STUDIES ON WORT TURBIDITY, VISCOSITY AND DEGREE OF ATTENUATION IN BARLEY MALT

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STUDIES ON WORT TURBIDITY, VISCOSITY AND DEGREE OF ATTENUATION IN BARLEY MALT

INTRODUCTION

Malting barley is judged principally by its potentialities for making good beer. Barley is malted to increase the amounts of soluble substances in the kernels. These substances are extracted with water to form a wort from which the beer is made. The quality of beer is thus a function of the amounts and proportions of the potentially soluble substances in the barley.

To make malt, the grain is steeped in water and allowed to germinate, after which the sprouted barley is dried to arrest growth and to facilitate handling and storage. Germination permits the development of enzymes that cause changes in the constituents of the grain and that are also utilized in the brewing process. The first stages of the brewing process involve grinding the malt and mashing the grist with water under specified conditions of time and temperature. During this process, the enzymes in the malt render a high proportion of the kernel soluble. The liquid from the mash, called the wort, is then drained off and boiled with hops. The wort is cooled and yeast is added to start the fermentation process. The yeast converts the sugars into alcohol and when fermentation has reached the desired stage, the beer is filtered off and stored. Thus only that part of the malt that is extracted and drained off in the wort is used by the brewer and the amount and quality of this material determine

the quality of the beer, and thus the quality of the original malt and barley. The studies described in this thesis deal with several aspects of wort quality.

A primary function of the malting chemists in Canada is to provide assistance in the development of better barley. This involves evaluation of the qualities of the various strains that are produced by plant breeders. The laboratory malting system for testing malting quality of barley that has been developed in Canada was described by Anderson and Rowland in 1937 (6), and by Meredith in a thesis submitted to the University of Manitoba in 1938 (63). A review of the principles and applications of the system of testing was published in 1943 by Anderson, Meredith and Sallans (4). In brief, malting chemists make measurements of barley and malt properties and attempt to relate varietal differences in these properties to the known requirements in malting barley. Thus, a continuous phase of malting research is the development of methods of analysis, the application of these to the study of varieties, and the determination of their significance.

A considerable body of information on many properties of barley varieties grown in Canada has been published by Canadian malting chemists (4,7,75 and previous papers in their respective series). A summary of one phase of the work was published in 1941 by Anderson, Sallans, and Meredith (7). This summary did not deal with varietal differences as such, but with the interrelations among the values for 18 barley, malt, and malting properties. It illustrates the mode of attack developed in Canada for the evaluation of properties that have significance and which may help to elucidate the nature of malting quality in barley. Eleven barley and six malt properties were examined in these studies, but only one

wort property, wort nitrogen, was included. As wort is the material used for fermentation, it was considered advisable to study wort properties, and this thesis deals with certain measurements made on worts and the relations between these properties and other barley and malt properties.

Among the characteristics of wort that are closely observed by brewers there are three, viscosity, degree of attenuation, and turbidity, that can be accurately and objectively measured in the laboratory. But varietal differences in these qualities have not yet been adequately studied. Viscosity can be readily measured, and the viscosity of wort, according to Hopkins and Krause (50, p.193) is influenced by the quantity and quality of carbohydrate. Despite the simplicity of the measurement of viscosity, and the fact that variation in viscosity is of considerable interest to the brewer, investigations of this property are extremely few (50, p.192). Degree of attenuation is a measure of the amount of sugars that can be converted into alcohol by fermentation and, although it is of great importance to brewers, investigations of it have been confined mainly to studies of the effect of mash temperature (50, p.198) yeast type (50, p.280; 47, p.778), mineral content (47, p.572), and fermentation system (47, p.808). Turbidity or haze in wort and beer has received a great deal of attention as is evidenced by the discussion by Hind (47, p.707) and others (31,39,42,43,51,52,56). But information on the effect of variety is very scarce, though Hopkins and Krause state (50, p.181) of turbidity measurements made by means of a Nephelometer, "The barley variety is of great importance, poor malting varieties always give much higher figures than fine malting barley". There also appears to have been no systematic study of the relations between viscosity, degree of attenuation, and turbidity. Hopkins and Krause quote one investigation (50, p.207) in which all three

measurements were made, but this study involved mainly comparisons of pH of water used for mashing.

Studies of viscosity, degree of attenuation, and turbidity of worts obtained from experimental malts made from 24 varieties of barley were therefore undertaken, and the results are presented in this thesis. The first section is a review of the existing knowledge of chemistry of wort. The second section describes materials and methods used in the study. The results of the measurements made on the worts and the significance of varietal differences are discussed in the third section. In the fourth section, the relations between wort properties, and between these and other barley and malt properties, are considered. Studies on materials contributing to wort viscosity are discussed in the fifth section. Finally, all the relevant information is reviewed and conclusions are drawn regarding the utility of measurements made on wort in the differentiation of varieties and regarding the contribution that such measurements make towards elucidation of factors responsible for malting quality.

REVIEW OF WORT PROPERTIES

Wort is obtained from malt by extraction with water at temperatures that facilitate enzymatic conversion of the insoluble starch, protein and other substances, into soluble materials. The series of changes that are brought about in the barley kernel during malting are thus continued during mashing, and about 75 per cent of the malt is converted into water-soluble materials. Wort contains fermentable and unfermentable carbohydrates, proteins and their degradation products, tannins, resins, minerals, and some enzymes and vitamins. However, as stated by Hind (47, p.575), "the composition of wort is so complex and imperfectly known that no methods of analyses suitable for routine control are entirely satisfactory".

Nevertheless, although the chemical nature of many of the wort constituents are not known, there are many properties of wort that are subject to measurement. But some of these properties can be altered by change in processing condition. For example, the ratio of fermentable to non-fermentable sugars and the amount of nitrogen in wort can be altered by changes in mashing temperature and duration. Accordingly, in order to have a common basis for evaluation for malt, certain standard systems of laboratory mashing and analysis have been developed. The Congress method (1) for extract determination is used on this continent. This was developed in Germany and differs considerably in technique and conditions from the British system. It was designed to give information to those using the continental brewing system, which is the basis for most brewing processes on this continent.

In the Congress determination, 50 g. of malt are ground to a fine

grist and 200 ml. of distilled water at 46°F. are added to the ground malt in a mash beaker. The beaker is then placed in a mash bath at 45°C., and the mash is stirred continuously during the mashing process. The mash is held at 45°C. for 30 minutes, the temperature is then raised to 70°C. at the rate of 1° per minute. When the mash has reached 70°C., 100 ml. distilled water at 70°C. are added and the mash is held at 70°C. for 60 minutes. The mash is then quickly cooled to about 20°C., the stirring blade is rinsed into the beaker, and the contents of the beaker are made up to 450 g. by the addition of cold distilled water. The liquid is then separated from the solids by filtration. The resulting wort is used for a variety of determinations of which the most important is specific gravity; this serves for the calculation of the amount of material extracted from the malt.

By the use of this standard method for making laboratory mashes of malt, different laboratories are able to express results on a uniform basis and are also able to obtain a fair degree of uniformity of results in terms of extract yield, color and clarity of wort, and wort nitrogen content, etc. The relative importance attributed to the different characteristics of wort varies with the individual making the assessment, and all properties must be interpreted according to the process for which the malt is intended. Some brewers may desire higher wort nitrogen content than others, some may prefer light color in worts, and such requirements can be multiplied almost indefinitely. Nevertheless, on the whole, the Congress worts, although they are not boiled and hopped, appear to yield information that can be interpreted in operational terms for the brewer. There are, naturally, some conflicting opinions on this generalized assumption on the use of Congress worts and these must be considered. Before doing that, however, it is advisable to consider some more general aspects of malt quality.

According to Anderson, Meredith and Sallans (4), malt quality in barley is not easy to define. It depends on the malting method to be adopted, the brewing process in use, and the type of beer required. As all these differ in different countries and are superimposed on different types of barley, it is obvious that no single definition of malting quality can be universally applied. Thus, differences of opinion on what constitutes quality in malt may be expected. Outstanding examples of these differences are evident in the papers presented at the Malting and Brewing Section of the 6th International Technical and Chemical Congress of the Agricultural Industries in 1939. The general topic was, "What are the qualities of the good malt, and what relation do they bear to the quality of the beer obtained?", and many conflicting opinions were expressed.

Bishop (13) in reviewing the proceedings of this section of the Congress suggests that at first sight the results are discouraging. Some hold to the old adage that "The malt is the soul of the beer". Others say that "This was only true in the old days". It actually appears that these conflicting opinions arise from the diversity of materials that are used and the adaptation of methods of analysis to local problems. Bishop (9,10, 11) was the first to show that the composition of barley largely determines the composition of the malt, and this relation is now widely accepted. However, the skill of the malster will determine whether the barley is malted to best advantage. This change from barley to malt is generally called "modification", and it also seems agreed that a well modified malt will produce a better beer than will a poorly modified malt. The main problem is created by the fact that maltsters cannot agree how to measure modification. At the close of the Congress, it was agreed that further

work was required to find a basis for agreement and to obtain additional measurements of modification. This implies that there is a strong belief that beer quality is related to malt quality.

The measurements of modification that were discussed included those of Enders (33), Hartong (41), and Kolbach (54). Enders (33) described a machine, for grinding malt, to which is attached a dynamometer; the force required to grind the malt to uniform size is measured and registered by means of a kymograph. The results are reported in arbitrary units of hardness, and low values are desirable. This method is based on the fact that during the change from barley to malt there is a softening of the endosperm structure due to enzymatic disintegration of cell walls and other rigid structures such as aggregates of starch granules. Thus malt is more easily crushed than barley. Some poor malts have steely tips that have not been changed during malting and these create difficulties in grinding and mashing.

Hartong (41) discussed results obtained with his four-mash system. In this method, aliquots of malt are mashed at 25°, 45°, and 65°, and 85°C., and the extract yields are measured and reported in percentage of that obtained by the Congress method. The four results are averaged and the excess over 60 per cent is called "solution number". A desirable value is 5 and lower values indicate poor modification. Hartong comments that both enzymatic activity and attackability of substrate are involved in this test.

Kolbach (54) favours the old system of measuring the difference between extract from fine and coarse grists, which involves solution of enzymes and attackability of substrates. The older procedures are subject to high analytical errors, and Kolbach suggests procedures that improve

the reproducibility of results.

The determination of wort viscosity as suggested by Piratzky and Wiecha (70) is also considered by Kolbach (54) as a useful method of measuring the state of degradation of malt. Piratzky and Wiecha (70) had found that worts from malts that have been grown only a few days were high in viscosity.

Another method for determining malt modification, and one that is favored by Bishop (12), is the determination of soluble nitrogen in wort and the expression of the results in terms of percentage total nitrogen in the malt. This index appears to be a varietal characteristic and the optimum value may vary with variety (58,64,82). Fink (35) concludes that its general use is not justified, but Luers (57) considers this index important, and the consensus is that it is useful, provided that its limitations are recognized.

Actually, the diversity of methods for assessing malt modification indicate the different approaches that have been taken to the subject. There is little doubt that these approaches are the result of practical experience--the association of brewing results with malt analysis--and they emphasize the difference between areas in barley type, malting and brewing processes, and final products. It is also interesting to observe that comparisons of extracts from mashes made at different temperatures and from different grists, and the determination of wort nitrogen content and viscosity are all useful indicators of the conditions that may have to be used in plant operation. Thus results of the various determinations can be used to determine whether a malt sample should be rejected and may also indicate the steps to be taken during mashing to overcome faults that can be partially corrected. Thus, it is evident that systems of malt analysis

were developed from old rules of thumb that existed in the malting and brewing industry. Although most of the determinations have operational significance, they are empirical and frequently fail to produce information on fundamental characteristics of quality.

Luers (57) is quite definite in his statements that malt quality should be considered in determining mashing conditions. He also states that inherent quality governs many factors of importance during fermentation, and of the beer, including the tendency for the beer to become hazy. He further suggests that new methods for determining malt quality should be developed and that these should be applied to studies of barley varieties and of environmental factors.

Tombeur and de Clerck (83) join Luers in asking for better methods of analysis. They found that, although in general a well modified malt is known to give a better beer, no method is known for actually establishing a definite relation between analysis of malt and stability of beer--"the road between the malt and beer was too long"--and many factors can interfere during the course of manufacture. However, they appear to consider that stability of beer is caused by factors that are inherent in the malt, and they suggest that the factors leading to stability in beer should be isolated and traced back through mashing and malting. They conclude that malt analysis can forewarn them of serious faults in the malt but that minute details of analysis do not yield further useful information.

A considerable body of literature exists on the development of turbidity or haze in worts and beers. Since this thesis deals in part with turbidity, the principal papers are reviewed in some detail. Krauss (55) dealt with haze in beer and stated that malt modification, except when grossly abnormal, is no guide to haze portentialities of wort and beer;

further, there was no relation between haze in Congress worts and brewhouse worts. Krauss also states that the differences in viscosity in laboratory worts, brewhouse worts and beers, are without significance, but did not offer any constructive explanations or suggestions.

It is evident from the comments of Luers (57) and Tombeur and de Clerck (83) that development of haze in beer is a hazard that they would like to be able to predict and prevent. This implies that they consider that haze should be predictable, though the means were not available to them. It is also evident that they consider that this is a property that reflects degree of malt modification. Haze in wort and beer has been reviewed by many authors (31,39,42,43,46,47,50,51,52,56) and it is agreed that it can be caused by many factors. The most important haze is that developed on chilling of beer and this is usually reversible. The meterial that actually causes this chill haze has been the subject of numerous investigations and analysis, and it is now definitely established as a material of high molecular weight that is derived from protein and tannin (31,32,34,40,42,43,56). Other materials have also been found in the material separated from turbid wort and beer. Enders found pectin (31), Hartong found silicic acid (40), and Helm found silicic acid and carbohydrates, including pentosans and dextrins (43). The composition of the material is likely to be somewhat variable, depending on barley, environment, and malting and brewing process, but workers in Sweden have now shown that it is primarily a protein-tannin complex, and that the protein moiety is derived from the beta globulin of barley.

Quensel (73) reported in 1942 on studies of separation of barley globulins by progressive precipitation with ammonium sulfate and isolation and identification by special techniques involving use of the ultra-centrifuge

and the Tiselius electrophoresis apparatus. Four fractions were found and these were named, alpha, beta, gamma, and delta globulins, in order of increasing molecular weights. The alpha, beta and gamma fractions were found in appreciable amounts, but the delta fraction was found in low concentrations.

Saverborn, Danielson and Svedberg (79) re-investigated these globulins and found that the beta globulin is characteristic of barley and is present in only trace amounts, if at all, in wheat or rye or oats. The alpha and beta globulins are found in the aleurone layer and the gamma globulin is found in the embryo. These authors (79) also traced the globulins through malting and found less of the gamma globulin in malt than in barley. The amount of alpha globulin was slightly reduced, but the amount of beta globulin was not affected by malting. The beta globulin was also more resistant to mashing, and only beta globulin was found in worts prepared by the Congress method. The destruction of alpha and gamma globulins is not due entirely to enzymes; tests on solutions of the pure globulins showed that beta globulin was resistant to heat changes at the temperatures used in the Congress system of mashing; the alpha and gamma fractions were heat labile to some extent, and the gamma component was more resistant to heat than the alpha component. Wort contains only beta globulin, but boiling the wort degrades the beta globulin. Thus, none of the barley globulins are found in commercial worts that have been boiled.

Sandegren (78) reviewed the work of the Uppsala group and makes the additional comment that beta globulin can be decomposed by papain when activated with hydrogen cyanide and that the alpha and gamma fractions are more resistant to the attack of this enzyme. He also describes experiments to determine the material that causes chill haze.

Pilsener type beer was cooled and the chill haze was removed in a centrifugal type separator. The beer became more stable towards chill haze, but when some of the separated material was replaced, the beer became quite turbid. The chill haze material contained about 10 per cent nitrogen, corresponding to about 60 per cent protein, and gave qualitative tests for tannins. The molecular weight of this material as determined by sedimentation constant was approximately 40,000. A comparable figure was obtained on artificially prepared protein-tannin complexes in which the ratio of protein to tannin was the same as in the natural complex. The protein moiety in the artificial complexes had a molecular weight of 30,000. Thus, it appears that the protein component of the chill haze material also has a molecular weight of about 30,000.

The chill haze was investigated further by hydrolyzing it with sulfuric acid, after which it was found to contain 3.4 per cent glucose. If the tannin is penta-digalloyl-glucose, the theoretical amount of glucose is 3.5 to 4 per cent. Although this conformity in glucose content is striking, Sandegren warns that there is no proof that the tannin is really penta-digalloyl-glucose. However, the results do indicate that there are no appreciable amounts of dextrins of high molecular weight in the chill haze complex.

Additional work of Saverborn on chill haze material was also reviewed by Sandegren. A beer was treated with Bentonite to remove material likely to give rise to turbidity, and the beer was then boiled with some barley beta globulin. After cooling, this mixture was added to another portion of Bentonite-treated beer and the entire mixture was filtered. This gave a clear solution, but a typical reversible chill haze appeared when the beer was cooled. Similar tests with alpha and

gamma globulins produced inreversible hazes that coagulated and precipitated rapidly. Thus the beta globulin cleavage products are regarded as those responsible for this chill haze. The original molecular weight of the beta globulin is around 100,000, so that particules of molecular weight of 30,000 are formed during degradation of this globulin.

