PROPERTIES OF BARLEY GUMS OF REDUCED NITROGEN CONTENT

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INTRODUCTION

Plants and their fruits are composed principally of carbohydrates and proteins. The composition and structure of these materials have been studied for many years. For some time carbohydrates were regarded mainly as reserve food material, although certain carbohydrates were classed as structural material. Recent advances, made possible by the development of new techniques, have indicated that carbohydrates, as well as proteins, may play an important role in the properties of plants and their products. As a result, the composition and structure of plant carbohydrates are now being investigated intensively. Although many non-starch polysaccharides have been recognized in plant material, study of these lagged until a clear picture of the structure of starch -- the main plant polysaccharide -- was attained. The techniques that were used to determine starch structure are now being applied to non-starch polysaccharides, the most prominent of which are the plant gums.

Many materials are classed as plant gums. Some are produced as exudates from cut surfaces, whereas others are an integral part of plants or their fruits. Although gums obtained from different sources vary in composition and structure, they usually contain hexose residues, uronic acid residues and pentose residues, and produce highly viscous aqueous solutions. Detailed study of these complex polysaccharide materials was greatly assisted by the development of chromatographic procedures for isolation and identification of organic materials. The progress in studies of plant gum composition and structure was reviewed by Jones and Smith (20), and has assisted studies of other complex polysaccharides. Among these are the non-starch polysaccharides obtained from cereals. These carbohydrates have been recognized for many years, but were not amenable to detailed study. Investigation of these materials has been revived (28) and they now appear to be related to the plant gums.

Gum isolated from barley is one of several cereal gums that have been investigated. Barley gums have been known for seventy years, but their composition and structure could not be determined. Earliest investigation (32) indicated that these gums consisted of polymerized carbohydrate material, but no methods were available to study the products further. Later work (5, 11, 28) showed that crude barley gums also contain nitrogenous material that was believed to have a significant influence on gum properties. Suggestions have been made that some of the barley gum nitrogen may be part of enzymes which degrade the gum (28), and that at least some of the nitrogen may be part of a protein-carbohydrate complex (11, 28). Barley gums were therefore investigated in an attempt to ascertain the extent to which nitrogenous material contributes to gum properties.

Interest in barley gums was aroused in this Laboratory by results of studies on wort. Certain wort properties are believed to be influenced by a component containing carbohydrates and nitrogenous material. Wort viscosity, a characteristic wort property, decreases as the amount of this complex material in wort decreases. As viscosity of wort is an useful measure of malt quality, investigation of the origin of the viscous principle of wort was undertaken.

Isolates similar to barley gum can be obtained from malt, wort and beer (26, 28, 42). However, gums from the latter materials have not been investigated intensively, and consequently the relation between the four gums is indefinite. It was believed a systematic study of the four gums should begin with the product isolated from barley. Since the gums are presumably interrelated, it will be useful to review briefly the malting process and some brewing terms.

There are four stages through which barley must be passed before it is ready to be utilized for the production of beer. The grain must first be cleaned and stored. Freshly harvested barley has a poor germinative capacity and, therefore, should be stored for a few months before malting. After storage, the barley is steeped or soaked in water until the moisture content of the grain is about 45%. Steeping is necessary to allow germination to take place.

The third, and most important, part of the malting process is germination. Changes that take place in the kernel during this stage are: (a) formation and liberation of enzymes, (b) growth of plumule and rootlets, (c) disappearance of endosperm cell walls and subsequent softening of the grain, and (d) degradation of proteins and starch. The development of enzymes is the primary change and all the other changes are, to some extent, a result of enzyme activity. When the plumule, which develops during germination, breaks through the hull, enzymatic activity is usually a maximum.

Kilning is the final stage of malting; the green malt is dried by a stream of air to inhibit germination. The temperature of air is increased stepwise from 85°F. to 170°F. This drying process determines the enzymatic activity of malt and the final flavor and color of beer. Kilned malt is ground and the grist extracted with water. The mash is filtered, and the filtrate or wort is the basic material in the production of beer.

Several chemical changes occur in the barley kernel during malting; the most important changes are the enzymatic degradations of carbohydrates and proteins. Enzyme hydrolysis of these materials is essential to attain the greatest potential of malt extract. Modification is a general term used to describe the conversion of insoluble material into water-extractable products during malting. Degree of modification is defined as a measure of malt quality, and is one of the most important factors governing the brewing value of malt. Brewers use the terms "over-", "under-" and "normal-modification", when speaking of malt quality. However, normal modification is difficult to define because required characteristics vary with brewing methods used and the type of beer desired.

Several procedures have been proposed for measurement of degree of modification (14), but none is completely satisfactory as shown by the diversity of the methods. One of the most recent and more practical methods suggested is that of wort viscosity (38); viscosity of wort decreases with length of barley germination time. It was through this property that investigation of barley gums began.

Barley gums are believed to be the precursor of at least part of malt gums (28), and hence the former gums may be indirectly responsible for certain wort properties. Barley gum and hemicellulose are chemically similar and both are believed to be present in barley cell walls. The presence of nitrogenous material in hemicellulose has not been determined, but nitrogen has been shown to be present in barley gum either as a constituent or as a contaminant. Gum that is free from extraneous nitrogenous material would be of value in studying gum structure. Therefore, gums from which nitrogen was removed in a systematic manner were studied in order to elucidate the effect of nitrogen on gum properties.

Results of studies of properties of barley gums of reduced nitrogen content are presented in this thesis. The first section presents some information on the non-starch polysaccharides contained in barley, malt and wort. The carbohydrate composition of these materials shows their relation to plant gums. Precipitation and extraction methods used to reduce the nitrogen content of barley gums are described in the second section. The products were characterized by determination of solution viscosity, solution instability, nitrogen content, carbohydrate components and amino acid components of gum. The significance of these gum characteristics is discussed in the third section. Finally, all the relevant information is reviewed and conclusions are drawn regarding the relation of nitrogenous material present in barley gums to barley gum properties.

REVIEW OF BARLEY, MALT AND WORT GUMS

Although barley gums have been recognized for a long time, it is only with the development of modern techniques that the significance of barley gums in relation to malting has become apparent. Consequently, the bulk of the work done on these polysaccharide materials is relatively new and not very extensive. Late in the nineteenth century several workers described gums obtained from barley, but no investigation was made at that time concerning the function of these gums in the grain kernel. The early literature cites a few preparations of gums from cereal grains other than barley, but the work was of a general nature and no significance was attached to the products. It was not until Piratzky and Wiecha (39) in 1938 obtained a polysaccharide material from wort of short-grown malt that an indication was given of the importance of these materials in the malting process. This information created a new interest in barley and malt gums, and most recent work has been directed towards investigation of the occurrence of these materials in barley.

One of the earliest workers in the field of barley gums was O'Sullivan who reported (32) two fractions of carbohydrate material obtained from barley by water extraction. O'Sullivan believed the fractions to be derived from starch and, therefore, named the materials "amylans". The two amylan fractions differed in water solubility. Part of the amylan material was insoluble in water and was designated "α-amylan", whereas the water-soluble fraction was called "β-amylan".

The name "amylan" is unfortunate for it was later shown (39) that O'Sullivan's β-amylan contained pentosan material and, so, could not be considered to be a starch derivative. Preece (41) found this water-soluble fraction was similar to plant gums and so designated it "barley gum".

During the separation of protein fractions of barley meal Osborne (31) found that gum was extracted from barley by salt solution. However, Osborne was primarily interested in preparing pure protein fractions and did not investigate the barley gum contaminant. Lindet (23) isolated gum similar to that obtained by O'Sullivan. Later Brown, Escombe, McMullen and Miller (5) extracted a barley gum, the amylan content of which corresponded with the estimated quantity of hemicellulose in barley. Preece, Ashworth and Hunter (43) point out, however, that the amylan material of Brown et al. may have been highly contaminated with hemicellulosic material by virtue of their extraction method.

The early workers gave no indication of the origin of the complex polysaccharides in the barley kernel, although O'Sullivan believed α- and β-amylans were degradation products of starch. However, Brown et al. state that the "amylans" have no genetic connection with starch. At the same time, these workers perceived that barley gums played an important role in the malting process. Hind (16, p. 70) in a summary of the early work on barley gums writes that, according to Brown, solution of barley gum is one of the most significant changes that occurs when barley is converted into malt. However, it was not until thirty years later that investigations of this evident change were undertaken.

In general, there are two methods by which polysaccharide material may be prepared from cereal products. These are:

(1) extraction of the cereal with water or dilute salt solution, or

(2) extraction with dilute sodium hydroxide

The first method was employed by O'Sullivan and some other early workers, and yields what are now called cereal gums. Alkaline extraction, on the other hand, yields hemicelluloses. The two products are chemically similar, although they differ in initial water-solubility in the grain. As little is known about the structure of hemicelluloses, they are very inadequately defined. Preece (41) defines hemicelluloses as cell wall materials that are insoluble in water, but which are fairly soluble in 4% sodium hydroxide. Hind (16, p. 70) broadens this definition by stating that hemicelluloses are more readily attacked by enzymes than is cellulose; hemicellulose-splitting enzymes are called hemicellulases or cytases. Hemicelluloses have been isolated from a variety of sources such as woods, straws and seeds, and according to Preece (41) many hemicelluloses obtained from such materials yielded xylose and arabinose after hydrolysis. Hemicellulosic material may also contain hexose sugars and uronic acids. Larmour (22) obtained gummy substances, which contained glucose and appreciable amounts of pentose sugars, from alkaline extracts of several cereal grains. Thus, two different, though somewhat similar, materials can be obtained from cereal products depending on the method of extraction. Cereal gum is in an initially water-soluble state in the grain, while hemicellulose material is extractable only with alkaline solution. On this basis Preece (41) declares that O'Sullivan's amylans are barley gums and not hemicelluloses as Hind (16, p. 71) previously named them. It is generally agreed that the source of both gum and hemicellulose is in the cell wall of the kernel.

What happens to hemicelluloses during malting presents a complex problem to study, and one about which there has been much speculation. Hopkins and Krause (17, p. 132) state that during malting hemicellulases dissolve material in the cell walls of the grain which results in softening of the kernel. Kernel softening is said to be the cause of the most evident feature of malt modification. This view is supported by Preece (40) in an address to the Joint Meeting of the Institute of Brewing and the Incorporated Brewer's Guild. He states that Windisch and van Waveren found softening of the grain is accompanied by an increase in the soluble pentosans of the corn. The important role that cytolysis plays in malting is emphasized by Helm (14). Decomposition of the cell walls permits amylases to come in contact with starch and so make saccharification possible during mashing. Hemicellulosic material of cell walls is broken down by enzymes during malting.

Enzymatic degradation of cell wall material is the central point in the modification problem. Modification is essential in the production of malt, and there have been several methods proposed to measure degree of modification. As the quality of malt is reflected in the quantity and quality of malt extract, many procedures utilize wort quality as a measure of modification. Two such measurements are determinations of wort nitrogen and wort turbidity. In 1936 wort viscosity was proposed as a method for determining degree of modification. Recently Feys (9), after studying five methods of measuring degree of modification, recommended the determination of wort viscosity as an index of modification. This method is now used quite extensively, as it is easily and quickly done. However, studies on variations of wort viscosity are few as Hopkins and Krause (17, p. 192) indicate.

Piratzky (37) found that short-grown malt gave more viscous solutions than malt grown a normal length of time. He therefore suggested that high wort viscosity was an indication of imperfect modification. Later, Piratzky and Wiecha (38) indicated this property could be used as a measure of modification. They concluded that when malts produce worts that have minimum viscosity, they are well modified and are in a condition where all the convertible hemicelluloses in the worts have been converted.

Wort viscosity is an important factor to be considered in the production of beer, for, according to Helm (14), beer viscosity is dependent on the viscosity of the extract. It is believed by some brewers that high viscosity is a governing factor of head retention and probably of palatefulness of beer. However, "high viscosity" is not defined and it remains a rather uncertain term.

As the importance of wort viscosity in brewing became more apparent, not only as a measure of wort quality but also as a factor affecting beer quality, the nature of the "viscosity-giving compounds" in wort was investigated. In a paper by Meredith and Sallans (29) the authors state that wort viscosity is a measure of the extent of degradation of starch and protein material, and is attributed to the combined effects of high molecular weight fractions of these materials. Later, the same workers reported (30) that an increase in wort nitrogen was accompanied by a decrease in viscosity. This inverse relationship is the result of incomplete degradation of protein material resulting in the presence of colloidal protein particles. After studying the effect of temperature on wort viscosity and wort nitrogen, Meredith (25) suggested that wort viscosity was due to some factor other than nitrogen.

Piratzky and Wiecha (39) indicated that wort viscosity is caused by carbohydrate material. These workers isolated a viscous polysaccharide from wort of barley that was germinated for three days. The material was not obtained from fully germinated barleys, however, and it was suggested that the polysaccharide is broken down during the latter stages of malting. Preece (41) agrees with this suggestion when he writes that full modification of barley probably results in complete hydrolysis of the viscous material of barley. If this statement is accepted, the low viscosity of wort from fully modified barley can be explained. Hopkins and Krause (17, p. 193) mention that wort viscosity is mainly determined by the quality and quantity of carbohydrate material present in the extract, thus indirectly, indicating their agreement with the suggestion of the German workers.

The product of Piratzky and Wiecha resembled O'Sullivan's α-amylan; it was only slightly soluble in water and was reported to be nitrogen-free. A solution of the gum was quite viscous; 1% solution had a viscosity of over 4.0 cp. The polysaccharide material was starch-free, but on enzyme hydrolysis yielded only glucose and maltose. This result implied that the material was comprised of high molecular weight dextrins.

Helm (14) agrees that the wort gum of Piratzky and Wiecha is responsible, at least in part, for high wort viscosity. It is known that maltose imparts to wort a higher viscosity than to water, but, as Helm (14) points out, wort viscosity is considerably higher than that of a maltose solution of the same density. This would indicate that there is another factor contributing to wort viscosity.

