# THE INFLUENCE OF ALPHA AMYLASE AND PROTEASE ACTIVITY ON THE BAKING QUALITY OF FOUR SECONDARY HEXAPLOID TRITICALES

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Lori JoAnne Macri

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TABLE OF CONTENTS

PAGE

PAGE

# LIST OF FIGURES

FIGURE PAGE

# LIST OF TABLES

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# ABSTRACT

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The influence of α-amylase activity, exoprotease activity, endoprotease activity, and gluten protein content on the baking performance of four secondary hexaploid triticales (4T, 11T, Impala, and Carman) was examined. One hard red spring wheat (cv. Marquis) was included in the study for comparison. Cultivars were grown in dryland field plots during the 1983 and 1984 growing seasons.

Enzyme activities were followed from 21 days post anthesis to full maturity. Alpha-amylase activity of the triticales generally increased during kernel development, while exoprotease and endoprotease activity generally decreased. At full maturity, the triticales had higher whole grain α-amylase, exoprotease, and endoprotease activity than the Marquis wheat check grown in the same year. Differences in α-amylase and protease activity due to cultivar and year were observed.

For any given cultivar, the α-amylase activity, exoprotease activity, endoprotease activity, and protein content (N X 5.7) were higher in the mature grain samples

than in the corresponding flours. Compared to the Marquis wheat flours, the triticale flours contained higher levels of α-amylase and exoprotease activity, similar levels of endoprotease activity, and lower levels of protein.

The weight of dry gluten recovered from the triticale flours varied widely, and protein analysis of the glutens showed that all triticale flours contained significantly lower amounts of gluten protein than the Marquis wheat flours. The lower gluten protein content of the triticale flours was due in part to their lower protein content, and in part to their lower percentage of total flour protein as gluten-like protein.

The loaf volume of the triticale breads varied widely under the conditions of the A.A.C.C. straight dough bake test, and breads baked from the Marquis flours were superior in overall quality to breads baked from the triticale flours. The loaf volume of the triticale breads was significantly correlated with the gluten protein content of the flours ($r = +0.879$, d.f. = 6, $P = 0.01$). In contrast, the correlations between loaf volume and protease activity of the flours were nonsignificant and there were no apparent differences in exoprotease or endoprotease activity that could account for differences in the baking performance of the triticale flours. In spite of the relatively high α-amylase activity in all triticale flours, only cultivar 4T produced breads with a gummy crumb texture. The low tolerance of the 4T

doughs towards α-amylase activity was attributed to their low gluten protein content and high level of damaged starch.

It was concluded that the gluten protein component was the major factor controlling the baking potential of the secondary triticales, while endogenous α-amylase and protease enzymes had only a minor effect on loaf quality. Accordingly, high gluten protein content should be an important criterion in a breeding program when selected for triticales with improved baking quality.

# I. INTRODUCTION

Cereal grains contribute approximately 50% of the protein and 70% of the calories in human nutrition, and bread provides more nutrients than any other single food source (Lorenz and Lee 1977). As the world population rises, there is a growing need to increase the total amount of cereal grains available for human consumption. Hexaploid triticale (X Triticosecale Wittmack) is a man made cereal produced by cross breeding tetraploid wheat (Triticum) and rye (Secale) that has shown promise as a nutritious cereal crop that could supplement existing grain supplies.

Triticale is highly adaptable to sandy or acid soils that are unsuitable for wheat (Bushuk and Larter 1980, Slootmaker 1974) and is superior to wheat in nutritional quality (Kies and Fox 1970, Villegas et al. 1970). In spite of these advantages, commercial utilization of triticale for human consumption is relatively limited. One of the major reasons is that triticale is generally inferior to wheat for bread production (Haber et al. 1976, Lorenz et al. 1972, Tsen et al. 1973). In fact, the "triticale" bread available in the supermarket today is usually made with 60% wheat flour and only 40% triticale flour.

It is generally accepted that protein and starch play

an important role in determining the baking quality of a flour. When wheat flour is mixed with water, the water-insoluble gluten proteins hydrate and form an elastic dough that can entrap gases during fermentation, allowing the dough to rise and expand. In the oven, starch granules will gelatinize and take up water from the gluten proteins, allowing the gluten proteins to set and become rigid.

Flour also contains many enzymes, of which the starch degrading amylases and protein degrading proteases are the most important technologically. Excessive levels of α-amylase will dextrinize a large portion of the starch, reducing its water binding capacity and resulting in bread with a wet, gummy crumb. Excessive levels of proteases may weaken the dough structure to a point where the dough is unable to retain the gases produced by the fermenting yeast, which will result in a bread with a reduced loaf volume and a dense crumb structure. Triticale flours generally contain higher levels of α-amylase activity (Klassen and Hill 1971, Pena and Bates 1982, Welsh and Lorenz 1974) and protease activity (Madl and Tsen 1973, Singh and Katragadda 1980) than wheat flours, and past research would suggest that both the α-amylase and protease activity contribute to the generally inferior quality of triticale flours (Lorenz 1974).

In recent years, plant breeders have attempted to improve the quality of triticale by cross breeding hexaploid triticale and bread wheat. A few such crosses have produced

hybrids (secondary triticales) such as Rahum and Impala that will produce acceptable bread from 100% triticale flour with only minor modification to standard breadmaking methods (Lorenz and Welsh 1977, Pena 1984).

The main objective of the present study was to evaluate the influence of endogenous α-amylase and protease enzymes on the baking performance of four secondary hexaploid triticales. The advanced lines of secondary triticale used in this study (4T, 11T, Impala, and Carman) were chosen because of their diverse baking quality. Alpha-amylase and protease enzymes are present in grain throughout kernel maturation, and it is therefore possible that the baking potential of a cultivar may be altered if endosperm starch and storage proteins are modified in situ (i.e. before the endosperm is milled into flour and processed for bread production). For this reason, the α-amylase and protease activity of the triticales was examined in developing kernels during the latter stages of grain maturation (21 days post anthesis to harvest ripeness) as well as in the milled flours.

Both exoproteolytic and endoproteolytic enzymes occur in cereals, but studies of Hanford (1967) and Redman (1971) would indicate that it is the endoprotease enzymes which cleave peptide bonds in the middle of proteins, that are primarily responsible for the changes in the physical properties of gluten proteins. Both the exoprotease (hemoglobinase) and endoprotease (azocaseinase) activity was assayed in the present study. To date, there has been no report in

the literature on the azocaseinase activity of triticale.

A second objective follows from the observation that wheat flours with higher gluten protein content generally have stronger mixing characteristics and tend to tolerate higher levels of α-amylase activity without serious bread quality deterioration. Accordingly, the second aim of this study was to investigate the effect of gluten protein content on the rheological properties and baking performance of the triticale flours.

# II. LITERATURE REVIEW

## A. The Breeding and Cytology of Secondary Triticales

Almost all triticales developed for commercial use today are secondary hexaploid triticales (Bushuk and Larter 1980) and two major steps are commonly used in their synthesis:

a) production of a tetraploid durum wheat (AABB) X rye (RR) hybrid, followed by chromosome doubling with colchicine to produce a fertile AABBRR amphiploid (called the primary hexaploid triticale), and

b) improvement through breeding by crossing the primary hexaploid triticale with a hexaploid bread wheat (AABBDD); the progeny of this cross are known as secondary hexaploid triticales.

Durum wheats used in the synthesis of primary triticales are high yielding but lack the D genome chromosomes that are responsible for the baking quality of the hexaploid bread wheats (Kerber and Tipples 1969). Merker (1976) has stated that the superior performance of secondary triticales can be attributed to the combined action of D genome chromosome substitutions, introduction of the hexaploid wheat cytoplasm, and gene recombination.

## Chromosome Substitutions and Translocations

In the late 1960's, breeders at CIMMYT (International Maize and Wheat Improvement Center, Mexico) selected a strain of triticale having high fertility, day length insensitivity, high yield, early maturity, and one gene for dwarfness (Zillinsky and Borlaug 1971). Gustafson and Zillinsky (1973) later reported that this so-called Armadillo strain of triticale had wheat chromosome 2D substituted for rye chromosome 2R, and suggested that the favorable agronomic characteristics of the Armadillo triticales were "accidentally" introduced by a spontaneous outcross between a primary hexaploid triticale and a Mexican dwarf bread wheat. Since this discovery, several improved strains of hexaploid triticale have been produced from artificial crosses between primary hexaploid triticale and bread wheat.

When a primary triticale (AABBRR) and a bread wheat (AABBDD) are crossed, the $F_1$ hybrid will have the genomic formula AABBDR. After a number of generations of self-pollination, the ultimate result should theoretically be a multitude of plants with a wide range of R and D chromosome mixtures (Muntzing 1979). In practice, certain substitutions are much more frequent than others, owing in part to natural selection and in part to breeder selection for agronomic performance.

Rye chromosomes differ from wheat chromosomes in having large blocks of telomeric heterochromatin and a higher DNA content per chromosome. Gustafson and Bennett (1976)

have suggested that these differences in size and/or DNA content could lead to natural selection against substitutions involving those rye chromosomes least compatable with wheat. Indeed, in hexaploid triticale X bread wheat crosses where no breeder selection is applied, the largest rye chromosome 2R has a strong tendency to disappear from triticale and be replaced by its homoeologous wheat chromosome 2D, while the smallest rye chromosome 1R is usually the last to be substituted (Lukaszewski et al. 1982, Gustafson and Zillinsky 1978).

Since the A and B genomes have the possibility of full pairing at meiosis in the triticale X bread wheat hybrid, it is more probable that substitutions take place between the R and D genomes. However, chromosomes of the A and B genome occasionally can be found as univalents in such hybrids (Sanchez-Monge and Sanchez-Monge Jr. 1977), and substitution of D genome chromosomes for A and B genome homoeologues cannot be ruled out. Such substitutions might be used to improve the quality of some triticales while still retaining full rye complement (Larter and Noda 1981).

Gregory (1974) found that in addition to substitutions involving entire chromosomes, translocation of single arms or even segments of D genome chromosomes are possible via the triticale X bread wheat cross. Kaltsikes et al. (1968a, 1968b) reported that translocation of the long arm of chromosome 1D into an AABB tetraploid wheat considerably

improved its baking quality, and similar translocations may be responsible for the improved quality of some secondary triticales.

## Cytoplasmic Factors

In a few cases, new secondary hexaploid triticales such as CIMMYT's Beagle and DR-IRA strains are entirely free from substitutions but still have improved vigor, fertility, and seed type (Muntzing 1979). However, these strains do have a bread wheat cytoplasm and Hsam and Larter (1974a, 1974b) have found that the cytoplasm of hexaploid wheat is superior to the cytoplasm of tetraploid wheat for triticale in many respects. In addition to improved fertility, yield, and protein content, triticales with hexaploid wheat cytoplasm may also have significantly lower α-amylase levels (Hsam 1974).

## Genic Recombination

Although tetraploid and hexaploid wheats share an AABB component in their genomic formula, the A and B genomes of tetraploid wheat are not identical to those of hexaploid wheat (Kerber 1964). Presumably the A and B genomes of bread wheat have become adapted to the hexaploid level, making them cooperate better with the rye genome in hexaploid triticale (Thomas and Kaltsikes 1972).

In the triticale X bread wheat hybrid, the A and B genome chromosomes can pair at meiosis and recombine. This recombination can lead to the introduction of favorable A

and B genome chromosomes from hexaploid wheat into triticales.

## B. Flour Components That Influence Baking Quality

During grain maturation, the bulk of the protein and starch in the seed are stored in the endosperm. When grain is milled, the germ and outer bran are removed and the starchy endosperm is then ground into flour. A typical wheat flour will contain about 70% starch, 12% protein, 2% lipid, 2% pentosans, 0.5% mineral, and 12% moisture by weight (Birnbaum 1977). Triticale flours have a similar composition (Farrell et al. 1974).

Because the main components of flour are also enzyme substrates, enzymes such as the starch degrading amylases and protein degrading proteases can affect the baking potential of a flour. Most enzyme levels in sound (ungerminated) wheat are very low and, as such, are of little consequence in altering the baking potential of flours milled from such wheats. Triticale, however, generally possesses higher levels of α-amylase activity (Klassen and Hill 1971, Lorenz and Welsh 1977, Pena and Bates 1982, Welsh and Lorenz 1974) and protease activity (Madl and Tsen 1973, Singh and Katragadda 1980) than wheat, even in the absence of visible sprouting. These enzymes may alter the functional properties of a triticale flour, making it unsuitable for baking.

This section reviews the structure and function of four important components of triticale flour - protein, starch,

amylase enzymes, and protease enzymes - and their role in bread production. Because triticale is a relatively new cereal species, reports on the composition of triticale flours are limited. Accordingly, relevant literature on related cereal species such as wheat and rye will also be discussed.

## Protein

Both protein quantity and quality are major determinants of the breadmaking potential of a flour. Protein quantity depends not only on the variety but on the environment in which that variety is grown. Protein quality is heritable and is assessed by various rheological and baking tests. A high quality flour produces a good bread over a fairly wide range of protein levels, while one of low quality produces relatively poor bread even when its protein content is high.

Wheat Gluten Proteins. Gluten is the cohesive, elastic substance obtained by washing a dough in a continuous stream of water to remove the starch and water solubles. When dried, wheat gluten contains over 80% protein (Kasarda et al. 1971); the remainder is lipid and carbohydrate.

Over 40 years ago, Finney (1943) demonstrated that differences in baking quality between wheat varieties were due to differences in the gluten component of the flour. Subsequent studies have shown that differences in baking quality cannot be ascribed to the gluten lipids (Fisher

et al. 1966, MacRitchie 1978) and that gluten protein is invariably responsible (Booth and Melvin 1979, MacRitchie 1980).

The gluten proteins can be conveniently divided into three groups: the alcohol-soluble gliadins, the acid-soluble glutenins, and the acid-insoluble glutenins (residue protein). The gliadins consists of single polypeptides with molecular weights below 130,000 (Pyler 1983). The glutenins have molecular weights ranging from 100,000 to many millions, and consist of smaller peptide subunits (10,000 to 130,000 daltons) linked by disulfide bonds (Greenwood and Ewart 1975). The gluten proteins are characterized by an unusually high content of glutamine (just over 30%) and proline (about 14%) (MacRitchie 1980).

In typical bread wheats, the gluten proteins account for about 78 to 85% of the total protein in the flour (Pence et al. 1954). The remainder of the flour proteins are the soluble (non-gluten) proteins, which include the metabolic proteins such as the amylase and protease enzymes (Bushuk and Lee 1978). The soluble proteins are not essential to produce a normal loaf of bread (Hoseney et al. 1969) but are nutritionally important because they contain relatively high levels of lysine, the first limiting amino acid in most cereals (Huebner 1977).

The amino acids which occur in gluten play a critical role in protein properties and interactions. For example, the formation of disulfide crosslinks between cysteine resi-

dues can covalently join proteins, imparting strength to a dough (Bloksma 1975). Glutamine is a hydrophobic amino acid, and because of its high level in gluten proteins, it will often occur on the surface of a protein. The strong tendency of the amide group of glutamine to participate in hydrogen bonding is thought to be responsible to a large extent for the cohesiveness of gluten proteins (Beckwith et al. 1963, Birnbaum 1977). Residues such as proline and phenylalanine may produce hydrophilic regions that can associate with lipids (Kasarda et al. 1971). The simultaneous binding of lipids to both gliadin and glutenin may contribute to the gas-retaining ability of gluten (Hoseney et al. 1970).

Triticale Gluten Proteins. Various investigators (Chen and Bushuk 1970, Pena 1984, Wall et al. 1972) have found that triticale flours have a considerably higher percentage of their total flour protein as soluble (non-gluten) protein than wheat. The higher levels of lysine-rich soluble proteins contribute to the nutritional superiority of triticale over wheat, but Tsen (1974) has suggested that this deficiency in gluten-like protein in triticale is partially responsible for the poorer rheological properties and baking quality of this cereal.

Triticale flours also have a lower percentage of their total flour protein as acid-insoluble residue protein compared to wheat (Chen and Bushuk 1970, Pena 1984, Wall et

al. 1972). Orth and Bushuk (1972) reported that the amount of acid-insoluble residue protein correlated positively with loaf volume in wheats of diverse baking quality and it is possible that this deficiency of residue protein in triticale gluten also contributes to the generally inferior baking quality of triticale.

Wall et al. (1972) reported that gluten isolated from the flour of triticale 6A204 contained 70% protein and accounted for 81% of the total protein in the flour. In comparison, gluten from a hard red spring wheat flour contained 78% protein, which represented 84% of the total flour protein. The triticale yielded a weak, soft gluten, and triticale gluten was unsatisfactory as an additive for improving the baking performance of a low protein flour.

Pena (1984) examined the mixing and baking characteristics of four advanced lines of secondary hexaploid triticale (4T, 11T, Impala, and Carman). The gluten content and gluten strength varied widely among the triticales. All four triticales had low gluten content and less gluten protein as a percentage of the total flour protein than a typical bread wheat. The mixing and baking properties of flours reconstituted to an equal gluten level (11.5% on a dry moisture basis) showed that the only triticale to have gluten quality comparable to the bread wheat was the Impala triticale. Interchange of gluten between Carman triticale and Marquis wheat resulted in an interchange of mixing and

baking properties, which suggests that differences in baking quality among triticales can be attributed to the gluten component of the flour.

Although there is no general agreement on the reason for differences in triticale gluten quality, the answer must ultimately lie in the way that gluten proteins interact with one another and with other macro-molecules such as lipid and polysaccharide. Both the total content of glutamic acid and aspartic acid, as well as the degree of amidation of these residues, has been shown to be lower in triticale protein than in bread wheat protein (Ahmed and McDonald 1974, Chen and Bushuk 1970, Tkachuk and Irvine 1969). Considering the importance of amide side chains in hydrogen bonding, and the fact that MacRitchie (1979) has shown a positive correlation between loaf volume and the primary amide (glutamine + asparagine) content of gluten proteins in wheat, the amide content of triticale gluten may prove to be an important factor in controlling gluten strength. Tsen (1974) reported that triticale protein also had higher sulfhydryl contents (i.e. fewer cysteine residues participating in disulfide crosslinks) than bread wheat protein, which could also be partially responsible for triticale glutens being weaker than wheat glutens.

Starch

In the wheat or triticale endosperm, starch occurs as discrete granules embedded in a protein matrix. Two types

of starch granules exist. The larger (earlier synthesized) primary granules are lenticular in shape; the smaller secondary granules are spherical and synthesized at a later stage in the spaces between the large granules (MacRitchie 1980). There is a continuum of granule sizes from the small spherical to the large lenticular, but wheat and triticale both show a bimodal distribution of granule size (Berry et al. 1971).

The starch granule contains two carbohydrate polymers. Amylose is an essentially linear polymer of α-1,4 linked glucose. Amylopectin is a much more highly branched glucose polymer; α-1,4 linkages predominate but branching is introduced by α-1,6 linkages.

When grain is milled into flour, a portion of the starch granules may be cracked, fractured, or otherwise damaged. Intact starch granules absorb about one-half their own weight in water, while damaged starch granules absorb almost two times their own weight in water (Bushuk 1966). As the level of damaged starch in a given flour is increased, more water is therefore required to produce a dough of constant consistency.

In the oven, starch granules undergo marked changes. As the temperature in the dough increases, granules begin to swell and gelatinize (Hoseney et al. 1978). The starch granules continue to take up water from the gluten during baking, allowing the gluten to set and become rigid (Tipples

1982). For this reason, starch granules are important to crumb grain and texture.

In a study of various non-wheat starches in breadmaking, Hoseney et al. (1971) found that wheat starch could be replaced by barley or rye starch with no adverse effect on loaf quality, indicating that wheat starch is not entirely unique in its baking properties. The general conclusion seems to be that, although contributing to water absorption and dough consistency, starch is not as critical as the gluten proteins for optimum loaf volume. Prior to gelatinization, the role of starch is to dilute the gluten to an optimum level, and provide yeast with fermentable sugars through amylase activity.

## The Amylase Enzymes

There are two main starch degrading enzymes in cereals. Alpha-amylase is an endoenzyme that hydrolyzes the α-1,4 glucosidic linkages at random within linear regions of starch molecules, producing lower molecular weight dextrins. Beta-amylase (which by itself is incapable of attacking starch granules) further degrades these dextrins by progressively removing maltose from the non-reducing end.

There are at least three areas in which starch and amylases are important in breadmaking, and these are:

a) in the production of fermentable carbohydrates that will support yeast growth,

b) in the determination of baking absorption, and

c) in the production of dextrins during baking.

Production of Fermentable Sugars. During dough fermentation, α-amylase and β-amylase work together, with α-amylase solubilizing the granular starch and opening up new sites of attack for β-amylase. At optimum levels, these enzymes will provide fermentable sugars that will support yeast growth and ensure adequate carbon dioxide production for good sized, well aerated bread (Tipples 1982).

