T H E S I S

on

THE APPLICATION OF CHEMICAL AND BIOLOGICAL METHODS TO THE EVALUATION OF OREGON HOPS

Submitted to the

OREGON STATE AGRICULTURAL COLLEGE

In partial fulfillment of
the requirements for the
Degree of

MASTER OF SCIENCE

By

Carl F. Whitaker

APPROVED:

[redacted]

Professor of Agricultural Chemistry.

In Charge of Major.

[redacted]

Chairman of Committee on Graduate Study.

ACKNOWLEDGEMENTS

In presenting this paper I am indebted:

To Professor J. S. Jones for making possible this piece of work by providing laboratory facilities.

To Dr. E. N. Bressman for providing the material and for advice and assistance in writing this report.

To Professor J. E. Simmons for advice in the bacteriological work.

TABLE OF CONTENTS

THE APPLICATION OF CHEMICAL AND BIOLOGICAL METHODS TO THE EVALUATION OF OREGON HOPS

INTRODUCTION

Since 1930 the United States Department of Agriculture has appropriated funds for the purpose of making studies on the control of downy mildew on Willamette Valley hops. The investigation included chiefly pathological studies for the control of the mildew and the breeding of resistant varieties of hops. As the problem advanced it became evident that insofar as the plant breeding and related trials were concerned, anything that might be accomplished would not be of full value if the new developments were unsuited for brewing.

That different varieties of hops and even the same variety handled under different conditions, vary considerably in brewing value is well known. It is well known that in this country at least, the method for evaluating different samples of hops has been for buyers to grade them on physical characteristics alone, such as color, odor, lupulin content, dryness, foreign material and so forth. That this method of judging hops was inadequate was voiced as long ago as 1907 when Dr. W. W. Stockberger (1) said, "In determining the quality of marketable hops, each consumer has been a law unto himself with the frequent result that individual preference instead of intrinsic value decides

the choice. Thus, it actually occurs that a hop rejected by one consumer will be readily purchased by another; a state of affairs which is directly responsible for the sentiment sometimes voiced among hop growers that 'No matter how inferior the hops may be, someone will be found who will buy them'."

A group of English workers have since 1920 given considerable thought and work to the development of chemical and biological methods for the determination of the actual brewing value of hops and to all appearances have succeeded to a very marked degree. It is the application of these methods to the problem at hand with which this paper is concerned.

REVIEW OF LITERATURE

Except in a few cases, the literature prior to 1925 has not been examined due largely to the limitations of the library at my disposal. Furthermore, the references are restricted almost wholly to an English publication, the Journal of the Institute of Brewing, because it contains the reports of the "Research Scheme" promulgated by the Institute in 1920 for the purpose of studying the preservative properties of hops. Up to the beginning of 1932, twelve reports have been made to the Institute on this research problem. In addition to the research sponsored by

the Institute of Brewing, there have been a number of independent English workers together with German, French and Russian workers interested in the problem.

It is well known that the chief value of hops in brewing is the inhibition of undesirable bacterial action during and after brewing. Also that the antiseptic or preservative properties of hops are tied up with the so-called soft resins or the portion of the total resins that is soluble in light petroleum, and that hops of high soft resin content are likely to have a high preservative value. The preservative value and the soft resin content, however, do not run parallel because the soft resins consist of a mixture of compounds of different activities in varying proportions. As long ago as 1888 it was shown by Hayduck that the soft resin could be separated into at least two fractions by treatment with alcoholic lead acetate. The α- fraction was precipitated as a sparingly soluble lead salt while the β- fraction was not so precipitated. Since that time, much work has been done toward devising simple and accurate chemical and biological methods for determining the preservative value of hops.

In 1923, Walker (3) published an article on the nature of the preservative principles of hops. He stated that hops are used in brewing for the purpose of (1) imparting flavor, (2) acting as a preservative, and (3) as-

sisting in classification. The resins may be divided into two major divisions. The so-called hard resins that are valueless from a brewing standpoint and the so-called soft resins in which, it is believed, all of the preservative power rests. The soft resins may be separated into two fractions. The α - fraction which contains α - acid or humulon and the β - fraction which contains β - acid or lupulon.

In 1924, Walker (6) published a method for the extraction of crystalline lupulon from crude soft resins. β - resin was found to contain 26 - 33% lupulon. Whole hops extracted with light petroleum gave 3% β - resin, lupulin gave 10.6% β - resin.

In 1924, Walker (7) found lupulon to be a monobasic, unsaturated hydrocarbon, having the formula $C_{25}H_{36}O_4$, M.P. 94.5° - 95.5°, Mol. weight 400. It is insoluble in water, but soluble in the usual organic media though only slightly so in light petroleum. Forms metallic salts, and bromine addition products. Oxidation or treatment with aqueous or alcoholic potash produces valeric acid.

P. Kolbach (10) reviewed our present knowledge of the hop resins. The α - bitter and β - bitter acids, termed humulon and lupulon respectively, being crystalline substances should not be designated as resins. These by polymerization or hydrolysis form α and β soft resins

and going finally to α_1 and α_2 hard resins and β_1 and β_2 hard resins. α_2 and β_2 hard resins are insoluble. α_1 and β_1, hard resins are soluble in ethyl ether. The soft resins and acids are soluble in petroleum ether. The α- bitter acid is precipitable by lead acetate.

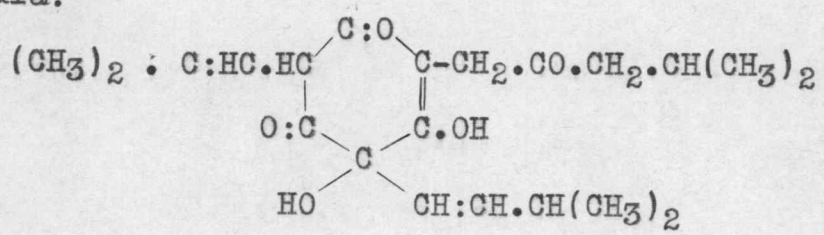
In 1925, Windisch, Kolbach, and Grohn (11) studied the effect of hydrogen ion in aqueous boiling solution on the transformation of humulon. Solutions ranging in hydrogen ions from pH 3.6 to pH 9.18 were used. The smallest amount of decomposition took place at pH 6.5, 18% in an hour's boiling. At pH 8.5 there was 45% decomposition indicated in an hour of boiling. When the humulon is boiled for periods longer than an hour it was found that the transformation took place at a somewhat slower rate as duration of boiling is increased.

Wollmer (12), in valuating a series of hops, prepared from each of the hop resins, three separate fractions; (1) α- bitter acid, (2) β- fraction consisting of that portion of the soft resins not precipitated by lead acetate, and (3) the γ - resin or hard resin, insoluble in petroleum ether. The separate fractions were boiled with wort, the liquid fermented, and the taste and stability of the resulting beers examined. The bitter flavors imparted by the resin fractions were judged by several persons, while

the length of time the beers kept sound was taken as a criterion of the antiseptic power. The γ - resin imparted no bitter taste to the beer, the β - fraction a slight bitterness, while with the α - acid an extremely bitter flavor resulted. That the antiseptic power resided in the α - acid was proved when beers treated with this fraction kept sound for months, whereas beers hopped with the β - fraction or γ - resin soon went sour. The author suggested that the brewing value of hops be taken as 7/6 x humulon, or humulon – 1/9 x β - fraction.

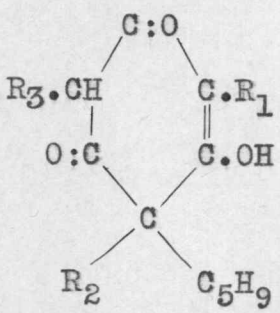
In 1925, Wieland (14) stated that humulon, $C_{21}H_{30}O_5$, is a weak acid owing its acidity to an enol group and is capable of forming di-metallic salts with heavy metals. Humulon is readily decomposed by alkalis into humulinic acid, $C_{15}H_{22}O_4$ and either a C_6 - acid or a mixture of isobutyraldehyde and acetic acid. Wollmer is quoted as stating that on catalytic hydrogenation with the aid of palladium, humulon takes up six atoms of hydrogen and yields isopentane and an acid substance, $C_{16}H_{24}O_5$ which is a derivative of hydroquinone.

Wieland suggests the following as the constitutional formula:



Wollmer (15) also in 1925 described lupulon as a monobasic acid without containing a hydroxyl group, but does not form di-metallic salts with heavy metals. It is much more stable than humulon toward alkalis. On hydrogenation lupulon will take up 8 atoms of hydrogen and yields the same pentane as humulon together with a resinous residue, $C_{21}H_{34}O_4$ which on oxidation in alcoholic solution in the presence of lead acetate produces a substance differing from humulon in composition only by containing 4 more hydrogen atoms in the molecule (two double linkages less). An analagous constitution is assigned and the compound termed tetrahydro humulon.

The suggested constitutional formula for lupulon is:



In which $R_1 + R_2$ may equal $C_{10}H_{20}O$ or $C_{10}H_{16}O$ or $C_{10}H_{18}O$ and R_3 may be C_5H_{11} or C_5H_7 or C_5H_9.

In 1925, Kolbach (16) writing on the problems of hop chemistry, discussed the limitations of hop analysis from the standpoint of grading. He suggested the use of iodine absorption as a means of measuring deterioration of hops during storage. The Versuchs und Lehranstalt in Berlin grades hops on the following basis:

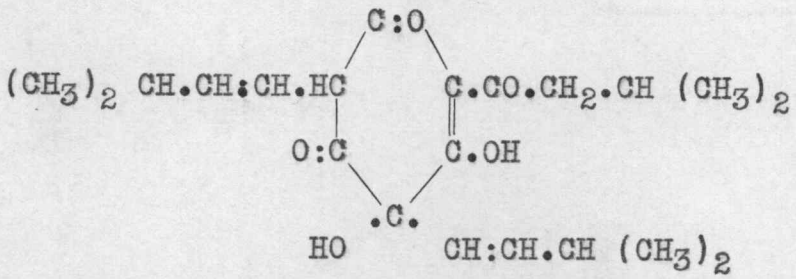
Development of cones	23.8%
Lupulin	23.8%
Aroma	23.8%
Color and lustre	15.9%
Dryness	7.9%
Picking and sorting	4.8%
	100.0%

He discussed the possibility of substituting analytical standards for the more or less empirical methods now in use.

Walker (18) believed that the large part of the resin fractions in hops are derived from humulon and lupulon through polymerization, hydrolysis or oxidation or all three together. When the lead acetate precipitate from the soft resins is treated with sulfuric acid or hydrogen sulfide a resin is obtained which is a mixture of humulon (which partly crystallizes out) and an amorphous derivative of humulon which cannot be obtained crystalline. This latter body is the true α - resin. Walker obtained about 40% humulon from this fraction. Since a small amount of the lead salt is soluble in the alchol the β - fraction will always contain a certain amount of resin derived originally from humulon. In addition the β - fraction also contains 15 to 33 per cent lupulon, resins derived from lupulon, and about 40% of a neutral material which appears not to originate from either humulon or lupulon. The γ - resin or hard resin is insoluble in light petroleum and is probably made up of further decomposition pro-

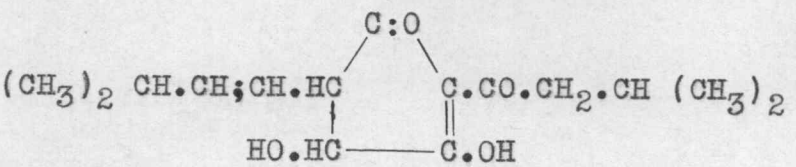
ducts of humulon and lupulon together with substances derived from the neutral portion of the β- fraction. The γ- resin has no antiseptic value.

