Almond Harvesting, Processing, and Microbial Flora

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This survey was set up on a statistical sampling plan to determine the microbial quality of almonds as they are received at the processing plant. The total aerobic bacterial count and yeast and mold count distribution were skewed by a few high counts compared with the majority of relatively low counts. Hard shell varieties of almonds had lower counts than did soft shell, and almonds with complete shells had lower counts than shelled almonds. Almonds harvested onto canvas had lower counts than those harvested by knocking onto the ground. Nuts with the least amounts of foreign material mixed into the sample had the lowest counts, as did nuts with the least amount of insect damage. Coliforms, Escherichia coli, and Streptococcus were isolated from the nuts, and their presence was correlated with soil contamination. When almonds are stored, the total plate count, the Streptococcus count, and the E. coli count after an initial drop remain nearly constant for more than 3 months. In addition to the indicator organisms, several genera of bacteria were isolated including Bacillus, Xanthomonas, Achromobacter, Pseudomonas, Micrococcus or Staphylococcus, and Brevibacterium.

Edible sweet almonds (Prunus amygdalus) have three distinct parts: the inner kernel or meat, the middle shell portion, and an outer hull. Almond varieties vary in shell texture; therefore, they are termed hard or soft shelled. The harvesting procedure starts when the almonds are partly dried on the trees. They are shaken down to collecting sheets or onto the ground and are mechanically picked up after further drying. A hulling operation then removes the outer hulls. During this operation, some nutmeats are inadvertently removed from the shell. The hulled nuts are then sent to the processing plant where the nuts are fumigated to destroy insects and eggs before shelling. After the shell has been removed, the nutmeats are processed into graded meats or almond products.

This research was conducted at the request of the almond industry to determine the microbial quality of almonds as received at the processing plant. A preliminary report of this research has been published (5). There has been a continuing interest in coliforms and Escherichia coli in relation to almond contamination (7, 9, 13).

MATERIALS AND METHODS

Sampling plans. A preliminary survey showed that 10% of the almond samples were contaminated with coliforms. Therefore, our statistical sampling plan in 1966 was set up to detect coliform contamination on nutmeats in 10% of the lots with 95% confidence. A 0.25- to 0.5-lb sample (113 to 227 g) was collected from every 200th lot of nuts received at the California Almond Growers Exchange plant in Sacramento for a total of 172 samples. It was shown in a separate preliminary unreported study that samples so collected would represent receipts at all almond processing plants because samples from all growing areas would be included.

Samples used were subsamples of those taken by automatic sampling devices used to grade each lot. The samples were carefully packaged in sterile plastic bags to prevent additional contamination. They were taken immediately upon receipt at the plant and refrigerated until transported to the laboratory where they were stored at 2 C in metal containers to prevent moisture pickup.

During the 1967 season, the sampling plan was changed to include samples that contained such foreign material as soil and sticks, as well as those samples representative of harvesting methods, varieties, and growing areas. A total of 99 samples was selected.

Microbial analyses. Nutmeats were removed from the sample bag or from the shell in an aseptic manner and placed in a sterile bottle. Hard-shelled varieties were cracked with a small hammer before the meat was removed with forceps. An equal weight of water was added to the approximately 10 g of nutmeats in the bottle, and the sample was shaken to wash the surface of the nutmeat. After 5 min, the sample was again shaken, and portions were removed for dilution in 0.1% peptone water (12) or inoculation of plates for microbial counts.

Bacteria counts were made with plate count agar containing 100 µg of cycloheximide (Upjohn) per ml added to suppress mold growth. Yeast and mold counts were made with potato dextrose agar acidified to pH 3.5 with tartaric acid.
Coliform counts were carried out by using Violet Red Bile Agar with an agar overlay (2). Positive cultures were transferred to Lauryl Tryptose Broth. From tubes showing gas, cultures were transferred to Boric Acid Lactose Broth (BALB), and a Gram stain was made. Cultures positive on BALB were streaked onto Levine’s Eosin Methylene Blue plates which, when positive, were confirmed as *E. coli* by procedures suggested by the American Public Health Association (1, 2).

**Streptococcus** counts were made on KF *Streptococcus* agar (8) containing 0.01%, 2,3,5-triphenyltetrazolium chloride. During the 1966 season, all positive cultures were transferred to Azide Dextrose Broth. Those positive on this medium were transferred to Ethyl Violet Azide (EVA) Broth. Of 22 samples (13% of all samples) positive on KF *Streptococcus* agar, all except one were positive on EVA Broth. Because of the selectivity of the KF *Streptococcus* agar, the Azide Dextrose and EVA Broth tests were not used in 1967.

