YEASTS OCCURRING ON DATES
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Dates sometimes undergo a yeast fermentation even though they appear to be sufficiently low in moisture and high in sugar content to inhibit the development of most spoilage organisms. In the packing of dates the procedures followed, as outlined by Sievers and Barger (1930) do not involve the use of preservatives or of sufficient heat to prevent yeast growth. In fact, yeast spoilage has been observed at all stages of handling from harvesting to packing and distribution. Fermentation and yeast growth on dates are usually slow and frequently overlooked although undesirable odors are commonly present. Occasionally, however, fermentation is accompanied by the formation of subcutaneous gas pockets or rupturing of the skin.

Several investigators have given consideration to the morphology and taxonomy of yeasts obtained from dates, although in most instances relatively few organisms were studied. Rodio (1924) observed the formation of a white crust in the wrinkles of the pericarp and particularly in the cracks of the epidermis. Yeasts isolated from this white material were described as *Zygosaccharomyces cavae*. Later Beauverie (1929) also isolated this organism as well as *Z. cavae* var. beauverie from the skin scrapings of Tunisian dates. Verona and Valleggi (1933) isolated and described *Hansenula fermentans* characterized by the fermentation of both maltose and lactose, but unfortunately this unusual yeast was lost. Esau and Cruess (1933) obtained two distinct groups of yeast from fermented California dates; one termed *Saccharomyces* because of the strong alcohol-forming powers, and a second termed "torula yeast" because of the moderate production of alcohol. Melliger (1931) studied yeasts isolated from two distinct types of Egyptian dates which were char-

1 Part of nontechnical assistance supplied by W.P.A. Official Project 65-1-08-91-Unit B-5.
acterized as follows: those containing sufficient sugar to prevent fermentation termed "amhat," and red dates containing insufficient sugar to prevent fermentation termed "hayami." Melliger obtained the amhat dates from markets in Marseille and Geneva, and the hayami dates directly from Egypt. *Z. cavaræ* and several unidentified species of *Zygosaccharomyces* and *Saccharomyces* were obtained from the amhat dates. *Hanseniaspora melligeri*, *Torulopsis pulcherrima* and some unidentified species of *Torula* and *Mycoderma* were isolated from the hayami dates.

Postlethwaite (1927) attributed the souring and fermentation of dates to yeasts and enzymes and suggested the use of dehydration as a means of control. Freeman (1911) indicated that the use of temperatures below 120° F. for the processing of dates by incubation prolonged the treatment period and thus increased the liability of the fruit to become sour. Fellers and Clague (1932) induced souring by placing pure cultures of yeasts isolated from sour dates in contact with sound pasteurized dates containing about 25 per cent moisture. In 1933 Clague and Fellers identified "torulae" with the souring of dates. This "souring" is not likely to be the result of independent action of yeasts but rather an association between yeasts and acetic acid bacteria or independent activity of lactic acid bacteria. Studies by Vaughn (1938) have shown that association between *Acetobacter* and certain species of *Saccharomycetaceae* causes rapid acetification in wine. The authors have noted that species of *Acetobacter* as well as yeasts are prevalent in all samples of sour dates observed. Studies are in progress to determine the role of various yeasts and *Acetobacter* in the fermentation and souring of dates. Fellers (1930) and Clague (1936) made counts of yeast occurring on dates and other dried fruits and in most instances found relatively few present.

It is apparent that the literature concerning the taxonomy of yeasts occurring on dates is fragmentary. In view of this a study has been made to extend the knowledge in this field.

**EXPERIMENTAL PROCEDURE**

Nineteen samples, each containing several spoiled dates were collected in 1938 and 1939 at various date gardens and packing
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plants in Coachilla Valley in California, the center of the American date-growing industry. Two samples of Saidy (amhat) dates were obtained from Egypt in hermetically sealed cans. They were discolored and covered with sugar crystals when removed from the cans, a condition not observed on the California dates. Yeasts were obtained from 14 California and the 2 Egyptian samples whereas bacteria were isolated from all.

In all instances dates were suspended in 20° Balling wort for 2 to 3 days before plating. Purification was accomplished by use of 3 or more subsequent platings from the enrichment cultures. Bacteria as well as yeasts were isolated with the view of conducting future studies on the symbiotic relationships of yeasts and bacteria in the souring of dates. The procedures of Stelling-Dekker (1931), Lodder (1932 and 1934), Langeron and Guerra (1938), and Custers (1940), were used for identification. Color standards were those of Maerz and Paul (1930).

