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RELATIONSHIP OF WINTER HABIT AND MALTING
QUALITY IN WINTER X SPRING BARLEY CROSSES

Thesis for the Degree of Ph. D.

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David H. Smith, Jr.

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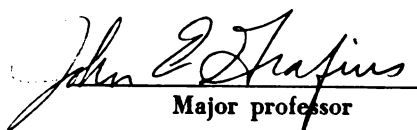
RELATIONSHIP OF WINTER HABIT AND MALTING QUALITY
IN WINTER X SPRING BARLEY CROSSES

presented by

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ABSTRACT

RELATIONSHIP OF WINTER HABIT AND MALTING QUALITY IN WINTER X SPRING BARLEY CROSSES

by David H. Smith, Jr.

Twelve crosses of winter x spring barley were made. F_3 rows of the progenies of these crosses were classified as spring, winter and intermediate based on heading date. Bults of each growth habit class were made and malted in micro-malting equipment designed and built during the course of this research. Quality evaluation of the bulk malts show that malt quality is independent of growth habit.

Micro-malts of the progeny of a cross between Kindred and Hudson were subjected to cold water extraction. Lines of high, low and intermediate cold water extract values were tested for cytolytic activity on gums isolated from the parental lines. Cytolytic activities of all lines was lower on Hudson gum than on Kindred gum indicating a difference in gum structure. The correlation of cold water extract with cytolytic activity was not statistically significant.

Fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$ of water extracts of grist, alcohol refluxed, of the two parents were made which gave further evidence of a structural difference between the two gums.

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By

David H. Smith, Jr.

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INTRODUCTION

The possibility of a winter barley variety which would be acceptable to the Malting and Brewing Industry is of interest both from the academic and the economic viewpoint. Since winter barley, in contrast to spring barley, is adapted to the climatic areas surrounding population centers such as Detroit, Chicago and New York, a winter type malting barley might prove advantageous to both the farmer and the industry.

The reason why such a variety has never been developed may be traced to custom, lack of interest, and to a conservative attitude which maintained that since it had not been done it could not be done. Whether or not it can be done is still a matter of conjecture, but this thesis allows at least one step towards such a goal.

It was the intent of this research to investigate the degree of independence of factors associated with malting quality and growth habit. In brief, was there any real bar to the production of a winter barley with a spring type quality pattern? Comparison of the malting characteristics of the three growth habit categories, spring, winter and intermediate, within crosses of winter x spring varieties when vernalized and grown under the same conditions should furnish some answers to the problem.

Malting in very simple terms involves germination of barley under closely controlled conditions which is terminated at the time the acrospire reaches the length of the kernel. During germination a number

of changes occur within the seed most of which are directed toward the breakdown of the complex storage forms of energy-containing substances into simpler forms. This breakdown is largely enzymatic and involves a host of enzymes of varying specificities. Modification is a term used to describe the overall breakdown of the contents of the endosperm into simpler and more soluble forms. Poorly modified kernels will be less friable and will have a lower quantity of extractable material than kernels that are well modified. In general winter barley varieties tend to produce poorly modified malts under commercial malting conditions. Dicktoo is one of the poorer in this respect while Hudson is one of the better varieties. It would appear that differences in modifiability are related to the relative amounts of gum and cytolytic enzymes present in the seed.

Enzymes known as cytases are those which in effect open the door to other digestive enzymes. The cytases are those which have been recognized and defined as involved with cell wall degradation. Rupture of the cell wall must occur in order for further degradation of the cellular contents to be brought about by other digestive enzymes.

In order to study the problems inherent in the development of a winter type barley for malting purposes, the differences in the chemistry between the spring and winter types must be taken into account. Certain physiological differences between the two types should also be considered.

LITERATURE

Malting quality is a complex of a number of chemical and morphological traits. These traits are varietal and therefore heritable, but the inheritance is mainly quantitative. Grafius (12), Luedders (17), and Whitehouse et al. (30) working with barley, oats and wheat, respectively, have shown that the components of the complex trait yield can be predicted in progenies from mid-parental values. Dickson and Grafius (8) have found a high correlation between progeny mean malting characteristics and midparent values. Any substance which prevents or slows down cytolytic processes should have a profound effect on the overall modification of the endosperm contents from barley substance to malt substance. Materials of this nature either pre-existing or derived from cell wall hemicelluloses have been recognized as occurring in especially large amount in barleys that are difficult to malt while easily modified barleys contain much smaller amounts (19). These non-starchy water soluble polysaccharides are known as gums or mucilages, and the non-starchy water insoluble polysaccharides which dissolve in 4% sodium hydroxide are hemicellulose (27). Hemicelluloses play an unusual role in cereal endosperms because little or no cellulose and no pectins are present (7) (9, 10, 11). It has been suggested that they function along with protein as the cementing agent between endosperm cells (18) (31).

O'Sullivan (22) isolated two gums from barley flour extracted with water which he called alpha and beta amylan. Alpha amylan is now

called beta-D-glucan. Methylation studies have shown that beta-D-glucan is a long unbranched chain of D glucopyranose units (27) (2) made up of 1→3 and 1→4 linkages of approximately equal proportions. Whether the beta-D-glucan molecule is cyclic i.e. in the form of a loop (2) or strictly linear (27) has not been resolved.

Aqueous solutions of beta-D-glucan have very high specific viscosities (26) (20), but according to Preece and McKenzie (26) the beta-D-glucan disappears rapidly during malting and is almost absent from finished malt. Thus wort viscosities are not due to the presence of beta-D-glucan. Harris and McWilliam (15) have shown that the amount of water soluble glucose polymers that are insoluble in 80% ethyl alcohol rises during steeping and decreases as germination begins. Since Hall et al. (14) have found little change in starch content during steeping, this rise and fall in amount of such polymers is related to their liberation from insoluble non-starchy material and subsequent hydrolysis to less complex forms. Preece and Aitkin (23) have found a similar increase in beta-D-glucan content measured at intervals in barley grists treated with water followed by a decrease in the amount of gum. Varieties which are poor in malting quality were found to release gum during the entire period of extraction while gum quantity from good malting varieties increased for 2 hours and then decreased (23).

Two cytases have been found in barley and green malt which attack beta-D-glucan, endo-beta-glucosylase (24) or endo-beta-glucosidase (4) and exo-beta glucosylase (24) and exo-beta-glucosidase (4). Endo-beta-glucosylase attacks the polymer at points remote from the ends of the chain thus producing a marked decrease in viscosity

without increasing reducing power significantly (4, 24). Exo-beta-glucosidase attacks the ends of the polymer splitting off units of cellobiose (4, 24).

Bass and Meredith (5) and Bass, Bendelow and Meredith (3) have shown a relationship between green malt α -glucosidase activity on beta-D-glucan and malting quality. α -glucosidase activity of most of the lines tested correlated well with malt quality although 3 lines of low α -glucosidase activity were of satisfactory quality and 2 lines with high enzyme activities were unsatisfactory in one or more malt properties. It was also reported (3) that cold water extract is the best single criterion of overall malt quality currently available because of the high correlations found between cold water extract and high levels of malting quality traits.

Other seed gums have been isolated from water extracts of ground barley. Alpha-D-glucan and an arabinan, a branched pentosan have been found (27). Smith and Montgomery (27) state that the viscosity of gum preparations of mixtures of pentosan and hexosan is due to beta-D-glucan. Table 1 is taken from Preece and Hobkirk (25). It shows the composition of gums from different cereals as isolated using different concentrations of $(\text{NH}_4)_2\text{SO}_4$.

The velocity of enzyme catalyzed reactions is a function of substrate concentration only when the concentration of substrate is less than that which allows maximum velocity to be obtained. When sufficient substrate is present to allow maximum velocity the reaction curve becomes that of a zero order reaction as shown in Figure 1.

TABLE 1.--Composition of the principal cereal gum fractions (25)

Precipitation Level (NH ₄) ₂ SO ₄ %	Sugar Unit	Rye	Wheat	Barley	Oats	Maize
20	Glucose	-	-	100	100	-
30	Glucose	17	11	96	93	-
	Xylose	45	64	2	1	-
	Arabinose	38	25	2	6	-
40	Glucose	0	6	12	88	40
	Xylose	61	61	56	8	40
	Arabinose	39	33	32	4	19 ^a
50	Glucose	0	23	7	-	92
	Xylose	55	47	65	-	0 ^b
	Arabinose	45	30	28	-	6
60	Glucose	2	56	-	-	-
	Xylose	68	24	-	-	-
	Arabinose	30	20	-	-	-
Saturation	Glucose	-	-	17	61	-
	Xylose	-	-	61	20	-
	Arabinose	-	-	9	19	-
	Mannose	-	-	13	0	-
Mother liquor	Glucose	25	18	15	15	66
	Xylose	9	9	10	14	7
	Arabinose	42	47	62	40	19
	Galactose	22	26	13	31	8

^aContains approximately 1% Galactose.

^bContains approximately 2% Galactose.

