The effect of the waxy endosperm, short awn, and hulless seed genes upon the biochemical and physiological seed characteristics important in barley (Hordeum vulgare L.) utilization
by Gregory Joseph Fox

A thesis submitted in partial fulfillment for the requirements for the degree of DOCTOR OF PHILOSOPHY in CROP AND SOIL SCIENCE
Montana State University
© Copyright by Gregory Joseph Fox (1981)

Abstract:
The effect of the waxy endosperm, short awn, and hulless seed genes upon alpha-amylase activity in ungerminated seed, starch digestibility, starch and protein content, beta-glucan viscosity, and kernel weight was examined in the cultivars 'Compana', 'Titan', and 'Betzes'.

A gel-diffusion assay for alpha-amylase, based on a chromogenic substrate (amylose-azure blue) suspended in an agar medium was developed. Activity was measured as the diameter of digestion rings around sample wells into which a flour paste or enzyme extract was placed. The area of the digestion rings was proportional to the log10 of actual enzyme activity (r = .99**). Hundreds of samples could be assayed daily.

No unusual amount of alpha-amylase in nongerminated seed was found in any isotype within any cultivar. Some activity was found in all 50 barley cultivars and isotypes tested. No unique isozymes for alpha- and beta-amylase were found in any of the waxy, short-awn, hulless isotypes of Compana. However, the presence of the waxy gene was found to increase starch digestability. This was evident when upon malting, waxy isotypes produced higher levels of reducing substances (maltose) at a more rapid rate than normal isotypes.

Kernel weights were reduced for all mutant isotypes while beta-glucan viscosity was increased. In nonwaxy isotypes, these increases were proportional to the decreases observed for kernel weight. Waxy isotypes had viscosity levels significantly higher than that predicted by kernel weight reductions indicating they were accumulating beta-glucans beyond the simple concentration effect of reduced kernel weight. Waxy isotypes had lower extract (starch content) and higher protein.

All isotypes responded across environments and genotypes in a relatively uniform manner for extract and protein. Regression analysis demonstrated that the background genotype and environment can modify the kernel weight and beta-glucan response of a particular isotype. When tested in the Brabender Amylograph, waxy, waxy hulless, waxy short-awn, and waxy short-awn hulless isotypes within Titan, Betzes, and Compana pasted in the same temperature range (65-75 C). Pasting peak viscosity was variable, but related to alpha-amylase activity and beta-glucan viscosity of the barley flour samples (r = .96**).

THE EFFECT OF THE WAXY ENDOSPERM, SHORT AWN, AND HULLESS SEED GENES UPON BIOCHEMICAL AND PHYSIOLOGICAL SEED CHARACTERISTICS IMPORTANT IN BARLEY (*HORDEUM VULGARE* L.) UTILIZATION

by

GREGORY JOSEPH FOX

A thesis submitted in partial fulfillment
for the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

CROP AND SOIL SCIENCE

Approved:

Robert F. Eslick
Chairperson, Graduate Committee

Dwane H. Miller
Head, Major Department

Graduate Dean

MONTANA STATE UNIVERISTY
Bozeman, Montana

December, 1981

ACKNOWLEDGEMENTS

I would like to thank the following people and agencies for technical contributions made to this project:

Professor R. F. Eslick and Dr. E. A. Hockett, who provided the isogenic barley lines essential for the studies contained in this dissertation.

Dr. Sandy MacGregor, who performed the IDC alpha-amylase analyses in Chapters 3 and 4, and the alpha- and beta-amylase isozyme analysis (isoelectric focusing) in Chapter 4.

Mrs. Bernice DeHaas, for performing the CBA alpha-amylase assays in Chapters 3 and the 3,5-dinitrosalicylic acid maltose assays in Chapter 4.

Dr. Wynn Raymond, who performed the Brabender Amylographs in Chapter 8.

The Madison Malt Laboratory, for providing micro-malt analyses in Chapter 5.

This research was supported in part by the Montana Wheat Research and Marketing Committee.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	ix
LIST OF FIGURES	xiii
ABSTRACT	xix
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	3
The Use of Washonupana Barley in High Maltose Syrup Production	3
The Pasting Characteristics of Starch as Measured by the Brabender Amylograph	5
Genetic Components of Washonupana Barley	6
Waxy Endosperm	7
Short Awn	11
Hulless Seed	12
Alpha-Amylase in Barley	13
Beta-Glucan in Barley	17
Starch and Protein Accumulation in Developing Barley	23
The Effect of Environment on Genotypes and Isotypes	25
Isotypes and Name Coding	27
CHAPTER 3: RAPID DETERMINATION OF CEREAL ALPHA-AMYLASE USING AMYLOSE-AZURE BLUE AGAR PLATES	30
Introduction	30
Materials and Methods	30
General	30
Study I: The BPA Assay vs. the Cibacron Blue-Amylose Dye Release Assay	32

Page

Study II: The BPA Assay vs. the Iodine Dextrin Color Loss Method 33
Study III: The BPA Assay vs. Degrees Lintner Reducing Power of Malt Extract 33
Study IV: Among Plate Variability of the BPA Assay 34
Study V: The Effect of Time and Temperature on the Ability of the BPA Assay to Measure Alpha-Amylase 35
Study VI: Alpha-Amylase Activity Measured by the BPA Assay vs. Germination Time 35
Study VII: Alpha-Amylase Activity Measured by the BPA Assay vs. Percent Sprouted Seed. . 36
Study VIII: The Use of the BPA Assay in Distinguishing Between the Malt Alpha-Amylase Activity of Feed and Malting Barley 37
Results and Discussion 38
General 38
Study I: The BPA Assay vs. the Cibacron Blue-Amylose Dye Release Assay 39
Study II: The BPA Assay vs. the Iodine Dextrin Color Loss Method 45
Study III: The BPA Assay vs. Degrees Lintner Reducing Power of Malt Extract 45
Study IV: Among Plate Variability of the BPA Assay 52
Study V: The Effect of Time and Temperature on the Ability of the BPA Assay to Measure Alpha-Amylase 55
Study VI: Alpha-Amylase Activity Measured by the BPA Assay vs. Germination Time . . 66
Study VII: Alpha-Amylase Activity Measured by the BPA Assay vs. Percent Sprouted Seed. . 66
Study VIII: The Use of the BPA Assay in Distinguishing Between the Malt Alpha-Amylase Activity of Feed and Malting Barley 70
Summary 73

