CONTROLLING FERMENTATION WITH VITAMIN K_5

by

JOHN NICHOLAS CASPAR

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APPROVED:

Associate Professor of Food Technology

In Charge of Major

Head of Department of Food Technology

Chairman of ~~School Graduate~~ Committee

Dean of Graduate School

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CONTROLLING FERMENTATION WITH VITAMIN K_3

INTRODUCTION

Since earliest historical times, wine has been one of the most important beverages of man. The production of wine is of great importance to the economy of a number of European countries, and to a lesser extent to the United States.

One of the main problems in wine making is secondary fermentation. First stage fermentation may be controlled by use of special yeast cultures and sulfur dioxide. However, some types of wines, where there is unfermented sugar left or added, can be subsequently contaminated with yeasts and begin a secondary fermentation. This results in gas, turbidities, and sediment, making the wine unmarketable. (21,p.134).

The over-development of volatile acids is another problem which the wine manufacturer may encounter. This may be controlled by strict cleanliness, pasteurization, absence of oxygen, and the addition of sulfur dioxide. Should the wine subsequently come in contact with the atmosphere for an extended period of time, the over-development of volatile acids may occur. This produces a vinegar-like product which is unmarketable as wine.

At the present time, even though it possesses certain disadvantages, sulfur dioxide is the only legally permitted preservative to be used in wines, the most serious disadvantage being undesirable flavor and interference with proper aging. In some wines, such as apple, an undesirable bleaching action produces a pale-colored wine.

In recent years the development of new chemicals and antibiotics has offered new possibilities of preservative agents for wines. Athanassiadis (4,p.34) showed that vitamin K_5 was effective in inhibiting secondary fermentation in light sweet tokay wines.

This investigation dealt with the use of vitamin K_5 as a possible preservative for wines and had four purposes--to pinpoint the amount of vitamin K_5 necessary to inhibit secondary fermentation in light sweet tokay wine, study the effect of vitamin K_5 to prevent development of volatile acids in apple wine, study the inhibitory effect of vitamin K_5 in light sweet apple wine by following the increase or decrease of microorganisms by plate counting, and study the effect the addition of vitamin K_5 has on the color of apple wine.

In all studies sulfur dioxide was used as a reference standard. This investigation has been made with

the hope of establishing vitamin K_5 as an effective preservative for wine.

REVIEW OF LITERATURE

There has been a constant search for different and new ways to preserve wines. A number of chemicals have been tried and abandoned for various reasons (2,p.316). At the present time sulfurous acid or molecular sulfur dioxide is the only preservative that is legally permitted to be used in wines.

There has been extensive studies on the effect of sulfurous acid or molecular sulfur dioxide on wines (5,p.170-174)(21,p.134-38,157) and (9,p.18-21). Free sulfur dioxide has a powerful antiseptic action on most spoilage microogranisms in wine. When used in the right concentrations, it tends to protect wine from excessive oxidation, stabilizes the coloring matter present, and prevents the formation of turbidities, clouds, and sediments (2,p.311-315). However, when used in excess, it interfers with the proper aging and causes a detraction from the original flavor of the wine. Undesirable turbidities and deposits tend to be produced when copper salts are present. Excessive amounts of sulfur dioxide in wines over long periods of time may also lead to the formation of excessive quantities of sulfate (2,p.89-90). Yang and Wiegand (21,p.134) mention the possibility of the yeast becoming resistant to sulfur dioxide.

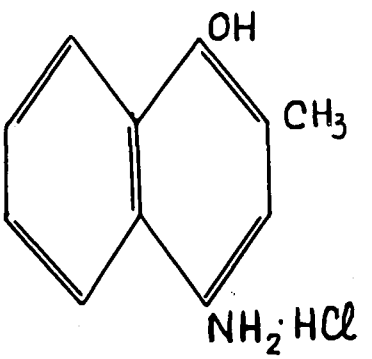
The recent development of new chemicals, which possess preservative action, has brought new possibilities for the replacement of sulfur dioxide as a wine preservative. One of these chemicals, an analog of vitamin K, vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride), was shown by Pratt et al. (11,p.127-134) to exhibit a marked inhibitory activity toward a number of microogranisms, including Saccharomyces cerevisiae, the common brewer's yeast.

The term vitamin K derived from the German expression, "Koagulations-Vitamin", has been applied to the compound recognized by Dam (6,p.475-80) in 1929 as specifically decreasing blood clotting time and identified independently by Dam (7,p.652) and Almquist and Stockstadt (1,p.105-110) in 1935 as 2-methyl 3-phytyl-1-4, napthoquinone. With recognition of the fact that other related compounds possess similar antihemorrhagic properties, this compound was designated vitamin K_1.

Vitamin K occurs in leafy vegetables and other natural sources. Several strains of bacteria, notably of the Bacillus subtilis and B. brevis groups also biosynthesize napthoquinones endowed with vitamin K activity (11,p.127). Several analogs of vitamin K have been synthesized and one of these, vitamin K_5, was found to be not only a superior antihemorrhagic agent for clinical

use, but also to exhibit marked inhibitory activity toward a number of parasitic and saprophytic microorganisms (11,p.134).

Vitamin K_5 with the following structure (19,p.77),



is soluble in water and has a low order of toxicity, the acute LD_{50} for mice being approximately 750 mg./kg. when administered orally or intraperitoneally (12,p.324). Shwartzman (15,p.376-378) shows that vitamin K_5 possesses marked antibacterial action against gram positive and gram negative microorganisms. Pratt et al. (12,p.328) report yeast species were completely inhibited in glucose solution containing 0.0075 to 0.01 per cent of vitamin K_5, but in certain types of wine were killed by 0.001 per cent. Verona (20) found vitamin K_5 and K_3 to possess antibiotic powers at concentrations ranging from 0.05 per cent to 0.005 per cent when used on many different yeasts grown in a synthetic medium.

In studying vitamin K_5 as a food preservative, Faggioli (8) found a 0.1 per cent concentration of vitamin K_5 had the inhibitory effect of 1 per cent salicylic acid and 0.5 per cent methyl-p-hydroxybenzoate. Tanaka and Kawakita (16) found that a concentration of 20 ppm of vitamin K_5 was an effective preservative agent for soy sauce. This concentration did not influence the flavor and taste. Tongerdy and Francia (18) found that vitamin K_5 at a concentration of 0.6 gm. to 1.0 gm./liter did not change the taste or produce any toxic effects when used as a beer preservative.

Ribereau-Gayon and Peynaud (13) in studying the effects of vitamin K_5 added to grape musts, found that concentrations of 5 mg. to 300 mg./l. delayed onset of fermentation (at 25° C.) by 1-28 days. They mention that in preserving sweet wines 10 mg. of vitamin K_5 with 30-40 mg. of sulfur dioxide per liter should give improved resistance. Athanassiadis (4,p.34) in studing the controlling of secondary fermentation of wines with new preservatives, found vitamin K_5 to be more effective than penicillin, bacitracin, patulin, tetracycline hydrochloride, aureomycin hydrochloride, oxamycin, neomycin, and streptomycin. Secondary fermentation was completely inhibited by 20 ppm of vitamin K_5. Laufaurcade (10) showed vitamin K_5 would completely stop fermenting wine and that

5 mg./l. would offer protection for some types of French sugared wines. Vitamin K_5 appears to prevent undesired secondary fermentation in sweet meads and to stop it in weakly alcoholic meads with 10 mg./l. Concentrations of 5 mg./l. suppresses acetification (14,p.174). Tanaka and Kawakita found 0.0007 per cent of vitamin K_5 would inhibit the growth of Bacillis saprogenes which causes putrefaction of sake.

Faggioli (8) mentions the oxidation by atmospheric oxygen causes a darkening effect. If carefully protected from sunlight, vitamin K_5 is very stable. The slightly pinkish color which sometimes develops on standing does not alter the biological properties of the vitamin. Preparations containing vitamin K_5 do not lose microbic activity upon autoclaving or upon storage for several weeks at temperatures as high as 104° F. (12, p.128). The antibacterial activity of the vitamin appears to depend on an intermediate product of oxidation by air which is formed immediately upon dissolving the compound (15,p.377).

MATERIALS AND METHODS

The experimental work in this investigation is divided into four main parts. The first is a study of the inhibitory effect of vitamin K_5 to control the secondary fermentation in wine. The second is a study of the inhibitory effect of vitamin K_5 to control the development of volatile acids in wine. The third deals with following the inhibitory effect of vitamin K_5 in wine by plate counting, while the fourth is a study of the color change in apple wine due to the addition of vitamin K_5 as a preservative.

Preparation of Dextrose-Peptone Agar Media

The following dextrose-peptone agar media was used throughout this work:

15 grams Bactoagar	(Difco Laboratories)
10 grams Bactopeptone	(Difco Laboratories)
20 grams Crystalline dextrose	(Baker Chemical Co.)
950 ml. Distilled water	

The pH of the media was adjusted to 3.6 by the addition of a 15 per cent solution of tartaric acid. Fourteen milliliters of this solution gave the desired pH. This adjustment was done to give the agar media a pH similar to that of the wine used.

Yeast Culture

A champagne strain yeast culture (*Saccharomyces ellipsoideus*) that was used in this work was obtained from the Enology Laboratory of the Food Technology Department of Oregon State College. This culture is the one distributed by the above laboratory to the wineries in Oregon, and is cultured on dextrose-peptone-agar media.

Apple Juice

Apple juice was used as the raw product throughout the investigation for all studies except secondary fermentation. It is generally recognized that apple wine is more susceptible to the development of volatile acids than most other types of wines. Also, apple wine is ideal for color change studies due to its characteristic pale color.

