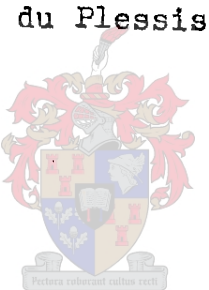
ION EXCHANGE IN WINE MAKING WITH SPECIAL REFERENCE TO THE HYDROGEN CYCLE TREATMENT OF WHITE MUSTS.

by

C. S. du Plessis.



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CHAPTER I.

A. INTRODUCTION.

(a) The Ion Exchanging Resins.

The principle of ion exchange was applied unknowingly by many ancient peoples. The miracle supposedly performed by Moses as he led the Israelites through the wilderness suggests the possibility of ion exchange. In order to make the bitter water at Marah potable, Moses had a tree cast into the waters; "the waters were made sweet". It has been suggested that the oxidized cellulose of the tree entered into an exchange reaction with the bitter electrolytes of the water rendering the water potable. However, the credit for recognition is generally attributed to Thompson and Way (1850). Only about eighty years later did Adams and Holmes (1935) succeed in synthesizing a stable resin which could exchange cations and another which could exchange anions.

The usefulness of resins as well as the possibility of manufacturing resins for specific purposes was immediately realized and exploited.

Definition of Ion Exchange.

Ion Exchange can be defined as a reversible exchange of ions between a liquid phase and a solid body which does not involve any radical change in the structure of the solid.

The Cation and Anion Exchanging Resins.

1. The Anion Exchange Resins.

The Anion Exchange resins are basic resins and of varying basisities. They can exchange anions as in the following examples:-

$$Cl^- + OH^-R \rightleftharpoons Cl^-R + OH^-$$

$$Cl^- + F^-R \rightleftharpoons F^-R + Cl^-R.$$

2. The Cation Exchange Resins.

The cation exchange resins are acid resins and of varying acidities. The resin used in this experiment was of the strong acid type, containing only one functional group viz. the sulfonic group. When such a group is made into sulfonic acid i.e. hydrogen ions are made available to the $-SO_3^-$ anions, ($R-SO_3H$) then it is able to function in the hydrogen cycle.

Structure of an Organic Cation Exchange Resin.

The inactive part or the resin skeleton of a mono-functional sulfonic cation exchanging resin consists of two basic compounds viz. styrene and divinyl benzene. When styrene is polymerised, long linear chains of polystyrene are formed having the following general formula:-

$$H-\left[\begin{matrix}CH-CH_2\\ |\\ C_6H_5\end{matrix}\right]_n-\begin{matrix}C=CH_2\\ |\\ C_6H_5\end{matrix}$$

However, such a polymer is still soluble in aromatic hydrocarbons and many esters. Introduction of divinyl benzene into the polystyrene molecule leads to products with decreased solubility. They have the same general chemical structure except that the linear polystyrene molecules are now bound or linked together into one vast molecular network at more or less infrequent intervals by divinyl bezene units. This is known as cross-linking. The percentage of divinyl bezene contained by a resin is also expressed as the percentage cross-linkage.

The copolymer of styrene and divinyl benzene is as such an inactive compound. In point of fact it is only attacked by nitric acid. To this skeleton can be attached

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various ionic groups which will determine its properties, in fact the ionic character of the group is the same as it is in a simple organic compound. A resin in which the sulfonic acid group is incorporated would exhibit relatively strong acidic properties. Similarly, by incorporating an aliphatic amine in the resin, a basic resin will result. Figure 1 gives a schematic illustration of a sulfonic acid resin.

It has been shown by X-Ray diffraction that the ionic groups are randomly dispersed throughout the interior of the resin particle. The majority of the ionic groups or exchange sites are situated within the particle. However, the sulfonic anions ($-SO_3^-$) are free to move with the network and to rotate and vibrate about the network but their motion away from the structure is limited by the immobility of the network as a whole.

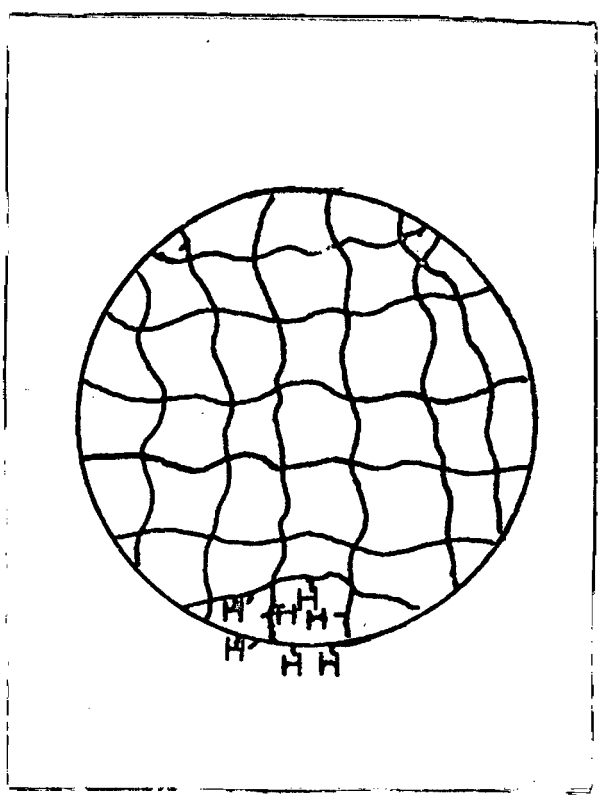

Figure 1. Schematic illustration of the basic structure of a sulphonic acid rosin. The short strokes to the hydrogen ions in the completed portion represent the $-SO_3^-$ anions.

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It is logical to suppose that the more reactive groups in a resin the higher will be its capacity per unit volume. However, the more reactive groups added the more they will tend to solubilize the resin and the more the resin will swell. Excessive swelling will greatly impair its exchanging properties. To prevent an increase in swelling more cross-linkages have to be brought in i.e. a higher percentage of divinyl benzene. So it will be seen that to increase the exchange capacity one would have to increase the cross-linkage. The overall effect is a denser resin. Now since exchange of ions is primarily within the resin particle, the ions move or diffuse into the structure by way of the molecular channels or pores to effect exchange. Therefore, if these passage-ways are decreased in size (by a higher degree of cross-linking) ultimately only the smallest of ions would be able to enter. The progressive tightening of the structure also slows down the diffusion rate of exchanging so that the ion exchange rate can also decrease as the ion exchange capacity increases. Generally speaking one could compare the higher cross-linked resins to smaller mesh sieves.

Factors Affecting and Controlling Exchanging Properties.

(i) Factors Controlling Exchange Rate.

The factors controlling exchange rate can be divided into five steps.

Taking the equation $K^+ + RH^+ \rightleftharpoons RK^+ + H^+$ as an example the steps can be formulated as follows:-

1. Diffusion of the potassium ions through the solution to exchanger particles.
2. Diffusion of the potassium ions through the molecular pores of the particle to the exchanging groups.

3/...

3. Chemical exchange between the potassium ions and hydrogen ions at the exchanging sites within the resin.
4. Diffusion of the displaced hydrogen ions to the surface of the exchanger.
5. Diffusion of the hydrogen ions away from the resin particle.

The slowest step of the above five will control the rate of exchange. Under different conditions the step controlling exchange rate will differ but in most cases the diffusion of the exchanging ion through the resin particle (Step 2) will be rate controlling.

(ii) Electrical Charge and Radius of Hydrated Ion.

The adsorption affinities of various ions have been shown by Boyd (1947) to be determined by magnitude of the charge (or valence) and radius of the hydrated ion.

The importance of charge indicates that adsorption is largely controlled by electro-static forces. Trivalent ions are held more firmly than divalent ions which are in turn adsorbed to a greater extent than monovalent ions. For ions of the same valence adsorbability usually increases with a decrease in the radius of the hydrated ion.

The series of adsorbability for cations usually found in must is as follows:-

Divalent cations $Ca^{++} > Mg^{++}$

Divalent heavy metal cations $Cu^{++} > Fe^{++}$

Monovalent cations $K^{+} > NH_4^{+} > Na^{+} > H^{+}$

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(iii) Size of the Organic Ion.

Kunin (1958) has found that the capacities of various cation exchangers decrease as the ionic size of the cation attains a threshold value. See Figure 2.

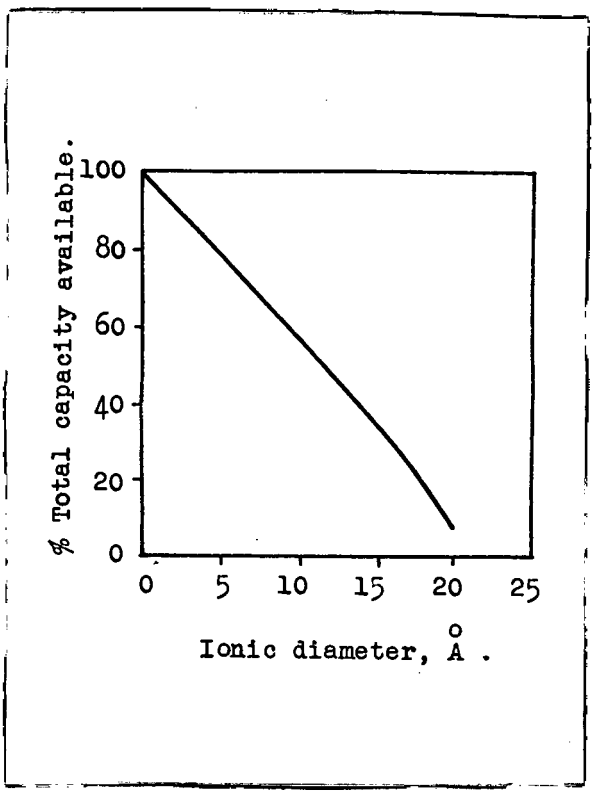

Figure 2. Effect of ionic diameter on total available exchange capacity of a sulphonic acid cation exchange resin.

Gregor, Kressman and Kitchener (1952, 1955) have found that the rate of diffusion of large ions into and through ion exchange resins proceeds very slowly. In the case of ordinary dyes, rates of diffusion are so slow that we may conclude that the ordinary resin (±8% divinyl benzene) has a low capacity for very large ions. Where the ionic size of the exchanging substance is of such magnitude that it exceeds the distance between the polymer chains or cross-links of the resin matrix no diffusion into the interior of the resin can occur. It is thus feasible that ionic separations can be achieved by utilization of an exchanger whose structure is such that is will only allow penetration of small ions. Large ions may however still be adsorbed and exchanged at the exchanger surface (Figure 1), but, since the majority of exchanging sites are within the

particle/...

particle, the latter phenomena of surface contribution to the total capacity is small. Moreover, if the resin particles are of a relatively large mean diameter, surface adsorption area will be relatively small.

It has been found that a considerable decrease in capacity of an exchange resin can be caused by large organic ions. Apparently, these ions become firmly wedged in the molecular channels of an exchanger and can considerably reduce the capacity of a new resin. Normal regeneration is of little use in restoring the resin to its initial capacity. In pineapple juice the main constituent of such a blocking group was isolated and showed to be a polypeptide fraction (Felton 1949). The practical importance of resin blockage is obvious.

(iv) Flow Rate.

For a strong acid resin exchange is very fast. It has been found that rate of diffusion, which in this case was exchange rate controlling, of HCl and NaOH through Dowex 50 (a unifunctional sulphonic resin) to be about one-fifth as great as in dilute aqeous solutions. It is apparent that for the exchange of Na^+ and H^+, a high flow rate would not materially affect the issue.

The following graph (Figure 3) illustrates the rate of adsorption of a small ion and large ions on a sulphonic acid cation exchange resin.

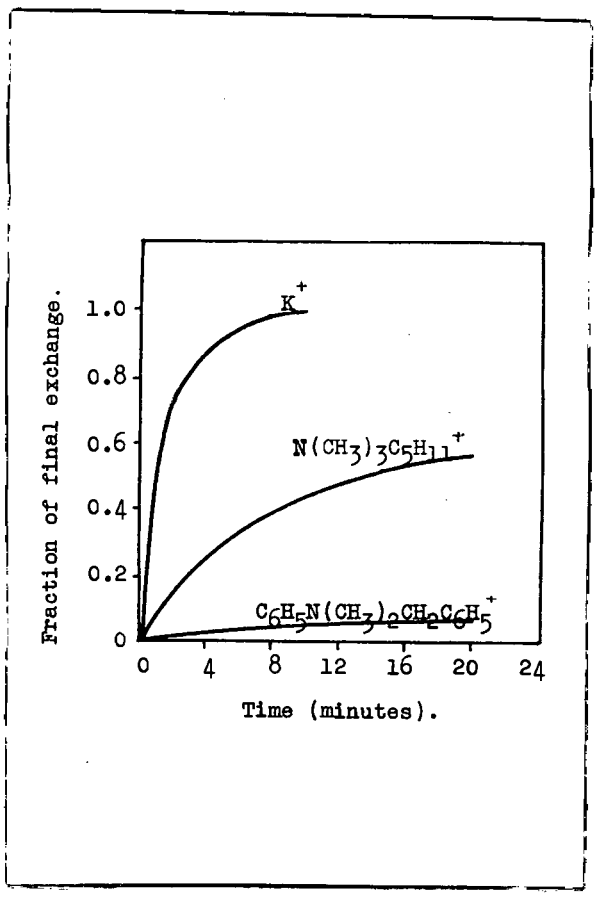

Figure 3. Rate of adsorption of large ions by a sulphonic cation exchange resin. Data of Kressman and Kitchener (1949).

(v) Ion Exchange in Column Operation.

Upflow and Downflow.

The process of ion exchange can be applied by two general methods. The first is the batch process which is the addition of, usually a predetermined weight of resin to a solution. The mixture is stirred or roused until the required reaction stage has been reached. The second process is known as column operation and may be divided into two parts viz. upflow and downflow. In the former the influent is flowed upwards through the vertical resin column whilst in the latter the direction of flow is reversed. In upflow, the resin bed is elongated within the confines of the column, turbulence is set up and channeling occurs. In downflow the exchanging ions flow through a closely packed resin bed. During the latter process the maximum exchange capacity will be attained sooner and, furthermore, exchange will usually be of a higher order.

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The following graph in Figure 4 illustrates the fluctuations in pH during a run of grape must caused by altering the direction of flow.

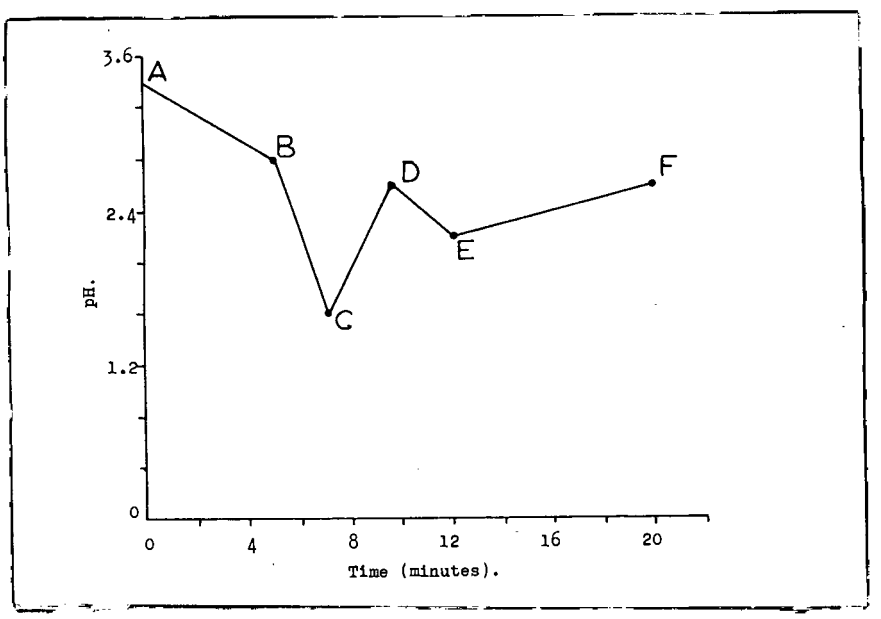

Figure 4. Fluctuation of pH of a grape must caused by upflow and downflow through a sulphonic acid cation exchange resin. (AB upflow, BC downflow, CD upflow, DE downflow).

(b) Quality in Dry White Table Wines.

The wine industry has two important problems; maintaining and improving quality and surplus production. These are interrelated, for should only quality be increased it is logical to presume that under normal conditions surplus would decrease. Local and export markets are highly competitive and discriminating; it is in the interest and to the benefit of the industry and country that wines of quality be marketed.

Wine quality is a term of many facets and there are consequently many possible ways to better it. The most economical and certainly the best method is the cultivation of varieties which have the proper composition and character for the type of wine that is to be produced.

But to this must be added that for the best results the right variety must be cultivated in the right environment. The availability of new suited varieties and the knowledge of where to grow them and existing varieties to the best advantage is a problem of South African viticulture and, one to which a quick solution is not readily envisaged. By better viticultural practices a relatively small crop of high quality could be achieved. Under present, and perhaps future circumstances few producers will follow such a system. A third possibility is in the wine-making procedure. Here much has already been done; one need only look back to what cellar conditions and practices were and to what they are to-day with better facilities, cooler fermentations, pure yeast cultures etc. It can not be said that the "end of the line" in wine-making procedure has been reached. This procedure is one facet which appears to hold some promise in South Africa.

The consumer wants a wine that is fresh and fruity i.e. a wine in which the term quality is functional. This term is multidimensional and embraces many individual factors of which bouquet, taste, colour and clarity are important. In white wines it is not envisaged that vast improvements can be achieved by wine-making procedures alone. However, it is not impossible that some present day methods can be bettered upon.

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B. THE PURPOSE AND DESIGN IN THE ION EXCHANGE TREATMENT (HYDROGEN CYCLE) OF WHITE MUSTS.

It is generally conceded that South African musts and especially those from which white table wines are made are high in pH (and low in total acidity). It is also commonly accepted that a positive correlation exists between low pH and quality in wines. The pH of South African musts are often decreased by addition of tartaric acid; the advantages gained thereby are manifold. A wine of low pH tasts fresh and tart whereas those of high pH are flat and insipid; colloidal clarity of wines are undoubtedly bettered and bacterial infection is suppressed in a low pH must. Although much is to be gained by this practice it would be better if it could be improved upon or circumvented for it is not a practice which is suited to the production of the highest-quality wines.

The recent introduction of ion exchange as a unit process and its use in many food industries suggests itself as a possible substitute for tartaric acid as a means to decrease pH. Apart from the latter possibility there are also others by which the ion exchange process may theoretically at least be to the advantage of must and wine.

Since markets are so competitive, wines, irrespective or origin or composition are cold stabilized. Although this practice has certainly removed potassium bitartrate precipitation as a problem there are indications that what is gained in potential clarity is sometimes more than lost in detrimental effect upon the wine. The delicate white table wines are often the worst sufferers. By ion exchange (in the hydrogen cycle) a portion of the potassium ions (and other cations) are exchanged for hydrogen ions. It follows that potassium ion concentration

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will decrease and hydrogen ion concentration increase (pH decrease); therefore, should exchange proceed far enough both pH and potassium ion concentration will materially inhibit potassium bitartrate precipitation. It is further possible that organoleptically ion exchange could be an improvement on cold stabilization.

The sulphonic acid of a cation exchange resin is a stronger acid than tartaric acid. Therefore, if in two portions of a must X, pH is decreased to a set value in (a) by ion exchange and in (b) by tartaric acid then it is possible that the fixed acidity in (a) will be lower than that in (b). According to Amerine and Joslyn (1951),"the titratable acidity is a better indication of acid taste than the pH is." Logically and generally then, the lower the total acidity, for the same pH the less the acid taste (hardness).

During the passage of must through a cation exchange resin, cations and those ampholytes which would react as cations are held by the resin. Amongst these cations are ammonium ions and amino acids and since they are yeast nutriments a decrease of them will affect both fermentation and fermentation products. Similarly the corresponding increase in hydrogen ions (or pH decrease) will also influence fermentation and its products.

The object of this project may be briefly summarized as follows:-

By the treatment of must by a cation exchange resin, hydrogen cycle, an improvement of South

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African white wines, with special regard to acidity and pH is to be attempted. Chemical analysis of treated musts and wines are to be done and the influence of treatment on fermentation of musts studied.

CHAPTER II.

USES OF ION EXCHANGE RESINS IN THE WINE INDUSTRY.

1. Prevention of Potassium Bitartrate Precipitation.

One of the most widely acknowledged and applied uses of the cation exchange resins to-day is the potassium bitartrate stabilization of wines. The process is briefly the substitution of a portion of the potassium ions (and other cations) for sodium ions. Since sodium bitartrate is more soluble than potassium bitartrate in wine and if enough potassium ions have been replaced then precipitation of potassium bitartrate will not occur.

The economics of the ion exchange process compares very favourably with the cold stabilization process; in fact capital investment and time costs are very much less and labour and operating costs equal or less.

Quality of treated wines have not been noticeably affected in all cases. It has been noted in Italy (Agostinis, 1958) that certain treated dry white wines tend towards a sherry or oxidized character. McGarvey (1958) and Percival (1957) fully described the chemical changes which occurred, however, they made no mention of the influence of the process on wine quality. It is generally assumed that small wines, for quick consumption are not unduly affected by this process

2. Removal of Heavy Metal Ions notably Iron and Copper.

Iron and Copper can be removed from wines by both anion and cation exchange resins. Since these two elements are cations, the anionic removal indicates

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that they are in complex form and that the complex is of anionic character. If this is true for anion exchange resins then the same is true for cation exchange resins. It will be seen that iron (and copper) can be removed by cation exchange as Fe^{++}, Fe^{+++} or Fe^{c}(complex). Since wines and musts differ widely as to their heavy metal content and the state in which these metals exist therein, it follows that the removal by a cation exchange resin of iron or copper will also differ from wine to wine and must to must. With respect to iron, the following table (Table 1) clearly illustrates this point. The same general fluctuation has been noted in copper removal by cation exchange resins.

TABLE 1.

Iron Removal from Wines by Various Cation Exchanging Resins.

Resin.	Cycle.	Initial iron conc. (ppm.)	Residual iron conc. (ppm.)	Max. % iron removed.
ZK 225[a]	Na	3.5	3.0	14
ZK 225[a]	H	9.2	6.6	28
Dowex 50[a]	Na	3.2	1.9	41
Amberlite IR-120[a]	Na	3.2	3.2	Nil
Amberlite IR-120[b]	Na	6	±3	±50
KU-1[c]	Na	78	5	94
KU-1[c]	Na	59	1	98

a Rankine (1955), b Percival and McGarvey(1957)
c Legunova (1957).

Patou (1959) has found that by progressively decreasing pH of a wine prior to cation exchange in the sodium cycle, a progressive removal of both iron and copper occurred. This indicates pH of the wine as a functional factor and also partially bears out the previous supposition.

Joslyn/...

Joslyn and Lukton (1953) adequately sum up the present position by stating that "none of the ion exchange resins tested"(by them) "was a suitable substitute for ferrocyanide either in efficiency or in effects on the organoleptic qualities of wines. The wine passed through the cation exchange resins in the hydrogen form became objectionably sour and lost most if not all of its original fruity bouquet."

3. Decrease in Acidity.

Acid decreasing in musts or wines is unimportant in the warm wine producing countries whilst the opposite is true of the colder ones. The basic anion exchange resins can be utilized to decrease the fixed acidity in musts or wines. Since the influence of anion exchange on the organoleptic properties of wines appeared to vary, the older method of calcium carbonate addition is still commonly employed.

4. Increase in Acidity.

It is the aim of this project to increase acidity of a must, by ion exchange prior to fermentation. To the writer's knowledge no detailed work in this field on musts or wines has been reported. Joslyn and Lukton (1953) found that bouquet of wines were adversely affected in the hydrogen cycle.

CHAPTER III.

EQUIPMENT, MATERIALS AND METHOD OF TREATMENT.

(a) Equipment.

(i) Resins.

Three monofunctional (sulphonic group) cation exchange resins, Zeo-Karb 225, Amberlite IR - 120 and Lewatit S - 100 were used. These resins were all in bead form (spheres) and had a cross-linkage of approximately 8%. Table 2 supplies pertinent details.

TABLE 2.

Bead Size, Capacity and pH Operating Range of Various Cation Exchanging Resins.

Resin.	Bead size mm.	Cap. in meq. /dry gm.	pH operating range.
ZK 225	0.3 -1	5.0	1-14
Amb.IR-120	0.45-0.6	4.6	0-14
Lew. S-100	0.3 -1	5.0	0-12

Conditioning and Regenerating of Resins.

Prior to use resins were conditioned by alternate washes of approximately 4% NaOH solution and approximately 2N HCl.

The resin used in the Franschhoek Co-operative Cellar was regenerated with a mixture of 12 gallons concentrated commercial HCl and 40 gallons tap water. Residual HCl was removed by washing with tap water.

The resin used in the Elsenburg cellar was regenerated with a mixture of 4.5 gallons concentrated chemically pure HCl and 18 gallons tap water. Residual HCl was removed by washing with tap water.

The resins used in the laboratory were regenerated with 500 ml. of 2N HCl (distilled water andchemically pure HCl).

Regeneration/...

Regeneration was always downflow and flowrate slow. Laboratory resin flowrate was 3.2 ml./sq. cm. resin/min.

(ii) Ion Exchange Apparatus.

Two industrial and one laboratory ion exchange apparatus were used. Figure 5 is a photographic reproduction of the laboratory set-up. The further reservoir contained distilled water, the center one 2N HCl and the third was for must. By manipulation of reservoir and feed line cocks it was a simple matter to wash, back-wash and regenerate. The apparatus was so arranged that by closing and opening the correct clamps the influent could flow through the column either upwards or downwards. The glass column contained 100 gm. of resin resting on a pad of glass wool.

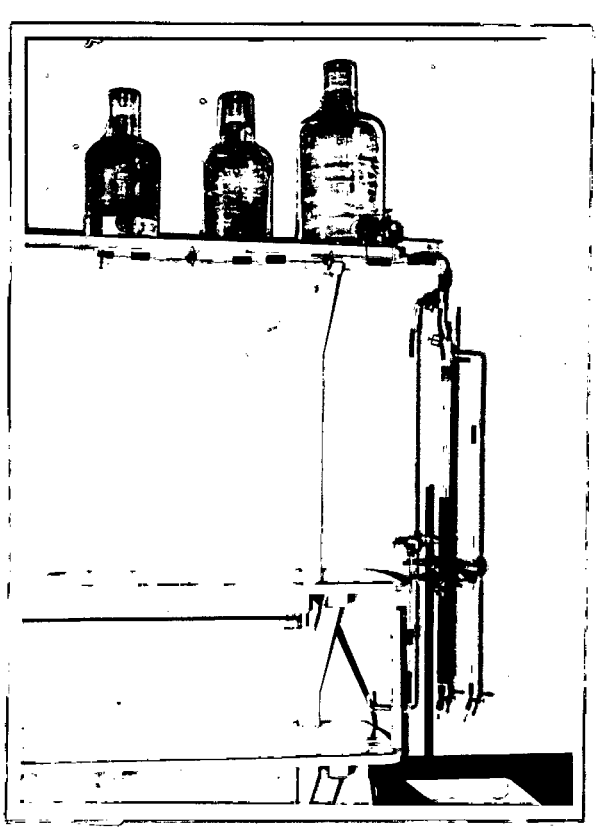

Figure 5. Laboratory set-up used for treating musts by the ion exchange process.

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In the cellar two industrial plants, an Enopila and a Berkefeld were used. The Enopila plant which was used at the Franschhoek Co-operative Cellar consisted of 2 pairs of squat chromed metal columns interconnected with inert pliable plastic hose. Two multiport cocks controlled direction of flow and could also direct flow to any of the two pairs of columns. Meters which controlled flow were incorporated into the apparatus. Each column contained 70 lbs. of resin which nearly filled it. The Berkefeld plant which was used at the Elsenburg cellars consisted of two long mild steel enamelled columns of equal length but different diameter. The smaller column was used; it was filled to approximately half way with 56 lbs. of resin. By a system of plastic cocks and inert rigid plastic hose the direction of flow could be controlled. The apparatus was fitted with a pressure gauge and a rotameter (flow meter).

(iii) Fermentation and Storage Containers.

In the laboratory, fermentation was carried out in liter and 500 ml. reagent bottles at 25°C. Figure 6 shows a 500 ml. fermentation unit. At Franschhoek Co-operative Cellars musts were fermented in 12 leaguer tanks. In the Elsenburg cellar wines were fermented in horizontally placed 40 gallon stainless steel drums of which the bunghole was closed with a cotton wool wad. Each drum contained 25 gallons of must. Inoculation followed the standard procedure as outlined in the relevant section. Immediately after inoculation and for each successive day, degree Balling and temperature readings were

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taken of the contents of each drum until the former readings showed no difference on two consecutive days. At that stage the contents underwent their first racking into glass containers which are described in a later paragraph.

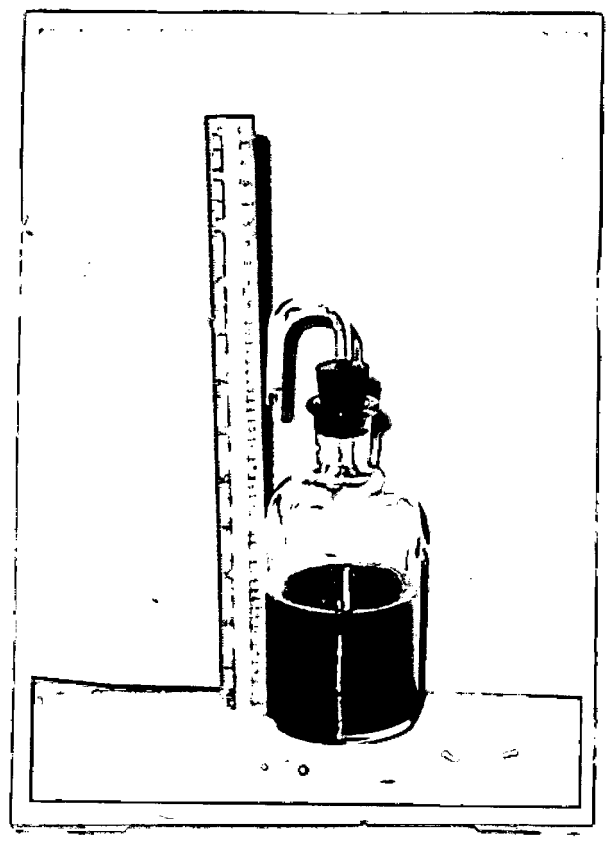

Figure 6. 500 ml. fermentation unit.

Fermentation in the containers was allowed to proceed longer than is normally the case, primarily, to obtain complete fermentation graphs. High temperatures and air contact presented no problem as ambient temperatures were low and due to the type of container and closure used a good head of carbon dioxide covered the wine.

In the procedure followed these cellar wines received three rackings instead of the usual four. The rackings were given as follows:-

(a) First racking; upon cessation of fermentation into unsulphured containers.

(b) Second racking; one month after the first into lightly sulphured containers.

(c)/...

(c) The final racking was given after the winter into lightly sulphured containers.

Bottling was carried out by siphoning into pint bottles four months after the final racking.

After racking, wines were transferred to ten gallon glass containers. These containers were set up to counteract expansion and contraction of the wine which could have fractured it. Figure 7 is a photographic reproduction of the apparatus.

Figure 7. Container in which the dry white wine made from ion exchange treated must was matured.

A thin glass tube connects the large container with a small reservoir. This tube projects approximately three inches past the cork of the large container into the wine and also reaches to the bottom of the small reservoir. The reservoir is a 500 c.c. reagent bottle approximately half filled with wine and 50 ml. of liquid paraffin which covered the wine surface to a depth of one-quarter inch. In this way the wine could expand and contract out of contact with air. As a further safeguard against contamination and air contact a fairly tightly packed

cotton/...

cotton wool filter served as air vent and carbon dioxide was used to displace the air above the liquid paraffin layer. The carbon dioxide atmosphere was replenished weekly.

