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This investigation was undertaken to determine the microbiological flora of a number of varieties of grapes from the Oregon State College farm, and the suitability of this flora for commercial wine production. An incidental study was made to determine the potential values of several varieties of grapes for the manufacture of wine.

The representative microorganisms were isolated from 18 varieties of grapes. These many isolates were classified as 15 different organisms according to their differences in morphology, cultural characteristics, sugar fermentation, alcohol production and sporulation.

Seven of the 15 different microorganisms produced spores. These 7 were classified as true yeasts, genus Saccharomyces. Four of the true yeasts were classified as strains of wine yeasts, the species Saccharomyces ellipsoideus. The other 3 were classified as Saccharomyces apiculatus.

The 8 non-sporulating microorganisms were classified as false yeasts, Pseudosaccharomyces sp.

Experimental production of a dry white type of wine with the isolated strains of Saccharomyces ellipsoideus resulted in a product which was very similar to wines made with recognized strains of wine yeasts.

Wines resulting from a blend of different musts were shown in several cases to be superior to the wine produced from either of the component musts.

It was shown that there are few if any differences in flavor and aroma between wines produced from the same must by different strains of Saccharomyces ellipsoideus.

Due to the lack of aging of the wines it was not possible to draw definite conclusions as to the potential values of the different varieties of grapes for the manufacture of wine. Further analyses after the experimental wines have been properly aged should, however, indicate whibh varieties of grapes are suitable for the production of a dry white wine.

A STUDY OF SOME YEASTS OCCURRING ON OREGON GRAPES

by

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A STUDY OF SOME YEASTS OCCURRING ON OREGON GRAPES

INTRODUCTION

The microorganisms present on the surface of grapes and other fermentable fruits have been shown to vary with the locality and conditions of culture (2). The famous wine districts of Germany, France, Eastern United States and California produce fruit on which the predominating yeast flora is usually an excellent strain of the species Saccharomyces ellipsoideus or wine yeast (2) (5). In localities where grape culture is more recent and the vineyards are small and scattered there is variation in the predominating types of microorganisms on the grapes of each vineyard. Certain sections have a flora that is largely wine yeasts (Saccharomyces ellipsoideus) whereas the flora of others contains an excess number of "wild" yeasts which produce undesirable flavors and very little alcohol (2). This investigation was undertaken to determine the flora on several varieties of grapes grown on the Oregon State College farm and to determine the suitability of this indigenous flora for commercial wine production. Incidental to the investigation of the yeasts was a study undertaken to determine the potential values of a number

of varieties of grapes for the manufacture of wine.

EXPERIMENTAL OUTLINE

The investigation of yeasts occurring naturally on Oregon grapes consisted of isolation, classification, and experimental wine production.

The microorganisms representative of the vineyard on the Oregon State College farm were isolated from a number of varieties of grapes. The morphology, colony characteristics, sugar fermentations, alcohol production and spore formation were determined for the many isolates. These characteristics were used as criteria for classification.

The yeasts classified as Saccharomyces ellipsoideus were subjected to further studies relative to their suitability for wine production.

The experimental wine was made from a number of varieties of grapes to determine their relative potential values for the production of wine. This experimental wine was of secondary importance to the investigation. The main object was to study the several types of yeasts and related microorganisms found on the grapes by using the following procedures.

ISOLATIONS

Isolations were made of the microorganisms present on the following varieties of grapes grown at the Oregon State College farm.

1. Salem
2. Agawan
3. Concord
4. Campbell's Early
5. Niagara
6. Diamond
7. Golden Muscat
8. Urbana
9. Keuka
10. Italian Coloring
11. Catawba
12. Tokay
13. Delaware
14. Carmen
15. Black July
16. Beclan
17. Moore's Early
18. Thompson's Seedless

The microbiological flora of each variety was determined by crushing about twenty sound grapes in a sterile flask and plating out in various dilutions portions of the must (fresh pressed juice). The plates were poured with Sabouraud's agar. This culture medium supports a good growth of yeast and is a poor medium for bacterial growth because it contains no beef extract and has an acid reaction.

Composition of Sabouraud's Agar

Peptone	10 g
Dextrose	40 g
Agar	15 g
Water	1 liter
pH	5.6

The must was then incubated at 25° C. and after three to five days a second series of plates was made in a similar manner. A third set of plates was made after six or eight days. If fermentation was still evident an additional set of plates was made after two weeks incu-

bation.