Confirmation that the chill haze material is derived from beta globulin was also obtained by chemical analysis. The alpha and gamma fractions contain only 1.6 per cent sulphur. The beta fraction contained 1.9 per cent sulphur, and the chill haze material contained about 2 per cent sulphur. This high sulphur content suggested the presence of amino acids, such as cysteine, that can be oxidized. Additional tests showed that turbidity increased with oxygen up-take. The removal of this chill haze material had no effect on the foaming capacity and foam retension of beer. The surface active nitrogenous materials that contribute to foam in beer were found to have a molecular weight of about 10,000. The haze forming capacity of beer is thus traced back through wort and malt to barley.

Enders (31) and Hartong (39,40) suggested in 1937 that turbidity was caused by properties inherent in barley and was controlled more by variety and season than by malting condition. Sandegren's review of Saverborn's work does not deal with variety, but it does indicate the effect of season. There were variations from year to year in the relative amounts of the alpha and gamma globulins of barley (both of which exceed the beta fraction in amount) but the amount of the beta component was more constant. However, as salt soluble nitrogen content varies among varieties (29,11,48,49) it may be expected that the beta globulin content also varies.

Hartong (40) also believes that the materials responsible for turbidity in unboiled worts are similar to those that caused turbidity in beers. This statement may be difficult to reconcile with the fact that Saverborn's beta globulin was clear in dilute saline solution and gave haze after boiling. It is quite likely that the results of the Upssala group present an oversimplification of the problem, although they point the way to more complete understanding of wort turbidity. The techniques and equipment for separating the globulin components are so complicated and expensive that it will be some time before they can be applied adequately to studies of different varieties of barley and to the malts made from them.

The importance of haze in wort is best summarized by the statement of Hopkins and Krause (50, p.180), "On the whole, it [haze] is an indicator of the actions of all dissolving enzymes in the malt such as diastase, hemicellulases and proteinases". Hind (47, p.565) states that, "persistent haze in the stronger worts suggests inadequate conversion of steely malts". These statements are based on many observations and much experience, and indicate that brewers anticipate trouble from malts that give hazy worts. It may well be that the composition of haze in beers differs from that of haze of worts but that both arise from a similar source or at least trace to a common cause. Sandegren mentions that although the barley globulins could not be found in wort, high molecular components were found; but these were present in such small concentrations that it was impossible to obtain exact data on them through investigations in the ultra centrifuge.

By comparison with the extensive studies on haze formation comparatively little work has been done on degree of attenuation, which is the percentage of wort solids that is removed by fermentation.

Hopkins and Krause mention studies on the effects of mash temperature (50, p.198) and yeast type (50, p.280) on degree of attenuation, while Hind discusses the effects of yeast type (47, p.778), mineral content (47, p.572) and fermentation system (47, p.808). There has been considerable interest in methods for determining degree of attenuation in wort and beer. Silbereisen (80) developed a rapid method for determining degree of attenuation of wort. Other methods have been developed by Bishop (14) and Bishop and Whitley have also studied the effects on attenuation of wort flocculum (18), agitation (19), yeast type (20) and design of fermentation vessel (21). All these studies deal with the mechanisms of fermentation and there is no published information on the effect of barley variety on degree of fermentability.

The degree of attenuation to which a wort will ferment depends on the amounts and types of the wort sugars that can be fermented by the growing yeasts. Malt contains sucrose, glucose, fructose, possibly some trisaccharides, and some soluble products from hemicelluloses (46, p.191). Wort contains these same sugars in addition to maltose, dextrins, pentoses and pentosans (50, p.242) and some trisaccharides (23). Sucrose, maltose, glucose and fructose are fermented by brewers' yeast, but the other carbohydrates in wort are not fermented by this type of yeast.

The ratio of the fermentable sugars in wort to the non-fermentable carbohydrates, chiefly dextrins, can be varied by changes in mashing procedure. However, a high amount of potentially fermentable material is desirable and this is dependent on the enzymatic activity as well as on the content of carbohydrate material. Attenuation studies provide information only on the total amount of fermentable sugars and provide no information on the amounts of the individual sugars. This is not a serious

deficiency of information for purposes of brewing operations as the main interest is the production of the required amount of alcohol. Nevertheless, it is desirable that more should be known about the carbohydrates present in malt and wort in order that their origin may be traced. Further aspects of these carbohydrates are discussed in dealing with wort viscosity.

The viscosity of wort has received considerable attention. It is first mentioned as a measure of wort quality by Hopkins and Krause (50, P.193). They indicate that wort viscosity is mainly determined by the quality and quantity of carbohydrates, but that beer viscosity is determined by the content of alcohol and nitrogenous substances. Helm (45) elaborates on these points; he considers that the viscosity of wort can provide useful information on malt modification. The maltose content of wort influences viscosity, but the viscosity of wort is considerably higher than that of maltose solution of the same specific gravity. Helm also states that proteins have no noticeable influence on viscosity. The viscosity of the beer depends on that of the wort as the compounds causing viscosity are unferementable and are not degraded during fermentation. The effect of maltose on viscosity is removed by fermentation of this sugar, but the final viscosity of the beer is but little lower than that of wort as the effect of alcohol on viscosity compensates for the loss of maltose. The factors causing high viscosity are important in retention and palatefulness of beer. The actual factors that cause high viscosity in wort and beer have not been widely investigated. The main studies on this factor are those of Piratzky and Wiecha (71), though these in turn trace back to investigations of barley gums made by O'Sullivan, Lintner, and Brown. These early studies are reviewed by Hind (46, p.70).

Piratzky (69) reported that worts prepared from malt that have

been grown only three to four days showed no break in boiling for 1.5 hours; the worts also filtered very poorly. Although the symptoms were indicative of poor starch conversion, the worts gave no blue color with iodine, which showed that the starch had been degraded. The worts were very viscous and were only reduced to normal viscosity by boiling under a reflux for five hours, or by adding trichloracetic acid and filtering off the precipitate. This latter material contained much less protein than was found in precipitates from normal worts treated in the same way. Piratzky concluded that the material causing high viscosity was not pentosans, dextrins, or proteins, but that it might be a complex containing fatty material. Normal wort viscosities were not obtained until the malts had been grown at least five days, and it was suggested that high wort viscosity was a sign of imperfect mofification.

Piratzky and Wiecha (70) subsequently studied the enzymatic reduction of high viscosity in worts from malts grown for only three to four days. Proteolytic enzymes, such as papain and pepsin, had no effect on wort viscosity, but Filtragol-N (a pectin splitting enzyme) and Merck's "Diastase puriss" reduced viscosity. Of these, the Filtragol-N was more active. Studies of pH and temperature optimum indicated that the enzyme causing the reduction in viscosity was similar to cytase in its requirements for optimum activity. Barley and malt extracts also reduced viscosity, but these were only 1/30 to 1/50 as active as the enzymatic preparations. Kilning the malt reduced the activity of the extracts. They concluded that the enzymes responsible for the reduction in viscosity were present in barley and malt in very small concentrations and that they acted on hemicelluloses. As the optimum activity of the enzymes was found to be at pH 4.5 to 4.7 and at 45° to 50°C., they suggested that

modification of the hemicelluloses could best be done during malting rather than during mashing. Data on wort viscosity for 180 samples of malt prepared during the period from 1933-36 were presented. The mean and maximum values varied, but the yearly minimum values, about 1.6 centipoises, were identical. Piratzky and Wiecha concluded that these minimum values represent well modified malts in which all the convertible hemicelluloses have been converted.

Helm (44,45) confirmed the production of viscous worts from malts that have been grown for short periods, and showed that viscosity fell sharply up to five days growth and then decreased slowly to 12 days growth. The viscosity was about 1.6 centipoises for normal worts. Worts from three-day malts had relative viscosities of 2.5, and this value was reduced to 1.8 at five days growth and to 1.4 for 12 days growth. He also gave data showing the close relations among viscosities of Congress worts, brewhouse worts and beer.

Piratzky and Wiecha (71) continued their studies on wort viscosity and isolated from a three day malt a material that they considered to be principally responsible for wort viscosity. It was found to be an amylan, not a pentosan, as it produced maltose on enzymatic hydrolysis and glucose on acid hydrolysis. The yields of sugar indicated that only glucose units were present, and they concluded that the material was a polysaccharide of glucose units.

The amylan was not homogeneous, and molecular weight and viscosity varied according to method of isolation. Viscosity increased with increasing molecular weight; the material of highest molecular weight contained 400 glucose units and produced a viscosity of 4 centipoises in a 1 per cent aqueous solution. Material of similar type was found in

barley but not in malt. The amylan is thus different from the gums reported by earlier workers, as these gums contained pentosans and were much less viscous in solution. Piratzky and Wiecha prepared gums according to the directions of previous workers and found that the isolates from barley could be fractionated into amylans and pentosans but the isolates from malt contained no amylan.

It thus appears that the amylan that produces a very viscous solution is a contributing factor to wort viscosity only in abnormal malts that are typified by short growth. The pentosan material is found in barley, normal malts and worts, and in beer, but all but one of the pentosan products obtained by Piratzky and Wiecha failed to produce high viscosities in solutions. However, they isolated a pentosan material of high viscosity in low yield (4 g. per hectolitre) from the last washings of spent grains. They state "Über den Verbleib und die Funktionen dieser in so geringer Menge in die Bierwürze gelangenden Körper könnte man zunächst nur Vermutungen anstellen, deren Unterlagen mehr als unsicher sind." It is therefore apparent that Piratzky and Wiecha are not prepared to discuss this material.

As it is apparent that Piratzky's and Wiecha's amylan contributes to viscosity only when the malts are grossly undermodified, it is advisable to consider the origin of materials contributing to viscosity in normal worts. Piratzky and Wiecha admit they got a pentosan material from beer and, although they do not discuss it, the implication of this discovery cannot be ignored. As quoted by Hind (46, p.70) O'Sullivan and Brown discussed barley gums many years ago, but little has since been done about these. According to Hind (46, p.70), the quantity of these gums or amylans is roughly measured by the difference between extract obtained

from barley and the sum of the proteins, sugars and starch in barley. Hind states that, according to H. T. Brown, the solution of the highly colloidal gum of barley is one of the most significant changes made during conversion into malt. These gums represent the hemicelluloses of barley and, according to Hopkins and Krause (50, p.132), the action of cytase on the hemicelluloses in the cell walls of the endosperm is the cause of the softening of the grains, which is the most evident physical change during malt modification. This cellular modification was studied by Dickson and Shands (30). These authors presented photomicrographs showing the structural changes in barley during germination.

Hind (46, p.67) and Hopkins and Krause (50, p.114) report that barley contains about 9 per cent pentosans. Hind reports the figure as hemicelluloses, but Hopkins and Krause actually call them pentosans. Piratzky's and Wiecha's discussion of their amylan is therefore somewhat unfortunately phrased as it has tended to obscure the role that pentosans may play in determining or influencing malt modification.

Some of the confusion in the literature arises from the changes in terminology that have accompanied advances in the knowledge of carbohydrate structure. The studies discussed by Hind (46, p.70) were made forty to seventy years ago. O'Sullivan in 1882 extracted laevorotary carbohydrates from barley to which he gave the names alpha and beta amylans. Lintner reported his gum in 1890. Brown also used the term amylan for his gummy material prepared in 1906. The term amylan is actually suggestive of a hexosan similar to starch, but Piratzky and Wiecha find that compounds prepared by the methods of O'Sullivan and Lintner are mixtures that contain pentosans. Hind considers that these gums should be regarded as hemicelluloses.

Preece (72) has recently reviewed the available information on pentosans in barley and malt. He suggests that there is little doubt of the importance of cytolysis of the hemicelluloses during malting and perhaps even during further stages in processing. But there is little precise information available on the subject. He considers that the barley gums appear to have some of the general characteristics of hemicelluloses, including a hexosan fraction. However, these gums differ from the common hemicelluloses in solubility. The resemblance between the barley gums and the hemicelluloses may be fortuitous but it is important to investigate the chemical constitution of the gums.

The protein and other nitrogen compounds in wort are of great importance in brewing and have been widely studied. The obvious need for nitrogen compounds in wort is for yeast nutrition during fermentation, and the simpler compounds are used for this purpose (81). However, the definition of optimum amount of wort nitrogen is fraught with many difficulties, and clear cut statements are not yet possible. There has been more written on the subject of nitrogen in barley, malt and wort than on any other subject in malting and brewing, but the issue is still unsettled. The nutrition requirements of yeast are not yet fully known, although Thorne (81) has made significant contributions to this subject. However, amount of wort nitrogen seems to be regarded more as an indication of malt characteristics, and as an indication of the processes by which the wort has been derived from malt and barley, than as a measurement of nitrogen per se.

The total amount of nitrogen compounds that can be obtained from any malt can be altered by changes in mashing temperature and duration, and the relative amounts of the amino, amide and peptide nitrogen are also

changed as shown by Bishop (12). The sum of the ammonia N., amino N., amide N., and peptide N. accounts for about 55% of the total nitrogen, so that about 45% of the nitrogen is not accounted for, or to use Bishop's own expression is "undetermined nitrogen". This "undetermined nitrogen" includes the complex substances that contribute to palatefulness of beer and which may cause a tendency towards haziness.

In recognition of the differences that can be brought about in amount, and perhaps quality, of wort nitrogen by changes in mashing conditions, and in order to have a useful basis by which to make comparisons, most workers have confined studies to wort prepared under standard conditions, such as the English or Congress methods of mashing malt. Such studies have been used to determine the relations between barley nitrogen content and wort nitrogen content.

The barley protein fractions have been shown by Bishop (9,10,11,12) and others (2,48,49) to follow a regular pattern with increase in total nitrogen content. This generalization applies only within a variety. However, the relative proportions of the different fractions differ among varieties, even when they are compared at equal barley nitrogen contents. Bishop (12,16) and others (3,5,58,64,82) have also shown that within varieties the amount of wort nitrogen determined under standard conditions of malting and analysis is related to original barley nitrogen content. Again however, there are varietal differences in percentage of total barley nitrogen that appears in the wort. An important point to note is that this ratio of wort nitrogen to barley nitrogen, which is called by Bishop "index of nitrogen modification," decreases with increase in barley nitrogen content when comparing samples of a single variety that differ in barley nitrogen content. It has been suggested that with increase in barley

nitrogen content, the protein complexes become more resistant to enzymatic degradation during malting and mashing.

Fractionation of wort nitrogen into various components has not proved of much value in determining the "quality" factors in wort nitrogen. Lundin (58) has shown that the traditional fractions--tannin and phosphomolybdate nitrogen--show the same relations to total nitrogen as does total soluble nitrogen. Burkhart and Dickson (26) studied laboratory worts, brewery worts, and beer, and showed that the amounts of many of the fractions were closely related, not only within series of worts and beers but between the worts and beers. A few fractions showed only loose relations to total wort nitrogen, but Burkhart and Dickson failed to find any significant effects on the worts and beers of variations in these fractions.

Bishop (12) regards the "index of nitrogen modification" as an important aspect of malt quality. Under English conditions, he considers that values of 32 per cent (30 to 33 per cent) imply under-average modification, values of 35 per cent (34 to 36 per cent) show average modification, and values of 38 per cent (37 to 40 per cent) imply over-average modification. These values are for malt from two-rowed barley of about 1.6 per cent nitrogen; the comparable average figure for a six-rowed barley malt is 29 per cent.

The English and Congress mashing systems produce different amounts of nitrogen in wort, so that Congress worts give average values of 40 per cent and 36 per cent respectively. Further, the English workers base their ratio on permanently soluble nitrogen and most other workers determine only total wort nitrogen. Burkhart and Dickson (26) have shown that total and permanently soluble nitrogen are closely

related and Hind (46, p.249) estimates that the amount of total wort nitrogen is 5 per cent higher than the amount of permanently soluble nitrogen. Accordingly, the average figures given last can be raised again to 42 and 38 per cent respectively.

American malts are much higher in nitrogen content than English malts so a direct application of English experience is impractical. The situation is further involved by differences in malting and brewing procedures. To these limitations must be added those imposed by the varietal differences in index of nitrogen modification that have been found by Thunaeus and Schroderheim (82) and others (3,5,58,64).

Differences of opinion in the use of this index of nitrogen modification have been mentioned previously. Current opinions on this determination are perhaps best summarized by Helm (45) who suggests that the results may be of most value when they are in conjunction with other analytical figures. Brown (24) indicates that under English systems of mashing the amount of wort nitrogen that is obtained in the brewhouse varies according to mashing conditions and always exceeds that obtained in laboratory worts. He found that the average increment in one year was 15 per cent and that the increase varied from 4 to 32 per cent. Hopkins and Krause (50, p.193) state that, with normal malts, particularly when using the infusion mashing system, proteolysis cannot be carried too far, but that a certain caution is advisable when mashing over-modified malts by the decoction system. It is thus apparent that wort nitrogen content is important as an indication of other factors such as barley type, processing conditions etc., as well as a measure of protein degradation in the wort.

Most studies on the quality of wort have been carried out on

worts prepared by the standard system of analysis of the English or the Congress mashing procedures. There appears to be unanimity of opinion in considering that these standard worts yield information of value to the brewer, even through the information and results are of an empirical nature. There is less unanimity of opinion on the use of laboratory worts for predicting haze in beer, but the suggestion is that haze in worts indicates that some factors are out of balance.

Ideally, it is desirable to analyze a malt but, as its constituents must be brought into solution, this cannot be done without grinding and solution, which is almost invariably accompanied by enzymatic activity. As the laboratory mashing procedures are designed to simulate more or less average conditions of mashing in the brewhouse, the study of laboratory worts is likely to provide both fundamental and functional information on malt and wort quality. Wort is essentially a dynamic system and the chemistry of wort requires applications of knowledge and techniques from many fields of chemistry.

