THE BACTERIOLOGY OF DEHYDRATED VEGETABLES

By J. RISHBETH, *Ministry of Food, Department of Pathology, Cambridge*

(With 3 Figures in the Text)

I. BACTERIOLOGICAL ASPECTS OF THE DEHYDRATION PROCESS

INTRODUCTION

During the development of vegetable dehydration in Britain from 1940 to 1945 attention was given to problems of bacteriological control. Prescott (1920) had shown in America that dehydrated vegetables might have high bacterial counts, and that for certain micro-organisms there were limiting moisture contents below which marked growth on the vegetables would not take place. On the basis of experience gained at the pilot dehydration plant set up by the Ministry of Food many recommendations for observance in factories were made (Haines & Elliott, 1944). It was stressed that opportunities for reinfection from human sources after preliminary cleaning should be kept to a minimum, and that processing and drying should be carried out under conditions allowing no multiplication of bacteria and hence no formation of toxin. In the course of further work, which is described here, the extent and origin of bacterial contamination during the dehydration process were studied and a method for reducing it devised. The conditions under which growth of micro-organisms might take place were also investigated. The centre for this work was the Ministry of Food's bacteriological laboratory at the Department of Pathology, Cambridge.

SAMPLING AND BACTERIOLOGICAL TECHNIQUE

Samples of dehydrated vegetables were collected at the factories in sterile tins and sent to the central laboratory. Since some of the micro-organisms were found to die in a few weeks at room temperature, samples were stored at 0° C. if examination had to be delayed. During factory visits the examination of wet vegetables was carried out directly after sampling. Replication of vegetable samples was necessary because the degree of contamination of the strips or shreds varied considerably.

In the central laboratory suspensions were prepared by shaking 5 g. vegetable with 95 ml. quarter-strength Ringer in a screw-topped bottle for 5 min. Further details of this method will be given in Part II (p. 40). For the examination of wet samples during factory visits, suspensions were made by hand-shaking 10 g. vegetable with 90 ml. 0·9 % saline for 2 min. A full examination consisted of making plate counts at 37° C. with nutrient agar, at 55° C. with dextrose tryptone agar, and at 22° C. with malt agar. Counts were made after incubation at 37 and 55° C. for 2 days, and at 22° C. for 3–4 days. The approximate level of coliform contamination was estimated by inoculating tubes of MacConkey broth with dilutions of the suspension. Deep glucose agar shakes, heated at 80° C. for ½ hr., were prepared to detect sporing anaerobes, and blood agar plates were poured for streptococci and staphylococci. In selected cases examination for food-poisoning organisms was carried out by incubating the vegetable overnight in tetrathionate broth and subculturing on to a selective medium. For the detection of toxins, enrichment cultures were inoculated into mice. The predominant types of organism were noted.

THE MICROFLORA

(*a*) *Types of organism found*

The flora of dehydrated vegetables was varied and often characteristic of the particular factory producing them. Most commonly, the majority of bacteria were cocci, usually small micrococci or enterococci (Lancefield group D). *Viridans* streptococci were isolated from cabbage made on the pilot plant in 1943, but have not been recorded since. Species of *Leuconostoc* and *Sarcina* and a few strains resembling *Staphylococcus albus* were found, but *Staph. aureus* was not isolated. Gram positive sporing rods and coccal rods were frequent; non-pathogenic species of *Corynebacterium* were sometimes present. Coliforms were of widespread occurrence but usually comprised less than 1 % of the flora. *Bacterium coli* was the most common but *Bact. aerogenes*, intermediate and irregular types, and various late lactose fermenters were also often present (Table 1). Species of *Achromobacter* were fairly abundant. Organisms of the *Salmonella* group were never found.

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The fairly regular appearance of *Bact. coli* is striking; the probable reason for its appearance is discussed later (see p. 36). Flat sour organisms were the most characteristic thermophilic bacteria and were frequently present on dehydrated cabbage.

Anaerobic sporing bacteria were found on the majority of samples. Facultative types were very common and, in addition, a variety of strict anaerobes were isolated, e.g. *Clostridium sporogenes*, *Cl. tertium*, *Cl. histolyticum*, *Cl. multifermentans*, and *Cl. bifermentans* (including some toxin-producing strains). *Cl. botulinum* was not found, though the appearance of related soil types in the final product suggests that its spores would be capable of surviving the dehydration process. Fungal spores were commonly present, sometimes up to 10^5 per g. in number. Species of *Penicillium* were very frequently present and species of *Dematium*, *Aspergillus* and *Neurospora* were also found. Yeasts were widespread in occurrence and were sometimes more numerous than moulds. *Actinomyces* species were sometimes present in small numbers.

Of the various micro-organisms recorded above, the great majority have no pathogenic significance. *Bact. coli* has seldom been found associated with cases of food poisoning, and the bulk of cocci are probably harmless. *Viridans* streptococci occasionally cause poisoning (Jordan & Burrows, 1935) but have only been isolated once during this work. Of the anaerobes found, *Clostridium bifermentans* was recorded as the causal organism in a mild outbreak of food poisoning (Duncan, 1944); on this occasion the spores survived a two-stage cooking of raw potatoes used in the preparation of meat and potato pies. Because of the rarity of toxin-producing organisms on dehydrated vegetables, toxin production is very improbable, but the souring organisms commonly present are likely to cause spoilage whenever conditions permit considerable multiplication.

Table 1. *Number of samples of dehydrated potato and cabbage from which coliforms were isolated in* 1944

	Total no. of samples examined	No. with coliforms	No. with the following types					
			Coli I	Coli II	Int. I	Int. II	Aerog. I	Irreg. I
Potato	34	26	14	.	2	.	13	.
Cabbage	60	53	47	1	4	2	23	1

Table 2. *Summarized results of the distribution of bacterial counts of vegetable samples received from eighteen factories*

(*a*) Plate counts, bacteria per g.

Vegetable	Temp. of incubation ° C.	Counts							
		< 10^2	10^2	10^3	10^4	10^5	10^6	10^7	10^8
Potato	37	.	10	27	26	17	2	.	.
Cabbage	37	1	5	7	17	45	49	29	3
Potato	55	76	6	.	.	.	.	.	.
Cabbage	55	73	59	24	.	.	.	.	.