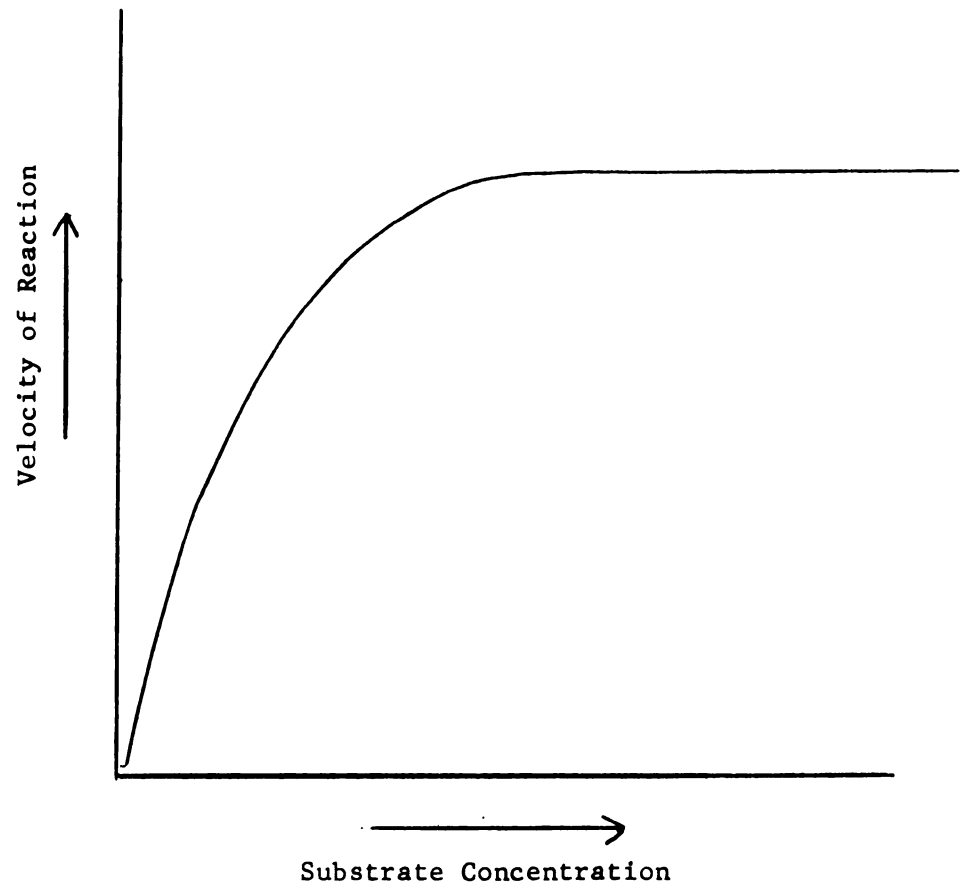


Fig. 1.--Effect of substrate concentration on the velocity of an enzyme reaction (16).

MATERIALS AND METHODS

Twelve crosses were made using spring and winter barleys as parents (Table 2).

TABLE 2.--Parentage of winter x spring barley crosses made in 1959 and 1960 at East Lansing

Cross Number	Winter Parent	Spring Parent
59 301	Dicktoo CI 5529	(Moore x Anoidium) x Montcalm
59 302	Dicktoo CI 5529	(Moore x Anoidium) x Montcalm
59 303	Dicktoo CI 5529	Moore x ² Montcalm
59 304	Dicktoo CI 5529	Liberty x Kindred
59 305	Dicktoo CI 5529	Moore x ² Montcalm
59 306	Dicktoo CI 5529	Moore x ² Montcalm
59 307	Dicktoo CI 5529	Moore x (Kindred x Bay)
59 308	Dicktoo CI 5529	Moore x (Kindred x Bay)
60 301	Dicktoo CI 5529	Montcalm CI 7149
60 302	Hudson CI 8067	Kindred CI 6969
60 303	Dicktoo CI 5529	Kindred CI 6969
60 304	Hudson CI 8067	Traill CI 9538

Spring parents were selected for their superior malting qualities as shown by quality evaluations made at the USDA Barley and Malt Laboratory in Madison, Wisconsin. Winter parents were picked for their agronomic characteristics, particularly winter hardiness.

Progenies of these crosses were grown in the greenhouse for 2 generations and then seed from each F₂ plant was planted in a single hill in the field. Field plantings were made in the early spring when the soil was thawed only to a depth of about 1 inch. This early planting gave sufficient cold temperature during the early development of the plants to vernalize the winter types in the population. Each hill was harvested and threshed individually. Such a procedure made

it possible to obtain seed for malting from all growth types, winter, intermediate and spring, grown in the same environment. This nursery will henceforth be referred to as the vernalized nursery.

Since all seed was vernalized in the experimental planting, it was necessary to plant a nursery at a time when vernalization could not occur of the same material to determine the growth habit of the plants in each row. In the spring of 1962 seed samples were taken from each line and approximately 30 seeds of each sample were planted on May 16, 1962 in 3 foot rows in a nursery. Heading notes were taken and the rows in each cross were classified into growth habit categories: spring, intermediate and winter. Spring habit included all uniform rows heading at the same time as or before the spring parent of the cross. Intermediate included all uniform plants in rows heading later than the spring parent and winter all uniform plants in rows failing to head. Rows of segregating plants were not included in the three groups. Plants in this nursery were not harvested, but used only for indication of the types of growth. Each growth type for each cross from the nursery where plants were vernalized was bulked by taking an equal amount of seed from each line within a growth habit type for each cross. In two crosses there was insufficient seed to group into the three categories and these crosses were analyzed as bulks ignoring growth type. All of the seed in each cross was produced in the same year in the same nursery i.e. the spring, intermediate and winter types were all grown under conditions as close to identical as is possible in the field.

Three bulks, winter, intermediate, spring, and the two parental lines for each cross were malted using a modification of the technique

of Whitmore and Sparrow (30) described in the appendix.

All but two crosses contained over 100 lines making a total of about 1300 lines for the group. Detailed analysis of this many lines appeared to be an impossibly long task. It was decided that one cross should be selected for such analysis. The selection was made by calculating the mid-parental values for a set of malting characteristics for each cross and choosing the cross having a midparent most closely approaching a typical spring quality pattern. The midparents are shown in Table 3. Cross number 60 302, Hudson x Kindred, appeared to be the best of the group by this method of prediction. The individual lines within 60 302, Hudson, Kindred, and a standardized check were malted in large test tubes according to the technique of Whitmore and Sparrow (30) outlined in the appendix. Prior to kilning, five grams of green malt were taken from each tube in the group and stored at 0° F. in a deep freeze.

Individual kilned malts were ground in a Wiley mill using a #40 sieve. Cold water extracts were made on five grams of seed from each line. The ground malt was mashed with 39 ml of deionized water and 1 ml of 0.05 molar mercuric chloride at 18° C. for 120 minutes. The mixture was then filtered and the specific gravity of the filtrate determined using a 10 ml specific gravity bottle. The procedure followed was that of Bass, Bendelow and Meredith (3) except that they used 25 grams of malt and proportional amounts of water and mercuric chloride.

Using these specific gravities as an index of modification (3, 6) seed from lines of high, low and intermediate extract were chosen for analysis of their cytolytic activity contained in their green malts.

TABLE 3.--Midparental malting trait values for 12 spring x winter barley crosses compared to the spring variety Traill

	60 301	60 302	60 303	60 304	59 301	59 302	59 303	59 304	59 305	59 306	59 307	59 308	Traill
Z ^a	27.8	29.6	28.6	28.7	28.8	29.1	26.6	28.8	26.7	27.1	28.9	26.8	31.5
% 6/64	40.6	47.4	38.2	29.6	34.5	42.9	29.1	37.6	25.2	31.4	29.7	26.4	37.2
Ext	75.0	76.4	75.4	77.0	73.9	75.0	75.3	75.2	75.1	75.0	72.9	75.7	77.8
WN	.659	.719	.696	619	610	.693	.668	.593	.673	.664	.655	.675	.678
MN	2.00	1.87	1.97	1.63	1.85	1.96	1.86	1.89	1.85	1.90	2.07	1.85	1.76
W/M	33.0	38.4	35.3	37.9	32.9	35.4	35.9	31.4	31.4	34.9	31.6	36.5	38.5
DP	163	190	175	157	160	157	158	139	145	146	130	156	183
β	504	510	553	471	511	479	506	467	444	461	389	478	575
α	45.0	55.6	45.1	45.8	40.7	45.1	39.8	28.4	42.6	39.1	41.3	45.0	45.7
β/α	11.2	9.2	12.3	10.3	12.7	10.6	12.7	16.4	10.4	11.0	9.4	10.6	12.6

^aMalting traits: seed weight, % plump, % extract, wort nitrogen, malt nitrogen, ratio of wort nitrogen to malt nitrogen, diastatic power, β amylase, α amylase, ratio β amylase to α amylase respectively.