Meredith (26) in a further effort to isolate the viscous principle of wort, obtained a gum from wort by alcoholic precipitation. This wort gum, which contained pentosan material, was obtained from an extract of well-modified malt. Since the material contained pentosans, Meredith considered it to be derived from the pentosans of barley. It has been mentioned that Windisch and van Waveren found that soluble pentosans of barley increase in amount with increase in length of germination time.

Helm (14) acknowledges that barley contains pentosan material which is attacked during cytolysis. He writes that cytase, which includes enzymes capable of hydrolyzing pentosans and similar compounds, attacks the hemicelluloses of endosperm during modification. The products of decomposition of pentosans in wort are likely to be more complex than high molecular weight dextrins. Therefore, as noted by Meredith (26), it seems reasonable to expect that pentosans or similar carbohydrates are responsible for wort viscosity.

Several workers (11, 31) have reported the preparation of gums from cereal products by salt extraction. The gums obtained from barley were found to contain pentosan material and, according to Freeman and Gortner (11), "they consist largely of mixed polymers of xylose and arabinose admixed with some protein material". Perlin (35) describes gum isolated from wheat flour, and reports that one fraction of it contained principally xylose and arabinose, while a second fraction was hexosan in nature. A similar polysaccharide material had been found associated with β-amylase prepared by extraction with alcohol from flour of ungerminated wheat (10).

More recent work on gums has been directed towards determining the relation between wort gums and barley gums. Cytolysis involves the water-soluble barley gums that make some contribution to viscosity in barley extracts. Preece, Ashworth, and Hunter (43) isolated gum from barley that had been boiled with alcohol to inactivate enzymes. The gum, recovered by acetone precipitation, contained varying amounts of glucose, arabinose and xylose as determined by chromatographic analysis of a fraction of acid hydrolysate. The barley gum obtained by Preece et al. corresponds to O'Sullivan's β-amylan, and both have been established to be non-starch polysaccharides (39, 43). Preece et al. report that their material is viscous in aqueous solution, but as yet no definite connection has been shown to exist between the viscous polysaccharide of barley and the viscous principle of wort. However, Meredith (26) believes the factors causing high wort viscosity are complex carbohydrates. He postulates further that the material in barley which gives rise to compounds responsible for wort viscosity is a protein-carbohydrate complex. This view is emphasized in a later paper (28).

Gums from raw barley flour, malt grist and wort were prepared by Meredith, Bass and Anderson (28) to study the characteristics of the three products. These workers reported that solutions of barley gums are unstable, and suggested that instability may be due to gum-splitting enzymes that are precipitated with the gum. When hydrolyzed with acid, the gums yield glucose, arabinose and xylose as principal sugars with traces of galactose and mannose. Meredith et al. (28) report that gum which is stable in solution can be prepared by refluxing barley gum with dilute alkali. Hydrolysates of alkali gum contain the same sugars as hydrolysates of the parent gum, but in different proportions.

Early workers who investigated the composition of barley gums were not in agreement concerning the nitrogenous content of gums. O'Sullivan (32) indicated that his products were nitrogen-free, while Brown et al. (5) reported that their crude gum contained 0.1 to 0.8% nitrogen. Meredith et al. (28) were the first to attach any significance to nitrogen content. They suggested that nitrogen appears to be a critical factor affecting gum stability, since the writers found unstable barley gum contained about 3.0% nitrogen. Unfortunately, Preece et al. (43) did not report nitrogen content of their barley gums.

It has been reported that alkali treatment of gum will reduce (26) or remove (28) the nitrogenous material and produce a stable polysaccharide. Larmour (22) also found that gum recovered after alkali extraction was low in nitrogen. Piratzky and Wiecha (39) reported a nitrogen-free gum. This product was obtained from alkaline solution which may have been responsible for the removal of the nitrogenous constituents.

After heat-coaguable protein had been removed, the protein nitrogen content of barley gum was 7.25%, according to Freeman and Gortner (11). They suggested that the protein material was admixed with polymers of pentosans. Similarly, Meredith (26) made the suggestion that nitrogen content may be due to a protein-carbohydrate complex. However, the function of nitrogen in barley gums remains in doubt (2, p. 31) and as yet little work has been reported on the problem. As Meredith (26) writes concerning alkali gums, "although there is a direct relation between nitrogen content of product and viscosity of solution, the role of nitrogen in the properties of the material is not clear".

From the available literature it is evident that knowledge concerning the chemistry of barley gums is quite limited, although there has been considerable speculation about these materials. The problem of the relation between the gums from barley, malt and wort is almost untouched. Preece _et al._ (43) believe that water-soluble malt gum is formed from hemicelluloses by cytolytic action. However, other workers (28) suggest that at least some of the malt gum is derived from barley gum. Little information is available concerning the composition and structure of these gums, and until such work is done, the relation between the gums will remain obscure. To this end it was believed a worthwhile contribution could be made by the preparation of pure barley gum in which the material contributing to viscosity is concentrated. The isolation of such a gum may also shed some light on the problem of the role of nitrogen in barley gum.

EXPERIMENTAL

MATERIALS

The material used for the preparation of barley gums was Montcalm barley grown at the University of Manitoba in 1949. The grain was stored in bulk in sealed containers from which aliquot samples were obtained with a Boerner sampler. The barley contained 11.3% protein based on 14% moisture. Barley grist was obtained by grinding 500 g. lots of barley in a Wiley mill with 1 mm. sieve.

Studies were made using both raw barley grist and alcohol-treated barley grist. Preece et al. (43) reported the preparation of gum from ground barley that had been refluxed twice with 85% ethyl alcohol to inactivate enzymes. A similar procedure, in use by Meredith (27), was utilized in this study. Ground barley was boiled under reflux for 30 mins. with 5 parts ($^{w}/_{v}$) of ethanol (sp. gr. 0.85). Specific gravity of alcohol must be adjusted carefully, as it has a significant influence on properties of the final gum product. The mixture of alcohol and barley was centrifuged and the grain air-dried.

METHODS

Gum Preparation

The procedure for fractionation of gums described by Meredith et al. (27, 28) was modified slightly for the preparation of further barley gum fractions. The modified method involved treatment of the mash with various protein-active reagents, either after or during extraction.

Two general methods for preparation of gum fractions from both raw barley grist and alcohol-treated barley grist are described below. Details regarding reagents will be found in a separate section. The procedure designated "precipitation method" was used to remove nitrogenous material after autolysis had occurred. The second procedure, "extraction method", consists of the removal of nitrogen-containing material before hydrolysis had taken place to any great extent.

Precipitation Method. Grist was extracted for 2 hrs. with distilled water by continuous stirring of the mash in a water bath at room temperature. Ratio of ground barley to water was 1:8 ($^{w}/_{v}$). Spent grains were removed by centrifuging and the reagent solution was added to supernatant liquor. Material that was precipitated at this point was removed by centrifuging.

The liquor was added with constant stirring to 3 volumes of distilled ethyl alcohol to precipitate gum material. After settling for 1 hr., the precipitate was recovered by centrifuging. The gum was dried with alcohol, acetone and ether in that order. This method of drying is subsequently referred to as "drying by solvent exchange". The product was then air-dried and pulverized.

Extraction Method. Ground barley was extracted directly for 2 hrs. with 8 volumes reagent solution ($^w/v$) by continuous stirring of the mash at room temperature. Spent grains were removed by centrifuging. The clear supernatant liquor was added to 3 volumes of ethyl alcohol, and gum was recovered and dried as described under "Precipitation Method".

Reagent Solutions. The reagent solutions used in the preparation of barley gums were:

Trichloracetic acid

Trichloracetic acid was dissolved in distilled water so that final concentration of acid after addition to mash or extract liquor was 6%.

Tannic acid

Twenty grams tannic acid and 10 ml. concentrated sulphuric acid were dissolved in 400 ml. distilled water (46). An aliquot of this solution was diluted to yield a final tannic acid concentration of 2% after addition to mash or extract liquor.

Ficin

Powdered ficin was ground into solution with distilled water. Undissolved ficin was removed by centrifuging. After addition of this solution to mash or extract liquor, final ficin concentration was 0.25% assuming all ficin was in solution. After 2 hrs. of mashing, or 15 mins. in contact with extract liquor, ficin was precipitated in 6% trichloracetic acid. The precipitate was removed by centrifuging.

Papain

Papain solution was prepared in the same way as ficin solution. However, assuming all papain was dissolved, the final concentration of enzyme in mash or extract liquor was 1%.

Gum Characterization

Viscosity and Concentration. Viscosity may be used as a measure of relative molecular lengths of polysaccharide material in solution. Viscosity measurements were made with fast-flowing Ostwald viscometers; water flow-time of viscometers was 13 to 20 sec. Water viscosity was assumed to be 1 cp. for relative viscosity claculations, although temperature of the water bath was 30° ± 1°C. However, since the gum solutions were very viscous, this error was negligible.

Solution concentration was obtained by heating an aliquot of solution in a tared dish at 105°C. and calculating from difference in weight.

Nitrogen Determination. The technique used for nitrogen determination was a modified combination of two micro-Kjeldahl methods (15, 45, p. 78). A sample containing 0.2 to 2 mg. nitrogen was digested for 60 mins. with 1 g. sodium sulfate, 0.3 g. mercuric sulfate and 2 ml. sulphuric acid (sp. gr. 1.84). When the mixture was cool, 3 to 4 ml. water were added to the digestion flask. The solution was transferred to a distillation apparatus similar to that described by Pregl (45, p. 80). Fifteen ml. of 30% sodium hydroxide solution containing 5% sodium thiosulfate was added to the apparatus and the mixture steam distilled. The distillate was passed into a suitable volume of 0.01 N hydrochloric acid containing one drop of 0.2% water solution of methyl red as indicator. After distilling the solution for 5 mins., the receiver was lowered and distillation was continued for a further 2 mins. to rinse out the delivery tube. The excess acid was titrated with 0.01 N sodium hydroxide. A reagent blank was done on each run as the blank was found to vary considerably from day to day.

The method was tested with 71.4 mM. ammonium chloride (1 ml. = 1.0 mg. N), and the experimental error in the recovery of nitrogen was within ±4%. Treffers (47, p. 71) states that when samples containing 0.1 to 1.0 mg. of nitrogen are determined with micro-Kjeldahl procedure, the reproducibility of results is 5% or better.

Sugar Components. Gums were hydrolyzed for 16 hrs. with N sulphuric acid in sealed glass tubes of 4 mm. bore at 100°C. Concentration of gum was 3%. The hydrolysate was neutralized by passage through a column packed with activated Duolite A-7 resin. Percolate from the column was distilled under reduced pressure and the residue of mixed sugars retained.

The apparatus and method used for chromatography were based on those originally described by Consden, Gordon and Martin (7) and incorporated refinements suggested later by Jermyn and Isherwood (18). The cellulose base for partition of solute was Whatman No. 1 filter paper.

Six µl. of a 15% mixed sugar solution were applied to a pencilled starting line, 3 inches from one end of the filter-paper strip. Solution spots were placed 2.5 cm. apart on the base line. The chromatogram was placed in the glass tank and equilibrated for a few hours with the aqueous phase of the solvent system, ethyl acetate/pyridine/water in the ratio 5:2:5 (18, 19). The solvent phase was then added to the trough and the chromatogram allowed to develop for 20 to 24 hrs., which was sufficient time for resolution of galactose, glucose, arabinose and xylose.

The paper was air-dried and sprayed with aniline phosphate in butanol as described by Bryson and Mitchell (6). These workers claim that this spray gives a more compact spot than that obtained with Partridge's aniline phthalate (34). The chromatogram was finally dried at 90°C. for 10 mins.

When sprayed with aniline phosphate, hexose sugars show up as brown spots, while pentose sugars are indicated by a characteristic red-brown color. Rf value, as defined by Consden et al. (7), is usually used to identify sugars separated by filter-paper chromatography. Calculation of Rf involves knowledge of the distance travelled by the solvent front during development of the chromatogram. However this measurement is often difficult to obtain. Therefore, the components of unknown sugar solutions were identified by reference to a mixture of known sugar composition that was applied to the same chromatogram. The concentration of each reference sugar on the chromatogram is around 0.2 mg.

Amino Acid Components. Gums were hydrolyzed with N hydrochloric acid in the same way as described under sugar chromatography. Salts, which interfere with mobility of amino acids during development, were removed by repeated reduced pressure distillation. Water was added to the residue to form a 30% solution, 6 µl. of which were chromatographed on Whatman No. 1 filter paper (18 x 22 inches).

McLeod (24) suggested one dimensional chromatography was best when several amino acids were possibly present in the solution to be chromatographed. This procedure was considered adequate, when control solutions of amino acids were chromatographed and used as reference for comparison of hydrolyzed solutions. Control solutions of amino acids in ethyl alcohol-hydrochloric acid were prepared according to Dent (8). This worker states that solutions of amino acids in alcohol are stable for several months.

The chromatographic apparatus is described in the Twenty-fourth Annual Report of the Board of Grain Commissioners (3, p. 31). Resolution of most amino acids was obtained with the solvent system butanol/acetic acid/water in the ratio 4:1:5 by volume (24, 33). After development with the solvent, the air-dried paper was sprayed with 0.1% ninhydrin in butanol (7). Color was developed by oven-drying the paper for 10 mins. at 90°C. After heating with ninhydrin, most amino acids give a color ranging from blue to purple. On standing for several hours, the color deepens and detection of traces of acids is facilitated. With ninhydrin spray, proline produced a light yellow color that was often difficult to detect. A specific proline color developer of isatin in acid butanol was described by Acher, Fromogeot and Jutisz (1). This spray with proline gave a characteristic blue color on a yellow background.

Some amino acids are "fast-moving" while others are "slow-moving" on paper chromatograms. The more mobile acids can be resolved by 24 hour-development, while papers bearing the less mobile acids must be developed 48 hrs. for resolution. The solvent system butanol/acetic acid/water does not resolve leucine, iso-leucine and phenylalanine, but these acids are nicely separated with butanol/benzene/pyridine/water in the ratio of 4:2:2:5 by volume.

