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Microbial Spoilage of Dried Prunes

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Yeasts and Molds Associated with Spoiled Dried Prunes

Yeasts and molds isolated from spoiled dried prunes were identified. Sixty-two strains of yeasts included 21 of *Saccharomyces rouxii,* 11 of *S. mellis,* eight of *Torulopsis magnoliae,* five of *T. stellata,* four of *Candida krusei,* three of *Trichosporon behrendii,* two of *Pichia fermentans,* two of *P. membranaefaciens,* two of *C. chalmersi,* and one each of *S. rosei, S. cerevisiae, Sporobolomyces roseus,* and *C. parapsilosis.*

One hundred and twenty-four strains of molds which were identified included 56 strains of *Aspergillus glaucus,* 18 of *A. niger,* 41 of *Penicillium* spp., four other *Aspergillus* spp., two *Alternaria,* and one each of *Monilia* sp., *Chaetomella* sp., and *Mucor* sp.

Studies of the Osmophilic Nature of Spoilage Organisms

Eleven strains of yeast, representing species most frequently isolated from spoiled dried prunes, and 124 strains of molds isolated from the same source were studied for their osmophilic character.

Strains of *Saccharomyces rouxii, S. mellis,* and *Torulopsis stellata* were able to ferment in a medium containing 70 per cent soluble solids, but failed to grow in two weeks in a medium containing 75 per cent soluble solids. Strains of *T. magnolieae* and *S. rosei* were able to ferment in 65 per cent soluble solids but not in 70 per cent.

The growth rate of *Saccharomyces rouxii* was suppressed considerably when soluble solids were increased from 40

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III. Relation of Equilibrium Relative Humidity to Potential Spoilage[1,2]

INTRODUCTION

Very few studies have attempted to determine what conditions favor microbial spoilage of prunes. It is known, however, that the equilibrium relative humidity (ERH) is one of the best indicators of possible spoilage.

Reported studies on mold growth or spore germination under controlled humidities and temperatures have been based on the following principles: (1) addition of solutes to the growth medium (Heintzeler, 1939; Burcik, 1950); (2) equilibration with humidity-controlling solutions, such as sulfuric acid (Walter, 1924; Heintzeler, 1939) and saturated salts (Mossel, 1951); and (3) determination of the water-sorption isotherm (Scott, 1953; Christian and Scott, 1953).

Previously, 62 strains of yeasts and 124 strains of molds were isolated from spoiled prunes and identified (Tanaka and Miller, 1963*a*). The osmophilic characteristics of the isolated strains were studied, and the most representative isolates were then compared on laboratory media (Tanaka and Miller, 1963*b*).

The present paper describes studies of (1) the soluble-solids content of fresh prunes at different stages of maturity; (2) changes in the moisture content of dried prunes stored under various controlled relative humidities, and (3) results of inoculations of selected osmophilic yeasts and molds on prunes equilibrated in atmospheres of controlled relative humidities.

MATERIALS AND METHODS

Soluble-solids Content

The soluble-solids content of fresh prunes was determined by measuring the refractive index in a Zeiss Opton refractometer. Soluble solids were expressed as per cent sucrose at 20° C; the percentages are on a weight basis.

The soluble-solids content was determined on samples of fresh prunes taken from the same trees on different dates and thus at different stages of maturity.

French prunes were harvested by hand on August 14 and 25, and September 12, 1958. The fruits were carefully sorted for size and color. Fifty fruits for analysis were taken at random from each sample. Counts were made of the number of fruits that had soluble solids in the range of 10 to 35 per cent.

Moisture Content

The average moisture content of the prunes was determined by the AOAC vacuum-oven method (Association of Official Agricultural Chemists, 1955). A sample of about 100 gm of pitted prunes was ground, and portions of

[1] Submitted for publication May 4, 1961.

[2] A report of work done under contract with the U.S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Western Utilization Research and Development Division of the Agricultural Research Service.

about 2 gm were used. All determinations were made in duplicate. Changes in moisture content were followed for 25 weeks.