(b) Materials.

Musts and Yeasts.

Since clear musts were essential for ion exchange treatment it was necessary that they first be clarified. Settling by the sulphur dioxide method and/or refrigeration was done on all musts prior to passage through the resin columns.

In the Franschhoek experiment the must, a mixture of Green grapes and White French grapes was settled by an adequate addition of meta and then sentrifuged, whilst in the Elsenburg cellar experiment Riesling and Stein musts were settled with 4 ozs. meta/leaguer. In laboratory tests, which were done on St. Emillion, Colombard, Stein and Riesling musts the procedure varied. Since laboratory musts were preserved in cold storage at 25°F clarification normally occured, however, those musts which were sterilized (heat) did not receive sulphur dioxide whilst those that were not sterilized did.

In all experiments except the Franschhoek one, Saccharomyces cerevisiae var. ellipsoideus (Strain 13) was employed as the fermenting yeast. At Franschhoek the naturally occurring yeast was used. All inoculations were 2% of actively fermenting must. An inoculation of 5% of a pure yeast into a standard sterilized must and held at 25°C for three days served as inoculant in all laboratory tests.

(c)/...

(c) Method of Treatment.

Columnar exchange was considered the most suited method as it is the most commonly used, the most practical system and the one in which exchange reactions are driven to completion. However, a choice still had to be made between upflow and downflow techniques. The experience gained at Franschhoek indicated that upflow would give practical difficulties, in fact, it was found that under the incident conditions of this experiment pH 2.6 was not easily reached; three regenerations totalling 36 gallons of commercial concentrated HCl were necessary to obtain 1390 gallons of pH 2.6 must. The data in Table 3 gives pH values attained for each regeneration run.

If a must of pH higher than 3.4 were used, the decreasing of pH to 2.6 would have been still more difficult and time consuming. With a microbiologically unstable compound such as must, time is an important factor. Furthermore, the fluctuating chemical composition of the upflow effluent would make it difficult to obtain fixed volumes of set pH and more so without admixture of varying volumes of treated and/or untreated must. It follows that analysis of must pH groups, to which the pH of many control musts were to be lowered would entail considerable analytical work and certainty as to the concentration of micro components (e.g. vitamins, trace elements) be suspect.

By downflow exchange these disadvantages are overcome; a minimum pH of approximately 2 is quickly attained and remains constant for a relatively large volume of must. Downflow, however, also has its disadvantages. Since, by the latter procedure the must effluent pH is low, (approximately 2) the probability of hydrolytic and other changes occurring

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in the chemical constituents of the must can not be excluded. Another disadvantage is that if a very turbid must is flowed down a column the insoluble grape particles could mechanically block the resin bed. However, from a scientific and practical point of view the advantages of downflow over that of upflow justified its use.

TABLE 3.

pH Values of a Grape Must during passage through a Sulphonic Acid Resin Column.

Time (mins.)	Must pH.	Flow Direction.
First Regeneration.		
-	3.4	-
5	2.61	Bottom to top
15	2.61	"
25	2.59	"
35	2.56	"
45	2.58	"
60	2.6	"
65	2.75	"
Second Regeneration.		
5	2.56	Bottom to top
15	2.7	"
25	2.65	"
27	2.69	"
35	2.68	"
Third Regeneration.		
5	2.8	Bottom to top
7	1.6	Top to bottom
10	2.56	Bottom to top
12	2.25	Top to bottom
20	2.6	"
25	2.6	"
30	2.65	"
37	2.65	"

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The diagram in Figure 8 illustrates schematically the system and also the set pH values to which must pH values were reduced in Experiments II - X and the cellar experiments.

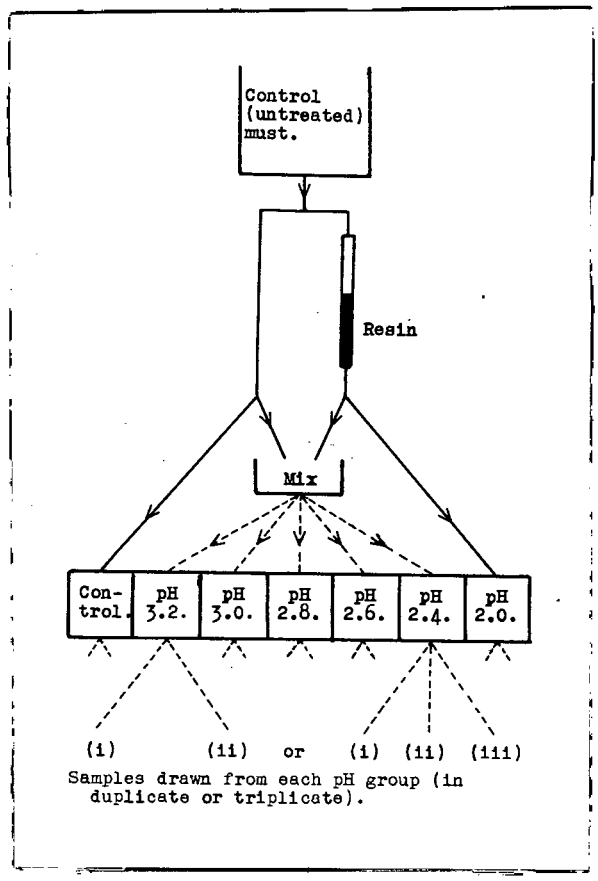

Figure 8. Schematic illustration of system by which must pH was reduced to set values.

It will be seen that only two must pH groups are used viz. control (untreated) and pH 2 (treated) and obtaining various pH values was a simple procedure. In the later experiments 20 ml. of control must was taken and increasing quantities of pH 2 must added until the desired pH was reached. By means of this control:pH 2 ratio, larger quantities were blended.

The process of ion exchange, as utilized in this work, has two major effects, these are:-

(a) the removal (or adsorption) by the resin of must cations (other than hydrogen ions) and ampholytes from the must and,

(b)/...

(b) the equivalent substitution of hydrogen ions to the must (decrease of pH).

The two final experiments, IX and X, were done in an attempt to differentiate between these two influences. In effect, the lower pH values of portions of treated musts were increased to the pH value of their untreated (control) must by the addition of potassium hydroxide.

By this technique any differences manifested between a specific treated must or wine and its increased pH counterpart would then have been due largely to effect (a) or (b).

The following scheme gives the setting out of experiments IX and X and also the designation of the different pH groups:-

(a) Control must (untreated).

2 x 250 ml. samples + C ml. water added to each sample.

(b) (i) pH of control must decreased to pH 3.2, 3.0 and 2.8.

pH 3.2 must, 2 x 250 ml. samples)
pH 3.0 " , 2 x 250 ml. ") C ml. water added to each sample.
pH 2.8 " , 2 x 250 ml. ")

(ii) Portion of (b)(i) musts but with pH increased back to that of control sample by addition of potassium hydroxide solution.

pH 3.2 must, 2 x 250 ml. samples + a ml. KOH solution. + c - a ml. water added to each sample.

pH 3.0 must, 2 x 250 ml. samples + b ml. KOH solution. + c - b ml. water added to each sample.

pH 2.8 must, 2 x 250 ml. samples + c ml. KOH solution.

(c) pH of control must decreased to approximately 2 (minimum value) and brought back to control pH by addition of potassium hydroxide solution.

The potassium hydroxide solution was more concentrated here than in (b)(ii) and volumes added were near enough equal to c ml.

The/...

The wines of (b)(i) as of all wines from must groups of experiments I to VIII are designated as pH 3.2 wine, pH 3.0 wine etc., whilst the wines of (b)(ii) are designated as pH 3.2 (control), pH 3.0 (control) etc. The wines of group (c) are designated as pH 2.0 (control) wines.

(d) pH of control must increased to pH 4.5 by the addition of potassium hydroxide solution.

This group was included to note the influence of a pH increase in a control (untreated) must and its wine.

The volumes of potassium hydroxide solution added were near enough equal to c ml. The wines of these musts are designated as pH 4.5 wines.

CHAPTER IV.

METHODS OF ANALYSIS.

Wine and must samples were analysed by the following methods:-

1. Specific gravity - picnometrically.
2. Extract - "
3. Alcohol - "
4. pH - glass electrode with saturated calomel electrode as reference electrode. Buffers were periodically made up from certified buffer tablets and also checked against M/20 potassium hydrogen phthalate solution.
5. Total Acidity. 10 ml. of must or wine to which 100 ml. of boiling distilled water was added was titrated rapidly to a phenolphthalein endpoint with 0.1N NaOH (carbonate - free).
6. Volatile Acidity was determined by steam distilling (Cash volatile acidity assembly) wine or must and titrating the heated distillate to a phenolphthalein endpoint with 0.1N NaOH.

The pH, total acidity and volatile acidity wine analyses were always done on 50 ml. of degassed wine. The degassing procedure was carried out by placing 50 ml. of wine into a 500 ml. filtration flask and exhausting the air by means of a filter pump whilst simultaneously shaking the flask. After one minute the filtration flask was sealed off from the pump and shaking continued for another minute. In this manner most of the carbon dioxide was removed.

7./....

7. Esters. Method of the A.O.A.C. (1950). The method is essentially the neutralization of an aliquot wine distillate to a phenolphthalein endpoint with 0.1N NaOH, addition of a known excess 0.1N NaOH, refluxing, cooling and back titrating the surplus NaOH with 0.1N H_2SO_4.

8. Aldehydes. The method of Guymon and Nakagiri (1957) The procedure is based upon the stability of the aldehyde-bisulphite complex at various pH values. The final step in this determination is the splitting of this complex in an alkaline medium (pH 8.8 - 9.5) and titration with a standard iodine solution.

9. Reducing Sugars. Volumetric method of G. Bruhns.

10. Sulphur Dioxide. (Free and Total). Volumetric method of Ripper.

11. Ash determined as described by Amerine (1955).

12. Alkalinity of the Ash determined as described by Amerine (1955).

13. Total Tartrates. Two methods were used in the determination of tartrates. The first was an adaptation, as given by Amerine (1955) of the well-known von der Heide-Schmitthenner potassium bitartrate precipitation procedure. The second method was a colorimetric one in which the red colour of a pervanadyl-tartaric acid complex, developed with sodium metavanadate is photometrically determined (Matchett, 1944). Although normally the von der Heide method gave reproducible results there was some doubt as to whether low pH values of treated wines would influence precipitation of potassium bitartrate. In the sodium metavanadate method pH is corrected

during/..

during the preparation of the sample and, therefore, of little consequence. Furthermore, the older method is empirical to a degree in that a standard correction figure has to be added whilst in the newer method this is not so, in fact, the colour developed is, within limits, directly proportional to the tartrate content. The accuracy of the sodium metavanadate method is also not affected by a turbid must or wine as is the other one. In effect, a clear must or wine is a requisite of the von der Heide procedure.

Table 4 gives tartrate contents of various wines determined by both methods. The dual determinations are not duplicates but single determinations on two wines made separately from the same must and under identical conditions.

TABLE 4.

Total tartrate content of various Wines, determined by the von der Heide and Sodium Metavanadate Procedures.

Experiment A.

Wine.	pH.	Tartaric Acid gm/L.	
		$NaVO_3$ method.	v. d. Heide method.
A. (i)	3.55	2.78	2.92
(ii)	3.5	2.83	2.92
B. (i)	3.27	5.33	5.36
(ii)	3.21	5.33	5.47
C. (i)	3.25	2.83	2.92
(ii)	3.22	2.83	2.94
D. (i)	3.09	2.90	2.95
(ii)	3.08	2.90	2.94
E. (i)	3.01	7.55	7.49
(ii)	3.05	8.13	7.87
F. (i)	2.98	2.78	2.93
(ii)	2.96	2.78	2.92
G. (i)	2.95	11.90	11.62
(ii)	2.93	12.33	11.69

Table 4 cont./...

TABLE 4. (Cont.).

Experiment B.

Wine.		pH.	Tartaric Acid gm/L.	
			$NaVO_3$ method.	v. d. Heide method.
H.	(i)	3.55	2.23	2.47
	(ii)	3.53	2.20	2.47
I.	(i)	3.27	4.90	5.19
	(ii)	3.26	4.90	5.23
J.	(i)	3.22	2.41	2.47
	(ii)	3.22	2.33	2.47
K.	(i)	3.02	8.05	8.32
	(ii)	3.03	8.11	8.35
L.	(i)	3.01	2.32	2.49
	(ii)	3.02	2.30	2.49
M.	(i)	2.9	2.23	2.39
	(ii)	2.89	2.21	2.39
N.	(i)	2.86	11.9	11.92
	(ii)	2.87	11.94	11.99

The sodium metavanadate results are generally slightly lower than the other but otherwise they agree very well.

The sodium metavanadate method proved to be the more laborious of the two; it was used in only one portion of this work (Table 23) whilst the von der Heide method was used in all the other.

14. Total Nitrogen. Method of Kjeldahl.

15. Iron. was determined by a colorimetric procedure based on the formation of a red coloured ferric thiocyanate complex. (Nobile 1954).

16. Ammonia.

(a) In Must.

Ammonia was determined by a slightly altered Boussingault procedure (Amerine 1955). The variation amounted to a substitution of a 4% boric acid solution for an 0.05N sulphuric acid solution as the ammonia binding solution and the use of a mixed indicator instead of the recommended methyl red. The mixed indicator contained

0.125 gm. methyl red and 0.083 gm. methylene blue per 100 ml. absolute ethanol. One ml. of this indicator was added to 50 ml. of boric acid solution and the bound ammonia titrated with 0.05N sulphuric acid.

This method was checked as to reliability and with a view to later determining ammonia in only control and pH 2 musts and working out the ammonia content of intermediate pH groups on the ratio of mixtures without actual analysis.

A Ferdinand de Lesseps must was found to contain 4.66 mg. ammonia per 50 ml. must (92.8 mg. ammonia/liter) and an ammonium chloride (A.R.) solution 0.775 mg. ammonia per ml. solution. 5 ml. of this solution (3.87 mg. ammonia) was added to 50 ml. of the Ferdinand de Lesseps and ammonia then found to be 8.3 mg. (165.9 mg. ammonia/liter). This showed a small loss of 0.23 mg. ammonia per 50 ml. A White French must was treated by ion exchange and the following ratios of control and pH 2 musts found to be necessary to decrease pH to set values (Table 5):-

TABLE 5.

Percentage of Untreated and Ion Exchange Treated White French Must contained in pH 3.2, 3.0 and 2.8 Must Groups.

% Control* must in mixture.	Final pH.
64.7	3.2
51.5	3.0
40.0	2.8

* Untreated.

The/...

The following table (Table 6) gives analytically and theoretically determined ammonia concentrations in the set pH groups of the White French must:-

TABLE 6.

Ammonia contents of Untreated and Ion Exchange treated Groups of a White French Must.

Must.	Analyt. det. NH_3 mg/L.	Theor. det. NH_3 mg/L.	"Loss" on analysis mg/L.
Control*	62.2	-	-
pH 3.2	37.4	39.8	2.4
pH 3.0	29.6	32.3	2.7
pH 2.8	23.2	24.9	1.7
pH 2.	Nil	-	-

* Untreated.

The agreement between analytically and theoretically determined values was good and hence all further determinations were done only on control and pH 2 musts and intermediate values determined on a pro rata basis.

(b) In Wines.

Ammonia was also determined in wines by the Boussingault procedure but since it is normally present in low concentrations (or not at all) an adaption of this method had to be used. The variation of the above procedure amounted to titration to a potentiometric endpoint (pH meter) rather than an indicator one.

The ammonia from 50 ml. of wine was distilled into 50.0 ml. 4% boric acid solution (plus 1 ml. indicator) and the final volume of the distillate brought to 180 ml. With each distillation batch duplicate blanks were done. The pH of the blanks were measured and titration of the other samples carried out with 0.01N sulphuric acid back

to the pH of the blank.

The method was checked and the following table (Table 7) gives ammonia values of various wines determined by this procedure. The dual determinations are not duplicates but single determinations on two wines made separately from the same must under identical conditions

TABLE 7.

Ammonia content of various Wines determined by a modified Boussingault procedure.

Wine Group.		pH of distillate.	0.01104N H_2SO_4 ml.	NH_3 mg/50 ml.	NH_3 mg/L.
Blank		4.8	-	-	-
		Stein.			
Control	(i)	6.35	2.55	0.48	9.6
	(ii)	6.35	2.5	0.47	9.4
pH 3.2	(i)	6.05	1.4	0.26	5.2
	(ii)	5.95	1.25	0.23	4.6
pH 3.0	(i)	5.95	1.1	0.20	4.0
	(ii)	5.95	1.1	0.20	4.0
pH 2.8	(i)	5.8	0.8	0.15	3.0
	(ii)	5.8	0.8	0.15	3.0
		Riesling.			
Control	(i)	6.2	4.3	0.8	16.0
	(ii)	6.3	4.2	0.79	15.8
pH 3.2	(i)	5.75	0.9	0.17	3.4
	(ii)	5.65	0.8	0.15	3.0
pH 3.0	(i)	4.9	0.6	0.11	2.2
	(ii)	5.6	0.7	0.13	2.6
pH 2.8	(i)	5.1	0.8	0.15	3.0
	(ii)	-	0.6	0.11	2.2

The last two wines, viz. Riesling pH 3.0 and pH 2.8, were the first attempted and erratic results must be ascribed to faulty technique. The wines used in this determination had been left on their lees for a lengthy period. However, the method worked well as such and was employed for all further ammonia determination on wines.

17/...

17. Higher Alcohols (Fusel oils) were determined by the method of Guymon and Nakagiri (1952). The determination is a colorimetric one in which colour is developed with p-dimethylaminobenzaldehyde in a sulphuric acid solution.

18. Amino Acids. Prior to determination of amino acids by a paper chromatographic technique it was essential that interfering substances be removed. In must, one such substance was sugar which apparently acted as a mechanical barrier in the paper causing streaking and "tailing" and the running of a clear chromatogram an impossibility. Furthermore, the non-volatile nature of sugar and its high concentration made the spotting of relatively large quantities of must on a small diameter spot impossible. Figure 9 is an accurate tracing of a single dimension chromatogram where spot 1 is a natural must and spot 2 the identical must but with sugar removed. Although the amino acids spots in 2 are clearly marked this was not wholly the case for mingling still occurred; development in a second dimension would have resulted in a good chromatogram whilst it would not have been the case with the natural must.

Although dry white wines contained little sugar it was nevertheless found that the colour which they contained quickly filled the initial spot and made the spotting of relatively large quantities of wine on a small diameter spot a difficult and lengthy procedure. For this reason it was advisable that colour be at least partly eliminated. Proteins (and peptides) give positive ninhydrin reactions and it was also necessary that some if not all be removed with the sugar.

The/...

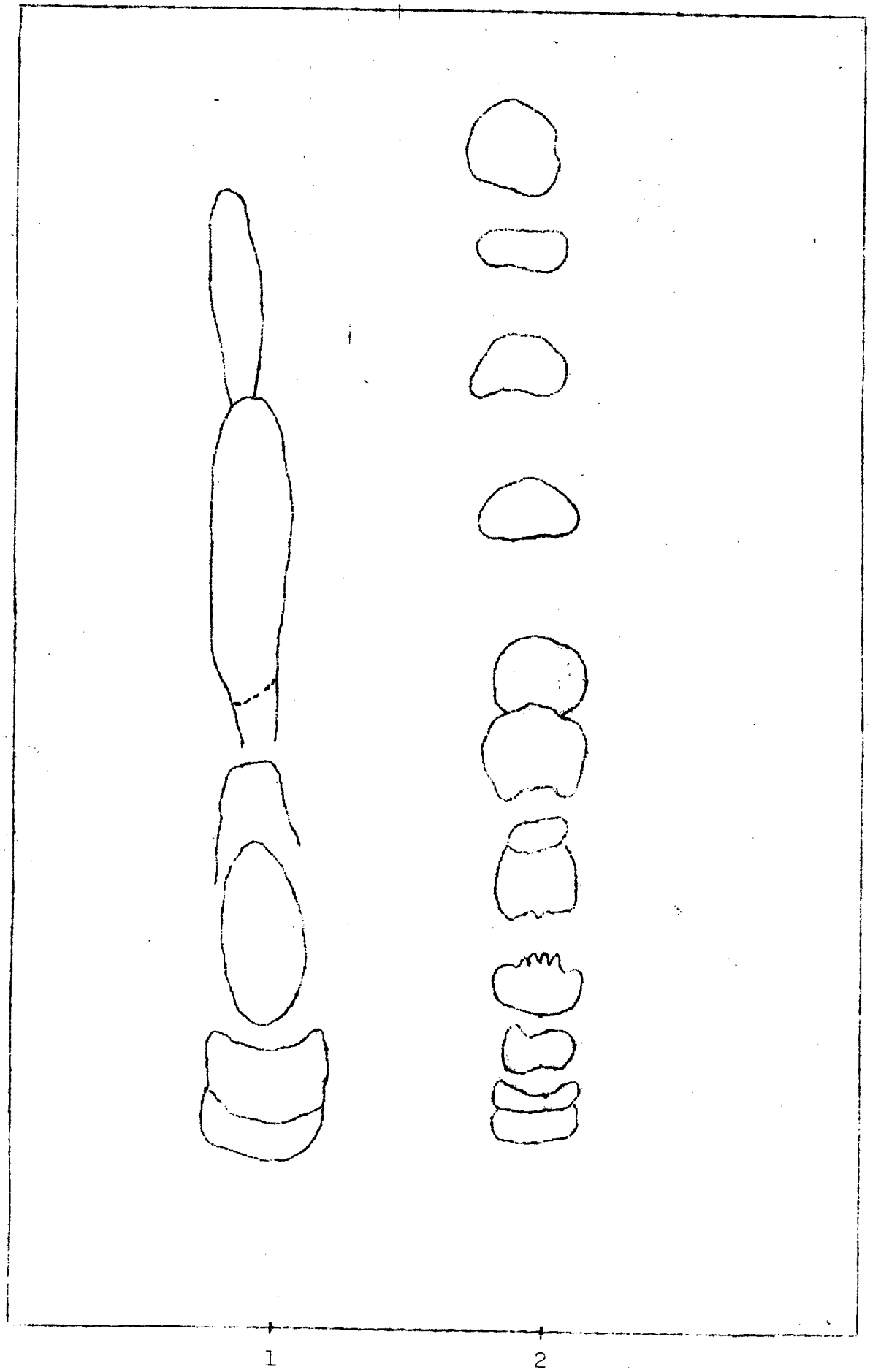

Figure 9. Single dimension paper chromatogram of amino acids of a grape must. Spot 1 contained the original must, spot 2 contained the same must but with the sugar removed by means of a sulphonic acid resin.

The ion exchange resins are currently used to fractionate amino acid mixtures, in effect, the amino acid, being electrolytes are held by the resin. This fact was applied in removing non-electrolytes from musts and wines.

The prepared wine sample was flowed slowly down a cation resin column whereupon cations were absorbed and sugars were not. Colour bodies were found to be strongly held by the resin and eluting released only a small fraction thereof. The colour of the eluate was decidedly less than that of the original wine, in fact, the upper portion of the resin bed being permanently discoloured (See figure 10).

Proteins are large molecules and their adsorption by a normal resin is low (Figure 3) and rate of uptake decreased with increasing ionic volume and increasing crosslinkage. A large portion of the proteins are thus not removed from the must or wine by the resin.

Prior to passage through the resin column proteins of musts and wines were denaturized in an 80 vol. % ethanol solution and were removed with those salts which were insoluble in this medium. In beer it has been found that of the material precipitated in 80 vol. % ethanol, tannin precipitable material (Lundin fraction A) was distributed between the filtrate and precipitate in the ratio 2:1 (Ruch 1958). However, in actual chromatograms unidentified spots, probably proteins and/or peptides were of little consequence, in fact, only 7 unidentified substances were noted in more than 40 chromatograms.

The/...

The Method.

It must be stated here that the object of this portion of the work was only to find a suitable method to remove interfering substances and not the perfecting of it for, to be on the safe side eluant volumes and normalities, resin volumes, flow rates, etc., were chosen as to be well within the minimum limits.

(a) Removal of Ethanol Insoluble Substances.

20 ml of wine or must was fortified to 80 vol. % with absolute ethanol and heated for 15 - 20 minutes at approximately 60°C. The insoluble matter was filtered off, the filtrate corked and left overnight. A crystalline precipitate settled out. The solution was filtered and evaporated in vacuo at 40°C to approximately half its original volume. The alcoholic strength of the sample decreased to approximately 50 vol. % and the next step was to pass it through the resin column.

(b) The Resin Column and its Operation.

Although initial experimental work was carried out with Amberlite IR - 120 all the later separations were done with Dowex 50 W(H) x 8. The sodium form of the resin was ground and sieved (60-85 mesh, B.S. No. 410/1943), settled in distilled water and fines siphoned off until supernatant water remained clear. The resin was air-dried, 5 grams weighed off and slurried into the column. This amount of resin was determined to be approximately five times the required

amount/...

amount. The under-water volume of the resin bed was 6 cc. and the tube specifications were:- length 32.5 cm., internal diameter 0.75 cm. Four columns of construction as shown in Figure 10 were set up in a manifold as shown in Figure 11.

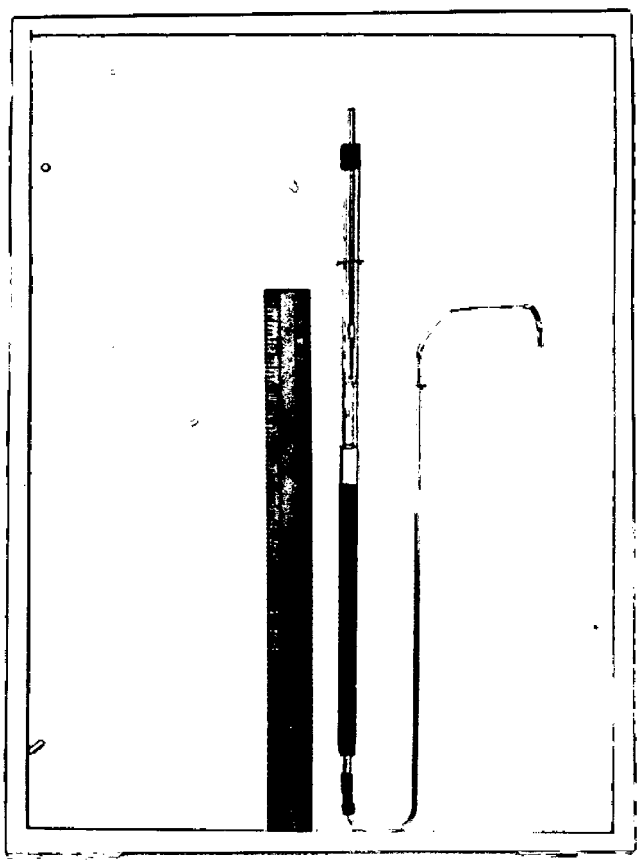

Figure 10. Photograph of ion exchange column used for separating amino acids from sugars.

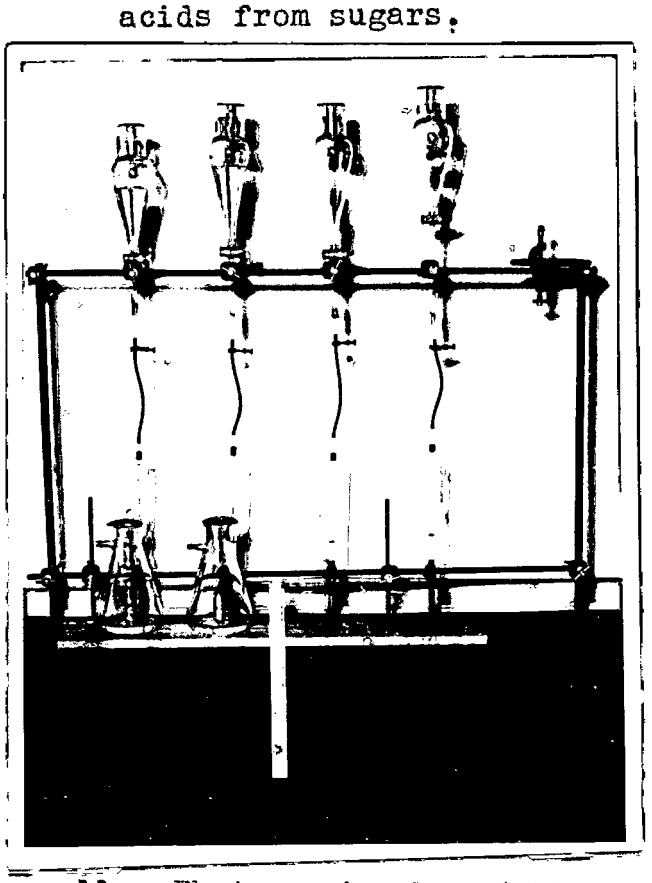

Figure 11. Photograph of manifold of four ion exchanging columns.

After/...

After conditioning the resin, 100 ml of approximately 2N HCl was flowed down the column at approximately 1 ml. per minute (2 ml. per sq. cm. resin/minute). The excess HCl was washed out with 50 ml. 25 vol. % ethanol and 50 ml. 50 vol. % ethanol. This latter step was also to condition the resin for the alcoholic sample.

The alcoholic wine or must sample was placed into the column reservoir and flowed down the column at approximately 0.4 ml. per minute (0.8 ml./sq.cm. resin/minute). The hydrogen form resin colour was slightly off white and the darker descending front of the wine or must cations could be clearly seen. Effluent fractions were tested for amino acids but gave negative results. On completion of the run the resin was washed with 50 ml. 25 vol. % ethanol followed by 50 ml. distilled water.

The eluant solution was approximately 5N NH_4OH of which 150 ml. per column was used. Initial flowrate was very slow, approximately half of the normal rate (0.2 ml/minute) and continued until ammonium ions had progressed the length of the bed. The darker advancing front of the ammonium form could be seen. If an initial normal flow rate is used gas development occurs within the bed and usually breaks it about three-quarter way down. The eluate was collected in a 500 ml. filtration flask

and/...

and evaporated to dryness in vacuo at 40°C. The residue was taken up with approximately 0.01N HCl and final volume brought to 10 ml. in a volumetric flask. The amino acids of 20 ml. must or wine were now contained in a 10 ml. sample.

To test completeness of elution a further 100 ml. of eluant was flowed down the column and eluate similarly treated. 100 microliter (0.1 ml.) of the condensate was placed on a 3 mm. spot and ascendingly paper chromatographed with a n-butanol:acetic acid:water (4:1:5) solvent. The chromatogram showed no spots by reflected light although by direct light very faint spots were discernible. The concentrations were, however, so low as to be negligible.

(c) Separation and Identification of Individual Amino Acids.

Although there are several methods available by which individual amino acids may be determined, two dimensional paper chromatography appeared the best suited to this purpose. Although hundred of studies on this technique have been done not many are specific for wines and musts and those that are did not appear to be entirely satisfactory. A good procedure had to be found and it was to this end that various procedures were tested.

It has been shown that by buffering of a solvent and the paper at a suitable pH (Landua, 1951) spots are more compact and an improvement in separation can be achieved. The initial method tested was that of Berry and Cain (1949) which was altered in that the citrate-phosphate buffer (pH 6.1) incorporated in the phenol solvent was used to buffer the paper also.

Ten (

Ten micrograms of 20 amino acids, normally found in must were individually spotted on 3 mm. diameter spots and developed in an ascending direction with the phenol solvent. From this one dimension chromatogram it was seen that phenyl alanine and arginine spots were very weak, histidine streaked badly and tyrosine, tryptophane and lysine "lost". Furthermore, from the known Rf values of the first and second dimension solvents it could be deduced that serine & glycine and glutamic acid & threonine would probably not separate. As a result of these and other practical disadvantages this method was not used.

