Effects of Genotype and Environment on Polyphenol Oxidase Activity and Related Properties of Red and White Wheats

by
Daniel Vázquez

A Thesis
Submitted to the Faculty of Graduate Studies
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Department of Foods and Nutrition
University of Manitoba
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ABSTRACT

The color of wheat end-products depends on flour characteristics, including polyphenol oxidase (PPO) level, in addition to processing. PPO has been related to non-desirable discoloration in end products but it is a poorly understood component. The purpose of this study was to evaluate the methodologies used to determine PPO activity, to compare the effects of genotype and environment on PPO, and to determine the relationship of PPO to end-product discoloration. A set of double haploid experimental lines, including red and white kernel genotypes and agronomical checks, were grown at four locations in western Canada during two years. PPO activity was analyzed in grain and flour by the oxygen consumption method with catechol as substrate, and by spectrophotometric methods. Color values and discoloration after 24 hours were determined in salted (Udon) and alkaline (Kansui) type noodle sheets. A simple fast spectrophotometric whole kernel method using tyrosine as a substrate was well correlated with results of the oxygen consumption method ($r=0.813$, $P=0.001$). PPO activity can also be categorized visually using this whole kernel procedure. This is a simple rapid method for detecting high PPO activity genotypes. Milling and flour properties of the samples were affected significantly by the environment. The genotypes analysed formed two distinct groups according to their PPO activity. A high degree of genetic control was evident for this enzyme: 80% of the grain PPO activity was attributed to genetic factors. The environmental influence was greatest for the high PPO cultivar and low PPO genotypes were the least influenced by environment. PPO activity was independent of kernel color. The color of alkaline noodles after 24 hours and

discoloration of both noodle types were influenced significantly by PPO group. Noodle color and discoloration were strongly influenced by the growing environment of the wheat sample.

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ABBREVIATIONS

Aa0	Alkaline noodle redness (a*) immediately after preparation
Aa24	Alkaline noodle redness (a*) 24 hours after preparation
AaC	Alkaline noodle redness (a*) change after 24 hours
Ab0	Alkaline noodle yellowness (b*) immediately after preparation
Ab24	Alkaline noodle yellowness (b*) 24 hours after preparation
AbC	Alkaline noodle yellowness (b*) change after 24 hours
AL0	Alkaline noodle brighhness (L*) immediately after preparation
AL24	Alkaline noodle brightness (L*) 24 hours after preparation
ALC	Alkaline noodle brightness (L*) change after 24 hours
ANOVA	Analysis of variance
APH	Australian Prime Hard
ASW	Australian Soft Wheat
AU	Absorbance units
CPS	Canadian Prairie Spring
CV	Coefficient of variation
CWRS	Canada Western Red Spring
CWSWS	Canada Western Soft White Spring
DH	Double haploid
EU	Extraction unit
Env #1	Environment #1, Brandon 1998
Env #2	Environment #2, Morden 1998
Env #3	Environment #3, Swift Current 1998
Env #4	Environment #4, Glenlea 1998
Env #5	Environment #5, Brandon 1999
Env #6	Environment #6, Glenlea 1999
Env #7	Environment #7, Melfort 1999
Env #8	Environment #8, Portage 1999
Fa	Flour redness (a*)
Fb	Flour yellowness (b*)
FL	Flour brightness (L*)
FPPO	Flour polyphenol oxidase, determined with the oxygen consumption method

GPPO	Grain polyphenol oxidase, determined with the oxygen consumption method
PPO	Polyphenol oxidase
%PPO	Flour PPO percentage of grain PPO
Sa0	Salted noodle redness (a*) immediately after preparation
Sa24	Salted noodle redness (a*) 24 hours after preparation
SaC	Salted noodle redness (a*) change after 24 hours
Sb0	Salted noodle yellowness (b*) immediately after preparation
Sb24	Salted noodle yellowness (b*) 24 hours after preparation
SbC	Salted noodle yellowness (b*) change after 24 hours
SD	Standard deviation
SL0	Salted noodle brightness (L*) immediately after preparation
SL24	Salted noodle brightness (L*) 24 hours after preparation
SLC	Salted noodle brightness (L*) change after 24 hours
SQALC	Square root transformation of ALC
SQFPPO	Square root transformation of FPPO
SQGPPO	Square root transformation of GPPO
SQSLC	Square root transformation of SLC
SQWK	Square root transformation of WK
WK	Grain polyphenol oxidase, determined with the selected whole kernel method

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INTRODUCTION

Wheat is the most cultivated cereal in the world; over 500 million metric tonnes are harvested each year (Canadian Wheat Board 1996-97). Production of wheat has risen continuously during the last 50 years (Conca-Torres 1998) and according to the tendency of the last 10 years (Canada Grain Council 1999) production will be over 600 million metric tonnes in the next decade. Much of this wheat is used as human food. Wheat is a basic dietary constituent of many regions and cultures due to its agronomic adaptability, good storage properties and valuable nutritional properties. In addition, wheat is used in a wide diversity of diets because it is versatile and can be adapted to many different forms of foods depending on the kernel hardness, protein characteristics and enzyme levels. Wheat flour can produce dough with unique and varied viscoelastic properties. (Orth and Shellenberger 1988).

Canada is one of the most important wheat exporters (Canadian Wheat Board 1996-97). Wheat is the main crop in Canadian Prairies, occupying about a half of the cultivated land. The Canadian export strategy for wheat, the main crop in Western Prairies, depends on ensuring customers of consistent high quality.

Quality requirements vary widely among markets, and depend on the end products. In Asia, 40% of wheat consumption is as noodles, and production of these wheat derivatives is forecast to increase in the near future (Hatcher 1990). In the last 10 years, China, Japan and Korea imported more than one million of metric tonnes of wheat per year from Canada (Canada Grain Council 1999). The total

imports for the three countries is now more than 20 million metric tonnes per year (Canada Grain Council 1999).

Color is one of the most important considerations in assessment of food quality. This factor is particularly important in some wheat end-products like Asian noodles. Wheat end-product color depends on the grain properties, milling procedure, industrial process and several flour characteristics. In particular, kernel color has been related with products color and many markets have preference for white wheats.

Some of the color problems and discoloration may be solved at least partially with the use of additives. But there is a tendency to avoid the use of these products due to consumers concerns. Therefore, it is necessary to develop more knowledge about different color and discoloration components. This knowledge will allow breeders to develop new varieties with improved characteristics.

The effect of polyphenol oxidase (PPO) activity on end-product is not fully understood. This enzyme has been implicated in enzymic browning of wheat end products during processing and storage which makes products less acceptable to consumers. The development of procedures suitable for breeding programs to detect genotypes low in PPO and the understanding of the influence of environment on this enzyme will contribute to the improvement of the wheat properties.

In this research, several PPO methods were evaluated, as well as the influence of genotypes and environments on this enzyme activity. The relationship between PPO activity and kernel color with the color of the two types of Asian

noodles was studied. The main objectives were:

1) To evaluate the methodology used to determine PPO activity in wheat, and to determine the most suitable method for breeding programs.

2) To compare the effects of genotype and environment on PPO activity and noodle color and discoloration.

3) To evaluate the influence of kernel color and PPO activity on product color.

2. LITERATURE REVIEW

2.1. COLOR

Color is one of the most important considerations in assessment of any food product. It is not only the first quality parameter perceived by the consumer, but also it is the most influenced by the consumers cultural background. These considerations are especially valid for wheat and wheat products: color is a key parameter for some wheat flour derivative products such as Asian noodles (Morris 1998). The term "color" as a quality parameter is used to refer to wheat kernel color, flour color and end-product color.

2.1.1. Wheat kernel color

Common wheat cultivars are typically classed as "red" or "white" depending on the pericarp color. Reddish pigments in the pericarp result in a rusty red coloration. Absence results in a creamy appearance termed "white".

The nature of the reddish pigments is not fully understood. They are biosynthesized starting from the aminoacids tyrosine and phenylalanine. McCallum and Walker (1989) found higher concentrations of phenylalanine ammonia lyase (PAL) in red wheats than in white wheats. This enzyme produces cinnamic acid from phenylalanine, which is the first step in the synthesis of the pigment. The increase in PAL activity seems to be a determinant in the formation of these compounds.

The visual differentiation between white and red kernels is not always possible due to environmental effects on the kernels texture. As the red pigments

are more intense in high pH, the difference can be enhanced by alkaline conditions. Therefore, different methods using sodium hydroxide solutions have been proposed to determine genotypic kernel color (Corpuz et al 1983, De Pauw and McCaig 1988).

Pericarp color is controlled by three independent genes (R_1, R_2 and R_3) located on homologous chromosomes (3D, 3A and 3B, respectively). Red color is partially dominant. The presence of only one red allele will cause red colored kernels (Corpuz et al 1983, Cooper and Sorrells 1984, DePauw and McCaig 1988, Morris 1998), but the more red alleles, the more intense is the red color (Corpuz et al 1983). The red coloration is under maternal inheritance (Corpuz et al 1983).

Sprouting susceptibility has been related to white wheats. Sprouted kernels have higher content in enzymes, particularly amylases, that make them unsuitable for industrial purposes (McCrate et al 1981). Paulsen et al (1983) compared the sprouting susceptibility of white and red wheats. They did not observe significant differences in sprouting susceptibility between white and red siblings and concluded that there is no barrier to development white wheats production.

Many markets prefer white wheat to red wheat. White grains are preferred over red ones in most countries (Morris 1998), with very strong preference in several countries, such as Pakistan and India (Paulsen et al 1983). Paulsen et al (1983) investigated the feasibility of producing hard white wheat in United States, and concluded that the reason of the actual predominance of red wheats is tradition, not technology.

2.1.2. Flour color

In 1895, William Jago stated "Color is probably the most difficult and the most important test to be made on flour" (Oliver et al 1992). More than 100 years later difficulties in measurement persist but it has been proved that there is no relationship between color and rheological properties. In 1929 Ferrari and Bailey wrote "Color is regarded as a factor whose importance is scarcely second to other considerations more fundamental in their relation to the potentialities of the flour in baking..." (Oliver et al 1993). This consideration is entirely valid today.

Flour color is affected by wheat characteristics and the industrial milling process. The wheat characteristics that influence the flour color include grain soundness and cleanness, kernel pericarp color, endosperm pigments and PPO activity (Baik et al 1994a). Other components like protein content and starch damage also have an impact on flour color (Baik et al 1994a, Baik et al 1995, Miskelly 1984).

The absence of red pigments in white wheats allows them to produce flour of the same color but with higher extraction yield than with the red ones (Paulsen et al 1983). Up to 8% of higher yields for white wheats than for red wheats can be obtained while retaining flours with the same color have been reported (Li and Posner 1989).

The endosperm pigments are independent of the pericarp ones. The main colored compounds of endosperm are xanthophylles, flavones and, in smaller percentage, carothenes (Kruger and Reed 1988). Flavones or flavonoids are responsible of the flour yellow to creamy color (Baik et al 1995). The flour flavones content increases considerably if there is a contamination with germ (Baik et al

1995), since the flavone percentage in embryo is more than 50 times the endosperm percentage (Kruger and Reed 1988). The flavonoid compounds are the same in different wheat classes, including red and white cultivars; the difference in the coloration is caused by a difference in the amount (Feng and McDonald 1989). Usually, it is preferred that hexaploid wheats have low levels of endosperm pigments, so the end-products are as white as possible.

The effect of the industrial milling process depends on the method and the extraction rate (Kruger and Reed 1988). The most important factor is the bran contamination: the higher the amount of bran contaminants, the darker the flour. Therefore, the proper flour extraction should minimise the bran contamination. This problem is more evident for red wheats: the presence of bran containing reddish pigments causes a darker color, which is disadvantageous. This is the reason why the white color pericarp is preferred in the majority of the markets (Morris 1998). The extraction rate is one of the most effective ways to manage the flour color. Kruger and Reed (1988) reported that as extraction was increased up to 70%, there was no change in color, but as extraction increased from 70 to 82%, the higher the extraction rate, the more the darkening increased (Kruger and Reed 1988). Other factors have a minor influence on flour color. For example, the smaller the particle size, the whiter is the flour (Kruger and Reed 1988).

2.1.3. End-product color

Color is a very important characteristic for all wheat derivatives, but it is an especially critical attribute for certain products, like Asian noodles (Bhattacharya et al 1999, Morris 1998). The end-product color is affected by the flour color

(pigments), other flour components and the industrial process (Kim 1996, Baik et al 1995).

The darker the flour, the darker will be the end-product. Multiple factors, including gluten content, milling extraction rate, endosperm and pericarp pigments and starch damage, cause the difference in color of noodles. The red wheat pigments are even darker at the high pH of alkaline noodles (Morris 1998). The influence of protein on noodle color (Baik et al 1995; Miskelly 1984) leads to a dilemma. Higher protein content results in better eating quality, but affects the color adversely (Miskelly and Moss 1985, Baik et al 1995). Not just the protein quantity but also the protein quality affect the end-product color. Moss (1971) found that the variability in color of gluten from different Australian varieties was less within a genotype, but was still significant.

Another important flour component that affects the end-product color is the polyphenol oxidase (PPO) activity. The oxidation of phenols caused by PPO and the consequent reactions may cause grey discoloration of end-products (Baik et al 1995). This is especially important in noodles; these products are object of a darkening process due to the presence of small bran particles which are subject of browning by the action of PPO (Miskelly 1996). This enzyme has been the least studied color component (Baik et al 1994a).

Different process parameters may influence end-product color. In noodles, water activity is an important issue. A higher water activity in noodle causes lower brightness. Increasing the added water from 31 to 35% caused decreases in L* values (Baik et al 1995). The storage temperature also affects the end-product color. Baik et al (1995) observed faster discoloration of different oriental noodles at

23°C than at 4°C. The change in brightness (L*) at 23°C was significant even at short periods of time (15 hours).

2.1.4. Influence of kernel color on end-product quality

The presence of bran with reddish pigments causes darker color in flour, which is disadvantageous. White wheats also proved to produce lighter end-products. Bran is lighter in white wheats than in red (Miller 1979). Watts et al (1999) studied the flours obtained at different extraction rates of near-isogenic lines of hard red and hard white wheat. Using a Kent-Jones color grader, a Minolta chromameter and a sensory evaluation panel, they observed that white lines produce lighter flours, even with a 2-4% higher extraction yield. Using both physical methods and panellists, Lang and Walker (1990) proved that whole wheat hamburger buns were lighter than those obtained from red wheat. For both whole wheat and white flour breads, a panel concluded that products obtained from white wheat were lighter than those obtained from red wheat (McGuire and O'Palma 1995).

Different characteristics are influenced by the pericarp color. Comparing whole wheat hamburger buns made from red and white wheats, panellists showed no preferences for either group (Lang and Walker 1990). Miller (1979) did not observe a significant difference in flavour of bran from white and red wheats. Comparing sibling lines, Paulsen et al (1983) observed that white wheats had higher flour extraction rates, more nutritive bran, higher protein content and better flour color than their red siblings. Preparing whole wheat bread, white wheat had a sweeter flavour, whereas red wheat was more bitter (McGuire and O'Palma 1995). Comparing crust flavours, Chang and Chambers IV (1992) observed that the red

wheat breads were more astringent and the white one had a toasty flavour; meanwhile, the red wheat crumb was sweeter than the white one, and the white wheat crumb had a "phenol like" flavour. Chang et al (1995) were not able to find differences between volatile flavour components of breads made from red and white wheats.

2.1.5. Genotypical and environmental influence on product color

There is little information about the effects of environment and genotype on end-product brownness. It has been observed that brownness is much higher in Mediterranean varieties than in American ones, and Matsuo (1987) proposed that this is mainly due to a genetic factor. The phenomena called "dark crumb" varies with environment, genetics and is higher early in the season (McCallum and Walker 1990).

In flour, both genetic and environmental factors contribute to the color variability, but it was observed that the greatest contribution is from the germplasm (Miskelly 1984).

2.1.6. Color determination

Two factors, completely independent of each other, are the components of flour whiteness: brightness and yellowness. Brightness is due principally to the bran, extraneous materials and particle size effects, whereas yellowness is caused by the endosperm pigments (Oliver et al 1992, Oliver et al 1993).

Traditionally the flour color has been measured using a Kent-Jones color grader. It measures the reflectance of a slurry prepared by a standard procedure.

The wavelength used is 540nm, which is close to 555nm, the human eye maximum visibility. Therefore, this method actually measures brightness (Oliver et al 1992). The following limitations of the applicability of the Kent-Jones color grader were stated by Kruger and Reed (1988). Buyers are actually interested in the dry flour color, not in the slurry color, which is the parameter measured by the Kent-Jones colorimeter (Oliver et al 1992). Also, the paste reflectance of the endosperm affects the color grade (Barnes 1986), specially in low color grades and for some cultivars (McCallum and Walker 1990).

A group of instruments has been developed to duplicate the response of the human eye using the standard curves of the Commission Internationale de L'Eclairage (CIE). These tristimulus instruments use built-in standard illuminants to measure color on dry products. Color can be measured using the x, y and z parameters of the CIE system, or transformed to L*, a* and b* values. The L* value measures brightness and is always positive; a value of 0 indicates black and 100 indicates white. The a* value is a function of the red-green differences, with positives values for red and negative values for greenness. Finally, b* is a function of the yellow-blue differences; positive values denote yellowness, and negative denote blueness (Oliver et al 1992).

The advantages of the CIE system include no sample preparation, non-destructiveness, speed, ease of use and that it provides three results simultaneously (Oliver et al 1993). Flour L* was correlated with ash content ($r=0.84$, $P< 0.05$) and Kent-Jones grade ($r=0.84$, $P<0.01$; Oliver et al 1992). Flour b* was correlated with extractable yellow pigments ($r=0.78$, $P<0.001$, Miskelly 1984; $r= 0.72$, $P<0.05$, Oliver et al 1992). A ranking obtained with a flour color index, defined

as the difference between L* and b*, was very similar to a visual assessment ranking. (Oliver et al 1992). A disadvantage of the CIE measurement on dry flour should be noted: the particle size affects the results, as it does with the human eye. Measuring color on a slurry solves this problem (Oliver et al 1993).

The CIE systems have been used extensively for noodle color and discoloration measurement (Miskelly 1984, Kruger et al 1992, Kruger et al 1994, Baik et al 1995, Crosbie et al 1995, Downing et al 1995, Martin et al 1995, Vadlamani et al 1996, Corke et al 1997, Bhattacharya et al 1999, Hatcher et al 1999, Morris et al 2000). Martin et al (1995) observed a good accuracy in noodles L*, a* and b* measures after two hours, and L* measures after 24 hours. The variability of a* and b* after 24 hours showed low level of precision.

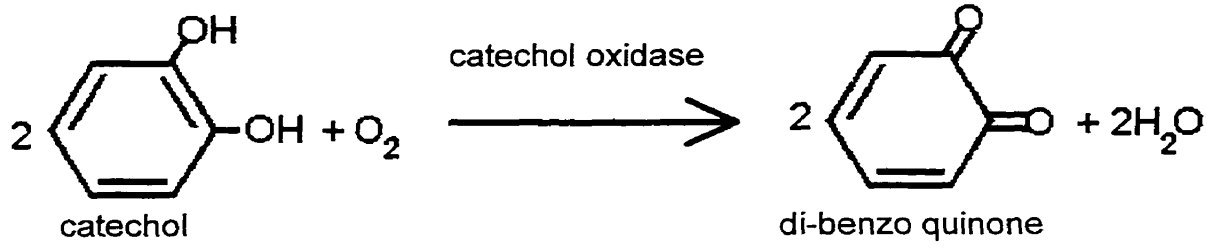
2.2. POLYPHENOL OXIDASE

PPO is the key enzyme responsible for browning reactions in foods. This enzyme causes changes in color of tea, grapes, cherries, bananas, tobacco, peaches, apples, potatoes, mushrooms, sugarcane, etc. (Taneja and Sachar 1974, Mayer and Harel 1979, Interesse and Ruggiero 1980, Bucheli and Robinson 1994, Park et al 1997). The discoloration is desirable in some food systems, but may also lead to a low value end-product. High PPO activity is undesirable when white or

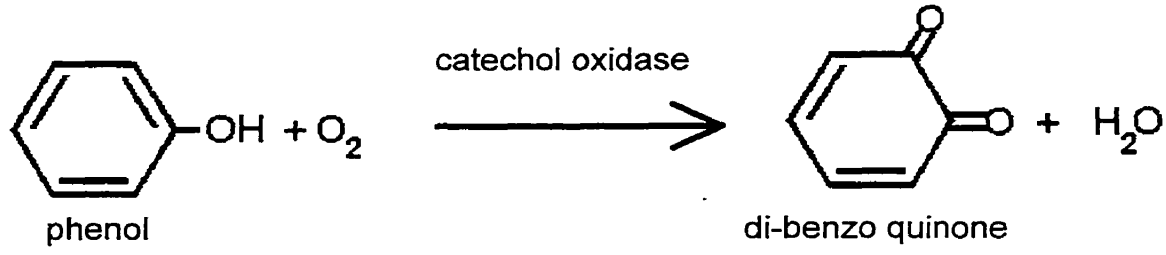
light colored end-products are preferred and are a determinant of acceptable quality.

2.2.1. The enzyme

According to IUBMB (International Union of Biochemistry and Molecular Biology, Nomenclature Committee 1992), the enzyme 1.10.3.1, catechol oxidase, also named polyphenol oxidase, diphenol oxidase or diphenolase, catalyses the following reaction:



An ortho-diphenol (catechol in this example) in the presence of oxygen is oxidized to the corresponding di-benzo-quinone. Any diphenol oxidation may be catalyzed by this enzyme. The same enzyme also catalyses the reaction:



A monophenol (phenol in this example) in presence of oxygen is oxidized to the corresponding di-benzo-quinone.

Another enzyme, named monophenol monoxygenase (1.14.18.1, International Union of Biochemistry and Molecular Biology, Nomenclature Committee 1992), catalyzes only the second reaction. This enzyme is also called tyrosinase, monophenolase, phenolase or cresolase (Stauffer 1987, International Union of Biochemistry and Molecular Biology, Nomenclature Committee 1992).

The terms "polyphenol oxidase" or "PPO" have been used in the bibliographical sources for both enzymes, either separately or together (Tikoo et al 1973, Kruger 1976, Mayer and Harel 1979, Lamkin et al 1981, Marsh and Galliard 1986, Hatcher and Kruger 1993, Baik et al 1994a, Kruger et al 1994, Park et al 1997, Morris et al 1998, Bhattacharya 1999). These terms will be used in the same way in this thesis.

In both reactions, the substrates are a phenol (mono- or di-) and oxygen, and both produce di-benzo-quinones. These compounds result in brown colored polymeric polyphenols, produced rapidly by non-enzymic reactions, including rearrangement, oxidation and polymerization (Stauffer 1987). These brown products can be responsible for an undesirable darkening of wheat products, and are the reason why PPO is an important factor in wheat quality (Miskelly 1984). Discoloration is an important quality factor for almost any end-product obtained from flour, but is particularly important for products like Asian noodles.

Polyphenol oxidases are copper proteins with tetrameric nature that occur widely in plants (Stauffer 1987, Wong 1989). The oxidation mechanism involves two copper atoms for both mono- and di-phenol oxidation. The monophenol oxidation

requires an initial reaction with the oxy-form of the enzyme in order to be hydroxylated (Wong 1989).

The substrate specificity is broad, with big differences among sources. The substrates used most frequently to determine activity are tyrosine, catechol, DOPA (3,4-dihydroxyphenylalanine), catechin and dopamine pyrogallol (Wong 1989).

Despite the fact that this enzyme is one of the oldest known (Mayer and Harel 1979) the physiological function is still not clear. Therefore, different possibilities have been suggested. It has been proposed that plants need PPO to synthesise *o*-diphenols or as electron transport. It was also observed that it regulates the plant growth. A strong interaction between plant PPO activity and fungi infection has been proven, suggesting a disease resistance role. Activity on catechol increases during infection with virus, bacteria, fungi, nematodes and even mechanical injury. The resistance mechanism proposed was based in either the reduction of the oxygen concentration in the plant cell or the decreased nutritive value of the proteins due to the reaction with quinones (Mayer and Harel 1979, Bernier 1998).

2.2.2. PPO in wheat

The wheat PPO system is complex and still not fully understood. The first PPO reported in wheat was a tyrosinase in 1907 (Lamkin et al 1981). Since then, diphenolase activity has been reported in embryo and bran, and mono-phenolase activity just in bran (Taneja et al 1974). Several research groups have reported the presence of a number of isoenzymes in wheat (Stauffer 1987, Taneja and Sachar 1974, Tikoo et al 1973). Up to 16 isoenzymes with PPO activity have been

identified. Difference in isoenzyme structure may be largely in the number of polypeptide chains (Stauffer 1987). However, the large differences observed between some plant PPO isoenzymes cannot be explained just by differences in the number of polypeptides chains (Lamkin et al 1981).

Taneja et al (1974) found a lack of synchrony in the increase of activity on tyrosine and catechol, and they proposed that this might be due to the presence of two different enzyme complexes. Taneja and Sachar (1974) observed 7 electrophoretic bands with PPO activity when tested with different substrates. Some bands reacted with DOPA, some of them with a monophenol (tyrosine) and one with both diphenol and monophenol. It is clear that the increase in PPO activity in sprouted grains is due, at least partially, to isoenzymes which are different from those present in the mature not germinated grains. Therefore, there is PPO *de novo* synthesis after germination (Stauffer 1987). Also, different parts of the grain produce different isoenzymes, as was verified by electrophoresis (Kruger 1976).

The level of PPO activity varies with stages of kernel development. During the early kernel development the catecholase activity is higher than in the mature kernel (Taneja et al 1974). During sprouting the PPO activity increases, but in contrast to α-amylase which increases up to several thousand-fold, PPO increases less than 2.5-fold (Kruger and Hatcher 1993). During germination the activity of enzymes, such as α-amylase, increases in the endosperm. The PPO activity in the endosperm is a small percentage of the grain activity, and after germination it remains as just 1% of the total grain activity (Kruger and Hatcher 1993).

There is little knowledge about PPO genetics. It was proposed that group 2 chromosomes carry the darkening gene or genes (Morris 1998). Bernier (1998) identified two genes, one on the 2DS and the other on the 2A chromosome. The one on the 2DS was stronger than the other; they interacted epistatically. This information is in conflict with previous observations (Bernier 1998). Despite differences among these proposals, all of them propose a simple genetic system.

Hexaploid wheats usually have higher PPO activity than tetraploid wheats (Lamkin et al 1981, Bernier and Howes 1994) and this difference has been the basis for tests of pasta products to detect presence of *Triticum aestivum* (hexaploid). In a number of countries, like Italy and France, pasta must legally be 100% *Triticum durum* (tetraploid). Feillet et Kobrehel (1974) developed a electrophoretic method to detect common wheat flour contaminating durum wheat derivatives. Mahoney and Ramsay (1992) proposed a simpler one, to detect hexaploid grains contaminating tetraploid samples.

2.2.3. PPO in flour

Flour should be mainly composed of endosperm, and there is no evidence of PPO activity in the endosperm. Therefore, PPO activity in flour is due to bran contamination, which is always present to some extent but varies depending on the extraction rate, flour yield and milling stream.

Working with five wheat samples from five different Canadian classes, Hatcher and Kruger (1993) found that bran and shorts duster flours were the milling streams with higher PPO activity. The difference in activity between bran and flour was noticeable. This observation is coincident with the anatomical location of the

enzyme in the kernel. The higher the flour extraction rate, the higher the PPO percentage relative to the total activity in the grain, and therefore, the higher PPO activity in flour. The percentage of PPO found in flour at an extraction rate of 75% was approximately 3, 4 and 5% at cumulative flour yields of 50, 60 and 70%. For an 85% extraction the PPO levels were 4, 5 and 6% at cumulative flour extraction yields of 50, 60 and 70%. Therefore, if wheat is milled to a "high" extraction rate, the lower-ash flours (i.e. patent) will have higher PPO activity. The authors stated that this high PPO content can be avoided with certain industrial processes. All the hard cultivars had similar percentage of wheat PPO activity in flour at a particular extraction rate. The soft samples had higher percentages due to higher bran contamination. Hatcher and Kruger (1993) also stated that these results may be extrapolated to flours obtained from other wheat samples with similar procedures. They concluded that PPO is closely associated with bran.

In a study by Baik et al (1994a) it was reported that the PPO activity of flours increased with the extraction rates from 72 to 83%. The difference was higher when the rate increased from 76 to 83% than from 72 to 76%. This was explained as a higher inclusion of bran in the 76-83% range, based in the assumption that the PPO activity is concentrated in the bran. This assumption was confirmed by a higher increase also in ash content from 76 to 83% than from 72 to 76%.

Flour PPO activity depends on grain PPO activity, but the relationship between them is not always clear. Baik et al (1995), working with different wheat classes, observed that the average flour PPO activity was 3% of the wheat PPO activity, with higher variability of PPO activity in flour than in wheat. Despite this variation, the flour and wheat PPO data for genotypes were highly correlated

($r=0.906$, $P<0.01$). However, the correlation was not significant (r values are 0.093, -0.711 and -0.109 for cultivar Klassic and lines ID00377S and K9205117 respectively) when the effect of different growing conditions on the same genotype were analyzed. The correlation between flour and wheat PPO for different cultivars grown in the same environment was significant but low ($r=0.53$, $P<0.05$) in a previous report (Baik et al 1994a).

Therefore, if mature, not sprouted, kernels are used, and the separation of bran from endosperm during the industrial milling process is done carefully, the PPO content of the flour should be only a small percentage of the total wheat PPO activity. Baik et al (1995) compared different wheat samples and observed that the ratio of flour PPO to wheat PPO was lower in hard samples. More than ninety percent of the PPO is removed when a 70% extraction rate flour is obtained. The influence of this low percentage on the end-product color and discoloration will depend on the whole wheat PPO activity, enzyme substrate concentration in the product, the product itself and the process used to obtain it. Even with a good milling process, if the starting material is a high PPO activity wheat the most sensitive end-products may be affected (Hatcher and Kruger 1993, Baik et al 1994a).

2.2.4. Wheat PPO properties

A wide range in the optimum pH for PPO activity has been reported, depending on the substrate used for the determination. The optimum is in a range that goes from 6 to 7 for catechol according to Marsh and Gaillard (1986). Lamkin et al (1981) observed a pH optima of 8.3 for catechol and pyrogallol; they observed no

peak below pH 9.0 for L-DOPA, with activity increasing at high values. Using 4 methyl-catechol, McCallum and Walker (1990) obtained an optimum pH of 5.6. Interesse and Ruggiero (1980) observed two different pH optima (the main at 6.9, and a secondary one at 5.3); this last observation is in accordance with pH optima of PPO from other plants.

No temperature optimum was observed in the range 25-40°C, with activity increasing at the higher temperatures (Lamkin et al 1981). Lamkin et al (1981) proposed using 37°C when measuring PPO activity. Marsh and Galliard (1986) estimated the K_m value for catechol of 5mM at 25°C, pH 6.8. The heat stability varies widely with the wheat genotype. After one hour at 60°C different samples retained from 48 to 85% of PPO activity (Hatcher 1990).

Lamkin et al (1981) measured the PPO activity on several wheat samples using nine different substrates, including mono-, di- and triphenols. They observed differences in the specificity among different substrates; these differences were not related to the number of hydroxy groups (i.e., mono-, di- or triphenols). For example, activity on phenol (a monophenol) was more highly correlated with catechol (a diphenol; $r=0.884$, $P<0.001$) than with tyrosine (a monophenol; $r=0.677$, $P<0.001$). Other chemical groups seem to have more influence on the affinity of enzyme to substrate; for example, the aminoacids tyrosine and DOPA (a diphenol) had a correlation coefficient of 0.889 ($P<0.001$), whereas DOPA and catechol (both o-diphenols, but with different chemical structure) had a correlation coefficient of 0.821 ($P<0.001$).

In a wheat sample smaller grains have lower PPO activity, both on weight and on kernel basis. This observation is interesting since small kernels have higher amount of bran (Baik et al 1994a).

2.2.5. Relationship with other parameters

Correlation of PPO activity with other parameters was recorded in several studies. In a study by Baik et al (1994a) PPO activity was negatively correlated with 1000-kernel weight. Working with 16 cultivars from different classes grown in two locations the correlation coefficient was -0.46 ($P<0.01$). Park et al (1997) observed significant correlation between 100-kernel weight and flour PPO ($r=-0.39$, $P<0.01$ for hard white wheat, $r=-0.79$, $P<0.01$ for hard red wheat), but the correlation with wheat PPO was not significant. This could have resulted from higher PPO activity in shrunken kernels or from cultivars with low 1000-kernel weights.

Both wheat and flour protein content were correlated positively with PPO activity; the correlation coefficients were 0.571 ($P<0.05$) and 0.832 ($P<0.001$) (Baik et al 1994a). Others researchers (Park et al 1997) observed negative correlation between PPO and flour protein ($r=-0.25$, $P<0.05$ for hard white wheat; $r=-0.41$, $P<0.05$ for hard red wheat).

Ash percentage is a measure of bran contamination. Therefore, there is a positive correlation between ash and PPO content in flours. The values obtained from the same wheat samples were in the range 0.997-0.999 (P value not reported) (Baik et al 1994a). The correlation dropped to 0.548 (P value not reported) when different wheat varieties were pooled by the same researchers (Baik et al 1994a). Hatcher and Kruger (1997) also observed high correlation between PPO activity

and ash content where flours were obtained from the same sample. The correlation coefficients were higher than 0.90 ($P<0.05$) for Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Spring (CWRS) and Canadian Western Red Winter (CWRW). The correlation was lower ($r=0.78$) for CWES (Canadian Western Extra Strong). Consistently, Park et al (1997) observed a significant correlation ($r=0.63$, $P<0.05$) between ash and PPO activity comparing flours from hard red wheat, but it was not significant using a set of hard white wheat samples.