Cruess (2) and Henry (4) found that the predominating flora in many California and Washington musts consisted of undesirable yeasts, whereas the desirable forms, strains of Saccharomyces ellipsoideus, were present only in limited numbers. It was therefore necessary to make plate cultures after different times of fermentation, allowing any Saccharomyces ellipsoideus which were present to gain predominance by producing sufficient alcohol to inhibit the undesirable yeasts and other types of microorganisms. Following this procedure it was possible to obtain well isolated colonies which were representative of the many types of microorganisms present on the grapes.

A plate having a large number of well isolated colonies was selected from each set of dilutions and the representative types of colonies were streaked on Sabouraud's agar slopes. The source and series were recorded to identify each isolate.

The cultures were reisolated from loop dilution plates to insure their purity. The size, shape, color and general appearances of the colonies of the reisolated cultures were observed and recorded.

Isolations were also made from a sample of fermenting prune juice which was brought to the laboratory during the early part of this work. These isolates were grouped with

those from the grapes throughout the remainder of this study.

CLASSIFICATION

Morphology.

The relative sizes and general morphology of the many isolates were determined by microscopical observation of wet mounts made from 24 hour dextrose nutrient broth cultures and stained with methylene blue. The average diameters of the living (unstained) cells were measured in microns with an ocular micrometer. Many of the microorganisms were morphologically similar.

Sugar Fermentations.

Hansen (3) has reported that yeasts may be divided into six groups according to their ability to ferment dextrose, sucrose, maltose and lactose. These groups are shown in Table I.

TABLE I

HANSEN'S DIVISION OF THE GENUS SACCHAROMYCES

Group	Dextrose	Sucrose	Maltose	Lactose
1.	X	X	X	-
2.	X	X	-	-
3.	X	-	X	-
4.	X	-	-	-
5.	X	-	-	X
6.	-	-	-	-

X indicates gas production
- indicates growth without gas production

The sugar fermentations of the many isolates were determined by inoculation into Durham fermentation tubes containing 5 per cent nutrient broth solutions of the different sugars.

Composition of Sugar Nutrient Broth

Beef Extract	3 g
Peptone	5 g
Water	1 liter
pH	7.0

The desired sugar was added to give the stated concentration.

Gas production was observed and recorded after 48 hours incubation at 25° C. The amount of growth in the tubes as evidenced by the sediment varied with different cultures, therefore a quantitative estimate was made of the relative amounts of sediment in the different tubes. All of these tests were performed in duplicate.

Many of the isolates failed to produce gas from any of the sugars. These either belonged to group six in Table I or were organisms other than true yeasts. Carbon dioxide and alcohol are always produced simultaneously in an alcoholic fermentation by wine yeasts, therefore those isolates not producing any gas in any of the sugars were discarded as being useless in the production of wine.

Table II and Table III show the sugar fermentations of the yeasts from the first and second series of isolations, or at the start and after 4 days of fermentation. All of the microorganisms obtained from the third and

fourth series or after eight days failed to produce gas from any of the sugars; therefore they were not included in the sugar fermentation tables. Molds were the predominant organisms on the plates of the third and fourth series.

TABLE II

SUGAR FERMENTATIONS OF FIRST SERIES OF ISOLATIONS

Yeast No.	Source	Sugars Fermented			
		Dext.	Sucr.	Malt.	Lact.
2	Golden Muscat	X	-	-	-
4	" "	X	X	X	-
4a	" "	X	X	-	-
5	Concord	X	-	-	-
6	"	X	-	-	-
7	"	X	-	-	-
8	"	X	-	-	-
9	Agawan	X	-	-	-
10	"	X	-	-	-
11	"	X	-	-	-
12	"	X	-	-	-
13	"	X	-	-	-
14	Diamond	X	-	-	-
15	"	X	-	-	-
17	"	X	-	-	-
18	"	X	-	-	-
19	Campbell's Early	X	-	-	-
20	" "	X	-	-	-
21	" "	X	-	-	-
23	" "	X	-	-	-
25	Niagara	X	-	-	-
26	"	X	-	-	-
27	"	X	-	-	-
31	Urbana	X	-	-	-
32	"	X	-	-	-
33	Keuka	X	-	-	-
37	Salem	X	-	-	-
38	"	X	-	-	-
39	"	X	-	-	-
49	Thompson's Seedless	X	-	-	-
72	Tokay	X	-	-	-
82	Black July	X	-	-	-
97	Catawba	X	-	-	-
98	"	X	-	-	-
103	"	X	X	X	-
103a	Beclan	X	-	-	-
119	Prunes	X	X	X	-

X indicates gas production
- indicates growth without gas production

TABLE III

SUGAR FERMENTATIONS OF SECOND SERIES OF ISOLATES

Yeast No.	Source	Sugars Fermented			
		Dext.	Sucr.	Malt.	Lact.
45	Salem	X	-	-	-
46	"	X	-	-	-
54	Urbana	X	-	-	-
55	"	X	-	-	-
59	Niagara	X	-	-	-
60	Concord	X	-	-	-
61	"	X	-	-	-
62	"	X	-	-	-
65	Agawan	X	-	-	-
77	Keuka	X	-	-	-
88	Thompson's Seedless	X	-	-	-
120	Prunes	X	X	X	-

X indicates gas production
- indicates growth without gas production

Alcohol Production.