The following method tested was that of Levy and Chung (1953). The first dimensional solvent was n-butanol:acetic acid:water (4:1:5) and the second dimension solvent a phenol:m-cresol mixture (1:1) buffered at pH 9.3. The paper for the second dimension also buffered at pH 9.3. This method was also found to be unsatisfactory as some amino acids when present in concentrations of 10 micrograms or less per 3 mm. initial spot were "lost".

The method finally used was one which was developed at the Viticultural Oenological Research Institute.

The following are pertinent data:-

Direction of flow - descending.

Effective flow distance - 14" either dimension.

Paper - Whatman No. 1 (chromatographic grade).

Volume of sample - 100 microliters (=200 microliters of must or wine).

Reagents.

First/...

(i) First dimension solvent (machine direction)

Solvent I - Butanone:propionic acid:water (15:5:6) (Clayton, 1954).

(ii) Second dimension solvent

Solvent II - n-Butanol:acetone:water: dicyclohexylamine (10:10:5:2) (Hardy, 1955).

Characteristic colours were produced in specific amino acids by inclusion of dicyclohexylamine:-

Phenyl Alanine	- gray.
Tyrosine	- light yellowish.
Threonine	- gray.
Glycine	- reddish.
Aspartic Acid	- light blue.
Serine	- gray-purple.
Histidine	- gray.
Asparagine	- yellow.

(iii) Ninhydrin solution.

0.25% ninhydrin in acetone which contained 7% acetic acid.

The following is a typical example of one run of four chromatograms. Cabinets were saturated with solvent I or II:-

4 chromatograms per cabinet (3 wines and 1 reference of 20 amino acids.)

First dimension - solvent I.

Chromatogram in cabinet 8.30 - 10.30 a.m.

Start - 10.30 a.m.)
) 5 hours.
End - 3.30 p.m.)

Temperature 24°C.

Dried at 75°C (15 minutes).

1" trimmed of bottom and 2" below front.

Second/...

Second dimension - solvent II.

Chromatogram in cabinet overnight.

Start - 8.30 a.m.)
) 7½ hours.
End - 4.0 p.m.)

Temperature 24°C.

Dried at 75°C (15 minutes).

The chromatograms were each sprayed with 30 ml. of ninhydrin solution and dried at 75°C (5 minutes). Chromatograms were placed on an X-Ray viewer and spot boundaries outlined in pencil. Spots of low colour intensity were outlined in dotted pencil lines.

The initial spotting technique was originated by van Wyk at the Viticultural Oenological Research Institute (unpublished data). The samples to be chromatographed were micropippeted on a 2 mm. square of Whatman No. 3MM paper, dried and pressed into a 2 mm. square hole cut out of the chromatogram paper. Since initial spots were all of uniform size and volumes of samples equal, the size (and concentration of colour) of a specific amino acid spot in different chromatograms could be visually compared for concentration of this amino acid in the different samples.

The method had some faults, such as "frosting" of cabinet interior and double front formation, but these did not appear to materially affect the chromatograms.

The/...

The amino acids investigated and the abbreviations used for them:-

Arginine	Arg.
Lysine	Lys.
Aspartic Acid	Asp.
Glutamic Acid	Glu
Asparagine	$AspNH_2$
Glycine	Gly.
α-Alanine	Ala.
Proline	Pro.
α-Amino Butyric acid	Am. But.
Methionine	Met.
Leucine-iso-Leucine	Leu.-iso-Leu.
Phenyl Alanine	Phe.
Serine	Ser.
Histidine	His.
Threonine	Thre.
Valine	Val.
Tryptophane	Try.
Tyrosine	Tyr.
Cystine	Cys.

19. Fermentation.

Weight losses of samples in fermentation bottles (Figure 6) were recorded daily

20. Thermolabile and Tannin precipitable Fractions were determined according to the specifications of Berg (1953).

Thermolabile fraction: A filtered wine in a full and tightly corked bottle was held at 60°C for 24 hours. The wine was then removed and held a further 24 hours at room temperature. The turbidity in the wine was measured against that of the same wine which differed only in that it was not heated.

Tannin/...

Tannin precipitable fraction: A filtered wine (250 ml.) was aerated by a constant stream of fine air bubbles. 0.05 gm. of tannin was dissolved in 100 ml. of this wine and held in full and corked bottles for 24 hours at room temperature. The turbidity of the wine was measured against its counterpart which did not receive tannin.

21. White Wine Colour.

Prior to measurement of colour each sample received a standard filtration (suction). 0.25 gm. portions of a No. 7 filter pulp was slurried onto similar sintered glass crucibles and washed well with distilled water. 25 ml. of a 5% tartaric acid solution was passed through the filter and washed with 100 ml. of distilled water. Three portions (10 ml. each) of the wine to be filtered were then filtered, to be followed by a further 15 ml. of this wine which was collected and used for colour measurement.

22. The actual measuring of wine colour was carried out by means of a "Spekker" photometer using 2 cm. cell and a blue filter. Turbidity was determined with the same instrument also using a 2 cm. cell but with a gray filter (H.508).

CHAPTER V.

A. RESULTS.

The Franschhoek Experiment.

The initial experiment in this project was carried out in the Franschhoek Co-operative Cellars but, due to several uncontrollable factors was not successful. In the first instance, the maximum capacity of the ion exchange plant used did not permit sufficient must of each of the four set pH groups to be treated; fermentation set in before completion of the treatment series. In fact, only one group, viz. pH 2.6 (1,390 gallons) could be prepared. Furthermore, to retard the initial fermentation 500 mg/L sulphur dioxide was added to the must and this was no doubt the cause of lengthy and incomplete fermentation.

Routine analysis and tasting of the treated and untreated (Control) wines showed some differences but due to abnormal fermentation they could not be accepted as fully indicative of the treatment effects.

The analysis of magnesium, manganese, copper and iron in treated and untreated wines is given in Table 8.

TABLE 8.

Magnesium, Manganese, Copper and Iron contents of Wines made from an untreated and ion exchange treated must.

Wine.	Magnesium ppm.	Manganese ppm.	Copper ppm.	Iron ppm.
Control*	92	0.79	1.1	4.3
Treated	58	0.46	3.4	3.4
% Decrease	37	71	-	20
% Increase	-	-	209	-

* Untreated.

It/...

It will be seen that magnesium, manganese and iron removals were recorded but that copper concentration increased markedly. Copper contamination had undoubtedly occurred and the most obvious contaminant was the commercial hydrochloric acid which was used for regenerating. On analysis the acid was found to contain approximately 40 ppm. copper, hence all further experiments were done with chemically pure (C.P.) hydrochloric acid.

Laboratory and Elsenburg Cellar Experiments.

Ten laboratory (I - X) and two cellar experiments were done and the analysis of the five musts that were used there are tabulated in Table 9.

The Riesling and Stein musts treated in the cellar were identical to those used in laboratory experiments IX (Riesling) and X (Stein).

unst. below an experiment number signifies no sterilization.

st. below an experiment number signifies sterilization (heat) of that must after treatment.

TABLE 9.

Composition of the various Musts used in Ion Exchange Experiments I to X and the Cellar Experiments.

Analysis.	St.Emillion.	Colombard.	Stein 1958.		Riesling.	Stein 1959
	I II. unst.	III IV. st.	V,VI st.	VII,VIII unst.	IX Cellar. unst.	X Cellar. unst.
pH.	3.2	3.13	3.42		3.73	3.58
Specific Gravity.	-	1.0743	1.0806		1.0820	1.0825
Extract gm/L.	-	199.5	215.8		219.1	220.5
Total Acid. (as Tartaric Acid) gm/L.	8.3	8.0	5.93		5.85	6.3
Volatile Acidity (as Acetic Acid) gm/L.	0.12	0.14	0.12		0.14	0.3
Reducing Sugar gm/L.	187.0	170.7	178.0		-	-
Balling Degrees.	-	-	-		19.5	20
Ammonia mg/L.	-	-	-		115.6	67.5

Tables/...

Tables 10 to 17 are analyses of wines of experiments I to VIII. The data of experiments IX and X and the two carried out in the cellar will be given in the appropriate sections.

TABLE 10.

Analyses of Wines made from an Untreated and Ion Exchange Treated Must.

EXPERIMENT I.

Analyses.	Control*	Ion Exchange Treated.		
Must pH	3.2	3.2	2.95	2.65, 2.35 2.05
pH	3.38	3.29	3.03	
Specific Gravity	0.9926	0.9930	0.9928	
Alcohol Vol. %	11.65	11.57	11.48	
Extract gm/L.	24.8	25.6	24.1	
Reducing Sugar gm/L.	1.5	1.06	1.84	
Sugar-Free Extract gm/L.	23.3	24.5	22.6	
Sulphur Dioxide Free mg/L.	4.8	5.8	4.1	
Sulphur Dioxide Total mg/L.	34.3	34.0	33.0	
Total Acidity Must gm/L. (tartaric acid)	8.3	-	-	
Wine gm/L. (tartaric acid)	7.95	9.2	10.4	
Volatile Acidity gm/L. (acetic)	0.38	0.37	0.61	
Fixed Acidity Wine gm/l (tartaric acid)	7.5	8.8	9.6	
Total Tartrates gm/L. (tartaric acid)	3.7	3.6	3.7	
Esters mg/100 ml. (ethyl acetate)	6.9	6.9	16.2	
Aldehydes mg/100 ml. (acetaldehyde)	7.5	4.7	5.7	
Ash. gm/L.	2.276	2.856	2.311	
Alkalinity of Ash ml. 0.1N acid/100 ml.	13.6	31.6	20.6	
as potassium mg/L.	530.4	1232.4	803.4	
% Dissociation of Fixed Acidity	0.5	0.5	1.0	

Did not Ferment.

* Untreated.

TABLE 11.

Analyses of Wines made from an Untreated and Ion Exchange Treated Must.

EXPERIMENT II.

Analyses.	Control*	Ion Exchange Treated.			
Must pH	3.2	3.0	2.8	2.6	2.4
pH	3.39	3.16	2.93	2.69	
Specific Gravity	0.9924	0.9920	0.9919	0.9931	
Alcohol Vol. %	11.42	11.32	11.26	11.05	
Extract gm/L.	23.5	22.3	22.9	24.5	
Reducing Sugar gm/L.	1.1	1.3	1.5	3.3	
Sugar-Free Extract gm/L.	22.4	21.0	21.4	21.2	
Sulphur Dioxide Free mg/L.	3.2	4.2	5.1	5.7	
Sulphur Dioxide Total mg/L.	33.3	30.6	28.7	33.3	
Total Acidity Must gm/L. (tartaric acid)	8.1	-	-	-	
Total Acidity Wine gm/L. (tartaric acid)	7.4	8.3	9.5	10.9	
Volatile Acidity gm/L. (acetic)	0.39	0.38	0.48	0.91	
Fixed Acidity Wine gm/L. (tartaric acid)	6.9	7.8	8.9	9.8	
Total Tartrates gm/L. (tartaric acid)	3.2	3.2	3.2	3.2	
Esters. mg/100 ml. (ethyl acetate)	5.3	6.9	7.9	9.9	
Aldehydes. mg/100 ml. (acetaldehyde)	7.1	11.0	8.4	7.0	
Ash gm/L.	2.260	1.752	1.472	1.064	
Alkalinity of Ash ml.0.1N acid/100 ml.	31.9	22.6	14.4	8.4	
Alkalinity of Ash as potassium mg/L.	1244.3	881.2	561.2	327.4	
% Dissociation of Fixed Acidity	0.5	0.7	1.1	1.7	

Growth of Candida Mycoderma Film upon Must.

* Untreated.

TABLE 12.

Analyses of Wines made from an Untreated and Ion Exchange Treated Must.

EXPERIMENT III.

Analyses.	Control*	Ion Exchange Treated.			
Must pH	3.13	3.0	2.8	2.6	2.4
pH	3.17	3.1	2.83	2.66	2.51
Specific Gravity	0.9924	0.9921	0.9946	1.0010	1.0366
Alcohol Vol.%	11.39	11.48	11.05	10.13	5.15
Extract gm/L.	23.7	23.5	28.4	42.2	118.1
Reducing Sugar gm/L.	2.3	2.5	7.9	20.6	93.6
Sugar-Free Extract gm/L.	21.4	21.0	20.5	21.6	24.5
Sulphur Dioxide Free mg/L.	-	-	-	-	-
Sulphur Dioxide Total mg/L.	-	-	-	-	-
Total Acidity Must gm/L. (tartaric acid)	8.0	8.3	8.9	9.3	9.8
Total Acidity Wine gm/L. (tartaric acid)	8.1	8.3	9.3	10.0	11.2
Volatile Acidity gm/L.(acetic)	0.24	0.26	0.4	0.64	1.32
Fixed Acidity Wine gm/L. (tartaric acid)	7.8	8.0	8.8	9.2	9.6
Total Tartrates gm/L.(tartaric acid)	4.2	4.3	4.0	4.0	4.0
Esters. mg/100 ml. (ethyl acetate)	8.5	9.0	10.3	11.9	28.0
Aldehydes mg/100 ml. (acetaldehyde)	2.9	2.8	3.2	4.9	15.5
Ash gm/L.	1.972	1.788	1.360	0.996	0.712
Alkalinity of Ash ml 0.1N acid/100 ml.	27.2	23.9	14.3	10.0	6.2
Alkalinity of Ash as potassium mg/L.	1060.	932	557	390	243
% Dissociation of Fixed Acidity	0.7	0.8	1.4	1.9	2.6

*Untreated.

TABLE 13.

Analyses of Wines made from an Untreated and Ion Exchange Treated Must.

EXPERIMENT IV.

Analyses.	Control*	Ion Exchange Treated.		
Must pH	3.0	2.8	2.6	2.4
pH	3.2	2.95	2.65	2.5
Specific Gravity	0.9930	0.9947	1.0177	1.0518
Alcohol Vol. %	11.05	10.72	7.6	2.87
Extract gm/L.	24.0	27.6	76.8	149.7
Reducing Sugar gm/L.	3.6	5.7	52.1	159.6
Sugar-Free Extract gm/L.	20.4	21.9	24.7	-
Sulphur Dioxide Free mg/L.	-	-	-	-
Total mg/L.	-	-	-	-
Total Acidity Must gm/L. (Tartaric acid)	8.1	8.6	9.2	9.5
Wine gm/L. (tartaric acid)	8.0	8.9	9.7	9.9
Volatile Acidity gm/L. (acetic)	0.37	0.47	0.72	0.82
Fixed Acidity Wine gm/L. (tartaric acid)	7.5	8.3	8.8	9.0
Total Tartrates gm/L. (tartaric acid)	3.0	2.6	2.5	2.9
Esters. mg/100 ml. (ethyl acetate)	13.2	10.7	14.7	18.6
Aldehydes mg/100 ml. (acetaldehyde)	3.8	3.8	6.1	7.0
Ash gm/L.	1.656	1.184	0.784	0.536
Alkalinity of Ash ml.0.1N acid/100 ml.	21.2	14.2	8.8	6.0
as potassium mg/L.	827	555	343	234
% Dissociation of Fixed Acidity.	0.7	1.1	2.1	2.8

* Untreated.

TABLE 14.

Analyses of Wines made from an Untreated and Ion Exchange Treated Must.

EXPERIMENT V.

Analyses.	Control*	Ion Exchange Treated.			
Must pH	3.43	3.2	3.0	2.8	2.6
pH	3.55	3.34	3.14	2.8	2.63
Specific Gravity	0.9920	0.9915	0.9911	0.9970	1.0130
Alcohol Vol.%	11.87	11.86	11.88	10.98	8.77
Extract gm/L.	23.6	22.4	21.5	34.2	67.6
Reducing Sugar gm/L.	0.5	0.5	1.2	13.1	44.6
Sugar-Free Extract gm/L.	23.1	21.9	20.3	21.1	23.0
Sulphur Dioxide Free mg/L.	-	-	-	-	-
Sulphur Dioxide Total mg/L.	-	-	-	-	-
Total Acidity Must gm/L. (tartaric acid)	6.0	6.7	7.3	8.0	8.3
Total Acidity Wine gm/L. (tartaric acid)	7.1	7.7	8.3	9.2	9.6
Volatile Acidity gm/L. (acetic)	0.30	0.39	0.48	0.73	1.01
Fixed Acidity Wine gm/L. (tartaric acid)	6.7	7.2	7.7	8.3	8.4
Total Tartrates gm/L. (tartaric acid)	3.7	3.7	3.8	3.7	3.6
Esters. mg/100 ml. (ethyl acetate)	15.0	15.0	15.1	19.4	29.5
Aldehydes. mg/100 ml. (acetaldehyde)	9.3	8.3	9.7	7.2	12.7
Ash gm/L.	2.525	2.103	1.602	1.049	0.932
Alkalinity of Ash ml.0.1N acid/100 ml.	35.1	26.0	18.2	9.4	7.2
Alkalinity of Ash as potassium mg/L.	1369	1014	710	367	281
% Dissociation of Fixed Acidity	0.4	0.5	0.7	1.5	2.2

* Untreated.

TABLE 15.

Analyses of Wines made from an Untreated and Ion Exchange Treated Must.
Experiment VI.

Analyses.	Control*	Ion Exchange Treated.			
Must pH	3.43	3.2	3.0	2.8	2.6
pH	3.48	3.27	3.07	2.85	2.63
Specific Gravity	0.9917	0.9913	0.9910	0.9934	1.010
Alcohol Vol.%	11.83	11.87	11.85	11.42	9.08
Extract gm/L.	23.2	22.1	21.5	26.1	62.8
Reducing Sugar gm/L.	0.5	0.9	1.4	6.3	40.3
Sugar-Free Extract gm/L.	22.7	21.2	20.1	19.8	22.5
Sulphur Dioxide Free mg/L.	-	-	-	-	-
Sulphur Dioxide Total mg/L.	-	-	-	-	-
Total Acidity Must gm/L. (tartaric acid)	5.9	6.8	7.4	7.8	8.1
Total Acidity Wine gm/L. (tartaric acid	6.9	7.5	8.2	8.8	9.6
Volatile Acidity gm/L. (acetic)	0.39	0.39	0.49	0.62	1.07
Fixed Acidity Wine gm/L. (tartaric acid)	6.4	7.0	7.6	8.0	8.3
Total Tartrates gm/L. (tartaric acid)	3.4	3.5	3.6	3.6	3.5
Esters. mg/100 ml. (ethyl acetate)	12.5	12.1	13.2	16.9	32.8
Aldehydes. mg/100 ml. (acetaldehyde)	9.1	7.7	8.8	5.8	11.2
Ash gm/L.	2.416	1.935	1.488	1.113	0.90
Alkalinity of Ash ml.0.1N acid/100 ml.	34.2	24.6	17.6	11.9	7.5
Alkalinity of Ash as potassium mg/L.	1334	959	686	464	293
% Dissociation of Fixed Acidity	0.4	0.6	0.9	1.4	2.3

* Untreated.

TABLE 16.

Analyses of Wines made from an Untreated, Ion Exchange Treated and Tartaric Acid Addition Must.

Experiment VII,

Analyses.	Control.*	Must pH decreased by:-					
		Ion Exchange to:-			Tartaric Acid to:-		
	3.5	3.2	3.0	2.8	3.2	3.0	2.8
pH	3.53	3.24	3.09	2.97	3.24	3.03	2.94
Specific Gravity.	0.9908	0.9903	0.9903	0.9898	0.9920	0.9931	0.9954
Alcohol Vol. %	11.65	11.65	11.57	11.65	11.61	11.48	11.57
Extract gm/L.	20.2	19.0	18.8	17.7	23.2	26.0	31.7
Reducing Sugar gm/L.	0.7	0.5	0.9	0.4	0.4	1.0	1.6
Sugar-Free Extract gm/L	19.5	18.5	17.9	17.3	22.8	25.0	30.1
Sulphur Dioxide							
Free mg/L.	7.5	6.9	7.2	5.1	8.4	8.1	4.2
Total mg/L.	26.9	35.4	45.7	42.0	30.3	34.5	27.9
Total Acidity							
Must gm/L.(tartaric acid)	5.5	6.4	6.9	7.1	8.0	10.9	14.1
Wine gm/L. (tartaric acid)	6.3	7.1	8.0	8.3	8.8	11.7	15.0
Volatile Acidity gm/L. (acetic)	0.38	0.41	0.74	0.76	0.45	0.75	0.83
Fixed Acidity							
Wine gm/L. (tartaric acid)	5.8	6.6	7.1	7.5	8.3	10.7	14.0
Total Tartrates gm/L. (tartaric acid)	2.9	2.9	2.9	2.9	5.4	7.7	11.7
Esters.							
mg/100 ml. (ethyl acetate).	10.0	9.2	10.4	11.8	11.6	12.0	12.6
Aldehydes.							
mg/100 ml (acetaldehyde)	-	-	-	9.5	-	-	10.4
Ash gm/L.	2.001	1.594	1.312	1.046	1.990	1.704	2.21
Alkalinity of Ash							
ml.0.1N acid/100 ml.	26.9	18.1	13.0	8.4	27.3	22.9	28.6
as potassium mg/L.	1049	706	507	328	1065	893	1115
Dissociation of Fixed Acidity	0.4	0.7	0.9	1.2	0.6	0.7	0.6

* Untreated.

TABLE 17.

Analyses of Wines made from an Untreated, Ion Exchange Treated and Tartaric Acid Addition Must.

Experiment VIII.

Analyses.	Control.*	Must pH decreased by:-					
		Ion Exchange to:-			Tartaric Acid to:-		
	3.52	3.2	3.0	2.8	3.2	3.0	2.8
pH	3.54	3.22	3.02	2.9	3.27	3.03	2.87
Specific Gravity.	0.9905	0.9901	0.990	0.9906	0.9919	0.9937	0.9962
Alcohol Vol. %	11.7	11.7	11.63	11.48	11.62	11.53	11.41
Extract gm/L.	19.o	18.5	18.0	19.0	23.2	27.4	33.7
Reducing Sugar gm/L.	Nil	Nil	1.2	2.3	0.5	0.9	2.0
Sugar-Free Extract gm/L.	19.6	18.5	16.8	16.7	22.7	26.5	31.7
Sulphur Dioxide Free mg/L.	7.8	6.7	7.2	7.0	8.1	8.4	8.1
Sulphur Dioxide Total mg/L.	23.5	27.3	33.0	37.8	26.4	27.3	25.2
Total Acidity Must gm/L. (tartaric acid)	5.4	6.2	6.7	7.1	8.0	11.1	14.9
Total Acidity Wine gm/L. (tartaric acid)	6.1	7.0	7.8	8.3	8.9	12.3	16.6
Volatile Acidity gm/L. (acetic)	0.33	0.33	0.53	0.9	0.51	0.65	0.96
Fixed Acidity Wine gm/L. (tartaric acid)	5.7	6.6	7.1	7.2	8.3	11.5	15.4
Total Tartrates gm/L. (tartaric acid)	2.5	2.5	2.5	2.4	5.2	8.4	12.0
Esters mg/100 ml. (Ethyl acetate)	9.6	8.6	9.7	15.1	10.0	12.3	14.7
Aldehydes mg/100 ml. (acetaldehyde)	12.1	9.5	9.7	6.2	11.1	8.1	8.4
Ash gm/L.	1.870	1.382	1.086	0.956	1.971	1.988	2.076
Alkalinity of Ash ml.0.1N acid/100 ml.	24.5	14.7	10.0	6.9	24.6	25.2	25.2
Alkalinity of Ash as potassium mg/L.	956	573	390	269	959	983	983
% Dissociation of Fixed Acidity	0.4	0.7	1.1	1.5	0.5	0.7	0.7

* Untreated.

B. DISCUSSION OF RESULTS.

Influence of ion exchange treatment (hydrogen cycle) upon certain chemical constituents, phenomena and conditions of white musts and their wines.

In Experiment I pH was decreased by 0.3 units to a maximum of 2.05 as a pilot experiment but in all later experiments pH was decreased to the given set values although the pH 2.6 and 2.4 groups were later discarded.

The pH 3.2 treated must (Experiment I) was obtained by allowing the control must to flow through the column until the effluent attained the control pH (3.2). The pH 2.95 must also contained a portion of the pH 3.2 treated must. It will be seen from Table 10 that the ash contents of the pH 3.2 and pH 2.95 wines are higher than that of control. Expressing the alkalinity of the ash as potassium it will also be seen that a considerable increase of cations occurred in the pH 3.2 wine and a somewhat smaller increase in the pH 2.95 wine. The reason for this is that desorption of resin-held cations by the influent (control must) occurred, which resulted in an effluent of higher cation concentration than the control. It is of interest to note that the cation increase, no doubt due largely to potassium ions, did not manifest undue effect upon the determined components of the pH 3.2 wine.

pH.

All wines except one, which finished fermentation with its must pH showed an increase in pH. The influence of pH is manifested by many components and its effect will be examined when these substances are discussed.

(a)/...

(a) Alcohol.

Where fermentation was complete, as shown by residual sugar content, alcohols did not markedly differ.

(b) Specific Gravity, Sugar-free Extract, Ash and Alkalinity of the Ash.

Specific gravity and sugar-free extract do not reflect significantly large differences, a better indication of the effect of ion exchange is given by ash and alkalinity of the ash figures (Tables 10 to 17). Progressive exchange shows a clear decrease in ash content, i.e. inorganic cation material. In expressing the alkalinity of the ash as potassium the effect of ion exchange on the removal of cations is further illustrated.

(c) Total Acidity.

Alkalinity of the ash is a measure of the organic acid salts present in the wine and this figure will be seen to decrease with increased ion exchange treatment (Tables 10 to 17). In effect, the acid content of a must is increased by the treatment at the expense of the organic acid salts; notably potassium bitartrate. It is obvious that pH can be comparatively easily decreased by this means with but a relatively small increase in the fixed acidity. If tartaric acid is added to must, relatively large quantities will be required to decrease pH to the set values. Table 18 illustrates the latter and the former points.

Table 18./...

TABLE 18.

Comparison of the Increases in Total Acidity of a Must, caused by Tartaric Acid Addition and Ion Exchange Treatment.

Must pH.	Tartaric Acid Addition.		Ion Exchange Treatment.	
	Total Acidity of Must gm/L. (Tartaric Acid).	Increase in Total Acidity gm/L. (Tartaric Acid).	Total Acidity of Must gm/L. (Tartaric Acid).	Increase in Total Acidity gm/L. (Tartaric Acid).
Experiment VII.				
Control*- 3.5	5.5	-	5.5	-
3.2	8.0	2.5	6.4	0.9
3.0	10.9	5.4	6.9	1.4
2.8	14.1	8.6	7.1	1.6
2.6	20.2	14.7	7.4	1.9
Experiment VIII.				
Control*- 3.52	5.4	-	5.4	-
3.2	8.0	2.6	6.2	0.8
3.0	11.1	5.7	6.7	1.3
2.8	14.9	9.5	7.1	1.6

* Untreated.

It will be seen from Table 18 that the increase in acidity of must by addition of tartaric acid and ion exchange differ widely.

In the wines of Experiments VII and VIII the effectiveness of the fixed acidity of ion exchange treated musts over those to which tartaric acid was added is given in Table 19.

Table 19/...

TABLE 19.

Comparison of Per Cent Dissociation of Fixed Acidity of Wines whose Must Acidity was increased by Tartaric Acid addition and Ion Exchange Treatment.

Wine Group.	Per Cent Dissociation of Fixed Acidity.	
	Tartaric Acid Addition.	Ion Exchange.
Experiment VII.		
Control*	0.4	0.4
pH 3.2	0.6	0.7
pH 3.0	0.7	0.9
pH 2.8	0.6	1.2
Experiment VIII.		
Control*	0.4	0.4
pH 3.2	0.5	0.7
pH 3.0	0.7	1.1
pH 2.8	0.7	1.5

* Untreated.

(d) Total Tartrates.

Tartrates are closely related to fixed acidity and pH of wines and musts and it is important to note the effect of ion exchange upon this component. In Experiments I to VIII (wines) the total tartrates do not show large differences, in fact allowing for errors in the method of analysis they show no variation at all. Theoretically a larger percentage of tartrates should have been precipitated in the control than the ion exchange samples (Tables 10 to 17). In Experiments VII to VIII it became clear that in all probability no tartrates were precipitated at all. Except in one case (Experiment VII, pH 3.0 tartaric acid group) where a visible crystalline precipitation occurred, the tartaric acid added to the must was fully recovered in the wine (Table 20).

TABLE 20.

Total Tartrate Content of Musts to which varying quantities of Tartaric Acid had been added and the Total Tartrate Content of their Wines.

Wine Group.	Tartaric Acid added to Must. A. gm/L.	Tartrate Content of Control Wine. B. gm/L.	Theoretical Tartrate Content of Wine. A.+ B.gm/L.	Actual Tartrate Content of Wine. gm/L.
Experiment VII.				
Control*	Nil	2.9	-	-
pH 3.2	2.5	-	5.4	5.4
pH 3.0	5.5	-	8.4	7.7
pH 2.8	8.9	-	11.8	11.7
Experiment VIII.				
Control*	Nil	2.5	-	-
pH 3.2	2.7	-	5.2	5.2
pH 3.0	5.9	-	8.4	8.4
pH 2.8	9.7	-	12.2	12.0

* Untreated.

However, on storage (maturation) and racking (clarification) an argol precipitation should occur. The Stein and Riesling wines, fermented in the cellar in stainless steel drums showed large quantities of crystalline precipitate on the inner surfaces of the drums. Upon storage for approximately one year in large glass containers a further visible crystalline precipitation occurred in all the wines.

TABLE 21.

Total Tartrate Content of the Wines, made from Untreated and Ion Exchange Treated Riesling and Stein Musts, after one year maturation period.

Wine Group.	Riesling.	Stein.
	Total Tartrates. gm/L. (Tartaric Acid).	Total Tartrates. gm/L. (Tartaric Acid).
Control*	2.4	1.9
pH 3.2	3.6	3.2
pH 3.0	4.4	4.3
pH 2.8	4.9	4.8

* Untreated.

From the data contained in Table 21 a progressive retention of tartrates will be noted; even although precipitation occurred in all these wines the pH 2.8 wine retained far more tartrates than did the control wine. It is obvious that since these tartrates are also largely in the form of tartaric acid and more so in the lower pH groups, the fixed acidity values will also be and remain relatively large. This is born out by the data in Table 22 (cellar wines) where it will be seen that large differences occurred in the fixed acidity of control musts and their wines and only slight differences in the fixed acidity of ion exchange treated musts and their wines after a maturation period of approximately one year.

TABLE 22.

Fixed Acidity of the Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts after a one year maturation period.

Wine Group.	Riesling. Fixed Acidity of Must. gm/L. (Tartaric Acid).	Riesling. Fixed Acidity of Wine. gm/L. (Tartaric (Acid).	Stein. Fixed Acidity of Must. gm/L. (Tartaric Acid).	Stein. Fixed Acidity of Wine. gm/L. (Tartaric Acid).
Control *	5.7	3.5	5.9	3.7
pH 3.2	7.2	6.7	6.9	6.6
pH 3.0	7.8	7.6	7.7	7.8
pH 2.8	8.6	8.6	8.3	8.4

* Untreated.

Due to practical difficulties, pH of must could not also be decreased in the cellar by tartaric acid addition, however, factual evidence is available (van Wyk, (1958) Rabie, (1950) to show that appreciable decreases in fixed acidity does occur during the maturation of wines whose must pH was decreased by addition of tartaric acid. Accepting this as fact it is evident that by the ion exchange process the fixed acidity can be better held in a wine than would be the case if tartaric acid were added.

The tartrate investigation was, however, carried a step further; the laboratory wines of Experiments VII and VIII were subjected to cold stabilization in place of a lengthy maturation period. A fixed volume of each wine of both Experiments (VII and VIII) was chilled at 0°C for six weeks in stoppered sentrifuge tubes. The wines were then sentrifuged clear and tartrate analysis done on them.