Equilibration

Prunes for the equilibration studies were the French variety, selected for uniform size and maturity from four trees in the University orchard at Davis. They were dehydrated to approximately 18 per cent moisture content under conditions carefully controlled to prevent incipient heat damage during drying. The drying schedule simulated counter-current conditions found in the commercial dehydration of prunes. The initial dry-bulb temperature of 135° F was gradually increased to a maximum of 165° F. After the dried prunes were sterilized by fumigation with propylene oxide, they were stored in 5-gallon containers sealed with friction lids.

An experiment was conducted to determine the rate of moisture uptake by dried prunes in atmospheres of various relative humidities, and the time necessary under those conditions for microbial spoilage to occur.

A solution saturated with a salt with an excess of the solid phase (International Critical Tables, 1926) was put into a desiccator, and the atmosphere was allowed to equilibrate with the salt solution for two days at the desired temperature (20° or 30° C). This type of solution was used in preference to sulfuric acid solutions to insure that the concentrations were not changed either by moisture absorbed from the prunes or by the prunes absorbing moisture from the solution. Such a change in concentration would affect the ERH. A saturated salt solution, on the other hand, does not change its ERH provided an excess of the solid phase is present in the solution and the solution is stirred or agitated periodically. The table below lists the relative humidities of the saturated salt solutions used, at 20° and 30° C.

TEMPERATURE	RH	SOLID PHASE
	per cent	
20°C	52	$NaHSO_4$
	63	NH_4NO_3
	76	$NaNO_3$
	87	KCl
	93	Na_2SO_4
30°C	60	NH_4NO_3
	68.6	NH_4Cl plus KNO_3
	76	NaCl
	85	KCl
	97	K_2SO_4

About 300 gm of dried prunes with a moisture content of 15.6 per cent were transferred, by means of sterile glass rods, from a storage container to the sterile desiccator.

The weight of the prunes in the desiccators was measured weekly, and water sorption or desorption was calculated by weight changes.

Inoculation

Prunes equilibrated in the experiments described above were inoculated with test organisms, and the appearance of spoilage was followed at 20° and 30° C.

The most osmophilic strain was selected from each of the four groups of organisms most frequently isolated: *Saccharomyces rouxii* (59–4), *Aspergillus glaucus* (59–16), *A. niger* (59–17), and *Penicillium* sp. (59–18) (Tanaka and Miller, 1963*a*).

Wide-mouth, 1-gallon jars with tight lids were used as humidity chambers. Four small glass containers (volume approximately 100 ml) were placed in each of the gallon jars. Each small vessel contained three prunes from equilibrated samples inoculated with one of the four test organisms (fig. 1). The inocula were either conidia of molds or vegetative yeast cells, which were applied to the surfaces of the prunes. Uniformity of all inoculations was attempted. Metal foil caps were placed over each small container to minimize cross-contamination in the humidity chamber. The relative humidity inside each jar was maintained by the same

