for the first pasteurization, generally between 160° and 180° F. The first pasteurization kills the yeast cells in order to stop the fermentation, precipitate the proteinous materials, and hydrolyze the excessive pigment, which, if not removed before bottling, will separate out in the bottles (*6*).

The second pasteurization, immediately before bottling, employs a lower temperature, usually about 140° F.

Summary

The following points should be observed for the successful fermentation of fruit wines.

1. Use sound, ripe fruit.
2. Use fast-fermenting yeast strains.
3. Keep the initial sugar content of the must below 16%.
4. Maintain the fermentation temperature at about 68° F.
5. Add about 0.1% urea to supplement the nitrogen.
6. Avoid excessive use of sulfur dioxide; about 100 p.p.m. is recommended.
7. Aerate the must to maintain the maximum yeast activity.
8. Regulate a good sugar-acid ratio, about 10 to 1.
9. Do not age the wine too long. Six months to 1 year is sufficient.
10. Pasteurize the wine twice, after fermentation and before bottling.

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MALODOROUS FERMENTATION

Acidic Constitutents of Zapatera of Olives

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In an attempt to find the microorganisms responsible for the malodorous fermentation of olives, known as "zapatera" spoilage in the California industry, it became necessary to determine the acidic end products of this spoilage. Conventional chromatographic methods were used to separate and identify the acids that had been recovered from the olive brines by ether extraction. Normal brines contained only acetic and lactic acids, whereas zapatera brines also contained formic, propionic, butyric, and succinic acids.

ZAPATERA SPOILAGE, whose presence in Spanish green olives was first recorded by Cruess (*10*) in 1924, is a malodorous fermentation also found in storage fruit used for ripe process, Sicilian, and Spanish-type olives in California (*11, 21, 22*). This abnormality is characterized by the development of a very penetrating, unpleasant odor in olives undergoing fermentation. In the early stages of spoilage the odor is usually described as cheesy or sagey but, as deterioration progresses, it develops into a foul, fecal stench. In other types of cured olives, such as the "Greek style," lactic acid fermentation does not occur.

Under California conditions "zapatera" spoilage, unlike butyric fermentation (12), occurs when the desirable lactic acid fermentation is allowed to cease before the pH of the brine has decreased below 4.5. At the onset of spoilage, the pH of the affected brine increases while the titratable acidity decreases. There is a continuous loss in acidity as the spoilage progresses.

The cause of zapatera is obscure. Smyth (*18*), apparently the only one to report on the bacteriology of this spoilage, concluded that it was due "to one or more of a group of spore-forming, proteolytic, facultative rods normally present in the soils of Andalusia." Persistent inability to isolate bacteria capable of causing this spoilage prompted studies of the acidic end products of this spoilage, because it was believed that the possession of such knowledge would simplify the search for the causative organisms.

Materials and Methods

The samples examined included normal, suspected, and known spoiled brines from Spanish-type olives collected in California as well as imported brines from Spanish green olives.

Each brine was subjected to clarification with zinc hydroxide, allowed to stand overnight, and filtered before removal of the acids by conventional liquid-liquid extraction with ethylether for 30 hours. After removal of the ether, the total acids were determined by titration with 1 N sodium hydroxide to the phenol red end point and the neutralized solution was evaporated to dryness and dried overnight in a desiccator.

For acids below butyric, the salts were dissolved in sufficient hydrochloric acid to give a solution between 0.1 and 0.2 N with respect to total acid as hydrochloric acid and with sufficient extra to make 0.01 N hydrochloric acid in excess. The mixture of acids in each brine so treated then was separated by partition on a silica gel column, using chloroform and various mixtures of 1-butanol in benzene

