CYTOLYSIS FACTORS AS CRITERIA IN THE EVALUATION OF MALTING QUALITY IN BARLEY BREEDING PROGRAMS

by

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ABSTRACT

Cytolysis, the enzymic degradation of the grain endosperm cell-walls, is the initial activity in malting. The hydrolysis of gums, viscous polysaccharides of cell-wall origin, exposes various substrates to attack by appropriate enzymes and thereby sets in motion the modification of barley to malt. In the past, the lengthy germination time in malting permitted extensive enzyme synthesis and gum degradation, and consequently rate of cytolysis was not a limiting factor. Presently, trends in the malting and brewing industries that include accelerated and continuous processing, further increases in the use of adjunct cereals and the advent of materials such as barley syrups, are focussing attention on gum content and rapid development of cytolytic activity. Therefore, the cytolysis properties of present barley cultivars, and those under development in plant breeding programs, are important criteria of quality, and can be expected to have some influence on the course of technological changes in industry. This study was undertaken with the object of assisting Canadian barley breeders to develop cultivars having the quality characteristics that are foreseen.

Varietal and environmental differences in barley gum content and cytolytic activity developed during malting were investigated and these were shown to be associated with quality measurements. A range in these cytolytic properties was found within, and notably between, the six-row and two-row species. It is evident that there are sources of desirable characteristics whose use in plant breeding should result in quality enhancement in the barley crop, particularly in two-row

cultivars. These findings were augmented by study of the changes in cytolysis factors that occur early in the malting process and interactions of enzyme systems with gibberellic acid used to stimulate their synthesis in malting. The results, along with those of a study of thermal properties of the cytolytic enzyme system, revealed additional differences in the behavior of barley species and cultivars during malting and provided information useful in the evaluation of quality. Finally, new techniques for gum determination and automated assays of enzyme activities were developed. They will make possible the efficient selection of high quality lines in plant breeding programs.

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SECTION ONE

PERSPECTIVE

INTRODUCTION AND OBJECTIVES

For centuries beer has been made by the action of endogenous enzymes, developed during the germination of barley, that modify grain constituents in malting and produce soluble, fermentable materials in brewery mashing. Malting and brewing have grown from cottage crafts into today's industrial complexes - but the raw material has remained unchanged. At the present time, however, there are several proposals for changes in methods of beer production, chiefly from the economic viewpoint. In malting, attention is focussed on continuous operations combined with acceleration in germination and on the reduction of costly losses due to respiration and growth. In brewing, there are indications that the use of raw cereals as adjuncts to malt, long-established in North America, will increase and also become the normal practice in other regions of the world. Enzyme sources other than traditional malt are being explored. Thus the technologies of malting and brewing are in a transition state.

It is difficult to forecast the eventual nature of the brewer's raw material. A syrup made from grain, not necessarily barley, converted by enzymes of bacterial or fungal origin appears to be economically attractive. On the other hand, the accelerated production of green-malt enzymes may be competitive with the microbiological sources. The use of green-malt and raw barley has the advantage of similarity to traditional material and could have more appeal than unfamiliar products that may be difficult to control. It appears at first sight that the most suitable green-malt will be high in enzyme activity. However, it is uncertain which enzyme systems are the most important and in what order their

synthesis is desirable in malting. Universal high levels of activity may not be necessary. Equally uncertain is the effect of external stimuli on the order and extent of synthesis. Substrate degradation during malting, or in the conversion of raw grain by green-malt or other enzymes, is important, but again it is difficult to define optimum levels. More certain is, that whatever the optima are, they must be reached rapidly, and this depends largely on the varietal characteristics of the barley used for malting.

It is not unlikely that the present concepts of malting quality will undergo revision as more information becomes available. At the same time there is an urgent need for more information on barley feed quality and its relation to malting quality. There are indications that the two qualities are not incompatible. In barley high feed energy arises from the same source as high malt extract and in each case rapid enzymic conversion is desirable. It is suggested that the study of varietal characteristics should proceed with both malting and feed qualities in mind and with a view to examining the hypothesis that they are not dissimilar.

It is recognized that the progress and extent of modification or conversion of barley to malt can be monitored by the measurement of the viscosity of extracts of the grain. Viscosity is mainly due to non-starch polysaccharides. These materials originate in the endosperm cell-walls where they are probably associated with protein which may indirectly influence viscosity (18). The softening and eventual dissolution of the cell-walls by enzyme systems that develop during germination, is the key process in modification. The cell contents, the substrates

of other enzymes that are being synthesized, are rendered accessible to degradation in malting, and to conversion to soluble matter in subsequent mashing. Extract viscosity is an index of cell-wall degradation and therefore of the modification that depends on this preparative step.

The first study of viscous barley components or gums was made by O'Sullivan in 1882. He isolated from barley two polysaccharides that were soluble in water at 35-38°, one predominantly glucosan in nature, the other a hexosan-pentosan complex. These he referred to as alpha- and beta-amylan. Between 1890 and 1906, the barley gums were further investigated by Lintner and by Brown. It was Brown who observed that the dissolution of the highly colloidal gum of barley is one of the most significant changes made during the conversion of barley to malt. Interest in the gums was revived in 1938 when Piratzky and Wiecha (23) described a viscous material corresponding to O'Sullivan's alpha-amylan but uncontaminated by pentosan. This material was present in a 3-day malt but not in fully modified malt. These workers had previously observed that the viscosity of malt extracts, or worts, decreases with the progress of germination, and they related modification to the fall in wort viscosity and the enzymic hydrolysis of barley gums.

In 1945, Meredith and Sallans (19) suggested that wort nitrogen appeared to play a major role in determining wort viscosity and that the relation between the two properties was inverse. In a detailed study of wort properties, undertaken with the object of assisting Canadian barley breeders to develop high quality cultivars, Meredith (18) showed that wort viscosity reflected malting modification,

a finding in accord with European results. He also found that the degradation of carbohydrates was the main factor influencing wort viscosity and isolated the viscous principle. He suggested that while nitrogen compounds do not have a direct effect on wort viscosity, they are indirectly involved, as proteins and pentosans degrade simultaneously during malting. This led to his hypothesis that the material in barley that gives rise to compounds responsible for high viscosity is a protein-carbohydrate complex.

Between 1950 and 1960 there was considerable interest in the gums and gum-degrading enzymes. Two groups of workers, Meredith in Canada and Preece in Scotland, were especially active. By 1953 each group had isolated a natural substrate, of cell-wall origin and now known as beta-glucan, that was suitable for the study of enzyme activity and preferable to the artificial substrates used earlier by Swedish workers (28). Aspinall and Telfer (1) showed this material to be a polymer of mixed beta-1,3 and beta-1,4 glucose linkages. Preece and Ashworth (24) proposed a dual mechanism for the action of enzymes in germinating barley on gums which they termed cytoclastic, or viscosity decreasing, and cytolytic, the release of reducing sugars. Later Preece (25) referred to these two classes of enzymes as endo-beta-glucosanase and exo-beta-glucosanase. Though they differed on nomenclature, the Scottish and Canadian groups were in agreement on the mode of enzymic degradation of the gums (5, 25). Only the endo-enzyme can attack the whole initial material. The function of the exo-enzyme was suggested as the removal of inhibiting high-molecular weight polysaccharides resulting from initial attack of the endo-enzyme on the closely packed

molecules of the cell-wall (5). A third component, a disaccharase, was later postulated by both groups (7, 26).

Cytolytic activity was shown to increase during malting (4, 26), and as a result it was suggested that green-malt was a better source of enzymes for the study of their mechanisms and of varietal differences in their activities (6). Studies of two-row barleys in Europe in 1951-1953 showed that barley cytolytic activity had little relation to malting quality. A Canadian study on six-row barleys in 1957 (3) provided strong evidence that the cytolytic activity of green-malt was related to modification and malting quality and an important component of varietal differences in these properties.

There is little doubt that the researches of Meredith and Preece stimulated other investigations on the mechanism and categorization of the cytolytic enzymes and the structure of their substrates. Luchsinger (14) has reviewed the structural properties of gums, and Steiner (30) has summarized what is presently known about the role of pentosans in brewing. Studies on the enzyme systems are numerous but so far not really definitive with respect to in vivo action, as reported in the recent contribution of Manners and Marshall (17).

With the termination of the work of Preece and Meredith a few years ago, interest in the practical application of their results appeared to decline. However, the technical importance of wort viscosity, beta-glucan and beta-glucanases is presently being emphasized, undoubtedly as a result of changes in malting technology. Acceleration of the malting process by reducing the germination time results in reduced enzyme synthesis and in less degradation of cell-wall materials.

The consequent higher viscosity in malt extracts causes many difficulties in the brewing process. Further, low fermentability may result if insufficient cell-wall degradation impedes the action of alpha-amylase.

High viscosity has been found to effect the rate of mashing operations adversely (8, 29) and to cause difficulties in mash filtration (10, 29). Erdal and Gjertsen (9) in Denmark found that beta-glucans were not dissolved in mashing until the starch gelatinized at about 60° and that endo-beta-glucanase was inactivated at 65°. Wort viscosity and glucan content were therefore lower when the mash program included an initial mash at 35-55° than when the malt was mashed directly at 65°. Actually, Meredith obtained similar results in 1949 (18). He found that viscosity and gum content of wort were unaffected by changes in mashing temperature from 20-55° but increased sharply as the temperature was further raised. Schuster et al. (29) in Germany have also obtained similar results on the effect of mashing temperature on gum content, and they noted that low mash concentrations produce high wort gum content. They found barley gum content to be influenced more by cultivar than by environment and that within a cultivar hot, dry growing conditions increase the gum content. Schuster also reported that the increase in synthesis of glucanase enzymes due to gibberellic acid treatment in malting is inversely related to the initial activity. Gums affect the end-product of the brewing process, and in Japan, Igarashi and Amaha (13) have identified the material that precipitates when beer is frozen and causes cloudiness on thawing as beta-glucan of endosperm origin.

The problem is, therefore, to effect economies in manufacture

while producing wort of minimal viscosity, that is, gum content, and high fermentability. The rate of modification may be increased by higher germination temperatures and the manipulation of air-water-temperature interactions in steeping. Malting losses can be reduced by inhibiting rootlet growth, and the most useful technique for this appears to be a re-steep of the barley in water early in the germination process. A pre-germination treatment with gibberellic acid in otherwise conventional malting accelerates enzyme synthesis. Thus there are several techniques that may be exploited and the final solution may well be a combination. Much will depend on studies of the enzyme systems, on their relative rates of development and the degree to which they are affected by the various treatments.

The use of gibberellic acid (GA) to stimulate enzyme synthesis in malting is an established practice that is well-founded. There is considerable evidence, reviewed by MacLeod (15), that gibberellin-like materials are important in regulating the production of enzymes in germinating grain. They are thought to be released by the barley embryo during early germination and to migrate to the aleurone layer where they initiate the re-synthesis of a variety of hydrolytic enzymes. Briggs (9) stated that the addition of GA_3 to the whole grain augments the supply of endogenous gibberellins, and that the large response of enzyme systems to GA treatment indicated that the endogenous gibberellins do not saturate the enzyme synthesis mechanisms of the aleurone. Groat and Briggs (12) studied the quantitative relationships of the "gibberellin" content of barley, added GA and the production of alpha-amylase. The results strongly supported the concepts that the synthesis of the

enzyme is controlled in the main by gibberellin-like materials and that gibberellins are the most important, if not the only, activators of enzyme synthesis in the barley aleurone.

Exogenous GA apparently enters the whole barley kernel only through a micropyle-like area in the embryo (21) and the pericarp is impermeable. Crushing the grain to rupture the pericarp, and abrasion of the distal end (21) have been shown to be effective ways to increase the contact between exogenous GA and the aleurone layer and thereby initiate enzyme synthesis more rapidly. A recent report (22) on the abrasion treatment indicated that it reduced malting time by 25% and had no adverse effects on pilot plant brewing or beer quality. These results were obtained on one cultivar of barley and no attempt to study varietal differences seems to have been made. Varietal differences in response to GA treatment have been found. Schuster et al. (29) observed that cultivars low in glucanase activity showed a greater response than those with initially high activity. Atanda and Miflin (2) noted that varietal differences in alpha-amylase activity diminished in the presence of GA, and they suggested that part of the difference in activity between untreated barleys may be due to the gibberellin production and dissemination systems. It seems reasonable to associate differences in GA response with endogenous gibberellin. On the other hand, physical differences in cultivars that regulate the penetration of exogenous GA may be more significant.

The essential factor in malting is the balance of enzyme activity, so that the order in which the enzyme systems respond to GA and the extent of the response are important. It is evident that all

enzyme systems do not show the same response, and it is desirable that they should not. For example, proteolytic activity is increased by GA treatment, possibly more so than other enzyme systems, and this results in unwanted high levels of soluble nitrogen in the wort. It is possible to regulate some of the expected departures from traditional wort composition, although whether this is necessary is questionable. Provided that, in the consumers' opinion, the end-product retains its traditional character, variations in analytical data seem to be unimportant.

MacLeod et al. (16) found that the pattern and extent of enzyme formation in GA treated and untreated Proctor barley was the same. In both, endo-beta-glucanase, alpha-amylase and protease activities developed sequentially, though production was about ten hours earlier in the treated grain. This is useful information and it showed that GA treatment did not affect the required sequence of cytolysis (or at least one of the cytases) and amylolysis.

Cytolysis is the essential initial activity in malting. Therefore, acceleration of the malting process depends on early initiation and rapid development of the cytase system of enzymes. Ideally, the enzyme balance developed during malting should include high levels of cytase, possibly even at the expense of some other enzymes operating in mashing. GA treatment accelerates the synthesis of enzymes but it is uncertain whether the relative rates of increase are appropriate, though admittedly these are difficult to specify. Nevertheless, advances in malting technology are dependent on increased knowledge of the enzyme systems and methods of control or enhancement of them. GA treatment

is known to be effective and therefore will continue to be a factor in malting.

It seems likely that an appropriate combination of new steeping and germination procedures and GA treatments will accelerate the malting process and effect the necessary economy in wort production. However, efficiency will be influenced by the characteristics of the barley. The extent to which cultivars in present production, whose quality was defined on traditional grounds, will respond to new techniques is uncertain.

No selection for cytolytic properties has been made so far in Canadian barley breeding programs. Nevertheless, the barley project at the Department of Agriculture station at Brandon, Manitoba, has produced a succession of cultivars that, in addition to progressively better quality as defined in traditional terms, have shown a steady improvement in modification ability. This can be interpreted as an improvement in cytolytic properties, and it has occurred without deliberate selection. However, these are six-row types and, as such, have only limited acceptance in export markets. Further, the amount of two-row barley used by domestic industry is increasing. These considerations were a reason for a recent re-alignment of Canadian barley breeding in favor of the improvement of two-row barleys.