There are great variations in alcohol production between different species of yeasts. To determine these variations the isolated yeasts were inoculated into 15 per cent dextrose nutrient broth. The maximum theoretical yield of alcohol from this medium is about 7.5 per cent as calculated from the theoretical equation of Gay-Lussac. The transformation of a hexose sugar into carbon dioxide and ethyl alcohol is represented as

$$C_6H_{12}O_6 = 2\ CO_2 + 2\ C_2H_5OH$$

Alcohol production was determined after 30 days incubation at 25° C. by the boiling point method using a Du Jardin ebulliometer (7).

The yeasts were divided into two groups according to their alcohol production. The low alcohol producing group was characterized by the production of less than 3 per cent alcohol in most cases. The high alcohol group, however, formed nearly the theoretical maximum yield, indicating that in the presence of more sugar they would probably have produced more than 7.5 per cent alcohol.

The high alcohol producing yeasts were inoculated into 40 per cent dextrose nutrient broth to determine their maximum alcohol production. Duplicate inoculations were incubated at 20° C. and 30° C. until the fermentations had ceased. The amount of alcohol produced was determined

with an ebulliometer.

The maximum alcohol production of the different yeasts, as shown in Table IV, is valid only for the conditions which have been specified. The per cent alcohol produced by these yeasts during a wine fermentation would probably not be in agreement with these results due to the environmental differences in the grape must.

TABLE IV

ALCOHOL PRODUCTION OF HIGH ALCOHOL PRODUCERS AT DIFFERENT TEMPERATURES

Yeast No.	Per cent Alcohol	
	20° C.	30° C.
4.	16.5	16.8
98.	5.0	6.3
103.	20.0	17.8
119.	15.6	15.6
120.	17.4	17.6

Sporulation.

All true yeasts, genus Saccharomyces, are defined as producing spores. Sporulation is therefore an important criterion of classification.

Factors that are necessary for sporulation are free access to the air, a favorable temperature (which is usually 25° C.), an abundance of moisture and a poor food supply (3). The yeast cells used for the determination of sporulation should be from a young culture which has

been transferred frequently in a favorable medium. These frequent transfers allow the cells to acquire a sufficient reserve in their protoplasm to assure the formation of spores (3).

The technique used for determining spore formation consisted of spreading 0.5 cc. of an actively growing yeast culture on the smooth surface of a small piece of clay brick which had been autoclaved in a petri plate. Sterile water was added to each plate to insure a sufficient supply of moisture. Incubation was at 25° C.

Microscopic slides were made from the brick surfaces after 24 and 48 hours. These were stained by Levine's (6) technique with hot carbol fuchsin for 5 minutes, decolorized with 5 per cent acetic acid for about 2 seconds, then counterstained with 1 per cent methylene blue. This spore stain gave excellent results, the spores being bright red against the blue background of the vegetative cell.

Spores were formed by seven of the 15 different yeasts. These showed sporulation after 24 hours incubation as shown in Table V. All spore forming non-mycelium producing yeasts were assumed to belong to the genus Saccharomyces.

TABLE V

SPORE FORMATION OF DIFFERENT CULTURES

Yeast No.	24 hours	48 hours
4	X	X
4a	-	-
6	-	-
23	-	-
49	X	X
98	X	X
103	X	X
103a	X	X
119	X	X
120	X	X
2	-	-
15	-	-
25	-	-
32	-	-
97	-	-

X indicates spore production
- indicates no spores formed

Carrot infusion agar and raw carrot slices were also used as sporulating media in an attempt to confirm the results obtained on the brick surfaces. None of the yeasts had formed spores on either of these media after 15 days.

Differentiation of species.

Classification according to their cultural characteristics, morpholoty, sugar fermentations, alcohol production and sporulation grouped the many yeasts into 15 different strains of 3 species.