In order to determine cytolytic activities, a suitable substrate must be available. Meredith, Watts and Anderson (20) and Bass and Meredith (4) have outlined a method for the preparation of a gum substrate containing beta-D-glucan from spring barley. Using this technique 200 grams of Hudson and Kindred barley were ground separately in a Wiley mill using a 1 mm sieve. The ground barleys were refluxed for 30 minutes with 5 parts of 85% ethyl alcohol, centrifuged and air dried. The alcohol treated grist was mashed in 8 parts of 0.025% aqueous papain solution for 2 hours. The papain was precipitated by adding 200 ml of water containing 108 grams of trichloroacetic acid. The mixture was centrifuged and filtered. The gum was precipitated from the filtrate by adding 30 grams of $(\text{NH}_4)_2\text{SO}_4$ per 100 ml of filtrate and allowing the salted filtrate to stand overnight in a refrigerator. $(\text{NH}_4)_2\text{SO}_4$ was washed from the precipitate using 50% ethyl alcohol until the presence of the salt could no longer be detected using barium chloride. Then the precipitate was washed with absolute alcohol, acetone and ether. The precipitated gum was air dried and ground in a mortar and 2 grams dissolved in 175 ml 0.1 molar sodium chloride (3) and heated in a boiling water bath for 30 minutes. The hot gum solution was suction filtered through #41 H Whatman filter paper. The solution was cooled and 6 drops of toluene added as a preservative and stored in a refrigerator.

Enzyme preparations from green malts were made according to the technique of Bass, Bendelow and Meredith (3). Two grams of green malt were blended with 80 ml of 0.1 molar sodium acetate-acetic acid buffer (pH 4.4) for 2 minutes, then allowed to stand in the blender for five minutes and blended again for 2 minutes. The blended mixture

was centrifuged for 5 minutes at 1500 rpm and filtered through #12 fluted Whatman filter paper. Seven ml of filtrate were diluted to 100 ml with deionized water.

All solutions were adjusted to 30° C. in a water bath. The reaction mixture contained 1 ml of green malt enzyme solution, 2 ml of sodium acetate-acetic acid buffer (0.1 molar) and 7 ml of gum solution. Five ml of the reaction mixture were pipetted to an Ostvald viscometer and the time of fall was determined during 1 hour at 8 to 10 minute intervals.

Relative viscosities were calculated by dividing the time in seconds of each viscometer reading during the reaction by the time in seconds for water. Specific viscosity is equal to the relative viscosity minus 1. Enzymatic activity has been defined by Bass, Bendelow and Meredith (3) as 120 times the slope of the linear reaction curve of the reciprocal of specific viscosity plotted against time elapsed after mixing the enzyme, buffer and substrate.

Small samples of grist, alcohol refluxed, of Hudson and Kindred were mashed with water containing no papain for 2 hours at room temperature. The mixtures were centrifuged and filtered and then fractionally precipitated with 10% increments of $(\text{NH}_4)_2\text{SO}_4$ added at 24 hour intervals. The salted mixtures were maintained in a refrigerator. Prior to adding the next higher amount of the salt the precipitate was centrifuged from the supernatant mother liquor and washed with 50% ethyl alcohol to remove the $(\text{NH}_4)_2\text{SO}_4$. Thus a series of precipitates was obtained. These along with samples of the gum previously prepared from Hudson and Kindred, using papain, were dried in a vacuum oven. The dried residues were hydrolyzed with 88% formic

acid under reflux and dried on a steam table. Residues were taken up in water and spotted on #1 Whatman filter paper along with knowns of glucose, xylose and arabinose and subjected to descending chromatographic separation in a solvent system of benzyl alcohol, n-propyl alcohol, formic acid and water for 30 hours. The paper was dried and developed with p-anisidine hydrochloride. The remaining portions were taken to dryness and treated with a mixture of concentrated sulfuric acid (ten parts) and phenol (80% aqueous solution by weight, one part) as a test for the presence of pentosans.

RESULTS

Growth Habit Distribution Within Crosses

Table 4 shows the number of lines in each growth habit grouping for the 12 spring x winter barley crosses.

TABLE 4.--Number of rows of each category of growth habit of 12 winter x spring barley crosses grown at East Lansing in 1962

Cross Number	Habit of Growth			
	Spring	Intermediate	Winter	Segregating
59 301	15	59	25	24
59 302	19	57	12	14
59 303	28	58	10	19
59 304	47	54	11	11
59 305	11	18	0	13
59 306	19	26	5	35
59 307	3	19	6	4
59 308	38	36	18	15
60 301	8	10	1	2
60 302	56	73	13	20
60 303	87	50	7	33
60 304	46	72	12	25

Progeny versus Mid-parent Comparison

The relationship between mid-parent and progeny is borne out in Table 5 where it can be observed that the progeny means do tend to regress towards the mean of the two parents. The average r value for all eleven crosses is .500 (Table 6) which is highly significant, although smaller in size than that obtained by Dickson and Grafius (8). Presumably some of the lack of agreement could be due to the effect of the winter habit gene which even when the plants were vernalized tended to delay heading well beyond the average for the spring isolates.

TABLE 5.--Malting traits of progeny mean, mid-parent, parents, (P_1 to P_2) progeny mean percentage, and mid-parent percentage for 11 spring x winter crosses

Malting Trait	Progeny Mean	Mid-Parent	Cross Number 60 301		Progeny Mean %	Mid-Parent %
			Mont-calm (P_1)	Dick-too (P_2)		
Extract	72.4	72.6	74.4*	70.8	99.6	99.8
Wort Nitrogen	1.10	.982	.977	.987*	111.9	100.0
Malt Nitrogen	2.74	2.65	2.42*	2.88	106.6	103.1
Diastatic Power	259	252	285*	219	97.8	95.1
beta-amylase	802	791	910*	672	95.6	94.3
alpha-amylase	71.1	66.0	69.8*	62.1	96.5	89.6
Malting Trait	Progeny Mean	Mid-Parent	Cross Number 60 302		Progeny Mean %	Mid-Parent %
			Kin-dred (P_1)	Hudson (P_2)		
Extract	72.4	72.7	72.7*	72.7*	99.6	99.9
Wort Nitrogen	1.06	.985	1.03*	.940	107.9	100.3
Malt Nitrogen	2.60	2.62	2.66	2.58*	101.2	101.9
Diastatic Power	274	279	299*	259*	103.4	105.3
beta-amylase	843	850	914*	785	100.5	101.3
alpha-amylase	76.0	78.7	82.4*	74.9	103.1	106.8
Malting Trait	Progeny Mean	Mid-Parent	Cross Number 60 303		Progeny Mean %	Mid-Parent %
			Kin-dred (P_1)	Dick-too (P_2)		
Extract	71.8	71.8	72.7	70.8	98.7	98.7
Wort Nitrogen	.963	1.01	1.03*	.987	98.0	102.8
Malt Nitrogen	2.71	2.77	2.66*	2.88	105.5	107.8
Diastatic Power	273	259	299*	219	103.0	97.8
beta-amylase	864	793	914*	672	103.0	94.5
alpha-amylase	68.8	74.8	82.4*	62.1	93.4	101.5
Malting Trait	Progeny Mean	Mid-Parent	Cross Number 60 304		Progeny Mean %	Mid-Parent %
			Trail (P_1)	Hudson (P_2)		
Extract	72.5	73.3	73.9*	72.7	99.7	100.8
Wort Nitrogen	.983	.945	.950*	.940*	100.1	96.2
Malt Nitrogen	2.63	2.48	2.38*	2.58*	102.3	96.5
Diastatic Power	263	276	292*	259*	99.3	104.2
beta-amylase	783	845	905*	785	93.2	100.7
alpha amylase	79.4	76.4	77.9*	74.9	107.8	103.7

*The more desirable parental value.

TABLE 5.--Continued

Malting Trait	Progeny Mean	Mid- Parent	Cross Number 59 301		Progeny Mean %	Mid- Parent %
			-568 (P ₁)	Dick- too (P ₂)		
Extract	72.1	71.9	70.6	70.6	100.3	101.8
Wort Nitrogen	.773	.815	.830	.799	88.5	93.3
Malt Nitrogen	2.52	2.53	2.49	2.56	98.4	98.8
Diastatic Power	236	238	277*	199	100.9	101.7
beta-amylase	770	747	878*	615	107.4	104.2
alpha-amylase	54.7	63.4	70.0*	56.8	81.7	94.7
Cross Number 59 303						
Malting Trait	Progeny Mean	Mid- Parent	Cross Number 59 303		Progeny Mean %	Mid- Parent %
			-26 (P ₁)	Dick- too (P ₂)		
Extract	72.2	71.7	72.5*	70.8	99.4	98.7
Wort Nitrogen	806	808	857	759	97.6	97.8
Malt Nitrogen	2.50	2.56	2.58	2.54	101.6	104.1
Diastatic Power	203	214	245*	182	94.0	99.1
beta-amylase	642	685	804	566	95.4	101.8
alpha-amylase	53.4	53.2	55.3	51.0	89.9	89.6
Cross Number 59 304						
Malting Trait	Progeny Mean	Mid- Parent	Cross Number 59 304		Progeny Mean %	Mid- Parent %
			-3584 (P ₁)	Dick- too (P ₂)		
Extract	71.7	72.0	73.1*	70.8	98.7	99.1
Wort Nitrogen	.751	.810	.861*	.759	91.0	98.1
Malt Nitrogen	2.50	2.50	2.46*	2.54	101.6	101.6
Diastatic Power	196	202	221*	182	90.8	93.5
beta-amylase	628	609	651	566	93.3	90.5
alpha-amylase	50.4	60.9	70.8	51.0	84.9	102.6
Cross Number 59 305						
Malting Trait	Progeny Mean	Mid- Parent	Cross Number 59 305		Progeny Mean %	Mid- Parent %
			-2 (P ₁)	Dick- too (P ₂)		
Extract	73.8	72.1	73.4*	70.8	101.6	99.3
Wort Nitrogen	.876	.858	.957	.759	106.1	103.9
Malt Nitrogen	2.56	2.48	2.41	2.54	104.1	100.8
Diastatic Power	202	216	249*	182	93.5	100.0
beta-amylase	587	668	769*	566	87.2	99.3
alpha-amylase	66.2	59.7	68.3*	51.0	111.5	100.5