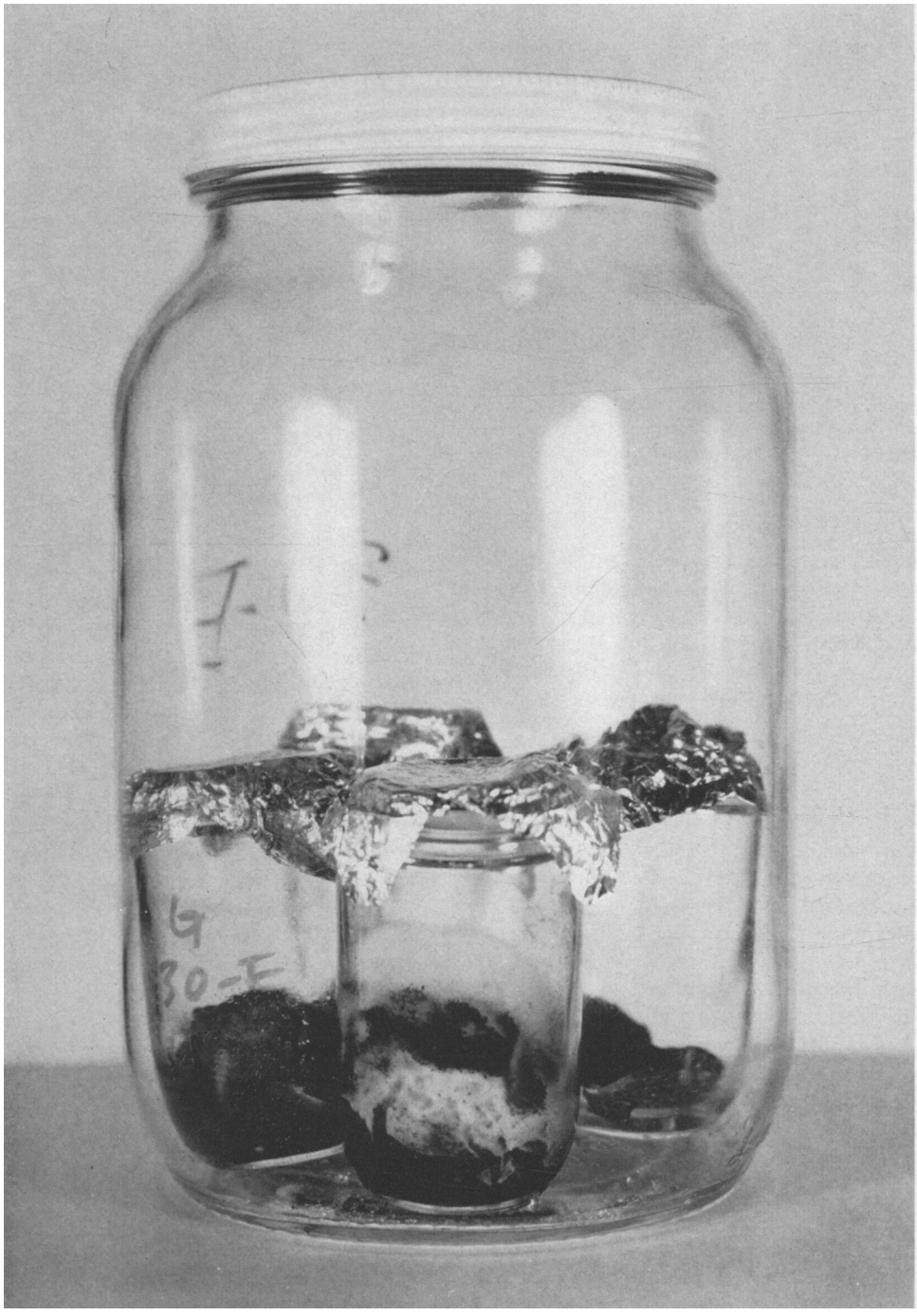

Figure 1. *Saccharomyces rouxii, Aspergillus glaucus, A. niger,* and *Penicillium* spp. grown on prunes, under controlled relative humidity, in 1-gallon jar.

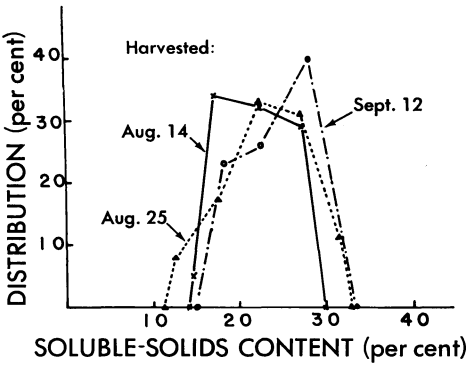

Figure 2. Distribution of soluble-solids content in fresh prunes harvested at three stages of maturity. At each stage, prunes were selected by hand, for uniformity of size and color.

saturated salt solution used for the equilibrium study (table, p. 184). For investigation of the contribution of mechanical injury to the growth of molds and yeast, skins of a duplicate series of prunes were incised repeatedly with a sterile knife before the prunes were inoculated.

RESULTS

The percentage distribution of fruit population was plotted as a function of the average value of the soluble-solids contents of the fruits in each stage of maturity (fig. 2).

Soluble-solids Contents of Fresh Prunes

Averages of soluble-solids contents for the total samples collected on the various dates were: 21.7 per cent for August 14; 23.4 per cent for August 25; and 26.2 per cent for September 12. Although these fruits were picked by hand and carefully sorted for size and color, the range of soluble-solids content was large: 14.3 to 31.6 per cent for August 14; 11.5 to 32.1 per cent for August 25; and 15.3 to 33.5 per cent for September 12. Thus, the stability of individual fruits after they have been dried and processed may vary considerably.