A similar relationship was observed between flour color and PPO activity. Comparing different flours obtained from the same sample, flour color is a measurement of bran contamination. Hatcher and Kruger (1993) obtained flours with different procedures and at different extraction rates. Within each of the five wheat samples used, they observed high correlation coefficients ($r=0.78$-0.93, $P<0.001$) between flour color (Kent-Jones values) and PPO activity.

The reported differences in PPO activity between red and white kernels are consistent. Park et al (1997) observed higher activity in red kernel cultivars (on average, 1268 units) than in white ones (on average, 536 units). Baik et al (1994a) reported PPO activity of two red and two white cultivars grown in USA; both red samples had higher PPO activity. Lamkin et al (1981) studied red and white wheats from different classes. Working with cultivars from New Zealand, PPO activity was lower for the two white varieties analysed, compared with four red wheats (McCallum and Walker 1990). The averaged PPO activity on catechol of white samples was lower (736 O_2 nmol/g/min) than the red one (1175 O_2 nmol/g/min), but there were some red wheat samples with lower activity than some white ones.

There are other reports of white wheats with PPO activity considered high. Baik et al (1995) measured PPO activity in several samples from different classes, including a hard white with 1293 O_2 nmol/g/min and a soft white wheat with 1025 O_2 nmol/g/min.

2.2.6. Related components

Different reports suggest a relationship of PPO effect on end-products and other related components, such as phenolic compounds and peroxidase. The functionality proposed for phenols is variable, and closely related to PPO. They were proposed to contribute to dormancy, coat pigmentation, fungal resistance and properties of products (McCallum and Walker 1989).

2.2.6.1. Phenolic compounds

More than 80% of flour phenolic components in Canadian wheat classes are insoluble bound phenolic acids. Hatcher and Kruger (1997) compared flours of different extraction rates, and reported that ash and flour content were highly correlated with insoluble phenolic content for five different wheat samples ($r>0.93$, $P<0.05$ and $r>0.87$, $P<0.05$, respectively). The correlations were also significant for estherified phenolic acids ($r>0.76$, $P<0.05$ and $r>0.68$, $P<0.05$ respectively) and free phenolic acid ($r>0.87$, $P<0.05$ and $r>0.90$, $P<0.05$, respectively). Overall, more than 80% of all the phenolic compounds content was in the form of ferulic acid. Comparing five different wheat classes, these authors observed than the softest class contained highest ferulic acid content; the medium hardness sample had medium phenolic content and the hardest classes had lower content. In other

research it was reported that the amount of the most important phenol (soluble and bound trans-ferulic acid, the natural substrates for PPO) did not differ significantly between red and white cultivars (McCallum and Walker 1991).

Correlation between PPO activity and total phenolic compounds has been reported to be highly significant ($r=0.79$-0.94, $P<0.001$) for different Canadian wheat classes (Hatcher and Kruger 1997). The correlation was similarly significant ($r=0.76$-0.98, $P<0.001$) whether insoluble bound, soluble estherified or free phenolic contents were compared. This close relationship between phenol and PPO content suggests a synergistic role during enzymic darkening in end-products.

2.2.6.2. Peroxidase

Peroxidase activity also may contribute to the enzymic browning process. As PPO, this enzyme has different isoenzymes, the activity is high during the kernel maturation and after sprouting, and it is lower in a sound mature kernel (Kruger and LaBerge 1974b, Stauffer 1987).

2.2.7. Relationship of PPO activity to end product discoloration

PPO activity is present in many plants, and causes enzymic browning in different food materials (Marsh and Galliard 1986). In wheat, the PPO activity may be related to undesirable "brown" or "grey" discoloration of end-products (Moss 1971, Matsuo 1987, Taha 1990, Park et al 1997, Bernier 1998, Bhattacharya et al 1999, Morris et al 2000). The grey discoloration is attributed to the oxidation of wheat phenols, like tyrosine (Baik et al 1995, Bhattacharya et al 1999) or ferulic acid (Drapron and Godon 1987). It has also been claimed that PPO affects the color of

products like yeast bread (McCallum and Walker 1990) chapattis (Singh and Sheoran 1972, Tikoo et al 1973, Lamkin et al 1981, Marsh and Galliard 1986, Kruger et al 1994), Middle East flat breads (Kruger et al 1994), steamed bread (Kruger et al 1994), pasta (Marsh and Galliard 1986, Taka 1990) and noodles (Miskelly 1984, Kruger et al 1992, Kruger et al 1994, Bernier and Howes 1994, Downing et al 1995, Corke et al 1997). Discoloration is more evident when high extraction flours are used.

2.2.7.1. Asian noodles

The darkening process is a very important issue for certain Asian noodles (Kruger et al 1992, Hatcher and Kruger 1993, Baik et al 1994b, Martin et al 1995, Corke et al 1997, Bhattacharya et al 1999, Hatcher et al 1999, Morris et al 2000), since they may not be consumed up to 24 hours after their production (Miskelly 1996), without any heat treatment to prevent it (Corke et al 1997). This darkening process, also called browning or grey discoloration, is accompanied by an increase in absorbance in all the visible spectrum (Kruger et al 1992). This discoloration may occur during preparation, during storage or both, depending on product. For example, Cantonese noodles are usually stored uncooked for up to 24 hours (Kruger et al 1994). This long storage time may lead to darkening in presence of high PPO activity, especially in alkali conditions (Miskelly 1984). Darkening of cooked noodles is not related to PPO since boiling inactivates the enzymes involved in discoloration (Kruger et al 1992, Bhattacharya et al 1999).

The discoloration is more evident for Kansui noodles (Cantonese alkaline) than for others like salted noodles, due to a higher activity of PPO at high pH's

(Edwards et al 1989). The higher intensity of pericarp pigments color at alkaline pH's (Corpuz et al 1983, DePauw and McCaig 1988) may play an important roll as well.

Different researchers have studied the relationship between the wheat PPO activity and the discoloration of noodles. Kruger et al (1994) used 22 Canada Prairie Spring experimental lines to study the relationship of PPO with enzymic darkening in Cantonese noodles. They measured L* right after the dough preparation, 4 hours later and 24 hours later. They obtained a Pearson correlation coefficient of 0.84 and 0.87 (significant at $P<0.001$) between grain PPO activity (determined by the oxygen consumption method) and the rate of decrease in brightness (L*) after 4 and 24 hours, respectively. The correlations were lower but still significant using a whole kernel method to determine PPO activity and change in noodle yellowness (b*).

Baik et al. (1995) prepared three different types of noodles with flours obtained from wheats of different classes. The authors measured color in noodles at different times. They observed a significant correlation between PPO activity and discoloration, but the relationship between protein content and brightness parameters was better. They obtained the correlation coefficients between change in L* after 75 hours (discoloration) and flour PPO activity using an oxygen consumption method. The values obtained were 0.78 ($P<0.01$), 0.75 ($P<0.01$) and 0.88 ($P<0.001$) respectively for Udon, Cantonese and instant noodles ($n=12$). The correlation with discoloration was also significant ($P<0.01$) for wheat PPO (0.69, 0.81 and 0.83 respectively). Besides, brightness after 75 hours was correlated significantly with both wheat and flour PPO. These values are consistent with the hypothesis that PPO affect discoloration. However, the correlation of both L* at 75

hours and discoloration (change in L*) of the three noodle types with protein content was higher in all cases (from 0.84 to 0.97; $P<0.001$). Working with samples of the same cultivar grown in different locations, there was no correlation between L* at 75 hours or change in L* and PPO activities, but it was significant when brightness parameters were compared with protein content. The authors suggested that, among different samples of one cultivar, protein content has a higher influence on discoloration than PPO. It may be explained by three main reasons. Firstly, it may be caused because the protein content is a marker for other components. Another explanation was that the protein affects the hardness of the kernel and, therefore, the starch damage and particle size. Finally, the rate of water binding at different protein levels can cause a differential discoloration. They also explained this lack of correlation based on the small differences in PPO activities among the samples analysed.

In the same research, variation in a* (redness) and change in a* was slight or insignificant. The variation in b* (yellowness) and change in b* was more evident, but not consistent.

Park (1996) found a relationship between PPO and decrease in brightness and increase in yellow color. This relationship was different among sample sets. They used a spectrophotometric method to measure PPO activity in a wheat extract. It should be mentioned that according to Baik et al (1994a) PPO based in colorimetric assays are of dubious validity. Besides this remark, it is evident that the observations reported emphasize the importance of PPO. The correlation between PPO and discoloration is not always significant (Kruger et al 1992).

2.2.7.2. Other end-products

Discoloration due to PPO is also important in other products than noodles. Using 8 New Zealand wheat cultivars, McCallum and Walker (1990) observed that both flour and grain PPO activity showed significant correlation with bread crumb color measured in a Kent-Jones color grader ($r=0.751$, $P<0.001$ and $r=0.636$, $P<0.001$, respectively).

2.2.7.3. Other effects of PPO

PPO may cause other effects than changes in color (Matheis and Whitaker 1984). Working with mushroom extract, Kuninori et al (1976) reported that the oxidative effect of PPO can make dough less extensible. The strengthening of the dough may be explained by two mechanisms (Drapron and Godon 1987). In one, the oxidation of ferulic acid and relative compounds estherified to pentosans induces gelation. In the other, a change in the gluten structure through oxidation of tyrosyl groups or conjugation of oxidation products and thiol groups is the mechanism. These effects are not relevant in the selection of low PPO lines since strengthening is noticeable only with a PPO concentration much more higher than that present in high PPO activity wheat. Modification of protein by PPO may lead also to a change in nutritional properties due to cross linking (Matheis and Whitaker 1984).

2.2.7.4. Inactivation of PPO action

Different attempts have been made to minimise PPO activity in order to avoid enzymic browning. As PPO is a copper enzyme, its activity may be inhibited by

chelating agents or by zinc and calcium ions. The disadvantage of this is that the agents may affect flavour (Vadlamani and Seib 1996). Sulphite is used to denature PPO in other food products, but it destroys the elasticity of wheat (Vadlamani and Seib 1996). The capacity of several reducing agents to limit discoloration was evaluated by Baik et al (1995). Ascorbic acid proved to be effective, to different extents for different noodle types. Ascorbic acid was proposed by other authors as well (Bhattacharya et al 1999). Taha (1990) observed that with radiation (5kGy) the pasta color could be improved. The author explained this observation by the lowering effect of radiation on PPO activity. Vadlamani and Seib (1996) found that by heating and cooling the tempered wheat grains very rapidly, the PPO activity could be reduced up to 50% without causing damage to the gluten proteins. Other processes proposed are: controlling the calcium concentration in products (Matsuo 1987), modified atmosphere packaging (Vadlamani and Seib 1996 and Bhattacharya et al 1999) and addition of specific proteases (Vadlamani and Seib 1996). PPO inhibition may be good way to avoid undesired browning (Mayer and Harel 1979).

2.2.7.5. Other factors that cause discoloration

Other factors than PPO affect discoloration (Edwards et al 1989, Crosbie et al 1995). Peroxidase, an enzyme related to PPO, is one of them (Kasatkina et al 1986, McCallum and Walker 1990, Vadlamani and Seib 1996, Fraignier et al 2000), but the browning may occur only in some specific situations (McCallum and Walker 1990). Breadmaking with flour high in peroxidase activity may cause browning since this enzyme is relatively stable up to 80°C (Kruger and LaBerge 1974b).

Bhattacharya et al (1999) suggested that "other enzymes" than PPO influence the browning effect. Non enzymic changes, like Maillard reaction, also may cause noodle browning (McCallum and Walker 1990, Vadlamani and Seib 1996). Edwards et al (1989) proposed that proteolytic activity influences enzymic browning because most phenols are linked to proteins and so proteases increase the availability of PPO substrates, causing discoloration.

2.2.8. Genotypical and environmental influence on PPO activity

There has not been any study to compare the influences of genotype and environment on PPO activity. However, researchers who had studied different genoytpes in different environments agree that the main component affecting PPO is genetic.

Lamkin et al (1981) observed a very strong influence of genotype on PPO activity. They studied the difference in substrate specificity among wheat varieties. They tested five American wheat classes: hard red winter, soft red winter, hard red spring, white common, club and durum wheat. Five varieties were cultivated two different years (1977 and 1978), and one genotype was cultivated in two different locations the same year. PPO activity was mesured by oxygen consumption with nine different substrates, including mono- and di-phenols. The authors observed a good relationship between the varieties and the PPO activity. They proposed that differences in activity may be useful to distinguish among wheat cultivars, and even among wheat classes. The difference in PPO activity between two environments for the six varieties analysed under these conditions was small. Therefore, they concluded that different samples of the same cultivar from different growing

locations and crop years gave comparable activities. These conclusions cannot be extended to other environments due to the limited conditions evaluated. The difference of activities among genotypes was evident: the range obtained for bread wheat using catechol was 347-1477 O_2 nmol/g/min.

Baik et al (1994a) concluded that both genotype and environment had a significant effect on this enzyme. They analysed PPO activity in American and Australian wheats. They evaluated 16 varieties from different classes in two environments: one in Australia and the other one in USA. They also used the oxygen consumption method, with catechol as substrate. The range of PPO activity was 431-1443 O_2 nmol/g/min. This report did not include an ANOVA table, or other statistical details, but they reported significant differences: P>F was 0.0008 (d.f.=1) for location effect and 0.0001 (d.f.=15) for cultivar effect. These values show a strong influence of both environment and genotypic on PPO activity. The authors reported a cultivar by location interaction, but the correlation of PPO activities between the locations was not significant.

Working with different wheat classes, Baik et al (1995) suggested that PPO variation is governed by genetic factors. They studied three wheat lines in several growing locations and observed no significant differences among those environments.

Park et al (1997) studied the genotypic and environmental effects on levels of PPO in hard red and hard white wheats and concluded that "enhancement of wheat-based end product quality is possible through the wheat breeding and selection strategies". Forty experimental lines grown in two different locations and 10 hard red experimental lines grown in 3 locations were analyzed. A

spectrophotometric after extraction method was used with DOPA (di-phenol) as substrate to measure PPO activity in both flour and ground wheat. A significant effect of environment (location), genotype (population) and genotype x environment interaction for the hard white wheat grain and flour was observed. The same results were obtained for the hard red wheat flour, but the effects were not significant for the hard red wheat grain. In grain PPO activity, it was observed that environment contributed more to variability than genotype. The conclusions of Park et al (1997) should be used with caution, since the results obtained with both groups of samples are different and the method used to determine PPO is not a recommended one (Marsh and Galliard 1986). The range of PPO activity was 267-1505 units (these units were defined by the authors and have no relationship with O_2 nmol/g/min).

2.2.9. Methods to measure PPO activity

The methods used to determine PPO activity measure either the oxygen consumption or the colored products. The spectrophotometric methods are based in the fact that the products of the enzymic reaction are colored. These methods are more convenient, but also more inaccurate (Mayer and Harel 1979). There are two types of spectrophotometric methods. In one, the activity is measured using a ground wheat or flour extract. In the other one, the activity is determined on whole kernels, with no prior preparation. Several substrates can be used in each method.

2.2.9.1. Substrates – general considerations

The substrate to be used in any method should be selected according to the convenience of use (stability, solubility, etc.) and it should have non detectable non

enzymic oxidation in the analysis conditions (Marsh and Galliard, 1986). Lamkin et al (1981) evaluated several substrates. Marsh and Galliard (1986) concluded from the Lamkin et al (1981) results that catechol (diphenol) is the most suitable substrate. In addition, they observed that the autoxidation at pH values below 7 is very low. They discarded tyrosine because of its low solubility. However, Marsh and Galliard (1986) indicated that it is important to use tyrosine since it is an endogenous substrate that may be responsible for the enzymic darkening.

2.2.9.2. Spectrophotometric using enzyme extract

In this method, ground grain or flour is extracted in water or buffer. In order to measure PPO activity, the extract is incubated in a buffered media with substrate while the absorbance is measured (Singh and Sheoran 1972, Interesse and Ruggiero 1980, Park et al 1997). The solution must be clear to measure absorbance accurately. The rate of increase in absorbance is proportional to the amount of enzyme.

Different PPO extraction methods have used, but the authors did not report that they had checked the extractability of the enzyme. Marsh and Galliard (1986) evaluated six different extraction procedures. They added sodium dodecyl sulphate, Triton X-100 and lactic acid to the buffer media as solubilizing agents in order to try to improve the solubility of the enzyme, but they observed that none of the methods resulted in complete extraction. The best extraction reported, 55%, was using Triton X-100 0.1%. In addition to the low extraction percentage observed, the reproducibility of the extraction it was not reported. Extraction rates may be influenced by different genotype and environment.

For this method, the substrate should be soluble, since the absorbance cannot be measured accurately on a suspension. The substrates that have been used include catechol, catechin, gallic acid, pyrogallol, caffeic acid, L-DOPA (Park et al 1997; Kruger 1976). The substrate solutions should be freshly prepared since autoxidation may interfere with the results (Kruger 1976).

2.2.9.3. Spectrophotometric using whole kernel

In order to measure PPO using whole kernels the grains are incubated under controlled conditions in a solution containing substrate and the resulting darkening is evaluated (Crosbie et al 1995, Kruger et al 1994, Bernier and Howes 1994, Morris et al 1998, Mahoney and Ramsay 1992). Both kernel and solution darkening may be evaluated. The color may be spectrophotometrically measured (Kruger et al 1994, Bernier and Howes 1994, Morris et al 1998b) or visually assessed (Mahoney and Ramsay 1992, Crosbie et al 1995). For a proper spectrophotometric measure, the substrate solution must be transferred to another plate (Kruger et al 1994).

PPO is located near the surface of the kernel and, therefore, is accessible to the substrate (Hatcher and Kruger 1993, McCaig et al 1999), or else it is partially leached out during kernel incubation in solution (Kruger et al 1994, McCaig et al 1999). Therefore, after specific incubation conditions, the solution absorbance will be proportional to the PPO activity (Bernier and Howes 1994, Kruger et al 1994). The assay may be done on individual (Bernier and Howes 1994) or several kernels (Mahoney and Ramsay 1992, Kruger et al 1994).

Different substrates have been utilized. Kruger et al (1994) compared two monophenolic substrates, phenol and tyrosine, with catechol. They found that the

monophenols produced less color and the rates were lower than the diphenol utilized. Besides, catechol is the substrate used in the oxygen consumption method, which is considered the standard one. Therefore, they decided to choose catechol as substrate for the whole kernel method that they developed. The absorbance used was 405nm. Other authors (Bernier and Howes 1994, Mahoney and Ramsey 1992) selected tyrosine. This substrate has low solubility at neutral pH, therefore they proposed to use a pH 9.0 Tris buffer. For this substrate, the absorbance is measured also at 405 nm. Bernier et Howes (1994) checked also phenol as substrate, measuring absorbance at 340nm. Morris et al (1998) used DOPA (di-hydroxyl phenyl alanine), measuring absorbance at 475nm.

Different variations were attempted by Kruger et al (1994). They added a previous step. They observed that adding a previous overnight steep in water made it easier to distinguish among samples with different PPO activity. They also evaluated the effect of the use of oxygen saturated solution. They observed a higher reaction rate, but not improvement in differentiation among samples. They decided not to steep kernels in order to simplify the process. Bernier and Howes (1994) suggested the addition of Tween 80 to the buffer in order to ensure a complete wetting of the kernels.

Kruger et al (1994) compared the results using their method with those obtained using the oxygen consumption method, based on 22 individual Canada Prairie Sring breeder lines. They observed a Pearson correlation of 0.85 ($P<0.001$) between the two methods indicating that the whole kernel method was a good predictor of the PPO activity. They concluded that the method proposed is useful for the CPS class of wheat, but modification might be needed for other classes.

McCaig et al (1999) compared the whole kernel procedures proposed by Kruger et al 1994 (catechol as substrate) and Bernier et al 1994 (tyrosine as substrate). Their objective was to determine if one of these methods was more suitable for use in a breeding program. They observed a high correlation between both assays ($r=0.85$, $P<0.01$); therefore, concluded that either method could be used. Results for catechol were closer to a normal distribution, and catechol is more soluble. In the other hand, tyrosine is safer to handle than catechol and the procedure is not destructive.

There are some reports about the non-destructive nature of this method: after the assay, the grains are suitable to be planted (Kruger et al 1994, Bernier and Howes 1994, Bernier 1998). McCaig et al (1999) observed above 95% germination rate after the assay with tyrosine. Using catechol, germination was not affected with concentrations up to 30mM (0.33%). This may be a substantial advantage in its use for breeding programs (Kruger et al 1994).

The advantages of whole kernel methods are that they are simple, rapid, non-destructive, need small sample and no sample preparation is required. Up to 96 samples can be monitored simultaneously (Kruger et al 1994). Therefore, it is suitable for breeding program screenings.

The reproducibility and accuracy are the main disadvantages. Some interferences were reported; some cultivars develop a yellow pigment in the substrate solution, independently of the addition or not of substrate (Bernier and Howes 1994).

2.2.9.4. Oxygen consumption

This method is considered the standard one (D. Hatcher, personal communication). It was firstly proposed by Lamkin et al (1981). The rate of oxygen consumption is measured by a biological oxygen monitor in a suspension with substrate, oxygen saturated buffer and ground sample. A water bath is used to ensure constant temperature (37.0°C). The oxygen is provided by the saturated buffer, and its concentration is measured by an electrode. This concentration is registered versus time. As oxygen is one of the enzyme substrates, its consumption rate will be proportional to the amount of enzyme (Lamkin et al 1981, Marsh and Galliard 1986, Kruger et al 1994).

The sample for analysis may be ground wheat or flour (Marsh and Galliard, 1986; Kruger et al 1994). The linearity of the method goes from 0 to 400mg of ground sample (Marsh and Galliard). Several substrates can be used, including di-phenols, like catechol, or mono-phenols, like tyrosine. The substrates are not necessarily very soluble in the buffer, since they can be added as solid (Lamkin et al 1981).

Some precautions should be taken using this method. The sample may consume oxygen by other means than through PPO activity. The most important one is the peroxidation of polyunsaturated fatty acids catalyzed by lipoxygenase. Therefore, the oxygen consumption of ground wheat is much higher than the flour one. This effect is minimized if the sample is freshly milled (Marsh and Galliard 1986).

The main advantages of this method are accuracy and reproducibility. Under controlled condition of temperature and pH, the measurement can be both accurate

and precise. Another important advantage is that no extraction is necessary, since the ground sample can be used directly.

Disadvantages are related to the sophistication of this method, which requires specialized equipment. The method is also time consuming: over 30 minutes for a sample with 3 reps. This fact limits severely the numbers of samples that can be processed in a given time. Therefore, it makes the method unsuitable when large amounts of samples need to be analysed. For example, it is not suited to be used in a wheat breeding program (Kruger et al 1994).

2.2.9.5. Methods comparison

Spectrophotometric methods using aqueous extracts of wheat have been used extensively to measure PPO activity (Tikoo et al 1973; Taneja and Sachar 1974; Taneja et al 1974; Kruger 1976). Lamkin et al (1981) proposed using a polarographic electrode to determine the rate of oxygen consumption as a measure of the PPO activity. Marsh and Galliard (1986) compared extensively the methods based on oxygen consumption and spectrophotometry. They concluded "enzyme assays that measure oxygen uptake by aqueous suspensions of finely ground wholegrain are preferred to spectrophotometric assays". Since then, most research conducted to study wheat PPO has used this method (Hatcher 1990, Hatcher and Kruger 1993, Baik et al 1994; Kruger et al 1994; Hatcher and Kruger 1997; Bhattacharya et al 1999) and it is considered the standard one (Hatcher, D.; personal communication).

Different spectrophotometric methods, using whole kernel or an enzyme extract, have been proposed as fast assays, but they have not been compared among each other or with the oxygen consumption method.

2.3. NOODLE METHODS

This discussion will focus on Asian noodles which can be classified into two main groups: white salted noodles and yellow alkaline noodles. The inclusion of alkaline salts in the latter group produce their typical yellow color (Morris et al 2000). In all types of noodles and regions, a grey discoloration is an undesirable characteristic (Moss 1971, Park et al 1997, Bhattacharya et al 1999, Morris et al 2000). Ingredients and preparation procedures vary for different types of noodles, resulting in different textures, color and flavors (Baik et al 1994b).

Laboratory scale tests, developed to evaluate the quality of ingredients, proportion in formulations,and procedure for making noodles, have not yet been standarized. The extent of the variations in the method used can be seen from the following summary.

Ingredients always include flour, water and salts, however the salts used were sodium chloride, sodium carbonate, potassium carbonate or a combination of at least two of them (Miskelly 1984, Miskelly and Moss 1985, Kruger et al 1992, Baik et al 1994b, Kruger et al 1994, Downing et al 1995, Bhattacharya et al 1999). The concentration of these salts also varied widely; sodium chloride was used from

0.5% to 6.25% (Miskelly 1984, Baik et al 1994b, Downing et al 1995, Bhattacharya et al 1999); carbonates varied from 0.52% to 3% (Miskelly 1984, Miskelly and Moss 1985, Kruger et al 1994, Baik et al 1994b). The water absorption used was from 32% to 38% (Miskelly 1984, Miskelly and Moss 1985, Baik et al 1994b, Allen et al 1995, Downing et al 1995, Vadlamani and Seib 1996).

Ingredients were mixed, sheeted and the color measured on the dough sheet obtained, however the equipment used differed from study to study. Dough was prepared using a farinograph (Downing et al 1995), Hobart mixer (Miskelly and Moss 1985, Baik et al 1994b, Kruger et al 1994), Waring blender (Kruger et al 1992), Kenwood mixer (Miskelly 1984), and even a spatula (Vadlamani and Seib 1996) or manually (Bhattacharya et al 1999). Mixing time used was from 3 to 6 minutes, at constant or variable speed and with or without resting periods (Miskelly 1984, Miskelly and Moss 1985, Kruger et al 1992, Kruger et al 1994, Morris et al 2000).

Dough thickness used was from 0.75 to 3.0mm, in one or several consecutive steps reducing the gap between one step and the next (Miskelly 1984, Miskelly and Moss 1985, Kruger et al 1992, Baik et al 1994b, Downing et al 1995, Vadlamani and Seib 1996, Bhattacharya et al 1999, Morris et al 2000). Rolling was done with regular sheeters or with noodle specific equipments like pasta maker or Othake noodle machine (Kruger et al 1992). The dough thickness has a very significant effect, and therefore it should be carefully controlled.

In these research studies, color was measured once, twice or several times, at different periods from immediately after the preparation until up to 75 hours later (Kruger et al 1992, Kruger et al 1994, Downing et al 1995, Bhattacharya et al 1999,

Morris et al 2000). The measurement of noodle color and darkening using CIE parameters (L*, a* and b*) was preferred by most researchers (Kruger et al 1994, Allen et al 1995, Crosbie et al 1995, Downing et al 1995, Martin et al 1995, Baik et al 1995, Vadlamani and Seib 1996, Corke et al 1997, Bhattacharya et al 1999, Morris et al 2000). However, other methods have been used. Hatcher et al (1999) observed that darkening appears accelerated in some localized areas. These areas are those that contain bran particles. Therefore, the final product has a mottled appearance. They developed a method that measures the appearance of discolored spots using image analysis. Other methods, like the Pekar slick test, have proved to be unsuitable (Bhattacharya et al 1999).

Different attempts were performed to find the best method to measure noodle color, discoloration or both. Morris et al (2000) evaluated different method to measure final color of yellow alkaline noodles. They compared water absorbance from 33% to 39%, different salt (sodium chloride) concentrations (with constant sodium carbonate concentration), mixing time from 2 to 6 min, with and without resting periods, noodle dough thickness from 0.75-2.00 and color measurements at 0 (immediately after preparation) and 24 hours. They observed that higher absorptions gave better discrimination among the cultivars used, but that too much water may lead to a slack dough, difficult to handle. The salt concentration and mixing time had little effect. The resting period had no effect, so the authors proposed to eliminate it in order to improves time efficiency of the final method. The best separation of flours color was obtained with a dough thickness of 1.5mm. Color at 24h was selected because of convenience (just one measurement) and better separations among samples. This is in agree with Hatcher et al (1999), who found

significant differences among cultivars only after 24 hours. Change in color during short periods may be misleading since the water distribution changes during the first hour, causing the consequent change in color (Kruger et al 1992).

The method used to prepare the noodle influences the color, but the ranking of the samples did not vary (Kruger et al 1992). Bhattacharya et al (1999) compared color of salted and alkaline noodles of different samples and observed that different formulations have different effects on dough color, specially in brightness. They recommended to measure color of both alkaline and salted noodles.

Alkaline noodles can be made with different Kansui formulations. These formulations are prepared with sodium and/or potassium carbonates and may contain other components like sodium chloride or hydroxide. Kruger et al (1992) compared the results with different Kansui formulations and found that, with the exception of sodium hydroxide, all formulas had similar effects on noodle brightness and yellowness.

3. MATERIALS AND METHODS

3.1. WHEAT SAMPLES

3.1.1. Method comparison

A set of 18 wheat samples from different Canadian and Australian classes, grown in Lethbridge, Alberta (L); Swift Current, Saskatchewan (SC) or both in 1997, were used to evaluate different methods to measure PPO activity. These samples

Table 3.1.Samples used for methods comparison

Cultivar/line	Location	Class
AC Domain	Lethbridge	CWRS
AC Majestic	Lethbridge	CWRS
AC Reed	Lethbridge	CWSWS
AC Vista	Lethbridge	CPS
Banks	Lethbridge	ASW
Avonlea	Lethbridge	Durum line
Genesis	*	CPS
Glenlea	Lethbridge	CWES
Glenlea	Swift Current	CWES
Hartog	Lethbridge	APH
Janz	Lethbridge	APH
Katepwa	Lethbridge	CWRS
Katepwa	Swift Current	CWRS
Leader	Lethbridge	CWRS
Leader	Swift Current	CWRS
Suneca	Lethbridge	* (Australian)
Suneca	Swift Current	* (Australian)
AC Nanda	Lethbridge	CWSWS

* Information not available.
CWRS (Canada Western Red Spring), CPS (Canada Prairie Spring), CWSWS (Canada Western Soft White Spring), ASW (Australian Standard White), APH (Australian Prime Hard)

are described in Table 3.1. All samples were harvested at optimal maturity, threshed, cleaned and stored at 15°C.

3.1.2. Genotype by environment study

The Canada Western Red Spring wheat variety AC Domain was crossed with the hard white wheat Australian Sun 251 and double haploid lines (DH) were produced. Some of these lines were inter-crossed to obtain a second generation of DH. Fifty-six DH obtained from these crosses and eight agronomical checks (Katepwa and AC Domain from two different sources, SD 3055, AC Roblin, AC Splendor and AC Majestic) were grown in four locations in western Canada in 1998 (Table 3.2). DH lines are described by a code that includes the origin of the seed (BW or L), a program code (1459-1567), the population name (95B02, 96F29, 96F30, 96F31, 96F32, 96F34, 96F35 and 96F39; each population is composed of the lines obtained from the same cross) and the plant selected (alphanumeric code).

From this set of 64 lines, 17 DH and 3 checks (AC Domain, SD 3055 and Katepwa) were selected in order to have a wide variation of PPO activity and agronomic yield (Table 3.3). The selected genotypes were grown in four locations in 1999. In 1998, the locations chosen were Glenlea, Morden, Brandon (MB) and Swift Current (SK). In 1999, the locations cultivated were Glenlea, Brandon, Portage (MB) and Melfort (SK) (Figure 3.1). All samples were harvested at optimal maturity, threshed, cleaned and stored at 15°C.