TABLE 5.--Continued

	Progeny Mean	Mid- Parent	Cross Number 59 306		Progeny Mean %	Mid- Parent %
			-29 (P_1)	Dick- too (P_2)		
Extract	72.3	71.9	73.2*	70.6	100.6	100.0
Wort Nitrogen	.853	.874	.948*	.799	97.7	100.1
Malt Nitrogen	2.66	2.58	2.60*	2.56	103.9	100.8
Diastatic Power	246	233	266*	199	105.1	99.6
beta-amylase	788	735	855*	615	109.9	102.5
alpha-amylase	60.7	60.6	64.4*	56.8	90.6	90.5
Cross Number 59 307						
			Dick-			
			-461 (P_1)	too (P_2)		
Extract	67.5	70.0	69.3	70.6	93.9	97.4
Wort Nitrogen	.978	.832	.866	.799	112.0	95.3
Malt Nitrogen	2.87	2.64	2.72	2.56	112.1	103.1
Diastatic Power	200	203	207	199	85.5	86.8
beta-amylase	535	605	594	615	74.6	84.4
alpha-amylase	79.2	63.9	70.9	56.8	118.2	95.4
Cross Number 59 308						
			Dick-			
			-413 (P_1)	too (P_2)		
Extract	72.7	72.8	75.0*	70.6	101.1	101.3
Wort Nitrogen	.915	.868	.937*	.799	104.8	99.4
Malt Nitrogen	2.59	2.53	2.50*	2.56	101.2	98.8
Diastatic Power	234	236	273*	199	100.0	100.9
beta-amylase	706	722	838*	615	98.5	100.7
alpha-amylase	70.1	67.3	77.8*	56.8	104.7	100.5

TABLE 6.--Average correlation of midparent with progeny mean for 6 malting traits

Cross Number	n	n-3	r	z	(n-3)z Weighted z
59 301	6	3	.911	1.52752	
59 303	6	3	.791	1.07143	
59 304	6	3	.071	.07112	
59 305	6	3	.094	.09024	
59 306	6	3	.896	1.47222	
59 307	6	3	.787	1.04537	
59 308	6	3	-.389	-.41180	
60 301	6	3	.746	.97295	
60 302	6	3	-.024	-.02000	
60 303	6	3	.057	.06007	
60 304	6	3	.171	.17167	
Total		33			18.14937
Average			.500**		.54998

Table 5 contains the quality data for six malting traits for the two parents in each cross, the mid-parent, and the progeny means. Progeny means were calculated by averaging the values obtained from the bulk malts for each of the growth habit classes within a cross. Crosses 60 301, 59 305, and 59 307 did not have sufficient seed to allow malting of bulks with growth habit classes and hence, in these cases the progeny values were determined on a bulk containing samples of each non-segregating row in the cross. The asterisks in Table 5 indicate the more desirable parental value for each of the six traits. In cases where the parental values are equally good both are starred. Most of the spring type parents were superior to Dicktoo for the six traits.

The average mid-parent progeny mean correlation was calculated (Table 6) using the method in Snedecor (28) after first converting the values for the various malt characters to percentages of the mean for the trait for the entire group of malts in a particular lot.

Cold Water Extract as a Measure of Modification

Cold water extract percentages relate well to modification and to overall malt quality in most of the spring lines tested by previous workers (3, 23). The cold temperature (18° C.) at which the extraction is made gives little opportunity for further enzymatic degradation during the extraction process and thus gives a better index of the degree of cytolysis and subsequent modification of the endospermic contents during germination than is obtained from ordinary extract percentages which are determined at much higher temperatures (1).

Figure 2 shows the frequency distribution of cold water extracts in terms of specific gravity for the three growth habit classes, spring, intermediate and winter. The differences between the means of the three groups are within the experimental error and are thus not significant, indicating that winter habit is not detrimental in itself to modification.

Cytase Activity

The relative cytase activity of green malt extract of the selected lines of the progeny cross number 60 302 was the same on the two substrates as shown in Table 7. A correlation of .9666** was obtained for the cytase activities of the lines on the 2 substrates.

Table 7 also shows the relationship between cytase activity of the selected lines on Kindred gum and cold water extract to be $r = .3243$ which is not statistically significant.

Barley Gums

A 0.5% yield of beta-D-glucan containing gum was isolated from the spring variety Kindred. This gum was identical in behavior to that

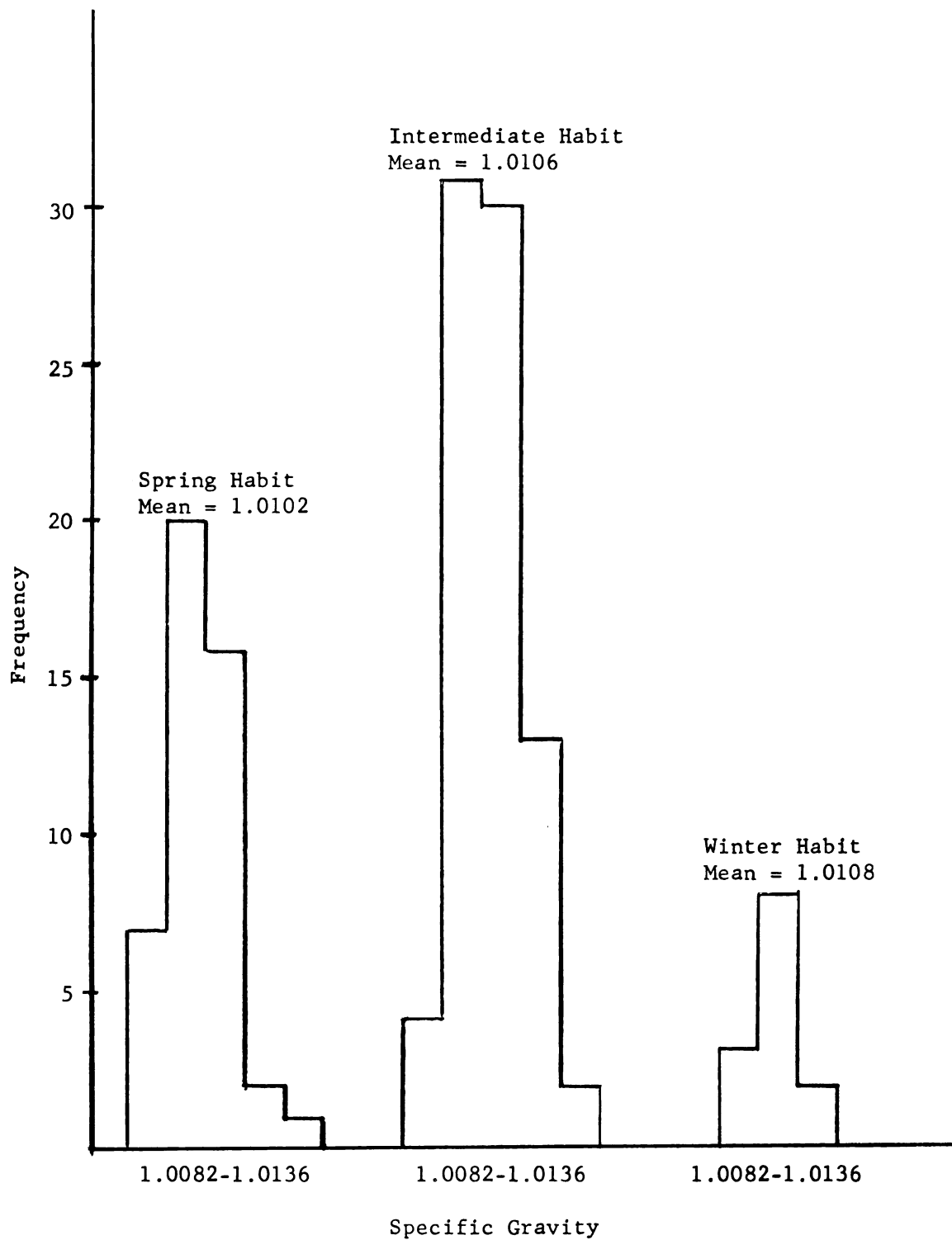


Fig. 2.--Frequency distribution of cold water extract specific gravities for spring, winter, and intermediate growth habits for the progeny of the cross of Hudson x Kindred, number 60 302.

isolated from the spring variety Montcalm by Bass and Meredith (5). The gum solution was highly viscous and its viscosity decreased rapidly when degraded by extracts of green malts. Enzyme activity of extracts of Kindred green malts gave the same order of cytase activity as found by the Canadian workers (3, 4, 5) (Table 7). The gum solution remained stable for over seven days.

