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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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II. Studies of the Osmophilic Nature of Spoilage Organisms[1,2]

INTRODUCTION

Drying preserves unsulfured fruits primarily by concentrating their soluble solids. Since properly dried, unsulfured fruits very rarely undergo microbial spoilage, only microörganisms of osmophilic characteristics should be able to initiate such spoilage of dried prunes.

Ingram (1949, 1950) isolated osmophilic yeasts, from concentrated orange juice, that could tolerate a sugar concentration of 70 per cent. Schelhorn (1950) investigated the osmophilic nature of *Zygosaccharomyces barkeri* (*Saccharomyces rouxii* var. *polymorphus*). He found that this yeast grew very slowly in a fructose syrup that was in equilibrium with an atmosphere having a relative humidity (RH) of 62 per cent. Mossel (1951) isolated osmophilic yeast from samples of sugared fruits containing "at least" 70 per cent solids. The yeast was capable of fermentation in a 70 per cent fructose medium. English (1953) studied the osmophilic nature of a strain of *S. rouxii*. According to her estimate, the medium equilibrium relative humidity (ERH) at which growth could still occur in malt extract was about 73.3 per cent.

The present paper reports studies of the osmophilic character of yeasts and molds isolated from spoiled dried prunes. The organisms studied were 11 strains of yeast species isolated most frequently from the prunes, and all of the molds previously described by Tanaka and Miller (1963).

MATERIALS AND METHODS

Processed prunes in California contain 38.4 to about 51 per cent reducing sugars (glucose and fructose) and 0.6 to 5.5 per cent sucrose, on the basis of a 20 per cent moisture content (Mrak, Smith, and Henriques, 1933). In the present study, sucrose was used to prepare media of high soluble-solids content. Glucose could not be used because the maximum solubility in aqueous media at room temperature is about 45 per cent. When sucrose is dissolved by heating, it is possible, by partial hydrolysis, to make media containing 70, 75, and even 80 per cent soluble solids. The media with soluble solids above 80 per cent, however, formed crystals of sucrose in about one week. Media of 70 and 75 per cent soluble solids maintained their soluble-solids content during the experiments without producing crystals, probably because invert sugar formed in the media promoted the solubility of sucrose.

Preparation of Liquid Media

Liquid media were prepared, containing 40, 50, 60, 65, 70, 75, and 80 per

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[2] 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.

cent soluble solids. For 100 gm of a medium containing x per cent solids, the following were mixed and heated to dissolve the sugar: 1 gm yeast autolysate (Albimi); 0.5 gm glucose; $(x - 1.5)$ gm sucrose; and $(100 - x)$ gm water. The soluble-solids content was checked with a Zeiss Opton refractometer and adjusted to the desired concentration with sucrose or distilled water. Soluble-solids content is expressed as per cent sucrose at 20° C. All content percentages given are on a weight basis.

Media with soluble-solids contents of 60 per cent or lower were sterilized by filtration through a Zeiss filter; the other media were pasteurized in test tubes by heating for 3 minutes in an autoclave at 120° C. Partial hydrolysis of sucrose to invert sugar during autoclaving was necessary to prepare media containing the higher concentrations of soluble solids.

The sterilized media were poured into test tubes provided with screw caps. An inverted vial was placed in each tube, and the air was expelled by autoclaving the tubes for 3 minutes at 120° C. For some experiments, the media were dispensed in flasks with Klett tube sidearms.

The media were not given forced aeration, since the static condition more closely resembles conditions during actual spoilage of prunes.

The 11 strains of osmophilic yeasts included four of *Saccharomyces rouxii*, four of *S. mellis*, and one each of *Torulopsis magnoliae*, *T. stellata*, and *S. rosei* (Tanaka and Miller, 1963). They were tested for their ability to grow and ferment in the media, which were inoculated with cells from 24-hour-old yeast cultures that had been grown on 10 per cent malt extract, 10 per cent sucrose agar. Degree of fermentation was determined daily by the volume of carbon dioxide trapped in the vial.