RESULTS AND DISCUSSION

SYSTEMATIC PREPARATION OF BARLEY GUMS

Barley, malt and wort gums were studied by Meredith *et al.* (28) in an attempt to find a relation between the three polysaccharide materials. Preliminary investigation indicated that the problem was very complex, and that a systematic investigation of the three gums should begin with a detailed study of barley gum.

Crude barley gum was described by Meredith *et al.* Later studies indicated that treatment of barley with boiling alcohol prior to extraction with water produces a gum that is more viscous in solution and is lower in nitrogen content than crude barley gum (27). Both gums are composed principally of glucose, arabinose and xylose. Crude barley gum and gum obtained from alcohol-treated barley are unstable in aqueous solution. Solution instability was suggested to be caused by enzymes that were precipitated with the barley gums (28). However, the role of nitrogen in barley gums was not clearly understood. Therefore, properties of barley gums of reduced nitrogen content were studied in an attempt to elucidate the function of nitrogen in barley gum. The results of this study are reported and discussed in the following sections.

Nitrogenous material was systematically removed from barley gum by treatment of barley and barley extracts with protein precipitants and proteolytic enzymes. Gums prepared by these methods were compared with crude barley gum and gum extracted from alcohol-treated barley with water.

Gum preparations were characterized by the determination of several gum properties. These were: viscosity of gum solution, degree of solution instability, nitrogen content, sugar composition and amino acid composition. Concentration influences gum solution viscosity appreciably, therefore, viscosity values reported here are accompanied by the corresponding solution concentration. Degree of instability or viscosity reduction was calculated by dividing the change in gum solution viscosity in centipoises after 24 hrs., by initial relative viscosity in centipoises; the value was expressed as per cent. These properties were considered adequate to characterize most of the gum preparations.

Gums Prepared by Water Extraction

Gums were first prepared by water extraction of raw barley and alcohol-treated barley. These preparations were subsequently used as a basis for comparison of other barley gums. Some properties of raw barley gums are given in Table I.

The analytical data contained in Table I are similar to the results reported by Meredith (27) and Meredith et al. (28). Alcoholic digestion of barley prior to extraction results in an appreciable reduction in gum yield and an increase in gum solution viscosity. These results are probably due to prevention of internal changes in barley grist as a result of alcoholic treatment. Preece and Ashworth (44) suggest that raw barley contains two fractions of pentosans. Some pentosans are initially water-soluble, whereas other pentosan material is made soluble by enzyme action during extraction. When barley is digested with alcohol, the action of this enzyme system is inhibited, and thus the yield of gum obtained from alcohol-treated barley is reduced. The enzyme system is probably only partially inactivated, as an enzyme in the presence of its

TABLE I

Data on Crude Barley Gums

	Gum from Raw Barley	Gum from Alcohol-Treated Barley
Yield, %	3.4	1.1
Nitrogen, %	3.00	0.46
Viscosity of aqueous solution, cp.	2.34	48.2
Viscosity reduction after 24 hrs., %	50	62
Concentration of aqueous solution, %	0.64	0.79
Amino acid components:		
(a) alanine, glutamic acid, glycine, serine, aspartic acid, arginine, lysine	+	+
(b) leucine, iso-leucine, valine, proline	+	-

substrate is more stable to inactivation treatment. Preece and Ashworth suggested further that this cytoclastic enzyme system may also decrease the molecular complexity of barley gum. Therefore, gum obtained from barley in which the gum degrading enzyme has been partially inactivated should be more viscous in solution. Fuoss (12) states that increased solvent viscosity is an indication of a polymeric structure of the solute. Thus, barley gum may consist of a polymeric polysaccharide structure. Alcoholic digestion of barley appears to cause the retention of a high degree of polymerization in barley gum.

Although gum obtained from alcohol-treated barley may be more complex, both gums possess about the same degree of instability in solution. The separation of white material from solutions of raw gums from untreated and alcohol-treated barley, when the solutions were stored for 24 hrs., is believed to be a result of solution instability. Crude gum solution yielded a greater volume of precipitate. The cause of instability was suggested (28) to be a degradative enzyme system that is precipitated with the gum. Thus, a relation between nitrogen content and gum stability is implied. Alcoholic treatment of barley reduced nitrogen content of gum by about 2.5 percentage units, but did not increase stability.

Although amino acid composition of the nitrogenous material was not reported previously, it was found useful for gum characterization. Seven amino acids were common to both gums, and leucine, iso-leucine, valine and proline were present only in crude gum. Alcoholic treatment of barley must remove these four amino acids from crude gum. Other amino acids may have been present in the gums but in insufficient quantities to give a color with ninhydrin. Chromatograms of acid hydrolysates of both gums bear diffuse spots at the upper end. These spots were attributed to peptide fragments resulting from incomplete hydrolysis of protein material. The composition of the peptide fragments was not investigated.

A gum which is quite viscous in aqueous solution can be extracted from barley that has been pretreated with boiling alcohol. The viscous product is precipitated from the extract liquor by alcohol. Preece _et al._ (43) extracted alcohol-treated barley with water and precipitated the gum with acetone. The material obtained after acetone precipitation yielded a comparatively low viscosity solution; a 0.5% gum solution had a viscosity of 1.28 cp. at 25°C. As a preliminary experiment in this study, alcohol-treated barley grist was extracted with water and gum precipitated from the extract in 3 volumes of acetone. Aqueous solution viscosity of the "acetone gum" was 5.13 cp. at 0.82% concentration. The gum solution was unstable. As a more viscous gum can be obtained by alcoholic precipitation of the extract liquor, neither the "acetone gum" nor its method of preparation were investigated further. Alcoholic precipitation was used for the preparation of subsequent gum fractions.

Digestion of barley grist with alcohol prior to extraction with water results in a gum product of reduced nitrogen content that is quite viscous in aqueous solution. Solutions of crude gum and gum obtained from alcohol-treated barley are unstable. As both gums contain nitrogenous material and are unstable, they are not completely satisfactory for detailed study. However, the products were used as a basis for comparison of gums that were prepared by treatment of barley and barley extracts with protein-active reagents.

Gums Prepared by Acid Treatment

Acid protein-precipitants were used in an attempt to reduce the nitrogen content of barley gums obtained from raw barley and alcohol-treated barley. The acid reagents employed were 6% trichloracetic acid solution and 2% tannic acid solution. The pH of both acid solutions was about 1.0. Gums were first prepared by the precipitation method using acid protein-precipitants on raw and alcohol-treated barley. The gum products obtained after precipitation of barley extracts with tannic acid were highly colored and difficult to handle. This method of gum preparation was not satisfactory and so was abandoned. The acid reagents, in addition to removing protein material, may dissolve some of the gum. To determine the effect of pH on gums during recovery from acid solution, raw and alcohol-treated barley extracts were treated with hydrochloric acid solution of pH 1.0. The gums were recovered from the hydrochloric acid solution by the usual procedure.

Some properties of gum fractions obtained by trichloracetic acid and hydrochloric acid treatment of aqueous extracts of untreated and alcohol-treated barley were determined. Results of the determinations are given in Table II.

Yields of gums obtained after acid treatment of extracts of raw barley were lower than the yields of gum from untreated extracts of the same barley. The difference in yields between trichloracetic acid-gum and crude gum is partially accounted for by the yield of precipitate obtained when trichloracetic acid was added to the aqueous extract. The total yield of trichloracetic acid-gum plus protein precipitate is 2.8%. No precipitate resulted when hydrochloric acid was added to the aqueous extract of raw barley, and yield of gum recovered by alcoholic precipitation

TABLE II

Data on Gums Obtained from Acidified Aqueous Extracts of Barley

	Raw Barley Extract		Alcohol-Treated Barley Extract	
	Trichloracetic Acid	Hydrochloric Acid	Trichloracetic Acid	Hydrochloric Acid
Yield, %	2.1	2.7	1.1	1.1
Nitrogen, %	0.50	2.14	0.50	0.30
Viscosity of aqueous solution, cp.	3.55	2.77	64.1	33.3
Viscosity reduction after 24 hrs., %	1.7	1.1	61	3.0
Concentration of aqueous solution, %	0.79	0.71	0.75	0.56

was 2.7%. Therefore, acid treatment of liquors has probably extracted some gum material. Nitrogen determinations on these fractions indicate that the material removed from gum by acid treatment of liquor is nitrogenous.

Gum yields from trichloracetic acid- and hydrochloric acid-treated extracts of pretreated barley were the same as the yield of gum obtained by direct water extraction of alcohol-treated barley. When trichloracetic acid was added to an aqueous extract of alcohol-treated barley, a slight amount of material (0.3% of barley) was precipitated. Digestion of barley with alcohol prior to extraction removes most of the precipitable protein of crude barley gum, since further treatment of the gum with a protein precipitant has no effect on the nitrogen content. Similarly, no precipitate was obtained when hydrochloric acid was added to the extract liquor. Thus, yields of all gums prepared from alcohol-treated barley are the same.

Comparison of yields and nitrogen contents of gums obtained after acid treatment of aqueous extracts of both barleys indicates that at least three fractions of nitrogenous material are associated with crude barley gum. These fractions are: material that is soluble at pH 1.0; precipitable protein material; and, finally, material that is more closely associated with the gum.

Gums obtained from acid-treated extracts of raw barley have approximately the same viscosity in solution as gum extracted with water from untreated barley. Solutions of all acid-gums obtained from raw barley extracts, however, were much more stable. Hydrochloric acid treatment of aqueous extracts of alcohol-treated barley tended to stabilize gum, whereas trichloracetic acid had no effect on gum stability.

All gums obtained from acidified aqueous extracts of both barleys contain glucose, arabinose and xylose. The same sugars were identified previously in barley gums (28, 43) and in wheat gums (35). Qualitative comparison of the color density of sugar spots on chromatograms of hydrolysates of the acid-gums indicated the proportion of glucose was greater than that of the pentose sugars. The relative proportion of the three sugars appeared to be the same as in crude gums extracted with water from both untreated barley and alcohol-treated barley.

Although a mixture of three sugars is present in barley gum, only glucose was detected in the hydrolysates of the precipitates that resulted from the addition of trichloracetic acid to aqueous extract liquors. Thus, this precipitant removed some carbohydrate material as well as protein from the barley gums. The glucose-containing material may be an integral part of the precipitated protein, or it may be coprecipitated.

Addition of trichloracetic acid to extract liquors of untreated and alcohol-treated barleys does not remove all protein material from gum. Amino acid composition of gums obtained by acid precipitation methods on untreated and alcohol-treated barley extracts was determined and the results are given in Table III.

Acid-gums prepared from untreated barley by the precipitation method using either trichloracetic acid or hydrochloric acid contained essentially the same amino acids as those found in the corresponding crude gum. Insufficient trichloracetic acid-gum from extract of alcohol-treated barley was available to determine its amino acid composition. However,

TABLE III

Amino Acid Components of Gums Obtained from

Acidified Aqueous Extracts of Barley

	Raw Barley Extract		Alcohol-Treated Barley Extract
	Trichloracetic Acid	Hydrochloric Acid	Hydrochloric Acid
leucine	+	+	+
iso-leucine	+	+	+
phenylalanine	-	-	-
valine	+	+	+
proline	-	-	-
alanine	+	+	+
glutamic acid	+	+	+
glycine	+	+	-
aspartic acid	+	+	+
lysine	+	+	+
arginine	+	?	+
serine	+	+	-

+ denotes identification
- denotes not detected
? denotes trace

evidence discussed later indicates that the amino acid content of this gum is likely similar to that of the corresponding product obtained from raw barley extract. With the exception of glycine and serine, the amino acids contained in the hydrolysate of hydrochloric acid-gum obtained from extract of treated barley were the same as those contained in the acid-gums from raw barley extract. Lack of detection of amino acids does not necessarily mean that they are absent from the gum; they may be present, but in quantities below the minimum required for chromatographic detection. The occurrence of leucine, iso-leucine and valine in the hydrochloric acid-gum from extract of treated barley makes this gum significantly different from gum extracted with water from the same barley. The explanation for this difference will be discussed later.

Acid treatment of aqueous extracts of raw and alcohol-treated barleys produces gum fractions that are more stable in solution than solutions of the corresponding crude gums. The nitrogen content of gum from an acid-treated extract of raw barley is reduced, whereas acid treatment has no effect on nitrogen content of gum obtained from an extract of pretreated barley. Gums prepared by acid treatment of aqueous extracts have the same sugar composition as that of crude gums, but differ in amino acid content.

Gums prepared by addition of acid reagents to the extract liquor of raw barley grist have lower aqueous solution viscosity than gums prepared by acid treatment of alcohol-treated barley extracts. The lower solution viscosity of acid-gums from raw barley extracts suggest that gum degradation has taken place during extraction of raw barley. In an attempt to prepare gum that had been subjected to limited degradation during extraction, raw barley and alcohol-treated barley were mashed with acidic protein-active reagents. Extraction with tannic acid yielded unsatisfactory gums, and the products were not investigated. Table IV shows results of some determinations made on the material isolated by trichloracetic acid and hydrochloric acid extraction of untreated and alcohol-treated barley.

The yield of gum obtained by hydrochloric acid extraction of raw barley is greater than that of gum obtained by trichloracetic acid extraction, although yields of both acid-gums are lower than the yield of crude barley gum. Similar gum yields were obtained when the corresponding precipitation methods were applied to untreated barley extracts. During the discussion of gums prepared by the acid-precipitation method, the suggestion was made that trichloracetic acid removes precipitable protein material as well as acid-soluble nitrogenous substances from crude gum, whereas hydrochloric acid removes only acid-soluble nitrogenous material. The same explanation would account for the difference in yields of gums obtained from raw barley by acid extraction. Yields of gums isolated from alcohol-treated barley by acid extraction are greater than the yield of gum obtained by aqueous extraction of the same barley. A low pH of the mash liquor may be responsible for releasing more gum from alcohol-treated grain, but this facet of acid extraction was not investigated.