Preservatives Used

1. Vitamin K_3 (Nutritional Biochemical Co.)

No specifications were given by the manufacturer. It is water soluble, has a low toxicity, and does not affect the flavor of the product to which it is added (12,p.323-4). The prepared solutions gave final

concentrations of 200 ppm, 100 ppm, 90 ppm, 80 ppm, 70 ppm, 60 ppm, 50 ppm, 40 ppm, 30 ppm, 20 ppm, 5 ppm, 1 ppm, and 0.1 ppm.

2. Potassium metabisulfite, crystal (J. T. Baker Company)

Throughout this investigation this chemical was used as a comparative standard. The prepared solutions gave final concentrations in terms of sulfur dioxide of 300 ppm, 200 ppm, 150 ppm, 100 ppm, and 75 ppm.

PART I

Preparation of Wine for Secondary Fermentation Studies

Fresh grapes (*Vitis vinifer* var. Tokay) were obtained from Lodi, California, and frozen at 0° F. until used.

For wine making a small quantity of frozen grapes, approximately 3 pounds, were thawed and crushed by hand. A wine yeast culture (*Saccharomyces ellipsoideus*) was then introduced into the crushed grapes and the preparation incubated for 48 hours at 28° C. After incubation the fermenting must was mixed with approximately 17 pounds of newly thawed and crushed grapes. One gram of technical urea was added to the must to facilitate fermentation. This preparation was incubated and allowed to ferment for a period of 7 days. The fermentation was

closely observed and when no further alcohol was produced the wine was strained through cheese cloth. The wine was stored at 36° F. for a period of two weeks. The wine was siphoned to remove the sediment formed and samples taken for analysis which appear in Table 1.

Table 1

WINE ANALYSIS

Degrees brix at 20°/20°	-1.8
Specific gravity at 20°/20°	0.9932
Per cent alcohol by volume	11.60
Total acids as tartaric gm./100 ml.	0.533
Volatile acids as acetic gm./100 ml.	0.018

The wine was not pasteurized and 7 per cent by weight of dry commercial sucrose was added to the wine prior to its experimental use to favor conditions for the initiation of a secondary fermentation. Wines made for subsequent experimental work were of similar analysis with the alcohol contents adjusted by blending when needed to 11.60 per cent by volume for ease of comparison.

Methods of Testing Preservative Effect in Wine

Athanassiadis (4,p.34) had shown that secondary fermentation in similar wine could be inhibited

completely with concentrations of 20 ppm of vitamin K_5.

In a series of two experiments vitamin K_5 was added to the wine in various concentrations designed to pinpoint the concentration of vitamin K_5 which would inhibit alcoholic production completely for a period of 30 days. As a comparison sulfur dioxide in the form of potassium metabisulfite was tested simultaneously in the final experiment.

The procedure for testing the effectiveness of vitamin K_5 was as follows: Three bottles each containing 149.0 ml. of wine for each concentration, plus three controls with no preservative were used. The preservatives were added from stock solution of such concentrations that the addition of 1.0 ml. brought the final volume to 150.0 ml. at the desired concentration. The concentrations used appear in Table 2.

Table 2

CONCENTRATIONS USED IN EXPERIMENT 1

Preservative	Concentration in ppm			
Vitamin K_5	0.1	1	5	10

The results from experiment 1 indicated that the concentration needed was between 5 and 10 ppm of vitamin K_5. The concentration used in the second experiment appears in Table 3.

Table 3

CONCENTRATIONS USED IN EXPERIMENT 2

Preservative	Concentration in ppm				
Vitamin K_5		6	7	8	9
Sulfur dioxide	75	100	150	200	300

All bottles were stored at room temperature. One bottle of each set was analyzed for alcohol content at 10-, 20-, and 30-day intervals. The increase in the alcohol content was the index used for measuring the secondary fermentation in the wine.

The alcohol was determined in all the samples according to the procedure described by the Association of Official Agricultural Chemists (3,p.170).

PART 2

Preparation of Apple Juice

Fresh apples of sweet and sour varieties were obtained locally. When received they were inspected and any spoiled apples removed. They were thoroughly washed and pulverized in a commutative machine. The pulverized apples were then pressed and the juice stored in clean gallon jugs. Analysis of the juice is shown in Table 4. The juice that was not immediately used was stored at 32° F.

Table 4

ANALYSIS OF APPLE JUICE

	Brix	Total acids as malic gm./100 ml.	pH	Specific gravity at 20°/20° C.
(1)	14.7	0.510	3.46	1.0598
(2)	14.7	0.506	3.51	1.0598
(3)	14.7	0.508	3.53	1.0598
(4)	14.7	0.516	3.42	1.0598
Average	14.7	0.510	3.48	1.0598

Preparation of High Alcohol Apple Wine

For producing wine with the higher alcoholic content (11-12 per cent by volume), a small quantity of juice, about 3/4 gallon, was inoculated with a wine yeast culture (*Saccharomyces ellipsoideus*) and the preparation was incubated at 28° C. for 48 hours. The rapidly fermenting juice was added to approximately 5 gallons of juice. Five grams of technical urea was added, and the preparation incubated for 72 hours at this temperature. At this time 7 per cent by weight of commercial dextrose was added so that the final concentration of alcohol would be around 11 per cent by volume. The juice was again incubated at this temperature and allowed to ferment for 8 days. The fermentation was closely observed and when no further activity could be detected the wine was strained

through cheese cloth. The young wine was stored at 32° F. for a period of 2 weeks. Cold storage is advantageous since a clearer wine is produced as well as inhibiting growth of microorganisms.

The analysis of the wine before and after storage is shown in Table 5.

The wine was removed from cold storage and siphoned to remove the sediment that was formed. The siphoned wine was thoroughly mixed and samples taken for analysis which appears in Table 5.

Table 5

WINE ANALYSIS BEFORE AND AFTER STORAGE

Determinations	Before Storage	After Storage
Per cent alcohol by volume	11.7	11.7
Total acids as tartaric gm./100 ml.	0.0	0.0
Volatile acid as acetic gm./100 ml.	0.054	0.060
Degrees brix 20°/20° C.	-1.7	-1.8
Specific gravity at 20°/20° C.	0.9936	0.9932
pH	3.47	3.43

Methods of Determining Inhibition of Volatile Acids Production in High Alcohol Wine

The procedure for testing the inhibition of volatile acid production in the higher alcoholic wine was as

follows: Three samples for each concentration were used. Each sample consisted of 4/5 quart screw-capped bottle containing 598 ml. of wine. The preservatives were added so that 2.0 ml. of the stock solution would bring the final volume to 600 ml. at the desired concentration. The concentrations used are shown in Table 6. Three control samples with no preservatives added were also used.

Table 6

CONCENTRATIONS USED IN HIGH ALCOHOL WINE

Preservative	Concentration in ppm		
Vitamin K_5	20 80	40 100	60 200
Sulfur dioxide	100	200	300

All bottles were stored at room temperature with the caps loosely fitted. All three bottles at each concentration were analyzed for volatile acid content at 30, 60, 90, and 120 days.

The volatile acid content in all cases was determined by steam distillation according to the procedure described by the Association of Official Agricultural Chemists (3, p.173-4).

Macroscopic Surface Growth in Wine

At the end of the 120-day period, a slight amount of surface growth resembling mold was observed. As this may have affected further analysis, the experiment was discontinued. The growth was then observed and recorded at 15, 30, 45, 60, 75, and 90 days.

Preparation of Low Alcohol Wine

For this study juice was obtained using the same procedure as was used in preparation of the higher alcoholic wine.

Approximately 5½ gallons of juice was removed from storage and placed in two 3-gallon containers and samples removed for analysis which appears in Table 7. Clean cloths were placed over the tops of the containers and juice was allowed to ferment at room temperature without the addition of any chemicals or yeast culture.

Table 7

ANALYSIS OF APPLE JUICE

	Degrees brix at 20°/20° C.	Total acids as malic gm./100 ml.	pH	Specific gravity at 20°/20° C.
(1)	14.6	0.514	3.48	1.0594
(2)	14.7	0.516	3.52	1.0598
Averages	14.65	0.515	3.50	1.0596

The fermentation and the development of volatile acids were followed closely. After 0.063 gm./100 ml. of volatile acid had developed in the young wine, it was strained through cheese cloth and thoroughly mixed. Samples were removed and analyzed as reported in Table 8.

Table 8

ANALYSIS OF FERMENTED JUICE

Degrees brix at 20°/20°	-0.2
Specific gravity at 20°/20°	0.9993
Volatile acids as acetic gm./100 ml.	0.063
pH	3.59
Total acids as tartaric acid gm./100 ml.	0.563
Per cent alcohol by volume	7.1

Methods of Testing Inhibition of Volatile Acid Production in Low Alcohol Wine

The procedure for testing inhibition of volatile acid production in low alcohol wine was as follows: Three samples for each concentration were used. Each sample consisted of a 4/5 quart screw-capped bottle containing 449 ml. of wine. The preservatives were added so that the addition of 1.0 ml. of the stock solution brought the final volume to 450.0 of the desired concentration. The concentrations used appear in Table 9. Three controls with no preservatives were also used.

Table 9

CONCENTRATIONS USED IN LOW ALCOHOL WINE

Preservative	Concentration in ppm					
Vitamin K_5	10	20	30	40	50	60
	70	80	90	100		
Sulfur dioxide	100	200	300			

All bottles were stored at room temperature with the caps loosely fitted. Each bottle was analyzed for volatile acid content at 2-, 4-, 6-, 8-, 10-, 13-, 15-, 17-, 21-, 25-, and 30-day storage periods.