(*b*) Highest dilution at which samples gave growth

Vegetable	Tested at 37° C. for	Dilutions					
		0	1/2	1/20	1/200	1/2000	1/20,000
Potato	Coliforms	65 (0 and 1/2)		12	5	.	.
Cabbage	Coliforms	34 (0 and 1/2)		19	20	30	53
Potato	Anaerobes	60	15	7	.	.	.
Cabbage	Anaerobes	24*	78	49	.	.	.

* Five samples unrecorded.

Total number of samples examined: Potato 82. Cabbage 156.

(*b*) *Numbers present*

Table 2 records the number of bacteria present on samples of dehydrated vegetable produced in Britain during 1944–5. The few samples of carrot examined had counts similar to those of potato from the same factories. Cabbage tended to be the most heavily contaminated product and often had about a hundred times as many bacteria as potato.

Analysis of the species of the microflora suggests that the extremely low number of potential pathogens in the product was satisfactory. Nevertheless,

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the large number of other bacteria present shows clearly that contamination during the process was in many cases serious.

THE DEHYDRATION PROCESS AND PLANT HYGIENE

(a) *Outline of the process*

The standard British dehydration process has recently been described by the technical staff of the Ministry of Food, Dehydration Division (1945 and 1946). During preparation, potatoes and carrots are washed and peeled mechanically, and then trimmed by hand. Commonly, part is processed at once and the remainder fed into temporary storage tanks, where it is kept under water until required. The trimmed vegetables are cut into strips mechanically. Cabbages are trimmed, cored, and shredded; trimmed uncored cabbages are stored, when necessary, in a cool place.

The strips or shreds are washed and then pass to a rotary scalder, which is heated by direct steam injection and kept about one-third full of liquor. This liquor is normally used without emptying throughout the week. Vegetables are scalded for about 3 min. at a temperature of 99° C., and are then delivered on to a moving belt. The vegetable layer on this belt is subjected to an upward blast of air from fans, which checks over-cooking and cools the vegetable sufficiently for tray spreading. A hand-operated 'strickling-box' delivers the vegetable to the trays. In some plants, a rubber conveyor belt delivers the vegetable from the end of the cooler to the strickling gear. The strips or shreds are spread by hand, and the trays placed in racks on trolleys.

Two trolleys are inserted at intervals of 30 min. into the drier, which consists of two tunnels side by side, the first or 'wet' section with concurrent air flow and the second or 'dry' section with counter-current air flow. The total drying time is about 6½ hr. A brief inspection follows, and the vegetables are either packed directly into cans or are first compressed into blocks. In the preliminary 'conditioning' of cabbage before compression, shreds from the drier are heated in humid air for 10 min. to give a moisture content of about 8 %. After compression the moisture content is reduced to the specified level by drying the blocks for 12 hr. Carrot and cabbage are packed in cans in an atmosphere of nitrogen.

(b) *Variation in the number of bacteria at different processing stages*

Samples of vegetables were taken at successive stages of the dehydration process in several factories and the sequence of counts obtained in each case was very similar. The raw vegetable after being cut into strips or shreds had a count of about 10^4–10^5 bacteria per g. and washing only slightly affected this. After scalding, the vegetable was nearly sterile. During cooling, vegetable in contact with the mesh of the conveyor belt was contaminated, often heavily, but the bulk of the vegetable remained almost sterile. Vegetable picked up bacteria during tray spreading and commonly had 10^4 per g. at this stage. Where the tray spreading gear was fed by a subsidiary rubber conveyor belt, the cooled vegetable was even more heavily contaminated. There was no multiplication in the relatively short period before drying. Some destruction of bacteria commonly took place during drying. Subsequent operations such as conditioning, compressing and packing did not appear to affect the number of bacteria appreciably.

The variation, described above, in the number of bacteria on vegetables is illustrated in Fig. 1. These results were obtained from three factories each of which was processing a different vegetable; about twelve samples were taken at each processing stage. The number of bacteria is expressed per unit weight of the raw, scalded or dried material, and the value plotted for each stage is the mean of the logarithmic count of the samples. The samples of potato and cabbage taken after cooling carried more bacteria than those of carrot. This was because the former were taken only from vegetable in direct contact with the mesh of the cooler belt, whereas the latter were taken as the vegetable fell off the end of the belt and therefore contained a proportion of strips that did not touch the mesh. After tray spreading, the number of bacteria on potato and cabbage was greater than the number on carrot. This may be explained by the presence of a subsidiary conveyor carrying vegetable to the tray spreading gear in the factories processing the first two vegetables, and by its absence in the factory processing carrot.

The proportion of bacteria surviving drying can be calculated from the counts plotted in Fig. 1 by allowing for the weight lost during this operation. In the case of carrot, the dry weight was 1/11 of the scalded weight, and thus the expected log-plate count of the dried vegetable is $4{\cdot}6+\log 11$ or $5{\cdot}6$. The actual value obtained was 3·6, and therefore it is probable that in this case about 1 % of the bacteria survived drying. When a similar calculation is made for cabbage, the destruction of bacteria during drying is found to be small. The probable reason for this is discussed later (see p. 37).

The number of bacteria on vegetables at any one stage fluctuated widely: for example, sixteen samples taken from a subsidiary conveyor belt had counts ranging from 6×10^4 to 1×10^6 bacteria per g. This would perhaps be expected from the types of contaminating surface involved. However, the sequences of counts described suggest that each stage