Table I. Acids Identified in Good and Spoiled Samples

Sample	Acidity as Lactic, G./100 Ml.	pH	Acids Found
		Representative Good Samples	
C-1[a]	0.749	3.4	Lactic and acetic
C-2	0.662	3.5	Lactic and acetic
C-3	0.417	4.0	Lactic and acetic
C-4	0.572	3.9	Lactic and acetic
S-1[b]	0.486	3.5	Lactic and acetic
S-2	0.668	3.4	Lactic and acetic
S-3	0.723	3.6	Lactic and acetic
S-4	0.876	3.6	Lactic and acetic
		Representative Spoiled Samples	
C-1	0.235	4.5	Lactic, acetic, propionic, and butyric
C-2	0.226	4.6	Lactic, acetic, and propionic
C-4	0.584	3.8	Lactic, formic, acetic, propionic, and butyric
C-9	0.648	4.0	Lactic, acetic, and propionic
C-10	0.598	3.8	Lactic, acetic, propionic, and butyric
C-11	0.348	4.6	Lactic, formic, acetic, propionic, butyric, valeric, and caproic
C-12	0.384	4.5	Lactic, acetic, propionic, butyric, valeric, and caprylic
C-18	0.328	4.6	Lactic, formic, acetic, propionic, butyric, and valeric
S-1	0.244	4.7	Lactic, acetic, propionic, butyric, valeric, and caprylic
S-2	0.087	6.8	Traces of lactic, formic, acetic, propionic, butyric, valeric, and caproic
S-3	0.254	4.0	Lactic, formic, acetic, butyric, valeric, and caprylic
S-5	0.334	4.6	Lactic, acetic, propionic and butyric

[a]C, California production.
[b]S, Spanish importation spoiled before arrival at destination.

as directed by Neish (*16*) and Bullen *et al.* (*7*).

For acids above butyric, the salts were moistened with 2 to 4 drops of sulfuric acid (1 + 1). The liberated acids then were extracted with 5 ml. of iso-octane and shaken overnight, and 4 ml. of this preparation was partitioned on the column with iso-octane following the method of Ramsey and Patterson (*17*).

The individual acids found on partition were tentatively identified graphically by comparison with known acids treated in exactly the same manner. The tentative identification was confirmed by paper chromatography, using the methods of Brown (*5*) and Brown and Hall (*6*) for the volatile acids and those of Lugg and Overell (*13, 14*) for the fixed acids.

Results

Results of the investigation are summarized in Table I. The normal brines were found to contain acetic and lactic acids, whereas the suspected and obviously spoiled samples contained from one to several additional acids. Propionic acid occurred most frequently in the abnormal brines, followed by butyric acid. Succinic, formic, valeric, caproic, and caprylic acids were also found. These latter volatile acids, together with butyric, are partly responsible for the offensive odor characteristic of zapatera spoilage.

Discussion

The bacteriology of zapatera spoilage is complex. It has been established that the spoilage occurs only when the lactic acid fermentation of the olives is disrupted and that with the onset of spoilage the acidity decreases. Therefore, it seems probable that lactic and acetic acids furnish the energy for the bacteria which cause this abnormality.

The propionic acid bacteria utilize lactate very readily. Perhaps species of the genus *Propionibacterium* or the anaerobe *Clostridium propionicum* described by Cardon and Barker (*8, 9*) are responsible for the production of propionic acid.

It is known that *C. butyricum* and related species ferment lactic acid in vitro, if sufficient acetic acid is supplied simultaneously (*3, 20*). It also is possible that the higher volatile fatty acids may be formed by *Cc. kluyveri*, whose synthetic abilities have been studied by Barker and associates (*1, 2, 4, 19*). These or similar anaerobes may be involved in the spoilage. Gililland and Vaughn (*12*) have shown that the saccharolytic, spore-forming anaerobes may cause a butyric spoilage of olives during the primary stage of the fermentation when sufficient sugar is still available.

The presence of formic and succinic acids may or may not be significant. The coliform bacteria are common contaminants during the early stages of the olive fermentation, as shown by Vaughn and his students (*22, 23*) as well as by Martinez *et al.* (*15*). On the other hand, these compounds may also be produced by the anaerobes.

Finally, lipolytic bacteria also may be involved in the production of the malodorous compounds.

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