The advances that are being made in the knowledge of the constituents of wort by such specialized techniques as the use of the ultra-centrifuge and the Tiselius apparatus are indeed useful contributions. Nevertheless such techniques cannot be applied on a wide scale and must be used in conjunction with a considerable background of additional information on malt and wort properties. There is thus a need for the type of information on malt and wort quality that can be obtained by methods such as were used in the studies reported in this thesis. The commonly measured properties have been useful in assessing gross differences in malting quality, and measurements of wort properties will amplify this type of information and may well asist in providing a better definition of malting quality.

EXPERIMENTAL

MATERIALS

The investigation was carried out on a set of 24 varieties of barley, each of which was grown at six experimental stations in Canada in 1938 as part of agronomic trials known as the Uniform Variety Trials. The stations were: Lacombe, Alta.; Brandon, Man.; Winnipeg, Man.; Ste. Anne de la Pocatiere, Que.; Fredericton, N. B.; and Nappan, N. S.

Barley Type	Variety
Six-rowed, rough-awned, Manchurian.	O.A.C. 21, Mensury, Olli, Peatland, Pontiac.
Six-rowed, rough-awned, Coast.	Trebi.
Six-rowed, smooth-awned	Alberta 8 (Titan), Brandon 216, Byng, Newal, Nobarb, Ottawa E. 25, Plush, Prospect, Regal, Saskatchewan 264, Velvet, Wisconsin 38, York.
Two-rowed, rough awned	Charlottetown 80, Hannchen, Victory.
Two-rowed, smooth-awned	Rex, Sanalta.

METHODS

Malting Methods

The samples were malted in duplicate in the equipment previously described (6,63) and by the standard procedures used in the malting laboratory at the University of Manitoba. These methods have been described by Meredith (63).

Analytical Methods

Methods used in Routine Testing. The barleys and malts were analyzed in the course of routine malting tests for yield of heavy grade, 1000 kernel weight, and nitrogen content of the barley, hours steep and malting loss, malt extract, saccharifying activity, and wort nitrogen. The methods for these determinations have been described previously (6,63,64).

Additional Determination on Barley. At the time that the malts were made, the laboratory was working in close collaboration with the Malting Laboratory in the National Research Laboratories, directed by Dr. H. R. Sallans, and each laboratory assisted the other by making samples and data available. In return for certain determinations in which the Ottawa laboratory was interested, but which were more readily made in the Manitoba laboratory, the Ottawa laboratory made determinations of salt-soluble nitrogen (2), starch (8), extract (8), and saccharifying activity (74) on the barleys used in this study.

Wort Properties. Almost a year elapsed from the time that the samples were malted until the wort quality determinations were made, hence the data represent qualities of stable malts. Worts were prepared in the usual

way (1) and aliquots were withdrawn for the various analyses. Duplicate determinations were made on one-third of the samples, and the standard errors of analysis were calculated from the pairs of data thus obtained. The samples on which duplicate determinations were made were selected at random, after imposing the limitation that two samples of each variety and eight samples from each station, should be chosen. Analyses were carried out in random order at a rate of six samples per day on two days in each week.

The worts were analyzed for turbidity immediately after filtration (initial turbidity), turbidity after standing 24 hours at 10°C. (final turbidity), viscosity and degree of attenuation. Turbidity and viscosity are simple expressions and measurements are readily made, so these are described first. Degree of attenuation falls into a different class, both from ease of measurement and ease of interpretation. The method for this determination is described last. As the development of a suitable procedure for determining degree of attenuation required several months' study, some features of the method developed are discussed after the details of the analytical methods are given.

Viscosity. Viscosity was determined at 20°C. by means of an Ostwald viscosimeter. Three trials were made on each wort sample. The average value was used for conversion into relative viscosity in the usual way, using water as the standard. The mean relative viscosity was 1.52, and the standard error of the mean of duplicate determinations was 0.01 centipoises.

Initial Turbidity. Turbidity was determined on each wort immediately after filtration by means of a Zeiss Pulfrich Nephelometer. The results are reported in per cent of the standard aperture, using filters No. 4

and 12, and wedge illumination. The formula for converting relative turbidity into absolute turbidity is as follows:

$$\text{Absolute turbidity} = \frac{\text{relative turbidity} \times 0.0290.}{166.7}$$

The standard error of the mean of duplicate determinations was 2.6% and the mean initial turbidity was 37%.

Final Turbidity. The samples of wort for the determination of initial turbidity were poured into test-tubes, and the tubes were tightly stoppered. They were then placed in a water-bath maintained at 10°C. Turbidity measurements were repeated on the samples after 24 hours. The standard error of the mean of duplicate determinations was 18%, and the mean final turbidity was 162%.

Stability. The values for this determination were obtained by subtracting the values for initial turbidity from those for final turbidity, and subtracting these differences from 200. The final subtraction was made in order that higher stabilities would be indicated by an increase in numerical value. The standard error of the mean of duplicate determinations was 18, and the mean stability was 75 units.

Degree of Attenuation. One hundred and fifty ml. of wort was mixed to a smooth paste with 24 gm. of commercial bakers' yeast (supplied fresh each day) in a mash beaker. The beaker was then placed in a mash bath maintained at 20°C. and the contents were stirred constantly for 4.5 hours. The fermented wort was filtered overnight, and the specific gravity was determined and corrected by means of a blank determination. This is the method described by Silbereisen (80), except that no yeast fat was used. Apparent degree of attenuation was calculated by the formula given by

Pawlowski-Doemens (68) which is as follows:

$$\text{Apparent attenuation } \% = \frac{(\text{original extract} - \text{apparent extract in fermented wort}) \times 100}{\text{original extract}}$$

The extracts are found by determining specific gravity of the wort and referring to the Plato tables (1), which give amount of extract corresponding to various specific gravities. The results were left in terms of apparent degree of attenuation for all calculations and examination.

The mean apparent attenuation was 77.1%, and the standard error of the mean of duplicate determination was 0.6%.

Degree of attenuation is a measure of the extent to which sugars in wort are fermented by yeast and it can be measured in two ways. The original specific gravity of the wort is determined, from which the original amount of wort solids can be ascertained by reference to specific gravity tables for sugar solutions. The wort is fermented and the amount of alcohol produced by the yeast is determined directly by distillation, or indirectly by evaporating the alcohol, making the wort back up to original weight with water and determining specific gravity again. The amount of sugar fermented is determined by the amount of alcohol produced, or by the difference between original solids and solids remaining after fermentation, and the ratio of solids lost to original solids is called the real degree of attenuation. The second method involves determining the specific gravity of the fermented wort without further treatment. During fermentation, the specific gravity of wort drops because of removal of dissolved solids from and addition of alcohol to the solution. The specific gravity of a fermented wort is therefore lower than that of a water solution containing the same proportion of solids and the content of dissolved solids obtained by reference to

specific gravity tables is lower than the real value. The corresponding value for sugars removed by fermentation is therefore higher than the real value, but calculations are carried out as above and the result is called apparent degree of attenuation. The difference between real attenuation and apparent attenuation is dependent on the extract content before fermentation. By use of an appropriate factor, which is determined by reference to the original extract value, real attenuation may be calculated from apparent attenuation. Thus, the determination of apparent attenuation, which is much simpler to conduct than that for a real attenuation, provides an indirect determination of the latter.

Preliminary studies were necessary in order to develop a suitable method for the determination of apparent attenuation. An old standard method, given by Pawlowski -Doemens (68) was tested and found unsuitable, but an adaptation of a method described by Silbereisen (80) was found to be satisfactory. Tests comparing apparent and real attenuation using bakers' yeast were conducted. These showed that the formula given by Pawlowski for converting apparent attenuation to real attenuation was quite suitable for the samples examined in this study, except that the formula gave values that were 1.5 per cent lower than the real attenuation. The formula given by Pawlowski-Doemens is:

$$\text{Real attenuation} = \frac{\text{apparent attenuation}}{(q + 1)}$$

The value for q is dependent upon original extract content and is 0.228 for all samples in this investigation.

It may be noted that some criticism can be levelled at this method for determining degree of attenuation. The use of bakers' yeast instead of brewers' yeast may be viewed with suspicion, but bakers' yeast

was used as it was expected to be uniform over long periods. Moreover, it was supplied fresh each day. The uniformity of the bakers' yeast is evident by the low value (0.6%) for the standard error of the mean of duplicate determinations. Actually, investigations on attenuation were carried out over a period of about nine months, and the yeast was constant throughout as determined by check samples.

It is interesting to note that Bishop (14) states that most workers agree that different yeasts give the same limit of attenuation for a given wort, though the fermentation rate may vary. Although the literature that Bishop reviews (14) is concerned with various types of the so-called brewers' yeast, the bakers' yeasts are actually strains of brewers' yeast (Saccharomyces cerevisiae) that have been selected for high rates of fermentation. It therefore seems that it is in order to assume that the same limits of attenuation would have been reached had any other type of yeast been used, particularly when the relatively enormous proportion of yeast to wort is considered. The advantages of the bakers' yeast are that it is readily obtainable and uniform, and it produces a rapid fermentation.

As noted by Bishop (14), the method used in this study may be faulted in that alcohol may be lost by evaporation. As all samples were tested in the same way, the losses would be uniform, or at least proportional, so that all results are comparable. The main object was to determine whether varieties differ in extent to which they will ferment; it is the differences between varieties that are significant.

RESULTS AND DISCUSSION

DIFFERENCES IN WORT PROPERTIES

The data for the 144 samples that were analyzed are extensive and, as the variety and station means are of principal interest, only these are presented. The data for each determination were examined by means of analyses of variance (38) and the results of these were used to determine the significant levels for differences between variety means and between station means. The ranges among varieties and among stations were considerable and significant varietal and station differences were demonstrated in each property.

Differences between Varieties

The object of determining whether varieties differ in malting quality is to obtain information for the plant breeders who are producing new varieties of barley with superior yield, disease resistance, etc. Information on the malting quality of the standard varieties is required in order that the plant breeder may use these to best advantage as parental material. Similar information is required for the new hybrids that are obtained so that the producers may select and concentrate on those that are most promising. Plant breeders are always in need of as much information as they can obtain on varietal differences in malting quality. Thus, practical requirements are invariably ahead of quality research. What is known about quality at any time is used by plant breeders at that time even though the fundamental significance of certain varietal differences may not be fully understood. This situation exists in part

with respect to the wort properties studied in the present investigation. The first steps in deciding how to use information on wort properties thus requires that the errors of the determinations and the ranges of varietal differences be established.

The mean values, over six stations, for each variety are given in Table I, together with the necessary differences for the 5% level of significance obtained from analysis of variance. The varieties are listed in order of decreasing degree of attenuation. The ranges are: degree of attenuation, 4.8%; viscosity, 0.23 centipoises; initial turbidity, 84%; final turbidity, 161%; and stability, 93 units. The differences between varieties in these properties are therefore considerable, and these are discussed in more detail in the following sections.

The variety having the lowest degree of attenuation is Ottawa E.25 at 74.6%, and the highest value is that for Olli at 79.4%. The distribution of the values for the other varieties is normal as there is a smooth gradation from high to low over the series of values. The necessary difference between values is 1.7% and the standard error of the mean of duplicate determinations is 0.6%. These values indicate that the determination is relatively precise and that it is capable of significant differentiation between varieties. As a high amount of potentially fermentable sugar, represented by a high degree of attenuation, is desirable, it is of interest to note that O.A.C.21, which is the statutory standard of malting quality for six-row barley in Canada, is fourth highest of all varieties in degree of attenuation. No variety is significantly higher than O.A.C.21 in this property and seven varieties, Prospect, Victory, Byng, Wisconsin 38, Saskatchewan 264, Regal and Ottawa E.25, are significantly lower than O.A.C.21 in fermentable material.

TABLE I

Varietal Means for Wort Properties

Variety	Attenuation	Viscosity	Initial Turbidity	Final Turbidity	Stability
	%	centipoises	%	%	units
Olli	79.4	1.44	19	120	99
Brandon 216	78.7	1.45	28	142	86
Sanalta	78.4	1.51	39	140	99
O.A.C.21	78.2	1.46	18	130	88
Nobarb	78.0	1.54	29	169	60
Charlottetown 80	77.9	1.49	61	218	43
Velvet	77.8	1.48	32	148	84
Rex	77.7	1.46	18	119	99
Plush	77.6	1.57	47	188	59
York	77.6	1.58	102	262	40
Peatland	77.5	1.48	46	176	70
Newal	77.5	1.54	27	154	73
Mensury	77.4	1.48	18	134	84
Hannchen	77.2	1.50	18	101	117
Pontiac	77.0	1.50	22	123	99
Trebi	77.0	1.57	40	163	77
Alberta 8 (Titan)	76.8	1.52	27	164	63
Prospect	76.1	1.56	37	208	29
Victory	76.0	1.56	21	137	84
Byng	75.8	1.64	47	196	51
Wisconsin 38	75.7	1.59	73	238	34
Saskatchewan 264	75.4	1.48	27	121	106
Regal	75.1	1.49	38	116	122
Ottawa E.25	74.6	1.67	50	218	32
Mean over all varieties	77.1	1.52	37	162	75
Necessary difference, 5% level.	1.7	0.06	24	64	59

The determination of viscosity is also precise and useful for differentiating varieties. The necessary difference between varieties is 0.06 and the standard error of the mean of duplicate determinations is 0.01 centipoises, while the range between varieties is 0.23 centipoises. The variety with the lowest value is Olli, 1.44, and Ottawa E.25 is high with a value of 1.67. Again there is a smooth gradation from low to high values so that the distribution of values is normal. Viscosity of wort is a measure of the state of degradation of dissolved particles and the values are usually regarded as being related to molecular complexity. As O.A.C.21 has proved to be a suitable malting variety it is not surprising to find that O.A.C.21 produces a wort of low viscosity. Only the value for Olli is lower than that for O.A.C.21 and the difference is not significant. The values for eleven varieties are significantly lower than that for O.A.C.21. These varieties are Nobarb, Plush, York, Newal, Trebi, Titan, Prospect, Victory, Byng, Wisconsin 38, Ottawa E.25.

The measurements of turbidity fall into a completely different category than those for attenuation and viscosity. The necessary differences for initial turbidity, final turbidity, and stability are respectively 24%, 64%, and 59 units, and the standard errors of the mean of duplicate determinations are 2.6%, 18% and 18 units respectively. When these statistics are compared with the mean values of 37, 162, and 75, it is evident that the measurements are not very precise and that differentiation of varieties is difficult. Despite these limitations, varietal differences in these properties were found. The values for O.A.C.21 again fall in the desirable range, that is, low turbidities and high stability. Five varieties, Charlottetown 80, York, Byng, Wisconsin 38 and Ottawa E.25 are significantly higher than O.A.C.21 in both initial and final turbidity.

Two varieties, Peatland and Plush are significantly higher than O.A.C.21 only in initial turbidity, and Prospect is significantly higher than O.A.C.21 in final turbidity and significantly lower in stability.

The lack of precision in measuring turbidity in duplicate samples, and the high error in differentiating varieties, make these measurements less useful for variety classification than attenuation and viscosity measurements. However, the situation with respect to composition and origin of haze forming material (47, p.993) is so uncertain that the condition found in this study is not surprising. Haze can be caused by many factors, and the replicate samples were analyzed weeks apart, so that it is probable that some uncontrollable factors were operating to cause the differences between duplicate results. Further, the varieties were grown under a wide range of environmental conditions, which produced wide ranges in the mean turbidity values for stations, and the differential reactions of the varieties to these changes in growing conditions cause the high error in varietal differentiation.

The differential response of varieties to change in environmental conditions is the limiting factor in varietal differentiation for most properties (3,4,63,64,66), and when it is superimposed on a high laboratory error, as in the measurement of turbidity made in this study, differentiation of varietal mean values becomes very limited. But some form of varietal evaluation must be attempted in the course of making recommendations to plant breeders. There are no definite values for the wort properties that can be used as reference standards, although high values for attenuation and low values for viscosity and turbidity are desirable, as the ideal levels have not been determined. The practice throughout the evaluation of the results of the laboratory malting test

has been to use as criteria the values for material that is acceptable for commercial malting and brewing. These are represented in Canada by the values for O.A.C.21, which as well as being a satisfactory variety (4) is the statutory standard for six-row barley grades in Canada. This is by no means an ideal practice, but it is the only practical method available until more is known about the factors responsible for malting quality and the desirable levels for these. A result of this is that O.A.C.21 is included in all sets of varieties being grown for malting tests and the values for any variety are compared with those of O.A.C.21 grown under the same conditions. Comparisons between varieties with respect to malting quality cannot be made satisfactorily if they have been grown under different conditions, as environment during growth has a very pronounced effect on malting quality (3,4,5,63,64). The practice of using results of a comparable sample of O.A.C.21 as criteria of quality therefore eliminates the direct effect of environment from varietal comparisons and also provides an indication of commercial suitability for malting.

The data in Table I have been rearranged in Table II to provide ready comparison of the properties of the other varieties with those of O.A.C.21. A zero indicates that the value was not significantly different from that for O.A.C.21. Plus and negative signs indicate significantly higher and lower values.

