MICROBIOLOGY OF WINEMAKING[1]

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Contents

PREVIOUS REVIEWS

A number of reviews on the yeasts and bacteria involved in table and dessert winemaking[2] have been published in Europe. The last extensive review in English was that of Vaughn (290) on bacterial spoilage in 1955 which we take as the starting date of this review. There are also short reviews in English by Beech (10, 10a), Peynaud & Domercq (195), Lüthi (143), and Carr (32), and in the texts by Joslyn & Amerine (112) and Amerine et al. (4). Numerous reviews, both separately and in texts, may be found in the European literature since 1955 [See especially Böhringer (19a) and Schanderl (249a)]. Among the textbooks are those of Verona & Florenzano (293), Schanderl (249), Castelli (35, 36), Ribéreau-Gayon &

[1] The survey of the literature pertaining to the review was concluded in December 1967.

[2] Table wines, for the purpose of this review include all wines, still or sparkling and dry or sweet, with less than 14 per cent ethanol (by volume). Dessert wines usually contain 17 to 21 per cent ethanol and, except for a few sherry types, normally contain fermentable sugar. Must refers to unfermented grape juice.

Peynaud (234), Laho & Minárik (135), Bernaz et al. (16), and Rakcsányi (223). The texts by Verona & Florenzano, Castelli, and Schanderl are especially recommended, since they are entirely devoted to the subject under review. Among general review articles, the following may be noted: Tdintsova (265), Minárik (168), which is in two parts and covers the period 1956 to 1964, Castelli (37), which is especially interesting for its historical perspective, and Malan et al. (149) which also contains important original research.

There have been many specialized reviews. Of those on yeasts we note Castelli (34), Domercq (54), Lüthi (141), De Becze (48), Tarantola (264), and Van Kerken (285). Among those on bacteria see Lambion & Meskhi (136), Kushida (133), Tarantola (264), Bezzegh (17), Peynaud & Domercq (197), Radler (219–222), Fornachon (79), Husfeld (100), Peynaud & Dupuy (200), Tirdea (268), and the 7th edition of *Bergey's Manual* (23). The reports of the microbiology group of the Office International de la Vigne et du Vin (4a) are also apropos.

With some exceptions, particularly in eastern Europe, the modern yeast nomenclature generally follows the taxonomic studies of Guilliermond (90), Stelling-Dekker (259), Lodder (138), Diddens & Lodder (49), Wickerham (305), Lodder & Kreger-Van Rij (139),[3] Verona & Montemartini (295), and Kreger-Van Rij (125), Kudriavtsev (128) gives a somewhat different classification. The summary of De Becze (48) is useful for the clear photographs and the detailed taxonomic tables. The atlas of Kocková (121) should also be useful and also the photographs and measurements in a number of papers (5, 30, 89a, 167).

YEASTS

Natural Flora of Grapes, Musts, and Wines

The ecology of wine yeasts has been of great interest to enologists since the last part of the 19th century. Most of the studies predate the period under review but the recent studies reviewed here are especially thorough. The results reported here are typical, though a number of local studies have been omitted. We have summarized the data more or less by country, with some typical data in Table I. The general purpose of these investigations has been to identify the yeast flora of grapes, musts, fermenting musts, and new wines. The hope has been that this might give a clue as to the influence of different yeasts on the quality of the finished wine.

From ninety-six Czechoslovakian grape and must samples, Minárik et al. (172) isolated 1014 yeast cultures—358 before fermentation, 330 during the most active period of fermentation, and 326 at the end of the fermentation.

[3] In general, we have followed this text on taxonomical questions involving yeasts. When not given there the author's name is given.

TABLE I

YEASTS REPORTED IN VARIOUS MUSTS AND WINES[a] (as per cent)

	Italy (36)				Israel[c] (36)	Spain (40)		Bordeaux (54)				Czechoslovakia (172)			
								Red		White					
	Veneto	Toscana	Calabria	Sicily[b]		Rioja[d]	La Mancha	Musts[e]	Wine[f]	Musts[g]	Wine[h]	On grapes[i]	In musts	During[i] ferment.	New[i] wine
No. samples	19	103	26	51	10	24	29	58	17	38	53	96			
No. cultures	143	588	447	712	163	352	430	1070	54	953	153	358		330	326
Saccharomyces															
acidifaciens	—	—	—	—	—	—	—	—	29	8	92	3	—	—	—
bayanus	5	6	46	22	—	—	—	3	—	—	—	5	1	1	—
carlsbergensis	—	—	—	—	—	—	—	3	6	16	—	5	1	—	1
cerevisiae	100	72	94	100	100	100	100	98	100	100	32	97	33	65	80
chevalieri	21	1	38		10	32	59	31	18	34	—	2	—	—	—
elegans	—	—	—	—	—	—	21	—	23	11	4	2	1	—	—
exiguus	—	—	—	—	—	—	17	—	—	—	—	2	1	—	—
fructuum	—	—	—	—	—	—	—	3	—	8	—	1	—	—	—
heterogenicus	—	—	—	—	—	—	—	—	35	11	2	3	—	—	1
italicus	5	1	42	39	—	17	27	—	—	—	—	2	—	—	1
oviformis	5	2	4	71	40	21	34	19	88	71	100	21	3	3	5
rosei	16	20	15	20	—	58	38	12	—	21	—	13	6	—	2
uvarum	5	12	—	8	10	—	—	3	—	3	—	21	4	5	3
veronae	—	—	—	—	—	—	24	—	—	3	—	3	1	—	—
Hansenula anomala	—	—	—	—	—	—	—	3	—	—	—	5	3	—	—
Pichia fermentans	—	—	—	—	—	—	—	3	—	3	—	3	1	—	—
Torulopsis bacillaris	—	—	—	—	—	—	—	9	—	76	—	5	1	—	—
Candida pulcherrima	—	17	4	10	—	50	10	—	—	—	—	28	12	8	—
Kloeckera apiculata	94	87	50	49	60	92	86	95	—	89	—	65	33	13	1

[a] Samples are of musts unless otherwise indicated.
[b] Also *Hanseniaspora valbyensis*, 41%.
[c] Also *Hanseniaspora valbyensis*, 100%.
[d] Plus *Saccharomyces bisporus* and *Saccharomyces delbrueckii*, 4% each.
[e] Also *Kloeckera jensenii*, 2%, *Brettanomyces vini*, 3%, *Candida pulcherrima*, 3%, *Torulopsis famata*, 2%, *Rhodotorula mucilaginosa*, 2%, *Saccharomyces steineri*, 7%, *S. florentinus*, 2%, *Debaryomyces hansenii*, 5%, *Pichia membranaefaciens*, 3%, and *Kloeckera africana*, 2%.
[f] Also *Brettanomyces schanderlii*, 18%.
[g] Also *Saccharomyces steineri*, 24%, *S. florentinus*, 3%, *S. delbrueckii*, 3%, *Saccharomycodes ludwigii*, 3%, and *P. membranaefaciens*, 3%.
[h] Also *Saccharomycodes ludwigii*, 41%.
[i] Also *Saccharomyces steineri* and *S. willianus* 2 and 1% on grapes, respectively, *S. pastorianus*, 9% on grapes, and 1% and 2% in fermenting musts and new wines, respectively.

The isolates consisted of 19 species of 3 sporogenous genera and 6 species of 3 asporogenous genera. The report of considerable *Saccharomyces pastorianus* on northern grapes is normal (36), but the isolation of *S. acidifaciens* is apparently the first from fruit. The rapid disappearance of the asporogenous yeasts during fermentation is the usual case. Minárik (164) and Minárik et al. (172) reported cultures of *S. oviformis* which could ferment to 19 per cent ethanol and *Kloeckera apiculata* above 4 per cent, but strains of *Candida pulcherrima* could only ferment to 1 to 2 per cent ethanol. With the exception of a relatively high occurrence of *C. pulcherrima* on red grapes the flora of red and white grapes in Czechoslovakia appears to be very similar [See also Minárik (165)]. Except for *S. acidifaciens, S. elegans,* and *Torulopsis bacillaris,* all were glucophils, although *Hansenula anomala* and *C. pulcherrima* ferment fructose and glucose about equally rapidly.

In contrast to the results obtained in other regions of Czechoslovakia, Minárik (165) and Minárik & Laho (171) found only *Saccharomyces* in musts and wines of the Tokay district of the country. They reported an especially high percentage of *S. carlsbergensis.* In red wine fermentations (166), the normal succession of yeasts occurred.

In another study of the yeast flora of Czechoslovakian grapes, Minárik (167) isolated 3739 cultures from 285 grape samples and 980 strains from 162 wines. One of the purposes of the study was to determine if the widespread use of new fungicides and insecticides had changed the yeast flora. Apparently they have not. The familiar northern European pattern of a predominance of *K. apiculata* and *C. pulcherrima* (particularly on red grapes) at the start, *S. cerevisiae* var. *ellipsoideus* during the main fermentation, and *S. oviformis* at the end, was fully confirmed. Not all studies have shown such an initial predominance of *Kloeckera* and *Candida.* In southern regions, other yeasts may be equally or more important at the start. Many enologists report very rapid predominance of *S. cerevisiae* var. *ellipsoideus* (sometimes in a few hours). Sporogenous yeasts of lesser importance isolated were: *S. carlsbergensis* [the brewing bottom yeast which was found particularly in the Tokay region (164)], *S. uvarum* (in the Malé Karpaty region), *S. bayanus, S. willianus, S. pastorianus, S. coreanus, S. chevalieri* (in the Danube), *S. exiguus, S. italicus, S. heterogenicus, S. elegans, S. veronae, S. rosei* (in the Malé Karpaty region), *Pichia membranaefaciens* (especially in the Danube), and *Hansenula anomala.* Asporogenous yeasts, in addition to those mentioned above, were: *Torulopsis stellata* (probably *T. bacillaris*), *T. bacillaris, T. inconspicua, T. glabrata, T. anomala* (particularly in Bohemia), *T. versatilis* (in the Danube), *Candida mycoderma, C. krusei, C. parapsilosis, C. zeylanoides, Kloeckera africana,* and *Brettanomyces vini.* The spore-forming yeasts represented 60 to 70 per cent of the isolates. Minárik emphasized the importance of climate to the yeast flora. *Saccharomyces* species predominated in musts of warm dry years and *Kloeckera apiculata* in those of cool rainy seasons.

