Effects of Nitrogen Fertilization, Genotype and Environment on the Quality of Oats (*Avena sativa*) Grown in Manitoba

by

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

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ABSTRACT

Manitoba is a major producer of high quality oats (*Avena sativa*) destined for milling and food processing in domestic and export markets, particularly the United States. Strengthening the demand for Canadian oats requires continued improvement of oat cultivars to meet the changing needs of the agricultural and food industries. In order for plant breeders to achieve this, more information is needed regarding what factors affect variation in the milling, nutritional, functional and end-product quality of oats grown in western Canada. The objective of this study was to determine the relative effects of genotype, environment, nitrogen fertilization and their interactions on the quality of oats destined for human food. Replicated field tests were grown at each of six environments using a split plot design. Five genotypes were the main plots and four nitrogen fertilization treatments (0 to 120 kg/ha) were applied to the sub-plots. Hull content was significantly affected by a qualitative genotype-by-environment interaction, indicating the need for multiple testing sites. Growing conditions also had a strong influence on groat breakage, protein, and oil (36, 73, and 49 % of total variation respectively) but differences between genotypes remained consistent across environments. Genotype was the main factor affecting β-glucan content (78 % of total variation). Nitrogen fertilization had a greater impact on oat composition than on milling characteristics and in many cases the effect of nitrogen was dependant on the location. At sites where residual nitrogen was low (less than 36 kg/ha), fertilization resulted in increased levels of protein and β-glucan (by as much as 4.4% and 1 % respectively), while oil decreased slightly (less than 1 %). The effect of nitrogen on wholemeal pasting properties was also dependant on location. Investigation of oat starch characteristics and wholemeal pasting properties revealed many significant differences between genotypes, suggesting potential for the genetic improvement of oat functionality in food systems. Several of these properties also varied with environment, but for most, the ranking of genotypes across environments was stable. A laboratory scale oat conditioning and flaking process was developed, which allowed for the measurement of genotypic and environmental influences on flake granulation, water hydration capacity and cooked oatmeal texture. Overall, the results of this study indicate that the processing quality of Canadian produced oats can be optimized with plant breeding and crop management efforts.

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CHAPTER 1

INTRODUCTION

Oat (*Avena sativa*) production in Canada has a substantial economic influence on the agricultural industry. The estimated area seeded to oats was 1.82 million hectares in the year 2000 (Agriculture and Agri-Food Canada, 2001), making it the fourth most seeded crop in Canada. Currently, the majority of Canadian oat production occurs in western Canada. The end of the Western Grain Transportation Act in 1995 resulted in a shift in oat production from Alberta to eastern Saskatchewan and Manitoba due to their close proximity to markets located in the mid west United States (Agriculture and Agri-Food Canada, 2000). Seeded area to oats in Manitoba was 384 500 hectares in 2000 and the predicted seeded acreage was similar for 2001 at 374 300 hectares (Manitoba Agriculture and Food, 2001).

Dramatic increases in oat exports since the 1980's have made Canada a leader in oat export markets. Canadian oats satisfied an estimated 60 % of world demand in the 1999-2000 crop year. This share was considerably greater than that of other major oat producing countries like Sweden (14 %), Finland (8 %), and Australia (7 %). Canadian producers continue to grow more oats, which is helping to satisfy demands from the largest importer of oats in the world, the United States. An estimated 95 % of Canadian oat exports go to the north central United States; other customers include Japan. Relatively low transportation costs to the United States and supply of high quality oats are the main reasons why Canada has dominated world oat exports (Agriculture and Agri-Food Canada, 2000).