A highly osmophilic strain, *Saccharomyces rouxii* (59-4), was studied separately. Its growth rate was determined by changes in turbidity of media of various soluble-solids content. Two-day-old cells grown on 10 per cent malt extract, 10 per cent sucrose agar media were inoculated in flasks of liquid media containing 40, 50, 60, and 70 per cent soluble solids, and incubated at room temperature. The controls were flasks containing noninoculated media. Changes in the turbidity of inoculated and noninoculated media were followed for 25 days with a Klett-Summerson colorimeter (red filter #66). Cells were harvested, washed, and suspended in distilled water before being dried *in vacuo*.

Preparation of Solid Media

Solid media containing 40, 50, and 60 per cent soluble solids were prepared in the same manner as the liquid media except for the addition of 2 per cent agar. The media were pasteurized by immersion in boiling water for 10 to 15 minutes. Invert-sugar solid media containing 60 to 70 per cent soluble solids were prepared in the same way, except that invert sugar was used instead of sucrose.

Screening of mold strains. All molds (124 strains) isolated from spoiled prune samples were tested for their ability to grow on the solid media containing 40, 50, or 60 per cent soluble solids. All strains capable of growing on the most concentrated medium were tested with the invert-sugar media containing 60 and 70 per cent solids.

Pin-point inoculations were made with conidia from young cultures. Generally, four isolates were tested per plate. Colony diameters were measured daily for seven days. To minimize changes in the moisture content of the media, each petri dish was sealed with tape after inoculation (see figs. 1 and 2).

Growth-rate studies. The growth rates of mold strains in the three most osmophilic groups were compared on solid media containing 40, 50, and 60 per cent soluble solids. The technique was the same as that used in screening.

