POLYPHENOL OXIDASE AND PHENOLIC ACIDS: THEIR LEVELS AND INTERACTION IN COLOR PRODUCTION IN CANADIAN WHEAT FLOURS

By

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ABSTRACT

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Five Canadian varieties, representing different classes of Canadian wheat, were milled to 75, 80, and 85% extraction yields. Individual streams were pooled to produce representative 1st and 2nd patent, 1st and 2nd clear, and straight grade flours.

The level of the enzyme polyphenol oxidase (PPO) was determined in each mill stream and the pooled flours. Analysis of the pooled flours indicated low enzyme levels in all varieties 1st patent flours at 75 and 80% extraction rates but increasing rapidly as the flour quality decreased. The Hard Red Winter (HRW) wheat Norstar yielded the lowest enzyme levels in the majority of pooled flours at both the 75 and 80% extraction rates.

The pooled flours were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) to establish the endogenous levels of insoluble bound, soluble bound, and free phenolic acids. Only ferulic acid was detected in the insoluble bound category. The soluble bound phenolic acids accounted for a maximum of 17% of the total phenolic acid content with sinapic acid predominating. Wide variations in their levels within comparable flours were detected across

the varieties. Ferulic acid was the dominant free phenolic acid while sinapic acid was absent in all flours.

Significant correlations, $p < 0.05$, were detected between insoluble ferulic, all soluble bound acids, free ferulic and vanillic acids with both PPO and ash content.

Initial flour paste colors were determined by Kent-Jones, Agtron, and Hunter brightness (L^*), redness (a^*), and yellowness (b^*) values. Strong, significant correlations were observed between paste color values with ash, pigment, PPO total free, total soluble bound, insoluble, and total phenolic acids. Introduction of the alkaline Kan Sui reagent had minimal effect on the relationship of paste brightness or yellowness with these variables.

Each varieties flour pastes were analyzed for their changes in Hunter L^*, a^* and b^* values as a function of time. Differences were noted between varieties in their ability to distinguish between their flour paste colors on the basis of quality or extraction rate. The variety Norstar consistently displayed the brightest pastes undergoing relatively minimal color change.

The effect of temperature and free phenolic acid addition on flour pastes' L^*, a^*, and b^* values was investigated. No temperature effect was detected while caffeic acid and sinapic acids were found to influence the flour paste color.

Attempts were made to model the initial paste color and total color change over time using the factors determined in this study.

1.0 Introduction

The desire to establish Canadian wheats in non-traditional foreign markets has necessitated the need for information on the quality of Canadian wheats milled to higher than conventional extraction levels. Achieving these higher levels culminates in the elevation of bran contamination in the resulting flours. Concurrent with the increased bran content is the associated increase in phenolic acid levels, pigment content, and the amount of oxidative enzymes, in particular, polyphenol oxidase.

Consumers throughout the world assess their wheaten products on a number of criteria, particularly color. Processing of end-products using the high extraction flours has the associated problem of undesirable discoloration known as browning. Use of such flours is therefore subject to consumer acceptance of the final product.

Formation of "brown" pigments in wheat products is the result of two separate reactions. The common browning reaction associated with baking, the Maillard reaction, consists of the carbonyl group of sugars reacting, via Schiff base formation, with free amines to produce a colored polymer. The undesirable discoloration is enzymic in nature. Polyphenol oxidase is believed to be involved in the oxidation of endogenous wheat phenolics resulting in the production of very labile quinones. The quinones in turn can react with a number of compounds, amines and thiols, or

undergo self polymerization, to produce highly colored products.

The objective of this work was divided into three sections. Establish the polyphenol oxidase levels in the mill streams and combined flours of five varieties representative of Canadian wheat classes, under increasing mill extraction rates. Determine the individual simple phenolic acids contents in the combined flours of each variety. Subsequent to this, evaluate the role and the interaction amongst these factors in color production relevant to end-use products.

2.0 Literature Review

2.01 Polyphenol Oxidase (PPO)

The international nomenclature of polyphenol oxidase is under constant revision and at present consists of three classes. Monophenol monooxygenase (tyrosinase) is referred to as 1.14.18.1 while diphenol oxidase (catechol oxidase, diphenol oxygen oxidoreductase) as 1.10.3.2, and laccase as 1.10.3.1 (Mayer 1987).

Catechol oxidase is often known as; phenolase, polyphenol oxidase, tyrosinase, catecholase and cresolase. They refer to the enzyme's ability to oxidize mono and o-diphenols.

Laccase however is capable of oxidizing the same substrates as catechol oxidase but it is its ability to oxidize p-diphenols which sets it apart.

Catechol oxidase is a copper containing enzyme which has been shown to catalyze two separate reactions. The initial reaction is the addition of molecular oxygen ortho to the existing hydroxyl group in a monophenolic and the subsequent oxidation to quinone. This reaction is often referred to as cresolase.

The second reaction is the oxidation of an existing o-diphenolic compound to it's corresponding quinone. Using

this form of substrate the enzyme is said to be displaying catecholase activity.

Catechol oxidase has been found to be widespread throughout the plant kingdom and does not appear to be exclusive to any particular organ or tissue.(Mayer and Harel 1979) The enzyme's levels have been shown to change during plant development (Taneja *et al* 1974) and may be influenced by environmental conditions (Sharma and Tayal 1983). In their review of the enzyme, Mayer and Harel(1979) state that there is extensive evidence for the membrane-bound enzyme, originating in the chloroplasts and mitochondria. They further report that the strength of this binding varies from slight to requiring drastic conditions to release the enzyme.

2.02 Wheat Specifics

Polyphenol oxidase has been reported to be isolated from wheat by a number of researchers with varying degrees of success depending on the purification scheme employed. Tikoo *et al*(1973) using a semi-purified extract from both tall and dwarf wheats found three to five bands of PPO on acrylamide gel electrophoresis. They found that the specific activity of the dwarf variety was distinctly higher than the tall varieties. Substrate specificity was also shown when monophenols such as tyrosine and phenol did not serve as substrates. Diphenolic specificity was also displayed with

catechol being superior to dopamine and caffeic acid. Differences in heat stability were found between the dwarf and the tall variety. The enzyme from the dwarf variety was shown to retain 83-85% of its activity after 1 h at 60 C while that from the tall varieties dropped to 48-53 % under the same conditions. In both cases the majority of loss occurred within the first 10 min before levelling off. Marsh and Galliard (1986) report a 50% loss in activity at the same temperature after 5 min using a common bread wheat as the source of the enzyme.

Singh and Sheoran (1972) indicated that the major factor determining extensive browning of dough was the high activity of tyrosinase and the concentration of free tyrosine and total phenols present in the wheat. Abrol *et al* (1971) reported that some wheat varieties are less acceptable to the consumer due to dough darkening and the browning of chapatties. They used a phenol color reaction to screen for acceptable varieties based on tyrosinase activity.

Salt stress caused increased polyphenol oxidase activity in wheat with different responses between tolerant and resistant varieties. The salt sensitive varieties showed the greatest increases (Sharma and Tayal 1983).

Taneja and Sachar(1974) reported the electrophoretic isolation of a monophenolase and an o-diphenolase activity. In crude extracts they obtained one band which reacted with tyrosine and 6 bands which reacted with dihydroxy

phenylalanine (DOPA). The tyrosine reactive band was not associated with any of the 6 bands displaying diphenolase activity. The influence of the development stage was highlighted as the monophenolase activity appeared in the later stages of the maturing grain. There was no indication of the presence of both catalytic sites on the same enzyme. Their work supported the view that the two activities of polyphenol oxidase represent two distinct enzymes in wheat. Taneja *et al* (1974) showed o-diphenolase activity to be high in young seeds while monophenolase activity was practically undetectable throughout the major period of grain filling. The induction of monophenolase activity occurred with the onset of grain ripening while a corresponding 60- fold reduction was seen in o-diphenolase activity. This work was supported by Kruger (1976) who found similar results as well as discerning the major amount of activity in the immature durum kernel resided in the endosperm. Kruger found 12 different isozymes of the enzyme by polyacrylamide gel electrophoresis, PAGE. The anatomical dissection of the kernel indicated that different isozymes were present in different parts of the kernel. Kruger (1976) also found that enzyme activity increased 33 fold by 5 days of germination and then decreased. Nine to ten isoenzymes of o-diphenolase activity, but only one band of monophenolase activity, was found in the developing grain. Monophenolase activity was detected at 35 to 39 days reaching a maximum at 42 days after anthesis followed by a decline at maturity (Taneja and

Sachar ,1974). The appearance of tyrosine specific activity appears to be linked with the onset of maturation. The authors implied the change in seed color from green to brown may indicate that monophenolase has a possible role in the formation of pigments in the grain coat. The lack of synchronicity between the two activities during grain development suggested two distinct enzymes rather than the same enzyme complex.

Saluja *et al* (1989) found a significant increase in monophenolase activity in deembryonated half seeds of wheat after a two hour exposure to gibberellic acid. This response however occurred only after lowering the endogenous levels of phytohormone by leaching. Similar stimulation was induced by the administration of phosphate ions (75 mM). Simultaneous addition of gibberellic acid and phosphate ions had no cumulative affect on the activation of the enzyme. Gibberellic acid and phosphate ion activated enzyme showed altered molecular properties. An increased thermostability and a shift in pH optimum towards pH 9 were observed. The control pH optimum was pH 7.0. Gel permeation fractionation indicated a single molecular form of monophenolase (45,000) both in the control and gibberellic acid treated half seeds. The authors suggested that the monophenolase induction was due to possible structural modification of the enzyme without its oligomerization. They felt that the induction of the enzyme activity by gibberellic acid ,GA_3, to be a true primary response to the final hormone. Abscisic acid (100

uM) exerted a negative control on the gibberellic acid stimulated monophenolase activity. Cycloheximide did not inhibit the GA_3 mediated increase in monophenolase activity. This indicated the hormonal regulation of the enzyme was achieved by the activation of the enzyme at the post-translational level.

Interesse *et al* (1981) purified four isozymes of o-diphenolase from wheat with pI's of 3.60, 4.95, 6.80, and 9.60. The highest specific activity was found at pI 9.60. Further work by Interesse *et al* (1983) on this fraction found a molecular weight,(MW), of approximately 115 000. o-Diphenolase (o-DPO) from higher plants is thought to be aggregates of monomers. The isozyme, pI 9.60, when exposed to SDS- PAGE showed two subunits, one of 30,000 molecular weight and the other of 23 500. The ratio of material in the two subunits was approximately 3 to 1 respectively. They suggested the 30,000 MW protein was the basic subunit of the enzyme. The role of the smaller component is not clear. Interesse and co-workers suggested that the 115,000 MW protein, pI 9.60, is a tetramer of three 30,000 MW subunits and one 23,500 MW subunit. They found the copper content to be 3.6 atoms of copper per molecule of enzyme. They postulated that a small amount of copper was lost during purification thus implying one atom of copper per subunit. This agrees well with values found for mushrooms and bacteria. Amino acid analysis showed the basic and hydrophobic residue contents were 11.4% and 27.7%

respectively. Glutamic acid was the most abundant followed by aspartic acid and glycine. The acid values include glutamine and asparagine. Comparison with grape, spinach and potato enzyme composition shows similarity. The wheat enzyme was distinguished by greater glutamic acid and glycine composition. The authors reported comparison between wheat and fungal o-diphenolase amino acid compositions to be very different.

Previous work by Interesse *et al* (1980) indicated the preferred enzyme substrates to be 4-methylcatechol(4-MECAT) and epicatechin, followed by moderate activity towards DOPA and protocatechuic acid. Low activity was seen towards caffeic, chlorogenic, and p-hydroxycinnamic acids with no activity when tyrosine was used as the substrate. They also found when using 4-methylcatechol as the substrate, a marked inhibition at concentrations above 10 mM (Interesse *et al* 1981). The Km value was found to be 5.13 mM indicating that the isoenzyme had a low affinity for this substrate. Lamkin and coworkers (1981) using a Clark polarographic electrode investigated a number of wheat classes using a variety of substrates. High tyrosinase activity as well as an affinity for caffeic acid, dopamine, d-catechin and catechol was detected in over 30 different varieties. The authors stated that in many cases differences in polyphenol oxidase activities could be used to distinguish between wheat cultivars. They also found that hard red spring wheats tended to have activities close to those obtained for hard

red winter wheats although varietal differences did exist. Durum wheats could be distinguished from any other class based on polyphenol oxidase activity levels alone.

Interesse *et al* (1982) also investigated o-DPO in durum wheat and found that it displayed no creosolase activity. This lack of activity was attributed to structural modification taking place during the protein purification. The preferred substrate was 4-MECAT as was the case for the common wheat. The pH activity profile for the durum wheat also displayed two pH optimum peaks at 5.3 and 7.3. The pH 5.3 activity peak, however, showed only 55% of the activity of the pH 7.3 peak. Comparison to common wheat indicated an alkaline shift of 0.4 pH units for the major peak as well as a broader plateau between pH 7.3 to 7.7. Isoelectric focussing revealed only 3 active fractions with pI's of 5.4, 6.8, and 9.4. The alkaline fraction exhibited the greatest specific activity.

During the purification of both the common (Interesse *et al* 1980) and durum wheat enzyme latent enzymatic activity was observed after calcium phosphate gel treatment. This phenomena has been shown by this enzyme from a number of sources. Theories to explain this latency include activation by rearrangement of the enzyme's tertiary structure and the existence of latent form as an enzyme-inhibitor complex (Mayer and Harel 1979).

Marsh and Galliard (1986) found that 53% of the total enzyme activity remained in the insoluble residue after

extensive extraction with various aqueous buffer solutions. Their work indicated that even if solutions containing SDS or Triton X-100 were employed, the bulk of the activity remained insoluble. They also indicated a relatively broad pH profile ranging from pH 6.0 to 7.0. Their work indicated that the polyphenol oxidase activity in wheat grain was associated almost entirely with the bran fraction as minimal activity was found in the flour and none in the wheat germ. An important point observed was that material used by the oxygen polarograph method should be either freshly milled or stored frozen. This eliminated the interfering peroxidation of polyunsaturated fatty acids which accumulated during storage at ambient temperatures. They found a Km value of 5mM for catechol. Marsh and Galliard displayed that the polarograph method was linear over the 0-0.40g sample size per 4 ml range.

2.03 Alpha Amylase

Alpha amylases (1-4 glucan-4-glucanohydrolases, E.C. 3.2.1.1) are endoenzymes which hydrolyze the internal glycosidic linkages of starch yielding smaller glucose oligomers which have the alpha configuration at the anomeric carbon of glucose (Banks and Greenwood 1975). The primary role of this enzyme is to mobilize and solubilize the kernel's starch reserves thus providing an energy source for the germinating embryo.

There are two major groups of alpha amylase isozymes present in wheat. Isoelectric focussing indicates the alpha I group is acidic in nature with pI's ranging from 4.6-5.0 and is sometimes referred to as the "green" form. The alpha II group, found with the onset of germination, has a pI of 6.2. Although mainly appearing at germination, the alpha II group, contributes the bulk of the enzymatic activity. Isoelectric focussing (Nishikawa and Nobuhara, 1971, MacGregor, 1978) of each group has shown numerous electrophoretically distinct species. Marchylo *et al* (1980) displayed up to 22 separate alpha amylase components in wheat. The degree of multiplicity within a group was found to be cultivar dependant.

Investigation of cereal alpha amylase has shown it to be monomeric in nature with a molecular weight of 40-58,000 (Greenwood and Milne,1968, MacGregor 1978, Tkachuk and Kruger 1974 and Silvanovich and Hill 1977). The pH activity indicated pH 5.5 to be optimum although both alpha I and II groups required calcium in trace quantities to maintain their tertiary structure and maximum activity. The enzyme was thermally stable at 70 C for 15 min, but acid labile, (Kruger and Lineback 1987) being inactivated at pH 3.4.

Alpha amylase in immature wheat was found primarily in the pericarp, (Kruger 1972) with small amounts in the seed coat and aleurone. The enzyme was detected shortly after anthesis, increasing with development before decreasing at maturity. At maturity trace quantities of germination like

alpha amylase were detected in the endosperm, (Marchylo *et al* ,1980) seed coat, and the scutellum. The amount of enzyme present in the wheat kernel was dependant on the state of the seed. The amount of enzyme increased dramatically with the degree of germination. In germinated wheat the enzyme was found in the endosperm adjacent to the aleurone and scutellum. The aleurone was the main tissue in which alpha amylase was synthesized "de novo" although the scutellum was implicated as well. Sound wheat had 50-60% (Kruger and Tipples,1980) of it's amylase activity removed during the milling process. Removal of the final reduction flour streams to reduce the enzyme's presence (Kruger and Lineback,1987) was not economically feasible.

2.04 Phenolic Compounds

Phenolic compounds encompass a large array of chemical compounds usually thought to be secondary metabolites. The common phenolic constituents of plants fall into two broad groups: the phenolic acids and coumarins, or the flavonoid compounds including anthocyanidins.