Wieland (20) in 1926 published more material on the chemical nature of humulon and lupulon. His formula for humulon:

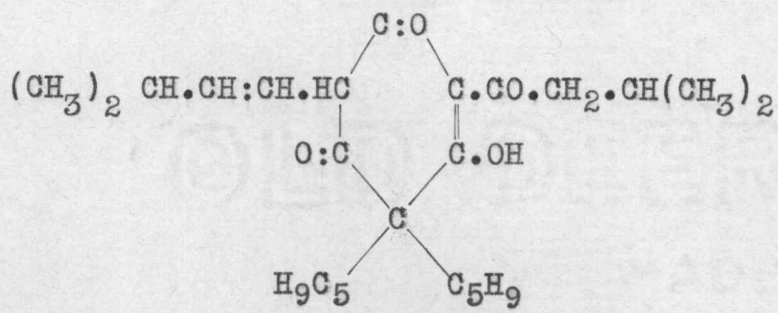


he felt was accurate except for the position of one of the double linkages. It is optically active by virture of the dissimilar groups attached to the lowest carbon atom as pictured.

When humulon is converted to humulinic acid



this lower carbon atom is removed and the other carbon atoms in the molecule are all subject to keto-enol transformations and therefore to racemisation, with the result the molecule is rendered inactive optically. Wieland's formula for lupulon is:



As in the case of humulon, the position of one of the double linkages is still in doubt. This compound is optically inactive because of the similarity of the groups attached to the lowest carbon atom.

In 1926, Windisch, Kolbach, and Banholzer (21) made a comparison of several analytical methods and the visual method of experts for the valuation of hops. Fourteen samples of hops were used.

In the chemical analysis the following values were determined: (a) the total resins representing the fraction soluble in ether and also in methyl alcohol; (b) the α - bitter acid, determined gravimetrically by precipitation with lead acetate; (c) the "soft resins" as determined by extraction with petroleum ether and weighing, also by alkaline titration; (d) the hard resin representing the difference between (a) and (c). The range of the series of 14 hops tested was 17.7% to 13.9% for (a), 7.1% to 5.0% for (b), 15.2% to 10.2% for (c) and 3.0% to 1.9% for (d). The lupulin content as estimated visually ranged from the maximum of 15 points down to 5 points. There was

no approach to satisfactory correspondence in grading between the visual and analytical methods. The order of the arrangement of the samples was also entirely different. The authors therefore conclude that visual examination of the amount of lupulin in hops appears to be a very unsafe criterion of their actual content of bitter substances.

In 1926, Windisch, Kolbach, and Yole (24) studied the effect of molecular oxygen on humulon. In the presence of air, humulon undergoes oxidation, although not as readily as lupulon, and yields resinous products. In the pure dry state, humulon is not readily altered even in the presence of oxygen. If oxygen is absent, it will remain unchanged for long periods even in the presence of water, and it undergoes very little change on heating for six hours at 100°C. It is more readily decomposed when heated in the air, but resinification is usually far from complete, as the resinous products tend to protect the unaltered humulon from further oxidation. In solution, its susceptibility to oxidation depends on its colloidal condition. In alcoholic solution it is only slightly attacked. During storage of hops, humulon undergoes resinfication much more rapidly than when in the pure state due to substances present that accelerate oxidation. Stored hops should not be heated because of the reaction of peroxides that may have been formed. On the other hand, fresh hops

can be heated to 80°C. with benefit, as volatile matters are thereby in part removed and in part oxidized beyond the stage at which they would accelerate the oxidation of humulon.

In 1926, Chapman (25) examined some very old cold-stored hops for resin content. After 30 years, cold storage, he found quite a high percentage of soft resins. The α- acid had completely disappeared. The author contends that his theory (rather rapid deterioration at first, after which a more or less stable condition obtains for long periods) was upheld.

Wieland and Martz (26) in 1927 stated that humulon on treatment with dilute alkalies yields humulinic acid, $C_{15}H_{22}O_4$, and this on reduction yields $C_{15}H_{28}$. The question of whether one of the side chains has the structure of (1) $(CH_3)_2C{:}CH.CH_2$. or (2) $(CH_3)_2CH.CH{:}CH$ seems to be solved in favor of (1) when acetone is formed by treatment of the above hydrocarbon with ozone and subsequently hydrolyzing the ozonides. If (2) were correct, isobutyraldehyde would be formed at this point.

In 1927, Pyman (27) reviewed the work on the chemical constitution of humulon and lupulon. Humulon was first seen in a crystalline state by Hayduck in 1888 and first isolated by Lintner and A. Bungener in 1891. In 1916, Wollmer isolated it in a pure state and found the

melting point to be 65° - 66.5°C. and to have the formula $C_{21}H_{31}O_5$. The determination of its constitution was accomplished mainly through the clever experimental work of Wollmer, and its brilliant interpretation by Wieland. Lupulon probably was first discovered by Lermer in 1863. It was rediscovered and characterized by M. H. Bungener in 1886 and its empirical formula established in 1926 by Wollmer as $C_{26}H_{38}O_4$. Its constitution has not been definitely established at this time.

Wiegmann (30) 1927, showed that spraying hops before, during, or after cone formation with bordeaux for mildew control has no apparent effect on flavor and brewing value. The ash and bitter substances were normal and the $CuSO_4$ content at .004% - .032%. On the other hand, if as little as .002% of $CuSO_4$ is added to the finished beer, a metallic flavor is imparted.

In 1927 Windisch, Kolbach, and Schleicher (32) studied the transformation of the α - bitter acid of hops in boiling solutions of different pH. They found that when humulon is boiled in slightly acid, neutral, or slightly alkaline liquids or in wort it undergoes decomposition and forms a mixture of resins part of which are insoluble in petroleum ether (hard resins) and part of which are soluble (soft resins). The soft resins formed are more acid than the original humulon, although it resembles it in

many of its reactions and in its bitter flavor. It is not precipitated by lead acetate. In strong alkaline solution, humulon is hydrolyzed to humulinic acid, a hexene acid, isobutyraldehyde, and acetic-acid. The formation of the hard resin is an oxidation process and does not occur when humulon is boiled with aqueous liquids to the exclusion of air. Soft resins, however, are formed even in the absence of air. Soft resin is thought to be an intermediate stage in the transformation of humulon to humulinic acid. With N/15 caustic soda humulon is completely transformed in 8 minutes in the absence of air to 91.4% soft resin and 8.6% humulinic acid. After two hours' boiling under same conditions the ratio is 25% soft resin and 75% humulinic acid. At the pH of brewery worts there is scarcely any humulinic acid formation. In a citrate buffer at pH 6.2, 33% soft resin is formed on two hours' boiling in absence of air.

In 1928, Stadnik (35) showed that the quality of hops depended on much more than the resin content. Among the compounds present in hops that may affect the flavor of beer are the tannins, the fatty compounds in seeds, the very slight solubility of hop oil, certain glucosides and organic sulfur compounds. The small amounts of waxy matter, oxalates, citrates, and sugar present are of no apparent significance. Certain nitrogenous compounds are concerned

with the occurrence of a disagreeable aroma.

In April 1928, Pyman (38), gave the extent of the knowledge of the preservative principles of hops in 1920 at the beginning of the Research Fund Scheme of the Institute of Brewing and gave a summary of what had been accomplished to date. This was followed by a discussion of the modified Ford and Tait gravimetric method, Chapman's plate method, and the B.delbruchii method all of which give comparable results. There was no bibliography of the early work.

In 1928, Hastings and Walker (40) studied the effect of special methods of drying on the preservative power of hops. The special methods used were (1) in a current of air at 40°C., (2) in a steam jacketed vacuum, the temperature was raised to 100°C., and then the steam shut off before introduction of the hops, (3) water heated vacuum at 40°C., (4) regular commercial method without use of sulfur. The conclusions are that the special methods of drying yield products of initially greater antiseptic power, but after six months of cold storage after drying the antiseptic value approximates that of the same variety of hops after treatment in the ordinary manner followed by six months' storage. The formula $\alpha + \beta/3$ cannot be used until after the storage period. The reason for these superior preservative powers is not definitely

known.

In a second part of this paper the authors studied the antiseptic material present in green hops and in hops dried under special conditions. Several methods of extraction were employed as well as several methods of treatment of the extracted products. The authors conclude that the extra antiseptic power of green hops and of low-temperature-dried hops, is due to the presence of a large proportion of crystalline lupulon in the β portions of their soft resins. This is partly destroyed when the hops are dried by the ordinary kilning process. Both humulon and lupulon suffer progressive destruction, which is more severe in the case of the latter substance, when hops are boiled in water or in wort in the brewery copper.

In December, 1928, Chapman (42) told of the essential oil of hops. The oil is obtained from glands surrounding the seeds at the base of the bracts in the flower of the common hop, by distillation. These glands are commonly known as lupulin and also contain a large portion of the preservative principles. The oil occurs in largest quantity in the fresh hops. With age it darkens, becomes viscous, resinifies. Fresh oil is only slightly soluble in water, but becomes increasingly more soluble in water-alcohol mixtures until in 95% alcohol it is completely so. At atmospheric pressure the oil starts to boil at 150°C.

the larger portion distilling between 230°C. and 270°C. Reduced pressure is necessary to obtain higher boiling fractions without decomposition. The chief functions of the oil in brewing are aroma and flavor. The quality of these characteristics seems to depend on age, origin of the hops, and proportions of the various constituents. Chapman has isolated and identified eight fractions from the essential oil, each of which he describes briefly. The author points out that it is useless for a brewer to purchase high grade hops if he does not take suitable precautions in its use in brewing. About 90% of the essential oil is volatilized on two hours' boiling in an open vessel.

In 1929, Behrendt (43) pointed out that hops dried in vacuum or in air yield products very similar in appearance and in their contents of total resins, soft resins, and humulon. Kiln-dried hops were of somewhat lower quality.

In November, 1929, Walker (47) presented a paper entitled "Some Recent Ideas on the Evaluation of Hops," which was followed by considerable discussion. See Walker (48).

In 1931, Walker and Hastings (55) published an outline of certain preliminary experiments on the copper boiling of hops. It was established that α- resin confers much

more bitter properties on wort than β - resin; that the bitter effect of α - resin increases progressively during boiling until a maximum is reached, after which a nauseous, unpleasant flavor is produced; that α - resin produces no aroma whatsoever in wort but that a pleasant aroma is produced by β - resin particularly when warm. Determinations on the rates at which hop antiseptics suffer change or destruction during the course of brewing operations was deferred until more delicate methods for estimation of antiseptic potency are developed.

In January, 1932, Walker (59) reviewed the progress of the work with which he has been associated since 1921. A recent modification of the recommended gravimetric procedure is given. The soft resin is passed through light petroleum before precipitation with lead acetate. This process eliminates an acidic substance which is soluble in the methyl alcohol but insoluble in light petroleum and which, when present, is precipitated as a lead compound by lead acetate.

Gravimetric Methods

Concerning the extraction of humulon and lupulon from hop cones, Walker (4) in 1923 devised a new method making use of methyl alcohol. In qualitative tests he found that light petroleum was unsuitable because (1) it was slow in

extractive action, (2)fails of complete extraction in the cold, and (3) hastens resinification of β - acid. Ethyl ether is a much better extractant, but is also an excellent solvent for wax. Methyl alcohol is much superior because (1) extraction is speedy and complete in the cold, (2) a minimum of time is required, (3) no tendency to cause resinification, and (4) solvent can be easily recovered. In quantitative tests, the minced hops were extracted for 24 hours with cold methyl alcohol. 100 cc. of the extract is shaken with 80 cc. light petroleum b.p. 35°-40° following which 200 cc. ice water are added. The petroleum layer is drawn off and two further extractions with 60 cc. petroleum each are made. The petroleum is evaporated and the residue dried to constant weight at 70°.

