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THE FERMENTATION ORGANISMS OF
CALIFORNIA GRAPES¹

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INTRODUCTION

The manufacture of wine, grape-juice, and raisins and the shipping of grapes depend very largely upon the control of micro-organisms, particularly of those occurring naturally upon the fruit. This economic fact and the scientific interest of the subject led to a study of the micro-organisms occurring on the grapes of California. The investigation was qualitative and quantitative, covering the effect on type and number of micro-organisms of (*a*) locality, (*b*) degree of ripeness, (*c*) shipment from vineyard to winery. It included studies of (*d*) the micro-organisms normally found on grapes as received at the winery, (*e*) their control in fermentation, and (*f*) their morphological and physiological characteristics.

I. GENERAL DISCUSSION OF GRAPE MICRO-ORGANISMS

Several groups of micro-organisms are normally found on grapes. These may be classified under the general terms: (1) Budding fungi, including molds, true yeasts which form spores, and pseudo-yeasts, which do not form spores; and (2) fission fungi, including bacteria (non-motile rods), bacilli (motile rods), the various forms of *Coccaceae*, and the fission yeasts.

Representatives of all the above groups except the bacilli, *Coccaceae*, and fission yeasts were found in California grapes. Most attention has been given to the fermentation organisms: *i.e.*, yeasts. The molds and bacteria have in most cases been merely listed.

The more important forms of organisms occurring on grapes are the following:

MOLDS

Penicillium.—This group includes a large number of varieties, which are the most widely distributed and common of all the molds. They are characterized by the method of formation and grouping of conidia shown in a typical manner in figure 1.

The most common mold of this group met with on grapes is *Penicillium glaucum* or *Penicillium expansum*. It is the common green mold and is the cause of moldy flavors in grapes, barrels, etc.

During the first stages of growth it appears as a cottony, white mass of mycelial threads. These white threads soon develop fructifications bearing large numbers of conidia that are green in young cultures and brown in old. These give a powdery appearance to the culture. The individual conidiophores are branching and present a broomlike appearance under the microscope.

It does not carry on alcoholic fermentation, but may destroy sugar by oxidation to CO_2 and H_2O or the formation of penicillic acid.

Aspergillus niger.—This is a black mold of very common occurrence on California grapes (see fig. 1). On grapes and must it forms a white mycelium from which spring short rods bearing aggregations of black cells. These conidiophores are not branching, thus differing from penicillium. These groups of cells are easily discernible with the unaided eye. On the grapes of some districts the spores or conidia may be broken away from the main growth of the mold during picking and rise as a black dust. At present it is not held to be very harmful in wine making.

Oidium or Powdery Mildew of the Vine.—This fungus is of more concern to the grape grower than it is to the wine maker and will be found fully described in Bulletin 186² of the University of California Agricultural Experiment Station (see fig. 1). This mold may prevent the grapes reaching maturity or may cause them to crack and thus to be liable to attack by penicillium. In both cases, the grapes affected may become unfit for wine making. The microscopical appearance of the summer form of the powdery mildew is shown in figure 1. It occurs most commonly as a downy white growth on the leaves, canes, and grapes during moist or foggy weather and is most prevalent during early summer. It forms winter spores or perithecia on the canes, in which form the organism lives through the winter.

²Bioletti, F. T. (*Oidium or Powdery Mildew of the Vine*), Univ. Calif. Exp. Sta., Bull. 186, pp. 317-327, 1907.

1. *PENICILLIUM* $\times 800$. 2. *ASPERGILLUS* $\times 800$. 3. *BOTRYTUS* $\times 800$. 4. *MUCOR* $\times 150$. 5. *OIDIUM* $\times 800$. 6. *BROWN MOLD* $\times 800$.

Figure 1

Molds from California grapes:

1. *Penicillium* variety from California grapes, magnified 800 diameters.
2. *Aspergillus* variety from California grapes; $\times 800$.
3. *Botrytis cinerea* from California grapes; $\times 800$.
4. *Mucor* variety from California grapes; $\times 150$.
5. *Oidium* or Powdery Mildew from California vine, summer form; $\times 800$.
6. Brown mold, parasitic fungus from California grapes; $\times 800$. Species and variety not determined.

Botrytis cinerea.—This mold is a parasite or facultative saprophyte occurring on grapes in moist climates (see fig. 1). It rarely develops on California grapes during the wine making season but is often found on grapes left on the vine during the winter. It appears as a gray, matted growth on the surface of the grapes. The individual fructifications may be seen with the naked eye on short upright conidiophores. Under the microscope, the conidia may be seen in grapelike clusters.

The mold did not grow readily in culture media, but will develop profusely on infected grapes in a moist jar.

In Europe, the *Botrytis* is considered beneficial in the Sauternes district, because it causes a concentration of the sugar content of the grapes by favoring evaporation of the moisture. It produces also an oxydase that acts vigorously upon the color of the grapes or wine.

Mucor.—*Mucor* mold will nearly always be found in an examination of California grapes (see fig. 1). Since it develops very slowly in comparison with the commoner molds and yeasts found on grapes it is not probable that it does very much harm in wine making.

In pure cultures, it produces a gray filamentous mass of mycelial threads, from which upright rods are given off, bearing at their uppermost ends spherical sporangia filled with large numbers of spores. These sporangia are easily seen with the unaided eye. Most of the *mucor* molds are capable of forming yeastlike cells and carrying on a feeble alcoholic fermentation in sugary liquids.

Monilia.—The monilia molds and especially *Monilia candida* occur very commonly on fruits of all kinds (see fig. 2). Most of the grapes examined in 1912 bore considerable numbers of the cells of this organism.

In young cultures, it forms colorless yeastlike cells and gives a feeble fermentation. The fermentation is followed by a moldlike growth on the surface of the grape must, etc., which in old cultures becomes olive green in spots.

TRUE YEASTS

Culture Yeasts.—(1) *Saccharomyces ellipsoideus* (True Wine Yeast). In general, the ellipsoideus yeasts are characterized by rapid growth in grape must with the production of a strong fermentation, yielding 10% to 16% of alcohol. They differ from the beer yeasts, *S. cerevisiae*, principally in their higher alcohol-forming power and

in the flavor of the fermented liquids, the wine yeast giving a vinous flavor to fermented liquids and the beer yeast a beer flavor. In grape must, the *S. ellipsoideus* forms a cloudy growth and a pasty or granular sediment in the bottom of the container during the main fermentation. After fermentation is complete the suspended cells settle

Figure 2

Molds and yeasts from California grapes:

1. Dematium variety from California grapes; $\times 800$.
2. Monilia variety from California grapes; $\times 800$.
3. Vinegar bacteria from California grapes; $\times 1000$.
4. Tourne bacteria from California wine; $\times 1000$.

out, giving a clear liquid and compact sediment. Under suitable conditions spores are formed. The usual shape of the cells is shortly ellipsoidal, although this will vary from spherical to elongate, with the different varieties. The shape of the spores is spherical. The appearance of several specimens of *S. ellipsoideus* from California grapes and of cells containing spores will be found in figures 5 and 6.

The main part of all wine fermentations is carried on by this yeast. There are a great many different varieties in this group and these vary considerably in their suitability for wine-making purposes. Modern methods of wine making aim to make use of the most desirable varieties.

2. *Saccharomyces cerevisiae* (Beer Yeast).—It is possible that these yeasts occur on grapes to a more or less limited extent, depending upon the proximity to vineyards of breweries or distilleries using these yeasts. No reference has been found in the literature describing their occurrence on grapes and none have been found on California grapes. There is probably little likelihood of their being present on grapes in sufficient numbers to influence in any way the fermentations. This yeast is worthy of mention in connection with wine making, however, because it has been used in the past to start wine fermentations. Where brewery yeast is so used, it produces a wine of beerlike flavor and low alcohol content and with an excess of unfermented sugar. Such a wine is not palatable and is very liable to be attacked by bacteria and to be lost through bacterial decomposition.

3. *Saccharomyces malei*.—This yeast occurs on apples and is similar to *S. ellipsoideus*, but usually forms less alcohol. It was not found on any samples of grapes examined, but probably occurs occasionally on grapes.

Wild Yeasts.—(1) *Saccharomyces pastorianus*.—This group of yeasts is characterized by its elongate or sausage-shaped appearance and its ability to form spores. Members of this group of yeasts have been found on two samples of California grapes. They form small amounts of alcohol in grape must and at the same time usually produce undesirable flavors and odors—usually a bitter flavor. The microscopical appearance of a culture of *S. pastorianus* yeast from California grapes is shown in figures 3 and 6.

2. *Saccharomyces anomalous* (*Willia Yeasts*).—The yeasts of this group are characterized by the production of hat-shaped spores. The appearance of such spores is shown in figure 7. The members of this group grow rapidly in must and form a wrinkled film. They carry on a weak fermentation with the production of small amounts of alcohol. Liquids fermented by these yeasts are high in aromatic compounds of various sorts and for this reason their use has been suggested as a means of flavoring various fermented beverages. Cells from a culture isolated from California grapes are shown magnified 1000 diameters in figures 3 and 6.

3. *Saccharomyces ludwigii*.—This yeast has been found on grapes grown in Europe. In microscopical appearance it resembles the *S. apiculatus* yeast, but is much larger. It was not found on California grapes.

4. *Saccharomyces marxianus*.—This yeast has been reported as being present on grapes. It is recognized by its kidney-shaped spores. It was not found on the samples of California grapes examined.

PSEUDO-YEASTS

Apiculatus Yeast.—The *apiculatus* yeast (*Saccharomyces apiculatus*, *Hansenia apiculata*) is recognized by the peculiarly pointed appearance of many of its cells (fig. 3). According to most authorities it does not form spores and so is placed in the group of pseudo-yeasts. According to Lindner, however, it forms spores in drop cultures, one spore per cell. It carries on a feeble bottom fermentation in grape must but does not have the power to ferment saccharose, maltose, or lactose. In must it gives from 0% to 6% alcohol and at the same time produces fruity flavors and odors. It settles more slowly and less completely than *S. ellipsoideus*. *Apiculatus* yeast probably does more harm in wine making than all other varieties of wild yeasts combined, because of its large numbers on grapes and its very rapid development after the grapes are crushed. It develops so rapidly that the first stages of most natural wine fermentations are carried on by this yeast and the preliminary fermentation of grapes is often spoken of as the "apiculate" stage. During this preliminary fermentation it produces undesirable flavors and aromas, destroys yeast food that should have gone to the true wine yeast, forms compounds deleterious to the vigorous development of the true wine yeast and gives a great many cells of low specific gravity that settle out slowly after the main fermentation is over. After the *S. ellipsoideus* yeast has formed 8 to 10 per cent alcohol the *Apiculatus* yeast is killed and will not be found in the living state in the finished wine.

Mycoderma Types.—The *Mycoderma* forms are known to all wine makers as "wine flowers" (fig. 4). They are present in considerable numbers on uncrushed grapes and have been found on nearly all samples of California grapes so far examined. In pure cultures in liquids, they appear as white films, usually more or less deeply wrinkled. From time to time portions of the pellicle are detached and fall to the bottom giving in time a large amount of sediment. A

feeble fermentation is carried on at the surface of the liquid, provided that it contains fermentable sugar. Under the microscope the yeast appears as cells of irregular shape and size, the usual form, however, being elongate or sausage-shaped. The cells tend to adhere together in groups and chains. It is thought that the cells are maintained at the surface of the liquid by means of small bubbles of air occluded between them.

In pure cultures in grape must, the *Mycoderma* yeasts are capable of destroying varying amounts of sugar with the production of small amounts of alcohol, in most cases, and obnoxious flavors and aromas. It is probable that in many natural wine fermentations they are more or less active at the beginning of the fermentation. They undoubtedly cause trouble in tanks of wine that are not kept well filled during storage. They are aerobic.

Torula Yeasts.—Under the heading of *Torula* are placed most of the yeasts that do not fit into other groups. Consequently, this name covers yeasts of wide variation in properties. As a group they do not form spores; they form small amounts of alcohol, do not normally develop as a film growth, and the form considered as typical is spherical, although there is considerable variation from the typical form. They are found on grapes fairly commonly, though less abundantly than *S. apiculatus* and *Mycoderma*. Two forms from California grapes are shown in figure 3.

II. PROPERTIES OF MOLDS AND BACTERIA FROM CALIFORNIA GRAPES

SOURCES OF CULTURES

In 1911 micro-organisms were isolated from grapes received at the winery of J. E. Colton, Martinez, California, and from grapes picked at the University Farm, Davis. Samples were obtained in 1912 from Fresno and El Centro. The properties of these micro-organisms were studied more or less in detail. Most attention was paid to the organisms and characteristics that were of importance in wine making.

Method of Separation.—The materials used in the separation of the various types of organisms present on the grapes were sterile grape must agar, sterile grape must, petri dishes, and a small platinum inoculating rod. The grape must agar was made by dissolving 20 grams of agar agar in 1000 c.c. of boiling water to which was added 60 c.c. of grape must after the agar had dissolved. It is necessary

Figure 3

Yeasts from California grapes magnified 1000 diameters:

- 35. *S. apiculatus* from Davis grapes.
- 72. *S. apiculatus* from Acampo grapes.
- 37. *Torula* from Davis grapes.
- 77. *Torula* from Contra Costa County grapes.
- 36. *S. pastorianus* from Davis grapes.
- 69. *Willia* species from Acampo grapes.

to use this high dilution of must in the agar in order that the agar will not be hydrolyzed by the acid of the must. At this dilution the agar contains about .05 per cent acid as tartaric. This should permit the growth of all forms. The hot agar solution was filtered through cotton wool and filled in 10 c.c. portions into test tubes plugged with cotton wool. It was sterilized at 15 pounds pressure in an autoclave. The grape must was clarified by boiling with the white of egg and filtration. The clear must was filled into plugged test tubes and sterilized at 100° C. The Petri dishes were sterilized in packages of three by dry heat. The type of dish used is shown in figure 3.

