1-1-1935

Studies on the microbiology of dried foods

John Albert Clague
University of Massachusetts Amherst

STUDIES ON THE MICROBIOLOGY OF DRIED FOODS

CLAGUE - 1935

STUDIES ON THE MICROBIOLOGY OF DRIED FOODS

By John Albert Clague

Thesis submitted for the degree of Doctor of Philosophy

Massachusetts State College

Amherst

May, 1 9 3 5

CONTENTS

INTRODUCTION

Although drying is one of the oldest methods of preserving food which has persisted up to the present day, yet the study of dried foods from the microbiological standpoint has received but very little attention in comparison with the study of other foods.

Dried foods have been used for years without any apparent ill effects to the consumers. The use of canned foods developed coincident with the growth of the science of bacteriology, and food bacteriologists were occupied with the study of methods for safely producing that type of food product. Consequently it was not until the world war created a demand for concentrated food products which could be easily handled, that interest in dried foods was revived and some studies on the microbiological aspects of these foods were made.

The obvious necessity for a safe milk and water supply in this country has been an incentive to the development of methods for determining the sanitary quality of these substances. As a result, there are now standard methods for bacteriological analyses of milk and water which provide laboratories all over the country with a uniform means of gauging the safety of milk and water supplies.

During the past few years there has been some agitation in public health circles for the development of similar standard methods for the examination of fresh, canned, frozen, and dried foods.

The object of this study is to investigate and develop methods for determining the number and kinds of microorganisms on dried foods; to study the nature and characteristics of the predominant types of microorganisms found on dried foods; and to study the effects of dehydration on the microbiological flora of fresh foods.

With these factors studied and considered together with the manner in which the foods are ultimately handled by the consumer the significance of the microbic flora of dried foods will be better understood and standards can be set which may be used to define a safe dried food.

In this paper, the term "dried" is applied to all dried products regardless of the method of drying; "dehydration" refers to drying by artificially produced heat. (2)

LITERATURE REVIEW

Aside from studies on dried eggs and dried milk

there has been comparatively little work reported in the literature. Prescott and his co-workers (18,19) found bacteria and molds normally present in commercially dried vegetables, peaches, and bananas. The numbers varied from less than 100 to over 1,000,000 per gram. The bacteria were similar to those found on fresh fruits or in soil and water. Both sporulating and non-sporulating organisms were found. Saprophytic molds were nearly always present on the samples tested, usually in the spore form which evidently had survived all pretreatment and dehydrating processes. Prescott found that the numbers of microorganisms on dried foods gradually decreased during storage, provided the moisture content of the foods remained approximately constant. Several laboratory and factory tests were made using fresh vegetables and fruits artificially inoculated with B. coli, B. paratyphosus A and B, B. enteritidis, B. suipestifer, B. typhosus, B. botulinus, Micrococcus pyogenes aureus and B. subtilis. No typical pathogen was recovered from the vegetables or fruits after thorough dehydration. Slip-cover cans were found to be the best containers for storage of dried products.

Nichols (15) found that no sample of dried fruit was sterile, but that many had only a few bacteria and

molds. No pathogens were found. He called attention to the possibility of contamination between the drying of the fruit and its sale to the consumer. Though the foods may be too dry to promote bacterial growth, bacteria may not die immediately but remain dormant until favorable conditions of moisture and temperature occur.

Hunwicke and Grinling (11) in England traced an outbreak of severe colitis to French packaged dates. The causative organism was called B. coli tropicalis. Eleven samples of French and English dates, both packaged and bulk, were examined bacteriologically, with the result that intestinal coliform organisms were isolated from six of the seven packaged samples. None of the bulk date samples showed any intestinal bacteria. It was concluded that dates may readily be contaminated during the repacking of the bulk dates into the smaller retail packages, and that the presence of coliform organisms on the surface of packed dates constitutes a health problem.

Fellers (7) examined dried fruits bacteriologically and found the average number of bacteria to vary from 0 to 18,000 per gram; yeasts 0 to 16,000 per gram; and molds from 0 to 42,000 per gram. Fruits treated with sulfur dioxide were found to have the

lowest bacterial counts. He advocated pasteurization as a means of eliminating pathogenic organisms and of decreasing the total number of all microorganisms present on dried fruits. Clague and Fellers (1) studied the time, temperature, and humidity relationships involved in the pasteurization of dates and found the best conditions to be heat treatment for 55 minutes at 77°C. with a relative humidity of 78 per cent. They (8) found that this treatment also effectively controlled yeasts which they found to be the cause of souring of dates.

A very good summary of the public health aspects of dried foods was presented by Nichols (16) who pointed out that the high acid and low protein content of dried fruits, and the manner in which dried vegetables and most dried fruits are prepared for consumption, are safeguards against infection or poisoning from these foods.

Several studies on the longevity of microorganisms on dried foods have been reported in the literature. Fellers (7) found the colon bacillus to die on prunes at room temperature in 15 days, and on raisins, dates, and figs in 30 days. Smeall (20) found the typhoid bacillus to remain viable for 68 days on dates. Naoum (14) found Esch. coli to survive on figs for

seven weeks when they were held at 34°F., and for five weeks when they were held at room temperature.

Moisture content has been found to be a most important factor in the spoilage of dried foods. Nichols (15) states that leafy vegetables showed active spoilage at 20 per cent of moisture, and starchy and sugary foods at 25 - 30 per cent. Gore and Mangels (9) found that unless vegetables were held at a moisture content of less that 8 per cent they deteriorated in storage although there was no active microbiological spoilage. McGillivray (13) set 25 per cent as the maximum permissible moisture content for dried apples.

Nichols, Powers, Gross, and Noel (17) concluded that..."to assure best keeping qualities the moisture content of fruits containing much sugar should not exceed 15 - 20 per cent while that of other fruits and vegetables should not exceed 5 - 10 per cent."

Lewis, Brown,and Barss (12), in their experimental work with prunes, adopted 17 - 18 per cent as the proper moisture content, but in some cases it ran as high as 22 per cent without apparent injury to the keeping quality of the fruit.

Cruess, Christie, and Flossfeder (4), and Cruess and Christie. (3), stated that grapes dried to 23 per cent moisture after treatment with sulfur dioxide would

keep indefinitely, but that those with higher moisture contents ultimately molded, as did prunes with more than 25 per cent moisture content.