The volatile acid content was determined by steam distillation in all samples according to the procedure described by the Association of Official Agricultural Chemists (3,p.173-4).

PART 3

Preparation of Wine for Plate Count Studies

Apple wine was made following the same procedure used in preparation of high alcohol wine (see page 15). When no further fermentation could be detected, the young wine was strained through cheese cloth and placed in cold storage at 36° F. After two weeks of storage the wine was removed, siphoned, and thoroughly mixed, and samples taken for analysis which are reported

in Table 10. Five per cent by weight of dry commercial sucrose was added to the wine to promote additional yeast growth.

Table 10

ANALYSIS OF WINE USED FOR PLATE COUNTING

Per cent alcohol by volume	11.5
Total acids as tartaric gm./100 ml.	0.570
Total volatile acids as acetic gm./100 ml.	0.030
Degrees brix at 20°/20° C.	-1.4
Specific gravity at 20°/20° C.	0.9947
pH	3.61

Methods of Following Inhibitory Effect of Vitamin K_5 in Wine by Plate Counting

The procedure for following effect of vitamin K_5 in wine by plate counting was as follows: Half-pint, screw-capped bottles, with cotton plugs were autoclaved, and 149 ml. of wine added by sterile pipettes to each bottle. The preservatives were added from stock solutions of such concentrations that the addition of 1.0 ml. brought the final volume to 150.0 ml. at the desired concentration. The concentrations used appear in Table 11.

The samples were incubated at 28° C. and plate counts made at 1, 2, 4, 6, 8, 10, and 12 days. Serial dilutions

using sterile water blanks were made. All plates were made using dextrose peptone agar (see page 9) and counted after 48 hours of incubation at 30° C.

Table 11

CONCENTRATIONS USED FOR PLATE COUNTING

Preservative	Concentration in ppm		
Vitamin K_5	10	20	40
Sulfur dioxide	100	200	300

PART 4

Preparation of Wine to Study Effect of Vitamin K_5 on Color

Apple wine was made following the same procedure used in preparation of high alcohol wine (see page 15). When no further fermentation could be detected the wine was strained through cheese cloth and placed in cold storage at 36° F. for a period of 4 weeks. The wine was then removed, siphoned, thoroughly mixed and samples taken for analysis, which are reported in Table 12.

Methods of Studying the Effect of Vitamin K_5 on Color of Apple Wine

The procedure for studying the effect of vitamin K_5 on the color of apple wine stored for one month was as

follows: Screw-capped, 4/5 quart bottles were thoroughly cleaned and 449.0 ml. of wine added to them. The preservatives were added from stock solutions of such concentrations that the addition of 1.0 ml. brought the final volume to 450.0 ml. at the desired concentration. Two bottles for each concentration and two with no additives for controls were used. The concentrations used appear in Table 13.

Table 12

ANALYSIS OF WINE USED FOR COLOR CHANGE STUDIES

Per cent alcohol by volume	11.7
Total acids as tartaric gm./100 ml.	0.578
Volatile acids as acetic gm./100 ml.	0.036
Degrees brix at 20°/20° C.	-1.3
Specific gravity at 20°/20° C.	0.9951
pH	3.58

Table 13

CONCENTRATIONS USED IN APPLE WINE STORED FOR ONE MONTH

Preservative	Concentration in ppm				
Vitamin K_5	10	30	50	80	100
Sulfur dioxide	100	200	300		

The same procedure was used for studying the color change in apple wine stored for 5 months. Similar wine

was used and the concentrations used appear in Table 14.

Table 14

CONCENTRATIONS USED IN APPLE WINE STORED FOR FIVE MONTHS

Preservative	Concentration in ppm			
Vitamin K_5	10	50	100	200
Sulfur dioxide	100	200	300	

All samples were stored at room temperature. At the end of the storage periods samples were taken and the extinction coefficient read on a Beckman Quartz Model DU spectrophotometer at wave lengths of 400, 425, 450, 475, 500, 525, 550, 575, 600, and 625 millimicrons.

RESULTS AND DISCUSSION

Effect of Vitamin K_3 to Inhibit Secondary Fermentation in Wine

The results of inhibitory effects of vitamin K_3 in the first experiment appear in Table 15 on page 27. The alcohol production inhibition in per cent was calculated according to the following equation:

$$q = \frac{a-b}{a} \times 100,$$

where "q" is alcohol inhibition in per cent, "a" is the alcohol increase in the control, and "b" is the alcohol increase in the sample containing the preservative. This allowed for a more accurate comparison between the samples.

Table 15, page 27, shows that 0.1 ppm of vitamin K_3 had no inhibitory effect on the production of alcohol, as the production of alcohol paralleled that of the control sample. The concentration of 1 ppm showed 50 per cent inhibition at 10 days, but only 35 per cent at 20 days, and no inhibition at 30 days. At the 5 ppm level 100 per cent inhibition was obtained, but this was reduced to 85 per cent at 20 days and finally to 79 per cent at 30 days of storage. This seems to indicate that effectiveness of vitamin K_3 diminishes with time,

perhaps due to the reactivation of yeast cells.

At a concentration of 10 ppm, complete inhibition was achieved and remained constant for the entire 30-day period. The results from this first experiment indicated that the concentration of vitamin K_5 that would completely inhibit alcohol production for 30 days was between 5 ppm and 10 ppm.

The results of Experiment 2 to pinpoint the concentration appear in Table 16, page 28. The alcohol production inhibition was calculated the same way as in Experiment 1 (see page 25).

Vitamin K_5

At a concentration of 6 ppm, vitamin K_5 showed 100 per cent inhibition at 10 days, 90 per cent at 20 days, and 82 per cent at 30 days. At a concentration of 7 ppm, 100 per cent inhibition was obtained for 20 days and 89 per cent inhibition for 30 days. Complete inhibition through the period of 30 days was shown by the 8 ppm and 9 ppm concentrations.

Sulfur Dioxide

Sulfur dioxide showed a distinct inhibitory effect as the concentration increased from 75 to 300 ppm. At the 100 and 75 ppm concentrations, no inhibition was

Table 15

Experiment 1
ALCOHOL CONTENT, AND ALCOHOL PRODUCTION INHIBITION, BY VITAMIN K_5

Vitamin K_5 Concentration ppm	10 days		20 days		30 days	
	% alcohol	"q"	% alcohol	"q"	% alcohol	"q"
0.1	12.60	0	13.60	0	14.63	0
1.0	12.10	50	12.90	35	14.43	0
5.0	11.60	100	11.90	85	12.20	79
10.0	11.57	100	11.60	100	11.60	100
Control	12.60	0	13.60	0	14.43	0