Gums prepared by acid extraction of raw barley are more viscous in solution than their counterparts obtained by precipitation methods. High solution viscosity of these gums indicates that acid reagents hinder degradation of the polysaccharide material during extraction. Some

TABLE IV

Data on Gums Obtained after Acid Extraction of Barley

	Raw Barley		Alcohol-Treated Barley	
	Trichloracetic Acid	Hydrochloric Acid	Trichloracetic Acid	Hydrochloric Acid
Yield, %	2.3	2.8	1.5	1.8
Nitrogen, %	0.73	1.97	0.50	0.82
Viscosity of aqueous solution, cp.	12.8	11.8	32.1	44.8
Viscosity reduction after 24 hrs., %	21	10	18	13
Concentration of aqueous solution, %	0.60	0.51	0.37	0.68

degradation has taken place, however, as the raw barley isolates are not as viscous as gums extracted with acid solution from alcohol-treated barley. Extraction of barley with hydrochloric acid has a greater effect on gum solution stability than trichloracetic acid extraction, even though gums prepared by the former method contain more nitrogenous material. This result is anomalous unless the degradation of polysaccharide material is due to enzymes that are partially inhibited by hydrochloric acid.

The composition of gums prepared by acid extraction is similar to that of the other acid-gums. The polysaccharide material of gums obtained by direct acid extraction of both barleys consists of glucose, arabinose and xylose. Gums isolated by acid extraction of untreated and alcohol-treated barley have the same amino acid composition as gums obtained by acid treatment of raw barley extracts. Amino acid composition of the latter gums was presented in Table III. As gums prepared from untreated barley by both acid methods and gums prepared by acid extraction of alcohol-treated barley contain the same amino acids, it seems likely that gums isolated after acid precipitation of alcohol-treated barley extract also would contain the same amino acids. That is, all acid-gums would be similar in amino acid composition. Acid-gums from alcohol-treated barley contain several amino acids that are not present in the corresponding gum prepared by water extraction (cf. Table I). The difference in amino acid composition is probably due to the different pH of the extract liquors.

Gums prepared by acid treatment of barley and barley extracts are similar not only in amino acid composition but also in sugar composition. Some of the nitrogenous material present in the gum may be part of a protein-carbohydrate complex as suggested by Meredith et al. (28), but the possibility also exists that the protein may be simply admixed with the gum polysaccharide material (11). Some of the residual nitrogen may be part of an enzyme system that causes gum instability in solution. All acid-gum solutions are unstable, but, as gum instability is decreased appreciably by hydrochloric acid treatment of barley and barley extracts, hydrochloric acid may partially inhibit enzymic degradative action. Acid treatment has been useful in removing some nitrogenous material from barley gums, however, the residual nitrogen is not passive and appears to affect gum properties. Therefore, other methods for removing nitrogen were tested.

Gums Prepared by Proteolytic Enzyme Treatment

Acid extraction of untreated barley and acid precipitation of aqueous extracts of untreated barley produced gums that had reduced nitrogen content, but that were still unstable in solution. Trichloracetic acid was more effective than hydrochloric acid for removal of nitrogen, and it is likely that all free protein material was removed from gum produced by trichloracetic acid methods. Neither acid was more effective than boiling alcohol in reducing the nitrogen content of crude barley gum. Thus, trichloracetic acid and boiling alcohol probably remove the same protein material from crude gum. If the residual nitrogen is part of bound protein material, then proteolytic enzymes may split the binding linkages and thus facilitate removal of the nitrogenous material from gum. Therefore, ficin, an active proteolytic enzyme, was employed in preliminary extraction experiments.

Alcohol-treated barley was extracted with ficin solution, as pretreated barley grist yields viscous gum of the lowest nitrogen content.

The product obtained after ficin extraction was much more viscous in solution than any barley gum prepared previously. A 0.85% ficin-gum solution had a viscosity of 282 cp. High solution viscosity indicates that ficin limited the degradation of the polysaccharide material during extraction. Nitrogen content of the gum was 0.34%. This nitrogen value is lower than that of other gum fractions prepared from alcohol-treated barley. The gum solution was not completely stable; viscosity decreased 8% after 24 hrs. Nevertheless, this ficin product had the highest solution viscosity and was the most stable gum prepared from alcohol-treated barley.

Ficin products prepared subsequently did not possess the same properties. Gum solutions were lower in viscosity, 184 cp. at 0.78% concentration, and were more unstable. Solution viscosity decreased 17% after 24 hrs. The apparent anomaly in properties is difficult to explain as all ficin-gums were prepared with the same enzyme preparation. The nitrogen content of the more unstable ficin-gums was 0.42%, and the increase in nitrogenous material may be responsible for the greater instability and lower viscosity of solutions of these gums.

Chromatographic analysis indicates that the "high-" and "low-viscosity gums" prepared by ficin extraction of alcohol-treated barley are similar in sugar composition. An hydrolysate of the more viscous gum contained glucose, arabinose, xylose and a low Rf sugar that appeared above glucose on the chromatogram. On hydrolysis, the low-viscosity ficin-gum yielded glucose, arabinose, xylose and galactose. Galactose has a lower Rf value than glucose, and separates above the latter sugar on the chromatogram. The concentration of glucose in ficin-gum was greater than the concentration of either of the pentoses, and galactose was present only as a trace. Although galactose was not detected in gums prepared by acid treatment nor in gum extracted from alcohol-treated barley with water, trace amounts of this sugar have been reported to be present in crude barley gums (28) and wheat gums (35).

The precipitate recovered after addition of trichloracetic acid to ficin extract liquor contained material which, when hydrolyzed, yielded glucose. The precipitate consists predominantly of ficin that is removed from the extract liquor by the protein precipitant. However, no sugars were detected in the hydrolysate of pure ficin. This indicates that either trichloracetic acid or ficin extracts some gum material which is then co-precipitated with the proteolytic enzyme by the protein precipitant. Glucose was also identified in the protein precipitate obtained when trichloracetic acid was added to aqueous barley extracts. Therefore, it appears more likely that trichloracetic acid rather than ficin liberates the saccharide material from gum. The glucose-containing material may be associated with protein that is removed, or it may be wholly carbohydrate material. The same material was obtained from preparations of gums of both high and low solution viscosity.

Insufficient ficin-gum of high solution viscosity was available to determine its amino acid composition. An hydrolysate of "low-viscosity gum" contained the same amino acids, with the exception of arginine, that were detected in the acid-gums.

Proteolytic enzyme extraction of alcohol-treated barley yielded a gum which was similar in amino acid composition to acid-gums, but which contained a new hexose sugar. This gum was somewhat unstable and contained nitrogenous material. Another proteinase may be more efficient than ficin for removal of the nitrogenous material. Therefore papain, a less active proteolytic enzyme, was used to prepare barley gums.

Before papain was used to prepare barley gum, its proteolytic effect on gums obtained from untreated and alcohol-treated barley was tested. One milliliter of 1% papain solution was added to an aqueous solution of crude gum extracted with water from untreated barley. A white substance separated from solution and the solution viscosity decreased 23% after 24 hrs. Viscosity of a control solution of crude gum decreased 29% in an equivalent time. The precipitate was removed and the gum recovered by alcoholic precipitation. Recovery was 76% of the crude gum. The nitrogen content of the crude gum was 2.91%, while that of the recovered gum was 0.46%. As papain is insoluble in alcohol, the recovered gum is probably contaminated with co-precipitated papain. Addition of 1% papain to a solution of gum obtained after water extraction of alcohol-treated barley resulted in the gum solution becoming cloudy, but solution instability was not increased. Therefore, papain does not degrade the polysaccharide material, but appears to reduce nitrogen content of crude gum effectively. These preliminary experiments indicated that preparation of gums by papain treatment of barley might be worthwhile.

Papain has a much lower proteolytic activity than ficin. According to the Klim Clotting Method for the determination of relative enzymatic activities, ficin is 5-20 times more active than papain[1]. Extraction of alcohol-treated barley with 0.25% ficin solution produced

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[1] Personal communication from Dr. R. B. Long, Chief Chemist, General Biochemicals, Inc., Chagrin Falls, Ohio.

gum that was the most stable and most viscous in aqueous solution of any gum thus far obtained. Therefore, papain solution of equivalent proteolytic activity might produce a gum possessing similar properties to those of ficin-gum. A 1% papain solution was believed to have maximum activity equivalent to a 0.25% ficin solution, and hence this concentration of papain was used in the precipitation and extraction methods.

The precipitation method was applied to both raw barley and alcohol-treated barley to determine the effect of papain on gum obtained from their aqueous extracts. When papain solution was stirred into either extract, grey-colored material was precipitated and was subsequently recovered. Yields of this material were 0.5% of barley. The precipitates were believed to consist mostly of protein material that was removed from gums by papain. However, hydrolysates of the precipitates from both barley extracts contained small quantities of galactose and glucose. Although the precipitates may be contaminated with papain, this proteolytic enzyme, on hydrolysis, contains no detectable sugars. Therefore, papain must remove some carbohydrate material from the extracts. The presence of galactose and glucose in the material precipitated from both extracts by papain is a significant difference from the presence of only glucose in the precipitate obtained from raw barley extract by trichloracetic acid treatment and in the precipitate obtained when papain is removed from extracts by trichloracetic acid. The bulk of these precipitates is protein, but the presence of sugars in them is suggestive of a protein-carbohydrate complex in gum. The presence of galactose in one precipitate is interesting, but for the present the similarities in the materials are more important than the differences.

Yields of gums obtained from papain-treated extracts of both barleys (Table V) are lower than yields of the corresponding gums recovered by acid treatment. Low yields are probably due to purity of the gums; these products are almost free of nitrogenous material. The residual nitrogen may be merely a contaminant or it may be an integral part of the gum.

Gum obtained from untreated barley extract by the papain precipitation method was very soluble in water, and the solution was correspondingly more viscous. Increased solubility may be due to the purity of the gum. Gum prepared from extract of alcohol-treated barley by the papain precipitation method was more soluble in water than gum obtained by direct water extraction. Further, the papain-gum solution was stable. Gums prepared by water extraction and acid treatment of barley contain various amounts of nitrogenous material and have various degrees of stability, whereas "nitrogen-free" papain-gums are stable. These two properties of papain-gums, stability and almost complete freedom from nitrogenous material, lend support to the hypothesis that degradation of gum in solution is caused by an enzyme or enzyme system. Degradation of gum during extraction is discussed later.

Sugar components of gums obtained from papain-treated extracts of both barleys were glucose, arabinose, xylose and an unidentified sugar. This sugar had the same Rf value as galactose which appeared to be a component of ficin-gum. The important point is that gums produced by proteolytic enzyme treatment of aqueous extracts of raw and alcohol-treated barley have the same sugar composition.

Gums obtained from papain-treated aqueous extracts of raw and alcohol-treated barleys contained some protein material. The amino acid

TABLE V

Data on Gums Obtained from Papain-Treated

Aqueous Extracts of Barley

	Yield	Nitrogen	Viscosity of Aqueous Solution	Viscosity Reduction after 24 hrs.	Concentration of Aqueous Solution
	%	%	cp.	%	%
Gum from raw barley extract	1.8	0.12	4.07	1.0	0.93
Gum from alcohol-treated barley extract	0.9	0.07	75.8	1.0	0.88

composition of these gums, however, is quite different from those of the other gums studied. Detailed results of amino acid chromatography of hydrolysates of gums obtained by the papain precipitation method will be presented and discussed along with results of the determination of the amino acid composition of gums prepared by papain extraction of barleys.

The effect produced on gum properties by direct papain extraction of barley was tested. Extraction of alcohol-treated barley with papain solution should produce a stable gum that has been degraded to a limited extent during mashing.

Before barley gums were precipitated by alcohol, papain was removed from the extract liquors by addition of trichloracetic acid to the solutions. The precipitates were recovered and hydrolyzed. Sugar analysis of the hydrolysates showed that they contained glucose. This glucose-containing material appeared to be similar to the products precipitated by trichloracetic acid from the liquor from ficin extraction of barley and the papain-containing barley extracts from the precipitation method. Presumably trichloracetic acid removes nitrogenous material from the solutions, but the precipitates contain carbohydrate material as well as protein. The carbohydrate material may be associated with protein and removed with it. Similar material was obtained from the papain extracts of either raw or alcohol-treated barley.

Table VI shows some properties of gums obtained after extraction of untreated barley and alcohol-treated barley with papain solution.

Yield of gum extracted with papain from raw barley is the lowest gum yield obtained by any extraction method. The polysaccharide material

TABLE VI

Data on Gums Obtained after Papain Extraction of Barley

	Yield	Nitrogen	Viscosity of Aqueous Solution	Viscosity Reduction after 24 hrs.	Concentration of Aqueous Solution
	%	%	cp.	%	%
Gum from raw barley	1.7	0.12	3.66	1.0	0.92
Gum from alcohol-treated barley	1.3	0.08	752	2.9	0.82

is more readily soluble in aqueous solution, thus increasing solution viscosity. The gum was white and pulverized to an extremely light powder. The appearance of this gum closely resembled that of the corresponding product obtained by the papain precipitation method. These properties, together with low nitrogen content, indicate that gum prepared by papain treatment is probably the least contaminated barley gum that has been prepared. Gum purity is defined in terms of the amount of nitrogenous material present in gum. Nitrogen content of papain-gum is low and the residual nitrogen may be partly due to papain contamination. Solution viscosity is reduced only 1% after 24 hrs. Solution stability may be associated with the removal of nitrogenous material from the gum. Low solution viscosity indicates the gum was degraded during mashing, which in turn indicates that papain does not inhibit initial degradative action during extraction of raw barley.

Degradation of barley gum during extraction is partially inhibited by pretreating the grain with boiling alcohol. In general, gums obtained from treated barley are more viscous in solution than their counterparts obtained from raw barley, but are still unstable. The results contained in Table VI show that extraction of alcohol-treated barley with papain produces a stable gum that is extremely viscous in 1% solution. Solution viscosity of this gum is much higher than that previously reported (27, 42) for barley gums extracted from alcohol-treated barley. As viscosity measurements were made in an Ostwald viscometer, the viscous solution of papain-gum required a long flow-time which resulted in reading and drainage errors. Jones and Stauffer (21) showed that drainage error in viscometry is negligible except for very viscous liquids. Calculation of viscosity of highly viscous solutions relative to the viscosity of water results in further error. Therefore, viscosity values of very viscous solutions are not considered to be accurate, but for purposes of comparison they are adequate.