TABLE 23.*

Influence of Chilling upon the Total Tartrates of Stein Wines whose Must pH was decreased by Ion Exchange Treatment and Tartaric Acid Addition.

Wine Group.	Experiment VII. Unchilled. Total Tartrates gm/L. (Tartaric Acid).	Experiment VII. Chilled. Total Tartrates gm/L. (Tartaric Acid).	Experiment VIII. Unchilled. Total Tartrates gm/L. (Tartaric Acid).	Experiment VIII. Chilled. Total Tartrates gm/L. (Tartaric Acid.
Control**	2.9	2.4	2.4	2.2
Ion Exchange Treatment.				
pH 3.2	2.6	2.6	2.6	2.6
pH 3.0	2.6	2.6	2.6	2.5
pH 2.8	2.6	2.6	2.4	2.3
Tartaric Acid Addition.				
pH 3.2	5.9	3.8	5.5	3.7
pH 3.0	9.1	7.0	9.2	7.1
pH 2.8	12.4	10.3	13.4	10.9

** Untreated, * Total tartrates determined by sodium metavanadate colorimetric procedure.

From Table 23 it will be seen that no relatively large tartrate decrease occurred in either control or ion exchange treated wines. In the tartaric acid addition wines a constant decrease of about 2 gm/L. occurred.

The failure of the tartrates to precipitate further in the tartaric acid addition group indicates chiefly insufficiently low temperatures and/or a low potassium ion concentration. The initial low concentration of tartrates in the control must is one possible reason for the control samples not manifesting a larger tartrate decrease.

Due to the practical facilities for controlled low temperatures not being available this portion of the work was not further investigated.

(e)/...

(e) Total Esters.

From Tables 10 to 17 it will be seen that some high ester values were found in Experiments .. III, V and VI. The majority of the remainder of the determinations also show increases but not as large as the former. Where tartaric acid was added to musts an increase in esters was also manifested.

Since the most significant esterification process in wine is that between acetic acid and ethyl alcohol, the volatile acidity content must also be taken into account when seeking an explanation of the ester increases. Gentilini (1947) found that as a general rule wines high in ethyl acetate were high in volatile acidity. From the data in Tables 24(a) and (b) it will be seen that as volatile acidity increased substantially, either as a result of ion exchange or tartaric acid addition, so do the esters increase. This is in agreement with the findings of Gentilini.

It is obvious that in these ion exchange wines volatile acidity concentration was a functional factor in ester formation and since a normal increase of esters occurred with volatile acidity increases it does not appear that the adsorption of must cations is of direct consequence in this process.

An inexplicable occurrence was the decrease in ester content of some wines in the sample immediately following the control. The fact that these increases happened immediately after the control indicates that volatile acidity must be present in relatively large quantities to ensure ester increments.

TABLE 24(a)and (b)

(a)

Volatile Acidity and Ester Content of different Wines whose Must pH values were decreased by Ion Exchange Treatment and Tartaric Acid Addition.

Must pH decreased by Ion Exchange Treatment.

Experiment.	Wine Group.	Volatile Acidity gm/l (Acetic Acid.)	Esters. mg/100 ml. (Ethyl Acetate.)
II	Control*	0.39	5.3
	pH 3.0	0.38	6.9
	pH 2.8	0.48	7.9
	pH 2.6	0.91	9.9
III	Control*	0.24	5.5
	pH 3.0	0.26	9.0
	pH 2.8	0.40	10.3
	pH 2.6	0.64	11.9
	pH 2.4	1.32	28.0
IV	Control *	0.37	13.2
	pH 2.8	0.47	10.7
	pH 2.6	0.72	14.7
	pH 2.4	0.82	18.6
V	Control*	0.3	15.0
	pH 3.2	0.39	15.0
	pH 3.0	0.48	15.1
	pH 2.8	0.73	19.4
	pH 2.6	1.01	29.5
VI	Control*	0.39	12.5
	pH 3.2	0.39	12.1
	pH 3.0	0.49	13.2
	pH 2.8	0.62	16.9
	pH 2.6	1.07	32.8

* Untreated.

TABLE 24(b).

Analyses.	Control*	Must pH decreased by:-					
		Ion Exchange Treatment.			Tartaric Acid Addition.		
		pH 3.2	pH 3.0	pH 2.8	pH 3.2	pH 3.0	pH 2.8
Experiment VII.							
Volatile Acidity. gm/L.	0.38	0.41	0.74	0.76	0.45	0.75	0.83
Esters. mg/100 ml.	10.0	9.2	10.4	11.8	11.6	12.0	12.6
Experiment VIII.							
Volatile Acidity. gm/L.	0.33	0.33	0.53	0.9	0.51	0.65	0.96
Esters. mg/100 ml.	9.6	8.6	9.7	15.1	10.0	12.3	14.7

* Untreated.

(f) Volatile Acidity.

From Tables 24(a) and (b) it will be seen that as the proportion of pH 2 must was substantially increased in the mixtures (pH groups) so did the volatile acidity of their wines.show a corresponding increase. The group immediately after the control did not always show an increase but groups thereafter did.

Certain microbes, other than yeast cells are known to facilitate the formation of volatile acidity in musts or wines. In Experiment II, pH 2.4 group, a film of Candida Mycoderma developed upon the must surface prior to fermentation and the possibility that volatile acidity increases could be at least partly due to the activities of these or other microbes could not be excluded.

The/...

The musts of Experiments III - VI were, therefore, sterilized (heat) and sterile conditions maintained throughout.

On examining the data in Table 24(a) and (b) it is apparent that whether must was sterilized prior to ferment or not the tendency of volatile acidity formation was not unduly affected, in effect, the presence of microbes, other than yeast cells, did not radically influence formation of volatile acidity. The cause of this phenomenon was undoubtedly due to the ion exchange treatment.

In the ion exchange treatment of must two major influences, viz. the removal of cations and the equivalent substitution of hydrogen ions are effected and it is necessary to establish which of these is responsible for volatile acidity increments. It will be shown in the section on fermentation that both of these factors influenced fermentation and it is, therefore, not impossible that the same could have been the case with volatile acidity formation.

It will be seen from graphs in Figures 12(a) and (b) where the dotted lines represent tartaric acid addition data and unbroken lines ion exchange data that volatile acidity still increased no matter by which of the two means pH reduction was effected. In the tartaric acid addition groups the increase in volatile acidity was the result of either an increase in hydrogen ions and/or an increase in tartaric acid. In Experiments IX and X where pH of ion exchange treated must was increased by addition of potassium hydroxide, the influence of pH was neutralized and the must cations removed by the resin replaced by potassium ions, which ions made out the major portion of the total must

must cations held by the resin.

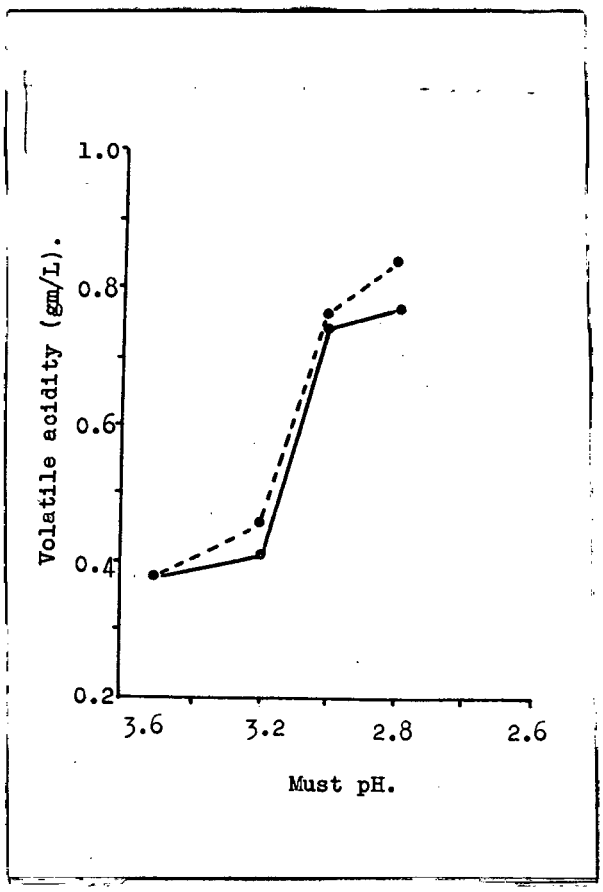

Figure 12(a). Influence of pH upon the formation of volatile acidity in the wines of Stein must (Experiment VII), whose pH was decreased by both ion exchange treatment (I.E.T.) and addition of tartaric acid (T.A.A.) to values of 3.2, 3.0 and 2.8. ———, I.E.T; ------, T.A.A.

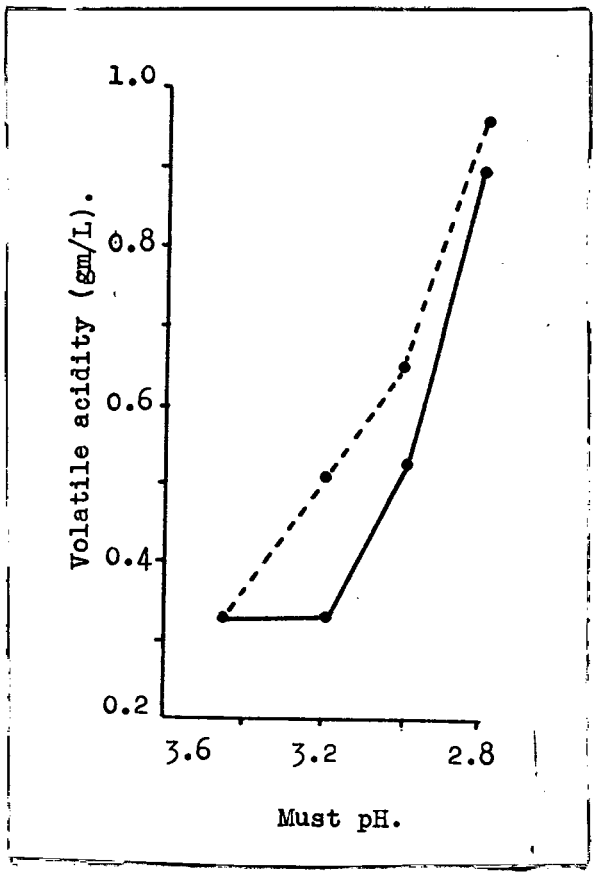

Figure 12(b). Influence of pH upon the formation of volatile acidity in the wines of Stein must (Experiment VIII), whose pH was decreased by both ion exchange treatment (IE.T.) and addition of tartaric acid (T.A.A.) to values of 3.2, 3.0 and 2.8. ———, I.E.T; ------, T.A.A.

From Table 25 and the graphs in Figures 13(a) and (b) where dotted lines represent the date of ion exchange (control) wines and the unbroken lines the usual ion exchange wine data it is apparent that the usual progressive increase of volatile acidity again occurred with increased ion exchange treatment. Where must pH and cation effect were neutralized, a relatively constant volatile acidity content resulted which was generally slightly lower than that of the control sample.

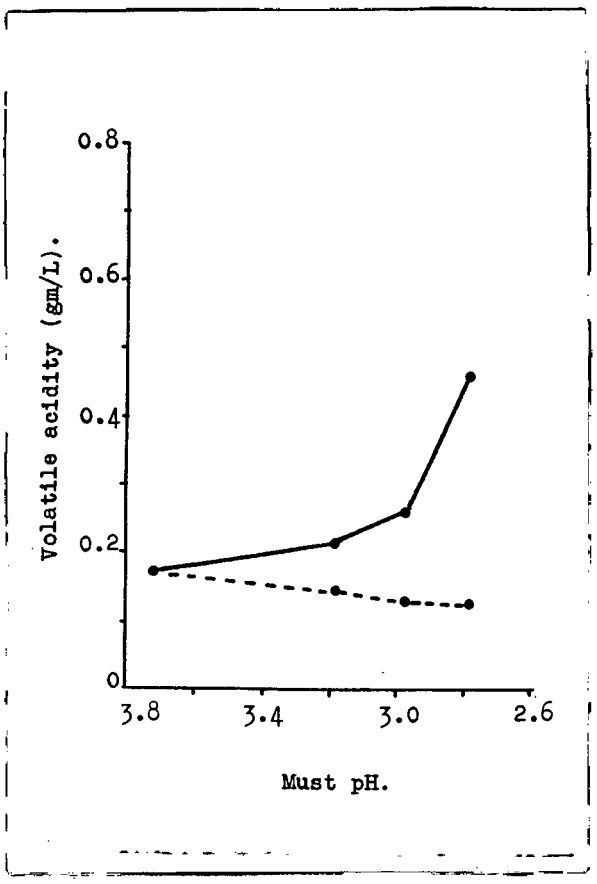

Figure 13(a). Influence of pH upon the formation of volatile acidity in the wines of an ion exchange treated Riesling must (Experiment IX). ————, must pH decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8; ------, must pH decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8 and then increased, by the addition of potassium hydroxide, to the control (untreated) values.

TABLE 25.

Influence of pH upon the Volatile Acidity formation in the Wines made from Ion Exchange Treated Riesling and Stein Musts.

Wine Group.	Must pH decreased by Ion Exchange.			
	pH increased to that of Control* Must.		pH as initially decreased.	
	Wine pH.	Volatile Acidity gm/L.	Wine pH.	Volatile Acidity gm/L.
Experiment IX (Riesling).				
Control* (i)	3.7	0.16	-	-
(pH 3.71) (ii)	3.72	0.18	-	-
pH 3.2 (i)	3.77	0.15	3.3	0.21
(ii)	3.76	0.14	3.29	0.21
pH 3.0 (i)	3.77	0.12	3.1	0.26
(ii)	3.79	0.13	3.1	0.24
pH 2.8 (i)	3.8	0.12	3.0	0.44
(ii)	3.8	0.12	3.0	0.46
Experiment X (Stein).				
Control* (i)	3.75	0.36	-	-
(pH 3.58) (ii)	3.8	0.34	-	-
pH 3.2 (i)	3.9	0.29	3.5	0.38
(ii)	3.9	0.30	3.52	0.36
pH 3.0 (i)	3.91	0.30	3.3	0.44
(ii)	3.92	0.30	3.32	0.42
pH 2.8 (i)	3.93	0.30	3.2	0.66
(ii)	3.91	0.33	3.18	0.66

* Untreated.

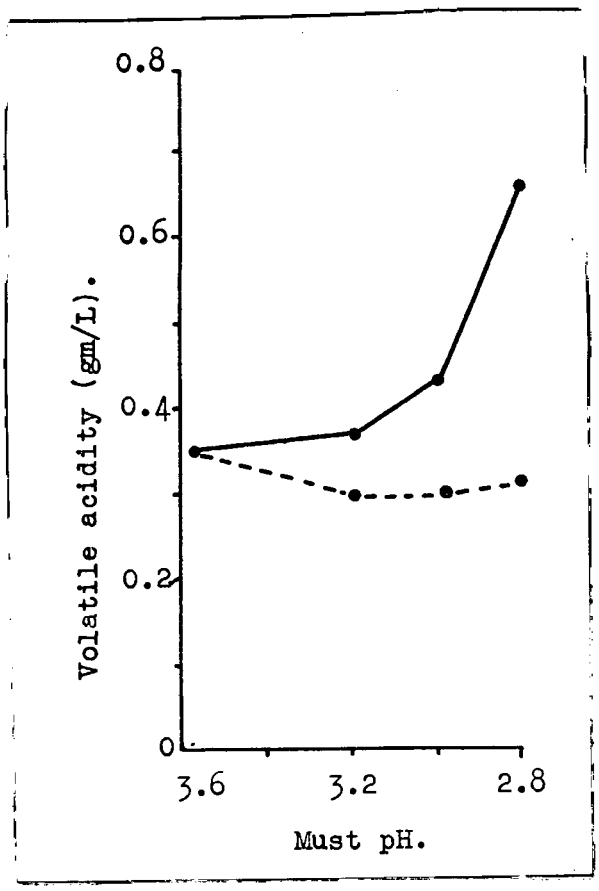

Figure 13(b). Influence of pH upon the formation of volatile acidity in the wines of an ion exchange treated Stein must pH (Experiment X). ———, must/decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8; -----, must pH decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8 and then increased, by the addition of potassium hydroxide, to the control (untreated) values.

From the data of Experiments I to X it is evident that the progressive increases in volatile acidity were due to pH. It was not the removal of must cations by the ion exchange resin but rather the addition of hydrogen ions which was the agency of volatile acidity increments.

(g) Total Aldehydes.

Aldehyde figures were most erratic and no correlation or tendency could be found with the treatment. Even duplicate samples often gave widely differing results although duplicate determinations on the same sample gave good results.

(h)/...

(h) Clarity of Wines.

The clarity of all wines can conveniently be divided into two major parts, viz. actual turbidity or clarity and potential turbidity. These two conditions are discussed separately.

It must be stated here that the chemical nature and properties of the organic non-crystalline substances which are factors in the clouding of wines are but slightly known and generally assumed to be of a proteinaceous nature. In this section, but more specifically in the sub-section on potential turbidity this assumption is accepted as fact and certain deductions based upon it.

Actual Clarity.

The turbidity tests on the ion exchange treated wines were for practical reason determined upon the cellar wines and immediately after bottling.

TABLE 26.

Turbidity of the Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts.

	Riesling.		Stein.	
Wine Group.	% Direct light.	% Turbidity*.	% Direct light.	% Turbidity*.
Control**	88.8	11.2	89.8	10.2
pH 3.2	97.9	2.1	90.0	10.0
pH 3.0	97.2	2.8	93.0	7.0
pH 2.8	98.5	1.5	94.9	5.1

* Table 26 gives two light values for each wine, the first is direct light, which was the experimentally determined value and the second reflected light (100% - % direct light) which is directly proportional to the concentration of suspended opaque particles and which was also termed turbidity.

** Untreated.

It/...

It will be seen from Table 26 that the Riesling control wine was more cloudy than the ion exchange treated wines; these treated wines were visually brilliant. The Stein wines were all visibly hazy having a fine milky suspension whose concentration showed a slight decrease with increased treatment.

Since it was not practically possible to increase pH of treated musts as was done in Experiments IX and X in similar large volumes as were treated in the cellar, the explanations of clarity differences must perforce be based upon theory and the known facts.

It has been shown by van Wyk (1958) that tartaric or citric acid addition to Riesling and St. Emillion musts resulted in relatively clear wines and more so than would have been the case had no tartaric or citric acid been added. Amerine (1953) has also shown that addition of tartaric acid to musts results in clearer wines. Furthermore, he has stated that wines from high acid, low-Balling grapes almost always clarify more easily than do those from low acid, high-Balling grapes. These effects of high acid musts upon clarity of their wines is largely due to pH.

It will be shown in the following section that the percentage large molecule adsorption by the resin is low and, therefore, this factor can not be credited as an influential one in wine clarity; pH is suggested as the operative factor, for, the hydrogen ion has a direct effect on many colloids, leading to their precipitation. The differences in actual clarity which occurred as a result of pH between the Riesling and Stein wines is ascribed to varietal effects and which could include, amongst others, oxidation potential and the nature and concentration of their colloids.

Potential/...

Potential Turbidity.

It was important to determine what the influence of the ion exchange treatment upon the potential turbidity was or in other words, what decrease occurred in the concentration of those proteinaceous substances which could later detrimentally affect wine clarity. In this respect, all wines have been the scene of a protein-tannin precipitation which stops at a certain point i.e. when the system has reached a certain state of equilibrium. Any disturbance of this equilibrium which can be affected by many factors may result in a new precipitation. Thus the protein-tannin reaction must be regarded as the principal cause of protein instability and all other factors affecting protein clouding regarded in the light of their effect on the said equilibrium (Berg, 1953). The actual and potential turbidities are thus also interrelated and many factors which are functional in the latter condition are also involved in the former.

The heat and tannin-precipitable fractions were determined upon Riesling and Stein cellar wines, pertinent data are given in Table 27(a) and (b).

It will be seen from Table 27(a) that for both Riesling and Stein wines the potential turbidity clearly decreased with increased treatment and that the pH 3.2 wines were more cloudy than the pH 3.0 and 2.8 wines which were about the same. In Table 27(b) the control wines also contained appreciably more tannin-precipitable matter than did the treated wines which in this case were of approximately equal clarity.

It is apparent that one or more factors of the ion exchange treatment was or were responsible for the decrease of proteins and, since these substances are of an ampholytic nature the one possibility of their adsorption by the resin is feasible. However, on closer scrutiny of the facts and

data/

TABLE 27(a) and (b).

Turbidity of Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts and caused by (a) Thermolabile and (b) Tannin-precipitable.Fractions.

(a) Thermolabile Fraction.

Wine Group.	Wine pH.	% Direct light.	% Turbidity*
Riesling.			
Control**	3.83	37.4	62.6
pH 3.2	3.22	87.6	12.4
pH 3.0	3.14	94.6	5.4
pH 2.8	2.87	96.3	3.7
Stein.			
Control**	3.73	66.8	33.2
pH 3.2	3.34	76.3	23.7
pH 3.0	3.0	89.8	10.2
pH 2.8	2.95	87.8	12.2

(b)

Tannin-precipitable Fraction.

Riesling.			
Control**	3.83	39.8	60.2
pH 3.2	3.22	94.8	5.2
pH 3.0	3.14	96.2	3.8
pH 2.8	2.87	96.0	4.0
Stein.			
Control**	3.73	64.7	35.3
pH 3.2	3.34	94.0	6.0
pH 3.0	3.0	96.0	4.0
pH 2.8	2.95	96.6	3.4

* See Turbidity, Table 26.

** Untreated.

data this phenomenon can be relegated to a position of little importance.

Proteins are macromolecules whose molecular weight can vary from say 16,000 to very much more. A single large amino acid like tryptophane has a molecular weight of 204. Egg albumen contains 300 equivalents amino acid per molecular weight of 45,000 and haemoglobin consists of about 580 amino acid molecules. Since the resin utilized had a divinyl benzene content of approximately 8% the molecular channels were relatively small. It is known that the capacity of various ion exchanging resins decrease as the ionic size of the cations increase and, furthermore, rates of diffusion are so slow that we may conclude that the ordinary resin (as used) has a very low capacity for large ions.

It has been stated that resins can be blocked by large organic molecules and the normal regeneration procedure have little effect on restoring the initial capacity. In this work and under the specified conditions this was not found to be so. A laboratory column containing 100 grams of resin (moist) was used over a period of 14 months to treat more than 20 gallons of turbid must and the total capacity loss of this resin was only approximately 6%. This is partial evidence that macromolecules were not firmly and substantially held by the resin.

The paper chromatographic analysis of pH 2 musts showed 15 amino acids to be present which, although present in only small quantities were still nevertheless identifiable. The fact that the comparatively small amino acid molecule were not quantitively held by the resin make the chanees for consequential protein adsorption small indeed.

Ruch and Block (1958) who treated beer with a similar resin as was used in this work, found that the major portion of tannin precipitable material (Lundin fraction A) appeared in the effluent whilst only negligible quantities were found in the eluate. This is largely in agreement with the facts and factual assumptions of the previous paragraphs.

It has been shown that it was unlikely that resin adsorption was responsible for substantial protein decreases in must, but, since decreases did occur it is necessary that an explanation be given of it. The first indications of the cause of this phenomenon was noted during the cellar treatment of musts, for, toward the end of a run of must through the cellar ion exchange plant it was noticed that pressure rose abnormally and flow decreased proportionately. Upon opening and examining the interior of the column a thick greenish cream scum was found on the liquid surface and in the upper portions of the resin bed. Its physical nature was definitely flocculant, which was not the case in the untreated must. Logically, this flocculant could only have been caused by the resin. When one considers that the effective pH value in the resin bed was 2 or less it is obvious that decrease of pH of the must in the column was the major causative factor of this phenomenon. The possibility, therefore, existed that a decrease in potential (and actual) turbidity of the resultant wines already occurred in the treated must and, moreover, the possible turbidity decrease was due to pH and not to resin adsorption. Furthermore, since it has been shown that:-

(a) it was unlikely that consequential protein adsorption was effected by the resin.

(b)/...

(b) acid addition to and naturally high acidity of musts is largely responsible for clearer wines and that the actual and potential turbidities are closely connected,

it is obvious that pH of the must or wine was no doubt largely responsible for a decrease in the concentration of the potential clouding substances.

It was not practically possible to increase pH of cellar treated musts as was done in Experiments IX and X, but it was possible to increase the pH of the treated wines, for, the possibility that the low pH of these wines could in itself affect the potential clouding still existed. Consequently, the pH of treated cellar wines were increased to that of their controls by addition of sodium hydroxide and subjected to the same treatments and tests as before. The relevant data are tabulated in Table 28(a) and (b).

In the thermolabile fraction section of Table 28 the turbidities of the treated Riesling wines do not have the same downward trend as do their counterpart wines (Table 27(a), the former wines are all more or less of the same turbidity. If one, however, compares the turbidity value of each wine with its counterpart then the differences are not large, in fact, they do not exceed a value of 4.7% and where the difference between identical controls is 3.6%. For the Stein wines in Tables 27(a) and 28(a) the same is the case for the pH 3.0 and 2.8 wines, but not for the pH 3.2 wine. Here a difference of 11.1% is significant but the reason for it is not clear and is inexplicable. In the (b) sections of Tables 27 and 28 it will be seen that the Riesling control wine turbidities differ widely,

by/...

TABLE 28(a) and (b).

Influence of pH of Wines, made from Ion Exchange Treated Riesling and Stein Musts, upon the Turbidity caused by (a) Thermolabile and (b) Tannin-precipitable Fractions.

(a) Thermolabile Fraction.

Wine Group.	Wine pH.	% Direct light.	% Turbidity.
Riesling.			
Control*	3.83	41.0	59.0
pH 3.2	3.83	92.2	7.8
pH 3.0	3.83	92.9	7.1
pH 2.8	3.83	91.6	8.4
Stein.			
Control*	3.73	70.3	29.7
pH 3.2	3.73	87.4	12.6
pH 3.0	3.73	92.5	7.5
pH 2.8	3.73	92.3	7.7

(b) Tannin-precipitable Fraction.

Riesling.			
Control*	3.83	24.6	75.4
pH 3.2	3.83	89.3	10.7
pH 3.0	3.83	89.5	10.5
pH 2.8	3.83	90.0	10.0
Stein.			
Control*	3.73	58.1	41.9
pH 3.2	3.73	88.7	11.3
pH 3.0	3.73	93.9	6.1
pH 2.8	3.73	93.0	7.0

* Untreated.

by approximately 15%, where they should have been similar. The reason for this is not known. However, the turbidity trend of the treated wines is similar and if the individual counterpart turbidity values of these wines are compared and taking into account the large difference between the controls it will be apparent that the differences between these values are relatively insignificant. In the (b) sections of Tables 27 and 28 the Stein control turbidities are similar and so are those of the treated wines which also show the same downward tendency of turbidity.

On overall comparison of the data contained in Tables 27(a) and (b) and 28(a) and (b) shows that but for one wine (Stein pH 3.2 - thermolabile fraction) the different values manifested in the treated counterpart wines do not, when compared to the values of their controls, differ radically. From this data, therefore, it is apparent that the final pH of the treated wines had but little effect on their potential clouding.

From all the data presented in this section it is indicated that the pH of the musts and/or wines was the most important factor in the decrease of their potential proteinaceous and actual turbidities and that the actual removal of these components occurred in the must or wine prior to bottling.

The reason for the decrease in the actual and potential turbidities of the treated wines by pH was no doubt due to the higher order of neutralization of the negative charge on the wine colloids (Malan, 1952) by the increased hydrogen ion concentration and, which resulted in a flocculation and subsequent precipitation of these substances. The differences between actual turbidities in Stein and Riesling wines has already been shown as

being/...

possibly due to varietal effects, which includes the nature and concentration of the inherent colloids and which could, therefore, react differently to an increase in hydrogen ion concentration. In the potential turbidities of Riesling and Stein wines (Table 27(a) and (b) the clouding was due more specifically to proteins and the neutralization of their charge within the given pH groups and in both wines reached its maximum effect in the thermolabile fraction in the pH 3.0 group and in the tannin-precipitable fraction in the pH 3.2 group.

(i) Colour of Wines.

The musts of some grape varieties (e.g. Riesling) are prone to browning and this occurrence is generally accepted to be detrimental to the quality of their wines. In this section an attempt was made to determine whether resin treatment (H Cycle) could decrease the colour content (actual or potential) of such musts.

The determinations were done on the wines of Experiments IX and X as well as the cellar wines and carried out as described under Methods of Analysis. The filter used for the wines of Experiments IX and X was one of 5150Å and although percentage transmissions (light) were obtained they were, however, not as significant as visual colour differences. For the colour measurement of cellar wines a more yellow-sensitive filter was used, viz. one of 4450Å.

From Table 29 it will be seen that the colour of control wines (Stein and Riesling) was approximately the same as their ion exchange treated (control) wines, which suggests the non-removal of colouring matter by the resin. However, in Experiment IX the ion exchange treated wines (pH 3.2, 3.0 and 2.8) show a decrease in colour to that of their control, which now indicates a possible colour removal.

But/...

TABLE 29.

Influence of pH upon the Colour of Wines made from Riesling and Stein Musts which received no Treatment, Ion Exchange Treatment and Potassium Hydroxide to increase its pH.

Wine Group.		Wine pH.	% Transmission.	Average % Transmission.	Average % Absorption.[a]	Visual Colour.
			Experiment IX (Riesling).			
Control[b]	(i)	3.7	78.0	78.6	21.4	light brown.
	(ii)	3.72	79.3			
pH 3.2	(i)	3.3	83.7	83.6	16.4	light straw.
	(ii)	3.29	83.4			
pH 3.0	(i)	3.1	85.5	85.7	14.3	" "
	(ii)	3.1	85.8			
pH 2.8	(i)	3.0	85.8	85.7	14.3	" "
	(ii)	3.0	85.6			
pH 4.5[c]	(i)	-	62.2	60.8	39.2	brown.
	(ii)	-	59.3			
pH 3.2 (control)[d]	(i)	3.77	76.4	76.9	23.6	light brown.
	(ii)	3.76	77.4			
pH 3.0 (control)[d]	(i)	3.77	76.9	77.8	22.2	" "
	(ii)	3.79	78.8			
pH 2.8 (control)[d]	(i)	3.8	74.7	73.5	26.5	" "
	(ii)	3.8	72.3			
			Experiment X (Stein).			
Control[b]	(i)	3.75	91.7	91.5	8.5	Light straw.
	(ii)	3.8	91.2			
pH 3.2	(i)	3.5	90.5	91.0	9.0	" "
	(ii)	3.52	91.4			
pH 3.0	(i)	3.3	91.1	90.4	9.6	" "
	(ii)	3.32	89.7			
pH 2.8	(i)	3.2	90.9	90.6	9.4	" "
	(ii)	3.18	90.3			
pH 4.5[c]	(i)	-	85.7	85.9	14.1	pink tinge.
	(ii)	-	86.1			
pH 3.2 (control)[d]	(i)	3.9	91.0	91.1	8.9	light straw.
	(ii)	3.9	91.2			
pH 3.0 (control)[d]	(i)	3.91	91.9	91.4	8.6	" "
	(ii)	3.92	90.9			
pH 2.8 (control)[d]	(i)	3.93	91.7	91.5	8.5	" "
	(ii)	3.91	91.2			

a This figure is arbitrarily obtained by subtracting the per cent light transmission from 100%. It is a smaller figure and is more indicative of actual colour, being directly proportional to colour intensity, whereas per cent transmission is inversely proportional.

b Untreated.

c Untreated must was increased in pH to a value of 4.5 by addition of potassium hydroxide.

d Must pH decreased by ion exchange treatment to set pH values and then increased in pH tc its control (untreated) value by addition of potassium hydroxide.