Samples of the grapes received at the winery were taken from the center of the boxes and crushed into sterile containers. A small amount of the must was in each case transferred by a sterile platinum wire to a tube of melted agar kept at 40° C to 45° C, and further dilutions were made by transfer to other tubes of agar.

The organisms were separated by growth on agar must. They were purified by replating on the same medium. Subcultures were then made in sterile must, on agar slants and permanent stock cultures were made in sterile 10 per cent cane sugar in Freudenreich flasks.

The pure cultures obtained in this way are discussed in groups, the members of each group having certain characteristics in common. No attempt has been made so far to study the molds and bacteria in detail and for this reason they have simply been listed with a few words of explanation.

MOLDS

Penicillium Species (probably *Penicillium expansum*).—Found on nearly all samples examined; produces fructifications bearing numerous round conidia as shown in figure 1; forms sclerotia, resistant vegetative forms, in old cultures; no fermentation in must, but grows vigorously in all culture media tested; giving a characteristic moldy odor.

Penicillium Species of Olive Green Color.—Forms ellipsoidal conidia; very common on California grapes and also found in samples of pasteurized unfermented grape juice that had molded after pasteurization.

Aspergillus.—Two forms: (a) produces small, smooth, black conidia; (b) produces large black conidia with spikelike projections. The appearance of the one yielding the smooth conidia is shown in figure 1.

Botrytis (probably *B. cinerea*).—Found on many samples of grapes

Figure 4

Yeasts from California grapes:

- 65. Mycoderma yeast from Acampo grapes.
- 68. Mycoderma yeast from Acampo grapes.
- 70. Mycoderma yeast from Acampo grapes.
- 71. Mycoderma yeast from Contra Costa Grapes.
- 73. Mycoderma yeast from Acampo grapes.
- 76. Mycoderma yeast from Acampo grapes.

after the early rains of 1912. It gives the characteristic *Botrytis* growth on the grapes but is very reluctant to grow on any of the ordinary culture media, the best artificial medium found so far being grape must gelatin made up by dissolving gelatin in undiluted grape must. The appearance of this mold from grapes grown at the vineyard of J. Swett and Son, Martinez, is shown in figure 1.

Mucor.—Color, gray; spores, shortly ellipsoidal. Does not produce fermentation in grape must. Its microscopical appearance is given in figure 1.

Dematium Variety.—Grows vigorously in must giving a slimy mass of colorless cells and a surface growth of black cells arranged in long chains. Found in large numbers on grapes examined in 1912. Does not cause fermentation in must. Microscopical appearance is given in figure 2.

Monilia Species.—Colonies on agar resemble yeast colonies with rootlike projections into the depths of the medium. Gives a feeble fermentation in grape must, followed by a mycelial growth on the surface of the liquid; in old cultures, part of the mycelium becomes olive green (fig. 2).

Dark Green Mold (unidentified).—Found growing profusely as a parasitic fungus on skins of grapes after the early rains in fall of 1912; grows equally well on artificial media in the laboratory, so may be termed a facultative parasite. It causes no visible fermentation in must and is odorless. Its microscopical appearance is given in figure 1. It may possibly be a *Cladosporium* form.

BACTERIA

Vinegar Bacteria.—The only forms of bacteria so far found on grapes examined at the laboratory or winery have been vinegar bacteria. They were observed on two different lots of grapes, both of which had been shipped long distances and were in a badly broken and moldy condition. Both cultures grew rapidly in grape must giving a heavy tough film. One culture gave bacteria of medium length that tended to form in chains, while the other developed in the form of very short rods usually grouped in pairs (fig. 2).

“Tourne” Bacteria.—The form shown in figure 2 is found in wines quite often, but has not been met with on California grapes. It grows in the absence of air and gives a “mousey” taste to badly affected wines (fig. 2). This organism is otherwise known as *Bacterium mannito-pocum*. It occurs in vinegar and fermented fruit juices.

Figure 5

Yeasts from California grapes:

- 78. *Mycoderma* yeast from Contra Costa County grapes.
- 64. *S. ellipsoideus* from Acampo grapes.
- 66. *S. ellipsoideus* from Davis grapes.
- 67. *S. ellipsoideus* from Contra Costa County grapes.
- 74. *S. ellipsoideus* from Acampo grapes.
- 75. *S. ellipsoideus* from: Contra Costa County grapes.

III. CHARACTERISTICS OF YEASTS FROM CALIFORNIA GRAPES

METHODS OF STUDY

The yeasts isolated from grapes in 1911 according to the methods given on preceding pages were studied in accordance with the following outline. From the tests made, it was possible to determine the specific character of the yeasts and to obtain a fair idea as to their suitability or unsuitability for wine making purposes.

Morphology.—(1) Macroscopical Appearance.—In must, beer wort, nutrient dextrose, saccharose, and lactose solutions; on agar must and on gelatin must.

(2) Microscopical Appearance.—Size, form, and general appearance from five days' growth in grape must. Microscopical appearance of agar colonies.

(3) Spore Formation.—Observed by placing the sediment from vigorous cultures on gypsum blocks partly immersed in water in wide mouthed bottles plugged with cotton wool. This arrangement gives the necessary conditions of aeration, poor food supply, and moisture. Tests were made of spore formation at 22° C and 28° C.

Fermentation Tests.—(1) Rates of Fermentation.—The rates of fermentation in grape musts of 23.01% and 29.75% Balling, in beer wort, nutrient³ dextrose solution, nutrient saccharose solution and nutrient lactose solution were determined on 100 c.c. portions of the above liquids by noting the loss in weight during fermentation after inoculation with pure cultures of the organism under observation. The tests were carried out at 33° C. The loss in weight is due to the formation of carbon dioxide during fermentation. The escape of this gas causes the change in weight by which the fermentation may be followed. The main chemical reaction involved is the following:

A check flask, which was not inoculated, was used as a means of obtaining the loss in weight due to evaporation. The figures given in the tables are corrected for this loss. Points representing the time in hours reckoned from the time of inoculation and also representing the

³ The nutrient solutions used for the dextrose, saccharose, and lactose consisted of .01 grams magnesium sulfate, .5 grams of dipotassium phosphate, 10 grams Witte's pepton dissolved in 1000 c.c. of water. To a portion of this solution was added 15% dextrose, to a second part 15% saccharose, and to the third, 15% lactose.

Figure 6

Yeasts from California grapes showing spore formation:

- 79. *S. ellipsoideus* from Contra Costa County, California.
- 66. *S. ellipsoideus* from Davis grapes showing spores.
- 75. *S. ellipsoideus* from Contra Costa County grapes, showing spores.
- 79. *S. ellipsoideus* from Contra Costa County grapes, showing spores.
- 36. *S. pastorianus* from Davis grapes, showing spores.
- 69. *Willia* from Acampo grapes, showing spores.

loss in weight have been plotted on diagrams and the individual points connected to give "fermentation curves." These curves show at a glance the character of the fermentations and serve as convenient means of comparison.

(1) Products of Fermentation.—The products of fermentation in the solutions given above were determined after the rates of fermentation were ascertained. Similar tests were made in grape must of 30°⁴ Balling fermented by the various yeasts at 24° C.

YEASTS STUDIED

The yeasts have been taken up in the following order: (1) *S. apiculatus* yeasts, (2) *Mycoderma* forms, (3) *Torula* yeasts, (4) *S. pastorianus* and *Willia* yeasts, and (5) *S. ellipsoideus* yeasts. The characteristics of each have been discussed according to the outline given above.

APICULATUS YEASTS FROM CALIFORNIA GRAPES

Two *apiculatus* yeasts were studied. One of these was isolated from grapes grown at Davis and the other from grapes grown at Acampo. The morphology and biological characteristics were studied as previously outlined.

Microscopical appearance is based on cells from five days' growth in grape must of 15% Balling. The agar used for plate cultures consisted of 2 grams of agar agar, 60 c.c. of must, and 1000 c.c. of water. The gelatin medium consisted of 15 grams of gelatin to each 100 c.c. of grape must.

Organism 35. Apiculatus from Grapes Grown at Davis

Morphology.—Microscopical Appearance.—Majority of cells lemon-shape. Cells broader and larger per cent apiculate-shape than in case of yeast no. 72.

Size.—Average, $4.5\mu \times 2.4\mu$. Maximum, $7.5\mu \times 4.5\mu$. Minimum, $3\mu \times 1.5\mu$.

Spore Formation.—No spores at 22° C or 28° C.

Colonies on Agar Agar.—Flat. Elevation uniform. Smaller than *S. ellipsoideus* colonies. Semitranslucent. Edges smooth and entire. Under microscope the edges of the colonies are entire.

⁴ Degrees Balling indicates total dissolved solids in grams per 100 grams, as cane sugar.

Colonies on Gelatin.—Small. Gelatin is slowly liquefied, giving craterlike depressions. Edges entire.

Growth in Liquid Media.—In grape must, beer wort, nutrient dextrose, and saccharose solutions, rapid but fermentation feeble. Growth in lactose slight and no fermentation. Sediment and growth in liquid, fine grained. Cells settle slowly. Liquid becomes fruity in flavor and odor.

Organism 72. Apiculatus from Grapes Grown at Acampo

Morphology.—Microscopical Appearance.—Majority of cells sausage-shaped or spherical. Only a few typical apiculate cells. Decidedly different from no. 35.

Size.—Average, $4.5\mu \times 2.7\mu$. Maximum, $6\mu \times 2.7\mu$. Minimum $2.2\mu \times 1.5\mu$.

Spore Formation.—No spores at 28°C or 22°C .

Colonies on Agar Agar.—Small. Edges undulate or indented. Centers slightly elevated. Opaque and porcelainous. Under low power, edges entire. Different from no. 35.

Colonies on Gelatin.—Small. Edges branchlike. Waxy luster. Edges under microscope lacerate, *i.e.*, saw-tooth appearance. Gelatin slowly liquefied to form craterlike depressions around colonies; whole plate liquefied in few days.

Growth in Liquid Media.—In grape must, beer wort, and nutrient dextrose, growth is rapid. Almost no growth in saccharose and lactose nutrient solutions. Deposit and growth in liquid fine grained. Settles very slowly. Fruity odor and flavor in fermented liquid.

Rates of Fermentation, Various Media.

The following solutions were used:

- (a) Grape must, 23.01° Balling.
- (b) Grape must, 29.75° Balling.
- (c) Sweet beer wort, 14° Balling.
- (d)⁵ Nutrient dextrose, 15% dextrose.
- (e) Nutrient saccharose, 15% saccharose.
- (f) Nutrient lactose, 15% saccharose.

⁵ The nutrient solutions (d), (e), (f), contained .01 grams MgSO_4 , .5 grams K_2HPO_4 , 10 grams Witte's pepton, per 1000 c.c. water, and 150 grams of the respective sugars per 1000 c.c.

One hundred cubic centimeters of the above solutions were sterilized in small plugged flasks. The solutions were inoculated in each instance with one platinum loopful of a vigorous culture. Non-inoculated checks were used to correct for loss due to evaporation. The inoculated flasks were placed in an incubator at 33° C.

The flasks were weighed at intervals during fermentation. The loss in weight corrected for loss due to evaporation was taken as a measure of the rate of fermentation.

TABLE 1
DATA ON RATES OF FERMENTATION OF YEASTS NUMBERS 35 AND 72 (*S. apiculatus*)
IN VARIOUS SUGAR SOLUTIONS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Concentrated must, loss in grams	Wort loss, in grams	Dextrose loss, in grams	Saccharose loss, in grams	Lactose loss, in grams
35	46	1.85	.33	.35	.45	.03	.00
35	73	2.84	1.10	.45	.65	.02	.00
35	96	3.26	2.05	.47	.79	.01	.00
35	118	3.42	2.53	.47	.93	.01	.00
35	167	3.54	2.90	.49	1.13	.00	.00
35	215	3.54	2.95	.57	1.35	.03	.00
35	239	3.57	2.98	.58	1.40	.00	.00
72	27	.18	.01	.35	.10	.00	.00
72	52	1.09	.19	.56	.33	.00	.00
72	77	1.92	.42	.59	.83	.00	.00
72	125	2.75	1.15	.69	.98	.00	.00
72	167	2.84	1.32	.96		.00	.00
72	244	3.18	2.05	.97	1.21	.00	.00
72	341	3.50	2.47	1.10	1.42	.00	.00
72	421	3.55	2.53	1.13	1.44	.00	.00

From an examination of the data given in table 1 and of the fermentation curves in the accompanying diagram, it is evident that both yeasts have very low fermenting power and slow rates of fermentation. In grape must at 33° C, yeast no. 35 produces a more rapid fermentation than yeast no. 72, but in beer wort and dextrose solutions the two yeasts are similar. The concentrated must of 29.75° Balling has a greater retarding effect on yeast no. 72 than on yeast no. 35. Neither yeast fermented saccharose or lactose. Because of their difference of morphology and in the character of their fermentations, it is justifiable to designate them as two different varieties of *apiculatus* yeast.

Attenuation and Alcohol Formation.—The liquids listed in table 2 were analyzed after fermentation. The term "attenuation" in the accompanying table refers to the loss in sugar during fermentation. The alcohol, theoretical and observed, is reported as volume per cent.

Fig. 7.—Fermentation curves of *S. apiculatus* yeasts from California grapes.