Changes in Moisture Content of Dried Prunes

Figures 3 and 4 show the weight changes in prunes at 20° and 30° C, respectively, during 25 weeks of storage in atmospheres of various relative humidities. The figures show that equilibrium between dried prunes and moisture in the atmosphere requires a long period. In 25 weeks at 20° and 30° C, equilibrium was reached if relative humidity was below 76 per cent.

Mold spoilage developed, after 20 weeks, on one prune in an atmosphere of 97 per cent relative humidity at 30° C. No other sample in the experiment molded during the 25 weeks of storage.

Growth of Organisms

Rate of growth differed considerably with the inoculum and the temperature of incubation. At 30° C and the highest relative humidity (97 per cent), growth of *Aspergillus glaucus* was noticeable in three days and of *A. niger* and *Saccharomyces rouxii* in four days. Growth proceeded very rapidly on these samples. At 85 per cent relative humidity, *S. rouxii* produced visible growth in 13 days, whereas the other organisms failed to grow in four months.

Penicillium behaved very differently on damaged and undamaged prunes at 30° C. Growth appeared on the incised prunes after six days, and developed rapidly at 97 per cent relative humidity. On the undamaged prunes, growth was detectable after eight days' incubation, and developed less extensively under the same conditions.

At 20° C, growth by *Aspergillus glaucus* occurred on the prunes equilibrated with the atmosphere of highest relative humidity (93 per cent) after six days. Growth of *A. niger* was detectable on the prunes under the same conditions after 15 days. *Penicillium* sp. again showed growth responses on the damaged and undamaged fruits

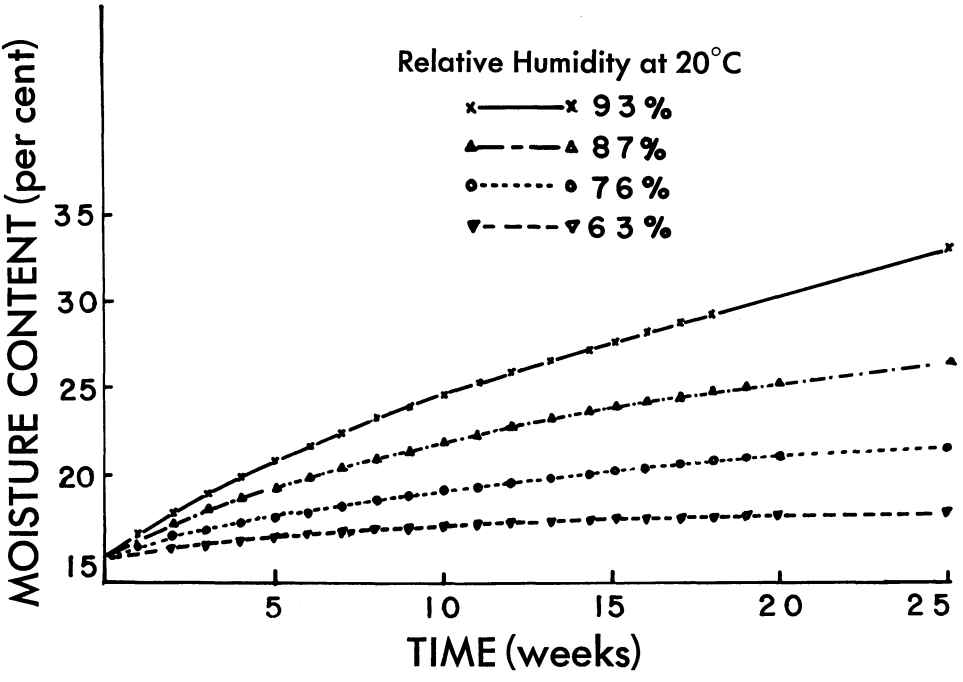

Figure 3. Changes in moisture content of dried prunes stored in atmospheres of various relative humidities, at 20° C.