Flours milled from sound wheat contain an abundance of β-amylase and a low variable level of α-amylase (Fox and Mulvihill 1982). For best baking results, it is common practice to supplement wheat flours with malt or fungal amylases to increase the amylase activity to an optimum level.

Determination of Baking Absorption. Water in dough is absorbed by various flour components. Bushuk (1966) has calculated that for a typical flour containing 14% protein and having 15% of its total starch granules as damaged starch, each of the following components - intact starch, damaged starch, gluten protein, and pentosans - will absorb about one-quarter of the total water in the dough.

Undamaged starch is relatively resistant to attack by α-amylase at fermentation temperatures, while damaged starch is very susceptible. When damaged starch granules are degraded by amylases, the water that was held by the granules

is released. As a consequence, fermenting doughs with high α-amylase activity and/or high starch damage may become sticky and difficult to machine (Bloksma 1971, Tipples 1982). Under such circumstances, the baker must either reduce the fermentation time (i.e. reduce the amount of time the amylases have to degrade the damaged starch) or reduce the amount of water added to the flour (the baking absorption) in order to avoid handling problems in the later stages of dough make-up. Reducing the baking absorption is the less desirable alternative in a commercial bakery since bread is sold on a weight basis and the more water that can be added, the greater the margin of profit.

Production of Dextrins. Although intact starch granules contribute to water absorption, they can be considered as an inert filler in fermenting dough. Once undamaged starch granules gelatinize in the oven, however, they become readily available for amylase degradation. It is only in the time interval between the onset of gelatinization and the thermal inactivation of amylases during baking that starch breakdown is greatly enhanced (Bloksma 1971).

At 75° C, β-amylase is completely inactivated while α-amylase still retains 50% of its original activity (Walden 1955). In the oven, the greater thermal stability of α-amylase allows it to act on gelatinizing starch another 2 or 3 minutes after β-amylase is inactivated. Instead of maltose being the main hydrolysis product, as is the case

when α- and β-amylases work together, there is an increase in the production of dextrins. A certain level of dextrin production will improve the crust color and flavor of bread through browning reactions that occur in the oven (Bertram 1953), but excessive α-amylase activity will produce an excessive level of dextrins resulting in a highly colored loaf with a wet and sticky crumb (Buchanan and Nicholas 1980).

Because undamaged starch is made available for amylolytic attack by the onset of gelatinization, the production of dextrins in the oven is a function of α-amylase activity alone and is not influenced by the level of damaged starch. Unlike baking absorption, dextrin production in the oven is therefore beyond the control of the baker.

The Effect of α-Amylase Activity on the Baking Quality of Triticale Flours. Triticale flours lack the gluten protein quantity and quality of wheat flours and therefore depend to a greater extent on the gelatinization characteristics of the starch for good baking performance (Welsh and Lorenz 1974). The crumb characteristics of a loaf of bread are determined in part by the amount of water bound by the starch during gelatinization, and because this water binding capacity is affected by α-amylase activity, the levels of α-amylase in a triticale flour can be especially critical in loaf quality.

Lorenz (1972) added an enzyme inhibitor to decrease the

α-amylase activity of two spring triticale flours (6TA204 and 6TA206), and found that bread baked with these flours showed an improvement in overall quality. Breads showed increased loaf volume and more uniform grain when α-amylase inhibitor was added to the flour.

Welsh and Lorenz (1974) examined the effect of location on the α-amylase activity and baking performance of the winter triticale TR131. This cultivar was grown at six different sites in Colorado, and the α-amylase activity of the flours was measured by peak amylograph viscosity (A.V.). Alpha-amylase activity of the triticale samples varied widely, with A.V.'s ranging from 20 to 840 BU. Two hard red winter wheat cultivars grown as checks at each site (Scout and Lancer) had much lower α-amylase activity; all wheat flours had A.V.'s exceeding 2000 BU. Triticale flours from all sites produced bread with a more open crumb and a coarser texture than wheat flours, and the two triticales with the highest α-amylase activity produced bread with a wet and gummy crumb. It is worthwhile to note here that wheat flour used for baking bread is usually malted to a level that will give an amylograph reading 500 to 600 BU (Watson 1984).

Lorenz and Welsh (1977) evaluated the α-amylase activity and baking performance of seven semi-dwarf secondary triticales developed at CIMMYT, along with the semi-dwarf hard red spring wheat, Colano. The triticale flours had much higher α-amylase activity (A.V.'s of 20 to 300 BU) than the

Colano wheat (A.V. of 2660 BU). Two triticale flours, Rahum and RF6012 (with A.V.'s of 120 and 300 BU, respectively) produced bread with very satisfactory volumes and good crumb characteristics in spite of their high α-amylase activity. The remaining five flours, however, produced bread with an open grain and gummy crumb.

## The Protease Enzymes

Analogous to the amylase enzymes, there are two main types of protein degrading enzymes in cereals. The endoproteases cleave a limited number of peptide bonds within proteins to produce shorter peptides. Exoproteases (both amino- and carboxypeptidases) will further degrade peptides by sequentially removing terminal amino acids.

Wheat Proteases. Glutens isolated from sound wheat become weaker after they are incubated with proteases (Hanford 1967, Kruger 1971, Redman 1971). Hanford (1967) found that the proteolytic activity measured by the release of trichloroacetic acid-soluble nitrogen from hemoglobin did not correlate well with gluten softening. It has since been shown that most of this hemoglobinase activity in wheat is due to the exoproteolytic action of carboxy peptidases (Kruger and Preston 1977). Redman (1971) reported extensive proteolytic softening of gluten even though very few peptide bonds were broken, and showed that this softening correlated well with protease activity measured on an azocasein substrate. Today it is generally accepted that the gluten

softening azocaseinases are actually endoprotease enzymes.

Kruger (1971) incubated glutens with various fungal, mammalian, and cereal proteases and recorded changes in the physical properties of the glutens. Increasing concentrations of proteolytic enzymes caused a progressive decrease in gluten consistency on the farinograph and reduction of bubble size on the alveograph. A number of other enzymes such as α-amylase and lipase had no effect on gluten properties. Although all tests were conducted on isolated gluten, Kruger suggested that similar changes would occur in doughs with incubation with proteolytic enzymes.

Triticale Proteases. Madl and Tsen (1973) determined the hemoglobinase activity of six triticales (including two advanced CIMMYT lines, Bronco and Armadillo), four wheats, and two ryes. The proteolytic activity of the triticale flours was higher than that of the wheat flours, and similar to that of the rye flours. All triticale, wheat, and rye cultivars had higher hemoglobinase activity in the bran than in the flour.

Singh and Katragadda (1980) determined the hemoglobinase activity and baking performance of six triticales, one wheat, and one rye. Results of the hemoglobinase assay were similar to those of Madl and Tsen (1973) in that triticale flours had higher hemoglobinase activity than the wheat flour. As well, all cereals had highest hemoglobinase activity in the bran, followed by whole grain and then flour.

Singh and Katragadda (1980) also found a significant negative correlation between loaf volume and flour hemoglobinase activity among the triticales ($r = -0.85$, $P = 0.05$), and suggested that triticale flours with low proteolytic activity will produce bread of acceptable quality.

To date, there are no reports in the literature on the "gluten softening" azocaseinases (endoproteases) of triticale, or on the effect of proteases on the physical properties of triticale gluten. Although Madl and Tsen (1974) reported that the pH optimum, heat stability, and molecular weight of a triticale homoglobinase enzyme were similar to values reported in the literature for wheat hemoglobinases, the triticale proteolytic enzymes remain largely uncharacterized.

## C. The Amylase and Protease Enzymes of Maturing Wheat and Triticale

### The Morphology of Developing Kernels

Triticale kernels closely resemble rye and wheat kernels in structure and general morphology (Simmonds 1974a). The embryo (germ) is attached through the scutellum to the endosperm. The embryo and endosperm are enclosed by the remains of the nucellar tissue and an outer pericarp, which surrounds the entire seed and adheres closely to it.

The nucellar tissue and pericarp, which comprise a major portion of the seed at anthesis, degenerate as the seed matures (Simmonds and O'Brien 1981). The nucellar

tissue is crushed by the enlarging endosperm between 6 and 8 days post anthesis (d.p.a.), and eventually forms the seed coat and pigment strand. Together, the seed coat and pigment strand form a waxy, water repellent layer completely surrounding the embryo and endosperm. The pericarp, initially a fleshy organ containing several layers of cells rich in starch, plays an important role in providing nutrients to the seed during its early development. Pericarp starch degradation and cell lysis begins at about 10 d.p.a. and is completed by about 22 d.p.a. The pericarp gives rise to the outer bran or husk in the mature seed.

Between 10 and 15 d.p.a., the outer layer of the endosperm begins to differentiate into the aleurone (Fulcher et al. 1972). By 30 d.p.a., the aleurone cell walls have thickened considerably, and the aleurone cells can be seen as a separate and clearly identifiable layer surrounding the endosperm. As in wheat, the aleurone layer of triticale is rarely more than one cell wide, although the aleurone layer of triticale can be much more irregular in shape than the aleurone layer of wheat (Simmonds 1974a).

At full maturity, only the aleurone layer and the embryo remain viable; the starchy endosperm and outer bran layers are dead tissue. During germination, gibberellins promote the <u>de novo</u> synthesis of α-amylases and endoproteases by the aleurone (Preston and Kruger 1979, Radley 1979). These enzymes are released into the endosperm, hydrolyzing the storage protein and starch into amino acids and

sugars which can then be translocated to support the growth of the developing embryo.

## The Amylase Enzymes

The Amylases of Maturing Wheat. There are two major groups of α-amylase in wheat. Ungerminated wheat contains "green" (α-I) α-amylases which appear in the pericarp in the early stages of kernel development and then decrease with maturation (Kruger 1972a). Presumably this pericarp α-amylase, along with β-amylase, digests the starch granules in the pericarp to provide sugars to the seed during its early development. When wheat is germinated, a second group of electrophoretically distinct "malt" (α-II) α-amylases appear in the kernel (Kruger 1972b, Olered and Jonsson 1970).

While the bulk of the α-amylase in immature wheat kernels is found in the pericarp, β-amylase is found mainly in the endosperm and increases in quantity during maturation (Kruger 1972a). Most of the β-amylase is bound in a latent, inactive form to glutenin (Rowsell and Goad 1962b). During germination, the increase in β-amylase activity in the endosperm occurs by release of pre-existent bound enzyme and not by de novo synthesis (Rowsell and Goad 1962a).

In addition to endosperm β-amylase, there is a minor β-amylase in wheat which appears in the pericarp along with the green α-amylase and then disappears in the later stages of kernel development (Kruger 1972a). As previously mentioned, it is presumed that this β-amylase will contribute

to starch breakdown in the pericarp.

The Amylases of Maturing Triticale. Triticale is generally higher in α-amylase activity throughout kernel development than wheat (Agrawal 1977, Dedio et al. 1975, Jenkins and Meredith 1975, Klassen et al. 1971, Lorenz and Welsh 1976, Pena and Bates 1982). Dedio et al. (1975) examined the distribution of α-amylase in the developing kernels of four triticales, two wheats, and one rye. The triticale cultivars included two primary hexaploids (6A190 and 6A250) and two advanced lines from the international screening nursery at CIMMYT (Beaver and Kangaroo). Alpha-amylase activity in the pericarp reached a maximum at approximately 10 to 15 d.p.a. in all cereals. However, the α-amylase activity in the pericarp of the triticales was higher and usually lasted for a longer period than the α-amylase activity in the pericarp of the wheat and rye cultivars. With the exception of primary triticale 6A190, α-amylase activity in the aleurone and endosperm of all the samples remained low throughout development. In triticale 6A190, α-amylase activity in the aleurone and endosperm increased dramatically between 20 and 25 d.p.a.

Silvanovich and Hill (1977) found that in addition to green (α-I) α-amylases, maturing triticale 6A190 contained malt (α-II) α-amylases which appeared as early as 10 d.p.a. These malt amylases were produced by the parental and durum and rye cultivars only after germination. Both α-I and α-II

amylases of triticale 6A190 were characterized and were very similar in pH optima, thermal stabilities, and isoelectric points to the corresponding enzymes in barley and wheat.

Most studies have indicated that immature wheat contains only green α-amylases (Kruger 1972a, Marchylo et al. 1976, Olered and Jonsson 1970). However, there is recent evidence that the immature kernels of many "normal" wheat cultivars (i.e. cultivars showing no visible signs of preharvest sprouting) also contain traces of malt α-amylases in the aleurone and endosperm (Daussant et al. 1980, Marchylo et al. 1980a). Marchylo et al. (1980b) have concluded that the immature wheat kernel is capable of synthesizing malt α-amylase, but that this synthesis is normally suppressed except for a small amount of "leakage" before germination. Apparently, this leakage is much more severe in triticales such as 6A190.

Gale et al. (1983) investigated the genetic and environmental factors that regulate this malt α-amylase production in maturing wheats. Several wheat varieties which were either resistant or susceptible to sprouting were grown in controlled environments under a slow drying treatment (96% relative humidity) and a fast drying treatment (56% relative humidity). The grains were sampled throughout maturation and tested for α-amylase content and distal half seed α-amylase production after incubation with gibberellic acid

(GA). Alpha-amylase production before maturity was enhanced by slow drying conditions, although increases in enzyme activity were less marked for sprouting-resistant varieties. The only wheat genotypes that did not show any premature α-amylase production were two Tom Thumb lines of dwarf wheat. The Tom Thumb wheats are known to carry a gene which renders the aleurone (and scutellum) relatively insensitive to GA (Gale and Marshall 1975). Based on their observations, Gale et al. proposed a model to account for malt α-amylase production in developing wheats, which involved three factors: a) the attainment of aleurone (and scutellum) competency to respond to GA during ripening, b) the availability of a promoter such as GA in the grain at the same time, and c) the availability of adequate grain moisture to allow the production of hydrolases to proceed.

Very little is known about the β-amylase levels in maturing triticale, although Agrawal (1977) reported that β-amylase activity was very high in the two triticales he studied. This finding is not surprising since ungerminated wheat contains an abundance of β-amylase at maturity (Fox and Mulvihill 1982).

The Regulation of α-Amylase Activity in Maturing Triticale. The de novo synthesis of α-amylase by the aleurone is promoted by GA and blocked by abscisic acid (ABA) in wheat (Radley 1979). King et al. (1979) investigated the possibility that α-amylase production in maturing triticale

6A190 may be regulated by a balance between endogenous GA and ABA levels. They observed that although ABA levels declined rapidly after 22 d.p.a. at a time when α-amylase activity was increasing, the levels of ABA were no lower than those reported for "normal" cereal cultivars. As well, stimulation of α-amylase production by exogenous GA was only evident in the almost mature grain (30 to 40 d.p.a.) and then only if the kernels were first artificially dried. King et al. did point out, however, that triticale 6A190 may be less sensitive to growth hormones than other triticale cultivars.

Inhibitors of native α-amylase may also play a regulatory role. Weselake et al. (1985) recently isolated a protein inhibitor from barley that could complex with malt (α-II) α-amylase, preventing the enzyme from attacking starch granules. This inhibitor showed partial immunochemical identity with a protein in a triticale cultivar, suggesting that the triticale also contained an endogenous α-amylase inhibitor. Warchalewski (1977) has suggested that the dissociation of α-amylase-inhibitor complexes already present in mature seeds might supplement de novo synthesis of this enzyme in the early stages of germination. Similarly, premature dissociation of such complexes could explain the appearance of malt α-amylases in some developing triticale kernels.

Microscopic examination of developing triticale 6A190

has shown that *in situ* degradation of starch granules due to premature release of α-amylase into the endosperm is quite localized and confined to the subaleurone layer (Dronzek et al. 1974). Furthermore, α-amylase damage in this triticale is often associated with specific lesions in the endosperm or aleurone cells (Dedio et al. 1975). Cytogenetic studies have shown that incompatability of wheat and rye chromosomes in the triticale genome can lead to mitotic division errors and patches of aberrant nuclei at the earliest stages of endosperm development (Bennett 1977). As a result, internal cavities form close to the surface under the aleurone as well as deep within the tissue of the endosperm. These abnormalities not only reduce the number of endosperm cells and limit the capacity of the kernel to accumulate as much starch and protein as possible, but may also give the triticale kernel a shrivelled appearance as the moisture content falls and the internal cavities collapse (Thomas et al. 1980). Pena et al. (1982) have suggested that collapse of the pericarp seed coat, and aleurone layers into the endosperm cavities during kernel dessication could physically disrupt aleurone cells, thereby releasing α-amylase to attack starch granules in adjacent endosperm cells.

## The Protease Enzymes

**The Proteases of Maturing Wheat.** In developing wheat kernels,

both the endoprotease and exoprotease activities increase in the outer kernel layers (pericarp, seed coat, and aleurone) until approximately 20 d.p.a., and then decline rapidly (Kruger 1973, Preston and Kruger 1976). Presumably this protease activity in the pericarp at this early stage of kernel development produces amino acids that are translocated to the endosperm for storage protein synthesis.

There are also exoproteases which appear in the endosperm about 16 d.p.a. and continue to increase (on a per kernel basis) until maturity (Kruger 1973, Preston and Kruger 1976). This exoprotease activity is due almost entirely to carboxy peptidases that are synthesized and deposited in the endosperm in the presence of endogenous inhibitors (Kruger and Preston 1977, Preston and Kruger 1976).

It is not known how these endosperm exoproteases are prevented from seriously degrading storage proteins in the later stages of kernel development. They may be deposited as inactive enzyme-inhibitor complexes or as inactive proenzymes that require limited proteolytic degradation for full activity. Kruger and Preston (1977) have suggested that, like the amylase system where limited attack of starch granules by α-amylase is required before the substrate is susceptible to β-amylase, the exoproteases may simply require some initial cleavage of storage protein by endoproteases before they are fully active.

Kruger (1973) reported that Prairie Pride, a poor baking quality wheat, had higher endoprotease activity throughout maturation than Marquis, a wheat of excellent baking quality. He suggested that high protease activity in developing kernels might alter the nature of storage proteins being laid down at the same time, and as a consequence, the ultimate quality of wheats such as Prairie Pride.

Bushuk et al. (1971) reported that the exoprotease (hemoglobinase) activity in maturing wheats declined gradually during maturation (on a dry weight basis). This trend is opposite to the previously discussed findings of Preston and Kruger (1976), who reported that the hemoglobinase activity in wheats increased with maturation. However, one must take into account that the results of Preston and Kruger were expressed in terms of activity per kernel. Because the dry weight of the kernel increases during maturation, the activity per unit weight might have been either constant or decreasing.

The Proteases of Maturing Triticale. Lorenz and Welsh (1976) found that the hemoglobinase (exoprotease) activity of two tall spring triticales, two semi-dwarf triticales, one rye, and two durum wheats decreased steadily from initial kernel development to full maturity (on a dry weight basis). A comparison of proteolytic activities at full maturity showed the rye to be highest, followed by the

triticales, and then the durum wheats.

To date, there is no report in the literature on the changes in azocaseinase (endoprotease) activity in maturing triticale, or on the location of proteolytic enzymes in developing triticale kernels.

# III. MATERIALS AND METHODS

## A. Grain and Flour Samples

Of the four secondary hexaploid (2n = 42) triticales used in this study, three (4T, 11T, and Impala) were obtained from CIMMYT, Mexico, and all three had wheat chromosome 2D substituted for rye chromosome 2R (Gustafson 1982). The fourth triticale was the licensed Canadian cultivar Carman, which has all seven pairs of rye chromosomes present. Carman was selected at the University of Manitoba from a triticale line (Beagle) introduced from CIMMYT in 1975 (Gustafson et al. 1982). Pedigrees of the triticales are listed in Table 1. One Canadian hard red spring wheat (cv. Marquis) was included in the study for comparison.

All cultivars were grown at the University of Manitoba during the growing seasons of 1983 and 1984. Five plots of each cultivar were planted, each plot consisting of four 3-meter rows. Approximately 1600 heads per cultivar were tagged on the day of anthesis, and 50 to 70 heads were collected randomly throughout the plots in the early morning at 21, 28, 32, 36, 40, and 44 days post anthesis. Ten grams of fresh seed (about 5 heads) were immediately threshed by hand for fresh weight and moisture determinations. The remaining heads were frozen (-20$^{o}$ C) and later freeze dried to a moisture content of 6%.

TABLE 1. Pedigrees of the triticale cultivars.[1]

| Cultivar | Pedigree |
|---|---|
| 4T | W74.104-Addax/Bgl"s" - $M_2A$ X IRA X-33470-c-1Y-3M-2Y-2M-0Y |
| 11T | Trr"r"-Mpe/Pnd"s" - $M_2A$ X IRA X-47220-A-2M-1Y-1Y-0H |
| Impala | Bgc - Bulk $E_2A$ X-11066-A-6M-100Y-100B-101Y-0Y |
| Carman | UM'5'/tc1 Bulk X 1503A X-12M-5N-1M-0Y |

[1]Pedigree identification system of CIMMYT
A = Armadillo (X308 outcross to bread wheat)
IRA = (Inia bread wheat X rye)$^2$ X Armadillo
$M_2A$ = Maya X Armadillo

Dried heads were threshed on a single head roller thresher and cleaned using an Erikson column blower. For each sample, 50 g of cleaned, dried kernels were ground in a Udy cyclone mill (1.0 mm screen), and the grist was then homogenized and stored at 4° C for protein and enzyme analysis.