The two-row cultivars presently grown in Canada, with one possible exception that has limited adaptation, do not appear to possess the anticipated quality properties. The six-row types may be much closer to what is required. Therefore, it seems reasonable to combine the useful attributes of both species and an extensive program

along this line has begun at the Department of Agriculture station at Winnipeg. Selection for agronomic characteristics can be made in the usual way, but quality criteria must be modified, and the present and anticipated industrial requirements taken into account. It is necessary to determine what properties are related to accelerated malting and the production of satisfactory wort. Secondly, it is important to investigate the extent to which present cultivars may be manipulated in order to achieve the desired objectives. Finally, early generation material in barley breeding programs must be assessed by simple tests that are suitable for the examination of large numbers of samples.

Accordingly, the present study dealt with three topics. The cytolytic properties of barley and green-malt and their relation to malting quality were investigated. Secondly, the effect of gibberellic acid and an alternate steeping process on the development of enzyme activity in a number of commercial Canadian cultivars was studied. Finally, analytical techniques for the determination of cytolytic and amylolytic activities were examined, and automated procedures, that are applicable to plant breeding, were developed.

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SECTION TWO

CYTOLYSIS AND MALTING QUALITY IN BARLEY

ABSTRACT

Barley gum content is reflected by the viscosity of aqueous extracts of barley and green-malt. Initially soluble gum can be extracted from barley at 40°. Extracts of green-malt made at 70° contain higher molecular weight gums that are solubilized during malting and are associated with maltability and the properties of brewery worts. Although the gum soluble at 40° is degraded early in the germination process, the viscosity of 40° extract of barley is closely related to that of 70° extract of green-malt. Gum content estimated by barley viscosity is a varietal characteristic and is influenced by environment.

Barley cytolytic reducing power activity is not related to malting quality, but the activity of green-malt extracts is useful in predicting malting modification. Kilned-malt congress extract depends partly on cytolysis during malting, though other factors are involved. The residual cytolytic activity after a heat treatment of green-malt extract at 45° for 20 minutes provides some information on the thermal stability of the enzyme system. There is some evidence of an inverse relation between cytolytic activity and the percentage of the activity that is heat-stable, though environmental effects are more significant than varietal differences.

The simple barley extract viscosity test and the automated determination of cytolytic activity of green-malt extract are readily applicable to the evaluation of barley breeding material, and their use should lead to the development of improved cultivars.

INTRODUCTION

Trends in the malting and brewing industries that include accelerated and continuous processing, further increases in the use of adjunct cereals and the advent of materials such as barley syrups, are focussing more attention on cytolysis, the enzymatic degradation of the barley endosperm cell-walls. This has led to an examination of the cytolytic properties of present commercial barley cultivars and material under development in plant breeding programs. There are indications that significant varietal improvements are possible.

It is well established that the modification of vitreous barley into friable malt results primarily from enzymatic hydrolysis of gums. Experimental evidence suggests that the barley gums are located in the central endosperm of the kernel and probably in the cell-walls (13, 14, 19). Preece and Hobkirk (19) showed that husk-type hemicelluloses had low viscosities in solution and high pentosan contents, whereas endosperm hemicelluloses were high in viscosity and glucosan content, properties similar to those of barley gums. MacLeod and Napier (14) found that the husk, embryo and aleurone accounted for all of the cellulose of the barley kernel. MacLeod (13) concluded that beta-glucan is associated with the endosperm cell-walls and that its progressive removal is linked with their changing physical nature during malting modification.

The structure of barley beta-glucan has been examined by several investigators. In 1954, Aspinall and Telfer (2) concluded that beta-glucan was composed of glucose units joined by beta-1,3 and beta-1,4

bonds in approximately equal proportions. Since then various attempts have been made to elucidate the structure. These were summarized by Luchsinger (12), who concluded that in view of conflicting results the question of the fine structure of beta-glucan could not be considered settled, but that it was apparent that the molecule is composed mainly of units containing two adjacent 1,4 bonds and units containing three adjacent 1,4 bonds, separated by isolated 1,3 bonds.

Meredith and Anderson (16) compared barley and malt gums. The gum in aqueous extracts of barley contains two components. One of these, about 60% of the total, is initially soluble in water; the other is solubilized by enzyme activity during extraction. Both are similar and contain 80-85% glucose. Kilned-malt also contains two components, but the soluble fraction is only 20% of the total. As barley and malt have the same gum content, more material has been solubilized during malting and some of the initially soluble barley gum has disappeared. Malt gums are much less viscous in solution than barley gums and their pentose content has increased from 15-20% in barley to 75%.

Cytolysis is the initial activity in malting. Cell-wall degradation exposes various substrate to attack by appropriate enzymes and thereby sets in motion the process of modification of barley to malt. Further hydrolysis of the cell-wall material is important, as its continued presence has several effects on the brewing process. High viscosity, indicative of high levels of undegraded gum, has been found to have an adverse effect on the rate of mashing operations (7, 20) and to cause difficulties in mash filtration (8). Oxidative haze and haze developed in beer stored at high temperatures has been associated with

barley gum (10). The material that precipitates when beer is frozen and causes cloudiness on thawing has been identified as beta-glucan (11). On the other hand, the gum polymers are believed to contribute to beer palatability and foam stability (8, 9), so that their complete absence is apparently not desirable.

Barley cultivars differ in gum content, gum composition and in the activity of gum-degrading enzymes. Various studies have been made on the relation between these varietal differences and general malting quality. Bass and Meredith (4) found no relation between the gum content or composition of 15 North American six-row barleys and parameters used to assess malting quality. A later study by Sparrow and Meredith (21) showed a positive association between the gum content of 12 two-row barleys of diverse origin and malt extract yield, and similar results were obtained by Schuster et al. in Germany (20). Bass et al. (3) found no relation between barley cytolytic activity and malting quality, but a significant association between green-malt cytolytic activity and malting properties. Sparrow and Meredith (21) obtained similar results, though in both studies anomalous behavior by certain cultivars was observed. Meredith has concluded (3, 4, 21) that the gums do not contribute much to the total extractable sugars in malt but they are important in the production of the extract, and that the activity of the cytolytic enzymes is more significant than barley gum content.

The gum-degrading enzyme system in barley and green-malt has been intensively studied. In 1950 Preece and Ashworth (18) proposed a dual mechanism for the action of the system in germinating barley.

Cytoclastic action reduced molecular size, and viscosity, and cytolytic action effected further degradation, with the production of reducing sugars. Meredith's group (5) postulated in 1953 that the gum-degrading system in green-malt consisted of endo- and exo-polyglucosidases and a disaccharase, a view that was accepted by Preece. Since that time many other studies on the enzyme system have been reported. So far the results are inconclusive and the original hypotheses of Meredith and Preece are undisputed - though the enzymes are now referred to as beta-glucanases. The most recent contribution is that of Manners and Marshall (15), who found that at least four enzymes are involved in the hydrolysis of barley beta-glucan. Viscosity reduction is mainly due to endo-beta-1,4- and endo-beta-barley-glucanase, while two glucosidases are major factors in the increase in reducing power. The barley beta-glucanase activity increases during germination, but that of the other three enzymes is not greatly affected. It is not possible to decide which of the activities are the most important in cell-wall degradation during malting.

No selection for gum content or cytolytic activity has been made so far in early generations of Canadian barley breeding programs. It is now apparent that these properties have become more important and that techniques for their selection are required.

Sparrow and Meredith (21) stated that cytolytic activity is almost certainly a more significant factor in determining malt extract, and that high levels of barley gum are no bar to good maltability provided that cytolytic activity is at a compensating level. On this basis the enzyme assay alone should suffice in screening barley breeding material, selecting only high activity lines. On the other hand, if

both gum content and enzyme activity are taken into account, medium levels in both properties may be interpreted as equivalent to high enzyme activity when it is accompanied by high gum content. This may result in an increase in the proportion of useful material selected, which is desirable because of the number of other plant attributes being sought.

The cytolytic activity assay described in Section Four of this study meets the requirements of a satisfactory screening technique. A simple method to estimate gum content is required. Published procedures are not readily applicable in barley breeding, though they measure absolute amounts. Relative values are equally useful in screening breeders' populations, and it was considered that they should be obtainable simply by comparing the viscosities of water extracts of the barley samples. The amount of gum in a barley extract depends on the amount and availability of gum originally present in a soluble form and on the activities of enzymes that convert originally insoluble gum to a soluble form and that degrade gum. Attempts to measure the soluble form only by using various enzyme inactivators and to extract more gum by increasing mashing temperature, were unsuccessful. The procedure described in the next section appeared to be the most promising compromise.

while producing wort of minimal viscosity, that is, gum content, and high fermentability. The rate of modification may be increased by higher germination temperatures and the manipulation of air-water-temperature interactions in steeping. Malting losses can be reduced by inhibiting rootlet growth, and the most useful technique for this appears to be a re-steep of the barley in water early in the germination process. A pre-germination treatment with gibberellic acid in otherwise conventional malting accelerates enzyme synthesis. Thus there are several techniques that may be exploited and the final solution may well be a combination. Much will depend on studies of the enzyme systems, on their relative rates of development and the degree to which they are affected by the various treatments.

The use of gibberellic acid (GA) to stimulate enzyme synthesis in malting is an established practice that is well-founded. There is considerable evidence, reviewed by MacLeod (15), that gibberellin-like materials are important in regulating the production of enzymes in germinating grain. They are thought to be released by the barley embryo during early germination and to migrate to the aleurone layer where they initiate the re-synthesis of a variety of hydrolytic enzymes. Briggs (9) stated that the addition of GA_3 to the whole grain augments the supply of endogenous gibberellins, and that the large response of enzyme systems to GA treatment indicated that the endogenous gibberellins do not saturate the enzyme synthesis mechanisms of the aleurone. Groat and Briggs (12) studied the quantitative relationships of the "gibberellin" content of barley, added GA and the production of alpha-amylase. The results strongly supported the concepts that the synthesis of the

the slope of the two timed reciprocal specific viscosities.

To measure green-malt extract viscosity, the sample was macerated with water at 70° for three minutes in a Waring blender. The mixture was transferred to mash beaker and mashed at 70° for 25 minutes at 100 rpm. Before filtering, the weight of the mash was adjusted so that the ratio of water to original barley was eight to one. The viscosity of the filtrate, which is stable, was measured at 25° in an Ostwald tube.

Analytical determinations related to malting quality were made on extracts of kilned-malt. The malt samples were prepared from 250 g. of barley, dry basis, in the laboratory malting equipment (17). Extracts of kilned-malt were made by three methods. The so-called congress extract, measured by the procedure of the American Society of Brewing Chemists (1), is an indication of total soluble material in the malt. Material solubilized by a cold water extraction of malt has been shown to be related to modification that has taken place during malting (3). Information on malting plus mashing modification is provided by an extraction of coarsely ground malt at 70° for one hour.

The analytical measurement usually made in these procedures is the specific gravity of the extract, or wort, from which an extract value is calculated, in terms of percentage. The pycnometric measurement of specific gravity is time-consuming. An auto-analyzer procedure method was developed that measures total hydrolyzable carbohydrate and reducing sugar contents of the wort. The relation between the total value, expressed as glucose, and wort specific gravity was as close as could be expected when the complex nature of wort solids is considered.

enzyme systems do not show the same response, and it is desirable that they should not. For example, proteolytic activity is increased by GA treatment, possibly more so than other enzyme systems, and this results in unwanted high levels of soluble nitrogen in the wort. It is possible to regulate some of the expected departures from traditional wort composition, although whether this is necessary is questionable. Provided that, in the consumers' opinion, the end-product retains its traditional character, variations in analytical data seem to be unimportant.

MacLeod et al. (16) found that the pattern and extent of enzyme formation in GA treated and untreated Proctor barley was the same. In both, endo-beta-glucanase, alpha-amylase and protease activities developed sequentially, though production was about ten hours earlier in the treated grain. This is useful information and it showed that GA treatment did not affect the required sequence of cytolysis (or at least one of the cytases) and amylolysis.

Cytolysis is the essential initial activity in malting. Therefore, acceleration of the malting process depends on early initiation and rapid development of the cytase system of enzymes. Ideally, the enzyme balance developed during malting should include high levels of cytase, possibly even at the expense of some other enzymes operating in mashing. GA treatment accelerates the synthesis of enzymes but it is uncertain whether the relative rates of increase are appropriate, though admittedly these are difficult to specify. Nevertheless, advances in malting technology are dependent on increased knowledge of the enzyme systems and methods of control or enhancement of them. GA treatment

RESULTS AND DISCUSSION

Barley gums

Barley and green-malt viscosities

Varietal differences in the viscosity of 40° extracts were observed in several groups of cultivars. Data on two of these are given in Table II. These results, and the simplicity of the test, suggested that barley viscosity may be a useful tool for the examination of barley breeding material. The utility of the technique was therefore investigated. Barley samples were steeped in water and in a 4 ppm. solution of gibberellic acid (GA) for 24 hours at 20°, and then germinated for four days at 15°. The viscosity of water extracts of the grain was determined at intervals during the malting process.

The results shown in Table I and Figure 1 on the cultivars Conquest and Betzes are typical of other six- and two-row barleys that were studied. The data show that significant changes occur during steeping and that substantial amounts of warm (40°) water-soluble gum are solubilized early in the steeping process. Gum-degrading action begins as soon as barley is soaked in water, as is shown by the barley extract viscosity test. It appears that solubilizing activity begins at the same time, and that it greatly exceeds degradation activity in the first half of the steep period. The activities were about equal at 16 hours, and thereafter, as the substrate of the solubilizing enzymes became exhausted, degradation was the main activity. After 72 hours, that is, 24 hours steep and 48 hours germination, the viscosity of 40° extracts was almost minimal and very little further reduction occurred as germination progressed. Samples steeped in GA solution

had lower 40° extract viscosities during steeping and early germination, indicating that degrading activity had been enhanced. It is possible that GA has little effect on solubilization.

Hot water (70°) treatment of dry and steeped barley resulted in an unmanageable product, and the first measurement of 70° extract viscosity was not made until the samples had been germinated for 48 hours. By this time the gum soluble in water at 40° had been extensively degraded, so that the viscosity of 70° extracts of green-malt is mainly due to other polysaccharides that are only brought into solution at the higher temperature. Degradation of these gums was rapid during the third 24 hours of germination, but the 3-day green-malts still contained appreciable amounts of viscous components. As in the case of the gums soluble at 40°, degradation of the 70°-soluble material was accelerated by GA.