In 1924 Ford and Tait (5) brought forth a method for the estimation of α - acid and β - resin. The hops are extracted with methylated ether. The ether evaporated in a current of CO_2 and the residue redissolved in methyl alcohol. The α - acid is precipitated with lead acetate at 50°-60°, filtered, dried, and weighed. The β - resin is obtained from the filtrate by addition of sulfuric acid and extraction with light petroleum. The extract is filtered and dried to constant weight. In testing these two fractions biologically against Bacterium X it was found

that α- acid was 5 times as powerful an antiseptic as β- resin. The formula $\alpha + \beta/5$ was suggested as indicating the preservative value.

In 1928, Hastings and Walker (33) proposed some modifications to the Ford and Tait method of chemical analysis. Extraction of the hops is made in a Soxhlet with methylated ether for three hours. The ether is evaporated and the residue extracted with methyl alcohol, filtered and made to volume. A portion of the methyl alcohol solution is precipitated in the usual way with lead acetate. The β - resin, however, is obtained indirectly by treating a second portion of the methyl alcohol solution with twice its volume of cold water and extracting the colloidal suspension formed with four portions of petroleum ether. The combined ether extractions are evaporated to dryness and the residue weighed and calculated as total soft resin. The α - resin, as calculated from the lead salt precipitate, is subtracted from the total resin to obtain the β - resin. In precipitating the α - resin with lead acetate, care must be exercised since the precipitate is soluble in an excess of the precipitating agent. 1 - 2 cc. excess is allowable, however,

In 1928, Van Laer (39) discussed the chemical method for valuating hops. He states that early gravimetric methods are unsatisfactory because of the variability of the components of β - resin. The biological tests are

uncertain due to variability of the organism used and furthermore they are more laborious of operation. The formula $\alpha + \beta/x$ is quite satisfactory except there is no unanimity as to the correct value of x; numbers ranging from 3 to 9 have been given by different workers. Windisch obtained a value of 10 for x from determinations of the preservative power before boiling, after boiling for two hours, and after boiling and fermentation, in worts of pH 8.2, 7.0, 5.6, and 4.3. It is suggested that a knowledge of the preservative value retained in the finished beer is of more interest to the brewer than that possessed by the hops. The contribution of the β- resin to the total preservative power is so small that variations in constitution are of little consequence.

In 1929 Hastings and Walker (41) further modified the Ford and Tait gravimetric method. In the place of methylated ether it is pointed out that complete extraction of the soft resins may be had with cold methyl alcohol in a period of 10 minutes. The authors again pointed out the necessity of carrying out the lead acetate precipitation at a temperature of 60°C. and further suggest a digestion of 3 or 4 minutes after completion of precipitation to assist in filtering when cold. The hop samples should not be minced until they can be analyzed. Minced hops that are allowed to stand around for a few days have

a tendency to form difficult emulsions when the petroleum ether extraction is made of the original methyl alcohol extract. Enzymic action may be the cause of this.

In 1929, Wollmer (44) described a method for total resins, soft resins, and humulon that he has used since 1921. The primary extraction is made by shaking the sample for 3 hours with cold ether; the ether is removed on a water bath in a current of carbon dioxide. Methyl alcohol dissolves the hot residue. Humulon is precipitated according to Siller and the total soft resins obtained by a single extraction with petroleum ether.

In 1929, Windisch, Kolbach, and Winter (46) made a study of the present methods of hop analysis. The authors suggest that due to the solubility of the lead salt in an excess of the precipitant, a series of test tube trials using varying amounts of lead solution be made to determine the correct amount to use. Precipitation is carried out by adding the required amount of lead solution to equal quantities of resin solution and methyl alcohol and heating in a gently boiling water bath for 5 minutes. After 10 minutes cooling the precipitate is filtered off. The lead content of the precipitate should be checked to detect contamination. With old hops, a substance is present that makes necessary the use of a large excess of

lead acetate. It can be removed by shaking the petroleum ether extract with an aqueous phosphate buffer of pH 6.4. The authors claim that lupulon is of very little value in brewing so that the brewing value of fresh hops can be judged practically from the humulon content alone. The changes in resins due to ageing are represented graphically in a table.

In 1930, Guthrie and Philip (49) proposed a colorimetric method for the evaluation of hops. This method is based on the intensity of color produced when uranyl acetate is added to an alcoholic solution of either the α- or β- soft resin. The α- resin produces a more intense color than the β- resin for the same concentration. It is claimed that the method is sensitive to 1 part α- resin in 700,000 parts methyl alcohol. The proceedure involves the extraction of the hops with methyl alcohol, shaking out with petroleum ether and making the ether to volume. For determination varying amounts are introduced into test tubes, the petroleum ether evaporated, the residue dissolved in methyl alcohol and the alcoholic solution of uranyl acetate added. Comparison was made in a Hellige colorimeter with a known solution of α soft resin. Excellent agreement was had with both Ford and Tait's (4) gravimetric method and Hastings and Walker's (32) modification.

In 1930, A. S. Basilewick (50) divided the resins of hops into two groups (1) the humulides, which are precipitable from solutions in dilute methyl alcohol by lead acetate, and (2) the lupulides which are not so precipitable. He distinguishes 4 humulides and 3 lupulides and considers that the former are probably convertible into the latter by oxidation or intermolecular transformation. From numerous analyses it is concluded that the proportions of the various constituents may vary according to the nature of the soil on which the hops are grown, according to the fertilizers employed, and according to the part of the plant from which the hops have been plucked.

Wollmer (51) in 1930 published a gravimetric method for hops that uses ether for the original extraction and hexane as solvent for the soft resins. When drying the resins for weighing the last traces of solvent are removed by a current of pure, dry, CO_2.

In 1931, French (53) proposed a simplified colorimetric method for the determination of the preservative value of hops, based on that of Guthrie and Philip (48). There is a saving of time and materials on the original method. Standards are made up with ferric chloride based on an original series made with humulon. Various grades of alcohol from pure to industrial methylated spirit can be used with equal success provided the standard is made

with the same alcohol. The determinations on the hops should be made as soon after extraction as possible. The uranium salt must be kept in amber bottles. The determination is made directly on the original alcohol extract.

In 1931, Wildner (54) analyzed a number of hop samples of various origin by most of the methods which have been proposed for the estimation of the bitter acids and resins. The results are set out in tables and compared, and the practical advantages and disadvantages of the various methods are summarized. The methods compared are those proposed by Stadwick, Wollmer, Siller, and Hastings and Walker.

In 1931, Heintz (37) stated that hops are liable to become rancid on long storage, especially in the warm, and that fats or fatty acids may be present. A study was made of the substances extracted from hops by petroleum ether and ethyl ether followed by alcohol, water and dilute alkali. It was observed that petroleum ether and ethyl ether extracted among other things certain non-resinous substances of an acid nature. These were thot to be fatty acids and the author devised a method of separation which proved the assumption to be true. About 90% of fatty acid is soluble in petroleum ether and has the general characteristics of oleic acid while the remainder is soluble in ethyl ether and resembles a saturat-

ed acid. These acids are not present as glycerides in the hop. After separation, the acids are titrated with standard alkali. No tannin was found present in hops.

In 1931, Heintz (58) described a volumetric method of analysis based upon the author's recent work as noted in the preceeding abstract. The hops are extracted first with petroleum ether and then with ethyl ether. The petroleum extract is treated to esterify the fatty acids, filtered from wax and titrated with alkali for determination of soft resins. The ether extract is esterified in the same way and titrated for hard resins. Another portion of the hops is extracted with ethyl ether and titrated without esterification. This latter titration less the two previous titrations gives the free fatty acids. The author recommends the removal of stalks from hop samples before analysis, because they contribute practically no valuable constituents, but their amount is so variable as to vitiate exact comparative studies on the resin content of different hops.

Biological Methods

In 1923 Brown and Clubb (2) carried on a number of experiments on a biological method for evaluating hops. A bacterium was isolated from beer wort which they chose to call Bacterium X and the ability of hop extracts to

suppress the activity of this bacterium was noted. The authors recognized that for comparable results the determinations should be made at, or about, the same time, using the same culture medium, and, so far as possible, cultures of Bacterium X of the same age and vigor.

In 1924 I. Stoleru (9) attempted to fix more or less precisely the conditions under which hops exhibit their antiseptic properties. It was found that the reaction of the culture medium must be acid. When the reaction was at pH 5.5 to pH 6.8 the best results were obtained. At neutrality or at pH 7.4 to pH 8.4 the antiseptic power was almost nil. Neutralization of the hop resins (which normally are acid) with sodium hydroxide had no influence on antiseptic strength providing the culture mediums were acid.

In 1925, Chapman (8) devised a biological method in which cultures of an organism isolated from beer wort were treated with varying amounts of an infusion of hops and the point of growth inhibition noted. Chapman also observed that hops deteriorate in preserving power more or less rapidly with age and method of storage. Less deterioration was noted with cold stored as against ordinary stored hops.

Kolbach (13) in the same year suggested a biological method of analysis that makes use of Bacillus delbruchi, a lactic acid forming bacillus. Since the bitter acids of

hops are only slightly soluble in water, it is suggested that an alcoholic extract be made in the cold and different amounts added to a series of tubes containing wort. A second series of tubes are prepared to which different amounts of pure humulon are added for comparison. Both series are sterilized and fermented with yeast followed by inoculation with B. delbruchi. Inhibiting amount of hop extract is determined by absence of lactic acid formation which can be detected by titration with alkali.

In 1925 Walker (17) studied the soft resin, as extracted from lupulin by alcohol, in detail. Alcoholic lead acetate separated the resin into an α - fraction and a β- fraction. These two fractions were further divided and each sub-fraction tested against four different organisms among which was the Bacterium X of Mr. J. S. Ford. Of the purified constituents, lupulon was found to be twice as powerful an antiseptic as humulon. The α- resin was found to have three times the antiseptic power of the β- resin.

In 1926 Ford and Tait (19) pointed out that while they had previously determined that the α- resin was five times as powerful an antiseptic as the β - resin more recent work had indicated the comparison figure to be 4.3 instead of 3 as declared by Walker (16). In any event, it is pointed out that when fresh hops are used, the difference

in antiseptic value calculated with any of the above figures is negligible. In conducting the biological tests, Bacterium X was used but difficulties were experienced because conditions could not be standardized to obtain reliable uniformity of development of the organism. Thus, each set of results would be comparable within itself but not necessarily with other sets. Close agreement was obtained between biological and chemical methods.

Van Laer, (22) in 1926, reviewed recent advances in the knowledge of hop resins. The biological methods come in for criticism by reason of the method of making the hop decoction and also for the difficulty with which bacterial cultures of the same virulence are obtained. The chemical method alone gives identical results in absolute value.

In 1926 Hasting, Pyman, and Walker (23) made comparisons of Ford and Tait's (4) gravimetric method, Chapman's (7) biological method, and the B. delbruchi method; all of which gave comparable results. Bacterium X had been found unsuitable, and definite reasons are given for its being dropped from consideration. The authors conclude that the gravimetric method is the best since it can be standardized and is absolute. The biological methods at present are hardly more than comparative.

In 1926 Chapman and McHugo (28) examined some green

and kiln-dried hops by the senior author's biological method. When the results were reduced to a moisture-free basis, it was shown that very little difference existed between distinctly green hops and fully ripe hops, but during the kiln-drying process 50% to 70% of the antiseptic value was lost.

Walker (36) in October, 1927, gave a lecture which was followed by a discussion on the more generally accepted gravimetric and biological methods for evaluating hops.