Culpepper and Caldwell (5) found that apples stored at relative humidities of 18.8 per cent or less retained their original color, odor, and flavor unchanged throughout a storage period of three years. Active growth of yeasts and molds did not occur until the relative humidity reached 80 per cent, but off-flavors and colors appeared at between 47 and 80 per cent.

Nichols (16) found that unprocessed prunes spoiled at moisture contents as low as 18 per cent, while processed prunes did not spoil at moisture contents less than 22.5 per cent.

The most complete description of methods for examining dried vegetables is given by Prescott, Nichols and Powers (19). They weighed 10 grams of the sample into a flask containing 200 cc. of sterile tap water. After thorough shaking this 1 to 20 dilution was incubated at 37°C. for two hours; the flask was again shaken by hand 25 times and the higher dilutions made. Both plain and dextrose agar were used. Incubation was for 48 hours at 37°C.

In another paper Prescott (18) reported also using litmus glucose agar and Czapek's (6) media for fungi.

Beer wort gelatin or agar was used for a time to test for yeasts, but was discarded because of negative results.

Fellers (7) used 10-gram samples of various fruits in 90 cc. of water. He shook the samples by hand, allowed them to stand one hour, and then violently agitated them for two minutes. Only nutrient agar prepared according to Standard Methods (21) was used; the incubation period was 72 hours at 30°C., but in some cases, due to overgrowths of molds, mold counts were made after 48 hours.

MEDIA AND METHODS

Media

The media which were used in this study were tomato agar, dextrose agar, and plain nutrient agar. The tomato agar was used primarily as a medium for fungi. In preliminary tests comparing tomato agar with dehydrated wort agar (Difco) it was found that the two were about on a par as regards yeast and mold growth. However, for laboratories which make their o own media, the tomato agar is probably the more satisfactory of the two because it is more easily prepared. The clear juice may be strained off from canned tomatoes, which can be bought anywhere in this country. The pH of the finished agar is around 4.5 without adjusting, making it satisfactory for the growth of most fungi, and at the same time inhibitory for the commoner bacteria.

The formula used in this study was as follows:

Tomato juice	400 cc.
Peptone (Difco)	10 gms.
Dextrose (Difco)	5 gms.
Water	600 cc.
Agar	20 gms.

The increase of agar content over that ordinarily used in media was to take care of hydrolysis caused by

the low pH. Sterilization was at 10 pounds steam pressure for 10 minutes.

Both dextrose and plain nutrient agar prepared according to Standard Methods (21) were used for the microorganisms other than fungi.

Procedure

Dried foods may be divided into two main groups on the basis of the method of testing them microbiologically. The first, whole or cut form group, would include for the most part dried fruits. The second group would consist of foods which are in the powdered form when marketed and would include chiefly the dried vegetables. There is one company producing dried foods in the form of films, but these products are not of commercial importance at the present time and are not considered in this paper.

In testing the whole and cut fruits, two methods were tried. One procedure was to take the whole fruit and aseptically cut off portions until a 10-gram sample was obtained. For example, if prunes were being tested, one-half of a sterile petri dish was tared on a balance and portions from each of 10 whole prunes would be cut into the dish until the proper weight was had. The

10 grams of the cut fruit was then transferred to a dilution bottle containing 90 cc. of sterile water.

The other procedure was to take a given number of whole pieces of the fruit and place them directly in the bottle containing 90 cc. of water. In testing prunes 5 whole prunes were taken as a sample; in testing apricots, 5 apricot halves were taken because dried apricots are marketed in halves. After shaking the bottle, one cc. of the suspension in the bottle was taken for plating directly, or for further dilution, depending on the amount of dilution desired. Thus, one cc. was considered as representing 5/90 or 1/18 of the total number of organisms per piece of fruit. The only reason for using 90 cc. of water in the dilution bottle rather than 100 cc. was that this practice eliminated the necessity of making separate dilution bottles for the whole and cut samples.

A comparison of the two methods would also give an indication of the surface contamination as compared with that distributed throughout the flesh of the fruit.

For making the initial suspension, wide mouth dilution bottles were used. See Plate I. Shaking of samples was all done by hand.

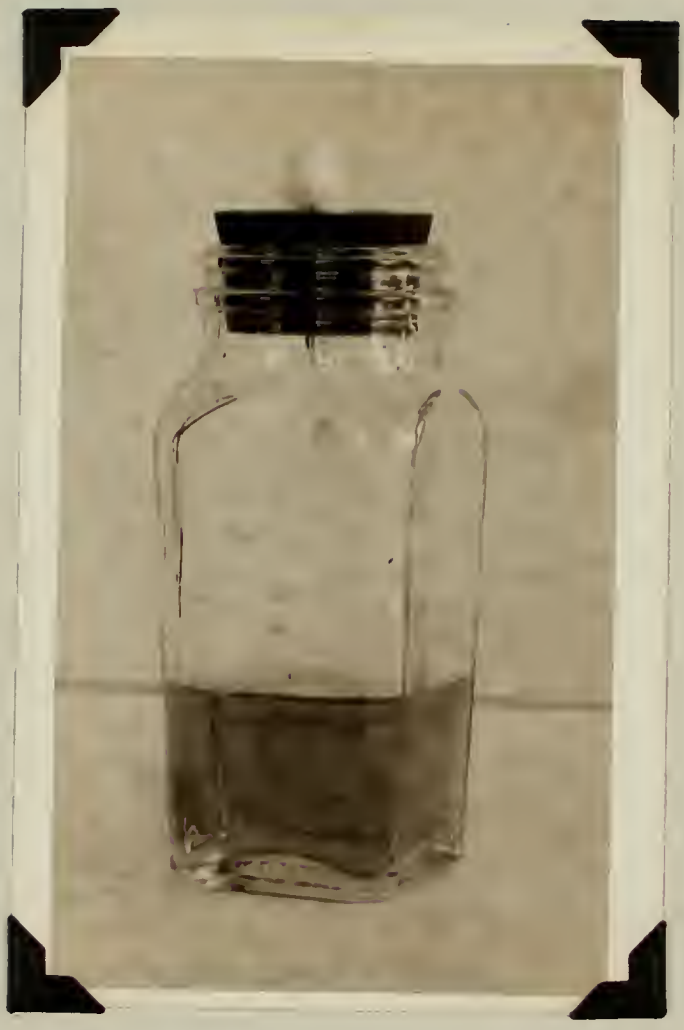

Plate I. Type of dilution bottle used for original 1-10 suspension capacity 350 cc.