EXPERIMENTAL RESULTS

Sixty-seven cultures of yeast isolated from spoiling California and Egyptian dates were found to be primarily species of Zygosaccharomyces and Hanseniaspora although nine cultures of Candida were obtained. A few cultures of Pichia, Saccharomyces, Hansenula and Torulopsis were also found.

Sporulating yeasts

Genus Zygosaccharomyces: Thirty-one cultures of Zygosaccharomyces were isolated from 12 samples of dates. The variety of yeast most commonly encountered was Z. japonicus var. soya (Saito) Takahashi and Yukawa. This was the only organism isolated from the Egyptian dates as well as the one most frequently obtained from California dates. The characteristics of the organisms isolated correspond in detail to those of Z. japonicus var. soya. The cells of one of these cultures showed a tendency to sporulate without first conjugating in pairs as is characteristic for the genus Zygosaccharomyces. This manner of ascospore formation is not very common in cultures of this genus when freshly isolated from nature although it has been observed occasionally in cultures held on artificial media for several years.
Nine cultures were identified as *Z. barkeri* Saccardo and Sydow. The giant colonies of these cultures showed surface variations ranging from smooth to corrugated and crumbly. Although these differences appeared to be considerable they are insufficient to warrant even varietal separation. Stelling-Dekker gave the characteristics of giant colonies but did not consider them sufficiently important to be included in the species or variety definition.

All cultures of *Z. barkeri* produced clumps of cells on old liquid wort cultures similar to the drawing of Fabian and McCullough (1934) of transition cells with gonidia. Krumbholz (1931) observed similar structures in *Z. variabilis* and termed them crown formations (Kronenbildung).

Single cultures of *Z. globiformis* Kroemer and Krumbholz and *Z. nadsonii* Guilliermond were also isolated. The culture of *Z. globiformis* was identical with the characters as given by Krumbholz (1931) except for the presence of a few very primitive pseudo-mycelia in old liquid wort cultures. This however was not discussed by Krumbholz. *Z. nadsonii* was identical with the description given by Stelling-Dekker although the cluster-like cells were present on old slants.

Genus *Hanseniaspora*: Eighteen cultures, identical in all respects with *Hanseniaspora melligeri* Lodder were isolated from 8 samples of California dates. Prior to the present study this organism had been isolated only from Egyptian hayami dates by Melliger (1931). Although sporulating apiculate yeasts of the genus *Hanseniaspora* were found to occur commonly on dates, corresponding non-sporulating forms of the genus *Kloeckera* were not encountered. Melliger likewise did not report the isolation of a single culture of *Kloeckera*. Mrak and Baker (1939) made similar observations on dried prunes and figs. In a recent survey of fresh figs, on the other hand, the writers have isolated about equal numbers of *Hanseniaspora* and *Kloeckera*. Mrak and McClung (1940) also found both genera equally well represented on fresh grapes. These observations tend to indicate the presence of certain factors in dried fruits limiting the growth of *Kloeckera* or favoring the development of the ascospore-forming
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It appears that the concentration of sugar present in dried fruits may be the factor inhibiting the growth of *Kloeckera*. *Hanseniaspora melligeri* is tolerant of relatively high concentrations of sugar as is shown in table 1.

**Genus *Pichia***: Three cultures isolated from 3 different samples resembled *Pichia chodati* (Zender) Dekker but differed by the formation of a thick film rather than islets on alcohol medium and the slow fermentation of glucose, fructose and mannose rather than the absence of fermentation. Fermentation characteristics of these cultures and of *P. chodati* were compared quantitatively in van Iterson-Kluyver fermentometers. The three cultures obtained from dates resemble *P. chodati* and are included in this species but differ sufficiently to be set apart as a new variety. They are described as *Pichia chodati* var. *fermentans* nov. var.