These results show that barley gum that is extracted at 40° makes only a minor contribution to the viscous properties of malt extracts, even when the germination time is as little as two days. The rapid degradation of this material during late steeping and early germination suggests that it has little direct effect on modification during germination, although changes that occur in early steeping may well influence subsequent developments.

The viscosity of malt extracts is mainly due to high molecular weight aggregates that are solubilized but not degraded during malting and are extracted from the malt when the mash temperature is raised to 70°. High viscosity values are associated with poor modification during malting, and the viscosity of 70° extracts of 3-day green-malt

has been used in Canadian barley breeding programs to select lines with good maltability (6). The substrate for cytolytic activity assay that is regarded as the most suitable is beta-glucan. This material is extracted from barley, that has been treated to inactivate degrading enzymes, by mashing in water at 40°. The source of enzyme activity in the assay is an extract of green-malt. The results of this study suggest that it would be of interest to investigate the utility of a cytase substrate prepared by 70° extraction of short grown green-malt.

In addition to its value as an index of modification, the viscosity of 70° extracts of malt provides information on the amount and molecular complexity of non-starch polysaccharides in the extract that influence brewery processing and beer properties. Consequently, the determination is a useful quality criterion in varietal differentiation.

The measurement of the viscosity of 40° extracts of barley is a much simpler procedure than the malt determination, and the two were therefore compared. Viscosity data on extracts of barley and 3-day green-malt are shown in Table II. The simple correlation coefficient for the relation between the properties is 0.64** in the six-row set and 0.85** in the two-row set, with an overall value of 0.78**. The partial correlation coefficient at constant cytolytic activity is 0.87**. These results show that the barley extract viscosity is a good indication of the amount of other gum components present in the barley that are involved in malting modification and extract quality. It is suggested that the test has additional value in that it can be applied to the examination of gum properties of unmalted cereals used as

brewing adjuncts.

Various anomalies have been observed in the relation between cytolytic activity and malting quality as defined by cold water and congress extracts (3, 21). High cytolytic activity was not invariably related to high quality, while in other cases high quality cultivars had only medium levels of enzyme activity. Inspection of the data shows that the anomalous relations may be at least partly explained by the gum content of the barley. However, in the present study multiple correlations involving barley extract viscosity, green-malt cytolytic activity and kilned-malt extracts (Table II) showed no improvement over the simple correlation between cytolytic activity and the extract values.

An estimate of gum content by the barley extract viscosity technique appears to complement the enzyme activity assay in examining cytolytic relations in barley. If consideration is given to the balance of the two properties when interpreting data on early generation breeding material, an improvement in the precision of selection of lines with satisfactory quality may be expected.

Effect of environment on barley gum content

As is evident from the data in Table II, there are varietal differences in gum content as expressed by barley extract viscosity. It was of interest to examine the effect of environment on this property. Viscosity determinations were made on several cultivars grown at various locations in 1968 and 1969. The results are given in Table III. The 1969 data show that gum content was higher under the relatively dry growing conditions at Saskatoon and Lethbridge than in the moister

climates of Winnipeg and Eastern Canada. The lower values on the 1968 samples seem to reflect the high rainfall in Western Canada in that year, though the possibility that the gum extractable from barley at 40° decreases with storage time cannot be discounted.

The data are limited, but they agree with the results of Schuster et al. (20) who reported that hot dry growing conditions increase total gum content. Mean values for barley extract viscosity and nitrogen content are given in Table III and they indicate that the environmental effect on gum content, as estimated by barley extract viscosity, parallels that on nitrogen content, although there is no intervarietal relation between the data in Table II on the two properties.

It is well known that environments that produce high levels of grain nitrogen are not usually suitable for the production of malting barley for commercial purposes, nor in fact for the quality evaluation of new cultivars. It would appear that nitrogen is not solely responsible for this situation, as a high level of gum is a barrier to modification. It is possible that, with further study, the barley extract viscosity test would be useful in intravarietal selection for malting suitability.

Cytolytic activity

Variation in cytolytic properties during plant growth

A plot of Conquest barley, grown at Winnipeg in 1969, was sampled at intervals from about six days after anthesis to the fully ripe stage. The material was freeze-dried and ground. The cytolytic reducing power activity of the samples was determined by the method

described in Section Four of this study. The variation in activity, mg. of glucose produced by 100 mg. of barley in 100 minutes, is shown in Table IV and Figure 2. Activity was highest in the early samples, declining to a level that showed little variation in the three weeks prior to maturity. Gum content was also estimated by the barley extract viscosity technique. The results indicated that the amount of barley gum soluble at 40° increased to a peak about July 23rd, and decreased during the following 10 days to a constant level. Kernel moisture content data were not available, but during this period a rapid reduction in moisture normally occurs. It appears that solubilizing activity is quickly arrested as moisture begins to fall, while degrading activity is less dependent on high moisture content. It would have been interesting to determine the enzyme activity in green-malts made from the samples, but all except the three most mature failed to germinate.

Varietal differences in barley and malt cytolytic activity

In studies of intervarietal relations between cytolytic viscosity reducing activity and malt quality factors, Bass et al. (3) found that differences in the barley enzyme activity were small and unrelated to quality. The enzyme activity of green-malt extract, on the other hand, was useful in differentiating barley cultivars and directly related to measures of malting modification made on kilned-malts. In the routine examination of varietal differences in cytolytic activity, the automated assay of reducing power activity is much more convenient than the determination of viscosity decreasing activity. Therefore, reducing power activity of barley was compared with that

of green-malt.

The data in Table V show that varietal differences in barley cytolytic activity are small, though the range is wider in the miscellany of two-row cultivars than in the North American six-row barleys that have a narrower genetic base. The correlation coefficient for the relation between barley and green-malt activities is -.21, which is not significant. Therefore, the determination of barley cytolytic reducing power activity is of no value as a selection tool in plant breeding.

The activity of the cytolytic enzymes in degrading cell-walls to expose the cell contents to the action of amylolytic and proteolytic enzymes is important in determining the rate of malting modification. Further hydrolysis of the cell-wall material is desirable to reduce the viscosity of brewery worts. Wort may be made by hot water extraction of kilned-malt or blends of malt and unmalted cereals or, possibly, by mashing green-malt with raw cereals. Therefore, the thermal properties of green-malt cytolytic enzymes, that is, the extent of inactivation during kiln drying and brewery mashing are important, and information on possible varietal differences in them is useful.

A study made on six commercially grown Canadian cultivars (Table VI) showed that the optimum temperatures for green-malt viscosity decreasing and reducing power activities on beta-glucan to be about 38° and 35°. The optima were unaffected by varietal differences in activity. Reducing power was inactivated by 7% by heating the extract at 35° for 60 minutes and by 90% by heating at 55° for 15 minutes. Further work showed that about half the reducing power activity

remained after heating the green-malt extract for 20 minutes at 45°, and this seemed to be a useful treatment for thermal studies.

Data on the cytolytic reducing power activity of extracts of green- and kilned-malts and heated extracts of green-malt are given in Table V. The kilned malts were made by the routine malting procedures used at the Winnipeg research station, which includes a germination period of 5 days at 12.5°, and the green-malts were prepared by germinating steeped barley for 3 days at 17°, as described in Section Four of this study. Therefore, the difference in the two malt activities in the table is not a direct measure of kilning inactivation. This was not important as the concern was with varietal differences.

The data indicate that there are small varietal differences in the heat stability of green-malt cytolytic enzymes and this suggests that cytolytic activity may be more meaningfully measured on heat-treated extracts. The correlation coefficients for the relation between kilned-malt activity and unheated and heat-treated green-malt activities are 0.92** and 0.96** respectively. Although that for the heat treatment relation is the higher, a test of significance showed that they do not differ. Therefore, it appears that a determination made on untreated green-malt extract is adequate in the prediction of varietal differences in kilned-malt cytolytic activity. However, in spite of the close statistical relation, an inspection of the data supports the proposal that green-malt activity assays should be made on heated extracts, although experience will eventually decide on the necessity of the heat treatment.

There is some suggestion that, in green-malt, the higher the

initial cytolytic activity, the greater the percentage inactivation by the heat treatment of the extract. Intervarietally and within stations the relation between the two properties is not significant for the data in Tables V and VII. Intravarietal data indicate that the effect is mainly environmental. Mean values in Table VII for cytolytic activity and percentage heat inactivation on a set of 16 cultivars grown at four stations in 1968 were 2.62 units, 47%; 2.79 units, 40%; 3.57 units, 56% and 4.42 units, 61%. A variance analysis of the data in Table VII shows that variability in the percentage of heat stable component due to stations is about ten times that due to cultivars.

Relations between cytolytic properties and kilned-malt extracts

The viscosity of 70° extracts and the cytolytic reducing power of 3-day green-malts were compared with extracts of kilned-malts made from the same barley samples by the standard laboratory procedure. The cold water and 70° coarse grind extracts (Table II) were used as measures of modification, and the correlation coefficients for the relation between them and cytolytic activity were 0.90** and 0.75** in the six-row group and 0.86** and 0.74** in the two-row group. These values agree closely with the correlation coefficient of 0.86** reported by Bass, Bendelow and Meredith (3) for the relation between cold water extract and cytolytic viscosity decreasing activity for a set of Canadian six-row cultivars. Bass et al. also found a good correlation (0.71**) between cytolytic activity and congress extract, which is a measure of total soluble material in the malt. In the present study, the correlation coefficient for this relation in the six-row set was 0.67**, which is similar to that quoted in the earlier work.

In the two-row group, the value was 0.55, which is only significant at the 5% level.

There was an inverse relation between green-malt extract viscosity and cold water, 70° coarse grind and congress extracts of the six-row group with correlation coefficients of -0.70**, -0.69** and -0.67**. In the two-row set the relation was also inverse but not significant. These results support the use of the 70° extract viscosity test in selecting for general malting quality in Canadian six-row breeding programs, but they advise that it is unsuitable when working with other material.

It seems reasonable to suppose that high viscosity in extracts of short-grown green-malt is due to initially high gum content in the barley. The two-row data in this study support the view of Sparrow and Meredith (21) that high levels of gum are no bar to good maltability, provided that enzyme activity is at a compensating level. However, the malt extract values used for comparison were traditional quantitative criteria that do not describe wort quality, and present concern is as much with the nature as with the amount of total extractable carbohydrate, especially when malting is accelerated.

The cytolytic reducing power activity determination is important in predicting modification. The green-malt extract viscosity test, or, as suggested, barley extract viscosity, should provide information on wort quality when short germination times are used. The quantity of congress extract of kilned-malt depends partly on cytolytic activity and partly on other factors such as alpha-amylase activity and barley nitrogen content.

Cytolysis in malt kilning and mashing

The extent of inactivation of cytolytic enzymes during kilning was not determined, as the green- and kilned-malts were made under different germination conditions. Kilning inactivation has been investigated by other workers (12, 15) who have reported the effects, under their particular conditions, of kilning on various enzyme components of the cytase system. In the present study, cytolytic activity of kilned-malt was found, as already observed, to be related to that of green-malt. This is useful information, but it applies to one kilning method, and whether the same relation would be found under the wide range of kilning conditions that are in use is unknown. Further, the relation was established for reducing power activity, because of the convenience of this assay in the examination of barley populations.

In brewery mashing, a reduction in the molecular complexity of the gum components of malt and adjunct cereals, that is, a decrease in viscosity, is important. Gums cannot be expected to contribute much to the total fermentable sugars, but their presence in an undegraded state creates brewhouse processing problems.

Cytolytic viscosity decreasing activity is more sensitive to heat than reducing power activity. When kilned-malt extracts, prepared for enzyme assays, were heated at 45°, their reducing power activity was decreased by 13% in 20 minutes and to about half the untreated value in 60 minutes. Kilning reduced but did not inactivate the viscosity decreasing activity of green-malt. About 20% of the residual activity after kilning was destroyed by heating kilned-malt extracts

at 45° for 10 minutes, and after 30 minutes no activity was detectable.

These observations are of interest in that they show that even after kilning there is still a heat-labile component present in a cold extract of malt, though it is readily inactivated at 45°, the initial congress mash temperature. However, the data are of limited value, as heating cold water extracts of malt is not analogous to brewery mashing. Information on varietal differences in cytolysis during mashing may be useful in the evaluation of barley brewing quality, and it could be obtained by the determination of residual enzyme activity, or viscosity, at intervals in the mash process. Cytolytic activity is influenced by temperature, but mash concentration, rate of agitation and the physical nature of the grain can also be expected to have some effect. Therefore, it may be difficult to devise a laboratory procedure that is representative of the varied mashing conditions used in industry. It is possible that the simple tests related to heat sensitivity of cytases, that can be made on extracts of green-malt, are adequate in selecting good quality lines in barley populations. Nevertheless, it is evident that more information on the effects of industrial mashing, and kilning, on cytolytic properties is required.

Table I

Variation in barley and green-malt extract viscosity during steeping and germination

Time from start of steep (hrs.)	Relative viscosity, cp.			
	40° extract		70° extract	
	Water steep	GA steep	Water steep	GA steep
Conquest				
6	1.99	1.93		
16	2.38	1.91		
24	2.31	1.88		
48	1.50	1.40		
72	1.27	1.29	2.66	1.64
96	1.25		1.71	1.45
120	1.23		1.45	1.30
Betzes				
6	3.43	3.05		
16	4.34	3.01		
24	4.14	2.85		
48	2.02	1.68		
72	1.33	1.33	4.94	2.83
96	1.30		2.26	2.03
120	1.25		1.78	1.50

6-24 hrs. - steeped barley
48-120 hrs. - germinating green-malt

Viscosity of dry barley extracts
Conquest - 1.60 cp.
Betzes - 2.08 cp.