The phenolic acids are subdivided into two classes based upon their basic backbone; either the benzoic acid based acids or the cinnamic acid based compounds. Both classes are usually found in either a conjugated or esterified form. The simple benzoic acid derivatives include p-hydroxy benzoic acid, protocatechuic acid, vanillic,

gallic, syringic acids and the o-hydroxy salicylic and gentistic acids. Caffeic, ferulic, sinapic and p-coumaric are the simple cinnamic acid based phenolic acids.(Deshpande *et al* 1986)

Phenolic acids are endogenous to the wheat plant (Bose,1972,) and wheat flour (Gallus and Jenning 1971, Maga and Lorenz,1974, Sosulski *et al* 1982, Fulcher,1982, Cherney *et al*,1989, Pussayanawin *et al* 1988, Seitz,1989). Ferulic acid, the most abundant, has been shown by Fulcher (1982) to be associated with aleurone cell walls. Polysaccharide esters of ferulic and p-coumaric acid have been identified by Hartley and Jones (1977). Subsequent release of ferulic acid bound to bran cell wall carbohydrate through the action of a cellulase by Smith and Hartley(1983) resulted in a single component, FAX, which accounted for all the ferulic acid esterified to the walls. RP-HPLC of FAX consistently gave two peaks in a ratio of 2:1 based upon area. Subsequent isolation of either peak followed by chromatography yielded two peaks suggesting a rapid equilibrium between the two forms. Analysis revealed FAX to be 2-O-[5-O-(trans-feruloyl)-B-L-arabino-furanosyl] D-xylopyranose. The two forms were thought to be trans and cis isomers of the molecule. Gubler *et al*(1985) showed the presence of two feruloyl containing compounds which were released into the incubation medium when isolated barley aleurone layers were treated with giberrellic acid. One compound was identified

as O-[5-O-feruloyl-L-arabino- furanosyl]-(1-3)-O-D-xylopyranosyl-(1-4)-D-xylopyranose.

Enzymic cleavage and subsequent alkaline hydrolysis of maize arabinoxylan fragments by Nishitani and Nevins (1989) indicated diferulic acid played an important role in the crosslinking of the oligosaccharides. One fragment resulting from the enzyme cleavage had 0.5% w/v ferulic acid present.

Using an alkaline hydrolysis of the wheat bran cv. Flanders, 6.6 mg/g. of ferulic acid, 34 ppm p-hydroxy benzoic acid, and 38 ppm p-coumaric acid were detected (Smith and Hartley,1983). Values for a second variety Huntsman yielded 3.8 mg/g ferulic acid while durum displayed 5.0 mg/g.

Schwartz *et al* (1989) characterized lignin from HRS wheat bran and found both trans ferulic acid and p-coumaric acids present in a ratio of 2.6:1. Further analysis of the lignin core by alkaline nitrobenzene oxidation revealed the presence of vanillin, syringaldehyde, p-hydroxybenzaldehyde, and p-hydroxybenzoic acid.

Seitz (1989) using dissected wheat kernels quantitated stanol and sterol esters of ferulic acid. He demonstrated that these esters were associated solely with the inner pericarp. Values listed for ground grain stanyl and steryl ferulates ranged from 62-64 ppm for white wheats to 123 ppm for the hard red spring wheat Oslo. Seitz reported that the solvent used for the initial extraction significantly affected the measured total concentration of the ferulate

esters. Initial extraction of wheat aleurone with acetone yielded 73 ppm while methanol extracted only 7.3 ppm., hexane removed 13 ppm, and chloroform 38 ppm for the wheat sample. However, for corn, acetone extracted only 32 ppm compared to hexane's 88 ppm. Seitz reported that campestanyl ferulate followed by sitostanyl ferulate were the major wheat stanol esters.

Fractionation of solubilized bound niacin from wheat bran has highlighted a series of phenolic-carbohydrate components. They were found to consist of a series of mono and oligosaccharide derivatives of 2-aminophenol (2-hydroxy aniline). Enzyme studies indicated the sugars were linked to the phenolic hydroxyl groups.

Maga and Lorenz (1974) showed the presence of 16 different simple phenolic acids in five different wheat flours with vanillic acid being the major component in each case. Ferulic and p-coumaric acids were also present in significant amounts. Surprisingly, the free acids contributed the bulk of the phenolic compounds. Total vanillic acid values for the wheat ranged from 36 to 41 ppm, while ferulic acid went from 25 to 32 ppm. p-Coumaric acid stayed relatively constant at 21 to 25 ppm. Similar trends and values were reported for the four triticale flours also investigated in their paper.

Sosulski *et al* (1982) showed that cis and trans-ferulic (1.2ppm), vanillic (0.6ppm) and syringic(0.5ppm) acids were the principal free acid phenolic compounds in the wheat

flour. The soluble bound phenolic esters consisted of ferulic (3.8 ppm), vanillic (3.0 ppm), and syringic(2.3 ppm) acids. Alkaline hydrolysis of the insoluble residue indicated the major component to be trans-ferulic acid at 58.6 ppm with 1.4 ppm syringic acid present. Ferulic acid accounted for 89.1% of the total phenolic acids found in their study. Other phenolic acids such as sinapic, caffeic, and protocatechuic were said to be present in trace amounts. Sosulski and his coworkers also highlight a major decline by approximately two-thirds in the phenolic acids of wheat flours stored for six months at room temperature.

Jackson (1983) found similar values to Sosulski with ferulic acid levels of 1.04, 3.8, and 36.1 ppm for the free, soluble, and insoluble phenolic acids respectively. He indicated that in overmixed doughs, the free and soluble forms dropped to one-third of their unmixed value. No other phenolics were reported except as trace amounts.

Kuninori and Nishiyama (1986) using a single methanol extract of a Canadian white wheat reported values for both free and soluble bound ferulic acid levels in the bran, germ and endosperm. The bran had 14.2 ppm free ferulic acid compared to 2.80 ppm in the germ. The endosperm showed a limited quantity as only 0.114 ppm free ferulic acid and 0.217 ppm soluble bound ferulic acid was found. The germ and the bran had significantly higher bound values of 31.3 and 29.4 ppm respectively.

Pussayanawin *et al* (1988) using a different extraction procedure found values considerably higher, ranging from 24.4 to 795 ppm for total ferulic acid in the milling fractions of four different HRW wheats. The extraction procedure entailed a rapid 30 min acid hydrolysis using 0.2 N sulfuric acid maintained in a boiling water bath. The resulting extract was then incubated with alpha amylase prior to dilution with distilled water. The diluted extract was utilized directly in subsequent HPLC analysis. Their HPLC values corresponded well with relative fluorescent intensity measurements of the same mill streams yielding a correlation coefficient of 0.97.

Fulcher *et al* (1988) using quantitative fluorescent microscopy was able to define and image a single whole aleurone cell and adjacent nucellar and pericarp walls based on ferulic acid emissions at 420 nm. They were able to show that the aleurone cytoplasm and starchy endosperm emitted little to no response.

2.05 Biosynthesis

Formation of the various cinnamic based phenolic acids starts with either phenylalanine or tyrosine which undergo deamination by their respective ammonia lyases to yield cinnamic or p-coumaric acids respectively. The cinnamic acid produced from phenylalanine is converted to p-coumaric acid by the action of cinnamate-4-hydroxylase. The production of

caffeic acid is accomplished by further hydroxylation by the microsomal enzyme p-coumarate-3-hydroxylase. All of the enzymes to this point are membrane associated, mixed function oxygenases. Conversion of caffeic acid to ferulic acid is by catechol O-methyltransferase using S-adenosyl-L-methionine as the methyl donor. Introduction of a third hydroxyl group to form 5-hydroxyferulic acid prior to a second methylation by catechol O-methyl transferase results in the formation of sinapic acid.

The most important mechanism for benzoic acid based phenolic biosynthesis is through side chain degradation, via acetate removal, to yield the corresponding acids. Hydroxylation and methylation of benzoic acid itself has also been shown to occur.

The toxicity of free phenolics may account for the fact that most of these compounds in living tissue are found as glycosides. In vivo synthesis of glycosides has a dual role, deactivation of the phenolic nucleus and providing greater water solubility.

One of the major biological properties of phenolic compounds is their antimicrobial activity. It is often assumed that this is one of their major roles in plants (Friend 1979). Methyl esters of ferulic and 3,4-dimethoxy cinnamic acid inhibit a wide range of rust fungi. In onions, catechol and protocatechuic acid act as inhibitors to certain spores. Chlorogenic acid, widely distributed in the plant kingdom has been associated with resistance to fungal

attack. The fungus responsible for brown rot in apples is inhibited by the oxidation of endogenous phenolics by polyphenol oxidase. A similar reaction is found in cotton and red clover (Friend 1979). Hydroxy benzoic acids, particularly p-hydroxy benzoic, salicylic and vanillic acid at phytotoxic levels have been found in apples and potatoes. Certain varieties of rice and potatoes resistant to plant pathogens have shown an increase in phenolic acid biosynthesis upon infection. Friend and Threlfall (1976) showed in infected plants that an increase in the production of polysaccharide bound phenols occurred. This observation in conjunction with oxidative cross linking of the cell wall pectin polymers rendered the cell wall impenetrable to fungal hyphae.

The role of phenolic compounds also have been found in dying plants to acts as reproductive inhibitors to herbivorous rodents feeding on these plants. Two phenolics in particular, ferulic and p-coumaric acid have been implicated (Harborne 1979). Harborne also discussed evidence for the role of free phenols from leaf washings acting as phytotoxins by acting to inhibit the growth of annual grasses in the plant's proximity.

Lignin is considered to contribute to the compressive strength of the cell wall. The cinnamyl alcohols are the primary building blocks of lignin being derived from phenylalanine via the corresponding cinnamic acid pathway. The various cinnamic acids are then activated to form

coenzyme A thioester by the enzyme 4-coumarate:CoA ligase. It has been suggested that there are two forms of this enzyme, each specific for either caffeic and p-coumaric acids, used in flavanol biosynthesis, and a second form for ferulic and sinapic acids. It has been observed that the percentage of syringyl residues in lignin increased with plant age. Reduction of the cinnamoyl CoA thioesters to their corresponding alcohols is specific to lignin biosynthesis. The two step process, involving the formation of the aldehyde and then the alcohol requires two molecules on NADPH. The enzymes responsible are cinnamoyl-CoA:NADPH oxidoreductase, followed by cinnamyl alcohol dehydrogenase. The resulting monolignols are then polymerized via the action of peroxidase. The initial reaction is the generation of mesomeric phenoxy radicals which couple to form a dilignol. This process continues yielding the macromolecule lignin.

Flavanoids consist of six major subgroups based upon a C 15 chemical backbone. Other than providing a color function , the flavanoids have also been suggested to be protection against UV light and infection by phytopathogenic organisms. The initial biosynthesis of all the subgroups is closely related. The carbon skeleton of rings A and C are derived from three acetate and 1 malonate acids. The third ring ,B, comes from the cinnamic acid pathway and is usually introduced as a coumaric or caffeic thioester of Coenzyme A. The intermediate in the initial stages of biosynthesis is

the chalcone. The reaction involving the incorporation of the thioester cinnamate derivative into the initial ring structure is accomplished by chalcone synthase. This reaction is considered to be the key step in flavanoid biosynthesis. It is from the basic chalcone structure that the other subgroups are synthesized. There are two pathways in the specific flavanoid biosynthesis. The chalcones lie in an equilibrium with the flavanones through the action of chalcone isomerase. From the initial chalcone structure flavones and isoflavones are produced. If the starting material is flavanone subsequent flavanols and anthocyanidins are formed. As the C ring of the anthocyandins undergoes increasing substitution the deeper the blue color. As the hydroxyl groups are methylated the red deepens.(DeMan,1976) As the pH becomes more alkaline the color of the anthocyanins lightens. Most flavanoids occur as glycosides with different combinations of sugars attached to their hydroxyl groups. The sugars are often further substituted by acyl residues such as p-coumarate, caffeate, and ferulate. The flavones and flavanols are so similar in structure except for the double bond in ring C that the same enzymes hydroxylate and methylate the B ring. Flavanoids from wheat bran have been identified by Feng *et al* (1988) and Feng and McDonald(1989) to be two di-C-glycosyl flavones. These are 6-C-pentosyl-8-C-hexosyl and 6-C-hexoxyl-8-C-pentosyl apigenins.

The glycosylation of the flavanoids also plays an important role in protection from oxidation by polyphenol oxidase. Phenolases from potato and tea did not oxidize flavonols glycosylated in the 3 position but did attack flavonols-7-glycosides. The reason for the protective effect of the 3-glycosylation may be due to flavanoid's structure. In order to be a substrate for the enzyme the molecule must have a planar structure. Substitution of the bulky sugar forced the flavanol to take a non-planar confirmation (Harborne 1979).

A criteria used by breeders in the wheat selection procedure against sprouting damage is grain coat redness (Gordon 1979). The role of the red pigment and dormancy has been suggested by Miyamoto and Everson (1958). Flavan-3-ols and flavan-3,4-diols have been found to be major precursors of phlobaphenes, Brown (1964), which have been shown to be present in the grain coat. Polymerization of these precursors to phlobaphene involves the enzyme polyphenol oxidase (Taneja and Sachar, 1974, Taneja *et al* 1974). Gordon,(1979), suggested that flavanols in vivo were not associated with embryo dormancy. However, comparison of the embryo dormancy development curves of white and red grained wheats indicated that the point of departure between the wheats corresponded with the formation of the red pigment.

2.05 Phenolic Acid Interactions

Baker *et al* (1943) reported aqueous extracts of wheat flour were capable of forming viscoelastic gels when treated with small amounts of oxidizing agent. This form of oxidative gelation occurred without heating or cooling and only in the presence of an oxidizing agent. Neukom and Markwalder (1978) stated no other natural polysaccharides displayed this property and it could not be explained by conventional polysaccharide chemistry. Ferulic acid was shown to be released from these polysaccharides upon alkali treatment. They were also able to show the presence of diferulic acid in the gel fraction. The dimer was formed by oxidative coupling of two adjacent ferulic acid residues causing crosslinking of the arabinoxylans. Neukom and Markwalder were able to artificially synthesize diferulic acid by reacting ferulic containing pentosans. Earlier work by Markwalder and Neukom(1976) had shown the existence of diferulic acid as a natural component of insoluble pentosans and they assumed that oxidative crosslinking took place sometime during kernel development.

Schroeder and Hoseney(1978) reported that a water soluble extract of wheat flour was responsible for dough breakdown during over mixing. The resulting gluten/starch fraction, GS, showed no breakdown during mixing. KIO_3 had no effect on the GS fraction but fumaric acid, a double bond compound, did. Addition of both KIO_3 and fumaric acid

accelerated the rate of dough breakdown. Similar results were obtained with either ferulic acid or NEMI, a sulfhydryl binding agent. Separation of the water soluble extract into fractions indicated a heat stable component was responsible, which the authors believed contained ferulic acid. They felt that the effect of activated double bond compounds on reducing mixing time and subsequent dough breakdown depended on some form of interaction between some group or radical created in the GS fraction of flour. The authors suggested that the role of the oxidants was to provide a functional group or radical that would interact with the added or indigenous activated double bond compounds, particularly ferulic acid. Support for the involvement of radicals was confirmed by the reversal of breakdown in the presence of radical scavengers butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA). Subsequent work by Sidhu *et al* (1980b) indicated that both ferulic acid and cinnamic acid could cause this effect at 250 ppm while other non-double bond phenols, (4-hydroxylphenyl)-3-propanoic acid and vanillic acid did not. Ferulic acid was considerably more active requiring 250 ppm to illicit a response while 2000 ppm were required for fumaric acid. Okada *et al* (1987a,b) confirmed the effect of the ferulic acid at this level.

Using C^{14} cysteine mixed with the water soluble extract and irradiated with UV light to generate radical formation, 67% of the radioactivity eluted in the same place as ferulic acid. A corresponding increase in protein was

also found at this point. The authors suggested that the irradiation mimics the formation of thiyl radicals caused by the rupture of disulfide bonds during mixing. The resulting radical combines with ferulic acid or other activated double bond compounds esterified to the water soluble pentosans thus covalently binding some proteins to pentosans during mixing. The introduction of the carbohydrate on the gluten protein would profoundly effect the dough's rheology. Graveland *et al*(1979) had claimed to have found carbohydrate bound to gluten protein.

Kuninori *et al* (1976) were able to demonstrate that a water extract of mushrooms and commercially obtained mushroom tyrosinase were found to have an oxidative effect on unfermented dough. In extensigraph response measurements 1.5 g of mushroom extract produced a response equivalent to 6 mg of potassium iodate.

Using C^{14} fumaric acid Sidhu *et al* (1980a) found most of the double bond acid associated with the gluten protein after dough mixing. Although most of the free sulfhydryl groups in flour are found in the water soluble fraction, only a small amount of C^{14} fumaric acid reacted with it. Hydrolysis of the gluten protein with pronase gave a single radioactive compound, S-succinyl-L-cysteine, the saturated acid counterpart to fumaric acid, covalently bonded to cysteine.

Jackson and Hoseney (1986a) reported that during mixing of wheat flour doughs, free and soluble bound ferulic acid

was lost from those fractions which initiated breakdown. They were able to show that free soluble ferulic acid levels declined from 1.0 to 0.3 ppm during mixing while the soluble bound ferulic acid dropped from 3.8 to 0.9 ppm. Surprisingly, the insoluble bound ferulic acid remained unchanged at 36 ppm. However, they stated that simple differences in endogenous concentration of water soluble ferulic acid was not sufficient to explain poor mixing tolerances between good and poor flours. This was confirmed when the water extract from a poor mixing tolerant flour was substituted for the same extract in a good flour without conveying its effect. The reverse substitution also did not impart improved tolerance upon a poor flour. They felt that the ferulic acid was necessary for the rapid dough breakdown process, but factor/s remaining in the gluten/starch fraction control the flours tendency to poor mixing tolerance.

Subsequent work by Jackson and Hoseney (1986b) artificially synthesized an adduct of ferulic acid and cysteine. The adduct itself had a negligible effect on dough rheology. The authors were able to indicate, in a pronase digest of dough, a small peak which eluted at the same retention time as the artificial adduct. They were unable to isolate this component, due to minimal concentration, to confirm its identity.