**Pichia chodati** var. *fermentans* nov. var.

Cells mostly cylindrical with rounded ends, also allantoid, ellipsoidal, globose, spherical or clavate. Cell size in 24-hour

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**TABLE 1**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>NUMBER OF CULTURES</th>
<th>CONCENTRATION OF DATE SYRUP (DEGREES BALLING)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td><em>Zygosaccharomyces barkeri</em></td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td><em>Zygosaccharomyces japonicus var. soya</em></td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td><em>Zygosaccharomyces nadsonii</em></td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Zygosaccharomyces globiformis</em></td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Saccharomyces carlsbergensis</em> var. polymorphus*</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Hanseniaspora melligeri</em></td>
<td>18</td>
<td>+</td>
</tr>
<tr>
<td><em>Pichia chodati</em> var. <em>fermentans</em></td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td><em>Hansenula subpelliculosa</em></td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida chalmersi</em></td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida sp.</em></td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td><em>Torulopsis dactylica</em></td>
<td>3</td>
<td>+</td>
</tr>
</tbody>
</table>
and 3-day wort cultures range \((2.25-5.25 \ \mu) \times (3-11.75 \ \mu)\) and average \(3 \times 7 \ \mu\). Pellicles form rapidly on wort, smooth, becoming finely wrinkled. Primitive pseudomycelium in wort. Asco-
spores hemispherical or hat-shaped, commonly containing an oil droplet. Four spores per ascus. Conjugation absent. Fer-
ments glucose, fructose and mannose slowly. Asparagin, am-
monium sulfate, urea and peptone utilized, nitrate not. Good
growth with formation of a thick smooth pellicle in alcohol me-
dium; slant culture cartridge buff, slightly verrucose, dull, dry,
and pulvinate with erose borders. Giant colony, fawn to olive
buff, irregular scales or crust-like, dull, dry and umbonate with
lobate lobulate borders. Gelatin liquified in 70 days.

Genus Saccharomyces: Only two cultures of Saccharomyces
were isolated although this genus is usually one of the most com-
mon yeast genera found in nature. This sparse occurrence can
be attributed to the high sugar concentration of dates. The
comparative sugar tolerances of the various species of yeasts
obtained from dates are given in table 1. One of the cultures
isolated was identical with S. carlsbergensis var. polymorphus
Dekker and the other with S. cerevisiae Hansen.

Genus Hansenula: A single culture of Hansenula similar to
H. subpelliculosa Bedford was isolated in 1939. This particular
organism recently described by Bedford (1941) has been isolated
from a number of fruits and food products in California. It is
characterized by the formation of an extremely thin film, hat-
shaped spores, fermentation of glucose, fructose, mannose, suc-
rose, maltose and raffinose \(\frac{1}{3}\) and the utilization of peptone,
asparagin, ammonium sulfate, urea and potassium nitrate and
growth without film formation in alcohol medium. It is not in-
cluded in Endomycopsis because of the absence of a true mycelium
and its ability to reduce \(\text{NO}_3\).

Non-sporulating yeasts

Twelve, or less than one fifth of the cultures studied, failed to
produce ascospores. Nine of the non-sporulating organisms iso-
lated were cultures of Candida, the remainder being Torulopsis.

Genus Torulopsis: Three cultures of Torulopsis isolated from
3 different samples are all similar and resemble, to some extent, *T. stellata* (Kroem. and Krumb.) Lodder. Our cultures differ from *T. stellata* by utilizing asparagin, ammonium sulfate and urea as sources of nitrogen and in the characteristics of the slant cultures. These differences are quite distinct and can be reproduced easily, so the cultures have been included in a new species, *Torulopsis dactylifera*.

*Torulopsis dactylifera* nov. sp.

In liquid wort cells ellipsoidal, globose, spherical and pyriform. Cell size in 24-hour wort culture (1.5–4.5 μ) x (2.0–5.3 μ), from 3-day wort cultures (2.5–5.2 μ) x (3.0–5.4 μ). Film absent, ring formation after 3 days. Pseudomycelium absent although cells adhere in clusters (Sprossverbände). Fermentation of glucose, fructose, mannose, sucrose and raffinose (½ only). Galactose, maltose and lactose not fermented. Asparagin, ammonium sulfate, urea and peptone utilized. Nitrate not utilized. No growth in ethyl alcohol medium. Glucose, fructose, mannose, sucrose and raffinose are respired but galactose, maltose and lactose are not respired. Slant cultures cartridge buff, smooth, dry, glistening, and convex with entire borders. Gelatin not liquified in 60 days.