CHAPTER 4: THE CHARACTERIZATION OF ALPHA-AMYLASE IN NON-GERMINATED GRAIN SAMPLES OF WASHONUPANA AND OTHER BARLEYS 75

	Page
Introduction	75
Materials and Methods	76
Study I: Alpha-Amylase Activity in Washonupana vs. Compana	76
Study II: Isozyme Analysis for Alpha- and Beta-Amylase Based Upon Isoelectric Focusing of Enzymes Derived from Washonupana Barley Grown at 11 Locations	77
Study III: Comparison of Washonupana Barley with Other Waxy, Short Awn, Hulless Isotypes of Compana Using the Isozyme Analysis for Alpha- and Beta-Amylase	77
Study IV: Alpha-Amylase Activity vs. Starch Digestibility in Waxy Isotypes	78
Study V: Alpha-Amylase Activity of 50 Barley Cultivars	79
Results and Discussion	80
Study I: Alpha-Amylase Activity in Washonupana vs. Compana	80
Study II: Isozyme Analysis for Alpha- and Beta-Amylase Based Upon Isoelectric Focusing of Enzymes Derived from Washonupana Barley Grown at 11 Locations	85
Study III: Comparison of Washonupana Barley with Other Waxy, Short Awn, Hulless Isotypes of Compana Using the Isozyme Analysis for Alpha- and Beta-Amylase	85
Study IV: Alpha-Amylase Activity vs. Starch Digestibility in Waxy Isotypes	91
Study V: Alpha-Amylase Activity of 50 Barley Cultivars	100
Summary	104
CHAPTER 5: THE EFFECT OF WAXY ENDOSPERM UPON MALTOSE PRODUCTION IN MALTED BARLEY	106
Introduction	106
Materials and Methods	107
General	107
Study I: The Influence of Germination Interval Upon Maltose Production in Waxy vs. Non-Waxy Compana	107

Page

Study II: The Influence of Germination Interval Upon Maltose Production in Waxy vs. Nonwaxy Isotypes of Three Cultivars . . . 109
Study III: The Influence of Waxy vs. Nonwaxy Endosperm in Three Cultivars Upon Maltose Production Within Finished Malt 110
Results and Discussion 112
Study I: The Influence of Germination Interval Upon Maltose Production in Waxy vs. Nonwaxy Compana 112
Study II: The Influence of Germination Interval Upon Maltose Production in Waxy vs. Nonwaxy Isotypes of Three Cultivars . . . 117
Study III: The Influence of Waxy vs. Nonwaxy Endosperm in Three Cultivars Upon Maltose Production Within Finished Malt 119
Summary 128

CHAPTER 6: THE EFFECT OF THE WAXY, SHORT-AWN, AND HULLESS GENES UPON SEED QUALITY CHARACTERISTICS IN THREE CULTIVARS 130

Introduction 130
Materials and Methods 131
Isotypes 131
Commercial Check Cultivars 131
Experimental Design and Analysis 133
Quality Determinations 135
Kernel Weight 135
Beta-Glucan Viscosity 136
Percent Protein 137
Percent Extract 137
Alpha-Amylase Activity 138
Results and Discussion 138
Analysis of Variance 138
Comparison of Means 144
Kernel Weight 144
Beta-Glucan Viscosity 148
Relationship Between Kernel Weight and Beta-Glucan Viscosity 152
Percent Protein 158

Page

Percent Extract 162
Alpha-Amylase Activity 165
Summary 171

CHAPTER 7: THE EFFECT OF ENVIRONMENT AND ISOTYPE UPON SEED QUALITY CHARACTERISTICS IN THREE CULTIVARS 174

Introduction 174
Materials and Methods 175
Results and Discussion 177
Interpretation of the Regression Analysis 177
Kernel Weight 180
Beta-Glucan Viscosity 186
Percent Extract 196
Alpha-Amylase Activity 199
Summary 199

CHAPTER 8: A RAPID SCREENING METHOD FOR BARLEY CULTIVARS SUITABLE FOR SYRUP PROCESSING 203

Introduction 203
Materials and Methods 203
Results and Discussion 205
Pasting Peak Temperature and Viscosity 205
Association Between Pasting Peak, Beta-Glucan Viscosity, and Alpha-Amylase Activity 205
Summary 216

CHAPTER 9: SUMMARY AND CONCLUSIONS FROM ALL STUDIES 218

LITERATURE CITED 224

APPENDICES 238

I. Expected Mean Squares for Waxy Isogenic Analysis Grown in 1979 239
II. Field Design of Waxy Isogenic Analysis Grown in 1979 240

LIST OF TABLES

Table		Page
2-1	Gene description, symbol, and name code prefix used in naming isogenics	28
2-2	Cultivars and their name code suffix used in naming derived isogenics	28
2-3	The waxy, short-awn, hulless isogenic series of Betzes, Compana, and Titan	29
3-1	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of 22 barley samples at 1 hour of incubation	43
3-2	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of 22 barley samples at 4 hours of incubation	43
3-3	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of 19 barley samples at 1 hour of incubation	48
3-4	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of 19 barley samples at 4 hours of incubation	48
3-5	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of five malt extract dilutions at 1 hour of incubation	51
3-6	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of five malt extract dilutions at 4 hours of incubation	51
3-7	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2) of four standard samples at 4 hours of incubation	54

Table		Page
3-8	Analysis of variance of BPA alpha-amylase area (mm^2) for five selected samples at six temperatures and 10 incubation times	56
4-1	BPA alpha-amylase activity of eight Compana-Washonupana pairs	81
4-2	IDC alpha-amylase activity of 11 Washonupana samples and a Conquest check	82
4-3	Reducing power alpha-amylase activity of eight Compana-Washonupana pairs	84
4-4	IDC alpha-amylase activity of six component isotypes of Washonupana and a Conquest check	90
4-5	BPA alpha-amylase activity of 16 waxy-nonwaxy Compana pairs	96
4-6	Reducing power alpha-amylase activity of 10 waxy-nonwaxy Compana pairs	97
4-7	Reducing power alpha-amylase activity of six waxy-nonwaxy pairs within three genotypes	99
4-8	Analysis of variance and coefficient of variation (C.V.) for BPA alpha-amylase area (mm^2/2 hours at 65 C) of 50 cultivars grown in three environments	101
4-9	Mean BPA alpha-amylase area and commercial use (malt = M; feed = F) of 25 six-row and 25 two-row barley cultivars	102
5-1	Analysis of variance for maltose produced (umoles maltose/g flour) by Compana and Wapana at four stages of micro-malting and comparison of isotype means at five stages of micro-malting	112
5-2	Alpha-amylase activity, malt extract, and diastatic power of micro-malts as determined by the Madison Malt Laboratory for Compana-Wapana pairs grown in 12 Montana environments	116