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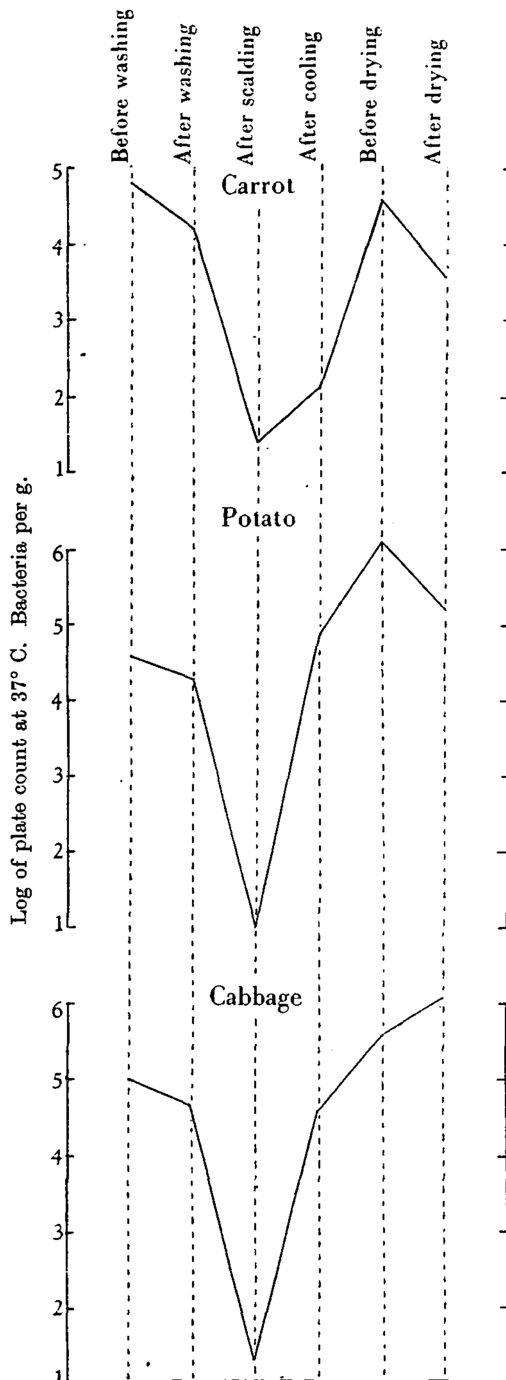

Fig. 1. Numbers of bacteria on vegetable at successive stages of the dehydration process.

from scalding up to the end of drying affected the general level of contamination. The composition of the flora also tended to change at successive stages.

(c) *Contamination and the effects of cleaning*

Liquor used for scalding vegetables has, in canning, sometimes been found a significant source of spore contamination (Anonymous, 1944), but the scalder used in the dehydration process did not accumulate spores greatly throughout the week. This may well have been associated with the relatively high overflow rate of the liquor. Kintner & DeLay (1943) have shown that, with poor equipment, a marked reduction in count during scalding may not occur.

Vegetable in contact with the conveyor belt of the cooler normally picked up 10^4–10^5 bacteria per g., but the number of bacteria on the remainder of the vegetable always remained low. Direct contamination by the cooling air was probably slight since in one factory vegetable strips touching the cooler belt, and hence receiving the full force of the draught, had an average count as low as 10^2 bacteria per g. over a period of 5 days following the introduction of routine cleaning. The mesh of the belt was very liable to become clogged with vegetable pulp, which carried an extensive flora of yeasts and bacteria and, in some cases, became interwoven with fungus mycelium. Water jets, where sufficiently powerful, restricted this accumulation but did not prevent it. Even when the mesh appeared to be clean, contamination of the scalded vegetable took place. Organisms capable of growing on the mesh arrived continuously from the cooling air and, to a smaller extent, from the scalder. Organisms characteristic of raw strips, including *Bacterium coli*, may have been transferred from other parts of the factory to the cooler during adjustment of conveyors. The regular occurrence of *Bact. coli* and enterococci on the belt in different factories and their almost consistent appearance in the product suggested that some types became so firmly established that cleaning did not remove them. It was shown that, although the regular week-end cleaning reduced numbers temporarily, there was an increase back to the original level within a day or two. It thus appeared likely that if a toxin-producing organism were to become established on the cooler belt, it would appear regularly on the dehydrated vegetables.

A number of methods of cleaning were suggested, but owing to the construction of the cooler only one was found to be practicable. This consisted of a preliminary scrubbing of the mesh with hot water and detergent, followed by hosing and treatment with hypochlorite. Good results depended on the thorough removal of organic matter during the preliminary treatment and on using relatively

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strong hypochlorite. Results were fairly satisfactory when hypochlorite of strength about 0·5% available chlorine was left in contact for 2 hr., but a milder treatment tried previously was relatively ineffective. Cleaning was carried out in the way described in a factory processing carrot and samples were taken from the cooler belt at intervals during the following 5 days (see Table 3).

(d) *Opportunities for growth of micro-organisms*

Overnight storage of carrot in buffer tanks was shown in one instance to lead to little or no increase in the number of bacteria on strips before scalding. In any case, a small increase at this stage would be unlikely to affect the final count. No marked bacterial growth took place when cooled vegetable, awaiting drying, was left for a few hours at a temperature of 22° C. (the normal period was less than 1 hr.).

Table 3. *Distribution of bacterial counts of samples of carrot strips in contact with the mesh of the cooler belt*

	Total no. of samples	10^1	10^2	10^3	10^4	Mean of log plate count
Before cleaning	9	1	2	5	1	3·3
After cleaning	21	8	9	3	1	2·1

Table 4. *Distribution of bacterial counts of samples of carrot strips after tray spreading*

	Total no. of samples	10^1	10^2	10^3	10^4	10^5	Mean of log plate count
Before cleaning	12	.	.	4	7	1	4·6
After cleaning	23	3	7	10	3	.	3·0

The main scraper rapidly accumulated pulp and was cleaned daily, but the belt was not treated. The method, although not altogether satisfactory, must have interrupted almost completely the continuity of microbial growth on the belt.

The rubber conveyor belt which, in some factories, led to the tray spreading gear, had a very thin contaminating film and could not be kept clean because of the numerous sources of infection. Even after dismantling, thorough scrubbing, and treatment of the belt with hypochlorite, contamination of the vegetables was only reduced to 10^5 bacteria per g. from an original level of about 10^6.

When a similar scrubbing and hypochlorite treatment were given twice daily to the tray spreading gear, the films of compacted vegetable were removed and the level of contamination lowered (Table 4).

Hand spreading involved some risk of contamination by staphylococcal or enteric carriers, but was continued chiefly because it was found that in no other way could the production rate be maintained at a satisfactory speed. Trays contaminated the vegetables little or not at all.