Table 3.2. Genotypes

BW	49 Katepwa (CWRS)	L	1507 96F32 * A13
BW	148 AC Domain (CWRS)	L	1509 96F32 * A14
L	1717 South Dakota 3055	L	1511 96F32 * A15
L	1719 Roblin	L	1513 96F32 * A18
L	1721 AC Splendor	L	1515 96F32 * A26
L	1723 Katepwa (CWRS)	L	1516 96F32 * A27
L	1727 AC Majestic (CWRS)	L	1519 96F32 * A42
L	1725 AC Domain (CWRS)	L	1221 96F32 * A76
L	1459 95B02 * A140	L	1523 96F32 * A79
L	1461 95B02 * B116	L	1525 96F32 * B4
L	1463 95B02 * B207	L	1527 96F32 * B10
L	1465 95B02 * C99	L	1529 96F32 * B19
L	1467 95B02 * C120	L	1531 96F32 * B21
L	1469 95B02 * D130	L	1533 96F32 * B30
L	1471 95B02 * D75	L	1535 96F32 * B54
L	1473 95B02 * D156	L	1537 96F32 * B67
L	1475 96F29 * A27	L	1539 96F34 * A11
L	1477 96F29 * A29	L	1541 96F34 * A39
L	1479 96F29 * A33	L	1543 96F34 * A52
L	1481 96F29 * B21	L	1545 96F34 * A67
L	1483 96F30 * A2	L	1547 96F34 * B43
L	1485 96F30 * A16	L	1549 96F34 * B43
L	1487 96F30 * A32	L	1551 96F34 * B85
L	1489 96F30 * A72	L	1553 96F34 * B86
L	1491 96F30 * B2	L	1555 96F34 * B88
L	1493 96F30 * B37	L	1557 96F34 * B106
L	1495 96F30 * B101	L	1559 96F35 * B2
L	1497 96F31 * B43	L	1561 96F35 * B114
L	1499 96F31 * A67	L	1563 96F39 * A160
L	1501 96F32 * A1	L	1565 96F39 * A164
L	1503 96F32 * A5	L	1665 96F39 * A34
L	1505 96F32 * A8	L	1567 96F39 * B62

BW and L refer to the origin of the seed; the number after it is a program code; the number before the star refers to the population; the letter after the star refers to the row selected; the last number refers to the plant selected

Table 3.3. Genotypes selected for the Genotype x Environment study

#	Genotype
1	BW 49 Katepwa
2	L 1665 96F39*A34
3	L 1717 SD 3055
4	L 1725 AC Domain
5	L 1459 95B02 * A140
6	L 1471 95B02 * D75
7	L 1473 95B02 * D156
8	L 1481 96F29 * B21
9	L 1483 96F30 * A2
10	L 1485 96F30 * A16
11	L 1487 96F30 * A32
12	L 1491 96F30 * B2
13	L 1513 96F32 * A18
14	L 1519 96F32 * A42
15	L 1221 96F32 * A76
16	L 1523 96F32 * A79
17	L 1525 96F32 * B4
18	L 1531 96F32 * B21
19	L 1533 96F32 * B30
20	L 1565 96F39 * A164

3.2. REAGENTS

Hydrochloric acid, sodium carbonate, sodium chloride, sodium hydroxide, sodium phosphate dibasic heptahydrate sodium phosphate monobasic dihydrate and Tween 80 (polyoxyethylene sorbitan mono-oleate) were obtained from Fisher

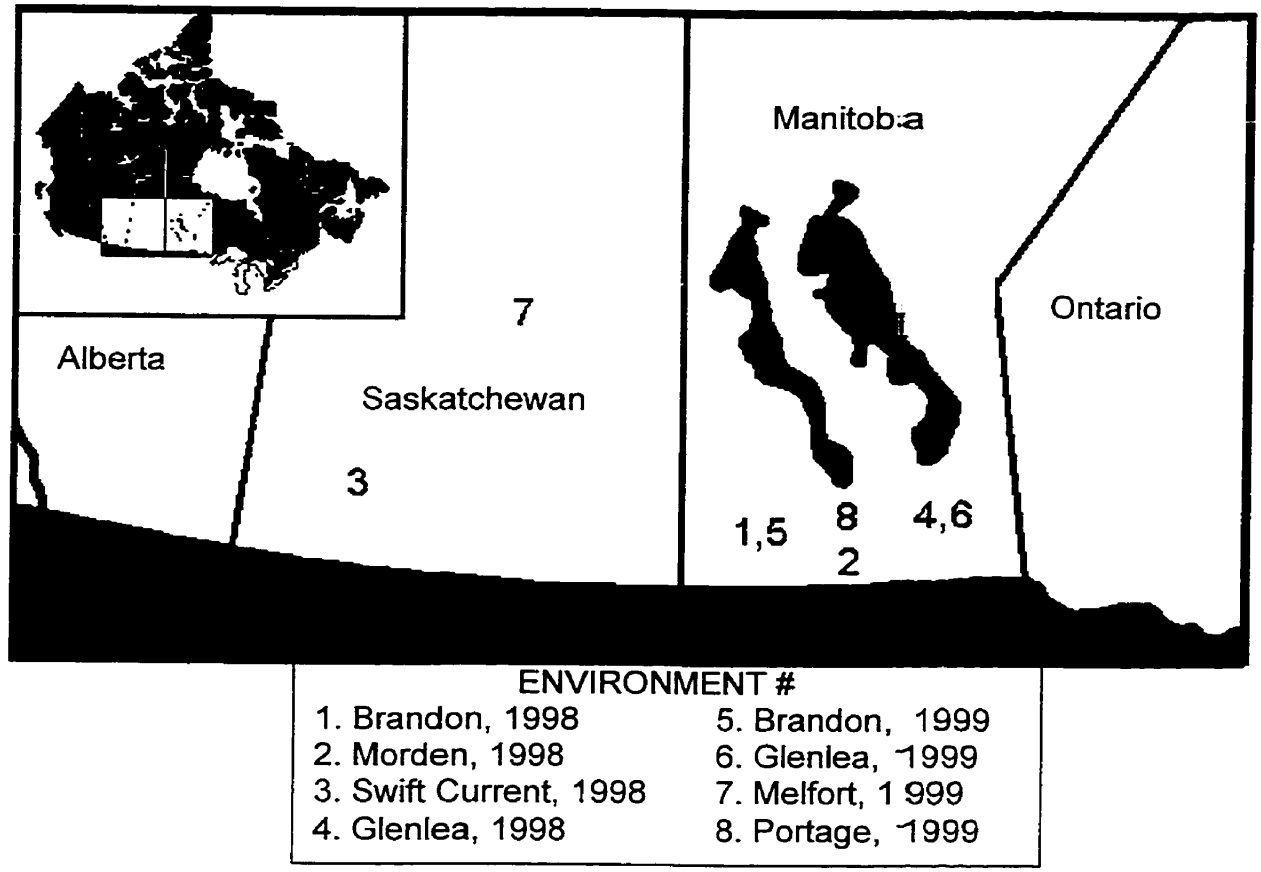


Figure 3.1. Distribution of environments

Chem. Co. (Fair Lawn, NJ, USA). Catechol, L-3,4-dihydroxyphenyl-alanine (DOPA), tris-(hydroxymethyl)-aminometane (Tris), Triton-X-114 (octyl-phenoxy-polyethoxyethanol) and L-tyrosine were purchased from Sigma Chem. Co. (St. Louis, MO, USA).

3.3. METHODS

3.3.1. Kernel color

Three kernels were soaked in 1 ml of 1M sodium hydroxide solution for 2 hours. The kernel color (red or white) was visually determined (De Pauw and McCaig 1988).

3.3.2. Sample preparation

Wheat samples were tempered to 15.5% moisture and milled to produce straight-grade flour in a Buhler experimental mill at 22°C. Flours were matured for at least two weeks before being used for the noodle dough preparation. Ground wheat was obtained in an Udy Cyclone mill with a 0.5mm mesh.

3.3.3. PPO activity

Methods for measuring PPO activity included a number of previously reported methods, and some modifications of theses methods. Activity was measured spectrophotometrically on buffer used to soak whole kernels, and on an extract of ground kernels. Activity was also measured using the oxygen consumption method on ground wheat and on flour.

3.3.3.1. Whole Kernel methods

Three reported methods (Kruger et al 1994, Bernier and Howes 1994, Morris et al 1998) that measure PPO activity using whole kernels were compared. These methods and some modifications are listed in Table 3.4. To test each method, 5

Table 3.4. Spectrophotometric whole kernels methods

Method	Steeping	Activity Buffer	Substrate	Time [a]
A [b]	No	Tris hydrochloric, pH 9.0	Tyrosine 0.1%	3 hours
B	No	Phosphate 0.05M, pH 6.8	Tyrosine 0.1%	3 hours
C	No	Tris Clorhydric, pH 9.0	Catechol 1%	3 hours
D	No	No	Catechol 1%	3 hours
E [c]	16h in water	No	Catechol 1%	30 min
F	16h in substrate	No	Catechol 1%	30 min
G	16h in buffer	Phosphate 0.05M, pH 6.8	Catechol 1%	30 min
H [d]	No	Phosphate 0.05M, pH 6.8	10mM DOPA	3 hours

[a] Referred just to incubation time; steeping time not included
[b] Bernier et al 1994
[c] Kruger et al 1994
[d] Morris et al 1998

kernels were incubated at 37°C in 1ml of substrate solution in a 24-well ELISA plate. The substrate was dissolved in the incubation buffer or water. In all cases, the incubation solution had Tween 80 at 0.2% (w/v). After incubation, an aliquot of the solution was taken and the absorbance was measured in a Titertek Multiscan MCC/340 plate reader with a 405nm filter. The PPO activity is reported as absorbance units (AU) measured in these conditions and represents the average of four replicates. After the selection of one method, the linearity of the PPO measurement was studied measuring activity on a Genesis sample (high PPO activity) from 30 to 240 minutes.

Darkening of all samples grown in 1999 were also visually scored after the incubation in tyrosine solution in the same conditions. In samples with high PPO activity, kernels and tyrosine solutions became very dark after incubation. The difference in darkening among samples was visually assessed. Both kernel and

solution darkening were determined in order to evaluate which of these parameters is the best estimator of PPO activity. Genesis kernels incubated at different times were used to establish a scale from 1 (no darkening) to 10 (very dark). A white background was used.

3.3.3.2. Extraction Method

Ground sample (200mg) was extracted in 1ml of phosphate buffer (0.05M, pH 6.8) plus Triton X-100 (0.1%) for two hours at 4°C, and then centrifuged at 8000g for 20 min (Marsh and Galliard 1986). Supernatant (200µl), catechol 10% (200µl) and phosphate buffer (2.00ml) were mixed by inversion and placed immediately in a quartz cuvette. Absorbance (430nm) was measured in a PYE UNICAM SP6-550 UV/VIS spectrophotometer (Phillips) at 0, 15, 30, 45, 60, 90, 120, 150 and 180 seconds at room temperature (20-21°C). The slope of the absorbance versus time line was calculated to obtain the change of absorbance per minute. Results were corrected for catechol autoxidation by measuring phosphate buffer (2200µl) and catechol 10% (200µl) in a quartz cuvette and substracting the slope of absorbance versus time line obtained from the value obtained for each sample. Linearity of the method was verified using dilutions of an extract from Genesis (high PPO sample; Figure 3.2). The PPO analysis of the selected samples was done in duplicate, using two separate freshly prepared extracts. The mean of the four values is reported. All samples and all replicates were analyzed the same day. PPO activity was expressed in "extraction units" (1 extraction unit = 1 EU = 1000 times change in absorbance per second per miligram in these conditions).

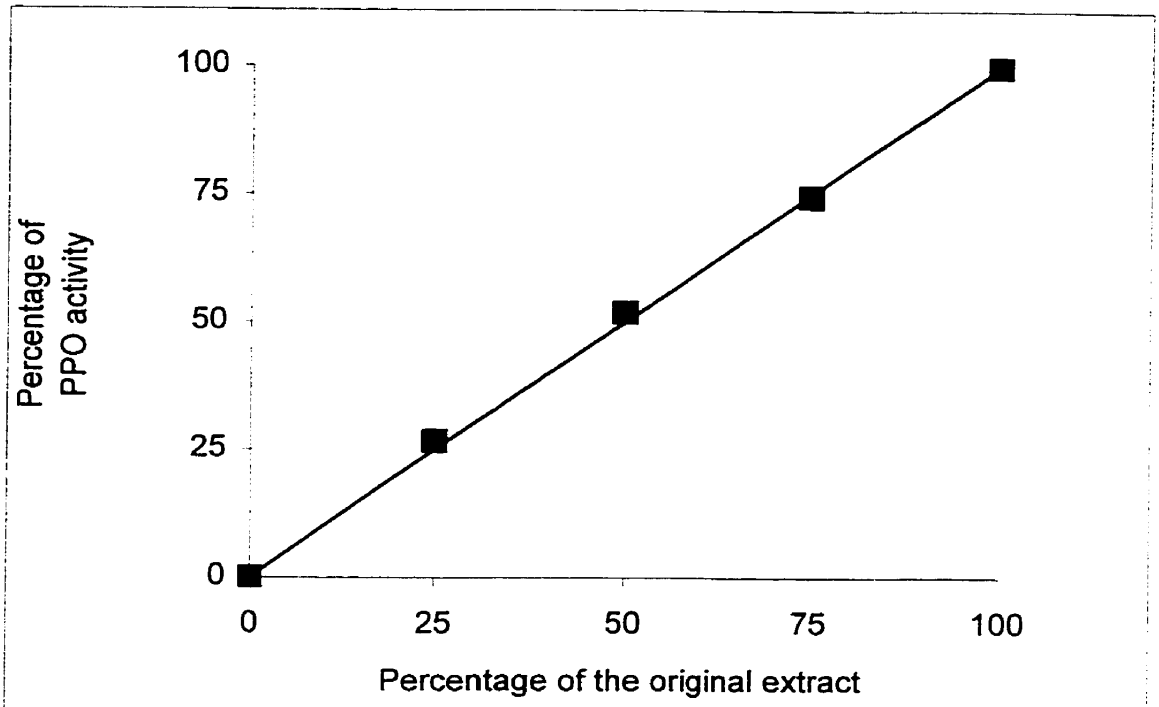


Figure 3.2. Linearity of extraction method

3.3.3.3. Oxygen Consumption Method

PPO activity was determined as described by Kruger et al. (1994) with minor modifications. Oxygen consumption was measured with an YSI model 5300 biological oxygen monitor (Yellow Spring Instrument Co., Yellow Spring, OH, USA), with a circulating water bath keeping the cell at 37.0 +/- 0.1°C. An air saturated phosphate buffer 0.05M, pH 6.8 was used. The samples were approximately 100mg of ground wheat or 500mg of flour. The substrate used was catechol for both ground sample (200µl of a 25% fresh solution) and flour (100µl of a 9% fresh solution). The samples, weighed with an accuracy of 0.1mg, were suspended in 4ml of buffer. The system was allowed to stabilize with stirring for three to five

minutes. All trapped air bubbles were removed by manipulation of the electrode plunger. The suspension was then monitored for 2 min to establish endogenous oxygen consumption. The substrate was added and the oxygen consumption was measured for 3 minutes. Results were corrected by endogenous oxygen consumption. Ground wheat results, which used more concentrated substrate, were also corrected for catechol auto-oxidation. Each sample was analysed in triplicate; and the results are presented as the average of the replicates. PPO activity was expressed as oxygen nanomoles consumed per gram per minute (O_2 nmol/g/min) under these conditions. The oxygen consumption method was used as the reference method (detailed procedure on Appendix II).

3.3.4. Flour color

Flour color was determined on dry samples using a Minolta Spectrophotometer CM-525i. Two reps were analysed and averaged.

3.3.5. Protein

Protein was determined in flour with a Dickey-John Instalab 800 NIR Product Analyzer, and was expressed on a 14.0% moisture basis.

3.3.6. Noodle dough color

Two different noodle types were prepared using two different solutions. Salted noodle doughs ("Udon" type) were prepared using a 2% sodium chloride solution. Alkaline noodle doughs ("Kansui" type) were prepared using a 1% sodium carbonate solution. For both noodle types, 10.0g of flour were mixed with 3.80ml of

the correspondent solution. A 10-gram mixograph was used to mix the ingredients for 3 minutes. Immediately after the mixing, the dough was passed once through a Somerset CDR-100S sheeter in the 1.5 position (approximately, 1.5mm clearance) and then the dough sheet was turned and passed through a second time, in a perpendicular direction. Dough color was measured immediately, and also 24 hours later, using a Minolta Spectrophotometer CR-210. Each sample was analysed twice and the mean reported.

3.4. STATISTICAL ANALYSIS

Correlation analysis (PROC CORR), ANOVA (PROC GLM), and components of variance estimation (PROC VARCOMP) were performed using SAS software (Version 6.12, 1996, SAS Institute, Cary, NC, USA).

The frequency of the grain PPO averages of the 20 genotypes in the 8 environments is presented in Figures 3.3 (oxygen consumption) and 3.4 (whole kernel method). The bi-modal distributions are due to 7 genotypes with low PPO content and 13 genotypes with high PPO content. The separation into two groups was observed in each one of the eight environments. Based on this observation the genotypes were treated as two separated groups in the statistical model used to analyse each parameter. To study the influence of kernel color, a specific factor was added to the model, with two levels: red and white. Genotype was included to evaluate other genetic factors than those mentioned previously. As genotype is nested within PPO level and kernel color, the factors PPO levels and kernel color

were evaluated using the genotype mean square as error term. The environment, defined as each location in each year, was also included as a factor. Finally, all the interactions were evaluated. Summarizing, the parameters measured were analysed as response to the following factors: PPO level (high or low), kernel color (red or white), genotype nested in kernel color and PPO level, environment and all possible interactions. For the ANOVA procedure, all effects were considered fixed. To run PROC VARCOMP, all effects should be considered random. The genotypes used can be considered a random sample of the double haploid population, and the eight environments a random sample of the Canada Prairies environments population. Therefore, these effects and their interactions were considered random effects. PPO level, kernel color and their interaction cannot be considered random effects. For them, the results obtained with the VARCOMP procedure should be interpreted as the influence of the respective effect on the total variation.

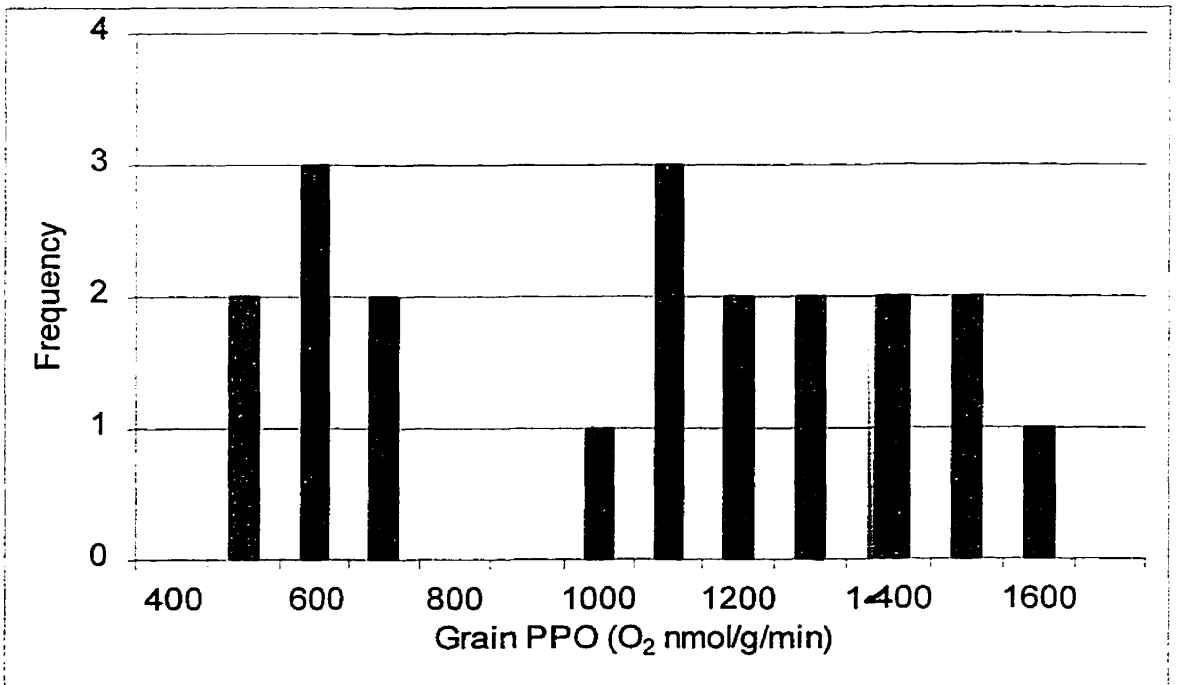


Figure 3.3. Frequency in grain PPO activity of the 20 genotypes. Average of 8 environments. Oxygen consumption method.

Using this model, the residuals were calculated and plotted versus the predicted values. The residuals of all parameters except PPO determined using the whole kernel method (WK), grain PPO activity determined using the oxygen consumption method (GPPO), flour PPO activity (FPPO), salted noodles L* change (SLC) and alkaline noodles L* change (ALC) showed independence from the residuals. Square root transformations were applied for these five parameters, obtaining SQWK, SQGPPO, SQFPPO, SQSLC and SQALC, respectively. The transformed parameters showed residual values uniformly distributed against the fitted values.

The uniformity of variance among environments of SQWK, SQGPPO, SQFPPO, flour L* (FL), salted noodles L* immediately after preparation (SL0),

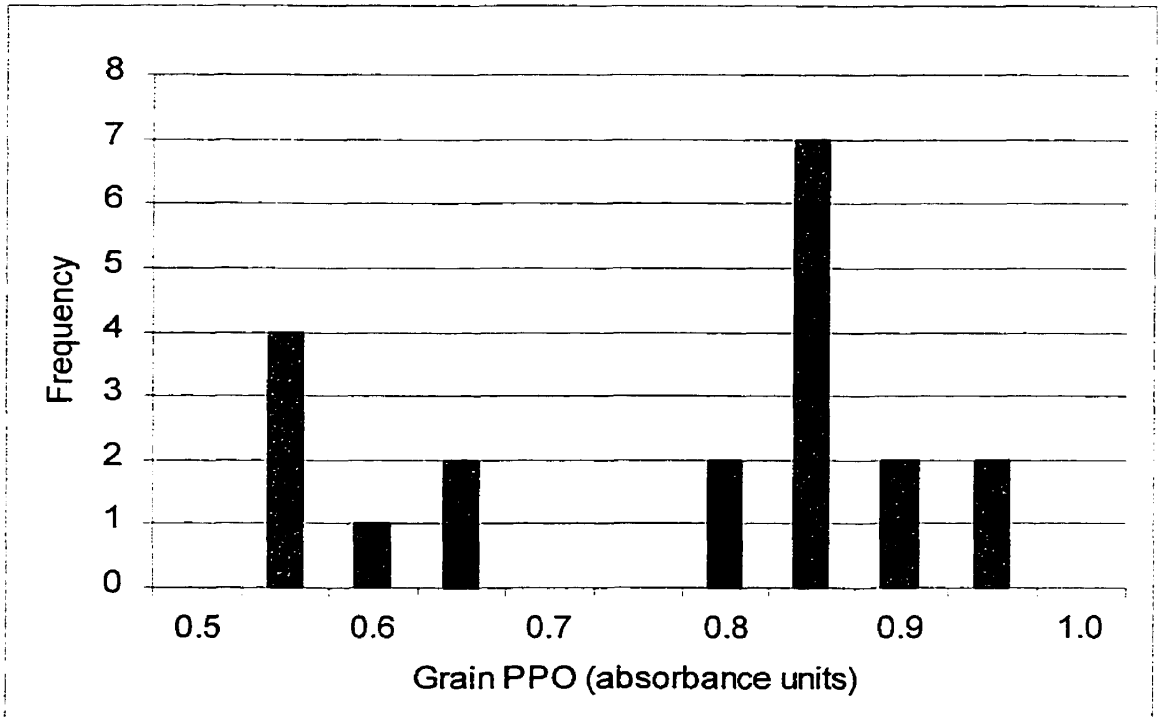


Figure 3.4. Frequency in grain PPO activity of the 20 genotypes. Average of 8 environments. Whole kernel method.

salted noodles L* 24 hours later (SL24), SQSLC, alkaline noodles L* immediately after preparation (AL0), alkaline noodles L* 24 hours later (AL24) and SQALC was evaluated by the F-max test. F-max is defined as the ratio between the highest variance estimator among the eight environments and the lowest one (Milliken and Johnson 1984). In all cases, the uniformity in variances for different environment could not be rejected with a P value of 0.01 (Table 3.5).

For each genotype, the grain PPO activity of each genotype was plotted versus the environment average for the 20 genotypes. The slope of the regression was defined as the stability of the genotype. The higher the slope, the higher is the response of the corresponding genotype to the environment effect. The lower the slope, the more stable is the genotype among the different environments (Lukow and McVetty 1991).

Table 3.5. Estimation of F-max

Parameter	Maximum variance estimator	Minimum variance estimator	F-max estimator
SQWK	0.0119	0.0047	2.51 **
SQGPPO	57.05	26.90	2.12 **
SQFPPO	1.5189	0.3849	3.95 **
FL	0.1934	0.0505	3.83 **
SL0	1.4904	0.4584	3.25 **
SL24	6.7417	1.2258	5.50 **
SQSLC	0.0678	0.0158	4.28 **
AL0	1.8093	0.4024	4.50 **
AL24	7.6953	1.8641	4.13 **
SQALC	0.1010	0.0330	3.06 **

** Significant at P<0.01

4. RESULTS AND DISCUSSION

4.1. PPO METHODS

4.1.1. Comparison of whole kernel methods and the oxygen consumption method

The seven variations of different spectrophotometric methods that use whole kernel to measure PPO activity presented in Materials and Methods (Table 3.4) were compared using 9 wheat samples from different classes. The oxygen consumption method on ground sample with catechol as substrate was used as reference. PPO activity results and Pearson correlation coefficients are presented in Table 4.1.

The results obtained with the oxygen consumption method showed a wide variation in PPO activity. The lowest activity corresponded to Avonlea (218 O_2 nmol/min/g), whereas the highest corresponded to Hartog (1205 O_2 nmol/min/g).

Both published whole kernel methods (Method A, Bernier and Howes 1994; Method E, Kruger et al 1994) and two variations (Methods B and G) were correlated significantly ($P<0.05$) with the oxygen consumption method. Method A, which uses tyrosine as substrate, had the highest correlation coefficient ($r=0.776$, $P=0.014$) with the reference method. The other method that used tyrosine (Method B) also had a highly significant correlation ($r=0.746$, $P=0.021$). There was a wide variation among the different correlation coefficients with the methods that used catechol (0.182-0.708). Among different variations, the method published by Kruger et al (1994)

Table 4.1. Results of PPO activity of spectrophotometric methods that used whole kernels, compared with oxygen consumption method results

	Spectrophotometric methods that used whole kernels [a]							
Wheat sample	A	B	C	D	E	F	G	Ox. cons. [b]
AC Majestic	0.384	0.426	0.365	0.306	0.324	0.716	0.420	620
Avonlea	0.144	0.063	0.294	0.267	0.254	0.340	0.417	218
AC Vista	0.435	0.416	0.327	0.295	0.263	0.668	0.450	902
AC Reed	0.220	0.148	0.282	0.294	0.257	0.553	0.337	270
AC Nanda	0.420	0.289	0.288	0.277	0.280	0.400	0.410	586
Banks	0.290	0.155	0.305	0.323	0.270	0.521	0.446	252
Hartog	0.407	0.375	0.339	0.302	0.333	0.606	0.471	1205
Janz	0.368	0.279	0.320	0.304	0.309	0.553	0.442	985
AC Domain	0.389	0.305	0.282	0.305	0.299	0.632	0.476	1100
r [c]	0.776	0.746	0.400	0.182	0.708	0.543	0.669	
P value	0.014	0.021	0.286	0.640	0.033	0.131	0.049	

[a] Results expressed in absorbance units

[b] Results expressed in nanomoles of oxygen per minute per gram

[c] Pearson correlation coefficient between the column method and the oxygen consumption results

had the highest correlation with the oxygen consumption method (r=0.708, P=0.033).

The PPO substrate specificity may vary among different genotypes (Lamkin et al 1981). Five out of the seven methods evaluated used catechol. It is the same substrate used in the reference method. The assays that used tyrosine (Method A and B; r=0.776 and 0.746 respectively) as substrate had higher correlation coefficients than those that used catechol (Methods C-G; r=0.182-0.708). According to these results, the difference in substrate specificity is minor compared to the effect of other factors.

Several factors should be considered in the process to select a substrate for a method to measure PPO. One of them is the non-enzymic oxidation rate in the

analysis conditions. Auto-oxidation is especially significant in di-phenol solutions (Kruger 1976). Catechol, as a di-phenol, has an auto-oxidation rate higher than tyrosine. This fact means that PPO activity may be overestimated when catechol is used. As an example, the results for Avonlea (the sample with lowest PPO activity according to the oxygen consumption method) were higher in all cases when catechol was used. In the whole kernel method, the incubation period should be carefully measured, especially when using catechol. It was observed that more than one hour after the proper incubation time, it was still possible to differentiate samples when tyrosine was used as substrate; when catechol was used, all sample solutions were very dark and no differences could be determined (data not shown). Catechol is suitable for the oxygen consumption method since the auto-oxidation rate can be determined and its effect eliminated.

The best results with catechol were obtained when a 16-hours steeping step was added. Sprouting may be promoted after an overnight incubation in water. During sprouting, PPO activity increases (Kruger and Hatcher 1993). This increase is due to *de novo* synthesis of the enzyme (Stauffer 1987). Therefore, the PPO activity after a steeping period is different from the activity of the un-sprouted kernel. As end-product discoloration increases during, a method with no steeping period is preferred. Using catechol, the lowest correlation coefficients were obtained when no steeping step was included.

It should be stated also that catechol presents some safety issues. Catechol is destructive to tissues of the mucous membranes, while tyrosine does not present those health concerns (http://msds.pdc.cornell.edu, McCaig et al 1999). The viability of seeds after the analysis is another important advantage of tyrosine. In the other

hand, McCaig et al (1999) observed problems with the normality of the distribution of results. Using catechol the distribution was closer to normal than using tyrosine. They concluded that the use of tyrosine may be conducive to less accurate statistical comparisons.

In summary, both published methods gave PPO activity values that correlated significantly with the oxygen consumption values. However, the method that used tyrosine had the highest correlation coefficient. In addition there are several disadvantages to use of catechol as substrate. Therefore, the Bernier et Howes (1994) method was selected for further work and will be referred to in the following discussion as the "whole kernel method".

When the absorbance versus time for the selected method was tested (Figure 4.1), the response was linear for 4 hours, an even longer period of time that the used in the proposed assay (3 hours). This means that initial conditions are present and therefore the absorbance is proportional to the enzyme activity.

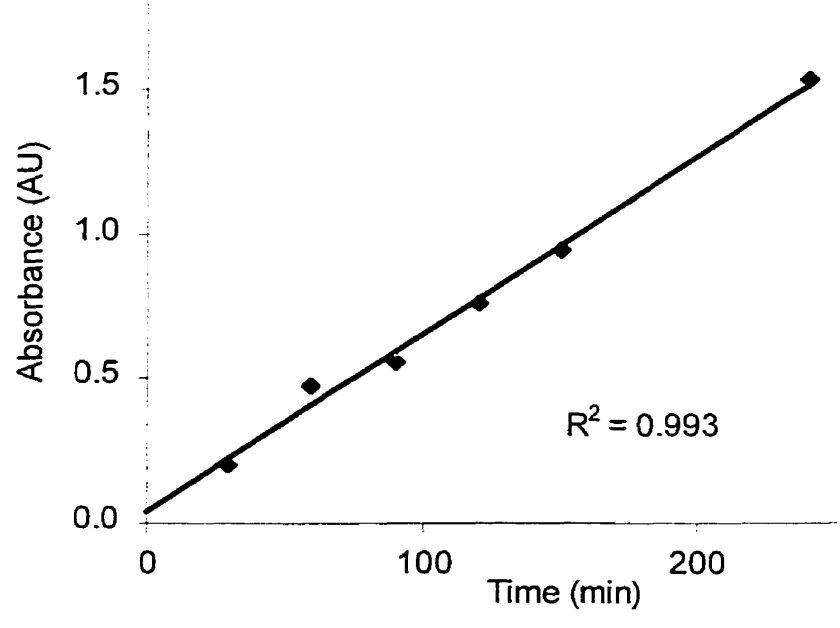


Figure 4.1. Linearity of spectrophotometric method using whole kernel method

4.1.2. Comparison of selected spectrophotometric methods and the oxygen consumption method

In a further experiment using a different sample set, two whole kernel methods and an extraction method were compared with the oxygen consumption method to confirm selection of the most valid, rapid test for PPO for breeding programs.

The selected whole kernel method, which uses tyrosine as substrate (method A, Table 3.4), another whole kernel method that uses DOPA as substrate (method H, Morris et al 1998) and one extraction method (enzyme extracted from ground sample) were compared with the oxygen consumption method, using 12 wheat samples from different classes and locations. In order to confirm the suitability of the PPO activity determination on the solution, the linearity of the PPO determined with the extraction method at different extract concentration was confirmed (Figure 4.2). Results of these methods and Pearson correlation coefficients with the oxygen consumption method are presented in Table 4.2. The range of PPO activity was even wider than for the prior set. Genesis had the highest value (2965 O_2 nmol/min/g) while Avonlea was the lowest (218 O_2 nmol/min/g). Using this set of samples, the correlation of whole kernel method A and oxygen consumption method had a higher significance ($r=0.813$, $P=0.001$) than with the other set. The correlations of the other methods compared were also significant, but the correlation was lower ($r=0.688$, $P=0.013$ for the extraction method; $r=0.695$, $P=0.012$ for the whole kernel using DOPA).

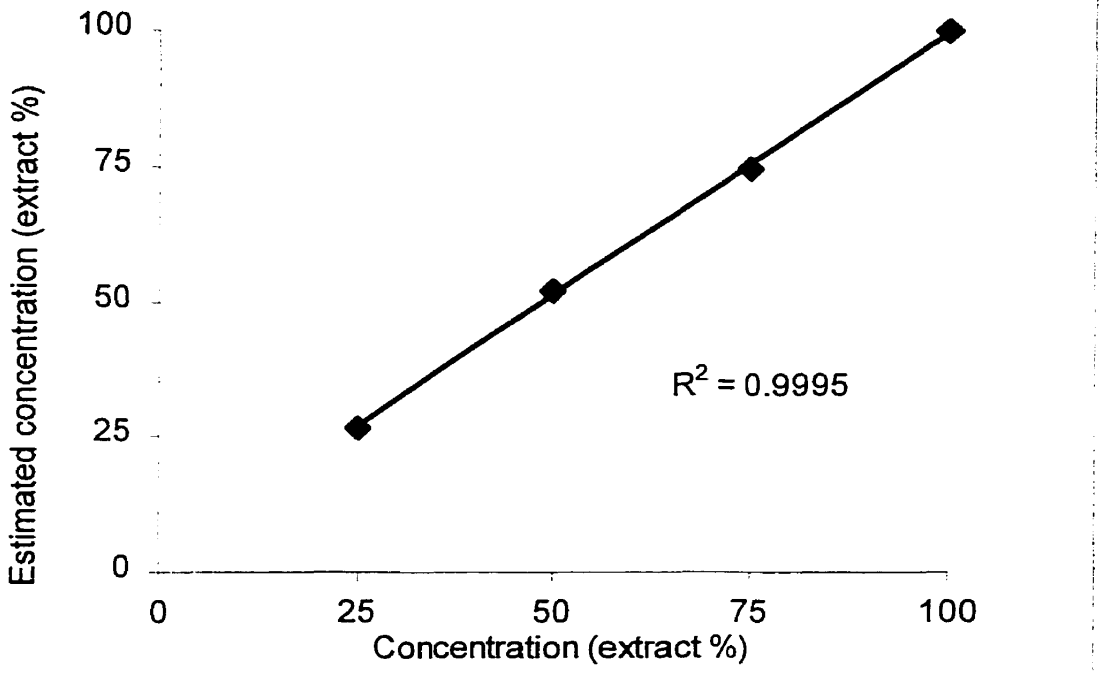


Figure 4.2. Effect of extract dilution on PPO activity. Extraction method.