TABLE 7. Cytase activity of green malt extracts on Hudson and Kindred gum compared to specific gravity of cold water extracts

Enzyme Source	Cold Water Extract Specific Gravity	Cytase Activity	
		Kindred Gum	Hudson Gum
Hudson	1.0098	.2779	.2304
Kindred	1.0107	.4032	.4560 ^a .3102
I ₅₇ ^b	1.0130	.3734	
I ₆₄	1.0087	.2520	
I ₁₂₀	1.0090	.1799	.1632
I ₁₆	1.0087	.2418	
I ₅₅	1.0088	.3840	.3024
W ₃₇	1.0105	.2878	.2148
W ₁₁₆	1.0114	.2316	.1548
S ₉₈	1.0084	.3168	
r = .3243		r = .9666**	

^aThis cytase activity determined on a 50:50 mixture of the two gums.

^bS, I, W refer to spring, intermediate and winter growth habits.

Gum was also prepared from Hudson a winter variety to discover if it had different properties from that prepared from spring barley. The Hudson gum was isolated with a yield of 1% which was twice as much as that from Kindred. The solution of Hudson gum was much less viscous than that of spring barley gum which indicates a difference in structure

between the two gums.

Cytase activity of green malt extract from both Hudson and Kindred was lower on the Hudson gum than on Kindred gum (Figure 3). In all cases cytase activity was lower on Hudson than on Kindred gum (Table 7) which confirms the graphs in Figure 3. Note the similarity of the slopes of the lines (Figure 3) of the reaction of the green malt extracts on the two substrates.

In order to test for the presence of an inhibitor in Hudson gum, cytase activity of Kindred green malt extract was determined simultaneously on Kindred gum, Hudson gum and a mixture of equal parts of the two gums. The cytase activity of Kindred green malt extract was higher on the gum mixture than either of the other two substrates (Figure 4) indicating that no highly specific inhibitor was present in the Hudson gum. It should be noted that the reaction rates of the Kindred green malt cytase were linear on all three substrates (Figure 4).

Fractional precipitation of water extracts of alcohol refluxed grist of Hudson and Kindred with $(\text{NH}_4)_2\text{SO}_4$ gave further evidence of a structural difference between the two gums. A floating precipitate or pellicle formed for both varieties, but the pellicle formation occurred in Kindred extract at 20% $(\text{NH}_4)_2\text{SO}_4$ concentration and at 30% for Hudson. The pellicle in Kindred extract formed rapidly and was complete and rigid within a few hours. Hudson extract pellicle formed very slowly and was not rigid in structure. Also less pellicle formed in the Hudson extract indicating a lower quantity of glucose polymer or a lesser degree of co-precipitation as compared to Kindred extract. The latter is supported by the results of testing for pentosan content

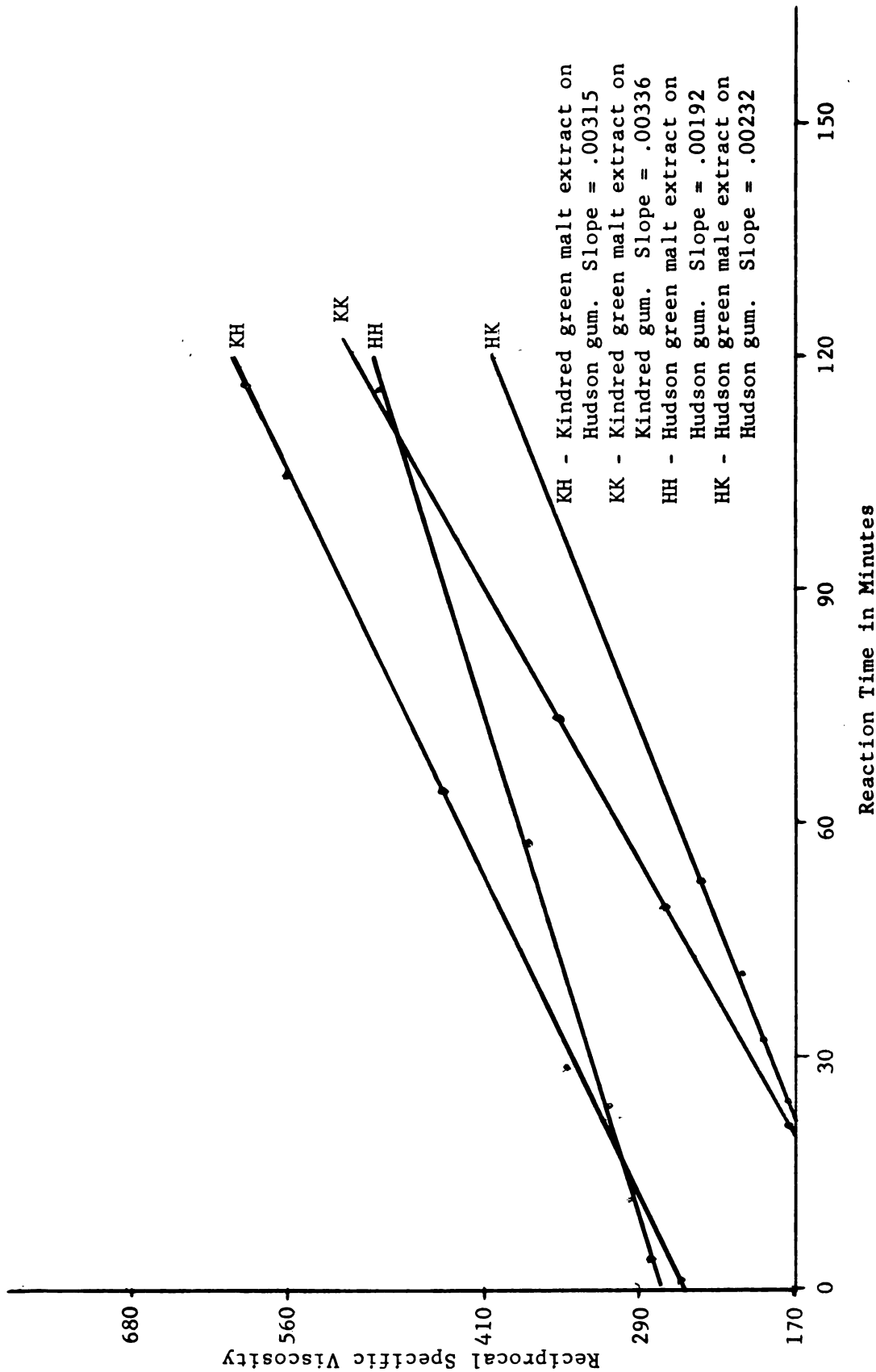


Fig. 3.--Reciprocal specific viscosity plotted against reaction time of Kindred and Hudson green malt extracts on Kindred and Hudson gums.

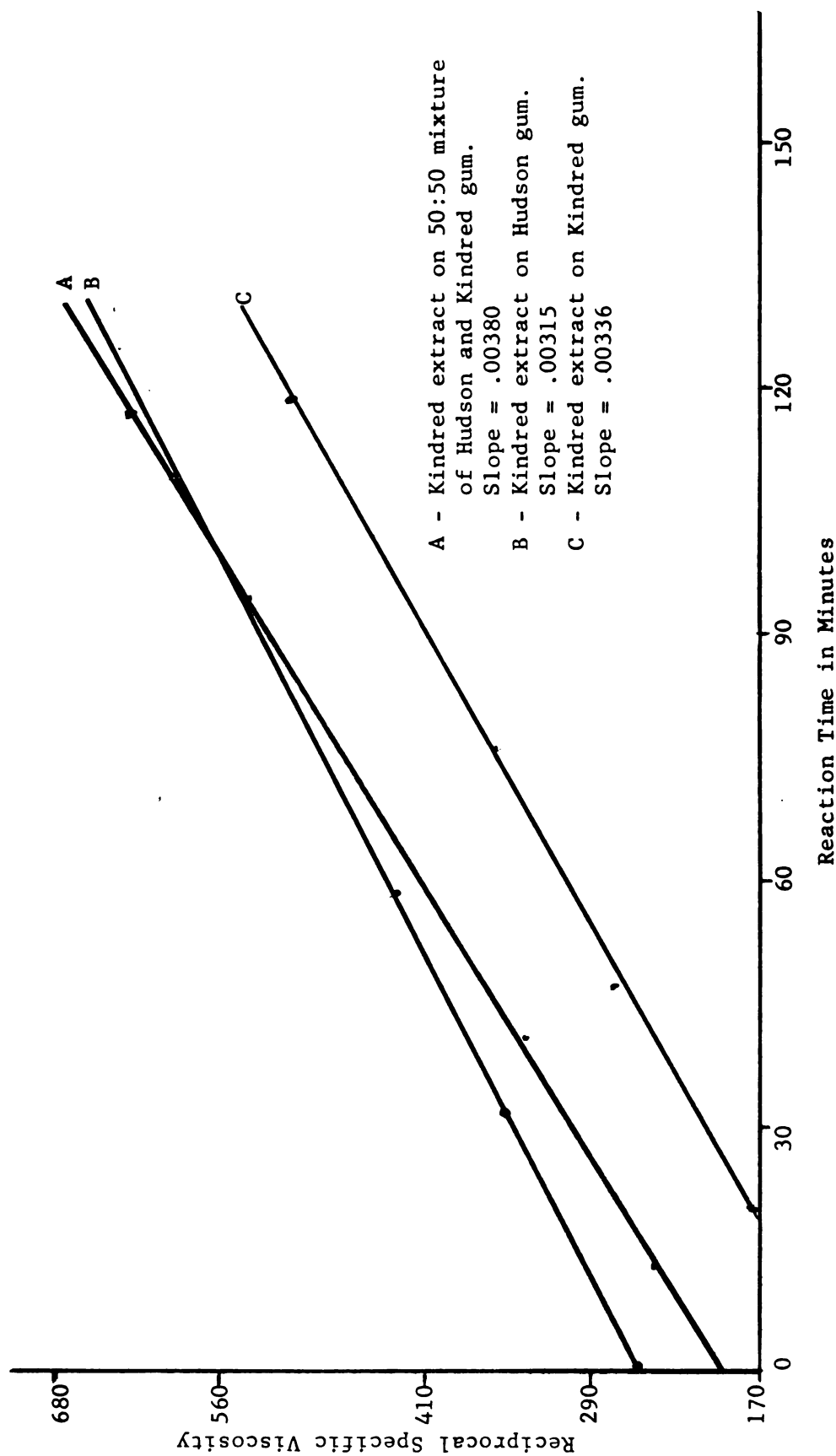


Fig. 4.--Reciprocal specific viscosity plotted against reaction time of Kindred green malt extract on Kindred gum, Hudson gum and a 50:50 mixture of the two gums.

of the dry residue of hydrolysis products from the fractional precipitation obtained using $(\text{NH}_4)_2\text{SO}_4$. More pentosan was found in Kindred pellicle than in Hudson.