But, since the treated musts of Experiment IX, i.e. the pH counterpart groups had identical ion exchange treatment, it follows that colour adsorption by the resin was not responsible for the observed colour decreases. In Experiment X, the colour of the ion exchange treated, ion exchange treated (control) and control wines were similar and it is apparent that colour matter was again not adsorbed by the resin to any significant degree.

The findings of the laboratory experiments IX and X are confirmed by the cellar wines, the data of which is given below in Table 30 where it will be seen that the Riesling control wine was also appreciably darker than the ion exchange treated wines and the Stein wine colour was also more or less constant for all the treated wines.

TABLE 30.

Colour intensities of Cellar Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts.

	Riesling.		Stein.	
Wine Group.	% Trans-mission.	% Absorption.	% Trans-mission.	% Absorption.
Control*	60.0	40.0	71.9	28.1
pH 3.2	68.3	31.7	70.5	29.5
pH 3.0	67.6	32.4	71.2	28.8
pH 2.8	65.7	34.3	70.5	29.5

* Untreated.

Furthermore, it will be seen that no marked progressive colour difference occurred in the ion exchange treated or ion exchange treated (control) wines. If the resin treatment had retained colour one could logically have expected a progressive colour decrease in the pH groups.

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The fact that such a decrease was not observed is also evidence that the resin was inactive as to colour matter adsorption from must.

From the aforesaid it is apparent that ion exchange treatment of white must (Riesling and Stein) did not substantially reduce colouring matter in it, colouring bodies had little or no affinity for the resin and were not adsorbed by it to any significant degree. From the former statement it follows that colour differences were rather an effect of the hydrogen ion concentration upon the latent colour of the must. This is confirmed by the marked dissimilarities in colour between the untreated wines, viz. control and pH 4.5, where there is a significant increase in colour with an increase in pH of the must. The positive development of colour in the wines of this experiment is, therefore, ascribed, directly or indirectly, to an increase in pH, and the decrease in colour, directly or indirectly to a decrease in pH of the must.

(j) Bacterial Infection of Wines.

The wines of Experiments IX and X exhibited marked dissimilarities in clarity. This turbidity was determined as described under Methods of Analysis and results tabulated in Table 31.

It was originally thought that non-microbial substances were responsible for the loss in clarity of these wines but microscopic investigation of all the wines revealed the cause of turbidity to be bacteria. These bacteria were initially thought to be of the acetic acid type but were later identified as lactic acid bacteria. The wines with a high percentage of turbidity were heavily infected whereas those that were relatively clear had but a few bacteria; a clear cut boundary existed between infected and uninfected wines.

The/...

TABLE 31.

Turbidity of Wines made from Riesling and Stein Musts which were Untreated, Ion Exchange Treated and had received Potassium Hydroxide. Excessive Turbidity signifies Bacterial Infection.

Wine Group.		% Turbidity[a] (% Reflected light).	Average % Turbidity.
Experiment IX (Riesling).			
Control[b]	(i)	64.1	63.2
	(ii)	62.2	
pH 3.2	(i)	17.8	18.9
	(ii)	19.9	
pH 3.0	(i)	19.3	19.2
	(ii)	19.0	
pH 2.8	(i)	17.2	17.1
	(ii)	16.9	
pH 4.5[c]	(i)	67.0	66.7
	(ii)	66.4	
pH 3.2 (Control)[d]	(i)	61.7	61.6
	(ii)	61.5	
pH 3.0 (Control)[d]	(i)	62.7	64.9
	(ii)	67.1	
pH 2.8 Control)[d]	(i)	64.7	63.3
	(ii)	61.8	
Experiment X (Stein).			
Control[b]	(i)	25.8	26.6
	(ii)	27.4	
pH 4.5[c]	(i)	61.2	61.1
	(ii)	61.0	

a Turbidity (See Table 26).

b Untreated.

c Untreated must was increased in pH to a value of 4.5 by addition of potassium hydroxide.

d Must pH decreased by Ion Exchange Treatment to set pH values and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

The infected wines of Experiment X (pH 4.5) and the whole of Experiment IX were discarded and a repeat experiment carried out. The wines of this repeat were racked two days after visual gas evolution had ceased and were not infected at that stage.

After the data for colour measurements were obtained, the 15 ml. filtered wine samples were held in 30 ml. corked test tubes at 25°C for 3 weeks. Turbidity again occurred in the same wine groups as before (Table 32) and microscopic examination of all the wines again revealed bacteria to be the cause of clouding. These were of the same morphological structure as before. It will be seen that infection of the susceptible Riesling (Experiment IX) and Stein(Experiment X) pH 4.5 wines occurred consistently, in fact, four times in four samples of the same pH group. Heavy inoculation of the healthy wines with the sentrifuged sediment of infected wines caused no infection even although the inoculated samples were held (with the un-inoculated checks) in an incubator at 25°C for 3 weeks.

TABLE 32.

Table illustrating Bacterial Infection of Wines made from Riesling and Stein Musts which were Untreated, Ion Exchange Treated and had received Potassium Hydroxide*.

Experiment.	Wine.															
	Control.		pH 3.2		pH 3.0		pH 2.8		pH 3.2 (Control).		pH 3.0 (Control).		pH 2.8 (Control).		pH 4.5	
	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)
IX (Riesling) Wine pH.	3.70	3.72	3.30	3.29	3.10	3.10	3.0	3.0	3.77	3.76	3.77	3.79	3.80	3.80	-	-
X (Stein). Wine pH.	3.75	3.80	3.50	3.52	3.30	3.32	3.20	3.18	3.90	3.90	3.91	3.92	3.93	3.91	-	-

* The dark shaded test-tubes are those whose contents showed bacterial infection whereas the unshaded test-tubes contained the

uninfected/...

uninfected wines.

The tabular columns headed by "pH 3.2 (control), pH 3.0 (control), pH 2.8 (control)"contains the data of those wines whose must pH was decreased by ion exchange treatment to the above set values and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

The tabular column headed by "pH 4.5" contains the data of the wines whose must consisted of control (untreated) must plus sufficient potassium hydroxide to increase its pH to a value of 4.5.

Two relevant factors which could possibly have influenced the bacterial infection of these wines were the amino acid and sulphur dioxide content. Both musts received 100 mg. per liter sulphur dioxide and aeration was as near as possible identical. Sulphur dioxide determinations were not done, as it is known that free sulphur dioxide decreases greatly during fermentation and that the effective sulphur dioxide concentration after fermentation is low. Since these laboratory wines received no sulphur dioxide bar that initially added to the must, the sulphur dioxide content of the wines can not be regarded as having significantly influenced bacterial infection. Individual amino acids and their concentrations were found to be more or less similar in both the musts and the wines. The amino acids and the sulphur dioxide content of the wines are, therefore, not regarded as decisive factors in this phenomenon.

From Table 32 it will be seen that infection occurred in Experiment IX in those wines whose pH exceeded 3.3 i.e. the control, pH 3.2 (control), pH 3.0 (control), pH 2.8 (control) and pH 4.5 wines. Since the healthy wines, viz. pH 3.2, pH 3.0 and pH 2.8 (Experiment IX) differed actively from their respective counterparts only in pH it is evident that pH was instrumental in inhibiting infection. In the Experiment X wines infection occurred only in the pH 4.5 wine, i.e. in

wines whose pH exceeded a value of 3.9. Here too, as in Experiment IX, pH was clearly the bacterial inhibiting factor.

Table 32 could, with an appropriate heading just as well have been placed in the section on colour. In other words, those wines of Experiments IX and X which manifested colour increases also manifested bacterial infection. It is obvious, therefore, that a close connection existed here between colour and bacterial development. It is generally conceded that the colour of a white wine is in itself a redox indicator; the darker the colour becomes the higher is the wines' rH. pH and rH are also closely connected, for, as the pH of a wine is increased so does its rH increase and vice versa. Furthermore, pH and rH can act either independantly or in conjunction as bacterial inhibitors (Schanderl, 1948).

From these facts and the data of this section it is indicated that rH could certainly have been of consequence in this phenomenon. However, it is clear that of the two primary influences of ion exchange (hydrogen cycle) upon these grape musts it was the hydrogen ion concentration which was directly or indirectly responsible for preventing the development of these bacteria. It is also obvious that this influence was not specific but was also dependant upon varietal effects.

(k) Fermentation of Musts.

It was one of the aims of this experiment to determine the influence of the ion exchange treatment upon fermentation of must. From the very first experiment fermentation differences were noted between the pH groups but the general trend of the fermentations were all similar whether the must was sterilized or not. Table 33 gives the averaged weight loss of each pH group up to the

seventeenth day (Experiment VI).

As the percentage of pH 2 must was increased so also did the fermentation velocity decrease and the cause of this could have been due to one or both of the major ion exchange effects (removal of must cations and equivalent substitution of hydrogen ions). From the data of Experiments VII and VIII where pH of one must was decreased by both ion exchange and tartaric acid addition it was possible to observe the effect of largely pH upon the ion exchange treated musts. The course of the fermentations are shown graphically in Figures 14(a) and 14(b) and the averaged weight losses recorded in Tables 34 and 35.

The graphs in Figure 14(a) where dotted lines represent tartaric acid addition data, show that these musts all fermented on a higher plane than did their ion exchange counterparts. Tabulating the analytical data (Experiment VII) which is often associated with fermentation the following is obtained:-

Wine Group.	pH.	Total Acidity gm/L. (Tartaric Acid).	Total Tartrates gm/L. (Tartaric Acid).	Volatile Acidity gm/L. (Acetic Acid).	Alcohol Volume %
I.E.T.[a] pH 2.8	2.97	8.3	2.9	0.7	11.7
T.A.A.[b] pH 2.8	2.94	15.0	11.6	0.81	11.6

a Ion exchange treated.

b Tartaric acid addition.

Here the pH influence was similar in both wines, i.e. it could in itself have had little relative bearing on this fermentation, but, the total acidity and the total tartrates of the "tartaric acid" wines were respectively almost double and four times that of the "ion exchange" wine. From these figures alone one would

normally/...

normally expect the fermentation to be retarded to a greater degree in the tartaric acid addition sample, but as shown, this was not the case and it already begins to appear that pH was not the retarding factor in the fermentation of this ion exchange treated wine.

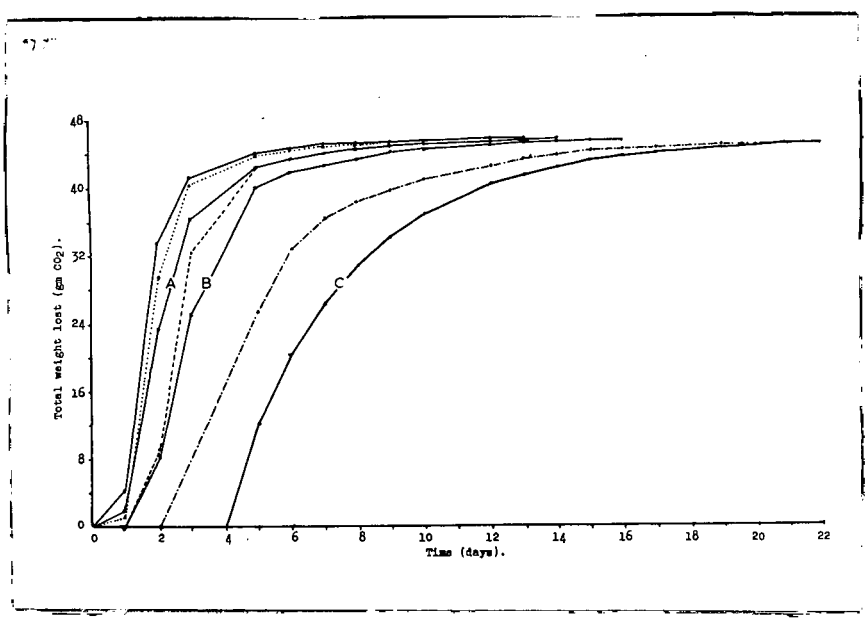

Figure 14(a). Influence of ion exchange treatment (I.E.T.) and the addition of tartaric acid (T.A.A.) upon the fermentation of a Stein must whose pH was decreased by both these means to values of 3.2, 3.0 and 2.8 (Experiment VII). ———, control (untreated); A, pH 3.2 I.E.T; B, pH 3.0 I.E.T; C, pH 2.8 I.E.T; ·····, pH 3.2 T.A.A; ----, pH 3.0 T.A.A; -·-·-·, pH 2.8 T.A.A.

In Figure 14(b) the fermentation differences between the counterpart musts is even more decided, in fact, the tartaric acid addition pH 2.8 must now fermented faster than did the ion exchange pH 3.0 must. Again grouping the relevant data (Experiment VIII) we have:-

Wine Group.	pH.	Total Acidity gm/L. (Tartaric Acid).	Total Tartrates gm/L. (Tartaric Acid).	Volatile Acidity gm/L. (Acetic Acid).	Alcohol Volume %.
I.E.T.[a] pH 3.0	3.02	7.7	2.5	0.5	11.6
T.A.A.[b] pH 2.8	2.86	16.6	11.9	0.95	11.4

a Ion exchange treated.

b Tartaric acid addition.

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TABLE 33.

The influence of progressive Ion Exchange Treatment of a Must upon the total Weight lost during fermentation.

Experiment V.

Days.	Control.* Total Weight Loss (gm.).	pH 3.2. Total Weight Loss (gm.).	pH 3.0. Total Weight Loss (gm.).	pH 2.8. Total Weight Loss (gm.).	pH 2.6. Total Weight Loss (gm.).
1	4.8	2.8	3.0	2.4	1.6
2	29.2	21.8	19.3	13.1	8.8
3	41.3	35.1	30.4	21.7	14.7
4	44.0	40.6	36.8	27.1	18.8
5	45.1	43.0	40.3	30.7	21.8
6	-	-	-	-	-
7	46.1	45.1	44.1	35.8	26.3
8	46.4	45.8	44.9	37.7	28.0
9	46.7	46.2	45.5	39.0	29.4
10	-	-	-	-	-
11	47.2	46.7	46.3	40.7	31.3
12	47.4	46.9	46.6	41.3	31.9
13	-	-	-	-	-
14	47.7	47.2	47.0	42.2	32.9
15	47.7	47.3	47.1	42.4	33.3
16	47.8	47.4	47.3	42.7	33.8

* Untreated.

It will be seen that in spite of the must and wine pH being appreciably lower, total acidity more than double and total tartrates more than quadrupled, the tartaric acid addition pH 2.8 must still fermented faster than did the ion exchange pH 3.0 must and, also in spite of this the volatile acidity of the faster fermenting must was almost double that of the other.

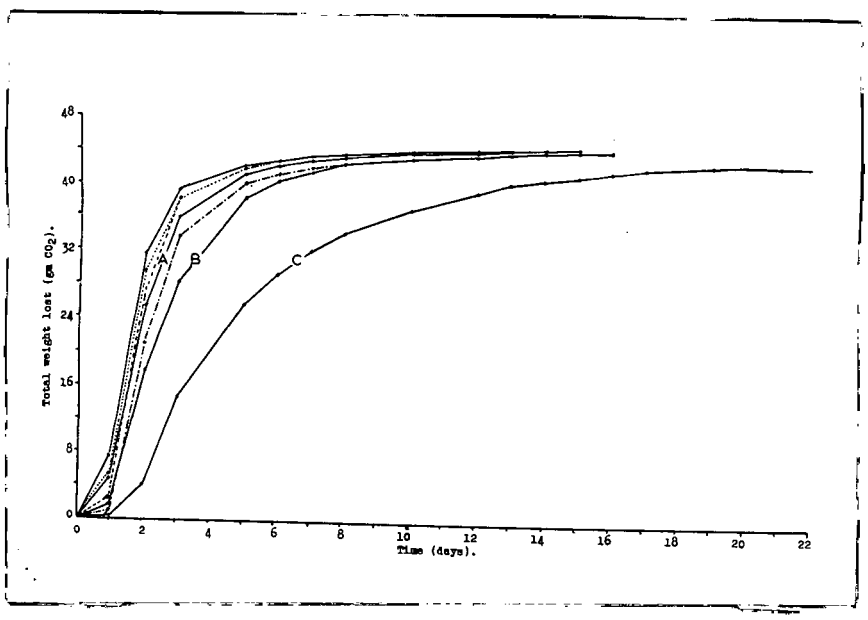

Figure 14(b). Influence of ion exchange treatment (I.E.T.) and the addition of tartaric acid (T.A.A.) upon the fermentation of a Stein must whose pH was decreased by both these means to values of 3.2, 3.0 and 2.8 (Experiment VIII). ———, control (untreated); A, pH 3.2 I.E.T; B, pH 3.0 I.E.T; C, pH 2.8 I.E.T; ····, pH 3.2 T.A.A; ----, pH 3.0 T.A.A; -·-·-·, pH 2.8 T.A.A.

To confirm these results, Experiments IX and X were done. As already stated Experiment IX became bacterially contaminated and was discarded; the fermentation weight losses were, however, recorded until constant weight and the graphs obtained were typical (not shown). The repeat of Experiment IX was held in the incubator until two days after all visual gas evolution had ceased and at that stage the wines were clear. The fermentation graphs are given in Figure 15 and averaged weight losses (of which the final figure for all the samples was between 22.2 and 22.4 gm.) are recorded in Table 36. The alcoholic contents were similar (See Table 44 Section (1)).

From/...

TABLE 34.

The influence of progressive Ion Exchange Treatment and Tartaric Acid Addition to a Must upon the Total Weight lost during fermentation.

Experiment VII.

Days.	Control.[a]	pH decreased by I.E.T.[b]			pH decreased by T.A.A.[c]		
		pH 3.2	pH 3.0	pH 2.8	pH 3.2	pH 3.0	pH 2.8.
	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.).
1	4.3	1.9	0.1	Nil	1.1	0.1	Nil
2	31.4	23.2	7.7	Nil	27.4	9.1	Nil
3	41.2	36.3	24.9	Nil	40.4	32.3	0.2
4	-	-	-	-	-	-	-
5	44.1	42.3	39.9	12.2	43.7	42.4	25.3
6	44.6	43.2	41.8	20.2	44.3	43.4	32.8
7	45.0	43.9	42.9	26.2	44.6	43.9	36.5
8	45.1	44.4	43.6	31.0	44.9	44.3	38.5
9	45.3	44.7	44.1	34.4	45.1	44.6	39.8
10	45.4	45.0	44.5	36.9	45.2	44.8	41.0
11	-	-	-	-	-	-	-
12	45.5	45.2	44.9	40.3	45.3	45.0	42.6
13	45.5	45.3	45.1	41.4	45.4	45.1	43.2
14	45.6	45.4	45.2	42.4	45.4	45.3	43.7
15	45.7	45.4	45.3	43.1	45.4	45.3	44.1
16	45.7	45.5	45.4	43.7			44.5
17		45.5	45.4	44.1			44.7
18				-			-
19				44.7			45.1
20				44.9			45.2
21				45.1			45.3
22				45.2			45.5
23				45.3			45.5
24				45.5			
25				-			
26				-			
27				45.7			

a Untreated.

b Ion Exchange Treatment.

c Tartaric Acid Addition.

TABLE 35.

The influence of progressive Ion Exchange Treatment and Tartaric Acid Addition to a Must upon the total Weight lost during fermentation.

Experiment VIII.

Days.	Control.	pH decreased by I.E.T.[b]			pH decreased by T.A.A.[c]		
		pH 3.2	pH 3.0	pH 2.8	pH 3.2	pH 3.0	pH 2.8.
	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.)	Total Weight Loss (gm.)	Total Weight Loss (gm.).	Total Weight Loss (gm.).	Total Weight Loss (gm.)
1	7.7	5.0	1.8	0.1	5.5	2.8	1.1
2	31.7	25.6	17.7	5.2	29.6	27.5	21.1
3	39.2	36.0	28.3	14.7	38.4	38.6	33.9
4	-	-	-	-	-	-	-
5	42.0	41.1	38.3	25.6	41.8	41.8	40.1
6	42.7	42.1	40.3	29.3	42.5	42.3	41.2
7	43.0	42.6	41.5	32.1	43.0	42.8	41.8
8	43.4	43.1	42.2	34.1	43.4	43.1	42.3
9	-	-	-	-	-	-	-
10	43.8	43.6	43.1	37.1	43.8	43.5	43.1
11	-	-	-	-	-	-	-
12	44.0	43.9	43.5	39.2	44.0	43.7	43.4
13	44.1	43.9	43.7	40.0	44.1	43.8	43.5
14	44.2	44.0	43.8	40.6	44.2	43.8	43.5
15	44.2		43.9	41.1	44.2	43.9	43.6
16			43.9	41.6			43.7
17				42.0			
18				-			
19				42.6			
20				42.7			
21				42.8			
22				42.9			
23				43.0			
24				-			
25				-			
26				43.3			
27				43.4			
28				43.5			

a Untreated.

b Ion Exchange Treatment.

c Tartaric Acid Addition.

From the fermentation graph (Figure 15) and weight loss table (Table 36) of Experiment IX, the pH 3.2 and 3.0 musts and their counterparts showed no differences in their fermentation and pH clearly did not have a significant effect on their fermentation. The pH 2.8 must and its counterpart, the pH 2.8 (control) must, however, did show a slight difference and since pH was the only active factor which differed in these two samples the recorded difference is, therefore, attributed to their pH values. What was found in the fermentation of the Experiment IX musts was also found in the Experiment X musts and the relevant graphs and data of the latter experiment are plotted in Figure 16 and tabulated in Table 37.

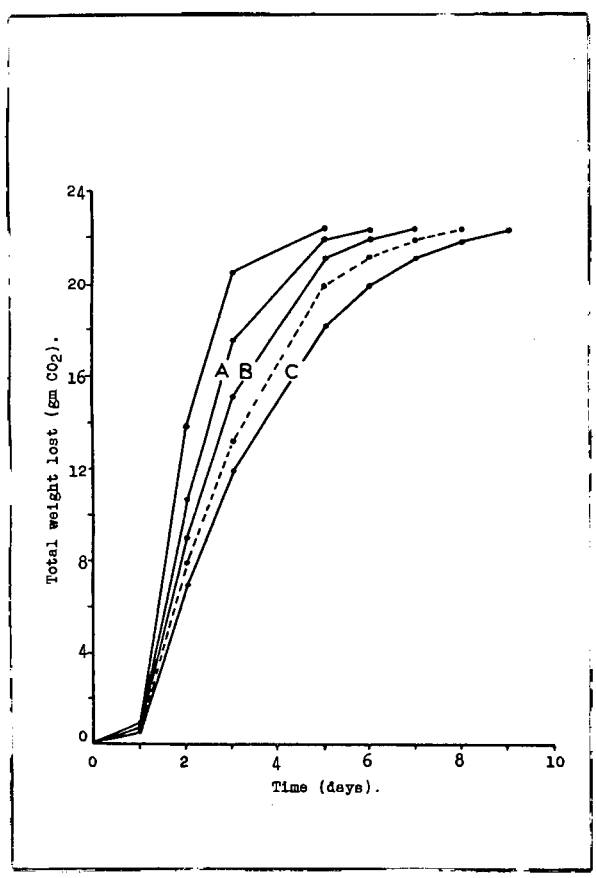

Figure 15. Influence of pH upon the fermentation of a Riesling must (Experiment IX) whose pH was decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8. (control) indicates that a treated must pH was increased, by the addition of potassium hydroxide, to the value of the control (untreated) must. ——, control (untreated); A, pH 3.2 and pH 3.2 (control) (Their data was similar); B, pH 3.0 and pH 3.0 (control) (Their data was similar); C, pH 2.8; ----, pH 2.8 (control).

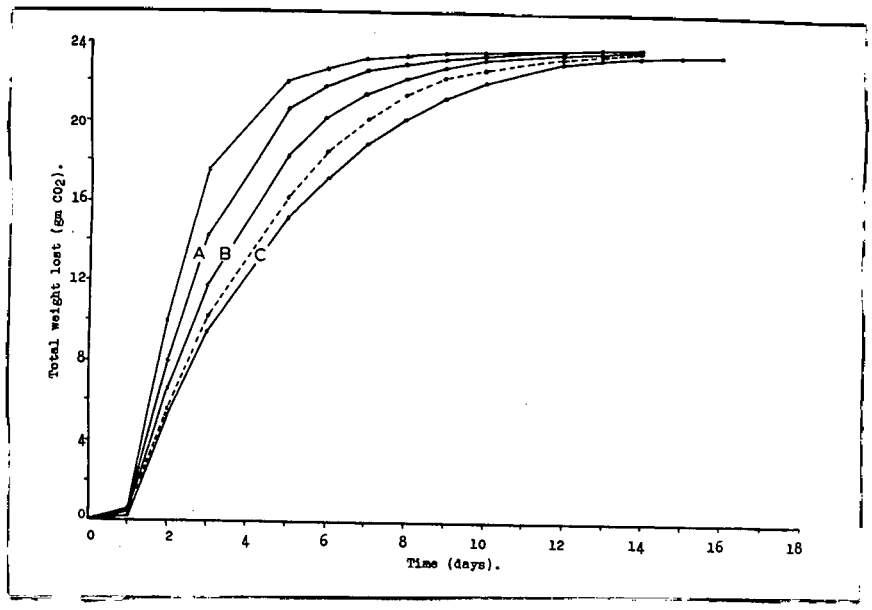

Figure 16. Influence of pH upon the fermentation of a Stein must (Experiment X) whose pH was decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8. (control) indicates that a treated must pH was increased, by the addition of potassium hydroxide, to the value of the control (untreated) must. ——, control (untreated); A, pH 3.2 and pH 3.2 (control) (Their data was similar); B, pH 3.0 and pH 3.0 (control) (Their data was similar); C, pH 2.8; ---, pH 2.8 (control).

The fermentation progress of the cellar wines was plotted with degrees Balling as a function of time(days) and typical curves obtained. Similar fermentation curves were obtained for the samples of the cellar wines which were held in an incubator.

It will be apparent that the general tendency of fermentation rate to decrease progressively with increased ion exchange treatment of must was consistently manifested and was not influenced by pH in the pH 3.2 and pH 3.0 groups and slightly in the pH 2.8 groups of Experiments IX and X. It is, therefore, obvious that in the pH 3.2 and pH 3.0 groups progressive resin adsorption was the factor which was influential in determining the fermentation rate and logically the fermentation of the pH 2.8 groups were influenced by this factor too.

But/...

But, since it has been shown that pH also influenced the latter group it is clear that the fermentation of this group was effected by both pH and resin adsorption.

It was also of primary importance to determine whether the progressive resin adsorption reached its maximum fermentation retarding effect in the pH 2 must or, in other words would the pH 2 must ferment if pH decrease was neutralized. It was to this end that the pH 2 musts were brought up in pH with a $\pm$5N potassium hydroxide solution to the control pH. The fermentation graphs of Experiments IX and X pH 2 (control) musts are given in Figure 17 and the averaged weight losses tabulated in Table 38. The corresponding control (untreated) must fermentation graphs are also given as reference in Figure 17. (Their weight loss data are given in Tables 36 and 37). The fermentation of the pH 2 (control) musts were extremely slow and in keeping with the previously found trend. It is thus indicated that excluding pH effect, progressive resin adsorption caused a progressive decrease in fermentation rate up to the maximum ion exchange treatment.

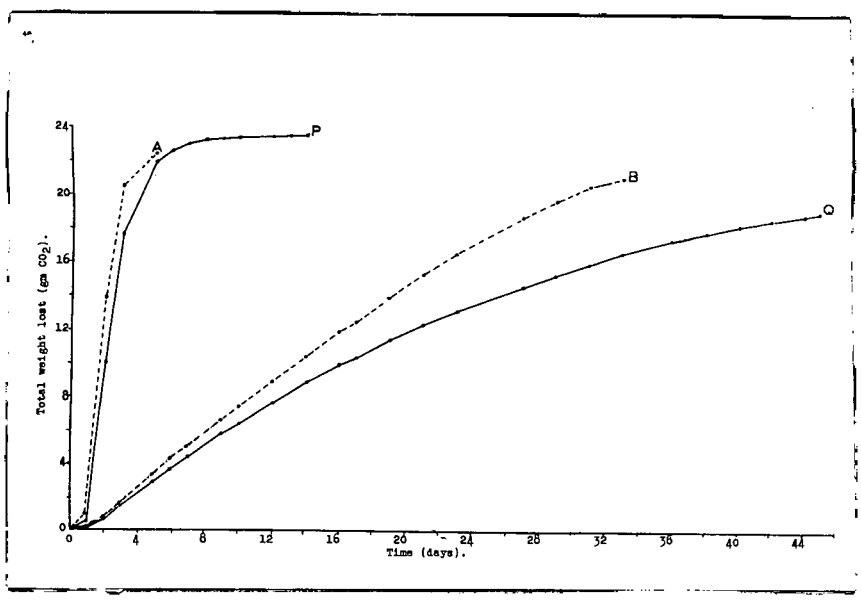

Figure 17. Influence of the maximum ion exchange treatment of a must upon its fermentation. (Control) indicates that a treated must pH was increased, by the addition of potassium hydroxide, to the value of its control (untreated) must. A, (Riesling, Experiment IX), control (untreated); B, (Riesling, Experiment IX), pH 2, (control); P, (Stein, Experiment X), (control (untreated); Q,(Stein, Experiment X) pH 2, (control).

TABLE 36.

The influence of pH of an Ion Exchange treated Riesling Must upon the total Weight lost during fermentation.

Experiment IX.

Days.	Control.[a]	pH 3.2.[b]	pH 3.0.[b]	pH 2.8.[b]	pH 3.2 (control).[c]	pH 3.0 (control).[c]	pH 2.8 (control).[c]
	Total Weight loss (gm.).	Total Weight loss (gm.).	Total Weight loss (gm.).	Total Weight loss (gm.)	Total Weight loss (gm.).	Total Weight loss (gm.).	Total Weight loss (gm.).
1	1.0	0.9	0.8	0.7	0.9	0.9	0.9
2	13.9	10.7	9.1	7.0	10.8	9.3	7.9
3.	20.5	17.6	15.1	11.9	17.6	15.3	13.2
4	-	-	-	-	-	-	-
5	22.4	21.9	21.2	18.2	21.9	21.1	19.9
6		22.4	21.9	19.9	22.3	21.9	21.1
7			22.4	21.1		22.4	21.9
8				21.9			22.3
9				22.3			

a Untreated.

b Must pH decreased by ion exchange treatment to set pH values.

c Must pH decreased by ion exchange treatment to set pH values and then increased in pH to its control (untreated) value by the addition of potassium hydroxide.

TABLE 37.

The influence of pH of an Ion Exchange Treated Stein Must upon the total Weight lost during fermentation.

Experiment X.

Days.	Control.[a]	pH 3.2.[b]	pH 3.0.[b]	pH 2.8.[b]	pH 3.2 (control).[c]	pH 3.0 (control).[c]	pH 2.8 (control).[c]
	Total Weight loss (gm.).	Total Weight loss (gm.).	Total Weight loss (gm.)	Total Weight loss (gm.).	Total Weight loss (gm.).	Total Weight loss (gm.).	Total Weight loss (gm.).
1	0.6	0.6	0.6	0.4	0.6	0.6	0.6
2	10.0	8.0	6.7	5.3	8.4	6.8	5.6
3	17.6	14.3	11.8	9.4	14.5	12.0	10.1
4	-	-	-	-	-	-	-
5	21.9	20.6	18.3	15.1	20.9	18.7	16.2
6	22.6	21.8	20.1	17.1	21.8	20.4	18.4
7	23.1	22.5	21.3	18.8	22.5	21.6	20.1
8	23.2	22.8	22.1	19.9	22.9	22.3	21.3
9	23.4	23.2	22.6	21.1	23.1	22.8	22.1
10	23.4	23.3	23.0	21.9	23.3	23.1	22.0
11	-	-	-	-	-	-	-
12	23.5	23.5	23.3	22.8	23.4	23.4	23.1
13	23.6	23.5	23.4	23.0	23.4	23.5	23.4
14	23.6		23.4	23.2		23.5	23.4
15				23.3			
16				23.4			

a Untreated.
b Must pH decreased by ion exchange treatment to set pH values.
c Must pH decreased by ion exchange treatment to set pH values and then increased in pH to its control (untreated) value by the addition of potassium hydroxide.