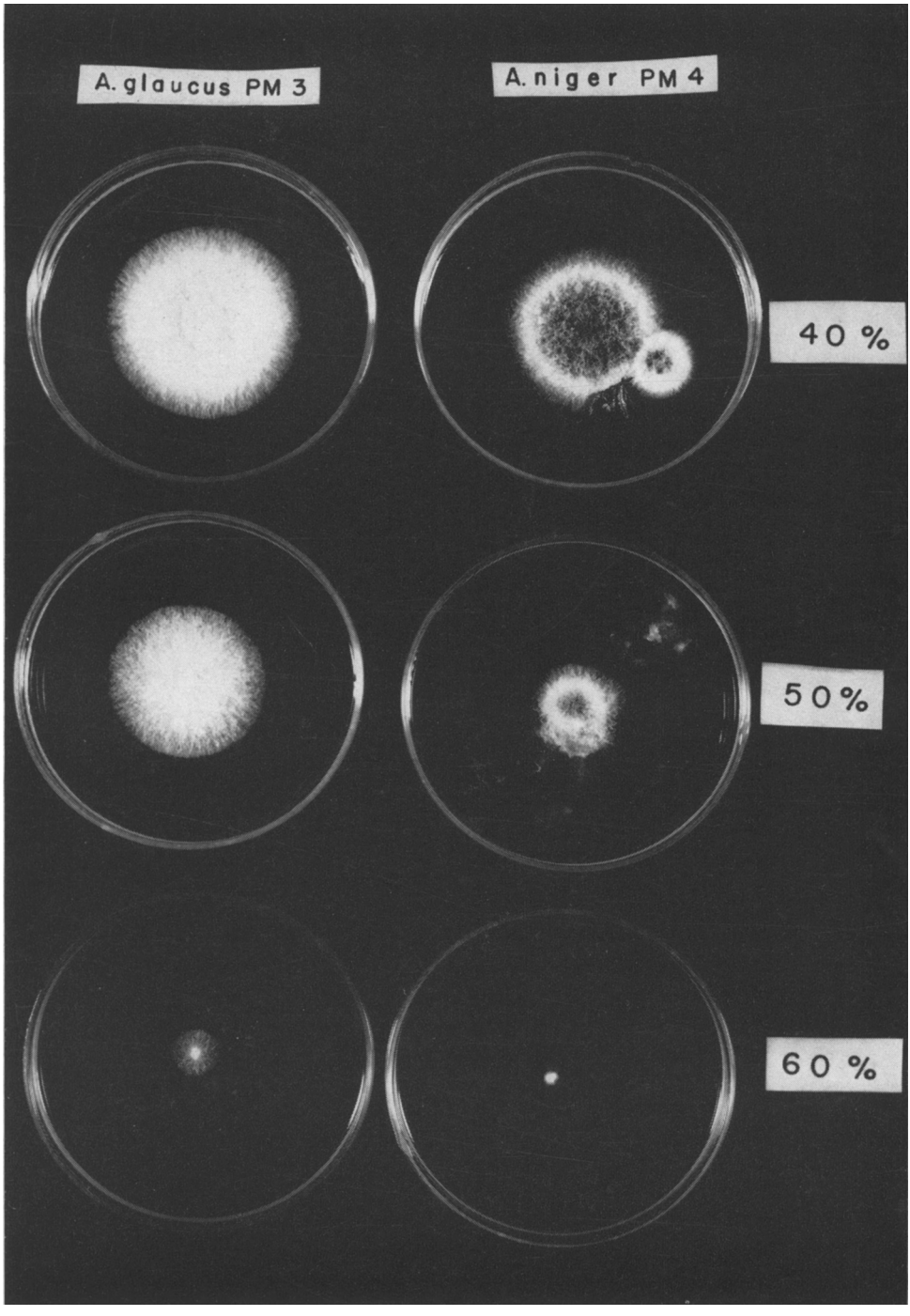

Figure 1. Growth of *Aspergillus glaucus* and *A. niger* after four days at room temperature (20° to 23° C) on media containing 40, 50, or 60 per cent (w/w) soluble solids.