Table		Page
5-3	Analysis of variance for maltose produced (umoles maltose/g flour) at four stages of micro-malting by nonwaxy-waxy pairs of three cultivars grown at three locations and comparison of isotype means at five stages of micro-malting	118
5-4	Analysis of variance for maltose produced (umoles maltose/g flour) in finished malt of nonwaxy-waxy pairs in three cultivars grown in eight environments	121
5-5	Analysis of variance for BPA alpha-amylase activity (ug dye released/mg flour/min at 65 C) produced in finished malt of nonwaxy-waxy pairs in three cultivars grown in eight environments	123
5-6	Analysis of variance for maltose produced (umoles maltose/g flour) in finished malt of seven cultivars in eight environments	124
5-7	Analysis of variance for BPA alpha-amylase activity (ug dye released/mg flour/min at 65 C) produced in finished malt of seven cultivars grown in eight environments	126
6-1	Abbreviated genetic symbols of the waxy, short-awn, hulless isotypes	132
6-2	Analysis of variance for kernel weight, beta-glucan viscosity, percent protein, percent extract, and alpha-amylase activity for six isotypes across three genotypes	139
6-3	Analysis of variance for kernel weight, beta-glucan viscosity, percent protein, percent extract, and alpha-amylase activity of five commercial cultivars, seven isotypes within Compana, and six isotypes within Titan and Betzes	141
8-1	Analysis of variance for pasting peak viscosity, beta-glucan viscosity, and alpha-amylase activity of unreplicated samples of four waxy isotypes within three cultivars	209

Table | Page

8-2 Mean pasting peak viscosity, beta-glucan viscosity, and alpha-amylase activity of three cultivars (across four isotypes) and four isotypes (across three cultivars) 210

LIST OF FIGURES

Figure		Page
3-1	A BPA assay plate (150 mm x 15 mm) with 14 samples ranging from sound seed to Klages malt and the resulting digestion diameters/areas after 4 hours of incubation at 65 C	40
3-2	Regression of BPA alpha-amylase area at 1 hour of incubation to CBA alpha-amylase activity for 22 barley flour samples	41
3-3	Regression of BPA alpha-amylase area at 4 hours of incubation to CBA alpha-amylase activity for 22 barley flour samples	42
3-4	Regression of BPA alpha-amylase area at 1 hour of incubation to IDC alpha-amylase activity for 19 barley flour samples	46
3-5	Regression of BPA alpha-amylase area at 4 hours of incubation to IDC alpha-amylase activity for 19 barley flour samples	47
3-6	Regression of BPA alpha-amylase area at 1 hour of incubation to reducing power alpha-amylase activity for five malt extract dilutions	49
3-7	Regression of BPA alpha-amylase area at 4 hours of incubation to reducing power alpha-amylase activity for five malt extract dilutions	50
3-8	Regression of BPA alpha-amylase at 4 hours of incubation to CBA alpha-amylase activity for four standard barley flour samples	53
3-9	BPA alpha-amylase area of five barley flour samples incubated at 25 C for 1-10 hours	58
3-10	BPA alpha-amylase area of five barley flour samples incubated at 35 C for 1-10 hours	59

Figure		Page
3-11	BPA alpha-amylase area of five barley flour samples incubated at 45 C for 1-10 hours	61
3-12	BPA alpha-amylase area of five barley flour samples incubated at 55 C for 1-10 hours	62
3-13	BPA alpha-amylase area of five barley flour samples incubated at 65 C for 1-10 hours	63
3-14	BPA alpha-amylase area of five barley flour samples incubated at 75 C for 1-10 hours	64
3-15	Comparison of BPA alpha-amylase area at 1 hour of incubation to alpha-amylase development during germination of two barley cultivars	67
3-16	Comparison of CBA alpha-amylase activity as measured by the BPA assay to alpha-amylase development during germination of two barley cultivars	68
3-17	Regression of BPA alpha-amylase area at 1 hour of incubation to the percent sprouted seed (1-6%) in two barley cultivars	69
3-18	Mean alpha-amylase as measured by the BPA assay of malted barley samples of four cultivars grown in eight environments	72
4-1	Alpha- and beta-amylase zymogram of Washonupana grown in 11 environments and a Conquest check after isoelectric focusing on a pH 4-8 gradient	86
4-2	Alpha-amylase zymogram of Washonupana grown in 11 environments and a Conquest check after isoelectric focusing on a pH 4-8 gradient	88
4-3	Alpha- and beta-amylase zymogram of all available waxy, short-awn, hulless isogenics of Compana after isoelectric focusing on a pH 4-8 gradient	92
4-4	Alpha-amylase zymogram of all available waxy, short-awn, hulless isogenics of Compana after isoelectric focusing on a pH 4-8 gradient	94

Figure		Page
5-1	Comparison of mean starch digestibility of Wapana and Compana grown in 15 environments at 0, 24, 48, 72, and 96 hours of malting	114
5-2	Comparison of mean starch digestibility of waxy and nonwaxy isotypes of Compana, Titan, and Betzes grown at three locations at 0, 24, 48, 72, and 96 hours of malting	120
5-3	Mean maltose content in finished malt of nonwaxy-waxy pairs of Compana, Titan, and Betzes and a Klages check grown in eight environments	125
5-4	Mean alpha-amylase activity in finished malt of nonwaxy-waxy pairs of Compana, Titan, and Betzes and a Klages check grown in eight environments	127
6-1	Mean kernel weight of six isotypes (across three genotypes) and three genotypes (across six isotypes) grown in eight environments	145
6-2	Mean kernel weight of six Betzes and Titan isotypes, seven Compana isotypes, and five commercial cultivars grown in eight environments	147
6-3	Mean beta-glucan viscosity of six isotypes (across three genotypes) and three genotypes (across six isotypes) grown in eight environments	149
6-4	Mean beta-glucan viscosity of six Betzes and Titan isotypes, seven Compana isotypes, and five commercial cultivars grown in eight environments	150
6-5	Comparison between mean kernel weight and mean beta-glucan viscosity of six isotypes across three cultivars grown in eight environments	153
6-6	Comparison between mean kernel weight and mean beta-glucan viscosity of seven isotypes within Compana grown in eight environments	154