**Identification of bacteria.** Routine methods were used to plate four samples of almond meats, two from almonds at the processing plant and two from pallets of bagged almonds ready for shipment after processing.

From each plate, 25 colonies were picked at random and transferred to BBL Trypticase Soy Broth. The tubes were incubated at 35°C for 72 hr, and the characteristics of the colonies were noted. A loopful of broth was then streaked on BBL Trypticase Soy Agar (TSA) plates to isolate any mixed colonies (incubated 72 hr at 35°C). Colony characters were noted, and the isolated organisms were transferred to TSA slants (incubated 24 hr at 35°C).

Appropriate descriptive tests were run on the isolates by use of standard methods (11). After these tests were completed, the organisms were grouped by genera (10).

**RESULTS**

**Microbial counts.** The total bacterial plate counts on the almonds were quite low, on the average, when compared with those of food items that are not dry processed. The highest value of bacterial counts is within the suggested limit of counts for many foods (4). During both seasons, the yeast and mold count plates contained mostly mold and very little yeast.

The data shown in Table 1 illustrate the wide range and distribution of microbial counts. The median is considerably lower than the mean, indicating that the majority of counts are low, with a relatively few high-count samples that skew the data. Ninety per cent of the values found are below the value listed as the ninth decile in Table 1.

To compare microbial contamination of the unprocessed nuts with that of processed nuts, a series of counts were made on processed almond meats. For 11 samples from 1966, the average count for bacteria was 580 per gram and for yeast and mold, 170 per gram. Comparable values from 1967 (the average of 12 samples) were 1,200 and 2,700 per gram, respectively. These values are somewhat lower than the values listed in Table 1 for receiving supplies, indicating a net drop in microbial count during the processing.

**Indicator organisms.** We examined the unprocessed nuts for coliform and streptococcus content as pollution-indicator organisms in almond processing. Both types were found on the nutmeats when delivered to the processing plant. Table 2 lists the counts obtained from the samples containing these organisms. From the 1966 season, all 22 streptococcus-positive samples were isolated from soft-shell varieties that had been shelled or had split shells. Coliforms were also present in 11 of these samples, but only two had *E. coli*. Of the 1967 samples, 51 (52%) had *Streptococcus* organisms present, only 17 having come from whole-shell nuts. In the 1967 tests, coliforms were also found in 33 of the 51 streptococcus-positive samples, but only 2 of the 33 had *E. coli*.

Of the 172 samples tested in 1966, the 42...
which had coliforms (24%) were the soft-shelled Nonpareil variety. Correlations shown in Table 3 for the 1966 data indicate that coliforms were mainly present in almonds shelled before ship-

ment to the processing plant and those with split shells. E. coli was present in seven (4.1%) of the samples, a value somewhat lower than that found by Kokal and Thorpe (7). A total of 40 of the 99 samples from the 1967 sampling had coliform organisms present. Of these, only nine contained E. coli. None of the correlations calculated for numbers of coliform organisms was significant. E. coli was isolated only from nuts that had the shell removed or had split shells.

**Correlations of microbial counts and test variables.** To give a more normal distribution to the data shown in Table 1, the microbial counts were expressed as logs from which geometric means and confidence intervals were estimated (3). The confidence intervals were based on Student's t distribution at the 95% probability level. Table 3 shows the correlations of microbial counts and some of the 1966 and 1967 test variables. Variables reported in per cent were trans-

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**Table 2. Numbers of indicator organisms per gram of nutmeats on positive samples for 1966 and 1967**

| Organisms          | 1966 | 1967 |
|--------------------|------|------|
| **Streptococcus**  |      |      |
| No. positive samples/total samples | 22/172 | 51/99 |
| Mean counts per gram | 26 | 14 |
| Range               | 1-345 | 1-124 |
| **Coliform**        |      |      |
| No. positive samples/total samples | 42/172 | 40/99 |
| Mean count per gram | 8 | 11 |
| Range               | 1-156 | 1-158 |

