MICROFLORA OF RASPBERRIES AND STRAWBERRIES

by

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TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

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INTRODUCTION

Delicate and perishable small fruits like strawberries and raspberries require extra care in handling, shipping and processing. Berry growers must put their product on the market in good condition, which requires careful handling during picking, storing and subsequent shipping to the market so that the fruits retain freshness and quality. On the other hand, the fruit preservation industries are interested in the quality of their finished product, which is largely dependent upon the quality of the raw berries. The consumer is interested in keeping quality and other considerations when selecting a frozen product. The initial microbial load on fresh berries is very important to the frozen food industry since the microbial content of the finished product depends upon it, which in turn influences the keeping quality, taste, flavour, etc. of the finished product. The method of food preservation at low temperatures is not new, but developed markedly with the introduction of new and efficient refrigeration units, where low temperatures can be attained very efficiently.

Foods preserved by refrigeration and freezing are not as sterile as are heat treated products in cans, and although the numbers of micro-organisms are reduced by prolonged low temperature treatment, some still remain viable. The microbial content of the fresh fruits may vary with such

factors as rainfall, weather conditions, location, variety of fruit, conditions of picking, handling and subsequent shipping. All these factors have an influence on the microbial content of the fresh fruit and on the quality of the finished product.

Microbiological assay of the fresh and finished product is important also from the health point of view. The keeping quality of a frozen product is mainly dependent upon its microbial content, both quantitative and qualitative. Fruits grown, collected, shipped and processed for freezing and cold storage under insanitary conditions can be a great health hazard, which demands a routine microbiological examination of fresh fruits and finished product, especially with reference to the fecal contaminants and the other spoilage organisms. Needless to add, that the spoilage organisms alter the texture, colour, flavour, and taste of the fresh and the frozen fruits. The fruit growers and processors should exercise great care in handling fruits and realize that microbiological examination of their products is an index of quality.

The aim of this project is first a study of the microbiological flora of freshly picked small fruits, strawberries and raspberries, as affected by variety, rainfall, location and pre-treatments, and secondly the effect of post-harvest storage treatments on the numbers and kinds of micro-organisms.

LITERATURE REVIEW

MICROBIOLOGY OF FRESH FRUIT

(a) Quantitative microbiology

Considering the manner in which strawberries and raspberries grow and their close proximity to soil (especially strawberries) their microbial flora is expected to consist largely of common soil organisms. However other sources of contamination are found before the fruit reaches the consumer even when all precautions are taken during and after collection. Wide variation in the microbial count of different lots of fresh material occur and Lochhead and Jones (1936); Mundt (1950) and Smart (1934) think that weather conditions at harvesting time are responsible. Lochhead and Jones (1936) reported that fresh strawberries and raspberries carried as many as 20,000 and 50,380 organisms per gram respectively. Strawberries in the fresh state carry comparatively large number of surface micro-organisms depending on methods of handling and condition of growth. Counts of 75,000 to 100,000 organisms per gram were obtained by Berry (1934) and of 19,000 to 800,000 per gram by Magoon (1931). Comparing these data to those reported for other fruits, Smart (1939) reported a count of 5,000 micro-organisms per gram of cultivated blueberries and Marshall and Walkley (1951b) reported that total count of 140,000 micro-organisms on the surface of apple was recorded.

Insects are considered responsible for infection of fruit with micro-organisms. Yeasts are found not only in the intestines of many insects but also are capable of multiplying there (Woll, 1956). Lüthi (1959) indicated the numbers of micro-organisms on the surface of the fruit rises as the result both of increasing opportunity for microbial reproduction offered by the ripening fruit and of augmented infection resulting from increased visits by insects.

Marshall and Walkley (1951a, 1951b) in their systematic studies of the occurrence of micro-organisms on apples found an enormous difference in the numbers of organisms present on healthy and damaged fruit. Niethammer (1942) examined a great quantity of plant material which includes apples, pears, cherries, and gooseberries and found that fungi far outnumbered all other organisms in healthy fruit and seeds.

A survey of the literature indicates that the great variations encountered in microbial load of fresh material, may be due to the influence of such variables, as weather conditions, condition of fruit, and method of picking the fruit.

(b) Qualitative microbiology

Smart (1934, 1935) reported that fruit on arrival at the factory carried on its surface many kinds of micro-organisms. Moulds of the genera Aspergillus, Penicillium, Mucor, Rhizopus, and Sterigmatocystis and yeasts such as Saccharomyces and

Torula were all common. Certain bacteria, Staphylococcus aureus, Bacillus termo and Bacillus subtilis also occur in abundance. Magoon (1931, 1932) observed on an average the following ratio of types on frozen fruits; 65% bacteria, 23% moulds, and 12% yeasts. The bacteria generally originate from the soil and are transferred to the product by hands and containers while yeasts are chiefly air-borne and mould spores are ubiquitous.

Numerous varieties of yeasts are found in all fruits. The yeast flora on apples and in apple juice were recently studied in detail by Marshall and Walkley, 1951a, 1951b, 1952a, 1952b; Clark, Wallace and Davis, 1954; Clark and Wallace, 1954. Clark, Wallace and Davis (1954) have shown that all yeasts which were isolated from apples in the Province of Quebec belong to the family Cryptococcaceae. The frequent appearance of specific species of yeasts (apiculated yeast) in certain years is a well known fact which has never been explained (Lüthi, 1959). Observations made by Marshall and Walkley (1951a) showed that the appearance of Mucor on fruits which were infected with Penicillium was quite frequent and concluded that Mucor and Penicillium alternate with each other. The same authors have ranked Aspergillus second in frequency to Penicillium in fruit juices. Among other genera which occur are Alternaria, Cladosporium, Botrytis, Oospora and Fusarium. Olliver and Rendle (1934) were able to show that while the moulds occurred frequently on strawberries, they were practically never found

on the other berries such as raspberries, loganberries or blackberries.

Smart (1934) isolated from fresh strawberries the following organisms:

Bacteria:

Achromobacter acidum	Bergy et al.
Achromobacter album	Bergy et al.
Achromobacter butyri	Bergy et al.
Aerobacter aerogenes	
Bacillus albolactis	Migula
Bacillus cereus	Frankland
Bacillus fluorescens	Ford
Bacillus megatherium	de Bary
Bacillus mycoides	Flugge
Flavobacterium annulatum	Bergy et al.
Flavobacterium butyri	Bergy et al.
Flavobacterium flavum	Bergy et al.
Sarcina sp	
Spirillum volutans	Ehrenberg
Staphylococcus albus	Rosenbach
Staphylococcus aureus	Rosenbach
Proteus vulgaris	Hauser

Yeasts:

Saccharomyces sp.

Torula colliculosa

Moulds: *Alternaria sp.*
Aspergillus sp.
Botrytis sp.
Cladosporium sp.
Monila sp.
Mucor sp.
Penicillium sp.
Oidium sp.
Rhizopus sp.
Stemphylium sp.

Borgstrom (1955) reported that on arrival at factory fruit carries on its surface large numbers of micro-organisms. Moulds of the genera *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, and *Sterigmatocystis* and yeasts such as *Saccharomyces*, *Torula* are most common. Certain bacteria, such as *Staphylococcus aureus*, *Bacillus termo*, *Bacillus subtilis*, also occur in abundance.

MICROBIOLOGY OF FROZEN FRUITS

(a) Quantitative microbiology

Preservation of food by low temperatures, although not new was in commercial use for more than 95 years, consequently refrigeration of foods has assumed new importance because of the great improvements made in equipment and in the

technique of refrigeration processes.

Low temperatures inhibit microbial growth, but frozen foods are not sterile in the same sense that canned products are, for they are not subjected to same temperature treatment. (Borgstrom, 1955; Prescott and Tanner, 1938). One factor which the food technologist should not overlook is that all bacteria, yeasts, and fungi even of the same species, will not behave alike at low temperatures. At the freezing temperature many are killed, but some surely survive (Prescott and Tanner, 1938). Berry (1946) and Hartsell (1951) have reported similar species variation in resistance to low temperatures in pathogenes. Prescott, et al. (1932) reported that yeasts and moulds are especially sensitive to low temperatures. In vegetative stage bacteria and fungi are sensitive to low temperatures, this effect is not always immediate and may require long periods of freezing temperature (Borgstrom, 1955), and one decisive factor is the freezing temperature, as reported by Prescott, et al. 1932; Swift, 1937 and Haines, 1938.

Prescott, et al. (1932); Haines (1934) reported that yeasts and moulds from berries and vegetables were especially sensitive to low temperature. They, however, reported that soil bacteria, which survived the freezing temperatures in nature were not very susceptible to cold. Moulds are particularly resistant to low temperatures; they are able to grow even at -6.6°C (20°F), Diehl, et al. 1934; Berry, 1934. Russian investigators also came to the conclusion that moulds

can develop at lower temperatures than other micro-organisms (Panassenko and Tatarenko, 1940).

Smart (1937) reported a 99% reduction in microbial content of fresh blueberries by storing at freezing temperatures for seven months, but there were sufficient numbers of bacteria, yeasts and moulds viable in the frozen berries to cause spoilage in the thawed berries in a short time at room temperature. Regarding general microflora of the frozen fruits and vegetables, pronounced decreases in number are obtained, varying with such factors as the nature of the product, temperature, and the type of container (Prescott, et al. 1932; Berry, 1933a). Haines (1938) reported that freezing is bactericidal to greatest extent if it takes place slowly and the products are afterwards stored at a comparatively high temperature. These findings are quite contrary to the demand of modern freezing technique, which dictates a rapid freezing and subsequent storage at lower temperature.

Earliest experiments on the effect of freezing upon suspensions of bacteria were those of Park (1901), where he froze suspensions of typhoid bacilli in distilled water at -5°C (23°F) and sterilization of the suspension required 22 weeks. Prescott (1931) reported a reduction in number of bacteria frozen in tap water in ice trays of an ordinary refrigerator. McCleskey and Christopher (1941) reported that Staphylococcus aureus and some Salmonella species survived in

unsliced strawberries stored for 14 months at - 18°C (0°F). In the same paper they reported that temperatures higher than 10°C (18°F) are more lethal to micro-organisms as the process of protein denaturation is less serious at lower temperatures. Death of frozen micro-organisms may be ascribed to the denaturation and subsequent flocculation of the cellular protein (Borgstrom, 1955). This concept is in opposition to the idea that death is due to the mechanical action of the ice crystals since the death is most common at -2°C (28°F) where such intracellular crystals do not form because of the salt content in the cell (Weisar and Osterut, 1945), and the immediate death seems to result principally from the mechanical action of extracellular ice. An extensive investigation on the occurrence of micro-organisms in frozen berries was made by Wallace and Tanner (1934; 1935). They reported that the number of micro-organisms increased during pretreatment and packaging, but deminished substantially during freezing and storage. Magoon (1932) reported that at lower temperature there is a very considerable reduction in count, but not complete sterilization. The viability of micro-organisms is greatly increased at low temperature, in food stuffs such as eggs, milk or juices, (McFarlane, 1940a, 1940b, 1941, 1942; Berry, 1932b, 1932c, 1932d, 1933b, 1935; Berry and Diehl, 1934). Smart (1934, 1935) reported in frozen strawberries a reduction of 99.3% in number of viable organisms after one year of storage. In spite of the high mortality rate, no less than 1,000,000 bacteria per gram of frozen berries remained. This high count after freezing

for such a long period can be attributed to initial high count, due to excessive handling, since the samples were taken from the processing line.

(b) Qualitative microbiology

Smart (1934) isolated 26 species of bacteria, yeasts and moulds from frozen strawberries, raspberries and cherries held at -9.4°C (15°F) for three years. Smart (1939) found the following micro-organisms repeatedly in commercially frozen fruits:

Bacteria:	Achromobacter butyri	Bergy et al.
	Bacillus mycoides	Flugge
	Bacillus aterrimus	Lehman and Neuman
	Pseudomonas syncyanea	(Ehrenberg) Migula
	Spirillum volutans	(Ehrenberg)
Yeasts:	Saccharomyces unisporus	Jorgensen
	Saccharomyces exignus	Pess-Hansu
Moulds:	Aspergillus sp.	
	Mucor sp.	
	Oidium sp.	
	Penicillium sp.	
	Rhizopus sp.	

Nickerson (1950) found Flavobacterium, Vibrio, Bacillus, Cellulomonas, Phytomonas, Pseudomonas, Micrococcus, Streptococcus (feacalis), Mycobacterium, Staphylococcus, Leuconostoc,

Achromobacter, Alcaligenes, Aerobacter, Erwinia, and Sarcina from frozen foods. However, Smart (1939) isolated most frequently bacteria belonging to the genera Sarcina, Flavobacterium, and Bacillus, from commercially frozen vegetables a result no doubt influenced by lower pH of fruits. Lochhead and Jones (1936) reported that bacteria were by far the most predominant organisms in frozen pack vegetables, whereas yeasts and moulds were relatively more numerous in acid fruits. In the same paper they reported that Micrococci and species of Flavobacterium withstand freezing better than other freeze-sensitive organisms. Hucker (1954) reported that the predominating types of organisms on frozen vegetables are gram-negative rods, resembling the Flavobacterium estereoromaticum. Small number of the isolates belong to Achromobacter sp. or to the species of Micrococcus. Berry (1933a) found that the growth of Cladosporium sp. at -2°C (28.4°F) and Oidium sp. and Torula sp. at -4°C (24.8°F) occured on small fruits in non-airtight containers. In the same paper he reported isolation of Torula sp. after four weeks storage and Monila sp. after six weeks storage from strawberries packed in 50% sucrose solution held at -4°C.