TABLE 2

YEASTS NUMBERS 35 AND 72 (*S. apiculatus*). ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c.	Theoretical yield of alcohol, per cent	Observed yield of alcohol, per cent	Per cent of theoretical yield obtained
72	Must of 23° Balling	3.81	2.50	1.00	40.0
72	Must of 29.75 Balling	1.20	.79	.30	37.9
72	Beer wort of 14.56 Balling	1.61	1.11	.30	27.6
72	Dextrose solution, 14.9 Ball.	2.73	1.79	.65	36.3
72	Saccharose sol., 13.73 Ball.	.78	.53	.00	.00
72	Lactose solution, 15.16 Ball.	.31	.20	.00	.00
35	Must of 23° Balling	8.50	5.58	3.05	54.5
35	Must of 29.75 Balling	6.15	4.03	2.35	58.3
35	Beer wort of 14.56 Balling	1.35	.93		
35	Dextrose solution, 14.9 Ball.	3.25	2.13	.65	30.5
35	Saccharose sol., 13.73 Ball.	.00	.00	.00	
35	Lactose solution, 15.16 Ball.	.00	.00	.00	

The results show that in most cases, less than 50% of the alcohol, theoretically obtainable from the sugar fermented, was actually formed by the two yeasts. For example, yeast no. 35 destroyed 3.81% sugar and should have produced 2.5% alcohol in must of 23° Balling, while the actual yield was only 1% or 40% of the amount that was theoretically possible. True wine yeasts of the *S. ellipsoideus* type produce at least 90% of the theoretical yield in grape must.

Since the yields of alcohol are so low and the flavor of the fermented liquids is not desirable, the two yeasts may be considered of little use for industrial purposes. Their importance lies in their power to cause trouble in wine making by growing in natural fermentations before the true wine yeast has developed. In this way they may injure the flavor and clearing quality of the wine and favor "stuck" tanks by the production of compounds that are injurious to the *S. ellipsoideus* yeast. Because of their very common occurrence in large numbers on California grapes, measures should always be taken to discourage their growth.

MYCODERMA FORMS FROM CALIFORNIA GRAPES

Under *Mycoderma* forms, seven film forming organisms have been described in the following pages. Some of these organisms are yeast-like in appearance and in their fermentation properties; other resemble molds more closely than they do yeasts. All have the common properties of forming heavy films on nutrient liquids and of not forming spores.

Organism 65. Mycoderma from Acampo Grapes.

Morphology.—Microscopical Appearance.—Sausage-shape to filamentous. Tends to grow in mycelial threads (fig. 4).

Size.—Average, $9\mu \times 2.5\mu$. Maximum, $40\mu \times 2.5\mu$. Minimum, $2\mu \times 1.5\mu$.

Spore Formation.—No spores at 22° C and 28° C.

Colonies on Agar Agar.—Small and growth slow. Powdery appearance. Color, white. Flat. Growth at surface only. Edges made up of mycelial threads. Under low power, colonies are made up of chains of long cells. These spread over the surface of the agar.

Colonies on Gelatin.—More vigorous than on agar. Concentric ring growth. Powdery white. Flat. Under microscope, same as on agar. Gelatin slowly liquefied.

Growth on Liquid Media.—Grows profusely on surface of grape must, beer wort, dextrose, saccharose and lactose solutions, but does not produce visible fermentation in any of these liquids. Film remains permanently on the surface. Does not sink. Film is very tough in texture.

Organism 68. Mycoderma from Grapes Grown at Acampo, California

Morphology.—Microscopical Appearance.—Predominating form sausage-shaped. Cells vary from spherical to long-rod shaped cells (fig. 4).

Size.—Average, $7.5\mu \times 3.75\mu$. Maximum, $15\mu \times 3.75\mu$. Minimum, $3\mu \times 3\mu$.

Spore Formation.—No spores formed at 22°C or 28°C .

Colonies on Agar Agar.—Large. Edges entire. Waxy luster. Centers of colonies slightly elevated. Edges entire. Opaque. Under low power of microscope, internal structure granular and edges are surrounded by long chains of cells of ellipsoidal shape.

Colonies on Gelatin. Filmy, translucent colonies, that rapidly cover the whole surface. Under microscope the colonies are made up of chains of cells.

Growth in Liquid Media.—Heavy growth in grape must, beer wort, and dextrose with formation of large sediment, the volume of which may be equal to 10 per cent of the total volume of the liquid. Growth slow in lactose and saccharose solutions. Visible fermentation very slow in grape must, beer wort and dextrose solution at first, but finally becomes vigorous in must and dextrose solutions. No visible fermentation in lactose and saccharose.

Organism 70. Mycoderma from Acampo Grapes

Morphology.—Microscopical Appearance.—Usual form, sausage-shape, but vary from spherical to sausage-shaped. Cells are usually grouped in branching chains (fig. 4).

Size.—Average, $7\mu \times 2\mu$. Maximum, $18\mu \times 2\mu$. Minimum, $1.5\mu \times 1.5\mu$.

Spore Formation.—No spores formed at 22°C and 28°C .

Colonies on Agar Agar. Large. Center elevated. Surface corrugated by ridges radiating from centers of colonies. Centers of colonies brown; edges yellowish white. Edges indented. Low power of microscope shows chains of cells growing from edges of colonies; this growth is beneath the surface of the agar (see plate 2).

Colonies on Gelatin.—Large. Centers convex. Colonies flatten near edges and become filmy in appearance. Edges entire under microscope. Gelatin slowly liquefied.

Growth in Liquid Media.—Heavy, wrinkled surface growth which frequently falls to bottom forming voluminous sediment. Fermentation slow. No fermentation in lactose.

Organism 71. Mycoderma from Contra Costa County Grapes

Morphology.—Microscopical Appearance.—Predominating form sausage-shaped. Varies from spherical to sausage-shape (fig. 4).

Size.—Average, $6.9\mu \times 2\mu$. Maximum, $18\mu \times 2\mu$. Minimum, $1.5\mu \times 1.5\mu$.

Spore Formation.—No spores at 22°C or 28°C .

Colonies on Agar Agar.—Large. Flat. Spreading. Edges lobate. Edges entire under microscope (see plate 2).

Colonies on Gelatin.—Flat. Filmy. Translucent. Rapidly cover entire surface of the gelatin. Slow liquefaction (see plate 2).

Growth in Liquid Media.—A wrinkled, loosely coherent film on all liquids tested. Films frequently sink and are quickly replaced by new growth. Fermentation in must, beer wort, and dextrose fairly vigorous, but extends over long period. No perceptible fermentation in saccharose and lactose.

Organism 73. Mycoderma from Grapes Grown at Acampo

Morphology.—Microscopical Appearance.—Usual form sausage-shaped. Varies from spherical to sausage-shaped (fig. 4).

Size.—Average, $6.7\mu \times 2.5\mu$. Maximum, $18\mu \times 3\mu$. Minimum, $3\mu \times 1.5\mu$.

Spore Formation.—No spore formation at 22°C and 28°C .

Colonies on Agar Agar.—Centers elevated. Edges flat and serrated. Shape irregular. Under the microscope chains of cells are seen to radiate from the edges of the colonies. The chains are made up of long cells with pairs of short ellipsoidal cells at the junctures of the long cells.

Colonies on Gelatin.—Flat. Smooth. Filmy. Translucent. Gelatin rapidly covered by a filamentous growth. Gelatin is slowly softened to a syrupy consistency.

Growth in Liquid Media.—Growth vigorous in all liquid media tested. Vigorous fermentation in grape must and dextrose solution.

During fermentation a heavy wrinkled film is formed with a moderate amount of sediment growth. After fermentation most of the film growth settles to the bottom. Fermentation is more vigorous and film formation is not so well developed as in no. 70.

Organism 76. Mycoderma from Grapes Grown at Acampo

Morphology.—Microscopical Appearance.—Sausage-shaped to filamentous (fig. 4).

Size.—Average, $10.5\mu \times 2.1\mu$. Maximum, $75\mu \times 2.5\mu$. Minimum, $3\mu \times 2\mu$.

Spore Formation.—No spores formed at 22°C or 28°C .

Colonies on Agar Agar.—Large. Flat. Surface smooth. Centers slightly elevated. Edges smooth and entire under microscope.

Colonies on Gelatin.—Large. Resinous. Translucent. Surface of colonies deeply corrugated. Mycelial projections from edges visible to naked eye. Surface glistening and slimy. Under microscope edges are fringed by branching chains of very long cells.

Growth in Liquid Media.—Growth in all media tested consists first of powdery white colonies. These coalesce to form a resinous heavy film resembling a growth of vinegar bacteria. The growth sinks after several weeks and is replaced by a second film. No visible fermentation in any liquids tested.

Organism 78. Mycoderma from Contra Costa County Grapes

Morphology.—Microscopical Appearance. — Sausage-shaped cells predominate. Very long filamentous cells not uncommon (fig. 4).

Size.—Average, $10.5\mu \times 3\mu$. Maximum, $37.5\mu \times 3\mu$. Minimum, $4.5\mu \times 2\mu$.

Spore Formation.—No spores at 22°C and 28°C .

Colonies on Agar Agar.—Large and flat. Centers slightly elevated. Waxy luster. Edges show hazy growth of mycelium. Under microscope colonies are seen to be surrounded by radiating mycelial threads.

Colonies on Gelatin.—Large. Flat. Smooth. Translucent. Rapidly cover the entire surface of the gelatin. Gelatin rapidly softened to a syrupy consistency (see plate 2).

Growth in Liquid Media.—In grape must, beer wort, and dextrose, solutions, growth is rapid and fermentation vigorous; in saccharose growth vigorous and fermentation slow; little growth in lactose. Consists chiefly of surface growth. Small sediment.

Fermentation Tests of Mycoderma Forms from California Grapes.

The data obtained by noting the loss in weight at 33° C of flasks containing 100 c.c. of grape must 23.01° Balling, grape must 29.8° Balling, beer wort 14° Balling, dextrose 15° Balling, saccharose 15° Balling, and lactose 15° Balling solutions, respectively, after inoculation with pure cultures of the above yeasts appear in table 3. The attenuation, representing the sugar destroyed, together with the yields of alcohol in the various sugar solutions tested are given in table 3. After each table the results of the tests are discussed. The nutrient solutions used were the same as those described for yeasts 35 and 72. See description of apiculatus yeasts.

TABLE 3
FERMENTATION TESTS OF MYCODERMA FORMS FROM CALIFORNIA GRAPES

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Must of 29° Balling, loss, in grams	Wort loss, in grams 14 per cent solution	Dextrose loss, in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
68	18	.00	.00	.04	.07	.00	.00
68	42	.70	.01	.21	.31	.10	.00
68	66	1.51	.24	.38	.93	.10	.00
68	92	2.45	.62	.37	2.10	.10	.00
68	123	3.35	1.32	.38	2.90	.10	.00
68	165	5.80	2.00	.40	5.89	.10	.03
68	236	8.45	3.30	.42	6.40	.10	.03
68	284	8.48	3.80	.50	6.40	.10	.03
68	314	8.48	3.80	.51		.10	.03
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65	18	.00	.00	.00	.00	.00	.00
65	42	.03	.00	.00	.00	.01	.00
65	66	.03	.00	.03	.00	.01	.00
65	92	.03	.00	.08	.04	.01	.00
65	123	.03	.04	.08	.09	.07	.03
65	165	.03	.04	.12	.09	.07	.03
65	236	.03	.04	.17	.09	.07	.03
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70	18	.02	.03	.00	.10	.00	.00
70	42	.10	.05	.06	.18	.20	.00
70	66	.38	.08	.33	.41	.20	.06
70	92	.80		.38	.42	.25	.05
70	123	1.90	.08	.80	1.30	.60	.20
70	165	2.47	.83	1.00	1.65	.90	.20
70	236	2.57	.95	1.00	1.65	.90	.30
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71	46	.85	.35	.25		.00	.00
71	73	1.85	1.17	.34	.46	.00	
71	96	2.70	2.19	.41		.00	
71	118	3.74	3.19	.42	2.31	.01	
71	167	5.47	3.89	.42	4.20	.00	.00
71	215	6.40	5.90	.44		.04	
71	239	6.85	6.40	.45		.04	

TABLE 3—(Continued)

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Must of 29° Balling, loss, in grams	Wort loss, in grams 14 per cent solution	Dextrose loss, in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
73	27	.20	.06	.18	.66	.10	.00
73	52	1.09	.61	.34	1.79	.28	.00
73	77	2.26	1.56	.49	3.21	.58	.00
73	125	4.14	3.34	.59	4.11	.93	.00
73	167	5.69	4.59	.61	5.72	1.19	.00
73	244	9.25	6.62	.72	7.07	1.41	.00
73	341	9.32	8.24	.87	7.35	1.52	.00
73	421	9.52	9.65	.92			.00
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76	27	.00	.04	.00	.00	.00	.00
76	52	.04	.08	.04	.07	.00	
76	77					.00	
76	125					.00	
76	167	.31	.13	.24	.20	.00	
76	244	.42	.39	.46	.41	.00	
76	341	.62	.69	.62	.50	.00	
76	421	.64	.69	.70	.54	.00	
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78	27	.23	.16	.23	.38	.23	.00
78	52	1.12	.44	.54	.80	.54	.00
78	77	2.37	1.64	1.75	2.05	1.37	.00
78	125	4.34	3.29	3.24	3.38	1.74	.00
78	167	6.05	4.60	4.52		1.95	.00
78	244	7.55	6.20	5.25	6.16	2.26	.00
78	341	9.50	7.50		6.98	2.50	.00
78	421	9.85	8.05		7.29	2.70	.00

The seven different varieties of mycoderma yeasts, whose rates of fermentation in normal must, concentrated must, beer wort, dextrose, saccharose and lactose solutions were studied gave great variation in the rate of fermentation and the amounts of sugar fermented. Organism 65 did not cause fermentation in any of the liquids tested, though it gave a vigorous growth. On the other hand, yeasts 68, 73, and 78, developed fairly strong fermentations in must and beer wort, destroying nearly as much sugar as the true wine yeasts. Organism 76, caused a very feeble fermentation in grape must but did not ferment any of the other liquids tested. The mycoderma forms did not ferment so rapidly in dextrose solution as in grape must. Contrasted with the mycoderma forms the true wine yeasts did not exhibit this difference. Lactose was not fermented by any of the mycoderma forms. Saccharose was attacked to a slight degree by forms 70, 73, and 78, the amount of sugar actually fermented in each case being small. In general, the fermentation curves of the forms shown in the diagrams

differ from those of the true wine yeasts shown in figures 12 to 15, by their low and gentle slope throughout. In most cases they show a slower start of fermentation than do the *S. ellipsoideus* yeasts. Be-

Fig. 8.—Fermentation curves of *Mycoderma* forms in must of 23° and 29.8 Balling and beer wort.

cause of their slow and incomplete fermentations the mycoderma forms discussed above are not desirable types to have in wine fermentations.