Figure		Page
6-7	Comparison between mean kernel weight and beta-glucan viscosity of six isotypes within Betzes grown in eight environments	155
6-8	Comparison between mean kernel weight and beta-glucan viscosity of six isotypes within Titan grown in eight environments	156
6-9	Mean predicted protein of six isotypes (across three genotypes) and three genotypes (across six isotypes) grown in eight environments	159
6-10	Mean percent protein for six isotypes of Betzes and Titan, seven isotypes of Compana, and five commercial cultivars grown in eight environments	161
6-11	Mean predicted percent extract of six isotypes (across three genotypes) and three genotypes (across six isotypes) grown in eight environments . . .	163
6-12	Mean percent extract of six Betzes and Titan isotypes, seven Compana isotypes, and five commercial cultivars grown in eight environments	164
6-13	Mean alpha-amylase activity in nongerminated samples of six isotypes (across three genotypes) and three genotypes (across six isotypes) grown in seven environments	166
6-14	Mean alpha-amylase activity of six isotypes of Betzes and Titan, seven isotypes of Compana, and five commercial cultivars grown in seven environments. .	168
6-15	Comparison between mean alpha-amylase activity and mean percent protein of six isotypes across three genotypes grown in seven environments	169
6-16	Comparison between mean alpha-amylase activity and mean percent protein of six isotypes within Betzes and Titan, and of seven isotypes within Compana grown in seven environments	170

Figure		Page
7-1	Kernel weight relationship between the means of Titan, Betzes, and Compana (all isotypes) and the mean of all entries grown in eight environments	181
7-2	Kernel weight relationship between the means of six isotypes of Titan, Betzes, and Compana (all environments) and the means of the six isotypes in all genotypes (all environments).	182
7-3	Kernel weight relationship between the means of five mutant isotypes (all genotypes) and the mean of the normal isotype (all genotypes) grown in eight environments	184
7-4	Kernel weight relationship between the means of six mutant isotypes of Compana and the mean of Compana grown in eight environments	185
7-5	Beta-glucan viscosity relationship between the means of Titan, Betzes, and Compana (all isotypes) and the mean of all entries grown in eight environments	187
7-6	Beta-glucan viscosity relationship between the means of six isotypes of Titan, Betzes, and Compana (all environments) and the mean of the six isotypes in all genotypes (all environments)	188
7-7	Beta-glucan viscosity relationship between the means of five mutant isotypes (all genotypes) and the mean of the normal isotype (all genotypes) grown in eight environments	190
7-8	Beta-glucan viscosity relationship between the means of six mutant isotypes of Compana and the mean of Compana grown in eight environments	191
7-9	Beta-glucan viscosity relationship between the means of five mutant isotypes of Titan and the mean of Titan grown in eight environments	193

Figure		Page
7-10	Beta-glucan viscosity relationship between the means of five mutant isotypes of Betzes and the mean of Betzes grown in eight environments	194
7-11	Predicted percent extract relationship between the means of five mutant isotypes (all genotypes) and the mean of the normal isotype (all genotypes) grown in eight environments	197
7-12	Predicted percent extract relationship between the means of five isotypes of Titan and the mean of Titan grown in eight environments	198
7-13	Alpha-amylase activity relationship between the means of Titan, Betzes, and Compana (all isotypes) and the mean of all entries grown in eight environments	200
8-1	Brabender amylograms of flour samples of four waxy isotypes of Compana	206
8-2	Brabender amylograms of flour samples of four waxy isotypes of Titan	207
8-3	Brabender amylograms of flour samples of four waxy isotypes of Betzes	208
8-4	Regression of $\log_e$ pasting peak viscosity to $\log_e$ BPA alpha-amylase activity for four waxy isotypes of Betzes, Compana, and Titan	212
8-5	Regression of $\log_e$ pasting peak viscosity to $\log_e$ beta-glucan viscosity for four waxy isotypes of Betzes, Compana, and Titan	213
8-6	Relationship between actual $\log_e$ pasting peak viscosity and a predicted $\log_e$ pasting peak viscosity	214

ABSTRACT

The effect of the waxy endosperm, short awn, and hulless seed genes upon alpha-amylase activity in ungerminated seed, starch digestibility, starch and protein content, beta-glucan viscosity, and kernel weight was examined in the cultivars 'Compana', 'Titan', and 'Betzes'.

A gel-diffusion assay for alpha-amylase, based on a chromogenic substrate (amylose-azure blue) suspended in an agar medium was developed. Activity was measured as the diameter of digestion rings around sample wells into which a flour paste or enzyme extract was placed. The area of the digestion rings was proportional to the $\log_{10}$ of actual enzyme activity ($r = .99^{**}$). Hundreds of samples could be assayed daily.

No unusual amount of alpha-amylase in nongerminated seed was found in any isotype within any cultivar. Some activity was found in all 50 barley cultivars and isotypes tested. No unique isozymes for alpha- and beta-amylase were found in any of the waxy, short-awn, hulless isotypes of Compana. However, the presence of the waxy gene was found to increase starch digestability. This was evident when upon malting, waxy isotypes produced higher levels of reducing substances (maltose) at a more rapid rate than normal isotypes.

Kernel weights were reduced for all mutant isotypes while beta-glucan viscosity was increased. In nonwaxy isotypes, these increases were proportional to the decreases observed for kernel weight. Waxy isotypes had viscosity levels significantly higher than that predicted by kernel weight reductions indicating they were accumulating beta-glucans beyond the simple concentration effect of reduced kernel weight. Waxy isotypes had lower extract (starch content) and higher protein.

All isotypes responded across environments and genotypes in a relatively uniform manner for extract and protein. Regression analysis demonstrated that the background genotype and environment can modify the kernel weight and beta-glucan response of a particular isotype.

When tested in the Brabender Amylograph, waxy, waxy hulless, waxy short-awn, and waxy short-awn hulless isotypes within Titan, Betzes, and Compana pasted in the same temperature range (65-75 C). Pasting peak viscosity was variable, but related to alpha-amylase activity and beta-glucan viscosity of the barley flour samples ($r = .96^{**}$).

CHAPTER 1

INTRODUCTION

Barley (Hordeum vulgare L.) is an important cereal grain in Montana and the Western United States. It is generally classified as a feed grain, and malting is the only premium market that exists. In order to improve the producer's cash return on barley, researchers at Montana State University have emphasized the development of alternate uses. One such use is the production of high maltose syrup from waxy, short-awn, hulless barley.

The purpose of these investigations was to determine how the waxy, short-awn, and hulless genes, separately and in combination, affect seed quality characteristics that influence high maltose syrup production. Characters such as alpha-amylase activity, starch digestibility, and beta-glucan viscosity, affect the ease of syrup production. The relative proportions of seed components such as protein, starch, and beta-glucan, influence the total amount of syrup produced. The effect of the three genes on these characters was examined within three genotypic backgrounds across a range of environments.