EFFECT OF pH ON MICROFLORA

Bread and Cleary (1932), reported that freezing temperatures, although not necessarily lethal to micro-organisms,

may markedly influence the action of deleterious agents present. There is an appreciable enhancement of the bactericidal effect of hydrogen ion at low temperatures. Smart (1939) found that defrosted vegetables became unfit for consumption in 24 hr at 30°C, while the fruits, aside from being softened in texture, were still edible, he attributed this difference in spoilage to the difference in acidity. The high acidity of fruits inhibits the growth of bacteria, while the pH of most vegetables is within the growth range for micro-organisms. Smart (1934) pointed out that yeasts and moulds were relatively more numerous in acid fruits, as compared to non-acid fruits and vegetables. Berry (1932a) reported that the acidity of strawberries has a decidedly bactericidal influence although moulds and spores were relatively resistant. Stille (1943) reported that more yeast cells were killed by cold temperatures at a low pH than at a higher pH and the same author (1950) reported that this was true for all micro-organisms.

According to Ko (1918), juices of half ripe fruit has greater bactericidal power than juice from ripe fruit. Lüthi (1959) indicated that acceleration of the death rate is also very greatly dependent upon pH, storage temperature and species of micro-organisms. It was pointed out that in apples and pears, malic and citric acids are the most common, while in berries citric acid can predominate. Bach (1932) reported that the undissociated part of organic acids is the active factor in germicidal action and actual pH has little effect.

McFarlane (1940a, 1940b) reported that frozen fruits and berries all have such a low pH that the substrate is unfavourable for most organisms that might be factors in spoilage during freezing. Douglas and Edin (1930) reported that apple juice with a pH of 2.0 killed Eberthella typhosa in 5-9.5 min, Salmonella paratyphi in three hours, and that Escherichia coli was not killed in 48 hr, Salmonella paratyphi in 24-30 hr, but Escherichia coli was not killed in 24 hr. In the same paper they reported that at pH values below 4.0, those bacteria, including the spore forming bacteria, which are not adapted to fruit juice, will not survive storage. Wallace and Park (1953a) reported that in frozen cherries and frozen cherry juice (pH 3.5) no organisms were found at the end of four week storage at -8°C or -17°C or - 40°C. An exceptional case was described by Wallace and Park (1953b), where the formation of toxin in strawberry and raspberry preserves took place. McFarlane (1942) reported that mortality of both Saccharomyces sp. and Escherichia coli frozen cells was greater at pH 3.6 to 3.7 than at higher pH values. Lochhead and Jones (1936) reported that with the more acid products, strawberries and raspberries, increase in number of micro-organisms after defrosting was slight at 5-10°C (41-50°F) but more pronounced at room temperature, though less striking than in the case of less acid vegetables, and in the same paper they reported that Coli-aerogenes types and anaerobic spores showed no indication of development in the fruit during the holding period.

FECAL CONTAMINANTS AND PATHOGENS

The literature of food microbiology contains many reports on the effect of freezing and low temperatures for different lengths of time on different pathogenic organisms and fecal contaminants.

The presence of Escherichia coli or other coliforms in a food product is considered as an index of fecal contamination. Burke-Gaffney (1932), referring to Escherichia coli quoted Theobald Smith's statement, "It is safe to infer that any organism so uniformally present in the intestinal tract ... really belongs there, and that its presence outside the intestines in the soil and water may be regarded as due to contamination with fecal discharges of men or animals". The same author in 1932 reported that such a statement may be applicable equally well to the enterococci, of the human and animal intestines, Kline (1935) substantiated this belief. Sherman (1937) stated that the presence of enterococci should be attributed to survival rather than growth. Slocum and Boyles (1941) reported that coliforms are more abundant on the vegetables the edible portion of which is in the ground than on those the edible portion of which is above the soil.

Straka and James (1932, 1933, 1935), James (1933) and Wallace and Park (1933a, 1933b), reported that the spores and toxin of Cl. botulinum are not destroyed by freezing and there is little danger of botulism from frozen foods if handled

properly and used immediately after defrosting. Allowed to thaw and stand for days at room temperature, however, foods containing spores of this organism may become dangerous. Prescott and Tanner (1938) reported that 99% of typhoid bacilli died when frozen in water, in the same paper they reported that there was little, if any, danger of typhoid from food substances frozen and stored at temperatures below 0°C unless the infection was massive. Prescott and Geer (1936) reported that very small numbers of Salmonella species and of Clostridium botulinum survive for periods of several weeks at temperatures as low as 20°C (4.0°F). They carried out experiments in which packaged spinach, heavily inoculated with detoxified spores of type A Clostridium botulinum and found that at temperatures considerably below 10°C, no development of toxin was produced during the period of one month in either inoculated or uninoculated samples. At 10°C no toxin development occurred within a period of 31 days. The production of certain acids by other bacteria normally occurring in the food, were found by the same authors to delay, or in some instances to inhibit, toxin production at 20°C. The same fact was noted with fruits or vegetables having pronounced natural acidity. McCleskey and Christopher (1941), Feller (1933) and James (1932) have discussed the health aspects of frozen fruits and vegetables and they considered frozen fruits and vegetables were recognized potential carriers of disease producing bacteria.

ARTIFICIAL INOCULATION EXPERIMENTS

The fate of micro-organisms in frozen foods has been studied by numerous workers, including Berry (1933a, 1933b), Prescott et al, (1932), Wallace and Park (1933b), Wallace and Tanner (1935), and Smart (1939). McCleskey and Christopher (1941) reported that certain pathogenic bacteria inoculated into sliced sweetened strawberries and held at 18°C (4°F) were recovered after varying periods of storage, as follows, Eberthella typhosa, six months; Staphylococcus aureus, five months; Salmonella aertrycke and Salmonella schottmulleri, one month; Salmonella paratyphi, was not recovered at any time from the frozen berries. Eberthella typhosa inoculated into unsliced but sweetened berries was still present in small numbers after 14 months storage at -18°C. The death rate of Eberthella typhosa in strawberries held at room temperature was very rapid, and such that heavily inoculated berries were free of living germs after 6 hrs. Held at 5°C (41°F) the death rate was such that about 98% were killed in one day and sterility reached in eight days. These results were substantiated by Wallace and Park (1933a, 1933b). McFarlane (1942) insisted upon the importance of pH of strawberries in such studies. Kiser (1943) reported a quantitative study on the rate of destruction of an Achromobacter sp by freezing and found that during the first 300 hr of freezing at -28°C the destruction of Achromobacter sp was proportional to the number of viable organisms present, whereas during subsequent periods

no such relation existed, and total sterilization did not always result. Tanner and Wallace (1931) reported that many frozen fruits and vegetables were not sterile even after storage at -16°C (3.2°F), although considerable reduction in the original numbers of micro-organisms was observed. In the same paper they reported that micro-organisms suspended in cherry juice and in strawberry juice, having characteristically low pH, decreased more rapidly in numbers than when in other menstura having a higher pH. Douglas and Edin (1930) and Scholz (1943) reported that pathogenic bacteria die off in apple juice within a few hours to a few days. Wallace and Park (1953a), reported that while the artificial infection of sour cherry preserves with colon-typhoid type bacteria showed that they could remain viable for only 4-7 weeks at this temperature. Prescott and Tanner (1938) reported that for certain strains or species of bacteria, however, it may happen that if the material is not actually frozen solid the organisms become adapted to low temperatures after a time and slow growth may take place, increasing materially with the lapse of time, until the number of organisms may reach very large figures. While this is characteristically true in the cold storage above the freezing temperature, it is not likely to occur at lower temperature ranges. As a group pathogenic bacteria seem to have less resistance to freezing storage than common saprophytes (American Society of Refrigerating Engineers, 1946).

INFLUENCE OF ANTHOCYANIN PIGMENT ON MICROFLORA

Blank and Suter (1948) investigated the effect of anthocyanin on pathogenic bacteria and reported that concentration of 0.02 and 0.1 M had no bactericidal action, and their work was substantiated by Mandrik (1953). Masquelier and Jensen (1953a, 1953b), studied the bactericidal action of red wines and reported that the pigment isolated from grapes to be used in red wines were not bactericidal, in the same papers they reported that on hydrolysis, a fraction containing partially demethoxylated oenidol (malvidin) had definite bactericidal activity. Masquelier and Jensen (1953a, 1953b) reported that as wine aged oenside (malvidin-3-monoglucoside) was converted to oenidol (malvidin), and the oenidol thus formed possessed a phenol coefficient of 33. Recently Masquelier (1958) has stated that cyanidin and pelargonidin were not bactericidal. Pratt, Powers and Somaatmadja (1960a) reported that strawberry and grape anthocyanines were pelargonidin 3-monoglucoside, cyanidin 3-monoglucoside, and delphinidin 3-monoglucoside, and influenced the growth of E. coli and L. acidophilus in the presence of both pelargonidin 3-monoglucoside and delphinidin 3-monoglucoside. Later Pratt, Powers and Somaatmadja (1960b) concluded that pelargonidin 3-monoglucoside and delphinidin 3-monoglucoside inhibited the growth of E. coli, 5-desoxy-3-methoxy-apigeninidin chloride-4-methyl ether, apiginidin-chloride-4-methyl ether and pelargonidin-3-monoglucoside, all inhibited the growth of Staphylococcus aureus. Jakovliv

(1948) reported that in citrus juice as in strawberries there was every evidence to indicate the presence of specific bactericidal substances.

METHODS

The committee on microbiological examination of foods has developed methods intended to be used for the preparation of samples of frozen foods. The following in the summary of the recommendation of the committee (American Public Health Association, 1946).

"A mechanical blendor (Waring-blendor, Turmix, etc.) should be used in the preparation of frozen fruit and vegetables. This conclusion has been arrived at after trying many different methods of preparation with frozen products. The mechanical blendor gives higher counts, but at any rate more uniform results are obtained. If the blendor is equipped with a variable transformer, it is advisable to increase the speed of the motor gradually to full speed. After blending allow the suspension to stand for 2-3 minutes to permit the foam to subside, and then make the further dilutions as desired. Pour melted tryptone glucose extract agar (pH 7.0) cooled to 45°C (113°F) into the Petri dishes immediately, and thoroughly mix the dilution water with agar by gently rotating the plates in a figure of 8 motion with slight tilting of the Petri dish. Cool to harden,

and incubate at 32°C (90°F) for 4 days. It is of primary importance that the agar is poured immediately after the inoculum is introduced, otherwise many bacteria will adhere to the glass and an inaccurate count will result."

Goresline (1948), reported that in the preparation of samples of frozen fruits the package should be held at room temperature for 1 - 2 hrs before opening, in order partially to defrost the contents and then portions are cut from various parts of the contents of the package with a sterilized scalpel. However Borgstrom (1955) reported that samples for the bacteriological investigation from the thawed product are not preferred. If the product has thawed, the water frozen in the product melts, as does the water which is retained as a coating during the preparation, consequently this water contains more bacteria per unit weight than the product itself. Therefore, a proportionate amount of water must be sampled but this is very difficult, hence sampling of an unthawed product is preferable. This recommendation was further endorsed by Nickerson (1950).

Devereux (1932a, 1932b) reported an yeast extract medium for the examination of milk, which would permit earlier detection of milk of poor quality. Devereux and Etchells (1933) developed a yeast extract medium to be used in standard plate method for determining the total viable count in milk. The American Public Health Association (1958) recommended tryptone glucose yeast extract agar medium for the total plate

counts in frozen fruits. This medium differs from nutrient agar in that yeast extract and peptonozed milk are substituted for meat extract and peptone, and dextrose is added. Counts at the end of 24 hr incubation were on the average comparable to nutrient agar counts made at the end of 48 hr, resulting in a saving of 24 hr for the completion of the test. Also counts at the end of 48 hr were on the average 45% higher than similar counts on nutrient agar. Food microbiologists need a selective medium that favours the growth of lactic acid bacteria and at the same time, inhibits or prevents the growth of all other micro-organisms. Recent descriptions of a liver infusion, sorbic acid medium (Vaughn and Emard, 1951, and Emard and Vaughn 1952) and "Tween 80" media by Rogosa, Mitchell and Wiseman, 1951, Evans and Niven, 1951 attained this objective. The former authors claimed that their medium for lactobacilli, does not need autoclaving and the heat necessary to dissolve the agar is sufficient to maintain the medium free of contamination for at least 6 months in the refrigerator. Briggs (1953) devised a practical medium for the growth of all varieties of lactobacilli, a tomato juice agar with Tween 80 incorporated. The use of anethol and thymol in media to favour the growth of lactic acid bacteria has been described by Marthinsen and Vaughn, 1958. The selective inhibition caused either by anethol or thymol was observed to be a function of concentration and was not enhanced by varying the pH value of the basal medium. The prospective usefulness of either of these compounds as selective

agents in the medium for isolation of lactic acid bacteria is restricted to conditions where these bacteria predominate.

Slocum and Boyles (1941) stated that brilliant green bile 2% was slightly more efficient than standard lactose broth and much superior to ricinoleate broth as a presumptive medium for the detection of all coliforms. The results indicated that there is an advantage in the use of a combination of brilliant green bile 2% and standard lactose broth for the detection of coliforms in food products. Gehm and Heukelekian (1935) indicated that the eosin methylene blue smear plate method is satisfactory for direct rapid E. coli enumeration. The outstanding advantages are:

(1) Confirmed results in 24 hrs.
(2) Less work involved.
(3) Medium cheaper and easy to prepare.
(4) Less equipment required.
(5) Gives counts comparable with those obtained with brilliant green broth.

The direct microscopic count shows great promise as a quality control test for frozen food. This method was proposed by the committee on Microbiological Examination of Foods (American Public Health Association, 1946). A great advantage of this procedure is the speed with which the test is conducted. A period of 15-30 min is required for the completion of various steps involved. The data obtained with the microscopic count,

however, must be properly interpreted. The direct count may be less accurate than the viable plate count when material which contains few bacteria is examined. However, the direct method will indicate whether or not the count is within a desirable range and this is adequate for some purposes. The culture media ordinarily used do not provide for the growth of certain types of bacteria which may be present in some food products in considerable numbers, such organisms may be detected with the direct microscopic count. The direct microscopic examination of food products is a tedious procedure and allows no differentiation between living and dead cells. With some products, such as corn, due to the presence of starch, it is extremely difficult to detect bacteria.