Oats have traditionally been used for animal feed, which remains the primary use today. However, the proportion of the world oat supply destined for human consumption has doubled to 24 % of total consumption since 1960 (Agriculture and Agri-Food Canada, 1998). Demand for oat based foods is expected to stay strong and increase, particularly as there are a growing number of consumers seeking functional foods with the ability to reduce the risk of chronic diseases. The cornerstone of this functional food market for oats is the health claim that states oat β-glucan lowers cholesterol and reduces the risk of cardiovascular disease. This health claim has increased the demand for milled oat products such as cut groats, flakes and flour, which are used in a wide range of products including hot cereals, granola, extruded cereals, cookies, muffins, breads, crackers, snacks, baby food, meat extenders and even beverages (Can-Oat Milling, 1998). Between 60 and 70 % of Canadian oats exported to the United States are for milling (Agriculture and Agri-Food Canada, 2000). Canadian millers also export groats, flakes, and meal. Manitoba's exports of these products were valued at approximately $36 million in 1999 (Manitoba Agriculture and Food, 2001). For example, Can Oat Milling Inc., located in Manitoba and Saskatchewan, has become the largest industrial supplier of oats in the world. It can process approximately 10,000 cwt of finished product per day, 95 % of which is exported to the United States, South and Central America, the Caribbean and Australia (Can-Oat Milling, 1998). There are also other potential uses of oats for food such as oat oil for cooking (Branson and Frey, 1989; Erazo-Castrejón et al., 2001) and oat extracts for use in functional foods. For example, Ceapro Inc., located in Alberta, uses fractionation technology to concentrate oat components such as β-glucan and antioxidants for use in nutraceuticals and non-food products (Agriculture and Agri-Food Canada, 2000). Oat β-glucan is thought to improve the health of skin and is used in the treatment of wounds. Oat preparations are used in a variety of lotions and cosmetics and in veterinary shampoo (Paton and Fedec, 1996). As innovative uses for oats are created and human consumption continues to increase, the demand for high quality oats will strengthen. Successful competition in domestic and international markets requires continued improvement of Canadian oat cultivars to meet the changing needs of the agricultural and food industries.

Oat breeding programs are in place in Canada to ensure the availability of cultivars that possess the characteristics desired by producers, millers, food and non-food manufacturers and consumers. Quality traits that have long been a priority to breeders include agronomic performance, disease resistance, yield, test weight and hull content. Traditionally, oats with high oil and protein were desired for high energy animal diets (Burrows, 1986). However, increased human consumption of oats has shifted breeding priorities to include the development of cultivars high in β-glucan and low in oil in order to meet low energy requirements for human diets. As food oat markets develop and oat research progresses in the areas of human nutrition and food processing, there is an opportunity for oat breeding programs to evolve even further. For example, it is essential to the success of a cultivar for it to perform well in an end-product. This concept is demonstrated in the breeding programs of other cereal crops, such as wheat, where achieving high bread loaf volume is an integral part of the screening process (Peterson et al., 1997). Currently, no end-product testing system exists for Canadian oat breeding.

Part of the reason lies in the lack of small scale methodology and equipment needed to process oats as is done in the industry. Prior to flaking or milling, oats are subjected to moist heat treatments and then dried down in a kiln. This conditioning process is controlled to inactive enzymes and alter the sensory qualities of the oats. Oats are briefly treated again with steam before flaking to impart resilience to the groat and thus reduce the production of fine particles under the pressure of rolling (Deane and Commers, 1986). Methodologies that mimic these complex processes need to be developed for use in genetic screening of oat end-product quality. Furthermore, this technology would open the door to studies on factors affecting the quality of oat foods and may justify the need to breed for specific oat starch and functional characteristics.

Successful introduction of novel or improved traits into adapted cultivars requires a good understanding of the factors that control the expression of the trait. For example, traits that are highly controlled by genetics can be manipulated by the plant breeder relatively easily, whereas those that are strongly influenced by environmental factors, like weather and soil conditions, cannot. Quantitative interactions can occur between the genotypic and environmental factors controlling a trait, meaning that the magnitude of the genotype response changes with different environments. Breeders are more interested in qualitative (also called cross-over) interactions, which are characterized by a change in the rank order of a number of genotypes when they are grown in different environments. This type of interaction would cause the choice of superior genotype to be different depending on the growing environment, thus greatly reducing the effectiveness of recurrent selection of the desired trait (Gail and Simon, 1985; Baker, 1988; Kang, 1990).

In order to assess relative effects of genetic and environmental factors, studies need to be conducted in the target growing region using genotypes of interest.

In addition to genetic improvement, controlling quality at the crop production stage may help provide the food industry with superior food oats. Specific environmental conditions, such as soil fertility, are largely controlled by the producer. For example, nitrogen fertilization is a common agricultural practice used to increase yield and test weight. The effect of nitrogen fertilization on other oat characteristics that are important to millers and the food industry need to be examined. Companies sourcing oats may be able to ensure desired oat quality by providing producers with specific nitrogen fertilization recommendations. Furthermore, breeders will benefit from information regarding interactions between genotype and nitrogen effects.

The goal of the research presented in this thesis was to provide oat breeders, millers and manufacturers of oat products with information regarding the variation that exists in the quality of oats grown in Manitoba. The specific objectives of the study were as follows:

1. To determine the relative effects of genotype, environment and genotype-by-environment interaction on a) the composition and physical attributes of whole and/or milled oats, b) the characteristics and functionality of oat starch and wholemeal and c) the quality of oat end-products processed by laboratory scale methodologies and equipment.