In 1929 chapman (45) attempted to standardize the strength of his Bacterium "C" against pure phenol. Nutrient agar in tubes containing from .5 cc. to 2.5 cc. of 1% phenol was inoculated with 3 drops of an 18 hour broth culture of Bacterium "C". Plates were poured and incubated at 37° C. for 48 hours. Evidence of growth was taken as the criterion. Bacterium "C" was found to be quite constant.

In 1931, Walker, Hastings, and Farrar (56) published a long and detailed account of a new biological method which made use of B. bulgaricus instead of B. delbruchi. It is claimed for this method that it is much more sensitive and that very small differences in the antiseptic potency of different hops can be detected. The method depends upon the production of definite and comparable amounts of lactic acid which are subsequently titrated

with alkali to the pH of the original wort. Specific and detailed technique is described. Comparison of results with those obtained by Ford and Tait's gravimetric method and Chapman's biological method show excellent agreement.

DISCUSSION OF GRAVIMETRIC METHOD FOR CHEMICAL EVALUATION OF HOPS

It has been known for some time that the preservative properties of hops are associated with the so-called "soft resins," the fraction of the total resins soluble in petroleum ether. But while hops containing a high proportion of soft resins are likely to have high preservative value, experience has shown that the two are not exactly parallel. As early as 1888 it was shown that the soft resin could be split into at least two fractions by treatment with alcoholic lead acetate.

Later Ford and Tait (5) developed a chemical method for comparing the antiseptic properties of different hops. The portion of the soft resin that precipitated with the lead acetate was termed the α- resin and the fraction not precipitated was termed the β- resin. Upon testing these fractions separately as to their power to suppress the growth of certain organisms in malt wort it was found that the α- fraction had 5 times the potency of the β- fraction, hence if one wished to express the value

of the particular hop in terms of the α - resin, it would be merely a case of dividing the percentage of β - resin by 5 and adding the result to the percentage of α - resin found. $\alpha + \beta/5$ is the graphic representation for the value of a hop in terms of its α - resin content. A little later, Walker (17) made a study of the soft resin constituents of hops. Humulon, a crystalline compound, was isolated from the α - resin and its preservative properties compared with those of the original α - resin. The properties of the humulon and the α - resin were so nearly alike that it was considered that the α - resin was made up largely of humulon and its degradation products. The β - resin was another problem. To fractionate this portion it was subjected to extraction with aqueous alkalies in increasing strengths as follows:

4% sodium bicarbonate extracted	7.5% of β - resin
1% ammonium carbonate extracted	5.8% of β - resin
1% sodium carbonate extracted	14.5% of β - resin
1% caustic potash extracted	30.2% of β - resin
Remainder of neutral material	42.0% of β - resin

The neutral material was found, for all practical purposes, to have no preservative value. Of the portion soluble in caustic potash about half of it could be obtained as crystalline lupulon which was subsequently proved to be largely responsible for the preservative power of the β - resin. The other half was a non-crystalline resinous material, probably a decomposition product of lupu-

lon, and it, together with the portions soluble in sodium carbonate and ammonium carbonate had a preservative value of about 1/4 that of the crystalline lupulon. The sodium bicarbonate fraction was of small account. The crystalline lupulon was found to have about twice the antiseptic power of crystalline humulon but the β- resin as a whole had only 1/3 the antiseptic power of the α- resin. Thus Walker proposed $\alpha + \beta/3$ as the formula for calculating the antiseptic power as against the $\alpha + \beta/5$ of Ford and Tait. Walker's results were obtained using the Bacterium X of Ford and Tait together with three other organisms obtained from A. C. Chapman.

Still later, Ford and Tait (19) again made an examination of resin constituents of hops and found that $\alpha + \beta/4.3$ was the correct formula to use when calculating the value of any particular sample. They point out, however, that when fresh hops are used the difference in antiseptic value calculated with any of the three formulas presented is negligible. In arriving at this new formula, Ford and Tait made use of the same Bacterium X as previously but they admit of difficulties due to their inability to standardize conditions for uniform development of the organism. Thus, while each set of results would be comparable within itself, they would not necessarily be comparable with other sets.

In the original Ford and Tait (5) method the hops were extracted for 5 hours in a Soxhlet with methylated ether and then allowed to stand over night. This was followed by more extraction until the run-off ether was colorless. The extract was evaporated in a current of CO_2 until free of ether and the residue dissolved in hot methyl alcohol, cooled, filtered and made to volume. The α - acid is determined by precipitation at 50° C. - 60° C. with methyl alcoholic lead acetate, the precipitate being allowed to settle over night before filtering and weighing. The β- resin is obtained by acidifying the filtrate from the lead acetate precipitation with sulfuric acid and extracting the mixture with petroleum ether. The extract being filtered, evaporated and dried to constant weight at 100° C. There is considerable time involved in this procedure and in 1928 Hastings and Walker (33) proposed some modifications designed to shorten the time appreciably and to reduce the limits of error. In the first place, it was found that three hours in the Soxhlet with methylated ether was sufficient for proper extraction. As a matter of fact their data showed that nearly all the material that is soluble in methyl alcohol is extracted in one hour. The ether is removed by distillation, the last 30 cc. under diminished pressure and the residue is extracted with successive portions of warm methyl alcohol until about

100 cc. have been obtained when all portions are mixed together, filtered and made to volume. The α - acid is obtained in the usual manner by precipitation with alcoholic lead acetate, however, one-half hour only is allowed for the precipitate to settle before filtration takes place. The β - resin is obtained indirectly by making a total soft resin determination during the time allowed for the α - acid lead salt to settle. This is accomplished by treating another portion of the methyl alcohol solution of resins with twice its volume of water and subsequently extracting the mixture with petroleum ether after which the petroleum is evaporated and the residue dried and weighed. The percentage of total soft resin less the percentage of α - acid gives the percentage of β - resin.

It was suggested by Hastings and Walker (33) that the reason for the discrepancy between Ford and Tait's formula $\alpha + \beta/4.3$ and Walker's formula $\alpha + \beta/3$ lies in the mode of extraction with petroleum ether. In the Ford and Tait method the only water added to the β - resin before extraction with light petroleum was that contained in the few cubic centimeters of sulfuric acid used to precipitate the lead. Under those conditions light petroleum does not give a complete extraction of the methyl alcohol solution. This latter fact was well known by Ford and Tait, but, under the conditions of their experiments at

that time, the residue left in the methyl alcohol was found by them to be without antiseptic value, and hence they neglected it. The addition of water to the methyl alcohol solution of resins throws these resins out of solution in a colloidal suspension which is suceptible to a rapid and complete extraction with light petroleum. Hastings and Walker (33) point out that in every case that has come to their attention, the β- resin obtained by the latter method has always, weight for weight, an antiseptic value of 1/3 that of humulon, while the β- resin obtained by the Ford and Tait method has an antiseptic value of 1/4.3 that of humulon. The proper amount of water to add to the methyl alcohol extract was found experimentally by Hastings and Walker to be in the ratio of 2 parts water to 1 part alcoholic extract. About 1% of common salt is added to the colloidal suspension before extraction with light petroleum to prevent emulsification troubles.

Not being satisfied with methylated ether as a primary solvent, Hastings and Walker (41) again changed the Ford and Tait method for the gravimetric analysis of the preservative properties of hops. Methylated ether besides being a solvent for the soft resins also is a solvent for the hard resins which, under normal conditions of drying and storage, may form a protective coating over

the soft resin. Ether also extracts other substances chiefly of a fatty nature which, being soluble later on in light petroleum, have the tendency to produce high results. The soft resins besides containing the antiseptic properties are described as the portion of the total resins soluble in light petroleum. It was found, however, that a primary extraction with light petroleum had the tendency to give too low results, probably because of the protective action of the hard resins. Methyl alcohol was considered and appeared to be the ideal solvent since it would dissolve both the hard resins and the soft resins and leave untouched the fatty material which was a hazard to accurate work. A soxhlet, however, cannot be used with methyl alcohol because the boiling point of the solvent is sufficiently high to cause serious changes in the composition of the soft resins. In the cold it was found that with mechanical agitation, a sample of ten grams of hops could be completely extracted with 100 cc. methyl alcohol in 3 minutes or if shaken by hand a period of 10 minutes should be allowed. The α- soft resin or α- acid is precipitated directly from the filtered methyl alcohol extract. There were no other changes in the procedure recommended but it is apparent that a great saving of time and an increase in accuracy is brought about by this modification. Particular emphasis is placed on the importance of the temperature at which precipitation with lead ace-

tate is carried out. 60° C. is the recommended temperature with 3 or 4 minutes digestion after precipitation is complete to insure a granular precipitate that will filter easily. Another note of warning is sounded when it is advised that the samples of hops be minced only so fast as they can be analyzed. If the hops are allowed to stand for several days in the minced condition, they may become troublesome through emulsification at the time of extraction with light petroleum. It is suggested that this condition may be brought about by the liberation of lipases, when the cells are bruised during mincing, which could then result in the saponification and oxidation of the esters of the fatty acids with the production of emulsifying agents.

In answer to an inquiry in the fall of 1931, Dr. Walker explained that a still further modification of the Ford and Tait gravimetric process had been made. Instead of precipitating the α - acid directly from the methyl alcohol extract, a portion is first diluted with 1% aqueous sodium chloride and extracted with light petroleum. After filtering and making to volume half of this petroleum extract is evaporated, the last few cc. under diminished pressure, the residue taken up with methyl alcohol and the α - acid precipitated in the usual manner. The remainder of the extract is dried and weighed. It was

found that certain hops contained an acidic substance which is not true α - resin, but which is soluble in methyl alcohol, and which is precipitated as a lead compound by lead acetate. This substance or mixture of substances is insoluble in light petroleum, hence the extraction with light petroleum before treatment with lead acetate. This modification has since been published in connection with a review of ten years' research work on the antiseptic constituents of hops (59).

The details of the gravimetric method as submitted by Walker follows: Mince 40 grams of carefully selected hops and mix well together. Stir 10 grams of the minced hops with 100 cc. of methyl alcohol for 10 minutes. Filter through paper. Transfer 40 cc. of the filtrate to a separating funnel, add 80 cc. of 1% aqueous common salt solution and extract four times with light petroleum, using 45 cc. of light petroleum for each separate extraction. Combine the petroleum extracts, filter into a 200 cc. graduated flask, wash the filter paper with a little light petroleum and make up the solution to the 200 cc. mark. Pipette 100 cc. of the light petroleum extract into a 200 cc. flask and evaporate, removing the last few ccs. under diminished pressure. The resin left behind is dissolved by gently warming with successive small portions of pure methyl alcohol using a total of about 30 cc. This is

placed in a beaker and the α - resin precipitated at 60° C. by the addition of a 1% solution of lead acetate in methyl alcohol. The proper amount of lead acetate solution to use is determined by bringing a drop of the clear supernatant liquid in contact with a drop of ammonium or sodium sulfide. The immediate appearance of a brown ring is evidence of excess. Because the α - resin lead acetate precipitate is soluble in an excess of precipitating agent not more than 2 cc. excess is allowable. At the completion of precipitation, the mixture is allowed to digest for 3 or 4 minutes before setting aside for half an hour to settle and cool. When cool, filter on a gooch, dry for half an hour at 100° C., cool and weigh. The total soft resin is determined by evaporating and drying the remaining 100 cc. of light petroleum extract. The β - resin is obtained by difference. α - resin lead salt x .631 = α - resin. The lead acetate used is the .3 H_2O hydrate. The methyl alcohol must be pure. The light petroleum should distill between 35° C. - 40° C. However, light petroleum boiling between 50° C. - 55° C. gives good results.