Burris' tube method is used primarily for determining the microbial content of milk, Burri (1928) and Cunningham and Andrews (1933). Details of the method are given in "Standard methods for the examination of the dairy products" (American Public Health Association, 1933). It provides an inexpensive and relatively accurate procedure for determining the microbial content of frozen and unfrozen (line samples) vegetables at the processing plant level. Another obvious advantage of the Burri technique is that by varying the medium and/or the incubation temperature it is possible to detect different types of bacteria. This is particularly useful when special contamination problems are encountered (Jones and Jean, 1959).

Wilson (1935) conducting researches concerning the accuracy and precision of bacterial counts in milk, summed up his work as follows: "It is impossible to avoid the conclusion that the bacterial count is an inaccurate and unreliable method of ascertaining the number of organisms in milk. The final result, which even with a standard technique is correct only within limits estimated at 90%, bears no constant relationship to the total number of bacteria, either alive or dead, in the sample. As a figure therefore, it possesses no special significance, and its value is purely relative." Wilson's work was carried out on milk, therefore the sampling error was probably not nearly so great as it would have been with certain other foods. Ziegler and Halvorson (1935) found that the standard plate count was more accurate and precise than the serial dilution method even when 10 tubes were used at each dilution. The standard plate count is more precise and accurate than the cotton swab technique and serial dilution described in "Recommended methods for the microbiological examination of foods" (American Public Health Association, 1958). Bacterial counts are not precise and vary with the methods and media used. In general, the methods for bacterial counts on frozen foods are very similar to those used for other food products, as described in "Standard methods for the examination of water and sewage" (American Public Health Association, 1936). However, certain features of the methods are modified for best results. An incubation temperature of 25°C (77°F) was found to give higher bacterial counts on frozen food products than either 20°C or

37°C. Petri dish cultures are incubated for 72 hr to obtain a maximum count when plating on nutrient agar.

To differentiate between food particals and bacterial colonies, techniques such as surface swab and serial dilution (most probable number, decimal dilution) were used previously. Angelloti, et al. (1958) evaluated the cotton swab technique with reference to the recovery of a known contamination and the precision between successive recoveries of known contamination and they concluded that the swab technique gave low recoveries and low precision. Goetz and Tsuneishi (1951) reported that 2,3,5-triphenyltetrazolium chloride, as a component of bacterial media, facilitated the detection and counting of micro-organisms but Weinberg (1953) reported that 2,3,5-triphenyl-tetrazolium chloride was a growth inhibitor of many organisms in concentrations as low as 0.04% and 0.05%. The ability of bacteria to reduce the 2,3,5-triphenyl-tetrazolium chloride to the insoluble, red coloured formazan could, therefore, not be utilized to obtain total counts if the indicator solution was incorporated into the growth medium (Weibull, 1953). With several food products, both after processing and in the raw sterile state, no reduction of indicator occurred when it was added to the product, and since 2,3,5-triphenyl-tetrazolium chloride solution is capable of diffusing through a thin layer of agar within a reasonable period of time the tetrazolium flooding technique was developed by Solberg and Proctor (1960). Incubated plates are flooded with 2 ml. of a 0.1% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride and excess fluid

removed by inverting the plates. After 3-5 hrs. at room temperature, the colonies take up a distinct red colour, and are easy to differentiate from food particles. The tetrazolium flooding technique offers investigators in the food field an opportunity to obtain more accurate estimates of the actual numbers of bacteria present in many food products of low bacterial count. The data of Ziegler and Halvorson, (1935), illustrated the superiority of the plate count method over the serial dilution method when accuracy is desired and the flooding technique with tetrazolium allows extention of the lower range of numbers in which the standard plate count is normally employed.

METHODS AND MATERIALS

SAMPLE SOURCES AND CHARACTERISTICS

Strawberry and raspberry samples were collected from the plantation at Macdonald College with the cooperation of the Department of Horticulture. Strawberry samples were also collected from the Experimental Farm, Canadian Department of Agriculture, L'Assomption with the cooperation and assistance of Dr. St. Marie. Both these plantations have commercial and experimental varieties under test, and from these four varieties of strawberries were collected from Macdonald College, i.e. Sparkel, Valentine, Sangua and Premier, while nine varieties were sampled at L'Assomption, i.e. Sparkel, Redcoat, Early Dawn, Cavalier, Senator Dunlap, Grenadier, Pocahantas, Guardsman and Armore. The raspberry samples were taken from four popular varieties of this area, namely, Latham, Viking, Newburg and September.

Strawberries were sampled at regular intervals from June 16th until the end of July. Raspberries were sampled from July 15th to August 15th. Records were obtained for mean daily temperature, amount of rainfall and hours of sunshine for this period of time at each station.

Strawberries from L'Assomption Experimental Farm were sprayed with a fungicide mixture of Captan and Malathion. The mixture consisted of 3 lb. of Captan per 100 gallons of water and one pint of Malathion per 80 gallons of water. The

strawberries were sprayed on May 20th and again on the 2nd of June at a rate of 200 gallons per acre.

METHOD OF SAMPLING

Samples were collected on alternate days, two varieties at a time, as far as possible, but times were changed to suit weather conditions. All samples were collected about 7.30 am and processed as soon as possible. Samples were collected either in round, half pint, wax paper containers made by Seal Right Canada Ltd or in 8 ounce capacity, sterile disposable plastic containers made by Falcon Plastic Company. The wax paper containers were tested for sterility and 10 boxes were tested. To each container 50 ml of sterile distilled water was added then agitated for one minute and was plated on each of the different media used in this study, and incubated for 48 hr at 30°C. The results indicated that the wax paper containers were sterile and fit for sampling the berries.

Samples from L'Assomption were collected by their staff in small berry boxes and were transported back to the laboratory as quickly as possible. Medium sized berries were selected and transferred to the sampling containers.

At Macdonald College the samples were collected in six containers pre-cooled at -15°C in a pail insulated with fiber glass. Before sampling, the fiber glass pail was packed

with crushed ice to keep the contents cool. The sampling took about an hour and during this time the temperature inside the container rose to about 15-20°C. Samples were collected from six parts of each row of each variety. Two samples were taken from the two ends of the row, leaving about 1.5 ft to eliminate the "end effect" and four samples were collected from the remaining part of the rows. Berries from at least four plants were included in a sample and only medium sized berries were collected, since very small or very large berries affected the weight to surface area relationship, and thus would affect the microbial counts. Berries lying on the soil were not included and only berries on the top of the bedding were selected. All feasible steps were taken to avoid possible sources of contamination, while sampling, but it was not possible to avoid the contaminating effect of strong winds. To avoid contamination by the picker, hands were washed thoroughly with cresol-soap solution and after picking every box, hands were wiped with alcohol and dried. The sample containers were not filled completely to avoid compression on the berries by the lid.

Samples were brought from the field and then placed in the cold room (16°C) until they were plated. Out of six boxes of a single variety, one box was used as the fresh sample, one was frozen at -17°C for 96 hr, one left at room temperature (25°C) for 48 hr, and two left in the refrigerator (10°C) for 48 hr and 96 hr respectively. Samples could not be kept at room temperature for 96 hr, as there was always too much mould

growth and the samples were not suitable for microbiological assays.

PREPARATION OF THE SAMPLE

Each lot of fruit was sampled three times. Ten grams were weighed into a 100 ml beaker with a Petri dish cover. The beaker was washed previously with soap and water and dipped in boiling water for three minutes and inverted on a clean sheet of paper to dry. After each weighing, the beaker and forceps were dipped in boiling water as before. The 10 gm sample was blended with 100 ml of autoclaved distilled water in a Waring blendor. The speed of the blendor was increased gradually with a variable transformer and then the suspension was allowed to stand for 2 min for the froth to subside (American Public Health Association, 1958). After each blending the jar was washed with cresol-soap solution and rinsed with sterile distilled water. Tests showed that this gave adequate sterility. These blended samples were then used for microbiological and other tests.

Frozen samples were treated similarly but were weighed before thawing (Borgstrom, 1955 and Nickerson, 1950) and blended as described above.

QUANTITATIVE DETERMINATION OF MICROORGANISMS

The samples were assayed for a total number of bacteria. In addition special groups of micro-organisms were counted including lactics, yeasts, moulds and coliforms. Dilutions made as follows were used in the determinations. From the blended samples three dilutions of 10^2, 10^3, 10^4 were made in distilled water. Aliquots were plated to give 10^3, 10^4 and 10^5 dilutions as desired. After placing the aliquots into each of the plates, the melted and cooled agar media were poured immediately and after distributing the sample were allowed to solidify. The colonies were counted with the aid of a Quebec colony counter after three days incubation at 30°C. The three plates were counted that contained from 30 to 300 colonies.

Total numbers

Total numbers were estimated by plate counts on a medium containing the following:

Tryptone (Bacto)	5.0 gm.
Yeast extract (Bacto)	2.5 gm.
Glucose	1.0 gm.
Agar (Bacto)	15.0 gm.
Distilled water	1000 ml.

(Final pH adjusted to 7.0 before autoclaving).

This medium encourages the growth of widely different types of

micro-organisms (mainly bacteria) and in replicate experiments gave relative results.

Yeasts

Yeasts were counted on potato dextrose agar ("Difco"). The reaction of the medium was adjusted to pH 4.5 before autoclaving as specified by American Public Health Association (1948).

Lactic acid bacteria

Freshly prepared tomato juice agar was used for the estimation of lactics and contained: (Society of American Bacteriologists, 1957)

Tryptone (Bacto)	10.0 gm.
Yeast extract (Bacto)	10.0 gm.
Agar (Bacto)	12.0 gm.
Distilled water	800 ml.

Heat to 100°C and adjusted to pH 7.2 before autoclaving, then add

Tomato juice	250 ml.

Tomato juice was taken from canned unseasoned tomatoes (commercial tomato juice preparations contain preservatives). It was prepared by filtering juice from a can through cheese cloth, refrigerating overnight, filtering and then autoclaving to precipitate proteins. After cooling and refrigerating for

12 hr the preparation was refiltered and adjusted to pH 7.0. The tomato juice agar was not heated excessively as this destroyed its solidifying properties. A small amount of precipitate appeared in the medium and this was dispersed throughout the medium by mixing before pouring the plates and did not effect the quality of the medium.

Moulds

Moulds were counted on Sabouraud dextrose agar ("Difco").

Coliforms

This group of bacteria was estimated as described (Recommended methods for the microbiological examination of foods, American Public Health Association, (1958)) by the most probable number (MPN) method. Determinations were made by inoculating five tubes of each of the following aliquots (10.0, 1.0 and 0.1 ml) of a 10^2 dilution of the sample. The aliquots were added to suitable volumes of brilliant green broth ("Difco") tubed with Durham tubes included to show the presence of gas. After incubation at 37°C for 48 hr the number of positive tubes was compared to the standard chart (Recommended methods for the microbiological examination of foods, American Public Health Association (1958)) and the number of coliforms recorded.

TETRAZOLIUM FLOODING TECHNIQUE

This method was originally tested as a means of differentiating colonies from fruit particles (Solberg and Proctor, 1960). Each plate after incubation was removed from the incubator and was flooded with 2 ml of a 0.1% aqueous solution of 2,3,5-triphenyltetrazolium chloride. The flooded plate was gently rocked back and forth and from side to side several times so that the entire area was covered by the solution. The excess solution was poured off, the Petri dish cover replaced and the plate inverted and allowed to remain at room temperature for 3-5 hr. The metabolizing bacterial colonies normally reduced the indicator solution within the first hour but the additional time was necessary to allow slower metabolizing colonies to reduce the tetrazolium and this resulted in colour intensification within all the colonies. In counting the colonies the Quebec colony counter was found satisfactory. The method was used for estimating total numbers and for lactics but was abandoned when repeated tests indicated that estimating the number of colonies before and after flooding with the dye gave similar results.

DETERMINATION OF pH OF THE FRUIT

The pH of the fruits was determined with a Beckman glass electrode pH meter. In the beginning of the study the pH determinations were made respectively on the whole fruit

suspension, on the supernatant, and on the sediment after centrifuging. But in the latter part of the study pH determinations were made only on the whole suspension, since simultaneous comparative determinations by the three methods agreed and the whole suspension method was easier. The pH meter was standardized with pH 7.0 buffer.

EXPERIMENTAL INOCULATION OF RASPBERRIES WITH ESCHERICHIA COLI

The inoculum of Escherichia coli (Mac No. 22) was grown in nutrient broth for 48 hr at 37°C, the cells were centrifuged and suspended in normal saline (0.9% NaCl). The berries were placed on a clean sheet of paper and spread in an even layer, then the suspension of Escherichia coli in normal saline was sprayed uniformally over the berries with an atomizer and covered with a clean plastic tray. After 3-4 hr at room temperature the berries were divided into three equal lots. One portion was treated as the 0 hr sample; one portion was placed in refrigerator (10°C) for 48 hr; the third portion placed in the deepfreeze (-17°C) for 96 hr. The determinations of most probable numbers of coliforms were made by inoculating in brilliant green bile broth as described previously.

EXTRACTION OF ANTHOCYANIN PIGMENT OF RASPBERRIES

The anthocyanin in the present study was isolated from fresh raspberries. The raspberries were macerated in a Waring blendor and the pulp removed from the juice by pressing through cheese cloth. The pigment was extracted following the method of Pratt, Powers and Somaatmadja, 1960. To the fruit juice was added acidified (1% HCl) N-butanol; 2 parts to 1 part of fruit juice. The pigment was extracted from the solvent into an aqueous phase by the addition of petroleum ether to the pigment-butanol solution. The aqueous phase was separated and concentrated to thick syrup in a rotary evaporator at a low temperature and used for various tests.