Attenuation and Alcohol Formation.—Analyses were made of the liquids fermented by the mycoderma forms (table 4).

TABLE 4

YEASTS NUMBERS 65, 68, 70, 71, 73, 76, AND 78. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
64	Must of 23° Balling			0.00	
65	Must of 29°8 Balling			0.00	
65	Beer wort of 14° Balling			0.00	
65	Dextrose solution, 15%			0.00	
65	Saccharose solution, 15%			0.00	
65	Lactose solution, 15%			0.00	
68	Must of 23° Balling	19.88	13.08	7.45	56.9
68	Must of 32° Balling	7.84	5.15	4.50	89.3
68	Beer wort of 14° Balling	.86	.62	.60	96.8
68	Dextrose solution, 15%	12.68	8.30	6.40	77.1
68	Saccharose solution, 15%	.03	.02	0.00	00.0
68	Lactose solution, 15%	.27	.19	0.00	00.0
70	Must of 23° Balling	6.66	4.38	2.55	58.2
70	Must of 29°8 Balling	2.05	1.35	1.00	74.5
70	Beer wort of 14° Balling	1.43	.96	.80	83.3
70	Dextrose solution, 15%	4.51	2.96	1.70	57.4
70	Saccharose solution, 15%				
70	Lactose solution, 15%	0.00	0.00	0.00	00.0
71	Must of 23° Balling	17.61	11.58	8.80	75.9
71	Must of 29°8 Balling			7.90	
71	Beer wort of 14° Balling	2.91	2.03	0.00	00.0
71	Dextrose solution, 15%	12.20	8.02	4.10	51.1
71	Saccharose solution, 15%	.08	.05	0.00	00.0
71	Lactose solution, 15%	.16	.11	0.00	00.0
73	Must of 23° Balling	12.01	7.90	7.50	94.9
73	Must of 29°8 Balling	14.75	9.70	6.80	70.1
73	Beer wort of 14° Balling	1.61	1.16	.52	44.8
73	Dextrose solution, 15%	12.60	8.29	5.60	67.5
73	Saccharose solution, 15%	2.08	1.50	1.50	100
73	Lactose solution, 15%	.35	.25	0.00	00.0
76	Must of 23° Balling			0.00	
76	Must of 29°8 Balling			0.00	
76	Beer wort of 14° Balling			0.00	
76	Dextrose solution, 15%			0.00	
76	Saccharose solution, 15%			0.00	
76	Lactose solution, 15%			0.00	
78	Must of 23° Balling	18.87	12.41	10.30	83.1
78	Must of 29°8 Balling	14.13	9.29	9.00	97.9
78	Beer wort of 14° Balling	12.15	7.90	7.20	91.1
78	Dextrose solution, 15%	13.22	8.69	6.20	71.3
78	Saccharose solution, 15%	3.93	2.86	1.40	48.9
78	Lactose solution, 15%	.36	.26	0.00	00.0

An examination of the data given in table 4 shows that in grape must, which was the most favorable medium for the activity of the yeasts under observation, amounts of alcohol varying from .00% to 10.3% were formed. Some of the organisms, namely, 73 and 78, gave good yields of alcohol for the sugar fermented in grape must, while others e.g., 68, 70, 71, gave low yields. Yeasts 65 and 76 gave no alcohol in any of the liquids in which they were grown, although they destroyed small amounts of sugar in some of the solutions. None of the organisms of this group fermented beer wort to any notable degree and the fermentations that did take place in this liquid probably represented the destruction of dextrose present, only, while it is probable that the maltose was not attacked. Saccharose likewise proved very resistant to fermentation by the above forms. Organisms 68, 71, 73, and 78 formed considerable amounts of alcohol in dextrose solution, indicating that this sugar is more easily attacked than cane sugar or maltose. These facts indicate that the yeasts under discussion are deficient in invertase and maltase, enzymes that are necessary to the fermentation of saccharose and maltose.

Since the mycoderma forms discussed above do not ferment beer wort or cane sugar, they would be useless in the manufacture of beer or of alcohol from materials containing cane sugar. The amounts of alcohol formed in grape must by several of the organisms might be sufficient for some purposes, but their use for the production of wine is excluded by the fact that all of them produce disagreeable tastes and odors in grape must, resulting in the formation of an undrinkable fermented liquid in all cases. Therefore, it is to the winemaker's interest to prevent the growth of these organisms.

TORULA FORMS FROM CALIFORNIA GRAPES

Two yeasts of the torula type were found on grapes examined in 1911, one from Davis grapes and the other from grapes grown in Contra Costa County, California.

Organism 37. Torula Yeast from Grapes Grown at Davis

Morphology.—Microscopical Appearance.—Varies from spherical to shortly ellipsoidal in form; latter form predominating. Cells tend to form chains of cells of various sizes (see fig. 3).

Size.—Average, $4.6\mu \times 3.7\mu$. Maximum, $15\mu \times 6\mu$. Minimum, $3\mu \times 3\mu$.

Spore Formation.—No spores formed at 22° C or 28° C.

Colonies on Agar Agar.—Almost flat, but centers, slightly elevated. White. Waxy luster. Edges entire. Colonies in the agar, spherical. Under microscope, edges of colonies entire.

Colonies on Gelatin.—Small. White. Waxy luster. Gelatin quickly liquefied.

Growth in Liquid Media.—Growth in grape must, dextrose, and beer wort, rapid, with formation of considerable sediment, and development of a feeble bottom fermentation. No film formation. No fermentation in lactose solutions, but good growth. Feeble fermentation and good growth in saccharose. Rancid taste and odor developed in fermented liquids.

Fig. 9.—Fermentation curves of *Mycoderma* forms in dextrose and saccharose solutions.

Organism 77. Torula from Contra Costa County Grapes

Morphology.—Microscopical Appearance.—Vary from ellipsoidal to sausage-shaped with ellipsoidal form predominating (see fig. 3). Tends to form short chains.

Size.—Average, $4\mu \times 2.7\mu$. Maximum, $8\mu \times 4.5\mu$. Minimum, $2.2\mu \times 1.5\mu$.

Colonies on Agar Agar.—Small. White. Convex. Waxy luster. Spherical colonies in the agar. Under the microscope colonies are found to be surrounded by straight chains of long cells. Most other yeasts examined that formed side chains in this manner produced branching cells.

Colonies on Gelatin.—Contoured surface with highest elevation at centers of colonies. Centers of colonies colorless. Chalklike at edges.

Undermicroscope, colonies are fringed with branching chains of long cells.

Growth in Liquid Media.—Growth in all liquids slow. Feeble fermentation in grape must, beer wort, and dextrose. No surface growth. No fermentation in lactose and saccharose.

Rates of Fermentation of Torulas 37 and 77 in Various Media.

Rates of fermentation in grape must of 23° Balling, and 29.8° Balling, beer wort of 14° Balling, and 15% solutions of dextrose, saccharose, and lactose were tested as previously described for apiculatus and mycoderma forms. The results are given in table 5 and figure 10.

TABLE 5
FERMENTATION TESTS AT 33° C OF TORULA FORMS FROM CALIFORNIA YFASTS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Must of 29.8° Balling, loss, in grams	Wort loss, in grams 14 per cent solution	Dextrose loss in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
37	18	.01	.00	.04	.12	.07	.02
37	42	.19	.07	.31	.54	.29	.07
37	66	.51		.45	.98	.38	
37	92	.63		.50	1.40	.43	.08
37	165	.87		.51	2.74	.54	.08
37	236	.87	.13	.51	3.64	.54	.09
77	46	.17	.01	.23	.23	.01	.03
77	73	.84	.15	.48	.48		
77	96	1.14	.42	.58	.58	.10	
77	118	1.30	.81	.68	.68		
77	167	1.37	.86	.78	.78	.10	
77	215	1.44	1.03	.83	.83		
77	239	1.48	1.08	.95	.95		.03

Torulas 37 and 77 were able to produce only very feeble fermentations in the liquids tested. Torula yeast 77 was not able to ferment must of 30° Balling, or saccharose solution. In must of 23° Balling, and in beer wort the fermentations of the two yeasts were about equal. In must, beer wort, and saccharose the fermentations were completed in a short time, about 120 hours. In comparison with the fermentation curves of the *S. ellipsoideus* yeasts (figs. 12 to 15), the yeasts above gave very weak and incomplete fermentations. The flavors of the fermented liquids were disagreeable. Therefore, the yeasts seem to have no practical value but may cause defective flavors in wine where their growth is not prevented.

Attenuation and Yields of Alcohol.—The fermented liquids were analyzed for alcohol with the results reported in table 6.

Fig. 10.—Fermentation curves of yeasts 37 and 77 in grape must, beer wort, dextrose and saccharose solutions.

TABLE 6
YEASTS 37 AND 77. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT,
DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c.	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
37	Must of 23° Balling	5.21	3.42	.60	17.6
37	Must of 29°8 Balling	.05	.03	.00	.0
37	Beer wort of 14° Balling			.20	
37	Dextrose solution, 15%	7.96	5.22	3.50	67.3
37	Saccharose solution, 15%	.64	.43	.40	93.02
37	Lactose solution, 15%	.06	.04	.00	.0
77	Must of 23° Balling	4.58	2.99	1.31	43.7
77	Must of 29°8 Balling	3.85	2.53	.65	25.7
77	Beer wort of 14° Balling	2.91	2.00	.30	15.0
77	Dextrose solution, 15%	4.55	2.99	.65	21.7
77	Saccharose solution, 15%			.00	.0
77	Lactose solution, 15%	.06	.04	.00	.0

Analyses of the fermented liquids from fermentation of *Torulas* 37 and 77 in grape must, etc., showed that the absolute amounts of alcohol formed were small and that the yields for each per cent of sugar destroyed were in all cases, except for yeast 37 in dextrose, very low. In the majority of the fermentations, the yields were less than 50 per cent of the theoretical. These yeasts have the defects of giving slow incomplete fermentations, low yields of alcohol for sugar fermented and of producing liquids of disagreeable odor and flavor.

PASTORIANUS AND WILLIA YEAST FROM CALIFORNIA GRAPES

One *S. pastorianus* and one *Willia* yeast were obtained from grapes in 1911. Both are true yeasts and form spores.

Organism 36. S. pastorianus Yeast from Grapes Grown at Davis

Morphology.—Microscopical Appearance.—Form varies from spherical to sausage-shaped. The ellipsoidal form predominating. Much smaller than true ellipsoideus yeast (fig. 3).

Size.—Average, $4.5\mu \times 3\mu$. Maximum, $7.5\mu \times 3\mu$. Minimum, $1.5\mu \times 1.5\mu$.

Spore.—Produced in great abundance in 24 hours on gypsum spore blocks. Spores also form in grape must cultures. Spores spherical in form. Number per cell, 2 to 4. Size of spores, $2\mu \times 2\mu$ (fig. 7).

Colonies on Agar Agar.—Round. Slightly convex. White. Waxy luster. Colonies in agar spherical. Under the low power, the colonies

are surrounded by chains of cells made up of long central cells with pairs of short ellipsoidal cells at the junctures of the larger cells.

Colonies on Gelatin.—Flat. Concentric rings of growth. Outer edges of colonies sharply indented. Gelatin slowly softened but not completely liquefied.

Growth in Liquid Media.—Heavy wrinkled surface growth which sinks to bottom frequently. No visible fermentation. Taste of liquids bitter and disagreeable.

Organism 69. Willia Species (Saccharomyces anomalus) from Grapes Grown at Acampo, California

Morphology.—Microscopical Appearance.—Size and shape very irregular. The larger cells usually sausage-shaped or ellipsoidal and the smaller ones spherical. Larger than typical *S. anomalus* in the University collection (fig. 3).

Size.—Average, $6\mu \times 3\mu$. Maximum, $14\mu \times 2.5\mu$. Minimum, $3\mu \times 3\mu$.

Spore Formation.—Spores are formed abundantly on gypsum blocks at 28°C and 22°C , in three days. Spores are of typical *Willia* form, i.e., hat-shaped (fig. 7). Number of spores per cell varies from 2 to 8. Size of spores, $4\mu \times 1.5\mu$.

Colonies on Agar Agar.—Medium size. Flat. White. Waxy luster. Edges entire. Colonies on the agar are spherical. Under the microscope the edges of the colonies are entire.

Colonies on Gelatin.—Large. Flat. White. Chalklike. Colonies spread rapidly and soon cover entire surface and the gelatin is rapidly liquefied.

Growth in Liquid Media.—A chalky white film develops on all liquids tested, with flocculent growth at bottom of flask. Prolonged top fermentation takes place in all liquids except lactose. Strong odor of ethyl acetate is developed. The fermented liquids possess a burning taste.

Rates of Fermentation of Yeasts 36 and 69.—These yeasts were tested for their rates of fermentation in various media as previously described for other organisms. The results are given in table 7 and figure 11.