The procedure with the powdered foods was to weigh 10 grams of the sample directly into the wide mouth bottle.

Prescott (19) recommended incubating dried food samples at 37°C. for two hours to soak out and free most of the microorganisms adhering to the food. Fellers (7) allowed the sample to stand for only one hour.

To test the effect of this incubation period on the count of some dried foods the following experiment was run.

Ten grams of the sample were weighed into a dilution bottle containing 90 cc. of water. Samples were taken from the dilution bottle as follows and plated on plain and tomato agar. For each product tested, the samples, taken at the intervals noted, were from the same bottle:

1. After shaking 50 times.
2. After incubation for one hour at 37°C.
3. After shaking 25 times more.
4. After shaking another 25 times.
5. After standing another hour at room temperature and shaking 25 times.

As will be noted in table 1, the differences in the numbers of organisms found before and after the incubation period mentioned above are not sufficient to warrant the added expenditure of time. The results also indicate that there is no appreciable increase in numbers in the dilution bottle after a standing period of two hours.

The procedure finally used in these tests was to shake all samples 100 times without incubation in order to insure ample separation of microorganisms present.

Table 1

Effect of Shaking and Incubation on the

Microbial Count of Dried Foods

Product Tested	After Shaking 50 Times		After Incubation of 1 Hour at 37°C		After Shaking 25 Times More		After Second Shake of 25 Times		After Standing at Room Temperature for Additional Hour	
	Bact./gram		Bact./gram		Bact./gram		Bact./gram		Bact./gram	
	Plain agar	Tomato agar	Plain agar	Tomato agar	Plain agar	Tomato agar	Plain agar	Tomato agar	Plain agar	Tomato agar
Banana powder	8300	6500	4700	6500	6000	5500 100 molds	6200	6200 100 molds	9800	6600 100 molds
Prunes	10	10 yeasts	10	10 molds	10	0	10	0	30	0
Apricots	130	0	100	0	80	0	140	0	120	0
Peaches	160	100	150	0	230	0	240	0	200	0
Apples	80	10 molds	50	0	20	10 molds	70	0	not tested	not tested

SURVEY OF DRIED FOODS

Effect of Drying

To determine just what effect the process of drying might have on the flora of dried foods, grapes, prunes, spinach, and carrots were dried after inoculation with test organisms.

Prunes: A 30-pound basket of California fresh prunes was bought in a retail market for the drying experiment and was kept at 34°F. until used.

A tunnel drier was employed for the drying (Plate II). Circulation of air was accomplished by means of a small electric fan, and three electric heating units were used for providing the heat.

Six samples of five prunes each were taken to determine the bacteria originally present on the fruit. Four of these were cut prune samples of ten grams each, and the other two were whole prune samples.

With the first lot of prunes dried, it was impossible to get the temperature in the drier above 120°F. Consequently it took about 6 days to dry the prunes. The counts obtained during various intervals of the drying process are shown in Table 2. While a

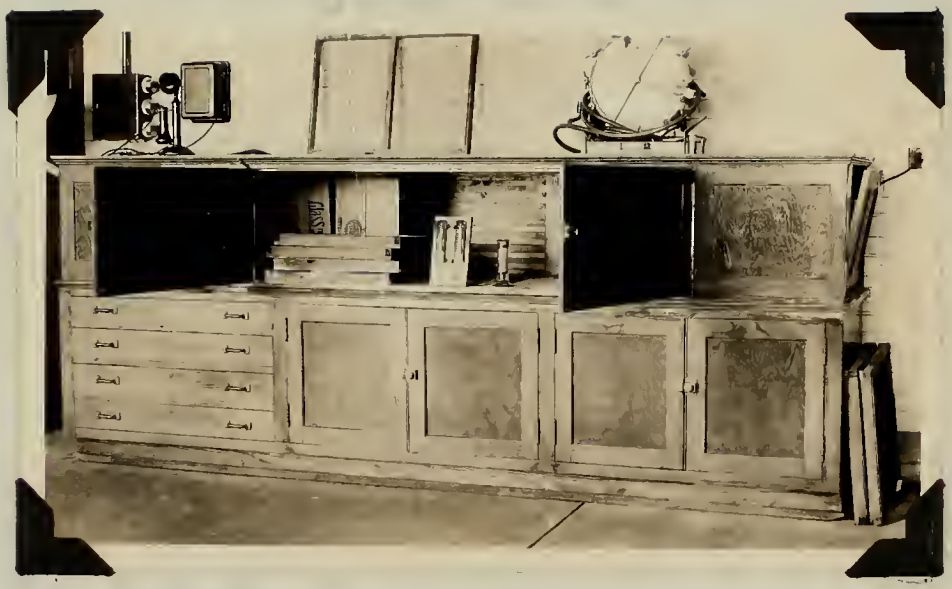

Plate II. Cabinet drier used for drying experiments

Table 2

Number of Microorganisms on Fresh Prunes and Prunes During Drying at 120°F.

	Number per Gram			Number per Prune		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
Fresh prunes	26,700	16,030	Overgrown	1,280,000	570,000	Overgrown
Prunes after washing	57,500	1,750	3,000	1,000,000	88,000	50,000
In drier 24 hrs.	2,000	200	2,900	20,900	Overgrown	450
In drier 48 hrs.	2,200	1,200	800	3,420	9,500	Overgrown
In drier 72 hrs.	70	635	30	5,890	1,900	133
In drier 144 hrs.	250	0	600	1,995	1,520	0

temperature as low as 120°F. would never be used in a commercial prune dehydrator, the conditions roughly approximate those occurring in the sun drying of prunes, so the results of this treatment are given here. Control of humidity was not attemped in this particular experiment.

The number of organisms on the fruit decreased rapidly during the first 24 hours of drying, and then the reduction was more gradual. The original typical flora of brown yeasts was supplanted by a yeast having a ray-like colony. This latter type persisted right through the drying process. It was not determined whether or not this ray yeast was a variant of the original yeast present on the fruit.