DETERMINATION OF BACTERICIDAL PROPERTIES OF RASPBERRY PIGMENT

The bactericidal property of the pigment was tested against Escherichia coli. The organism was grown in nutrient broth and seeded on Endo agar plates. The surface of the agar was dried by leaving the poured plate in the incubator at 37°C and then inoculum was spread uniformally with a glass rod. Filter paper discs impregnated with the pigment preparation were placed on the seeded agar surface and allowed to stand in the refrigerator for six hours, before incubating the plates at 37°C for 48 hr. The diameter of the inhibition zones was measured in millimeters.

COLLECTION AND MAINTENANCE OF CULTURES

During this study colonies were picked at random from the plates and isolated as a pure culture on the same media as they were growing initially. Then the bacteria were maintained on nutrient agar ("Difco") with 0.5% yeast extract (Bacto). The yeasts were maintained on Sabouraud maltose agar ("Difco") and the moulds on Potato Dextrose agar ("Difco"). All the cultures were stored at 16°C in a cold room and transfers made every three months of yeasts and bacteria, whereas the moulds were not transferred.

RESULTS

In this study 44 samples of strawberries and 23 raspberry samples were collected. Data concerning the time of sampling, the variety, the source and rainfall are given in Table I. As outlined in "Methods" section these samples were taken to the laboratory and assayed for total numbers of microflora, fungi, yeasts, lactobacilli and coliforms. The examinations were performed on the fresh (untreated) fruit and after storage at room temperature for 48 hours; after storage at 10°C for 48 hours, and 96 hours; and after storage at -17°C for 96 hours. The individual data for each sample are given in the Appendix Tables I-V. The effects of these storage conditions on each group in the microbial population are considered separately.

NUMBER OF MICRO-ORGANISMS ON FRESH STRAWBERRIES AND RASPBERRIES

Microbiological assay of strawberries from Macdonald College and Experimental Station, Canadian Department of Agriculture, L'Assomption indicated that the microbial content of different lots of fresh material varied over wide range. The average counts are given in Table II. The total microbial count on strawberries from L'Assomption was four times as high as on samples from Macdonald College ($P < 0.05$) which might be expected considering differences in sampling methods for the two sites. The other groups of organisms do not vary significantly on the strawberries from

TABLE I

INFORMATION CONCERNING INDIVIDUAL SAMPLES OF STRAWBERRIES AND RASPBERRIES

Sample No.	Date	Variety	Total Rainfall in the Last 3 Days (Inches)
Strawberries from Macdonald College			
1	17/6	Sparkel	0.7
2	21/6	Sangua	0.68
3	21/6	Premier	"
4	22/6	Premier	0.4
5	22/6	Valentine	"
6	22/6	Sangua	"
7	23/6	Redcoat	-
8	23/6	Sparkel	-
9	23/6	Valentine	-
10	24/6	Sparkel	0.58
11	27/6	Premier	0.6
12	27/6	Sangua	"
13	27/6	Valentine	"
14	2/7	Redcoat	0.12
15	2/7	Sparkel	"
16	3/7	Premier	0.02
17	8/7	Premier	0.08
18	8/7	Sparkel	"
L'Assomption Experimental Station			
19	21/6	Redcoat	0.67
20	21/6	Cavalier	"
21	21/6	Senator Dunlap	"
22	21/6	Grenadier	"
23	21/6	Pocahantas	"
24	21/6	Early Dawn	"
25	27/6	Grenadier	0.63
26	27/6	Armore	"
27	27/6	Sparkel	"
28	27/6	Cavalier	"
29	27/6	Early Dawn	"
30	27/6	Senator Dunlap	"
31	27/6	Pocahantas	"
32	27/6	Guardsman	"
33	27/6	Redcoat	"
34	27/6	Tenn Beauty	"

Table I (Continued)

INFORMATION CONCERNING INDIVIDUAL SAMPLES OF STRAWBERRIES AND RASPBERRIES

Sample No.	Date	Variety	Total Rainfall in the Last 3 Days (Inches)
L'Assomption Experimental Station			
35	1/7	Guardsman	0.16
36	1/7	Redcoat	"
37	1/7	Sparkel	"
38	1/7	Cavalier	"
39	8/7	Grenadier	0.56
40	8/7	Guardsman	"
41	8/7	Senator Dunlap	"
42	8/7	Early Dawn	"
43	8/7	Redcoat	"
44	8/7	Sparkel	"
Raspberries from Macdonald College			
1	15/7	Viking	-
2	18/7	Latham	0.5
3	25/7	Viking	1.72
4	26/7	Viking	1.07
5	26/7	Newburg	"
6	26/7	Viking	"
7	27/7	Newburg	-
8	28/7	Viking	0.06
9	28/7	September	"
10	28/7	Latham	"
11	29/7	September	"
12	29/7	Newburg	"
13	29/7	Viking	"
14	31/7	Newburg	0.16
15	31/7	September	"
16	31/7	Newburg	"
17	1/8	Latham	0.10
18	1/8	September	"
19	1/8	Latham	"
20	1/8	September	"
21	4/8	Latham	-
22	4/8	September	-
23	4/8	Newburg	-

TABLE II

MICROFLORA OF FRESH STRAWBERRIES AND RASPBERRIES

	Average Counts/Gram Wet Weight (Thousands)				
	Total Microbial Count	Fungi	Lacto-bacilli	Yeasts	Coli-forms
Strawberries from Macdonald College. (Average of 18 Determinations)	1542	666	241	805	0.010
Strawberries from L'Assomption. (Average of 26 Determinations)	6295	587	142	578	0.013
Raspberries from Macdonald College (Average of 23 Determinations)	900	490	176	760	0.0004

two locations. "Captan" spray does not influence significantly the fungal population of strawberries as show in Table III. The original fungal population was not significantly different in numbers nor was there a significant difference after storage at room temperature for 48 hours. Raspberries carry a smaller population of micro-organisms as compared to strawberries (Table II). The results in Table II show that there is not a great difference in numbers between the population of any of the groups of micro-organisms on strawberries and raspberries.

EFFECT OF STORAGE AT ROOM TEMPERATURE

The results presented in Table IV show that total number of micro-organisms on strawberries and raspberries increases and the numbers belonging to each group increase also, when held at room temperature for 48 hours. The results in Table III show that even "Captan" spray does not influence the increase in fungal population of strawberries during this storage treatment. The pH of strawberries rose slightly during this treatment from 3.3 to 3.5, whereas the pH of raspberries showed a slight decrease from 3.4 to 3.3. The raspberries carried comparatively fewer organisms than strawberries but demonstrated a similar increase in numbers.

EFFECT OF STORAGE AT 10°C

(a) 48 hours storage

During this storage treatment the total number of organisms on strawberries and raspberries decreased

TABLE III

EFFECT OF "CAPTAN" SPRAY ON FUNGAL POPULATION OF STRAWBERRIES

	Count/Gram Wet Weight. Fresh Strawberries. (Thousands).	Count/Gram Wet Weight After 48 Hr at (25°C) Room Temperature (Thousands)
Strawberries Sprayed with "Captan". (L'Assomption) (Average of 26 Determinations)	567	871
Strawberries Unsprayed. (Macdonald College). (Average of 18 Determinations)	677	888
Difference	110*	17*

* Not significant. P = 0.5

TABLE IV

EFFECT OF STORAGE AT ROOM TEMPERATURE (25°C)

	Strawberries		Raspberries	
	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)
Total Microbial Count	3918	7343	900	1565
Fungi	769	880	490	681
Yeasts	191	345	176	283
Lactobacilli	691	971	760	997
Coliforms	0.011	0.015	0.0004	0.0006

TABLE V

EFFECT OF STORAGE AT 10°C FOR 48 HOURS

	Strawberries		Raspberries	
	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)
Total Microbial Count	3918	2460	900	732
Fungi	769	678	490	457
Yeasts	191	147	176	140
Lactobacilli	691	506	760	622
Coliforms	0.011	0.0042	0.0004	0.00015

appreciably (Table V) as did the numbers of each microbial group, except the numbers of fungi increased significantly ($P < 0.001$) on strawberries from Macdonald College. This increase can be explained by the use of "Captan" spray on the L'Assomption plantation while at Macdonald College plants were not sprayed. The average pH of strawberries and raspberries increased from 3.3 to 3.55 and from 3.4 to 3.5 respectively. Raspberries carried fewer organisms as compared to strawberries except for lactobacilli.

(b) 96 hours storage

The data in Table VI indicate that the numbers of micro-organisms belonging to all the groups studied, decreased considerably after storage at refrigerator temperatures for 96 hours. Comparison of data (Table V and VI) showed that the berries carried fewer organisms after this storage treatment than at 10°C for 48 hours. The pH of strawberries and raspberries also increased from 3.3 to 3.5 and from 3.4 to 3.5 respectively.

EFFECT OF STORAGE AT BELOW FREEZING TEMPERATURES

The results (Table VII) show that the numbers of micro-organisms (total count, fungi, yeasts, lactics and coliforms) on berries decreased greatly, as result of storage at sub-freezing temperature (-17°C). The reduction varied from 62% of the yeasts to 90% of the coliforms, while the fungi and lactics were each reduced by 70%. After this

TABLE VI

EFFECT OF STORAGE AT 10°C FOR 96 HOURS

	Strawberries		Raspberries	
	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)
Total Microbial Count	3918	1875	900	628
Fungi	769	398	490	393
Yeasts	191	152	176	119
Lactobacilli	691	454	760	517
Coliforms	0.011	0.0017	0.0004	0.00007

TABLE VII

EFFECT OF STORAGE AT BELOW FREEZING TEMPERATURE (-17°C)

	Strawberries		Raspberries	
	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)	Count/Gram Wet Weight Control. (Thousands)	Count/Gram Wet Weight After Treatment (Thousands)
Total Microbial Count	3918	509	900	397
Fungi	769	231	490	326
Yeasts	191	71	176	80
Lactobacilli	691	208	760	309
Coliforms	0.011	0.0011	0.0004	0.00007

TABLE VIII

CLASSIFICATION OF MICRO-ORGANISMS ISOLATED FROM FRESH AND FROZEN BERRIES

A. STRAWBERRIES

Fresh Fruit	Numbers	Frozen Fruit	Numbers
BACTERIA		BACTERIA	
Bacillus cereus var. mycoides	9	Bacillus cereus var. mycoides	1
Flavobacterium diffusum	4	Flavobacterium diffusum	6
Alcaligenes spp	2	Alcaligenes sp	1
Actinomyces sp	1	Sarcina spp	5
Sarcina spp	2		
Spirillum spp	2		
Pseudomonas sp	1		
YEASTS		YEASTS	
Saccharomyces spp	6	Saccharomyces spp	2
Hansenula spp	2	Hansenula spp	3
Pichia sp	1	Pichia sp	1
Schizosaccharomyces sp	1	Schizosaccharomyces sp	1
		Candida spp	2
FUNGI		FUNGI	
Aspergillus spp	7	Aspergillus spp	7
Penicillium spp	5	Penicillium spp	9

TABLE VIII (Continued)

CLASSIFICATION OF MICRO-ORGANISMS ISOLATED FROM FRESH AND FROZEN BERRIES

A. STRAWBERRIES

Fresh Fruit	Numbers	Frozen Fruit	Numbers
FUNGI		FUNGI	
Alternaria spp	4	Alternaria spp	4
Oospora spp	2	Oospora sp	1
Cladosporium sp	1	Cladosporium spp	3
Fusarium spp	4	Fusarium spp	4
Dactylium sp	1		

B. RASPBERRIES

Fresh Fruit	Numbers	Frozen Fruit	Numbers
BACTERIA		BACTERIA	
Bacillus cereus var. mycoides	2		
Flavobacterium spp	2	Flavobacterium spp	2
Actinomyces sp	1		
Sarcina sp	1	Sarcina spp	2
Spirillum sp	1		
Pseudomonas sp	1		

TABLE VIII (Continued)

CLASSIFICATION OF MICRO-ORGANISMS ISOLATED FROM FRESH AND FROZEN BERRIES

B. RASPBERRIES

Fresh Fruit	Numbers	Frozen Fruit	Numbers
YEASTS		YEASTS	
Saccharomyces spp	5	Schizosaccharomyces sp	1
Candida spp	5	Candida sp	1
		Hansenula sp	1
		Pichia sp	1
FUNGI		FUNGI	
Aspergillus spp	8	Penicillium sp	1
Penicillium spp	8	Oospora sp	1
Alternaria spp	5	Alternaria spp	3
Oospora sp	1		
Cladosporium sp	1		
Fusarium spp	3		

treatment the pH of the strawberries dropped from 3.4 to 3.1 and from 3.4 to 3.2 in raspberries. Commercially frozen, unsliced and unsugared berries from the local markets were also assayed, and the results showed that the total numbers of micro-organisms and the numbers of the other groups of organisms were so low to be of any significance.

CLASSIFICATION OF MICRO-ORGANISMS ISOLATED FROM FRESH AND FROZEN BERRIES

Pure cultures of organisms were isolated and bacteria classified according to Bergey et al. (Bergey's Manual of Determinative Bacteriology, Williams and Williams, Baltimore, Seventh Edition, 1953), yeasts were classified according to Lodder and Kreger-Van Rij (The Yeasts, A Taxonomic Study, Interscience Publishers, New York, 1952), and fungi according to Gilman (A Manual of Soil Fungi, The Iowa State College Press, Ames, 1945).