TABLE 38.

The influence of the maximum Ion Exchange Treatment of Riesling and Stein Musts, upon the total Weight lost during their fermentation.

	Experiment IX (Riesling).	Experiment X (Stein).
Days.	pH 2 (control)* Must.	pH 2 (control)* Must.
	Total weight loss (gm.).	Total weight loss (gm.).
1	0.2	0.1
2	0.6	0.7
3	1.7	1.5
5	3.4	3.0
6	4.3	3.8
7	5.2	4.5
8	6.0	5.2
9	6.7	5.9
10	7.5	6.5
12	9.0	7.7
13	9.7	8.3
14	10.4	8.9
15	11.2	9.4
16	12.0	10.1
17	12.6	10.5
19	14.0	11.5
20	14.8	12.0
21	15.4	12.4
22	16.0	12.8
23	16.7	13.2
24	17.3	13.6
27	18.9	14.7
28	19.4	15.0
29	19.9	15.4
30	20.3	15.7
31	20.8	16.1
33	21.2	16.7
34	21.5	17.0
36		17.5
38		17.9
40		18.4
42		18.8
44		19.2
47		19.6
49		19.9
52		20.1
54		20.4
56		20.5

* Must pH decreased by ion exchange treatment to a minimum value of approximately 2 and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

The pH, volatile acidity and alcohol content of the pH 2 (control) wines are given in Table 39. The alcohol contents of the control to pH 2.8 wines in Experiment IX ranged from 11.6 - 11.8 volume % and in Experiment X from 12.0 - 12.1 volume % (See Table 44 section 1).

TABLE 39.

pH, Alcohols and Volatile Acidities of Riesling and Stein Wines whose musts received maximum Ion Exchange Treatment.

Wine Group.		Experiment IX (Riesling). pH.	Alcohol Volume %.	Volatile Acidity gm/L.	Experiment X (Stein). pH.	Alcohol Volume %.	Volatile Acidity gm/L.
pH 2 (control)*	(i)	3.78	10.97	0.42	3.53	10.21	0.45
	(ii)	3.78	10.88	0.42	3.53	10.21	0.45

* Must pH decreased by ion exchange to a minimum value of approximately 2 and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

The fermentation of these musts, with respect to alcohol did not proceed as far as that of the other groups; several possible reasons could be advanced for this. The lengthy fermentation period was no doubt also instrumental in the evaporation of at least a small portion of this alcohol. An interesting point which was not further investigated was the comparatively low volatile acidities; lower than would be normally expected for such a lengthy fermentation.

It has been established that excluding pH, resin adsorption of the must components was responsible for a decreased fermentation rate but, it has not been established which of these components were responsible for it. The most obvious are those of the nitrogeous group and particularly the ammonium, amido and amino forms

which/...

which are known to be yeast nutriments. The ammonia content of the Experiments IX and X musts were determined as described under Methods of Analysis and are given in Table 41.

The percentage of control and pH 2 musts contained in each pH group is given in Table 40. Upon these values the ammonia content of the pH 3.2, 3.0 and 2.8 must groups were determined.

TABLE 40.

Percentage of Untreated and Ion Exchange Treated Riesling and Stein Musts contained in pH 3.2, 3.0 and 2.8 Must groups.

	Experiment IX.		Experiment X.	
Must.	% control Must.	% pH 2 Must.	% control Must.	% pH 2 Must.
Control*	100	Nil	100	Nil
pH 3.2	67	33	71	29
pH 3.0	53	47	53	47
pH 2.8	41	59	41	59

* Untreated.

TABLE 41.

Ammonia contents of Untreated and Ion Exchange Treated Riesling and Stein Musts.

	Experiment IX.	Experiment X.
Must.	NH_3 mg/L.	NH_3 mg/L.
Control*	115.6	67.5
pH 3.2	77.4	47.9
pH 3.0	61.3	35.8
pH 2.8	47.4	27.7
pH 2.0	Nil	Nil

* Untreated.

The ammonia content of the wines of Experiments IX and X are given in Table 42 and is of value insofar that it shows that practically all the ammonia initially present in the musts was utilized during fermentation.

TABLE 42.

Ammonia content of Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts.

Wine Group.	NH_3 mg/50 ml. (i)	NH_3 mg/50 ml. (ii)	NH_3 mg/L. (average).
Experiment IX.			
Control[a]	0.1	0.09	2,0
pH 3.2	0.07	0.073	1.5
pH 3.0	0.07	0.07	1.4
pH 2.8	0.065	0.075	1.4
pH 3.2 (control)[b]	0.11	0.11	2.2
pH 3.0 (control)[b]	0.11	0.1	2.1
pH 2.8 (control)[b]	0.09	0.094	1.9
Experiment X.			
Control[a]	0.13	0.14	2.7
pH 3.2	0.1	0.09	1.9
pH 3.0	0.09	0.09	1.8
pH 2.8	0.09	0.09	1.7
pH 3.2 (control)[b]	0.12	0.11	2.3
pH 3.0 (control)[b]	0.09	0.09	1.8
pH 2.8 (control)[b]	0.09	0.09	1.8

a Untreated.

b Must pH decreased by ion exchange to set pH values and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

The accurate tracings of the scaled-down photographic reproductions of the original amino acid paper chromatograms are shown in Figures 18 to 26 and will be referred to in the relevant paragraphs. The dotted lines outlining certain spots indicate that they were barely visible and of low concentration, whereas the unbroken lines outline spots in which the opposite was the case. Each lot of chromatograms run (4) included one reference chromatogram containing only pure amino acids, each of known concentration; these reference chromatograms were, however, so similar that only one is shown (Figure 18).

The/...

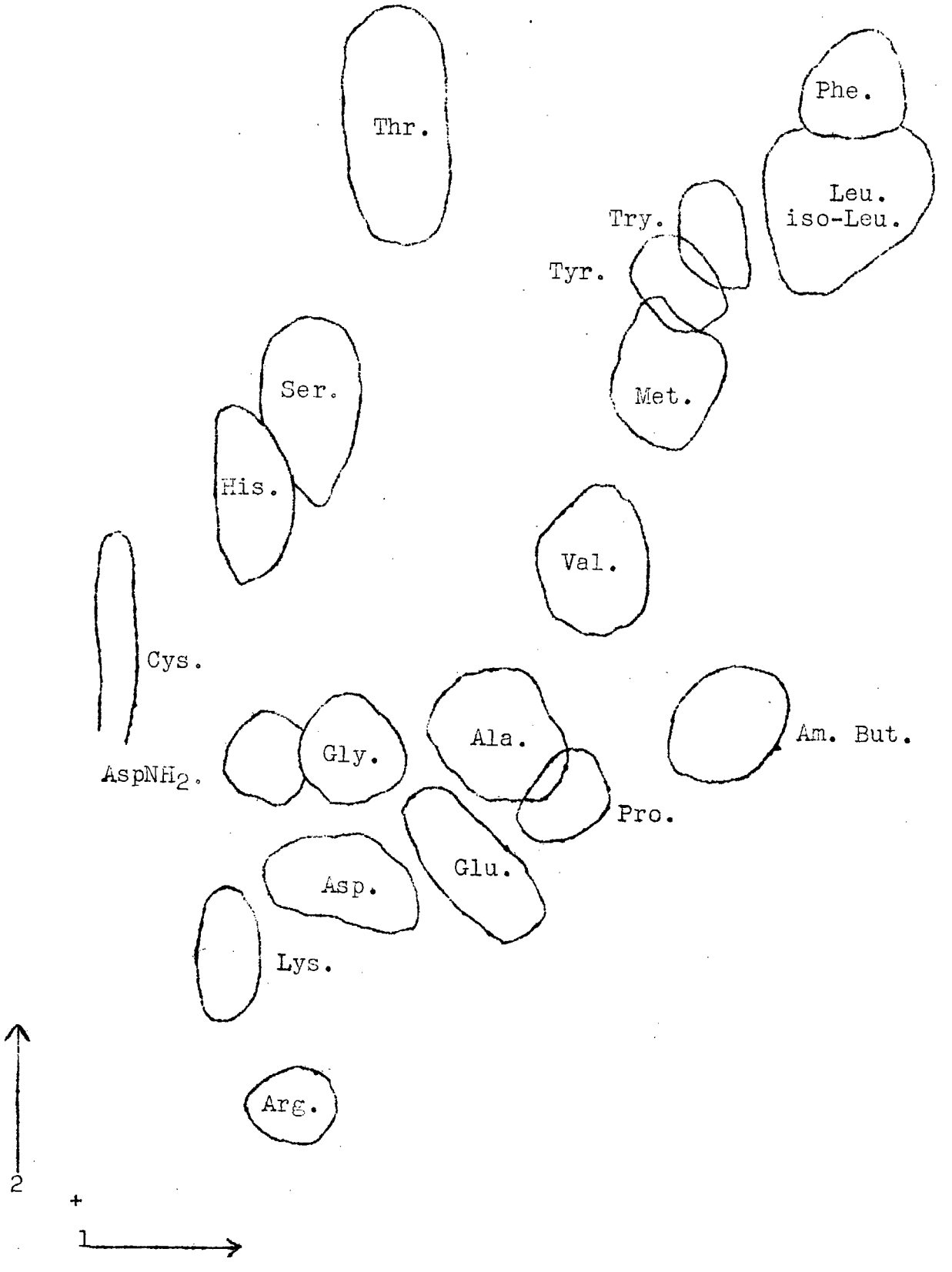

Figure 18. Two-dimensional paper chromatogram of 20 amino acids. The concentration of each amino acid is 5 micrograms except for histidine which is 10 micrograms.

The concentration of each amino acid was 5 micrograms with histidine at 10 micrograms. A paper chromatogram containing 25 micrograms of each amino acid (histidine 5 micrograms) still showed clearly visible spots. In some paper chromatograms spots appeared which could not be identified. Table 43 shows the amino acids which were found to be present in the control and pH 2 musts and their wines.

In the paper chromatograms of the Riesling (Experiment IX) and Stein (Experiment X) control musts (Figures 22 and 24) it will be seen that the amino acids alanine, proline, glutamic acid and lysine did not separate. These amino acids were, however, separated when a lower must concentration was spotted. As this latter concentration (100 microliters) asparagine was also identifiable.

It will be seen that in the paper chromatograms of the pH 2 musts of Experiments IX and X (Figures 19 and 20) traces of cystine appeared which did not appear in their control musts (Figures 22 and 24) as well as a tailing effect of arginine and lysine. These phenomena are inexplicable but it is not impossible that the cause was a slight hydrolysis of the proteins or peptides present in the must. It must also be stated here that although the spot of one amino acid was smaller than another one of the same type it did not necessarily mean that the concentration was lower, the intensity of the spot colour had also to be taken into consideration.

It is advisable at this point that the generally accepted view of the nitrogen metabolism of yeasts be briefly given and the relevant fermentation data of Experiments IX and X then examined in the light of these facts and theories.

Nitrogen/...

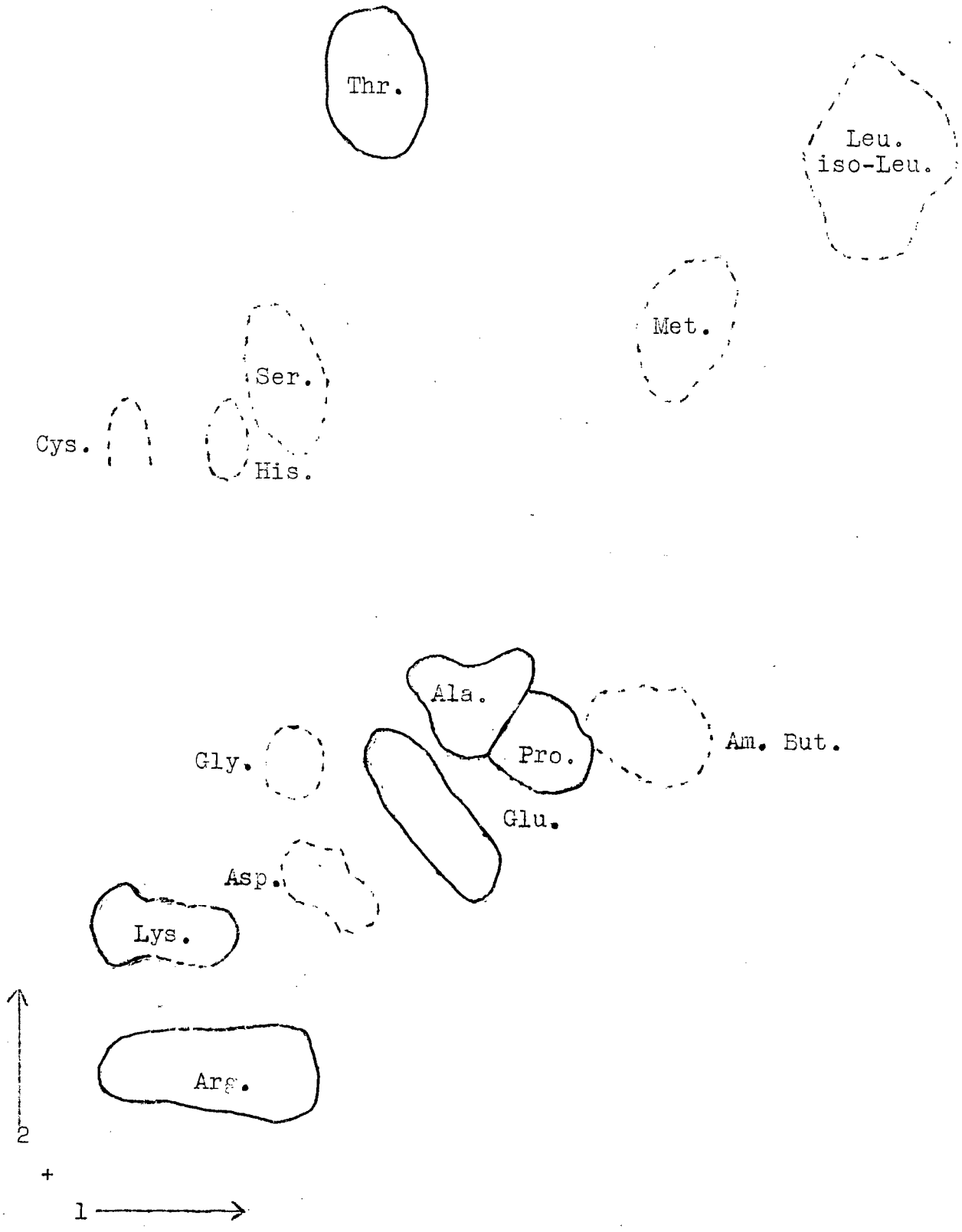

Figure 19. Two-dimensional paper chromatogram of amino acids of Riesling must (Experiment IX) which had received maximum ion exchange treatment. The pH value of this must was approximately 2.

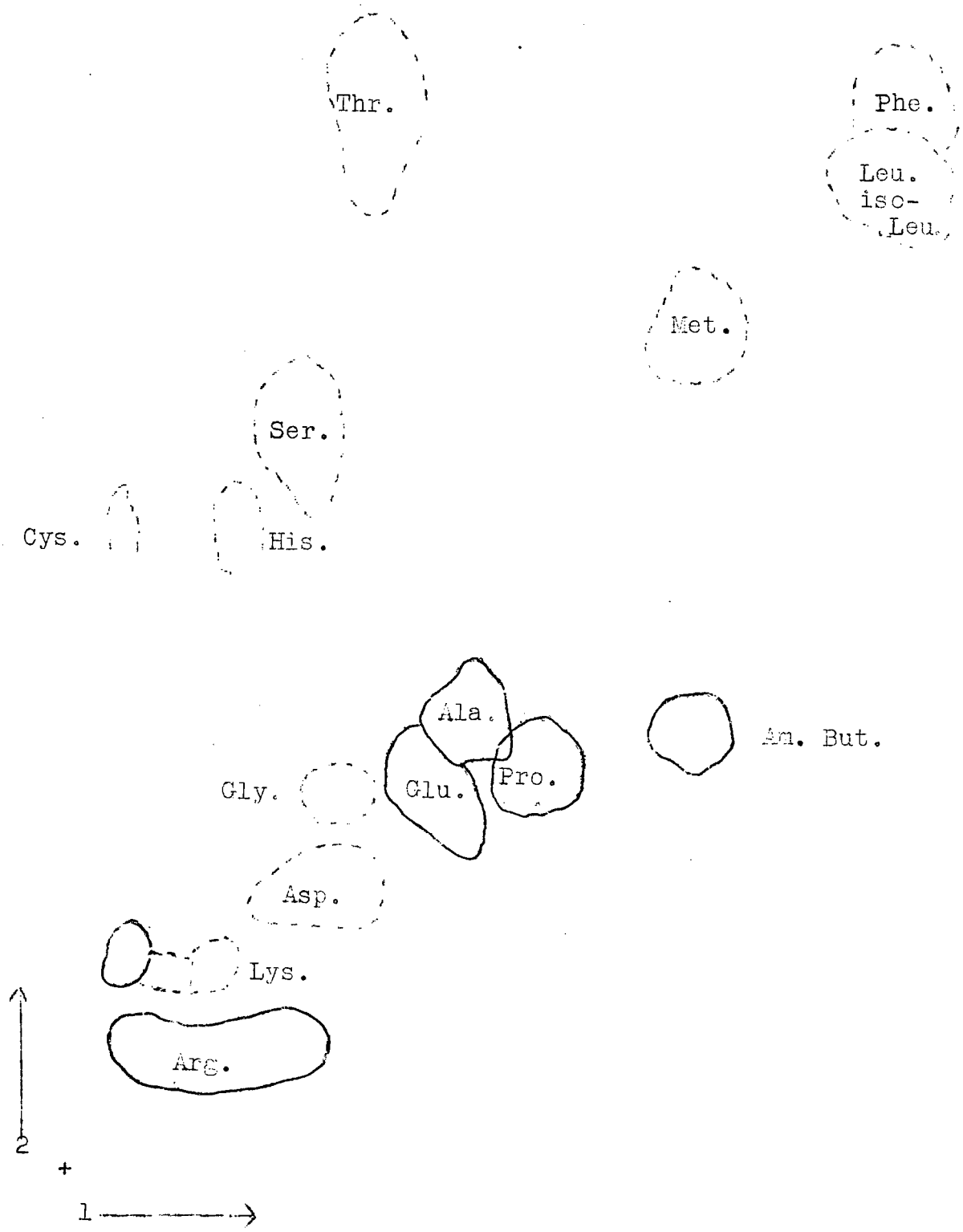

Figure 20. Two-dimensional paper chromatogram of amino acids of Stein must (Experiment X) which had received maximum ion exchange treatment. The pH value of this must was approximately 2.

TABLE 43.

Amino Acids found to be present in Untreated and Ion Exchange Treated Riesling and Stein Musts and in the Wines of the Ion Exchange Treated Musts.

Control Must.[a]	pH 2 Must.[b]	pH 2 (control) Wine[c]
Experiment IX (Riesling).		
Arginine	+	+
Lysine	+	+
Aspartic Acid	+ (T)	+ (T)
Glutamic Acid	+	+
Asparagine	-	-
Glycine	+ (T)	+
α-Alanine	+	+
Proline	+	-
α Am. Butyric Acid	+ (T)	+ (T)
Methionine	+ (T)	+ (T)
Leucine-iso-leucine	+ (T)	+ (T)
Phenyl Alanine	-	-
Serine	+ (T)	+ (T)
Histidine	+ (T)	-
Threonine	+	-
Experiment X (Stein).		
Arginine	+	+ (T)
Lysine	+	+ (T)
Aspartic Acid	+ (T)	-
Glutamic Acid	+	+
Asparagine	-	-
Glycine	+ (T)	+ (T)
α-Alanine	+	+
Proline	+	+ (T)
α Am. Butyric Acid	+	+ (T)
Methionine	+ (T)	-
Leucine-iso-leucine	+ (T)	-
Phenyl Alanine	+ (T)	-
Serine	+ (T)	-
Histidine	+ (T)	-
Threonine	+ (T)	-

a Untreated.

b Must pH decreased by ion exchange treatment to a minimum value of approximately 2.

c Must pH decreased by ion exchange treatment to a minmum value of approximately 2 and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

(T) signifies traces.

Nitrogen is indispensable for yeast growth, for, from it the yeast cell synthesizes the proteins which are an integral part of its structure. In this respect ammonia is acknowledged as being a most important nitrogen constituent of must; it is regarded as being taken up directly by the yeast cell and, furthermore, certain amino acids are deaminated by at least two processes, viz. the Ehrlich (1907, 1912) and Stickland (1934, 1935) mechanisms to supply the necessary ammonia. It is also known that apart from ammonia, aspartic acid and its amide asparagine are also very readily accessible to the yeast cell (Hartelius, 1939 and Thorne 1949a) and, if an abundance of the latter substances be present in a suitable medium it will lead to a fast assimilation of them with a corresponding rapid yeast multiplication. Cantarelli (1957) has shown that in grape juice, ammonia, aspartic acid and asparagine effectively contributed to the speed of fermentation. In effect, the rate of fermentation of a must would be largely influenced by the availability of suited nitrogenous substances. Therefore, in the earlier stages of fermentation the more readily assimilable amino acids and ammonia are rapidly taken up by the yeast and a high fermentation rate manifested. At the end of this stage of rapid assimilation a second phase of markedly slower assimilation sets in and there several probable reasons which can account for this.

As the yeast selects out the more suited amino acids it will leave in the medium a progressively more inadequate mixture and, in order to restore the increasing deficiency of nitrogen, those remaining amino acids will have to be deaminated. This process with this group of amino acids does apparently not occur readily for their nutrient values are also low. It follows that the utilization of these

amino/...

amino acids must constitute a check upon the rate of nitrogen assimilation.

If the concentration of free amino acids is low further amino acids can only arise from the hydrolysis of simple polypeptides and proteins present in the must and autolysis products of the yeast. It has been shown that the rate of yeast growth in dipeptides tends to be slower than in corresponding binary mixtures of amino acids (Thorne 1949b) and this must also retard nitrogen assimilation.

If the analytical data of the pH 2.0 must of Experiments IX and X (Tables 40 and 43) are examined it will be seen that ammonia and asparagine are absent and that the relative total concentrations of its amino acids are so low that an initial rapid assimilation, such as manifested in the control must appears improbable. The graphs (Figure 17 - pH 2 (control) must) in fact, show no fast initial fermentation but rather gradual and constant fermentations which are almost straight lines. It is thus indicated that from the beginning of fermentation of these musts the yeast cells were forced to degrade those amino acids which normally would have been but slightly utilized and hydrolyse peptides or proteins to augment their nitrogen requirements. The net effect of these activities would be to retard fermentation seriously and which was actually the case. If the chromatograms of the control wines and musts (Figures 21 to 24) are examined it will be seen that proline is present in both in similar large quantities, which indicates that it has undergone little change during fermentation and which Castor (1956) has also found to be true. But in the pH 2 (control) wines chromatograms (Figures 25 and 26) proline concentration decreased largely in the wine

of/...

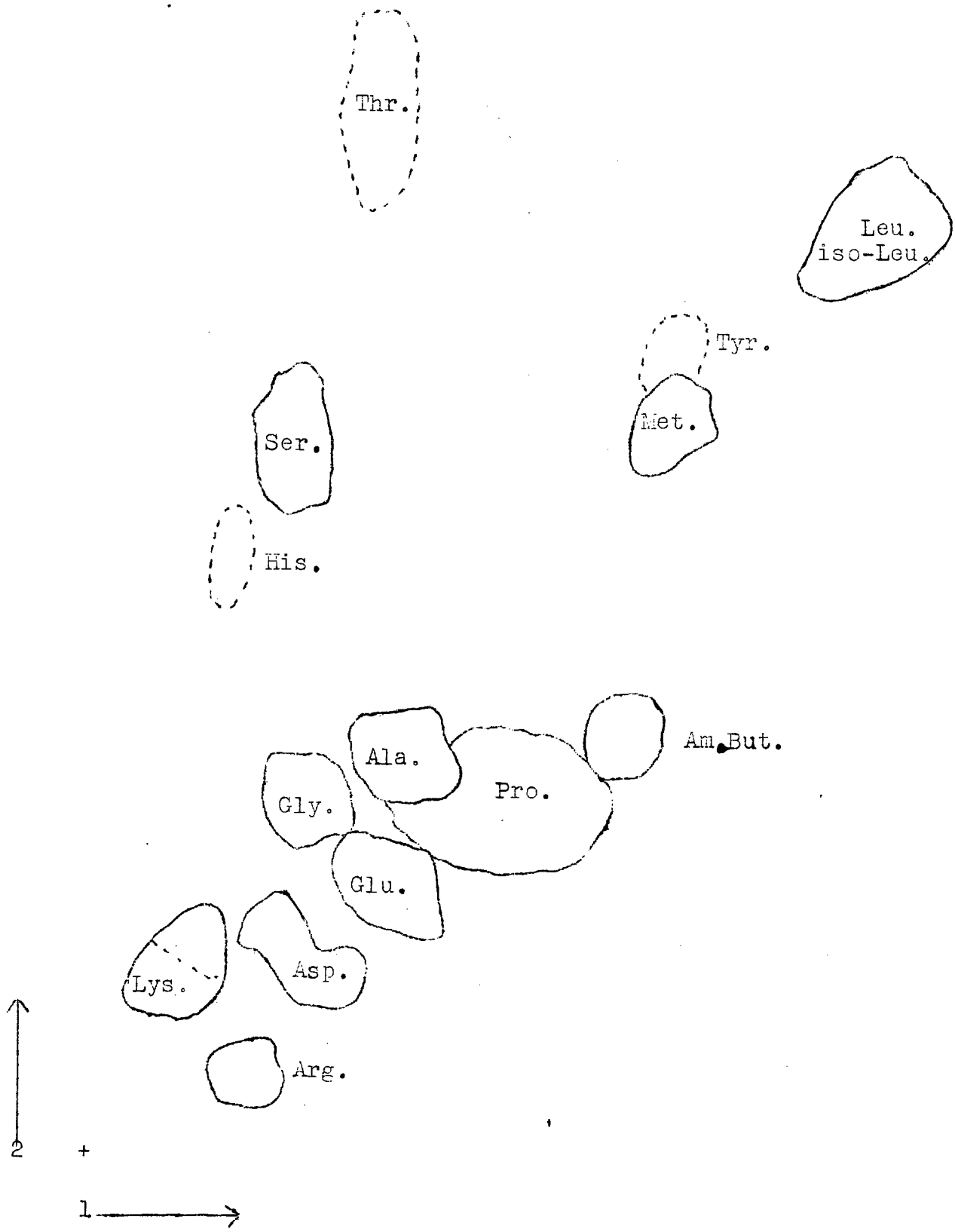

Figure 21. Two-dimensional paper chromatogram of amino acids of Riesling control (untreated) wine (Experiment IX).

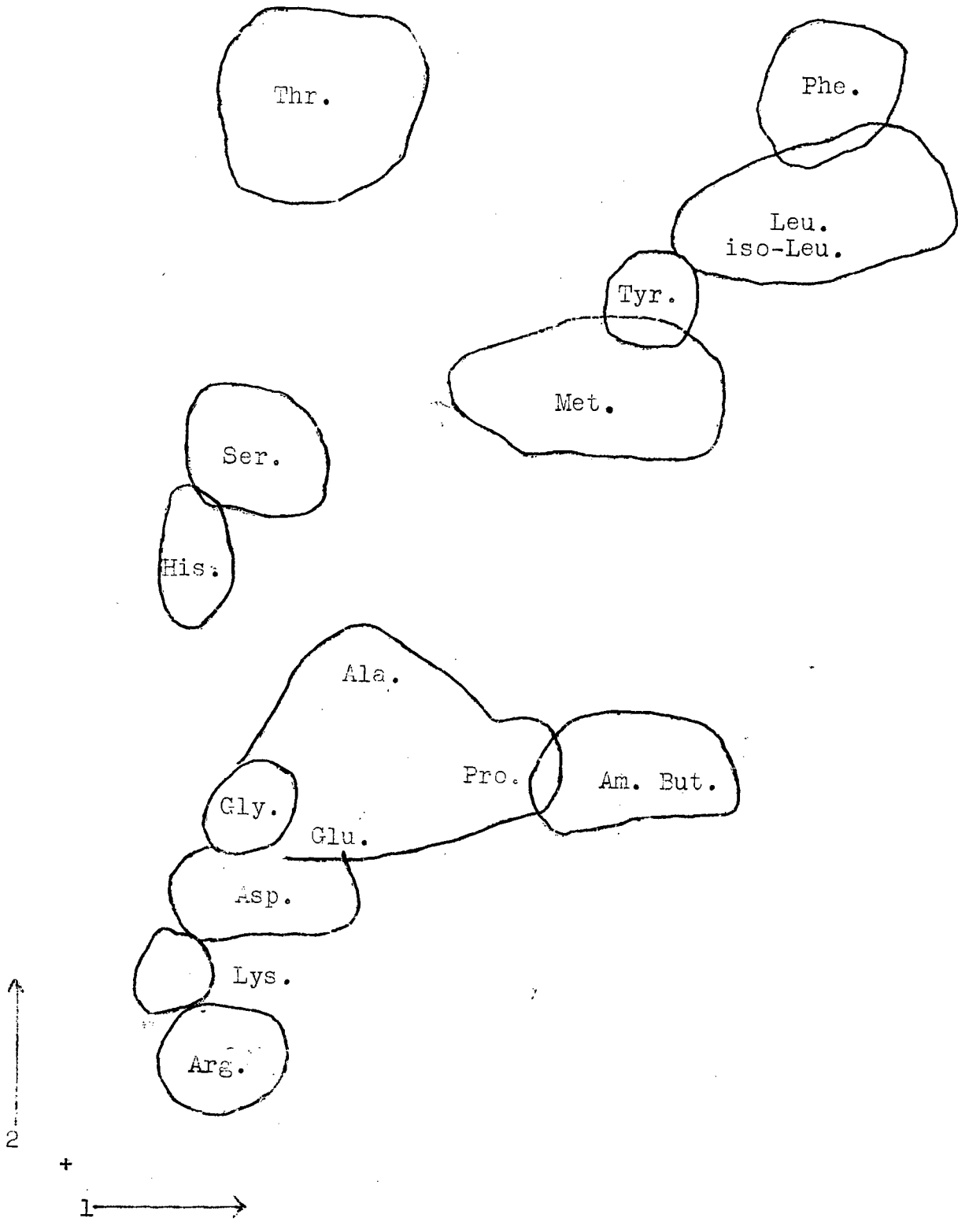

Figure 22. Two-dimensional paper chromatogram of amino acids of Riesling control (untreated) must (Experiment IX).

