles the fresh fruit (control) with little matrix between the generally intact and unaffected cells. The anthocyanin deposits in the subepidermal layers are somewhat heavier than in other berries of the same maturity. Pigmentation is heavy, some being present even in the second subepidermal layer. The second subepidermal layer resembles in many places the parenchymatous cells of the flesh.



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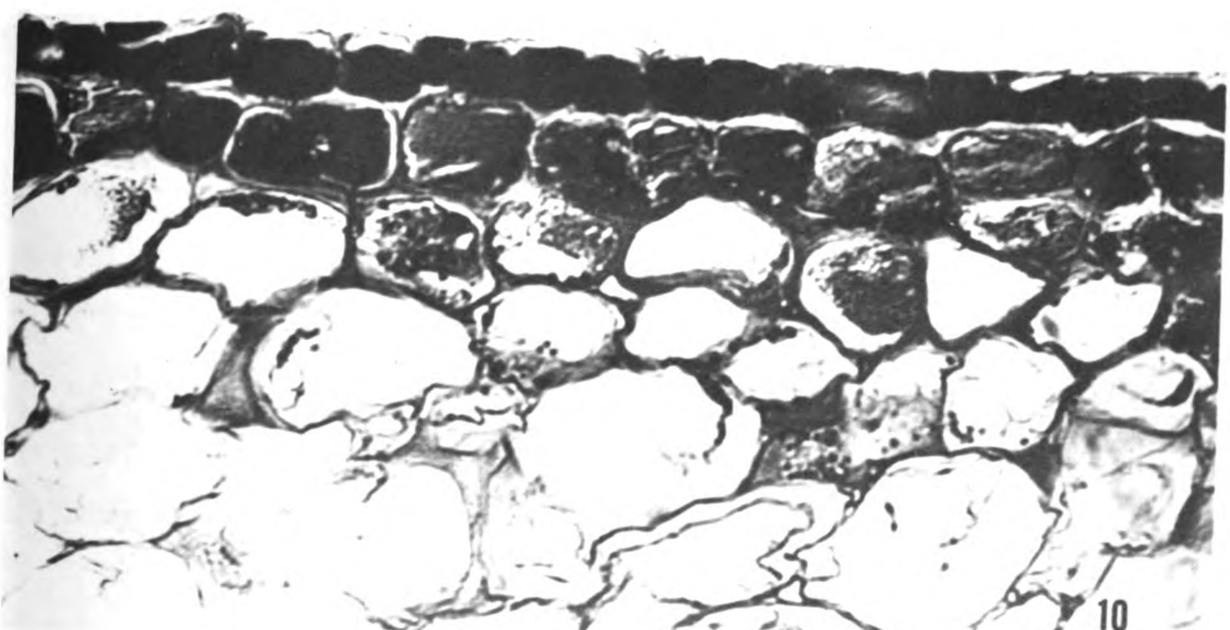

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DISCUSSION

The changes responsible for deterioration of the edible qualities of blueberries during storage were found to be primarily of a physical nature; also there were marked changes in flavor and decay development.

Storage of Fresh Blueberries

Freshly harvested blueberries have the desirable physical characteristic of crispness, apparently because of the combination of firm, turgid flesh with tender skins. This should be preserved during long-term storage if the berries are to be of any value. However, the most prominent changes during storage are toughening of the skin, whereby it becomes leathery, and the development of either a "woody" consistency of the flesh, or a disintegration of the flesh to a mealy or even mushy structure.

The original flavor should be maintained and there should be no development of off-flavors in fresh berries. Frozen berries should hold their flavor, the syrup should remain colorless, the berries crisp and firm, but not hard or woody.

Blueberries may be stored for approximately 2 weeks at 32°F. For longer storage, a supplement such as the addition of CO_2 to the storage atmosphere should be advantageous.

The distinction of three maturities helped to determine whether or not the grower should be more particular about the stage of maturity of the fruit for storage purposes than for fruit harvested for immediate marketing. Definite differences were noted in the quality of the fruit, especially when one compared the "hard ripe" berries (less than 6 days blue) with the "ripe" berries (over 13 days blue). In general, the "hard ripe" berries retained more flavor than the others throughout the storage period. The "hard ripe" fruit were slightly more tart than is probably desired.

Best storage was obtained at 32°F, yet those stored at 40° in 5% CO_2 and 2% O_2 did not become tough in texture or strongly fermented in flavor. However, since a slightly alcoholic flavor developed in the "ripe" and "firm ripe" berries, this storage condition is of questionable value for the riper fruit. The "hard ripe" fruit did not develop an alcoholic flavor, but remained rather sour, apparently because of their deficient maturity.