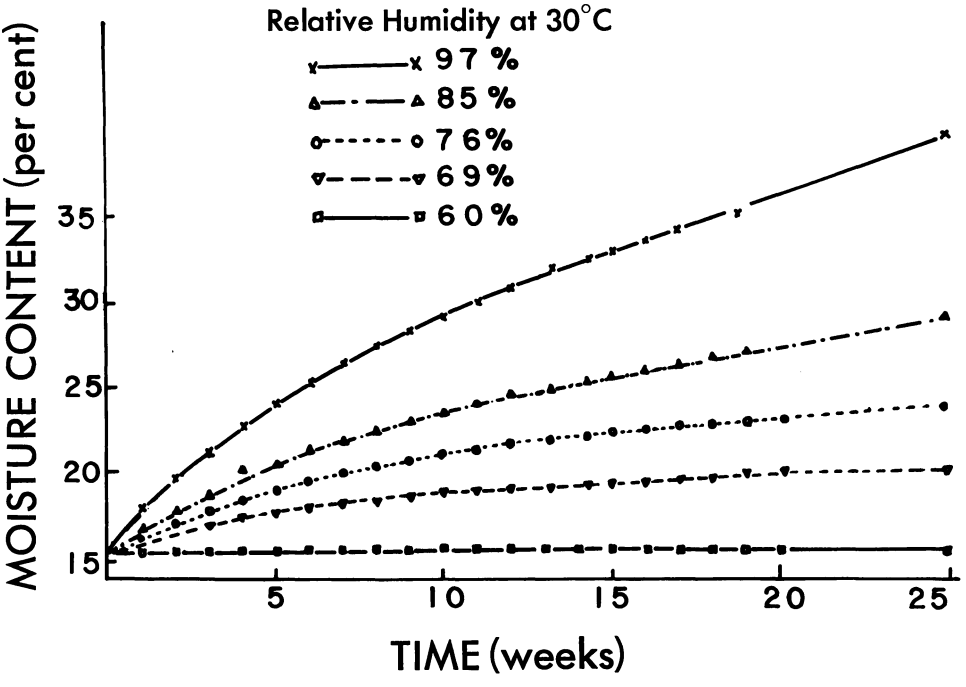

Figure 4. Changes in moisture content of dried prunes stored in atmospheres of various relative humidities, at 30° C.

similar to those at 30° C. Growth was slower on the undamaged than on the damaged fruits; however it eventually covered the skin surfaces of both samples. *Saccharomyces rouxii* had visible growth after 19 days on prunes equilibrated with the atmosphere of 93 per cent relative humidity. In contrast to the rapid growth that occurred at 30° C, the growth of molds and yeasts at 20° C proceeded rather slowly, but steadily.

Growth by *Aspergillus niger* and *Penicillium* was inhibited on prunes at 87 per cent relative humidity. However, *A. glaucus* and *Saccharomyces rouxii* showed visible growth on prunes equilibrated in the atmosphere of 87 per cent relative humidity after 19 days. They also eventually produced visible growth on the prunes equilibrated with the atmosphere of 76 per cent relative humidity after an incubation period of four months (fig. 5).

Except as noted with *Penicillium* sp., the presence or absence of mechanical injury to the skin did not change the growth picture for the other organisms tested.

DISCUSSION

The concentration of soluble solids in the fruit is the most important factor affecting resistance to microbial deterioration after the prunes are dried and processed.

The great variation in soluble-solids content among the fresh prunes of each sample of uniform size and color indicates that even more variation may be expected in the soluble-solids content of