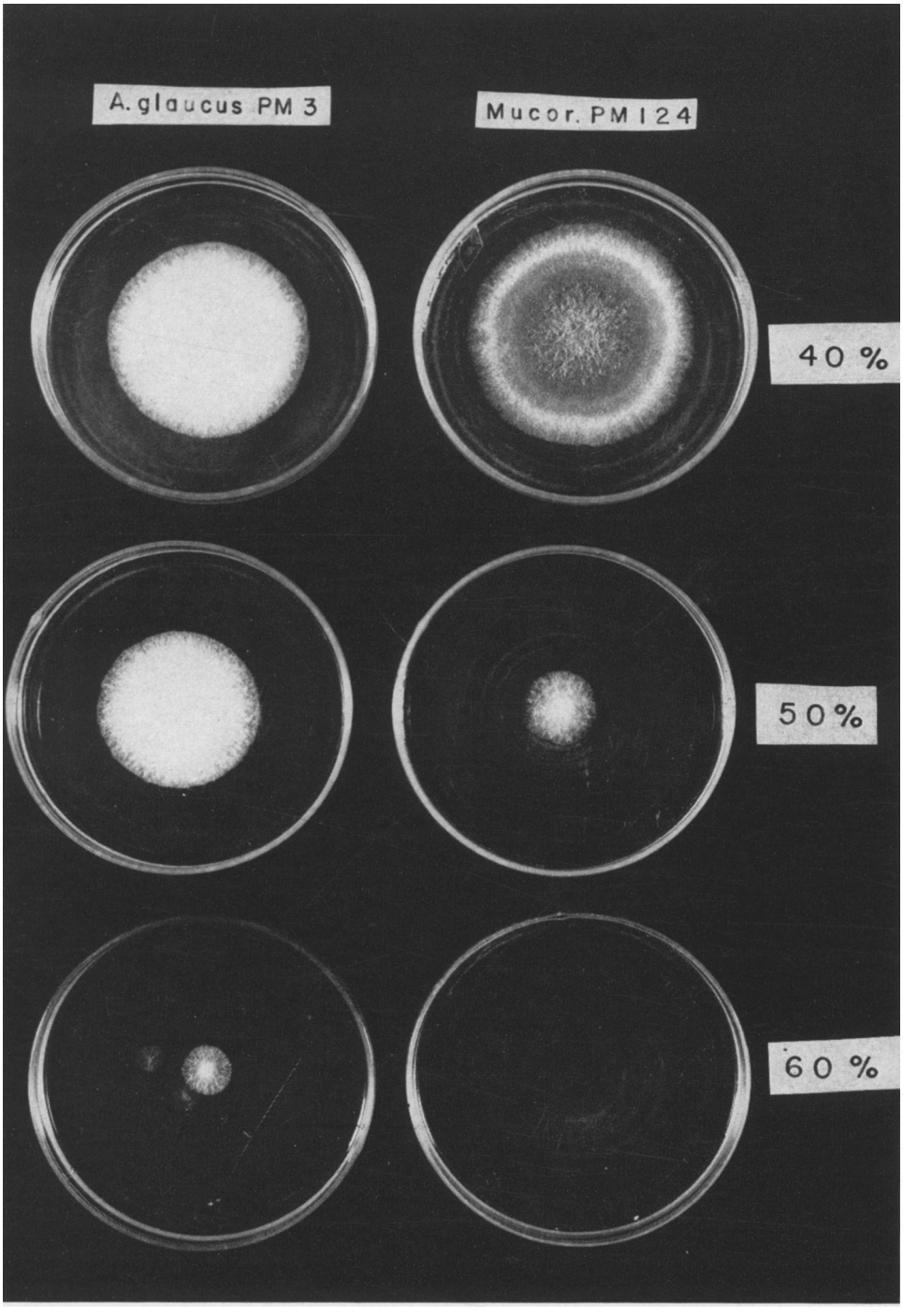

Figure 2. Growth of *Aspergillus glaucus* and *Mucor* spp. after four days at room temperature (20° to 23° C) on media containing 40, 50, or 60 per cent (w/w) soluble solids.

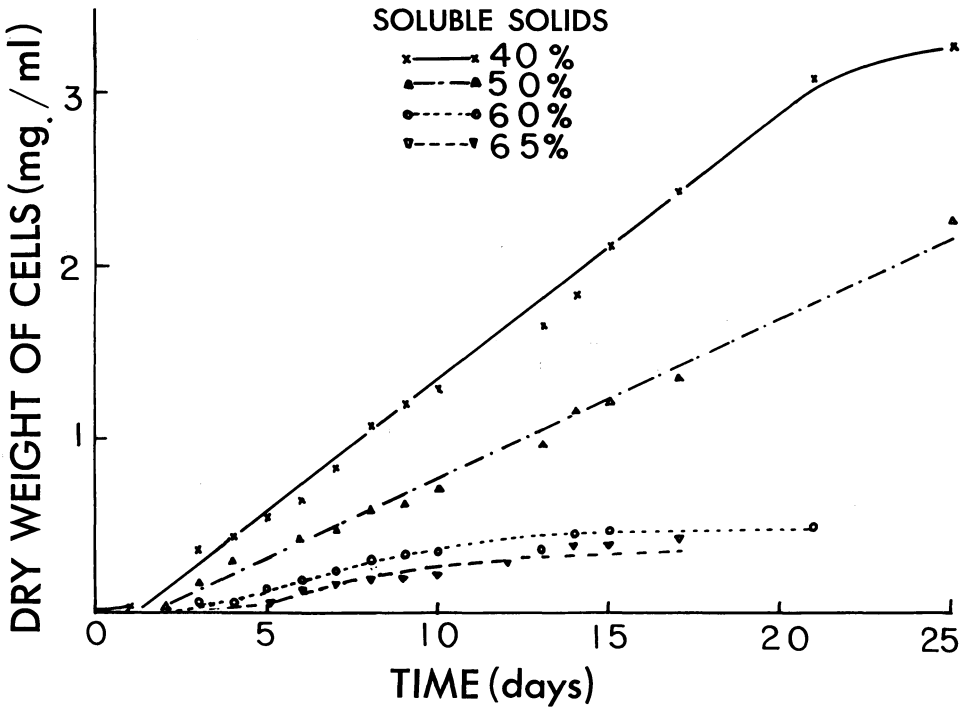

Figure 3. Growth of *Saccharomyces rouxii* (59-4) on various media of high soluble-solids concentration.

The strains tested were 12 of *Aspergillus glaucus,* 8 of *A. niger,* and 13 of *Penicillium* spp.

Colony diameter was measured daily, and the average for all strains of each group tested was plotted as a function of time.