By recirculating the air in the tunnel drier it was possible to more nearly approximate conditions found in a commercial dehydrator. The second lot of prunes was dried for 24 hours at a temperature of 130°F. for the first few hours and then the heat was gradually increased until a temperature of 165°F. was attained. The relative humidity of the cabinet was held at 16 per cent during the process in order to prevent too rapid drying on the surface and subsequent case hardening, which would hinder the loss of moisture from the inside of the fruit.

In actual commercial practice, the relative humidity in the drier is about 60 per cent at the start of the process and 25 per cent at the finish. It was not possible to maintain as high a humidity in the small cabinet drier as is used in the large commercial dehydrators. However, as was shown by Clague and Fellers (1) in their studies on the pasteurization of dates, heat treatment at a given temperature coupled with a high relative humidity is more destructive to microorganisms than treatment at the same temperature in an atmosphere with a lower relative humidity. Consequently the number of microorganisms found on prunes after drying under the conditions of this experiment would probably indicate that under commercial conditions where the same temperature and a higher humidity was maintained, the number of microorganisms surviving the process would not be more than those found in these tests.

After commercial drying is completed the prunes are stacked in large piles to"sweat," so that the moisture will become evenly distributed throughout all the fruit. In this experiment the prunes were put in sterile glass jars for the sweating process.

After the commercial sweating process the prunes are steamed for from 2 to 5 minutes, or are run under

sprays of hot water. The bulk prunes are then pressed into boxes holding 25 pounds each right after the heat treatment. The temperature at the center of the packed boxes is about 180°F. To simulate this procedure the experimental pack was dipped in boiling water for 2 minutes.

Bacterial counts made at various steps in the process as outlined above are shown in Table 3. The results of this experiment indicate that the dehydrating process as used in tunnel driers was very efficient in reducing the number of microorganisms on prunes. The temperature used (165°F.) was sufficient to kill off yeasts and probably some molds, and the high acid content in combination with the temperature apparently eliminated many bacteria.

Grapes: The grapes were dried by the same method as that used for the prunes, except that the skins of the grapes were checked with hot sodium hydroxide solution before putting them in the drier. Most of the raisins produced are sun dried, but as it was not possible to use that process here, this experiment was run under much the same conditions as would be used for the commercial dehydration of grapes.

The purpose of the "checking" of the grapes in the hot lye solution is to make the skins of the grapes

Table 3

Numbers of Microorganisms on Prunes

Dried at 165°F.

	Number per Gram			Number per Prune		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
Fresh prunes	26,700	16,030	Overgrown	1,280,000	570,000	Overgrown
Prunes after washing	57,500	1,750		1,000,000	88,000	50,000
Prunes after drying	30	0	10	380	0	0
Prunes after sweating	10	0	0	76	0	760
Prunes after hot water dip	30	0	0	95	0	0

more permeable and thus facilitate evaporation of moisture from the inside of the fruit.

Emperor variety grapes were used for the drying experiments as this was the only type available at the time of year at which the experiment was run. The grapes were purchased in the retail market and held at 34°F. until they were used.

Twenty-four samples of the fresh grapes were tested bacteriologically to determine the flora occurring on the fresh fruit as purchased in the market. It was deemed advisable to inoculate some of the fresh fruit with two test organisms in order to see just what effect the drying process might have on these organisms. Escherichia coli was chosen because it is indicative of pollution and resembles many of the intestinal pathogens in its physiology and reaction to destructive agents. Yeast was used because it is important in the spoilage of fruits high in sugar content.

After being dipped for from 10 to 15 seconds in the hot lye solution the grapes were immediately put into cold water and thoroughly rinsed. Part of the fruit was then sprayed with a suspension of Escherichia coli, part with a suspension of yeasts, and the

remainder was left untreated. The grapes were then put on trays in the drier and dried for 24 hours at a temperature of 130°F. for the first 16 hours, and then the temperature was raised to 165 - 170°F.

Results of this experiment are shown in Table 4. The number of microorganisms per gram was determined from a suspension in 90 cc. of water of ten grams of fresh or dried grapes cut from five or more grapes. The number per grape was determined on the basis of five whole grape berries in 90 cc. of water. Results with the uninoculated grapes are from three separate tests, and results with the yeast and Escherichia coli inoculated samples are from two tests.

The counts in Table 4 indicate that the process used for dehydrating grapes was very efficient in reducing the total number of bacteria, yeasts, and molds present on the fresh fruit. Dehydration effectively destroyed E. coli and yeasts on grapes, so that the treatment was really equivalent to pasteurization.

Carrots and Spinach: The procedure used commercially in drying vegetables differs in several respects from that used for fruits. The vegetable is blanched, that is, either dipped in boiling water for 2 or 3 minutes or steamed for 4 or 5 minutes. The object of this treatment is to inactivate tissue enzymes which might

Table 4

Number of Microorganisms on Fresh Grapes and Grapes Dehydrated at 130° - 165°F.

	Number per Gram			Number per Grape		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
Fresh grapes	340	4,720	170	11,276	40,276	2,461
Uninoculated grapes after drying	80	0	0	19	0	0
Yeast inoculated grapes after drying	0	0	10	0	0	0
E. coli inoculated grapes after drying	30*	0	10	0	0	190

* Not *E. coli*. There was no growth in lactose broth tubes inoculated from the dilution bottle.

cause off-flavors, and to set the color of the vegetable. Drying of vegetables is accomplished at a temperature lower than that employed for fruits, and as the particles of vegetables are much smaller, it is not necessary to have a humid atmosphere in the dehydrator to eliminate case hardening.

Carrots are dried in either the sliced or cubed form. In this experiment the carrots were peeled and sliced transversely into discs about 1/16 of an inch in thickness. They were then put on screen trays and steamed for 5 minutes, after which they were sprayed with a suspension of E. coli and put in the tunnel drier. They were left in the drier for 13 hours. The temperature was held at 120°F. for the first 7 hours and then it was raised gradually to 170°F. Although this latter temperature is higher than that ordinarily used for dried vegetables, the product was in good condition when it was finished so that apparently there was no deleterious effect.