Saccharomyces ellipsoideus was the species of most interest since it is the true wine yeast. The ellipsoi-

deus yeast is characterized by a strong fermentation yielding about 15 per cent alcohol. The usual shape of the cells is shortly ellipsoidal, although this may vary from elongate to nearly spherical. Spores are formed within the cell. These are spherical and may vary in number from 1 to 4. Gas (CO_2) is produced from the fermentations of dextrose, sucrose and maltose.(3).

The yeasts which were classified as being typical ellipsoideus yeasts are shown in Table VI. This table also shows the colony characteristics upon which the strain separations were based.

TABLE VI

CHARACTERISTICS OF THE YEASTS CLASSIFIED AS SACCHAROMYCES ELLIPSOIDEUS

Yeast No.	Sugar Fermentations				Spores Formed	Est.Ave. Cell Size in microns	% Alc	Colony Characteristics
	Dex.	Suc.	Mal.	Lac.				
4	X	X	X	-	*	2.2x3.0	16.6	tan, dense center round, flat top beveled edges.
103	X	X	X	-	*	3.0x4.5	18.9	white with dark edges, round, top convex.
119	X	X	X	-	*	3.0x4.5	15.6	white, round, top convex, surface rough and shiny.
120	X	X	X	-	*	3.0x3.8	17.5	white, round, top flat with rounded edges, translucent.

X indicates gas production
- indicates growth without gas production
* indicates spores formed

Yeasts which produce only small amounts of alcohol from dextrose and fail to invert sucrose are classified under the species Saccharomyces apiculatus. The apiculatus yeasts rarely produce more than 3 per cent alcohol although they resemble Saccharomyces ellipsoideus in many respects (3). The characteristics of the yeasts classified as Saccharomyces apiculatus are shown in Table VII.

TABLE VII

CHARACTERISTICS OF THE YEASTS CLASSIFIED AS SACCHAROMYCES APICULATUS

Yeast No.	Sugar Fermentations				Spores Formed	Est.Ave. Cell Size in microns	% Alc	Colony Characteristics
	Dex.	Suc.	Mal.	Lac.				
49	X	-	-	-	*	2.2x3.0	2.8	white, round, top flat with dense pointed center.
98	X	-	-	-	*	3.0x3.0	5.7	white, round, convex with dense pointed center.
103a	X	-	-	-	*	3.0x4.5	trace	white with dark edges, round, top convex.

X indicates gas production
- indicates growth without gas production
* indicates spores formed

A majority of these yeasts failed to produce spores; which indicated they were false yeasts, members of the genus Pseudosaccharomyces (3). Their characteristics are shown by Table VIII.

TABLE VIII

CHARACTERISTICS OF THE YEASTS CLASSIFIED AS PSEUDOSACCHAROMYCES SP

Yeast No.	Sugar Fermentations Dex.	Suc.	Mal.	Lac.	Spores Formed	Est. Ave. Cell Size in microns	% Alc	Colony Characteristics
2	X	-	-	-	None	1.5x2.2	trace	cream, dark center round, sharp-edges smooth, conical.
4a	X	X	-	-	None	2.2x3.0	trace	tan, dense center, round, flat top, beveled edges.
6	X	-	-	-	None	3.0x4.5	2.5	cream, uniform density flat top, beveled edges.
15	X	-	-	-	None	1.5x2.2	trace	light reddish brown top convex, edges scalloped.
23	X	-	-	-	None	1.5x2.2	3.0	shadowy with dense center feathery edges.
25	X	-	-	-	None	1.5x2.2	trace	small, darkccream, hemispherical, sharply defined boundaries.
32	X	-	-	-	None	0.7x1.5	trace	white, edges ragged, top conical, surface wrinkled and crusted.
97	X	-	-	-	None	1.5x3.0	trace	white, round flat top, edges beveled, pointed center

X indicates gas production
- indicates growth without gas production

PRODUCTION OF EXPERIMENTAL WINE

The isolated strains of Saccharomyces ellipsoideus were used for experimental wine production to determine their suitability for such fermentations. A dry white wine was selected as the type best suited for experimentation due to its ease of production on an experimental scale.

A dry wine is one in which practically all of the sugar has been converted by fermentation into alcohol. Usually it is of comparatively low alcoholic content, from 8 to 12 per cent.

A white wine is produced by fermentation of must pressed from fresh grapes. The marc (skins, stems, etc.) is removed before fermentation. Riesling, Sauterne, Moselle and Chablis may be cited as examples (5). A colored wine made from red fleshed grapes is classed as a white wine if it has been made from must pressed before fermentation.