Fifteen varieties differ significantly from O.A.C.21 in one or more properties. Seven differ in only one property, three in two properties, one in three properties and four in four properties. Although the varieties may be discussed in any sequence, it is appropriate to discuss them by groups listed in the section on materials (p.27) as these

TABLE II

Comparison of Varietal Means with those for O.A.C.21, in Terms of Significance of Difference[1]

Key No.	Variety	Attenuation	Viscosity	Initial Turbidity	Final Turbidity	Stability
		%	centipoises	%	%	units
1	O.A.C.21	78.2	1.46	18	130	88
2	Mensury	0	0	0	0	0
3	Olli	0	0	0	0	0
4	Peatland	0	0	+	0	0
5	Pontiac	0	0	0	0	0
6	Trebi	0	+	0	0	0
7	Alberta 8 (Titan)	0	+	0	0	0
8	Brandon 216	0	0	0	0	0
9	Byng	-	+	+	+	0
10	Newal	0	+	0	0	0
11	Nobarb	0	+	0	0	0
12	Ottawa E.25	-	+	+	+	0
13	Plush	0	+	+	0	0
14	Prospect	-	+	0	+	-
15	Regal	-	0	0	0	0
16	Saskatchewan 264	-	0	0	0	0
17	Velvet	0	0	0	0	0
18	Wisconsin 38	-	+	+	+	0
19	York	0	+	+	+	0
20	Charlottetown 80	0	0	+	+	0
21	Hannchen	0	0	0	0	0
22	Victory	-	+	0	0	0
23	Rex	0	0	0	0	0
24	Sanalta	0	0	0	0	0

[1] 0 denotes non-significance of difference.
- denotes significantly lower than O.A.C.21.
+ denotes significantly higher than O.A.C.21.

represent a commonly used classification of the varieties. Anderson, Meredith and Sallans (4) have discussed the reasons for assuming that definite inferiority in any one major property is adequate grounds for rejection of a variety and this practice is also followed in making varietal comparisons for the wort properties.

Olli and Mensury, which are acceptable to Canadian maltsters and are eligible for the malting grades, cannot be differentiated from O.A.C.21 in wort quality. This suggests that varieties acceptable in commerce because of similarities in barley and malt qualities are also similar in wort properties. Pontiac, which is derived from the same stock as O.A.C.21 but which has never been widely grown, is not regarded as equal to O.A.C.21 in malting quality owing to deficiencies in malt extract and enzymatic properties (4,66). Pontiac is not significantly different from O.A.C.21 in any wort property but it tends to be low in degree of attenuation and high in viscosity so that it does not appear as satisfactory as O.A.C.21 in wort quality. Peatland is a rust resistant variety that was removed from the list of varieties eligible for the malting grades when it was shown to have enzymatic deficiencies. It produced a wort with higher initial turbidity than that of O.A.C.21, and the remaining wort properties of Peatland, although not significantly different from those of the standard, also indicate that its wort quality is lower than that of O.A.C.21. Trebi was higher than the standard variety in wort viscosity, and its initial wort turbidity just fails to be significantly higher than that for O.A.C.21. Thus Trebi also possesses inferior wort qualities. This variety has been known to maltsters for many years and it is avoided by them for many reasons, the most general of which are its ragged growth and difficulty in

obtaining proper modification.

Among the six-rowed smooth-awned varieties, only Brandon 216 and Velvet do not differ significantly from O.A.C.21 in wort properties. Brandon 216 is closer to O.A.C.21 in properties than is Velvet; the differences from O.A.C.21 for both varieties are of low order except perhaps for initial wort turbidity in which both are higher, though not significantly so, than O.A.C.21. Nobarb, Newal, and Titan (Alberta 8) differ significantly from O.A.C.21 only in viscosity, but the degree of attenuation of Titan tends to be low. Saskatchewan 264 and Regal are definitely low in degree of attenuation and can be considered inferior in wort quality. Plush is high in viscosity and initial turbidity of wort, and the value for final turbidity approaches significance, so that Plush is considered definitely inferior in wort quality. This variety is not liked by maltsters, though it is widely grown in Western Canada. York, which is a variety that has not come into prominence, is inferior to O.A.C.21 in wort viscosity, initial turbidity, and final turbidity, and its value for stability tends to be low; its inferiority in wort quality is obvious. Prospect, Byng, Wisconsin 38, and Ottawa E.25, differ so widely from O.A.C.21 in four properties that their inferiority in wort quality is beyond dispute.

The rating of Wisconsin 38 in this study is of particular interest because this variety is widely used for malting and brewing in the United States. The results of tests carried out by Malt Research Institute in the United States on comparable samples of Oderbrucker, a sister strain to O.A.C.21, and Wisconsin 38 from four crops have been published (59,60,61,62), and the authors suggest that Wisconsin 38 is as acceptable for brewing as Oderbrucker. It does not appear advisable

to carry the interpretation of the results presented here too far, but under Canadian conditions it appears that Wisconsin 38 is by no means equal to O.A.C.21 in wort quality as defined by the measurements under discussion. It is worth while noting, however, that at the present time the trend in the United States is definitely towards the production of varieties with qualities that are more similar to those of O.A.C.21 than to those of Wisconsin 38.

The five varieties in the two-rowed group exhibit as wide ranges in wort quality as the six-rowed varieties. Owing to distinct differences between the groups in malting characteristics, it is not advisable to lay too much stress on the direct comparisons between the two-rowed and six-rowed barleys. Comparisons within the two-rowed group should be made with Hannchen. This variety is the most widely known of the group and it is used to a limited extent for malting and brewing in the United States. In general, Hannchen appears to be quite similar to O.A.C.21 in wort quality, although it tends to be lower in degree of attenuation and higher in viscosity. As all varieties were malted under identical conditions, which actually were developed for six-rowed varieties and do not favour the two-rowed varieties, the differences between O.A.C.21 and Hannchen are about as would be expected as a result of inadequate modification of the latter variety. The wort properties for Rex and Sanalta, which are smooth-awned varieties, are similar to those of Hannchen, although those from Sanalta tend to be more turbid. These two varieties have been fairly widely grown in recent years, but they are not considered suitable for malting owing to low extract (4,66). The wort for Charlottetown 80 is much more turbid than those for Hannchen, and should be regarded with suspicion. Victory produced wort that tends to

be less fermentable and more viscous than that for Hannchen and it must be considered inferior in quality.

Differences between Stations

The mean values for station, over all varieties, for the wort qualities are give in Table III. These show wide ranges in each property, and the differences between stations are highly significant. It is therefore apparent that the environment under which barley is grown produces a considerable effect on each wort property. This is to be expected in view of the results obtained with more commonly determined barley and malt properties (63,64), and it does not seem necessary to discuss the station effects further.

Analyses of Variance

It was necessary to carry out analyses of variance on the 144 results for each determination in order to assess the significance of differences between varietal means and between station means. The variance for each of the determinations was separated into portions due to (1) average differences, over all stations, between varieties; (2) average differences, over all varieties, between stations; and (3) differences in the relative placings of the varieties at different stations. The results of these analyses are given in Table IV. The necessary differences listed in Tables I and III were calculated for the 5 per cent level of significance in the usual manner (38) from the data in Table IV.

TABLE III

Station Means for Wort Properties

	Attenuation	Viscosity	Initial turbidity	Final turbidity	Stability
	%		%	%	%
Brandon	79.2	1.47	26	172	54
Fredericton	77.1	1.52	39	167	72
Lacombe	77.1	1.54	23	164	59
Winnipeg	77.0	1.49	25	159	66
Nappan	76.8	1.67	38	125	113
Ste. Anne de la Pocatiere	75.4	1.56	70	184	86
Mean over all stations	77.1	1.52	37	162	75
Necessary difference 5% level	0.8	0.03	12	32	30

TABLE IV

Analyses of Variance for Wort Qualities: Mean Squares

Quality	Variance due to Varieties	Stations	Interaction	Standard error of mean of duplicates
Attenuation, %	8.5**	35.1**	2.3	0.6
Viscosity	0.02**	0.04**	0.003	0.01
Initial turbidity, %	2420.5**	7577.6**	468.6	2.6
Final turbidity, %	11071.7**	9642.9*	3127.5	18
Stability, units	4431.0*	11479.0**	2703.0	18
Degrees of freedom	23	5	115	48

Note: In this and later tables * denotes that the 5% level, and ** that the 1% level, of significance is attained.

RELATIONS AMONG WORT QUALITY

The data in Table I suggest that inter-varietal relations exist between the various wort properties. In order to evaluate these trends, the correlation coefficients among the properties were calculated. These are given in Table V and discussed in this section.

TABLE V

Simple Intervarietal Correlation Coefficients Among Wort Properties

Quality	Viscosity (v)	Initial Turbidity,% (t_1)	Final Turbidity,% (t_2)	Stability (s)
Attenuation, % (a)	-.606**	-.183	-.222	-.217
Viscosity (v)		.532**	.678**	-.685**
Initial turbidity, %(t_1)			.875**	-.646**
Final turbidity, %(t_2)				-.934**

Simple Correlations

Degree of attenuation is significantly correlated with viscosity only, and the relation is inverse. Viscosity in wort may be attributed to the combined effects of fractions of starch and protein material of high molecular weight. Degree of attenuation is a measure of the amount of fermentable materials, which are products of starch degradation of low molecular weight. The inverse relation between degree of attenuation and viscosity is therefore to be expected. As degree of attenuation increases, the amount of residual, unfermentable material decreases, and viscosity also decreases. The results of this investigation suggest that, between

varieties, worts of high viscosity will not ferment to as great an extent as those with lower viscosity. But it should be noted that, as only a few suitable varieties are malted and eventually brewed, it is unlikely that the range of viscosity observed in this study would be encountered in commercial brewing.

Significant correlations exist between viscosity and each of the other qualities. These may also be explained by the presence of colloidal fractions remaining after the degradation of starch and protein. The relation between the two turbidity measurements is in accord with expectation, and the fact that the initial and final turbidity are quite closely related is of definite value in the laboratory. Between varieties, the initial measurement of turbidity is a good indication of final turbidity and of stability. The higher the initial turbidity, the greater is the final turbidity and the lower the stability value.

There appear to be no definite relations between attenuation and turbidity. This seems surprising, as degree of attenuation is an inverse expression of the amount of high molecular weight degradation products, and is inversely related to viscosity. However, the degree of attenuation does not indicate qualitative differences in residual, non-fermentable material, and this may be the reason for the lack of relation with turbidity.

It must also be pointed out that the failure to demonstrate relations between degree of attenuation and turbidity is not in accord with the results of Bishop and Whitley (18). They found that, as turbidity increased, degree of attenuation decreased owing to removal of yeast from the reactions. They examined 16 worts that were fermented for 120 hrs. at a pitching rate of 4 to 5 gm. per litre. The worts were prepared from a single sample of malt and the differences in turbidity were brought about

by combinations of differences in the rate of cooling the wort and differences due to sedimentation of the flocculum. In our investigation all worts were prepared by one method but from different malts. Bishop and Whitley demonstrated a relation between turbidity and attenuation among worts prepared by different methods from one sample; but it does not follow that there is a corresponding relation among worts prepared by one method from samples of different varieties. The results of the present investigation suggest that no such relation exists.

Partial Correlations

The simple correlations discussed in the previous section are subject to the limitation that two factors may appear to be related whereas this effect may be merely a reflection of the relations between a third factor and each of the two factors in question. Partial correlation coefficients in which the effects of other terms are removed, are therefore more stringent tests of relations than simple correlation coefficients. In order to determine whether the relations discussed in the previous section were real and independent, a number of partial correlation coefficients were computed. With one exception, these coefficients confirmed the relations already discussed. As no additional useful information was obtained, it is not necessary to report all of them.

The exception was the relations between viscosity (v), initial turbidity (t_1), and final turbidity (t_2). Neither partial coefficient ($r_{vt_1 \cdot t_2} = -.04$, or $r_{vt_2 \cdot t_1} = .39$) is significant. This is not surprising in view of the close relation between initial and final turbidities. However, the partial correlation between viscosity and final turbidity, independent of initial turbidity ($r_{vt_2 \cdot t_1}$), approaches the 5 per cent level of significance. This suggests that the material causing final turbidity is

mainly, though not solely, responsible for the association between viscosity and turbidity, and that the relation between viscosity and initial turbidity results from the association between the latter and final turbidity.

Graphical Representation of Relations

While the correlation coefficients shown in Table V present information on the degree of scatter of the points about the regression lines, they do so only in a general way. In order to illustrate the relations and to bring out some points that are not shown by the correlation coefficients, scatter diagrams for the relations between viscosity and other properties, and between initial and final turbidities are given in Fig. 1.

Fig. 1A shows that the points for two varieties, Regal and Saskatchewan 264 (nos. 15 and 16), fall farther from the regression line than those for any of the other varieties. The intervarietal correlation coefficient, when these two varieties are omitted from the calculations, is $r = -.814$, as compared with $r = -.606$ when they are included. For both varieties, the degree of attenuation is considerably less than would be expected on the basis of their viscosities. It may also be noted that the values for the stabilities of these varieties are much higher than those of any other six-rowed variety. These abnormalities suggest distinct qualititative differences between these and the other varieties in residual non-fermentable material. Judged on the basis of degree of attenuation, Regal and Saskatchewan 264 contain relatively large amounts of non-fermentable material; in fact, only one variety, Ottawa E.25, exceeds them in this respect. However, the viscosity and stability data suggest that the constituents of the residual non-fermentable material

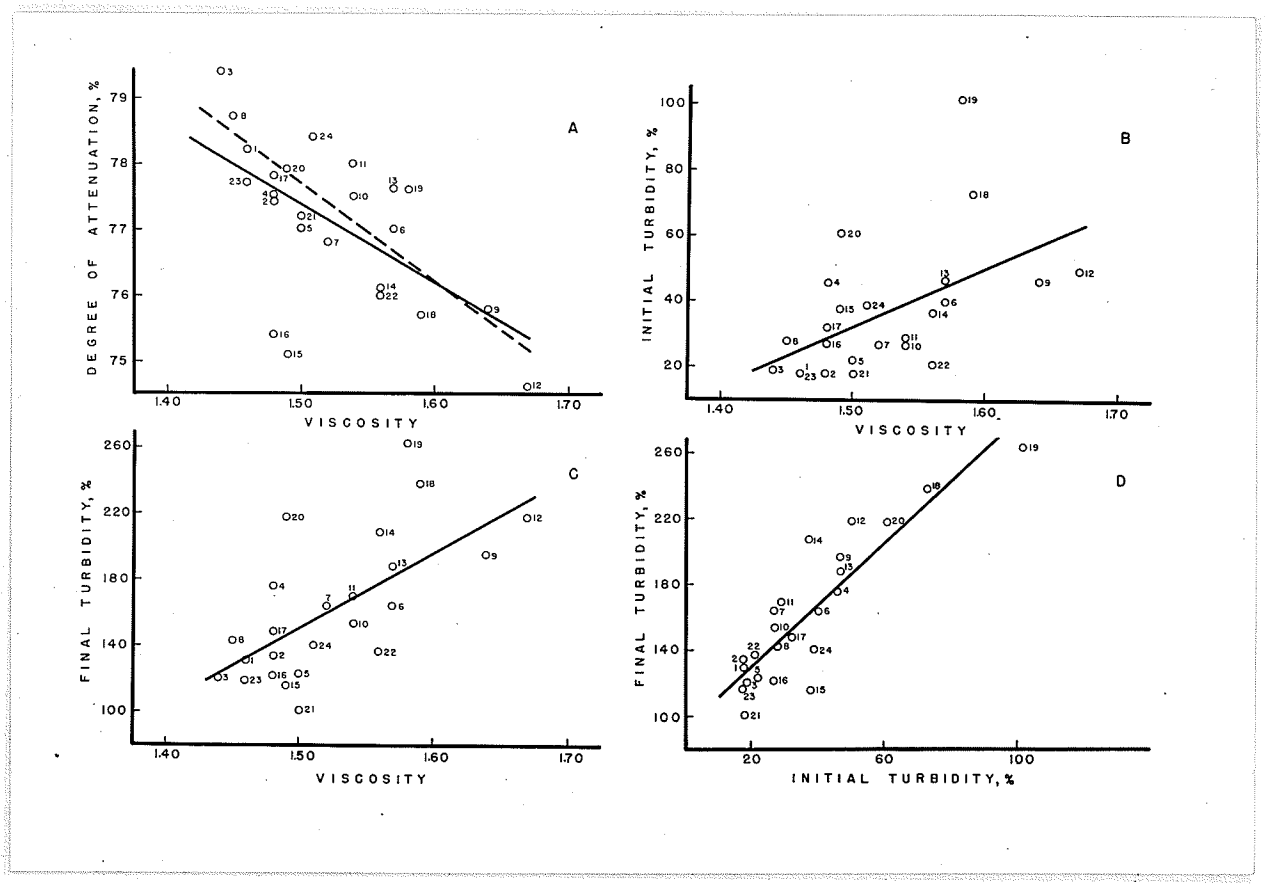

Figure 1.

Scatter diagrams for varietal means showing the relations between wort properties. The key to varieties is given in the first column of Table II. In 1A the dotted regression line is that over 22 varieties. The heavy regression line in 1A and those in 1B, 1 C, and 1D represent the regressions over all varieties.

derived from these two varieties are of lower average molecular size than those obtained from the other varieties, and this appears to account for their lower viscosity and higher stability values.

The remaining three graphs in Fig. 1 show that the scatter of the points about the regression lines is fairly uniform, with the exception of the position of No. 19 (York) in Fig. 1B.

As only six stations were represented in the investigation, the data are inadequate for examination of intravarietal relations.

RELATIONS BETWEEN WORT PROPERTIES AND BARLEY, MALTING, AND MALT PROPERTIES

The discussion in the previous sections dealt with the quality of the wort and the interrelations among the various properties involved. As quantity of malt extract is very important, it is logical to examine the relations between the quantity and quality of malt extract (or wort solids), and also between the enzymatic properties of the malt and wort properties. In the latter study enzymatic properties are represented by Lintner values and wort nitrogen content. As malt extract and Lintner values have been shown to be closely related to barley properties (7,74,76, 77) it is also logical to examine the relations between wort properties and barley starch, extract, Lintner value after activation by papain, and salt-soluble nitrogen. Determinations on those properties were made in the Ottawa laboratory under the direction of Dr. H. R. Sallans and the data were made available to the writer. As steeping time and malting loss have also been shown to be related to enzymatic activity (76), the examination of the relations between these and the wort properties is also advisable. All these various relations are discussed in this section. The object is to determine the extent to which wort qualities may be predicted from barley and malt properties and to offer some explanation of varietal differences in wort quality.