In the dry sandy soil areas of Czechoslovakia, Minárik & Nagyová (173) reported a relatively high amount of *S. carlsbergensis* in the musts and of *S. chevalieri* in the wines. The typical association of *K. apiculata* and *C. pulcherrima* in the freshly crushed musts, and of *S. cerevisiae* var. *ellipsoideus* and *S. oviformis* in the fermenting musts, held here. In the new wines, 35 to 47 per cent of the yeasts were *S. cerevisiae* var. *ellipsoideus,* 13 to 17 per cent *S. oviformis,* 4 to 11 per cent *S. chevalieri,* 21 to 28 per cent, *C. mycoderma,* and 2 per cent of *C. zeylanoides.* To prevent growth of the latter two and of other film yeasts, they recommended strict anaerobic conditions for the wine. The tendency of white wines to form films Minárik believed was associated with their higher percentage of asporogenous yeasts and yeasts with an oxidative mechanism: *C. mycoderma, C. zeylanoides,* and *P. membranaefaciens.*

S. oviformis had the highest alcohol-forming ability. In general, yeasts isolated from fermenting wines had a higher alcohol-forming power than those from grapes or unfermented musts. They seldom isolated *S. coreanus, Brettanomyces vini,* or *Torulopsis versatilis.* The characteristics of the yeasts isolated did not all conform to those of the standard species, which is not uncommon. Minárik noted the discrepancies in the literature of the taxonomic descriptions of certain yeasts.

Habala & Švejcar (91) summarized the chronological succession of yeasts in Czechoslovakia as follows: at the start, *K. apiculata* and *C. pulcherrima;* as fermentation starts, *S. cerevisiae* var. *ellipsoideus,* occasionally with *S. oviformis;* at full fermentation, the last two plus *S. uvarum* and *S. pastorianus;* and at the end, *S. oviformis.* The film yeasts *C. mycoderma* and *C. zeylanoides* may then develop, and later, *T. bacillaris.*

The most important study of the flora of French vineyards and wines is that of Domercq (54). This study is important not only for its completeness but also for the historical information it gives, not only for France but also for other countries. A summary of the yeasts isolated from Bordeaux red and white grapes and wines is included in Table I. Clearly, *K. apiculata* and *S. cerevisiae* var. *ellipsoideus* predominate in Bordeaux musts, whether red or white. The other major yeasts were *S. oviformis* and *T. bacillaris* (particularly from the whites and especially from grapes infected with the fungus *Botrytis cinerea*), and *S. chevalieri, S. fructuum, S. carlsbergensis, S. steineri,* and *S. rosei.* It may be significant that *S. acidifaciens,* which was isolated only from white musts, was very resistant to sulfur dioxide, and fermented fructose faster than glucose (as did also *T. bacillaris* and *S. elegans*).

Bordeaux wines, in contrast to the regions mentioned above, contained only sporogenous yeasts, the most important being *S. cerevisiae* var. *ellipsoideus, S. oviformis, S. acidifaciens* and *Saccharomycodes ludwigii.* The latter three were especially prominent in sweet white wines and they were considered to be undesirable yeasts by Domercq (54). *S. chevalieri, S. carlsbergensis,* and *Brettanomyces schanderlii* were seldom found and only from red

wines. For further information on *Brettanomyces* in France see Peynaud & Domercq (194) and Barret et al. (9).

In the Beaujolais region, northeast of Bordeaux, Bréchot et al. (22) reported *K. apiculata* in only 13 per cent of the musts (as compared with over 50 per cent in Bordeaux musts and 75 per cent in Italian musts). *S. cerevisiae* var. *ellipsoideus* was the dominant yeast followed by *S. steineri*. Very few *S. oviformis* were found. In comparison to Bordeaux, there were relatively more species of *Hansenula, Brettanomyces, Candida, Endomyces, Rhodotorula,* and *Torulopsis*.

Castelli (38) notes that his extensive research on the ecology of wine yeasts dates from 1933. He emphasizes again that *Hanseniaspora* spp. predominate in southern Italy (including Calabria and Sicily), Israel, Iraq, Malta, and Spain, while they are rare in France, Germany, Czechoslovakia, and Jugoslavia. In Sicily, for example, it was practically the only yeast found in the vineyards of Etna up to 100 meters altitude, whereas at 700 to 800 meters only *Kloeckera* spp. were found. Actually, *Kloeckera* is the asporogenous form of *Hanseniaspora*. *S. pastorianus* is only sporadically found in Italy, except in the cool region north of Venice where it greatly exceeded *S. cerevisiae* var. *ellipsoideus*. Because of its adaptation to cold he recommends it for fermentation of sparkling wines in bottles or in tanks. Castelli also confirmed the results of Peynaud & Domercq (195), of Martini (155) and Minárik (167), in that in young wines of three to four months of age, *S. oviformis* is often the major yeast. Castelli also noted that *S. rosei* usually produces a very low amount of volatile acidity. He therefore recommended it for white musts where a neutral wine is desired. He preferred using *S. rosei* to start the fermentation and after four or five days to add *S. cerevisiae* var. *ellipsoideus* or another yeast of high fermenting ability to complete it. He noted that the predominance of *Saccharomycodes ludwigii* in fermentations of highly sulfited musts was observed as early as 1911 by Mensio. Castelli confirmed this for some wines of Malta and also reported *Schizosaccharomyces pombe*. On the other hand, he considered *C. pulcherrima, S. acidifaciens,* and *Brettanomyces* spp. as undesirable yeasts. From the taxonomic point of view, Castelli agreed with Van der Walt that a new genus, *Dekkera,* should be used for the *Brettanomyces* found in wine.

In the Cortese area of the Piedmont region of Italy, Malan & Cano Marotta (148) found in the initial must 44 per cent *K. apiculata,* 40 per cent *C. pulcherrima,* and 12 per cent *S. rosei*. If the must was not sulfited *S. rosei* was primarily responsible for the fermentation. If the musts were sulfited (the usual case) *S. chevalieri, S. uvarum,* and, especially, *S. cerevisiae* var. *ellipsoideus* were found. *S. uvarum* was sometimes associated with the final stages of the fermentation. In cases of high ethanol production, *S. italicus* and *S. oviformis* were isolated. In contrast to some other reported work, viable cells of *K. apiculata* survived fermentation.

Capriotti (30) includes a review of the Italian studies on the yeast flora

of various regions including excellent photographs of many of the genera and species isolated. He made a detailed study of the flora of Sardinian musts. Of 37 strains of *S. cerevisiae* var. *ellipsoideus* isolated, some produced large amounts of volatile acidity. Strains of *S. rosei, S. veronae,* and *S. oviformis* generally produced little volatile acidity. Surprisingly, Capriotti also isolated strains of *S. cerevisiae* var. *ellipsoideus, S. oviformis, S. mangini* (probably *S. chevelieri*), and of *S. veronae* which gave a large increase in fixed acidity during fermentation. Yeasts of this type might be very useful in regions of low natural acidity. On the other hand, *K. apiculata* not only produced considerable volatile acidity but the fixed acidity was reduced. Less common isolates included *S. rouxii, S. mellis, Candida krusei* and *Cryptococcus albidus.* Capriotti (30) stated that under the warm climatic conditions of Sardinia his objectives were to secure strains capable of producing a high ethanol content, low volatile acidity, increased fixed acidity, and with a high optimum fermentation temperature. See also Castelli & Terzaroli (41) and Martini (155).

A detailed study of 14 strains of wine yeasts (all classified as *S. cerevisiae* var. *ellipoideus*) from the port-producing district of Portugal, has been made (152). Since port wines are fortified with spirits during fermentation, it would not be expected that the yeasts would have a marked effect on wine quality. This was essentially true. One strain produced more volatile acidity and one (recommended) produced an exceptionally adherent deposit which resulted in brilliant wines at an early stage of aging. The differences in other components and characteristics were negligible as far as sensory detection is concerned.

Numerous studies on Spanish yeasts were made by Marcilla and his coworkers (150). Insofar as these affect sherry production see p. 333. Castelli & Iñigo Leal (39, 40), Table I, give a summary of the distribution of yeasts in the La Mancha and Rioja regions of Spain. Note especially the high frequency of *S. exiguus, S. elegans,* and *S. veronae* in the La Mancha musts. On the other hand, the absence of *Hanseniaspora* is noteworthy, particularly since the La Mancha region has a very warm climate.

Iñigo Leal et al. (104) reported *K. apiculata, Hanseniaspora valbyensis,* and *Hansenula subpelliculosa* as initial-phase yeasts in Spanish fermentations, producing little ethanol but considerable volatile acidity. Intermediate-phase yeasts were *S. veronae* and *S. rosei,* with more ethanol production and low volatile acidity. In the final stage, *S. mangini* (no doubt *S. chevalieri*), *S. oviformis, S. pastorianus,* and *S. italicus* predominated. These had the highest ethanol yield per unit of sugar fermented. In a film stage, *S. beticus* (*fermentati?*) and *S. cheresiensis* (*oviformis?*) (see p. 333) predominated.