The results in Table VIII gives the names of organisms selected at random from fresh strawberries. Amongst the bacteria, Flavobacterium spp, Bacillus cereus var. mycoides, Sarcina spp, and Spirillum spp were the most common. The yeasts isolated included Saccharomyces spp, Hansenula spp, Pichia sp and Schizosaccharomyces sp. The fungi isolated were species of Aspergillus, Penicillium, Alternaria, Oospora, Cladosporium, Fusarium and Dactylium. The bacteria from frozen strawberries were classified as Bacillus cereus var. mycoides, Flavobacterium diffusum and Sarcina spp. Yeasts

isolated belonged to the same genera as those found on fresh strawberries but in addition Candida spp were isolated from frozen strawberries. The fungi isolated were species of Aspergillus, Penicillium, Alternaria, Oospora, Cladosporium and Fusarium.

Fresh raspberries carried bacteria (Table VIII) belonging to Flavobacterium spp, Bacillus cereus var. mycoides, Spirillum sp, Sarcina sp and Pseudomonas sp. Two species of yeasts were isolated, Candida and Saccharomyces. The fungi were classified as species of Cladosporium and Oospora. Frozen raspberries, carried only two species of bacteria, Sarcina and Flavobacterium and four species of yeasts namely, Candida, Schizosacchromyces, Hansenula and Pichia while three species of fungi were isolated, Alternaria, Penicillium and Oospora.

RELATIONSHIP BETWEEN MICROFLORA AND VARIETY OF FRUIT

The results presented in Figs. I, II, III (Appendix Table VI) indicate the variety of the berries has little influence on the microbial population of strawberries and raspberries. All groups of organisms behave the same way under all the storage conditions studied on these varieties, however, minor differences are apparent eg. the initial load varies considerably; the effect of storage at room temperature on the numbers of fungi shows a marked increase with some varieties and a very small increase with others, etc. These

FIG. I

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

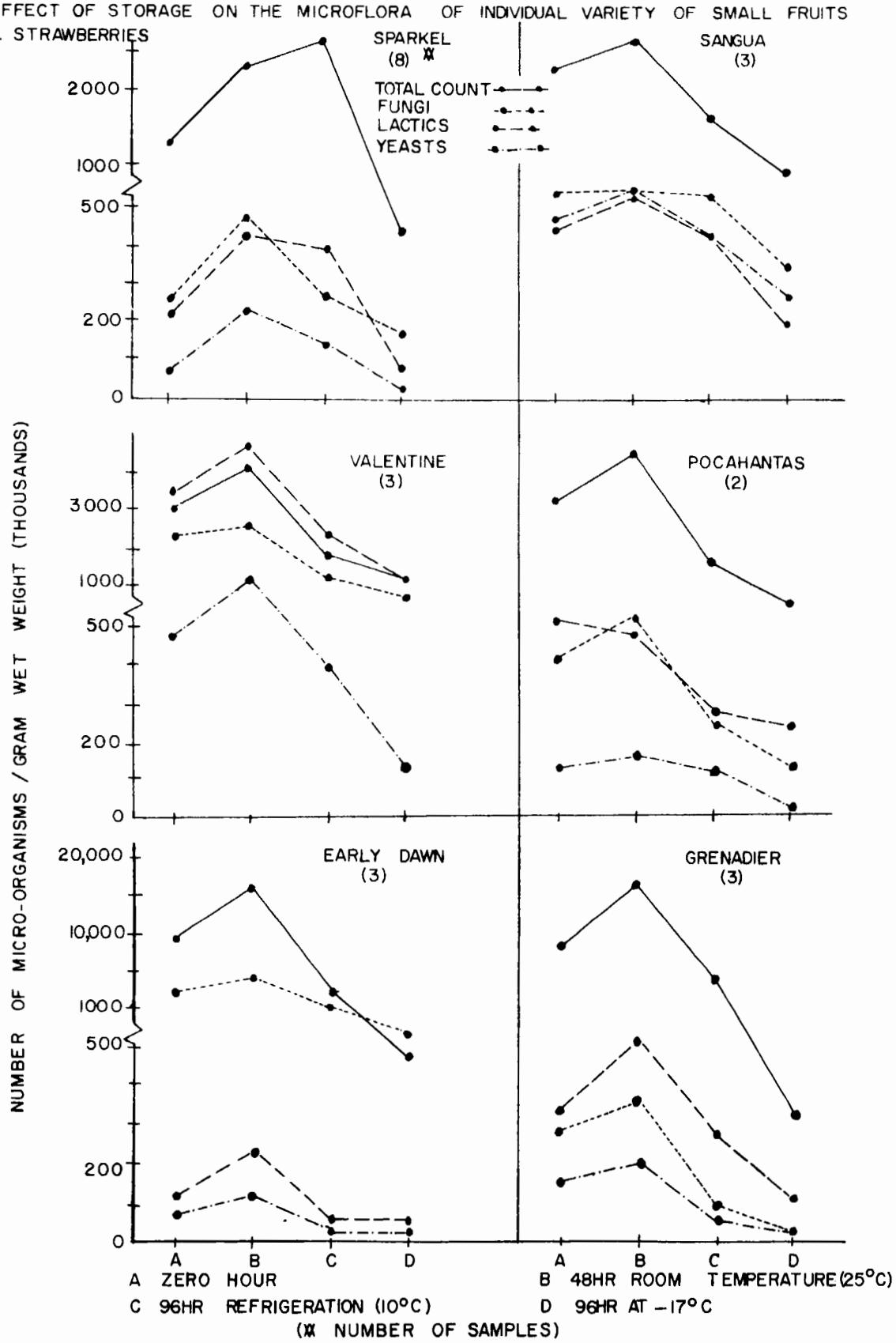


FIG. II

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

A. STRAWBERRIES (CONTINUED)

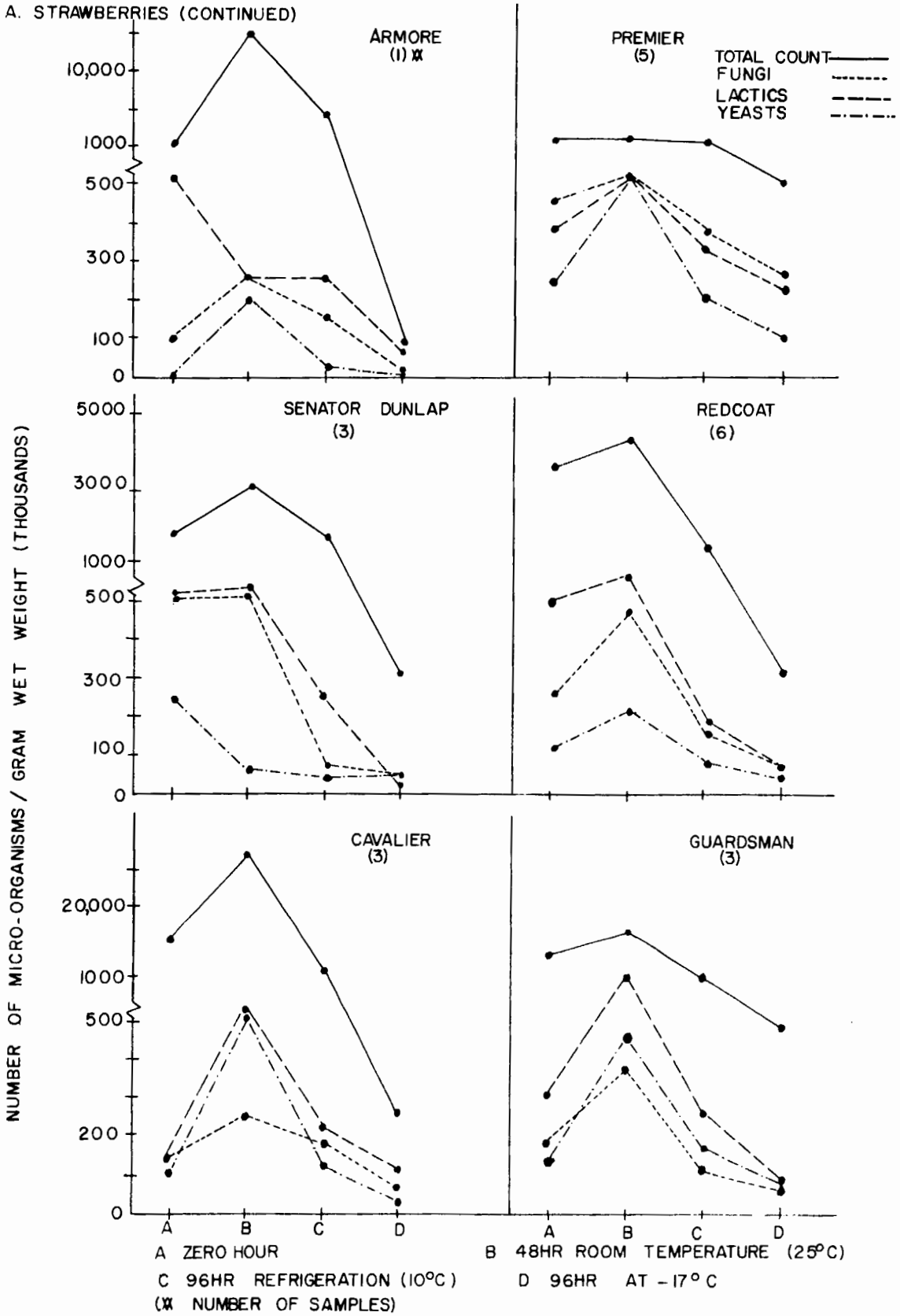
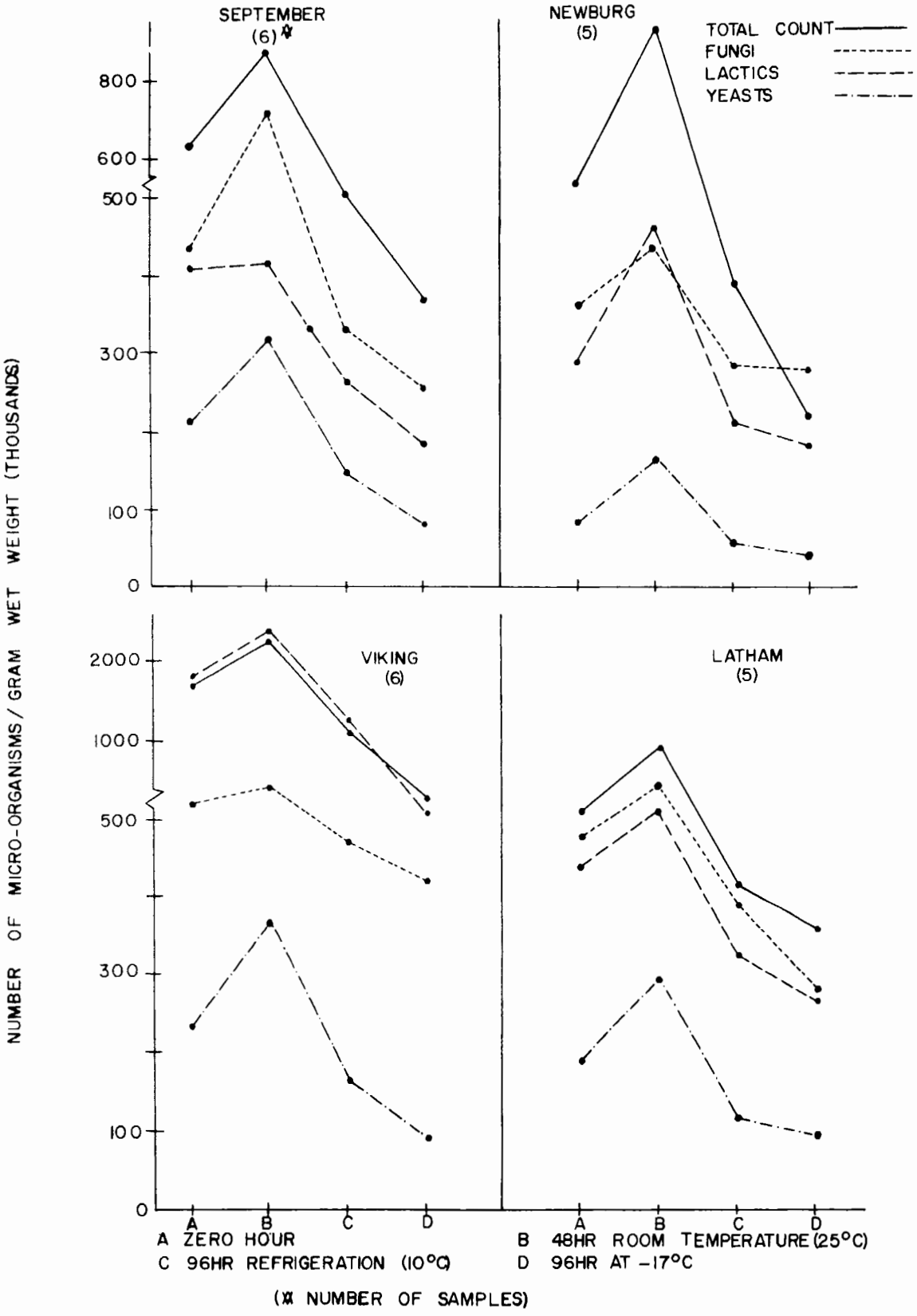


FIG. III

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

B. RASPBERRIES



data indicate that these varieties behave differently as regards their microbial flora but the tests are too limited to demonstrate the significance of this difference.

INFLUENCE OF RAINFALL ON THE TOTAL MICROFLORA OF STRAWBERRIES AND RASPBERRIES

The data presented in Figs. IV and V indicate that the amount of rainfall bears no exact relationship to microbial population of strawberries and raspberries. The results showed that the splashing and washing effects of rain compensate each other with strawberries, whereas in raspberries (Fig. V) washing effect is more apparent. The splashing effect is more marked in strawberries, whenever two successive rainfalls are separated by a dry spell.

ARTIFICIAL INOCULATION OF RASPBERRIES WITH ESCHERICHIA COLI (MAC# 22)

The results Fig. VI (Appendix Table VII) showed that 56% of the Escherichia coli were nonviable after holding the berries for 48 hours at 10°C, while 86% were nonviable after storage at -17°C for 96 hours.