Papain-gum extracted from alcohol-treated barley was a white, fibrous material; fibrous appearance and high solution viscosity are indicative of a complex, polymeric gum structure. Viscosity of gum solution decreased 2.9% after 24 hrs., but the decrease probably is not due to enzymatic degradation, as viscosity changed only slightly after another 24 hours. Stability of gum solutions accompanies removal of the bulk of nitrogen from gums obtained by papain treatment of barley and barley extracts. It is quite possible, therefore, that instability is a function of the nitrogenous material present in barley gums. Some of the nitrogen may be part of enzymes that cleave the polysaccharide material, and thus cause a decrease in solution viscosity.

The principal sugars present in gum extracted with papain from raw barley and alcohol-treated barley were glucose, arabinose and xylose. Trace amounts of a fourth sugar were also detected. This sugar yielded a spot of low Rf value on the chromatogram. On some chromatograms of papain-gum hydrolysates, the sugar spot of low Rf value was replaced by a spot that was identified as galactose. Similar results were obtained previously when hydrolysates of gums prepared by proteolytic enzyme action were chromatographed. The sugar responsible for the spot of low Rf value is probably an oligosaccharide resulting from incomplete acid hydrolysis of gum. The oligosaccharide may contain only galactose units, or galactose combined with at least one of the other three sugars.

Glucose was present in the material precipitated when trichloracetic acid was added to papain extracts of raw and alcohol-treated barley. Glucose was also present in other proteolytic enzyme precipitates obtained in the same way from various liquors. The suggestion has been made that carbohydrate-containing material is associated with the material that is removed from extracts by trichloracetic acid.

Most of the nitrogenous material contained in crude barley gums is removed by papain treatment of barley and barley extracts. Some nitrogen remains in the gums, however, either as a contaminant or as part of the gum structure. Amino acid composition of papain-gums prepared by the precipitation method and the extraction method are much alike and hence are discussed together. Data on amino acid components of these gums are given in Table VII.

The amino acid compositions of the four papain-gums are striking in their similarity to that of gum extracted from alcohol-treated barley with water (cf. Table I). The amino acids that are chromatographically more mobile, leucine to proline in Table VII, are absent from all papain-gums. Several of the "slower-moving" amino acids were not detected, or were present only in trace amounts. Lysine and serine were the only amino acids that were present in all four papain-gums. Lack of detection of other amino acids, however, does not exclude the possibility that these were present in non-detectable quantities. The presence of amino acids in papain-gum hydrolysates indicates that the residual nitrogen is probably part of protein material and not of other nitrogenous compounds.

Barley gum, that is almost free of extraneous nitrogenous material and which has undergone minimum degradation during mashing, has been prepared by papain extraction. Possibly this gum bears the closest resemblance to the material in barley that is the precursor of

TABLE VII

Amino Acid Components of Barley Gums Prepared by Papain Treatment

	Raw Barley		Alcohol-Treated Barley	
	Precipitation Method	Extraction Method	Precipitation Method	Extraction Method
leucine	-	-	-	-
iso-leucine	-	-	-	-
phenylalanine	-	-	-	-
valine	-	-	-	-
proline	-	-	-	-
alanine	-	+	+	-
glutamic acid	-	?	-	-
glycine	+	-	-	+
aspartic acid	?	-	+	?
lysine	+	+	?	+
arginine	-	-	-	-
serine	+	?	+	+

+ denotes identification
- denotes not detected
? denotes trace

other gums. The nitrogenous material, which is removed from barley gum by papain, is probably protein material that is released from the gum by proteolytic enzyme action. The residual nitrogen may be due to papain contamination, or it may be an integral part of the gum structure. If the protein material was papain that was co-precipitated with the gum, it seems likely that the quantity of nitrogenous material precipitated would vary within fairly wide limits from one preparation to another. However, the nitrogen content of several gums extracted with papain from alcohol-treated barley was consistently between 0.07% and 0.11%.

Gum extracted from alcohol-treated barley with papain was reprecipitated in an attempt to remove the residual nitrogenous material. A 0.15% aqueous solution of papain-gum was mechanically stirred until the gum appeared to be completely dissolved. Gum concentration of such a solution is equivalent to that of an extract liquor obtained by papain extraction of barley. The gum solution was acidified with trichloracetic acid to a final acid concentration of 6%. The solution was filtered, and the filtrate added to 3 volumes of ethanol. The precipitate was recovered, dried by solvent exchange and then air-dried.

Recovery of reprecipitated gum was 83%. The original gum did not dissolve completely thus reducing final gum yield. Nitrogen content of papain-gum was not reduced by reprecipitation from trichloracetic acid solution. The gum was fibrous and had a viscosity of 546 cp. in a 0.78% solution. This viscosity value is similar to the viscosity of 476 cp. yielded by a 0.76% solution of the crude papain-gum. The difference in solution viscosities is probably due to the slight difference in gum concentration. A small change in concentration of gum solutions results in a marked change in viscosity. Solution viscosity of papain-gum is plotted against concentration in Figure 1. The slope of the viscosity-concentration curve increases rapidly with increasing concentration. Both physical and chemical properties of reprecipitated gum are essentially the same as those of crude papain-gum. Unfortunately, there was not sufficient material available to do sugar analysis on the reprecipitated gum. However, as solution viscosities of crude and reprecipitated papain-gums are the same, probably neither sugar composition nor gum structure has changed.

Purification of papain-gum did not remove the nitrogenous material. Hence, the nitrogenous residue present in gum is probably not a contaminant, but may be part of the gum structure. As amino acids were detected in papain-gum hydrolysate, the non-carbohydrate fraction of gum is proteinaceous. The protein material may be part of a gum degrading enzyme system that accompanies the gum. If an enzyme is present in barley gum, it must be inactivated by papain, as solutions of papain-gum, unlike raw gum solutions, are stable. The suggestion of Meredith et al. (28) that some of the nitrogen in barley gum is part of a protein-carbohydrate complex also appears plausible. A nitrogenous residue of 0.08% represents about 0.46% protein. If the protein material is unbound, it should be removed by trichloracetic acid. The alternative possibility is that the protein is attached to the carbohydrate material and the protein-carbohydrate linkage is not broken by papain treatment. The protein may be bound to the carbohydrate material in such a way that it acts as a cement to hold small polysaccharide fragments together as a large unit. The enzyme system that degrades

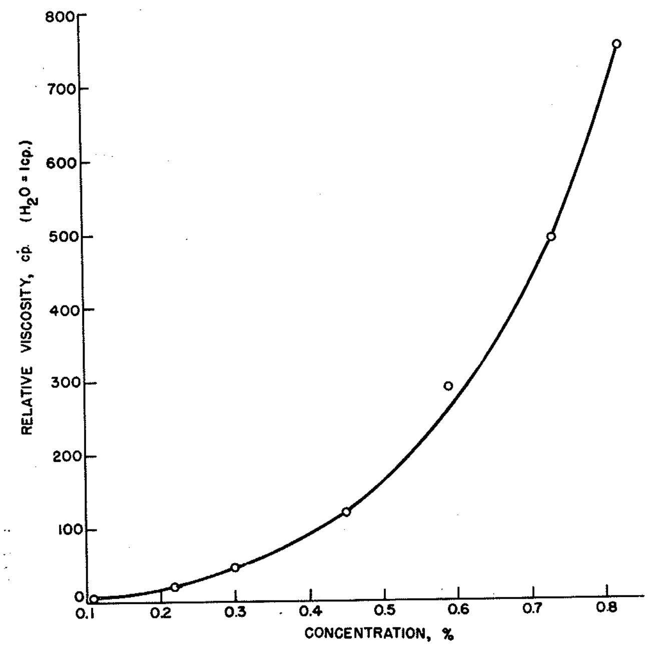

Figure 1.

Curve showing relation between relative viscosity and concentration for papain-gum.

crude barley gums may alter the linkage of the protein-carbohydrate complex, causing the liberation of the smaller fragments constituting the polysaccharide unit. The polymeric structure of barley gum may thus be due to polysaccharide fragments that are cemented together by protein material.

In general, materials of polymeric structure increase the viscosity of a solvent. Valuable information concerning the type of polymeric molecules being studied can be deduced from viscosity curves. If weight intrinsic viscosity $(\frac{\eta r - 1}{c})$ is plotted against concentration (c), Fuoss (12) states that "for neutral polymers, the data lie on a straight line which climbs with increasing concentration. For ... polyelectrolytes, the data give a curve which climbs very sharply with decreasing concentration". Polyelectrolytes are high molecular weight substances that are simultaneously electrolytes. Weight intrinsic viscosity is plotted against concentration in Figure 2 for papain-gum obtained from alcohol-treated barley. A minimum occurs in the curve at about 0.05% concentration. The shape of the curve suggests that papain-gum is a polyelectrolyte. Meredith _et al._ (28) found that crude barley, malt and wort gums appeared to be polyelectrolytes. These workers found the minima of intrinsic viscosity-concentration curves for these gums occurred at about 0.4% to 0.6% concentration. The minimum in the curve for papain-gum may lie at a lower concentration because of the purity of papain-gum. Polar groups causing polyelectrolyte behavior in papain-gum may be attributed to protein material, polar sugar derivatives, or a combination of both.

To determine whether the proteolytic enzyme action of papain liberated more gum from ground barley than was obtained by direct water

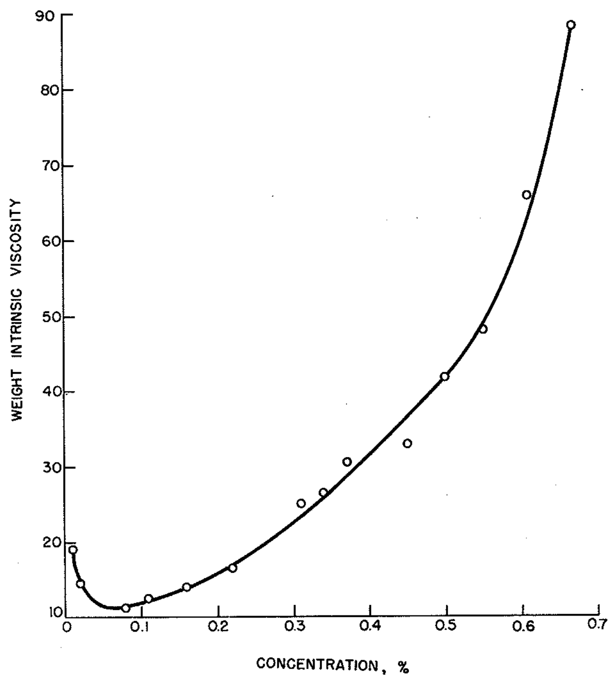

Figure 2.

Curve showing relation between weight intrinsic viscosity and concentration for papain-gum.

extraction of the grain, a series of extractions was made on alcohol-treated barley grist. The grist was first extracted for two hours with 8 parts of distilled water at room temperature. The spent grains were recovered by centrifuging the mash, and were washed twice with water. The spent grains were then extracted twice more under the same conditions with 8 parts of water. Gum was precipitated from each of the aqueous extracts and washings by 3 volumes of alcohol. The products were dried by solvent exchange and air-dried. The spent grains were then re-extracted for 2 hours with 8 parts of 1% papain solution. The mash was centrifuged and trichloracetic acid added to the liquor. The precipitate was removed by centrifuging and the supernatant liquor added to 3 volumes of alcohol. A slight precipitate was recovered from solution that was stored overnight. The product was dried by solvent exchange and air-dried.

Viscosity determinations were made on solutions of gum fractions that were isolated by successive extraction of alcohol-treated barley. Table VIII contains data on these fractions.

Yields of isolates obtained after successive water extraction of barley are much lower than yield of the crude isolate obtained after the first water extraction. The low yield of isolate from papain extract liquor indicates that proteolytic enzyme action does not free an appreciable amount of gum from spent grains. Gum obtained after papain extraction of spent grains is probably water-soluble material that was not removed by water extraction. Gum precipitated from washings of spent grains and from second and third water extracts were coarse, grey, powdery products. Their appearance and low solution viscosity resemble the corresponding properties of crude gum obtained

TABLE VIII

Data on Gum Fractions Obtained by Successive Extraction of Alcohol-Treated Barley

	Yield	Viscosity of Aqueous Solution	Concentration of Aqueous Solution
	%	cp.	%
Isolate from 1st water extract	1.2	134	0.80
Isolate from washings of spent grains	0.9	12.2	0.89
Isolate from 2nd water extract	0.4	3.05	0.38
Isolate from 3rd water extract	0.3	2.15	0.69
Isolate from 1% papain extract	0.1	1.47	< 0.60

from untreated barley grist. The final gum obtained after papain extraction of spent grains also was not viscous in aqueous solution. The four isolates obtained after water extraction of treated barley grist were unstable in solution, whereas the product obtained by papain extraction was stable. Thus, although papain does not increase gum yield, it does have a stabilizing effect on any water-soluble barley gum material.

As papain treatment of barley had proved useful in the preparation of a stable gum, additional experiments on gum preparation by this procedure were undertaken. The first gums prepared by papain extraction of alcohol-treated barley had low nitrogen contents and were very viscous in aqueous solution. However, papain obtained from various supply houses produced considerable variations in solution viscosity of gum obtained from alcohol-treated barley. Several of the enzyme preparations produced gums that had comparatively low viscosity in solution. Results of the effect of various papain preparations on gum viscosity are given in Table IX.