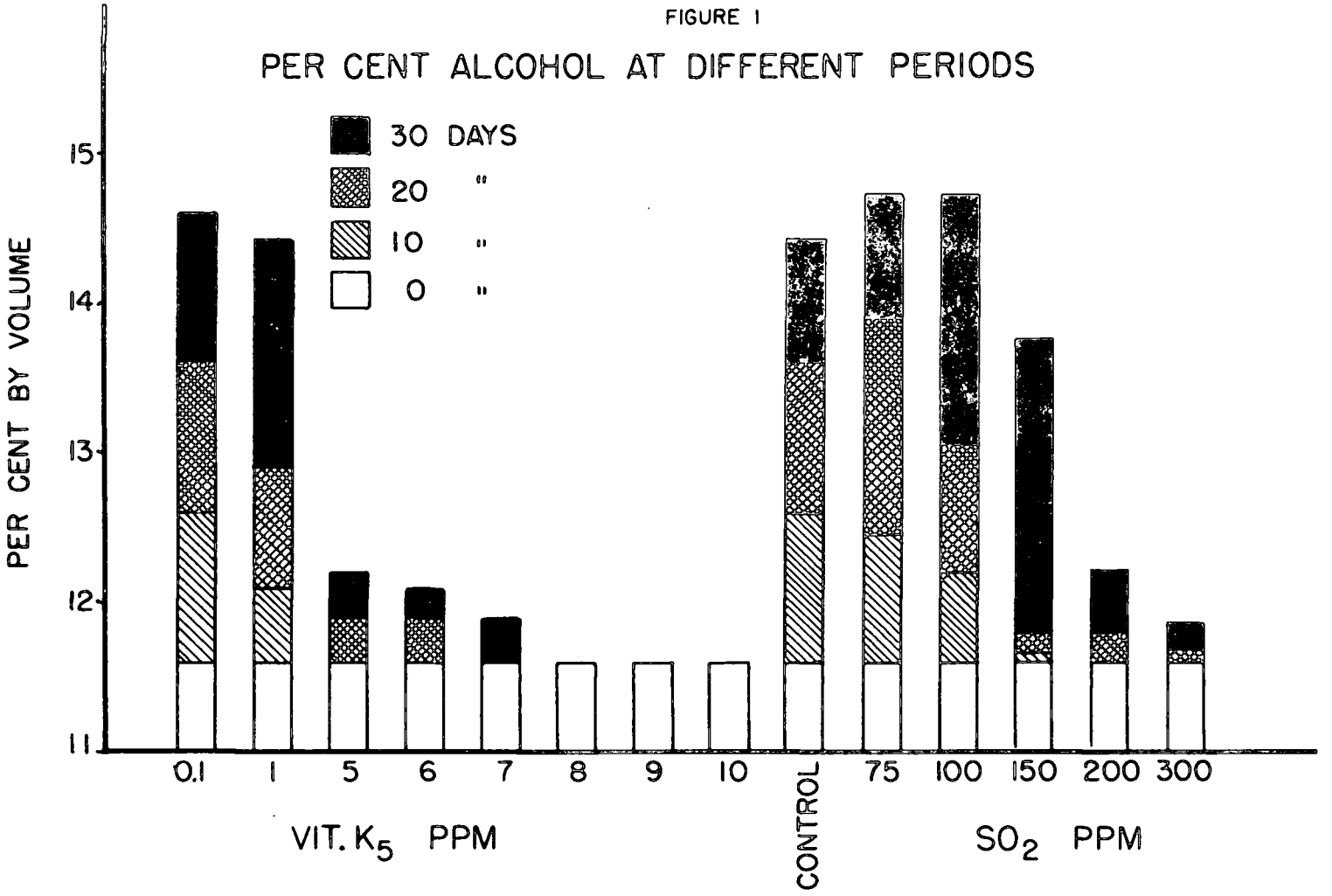
Table 16

Experiment 2

ALCOHOL CONTENT, AND ALCOHOL PRODUCTION INHIBITION, BY VITAMIN K_5 AND SULFUR DIOXIDE

Preservative and Concentration	10 days		20 days		30 days	
ppm	% alcohol	"q"	% alcohol	"q"	% alcohol	"q"
Vitamin K_5						
6	11.60	100	11.80	90	12.10	82
7	11.55	100	11.55	100	11.90	89
8	11.60	100	11.58	100	11.60	100
9	11.60	100	11.57	100	11.60	100
Sulfur Dioxide						
75	12.48	12	13.88	0	14.73	0
100	12.20	40	13.08	26	14.73	0
150	11.65	95	11.80	90	13.73	25
200	11.60	100	11.82	89	12.23	78
300	11.60	100	11.70	95	11.86	91
Control	12.60	0	13.60	0	14.43	0

FIGURE 1


PER CENT ALCOHOL AT DIFFERENT PERIODS
30 DAYS
20 "
10 "
0 "
PER CENT BY VOLUME
15
14
13
12
11
0.1
1
5
6
7
8
9
10
CONTROL
75
100
150
200
300
VIT. K_5 PPM
SO_2 PPM

obtained at periods of 20 and 30 days. At the 150 ppm concentration, 95 per cent and 90 per cent inhibition was obtained at the end of 10 and 20 days respectively, while only 25 per cent inhibition was obtained at the end of 30 days. The two highest concentrations of 200 and 300 ppm resulted in no increase in alcohol at the end of 10 days, showing a 100 per cent inhibition. However, these two concentrations exhibited lower inhibitory effects of 78 per cent and 91 per cent respectively on the 30th day, indicating that sulfur dioxide does not possess a lasting action. This is also indicated by the greater decrease in inhibitory power noted with the lower concentrations at the end of the 30-day period.

Comparison of Vitamin K_5 with Sulfur Dioxide

In comparison, the inhibitory power of 7 ppm of vitamin K_5 is greater than 300 ppm of sulfur dioxide at a concentration of 6 ppm, the effectiveness of vitamin K_5 is comparable to 200 ppm of sulfur dioxide.

Effect of Vitamin K_5 in Inhibiting Volatile Acids Production in High Alcohol Wine

Results obtained from the experimental work on high alcohol wine are reported in Tables 17 and 18, pages 32 and 33, and shown in Figure II. In all cases the total volatile acids is expressed as acetic acid. All results

are arithmetic means of three determinations for each concentration used. This method was used due to the small variation, never more than 0.006 gm./100 ml., between the highest and lowest determination. The volatile acids production inhibition in per cent was calculated according to a similar formula used for calculating alcohol production inhibition:

$$q = \frac{a-b}{a} \times 100,$$

where "q" is volatile acids production in per cent, "a" is the volatile acids produced in the control, and "b" is the volatile acids produced in the sample containing the preservative.

Vitamin K_5

Tables 17 and 18 show that 20 ppm of vitamin K_5 was not very effective in inhibiting the development of volatile acids. At the 30-day level 45 per cent inhibition was shown, while at 90- and 120-day levels only 24.1 and 24.3 per cent inhibition, respectively, was shown. Effective inhibition was shown with 40 ppm and 60 ppm at the 30-day level, but this inhibition decreased to 36.5 per cent and 60.8 per cent inhibition, respectively, at the 120-day level. At 80 ppm, there was no volatile

Table 17

VOLATILE ACIDS PRODUCTION IN HIGH ALCOHOL WINE

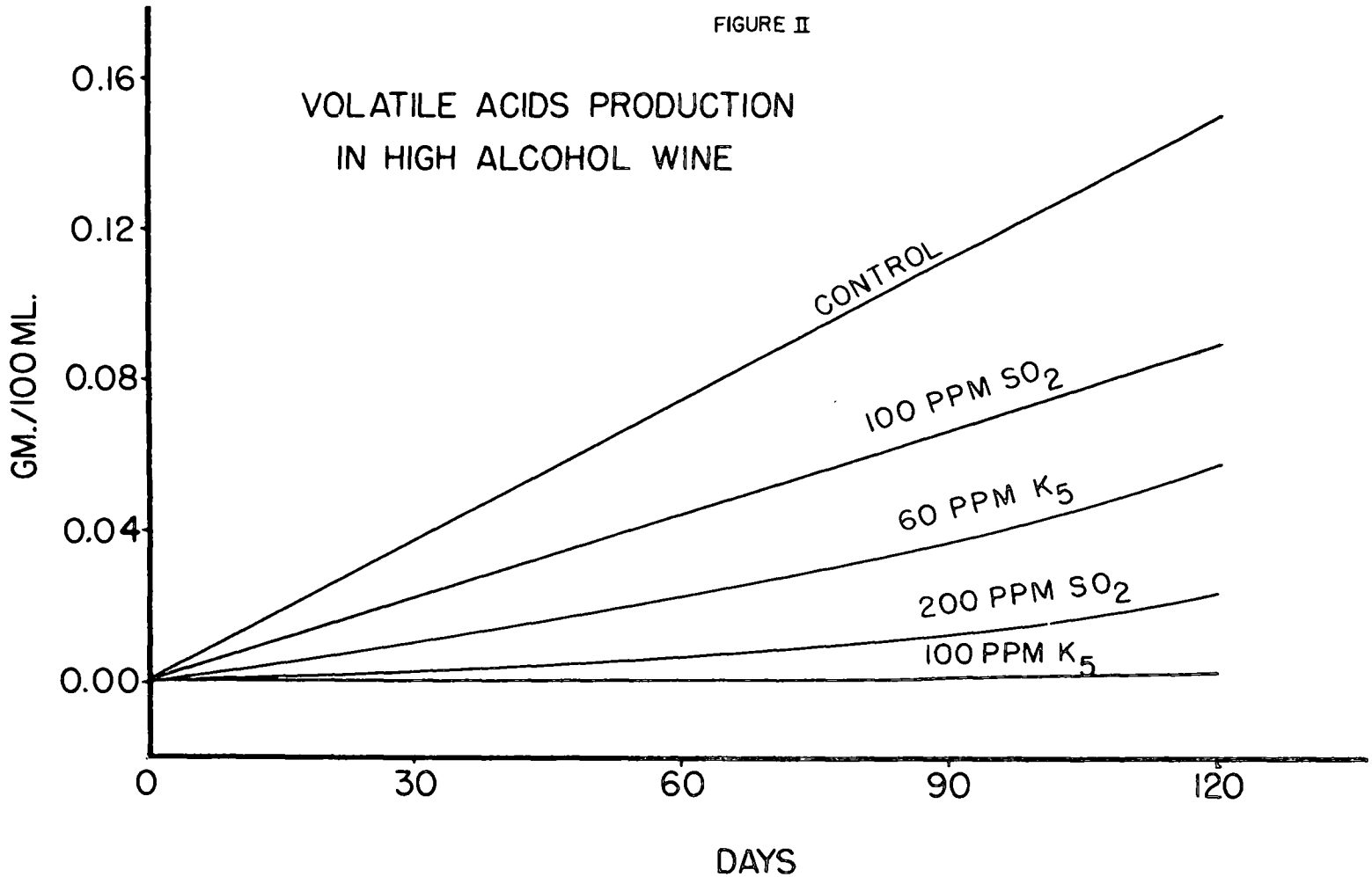
Preservative and Concentrations	Gm./100 ml.			
	30 days	60 days	90 days	120 days
Vitamin K_5				
20 ppm	0.022	0.058	0.088	0.112
40 ppm	0.004	0.024	0.070	0.094
60 ppm	0.002	0.010	0.034	0.058
80 ppm	0.000	0.003	0.009	0.024
100 ppm	0.000	0.000	0.000	0.003
200 ppm	0.000	0.000	0.000	0.000
Sulfur Dioxide				
100 ppm	0.018	0.046	0.064	0.090
200 ppm	0.006	0.090	0.012	0.022
300 ppm	0.000	0.000	0.000	0.000
Control	0.040	0.074	0.116	0.148

Table 18

PER CENT VOLATILE ACIDS PRODUCTION INHIBITION
IN HIGH ALCOHOL WINE

Preservative and Concentrations	30 days	60 days	90 days	120 days
Vitamin K_5				
20 ppm	45.0	21.6	24.1	24.3
40 ppm	90.0	67.6	39.7	36.5
60 ppm	95.0	86.5	70.7	60.8
80 ppm	100.0	96.0	92.2	83.8
100 ppm	100.0	100.0	100.0	98.0
200 ppm	100.0	100.0	100.0	100.0
Sulfur Dioxide				
100 ppm	30.0	37.8	44.8	39.2
200 ppm	85.0	87.8	94.5	85.1
300 ppm	100.0	100.0	100.0	100.0
Control	0.0	0.0	0.0	0.0

FIGURE II


VOLATILE ACIDS PRODUCTION
IN HIGH ALCOHOL WINE
GM./100 ML.
0.16
0.12
0.08
0.04
0.00
CONTROL
100 PPM SO2
60 PPM K5
200 PPM SO2
100 PPM K5
0
30
60
90
120
DAYS

acids production, or 100 per cent inhibition, at the 30-day level. This concentration obtained 96, 92.2, and 83.8 per cent inhibition at 60-, 90-, and 120-day levels. The two highest concentrations, 100 ppm and 200 ppm, obtained 100 per cent inhibition at all time-levels, except at the 120-day level, where 98 per cent inhibition was obtained with the 100 ppm concentration.

