MICROBIOLOGICAL RESEARCH ON RAISINS AND ALMONDS

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In the food industry there is an increasing awareness of microbiology particularly with respect to sanitation. This is manifest by clauses written into purchase specifications that state, for instance: Escherichia coli negative, presumptive coliform less than 10 per gram, bacterial count 10,000 maximum. Another reason for the interest in microbiology of processed foods is the desire for compliance with various state or federal food and drug laws. Of late there has been emphasis on the Salmonellae bacteria. This is due to recent seizures of certain food products because of alleged Salmonellae contamination. Also, certain buyers are insisting that all products for remanufacture be free of these bacteria. The combination of these several factors plus a desire to produce the best possible product have raised questions about the lack of firsthand knowledge of the microbiology of individual food products.

The reason that these two bacteria (E. coli and Salmonellae) are specified is due to their importance in sanitation and public health. Escherichia coli and Salmonellae are found in the intestinal tract of animals. E. coli is always present in large numbers while Salmonellae organisms are only present occasionally. The presence of E. coli, which is a member of the coliform group of bacteria, in food or water is assumed to be indicative of fecal contamination and thus the possible presence of fecal pathogenic or disease-causing bacteria. The coliform bacteria are easy to detect, and as they are present in large numbers, are used as an indicator of fecal contamination. Escherichia coli is normally not a pathogenic bacterium.

Under normal conditions Salmonellae organisms are not present or are present only in small numbers in feces. Certain species in the Salmonella genus are very pathogenic to man such as S. typhosa which is the causative agent in typhoid fever. Other species of Salmonella can produce food poisoning or other types of disease that can be fatal. Some other fecal transmittable diseases include cholera, brucellosis and tuberculosis.

The primary method of control of these diseases is by sanitation. Sanitary processing of foods is a major link in the public health processes whereby our food and water supplies are ensured of being wholesome and fit for consumption.

The current Food and Drug standards state that no Salmonellae shall be present in food products which means that sophisticated methods of analysis must be employed to detect small numbers of organisms if present in foods. The analysis to determine a limited number of these organisms requires several steps to encourage growth of organisms in order to increase the total numbers of bacteria present as well as the Salmonellae and then the use of selective and differential media to isolate Salmonellae from other organisms present (1). A typical analysis is shown in Figure 1.

1st Day - Place weighed sample in a non-selective pre-enrichment broth (lactose broth) and incubate 16-24 hours at 35° C.

2nd Day - Transfer a small portion of the lactose broth culture to selenite-cystine broth which is a selective enrichment medium. Incubate 16-24 hours.

3rd Day - Inoculate Petri plates containing the selective media SS agar, bismuth sulfite agar, or brilliant green agar. Incubate 24 hours.

4th Day - Transfer typical colonies from these plates to the differential medium triple sugar iron agar (TSI) and incubate for 24 to 48 hours.

5th Day - Examine TSI tubes for the typical reaction of Salmonellae organisms: This constitutes a presumptively positive test.

For confirmation of an organism as Salmonella it is necessary to use several differential media. The exact identification of Salmonella to a particular type requires also various biochemical and serological tests. A brief description of this technique is included to illustrate the complex nature of the analysis for Salmonellae and the length of time involved. As the procedure now stands at least one week is required for the presumptive test. In addition specialized skills, techniques and equipment are required. Therefore, it is impractical to analyze for Salmonellae on a routine basis such as would be required for quality control.

For the past few months we have been examining almonds, as received at the processing plants, for various microorganisms. When these analyses are complete the data will be analyzed statistically to determine, if possible, what may be important in terms of microbial quality of the nuts. The analyses have been for the content of aerobic bacteria, yeast and mold, as well as the fecal indicator organisms, and the coliform group including E. coli.

The data obtained from the analysis have in part been statistically analyzed and related to certain handling practices as well as varietal characteristics.

For instance, the effect of the shell condition upon the aerobic bacterial and yeast and mold content of Neplus, Nonpareil and Mission almonds is shown in Table 1.