In the Rioja (a northern region), *K. apiculata* was the main asporogenous yeast and *S. rosei* was found in 58 per cent of the fermenting musts (40). The high frequency of *C. pulcherrima* is also notable. This report closely resembles many others in showing the great variation in ethanol production by strains of the same yeast:

Yeast	No. of strains	Per cent ethanol produced	
		Minimum	Maximum
Saccharomyces cerevisiae var. *ellipsoideus*	142	8.5	15.0
Kloeckera apiculata	95	1.1	7.5
Saccharomyces rosei	48	4.0	8.5
Candida pulcherrima	24	0.1	0.7
Saccharomyces pastorianus	17	5.0	12.5
Saccharomyces chevalieri	11	10.0	13.7
Saccharomyces oviformis	9	10.9	12.9
Saccharomyces italicus	4	10.0	12.5

A number of studies on the microflora of Greek musts and wines have been made (162, 207, 294), particularly of those from Peloponnesus and Crete. In the early study (294), in addition to the usual *Saccharomyces* species, *Saccharomyces kluyveri* Phaff, Miller, Shifrine 1956 (205) was identified. Also isolated were *P. fermentans, K. apiculata* and *T. bacillaris*. The comparatively small number of species and the predominance of *S. carlsbergensis* and *S. kluyveri* (after *S. cerevisiae* var. *ellipsoideus*) should be noted. It is also of interest that most of the strains were auxo-heterotrophic and addition of biotin was advantageous. In the second study (162), the following were added: *S. exiguus, S. fermentati, S. delbrueckii, S. florentinus, S. fructuum, S. veronae, Hansenula anomala, Candida pulcherrima, C. krusei, C. tropicalis, C. solani, C. melinii, Rhodotorula mucilaginosa* and *Trichosporon hellenicum*. In a third study (207), *Saccharomyces microellipsoideus, S. steineri, S. transvalensis* Van der Walt 1956 (274a), *Kloeckera jensenii,* and *Trichosporon behrendi* Lodder et Kreger-Van Rij 1952 (139) were added.

They conclude that some quite remarkable differences in the yeast flora exist from year to year in fermenting Greek musts. There were also marked differences in the amount of volatile acidity and ethanol produced by different strains of the same species, and there were some overall differences in ethanol yield between seasons. Whether these are a function of the climate or reflect differences in the level of must sugar is not clear. The authors clearly feel that climate is the most important determining factor, but it can, of course, change the composition of the musts from season to season or even region to region.

A number of important studies on yeast flora have been made in South Africa (279, 280, 285, 287, 289). On vines and ripening grapes the predominant species were: *Kloeckera apiculata, Rhodotorula glutinus* and *C. krusei*. Very few *Saccharomyces* spp. were isolated, however, in crushed grapes; *S. cerevisiae* var. *ellipsoideus* and *S. oviformis* were the major species. The low frequency of *K. apiculata* and *S. rosei* was attributed to the general use of sulfur dioxide. Species of *Schizosaccharomyces, Hanseniaspora, Pichia,*

Candida, and *Kloeckera* were also reported. The isolates of Van Kerken (285) are among the most complete for a given region and are summarized here: *Candida albicans, C. boidini, C. ingens, C. krusei, C. lipolytica, C. melinii, C. mycoderma, C. parapsilosis, C. pelliculosa, C. pulcherrima, C. rugosa, C. sorbosa, Cryptococcus diffluens, C. laurentii, C. luteolus, Debaryomyces hansenii, D. vini, Hanseniaspora uvarum, H. valbyensis, Hansenula anomala, Kloeckera apiculata, K. magna,*
(includes *P. alcoholphila*), and the following species of *Saccharomyces: acidifaciens, capensis* Van der Walt et Tscheuschner 1956 (277), *cerevisiae,*[4] *chevalieri, vanudenii* Van der Walt et Nel 1963 (276), *elegans, fructuum, florentinus, italicus, oviformis, rosei,* and *veronae, Saccharomycodes ludwigii, Torulopsis bacillaris, T. cantarelli,* and *T. domercqii.* In addition, she isolated the first three of the *Brettanomyces* listed below. Van Kerken had difficulty separating *S. acidifaciens* and *S. elegans.*

The most interesting feature of these studies is the widespread occurrence of *Brettanomyces* spp. as spoilage organisms in finished wines: *B. claussenii, B. intermedius, B. lambicus* and *B. schanderlii*[5] Peynaud et Domercq 1956 (194). They were the main cause of cloudiness in finished wines, although *S. italicus, S. acidifaciens, S. elegans, S. oviformis, S. cerevisiae, Saccharomycodes ludwigii, P. membranaefaciens* (includes *P. alcoholphila*), *P. fermentans, Candida mycoderma, C. krusei, C. melinii, Cryptococcus laurentii, C. diffluens, C. luteolus, T. bacillaris, Debaryomyces hansenii,* and *D. vini* were sometimes involved in clouding. Schanderl (249) reported a serious contamination of German sparkling wine by *Brettanomyces* spp.

Recently, several very interesting papers reporting the use of new techniques for isolation have appeared in Germany (12, 13). These have attempted to relate soil type, micro-climate, variety, and other factors to the yeast flora. The most surprising result was that the fungus *Dematium pullulans* (now *Aureobasidium pullulans*) was of very general occurrence and appeared to be in competition with the wine yeasts. The yeasts most often found on the grapes were: *K. apiculata, T. bacillaris, C. pulcherrima,* and *S. cerevisiae* var. *ellipsoideus.* No correlations of yeasts and varieties were found except that *Vitis vinifera* varieties appeared to have more *K. apiculata, T. bacillaris,* and *Saccharomyces* spp. than hybrids of *Vitis vinifera* and the American species of grapes, *V. labrusca.* In the warm year of 1959, there were more *Dematium pullulans* and *K. apiculata,* whereas in the cool 1960, there were more *C. pulcherrima* and *Saccharomyces* spp. Among the less common species isolated were *S. rouxii* var. *polymorphus* and *S. delbrueckii* var. *mongolicus, Torulopsis glabrata,* and *T. stellata* (probably *T. bacillaris*), *T. burgeffiana* Benda 1962 (13), *P. fermentans, R. glutinus, Candida krusei, Cryptococcus albidus, K. magna,* and *Debaryomyces nicotianae.*

[4] Van Kerken (285) and some other taxonomists do not distinguish between *S. cerevisiae* and *S. cerevisiae* var. *ellipsoideus.*

[5] Van der Walt (275, 280) includes *B. schanderlii* in *B. intermedius.*

Benda & Wolf (15) were able to isolate two strains of *S. cerevisiae* var. *ellipsoideus* based on preference of *Drosophila melanogaster* for one. One was a slow-growing haploid and the other a diploid. It is surprising that the haploid fermented to the higher ethanol content. Wolf & Benda (306, 307) also used the fruit fly to differentiate strains of *Schizosaccharonyces pombe* from *S. malidevorans* Rankine et Fornachon 1964 (228a). This research might well be extended to other regions. It is of interest that Stević (260) considers bees and wasps to be important vectors of yeasts. He even suggested using them to disseminate desirable yeasts.

On green New Zealand grapes *Cryptococcus albidus, C. diffluens, Candida mycoderma, C. scottii, Rhodotorula mucilaginosa,* and *R. minuta* were found as well as the fungus *Dematium pullulans* (190). As the grapes ripened, more of *K. apiculata* and *S. cerevisiae* var. *ellipsoideus* appeared, the former predominating early in the fermentation and the latter thereafter.

In contrast to most other countries, in Japan, molds are a major competing factor in vineyards. Shimatani & Nagata (254) reported widespread *Penicillium* spp. and *Aspergillus* spp. At the harvest, *Dematium pullulans* was found throughout the vineyard on damaged grapes. *K. apiculata, Candida mycoderma, C. krusei, P. membranaefaciens, P. fermentans, T. famata,* and *Cryptococcus laurentii* but no *Saccharomyces* spp. were isolated from grapes in the vineyard. During crushing and in the new wines *S. oviformis, S. mellis, H. anomala, Candida guilliermondii, C. parapsilosis,* and *C. pulcherrima* were found.

The distribution of yeasts was reported to vary between two regions of Uruguay (26). In one, *S. cerevisiae* var. *ellipsoideus, S. carlsbergensis, K. apiculata, S. fructuum, S. chevalieri, S. rosei, S. oviformis, P. membranaefaciens, C. mycoderma,* and *C. krusei* were found. In the other, a region where grapes have been grown only ten years, only the first three were isolated.

In a lengthy study of the microflora of grape flowers in the São Paolo district of Brazil (297) no *Saccharomyces* spp. were isolated. Yeasts that were found included: *H. anomala, Cryptococcus laurentii, R. rubra, R. mucilaginosa, Candida brumptii, C. solani, C. pulcherrima, C. stellatoidea, C. guilliermondii, C. guilliermondii* var. *membranaefaciens, C. intermedia* var. *ethanophila* Verona et Toledo, *K. africana, Trichosporon pullulans, Sphaerulina intermixta* and probably *Anthoblastomyces saccharophilus, A. campinensis,* and *A. cryptococcoides.* In a later study on Brazilian musts, fermenting musts, and new wines a wide range of the usual yeasts were isolated by Toledo et al. (269). *S. elegans* var. *intermedia* Verona et Toledo was found on grapes. They also reported *Sphaerulina intermixta* (in musts and fermenting musts), *Trichosporon cutaneum* (de Beurm. et al.) Ota (in musts), *Endomyces lindneri* (*fibuliger?*) (in musts and fermenting musts), *Trigonopsis variabilis* Schnachner (in musts), and *Debaryomyces* spp. (in fermenting musts). *B. bruxellensis* var. *nonmembranaefaciens* was found in fermenting musts and new wines. In musts, *P. fermentans* and *K. apiculata* accounted

for over 60 per cent of the isolates. In fermenting musts they, plus *S. carlsbergensis,* accounted for 60 per cent, and in new wines *S. cerevisiae* var. *ellipsoideus,* and *S. carlsbergensis* represented 82 per cent of the isolates.