The results of cleaning experiments as a whole suggest that an improvement at any one stage did not necessarily give the final product a lower count; this was likely to happen only when attention was paid to all sources of contamination. It can be seen that the procedure tried by no means eliminated these sources; it did, however, reduce them, and also prevented vegetable pulp from accumulating on the equipment over long periods.

It has not been found practicable to dry vegetables throughout at a temperature above which significant bacterial growth is unlikely to occur: Table 5 gives some of the temperatures commonly used. Vegetable in the wet tunnel reached the wet bulb temperature of the vent in a few minutes. During its passage through the wet and dry sections of the tunnel the vegetable gradually increased in temperature to the dry tunnel inlet value. Under these conditions there was no evidence of bacterial growth, though possibly this might have taken place for the short period before the temperature rose to about 50° C. On the contrary, allowing for the difficulty of obtaining accurate counts for dried material, there was a marked reduction in the number of bacteria in the case of carrot and potato (see Fig. 1). There was no evidence that cabbage picked up more bacteria during previous stages than other vegetables, and therefore the greater bacterial load and higher incidence of coliforms on dehydrated cabbage (see Table 2) suggests that the final temperature of drying was an important factor. The

Table 5. *Temperatures of the drying tunnel* (° C.)

	Wet tunnel vent Wet bulb	Dry tunnel entry Dry bulb
Cabbage	41	63
Carrot	41	71
Potato	43	68

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appearance of coliforms on dehydrated vegetables supports the findings of Clague (1936) who showed that these bacteria were destroyed by scalding but survived drying at a higher temperature and for a longer time than our own.

The drying of carrot and potato was uniform with very few exceptions, but that of cabbage was sometimes uneven. Owing to heavy loading or uneven spreading some vegetable passed through the tunnel with little loss of water. The resulting wet patches were sometimes sour smelling and carried up to a thousand times more bacteria than normally dried material. This occurred from time to time in several factories, and to overcome it particular care in tray loading was found to be necessary.

Results obtained by Haines & Elliott (1944) suggest that bacterial growth on dehydrated vegetables is unlikely below 90% relative humidity. Little work has been carried out on the incidence of mould growth on vegetables at different humidities, but Wright (1940) found that samples of artificially dried grass stored at 70% R.H. showed no signs of moulding for almost a year, after which slight growth took place. Snow, Crichton & Wright (1944) found that moulds developed fairly rapidly on animal feeding-stuffs stored at 100 to 75% R.H. At humidities below 75% R.H. mould growth was not entirely prevented but took place after a very long latent period. Growth was not found to take place below 65% R.H. Gane (1943) gives figures for the water relations of air-dried scalded vegetables. When at equilibrium with a relative humidity of 30%, carrot and cabbage have a moisture content of 4·9% and potato one of 7·4% (figures at 37° C.). Similar results were obtained by Makower & Dehority (1943). A moisture content for cabbage and carrot of 5% and, for potato, of 7–8%, values usually attained in the dehydration process, will therefore prevent microbial growth during storage.

Spoilage may take place when vegetables at the time of packing have moisture contents considerably higher than the limits just given (i.e. equivalent to about 75% R.H.), or when moisture enters as a result of damage to cans. Spoiled dehydrated vegetable, with or without visible mould growth, was occasionally found during the examination of stored material. Many types of bacteria, including coliforms, had multiplied. Growth of strict anaerobes in cans during storage might perhaps be possible in view of the nitrogen pack given to cabbage and carrot, but there was little evidence that this took place. Owing to the infrequency of toxin-producing strains of bacteria on dehydrated vegetables, toxin formation is less likely to accompany isolated cases of bacterial growth during storage than in the case of some other dehydrated products.

A few experiments have shown that bacteria normally die out during storage, and that the rate of destruction is largely determined by temperature. Fig. 2 shows the results of one trial with dehydrated cabbage packed in nitrogen. Coliforms were still present at 0 and 15° C. at the end of a year, but died out rapidly at 28 and 37° C.; the only bacteria surviving 2 months' storage at this last temperature were Gram-positive sporing rods and a few micrococci. The results for potato were very similar, the counts of nitrogen-packed material being throughout slightly greater than those of air-packed.

Opportunity for the growth of micro-organisms may occur during rehydration. The recommended procedure in which the vegetable is covered with hot water and boiled for a variable length of time leads to the destruction of all vegetative bacteria and most of the spores. Growth is therefore only possible if the vegetable is subsequently left standing for some hours at a temperature above about 15° C. Experiments with *Clostridium bifermentans* showed that the liquor in which vegetables had been rehydrated was a good medium for the growth of this organism at temperatures between 22 and 37° C. Since anaerobic spores such as those of *Cl. bifermentans* are normally present on dehydrated cabbage and have been shown to survive the standard cooking procedure, there is a possibility of anaerobes multiplying during subsequent standing. This is particularly likely to happen when meat has been mixed with the vegetable. These findings suggest that dehydrated vegetables should be consumed soon after cooking.

In the alternative method recommended for the rehydration of carrot, the vegetable is soaked overnight in cold water. When this method was used, the number of bacteria developing was shown to be related to the number initially present on the vegetable, to the temperature, and to the length of soak. At 0 and 10° C. growth either did not occur or was exceedingly slow. At 15° C. the count sometimes increased ten to a hundred times in 24 hr., but no appreciable souring occurred. At 22° C., rapid multiplication (logarithmic phase) took place within 4–10 hr., whereas at 28° C. and higher temperatures this started at once or was delayed for 1–3 hr. only. Rapid bacterial growth during rehydration at room temperature has been noted by Jones (1943), who stressed that initially high counts would lead to earlier spoilage under these conditions. Although vegetative bacteria are destroyed by the short period of boiling necessary for cooking after the soak, it is possible that if toxin had been formed some might remain. As already noted, toxin-producing strains are very uncommon on dehydrated vegetables, but spoilage is likely under conditions favourable for growth owing to the widespread occurrence of souring organisms on the vegetables.