In these results, as was shown in the inhibitory effect of vitamin K_5 in controlling secondary fermentation, a relationship exists between vitamin K_5 and time. In all cases excepting for the 90- and 120-day level of the 20 ppm concentrations, all successive time levels resulted in a lower per cent inhibition; this of course was not true where 100 per cent inhibition was obtained throughout the different time levels.

Sulfur Dioxide

Sulfur dioxide showed a distinct inhibitory effect as the concentration increased from 100 to 300 ppm. At the 120-day level no volatile acids were produced, or 100 per cent inhibition was obtained, with the 300 ppm concentration. At the 200 ppm and 100 ppm concentrations, 85.1 and 39.2 per cent inhibition was shown at the 120-day level.

Comparison of Vitamin K_5 with Sulfur Dioxide

Vitamin K_5 proved to be more effective than sulfur dioxide as an inhibitory agent for production of volatile acids. At the 30-day level 20 ppm of vitamin K_5 showed 45.0 per cent inhibition, while 100 ppm of sulfur dioxide obtained 30.0 per cent inhibition. Also at this time level, 40 ppm of vitamin K_5 obtained more effective inhibition than did 200 ppm of sulfur dioxide, and 80 ppm of the vitamin compared with 300 ppm of sulfur dioxide. At the 60-day level, 20 ppm proved less, while 40 ppm of the vitamin, proved more effective than 100 ppm of sulfur dioxide. Also, at this time level, 60 ppm proved more effective than did 200 ppm of sulfur dioxide, while 100 ppm of the vitamin was as effective as 300 ppm of sulfur dioxide, both obtaining 100 per cent inhibition. At the 90-day level, 60 ppm of vitamin K_5 had much greater inhibitory power than did 100 ppm of sulfur dioxide, while 80 ppm had more power than did 200 ppm of sulfur dioxide. Also, at this time level, 100 ppm of the vitamin proved as effective as 300 ppm of sulfur dioxide. At the 120-day level, 60 ppm of the vitamin proved more effective than 100 ppm of sulfur dioxide, while the effectiveness of 80 ppm compared with 200 ppm of sulfur dioxide. Also, at this time level, 200 ppm of vitamin K_5 proved as

effective as 300 ppm of sulfur dioxide, both obtaining 100 per cent inhibition.

Macroscopic Surface-Growth in Wine

The amount of white mold-like surface growth observed growing in the high alcohol wine after 120 days appears in Table 19, page 38.

The results show that no surface growth was observed at the lowest concentration, 20 ppm, of vitamin K_5. This was true for all vitamin K_5 samples and thus the higher concentrations 70, 80, 90, and 100 ppm are omitted from the table.

Sulfur dioxide showed a distinct inhibitory effect as the concentration increased from 100 to 300 ppm. Slight growth appeared at the 135-day level in the 100 ppm sample, while slight growth did not appear in the 200 and 300 ppm samples until the 150- and 165-day levels, respectively. Moderate growth appeared in the 100 ppm sample at 150-day level, while in the 200 and 300 ppm samples appeared at 165- and 180-day levels, respectively. Heavy growth appeared in the 100 ppm sample at the 180-day level and appeared at the 210-day level in both 200 and 300 ppm concentrations. The control samples obtained heavy growth at the 150-day level.

Table 19

MACROSCOPIC SURFACE-GROWTH IN WINE

Preservative and Concentration	Amount of Growth					
	135 Days	150 Days	165 Days	180 Days	195 Days	210 Days
Vitamin K_5						
20 ppm						
1	none	none	none	none	none	none
2	none	none	none	none	none	none
3	none	none	none	none	none	none
40 ppm						
1	none	none	none	none	none	none
2	none	none	none	none	none	none
3	none	none	none	none	none	none
60 ppm						
1	none	none	none	none	none	none
2	none	none	none	none	none	none
3	none	none	none	none	none	none
Sulfur Dioxide						
100 ppm						
1	slight	medium	medium	heavy	heavy	heavy
2	none	slight	medium	medium	medium	heavy
3	none	slight	slight	slight	heavy	heavy
200 ppm						
1	none	slight	slight	medium	medium	heavy
2	none	slight	medium	medium	medium	heavy
3	none	none	slight	medium	medium	medium
300 ppm						
1	none	none	slight	slight	medium	heavy
2	none	none	none	slight	slight	slight
3	none	none	none	medium	medium	heavy
Control						
1	medium	heavy	heavy	heavy	heavy	heavy
2	slight	medium	medium	medium	heavy	heavy
3	none	medium	medium	heavy	heavy	heavy

In comparing the inhibitory effects of vitamin K_5 with sulfur dioxide to control this surface growth, it was obvious that vitamin K_5 was by far more effective. The results indicate that sulfur dioxide seems to lose its inhibitory effect more rapidly than does vitamin K_5.

Effect of Vitamin K_5 to Inhibit Volatile Acids Production in Low Alcohol Wines

The results obtained from the experimental work on low alcohol wine are reported in Tables 20 and 21, pages 40 and 41 and shown in Figure III. The same procedure used for reporting volatile acids production and calculating per cent inhibition is used here, as was used in high alcohol wines (see page 30).

Vitamin K_5

At a concentration of 10 ppm, at the 2-day level, no volatile acids were produced, or 100 per cent inhibition was obtained. The inhibition was reduced in the following days, and at the 30-day level only 50.6 per cent was obtained. The concentration of 20 ppm showed 100 per cent inhibition at the 4-day level, 93.8 per cent at the 6-day level, and finally 38.4 per cent at the 30-day level. This general trend appears throughout the results. As the concentration increased, 100 per cent inhibition was obtained for a longer period of time.

Table 20

VOLATILE ACIDS PRODUCTION IN LOW ALCOHOL WINE

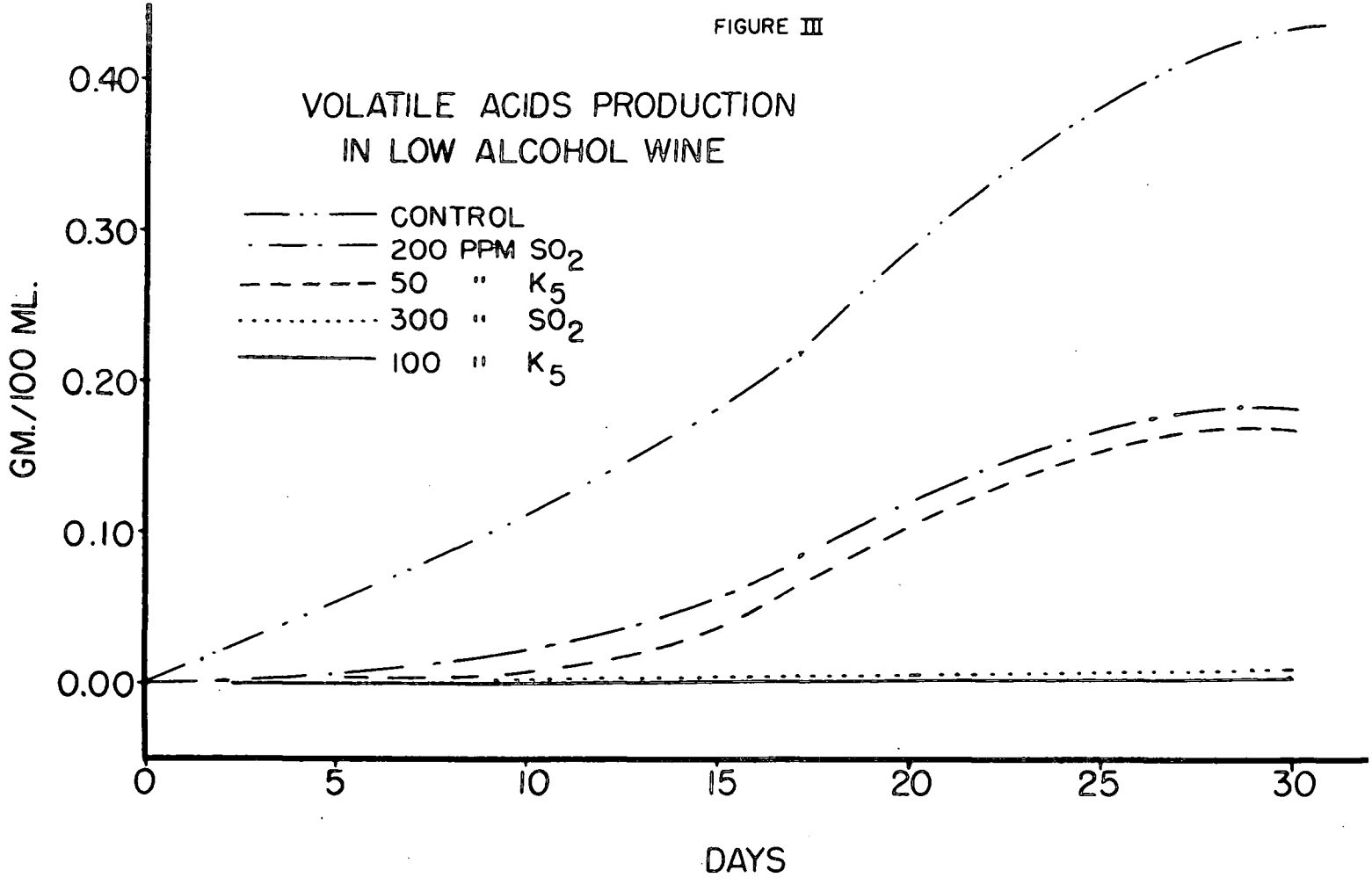
Preservative and Concentrations	Gm./100 ml. Days Storage										
	2	4	6	8	10	13	15	17	21	25	30
Vitamin K_5											
10 ppm	0.000	0.012	0.024	0.042	0.060	0.087	0.108	0.144	0.204	0.246	0.300
20 ppm	0.000	0.000	0.006	0.018	0.024	0.052	0.088	0.111	0.170	0.230	0.266
30 ppm	0.000	0.000	0.003	0.006	0.021	0.030	0.060	0.086	0.145	0.208	0.252
40 ppm	0.000	0.000	0.000	0.006	0.020	0.024	0.042	0.075	0.130	0.165	0.174
50 ppm	0.000	0.000	0.000	0.000	0.003	0.015	0.036	0.066	0.123	0.153	0.165
60 ppm	0.000	0.000	0.000	0.002	0.002	0.006	0.009	0.015	0.036	0.060	0.093
70 ppm	0.000	0.000	0.000	0.000	0.000	0.003	0.009	0.012	0.024	0.036	0.054
80 ppm	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.012	0.021	0.036
90 ppm	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.006	0.012
100 ppm	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.003	0.003
Sulfur Dioxide											
100 ppm	0.000	0.006	0.015	0.036	0.066	0.090	0.111	0.138	0.168	0.222	0.258
200 ppm	0.000	0.000	0.006	0.009	0.015	0.036	0.054	0.087	0.135	0.168	0.180
300 ppm	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.003	0.006	0.006
Control	0.006	0.024	0.096	0.120	0.144	0.156	0.180	0.216	0.312	0.384	0.432