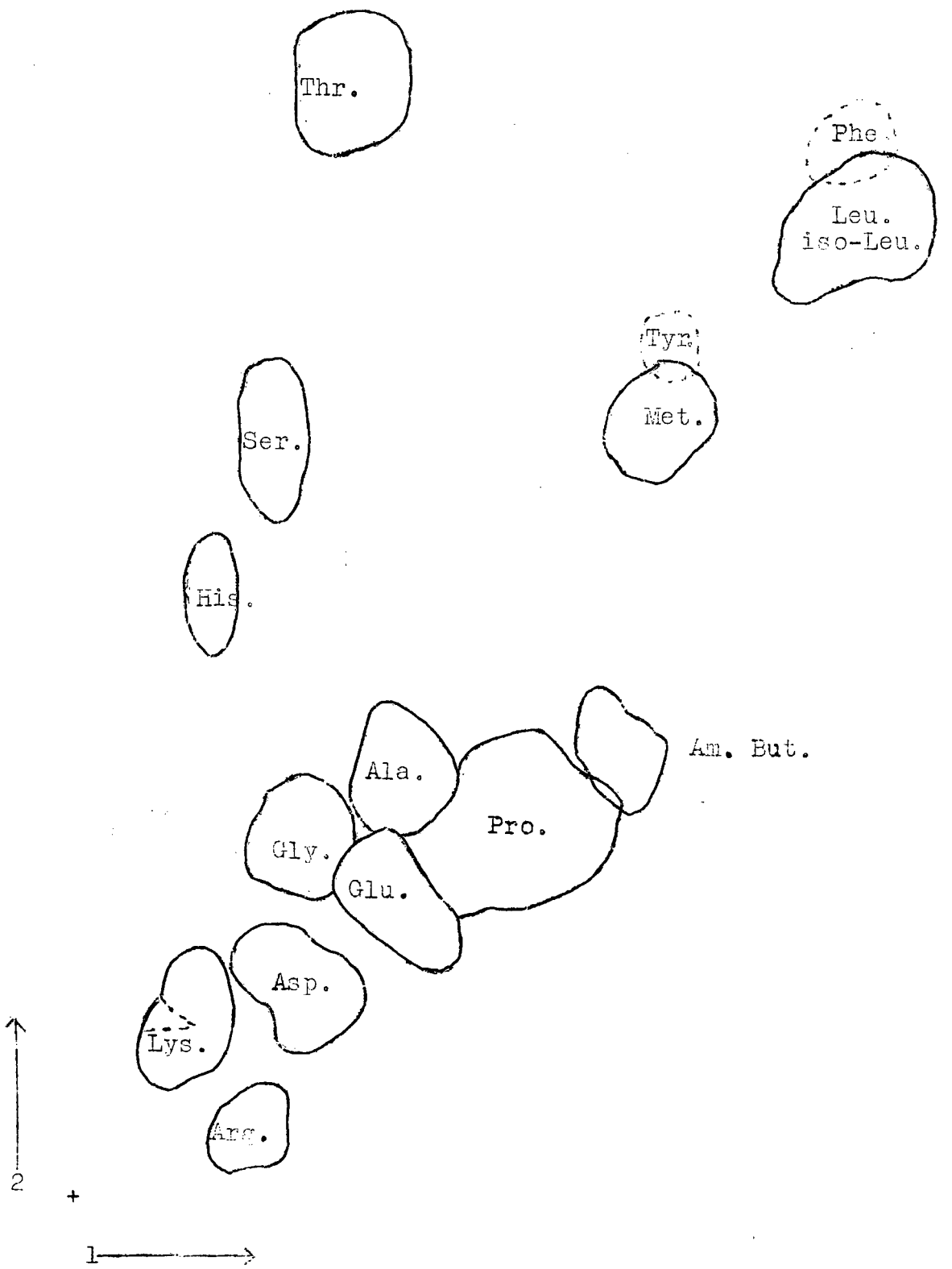

Figure 23. Two-dimensional paper chromatogram of amino acids of Stein control (untreated) wine (Experiment X).

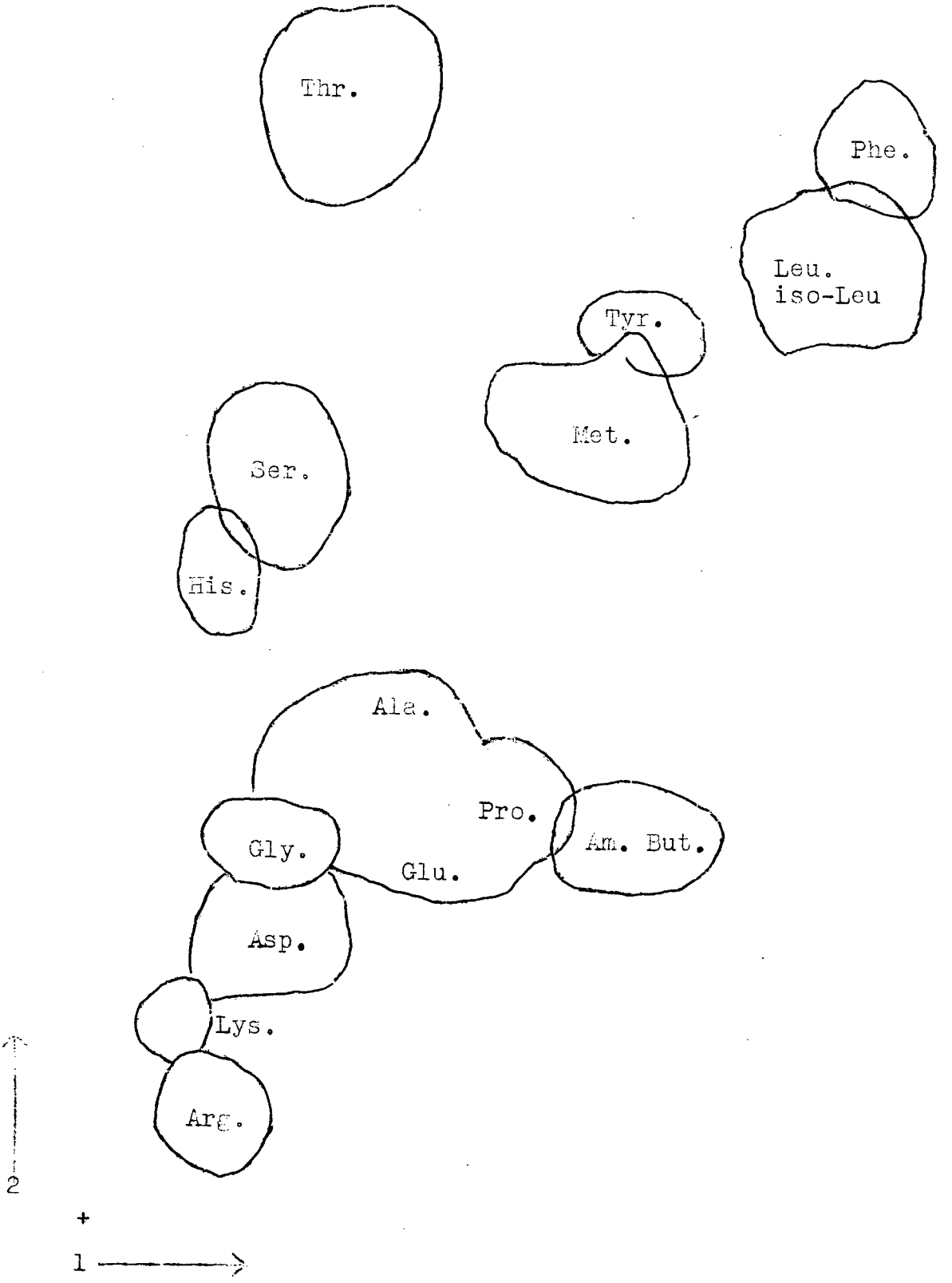

Figure 24. Two-dimensional paper chromatogram of amino acids of Stein control (untreated) must (Experiment X).

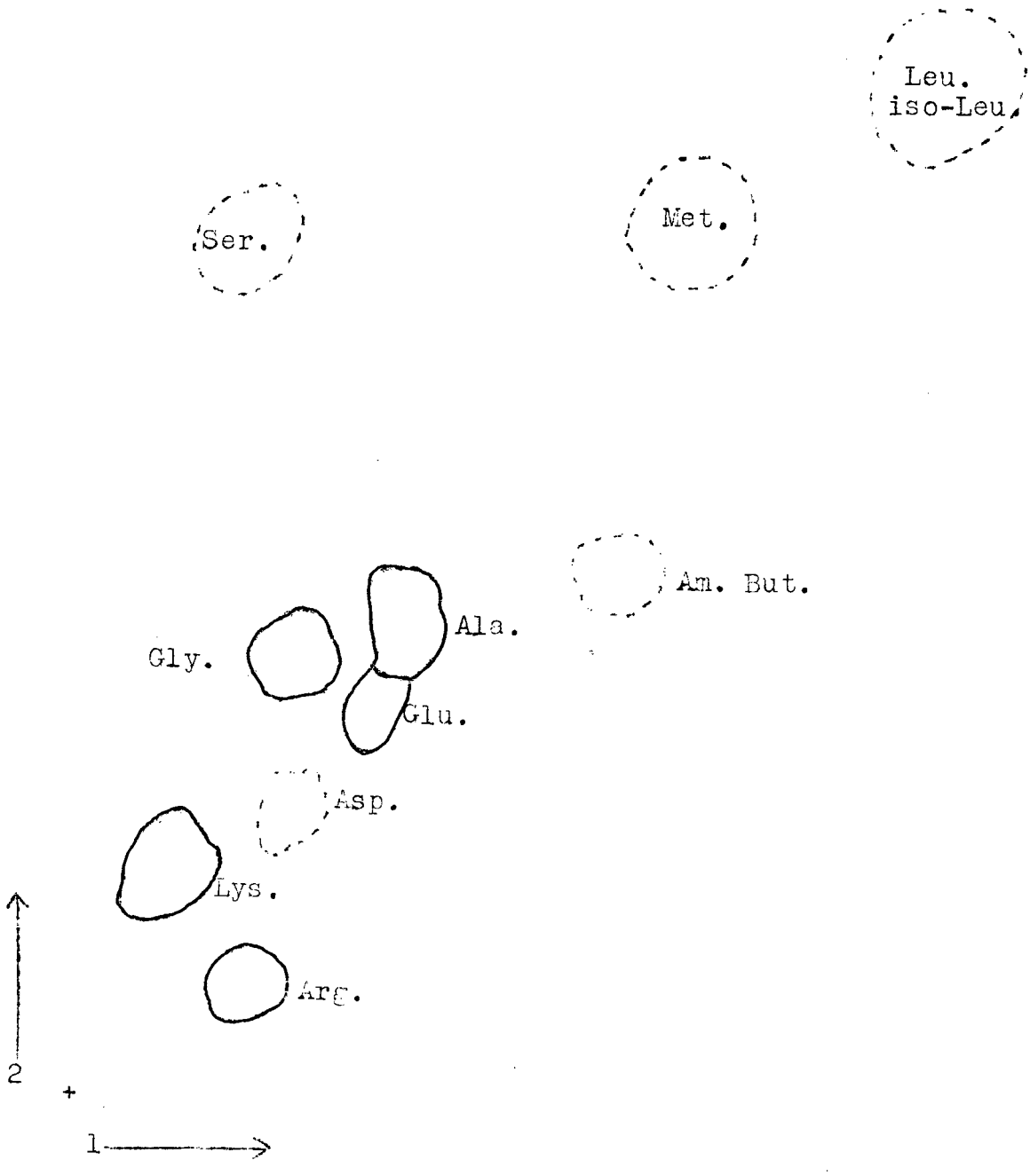

Figure 25. Two-dimensional paper chromatogram of amino acids of Riesling wine (Experiment IX) whose must had received the maximum ion exchange treatment. The pH value of this must was approximately 2 but was increased in pH, by the addition of potassium hydroxide, to the value of its control (untreated) must prior to fermentation.

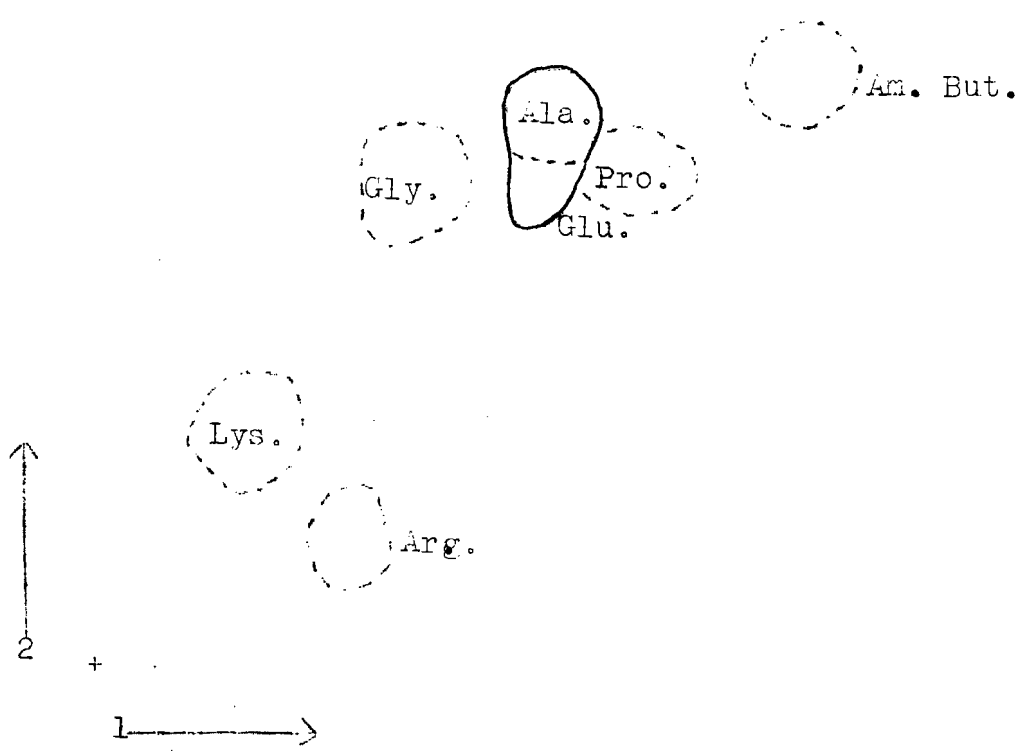

Figure 26. Two-dimensional paper chromatogram of amino acids of Stein wine (Experiment X) whose must has received the maximum ion exchange treatment. The pH value of this must was approximately 2 but was increased in pH, by the addition of potassium hydroxide, to the value of its control (untreated) must prior to fermentation.

of Experiment IX. This indicates that it was utilized by the yeast and was, no doubt, an agency which contributed to the slow fermentation of these musts. Furthermore, from Table 43 it is shown that in the pH 2 (control) wine of Experiment X aspartic acid, methionine, the leucines, phenyl alanine, serine, histidine and threonine did not appear, and, which indicates that they were assimilated by the yeast to satisfy their nitrogen needs. This, no doubt, also materially influenced the fermentation of these musts.

The influence of ammonia and amino acid removal on the overall fermentations of the pH 3.2, 3.0 and 2.8 musts was regarded as a pro rata decrease of these substances with a corresponding decrease in fermentation rates and which attains its maximum effect in the pH 2 (control) musts. The initial rapid assimilation and fermentation in the pH 3.2, 3.0 and 2.8 musts did occur, but a progressive decrease was manifested, for, although all of them contained ammonia and the known amino acids, which could influence fermentation rate, their concentration decreased with increased treatment.

The fermentation of the pH 2 (control) musts were regarded as the ultimate of a fermentation continuum of the progressive resin adsorption of ammonia and amino acids. But the position was possibly not quite as simple, for in all probability in the treated musts, perhaps more specifically the pH 2 (control) musts there could have been other factors which could have had a bearing on this phenomenon. Two possible factors are:-

(a) that trace elements may not have been present in sufficient quantities and

(b) that the bios factors, under which Nielsen and Hartelius (1938) also list glutamic acid,

aspartic/...

aspartic acid, asparagine, methionine, lysine, arginine, similarly not being present in sufficient quantities.

From the data in this section it is indicated that pH did not affect fermentation above pH 3.0 (must pH) but did affect it below this value. Furthermore, the progressive resin adsorption of the nitrogenous substances which consisted mainly of ammonia and amino acids was largely responsible for a corresponding decrease in the fermentation rate of all the treated musts.

(1) The Higher Alcohols (Fusel Oils).

The higher alcohol determinations were done upon the wines of Experiments VI to X, the results of which are tabulated in Table 44 and graphically illustrated (as a function of must pH) in Figures 27 and 28.

The present knowledge of the nitrogen nutrition of yeast is based largely upon the work of F. Ehrlich (1907). His initial interest was to determine the origin of the amyl alcohols which were formed during fermentation. In this respect he succeeded for he discovered that (in synthetic media) leucine was responsible for iso amyl alcohol and iso leucine for optically active amyl alcohol (Ehrlich, 1912a). The origin of iso butyl alcohol was also found by him to be in the amino acid valine. A general schematic formulation of the hydrolytic deamination and decarboxylation which occurs can be illustrated as follows:-

$$R \cdot CH_2 \cdot NH_2 \cdot COOH + H_2O \rightarrow R \cdot CH_2 \cdot OH + NH_3 + CO_2 \quad \cdots\cdots\cdots (1)$$

Similar reactions were found by Ehrlich to occur with phenyl alanine, tyrosine (Ehrlich 1912b) and tryptophane.

The/...

The mechanism of the deamination of amino acids is, however, not as simple as shown in equation (1). Later work by Neubauer and Fromherz (1910) and Neuberg and Hildesheimer (1911) indicated that an oxidative deamination of the amino acids occurred over an imino acid (equation (2)) leading to the formation of an ketonic acid (equation (3)) plus ammonia .

$$R\cdot CH\cdot NH_2\cdot COOH \xrightarrow{-2H} R\cdot C{:}NH\cdot COOH \quad \cdots\cdots(2)$$

$$R\cdot C{:}NH\cdot COOH + H_2O \rightarrow R\cdot CO\cdot COOH + NH_3 \quad \cdots\cdots(3)$$

This is followed by the decarboxylation of the keto acid with the formation of an aldehyde and carbon dioxide (equation (4)).

$$R\cdot CO\cdot COOH \rightarrow R\cdot CHO + CO_2 \quad \cdots\cdots(4)$$

Finally the aldehyde may be reduced to an alcohol to form one of the higher alcohols (equation (5)) or it may be oxidized to form an acid (equation (6)) as probably happens when succinic acid is produced from glutamic acid.

$$R\cdot CHO + 2H \rightarrow R\cdot CH_2OH \quad \cdots\cdots(5)$$

$$R\cdot CHO + O \rightarrow R\cdot COOH \quad \cdots\cdots(6)$$

The ammonia which is liberated in these reactions is utilized by the yeast as fast as it is formed for it does not appear in the fermenting medium at all. It also follows that if sufficient ammonia is available to the yeast cell the necessity of its deamination of amino acids will be decreased and consequently the formation of higher alcohols will also be decreased. This has been shown to be true (Villforth, 1954).

It has been shown that by the ion exchange process both ammonia and amino acids were removed from musts and as a result the higher alcohol contents of the treated musts' wines were bound to be affected, in fact from the relevant data and graphs this will be seen to have been the case.

It/...

It will also be seen that there existed a tendency of the higher alcohol (H.A.) values to attain a maximum in the pH 3.2 groups and then to decrease progressively with increased treatment. This phenomena could have been due to at least two factors:-

(a) If only ammonia was removed progressively by the resin then according to the Ehrlich mechanism a progressive increase in higher alcohols should have been manifested. But since amino acids were also progressively removed a corresponding decrease of higher alcohols could also occur. In the pH 3.2 groups thus the balance between ammonia and amino acids appeared to have reached that point where maximum higher alcohol formation occurred.

(b) But since this trend was consistently manifested in the pH 3.2 wines, whose must ammonia concentrations were, no doubt, dissimilar, (compare Table 40) it seems probable that another factor could have been responsible for this phenomena. The most obvious factor was pH.

The pH effect upon the formation of higher alcohols was examined firstly in Experiments VII and VIII where the pH of the same must was decreased by both ion exchange treatment and addition of tartaric acid to the set values. If pH played no role in higher alcohol formation then the concentrations of these substances (higher alcohol) should have been approximately similar, for, by addition of tartaric acid no radical change in ammonia and amino acid concentrations could have occurred. But the data (Table 44) and graphs (Figure 27) of the tartaric acid addition wines

show/...

TABLE 44.

pH, Total Alcohol and Higher Alcohol content of the Wines made from Untreated, Ion Exchange Treated and Tartaric Acid Addition Riesling and Stein Musts.

Wine Group.	pH.	Average Total Alcohol. Volume %.	Average Higher Alcohols. mg/100 ml.
Experiment VI (Stein).			
Control*	3.48	11.83	16.8
pH 3.2 (I.E.T.)	3.27	11.87	17.7
pH 3.0 (I.E.T.)	3.07	11.85	16.4
pH 2.8 (I.E.T.)	2.85	11.42	14.8
pH 2.6 (I.E.T.)	2.63	9.08	13.6
Experiment VII (Stein).			
Control*	3.53	11.65	21.1
pH 3.2 (I.E.T.)	3.24	11.65	21.6
pH 3.0(I.E.T.)	3.09	11.57	20.6
pH 2.8 (I.E.T.)	2.97	11.65	12.2
pH 3.2 (T.A.A.)	3.24	11.61	19.9
pH 3.0 (T.A.A.)	3.03	11.48	16.6
pH 2.8 (T.A.A.)	2.94	11.57	10.8
Experiment VIII (Stein).			
Control*	3.54	11.7	20.4
pH 3.2 (I.E.T.)	3.22	11.7	23.4
pH 3.0 (I.E.T.)	3.02	11.63	22.0
pH 2.8 (I.E.T.)	2.9	11.48	19.3
pH 3.2 (T.A.A.)	3.27	11.62	18.5
pH 3.0 (T.A.A.)	3.03	11.53	16.9
pH 2.8 (T.A.A.)	2.87	11.41	15.7
Experiment IX (Riesling).			
Control*	3.71	11.8	25.5
pH 3.2 (I.E.T.)	3.3	11.74	27.0
pH 3.0 (I.E.T.)	3.1	11.8	26.2
pH 2.8 (I.E.T.)	3.0	11.62	23.5
pH 3.2 (control) (I.E.T.)	3.77	11.8	28.2
pH 3.0 (control) (I.E.T.)	3.78	11.8	28.5
pH 2.8 (control) (I.E.T.)	3.8	11.74	31.3

Table 44 continued/...

TABLE 44 continued.

Wine Group.	pH.	Average Total Alcohol. Volume %.	Average Higher Alcohols. mg/100.ml.
Experiment X (Stein).			
Control*	3.78	12.0	33.8
pH 3.2 (I.E.T.)	3.51	12.0	33.6
pH 3.0 (I.E.T.)	3.31	12.0	32.4
pH 2.8 (I.E.T.)	3.19	12.0	28.0
pH 3.2 (control) (I.E.T.)	3.9	12.0	36.5
pH 3.0 (control) (I.E.T.)	3.92	12.0	38.2
pH 2.8 (control) (I.E.T.)	3.92	12.1	40.1

* Untreated.

I.E.T. Must pH decreased by ion exchange.

T.A.A. Must pH decreased by addition of tartaric acid.

(control) Must pH decreased by ion exchange treatment to set pH values and then increased in pH to its control (untreated) value by addition of potassium hydroxide.

show that pH did affect higher alcohol formation for, as pH decreased so did the higher alcohols decrease. Logically, therefore, if the higher alcohols of the latter groups of wines were affected by pH then also must the higher alcohols of the ion exchange treated wines have been similarly affected. The differences in higher alcohol content of the ion exchange treated and counterpart tartaric acid addition wines illustrate the working of the Ehrlich mechanism, for, the former group of wines (ion exchange) contained less ammonia than the latter. Since amino acid concentration also progressively decreased in the ion exchange wines the difference in higher alcohol content of e.g. the pH 2.8 counterpart wines was not expected to be large and this was also confirmed from the experimental data (Table 44 and Figure 27).

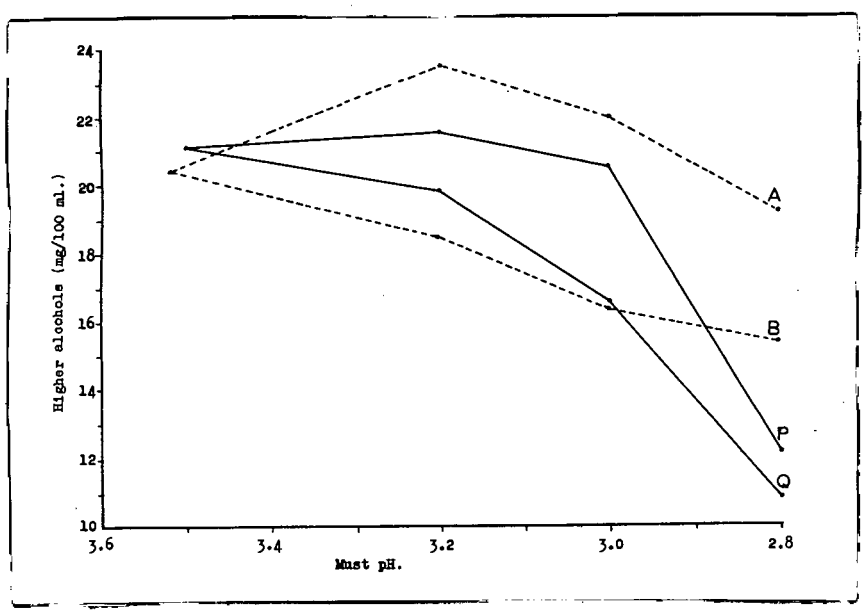

Figure 27. Influence of pH upon the formation of higher alcohols in the wines of a Stein must whose pH was decreased by both ion exchange treatment (I.E.T.) and addition of tartaric acid (T.A.A.) to values of 3.2, 3.0 and 2.8. A, (Experiment VII), I.E.T; B, (Experiment VII), T.A.A; P, (Experiment VIII), I.E.T; Q, (Experiment VIII), T.A.A.

Since it appeared that pH decrease did influence higher alcohol formation, this factor (pH) was effectively neutralized in the ion exchange treated musts of Experiments IX and X. The pertinent data and graphs are given in Table 44 and Figure 28 and it is evident what an important role pH played. The higher alcohol values of the pH 3.2, 3.0 and 2.8 (control) wines were now all in excess of their counterpart wines and also of their respective controls. These figures also bear out what was originally suspected viz. that below pH 3.2 the higher alcohol forming mechanism was progressively retarded. It is further apparent that although all of the counterpart wines showed differences in higher alcohol content, those of the pH 2.8 group were especially large; approximately 80 mg/L. in the wines of Experiment IX and approximately 120 mg/L. in the Experiment X wines. These differences were larger than expected.

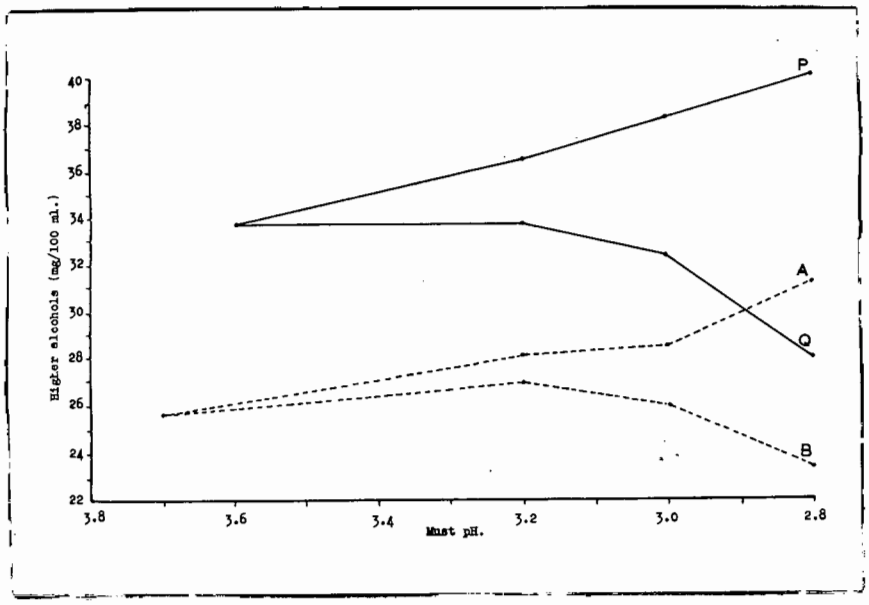

Figure 28. Influence of pH upon the formation of higher alcohols in the wines of a must, whose pH was decreased by ion exchange treatment to values of 3.2, 3.0 and 2.8. (control) indicates that a treated must pH increased, by the addition of potassium hydroxide, to the value of its control (untreated) must. A, (Riesling, Experiment IX), control, pH 3.2 (control), pH 3.0 (control) and pH 2.8 (control); B, (Riesling, Experiment IX), control, pH 3.2, pH 3.0 and pH 2.8; P, (Stein, Experiment X), control, pH 3.2 (control), pH 3.0 (control) and pH 2.8 (control); Q, (Stein, Experiment X), control, pH 3.2, pH 3.0 and pH 2.8.

The basic literature on the higher alcohols indicates that valine and the leucines are the respective precursors of iso-butanol and the amyl alcohols, which are claimed to be the chief components of the higher alcohols of fermentation. But the nature of the higher alcohol components is likely to depend on two factors, viz. the kind of amino acids present and the nature of the metabolic utilization of these nitrogen compounds by the yeast. In all the musts of Experiments IX and X no valine was found and since ammonia, particularly in the lower pH groups, was low the yeasts were forced to attend to their nitrogen needs by degradation of available amino acids and which included the leucines. Thus it does seem likely that a large percentage of the higher alcohols consisted of amyl alcohols. However, since the higher alcohol contents of the pH 2.8 wines differed widely their

leucines/...

leucines concentrations should also have differed by substantial amounts. But, if the chromatograms of pH 2.8 and pH 2.8 (control) wines of Experiments IX and X (Figures 29 to 32) are examined it will be seen that virtually no difference occurred in the concentrations of the leucines (and other amino acids) of these counterpart wines. In fact, if there was a difference it was so small that it certainly could not account for the 80 and 120 mg/L. higher alcohol differences manifested in these wines. It is thus indicated that a means other than the Ehrlich mechanism also operated and whereby higher alcohols were formed.

From the given data it is apparent that pH was the factor which was the major cause of the higher alcohol differences in the treated wines and their counterparts. Furthermore, it is indicated that, more specifically in the pH 2.8 wines and their counterparts, the formation of higher alcohol from the relevant free amino acids in the medium was not the sole means of its formation. Moreover, it is also indicated that the differences between the higher alcohol contents of the pH 2.8 wines was, at least, partly due to an alternate pathway of higher alcohol formation and that its formation by this means was affected by the pH of the medium.

The paper chromatograms of the amino acids of the pH 3.2 and 3.0 wines and their counterparts were not done as actual higher alcohol differences were relatively small and, consequently, differences in the relevant amino acid concentrations would also have been small and inferences drawn as to small differences in amino acid concentrations have been suspect. But it is not improbable that the differences in higher alcohol content of these wines could also have been similarly caused as in the pH 2.8 and pH 2.8 (control) wines.