The lower initial viscosity of the Hudson gum, the lack of evidence for the presence of some form of inhibition, the different amount of $(\text{NH}_4)_2\text{SO}_4$ to precipitate the pellicle, and the lower cytase activity of all green malt extracts on Hudson gum all indicate that the glucan from Hudson is different in structure from that of Kindred and also that this difference is one of more irregularity of structure in the Hudson polymer.

DISCUSSION

One of the most interesting results of this study was the difference found between Kindred and Hudson gums. Without the supporting evidence of the analysis of the bulk malts (Appendix) which show that there was no apparent association of malt quality and growth habit, and the cold water extracts that indicate the independence of winter habit and modification, it might be suggested that there is something i.e. the gum structure, inherent in winter barleys that would prevent their being accepted for malting. This was not true for Cross Number 60 302, Hudson x Kindred, which was analyzed in this research. The difference between the gums of Kindred and Hudson barley contributes to the complexity of developing high quality malting winter types from such crosses but does not mitigate against the eventual production of such lines.

Since the winter and spring beta-D-glucans were precipitated by different levels of $(\text{NH}_4)_2\text{SO}_4$, it would be fairly simple to test for the type of gum present in larger samples of populations from winter x spring crosses than was done in this thesis by extracting alcohol refluxed grist with water and adding 20% $(\text{NH}_4)_2\text{SO}_4$. If no pellicle formed at 20% then enough additional salt could be added to make the concentration 30% $(\text{NH}_4)_2\text{SO}_4$. This technique would lend itself well to analyzing the genetic control of gum production in populations from crosses involving winter parents.

Gums from both winter and spring types should be purified through

fractional precipitation and crystallization to yield pure beta-D-glucans. These could be used for enzymatic studies of cytase activity as well as for structural analysis of the glucans by means of methylation and periodate degradation. Comparisons should also be made of the physical properties of the purified glucans using osmotic pressure measurements, freezing point depression tests, ultracentrifugation, and viscosity measurements.

The role of pentosans and proteins associated with gums must also be illucidated. Since the beta-D-glucans from spring and winter barleys are different there might also be differences in the pentosans and proteins in each.

Since cytase activity is of a zero order reaction (Figure 1) and thus not a function of substrate concentration the reduction of cytase activity of Hudson gum (Figure 3) must be due to some sort of inhibitive action. Such action could be due to the nature of the gum as indicated by its lower initial viscosity, and the fact that in all cases the enzyme reaction rate is at maximum velocity. It is also possible that complexing of the Hudson gum with other substances occurred as a result of the addition of 30% $(\text{NH}_4)_2\text{SO}_4$ in its preparation. These substances could be other non-starchy polysaccharides such as pentosans as shown in Table 1 or possibly proteins or protein derivatives. A final possibility exists, a specific inhibitor could be present in the Hudson gum.

If the lower cytase activity of green malt extracts on Hudson gum were due to a complexing of the Hudson polymer with a pentosan in some way that the pentosan covered or protected sites on the glucan molecule from enzymic rupture, a sharp decrease in reaction rate on

the mixture of the gums would be expected. Such an effect would occur at low concentration of pentosan and increased amounts of the protective substance would not give increased lowering of enzymatic activity because there are limited numbers of sites within the polymer which can be attacked.

If the lowering of cytase activity on Hudson gum were due to an additive type of interference the reaction rate of the green malt extract on the mixture of the two gums should have fallen at the mean of the cytase activity on the two pure gum substrates.

The reaction rates of the Kindred green malt cytase were linear for all three reaction mixtures (Figure 4). If the lower activity on Hudson gum was due to the presence of a substance which had only a small effect at low concentrations and the effect increased exponentially with increasing concentration of the inhibitive substance the curve for cytase activity on the mixture of the two gums should have left a zero order type because as the reaction progressed the relative concentration of inhibitor would rise in the reaction mixture.

The cytase activity of Kindred green malt extract was higher on the gum mixture than either of the other substrates (Figure 4) indicating that no highly specific inhibitor was present in the Hudson gum. The reason for the higher cytase activity on the gum mixture is not clear. The result cannot be explained on the basis of the presence of other enzymes in the green malt extract simultaneously degrading substances other than beta-D-glucan because this same effect would be present when using the individual gums alone. Perhaps it is due to some interaction of the two gums which makes the mixture more easily attacked by the enzyme. Replication of this result is necessary before

its real meaning can be determined.

The simplest explanation of the lowered activity of green malt extract cytases on Hudson gum is that the Hudson polymer is more irregular in structure than that isolated from Kindred. The irregularity of structure of the gum of Hudson barley may not exist in all winter types. It would be interesting to isolate gums from other winter varieties and analyze them for such differences.

The lack of association of cold water extract and cytase activity (Table 7) is not in agreement with Bass, Bendelow, and Meredith (3) who found a highly significant relationship between these two phenomena in spring barleys. Perhaps this lack of agreement is due to the fact that the lines tested are from a winter x spring cross in which recombinations occur that are not common to progenies from spring x winter crosses.

Improved lines of hardy winter barley that have malting properties approaching a spring barley quality pattern have been produced at Michigan State University. These lines contain no spring barley germ plasm, but have been produced by recurrent selection and vector technique for parental choice (13). Gum analysis of these lines may provide some fascinating insights into the effects of selection and genetic recombination on the structure and types of gums present.

From the standpoint of malting, there is no apparent reason for preventing the use of improved winter barleys. Winter barleys, up to 20%, are being used in Canada in blends for malting. Malting of winter barleys may require some adjustment in technique on the part of the maltster as was the case with the micro-malting of the winter bulks

used to standardize the micro-malting equipment and procedures utilized in this study.

The micro-malts exhibited very high levels of modification thus indicating that with proper technique winter barleys can be successfully malted.

Acceptance of winter barley malts by brewers will depend on the quality of beer which can be made from them. A few micro and pilot scale brewing tests of winter barleys have been made in the past and the results have been inconclusive. Work on improving winter lines for better brewing properties must be intensified to determine if such lines are possible of development.

SUMMARY AND CONCLUSIONS

1. Micromalting of bulks within growth habit classes has been shown to be of use for screening early generation progenies of crosses made for malting quality.

2. Winter growth habit has been shown to be independent of any association detrimental to malting quality of eleven spring x winter barley crosses.

3. Modification of progeny lines of a spring x winter cross appears to be well independent of cytase activity on Kindred gum.

4. Polysaccharide gums isolated from Kindred and Hudson barleys have been shown to have structural differences which effect cytase activity of green malt extracts reacted on these gums.

5. The structural difference between the gums is in the direction of more irregularity of the molecule on the part of the gum isolated from the winter variety Hudson as shown by differences in viscosity, fractional precipitation, and lower cytase activity of green malt extracts on Hudson gum.

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APPENDIX

The second major objective of this study was to develop micro-malting equipment capable of handling large numbers of early generation samples. Seed supplies of such material are generally very small and this was also taken into consideration.

The equipment designed in this study was built to accommodate malting trays of stainless steel which are capable of handling 20 grams of each of 100 lines of barley at a time. Trays are divided into 100 2" x 2" x 3" compartments and have stainless steel screen covering the bottom of the entire tray. This design allows malt to be produced under conditions similar to commercial pneumatic malting. The 3 pieces of equipment are: steep tank, germinator, and kiln. The steep tank is of heavy #16 galvanized metal and is large enough to hold one tray. The tray is supported on hangers so that it is about 4 inches off the bottom of the tank. Two methods of steeping were used. With spring barleys 20 grams of grain were placed in each compartment of the tray which was covered with a piece of screen to prevent seeds from floating out and becoming mixed. The covered tray was then lowered into the steep tank. With winter barleys, malted in test tubes and cottage cheese dishes, 20 grams of grain were placed in small cheesecloth bags, one bag per cubicle. The bags facilitated the transfer of the steeped grain from the tray to the test tubes and cottage cheese dishes. The steep tank was maintained at 16° C. in the germinator. In the early phases of the work the steep tank temperature was maintained by running

tap water through the grain. This proved to be unsatisfactory as the temperature of the tap water was not sufficiently uniform.