Data

The results of the test on the various barley and malt properties have been published elsewhere (4,66) but for convenience the data are given in Tables VI and VII.

TABLE VI

Varietal Means for Barley Properties

Class	Variety	Total nitrogen, %	*Salt-sol. nitrogen, %	*Starch %	*Extract %	1000-kernel wt., gm.	*Sacch., act., °L.	Plump barley, %
Rough-awned 6-rowed malting	1. O.A.C.21	2.08	.54	54.1	75.9	31.8	186	76.9
	2. Mensury	2.06	.53	54.0	75.8	31.9	194	78.4
	3. Olli	2.00	.54	54.6	77.2	29.6	216	68.0
Rough-awned 6-rowed non-malting	4. Peatland	2.34	.56	53.6	75.8	29.2	221	72.7
	5. Pontiac	2.04	.50	53.5	74.9	31.8	193	80.6
	6. Trebi	1.88	.46	56.2	76.8	40.0	193	87.7
Smooth-awned 6-rowed	7. Alberta 8	2.03	.46	54.7	74.8	33.6	153	82.6
	8. Brandon 216	1.90	.47	55.6	76.7	24.4	142	45.3
	9. Byng	1.90	.43	55.6	77.6	33.9	179	87.7
	10. Newal	2.17	.50	52.6	75.5	34.2	276	95.2
	11. Nobarb	1.91	.44	55.6	76.9	32.6	158	79.3
	12. Ottawa E.25	2.20	.44	52.3	74.6	37.6	195	94.0
	13. Plush	1.97	.41	54.8	75.9	33.8	142	81.4
	14. Prospect	2.03	.45	52.4	74.5	35.0	170	92.0
	15. Regal	2.07	.48	53.3	74.8	31.2	133	75.2
	16. Sask. 264	2.07	.49	53.6	75.6	32.2	159	60.5
	17. Velvet	2.10	.47	53.6	74.9	30.6	177	82.0
	18. Wisconsin 38	2.05	.44	53.2	74.6	32.8	167	90.5
	19. York	2.08	.48	54.5	76.3	31.4	209	78.8
Rough-awned 2-rowed	20. Charlottetown 80	2.05	.52	56.9	78.6	33.8	169	82.7
	21. Hannchen	1.99	.50	57.3	79.3	32.6	174	69.4
	22. Victory	1.96	.49	57.1	79.1	34.4	152	79.0
Smooth-awned 2-rowed	23. Rex	2.17	.49	54.4	76.5	36.7	179	82.2
	24. Sanalta	2.13	.47	55.6	77.4	39.2	180	95.6
Mean over all varieties		2.05	.48	54.6	76.3	33.1	180	79.9
Necessary difference, 5% level		0.12	.03	1.2	1.0	2.1	21	10.9

*Data provided by Dr. H. R. Sallans.

TABLE VII

Varietal Means for Malting and Malt Properties

Class	Variety	Malting property			Malt property		
		Steeping time hr.	Malting loss, %	Sprouts, %	Extract, %	Wort nitrogen, %	Sacch. activity, °L.
Rough-awned 6-rowed malting	1. O.A.C.21	52	8.2	3.8	75.0	1.06	116
	2. Mensury	55	7.7	3.6	75.0	1.07	118
	3. Olli	38	8.4	4.0	77.1	1.11	143
Rough-awned 6-rowed non-malting	4. Peatland	59	8.1	3.8	74.3	1.10	124
	5. Pontiac	56	7.7	3.4	74.0	1.02	124
	6. Trebi	64	6.6	2.6	74.2	0.80	92
Smooth-awned 6-rowed	7. Alberta 8	60	7.5	3.4	73.6	0.96	89
	8. Brandon 216	43	8.2	4.2	76.2	1.04	106
	9. Byng	55	6.3	2.6	74.6	0.76	74
	10. Newal	53	6.6	2.6	72.8	1.00	149
	11. Nobarb	59	7.2	3.2	74.2	0.83	93
	12. Ottawa E.25	56	6.3	2.8	71.3	0.87	96
	13. Plush	58	6.4	2.7	73.4	0.81	76
	14. Prospect	56	6.9	3.0	71.9	0.84	91
	15. Regal	55	7.2	3.0	72.8	0.95	77
	16. Sask. 264	50	8.6	4.0	74.5	1.08	100
	17. Velvet	56	7.4	3.4	73.7	1.04	114
	18. Wisconsin 38	62	7.4	3.1	71.8	0.86	93
	19. York	66	6.5	2.4	73.8	0.90	101
Rough-awned 2-rowed	20. Charlottetown 80	56	8.5	4.0	77.0	0.96	94
	21. Hannchen	52	8.3	3.6	77.4	1.00	111
	22. Victory	56	7.9	3.6	76.8	0.86	93
Smooth-awned 2-rowed	23. Rex	58	8.0	3.6	75.4	1.12	119
	24. Sanalta	48	7.7	3.3	75.6	1.04	112
Mean over all varieties		55	7.5	3.3	74.4	0.96	104
Necessary difference, 5% level		6	0.6	0.4	1.1	0.06	12

The relations between the properties were examined by means of correlation studies using the values for variety means. The small number of stations included in the study is insufficient for study of intra-varietal relations.

Correlation Coefficients

The simple intervarietal correlation coefficients between wort properties and barley or malt properties are given in Table VIII. The simple correlation coefficients between the malt and barley properties themselves are given in Table IX. Some of the latter are of value in explaining certain factors contributing to wort quality.

Of the 55 correlation coefficients involving wort qualities (Table VIII), 13 exceed the 1% level of significance, 10 exceed the 5% level, 5 just fail to attain the 5% level, and 27 are not significant.

Table VIII shows that significant correlations occur much more frequently between wort properties and malting or malt properties, than between wort properties and barley properties. Thus it appears that wort quality is related to the amounts of extract and enzymes actually developed during malting and not to the amounts of extract or B-amylase that are potentially available in the barley. In other words, wort qualities reflect the extent to which the barleys are modified in the production of malt. This point will be discussed in greater detail after an examination of the relations involving each wort property.

Wort attenuation is significantly and directly related to barley salt-soluble nitrogen, malt extract, malt saccharogenic activity, and wort nitrogen content; also, the correlation coefficients for steeping time and malting loss with degree of attenuation approach the 5% level of significance. As salt-soluble nitrogen, steeping time, and malting loss

TABLE VIII

Simple Intervarietal Correlation Coefficients Between Wort Properties and Barley, Malting, and Malt Properties

Property	Wort properties				
	Attenuation, %	Viscosity	Initial turbidity, %	Final turbidity, %	Stability units
Barley					
Total nitrogen, %	-.072	-.132	.112	.057	-.008
Salt-soluble nitrogen, %	.424*	-.671**	-.316	-.424*	.439*
1000-kernel wt., gm.	-.304	.489*	.064	.150	-.187
Starch, %	.329	-.103	-.080	-.164	.200
Extract, %	.334	-.108	-.131	-.214	.236
Saccharogenic activ., °L.	.251	.042	.050	.088	-.100
Malting					
Hours steep	-.391	.382	.534**	.559**	-.492*
Malting loss, %	.370	-.810**	-.439*	-.550**	.543**
Malt					
Extract, %	.572**	-.559**	-.363	-.487*	.503*
Saccharogenic activ., °L	.550**	-.520**	-.375	-.398	.355
Wort nitrogen, %	.461*	-.842**	-.438*	-.594**	.614**

NOTE: In this table and Table IX * denotes that the 5%, and ** that the 1%, level of significance has been attained.

TABLE IX

Simple Intervarietal Correlation Coefficients Between Barley, Malting, and Malt Properties

Property	Malt properties		
	Extract,	Saccharogenic activity, °L.	Wort nitrogen,
Barley			
Total nitrogen, %	-.282	.448*	.517**
Salt-soluble nitrogen, %	.496*	.690**	.554**
1000-kernel wt.,gm.	-.211	-.211	-.394
Starch, %	.785**	-.227	-.163
Extract, %	.854**	-.098	-.042
Saccharogenic activity, °L.	-.088	.753**	-.274
Malting			
Hours steep	-.485*	-.433*	-.519**
Malting loss, %	.693**	.388	.736**
Malt			
Extract, %	—	.260	.395
Saccharogenic activity, °L.	.260	—	.743**
Wort nitrogen, %	.395	.743**	—

are associated with malt extract, malt saccharogenic activity, and wort nitrogen content (see Table IX), it is evident that all these properties influence or reflect enzymatic development; this, in turn, effects the quantity of fermentable material in wort as measured by degree of attenuation. Among these complex factors, malt extract and malt saccharogenic activity appear to be the most important single factors in determining degree of wort attenuation.

Wort viscosity exhibits relations similar to those for degree of attenuation, and the same properties are involved. In general, the correlation coefficients are somewhat higher than those for degree of attenuation. This may be explained on the basis that wort viscosity measures less complex factors than does fermentability. The relation involving 1000-kernel weight with attenuation is not significant, but that for 1000-kernel weight and viscosity is significant. The same conclusions as were drawn in the previous section, with respect to the effect of modification of the barley on wort attenuation, apply to the relations involving wort viscosity. In fact, additional confirmatory evidence is obtained from the significant direct correlation between kernel size and viscosity. It is generally accepted that small kernels modify more readily than larger kernels, and the statistics indicate that with increase in kernel size there is an accompanying increase in wort viscosity. Hence there is further reason for associating wort viscosity with extent of malt modification. Among the factors contributing to wort viscosity, malt extract and wort nitrogen content appear to be the most important.

Initial turbidity, final turbidity, and stability are very similar in their relations with the other properties, and these relations resemble those for degree of attenuation and wort viscosity. There are no

outstanding features about the relations except that they provide additional evidence that wort quality is dependent on the enzymes that produce growth, and hence on the degree of modification of the malts. The turbidities decrease and stability increases with increase in salt-soluble nitrogen, malting loss, malt extract, malt saccharogenic activity, and wort nitrogen content, and the reverse occurs with increase in steeping time.

Discussion of Relations among Properties

The correlation coefficients given in Table VIII show that wort quality is associated with malt extract, saccharogenic activity and wort nitrogen content, which are the commonly measured malt properties. However, with one exception, the correlations are not such that any one of these properties can be considered to control any one wort quality. The exception is wort nitrogen content, which accounts for about 70% of the variations in wort viscosity. Partial correlation coefficients were calculated, but they do not contribute to the study except to emphasize the interlocking relations among malt properties and wort qualities. For this reason, and because they may be readily calculated from the simple correlation coefficients, the partial coefficients are not reported.

It is of interest to note that improvements in malt extract, saccharogenic activity, and wort nitrogen, are accompanied by improvements in wort quality. The increase in wort nitrogen content must be regarded as a qualitative improvement rather than as a strictly quantitative improvement. Low wort nitrogen in a variety is generally accompanied by deficiencies in enzymatic activity. Therefore, among varieties, as enzymatic activities increase, malt extract, saccharogenic activity, and wort nitrogen also increase. These increases cause an improvement in wort quality.

There are certain implications of the relations between wort nitrogen and wort quality that are now becoming apparent. As noted above, low wort nitrogen content in a variety is generally accompanied by deficiencies in enzymatic activity, but the interpretation of high wort nitrogen content is by no means clear. The indications are that a new variety that is higher than O.A.C.21 in wort nitrogen content at the same level of barley nitrogen would be lower than O.A.C.21 in viscosity; moreover, the nitrogen in the new variety would be in simple form. There is a general assumption that the body, foam, aroma and character of the beer are associated with the colloidal state of the nitrogenous complexes in the wort (50, p.191). Thus it is possible that a variety that is high in wort nitrogen content because of extensive degradation of the nitrogen complexes during malting and mashing would produce a rapidly fermenting beer that might be abnormal; it would be thin and would lack body, character and aroma. Very little is known about this aspect of wort nitrogen, but some recent work by Witt and Fitzsimons in the United States supports such hypotheses (84).

The relations of wort quality to malt extract and saccharogenic activity are particularly interesting in view of the fact that both these properties can be predicted from barley properties. Starch and barley extract are closely related to malt extract, while saccharogenic activity of the barley after extraction with papain is closely related to saccharogenic activity of the malt (cf. Table IX and 74,76). However, neither starch, extract, nor saccharogenic activity of barley shows a significant association with any wort quality. That is, there are factors contributing to wort quality that are not measured by determinations of barley extract or saccharogenic activity. But these factors are measured to some extent by

malt extract and enzymatic activity. An examination of the relations between barley and malt properties throws some light on the reasons for this.

The determination of starch and extract content of barley provides information on the available material that can be converted into malt extract. Accordingly, there is a close relation between these barley properties and malt extract. However, the differences between varieties in potential extractives do not account for all of the differences between varieties in malt extract. This failure is caused by the differences between varieties in enzymatic activity. As noted previously, various properties have therein been examined to determine whether they can be applied to the prediction of malt extract as indices of enzymatic activity (77). This failure of barley properties to reflect enzymatic properties is also reflected in the relations between the various saccharogenic activities. The saccharogenic activity of barley after activation by papain is attributed solely to the action of β -amylase. While this activity is closely related to the saccharogenic activity of the ensuing malt, it may be expected to be more closely related to β -saccharogenic activity of the malt than the total saccharogenic activity of the malt (cf. 65). Similarly, while the saccharogenic activity of barley is related to malt α-amylase activity, the relation is not as close as that between the saccharogenic and the α-amylase activities of malt (7). That is, only part of the saccharogenic activity of malt can be predicted from the saccharogenic activity of the barley, since the latter does not provide an adequate estimate for malt α-amylase activity.

The complexity of the factors influencing wort quality is evident from the low values of the various correlation coefficients that

are statistically significant (Table VIII); no individual barley or malt property can be considered to control any one wort quality, though wort nitrogen content appears to play a major role in determining wort viscosity.

Scatter diagrams illustrating the relation between the degree of attenuation and malt extract, malt Lintner value, and wort nitrogen content were prepared. They were unusual in that the degree of scatter about the regression line was not uniform, and the correlation surfaces appeared to be made up of several swarms. The relation between malt extract and degree of attenuation ($r = .572^{**}$) is illustrated in Fig. 2A. This scatter diagram is the most interesting of the three prepared, and is the only one reproduced in this thesis. The 24 varieties appear to be divided into three distinct groups, among which the slopes of the regression lines are similar. The grouping of the varieties is not in accord with any one characteristic; however, inclusion of malt saccharogenic activity in a multiple correlation modifies the relation appreciably. The multiple correlation coefficient of malt extract and Lintner value with degree of attenuation is $R = .710^{**}$; the regression equation is:

Degree of attenuation =
49.4 + .33 malt extract + .03 malt Lintner value.

The calculated values for degree of attenuation, obtained from the above equation, are plotted against actual values in Fig. 2B. The scatter is considerably reduced from that in Fig. 2A. The varieties responsible for the largest residual deviations from the regression line are: Plush, Nobarb, and Brandon 216, for which the actual degree of attenuation is higher than the estimated value; and Ottawa E.25, Saskatchewan 264, Hannchen, and Victory, for which the actual degree of attenuation is lower than the estimated value. None of the available data will satisfactorily

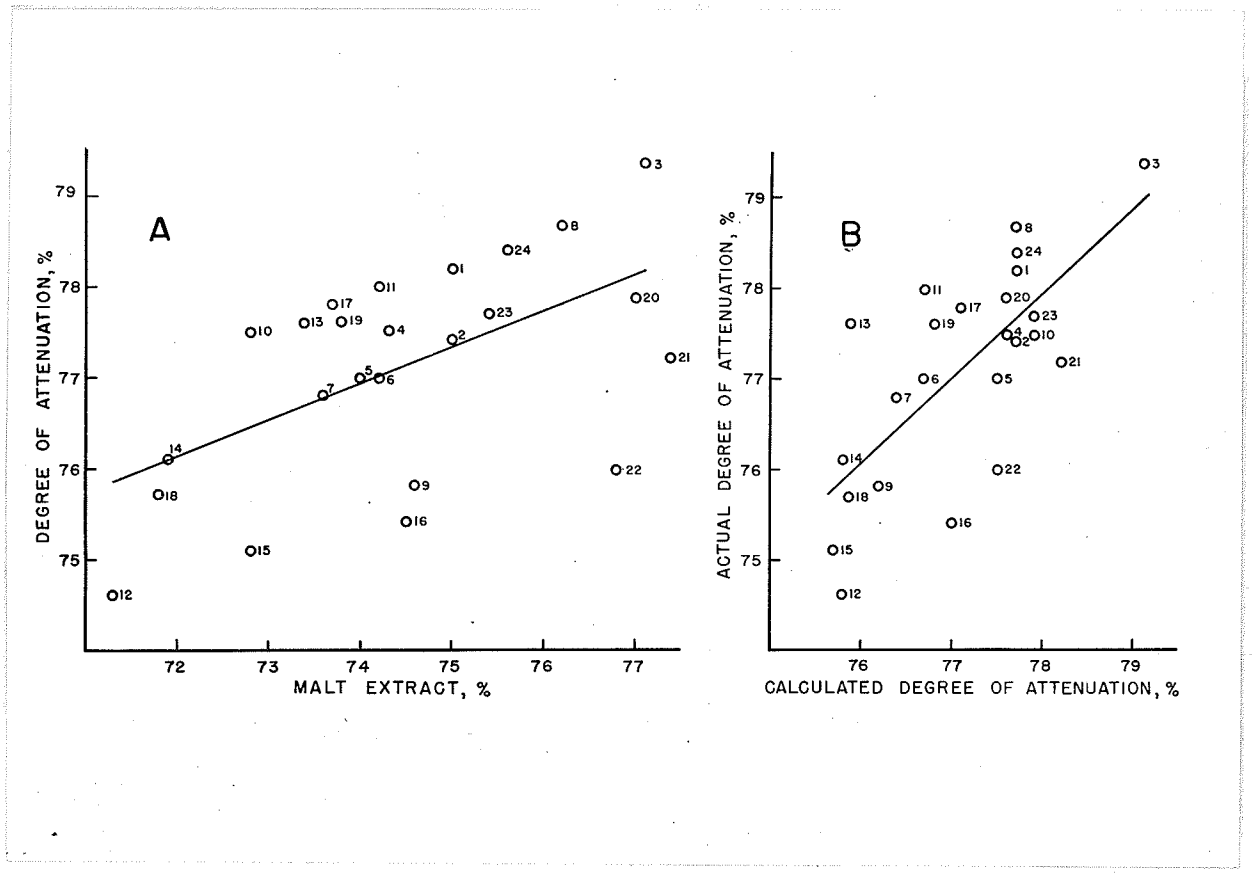

Figure 2.