BACTERICIDAL PROPERTIES OF THE ANTHOCYANIN PIGMENT OF RASPBERRIES

Endo agar (Difco) plates seeded with Escherichia coli (Mac # 22) showed (Plate I) that after incubation at 37°C for 48 hours, very distinct zones of inhibition were produced around filterpaper discs impregnated with extracted pigment. The diameter of the inhibition zones varied from

FIG. IV

EFFECT OF RAINFALL ON TOTAL MICROFLORA OF STRAWBERRIES

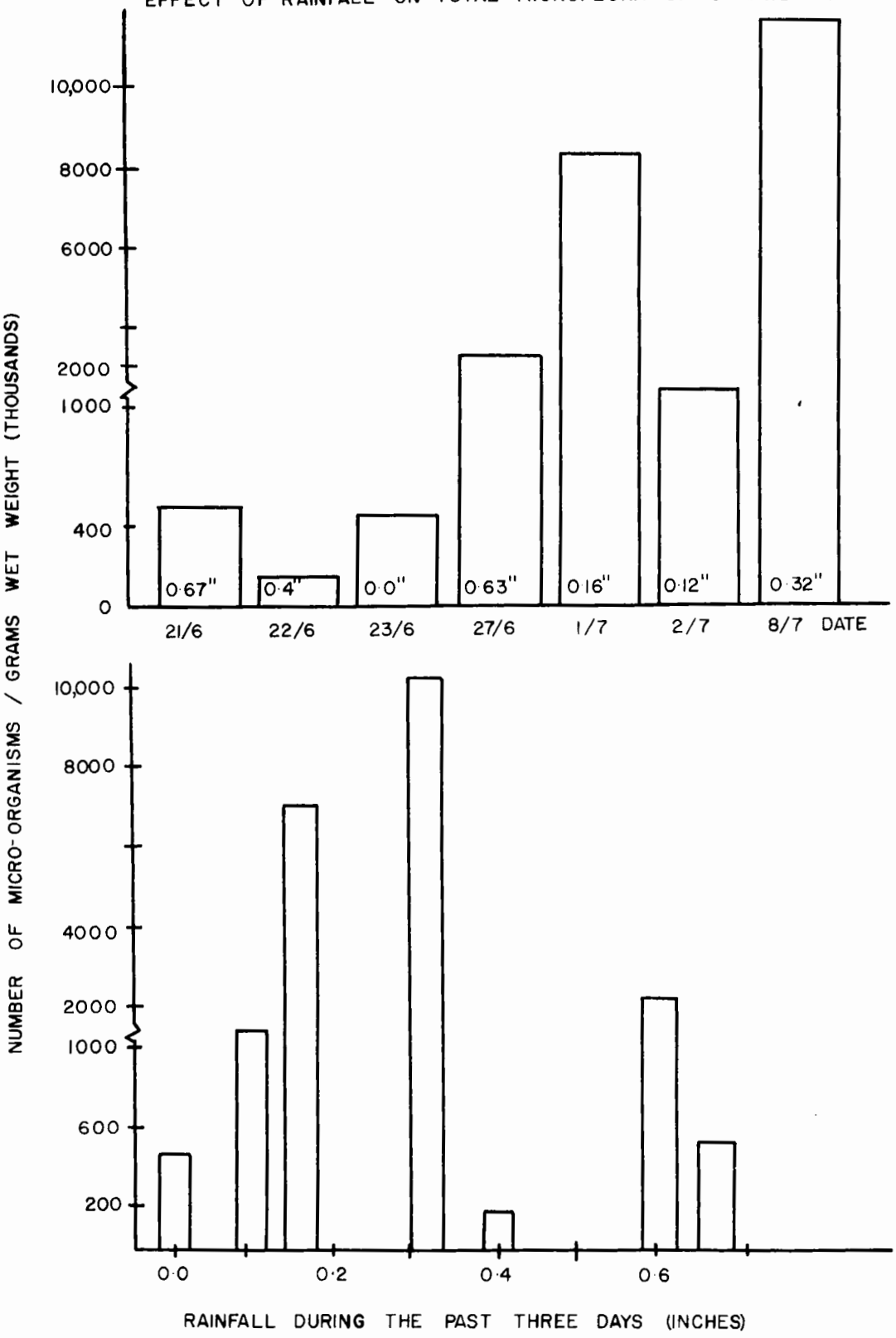


FIG.V

EFFECT OF RAINFALL ON TOTAL MICROFLORA OF RASPBERRIES

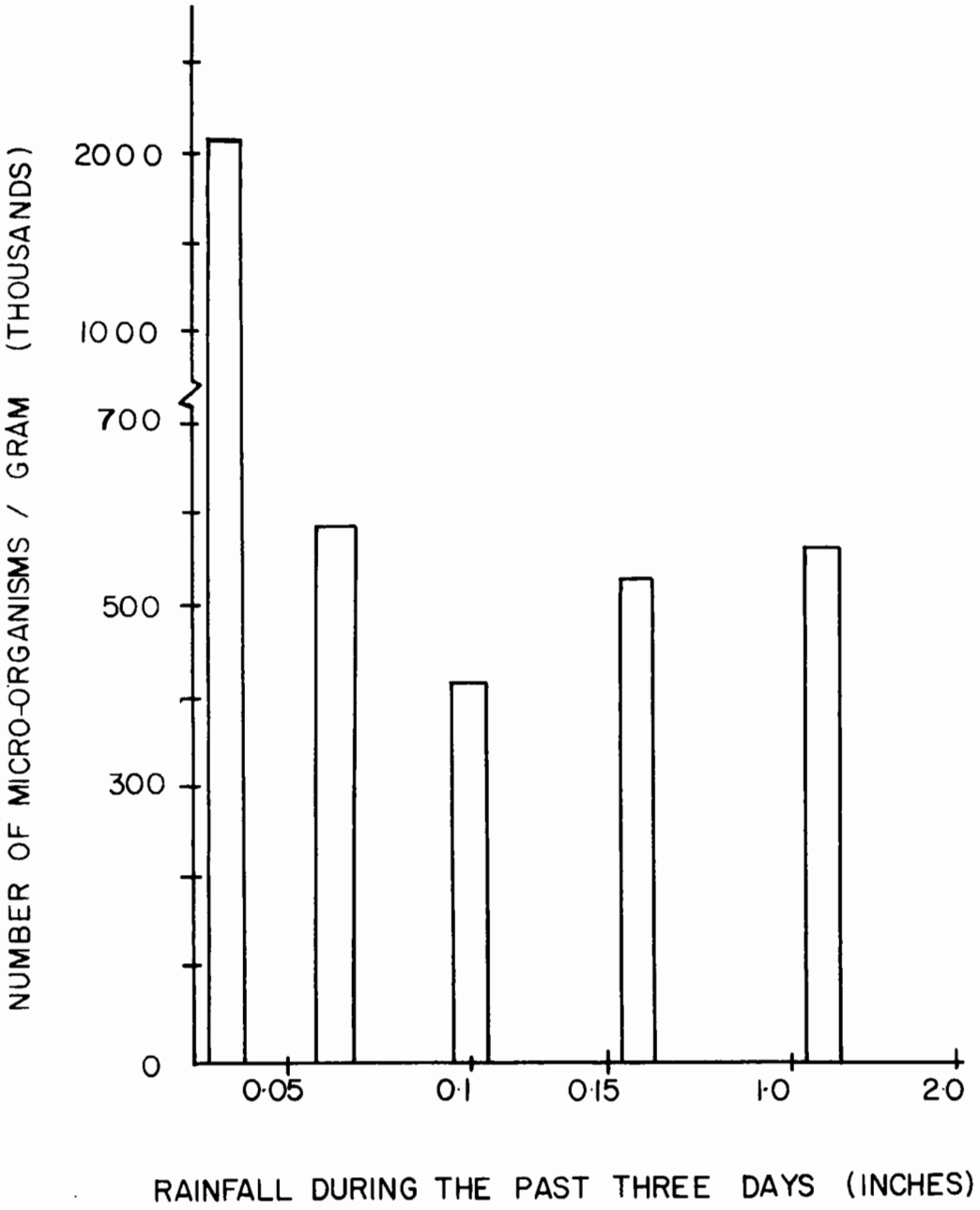
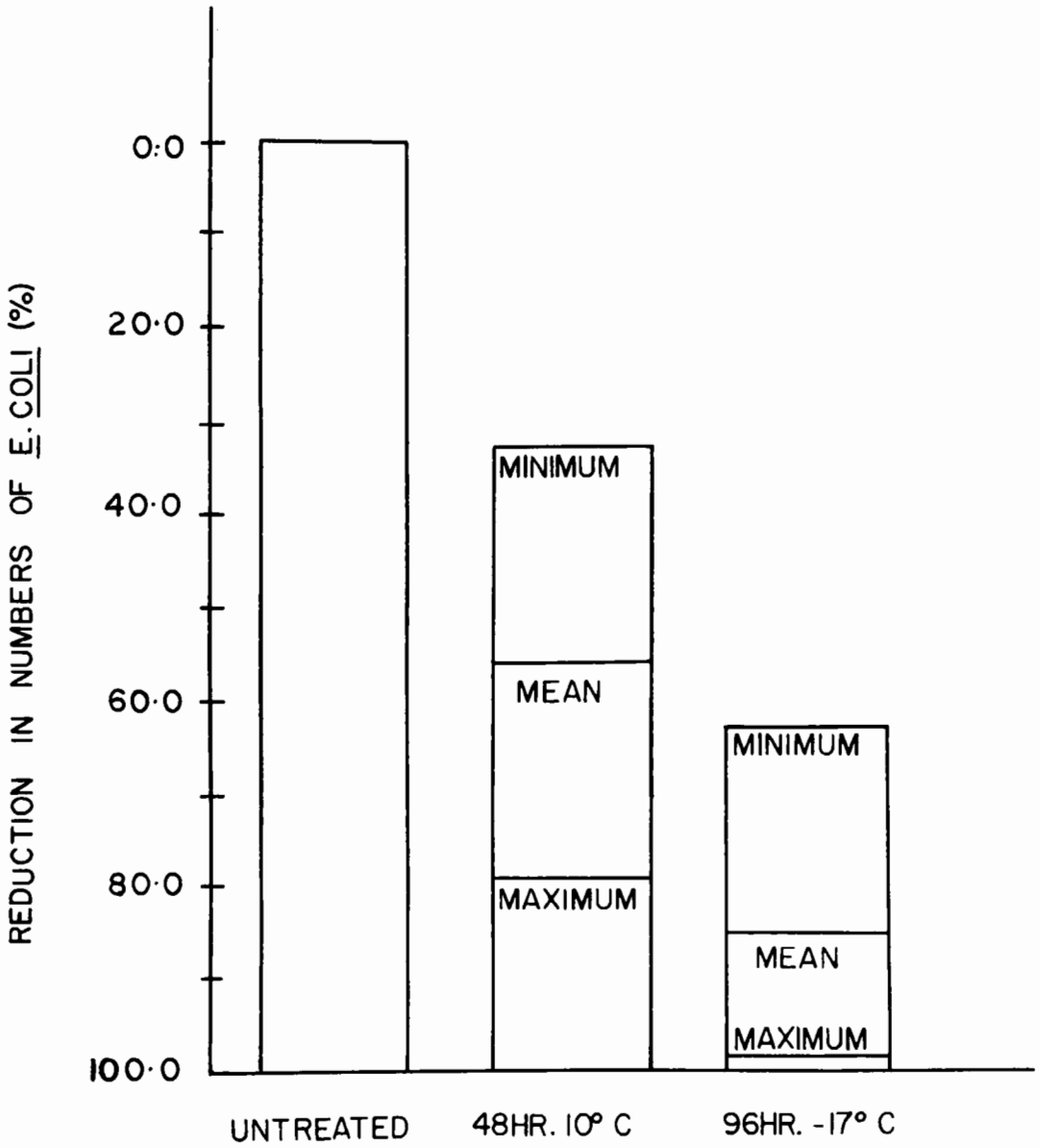


FIG. VI

ARTIFICIAL INOCULATION OF RASPBERRIES WITH E. COLI



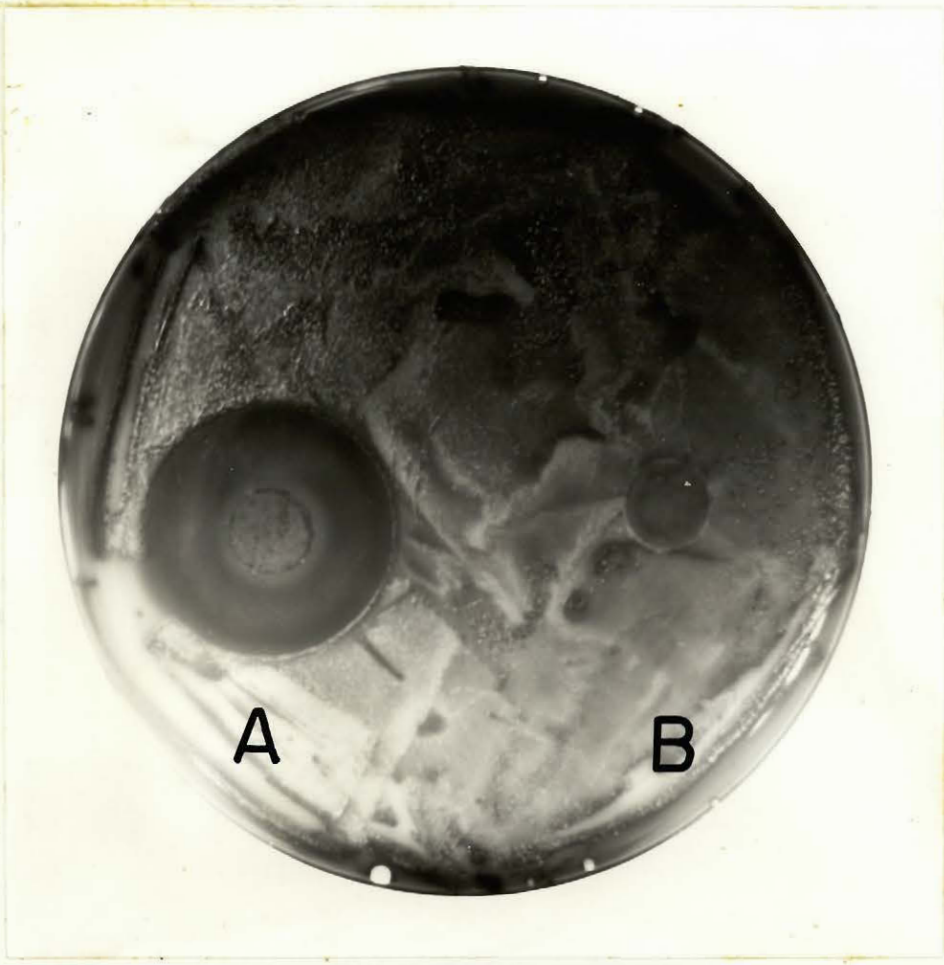


Plate.1. Bactericidal property of Anthocyanin pigment

(A) pH of the pigment extract 3.8

(B) pH of the pigment extract 6.8

24mm to 30mm. This property of the pigment is greatly influenced by the pH of the preparation, as shown in Plate I the activity of the pigment is lost when the pH is raised to 4.0 but is active below pH 3.5.