This type of wine had several experimental advantages over red wine which is produced by allowing the initial fermentation to take place on the marc. The must was pressed from the grapes without any fermentation on the marc, thus saving extra containers. Inoculation with a pure culture of a known strain of yeast was more effective because most of the natural microbiological flora remained

in the marc (5), thus giving more nearly the effect of a wine produced by a pure culture of Saccharomyces ellipsoideus.

The experimental wines were produced from the following varieties of grapes furnished by the Oregon State College Horticulture Department from the same experimental vineyard from which the studied yeasts had been isolated.

1. Niagara
2. Delaware
3. Alicante Bouschet
4. Champagne
5. Black July
6. Isabella
7. Savignon Vert
8. Goethe
9. Green Chaislas
10. Hungarian Muscat
11. Keuka
12. Agawan
13. Concord
14. John Hubbard
15. Cinsault
16. Urbana
17. Traminer
18. Catawba

The grapes were pressed a bushel at a time in a hand operated screw type wine press. The must from each variety of grape was measured into tared gallon glass jugs. Each jug was weighed again after filling and inoculating so that the fermentation could be followed by the loss of weight. Carbon dioxide from the fermentation was allowed to escape through a water seal. A refrigerating machine was used to maintain the storage room temperature between 15° C. and 18° C.

Inoculation.

Each jug was inoculated with a 100 cc. grape must culture of Saccharomyces ellipsoideus. Two of the strains were laboratory cultures, Saccharomyces ellipsoideus-Burgundy and the other Saccharomyces ellipsoideus (Tanner).

Several inoculations were made with the strains of Saccharomyces ellipsoideus isolated during this study to compare their wine production with the results obtained with the recognized wine yeasts.

Inoculation of the must soon after pressing probably enabled the desired yeast to outgrow the microorganisms naturally on the grapes and produce its own characteristic fermentation. Sterilization before inoculation was not attempted because the effect on the flavor of the finished wine is a disputed point.

Blending.

Several of the musts were very acid or highly astringent due to the tannins. Other musts lacked acidity and astringency. Several musts were blended in varied proportions in order to utilize the best properties of each and to compensate for what seemed to be undesirable qualities. Controls of the several musts were inoculated without blending so that its effects on the finished wine could be determined.

Chemical Analysis.

The different musts were analyzed for per cent sugar so that their sugar concentrations could be made comparable. Total acidity and pH determinations were for comparison between the musts and the finished wines. The

methods of analysis are given later under Analysis of the Wines.

The results of these analyses are shown in Table IX.

TABLE IX

CHEMICAL ANALYSIS OF GRAPE MUSTS

Variety	Per cent Sugar	pH	Total Acidity % tartaric acid
1. Delaware	21.2	3.04	0.848
2. Niagara	17.7	2.80	0.855
3. Catawba	16.5	3.02	0.846
4, Urbana	15.75	2.98	0.872
5. Black July	15.0	2.92	0.923
6. Concord	15.0	3.02	0.842
7. Isabella	14.2	3.04	0.850
8. Champagne	14.1	3.05	0.938
9. Agawan	13.4	3.02	0.846
10.Green Chaislas	13.2	2.98	0.85
11.John Hubbard	13.2	3.00	0.936
12.Savignon Vert	13.1	2.78	0.997
13.Keuka	13.0	3.08	0.848
14.Hungarian Muscat	12.8	3.00	0.845
15.Goethe	12.6	2.96	1.08
16.Traminer	10.4	2.96	0.895
17.Cinsault	10.2	2.85	1.16
18.Alicante Bouschet	9.6	2.70	1.39

The normal acidity of the must should be 0.5 - 1.5 per cent expressed as tartaric. A proper sugar content for a good wine grape should be between 20 and 28 per cent. (5).

The results of the chemical analyses in Table IX show that the acid concentrations of the different musts were about average. The sugar concentrations, however, were below those expected for commerical wine grapes ex-

cept for the variety Delaware. Sufficient dextrose was added to each must to give it a total sugar concentration of 25 per cent, in order that the final fermentation product would contain the desired 12 per cent alcohol.

Racking.

The wines were racked (carefully siphoned off from the sediment into clean containers) once before the addition of the sugar and again before bottling. The first racking was accomplished by siphoning the wine from one jug into another with as little contact with the air as possible. The second racking was part of the bottling process. The finished wines were siphoned into sterile bottles and immediately sealed with screw caps. The bottles and caps were sterilized by autoclaving.

Several bottles were kept of each wine, one for immediate analysis and the others for determining the effects of aging upon the wines.

ANALYSIS OF THE WINES

The finished wines were subjected to several chemical and tasting tests to determine their properties.

Chemical Methods.

The chemical methods used for analysis of the grape musts and the resulting wines are listed below.