The spinach was treated in much the same way as the carrots. After washing and trimming the spinach was put on the trays, steam-blanched, inoculated, and put in the drier with the carrots.

Tests with the first lot of spinach and carrots showed that E. coli still survived after drying, so

in the second experiment the inoculation was made before blanching to note the effect of this part of the process on the survival of E. coli. Results of both of these experiments are shown in Table 5.

As is noted above E. coli was able to survive the drying process when it was inoculated after the vegetables had been blanched. However, when the vegetables were sprayed with the test organism before blanching no survival was found.

Table 5

Number of Microorganisms on

Carrots and Spinach Dehydrated

at 120 - 170°F.

	CARROTS Number per Gram			SPINACH Number per Gram		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
After washing and trimming	2300	0	0	8700	0	0
After inoculation with *E. coli*	20,100,000	0	0	37,000,000	0	0
Dried, inoculated after blanching	34,000*	0	400	500,000*		
Dried, inoculated before blanching	50,000#	0	0	301,000#	0	0

* *E. coli* surviving

\# *E. coli* not surviving

Market Products

It was thought best in the study of commercially dried foods to make a detailed investigation of a few products rather than to less thoroughly examine many.

With dried fruits, examination was made of both the whole and cut samples by the method described earlier in this paper. As most commercially dried vegetables are cut in their preparation, it was not possible to include the whole form in the determination of microorganisms occurring on them.

Two 10-gram samples were taken from each lot examined. These were put into wide mouth dilution bottles containing 90 cc. of water each, shaken 100 times, and further dilutions made from this suspension. Two plates were made from each dilution. Media used were tomato, plain, and dextrose agars.

Fruits examined were prunes, raisins, and apricots. Vegetables studied included carrots, spinach, dill, onions, garlic, asparagus, beets, okra, and cauliflower.

Prunes: Two samples of bulk, and one of packaged prunes were examined. All were bought at different retail stores in Amherst. One bulk sample was taken from a bin, and the other from a closed glass jar on

the grocer's counter.

Results of the microbial counts on the samples of prunes are shown in Table 6. The number of microorganisms found was very low, 80 bacteria per gram being the highest count obtained. Molds were present on all of the samples, but yeasts were found on only one sample. The nature of the colonies, whether bacteria, yeasts or molds, was determined by observation, and in case of doubt, stained slides were made from the colonies in question.

Apricots: One sample of bulk apricots and two of the packaged were studied in this test.

The method of sampling was essentially the same as was used in the tests with the prunes except that 5 apricot halves were taken as the whole or uncut sample.

Table 7 shows the results of this experiment. The number of microorganisms which developed from dried apricots was very low. Yeasts were not encountered on any of the samples, and molds were found on only one sample. It must be remembered that dried apricots are treated with sulfur dioxide which has a distinctly inhibitory action on microorganisms. (10)

The bacterial count per gram was higher in proportion to the count per half apricot than would be expected on the weight basis. The probable reason for

Table 6

Numbers of Microorganisms on Dried

Prunes as Purchased in Retail Stores

		Tomato			Plain			Dextrose		
		Bact.	Yeasts	Molds	Bact.	Yeasts	Molds	Bact.	Yeasts	Molds
Bulk Sample No. 1	I *	0	0	72	2030	0	4	2070	0	4
	II	0	0	5	80	0	0	70	0	3
Bulk Sample No. 2	I	38	19	95	594	0	38	1062	0	31
	II	0	0	12	45	0	12	50	0	5
Packaged Sample	I	0	0	9	54	0	0	45	0	0
	II	0	0	0	40	0	5	55	0	0

* I = Number per prune

II = Number per half apricot

Table 7

Number of Microorganisms on Dried

Apricots as Purchased in Retail Stores

		Tomato			Plain			Dextrose		
		Bact.	Yeasts	Molds	Bact.	Yeasts	Molds	Bact.	Yeasts	Molds
Bulk Sample	I	0	0	42	198	0	27	240	0	40
	II	0	0	8	107	0	0	137	0	0
Packaged Sample No.1	I	0	0	0	136	0	9	99	0	0
	II	0	0	0	177	0	0	135	0	0
Packaged Sample No.2	I	0	0	0	1422	0	0	1008	0	0
	II	0	0	0	850	0	0	730	0	0

I = Number per half apricot

II = Number per gram

this difference is the lower pH resulting in the agar when the more concentrated sample from the suspension of the whole or uncut fruit is mixed with the medium. This difference was found to be a pH value of about 0.6 which could possibly be significant in inhibiting the growth of some bacteria.

Raisins: Two samples of seedless, and one of seeded raisins were tested. Because of the smallness of the fruit, ten raisins each were taken for the whole or uncut samples instead of the 5 pieces that were used in the tests with the prunes and apricots. Otherwise the procedure was the same as in the two previous experiments.

As will be seen from the results in Table 8, more molds were found from raisins than from either prunes or apricots. Yeasts were not found on the raisins tested, although the composition of the fruit should be conducive to their presence.

The seeded raisins were much lower in mold content. This can probably be explained by the fact that dried raisins are steamed to soften them and facilitate the extraction of the seeds.

Table 8

Numbers of Microorganisms on Raisins

as Purchased in a Retail Store

		Tomato Agar			Plain Agar			Dextrose Agar		
		Bact.	Yeasts	Molds	Bact.	Yeasts	Molds	Bact.	Yeasts	Molds
Seeded Raisins	I	108	0	0	279	0	4	234	0	4
	II	580	0	0	1390	0	5	1180	0	7
Seedless Raisins No. 1	I	0	0	121	315	0	68	234	0	90
	II	0	0	300	380	0	290	380	0	50
Seedless Raisins No. 2	I	0	0	135	315	0	108	315	0	117
	II	0	0	190	815	0	175	730	0	210

I = Number per raisin

II = Number per gram

Vegetables: Dried vegetables are not available to any large extent in the retail markets of this country, and the samples tested in this experiment were all obtained directly from the manufacturers. As was explained at the beginning of this section, dried vegetables are not prepared in the whole form, so only the count per gram was made on these samples.