DISCUSSION

The results of this study indicate a wide variation in the microbial content of fresh strawberries and raspberries, due to their close proximity to soil, weather conditions and other sources of contamination, these results are substantiated by the previous workers Lochhead and Jones (1936) and Mundt (1950). Numbers of micro-organisms on strawberries varied from 1.5 to 6.2 million per gram wet weight and these figures are higher than those obtained by Berry (1934) and Magoon (1931). Quantitatively raspberries carried fewer micro-organisms than strawberries, which may be attributed to the latters proximity to soil, and this did not influence any particular group of organisms. Raspberries carried a great number of fungi, an observation which does not support Oliver and Rendle (1934), who claimed that fungi were never found on raspberries. Numbers of micro-organisms on strawberries increased after rainfall, this finding is substantiated by previous work (Mundt, 1950), whereas the numbers of micro-organisms decreased when ever the dry spell was short between the periods of rainfall. This indicates that washing and splashing effects of rain compensate each other. Conversely, the numbers of micro-organisms on raspberries decreased after rainfall, which was a constant feature and could be attributed primarily to the washing effect of rain and their distance from soil in contrast to

strawberries. The numbers of micro-organisms on strawberries from L'Assomption were significantly higher as compared to strawberries from Macdonald College, which may be either due to the influence of location, the way the samples were picked, the mannuring of the plantation at L'Assomption or the combination of all these factors. It was observed that the variety of the berries influenced the microbial content of the fruit and that all groups or organisms behaved the same way under different storage treatments. From these results no generalization could be made, as to which variety is superior to the other. Results indicated an increase in numbers of micro-organisms on strawberries and raspberries after storage for 48 hours at room temperature (25°C), due to ripening of berries and congenial conditions for the development of micro-organisms at this storage temperature. This was supported by previous work of Lochhead and Jones (1936) and Lüthi (1959).

The numbers of micro-organisms decreased when berries were held at 10°C (Refrigeration) for 48 hours, except for an increase in the numbers of fungi on strawberries from Macdonald College. This may be attributed either to the condition of the berry plantation at Macdonald College, which was weedy, had infection of black root rot and was not sprayed by any fungicide, whereas the plantation at L'Assomption had no weeds, was more vigorous and had no incidence of black root rot, and was sprayed with fungicide "Captan".

Numbers of micro-organisms decreased considerably after 96 hours storage at 10°C (Refrigeration), which is connected with the prolonged action of low temperature (Borgstrom, 1955) and low pH of berries, apparently unfavourable conditions for organisms to develop. A great decrease in the numbers of micro-organisms occurred when berries were held at -17°C for 96 hours, similar results were obtained by previous workers (Park, 1901; Prescott and Tanner, 1938; Berry, 1933a; Smart, 1934 and Magoon, 1932). The reduction in the numbers of micro-organisms on berries after storage at -17°C for 96 hours is attributed to sub-freezing temperature and to the low pH of the berries. These conclusions are substantiated by previous workers (Berry, 1932a; Beard and Cleary, 1932; McFarlane, 1940; Stille, 1950 and Lüthi, 1959). The results showed that storage at -17°C for 96 hours reduced the pH of berries. Another factor, is the presence of the bactericidal anthocyanin pigment in strawberries and raspberries. The bactericidal properties of anthocyanin have been reported previously by Pratt et al. (1960a; 1960b). Organisms surviving storage at subfreezing temperature may be members of species which are extremely psychrophilic as reported by Prescott et al. (1932) and Haines (1934). The results showed that the microbial population of frozen berries mainly consists of bacteria belonging to genera Flavobacterium, sarcina and Bacillus; yeasts belonging to genera Sacchuromyces, Hansenula, Candida and Pichia

and fungi belonged to genera Aspergillus, Alternaria, Penicillium and Fusarium. These results are substantiated by the previous workers (Nickerson, 1950; Hucker, 1954; and Smart, 1934).

Storage of artificially inoculated raspberries with E. coli at 10°C for 48 hours and -17°C for 96 hours showed that 56% and 86% of initial E. coli content was non viable at these storage treatments, respectively. These results are substantiated by previous workers (McCleskey and Christopher, 1941). The results indicated that low temperature markedly influenced the deleterious action of low pH and anthocyanin pigment. Experiments with anthocyanin pigment extracted from raspberries show that it had a bactericidal action against Escherichia coli and the results further pointed that bactericidal property of the pigment was lost as soon as the pH of the pigment extract was raised above 3.8, the natural pH of the raspberries. These results suggested the influence of low pH on the bactericidal properties of anthocyanin pigment. The results show that low temperature, pH and anthocyanin pigment, exert their influence on microbial flora of raspberries and strawberries, apart from other factors such as the location, condition of the plantation, the method of harvesting, fungicide sprays and storage treatments, which exert their influence on the microbial flora of raspberries and strawberries.

SUMMARY

The microbial flora of the small fruits, strawberries and raspberries, were investigated. Total numbers of micro-organisms and special groups, namely lactics, yeasts, fungi and coliforms were determined on the fresh berries and the berries after various treatments. The microbial-content of berries was influenced by the location of the plot, by rainfall, and by the method of harvesting. The variety of the berries influenced the microbial content but the data were not sufficient to draw any firm conclusions. Numbers of micro-organisms increased on strawberries after a rainfall and decreased on raspberries, which can be explained by the washing and splashing effects of the rain. Raspberries were found to have fewer micro-organisms than strawberries.

Storage at room temperature for 48 hours increased the numbers of micro-organisms on both strawberries and raspberries, while storage at 10°C for 48 hours and 96 hours decreased the numbers. Storage at sub-freezing temperature (-17°C) for 96 hours markedly lowered the numbers of micro-organisms. Raspberries were artificially inoculated with Escherichia coli but the numbers fell rapidly after storage at 10°C for 48 hours and -17°C for 96 hours. The anthocyanin pigment extracted from raspberries had bactericidal properties which were influenced by the pH. At low temperatures, the acid pH of berries and the anthocyanin pigment

have a decided influence on the microflora during storage.

Microbial isolates selected at random were classified and no particular group of organisms was predominant although after storage under some conditions some of the types appeared to be absent. Representative bactera, veasts and fungi were identified.

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Appendix Table I

EFFECT OF STORAGE CONDITION ON THE TOTAL NUMBER OF MICRO-ORGANISMS OF SMALL FRUITS

A. STRAWBERRIES FROM MACDONALD COLLEGE

		48 Hour Treatment		96 Hour Treatment	
Sample	Untreated	Room Temp. (25°C)	10°C	10°C	-17°C
1	133	146	265	-	20
2	1263	1670	1033	650	633
3	1216	1360	4100	3000	92
4	99	121	80	68	67
5	106	150	90	75	74
6	110	147	91	66	61
7	112	135	96	83	90
8	159	720	450	516	106
9	990	1933	723	606	546
10	221	281	188	160	176
11	3430	4660	1560	900	1833
12	5630	7760	4730	4060	2300
13	8000	10200	6760	5230	2533
14	316	356	243	156	173
15	2830	4860	2900	3660	180
16	2130	2760	3600	2000	165
17	506	620	450	316	375
18	520	723	406	353	266
Mean	1542	2144	1375	1288	536

(Counts/Gram Wet Weight, in Thousands)

Appendix Table I (Continued)

EFFECT OF STORAGE CONDITION ON THE TOTAL NUMBER OF MICRO-ORGANISMS OF SMALL FRUITS

B. STRAWBERRIES FROM L'ASSOMPTION

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
19	736	876	353	433	154
20	173	227	153	144	41
21	99	134	81	68	41
22	69	300	663	121	6
23	93	123	36	100	26
24	36	43	260	136	7
25	6300	9600	3950	8600	216
26	1230	15000	4000	4600	90
27	196	383	123	63	93
28	7060	9830	5830	3700	49
29	1900	3000	1500	900	1246
30	2750	4000	9960	3700	760
31	6400	8730	4400	3130	1680
32	1246	1503	996	720	650
33	183	183	130	93	104
34	64	18860	400	69	54
35	1960	2860	1400	1000	105
36	2400	4730	18600	2200	197
37	1560	6060	12860	11320	122
38	28100	88000	2830	1860	706
39	17530	38300	130	3000	713
40	26030	37000	3260	2130	713
41	2700	4900	2530	1330	118
42	27060	42300	7900	6630	211
43	18400	23300	6830	5900	1190
44	4900	5860	3400	2100	2553
Mean	6295	12542	3545	2462	455

(Counts/Gram Wet Weight, in Thousands)

Appendix Table I (Continued)

EFFECT OF STORAGE CONDITION ON THE TOTAL NUMBER OF MICRO-ORGANISMS OF SMALL FRUITS

C. RASPBERRIES FROM MACDONALD COLLEGE

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
1	8000	10200	6430	5230	2533
2	506	620	450	316	373
3	336	523	166	133	217
4	520	693	406	320	266
5	593	806	383	350	340
6	570	913	460	356	216
7	620	923	516	430	35
8	606	753	496	426	446
9	620	883	513	436	410
10	663	920	566	503	423
11	533	733	673	610	326
12	520	750	460	460	246
13	626	873	503	496	350
14	470	580	390	280	260
15	950	1160	900	830	520
16	210	310	150	106	140
17	480	740	390	320	280
18	230	340	186	171	154
19	570	700	500	450	340
20	1190	1850	950	900	690
21	720	1630	620	480	340
22	260	380	103	123	117
23	916	2280	730	720	310
Mean	900	1565	732	628	397

(Counts/Gram Wet Weight, in Thousands)

Appendix Table II

EFFECT OF STORAGE CONDITIONS ON NUMBERS OF FUNGI ON SMALL FRUITS

A. STRAWBERRIES FROM MACDONALD COLLEGE

		48 Hour Treatment		96 Hour Treatment	
Sample	Untreated	Room Temp. (25°C)	10°C	10°C	-17°C
1	21	29	13	14	10
2	53	63	6530	270	206
3	250	590	83	430	37
4	463	620	236	263	239
5	124	144	110	81	82
6	76	88	55	40	44
7	38	54	27	16	23
8	69	943	186	220	42
9	913	1250	736	576	470
10	74	104	54	42	47
11	440	596	346	250	273
12	1480	1646	1313	1236	760
13	5660	6600	5030	3230	2123
14	226	366	186	153	161
15	290	366	335	383	161
16	273	316	353	250	158
17	870	1063	776	770	610
18	883	1163	786	593	523
Mean	666	889	953	489	331

(Counts/Gram Wet Weight, in Thousands)

Appendix Table II (Continued)

EFFECT OF STORAGE CONDITIONS ON NUMBERS OF FUNGI ON SMALL FRUITS

B. STRAWBERRIES FROM L'ASSOMPTION

		48 Hour Treatment		96 Hour Treatment	
Sample	Untreated	Room Temp. (25°C)	10°C	10°C	-17°C
19	66	326	43	55	37
20	75	127	60	43	12
21	73	80	53	48	20
22	39	41	23	10	14
23	390	430	323	313	96
24	13	21	0	0	10
25	53	123	10	96	18
26	100	250	0	153	1
27	130	280	86	56	83
28	253	370	170	113	166
29	140	226	86	63	73
30	563	580	136	110	153
31	420	640	316	186	166
32	343	633	236	110	160
33	136	236	120	66	57
34	10	71	80	176	1
35	27	213	166	13	18
36	183	866	132	340	193
37	150	313	102	623	134
38	100	263	806	416	11
39	790	936	170	143	8
40	186	270	246	190	17
41	1386	1503	170	53	2
42	8300	12310	6400	4200	2180
43	940	1003	410	326	26
44	395	536	173	110	263
Mean	587	872	404	308	151

(Counts/Gram Wet Weight, in Thousands)

Appendix Table II (Continued)

EFFECT OF STORAGE CONDITIONS ON NUMBERS OF FUNGI ON SMALL FRUITS

C. RASPBERRIES FROM MACDONALD COLLEGE

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
1	300	660	503	323	212
2	870	1063	776	770	613
3	320	493	156	110	186
4	883	1163	886	593	523
5	893	1040	666	506	683
6	870	950	720	636	580
7	603	533	710	650	613
8	760	863	590	536	550
9	636	960	636	546	416
10	723	870	636	546	446
11	736	930	883	856	476
12	260	333	226	220	163
13	936	103	810	743	563
14	220	310	170	130	130
15	660	1320	650	600	470
16	120	220	90	86	60
17	180	240	140	120	70
18	190	210	150	120	90
19	190	270	170	130	80
20	310	670	300	200	310
21	470	1110	430	380	170
22	130	200	100	116	58
23	123	240	120	130	46
Mean	490	681	457	393	326