Table 1

Average Plate Counts of Organisms Found on Almond Varieties With Different Shell Conditions

	Whole Shell		Split Shell		Shelled	
Variety	Bacteria[a]	Yeast & mold[b]	Bacteria	Yeast & mold	Bacteria	Yeast & mold
Neplus	5,800 n=3	750	3,600 n=5	1,400 n=4	11,000 n=4	5,800 n=4
Nonpareil	960 n=4	1,100 n=4	1,900 n=4	920 n=9	2,900 n=7	1,400 n=6
Mission	200 n=20	200 n=20				

[a] Total aerobic plate count - plate count agar, 48 hours, 30°C. [b] Yeast and mold plate count - acidified potato dextrose agar, 24 hours 30°C. n = Number of counts averaged.

These data show that the semihard shell Mission variety has very low counts that are less than either paper shell Nonpareil or softshell Neplus, indicating that the shell helps protect the nuts from microbial invasion. There is apparently not much difference in microbial counts between nuts that have whole shells and those that have the shell partly split. However, the nuts that are received already shelled have a higher number of microorganisms probably due to increased exposure to dust and dirt. It is interesting that from the 168 samples analyzed, only 7 had confirmed E. coli contamination and these were all shelled Nonpareil. In addition, 3 other cultures isolated from whole shell and split shell nuts might have E. coli but these have not been confirmed yet.

The storage stability of E. coli on almonds is shown on Figure 2. The almonds were inoculated with E. coli cells at an original concentration of 170 per gm. and tested for survivors with time of storage. As shown in Figure 2 there is an initial rapid decline in the number of cells present but even after 68 days the cells still persist and are recoverable. It has been reported that E. coli persists on pecans (2) for 68 days and on walnut meats (3) for 240 days. The total aerobic counts on the almonds as shown in Figure 2 indicate that the population does not decrease markedly with time of storage up to 68 days at 34°F.

Almonds were infected with 100 Salmonella typhimurium per gram and treated with propylene oxide at a concentration of 0.5 ml. per 250 grams in a flask. After 5 days storage no Salmonella were recovered from the propylene oxide treated almonds; however, they were recovered from an untreated control. Thus propylene oxide can be used to destroy these microorganisms on almonds. Analysis for propylene chlorohydrin formation showed that none formed from the propylene oxide.

Experiments with almonds taken before and after commercial methyl bromide fumigation are shown in Table 2. The fumigated nuts had 50% or fewer microorganisms than the nonfumigated, both in terms of aerobic bacteria and yeast and molds, indicating the commercial fumigation of nuts reduces the normal content of microorganisms. However, the treatment is probably not as effective as propylene oxide in decreasing the numbers of microorganisms.

Table 2

Methyl Bromide Fumigation of Almonds

	Aerobic Bacteria	Yeast and mold
Fumigated	670	190
Nonfumigated	1,590	800

We also have been investigating certain aspects of the microbiology of dried fruits. Table 3 shows the aerobic bacteria and yeast and mold plate counts on raisins collected in retail packages from processors. The variation of the counts obtained is indicated by the figures showing the highest and lowest counts of the twelve samples analyzed thus far and the average counts.

The wide range of counts is interesting and shows the variation that is present due to handling techniques of the raw product. The low bacterial count by comparison with other food products may be due to the acidity of the raisins and the higher sugar content.

Table 3

Average Counts on Retail Packaged Raisins - 30 Samples

	Aerobic Bacteria	Yeast and mold
Highest count	1,400	15,000
Lowest count	50	20
Average	343	3,308

Attempts to isolate Salmonella from 30 samples of commercially packed raisins have all been negative, indicating there is not a gross contamination by these microorganisms. In other tests with experimentally inoculated raisins we have developed techniques for recovering Salmonella in small numbers and are investigating the longevity of these bacteria under storage conditions.

Conclusion

Currently, experiments are being conducted to examine almonds and raisins for their microbial content with emphasis on sanitary bacteriology. The total counts examined thus far indicate the microbial content of these products is reasonably low compared with other food products. No Salmonellae have been isolated from 30 samples of raisins examined. Propylene oxide effectively destroys Salmonella on almonds and methyl bromide reduces the microbial population. Escherichia coli have been recovered from 7 of 168 samples tested and persist on artificially contaminated almonds for at least 68 days.