In summarizing the ecological studies we note that a wide variety of techniques were used for making the isolations: more or less enrichment of the media, shorter or longer periods before plating, use of antiseptics, etc. We should also note that it is of greater importance to consider the growth characteristics of a yeast and its tendency to dominate rather than the percentage of it present in a must. In our experience, after the start of the fermentation, *Saccharomyces* spp. tend to overgrow most other yeasts, even when the other yeasts have been added in large amounts. We should like to see the ecological studies extended to determine how much, if any, influence on flavor each of the yeasts isolated may have—both in pure and mixed cultures. Careful sensory analysis of the results would, of course, be essential.

Sherry Film Yeasts

The correct classification of the film-forming yeasts used in the sherry district of Spain and elsewhere is still an unsolved problem. An excellent history from the Russian point of view is given by Kudriavtsev (128) and Saenko (241). The former preferred to classify the main Soviet film-former as *Saccharomyces oviformis* var. *cheresiensis,* although earlier Soviet microbiologists preferred *S. cheresiensis* var. *armeniensis* and the first Spanish classification was *S. beticus.* However, Saenko & Sakharova (244), Tsyb (271), and Shakhsuvaryan (253) successfully used *S. oviformis* as a film former. In fact, most of the strains isolated from spontaneous fermentations were of this species in the latter's work. Zhuravleva & Timuk (312) found 12 per cent of the film yeasts in Turkmenia were *Pichia* spp. and 88 per cent *Saccharomyces* spp. Of the latter, 74 per cent was *S. oviformis* and 26 per cent *S. cerevisiae* var. *ellipsoideus.* Saenko (240) adapted sherry yeasts to grow rapidly at 16 to 17 per cent ethanol. The original Spanish classification of sherry film yeasts was by Marcilla Arrazola et al. (150). Schanderl (249) gives only *S. cerevisiae* var. *ellipsoideus* although he found that a number of species of *Saccharomyces* easily form films. Van Zyl (286), working in Schanderl's laboratory, gave no information on this subject.

Iñigo Leal et al. (107) studied the flora in the classical film-yeast districts of Jerez de la Frontera and Montilla in Spain. In the former, the predominant species were *S. cerevisiae* var. *ellipsoideus, S. italicus,* and *S. mangini* (no doubt *S. chevalieri*). From 20 musts only one isolate of *S. oviformis* was reported. In contrast, in Montilla, *S. oviformis* appeared in half the musts. *Saccharomycodes ludwigii* and *Saccharomyces delbrueckii* were isolated for the first time from Spanish musts. In an earlier publication, Iñigo Leal et al. (104) considered *S. beticus, S. cheresiensis, S. montuliensis,* and *S. rouxii* as the most suitable for film formation. Castelli (36) considered the film to be *S. oviformis* or, rarely, *S. rouxii.*

In Japan, Ohara et al. (182) used a yeast strain from Spain, Jerez-5 (*S.*

beticus) and *S. fermentati.* Since Ohara's laboratory is a center for film yeast study, their *S. beticus* is clearly not *S. fermentati.* Their *S. beticus* may be *S. cheresiensis, S. oviformis,* or itself. Iñigo Leal & Bravo Abad (104a) specifically differentiate between *S. beticus* and *S. cheresiencis.*

In some of the early California work (84), a film yeast strain from Spain was classified as *S. cheresiensis* while in other work (42) it was classified as *S. beticus.* However, recent work in this laboratory (R.E.K.) indicates that the strain here is *S. fermentati.* More work, such as Van Zyl's (286), on the morphology and comparative fermentation characteristics of film yeasts, needs to be done. The role of *Pichia* spp., *Hansenula* spp., and *Candida* spp. in the production of the sherry flavor must also be considered. Yokotsuka & Goto (309), for example, reported distinct sherry flavor formation by *C. mycoderma.*

The traditional process for growing film yeasts on the surface has been described frequently (77, 112, 238). Accumulation of acetaldehyde is an important characteristic of the system. For detailed descriptions of the process as used in the Soviet Union and elsewhere, see (4, 241). Soviet work on yeast strains and the process are also summarized (2, 109, 123, 176, 243, 245). It is of interest that the general nature of the sherry characteristics is not much affected by the strain (109) or substrate (262)—even on Finnish berry wines the general products were the same as in Spanish sherries.

The submerged culture process for growing these yeasts has been described in Canada (45–47), California (3, 65, 183, 185, 186), New York (144), and the Soviet Union (153). Continuous-flow modifications of the discontinuous film process have been proposed in the Soviet Union (8, 154, 212, 272). While these appear to increase the yield, few comparative sensory data with wines prepared by the traditional film yeast process are available.

There have been several related reports from Italy and Spain on the use of film yeasts to age red wines more rapidly (29, 36). *Saccharomyces oviformis* is used as a film on red wines for a limited period (three weeks) to reduce volatile acidity, produce esters, and some acetaldehyde. The latter results in loss of tannin and color and was one procedure (27, 28) recommended. While the analytical objectives were achieved, no statistical data on the sensory differences of the treated and untreated wines were given. A similar procedure was used in Spain (239). In another procedure (274), *C. mycoderma* was used in closed fermenters with control of oxygen. Again, the claim of producing high quality wine is made.

When *S. oviformis* was used to form the film, Iñigo Leal (103) reported that addition of other yeasts after three to six days hindered film formation; in fact, addition of *S. veronae* prevented film formation.

New Species

New species of wine yeasts isolated between 1952 and 1958 were summarized by Verona & Montemartini (295). We note here: *Candida boidini* Rami-

rez 1954 (224) originally from tannates; *C. ingens* Van der Walt et Van Kerken 1961 (284) recovered from a winery; *C. intermedia* var. *ethanophila* Verona et Toledo 1954 (297) isolated from grape flowers; *S. vanudenii* Van der Walt et Nel 1963 (276) reported from a winery; *T. cantarelli* Van der Walt et Van Kerken 1961 (283) from industrial grape musts; *T. vanzylii* Van der Walt et Van Kerken 1961 (283) from mold from the floor of a refrigerated wine cellar; *T. capsuligenus* Van der Walt et Van Kerken 1961 (283) isolated from a culture from a winery; *T. domercqii* Van der Walt et Van Kerken 1960 (281) from vineyard soil (278) is really *T. osmophila* (205); *S. capsensis* Van der Walt et Tscheuschner 1956 (277) found in the winery and in musts; *S. prostoserdovi* Kudriavtsev (128) from several quite different wines; and *Trichosporum hellenicum* Verona et Picci 1958 (296) from fermenting Greek musts. The correct identity of the *jerezanus* varieties of *S. italicus, S. rouxii,* and *S. pastorianus* isolated from sherry wines by Zajára Jiménez (310) is still not clear. Joly (111) believes *P. membranaefaciens* and *P. alcoholophila* should remain separate—based on size and amino acid requirements. For some uncommon isolates from Brazilian grapes or fermenting musts see p. 332.

Distribution of Yeasts in Wineries

Peynaud & Domercq (195) reported a varying microflora in a Bordeaux winery. From the outside of the barrels they isolated *S. oviformis,* 6, *S. cerevisiae* var. *ellipsoideus,* 3, *C. mycoderma,* 13, and *Pichia* spp., 1 (numbers following species name refer to the number of isolates). At the bungs they reported *S. elegans,* 7, *S. acidifaciens,* 2, and *C. mycoderma,* 2. From bottling equipment they obtained *S. oviformis,* 4, *S. acidifaciens,* 4, *C. mycoderma,* 10, and *Brettanomyces* spp., 5. From the floors they reported *S. cerevisiae* var. *ellipsoideus,* 1, *Pichia* spp., 7, and *C. mycoderma,* 7.

A detailed study of the yeasts present in various parts of three Czechoslovakian wineries was made by Minárik (169). A summary of his results are given in Table II. Minárik indicates the importance of finding viable cells of *Candida* and *Hansenula* in many wines. In Czechoslovakia, at least, the predominant wine yeasts are *S. cerevisiae* var. *ellipsoideus* and *S. oviformis. Torulopsis* spp. and *Rhodotorula* spp. are rare.

Ribéreau-Gayon & Peynaud (234) also studied the distribution of yeasts within the winery: outside of casks, inside of casks after washing, racking equipment, bottling equipment, floors, walls, etc. Most widely distributed were *S. oviformis,* 19; *S. cerevisiae* var. *ellipsoideus,* 18; *S. acidifaciens,* 14; *S. elegans,* 11; and *C. mycoderma,* 38. Also reported were *S. chevalieri,* 5; *Pichia* spp., 10; and *Brettanomyces* spp., 17. Van Kerken (285) emphasized that many more species are found in musts in the winery than from aseptically handled grapes. The winery is the obvious source, and adequate sanitation measures are essential to prevent growth of undesirable yeasts—some of which cause haze formation in wine.