Gums prepared in the study of the effect of papain on gum viscosity were extracted from alcohol-treated barley, as gum from this barley is of most interest for future investigation. All papain preparations produced gums that were stable in solution and had a nitrogen content of about 0.10%. The papain-gums all contained glucose, arabinose, xylose and either galactose or a sugar of low Rf value. Variations in solution viscosities as shown in Table IX indicate that gums were subjected to various degrees of enzymatic degradation during extraction. Unfortunately, time did not allow investigation of the mode of action of papain on barley gums. The use of papain obtained

TABLE IX

Effect of Various Papain Preparations on Viscosity of Gums from Alcohol-Treated Barley

Source	Viscosity	Viscosity Reduction after 24 hrs.	Solution Concentration
	cp.	%	%
City Chemical Corporation, New York #1	346 211 289	2.3	0.72 0.73 0.70
City Chemical Corporation, New York #2	72.1 58.3	3.2	0.87 0.75
City Chemical Corporation, New York #3	148		
General Biochemicals, Inc., Chagrin Falls, Ohio	118	1.7	0.88
Nutritional Biochemicals Corporation, Cleveland, Ohio	752 445 546	2.9 2.0	0.82 0.65 0.78

from Nutritional Biochemicals Corporation resulted in the preparation of a gum that was extremely viscous in solution. This papain preparation was, therefore, used to prepare papain-gum for further studies of barley gum properties.

ISOLATES FROM NEUTRALIZED ACID-ALCOHOL LIQUORS

Gums prepared by either acid treatment or proteolytic enzyme treatment of barleys and barley extracts were precipitated in an acid-alcohol medium. After barley gum was removed from the acid-alcohol liquor, a second fraction was precipitated when the liquor was made alkaline with 1 N sodium hydroxide. This material was recovered by centrifuging, dried by solvent exchange with alcohol, acetone and ether, and then air-dried. When dry, precipitates recovered from all neutralized acid-alcohol liquors were cream to dark brown powders. The final color of the material depended on the volume of sodium hydroxide added in excess of neutrality. When the acid-alcohol liquor was made just alkaline to litmus paper, the dried products were cream colored.

As the isolates are precipitated from solution by neutralization of the liquors, the products probably contain high concentrations of salts. However, they were not purified, and physical and chemical properties were determined directly on the crude precipitates. Table X contains data on some properties of the precipitates obtained from various neutralized acid-alcohol liquors.

Yields of precipitates varied considerably among preparations. Variations in yields probably depend on the extent of salt contamination of the products. In general, the yields of products obtained by tannic acid precipitation and extraction methods are exceptionally high. These

TABLE X

Data on Isolates from Neutralized Acid-Alcohol Liquors

	Yield	Nitrogen	Viscosity of Aqueous Solution	Concentration of Aqueous Solution
	%	%	cp.	%
<u>Raw Barley</u>				
Trichloracetic Acid Precipitation Method	1.1	0.18	1.08	0.55
Hydrochloric Acid Precipitation Method	1.2	1.76	1.16	0.67
Tannic Acid Precipitation Method	6.1	0.11	1.01	0.91
Trichloracetic Acid Extraction Method	2.3	0.17	1.04	0.53
Hydrochloric Acid Extraction Method	2.5	0.95	1.14	0.70
Tannic Acid Extraction Method	5.9	0.18	1.07	0.79
Papain Extraction Method	1.2	0.45	1.06	0.50
<u>Alcohol-Treated Barley</u>				
Trichloracetic Acid Precipitation Method	0.5		1.08	0.55
Hydrochloric Acid Precipitation Method	0.3		1.85	0.57
Tannic Acid Precipitation Method	0.4		1.14	0.75
Trichloracetic Acid Extraction Method	2.3	0.21	1.05	0.65
Hydrochloric Acid Extraction Method	2.1	0.50	1.06	0.34
Tannic Acid Extraction Method	9.1	0.11	1.01	0.86
Papain Extraction Method	0.6		1.25	0.37

isolates are highly contaminated with sodium sulfate. Also, yield of an isolate obtained after the precipitation method was applied to barley is generally much lower than the yield of material obtained after extraction of barley. Solution viscosity of these products is low compared to the viscosity of barley gum solution, and does not appear to differ among preparations. Pretreatment of barley with alcohol does not affect the viscosity of solutions of these precipitates. Although apparent concentrations of solutions of precipitates appear to be high, they are probably increased because of salt contamination of the products. Nitrogen content of the products varies considerably, the highest being contained in isolates from neutralized hydrochloric acid-alcohol liquors. No attempt was made to investigate the form in which the nitrogenous material is present in the precipitates.

In addition to nitrogenous material the products contain sugar components. Acid hydrolysis of these materials produced galactose, glucose, arabinose and xylose in about equal proportions. Galactose was present in all precipitates obtained after neutralization of acid-alcohol liquors, whereas this sugar was detected only in gums prepared by proteolytic enzyme treatment of barley. However, in general, the sugars contained in barley gums are the same as those found in the products from neutralized acid-alcohol liquors.

The precipitates resulting from neutralization of acid-alcohol liquors were isolated incidently during investigation of methods of preparation of barley gums, and the products were not investigated further. However, the results of more intensive study of these fractions may be helpful in completing the picture of the structure of barley gum.

FURTHER PROPERTIES OF BARLEY GUMS

Gum extracted from alcohol-treated barley with papain solution is almost completely free of non-carbohydrate material, and is very viscous and stable in aqueous solution. Thus, of the several barley gums prepared, papain-gum is the most suited to studies of barley gum structure. Although investigation of structure is beyond the scope of this study, several other properties of papain-gum have been determined, and knowledge of these may contribute to the elucidation of the structure of barley gums.

Effect of Sodium Hydroxide on Barley Gum

Sodium hydroxide has an important use in carbohydrate chemistry as an extraction medium. Cell-wall polysaccharide material, hemicellulose for example, is separated from cellulose by virtue of the insolubility of the latter polysaccharide in alkaline solution. Barley gums and hemicellulose are defined by Preece (41) in terms of their solubility in sodium hydroxide. Barley gums are soluble in alkali as well as in water, whereas hemicellulose is soluble only in alkali. It is generally believed that alkali treatment of these polysaccharide materials only dissolves them, and does not affect their chemical structure.

Barley gums have a maximum solubility in water of around 0.7%, whereas they readily form 1% solutions in 1 N sodium hydroxide. Meredith _et al_. (28) found barley, malt and wort gums that had been refluxed with alkali were more soluble and more viscous in water than the corresponding crude gums. These workers reported also that alkali-treated gums contained no nitrogen and were stable in solution.

It has been observed during the study of gums of reduced nitrogen content that as nitrogenous material is removed progressively from gum, gum solubility in water increases. Hence, greater water solubility of alkali-treated gums may be due to maximum removal of nitrogen from the gums by alkali treatment. In any case, properties of alkali-treated gums reported by Meredith et al. opened up a field for considerable speculation. Therefore, a preliminary study of the effect of alkali on barley gum fractions obtained from alcohol-treated barley was undertaken.

Treatment, less drastic than alkali digestion, was applied to several gums obtained from alcohol-treated barley. The polysaccharide material was usually dissolved in cold 1 N sodium hydroxide to form 1% solutions. In some tests, however, gum solution was heated to boiling. Both hot and cold 1% solutions of crude barley gum and gum obtained after trichloracetic acid precipitation of aqueous extract, and a cold 1% solution of gum prepared by papain extraction of grist were prepared.

The gums were readily soluble in sodium hydroxide solution at 1% gum concentration. Viscosity determinations were made on aliquots of the gum solutions. Gum viscosity values were calculated relative to the viscosity of 1 N sodium hydroxide solution. Heated crude gum solution had a viscosity of 9.85 cp. and cold gum solution had a viscosity of 9.32 cp. As all gum was dissolved, the maximum viscosity of a 1% alkaline solution of raw gum obtained from alcohol-treated barley is around 10 cp. This viscosity value, however, is much lower than the viscosity of an aqueous solution of the same gum. Aqueous raw gum solution has a viscosity of 48.2 cp. at 0.79% gum concentration.

Similar results were obtained with alkaline solutions of gum obtained after trichloracetic acid precipitation of aqueous barley extract. Viscosities of hot and cold 1% solutions of this gum were 9.03 cp. and 15.7 cp. respectively. These values are again much lower than the aqueous solution viscosity of 64.1 cp. at 0.75% gum concentration. The four alkaline solutions were unstable, their viscosities being decreased from 10 to 15% after 24 hours. A stable solution resulted when gum that was obtained by papain extraction of pretreated barley was dissolved in sodium hydroxide. A cold 1% solution of this gum had a viscosity of 35.8 cp. as compared to an aqueous solution viscosity of 752 cp. at 0.82% gum concentration. Thus, gums obtained from alcohol-treated barley impart a lower viscosity to alkaline solution than to aqueous solution, and those gums that are unstable in aqueous solution retain their instability in alkali.

The lack of stability of solutions of certain gums appears to be caused by several factors. Firstly, gums from raw and alcohol-treated barleys may be degraded during extraction and in aqueous solution. Two or more enzyme systems may be involved. The simplest condition is that in papain-gum obtained from alcohol-treated barley, as degradation of this gum is prevented by extraction with papain. Thus, one enzyme system may be inactivated by boiling 85% alcohol and another by papain. Papain also stabilizes gum from raw barley, but as this gum produces a solution of low viscosity, it must have been degraded to an appreciable extent during extraction. This partial degradation may be a contributing factor to further degradation of the gum when in aqueous solution.

The predisposition of certain barley gums towards instability in solution is further emphasized by alkali treatment of gums. Raw gums that are unstable in aqueous solution are also unstable in alkali solution, whereas stable gums retain their stability in alkali. However, all gums possess lower viscosities in alkali than in water. It is most unlikely that enzymes would be active in 1 N sodium hydroxide, so the instability of certain gums must be regarded as inherent. The difference in viscosity between aqueous gum solutions and alkaline gum solutions is another matter. It appears that sodium hydroxide must react with the gum so as to reduce molecular complexity. This may well be accomplished by the scission of weak bonds that hold polysaccharide molecules together. A further experiment directed towards elucidating this point was undertaken.

To determine the effect of boiling alkali on barley gum, papain-gum extracted from alcohol-treated barley was dissolved in boiling 1 N sodium hydroxide to yield a 1% gum solution. The solution was cooled and added to 3 volumes of alcohol. The bulky precipitate was recovered. The product from the sodium hydroxide-alcohol liquor was then dissolved in water and reprecipitated by alcohol. The precipitate was centrifuged and washed with alcohol until the washings were neutral to litmus paper. The final product was dried by solvent exchange, followed by air-drying. Gum recovery from the second precipitation was 80%. The final product yielded readily a 1% aqueous solution that had a viscosity of 3.70 cp. relative to the viscosity of water. The solution was completely stable. A nitrogen determination on the isolate showed that the reprecipitated gum contained 0.06% nitrogen.

Papain-gum precipitated from boiled alkali solution was less viscous in aqueous solution than pure papain-gum dissolved in cold sodium hydroxide solution. The nitrogenous material was not removed completely from the gum by more drastic alkali treatment; nitrogen content was reduced only 0.02%. The residual protein of pure barley gum may function as a binding agent, holding smaller polysaccharide fragments together as a unit. Sodium hydroxide may then break this cementing protein material and release the smaller carbohydrate fragments. Reduction in size of the gum complex would account for the low viscosity of alkaline gum solution. The same cementing material could be present in the other gums, but would be associated with extraneous protein material.

Although the results of alkali treatment of barley gums do not provide explicit information on the influence of nitrogen on gum properties, they suggest strongly that a protein-carbohydrate complex exists in barley gum. Additional tests on gum cleavage were therefore undertaken.

Effect of Crude Barley Gum Solutions on Papain-Gum

A characteristic property of gums isolated after papain treatment of raw and alcohol-treated barley grists is their stability in aqueous solution. Crude gums and acid-gums obtained from these barleys have various degrees of instability. Meredith et al. (28) suggested that instability of solutions of crude gum extracted from raw barley may be the result of enzymatic cleavage of the polysaccharide complex. Thus, these authors implied a relation between instability and nitrogen content of gum. The existence of such a relation is more evident when the properties of recently prepared gum fractions are considered. Papain-gums obtained from both barleys contain around 0.1% nitrogen, whereas acid-gums and crude gums have nitrogen contents of 0.4% to 3.0%. If degradation of crude gum solution is due to enzymatic action and the enzyme system is removed or its action is inhibited by papain treatment, then a solution of crude gum obtained from either untreated or alcohol-treated barley should degrade a solution of papain-gum.

To determine the effect of a solution of crude gum on stability of papain-gum solution, 1 ml. of a 1% solution of crude gum obtained from raw barley was mixed with 7 ml. of papain-gum solution that had a viscosity of 180 cp. A dilute solution of papain-gum was used so that viscosity would be lower and, consequently, the time of flow of solution in the viscometer would be comparatively short. The initial viscosity of the mixture was 68.8 cp. The large initial reduction in viscosity is attributed mainly to dilution of the viscous solution. However, papain-gum was degraded rapidly and in 30 mins. solution viscosity was reduced to 58.8 cp. Similar results were obtained when a solution of raw gum extracted from alcohol-treated barley was added to papain-gum solution. Initial viscosity of the mixture was 140 cp. Two hours after mixing, solution viscosity was 123 cp; gum degradation was almost complete after 24 hrs. If instability of crude gum solution is attributed to degradative action of an enzyme system accompanying the gum, then this enzyme system must also attack papain-gum.