Although these investigations have focused on high maltose syrup production, they may also provide useful information for other industrial and domestic uses of barley, such as, malting and brewing,

milling and baking, ethanol production, and human and animal nutrition.

CHAPTER 2

LITERATURE REVIEW

The Use of Washonupana Barley in High Maltose Syrup Production

The barley cultivar 'Washonupana' is a triple isogenic of the cultivar 'Compana'. It was developed by the selection of waxy endosperm, short-awn, hulless lines from the cross, waxy 'Oderbrucker'/7*Compana/2/short awn hulless/7*Compana (Goering and Eslick, 1976). Thus, the name Washonupana is an acronym for waxy (wa), short awn (sho), nude (nu), Compana (pana).

The starch extracted from Washonupana barley was reported to be unique because it retained a sufficient amount of alpha-amylase to liquefy itself in a Brabender Amylograph at 70 C (Goering and Eslick, 1976). This liquefaction occured without the addition of any amylolytic enzymes. This is unique because sound barley is reported to have little or no alpha-amylase at harvest (Kneen, 1944). The self-liquefying property of Washonupana barley prompted its use as the first experimental high maltose syrup barley (Goering et al., 1981).

The high maltose syrup process (Goering et al., 1981) initially separates Washonupana barley grain into the various seed components. The grain is first milled to produce flour which is then suspended in warm water (40-45 C). The crude fiber and aleurone protein fraction

is screened off. The crude starch fraction which consists of starch, endosperm storage protein, and the warm water soluble gums (beta-glucans and arabinoxylans derived from cell wall material) is re-suspended. This mixture is pasted (10 minutes at 75 C) to disrupt all the starch granules then cooled to 40 C in order to add green malt which saccharifies the starch. After four hours the starch is solubilized and the suspended protein and beta-glucan material is removed from solution by centrifugation of the solids. The supernatant is then heated to 60 C and conversion to sugar is complete within 24-36 hours.

The syrup making process is similar to the production of sweet wort in the brewing industry. The main difference is that it produces high maltose syrup, 70% protein, bran fiber plus protein, and possibly beta-glucan as separate end-product fractions (Goering et al., 1981). The brewer, on the other hand, produces a heterogeneous sweet wort that contains sugars, dextrins, proteins, amino acids, and gums (Palmer and Bathgate, 1976). The solid residue (spent grain) from the brewing process is an equally heterogenous mixture of starch, gums, protein, crude fiber, and sugars which is usually dried and marketed as animal feed (Pomeranz, 1973).

One problem encountered in the Goering syrup process is the occurence of high levels of beta-glucans or gums in Washonupana barley

grain (Goering and Chapman, personal communication). In the initial isolation of the crude starch fraction, the gum becomes dissolved or suspended in the warm water mixture, increasing the viscosity and impeding starch separation. Thus, in order to effect efficient starch separation, it has been necessary to add beta-glucanase to reduce the viscosity of the warm water grist (Goering et al., 1981). These gums have potential as industrial gelling agents (Wood, personal communication), however, it is difficult to separate them from the protein. Thus, it is not clear whether they should be separated with the protein from the crude starch or simply hydrolyzed and allowed to be a component of the finished syrup (Chapman, personal communication).

The Pasting Characteristics of Starch as Measured by the Brabender Amylograph

Starch granules are insoluble in cold water, but as starch and water is heated, the granules absorb water and swell. This swelling is reversible until the gelatinization temperature is reached. Structural integrity of the granule is lost at this temperature and material is leached from the granule. Gelatinization is followed by loss of birefringence, increased susceptibility of the granule to enzymatic degradation, and increased viscosity of the starch solution. After reaching peak viscosity, the granules completely break down and viscosity is greatly reduced (Greenwood, 1976).

The swelling and subsequent disruption of granules is referred to as "pasting" and is measured in a Brabender Amylograph (Smith, 1964). This instrument measures the viscosity (as Brabender Units, B.U.) of an aqueous starch suspension as it is mechanically stirred and heated according to a preset time/temperature schedule. The temperatures which bring about initial gelatinization, pasting peak, and liquefaction are determined with this instrument (Smith, 1964 and Goering et al., 1973a).

In addition to purified starch, crude flour (Goering et al., 1981) and malt flour (Yoshida and Yamada, 1970) have been studied with the Brabender Amylograph. Adding alpha-amylase to crude barley flour and purified barley starch greatly altered the initial pasting temperature, pasting peak temperature, and final liquefaction temperature and viscosity (Yamada and Yoshida, 1973). The addition of beta-1, 4 glucanase to crude barley flour greatly reduced the overall viscosity of the mash, while the addition of protease had no appreciable effect. Goering and Eslick (1976) observed that the presence of alpha-amylase with Washonupana starch reduced peak viscosity and resulted in complete liquefaction of barley starch (B.U. = 0). The observed initial pasting temperature was not affected.

Genetic Components of Washonupana Barley

The three genes that were introduced into Compana barley to produce Washonupana barley are all recessive and located on chromosome 1.

The waxy gene (wxwx) is located on the short arm approximately 50 recombination units from the centromere (Nilan, 1964). The short-awn (lk2lk2) and the hulless gene (nn) are linked characters located on the long arm. The hulless gene is about 7 recombination units from the centromere while the short-awn gene is about 15 recombination units from the centromere (Eslick et al., 1972).

Waxy Endosperm

The waxy endosperm gene was introduced into syrup barley cultivars because of the observation that waxy starch is more readily modified by enzymes and chemicals than normal starch (Cowie and Greenwood, 1957; Buttrose, 1960; Leach and Schoch, 1961; Sandstedt et al., 1968; Goering et al., 1973a; Sullins and Rooney, 1975; Tovar et al., 1977; and MacGregor and Ballance, 1980). Also, it has been found that waxy barley starch will paste at a temperature 20 C below normal starch in a Brabender Amylograph (Goering et al., 1973a; Goering and Eslick, 1976; and Goering et al., 1981). This represents savings in energy since starch must be pasted before it can be converted to sugar.

Normal starch found in barley and other small grains is a mixture of two polymers: a helical straight-chained polymer called amylose in which glucose units are joined by alpha-1, 4 linkages; and a branched polymer called amlopectin in which glucose units are joined by alpha-1, 4 and alpha-1, 6 linkages. The branches of amylopectin are formed by

alpha-1, 6 linkages and are 20-24 glucose molecules long (Harris, 1962a; Banks and Greenwood, 1975; and Greenwood, 1976).