TABLE II

OCCURRENCE OF YEASTS IN WINES AND WINERIES

Location	Winery I	Winery II	Sparkling Wine
White wine in cement tank	1, 2, 3, 4, 5[a]	1, 2, 4	4, 5
White wine after Kieselgur filtration	1, 2, 3, 4	—	—
White wine after filtration	1, 2, 4	1, 4, 6	1, 2, 3, 4[b]
White wine after filtration (2 weeks)	—	2, 4, 5, 7	1, 2
White wine bottled (5 months)	2, 4	2, 4, 5	1, 2
White wine bottled (18 months)	—	—	2

[a] 1. *S. cerevisiae* var. *ellipsoideus;* 2. *S. oviformis;* 3. *S. carlsbergensis;* 4. *C. mycoderma;* 5. *C. zeylanoides;* 6. *Rhodotorula* spp.; 7. *S. rosei.*

[b] Bottled and yeasted.

SPOILAGE YEASTS

Peynaud & Domercq (195) have emphasized that the same yeast may be desirable or undesirable under different conditions. Thus, *S. oviformis,* which is very useful in producing dry wines, is harmful if used in cases where residual sugar is desired. This is by no means true of many of the other yeasts. *Brettanomyces* spp. seem clearly to be wholly spoilage microorganisms. For the extensive pre-1955 literature, see Amerine et al. (4). In South Africa, Van der Walt & Van Kerken (282) showed that the source of *Brettanomyces* spp. contamination was due to latent infection in the winery, see also p. 331). Van Zyl (287) reported that appearance of *Brettanomyces* spp. was of recent origin in South Africa and is confined to a 40-mile radius around Cape Town. These yeasts require no extra vitamins or amino acids. Lowering the pH or adding 60 mg per l or more of sulfur dioxide retarded or prevented growth. He recommended sterilization filtration or pasteurization (presumably in addition to sulfur dioxide).

The "black mold" *Rhacodium cellare* Pers., present in many cellars, has been best studied by Schanderl (249). Difficult taxonomical problems remain (44).

PURE YEAST CULTURES VERSUS MIXED CULTURES

A major problem of enologists in the 20th century has been the selection of the proper yeast for fermentation. This research has largely been devoted to (*a*) the species of *Saccharomyces;* (*b*) the strains of *S. cerevisiae* var. *ellipsoideus;* (*c*) the use of more than one strain; (*d*) the use of more than one species; and (*e*) the use of one or more species of *Saccharomyces* plus one or more of the other genera of wine yeasts.

The possibility that the apiculate yeasts may make an important contri-

bution to wine quality was considered by many early enologists [see Picci (206)]. The source of apiculate yeasts which are found on the grapes has been the subject of surprisingly few studies. Feduchy (66) isolated viable *Kloeckera* spp. from grape leaves early in the season and long after the harvest.

While the pure culture technique was enthusiastically and probably justifiably accepted by the beer industry, it did not, and has not, achieved the same acceptance by the wine industry in many parts of the world. The wines produced with pure culture techniques have been described as not "completo." Some of the discrepancies between pure and mixed culture techniques have apparently arisen because one or the other was conducted under laboratory conditions which are not always comparable to plant fermentations. Ribéreau-Gayon & Peynaud (234) cite, with apparent approval, the case of a wine produced with indigenous yeasts compared to a pure culture of a single yeast, to the advantage of the former. As early as 1942, Castelli (33) recommended *S. rosei* for musts low in sugar, because of its low production of volatile acids. Ribéreau-Gayon & Peynaud (233) recommended *S. oviformis* for high sugar musts.

It is clear that such special uses of yeast cultures other than *S. cerevisiae* var. *ellipsoideus* appear logical. The results with strains of the latter have been much less convincing. Rankine (225), however, estimated that three fourths of the Australian wine fermentations were conducted with pure yeast cultures. He noted that no increase in maximum alcohol attainable was achieved by using two strains of *S. cerevisiae* var. *ellipsoideus*. Enologists generally consider pure yeast cultures essential (249a) for special fermentations (sparkling wines, low-temperature, when clarified musts are used, submerged cultures, etc.) or for musts from grapes in poor condition. Some (137) even consider them universally valuable. The various chemical differences detected by Van Zyl et al. (288a) with two strains of *S. cerevisiae* var. *ellipsoideus* certainly indicate that more work should be done.

The use of selected strains of *S. cerevisiae* var. *ellipsoideus* as a major factor in changing the quality of wines in normal fermentations is less clear as Ribéreau-Gayon & Peynaud (234) have concluded:

> Nous avons conduit à ce sujet, un certain nombre d'essais qui consistaient à faire fermenter le même moût dans les conditions de la pratique, avec des souches de *Sacch. ellipsoideus* provenant de divers pays viticoles. Les différences constatées, qui pouvaient apparaître dans le cours même de la fermentation; étaient faibles et ne persistaient pas après deux soutirages. Au mois de mars, tous les vins se goûtaient identiquement où les différences constaté es n'avaient aucune valeur commercial. D'autres auteurs ont obtenu les mêmes résultants, nous l'avons vu.

Various reports recommending mixed cultures or variant yeasts have appeared (74, 147, 175). The latter recommended *S. bailii, S. eupagicus,* and *Hanseniaspora apiculata* (probably *valbyensis* or possibly *uvarum*) to improve flavor while the former (74) favored *S. rosei* or even *K. apiculata* if an incomplete fermentation was desired. This paper reported wide variations in

the ethanol content and odor and taste of wines made by different strains of the same species. Although Kir'ialova (118) recommended mixed cultures, she also showed that *S. cerevisiae* var. *ellipsoideus* tended to overrun other yeasts. The rate at which this occurred depended on the sugar concentration and species. Contrariwise, Iñigo Leal (103) reported that other yeasts may markedly slow the growth of *S. cerevisiae* var. *ellipsoideus*. For example, *C. pulcherrima* inhibited its growth when added early in the fermentation (three to six days) but not after 20 days (when presumably the fermentation was nearly complete). These reports of the advantages of mixed cultures would be more convincing if detailed results on the nature of the flora at each stage of the fermentation were presented and if some verifiable sensory data on the comparative quality of the products were made available. The unsupported statement that *S. rosei* gave a very palatable and flavorous wine is not convincing.

There are few statistically verifiable sensory data that pure culture, mixed culture, or successive culture techniques produce better wines so that at this stage it is difficult to determine which procedure is best.

It has been shown that strains of yeast suitable for batch fermentation might not be satisfactory for continuous systems. Egamberdiev (63) reported the strain Parkentskayia-1 superior to the usual Rkatziteli-6 (both *S. cerevisiae* var. *ellipsoideus*) for continuous fermentation of musts.

Ásvány (6) recommended and isolated sulfite-tolerant yeasts (called *Gyöngyös* in Hungary). He reported that both free and total sulfur dioxide influenced yeast multiplication. Use of low-temperature fermenting yeasts has been recommended for many years by enologists. For yeasts acclimated to 6° to 9° C see (99, 237). For wines high in sugar, Mosiashvili (174) recommended simultaneous addition of cold- and warm-acclimated yeasts. Less residual sugar and superior flavor were claimed. More comparative sensory data on the products would be useful. One case in which special yeasts are obviously desirable is when high ethanol yields are desired (170). There are many other cases in the earlier literature.

Yeast Fermentation of Malic Acid

Recently, interest in the possible use of mixed yeast cultures has been stimulated by the rediscovery of the ability of *Schizosaccharomyces pombe* to ferment malic acid during alcoholic fermentation and reduce the titratable acidity. Gandini & Tarditi (85) have reviewed the history of these recent investigations. They recommended *S. rosei* plus *S. cerevisiae* var. *ellipsoideus* (or plus *S. oviformis*) and *Schizosaccharomyces pombe* plus *S. cerevisiae* var. *ellipsoideus* (or plus *S. oviformis*). The weakness of these results is that no statistically verifiable sensory evaluation is presented, although the results are said to be "disarmonico."

For other studies on the use of *Schizosaccharomyces pombe* to reduce the malic acid content see (24, 50–52, 158, 204, 228, 235). This would appear to be a desirable yeast for high-malic acid low-sugar musts, but yields generally poor

results as far as the quality of the wine is concerned (14, 50, 156). The metabolic pathway is not clear, some results indicating that about half the malic acid is converted to ethanol while others report carbon dioxide and water to be the primary products. Ribéreau-Gayon & Peynaud (235), as well as many earlier investigators, have reported that *Saccharomyces* spp. also reduce malic acid but that they accomplish this best under aerobic conditions. In addition to *S. pombe* (includes *S. liquefaciens*), *S. acidodevoratus* [now *pombe* (85)], *S. versatilis* and *S. octosporus* are effective in more or less completely metabolizing malic acid, either under aerobic or anaerobic conditions.

Other yeasts may utilize or reduce the acidity or raise the fixed or volatile acidity, or both (57, 105, 106). Increasing or decreasing the fixed acidity by the use of special yeasts for musts would appear to be a fruitful field for investigation.

BACTERIA IN WINE

Bacterial growth in wines is not uncommon. The effect on the wine of growth of bacteria is sometimes acceptable, or even desirable. However, this is not always the case; bacteria also cause spoilage of wine. When modern microbial technology is understood and practiced, the latter situation is rare and usually inexcusable. The level of ethanol and sulfur dioxide in wine and its high acidity and low content of nitrogenous material make it a hostile environment for all but a few kinds of bacteria. The kinds of bacteria which have been isolated from wine are bacilli and lactic acid and acetic acid bacteria. In properly stored table wines, when the oxygen concentration is kept low, only the lactic acid bacteria have been found. The latter can be classified as desirable whenever they bring about pleasant flavor changes or needed changes in acidity, as in malo-lactic fermentation (see below). Even in these cases, however, there may be undesirable side effects, such as an increase in turbidity from the bacteria themselves, which must be corrected, or of carbon dioxide accumulation in otherwise still wines. Acetic acid bacteria grow whenever improper storage practices allow contact of oxygen and table wines. Because of the formation of acetic acid which results, the acetic acid bacteria are always considered spoilage organisms in wine. In dessert wine, the high concentration of ethanol prevents development of acetic acid bacteria and nearly all lactic acid bacteria; however, some bacilli, lactobacilli, and pediococci have been isolated from spoiled dessert wine (82, 89). One source of the origin of bacteria in table wine seems to be from the skins of the grapes [cf. (129)]. Zhuravleva (311) isolated bacteria belonging to the genera *Pseudomonas, Micrococcus, Bacterium, Chromobacterium,* and *Bacillus,* as well as lactic acid bacteria, from grape juice; but apparently only the lactic acid bacteria survive the inhibitory surroundings of fermenting musts. [Radler (215, 222) was unable to isolate lactic acid bacteria from fresh grape juice, although he did detect them on grape leaves (215)]. Webb & Ingraham (301) and Ribéreau-Gayon & Peynaud (234) showed that wine cellars, themselves, might also be the source of lactic acid organisms. Although

bacteria are found in must, bacterial growth, when it occurs, is usually noticed only sometime after alcoholic fermentation is completed—usually many months later.