2. To determine the relative effects of nitrogen fertilization rate, genotype, environment and interactions amongst these factors on whole and/or milled oat physical attributes, composition and wholemeal pasting characteristics.

CHAPTER 2

REVIEW OF LITERATURE: FACTORS AFFECTING VARIATION IN OAT QUALITY CRITERIA

2.1 PHYSICAL OAT PROPERTIES

Physical oat properties, such as hull content and test weight, have long been a measure of oat quality at grain elevators and mills. The increasing usage of oats for human foods has strengthened the need for oats with superior milling quality. In addition to traditional quality parameters, newly studied traits such as groat breakage are beginning to shape the definition of high quality milling oats.

2.1.1 Hull Content

Whole oats, in the state they are harvested, consist of a caryopsis, or groat, enclosed in a fibrous covering called a hull, which is made up of the lemma and palea. Although the hull protects the groat from damage during seed development and grain handling, it is of minor economic value to millers compared to the groat because it is inedible. Thus, the first step of the oat milling process is removal of the hull from the groat. Naturally, whole oats that have a minimum proportion of hull to groat content provide millers with a greater recovery of usable product. Oats with high proportions of hull can be bulky, thereby increasing storage space and transportation requirements. Hull content is also negatively correlated with test weight (Asp et al., 1992; Doehlert et al., 1999), which is an important grading factor. Oats receiving a low grade due to low test weight at the point of sale earn a reduced price for producers and are generally used for animal feed rather than enter the food market. Due to the economic importance of this characteristic, reducing the hull content of registered oat cultivars is a major goal of Canadian oat breeding programs.

Hull content is measured as the ratio of the weight of the hull compared to the weight of the whole grain sample and is expressed as a percentage. Conversely, groat percentage of an oat sample can be measured, in which case a high value is desirable. Based on these definitions, it follows that the physical basis for a change in hull percent can be due to a change in the quantity or thickness of the hull, the plumpness of the groat, and the occurrence of tertiary or bosom kernels, which tend to have relatively high amounts of hull. The most accurate measurements of hull content are achieved with hand separation but more practical mechanical methods are available. Compressed air and impact dehulling machines are useful for larger sample sizes especially when followed by removal of remaining hulls in the final groat sample (Doehlert et al., 1999).

Hull and groat percentages of oats vary significantly with genotype as well as the environmental conditions in which the oats are grown. For example, one hundred Swedish oat samples showed a range in hull content from 23.2 to 35.0 % (Asp et al., 1992). Oat genotypes originating from Australia (Zhou et al., 1998b; Zhou et al., 1999a), Canada (Humphreys et al., 1994a; Ronald et al., 1999) and the United States (Doehlert et al., 1999) also varied significantly ($P \leq 0.01$) in hull and groat content. Three growing locations in North Dakota, USA resulted in significantly different ($P \leq 0.01$) groat percentages (Doehlert et al., 1999) as did eight locations in New South Whales, Australia (Zhou et al., 1999a). Zhou et al. (1999a) also found that the mean groat percentage for eight genotypes was different depending on the growing year and that location influenced groat percentage more than genotype in both of the two growing years. In contrast, Ronald et al. (1999) studied the heritability of hull content in oats derived from three crosses between Canadian cultivars grown in several locations and found genotype to be the dominating influence on hull percentage. Location effects were significant ($P \leq 0.001$) but the component of variation for genotypes was greater. Genotype-by-environment interaction effects were found to be significant in all three studies. Doehlert et al. (1999) and Zhou et al. (1999a) thus concluded that multiple growing sites would be required for breeding purposes. However, Ronald et al. (1999) determined that the interaction effect contributed little to total variation compared to the effects of genotype, location and experimental error, indicating that few growing sites would be required to breed for hull content using the genotypes in the study. These findings, in addition to broad-sense heritability estimates of 0.35 to 0.90 (towards high range with more diverse parents), indicate confidence in the ability to select for oats with low hull content.

The effects of agricultural management practices, such as nitrogen fertilizer application, on oat hull and groat content have also been studied. An experiment involving five Australian cultivars and six nitrogen fertilizer rates ranging from 0 to 100 kg/ha found no significant effect of fertilizer rate on groat percentage (Zhou et al., 1998b). Another study conducted in Eastern Canada examined the effect of applying 40 kg/ha of nitrogen fertilizer at seeding versus the same treatment plus 20 kg/ha at a later stage in the crop's development. An observed decrease in hull percent with the higher level of nitrogen was thought to be associated with the observed increase in plump grain and decrease in bosom grain (double kernels high in hull content). Further study is required to confirm this finding, considering that the decrease in hull content observed with nitrogen fertilization was so small (25.7 to 25.2 % hull) and was only significant ($P \leq 0.05$) at one out of four growing environments studied (Humphreys et al., 1994a).