RESULTS

Yeasts

All four strains of *Saccharomyces rouxii* showed similar osmophilic characteristics. In 40 per cent soluble solids, fermentation started two days after inoculation, and the vials were filled with gas in six days. In 50 per cent soluble solids, fermentation was slightly weaker, although three of the four strains filled the vials in six days. In 60 per cent soluble solids, growth and fermentation were much weaker. Fermentation generally started three days after inoculation, but was so weak that carbon dioxide only half filled the inverted vial in 13 days (except for one strain that filled the vial in that time). In 65 per cent soluble solids, growth and fermentation started three to four days after inoculation, and fermentation was suppresed even more. At 13 days, gas filled only one quarter (three strains) or one half of the vial (one strain). All strains showed apparent growth in 70 per cent soluble solids, but no gas was trapped by the vial. No growth occurred in 75 per cent soluble solids.

Of the four strains of *Saccharomyces mellis* tested, two showed an osmophilic nature similar to that of the *S. rouxii* strains, and two displayed weaker osmophilic characteristics, indicating some diversity in this particular within the same species. The strains of the more osmophilic group were able to carry out a fermentation at 70 per cent soluble solids, but no growth was detectable in two weeks at 75 per cent. The less osmophilic strains took five days to initiate fermentation in media containing 40

and 50 per cent soluble solids. The inverted vials were not gas-filled 13 days after inoculation. At 70 per cent soluble solids, growth and fermentation, although slow, were distinct after 13 days.

The strains of *Torulopsis magnoliae, T. stellata,* and *Saccharomyces rosei* possessed typical osmophilic natures. *T. stellata* fermented and showed definite growth in media with 70 per cent soluble solids. *T. magnoliae* and *S. rosei* did not grow at 70 per cent soluble solids, but fermented and showed definite growth at 65 per cent.

Figure 3 shows the growth of *S. rouxii* (59-4), expressed as dry weight of cells per ml of medium. Growth showed a linear rather than a logarithmic relationship with incubation time (fig. 3), and was considerably suppressed as the soluble solids were increased. At 70 per cent soluble solids, there was no detectable change in optical density or in dry weight of cells.

Molds

Since the samples of spoiled prunes were often in an advanced state of deterioration, and sometimes not in the original packages, mold contaminants not associated with spoilage may have been included among the isolates. Strains capable of growing under low water activity probably would be the primary spoilage organisms. Therefore, the mold isolates were screened for strains that could grow well on media containing high percentages of soluble solids. The growth rates of those strains were then studied.

Figure 4 shows the averages and ranges of colony diameters achieved in three days by several groups of molds on media of 40, 50, and 60 per cent