The most effective method of producing foods of low microbial content is strict adherence to sanitation in processing. These sanitary procedures should be incorporated in the handling of foods from the fields through the processing plants and into the final package. All the numerous steps involved should be evaluated in terms of sanitation and elimination of any possible sources of contamination such as equipment or handling procedures.

References

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(3) Kokal, D. 1965. Viability of Escherichia coli on English Walnut Meats (Juglans regia). J. Food Sci. 30: 325-332.

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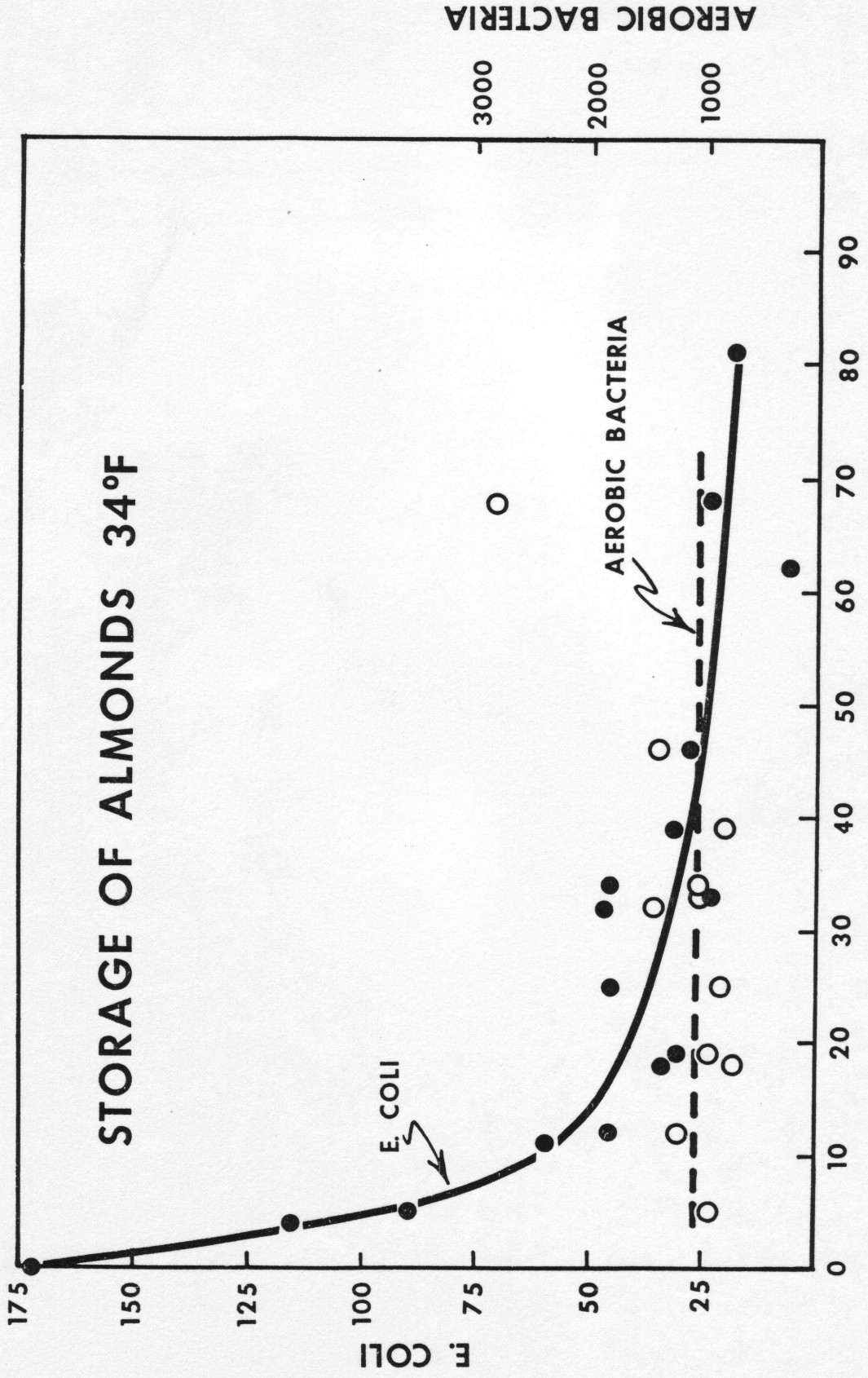

Figure 2. Effect of time at 34°F. on total aerobic plate count and artificially inoculated E. coli on almonds.