1. Sugar--per cent sugar was determined by the Lane-Eynon method for dextrose (A.O.A.C.)(1).
2. Total acidity--the total acidity expressed as per cent tartaric acid was determined by titration of a 20 cc. sample with 0.1 N sodium hydroxide using phenolphthalein as an indicator (A.O.A.C.)(1)
3. Volatile acids--volatile acidity was determined by steam distillation and titration with 0.1 N sodium hydroxide. The results were expressed as per cent acetic acid (A.O.A.C.)(1).
4. Alcohol--per cent alcohol by volume was determined with a Du Jardin ebulliometer (7).
5. pH--a glass electrode was used to measure pH.

The chemical analysis, initial composition, and cellar treatment of each wine is shown in Table X.

The content of alcohol is nearly the same for all the wines. The differences may be due to differences in the musts.

Differences in total acidities between two wines from the same must are probably due to differences in the amounts of acid used by the two strains of yeasts. This consumption of acid by the yeast is largely responsible for the drop from about 0.5 - 1.5 per cent to 0.3 - 0.8 per cent acid expressed as tartaric (5).

All the wines were well inside the legal limit of 0.15 per cent volatile acids expressed as acetic.

TABLE X

ANALYSIS OF ALL THE WINES

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol. Acids % Acetic	Total Acids % Tartaric	pH
1.	Niagara, 3600 cc. 17.7 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 263 g. dextrose added 5-20-39 Racked and bottled	12.3	.0465	.488	3.62
2.	Niagara, 3600 cc. 17.7% sugar Yeast # 6	10-7-38 Inoculated 12-3-38 Racked and 263 g. dextrose added 5-20-39 Racked and bottled	12.3	.056	.635	3.62
3.	Niagara, 1750 cc. Delaware, 1750 cc. 19.4% sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 196 g. dextrose added 5-20-39 Racked and bottled	12.3	.03	.56	3.70

Yeast # 1 --- Saccharomyces ellipsoideus-Burgundy
Yeast # 6 --- Saccharomyces ellipsoideus (Tanner)
Yeasts # 4, # 119, and # 120 --- Isolated strains of Saccharomyces ellipsoideus

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol.Acids % Acetic	Total Acids % Tartaric	pH
4.	Niagara, 2200 cc. Delaware, 1100 cc. 18.9 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 208 g. dextrose added 5-20-39 Racked and bottled	12.3	.042	.60	3.56
5.	Delaware, 3600 cc. 21.2 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 137 g. dextrose added 5-20-39 Racked and bottled	12.3	.039	.675	3.72
6.	Delaware, 3600 cc. 21.2 % sugar Yeast # 6	10-7-38 Inoculated 12-3-38 Racked and 137 g. dextrose added 5-20-39 Racked and bottled	12.3	.042	.785	3.68
7.	Delaware, 1750 cc. Alicante Bouschet 1750 cc. 15.4 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 346 g. dextrose added 5-20-39 Racked and bottled	11.9	.041	.825	3.45
8,	Alicante Bouschet 3600 cc. 9.6% sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 555 g. dextrose added 5-20-39 Racked and bottled	12.1	.039	.488	3.26

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol. Acids % Acetic	Total Acids % Tartaric	pH
9.	Alicante Bouschet 3600 cc. 9.6 % sugar Yeast # 6	10-7-38 Inoculated 12-3-38 Racked and 346 g. dextrose added 5-20-39 Racked and bottled	12.1	.039	.488	3.25
10.	Delaware, 2200 cc Alicante Bouschet 1100 cc. 17.3 % sugar Yeast # 6	10-7-38 Inoculated 12-3-38 Racked and 262 g. dextrose added 5-20-39 Racked and bottled	12.0	.036	.56	3.54
11.	Alicante Bouschet 1750 cc. Niagara, 1750 cc. 13.7 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 396 g. dextrose added 5-20-39 Racked and bottled	12.1	.044	.90	3.30
12.	Black July, 3500 cc. 15.0 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 350 g. dextrose added 5-20-39 Racked and bottled	12.4	.058	.75	3.54
13.	Champagne, 2400 cc. 14.1 % sugar Yeast # 4	10-7-38 Inoculated 12-3-38 Racked and 156 g. dextrose added	5-20-39 still fermenting			