After plots had been sampled for the maturation study, the remaining grain was harvested by combine at full maturity (harvest ripeness). Seed was cleaned on a Carter dockage tester using a 6/64 (.094) X 3/4 inch sieve. Fifty gram samples from each line of bulk seed were ground in the cyclone mill as described above for protein and enzyme analysis. Two kilogram samples of bulk seed were then milled into flour on a Buhler pneumatic laboratory mill. Triticales and wheat were tempered overnight to 14.5 and 15.5% moisture, respectively before milling, and flour was rebolted through a 70 gg (236 μ) screen and blended prior to use.

## B. Moisture

The moisture content of the freshly harvested kernels and whole grain meal was determined by drying to constant weight in a 110° C oven. The moisture content of the bulk seed (250 g samples) was determined with a CAE model 919 moisture meter. The moisture content of the flour was determined on the Brabender Rapid Moisture Tester (130° C for 1 h).

## C. Protein

Protein (N X 5.7) was determined by the macro Kjeldahl method of Williams (1973) using a $TiO_2$ containing catalyst and 1.0 g samples of flour or whole meal.

## D. Alpha-Amylase Activity

### Instrument Operation

The Perkin-Elmer model 191 Grain Amylase Analyzer was operated according to the method of Campbell (1980) as modified by Kruger and Tipples (1981). Substrate (beta limit dextrin), standard enzymes, extracts, and all dilutions described below were prepared with 0.05 M sodium acetate buffer (pH 5.5) containing 1 mM $CaCl_2$.

### Preparation of Beta Limit Dextrin

Beta limit dextrin was prepared according to the method of Kruger (1972a), using amioca pearl starch (American Maize Products). Prior to freeze drying, the dextrin solution was boiled for 5 min to inactivate the sweet potato β-amylase (Sigma). Working substrate was prepared by adding 0.5 g of beta limit dextrin per 100 ml of acetate buffer and bringing the solution to a full boil. The substrate was then cooled to room temperature with stirring and centrifuged at highest speed in a bench top clinical centrifuge for 5 min. Substrate solutions prepared this way and stored overnight at $4^{o}$ C had nephelos readings of 35 to 40, and were prepared fresh daily.

Enzyme Extraction

Whole meal or flour samples (1.0 g) were extracted in 5.0 ml of acetate buffer at room temperature for 1 h on a model 150 V variable speed multipurpose rotator (Scientific Industries) at 9 r.p.m. Suspensions were filtered (Whatman GF/C glass fibre paper) and filtrates were stored overnight at 4° C. Appropriate dilutions of filtrates were then analyzed. Because the nephelos reading of the substrate varied slightly from day to day, fungal α-amylase (Aspergillus oryzae, Miles Scientific) was used as a reference. When stored in the freezer, this enzyme is known to be very stable for long periods of time. Standard curves were linear up to 0.4 µg fungal amylase/ml (0.08 µg/200 µl).

## E. Exoprotease Activity

Exoprotease (hemoglobinase) activity was determined by a modification of the method of Bushuk et al. (1971). The substrate was 1% hemoglobin (bovine type II, Sigma) in 0.02 M sodium acetate buffer (pH 4.5). Whole meal or flour samples (0.1 g) were suspended in 5.0 ml of substrate and incubated at 37° C for 2 h with vortexing at half hour intervals. The reaction was stopped with 5.0 ml of cold 10% trichloroacetic acid (TCA) and clarified by centrifugation (27,000 X *g* for 10 min). Appropriate aliquots of supernatant were made up to 2.0 ml with the same acetate buffer, and analyzed for TCA-soluble nitrogen according to the method of Moore and Stein (1954), using 2.0 ml of ninhydrin solution.

The ninhydrin solution was made using commercially available ninhydrin (Pierce) and hydrindantin (Pierce). Blanks were determined by incubating samples (0.1 g) in 5.0 ml of 10% TCA, adding 5.0 ml of substrate after 2 h and proceeding as for enzyme digests. Nitrogen content of the diluted supernatants was determined using glutamate (0-30 µg/2 ml acetate buffer) to obtain the standard curve.

## F. Endoprotease Activity

Endoprotease (azocaseinase) activity was determined by a modification of the method of Preston et al. (1978). The substrate was 1.4% azocasein (Sigma) in 0.05 M McIlvaines citric acid-disodium phosphate buffer (pH 6.0). Substrate was dialyzed overnight at 4° C against the same McIlvaine's buffer (approximately 10X the volume of the substrate) before use to reduce the substrate blank. Whole meal or flour samples (1.2 g) were extracted in 7.0 ml of 0.05 M sodium acetate buffer (pH 5.5) at 4° C for 1 h on a variable speed multipurpose rotator (9 r.p.m.). Suspensions were filtered (Whatman GF/C paper) and 0.5 ml aliquots of the filtrate were immediately incubated with 3.5 ml of dialized substrate at 40° C for 4 h, with vortexing at 1 h intervals. The reaction was stopped with 5.0 ml of cold 10% TCA. After centrifugation (24,000 X *g* for 15 min), cold 0.5 N sodium hydroxide was added to an equal volume of supernatant and the solution was allowed to sit for 20 min before reading the absorbance at 440 nm. Blanks

were determined by incubating enzyme and substrate solutions separately, adding TCA to the enzyme solution, adding the substrate solution, and proceeding as above. Azocaseinase activity is described as the change in absorbance at 440 nm per h per g of dry sample. According to Kruger (1973), this assay is linear only up to 0.1 absorbance units.

## G. Additional Tests on Bulk Grain Harvested at Maturity

### Test Weight

Test weight was determined using an Ohaus Test Weight Apparatus and 0.5 liter measure. Results are given in kilograms per hectaliter and are reported on an "as is" moisture basis.

### Thousand Kernel Weight

Thousand kernel weight (MKW) was determined by counting the number of seeds in 20 g of grain from which all broken seeds and foreign material had been removed. The weight if 1000 kernels was calculated and is reported on a 14% moisture basis.

### Kernel Hardness

Grain was equilibrated at 21$^{o}$ C, 64% relative humidity, for two weeks. Twenty gram samples were ground for 20 seconds on a Strong Scott barley pearler as described by Obuchowski and Bushuk (1980). The pearling resistance index (PRI) is the weight of the pearled grain in grams.

Falling Number

Falling numbers of whole meal samples were determined according to A.A.C.C. method 56-818 (1976).

## H. Additional Tests on Flours

Ash

Ash content was determined according to A.A.C.C. method 08-011 (1976).

Damaged Starch

Damaged starch was determined according to the method of Farrand (1964).

Sedimentation Test

Zeleny sedimentation values were determined according to A.A.C.C. method 56-60 (1976).

Farinograph Test

Farinograms were obtained according to A.A.C.C. method 54-21 (1976) using a constant flour weight of 50 g (14% moisture basis).

Extensigraph Test

Extensigrams were obtained according to A.A.C.C. method 54-10 as modified by Holas and Tipples (1978). Water was added to equal farinograph absorption, and triticale and wheat doughs were mixed for 2 and 5 min, respectively, in a large farinograph bowl. Doughs were stretched at 45 and 135 min, but measurements are reported only for the 135 min

curves. Areas under the curves were measured with a compensation polar planimeter (Sokkisha Limited of Tokyo).

Straight Dough Bake Test

Baking performance was evaluated by the A.A.C.C. straight dough method 10-10 (1976). The baking formula included 100 g flour (14% moisture basis), 3 g fresh yeast, 1 g NaCl, and 5 g sucrose. No malt was added. Water was added to farinograph absorption, and triticale and wheat doughs were mixed for 2 and 5 min, respectively, in a GRL mixer. These conditions were considered to be optimum for dough development and handling. Loaf volume was determined by rapeseed displacement.

Gluten Content

Glutens were isolated from flours using a Glutomatic 2100 (Falling Number of Sweden) and 80 micron sieve. Flour (10.0 g) and a 2% NaCl solution (4.6 ml for 4T, 11T, and Carman; 4.9 ml for Impala and Marquis) were mixed into a dough for 20 sec. Doughs were then washed for 12 min using tap water (approximately 500 ml in total) to remove starch and water solubles.

Glutens from three 1984 triticale samples - 4T, 11T, and Carman - could not be isolated using the Glutomatic. Doughs of these samples were prepared on the Glutomatic as described above but were washed by holding the mixing chamber under a slow drip of tap water while hand kneading.

For dry weight determination, gluten balls were first

freeze dried and then dried to a constant weight in a 110° C oven. Dried gluten balls were crushed in a Carver press and ground to a fine powder with a mortar and pestle. This powder was dried overnight at 110° C and 0.25 g samples were analyzed for protein as described in Section C.

## I. Statistical Analysis

Analysis of variance was used to test the data. Least significant differences (LSD) were computed at the 5% confidence level. Differences among means were compared by using the Duncan's multiple range test. All mathematical formulas used in the calculations were obtained from the text of Little and Hill (1978).

# IV. RESULTS AND DISCUSSION

## A. Changes in the Developing Triticale and Wheat Kernels

### Moisture Content

The moisture content of the developing kernels is shown in Figure 1. Moisture loss from the grain was more rapid in 1983 than in 1984 for any given cultivar. This was probably due to the lower amount of rainfall in the 1983 growing season (Table 2).

### Fresh Weight Per Kernel

The fresh weight of the developing kernels is shown in Figure 2. In the dry 1983 growing season, the fresh weight per kernel decreased steadily from 21 to 36 days post anthesis (d.p.a.) and remained relatively constant thereafter. In 1984, the fresh weight per kernel reached a maximum at 28 d.p.a. and then decreased steadily from 28 d.p.a. to 44 d.p.a.

### Whole Grain Protein

The protein content of the developing kernels is shown in Figure 3. Accumulation of protein in the grain ceased at about 32 d.p.a. in both 1983 and 1984. Although reported here on a dry weight basis, the same result was found when protein values were calculated on a per kernel

Figure 1. Changes in moisture content during kernel development.

Triticale

4T

Carman

11T

Impala

Hard red spring wheat

Marquis

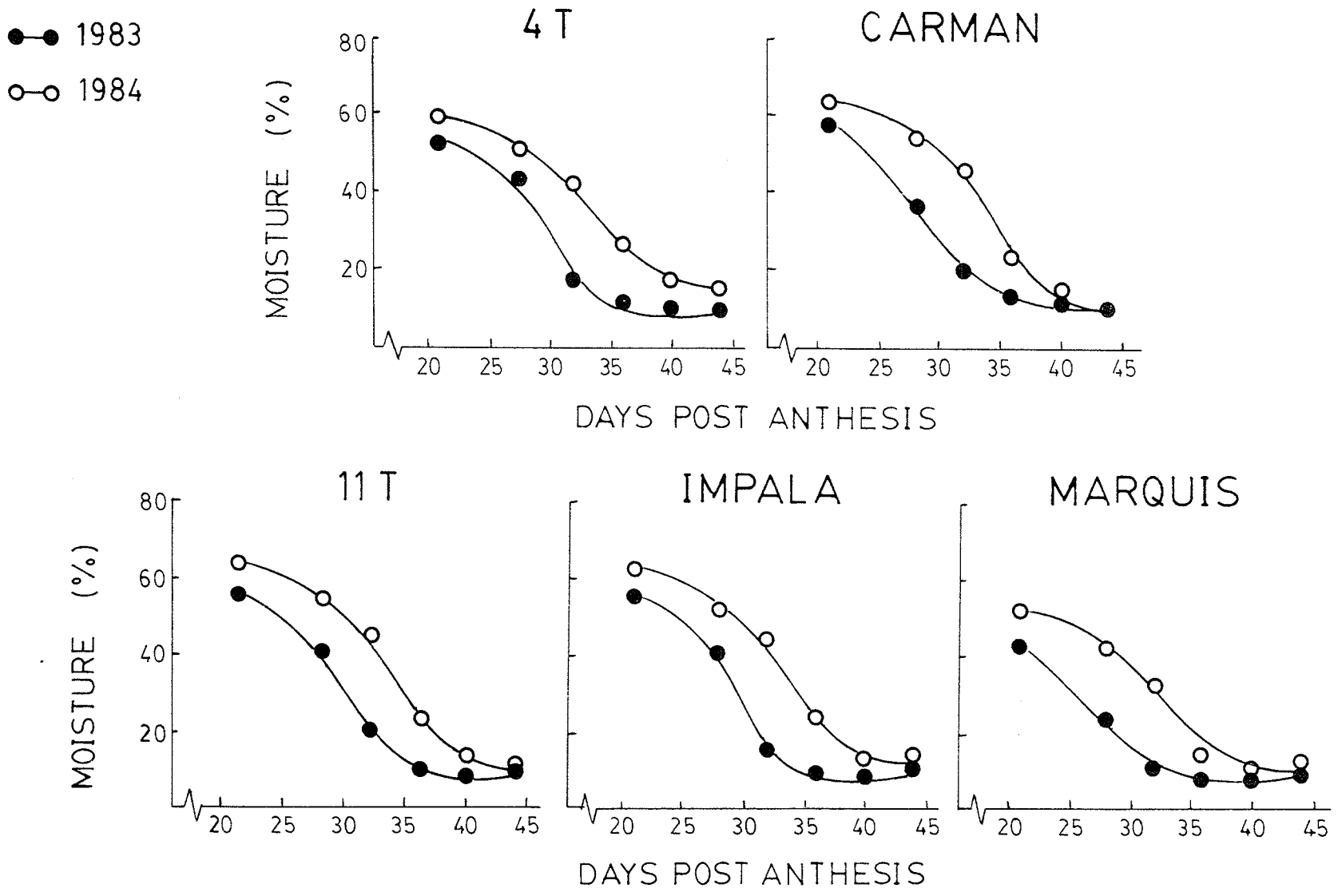

1983
1984
4 T
CARMAN
11 T
IMPALA
MARQUIS
MOISTURE (%)
80
60
40
20
20 25 30 35 40 45
DAYS POST ANTHESIS

TABLE 2. Temperature and rainfall data for the 1983 and 1984 growing seasons.[1]

| | Temperature (°C) | | |
|---|---|---|---|
| | Maximum | Minimum | Rainfall (mm) |
| 1983 | | | |
| May | 16.0 | 1.5 | 23.0 |
| June | 23.6 | 12.1 | 95.5 |
| July | 28.4 | 16.9 | 41.8 |
| August | 30.8 | 17.5 | 37.2 |
| | | | 197.5 |
| 1984 | | | |
| May | 18.6 | 5.4 | 28.2 |
| June | 24.0 | 13.7 | 226.8 |
| July | 28.0 | 16.0 | 31.5 |
| August | 29.8 | 15.8 | 20.6 |
| | | | 307.1 |
| Normal[2] | | | |
| May | 18.0 | 4.5 | 63.2 |
| June | 23.1 | 10.5 | 80.1 |
| July | 25.9 | 13.3 | 75.9 |
| August | 24.7 | 11.8 | 75.2 |
| | | | 294.4 |

[1]Approximate dates (all lines included):
planting - May 5
heading - July 1-10
anthesis - July 5-13
harvest - August 23

[2]Environment Canada Annual Meteorological Summary (1984) for Winnipeg, Manitoba.

Figure 2. Changes in fresh weight during kernel development.

Triticale

4T

Carman

11T

Impala

Hard red spring wheat

Marquis

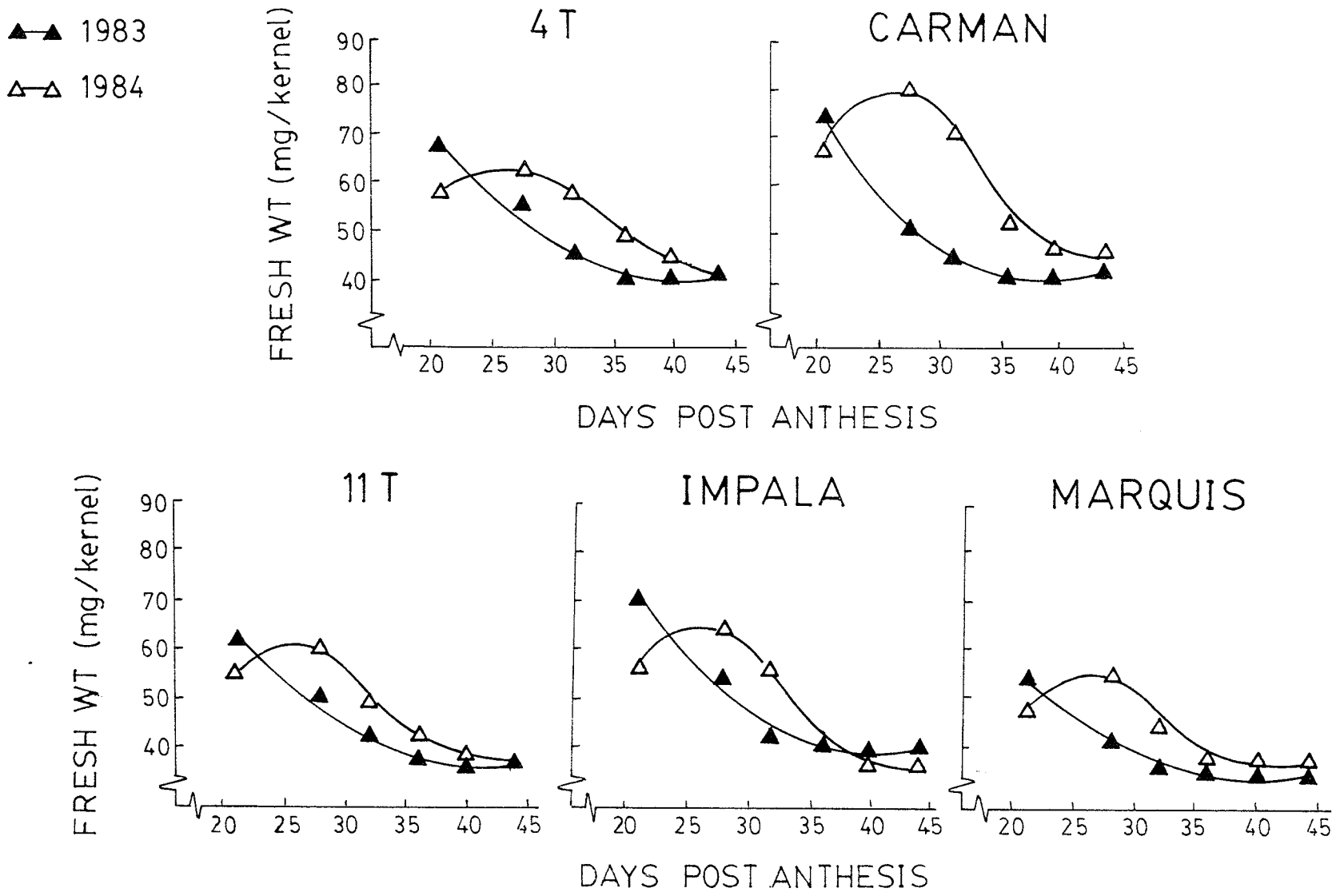

1983
1984
4 T
CARMAN
11 T
IMPALA
MARQUIS
FRESH WT (mg/kernel)
DAYS POST ANTHESIS
90
80
70
60
50
40
20
25
30
35
40
45

Figure 3. Changes in whole grain protein (N X 5.7) content during kernel development.