The procedure was to weigh out two 10-gram portions of each sample into two wide mouth dilution bottles containing 90 cc. of distilled water. The samples were shaken 100 times by hand and further dilutions were made from these bottles. Duplicate plates were made of each dilution using tomato, plain nutrient, and dextrose agars. Incubation of the plates was at 30°C. for 48 hours.

Bacterial counts on the vegetables were much higher than the counts on the fruits as is evident from Table 9, the number of bacteria being over 500,000 per gram except on the dill and garlic. It is interesting to note that the latter products contain essential oils which may have exerted an inhibitory action on the bacterial growth. Molds were found much less frequently on vegetables than on fruits. Yeasts were observed on only the dill sample.

Table 9

Number of Microorganisms on Commercially Dehydrated Vegetables

	Number per Gram								
	Tomato Agar			Nutrient Agar			Dextrose Agar		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
Carrot 1	2,830,000	0	0	3,950,000	0	0	3,780,000	0	0
Carrot 2	2,725,000	0	40,000	4,750,000	0	0	4,400,000	0	0
Beets 1	200,000	0	0	17,500,000	0	0	14,400,000	0	0
Beets 2	4,700,000	0	0	26,800,000	0	0	24,300,000	0	0
Onions 1	100,000	0	0	2,500,000	0	0	800,000	0	0
Onions 2	4,000,000	0	0	9,700,000	0	0	9,200,000	0	0
Dill	22,500	2,500	10,500	177,000	0	0	120,000	7,500	2,500
Asparagus 1	256,500	0	0	550,000	0	0	925,000	0	0
Asparagus 2	200,000	0	0	1,640,000	0	0	1,380,000	0	0
Garlic 1	0	0	50	5,850	0	0	6,425	0	0
Garlic 2	0	0	50	550	0	0	375	0	0
Okra	13,300,000	0	75,000	28,100,000	0	0	20,570,000	0	0
Cauliflower	500,000	0	0	2,057,000	0	0	1,867,000	0	0

Miscellaneous Products

Table 10 shows the numbers of yeasts, molds, and bacteria found on various fruit and vegetable products which were not studied in as much detail as those previously reported. These products were examined in an attempt to get a general idea of the number of organisms on dried foods. The microbial counts do not represent an average of as many samples as do the more detailed counts, but give a very good indication of the total numbers of microorganisms on the foods. No outstanding differences were noted between these counts and those reported previously in this paper.

The banana powder had a higher total count than the other products but it had picked up moisture from the container a few days before sampling and this increased moisture content may have made conditions favorable for growth of some of the organisms. The banana powder, however, was still in good condition after storage for a year, so that no active spoilage had occurred. The dried pumpkin had a much lower microbial count than did the other samples of dried vegetables.

Table 10

Microorganisms on Miscellaneous Dried Foods

	Number per Gram					
	Tomato Agar			Plain Agar		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
Adriatic figs	0	20	0	2,600	0	0
Calimyrna figs	0	0	10	20	0	0
Dates, unpitted bulk	0	0	440	0	0	520
Mission figs	0	20	0	0	0	0
Dried apples	0	0	10	58	0	0
Banana powder	6,260	7,400	200	7,000	0	100
Prunes	0	10	10	120	0	0
Apricots	0	0	0	114	0	0
Peaches	0	0	0	196	0	0
Dates, packaged pasteurized	600	600	150	605	700	70
Prunes from bulk box	0	10	1	14	0	0
Dried orange	10	0	0	400	0	0
Dried pumpkin	0	0	0	540	0	0
Australian raisins, seeded	70	0	300	350	0	70
Australian raisins, seedless	0	630	250	1,120	0	70

General Types of Microorganisms Present on the Dried Foods

Gram stains were made of typical organisms occurring on representative varieties of dried foods. Table 11 gives the results of this experiment. Of 28 cultures of organisms obtained from dried vegetables, 23 were Gram-positive and five were Gram-negative. Four of the 28 types were cocci, the rest were all rods with the exception of one filamentous type obtained from the tomato sample.

Only two Gram-negative organisms were found among the 24 bacterial cultures obtained from the dried fruit samples. Twenty-two of the types were rods and two were cocci.

Representative vegetables were also tested for the presence of proteolytic types. Suspensions of 16 different dried vegetables were inoculated into tubes of melted gelatin and incubated at 30°C. for a period of a week. Observations for liquefaction were made every twenty-four hours. Liquefaction of the gelatin had occurred in all the samples at the end of five days with the exception of one sample of garlic. In this sample there was no evidence of liquefaction even after two weeks incubation. As

mentioned previously in this paper, garlic seemed to exert an antiseptic action.

Inoculation of a suspension of dried vegetables into lactose broth tubes showed lactose fermenting organisms to be present in the flora from six dried vegetables. Transfers from the fermentation tubes to Endo's medium indicated that none of these were of the coli group.

Table 11

Types of Bacteria Isolated

from Dried Foods

Product	Morphology	Gram Staining Characteristic
Asparagus	Short plump rods	+
"	Very small rods	-
"	Large staphylococci	+
"	Very large rods, spore formers, occur in chains	+
Beets	Very large rods	+
"	Large rods, occurring in chains	+
"	Very small rods, almost cocci-like	-
"	Spore forming rods	+
"	Short rods	+
"	Large spore forming rods	+
Carrots	Long slender rods, spore former	+
"	Very small cocci	+
"	Short plump rods	+
"	Very large thick rods occurring in chains	+
"	Short plump rod	+

Table 11 cont.

Product	Morphology	Gram Staining Characteristic
Cauliflower	Medium large, long, narrow rods	+
"	Small plump rods	-
"	Medium large rods	+
"	Short rods	-
"	Large staphylococci	+
"	Medium large, spore forming rod	+
"	Large cocci	+
Onions	Large, spore forming rods Note: At the dilution tested this was the only type of organism on the plate.	+
Tomato	Very small rod	-
"	Large filamentous growth, probably octinomyctes	+
"	Large spore forming rods, occurring in chains	+
"	Medium large rods	+
"	Very large rods, spore formers, occurring in chains	+

Table 11 cont.