Table 21

PER CENT VOLATILE ACIDS PRODUCTION INHIBITION IN LOW ALCOHOL WINE

Preservative and Concentrations	Days Storage										
	2	4	6	8	10	13	15	17	21	25	30
Vitamin K_5											
10 ppm	100.0	50.0	75.0	68.3	58.3	44.2	40.0	33.3	34.6	35.9	30.6
20 ppm	100.0	100.0	93.8	85.0	83.3	66.7	51.1	48.6	45.6	40.1	38.4
30 ppm	100.0	100.0	96.9	95.0	85.4	80.8	66.7	60.2	53.5	70.8	41.7
40 ppm	100.0	100.0	100.0	95.0	86.1	84.6	76.7	65.3	58.3	75.7	59.7
50 ppm	100.0	100.0	100.0	100.0	97.9	90.4	80.0	69.4	60.6	60.2	61.8
60 ppm	100.0	100.0	100.0	98.3	98.6	96.2	95.0	93.1	88.5	84.4	78.5
70 ppm	100.0	100.0	100.0	100.0	100.0	98.1	95.0	94.4	92.3	90.6	87.5
80 ppm	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.6	96.2	94.5	91.7
90 ppm	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.0	98.4	97.2
100 ppm	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	99.2	99.3
Sulfur Dioxide											
100 ppm	100.0	75.0	84.4	70.0	54.2	42.3	38.3	36.1	46.2	42.2	40.3
200 ppm	100.0	100.0	93.8	92.5	89.6	76.9	70.0	59.7	56.7	56.3	81.5
300 ppm	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.1	99.0	98.4	98.6
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

FIGURE III


VOLATILE ACIDS PRODUCTION
IN LOW ALCOHOL WINE
CONTROL
200 PPM SO_2
50 " K_5
300 " SO_2
100 " K_5
GM./100 ML.
0.40
0.30
0.20
0.10
0.00
0
5
10
15
20
25
30
DAYS

The two highest concentrations 90 and 100 ppm produced 100 per cent inhibition at the 17-day level, and obtained 97.2 and 99.3 per cent inhibition, respectively, at the 30-day level. The same relationship between the vitamin and time was noticed in the inhibiting effect of vitamin K_5 in controlling secondary fermentation and in preventing the development of volatile acids in high alcohol wine (see pages 25 and 30).

Sulfur Dioxide

This preservative showed a distinct inhibitory effect as the concentration increased from 100 to 300 ppm. All concentrations obtained 100 per cent inhibition at the 2-day level. Again the trend of higher concentrations to show 100 per cent inhibition for longer periods of time was shown. The concentration of 200 ppm showed 100 per cent inhibition at the 4-day level, while 300 ppm showed this inhibition at the 15-day level. At the 30-day level, 100 ppm showed 40.3 per cent inhibition, while 200 and 300 ppm showed 81.5 and 98.6 per cent, respectively.

Comparison of Vitamin K_5 with Sulfur Dioxide

Throughout the experiment 30 ppm of vitamin K_5 showed greater inhibition than did 100 ppm of sulfur

dioxide. The 30-day inhibition for the vitamin being 41.7 per cent, while 40.3 per cent was obtained for sulfur dioxide. The concentration of 80 ppm of vitamin K_5 proved more effective at all time levels than did 200 ppm of sulfur dioxide. The 30-day level inhibition for the vitamin being 99.3 per cent, while 98.6 per cent was obtained with sulfur dioxide. At a concentration of 100 ppm the vitamin proved more effective than did 300 ppm of sulfur dioxide; the 30-day level of inhibition being 99.3 and 98.6 per cent, respectively.

Inhibitory Power Shown by Vitamin K_5 in Reducing Number of Microorganisms in Light Sweet Wine

The total number of colonies developing per ml. of wine upon plating is shown in Table 22, page 45. The increase and decrease in relation to the initial count was calculated and appears in Table 23, pages 46 and 47, and in Figure IV.

The results show that all concentrations of vitamin K_5 used produced a 100 per cent decrease in colonies developing per ml. of wine. At a concentration of 10 ppm the vitamin showed this decrease at the 4-day level, while this decrease was obtained at the 1- and 2-day levels with 40 ppm and 20 ppm, respectively. Further plating after these time levels showed no colonies developing. Sulfur dioxide showed a 100 per cent decrease

Table 22

PLATE COUNTS OF LIGHT SWEET WINE AT DIFFERENT PERIODS

Preservative and Concentrations	Per Cent Increase or Decrease						
	1 day	2 days	4 days	6 days	8 days	10 days	12 days
Vitamin K_5							
10 ppm	-34.2	-78.2	-100	0	0	0	0
20 ppm	-53.2	-100	0	0	0	0	0
40 ppm	0	0	0	0	0	0	0
Sulfur Dioxide							
100 ppm	703	1,611	3,497	9,374	17,575	37,619	56,304
200 ppm	307	532	1,319	2,849	6,026	14,655	26,343
300 ppm	-100	0	0	0	0	0	0
Control	2,903	9,700	15,220	17,829	25,367	48,833	67,279

Table 23

NUMBER OF COLONIES DEVELOPING IN LIGHT SWEET WINE AT DIFFERENT PERIODS

Preservative and Concentrations	Number of Colonies Developing per Ml.				
	Initial	1 day	2 days	4 days	6 days
Vitamin K_5					
10 ppm	2.68×10^5	1.8×10^5	5.93×10^4	0	0
20 ppm	2.37×10^5	1.11×10^5	0	0	0
40 ppm	2.05×10^5	0	0	0	0
Sulfur Dioxide					
100 ppm	2.28×10^5	1.83×10^6	3.9×10^6	8.2×10^6	2.15×10^7
200 ppm	2.53×10^5	1.03×10^6	1.6×10^6	3.59×10^6	7.46×10^6
300 ppm	2.15×10^5	0	0	0	0
Control	2.25×10^5	9.0×10^6	2.205×10^7	3.402×10^7	4.034×10^7

Table 23 (Contd)

NUMBER OF COLONIES DEVELOPING IN LIGHT SWEET WINE AT DIFFERENT PERIODS

Preservative and Concentrations	Number of Colonies Developing per Ml.			
	Initial	8 days	10 days	12 days
Vitamin K_5				
10 ppm	2.68×10^5	0	0	0
20 ppm	2.37×10^5	0	0	0
40 ppm	2.05×10^5	0	0	0
Sulfur Dioxide				
100 ppm	2.28×10^5	4.03×10^7	8.62×10^7	1.286×10^8
200 ppm	2.53×10^5	1.55×10^7	3.733×10^7	6.69×10^7
300 ppm	2.15×10^5	0	0	0
Control	2.25×10^5	5.73×10^7	9.66×10^7	1.516×10^8