Neither the extraction method nor the whole kernel-DOPA method proved to be an improvement on the selected whole kernel-tyrosine method. The extraction method is more tedious and time consuming that the whole kernel method and it did not give better results. Moreover, using the 12-samples set, the correlation between the selected whole kernel method was significant at P=0.001 (r=0.813), while the significance between the extraction and the oxygen consumption methods was only at higher P values (r=0.688, P=0.013). The whole kernel-DOPA method had similar values (r=0.695, P=0.012).

Table 4.2. Results of PPO activity using different spectrophotometric methods

Wheat sample	Whole kernel Method A [a]	Extraction method [b]	Whole kernel method H [a]	Oxygen consumption [c]
Avonlea	0.126	1.987	0.091	218
AC Reed	0.233	2.507	0.145	270
Banks	0.298	2.609	0.156	252
Genesis	1.052	3.576	0.229	2965
Glenlea (L)	0.605	3.208	0.192	941
Glenlea (SC)	1.014	5.197	0.250	1667
Katepwa (L)	0.579	4.255	0.168	1023
Katepwa (SC)	0.666	4.385	0.227	1405
Leader (L)	0.644	5.649	0.214	1666
Leader (SC)	0.727	5.334	0.191	2685
Suneca (L)	0.666	2.682	0.178	668
Suneca (SC)	0.452	3.311	0.164	1317
r [d]	0.813	0.688	0.695	
P value	0.001	0.013	0.012	

L = Lethbridge, S = Swift Current

[a] Results expressed in absorbance units

[b] Results expressed in EU

[c] Results expressed in nanomoles of oxygen per minute per gram

[d] Pearson correlation coefficient of the column method and the oxygen consumption results

4.2. GENOTYPE BY ENVIRONMENT STUDY

Detailed results for each parameter of each genotype in each environment are presented in Appendix I. All data is presented with means and standard deviations (SD) for environments and genotypes. Summaries of the results are

presented averaged by genotypes (Tables 4.3, 4.4 and 4.5) and environments (Tables 4.6, 4.7 and 4.8). The coefficients of variation (CV) of each parameter among genotypes and environments are given in Table 4.9.

In this section, the discussion will describe milling and flour properties of the samples used. Then, the influence of the genotype and environment on PPO activity, flour color and noodle color is discussed.

Table 4.3. Genotype means for flour yield, flour color, protein and PPO activity of flour and grain

	Parameter								
Genotype #	FY	FL	Fa	Fb	FP	WK	GPPO	FPPO	%PPO
1	71.3	93.8	-0.46	9.3	14.0	0.77	1330	40	3.0
2	69.3	93.7	-0.44	9.1	13.2	0.84	1491	43	3.0
3	72.1	93.9	-0.63	9.1	14.4	0.79	1480	55	3.7
4	72.5	93.6	-0.45	8.8	15.0	0.77	1287	50	4.0
5	73.5	94.0	-0.58	8.7	14.3	0.50	480	30	6.1
6	73.9	93.8	-0.53	8.7	12.6	0.54	525	37	7.1
7	72.8	93.7	-0.34	8.5	14.7	0.56	454	25	5.6
8	71.1	93.8	-0.44	9.1	12.2	0.52	569	31	5.4
9	73.3	94.0	-0.56	8.3	13.6	0.79	1315	49	3.8
10	68.8	93.8	-0.56	9.2	13.7	0.62	607	30	5.2
11	71.7	94.3	-0.62	8.3	13.5	0.53	535	28	5.3
12	70.1	93.9	-0.68	9.0	14.3	0.60	695	35	5.2
13	73.2	94.4	-0.53	8.3	13.1	0.81	1027	32	3.2
14	74.2	94.1	-0.59	8.3	13.0	0.87	1085	43	3.9
15	72.8	94.3	-0.53	8.2	12.9	0.81	974	39	3.7
16	73.3	94.3	-0.53	8.2	13.2	0.88	1063	36	3.4
17	72.1	94.1	-0.48	8.3	13.9	0.75	1203	39	3.2
18	74.5	94.0	-0.69	9.1	13.3	0.82	1143	44	3.9
19	73.4	94.2	-0.43	7.9	13.7	0.74	1185	33	2.9
20	72.4	94.1	-0.44	8.0	13.1	0.80	1575	49	3.2

FY = flour yield, FL = flour L*, Fa = flour a*, Fb = flour b*, FP = flour protein, WK = grain PPO, determined by whole kernel method, GPPO = grain PPO activity, determined by oxygen consumption method, FPPO = flour PPO activity, determined by oxygen consumption method, %PPO = flour PPO percentage of grain PPO

4.2.1. Milling and flour properties

4.2.1.1. Flour extraction yield

The samples grown in Brandon in 1999 (Environment #5) had the lowest average flour yield among the eight environments, 67.1%, compared to 71.2 to 74.0 for the rest of environments. In fact, all but one cultivar gave the lowest flour yield when grown at Brandon 1999 (Appendix I). This may be explained by a lack of filling of the kernels, evident in the physical appearance of these samples. Brandon

Table 4.4. Genotype means for alkaline noodles color and discoloration parameters

	Parameter								
Genotype #	AL0	AL24	ALC	Aa0	Aa24	AaC	Ab0	Ab24	AbC
1	82.3	67.8	14.50	0.30	2.71	-2.41	19.4	23.3	-3.95
2	81.9	66.8	15.12	0.35	3.05	-2.71	19.7	24.6	-4.96
3	82.0	69.0	13.00	0.31	2.61	-2.30	19.7	23.7	-4.03
4	82.0	68.3	13.77	0.65	3.17	-2.52	17.6	21.3	-3.67
5	82.2	70.6	11.58	0.28	2.97	-2.69	18.7	23.5	-4.81
6	81.3	69.6	11.78	0.44	3.13	-2.70	19.8	24.2	-4.44
7	82.3	70.9	11.36	0.42	2.93	-2.51	17.9	22.1	-4.28
8	82.9	70.6	12.32	0.05	2.80	-2.75	18.8	24.9	-5.47
9	82.7	70.2	12.48	0.23	2.55	-2.32	17.7	21.1	-3.41
10	83.2	70.9	12.24	0.02	2.82	-2.80	18.5	22.7	-4.17
11	82.1	70.8	11.39	0.32	2.93	-2.61	18.4	22.4	-4.00
12	83.1	71.1	12.03	0.05	2.72	-2.67	18.6	22.6	-4.01
13	83.0	70.4	12.58	-0.10	2.32	-2.42	18.9	23.7	-4.76
14	82.0	69.3	12.67	0.27	2.88	-2.61	19.0	22.6	-3.57
15	83.1	69.7	13.39	-0.20	2.29	-2.49	18.5	23.1	-4.61
16	82.8	69.7	13.10	-0.11	2.40	-2.51	18.7	23.1	-4.39
17	82.7	69.7	13.01	0.05	2.45	-2.40	17.9	21.8	-3.93
18	82.6	69.3	13.24	-0.08	2.66	-2.75	19.6	23.1	-3.51
19	82.7	70.1	12.66	0.16	2.36	-2.20	16.8	20.6	-3.72
20	82.1	67.7	14.40	0.60	3.01	-2.40	18.2	21.5	-3.34

AL0 = L* value at time 0, AL24 = L* value after 24 hours, ALC = L* value change after 24 hours, Aa0 = a* value at time 0, Aa24 = a* value after 24 hours, AaC = a* value change after 24 hours, Ab0 = b* value at time 0,

samples (Environment #1) also had the lowest flour yield of the 1998 samples. At the other extreme, locations from Saskatchewan (Swift Current and Melfort, environments # 3 and #7) had the highest means in both 1998 and 1999 (74.2% and 74.0% respectively). The range between the highest and the lowest extraction rates for genotypes (68.8%, #10 - 74.5%, #18) was smaller than the range for environments (67.1%, #5 - 74.2, #3). There was no clear tendency of high or low extraction yield values among the genotypes (Appendix I). The variability among

Table 4.5. Genotype means for salted noodles color and discoloration parameters

	Parameter								
Genotype #	SL0	SL24	SLC	Sa0	Sa24	SaC	Sb0	Sb24	SbC
1	83.7	72.8	10.95	0.69	2.93	-2.24	17.6	21.3	-3.73
2	82.4	70.6	11.79	0.95	3.57	-2.61	18.5	22.0	-3.58
3	83.3	73.2	10.15	0.70	2.60	-1.90	17.9	21.9	-3.96
4	83.7	73.1	10.68	1.06	3.26	-2.21	15.9	19.9	-4.02
5	83.9	74.3	9.62	0.77	3.00	-2.23	16.7	21.4	-4.73
6	82.5	72.7	9.87	1.00	3.45	-2.46	17.8	21.6	-3.77
7	84.2	75.3	8.97	0.88	2.85	-1.97	15.5	19.8	-4.39
8	83.4	74.2	9.21	0.72	3.16	-2.44	17.9	21.7	-3.82
9	84.3	73.9	10.41	0.71	2.68	-1.96	15.4	19.0	-3.58
10	84.3	74.0	10.34	0.63	3.07	-2.44	15.9	19.7	-3.88
11	83.9	74.2	9.77	0.62	2.79	-2.17	16.3	19.8	-3.52
12	84.4	74.4	10.05	0.59	2.93	-2.34	16.0	19.9	-3.91
13	84.3	74.6	9.74	0.40	2.44	-2.05	16.8	20.7	-3.90
14	83.5	73.6	9.87	0.76	2.81	-2.05	16.8	20.8	-3.94
15	84.2	74.0	10.15	0.39	2.47	-2.08	16.8	20.8	-4.04
16	84.0	74.2	9.81	0.39	2.49	-2.10	16.8	20.5	-3.72
17	84.4	74.3	10.07	0.58	2.65	-2.08	15.8	19.8	-4.01
18	83.8	73.8	9.97	0.41	2.64	-2.22	17.8	20.4	-2.65
19	84.9	75.3	9.53	0.51	2.25	-1.74	15.0	18.9	-3.91
20	83.0	72.2	10.82	1.07	3.21	-2.14	17.0	19.8	-2.81

SL0 = L* value at time 0, SL24 = L* value after 24 hours, SLC = L* value change after 24 hours, Sa0 = a* value at time 0, Sa24 = a* value after 24 hours, SaC = a* value change after 24 hours, Sb0 = b* value at time 0, Sb24 = b* value after 24 hours, SbC = b* value change after 24 hours.

environments (coefficient of variation, CV=3.2%; Table 4.9) was also higher than the one among genotypes (CV=2.2%).

Table 4.6.Environmental means for flour yield, flour color, protein and PPO activity of flour and grain

	Parameter								
Env. #	FY	FL	Fa	Fb	FP	WK	GPPO	FPPO	%PPO
1	71.2	93.7	-0.55	8.6	14.9	0.81	974	32	3.9
2	72.5	94.2	-0.63	8.4	13.5	0.67	864	30	3.6
3	74.2	94.2	-0.68	8.9	12.8	0.60	745	36	5.4
4	72.9	94.3	-0.54	8.5	12.8	0.79	789	26	3.6
5	67.1	93.6	-0.42	8.9	14.6	0.71	1426	62	5.0
6	73.1	93.6	-0.39	8.4	13.2	0.84	1239	50	4.6
7	74.0	94.1	-0.52	8.8	14.1	0.62	816	33	4.2
8	73.5	94.3	-0.48	8.3	12.8	0.69	1155	38	3.5
Means	72.3	94.0	-0.53	8.6	13.6	0.72	1001	38	4.2

FY = flour yield, FL = flour L*, Fa = flour a*, Fb = flour b*, FP = flour protein, WK = grain PPO, determined by whole kernel method, GPPO = grain PPO activity, determined by oxygen consumption method, FPPO = flour PPO activity, determined by oxygen consumption method, %PPO = flour PPO percentage of grain PPO

Table 4.7. Environmental means for alkaline noodles color and discoloration parameters

	Parameter								
Env. #	AL0	AL24	ALC	Aa0	Aa24	AaC	Ab0	Ab24	AbC
1	81.9	72.0	9.87	1.0	3.3	-2.28	17	21	-3.8
2	82.7	72.4	10.24	0.7	3.1	-2.41	18	22	-4.2
3	83.9	73.9	9.95	0.3	2.3	-1.93	19	24	-4.8
4	84.0	73.1	10.90	0.2	2.1	-1.96	18	23	-4.9
5	80.2	62.7	17.50	0.2	3.9	-3.68	21	23	-2.6
6	80.8	65.4	15.46	0.2	3.1	-2.97	18	20	-2.4
7	83.1	69.3	13.81	-0.5	2.0	-2.44	19	25	-6.1
8	83.0	68.1	14.91	-0.6	2.1	-2.65	20	24	-4.4
Means	82.5	69.6	12.83	0.2	2.7	-2.54	19	23	-4.2

AL0 = L* value at time 0, AL24 = L* value after 24 hours, ALC = L* value change after 24 hours, Aa0 = a* value at time 0, Aa24 = a* value after 24 hours, AaC = a* value change after 24 hours, Ab0 = b* value at time 0,

Table 4.8.Environmental means for salted noodles color and discoloration parameters.

	Parameter								
Env. #	SL0	SL24	SLC	Sa0	Sa24	SaC	Sb0	Sb24	SbC
1	82.5	73.4	9.07	1.1	3.2	-2.10	16	19	-3.0
2	83.3	74.2	9.11	0.7	2.9	-2.20	17	21	-3.3
3	84.4	75.4	8.98	0.3	2.0	-1.65	18	23	-4.5
4	84.7	75.4	9.33	0.2	2.1	-1.88	17	22	-4.4
5	82.3	68.8	13.52	1.2	4.3	-3.08	17	20	-2.8
6	83.4	72.6	10.79	1.2	3.6	-2.41	15	18	-3.4
7	85.3	75.8	9.59	0.2	1.9	-1.61	16	22	-5.4
8	84.6	74.3	10.30	0.6	3.0	-2.43	16	20	-3.4
Means	83.8	73.7	10.09	0.7	2.9	-2.17	17	20	-3.8

SL0 = L* value at time 0, SL24 = L* value after 24 hours, SLC = L* value change after 24 hours, Sa0 = a* value at time 0, Sa24 = a* value after 24 hours, SaC = a* value change after 24 hours, Sb0 = b* value at time 0, Sb24 = b* value after 24 hours, SbC = b* value change after 24 hours.

4.2.1.2. Flour color

Overall, the difference in flour color among samples, environments and genotypes was small. For most genotypes, flours from environments #1, #5 and #6 were darker than others. This was reflected in lower L* values for most samples (Appendix I) and means (Table 4.6). This consideration was specially significant for Brandon samples (environments #1 and #5) since they produced not just less flour, but also flours of lower quality, because they were darker. There was no evident difference in brightness among the other environments (#2, #3, #4, #7 and #8). The range in L* environment means (93.6-94.3) was similar to the range in genotype means one (93.6-94.4); the variability among environment (CV=0.32%; Table 4.9) was higher than among genotypes (CV=0.25%). Genotype #15 had the highest L* mean (Table 4.3) and the highest value in the best environments (#2, #3,

Table 4.9. Coefficients of variation of different parameters for environment and genotype

	Environment	Genotype
FY	3.2	2.2
FL	0.32	0.25
Fa	18.5	17.1
Fb	2.8	5.1
FP	6.2	5.3
WK	12.8	18.1
GPPO	24.6	37.3
FPPO	31.3	21.6
%PPO	17.1	28.7
SL0	1.33	0.76
AL0	1.67	0.60
SL24	3.1	1.5
AL24	5.8	1.7
SLC	15.1	6.3
ALC	23.1	8.1
Sa24	29.9	12.2
Aa24	26.4	10.1
SaC	22.1	9.8
AaC	22.5	6.7
Sb0	5.7	5.8
Ab0	7.2	4.2
Sb24	7.2	4.5
Ab24	7.6	5.1
SbC	23.5	12.0
AbC	29.5	13.6

FY = flour yield, FL = flour L*, Fa = flour a*, Fb = flour b*, FP = flour protein, WK = grain PPO, determined by whole kernel method, GPPO = grain PPO activity, determined by oxygen consumption method, FPPO = flour PPO activity, determined by oxygen consumption method, %PPO = flour PPO percentage of grain PPO, AL0 = L* value at time 0, AL24 = L* value after 24 hours, ALC = L* value change after 24 hours, Aa24 = a* value after 24 hours, AaC = a* value change after 24 hours, Ab0 = b* value at time 0, Ab24 = b* value after 24 hours, AbC = b* value change after 24 hours. SL0 = L* value at time 0, SL24 = L* value after 24 hours, SLC = L* value change after 24 hours, Sa24 = a* value after 24 hours, SaC = a* value change after 24 hours, Sb0 = b* value at time 0, Sb24 = b* value after 24 hours, SbC = b* value change after 24 hours.

#4, #7 and #8). The variation in redness (a*) was similar in genotypes (CV=17.1%) and environments (CV=18.5%). In yellowness (b*), there was higher variation due to genotype (CV=5.1%) than to environments (CV=2.8% for b*).

4.2.1.3. Protein content

There was a significant variation in protein content among genotypes, and even more among environments. The environment that resulted in highest protein content was #1 (Brandon in 1998; Table 4.6). The lowest level was observed in environments #3 (Swift Current in 1999), #4 (Glenlea in 1999) and #8 (Portage in 1999). The highest protein content genotype was AC Domain, a well-adapted CWRS cultivar. The lowest value corresponded to genotype #8, a double haploid experimental line (Table 4.3). Variability among environments (CV=6.2%; Table 4.9) was higher than among genotypes (CV=5.3%).

4.2.1.4. Milling and flour properties: general discussion

Milling and flour properties showed substantial environmental effects on wheat samples. The variability among environments was larger than among genotypes in flour yield, protein content and flour brightness as is shown by the coefficients of variation. The difference in some properties was very large between certain environments. In environment #5 (Brandon 1999) the mean flour yield (67.1%) was much lower than in environment #3 (74.2%, Swift Current 1998). The difference in flour protein within the same environments was also important: almost 2% (protein content is 14.6 and 12.8, respectively). Environmental effect was

visually evident in the kernels; most wheat samples from environment #3 were sound, whereas samples from environment #5 were shrunken. Although high protein is an important issue in flour quality, it is relevant only among grain samples with sound physical properties. Wheat from environment #5 presented poor milling quality characteristics, making it undesirable (lower grade) in the market. Therefore, the high protein content of environment #5 flours are not indicative of higher quality samples.

4.2.2. Polyphenol oxidase activity

4.2.2.1. Characteristics of the sample set

There was a wide variation among environments and genotypes in grain PPO activity (Tables 4.3 and 4.6). The variation was greater among genotypes than among environments. Using the oxygen consumption method (GPPO), genotype means were from 454 to 1575 O_2 nmol/g/min (CV=37%, Table 4.9), while environmental means were from 745 to 1426 O_2 nmol/g/min (CV=25%). Using the whole kernel method (WK) the ranges were 0.50-0.88 AU (CV=18%) and 0.60-0.84 AU (CV=13%) respectively. The locations with lowest activity each year were from Saskatchewan (Swift Current and Melfort; environments #3 and #7, respectively). Although 1998 Glenlea (environment #4) samples had, on average, lower activity than 1999 Melfort (environment #7) with the oxygen consumption method, for both grain PPO methods. Among genotypes, there was a clear tendency of some lines to have lower activity than others. The variation in flour PPO among environments (26-

62 ox. nmol/min/g, CV=31%) was greater than the variation among genotypes (26-55 ox. nmol/min/g, CV=22%).

PPO in flour as a percentage of grain PPO when measured with oxygen consumption method varied from 1.5 to 11.5 (Appendix I, Table AI.27). The variation among genotypes (CV=29%; Table 4.9) was higher than among environments (CV=17%). The environment with highest value was #3 (Table 4.6). The genotypes with %PPO over 5% were #5, 6, 7, 8, 10, 11 and 12.

The environment with highest mean of grain PPO determined with oxygen consumption was #5 (Brandon, 1999), whereas the lowest was #3 (Swift Current, 1998). In other words, the environment that produced samples with better milling properties (Swift Current, 1998: 74.2%; Appendix I, Table AI.1) was the one that produced samples with the lowest PPO activity (745 O_2 nmol/g/min; Appendix I, Table AI.7), and the one that produced samples with lowest flour yield (Brandon 1999, 67.1%) also produced samples with the highest PPO activity (1426 O_2 nmol/g/min). This environment was also the one for which the percentage of flour PPO based on grain PPO was the highest. Therefore, the environment that produced the grain with the best physical properties, also produced the wheat that resulted in lowest PPO activity. This constitutes an important advantage for wheat production since the environment that is the best for one characteristic (physical properties), is also the best for the other (PPO activity).

4.2.2.2. Groups according to PPO activity

As it was previously mentioned, the genotypes used in this research can be divided into two groups according to the PPO activity level, a low PPO group (less

than 700 O_2 nmol/g/min) and a high group (more than 1000 O_2 nmol/g/min). The separation is evident with both oxygen consumption and whole kernel methods (Figures 3.3 and 3.4). The genotypes included in the low PPO group were #5, 6, 7, 8, 10, 11 and 12; whereas in high PPO group they were #1, 2, 3, 4, 9, 13, 14, 15, 16, 17, 18, 19 and 20. The genotypes were sorted for each environment from low to high PPO activity in Tables 4.10 (oxygen consumption method) and 4.11 (whole kernel method). Using the first method, all seven genotypes that belong to the low PPO group had lower PPO activity than the thirteen genotypes that belong to the high PPO group, in each one of the eight environments. Using the second method, this separation was verified in six out of the eight environments. Genotype #12 was the only one that belongs to the low PPO group and was higher than some genotypes of the high PPO group. This fact was verified in two environments (#7 and 8).

The means and standard deviations of each PPO group for different parameters are presented in Table 4.12. The PPO group had a significant influence on all PPO activity determinations (grain PPO with both methods and flour PPO, $P<0.001$), discoloration of both salted ($P<0.05$) and alkaline noodle doughs ($P<0.001$) and alkaline noodle dough brightness after 24 hours ($P<0.01$).

4.2.2.3. Influence of different factors on grain PPO activity

The separation into two groups was included as a two-effects factor in the statistical model used in the genotype by environment study. In the ANOVA tables obtained with this model (Tables 4.13 and 4.14), it is clear that the PPO

Table 4.10. Genotypes by number[a] sorted in order of grain PPO[b] content

	Environment #								
	1	2	3	4	5	6	7	8	
LOW	**7**	**7**	**10**	**5**	**7**	**11**	**7**	**7**	LOW
	5	**10**	**7**	**7**	**6**	**5**	**6**	**5**	
	8	**8**	**5**	**6**	**5**	**8**	**5**	**6**	
	11	**5**	**11**	**11**	**11**	**7**	**10**	**11**	
	10	**11**	**8**	**8**	**8**	**6**	**11**	**10**	
	6	**6**	**6**	**10**	**12**	**10**	**12**	**8**	
	12	**12**	**12**	**12**	**10**	**12**	**8**	**12**	
	13	15	13	15	15	13	18	16	
	14	13	16	13	4	18	16	15	
	15	19	15	16	19	15	14	14	
	17	16	9	9	18	14	15	13	
	4	14	17	18	14	16	17	18	
	19	17	19	14	16	17	1	17	
	16	1	14	1	1	19	4	19	
	18	9	18	17	13	3	13	4	
	9	4	3	19	2	9	19	3	
	1	18	1	3	20	2	9	9	
	3	2	4	2	9	20	2	1	
	2	20	2	4	17	4	3	2	
HIGH	20	3	20	20	3	1	20	20	HIGH

[a] In bold, genotypes from the low PPO group.

[b] Oxygen consumption method

group factor contributes significantly to the grain PPO variability, measured with both whole kernel and oxygen consumption methods. This contribution was not only significant, but also the PPO group factor is the main component of the variation. It is 66.4% for the whole kernel method and 75.2% for the oxygen consumption method (Table 4.15). The difference in these values is explained by the higher error of the whole kernel method, compared to the very accurate oxygen consumption method. The contribution of the error term is smaller for oxygen consumption method (3.5% of the total variability) than for whole kernel method (13.2%). While

Table 4.11. Genotypes by number[a] sorted in order of grain PPO[b] content

	Environment #								
	1	2	3	4	5	6	7	8	
LOW	**5**	**8**	**6**	**8**	**5**	**8**	**5**	**5**	LOW
	6	**11**	**11**	**5**	**11**	**6**	**11**	**7**	
	7	**12**	**5**	**11**	**8**	**12**	**6**	**8**	
	8	**10**	**12**	**12**	**12**	**7**	**8**	**6**	
	11	**6**	**8**	**6**	**7**	**11**	**10**	**10**	
	12	**7**	**7**	**7**	**10**	**10**	**7**	**11**	
	10	**5**	**10**	**10**	**6**	**5**	17	3	
	19	17	3	19	9	18	2	9	
	1	1	13	15	4	15	13	4	
	18	19	4	14	13	9	15	**12**	
	17	2	17	1	15	19	4	17	
	20	20	1	20	19	20	**12**	19	
	2	16	19	9	3	3	1	1	
	15	4	2	4	16	13	3	20	
	3	18	18	16	17	17	9	2	
	16	14	14	17	1	4	14	18	
	13	9	9	18	18	14	19	13	
	4	13	20	2	14	1	16	16	
	14	15	16	13	2	2	18	14	
HIGH	9	3	15	3	20	16	20	15	HIGH

[a] In bold, low PPO level group genotypes.

[b] Whole kernel method

the conclusions are stronger using the first method, results obtained with the whole kernel method fully confirm these conclusions.

Kernel color did not contribute significantly to PPO group (Tables 4.13 and 4.14), with a minor contribution of the interaction between PPO group and kernel color (1.7% using the oxygen consumption method, not significant for the whole kernel method, Table 4.15). It can be interpreted that there is no relationship between the pericarp color and PPO activity. It is possible to observe both white and red cultivars with low or high PPO activity. In most published papers (Lamkin et

Table 4.12. Mean +/- standard deviation at each PPO effect level

PPO group	LOW	HIGH	
FL	93.90 +/- 0.46	94.05 +/- 0.43	n.s.
SQWK	0.741 +/- 0.063	0.793 +/- 0.077	***
SQGPPO	23.22 +/- 3.61	34.89 +/- 5.09	***
SQFPPO	5.44 +/- 1.10	6.40 +/- 1.25	***
SL0	83.82 +/- 1.51	83.81 +/- 1.37	n.s.
SL24	74.13 +/- 2.85	73.51 +/- 2.87	n.s.
SQSLC	3.10 +/- 0.30	3.20 +/- 0.29	*
AL0	82.45 +/- 1.72	82.46 +/- 1.59	n.s.
AL24	70.64 +/- 3.95	69.08 +/- 4.49	**
SQALC	3.42 +/- 0.40	3.63 +/- 0.47	***

* Difference significant at P<0.05

** Different significant at P<0.01

*** Difference significant at P<0.001

n.s. Difference not Significant

FL = flour L*, SQWK = grain PPO, whole kernel method, square root transf., SQGPPO = grain PPO, oxygen consumption method, square root transf., SQFPPO = flour PPO, oxygen consumption method, square root transf., SL0 = salted noodles L* value at time 0, SL24 = salted noodles L*value at 24 SQSLC = salted noodles L* value, square root transf., AL0 = alkaline noodles L* value at time 0, AL24 = alkaline noodles L* value at 24h, SQALC = alkaline noodles L* value, square roor transformed.

al 1981; McCallum and Walker 1990; Park et al 1997; Baik et al 1994a; Baik et al 1995), white cultivars had lower activity than red ones. The results obtained in this research proved that those observations cannot be generalized. The results observed in other studies may be explained by a more specific selection of good end-product color in white genotypes. As end-product color is related to discoloration, and discoloration is related to PPO activity, the selection of good end-

Table 4.13. ANOVA for PPO activity[a] (square root transformation)

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [b]	1	0.0137	0.0137	2.77	0.116
PPO level [b]	1	0.7714	0.7714	155.81	0.000
Kernel color * PPO level [b]	1	0.0000	0.0000	0.01	0.931
Genotype (kernel color * PPO level)	16	0.0792	0.0050	2.24	0.007
Environment	7	0.4060	0.0580	26.30	0.000
Kernel color * Environment	7	0.0297	0.0042	1.92	0.090
PPO level * Environment	7	0.0209	0.0030	1.35	0.154
Kernel color * PPO level * Env.	7	0.0216	0.0031	1.40	0.212
Error	112	0.2470	0.0022		
Corrected total	159	1.6601			

[a] Whole kernel method

[b] F value calculated using Variety (kernel color * PPO level) as the error term

Table 4.14. ANOVA for grain PPO activity[a] (square root transformation)

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [b]	1	0.0268	0.0268	0.00	0.980
PPO level [b]	1	5112.82	5112.82	126.25	0.000
Kernel color * PPO level [b]	1	183.359	183.359	4.53	0.049
Genotype (kernel color * PPO level)	16	647.984	40.499	12.15	0.000
Environment	7	1521.095	217.299	65.20	0.000
Kernel color * Environment	7	67.62	9.66	2.90	0.009
PPO level * Environment	7	130.35	18.62	5.59	0.000
Kernel color * PPO level * Env.	7	22.1741	3.1677	0.95	0.471
Error	112	373.275	3.333		
Corrected total	159				

[a] Oxygen consumption method

[b] F value calculated using Variety (kernel color * PPO level) as the error term

Table 4.15. Components of variance (%) of PPO activity determinations

	SQWK	SQGPPO	SQFPPO
Kernel color	0.6	0.0	0.0
PPO level	66.4	75.2	23.3
Kernel color * PPO level	0.0	1.7	4.0
Genotype (kernel color * PPO level)	1.8	4.9	6.4
Environment	15.9	12.2	39.5
Kernel color * Environment	1.1	0.7	0.0
PPO level * Environment	0.2	1.8	0.6
Kernel color * PPO level * Env.	0.7	0.0	0.0
Error	13.2	3.5	26.4

SQWK = whole kernel method, square root transformation
SQGPPO = det. on grain, oxygen cons. method, square root transformation
SQFPPO = det. on flour, oxygen cons. method, square root transformation

product color may lead to low PPO varieties, as the white wheats analysed in the mentioned papers.

Wheat genotype influenced grain PPO activity, but to a minor degree. With the statistical model used, the interpretation of the genotype factor is that it includes all other genetic components but the related to PPO group and kernel color. Using the oxygen consumption method, the PPO group, a genetic factor, contributed 75.2%, while the contribution of genotype, nested in PPO group and grain color, contributed just 4.9% (Table 4.15). Therefore, there is genetic variability for PPO activity in each one of the PPO groups, but the difference between groups is more than 15 times the variability due to other genetic components.

Environment is the second major component influencing grain PPO variation. Using the oxygen consumption method, it accounted for 12.2% of the total (Table 4.15). This value is low when it is compared with the contribution of the genetic components. This observation becomes more relevant since, according to the

milling and flour properties, there was a large influence of these environments on wheat samples. All the genetic components (grain color, PPO group, their interaction and genotype) summed up to 81.8% of total variation, almost 7 times the contribution of environments. In other words, by selecting cultivars, it is possible to control more than 80% of the PPO activity. The interactions of environment with genetic components contributes in low percentages: 2.5% in total. According to these percentages it is concluded that by selecting cultivars according to their PPO content it is possible to ensure grains with low PPO content.

Using the whole kernel method, the contribution of the environment to grain PPO activity is higher: 15.9% (Table 4.15). The difference can be explained by the difference in the methods. For the whole kernel method, the environmental factors influences not only PPO activity, but also the accessibility of the enzyme to the substrate in the kernel. Using the oxygen consumption method, this factor is minimised by grinding the sample.

4.2.2.4. *Influence of different factors on flour PPO activity*

Importance of factors differed between flour and whole wheat, as is shown by the ANOVA table (Table 4.16). Kernel color was still not significant, confirming the previous conclusion that this factor does not affect PPO activity. The PPO group was significant, but its contribution to the total variability was much lower than for grain PPO: 23.3% (Table 4.15). Environmental influence was increased (39.5%) as

Table 4.16. ANOVA of flour PPO activity[a] (square root transformation)

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [b]	1	0.6402	0.6402	0.40	0.152
PPO level [b]	1	39.609	39.609	24.68	0.000
Kernel color * PPO level [b]	1	4.4128	4.4128	2.75	0.007
Genotype (kernel color * PPO level)	16	25.681	1.605	2.77	0.001
Environment	7	104.901	14.986	25.90	0.000
Kernel color * Environment	7	1.7565	0.2509	0.43	0.879
PPO level * Environment	7	4.0738	0.5820	1.01	0.431
Kernel color * PPO level * Env.	7	2.1951	0.3136	0.54	0.801
Error	112	64.8138	0.5787		
Corrected total	159	261.0936			

[a] Oxygen consumption method

[b] F value calculated using Variety (kernel color * PPO level) as the error term

was the error (26.4%). These increases can be explained by the differences in the flour yield. Milling properties were affected significantly by environments, as indicated by the extraction yields obtained. Different flour yields and milling properties resulted in different percentages of flour PPO derived from grain PPO. Therefore, the environment influence was stronger on flour PPO than on grain PPO. Simultaneously, as flour PPO is only a small percentage of the grain PPO, a small change in the kernel PPO caused a big change in flour PPO. It should be noted that although the genotypes of the low PPO group had higher percentage of flour PPO out of grain PPO in all cases (Table 4.3), their flour still had a significantly lower activity than the flour obtained from genotypes of the high PPO group (Table 4.12 and 4.16). The contributions of other components were unimportant.

* * *

Summarising, the genetic components that determine whether the genotype belongs to the high or low PPO group are the main factor that affect grain PPO. Overall, genetic components contributed 80% of the grain PPO variability. The influence of environment was more important on flour PPO, but the PPO group effect was still the most significant component.

4.2.2.5. Spearman correlations

The importance of the contribution of genetic factors is confirmed by Spearman rank correlation results. As can be seen in Table 4.17, all correlation coefficients were significant (P<0.05), and most of them were very significant

Table 4.17. Correlation of PPO[a] genotype rankings[b] by environments.