FIGURE I

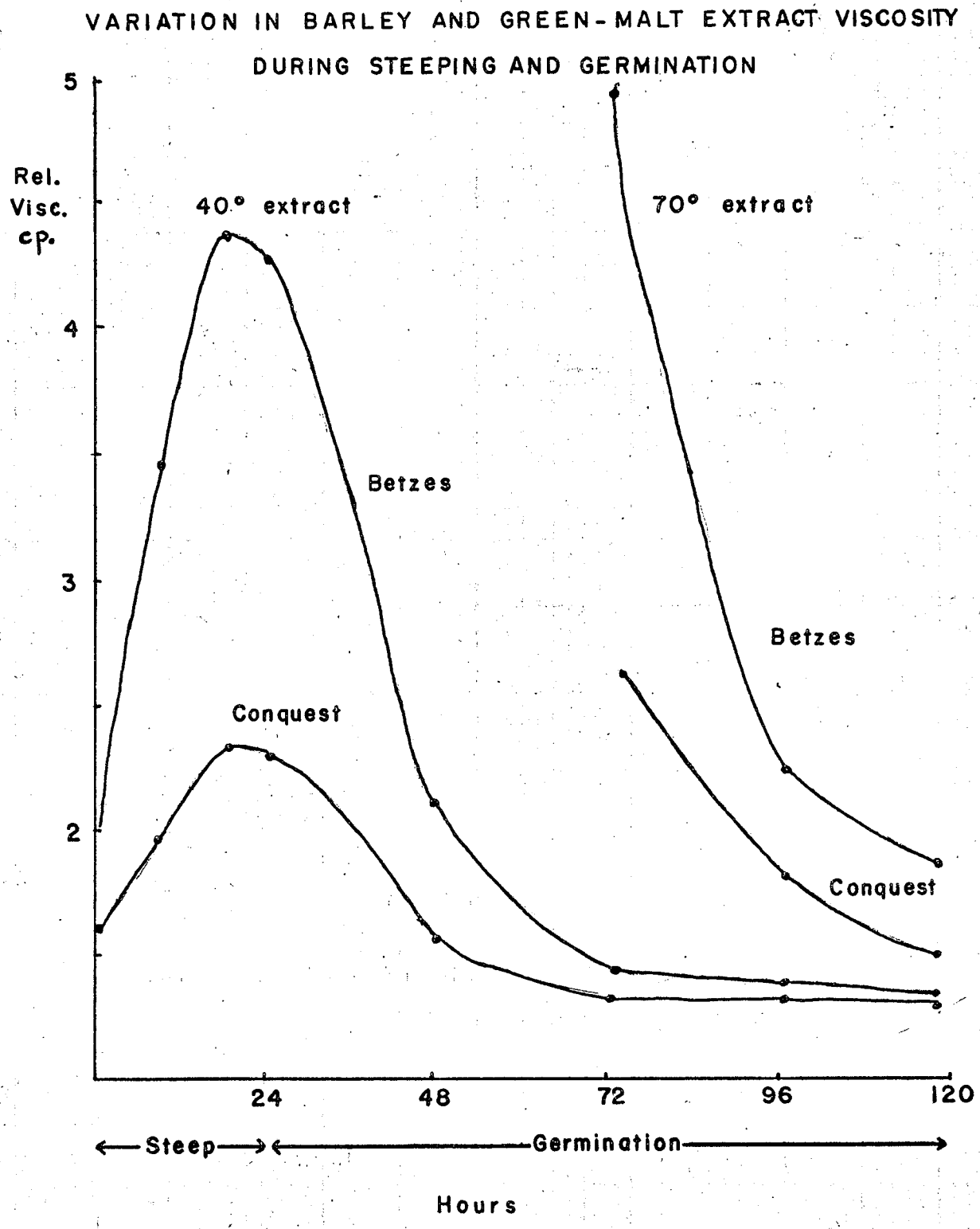
VARIATION IN BARLEY AND GREEN-MALT EXTRACT VISCOSITY
DURING STEEPING AND GERMINATION
Rel.
Visc.
cp.
5
4
3
2
40° extract
70° extract
Betzes
Conquest
Betzes
Conquest
24
48
72
96
120
Steep
Germination
Hours

Table II

Cytolytic properties of barley and green-malt and kilned-malt extracts

Cultivar	Barley		Green-malt		Kilned-malt extracts		
	Nitr. %	Visc. cp.	Visc. cp.	Cytase act. units	Total glucose, g./100 ml. Cold	70° coarse	Congress
Six-row barley							
Barbless	2.34	2.43	2.86	1.80	1.16	5.75	6.22
Minn M9	2.33	2.16	2.38	2.38	1.19	5.85	7.08
SD 178	2.13	3.33	2.48	4.30	1.45	6.31	7.20
Minn M11	2.27	2.23	2.05	4.25	1.45	6.54	7.35
Larker	2.25	3.56	2.44	4.40	1.49	6.46	7.46
ND 140	2.36	1.59	1.64	5.36	1.55	6.88	7.46
ND 139	2.30	2.00	1.90	4.12	1.60	6.62	7.54
Wisc 993	2.17	2.10	1.68	5.70	1.58	6.54	7.35
SD 640	2.49	2.17	1.98	4.40	1.62	6.77	7.30
ND 130	2.27	3.25	2.90	4.18	1.45	6.19	7.00
Minn M12	2.05	2.11	1.99	4.71	1.53	6.69	7.23
Wisc 1217	2.66	3.00	2.13	5.15	1.62	6.08	7.08
Primus	2.17	2.13	1.70	5.70	1.64	6.54	7.23
Coho	2.58	2.00	1.62	4.77	1.59	6.54	7.38
Wisc 1068	2.37	2.25	1.95	5.28	1.59	6.81	7.25
Conquest	2.42	2.83	2.00	5.36	1.71	6.78	7.30
Two-row barley							
WI 2136	2.28	2.08	2.28	1.80	1.18	5.70	7.04
B 2022	2.66	1.89	1.78	3.05	1.32	5.62	7.04
Valticky	2.54	2.59	2.43	3.62	1.28	5.87	7.20
Nosovski	2.60	3.22	2.83	2.24	1.30	5.70	7.04
Cern. 5	2.44	2.89	2.68	2.70	1.27	5.70	7.20
Betzes	2.44	2.34	2.36	3.07	1.30	5.79	7.20
Sloven 822	2.46	1.89	1.78	3.14	1.28	6.29	7.48
Slovensky	2.22	1.94	1.86	3.46	1.49	6.50	7.71
Hellas	2.67	1.76	1.71	2.24	1.28	5.62	6.86
Beka	2.29	2.31	2.07	3.03	1.33	6.16	7.39
Zephyr	2.25	2.10	1.80	4.18	1.45	6.00	7.36
IB 6-3	2.42	2.60	2.50	3.40	1.36	6.00	7.26
Cern.	2.48	3.72	2.87	2.54	1.42	5.79	7.17
MFB 104	2.27	3.33	2.40	4.00	1.44	6.08	7.36
IB 6-29	2.36	2.04	1.90	3.77	1.50	5.96	7.17
Bran. 130	2.28	2.80	2.16	4.73	1.60	6.34	7.30
Bran. 131	2.38	2.81	1.98	5.05	1.60	6.46	7.35
Akka	2.29	2.04	1.51	4.27	1.55	6.38	7.36
Unzyozny	2.26	3.70	2.77	4.58	1.52	6.21	7.35

Table III

Effect of location on the viscosity of 40° extracts of barley

Cultivar	Relative viscosity, cp.				
	Winnipeg	Saskatoon	Lethbridge	Macdonald College	Charlotte-town
1969 crop					
Conquest	1.83	2.13	2.11	1.60	1.65
Betzes	2.12	3.62	2.38	2.08	2.60
Herta	2.78	3.60	3.72	2.60	2.54
Hellas	1.66	2.34	2.45	1.70	1.87
DB 5-120	2.06	2.65	2.47	1.70	1.60
Means	2.09	2.87	2.63	1.94	2.05
Mean nitrogen content, %	2.33	2.45	2.52	1.80	1.93
	Portage la prairie	Beaver-lodge	Swift Current		
1968 crop					
OAC 21	1.42	1.96	1.82		
Parkland	1.32	1.62	1.73		
Conquest	1.30	1.44	1.55		
Paragon	1.34	1.51	1.46		
Galt	1.33	1.59	1.57		
Bonanza	1.42	1.49	1.59		
Means	1.36	1.60	1.62		
Mean nitrogen content, %	2.09	2.17	1.97		

Table IV

Harvest date and cytolytic properties of Conquest barley

Date		Barley cytolytic activity units	Barley extract viscosity cp.
June	30	1.45	
July	2	1.29	
	4	1.34	
	7	1.30	
	9	1.40	1.28
	11	1.26	1.35
	14	1.18	1.42
	16	1.15	1.42
	18	0.82	1.46
	21	0.65	1.63
	23	0.58	1.85
	25	0.52	1.75
	28	0.52	1.50
	30	0.64	1.63
August	1	0.54	1.42
	5	0.60	1.35
	11	0.52	1.38
	14	0.52	1.28

FIGURE 2

VARIATION IN BARLEY CYTOLYTIC REDUCING ACTIVITY AND BARLEY VISCOSITY DURING GROWTH OF PLANT

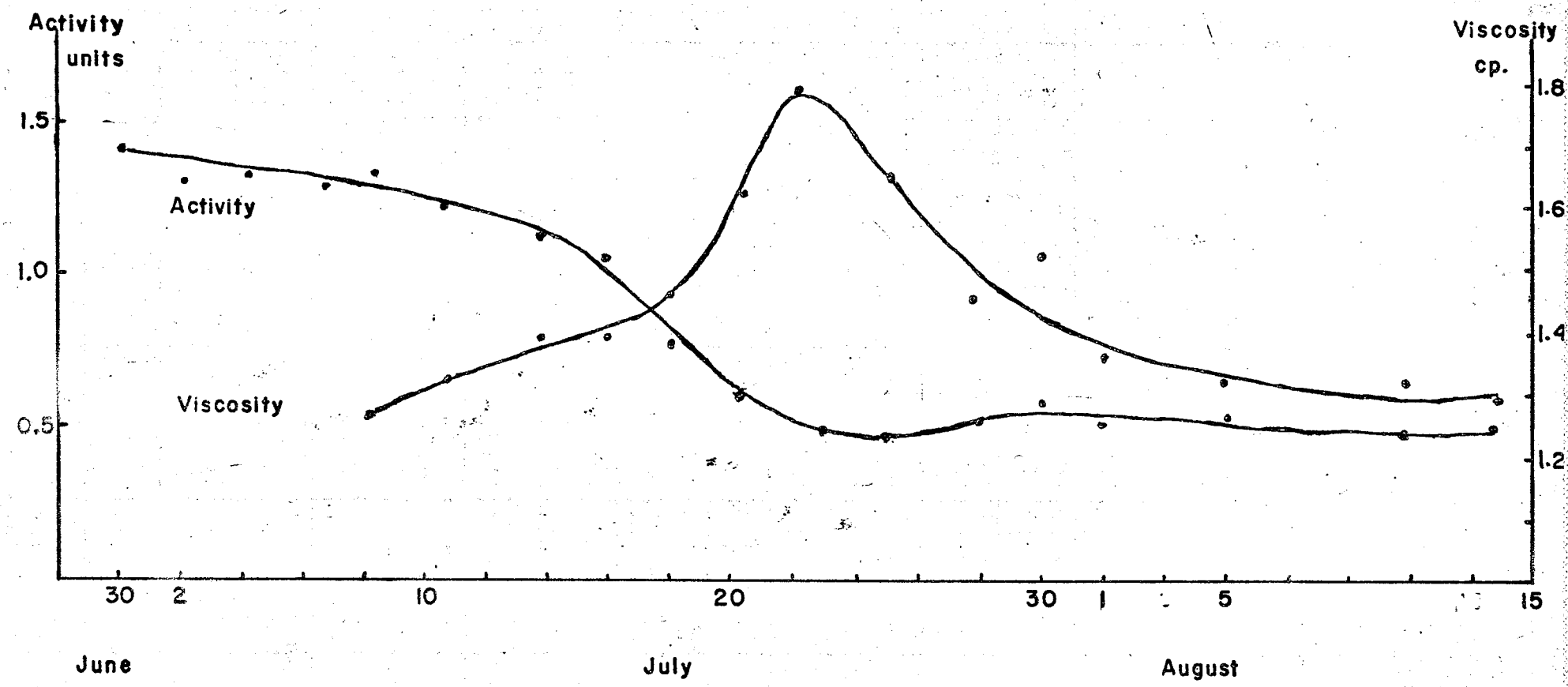

Table V

Cytolytic reducing power activities of barley, green- and kilned-malts

Cultivar	Cytolytic activity, units				% heat inact. of green-malt
	Barley	Green-malt	Green-malt heated	Kilned-malt	
Mississippi valley test					
Conquest	0.66	5.36	2.68	2.24	50
Dickson	0.69	3.93	1.96	1.69	50
ND 130	0.64	4.18	1.96	1.65	53
Wisc. 1068	0.47	5.28	2.27	1.83	57
ND 133	0.61	5.07	2.48	2.08	55
ND 134	0.59	5.54	2.66	1.98	52
Primus	0.59	5.70	2.62	2.09	54
Coho	0.59	4.77	2.50	1.83	48
SD 178	0.59	4.30	2.21	1.76	49
Wisc. 1217	0.62	5.15	2.40	1.91	53
Minn. M 9	0.59	3.11	1.40	1.33	55
Minn. M11	0.64	4.25	2.04	1.67	52
ND 140	0.59	5.36	2.84	2.08	47
Minn. M12	0.72	4.71	2.36	1.86	50
Two-row introductions					
Hellas	0.88	2.24	1.38	1.28	40
Zephyr	0.65	4.18	1.96	1.54	53
B 2022	0.64	3.40	1.46	1.15	57
Beka	0.58	3.03	1.30	1.36	57
Akka	0.71	4.27	2.09	1.58	51
IB 6-3	0.66	3.40	1.53	1.26	55
Betzes	0.49	3.07	1.38	1.26	55
MFB 104	0.53	4.00	1.72	1.36	57
Cern. 5	0.69	2.70	1.30	1.26	52
Sloven. 822	0.60	3.14	1.54	1.26	51
Erbet	0.80	3.42	1.64	1.40	52
Kristina	0.69	2.88	1.53	1.26	47
Diamant	0.64	3.38	1.54	1.26	55
Coho	0.47	4.07	2.28	1.67	44
Herta	0.48	2.83	1.30	1.26	54
Bran. 130	0.79	4.73	2.27	1.66	52
Slovensky	0.54	3.46	1.83	1.53	47
Unzyozny	0.42	4.58	2.06	1.62	55
IB 6-29	0.66	3.77	1.73	1.46	54
PR 28	0.66	4.00	1.88	1.60	53
Conquest	0.52	4.45	2.36	1.76	47

Table VI

Thermal properties of green-malt cytolytic activity

(i) Effect of reaction temperature

Temperature °C	Relative activity*	
	Viscosity decreasing	Reducing power
25	1.00	1.00
30	1.36	1.30
35	1.90	1.47
40	2.15	1.10
45	0.80	0.57
50	0.22	0.40

(ii) Thermal inactivation

Treatment		Loss of activity, %*	
Temp. °C	Time min.	Viscosity decreasing	Reducing power
35	60	7	7
40	20	21	19
	40	42	35
	60	55	40
45	10	45	39
	20	70	54
	40	88	34
	60	-	75
55	10	100	75
	15	-	90

* Mean values for six cultivars

Table VII

Effect of environment on heat stability of cytolytic activity of green-malt extracts

Cultivar	Initial activity, units				Heat inactivation, %			
	Lacombe	Beaver-lodge	Melfort	Swift Current	Lacombe	Beaver-lodge	Melfort	Swift Current
OAC 21	3.06	3.36	3.86	5.41	54	49	61	68
Parkland	2.62	2.88	3.68	4.41	48	38	56	65
Conquest	3.00	3.04	3.78	4.69	43	38	53	54
Paragon	2.62	2.84	3.68	4.21	45	37	57	64
Galt	2.08	2.64	3.24	3.86	43	41	58	63
BT 104	3.60	3.52	4.79	6.28	53	40	61	64
BT 105	1.78	2.00	2.53	2.79	36	34	50	59
BT 106	2.63	2.92	3.70	4.55	54	38	54	63
BT 107	2.53	2.96	3.54	3.97	48	47	56	61
Bonanza	3.18	3.20	4.28	5.31	50	41	58	62
BT 310	2.94	2.68	4.00	4.34	54	51	60	56
BT 311	2.48	2.24	2.84	4.41	54	37	49	61
BT 405	2.10	2.56	3.40	4.31	40	37	52	60
BT 406	3.04	3.12	3.70	4.69	50	42	56	60
BT 605	2.10	2.24	2.74	3.38	38	48	53	62
BT 805	2.22	2.40	3.43	4.41	40	32	54	59
Means	2.62	2.79	3.57	4.42	47	40	56	61

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SECTION THREE

EFFECT OF GIBBERELLIC ACID ON THE DEVELOPMENT OF ENZYME ACTIVITY IN BARLEY MALTING

ABSTRACT

Enzyme systems in green-malt were not affected by pre-germination treatment with gibberellic acid (GA) to the same extent. The increase in cytolytic activity was less than that in the proteolytic system, as indicated by the alpha-amino nitrogen content of extracts, but greater than the response of alpha-amylase activity. The relative activity of the components of the cytolytic system was not constant during germination and GA did not affect them equally. Germination and GA had little effect on glucosidase. The proportion of heat-labile component increased in control samples during germination, while the increment due to GA contained progressively higher proportions of heat-stable component.