An alternative model for the involvement of ferulic acid was proposed involving the oxidized phenolics reacting

with the thiols of proteins. Brenna *et al* (1988) using artificially synthesized pentosans esterified with ferulic acid, 3,4-dimethoxy cinnamic acid, and 3-(4-hydroxyphenyl) propionic acid found that only 3,4-dimethoxy cinnamic acid did not undergo gelation in the presence of both peroxidase and polyphenol oxidase. Further work with these esterified pentosans in wheat gluten showed that development time was shortened for both ferulic acid and the 3-(4-hydroxyphenyl) propionic acid esters. The volumes and density of the bread was normal and the texture fine and regular. The authors stated that this research demonstrated it was the hydroxyl group which was the determinant.

The number of reactions in which an oxidized phenol can take place are numerous. Of particular relevance to the wheat gluten sulfhydryl involvement is an alternative reaction which has been demonstrated to occur in wine (Singleton, 1987). The oxidized quinone reacted with the sulfhydryl to generate a thioester. The bond was to the aromatic ring rather than the double bond as proposed by Jackson and Hoseney (1986b). This reaction in wine between caftaric acid (caffeoyl tartaric acid) and glutathione was confirmed by the isolation of the reaction product (Cheynier *et al*. 1989). Pierpoint (1969) was also able to demonstrate a similar product formation using chlorogenic acid and cysteine. This form of the reaction product would not deviate from the reported results of Hoseney over the years but only with the resulting adduct's structure.

The influence of polyphenol oxidase on sulfhydryl involvement had also been shown by Pierpoint (1969) to generate a disulfide linkage.

It should be recognized that the quinone may also react with the amine groups of a protein. These include a Michael addition of the E-amino group of a lysyl residue to the quinone ring, confirmed by Kalyanaraman *et al* (1988), or by the formation of a Schiff base by the reaction with the keto group.

The products formed by the crosslinking of proteins with benzoquinones are brown and have a reduced solubility and nutritive value.

2.07 Phenolic Color Production

Phenols, especially o-diphenols, are well known to polymerize as the result of oxidation to produce colored products (Singleton, 1987). The mechanism of the polymerization can be divided into two categories; generation of the reactive components and polymerization itself. In auto-oxidation, the presence of oxygen causes the generation of two semiquinone radicals. These semiquinone radicals then proceed to form a quinone and the initial diphenol, or they can combine to generate a shared covalent bond. Hydrogen movement across the molecule by isomerization results in the regeneration of a linked dimer diphenol. The

production of the initial semiquinone radicals can also be accomplished by the interaction of a source independent quinone and a diphenol. A quinone can also react with nucleophilic centers, such as meta polyphenols, like phlorglucinol, or the A ring of flavanoids. Such a flavanoid reaction would incorporate into the polymer a phenolic compound not originally involved in the oxidation process.

Once the initial polymerization begins the resulting product usually undergoes further polymerization. The redox potential of the polymer is lower than the original phenol making it more susceptible to reaction than the initial compound. The resulting diphenyl quinoid absorbs more intensely in the visible spectrum and to the eye becomes browner. An example, catechol, displays a maximum molar extinction coefficient at 214 nm of 6300. The resulting para-linked dimer had a very noticeable color with an extinction coefficient of 69,000 at the visible wavelength of 400 nm (Singleton 1987). The auto-oxidation process appeared to be a cascading effect as each resulting product falls down the redox potential "hill" speeding up the ease of future oxidation (Singleton 1987). Since the resulting polymer is more susceptible to oxidation than its precursors, the survival of the initial components to some extent usually occurs.

The role of the enzyme polyphenol oxidase was to generate the initial quinone at a rate far faster than in auto-oxidation. All the further reactions resulting in

"brown pigments" and other products were nonenzymic in nature (Singleton 1987).

The quinones produced by either means were found to be very reactive and interact with a number of different compounds other than phenols (Singleton *et al* 1984, 1985, Singleton 1987). The wine industry has particular interest in the reaction involving caftaric acid (caffeoyl tartaric acid), the major phenol in grape juice. The compound could be easily oxidized by endogenous polyphenol oxidase. Extensive study (Singleton *et al* 1984,1985, Singleton 1987) indicated that differences between grape varieties' color production was due not only to the enzyme levels, but also glutathionine concentration. The resulting quinone did not polymerize, but rather reacted with glutathionine to produce a colorless product. The o-quinone reacted with the glutathionine through a 1,4 addition of the sulfhydryl to make a thioether link with the ring. This may explain the inhibition of browning enzymes by sulfhydryl compounds. The reaction of a o-quinnone with a -SH group yields a colorless product while reaction involving the E-amino group of amino acids produces a red or brown compound (Pierpoint 1969). Taylor and Clydesdale (1987) examined colors produced by reacting a number of phenolics in the presence of polyphenol oxidase with both peptides and amino acids. Reactions with proline yielded a bright purple while alanine, glutamine, arginine, gelation and casein hydrolysate yielded orange-brown colors.

2.08 Flour Color

Other than clear liquids, the color of foods is mainly a matter of reflection rather than transmission. Reflectance is the ratio of the amount of light reflected divided by the amount of incident light applied. White materials reflect equally over the entire visible wavelength spectrum. Grey and black materials also reflect equally over the spectrum but to a lesser extent. Our perception of color is based upon the degree of reflection at various wavelengths. The color of a surface can be expressed in terms of reflectance in each spectral component ranging from 400-700 nm. Objects appearing red to the human eye reflect light in the higher wavelengths, 630-700 nm, while absorbing the others. Blue colored objects do the exact opposite, reflecting in the low wavelengths and absorbing in the high region.

The Hunter system of color measurement is based upon the opponent color theory of human color perception. It assumes there is an intermediate signal switching stage between light receptors in the retina and the optic nerve which transmits color signals to the brain. In this switch, red responses are compared with green responses and result in a red-green dimension, designated (a^*). The green response is compared with the blue to also yield a yellow-blue color dimension designated (b^*). Brightness, L^*, is the third dimension. The values of the Hunter system can be

converted to CIE values. The change in color of a material can be observed and monitored by utilizing the L^*, a^*, and b^* values. However, our own perception of color does not discriminate each of these variables, rather a total color change is noted. Total color change, Eab, in a substance can be expressed by the following equation.

$$Eab = (L^{*2} + a^{*2} + b^{*2}) ^{0.5}$$

Quantitation of flour color has generally been based upon the degree of reflectance of the sample flour or slurried flour paste at 530 nm. The Kent-Jones and Martin flour grader are the standard instruments with grade color figure values (GCF) being assigned on an scale of -5 to +18. The lower the GCF values the brighter and whiter the sample. Over the years the GCF had been used by millers to indicate the degree of bran contamination in their flour samples.

However, Barnes (1986) using dissected endosperm from two different samples of wheat kernels of the variety Flanders, found the GCF to differ by 2.0 GCF units. The corresponding flours from the two samples had GCF differences of 2.4. Variation in greyness of dissected endosperm pastes were examined on 12 separates varieties. The range of values extended from -1.3 to +3.1 GCF units. Barnes attributed the differences to an intrinsic character found in the endosperm of the variety and not due to the bran content. He found that endosperm greyness could account for three out of four units by which flours varied leaving only one unit to be accounted for by the difference

in bran content. Barnes stated this finding contradicts the original premise used over the years that the major factor causing differences in GCF is due to the bran content. It was also of interest that unpigmented white wheats had endosperm with greyness values similar to those found in red wheats. He went further to suggest that the endosperm paste reflectance was influenced by the endosperm protein content. Grain grown at the same site but with different fertilizers levels resulted in varying protein values. Plotting endosperm reflectance at 540 nm versus protein content for the variety Copain revealed a linear relationship . Further evidence for protein involvement was shown by using dissected endosperm from mealy endosperm and vitreous endosperm grains of the same variety. Barnes was able to show a negative relationship between protein content and endosperm paste reflectance.

The other standard measurement of flour color is the Agtron test for flour (AACC Method 14-30,1983) which in the green mode measures, via reflectance, the presence of non-carotenoid pigments.

A number of different factors influence the final color of a flour sample. Aside from the key varietal differences, the degree of milling or extraction percentage of the flour from the initial wheat is very important. It has been shown by numerous authors,(Yasunaga and Uemura 1962,Barnes 1986) that extraction rate influences the color of the product. Generally, extraction of up to 70% has little influence on a

specific wheat sample's flour color. However as the percentage of flour increases past this point the GCF increases rapidly as the higher extraction results in a perceptible darker flour. Kruger and Reed (1988) reported that the typical patent flours have GCF values of 0-4.0 and straight grade flours of up to 72 % extraction, 4.5-6.5 GCF. As the extraction percentage on a straight grade flour increases to 85% they reported GCF values ranging from 9-13.5 while at 90% extraction the GCF rose to 13.0-15.0.

Yasunaga and Uemura (1962) found the flour color to be the most important criterion for assessing whiteness and brightness of noodle products. They stated that flour color was affected by grade, extraction rate, and the color characteristics of the endosperm.

Hook (1985) investigated the relationship between GCF and various Hunterlab values. He found that not only was the GCF related to the whiteness using the L^* scale, but also to the redness (a^*) value. The overall correlation between GCF and (a^*) value were variable but very good correlations were seen for individual samples. Using the variety Flanders he was able to show 3 straight-line relationships with correlation coefficient ranging from 0.93-0.98 for the variety grown on 3 different sites. The overall correlation coefficient however dropped to 0.63. Hook (1985) reported similar results for nine other varieties as well. Miyamoto and Everson (1958) had found a positive correlation between kernel color and the quantity of catechin and catechin

tannin present, particularly the reddish brown pigment phlobaphene, in the seed coat. Hook (1985) utilized this relationship to confirm that an increase in GCF is in part due to bran content. Careful examination of increasing extraction rate flour showed that they pass through a breakpoint were there is a rapid increase in the rate of change in bran content. Hook (1985) estimated that past this break-point the bran content increases on average 15 times greater than before it. He pointed out that the break-point differs considerably for different wheats and as such the position of the break-point when assessing the milling value of a wheat is very important. Comparison of the break-point for ash values versus extraction rates indicated that they were not the same as breakpoint between GCF and extraction rate. In the 18 wheats investigated by Hook (1985) the break-point for ash occurred at a lower extraction rate than for color and varied by up to 3.5% on the extraction rate axis. The differences in the break-points may reflect the differences in factors responsible. The color component is thus supplied by the bran while the ash reflects the influence of the aleurone. Considerable differences were found in the rate of change of bran content in flours after break-point as on average approximately 15 time greater than before it. Hook reflects that one must consider both the break-point and the rate of incorporation of bran in flour after this point when assessing the milling value of a wheat.

Examination by Hook (1985) of the xanthophyll content of both wheat and mill streams indicated that the pigment content was a varietal characteristic and was site independent. Furthermore, examination of a variety's mill streams indicated no significant differences in xanthophyll content as the extraction rate was increased although large increases in GCF were found.

Particle size of dry flour affects reflectance as larger particles appear darker due to casting shadows. Conditioning of wheat prior to milling, in particular conditioning temperature, influenced the final flour color (Robinson *et al* 1984).

Environment can also influence the color of flour as seen by Shuey (1976). Wheat plots at four different locations over two years were investigated. Examination of two varieties grown both years, Chris and Kitt, indicated inconsistencies in flour extraction, ash, and color amongst varieties and between stations. Chris, although displaying essentially the same average flour extraction and ash between years showed marked differences in flour color as determined by Agtron readings. Kitt on the other hand had crop year averages for ash and flour color in general agreement, yet an individual site, Minot, had data inconsistent with the averages. Shuey summarized by stating "...do indicate that the year, location, entry, or cultivar and the interaction of these factors can influence flour color as well as flour ash."

Irvine and Anderson (1952) established the correlation between flour brightness and crumb brightness in the final product using visual scoring. Pomeranz (1960) using the Kent-Jones and Martin color grader confirmed the good agreement between flour color grade and the resulting bread crumb color value.

Wheat yellowness has an important role in the production of acceptable white flour and the final end product. Researchers have found that wheats under different environmental conditions contain varying amounts and composition of yellow pigment. Initial study of the yellow pigments in wheat were wrongly attributed to carotene and subsequent study has found that principal pigments in wheat flour are free and esterified xanthophyll compounds which have an absorption band at 435.8 nm.

2.09 Noodles

Noodles are a form of pasta made from flour, rather than semolina or farina, containing salt and water. Oriental noodles, other than won ton, contain no eggs, and account for 40% of the wheat consumption in Asia (Hoseney 1986). A variety of noodle types exist depending upon the ingredients, percent moisture, and the degree of cooking prior to sale. The Japanese and the Chinese both produce raw noodles although in some cases, the addition of alkaline salts in the Chinese (Hokkien Mee) noodle is used to produce

a distinct yellow color. The Japanese noodles are usually thicker than the Chinese counterpart but both are sold either raw or dried. The Japanese Instant noodle has gained considerable useage in the past 25 years in S.E. Asia . The noodle is made in the same manner as the raw noodle but then steamed for 1-3 min prior to drying or deep frying.

Yasunaga and Uemura (1962) found that increased extraction rate was the major cause of color deterioration in Japanese noodles. Japanese instant noodle brightness and yellowness were also correlated with extraction rate by Moss (1982). Moss (1971) found that for Japanese noodles the brightness was inversely proportional to the flour's protein. He was able to show that the discoloration was based upon the degree of darkening developed by gluten washed from the respective flour. Subsequent work by Moss (1971) on flour streams showed that glutens prepared from different mill streams gave similar color readings for a particular variety but marked differences between glutens from different varieties. Miskelly (1984) working with Australian wheats, showed that flour brightness was negatively correlated with mineral content, protein content, and the degree of starch damage. Mineral content exerted the greatest influence on flour brightness. Flour paste brightness was also correlated with both brown and yellow pigment content. Moss (1989) stated that brightness appears to be the more significant component of color over yellowness in noodle acceptance by the consumer.

The flour yellowness, as measured by a Hunter Color Difference Meter was highly correlated with the yellow pigment (Miskelly 1984). Flour carotenoids, especially xanthophyll, along with flavones, were responsible for the yellow color of the flour. Tricin was suggested to be the major wheat flavone component. Moss (1967) found significant varietal differences in the yellow pigment content of flours milled from Australian wheat varieties. Although Moss reported no significant relationships were found to exist between the yellow pigment content and protein or extraction rate Miskelly (1984) indicated that for Chinese noodles, both yellowness and brightness, were related to flour extraction rate over the 60-72% range. Australian soft wheats were found to have more yellow pigment than the red wheats (Miskelly 1984). The degree of yellowness may be governed by the extent of contamination by the flavone rich germ (Moss 1974). Miskelly (1984) found that the flour's brightness was inversely related to its yellowness. Moss (1971) stated that although Australian wheat varieties differ in their yellow pigment content this influence was not apparent in their respective noodles except for highly pigmented varieties.

The normal pH of Chinese raw noodles is pH 9-11. Although alkaline ingredients toughen the dough and alter the paste properties, they also serve to inhibit enzyme activity and suppress enzymic darkening (Moss *et al* 1986). Moss and his coworkers were able to show that the color of

the prepared noodle was the result of the nature of the alkaline component. Noodles prepared with only NaCl were white to cream, but alkaline salts induced the yellow color. The use of potassium salts in the preparation induced a green hue while sodium salts resulted in a clear yellow product. Using two different mixtures of $NaCO_3$ and K_2CO_3 in the Kan Sui reagent yielded minor differences in the brightness as well. Addition of NaOH as the alkaline component at levels above 1% were found to eliminate the normal discoloration of raw noodles on standing. A side effect of the nontraditional use of NaOH is that although making the noodles a brighter yellow, NaOH accelerates the noodles' breakdown after cooking.

Protein content ranked second outside of actual flour color, as the most important factor affecting the color of the fresh Japanese noodle. Flours with lower protein gave the whitest noodles. Moss (1971) stated that at the same protein level, some varieties produce a brighter noodle than others, but the level of protein has a much greater effect than varietal differences. The preferred protein level for Japanese noodles is 9-9.5% (Moss 1982) while for Chinese noodles 9.5-13.3%. Although lower protein levels are preferred a compromise must be struck as sensory eating quality decreases with decreasing protein levels. Once the noodle was boiled the color became inversely related to the yellow pigment and the protein correlation was no longer significant (Miskelly 1984). Raw Chinese noodle brightness

was inversely related to the protein and brown pigment content as determined by Matsuo and Irvine (1967). Matsuo and Irvine found that the brown pigment was associated with a water soluble copper containing protein. Boiling removed the brown pigment as a contributing factor to color as the material dissolved in the cooking water. The initial flour's yellowness and the yellowness derived from the Kan Sui reaction however remained a factor.

3.0 MATERIAL AND METHODS

3.01 Wheat Samples

The wheat varieties selected were chosen to represent five distinct classes of Canadian wheats. The varieties picked were Katepwa (Hard Red Spring), Glenlea (Canadian Utility), HY320 (Canadian Prairie Spring), Norstar (Hard Red Winter) and Fielder (Soft White Spring). Purity of the variety was ensured by selecting from certified pedigree seed. Samples of Fielder and Norstar were purchased from the Alberta Wheat Pool while the remaining varieties were supplied by local Manitoba dealers. One tonne of each variety was purchased from the certified dealers, cleaned, and thoroughly mixed to ensure a homogeneous stock prior to milling. Specific details characterizing the individual wheats can be found in Table 1.