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**Table 3. Correlation coefficients of microbial data with individual sample test criteria**

| Year | Determination                          | Aerobic plate count | Yeast and mold count | Streptococcus count | Coliform count | E. coli* |
|------|---------------------------------------|---------------------|----------------------|---------------------|----------------|---------|
| 1966* | Per cent nuts in shell                | -0.175*             | -0.120               | -0.251**            | -0.302**       | -0.442** |
|      | Per cent shelled meats                | 0.398**             | 0.285**              | 0.260**             | 0.340**        | 0.494** |
|      | Per cent shells                       | -0.513**            | -0.438**             | -0.295**            | -0.336**       | -0.377** |
|      | Shell condition                       | 0.576**             | 0.479**              | 0.266**             | 0.333**        | 0.364** |
|      | Type of shell                         | -0.637**            | -0.529**             | -0.198**            | -0.149**       | -0.120** |
|      | Per cent sticktight hulls             | -0.221**            | -0.135               | -0.211**            | -0.214**       | -0.206** |
|      | Per cent foreign material             | 0.273**             | 0.152*               | 0.126               | 0.033          | -0.173* |
|      | Per cent reject (inedible)            | 0.303**             | 0.184*               | 0.181*              | 0.298**        | 0.239** |
|      | Aerobic plate count                   | 0.506**             | 0.299**              | 0.179*              | 0.198*         | 0.150   |
|      | Yeast and mold count                  | 0.197               | 0.197*               | 0.177*              | 0.461**        |         |
|      | Streptococcus count                   | 0.461**             |                     |                     |                |         |
|      | Coliform count                        |                     |                      |                     |                |         |
| 1967* | Per cent nuts in shell                | -0.358**            | -0.131               | -0.484**            | -0.394**       | -0.228** |
|      | Per cent shelled meats                | 0.254*              | 0.039                | 0.373**             | 0.371**        | 0.156   |
|      | Shell condition                       | 0.308**             | 0.083                | 0.349**             | 0.376**        | 0.152   |
|      | Type of shell                         | -0.357**            | -0.194               | -0.048              | -0.171         | 0.017   |
|      | Per cent sticktight hulls             | -0.357**            | -0.077               | -0.413**            | -0.336**       | -0.201* |
|      | Per cent foreign material             | 0.418**             | 0.192                | 0.162               | -0.041         | 0.189   |
|      | Per cent reject (inedible)            | 0.391**             | 0.320**              | 0.224*              | 0.252*         | 0.063   |
|      | Harvest method                        | 0.296**             | -0.145               | 0.156               | 0.052          | 0.055   |
|      | Insect damage                         | 0.476**             | 0.238*               | 0.421**             | 0.366**        | 0.059   |
|      | Aerobic plate count                   | 0.201*              | 0.479**              | 0.439**             | 0.196*         |         |
|      | Yeast and mold count                  | 0.150               | 0.129                | 0.120               | 0.364**        |         |
|      | Streptococcus count                   |                     |                      |                     | 0.475**        | 0.332** |
|      | Coliform count                        |                     |                      |                     |                |         |

* No. = 150.
b No. = 169.
^c No. = 99.
* Significant at 5% probability.
** Significant at 1% probability.
formed by arc sine square root and count data by logs. No influence of growing areas upon the microbial population was found.

**Variety.** There were differences in microbial content among individual varieties that appear to be a reflection of the shell type or condition, rather than a difference in chemical composition among the varieties.

**Shell type and shell condition.** Almond shells vary in texture from fragile to hard, depending on the variety. The varieties we studied were classified as hard or soft shell. Soft-shelled varieties were IXL, Jordonalo, Neplus, and Nonpareil; hard-shell varieties were Davey, Drake, Llewellyn, Mission, and Peerless. The completeness of shell is related to its hardness (Fig. 1).

The influence of shell type and condition upon the microbial counts of almond meats is shown in Table 4. In general, the hard-shelled almonds had lower counts than the soft-shelled varieties. The more complete the shell, the lower the microbial content of the meat. These data illustrate the importance of a complete shell for preventing contamination. The fact that the shells frequently crack and meats are exposed during drying on the tree, particularly with soft-shelled varieties, indicates the need to maintain relatively clean conditions during all handling steps in order to minimize contamination of the nut-meat.

**Method of harvest.** During the 1967 season, we obtained samples that had been harvested by knocking the nuts either onto some type of ground cover (such as canvas) or onto the soil, which usually is carefully rolled and prepared in advance. Nuts without contamination, harvested by either method, were statistically compared (Table 5). Those harvested on ground cloths had significantly lower bacterial and yeast and mold counts than those picked from the ground.

**Contamination materials.** During the 1967 season, some samples were selected from incoming shipments of almonds that contained noticeable dirt. These samples were classified and analyzed by the kind of foreign material present. The classes were: no noticeable contamination, mud balls and pieces of dried manure, mud balls only, and other foreign material (lint, ground in soil on meats, worms, rubber, rocks, etc.). The analysis shown in Table 6 illustrates the influence of the various contaminants upon the microbial counts.