Product	Morphology	Gram Staining Characteristic
Prunes	Large staphylococci	+
"	Spore forming rods, occurring in chains	+
"	Very large sporulating, chain-forming rods	+
"	Small rods	+
"	Large sporulating rods	+
"	Very small rods	+
Raisins	Large sporulating, chain-forming rods	+
"	Small sporulating rods	+
"	Very small rod	-
"	Sporulating rods	+
Figs	Small rods	+
"	Sporulating rods	+
"	Small sporulating rods, chain-formers	+
"	Long, thin rods	+
"	Small sporulating rods	+
"	Large sporulating rods	+

Table 11 cont.

Product	Morphology	Gram Staining Characteristic
Apples	Large sporulating rods	+
"	Slender sporulating rods	+
"	Large chunky rods	+
Banana	Staphylococci	+
"	Very small rod	+
"	Small sporulating rod	+
"	Very small rod	+
"	Very small rod	-

GENERAL DISCUSSION

The results of this investigation tend to substantiate the findings of Prescott et al. (17,18) that dehydration is a safe process for the preservation of vegetables and fruits. By dehydration is meant drying of foods by artificial means as contrasted to sun-drying. The latter method subjects the foods to considerable contamination and the temperature attained during the sun-drying process never gets as high as that commonly employed in a commercial dehydrator. However, lye-dipping, treatment with sulfur dioxide, or treatment with boiling water or steam after drying, all help to reduce the number of microorganisms on the sun-dried fruits.

Seedless and cluster raisins, and dates are usually sun-dried without receiving any of these treatments. It is important to note that these fruits are also eaten uncooked, whereas dried seeded raisins, prunes, apricots, peaches, apples, and dried vegetables are nearly always cooked before they are eaten. Pasteurization of dates, as advocated by Fellers (7) and Clague and Fellers (1), is an accomplished fact in two of the largest date repacking concerns in this country. It would probably be advisable to study

the application of pasteurization to the seedless and cluster raisins also.

As was noted previously, Escherichia coli survived on carrots and spinach when they were dried. The organism was still viable on those vegetables after 7 weeks storage. However, when Escherichia coli was sprayed on carrots and spinach before they were blanched, no viable organisms were found on the vegetables after drying. These results would indicate that blanching of vegetables for 1 - 4 minutes probably effectively eliminates the intestinal pathogenic types of organisms. Chances of infection between the blanching process and insertion in the drier are very remote. Furthermore, it is unlikely that dried vegetables would be eaten in the uncooked form unless they were used for seasoning, as might occur with dried onion, or garlic. Another factor which might have public health significance would be the growth of microorganisms or even the possibility of their elaborating toxins during the soaking process which usually precedes cooking. But here again, the cooking process would undoubtedly serve as a safeguard, and with most vegetables a soaking process is not always necessary in preparing the food for cooking.

The study of dried fruit and vegetable market products indicated that the dried fruits as purchased have a very low microbial count considering the manner in which they are handled; as, for example, bulk prunes in the grocer's bin. As the tendency at present is to market dried fruits in the packaged form, the principal chances for contamination after drying would be from the hands of the person filling the package, if filling is done by hand, or from the package itself. Here again, to absolutely assure freedom from intestinal pathogenic organisms, some heat treatment such as pasteurization would be the best solution, although it is probably not necessary in the case of some of the dried fruits. Microbial counts on the commercially dried vegetables were much higher than those found by Prescott et al. (18,19). They stated that the number of organisms on dried vegetables decreased in storage. The products tested in this experiment had been in storage at 34°F. for several months before they were sampled, but, even so, the bacterial count was high although active spoilage was not evident. As it was not possible to visit the concerns manufacturing the dried vegetables, it would not be justifiable to say whether or not the high bacterial content is indicative of careless handling.

The best preventive against microbial spoilage of dried foods is maintenance of a low moisture content in the food during storage. Of course, the fewer the spoilage organisms on the foods at the time of packing, the less likelihood there is of spoilage; but it is not feasible to eliminate all the bacteria, yeasts, and molds which might cause deterioration in the dried foods, and still produce a marketable product.

Detailed study of all the types of organisms occurring on dried foods was not possible in this study. Bacteria were for the most part spore formers, as judged from observation of the colonies and some microscopic examinations; cocci were often noted, and actinomyces occasionally. The morphology of some of the organisms observed microscopically was not sufficiently distinctive to permit their identification, and may have indicated merely variation from typical forms. Penicillia and Aspergilli were the most common types of molds found, although other types were occasionally present. Lactose fermenters were often found on vegetable samples, but no definite coliform types were observed.

In regard to a standard method for the examination of dried foods, the question arises as to just what value such an examination would have. Microbial

counts on plain agar, and on tomato agar or some other medium specific for fungi, would give an idea of the total flora present on the product examined. Inoculation from the original suspension into lactose tubes would indicate the presence of lactose-fermenting organisms which could be confirmed on Endo's medium and by other tests as to their status as coliform organisms.

Incipient spoilage might be detected by plating out samples of dried foods, such as figs or dates, which are undergoing souring by yeasts, but ordinarily when moisture conditions became favorable enough for yeast growth, the proliferation of molds would be sufficient indication that the product was unfit for food.

Judging from the number of microorganisms found on the dried fruits such as dates, prunes, figs, apricots, peaches, apples and raisins, it is believed that a reasonable standard could be set for these products after a sufficient number of samples had been studied.

Microbial counts on dried vegetables, on the other hand, were so high without any apparent indication of spoilage that it is questionable whether standards should be set for such products. To attach any practi-

cal significance to these high counts would necessitate a much more detailed investigation of the manner in which the dried vegetables are prepared and handled. It is the belief of the author that dried vegetables should not be judged on the basis of the number of microorganisms on them, but rather on the types of organisms found. The presence of Escherichia coli and of course of any pathogenic organisms on dried products of any kind should be considered evidence enough to seriously question the advisability of using, and perhaps even to condemn the product, even though the food were to be cooked before it was eaten. Compliance with such a standard should not impose too great a hardship on the manufacturer and should give reasonable protection to the consumer.

As to standard methods of examination, if such should be deemed advisable, the following suggestion is offered: Total microbial counts should be made on plain nutrient agar, and on tomato agar or some other medium, such as wort agar, suitable for the growth of fungi.

The difference in microbial counts between plain agar and dextrose agar was not sufficient to warrant the use of both media. Growth of the colonies was much more rapid on the dextrose agar, but with some

colonies the growth was so rapid as to cover up smaller colonies in 24 hours. For this reason the plain nutrient agar would probably be the best medium for organisms other than fungi.