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(e) Variation in the numbers of bacteria on the dehydrated product

Samples were received at intervals from nearly all the factories. These were taken, in the main, from the production of successive shifts, and therefore any considerable fluctuation in numbers should have been evident. The general level in each factory, however, seemed to be almost unaffected by sampling on different shifts, days, or even weeks. It was interesting to find to what extent the results supported the conclusions drawn from factory visits as to the sources of contamination. Table 6 records the number of factories whose samples, usually six in number, had an average log count in the groups shown.

the highest group had a subsidiary conveyor belt after cooling and before tray spreading; this was also present in the three factories giving rise to the highest counts for potato. The level of coliform contamination followed the plate count fairly closely. The general conclusion is, once again, that the number of bacteria on the final product was markedly influenced by factory equipment, and that contamination was greatest when the vegetables came in contact with conveyors and other surfaces.

In view of the present diversity of equipment it would seem premature to insist on standards for the plate count of dehydrated vegetables. It is evident that if the question of design of dehydration plants arises in the future, there is a good case on hygienic

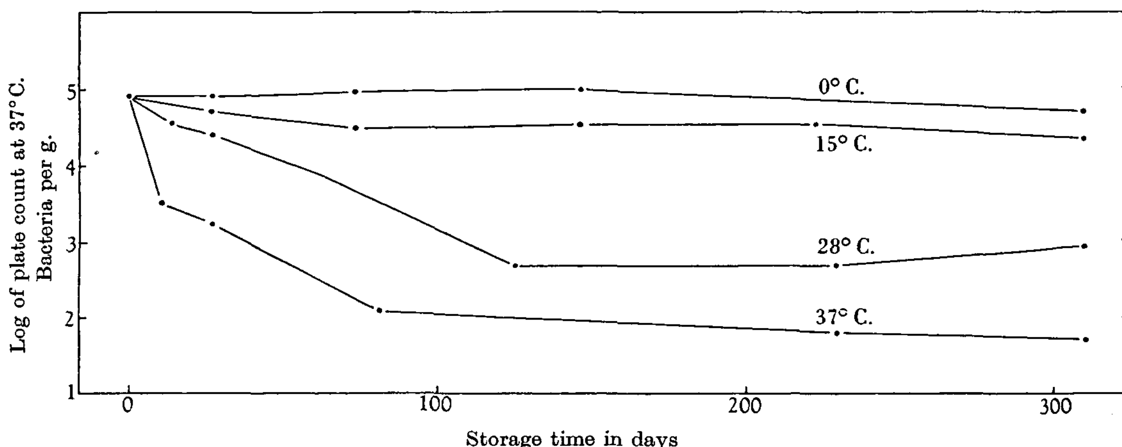

Fig. 2. Numbers of bacteria on cabbage during storage at different temperatures.

Table 6. *Distribution of bacterial counts of dehydrated vegetables from British factories*

	Total no. of factories	10^2	10^3	10^4	10^5	10^6
Carrot	3	.	3	.	.	.
Potato	10	1	3	3	3	.
Cabbage	17	.	1	2	4	10

The factory with the lowest count in the case of cabbage and potato had a non-standard plant: steam scalding was used and there was no cooler belt. Cabbage produced there carried somewhat fewer bacteria than 2000 per g. which was the level Kintner & DeLay (1943) thought American factories should attain when scalding was adequate and the product was subsequently handled with care. The two factories in the 10^4 group for cabbage had no cooler belt, whereas those in the next group had a standard lay-out. Six out of the ten factories in

grounds for the adoption of scalding on trays so that, after this, vegetables do not have to pass over conveyors. If water scalding is found to be desirable, then it should be possible with the knowledge now available to design a cooler which does not involve large contaminating surfaces and which is more accessible for cleaning. With a better factory lay-out it should be possible to dispense with subsidiary conveyors.

SUMMARY AND CONCLUSIONS

Bacteriological work on the dehydration process was designed to show the extent and origin of contamination, and to devise methods for reducing it.

An outline of the sampling methods and bacteriological technique is given.

The microflora of the vegetables was varied; coliforms were abundant but potential pathogens were very rare. The majority of samples of potato

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and carrot had counts of from 10^3 to 10^4 bacteria per g. and those of cabbage from 10^5 to 10^6 per g. An account is given of the dehydration process and associated problems of hygiene. The vegetables were nearly freed from bacteria by the scalding treatment, but subsequent passage over any conveyors and other surfaces present led to considerable increases in the number of bacteria on the vegetables. It was found difficult to reduce this contamination effectively; a method of cleaning which gave some improvement is described.

Drying normally caused some reduction in the number of bacteria, but uneven drying of cabbage was occasionally accompanied by bacterial multiplication. Bacteria normally died out during storage, the more rapidly the higher the temperature. In general, the moisture content of vegetables attained by the dehydration process effectively prevented growth during storage, but in some instances, when damp vegetable had been packed, growth of bacteria and moulds took place. Rehydration and cooking of the vegetables destroy the majority of the bacteria, but the use of long soaking periods may lead to souring.

Analysis of samples from different factories confirmed that the number of bacteria on the product was markedly influenced by factory equipment. It is suggested that with future designs of dehydration plant there is a strong case for the adoption of scalding on trays, so that subsequent passage of vegetables over conveyors is avoided.

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II. INTERPRETATION OF THE PLATE COUNT

INTRODUCTION

In the course of bacteriological work on the vegetable dehydration process (see Part I) the plate count method was used to determine the number of bacteria on samples of dehydrated vegetable. It was realized at an early stage that this method was subject to considerable errors.

The magnitude of the plate count depends on the number of bacteria, capable of forming visible colonies under the conditions of incubation, which are present in the sample of suspension prepared from the vegetable at the time of pouring the plates. Variation in plate count might be expected to arise in several ways during the examination of a particular sample of dehydrated vegetable.

(*a*) Different methods of preparing suspensions might lead to the loosening of varying numbers of bacteria from the surface of the vegetable, and this loosening might itself depend, for example, on the degree of rehydration, hydrogen-ion concentration, or temperature.

(*b*) Bacteria might multiply or lose viability during the preparation of suspensions and plating.

(*c*) The volume of suspensions is reduced owing to rehydration of the vegetables, and this might lead to over-estimation of the numbers of bacteria present.

(*d*) There are in addition errors of sampling: the number of bacteria present on individual strips or shreds might vary from sample to sample.