Although degradation of barley gum solution by enzyme action appears likely, the possibility that crude gums are inherently unstable and degrade naturally is not excluded. In an attempt to verify which of the possibilities is true, completely degraded crude gum solution was added to a viscous papain-gum solution. Two 1% solutions of crude gum extracted from alcohol-treated barley were prepared by dissolving the gum in hot and cold water. Two drops of toluene were added to each solution, and the solutions were stored for 7 days at room temperature. Maximum degradation of crude gum was attained within 7 days, and the solutions had viscosities of 1 to 2 cp. At the expiration of this time, 1 ml. of each of the heated and unheated solutions of crude gum was added to two 5 ml. aliquots of papain-gum solution that had a viscosity of 285 cp. The crude gum solutions were enzymatically active and were effective in degrading papain-gum solution. Initial viscosities of the mixtures containing heated crude gum solution and unheated crude gum solution were 110 cp. and 104 cp. respectively. These viscosity values were not corrected for change in concentration caused by dilution. The papain-gum solutions were degraded considerably; after 24 hrs. the mixture containing heated crude gum solution had a viscosity of 43.7 cp., while that containing unheated crude gum solution had a viscosity of 47.6 cp. Initial heating of crude gum solution does not affect its degradative action. This is not surprising since the enzyme system that causes gum degradation during extraction was not completely inactivated by digestion of barley with boiling alcohol. As papain-gum solution is degraded by crude gum solutions which have been completely self-degraded and have attained equilibrium, the conclusion can be drawn that gum instability is not an inherent barley gum property, but is caused by a heat-stable enzyme system.

Partition chromatography was employed in an attempt to detect sugar fragments resulting from degradation of papain-gum. The reaction mixture of papain-gum solution and unheated crude gum solution was evaporated to dryness by reduced pressure distillation. The residue was dissolved in 0.2 ml. water and an aliquot of the solution was chromatographed. No free sugars or polysaccharide fragments were detected. Lack of detection of polysaccharide degradation products may be because the quantity of reaction products formed is below the minimum amount required for detection by chromatography.

Results of these experiments support the suggestion made by Meredith _et_ _al_. (28) that gum instability is caused by enzyme activity. Stable papain-gum solution is degraded appreciably by solutions of crude barley gum extracted from either raw barley grist or alcohol-treated barley grist. Although no reaction products were detected, it is evident from the reduction in solution viscosity that the polysaccharide material of papain-gum is cleaved into smaller fragments by some agent present in crude gum solution. Degradation is not a result of inherent gum instability, but appears rather to be caused by a degradative enzyme system that is precipitated with crude barley gum.

Effect of X-Enzyme on Papain-Gum

Recent investigation of barley and malt gums showed that viscosity of aqueous solutions of these polysaccharide materials could be reduced by a commercial preparation of α-amylase and also by an extract of green malt (2, p. 32). Further studies indicated that a solution of inactivated α-amylase was also capable of reducing gum solution viscosity. This result indicated that a degradative enzyme system, other than α-amylase, was present in the commercial preparation. The carbohydrase system was subsequently isolated from both a bacterial source and green malt, and was tentatively named X-enzyme (4).

Bass, Meredith and Anderson (4) used a modified raw gum obtained from untreated barley as substrate for their studies of X-enzyme. Modified gum was prepared by digestion of crude barley gum with water. The modified product contained 0.7% nitrogen. This gum was not entirely satisfactory for enzyme kinetic studies, as it was low in viscosity and unstable in solution. Therefore, kinetic studies of X-enzyme were discontinued until a more satisfactory gum was prepared. If the viscous and stable gum extracted with papain from alcohol-treated barley is degraded by the carbohydrase system, this polysaccharide material would be satisfactory as a substrate for X-enzyme kinetic studies.

To determine the effect of X-enzyme on papain-gum, aliquots of X-enzyme solution prepared from Wallerstein's "α-Amylase Special for Analytical Purposes", and buffered at pH 6.5, were added to viscous gum solutions. One milliliter of X-enzyme solution was added to 7 ml. papain-gum solution that had a viscosity of 300 cp. Solution viscosity measured immediately after mixing was 3.83 cp. X-enzyme must rapidly cleave the polysaccharide material to result in such a marked decrease in viscosity, although part of the viscosity change is due to dilution of gum solution. After 3 hrs. in contact with the carbohydrase, gum viscosity was reduced to 1.35 cp. Within 24 hrs. a white precipitate separated from the mixture. Smaller aliquots of X-enzyme were equally effective for gum degradation and resulted in negligible dilution of gum solution; 0.01 ml. X-enzyme caused 89% degradation of gum in 2 hrs.

The enzymatic reaction proceeded until the solution viscosity was around 2 cp. Figure 3 shows the rate of viscosity reduction of papain-gum solution caused by 0.01 ml. X-enzyme. The curve emphasizes the initial rapid decrease in solution viscosity.

Although 0.01 ml. and 1.0 ml. of X-enzyme caused equivalent decreases in solution viscosity, the quantity of material that separated from the two solutions after 24 hrs. reaction time was not the same. The precipitate resulting from the addition of 1.0 ml. of X-enzyme to papain-gum solution was more voluminous. The precipitate recovered from the reaction mixture contained glucose, arabinose and xylose. The precipitated material could be either a part of the enzyme material or a degradation product of the substrate. The latter alternative seems more likely, as a similar precipitate was obtained from the reaction mixture of papain-gum solution and crude gum solution. Papain-gum is degraded more slowly by crude gum solutions than by X-enzyme. These results are further evidence that crude barley gums contain a gum-degrading enzyme system.

Viscosity reduction of gum solution indicates that the carbohydrase system cleaves the carbohydrate material into smaller polysaccharide fragments. The detection and identification of these fragments would yield some useful evidence for the problem of barley gum structure. Therefore, although Bass et al. (4) did not detect any free sugars or sugar fragments in the reaction mixture of X-enzyme and modified crude barley gum, it seemed worthwhile to chromatograph the reaction mixture of X-enzyme and papain-gum.

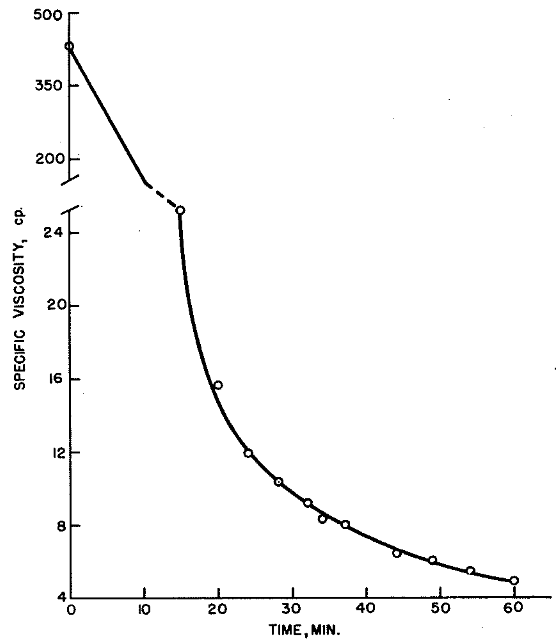

Figure 3.

Curve showing relation between specific viscosity and time for the reaction mixture of X-enzyme and papain-gum.

One milliter of X-enzyme solution was mixed with 10 ml. of 1% solution of gum extracted with papain from alcohol-treated barley, and the reaction was allowed to proceed for 36 hrs. at room temperature. Toluene was added to the reaction mixture to prevent bacterial growth. A white precipitate was removed from the solution, and the liquor was evaporated to dryness by reduced pressure distillation. The residue was dissolved in 0.1 ml. water and 15 μl. of the solution were chromatographed for sugars.

When sprayed with aniline phosphate, three spots were visible on the chromatogram. One of the three sugar spots was at the starting line on the chromatogram; this spot had the characteristic color of a pentose sugar. The other two sugar spots were lower on the chromatogram and had Rg values of 0.14 and 0.40. Rg is the ratio of the distance the unknown sugar travels to the distance travelled by glucose on the same chromatogram. This value is used to replace Rf (36), as calculation of the latter value requires measurement of the distance the solvent front travels on the chromatogram during development. However, to ensure sufficient resolution of these polysaccharides with the solvent system ethyl acetate/pyridine/water, the chromatogram must be developed for 48 hrs., in which time the solvent front runs off the filter paper. Both spots of low Rg value had colors characteristic of hexose sugars. The slight movement on the chromatogram of these three fragments relative to glucose indicates that they are definitely of greater complexity than simple sugars, and are probably even more complex than di- or trisaccharides. No free sugar fragments were detected in either gum solution or X-enzyme solution, therefore, the oligosaccharide fragments must be cleavage products from papain-gum.

The components of these three oligosaccharides were identified by hydrolysis of the polysaccharide fractions and subsequent chromatography of the hydrolysates. To obtain sufficient quantities of the oligosaccharides for hydrolysis, a streak of a solution of the residue from the reaction mixture was placed 3 inches from the end of a sheet of Whatman No. 1 filter paper (6 x 22 inches). The streak was 5 inches in length and was placed one-half inch from each side of the paper. The chromatogram was developed for 48 hrs. with the solvent system ethyl acetate/pyridine/water. The paper was air-dried after development. One inch strips were cut from each lengthwise edge of the chromatogram and sprayed with aniline phosphate. The sugar spots on the filter-paper strips were used as guides to determine the location of the sugars on the unsprayed part of the chromatogram. The sections containing the oligosaccharides were then cut from the paper. An equivalent section of filter paper was used as a cellulose blank. The oligosaccharides were eluted from the filter-paper sections with two 10 ml. portions of water. The solutions were filtered through glass wool and evaporated to dryness by reduced pressure distillation. The residues were then hydrolyzed with sulphuric acid. After neutralization and reduced pressure distillation, the residues from hydrolysis were dissolved in 0.1 ml. water, and 15 µl. of the solutions were chromatographed. The chromatograms were developed for 24 hrs. Color of the sugar spots was again developed with aniline phosphate spray.

Results of chromatography of the oligosaccharide hydrolysates show that the three sugar fragments consist of two glucosans and an arabo-xylo-glucosan. The base line sugar spot contained glucose, arabinose, xylose and a sugar that yielded a brown colored spot of Rg 0.27. This spot of low Rg value was unidentified, but may be due to a product resulting from incomplete hydrolysis of the oligosaccharide. The position of this spot on the chromatogram corresponds to the spot of low Rf value that was frequently found when the hydrolysate of papain-gum was chromatographed. The other two oligosaccharides yielded comparatively heavy concentrations of glucose. The hydrolysate of the cellulose blank contained no sugars. Figure 4 shows a chromatogram bearing the three oligosaccharides and their respective hydrolysis products.

Three of the four sugar spots below A in Figure 4 represent oligosaccharides; the fourth and lowest sugar spot is a glucose reference spot. The heavy-colored spots on either side of B and D represent glucose, arabinose and xylose, in descending order. These reference sugars aid the identification of unknown products. The faint spot appearing above glucose is a ghost spot that is associated with this sugar in the solvent used for development. The sugars below B, C and D are the hydrolysis products of the oligosaccharides. The sugars below B are contained in the hydrolysate of the base line oligosaccharide, while the sugars below C and D are the respective hydrolysis products of the lower oligosaccharides.

The same oligosaccharides were obtained by the action of X-enzyme on either high or low "viscosity-giving" papain-gums. However, the chromatogram of one reaction mixture contained a pentose-colored base line spot and three hexose-colored oligosaccharide spots. The Rg values of the latter spots were 0.05, 0.19 and 0.40. On hydrolysis, only two of these three low Rg spots yielded glucose; the third spot

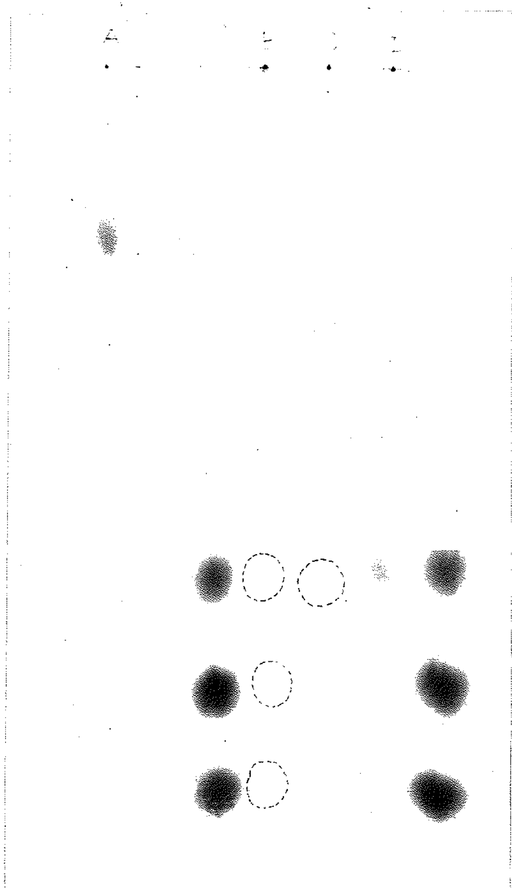

Figure 4.

Chromatogram showing the composition of oligosaccharides isolated from the reaction mixture of X-enzyme and papain-gum.

A = oligosaccharides of Rg 0.0, 0.14 and 0.40 and glucose.

B = hydrolysis products of oligosaccharide of Rg 0.0: glucose, arabinose and xylose in descending order.

C = hydrolysis products of oligosaccharide of Rg 0.14: glucose.

D = hydrolysis products of oligosaccharide of Rg 0.40: glucose.

gave no sugars. This third spot may have been produced by a contaminant on the paper.

The degradative action of X-enzyme on papain-gum extracted from alcohol-treated barley frees at least three soluble oligosaccharide fractions from the gum. One of these fractions is an arabo-xylo-glucosan. Associated with this complex fraction is another saccharide that may be galactose. The other two oligosaccharide fractions are glucosans. The composition of the three oligosaccharides obtained from papain-gum is analogous to the composition of three polysaccharide fractions isolated by Gilles, Meredith and Smith (13) from raw gum extracted from alcohol-treated barley. These workers found that methylated raw gum contained three polysaccharide fractions which, when hydrolyzed, yielded methylated simple sugars. Component (a) on hydrolysis gave 2, 3, 5-trimethyl arabinose, 2, 3-dimethyl xylose, 2-methyl xylose and simple xylose in the molecular ratio of 1:12:4:2 respectively. Component (b) on hydrolysis gave tetra-, tri- and di-methyl glucose in the molecular ratio of 1:6:1 respectively. Component (c) on hydrolysis gave 2, 3, 6-trimethyl glucose. As only one methylated sugar was obtained from component (c), this polysaccharide fraction appears to be structurally related to cellulose. Gilles et al. obtained an arabo-xylan fraction and two glucosan fractions from raw gum extracted from alcohol-treated barley. Thus, both raw gum and papain-gum extracted from alcohol-treated barley contain three similar polysaccharide fractions.