Lactic Acid Bacteria and Malo-Lactic Fermentation

Lactic acid bacteria are acid- and ethanol-tolerant, facultative anaerobes, and as such are often found in wine. These organisms produce lactic acid from carbohydrates. A special class, the malo-lactic bacteria, produce lactic acid also from malic acid. There is a great deal of confusion in the taxonomy of lactic acid bacteria isolated from wine, but apparently three genera of the Lactobacillaceae family are important: *Pediococcus, Leuconostoc,* and *Lactobacillus.* A large part of the information available about lactic acid bacteria from wine has been obtained from studies on malo-lactic bacteria. It will be convenient to discuss the lactic acid bacteria as a group, making distinctions where necessary between the malo-lactic and nonmalo-lactic bacteria. Discussions of lactic acid bacteria and malo-lactic fermentation are given in the enology texts mentioned at the beginning of this review as well as in the following articles: Suverkrop & Tchelistcheff (263), Vaughn (290), Vaughn & Tchelistcheff (291), Lambion & Meskhi (136), Lüthi (141), Sudraud & Cassignard (261), Fell (67), Peynaud & Domercq (197), Fornachon (79), Bezzegh (17), Rankine (226), Radler (219–222), and Kunkee (129). The last two articles listed are comprehensive reviews of malo-lactic fermentation and the last one includes a history of malo-lactic fermentation over the last one hundred years.

Most of the modern reports of lactic acid bacteria isolated from wine [Du Plessis & Van Zyl (60); Iustratova (108); Poittevin et al. (211); Pilone et al. (210); Radler (222); Peynaud (193a); Peynaud & Domercq (198, 199)] place the organisms in the above genera. They are Gram-positive, catalase-negative, nonmotile, nutritionally fastidious, microaerophilic cocci and rods which produce lactic acid as a major end product of carbohydrate fermentation. Difficulties in generic classification have been noted because of confusion in differentiation between short rods and elongated cocci and between heterolactic and homolactic fermentations (25, 129, 210, 290). Greater difficulty has been experienced in specific classification with the use of standard systematics, i.e., *Bergey's Manual of Determinative Bacteriology* (23). Peynaud & Domercq (198) have followed a later system, of Rogosa & Sharpe (236), for the classification of the homolactic rods. In this system, the genus *Lactobacillus* is divided into three subgenera: *Thermobacterium, Streptobacterium,* and *Betabacterium.* Peynaud & Domercq (198) go a step further and use *Streptobacterium* as a genus name for some homofermentative rods. Even more difficulty has been experienced with the heterofermentative cocci which are classified, in part, by their ability to ferment various hexoses and pentoses. Because of the fastidious nature of lactic acid bacteria, it is often difficult to obtain growth on defined media which is needed for testing sugar fermentations. One example of the difficulty in obtaining good

correspondence between the bacteria and standard classification is that of *Leuconostoc mesenteroides* isolated by Fornachon (81). This organism, a heterofermentative coccus, utilizes sucrose and pentoses, and thus is properly classified as *L. mesenteroides*. However, it does not produce dextran from sucrose, which is supposed to be characteristic for this species. After studying over 700 isolates of lactic acid bacteria from wine, Peynaud (193a) suggested new specific names for heterolactic cocci: *L. oenus* for those fermenting pentoses and *L. gracile* for the others. Earlier, Lambion & Meskhi (136) made a similar suggestion in the proposal to name pentose-positive heterolactic cocci *L. mesenteroides* var. *gracile*. Classic appellations, such as *Bacterium gracile* and *B. intermedium* are still being used (67, 72, 251).

Nakagawa & Kitahara (177) suggested a new key for pediococci. They divided the genus into five species. Of the three species which thrive at low pH, they separated anaerophilic *Pediococcus cerevisiae* from the microaerophilic *P. pentosaceus* and *P. acidilactici*. Furthermore, even though all of these bacteria were homofermentative, they added *L. citrovorum* as a variety of *P. pentosaceus*, and they classified a standard strain of *L. mesenteriodes* (P-60) as *P. acidilactici*. Peynaud & Domercq (199) designated all the homolactic bacteria isolated from wine as *P. cerevisiae*. The application of numerical taxonomy, which has been applied to some lactic acid bacteria (75), may help to bring bacteria isolated from wine into some standard classification with the others. In spite of difficulties in classification, the following list, given and annotated by Radler (222), seems to be composed of *bona fide* species of lactic acid bacteria: *Lactobacillus brevis, buchneri, casei, delbrueckii, fermenti, hilgardii, leichmannii, pastorianus, plantarum,* and *trichodes; Leuconostoc citrovorum, dextranicum,* and *mesenteroides;* and *Pediococcus cerevisiae*. *L. trichodes* and *L. hilgardii* are new species (82, 290, 292); they were not, however, accepted species for the latest edition of *Bergey's Manual* (23). Some strains of the above species are malo-lactic bacteria, and others are not.

Studies on nutritional requirement of lactic acid bacteria show the stimulating influence on growth by materials found in grape and other fruit juices (92, 129, 180, 257), in yeast extract (145, 146), and in protein hydrolysates (62, 72). Borroughs & Carr (20) showed that traces of amino acids resulting from yeast autolysis stimulated growth. The mineral content (19, 180, 222, 313), as well as the amounts and kinds of nitrogenous material, are also important. In studies with synthetic media, Radler (216) and Du Plessis (58) reported at least 18 common vitamins and amino acids necessary for growth of the bacteria they isolated. It is difficult to obtain good growth for some of the bacteria on any defined media, no matter how complex (129).

Krasil'nikova (124) found some growth of *Lactobacillus delbrueckii* in the absence of sugar if glycerol, fumaric acid, and pyruvic acid were present. In wine, lactic acid bacteria more often use the residual sugar as their source of carbon and energy—even dry table wine contains 0.1 per cent reducing sugar. Melamed (160, 161) showed decreases in sugar of dry wine after

growth of lactic acid bacteria. The decreases were greatest with glucose and arabinose, but he hesitated to conclude that the concentration or kind of sugar in wine was a decisive factor in favoring the secondary fermentation. Melamed also found (161) that xylose was poorly fermented by the natural malo-lactic bacteria; but Yoshizumi (308) reported that arabinose and ribose were fermented more vigorously than hexoses. Iustratova (108) studied the carbohydrate utilization of three lactic acid bacterial species and found wide variability in their utilization. She tested 18 different carbon sources. Trace amounts of sugars could be influential in growth of the bacteria. Melamed (161) found that arabinose consumption was increased in the presence of glucose; Stamer & Stoyla (258) noted a great growth stimulatory effect by small amounts of fructose, with arabinose and glucose as the main carbon sources. The kinds of sugar fermented are important not only from a standpoint of the vigor of the fermentation but also of the products formed. The latter can have an important effect on wine quality. For example, acetic acid formation by lactic acid bacteria comes largely from pentose fermentation (161); and mannitol results from reduction of fructose [cf. (61)]. Esau & Amerine (64) detected heptuloses and other sugars larger than hexoses in wine; unfortunately, little is known about the metabolism of these sugars by lactic acid bacteria. During malo-lactic fermentation, Tsyb (271) noted the reduction of aldehydes in sherry (containing 15 per cent ethanol).

In addition to the influence of the growth factors and carbon sources on growth of lactic acid bacteria, materials in the wine will have an inhibitory effect. In general terms, inhibition of growth is noticeable at 6 per cent ethanol, 75 mg per l sulfur dioxide, and at pH below 3.4, but higher concentrations of ethanol, sulfur dioxide, and acidity are required for complete inhibition (78, 129, 217, 308). Malo-lactic fermentation rarely occurs in dessert wine (76). Ingraham et al. (102) noted that the optimal pH's for the lactic acid bacteria isolated from wine were lower than those of other lactic acid bacteria. Saenko et al. (242) mentioned isolation of lactic acid bacteria which thrived at pHs as low as 1.1–3.3. Ribéreau-Gayon Peynaud (234) pointed out that inhibitory material may be excreted by the yeast that carry out the primary fermentation. Other natural materials inhibitory to lactic acid bacteria, such as discovered by Fleming & Etchells (69) in green olives, may also be present. Apparently, tannins found in wine are not inhibitory to these bacteria [(76), cf. (129)].

In spite of these unfavorable conditions, growth of lactic acid bacteria does take place, however slowly. Adaptation of the bacteria to the hostile environment certainly occurs. Flesch (70) and Flesch & Jerchel (73) were able to adapt malo-lactic bacteria to adverse surroundings by stepwise modification of conditions.