Scatter diagrams for varietal means showing the relations between degree of attenuation and malt properties. In Fig. 1B the relation is that between actual degree of attenuation and values computed from the equation: Attenuation = 49.4 + .33 malt extract + .03 Lintner value. The key to varieties is given in Table VI.

account for the greater deviations of these particular varieties from the regression line. It should be noted, however, that it is an enzymatic property that is applied to the data represented in Fig. 2A to obtain the better estimate of degree of wort attenuation from malt properties shown in Fig. 2B.

Several additional multiple correlation studies were made, and two of these merit discussion. The multiple correlation coefficient of malt extract and saccharogenic activity with degree of attenuation is $R = .710^{**}$, while that of malt extract and wort nitrogen with viscosity is $R = .873^{**}$. The addition of wort nitrogen to the former and of saccharogenic activity to the latter multiple relation resulted in no significant increases in the coefficients.

The best estimates of initial and final turbidity, and of stability, from malt properties are obtained from the simple regression coefficients involving these properties and wort nitrogen content. The additions of malt extract and saccharogenic activity to the estimation of these did not improve the estimates significantly. The associations of wort nitrogen content with turbidity and stability are loose, though significant. As low wort nitrogen content is regarded as an indication of poor modification (12), it is probable that some particles of nitrogenous material are only partially degraded in worts that are low in nitrogen. The loose associations between wort nitrogen content and turbidity are thus in accord with the belief that protein material of high molecular weight is partially responsible for turbidity (31,32,34, 40,42,43,56).

The absence of significant relations between barley extract and wort properties was noted in previous paragraphs. In a sense, this

finding is expected as wort properties are a function of the physical and chemical changes that take place during malting and mashing. However, there are some curious aspects of correlation coefficients between barley and wort properties. The barley properties determine the malt properties (7,12,16), and the malt properties are related to the wort properties, as is shown in this study. Therefore, there must be missing factors in barley or malt analysis of which wort quality is a function.

It is noteworthy that the inverse relation between wort viscosity and wort nitrogen content is very pronounced in this study. Yet, this finding is contrary to the beliefs of Hopkins and Krause, who state (50, p.193) "that wort viscosity is caused by the quantity and quality of carbohydrate material", and also to that of Helm (45) who states "that proteins have no noticeable influence on wort viscosity". The implications of the hypothesis of Piratzky and Wiecha (71), who attribute wort viscosity of under modified malts to an amylan, must also be considered. Finally, the suggestions of Preece (72) that the role of the pentosans in barley and malt should be clarified also have some bearing on this problem. Nevertheless, it is useful to recall that wort nitrogen content is considered by some authorities to be the best single measure of malt modification, so that it may well be that the factors contributing to high wort nitrogen content also contribute to low wort viscosity.

As the combination of malt extract and wort nitrogen content gave a better estimate of wort viscosity than wort nitrogen alone, it appears that malt extract is related to wort viscosity in a manner that is independent of wort nitrogen content. Further relations involving malt extract were therefore examined and the results proved of value in elucidating at least one of the missing factors.

The combination of malt extract and barley extract was much more closely related to wort viscosity than was any other single factor. The multiple correlation coefficient is .900**, as compared with coefficients of -.559** and -.108 respectively, for viscosity with malt extract and with barley extract. Wort nitrogen content was then added to the multiple relation by the inverse matrix method (29) and the correlation coefficient was increased to .919**. This is not significantly higher than the multiple coefficient (.900) obtained from barley and malt extracts. Thus, wort nitrogen content is related to viscosity only in an indirect manner.

The relation of barley and malt extracts to wort viscosity was studied further and the regression equation was found to be

Wort viscosity =
A +0.057 barley extract -0.060 malt extract

The two regression coefficients were compared by the inverse matrix procedure (29), and no significant difference was found between them. Therefore, the factor that is related to wort viscosity is the difference between barley and malt extracts, which appears to be another single measure of modification. As the difference between extracts increases, viscosity also increases. Thus, it appears that wort nitrogen content and the difference between barley and malt extract are both measures of a factor in malt modification that determines wort viscosity. The difference in extracts is the best measure of this quality factor. This suggests that the material responsible is a carbohydrate.

Varietal differences in conversion of potential extracts of barley into malt extract may be due to differences in enzymatic activity or to differences in carbohydrate composition. Bishop and Marx (17) and Bishop (16), have shown the importance of "insoluble carbohydrates" and

pentosans in determining the extract obtained by malting, and their studies also indicated varietal differences in these constituents. The possibility of similar differences between the varieties used in this study are appreciable, but no such analyses were made on the barleys. Varietal differences in enzymatic activity are also likely to be involved in failure to obtain potential extract. The addition of both barley saccharogenic activity and malt saccharogenic activity to the multiple relation involving barley extract, malt extract, and wort viscosity, did not significantly increase the correlation coefficient. Unfortunately, neither alpha amylase activity nor autolytic saccharogenic activity determinations were made on the malts used in this study. All that can be concluded is that either barley composition or enzymatic activity inherently present in barley or developed during malting (or most likely a combination of both factors) determine wort composition and wort viscosity. Wort viscosity does reflect malt modification, which is the extent and degree of change from barley to malt, and this finding is in accord with the statements of Helm (45), Kolbach (54) and Piratzky and Wiecha (70).

The differences between barley and malt extract for each variety are plotted against wort viscosity in Fig. 3. This scatter diagram suggests that the relation between the two factors is curvilinear (dotted line), and various methods for reducing the relation to a straight line were tried. The best linear fit was obtained when the differences in extract were squared, and the resultant correlation coefficient was .934**. This coefficient is of a high magnitude for a biological series; the error of estimate of viscosity from the squared differences is only $\pm$0.02, which is not much greater than the standard error of the mean of

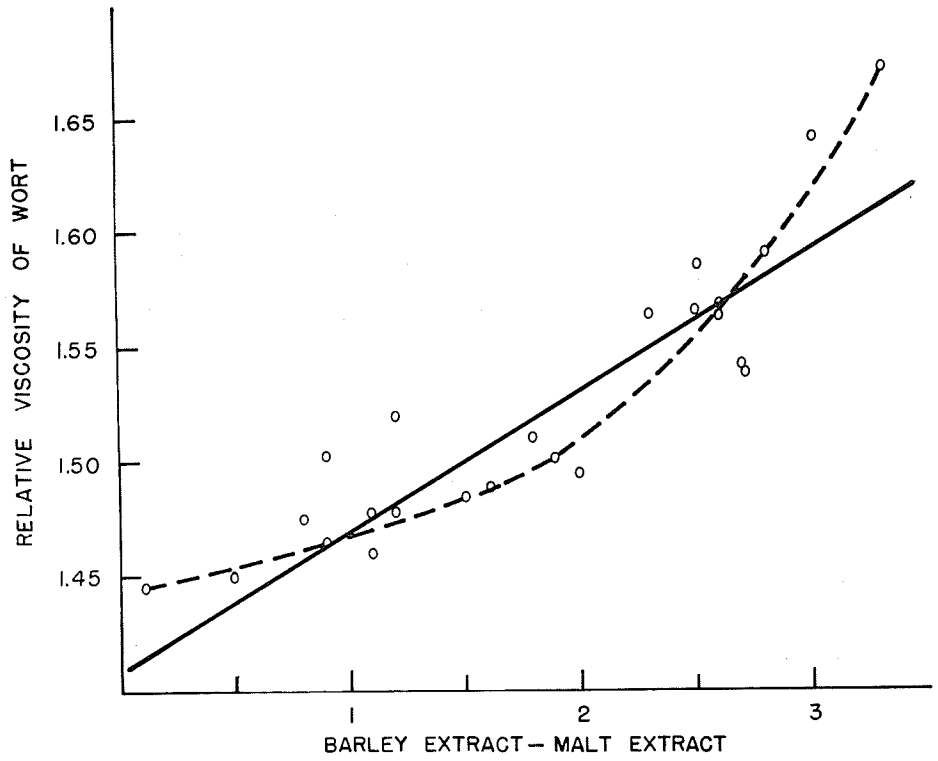

Figure 3.

Scatter diagram for varietal means showing relation between wort viscosity and difference between barley and malt extract. The solid line is the linear regression. The dotted line is an approximation to a curve.

duplicate viscosity determinations, ±0.01. However, though such a prediction is satisfactory, it is not practical. There is no point in predicting wort viscosity from a series of determinations that involve production of wort, more especially as the time consuming determination of barley extract is also required. Nevertheless, the relation between these properties is useful as it supplies some fundamental information on the cause of wort viscosity.

If barley extract is considered as a constant, an increase in the difference between this and malt extract requires a decrease in the latter. From this viewpoint, increased viscosity is associated with decreased malt extract. This is reasonable only if, as the amount of soluble material decreases, it also changes in character; for if no such change occurred the association would be reversed. In other words, varieties that contain greater amounts of intractable material, either as actual complexes or because of enzymatic deficiencies, yield more complex soluble substances that increase wort viscosity.

The main constituents of the solubles in barley and malt are carbohydrates, and it may be inferred that the materials contributing most to viscosity of worts are the complexes derived from the less soluble carbohydrate fractions of barley. Piratzky and Wiecha conclude that this is so when dealing with definitely under-modified malts (70). Although only a few varieties in this study are suitable for malting, and the non-malting varieties were under-modified, these latter are not as seriously under-modified as the short grown malts of Piratzky and Wiecha (70,71) and of Helm (44,45). Thus, it is not advisable to conclude that the viscosity is caused by an amylan similar to that of Piratzky and Wiecha. Further investigations were undertaken in order to determine whether carbohydrates were actually involved, and these are discussed in the following sections.

STUDIES ON MATERIALS CONTRIBUTING TO WORT VISCOSITY

INDIRECT CHARACTERIZATION OF MATERIAL: REDUCTION OF VISCOSITY BY ENZYMES

A commercially prepared malt was extracted for two hours at 70°C. with water, using the same ratios of malt and water as in the Congress procedure, and the mash was filtered in the usual way. Aliquots of the wort were treated with small amounts of commercially prepared proteolytic and amylolytic enzymes, and with sulphuric acid. The treated worts were maintained at room temperature overnight and viscosity measurements were then made. The results are given in Table X, and show that the alpha amylase preparation was the most effective agent in reducing wort viscosity, aside from boiling with acid. Extract of green malt was also very effective, but this extract contains many enzymes, some of which are not found in malt and others are greatly reduced in activity by kilning.

These data suggest that the material causing viscosity is a carbohydrate complex. It would be unwise to state that it is degraded by alpha-amylase as, although the alpha-amylase preparation is free from beta amylase, it was obtained from bacteria and may contain other enzymes such as the glucosidase described by Kneen (53).

These results suggest that the factors responsible for the differences in viscosity that were found in the studies of varieties are likely to be carbohydrate complexes. The enzyme preparation that was used for the determination of barley extract was of similar origin and prepared by the same firm as the alpha-amylase preparation that reduced viscosity of this study. It therefore seems likely that in preparation of barley

extract this enzyme system released some soluble carbohydrates from the barley that are not degraded completely by the enzyme systems developed during malting and active during mashing. The varieties may well differ in amount or activity of this particular enzyme.

TABLE X

Effect of Added Enzymes on Wort Viscosity

Treatment	Relative Viscosity	Treatment	Relative Viscosity
Check	1.60	Check	1.57
.1% β amylase[1]	1.48	.1% α amylase	1.28
.1% diastase[2]	1.46	.1% papain	1.42
.1% α amylase[3]	1.33	.1% α amylase and .1% papain	1.31
.1% papain[4]	1.44	Boiled and filtered	1.50
5% H_2SO_4 (cold)	1.54	5% H_2SO_4 (boiled 2 hrs. and filtered)	1.34
5% H_2SO_4 (boiled 2 hrs. and filtered)	1.19	25% extract of green malt (10% mash)	1.32

1. Beta-amylase for analytical purpose (determination of alpha-amylase) Wallerstein Laboratories.
2. Malt diastase, special for analytical purposes. Wallerstein Laboratories.
3. Alpha-amylase, special for analytical purposes. Wallerstein Laboratories.
4. Papain. City Chemical Corporation, New York.

Bishop and Marx (17) have shown that, as total carbohydrates in barley and the malt extract from the derived malt decrease, the amounts of cellulose and pentosans in the barley increase. Pentosans are the characteristic components of many plant gums and their cleavage products in malt and wort are likely to be more complex than starch derivatives; they would thus tend to produce an increase in viscosity with decrease in extract. Accordingly, it seems reasonable to suppose that pentosans

or similar carbohydrates may be responsible for wort viscosity. This suggestion is in accord with the beliefs of Piratzky and Wiecha (71) who isolated a viscous amylan from barley though they failed to find this in malt.

ISOLATION OF A VISCOUS PRINCIPLE FROM WORT

In order to investigate the hypotheses that certain carbohydrates not derived from starch are responsible for wort viscosity a fractionation study was undertaken.

To obtain preliminary experience, a sample of barley was treated by the method of Piratzky and Wiecha (71) for the extraction of the amylan. Repeated attempts were successful in isolating a gummy material that was viscous in solution and that gave negative tests for pentosans; but every attempt to purify the material caused the loss of the viscous properties. This is not surprising in view of the comments of Piratzky and Wiecha, "Vor allem trat dies hervor bei der uns wichtigsten Eigenschaft, der Viskosität in wässeriger Lösung. Je nach de Bedingungen der Gewinnung und Reinigung bekamen wir Zähigkeiten der 1 %ignen Lösung von 4,0 bis herunter zu 1,2 cp." Differences between barley types are also likely to have caused further difficulties in isolating the barley gum; Piratzky and Wiecha must have used a European two-rowed barley whereas a Canadian six-rowed barley was used in the present studies. It was, therefore, considered more advantageous to attempt to isolate complexes directly from wort.

Material and Methods

A commercially prepared malt was thoroughly mixed and split into a number of 1 lb. samples with a Boerner sampler. These samples were placed in airtight containers and stored at room temperature.

Mashes were made at temperatures ranging from 20-80°C. in 5° intervals. Fifty grams of malt were ground in a malt mill, and 250 ml. distilled water at mash temperature was added. The mash beaker was immediately placed in a mash bath maintained at the desired temperature, and the mashes were stirred continuously for two hours. The mashes were then cooled to about 20°C., and the beaker contents were made up to 450 grams as in the Congress procedure (1). The mashes were filtered through Eaton and Dikeman #509 filter paper in the usual way.

Viscosity measurements were made at 20°C. with an Ostwald viscosimeter on each wort, and specific gravity determinations were also made in the usual way. Total nitrogen and alcohol soluble nitrogen on each wort were determined on a 25 ml. aliquot. The alcohol soluble nitrogen is that remaining in solution after 70 ml. of alcohol is added to 25 ml. of wort and the mixture is allowed to stand overnight and is then filtered. It may be more accurately defined as nitrogen soluble in 70 per cent ethyl alcohol.

The addition of the alcohol to the wort produced a gummy precipitate that contained the material which contributed most to wort viscosity. An additional 25 ml. preparation of wort was treated with 70 per cent alcohol, and the mixture was centrifuged after 30 minutes. The supernatant liquid was removed by decanting and the residue was redissolved in 25 ml. of distilled water. Viscosity determinations were made on this solution of "gums", and the concentration of the material was determined by evaporating an aliquot to dryness and weighing the residue. The nitrogen content of this alcohol-insoluble gummy material is obtained by the difference between total and alcohol-soluble nitrogen in the wort.

Results and Discussion

The results of the various determinations are given in Table XI, and are shown graphically in Figs. 4A and 4B.