3.02 Alpha Amylase Assay

The method employed for the determination of alpha amylase levels in both the flour and ground grain was the nephelometric method American Association of Cereal Chemists, (AACC) 22-07 as modified by Kruger and Tipples (1980). One gram of sample was extracted for 1 h with 5 mls of 0.2M sodium acetate, 0.001 M $CaCl_2$, pH 5.5, with constant agitation. The mixture was centrifuged for 20 min at 15,000 G and the resulting supernatant filtered through

Table #1

Characterization Of Wheats Used In This Study

VARIETY	FALLING NUMBER (SECS.)	MOISTURE %	PROTEIN (13.5%) m.b.
Katepwa	405	11.8	15.7
Glenlea	385	11.5	13.1
Norstar	375	11.9	12.0
HY320	335	12.0	11.4
Fielder	380	11.8	9.0

mb = moisture basis

glass wool. A 0.200 ml sample of extract or diluted extract was mixed with 3 mls of waxy maize B-limit dextrin, preincubated at 37°C, and monitored on a Perkin Elymer Model 191 Alpha Amylase Analyzer. Alpha amylase values were calculated against a fungal amylase standard curve and reported in $x10^{-3}$ mg maltose/min /g sample.

3.03 Polyphenol Oxidase Assay Conditions

For the analysis of polyphenol oxidase the procedure of Marsh and Galliard (1986) was employed with minor modifications to assay temperature to maximize enzyme detection. A Yellow Springs Model 5300 Biological Oxygen monitor with a standard membrane was employed. The temperature was maintained at 37°C by a circulating water bath. The assay medium consisted of 4 ml of air saturated 0.01M McIlvaines buffer pH 6.8 (Kruger 1976) equilibriated at 37°C. The system was allowed to stabilize with stirring prior to the introduction of 10 to 200 mg of ground wheat or flour sample. A homogeneous solution was ensured by rapid stirring of the solution with a dissection pick before sealing with the electrode plunger. All trapped air bubbles were removed by manipulation of the electrode plunger. The suspension was then monitored for 5 min to establish endogenous oxygen consumption. A fresh substrate solution of 0.8 M catechol in 0.01 M McIlvaines buffer was prepared for each 15 assays. A 0.100 ml aliquot of the 0.8 M catechol

solution was injected into the assay cell and the oxygen consumption monitored as a recorder trace for 3 to 5 min. Blanks using only the assay medium and the substrate aliquot were run at the beginning and end of a sample series to determine substrate auto-oxidation values. Oxygen consumption was corrected for both endogenous and substrate auto-oxidation prior to conversion to a per gram basis. Results were reported on a nmoles O_2 consumed / g/ min

Where enzyme levels were extremely high the assay volume was increased to 8 mls of buffer and 0.200 ml of 0.8 M catechol were injected.

All samples were assayed a minimum of three times.

3.04 HPLC System and Conditions

Analysis was carried out on a Waters (Milford, MA.) 860 Chromatography Data System consisting of two M 510 pumps, a WISP autoinjector, and a M490 multiple wavelength detector all controlled by a Digital (Digital Equipment Corporation) MicroVax 2000 computer. The analytical column was a reversed-phase Supelco (Bellefonte,PA.) LC-18, (3.3cm x 4.6 mm) preceded by a 2 cm Supelguard column of the same material. The solvent system was a linear gradient from 100% A:0% B to 82%A :18% B, over 30 mins.. Solvent A consisted of 0.1% (v/v) trifluoracetic acid (Pierce,ILL.) in water while B was 0.1% (v/v) trifluoracetic acid in HPLC

grade acetonitrile (Fisher, Rockford N.J.). The flow rate was maintained at 1.0 ml/min and peak detection was carried out simultaneously at 280, 254, and 320 nm. The HPLC analysis was done at room temperature. Calibration standards consisting of protocatechuic, vanillic, caffeic, syringic, coumaric, ferulic and sinapic acids were run with each series of samples. Each acid displayed a linear response, r > 0.98, over the 0.02 - 0.40 ug calibration series on every occassion.

3.05 Phenolic Acid Extraction

The phenolic acid composition was determined through a method similar in nature to that outlined by Krygier *et al* (1982a). The phenolic acids were separated into three forms; the soluble free phenolic acids, soluble bound acids, and the insoluble bound acids.

The initial starting material was 15 g of wholemeal or flour mixed with 100 ml of nitrogen saturated 80% acetone in a 250 ml centrifuge bottle. A stirring bar ensured constant agitation and a nitrogen blanket was layered onto the mixture prior to capping. The sample was mixed for 15 min at room temperature before centrifugation at 15,000 G for 10 min The supernatant was drawn off and placed in a sealed nitrogen environment. The process was repeated a second time using 75 ml of the 80% acetone and the supernatants were pooled.

The residual pellet was then extracted by the same two step process using nitrogen saturated 80% methanol. The resulting grist was frozen, lyophilized, and stored under nitrogen in a freezer for later analysis of the insoluble bound phenolic acids.

The acetone fraction was rotary evaporated under reduced pressure at 37°C until all the acetone was removed. The methanol extract was then added and the process repeated. The reason for keeping the extracts separate was to minimize the evaporation time as material flocculated out of the methanol extract early in the evaporation process.

The resulting aqueous solution contained both the free and soluble bound phenolic acids and was acidified to pH 2.0 with HCl. The aqueous phase was then extracted with four aliquots of an ethyl ether:ethyl acetate (1:1) mixture of volumes 100, 75, 75, and 50 ml The organic phase drawn off at each stage was pooled in a closed flask under a nitrogen, and dried over sodium sulfate. This extract contained the free phenolic acids.

The remaining aqueous phase was rotary evaporated to remove any residual organic solvent before being hydrolyzed in 2 N NaOH, under nitrogen, for 4 h at room temperature. The solution was then brought to pH 2.0 with HCl and extracted with four volumes of the ethyl ether:ethyl acetate mixture, stored, and dried as described previously. This fraction contained the soluble bound phenolic acids.

The phenolic acid fractions were filtered to remove the sodium sulfate and rotary evaporated at 37°C. The phenolic acids were solubilized in 5 ml of 100 % methanol, filtered through a Millipore 0.45 uM filter, and a 0.005-0.010 ml aliquot was injected onto the HPLC column.

The lypholized grist containing the insoluble bound phenolic acids was ground to a fine powder in a coffee grinder. A 2.00 g sample was hydrolyzed, under nitrogen, in 100 mls of 2N NaOH , for 4 hrs., with continuous stirring. An 80 ml aliquot of water was added to the hydrolyzate and the solution brought to pH 5.0 with HCl. The solution was then centrifuged at 15,000XG for 15 min and the supernatant carefully removed. The pellet was resuspended in 20 ml of water and recentrifuged. The supernatants were pooled and made up to 250 ml in a volumetric flask. A 100 ml aliquot of this fractions was adjusted to pH 2.0 with HCl. The aliquot was then slowly applied to a 1.0 g Supelcoclean (Supelco Inc. Bellefonte,PA.) C-18 solid phase extraction column (Seo and Morr 1984, Jaworski and Lee 1987) previously wetted with methanol and washed extensively with 0.05 N HCl. After the addition of the sample the column was washed with 25 ml of 0.05 N HCL. The phenolic acids were then eluted from the column by rinsing the column with 4 ml of 100% methanol. The eluant was made up to 5 ml in a volumetric flask and passed through a Millipore 0.45 uM filter prior to HPLC analysis.

All flour and wholemeal samples were analyzed in duplicate. An example of the standard phenolic acid series and an experimental chromatogram can be viewed in Fig. 1

3.06 Agtron Colors

Flour color, from sources other than carotenoids, was determined through the use of an Agtron Color Monitor, Model 500A, utilizing the AACC method 14-30. A 20 g sample, corrected to a 14% moisture basis, was mixed with 25 ml of distilled water in a clean Agtron cup. The mixture was stirred for 2 min to ensure a homogeneous slurry before being allowed to rest for 5 min . The Agtron monitor was standardized against a #63 and #85 color disk and at 7 min from the initial water addition, readings were taken at 546 nm.

3.07 Carotenoid Determinations

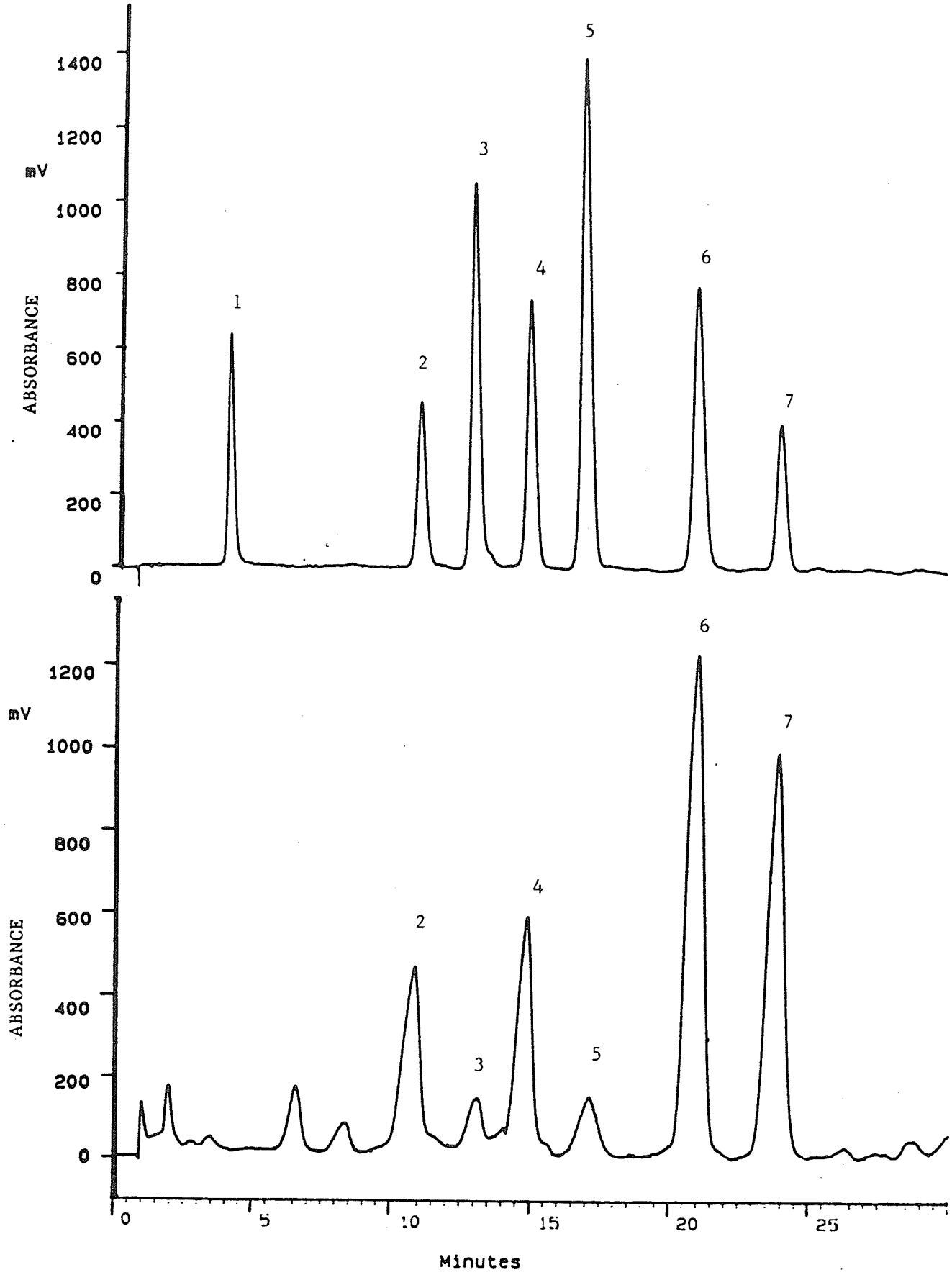
A 8.0 g sample of wholemeal or flour was mixed with 40 mls of water saturated butanol in a stoppered flask. The sample was agitated and left overnight. The sample was filtered through Whatman 1 filter paper and the filtrate's optical density read at 435.8 nm using a 1 cm cuvette. The optical density value was converted to parts per million by

Figure 1. Representative RP-HPLC Chromatograms

A. Phenolic Acid Standards

B. Katepwa 75% Extraction Straight Grade Flour Total Soluble Bound Phenolic Acids

1. Protocatechuic Acid
2. Vanillic Acid
3. Caffeic Acid
4. Syringic Acid
5. p-Coumaric Acid
6. Ferulic Acid
7. Sinapic Acid


mV
1400
1200
1000
800
600
400
200
0
ABSORBANCE
1
2
3
4
5
6
7
mV
1200
1000
800
600
400
200
0
ABSORBANCE
2
3
4
5
6
7
0
5
10
15
20
25
Minutes

multiplying by 30.05 and corrected to a constant moisture basis.

3.08 Protein Determination

Protein contents of flour and wheat samples were determined in duplicate by Kjeldahl analysis (Williams,1973) on 1 g samples. Moisture content was determined by AACC method 44-19 and protein content was expressed on a 14.0% moisture basis.

3.09 HunterLab Color

Three color components; brightness (L^*), redness (a^*), and yellowness (b^*), were determined on individual samples utilizing a HunterLab LabscanII Spectrocolorimeter (Hunter Assoc. Inc. Reston, VA.). The colorimeter was equipped with a 44 mm viewport and a light source filtered to approximate the CIE illuminant D65. The spectrocolorimeter was standardized every 2 h. All tristimulus values were based upon a 31 point spectral reflectance algorithm.

In all HunterLab paste analysis 10 g of sample were mixed with 25 ml of water or Kan Sui reagent adjusted to volume with respective aliquots of dissolved enzyme or phenolic compounds. The Kan Sui reagent consisted of 0.1 g of a 9:1 mixture of sodium carbonate and potassium carbonate made to 25 ml in a volumetric flask. The flour sample was

placed in an Agtron measuring cup, the aqueous solution added, and immediately stirred with a rubber tipped rod to form a homogeneous paste free of lumps. The sample was positioned on the colorimeter for measurement within 45 sec of addition of the reagent. A modified stirring paddle, suspended above the cup, was inserted into the paste to ensure a homogeneous solution while measurements were taken. Four measurements of the paste, each taking approximately 4 sec, were done on each sample, and automatically averaged.

The paste was then transferred to a covered beaker containing a stirring bar. The material was continuously stirred until the next timed reading.

3.10 Milling

The various wheats used in this study were milled in the Grain Research Laboratory's Pilot Mill. The mill consisted of 3 break rolls, 1 cleanup roll, 2 sizing rolls, one corrugated, one smooth, and 6 reduction rolls. An extra reduction pass was achieved by having the combined overs of the 8XX sieving from the 1st break to 6th middling being given an extra reduction on a 10" Ross mill. The mill was set up to optimize performance based upon the hard red wheat samples with minor modifications being made for the soft white wheat, Fielder. Modifications to tempering and feed rate were done for the respective millings.

In order to achieve higher extraction rates a number of changes had to be made to the mill flow. The differentials

on all four break rolls were changed from the 2:1 setting used in the 75% extraction, to 2.5:1 for both the 80 and 85 % extraction runs. Clothing changes were also made to the sieves, as the #132, was replaced by #183 Nitrex on M4-6, and B4 for the 80% runs. Due to insufficient yield the 4B and 5M shorts, 6M coarse shorts, red dog, and all the rebolt overs, excluding the bran flour were combined and fed over the 4th break. The combined overs from the 1st break to 6th middling inclusive were given three reductions over the 6" Allis-Chalmers mill. The shorts duster overs were added to the shorts to be fed to the 4th break rolls.

The highest extraction levels, 85%, were achieved by changing all flour sieves to #183 Nitex on all sifters except for the 4th Break, 5th Middling, and the 6th Middling which have the wider #202 Nitex. Elimination of sieves and changing the top sieve on the 4th break to #560 Nitex yields material containing some endosperm which would otherwise be lost.

The air to the purifiers was turned off and the last sieve of each purifiers and deck was removed in order to send more stock to the sizing roll. The mill flow was lengthened by using a portable vibratory feeder to resupply bran and 5 middling shorts back over the 4th break.

In all millings each mill streams was kept separate and pooled, as per Appendix A, to form their respective flours. **Chinese standard flour was made up as per Dexter et al (1984).**

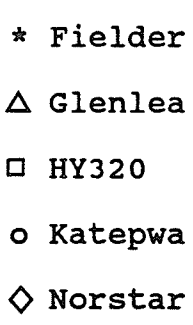
4.0 Results and Discussion

4.01 pH Activity Profile and Affinity Constants

Although the quantitative amounts of activity varied with variety, the five wheats displayed similar pH profiles as seen in Fig.2. The pH optimum was quite broad extending from pH 6.5 to 6.9. This value agreed with that reported by Marsh and Galliard (1986) who indicated a pH optimum of 6.8. The dual optima reported by Interesse *et al.* (1980) for purified o-diphenolase was not seen in these wheat samples. The alkaline optimum reported by Lamkin *et al* (1981) was not observed although considerable difficulty occurred in attempting to obtain accurate activity values above pH 7.4 due to the extreme rate of substrate auto-oxidation. The results of these analysis suggested a pH of 6.8 as the optimum for all further enzyme assays.