Waxy and normal grain differ in that the waxy type produces little (0-3%) of the straight chained amylose (Goering et al., 1973a and Briggs, 1978). Badenhuizen (1955, 1963) reported that in waxy maize a very small amylose portion was localized in the center of the granule. Normal barley starch consists of 15-25% amylose and 75-85% amylopectin and is not markedly affected by environment (Goering et al., 1957). This ratio is typical for most cereals (Harris, 1962a; Creech, 1968; and Banks and Greenwood, 1975).

Waxy starch can be distinguished from normal starch by iodine staining. Iodine reacts with the long chained amylose to produce a deep blue color while the reaction with the short chained (20-24 glucose residues) amylopectin produces a red color (Harris, 1962a; Creech, 1968; and Banks and Greenwood, 1975).

The occurrence of waxy endosperm starch in corn and rice is closely associated with an observed deficiency or complete lack of granule-bound UDP-G and ADP-G starch synthetase. Waxy corn and rice do possess soluble starch synthetase (Nelson and Rines, 1962; Nelson and Tsai, 1964; Akazawa and Murata, 1965; Murata et al., 1965; Frydman, et al., 1966; Murata and Akazawa, 1966; and Creech, 1968).

Starch granule synthesis occurs in amyloplasts within the barley

or cereal endosperm. The starch granules are laid down by apposition, layer by layer (Badenhuizen and Dutton, 1956 and May and Buttrose, 1959), forming a semi-crystalline structure with concentric amorphous and crystalline portions (Buttrose, 1960; Banks and Greenwood, 1975; and Greenwood, 1976).

Starch granule ultrastructure is solely dependent upon amylopectin. Amylose can be completely leached from starch granules allowed to remain in an aqueous solution at or near the gelatinization temperature (barley = 65 C). Under these conditions, the amylose will dissolve into the water while the crystalline structure of the granule remains intact. Waxy granules, even though completely lacking in amylose, form normal semi-crystalline granules (Montgomery and Senti, 1958; Banks and Greenwood, 1975; and Greenwood, 1976). Buttrose (1960) has noted that amylose has the effect of hardening the amylopectin granule against acid hydrolysis. It appears to intermesh with the granule-forming amylopectin to produce a more tightly packed starch granule.

The waxy gene produces no gross noticeable difference in crop appearance except that it imparts to the grain an opaque hue in contrast to the more vitreous appearance of normal grain (Ericksson, 1969). This characteristic is not readily apparent in hulled barley but is easily observed in hulless types.

Some physiological differences between normal and waxy types have been observed. Creech (1968) reported that waxy maize isotypes had reduced dry matter accumulation at all stages of development. Also, reduced endosperm starch accumulation was observed but not to a significant level. Eslick (1979) observed slight but insignificant yield reductions for the waxy barley types when compared to normals. When waxy corn isotypes were first introduced there were significant yield reductions. Improving the genetic background of the waxy lines resulted in yield levels comparable to normal maize cultivars (Crookston, 1979).

Bruckner and Eslick (1979) observed that the waxy isotype of Compana had less field dormancy and higher levels of alpha-amylase at all stages of seed development. MacGregor and Ballance (1980) reported that waxy starch granules were more susceptible to hydrolysis than normal starch granules. They suggested that the unique attached enzyme phenomenon of Washonupana barley was due primarily to the waxy nature of the starch.

Waxy grains have been evaluated for feed value since waxy starch was shown to be more digestible in vitro than normal starch (Sullins and Rooney, 1975). In studies with sorghum, advantages in feed efficiency of waxy grain compared to normal sorghum were observed for sheep (Nishimuta et al., 1969) and steers (Sherrod et al., 1969).

However, Cohen and Tanksley (1973) found no difference in the performance of swine fed waxy vs. nonwaxy grain sorghum. Superior performance of swine (Hanson, 1946) and ruminants (Braman et al., 1973) raised on waxy corn has been reported. But, other investigations have shown no appreciable advantage for ruminants (McCormick and Farlin, 1974 and Robinson et al., 1974) fed waxy vs. nonwaxy corn. Swine (Calvert et al., 1977), dairy cattle, beef cattle, and sheep (Moss et al., 1980), performed equally when fed waxy and nonwaxy barley rations.

Short Awn

Awns are active photosynthetic organs in small grains (Grundbacher, 1963). As awn length is reduced, photosynthetic rate declines (Johnson et al., 1975). Shannon and Reid (1976) reported yield reductions in comparisons of awnless vs. full-awned isogenic lines of winter barley. In studies with full-awned, half-awned, quarter-awned, and awnless isogenic barley lines, Schaller et al. (1972) observed kernel weight reductions proportional to the reduction in awn length. Significant yield reductions were also observed but only within high yielding environments. In comparison of short-awn (lk2lk2) vs. long-awn (Lk2Lk2) isotypes of hulled and hulless barley, McGuire and Hockett (1981) found that kernel weights were significantly reduced in the short-awn isotypes. However, awn length had no significant effect on

yield. Eslick (1979) found no yield differences in isogenic comparisons of long-awn vs. short-awn isotypes grown in Montana environments. He suggested that while kernel weight is reduced in short-awn isotypes, yield remains unchanged because these isotypes compensate by producing more seed.

Barley endosperm contains a bimodal distribution of starch granules (May and Buttrose, 1959 and Goering et al., 1973b). MacGregor (1979) divided the two groups of granules using a 5.3 um diameter as the dividing point, with large granules being greater than 5.3 um and small granules smaller than 5.3 um. The small granules are difficult to separate in the Goering syrup process because they can be washed away in the mill water or remain trapped in the gum-protein complex (Goering et al., 1981). In general, 90% of the starch granules are the small type but they represent only 10% of the starch present in the barley (May and Buttrose, 1959 and Goering et al., 1973b). The short-awn trait was included in the development of the syrup barley Washonupana because the short-awned isotype of Compana contained a high proportion of easy to process large starch granules (Goering et al., 1973b).

Hulless Seed

In crops such as barley and rice, the flowering glumes (the lemma and palea of the barley flower) are cemented to the caryopsis

to produce a hulled grain. In hulless isotypes, the hulls are not cemented to the seed (Briggs, 1978). Kernel weight and yield reductions of hulless isotypes are proportional to at least the weight of the hulls (8-16% of the kernel weight) (Briggs, 1978 and Eslick, 1979). Since the hulls are left in the field, the proportion of other seed components changes accordingly. The fat, protein, and starch content of the seed increase due to the reduction in the crude fiber represented by the hulls (McGuire and Hockett, 1981).

The hulless-seed trait has appeal in the barley syrup process because of reduced amounts of harsh lignified fiber, most of which is contained in the hulls of barley (Briggs, 1978). Goering et al. (1981) stated that the hulls only serve as an unwanted nuisance in the production of syrup from barley.