Barley cultivars differed in the response of their enzyme systems to GA treatment during malting, and the response was generally greater in the two-row species. There was evidence of an inverse relation between response and initial activity, and high increments in cytolytic activity were mainly heat-labile. The apparent benefit of a large response in cytolytic activity was therefore offset to some extent. The reaction of the cytolytic system to GA is suggested as a useful tool in the evaluation of barley malting quality.

A version of the re-steep technique that is used as a means of reducing malting loss showed further differences in enzyme and varietal behavior. Re-steeping decreased cytolytic and alpha-amylase activities, but had little effect on alpha-amino nitrogen. In most of the cultivars studied re-steeping did not affect the enzyme response to GA, but there

was an adverse effect on Conquest, indicative of unsuspected weaknesses in that cultivar. Varietal differences in the nature of the decrease in cytolytic activity were observed. In one case the decrease was largely thermally unstable enzyme, but in Betzes barley re-steeping caused an appreciable loss of heat-stable activity. Re-steeping may be a useful technique for varietal differentiation.

INTRODUCTION

The use of gibberellic acid (GA) to supplement the endogenous gibberellins of barley is a well established practice in the malting industry. The time required to achieve the desired modification of barley to malt is reduced by the acceleration of the synthesis of enzyme systems by exogenous GA. Although knowledge of the enzyme mechanisms operative in malting is incomplete, the significance of cytolysis, the essential initial degradation of the endosperm cell-walls, and amylolysis, the degradation of starch, is recognized. Advances in malting technology depend on an increased knowledge of these enzyme systems and methods of their control or enhancement. The development, by plant breeding, of improved cultivars requires the study of genetic variability in properties related to technical advance.

There is an extensive literature on the mechanism of gibberellin action, including the general effect of exogenous GA in malting. However, information is lacking on varietal differences in the response of enzymes to GA, on the relative rates at which enzymes respond and on whether the treatment accelerates the synthesis of all components of, for example, the cytolytic enzyme system, to the same extent.

MATERIALS AND METHODS

The barley samples used in this work were grown in test plots at various stations of the Canada Department of Agriculture Research Branch, except for a group of two-row barleys that were obtained from Sweden. Gibberellic acid (GA) was a commercial grade of 90% biologically active material and was supplied by the Dominion Malting Co., Ltd.

Green-malt samples, and extracts used for analytical determinations, were prepared as described in Section Four of this study. In pre-germination treatment of barley with GA, steeping conditions were as described except that water was replaced by a GA solution during the last 12 hours of the process.

Cytolytic reducing power and alpha-amylase activities were assayed by the methods described in Section Four. In tabulated data, cytolytic activity units are mg. of glucose produced by 100 mg. of barley in 100 minutes and alpha-amylase activity units are mg. of maltose produced in the assay by 100 mg. of barley. Cytolytic activity was partially inactivated by heating green-malt extract at 45° for 20 minutes and by including glucono-1,5-lactone in the extract-substrate reaction mixture at a concentration of 2×10^{-3} M. The alpha-amino nitrogen content of green-malt extracts was assumed to be an indication of proteolytic activity and was determined by an auto-analyzer procedure. Barley and green-malt extract viscosities were measured by the methods described in Section Two of this study.

RESULTS AND DISCUSSION

Enzyme response to GA treatment

It was necessary to determine an optimum level of GA treatment for use in this study and future application in routine laboratory work. Samples of 3 six-row and 3 two-row barleys were steeped in water and in 1.5, 2.5, 4.0, 5.0 and 6.0 ppm. solutions of GA as described in the Materials and Methods section. The steeped samples were germinated for 72 hours at 17° and the alpha-amylase and cytolytic reducing power activities of extracts of the resulting green-malts were determined. The maximum response of the enzymes was at 5 ppm. in the two-row set and at 4 ppm. in the six-row barleys. In each case the 6 ppm. treatment resulted in a decline from the maximum. It was decided that the 4 ppm. solution would be suitable for further work.

The development of enzyme activity during the germination of GA treated barleys was studied. Samples of several cultivars were assayed after 2, 3, 4 and 5 days of germination at 12.5°. Results obtained on a group of six barleys are given in Table I. There was a sharp increase in the enzyme activities and the alpha-amino nitrogen content of the extracts of untreated samples between the second and third days of germination as the values approximately doubled. The increase continued on the fourth day and showed some sign of levelling off by the fifth. The response to the 4 ppm. GA treatment, shown by the percentage increase data in the table, differed with enzyme and barley species. As germination progressed, the percentage increase in cytolytic and alpha-amylase activities declined. The increase in amino

nitrogen content, assumed to reflect proteolytic activity, remained more or less constant, and generally at a higher level than the other activities, especially on the fourth and fifth days. The response of the two-row barleys was greater, in all properties, than that of the six-row types. The difference between the species exceeded varietal differences within each species.

Effect of GA on cytolytic activity

Cytolytic reducing power activity of green-malt extracts was compared with the activity of the extracts following treatment with heat and glucono-1,5-lactone. The purpose was to determine whether the proportion of components of the cytase system remained constant as activity increased during germination and with GA treatment. Data on four cultivars are given in Tables IIa and IIb. The calculated data (Table IIb) show the percentage increase in total activity due to GA treatment in samples taken after 2, 3, 4 and 5 days germination at 12.5°. The increment due to GA is the difference between GA and control activities. The total activity less the residual activity after inactivation treatment is a measure of inactivation loss, and the difference between GA and control samples indicates the proportion of the GA increment that was inactivated. This is expressed in Table IIb as a percentage for each inactivation treatment. Data on one cultivar are shown graphically in Figure 1.

The results show that during germination the increase in cytolytic activity of the control samples was accompanied by an increase in the proportion of heat-labile components. The components inhibited by the lactone, presumably glucosidases, remained more or less constant

and consequently their proportion of the total activity declined sharply.

The response of the enzyme system to GA treatment decreased and the additional activity contained lower proportions of inactivated components as germination progressed. By the fifth day, the increment due to GA contained 70% heat-stable glucanase and no glucosidase.

The composition of the cytase system in green-malt was not constant during germination, and GA did not affect all components of the system equally. Neither germination nor GA had much effect on glucosidase activity. It is interesting that one effect of GA was to increase slightly the proportion of heat-stable enzyme in the total activity. A pre-germination treatment with GA not only accelerated the synthesis of cytolytic activity but appeared to have a favorable effect on its thermal stability.

Varietal effects on the response of enzymes to GA treatment

Cytolytic reducing power and alpha-amylase activities of extracts of green-malts made from barleys steeped in water and in 4 ppm. GA solution were compared in several groups of cultivars. The data in Table III show a range in initial cytolytic activity and in response to GA treatment. In general, there is an inverse relation between response and initial activity, though in each group there are cultivars with approximately the same initial activity that show quite different responses to GA. As observed in Section Two of this study, varietal differences in barley cytolytic activity are small, and become significant only when the barley is malted, presumably due to the action of endogenous gibberellins. A treatment with exogenous GA supplements

the endogenous hormone and reduces the range of activity in a set of cultivars. Varietal differences, however, are still apparent, indicating that factors in addition to gibberellins are involved.

The high response to GA treatment shown by the low activity barleys did not necessarily increase their activity to the level of untreated high activity cultivars. It is possible that higher concentration of GA solution, longer treatment time or higher steep temperature would further increase the low activities. These treatments were not investigated. The extent of the response of enzyme synthesis to pre-germination treatment with GA could be indicative of varietal differences in the level of endogenous gibberellin. However, it seems likely that response may also be influenced by physical differences in barley species and cultivars that affect the penetration of GA or the rate at which it reaches the aleurone layer.

Results obtained in a previous experiment (Table IIb) indicated that GA treatment did not affect all components of the cytolytic system to the same extent. The data in Table IIb associate higher proportions of heat-stable components in the increment in cytolytic activity due to GA with lower response to the treatment. The residual activity after heating green-malt extract at 45° for 20 minutes was determined for some of the cultivars listed in Table III. Data were calculated in the same way as those in Table IIb and the results are shown in Table IV. In general, high increments in activity were mainly heat-labile in nature. Within groups of cultivars of similar background and grown at the same location, there was an inverse relation between the response to GA and the proportion of heat-stable component in the increment. Therefore,

the apparent benefit of a large increase in cytolytic activity as a result of GA treatment appeared to be offset to some extent by the heat-lability of the increment. The heat-stable proportion of the total cytolytic activity of green-malt is particularly important in view of subsequent kilning and malt-mashing operations. The comparison of the activities of heat-treated and untreated extracts of green-malt made from GA-treated and untreated barley is a guide to thermal properties, and the procedure could be useful in the evaluation of varietal differences in GA response.

The response of alpha-amylase to GA treatment (Table III) was less than that of the cytolytic system, and varietal differences were less striking, though the tendency of low activity cultivars to show higher response was also evident. The difference in response may be associated with the relative simplicity of alpha-amylase compared with the multi-component cytolytic system, or it may be that the mechanisms of alpha-amylase synthesis in the aleurone layer are more readily accessible to endogenous gibberellin.

The extent of degradation of the endosperm cell-walls largely determines the course of malting modification. Alpha-amylase functions, along with other hydrolytic enzymes, in the subsequent solubilization of starch and the production of fermentable extract. There is a close relation between alpha-amylase activity and the amount of soluble material that is extracted by mashing malt, and enhanced activity could be expected to result in an increase in malt extract. Pre-germination treatment with GA increases both enzyme activity and malt extract, but it has not been shown that higher amylase activity alone is responsible

for increased extract. It could be hypothesized that the beneficial effects of GA treatment in accelerating the malting process and in increasing malt extract yield mainly depend on the stimulation of the cytolytic enzyme system, and that the effect on other enzymes is of relatively minor importance.

The reaction to GA treatment should be considered in the evaluation of the suitability of barley cultivars for malting. The response of cytolytic reducing power activity is suggested as a useful selection tool in barley breeding. It seems to be particularly significant in differentiating in populations derived from two-row parents. In inter-specific crosses where the desired quality is that characteristic of six-row barley, GA response may be a useful index of progress towards this objective.

GA treatment and re-steeping

The technique known as re-steeping has been suggested as a means of reducing rootlet growth and consequently malting loss. The system involves a cold water steep in the normal way followed by a short germination period, then a re-steep in warm water to inhibit growth, after which germination is resumed and completed. The effects of this procedure on enzyme activities in green-malt and on the response to GA treatment were investigated.

In the first experiment the cultivars Conquest, Betzes and Hellas, grown at Winnipeg and Charlottetown in 1969, were used. These barleys had been found to show small, medium and large relative response to GA treatment. The samples were steeped for 40 hours at 12°, with and without treatment in a 4 ppm. solution of GA. Samples for re-steeping

were removed from the germinator after 24 hours and soaked in water at 32° for one hour. Excess water was shaken from the samples and they were returned to the germinator for a further 48 hours, at 17°. The resulting green-malts were assayed for cytolytic reducing power and amylase activities, and alpha-amino nitrogen content of the extracts was measured as an indication of proteolytic activity. Results are given in Table V.

The results indicated a tendency to lower values for the enzyme properties due to re-steeping of non-GA treated samples. This was more pronounced in the alpha-amylase and cytolytic activities of Conquest. Re-steeping of the GA treated samples caused a marked reduction in the response of the enzymes of Conquest and Hellas to GA, though Betzes was only slightly affected. This apparent varietal difference was investigated on a second set of three six-row and three two-row cultivars grown at Brandon in 1969. Again the results (Table VI) showed the tendency towards lower activity in the re-steeped samples, particularly in the alpha-amylase and cytolytic activities of Conquest. Reduced response to GA in re-steeped samples was confined to the six-row cultivars; Herta, Betzes and 2128, a selection of which Betzes is a parent, were unaffected.

The reduction in GA response was thought to be caused by a leaching of the acid from the grain before it had had time to exert its effect, that is, that the 24 hours between completion of steep and the re-steep treatment was too short a period.

In a third experiment total germination time was extended to 96 hours at a reduced temperature, 12.5°. Samples were re-steeped 48 hours after completion of initial steeping and an additional re-steep treatment of one hour at 40° was included. Analytical measurements included a

determination of the viscosity of 70° extracts of green-malt. Data on the cultivars Conquest, Paragon, Betzes and Centennial are given in Table VII.

The results showed that alpha-amylase and cytolytic activities were decreased by the re-steep treatments, while amino nitrogen and beta-amylase (not shown in the table) were only slightly affected. Re-steeping did not affect the response of enzyme activities to GA treatment in Paragon, Betzes and Centennial but response was reduced in Conquest. It may be that the earlier observed loss of GA response was due to re-steeping too soon in the germination period, but it seems likely that another mechanism, a varietal characteristic, is also involved.