	Env#1	Env#2	Env#3	Env#4	Env#5	Env#6	Env#7	Env#8
Env#1		0.94***	0.90***	0.87**	0.83**	0.89***	0.85**	0.93***
Env#2	0.94***		0.94***	0.89***	0.83**	0.85**	0.83**	0.90***
Env#3	0.90***	0.94***		0.94***	0.72*	0.89***	0.82**	0.92***
Env#4	0.87**	0.89***	0.94***		0.79**	0.91***	0.88***	0.92***
Env#5	0.83**	0.83**	0.72*	0.79**		0.77**	0.87**	0.85**
Env#6	0.89***	0.85**	0.89***	0.91***	0.77**		0.84**	0.93***
Env#7	0.85**	0.83**	0.82**	0.88***	0.87**	0.84**		0.94***
Env#8	0.93***	0.90***	0.92***	0.92***	0.85**	0.93***	0.94***	
Means	0.887	0.883	0.875	0.886	0.808	0.866	0.861	0.913

* Significant at P<0.05

** Significant at P<0.01

*** Significant at P<0.001

[a]Oxygen consumption method

[b]Spearman correlation coefficients

($P<0.01$ or $P<0.001$). The Spearman correlation coefficients correlate the ranking of different genotypes among environments. High values indicate that the ranking is similar from one environment to other. Therefore, genotypes with low PPO content in one environment will be likely low in most other environments. Environment #5 (Brandon 1999) was the one where the mean of the seven Spearman correlation coefficients was the lowest. This environment was also the one with the lowest extraction yield and lowest flour brightness, which can be interpreted as the one where the grain samples were most adversely affected. Then, it is not surprising that this environment had a major impact on quality parameters.

4.2.2.6. Stability

In a breeding program, it is important for quality parameters not only to indicate good overall quality, but also to ensure that those values are consistent when cultivars are grown in different environments. One way to measure the amount of change in the parameter among environments is to determine stability as it was defined in Materials and Methods. Two examples are presented in Figure 4.3. The stability results (Table 4.18) show that the genotypes of the low PPO group are generally the more stable. Genotypes with low PPO activity may be genotypes that cannot produce high PPO activity, even when environmental conditions are the optimal for production of this enzyme.

Therefore, ranking among varieties should remain virtually unchanged from one environment to other, and low PPO varieties should be fairly stable. Analysing samples from one environment should be sufficient to select genotypes with low stable PPO activity.

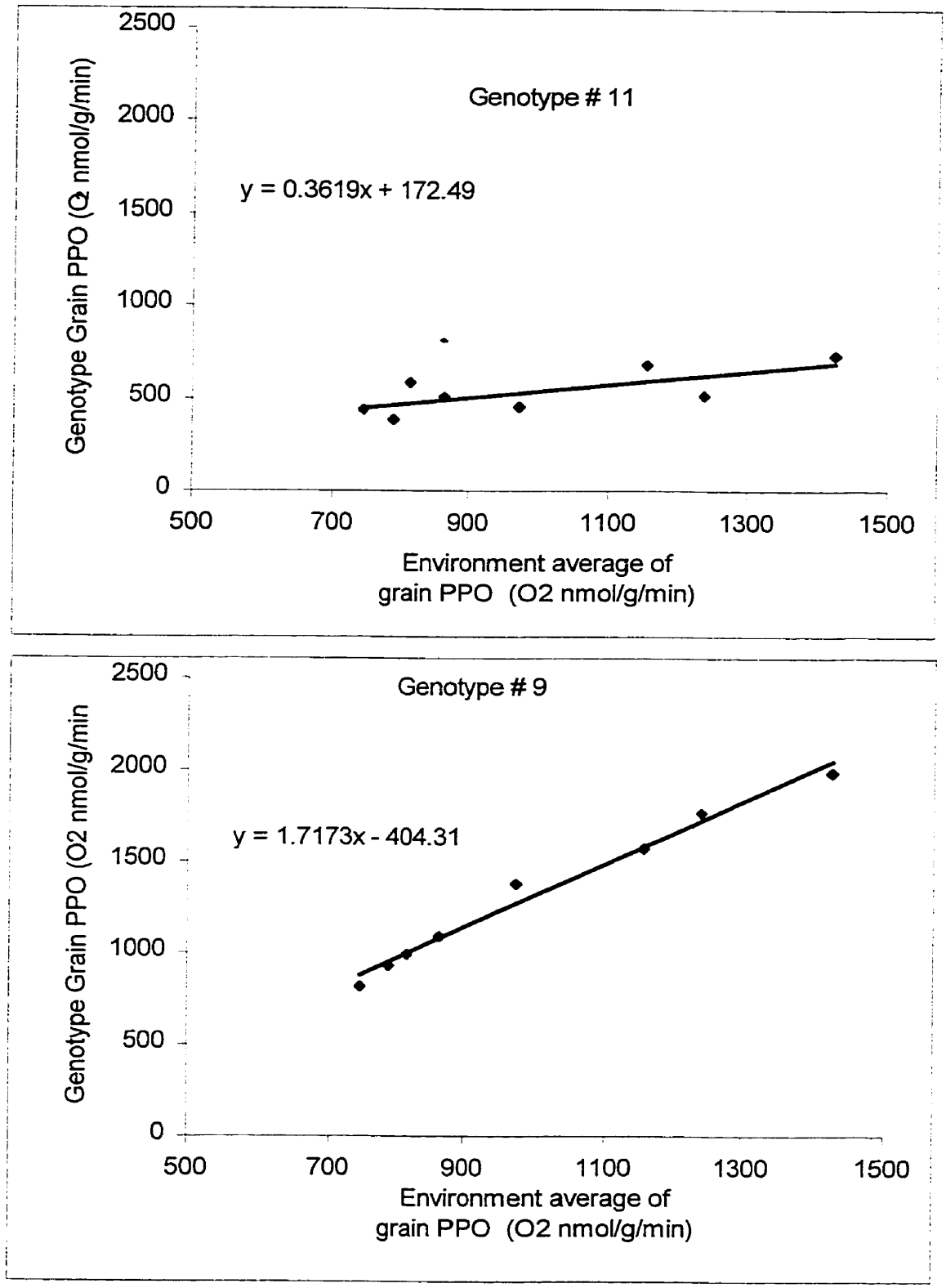


Figure 4.3. Examples of stability of grain PPO response of two genotypes

Figure 4.18 Grain PPO[a] stability

Genotype	Stability	PPO group
11	0.362	LOW
6	0.381	LOW
5	0.447	LOW
7	0.486	LOW
12	0.514	LOW
8	0.552	LOW
15	0.958	HIGH
4	0.970	HIGH
18	0.970	HIGH
14	1.085	HIGH
10	1.098	LOW
20	1.099	HIGH
2	1.203	HIGH
19	1.227	HIGH
16	1.228	HIGH
13	1.319	HIGH
17	1.403	HIGH
3	1.468	HIGH
1	1.512	HIGH
9	1.717	HIGH

4.2.3. Influence of different factors on flour color

The ANOVA table of the effect of different parameters on flour brightness is presented in Table 4.19, while the components of variance are in Table 4.20. Flour color was mainly affected by the environment, but some genetic components also influenced brightness significantly.

Environment was the main factor influencing the variance of the L* value. It contributed 39.4%. Grain soundness and cleanness are affected by environment,

Table 4.19. ANOVA of flour L*

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	1.4446	1.4446	4.48	0.050
PPO level [a]	1	0.2666	0.2666	0.83	0.376
Kernel color * PPO level [a]	1	0.4340	0.4340	1.35	0.263
Genotype (kernel color * PPO level)	16	5.1576	0.3224	4.10	0.000
Environment	7	10.9447	1.5635	19.90	0.000
Kernel color * Environment	7	0.2051	0.0293	0.37	0.916
PPO level * Environment	7	0.1900	0.0271	0.35	0.912
Kernel color * PPO level * Env.	7	1.4992	0.2142	2.73	0.012
Error	112	8.7220	0.0786		
Corrected total	159	31.2481			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

Table 4.20. Components of variance (%) of flour brightness

	Flour brightness
Kernel color	8.1
PPO level	0.0
Kernel color * PPO level	2.7
Genotype (kernel color * PPO level)	13.4
Environment	39.4
Kernel color * Environment	0.0
PPO level * Environment	0.0
Kernel color * PPO level * Env.	0.3
Error	36.2

and are very important determinants of the milling properties (Baik et al 1994a). Large differences in grain soundness were observed in this set of samples. These differences affected the milling properties and therefore the flour color.

Of the genetic factors, genotype (nested in kernel color and PPO group), contributed the highest percentage (13.4%) of the variance. It was previously mentioned that the factors that affect flour color include the protein quality and quantity and, mainly, endosperm pigments (Baik et al 1994a). These factors have genetic components, which may be responsible for this 13.4%.

The effect of kernel color on flour color has already been shown by several researchers (Paulsen et al 1983; Li and Posner 1989; Watts et al 1999). It was verified in this work. The contribution was minor (8.1%; Table 4.20), but still significant (P=0.05).

As expected, PPO group did not contribute to the flour brightness variability (Table 4.19). Enzyme activity is not initiated until water is added to the flour.

4.2.4. Noodle color

4.2.4.1. Characteristics of the samples set

Color and discoloration were affected mainly by the environment. The brightness (L*) of both salted and alkaline noodles immediately after the preparation (SL0 and AL0, respectively) were more influenced by environment (CV=1.33% and 1.67%, respectively, Table 4.9) than by genotype (CV=0.76% and 0.60%, respectively). Similarly, the noodle color after 24 hours for both types (SL24 and

AL24, respectively) had a higher CV (3.1% and 5.8%, respectively) for environment than for genotype (1.5% and 1.7%, respectively). The variation for change in color was higher for both salted and alkaline noodles (SLC and ALC, respectively), but environmental variation (CV=15.1% and 23.1%, respectively) was still more important than genotypical variation (CV=6.3% and 8.1%, respectively). The impact of environment was evident analysing the components of variance. This effect contributed more than 50% (Table 4.21) of the total variability for salted noodles, and more than 60% (Table 4.22) for alkaline noodles. The high contribution is explained by the large differences among environments.

Environment #5 (Brandon in 1999) produced both salted and alkaline noodle sheets that were darker than those from the rest of the environments (Tables 4.7 and 4.8). This observation is in agreement with the flour color, and it is specially important since it is also this environment that produced the lowest flour yield and

Table 4.21. Components of variance (%) for salted noodle doughs brightness (L*)

	SL0	SL24	SQSLC
Kernel color	0.0	0.0	0.0
PPO level	0.0	0.7	4.0
Kernel color * PPO level	0.0	0.0	0.0
Genotype (kernel color * PPO level)	15.2	8.7	3.3
Environment	55.7	56.1	51.9
Kernel color * Environment	0.1	0.1	0.4
PPO level * Environment	0.0	0.0	0.7
Kernel color * PPO level * Env.	0.0	0.0	0.2
Error	29.0	34.4	39.6

SL0 = salted noodles, L*, immediately after preparation
SL24 = salted noodles, L*, 24 hours after preparation
SQSLC = salted noodles, change in L*, square root transformed

Table 4.22. Components of variance (%) for alkaline noodle doughs brightness (L*)

	AL0	AL24	SQALC
Kernel color	3.7	0.0	0.0
PPO level	0.0	5.2	9.0
Kernel color * PPO level	0.0	0.0	0.0
Genotype (kernel color * PPO level)	2.9	2.0	2.1
Environment	63.2	72.1	67.6
Kernel color * Environment	0.0	1.1	1.6
PPO level * Environment	0.0	1.0	2.4
Kernel color * PPO level * Env.	0.0	0.0	0.0
Error	30.2	18.7	17.3

AL0 = alkaline noodles, L*, immediately after preparation
AL24 = alkaline noodles, L*, 24 hours after preparation
SQALC = alkaline noodles, change in L*, square root transformed

the highest discoloration. In general, all samples grown in 1999 had higher discoloration than those grown in 1998. Similar influences of environment and genotype were observed for redness (a*) and yellowness (b*).

Alkaline noodles are called also "yellow noodles", while salted ones are named "white noodles". These names are in agreement with the results obtained for b* value or yellowness. Alkaline noodles had higher values than salted noodles immediately after preparation (means were 18.6 and 16.7 respectively) and 24 hours later (means were 22.8 and 20.5, respectively; Tables AI.21, AI.22, AI.23 and AI.24, Appendix I).

4.2.4.2. Influence of different factors on noodle brightness

The ANOVA tables that describe the effect of different factors proposed in the model on brightness immediately after the preparation, 24 hours later and

discoloration are presented respectively in Tables 4.23, 4.24 and 4.25 for salted noodles, and in Tables 4.26, 4.27 and 4.28 for alkaline noodles.

Table 4.23. ANOVA for salted noodle doughs brightness

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	1.9439	1.9439	0.52	0.479
PPO level [a]	1	0.0024	0.0024	0.00	0.980
Kernel color * PPO level [a]	1	1.4232	1.4232	0.38	0.544
Genotype (kernel color * PPO level)	16	59.261	3.704	5.70	0.000
Environment	7	155.617	22.231	34.22	0.000
Kernel color * Environment	7	4.8656	0.6951	1.07	0.382
PPO level * Environment	7	4.6778	0.6683	1.03	0.409
Kernel color * PPO level * Env.	7	3.3548	0.4793	0.74	0.635
Error	112	72.1155	0.6497		
Corrected total	159	320.085			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

Table 4.24. ANOVA for salted noodle doughs brightness 24 hours after preparation

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	2.1952	2.1952	0.22	0.649
PPO level [a]	1	16.015	16.015	1.57	0.228
Kernel color * PPO level [a]	1	0.7606	0.7606	0.07	0.788
Genotype (kernel color * PPO level)	16	162.94	10.184	3.24	0.000
Environment	7	601.97	85.995	27.35	0.000
Kernel color * Environment	7	27.260	3.8943	1.24	0.282
PPO level * Environment	7	16.813	2.4019	0.76	0.613
Kernel color * PPO level * Env.	7	19.183	2.7405	0.87	0.525
Error	112	348.97	3.1439		
Corrected total	159	1308.61			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

Table 4.25. ANOVA for salted noodle doughs change in brightness after 24 hours, square root transformation

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	0.0010	0.0010	0.01	0.905
PPO level [a]	1	0.4211	0.4211	6.46	0.022
Kernel color * PPO level [a]	1	0.1014	0.1014	1.56	0.231
Genotype (kernel color * PPO level)	16	1.0427	0.0652	1.68	0.058
Environment	7	6.4149	0.9164	23.57	0.000
Kernel color * Environment	7	0.3521	0.0503	1.29	0.254
PPO level * Environment	7	0.2874	0.0411	1.06	0.390
Kernel color * PPO level * Env.	7	0.2347	0.0335	0.86	0.532
Error	112	4.3154	0.0389		
Corrected total	159	14.11			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

Table 4.26. ANOVA for alkaline noodle doughs immediately after preparation

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	11.5111	11.5111	7.39	0.015
PPO level [a]	1	0.0001	0.0001	0.00	0.995
Kernel color * PPO level [a]	1	3.2357	3.2357	2.08	0.169
Genotype (kernel color * PPO level)	16	24.9318	1.5582	1.60	0.081
Environment	7	223.4759	31.925	32.72	0.000
Kernel color * Environment	7	2.6897	0.3842	0.39	0.904
PPO level * Environment	7	2.9553	0.4222	0.43	0.880
Kernel color * PPO level * Env.	7	4.4802	0.6400	0.65	0.709
Error	112	109.2827	0.9757		
Corrected total	159	421.68			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

Table 4.27. ANOVA for alkaline noodle doughs 24 hours after preparation

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	6.0823	6.0823	0.77	0.393
PPO level [a]	1	88.0857	88.0857	11.15	0.004
Kernel color * PPO level [a]	1	0.1013	0.1013	0.01	0.911
Genotype (kernel color * PPO level)	16	126.46	7.9035	1.94	0.022
Environment	7	1824.2	260.5949	64.10	0.000
Kernel color * Environment	7	43.353	6.1933	1.52	0.162
PPO level * Environment	7	46.549	6.6498	1.64	0.129
Kernel color * PPO level * Env.	7	22.597	3.2281	0.79	0.588
Error	112	451.26	4.0654		
Corrected total	159	3028.46			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

Table 4.28. ANOVA for alkaline noodle doughs change in brightness after 24 hours of preparation

Source	DF	Sum of squares	Mean square	F Value	P > F
Kernel color [a]	1	0.0189	0.0189	0.16	0.647
PPO level [a]	1	1.6345	1.6345	14.25	0.001
Kernel color * PPO level [a]	1	0.045	0.045	0.39	0.536
Genotype (kernel color * PPO level)	16	1.84	0.1147	2.79	0.012
Environment	7	19.3	2.7623	67.12	0.000
Kernel color * Environment	7	0.560	0.0800	1.94	0.081
PPO level * Environment	7	0.624	0.0891	2.17	0.041
Kernel color * PPO level * Env.	7	0.234	0.0335	0.81	0.572
Error	112	4.57	0.0412		
Corrected total	159	32.61			

[a] F value calculated using Genotype (kernel color * PPO level) as the error term

The influence of kernel color on flour color is extended to noodle dough brightness immediately after preparation only in the alkaline type. Doughs were prepared mixing flour and a sodium chloride or sodium carbonate solution. As kernel color affected flour color, it would be expected that dough color immediately after preparation would be affected as well. But the effect of the kernel color factor was not significant in noodle doughs (Table 4.23). Factors other than flour color affected noodle color. Particle size, protein quantity and quality have been proposed to contribute as well (Baik et al 1995). These factors may interfere with the kernel color components causing a not-detectable factor effect. The interferences were not avoided with this samples. To avoid them, a study may be conducted with near isogenic lines. Using genotypes with differences only in the genes that determine the pericarp color, the actual effect of kernel color on salted noodle dough could be properly studied.

In alkaline noodles, kernel color affected brightness significantly, immediately after preparation ($P=0.015$; Table 4.26). It was mentioned previously that red pigments are more intense at high pH (Corpuz et al 1983, De Pauw and McCaig 1988). Therefore, in alkaline conditions the difference between bran contamination from red and white wheats is enhanced, and the interferences are not enough to mask it.

After 24 hours of storage, more factors affect product color. Other components, like enzymic browning, are added to the effects of kernel color, protein, endosperm pigments and other parameters of flour color, minimising their effect. This observation explains the lack of significance of kernel color on noodle dough brightness after 24 hours of preparation in both salted (Table 4.24) and

alkaline noodles (Table 4.27). The change in color after 24 hours was not affected by kernel color (Tables 4.25 and 4.28). It confirms the independence of kernel color and browning processes.

PPO has been proposed to increase the concentration of brown components (Baik et al 1995). When color is measured immediately after noodle dough preparation, PPO cannot affect noodle color. This observation is in agreement with the non significant effect of PPO group on noodle color immediately after preparation (Tables 4.23 and 4.26). After 24 hours, PPO group had a significant effect on alkaline noodles brightness (P=0.004; Table 4.27), but not on salted noodles (Table 4.24). Again, other factors are likely masking the effect and were more important in salted noodles. Since polyphenol colors are more intense at high pH, it was possible to detect a significant effect. Other authors have observed that discoloration is more important in alkaline noodles than in salted noodles. This could be due to increased activity at high pH (Edwards et al 1989), but the pH optimum determined are inconsistent (Interesse and Ruggieron 1980, Lamkin et al 1981, Marsh and Gaillard 1986, McCallum and Walker 1990). The relationship between grain PPO and color after 24 hours is plotted in Figure 4.4. Among samples from the low PPO group, there is no relationship between both parameters for both alkaline and salted noodles. Lower brightness values are shown with higher PPO activity among the high PPO group genotypes.

The significant effect of PPO group on both salted (P=0.022; Table 4.25) and alkaline (P=0.001; Table 4.28) dough discoloration proves the importance of the enzyme in browning processes. The dispersion plot of Figure 4.5 shows that the increase of PPO activity is usually associated with an increase in change in color.

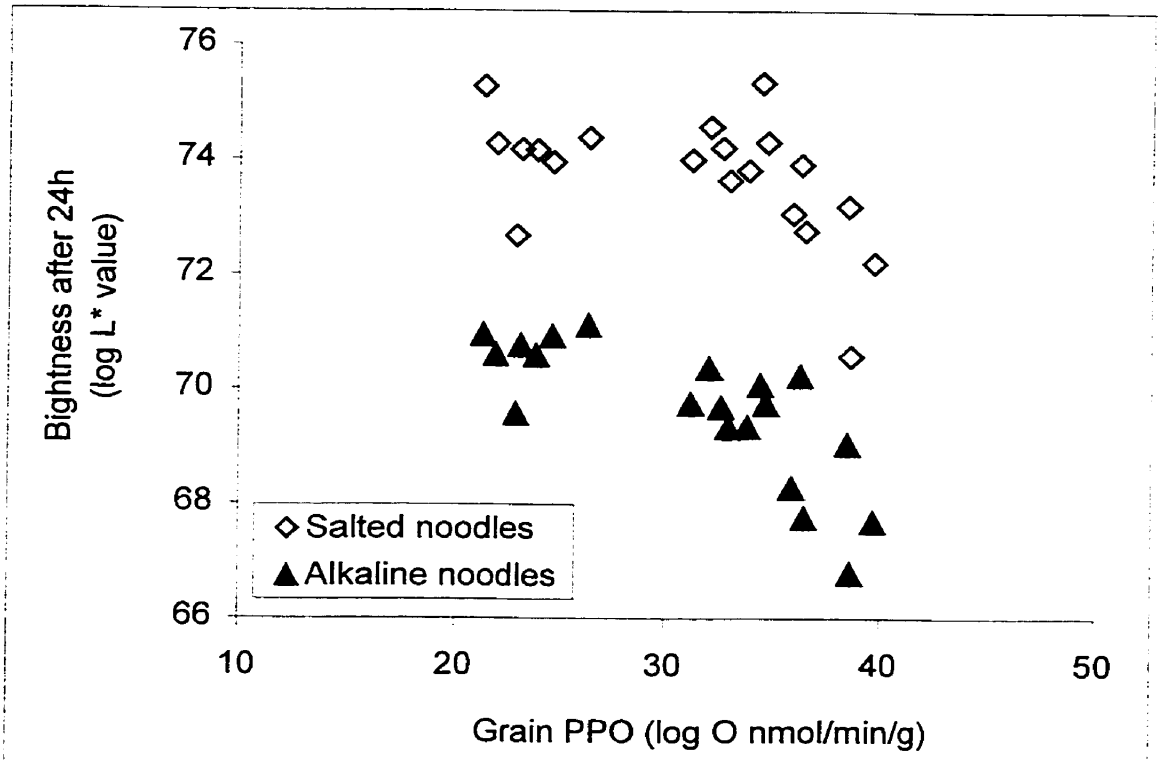


Figure 4.4. Brightness after 24 hours vs. grain PPO. Transformed values. Average of 8 environments.

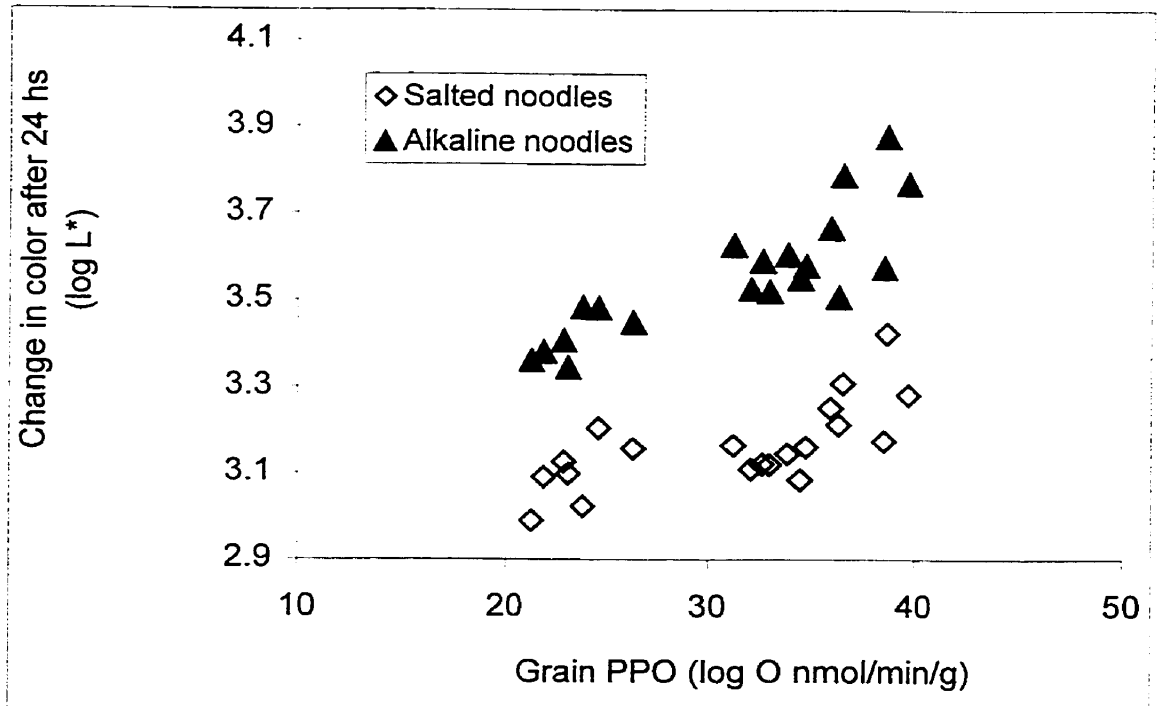


Figure 4.5. Change in brightness vs grain PPO. Transformed values. Averages of 8 environments.

Other genetic factors also had significant effect on noodle color or discoloration. The importance of such factors on salted noodles is evident in the significance of the genotype factor on dough color immediately after preparation ($P<0.001$; Table 4.23) and 24 hours later ($P<0.001$; Table 4.24). Genotype (nested in PPO and kernel color) was not significant effect on alkaline noodle color immediately after preparation (Table 4.26). After 24 hours, some genetic factors significantly effected on alkaline dough brightness. These factors may be related to browning factors, including peroxidase and phenol content (Kasatkina et al 1986, Edwards et al 1989, McCallum and Walker 1990, Crosbie et al 1995, Vadlamani and Seib 1996, Bhattacharya et al 1999, Fraignier et al 2000).

Genetic factors contributed much less than environmental ones to most parameters perhaps due to the very wide range of environmental conditions in the study, which affected wheat quality. These differences influenced all color components. It is expected that using just sound grain samples, the environment influence would be significantly lower. This hypothesis could not be verified in this research due to the lack of several environments with these characteristics. But comparing noodle dough brightness immediately after preparation of environments #3 (Swift Current 1998) and #7 (Melfort 1999), an important tendency is shown. The range in L* values for salted noodles among all environments was 82.3-85.3, but between the two environments with sound samples was much lower: 84.4-85.3. For alkaline noodles, the ranges were 80.2-84.0 and 83.1-83.9, respectively.

Interactions among the different factors analysed were not significant, except for PPO group by environment, which has little contribution (2.4%, Table 4.22). It

should be possible to select genotypes with improved color properties without the necessity of using a complex set of environmental conditions.

Comparing the three genetic factors considered in the model, genotype was much more important than kernel color and PPO group in salted noodle color, both immediately after preparation and 24 hours later. This means that other genetic factors than kernel color and PPO group should be considered to improve salted noodle color. Using alkaline noodles, kernel color was more important (component of variance = 3.7%; Table 4.22) than other genetic factors at time 0 (in total, component of variance = 2.9), but after storage, the effect of PPO group (component of variance = 5.2%) was more than the double the effect of the rest of genetic factors (in total, component of variance = 2.0%). As the product is sold after a storage period, it is very important to consider the 24 hours color reading. PPO was the main component of 24 hours color. Therefore, by selecting low PPO genotypes in a breeding program, end-product color after storage can be improved.

The effect of PPO on discoloration is not only significant, but also it is more important than the rest of the genetic components. For salted noodles, no other genetic factor significantly affected the change in brightness after 24 hours. For alkaline noodles, the component of variance of PPO group was 9.2%, while the component of variance for genotype was 2.1%. Other genetic components (kernel color and interactions) were not significant (Table 4.22). Therefore, the magnitude of the effect of PPO is more than 4 times this of other genetic factors. This observation enhances the importance of low PPO selection.

Significant correlations between PPO activity and discoloration were previously published (Kruger et al 1994, Baik et al 1995, Park et al 1996). In this

research, not only was the impact of PPO on discoloration confirmed, but also it was shown to be a simple component. More importantly, different genetic factors were quantified. When alkaline noodle were analysed, the contribution of PPO group was more than 4 times the contribution of all other genetic factors.

4.3. VISUAL SCORE

PPO group is mainly a genetic factor, and it is the most important in determination of color after storage of alkaline noodles and discoloration in both salted and alkaline noodles. Therefore, a rapid and simple method to determine PPO level is essential for a wheat breeding program that has as objective to select genotypes with good color properties.

The whole kernel method selected has these requirements. In order to make it even faster and simpler, visual darkening scoring was evaluated. The PPO activity was visually determined for all samples grown in 1999 (environments #5-8). Kernel darkening scores are reported in Table 4.29 and solution darkening scores in Table 4.30.

Genotypes were sorted from low to high score according to the evaluation of the grain (Table 4.31) and solution darkening (Table 4.32). The separation between low and high PPO groups was not possible analysing the solution darkening. Scoring grain darkening, the proper division of genotypes between low and high PPO groups was possible in three out of four environments evaluated.

Table 4.29. Grain darkening visual score for PPO activity.

Genotype #	Environment #				Avg
	5	6	7	8	
1	8	9	5	10	7.3
2	7	8	5	9	6.7
3	10	9	5	7	8.0
4	8	8	5	8	7.0
5	5	5	2	6	4.0
6	5	4	1	4	3.3
7	7	4	2	5	4.3
8	5	3	1	3	3.0
9	8	9	3	6	6.7
10	4	4	1	3	3.0
11	3	2	2	2	2.3
12	4	3	2	3	3.0
13	8	6	2	6	5.3
14	8	6	4	8	6.0
15	8	6	3	9	5.7
16	7	9	3	6	6.3
17	7	5	3	7	5.0
18	7	6	3	6	5.3
19	7	9	3	5	6.3
20	7	9	4	9	6.7
Avg	6.7	6.2	3.0	6.1	5.2

Table 4.30. Solution darkening visual score for PPO activity.

Genotype #	Environment #				Avg
	5	6	7	8	
1	9	8	5	4	6.5
2	7	8	6	4	6.3
3	8	6	8	4	6.5
4	7	7	5	4	5.8
5	6	4	5	3	4.5
6	6	4	4	1	3.8
7	6	3	6	1	4.0
8	4	4	4	1	3.3
9	9	5	7	4	6.3
10	5	4	4	1	3.5
11	4	5	4	3	4.0
12	4	5	5	3	4.3
13	7	6	6	3	5.5
14	6	9	7	5	6.8
15	5	9	5	4	5.8
16	9	6	6	2	5.8
17	8	5	6	2	5.3
18	7	6	6	2	5.3
19	8	5	6	2	5.3
20	8	8	6	4	6.5
Avg	5.6	6.7	5.9	2.9	5.2

Table 4.31. Genotypes #* sorted by grain PPO content (grain darkening visual assessment)

	Environment				
	5	6	7	8	
LOW	**11**	**11**	**6**	**11**	LOW
	10	**8**	**8**	**8**	
	12	**12**	**10**	**10**	
	5	**6**	**5**	**12**	
	6	**7**	**7**	**6**	
	8	**10**	**11**	**7**	
	7	**5**	**12**	19	
	2	17	13	**5**	
	16	13	9	9	
	17	14	15	13	
	18	15	16	16	
	19	18	17	18	
	20	2	18	3	
	1	4	19	17	
	4	1	14	4	
	9	3	20	14	
	13	9	1	2	
	14	16	2	15	
	15	19	3	20	
HIGH	3	20	4	1	HIGH

* In bold, low PPO level group genotypes.

According to these results, it is possible to eliminate wheat experimental lines with high PPO content by a simple visual assessment of the grain darkening in the described conditions. This avoids the use of a spectrophotometer, making the method simple and fast.

Table 4.32. Genotypes #* sorted by grain PPO content (solution visual score)

	Environment #				
	5	6	7	8	
LOW	**1**	1	4	1	LOW
	2	**5**	1	2	
	3	**6**	2	3	
	5	2	3	4	
	6	13	**7**	**8**	
	7	3	**5**	9	
	12	4	**6**	**10**	
	13	**7**	**8**	14	
	17	20	**10**	**5**	
	4	9	16	**6**	
	8	**11**	9	**7**	
	9	**12**	**11**	**11**	
	10	15	14	**12**	
	11	**8**	18	13	
	14	**10**	**12**	15	
	15	18	15	16	
	19	19	17	17	
	16	14	19	18	
	20	16	13	19	
HIGH	18	17	20	20	HIGH

* In bold, low PPO level group genotypes.

4.4. GENETICAL CONSIDERATIONS

Researchers disagree on the details of the PPO system genetics (Bernier 1998), but all of them agree that it is a simple system, with no more than two genes involved. The general concept is in agreement with the observation of this research. As the factor PPO group is much more important than other genetic components, the differences among the alleles present in the samples used can be

attributed to a qualitative difference more than to a quantitative one. The dispersion plot of PPO activity versus change in color (Figure 4.5) shows two clearly separated groups according to PPO activity.