Alpha-Amylase in Barley

Alpha-amylase is the main saccharifying enzyme of cereals. It hydrolyzes the alpha-1, 4 linkage of both amylose and amylopectin in a random manner. The importance of alpha-amylase lies in the fact that it is the only enzyme capable of digesting starch granules (Harris, 1962b; Dunn, 1974; and Maeda et al., 1978).

Under natural conditions, alpha-amylase does not work alone. It functions in breaking the integrity of the starch granule and reduces the starch into segments of 6-8 glucose molecules. Two other enzymes

are necessary to complete the conversion of starch into maltose and other sugars. Beta-amylase, which attacks a nonreducing end and breaks off two glucose molecules (maltose) at a time, converts the fragments produced by alpha-amylase to maltose. The third enzyme, pullanase, is necessary to hydrolyze the alpha-1, 6 linkages of amylopectin before complete starch hydrolysis can occur (Harris, 1962b; Dunn, 1974; and Palmer and Bathgate, 1976).

Alpha-amylase is a unique enzyme in that it has a remarkable temperature range. Other hydrolytic enzymes of barley (beta-amylase, beta-glucanase, protease, and pullanase) have their optimum temperature range from 40-50 C. The optimum range for alpha-amylase is 50-55 C, and the enzyme will function quite well at 70 C (although at reduced efficiency), a temperature at which all the other hydrolytic enzymes are completely denatured (Olson et al., 1943; Harris, 1962a; Harris, 1962b; and Palmer and Bathgate, 1976).

Alpha-amylase is a complex mixture of isozymes. The number of isozymes detected often depends on the barley cultivar examined, the environment in which it is grown, and the method used to separate the isozymes (MacGregor, 1978 and MacGregor and Dussant, 1979). However, there are two basic types of alpha-amylase that have a physiological as well as chemical distinction. Green alpha-amylase, or alpha-amylase I, is primarily found in developing seed. It develops in the pericarp

prior to anthesis and reaches a peak at 2-10 days after anthesis. The level of alpha-amylase activity in the developing seed never exceeds more than 1-5% of the activity of malted grain. As the seed reaches physiological maturity the activity is rapidly reduced until at harvest only small amounts of activity remain. The function of alpha-amylase I is unknown, but it has been suggested that starch granules found in the green pericarp tissue are hydrolyzed to provide energy for growing kernels or produce short chain primers to be used in endosperm starch synthesis (MacGregor et al., 1971a and MacGregor et al, 1972).

The other major component of alpha-amylase is germinative alpha-amylase or alpha-amylase II and III. These isozymes are produced de novo by germinating seed (Varner and Chandra, 1964; MacGregor et al., 1971b; and MacGregor, 1976). In order for germination to occur the moisture level of the seed must reach 35%. At this point the embryo begins to respire and secretes gibberellic acid which moves to the aleurone layer where it triggers the production of alpha-amylase. This is the sequence of events in the production of all the major hydrolytic enzymes except beta-amylase which is preformed in the endosperm as part of the protein matrix (Harris, 1962a; Harris, 1962b; and Palmer and Bathgate, 1976). The main purpose of malting in the brewing industry is the production of these hydrolytic enzymes, es-

pecially alpha-amylase, through controlled steeping and germination (Pomeranz, 1973).

Numerous methods have been used to measure alpha-amylase in cereals, but they are all based upon the same principle, action of alpha-amylase enzyme upon a substrate. The reduction in viscosity of a starch or flour solution can be measured using the Falling Number Method (Hagberg, 1961 and Perten, 1964). Particle size reduction of a dextrin substrate can be measured optically using the Nephalometric technique (O'Connell et al., 1980). Decreased iodine color of an iodinated beta-limit dextrin can be measured colorimetrically with the Iodine Dextrin Color Loss Method (Sandstedt et al., 1939). The increase in reducing power that occurs with hydrolysis of alpha-1, 4 linkages of starch can be measured by titration or quantitative dye attachment to the reducing ends of dextrin fragments with the Reducing Sugars Method (Hodge and Hofreiter, 1962).

In the last 10 years, cross-linked dye-labelled starch substrates that are quite specific for alpha-amylase have become available (cibacron blue amylose, amylose-azure blue). These substrates are dissolved in solution. With the addition of amylase, the starch is hydrolyzed and the dye released. The amount of dye released is quantitatively related to the alpha-amylase activity and can be measured with a spectrophotometer (Klein et al., 1969; Barnes and Blakeney,

1971; and Mathewson and Pomeranz, 1977). These chromogenic substrates can also be employed in gel-diffusion assays in which activity diameters are related to enzyme activity (Hejgaard and Gibbons, 1979).

Beta-Glucan in Barley

The starchy endosperm cell walls of barley are composed largely of a polymer called beta-glucan (75%) (Fincher, 1975; Forrest and Wainwright, 1977a; and Forrest and Wainwright, 1977b). Beta-glucans are straight chained beta-linked glucose units linked at the 1,4 position (as in cellulose) and at the 1,3 position. The average proportion of 1,3 to 1,4 linkages is 3:7 (Parish et al., 1960 and Bourne and Pierce, 1972).

The other polysaccharide components of the cell wall are pentosans, such as, arabans, xylands, and arabinoxylans. These polysaccharides are similar to the beta-glucans but the components are 5 carbon sugars and there is some evidence of branching within the polymer. Pentosans are the principle cell wall constituent in the aleurone layer (McNeil et al., 1975 and Palmer and Bathgate, 1976).

There is also a protein fraction within the cell wall material. Since calcium pectate is lacking in the barley endosperm it has been suggested that cell wall protein serves as the cementing substance which binds endosperm cells together (MacLeod and McCorquodale, 1958 and Palmer and Bathgate, 1976).

In the past, the beta-glucans and pentosans were classified by their solubility properties. Components which dissolved in warm water are called gums; and those that were extracted by 4% sodium hydroxide solution were called hemicellulose (Harris, 1962a and Palmer and Bathgate, 1976). Current thinking is that gums and hemicellulose are all derived from the same basic source, cell wall polysaccharides. The difficulty of extraction is simply a measure of polymer chain length, difference in the proportion of beta-1,3 and beta-1,4 linkages, and/or location within the cell wall (Fleming et al., 1974; Bathgate and Dalgliesh, 1975; and Forrest and Wainwright, 1977a).

Beta-glucans have presented problems in the brewing industry by causing increased viscosity to the sweet wort which causes filtration problems. Excessive beta-glucan can cause haze formation and palatability problems in beer. Conversely, a certain amount of this substance is desirable in the beer since it provides body and foam stability (Forrest and Wainwright, 1977a).