The heat stability of the increments of cytolytic activity due to GA treatment was determined and calculated by the method already described. The proportion of heat sensitive enzyme in the increment ranged from 48% in Conquest to 56% in Centennial and appeared to be of no particular significance in this case. The decrease in activity due to re-steeping was more interesting and calculation showed that about 85% of the reduction in the activity of Paragon was thermally unstable, that is, of the type that is inactivated by subsequent kilning and mashing of malt. On the other hand, the value for Betzes was about 60%, indicating that the re-steep treatment caused the loss of an appreciable amount of stable activity. In between were Centennial and Conquest with values of 80% and 73%.

An estimate of relative gum content of the four cultivars was made by determination of the viscosity of 40° extract of barley. The values were: Betzes, 4.60; Paragon, 4.50; Centennial, 3.75 and Conquest,

2.89. The viscosity of 70° extracts of green-malt was also determined and the results are given in Table VII. Plots of reciprocal specific viscosity and cytolytic reducing power activity gave different slopes and intercepts for each cultivar, but within the cultivar there was a close relation between the two properties. The increase in relative viscosity appeared to be a direct consequence of the loss of cytolytic activity and emphasized the serious nature of this loss, particularly in cultivars initially high in gum content.

There was no intention to study or evaluate the commercial application of re-steeping, and the results of this work are not a basis for criticism of the process. The treatments were uniform but probably not optimal for the cultivars used, the sample size was small and the malts were not kilned. These conditions were suitable for the intended purpose, which was to investigate possible varietal differences in reaction to an arbitrary version of the re-steep technique in combination with GA treatment. The reasons for the varying behavior may be associated with the complex air-water-temperature interactions in steeping, that are appreciated but not understood, or possibly the higher temperature in the re-steep solubilized or activated inhibitors that retard enzyme synthesis. The looseness of the hull of Paragon and Conquest may be a contributory factor.

The techniques used in this study revealed hitherto unsuspected weaknesses in the cultivar Conquest, and possible plus factors in Centennial. Re-steeping at 40° affected cytolytic and alpha-amylase activities to an extent where reduced activity was not restored by GA treatment, except perhaps in Centennial. Re-steeping at 32° was less

destructive than the higher temperature and, except for Conquest, the deleterious effects were counteracted by GA. However, it seems clear that the beneficial effects of a GA treatment on the enzyme systems that are most directly concerned with malting modification, are offset to a significant extent by the re-steep treatment.

Elucidation of the mechanism responsible for the effects observed in this study and further investigation of their significance would contribute to the understanding of malting quality. Meanwhile, it appears that the treatments used in this work provide information on varietal differentiation. Techniques that reveal differences, whether or not their significance is fully understood, are useful in selecting in plant breeding.

Table I

Effects of germination and GA treatment on green-malt enzyme activities

Cultivar	Germ. days	Controls: Cytolytic: Visc. decr. units	Controls: Cytolytic: Red. power units	Controls: Alpha-amylase units	Controls: Amino N mg/100 ml.	Increase due to GA: Cytolytic: Visc. decr. %	Increase due to GA: Cytolytic: Red. power %	Increase due to GA: Alpha-amylase %	Increase due to GA: Amino N %
Conquest	2	0.18	1.16	6.0	7.0	33	25	12	20
	3	0.76	2.65	13.2	15.2	16	22	16	15
	4	1.70	4.54	18.8	20.0	3	16	14	29
	5	1.80	5.10	23.0	23.4	0	8	14	26
Parkland	2	0.14	0.90	4.4	7.0	43	30	14	24
	3	0.78	2.15	11.6	13.8	32	26	14	20
	4	1.52	3.95	17.6	20.0	32	23	13	28
	5	1.70	4.54	21.2	24.0	18	15	13	28
Keystone	2	0.08		3.0		0		14	
	3	0.31	1.20	6.0	10.0	10	25	14	15
	4	0.82	2.52	11.0	11.0	29	24	12	27
	5	1.00	2.60	11.8	12.2	20	20	12	32
Herta	2	0.12	0.85	5.0	6.0	50	50	40	40
	3	0.39	1.72	10.0	12.8	79	62	30	37
	4	1.05	2.90	12.4	17.5	52	47	28	42
	5	1.30	3.35	16.2	20.0	50	31	22	41
Betzes	2	0.15	0.80	6.4	6.0	80	75	47	60
	3	0.56	2.12	13.2	14.5	64	60	32	44
	4	1.14	3.58	15.2	19.5	54	52	24	50
	5	1.40	3.82	18.2	21.0	54	48	22	46
Diamant	2	0.16	0.90	5.0	6.8	56	33	52	32
	3	0.45	1.85	10.4	14.0	73	72	46	28
	4	1.27	3.35	15.0	20.0	65	60	27	48
	5	1.54	3.72	18.0	22.2	59	40	23	45

Table IIa

Effects of germination and GA treatment on cytolytic activity

Experimental data

Cultivar	Germ. days	Total activity		Resid. activ. after trmt.			
				Control		GA trmt.	
		Control units	GA trmt. units	Heat units	Lactone units	Heat units	Lactone units
Conquest	2	1.44	1.78	0.94	-	1.09	-
	3	3.60	4.25	1.84	2.38	2.28	2.98
	4	4.45	5.12	2.09	3.16	2.56	3.83
	5	5.00	5.59	2.35	4.05	2.81	4.64
Paragon	2	1.45	2.00	0.90	-	1.12	-
	3	3.18	4.28	1.43	2.00	1.87	2.85
	4	3.86	4.79	1.66	2.74	2.20	3.59
	5	4.79	5.59	1.87	3.74	2.44	4.54
Centennial	2	1.78	2.85	0.80	-	1.44	-
	3	3.25	4.68	1.30	2.14	2.01	3.40
	4	4.36	5.47	1.61	3.01	2.28	4.08
	5	5.38	5.83	1.88	4.30	2.16	4.75
Betzes	2	1.25	2.00	0.69	-	0.99	-
	3	2.97	4.11	1.28	1.75	1.86	2.53
	4	3.54	4.66	1.42	2.37	2.11	3.17
	5	3.86	4.91	1.54	3.20	2.24	4.16

Table IIb

Effects of germination and GA treatment on cytolytic activity

Calculated data

Cultivar	Germ. days	Incr. activ. due to GA %	Increment due to GA			Inactivation					
			Total units	Inactivated		Control		GA increment		GA trmt.	
				Heat units	Lactone units	Heat %	Lactone %	Heat %	Lactone %	Heat %	Lactone %
Conquest	2	24	0.34	0.14	-	35	-	41	-	49	-
	3	18	0.65	0.21	0.06	49	34	32	9	46	30
	4	15	0.67	0.20	0	53	29	30	0	50	25
	5	11	0.59	0.13	0	53	19	22	0	50	17
Paragon	2	38	0.55	0.33	-	38	-	60	-	44	-
	3	34	1.10	0.66	0.25	55	37	60	23	57	33
	4	24	0.93	0.39	0.08	57	29	42	9	54	25
	5	17	0.80	0.23	0	61	22	29	0	56	19
Centennial	2	60	1.07	0.43	-	55	-	40	-	49	-
	3	40	1.43	0.72	0.17	60	34	50	12	57	27
	4	25	1.11	0.44	0	63	31	40	0	58	25
	5	8	0.45	0.16	0	65	20	36	0	63	19
Betzes	2	60	0.75	0.45	-	45	-	60	-	50	-
	3	38	1.14	0.56	0.36	57	41	49	32	55	38
	4	31	1.12	0.43	0.22	60	33	38	20	55	32
	5	27	1.05	0.35	0.09	60	17	33	9	54	15

FIGURE 1

DEVELOPMENT OF CYTOLYTIC REDUCING POWER ACTIVITY DURING GERMINATION PARAGON BARLEY

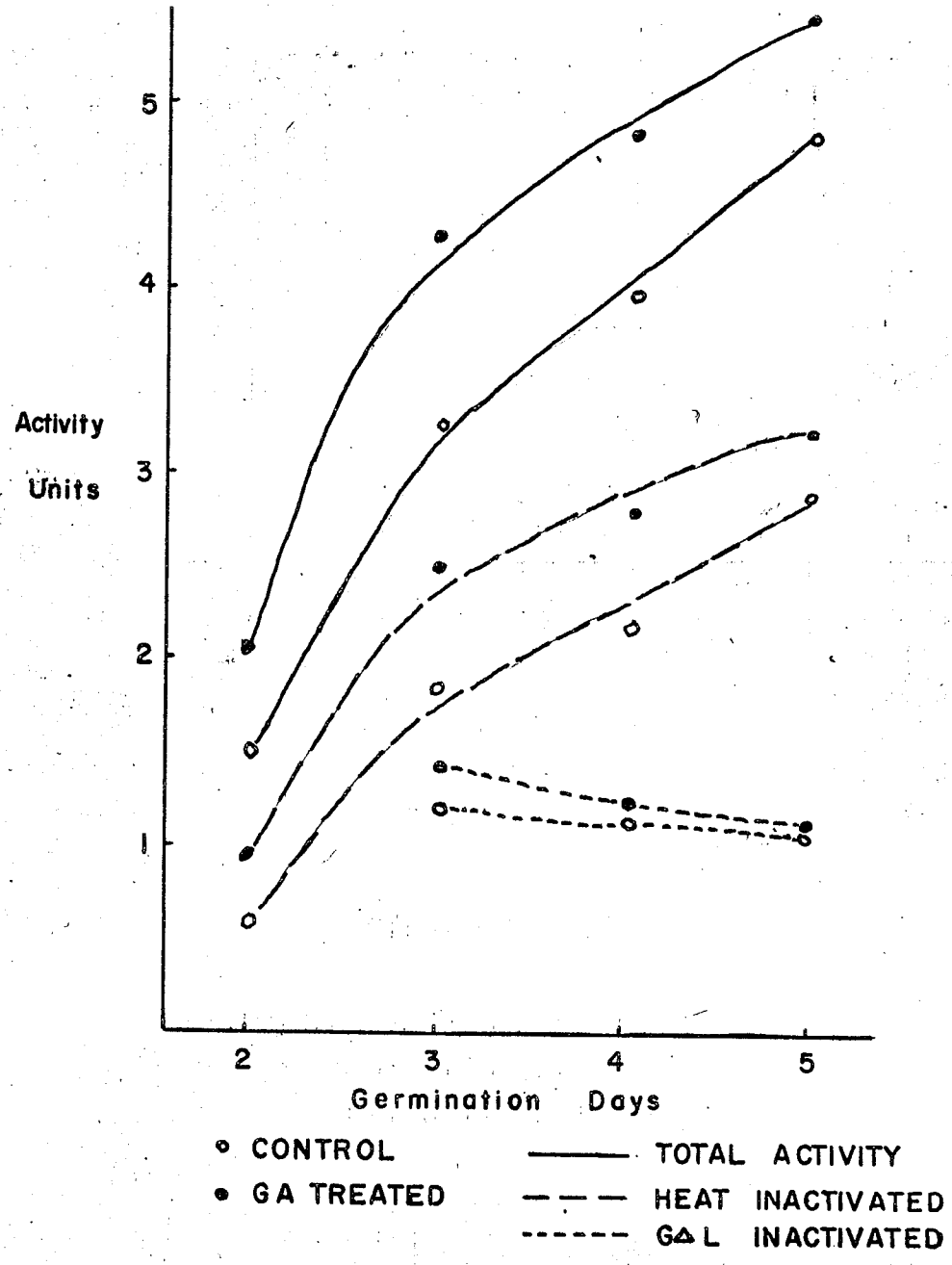


Table III

Varietal differences in the response of cytolytic and alpha-amylase activities of green-malt to GA treatment

Cultivar	Cytolytic			Alpha-amylase		
	Initial units	GA trmt. units	Response %	Initial units	GA trmt. units	Response %
a) Maritime 2-row, grown at Winnipeg						
Hellas	2.24	3.99	78	17.0	21.9	29
Kristina	2.88	4.64	61	18.0	23.9	33
Herta	2.93	4.60	57	18.0	24.1	34
Beka	3.03	4.24	40	17.0	22.6	33
Zephry	4.18	6.02	44	17.4	21.9	26
Akka	4.27	6.49	52	20.0	26.0	30
b) Maritime 2-row, grown in Sweden						
6202	1.85	3.87	109	18.8	24.1	28
6111	1.89	3.33	76	16.4	21.5	31
Sarla	2.10	3.86	84	21.2	26.7	26
6203	2.23	3.84	72	21.2	26.3	24
6199	2.27	3.86	70	24.6	28.3	15
6204	2.48	4.46	80	22.8	26.9	18
Ingrid	2.56	4.33	69	21.2	26.9	27
6171	2.61	3.29	26	22.6	26.4	17
6165	2.65	3.28	24	17.6	25.3	44
6168	2.65	4.24	60	21.2	25.0	18
6179	2.69	3.74	38	23.2	28.3	22
6194	2.90	4.41	52	24.2	27.8	15
Cilla	3.15	4.76	51	21.2	27.8	31
6187	3.36	4.37	30	28.2	30.2	7
c) North American 6-row, grown at Brandon						
Minn. M 9	2.38	3.24	36	15.0	18.0	20
ND 130	4.18	5.35	28	21.0	25.2	12
Minn. M11	4.25	5.02	18	18.4	21.7	18
SD 178	4.30	5.25	22	17.8	21.0	18
SD 640	4.40	5.46	24	20.2	22.6	12
Minn. M12	4.71	5.46	16	21.4	24.0	12
Conquest	5.36	6.00	12	23.4	25.7	10
ND 140	5.36	5.79	8	20.4	22.0	8
Wis. 993	5.70	6.27	10	20.4	23.7	16
Primus	5.70	6.27	10	22.0	23.3	6
d) Continental 2-row, grown at Winnipeg						
Nosovski	2.24	3.85	72	16.2	20.3	25
Cern. 5	2.70	4.37	62	16.4	20.0	22
B 2022	3.05	4.94	62	12.6	18.0	43
Betzes	3.07	4.39	43	16.6	20.6	24
Sloven.822	3.14	3.99	27	16.6	19.6	18
IB 6-3	3.40	4.93	45	17.8	21.5	21
Valticky	3.62	5.79	60	16.2	18.8	16
MFB 104	4.00	5.36	34	18.6	22.7	22
Unzyozny	4.58	5.86	28	20.0	23.4	17
Bran. 130	4.73	5.49	16	19.6	22.1	13