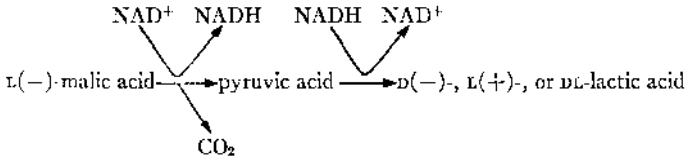
This discussion serves to emphasize the difficulties in prediction of the kinds of secondary fermentations which might occur in a given new wine. Not only would one need to know the nutritional requirements of the native organisms, but also the carbonic and nitrogenous complement of the wine

itself. Practically speaking, none of these would be known. In addition, one must consider the extent of the inhibitory effect of the wine and its storage on the growth and adaptive potentials of the organisms.

Deacidification by Malo-Lactic Bacteria

The bacterial metabolism of organic acids in wine can have profound influence on wine flavor. The products of fermentation of organic acids can contribute to the sensory characteristics of the wine, but usually of more importance is the resulting change in acidity. About half of the titratable acidity of grapes is due to malic acid (4, 119, 234), thus the decarboxylation of malic acid to lactic acid during malo-lactic fermentation results in substantial reduction in the acidity of the wine. The degree of loss of acidity is, of course, dependent on the initial concentration of malic acid, but the change in pH depends on the initial pH and amounts of other buffering agents present.

Malo-lactic conversion has been well studied but only in a few organisms. The following presentation of the mechanism is considered to be the general situation at our present state of information. Schmidt et al. (251) and Du Plessis (59) showed that malic acid is nearly stoichiometrically converted to lactic acid. The reaction, involving oxidation and reduction with NAD as coenzyme, is given as:



Studies by Kaufman et al. (113), Korkes & Ochoa (122), and Ochoa et al. (181) showed that the first step was catalyzed by "malic" enzyme [malate:NAD oxidoreductase (decarboxylating)] and the second by lactate dehydrogenases [L- *or* D-lactate:NAD oxidoreductases]. "Malic" enzyme is an inducible enzyme in some organisms and constitutive in others. The inducibility of "malic" enzyme in malo-lactic bacteria has been studied (18, 59, 71, 178, 304).

Considerations of the thermodynamics of the reaction by Schmidt (250) and Kunkee (129) revealed that under standard conditions the reaction is exergonic, even though it is endothermic (249). Nevertheless, the potential energy of the reaction is not biologically available. Apparently the intermediate, pyruvic acid, remains tightly associated with the enzyme complex and cannot be used for other energy-yielding reactions (113). Furthermore, there is no net change in redox state of the coenzyme. This explains why malic acid is not an energy source for malo-lactic bacteria, and why carbohydrates must be supplied for malo-lactic fermentation (217).

Many malo-lactic bacteria can also ferment citric acid, utilizing it as an

energy source (59). Wejnar (303) showed that in these cases malic acid is degraded before the degradation of citric acid. The fermentation of citric acid probably involves either the initial splitting of the substrate to oxaloacetic acid and acetic acid or decarboxylation of citric acid to give citramalic acid (α-hydroxy-α-methyl aspartic acid). Carles et al. (31) speculated that this is the source of citramalic acid in wine. Tartaric acid is, for all practical purposes, microbiologically stable in wine (126, 127).

Malo-lactic fermentation occurs in practically all winemaking areas of the world [cf. (129)]. The deacidification resulting from malo-lactic fermentation has been praised and declared an essential ingredient for premium quality wine, especially in cooler regions which produce wine with high acidity (68, 151, 196, 232, 234, 270). In warmer regions, where the acidity is generally lower, the deacidification would not be so beneficial. Nevertheless, malo-lactic fermentation is usually considered desirable in wines, especially premium quality red wine, from the warmer areas, as well (101). End products of malo-lactic fermentation may bring about desirable flavor and odor changes in wines, adding a distinctiveness and complexity they would not otherwise have (101, 151, 261, 263, 291, 300, 302). Sensory examination of wines in which the secondary bacterial fermentations were induced by inoculation with various malo-lactic bacteria, added some support to this contention (209, 210). No doubt of the desirability of malo-lactic fermentation exists in Europe (68, 234, 270). Biological stability, arising from removal of fermentable substrates, is another advantage of malo-lactic fermentation. Care must be taken whenever very young wines, which have not undergone malo-lactic fermentation, are sold, to prevent the secondary fermentation from occurring after bottling. Failure of control leads to gassy and turbid wine. Kunkee (129) discussed some of the undesirable secondary effects of deacidification.

The major end products of malo-lactic fermentation are carbon dioxide and lactic acid. L-Lactic acid is the predominant form of lactic acid found in wines which have undergone malo-lactic fermentation (21, 201–203), even though most malo-lactic bacteria produce D-lactic acid or a mixture with carbohydrates as substrates, but not necessarily with malic acid as the substrate (203).

Increase in volatile acidity and content of diacetyl (or diacetyl plus acetoin) is common in wine after malo-lactic fermentation, especially if citric acid has also been fermented (43, 53, 79, 83, 132, 218, 234). Pilone (208) showed that the increase in volatile acidity was due to acetic acid, and not to lactic acid which is formed in relatively large amounts. Diacetyl is highly flavorful and odoriferous and, unpleasantly so in large amounts. However, at low concentration, it, together with other products formed in trace amounts during malo-lactic fermentation, e.g., diethyl succinate, may be beneficial to the sensory quality (79, 210, 218).

Control of Malo-Lactic Fermentation

Malo-lactic fermentation is hard to control, and the decision by the wine-

maker to encourage or discourage it is difficult to put into practice. Highly acid wine which needs the fermentation to decrease its acidity is inherently inhibitory to the bacteria, because of its low pH, and vice versa. Stimulation of malo-lactic fermentation in wine has been achieved in laboratory studies by the addition of bacteria to wine or must (55, 67, 79, 131, 151, 196, 209, 261, 300, 301). There are very little data to guide one in the use of this procedure on a commercial scale. Kunkee (129) has suggested methods to be used, but they are empirical at best. The organism for inoculation must be carefully selected. It would seem that *Leuconostoc* spp. would be the cultures of choice because of their greater tolerance to low temperature and pH [cf. (79, 129)]; however, these bacteria may also produce larger amounts of diacetyl, dextrans, and mannitol than do other lactic acid bacteria. Inhibition of malolactic fermentation is best brought about by the judicious addition of sulfur dioxide and acid, and by heavy fining and filtering [cf. (129, 130)]. Pasteurization of table wine is usually not an acceptable practice because of the unfavorable effect on wine quality.

Bacterial Spoilage of Wine

Very few papers about bacterial spoilage have appeared since Vaughn's review (290) of the subject thirteen years ago, and the latter is still recommended study for those interested in wine spoilage. This attests not only to the completeness of Vaughn's article, but also to the technical advances which have been made in cellar practices. Modern winemakers are so sophisticated in their use of sulfur dioxide and storage of wine that it is now difficult in most wine regions of the world to purchase a bottle of bacterially spoiled wine. Vaughn's (290) concern with the improper use of old terms such as *tourne, pousse,* or *amertume* to describe, inadequately, certain types of wine spoilage is probably no longer of consequence. Even the terms "diseased wine" or "wine sickness" for spoiled wine are now rarely heard. Other important sources of information about bacterial spoilage should also be consulted (4, 143).

Of course, a watchful eye is important in the prevention of bacterial spoilage of wine. Even so, from time to time it may occur. The following kinds of spoilage are of bacterial origin:

Acetic or acescent spoilage (also called acetic souring or vinegar souring) results from acetic acid bacteria in table wine which has not been properly stored. Under aerobic conditions, these bacteria use ethanol or acetaldehyde as carbon sources and oxidize them to acetic acid. The spoilage is prevented by the presence of sulfur dioxide and especially by maintenance of anaerobic conditions, either by continuous blanketing of the wine with carbon dioxide, or other gas, or by storage in completely filled containers. Filled containers must be examined often to prevent ullage and, thus, oxidation. The odor from acetic spoiled wine is not only from acetic acid, which is measured in volatile acidity determinations, but also from the ester of acetic

acid and ethanol. Acetic acid bacteria are completely inhibited by about 15 per cent ethanol, thus, this kind of spoilage does not occur in dessert wine.

Vaughn (290) described acetification of musts. Here, the bacteria utilize glucose as an energy source, converting it to gluconic acid. In "rapid acetification" spoilage, acetic acid bacteria grow in association with wine yeast during alcoholic fermentation. They oxidize acetaldehyde and ethanol as they are formed, producing a large amount of acetic acid. This is not common now because of the ubiquitous use of sulfur dioxide and the maintenance of anaerobic conditions. The acetic acid bacteria, some of which are also used for vinegar production, are all classified in *Bergey's Manual* (23) as members of the genus *Acetobacter*. Reassessments of the classification by Shimwell & Carr (255, 256) divide the bacteria into two genera. By the latter classification, *Acetobacter* spp. are those which further oxidize acetic acid from ethanol to carbon dioxide and water. Morphologic studies showed that these organisms possess peritrichous rather than polar flagella (255) and thus should be removed from the family Pseudomonadaceae. Shimwell (255) also pointed out the great instability of some of the characteristics of these bacteria, making species classification practically impossible. Acetic acid bacteria which oxidize ethanol only as far as acetic acid remain in the Pseudomonadaceae as *Acetomnnas* spp. (256). If we use this classification, we should call acetic acid bacteria or vinegar bacteria *Acetomonas* rather than *Acetobacter*.