The germinator is a large plywood box with a hinged cover. It is equipped with a double squirrel cage fan which blows air taken into the box from the room over a refrigerator coil. After leaving the refrigeration coil the air flows over a furnace humidifier which saturates the air with water. The saturated air is evenly distributed by means of internal baffles in the box and passes up through the bottom of the tray and out a 6" hole in the back of the cover. The refrigeration coil is connected to a compressor through an evaporator pressure regulating valve. The compressor runs continuously and the coil temperature is regulated by setting the evaporator pressure valve. Two heaters are used; a 600 watt cone heater located at the air intake of the fan controlled by a Fenwal thermostat, and an electric stove element located under the water reservoir of the humidifier controlled by a variable transformer. Heating the water in the reservoir produces finer droplet size from the mechanical disperser as well as warming the air. The various controls make this unit highly flexible.

The kiln is also a plywood box equipped with a squirrel cage fan that blows air over a group of thermostatically controlled cone heaters and up through the grain which is in a tray sitting on the top of the kiln.

Originally these 3 pieces of equipment were standardized using a lot of plump, bright Traill barley furnished by Dr. A. D. Dickson of the USDA Barley and Malt Laboratory. This sample of Traill had been malted and evaluated at the Barley and Malt Laboratory and was of excellent quality. The malting schedules used in this work are shown in Table I.

TABLE I.--Comparison of malting schedules used for spring and winter barleys malted in micro malting equipment at Michigan State University.

	Spring Barley	Winter Barley
Steeping		
temperature control	running tap water	germinator
	15 - 20° C.	16° C.
time	48 hrs.	40 hrs.
final moisture	42 - 44%	42 - 44%
Germination		
temperature	16° C.	16° C.
time	2-1/2 days	8 days
container	compartmented trays	test tubes and cottage cheese dishes
Kilning		
time and temperature	24 hrs. at 45° C.	24 hrs. at 45° C.
	8 hrs. at 55° C.	8 hrs. at 55° C.

Three lots of Traill were malted according to the spring schedule in Table I. Lot 3 was deliberately oversteeped (60 hrs.). Two samples of each of the three lots were analyzed by the Barley and Malt Laboratory (Tables II and III). The quality of Lot 3 was lower than Lots 1 and 2 which were satisfactory.

The results of malting winter barley lines and growth habit bulks of crosses (Tables IV and V) indicated that changes in technique were needed. A bulk lot containing more than 40 lines of winter barley was germinated according to the two schedules in Table I. Both samples of the bulk were steeped for 40 hours in cheesecloth bags. Part of the grain was placed in the compartments of the malting tray and germinated for 3 days, the remainder being placed in large (5" x 1") test tubes, 30 grams of steeped grain per tube. The tubes were equipped with single hole rubber stoppers and germination was allowed to proceed for 8 days. This follows the method of Whitemore and Sparrow (31). Both samples

TABLE II.--Traill malt physical analysis on samples malted at East Lansing, Michigan in 1961^a

Lot sample no.	Wt. un- cleaned	Wt. cleaned	Root- let loss	H ₂ O %	Kwt mgm as is	Kwt mgm D.B.	Growth of acrospire				Over	Growth index
							0-1/4	1/4-1/2	1/2-3/4	3/4-1		
1-1	112.3	105.4	6.2	8.3	30.9	28.3	0	6	7	76	11	95.25
1-2	114.3	109.2	4.5	7.0	30.9	28.8	2	7	8	82	1	93.0
2-1	110.5	104.9	4.7	6.1	30.1	28.2	6	10	7	63	14	88.75
2-2	104.4	99.5	4.7	6.0	30.8	29.0	2	10	14	72	2	90.0
3-1	104.1	100.9	3.1	6.1	30.8	28.9	7	6	19	67	1	87.0
3-2	113.0	109.3	3.3	5.9	31.5	29.7	5	8	16	71	0	88.25

^aQuality data furnished by Dr. A. D. Dickson, USDA Barley and Malt Laboratory. Madison, Wisconsin.

TABLE III.--Traill malt chemical analysis on samples malted at East Lansing, Michigan in 1961^a

	Malt mois- ture %	Extract dry basis %	Con- version time min.	Color Lov. 52	Clarity of wort	Wort N. %	Malt N. total %	Ratio wort malt N. %	Dia- static power °L	Beta- amylase maltose equiv.	Alpha- amylase 20° dext. units	Ratio beta alpha- amylase
Traill Lot 1												
Sample 1	8.3	75.8	<5	1.6	Clear	.944	1.98	47.7	231	765	46.6	16.4
Traill Lot 1												
Sample 2	7.0	75.2	<5	1.5	Clear	.901	2.02	44.6	229	772	42.2	18.3
Traill Lot 2												
Sample 1	6.1	75.6	<5	1.6	Clear	.916	2.04	44.9	241	796	53.2	15.0
Traill Lot 2												
Sample 2	6.0	75.5	<5	1.6	Clear	.943	2.01	46.9	239	792	51.7	15.3
Traill Lot 3												
Sample 1	6.1	73.3	7-10	1.4	Hazy	.705	2.03	34.7	190	690	23.0	30.0
Traill Lot 3												
Sample 2	5.9	73.8	7-10	1.4	Hazy	.752	2.06	36.5	196	709	24.5	28.9

^aQuality data furnished by Dr. A. D. Dickson, USDA Barley and Malt Laboratory, Madison, Wisconsin.

TABLE IV.--Kernel weights and growth of malts prepared at Michigan State University from bulk barley selections and check varieties^a

Variety	Moisture of dried malt	Kernel weight of malt D.B.	Growth of Malt				Overgrown	Growth index
			0-1/4	1/4-1/2	1/2-3/4	3/4-1		
59-302								
A	7.1	27.9	17	24	10	49	0	72.75
B	7.1	28.2	11	14	17	54	4	80.50
C	7.3	28.5	8	19	17	56	0	80.25
D	7.3	29.1	13	26	17	43	1	73.00
59-303								
A	7.3	28.7	25	25	11	39	0	66.00
B	7.4	28.9	14	27	10	49	0	73.50
C	7.1	30.9	15	25	12	48	0	73.25
D	7.3	29.3	16	12	7	64	1	80.25
59-304								
A	7.2	27.0	23	13	14	46	4	72.75
B	7.2	28.7	12	17	10	61	0	80.00
C	7.1	28.1	16	19	19	45	1	73.75
D	7.2	30.1	18	12	19	48	3	75.75
Hudson	7.1	28.2	8	7	22	59	4	85.00
Dicktoo	7.3	28.2	22	34	13	31	0	63.25
Trail	7.1	30.5	27	16	14	43	0	68.25
Kindred	7.3	29.1	19	28	19	32	2	67.00

^aQuality data furnished by Dr. A. D. Dickson, USDA Barley and Malt Laboratory, Madison, Wisconsin.

TABLE V.--Malt quality for bulk barley selections grown and malted at East Lansing, Michigan^a

Variety	Growth Habit Group	Malt moisture %	Malt extract dry %	Conversion time min.	Wort color Olov 52	Clarity of wort	Wort N %	Malt N %	Wort N malt N %	Dia-static power degrees	Beta-amylase maltose equiv.	Alpha-amylase 200dext units	Ratio beta alpha-amylase
59-302	A	7.1	67.2	10-15	1.4	Hazy	.592	2.64	22.4	170	633	14.7	43.0
59-302	B	7.1	67.7	10-15	1.4	Hazy	.633	2.59	24.4	181	679	14.1	48.2
59-302	C	7.3	67.7	10-15	1.2	Hazy	.590	2.58	22.9	164	623	11.2	55.6
59-302	D	7.3	67.3	10-15	1.3	Hazy	.566	2.65	21.4	175	659	12.9	51.1
59-303	A	7.3	64.8	10-15	1.2	Hazy	.507	2.75	18.4	167	639	9.1	70.2
59-303	B	7.4	65.2	10-15	1.3	Hazy	.510	2.73	18.7	165	629	10.4	60.5
59-303	C	7.1	65.4	10-15	1.2	Hazy	.512	2.69	19.0	165	632	8.9	71.0
59-303	D	7.3	64.2	10-15	1.2	Hazy	.525	2.78	18.9	165	629	9.8	64.2
59-304	A	7.2	68.0	10-15	1.2	Hazy	.566	2.58	21.9	179	659	17.0	38.8
59-304	B	7.2	68.2	10-15	1.2	Hazy	.562	2.59	21.7	165	592	19.9	29.7
59-304	C	7.1	68.0	10-15	1.2	Hazy	.593	2.55	23.3	159	551	24.7	22.3
59-304	D	7.2	67.2	10-15	1.2	Hazy	.524	2.63	19.9	152	574	11.4	50.4
Hudson		7.1	69.2	10-15	1.1	Hazy	.562	2.55	22.0	164	599	17.2	34.8
Dicktoo		7.3	66.7	10-15	1.3	Hazy	.544	2.70	20.1	140	516	14.0	36.9
Traill		7.1	67.6	10-15	1.2	Hazy	.533	2.39	22.3	184	705	10.4	67.8
Kindred		7.3	66.5	15-20	1.1	Hazy	.518	2.62	19.8	164	638	6.1	104.6

^aQuality data furnished by Dr. A. D. Dickson, USDA Barley and Malt Laboratory, Madison, Wisconsin.

were kilned according to the same schedule (Table I). The results (Table VI) indicate a much higher level of modification using the test tubes despite the fact that the growth indices were of the same order.