The development of the woody characteristic of the flesh was favored by storage at high temperatures and in relatively high levels of CO_2. Woodiness was most pronounced after storage at 50° in an atmosphere of 16% CO_2 with 5% O_2.

Similarly, a development of a tough skin was noted, especially after 6 weeks storage at 50°F in an atmosphere of 5% CO_2 and 2% O_2. No clear correlation between the woodiness and any anatomical change could be established, whereas a tough skin was indicated by the so-called "corrugation" which will be discussed in more detail in context with the anatomical studies.

Mold growth inhibition in controlled atmospheres apparently depends on the oxygen content of the atmosphere. Practically no mold developed in 5% CO_2 + 2% O_2, and in 16% CO_2 + 5% O_2 at both 40° and 50°; whereas, the combination of 11% CO_2 and 10% O_2 did not prevent mold growth. An inhibition of mold growth by high CO_2 concentration seems to have taken place in the 25% CO_2 lot in which the O_2 content was estimated to have been 12-15%.

Comparison of the results obtained in 1953 and 1954 by DEWEY with the ones reported here show that the response of the berries in flavor and skin condition to certain storage conditions were generally similar. The differences which occurred can probably be attributed to a considerable extent to the subjective methods of evaluation employed. In addition, DEWEY did not separate the berries by degrees of ripeness, and as found here, wide differences in flavor occurred for berries of the maturity stages II and III. A real difference, however, seemed to occur in fruit texture for berries stored in 25% CO_2. The fruit was described as "pulpy" in 1953, whereas, in 1955 it was described as "soft with a tough skin". Good agreement was attained in respect to decay and its control by low temperature and the use of controlled atmospheres.

Influence of Previous Storage Treatments on the Quality of Frozen Blueberries

Evaluation of the berries frozen after storage confirmed the observation of quality of the fresh berries at the end of the respective storage periods and prior to freezing.

Breakdown of the epidermal tissue, which was not observed in the fresh fruit, was clearly evidenced after freezing by the leaching of the anthocyanin pigments into the syrup. Fruit stored at 40° in air was rated as "tough" upon inspection after 4 weeks of storage; yet, this fruit lost considerable pigment to the syrup upon freezing. The failure to detect the breakdown of the epidermis during storage was probably due to subjective judgment of the skin in relation to the partly decomposed flesh tissue. In the 72° sample with 25% CO_2 there was a visible rupturing of the skin of the fresh fruit, and this resulted in red coloration of the syrup when these berries were frozen. The samples stored fresh in 11% CO_2 at both 40° and 50° lost considerable pigment as a result of freezing. These berries were infected with molds, and it is likely that berries with invisible infections were inadvertently placed into the freezing pack, although a rigid inspection for any kind of defects was applied before freezing.

This may explain why some of the pigment was lost to the syrup solution.

The best texture for frozen blueberries resulted from storing the berries at 50°F in 5% CO_2 for 4 weeks prior to freezing. These berries, however, were unsuitable for consumption because of their strong alcoholic flavor. If it were possible to prevent or to remove this undesirable flavor characteristic, this storage treatment prior to freezing would be beneficial in providing firm frozen blueberries.

According to the results reported here, blueberries should be frozen immediately after harvest. If storage is inevitable, it should be for no longer than 4 weeks, even at 32°F.

Microscopical Examinations

The observation that there is generally less anthocyanin in "hard ripe" than in the "ripe" berries, indicates that the anthocyanin deposition in the blueberry is a very rapid process. This was noted by YOUNG (1952) who observed a quick change of the fruit color on the bush from green to a dark blue-violet. Correspondingly, berries with anthocyanin deposits in both epidermal and hypodermal layers are occasionally found even in the "hard ripe" berries, although normally in this group only the epidermis carries pigment deposits.

The "matrix" in the more mature berries is wider than in the less mature ones.

The "corrugation" of cell walls observed on tough berries may be the result of a dehydration of the cell wall through moisture loss during storage or it may be caused by acidity change as a result of dissolution of some of the atmospheric CO_2 in the cell sap.

Table 3 shows the parallel occurrence of skin toughness and "corrugation". The woodiness of the berry could not be related to any particular microscopic observation.