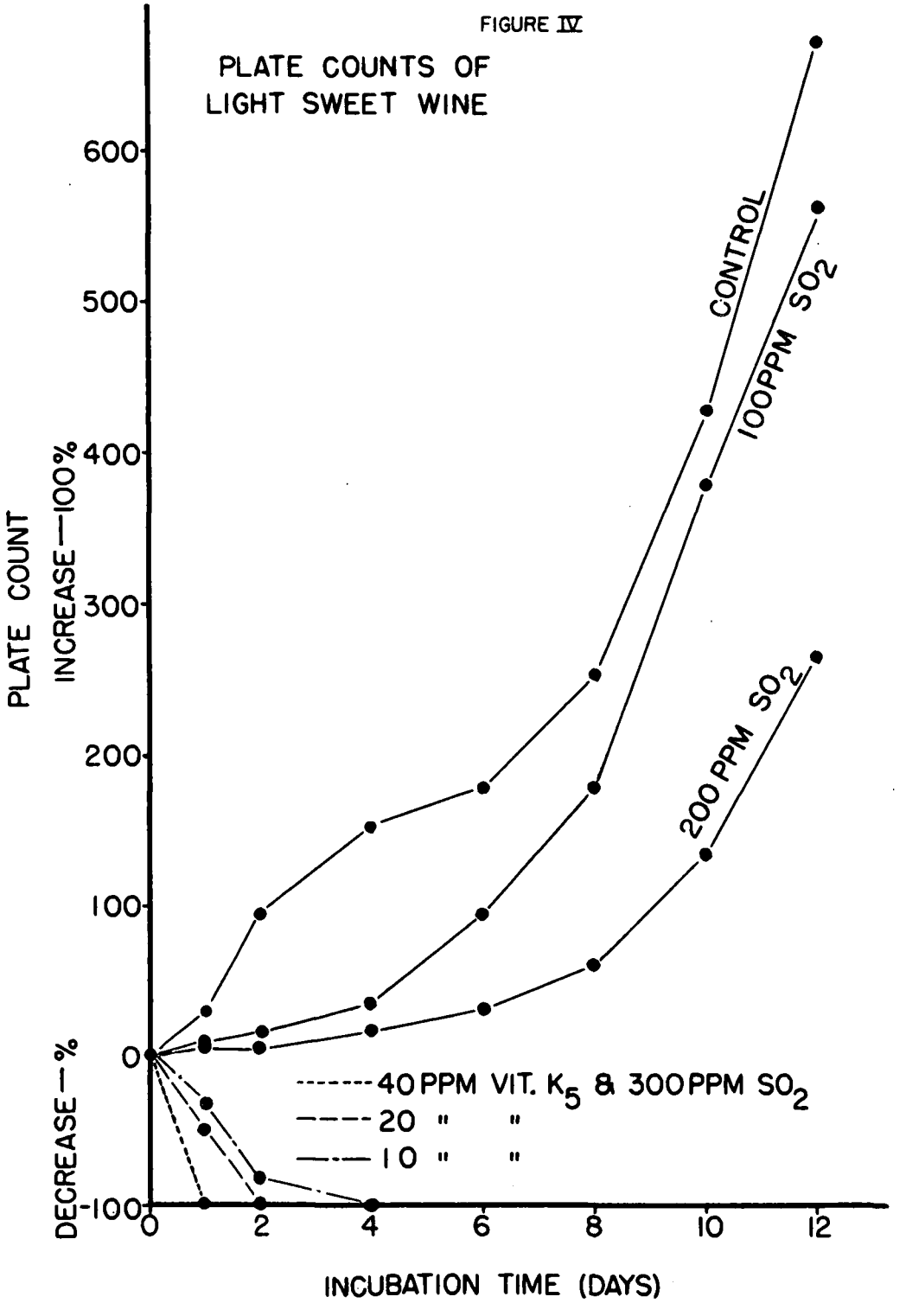


FIGURE IV
PLATE COUNTS OF
LIGHT SWEET WINE
PLATE COUNT
INCREASE—100%
DECREASE—%
600
500
400
300
200
100
0
-100
0
2
4
6
8
10
12
INCUBATION TIME (DAYS)
CONTROL
100PPM SO2
200 PPM SO2
40 PPM VIT. K5 & 300PPM SO2
20 " "
10 " "

at the 300 ppm concentration, which was obtained at the 1-day level. Throughout the entire incubation period 200 ppm showed a greater inhibitory power than did the 100 ppm concentration of sulfur dioxide. In comparing the two preservatives, 10 ppm of the vitamin proved more effective than did 100 and 200 ppm of sulfur dioxide, while 40 ppm proved as effective as 300 ppm of sulfur dioxide.

Effect of Vitamin K_5 on the Color of Apple Wine

Wine with One Month Storage: The extinction coefficient or E value for the different concentration levels of preservatives appear in Table 24, page 50 and shown in Figure V.

The results show that vitamin K_5 had a pronounced darkening effect, while sulfur dioxide had a bleaching effect. This was especially shown at the shorter wave lengths. At 400 mu, the E value for the control was 0.606, while for the 100 ppm concentration of vitamin K_5 and sulfur dioxide it was 1.16 and 0.452, respectively. At the higher wave lengths this difference was not as noticeable, the E value at 625 mu being 0.013 for the control, 0.009 and 0.037 for 100 ppm of sulfur dioxide and vitamin K_5. As the concentration of sulfur dioxide increased from 100 to 200 ppm there was not an appreciable difference

Table 24

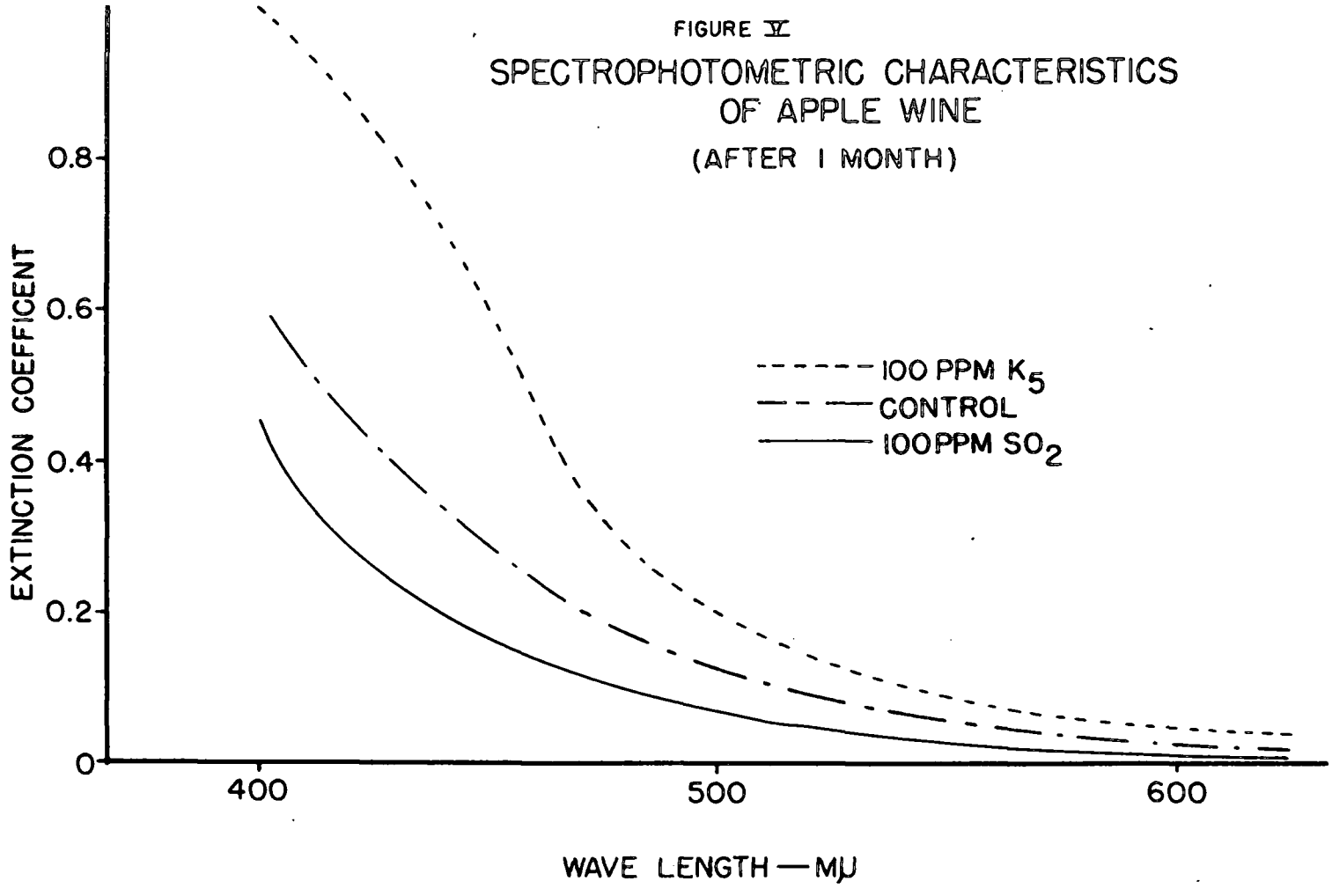
SPECTROPHOTOMETRIC CHARACTERISTICS OF
APPLE WINE AFTER ONE MONTH STORAGE

Preservative and Concentrations	Extinction Coefficient									
	Wave Length-Millimicrons									
	400	425	450	475	500	525	550	575	600	625
Vitamin K_5										
10 ppm	0.878	0.692	0.497	0.246	0.130	0.081	0.052	0.031	0.021	0.013
50 ppm	1.080	0.900	0.623	0.308	0.165	0.105	0.074	0.031	0.037	0.027
100 ppm	1.160	0.845	0.602	0.318	0.178	0.120	0.085	0.061	0.046	0.037
200 ppm	1.160	0.895	0.658	0.328	0.182	0.124	0.090	0.065	0.051	0.044
Sulfur Dioxide										
100 ppm	0.452	0.260	0.169	0.112	0.073	0.047	0.030	0.016	0.012	0.009
200 ppm	0.434	0.255	0.154	0.100	0.164	0.040	0.025	0.016	0.012	0.009
300 ppm	0.457	0.254	0.166	0.112	0.077	0.051	0.038	0.027	0.019	0.018
Control	0.606	0.424	0.294	0.177	0.108	0.070	0.046	0.031	0.019	0.013

FIGURE V

SPECTROPHOTOMETRIC CHARACTERISTICS OF APPLE WINE

(AFTER 1 MONTH)



in the bleaching action. Vitamin K_5, likewise, did not show an appreciable darkening effect when the concentration increased from 100 to 200 ppm.