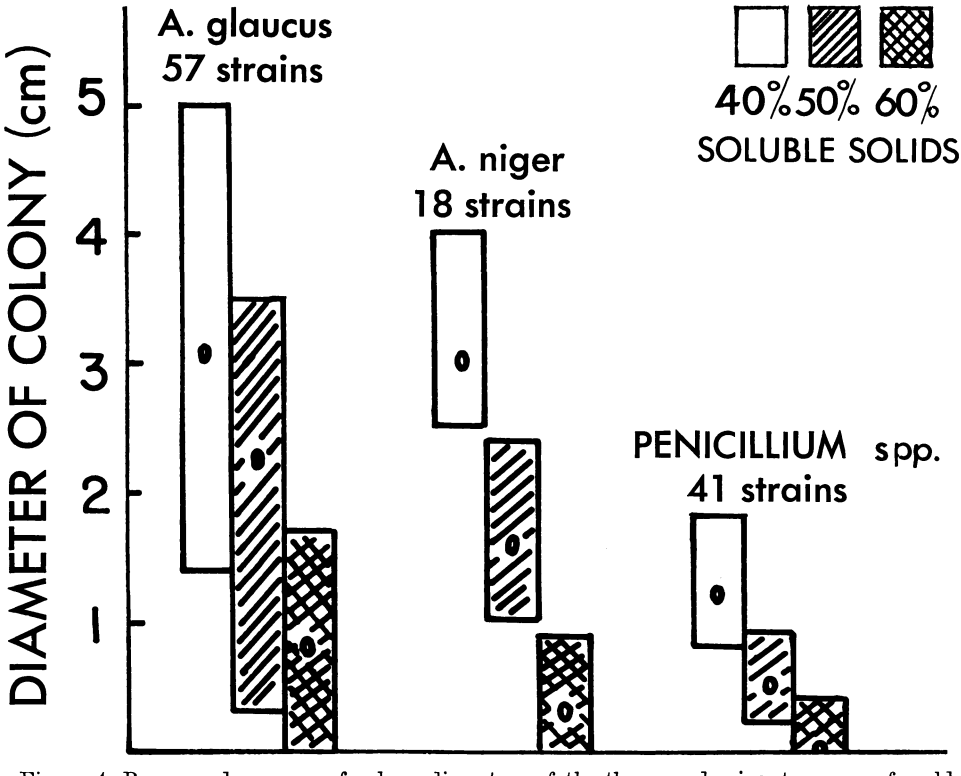

Figure 4. Range and average of colony diameters of the three predominant groups of molds isolated from dried prunes and grown for three days on media of high soluble-solids content. Average diameter is shown by circle in each range bar.

soluble solids. A longer growth period allowed some colonies on 40 per cent soluble solids to overlap, making measurement difficult.

Table 1 compares strains belonging to three groups of osmophilic molds on the basis of colony growth (diameter) in three days. In each group, separate count was made of the number of strains having colony diameters ranging from 0.5 to 5.0 cm.

The strains of *Alternaria* and *Monilia* did not grow on any of the media in seven days. The strain of *Chaetomella* grew very slowly on media with 40 per cent soluble solids, but failed to grow on 50 or 60 per cent soluble solids. The single strain of *Mucor* grew well on 40 per cent soluble solids, poorly on 50 per cent, and not at all on 60 per cent (fig. 2).

Table 2 shows growth rates on the different media of high soluble-solids content. The rates were calculated from the steepest part of the curves shown in figures 5, 6, and 7.

The soluble-solids content of the media affected both the length of the lag phase before appreciable growth had occurred, and the rate of growth. With strains of *Aspergillus glaucus,* increasing the soluble solids from 40 to 50 per cent increased the average lag phase about half but did not affect growth rate significantly. Increasing the soluble solids from 40 to 60 per cent

TABLE 1

DISTRIBUTION OF OSMOPHILIC MOLD STRAINS GROWN IN MEDIA OF VARIOUS SOLUBLE-SOLIDS CONCENTRATIONS

Mold	Media	Distribution of strains*					
		Colony diameter (cm) after 3 days' growth					
		0–0.5	0.6–1.0	1.1–2.0	2.1–3.0	3.1–4.0	4.1–5.0
	per cent sol. solids	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Aspergillus glaucus							
(41 strains)	40	0	0	12	38	<u>46</u>	5
	50	2	2	25	<u>61</u>	10	0
	60	24	<u>46</u>	30	0	0	0
Aspergillus niger							
(15 strains)	40	0	0	0	<u>50</u>	<u>50</u>	0
	50	7	7	<u>50</u>	36	0	0
	60	<u>64</u>	36	0	0	0	0
Penicillium spp.							
(25 strains)	40	0	<u>72</u>	28	0	0	0
	50	<u>58</u>	42	0	0	0	0
	60	<u>100</u>	0	0	0	0	0

* As percentage of total strains in the group. Underlined percentages indicate the range of growth achieved by the majority of the mold strains tested.

TABLE 2

AVERAGE GROWTH RATES (COLONY DIAMETERS) OF OSMOPHILIC MOLDS

Media	Mold group		
	Aspergillus glaucus (12 strains)	*Aspergillus niger* (8 strains)	*Penicillium* spp. (13 strains)
per cent soluble solids	*cm/day*	*cm/day*	*cm/day*
40	1.8	1.7	0.6
50	1.8	1.0	0.3
60	0.9	0.6	0.1