Triticale

4T

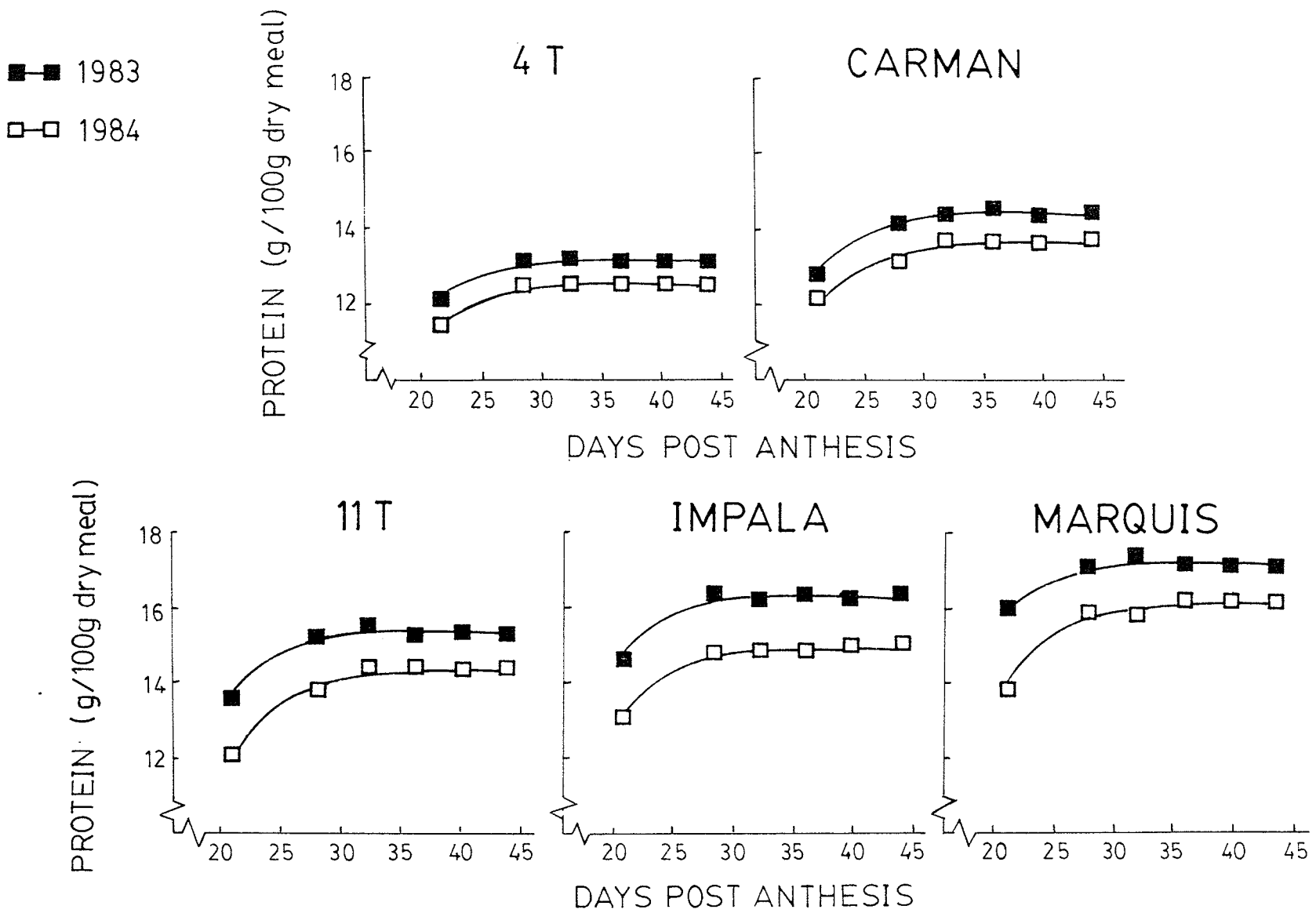
Carman

11T

Impala

Hard red spring wheat

Marquis


1983
1984
4 T
CARMAN
11 T
IMPALA
MARQUIS
PROTEIN (g/100g dry meal)
DAYS POST ANTHESIS
18
16
14
12
20
25
30
35
40
45

basis. Whole grain protein contents were higher in 1983 than in 1984 for any given cultivar.

Protein values shown in Figure 3 are total nitrogen multiplied by 5.7. No attempt was made in the present study to separate total nitrogen into protein incorporated versus nonprotein nitrogen, although it was realized that early samples in particular would contain higher levels of free amino acids. Jennings and Morton (1963) reported that non-protein nitrogen in wheat accounted for about 15% of the total nitrogen at 19 d.p.a., and then decreased slowly to about 3% at maturity.

Dry Weight Per Kernel

Dry weight per kernel (data not shown) increased at the same rate as protein and peaked between 28 and 32 d.p.a. in both 1983 and 1984. This result is in general agreement with the findings of Donovan et al. (1977), who reported that total nitrogen increases in developing wheat kernels paralleled dry weight increases, and that protein accumulation ceased when dry weight reached its maximum.

For the triticale cultivars, dry kernel weights at various stages of maturation were almost identical in 1983 and 1984. In contrast, dry weights of the developing Marquis wheat kernels were distinctly lower in 1983 than in 1984.

Alpha-Amylase Activity

The α-amylase activity in the developing kernels is shown in Figure 4. The α-amylase activity in the Marquis wheat checks decreased from 21 to 28 d.p.a. and remained very low (below 1 μg α-amylase/g dry meal) thereafter. In contrast, the α-amylase activity in the triticales generally increased from 21 d.p.a. to full maturity (44 d.p.a.). At full maturity, α-amylase activity in the triticales was 10 to 200 times higher than α-amylase activity in the Marquis wheat check grown in the same year.

The levels of α-amylase activity in developing Carman and 11T kernels were relatively stable from year to year. In contrast, the levels of α-amylase activity in developing 4T and Impala kernels were much higher in 1984 than in 1983. In 1984, the α-amylase activity in both these triticales increased sharply after 32 d.p.a., peaked at 36 d.p.a., and then decreased in the final stages of grain maturation. Similar increases in α-amylase activity in the final stages of grain maturation have been reported for many other triticale cultivars, including the primary triticale 6A190 (King et al. 1979) and the secondary triticale Rahum (Pena and Bates 1982).

The sudden increase in α-amylase activity in the 1984 4T and Impala samples occurred even though grain was not wetted by rainfall. It was noted, however, that the moisture content of 4T and Impala kernels at 32 d.p.a. was

Figure 4. Changes in whole grain α-amylase activity during kernel development.

Triticale

4T

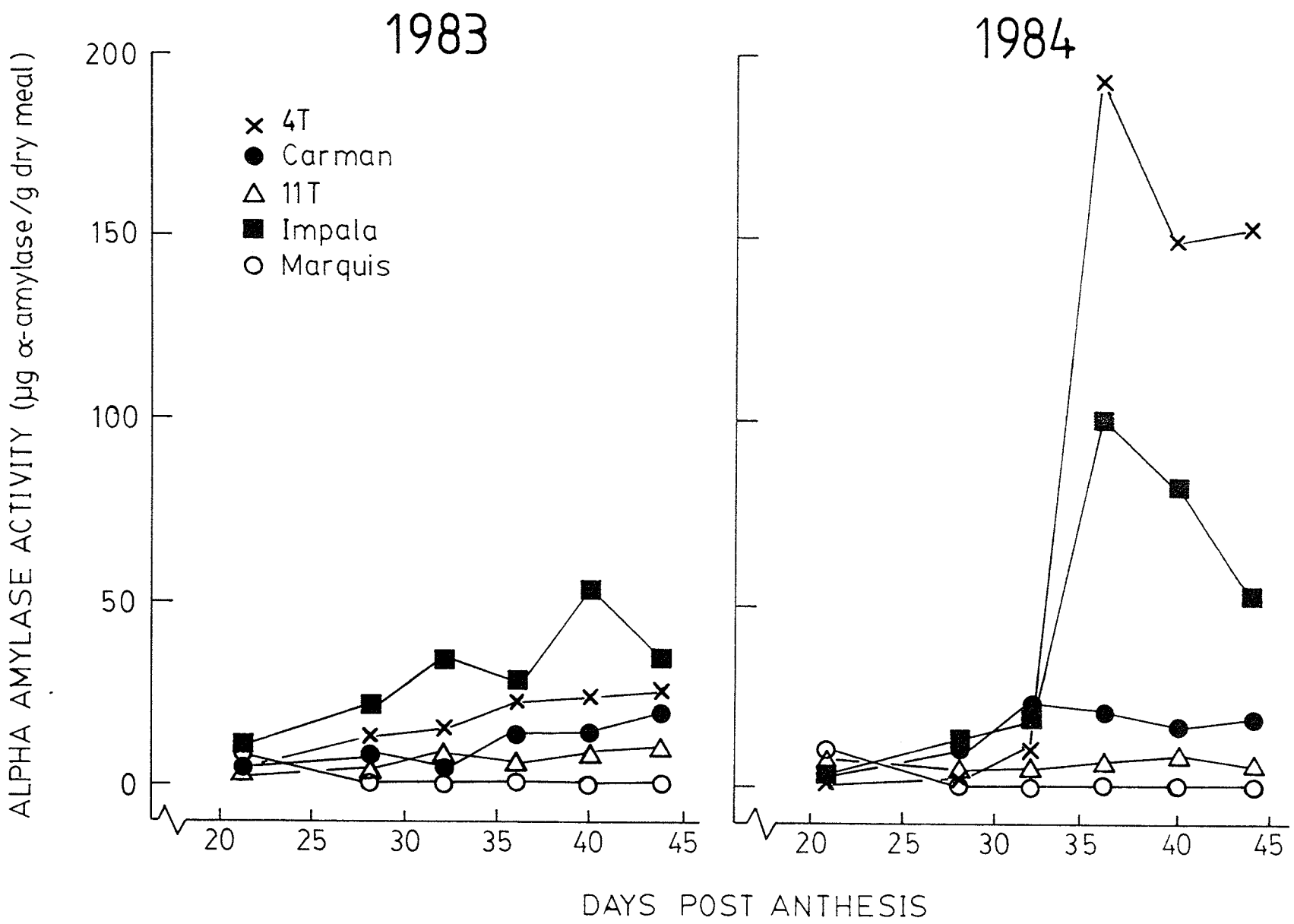
Carman

11T

Impala

Hard red spring wheat

Marquis


1983
1984
4T
Carman
11T
Impala
Marquis
ALPHA AMYLASE ACTIVITY (µg α-amylase/g dry meal)
200
150
100
50
0
20
25
30
35
40
45
DAYS POST ANTHESIS

approximately 30% higher in 1984 than in 1983 (Figure 1). Gale et al. (1983) reported that the production of α-amylases in maturing wheats just prior to harvest ripeness can be enhanced by "slow" drying conditions. It is, therefore, possible that the production of α-amylases in developing 4T and Impala kernels is dependent to some extent on the availability of adequate grain moisture.

Exoprotease (Hemoglobinase) Activity

A preliminary study in 1983 showed that the optimum pH for hemoglobinase activity in 4T (28 d.p.a.) and 11T (36 d.p.a.) whole meal suspensions was 4.5. This result agrees closely with the findings of Madl and Tsen (1973), who reported that hemoglobinase activity in triticale flour suspensions had a pH optimum of 4.45.

The exoprotease activity in the developing kernels is shown in Figure 5. The exoprotease activity of the triticale and wheat samples generally decreased from 21 to 32 d.p.a., and then remained at relatively constant levels throughout the final stages of grain maturation. This is in general agreement with the findings of Lorenz and Welsh (1976) who reported that the hemoglobinase activity of two semi-dwarf Armadillo triticales, two tall spring triticales, and two durum wheats decreased from initial kernel development to full maturity.

All triticales examined had higher levels of exoprotease activity throughout kernel development than the Marquis wheat

Figure 5. Changes in whole grain exoprotease (hemoglobinase) activity during kernel development.

Triticale

4T

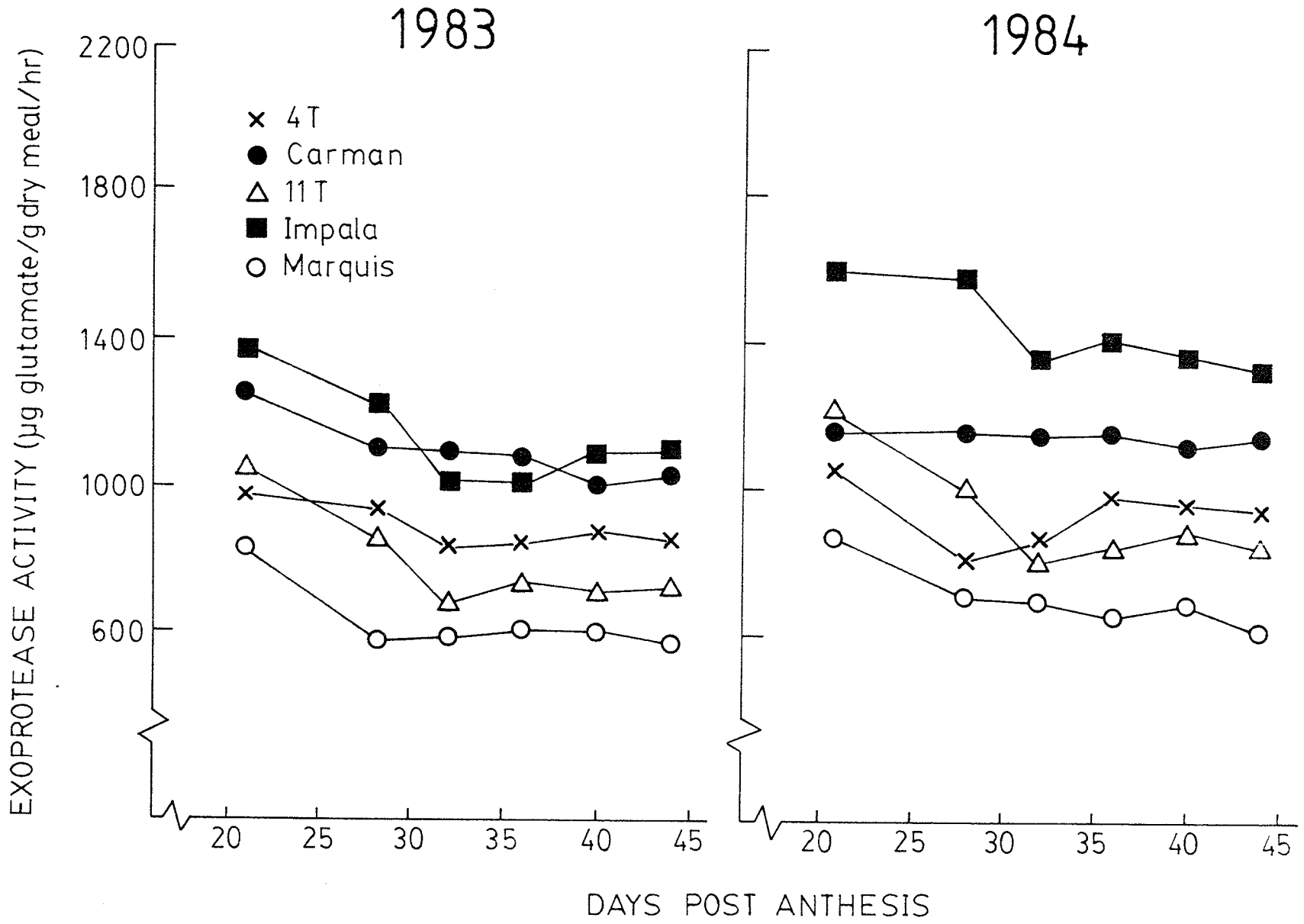
Carman

11T

Impala

Hard red wpring wheat

Marquis


1983
1984
EXOPROTEASE ACTIVITY (µg glutamate/g dry meal/hr)
2200
1800
1400
1000
600
4T
Carman
11T
Impala
Marquis
20
25
30
35
40
45
DAYS POST ANTHESIS

check grown in the same year. At full maturity, exoprotease activity in the triticales was 1.2 to 2.0 times higher than exoprotease activity in the Marquis wheat check grown in the same year.

Although exoprotease levels were slightly higher in the wetter growing season of 1984, ranking of the cultivars in terms of their exoprotease activity at full maturity gave the same result for both 1983 and 1984: Impala > Carman > 4T > 11T > Marquis. With few exceptions, this ranking held throughout the entire period of kernel development examined. This would suggest that the exoprotease activity of the cultivars was quite stable with respect to environment, at least when compared with the α-amylase activity of the 4T and Impala triticales.

Endoprotease (Azocaseinase) Activity

The endoprotease activity in the developing kernels is shown in Figure 6. The endoprotease activity in the developing triticale and wheat kernels decreased steadily from 21 d.p.a. to full maturity. The triticales generally had higher levels of endoprotease activity at all stages of maturation than the Marquis wheat check grown in the same year. At full maturity, endoprotease activity in the triticales was 1.5 to 2.3 times higher than endoprotease activity in the Marquis wheat check grown in the same year.

The levels of endoprotease activity in the developing kernels were distinctly higher in 1984 than in 1983, suggesting

Figure 6. Changes in whole grain endoprotease (azocaseinase) activity during kernel development.

Triticale

4T

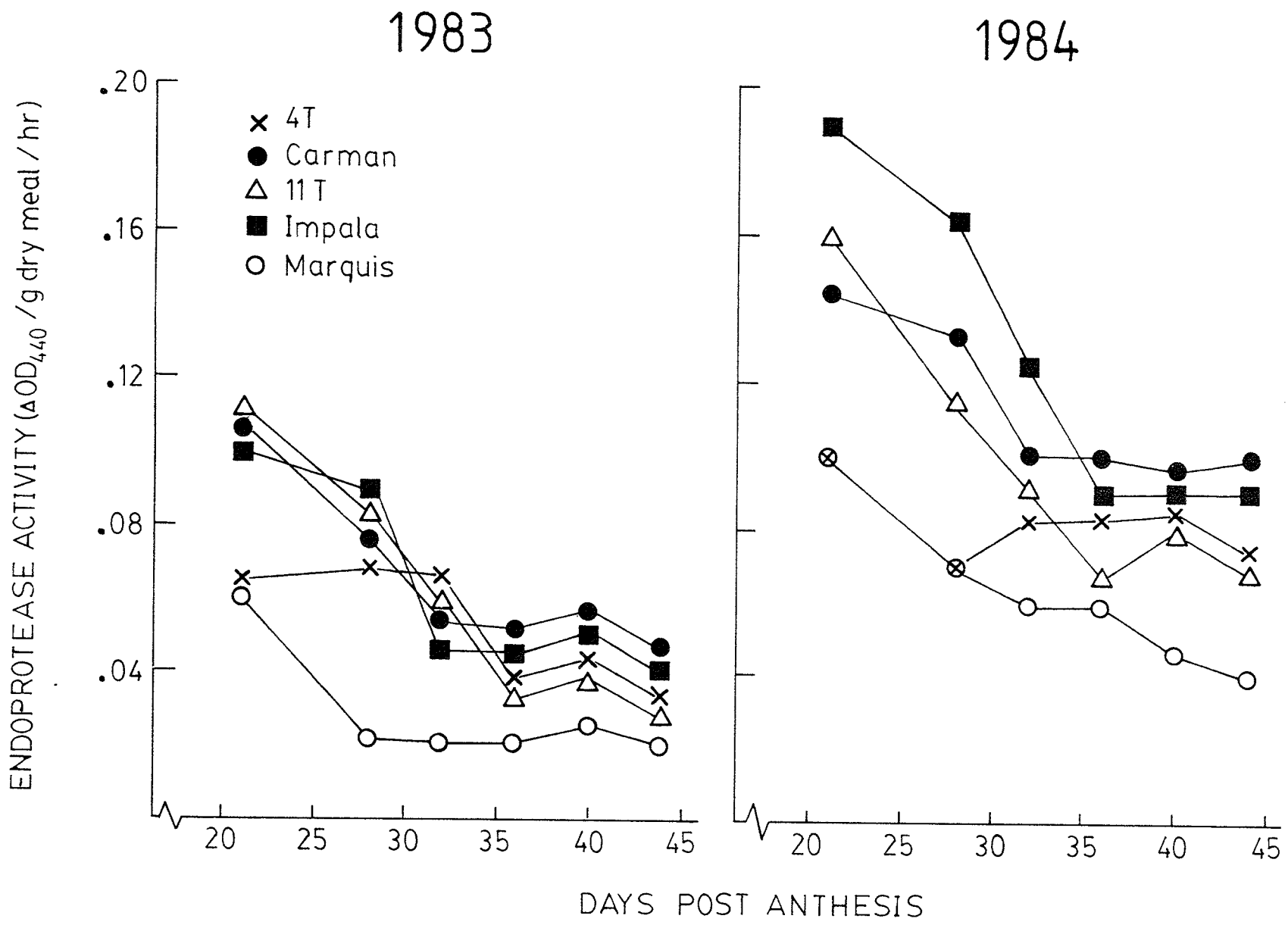
Carman

11T

Impala

Hard red spring wheat

Marquis


1983
1984
.20
.16
.12
.08
.04
4T
Carman
11T
Impala
Marquis
ENDOPROTEASE ACTIVITY (ΔOD440 /g dry meal / hr)
20
25
30
35
40
45
DAYS POST ANTHESIS

that endoprotease activity of the cultivars was influenced to some extent by environment. It is interesting to note that levels of endoprotease activity in developing kernels of the 1983 triticale samples were comparable to levels of endoprotease activity in developing kernels of the 1984 Marquis wheat check.

Ranking of cultivars in terms of their endoprotease activity at full maturity gave the same result for both 1983 and 1984: Carman > Impala > 4T > 11T > Marquis. It should be recalled that the cultivars were ranked in a similar fashion in terms of their exoprotease activity, except that the order of Carman and Impala was reversed. This raises the possibility that triticale samples having relatively high levels of endoprotease activity (e.g. the 1984 Impala sample) may have had hemoglobinase activity enhanced by endoproteases opening up new sites of attack for exoproteases along the hemoglobin molecule. In such an instance, hemoglobinase activity would not accurately reflect exoprotease activity.

B. Characteristics of Bulk Grain Harvested at Maturity

All cultivars examined in the present study were considered to be "harvest ripe" at approximately 44 d.p.a., and plots of each cultivar were harvested in bulk shortly after sampling for the maturation study was completed. The results discussed in this section refer to the cleaned bulk grain samples. The α-amylase, exoprotease, and endoprotease

activity of the mature bulk grain samples are discussed in detail in Section G.