From the data given in table 7, yeast 36 is seen to give scarcely any fermentation in any of the liquids tested, the maximum loss in weight per 100 c.c. being .68 grams in must of 29.8° Balling. On the other hand, yeast 69 gave fairly strong fermentations in must, beer wort, dextrose, and saccharose solutions. Neither yeast fermented lactose,

TABLE 7

DATA OF RATES OF FERMENTATION OF YEASTS NUMBERS 36 AND 69 IN VARIOUS SUGAR SOLUTIONS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Concentrated must, loss in grams 29°8 Ball.	Wort loss, in grams 14 per cent Balling	Dextrose loss, in grams 15 per cent solution	Saccharose loss, in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
36	46	.03	.03	.03	.03	.00	.00
36	73	.04	.04	.03	.06	.00	.00
36	96	.09	.11	.03	.08	.00	.01
36	118	.24	.16	.13	.13	.00	.01
36	167	.33	.43	.13	.19	.00	.02
36	215	.45	.63	.13	.26	.00	.02
36	239	.46	.68		.28	.00	.02
69	18	.01	.10	.13	.01	.10	.03
69	42	.58	.18	.46	.13	.28	.14
69	66	1.10	.23	.56	.77	.86	.14
69	92	1.79	.80	1.71	1.77	1.90	
69	123	2.49	2.20	1.75	2.87	2.88	
69	165	3.22	2.50	1.81	3.99	3.78	
69	236	4.02	4.88	1.92	5.31	4.62	.14
69	283	4.49	5.55	1.93	5.77	4.98	
69	314	4.62	5.75		5.79	5.03	

while yeast 36 did not attack saccharose. Although yeast 69 gives more vigorous fermentations than does yeast 36, still it does not approach the *S. ellipsoideus* yeasts from California grapes in rate of fermentation or fermentative power.

Attenuation and Alcohol Yields of Yeasts 36 and 69.—The fermented liquids were analyzed with the results given in the accompanying table.

TABLE 8

YEASTS NUMBERS 36 AND 69. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
36	Must of 23° Balling	2.49	1.95	.30	15.3
36	Must of 29°8 Balling	1.65	1.08	.00	.0
36	Beer wort of 14° Balling	.31	.20	.00	.0
36	Dextrose solution, 15%	1.95	1.28	.24	18.9
36	Saccharose solution, 15%	.02	.67	.00	
36	Lactose solution, 15%	.11	.07	.00	
69	Must of 23° Balling	11.12	7.30	1.25	17.1
69	Must of 29°8 Balling	2.92	1.90	1.00	52.6
69	Beer wort of 14° Balling	1.40	.92	.50	54.3
69	Dextrose solution, 15%	11.03	7.25	3.10	42.6
69	Saccharose solution, 15%	7.73	5.09	2.60	51.2
69	Lactose solution, 15%	1.25	.80	.00	.0

Fig. 11.—Fermentation curves for yeasts 36 and 69 in grape must, beer wort, dextrose, and saccharose solution at 33°C.

Yeast 69 destroyed larger amounts of sugar than did yeast 36 and formed greater amounts of alcohol, but both yeasts gave very low yields of alcohol compared to the amount of sugar fermented. In most cases the yield was below 50% of the theoretical, based on the sugar destroyed. Yeast 69 formed 3.1% alcohol in dextrose solution, this being the largest amount of alcohol produced by either yeast.

Because of its disagreeable flavor and low alcohol formation, yeast 36 is worthless for fermentation purposes. Yeast 69 produces a very aromatic odor and flavor in liquids in which it grows. These fermented materials are not pleasing to drink by themselves but when added in small amounts to new wine they have the property of "aging" the new wine so treated. Therefore it may be possible to use this yeast as an aid to aging wine either by adding some of the liquid fermented by it to new wine or by growing it directly in new wine, and in this latter case, allowing the desired flavors to be developed in the wine itself. Yeasts of this same group are used to some extent in the aging of "sake" (Japanese beer).

TRUE WINE YEASTS, *SACCHAROMYCES ELLIPSOIDEUS* FROM CALIFORNIA GRAPES

Six kinds of true wine yeast, *S. ellipsoideus*, were isolated from grapes in 1911 and have been discussed as a group in the following pages.

Organism 64. Saccharomyces ellipsoideus from Grapes *Grown at Acampo*

Morphology.—Microscopical Appearance.—Form varies from shortly ellipsoidal to spherical. Cells shorter than those of no. 74 (fig. 5).

Size.—Average, $7.5\mu \times 6\mu$. Maximum, $12\mu \times 3\mu$. Minimum, $3\mu \times 3\mu$.

Spore Formation.—Abundant spore formation at 22° C and 28° C. Usually 3 spores per cell. Shape spherical. Size, $3\mu \times 3\mu$.

Colonies on Agar Agar.—Medium size. Round. Moderately convex. White. Waxy luster. Edges entire. Under microscope edges are entire.

Colonies on Gelatin.—Large. Centers elevated. Radiating ridges from center. Centers slightly elevated. Edges of colonies are undulate in appearance. Gelatin slowly softened but not liquefied. Edges of colonies entire under microscope.

Growth in Liquid Media.—Rapid in grape must, beer wort, dextrose, and saccharose solutions with vigorous fermentation and formation of fine grained sediment. No fermentation in lactose, but fair growth. Characteristic vinous odor and flavor developed.

*Organism 66. Saccharomyces ellipsoideus from Grapes
Grown at Davis*

Morphology.—Microscopical Appearance. — Predominating form oblong with tendency to be pointed at one end. Resembles *S. cerevisiae* in this regard (fig. 5).

Size.—Average, $9\mu \times 5.7\mu$. Maximum, $13.5\mu \times 4\mu$. Minimum, $4\mu \times 3\mu$. Considerably larger than most varieties of *S. ellipsoideus* from California grapes.

Spore Formation.—Spores are formed abundantly at 22°C and 28°C but more rapidly at the lower temperature. Number per cell 2 to 3. Size, $3\mu \times 3\mu$.

Colonies on Agar Agar.—Size, medium. Convex. Circular. White. Waxy luster. Edges entire macroscopically and microscopically. Colonies in the agar are usually lens-shaped.

Colonies on Gelatin.—Colonies more flat than on agar. Smooth. Under microscope edges of colonies appear bordered with branching chains of ellipsoidal cells. Gelatin slowly softened but not liquefied.

Growth in Liquid Media.—Rapid with vigorous fermentation in all solutions tested except lactose. Grows in lactose. Sediment fine grained. Settles quickly after fermentation. Typical vinous flavor developed in fermented liquids.

*Organism 67. Saccharomyces ellipsoideus from Grapes Grown
in Contra Costa County*

Morphology.—Microscopical Appearance.—Ellipsoidal to spherical, former type predominating. Resembles yeast 74 in general appearance (fig. 5).

Size.—Average, $6.75\mu \times 5.25\mu$. Maximum, $9\mu \times 6.75\mu$. Minimum, $3\mu \times 3\mu$.

Spore Formation.—Large numbers of spores are formed at 22°C and 28°C in 24 hours on spore blocks. Number per cell, 2 or 3. Size, $3\mu \times 3\mu$.

Colonies on Agar Agar.—Small. Convex. White. Waxy luster. Colonies in agar are spherical. Edges of colonies entire, both macroscopically and microscopically.

Colonies on Gelatin.—Large. Centers of colonies slightly depressed. Edges are undulate. Under microscope, colonies are fringed with short straight chains of ellipsoidal cells. The gelatin is not liquefied or softened.

Growth in Liquid Media.—Growth rapid and fermentation vigorous in grape must, beer wort, dextrose, and saccharose. Growth slight and fermentation absent in lactose. Fermentation more rapid at 20° C than at 33° C. Sediment fine grained. Typical vinous flavor and

Organism 74. Saccharomyces ellipsoideus from Grapes Grown at Acampo

Morphology.—Microscopical Appearance.—Usual form oblong rather than shortly ellipsoidal. Decidedly different in form than yeast 64 from the same grapes (fig. 5).

Size.—Average, $6.6\mu \times 4.6\mu$. Maximum, $9\mu \times 4.5\mu$. Minimum, $3\mu \times 3\mu$. Smaller than yeasts 66 and 79, which it resembles in shape.

Spore Formation.—Few spores only at 28° C in 48 hours and none in 24 hours. More formed at 22° C in 48 hours. Number per cell, 2. Size, $3\mu \times 3\mu$ (fig. 6).

Colonies on Agar Agar.—Large. Round. Centers elevated. Colonies in agar lens-shaped. Edges of colonies entire both macroscopically and microscopically.

Colonies on Gelatin.—Large. Low elevation. Colonies in the gelatin spherical. Edges of colonies entire both macroscopically and microscopically. No liquefaction.

Growth in Liquid Media.—Rapid with vigorous fermentation in must, beer wort, dextrose, and saccharose solutions. Small growth and no fermentation in lactose. Typical vinous taste and odor.

Organism 75. Saccharomyces ellipsoideus from Grapes Grown in Contra Costa County

Morphology.—Microscopical Appearance.—Spherical to shortly ellipsoidal (fig. 5).

Size.—Average, $6.3\mu \times 5.5\mu$. Maximum, $8\mu \times 8\mu$. Minimum, $3\mu \times 3\mu$.

Spore Formation.—No spores formed at 28° C at 72 hours, but abundantly after 144 hours. This differs from yeast 67 from the same grapes; 67 forms spores in 24 hours at 28° C. Numbers of spores per cell, 2. Size, $3\mu \times 3\mu$ (fig. 6).

Colonies on Agar Agar.—Medium size. Round. Convex. White. Waxy luster. Colonies in the agar are lens-shaped. Edges of colonies entire, both macroscopically and microscopically.

Colonies on Gelatin.—Large. Low and of even elevation. Surface radiately ridged. Edges undulate. Under microscope colonies are bordered by short straight chains of ellipsoidal cells.

Growth in Liquid Media.—Rapid and fermentation vigorous in grape must, beer wort, dextrose, and saccharose. Slight and no fermentation in lactose. Typical vinous flavor and odor in fermented liquids.

Organism 79. Saccharomyces ellipsoideus from Grapes Grown in Contra Costa County

Morphology.—Microscopical Appearance.—Cells large and oblong. Not strictly ellipsoidal in form (fig. 6).

Size.—Average, $8.25\mu \times 5\mu$. Maximum, $12\mu \times 6\mu$. Minimum, $4.5\mu \times 3\mu$.

Spore Formation.—Spores are formed abundantly in 24 hours at 28°C . Number per cell, 2 to 4. Size, $2\mu \times 2\mu$ and $3\mu \times 3\mu$ (fig. 6).

Colonies on Agar Agar.—Medium size. Spherical. Convex. White. Waxy luster. Colonies in agar lens-shaped. Edges entire both macroscopically and microscopically.

Colonies on Gelatin. Flat. Edges hazy. White. Waxy luster. Under microscope edges are bordered by branching chains of ellipsoidal cells.

Growth in Liquid Media.—In grape must, beer wort, dextrose, and saccharose, rapid and fermentation vigorous. In lactose, slow and no fermentation. Typical vinous taste and odor developed.

Fermentation Records at 33°C .—Fermentations were carried out in 100 c.c. portions of various liquids at 33°C and records of the rates of fermentation were kept as previously explained.

All of the yeasts with the exception of yeast 67 show rapid fermentations in must of 23°Balling , and 30°Balling , beer wort, dextrose and saccharose solutions, there being little to choose between them in the rapidity of fermentation and the amount of sugar destroyed. The yeasts show considerable variation in the fermentation of beer wort. Apparently some of the yeasts ferment only the dextrose of the beer wort and not the maltose. Yeast 67, however, is very strongly inhibited in its activity by the moderate temperature of 33°C , because it causes only very feeble and incomplete fermentations at this tem-

TABLE 9

DATA ON RATES OF FERMENTATION OF YEASTS NUMBERS 64, 66, 67, 74, 75, AND 79
AT 33° C IN VARIOUS SUGAR SOLUTIONS

Yeast number	Time in hours	Must of 23° Balling, loss in grams	Must of of 29° 8 Balling loss in grams	Wort loss, in grams 14° Ball.	Dextrose loss, in grams 15 per cent solution	Saccharose loss in grams 15 per cent solution	Lactose loss, in grams 15 per cent solution
64	18	1.42	.00	.87	.49	.68	.03
64	42	4.18	.66	2.57	1.95	2.43	
64	66	6.72	4.33	2.75	2.32	3.96	
64	92	8.09	7.90	2.94	4.74	5.35	
64	123	8.26	8.70	2.94	5.84	6.30	
64	165	8.33	9.70	3.04	6.49	6.88	.07
64	236	8.88	10.00	3.16	6.77	7.30	.07
66	27	1.83	.05	.06	.44	.60	.00
66	52	5.92	.69	.55	2.13	1.89	.00
66	77	8.49	4.11	2.49	5.23	2.91	.00
66	125	10.09	9.31	6.64	6.81	4.59	.00
66	167	10.44	11.71	8.69		5.56	.00
66	244	10.98	11.85		7.54	7.38	.00
66	341	11.68	12.59			8.35	.00
66	421		12.82			8.55	.00
67	27	.14	.04	.33	.66	.31	.00
67	52	.89	.14	.53	1.80	.42	.00
67	77	1.99	.92	.64	3.21	.64	.00
67	125	2.79	1.57	.67	4.11	.83	.00
67	244	2.99	1.84	.72	5.72	.84	.00
67	341	3.33	2.40	.75	7.07	1.13	.00
67	421	3.50	2.85	.75	7.35	1.30	.00
67		4.59	2.98			1.39	.00
74	27	2.69	.16	1.76	.83	.93	.00
74	52	5.71	2.89	2.54	3.52	2.74	.00
74	77	7.81	6.15	3.99	5.52	4.42	.00
74	125	9.14	9.77	4.27		6.59	.00
74	167	9.31	11.87	4.32	7.23	7.09	.00
74	244	10.87	12.38	4.65		7.62	.00
74	341	11.50	12.53	5.00		7.87	.00
74	421		12.65	5.15		7.97	.00
75	27	2.08	.08	.66	.38	.61	.00
75	52	4.72	.67	1.29	.80	1.57	.00
75	77	6.74	4.05	1.89	2.05	2.47	.00
75	125	8.06	6.89	2.64	3.38	4.04	.00
75	167	8.74	7.94	3.12	6.17	5.17	.00
75	244	9.85	8.88	3.40	6.98	6.78	.00
75	341	10.97	10.66	3.55	7.29	7.60	.00
75	421	11.35	10.66	3.65		7.90	
79	46	4.96	3.21	1.81	1.79	1.80	.00
79	73	7.77	6.97	2.42	3.37	3.66	.00
79	96	8.40	8.84	2.63	4.52	4.77	.00
79	118	8.74	10.14	2.83	5.66	5.71	.00
79	167	8.88	10.83	2.83	6.38	6.40	.00
79	215	9.07	11.27	3.07	6.66	6.55	.00
79	239	9.10	11.40	3.07	6.68	6.60	.00

perature. The presence of such yeast as this in natural wine fermentations would render such fermentations very liable to "sticking" if the temperature arose much above 33°C . It is possible that the

Fig. 12.—Fermentation curves of true wine yeasts from California grapes.

reason that some wines will ferment completely even at temperatures above 38°C , and others stick with unfermented sugar is because of the difference in the character of the natural wine yeast present. The possibility of a yeast similar to yeast 67 being the only wine yeast present in a natural wine fermentation makes it very desirable to

insure good fermentations by the use of pure and selected varieties of wine yeast.