By visual inspection the wine containing the vitamin had a richer and darker color than did the control or wine containing the sulfur dioxide. The control possessed a darker and more acceptable color than did the wine with the sulfur dioxide.

Wine with Five Months Storage: The extinction coefficient or E values for the different concentration levels appear in Table 25, page 53 and shown in Figure VI.

As in the results for the wine with one month storage the samples containing vitamin K_5 possessed a darker color as shown by the higher E values. Here again this was more noticeable at the shorter wave lengths. At 400 mu the E value for the control sample was 0.296, while for the 100 ppm sample of vitamin K_5 and sulfur dioxide it was 0.655 and 0.203. At 625 mu the E value for the control was 0.029 and for the 100 ppm samples of vitamin K_5 and sulfur dioxide it was 0.018 and 0.027, respectively. The effect of bleaching or darkening as the concentration of preservative increased was not as noticeable as it was in the wine stored for one month. This was shown by the smaller differences in E values.

Table 25

SPECTROPHOTOMETRIC CHARACTERISTICS OF
APPLE WINE AFTER FIVE MONTHS STORAGE

Preservative and Concentrations	Extinction Coefficient									
	Wave Length-Millimicrons									
	400	425	450	475	500	525	550	575	600	625
Vitamin K_5										
10 ppm	0.559	0.442	0.315	0.152	0.083	0.057	0.024	0.033	0.023	0.017
30 ppm	0.573	0.452	0.317	0.148	0.078	0.050	0.030	0.028	0.020	0.014
50 ppm	0.628	0.498	0.354	0.179	0.103	0.074	0.056	0.049	0.039	0.034
80 ppm	0.609	0.467	0.319	0.155	0.082	0.054	0.038	0.028	0.017	0.015
100 ppm	0.635	0.476	0.324	0.161	0.087	0.058	0.024	0.029	0.018	0.018
Sulfur Dioxide										
100 ppm	0.230	0.143	0.105	0.079	0.061	0.047	0.039	0.035	0.031	0.027
200 ppm	0.199	0.114	0.077	0.051	0.034	0.023	0.017	0.013	0.012	0.011
300 ppm	0.203	0.117	0.077	0.052	0.036	0.024	0.019	0.015	0.012	0.111
Control	0.296	0.198	0.142	0.099	0.073	0.057	0.046	0.039	0.030	0.029

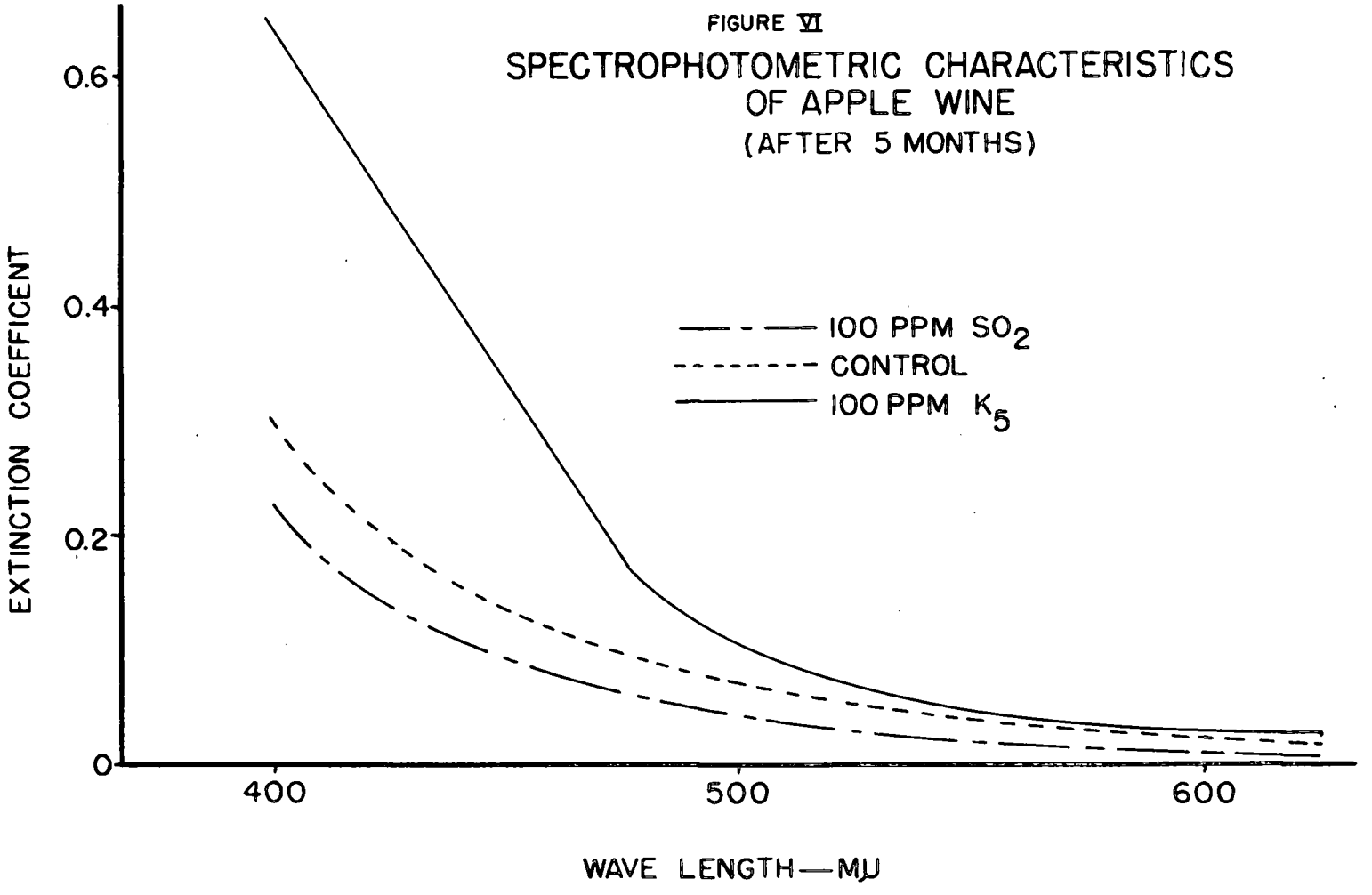


FIGURE VI
SPECTROPHOTOMETRIC CHARACTERISTICS
OF APPLE WINE
(AFTER 5 MONTHS)
100 PPM SO_2
CONTROL
100 PPM K_5
EXTINCTION COEFFICENT
0.6
0.4
0.2
0
400
500
600
WAVE LENGTH—MU

The control sample appeared lighter, which seemed to indicate that the wine had bleached during storage.

By visual inspection the vitamin K_5 samples did not possess as dark color as did the wine stored for one month. The color, however, was darker and richer than the control and sulfur dioxide samples by a considerable extent.

From the results obtained in this study it would indicate that vitamin K_5 would be very beneficial for wines such as apple, that lack much appeal due to their pale appearance. However in wines where bleaching is desirable, the addition of vitamin K_5 would prove harmful.

SUMMARY AND CONCLUSIONS

The first section of this work dealt with pinpointing the amount of vitamin K_5 needed to completely inhibit secondary fermentation. A series of two experiments was run using different concentrations. Sulfur dioxide was used as a comparative standard. Prior to the addition of the preservatives, 5 per cent of commercial sucrose was added to the wine after completion of fermentation. The alcohol production was used as the index of the effectiveness of the preservatives to inhibit secondary fermentation.

The second section consisted of investigating the inhibitory effect of vitamin K_5 to control the production of volatile acids in high and low alcohol apple wines. Sulfur dioxide was used again as a comparative standard. The amount of volatile acids produced was used as the index of effectiveness.

The third section consisted of following the inhibitory effect of vitamin K_5 in wine by plate counting. Sulfur dioxide was used as a comparative standard. Before the addition of the preservatives, 5 per cent commercial sucrose was added to the wine. Plate counts were made of wine at different periods.

The last section dealt with the study of the effect of vitamin K_5 on the color of apple wine after 1- and

5-month storage periods. Here again, sulfur dioxide was used as a comparative standard. The color was measured with a spectrophotometer.

The results indicated the following conclusions:

1. Vitamin K_5 completely inhibited secondary fermentation for a 30-day period at concentration of 8 ppm. The inhibitory power of 7 ppm of vitamin K_5 was greater than 300 ppm of sulfur dioxide.

2. Vitamin K_5 proved more effective than sulfur dioxide in inhibiting volatile acids production in both high and low alcoholic apple wine. At a concentration of 30 ppm, vitamin K_5 was more effective than 100 ppm of sulfur dioxide.

3. All concentrations of vitamin K_5 (i.e. 10, 20, and 40 ppm) showed a 100 per cent decrease of colonies of microorganisms developing in plates made from light sweet apple wine samples. A concentration of 40 ppm of vitamin K_5 proved as effective as 300 ppm of sulfur dioxide, both obtaining a 100 per cent decrease at the 1-day period.

4. Vitamin K_5, when added to apple wine, produced a darker and richer color than was observed in the control and sulfur dioxide samples. This was true in both the 1- and 5-month storage periods.

Throughout this entire investigation, vitamin K_3, disregarding cost, proved superior to sulfur dioxide in all ways. It imparted a richer color to apple wine and showed a much stronger antimicrobial power, when compared on concentration basis.

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