TABLE XI

Data on Gum Isolates from Worts Mashed at Various Temperatures

Temperature °C	Viscosity Wort	Viscosity Gums	Gum Concentration %	Nitrogen %	Extract % Wort	Extract % Malt
20	1.17	1.22	0.20	9.44	2.26	20.0
25	1.17	1.24	0.24	9.05	2.50	22.3
30	1.19	1.23	0.22	8.71	2.59	23.2
35	1.19	1.23	0.24	8.88	2.79	25.0
40	1.19	1.22	0.24	8.25	3.00	26.2
45	1.20	1.19	0.26	6.65	3.89	30.6
50	1.26	1.20	0.28	6.19	4.16	37.7
55	1.33	1.22	0.32	4.70	5.60	52.4
60	1.43	1.26	0.33	3.88	7.73	74.5
65	1.52	1.28	0.38	2.46	8.04	76.0
70	1.68	1.38	0.58	1.51	7.94	75.6
75	1.74	1.44	0.77	0.90	7.84	74.0
80	1.88	1.60	1.24	0.77	7.74	73.0

Fig. 4A shows that changes in mashing temperature did not effect wort viscosity to any marked extent until 50°C. was reached, but that further increases in temperature caused a considerable and steady increase in viscosity. The percentage of wort solids increased sharply after 55°C., and these contribute to viscosity. It seems best to discuss the curve in Fig. 4B before discussing the second curve in Fig. 4A. Fig. 4B shows the curve for maltose solutions of equal specific gravities to worts represented by corresponding points in Fig. 4A. Accordingly, the difference between the wort curve (Fig. 4A) and the maltose curve (Fig. 4B) shows the extent to which the worts are more viscous than maltose solutions of equivalent concentrations. The curve for the gum solutions

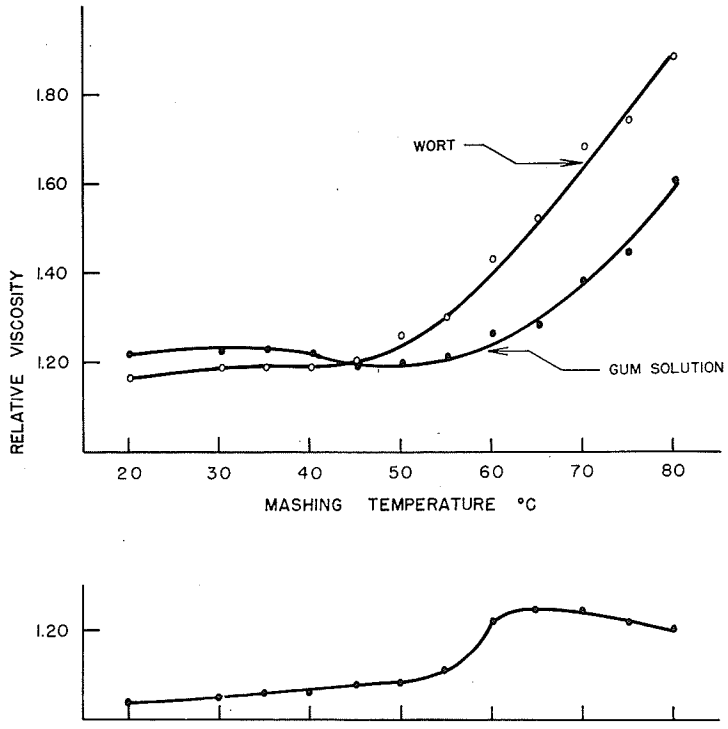

Figure 4.

Curves showing effect of mashing temperature on viscosity of wort and gum solutions (A), and contribution of wort solids, as maltose, to viscosity of wort on same ordinate (B).

in Fig. 4A indicates that this is the principal material causing the difference in viscosity between worts and maltose solutions of equivalent density. The concentrations of the gum ranges from 0.2% at 20°C. to 0.77% at 75°C. and 1.24% at 80°C.

The viscosities of the gum solutions derived from extraction at temperatures between 20° and 40°C. are higher than those for the corresponding worts. After 45°C., the gum solutions were lower in viscosity than the worts and the difference increased with increase in viscosity. These conditions are probably the result of some changes that take place in the composition of the precipitate with increase in mashing temperature. There was a slow decrease in nitrogen percentage of precipitates from 20° to 40°C., and then a sharp and steady decrease to 75°C. Although this decrease in nitrogen percentage is caused partly by increase in amount of material precipitated, the absolute amount of the nitrogen precipitated also decreased. Aside from these changes in chemical composition, the material also changed in properties. The precipitates from worts prepared from 20° to 40°C. and at 80°C. produced opaque aqueous solutions. These were centrifuged before viscosity determinations were made, but were still opaque as only minute amounts of material were actually thrown down by the process. Since the materials from the low temperatures of extraction were very high in nitrogen content, it seems safe to conclude that they were mainly proteins that had been denatured by the alcohol yet still retained a hydrophilic capacity. The opacity of the solution of material derived from 80° extract may be well attributed to long chain dextrins and denatured proteins. At this temperature, the saccharifying enzymes would be almost completely inactivated so that starch hydrolysis would be due mainly to effect of

temperature alone. There was also a sudden increase at 80°C. in amount of nitrogen precipitated. These results show that the isolates causing wort viscosity contain nitrogen, a considerable amount when isolates are derived from low temperature mashes; but the variations in nitrogen content are inversely related to viscosity of wort and of solutions of the isolate so that the complex causing viscosity is not likely to be a protein.

Larger scale isolations were undertaken in order to study the material precipitated by alcohol. As the material precipitated from mash at 70°C. was low in nitrogen and produced highly viscous aqueous solution, and as the wort filtered rapidly, mashing at 70°C. was selected as a standard condition for the larger scale experiments. Sufficient malt was mashed to provide 3 litres of wort and to this was added three volumes of ethyl alcohol. After standing overnight, the liquid was centrifuged and the precipitate was washed twice with alcohol and once each with ethyl ether and petroleum ether. The product, a brownish gummy mass, was air-dried and ground.

The product was subjected to nitrogen and viscosity determinations, to tests for starch, to the Molisch test for carbohydrates, and to the orcinol test for pentosans (67). The results of tests on three isolates are given below (Table XII). The tests for starch were negative but the presence of carbohydrates and pentosans were indicated. It is unlikely that the material contains dextrins, since these are not precipitated by a concentration of 70% alcohol (15).

The concentration of the material is quite low and the yields are variable, but the viscosity of the aqueous solutions and nitrogen content are inversely related to yield. It thus appears that further

work on the standardization of the methods of isolation is required. The yield of product No. 2 is very low, but it was observed during preparation that the alcohol was impure and this may have been a contributing factor in lowering yield. The maximum solubility of all products was about 1% and the solutions were opaque. Although there is a direct relation between nitrogen content of product and viscosity of solution, the role of nitrogen in the properties of the material is not clear. Papain did not materially reduce the viscosity of product 1A, but an alpha amylase preparation caused a considerable reduction in viscosity.

TABLE XII

Characteristics of Isolates of Gum

	Isolate 1A	Isolate 2A	Isolate 3A
Yield from 3 l. wort	12 g.	5.3 g.	10 g.
Relative viscosity of aqueous solution	1.57 c = 0.8%	1.95 c = 0.63%	1.88 c = 0.8%
Relative viscosity after treatment with —			
0.1% α amylase	1.18		
0.1% papain	1.41		
Nitrogen content, %	2.18	3.20	2.40
Molisch Test	+	+	+
Pentosan Test	+	+	+
Starch Test	-	-	-

In order to remove nitrogen from the material, the products digested with 30 per cent NaOH (25 ml. per g.) for two hours at 100°C. The solutions were quite brown during early stages of digestion, but they later became light yellow with a brown flocculent precipitate. This

precipitate disappeared when the solution was cooled and neutralized with HCl. The solutions were filtered to remove any undigested particles and three volumes of alcohol was added. The resultant precipitate was recovered in the same way as the original product. These products were lighter in color than the original product, indeed 1 B-2 was almost pure white. All products gave positive tests for pentosans. Nitrogen, viscosity, and other data are given below. They were all very low in nitrogen content and much more viscous than the original products. In order to obtain information on the type of the material, viscosity measurements were made at various dilutions and the results are included in Table XIII. It is also interesting to observe that the viscosity of 3B was considerably reduced by an alpha amylase preparation.

TABLE XIII

Characteristics of Gum Isolates after Alkali Treatment

	Isolate							
	1-B1		1 B-2		2B		3B	
Yield, %	37.5		25.0		64		16	
Nitrogen, %	0.26		0.52		0.20		0.39	
	Vis.	Conc.	Vis.	Conc.	Vis.	Conc.	Vis.	Con
	2.62	.90	3.43	.93	1.89	.44	5.07	.90
	2.07	.72	1.73	.46	1.51	.22	4.09	.72
	1.76	.54	1.42	.23	1.25	.11	2.97	.54
	1.57	.36	1.26	.12	1.13	.05	2.00	.36
	1.36	.18	1.18	.06	1.08	.025	1.40	.18
	1.21	.09					1.22	.09
							1.12	.04
pH 1% soln.			6.4		9.2		4.5	
Viscosity of 1% solution after digestion with alpha-amylase preparation							1.34	

The yields of the products are variable, as are the pH values of the solutions. This further emphasizes the need for careful standardization of isolation technique. There is, however, an inverse relation between yield and viscosity which suggests that the products obtained in highest yield are still impure. Relative viscosity is plotted against concentration for each isolate in Fig. 5A. The four figures appear to belong to one family and the single curve in Fig. 5B was constructed by adjusting the isolates 1-B1, 1 B-2 and 2B using the curve 3B as a reference. For example, the 0.93 concentration 1 B-2 is equivalent in viscosity to an 0.62 concentration of 3B, so that all concentrations of 1B were adjusted by this ratio. The curve in Fig. 5B is artificial but it indicates that the isolates correspond quite well in viscous properties. There is some scatter of points up to about 0.2% concentration but this is to be expected when dealing with such dilute concentrations; moreover, at such low viscosities, the electro-viscous effect is most pronounced, (Bull, 25. ch. 13).

The use of viscosity measurements as an indication of chemical constitution has been reviewed by Fuoss (36) who discusses the great increase in viscosity of liquids caused by polymers in dilute solutions. It is thus safe to assume that the isolates are polymers. Low nitrogen contents suggest that the isolates consist principally of polysaccharides. High viscosity is characteristic of the plant gums, the constituents of which are pentosans, often in conjunction with uronic acids (37, p.649); so that it appears likely that this material is derived from the pentosans of barley. Fuoss (36) states that useful information on the type of polymers can be obtained by plotting ratio of viscosity increment over concentration against concentration $\left(\frac{\eta r - 1}{c}\right.$ against c.$\left.\right)$. He writes, "For neutral

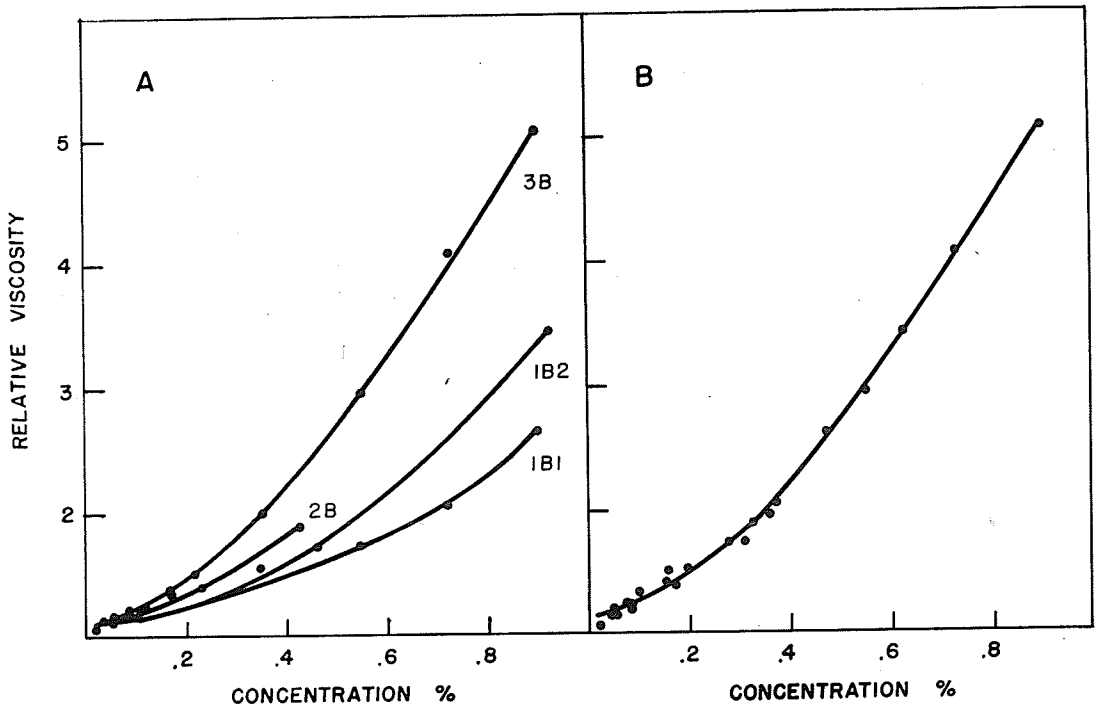

Figure 5.

Curves showing relation between viscosity and concentration of solutions. In Fig. 5A separate curves for each isolate are given. Fig. 5B shows a composite curve, after adjusting concentrations of all isolates in reference to isolate 3B.

polymers, the data lie on a straight line which climbs with increasing concentration. For polyelectrolytes, the data give a curve that climbs very sharply with decreasing concentration." This function of viscosity and concentration discussed by Fuoss is plotted for each isolate in Fig. 6A. The curves have similar shapes and these resemble that of a polyelectrolyte system. Data for the composite curve in Fig. 5B are also plotted in a similar manner in Fig. 6B; the resulting curve indicates a polyelectrolyte. It is therefore likely that the material responsible for viscosity is a polysaccharide that contains uronic acid groups. The pH values of the solutions confirm this conclusion. Moreover, there is a direct relation between acidity of product and viscosity. The role of nitrogen in the product is not clear. There was a considerable reduction in nitrogen content from the original product to the final product, and this was accompanied by an increase in viscosity. But it may well be that the basic material from which the viscous principle is derived is a protein-carbohydrate complex.

It seemed worthwhile to construct a balance sheet for viscosity from wort to final product, and this is given in Table XIV. The data indicate that the variation in yields is practically counterbalanced by the viscosity of the material obtained. Thus, each of the four final products that were obtained account for the fairly high proportion of the original viscosity. Agreement between the calculated contribution of the gums and the actual contribution of the products obtained is best when the gum was not actually purified, that is, when it was redissolved without drying. The agreement is not so good when the products were isolated, dried, treated, etc. When all manipulations, and probable losses due to incomplete precipitation, are taken into account, the agreement

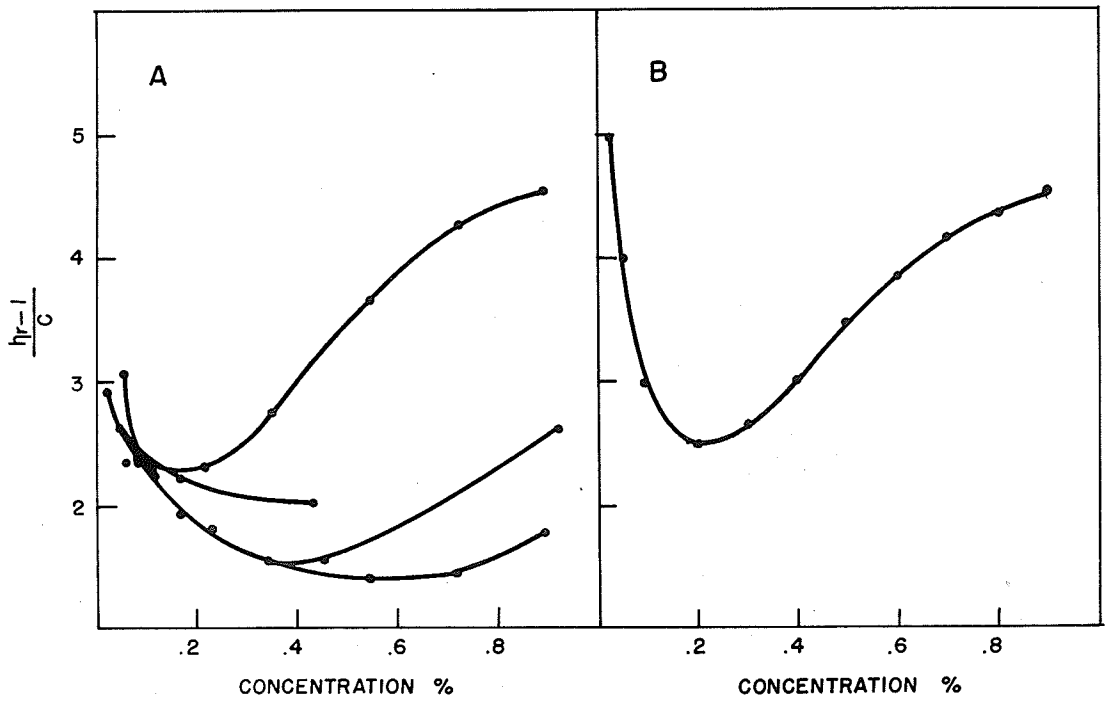

Figure 6.

Curves showing relation between viscosity increment per unit concentration and concentration. In 6A separate curves for each isolate are given. A composite curve derived from Fig. 5B is given in 6B.

is satisfactory for the present stage of investigations. The concentration of the viscous principle is about 0.1% in the wort, which represents about 1% of the malt.

The viscous principle of wort is concentrated by precipitation with alcohol, and further concentrated by digestion with alkali followed by acidification and precipitation with alcohol. The yields are low and variable, but there appears to be a balance between yield and viscous properties of the isolates. This is the stage at which the work discussed in this thesis was terminated. The material precipitated from wort by 70% alcohol must be a complex of several different compounds, and plans for further research include studies diverted towards improvements in technique of isolation of the compounds. When these improvements have been made, efforts will be made to determine the constitution of the compounds.