Lactose supported the growth of the *S. ellipsoideus* yeasts discussed above, but was not fermented by any of them.

Attenuation and Alcohol Formation at 33° C.—The fermented liquids were analyzed with the results in table 10.

TABLE 10

YEASTS NUMBERS 64, 66, 67, 74, 75, 79. ATTENUATION AND YIELDS OF ALCOHOL IN MUST, BEER WORT, DEXTROSE, SACCHAROSE, AND LACTOSE SOLUTIONS

Yeast No.	Medium	Attenuation in grams per 100 c.c	Theoretical yield of alcohol	Observed yield of alcohol	Per cent of theoretical yield obtained
64	Must of 23° Balling	19.70	12.80	11.75	93.4
64	Must of 29°8 Balling	21.40	13.90	12.80	92.1
64	Beer wort of 14° Balling	6.16	4.39	3.40	77.4
64	Dextrose solution, 15%	11.69	7.60	6.00	79.0
64	Saccharose solution, 15%	12.23	8.80	7.15	81.4
64	Lactose solution, 15%			.00	.0
66	Must of 23° Balling	19.41	12.70	11.40	89.7
66	Must of 29°8 Balling	19.75	12.90	11.40	88.4
66	Beer wort, of 14° Balling			5.70	
66	Dextrose solution, 15%	13.20	8.60	7.95	92.4
66	Saccharose solution, 15%	12.03	8.30	7.55	90.9
66	Lactose solution, 15%			.00	
67	Must of 23° Balling	5.41	3.55	2.00	56.4
67	Must of 29°8 Balling	3.85	2.53	1.30	51.3
67	Beer wort of 14° Balling	1.61	1.11	.65	58.6
67	Dextrose solution, 15%	12.75	8.38	7.95	94.7
67	Saccharose solution, 15%	2.82	1.94	1.00	51.5
67	Lactose solution, 15%	.36	.24	.00	.0
74	Must of 23° Balling	19.31	12.70	11.40	89.7
74	Must of 29°8 Balling	20.95	13.70	11.40	83.2
74	Beer wort, of 14° Balling	7.96	5.30	4.10	75.3
74	Dextrose solution, 15%	11.64	7.60	5.70	75.0
74	Saccharose solution, 15%	11.83	8.18	7.95	97.2
74	Lactose solution, 15%			.00	
75	Must of 23° Balling	18.31	12.03	8.01	66.6
75	Must of 29°8 Balling	22.10	14.53		
75	Beer wort of 14° Balling	6.07	4.17	2.34	57.3
75	Dextrose solution, 15%	12.55	8.60	6.40	74.4
75	Saccharose solution, 15%	12.62	8.70	8.60	98.8
75	Lactose solution, 15%	.36	.25	.00	.0
79	Must of 23° Balling	18.51	12.17		
79	Must of 29°8 Balling	18.54	12.20	11.45	93.8
79	Beer wort of 14° Balling	6.36	4.38	2.35	53.7
79	Dextrose solution, 15%	12.60	8.20	6.00	72.9
79	Saccharose solution, 15%	11.43	7.87	6.00	76.2
79	Lactose solution, 15%	.06	.04	.00	.0

Fig. 13.—Fermentation curves of true wine yeasts from California grapes.

The effect of a moderately high temperature on yeast 67 is shown by the analyses of the fermented liquids, from which it is seen that the absolute amounts of alcohol formed by this yeast are small and that the per cents of the theoretical yields obtained were very small. Thus at 33° C it not only gives very incomplete fermentations, but is also very wasteful of the sugar that it ferments. Yeast 75 gave the highest yield of alcohol, 14.53 per cent. None of the yeasts gave as much alcohol in must of 30° Balling, at 33° C as they did at 24° C. Contrasted with the yeasts discussed in the previous pages the *S. ellipsoideus* yeasts gave larger yields of alcohol and in general a larger per cent of the sugar destroyed was transformed into alcohol.

COMPARISON OF FERMENTATIONS AT 33° C AND 24° C (ALL VARIETIES)

The nineteen fermentation organisms discussed in the preceding pages were transferred to grape must of 29.8° Balling, and the products of fermentation at 24° C and 33° C studied and compared. The results appear in table 11.

TABLE 11
COMPARISON OF PRODUCTS OF FERMENTATION OF CALIFORNIA YEASTS IN GRAPE
MUST OF 30° BALLING FERMENTED AT 24° C AND 33° C

Organism	Attenuation in grams per 100 c.c.	Theoretical yield of alcohol, volume, per cent	Alcohol obtained, volume, per cent	Per cent of theoretical yield obtained	Temperature
35. <i>S. apiculatus</i>	8.31	5.46	2.60	47.6	24° C
72. <i>S. apiculatus</i>	6.80	4.47	3.20	72.7	24° C
65. <i>Mycoderma</i> species	2.70	1.70	0.00	00.0	24° C
68. <i>Mycoderma</i> species	4.20	2.70	1.20	44.4	24° C
70. <i>Mycoderma</i> species	3.40	2.20	.60	27.3	24° C
71. <i>Mycoderma</i> species	8.20	5.40	3.20	59.2	24° C
73. <i>Mycoderma</i> species	23.90	15.70	13.50	86.0	24° C
76. <i>Mycoderma</i> species	3.20	2.10	.65	30.9	24° C
78. <i>Mycoderma</i> species	10.10	6.64	4.05	60.9	24° C
37. <i>Torula</i> species	3.70	2.43	.30	12.3	24° C
77. <i>Torula</i> species	6.47	4.25	1.05	24.7	24° C
36. <i>S. pastorianus</i>	2.80	1.85	0.00	00.0	24° C
69. <i>Willia anomala</i>	5.11	3.35	1.05	31.3	24° C
64. <i>S. ellipsoideus</i>	24.60	16.20	15.60	96.3	24° C
66. <i>S. ellipsoideus</i>	22.00	14.40	14.10	97.9	24° C
67. <i>S. ellipsoideus</i>	24.01	15.80	15.30	96.8	24° C
74. <i>S. ellipsoideus</i>	22.50	14.80	14.20	95.2	24° C
75. <i>S. ellipsoideus</i>	22.70	14.90	14.40	96.6	24° C
79. <i>S. ellipsoideus</i>	25.30	16.60	15.55	93.7	24° C
35. <i>S. apiculatus</i>	6.15	.79	.30	37.9	33° C
72. <i>S. apiculatus</i>	1.20	4.03	2.35	58.30	33° C
65. <i>Mycoderma vini</i>	0.00	0.00	0.00	00.0	33° C
68. <i>Mycoderma vini</i>	7.84	5.15	4.50	89.30	33° C
70. <i>Mycoderma vini</i>	2.05	1.35	1.00	74.50	33° C
71. <i>Mycoderma vini</i>			7.90		33° C
73. <i>Mycoderma vini</i>	14.75	9.70	6.80	70.10	33° C
76. <i>Mycoderma vini</i>	00.00	0.00	0.00	00.00	33° C
78. <i>Mycoderma vini</i>	14.13	9.29	9.00	97.90	33° C
37. <i>Torula</i> species	.05	.03	0.00	00.00	33° C
77. <i>Torula</i> species	3.85	2.53	.65	25.70	33° C
36. <i>S. pastorianus</i>	1.65	1.08	.00	00.00	33° C
69. <i>Willia Anomala</i>	2.92	1.90	1.00	52.60	33° C
64. <i>S. ellipsoideus</i>	21.40	13.90	12.80	92.10	33° C
66. <i>S. ellipsoideus</i>	19.75	12.90	11.40	88.40	33° C
67. <i>S. ellipsoideus</i>	3.85	2.53	1.30	51.30	33° C
74. <i>S. ellipsoideus</i>	20.95	13.70	11.40	83.20	33° C
75. <i>S. ellipsoideus</i>	22.10	14.53			33° C
79. <i>S. ellipsoideus</i>	18.54	12.20	11.45	93.80	33° C

TABLE 11—(Continued)

Organism	Volatile acid, grams per 100 c.c.	Loss in fixed acid during fermentation, grams per 100 c.c.	Temperature
35. <i>S. apiculatus</i>	.110	—,10	24° C
77. <i>S. apiculatus</i>	.100	.05	24° C
65. <i>Mycoderma vini</i>	.020	.06	24° C
68. <i>Mycoderma vini</i>	.050	—,19	24° C
70. <i>Mycoderma vini</i>	.030	—,10	24° C
71. <i>Mycoderma vini</i>	.043	—,07	24° C
73. <i>Mycoderma vini</i>	.097	.02	24° C
76. <i>Mycoderma vini</i>	.020	.04	24° C
78. <i>Mycoderma vini</i>	.056	—,08	24° C
37. <i>Torula species</i>	.084	.06	24° C
77. <i>Torula species</i>	.020	—,02	24° C
36. <i>S. pastorianus</i>	.030	—,04	24° C
69. <i>Willia anomala</i>	.080	—,06	24° C
64. <i>S. ellipsoideus</i>	.060	.06	24° C
66. <i>S. ellipsoideus</i>	.050	.10	24° C
67. <i>S. ellipsoideus</i>	.080	.12	24° C
74. <i>S. ellipsoideus</i>	.078	.11	24° C
75. <i>S. ellipsoideus</i>	.070	.06	24° C
79. <i>S. ellipsoideus</i>	.090	.02	24° C

Discussion of Results in Table 11.—Organisms 35, 72, 73, 76, 37, 64, 66, 67, and 74 gave higher percentages of the theoretical yield at 24° C than at 33° C. Organisms 68, 70, 77, and 69 gave higher percentages of the theoretical at 33° C than at 24° C and organism 79 gave approximately the same percentages at 33° C and 24° C, while organisms 65 and 36 gave no alcohol at either temperature.

In regard to the absolute amounts of alcohol formed, the relations are somewhat different. Yeasts 68, 70, 71, and 78 gave higher yields of alcohol at 33° C than at 24° C. All of these are *Mycoderma* forms. This might indicate that these forms have a higher optimum than the other forms tested. All other forms tested gave higher yields of alcohol at 24° C than at 33° C. The results are especially striking with *S. ellipsoideus*, number 67, in this respect. It gave 1.3% alcohol at 33° C and 15.3% at 24° C.

The wild yeasts, *i.e.*, the *S. apiculatus*, *Mycoderma*, *Torula*, *S. pastorianus*, and *Willia* yeasts, with the exception of organism 73, all gave small amounts of alcohol and were very wasteful of the sugar fermented. The percentage of the theoretical yield varied from 0% to 72%. On the other hand, the *S. ellipsoideus* yeasts gave fairly high amounts of alcohol, varying from 14.1% to 15.6% at 24° C, and all gave over 93% of the theoretical yield of alcohol from the sugar destroyed at 24° C. These facts emphasize the necessity of favoring

the growth of the true wine yeasts and discouraging the development of the wild yeasts.

It is commonly held by enologists in general that wild yeasts as a rule destroy large amounts of tartaric acid and form high amounts of volatile acid during fermentation. To test this belief with the California wild yeasts under observation, analyses of the original unfermented must used in the above tests and of the fermented liquids were made. Contrary to expectations they did not destroy abnormally large amounts of fixed acid during fermentation, so that it is possible that the low yield of fixed acid in naturally fermented wines compared to those fermented with pure yeast and sulfurous acid may be due to other causes than the development of the wild yeasts. For example, it may be due to bacteria, or the higher yield of fixed acid in wines in which sulfurous acid has been used may be due to the chemical effect of the sulfurous acid on the cream of tartar which it may protect from precipitation by the alcohol of the wine. The fact that the use of sulfurous acid in wine making gives a higher yield of fixed acid in the finished wine made from the same grapes by natural fermentation, and the results of the above tests, simply cast doubt on the theory held that the low yield of fixed acid in naturally fermented wines is due to the activity of the wild yeasts. They do not necessarily prove the contrary view, because of the small number of yeasts under observation.