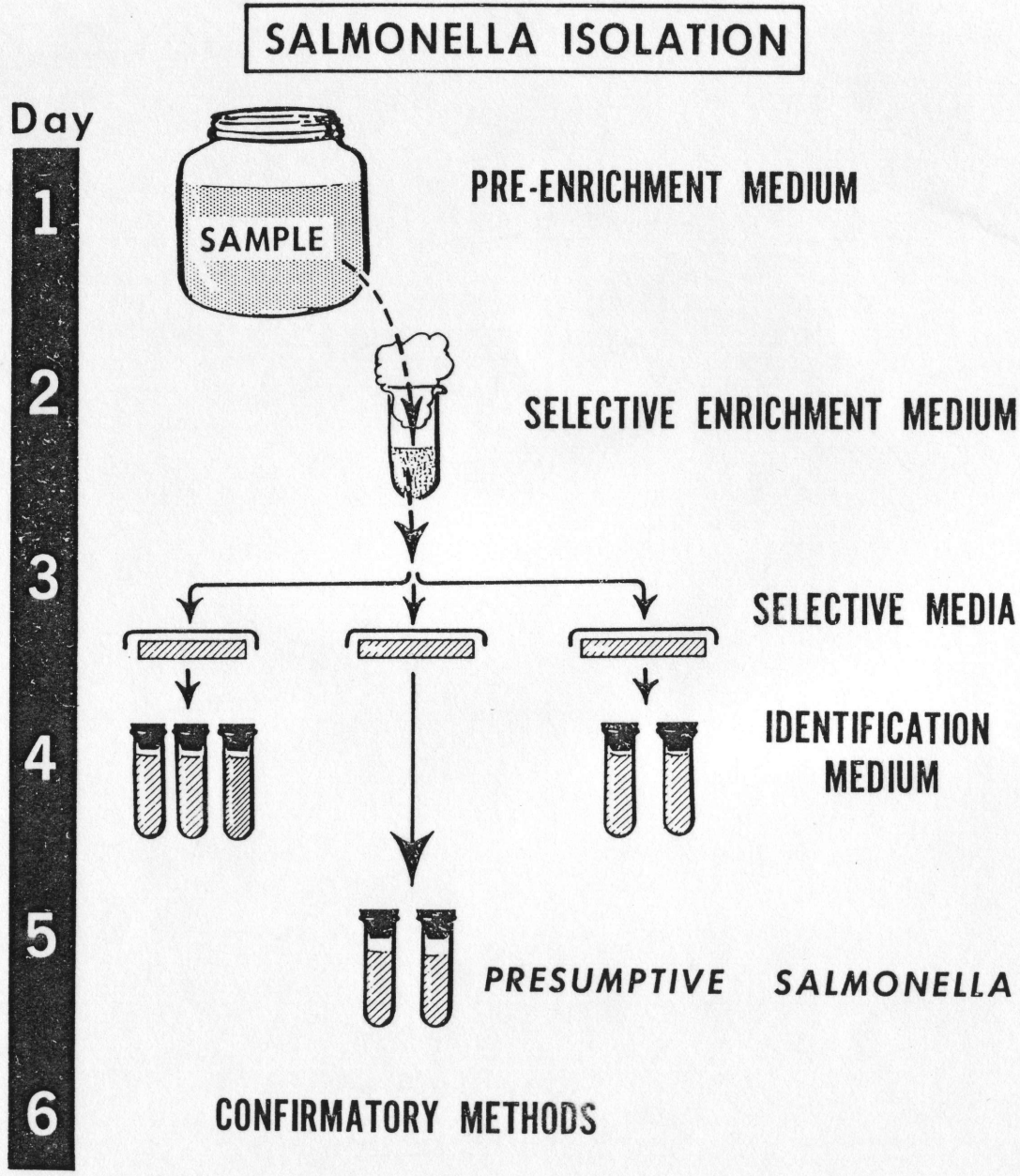

Figure 1. Schematic of Salmonellae isolation procedure.