TABLE 3

COMPARISON OF THE PROPERTIES OBSERVED WITH THE ANATOMICAL NATURE OF STORED BLUEBERRIES

Treatment	Properties	Anatomical Observations
Control "Ripe" (no storage)	Desirable blueberry texture and flavor	Wide matrix, smooth cells
50°, 4 wks, aerated "Ripe"	Mostly decayed (mold)	Slight disintegration of cell walls; elongation of subepidermal cells; limited width of matrix; slight "corrugation"
50°, 8 wks, 5% CO_2, "Ripe"	Some toughness, mostly woody	Limited width of matrix, pronounced "corrugation"
50°, 8 wks, 16% CO_2, "Ripe"	Very characteristic woodiness, skin fair	Matrix similar to control, but less in width. Only few spots of "corrugation"
Control "Hard Ripe" (no storage)	Desirable blueberry texture; flavor somewhat tart	Only a thin matrix to be observed in a few locations, narrow epidermal cells
50°, 6 wks, aerated, "Hard Ripe"	Many hard and tough, some decay present	Only very little matrix, rigid cell walls, some "corrugation"
50°, 8 wks, 5% CO_2, "Hard Ripe"	Mostly woody, some toughness	Corrugation present, but less than in "ripe" one from same treatment
50°, 8 wks, 16% CO_2 "Hard Ripe"	Woody, but skin normal	Skin structure similar to control

In the aerated samples of both maturity groups the berries released some of the red pigment into the 40% sucrose syrup used for freezing, while all the other treatments suffered no loss of pigment.

SUMMARY AND CONCLUSIONS

Rubel blueberry fruit were examined microscopically to determine the changes in anatomical structure associated with ripening and with storage following harvest at several temperatures and in controlled atmospheres.

The berries were harvested on July 28, 1955, from a commercial plantation near South Haven, Michigan, at three stages of maturity, classified as "hard ripe", "firm ripe", and "ripe", according to the length of time they had been completely blue in skin color. Similar fruit, of mixed stages of maturity, were harvested on the same day for processing by freezing.

The fruit was prepared for sectioning immediately after harvest and after storage periods of 2, 4, 6 and 8 weeks. Storage temperatures of 32, 40, 50 and 72°F were employed in conjunction with controlled atmospheres of several combinations of carbon dioxide and oxygen. For freezing studies, 10 oz. of fruit from each of the storage treatments was frozen with 4 oz. of 40% syrup.

Deterioration of fresh blueberries during storage was associated with skin toughening, and with changes of a woody consistency. Development of decay organisms and the formation of off-flavors occurred also.

Blueberries harvested 6 to 13 days after turning completely blue in color ("firm ripe") were of better storage quality than less mature or riper fruit. "Hard ripe" berries retained their original flavor, but remained undesirably tart. "Ripe" berries became alcoholic in flavor. These characteristics were retained upon processing by freezing.

Physical changes in the fruit, considered desirable for yielding a suitable texture of the frozen product, occurred after a storage period of 4 weeks at 50°F in 5% CO_2 and 2% O_2. After storage this fruit was described as slightly tough, firm, of a slightly fermented and flat flavor, and free of mold infection. After freezing this fruit had an agreeable texture, but was unsuitable for consumption because of its marked fermented flavor.

The changes in the texture of the fruit could not be definitely associated with the changes in anatomical structure. It was generally possible to distinguish whether it was the skin property or the flesh property which determined the toughness or the woodiness of the berries after they had been stored for 6 and 8 weeks. The toughness of the skin could then be correlated to a corrugated appearance of the hypodermal and epidermal cell walls. The cells lost their regular and smooth shape and became flattened. They appeared shriveled. No changes in the cells of the flesh were observed in the anatomical examinations even though the flesh seemed to have become more firm during storage.

The "corrugation" of the cell walls in the outer layers was attributed to dehydration during storage or to changes associated with the increased carbon dioxide content of the atmosphere.

The undesirable texture of frozen blueberries may be caused by the skin becoming tough more rapidly than the occurrance of breakdown or disintegration of the flesh parenchyma. It is a relative effect, and the primary value of certain storage treatments apparently is to retard the changes of the epidermal cells responsible for toughening.

It was observed that mold development was inhibited considerably by the use of 5% CO_2 and 2% O_2 or of 16% CO_2 and 5% O_2.

It was concluded that the controlled atmosphere storage of blueberries to be used for fresh consumption or for freezing cannot be recommended presently. Although desired physical changes were brought about which improved the texture of the frozen fruit, the treatment is considered to be of no practical value since it also caused serious flavor deterioration and the formation of off-flavors in both the fresh and processed berries.