These data indicate that bacterial counts are related to the amount of soil mixed with the sample. The three contaminated categories (Table 6) had significantly higher bacterial counts than did samples with no foreign material. Although not shown in the table, the highest counts that we noted were obtained from samples

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**Table 4. Geometric means and their confidence intervals for microbial counts by shell type and condition for 1966 and 1967**

| Shell condition | Bacteria | Yeast and mold |
|----------------|----------|----------------|
|                | Soft shell | Hard shell     | Soft shell | Hard shell |
|                | No. | Geometric mean | Confidence interval (95%) | No. | Geometric mean | Confidence interval (95%) | No. | Geometric mean | Confidence interval (95%) |
| 1966           |     |                |                    |     |                |                    |     |                |                    |
| Whole          | 16  | 1,510          | 640-3,550          | 40  | 161           | 97-267             | 16  | 374           | 152-924             | 40  | 39           | 18-85               |
| Split          | 87  | 2,030          | 1,690-2,430        | 21  | 430          | -a                  | 87  | 620           | 460-837             | 2    | 257          | -a                  |
| Removed        | 26  | 3,770          | 2,540-5,610        | 19  | 700          | -a                  | 26  | 1,030         | 636-1,650           | 1    | 7,900        | -a                  |
| 1967           |     |                |                    |     |                |                    |     |                |                    |
| Whole          | 8   | 1,610          | 420-6,190          | 8   | 1,080         | 292-4,010          | 8   | 22,100        | 5,850-83,800        | 18   | 4,000        | 6,430-54,800        |
| Split          | 23  | 4,450          | 3,000-6,590        | 18  | 1,390        | 716-2,690          | 23  | 33,800        | 23,700-48,300       | 18   | 100          | 10,000-36,200       |
| Removed        | 29  | 6,260          | 3,630-10,800       | 11  | 2,280        | 881-5,910          | 29  | 29,900        | 19,600-45,600       | 11   | 21,300       | 10,000-46,100       |

*a Insufficient data.
with soil ground into the nut meats and not easily removed. The yeast and mold count did not follow this pattern and appeared to be unrelated to such contamination.

**Insect damage.** The 1967 crop had an unusually large amount of insect damage, primarily due to the navel orange moth. Samples were visually graded with respect to the amount of insect damage on the nut meat and were labeled from 1 to 5, to indicate no damage through increasingly high damage. The data (Fig. 2) show a positive relationship between the amount of insect damage and aerobic plate count (note a correlation of 0.747 from Table 3). Three samples with least insect damage had significantly lower bacterial counts than the two with the most damage. For yeast and mold content, sample categories 1 and 3 were significantly lower than 5.

**Types of bacteria isolated from almond meats.** To determine the types of aerobic bacteria present on almond meats, a total of 25 colonies were isolated from each of two processed and two unprocessed samples. The colonies were classified as to genus by the method of Skerman (10). Most (60) of the cultures were *Bacillus* species, gram-positive, and catalase-positive with endospores.

Numerically, the second most important group (16 isolates) was the gram-negative, catalase-positive, motile, and rod-shaped bacteria. Of these, two seemed to fit the classification of the genus *Xanthomonas* and two *Achromobacter*. Except for two nonmotile cultures, the remaining bacteria had polar flagella but could not be easily placed in a genus, although they were related to *Pseudomonas* as were two nonmotile cultures.

The third group, containing 15 isolates, consisted of gram-positive cocci that fit into the classification of *Micrococcus* or *Staphylococcus*. The last bacterial group of seven isolates can be described as gram-positive, catalase-positive, having nonmotile rods that do not form endospores, and loosely classified in the genus *Brevibacterium*.

**Survival studies.** Figure 3 shows the survival of total bacteria, *E. coli*, and *Streptococcus* species on almond meats stored in plastic bags at 2 or 24 C. The total bacterial counts were made on normal samples of almonds; for the counts of *E. coli* and *Streptococcus* species, the organisms were

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**TABLE 5. Geometric means and their confidence intervals for microbial counts based on harvest methods for 1967**

| Harvest method | Bacteria | Yeast and mold |
|----------------|---------|----------------|
|                | No.     | Geometric mean | Confidence interval (95%) | Geometric mean | Confidence interval (95%) |
| Sheets .......... | 13      | 700            | 360–1,370                  | 40,900         | 22,200–75,200             |
| Ground .......... | 39      | 1,970          | 1,330–2,910                | 18,500         | 12,500–27,400             |