THE PRESENT INVESTIGATIONS ON THE GRAVIMETRIC METHOD

When the work involved in this paper was started, the recent modification as suggested by Walker (59) had not been made public and since there was no intention of devising a scheme of analysis but to attempt to put into practice one that had already been developed and recommended, it was assumed that the 1929 method, Hastings and Walker (41), was entirely adequate. Some difficulty was experienced with the precipitation and filtration of the ∝- resin lead salt. The precipitate came down slowly and in very finely divided condition. Uncertainty was experienced as to the proper amount of lead acetate solution to add. This was due no doubt to the finely divided condition of the precipitate which retarded or prevented settling so that small particles were carried out onto the test plate with the drop of supernatant liquid, the particles of the precipitate producing the brown color with the sulfide indicative of excess of lead acetate. When the mixture had cooled, it was found that the precipitate had the tendency to adhere very tenaciously to the sides of the beaker. So hard did it adhere that rubber policing made no impression and it was necessary to resort to the use of a small knife to scrape the precipitate loose. And in the filtering process the finely divided precipi-

tate had a tendency to pass through the asbestos felt or, failing that, filled the pores of the felt making it practically impervious, thus consuming large amounts of time in the filtering process.

All of these difficulties were overcome with the exception of precipitation, with the advent of the recent modification whereby the resins are passed through light petroleum before treatment with lead acetate. In an effort to determine the proper amount of alcoholic lead acetate for precipitating the α- resin in an average hop tests were made using varying amounts of the lead acetate solution. The amounts varied by 5 cc. from 5 cc. to 20 cc. The results of the tests indicated there was an increase in α- resin lead salt formation up to the addition of 15 cc. lead acetate solution, but remained constant thereafter. In other words, the amount of precipitate formed by 20 cc. of the lead acetate solution was comparable with that formed by 15 cc. while less than 15 cc. produced a diminished quantity of the precipitate. Therefore, in all of my analyses, 15 cc. of lead acetate solution were used to precipitate the α- resin except in cases that were obviously of very poor quality. In this procedure, the lead acetate solution is added at once instead of dropwise and a flocculent precipitate was produced which settled easily, did not adhere to the sides of the beaker, and filtered

easily. The temperature was raised to 60° C. before addition of the lead acetate solution and maintained for several minutes thereafter to assist in the coagulation.

As stated by Walker (59) it was observed that the results for α - resin using the 1929 method were somewhat higher than the results obtained with the recent (1932) modification. Furthermore, the α - resin lead salt precipitate from the 1929 method changed color from a dirty greenish-yellow to a brown upon heating in the oven while the bright greenish-yellow color of the precipitate from the recent modification remained unchanged even after over-night drying at 100° C. The color change may be accounted for by the presence of certain non-resinous materials which are present in the methyl alcohol extract.

The full details of the procedure used in making the analyses are as follows:

About 30 grams of hops are put through a coarse food chopper. The first 5 grams to pass through are discarded since a certain amount of the resins will adhere to the chopper and introduce an error in the determination. After 5 grams have passed through, it is assured that the amount adhering to the chopper will remain constant, and thus cause no further error. The sample is thoroughly mixed and 10 grams of the minced hops are shak-

en with 100 cc. methyl alcohol for 10 minutes. The extracted hops are filtered through paper with suction and washed by breaking the suction, saturating the packed mass with methyl alcohol and then sucking dry again. This process is repeated 3 times by which time the alcohol comes through nearly colorless. The filtrate is transferred to a 200 cc. volumetric flask and made to the mark.

50 cc. of the methyl alcohol extract are transferred to a separatory funnel and 100 cc. distilled water containing about 1% sodium chloride are added at once. The mixture is extracted 4 times with petroleum ether, using about 45 cc. for each extraction. The combined petroleum extracts are poured through filter paper into a 200 cc. volumetric flask, the paper washed with more petroleum ether and the flask filled to the mark. 100cc. are withdrawn and placed in a 400 cc. flask from which all but 10 cc. of the ether are distilled. The remaining 10 cc. are removed in a vacuum oven regulated to 40° C. The time was watched to prevent unnecessary heating, 10 - 15 minutes usually sufficing. The residue is dissolved with a small amount of methyl alcohol and washed into a 50 cc. beaker until 25-30 cc. are obtained. The beakers are set in a water bath maintained at 60° C. When the temperature of the contents has reached that of the water 12-

15 cc. of a 1% lead acetate solution in methyl alcohol are added at once. The precipitate is digested for 5 minutes to assist in coagulation and settling. After cooling for half an hour the precipitate is collected on a gooch washed with methyl alcohol, and dried over night at 80° C.

The remaining 100 cc. of petroleum ether extract are placed in a 400 cc. fat extraction flask; the petroleum is distilled off and the residue dried, first in the vacuum oven and finally for half an hour at 80°, and weighed as total soft resin. The β- resin is obtained by difference.

α- resin lead salt x .631 = α- resin

Total soft resin - α- resin = β - resin

Methyl alcohol = Baker's Absolute - acetone free

Petroleum ether = Baker's C. P. Boiling range 30° C. - 60° C.

When the methyl alcohol extract is obtained, the remainder of the determination should be completed as soon as possible to forestall excessive deterioration of the resin while in solution. At the beginning of this work a number of trials were made to become familiar with the methods at hand. During the course of those trials it was observed that not the slightest agreement could be obtained between aliquots drawn from a methyl alcohol extract if they were analyzed a day or two apart. The older the alcohol extract the less was its antiseptic value as evid-

enced by the diminished quantity of the α - resin lead salt. No difficulty was experienced in obtaining comparable results on fresh methyl alcohol extractions.

Another point of interest, not noted in the literature, concerns the solubility of the α - resin lead salt after it has been once dried. It was thought that as a convenience and time saving feature that successive filtrations could be made on the same gooch without alteration of the asbestos pad or the previous precipitates. Filtration proceeded in a satisfactory manner, but agreement between duplicates was not satisfactory and each succeeding collection of precipitate was reduced. The preparation of fresh asbestos pads for each determination eliminated the above mentioned difficulties. I am not prepared to state definitely to what the discrepancies are due. However, a slight cloudiness in the filtrate would seem to indicate that fine particles of the precipitate or possibly of asbestos had been carried through, since the cloudiness did not show up until the second and succeeding filtrations. If drying breaks up the original particles so fine that portions may be carried through the filter, then it seems possible that an increase in solubility is brought about. Freshly prepared gooches are necessary for accurate results.

DISCUSSION OF THE BIOLOGICAL METHODS FOR EVALUATING HOPS

The biological methods, while they are too cumbersome for routine analyses are nevertheless indispensable, particularly for checking on the accuracy of the chemical methods. They are based upon the ability of hops or decoctions of hops to inhibit the growth or development of certain bacteria.

Among the first to do work along this line were Brown and Clubb (2) who isolated an organism from beer wort which they called Bacterium X. Their experiments were rather extensive and they came to the conclusions that to be strictly comparative the tests should be made at, or about, the same time, using the same culture medium, and so far as possible, cultures of Bacterium X of same age and vigor.

Some time later in 1925 Chapman (8), in an attempt to overcome some of the difficulties of previous workers, devised a method in which an organism isolated from raw sugar was used. Inhibition of growth was taken as the measure of the antiseptic power.

Ford and Tait (19) also used Bacterium X to determine the relative antiseptic values of α and β - resins. They had the same difficulties experienced by Brown and Clubb.

When Hastings, Pyman, and Walker (23) undertook the study of this method of analysis, they made a thorough investigation of several methods and compared them one with the other. Bacterium X was given a thorough trial and finally discarded for the same reasons as given above, plus the fact that it produced only small amounts of lactic acid and evidence of growth was difficult to distinguish. In its place Bacterium delbruchii was suggested by the fact that it produced large quantities of lactic acid in normal growth and was particularly sensitive to the inhibiting powers of hop infusions. In using B. delbruchii, the suppression of growth as measured by the formation of lactic acid is taken as the criterion. The acid is titrated with N/40 alkali.

For comparative purposes the authors conducted experiments using Ford and Tait's gravimetric method, Chapman's plate method and the above mentioned B. delbruchii method. Good agreement was obtained from all the methods and it was concluded that satisfactory results could be had with any or all of the methods tried. In discussing the two biological methods the authors seem to favor Chapman's plate method since the bacterium used is more easily maintained in a viril condition than B. delbruchii, and it appears to be more sensitive to the antiseptic action of hops. In working out a biological method, how-

ever, it seems desirable to have the conditions as nearly like those encountered in actual brewing practice as possible. From that standpoint, the B. delbruchii method most nearly fulfills the requirements. The organism was obtained from wort, the medium is liquid and the formation of lactic acid is suppressed. Studies have shown that growth and acid formation do not go hand in hand since there is a certain lag period in the growth curve before any appreciable amount of acid is formed. Very little acid is formed during the first 18 hours. The rate of formation increases up to 24 hours where it remains constant until 40 hours from time of inoculation after which the rate decreases until the amount of acid becomes nearly constant. Thus a titration which indicates there has been no acid formation does not necessarily mean there has been no growth.

At the time the paper of Hastings, Pyman, and Walker (23) was written, the gravimetric method required a longer period of time to complete than either of the biological methods. It was, however, considered the most susceptible of standardization and for that reason is recommended as the most preferred of the three in spite of the time factor. Subsequent improvements in the gravimetric method have cut the time from 55 hours to 8 hours or less, and increased the accuracy so that it is even

more to be preferred at the present time.

The biological method has its place in hop investigations, however, as a check on the gravimetric method and as a scheme for testing the actual preservative properties of hops.

Chapman (45) in an attempt to standardize his plate method tested his Bacterium "C" against pure phenol. He found that the organism could be cultivated over quite long periods of time at a fairly constant level of virility.

Late in 1931 Walker et al (56) published the details of a method making use of B. bulgaricus, for which is claimed a much higher degree of sensitiveness than the B. delbruchii method and a considerable economy of time. While the B. delbruchii method was considered suitable for routine determinations for the antiseptic potency, when it came to the changes undergone during brewing it was found inadequate. For this purpose a method using B. bulgaricus was worked out. This method is termed the Log-Phase method, since it depends on the fact that when, under specified conditions, the antiseptic is added to the cultures at an early stage in the logarithm phase of growth, the relationship between dose and effect is shown by the expression: Percentage restriction of acid formation $\div$ percentage concentration of antiseptic substance = K (a

constant value).

In studies on the growth of bacteria it has been found that a succession of phases are passed through. There is an initial lag period during which the number of cells increase very slowly, a period of maximal growth, a stationary or resting period, and a period during which the number of living organisms progressively diminishes until all have died. With acid-forming bacteria the production of acid parallels the growth curve to the beginning of the third or resting period. From that point the production of acid continues at a much slower rate until a maximum is reached, whence it becomes stationary. The fact that the maximum acid content of the medium is not reached until some little time after the maximum bacterial population has been attained has been explained as due possibly to bacterial enzymes which continue to function even when the cells are dying or in the process of autolysis. B. bulgaricus was found to develop according to the above outline and to produce large quantities of lactic acid when grown on sterile malt-wort. When a quantity of humulon is added to some malt-wort which is subsequently inoculated with B. bulgaricus, the quantity of acid which the latter produced in a given interval of time was regulated to an extent depending on the quantity of the antiseptic present. But it was found that different amounts

of acid might be produced when using the same quantity of antiseptic if the antiseptic was added at different stages in the development of the organism. The principle of the method is best summed up in the words of the authors thus: "Addition of an appropriate quantity of humulon to a maltose solution undergoing fermentation by lactic acid bacteria of the type in question, when made at a definite stage in the logarithmic phase, results in a reduction of the rate of acid formation and, within certain ascertained limits, the percentage of such restriction is in a fixed ratio to the quantity of humulon added."