The viscosity of a warm water beta-glucan extract prepared from barley flour is not strictly related to the beta-glucan viscosity of the sweet wort which is derived from malt flour. The malting process releases additional beta-glucans as cell walls are hydrolyzed and it is this material that creates a viscous sweet wort (Harris, 1962a; Scott, 1972; Bourne and Pierce, 1972; and Palmer and Bathgate, 1976).

The amount of beta-glucan that is found in barley grain has a very strong genetic and environmental component. While there is a great deal of variability across environments the relative performance of genotypes is constant (Bourne and Pierce, 1970; Bourne and Pierce, 1972; and Coles, 1979). The high lysine mutant 'Riso 1508' has extremely low levels of beta-glucan and is almost insensitive to normal environmental variation (Coles, 1979). The high protein oat (Avena sativa L.) cultivar, 'Hinoat', has abnormally high levels of beta-glucan (Wood et al., 1977).

Elevated beta-glucan content is associated with xerophytic conditions (Whitmore and Sparrow, 1957; Preece et al., 1958; and Bendelow, 1975). Coles (1979) reported that under drought conditions beta-glucan synthesis is favored over starch. When the moisture level of the developing seed is above 45%, the synthesis of starch from glucose is the dominant glucan consuming process. However, when the moisture level falls below 45%, starch synthesis is halted while beta-glucan synthesis increases sharply. In rain (sprinkler) vs. root irrigated plants, beta-glucan content of the grain of the latter is higher throughout development (Coles, 1979). No evidence of beta-glucan degradation in the seed of rain irrigated plants suggests that less beta-glucan is synthesized (Aastrup, 1979). This difference is

attributed to seed dessication which inhibits starch synthesis and favors synthesis of beta-glucans.

Aside from providing cell wall material to the endosperm, beta-glucan molecules can also serve as an alternate storage polysaccharide and as a water-conserving mechanism (Aastrup, 1979). Beta-glucan is hydroscopic (Preece and MacKenzie, 1952 and Greenberg, 1974) and up to 50% of the beta-glucan present in barley can be leached out before cell wall disintegration becomes apparent (Forrest and Wainwright, 1977b).

Gohl (1977) and Aastrup (1979) found that barley seed harvested at yellow ripeness had a more viscous extract than barley seed harvested at complete ripeness. However, total beta-glucan content did not change. Soluble beta-glucan decreased while the insoluble beta-glucan content increased (insignificantly). This suggests that as seed ripens the soluble beta-glucans which are the source of extract viscosity become more tightly bound within the cell wall and are rendered insoluble

The amount of beta-glucan reported in barley seed varies from 1.5 - 8.0% (Prentice et al., 1980). This variation is partly due to the assay used to measure this nonstarchy polysaccharide. Extraction procedures vary from assay to assay. If only the hot water soluble gums are extracted, the beta-glucan estimates tend to be low. Esti-

mates increase if both the water soluble fraction and the insoluble fraction are extracted using strong acid or basic solutions (Forrest and Wainwright, 1977a and Wainwright, 1978). Temperature of extraction also influences the estimation of beta-glucan content. Extraction in basic solution at 80 C solubilizes three times as much beta-glucan as extractions performed at 45 C (Prentice et al., 1980).

Basically there are two methods used to measure beta-glucan content of barley grain: (1) extraction of the beta-glucan with subsequent hydrolysis of the polysaccharide to glucose; and (2) extraction of the beta-glucan in a highly acidic or basic solution and measurement of viscosity of the extract (Wainwright and Buckee, 1977). The first method is the most accurate. Glucose content is measured optically (600 nm) by addition of a glucose oxidase reagent, and beta-glucan content is determined on a percentage basis. The actual hydrolysis of beta-glucan to glucose is achieved through glucanase enzymes (Prentice et al., 1980), or a combination of acid hydrolysis and enzyme hydrolysis (acid hydrolysis-amyloglucosidase procedure) (Fleming et al., 1974 and Wood et al., 1977).

The second method, viscosity, although less accurate, is much faster. The amount of beta-glucan is not measured directly, but as the amount of viscosity produced by a beta-glucan extract (Greenberg and Whitmore, 1974 and Morgan and Gothard, 1977). Vis-

cosity is related to actual beta-glucan content in a logarithmic fashion. Viscosity increases logarithmically as beta-glucan content increases arithmetically (Greenberg, 1974). Unfortunately, both quantity and quality of beta-glucan can affect viscosity. The larger molecules of beta-glucan have greater influence on viscosity than the small types (Bourne and Pierce, 1970). Also, the pentosan fraction influences viscosity (Bourne and Pierce, 1970; Bourne and Pierce, 1972; and Bathgate and Dalgliesh, 1975). Researchers using this method in the past, have found it to correlate well with actual beta-glucan content ($r = .89$) (Greenberg, 1974). However, this method is more appropriate when the experimenter is interested in viscosity rather than actual beta-glucan content of barley grain.

Recently an infrared reflectance procedure has been introduced for beta-glucan analysis using a Technicon Infraalyzer 400. This highly automated method determines the beta-glucan content by scanning ground flour samples. This method correlates well with both viscosity ($r = .81$) and actual percentage beta-glucan ($r = .87$). While this technique is recommended only for the screening of barley material, its potential as an analytical tool should not be overlooked (Allison et al., 1978).

Starch and Protein Accumulation in Developing Barley

Dry matter accumulation in developing barley seed closely follows the accumulation of the two major components of the endosperm: protein and starch (MacGregor et al., 1971a). The endosperm is primarily a storage organ which, with the exception of the aleurone layer, is dead tissue at maturity. The endosperm consists of starch granules (large and small) and protein bodies embedded in a protein matrix surrounded by a beta-glucan/arabinoxylan cell wall (Palmer and Bathgate, 1976 and Briggs, 1978). The cell walls of the endosperm function primarily as a structural component, however, as mentioned previously, they may function as carbohydrate storage components under certain environmental conditions (Coles, 1979). Starch, the chief source of stored reserves, comprises 50-65% of the seed weight. Overall yield is primarily determined by this seed component (Palmer and Bathgate, 1976 and Briggs, 1978).

Protein has several functions within the endosperm. The metabolically active proteins of the aleurone layer are primarily enzymes and enzyme precursors consisting of water soluble albumins and salt soluble globulins (Simmonds and Orth, 1973). Within the starchy endosperm there exists two types of protein. The 70% ethanol-soluble hordeins, which are purely storage proteins, contain a preponderance of glutamic acid and proline. Hordeins are the main