**TABLE 6. Geometric means and their confidence intervals for microbial counts by type of contamination in 1967 nut delivery**

| Contamination       | Bacteria | Yeast and mold |
|---------------------|----------|----------------|
|                     | No.      | Geometric mean | Confidence interval (95%) | Geometric mean | Confidence interval (95%) |
| None ..........      | 52       | 1,500          | 1,070–2,160                | 22,600         | 16,100–31,600            |
| Manure and mud %    | 12       | 4,100          | 1,670–10,100               | 24,100         | 14,300–40,600            |
| Mud balls .......... | 26       | 6,200          | 3,830–10,100               | 36,500         | 23,300–57,000            |
| Other .......       | 9        | 13,900         | 7,830–24,800               | 23,600         | 10,500–53,100            |

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FIG. 2. Geometric means and their 95% confidence intervals for severity of insect damage to almond meats in storage. The amount of insect damage increases from none (1) to severe (5).
inoculated on almonds before storage. Figure 3 indicates that after an initial drop, the total aerobic count is nearly constant with time and shows no marked decline in 225 days at 2 C. Also, there is an initial decrease in the E. coli count which precedes a gradual leveling off. After 225 days of storage, E. coli was still present. Almonds inoculated with Streptococcus species and stored at both 2 and 24 C show curves similar in shape to those of total bacteria and E. coli survival in cold storage. The data show an initial steep drop in the count in the first several days and then a leveling off. At room temperature, the organism dies off more rapidly than at cold temperatures.

Salmonella typhimurium also was inoculated on whole almond meats that were stored at 2 C. After 190 days, the organism could still be recovered, indicating a long survival time on almond meats. Thus, if the nut meats are contaminated, the bacteria can persist for a long time.

Kokal (6) reported that the tannins of walnut skins kill E. coli. In experiments with ground almond skins added to the growth medium, we were unable to demonstrate a similar influence; in fact, the unwashed skins had a stimulatory influence on E. coli, further indicating the non-antagonistic aspect of the almond to bacteria.

**DISCUSSION**

The data reported in this paper reflect the microbial population on the surface of almond meats. A portion of the bacterial contamination stems from soil and dust contact with nutmeats. This is shown by the lower counts for hard-shelled varieties which have a more complete shell and less chance for soil contamination. The effect of soil contact is also reflected in the lower counts for nuts harvested on cloths as opposed to those collected from the ground.

Nuts with the lowest amount of contaminating foreign material had lower counts than those with large amounts, particularly if pieces of soil were ground into the meats. Portable catching frames or ground cloths would lessen soil and microbial contact. The increase in microbial counts with increased insect damage illustrates the need for better insect control both in the orchard and after harvest.

The yeast and mold surface counts correlate with shell factors during the 1966 season but not in 1967. The correlation with rejected material is an indication of the type of spoilage included in this classification. The correlations do not explain the wide range of yeast and mold counts observed. The lack of correlations in 1967 is probably a result of the different sampling plan used that season.

The indicator organisms (coliforms, E. coli, and Streptococcus) were associated with soil contamination of nutmeats, as were the total bacteria. Kokal and Thorpe (7) showed that the incidence of E. coli on almonds was associated with processing operations after harvesting, although some E. coli cells were found on nuts before harvest. The present findings indicate contamination of nutmeats before processing, so their presence on processed nuts does not necessarily indicate poor manufacturing practice.

We had hoped to determine if Streptococcus or E. coli was the best indicator organism to use for measuring contamination of almonds. They both appear to be present in similar numbers and are associated with the same kind of contamination. Neither test appears to be superior, except that the Streptococcus test is easier and less time consuming.

The genera of bacteria isolated from the nut kernel, Bacillus, Xanthomonas, Achromobacter, Pseudomonas, Micrococcus, and Brevibacterium, would be expected to be associated with soil or plant material and to survive on the kernel. The survival of heat resistant Bacillus species on almonds could be of consequence if almonds are used as an ingredient for heat-processed food products. Survival tests for total bacterial count, streptococci, S. typhimurium, and E. coli indicate that the almond does not exert an inhibitory effect and that organisms can persist on the nuts for long periods of time. This illustrates the importance of preventing contamination of the nutmeat, particularly during normal processing when there is only a dry cleaning operation; it is not common practice to wash nutmeats.

The data show that the microbial quality of the almond as it is received at the processing plant is generally good. However, the surface contamination of the nutmeat can be partially controlled.
by lessening the degree of soil or dust contact and thereby improving the quality of the product for further processing.

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