Table IV

Heat stability of increment in cytolytic activity due to GA treatment

Cultivar	GA response %	Heat-stable fraction of increment %
b) Maritime 2-row, grown in Sweden		
6171	26	69
6179	39	64
6194	52	40
Ingrid	69	38
Sarla	84	28
6202	109	34
c) N. American 6-row, grown in Canada		
Minn. M12	16	83
ND 140	8	75
Conquest	12	55
SD 640	24	44
ND 130	28	39
Minn. M 9	36	40
d) Continental 2-row, grown in Canada		
Bran. 130	16	77
MFB 104	34	34
Unzyozny	28	29
Cern. 5	62	26
Nosovski	72	24

Table V

Effects of re-steep and GA treatments on green-malt properties

Cultivar and treatment*	Cytolytic Control units	Cytolytic GA incr. %	Alpha-amylase Control units	Alpha-amylase GA incr. %	Alpha-amino N Control mg/100 ml	Alpha-amino N GA incr. %
Conquest - Lethbridge						
0	5.39		21.2		26.6	
0 RS	4.43		18.6		25.8	
GA	6.25	16	23.7	12	34.6	30
GA RS	4.74	7	19.5	5	29.4	14
Conquest - Charlottetown						
0	4.57		18.9		17.4	
0 RS	3.86		15.9		17.0	
GA	5.60	23	21.5	14	22.0	26
GA RS	3.96	3	17.0	7	20.0	18
Betzes - Lethbridge						
0	4.43		17.8		19.2	
0 RS	4.36		17.0		18.3	
GA	5.68	28	22.7	28	32.2	68
GA RS	5.41	24	20.4	20	27.4	50
Betzes - Charlottetown						
0	4.03		15.0		16.6	
0 RS	3.82		14.9		15.6	
GA	4.75	18	18.7	25	21.2	28
GA RS	4.46	17	18.2	22	20.0	28
Hellas - Lethbridge						
0	4.68		20.6		19.0	
0 RS	4.43		19.7		18.0	
GA	6.43	37	28.8	40	27.4	44
GA RS	5.28	19	23.2	18	23.2	29
Hellas - Charlottetown						
0	3.82		15.5		13.0	
0 RS	3.14		14.3		13.0	
GA	5.57	46	20.4	31	18.4	42
GA RS	3.79	21	15.9	11	16.2	25

* 0 - Control steep, GA - GA treatment
RS - re-steep one hour at 32°

Table VI

Effects of re-steep and GA treatments on green-malt properties

Cultivar and treatment*	Cytolytic		Alpha-amylase		Alpha-amino N	
	Control units	GA incr. %	Control units	GA incr. %	Control mg/100 ml	GA incr. %
Paragon						
0	4.44		19.8		17.5	
0 RS	4.20		18.8		17.1	
GA	5.56	25	23.5	19	21.5	23
GA RS	5.06	21	22.5	20	20.9	22
Betzes						
0	4.16		21.8		18.3	
0 RS	4.04		21.2		18.7	
GA	5.76	38	25.2	16	24.5	34
GA RS	5.60	39	24.7	17	24.8	33
6624 (six-row)						
0	4.88		23.9		18.8	
0 RS	4.88		23.9		19.8	
GA	6.40	31	29.4	23	26.4	40
GA RS	6.00	23	28.7	20	26.6	34
Herta						
0	4.36		20.3		15.9	
0 RS	4.18		19.9		16.4	
GA	5.76	32	23.5	16	19.1	20
GA RS	5.44	30	23.9	20	19.9	21
2128 (two-row)						
0	4.20		17.2		19.4	
0 RS	4.08		16.8		19.1	
GA	5.76	37	21.0	22	25.0	29
GA RS	5.52	35	21.8	30	25.9	36
Conquest						
0	5.44		23.0		18.8	
0 RS	5.08		21.0		19.7	
GA	6.12	13	25.5	11	23.4	24
GA RS	5.32	5	21.8	4	23.4	19

* Treatments as in Table V

Table VII

Effects of re-steep and GA treatments on green-malt properties

Cultivar and treatment*	Enzyme activity		GA response		a-amino N	70° extract viscosity
	Cytase units	a-amylase units	Cytase %	a-amylase %	mg/100 ml	cp.
Centennial						
0	4.39	27.4			15.4	1.50
32 RS	3.33	26.2			14.9	1.62
40 RS	2.56	23.4			15.0	2.19
GA	5.71	34.0	30	24	29.0	1.48
32 RS	4.88	33.2	47	27	26.8	1.36
40 RS	3.29	29.8	29	27	28.0	1.38
Betzes						
0	3.50	25.0			16.2	1.85
32 RS	2.48	21.0			14.8	3.13
40 RS	1.42	16.8			15.0	4.75
GA	4.55	29.0	30	16	26.4	1.51
32 RS	3.25	25.8	31	23	24.0	1.61
40 RS	1.97	20.8	38	24	24.6	2.13
Paragon						
0	3.50	21.0			15.2	1.78
32 RS	3.01	19.9			13.2	3.27
40 RS	1.68	13.4			13.6	3.64
GA	4.96	26.2	42	25	21.8	1.58
32 RS	4.15	25.8	39	30	18.4	1.56
40 RS	2.40	16.6	42	24	19.0	2.26
Conquest						
0	4.39	24.8			14.0	1.36
32 RS	2.60	20.2			14.0	1.75
40 RS	1.92	14.0			14.4	1.85
GA	5.41	29.8	23	20	22.0	1.40
32 RS	2.92	22.0	11	11	18.2	1.69
40 RS	2.40	15.4	12	11	20.2	1.90

* 0 - control steep, GA - GA treatment
32 RS - re-steep one hour at 32°
40 RS - re-steep one hour at 40°

SECTION FOUR

AUTOMATED METHODS FOR THE SELECTION OF CYTOLYTIC AND AMYLOLYTIC ACTIVITIES IN BARLEY BREEDING PROGRAMS

ABSTRACT

Automated methods for the determination of two key properties related to modification of barley during malting were developed and are described. Cytolytic reducing power and alpha-amylase activities are assayed by measurement of reducing sugar produced by the action, under standard conditions, of a green-malt extract on barley beta-glucan and starch substrates respectively. The assays are especially useful in early generation selection for quality in barley breeding programs.

INTRODUCTION

Efficiency in cereal breeding programs depends on the selection of promising lines as early as possible. Consequently, considerable effort has been directed toward the development of techniques related to quality that can be applied to large numbers of small samples. In Canada, selection for malting quality in early generations of barley breeding programs was initially based upon determinations made on unmalted barley that are related to the traditional criteria of malt extract and diastatic power. Later, these tests were augmented by others that made use of small samples of green-malt, that is, germinated barley, though the quality criteria remained unchanged. At the present time advances in malting and brewing technology necessitate a re-appraisal of quality. The trend towards accelerated and continuous processing and the use of higher proportions of unmalted cereal adjuncts has focussed attention on enzyme synthesis and activity.

It is clear that industrial acceptance of new barley cultivars will be influenced by their ability to modify rapidly during malting. Accordingly the enzyme systems that are associated with modification are important factors in screening barley hybrid populations, and techniques for their selection are required.

Although knowledge of the enzyme mechanisms operative during malting and brewery is not complete, the significance of the cytolytic, gum-degrading enzymes and of alpha-amylase is recognized. Gum-degrading activity is present in unmalted barley, but the small varietal differences have been found to be unrelated to malting quality. Alpha-amylase

activity is not detectable in barley by ordinary techniques. However, both activities increase markedly during germination and there are significant varietal differences in the cytase and amylase activities of extracts of green-malt. Present methods for the assay of these enzymes are time-consuming and of limited value in the examination of early generation plant breeding material.

An instrument for the acceleration of chemical analyses that has gained a good deal of interest in recent years is the Technicon Auto-Analyzer. The general principles and component parts of this equipment are fairly well known, so that a detailed description is not required. In brief, in this system the reaction of a sample under test is compared with that of a standard undergoing exactly the same treatment. Reactions need not be complete, nor need equilibrium conditions be attained, so that analysis time is considerably reduced. The system is applicable to any analytical determination that is based on colorimetry. Therefore, it is advantageous in any laboratory engaged in the routine testing of large numbers of samples to develop analytical methods that depend on colorimetric reactions.

In their studies of the relation between the cytolytic activity of green-malt extracts and malting quality, Meredith et al. (2, 3, 4, 11) determined the enzyme activity by a viscosimetric procedure. The decrease in viscosity of a mixture of barley beta-glucan substrate and enzyme extract was measured at timed intervals and the result, calculated from the slope of a plot of reciprocal viscosity against time, was regarded as the best measure of cytolytic activity. An increase in reducing power activity accompanies the viscosity reduction and the two phenomena

have been attributed to different enzyme systems. The viscosity decrease is due to an endo-beta-glucanase system and reducing power activity to a combined exo- beta-glucanase and disaccharase action. Though viscosity reduction is the prior, and faster, reaction, there are indications that the two systems are positively related. Therefore, the automated reducing power determination was compared with the viscosity technique.

Alpha-amylase activity is usually assayed by measuring either the reduction in the viscosity of a starch paste or the extent of starch dextrinization. It may be possible to automate the determination of dextrinizing activity, but it is simpler to utilize the enzyme's saccharogenic property. Saccharification of starch is a dual function of both alpha- and beta-amylases, but it is known that the beta-action can be inhibited. The use of the saccharifying property to assess alpha-amylase activity is open to question and does not appear to have wide acceptance. Nevertheless, the determination should provide a guide to the relative alpha-amylase activity within a barley breeding population, and therefore be useful in selecting high activity lines.

MATERIALS

The barley samples used in this study were grown in test plots at various stations of the Canada Department of Agriculture Research Branch in 1969. Substrates for enzyme activity studies were obtained from several sources: pachyman from the Sam-ae Trading Co., Seoul, Korea; cellobiose, carboxymethylcellulose and laminarin from the Nutritional Biochemical Corporation, Cleveland, Ohio; amygdalin, salicin, chitin and p-nitrophenyl-beta-glucoside from Mann Research Laboratories, New York; starch from Merck and Co., New Jersey. Before use, the pachyman was finely ground and de-fatted by a chloroform-alcohol treatment; the other materials were used as supplied.

Barley beta-glucan was prepared in the laboratory by the method of Bass and Meredith (2). Ground barley that had been refluxed with boiling 85% alcohol was dried and extracted for two hours at 40° with a .025% aqueous solution of papain. After precipitation of proteins by trichloracetic acid, the extract was filtered and 30 g. of ammonium sulfate added to each 100 ml. of filtrate. The precipitate was washed with alcohol and freeze-dried. Working solutions were prepared by dissolving glucan in hot 0.01 M acetate. Stored at 5°, the solution was stable for at least 7 days.

The sources of enzyme activity were extracts of green-malt. This material was prepared in the laboratory malting equipment (9). The germinator unit of this equipment was fitted with racks that hold 48 perforated, wide-mouth, 8-ounce polyethylene bottles. In these containers, samples of 15 g. of barley, dry basis, were steeped in water at 12°

until the moisture content reached 44%, usually about 40 hours, with a water change and air rest of 90 minutes every 8 hours. Excess surface water was removed by shaking and the bottles were transferred to the germinator. After germination at 17.5° for 72 hours, the green-malt samples were either quickly frozen or extracted immediately.

Green-malt extracts were made by macerating the whole sample with 0.5% sodium chloride, buffered with acetate to pH 4.7, in a Waring blendor for 0.5 minute at low speed followed by 2.5 minutes at high speed. The ratio of extractant to solid was ten to one, so that the weight of extraction mixture was 165 g. After filtration for 30 minutes the extract was used in analytical determinations, or stored at 5° if necessary.

EXPERIMENTAL

Cytolytic activity

Substrates

Barley beta-glucan is a natural substrate for the cytolytic enzymes of barley and malt, and it is regarded as the most suitable substrate for the study of the enzyme system. However, this material cannot be purchased from supply houses. As its laboratory preparation is somewhat laborious, the possibility of using alternate substrates for the determination of reducing power activity was investigated. The beta-linked carbohydrates that were commercially available were pachyman (1, 3), laminarin (1, 3), carboxymethylcellulose (1, 4), cellobiose (1, 4), chitin (1, 4) amygdalin (1, 6), salicin and p-nitrophenyl-beta-glucoside. The rate of reducing sugar production by the action of green-malt extracts on these materials was determined. All were hydrolyzed, but the rate of hydrolysis of carboxymethylcellulose, chitin, amygdalin and salicin was too slow to be of any practical value.

The chromogenic compound, p-nitrophenyl-beta-glucoside, was effective in a colorimetric test that required only 20 minutes incubation of substrate and extract at 30°. However, differences between the activities of green-malt extracts made from barley cultivars, that had shown a range of activity on beta-glucan, were very small. It was concluded that this substrate is of no value in screening barley breeding material. Pachyman is insoluble and must be handled in suspension, which is a disadvantage in that it precludes the use of automatic

dispensing equipment. Cellobiose had a high blank value in the assay used, and while differences in activity between green-malt samples were observed, the assay took twice as long as the determination made on glucan. The laminarin substrate appeared to be the most useful.

The effectiveness of laminarin, pachyman and cellobiose was further studied by the use of enzyme inactivation treatments. Data on the effects of various inhibitors on the action of green-malt extract on four substrates are given in Table I. On the basis of these results three inhibitors, $2x10^{-3}$ M glucono-1,5-lactone (GL), $2x10^{-4}$ M phenyl mercuric acetate (PMA) and $2.5x10^{-4}$ N-bromosuccinimide (NBS), together with a heat treatment of the green-malt extract at 45° for 20 minutes, were selected for comparison of the substrates. The effects of the treatments, and combinations of them, are shown in Table II. The inactivation values are the means for 20 green-malts.

It was apparent that none of the three artificial substrates was a reasonable alternative to barley beta-glucan. The heat treatment alone destroyed about half the activity of the extracts and very little of this was measurable on the other substrates. A set of equations was derived from the data and calculation indicated that the activity of green-malt extracts on laminarin, pachyman and cellobiose represents about 10%, 10% and 6% of the activity on beta-glucan. Two other observations provide further evidence of the ineffectiveness of the artificial substrates. A pre-germination treatment with gibberellic acid stimulated the activity of extracts on beta-glucan by 23% and 46% in two sets of barleys. The increase in activity on the other substrates was 4% and 12%. Data on a set of 15 samples, that showed regular increments in activity on

beta-glucan, were compared. The rank correlation coefficients for the relations between the activity on glucan and that on cellobiose, pachyman and laminarin were 0.50, 0.49 and 0.30.