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol. Acids % Acetic	Total Acids % Tartaric	pH
14.	Niagara, 1700 cc. Delaware, 1100 cc. Alicante Bouschet 600 cc. 16.9 % sugar Yeast # 1	10-7-38 Inoculated 12-3-38 Racked and 284 g. dextrose added 5-20-39 Racked and bottled	12.3	0.040	.785	3.45
15.	Niagara, 3600 cc. 17.7 % sugar Yeast # 119	10-7-38 Inoculated 12-3-38 Racked and 263 g. dextrose added 5-20-39 Racked and bottled	12.3	.0368	.56	3.50
16.	Niagara, 3600 cc. 17.7 % sugar Yeast # 120	10-7-38 Inoculated 12-3-38 Racked and 263 g. dextrose added 5-20-39 Racked and bottled	12.3	.0285	.675	3.56
17.	Savignon Vert 3600 cc. 13.1 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 428 g. dextrose added 5-20-39 Racked and bottled	12.2	.083	1.05	3.40
18.	Savignon Vert 3600 cc. 13.1 % sugar Yeast # 6	10-18-38 Inoculated 12-3-38 Racked and 428 g. dextrose added 5-20-39 Racked and bottled	12.2	.080	.635	3.48

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol Acids % Acetic	Total Acids % Tartaric	pH
19.	Isabella, 3600 cc. 14.2 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 399 g. dextrose added 5-20-39 Racked and bottled	12.4	.045	.785	3.46
20.	Isabella, 3600 cc. 14.2 % sugar Yeast # 6	10-18-38 Inoculated 12-3-38 Racked and 399 g. dextrose added 5-20-39 Racked and bottled	12.4	.044	.86	3.45
21.	Goethe, 3300 cc. 12.6 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 409 g. dextrose added 5-20-39 Racked and bottled	12.3	.065	.75	3.56
22.	Green Chaislas 3600 cc. 13.2 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 425 g. dextrose added 5-20-39 Racked and bottled	12.3	.058	.71	3.44
23.	Hungarian Muscat 3600 cc. 12.8 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 440 g. dextrose added 5-20-39 Racked and bottled	12.4	.060	.75	3.44

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol.Acids % Acetic	Total Acids % Tartaric	pH
24.	Savignon Vert 1750 cc. Isabella, 1750 cc. 13.7 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 396 g. dextrose added 5-20-39 Racked and bottled	12.3	.063	.71	3.38
25.	Savignon Vert 2200 cc. Isabella, 1100 cc. 13.5 % sugar Yeast # 1	10-18-38 Inoculated 12-3-38 Racked and 391 g. dextrose added 5-20-39 Racked and bottled	12.2	.0455	.71	3.42
26.	Savignon Vert 3600 cc. 13.1 % sugar Natural yeasts	10-17-38 Juice pressed 12-3-38 Racked and 393 g. dextrose added 5-20-39 Racked and bottled	12.1	.046	.65	3.46
27.	Isabella, 3500 cc. 14.2 % sugar Natural yeasts	10-17-38 Juice pressed 12-3-38 Racked and 378 g. dextrose added 5-20-39 Racked and bottled	12.4	.078	.86	3.26
28.	Traminer, 3600 cc.8 10.2 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 526 g. dextrose added 5-20-39 Racked and bottled	12.0	.075	.488	3.82

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol.Acids % Acetic	Total Acids % Tartaric	pH
29.	Traminer, 1750 cc. Keuka, 1750 cc. 11.7 % sugar Yeast # 6	10-21-38 Inoculated 12-3-38 Racked and 465 g. dextrose added. 5-20-39 Racked and bottled	12.1	.0615	.60	3.68
30.	Keuka, 3600 cc. 13.0 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 420 g. dextrose added 5-20-39 Racked and bottled	12.2	.063	.635	3.58
31.	Catawba, 3600 cc. 16.5 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 298 g. dextrose added 5-20-39 Racked and bottled	12.0	.045	.675	3.55
32.	Urbana, 3500 cc. 15.75 % sugar Yeast # 6	10-21-38 Inoculated 12-3-38 Racked and 324 g. dextrose added 5-20-39 Racked and bottled	12.4	.033	.60	3.56
33.	John Hubbard, 3500 cc. 13.2 % sugar Yeast # 6	10-21-38 Inoculated 12-3-38 Racked and 414 g. dextrose added 5-20-39 Racked and Bottled	12.1	.043	.635	3.56