A modification of the method of Whitmore and Sparrow (31) involving covered plastic cottage cheese dishes was used to malt larger samples of parental materials and bulk samples of groups of lines within growth habit categories in a cross. Four holes were punched in the sides of the plastic dishes just below the rim. The covers were not punched. These dishes were used to malt samples of the 40 line winter barley bulk used above as a standard check for each lot of malt. Quality evaluations of malts produced with this method are presented in Tables VII, VIII, and IX.

TABLE VI.--Comparison of 2 samples of a bulk of winter varieties malted at high levels of aeration and moisture and at low levels of aeration and moisture^a

	Sample No. 1 High Aeration High Moist	Sample No. 2 Low Aeration Low Moist
Malt Moisture %	5.1	4.8
Extract Dry Basis %	72.0	73.6
Time of Conversion min.	7-10	5-7
Color (Lov. 52)	1.6	1.8
Clarity of Wort	Hazy	Clear
Wort Nitrogen %	.616	.740
Malt Nitrogen, Total %	1.93	1.93
Ratio, SN/TN Nitrogen %	31.9	38.3
Diastatic Power, Deg.	123	161
Diast, Power Maltose EQ.	.492	.644
B-Amylase Maltose Equiv.	428	530
-Amylase Maltose Equiv.	64	114
-Amylase 20° dext units	19.1	34.8
Ratio, B/ -Amylase	22.4	15.2
Growth Index	85	89

^aQuality data furnished by Dr. A. D. Dickson, USDA Barley and Malt Laboratory, Madison, Wisconsin.

TABLE VII.--Quality evaluation of bulk malts of growth habit classes, and parental lines of crosses 60 301, 60 302, 60 303, 60 304, and a standardized check sample of a bulk of 40 winter varieties

Sample ^a	Malt moisture %	Malt extract (dry) %	Time of conversion min.	Wort color olov 52	Clarity of wort	Wort N %	Malt N %	Wort N malt N %	Dia-static power degrees	Beta-amylase maltose equiv.	Alpha-amylase 20° dext units	Ratio beta alpha amylase
60 301-Bulk	5.2	72.4	<5	2.0	Clear	1.10	2.74	40.1	259	802	71.1	11.3
60 302-S	4.7	72.9	<5	1.7	Clear	1.01	2.60	38.8	265	806	76.0	10.6
60 302-W	4.6	71.5	<5	1.9	Clear	1.04	2.54	40.9	283	878	75.9	11.6
60 302-I	5.0	72.9	<5	1.9	Clear	1.12	2.66	42.1	275	845	76.2	11.1
60 303-S	5.0	70.8	<5	1.7	Clear	1.01	2.78	36.3	282	877	74.9	11.7
60 303-W	5.1	72.4	<5	1.6	Clear	.923	2.67	34.6	260	819	66.4	12.3
60 303-I	4.9	72.1	<5	1.6	Clear	.957	2.67	35.8	278	895	65.2	13.7
60 304-S	5.0	72.4	<5	1.8	Clear	.994	2.60	38.2	262	775	80.8	9.6
60 304-W	4.8	72.1	<5	1.7	Clear	1.01	2.69	37.5	275	818	82.4	9.9
60 304-I	4.9	73.0	<5	1.6	Clear	.946	2.59	36.5	251	754	74.9	10.1
Montcalm	5.1	74.4	<5	1.7	Clear	.977	2.42	40.4	285	910	69.8	13.0
Kindred	4.9	72.7	<5	1.8	Clear	1.03	2.66	38.7	299	914	82.4	11.1
Trail	5.1	73.9	<5	1.6	Clear	.950	2.38	39.9	292	905	77.8	11.6
RRWB	4.9	73.4	<5	1.9	Clear	.834	2.08	40.1	228	912	74.9	9.5
Dicktoo	5.0	70.8	<5	1.8	Clear	.987	2.88	34.3	219	672	62.1	10.9
Hudson	5.0	72.7	<5	1.8	Clear	.940	2.58	36.4	259	785	74.9	10.5

^aS = Spring, W = Winter, I = Intermediate.

TABLE VIII.--Quality evaluation of bulk malts of growth habit classes, and parental lines of crosses 59 302, 59 303, 59 304, 59 305, and a standardized check sample of a bulk of 40 winter varieties

Sample ^a	Malt moisture %	Malt extract (dry) %	Time of conversion min.	Wort color olov 52	Clarity of wort	Wort N %	Malt N %	Wort N malt N %	Dia-static power degrees	Beta-amylase maltose equiv.	Alpha-amylase 20° dext units	Ratio beta alpha amylase
-2	4.9	73.4	<5	1.9	Clear	.957	2.41	39.7	249	769	68.3	11.3
-26	4.8	72.5	<5	1.7	Clear	.857	2.58	33.2	245	804	55.3	14.5
-568	5.1	72.7	<5	1.7	Clear	.822	2.55	32.2	268	863	64.3	13.4
-3584	4.8	73.1	<5	1.6	Clear	.861	2.46	35.0	221	651	70.8	9.2
59 302-I	5.0	72.7	<5	1.7	Clear	.734	2.49	29.5	202	665	46.4	14.3
59 302-S	4.9	72.3	<5	1.6	Clear	.755	2.48	30.4	205	662	50.5	13.1
59 302-W	5.0	72.8	<5	1.7	Clear	.755	2.43	31.1	204	654	51.8	12.6
59 303-I	5.1	72.8	<5	1.7	Clear	.758	2.41	31.5	221	715	54.0	13.2
59 303-S	5.1	72.3	<5	1.7	Clear	.785	2.63	29.8	174	534	51.8	10.3
59 303-W	4.8	71.6	<5	2.0	Clear	.877	2.48	35.4	213	680	54.6	12.5
59 304-I	4.9	71.6	<5	1.5	Clear	.748	2.50	29.9	232	760	53.8	14.1
59 304-S	4.9	71.9	<5	1.5	Clear	.751	2.54	29.6	183	578	49.2	11.7
59 304-W	4.9	71.7	<5	1.6	Clear	.755	2.48	30.4	174	547	48.2	11.3
59 305-Sng.	4.9	73.8	<5	1.7	Clear	.876	2.56	34.2	202	587	66.2	8.9
Dicktoo	4.7	70.8	<5	1.6	Clear	.759	2.54	29.9	182	566	51.0	11.1
RRWB	4.5	73.7	<5	2.0	Clear	.823	2.03	40.5	194	560	64.9	8.6

^aS = Spring, W = Winter, I = Intermediate.

TABLE IX.--Quality evaluation of bulk malts of growth habit classes, and parental lines of crosses 59 301, 59 306, 59 307, 59 308, and a standardized check sample of a bulk of 40 winter varieties

Sample ^a	Malt moisture %	Malt extract (dry) %	Time of conversion min.	Wort color olov 52	Clarity of wort	Wort N %	Malt N %	Wort N malt N %	Dia-static power degrees	Beta-amylase maltose equiv.	Alpha-amylase 20° dext units	Ratio beta alpha amylase
59 301-I	5.1	72.2	<5	1.6	Clear	.782	2.56	30.5	242	787	56.9	13.8
59 301-W	5.1	71.8	<5	1.6	Clear	.782	2.59	30.2	241	795	54.0	14.7
59 301-S	5.2	72.6	<5	1.5	Clear	.756	2.43	31.1	225	731	53.4	13.7
59 306-I	5.1	72.3	<5	1.6	Clear	.815	2.65	30.8	235	731	63.2	11.6
59 306-W	5.1	72.9	<5	1.6	Clear	.869	2.71	32.1	269	872	62.2	14.0
59 306-S	5.0	71.9	<5	1.7	Clear	.876	2.64	33.2	236	763	56.8	13.4
59 308-I	5.0	73.8	<5	1.7	Clear	.919	2.51	36.6	231	686	72.2	9.5
59 308-W	4.8	71.8	<5	1.9	S1 Hazy	.993	2.65	37.5	242	714	76.1	9.4
59 308-S	5.2	72.8	<5	1.7	Clear	.835	2.62	31.9	231	719	62.2	11.6
59 307-Sng.	4.8	67.5	<5	1.9	S1 Hazy	.978	2.87	34.1	200	535	79.2	6.8
Dicktoo	5.0	70.6	<5	1.6	Clear	.799	2.56	31.2	199	615	56.8	10.8
Rod Row	4.7	73.5	<5	1.9	Clear	.828	2.06	40.2	203	596	65.1	9.2
-29	5.3	73.2	<5	1.8	Clear	.948	2.60	36.6	266	855	64.4	13.3
-413	5.2	75.0	<5	1.7	Clear	.937	2.50	37.5	273	828	77.8	10.6
-461	5.0	69.3	<5	1.7	S1 Hazy	.866	2.72	31.8	207	594	70.9	8.4
0568	5.3	73.2	<5	1.7	Clear	.830	2.49	33.3	277	878	70.0	12.5

^aS = Spring, W = Winter, I = Intermediate.

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