The largest amount of volatile acid was formed by the *S. apiculatus* yeast, which gave 0.11%. This, in itself, is considerably below the legal limit of 0.140% for red wines and 0.120% for white wines. The real point to note, however, is that the *apiculatus* yeast develops in natural wine fermentations before the true wine yeast has had a chance to carry on any great amount of fermentation, so that the volatile acid formed by the *apiculatus* yeast is added to that later formed by the true wine yeast and other yeasts. In this way the volatile acid may rise dangerously high. Added to this, is the fact that the *apiculatus* yeast produces compounds during fermentation that are prejudicial to the activity of the true wine yeast and so cause it to behave abnormally and produce larger amounts of volatile acid than it otherwise would. It may be stated that wine ceases to be commercially salable when it contains more than 0.14% volatile acid.

From the data and discussions given in the foregoing pages on the properties of California yeasts, it may be said that the yeasts of the *S. ellipsoideus* or true wine yeast type are the only ones so far found

Fig. 14.—Fermentation curves of true wine yeasts from California grapes.

that are suitable for the production of wine. Although several of the wild yeasts produce fairly large amounts of alcohol and low volatile acid during fermentation, they can not be used in wine making, because they produce undesirable flavors and odors. An objection to the California *S. ellipsoideus* yeasts is the fine grained character of their sediments, giving slow clearing after fermentation and difficulty in racking. Another defect is that some of them seem to be weakened very perceptibly by such a moderate temperature as 90° F (32° C).

Fig. 15.—Fermentation curves of true wine yeasts from California grapes.

Several varieties of *S. ellipsoideus* isolated in France and cultures of which are in the University collection give much better results than any of the yeasts isolated from California grapes in this laboratory. Consequently it seems desirable to use one of the French varieties rather than any of the above California yeasts for commercial fermentation of grapes for wine.

IV. INFLUENCE OF LOCALITY ON THE CHARACTER OF THE MICRO-ORGANISMS ON GRAPES

METHODS OF TAKING SAMPLES⁶

Samples of grapes were obtained from Davis, Fresno, and El Centro, all localities in California. Two Mason fruit jars of one quart capacity each were plugged with cotton wool, packed into a small box with excelsior, and the box and contents were sterilized at 150° C in a dry heat sterilizer. Two such jars were sent to each of the above localities. Several small bunches of grapes were cut and allowed to fall into the open jars. The cotton plugs were then replaced. The grapes were sent to Berkeley where the numbers of the different groups of yeasts present were determined. Samples from Contra Costa County, California, were taken in sterile paper bags. The organisms on grapes from Ripon, San Joaquin County, California, were determined at the vineyard.

METHOD OF COUNTING LIVING CELLS ON GRAPES

The same sort of grape must agar, and sterile petri dishes previously described were employed in the counting tests. In addition to the above apparatus a number of sterile one c.c. pipettes, and test tubes, each containing 9 c.c. of sterile water were necessary. On arrival at the laboratory, the grapes were crushed in the jars by means of a sterile pestle. One cubic centimeter of the fresh juice was removed by means of a sterile 1 c.c. pipette and transferred to a tube of 9 c.c. sterile water. With another sterile 1 c.c. pipette one cubic centimeter of the liquid in the first tube of water was transferred to a second tube. This process was carried progressively to a third, fourth, and occasionally a fifth tube, depending on the condition of the grapes. This gave a dilution of 1:10 in the first tube, 1:100 in the second tube, 1:1000 in the third, and 1:10,000 in the fourth. From each of these tubes one cubic centimeter of the liquid was removed to separate petri dishes. Melted agar must at 40° C to 45° C was poured into each plate and the plates set away to allow development of the individual cells into colonies. Counts of the number of colonies and notes of the numbers of each type of organism present were made. From the dilutions used it was possible to calculate the number of active cells of each kind of micro-organism present in a unit volume of the original liquid (see plate 1).

⁶ The writer wishes to express his appreciation of the aid given him by Messrs. Flossfeder, Way, and Packard in taking samples.

RESULTS OF COUNTING

The results of the countings made, appear in the following tables:

TABLE 12
MICRO-ORGANISMS ON GRAPES FROM DAVIS

Organism	Sample number 1, first crop, Muscat grapes, cells per c.c.	Sample number 2, second crop, Muscat grapes, cells per c.c.
Penicillium, green mold (probably <i>glaucum</i>)	none	none
Aspergillus mold (probably <i>niger</i>)	none	1,000
Mucor mold	20,000	none
Dematium mold	130,000	105,000
Powdery white mold	none	3,000
<i>S. apiculatus</i> (a wild yeast)	less than 100*	less than 100
Other wild yeasts	200,000	
True wine yeast (<i>S. ellipsoideus</i>)	none	none

* The dilutions below 1:100 were so badly overgrown with the dematium mold that it was not possible to differentiate the yeasts present, so that "less than 100 per c.c." may mean from none to 100 per c.c. It signifies that no *S. apiculatus* or true wine yeasts appeared on the dilution of 1:100. There were no true wine yeast cells present, however, because all of the samples were plated out again during fermentation and in this way true wine yeast cells were proven to be absent. Added proof of the absence of true wine yeast is given by the fact that the crushed grapes did not undergo true wine yeast fermentation but after a feeble wild yeast fermentation became moldy and rotted.

TABLE 13
MICRO-ORGANISMS ON GRAPES FROM EL CENTRO

Organism	Sample number 1, cells per c.c.	Sample number 2, cells per c.c.
Penicillium (olive green mold)	2,400,000	20,000,000
Penicillium (blue green mold)	none	50,000
Fusarium-like mold	30,000	none
Aspergillus mold	40,000	100,000
<i>S. apiculatus</i>	less than 100	less than 100
Wild yeasts, <i>Mycoderma vini</i> type	400,000	8,000,000
True wine yeasts (<i>S. ellipsoideus</i>)	none	none

There were no true wine yeasts present because the grapes did not undergo a wine yeast fermentation but simply became very moldy and finally rotted.

TABLE 14
MICRO-ORGANISMS FROM GRAPES GROWN AT FRESNO

Organism	White grapes, cells per c.c.	Red grapes, cells per c.c.
Penicillium (olive green mold)	10,000	15,000
Aspergillus mold	10,000	1,000
Fusarium-like mold	10,000	4,000
Dematium mold	7,500	560,000
Mucor mold	200	less than 100
<i>S. apiculatus</i>	less than 100	less than 100
True wine yeast (<i>S. ellipsoideus</i>)	less than 100	less than 100

These grapes underwent true wine yeast fermentation, proving the presence of a few *S. ellipsoideus*.

TABLE 15

MICRO-ORGANISMS ON GRAPES FROM VINEYARD OF J. SWETT & SON, MARTINEZ

Organism	Alicante Bouschet grapes, cells per c.c.	Sauvignon verte grapes, cells per c.c.	Isabella grapes, cells per c.c.
Penicillium (green mold)	5,000	3,000	15
Mucor (gray mold)	2,000	none	none
Dematium (tree mold)	17,000	22,000	18,000
Aspergillus mold	none	none	200
Monilia mold	12,000	30,000	10,000
Wild yeasts	14,000	36,000	100
True wine yeast (<i>S. ellipsoideus</i>)	a few	a few	a few

These grapes underwent normal alcoholic fermentation, proving the presence of *S. ellipsoideus*.

TABLE 16

MICRO-ORGANISMS FROM GRAPES GROWN AT RIPON*

Organism	Cells per c.c.
Penicillium (blue green mold)	1,700
Mucor mold	100
Aspergillus mold	less than 100
Wild yeasts	2,600
True wine yeasts	less than 100 but more than 0

* The above counts were made by plating the grapes in the vineyard.

YEASTS FROM THE TULARE EXPERIMENT STATION

In 1906 grapes were gathered at the Tulare substation by Professor Bioletti⁷ under conditions that precluded contamination by organisms not present on the grapes. The yeasts present on them were isolated by H. C. Holm and their properties studied. All of them were found to be wild yeasts of very low fermenting power and all gave undrinkable fermented musts. No true wine yeast was found.

DISCUSSION OF TABLES 12 TO 16, INCLUSIVE

The grapes from Davis, Fresno, El Centro, Ripon, and Martinez were all allowed to ferment after crushing and plating tests were made during fermentation in order to obtain the true wine yeast if present. The Davis grapes and the El Centro samples did not undergo true yeast fermentations, but after a short wild-yeast fermentation, became very moldy and finally rotted. The grapes from the other localities underwent normal true wine yeast fermentations and the yeasts of this type present were found on dilutions in agar must made during fermentation.

⁷ Bioletti, F. T., and Holm, H. C., Calif. Exp. Sta. Bull. 197, pp. 169, 175, 1908.

Wine is not made in the regions in the immediate vicinity of Tulare and El Centro, and none is made at the University Farm, Davis. The grapes from Fresno, Ripon, and Martinez came from places near wineries and on all of these samples true wine yeasts were found. These tests indicate that grapes from regions remote from wineries have smaller numbers of true wine yeast cells on their surfaces than grapes from wine making regions. Reasoning from these facts it is probable that the grapes gathered during the beginning of the season will have fewer true wine yeast cells than those picked later in the crushing season. Thus the use of pure yeast is more necessary on grapes from regions distant from wineries and on grapes gathered during the forepart of the season than it is on those picked in wine making regions after the season has progressed for several weeks. The figures given in Part IV indicate the influence of the time of picking on the numbers of yeasts, etc., present.

All the samples tested above gave a great preponderance of molds and wild yeasts over the true wine yeasts. It can easily be seen that if there are several hundred thousand wild yeasts and a million or two mold cells for each one to a hundred true yeast cells, as was actually the case in several instances, the true wine yeasts will have overwhelming odds against which to develop. Where such a condition of affairs exists the wild yeasts must of necessity cause considerable damage. It is not probable that the molds do a great deal of damage after crushing because the conditions are not very favorable for their growth in the crushed grapes, but they undoubtedly depreciate the value of grapes for wine making during long shipments.

In summarizing the above data it may be stated that grapes, both from wine making regions and from those in which wine is not made, are deficient in true wine yeasts and carry a large number of molds and wild yeasts prejudicial to the making of the best wines. Grapes from regions remote from wineries seem to bear smaller numbers of the true wine yeasts than those from wine making localities.

V. INFLUENCE OF THE STAGE OF RIPENESS ON THE CHARACTER OF THE MICRO-ORGANISMS ON GRAPES

SAMPLING

Grapes were gathered in sterile paper bags from a Zinfandel vine in a large vineyard at Muir Station in Contra Costa County, California, in 1912. One sample was taken when the grapes were green, a second when they had begun to turn, and the third in the middle of

September when the grapes were ripe. Since the samples were all from the same vine, they may be taken as being more or less comparative. A sample was taken from an Alicante Bouschet vine in the same vineyard as the Zinfandel when the grapes were green and a second sample in the middle of September from another vine of the same variety in the vicinity of the first one.

The grapes were crushed in the laboratory, using precautions against outside infection, and counts of the active cells present were made as in the tests recorded in Part III.

RESULTS

The results of the tests appear in the accompanying tables.

TABLE 17

NUMBERS OF MICRO-ORGANISMS ON GRAPES AT DIFFERENT STAGES OF RIPENESS

Ripeness of grapes at sampling	Type of organisms found	Cells per c.c. of must, Zinfandel grapes	Cells per c.c. of must, Alicante Bouschet grapes
Hard, green	Molds	1,040,000	100
	Wild yeasts	less than 10	less than 10
	<i>S. ellipsoideus</i>	none	none
Beginning to color	Molds	1,000,000	
	Wild yeasts	175,000	
	<i>S. ellipsoideus</i>	none	
Ripe	Molds	190	22,000
	Wild yeasts	3,360	26,000
	<i>S. ellipsoideus</i>	less than 1, but present	a few

The crushed samples were allowed to stand in sterile jars plugged with cotton. Samples taken when the grapes were hard green and beginning to color, molded and putrefied, but did not ferment, indicating absence of *S. ellipsoideus*. The samples of ripe grapes both fermented, showing presence of *S. ellipsoideus*.

On the green grapes molds were about the only kind of organisms present. When the grapes began to turn in color the wild yeasts had made their appearance in large numbers, but no true yeasts were in evidence. On the ripe grapes were found chiefly wild yeasts and molds and in addition to these a few *S. ellipsoideus* cells, not enough to develop on the plates of dilution 1:10, but still enough to cause the grapes to undergo a true yeast fermentation after standing several days in a sterile flask plugged with cotton wool.

The surfaces of the hard green grapes are very poor places for the development of micro-organisms; and molds predominate probably because they are merely present in the dust on the surface of the grapes. As the grapes soften during ripening some of them are broken by birds and insects and the yeasts, etc., develop in the broken berries. From these the cells are carried to the surfaces of other grapes by insects, etc., so that as the season advances the numbers of yeasts increase. Even on the ripe grapes, however, the numbers of the true wine yeast cells, *S. ellipsoideus*, were small and were greatly exceeded by those of the molds and wild yeasts. The data of this table do not confirm the statement often made that the numbers of micro-organisms increase during ripening.

VI. CHANGES IN THE NUMBERS AND CHARACTER OF THE MICRO-ORGANISMS ON GRAPES DURING SHIPMENT FROM VINEYARD TO CELLAR

Burger grapes were picked at the vineyard of J. Swett & Son, Ripon, California, and dilution plates on agar were made at the vineyard on the freshly picked grapes. Counts were also made on grapes from the same block of the vineyard after they had stood about eighteen hours in boxes. The sample in this latter case was taken from a lot of two boxes that were crushed into a sterile barrel. After the grapes arrived at the winery in Martinez three days later an average sample was taken and counts of the micro-organisms present again made.

Alicante Bouschet grapes were plated at the vineyard and on arrival three days later at the winery. The results of the above tests appear in tables 18 and 19.