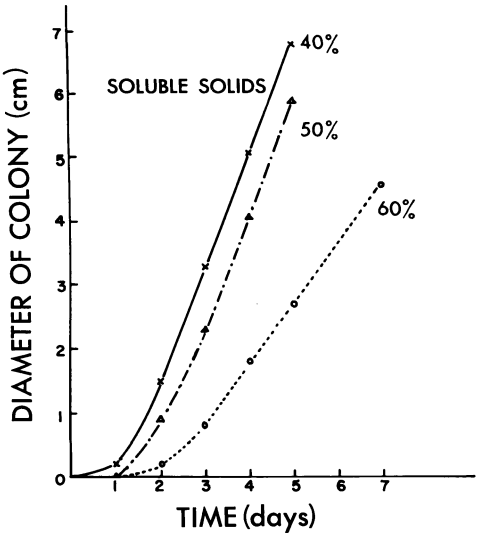

Figure 5. Average diameter of colonies of *Aspergillus glaucus* (12 strains) as a function of time during growth on media of various soluble-solids content. Temperature was 20° to 23° C.

roughly doubled the lag phase and halved the growth rate.

With *Aspergillus niger,* increasing the soluble solids from 40 to 50 per cent about doubled the lag phase and halved the growth rate. An increase from 40 to 60 per cent about tripled the lag phase and slowed the growth rate to about one third.

With *Penicillium* spp., the effect of increasing the soluble-solids content was more striking. An increase from 40 to 50 per cent more than doubled the lag phase (to about 2.5 times as long) and slowed growth to one third as fast. An increase from 40 to 60 per cent made the lag phase about six times as long, and growth rate about one sixth as fast.

The less osmophilic the species characteristics, as in *Mucor* sp., the more strikingly did soluble-solids content affect growth. Figures 1 and 2 compare *Aspergillus glaucus* with *A. niger* and *Mucor* sp. on media containing various concentrations of soluble solids.

DISCUSSION

Previous findings indicate that yeasts continue to grow in these liquid media for a long time. *Saccharomyces rouxii* inoculated on prunes showed visible growth after about four months at 20° C and 76 per cent RH (Miller and Tanaka, 1963). This is comparable with the RH of the atmosphere in equilibrium with an invert sugar-sucrose medium of 70 per cent soluble solids. Schelhorn (1950) reported that the cell concentration of a strain of *Zygosaccharomyces barkeri (Saccharomyces rouxii* var. *polymorphus)* in a fructose syrup which was in equilibrium with an atmosphere having 62 per cent ERH increased by a factor of 2.3 in two months, and tenfold in six months.

Results of the present study show that osmophilic yeasts are capable of growth and would have the ability to spoil prunes with a soluble-solids content of 60 per cent or lower. Since prunes vary in soluble-solids content, some prunes in a sample might be susceptible to spoilage while others could resist.

Among the 124 strains of mold isolated from spoiled dried prunes, strains

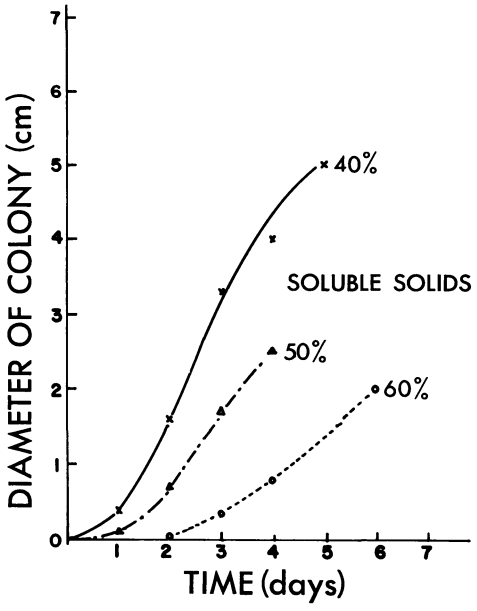

Figure 6. Average diameter of colonies of *Aspergillus niger* (8 strains) as a function of time during growth on media of various soluble-solids content. Temperature was 20° to 23° C.