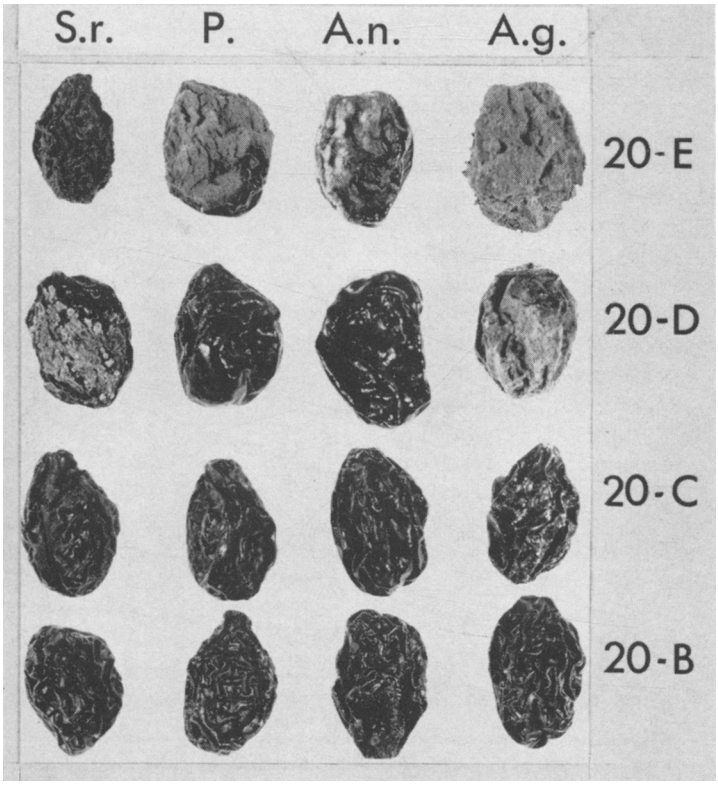

Figure 5. Microbial development, after four months, on prunes equilibrated with atmospheres of various relative humidities at 20° C. 20-E = 93% relative humidity; 20-D = 87%; 20-C = 76%; 20-B = 63%. S.r. = *Saccharomyces rouxii;* P. = *Penicillium* sp.; A.n. = *Aspergillus niger;* A.g. = *A. glaucus.*

commercially dried fruits, in which the fresh fruits are not selectively sorted. The susceptibility of such prunes to microbial spoilage should also vary as greatly since it, too, is influenced by the soluble-solids content.

The attainment of equilibrium between whole prunes and the atmosphere takes a long time even when the relative humidity of the atmosphere is high. Mossel (1951) found that equilibrium was not attained in three or four weeks in a layer of fruit syrup less than 2 mm thick. Scott (1957) pointed out that the diffusion of water molecules is the main mechanism for equilibrium in closed containers—a very slow process. Skins of dried prunes may be barriers against the diffusion of water molecules into the flesh. Diffusion within the prune itself may be very slow. The curves in figures 3 and 4 indicate that the main rate-limiting factor is the diffusion, and that the prunes have a sigmoid type of water-sorption isotherm. Moisture content increased sharply as relative humidity approached 100 per cent. Prunes in atmospheres of 85 per cent relative humidity or higher were still increasing in moisture content in a nearly linear fashion after 25 weeks (figs. 3 and 4).

Inoculation and incubation of equilibrated prunes in atmospheres of various relative humidities gave results in agreement with those obtained in the screening process for osmophilic organisms (Tanaka and Miller, 1963*b*). This may be seen by a comparison of the relative humidities of the atmospheres which would be in equilibrium with the screening media of various soluble solids. At 30° C, *Saccharomyces rouxii* grew on prunes equilibrating in an atmosphere of 85 per cent relative humidity, but not at 69 per cent. These relative humidities are similar to those found in the atmospheres over media having soluble-solids contents of 64 and 76 per cent, respectively, as invert sugar-sucrose mixtures (Schachinger and Heiss, 1951). In the screening process, *S. rouxii* grew at 70 but not at 75 per cent soluble solids. Reason for the failure of the molds (especially *Aspergillus glaucus*) to grow at 85 per cent relative humidity is not known. All grew on prunes at 97 per cent relative humidity. At 20° C, *S. rouxii* as well as *A. glaucus* slowly grew on prunes equilibrating at 76 per cent relative humidity, which is similar to that of an atmosphere over a medium of approximately 70 per cent soluble solids. The two most important spoilage organisms of dried prunes are *S. rouxii* and *A. glaucus*. Also important are yeasts with osmophilic properties similar to those of *S. rouxii*, for example, *S. mellis, Torulopsis stellata, T. magnoliae,* and *S. rosei. A. niger* and *Penicillium* species are considered less important than the above-named osmophilic yeasts or *A. glaucus,* since they did not cause spoilage of equilibrated prunes in atmospheres below 85 per cent relative humidity at 30° C and 87 per cent relative humidity at 20° C.