Genus *Candida*: This genus comprises a group of organisms commonly and incorrectly termed *Monilia* by the medical mycologists and *Mycoderma* or *Torula* by the technical mycologists. This may be explained by the poor taxonomic delimitation that existed until Berkhout (1923) defined the genus *Candida*. The genus *Candida* is differentiated from other non-carotinoid-producing imperfect yeast genera primarily by the formation of a pseudomycelium. Although this genus has been isolated from animal sources frequently there is very little information concerning its distribution on plant and food materials. Undoubtedly many cultures have been isolated from food materials and included in other genera, most probably in *Mycoderma* or *Torula*. For this reason the isolation of 9 cultures of *Candida* from 9 different samples of California dates is of interest. Studies of the possible pathogenicity of these cultures have not been made, although such
experiments are planned in collaboration with a medical laboratory. The system of Langeron and Guerra (1938) was used for the identification of these cultures because of its convenience for use in a non-medical laboratory and because of its similarity to other reliable systems. The cultures isolated were also placed in groups defined by Diddens and Lodder (1939) when possible.

Five of the 9 cultures of Candida isolated were similar to C. chalmersi Castellani and also fit into group 4 (Brettanomyces group) of Diddens and Lodder. The cultures isolated from dates utilized urea as a sole source of nitrogen, whereas, according to Langeron and Guerra C. chalmersi does not utilize this substance. It was found however that one of Langeron’s cultures of C. chalmersi, obtained from Dr. E. E. Baker, was able to utilize urea. Our cultures isolated from dates were observed to ferment raffinose slowly in a van Iterson-Kluyver fermentometer. Langeron and Guerra report a negative fermentation of this sugar by C. chalmersi. This inability to ferment raffinose was verified by use of one of Langeron’s cultures of C. chalmersi and a van Iterson-Kluyver fermentometer. The fact that our cultures differed from C. chalmersi only in the ability to ferment raffinose slowly was not considered sufficient to warrant taxonomic separation. One of our cultures of C. chalmersi produced acid from glucose and clarified yeast water glucose chalk agar (0.5 per cent chalk) in about 10 days. This single character however is not sufficient for inclusion in the genus Brettanomyces as defined by Custers (1940).

The cultures of C. chalmersi were able to respire and assimilate maltose in all instances, although no fermentation of this disaccharide was observed even when the quantitative van Iterson-Kluyver fermentometers were used. Since this yeast ferments glucose quite readily one might be inclined to believe that it respires maltose directly, i.e., without previous hydrolysis by the enzyme maltase. Kluyver and Custers (1940) have shown that several yeast species possessing these same properties do contain to a greater or lesser extent the corresponding hydrolases. Under anaerobic conditions these hydrolases are inactivated either completely or at least to such an extent that the fermentability of the
disaccharide is not detected by the relatively insensitive routine methods ordinarily used. Kluyver and Custers offer two possible explanations for this phenomenon; a decrease in permeability of the cell in the absence of air, or more probably the reversible inactivation of the hydrolase itself when an increased state of reduction in the cell is attained.

The morphological characteristics of the streak cultures of *C. chalmersi* gradually changed during storage from smooth glistening to dull rough forms.

Two cultures were identified as *C. tropicalis* (Castellani) Berkhout although they differed from the characters given by Langeron and Guerra by utilizing urea and fermenting raffinose slowly. An authentic culture of *C. tropicalis* obtained from Langeron, through Dr. E. E. Baker, was examined in regard to the last 2 characters and found to utilize urea and ferment raffinose (½ only) when auxanogram plates and van Iterson-Kluyver fermentometers were used for the respective tests. The cultures isolated from dates cannot be included in any of the groups given by Diddens and Lodder. They are, however, quite similar to group 1 except that they form films.

A single culture belonging to group 2 of Diddens and Lodder was identified as *C. krusei* (Castellani) Berkhout. The characteristics of this organism agreed well with those given by Langeron and Guerra.