TABLE XIV

Balance Sheet for Wort Viscosity

Viscosity of wort prepared at 70°C.	1.68
Viscosity of maltose solution of equivalent density	1.24
∴ viscosity of additional material	1.44
Viscosity of gum solution obtained from 70°C. wort (direct solution after precipitation).	1.38

Isolate	Conc. A.	Yield B	Final Concentration B	Viscosity at Final Concentratio
1	.4	38%	.15%	1.30
	.4	25%	.10%	1.25
2	.18	64%	.12%	1.30
3	.33	16%	.05%	1.20

GENERAL DISCUSSION

The looseness of the inter-varietal relations among the wort properties is an indication of the complexity of the factors governing wort quality. Although, in general, poor attenuation is accompanied by high wort viscosity, and high viscosity is accompanied by high wort turbidity and poor stability, the relations are such that one must conclude that many factors are involved. The point is further emphasized by the generally low magnitudes of the correlation coefficients between the wort properties and the barley, malting and malt properties.

Among the 24 varieties studied, the wort qualities--degree of atenuation, viscosity, and turbidity--are related to malt extract, malt saccharifying activity, and wort nitrogen, and to barley salt-soluble nitrogen, steeping time, and malting loss. The relations are not particularly close, but they are statistically significant. Further, the wort qualities are not related to barley extract, starch, or β-amylase activity after activation by papain. Therefore, the wort qualities appear to be related to enzymes other than β-amylase that are developed during malting and that change the barley materials into malt extract during mashing. These wort qualities also reflect the extent to which starch and protein are degraded in the wort and are indicative of molecular size and type. That is, the wort properties reflect the degree of modification of malt.

The outstanding feature of the correlation studies is that wort viscosity can be almost completely accounted for by an "extract factor". This is the difference between amount of extract obtained by mashing

barley with preparations of alpha-amylase and beta-amylase, and the amount of extract obtained by mashing the malt obtained from the barley by the regular procedure. The discussions of Bishop (16) and Bishop and Marx (17) emphasize the importance of quality of the carbohydrates of barley in determining the amount of extract obtained from the corresponding malt. They indicate the importance of the "insoluble" carbohydrates and the pentosans in reducing malt extract.

The present studies suggest that not all the carbohydrates that are degraded by the two enzyme concentrates used in preparing barley extract appear as soluble derivatives in malt. This failure to obtain potential extract has also been studied by Le Corvaisier (27) and Le Corvaisier and Duvivier (28). These workers eliminated the effects of intrinsic saccharogenic activity in the malt, fineness of grind, etc. by autoclaving a malt mash and then mashing with enzymes as in the determination of barley extract. The extract yields obtained were invariably higher, and frequently much higher, than those obtained by the regular mashing procedure. They conclude that optimum modification is obtained when similar results are obtained by mashing malt by both procedures.

The failure to obtain from the malt the potential extract in the barley may be caused by enzymatic deficiencies or by "intractable" carbohydrates. The present study has shown that the carbohydrate material in the barley that is not made soluble during malting and mashing has a marked influence on wort viscosity. Further, the material causing high viscosity in worts made at high temperatures can be degraded by the alpha-amylase preparation that is used in the determination of barley extract. This alpha-amylase preparation is obtained from bacteria and it may well be that it contains other carbohydrates, such as the limit dextrinase

discussed by Kneen (53).

Varieties may therefor differ in content of this enzyme system, or of some such similar system, or in the carbohydrate complexes in the barley. The results of the present studies do not provide definite information as to which condition actually exists. Nevertheless, there is a strong suggestion that wort viscosity is caused by a complex polysaccharide that probably contains acidic groups. This is shown by the data obtained from the material isolated from wort. There is, however, another indication that the cause of wort viscosity is a polyelectrolyte system.

It was found that a curvilinear relation was obtained when the wort viscosities of different varieties were plotted against differences between barley and malt extract. This curve is typical of that found when viscosity is plotted against concentration, and it suggests that when more "intractable" materials are present in the barley the resultant wort of corresponding malts contains more complex carbohydrates. Thus, complexity of wort carbohydrates is a direct function of the amount of resistant carbohydrates in the barley.

It seems worthwhile, therefore, to consider the differences between barley and malt extracts as representing concentrations of some factors in the wort, and to plot viscosity increments per unit concentration against concentration. This curve was shown in Fig. 7 and is a striking expression of a polyelectrolyte system. This result may be fortuitous, but when considered in conjunction with data obtained on the isolates from wort, it strongly suggests that the viscous principle of wort is a polysaccharide that contains uronic acids.

The origin of this viscous principle has yet to be determined,

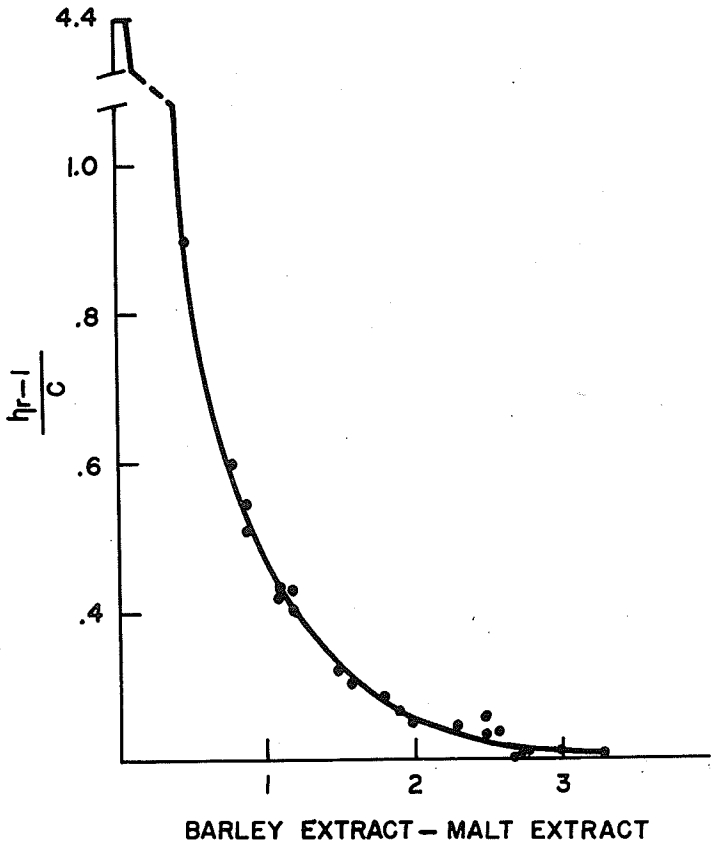

Figure 7.

Scatter diagram for varietal means showing relation between viscosity increment per unit and difference between barley and malt extract.

but some information is available on the probable source. The cell walls surrounding starch granules are digested by cytase during malting, and the starch is then exposed to attack by amylases. This cell wall material is considered by Preece (72) to be similar to hemicelloses and he has shown that spent Brewers' grains contain two urono-xylans, a urono-araban and a hexosan. Some soluble products will probably be obtained from these compounds during malting and mashing. It is recognized that wort does contain pentosans and pentoses (47, p.665; 50, p.242), but no particular properties have been assigned to them except the generalization that they may have some influence on palatefulness and foam retention of beer.

Preece (72) also suggests that the increase of uronic acids during malting is lost during kilning and that this is probably due to decarboxylation. An apparent increase in pentosans in malt would thus occur, as the decarboxylation would give rise to pentosans. Acid treatment of hexuronic acids also produces furfuraldehyde, which is characteristic of pentosans, so that positive tests for pentosans are obtained from hexuronic acids.

The identification of the constituents of the hemicelluloses or gums that exist in barley, malt and wort, requires the isolation and identification of each component by special technique. Preece (72) indicates that he is working on this problem. As the studies presented in this thesis show that the gums play a more important role in determining malt quality than has been previously assigned to them, plans for future research include further studies on the barley gums.

The relations between soluble nitrogen and wort viscosity suggested at first that complex nitrogenous compounds determine wort viscosity. Detailed examination of the available data revealed that

complex carbohydrates, as indicated by differences between barley and malt extracts for twenty-four varieties, were the main factor influencing viscosity and that the effect was independent of wort nitrogen content. Nevertheless, there appears to be an interrelation between protein and carbohydrate that is also shown by the high nitrogen content of the material precipitated from viscous worts by alcohol. Part of the protein is probably extraneous and does not belong to the complex that causes viscosity, but the final products were not entirely free from nitrogen. Though it appears that the protein plays no great role in determining viscosity, some other factors are worth considering.

The present studies have shown that the degradation of protein and carbohydrates appear to take place simultaneously with decreases in wort viscosity, but that the degradation of carbohydrates is the main factor influencing wort viscosity. Le Corvaisier and Duvivier (28) state that during malting, "Le solubilisation des pentosans suit celle des albuminoides, suivant les differents malts; il y a neanmoins quelques ecarts de proportions pour certain malts". Proteolytic enzymes are developed during the early stages of malting and production is about maximum by the fourth day of growth (50, p.134). Preece (72), in discussing pentosan development, indicates that total soluble pentosans increase sharply from germination to about five days' growth and then the amount levels off. Thus the cytases and proteolytic enzymes develop simultaneously. There is however another possibility that should be considered.

Protein is distributed throughout the endosperm of grain, and, as cell wall material is known to be attacked during the malting process, there is a possibility of the existence of a complex containing both protein and hemicelluloses. The degradation of this complex may yield

both protein and pentosan cleavage products. Earlier work by Sallans and Anderson (76) has shown that rate of absorption of water during steeping of barley is directly related to amount of salt soluble nitrogen. Thus protein appears to play some role in diffusion throughout the kernel. The highly viscous gums may also play some role in determining rate of diffusion. But whether this role is independent of that played by protein must in the meantime remain a moot point. The identification of carbohydrate complexes has reached a higher stage of development at the present time than the identification of protein complexes, so that the question of possible protein-carbohydrate complexes must await identification of the carbohydrate complexes.

The inter-relations among the various barley, malting, malt and wort properties have been discussed as contributions towards an improved understanding of the chemistry of the materials and processes involved in malting and mashing. Much remains to be accomplished in this field, especially in determining the changes in the complex carbohydrates and the effects of the degradation products on malt and wort quality. Indeed, it is likely that some of the effects will not be demonstrable without access to brewing trials. These are not yet available in Canada. Nevertheless, the studies reported herein provide some information that is of immediate use to plant breeders.

Differences between varieties were shown to exist in degree of atenuation, viscosity and turbidity of wort made from barley malt. O.A.C.21, Mensury and Olli, which are acceptable malting varieties, produced worts that were high in degree of attenuation and low in viscosity and turbidity, which are desirable features. Only two of the varieties that are not acceptable in malting quality by the general standards were similar to

O.A.C.21 in wort properties. The results indicate that the use of wort quality determinations provide a more rigorous test by which to screen varieties for suitability as parental material in barley breeding programmes. The determinations on wort may also be used in selecting the best parental material from a group of varieties that are equally poor in malting quality as judged by the commonly measured properties but which are the sole source of some particular factor that a plant breeder wishes to incorporate into his hybrids.

There is good reason to believe that when a variety is shown to be unsatisfactory in malting quality by laboratory tests it will also be unsatisfactory for commercial malting and brewing. However, when a variety is promising in laboratory tests there is no guarantee that it will be satisfactory for commercial purposes; for the laboratory malting test may fail to discover factors that may later militate against the use of the variety by the malting and brewing industries. Accordingly, additional criteria of malt quality, especially those that have a bearing on practical malting and brewing, are required in the laboratory test.

The study of the relations between the various properties has produced information that wort quality depends on degree of malt modification. If the molecular size and type of the wort solids are dependent on the complexes present in the barley and the changes that are made in these by the enzymes present in the barley or developed during malting, a similar condition should obtain in commercial worts. It seems likely that quantitative and qualitative differences, particularly in fermentability and viscosity, would persist in some degree through boiling, hopping and fermentation, into the beer. There is thus good reason to believe that analytical results for laboratory worts made from laboratory

malts provide a practical basis for making reasonably accurate predictions about the commercial acceptability of new varieties.

SUMMARY

(1) Determinations of barley, malt, malting and wort properties were made on 144 samples of barley representing 24 varieties grown at each of six widely scattered points in Canada.

(2) Studies were focused mainly on wort properties. Methods for measuring turbidity, viscosity and degree of attenuation of wort were improved and standardized. Turbidity was measured immediately after wort filtration (Initial turbidity) and again after wort had been kept at 10°C. for 24 hours (final turbidity) by means of a Zeiss Pulfrich Nephelometer. Viscosity was measured at 20°C. in an Ostwald viscosimeter. Degree of attenuation, which is a measure of fermentability of wort, was measured by determining specific gravity of wort before and after fermentation by yeast.

(3) Varietal and station differences were demonstrated in these wort qualities. Such varietal differences have not been shown previously. The mean values and ranges for the 24 varieties were: initial turbidity, 37% and 84%; final turbidity, 162% and 161%; viscosity, 1.52 and 0.23 centipoises; degree of attenuation, 77.1% and 4.8%.

(4) Inter-varietal relations between the wort properties were examined by means of correlation studies. The significant relations were: degree of attenuation and viscosity were inversely related ($r = -.606^{**}$); viscosity was directly related to initial ($.532^{**}$) and final turbidity ($.678^{**}$); initial and final turbidity were closely and directly related ($.875^{**}$). The correlation coefficients,

with the exception of that between initial and final turbidity, were not of high magnitude. This indicates the complexity of the factors contributing to wort quality.

(5) The following determinations were also made: percentage heavy grade, 1000 kernel weight, total nitrogen, salt-soluble nitrogen, starch, extract and total saccharogenic activity of the barley; steeping time and loss in weight during malting; extract and saccharogenic activity of malt and wort nitrogen content.

(6) The relations between the wort properties and the barley, malting and malt properties were examined by means of correlation studies. Significant correlations were found more frequently between the wort properties and the malting and malt properties than between the wort properties and the barley properties.

(7) Initial wort turbidity was directly related to length of steep required during malting (.534**) and inversely related to malting loss (-.439*) and wort nitrogen content (-.438**). Final wort turbidity was directly related to length of steep (.559**) and inversely related to salt-soluble nitrogen in the barley (-.424*), malting loss (-.550**), malt extract (-.487*) and wort nitrogen content (-.594**). The correlation coefficients involving wort nitrogen content are in accord with existing opinion that wort turbidity is an expression of high molecular weight nitrogenous complexes.

(8) Degree of attenuation was directly related to salt-soluble nitrogen in the barley (.424*), malt extract (.572**) and saccharogenic activity (.550**) and wort nitrogen content (.461**). The multiple correlation coefficient involving degree of attenuation, malt extract

and malt saccharogenic activity was significant (.710**). Degree of attenuation is thus shown to be related to both amount of extract and degree to which starch may be degraded by the amylases.

(9) Wort viscosity was inversely related to salt-soluble nitrogen in the barley (-.671**), to malting loss (-.810**), to extract (-.559**) and saccharogenic activity of the malt (-.520**), and to wort nitrogen content (-.842**). The highest correlation coefficient was that between viscosity and nitrogen content of wort. However, further examination of the data revealed that differences in wort viscosity was most closely related to the varietal differences between barley and malt extracts (.900**), and this relation was independent of wort nitrogen content.

(10) It is inferred from these studies that the wort qualities are measures of the changes in the kernel constituents that take place during malting and mashing. They are therefore a measure of malt modification. Among the varieties studied, as wort nitrogen content increases its state of subdivision also increases and this produces a more stable wort. Similarly, as more extract is produced, greater amounts of the simple sugars are formed and these are more readily fermented by yeast. The changes in total extract also influences wort viscosity. As amount of malt extract obtained approaches the potential extract in the barley, the extractables appear to be transformed into simpler compounds and wort viscosity is thus reduced.

(11) As the solubles of both barley and malt are predominantly carbohydrates, it is suggested that the principal factors causing high wort viscosity are complex carbohydrates. Additional studies

of wort viscosity were made on a commercial sample of malt. Wort viscosity increased with increase in temperature of mashing. Treatment of such viscous worts with papain, a proteolytic enzyme, did not reduce viscosity. Treatment with an alpha-amylase preparation caused a marked decrease in viscosity. This evidence is consistent with the hypotheses that the viscosity of worts is due to carbohydrate complexes.

(12) Worts prepared at temperatures ranging from 10°C. to 80°C. were fractionated by making up to 70% alcohol concentration. A highly viscous gum was precipitated by this procedure. When the gums were redissolved in water to the original concentration in wort, the solutions were almost as viscous as the original worts.

(13) Viscous gums were obtained in the dry state after fractionating wort from mashes made at 70°C. The gum contained about 3% nitrogen and was degraded by an alpha-amylase preparation. The gum was digested with sodium hydroxide and reprecipitated from acid solution. A second product was obtained that contained only about 0.3% nitrogen. This appears to be the factor mainly responsible for wort viscosity. It contains no starch or dextrins, gives positive tests for pentosans, and is also degraded by an alpha-amylase preparation.

(14) The characteristics of this final product were studied by means of viscosity measurements at various low concentrations. The viscosity-concentration curves suggest that the material is a polyelectrolyte. It is probably a complex of hexosans, uronic acids, and pentosans, that is derived from the barley gums. The significance of these barley gums in relation to malt quality has not been adequately established and no significant role in influencing wort quality has previously been assigned to pentosans.

(15) In spite of the indications that nitrogen compounds do not have a direct effect on wort viscosity, it is suggested that they are indirectly involved. Degradation of proteins and pentosans take place simultaneously during malting with an accompanying decrease in wort viscosity. It is suggested that the material in barley that gives rise to compounds responsible for high wort viscosity is a protein-carbohydrate complex.

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