Utilizing 5 initial catechol solutions of 0.05, 0.1, 0.2, 0.4, and 0.8 M, the maximum enzyme rate and affinity constants for individual ground grain samples were determined. The Lineweaver-Burk plot for the individual varieties can be seen in Fig. 3 and the calculated results are displayed in Table 2. It should be noted that analysis of enzyme kinetics using ground grain will not truly reflect the pure enzyme's kinetic nature but is used in this manner

Figure 2. Polyphenol Oxidase pH Acitivity Profile in Selected Wheats



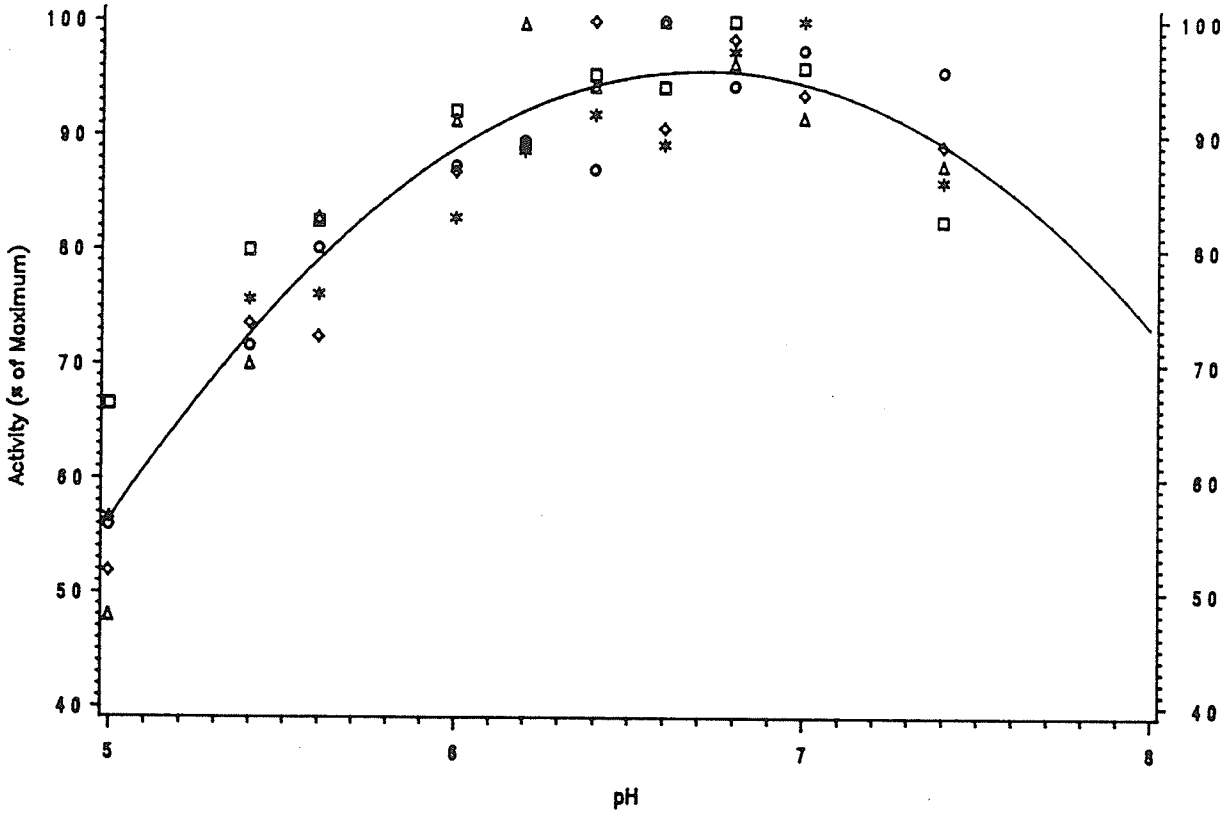

Activity (% of Maximum)
100
90
80
70
60
50
40
5
6
7
8
pH

Figure 3. Lineweaver-Burk Plots for Wheat Varieties Used in This Study (0.20 g samples)

* Fielder

△ Glenlea

□ HY320

o Katepwa

◇ Norstar

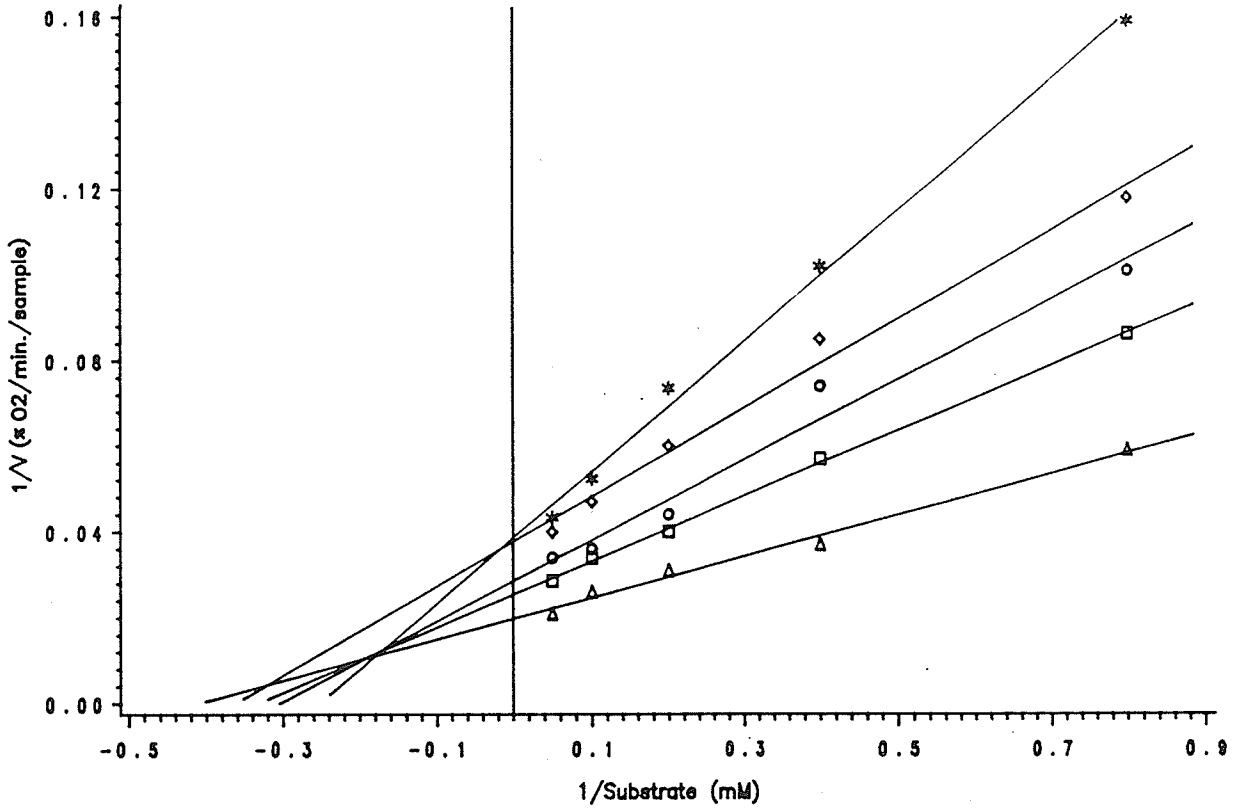

0.16
0.12
0.08
0.04
0.00
1/V (x O2/min./sample)
-0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
1/Substrate (mM)

Table 2

Lineweaver-Burk Results

Variety	V Max nmoles O_2 /g /min. Extrapolated	V Max nmoles O_2 /g /min. Experimental	Km (mM)
Glenlea	2000	1767	2.58
HY320	1561	1369	3.23
Katepwa	1372	1266	3.26
Norstar	1052	1011	2.82
Fielder	1026	864	3.84

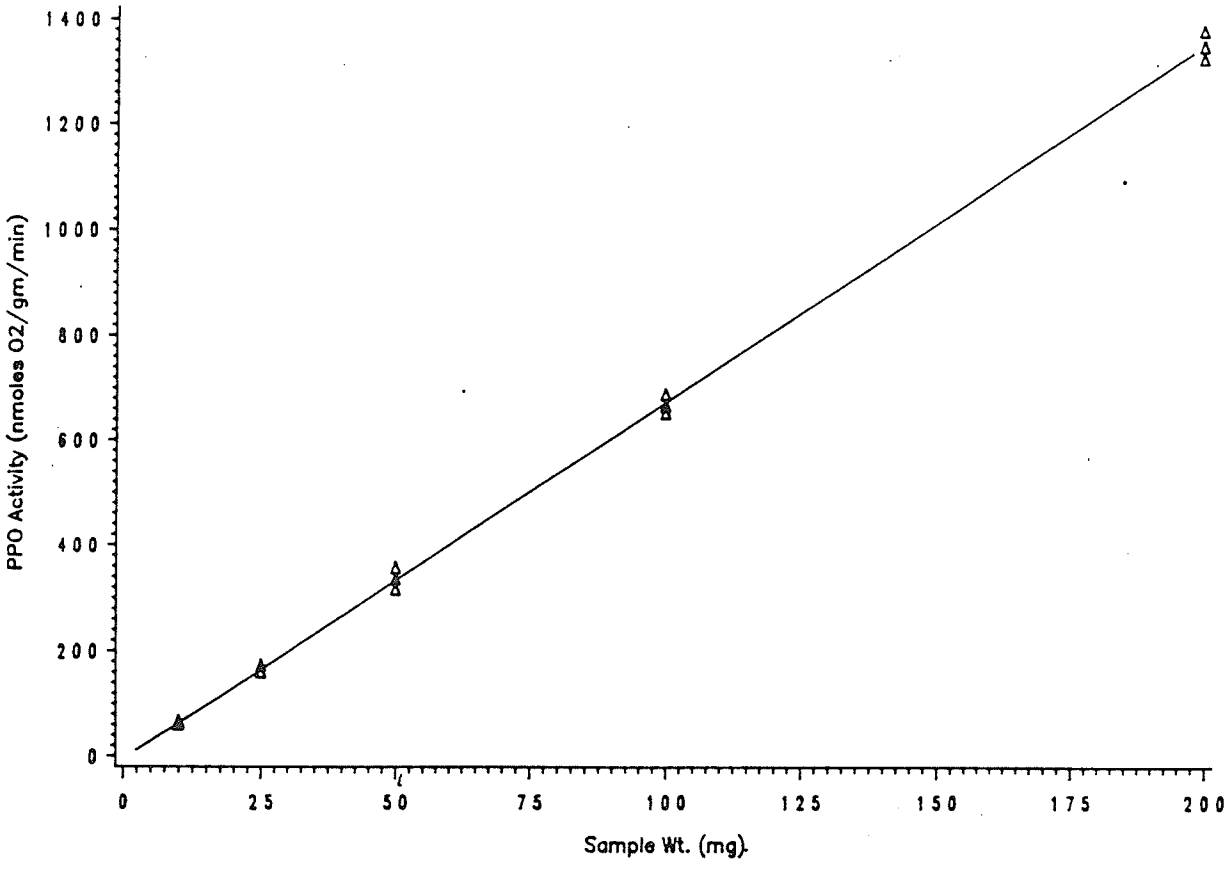
only to compare the varieties. The affinity constants agree with that of less than 5 mM reported by Marsh and Galliard (1986). All 5 varieties had similar Km values yet their maximum reaction rates varied considerably. Glenlea displayed a maximum velocity approximately double that observed for Fielder. HY320 showed a high reaction rate which surpassed the other harder wheats and was unexpected due to its softer nature. The extrapolated reaction rate values were similiar to those determined experimentally.

4.02 Polyphenol Oxidase Analysis

4.02.1 Assay Linearity

Due to the expected wide range in enzyme levels preliminary work was done to ensure a linear response between oxygen consumption and the amount of sample. Using Udy Cyclone ground Neepawa grain, a linear relationship was observed , Fig.4, between the amount of ground grain added and resulting oxygen consumption. The relationship remained linear over the 0.010 to 0.200 g sample size employed in this initial work. Marsh and Galliard (1986) also reported a similar linear response with sample size. Subsequent analysis of all of the mill streams for each variety utilized samples within this range.

Figure 4. Relationship Between Sample Size and PPO Oxygen Consumption Using a Biological Oxygen Monitor


1400
1200
1000
800
600
400
200
0
PPO Activity (nmoles O2/gm/min)
0
25
50
75
100
125
150
175
200
Sample Wt. (mg)

4.02.2 Individual Mill Stream Analysis

The lowest PPO levels in all five varieties under the three different extraction rates were found in the second sizings stream. Values extended from 0 for the 80 % Katepwa component to 31.8 nmoles O_2/g/min for the corresponding 85% Katepwa stream. Please refer to Appendix B for individual stream enzyme levels. All other varieties did not exceed an activity level of 20.0 nmoles O_2/g/min at any of the three extraction rates.

The maximum activity of an individual mill stream was found in the 85% extraction shorts duster flour of Glenlea. This stream's enzyme activity, 7960 nmoles O_2/g/min, represented a 4.5 fold increase over the ground grain, and was considerably higher than the second highest value, 5142 nmoles O_2/g/min, found in Katepwa's 85% extraction bran flour. The 85% extraction 9th middling stream was the most active for Fielder, Norstar, and HY320 with enzyme levels of 3502, 3662, and 4537 nmoles O_2/g/min respectively. These activity levels were 3.3 to 4 times greater than the enzyme levels found in their corresponding ground grain. The 9th middling stream had the second highest activity levels for both Glenlea and Katepwa at 4378 and 4195 nmoles O_2/g/min

In general terms, the relative order of the streams based upon enzyme levels, from lowest to highest, was; the break flours, followed by the remaining middling streams and

finally the bran flours and shorts duster flours. On the highest extraction runs, 85%, the most active stream was generally observed to be the 9th middling.

4.02.3 Enzyme Levels and Ash Content

All five varieties displayed a high degree of linearity between enzyme activity and ash content, independent of extraction rate, until approximately 2.5% ash. The enzyme levels for each variety then increased noticeably, indicative of a curvilinear relationship. The association between individual mill streams and ash content is seen in Figs.5 -9. Under normal milling conditions the blended flours produced by a mill using any of these wheats would not be expected to exceed 1.20%, representing a Chinese Standard Flour. The linear relationship observed between ash content and polyphenol oxidase would thus allow the miller and baker to anticipate for a wide range of prepared flours the degree of enzyme activity based upon ash content alone.

4.02.4 Alpha Amylase in Individual Mill Streams

Alpha amylase is primarily located in the aleurone-pericarp layers of the sound wheat kernel. Polyphenol

Figure 5. Relationship Between PPO Levels and Ash Content in Katepwa (HRS)

A. Over the entire ash range

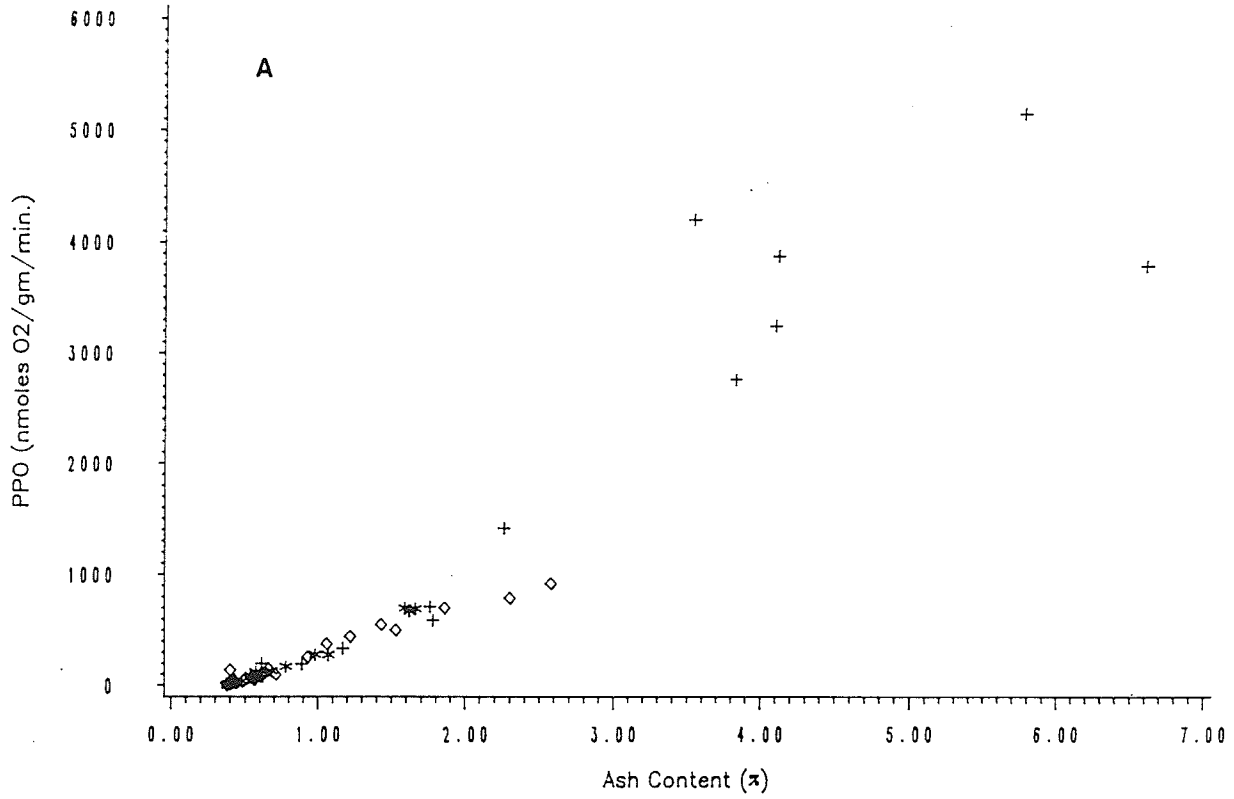
B. Limited to a maximum of 2.5% ash content