2.1.2 Groat Breakage

Another physical oat characteristic that affects milling product recovery upon dehulling is resistance to breakage. The mechanical stress that oats are subjected to during removal of the hull causes some of the groats to break. Broken groats cannot be processed into end products such as bran and whole flakes and thus represent an economic loss to millers.

It is only recently that research into the factors that cause oat groat breakage has been published. Doehlert et al. (1999) measured the breakage of ten oat genotypes (including some Canadian cultivars) grown at three locations in the United States. The type of hull removal system influenced the overall level of groat breakage, but within the range of each, there were significant differences among the genotypes and growing environments. Significant genotype-by-location interactions were observed in which the genotype response to environment varied in magnitude and resulted in rank order differences. Furthermore, Doehlert and McMullen (2000) reported that interaction effects may have been influenced by disease resistance since they observed higher breakage levels (up to 20 %) at a location heavily infected with crown rust. In both of these studies, oats with low amounts of breakage also tended to have high hull content suggesting that the hull provided protection against breakage. Breakage was also found to be influenced by the hardness of the groats (Doehlert and McMullen, 2000), which may be due to high amounts of bran or strengthened internal bonds due to the presence of phenolic compounds such as ferulic acid (Engleson and Fulcher, 2001).

2.2 OAT COMPOSITION

Major components in oats include β-glucan, protein, oil and starch. The levels of these components desired in a registered cultivar depend on the end-use. For example, oats used for animal feed ideally contain high fat and low fibre for maximum feed efficiency. Now that more oats are being milled for human consumption, it is necessary to develop cultivars that contain nutrients in proportions that are conducive with the low fat, high fibre diets recommended by nutrition authorities (Health and Welfare Canada, 1990). In other cases, it may be desirable to have levels of certain nutrients above dietary requirements in order to fractionate for use in nutraceutical and non-food industries and to blend with sources of oats having low levels of desired nutrients.

2.2.1 β-Glucan Content

The majority of the soluble dietary fibre fraction in oats is comprised of the unbranched polysaccharide (1→3)(1→4) β-D-glucan. This component is highly desirable in oats destined for human consumption because it is believed to be responsible for lowering total and low density lipoprotein cholesterol without decreasing high density lipoprotein cholesterol in both animals and humans (Klopfenstein, 1988; Wood et al., 1989; Mälkki et al., 1992; Newman et al., 1992; Kahlon et al., 1993; Braaten et al., 1994). This information prompted the United States Food and Drug Administration to allow health claims regarding the ability of oat soluble fibre to reduce the risk of cardiovascular disease to be printed on food packaging. To qualify for this claim, the food product must contain a minimum of 0.75 g/serving of β-glucan from oat bran, rolled oats or whole oat flour. This amount is based on oat bran containing at least 5.5 % β-glucan and the rolled oats and whole oat flour containing at least 4.0 % (Food and Drug Administration, 1996). Oat products meeting these requirements may satisfy increasing consumer demands for functional foods that help prevent heart disease. There is no Canadian counterpart to the US health claim on oats as of yet, but groups in Canada are pursuing such legislation (Fitzpatrick, 2001). Regardless, the majority of Canadian oats are exported to the United States, therefore it is essential from a competitive standpoint that newly registered Canadian oat cultivars meet industry specifications for high β-glucan content.

A survey of the literature reveals that oat β-glucan content can range from as low as 1.8 % (Miller et al., 1993b) to greater than 7.0 % (Peterson, 1991). Variation in β-glucan content of oat genotypes from several origins have been reported by many researchers (Asp et al., 1992; Lim et al., 1992; Cho and White, 1993; Miller et al., 1993b; Humphreys et al., 1994b; Lee et al., 1997). Peterson (1991) tested the β-glucan content of 12 oat genotypes grown at ten locations in the United States. He found that genotype, location and genotype-by-location interaction effects were all significant ($P \leq 0.01$) but that the variance ratio for the interaction effect was less than that of the main effects. This was in agreement with a study by Miller et al. (1993a) who found a significant genotype-by-location interaction to be of little practical importance. Furthermore, they determined that genotypic variation among a group of six Canadian cultivars and seven breeding lines to be greater than the variation observed among five growing sites in Eastern Canada in two out of three growing years. Brunner and Freed (1994) did not find