LITERATURE CITED

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS
1955. Official methods of analysis of the Association of Official Agricultural Chemists. (8th ed.) Washington 4, D.C.: The Association of Official Agricultural Chemists. 1008 pp.

BURCIK, E.
1950. Über die Beziehungen zwischen Hydratur und Wachstum bei Bakterien und Hefen. Arch. Mikrobiol. **15**:203–35.

CHRISTIAN, J. H. B., and W. J. SCOTT
1953. The influence of nutrition on water relation of *Salmonella oranienburg*. Jour. Biol. Sci. **8**:75–82.

HEINTZELER, I.
1939. Das Wachstum der Schimmelpilze in Abhängigkeit von den Hydraturverhältnissen unter verschiedenen Aussenbedingungen. Arch. Mikrobiol. **10**:92–132.

INTERNATIONAL CRITICAL TABLES
1926. New York: McGraw-Hill Book Company, Inc., Vol. **1**, p. 67.
MOSSEL, D. A. A.
1951. Investigation of a case of fermentation in fruit products rich in sugar. Antonie van Leeuwenhoek **17**:146–52.
SCHACHINGER, L., and R. HEISS
1951. Osmotischer Wert und Mikroorganismenwachstum in Zuckerlösungen. Arch. Mikrobiol. **16**:347–57.
SCOTT, W. J.
1953. Water relation of *Staphylococcus aureus* at 30°C. Australian Jour. Biol. Sci. **6**:549–64.
1957. Water relation of food spoilage microorganisms. Adv. Food Res. **7**:83–127.
TANAKA, H., and M. W. MILLER
1963*a*. Microbial spoilage of dried prunes. I. Yeasts and molds associated with spoiled dried prunes. Hilgardia **34**(6):167–70.
1963*b*. Microbial spoilage of dried prunes. II. Studies of the osmophilic nature of spoilage organisms. Hilgardia **34**(6):171–81.
WALTER, H.
1924. Plasmaquellung und Wachstum. Z. Botan. **16**:353–417.

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to 60 per cent, was very slow at 65 per cent, and not detectable at 70 per cent soluble solids.

Colony diameters of the mold strains were measured daily for up to seven days, on solid media containing 40, 50, and 60 per cent soluble solids. Strains of *Aspergillus glaucus* showed pronounced osmophilic characteristics whereas those of *A. niger* and *Penicillium* spp. were somewhat less osmophilic. Strains in the genera *Alternaria, Monilia,* and *Chaetomella* showed no osmophilic characteristics. The single strain of *Mucor* grew luxuriantly on 40 per cent soluble solids, barely grew on 50 per cent, and not at all on 60 per cent.

The effect of increasing the soluble-solids content in a medium on the lag phase and the growth rate was least for strains of *Aspergillus glaucus,* confirming the definite osmophilic nature of this group of molds, and greatest for strains of *Penicillium. A. niger* strains were affected in an intermediate fashion on the same media.

Relation of Equilibrium Relative Humidity to Potential Spoilage

The soluble-solids content of fresh prunes was studied in fruits picked by hand at three stages of maturity. Although fruits were carefully sorted for uniformity of size and color, the soluble-solids content of individual fruits varied greatly at each stage of maturity.

Changes in the moisture content of dried prunes placed in atmospheres of various relative humidities at 20° and 30° C were followed for 25 weeks. Prunes attained equilibrium in 25 weeks in atmospheres of 76 per cent relative humidity or lower.

Dried prunes equilibrated in atmospheres of various relative humidities were inoculated with selected strains of *Aspergillus glaucus, A. niger, Penicillium* sp., and *Saccharomyces rouxii.* At 20° C the strains of *A. glaucus* and *S. rouxii* grew on prunes equilibrated at relative humidities as low as 76 per cent, but not at 69 per cent, in a four-month period. The strains of *A. niger* and of *Penicillium* sp. grew well on prunes equilibrated at 93 per cent relative humidity but failed to grow on those equilibrated at 87 per cent. At 30° C, only the strain of *S. rouxii* grew on prunes equilibrated at 85 per cent relative humidity. All strains tested, however, grew at 97 per cent.

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