PPO = 497.1 x (ASH) - 186.7

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate


A
PPO (nmoles O2/gm/min.)
6000
5000
4000
3000
2000
1000
0
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
Ash Content (%)

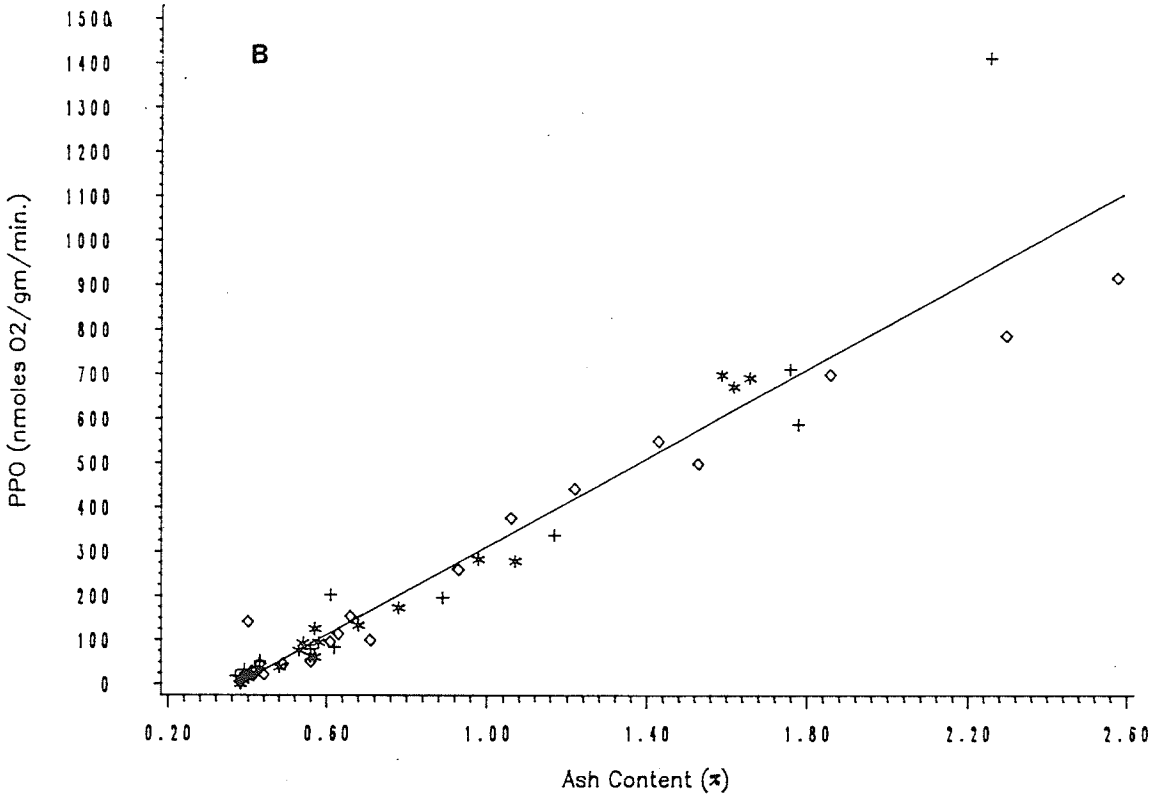
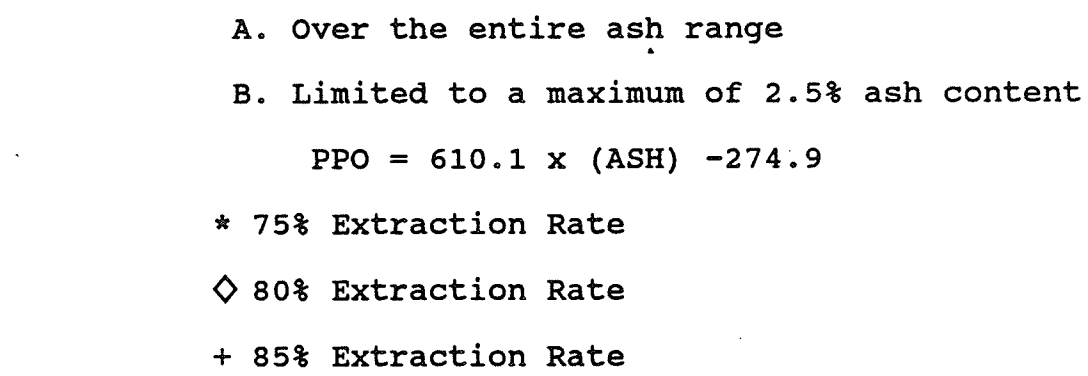
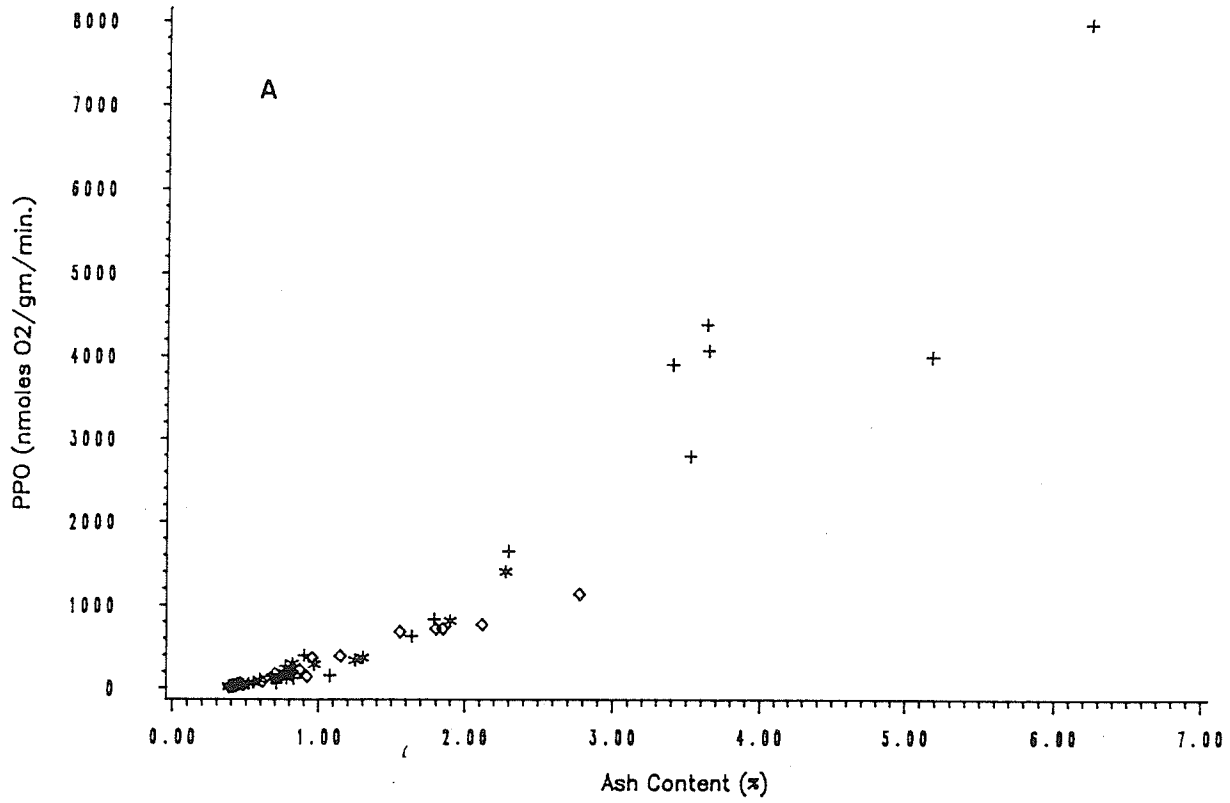

B
PPO (nmoles O2/gm/min.)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0.20
0.60
1.00
1.40
1.80
2.20
2.60
Ash Content (%)

Figure 6. Relationship Between PPO Levels and Ash Content in Glenlea (CU)




A
PPO (nmoles O2/gm/min.)
8000
7000
6000
5000
4000
3000
2000
1000
0
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
Ash Content (%)

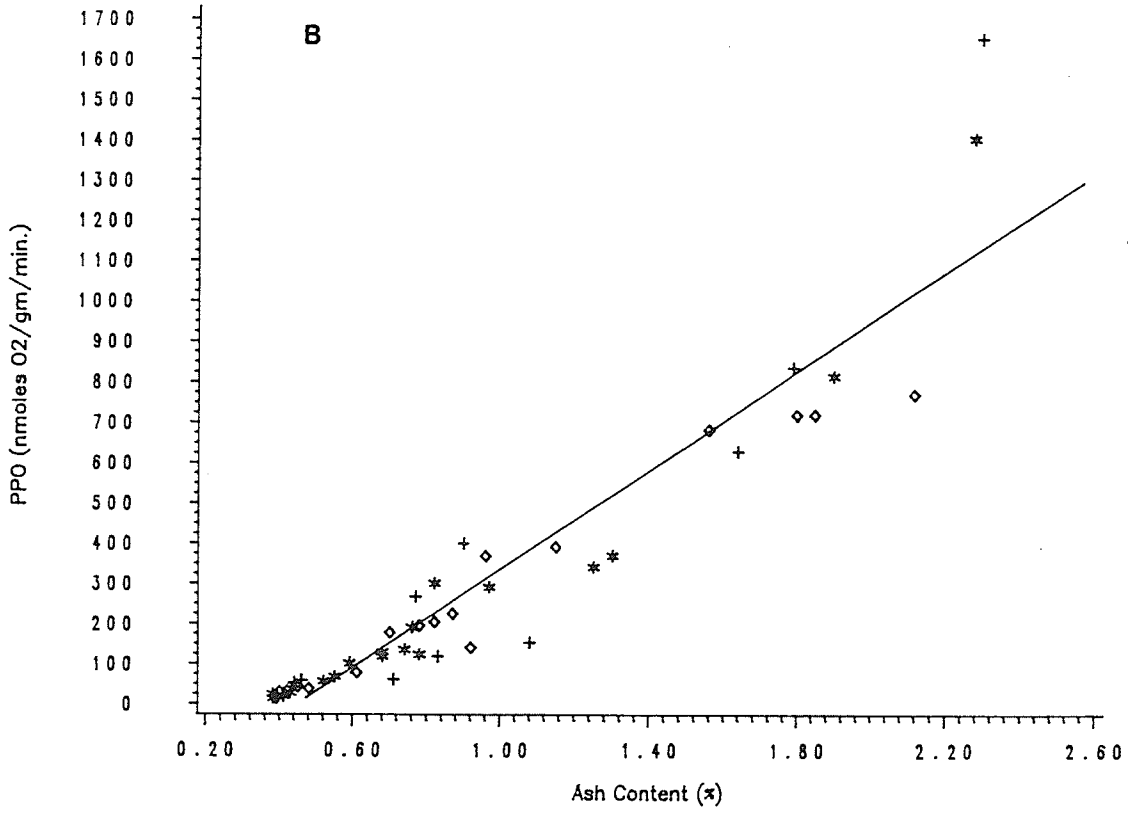

B
PPO (nmoles O2/gm/min.)
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0.20
0.60
1.00
1.40
1.80
2.20
2.60
Ash Content (%)

Figure 7. Relationship Between PPO Levels and Ash Content in Norstar (HRW)

A. Over the entire ash content range

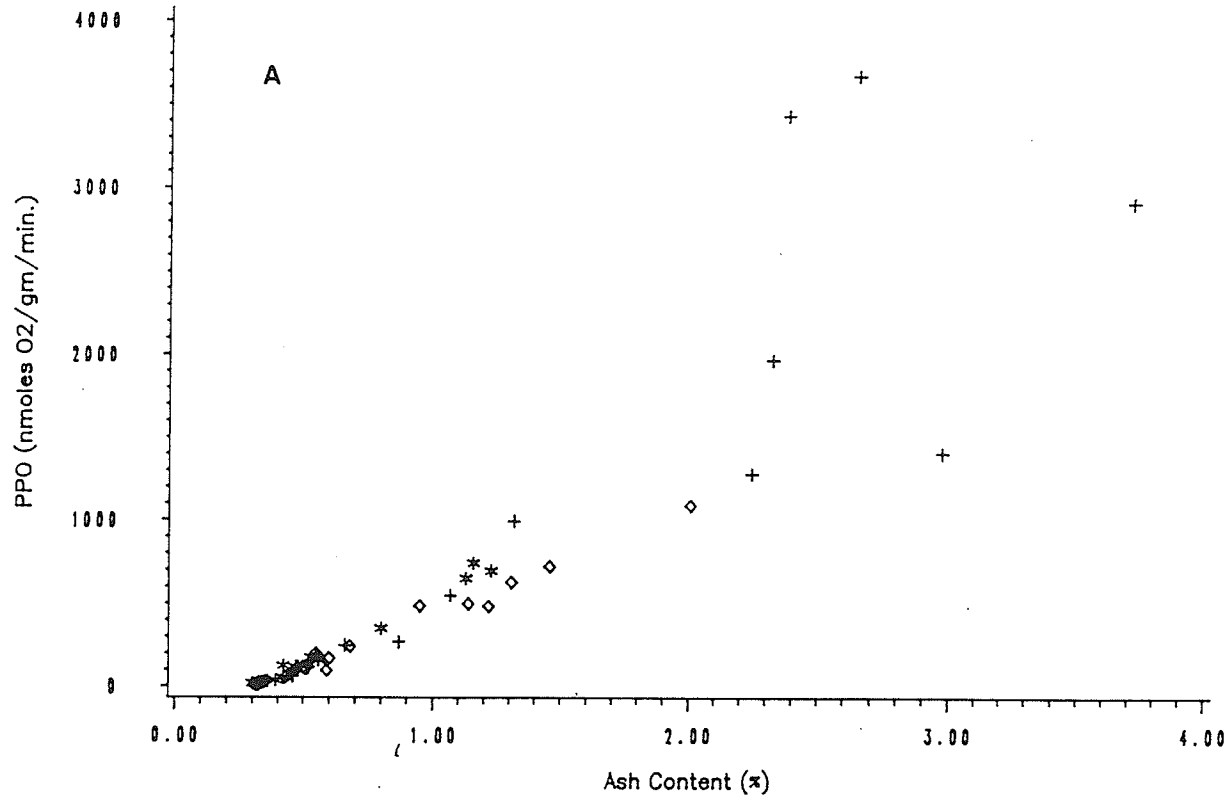
B. Limited to a maximum of 2.5% ash content

PPO= 961.0 x(ASH) - 369.7

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate


A
PPO (nmoles O2/gm/min.)
4000
3000
2000
1000
0
0.00
1.00
2.00
3.00
4.00
Ash Content (%)

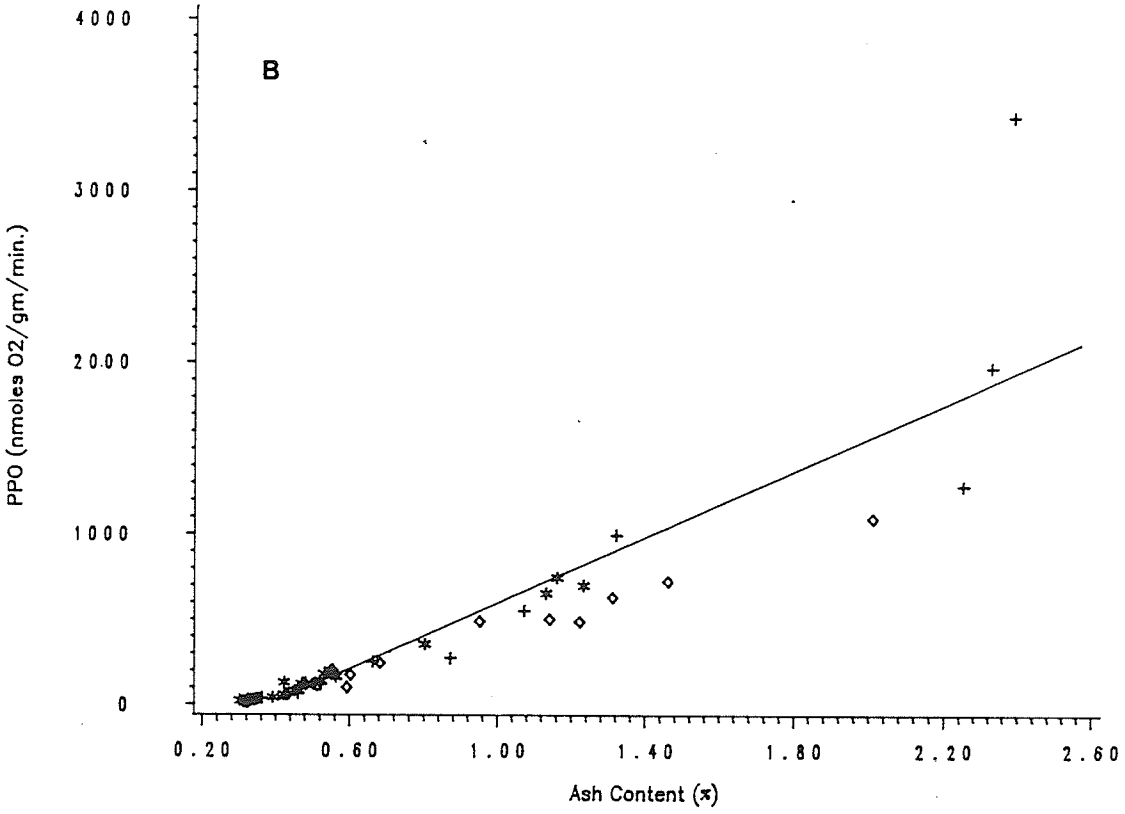

B
PPO (nmoles O2/gm/min.)
4000
3000
2000
1000
0
0.20
0.60
1.00
1.40
1.80
2.20
2.60
Ash Content (%)