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APPENDIX

TABLE I

THE AVERAGE LEVELS OF CO_2 AND O_2 MAINTAINED DURING STORAGE FOR THE 4 PERIODS OF 2 WEEKS EACH

Atmospheres (Desired):		CO_2 25%	O_2 *	CO_2 16%	O_2 5%	CO_2 11%	O_2 10%	CO_2 5%	O_2 2%
(Obtained)									
32°	0-2 wks.					12.7	9.0		
	2-4 "					11.8	8.6		
	4-6 "					11.3	11.4		
	6-8 "					11.9	5.6		
40°	0-2 "	25.0	--	17.5	4.5	12.0	9.4	7.1	2.2
	2-4 "	25.2	--	14.3	10.6	12.5	9.8	7.3	2.5
	4-6 "	24.8	--	12.7	13.0	10.5	13.2	6.7	3.2
	6-8 "	25.0	--	16.1	7.6	11.3	9.2	7.5	2.7
50°	0-2 "	26.1	--	17.4	4.0	12.4	7.9	7.3	2.5
	2-4 "	24.0	--	16.7	6.6	12.7	8.3	7.1	3.4
	4-6 "	23.5	--	16.7	6.1	12.9	8.1	7.0	3.2
	6-8 "	24.2	--	18.2	2.7	12.4	5.9	7.0	3.4
72°	0-2 "	26.4							
	2-4 "	25.2							

*The O_2 content could not be determined with the Hayes-Orsat gas analyzer.

TABLE II

OBSERVATIONS OF THE CHANGES IN FRESH BLUEBERRIES STORED FOR 4 WEEKS

Storage Temperature °F	Atmosphere CO_2:O_2 %	Stage of Maturity	Skin	Flesh	Flavor	Remarks
32	11:10	"ripe"	no change	no change	fairly fresh	
		"firm ripe"	" "	" "	" "	
		"hard ripe"	" "	" "	" "	
40	0.03:21 (air)	"ripe"	tough	a few soft, rest OK	flat taste	slight mold
		"firm ripe"	"	" "	" "	very slight mold
		"hard ripe"	"	" "	fairly fresh	some slight mold
	5:2	"ripe	no change	a few firm ones	very slightly fermented	
		"firm ripe"	" "	" "	" "	
		"hard ripe"	" "	" "	" "	
	11:10	"ripe"	" "	sl. firm	sl. fermented	
		"firm ripe"	" "	" "	" "	
		"hard ripe"	" "	" "	" "	
	16:5	"ripe"	" "	" "	" "	
		"firm ripe"	" "	" "	" "	
		"hard ripe"	" "	" "	" "	
	25:(15)	"ripe"	" "	sl. softened	flat	
		"firm ripe"	" "	" "	"	
		"hard ripe"	" "	" "	"	

TABLE II (Cont.)

Storage Temperature	Atmosphere $CO_2:O_2$	Stage of Maturity	Skin	Flesh	Flavor	Remarks
°F	%					
50	0.03:21 (air)	"ripe"	Mostly deteriorated by mold infection		(good berries) fair	
		"firm ripe"	" " "		" "	
		"hard ripe"	sl. tough	firm	fair	little mold
	5:2	"ripe"	sl. tough	firm	very sl. fermented, flat	no mold
		"firm ripe"	"	"	" " "	" "
		"hard ripe"	"	"	" " "	" "
	11:10	"ripe"	"	"	sl. fermented	" "
		"firm ripe"	"	"	" "	" "
		"hard ripe"	"	"	" "	" "
	16:5	"ripe"	"	"	fermented	" "
		"firm ripe"	"	"	"	" "
		"hard ripe"	"	"	"	" "
	25:(15)	"ripe"	"	"	strongly ferm.	" "
		"firm ripe"	"	"	" "	" "
		"hard ripe"	"	"	" "	" "
72	25:(15)	"ripe"	cracked or tough	many firm	fermented	
		"firm ripe"	mostly cracked mold	firm	"	
		"hard ripe"	" "	"	"	