Agronomic Performance/Spike and Kernel Characteristics

Typical spikes of mature triticale and wheat plants are shown in Figure 7. Carman, a triticale with a full complement of rye chromosomes, had longer spikes than the 2D/2R substituted Mexican triticales. However, the Mexican triticales produced more tillers per plant and were roughly equal or superior to Carman in yield (based on the weight of bulk seed harvested after plots had been sampled for the maturation study). Carman was also taller, and ripened 5 to 6 days after either the Mexican triticales or the Marquis wheat checks.

Grain samples from the 1984 harvest are shown in Figure 8. All triticales examined showed some degree of kernel shrivelling. Impala kernels were the most seriously shrivelled, while 4T kernels were relatively well-filled. There was no visible sign of sprouting in any of the cultivars.

Falling Number

The Hagberg falling number test is frequently used as a rapid method for estimating α-amylase activity in wheat and rye. Falling number values can range from 60 sec for flours or meal with very high α-amylase activity to more than 400 sec for flours or meal with very little activity (Watson 1984).

Figure 7. Spikes of mature plants from the 1984 growing season.

2D/2R Substituted semi-dwarf Mexican triticales

4T

11T

Impala

Tall spring triticale (all rye chromosomes present)

Carman

Hard red spring wheat

Marquis

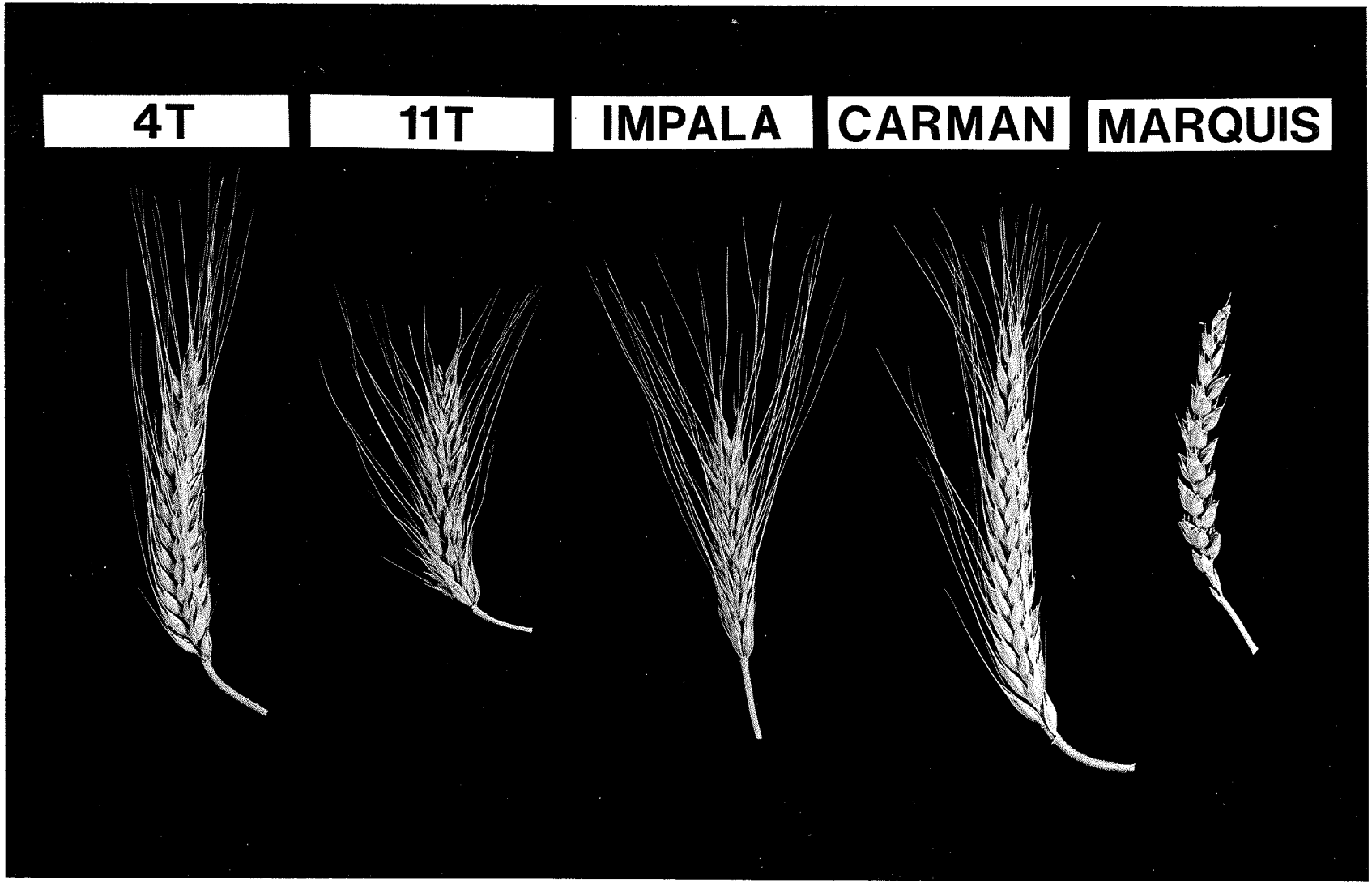

4T
11T
IMPALA
CARMAN
MARQUIS

Figure 8. Samples of mature bulk grain harvested in the 1984 growing season.

2D/2R Substituted semi-dwarf Mexican triticales

4T

11T

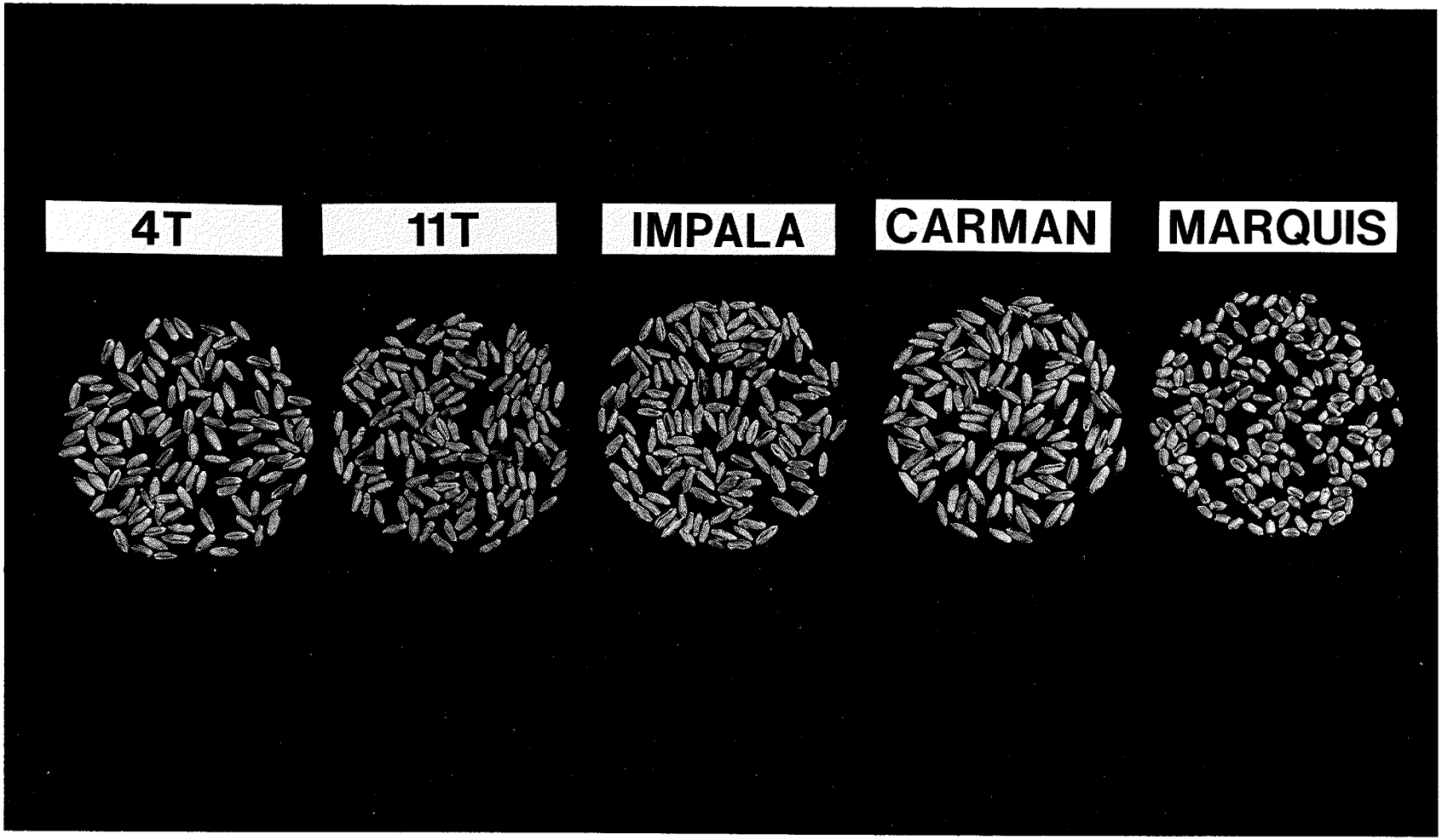
Impala

Tall spring triticale (all rye chromosomes present)

Carman

Hard red spring wheat

Marquis


4T
11T
IMPALA
CARMAN
MARQUIS

Falling number values can be influenced by a large number of factors, including the amount of starch damage in the sample and the inherent susceptibility of the starch to α-amylase. Therefore, whole meal falling number values (Table 3) were compared with whole meal α-amylase activities measured on the Grain Amylase Analyzer (GAA) (Table 3) to determine if the falling number test could accurately measure the α-amylase activity in the triticales.

There was a significant negative correlation found between whole meal falling number values and whole meal GAA α-amylase activity ($r = -0.748$, d.f. = 6, $P = 0.05$) for the eight triticale grain samples (four cultivars over 2 years), indicating that the falling number test gave a reasonable estimate of α-amylase activity. Kruger and Tipples (1982) examined 82 rye samples with falling number values ranging from 70 to 260 sec and reported that falling number values and GAA α-amylase activity gave a correlation of -0.75, which is very similar to the correlation found in the present study.

Thousand Kernel Weight/Test Weight/Protein Content

The mature grain of all triticales examined had higher thousand kernel weights, lower test weights, and lower whole grain protein contents than Marquis wheat checks (Table 3). As expected from the maturation study, whole grain protein content was higher in 1983 than in 1984 for any given cultivar.

TABLE 3. Characteristics of bulk grain harvested at maturity.

| | | Triticale | | | | Wheat |
|---|---|---|---|---|---|---|
| | Year | 4T | Carman | 11T | Impala | Marquis |
| Test weight (kg/hl)[1,2] | 1983 | 72.2$^{c}$ | 65.5$^{b}$ | 72.3$^{c}$ | 60.3$^{a}$ | 74.0$^{d}$ |
| | 1984 | 73.9$^{d}$ | 65.5$^{b}$ | 72.1$^{c}$ | 62.0$^{a}$ | 79.7$^{e}$ |
| | avg | 73.1 | 65.5 | 72.2 | 61.2 | 76.9 |
| MKW (g/1000 kernels)[1,3] | 1983 | 39.8$^{d}$ | 40.8$^{e}$ | 37.9$^{c}$ | 35.7$^{b}$ | 27.3$^{a}$ |
| | 1984 | 40.1$^{d}$ | 38.9$^{d}$ | 36.4$^{c}$ | 34.3$^{b}$ | 31.6$^{a}$ |
| | avg | 40.0 | 39.9 | 37.2 | 35.0 | 29.5 |
| Pearling resistance (g)[1] | 1983 | 11.6$^{c}$ | 10.0$^{b}$ | 10.0$^{b}$ | 8.1$^{a}$ | 12.4$^{d}$ |
| | 1984 | 11.4$^{c}$ | 8.7$^{b}$ | 8.0$^{a}$ | 8.6$^{b}$ | 11.5$^{c}$ |
| | avg | 11.5 | 9.4 | 9.0 | 8.4 | 12.0 |
| Falling number (sec)[1,2] | 1983 | 69$^{a}$ | 86$^{b}$ | 147$^{c}$ | 65$^{a}$ | 371$^{d}$ |
| | 1984 | 62$^{a}$ | 160$^{c}$ | 98$^{b}$ | 65$^{a}$ | 376$^{d}$ |
| | avg | 66 | 123 | 123 | 65 | 374 |
| Alpha-amylase activity[2] (μg α-amylase/g meal) | 1983 | 18.0 | 9.5 | 3.3 | 30.0 | 0.4 |
| | 1984 | 49.8 | 4.8 | 5.5 | 41.4 | 0.2 |
| | avg | 33.9 | 7.2 | 4.4 | 35.7 | 0.3 |
| % Protein (N X 5.7)[1,3] | 1983 | 11.4$^{a}$ | 12.6$^{b}$ | 13.3$^{c}$ | 14.4$^{d}$ | 14.8$^{e}$ |
| | 1984 | 10.9$^{a}$ | 12.0$^{b}$ | 12.5$^{c}$ | 13.2$^{d}$ | 14.0$^{e}$ |
| | avg | 11.2 | 12.3 | 12.9 | 13.8 | 14.4 |

[1]Average of duplicates; within each row, means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

[2]As is m.b.

[3]14% m.b.

## C. Characteristics of the Triticale and Wheat Flours

### Flour Yield/Ash Content

Flour yields of the triticales ranged from 58.2 to 68.3% and were lower than flour yields of the Marquis wheat checks (Table 4). This result is in general agreement with the findings of Unrau and Jenkins (1964), who obtained flour yields of 60.1 to 63.7% for four hexaploid triticales as compared with 69.6 to 71.4% for hard red spring and durum wheats.

Minerals are concentrated in the bran of triticale kernels (Lorenz et al. 1974) and flours with a high ash content are assumed to contain greater amounts of fine bran particles. Although ash content does not affect the baking performance of a flour per se, breads produced from flours of high ash content may be unacceptable to the consumer because of their dark color (Pratt 1971). Commercial hard red spring wheat flours generally have ash contents in the range of 0.41 to 0.49% on a 14% moisture basis (Zeigler and Greer 1971).

Ash content of the triticale flours (Table 4) ranged from 0.44 to 0.56%. The flour ash content found in the present study are similar to those reported by Farrell et al. (1974) for several triticales (0.42 to 0.58%), but slightly lower than those reported by Lorenz et al. (1974) for two spring and two winter triticales (0.51 to 0.63%).

TABLE 4. Characteristics of triticale and wheat flours.

| | Year | Triticale | | | | Wheat |
|---|---|---|---|---|---|---|
| | | 4T | Carman | 11T | Impala | Marquis |
| Flour yield (%) | 1983 | 68.0 | 64.7 | 67.8 | 58.2 | 71.1 |
| | 1984 | 67.6 | 63.7 | 68.7 | 59.0 | 72.3 |
| | avg | 67.8 | 64.2 | 68.3 | 58.6 | 71.7 |
| Ash (%)[1,2] | 1983 | 0.45[b] | 0.55[c] | 0.43[a] | 0.55[c] | 0.46[b] |
| | 1984 | 0.42[a] | 0.51[d] | 0.47[c] | 0.56[e] | 0.43[b] |
| | avg | 0.44 | 0.53 | 0.45 | 0.56 | 0.45 |
| % Damaged starch[2] (Farrand units) | 1983 | 24 | 16 | 11 | 14 | 17 |
| | 1984 | 23 | 14 | 10 | 14 | 17 |
| | avg | 24 | 15 | 11 | 14 | 17 |
| % Protein (N X 5.7)[1,2] | 1983 | 10.2[a] | 10.7[b] | 11.8[c] | 12.9[d] | 14.0[e] |
| | 1984 | 9.3[a] | 9.9[b] | 10.5[c] | 11.6[d] | 13.2[e] |
| | avg | 9.8 | 10.3 | 11.2 | 12.3 | 13.6 |
| Zeleny sedimentation (cc)[1] | 1983 | 26[a] | 29[a] | 26[a] | 40[b] | 58[c] |
| | 1984 | 26[a] | 25[a] | 30[b] | 39[c] | 59[d] |
| | avg | 26 | 27 | 28 | 40 | 59 |

[1]Average of duplicates; within each row, means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

[2]14% m.b.

Damaged Starch

The Farrand method of damaged starch determination measures the production of reducing sugars in the presence of a massive dose of α-amylase. Starch damage is reported in arbitrary units which, expressed on a percentage scale, gives an estimate of the portion of total starch that is damaged. The range for all types of flour is approximately 0 to 45%, but most bread wheat flours fall into a range of 15 to 30% (Farrand 1964).

The level of damaged starch in the Marquis wheat flours averaged 17%, while levels in the triticale flours ranged from 11 to 24% (Table 4). With the exception of 4T, all triticale flours had lower levels of damaged starch than the Marquis wheat flours. These results are in general agreement with the finding of Berry et al. (1971), who reported that the level of damaged starch in a triticale flour (14%) was lower than that in a hard red spring wheat flour.

In general, wheat kernels with softer endosperms incur less starch damage during milling than wheat kernels with harder endosperms (Simmonds 1974b). This relationship held for the triticales examined in the present study. Among the eight triticale samples (four cultivars over 2 years), kernel hardness (as measured by pearling resistance, Table 3) was significantly correlated with damaged starch levels ($r = +0.901$, d.f. = 6, $P = 0.01$). It is not surprising that

Carman, 11T, and Impala flours had lower damaged starch levels than Marquis flours, since the grain of these triticale cultivars also had softer kernels (i.e. showed less resistance to pearling) than the Marquis wheat.

Damaged starch levels were higher in triticale 4T flours than in Marquis flours even though the pearling resistance of these cultivars was similar. There are at least two possible explanations for this. Firstly, the amount of α-amylase added in the Farrand test is normally sufficient to overshadow any naturally occurring α-amylase in wheat, but the extremely high α-amylase activity in triticale 4T samples (Table 3) may have increased the production of maltose during the test, and hence the damaged starch value. Secondly, developing 4T kernels had much higher levels of α-amylase activity than developing Marquis kernels (Figure 4), and α-amylases may have been released into the endosperm of 4T kernels, resulting in <u>in situ</u> starch damage. This second explanation is less probable since damaged starch values in the 1983 and 1984 4T flours were almost identical, even though α-amylase activity in the developing 4T kernels was much lower in 1983 than in 1984. It should also be noted that pearling resistance can be affected to some degree by bran properties and endosperm characteristics (Obuchowski and Bushuk 1980). As such, a comparison of the pearling resistance between triticale 4T and Marquis wheat may not be valid.

## Protein Content

The protein content of the triticale and wheat flours is shown in Figure 9. The protein content of the mature bulk grain samples from which these flours were milled was previously discussed in Section B, but is included in Figure 9 for comparative purposes.

The protein content of the triticale flours ranged from 9.3 to 12.9% on a 14% moisture basis, and triticale flours had lower protein contents than the Marquis wheat flours. The flour protein content was higher in 1983 than in 1984 for any given cultivar.

## Zeleny Sedimentation

The Zeleny sedimentation test is a rapid method used for estimating the "strength" of a flour. Sedimentation values range from about 20 cc for a weak wheat flour to 60 cc or more for a strong wheat flour (Zeleny 1971).

All triticale flours examined had lower sedimentation values than Marquis wheat flours (Table 4). Flours of triticales 4T, Carman, and 11T had similar sedimentation values of about 27 cc. Impala flours had noticeably higher sedimentation values than other triticale flours. As such, one would expect Impala flours to have stronger mixing characteristics than other triticale flours.