Figure 8. Relationship Between PPO Levels and Ash Content in HY320 (CPS)

A. Over the entire ash range

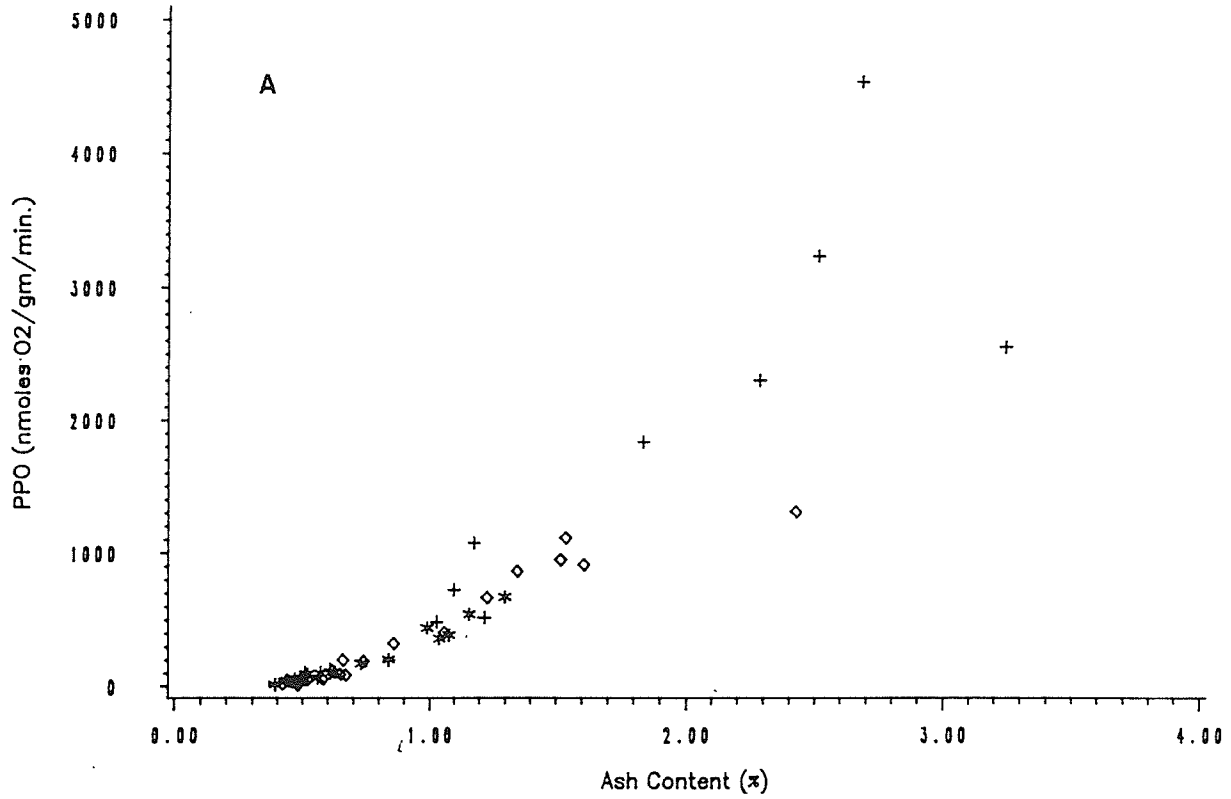
B. Limited to a maximum of 2.5% ash content

PPO = 1092.0 x (ASH) - 548.1

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate


A
5000
4000
3000
2000
1000
0
PPO (nmoles O2/gm/min.)
0.00
1.00
2.00
3.00
4.00
Ash Content (%)

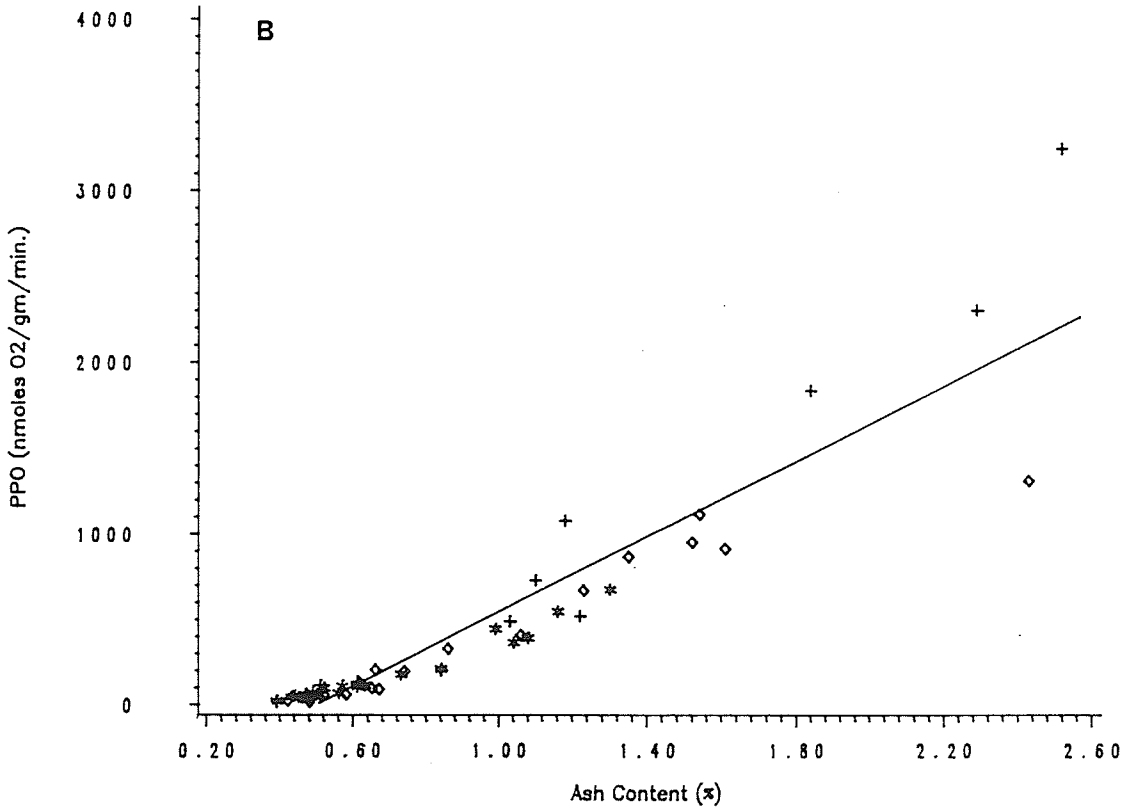

B
4000
3000
2000
1000
0
PPO (nmoles O2/gm/min.)
0.20
0.60
1.00
1.40
1.80
2.20
2.60
Ash Content (%)

Figure 9. Relationship Between PPO Levels and Ash Content in Fielder (SWS)

A. Over the entire ash range

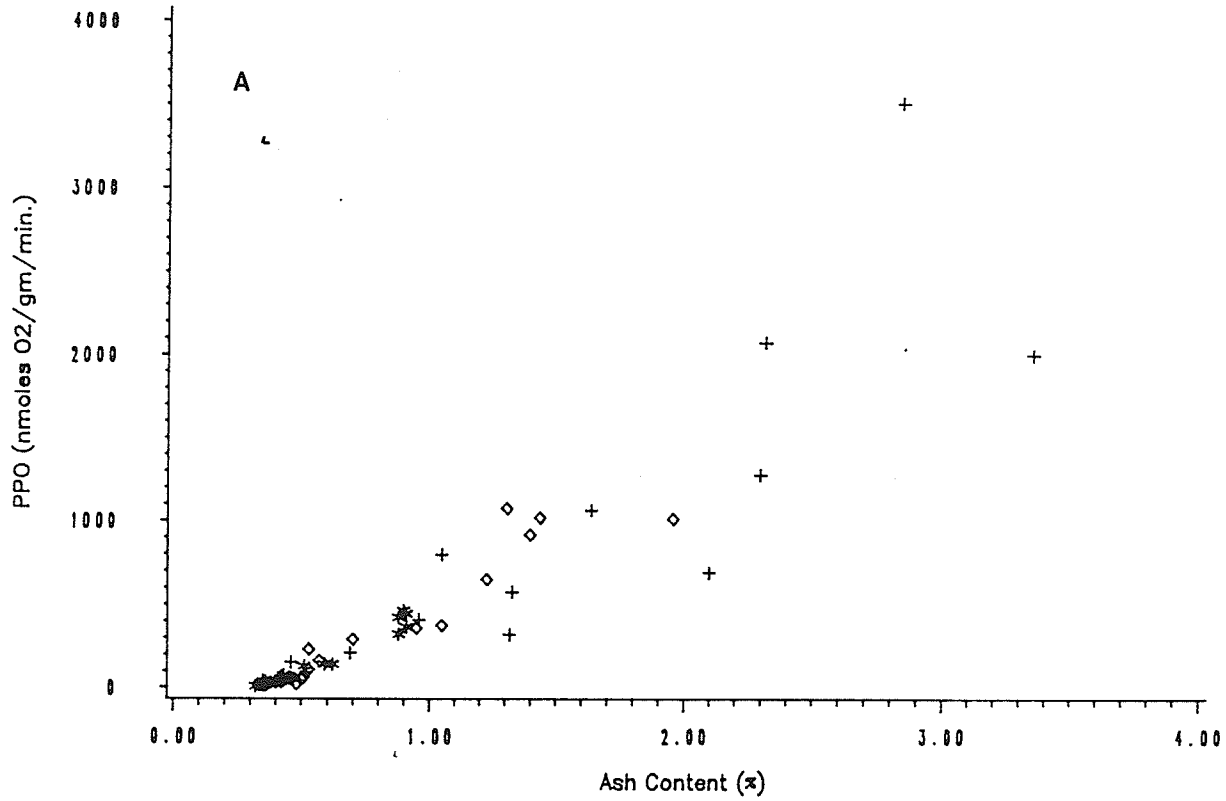
B. Limited to a maximum of 2.5% ash content

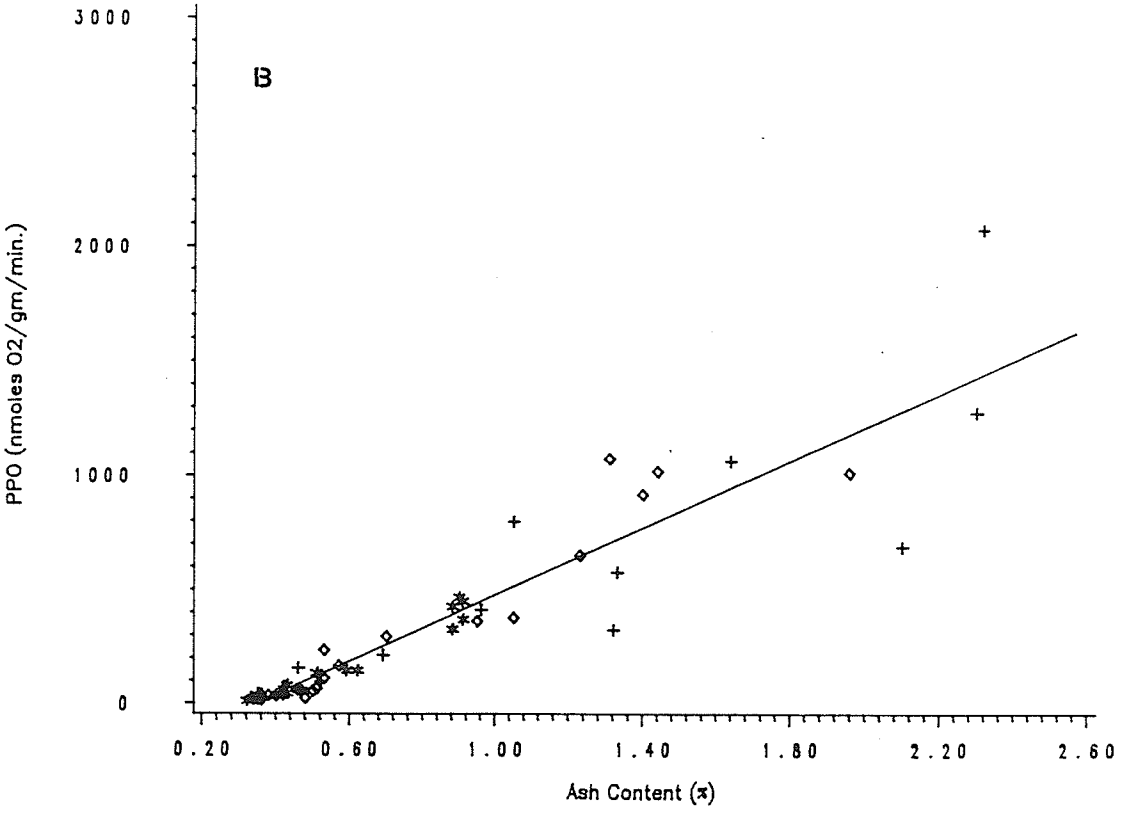
PPO = 729.7 x (ASH) - 253.8

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate


A
4000
3000
2000
1000
0
PPO (nmoles O2/gm/min.)
0.00
1.00
2.00
3.00
4.00
Ash Content (%)


B
3000
2000
1000
0
PPO (nmoles O2/gm/min.)
0.20
0.60
1.00
1.40
1.80
2.20
2.60
Ash Content (%)

oxidase is believed to be located in the outer layers of the kernel, in particular the bran. Wheat and wheat flour are normally assayed for their amylase content on a routine basis to assess the degree of sprout damage and the corresponding enzyme requirements in breadmaking. It would therefore be highly desirable to determine if there was a significant relationship between the two enzyme contents in the sound flour mill streams such that an assay for one enzyme would be indicative of the other enzyme's level.

An assortment of mill streams of 75 and 80% extraction levels were analyzed for alpha amylase content for each variety .

It was found that in the softer wheats, in particular Fielder, there was an excellent correlation between the alpha amylase levels and those of polyphenol oxidase. The correlation coefficient was found to be $r=0.83$ with a probability of significance in excess of 99%. A reasonably strong, significant relationship, $p < 0.05$, was also found between the two enzymes for the moderately hard wheat HY320 with $r=0.74$. However, as the wheats became harder, the relationship declined, Katepwa $r=0.62$, Norstar, $r=0.56$ and for the very hard Glenlea, $r=0.50$. Although the correlation had diminished, the probability of a significant relationship remained above 95% for these three varieties.

An observed factor which discouraged a strong correlation was the minimal alpha amylase content in all five varieties respective streams. The maximum level

attained in any stream was found to be 14.5 mg.maltose/min /g as observed in Fig. 11. The strongest correlation was observed in Fielder where there was a significant range of alpha amylase values. Evidence for this effect was noted in Figs. 10-14 relating PPO to alpha amylase in the mill streams. It was observed that in the harder wheats there was an insufficient range in amylase content within the streams to establish a meaningful relationship. In these wheats it was the single values at higher amylase values which made the relationship appear to be statistically significant when the relationship was tenuous at best. A germinated wheat sample would yield better amylase values to confirm this relationship.

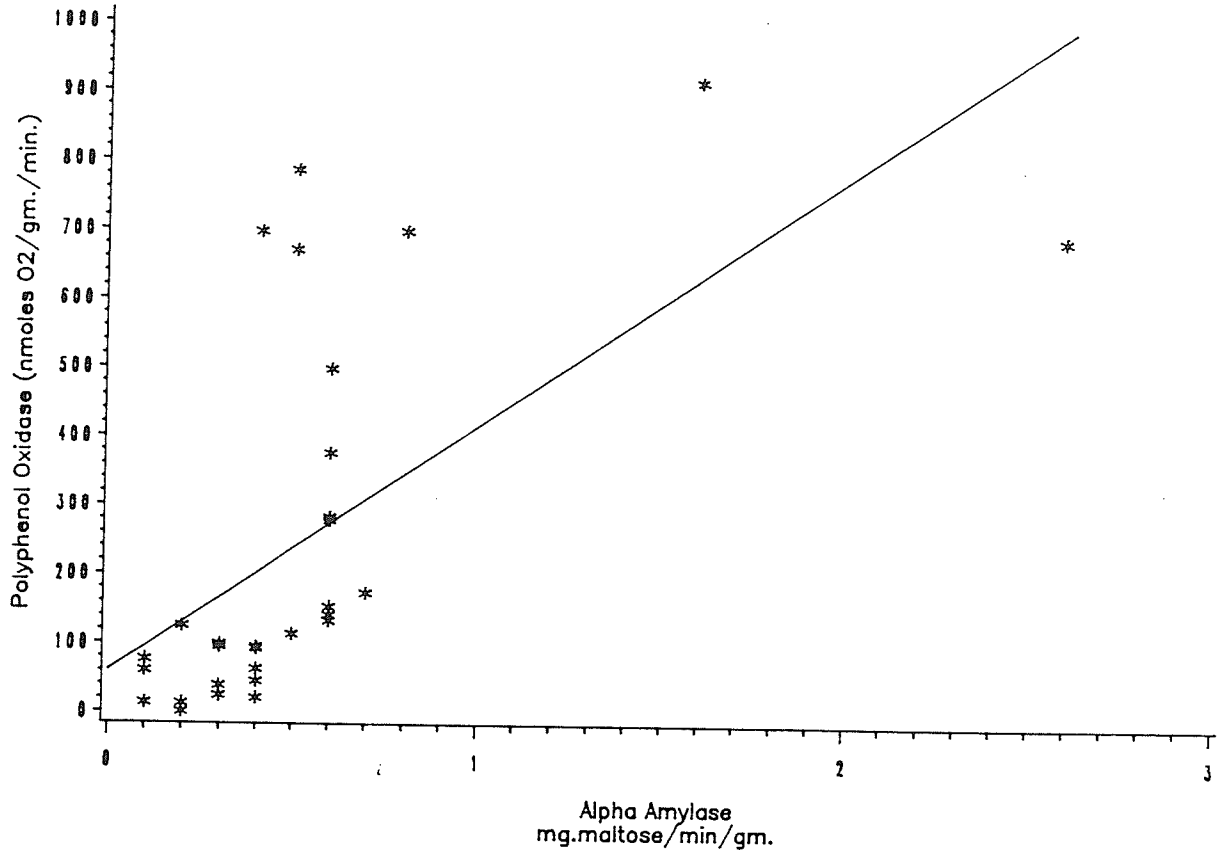
4.03. Polyphenol Oxidase Levels in Pooled Flours

4.03.1 1st Patent Flours:

The pooled 1st patent flours at both the 75 and 80% extraction rates, representing the initial 45% of the cumulative mill yield, displayed minimal amounts of enzyme activity. Values ranged from 16.7 nmoles O_2/g/min for the 75% extraction Norstar to 44.6 nmoles O_2/g/min in the 80% extraction Katepwa flour. These values agreed well with those reported by Marsh and Galliard (1986), of less than 30 nmoles O_2/min/g in a 1st patent flour. The samples also reflected the linear trend between PPO and ash content as minimal increases were observed in both components moving to the higher extraction rate.