The samples used in that figure can be grouped in populations according to the origin. The same dispersion plot for alkaline doughs, but showing the origin of each sample, is presented in Figure 4.6. The separation in sub-groups is evident, mainly in the high PPO group, with one exception. Both samples from 96F39 were the highest in PPO, and had a very high change in color. Checks are grouped in a second level, and the seven lines from the 96F32 population are together in the lowest PPO activity sub-group among high PPO group samples. In the low

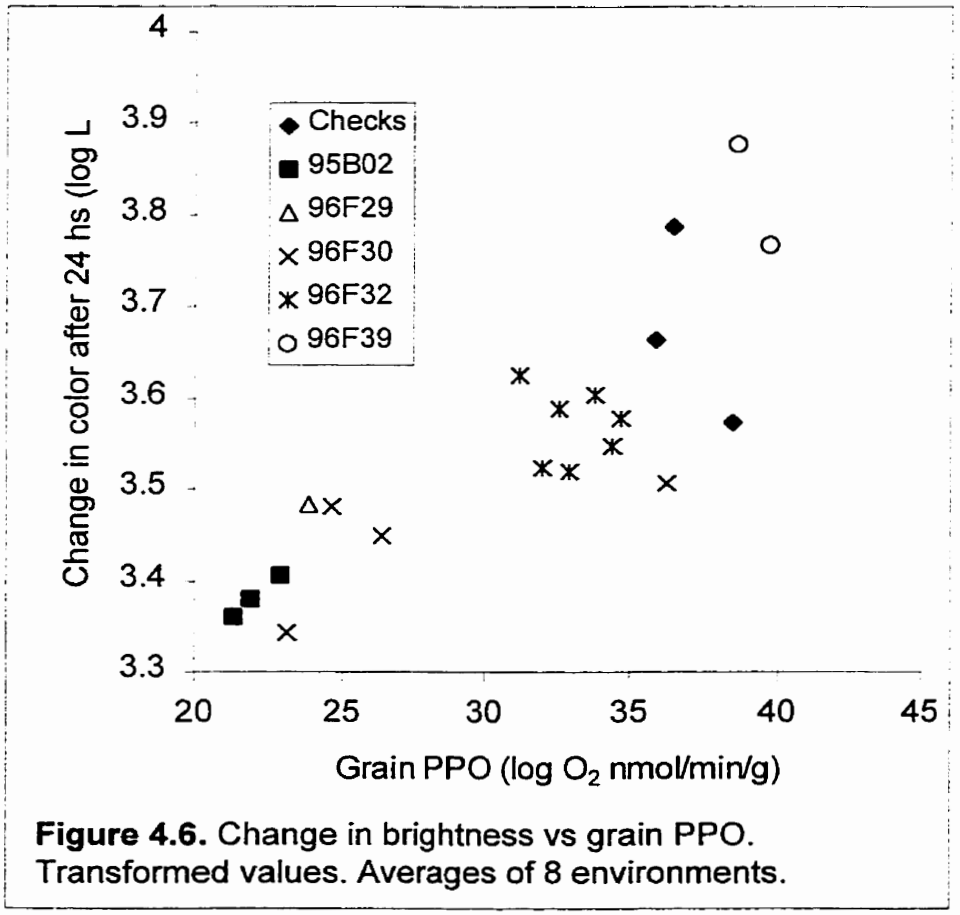


Figure 4.6. Change in brightness vs grain PPO. Transformed values. Averages of 8 environments.

PPO group, there is less separation in sub-groups. Moreover, population 96F30 has three samples in the low PPO group, and one in the high PPO group. It is not the only outstanding observation in this population. Genotype #10, the only one that belonged to the low PPO group and had low stability (Table 4.18), also is part of the 96F30 population. Although the mentioned exception, both PPO groups can be separated into sub-groups according to their genetic origin. Therefore, there are minor genetic components that determine little changes in PPO activity.

Figure 4.6 suggests a strong relationship between PPO activity and change in color of populations. Averaging the results for each of the five populations (Genotype #8 was not included, as is the only one in its population), the influence of the PPO on the change in color is evidenced by a not scattered dispersion plot (Figure 4.7). In Figure 4.7 populations were averaged for different genotypes and in several environments and so the factors that determined the values were primarily genetic. The relationship of PPO activity to discoloration is very strong. Therefore, it is concluded that the genetic components of change in color are closely associated to the PPO system.

In order to obtain a better understanding of the relationship between populations and PPO groups, the PPO activity obtained with whole kernels of the entire set of 64 genotypes grown in 4 environments in 1998 were analyzed (detailed results in Appendix III). The distribution of the means among the four environments is presented in Figure 4.8. The bimodal distribution, separating genotypes in two groups according to their PPO activity, was verified. According to this distribution, genotypes were grouped as "high PPO genotypes" (H) and "low PPO genotypes"

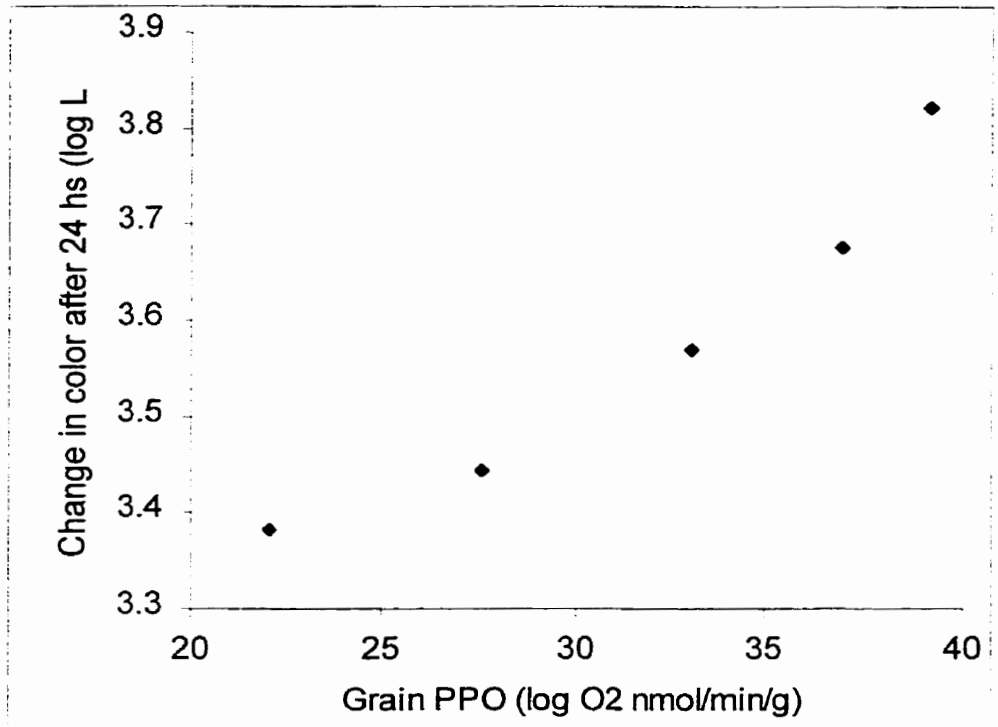


Figure 4.7. Change in color of alkaline noodles vs PPO activity (oxygen consumption method). Population averages. Transformed values.

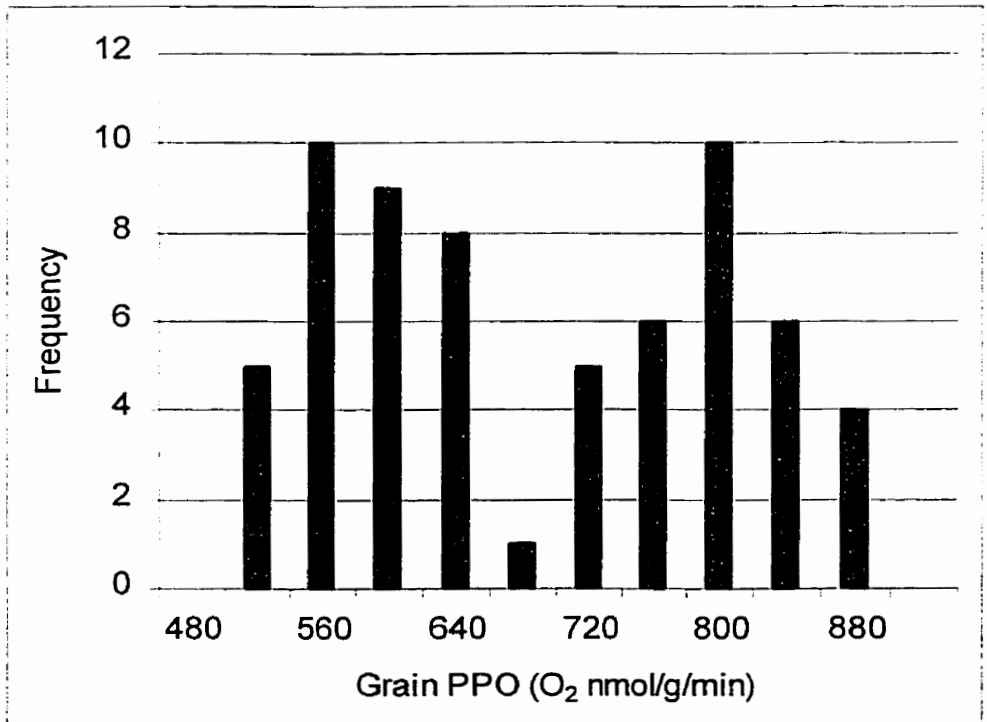


Figure 4.8. Frequency in whole grain PPO of the 64 genotypes. Mean of 4 environments.

(L) (Table 4.33). The genotype L 1497 96F31*B43 was between two groups, so no PPO group was assigned. All the checks were in the high PPO group. Also population 96F39 had all its genotypes in that group. Populations 95B02, 96F29 and 96F34 had all their wheat lines in the low PPO groups. In the other hand, populations 96F30, 96F32 and 96F35 had genotypes in both groups, with no clear predominance.

4.5. KERNEL COLOR

The pericarp color was determined in the 56 double haploids as well as in the 8 agronomic checks; results are presented in Table 4.34. All checks were red. Among the DH lines, most were white (39 genotypes). In some populations, all samples were white (96F31, 96F32, 96F35 and 96F39), while in other populations there were some white and some red genotypes (95B02, 96F29, 96F30 and 96F34).

The influence of kernel color on different parameters is summarized in Table 4.35. Kernel color has a significant influence ($P<0.05$) only on flour brightness (FL) and alkaline noodle dough brightness immediately after preparation (AL0). The effect of these factors was very low when compared with the standard deviation.

Table 4.33. Classification of genotypes according to their PPO group*

	GENOTYPE	GROUP		GENOTYPE	GROUP
W	49 Katepwa (#1)	H	L	1501 96F32 * A1	H
W	148 AC Domain	H	L	1503 96F32 * A5	H
L	1717 SD 3055 (#3)	H	L	1505 96F32 * A8	H
L	1719 Roblin	H	L	1507 96F32 * A13	H
L	1721 AC Splendor	H	L	1509 96F32 * A14	H
L	1723 Katepwa	H	L	1511 96F32 * A15	L
L	1727 AC Majestic	H	L	1513 96F32 * A18 (#13	H
L	1725 AC Domain (#4)	H	L	1515 96F32 * A26	H
L	1459 95B02 * A140 (#5	L	L	1516 96F32 * A27	H
L	1461 95B02 * B116	L	L	1519 96F32 * A42 (#14	H
L	1463 95B02 * B207	L	L	1221 96F32 * A76 (#15	H
L	1465 95B02 * C99	L	L	1523 96F32 * A79 (#16	H
L	1467 95B02 * C120	L	L	1525 96F32 * B4 (#17)	H
L	1469 95B02 * D130	L	L	1527 96F32 * B10	H
L	1471 95B02 * D75 (#6)	L	L	1529 96F32 * B19	H
L	1473 95B02 * D156 (#7	L	L	1531 96F32 * B21 (#18	H
L	1475 96F29 * A27	L	L	1533 96F32 * B30 (#19	H
L	1477 96F29 * A29	L	L	1535 96F32 * B54	L
L	1479 96F29 * A33	L	L	1537 96F32 * B67	L
L	1481 96F29 * B21 (#8)	L	L	1539 96F34 * A11	L
L	1483 96F30 * A2 (#9)	H	L	1541 96F34 * A39	L
L	1485 96F30 * A16 (#10	L	L	1543 96F34 * A52	L
L	1487 96F30 * A32 (#11	L	L	1545 96F34 * A67	L
L	1489 96F30 * A72	H	L	1547 96F34 * B43	L
L	1491 96F30 * B2 (#12)	L	L	1549 96F34 * B43	L
L	1493 96F30 * B37	L	L	1551 96F34 * B85	L
L	1495 96F30 * B101	L	L	1553 96F34 * B86	L
L	1497 96F31 * B43	-	L	1555 96F34 * B88	L
L	1499 96F31 * A67	H	L	1557 96F34 * B106	L
			L	1559 96F35 * B2	L
			L	1561 96F35 * B114	H
			L	1665 96F39 * A34 (#2)	H
			L	1563 96F39 * A160	H
			L	1565 96F39 * A164 (#2	H
			L	1567 96F39 * B62	H

*H = high PPO level, L = low PPO level

Table 4.34. Kernel color

Genotype	Color	Genotype	Color
W 49 Katepwa (#1)	RED	L 1507 96F32 * A13	WHITE
W 148 AC Domain	RED	L 1509 96F32 * A14	WHITE
L 1717 SD 3055 (#3)	RED	L 1511 96F32 * A15	WHITE
L 1719 Roblin	RED	L 1513 96F32 * A18 (#13)	WHITE
L 1721 AC Splendor	RED	L 1515 96F32 * A26	WHITE
L 1723 Katepwa	RED	L 1516 96F32 * A27	WHITE
L 1727 AC Majestic	RED	L 1519 96F32 * A42 (#14)	WHITE
L 1725 AC Domain (#4)	RED	L 1221 96F32 * A76 (#15)	WHITE
L 1459 95B02 * A140 (#5)	RED	L 1523 96F32 * A79 (#16)	WHITE
L 1461 95B02 * B116	WHITE	L 1525 96F32 * B4 (#17)	WHITE
L 1463 95B02 * B207	WHITE	L 1527 96F32 * B10	WHITE
L 1465 95B02 * C99	RED	L 1529 96F32 * B19	WHITE
L 1467 95B02 * C120	RED	L 1531 96F32 * B21 (#18)	WHITE
L 1469 95B02 * D130	RED	L 1533 96F32 * B30 (#19)	WHITE
L 1471 95B02 * D75 (#6)	RED	L 1535 96F32 * B54	WHITE
L 1473 95B02 * D156 (#7)	RED	L 1537 96F32 * B67	WHITE
L 1475 96F29 * A27	RED	L 1539 96F34 * A11	WHITE
L 1477 96F29 * A29	WHITE	L 1541 96F34 * A39	WHITE
L 1479 96F29 * A33	RED	L 1543 96F34 * A52	WHITE
L 1481 96F29 * B21 (#8)	WHITE	L 1545 96F34 * A67	RED
L 1483 96F30 * A2 (#9)	RED	L 1547 96F34 * B43	RED
L 1485 96F30 * A16 (#10)	WHITE	L 1549 96F34 * B43	RED
L 1487 96F30 * A32 (#11)	WHITE	L 1551 96F34 * B85	RED
L 1489 96F30 * A72	RED	L 1553 96F34 * B86	RED
L 1491 96F30 * B2 (#12)	WHITE	L 1555 96F34 * B88	RED
L 1493 96F30 * B37	RED	L 1557 96F34 * B106	WHITE
L 1495 96F30 * B101	WHITE	L 1559 96F35 * B2	WHITE
L 1497 96F31 * B43	WHITE	L 1561 96F35 * B114	WHITE
L 1499 96F31 * A67	WHITE	L 1665 96F39 * A34 (#2)	WHITE
L 1501 96F32 * A1	WHITE	L 1563 96F39 * A160	WHITE
L 1503 96F32 * A5	WHITE	L 1565 96F39 * A164 (#20)	WHITE
L 1505 96F32 * A8	WHITE	L 1567 96F39 * B62	WHITE

Table 4.35. Mean +/- standard deviation at each kernel color

PPO group	RED	WHITE	
FL	93.84 +/- 0.38	94.08 +/- 0.45	*
SQWK	0.815 +/- 0.106	0.8531 +/- 0.098	n.s.
SQGPPO	30.20 +/- 8.42	31.13 +/- 6.54	n.s.
SQFPPO	6.24 +/- 1.38	5.97 +/- 1.22	n.s.
SL0	83.69 +/- 1.35	83.88 +/- 1.46	n.s.
SL24	73.60 +/- 2.52	73.80 +/- 3.05	n.s.
SQSLC	3.17 +/- 0.28	3.16 +/- 0.31	n.s.
AL0	82.12 +/- 1.46	82.64 +/- 1.69	*
AL24	69.49 +/- 4.24	69.70 +/- 4.45	n.s.
SQALC	3.53 +/- .46	3.57 +/- 0.45	n.s.

* Difference significant at $P<0.05$

n.s. Difference not Significant

FL = flour L*, SQWK = grain PPO, whole kernel method, square root transf., SQGPPO = grain PPO, oxygen consumption method, square root transf., SQFPPO = flour PPO, oxygen consumption method, square root transf., SL0 = salted noodles L* value at time 0, SL24 = salted noodles L*value at 24 SQSLC = salted noodles L* value, square root transf., AL0 = alkaline noodles L* value at time 0, AL24 = alkaline noodles L* value at 24h, SQALC = alkaline noodles L* value, square roor transformed.

5. CONCLUSIONS

The objectives set forth in the introduction have been met. These were to evaluate PPO methods, to investigate the effects of genotype and environment on PPO activity, and to evaluate the influence of kernel color and PPO activity on product color. The results obtained from the research done to meet the objectives has provided important information on the role of PPO in noodle dough color and on the significance of genetic factors on its control and on the significance of genetic factors in controlling PPO.

The first objective of this research was to compare methods to determine PPO activity, and determine the most suitable method (or methods) to use in breeding program. As expected, the oxygen consumption method proved to be the most reproducible, with the smallest amount of error associated with the method. Therefore, this method was used as the standard one and other methods were compared to it. Of the nine spectrophotometric method variations tested, the one proposed by Bernier and Howes (1994), which uses whole kernel and tyrosine as substrate, gave the best results: the correlation with the oxygen consumption method was 0.813 (P=0.001). With this method, up to 96 samples can be tested simultaneously. Therefore, it is faster than the extract method. Besides, the results obtained proved that the whole kernel method is as good as the extract one, or better. Tyrosine is safer than catechol (McCaig et al 1999) and using this substrate the assay is non-destructive (Kruger et al 1994, Bernier and Howes 1994, Bernier 1998). The method is suitable to use with a wide spectrum of genotypes, since it was tested with samples from different classes as well as with the double haploid

wheat lines used in this research. Although spectrophotometer reading may enable better separation among genotypes, visual assessment of kernels is still suitable for identification of high PPO experimental lines. The rapid simple design of the test makes it suitable for wheat breeding program screening.

The second objective of this research was to investigate and compare the effects of genetic and environmental factors on PPO activity. The genetic factor was of overwhelming importance in determining PPO activity: 81.8% of the grain PPO variation was explained by genetic factors, while only 12.2% was explained by environmental factors. The genotypes used in this study formed two distinct groups based in the PPO activity, a high PPO group and a low PPO group. When factors affecting PPO were included in an analysis of variance using a nested design, the group effect accounted for 75.2% of the variance. The contribution of other genetic factors (genotype nested in PPO group and kernel color) was minor (4.9%) but significant. The strong influence of genetic factors was confirmed by high Spearman correlation coefficients among environments. Genotypes from the low PPO activity group were very stable across environments, in contrast to the genotypes from the high PPO group whose activities were highly influenced by environment. This research was done using samples from environments which produce a wide range in the quality of wheat, which was evidenced with the milling and flour properties. The influence of environment on flour PPO was more important (component of variance: 39.5%) than on grain PPO. PPO group influence on flour PPO was significant, but smaller (23.3%). The use of double haploid wheat experimental lines permitted grouping these 56 genotypes by populations according to their origin.

Most populations were composed of genotypes from low or high PPO group, but some populations (96F30, 96F32 and 96F35) had genotypes from both groups.

The third objective of the research was to evaluate the influence of kernel color and PPO activity on product color. Environmental effects dominated genetic effects in flour color and noodle color and discoloration. The influence of some genetic effects was minor, but significant.

The importance of the selection of white wheats was confirmed since the influence of kernel color on flour color was significant. Genotype, nested in kernel color and PPO group, also affected flour color significantly, but PPO group did not.

Among the genetic components, the gene or genes that determine PPO grouping explained most of the discoloration variation of both noodle types. The color of an end-product immediately after preparation is less important than the color of the product after a storage period. Color after 24 hours was used to estimate the relative color of the final product after discoloration. For alkaline noodle color after 24 hours, PPO was the most important genetic component with the high PPO group showing greater rate of color change. For salted noodles, other genetic components than kernel color or PPO level (genotype nested in kernel color and PPO group) were the most important factors. Kernel color had no significant effect on noodle discoloration and brightness after 24 hours.

The influence of kernel color on PPO activity was not significant. It was concluded that there is non significant relationship between the pericarp color and PPO activity.

6. RECOMMENDATIONS FOR FURTHER RESEARCH

Further research should be done in order to extend the conclusions of this research to a wider spectrum of genotypes, environments and wheat end-products.

In this research, a clear separation of genotypes into two groups according to their PPO activity was observed. This separation was used to prove the importance of genetic factors in determining PPO activity. The existence of these two groups in other genotypes should be confirmed using a set of samples with different genetic backgrounds. The classification can be confirmed with procedures similar to those used in this research. Other techniques can be added. Electrophoretic analysis with specific staining of the different PPO isoenzymes may be an useful tool to demonstrate the qualitative nature of the group separations.

The environments used in this research had a large influence on the samples. Some environments produced grains with good physical properties, while other produced samples with poor quality. Environments that produced grain with good physical properties tended to produce also low PPO activity. This tendency needs to be confirmed using a large number of varied environments.

Overall, the impact of the PPO genetic determinants on any color parameter was small compared with the environmental effect. It was due to the very wide range of environments used, including some that produced very poor quality grains. The incidence of the environmental effect may be lower among samples with good physical properties. To evaluate this, research should be conducted using a similar number of environments, but all of them should produce wheat samples with good physical properties. Using this design, the environmental effect would be minimized

and therefore, the genetic influence could be studied more effectively. The importance of the suggested study is enhanced when it is considered that, in Canada, grain with good physical properties is segregated and blended just with other samples that also have good quality.

The influence of PPO activity on end-product color and discoloration may extend to other products like bread. After the bread is baked, the enzyme is denatured and therefore, there is no discoloration problem during end-product storage. But during fermentation, the bread preparation conditions are optimum for the activity of PPO and other enzymes. A quantification of the discoloration during that step in bread and other products should be done in order to confirm the effect of the enzyme on color at sale point.

Final color of end-products is affected by discoloration. Among the genetic determinants of discoloration. PPO group was the most important, but it was responsible for a small percentage of the total variation. The influence on discoloration of other factors should be also considered in order to help to understand the browning reactions. These studies should include an investigation of the concentration of PPO substrates (phenols and phenol derivatives) and of peroxidase activity.

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APPENDIX I

Genotype by environment study: detailed data

Table A.1. Flour extraction yield (%)

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	69.9	69.9	72.0	71.5	68.5	69.8	77.4	71.8	71.3	2.73
2	66.5	68.7	71.6	72.8	62.6	73.8	69.0	69.7	69.3	3.61
3	69.7	72.5	72.2	71.9	69.9	75.0	74.5	71.2	72.1	1.90
4	70.8	75.2	74.1	73.8	68.8	72.4	71.3	73.4	72.5	2.07
5	71.5	75.0	76.1	74.4	69.3	74.0	73.7	74.0	73.5	2.13
6	74.5	75.2	75.8	74.4	66.9	73.0	76.9	74.3	73.9	3.07
7	70.1	72.6	76.2	74.4	70.4	73.1	72.4	73.2	72.8	1.99
8	69.1	68.6	71.7	72.0	65.6	72.2	75.4	74.0	71.1	3.17
9	72.0	74.5	75.7	73.1	67.9	73.2	74.4	75.7	73.3	2.53
10	67.6	67.9	73.0	67.5	62.3	69.6	71.1	71.1	68.8	3.29
11	70.2	72.4	74.8	71.9	65.7	72.5	74.0	72.3	71.7	2.77
12	71.2	73.1	73.0	68.7	63.1	70.5	71.0	69.9	70.1	3.19
13	74.0	71.1	74.3	74.5	64.6	75.9	74.7	76.2	73.2	3.81
14	73.0	74.4	76.0	74.9	67.5	75.2	76.5	76.1	74.2	2.92
15	70.4	73.0	74.6	73.1	65.4	74.1	75.8	76.2	72.8	3.51
16	72.6	71.7	75.2	74.1	67.7	74.5	75.9	74.9	73.3	2.67
17	70.0	71.8	73.3	74.3	67.6	71.4	74.0	74.3	72.1	2.39
18	75.5	73.6	75.0	n.r.	71.0	76.2	75.8	74.2	74.5	1.79
19	72.9	74.7	75.0	73.2	69.9	72.4	74.4	74.5	73.4	1.70
20	72.6	74.1	74.2	74.4	66.7	73.5	72.0	72.1	72.4	2.52
Mean	71.2	72.5	74.2	72.9	67.1	73.1	74.0	73.5	72.3	
SD	2.25	2.28	1.52	1.99	2.59	1.86	2.25	2.01		

n.r. : data not recorded

SD = standard deviation

Table AI.2. Flour protein percentage (on a 14% moisture basis)

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	15.1	12.9	13.2	12.7	14.9	14.2	15.4	13.5	14.0	1.06
2	n.r.	12.6	12.7	11.6	14.3	12.9	15.3	12.9	13.2	1.22
3	15.2	14.8	13.1	14.4	14.6	14.5	14.4	13.9	14.4	0.63
4	16.3	15.2	13.3	14.5	15.3	15.2	15.4	15.0	15.0	0.86
5	16.0	14.4	13.1	13.3	14.8	14.4	14.2	14.4	14.3	0.90
6	14.0	13.2	12.4	11.8	13.6	11.9	12.5	11.7	12.6	0.87
7	17.1	13.9	12.4	13.9	16.1	14.4	15.3	14.4	14.7	1.46
8	13.0	12.6	11.9	11.9	13.0	11.2	12.8	11.3	12.2	0.74
9	15.0	13.3	12.5	12.9	14.0	13.8	13.8	13.7	13.6	0.76
10	14.8	14.3	12.0	13.4	14.6	12.7	14.9	12.9	13.7	1.10
11	14.0	12.9	12.6	13.4	13.9	13.6	13.9	13.4	13.5	0.50
12	15.3	14.6	13.9	13.5	15.5	12.8	15.6	13.5	14.3	1.06
13	14.6	13.4	13.5	11.9	14.7	12.5	13.4	10.7	13.1	1.35
14	15.0	12.4	12.1	12.5	14.6	12.5	13.5	11.7	13.0	1.20
15	14.8	11.6	12.9	12.4	14.6	12.5	13.5	10.6	12.9	1.43
16	14.8	13.8	12.4	12.1	14.9	12.4	13.4	11.5	13.2	1.27
17	15.6	14.8	13.3	12.8	15.2	12.9	13.9	13.0	13.9	1.12
18	14.4	14.1	12.6	11.9	14.3	12.8	13.3	12.9	13.3	0.90
19	14.7	11.7	13.3	13.1	14.8	14.6	14.2	13.4	13.7	1.06
20	13.7	12.8	12.5	12.4	13.4	13.1	14.2	12.4	13.1	0.66
Mean	14.9	13.5	12.8	12.8	14.6	13.2	14.1	12.8	13.6	
SD	0.94	1.04	0.54	0.86	0.73	1.04	0.92	1.25		

n.r. : data not recorded

SD = standard deviation

Table AI.3. Flour L*

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	93.7	94.0	93.5	94.2	93.8	93.1	94.0	94.0	93.8	0.34
2	n.r.	93.9	93.2	94.2	93.6	93.5	93.8	94.1	93.7	0.36
3	93.8	94.0	94.6	94.0	93.5	93.1	94.0	93.8	93.9	0.43
4	93.6	93.6	94.2	93.7	93.4	93.2	93.4	93.8	93.6	0.31
5	93.8	94.2	94.5	94.2	93.4	93.7	94.1	94.2	94.0	0.37
6	93.1	94.1	94.2	94.2	93.3	93.9	93.9	94.0	93.8	0.41
7	93.1	93.9	93.6	93.9	93.5	93.6	94.0	94.1	93.7	0.33
8	93.6	93.9	94.1	93.7	93.1	93.5	94.1	94.0	93.8	0.36
9	94.1	93.9	94.0	94.6	93.4	93.6	94.2	94.5	94.0	0.41
10	93.4	93.9	94.3	94.0	93.4	93.1	93.9	94.2	93.8	0.42
11	94.5	94.6	94.6	94.7	94.3	92.5	94.4	94.5	94.3	0.74
12	93.6	93.8	93.8	94.4	93.5	93.8	94.2	94.4	93.9	0.36
13	94.0	94.6	94.7	94.8	94.1	93.8	94.4	94.6	94.4	0.36
14	93.5	94.5	94.3	94.7	93.5	93.9	94.1	94.6	94.1	0.47
15	93.8	94.8	94.9	94.8	93.3	93.9	94.4	94.8	94.3	0.62
16	93.7	94.6	94.8	94.8	93.9	94.0	94.3	94.3	94.3	0.42
17	93.5	94.4	94.4	94.4	93.9	93.8	94.3	94.3	94.1	0.35
18	93.9	94.0	94.4	94.1	93.6	94.0	93.9	94.1	94.0	0.23
19	93.9	94.5	94.4	94.5	94.3	93.5	94.1	94.4	94.2	0.35
20	93.9	94.0	93.7	94.1	94.2	94.2	94.2	94.3	94.1	0.21
Mean	88.8	89.6	89.7	89.8	89.2	89.2	89.7	89.9	94.0	
SD	0.34	0.34	0.45	0.36	0.36	0.41	0.25	0.26		

n.r. : data not recorded

SD = standard deviation

Table AI.4. Flour *a**

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	-0.38	-0.54	-0.41	-0.55	-0.49	-0.37	-0.53	-0.46	-0.46	0.07
2	n.r.	-0.46	-0.35	-0.55	-0.39	-0.50	-0.39	-0.49	-0.44	0.07
3	-0.76	-0.74	-1.05	-0.55	-0.52	-0.42	-0.61	-0.39	-0.63	0.22
4	-0.69	-0.65	-0.78	-0.36	-0.26	-0.19	-0.39	-0.33	-0.45	0.22
5	-0.69	-0.70	-0.89	-0.52	-0.39	-0.40	-0.59	-0.44	-0.58	0.18
6	-0.60	-0.67	-0.75	-0.53	-0.39	-0.43	-0.48	-0.41	-0.53	0.13
7	-0.26	-0.40	-0.35	-0.41	-0.26	-0.30	-0.46	-0.31	-0.34	0.07
8	-0.39	-0.51	-0.48	-0.46	-0.38	-0.36	-0.52	-0.46	-0.44	0.06
9	-0.78	-0.68	-0.68	-0.55	-0.43	-0.39	-0.52	-0.47	-0.56	0.14
10	-0.39	-0.59	-0.63	-0.59	-0.52	-0.54	-0.59	-0.63	-0.56	0.08
11	-0.83	-0.82	-0.84	-0.56	-0.52	-0.27	-0.62	-0.54	-0.62	0.20
12	-0.81	-0.77	-0.84	-0.62	-0.46	-0.68	-0.64	-0.65	-0.68	0.12
13	-0.41	-0.57	-0.62	-0.58	-0.53	-0.39	-0.60	-0.55	-0.53	0.09
14	-0.62	-0.84	-0.83	-0.59	-0.39	-0.37	-0.54	-0.52	-0.59	0.18
15	-0.35	-0.67	-0.72	-0.62	-0.41	-0.38	-0.55	-0.58	-0.53	0.14
16	-0.34	-0.60	-0.72	-0.62	-0.53	-0.41	-0.53	-0.51	-0.53	0.12
17	-0.33	-0.51	-0.62	-0.56	-0.41	-0.44	-0.54	-0.44	-0.48	0.09
18	-0.84	-0.80	-0.95	-0.72	-0.51	-0.53	-0.61	-0.56	-0.69	0.16
19	-0.38	-0.50	-0.54	-0.49	-0.40	-0.29	-0.45	-0.44	-0.43	0.08
20	-0.58	-0.69	-0.58	-0.37	-0.34	-0.27	-0.32	-0.39	-0.44	0.15
Mean	-0.55	-0.63	-0.68	-0.54	-0.42	-0.39	-0.52	-0.48	-0.53	
SD	0.20	0.13	0.20	0.09	0.08	0.11	0.09	0.09		

n.r. : data not recorded

SD = standard deviation

Table AI.5. Flour b*

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	9.15	9.07	9.46	9.29	9.69	9.31	9.51	9.10	9.32	0.22
2	n.r.	8.54	9.35	8.70	9.58	9.25	9.56	8.93	9.13	0.41
3	9.18	8.74	9.64	9.13	9.37	8.56	9.34	8.57	9.06	0.40
4	8.96	8.84	9.01	8.75	8.97	8.46	8.83	8.22	8.75	0.28
5	8.77	8.42	9.09	8.66	8.85	8.44	8.92	8.10	8.65	0.32
6	8.69	8.44	9.16	8.65	9.09	8.38	8.97	8.10	8.68	0.37
7	8.84	7.95	8.60	8.80	8.83	8.38	8.95	8.00	8.54	0.39
8	8.87	9.11	9.26	9.13	9.58	8.55	9.30	8.76	9.07	0.33
9	8.44	8.15	8.59	8.12	8.51	8.03	8.25	8.05	8.26	0.22
10	8.91	8.93	9.35	8.82	9.65	9.28	9.52	9.03	9.18	0.31
11	8.49	8.06	8.40	8.17	8.20	8.69	8.72	8.03	8.34	0.27
12	9.30	8.87	9.53	8.80	8.79	9.19	8.93	8.61	9.00	0.31
13	8.35	8.06	8.39	7.97	8.72	8.01	8.66	7.90	8.25	0.32
14	8.45	8.19	8.50	8.20	8.58	7.81	8.56	7.81	8.26	0.32
15	8.08	8.08	8.46	8.27	8.82	7.78	8.61	7.90	8.25	0.36
16	8.31	8.18	8.38	8.07	8.62	7.63	8.68	7.89	8.22	0.36
17	8.16	8.28	8.49	8.47	8.74	7.95	8.41	7.92	8.30	0.28
18	9.00	8.67	9.06	8.92	9.61	8.76	9.45	8.97	9.05	0.32
19	7.94	7.77	8.00	7.82	7.94	7.71	8.01	7.64	7.85	0.14
20	8.19	8.00	8.47	7.95	7.94	7.70	7.76	7.77	7.97	0.26
Mean	8.63	8.41	8.86	8.53	8.90	8.39	8.84	8.26	8.61	
SD	0.40	0.41	0.48	0.44	0.54	0.56	0.51	0.47		

n.r. : data not recorded

SD = standard deviation

Table AI.6. Grain PPO activity - whole kernel method (AU)