Figure 9. Protein content (N X 5.7) of mature bulk grain and flour samples:

Triticale

4T

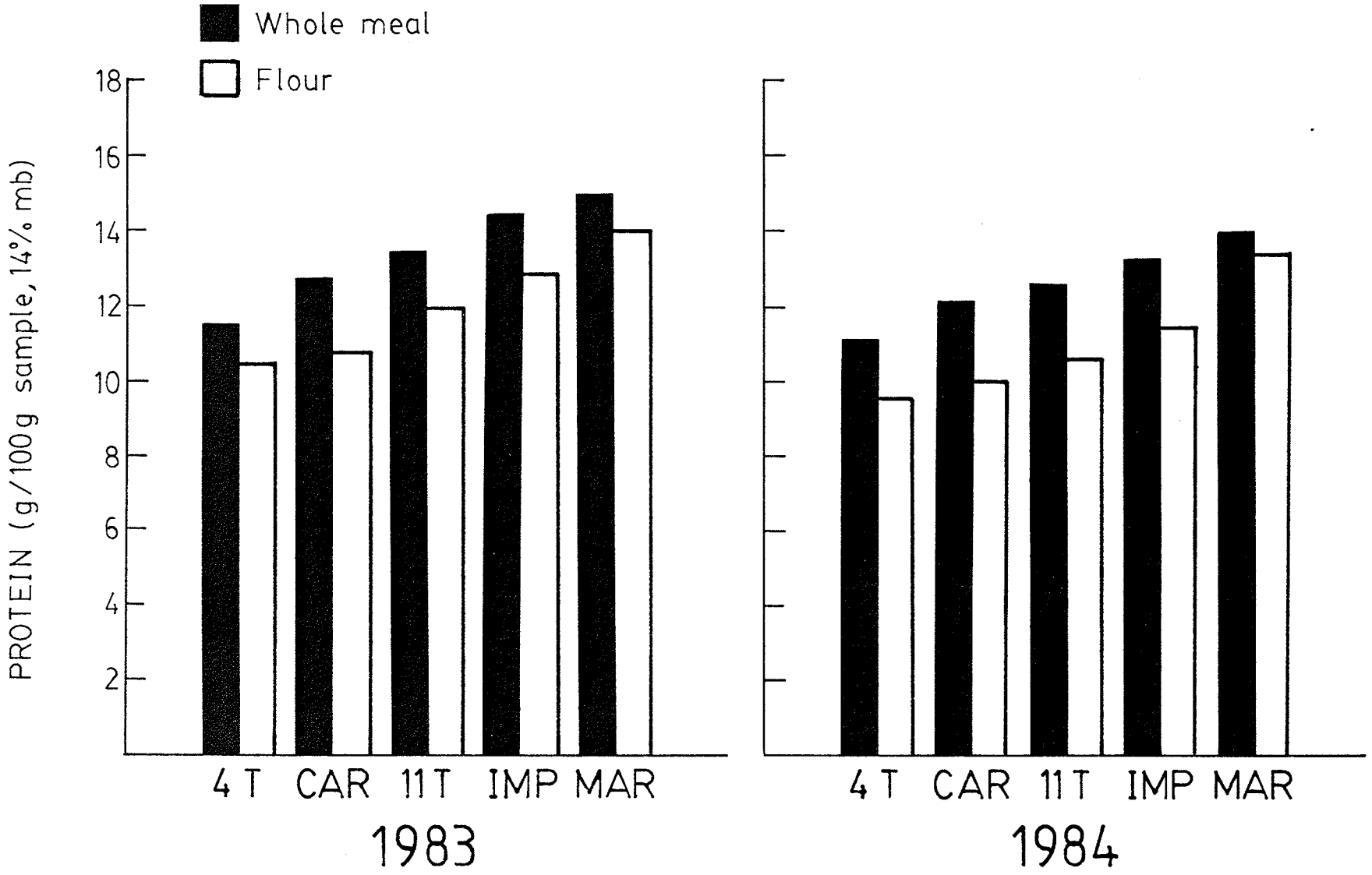
Car = Carman

11T

Imp = Impala

Hard red spring wheat

Mar = Marquis


Whole meal
Flour
PROTEIN (g/100g sample, 14% mb)
18
16
14
12
10
8
6
4
2
4 T CAR 11 T IMP MAR
1983
4 T CAR 11 T IMP MAR
1984

## D. Rheological Properties of the Triticale and Wheat Flours

### Farinograph Test

The farinograph is an instrument which measures the mixing characteristics of a flour. It may be used to determine a) the amount of water that must be added to the flour to produce a dough of the correct consistency for bread-making (flour absorption) and b) the mixing time that is required to achieve optimum gluten development (peak or dough development time). During a farinograph test, mixing is usually continued after peak consistency is reached in order to determine the tolerance of a dough to mechanical abuse.

Typical farinograms of the triticale and wheat flours are shown in Figure 10. Compared with Marquis wheat flours, the triticale flours had low farinograph absorptions, short dough development times, short stability times, and poor tolerance to mixing (i.e. high mixing tolerance indices) (Table 5). Several workers (Ahmed and McDonald 1974, Haber et al. 1976, Lorenz et al. 1972, Tsen et al. 1973, Unrau and Jenkins 1964) have noted similar farinograph characteristics for various triticale, rye, and durum wheat flours. According to Tsen (1974), short dough development and stability times are usually an indication of deficient gluten quantity and/or quality.

Protein and damaged starch absorb approximately 31%

Figure 10. Farinograms and extensigrams of the 1984 flour samples.

Triticale

4T

Carman

11T

Impala

Hard red spring wheat

Marquis

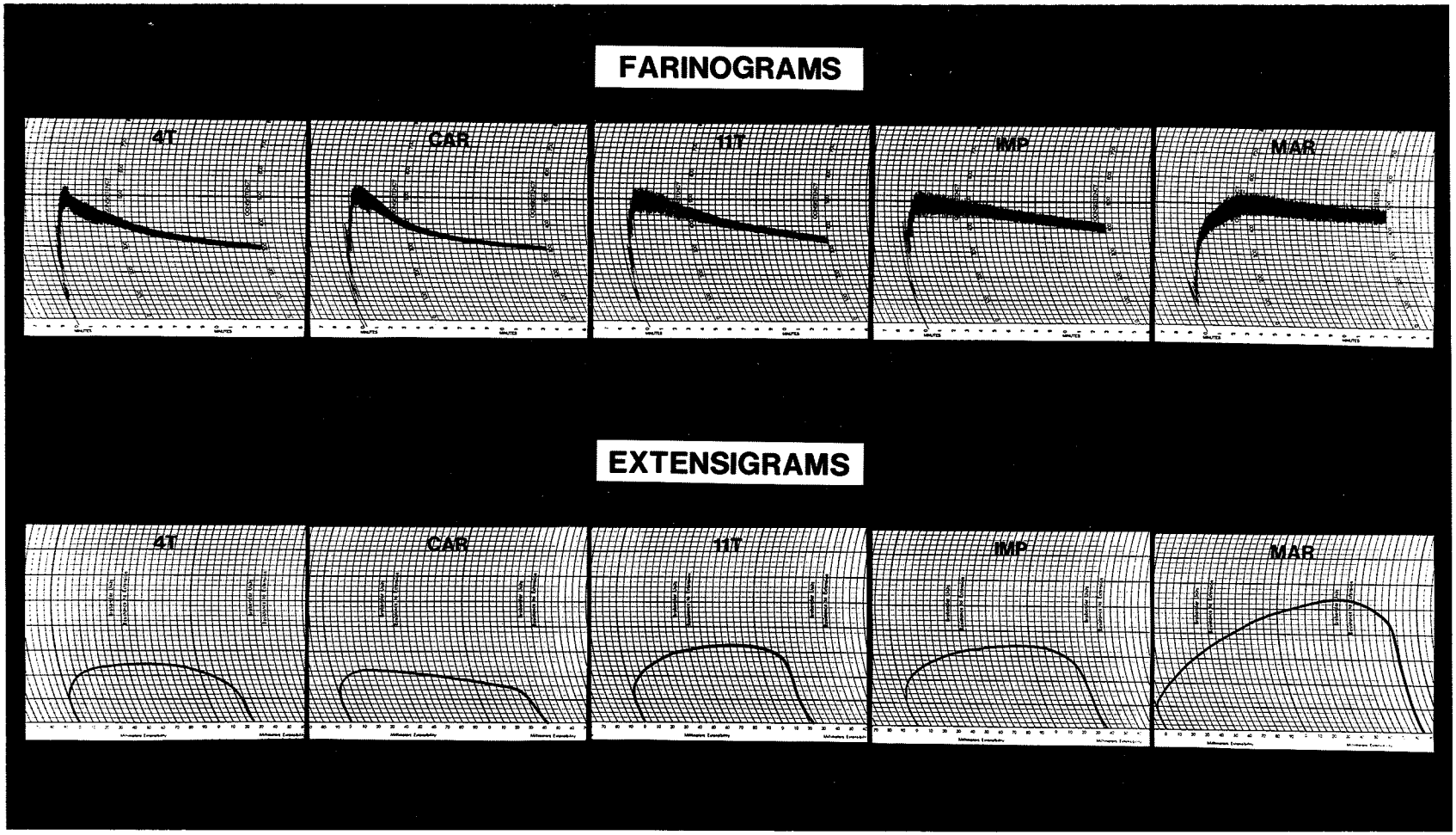

FARINOGRAMS
4T
CAR
11T
IMP
MAR
EXTENSIGRAMS
4T
CAR
11T
IMP
MAR

TABLE 5. Farinograph data for triticale and wheat flours.

| | | Triticale | | | | Wheat |
|---|---|---|---|---|---|---|
| | Year | 4T | Carman | 11T | Impala | Marquis |
| Absorption (ml)[1] | 1983 | 58.3 | 59.9 | 56.7 | 57.1 | 61.1 |
| | 1984 | 56.3 | 56.4 | 53.9 | 54.8 | 60.8 |
| | avg | 57.3 | 58.2 | 55.3 | 56.0 | 61.0 |
| Dough development time (min) | 1983 | 1.5 | 1.5 | 2.0 | 2.0 | 4.5 |
| | 1984 | 1.5 | 2.0 | 2.0 | 1.5 | 4.5 |
| | avg | 1.5 | 1.8 | 2.0 | 1.8 | 4.5 |
| Stability (min) | 1983 | 2.0 | 2.5 | 2.5 | 4.5 | 7.5 |
| | 1984 | 2.0 | 2.0 | 3.0 | 3.5 | 8.0 |
| | avg | 2.0 | 2.3 | 2.8 | 4.0 | 7.8 |
| Mixing tolerance index (BU) | 1983 | 90 | 110 | 90 | 40 | 30 |
| | 1984 | 120 | 140 | 70 | 60 | 20 |
| | avg | 105 | 125 | 80 | 50 | 25 |

[1]Calculated for 100 g flour (14% m.b.)

and 19%, respectively, of the total water in dough (Bushuk 1966). Triticale flours had lower protein content and generally lower levels of starch damage than Marquis wheat flours (Table 4) and it is probable that both these flour characteristics contributed to the relatively low farinograph absorption of the triticale flours.

Zeleny sedimentation values (Table 4) gave a reasonable prediction of mixing strength of the triticale flours. Impala flours had noticeably higher sedimentation values than other triticale flours and, as expected, produced doughs with longer stability times and lower mixing tolerance indices.

Extensigraph Test

It is generally accepted that the rheological properties of a dough influence the quality of the baked product. For example, a properly developed dough must be relatively extensible to allow expansion of gas cells during fermentation, but still have enough resistance to stretching to prevent the dough from being over-extended and collapsing during fermentation or baking. The extensigraph is an instrument which measures the elasticity and extensibility of a dough. The resting and stretching of a dough during an extensigraph test simulate the fermentation period of a bake test, interrupted by punching, and the extensigraph is often used to predict changes in dough properties which may occur during fermentation.

Typical extensigrams of the triticale and wheat doughs are shown in Figure 10. Compared with Marquis wheat doughs, triticale doughs generally had little resistance to stretching, small curve areas (a measure of the total energy required to deform the dough) and low $R_{max}/E$ ratios (Table 6). As such, one would expect that triticale doughs would generally have poorer gas retention and hence produce bread with lower loaf volumes, than Marquis wheat doughs.

Impala had the highest $R_{max}/E$ ratio and the largest extensigram curve area of the four triticale cultivars examined. Therefore, one would also expect that Impala flours would produce bread with a relatively high loaf volume in a bake test.

The continuous gluten matrix that is formed during mixing can be further developed by stretching, folding, and molding, and most bread wheat doughs become less extensible during fermentation (Pratt 1971). Although extensigraph curves of the 4T and Carman doughs showed little difference between the 45 min and 135 min stretches, 11T and Impala (as well as Marquis wheat) doughs showed a definite decrease in extensibility and increase in resistance to stretching at the 135 min stretch, i.e. 11T and Impala doughs became "tighter" with time. Protease treated doughs become more extensible with time (Pomeranz 1971) and one would have expected the triticale doughs to become more extensible if endogenous proteases were seriously

TABLE 6. Extensigraph data for triticale and wheat flours.[1]

| | Year | Triticale 4T | Triticale Carman | Triticale 11T | Triticale Impala | Wheat Marquis |
|---|---|---|---|---|---|---|
| Extensibility (mm) | 1983 | 113$^{a}$ | 148$^{c}$ | 113$^{a}$ | 134$^{b}$ | 172$^{d}$ |
| | 1984 | 121$^{a}$ | 142$^{b}$ | 123$^{a}$ | 140$^{b}$ | 193$^{c}$ |
| | avg | 117 | 145 | 119 | 137 | 183 |
| $Resistance_5$ (BU) | 1983 | 295$^{a}$ | 295$^{a}$ | 290$^{a}$ | 410$^{b}$ | 470$^{c}$ |
| | 1984 | 235$^{b}$ | 205$^{a}$ | 295$^{c}$ | 305$^{c}$ | 345$^{d}$ |
| | avg | 265 | 250 | 298 | 358 | 408 |
| $Resistance_{max}$ (BU) | 1983 | 300$^{a}$ | 310$^{a}$ | 305$^{a}$ | 485$^{b}$ | 695$^{c}$ |
| | 1984 | 235$^{b}$ | 210$^{a}$ | 315$^{c}$ | 325$^{c}$ | 535$^{d}$ |
| | avg | 268 | 260 | 310 | 405 | 615 |
| $R_{max}/E$ | 1983 | 2.63$^{b}$ | 2.09$^{a}$ | 2.70$^{b}$ | 3.60$^{c}$ | 3.91$^{c}$ |
| | 1984 | 1.95$^{b}$ | 1.47$^{a}$ | 2.56$^{d}$ | 2.33$^{c}$ | 2.78$^{e}$ |
| | avg | 2.29 | 1.78 | 2.63 | 2.97 | 3.35 |
| Area ($cm^2$) | 1983 | 50.8$^{a}$ | 68.3$^{b}$ | 50.9$^{a}$ | 91.6$^{c}$ | 162.0$^{d}$ |
| | 1984 | 42.4$^{a}$ | 44.3$^{a}$ | 59.5$^{b}$ | 67.5$^{c}$ | 139.9$^{d}$ |
| | avg | 46.6 | 56.3 | 55.2 | 79.6 | 151.0 |

[1]Average of duplicate doughs, 135 minute stretch; within each row, means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

degrading the gluten matrix within the 90 min rest period between stretches.

## E. Baking Performance of the Triticale and Wheat Flours

The loaf volume of the triticale breads varied widely under the conditions of the A.A.C.C. straight dough bake test, with average values ranging from 344 cc (4T) to 489 cc (Impala) (Table 7). Triticale breads had significantly lower loaf volumes than Marquis breads in either 1983 or 1984.

Crumb characteristics of the breads are shown in Figure 11. Breads produced from Carman and Impala flours had a noticeably darker crumb color than breads produced from 4T and 11T flours. This dark crumb color was probably related to the relatively high ash content of the Carman and Impala flours (Table 4).

Crust characteristics of the breads are shown in Figure 12. Upper crusts of the triticale breads were generally pitted and uneven, presumably because the triticale doughs were too weak to withstand oven spring (i.e. dough surfaces were ruptured by escaping gases in the oven). There was no discernable difference in crust color among the cultivars examined.

The loaf volume of the 4T breads was the lowest among the cultivars examined and 4T breads had a dense gummy crumb. Although Carman breads had significantly higher

TABLE 7. Characteristics of triticale and wheat breads.

| Characteristic | Year | Triticale 4T | Triticale Carman | Triticale 11T | Triticale Impala | Wheat Marquis |
|---|---|---|---|---|---|---|
| % Protein (NX5.7)[1,3] | 1983 | 10.2[a] | 10.7[b] | 11.8[c] | 12.9[d] | 14.0[e] |
| | 1984 | 9.3[a] | 9.9[b] | 10.5[c] | 11.6[d] | 13.2[e] |
| | avg | 9.8 | 10.3 | 11.2 | 12.3 | 13.6 |
| Loaf volume (cc)[2,3] | 1983 | 365[a] | 438[b] | 468[b,c] | 497[c] | 630[d] |
| | 1984 | 323[a] | 407[b] | 492[c] | 480[c] | 600[d] |
| | avg | 344 | 423 | 480 | 489 | 615 |
| Crumb[4] | 1983 | D,G,SDR | C,SO,DR | F,SO | F,SO,DR | F |
| | 1984 | D,G,SDR | C,O,DR | F | F,SO,DR | F |

[1]Average of duplicates, 14% m.b.

[2]Average of triplicates (three bakes, 1 loaf each)

[3]Within each row, means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

[4]D = dense; F = fine; SO = slightly open; O = open; C = coarse; G = gummy; SDR = slightly dark; DR = dark

Figure 11. Crumb characteristics of straight dough breads.

Triticale

4T

Carman

11T

Impala

Hard red spring wheat

Marquis

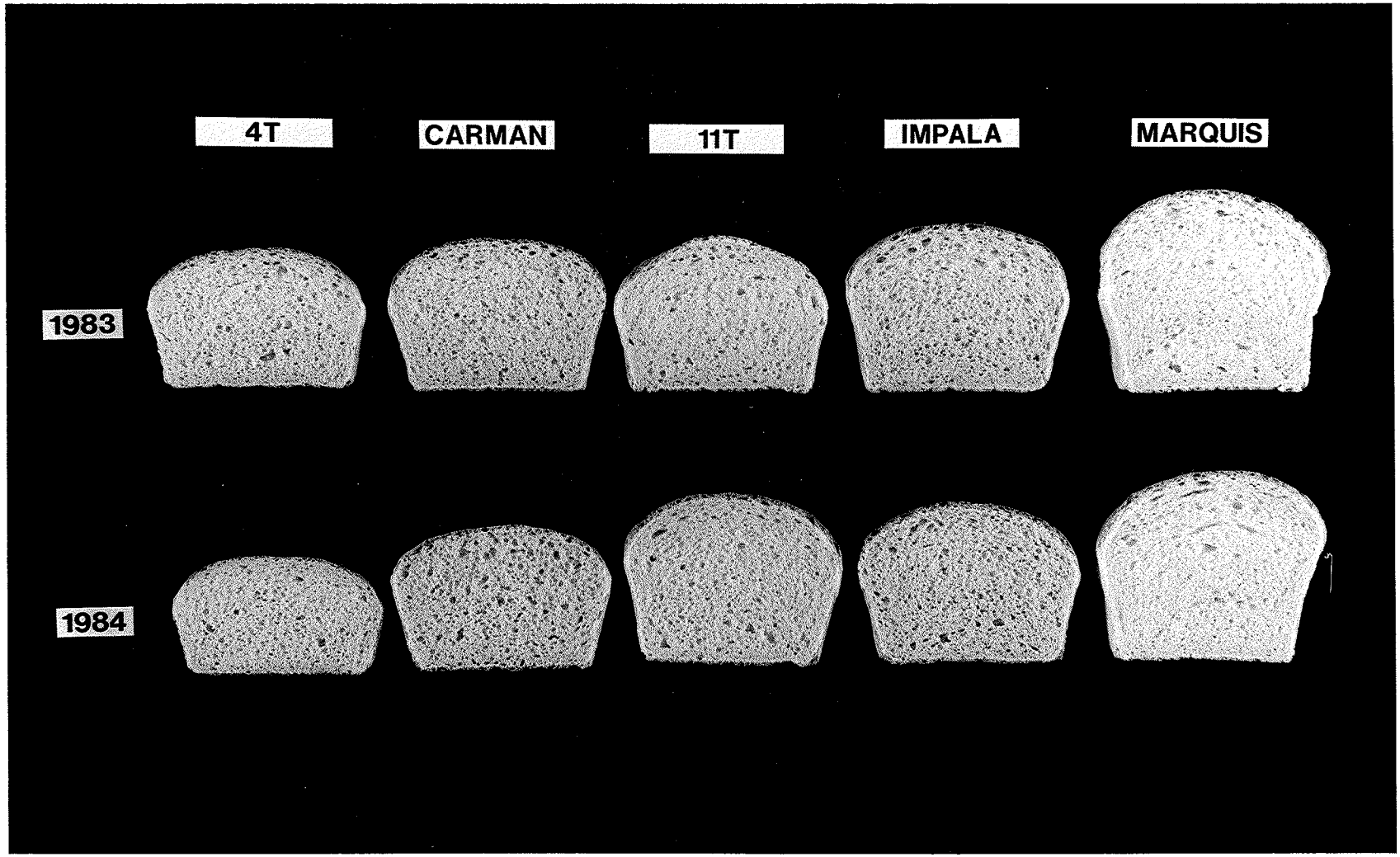

4T
CARMAN
11T
IMPALA
MARQUIS
1983
1984

Figure 12. Crust characteristics of straight dough breads.