(*e*) Further errors might arise in removing samples of suspension for plating. It has been assumed moreover that, of the bacteria present in the samples of suspension, the same proportion form visible colonies in each case. Variation in the composition of the nutrient agar has not been tried.

(*f*) Finally, when vegetables from different factories are compared, still more factors might be introduced.

Experiments have been made to determine the origin and magnitude of the errors arising from these various sources. The investigation was limited in scope and some of the deductions are necessarily tentative, but nevertheless it is thought that the results are worth recording, especially in view of their bearing on the bacteriological examination of other foods.

DESCRIPTION OF EXPERIMENTS

(*a*) *Preparation of suspensions*

Suspensions of comparable dehydrated vegetable were prepared in several ways and the number of bacteria estimated in each case.

(i) In the standard method used in this work, suspensions were prepared by shaking 5 g. vegetable with 95 ml. of quarter-strength Ringer (*p*H 7·0) in a screw-topped bottle for 5 min. A mechanical shaker with 200 to-and-fro cycles per minute was used. The suspension was normally kept at 20–24° C., but the temperature could be varied as required. The interval from first adding quarter-strength Ringer to the pouring of the plates was usually restricted to 20 min. and never exceeded 30 min. Nutrient agar was used, and counts were made after incubating the plates for 48 hr. at 37° C. In the

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majority of experiments suspensions were prepared from sixteen separate 5 g. samples of vegetable and 1 ml. of each suspension was plated. Distributions of plate counts of potato samples from a single can are given in Table 7.

When the standard method (A) was used, the mean value of the plate count was greater at higher temperatures over the range 10–40° C. and there was a corresponding increase in the turbidity of the suspensions. Experiments with other vegetables also showed that the temperature at which the suspension was made affected the plate count; the increase over the range 10–30° C. was often about fivefold.

(ii) When potato strips were soaked for 18 hr. at 0° C. and a suspension then prepared by shaking for 5 min. at 10° C. (B), the mean count was greater than that obtained by the standard method at 10° C. and the suspension was more turbid. Since other experiments showed that the bacteria present on these strips, when in suspension under comparable conditions at 0° C., tended to lose viability, it appears that the long period of soaking must have loosened a greater proportion of bacteria from the strips than was accomplished by the standard shaking alone.

Fewer bacteria were removed from the strips by the ½ min. mechanical shaking (C) than by the standard shaking of 5 min. Suspensions prepared from strips which had previously been ground in a sterile coffe-mill (D) gave lower counts than the standard method when samples were taken a minute or so after shaking, by which time the bulk of the vegetable particles had settled. Plates prepared from suspensions before the particles had settled were very difficult to count and gave values similar to those obtained by the standard method. Similar results were obtained by grinding other samples of potato and also carrot. This method of preparing suspensions was suggested by Haines (1943), but the lengthy procedure and difficult plate counting involved appear to be serious disadvantages, which are not compensated by any increase of accuracy.

The fact that plate counts obtained by grinding strips before preparing suspensions were not greater than those obtained by the standard method suggests that bacteria were restricted to the surface of the vegetables. This was likely to be the case since, during the dehydration process, vegetables were nearly freed from bacteria by the scalding treatment but were subsequently contaminated by contact with conveyors and other equipment.

Table 7. *Distributions of plate counts obtained from a single can of dehydrated potato by varying the method of estimation*

Method of estimation	Temperature of suspension (° C.)	No. of samples	Percentage of counts in groups 0–40	40–80	80–120	120–160	160–200	Over 200	Arithmetic mean of plate counts
A. Standard	10–11	92	83	14	2	.	.	1	24
Standard	20–24	110	32	44	14	4	1	5	73
Standard	31–33	77	2	21	32	19	3	13	132
Standard	40–41	32	.	16	25	3	16	40	204
B. 18 hr. soak at 0° C.	10	16	19	30	6	.	6	19	104*
C. ½ min. shake	20	16	88	12	.	.	.	.	17
D. Grinding	20	14	100	.	.	.	.	.	20
E. 15 min. shake	22	16	6	63	19	12	.	.	79
F. 30 min. shake	11	16	94	6	.	.	.	.	17
G. Detergent	22	16	37	44	.	19	.	.	59
H. Ringer, *p*H 6·0	23–24	32	13	55	13	13	.	6	91
I. 0·9 % saline	21	16	38	56	6	.	.	.	50

* Allowance made for change in volume of suspension (see p. 43).

(iii) A number of other variations in method gave mean counts little different from those obtained by using the standard method at similar temperatures. Longer periods of shaking were tried (E, F), and in another method (G) a small amount of detergent, 0·0025 % dodecane sodium sulphonate, was introduced before shaking. It had previously been shown that at this concentration the detergent did not affect the viability of the bacteria present in the suspension of potato. Quarter-strength Ringer of *p*H 6·0 and 0·9 % saline of *p*H 7·0 were substituted for quarter-strength Ringer of *p*H 7·0 in two other methods of making suspensions (H, I).

The fact that some methods of preparing suspensions gave high counts and others did not, suggests that some of the bacteria were firmly attached to the strips. No full explanation can be given why shaking at 40° C. removed far more bacteria than the shaking which followed a long soak at 0° C.,

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even allowing for the small loss of viability of the bacteria which probably accompanied this soak. However, the suspensions were more turbid at higher temperatures and thus it is possible that, during shaking, bacteria were set free from the surface of the vegetable by solution of carbohydrates or by some disintegration of the tissue, and that this process was more complete at higher temperatures. Part of the increase might conceivably have resulted from the breaking up of bacterial clumps, although longer shaking times would perhaps be expected to have given higher counts if this were so. Further work would evidently be required to elucidate the relation of the plate count to the temperature of the suspension.

(*b*) *Changes in the number of bacteria in suspensions*

It was found that the plate count might be increased by a short delay in taking samples of suspension. In one experiment 1 ml. samples were withdrawn consecutively from a suspension of dehydrated potato immediately after preparation by the standard method and plated in groups of five. Twelve such groups of plates were poured in 25 min.; the mean counts of these groups are given in Table 8. Despite some fluctuations, an increase can clearly be seen. This was an exceptionally high rate of

Table 8. *Changes in plate count of a dehydrated potato suspension at* 26° C.