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	0.81	0.66	0.62	0.83	0.86	1.05	0.64	0.72	0.77	0.15
2	0.89	0.72	0.64	0.95	1.08	1.07	0.59	0.76	0.84	0.19
3	0.90	0.87	0.60	0.97	0.80	0.90	0.66	0.60	0.79	0.15
4	1.03	0.76	0.62	0.87	0.66	0.94	0.63	0.67	0.77	0.16
5	0.46	0.53	0.45	0.56	0.41	0.73	0.43	0.45	0.50	0.11
6	0.54	0.51	0.41	0.66	0.62	0.63	0.45	0.53	0.54	0.09
7	0.56	0.52	0.52	0.70	0.54	0.68	0.52	0.45	0.56	0.08
8	0.56	0.46	0.49	0.56	0.50	0.61	0.50	0.48	0.52	0.05
9	1.18	0.80	0.68	0.87	0.65	0.83	0.72	0.63	0.79	0.18
10	0.76	0.51	0.53	0.75	0.58	0.73	0.51	0.57	0.62	0.11
11	0.57	0.49	0.43	0.61	0.44	0.72	0.43	0.57	0.53	0.11
12	0.69	0.50	0.48	0.63	0.53	0.66	0.63	0.67	0.60	0.08
13	1.01	0.81	0.61	0.96	0.70	0.90	0.62	0.87	0.81	0.15
14	1.08	0.79	0.67	0.83	1.02	0.96	0.72	0.91	0.87	0.14
15	0.90	0.82	0.79	0.78	0.72	0.80	0.63	1.01	0.81	0.11
16	0.96	0.75	0.76	0.89	0.81	1.26	0.73	0.91	0.88	0.17
17	0.85	0.59	0.62	0.93	0.84	0.91	0.57	0.68	0.75	0.15
18	0.81	0.78	0.66	0.94	0.91	0.80	0.79	0.85	0.82	0.09
19	0.77	0.68	0.64	0.75	0.78	0.86	0.73	0.70	0.74	0.07
20	0.86	0.74	0.70	0.86	0.82	0.87	0.84	0.76	0.80	0.06
Mean	0.81	0.67	0.60	0.79	0.71	0.84	0.62	0.69	0.72	
SD	0.20	0.14	0.11	0.14	0.18	0.16	0.12	0.16		

SD = standard deviation

Table AI.7. Grain PPO activity - oxygen consumption method (O_2 nmol/min/g)

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	1401	1084	953	957	1754	1925	951	1612	1330	397
2	1584	1347	1145	1126	1892	1812	1268	1755	1491	308
3	1533	1483	949	1083	2214	1701	1321	1556	1480	389
4	1162	1106	1080	1139	1428	1883	963	1533	1287	306
5	403	472	383	280	635	559	445	664	480	131
6	514	557	466	352	576	695	366	670	525	127
7	368	334	369	335	557	653	356	661	454	144
8	424	414	438	442	834	642	615	747	569	164
9	1380	1091	814	930	1984	1766	988	1567	1315	427
10	462	408	295	496	1217	755	498	722	607	290
11	453	509	433	384	728	510	581	681	535	121
12	577	628	571	620	873	868	587	835	695	137
13	901	758	652	810	1767	1102	966	1256	1027	356
14	915	959	898	946	1665	1280	812	1205	1085	283
15	1081	686	756	793	1398	1262	831	982	974	256
16	1178	868	736	896	1752	1289	811	972	1063	335
17	1139	1057	828	1030	2015	1335	927	1295	1203	370
18	1182	1226	929	942	1642	1210	729	1290	1143	278
19	1165	858	861	1033	1634	1661	971	1296	1185	321
20	1654	1444	1355	1189	1957	1878	1331	1792	1575	284
Mean	974	864	745	789	1426	1239	816	1155	1001	
SD	435	359	290	305	540	497	296	396		

SD = standard deviation

Table AI.8. Flour PPO activity - oxygen consumption method (O_2 nmol/min/g)

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	36	27	41	29	58	62	21	43	40	14.7
2	34	30	50	32	68	38	56	39	43	13.3
3	54	53	33	38	78	74	59	53	55	15.8
4	38	44	44	36	72	67	53	49	50	13.0
5	29	20	23	18	59	39	23	26	30	13.5
6	44	24	35	21	66	41	32	30	37	14.3
7	21	14	31	16	45	34	15	28	25	10.9
8	21	14	31	22	45	57	27	34	31	13.8
9	30	40	57	24	92	65	49	32	49	22.6
10	20	17	29	20	56	35	31	30	30	12.5
11	27	27	31	18	37	41	15	25	28	9.0
12	33	29	58	28	59	36	18	21	35	15.3
13	23	26	28	26	49	55	15	33	32	13.4
14	36	38	49	25	87	49	27	33	43	19.9
15	25	22	20	20	101	60	27	37	39	28.3
16	35	31	26	21	51	49	27	50	36	12.1
17	32	27	26	34	63	55	25	46	39	14.2
18	31	38	37	27	68	59	39	51	44	14.1
19	21	33	16	28	31	40	50	47	33	12.1
20	53	39	65	33	54	51	47	53	49	9.8
Mean	32	30	36	26	62	50	33	38	38	
SD	9.9	10.1	13.4	6.5	17.9	11.9	14.6	10.2		

SD = standard deviation

Table AI.9. Brightness (L*) of salted noodle doughs immediately after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	83.5	83.0	84.2	83.8	82.3	82.9	85.8	84.3	83.7	1.07
2	80.9	82.7	82.4	83.7	80.3	83.3	82.2	83.5	82.4	1.23
3	81.7	82.0	84.6	84.4	82.0	83.4	84.8	84.0	83.3	1.28
4	82.9	82.6	84.8	85.3	81.7	83.4	84.6	84.7	83.7	1.29
5	82.0	83.1	84.6	84.3	82.4	84.8	85.0	85.0	83.9	1.22
6	78.9	82.8	82.8	84.2	81.4	82.4	83.9	84.0	82.5	1.75
7	83.0	83.4	83.8	84.7	83.0	84.5	86.0	85.6	84.2	1.14
8	82.0	82.9	86.1	84.0	81.5	82.0	84.9	83.5	83.4	1.60
9	83.4	83.1	84.2	85.7	82.6	84.5	85.5	85.6	84.3	1.21
10	83.9	85.3	83.5	84.8	82.2	83.5	85.7	85.6	84.3	1.24
11	82.4	83.4	84.4	85.6	82.9	82.4	85.6	84.8	83.9	1.33
12	82.0	83.4	84.2	86.4	82.3	84.4	86.6	86.1	84.4	1.82
13	83.8	83.9	85.5	84.4	82.9	82.3	86.9	84.7	84.3	1.45
14	81.5	83.6	83.0	85.0	82.5	82.2	86.2	84.1	83.5	1.54
15	83.9	83.6	85.9	85.1	81.1	82.5	86.1	85.0	84.2	1.70
16	83.0	83.2	86.1	84.8	82.7	83.2	85.8	83.5	84.0	1.35
17	82.7	84.5	85.8	85.7	82.9	83.1	86.3	84.0	84.4	1.43
18	82.8	83.2	83.9	83.1	83.2	84.0	85.7	84.5	83.8	0.96
19	83.7	83.2	86.2	85.4	84.4	84.8	85.0	86.2	84.9	1.08
20	81.5	83.1	82.1	83.6	82.0	83.4	84.4	84.0	83.0	1.05
Mean	78.4	79.2	80.4	80.7	78.5	79.5	81.5	80.8	83.9	
SD	1.23	0.69	1.25	0.83	0.88	0.89	1.07	0.85		

SD = standard deviation

Table AI.10. Brightness (L*) of alkaline noodle doughs at 0 time

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	82.7	82.2	83.0	82.9	80.7	79.8	83.7	83.1	82.3	1.3
2	79.1	82.6	82.2	84.2	80.3	82.0	81.6	83.1	81.9	1.6
3	81.2	81.2	84.4	84.3	79.8	80.5	82.2	82.5	82.0	1.7
4	82.4	82.6	84.0	83.5	80.3	79.9	82.0	81.6	82.0	1.4
5	81.7	82.5	84.1	84.4	79.6	80.4	82.6	82.1	82.2	1.6
6	78.4	82.2	82.6	83.0	78.9	81.4	81.9	82.4	81.3	1.7
7	82.6	81.9	82.3	84.1	80.1	81.7	82.8	83.0	82.3	1.2
8	81.3	83.0	86.0	83.6	80.9	81.6	83.7	83.2	82.9	1.7
9	82.9	82.7	83.5	84.6	80.2	81.5	82.8	83.7	82.7	1.4
10	83.4	83.9	83.6	84.3	81.2	81.7	83.5	83.7	83.2	1.1
11	82.4	82.8	84.1	84.5	80.5	77.4	83.4	82.1	82.1	2.3
12	81.8	83.0	83.9	85.6	79.5	82.5	84.9	84.0	83.1	1.9
13	82.8	81.5	84.8	84.4	80.8	79.7	86.1	83.6	83.0	2.2
14	80.5	82.8	82.2	84.0	79.1	80.4	83.6	83.2	82.0	1.8
15	83.2	84.4	85.6	84.4	78.7	80.7	83.5	84.5	83.1	2.3
16	82.7	82.5	85.7	84.0	79.9	81.4	83.0	82.9	82.8	1.7
17	82.3	83.3	85.5	84.1	80.5	81.0	82.9	82.3	82.7	1.6
18	82.3	82.9	83.2	82.8	81.0	81.9	83.6	82.9	82.6	0.8
19	83.0	82.7	85.6	84.3	81.3	79.8	82.0	83.4	82.7	1.8
20	81.1	82.4	81.2	83.8	81.3	81.1	82.3	83.5	82.1	1.1
Mean	77.9	78.6	80.0	80.0	76.4	77.1	79.3	79.3	82.5	
SD	1.3	0.7	1.4	0.7	0.8	1.1	1.1	0.7		

SD = standard deviation

Table AI.11. Brightness (L*) of salted noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	73.5	72.3	72.2	73.7	70.9	71.6	75.4	72.5	72.8	1.4
2	67.6	72.0	69.7	74.3	65.2	72.5	69.5	73.8	70.6	3.2
3	71.9	72.8	77.1	75.1	68.2	73.4	74.4	72.5	73.2	2.6
4	74.3	74.9	76.6	74.7	68.9	70.8	72.5	71.9	73.1	2.5
5	72.8	74.5	76.2	75.1	68.5	75.9	76.4	74.7	74.3	2.6
6	69.6	75.0	74.6	75.4	66.6	71.6	73.5	75.2	72.7	3.2
7	75.5	75.3	74.0	75.5	71.7	76.4	77.3	76.6	75.3	1.7
8	75.3	72.9	78.7	74.5	69.5	71.1	76.3	74.9	74.2	2.9
9	75.1	73.8	73.6	76.5	67.1	75.1	74.6	75.6	73.9	2.9
10	74.0	76.6	72.8	75.1	68.0	72.4	75.8	77.1	74.0	3.0
11	75.6	75.6	76.5	76.5	68.7	68.5	77.6	74.4	74.2	3.6
12	74.3	74.4	74.3	76.6	67.7	73.5	78.0	76.2	74.4	3.1
13	74.8	74.1	77.5	75.7	70.4	70.2	79.3	74.7	74.6	3.1
14	73.0	76.1	74.2	75.6	66.4	72.3	77.5	74.0	73.6	3.4
15	72.6	74.2	78.6	76.8	65.5	71.1	77.7	75.5	74.0	4.3
16	72.2	74.1	79.1	76.4	71.3	71.7	77.0	71.8	74.2	2.9
17	73.8	74.2	78.2	76.7	69.6	71.9	77.7	72.6	74.3	3.0
18	73.9	73.3	75.6	75.2	69.1	73.5	76.7	73.3	73.8	2.3
19	75.6	72.9	77.6	75.6	74.3	75.2	75.0	76.5	75.3	1.4
20	72.5	74.7	71.1	72.2	68.4	72.5	73.1	73.1	72.2	1.8
Mean	73.4	74.2	75.4	75.4	68.8	72.6	75.8	74.3	73.7	
SD	2.0	1.2	2.7	1.1	2.2	2.0	2.3	1.6		

SD = standard deviation

Table AI.12. Brightness (L*) of alkaline noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	71.3	70.9	69.7	71.9	62.8	62.5	68.9	64.1	67.8	4.0
2	65.6	70.2	68.3	71.7	60.7	65.6	64.3	67.8	66.8	3.5
3	71.2	71.3	76.3	72.9	63.3	65.6	67.0	64.7	69.0	4.6
4	73.2	73.7	75.7	70.5	61.8	62.0	65.8	63.5	68.3	5.7
5	71.8	73.7	75.5	74.4	62.6	68.9	69.7	68.3	70.6	4.2
6	68.0	73.5	73.6	73.7	61.7	68.1	66.6	71.3	69.6	4.3
7	73.7	72.1	71.3	73.9	64.9	70.4	71.1	70.1	70.9	2.8
8	72.8	71.5	77.7	72.6	64.2	64.3	70.7	71.0	70.6	4.5
9	74.1	73.0	72.7	73.9	61.1	69.9	67.6	69.5	70.2	4.4
10	74.3	73.8	71.5	74.2	63.3	68.9	69.7	71.8	70.9	3.7
11	74.7	74.6	74.9	74.5	63.8	63.0	72.8	67.8	70.8	5.1
12	73.5	73.0	73.1	74.9	61.8	69.1	72.7	70.7	71.1	4.2
13	73.1	70.8	75.3	73.8	63.1	63.4	73.5	70.1	70.4	4.7
14	71.4	74.7	73.0	73.0	59.4	63.7	70.8	68.7	69.3	5.3
15	71.4	71.5	77.2	74.7	58.8	62.3	71.4	70.6	69.7	6.1
16	71.3	71.1	77.2	74.3	64.2	63.0	70.4	65.8	69.7	5.0
17	72.1	73.5	75.9	73.0	63.9	62.5	71.2	65.9	69.7	5.0
18	72.7	72.1	74.1	72.9	62.3	64.5	69.5	66.5	69.3	4.4
19	73.2	70.3	75.4	72.7	67.5	66.0	66.8	68.6	70.1	3.4
20	71.1	72.7	70.1	69.2	63.4	63.5	65.6	66.0	67.7	3.6
Mean	72.0	72.4	73.9	73.1	62.7	65.4	69.3	68.1	69.6	
SD	2.1	1.4	2.7	1.5	1.9	2.8	2.6	2.5		

SD = standard deviation

Table AI.13. Change in brightness (L*) of salted noodle doughs after 24 hours

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	9.9	10.7	11.9	10.1	11.4	11.3	10.4	11.9	11.0	0.8
2	13.2	10.7	12.7	9.4	15.1	10.8	12.7	9.7	11.8	2.0
3	9.7	9.2	7.4	9.3	13.8	10.0	10.4	11.5	10.2	1.9
4	8.6	7.6	8.2	10.7	12.8	12.6	12.1	12.8	10.7	2.2
5	9.2	8.5	8.4	9.2	13.9	8.9	8.6	10.3	9.6	1.8
6	9.3	7.8	8.2	8.8	14.8	10.8	10.4	8.8	9.9	2.2
7	7.6	8.1	9.8	9.2	11.3	8.1	8.7	9.0	9.0	1.2
8	6.6	10.1	7.4	9.5	12.0	10.9	8.6	8.6	9.2	1.8
9	8.4	9.4	10.6	9.2	15.5	9.4	10.9	10.0	10.4	2.2
10	9.9	8.7	10.7	9.6	14.2	11.2	9.9	8.5	10.3	1.8
11	6.8	7.7	8.0	9.0	14.3	13.9	8.1	10.4	9.8	2.9
12	7.7	9.0	9.9	9.8	14.6	11.0	8.6	9.9	10.1	2.1
13	9.1	9.9	8.1	8.7	12.5	12.1	7.6	9.9	9.7	1.8
14	8.4	7.5	8.8	9.4	16.2	9.9	8.7	10.1	9.9	2.7
15	11.3	9.4	7.2	8.2	15.7	11.4	8.4	9.6	10.2	2.7
16	10.8	9.0	7.0	8.4	11.3	11.4	8.8	11.7	9.8	1.7
17	8.9	10.3	7.6	9.0	13.4	11.3	8.7	11.5	10.1	1.9
18	8.9	9.9	8.3	8.0	14.0	10.5	9.0	11.2	10.0	2.0
19	8.1	10.3	8.6	9.7	10.2	9.6	10.0	9.8	9.5	0.8
20	9.0	8.4	11.0	11.4	13.6	10.9	11.4	10.8	10.8	1.6
Mean	9.1	9.1	9.0	9.3	13.5	10.8	9.6	10.3	10.1	
SD	1.5	1.0	1.6	0.8	1.7	1.3	1.4	1.2		

SD = standard deviation

Table AI.14. Change in brightness (L*) of alkaline noodle doughs after 24 hours

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	11.4	11.3	13.3	11.0	17.9	17.3	14.8	19.0	14.5	3.2
2	13.5	12.4	14.0	12.5	19.5	16.4	17.4	15.3	15.1	2.5
3	10.0	10.0	8.1	11.4	16.6	14.8	15.3	17.8	13.0	3.6
4	9.3	8.9	8.3	13.0	18.6	18.0	16.2	18.0	13.8	4.5
5	9.9	8.9	8.6	10.0	17.0	11.5	12.9	13.8	11.6	2.9
6	10.4	8.6	9.0	9.3	17.2	13.3	15.3	11.2	11.8	3.2
7	8.9	9.8	11.0	10.2	15.2	11.2	11.7	12.9	11.4	2.0
8	8.5	11.5	8.4	11.1	16.7	17.3	13.0	12.2	12.3	3.3
9	8.7	9.7	10.8	10.7	19.1	11.6	15.1	14.3	12.5	3.4
10	9.1	10.1	12.2	10.1	18.0	12.8	13.8	12.0	12.2	2.8
11	7.7	8.1	9.2	10.0	16.7	14.4	10.7	14.3	11.4	3.3
12	8.3	10.0	10.8	10.7	17.7	13.3	12.2	13.2	12.0	2.8
13	9.7	10.7	9.5	10.6	17.7	16.3	12.6	13.6	12.6	3.1
14	9.1	8.1	9.2	11.0	19.7	16.8	12.8	14.5	12.7	4.1
15	11.8	12.8	8.5	9.7	19.9	18.4	12.1	13.9	13.4	4.0
16	11.3	11.4	8.5	9.7	15.7	18.4	12.6	17.1	13.1	3.6
17	10.2	9.8	9.6	11.1	16.6	18.5	11.8	16.4	13.0	3.6
18	9.6	10.8	9.1	9.9	18.7	17.4	14.1	16.4	13.2	3.9
19	9.8	12.3	10.1	11.6	13.8	13.8	15.2	14.7	12.7	2.0
20	10.1	9.7	11.1	14.6	18.0	17.6	16.7	17.5	14.4	3.6
Mean	9.9	10.2	10.0	10.9	17.5	15.5	13.8	14.9	12.8	
SD	1.4	1.4	1.7	1.3	1.6	2.5	1.8	2.2		

SD = standard deviation

Table AI.15. a* of salted noodle doughs immediately after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	0.81	0.48	0.73	0.36	1.08	1.23	0.17	0.71	0.69	0.36
2	1.57	0.69	1.27	0.16	1.56	0.69	1.11	0.58	0.95	0.50
3	1.28	1.23	-0.33	0.28	1.12	1.10	0.25	0.72	0.70	0.58
4	1.02	1.30	0.40	0.55	1.70	1.51	0.83	1.15	1.06	0.45
5	1.28	1.11	0.18	0.31	1.38	0.94	0.12	0.83	0.77	0.50
6	1.89	1.14	0.90	0.22	1.55	1.20	0.52	0.59	1.00	0.56
7	1.10	0.80	0.89	0.53	1.46	1.23	0.24	0.84	0.88	0.39
8	1.12	0.60	-0.22	0.45	1.50	1.45	0.26	0.61	0.72	0.60
9	0.65	1.05	0.83	0.12	1.41	0.78	0.40	0.47	0.71	0.40
10	0.85	0.39	0.58	0.39	1.06	1.22	0.25	0.31	0.63	0.37
11	0.85	0.63	0.26	0.05	0.95	1.79	-0.15	0.63	0.62	0.61
12	1.11	0.91	0.48	-0.15	1.31	0.98	-0.12	0.20	0.59	0.56
13	0.74	0.35	-0.07	-0.02	0.81	1.39	-0.28	0.28	0.40	0.55
14	1.60	0.45	0.68	0.01	1.31	1.40	0.04	0.63	0.76	0.61
15	0.80	-0.04	-0.50	-0.13	1.66	1.30	-0.01	0.07	0.39	0.76
16	0.99	0.37	-0.63	-0.23	0.80	1.13	0.07	0.65	0.39	0.61
17	1.10	0.45	-0.20	0.08	1.22	1.12	-0.01	0.85	0.58	0.57
18	0.77	0.71	0.13	-0.14	0.79	0.54	0.07	0.43	0.41	0.35
19	0.84	0.45	-0.12	0.24	0.85	1.01	0.45	0.38	0.51	0.37
20	1.60	0.93	1.44	0.71	1.33	1.06	0.76	0.73	1.07	0.35
Mean	1.10	0.70	0.33	0.19	1.24	1.15	0.25	0.58	0.69	
SD	0.34	0.35	0.58	0.26	0.30	0.29	0.35	0.26		

SD = standard deviation

Table AI.16. a* of alkaline noodle doughs immediately after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	0.77	0.55	0.83	0.40	0.19	0.41	-0.36	-0.37	0.30	0.46
2	1.73	0.55	1.02	0.04	0.41	-0.24	-0.03	-0.72	0.35	0.77
3	1.25	1.33	-0.24	0.20	0.27	0.35	-0.42	-0.27	0.31	0.66
4	1.05	1.25	0.60	0.84	0.51	0.57	0.08	0.31	0.65	0.38
5	1.22	1.16	0.11	0.24	0.27	0.06	-0.74	-0.11	0.28	0.65
6	1.88	1.24	0.86	0.24	0.36	-0.13	-0.34	-0.61	0.44	0.84
7	0.92	0.72	1.01	0.49	0.58	0.07	-0.32	-0.14	0.42	0.49
8	1.00	0.52	-0.31	0.40	0.19	-0.07	-0.61	-0.74	0.05	0.59
9	0.65	1.12	0.83	0.13	0.14	-0.06	-0.33	-0.65	0.23	0.60
10	0.67	0.34	0.38	0.22	-0.08	-0.14	-0.43	-0.78	0.02	0.47
11	0.74	0.72	0.26	0.10	0.26	1.52	-0.71	-0.29	0.32	0.68
12	1.08	0.91	0.51	-0.10	0.39	-0.73	-0.76	-0.87	0.05	0.78
13	0.66	0.42	-0.09	-0.11	0.05	0.30	-0.92	-1.13	-0.10	0.63
14	1.51	0.48	0.67	-0.02	0.43	0.35	-0.57	-0.72	0.27	0.71
15	0.67	0.08	-0.67	-0.25	0.35	0.27	-0.70	-1.35	-0.20	0.67
16	0.73	0.32	-0.72	-0.25	0.18	0.19	-0.61	-0.73	-0.11	0.54
17	0.83	0.52	-0.24	0.10	0.13	0.12	-0.60	-0.45	0.05	0.48
18	0.72	0.72	0.22	-0.22	-0.15	-0.41	-0.73	-0.80	-0.08	0.59
19	0.70	0.44	-0.18	0.23	0.07	0.86	-0.31	-0.56	0.16	0.49
20	1.55	1.00	1.57	0.60	0.20	0.38	0.07	-0.52	0.60	0.73
Mean	1.01	0.72	0.32	0.16	0.24	0.18	-0.46	-0.57	0.20	
SD	0.39	0.36	0.61	0.29	0.18	0.47	0.28	0.37		

SD = standard deviation

Table AI.17. a* of salted noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	2.56	3.10	2.90	2.40	3.88	3.54	1.96	3.12	2.93	0.62
2	4.55	3.05	3.95	1.96	4.82	3.15	4.15	2.90	3.57	0.96
3	3.58	2.90	0.89	1.77	4.04	3.00	1.76	2.91	2.60	1.05
4	3.32	3.38	1.64	2.67	4.67	3.87	3.03	3.56	3.26	0.89
5	3.71	3.57	1.92	2.32	4.67	3.06	1.48	3.30	3.00	1.05
6	4.35	3.27	2.82	2.34	5.30	4.21	2.38	2.98	3.45	1.06
7	2.65	2.88	2.93	2.52	4.35	3.20	1.47	2.81	2.85	0.80
8	3.00	3.15	1.21	2.77	4.99	4.56	2.11	3.49	3.16	1.22
9	2.71	3.07	2.33	1.54	4.51	2.69	2.04	2.54	2.68	0.88
10	2.92	2.64	2.95	2.88	4.47	3.86	2.37	2.46	3.07	0.73
11	3.11	2.74	1.78	1.98	4.00	4.38	1.29	3.06	2.79	1.08
12	3.23	3.09	2.23	1.82	4.77	3.88	1.50	2.94	2.93	1.08
13	2.69	2.79	1.09	1.51	3.66	4.39	0.58	2.86	2.44	1.30
14	3.55	2.18	2.41	1.58	4.68	3.75	1.16	3.24	2.81	1.19
15	2.87	2.11	0.65	1.45	5.02	3.95	1.32	2.45	2.47	1.45
16	3.22	2.52	0.52	1.49	3.51	3.70	1.53	3.46	2.49	1.18
17	2.77	2.81	1.18	1.86	4.24	3.55	1.16	3.68	2.65	1.16
18	3.20	2.68	1.78	1.83	3.98	3.09	1.35	3.20	2.64	0.90
19	2.44	2.97	1.32	1.79	2.85	2.38	2.09	2.17	2.25	0.54
20	3.51	3.18	3.26	3.04	3.98	3.05	2.52	3.16	3.21	0.42
Mean	3.19	2.90	1.99	2.07	4.32	3.56	1.86	3.01	2.86	
SD	0.56	0.37	0.95	0.50	0.59	0.60	0.79	0.40		

SD = standard deviation

Table AI.18. a* of salted noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	2.85	2.97	2.88	2.41	3.43	3.00	1.92	2.25	2.71	0.48
2	4.48	2.95	3.29	2.07	4.71	2.34	2.91	1.67	3.05	1.09
3	3.75	3.28	1.18	1.92	3.58	3.13	2.01	2.07	2.61	0.94
4	3.55	3.48	2.02	3.09	4.09	3.49	2.79	2.82	3.17	0.63
5	3.88	3.72	2.07	2.28	4.09	2.96	1.89	2.87	2.97	0.85
6	4.66	3.72	3.02	2.12	4.58	2.92	2.52	1.54	3.13	1.12
7	2.83	3.09	3.32	2.55	3.83	3.08	2.01	2.74	2.93	0.54
8	3.33	3.06	1.90	2.52	4.15	3.36	1.99	2.10	2.80	0.80
9	2.88	3.43	2.64	1.69	3.94	2.65	2.05	1.12	2.55	0.92
10	2.52	3.28	2.38	2.72	4.35	3.25	2.00	2.09	2.82	0.78
11	3.48	3.02	2.34	2.20	4.08	4.14	1.29	2.93	2.93	0.98
12	3.49	3.34	2.43	1.73	4.50	3.18	1.46	1.67	2.72	1.08
13	2.63	3.04	1.77	1.56	3.29	3.68	0.87	1.75	2.32	0.98
14	3.64	2.45	2.89	1.79	4.48	3.46	2.21	2.12	2.88	0.92
15	2.50	2.49	1.08	1.52	4.45	3.40	1.51	1.39	2.29	1.16
16	2.82	2.92	0.89	1.66	3.14	3.45	1.79	2.54	2.40	0.87
17	2.87	2.81	1.90	2.09	3.43	2.85	1.39	2.25	2.45	0.66
18	3.45	2.95	2.18	1.88	3.82	2.74	1.97	2.35	2.66	0.70
19	2.54	2.95	1.48	1.94	2.68	2.95	2.40	1.95	2.36	0.53
20	3.73	3.55	3.44	2.75	3.72	3.01	2.53	1.35	3.01	0.81
Mean	3.29	3.12	2.25	2.12	3.91	3.15	1.97	2.08	2.74	
SD	0.63	0.35	0.75	0.44	0.54	0.40	0.51	0.53		

SD = standard deviation

Table AI.19. Change in a* of salted noodle doughs after 24 hours

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	-1.8	-2.6	-2.2	-2.0	-2.8	-2.3	-1.8	-2.4	-2.2	0.37
2	-3.0	-2.4	-2.7	-1.8	-3.3	-2.5	-3.0	-2.3	-2.6	0.47
3	-2.3	-1.7	-1.2	-1.5	-2.9	-1.9	-1.5	-2.2	-1.9	0.55
4	-2.3	-2.1	-1.2	-2.1	-3.0	-2.4	-2.2	-2.4	-2.2	0.48
5	-2.4	-2.5	-1.7	-2.0	-3.3	-2.1	-1.4	-2.5	-2.2	0.58
6	-2.5	-2.1	-1.9	-2.1	-3.8	-3.0	-1.9	-2.4	-2.5	0.64
7	-1.6	-2.1	-2.0	-2.0	-2.9	-2.0	-1.2	-2.0	-2.0	0.48
8	-1.9	-2.5	-1.4	-2.3	-3.5	-3.1	-1.9	-2.9	-2.4	0.70
9	-2.1	-2.0	-1.5	-1.4	-3.1	-1.9	-1.6	-2.1	-2.0	0.53
10	-2.1	-2.2	-2.4	-2.5	-3.4	-2.6	-2.1	-2.2	-2.4	0.44
11	-2.3	-2.1	-1.5	-1.9	-3.1	-2.6	-1.4	-2.4	-2.2	0.54
12	-2.1	-2.2	-1.7	-2.0	-3.5	-2.9	-1.6	-2.7	-2.3	0.63
13	-2.0	-2.4	-1.2	-1.5	-2.9	-3.0	-0.9	-2.6	-2.0	0.80
14	-1.9	-1.7	-1.7	-1.6	-3.4	-2.4	-1.1	-2.6	-2.1	0.70
15	-2.1	-2.1	-1.2	-1.6	-3.4	-2.7	-1.3	-2.4	-2.1	0.73
16	-2.2	-2.2	-1.1	-1.7	-2.7	-2.6	-1.5	-2.8	-2.1	0.61
17	-1.7	-2.4	-1.4	-1.8	-3.0	-2.4	-1.2	-2.8	-2.1	0.68
18	-2.4	-2.0	-1.7	-2.0	-3.2	-2.5	-1.3	-2.8	-2.2	0.62
19	-1.6	-2.5	-1.4	-1.5	-2.0	-1.4	-1.6	-1.8	-1.7	0.37
20	-1.9	-2.3	-1.8	-2.3	-2.7	-2.0	-1.8	-2.4	-2.1	0.32
Mean	-2.1	-2.2	-1.7	-1.9	-3.1	-2.4	-1.6	-2.4	-2.2	
SD	0.34	0.25	0.42	0.31	0.39	0.44	0.48	0.30		

SD = standard deviation

Table AI.20. Change in a* of alkaline noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	-2.1	-2.4	-2.1	-2.0	-3.2	-2.6	-2.3	-2.6	-2.4	0.41
2	-2.7	-2.4	-2.3	-2.0	-4.3	-2.6	-2.9	-2.4	-2.7	0.71
3	-2.5	-2.0	-1.4	-1.7	-3.3	-2.8	-2.4	-2.3	-2.3	0.60
4	-2.5	-2.2	-1.4	-2.2	-3.6	-2.9	-2.7	-2.5	-2.5	0.62
5	-2.7	-2.6	-2.0	-2.0	-3.8	-2.9	-2.6	-3.0	-2.7	0.58
6	-2.8	-2.5	-2.2	-1.9	-4.2	-3.1	-2.9	-2.2	-2.7	0.74
7	-1.9	-2.4	-2.3	-2.1	-3.3	-3.0	-2.3	-2.9	-2.5	0.48
8	-2.3	-2.5	-2.2	-2.1	-4.0	-3.4	-2.6	-2.8	-2.8	0.64
9	-2.2	-2.3	-1.8	-1.6	-3.8	-2.7	-2.4	-1.8	-2.3	0.71
10	-1.9	-2.9	-2.0	-2.5	-4.4	-3.4	-2.4	-2.9	-2.8	0.83
11	-2.7	-2.3	-2.1	-2.1	-3.8	-2.6	-2.0	-3.2	-2.6	0.64
12	-2.4	-2.4	-1.9	-1.8	-4.1	-3.9	-2.2	-2.5	-2.7	0.87
13	-2.0	-2.6	-1.9	-1.7	-3.2	-3.4	-1.8	-2.9	-2.4	0.69
14	-2.1	-2.0	-2.2	-1.8	-4.0	-3.1	-2.8	-2.8	-2.6	0.74
15	-1.8	-2.4	-1.7	-1.8	-4.1	-3.1	-2.2	-2.7	-2.5	0.82
16	-2.1	-2.6	-1.6	-1.9	-3.0	-3.3	-2.4	-3.3	-2.5	0.62
17	-2.0	-2.3	-2.1	-2.0	-3.3	-2.7	-2.0	-2.7	-2.4	0.47
18	-2.7	-2.2	-2.0	-2.1	-4.0	-3.1	-2.7	-3.1	-2.7	0.67
19	-1.8	-2.5	-1.7	-1.7	-2.6	-2.1	-2.7	-2.5	-2.2	0.43
20	-2.2	-2.6	-1.9	-2.2	-3.5	-2.6	-2.5	-1.9	-2.4	0.54
Mean	-2.3	-2.4	-1.9	-2.0	-3.7	-3.0	-2.4	-2.6	-2.5	
SD	0.33	0.22	0.26	0.23	0.49	0.40	0.31	0.41		