Triticale

4T

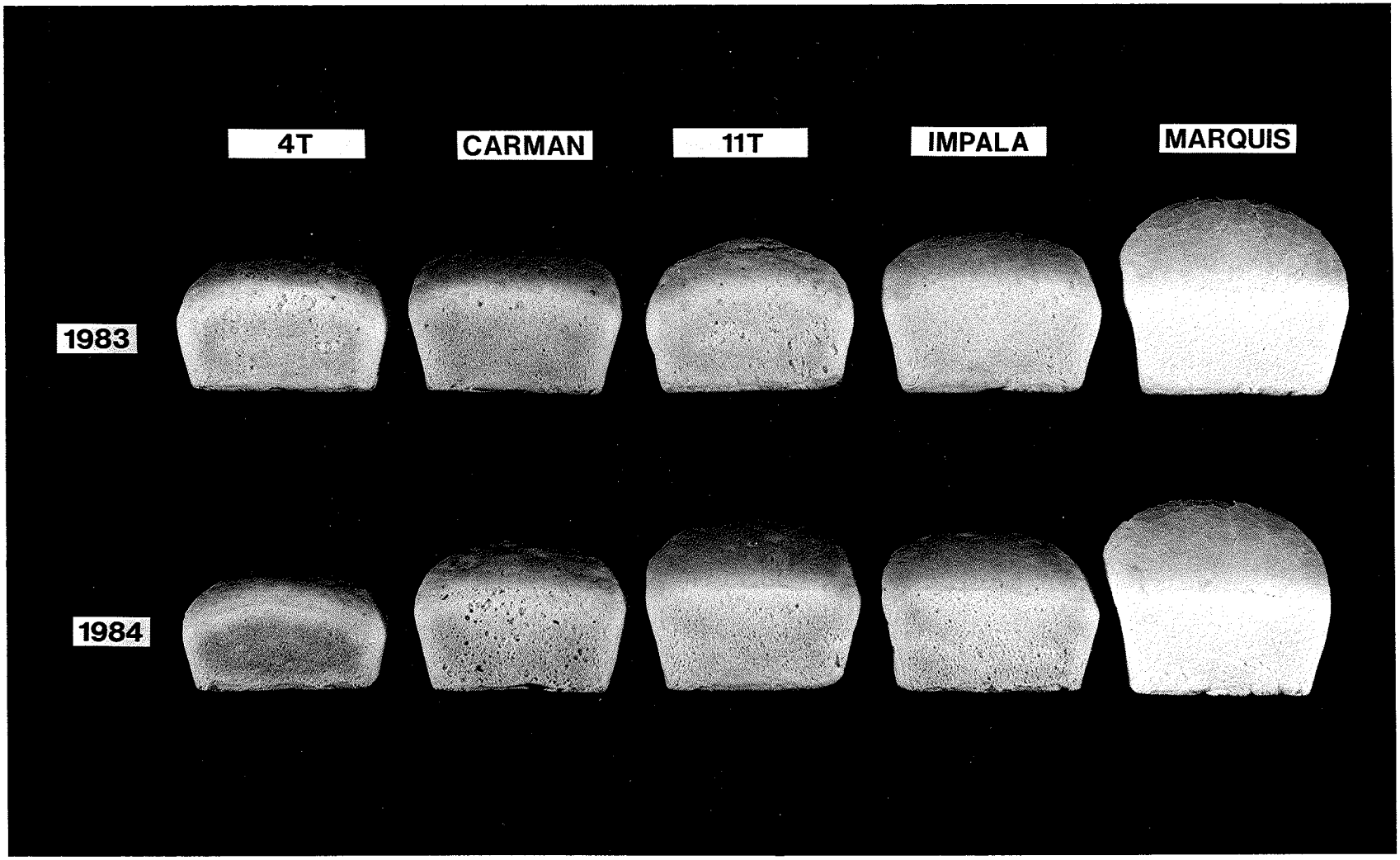
Carman

11T

Impala

Hard red spring wheat

Marquis


4T
CARMAN
11T
IMPALA
MARQUIS
1983
1984

loaf volumes than 4T breads in both 1983 and 1984, breads produced from Carman flours had a tough crust and coarse grain. Carman doughs showed little resistance to stretching in the extensigraph test (Figure 10) and, as expected, Carman doughs were more extensible and more difficult to sheet than other triticale doughs in the bake test.

The loaf volume of the 11T and Impala breads did not differ significantly in either 1983 or in 1984, and the loaf volumes of these breads were the highest among the triticale cultivars examined. Crumb structure of the 11T and Impala breads was generally satisfactory but slightly open in a few loaves. Impala doughs were more elastic than other triticale doughs and handled exceptionally well during dough make-up.

The loaf volumes of triticale breads generally increased as the Zeleny sedimentation value, farinograph stability time, mixing tolerance, extensigraph curve area, and $R_{max}/E$ value of the flours increased. However, none of the rheological tests used in the present study was entirely satisfactory in predicting the performance of the triticales in the bake test. For the eight triticale flour samples (four cultivars over 2 years), the correlation between loaf volume and Zeleny sedimentation value ($r = +0.654$) was non-significant at the 5% confidence level. Curve shapes of the 4T, Carman, and 11T farinograms were similar and gave no indication that 11T flours would perform so well in the bake

test. The extensigraph test provided more precise information on the physical properties of the doughs, but the changes that occur in a fermenting dough during a bake test (e.g. expansion of gas cells, changes in enzyme activities at elevated temperatures) are quite different from those that occur in a nonfermenting extensigraph dough, making predictions of baking quality from the extensigraph test somewhat uncertain.

The loaf volume of the triticale breads was closely related to the protein content of the flours. For the eight triticale flour samples (four cultivars over 2 years), there was a significant positive correlation between loaf volume and flour protein content ($r = +0.802$, d.f. = 6, $P = 0.05$). The flour protein content was higher in 1983 than in 1984 for any given cultivar and it was also observed that the loaf volume of the breads produced from these flours were generally higher in 1983 than in 1984.

## F. The Influence of Gluten Protein on the Baking Quality of the Triticale and Wheat Flours

It is generally accepted that gluten protein content is a major factor controlling the loaf volume potential of wheat flours. The loaf volume and protein content of the triticale flours examined in the present study showed a significant positive correlation ($r = +0.802$), suggesting that there was a similar relationship between gluten protein content and loaf volume potential of the triticale flours.

Therefore, glutens were isolated from the flours and analyzed for protein to determine if quantitative differences in gluten protein could account for differences in baking quality (as defined by loaf volume) of the triticale flours.

The glutens of triticales 4T, Carman, and 11T were difficult to isolate. The glutens isolated from 4T flours showed very little cohesion or elasticity. In contrast, glutens isolated from Impala flours were highly elastic and could be stretched into thin films without rupturing.

All triticale and wheat glutens contain over 80% protein (Table 8). Marquis gluten accounted for about 82% of the total flour protein, which falls within the 78 to 85% range that Pence et al. (1954) reported for typical bread wheats. The triticale glutens accounted for only 54 to 71% of the total flour protein.

The dry gluten content of the triticale flours ranged from 7.2 to 12.7% and triticale flours contained significantly lower amounts of gluten than Marquis wheat flours. These results are in general agreement with the findings of Pena (1984), who examined partially defatted flours of the same triticale cultivars used in the present study and reported that dry gluten content of the flours ranged from 6.3% (4T) to 10.0% (Impala). Pena also reported that total flour protein as gluten protein ranged from 49.9% (4T) to 69.2% (Impala), which is also in general agreement with the findings of the present study. It may be worthwhile to note

TABLE 8. Quantitative characteristics of triticale and wheat glutens.[1]

| | | Triticale | | | | Wheat |
|---|---|---|---|---|---|---|
| | Year | 4T | Carman | 11T | Impala | Marquis |
| % Protein in flour | 1983 | 11.9[a] | 12.4[b] | 13.7[c] | 15.0[d] | 16.3[e] |
| | 1984 | 10.8[a] | 11.5[b] | 12.2[c] | 13.5[d] | 15.3[e] |
| | avg | 11.4 | 12.0 | 13.0 | 14.3 | 15.8 |
| % Protein in gluten | 1983 | 80.3[a] | 82.2[a] | 81.5[a] | 83.9[a] | 84.6[a] |
| | 1984 | 82.4[a] | 82.8[a] | 82.3[a] | 82.1[a] | 86.4[a] |
| | avg | 81.4 | 82.5 | 81.9 | 83.0 | 85.5 |
| Dry gluten content (% of flour) | 1983 | 8.3[a] | 9.1[a] | 10.9[b] | 12.7[c] | 15.6[d] |
| | 1984 | 7.2[a] | 8.0[a] | 9.8[b] | 11.3[b] | 14.5[c] |
| | avg | 7.8 | 8.6 | 10.4 | 12.0 | 15.1 |
| Gluten protein content (% of flour) | 1983 | 6.6[a] | 7.5[b] | 8.9[c] | 10.6[d] | 13.2[e] |
| | 1984 | 5.9[a] | 6.6[a] | 8.1[b] | 9.2[c] | 12.5[d] |
| | avg | 6.3 | 7.1 | 8.5 | 9.9 | 12.9 |
| Total flour protein as gluten protein | 1983 | 55[a] | 61[b] | 65[b] | 71[c] | 83[d] |
| | 1984 | 54[a] | 57[a] | 66[b] | 68[b] | 81[c] |
| | avg | 55 | 59 | 66 | 70 | 82 |

[1]Average of triplicates, dry weight basis; within each row, means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

that the protein content of the Impala flour used in Pena's study (12.9% on a dry weight basis) was lower than the protein content of the Impala flours used in the present study.

The gluten protein content of the triticale flours ranged from 5.9 to 10.6%, and the triticale flours contained significantly lower amounts of gluten protein than the Marquis wheat flours. The lower gluten protein content of the triticale flours was due in part to the lower protein content of the triticale flours, and in part to the lower percentage of that flour protein as gluten-like protein.

Triticale flours with relatively high gluten protein content (e.g. Impala flours) produced bread of better overall quality than triticale flours with low gluten content (e.g. 4T flours). For the eight triticale flour samples (four cultivars over 2 years), there was a strong significant correlation between loaf volume and gluten protein content ($r = +0.879$, d.f. = 6, $P = 0.01$). These results would suggest that the baking performance of the triticale flours was highly influenced by the quantity of gluten protein in the flour.

The loaf volumes of the 11T and Impala breads were similar in both 1983 and 1984 (Table 7), even though the 11T flours had significantly lower quantities of gluten protein than the Impala flours. Apparently, the increase in loaf volume was greater for a unit of 11T gluten protein than for a unit of Impala gluten protein. This would suggest that

gluten protein quality, as well as quantity, controlled the loaf volume potential of the triticale flours.

## G. The Influence of α-Amylase and Protease Activity on the Baking Quality of the Triticale and Wheat Flours

Both α-amylase and protease activity affect bread quality. The crumb texture of a loaf of bread is partially determined by the amount of water taken up by starch granules during gelatinization and this water-binding capacity is affected by α-amylase activity. Excessive α-amylase activity will dextrinize an excessive portion of the starch, reducing its water-binding capacity and resulting in a wet, gummy crumb. Increased dextrin production may also result in a highly colored loaf through browning reactions that occur in the oven. Excessive proteolytic degradation of the gluten protein matrix may weaken the dough structure to a point where the dough is unable to retain the gases produced by the fermenting yeast, resulting in a collapsed loaf with a reduced volume and dense crumb structure. Therefore, flour samples were assayed for α-amylase, exoprotease, and endoprotease activity to determine if differences in endogenous enzyme activities could be related to differences in baking quality among the triticale flours. The levels of α-amylase and protease activity in the flour samples were compared with levels of activity in the bulk grain samples from which these flours were milled to determine the portion of whole grain enzyme activity that was

removed with the outer kernel layers during milling. Characteristics of these bulk grain samples were previously discussed in Section B.

Alpha-Amylase Activity

The α-amylase activity of the bulk grain and the milled flour samples is shown in Figure 13. Apparently, a large portion of the α-amylase activity was concentrated in the outer kernel layers of the triticale and wheat grain and was removed with the bran and germ during milling.

The triticale flours had significantly higher levels of α-amylase activity than the Marquis wheat flours in either 1983 or 1984 (Table 9). For example, the α-amylase activity in the 1984 Impala and 4T flours was 160 and 200 times higher, respectively, than the α-amylase activity in the 1984 Marquis flour. Alpha-amylase activity was higher in 1984 than in 1983 in all but one triticale cultivar. In the triticale Carman, the whole meal falling number value was lower in 1983 than 1984 (Table 3) and, as expected, the whole meal (and flour) α-amylase activity was higher in 1983 than in 1984.

Undamaged starch is relatively resistant to α-amylase attack at fermentation temperatures, and the release of bound water from starch is limited by the amount of available (damaged) starch. In the bake test, triticale doughs did not soften or become wet and sticky during fermentation in spite of the relatively high α-amylase activity of the

Figure 13. Alpha-amylase activity of mature bulk grain and flour samples.

Triticale

4T

Car = Carman

11T

Imp = Impala

Hard red spring wheat

Mar = Marquis

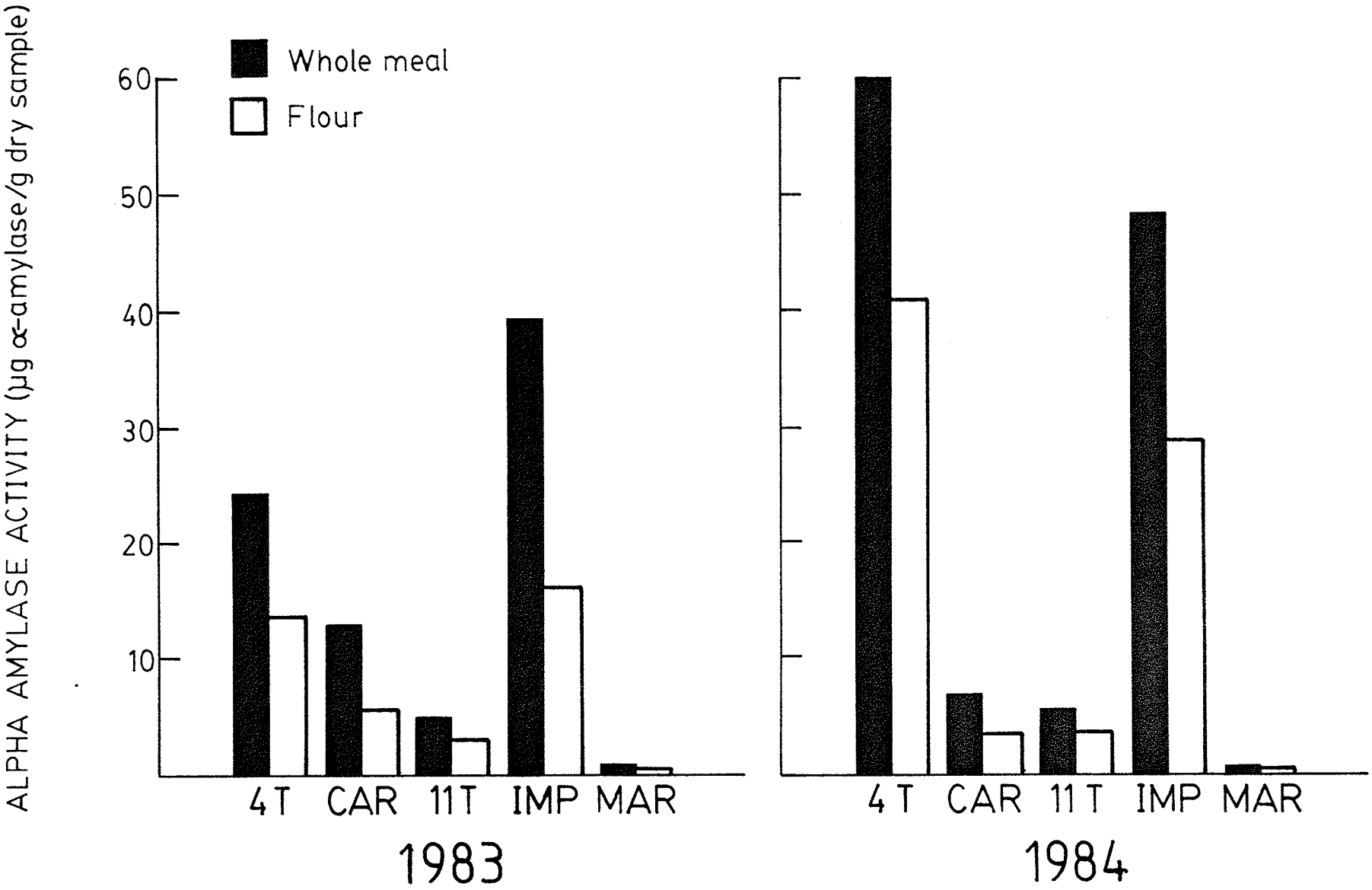

ALPHA AMYLASE ACTIVITY (µg α-amylase/g dry sample)
Whole meal
Flour
60
50
40
30
20
10
4T CAR 11T IMP MAR
1983
4T CAR 11T IMP MAR
1984

TABLE 9. Alpha-amylase and protease activities of triticale and wheat flours.[1]

| Enzyme activity | Year | Triticale | | | | Wheat |
|---|---|---|---|---|---|---|
| | | 4T | Carman | 11T | Impala | Marquis |
| Alpha-amylase[2] | 1983 | $12.7^{d}$ | $5.4^{c}$ | $2.9^{b}$ | $15.8^{e}$ | $0.2^{a}$ |
| | 1984 | $41.1^{d}$ | $3.0^{b}$ | $3.2^{b}$ | $29.1^{c}$ | $0.2^{a}$ |
| | avg | 26.9 | 4.2 | 3.1 | 22.5 | 0.2 |
| Exoprotease[3] | 1983 | $242^{b}$ | $308^{c}$ | $244^{b}$ | $494^{d}$ | $197^{a}$ |
| | 1984 | $271^{b}$ | $316^{c}$ | $325^{c}$ | $588^{d}$ | $212^{a}$ |
| | avg | 257 | 312 | 285 | 541 | 205 |
| Endoprotease[4] | 1983 | $0.017^{a}$ | $0.036^{b}$ | $0.015^{a}$ | $0.032^{b}$ | $0.017^{a}$ |
| | 1984 | $0.034^{a}$ | $0.032^{a}$ | $0.033^{a}$ | $0.035^{a}$ | $0.021^{a}$ |
| | avg | 0.026 | 0.034 | 0.024 | 0.034 | 0.019 |

[1]Average of duplicates; within each row, means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

[2]µg alpha-amylase/g dry flour

[3]µg glu/g dry flour/hr

[4]$\Delta OD_{440}$/g dry flour/hr

triticale flours. With the exception of 4T, the flours of the triticale cultivars had lower levels of starch damage than the flours of the Marquis wheat (Table 4), which might explain why triticale doughs did not become sticky during fermentation.

There were no discernable differences in crust color among the triticale breads even though α-amylase activity varied widely among the triticale flours. However, 5% sucrose was included in the baking formula, and it is possible that any increase in sugar production due to high α-amylase activity (and hence darkening of the crust) was masked by this additional sugar.

According to Bloksma (1971), addition of amylases is not expected to increase the gassing power of a dough if 3 to 6% sucrose is included in the formula. Because the baking formula used in the present study included 5% sucrose, it was assumed that the levels of α-amylase activity in the triticale flours had little effect on the loaf volume of the breads.

The only detrimental effect on bread quality that could be attributed to α-amylase activity was the gummy crumb texture of the 4T breads. Gummy bread crumbs are usually associated with increased dextrin production in the oven by α-amylases after thermal inactivation of β-amylases.

Exoprotease (Hemoglobinase) Activity

The exoprotease activity of the bulk grain and the

milled flour samples is shown in Figure 14. Exoprotease activity was similar to α-amylase activity in that a large portion of the exoprotease activity in the triticale and wheat grain was removed with the outer kernel layers during milling. The triticale flours had significantly higher levels of exoprotease activity than the Marquis wheat flours in either 1983 or 1984 (Table 9).

For the eight triticale flour samples (four cultivars over 2 years) the correlation between loaf volume and flour exoprotease activity was nonsignificant ($r = +0.571$, d.f. = 6, $P = 0.05$). In contrast, Singh and Katragadda (1980) found a significant negative correlation between loaf volume and flour hemoglobinase activity ($r = -0.85$, $P = 0.05$) for the four triticale cultivars they examined. It should be noted, however, that Singh and Katragadda also found a negative correlation between loaf volume and flour protein content ($r = -0.50$). In the present study, the loaf volume and flour protein content of the triticale samples were positively correlated ($r = +0.802$).

There was no apparent relationship between the exoprotease activity and the loaf volume potential of the triticale flours. For example, the 1983 4T and 11T flours had almost identical exoprotease activities but still produced breads with loaf volumes that differed by more than 100 cc (Table 7). Furthermore, Impala flours produced bread with higher loaf volumes than 4T or Carman flours, even

Figure 14. Exoprotease (hemoglobinase) activity of mature bulk grain and flour samples.