Mean plate counts of twelve groups of five samples, taken consecutively from the suspension over a period of 25 min.

1	2	3	4	5	6
70	74	110	102	115	108
7	8	9	10	11	12
100	102	126	130	141	140

increase; over a similar period it was commonly of the order of 10–20 % for suspensions at 20° C. and up to 50 % at 25° C. and higher temperatures. Not all samples of vegetable gave this immediate increase of count; for example, material stored for some months at room temperature seldom did so.

These increases of count might result from a progressive soaking off of bacteria from the vegetables, from multiplication of bacteria in the quarter-strength Ringer, which would contain extractives derived from the vegetable during shaking, or from some physiological change affecting the viability of the bacteria. Experiments were carried out in an attempt to show which factors were operative. In each experiment, a 200 ml. suspension of dehydrated potato, in contact with the strips, was incubated in a bottle at 37° C. and 1 ml. samples of the suspension plated at intervals. At the time of withdrawing these samples, 10 ml. aliquots were taken and incubated separately at the same temperature. Samples of these were also plated at intervals.

Suspensions were prepared (i) in distilled water, which is unfavourable to the preservation of viability in bacteria. In this experiment, when initial samples of suspension had been taken, the contents of the bottle were divided to give two portions of suspension and strips and two of suspension only.

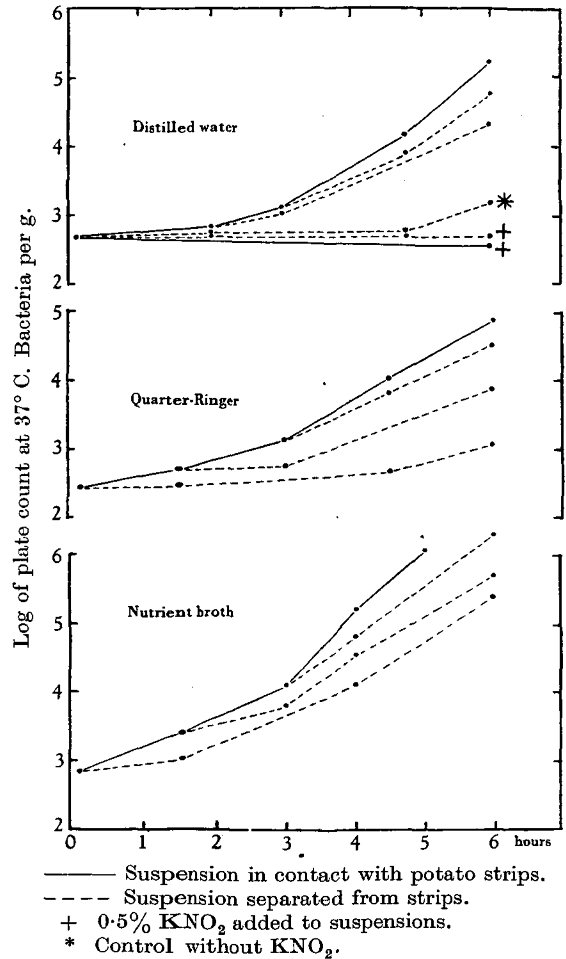

Fig. 3. Increases of bacterial count in suspensions of dehydrated potato during incubation at 37° C.

One portion of each type was incubated with 0·5 % potassium nitrite and the other without. Suspensions were also made (ii) in quarter-strength Ringer in which some increase of count had been observed in previous experiments, and (iii) in nutrient broth, to bring out the effects of any multiplication. The results of these experiments are given in Fig. 3.

(i) The fact that, after incubation for over 6 hr. with nitrite, there was little difference in count

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between the suspension in contact with strips and the suspension separated from them suggests that no appreciable soaking off of bacteria from the surface of the vegetable took place.

When suspensions were kept for 6 hr. in distilled water, the presence of vegetable strips led to large increases of count with successive samples. This suggests that the strips provided nutrient materials which enabled multiplication to take place in the suspensions. Some multiplication occurred in the absence of strips, perhaps owing to the presence of materials soaked from them during the preparation of the suspension.

(ii) A similar result was obtained with suspensions in quarter-strength Ringer. The rate of increase in count was low compared with some other samples of potato; this may have been associated with the fact that the one used in the experiment had been stored for several months.

(iii) In suspensions in nutrient broth, large increases in count resulted from incubation, presumably as a result of the multiplication to be expected in these circumstances. Even here, the presence of the strips gave apparently higher rates of multiplication, but the difference was less than in the previous cases, perhaps because the nutrient status of the medium was fairly high without them. This observation might have been attributed to some soaking off of bacteria from the strips in addition to multiplication, had not the experiments with nitrite discounted this possibility. The general tendency for the bacteria in suspensions separated from the strips to have a shorter lag phase in the nutrient broth than in Ringer or distilled water may also be explained by this medium being more favourable for growth.

The general conclusion is that increases of count in suspensions of dehydrated vegetables might be brought about by fairly rapid multiplication of bacteria in the presence of substances derived from the strips during preparation of the suspensions. It is possible too that there may be minor physiological changes affecting their viability, for loss of viability has already been mentioned as a possible result of prolonged soaking of vegetables at 0° C. (see p. 41).

(c) Changes in volume of the suspension

Rehydration of vegetables during estimation caused changes in the volume of suspensions. It was found that, after long soaking periods, there was a reduction in volume of about 12 % with suspensions of dehydrated potato, but one of less than 5 % when the standard method was used. Changes were more marked in the case of the other vegetables for, when the standard method was used, a reduction in volume of about 18 % took place with suspensions of carrot and one of up to 25 % with cabbage. Overnight soaking led to reductions in volume of over 30 % with both vegetables. Errors in the plate count could be avoided by noting such changes. The means given in Table 1 are comparable, although for the method in which there was a long soaking period (B), a corrected value is given. When a standard method is used results for a given vegetable should be comparable, since under these conditions the water uptake in different suspensions is similar.