One culture of *Candida* fitting into group 1 of Diddens and Lodder could not be included in any of the species given by Langeron and Guerra. It resembled *C. guilliermondii* Castellani although neither maltose respiration nor film formation occurred. In view of the difficulty in identifying this organism more closely with some of the imperfect yeasts, it was compared with some perfect yeasts.² *Saccharomyces exigus* (Rees) Hansen rarely sporulates and is identical physiologically with the forementioned unidentified culture. The two organisms are also very similar morphologically although the cells of *S. exigus* may be slightly

² A monograph on the genus *Candida* is being prepared by H. A. Diddens and J. Lodder in Holland. Until this work is published we do not feel justified in naming a new species of *Candida*. 
longer at times. It is quite possible that the unidentified organism may be a culture of *S. exigua* that has lost its ability to sporulate.

**TOLERANCE TO HIGH CONCENTRATIONS OF SUGAR**

California dates commonly contain as much as 65–70 per cent of sugar and most yeasts do not grow in media containing such a high concentration of sugar. Species of the genus *Zygosaccharomyces* however are notably tolerant to high concentrations of sugar, causing the spoilage of such products as honey and maple syrup. For an inclusive review of this subject see Henrici (1941). A series of tests were conducted in date syrups of concentrations ranging from 40 to 66° Balling to determine the relative tolerance to high concentrations of sugar. All of the yeasts isolated from dates were first inoculated in the 40° Balling syrup. If growth occurred at a certain concentration, the culture was transferred to a higher one. All cultures were incubated at 25°C. and examined periodically. The data obtained are summarized in table 1. In all instances the cultures belonging to the same species behaved in a similar manner when inoculated in the syrups. All species of *Zygosaccharomyces* except *Z. globiformis* fermented the 66° Balling date syrup within 48 hours. *Z. globiformis* and *Hansenula subpelliculosa* grew slowly in 60° Balling syrup but all other cultures failed to grow at this concentration even after 2 weeks of standing. This is rather interesting in view of the number of cultures of *Hanseniaspora mellei* isolated. The cultures of *Candida chalmersi* grew slowly in 50° Balling syrup whereas *C. tropicalis* and *C. krusei* grew in 40° but not in 50° Balling syrup. The 3 cultures of *Torulopsis dactyliclora* grew well in 50 but not in 60° Balling syrup. The results also indicate that the genus *Zygosaccharomyces* can be expected to survive on dates even though they may be relatively dry and rich in sugar.

**SUMMARY**

Sixty-seven cultures of yeast were isolated from California and Egyptian dates undergoing microbiological deterioration. Most of the organisms isolated were cultures of *Zygosaccharomyces, Hanseniaspora* and *Candida*. The species of *Zygosaccharomyces*
in order of occurrence were *Z. japonicus* var. *soya*, (twenty), *Z. barkeri*, (eight), *Z. globiformis* (one), and *Z. nadsonii* (one). All species of *Hanseniaspora* (eighteen), were identified as *H. melligeri*. Other perfect genera obtained are *Saccharomyces*, *Pichia* and *Hansenula*. The genus *Saccharomyces* was represented by two cultures, *S. cerevisiae* and *S. carlsbergensis* var. *polymorphus*. The three cultures of *Pichia* were described as *Pichia chodati* var. *fermentans*. A single culture of *Hansenula* was identified as *H. subpelliculosa*. Of the imperfect yeasts the genus *Candida* was most common. There were found five cultures of *Candida chalmersi*, two of *Candida tropicalis*, a single culture of *Candida krusei* and an unidentified species. In the genus *Torulopsis*, represented by three cultures, a new species is described as *Torulopsis dactylifera*. The sugar tolerance of all the yeasts isolated, was compared. All cultures of *Zygosaccharomyces* were more sugar tolerant than the other yeasts isolated.

REFERENCES


ESAU, P., AND CRUSS, W. V. 1933 Yeasts causing souring of dried prunes and dates. Fruit Products J., 12, 144–147.


KLUYVER, A. J., AND M. TH. J. CUSTERS. 1940 The suitability of disaccharides as respiration and assimilation substrates for yeasts which do not ferment these sugars. Anthonie van Leeuwenhoek, 6, 121-162.


POSTLETHWAITE, R. H. 1927 Treatment of dates to prevent souring and fermentation. Fourth Annual Date Growers Institute, Report 5-7.

RODIO, G. 1924 Di un saccharomicete del dattero (Zygosaccharomyces cavarae Nov. sp.). Bull. orto botan. r. univ. Napoli, 8, 1-12.