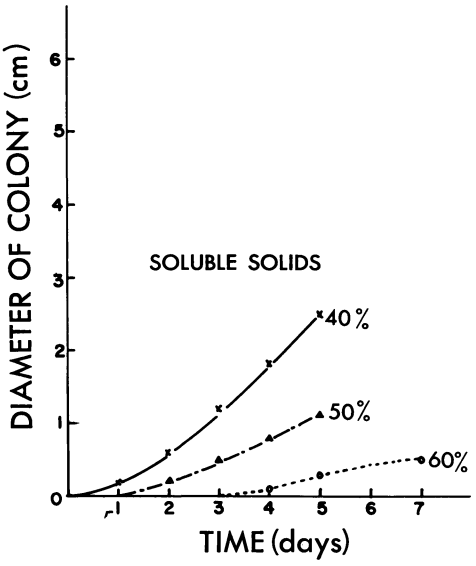

Figure 7. Average diameter of colonies of *Penicillium* spp. (13 strains) as a function of time during growth on media of variable soluble-solids content. Temperature was 20° to 23° C.

of *Aspergillus glaucus* were the most common (56 strains), and the most osmophilic. Included in this group are several species: *A. chevalieri, A. repens, A. ruber,* etc. All strains classified as *A. glaucus* showed distinct osmophilic characteristics. They were able to grow on a medium containing 60 per cent invert sugars. The RH of this medium (84 per cent) is similar to that of a medium containing 70 per cent sucrose (83 per cent) (Schachinger and Heiss, 1951). Members of this group are the most important fungi responsible for the spoilage of food products of low water activity. They cause spoilage in jellies, jams, soft sugars, honey, fruitcake, and the like. Thom and Raper (1941) stated that the molds present in moldy food products of minimum moisture content are likely to be members of the *A. glaucus* group.

Strains of *Aspergillus niger* and certain *Penicillium* spp. might also be considered to be potential spoilage organisms since they were isolated frequently from spoiled dried prunes and were found to be moderately osmophilic.

Strains of mold belonging to the genera *Alternaria, Chaetomella,* and *Mucor,* also isolated, are probably incidental contaminants, judging from frequency of isolation and their inabilitiy to grow on media containing high percentages of soluble solids. Reported studies on mold growth or spore germination under controlled humidities were based on the following principles: addition of solutes to the growth medium (Heintzeler, 1939; Burcik, 1950); equilibration with controlling solutions, such as sulfuric acid (Walter, 1924; Heintzeler, 1939) and saturated salts (Mossel, 1951); and determination of the water-sorption isotherm (Scott, 1953; Christian and Scott, 1953). The first principle was used in the present screening for osmophilic molds and experiments on the osmophilic nature of yeasts and molds.

The soluble-solids content of solid media affected the lag phase and rate of mold colony growth. The lag phase increased and rate of growth decreased as soluble-solids content was increased. This effect was less obvious with the more osmophilic strains. The changes in lag phase and in growth rate were least for *Aspergillus glaucus* and moderate for *A. niger*. Greater change was noted for *Penicillium* spp.

SUMMARY

Eleven strains of yeast were chosen from the species most frequently isolated from spoiled dried prunes. The ability of these strains to grow and ferment in media of high soluble-solids content was studied. 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. magnoliae* and *S. rosei* were able to ferment in 65 per cent soluble solids but not in 70 per cent.

The effect of soluble-solids content on the growth rate of *Saccharomyces rouxii* was studied in liquid media containing 40, 50, 60, 65, and 70 per cent soluble solids. Growth was suppressed considerably when soluble solids were increased from 40 to 60 per cent. Growth was very slow at 65 per cent soluble solids, and not detectable at 70 per cent.

Molds isolated from spoiled dried prunes were screened for osmophilic strains. Colony diameters of all the isolates 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. Those of *A. niger* and *Penicillium* spp. were somewhat less osmophilic. Strains in the genera *Alternaria, Monilia,* and *Chaetomella* showed no osmophilic characteristics. The isolated strain of *Mucor* grew luxuriantly on 40 per cent soluble solids, barely on 50 per cent, and not at all on 60 per cent.

The effect of a high content of soluble solids in a medium on the growth of *Aspergillus glaucus, A. niger,* and *Penicillium* spp. was studied. The lag phase for growth was extended and the growth rate decreased when soluble-solids content was increased. This effect was least for the strains of *A. glaucus,* confirming the definite osmophilic nature of this group of molds. Greater changes were noted for strains of *Penicillium. A. niger* strains were affected in an intermediate fashion on the same media.

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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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