Triticale

4T

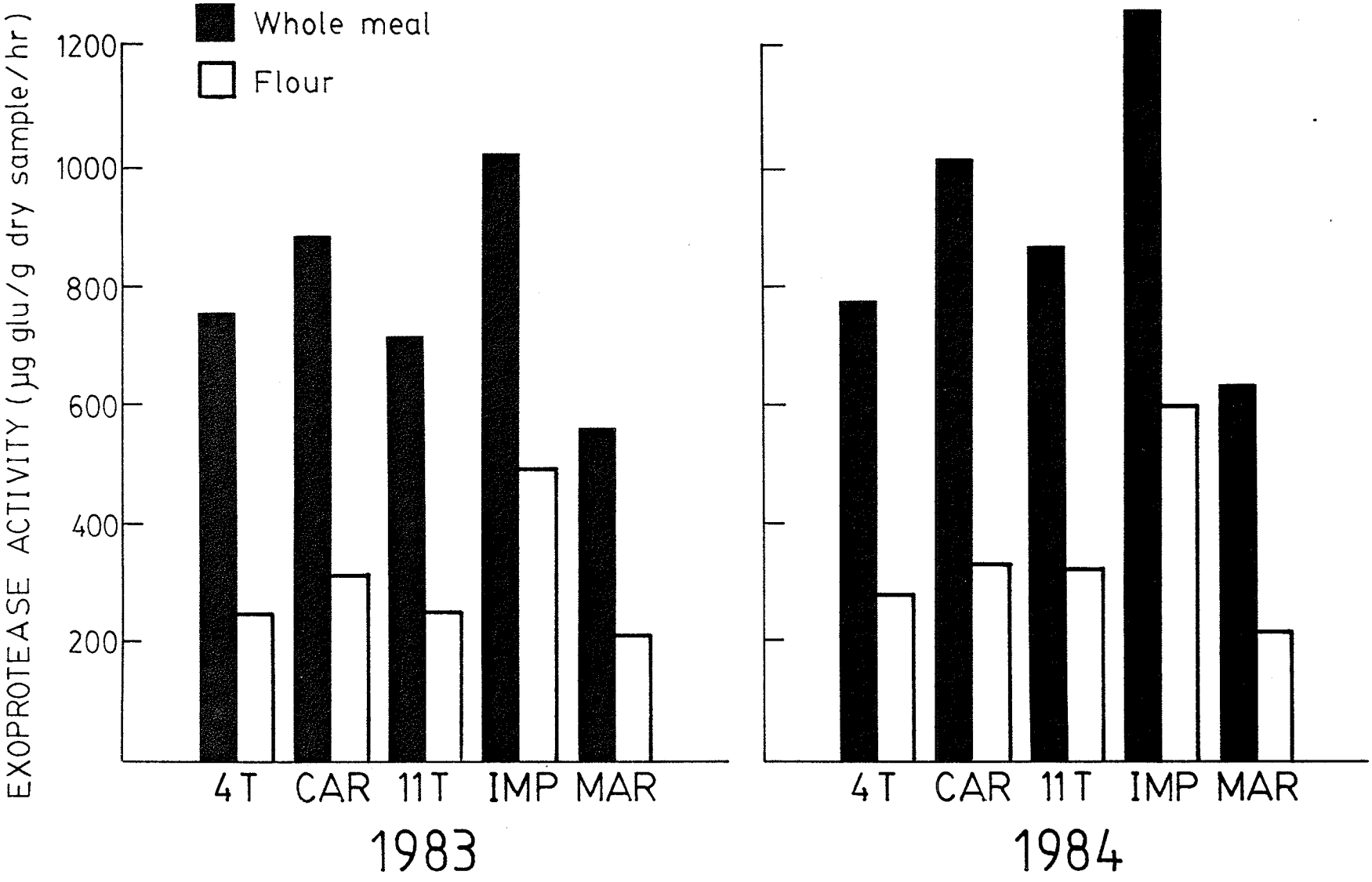
Car = Carman

11T

Imp = Impala

Hard red spring wheat

Mar = Marquis


Whole meal
Flour
EXOPROTEASE ACTIVITY (µg glu/g dry sample/hr)
1200
1000
800
600
400
200
4T CAR 11T IMP MAR
1983
4T CAR 11T IMP MAR
1984

though Impala flours had the highest level of exoprotease activity among the cultivars examined.

McDonald and Chen (1964) reported that hemoglobinase activity in wheat was reduced by 88% at the salt concentration normally used in doughs (0.05 M). Madl and Tsen (1974) demonstrated inhibition of hemoglobinase activity with salt concentrations as low as 0.01 M. Because salt was included in the baking formula used in the present study, it is probable that the exoprotease activity in the triticale doughs was similarly inhibited.

Endoprotease (Azocaseinase) Activity

The endoprotease activity of the bulk grain and the milled flour samples is shown in Figure 15. Endoprotease activity was similar to α-amylase and exoprotease activity in that a large portion of the endoprotease activity was removed with the bran and germ during milling. Although the triticale flours had significantly higher levels of α-amylase and exoprotease activity than the Marquis wheat flours, all triticale and wheat flours had similar levels of endoprotease activity.

There was no apparent relationship between the endoprotease activity and the loaf volume potential of the triticale flours. For the eight triticale flour samples (four cultivars over 2 years), the correlation between loaf volume and flour endoprotease activity was nonsignificant ($r = +0.119$, d.f. = 6, $P = 0.05$). In 1984, there was no significant

Figure 15. Endoprotease (azocaseinase) activity of mature bulk grain and flour samples.

Triticale

4T

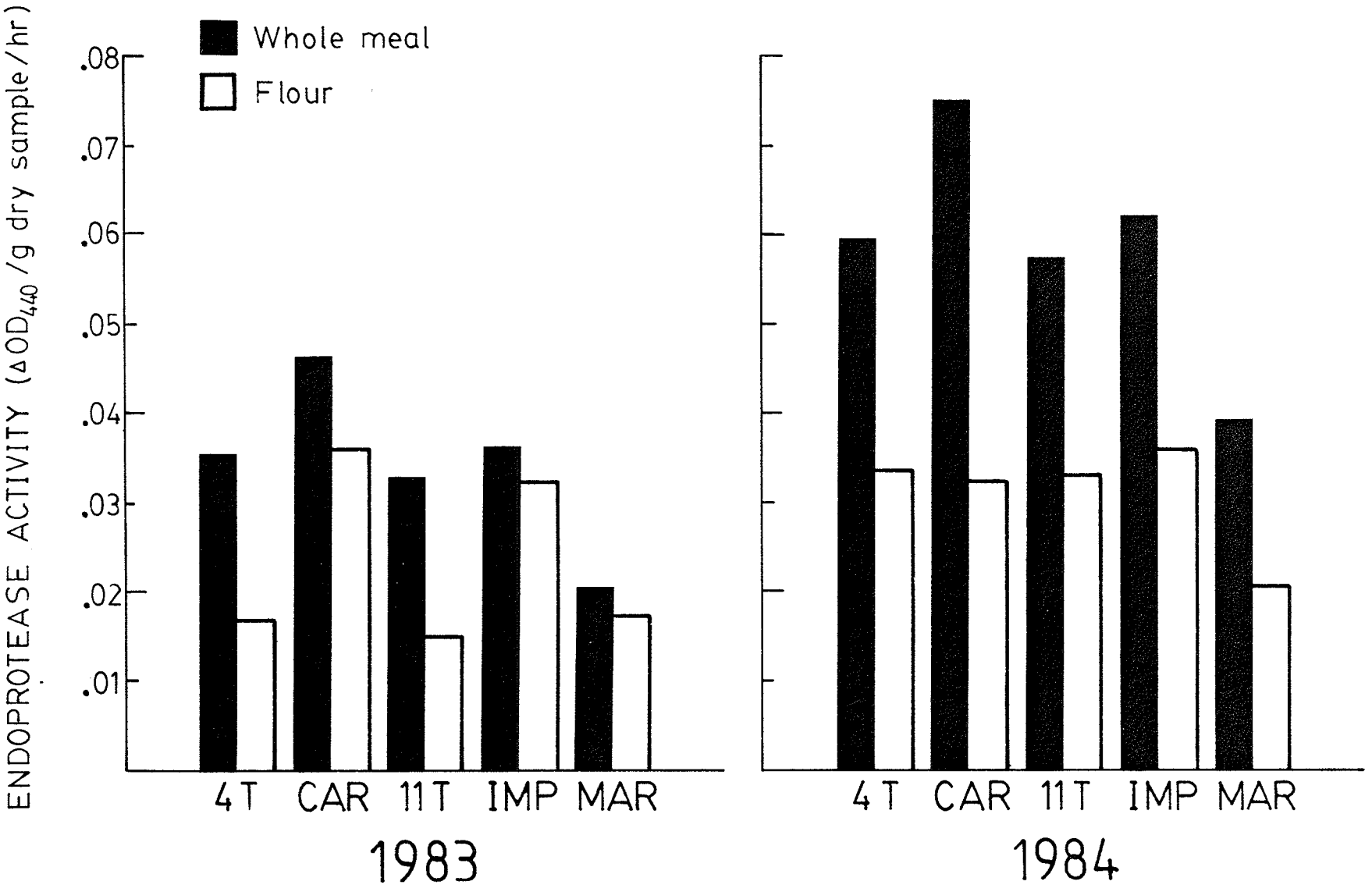
Car = Carman

11T

Imp = Impala

Hard red spring wheat

Mar = Marquis


ENDOPROTEASE ACTIVITY ($\Delta OD_{440}$ /g dry sample/hr)
Whole meal
Flour
.08
.07
.06
.05
.04
.03
.02
.01
4T CAR 11T IMP MAR
1983
4T CAR 11T IMP MAR
1984

difference in endoprotease activity among any of the triticale flours (Table 9) even though the loaf volume of the triticale breads produced from these flours varied widely.

In the bake test, triticale doughs did not soften or become slack during fermentation, suggesting that endoproteolytic cleavage of the gluten protein matrix during fermentation was limited. This does not, however, exclude the possibility that gluten proteins may have been altered *in situ* during kernel development. As previously mentioned, 11T and Impala flours produced breads of similar loaf volume even though Impala flours had significantly higher quantities of gluten protein than 11T flours. Developing Impala kernels also had higher levels of protease activity than developing 11T kernels (Figures 5 and 6), and it is, therefore, possible that the higher gluten protein content of Impala was offset by its higher *in situ* protease activity (with subsequent deterioration of gluten protein quality). This suggestion, however, is highly speculative. There are many other factors that may influence gluten protein quality (e.g. the portion of gluten protein as acid-insoluble residue protein, the primary amide content of the gluten protein, the extent of disulfide crosslinking) that were not investigated in the present study but which might have accounted for differences in gluten protein quality among the triticale cultivars examined.

# V. GENERAL DISCUSSION

In recent years, considerable progress has been made in improving the agronomic characteristics and yield potential of triticale. However, there must be a greater effort by breeders to improve the baking potential of the average population of high yielding triticale if this cereal is to meet industrial standards for bread production. The cereal chemist can assist the plant breeder in this regard by determining the factors that control the baking potential of triticale. The present study was undertaken to determine the influence of α-amylase activity, protease activity, and gluten protein on the baking performance of four secondary hexaploid triticales. The four triticale cultivars (4T, Carman, 11T, and Impala) were chosen because of their diverse baking quality. A hard red spring wheat (cv. Marquis) was included in the study for comparison.

Although triticale flours were generally higher in α-amylase and protease activity than Marquis wheat flours, endogenous enzymes appeared to have little effect on the baking quality of the triticale flours. For example, Impala flours produced breads with relatively high volumes and satisfactory crumb characteristics in spite of having high α-amylase and protease activity. There was no apparent relationship between the loaf volume potential and protease

activity of the triticale flours, and the only adverse effect to bread quality that could be attributed to high α-amylase activity was the gummy crumb texture of the 4T breads.

In contrast, gluten protein played a major role in determining the baking potential of the triticale flours. The loaf volume and gluten protein content of the triticale flours showed a significant positive correlation ($r = +0.879$, $d.f. = 6$, $P = 0.01$), and triticale flours with relatively high gluten protein content (e.g. Impala flours) generally had stronger mixing characteristics and produced breads of better overall quality than flours with low gluten protein content (e.g. 4T flours). This would suggest that the rheological properties and baking performance of the triticale flours were strongly influenced by their quantity of gluten protein. Differences in gluten protein quality, as well as quantity, were noted among the triticale cultivars. The factors responsible for differences in gluten protein quality were not determined, although results of the present study suggested that *in situ* proteolytic cleavage of storage protein might have contributed to deterioration of Impala gluten protein quality. To prove or disprove that the baking potential of triticale can be altered enzymatically during kernel development is certainly worthy of further research.

One cannot assume that elevated levels of α-amylase

and protease activity in developing kernels result in a concomitant breakdown of seed reserves. If such enzymes are confined to the outer kernel layers (e.g. the pericarp and aleurone) and are not released into the endosperm, then their presence in the developing kernel should not affect the baking potential of a given cultivar. While the four triticale cultivars examined in the present study had higher levels of α-amylase and protease activity than Marquis wheat checks throughout grain maturation, there are several observations that would suggest that <u>in situ</u> degradation of starch and protein reserves was limited. Firstly, levels of starch damage were generally lower in triticale flours than in Marquis wheat flours (Table 4). Secondly, there was a rapid decrease in kernel moisture content in the later stages of grain development (Figure 1), especially in the 1983 growing season. Accordingly, one would expect that the enzyme activity in the kernels would decrease with falling kernel moisture. Thirdly, the α-amylase, exoprotease, and endoprotease activity was noticeably higher in the whole grain than in the milled flour for any given cultivar (Figures 13, 14, and 15). This last observation would suggest that a large portion of the enzyme activity in the whole grain was confined to the outer kernel layers.

For any given flour, there is an optimum level of starch damage in terms of water absorption and bread quality, depending on the gluten protein content and the level of α-amylase

activity. Flours with higher gluten protein content seem to be able to tolerate higher levels of starch damage without serious bread quality deterioration (Pratt 1971, Tipples 1982). One might interpret this observation in another way: if starch damage is excessive and cannot be reduced by modifying the milling process, the adverse effects of α-amylase activity can be masked in part by increasing the quantity of gluten protein.

In the present study, the gummy crumb texture of the 4T breads was attributed to excessive α-amylase activity. However, Impala flours produced breads with good crumb texture, even though levels of α-amylase activity in Impala and 4T flours were similar (Figure 13). It is possible that Impala doughs were able to tolerate higher levels of α-amylase activity than 4T doughs because of the relatively low damaged starch levels (Table 4) and high gluten protein content (Table 8) of the Impala flours.

One of the most persistent problems in the development of triticale has been kernel shrivelling. Past research would suggest that kernel shrivelling in triticale is an indication of poor sink performance and an early termination of starch deposition in the endosperm (Thomas et al. 1980). Klassen et al. (1971) have reported that reduced starch accumulation may lead to low test weights. Since the protein content of mature grain essentially represents a ratio between the amount of protein and the amount of

starch per grain, reduced starch accumulation may also result in high protein content. Furthermore, shrivelled kernels usually have a higher ratio of bran to endosperm and thus contain more ash on a percentage basis and yield less flour than plump kernels (Zeleny 1971). The results of the present study would tend to confirm these relationships. For example, grain of the shrivelled triticale, Impala, had the lowest test weights and highest protein contents among the four triticale cultivars examined (Table 3), and yielded low amounts of high-ash flour (Table 4). In contrast, grain of the relatively well-filled triticale, 4T, had the highest test weights and lowest protein contents, and yielded high amounts of low-ash flour. Considering the significant positive correlation between loaf volume and protein content ($r = +0.802$, d.f. = 6, $P = 0.05$) for the triticale flour samples examined in the present study, the above results would suggest that breeder selection for plump kernels would tend to favor selection of triticales with low protein content and hence, low baking potential.

Klassen et al. (1971) proposed that starch breakdown by α-amylase activity in developing kernels may be one of the factors contributing to kernel shrivelling in triticale. In the present study, there was no evident relationship between kernel shrivelling and α-amylase activity. For example, both the well-filled triticale, 4T, and the

shrivelled triticale Impala both had relatively high levels of α-amylase activity. Furthermore, there was no evident relationship between the levels of α-amylase activity.and protease activity. Compared to the other triticale cultivars, Carman was low in α-amylase activity (Figure 4) but high in protease activity (Figures 5 and 6) throughout kernel development. In contrast, 4T was high in α-amylase activity but low in protease activity. It was also observed that the α-amylase activity of the triticales generally increased during kernel development, while protease activity generally decreased.

In bread wheat, the proteins which have the greatest influence on dough properties and baking quality (i.e. the high molecular weight gliadins and glutenins) are mainly coded by genes on chromosome 1D, with minor subunits coded by genes on chromosomes 1A, 1B, and 4D (Miflin et al. 1983, Wall 1979). It is therefore difficult to ascribe the satisfactory baking performance of 2D/2R substituted 11T and Impala triticales to the presence of 2D chromosome. Indeed, Carman triticale carried a full complement of rye chromosomes but still produced bread of better overall quality than the 2D/2R substituted 4T triticale.

The majority of the gluten proteins in wheat are encoded on the group 1 chromosomes, which may prove to be a problem in the breeding of secondary triticales with improved baking quality. Theoretically, a 1D/1R substitution in triticale should result in the improvement of

protein quality. As previously discussed, however, chromosome 1R is the smallest rye chromosome and is usually the last rye chromosome to be substituted in a triticale x bread wheat cross (Lukazewski et al. 1982, Gustafson and Zillinsky 1978). Improvement of the baking quality of secondary triticales through chromosome substitution may therefore be limited by the fact that certain substitutions are difficult to achieve. This does not, however, exclude the possibility of introducing 1D chromatin into secondary triticales via translocation of chromosome arms or segments. It is even possible that the satisfactory baking performance of triticale 11T and/or Impala is due to such a translocation.

# VI. SUMMARY AND CONCLUSION

Four secondary hexaploid triticales of diverse baking quality (4T, 11T, Impala, and Carman) were examined for differences in α-amylase activity, protease activity, and gluten protein content. The agronomic performance and rheological properties of the triticales were also evaluated. One hard red spring wheat (cv. Marquis) was included for comparison. All cultivars were grown in dryland field plots during the 1983 and 1984 growing seasons. Results of the study are summarized below.

1. The changes in α-amylase and protease activity were followed from 21 days post anthesis to full maturity. The α-amylase activity of the triticales generally increased during grain maturation, while the exoprotease and endoprotease activity generally decreased. At full maturity, all triticales had higher whole grain α-amylase, exoprotease, and endoprotease activity than the Marquis wheat check grown in the same year. Differences in α-amylase and protease activity due to cultivar and year were observed.
2. Evaluation of bulk grain samples harvested at maturity showed that the triticales had lower test weights, lower falling number values, lower whole grain protein content, and higher thousand kernel weights than the Marquis wheat checks. The whole grain protein content

of any given cultivar was higher in 1983 than in 1984.

3. Triticale grain samples also yielded less flour than the Marquis wheat checks. Compared with the Marquis flours, the triticale flours had lower Zeleny sedimentation values, lower farinograph absorptions, and shorter dough development times. Impala doughs had noticeably higher dough stability values and greater resistance to stretching than other triticale doughs.

4. The Marquis wheat breads were superior in overall quality to the triticale breads under the conditions of the A.A.C.C. straight dough bake test. The 4T and Carman flours produced breads with low loaf volumes and dense, compact crumbs. The 4T breads also had a gummy crumb texture. In contrast, the 11T and Impala flours performed relatively well in the bake test. The crumb texture and grain of the 11T and Impala breads were satisfactory and the loaf volumes of these breads were significantly higher than the loaf volume of either the 4T or Carman breads. With the exception of triticale 11T, loaf volumes were higher in 1983 than in 1984 for any given cultivar.

5. The weight of dry gluten recovered from the triticale flours varied widely (from 7.2 to 12.7 g dry gluten/100 g dry flour), and protein analysis of the dried glutens showed that all triticale flours contained significantly lower amounts of gluten protein than

the Marquis wheat flours. The lower gluten protein content of the triticale flours was due in part to their lower protein content, and in part to their lower percentage of total flour protein as gluten-like protein. For the eight triticale flour samples (four cultivars over 2 years), there was a significant positive correlation between loaf volume and gluten protein content ($r = +0.879$, d.f. = 6, $P = 0.01$). Differences in gluten protein quality among the triticale cultivars were also observed.

6. The triticale flours contained higher levels of α-amylase and exoprotease activity than the Marquis wheat flours. In contrast, the triticale and wheat flours contained similar levels of endoprotease activity.

7. There was no apparent differences in endogenous levels of exoprotease or endoprotease activity that could account for differences in baking quality among the triticale flours. Fermenting doughs showed no signs of proteolytic degradation (i.e. doughs did not become progressively softer during fermentation), and correlations between loaf volumes and flour protease activities were non-significant. In spite of the relatively high α-amylase activity in the triticale flours, only the 4T flours produced breads with a gummy crumb texture. The low tolerance of the 4T doughs to

α-amylase activity was attributed to their low gluten protein content and relatively high level of damaged starch.

In conclusion, the gluten protein component was the major factor controlling the baking potential of the secondary triticales, while endogenous α-amylase and protease enzymes had only a minor effect on loaf quality. By expanding the genetic base of secondary triticales and selecting strains with superior protein quantity and quality, it should be possible for breeders to further improve the baking quality of this promising new cereal.

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