TABLE 18
CHANGE IN MICRO-ORGANISMS ON BURGER GRAPES AFTER PICKING

Organism	Grapes in vineyard, cells per c.c. of must	Grapes after 18 hours in boxes, cells per c.c. of must	Grapes after 3 days on board cars, cells per c.c. of must
1. <i>Penicillium</i> mold	1,700	145,000	} 9,200,000
2. <i>Mucor</i> mold	100	50,000	
3. <i>Aspergillus</i> mold	none	none	
4. <i>Dematium</i> mold	less than 1	72,000	
5. <i>Mycodera</i> forms and <i>Torula</i> yeasts	2,500	160,000	4,700,000
6. <i>S. apiculatus</i> yeast	less than 1	less than 10	less than 1,000
7. True wine yeast (<i>S. ellipsoideus</i>)	less than 1	less than 100	500,000
8. Vinegar bacteria	none	none	800,000

TABLE 19

CHANGE IN MICRO-ORGANISMS ON ALICANTE BOUSCHET GRAPES DURING SHIPMENT

Organism	Grapes in vineyard, cells per c.c. of must	Grapes three days later at cellar, cells per c.c. of must
1. <i>Penicillium</i> mold	320,000	2,500,000
2. <i>Mycoderma</i> forms	125	5,400,000
3. Other wild yeasts	none	less than 1,000
4. True wine yeast (<i>S. ellipsoideus</i>).....	25	440,000
5. Vinegar bacteria	none	less than 100

The effect of standing over night in the boxes was to greatly increase the numbers of wild yeasts and molds present without materially affecting the quantity of true wine yeast present. No doubt a great deal of the mold and wild yeast cells came from the sides and bottoms of the boxes, where they had collected from broken and moldy berries during previous shipments. Thus, it may be seen how grapes almost free from micro-organisms could acquire large numbers by standing in boxes a few hours. Some of the increase is, of course, due to normal increase of the cells present on the surface of the grapes and to increase of organisms in the grapes broken during picking.

The grapes were three days on the road from the vineyard to the winery and arrived in a moldy condition, but in no worse state than other Burger grapes shipped under like conditions. The plating tests bore out what the appearance indicated, namely, the presence of great numbers of molds, 9,000,000 per cubic centimeter; wild yeasts, 4,700,000, and even large numbers of vinegar bacteria, 800,000 per cubic centimeter. The wine yeast was present in less numbers than any of the other organisms, there being only 500,000 of them per cubic centimeter.

The Alicante Bouschet grapes gave similar results, but being firmer grapes they arrived in better condition than the Burgers, as was shown by their appearance and by counting tests.

A method of controlling the micro-organisms on grapes during shipment is discussed under Part VII.

VII. CHARACTER AND NUMBERS OF MICRO-ORGANISMS ON GRAPES AS RECEIVED AT THE WINERY DURING THE SEASONS OF 1911 AND 1912

Samples of the freshly crushed grapes from two wineries in Contra Costa County were taken in 1911 and 1912 and counts of the active cells present were made with the results given in the following table:

TABLE 20
ORGANISMS ON GRAPES AS RECEIVED AT THE CELLAR IN SEASONS 1911 AND 1912

	Source and variety of grapes	Mold cells per c.c. of must	Apiculus yeast cells per c.c. of must	Other wild yeast cells per c.c. of must	Ellipioideus wine yeast cells per c.c. of must	Vinegar bacteria cells per c.c. of must
1911:						
1.	Palomino grapes, shipped 75 miles by rail from Acampo	97,600	7,341,000	*	58,500	-----
2.	Zinfandel from Acampo	1,600,000	2,830,000	30,000	20,000	-----
3.	Green Hungarian grapes from Acampo	29,200	6,603,000	-----	29,200	-----
4.	Zinfandel grapes picked near winery	59,000	28,000	-----	less than 100	-----
5.	Alicante Bouschet from Acampo	1,300	958,000	2,000	less than 100	-----
1912:						
6.	Mataro picked near winery	1,300,000	-----	650,000	a few	-----
7.	Zinfandel picked near winery	2,200,000	-----	1,300,000	a few	-----
8.	Burger from Ripon (3 days en route)	9,200,000	-----	4,700,000	500,000	800,000
9.	Alicante Bouschet from Ripon (3 days en route)	2,500,000	-----	8,440,000	440,000	-----

* Blank spaces in columns mean that no cells of the type listed in the respective column developed on plates of dilutions used. This does not indicate that no cells of this type were present.

These tests justify the statement that grapes ordinarily arrive at the winery with a preponderance of the undesirable organisms as compared with the desirable true wine yeast, *S. ellipsoideus*. The actual numbers of all molds, wild yeasts, etc., are less on grapes gathered near the winery than on those shipped considerable distances before crushing; but the ratio of molds and wild yeasts to true yeasts is greater on clean grapes than on moldy ones judging from the above tests. Therefore, there is just as much, or even a greater need for the use of pure yeast with clean grapes than with grapes in poor condition, although it is extremely desirable to check the growth of the undesirable and promote the development of the desirable types in both cases.

VIII. EXPERIMENTS UPON THE CONTROL OF MICRO-ORGANISMS ON GRAPES FOR WINE MAKING

BEFORE SHIPMENT

Burger and Alicante Bouschet grapes from the vineyard of J. Swett & Son were used in the tests discussed below.

Several boxes of Burgers were crushed into a barrel that had been sterilized with strong potassium metabisulfite solution and rinsed out with clean water to remove the metabisulfite. To the crushed grapes was added potassium metabisulfite at the rate of 12 ounces⁸ per ton of grapes. This was added in the form of a 10 per cent solution. Four clean Mason fruit jars were filled with the crushed grapes and sulfited at the rates of 6, 8, 9, and 14 ounces per ton. The metabisulfite is decomposed by the tartaric acid of the grapes to give sulfur dioxide and cream of tartar.

Several bunches of Alicante Bouschet grapes were crushed into a Mason jar and were treated at the rate of about eight ounces of metabisulfite per ton. The treated Burgers and Alicante Bouschet were shipped from Ripon to Martinez in the same car with untreated grapes. Counts of the micro-organisms on the grapes before treatment and on the treated and untreated grapes at the winery upon their arrival three days later were made.

⁸ Twelve ounces of $K_2S_2O_5$ (potassium metabisulfite) per ton corresponds to approximately 190 milligrams SO_2 (sulfur dioxide) per kilogram of grapes.

TABLE 21
EFFECT OF THE USE OF MODERATE AMOUNTS OF SULFUROUS ACID IN SHIPPING
GRAPES FOR WINE MAKING

	Grapes	Mold cells per c.c. of must	Wild yeast cells per c.c. of must	True wine yeast cells per c.c. of must	Vinegar bacteria cells per c.c. of must
1. Burgers before treatment		18,000	2,600	a few	none
2. Untreated Burger on arrival at cellar		9,200,000	4,700,000	500,000	800,000
3. Sulfited Burger in barrel on arrival at cellar		less than 1	less than 1	125,000	none
4. Alicante Bouschet before treatment		320,000	17,500	2,500	none
5. Untreated Alicante Bouschet on arrival at cellar		2,500,000	8,440,000	440,000	
6. Sulfited Alicante Bouschet on arrival at cellar		200	30,000	40,000	none

The Burger grapes in the jars all arrived in good condition, *i.e.*, with no visible mold growth and no fermentation; in fact, none of the treated grapes were moldy or fermenting. The untreated grapes of both varieties shipped in the ordinary way were visibly moldy and in some bunches the odors of fermentation and acetic acid were noticeable.

The amounts of sulfurous acid used were small and very much below the legal amount of 350 milligrams per liter allowed in finished wine in France. Analyses of the samples for sulfur dioxide on arrival at the cellar are tabulated in table 24. The grapes in the barrel were sulfited twelve ounces per ton, which is the equivalent to 190 milligrams of sulfurous acid per kilogram. On arrival at the cellar they contained only 110 milligrams of total sulfurous acid per liter. In other words almost one-half of the sulfurous acid had disappeared during shipment.

TABLE 22
SULFUROUS ACID IN TREATED GRAPES ON ARRIVAL AT THE CELLAR

Sample	Total sulfurous acid in milligrams per liter	Free sulfurous acid in milligrams per liter
1. Barrel of sulfited Burgers	110	45
2. Jar number 1, Burger.....	48	16
3. Jar number 2, Burger	60	20
4. Jar number 3, Burger	68	32
5. Jar number 4, Burger	160	64

It was thought possible that the sprinkling of a little dilute potassium metabisulfite solution over the grapes in the boxes before shipment might protect them against mold growth, etc. Accordingly, about 150 c.c. of a 5 per cent solution of potassium metabisulfite was sprinkled throughout the bottom, center, and top of each of several boxes of Burger grapes and these were shipped with the untreated grapes. Contrary to expectations, they arrived in about as poor condition as the untreated grapes. The cause for the ineffectiveness of the treatment probably was that the metabisulfite solution dried out rapidly during shipment and thus became of little use in checking mold growth.

Discussion of Tables 19, 20, and 21.—From the results of these tables it can be seen that the wild yeasts and molds increase enormously during shipment under the conditions of the above experiments, which represent a fair average of the present commercial methods of shipping. A moderate amount of sulfurous acid held the undesirable organisms in check so well that none of these could be found in one of the samples of treated grapes and only a few in the

other. The true wine yeasts were not entirely suppressed but actually increased in numbers, though fermentation had not started.

This method could be applied commercially by crushing the grapes into wooden or iron tank cars at the vineyard and at the same time adding sulfurous acid in the form of 6% sulfurous acid solution or potassium metabisulfite. For normal conditions and where it is desired to prevent all fermentation and growth of mold, etc., during shipping, it would be advisable to use from twelve to sixteen ounces of the metabisulfite or three-quarters to one gallon of a 6% sulfurous acid per ton of crushed grapes. If it should be desired to prevent only the growth of molds and wild yeasts, twelve ounces of the metabisulfite or three-quarters of a gallon of the sulfurous acid 6% solution per ton would be sufficient in all cases. If the grapes should undergo wine yeast fermentation no great harm would be done. In fact, one step further could be made and pure yeast as well as metabisulfite could be added, in this way making use of the cars as fermenters and insuring good fermentations. It is possible that too much tannin would be extracted in the shipment of white grapes by the crushing and sulfiting method, but if no fermentation should take place it is not likely that this would happen.

AFTER ARRIVAL AT CELLAR

From the tests recorded in Part VII, it may be seen that the grapes as ordinarily received at the cellar have on their surfaces large numbers of fermentation organisms, most of which are of no value in wine making, or are of no direct harm to the production of good wine. As the season progresses, the numbers of the true wine yeast may increase in dirty conveyors, in the boxes, etc.; but this is an uncertain method because the kind and the numbers of the yeasts present are not under control. After heavy rains such as in 1912, the numbers of all yeasts on the grapes are diminished by being washed off. Such grapes may undergo all manner of mold and wild yeast fermentations because of the lack of true wine yeast. This was actually the case in wineries in Sonoma County, California, in 1912.

For these reasons, it becomes advisable to apply methods of wine making that discourage the growth of the undesirable types and favor the development of the true wine yeast. Experiments carried out in 1911, 1913, and 1914 and fully discussed in Bulletin 230 and Circular 140 of the California Agricultural Experiment Station demonstrated that the use of moderate amounts of sulfurous acid and the subsequent application of selected pure yeast to the crushed grapes or defecated must gave uniformly good fermentations and sound wines.

SUMMARY OF PARTS II TO VIII

1. Nineteen different organisms from California grapes have been studied and their properties described. The majority of these were found to be types that are harmful in wine making. The true wine yeasts were not of the best varieties. If the yeasts so far studied are to be taken as representative of California yeasts in general, wine makers should not rely on natural fermentations for the production of their wines.

2. Grapes were gathered in vineyards at Davis, El Centro, Fresno, Ripon, and Martinez under conditions excluding, as far as could be, the possibility of outside contamination. The numbers and character of the micro-organisms present in a unit volume of the crushed grapes from each locality were determined. In all cases the undesirable organisms enormously outnumbered the true wine yeasts. Grapes from Tulare in 1906, Davis in 1912, and El Centro in 1912 contained no true wine yeasts, indicating that such yeasts are very rare in regions where wine making is not being carried on.

3. On the surfaces of the green grapes examined were found mostly molds with very few or no yeasts of any kind. As the grapes ripened wild yeasts made their appearance. The true wine yeasts were the last to appear.

4. As the grapes stand in boxes after picking in the vineyard, or on cars during shipment to the winery, the organisms on their surfaces greatly increase. During this increase in numbers of cells the molds and wild yeasts still remain greatly in excess of the true wine yeasts.

5. Grapes as received at two wineries in 1911 and 1912 under normal working conditions contained large numbers of molds and wild yeast cells and in one case vinegar bacteria, but there were very few wine yeasts (*S. ellipsoideus*) in all cases, compared to the numbers of other organisms present.

6. The molds, wild yeasts, and bacteria may be eliminated from the grapes during shipment by crushing the grapes at the vineyard and adding moderate amounts of sulfurous acid. The amounts of sulfurous acid necessary are far below the limits allowed by law in wine making countries and a great deal of this disappears during shipment and fermentation.

7. After the grapes have arrived at the cellar, the development of the undesirable types of fermentation organisms may be checked by the use of sulfurous acid and good fermentations assured by the use of pure selected yeast.

PLATE 1

Apparatus used in isolating micro-organisms from grapes: Mason jar plugged with cotton and sterilized. Sterile petri dishes. Sterile 1 c.c. pipettes. Sterile agar and sterile must in tubes.

PLATE 2

Colonies of yeasts 70, 71, and 78, on agar agar and gelatin. Upper row of plates agar agar; lower, gelatin. Reading from left to right colonies represent numbers 70, 71, and 78.