Development of the routine method for cytolytic activity

Reducing power activity was assayed by measurement of glucose production by a mixture of beta-glucan and green-malt extract. An attempt to use an autoanalyzer procedure similar to that for amylase activity, in which malt extract and starch substrate react in passage through glass coils, was unsuccessful. Higher reaction temperatures, up to 38°, and an increase in coil length to 160 feet did result in measurable hydrolysis of the beta-glucan, but the recorder chart pattern was poor and the procedure unreliable. Accordingly the incubation was separated from the auto-analyzer system, which was used only in sugar determination. Though the analyzer sampler module was fitted with a temperature controlled bath so that incubation could proceed in the cups in the sampler rack.

The decrease in optical density of potassium ferricyanide was used to measure reducing sugar. This reagent was made alkaline with sodium carbonate and the determination was sensitized by the addition of potassium cyanide to the sample diluting water. The optimum temperature for the reducing power action of green-malt extracts on beta-glucan was found to be 35°. Double reciprocal plots of reaction velocity and substrate concentration gave a Km value of 1.1 mg./ml. of reaction mixture, equivalent to 0.15% substrate concentration. Glucose production was strictly proportional to extract concentration.

Operational details

The Technicon auto-analyzer flow diagram is shown in Figure 1. A Fisher auto-dilutor, model 240, was used to mix 0.05 ml. of green-malt extract, prepared as described in the Materials section, and 2.45 ml. of 0.30% beta-glucan solution, buffered with acetate to pH 4.7. As the analyzer sampling rate was 40 per hour, the samples were mixed at 1.5 minute intervals. The first determination of reducing sugar content of the reaction mixture was made 5 minutes after mixing. In the analyzer system the sample was diluted with 0.5% potassium cyanide and then mixed with 0.04% potassium ferricyanide containing 2% sodium carbonate. After passage through a 40-foot coil at 95°, absorbance was measured at 420 mu. The analysis was calibrated with solutions of glucose of concentration 0.10 to 0.35 mg./ml. The test samples were incubated at 30° and a second glucose determination was made 125 minutes later. Activity was expressed as mg. of glucose produced by 100 mg. of original barley in 100 minutes, or under the particular conditions described, the difference between the two glucose concentrations multiplied by 40. When other incubation times were used, as was necessary in certain groups of barleys with low activity, the multiplier was 5000/ time in minutes.

Relation between reducing power and viscosity decreasing activities.

The viscosity assay was essentially as described by Meredith (2, 11). Fast-flowing Ostwald viscometers, with a water time of 0.25 minutes, were used and the reaction mixture was 5 ml. of 1% beta-glucan solution, buffered to pH 4.7, and 2 ml. of diluted (one in ten) green-malt extract. The viscometers were immersed in a water-bath at 25°.

Reaction was timed from the mixing of the reactants and the mixture was immediately transferred to the viscometer. Viscosity measurements were made at timed intervals until the relative viscosity decreased to half the initial value. Reciprocal specific viscosities were calculated and plotted against time. Activity was expressed as the slope of the linear plot multiplied by 100.

The correlation coefficient for the relation between the two enzyme activities determined on green-malt extracts made from 35 barley cultivars was 0.93**. The auto-analyzer procedure therefore provides a satisfactory estimate of cytolytic activity.

Alpha-amylase activity

An auto-analyzer procedure for the determination of diastatic power (saccharifying or total amylase activity) has been described by Saletan and Scharoun (10). This technique was modified to provide an estimate of alpha-activity alone. The principle was the differential inactivation of the beta-amylase component by a phenyl-mercury salt as in an earlier procedure reported by Bendelow (5). The inactivator may be 10^{-4} M p-chloromercuri-benzoate or a saturated solution of the very slightly soluble phenyl mercuric chloride. Both gave idential results, though the latter has some practical advantage. The assay was made on the green-malt extract prepared for cytolytic activity determination.

Operational details

The Technicon auto-analyzer flow diagram is shown in Figure 2. The green-malt extract was sampled and mixed with inactivator. A sample of this mixture was passed through a 40-foot coil in a 30° bath

along with an aliquot of 1% starch solution, buffered to pH 4.7 with acetate. This reaction was terminated by the introduction of 1.0 N sodium hydroxide into the system. The reducing sugar content of the alkaline hydrolyzate was determined by reaction with 0.037% potassium ferricyanide during passage through a 40-foot coil in a 95° bath. Absorbance was measured at 420 mu. and the analysis was calibrated by solutions of maltose of concentration 15 to 30 mg./ml. Activity was expressed as mg. of maltose produced by 100 mg. of original barley, which is quite satisfactory for the comparison of relative activity in a barley breeding population.

Relation to other methods

The total amylase determination was compared with the standard method of the American Society of Brewing Chemists (1) and a satisfactory relation was found, in agreement with the results of Saletan and Scharoun (10). No comparison of the alpha-amylase assay was made with the laborious standard method of the Society. Medcalf et al. (7) described a method for the determination of this activity in kilned-malt based on the falling number technique of Hagberg (6), and reported a highly significant correlation between their method and the standard method, though the two procedures measure different manifestations of the enzyme's activity, starch viscosity reduction and dextrinization. The auto-analyzer method, which measures saccharogenic activity, was compared with the falling number technique, using 50 samples of kilned-malt with a falling number range of 110 to 290. The correlation coefficient for the relation between the two methods was 0.88 and it was concluded that the auto-analyzer procedure provided

a useful indication of alpha-amylase activity.

An estimate of beta-amylase activity may be obtained by subtraction of the alpha value from the total activity. This is arbitrary and its inadequacies were discussed by Meredith (8). However, as no alternative procedure is available, it may be of some use in the comparison of barley hybrid lines.

Table I

Inhibition of green-malt cytolytic reducing power activity on various substrates

Inhibitor	Conc'n M	Percentage inhibition			
		Glucan	Laminarin	Pachyman	Cellobiose
Phenylmercuric acetate	10^{-4}	55	4	8	15
	$2x10^{-4}$	68	6	12	23
Phenylmercuric chloride	sat'd.	69	9	14	71
Chloromercuri-benzoate	10^{-4}	60	4	5	19
	$2x10^{-4}$	65	7	20	24
Iodoacetate Na	10^{-4}	0	0	7	0
	10^{-3}	0	0	10	5
EDTA	$5x10^{-3}$	0	0	30	0
	$2x10^{-2}$	0	0	46	0
Glucono-1,5-lactone	10^{-3}	25	0	25	78
	$2x10^{-3}$	35	0	38	95
N-bromo succinimide	10^{-4}	30			
	$2.5x10^{-4}$	86	40	25	80

Table II

Effects of inactivation treatments on green-malt cytolytic reducing power activity

Treatment	Percentage inactivation			
	Glucan	Laminarin	Pachyman	Cellobiose
Heat	51	5	6	0
GL	34	0	36	93
GL + heat	71	5		95
PMA	66	5	14	20
PMA + heat	79	10	26	20
PMA + GL	76	5		100
PMA + GL + heat	84	10	30	100
NBS	84	40	24	80

FIGURE 1

Auto-analyzer flow diagram for the measurement of reducing sugars in the cytolytic activity assay

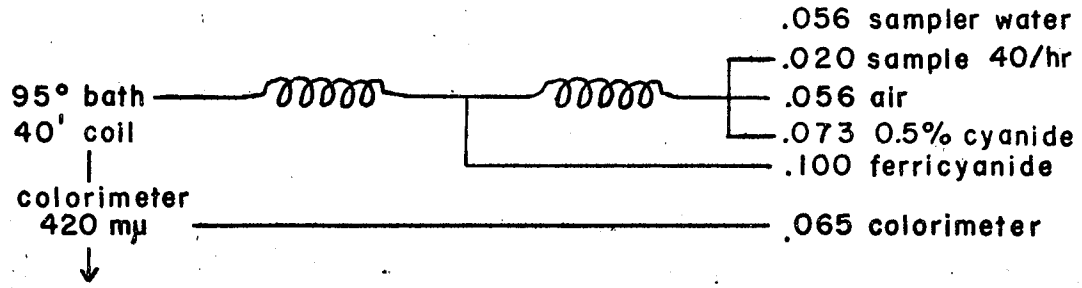
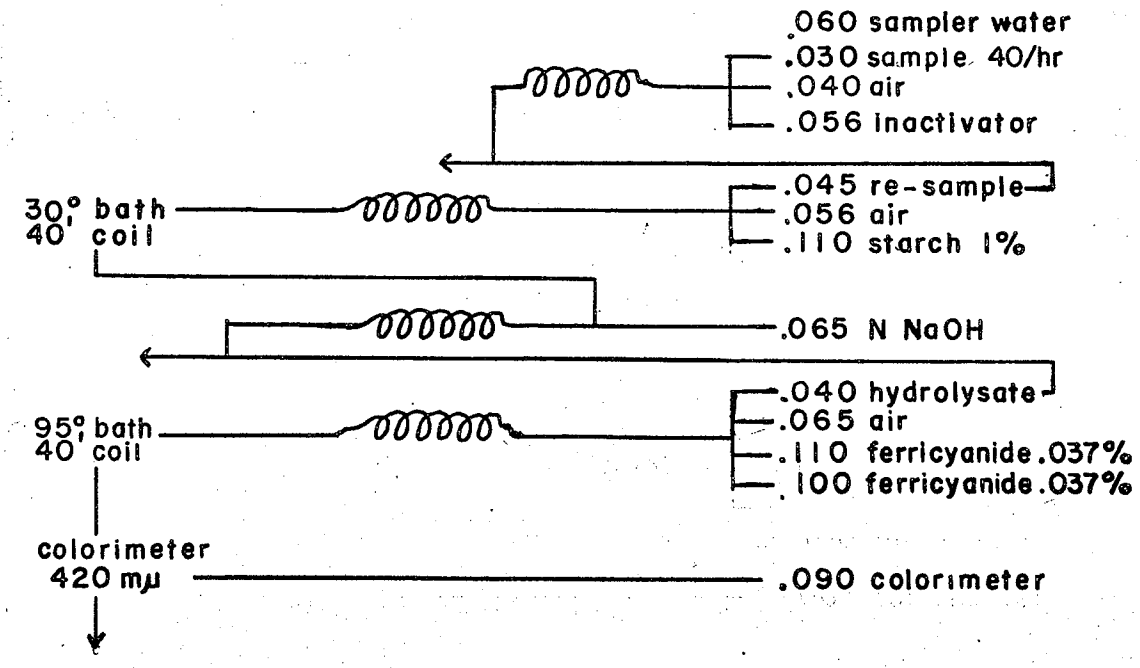


FIGURE 2

Auto-analyzer flow diagram for the determination of alpha-amylase activity in green-malt extracts



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DISCUSSION

"Consideration should be given to a re-inspection of the original structure and of the alterations in structure of the neutral, water-soluble polysaccharides of barley and malt using modern techniques and attempting to discern minor differences between analogous polysaccharide fractions from different varieties of barley, such as between six- and two-row barleys. The relationships between the genetic history of the plant and its carbohydrates have been a problem of interest to carbohydrate chemists for many years. Hence, information contributing to the elucidation of such relationships continues to be of great interest to this discipline and may eventually lead to a practical system of chemical taxonomy of assistance to plant breeders and those using plant products".

The above was a recommendation made in 1970 by McFarlane (3) in compliance with a request by the Brewing Industries Research Institute that he survey the fundamental research needs of the industry that complement the applied research of individual brewing and malting companies. McFarlane outlined the importance of the high molecular polysaccharides, gums, during the processing of barley and malt and their contribution to the unique character of beer. He stressed the need to elucidate the vaguely understood nature of these materials and the structural and physical alterations occurring at each stage in the malting and brewing processes, and concluded that, "it is necessary to relate the component in finished beer with its source material and meaningful results may only be obtained by initiating

the investigations at the first stage of the malting process, namely steeping and germination, and conducting a more extensive study of the enzymes which degrade the cell-walls of the barley kernel".

The concern of industry with the non-starch polysaccharides, that originate in the endosperm cell-walls of barley, is well founded. Their influence on the rate and extent of modification has been recognized for some time. Their effects on various operations of the brewing process and their contribution to the character of beer have become apparent more recently.

In the past, the lengthy germination time used in malting permitted extensive enzymic degradation of the cell-wall gums, and consequently cytolysis factors were of minor concern. Their present importance is due to the need to effect economies in the cost of production of brewery wort. Procedures that reduce malting time and malting losses are being investigated, and much effort is being spent on the study of wort production by a process in which raw, unmalted barley is converted by exogenous enzymes. The kilning of malt is expensive and therefore the use of green-malt in conjunction with unmalted cereals appears to be an attractive economic proposition.

The course that technological changes in the malting and brewing industries will take is uncertain, but it is safe to speculate that the characteristics, particularly the cytolytic properties, of available barley and of new cultivars produced by plant breeding will influence future developments. Acceleration of the malting process is dependent on rapid degradation of the endosperm cell-walls, the essential initial step. A low content of gummy cell-wall material in

barley and rapid synthesis of cytolytic enzymes early in malting may well enable malt of the traditional type to be competitive with alternate methods of wort production.

The use of barley as an adjunct in brewing is reportedly satisfactory (2) and has economic advantages in northern countries that do not produce corn or rice. The latter cereals contain only small amounts of gum and have little effect on brewing and beer properties. Barley used as an adjunct could be expected to have significant effects, depending on its gum content and the extent to which this is degraded during mashing.

The significance of the gum content of barley is not confined to malting and brewing. Recent work (1, 4) showed that poor growth in poultry fed on barley was associated with high viscosity in the small intestine caused by barley gums. Water treatment and enzyme supplements, of which malt is one source, hydrolyzed the gums, reduced the viscosity and resulted in improved performance. This indicates that gum content is a criterion of feed quality and is useful evidence of the compatibility of malting and feed qualities in barley.

The timely nature of the present study, which was begun before the publication of McFarlane's report, is evident from the above summary. As discussed in the introduction to the study, there was a sound foundation, provided principally by Meredith and Preece prior to 1960 and quoted extensively by McFarlane, on which to base a further investigation of the problem. This was undertaken, at the starting point subsequently suggested by McFarlane.

Varietal and environmental differences in barley gum content and cytolytic activity developed during malting were re-investigated. The major differences in these properties between Canadian six-row cultivars and two-row barleys are particularly significant. They show that there are genetic sources of desirable quality characteristics whose use in plant breeding should lead to improvements in cultivars of both the two-row and six-row species. These findings were augmented by study of the changes in cytolysis factors that occur early in the malting process and interactions of enzyme systems with gibberellic acid used to stimulate their synthesis in malting. The results, along with those of a study of thermal properties of the cytolytic enzyme system, revealed additional differences in the behavior of barley cultivars during malting and provided information useful in the evaluation of quality. Finally, a "practical system of chemical taxonomy of assistance to the plant breeder" was developed that will make possible the efficient selection in plant breeding programs of lines with the quality characteristics that are foreseen.

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