TABLE III

OBSERVATIONS OF THE CHANGES IN FRESH BLUEBERRIES STORED FOR 6 WEEKS

Storage Temperature	Atmosphere $CO_2:O_2$	Stage of Maturity	Skin	Flesh	Flavor	Remarks
°F	%					
32	11:10	"ripe"	no change	few soft berries	flat, sl. fermented	
		"firm ripe"	" "	" " "	" " "	
		"hard ripe"	" "	normal	" " "	
40	0.03:21 (air)	"ripe"	" "	hard, unless decayed	flat, no fermentation	some mold develop-ment
		"firm ripe"	" "	" "	" " "	
		"hard ripe"	some tough	some hard	sl. sour, no "	
	5:2	"ripe"	no change	" "	flat, sl. fermented	
		"firm ripe"	" "	few "	mild, " "	
		"hard ripe"	" "	some "	sour, " "	
	11:10	"ripe"	some wilting	some soft, few hard	flat, sl. fermented	
		"firm ripe"	" "	few " , some "	mild, " "	
		"hard ripe"	" "	some " " "	sl. sour, <u>no</u> fermentation	
	16:15	"ripe"	" "	" " " "	flat, sl. fermented	
		"firm ripe"	" "	" " " "	" " "	
		"hard ripe"	" "	" " " "	" , sour, sl. "	
	25:(15)	"ripe"	" "	mostly softened, few decayed	" , sl. fermented	no hard berries, flesh blue all over
		"firm ripe"	" "	" " "	" " "	
		"hard ripe"	" "	" " "	" " "	

TABLE III (Cont.)

Storage Temperature °F	Atmosphere $CO_2:O_2$ %	Stage of Maturity	Skin	Flesh	Flavor	Remarks
50	0.03:21 (air)	"ripe"	completely deteriorated due			
		"firm ripe"	to mold			
		"hard ripe"	some decay	many hard	fair, sl. fermented	
	5:2	"ripe"	sl. tough	firm	leathery, sl. fermented	
		"firm ripe"	" "	"	" " "	
		"hard ripe"	" "	"	" " "	
	11:10	"ripe"	" "	sl. firm	fair, sl. fermented	slight
		"firm ripe"	" "	" "	" " "	
		"hard ripe"	" "	firm	" some fermentation	mold
	16:5	"ripe"	fair	hard, few soft	fair, fermented	slight mold infection
		"firm ripe"	"	" " "	" "	very little mold
		"hard ripe"	"	" " "	" "	no mold
72	25:(15)	"ripe"	"	normal	strongly fermented	
		"firm ripe"	"	"	fermented	
		"hard ripe"	"	normal, a few hard ones	"	

TABLE IV

OBSERVATIONS OF THE CHANGES IN FRESH BLUEBERRIES STORED FOR 8 WEEKS

Storage Temperature	Atmosphere CO_2:O_2	Stage of Maturity	Skin	Flesh	Flavor	Remarks
°F	%					
32	11:10	"ripe"	no change	sl. softened	flat, sl. fermented	
		"firm ripe"	" "	" " and mealy	" " "	no mold
		"hard ripe"	" "	little change	sl. better than "firm ripe"	
40	0.03:21 (air)	"ripe" "firm ripe" "hard ripe"	(most berries decayed by mold infection)	rest: mostly softened, some toughened		heavy mold infection
	5:2	"ripe"	little change	firm	only sl. fermented	
		"firm ripe"	" "	"	" " "	
		"hard ripe"	somewhat tough	"	not fermented, sour	
	11:10	"ripe"	sl. tough	mealy or grainy	sl. fermented	
		"firm ripe"	" "	" " "	" " , sl. tart or sour	some mold
		"hard ripe"	little change	mostly firm (90%) some soft	" " , sour, fresh	
		(The commercial berries stored under identical conditions were mostly tough)				
	16:5	"ripe"	little change	partly soft, partly firm	sl. fermented	
		"firm ripe"	" "	partly soft, partly firm	sl. fermented, " sour	
		"hard ripe"	" "	mostly firm	" fermented, tart, sour	
	25:(15)	"ripe"	some tough	many soft	fermented	slight exudation at stem scar
		"firm ripe"	" "	" "	strongly fermented	slight exudation at stem scar
		"hard ripe"	mostly tough	" "	fermented	conspicuous exudation at stem scar

TABLE IV (Cont.)