The results of experimental work in connection with the development of this method follow:

1. B. bulgaricus develops freely on a malt-wort of sp. gr. 1.050 and, for the purposes of this work, pH of 5.0 and temperature of 30° C.

2. Using wort of sp. gr. 1.050 and pH 5.0 and incubating at 30° C., the logarithmic period of cell-multiplication terminated when there were present approximately 4×10^{8} organisms per cc., no matter to what extent the number introduced as inoculum was varied within the limits 4×10^{3} to 4×10^{6} organisms per cc.

3. The amount of acid produced was determined by titrating with N/10 sodium hydroxide, using the quin-

hydrone electrode and titrating to the pH possessed by the medium at the beginning of the experiment. The end of the logarithmic portion of the acidity-time curve is reached at the point at which 100 cc. of the culture required about 30 cc. N/10 sodium hydroxide to bring back its pH value to that of the original wort.

4. One part humulon in 500,000 causes approximately 50 percent restriction in the acid formation. The greatest restriction is obtained where the antiseptic is added at the beginning of the logarithmic phase of development or when the culture is about six hours old.

5. Acid production should be allowed to continue for as long a period as possible before estimation of acidity, but not beyond the end of the logarithmic period.

6. A quantity of antiseptic should be used so that the resulting restriction in acid formation lies between 20% and 50%.

7. Addition of a given dose of humulon to the culture medium early in the lag phase retards multiplication of the organisms to a relatively greater extent than it restricts acid formation.

The method as finally developed is quite empirical in that the details as laid down by the authors must be strictly adhered to if accurate results are to be obtained.

An outline of the method follows::

In all cases the medium used is a sterile malt-wort of sp. gr. 1.050 and pH 5.0. Incubation is at 30° C.

10 cc. of medium is inoculated with 2 loops of a 24-hours old culture of B. bulgaricus grown on the same medium and incubated for exactly eighteen hours. 1 cc. of this culture is diluted to 100 cc. with 99 cc. of the sterile medium. 200 cc. of the wort are placed in a 250 cc. flask and after warming to 30° C. is inoculated with 1 cc. of the 1-100 culture and then placed in the incubator for 6 hours. At the end of this time the antiseptic is added and incubation continued for an additional 17 hours. This latter time may be varied from 16 - 19 hours. At the end of the second incubation period the flasks are removed and an appropriate amount of quin-hydrone added immediately after which the titration may be leisurely accomplished. Care is exercised throughout that the temperature does not go below 30° C. during the entire incubation period. The addition of quin-hydrone puts an end to all fermentation. The results are calculated on the basis of the percentage restriction of possible acid production as determined in the absence of antiseptic. The sample causing the greatest restriction being assigned a value of 100 the others are compared proportionately to it.

THE PRESENT INVESTIGATIONS ON THE BIOLOGICAL METHODS

The amount of time available for conducting tests on the biological method was quite limited and for that reason no fair test could be made of that scheme for determining the antiseptic value of hops. At the time this project was started, the newer method making use of B. bulgaricus had not been published and it was not until some months later that it was called to my attention. Consequently what little work was done involved the B. delbruchii method only.

Through cooperation of the Department of Bacteriology at Oregon State College, space and equipment were provided for conducting the experimental work. A culture of B. delbruchii was obtained from "American Type Cultures" in Chicago. A standard wort broth was prepared, using "Difco" malt extract. The pH when first made was 6.1 but after a half dozen or so sterilizations the pH was reduced to 5.2. The specific gravity was not determined. At the commencement the organisms seemed to grow quite prolifically at 42° C., but on repeated transfers an apparent loss in virility was noted. The reason for this is not known.

Several determinations were made using a .25% decoction of the hops; 20 cc. of broth were used in each determination. After measured amounts of decoction are add-

ed to each tube, enough water to make a total of 30 cc. is added. The tubes are sterilized and one cc. of a 1-25 culture of B. delbruchii is added. The 1-25 dilution is made from a 24-hour old culture that has been sub-cultured for three or four successive days. After 40 hours incubation at 42° C. the tubes are removed and allowed to cool. Each determination is diluted to about 100 cc. with distilled water and the lactic acid present is titrated with N/40 alkali, using phenolphthalein as the indicator. A tube containing only broth and one containing broth and organisms only, were carried along with each series of determinations.

In titrating, much uncertainty was experienced in determining the end points, due to the dense color of the broth. The control without organisms was titrated first to ascertain the amount of acidity due to the broth alone and an attempt was made to match the end point color produced in the control tube. From the erratic results obtained it was obvious that either the growth or acid production of B. delbruchii was very erratic or very little success was experienced in matching end point colors. There is little doubt in the author's mind as to both of these conditions contributing to the difficulty in matching colors.

It may be that a fresh malt extract is sufficiently

light in color so as not to interfere with indicators, but whether it is or not, the suggestion in the lag-phase method of using the quin-hydrone electrode seems a good one.

As to the virility of the organism it seemed as time went on that it became progressively less virile with each succeeding transfer.

RESULTS OBTAINED ON THE 1931 CROP BY USE OF THE GRAVIMETRIC CHEMICAL METHOD OF EVAULATION

The results of the analytical work involved in this paper are recorded in Tables I to VI. They represent twenty-five seedlings and selections, three fertilizer trials of seventeen plots each and two samples from a dry ing experiment, all from the 1931 crop.

The recorded values for $\alpha - \beta/3$ were obtained by striking an average between the close results of two different methyl alcohol extractions. An exception to this procedure was necessarily practiced when the samples from the fertilizer trials conducted at the Ross Wood yard proved to be inadequate for more than single determinations.

The analytical work was accomplished in the chemistry laboratory of the Oregon Experiment Station which also supplied the necessary laboratory apparatus. All chemicals were furnished by the United States Dept. of Agri.

TABLE NO. I

Effect of Fertilizers on Antiseptic Value of Early Cluster Hops Grown at Ross Wood Yard, Dayton, Oregon, 1931

Plot No.	Treatment	Moisture	α- resin	β- resin	Tot. soft resins	$\alpha+\beta/3$
1	No treatment					
2	120# muriate of potash	8.18	6.33	8.57	14.90	10.19
3	66# treble phosphate 60# muriate of potash	6.80	6.63	8.47	15.10	9.45
4	133# treble phosphate	8.10	3.59	8.00	11.59	6.26
5	133# sodium nitrate 66# treble phosphate	7.85	4.92	7.70	12.62	7.49
6	No treatment	8.05	5.21	7.99	13.20	7.87
7	266# sodium nitrate	7.28	7.12	8.26	15.38	9.87
8	133# sodium nitrate 60# muriate of potash	7.08	6.47	8.71	15.18	9.37
9	266# sodium nitrate 120# muriate of potash 133# treble phosphate	7.30	6.73	6.65	13.38	8.95
10	100# sulfur	6.76	6.59	7.95	14.54	9.57
11	No treatment	7.10	7.37	6.58	13.95	9.56
12	120# muriate of potash 100# sulfur	8.20	5.73	8.19	13.92	8.46
13	133# treble phosphate 100# sulfur	8.90	6.80	8.57	15.37	9.66
14	266# sodium nitrate 100# sulfur	7.60	7.56	7.15	14.71	9.94
15	266# sodium nitrate 120# muriate of potash 133# treble phosphate 100# sulfur	9.50	7.16	7.68	14.84	9.72
16	No treatment	8.45	6.92	7.83	14.75	9.53
17	200# land plaster	9.95	5.80	7.58	13.38	8.33

Discussion of Results in Table I

The hops obtained from the fertilizer trial at the Ross Wood hop yard at Dayton, Oregon, were of the Early Cluster variety. This high producing yard is situated on good river bottom soil and the plots averaged over one ton of dry hops per acre. The differences in growth and yield of the variously treated plots were not great. However, some plots produced a little higher yield. None of the differences in yields, however, was significant as they were not equal to three times the probable error of a single determination.

The lack of significant results in the fertilizer trials as far as yields are concerned may be due to the excellent yield of these hops. Another factor may have been the dry weather conditions which followed the fertilizer applications. Of interest, however, is the difference in the antiseptic values of the hops picked from the various plots. In general, the values of the hops from this yard were not as high as those from the other two yards in trial. More than likely this is due to the variety which was Early Cluster as compared to Late Cluster in the other two yards. The average $\alpha + \beta/3$ value was 9.01. Both check plots averaged less than 8, indicating that the fertilizers may have had some value. As previously pointed out, however, because of the methods of

storage, differences too fine cannot be drawn between these results. The highest antiseptic value was obtained from the plot receiving 120 pounds of muriate of potash. However, two other plots which got this amount of potash plus other fertilizers did not run so high. The lowest antiseptic value was obtained from the hops grown on the plot treated with 133 pounds of treble phosphate. This plot was noticeably low in the α - resin.

In a comparison between yield and antiseptic value there appears to be no correlation; that is, some plots which yielded heavy had a low antiseptic value while others which yielded light had a low antiseptic value, and the same condition was true in regard to the heavier yielding plots.

TABLE NO. II

Effect of Fertilizers on Antiseptic Value of Late Cluster Hops Grown at Dubois Yard, Woodburn, Oregon, 1931

Plot No.	Treatment	Moisture	α resin	β resin	Tot. soft resins	$\alpha+\beta/3$
1	No treatment	8.26	8.61	8.84	17.45	11.56
2	120# muriate of potash	8.10	6.42	10.65	17.07	9.97
3	66# treble phosphate 60# muriate of potash	7.38	8.05	4.55	12.60	9.57
4	133# treble phosphate	7.56	7.74	4.53	12.27	9.25
5	133# sodium nitrate 66# treble phosphate	7.08	8.34	7.76	16.10	10.93
6	No treatment	6.82	7.08	8.44	15.52	9.89
7	266# sodium nitrate	8.28	6.53	10.22	16.75	9.94
8	133# sodium nitrate 60# muriate of potash	8.00	8.04	7.66	15.70	10.59
9	266# sodium nitrate 120# muriate of potash 133# treble phosphate	7.98	7.12	9.09	16.21	10.16
10	100# sulfur	7.16	7.20	8.60	15.80	10.07
11	No treatment	7.30	8.32	8.66	16.98	11.21
12	120# muriate of potash 100# sulfur	7.60	7.51	8.99	16.50	10.51
13	133# treble phosphate 100# sulfur	8.00	7.43	8.36	15.80	10.22
14	266# sodium nitrate 100# sulfur	8.14	6.20	9.60	15.80	9.40
15	266# sodium nitrate 120# muriate of potash 133# treble phosphate 100# sulfur	7.92	7.65	6.83	14.48	9.93
16	No treatment	7.88	7.53	5.36	12.89	9.32
17	200# land plaster	7.54	7.19	7.40	14.59	9.66

Discussion of Results in Table II

The hops which were analyzed for antiseptic value from the Frank Dubois hop yard at Woodburn, Oregon, were of the Late Cluster variety. The hops in this yard, which were grown on a heavy upland soil, did not yield so heavily as those in the Ross Wood yard. Yields, however, were quite good and averaged just about one ton of dry hops per acre. The differences in yield, as in the former case, were not very significant and this also may have been due to the good fertility of the soil or the dry weather conditions after the applications of fertilizer were made.

The average antiseptic value was 10.13, which indicates a high $\alpha + \beta/3$ value. The value of these hops varied all the way from 11.56 down to 9.25. It is of interest to note that in both the hops grown at the Ross Wood yard and the Dubois yard the plot which showed the lowest antiseptic value of the hops was the one treated with treble phosphate.