Tomato agar was useful in detecting yeasts, although they were not found on many samples of the dried foods examined. Molds also usually grew better on the tomato agar than on the other media, but were not so specific as the yeasts in their culture-medium requirements.

Inoculation from the original suspension of a dried food should be made into lactose broth for determination of lactose fermenting organisms which should be confirmed as recommended in Standard Methods for Water Analysis.

Where whole fruits such as prunes, dates, figs, and raisins, and half fruits such as apricots and peaches are tested, microbial counts could be reported as so many organisms per prune, etc. The microbial count on the whole prune samples was higher than that on the cut prune sample, figured on a weight for weight basis, as was the case with the raisins also, showing that the whole fruit count in general gives as accurate a count as the cut sample with these fruits. The results with the apricots were not in agreement, but

possibly may be explained on the basis of the pH change as reported in the discussion under apricots (see page 26). Vegetables, because of their condition after drying,can be tested only by weighing portions into dilution bottles, and reporting the number of organisms per gram of sample.

SUMMARY

The number of microorganisms on dried foods varies from a few thousand per gram as a maximum on dried fruits up to several millions per gram on dried vegetables.

Tests conducted with a small tunnel drier showed that artificial drying or dehydration effectively eliminated yeasts, and materially reduced the numbers of bacteria and molds on dried fruits. Escherichia coli inoculated onto the surface of grapes was destroyed by the drying process.

Reductions of the numbers of microorganisms during the dehydrating process was not so marked as with the fruits. In fact dehydration did not kill off Escherichia coli although the blanching process which ordinarily precedes dehydration did accomplish destruction of the organism.

Types of microorganisms found on dried foods were for the most part Gram-positive sporulating bacteria although Gram-negative bacteria, and yeasts and molds were occasionally observed. Lactose fermenters, not of the coli type, were found in dried vegetables.

Media suggested for a study of dried foods are: plain nutrient agar as recommended in Standard Methods, and tomato agar or a similar medium for yeasts and molds.

The methods used commercially in preparing dehydrated foods would probably result in a safe product, especially when it is considered that most of these foods are cooked before being consumed.

LITERATURE CITED

1. Clague, J. A. and Fellers, C. R. 1933
   Time, temperature and humidity relationships in the pasteurization of dates.
   Arch. für Mikrobiol., 4: 419-426.

2. Cruess, W. V. 1921
   "Commercial Fruit and Vegetables Products"
   McGraw-Hill Co., Inc., New York, N. Y.

3. Cruess, W. V. and Christie, A. W. 1921
   Dehydration of fruits (a progress report)
   Calif. Agr. Exp. Sta. Bull. 330.

4. Cruess, W. V., Christie, A. W., and Flossfeder, F. C. H. 1920
   The evaporation of grapes.
   Calif. Agr. Exp. Sta. Bull. 322.

5. Culpepper, C. W. and Caldwell, J. S. 1928
   The relation of atmospheric humidity to the deterioration of evaporated apples in storage.
   Jour. Agr. Res., 35: 880-906.

6. Czapek, F. 1901
   Beitrage z. chem. Physiol. u. Path., I: 538-560
   Cited from: E. B. Fred and S. A. Waksman.
   "Laboratory Manual of General Microbiology"
   McGraw-Hill Book Co., New York, N. Y. 1928.

7. Fellers, C. R. 1930
   Pasteurized dried fruits.
   Am. Jour. Pub. Health, 20: 175-181.

8. Fellers, C. R. and Clague, J. A. 1932.
   Souring of dates by yeasts.
   Jour. Bact., 23:63 (Abstract)

9. Gore, H. C. and Mangels, C. E. 1921
   The relation of moisture content to the dehydration of raw dried vegetables upon common storage.
   Jour. Ind. and Eng. Chem., 13: 523-24.

10. Hailer, 1911.
    Zeiter. Repert., 1911: 215
    Cited from: F. W. Tanner. "The Microbiology of Foods."
    Twin City Printing Co., Champaign, Ill. 1932.

LITERATURE CITED cont.

11. Hunwicke, R. F. and Grinling, G. N. 1928
A note on some intestinal bacteria isolated from dates.
Lancet (London) 214: 1071-1072. Cited from: Fellers, C. R. 1930 (7)

12. Lewis, C. I., Brown, F. R., and Barss, A. F. 1917
The evaporation of prunes.
Oregon Agr. Exp. Sta. Bull. 145.

13. McGillivray, C. S. 1917
Evaporated apples.
Canada Dept. Agr. Health An. Branch Bull. 24.
Cited from: Culpepper, C. W. and Caldwell, J. S. 1928 (5).

14. Naoum, A. 1934
Preservation of dried figs with special emphasis on pasteurization.
Master's thesis, Mass. State College.

15. Nichols, P. F. 1920
A brief summary of activities of the U. S. Department of Agriculture in dehydration.
Month. Bull., Dept. Agr. of Calif., 9: 133-136.

16. Nichols, P. F. 1934
Public health aspects of dried foods.
Am. Jour. Pub. Health, 24: 1129-1134.

17. Nichols, P. F., Powers, R., Gross, C. R., and Noel, W. A. 1925
Commercial dehydration of fruits and vegetables.
U. S. Dept. Agr. Bull. 1335.

18. Prescott, S. C. 1920
Some bacteriological aspects of dehydration.
Jour. Bact., 5: 109-125.

19. Prescott, S. C., Nichols, P. F. and Powers, R. 1922
Bacteria and molds in dehydrated vegetables.
Am. Food Jour., 17: 11-16.

20. Smeall, J. I. 1932
Bacteria on fruit.
Brit. Med. Jour. 1932. 917-919.

21. Standard Methods for the Examination of Water and Sewage. 7th Ed. 1933.
Am. Pub. Health Assn. 370 Seventh Ave., N. Y. C.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Bradley, Dr. Fellers and Dr. Fuller for their helpful advice and assistance; and to the many manufacturers of dried foods who so generously submitted samples of their products.

Approved by

Leon O. Bradley

C. R. Fellers

F. W. Morse

James E. Fuller

Date May 16, 1935