Wine No.	Initial Composition	Treatment	Final Chemical Analysis % Alc.	Vol. Acids % Acetic	Total Acids % Tartaric	pH
34.	Cinsault, 2200 cc. 10.2 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 553 g. dextrose added 5-20-39 Racked and bottled	12.3	.039	.86	3.38
35.	Cinsault, 2200 cc. Concord, 1100 cc. 11.8 % sugar Yeast # 6	10-21-38 Inoculated 12-3-38 Racked and 449 g. dextrose added 5-20-39 Racked and bottled	12.3	.036	.75	3.54
36.	Concord, 1575 cc. Apple juice, 175 cc. 14.5 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 184 g. dextrose added	5-20-39 still fermenting			
37.	Concord, 1320 cc. Apple juice, 330 cc. 14.0 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 182 g. dextrose added	5-20-39 still fermenting			
38.	Concord, 2700 cc. 15.0 % sugar Yeast # 1	10-21-38 Inoculated 12-3-38 Racked and 270 g. dextrose added 5-20-39 Racked and bottled	12.2	.067	1.12	3.60

Taste Analysis.

Only a person of long experience as a wine taster can tell all of the possibilities of a wine before it has been aged, therefore it was impossible to say definitely which wines showed promise. A record, however, was kept of the appearance of each wine and the differences in flavor and aroma which were marked enough to determine.

The noticeable differences between the wines from blended and unblended musts are shown in Table XI.

TABLE XI

RESULTS OF BLENDING

Varieties	Volume Ratio	Remarks
Niagara Delaware	1/1	More body than Delaware milder than Niagara.
Niagara Dela ware	2/1	Effect of Delaware not noticeable.
Delaware Alicante Bouschet	1/1	Less acid than Alicante Bouschet. Shows some promise.
Delaware Alicante Bouschet	2/1	Strong flavor foreign to either variety alone.
Niagara Alicante Bouschet	1/1	Very astringent, but shows some promise.
Savignon Vert Isabella	1/1	Milder than Isabella, more body and flavor than Savignon Vert.
	2/1	
Savignon Vert Isabella	2/1	Not enough body and flavor Inclined to be flat with too much acid.
Keuka Traminer	1/1	No improvement over either Traminer or Keuka.
Cinsault Concord	1/1	Cinsault adds acid and astringency which Concord lacks.

It was thought that there might be some noticeable differences in the flavors and aromas produced by the laboratory strains of wine yeasts and the strains of Saccharomyces ellipsoideus isolated as part of this study. Must from Niagara grapes was inoculated with the two laboratory strains of yeast and two of the isolated strains in order to determine any differences due to the yeast.

TABLE XIII

EFFECT OF DIFFERENT YEASTS
ON MUST FROM NIAGARA GRAPES

Yeast No.	Appearance	Remarks
1.	hazy	There were no noticeable differences in flavor or aromas.
6.	clear	
119.	hazy	
120.	clear	

As is shown in Table XIII there were no noticeable differences in flavors or aromas. Yeasts numbers 6 and 120, however, seemed to settle more rapidly and leave clearer more brilliant wines than yeasts 1 and 119. This difference between yeasts 1 and 6 was not consistent throughout all the other wines produced with these yeasts.

Further judging of the wines was of little significance because of the changes which would take place on aging. Several bottles of each wine were stored for future opening and analysis.

SUMMARY OF RESULTS

The microbiological flora was isolated from 18 varieties of grapes from the Oregon State College farm.

These many isolates were classified as 15 different microorganisms as the result of experiments to determine their differences in morphology, cultural characteristics, sugar fermentation, alcohol production and sporulation.

Seven of the 15 different microorganisms produced spores. These seven were classified as true yeasts, genus Saccharomyces.

Four of the true yeasts were classified as strains of wine yeasts, the species Saccharomyces ellipsoideus. Three were classified as strains of Saccharomyces apiculatus.

The eight non-sporulating microorganisms were classified as false yeasts, Pseudosaccharomyces sp.

Experimental production of wines with the isolated strains of Saccharomyces ellipsoideus resulted in a product which was very similar to wines made with recognized strains of wine yeasts.

Wines resulting from a blend of different musts were shown in several cases to be superior to the wine produced from either of the component musts.

It was shown that the strains of wine yeasts had very

little if any effect on the wines resulting from the same must.

CONCLUSIONS

This investigation has shown that true wine yeasts, strains of Saccharomyces ellipsoideus, occur naturally on grapes grown in Oregon, although their numbers are few in comparison with those of microorganisms unfavorable for wine production.

The wines produced by these yeasts compare favorably with the products of recognized strains of wine yeasts.

It was shown that there are few if any differences in flavor and aroma between wines produced from the same must by different strains of Saccharomyces ellipsoideus.

It was not possible to draw definite conclusions as to the potential values of the different varieties of grapes for the manufacture of wine. Further analyses after the experimental wines have been properly aged should, however, indicate which varieties of grapes are suitable for the production of a dry white wine.

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