The polysaccharide fragments contained in barley gum may be cemented together in a complex by protein material. X-enzyme apparently breaks the bonds of the protein-carbohydrate complex and liberates the polysaccharide fractions. No protein fragments or free amino acids were detected in the reaction mixture of X-enzyme and papain-gum. Lack of detection of nitrogenous material by chromatography may be due to the liberation of insufficient protein material from scission of the complex. Protein may not be removed completely from the complex. Some weak bonds of the protein-carbohydrate complex may be destroyed by X-enzyme, whereas stronger bonds remain unaltered and bind the bulk of the protein to some carbohydrate material.

Degradation of papain-gum by X-enzyme is indicated by the reduction in gum solution viscosity and the identification of cleavage products in the reaction mixture. Degradation liberates oligosaccharides from the gum. These oligosaccharides may be bound together by protein material in a protein-carbohydrate complex. The presence of X-enzyme may destroy the weak bonds of the gum complex and thus result in gum degradation.

GENERAL DISCUSSION

Results of experimental work show that papain extraction of alcohol-treated barley produces a non-starch polysaccharide that is very viscous and stable in aqueous solution. This material is almost completely carbohydrate, but contains about 0.1% nitrogen. Properties of this gum material can be compared to the properties of crude barley gum. The latter gum produces an unstable aqueous solution of low viscosity. The nitrogen content of crude gum is about 3.0%. Both papain-gum and crude barley gum contain approximately similar proportions of the same sugars. Therefore, the nitrogenous material of crude barley gum reflects the difference in properties between the two products.

Nitrogenous material was progressively removed from crude barley gum by various procedures. Acid reagents and alcoholic treatment of barley remove some nitrogen from gum, but the products are unstable and not very viscous in aqueous solution. The bulk of the residual nitrogen is removed by papain treatment of barley. Study of the properties of these gums of reduced nitrogen content has provided a key to the elucidation of the role played by nitrogen in barley gums.

The nitrogenous material that is removed most easily from crude barley gum is unbound or extraneous protein material. This material can be distinguished by treatment of barley and barley extracts with hydrochloric acid and trichloracetic acid. Hydrochloric acid removes acid-soluble protein from crude gum, while trichloracetic acid removes the residue of extraneous protein material as well as acid-soluble protein. These two fractions were removed from crude gum without affecting gum structure. However, yield and nitrogen content of gums recovered after acid treatment of barley and barley extracts were lower. This treatment was not as effective in reducing nitrogen content of barley gum as alcoholic digestion of barley prior to water extraction. Alcohol completely removes extraneous protein material from crude barley gum. When trichloracetic acid was added to an aqueous extract of alcohol-treated barley, only 0.3% protein material was recovered. However, alcoholic treatment of barley not only removes extraneous protein from gum but also affects gum properties.

Solution viscosity of gum was increased greatly as a result of alcoholic digestion of barley, whereas gum yield was reduced. The difference in yield between crude barley gum and gum obtained from alcohol-treated barley cannot be entirely attributed to removal of protein material by alcohol, but must be caused by some other factor.

Preece and Ashworth (44) suggested that there are two fractions of pentosans in barley. One pentosan fraction is present in a water-soluble state, whereas the second fraction is initially insoluble. However, the latter pentosans may be converted into water-soluble products by enzyme action during mashing of barley. If this enzymatic action were prevented, then insoluble pentosan material would not be extracted with water-soluble gum. Meredith (27) found that alcoholic treatment of barley prior to mashing considerably reduces the yield of gum. The decrease in gum yield could be attributed to prevention of the enzyme action that is responsible for the release of certain pentosan material from barley. Yields of gum prepared by hydrochloric acid, trichloracetic acid and papain treatment of raw and alcohol-treated barley offer confirmatory evidence for this hypothesis.

Alcoholic digestion of barley produces gums that are more viscous in aqueous solution than crude barley gum. Preece and Ashworth (44) suggested that crude barley gum may be degraded during extraction by a cytoclastic enzyme system. Gum structure appears to be polymeric and the polysaccharide molecules may be enzymatically cleaved to yield smaller fragments, thus resulting in lower solution viscosity. Digestion of barley with alcohol partially inhibits the action of this degradative enzyme system. Hence, the molecular complexity of gum from alcohol-treated barley is greater and solution viscosity is higher than that of crude gum.

Acid also appears to limit the action of the degradative enzyme system. Acid extraction of raw barley produces gum that is more viscous in aqueous solution than crude gum. The addition of acid to aqueous extracts of raw barley, however, has no effect on gum solution viscosity. This result is obtained because gums recovered from acid-treated aqueous extracts have been degraded during aqueous extraction, and so addition of acid does not affect a change in molecular complexity of the gums. These results imply that mashing at pH 1.0 partially inhibits the degradative action of the enzyme system. Extraction of alcohol-treated barley with acid does not result in an increase in solution viscosity of the recovered gum. Thus, an acid pH is no more efficient than alcoholic treatment of barley in inhibiting the action of the gum degrading enzyme system during extraction.

Extraction of alcohol-treated barley with papain effectively inhibits gum degradation during extraction and in aqueous gum solution. Solution viscosity of papain-gum was increased tenfold over that of raw gum obtained from alcohol-treated barley. Unlike raw gum solutions, solutions of papain-gums obtained from either raw barley or alcohol-treated barley were stable. Meredith et al. (28) suggested that instability of aqueous solutions of crude barley gum may be caused by cleavage of the gum polysaccharide material by enzymes precipitated with the gum. Results of papain treatment of barley support this viewpoint.

Barley gums that are unstable in aqueous solution are unstable in alkaline solution. It is most unlikely that instability in alkali is caused by enzyme action. Sodium hydroxide is commonly used in organic chemistry to remove nitrogen from carbohydrate materials, and polysaccharides are generally believed to be unaffected by sodium hydroxide treatment. However, degraded barley gums may contain a variety of polysaccharide molecules that can react at various rates with sodium hydroxide. The resultant changes in the gums resemble the phenomenon of enzymatic gum degradation.

Two enzyme systems that affect gum properties must be assumed to exist in barley. One enzyme system degrades the polysaccharide material during extraction of barley. This enzyme is partially inactivated by boiling 85% alcohol. The second enzyme system, which survives alcoholic treatment, is precipitated with the gum. This enzyme causes gum instability in aqueous solution, but it is inactivated by papain treatment of barley.

The mode of action of papain on alcohol-treated barley was not investigated, but a factor that prevents gum degradation appears to be present in some papain preparations. Several papain preparations did not prevent gum degradation, and the gum products produced solutions of viscosity comparable to viscosity of solutions of raw gum from alcohol-treated barley. Different papain preparations may have the same proteolytic activity, but contain another system present as a variable.[1] The effects of this variable have not been investigated. The presence or absence of the variable may well be the cause of the variations in molecular complexity of papain-gums obtained from alcohol-treated barley. The variable factor may be a substance that inhibits the action of the degradative enzyme, or it may be another enzyme system that destroys the degrading enzyme precipitated with the complex polysaccharide material of the gum. The source and mode of action of this variable in papain preparations should be investigated, if gum extracted from alcohol-treated barley with papain is to be studied further.

Although alcoholic treatment of barley, combined with papain extraction of the grist, removes the bulk of nitrogenous material from crude barley gum, some nitrogen is still associated with the polysaccharide. This nitrogenous material is bound quite firmly to the carbohydrate material. It is not a protein contaminant, as it was not removed by reprecipitation of gum from trichloracetic acid solution. The conclusion must, therefore, be drawn that the nitrogenous material is part of the gum structure.

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[1] Personal communication from S. M. Mann, Nutritional Biochemicals Corporation, Cleveland, Ohio.

The residual nitrogen resembles protein material, as an hydrolysate of the gum contained amino acids. Meredith et al. (28) suggested that some nitrogenous material of barley gum may be part of a protein-carbohydrate complex. The residual protein of papain-gum may form a complex with the polysaccharide material of the gum. The complex may not be chemically united, but may be bound together by weak bonds, such as hydrogen bonds.

The carbohydrate material of barley gum appears to be comprised of several polysaccharide fractions. These fractions may be cemented together by the protein material to form the complex gum. The extremely high solution viscosity of gum extracted with papain from alcohol-treated barley can be explained by this hypothesis. Extraction with papain prevents destruction of the weak linkages of the protein-carbohydrate complex, and thus the gum has a very branched structure.

Apparently the bonds binding the protein material to the carbohydrate fraction are susceptible to the action of the enzyme system that causes gum instability. Indeed, the destruction of this cementing material is probably the initial cause of the cleavage of gum in aqueous solution. Gums obtained from raw barley have had the protein-carbohydrate complex almost completely destroyed by enzyme action during extraction of gum from barley. The complex contained in gum obtained from alcohol-treated barley is only partially destroyed during gum preparation. However, destruction of the complex is completed in aqueous solution by the degradative enzyme system contained in unstable barley gums.

X-enzyme also degrades barley gum. This enzyme, or enzyme system, may not cleave the carbohydrate material directly. It may merely destroy the weak bonds cementing the protein and carbohydrate materials, and thus cause the liberation of smaller polysaccharide fragments. Three oligosaccharides were obtained by the action of X-enzyme on papain-gum. These were two glucosans and an arabo-xylo-glucosan. These oligosaccharides are similar in composition to three polysaccharides isolated by Gilles et al. (13) from gum extracted from alcohol-treated barley. The oligosaccharides, along with other polysaccharide material, may be bound together by a small proportion of protein to form a very complex gum.

Alkali treatment of barley gums offers further evidence for the presence of a protein-carbohydrate complex. When viscous papain-gum is dissolved in sodium hydroxide, viscosity of the solution is much lower than viscosity of an aqueous gum solution. The decrease in viscosity of alkaline gum solution indicates the gum has a much simpler molecular structure when it is dissolved in sodium hydroxide. Thus, alkali treatment must destroy the bonds binding the protein-carbohydrate complex and release the smaller polysaccharide fragments.

Crude barley gum contains a mixture of protein material. The bulk of the nitrogenous material is extraneous protein and merely contaminates the gum. Some of the protein material is enzymatic and appears to be more closely associated with the gum. This enzyme system apparently causes gum instability in aqueous solutions. Finally, the residual nitrogenous material forms a protein-carbohydrate complex, and is an integral part of barley gum structure. Thus, although there are three nitrogenous fractions associated with crude barley gum, only one of these fractions directly affects barley gum properties.

Study of properties of barley gums of reduced nitrogen content has provided information that indicates the role that nitrogen plays in barley gums. Knowledge of the role of nitrogen in barley gums will be of value in completing the picture of barley gum structure. Determination of the structure of barley gum is necessary before the relation between barley, malt and wort gums can be ascertained.

SUMMARY

(1) Certain properties of non-starch polysaccharides obtained from barley varied widely among preparations and appeared to be related to nitrogen content. To provide information on the role of nitrogen in barley gums, protein material was systematically removed from gums by various extraction and precipitation procedures. Properties of gums of reduced nitrogen content were studied.

(2) Crude gum obtained by water extraction of barley contains about 3.0% nitrogen and produces an unstable aqueous solution of low viscosity. Extraction of barley with aqueous 6% trichloracetic acid, of pH 1.0, removes more nitrogenous material from barley gum than extraction with hydrochloric acid of the same pH. Alcoholic digestion of barley was used previously to reduce nitrogen content of barley gums, but acid treatment of barley is no more efficient in removing nitrogenous material than boiling alcohol. Acid extraction of raw barley produces gum that is more viscous in solution than crude barley gum. ~~Acid~~ These gums are not completely stable in aqueous solution.

(3) Papain extraction of alcohol-treated barley produces gum that is stable and extremely viscous in aqueous solution. This gum has a polymeric structure and contains principally glucose, although arabinose, xylose, galactose and about 0.1% nitrogenous material are also present.

(4) Study of gums of reduced nitrogen content indicates that the bulk of the nitrogenous material contained in crude barley gum is extraneous protein.

(5) Preparation of gum from raw barley and alcohol-treated barley indicates that gum is degraded by an enzyme system during extraction. This enzyme, which is partially inactivated by alcoholic digestion of barley, reduces gum complexity and increases gum yield.

(6) Degradation of barley gum in aqueous solution appears to be the result of the action of an enzyme system that is precipitated with the polysaccharide material. Treatment of barley with papain removes the enzyme system and produces a stable gum.

(7) The residual protein material of barley gum appears to be part of a protein-carbohydrate complex that may be bound by weak bonds. The protein may bind polysaccharide molecules together to form complex gum molecules. The complex appears to be readily destroyed by alkali or enzymatic action.

(8) Solution of gum obtained by papain treatment of barley is degraded by aqueous solutions of crude barley gum and gum extracted from alcohol-treated barley with water. This lends support to the hypothesis of enzymatic degradation of gum in aqueous solution.

(9) The action of X-enzyme on gum obtained by extraction of alcohol-treated barley with papain yields at least three oligosaccharide fractions as gum degradation products. Two of these polysaccharide fractions are glucosans and the third is an arabo-xylo-glucosan.

(10) Alkali treatment of barley gum appears to decrease the complexity of gum structure, but does not completely remove nitrogen from the gum. Gums that are unstable in aqueous solution retain their instability in alkali.

(11) Neutralization of acid-alcohol liquor from which gum has been removed produces a second gum-like fraction. This material has low solution viscosity and contains galactose, glucose, arabinose, xylose and varying amounts of nitrogen. This is a further indication that the non-starch polysaccharide obtained from barley is very complex and may contain a protein-carbohydrate complex.

(12) Study of properties of barley gums of reduced nitrogen content has provided a key to the elucidation of the role of nitrogen in these materials. This information will be useful for studies of barley gum structure, knowledge of which is necessary to determine the relation between barley, malt and wort gums.

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