Other aerobes causing wine spoilage are *Bacillus* spp. Gini & Vaughn (89) isolated six species from spoiled dessert wine. Bacilli would not be found in table wine because it is normally kept under anaerobic conditions. Presumably, improper storage of table wine would result in acetic spoilage before *Bacillus* spoilage. The results of *Bacillus* spoilage are similar to those caused in dessert wine by anaerobic *Lactobacillus trichodes* (see below), but there does not seem to be any widespread occurrence of any of these kinds of spoilage. Nevertheless, winemakers ought to have knowledge of *Bacillus* spp. Semidry table wines are now being bottled under "aseptic" conditions. The equipment between the filtering and bottling apparatus is often sterilized by heat. The winery water may contain spore-forming bacteria, such as bacilli, and these organisms may appear in tests for sterility of the equipment.

Lactic acid bacteria, which can cause spoilage under anaerobic conditions, have already been discussed. Malo-lactic fermentation, itself, can be considered as spoilage if the deacidification is detrimental to the sensory quality, or if end products are formed which are particularly undesirable. It is not clear if the differences between desirable and undesirable lactic acid fermentations are due to the kinds of organisms, the kinds of substrates fermented, or the conditions under which the secondary fermentation occurs [cf. (59, 129)]. New fundamental information is obviously needed to fill this important gap in our knowledge of wine production. Some kinds of lactic acid spoilage are: "deacidification" or malo-lactic fermentation (see above); "lactic souring,"

the presence of end products of lactic acid fermentation, such as diacetyl, which give flavor and odor reminiscent of either cabbage and sauerkraut or "Spanish olives" (53); "mannite" or bitter, caused by formation of mannitol from fructose or acrolein from glycerol (4, 17, 290); and "ropy," a thickening of the wine caused either by glycerol or dextrans. The term "Fresno mold" has been used to describe the "flocculant precipitate of intertwined filaments" which is caused by growth of *L. trichodes* (and similar spoilage by *P. cerevisiae* and *Bacillus* spp.) in dessert wine with inadequate concentrations of sulfur dioxide (82, 89, 290). Microscopic examination of *L. trichodes* leads to the description of it as the "hair bacillus" or "cottony mold" (82). "Fresno mold" spoilage is now rare, but a report of it in Italian vermouths appeared in 1966 (147a).

The term "mousy" or "mousey" is commonly applied to some spoiled wine. The odor of these wines is said to be like that of mouse urine. There is confusion as to the cause of mousy wine. It has been described as resulting from the acidity caused by acetic acid spoilage of must (290), mannitol production by heterolactic bacteria (17), by the presence of acetamide [cf. (32)] or formic acid (4).

Bacterial spoilage is prevented by methods similar to those described for inhibition of malo-lactic fermentation. Winery equipment, especially cooperage, must be kept scrupulously clean and the wine must be examined often for free sulfur dioxide content. Sterilization is theoretically possible by filtration, especially with the use of small-pored membrane filters, but there are few data on the practical application of this for removal of bacteria from wine.

Vaughn (290) pointed out that no pathogenic bacteria have been isolated from either sound or spoiled wine.

BACTERIA AND YEAST INTERACTIONS

An area of microbiology of wine which must be mentioned but on which little information is available, concerns the synergistic, symbiotic, and inhibitory interactions between microbes in wine. Particular strains of yeast used for the alcoholic fermentation seem to have some effect on the secondary bacterial growth. Ribéreau-Gayon & Peynaud (234) explain the effects in terms of the relative amounts of micronutrients needed by bacteria taken up by the yeast, or by the excretion by the yeast of material inhibitory to bacteria. Lüthi (142) was not able to obtain certain characteristics in wine which was inoculated with bacteria originally isolated from wine having that particular chracteristic. He suggested that synergism between several organisms in the wine was important in producing the desired characteristics.

STABILIZATION OF SEMIDRY WINE

Semidry table wines (containing 1 to 3 per cent reducing sugar) have become increasingly popular. To prevent reoccurrence of yeast growth and fermentation, the wine must be treated by pasteurization, filtration, or the

addition of chemicals. As we have pointed out, pasteurization of wine is not considered to be an acceptable treatment.

Filtration

With the availability of membrane filters which allow a relatively high rate of flow and which have uniformly small pores, it is now possible to stabilize semidry wines by the removal of yeast. Theoretically, this is the most acceptable method of stabilization of these wines because of the negligible effect on flavor. However, these filters are screen filters and they tend to become clogged quickly; the wine must be carefully prefiltered. Furthermore, the line and equipment between the filter apparatus and the bottle and, the bottle itself, must be kept free of yeast. Little data are available on the results obtained with this kind of stabilization, but there is no doubt that some large wineries, at least in California, are using the method with confidence for the removal of yeast.

Addition of Chemicals

In the United States three chemicals may be added in limited amounts to wine as preservatives: sulfur dioxide, diethyl pyrocarbonate, and sorbic acid. Sulfur dioxide is added routinely to practically all musts and wines. There is general agreement among winemakers (4) as to its importance. Not only is it an inhibitor of microorganisms, but it is an antioxidant and inhibits browning. It would seem that sulfur dioxide is inhibitory because, in its bisulfite form, it complexes with acetaldehyde, an intermediate in the fermentation pathway, and it also reduces disulfide bonds of proteins (213). Rehm and co-workers showed that the addition compounds formed between bisulfite and various carbonyl compounds are, themselves, inhibitors—not only of alcoholic fermentation but also of yeast growth and respiration (229–231, 299). Scardovi (246–248) studied the adaptation of microorganisms to sulfur dioxide. Fornachon (80) pointed out that some bacteria can metabolize acetaldehyde that is bound to bisulfite and bring about an increase in concentration of free bisulfite. The inhibitory effect of sulfur dioxide at reasonable concentrations is not enough to stabilize semidry wines against continued fermentation. However, it can be effective when added with other compounds mentioned below, making lower concentrations necessary for each chemical (110, 187, 266, 288, 298). For more complete discussions on sulfur dioxide see (4, 18a, 81a, 114, 248, 252).

Diethyl pyrocarbonate, known in the United States as DEPC but also called PKE, Piref, and "Baycovin," is a nearly tasteless and odorless (see below) inhibitor of microorganisms, especially yeast. It was reported as a natural component of sparkling wine in 1951 by Parfent'ev & Kovalenko (189). This was questioned by Kielhöfer & Würdig (116) since no natural diethyl carbonate could be detected (see below). It was not until 1959 that Hennig (94) showed that it was a potent inhibitor of fermentation. Since then it has become legally acceptable in several countries for the stabiliza-

tion of wine [cf. (191)]. Several general discussions on the use of DEPC have appeared (1, 97, 120, 157, 191, 192, 227). The lethal effect of the chemical has been shown to be correlated with concentration of yeast cells present (1, 191, 273, 288). In aqueous solutions, DEPC is rapidly hydrolyzed to ethanol and carbon dioxide (95). Thus, during bottling operations, wine treated with DEPC must be bottled quickly before potency of the chemical is lost. This disadvantage, together with the relatively low solubility of DEPC, has resulted in the requirement for special methods for its continuous addition during bottling (93, 96, 97). In the presence of ethanol, DEPC breaks down [$(C_2H_5OCO)_2O + C_2H_5OH \rightarrow CO_2 + C_2H_5OH + (C_2H_5O)_2CO$] to carbon dioxide, ethanol, and diethyl carbonate (DEC) (97). The latter is stable and has a fruity odor and flavor. The sensory detection of DEPC, for which the 50 per cent threshold was determined by Ough (184) to be 280 mg per l, is actually based on the formation of DEC. Measurement of DEC by gas chromatography (86, 115, 214) can be used to determine the concentration of DEPC originally added to the wine and the effectiveness of the distribution of it during bottling (117). Diethyl pyrocarbonate inhibits many kinds of yeast (120, 157, 188, 191), but its inhibitory effect against wine bacteria is much less (157) and is not practical for the control of secondary bacterial fermentations. It would seem to satisfy the criteria suggested by Gillissen (88) as necessary properties of antiseptics for use in wine. These include lack of odor and taste, nontoxicity and nonsensitivity to man, specificity of microorganisms attacked, and lack of induction of resistance by microorganisms. To these criteria, one of us (M.A.A.) adds another: that the chemical should be easily quantitatively determined. The mechanism of activity of DEPC has not been elucidated, but it is apparently related to the reaction with amino and sulhydryl groups of proteins (87, 191), as well as with hydroxyl groups (56, 267). The chemical has been shown to have an inhibitory effect on several enzymes including alcohol dehydrogenase (98, 159, 236a).

Sorbic acid ($CH_3 - CH = CHCH = CHCOOH$) is an effective inhibitor of fermentation and has also been used to stabilize semidry wine. Although it does not deteriorate in wine and has this advantage over DEPC, it does have a higher threshold level of sensory detection. A complete discussion of the use and activity of sorbic acid will not be given here; instead, several useful articles will be listed: Auerbach (7), Bell et al. (11), Lück & Neu (140), Nomoto et al. (179), Ough & Ingraham (187), and Peynaud (193).

Also should be mentioned the alkyl esters of *p*-hydroxy benzoic acid, especially the *n*-heptyl ester (WS-7). These compounds have hardly been tested in wine as yet; and they cannot be added to wine legally, at least in the United States. However, they show great promise; brewers have found them to be effective against both yeast and bacteria at very low concentrations (134, 260a).

Because of lack of space we have omitted the following subjects: occur-

rence and pilot plant use of *Botrytis cinerea,* nutrient requirements of yeasts, control of by-product production (particularly of higher alcohol production) by the use of mutant yeasts or selected strains, introduction and use of pressed or dried yeasts as starters, the effect of various insecticides, fungicides, antiseptics, and antibiotics on yeast growth and alcoholic fermentation, use of sugar-alcohol relations to stabilize sweet table wines, biochemistry and process control of submerged culture of film yeasts, and continuous production of sparkling and other types of wines.

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