SD = standard deviation

Table AI.21. b* of salted noodle doughs immediately after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	16.7	18.9	18.0	19.3	18.3	15.7	16.5	17.1	17.6	1.26
2	17.5	17.1	18.7	18.0	19.8	18.2	19.4	19.1	18.5	0.92
3	18.7	18.3	20.5	17.5	18.3	15.4	17.3	17.4	17.9	1.45
4	16.1	16.7	17.4	15.4	17.0	14.5	15.1	14.8	15.9	1.10
5	17.2	17.4	18.5	18.2	16.1	13.6	17.1	15.1	16.7	1.64
6	17.4	17.8	20.1	17.9	16.6	17.3	17.8	17.3	17.8	1.03
7	15.0	14.8	17.6	16.7	16.1	13.5	15.6	14.4	15.5	1.31
8	17.5	18.8	17.1	18.7	18.2	16.6	17.8	18.4	17.9	0.77
9	16.7	16.4	17.4	15.7	14.3	13.0	15.4	14.7	15.4	1.43
10	14.6	14.6	20.4	15.1	16.1	14.0	16.3	15.9	15.9	2.00
11	18.0	17.4	17.8	15.6	16.1	12.8	16.8	15.5	16.3	1.68
12	17.1	16.9	18.7	16.0	14.8	13.6	15.7	15.2	16.0	1.56
13	15.5	17.2	17.9	18.2	17.3	15.8	15.6	17.2	16.8	1.06
14	16.2	18.0	19.1	17.4	15.6	15.4	15.7	17.0	16.8	1.31
15	14.9	18.4	18.3	18.1	16.0	15.5	16.0	17.2	16.8	1.37
16	15.8	17.7	18.1	17.8	17.1	14.8	16.3	16.7	16.8	1.11
17	15.3	15.7	17.6	15.9	16.2	15.0	15.1	15.9	15.8	0.82
18	16.9	18.4	20.3	18.5	16.8	17.2	16.7	17.6	17.8	1.23
19	14.6	17.1	15.6	15.7	15.4	12.8	15.0	14.1	15.0	1.26
20	17.0	16.8	18.8	17.1	17.6	15.9	15.6	17.1	17.0	0.99
Mean	16.4	17.2	18.4	17.1	16.7	15.0	16.3	16.4	16.7	
SD	1.18	1.18	1.25	1.27	1.30	1.56	1.13	1.38		

SD = standard deviation

Table AI.22. b* value of alkaline noodle doughs immediately after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	17.3	19.8	18.7	20.4	21.2	18.2	19.2	20.4	19.4	1.28
2	19.3	17.2	18.7	17.5	22.5	19.2	21.9	21.1	19.7	1.97
3	19.2	19.1	20.7	17.6	21.7	18.6	20.4	20.5	19.7	1.32
4	16.6	16.8	18.0	16.6	20.0	17.0	17.7	18.5	17.6	1.17
5	17.5	18.0	18.8	17.8	20.5	17.8	19.5	19.6	18.7	1.08
6	18.0	18.4	20.3	19.4	21.3	19.4	20.2	21.1	19.8	1.20
7	14.9	16.8	19.2	17.2	20.3	17.8	18.1	18.6	17.9	1.63
8	13.4	18.6	17.4	19.1	21.4	18.9	20.8	21.1	18.8	2.59
9	16.9	16.5	18.0	16.6	19.0	17.4	18.4	18.6	17.7	0.96
10	14.8	16.5	20.5	15.9	21.0	18.7	20.3	20.2	18.5	2.41
11	18.3	18.1	18.2	16.4	21.2	15.6	18.9	20.2	18.4	1.82
12	17.1	17.3	19.0	16.9	20.4	20.2	18.3	19.8	18.6	1.44
13	16.3	19.6	19.0	18.2	20.5	18.9	18.3	20.6	18.9	1.38
14	16.8	19.3	20.1	18.7	21.1	17.9	17.9	20.1	19.0	1.43
15	15.1	17.5	19.0	18.8	21.3	17.3	19.2	20.1	18.5	1.90
16	15.8	18.9	19.1	18.9	20.6	17.1	19.4	19.7	18.7	1.53
17	15.3	17.2	17.9	17.5	20.5	16.8	18.0	20.0	17.9	1.67
18	17.1	18.6	21.3	19.0	21.2	19.7	19.0	21.1	19.6	1.48
19	14.9	17.5	16.4	16.7	19.1	14.2	17.8	18.3	16.8	1.65
20	17.0	17.4	19.8	16.6	20.4	17.0	18.0	19.3	18.2	1.45
Mean	16.6	17.9	19.0	17.8	20.8	17.9	19.1	19.9	18.6	
SD	1.53	1.03	1.20	1.20	0.82	1.43	1.16	0.90		

SD = standard deviation

Table AI.23. b* of salted noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	19.4	22.4	22.7	24.0	19.9	17.8	22.9	21.2	21.3	2.10
2	21.6	20.5	24.1	22.7	21.4	21.0	23.0	22.1	22.0	1.17
3	23.7	21.5	26.0	22.3	20.4	17.6	22.2	21.5	21.9	2.45
4	19.2	19.2	22.8	20.6	19.7	18.1	20.3	19.2	19.9	1.38
5	21.5	21.1	23.6	23.9	20.0	17.4	23.4	20.2	21.4	2.22
6	21.1	21.4	24.0	22.0	20.3	20.0	23.2	20.5	21.6	1.43
7	17.9	18.2	23.0	21.8	19.9	16.8	22.2	18.9	19.8	2.28
8	19.7	23.4	22.3	24.0	20.7	19.5	23.6	20.3	21.7	1.86
9	18.9	20.0	20.9	20.3	17.9	15.1	20.6	18.5	19.0	1.92
10	18.3	17.9	26.4	18.5	19.8	18.8	20.5	17.9	19.7	2.85
11	18.9	20.2	22.4	19.8	18.9	19.2	20.9	19.0	19.8	1.28
12	19.2	20.9	22.0	20.0	19.8	18.7	20.8	17.9	19.9	1.32
13	18.2	20.5	23.9	22.7	18.7	19.9	21.7	20.3	20.7	1.95
14	19.7	20.5	22.9	22.7	19.4	18.9	22.2	19.8	20.8	1.59
15	18.6	21.9	23.1	22.6	19.4	18.8	21.9	20.4	20.8	1.77
16	18.9	20.7	22.8	21.8	19.0	18.7	22.3	19.7	20.5	1.65
17	17.9	19.9	22.2	20.2	19.0	18.8	20.8	19.9	19.8	1.32
18	19.9	21.3	22.9	20.1	19.5	18.6	21.9	19.4	20.4	1.46
19	17.3	20.9	19.4	20.9	17.6	17.5	19.8	18.3	18.9	1.51
20	19.0	18.9	21.5	20.9	19.1	18.5	20.6	19.9	19.8	1.10
Mean	19.4	20.6	22.9	21.5	19.5	18.5	21.7	19.7	20.5	
SD	1.53	1.35	1.56	1.63	0.90	1.28	1.15	1.14		

SD = standard deviation

Table AI.24. b* values of alkaline noodle doughs 24 hours after preparation

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	21.2	24.2	22.8	25.4	23.8	19.6	26.1	23.6	23.3	2.13
2	22.5	22.1	23.2	23.3	26.8	24.3	27.0	27.7	24.6	2.22
3	24.1	22.4	27.3	22.6	24.2	20.1	25.2	24.0	23.7	2.13
4	20.6	20.3	23.0	22.1	22.5	18.6	22.3	21.0	21.3	1.46
5	22.2	22.1	24.3	24.0	23.1	19.3	28.0	24.7	23.5	2.51
6	22.1	22.3	24.3	23.4	24.1	24.4	26.3	26.8	24.2	1.66
7	19.4	20.9	25.0	22.7	23.5	19.3	25.5	20.9	22.1	2.39
8	22.8	25.2	24.2	25.0	26.0	21.1	27.9	27.4	24.9	2.27
9	19.6	20.7	21.7	21.3	21.0	17.6	23.7	23.4	21.1	1.97
10	18.6	21.9	26.3	20.3	24.5	21.0	24.8	23.9	22.7	2.65
11	20.3	21.3	23.8	20.4	25.0	19.7	24.4	24.0	22.4	2.16
12	20.5	22.4	22.1	21.3	22.9	22.1	24.8	25.1	22.6	1.58
13	20.3	23.6	25.4	23.7	22.9	20.7	26.0	26.7	23.7	2.36
14	20.4	21.8	23.5	23.8	23.0	19.8	23.2	25.0	22.6	1.79
15	19.3	23.5	24.8	23.9	22.0	19.5	26.1	26.1	23.1	2.69
16	19.8	23.6	24.5	23.5	22.9	19.9	26.5	23.8	23.1	2.26
17	19.0	21.5	23.7	22.1	22.1	18.5	24.5	23.3	21.8	2.15
18	21.1	21.8	23.9	22.1	23.3	22.3	25.6	25.0	23.1	1.57
19	18.8	22.0	20.6	21.7	20.9	16.9	22.7	21.0	20.6	1.89
20	19.4	20.1	21.8	20.6	23.4	20.8	23.2	23.0	21.5	1.54
Mean	20.6	22.2	23.8	22.7	23.4	20.3	25.2	24.3	22.8	
SD	1.50	1.32	1.59	1.47	1.46	1.93	1.63	2.00		

SD = standard deviation

Table AI.25. Change in b* of salted noodle doughs after 24 hours

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	-2.7	-3.6	-4.6	-4.7	-1.7	-2.1	-6.4	-4.2	-3.7	1.56
2	-4.1	-3.3	-5.4	-4.7	-1.7	-2.9	-3.6	-3.0	-3.6	1.16
3	-5.0	-3.2	-5.5	-4.8	-2.1	-2.1	-4.9	-4.1	-4.0	1.33
4	-3.1	-2.5	-5.4	-5.2	-2.7	-3.6	-5.2	-4.5	-4.0	1.18
5	-4.3	-3.7	-5.1	-5.7	-3.9	-3.8	-6.3	-5.1	-4.7	0.96
6	-3.7	-3.6	-3.9	-4.1	-3.7	-2.7	-5.4	-3.2	-3.8	0.77
7	-2.9	-3.4	-5.5	-5.1	-3.8	-3.3	-6.7	-4.6	-4.4	1.29
8	-2.2	-4.7	-5.2	-5.4	-2.5	-2.9	-5.7	-1.9	-3.8	1.58
9	-2.2	-3.6	-3.5	-4.5	-3.6	-2.1	-5.2	-3.8	-3.6	1.06
10	-3.7	-3.3	-6.0	-3.4	-3.7	-4.8	-4.2	-1.9	-3.9	1.20
11	-1.0	-2.8	-4.5	-3.2	-2.8	-6.4	-4.1	-3.4	-3.5	1.57
12	-2.1	-4.1	-3.3	-4.0	-4.9	-5.1	-5.1	-2.7	-3.9	1.14
13	-2.7	-3.3	-6.0	-4.5	-1.4	-4.0	-6.1	-3.1	-3.9	1.61
14	-3.5	-2.5	-3.8	-5.3	-3.7	-3.5	-6.6	-2.8	-3.9	1.35
15	-3.8	-3.5	-4.8	-4.6	-3.4	-3.2	-5.9	-3.2	-4.0	0.95
16	-3.2	-3.0	-4.7	-4.1	-1.9	-3.8	-6.1	-3.0	-3.7	1.27
17	-2.7	-4.2	-4.7	-4.3	-2.9	-3.8	-5.7	-3.9	-4.0	0.97
18	-3.0	-2.9	-2.6	-1.7	-2.7	-1.4	-5.2	-1.8	-2.6	1.19
19	-2.6	-3.8	-3.8	-5.2	-2.1	-4.6	-4.8	-4.3	-3.9	1.06
20	-2.0	-2.1	-2.7	-3.8	-1.5	-2.6	-5.0	-2.9	-2.8	1.12
Mean	-3.0	-3.3	-4.5	-4.4	-2.8	-3.4	-5.4	-3.4	-3.8	
SD	0.93	0.62	1.03	0.92	0.98	1.20	0.84	0.92		

SD = standard deviation

Table AI.26. Change of b* values of alkaline noodle doughs after 24 hours

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	-3.8	-4.5	-4.2	-5.0	-2.6	-1.4	-6.8	-3.3	-3.9	1.63
2	-3.3	-4.9	-4.5	-5.8	-4.3	-5.1	-5.1	-6.7	-5.0	1.02
3	-4.9	-3.2	-6.7	-5.0	-2.5	-1.5	-4.8	-3.6	-4.0	1.65
4	-4.0	-3.5	-5.0	-5.5	-2.5	-1.6	-4.6	-2.5	-3.7	1.37
5	-4.7	-4.1	-5.6	-6.2	-2.6	-1.5	-8.6	-5.2	-4.8	2.16
6	-4.2	-3.9	-4.0	-4.1	-2.8	-4.9	-6.0	-5.7	-4.4	1.04
7	-4.5	-4.1	-5.8	-5.6	-3.2	-1.4	-7.3	-2.4	-4.3	1.93
8	-4.4	-6.6	-6.8	-5.9	-4.5	-2.2	-7.1	-6.3	-5.5	1.67
9	-2.6	-4.2	-3.8	-4.6	-2.0	-0.1	-5.2	-4.7	-3.4	1.72
10	-3.7	-5.3	-5.8	-4.4	-3.5	-2.3	-4.6	-3.7	-4.2	1.09
11	-2.0	-3.2	-5.6	-4.0	-3.9	-4.1	-5.6	-3.8	-4.0	1.18
12	-3.4	-5.1	-3.1	-4.5	-2.5	-1.9	-6.4	-5.2	-4.0	1.55
13	-4.0	-4.0	-6.4	-5.5	-2.4	-1.8	-7.7	-6.2	-4.8	2.05
14	-3.6	-2.5	-3.4	-5.1	-1.9	-1.8	-5.3	-4.9	-3.6	1.41
15	-4.2	-6.0	-5.8	-5.1	-0.7	-2.1	-6.9	-5.9	-4.6	2.15
16	-4.0	-4.7	-5.4	-4.6	-2.3	-2.8	-7.1	-4.2	-4.4	1.49
17	-3.7	-4.4	-5.8	-4.5	-1.6	-1.7	-6.5	-3.3	-3.9	1.75
18	-4.0	-3.3	-2.6	-3.1	-2.1	-2.6	-6.5	-3.9	-3.5	1.38
19	-3.9	-4.5	-4.3	-5.0	-1.9	-2.6	-4.9	-2.7	-3.7	1.18
20	-2.4	-2.7	-1.9	-4.0	-3.0	-3.8	-5.2	-3.7	-3.3	1.05
Mean	-3.8	-4.2	-4.8	-4.9	-2.6	-2.4	-6.1	-4.4	-4.2	
SD	0.74	1.04	1.37	0.77	0.92	1.25	1.15	1.32		

SD = standard deviation

Table AI.27. Percentage of flour PPO out of grain PPO (ox.cons.method).

	Environment #									
Genotype #	1	2	3	4	5	6	7	8	Mean	SD
1	2.5	2.5	4.3	3.1	3.3	3.2	2.2	2.7	3.0	0.7
2	2.2	2.2	4.3	2.8	3.6	2.1	4.4	2.2	3.0	1.0
3	3.5	3.6	3.4	3.5	3.5	4.4	4.4	3.4	3.7	0.4
4	3.3	4.0	4.1	3.2	5.1	3.5	5.5	3.2	4.0	0.9
5	7.1	4.3	6.0	6.4	9.3	6.9	5.1	4.0	6.1	1.7
6	8.5	4.2	7.5	5.9	11.5	5.9	8.8	4.5	7.1	2.5
7	5.6	4.3	8.3	4.8	8.1	5.2	4.3	4.2	5.6	1.7
8	5.0	3.5	7.1	4.9	5.3	8.9	4.3	4.5	5.4	1.7
9	2.2	3.6	7.0	2.6	4.6	3.7	4.9	2.0	3.8	1.7
10	4.3	4.1	9.8	3.9	4.6	4.7	6.3	4.2	5.2	2.0
11	6.0	5.2	7.2	4.6	5.1	8.1	2.5	3.7	5.3	1.8
12	5.8	4.6	10.1	4.5	6.8	4.1	3.1	2.6	5.2	2.4
13	2.5	3.5	4.2	3.2	2.7	5.0	1.5	2.6	3.2	1.1
14	3.9	3.9	5.5	2.6	5.2	3.8	3.3	2.8	3.9	1.0
15	2.3	3.2	2.6	2.5	7.2	4.8	3.2	3.8	3.7	1.6
16	3.0	3.5	3.5	2.4	2.9	3.8	3.3	5.2	3.4	0.8
17	2.8	2.6	3.2	3.3	3.1	4.1	2.7	3.6	3.2	0.5
18	2.6	3.1	4.0	2.9	4.1	4.9	5.3	4.0	3.9	0.9
19	1.8	3.8	1.9	2.7	1.9	2.4	5.2	3.6	2.9	1.2
20	3.2	2.7	4.8	2.8	2.7	2.7	3.5	2.9	3.2	0.7
Mean	3.9	3.6	5.4	3.6	5.0	4.6	4.2	3.5	4.2	
SD	1.8	0.8	2.3	1.2	2.5	1.7	1.6	0.8		

SD = standard deviation

APPENDIX II

PPO activity determination with the YSI Biological Oxygen Monitor: detailed procedure

II.1. EQUIPMENT

- YSI 5300 Biological Oxygen Monitor (completely assembled)
- Circulation water bath (37°C +/- 0.1)
- Recorder
- Pure air supply ("breathing air A1083K")

II. 2. REAGENTS

- Air saturated phosphate buffer 0.05 M pH 6.8 (it may take up to 30 min to saturate it). It will be referred as "buffer".
- Substrate solution: 25% w/v catechol solution for ground wheat or 9% w/v catechol solution for flour, prepared 30 minutes or less before. It will be referred as "substrate solution".

III.3. PROCEDURE

III.3.1. Preparation

1) Turn on the circulation bath and allow sufficient time for it to come to the desired temperature.

2) Turn on the monitor and the recorder.

3) Place 4.0 ml of buffer in a sample chamber and a magnetic stirrer (with the flat side up).

4) Insert the prepared standard probe into the chamber. Remove all the air from the sample chamber through the access slot. The solution level in the access slot should be between the lower end of the plunger and its overflow groove.

5) Start the stirrer. Allow 3 minutes for temperature equilibration and stop the stirrer.

6) Rotate the Function Switch of the monitor to AIR, and set the display to 100.0% with the CAL control. Turn the CAL control-locking knob clockwise to lock the knob.

7) Set the recorder to full scale.

8) Observe system stability as indicated by the recorder trace. The trace should be noise-free.

9) Probe test:

a. Unlock the CAL control knob, set the reading to 90.0%, and re-lock the knob.

b. Turn the Function Switch counter clockwise to the TEST position and wait for a steady trace.

c. The trace should be no lower than 87.0% of full scale after 2.5 min. If the probe does not meet this specification, the membrane should be replaced (see Instruction Manual). If performance still does not improve, the probe should be cleaned (see Instruction Manual).

10) After the probe test, unlock the CAL control knob and reset the display to 100.0%, then lock the CAL control knob.

III.3.2. Analysis

1) Weight exactly about 100mg of the ground wheat (ground in Udy with a 0.5 mm mesh) or 500mg of flour and introduce it into a sample chamber.

2) Add 4.0 ml of buffer and turn on the stirrer.

3) Allow 3 minutes for temperature equilibration.

4) Insert the probe into the chamber. Remove all the air from the sample chamber through the access slot. The solution level in the access slot should be between the lower end of the plunger and its overflow groove.

5) Start the recorder (2"/min for ground wheat or 1"/min for flour).

6) Record 1 or 2 minutes.

7) Add substrate solution (200µl for ground wheat or 100µl for flour) through the access slot using a pipette.

8) Record the oxygen percentage of the solution for at least 2 minutes for ground wheat or 4 minutes for flour.

III.3.3. Catechol autoxidation determination (just for ground wheat determination).

1) Repeat steps 2-8 with the concentrated catechol solution (25%).

IV. CALCULATION

1) Determine oxygen consumption by sample without added substrate (A), the slope obtained in step III.3.2.6 expressed in percentage of oxygen saturation per minute (%O_2/min).

2) Determine total oxygen consumption (B), the slope obtained in step III.3.2.8, expressed in $\%O_2$/min.

3) Determine oxygen consumed by catechol autoxidation (C), the slope obtained in step III.3.3.1 expressed in $\%O_2$/min.

4) Determine oxygen consumed by PPO:

$$D = B - A - C$$

(C is estimated zero for flour determination). The result is obtained in $\%O_2$/min.

5) Convert the D value to oxygen nanomoles per minute (O_2 nmol/min):

$$E = D * 7.96$$

6) Obtain the final result (F) expressed it in O_2 nmol/min/g

$$F = D/\text{weight (expressed in grams)}$$

APPENDIX III

PPO activity (whole kernel method) of the whole set of samples grown in 1998

Table AIII.1. PPO activity of the 64 genotypes using whole kernel method.

Environment #1: Brandon 1998

Genotype	AU [a]	Genotype	AU [a]
BW 49 Katepwa (#1)	0.811	L 1507 96F32 * A13	0.929
BW 148 AC Domain	0.994	L 1509 96F32 * A14	0.954
L 1717 SD 3055 (#3)	0.901	L 1511 96F32 * A15	0.557
L 1719 Roblin	1.117	L 1513 96F32 * A18 (#13)	1.009
L 1721 AC Splendor	0.851	L 1515 96F32 * A26	0.975
L 1723 Katepwa	0.841	L 1516 96F32 * A27	0.878
L 1727 AC Majestic	1.015	L 1519 96F32 * A42 (#14)	1.080
L 1725 AC Domain (#4)	1.026	L 1221 96F32 * A76 (#15)	0.898
L 1459 95B02 * A140 (#5)	0.462	L 1523 96F32 * A79 (#16)	0.964
L 1461 95B02 * B116	0.584	L 1525 96F32 * B4 (#17)	0.846
L 1463 95B02 * B207	0.756	L 1527 96F32 * B10	0.873
L 1465 95B02 * C99	0.610	L 1529 96F32 * B19	0.792
L 1467 95B02 * C120	0.689	L 1531 96F32 * B21 (#18)	0.811
L 1469 95B02 * D130	0.559	L 1533 96F32 * B30 (#19)	0.766
L 1471 95B02 * D75 (#6)	0.541	L 1535 96F32 * B54	0.496
L 1473 95B02 * D156 (#7)	0.559	L 1537 96F32 * B67	0.599
L 1475 96F29 * A27	0.515	L 1539 96F34 * A11	0.688
L 1477 96F29 * A29	0.648	L 1541 96F34 * A39	0.641
L 1479 96F29 * A33	0.697	L 1543 96F34 * A52	0.506
L 1481 96F29 * B21 (#8)	0.561	L 1545 96F34 * A67	0.786
L 1483 96F30 * A2 (#9)	1.177	L 1547 96F34 * B43	0.626
L 1485 96F30 * A16 (#10)	0.757	L 1549 96F34 * B43	0.572
L 1487 96F30 * A32 (#11)	0.573	L 1551 96F34 * B85	0.758
L 1489 96F30 * A72	0.964	L 1553 96F34 * B86	0.701
L 1491 96F30 * B2 (#12)	0.691	L 1555 96F34 * B88	0.656
L 1493 96F30 * B37	0.553	L 1557 96F34 * B106	0.741
L 1495 96F30 * B101	0.622	L 1559 96F35 * B2	0.535
L 1497 96F31 * B43	0.657	L 1561 96F35 * B114	0.551
L 1499 96F31 * A67	0.827	L 1665 96F39 * A34 (#2)	0.886
L 1501 96F32 * A1	0.854	L 1563 96F39 * A160	0.891
L 1503 96F32 * A5	0.983	L 1565 96F39 * A164 (#20)	0.857
L 1505 96F32 * A8	0.974	L 1567 96F39 * B62	1.158

[a] Absorbance units.

Table AIII.2. PPO activity of the 64 genotypes using whole kernel method.

Environment #2

Genotype	AU [a]	Genotype	AU [a]
BW 49 Katepwa (#1)	0.762	L 1507 96F32 * A13	0.790
BW 148 AC Domain	0.946	L 1509 96F32 * A14	0.597
L 1717 SD 3055 (#3)	0.979	L 1511 96F32 * A15	0.961
L 1719 Roblin	0.992	L 1513 96F32 * A18 (#13)	0.976
L 1721 AC Splendor	0.816	L 1515 96F32 * A26	0.860
L 1723 Katepwa	0.960	L 1516 96F32 * A27	0.827
L 1727 AC Majestic	0.872	L 1519 96F32 * A42 (#14)	0.781
L 1725 AC Domain (#4)	0.561	L 1221 96F32 * A76 (#15)	0.887
L 1459 95B02 * A140 (#5)	0.570	L 1523 96F32 * A79 (#16)	0.927
L 1461 95B02 * B116	0.569	L 1525 96F32 * B4 (#17)	0.711
L 1463 95B02 * B207	0.552	L 1527 96F32 * B10	0.835
L 1465 95B02 * C99	0.738	L 1529 96F32 * B19	0.942
L 1467 95B02 * C120	0.674	L 1531 96F32 * B21 (#18)	0.753
L 1469 95B02 * D130	0.658	L 1533 96F32 * B30 (#19)	0.653
L 1471 95B02 * D75 (#6)	0.699	L 1535 96F32 * B54	0.590
L 1473 95B02 * D156 (#7)	0.572	L 1537 96F32 * B67	0.671
L 1475 96F29 * A27	0.661	L 1539 96F34 * A11	0.699
L 1477 96F29 * A29	0.581	L 1541 96F34 * A39	0.594
L 1479 96F29 * A33	0.561	L 1543 96F34 * A52	0.659
L 1481 96F29 * B21 (#8)	0.866	L 1545 96F34 * A67	0.705
L 1483 96F30 * A2 (#9)	0.749	L 1547 96F34 * B43	0.587
L 1485 96F30 * A16 (#10)	0.612	L 1549 96F34 * B43	0.536
L 1487 96F30 * A32 (#11)	n.d.	L 1551 96F34 * B85	0.549
L 1489 96F30 * A72	0.626	L 1553 96F34 * B86	0.548
L 1491 96F30 * B2 (#12)	0.549	L 1555 96F34 * B88	0.597
L 1493 96F30 * B37	0.720	L 1557 96F34 * B106	0.693
L 1495 96F30 * B101	0.613	L 1559 96F35 * B2	0.556
L 1497 96F31 * B43	0.832	L 1561 96F35 * B114	0.961
L 1499 96F31 * A67	0.822	L 1665 96F39 * A34 (#2)	0.974
L 1501 96F32 * A1	0.769	L 1563 96F39 * A160	0.862
L 1503 96F32 * A5	0.829	L 1565 96F39 * A164 (#20)	0.819
L 1505 96F32 * A8	0.859	L 1567 96F39 * B62	0.610

[a] Absorbance units. n.d.: Not determined

Table AIII.3. PPO activity of the 64 genotypes using whole kernel method.

Environment #3

Genotype		AU [a]	Genotype		AU [a]
BW	49 Katepwa (#1)	0.710	L	1507 96F32 * A13	0.674
BW	148 AC Domain	0.641	L	1509 96F32 * A14	0.495
L	1717 SD 3055 (#3)	0.738	L	1511 96F32 * A15	0.606
L	1719 Roblin	0.691	L	1513 96F32 * A18 (#13)	0.669
L	1721 AC Splendor	0.689	L	1515 96F32 * A26	0.693
L	1723 Katepwa	0.789	L	1516 96F32 * A27	0.673
L	1727 AC Majestic	0.616	L	1519 96F32 * A42 (#14)	0.795
L	1725 AC Domain (#4)	0.448	L	1221 96F32 * A76 (#15)	0.765
L	1459 95B02 * A140 (#5)	0.533	L	1523 96F32 * A79 (#16)	0.619
L	1461 95B02 * B116	0.449	L	1525 96F32 * B4 (#17)	0.595
L	1463 95B02 * B207	0.469	L	1527 96F32 * B10	0.688
L	1465 95B02 * C99	0.521	L	1529 96F32 * B19	0.662
L	1467 95B02 * C120	0.530	L	1531 96F32 * B21 (#18)	0.636
L	1469 95B02 * D130	0.413	L	1533 96F32 * B30 (#19)	0.458
L	1471 95B02 * D75 (#6)	0.519	L	1535 96F32 * B54	0.505
L	1473 95B02 * D156 (#7)	0.535	L	1537 96F32 * B67	0.510
L	1475 96F29 * A27	0.457	L	1539 96F34 * A11	0.535
L	1477 96F29 * A29	0.482	L	1541 96F34 * A39	0.518
L	1479 96F29 * A33	0.486	L	1543 96F34 * A52	0.515
L	1481 96F29 * B21 (#8)	0.677	L	1545 96F34 * A67	0.534
L	1483 96F30 * A2 (#9)	0.532	L	1547 96F34 * B43	0.478
L	1485 96F30 * A16 (#10)	0.429	L	1549 96F34 * B43	0.496
L	1487 96F30 * A32 (#11)	0.714	L	1551 96F34 * B85	0.524
L	1489 96F30 * A72	0.479	L	1553 96F34 * B86	0.559
L	1491 96F30 * B2 (#12)	0.464	L	1555 96F34 * B88	0.465
L	1493 96F30 * B37	0.475	L	1557 96F34 * B106	0.492
L	1495 96F30 * B101	0.519	L	1559 96F35 * B2	0.445
L	1497 96F31 * B43	0.653	L	1561 96F35 * B114	0.705
L	1499 96F31 * A67	0.661	L	1665 96F39 * A34 (#2)	0.596
L	1501 96F32 * A1	0.647	L	1563 96F39 * A160	0.698
L	1503 96F32 * A5	0.682	L	1565 96F39 * A164 (#20)	0.752
L	1505 96F32 * A8	0.782	L	1567 96F39 * B62	0.471

[a] Absorbance units.

Table AIII.4. PPO activity of the 64 genotypes using whole kernel method.

Environment #4

Genotype		AU [a]	Genotype		AU [a]
BW	49 Katepwa (#1)	0.657	L	1507 96F32 * A13	0.741
BW	148 AC Domain	0.640	L	1509 96F32 * A14	0.781
L	1717 SD 3055 (#3)	0.871	L	1511 96F32 * A15	0.429
L	1719 Roblin	0.712	L	1513 96F32 * A18 (#13)	0.810
L	1721 AC Splendor	0.782	L	1515 96F32 * A26	0.687
L	1723 Katepwa	0.613	L	1516 96F32 * A27	0.640
L	1727 AC Majestic	0.678	L	1519 96F32 * A42 (#14)	0.793
L	1725 AC Domain (#4)	0.763	L	1221 96F32 * A76 (#15)	0.822
L	1459 95B02 * A140 (#5)	0.529	L	1523 96F32 * A79 (#16)	0.754
L	1461 95B02 * B116	0.473	L	1525 96F32 * B4 (#17)	0.594
L	1463 95B02 * B207	0.483	L	1527 96F32 * B10	0.696
L	1465 95B02 * C99	0.570	L	1529 96F32 * B19	0.734
L	1467 95B02 * C120	0.587	L	1531 96F32 * B21 (#18)	0.778
L	1469 95B02 * D130	0.610	L	1533 96F32 * B30 (#19)	0.684
L	1471 95B02 * D75 (#6)	0.513	L	1535 96F32 * B54	0.442
L	1473 95B02 * D156 (#7)	0.521	L	1537 96F32 * B67	0.577
L	1475 96F29 * A27	0.524	L	1539 96F34 * A11	0.588
L	1477 96F29 * A29	0.515	L	1541 96F34 * A39	0.603
L	1479 96F29 * A33	0.592	L	1543 96F34 * A52	0.478
L	1481 96F29 * B21 (#8)	0.462	L	1545 96F34 * A67	0.507
L	1483 96F30 * A2 (#9)	0.797	L	1547 96F34 * B43	0.502
L	1485 96F30 * A16 (#10)	0.510	L	1549 96F34 * B43	0.490
L	1487 96F30 * A32 (#11)	0.488	L	1551 96F34 * B85	0.510
L	1489 96F30 * A72	0.751	L	1553 96F34 * B86	0.540
L	1491 96F30 * B2 (#12)	0.500	L	1555 96F34 * B88	0.531
L	1493 96F30 * B37	0.489	L	1557 96F34 * B106	0.534
L	1495 96F30 * B101	0.524	L	1559 96F35 * B2	0.410
L	1497 96F31 * B43	0.511	L	1561 96F35 * B114	0.617
L	1499 96F31 * A67	0.693	L	1665 96F39 * A34 (#2)	0.724
L	1501 96F32 * A1	0.658	L	1563 96F39 * A160	0.654
L	1503 96F32 * A5	0.734	L	1565 96F39 * A164 (#20)	0.740
L	1505 96F32 * A8	0.612	L	1567 96F39 * B62	0.711

[a] Absorbance units.