It is of striking interest to note that the hops with the highest antiseptic value were grown on a plot which received no treatment. These hops showed a value of 11.56. The second highest plot was also a no treatment plot and this showed 11.21.

TABLE NO. III

Effect of Fertilizers on Antiseptic Value of Late Cluster Hops Grown on Ireland Yard, Corvallis, Oregon, 1931

Plot No.	Treatment	Moisture	α-resin	β-resin	Tot. soft resins	α+β/3
1	No treatment	7.30	6.17	7.52	13.69	8.68
2	120# muriate of potash	9.40	6.95	9.06	16.01	9.97
3	66# treble phosphate 60# muriate of potash	9.86	6.44	8.66	15.10	9.33
4	Treble phosphate	8.75	6.58	8.83	15.41	9.56
5	133# sodium nitrate 66# treble phosphate	7.25	5.73	8.07	13.80	8.42
6	No treatment	6.45	6.77	6.42	13.19	8.91
7	266# sodium nitrate	7.80	7.21	7.99	15.20	9.87
8	133# sodium nitrate 60# muriate of potash	8.40	7.88	7.73	15.61	10.46
9	266# sodium nitrate 120# muriate of potash 133# treble phosphate	7.55	6.83	8.06	14.89	9.52
10	100# sulfur	8.30	7.79	8.17	15.96	10.51
11	No treatment	7.15	8.07	7.83	15.90	10.68
12	120# muriate of potash 100# sulfur	8.10	9.03	9.68	18.71	12.29
13	133# treble phosphate 100# sulfur	7.10	7.92	7.83	15.75	10.53
14	266# sodium nitrate 100# sulfur	6.75	7.88	7.42	15.30	10.35
15	266# sodium nitrate 120# muriate of potash 133# treble phosphate 100# sulfur	7.00	9.26	8.22	17.48	12.00
16	No treatment	7.25	8.88	8.24	17.12	11.63
17	200# land plaster	9.40	8.24	8.97	17.21	11.23

Discussion of Results in Table III

The hops grown on the Ireland yard at Corvallis, Oregon, were of the Late Cluster variety. These hops were grown on good river bottom soil and yielded approximately one ton of dry hops per acre. The average antiseptic value of these hops was 10.23, indicating a good antiseptic value of the hops grown under these conditions. The plot treated with potash and sulfur gave the highest antiseptic value, and the highest value of any of the lots conducted. The value of this plot was 12.29. The lowest value in this trial was 8.42 per cent, and the hops were from the plot treated with sodium nitrate and treble phosphate. There were many high antiseptic value lots from this yard, and the results are not very significant in regard to the value of any particular fertilizer on the antiseptic value of the resulting crop.

TABLE NO. IV

Effect of Method of Drying on Antiseptic Value of Late Cluster Hops from F. Needham Dryers

Plot No.	Treatment	Moisture	α- resin	β- resin	Tot. soft resins	$\alpha+\beta/3$
Forced		7.48	6.57	5.04	11.61	8.25
Natural		7.28	6.08	3.57	9.65	7.27

Discussion of Results in Table IV

Late Cluster hops were obtained from the F. Needham yard at Salem, Oregon for this trial. Representative lots which were dried in the ordinary natural draft drier and those dried in the new type of drier which Mr. Needham has developed were compared. The latter drier makes use of forced draft and the time of drying is reduced materially. The results obtained are of interest for they show nearly one per cent difference in the antiseptic value of these two lots of hops. The moisture content of both these lots was practically the same. The forced draft drier gave a little higher Alpha resin content, but the Beta resin was quite a bit higher in the forced draft drier, giving an advantage of about one per cent. Of course, these treatments are limited and should be repeated. At any rate, it shows that the forced draft does not injure the hops in any way as far as antiseptic value is concerned and bears out the opinion of Mr. Needham who states that the hops dried with this new type of drier are superior.

TABLE NO. V

Yields and Antiseptic Values of Individual Hop Plants

Grown in Experimental Yard, Corvallis, Oregon, 1931

No.	Variety	Green Wt. Lbs.	Dry Wt. Lbs.	Moisture	α-resin	β-resin	Total Soft resins	$\alpha+\beta/3$
6-3	L.C.	6.9	1.6	8.03	6.77	10.06	16.83	10.12
6-4	L.C.	5.0	1.3	7.42	6.06	8.89	14.95	10.02
6-8	L.C.	4.8	1.2	7.56	7.45	10.55	18.00	10.97
6-9	L.C.	4.5	1.0	7.10	7.22	8.98	16.20	9.88
7-9	L.C.	6.2	1.3	7.38	5.07	5.82	10.89	7.01
8-4	L.C.	3.7	.9	7.04	6.90	10.31	17.21	10.34
8-9	E.C.	1.5	.4	7.08	6.59	7.14	13.73	8.97
8-10	E.C.	3.8	.9	7.76	7.00	6.90	13.90	9.30
8-11	L.C.	3.8	.9	7.72	6.60	6.78	13.38	8.86
8-12	E.C.	1.1	.25	7.40	7.63	7.67	15.30	10.19
11-3	F.	.3	.05	8.80	2.77	5.48	8.25	4.60
11-6	F.	.2	.05	8.17	3.30	4.15	7.45	4.68
11-8	F.	.6	.1	8.06	4.23	5.60	9.83	6.10
12-2	F.	.5	.1	8.86	2.62	5.63	8.25	4.50
12-4	F.	.3	.05	7.12	1.65	6.79	8.44	3.91
14-4	R.V.	3.4	.8	9.30	2.86	9.58	12.52	6.01
14-6	R.V.	.9	.25	7.66	5.27	6.62	11.89	7.48
15-3	R.V.	.8	.2	7.48	3.42	6.87	10.29	5.71
15-6	R.V.	1.9	.5	7.80	2.75	9.05	11.80	5.77
15-7	R.V.	.9	.2	10.38	4.25	7.56	11.81	6.77
16-2	Bav.	1.4	.6	9.32	7.22	7.56	14.78	9.74
16-6	Bav.	3.6	.9	9.10	8.10	6.20	14.30	10.17
17-7	Bav.	5.2	1.3	7.00	8.06	4.92	12.98	9.70
17-8	Bav.	5.2	1.2	8.54	8.62	6.86	15.48	10.91
17-9	Bav.	2.6	.7	6.60	7.48	8.02	15.50	10.15

Discussion of Results in Table V

Results reported herein are of interest because they indicate great differences in the antiseptic values of various hops. Similar to the results reported in the previous tables the Late Cluster varieties show a good antiseptic value. In contrast with the Late Clusters and also the Early Clusters, are the values of the Fuggles variety. This variety is extremely low, showing all the way from one-third to one-half the antiseptic values of the Cluster type of hops. The Red Vines also were rather low in antiseptic value but somewhat superior to the Fuggles variety. The Bavarian hop, which is very similar and may be the same as the Late Cluster, was high in antiseptic value. This table strikingly shows the low value of both the Fuggles and Red Vine varieties of hops from the standpoint of antiseptic value and the superiority of Late Clusters, Early Clusters, and Bavarians.

A seedling of the Late Cluster variety No. 6-8 had the highest antiseptic value, 10.97. It is of interest to note that this hop also was a good producer, giving 4.8 pounds of green hops and 1.2 pounds of dried hops for the plant. As far as yield is concerned, the hops giving the highest antiseptic values were also the highest yielding ones, as the Red Vines and Fuggles both produced less than a pound of dried hops per plant while the other varieties

produced one or more pounds per plant.

This is the type of work which must be followed to give the full information on both seedlings and selection of hop varieties. It willbe of interest to see whether or not the hybrids between Fuggles and the Cluster types of hops will give a high antiseptic and yielding value along with the resistance of the former variety. At any rate, this preliminary work indicates the wonderful opportunity for getting hops which are superior not only in yields but also in antiseptic values.

TABLE NO. VI

Summary of Antiseptic Values of Hops

Treated with Various Fertilizers. Oregon, 1931

Plot No.	Treatment	$\alpha+\beta/3$ Early Cluster Ross Wood's Yard	$\alpha+\beta/3$ Late Cluster Dubois' Yard	$\alpha+\beta/3$ Late Cluster Ireland's Yard	Average $\alpha+\beta/3$
1	No treatment		11.56	8.68	10.12
2	120# muriate of potash	10.19	9.97	9.97	10.04
3	66# treble phosphate 60# muriate of potash	9.45	9.57	9.33	9.45
4	133# treble phosphate	6.26	9.25	9.56	8.32
5	133# sodium nitrate 66# treble phosphate	7.49	10.93	8.42	8.95
6	No treatment	7.87	9.89	8.91	8.89
7	266# sodium nitrate	9.87	9.94	9.87	9.89
8	133# sodium nitrate 60# muriate of potash	9.37	10.59	10.46	10.14
9	266# sodium nitrate 120# muriate of potash 133# treble phosphate	8.95	10.16	9.52	9.54
10	100# sulfur	9.57	10.07	10.51	10.05
11	No treatment	9.56	11.21	10.68	10.48
12	120# muriate of potash 100# sulfur	8.46	10.51	12.29	10.42
13	133# treble phosphate 100# sulfur	9.66	10.22	10.53	10.04
14	266# sodium nitrate 100# sulfur	9.94	9.40	10.35	9.90
15	266# sodium nitrate 120# muriate of potash 133# treble phosphate 100# sulfur	9.72	9.93	12.00	10.55
16	No treatment	8.33	9.32	11.63	9.76
17	200# landplaster	9.53	9.66	11.23	10.14
	Average	9.01	10.13	10.23	9.80

Discussion of Results in Table VI

This summary table shows that the Late Cluster hops which were grown on the Dubois and Ireland yards were superior in antiseptic value to the Early Clusters which were grown at the Ross Wood yard. It is of interest to note that the average of the Late Clusters is almost the same at the two yards. There was a little greater variation in the Ireland yard but both yards produced hops of very good antiseptic value on all plots. In general, these hops were about 25 per cent better than what is considered a good hop for brewing purposes. It is of interest to note that the highest average was obtained from the plot which received a complete fertilizer, which included nitrate, potash, phosphate, and sulphur, and that the plot which received the treble phosphate treatment was the lowest for the average of all three trials.

Although the trials were limited in scope, there is some indication that good fertility means not only good yields but high antiseptic values of hops. If these trials can be continued, there appears to be some indication of getting valuable results.

SUGGESTIONS

Unfortunately the collection of the hop samples in the fall of 1931 were made without proper knowledge of the problems of handling and storing hops to preserve their antiseptic properties.

All of the seedling and selection samples were placed (after drying) loosely in burlap sacks with the result that some of the lupulin was shaken from the cones. These samples for the most part were so large that it was impossible to mince the whole sample so that the loose lupulin could be evenly distributed.

The other samples were also contained loosely but their containers were either paper or small flour sacks. In cases where the sample was small enough the whole of it was minced and the loosened lupulin recovered and thoroughly mixed with the whole before portions were taken for analysis. In some cases the samples were too large to admit of that procedure and while the lupulin could not sift through the sack the correct proportion could not be removed with the portion that was minced. Since lupulin contains about 28% soft resins as against about 15% for whole hops its importance in connection with the determination of the antiseptic power is readily realized. Theoretically, any loss of lupulin gives a result proportionately lower than the true antiseptic

power. The results, therefore, are too low in antiseptic value.

It is suggested that if a miniature baler could be provided so that the sample could be pressed into a more or less substantial block most of the errors of sampling would be eliminated.

The samples from one series of the fertilizer plots, namely the Ross Wood yard, were so inadequate that only single determinations could be made. Thus, too much credence should not be placed in the results obtained. For future work it is recommended that at least half a pound of properly selected hops be submitted for the analytical sample.