Storage Temperature	Atmosphere $CO_2:O_2$	Stage of Maturity	Skin	Flesh	Flavor	Remarks
°F	%					
50	0.03:21 (air)	"ripe"	Completely deteriorated by			
		"firm ripe"				
		"hard ripe"	mold development			
	5:2	"ripe"	much toughness	mostly woody	only sl. fermented	
		"firm ripe"	" "	" "	" " "	
		"hard ripe"	" "	" "	very sour	
	11:10	"ripe"	largely deter-	yet unaffected	only slightly	
		"firm ripe"	iorated by mold	berries:	fermented,	mold
		"hard ripe"	development	woody	flat taste	
	16:5	"ripe"	fair	very character-	sl. fermented,	almost mold-free
		"firm ripe"	"	istic woodi-	still fair	mold-free
		"hard ripe"	"	ness	blueberry flavor	almost mold-free
	25:(15)	"ripe"	fair, somewhat tough	firm	strongly fermented	
		"firm ripe"	poor appearance, some cracked epidermis	very firm	fermented	
		"hard ripe"	poor appearance, somewhat tough	firm, some soft breakdown	" , sour	

TABLE V

AVERAGE COMPRESSION OF FRESH BLUEBERRIES OF 3 MATURITY STAGES AFTER 8 WEEKS OF STORAGE AS DETERMINED WITH THE BOUYOUCOS-MARSHALL SMALL FRUIT TESTER USING POSITION 6 (550 grams)

Temp.	Atmosphere CO_2:O_2	Maturity Stage	Compression	No. of Tests
°F	%		mm	
40	0.03:21	"ripe"	7.7 ± 2.6*	19
		"firm ripe"	8.8 ± 1.6	16
		"hard ripe"	6.7 ± 2.3	15
	5:2	"ripe"	6.2 ± 2.6	25
		"firm ripe"	6.8 ± 2.3	25
		"hard ripe"	4.5 ± 1.9	50
	11:10	"ripe"	6.3 ± 2.2	38
		"firm ripe"	7.3 ± 2.0	33
		"hard ripe"	6.2 ± 1.9	34
	16:5	"ripe"	7.9 ± 2.9	27
		"firm ripe"	7.4 ± 2.7	32
		"hard ripe"	6.9 ± 1.6	27
	25:(15)	"ripe"	7.1 ± 1.7	21
		"firm ripe"	7.1 ± 1.8	26
		"hard ripe"	5.8 ± 1.1	44
50	0.03:21 (air)	"ripe"	no sample available	
		"firm ripe"	no sample available	
		"hard ripe"	no sample available	
	5:2	"ripe"	5.3 ± 2.2	19
		"firm ripe"	5.3 ± 2.4	23
		"hard ripe"	4.2 ± 2.4	42
	11:10	"ripe"	7.7 ± 1.8	14
		"firm ripe"	7.8 ± 1.8	13
		"hard ripe"	4.8 ± 2.6	42
	16:5	"ripe"	4.5 ± 2.5	27
		"firm ripe"	5.6 ± 2.7	33
		"hard ripe"	4.2 ± 2.6	43
	25:(15)	"ripe"	7.9 ± 1.9	21
		"firm ripe"	7.7 ± 1.5	13
		"hard ripe"	5.9 ± 2.0	27

*Standard deviation.

TABLE VI

DRAINED WEIGHT AND TENDEROMETER READINGS OF FROZEN BLUEBERRIES AFTER THAWING

Treatment Prior to Freezing		Duration of Storage							
		2 weeks		4 weeks		6 weeks		8 weeks	
Storage Temp. °F	CO_2:O_2 Relation %	Drained wt.	Tenderometer	Drained wt.	Tenderometer	Drained wt.	Tenderometer	Drained wt.	Tenderometer
32	11:10	10 1/4	33.5	10	35	10 1/4	34	10	34
40	0.03:21	10 1/4	33.5	10	34	10 1/8	32	10	33
	5:2	10 1/2	34	10 1/8	33	10 1/4	30.5	10	28
	11:10	10	35	9 5/8	32	--	--	9 7/8	31
	16:5	10 1/4	32.5	10 1/4	35	10	33	10	33
	25:(15)	10 3/8	31.5	10 3/8	34	10 1/2	33	10	70
50	0.03:21	9 7/8	31	9 3/4	34.5	--	--	--	--
	5:2	10 3/8	31	10	33.5	--	--	--	--
	11:10	10 1/2	31.5	10	31.5	10 1/8	32.5	--	--
	16:5	10 1/8	34	10	33	10 1/8	34	10	34
	25:(15)	10 1/8	30.5	10	37	10 1/4	45	9 3/4	45
72	25:(15)	10 1/8	32.5	--	---	--	--	--	--
Controls		9 1/4	34	--	--	--	--	--	--
(No treatment.		10 1/4	36	--	--	--	--	--	--
Frozen immediately		10 1/4	32	--	--	--	--	--	--
after harvest.)		10 3/8	35	--	--	--	--	--	--

Date Due

~~ROOM~~	~~USE~~	~~ONLY~~	
		1166	
ROOM	USE	ONLY	X

Demco-293