(Counts/Gram Wet Weight, in Thousands)

Appendix Table III

EFFECT OF STORAGE CONDITIONS ON NUMBERS OF LACTOBACILLI ON SMALL FRUITS

A. STRAWBERRIES FROM MACDONALD COLLEGE

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
1	22	30	140	195	14
2	39	100	300	293	13
3	433	526	120	630	83
4	206	223	162	149	144
5	189	217	158	136	93
6	72	97	56	40	41
7	32	33	25	16	13
8	36	986	700	210	28
9	916	1660	703	600	416
10	43	55	33	20	17
11	443	580	320	230	323
12	1226	1430	1373	903	516
13	9230	12230	7760	6130	2660
14	193	306	113	80	92
15	323	430	356	1660	166
16	196	360	413	180	160
17	706	930	616	530	440
18	310	486	306	250	180
Mean	805	1148	758	680	299

(Counts/Gram Wet Weight, in Thousands)

Appendix Table III (Continued)

EFFECT OF STORAGE CONDITIONS ON NUMBERS OF LACTOBACILLI ON SMALL FRUITS

B. STRAWBERRIES FROM L'ASSOMPTION

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
19	63	796	43	53	41
20	35	65	21	10	16
21	66	78	46	39	10
22	196	83	30	243	25
23	260	26	120	136	133
24	8	16	100	0	10
25	100	153	50	50	260
26	650	250	0	250	473
27	140	313	133	93	73
28	400	543	260	170	263
29	166	233	113	60	70
30	1023	590	300	250	80
31	760	916	553	400	340
32	440	520	283	193	193
33	180	246	123	93	97
34	20	1900	36	25	1
35	163	63	136	106	11
36	123	643	235	236	170
37	163	340	763	303	144
38	10	1706	270	456	70
39	706	1370	113	523	17
40	306	4000	610	493	56
41	8530	1650	960	476	6
42	156	406	180	123	97
43	2433	3000	636	573	74
44	640	736	490	346	310
Mean	578	794	254	228	117

(Counts/Gram Wet Weight, in Thousands)

Appendix Table III (Continued)

EFFECT OF STORAGE CONDITIONS ON NUMBERS OF LACTOBACILLI ON SMALL FRUITS

C. RASPBERRIES FROM MACDONALD COLLEGE

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
1	2230	12230	7760	6130	2660
2	706	930	616	530	473
3	273	426	153	150	150
4	310	486	306	250	180
5	373	523	296	223	310
6	343	453	273	206	193
7	380	493	253	196	205
8	386	446	310	276	196
9	403	510	340	256	236
10	423	486	353	286	236
11	376	486	613	573	256
12	260	366	206	193	170
13	390	473	326	313	190
14	190	240	140	130	103
15	480	570	330	280	220
16	130	280	120	93	90
17	260	360	220	190	170
18	170	260	130	100	100
19	170	230	150	120	80
20	430	570	380	340	290
21	670	1080	600	540	370
22	660	150	56	66	43
23	460	890	390	460	190
Mean	760	997	622	517	309

(Counts/Gram Wet Weight, in Thousands)

Appendix Table IV

EFFECT OF STORAGE CONDITIONS ON THE NUMBERS OF YEASTS ON SMALL FRUITS

A. STRAWBERRIES FROM MACDONALD COLLEGE

		48 Hour Treatment		96 Hour Treatment	
Sample	Untreated	Room Temp. (25°C)	10°C	10°C	-17°C
1	4	6	86	126	3
2	30	25	236	250	40
3	273	1400	80	430	13
4	116	146	92	80	72
5	143	193	121	96	82
6	69	97	53	44	34
7	23	34	16	13	11
8	79	520	233	16	33
9	327	530	230	163	146
10	70	79	51	41	35
11	306	456	206	146	130
12	1246	1663	1053	943	643
13	813	1153	643	593	260
14	106	240	63	56	53
15	76	176	186	250	46
16	100	166	203	126	43
17	436	510	30	230	243
18	126	256	100	66	76
Mean	241	425	204	203	109

(Counts/Gram Wet Weight, in Thousands)

Appendix Table IV (Continued)

EFFECT OF STORAGE CONDITIONS ON THE NUMBERS OF YEASTS ON SMALL FRUITS

B. STRAWBERRIES FROM L'ASSOMPTION

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
19	26	80	30	31	14
20	41	120	30	21	10
21	66	48	54	46	1
22	400	420	0	63	0
23	96	125	83	151	0
24	21	6	10	10	0
25	10	100	0	10	2
26	0	200	0	20	0
27	176	293	120	93	0
28	173	303	106	66	70
29	130	233	86	66	43
30	623	55	33	56	136
31	183	313	110	86	53
32	460	820	330	216	188
33	150	257	123	90	53
34	0	453	60	18	1
35	133	10	113	86	5
36	100	173	69	220	35
37	0	10	10	343	0
38	110	1403	153	326	31
39	10	80	66	110	1
40	153	550	306	196	21
41	76	30	173	100	3
42	73	113	40	33	20
43	313	540	153	120	88
44	183	303	96	76	90
Mean	142	266	90	102	33

(Counts/Gram Wet Weight, in Thousands)

Appendix Table IV (Continued)

EFFECT OF STORAGE CONDITIONS ON THE NUMBERS OF YEASTS ON SMALL FRUITS

C. RASPBERRIES FROM MACDONALD COLLEGE

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
1	813	1153	646	600	260
2	436	510	300	230	243
3	40	80	13	10	6
4	126	256	100	66	76
5	143	283	90	66	85
6	123	280	86	50	36
7	163	233	123	86	85
8	136	186	106	86	89
9	186	346	150	103	62
10	136	200	106	86	78
11	126	193	146	143	58
12	53	116	60	66	27
13	190	256	153	150	86
14	43	100	26	23	30
15	330	380	260	210	130
16	33	100	30	10	16
17	90	140	60	30	30
18	170	320	140	120	60
19	170	230	140	120	71
20	420	620	340	300	210
21	130	380	100	80	76
22	30	110	16	26	18
23	0	160	30	86	12
Mean	176	283	140	119	80

(Counts/Gram Wet Weight, in Thousands)

Appendix Table V

EFFECT OF STORAGE CONDITIONS ON THE NUMBERS OF COLIFORMS ON SMALL FRUITS

A. STRAWBERRIES FROM MACDONALD COLLEGE

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
1	1.8	1.8	0	1.8	0
2	0	0	0	0	0
3	0	1.8	3.6	3.6	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	1.8	5.4	1.8	0	0
8	1.8	3.6	3.6	0	0
9	0	1.8	3.6	0	0
10	0	0	0	0	0
11	1.8	0	0	0	0
12	1.8	0	0	0	1.8
13	1.8	0	1.8	0	0
14	1.8	0	0	0	0
15	1.8	0	0	0	0
16	1.8	0	0	0	0
17	0	0	0	0	0
18	1.8	3.6	0	0	1.8
Mean	1.0	1.0	0.8	0.3	0.2

(M.P.N./Gram Wet Weight)

Appendix Table V (Continued)

EFFECT OF STORAGE CONDITIONS ON THE NUMBERS OF COLIFORMS ON SMALL FRUITS

B. STRAWBERRIES FROM L'ASSOMPTION

Sample	Untreated	48 Hour Treatment		96 Hour Treatment	
		Room Temp. (25°C)	10°C	10°C	-17°C
19	3.6	5.4	3.6	1.8	1.8
20	1.8	1.8	0	1.8	0
21	0	0	1.8	0	0
22	0	1.8	0	0	0
23	1.8	3.6	1.8	0	0
24	1.8	5.4	1.8	1.8	0
25	1.8	3.6	0	0	0
26	0	1.8	0	0	0
27	0	0	0	0	0
28	0	0	0	0	0
29	1.8	0	0	0	0
30	0	1.8	0	0	0
31	0	0	0	0	0
32	0	1.8	0	0	0
33	1.8	3.6	0	0	0
34	3.6	5.4	0	0	1.8
35	3.6	7.2	1.8	1.8	1.8
36	0	0	0	0	0
37	1.8	3.6	0	0	0
38	0	0	0	0	0
39	0	0	0	0	0
40	0	0	0	0	0
41	0	0	0	0	0
42	1.8	0	0	0	0
43	1.8	3.6	0	0	0
44	0	0	0	0	0
Mean	1.7	2.8	1.03	0.48	0.3

(M.P.N./Gram Wet Weight)

Appendix Table V (Continued)

EFFECT OF STORAGE CONDITIONS ON THE NUMBERS OF COLIFORMS ON SMALL FRUITS

C. RASPBERRIES FROM MACDONALD COLLEGE

		48 Hour Treatment		96 Hour Treatment	
Sample	Untreated	Room Temp. (25°C)	10°C	10°C	-17°C
1	0	0	0	0	0
2	0	1.8	0	0	0
3	1.8	1.8	0	0	0
4	0	0	0	0	0
5	0	0	1.8	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	1.8	3.6	0	1.8	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	3.6	5.4	0	0	1.8
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	1.8	1.8	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	1.8	0	0
Mean	0.4	0.6	0.15	0.07	0.07

(M.P.N./Gram Wet Weight)

Appendix Table VI

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

A. STRAWBERRIES

Variety	0 Hour Control	Room Temperature. 48 Hr.	Refrigeration (10°C) 96 Hr.	96 Hr. -17°C	
CAVALIER	11777	32685	1901	265	Total Count
	142	253	190	63	Fungi
	148	773	212	116	Lactics
	108	608	137	37	Yeasts
SENATOR DUNLAP	1849	3013	1699	306	Total Count
	674	721	70	58	Fungi
	680	772	255	32	Lactics
	246	68	59	49	Yeasts
GRENADIER	7966	16066	3907	311	Total Count
	294	366	83	13	Fungi
	334	535	272	100	Lactics
	140	200	61	1	Yeasts
POCAHANTAS	3246	4426	1615	853	Total Count
	405	535	249	131	Fungi
	510	471	268	236	Lactics
	139	159	118	26	Yeasts
EARLY DAWN	2665	15114	2555	488	Total Count
	2817	4185	1421	754	Fungi
	110	218	61	59	Lactics
	74	117	36	21	Yeasts

(Counts/Gram Wet Weight)

Appendix Table VI (Continued)

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

A. STRAWBERRIES

Variety	0 Hour Control	Room Temperature. 48 Hr.	Refrigeration (10°C) 96 Hr.	96 Hr. -17°C	
SPARKEL	1314	2379	2596	439	Total Count
	251	466	255	157	Fungi
	209	422	384	81	Lactics
	89	205	126	35	Yeasts
SANGUA	2334	2684	1592	998	Total Count
	536	599	515	336	Fungi
	445	542	412	190	Lactics
	448	595	412	239	Yeasts
VALENTINE	3032	4094	1970	1051	Total Count
	2232	2664	1295	891	Fungi
	3445	4702	2288	1056	Lactics
	471	1027	395	139	Yeasts
PREMIER	1476	1904	1256	506	Total Count
	459	637	392	263	Fungi
	396	523	343	230	Lactics
	246	525	202	100	Yeasts
RED COAT	3691	4930	1477	318	Total Count
	264	475	159	82	Fungi
	504	837	175	81	Lactics
	119	220	88	42	Yeasts

(Counts/Gram Wet Weight)

Appendix Table VI (Continued)

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

A. STRAWBERRIES

Variety	0 Hour Control	Room Temperature. 48 Hr.	Refrigeration (10°C) 96 Hr.	96 Hr. -17°C	
GUARDSMAN	9745	13754	1283	489	Total Count
	185	372	104	65	Fungi
	303	1527	264	86	Lactics
	249	460	166	71	Yeasts
ARMORE	1230	15000	4600	90	Total Count
	100	250	153	1	Fungi
	50	250	250	73	Lactics
	0	200	20	0	Yeasts

B. RASPBERRIES

VIKING	1776	2325	1160	671	Total Count
	678	705	490	435	Fungi
	1822	2419	1220	594	Lactics
	238	368	160	92	Yeasts
LATHAM	587	922	415	351	Total Count
	486	710	389	275	Fungi
	445	617	333	265	Lactics
	192	292	109	99	Yeasts

(Counts/Gram Wet Weight)

Appendix Table VI (Continued)

EFFECT OF STORAGE ON THE MICROFLORA OF INDIVIDUAL VARIETY OF SMALL FRUITS

B. RASPBERRIES

Variety	0 Hour Control	Room Temperature. 48 Hr.	Refrigeration (10°C) 96 Hr.	96 Hr. -17°C	
SEPTEMBER	630	891	511	369	Total Count
	443	715	406	303	Fungi
	419	424	269	190	Lactics
	210	328	150	89	Yeasts
NEWBURG	554	941	391	221	Total Count
	369	446	287	282	Fungi
	298	465	212	178	Lactics
	72	165	56	42	Yeasts

(Counts/Gram Wet Weight)

Appendix Table VII

EFFECT OF STORAGE CONDITIONS ON ARTIFICIALLY INOCULATED COLIFORMS ON RASPBERRIES

Sample No.	0 Hour MPN Coliforms (Thousands)	% Reduced After 48 Hours Refrigeration (10°C)	% Reduced After 96 Hour Storage at -17°C
1	17	53.5	90.0
2	92	41.3	63.4
3	160	42.5	78.2
4	240	33.3	97.9
5	920	61.9	98.9
6	170	80.5	94.5
7	160	42.5	66.2
8	920	80.4	94.6
9	540	68.5	90.92
10	240	67.0	86.0
11	350	62.8	93.4
12	920	41.3	73.9
Mean	394	56.2 ±16.1	85.6 ±12.2