SUMMARY AND CONCLUSIONS

1. The literature as recorded in the Journal of the Institute of Brewing 1925 to date, is reviewed with respect to the preservative principles of hops, their chemical constitution and behavior, and methods of determining same.

2. Particular attention was paid to analytical procedures with the view of selecting a suitable method to be applied to Willamette Valley hops.

3. A gravimetric chemical method proposed by Ford and Tait (4) was subsequently modified by Hastings and Walker (32, 40), and Walker (58), and was found to be entirely adequate for the problem at hand.

4. Biological methods were proposed by Brown and Clubb (1), Chapman (7), Hastings, Pyman, and Walker (22), and Walker, Hastings, and Farrar (55). However, time did not permit of more than a tentative examination by one of the schemes proposed.

5. There were 78 samples of hops analyzed, for the most part in duplicate, representing 25 hybrids and seedlings from plant breeding experiments, 51 samples from three fertilizer trials of seventeen plots each and two samples dried under different conditions.

6. It is recommended that for future work the samples be preserved in small bales of one-half to one

pound in weight, and suitably wrapped in paper. It is thought that this scheme will tend to eliminate errors due to losses of lupulin such as obtains when the cones are stored loosely.

LITERATURE CITED

1. Improving the Quality of Domestic Hops. W. W. Stockberger. Paper read at a meeting of the American Brewing Institute, 1907.

2. On the Antiseptic Properties of Hops. Brown and Clubb. J. Inst. Brew. 19, 373, 1923.

3. II Report on the Preservative Principles of Hops. T. K. Walker. J. Inst. Brew. 29, 373, 1923.

4. III Report on the Preservative Principles of Hops. T. K. Walker. J. Inst. Brew. 29, 379, 1923.

5. Note on the Valuation of Hops for Antiseptic Properties. Ford and Tait. J. Inst. Brew. 30, 426, 1924.

6. IV Report on the Preservative Principles of Hops. T. K. Walker. J. Inst. Brew. 30, 570, 1924.

7. V Report on the Preservative Principles of Hops. T. K. Walker. J. Inst. Brew. 30, 712, 1924.

8. On the Preservative Properties of Hops. A. Chaston Chapman. J. Inst. Brew. 31, 13, 1925.

9. The Antiseptic Properties of Hops. I. Stoleru. (Ann. Brass. et Dist. 1924, 23, 132 - 136, 151 - 158) through J. Inst. Brew. 31, 87, 1925.

10. A Rational Nomenclature for the Bitter Substances of Hops. P. Kolbach. (Wochenschr. Brau. 1925, 42, 21 - 24) through J. Inst. Brew. 31, 142, 1925.

11. Transformation of the α- Bitter Acid of Hops on Boiling in Aqueous Solutions. W. Windisch, P. Kolbach and H. Grohn (Wochenschr. Brau. 1925, 42, 281 - 302) through J. Inst. Brew. 31, 143, 1925.

12 The Valuation of Hops. W. Wollmer (Wochenschr. Brau. 1925, 42, 1 - 3) through J. Inst. Brew. 31, 144, 1925.

13. Antiseptic Action of Hops and of the Bitter Substances of Hops. P. Kolbach (Wochenschr. Brau., 1925, 42, 61 - 75) through J. Inst. Brew. 31, 268, 1925.

14. Chemical Nature of the Hop Resin Acids. H. Wieland (Ber. 1925, 58, 102, cited in Wochenschr. Brau., 1925, 42, 105 - 107) through J. Inst. Brew., 31, 369, 1925.

15. Bitter Substances of Hops. W. Wollmer (Ber. 1925, 58, 672; cited in Wochenschr. Brau. 1925, 42, 112 - 113) through J. Inst. Brew. 31, 369, 1925.

16. Problems of Hop Chemistry. P. Kolbach (Wochenschr. Brau., 1925, 42, 157 - 159, 163 - 167) through J. Inst. Brew., 31, 526, 1925.

17. VI Report on the Preservative Principles of Hops. T. K. Walker. J. Inst. Brew., 31, 562 - 576, 1925.

18. The Nomenclature of Hop Resin Constituents. T. K.

Walker. J. Inst. Brew. 31, 576, 1925.

19. Evaluation of Varieties of Hops by Chemical, Biological and Dry Hopping Tests. J. S. Ford and Adam Tait. J. Inst. Brew. 32, 19, 1926.

20. Chemical Nature of the Hop Resin Acids. H. Wieland (Ber. 1925, 58, 2012; cited in Woch. Brau., 1925, 42, 325 - 326) through J. Inst. Brew., 32, 82, 1926.

21. Valuation of Hops. W. Windisch, P. Kolbach, and W. Banholzer (Woch. Brau., 1926, 43, 79 - 80) thru J. Inst. Brew., 32, 189, 1926.

22. The Antiseptic Value of Hops. M. Van Laer (Petit J. du Brass., 1926, 34, 254 - 257) through J. Inst. Brew., 32, 229, 1926.

23. VII Report on the Preservative Principles of Hops. J. J. H. Hastings, F. L. Pyman and T. K. Walker. J. Inst. Brew., 32, 484, 1926.

24. Resinification of the α - Bitter Acid of Hops (Humulon) by Molecular Oxygen. W. Windisch, P. Kolbach, and J. Yole. Woch. Brau., 1926, 43, 349 - 383) through J. Inst. Brew.) 32, 521, 1926.

25. Examination of Some Very Old Cold-stored Hops. A. Chaston Chapman. J. Inst. Brew., 32, 574, 1926.

26. Chemical Nature of Hop Resin Acids. H. Wieland and

E. Martz (Ber. 1926, 59, 2352 cited in Woch. Brau., 1927, 44, 30) through J. Inst. Brew., 33, 172, 1927.

27. Recent Work on the Chemical Constitutions of Humulone and Lupulone. F. L. Pyman. J. Inst. Brew. 33, 295, 1927.

28. On the Preservative Values of Some Green and Some Kiln-dried Hops. A. Chaston Chapman and C. W. McHugo. J. Inst. Brew., 33, 294, 1927.

29. The Institute of Brewing Research Scheme--Hops. Summary of the Progress of the Work, 1920-27. J. Inst. Brew., 33, 372, 1927.

30. The Brewing Value of Hops Attacked by Pseudoperonospora. D. Wiegmann (Z. ges Brauw., 1927, p. 73, cited in Petit J. du Brass., 1927, 35, 743-44) through J. Inst. Brew., 33, 401, 1927.

31. Antiseptic Action of the Bitter Substance of Hops. W. Windisch, P. Kolbach and W. Schuren. (Woch. Brau., 1927, 44, 285 - 350) through J. Inst. Brew., 33, 469, 1927.

32. Transformation of the α - Bitter Acid of Hops in Boiling in Aqueous Solutions of Various Reactions and the Nature of the Products Formed. W. Windisch, P. Kolbach, and R. Schleicher (Woch. Brau., 1927, 44, 453 - 459, 473 - 478, 485 - 490, 497 -

502) through J. Inst. Brew., 33, 584, 1927.

33. VIII Report of the Preservative Principles of Hops. J. J. H. Hastings and T. K. Walker. J. Inst. Brew., 34, 9, 1928.

34. Notes on the Extraction of α - Resin in the Gravimetric Estimation of Hop Resins. A. H. Burgess and H. Martin. J. Inst. Brew., 34, 13, 1928.

35. The Quality of Hops. Stadnik (Kvas, July 1927, No. 48; Cited in Brass. Malt, 1927, 17, 251 - 254) through J. Inst. Brew., 34, 43, 1928.

36. The Determination of Antiseptic Power, with Special Reference to Hops. T. K. Walker. J. Inst. Brew., 34, 61, 1928.

37. The Scientific Valuation of Hops. A. Chaston Chapman. Communication presented to the 6th Congress of Industrial Chemistry, 26th Sept. - 22nd Oct., 1926, Paris, through J. Inst. Brew., 34, 82, 1928.

38. The Investigations on the Preservative Principles of Hops. F. L. Pyman. J. Inst. Brew., 34, 447, 1928.

39. The Chemical Method of Determination of the Preservative Value of Hops. M. Van Laer (Petit J. du Brass., 1928, 36, 790) through J. Inst. Brew., 34, 454, 1928.

40. IX Report on the Preservative Principles of Hops. J. J. H. Hastings and T. K. Walker. J. Inst. Brew., 34, 556, 1928.

41. X Report on the Preservative Principles of Hops. J. J. H. Hastings and T. K. Walker. J. Inst. Brew. 35, 229, 1929.

42. The Essential Oil of Hops. A. Chaston Chapman. J. Inst. Brew., 35, 247, 1929.

43. Drying Hops in a Vacuum. K. Behrendt (Woch. Brau., 1929, 46, 86 - 87) through J. Inst. Brew., 35, 264, 1929.

44. Analysis of Hops. W. Wollmer (Woch. Brau., 1929, 46, 121 - 124) through J. Inst. Brew., 35, 268, 1929.

45. Standardization of the Strength of the Organism (Bacterium C) used in the Chapman Biological Method for the Determination of the Preservative Power of Hops. A. Chaston Chapman. J. Inst. Brew., 35, 363, 1929.

46. Analysis of the Bitter Substances of Hops. W. Windisch, P. Kolbach, and M. Winter (Woch. Brau., 1929, 46, 101 - 106, 111 - 117, 124 - 131) through J. Inst. Brew., 35, 269, 1929.

47. Some Recent Ideas on the Evaluation of Hops. T. K.

Walker. J. Inst. Brew., 36, 440, 1930.

48. Some Recent Ideas on the Evaluation of Hops--Discussion. T. K. Walker. J. Inst. Brew., 36, 444, 1930.

49. A Colorimetric Method for the Estimation of the Soft Resins of Hops. J. M. Guthrie and G. G. Philip, J. Inst. Brew, 36, 455, 1930.

50. Bitter Resins of Hops and Their Quantitative Separation and Estimation. A. S. Basilewich (Nauch. Agron. Zhur., 1929, 6, 415, cited in Woch. Brau., 1930, 47, 532,) through J. Inst. Brew., 37, 36, 1931.

51. Analysis of Hops. W. Wollmer (Allgem. Brauer-und Hoppen-Z. 1930, 70, 1531 - 1533) through J. Inst. Brew., 37, 81, 1931.

52. (3) Experiments on Preservative Power. T. K. Walker and J. J. H. Hastings. J. Inst. Brew., 37, 206, 1931.

53. Notes on the Colorimetric Method for Determination of the Preservative Value of Hops. J. M. French. J. Inst. Brew., 37, 436, 1931.

54. Estimation of the Bitter Substances of Hops. H. Wildner (Woch. Brau., 1931, 48, 221 - 225, 231 - 235, 241 - 244, 260 - 263) through J. Inst. Brew., 37,

440, 1931.

55. XI Report on the Preservative Principles of Hops. T. K. Walker and J. J. H. Hastings. J. Inst. Brew., 37, 509, 1931.

56. XII Report on the Preservative Principles of Hops. T. K. Walker, J. J. H. Hastings and E. J. Farrar. J. Inst. Brew., 37, 512, 1931.

57. Analysis of Hops. L. Heintz (Woch. Brau., 1931, 48, 315 - 320, 325 - 330, 335 - 338) through J. Inst. Brew., 37, 543, 1931.

58. Volumetric Estimation of Soft Resin, Hard Resin, and Free Fatty Acid in Hops. L. Heintz (Woch. Brau., 1931, 48, 369 - 372) through J. Inst. Brew., 37, 575, 1931.

59. A Review of Ten Years' Research on the Antiseptic Constituents of Hops. T. K. Walker. J. Inst. Brew., 38, 198, 1932.