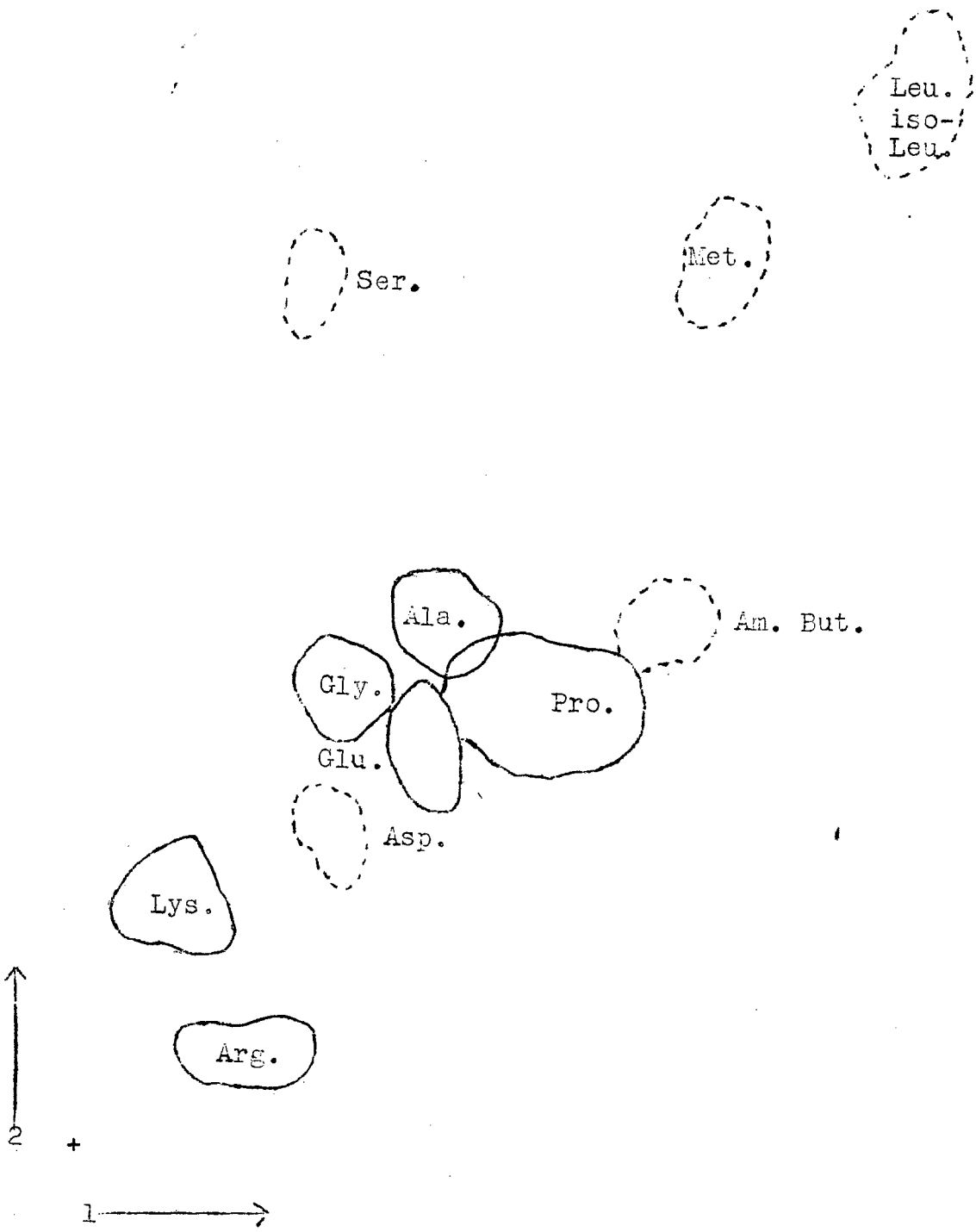

Figure 29. Two-dimensional paper chromatogram of amino acids of Riesling wine (Experiment IX) whose must pH was decreased by ion exchange treatment to a value of 2.8.

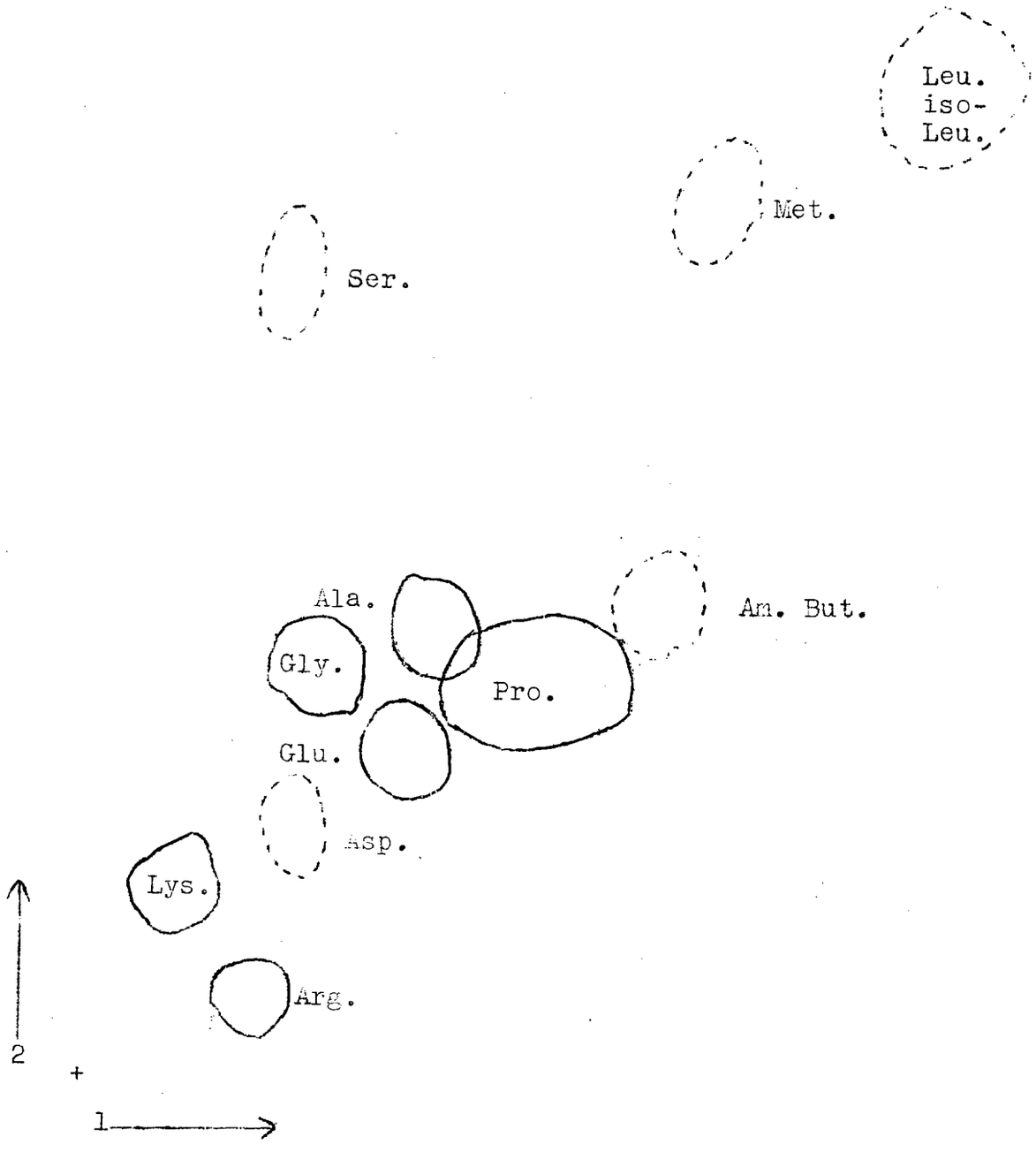

Figure 30. Two-dimensional paper chromatogram of amino acids of Riesling wine (Experiment IX) whose must pH was decreased by ion exchange treatment to a value of 2.8 and then increased, by addition of potassium hydroxide, to the value of its control (untreated) must.

A table was marked off into 24 blocks, which were numbered 1 to 24 and subdivided into two. Each block contained one pair. The order of the placings was randomized. Table 45 gives the sequence of the samples which was the same for both Riesling and Stein wines, bouquet and acidity.

The following information and instructions were given to the judges:-

(a) They were informed which wines were Riesling and which were Stein.

(b) They were informed that identical pairs occurred in the test but not how many and of which wines.

(c) They were asked to differentiate on the bouquet concentration and in the acidity tasting on the order of the acid taste.

(d) If one sample was found to be better than the other, to mark up a plus sign (+) in the space on their tasting sheet that was allocated to that sample; if no difference was found, an equal sign (=) in that block.

(e) They were asked to taste in numerical order, i.e. starting with the pair in block 1 and working up to block 24.

(f) They were asked always to taste the first sample in the block first; this sample would then also be regarded as a standard.

(g) They were asked to regard each pair as a unit which had no connection with any other pair on the table.

(h) They were asked to add any remarks which they wanted to make (e.g. malodours).

The/...

TABLE 45.

Chart giving the Tasting sequence of the Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts.

Block No.	Wine.	Block No.	Wine.
1	pH 3.0 Control	13	Control Control
2	pH 2.8 pH 2.8	14	pH 3.2 pH 2.8
3	pH 3.0 pH 2.8	15	Control pH 3.0
4	pH 2.8 pH 2.8	16	pH 3.2 Control
5	pH 2.8 Control	17	pH 2.8 pH 3.2
6	pH 3.0 pH 3.0	18	Control Control
7	pH 3.2 pH 3.2	19	pH 2.8 pH 3.0
8	Control pH 3.2	20	pH 2.8 pH 2.8
9	pH 3.2 pH 3.0	21	pH 3.0 pH 3.0
10	pH 3.0 pH 3.0	22	pH 3.0 pH 3.2
11	Control Control	23	Control pH 2.8
12	pH 3.2 pH 3.2	24	pH 3.2 pH 3.2

The data from these tests were analysed for significance by the chi square (χ^2) test. Chi square was determined as follows:-

If N represents the total number replications and x_1 the total number of times wine A is preferred over B (bouquet etc.) and x_2 the total number of times wine B is preferred over A then:-

$$\chi^2 = \frac{(x_1 - x_2 - 1)^2}{N}$$

If a preference occurred as a result of chance only once in 20 times then results are said to be significant (P = 0.05). If choice occurred by chance once in 100 times (P = 0.01) then the result is highly significant and it will be very highly significant if a preference occurred, by chance, once in a 1000 times (P = 0.001). The following values of χ^2 indicate significance where a standard exists:-

χ^2 = 2.71 - significant, P = 0.05
= 5.41 - highly significant, P = 0.01
= 9.55 - very significant, P = 0.001

(a) Bouquet of Wines.

Riesling.

In these wines a malodour was manifested but it was more pronounced in the control wine than in the treated wine. The cause of it could, therefore, not have been the result of the ion exchange treatment. However, the Riesling bouquet was not obscured by it. Furthermore, sulphur dioxide, apart from normal sulphuring of containers was increased in both Riesling and Stein by 40 ppm. prior to bottling. It was slightly noticeable in both wines especially the Riesling.

Tables/...

Tables 46(a) and (b) although given separately are actually integral parts of a single test. Table 46(a) gives identification of identical samples (e.g. A-A) whilst Table 46(b) gives preference for the wines of differing treatments (e.g. A-B).

The explanation of the signs in Tables 46 to 49 is as follows:-

In Tables 46(a) and 47(a) identical wines were compared; the correct judgment of a pair should, therefore, be equal (=); a plus sign (+) indicates an incorrect decision.

In Tables 46(b) and 47(b) "differing" wines were compared and a preference, if there was one, was required of the tasters. The signs are given in terms of the first wine of the pair in a column. For example in the A-B column (control-pH 3.2):-

(i) a plus sign (+) indicates that A was preferred (and B regarded as inferior to A).

(ii) an equal sign indicates no perceived difference.

(iii) a negative sign (-) indicates A inferior to B (and B regarded as the better one).

From Table 46(a) it will be seen that the panel as a unit could very significantly identify samples in which no difference occurred; it was acceptable on the 1% level (P= 0.01). These data indicated that differences in bouquet concentration were above threshold values and could be perceived. This is further born out by the data of Table 46(b).

Highly/...

TABLE 46(a).

Identification of Identical Riesling Wines by Tasters.

Taster.	A-A			B-B			C-C			D-D			P
	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	
I	+	=	=	=	=	=	=	=	=	=	=	=	0.01
II	=	=	=	=	=	=	+	=	+	=	=	=	0.05
III	=	=	=	=	=	=	=	=	=	=	=	=	0.001
IV	=	=	=	=	=	=	=	=	=	=	=	=	0.001
V	=	+	=	=	+	=	=	=	=	=	=	=	0.05
P	0.01			0.001			0.01			0.001			

TABLE 46(b).

Preference of Tasters for the Wines made from an Untreated and Ion Exchange Treated Riesling Must.

Taster.	A-B		A-C		A-D		B-C		B-D		C-D	
	i	ii	i	ii	i	ii	i	ii	i	ii	i	ii
I	=	=	+	+	+	+	+	=	+	=	=	=
II	+	+	+	+	+	+	+	=	+	+	=	+
III	+	+	+	+	+	+	=	=	=	=	=	=
IV	=	=	-	+	+	+	+	=	+	+	+	=
V	-	+	+	+	+	+	+	=	+	+	=	=
P	Not sig.		0.05		0.001		Not sig.		Not sig.		Not sig.	

Highly significant differences in bouquet concentration were perceived in the control(A) - pH 3.0(C) and control(A) - pH 2.8(D) pairs. It is evident that the ion exchange treatment of these musts influenced the bouquet concentration of the lower pH group wines and although not proved it would also appear as if this influence was progressive.

Stein/...

Stein.

Tables 47(a) and (b) are similarly arranged and integrated as the previous two.

TABLE 47(a).

Identification of Identical Stein Wines by Tasters.

Taster.	A-A			B-B			C-C			D-D			P
	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii	
I	=	+	+	=	=	+	+	=	=	=	=	=	Not sig.
II	+	=	+	+	+	=	=	+	=	=	=	+	Not sig.
III	=	+	=	=	+	=	=	+	=	=	=	=	Not sig.
IV	+	=	=	=	=	=	=	=	=	=	=	=	0.01
V	+	=	+	=	=	=	=	=	+	=	+	+	Not sig.
P	Not sig.			Not sig.			Not sig.			0.05			

TABLE 47(b).

Preference of Tasters for the Wines made from an Untreated and Ion Exchange Treated Stein Must.

Taster.	A-B		A-C		A-D		B-C		B-D		C-D	
	i	ii	i	ii	i	ii	i	ii	i	ii	i	ii
I	=	-	+	+	+	+	=	-	=	=	+	+
II	+	+	+	+	+	=	+	+	-	=	-	=
III	-	=	+	=	+	+	=	+	+	+	+	+
IV	=	=	+	=	+	-	-	=	=	+	=	+
V	-	-	=	=	-	-	=	=	+	+	-	+
P	Not sig.		Not sig.		Not sig.		Not sig.		Not sig.		Not sig.	

In Table 47(a) significant identification, by the panel of identical wines occurred only in the pH 2.8 (D) group. From these data it appeared that differences in bouquet between the treated wines was either small or non-existant. This was born out by the data of Table 47(b) where no significant difference in bouquet was found between any of the paired samples.

The difference in bouquet concentration between the Riesling control on the one hand and the pH 3.0 and pH 2.8

wines/...

wines on the other hand could have been due to pH and/or resin adsorption of must constituents. It was also possible that Stein bouquet could have been influenced by these factors. Consequently an attempt to determine the influence of pH upon bouquet was carried out upon the laboratory made wines of Experiments IX and X. The musts of these wines were identical to those used in the cellar experiments.

The pair sample design was again used. Identical wines were not included due to lack of material. The paired samples were the following:-

pH 3.2 (A) - pH 3.2 (control) (D).
pH 3.0 (B) - pH 3.0 (control) (E).
pH 2.8 (C) - pH 2.8 (control) (F).
pH 2.8 (control) (F) - pH 2.0 (control) (G).

Sample G was also included as representing the maximum ion exchange treatment. It was compared against the pH 2.8 (control) wine. The placings of the samples were randomized. Tasters were told which variety they were tasting and asked to differentiate between pairs, again upon bouquet characteristics. Furthermore, they were told that duplicate pairs occurred.

The Riesling pH 2.8 wine was unfortunately spoilt by a strong malodour and was useless for comparison. The results of this tasting is given in Table 48.

TABLE 48.

Preference of Tasters for the Wines made from an Ion Exchange Treated Riesling Must. Experiment IX Wines (Riesling).

Taster.	A-D		B-E		C-F		F-G	
	i	ii	i	ii	i	ii	i	ii
I	+	+	+	+	Discarded.		-	-
II	=	=	+	-			+	+
III	=	=	+	=			-	-
IV	∓	=	+	-				
V	=	-	-	-			+	+
VI	+	=	+	+			+	+
P	Not sig.		Not sig.				Not sig.	

From this data it appeared that no significant difference was caused in the bouquet concentration of the pH 3.2 and pH 3.0 wines by an increase in pH. The comparison of the pH 2.8 (control) (F) and pH 2.0 (control) (G) wines proved difficult for the latter wine had a distinctive nose which could not be recognized as a Riesling character. Three judges compared it to grenadilla (passion fruit) and a fourth to a malodour. One judge did not show his preference in this pair for to his interpretation they did not reflect a Riesling character.

Table 49 gives the results of the tastings of the Stein wines. The design and placings were similar to that employed in the previous test.

TABLE 49.

Preference of Tasters for the Wines made from an Ion Exchange Treated Stein Must.

Experiment X Wines (Stein).								
Taster.	A-D		B-E		C-F		F-G	
	i	ii	i	ii	i	ii	i	ii
I	=	=	=	-	+	=	+	+
II	=	=	=	=	=	=	+	+
III	+	=	+	=	=	=	+	+
IV	=	-	+	-	=	-	+	+
V	-	-	-	-	=	-	+	-
VI	+	-	=	=	-	-	+	+
P	Not sig.		Not sig.		Not sig.		0.01	

From these results no significance was established as to the influence of pH upon bouquet concentration in the pH 3.2, pH 3.0 and pH 2.8 wines and their counterparts. In the pH 2.8 (control) (F) - pH 2.0 (control) (G) pair, however, a significant difference was shown, the latter wine manifested a marked lack of bouquet.

In/...

In examining all the subjective data it would appear that the pH factor had relatively little influence upon bouquet concentration. In the Riesling wine, the pH 3.0 wine (Tables 46(b) and 48) it is indicated that resin adsorption of must components could have been responsible for bouquet decrease. In all the treated Stein wines (pH 3.2, 3.0 and 2.8) this latter factor appeared to have no influence, only in the maximum ion exchange treatment, viz. pH 2.0 (control) wine was a decrease in bouquet manifested. It is difficult to reconcile this lack of bouquet in this latter wine with the change of bouquet character in the Riesling pH 2.0 (control) wine. But since these two variaties were so different in other properties it is not impossible that they could also have differed in their bouquet forming substances. (The bouquet of the Riesling control wine was found to be of a higher quality than that of the Stein. The Riesling wine bouquet was the more delicate and fruity of the two). It must also be stated here that both the Riesling (Experiment IX) and Stein (Experiment X) pH 2.0 (control) wines had undergone lengthy fermentations and lees contact and which could have caused the bouquet change in the Riesling sample. Why the Stein pH 2 (control) wine did not also manifest a different bouquet is difficult to explain, but it again points to dissimilarities in these two varieties.

It would appear that the bouquet of some of the ion exchange treated wines were influenced to a greater degree by resin adsorption than pH. It is of interest to examine these wines in the light of present knowledge of the wine bouquet forming substances contained in musts and to attempt an explanation of this phenomena. F. Ehrlich stated that bouquet forming substances could result from the degradation or hydrolytic products of

grape/...

grape proteins. These products could certainly be amino acids. Kutal'ova (1931) by the use of synthetic media found glycine to give a yeast flavour, leucine a fruity flavour and alanine a wine or yeast flavour. Paris (1951) by addition of phenyl-alanine caused the rose like flavour of the Beaujolais wines to be manifested. Markh and Scherbakova (1958) concluded from their work that, of the wines they examined, those that had the highest organoleptic rating also contained the highest amount of amino acids.

In the Riesling wines there appears to be a connection between bouquet and amino acids concentration for as they were decreased below a certain level a decrease in bouquet concentration resulted (pH 3.0 and pH 2.8 wines). The amino acid concentration in the Riesling pH 2.0 (control) wine (Figure 25) and must (Figure 19) was low and the bouquet manifested by it was surely not of a Riesling type. It, therefore, does not seem illogical to suggest that the bouquet of this wine could also have been influenced by amino acids.

In the Stein pH 3.2, 3.0 and 2.8 wines the effect of amino acid removal upon bouquet concentration was not significant. However, in the pH 2 (control) wine the lack of bouquet was marked and, furthermore, the concentration of amino acids in this wine was indeed low (See Figure 26). It was found by the writer and two other persons that both the Riesling and Stein pH 2.0 (control) wines just after fermentation and racking definitely lacked in wine bouque in fact, they were just very ordinary wines. After approximately 7 months the Riesling sample manifested its "grenadilla" bouquet.

It/...

It would appear that there are indications that the amino acid removal in Riesling and Stein musts could effect a decrease or modify the wine bouquet. There are most certainly other components which are also influential or modifying in this respect; perhaps ammonia could be included in this category. Further study in the determination of the affect of amino acids in a must upon its wine bouquet would appear to hold promise.

(b) Acid Taste.

From the date of the laboratory experiments it has been shown that by the ion exchange process the pH of a must can be decreased without a relatively large change in total acidity. It seemed possible therefore, that the change of acid type (and modifying influences) by the ion exchange treatment could be either deterimental or advantageous to the acid taste of such wines. In this section the influence of ion exchange upon the organoleptic acidity is examined.

Table 50 gives the pH, total acidity and total tartrates of the Riesling and Stein cellar wines. The total acidities (as in all other cases) were determined by titration to a phenolphthalein endpoint ($\pm$ pH 8) and are, therefore, slightly higher than would be obtained by titration to a litmus endpoint ($\pm$ pH 7).

This part of the work was not the success it was hoped to be for the total acidities and pH values did not decrease as far as was expected and some of the treated wines were too acid in taste. It was found at the tastings that the inclusion of the high acid wines (pH 2.8) and low acid wines (controls) affected

the/...

TABLE 50.

pH, Total Acidity and Total Tartrate contents of the Wines made from Untreated and Ion Exchange Treated Riesling and Stein Musts after a one year maturation period.

Wine Group.	Riesling. pH.	Riesling. Total Acidity gm/L.	Riesling. Total Tartrates gm/L.	Stein. pH.	Stein. Total Acidity gm/L.	Stein. Total Tartrates gm/L.
Control*	3.85	4.2	2.4	3.7	4.3	1.9
pH 3.2	3.12	7.2	3.6	3.25	7.1	3.2
pH 3.0	3.01	8.1	4.4	3.03	8.3	4.3
pH 2.8	2.87	9.4	4.9	2.95	9.0	4.8

* Untreated.

the tasting; fatigue was appreciable.

The data of tasters preference and identification of identical samples which was identical in arrangement to the bouquet tasting design of cellar wines, is not given. In the identification of identical samples significance was achieved in the Riesling control and pH 3.2 pairs and in the Stein only in the pH 2.8 pairs. The Riesling and Stein control wines were, due to their relatively high pH, flat in comparison to the lower pH wines. In fact, this difference was so marked that in the identification of different pairs these controls were picked out 59 times out of a total of 60 replications. In the different pairs, pH 3.2 - 3.0, pH 3.2 - 2.8, pH 3.0 - 2.8 of the Riesling treated wines no significant differences were established and in the Stein significance was shown only in the pH 3.2 - 30 pair. From these findings it would appear as if total acidity or pH did not unduly influence the acid taste in the Riesling pH 3.2, pH 3.0 and pH 2.8 wines and in Stein pH 3.2 - 2.8 and pH 3.0 - 2.8 pairs. It was, however, felt that because of fatigue and sensory carry-over this data was of little value and deductions as to the influence of the treatment upon the acid taste could not be safely made.

As a further attempt to determine the hedonic quality of the acid, the low and high acid wines (control and pH 2.8) were discarded and the pH 3.2 and pH 3.0 wines placed before a panel of four competent judges who had much practical experience. These persons were asked to carry out an analytical tasting upon the pleasantness of the wine acidity. They were asked to compare it as such to that of an ideal hypothetical standard. Three judges, however, compared it to a wine which they considered to be very good in this respect. The following scheme gives a scale whereby points were to be allocated to these wines for their acidity on an hedonic basis:-

Acidity.	Points.
Hard or sharp	1-6
Good	7-11
Very good	12-15

Table 51 gives the points allocated to the wines by the four tasters.

TABLE 51.

Points allocated by Tasters on the Acid Taste of Wines made from Ion Exchange Treated Riesling and Stein Musts.

Taster.	Riesling.		Stein.	
	pH 3.2.	pH 3.0.	pH 3.2	pH 3.0.
I	5	3	4	2
II	5	2	3	1
III	8	6	4	2
IV	5	4	4	5

Tasters I, II, IV expressed the general opinion that these wines were hard. Taster III expressed the opinion that the Stein pH 3.0 wine would, perhaps, improve with further maturation. Furthermore, the analytical tasting required of the judges gave some difficulty in application and could have influenced the scores of these wines.

The/...

The results obtained here were disappointing. They showed no clear improvement upon the hedonic quality of acidity. These wines were inclined to be sharp. It was felt that maturation in another type of container (glass was used) or higher pH values would perhaps have given more satisfactory results.

CHAPTER VI.

SUMMARY AND CONCLUSIONS.

The results of all the aforegoing experiments can be summed up as follows:-

1. Where fermentation was complete specific gravity of the wine decreased with increased treatment.
2. Alcohol formation was not unduly influenced by by treatment between the must pH values of 3.8 and 3.0. Below the latter value a decrease in alcohol formation occurred.
3. Where fermentation was complete sugar-free extract decreased with increased treatment.
4. Fixed acidity was increased by this process by the must cations (other than hydrogen ions) being replaced by hydrogen ions. When the pH of a must was decreased to a set value by both ion exchange and tartaric acid addition the fixed acidity of the former must group was lower than that of the latter. The dissociation of the fixed acidity in the ion exchange treated must was thus larger.
5. Volatile acidity in wines increased progressively with increased treatment. Where a must was treated to a pH value of 2.8 and lower a marked increase in volatile acidity was manifested in its wine. The decreased pH of must was found to be the primary causative factor in increased volatile acid formation. When the pH of the 3.2, 3.0 and 2.8 ion exchange treated must groups was increased to the value of their controls the removal of amino acids and ammonia or their slower fermentation had very little effect upon volatile acidity formation

6/...

6. Ester contents showed increases with increased volatile acid concentration. The increase in ester content is ascribed to the formation of ethyl acetate . Since volatile acidity formation was shown to be a function of pH, ester formation was therefore also influenced by pH.
7. Aldehydes showed no significant changes with the ion exchange treatment.
8. Ash and alkalinity of the ash decreased strongly with increased treatment. Decreasing must pH by about 0.6 units generally caused the ash content of a wine to diminish by approximately half. Similarly, the alkalinity of the ash, expressed as potassium showed an approximate two-thirds decrease with a pH drop of 0.6 units.
9. The crystallization and precipitation of tartrates from wines fermented and held in glass did not occur readily either during fermentation or immediately thereafter. When a stock must received successively increased ion exchange treatment their matured wines showed a progressive retention of tartrates.
10. Fermentation velocities of treated musts were diminished by the adsorption of amino acids and ammonium ions by the resin. The minimum pH to which a must could be reduced to was approximately 2. The must which left the column at this pH contained no ammonium ions and a very much lower concentration of amino acids than did the untreated musts.

The control musts of both Riesling and Stein (Experiments IX and X) each contained 16 amino acids of which arginine, glutamic

acid, proline threonine and ∝ alanine occurred in relatively large concentrations. The pH 2 musts contained all the amino acids of the untreated musts except for asparagine.

The removal of amino acids and ammonia influenced fermentation velocity more than did pH in the 3.2 - 3.0 range. Below pH 3.0 i.e. at pH 2.8 pH also began to exhibit an influence upon fermentation rate.

When a must pH was decreased by both ion exchange and tartaric acid addition to 3.2, 3.0 and 2.8 the removal of ammonium ions and amino acids had a stronger retarding effect upon fermentation than did the tartaric acid.

11. Wine colour was more a function of pH than it was of resin adsorption of colour bodies from the must. Below pH 3.2 no progressively larger decreases in colour was manifested in either Riesling or Stein wines. The Riesling and Stein varieties differed in the effect of pH upon their colour. The colour components of the Riesling wines were more sensitive to pH changes than those in the Stein wines. Furthermore, under the incident conditions the Riesling variety also appeared to have a higher colour potential than the Stein wine.

12. The bacterial infection which occurred in both Riesling and Stein wines was identified as lactic acid bacteria. The infected wines were those that also manifested an increase in colour with an increase in pH.

In the treated Riesling and Stein wines whose must pH had been increased to that of their controls prior to fermentation, bacterial infection occurred only in the Riesling group.

When/...

When the pH of Riesling and Stein musts was increased to 4.5 bacterial infection occurred in their wines.

13(a). After one year of maturation all the treated Riesling wines cleared to a brilliant condition whilst all the Stein and the Riesling control wines remained hazy. pH was the factor which caused the clarification of the treated Riesling wines. Its influence upon the treated Stein wines was not as marked. The dissimilar effect of pH upon the clarities of treated Riesling and Stein wines was no doubt due to inherent varietal differences.

(b) Riesling control wines contained almost double the heat and tannin precipitable material than did the Stein control wines. The effect of pH upon the potential turbidities of both of these varieties was marked, more so than resin adsorption.

14. Where the pH of Riesling or Stein musts was decreased to a set value (3.2 - 2.8) by either ion exchange or tartaric acid a corresponding decrease in higher alcohol content occurred. Conversely, where the pH of an ion exchange treated must was increased to a set value (3.5 - 3.7) an increase in higher alcohol content resulted. Where a must pH was decreased to a set value (3.2 - 2.8) by both ion exchange and tartaric acid the wine of the ion exchange treated sample manifested a higher higher alcohol content than did its tartaric acid counterpart.

15. The organoleptic examination of ion exchange treated wines was divided into two parts viz:- (a) Bouquet and (b) Acidity.

(a)/...

(a) By the ion exchange treatment of Riesling and Stein musts to set pH values (3.2, 3.0 and 2.8) it was found that the former variety was more prone to reduction in wine bouquet by this process than was the latter. The Stein wine manifested no decrease in bouquet in any of the ion exchange treated wines (3.2, 3.0 and 2.8) whilst significant decreases in bouquet were found in the Riesling pH 3.0 and pH 2.8 wines. An attempt to determine whether pH decrease or resin adsorption was responsible for bouquet decrease indicated resin adsorption as the more functional of these two factors.

(b) It appeared that the decrease of must pH by the ion exchange process was inclined to produce a wine of sharp acid taste.

CONCLUSION.

It has been shown that many of the effects of ion exchange in the hydrogen cycle upon must and wine were primarily due to pH differences. The effects which were most strongly influenced by pH were:- color, protein decrease, fusel oil formation, bacterial contamination (lactic acid bacteria) and also clarity of wines. It follows that a decrease in pH of musts by another means could similarly affect the stated effects. Addition of e.g. tartaric acid could in these respects thus largely if not wholly fulfil the functions of a sulphonic acid resin must treatment.

It was found that the ion exchange treatment (hydrogen cycle) of Riesling must to pH 3.0 detrimentally affected its wine bouquet. Although no bouquet decrease effect was shown in any of the Stein treated wines (pH 3.2, 3.0 and 2.8) by this procedure it is still nevertheless not impossible that Stein must of another origin or vintage could have been detrimentally affected. It is known that the bouquet of a dry white table wine is not detrimentally affected by the addition of e.g. tartaric acid to its must, on the contrary, it may be improved. It is thus evident that as regards bouquet improvement, must pH decrease by the ion exchange process (hydrogen cycle) can not adequately replace the standard and well established must - pH decreasing procedures.

Fermentation velocities were decreased by this process but it is doubtful whether this fact is of much practical value, for, the decrease of must nitrogenous constituents, which may already be low could be the cause of incomplete fermentations.

Considering the cost of an ion exchange plant, the cost of the resin, the labour required, the possible

blockage/...

blockage of the column and the quality of the treated musts' wines against the cost of tartaric acid and known quality of tartaric acid(or citric acid) addition wines, the ion exchange procedure does not even appear to be an adequate replacement for the tartaric acid addition system. The improvements which this specific ion exchange procedure could cause e.g. the possible circumvention of cold stabilization, slower fermentation, retention of acidity are also not of such magnitude as to warrant its use in the prefermentation treatment (H cycle) of white musts.

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