(d) Variations between individual strips

Apart from the foregoing variations due to manipulation, it is to be expected that some would arise owing to the heterogeneity of the original material. During cooling in the standard dehydration process, some of the vegetable touched a contaminating surface and the remainder did not, and therefore individual strips carried varying numbers of bacteria after this operation. In some cases, subsequent operations tended to contaminate the vegetable more uniformly, but some variation in the number of bacteria on strips may well have persisted throughout the process. It is evident from the range of variation found within a single group of estimations, as shown in Table 7, that very high counts may occasionally be obtained. Although little direct work has been done in this connexion, such counts are most likely to be explained by sampling errors, since they lie altogether outside the range of variation to be expected from errors of the types discussed previously.

(e) Sampling of the suspensions

In addition, some variation in count might be expected to arise in the sampling of the suspensions. Some exceptionally high counts were probably to be explained on this basis, for in one case the distribution of the bacterial colonies on the plate indicated that fragmentation of a clump had taken place during pouring. The shaking of suspensions during their preparation by the standard method probably reduces this source of error, but prolonged shaking is of no advantage since multiplication takes place.

The standard deviation of counts of a series of samples from a single suspension was significantly less than that of counts from a series of suspensions prepared from different samples of the same lot of dehydrated vegetable. This shows that the error in sampling suspensions is of less importance than that in sampling different portions of the vegetable itself.

(f) Comparison of samples from different sources

Discussion has so far been confined to the sort of error which might occur in making plate counts of a single lot of dehydrated vegetables. When samples from different sources are compared, a number of

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other factors may influence the result: for example, different factories produce vegetables carrying different types of bacteria, and these may vary in behaviour during estimation and incubation.

Comparisons of plate counts on different types of nutrient agar might yield some information about this, but no such investigation has been made. However, indirect evidence of the reliability of making such comparisons is available from results obtained over a period of 18 months. The mean count of six or more samples from each of eighteen factories varied from 4×10^2 to 8×10^6 bacteria per g. In all the cases, ten in number, where two or more series of samples were received from the same factory, the mean counts of these did not differ from each other by more than a factor of five. It is clear that the variation of count between samples from the same factory was very much less than that between samples from different factories.

DISCUSSION

The results of the various investigations can now be briefly reviewed. It appears that the bacteria occur only on the surface of the vegetable, and that a similar proportion of bacteria may be removed even when suspensions are prepared in diverse ways. During the shaking of suspensions, some multiplication of bacteria may take place, but this is not likely to increase their number more than about 50 % in the short time allowed in the standard procedure (25 min.). It is unlikely that there would be any appreciable difference between suspensions in quarter-strength Ringer and distilled water. Errors due to changes in volume consequent on the rehydration of the vegetables are also small, of the order of 10–30 %, and can in any case be allowed for. Much larger variations may arise from sampling errors, but it is difficult to estimate the size of the contributions from different sources. It seems probable that differences in the number of bacteria borne on individual particles of vegetable are a major source of variation, and that imperfect distribution of bacteria in suspensions is less important.

The relation of the counts to the actual number of bacteria present on the vegetable is uncertain, but indirect evidence can be obtained from counts made on comparable lots of cabbage before and after drying. Previous experiments with undried vegetables had suggested that, before drying, the bacteria were attached loosely enough to be brought into suspension by the shaking given, so that counts on wet material could be regarded as accurate. When allowance was made for water loss, the plate counts were lower after drying: in one factory the mean count changed from 4×10^5 to 1×10^5 bacteria per g., and in another from 3×10^4 to 1×10^4. These differences might have arisen from death of the bacteria during drying, from the method giving too low a count after drying, or from a combination of both. If there were no death during drying, the results would indicate at the most an underestimate to the extent of about one-quarter of the number of bacteria on the dried vegetable.

For potato and carrot, the corresponding plate counts after drying are often only one-hundredth of those before drying, and this difference is so great that it must be largely due to destruction of bacteria during drying. This view is confirmed by the disappearance during drying of the great majority of heat-sensitive coliform organisms.

From the practical point of view, the source of the variations, and the value of the plate count as an index of the number of bacteria actually present, are less important than the question whether the total variation in the plate count is such as to render it useless as a general index of factory hygiene, a matter which may be considered in conclusion.

Coefficients of variation, calculated for each of twenty series of plate counts obtained from samples of dehydrated vegetable, were found to range from 23 to 83 % and to have a mean of 53 %. The counts from which these values were derived were all made by plating suspensions of a dilution 1/20; there was evidence from more heavily contaminated samples that the coefficient of variation might be somewhat greater when higher dilutions were plated. It is difficult to assign limits of error to plate counts because their distributions are not normal. It seems probable, however, that on any single plate count an allowance of about -65 % and $+150$ % should be made; for example, if a single count of 8×10^6 bacteria per g. was obtained from a vegetable, this might carry any number between about 3×10^6 and 2×10^7 per g.

The considerable variation in plate count of single samples shows clearly that this method can give only approximate values for the bacterial contamination of dehydrated vegetables. Nevertheless it is of value, for the error in counting a single sample (-65 % to $+150$ %) is appreciably smaller than that in counting successive samples from the same factory (about fivefold), and this in turn is much less than the variation from factory to factory (as much as a thousandfold). The plate count, despite its shortcomings, can therefore be used with some confidence as a general guide to the state of factory hygiene.

SUMMARY

Considerable errors arose when the plate count method was used to determine the number of bacteria on dehydrated vegetables. Bacteria probably occurred on the surface of the vegetables only.

Plate counts tended to be greater when suspensions were prepared at higher temperatures over the

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range 10–40° C. Counts often increased by 20% and sometimes by 50% in the short period allowed in the standard procedure (25 min.), probably owing to multiplication of bacteria. Changes in the volume of suspensions, caused by rehydration of vegetables, led to small errors for which allowance could be made. Larger variations in count arose from sampling errors through differences in the number of bacteria on individual strips and, to a lesser extent, through imperfect distribution of bacteria in suspensions. The relation of plate counts to the actual number of bacteria on the vegetables was uncertain.

However, the error in counting a single sample (−65% to +150%) was appreciably smaller than that in counting successive samples from the same factory (about fivefold) and this in turn was less than the variation between different factories (up to a thousandfold). The plate count could therefore be used with some confidence as a general guide to the state of factory hygiene.

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