Figure 10. Relationship Between PPO and Alpha Amylase in Katepwa

Figure 11. Relationship Between PPO and Alpha Amylase in Glenlea


Polyphenol Oxidase (nmoles O2/gm./min.)
1000
900
800
700
600
500
400
300
200
100
0
0
1
2
3
Alpha Amylase
mg.maltose/min/gm.

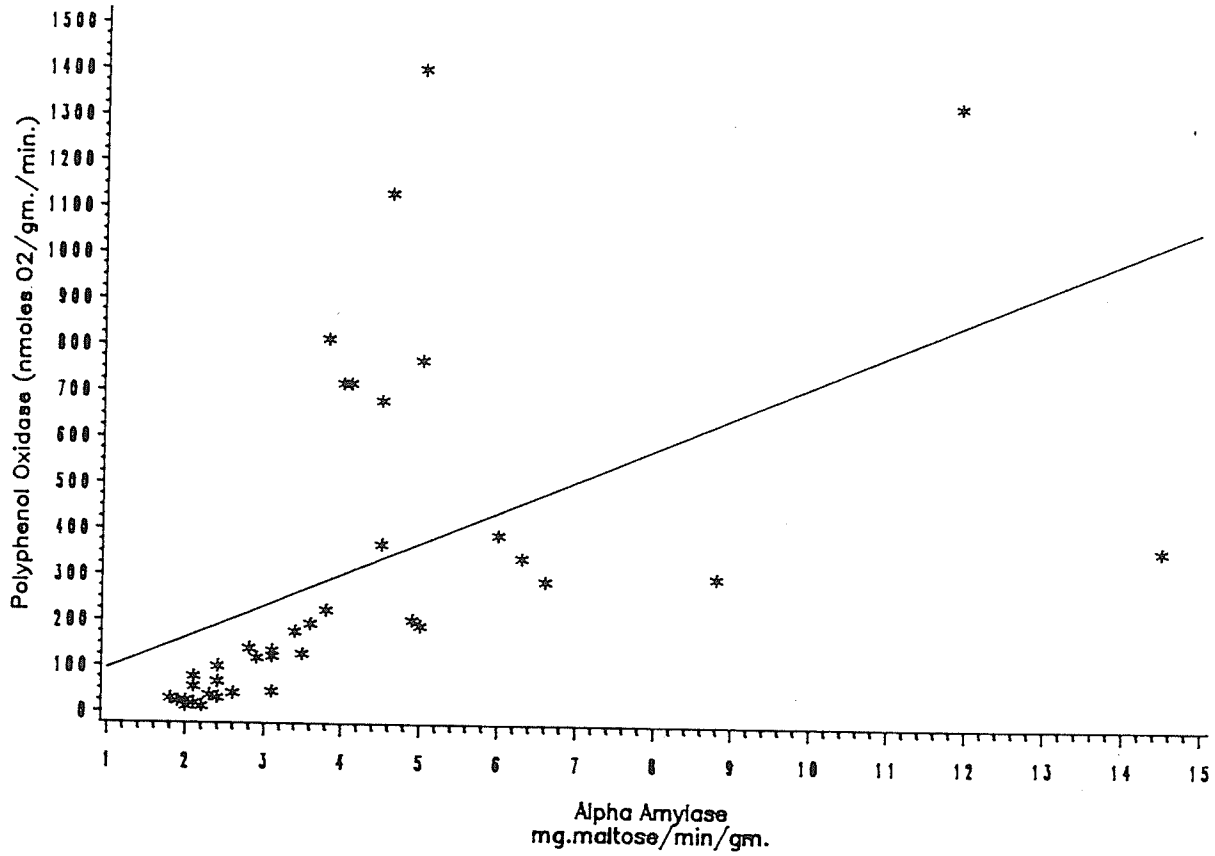
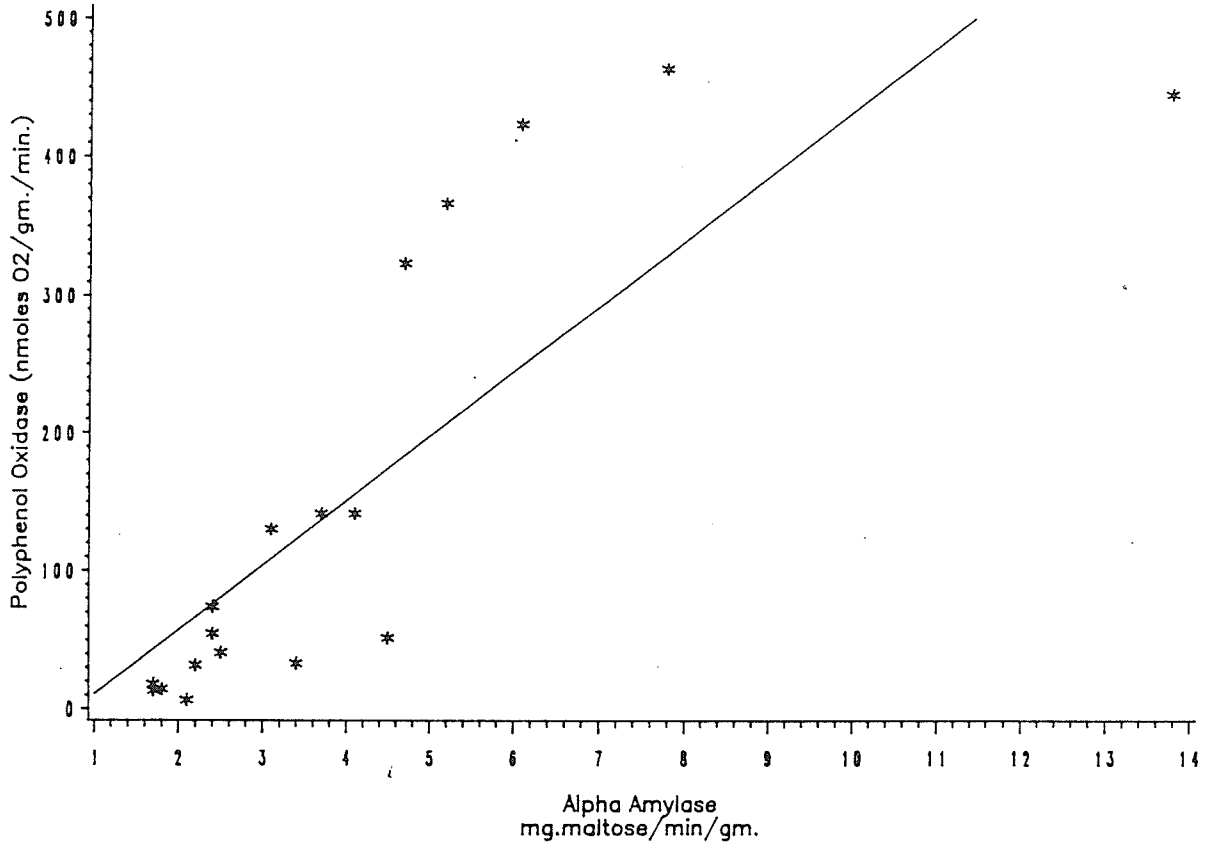

Polyphenol Oxidase (nmoles O2/gm./min.)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Alpha Amylase
mg.maltose/min/gm.

Figure 12 Relationship Between PPO and Alpha Amylase in Fielder

Figure 13 Relationship Between PPO and Alpha Amylase in HY320


Polyphenol Oxidase (nmoles O2/gm./min.)
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Alpha Amylase
mg.maltose/min/gm.

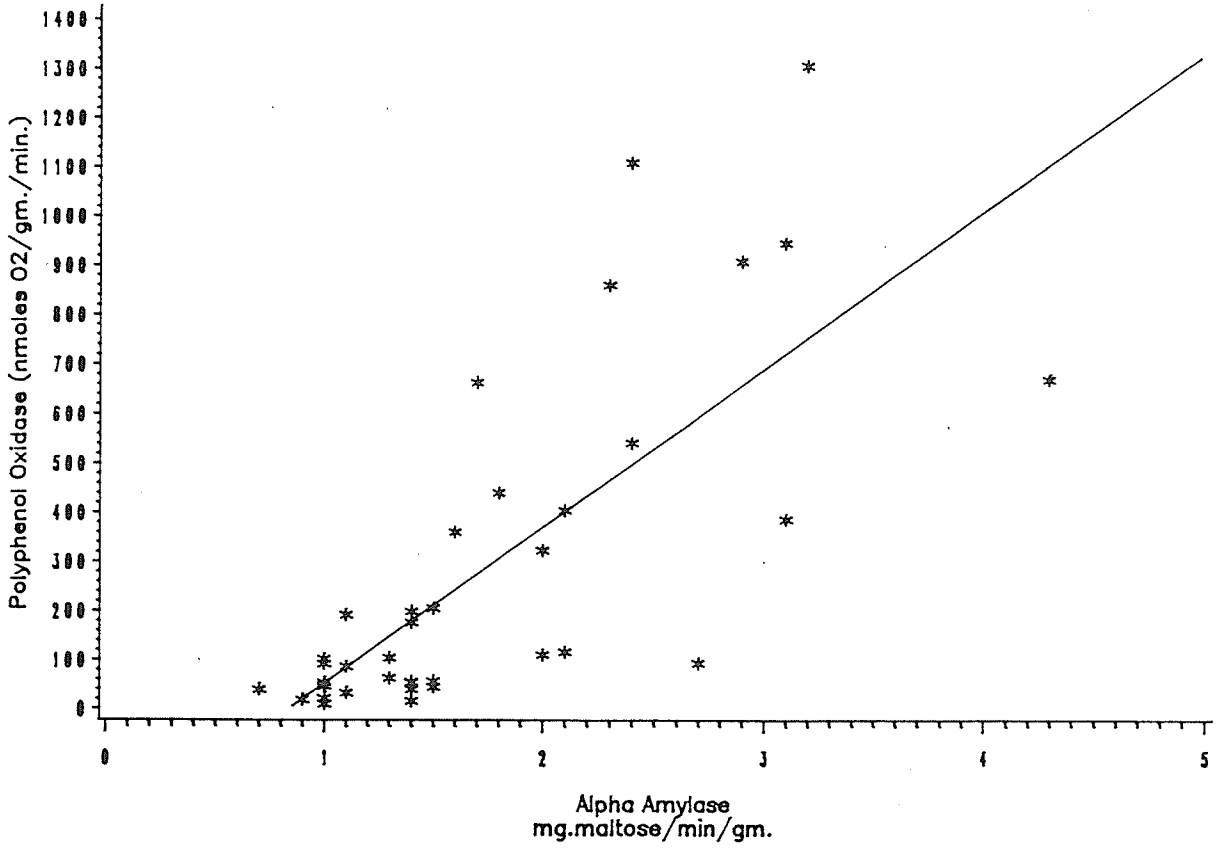
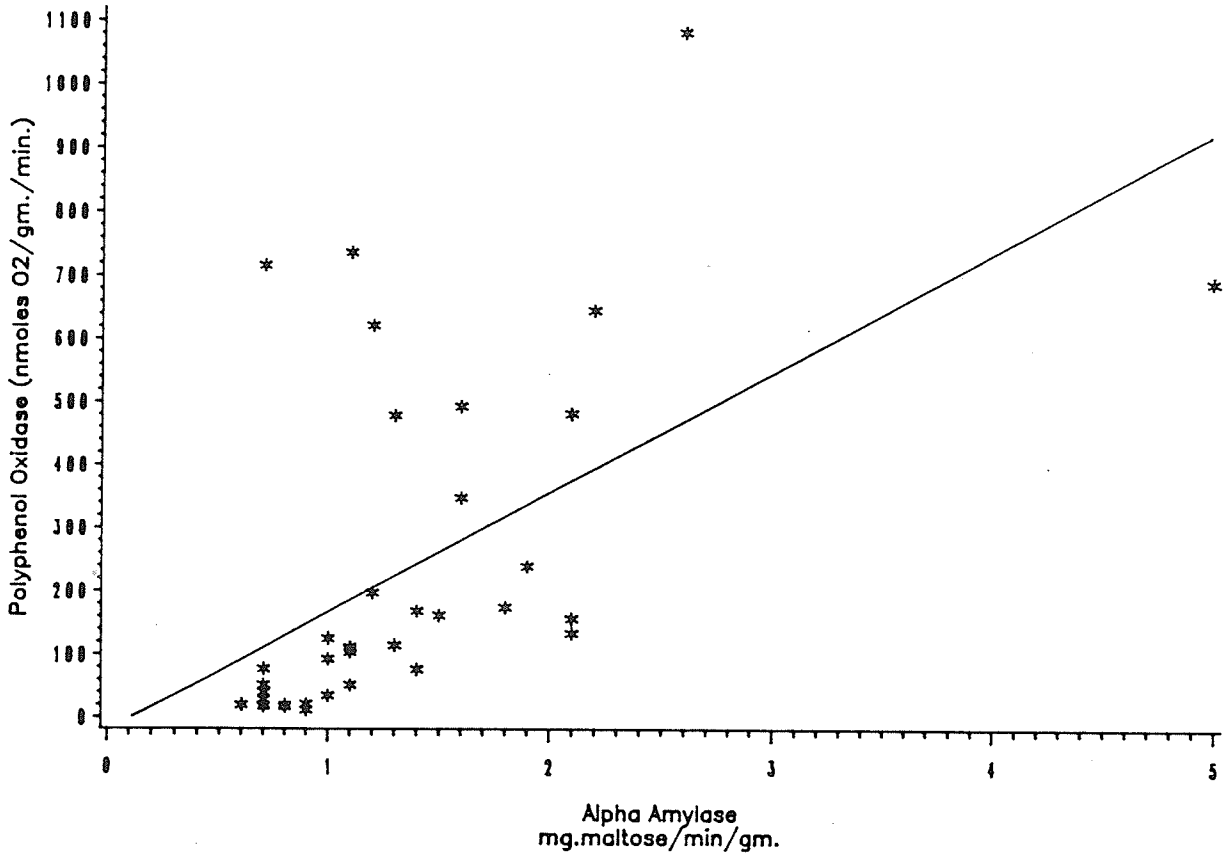

Polyphenol Oxidase (nmoles O2/gm./min.)
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Alpha Amylase
mg.maltose/min/gm.

Figure 14. Relationship Between PPO and Alpha Amylase in Norstar


Polyphenol Oxidase (nmoles O2/gm./min.)
1100
1000
900
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Alpha Amylase
mg.maltose/min/gm.

Analysis of the contribution of individual streams to the pooled flours indicated good agreement between estimated values and the experimentally observed activity levels. A comparison of these levels can be seen in Table 3 . It should be noted that at very low enzyme levels the additive error for individual streams made the estimation of the degree of agreement extremely difficult.

HY320 displayed the greatest activity in the 75 % extraction 1st patent flours at 43.8 nmoles O_2/g/min. This enzyme content was approximately twice that of the four other varieties. This finding had particular relevance as HY320 was developed specifically to cater to the non-traditional export markets. There was no significant, $p < 0.05$, difference between the remaining varieties with an average activity of 18.1 nmoles O_2/g/min. The greatest contribution of mass to the 75% 1st patent flours was supplied by the 1st middling stream, accounting for approximately 40% of the material. In all varieties but Fielder, this stream also supplied the largest component and greatest percentage of the overall enzyme activity. Fielder had the 4th middling stream included in the 1st patent flour . This stream was not present in the other varieties and although it accounted for only 10% of the mass, it contributed 30% of the overall enzyme activity.

In all varieties except Norstar and HY320, the corresponding 80% 1st patent flours displayed an approximate doubling in activity compared to their 75% extraction

Table 3 Actual versus Estimated Pooled Flours' Polyphenol Oxidase Levels

STREAM	OBSERVED KATEPWA	ESTIMATE KATEPWA	OBSERVED GLENLEA	ESTIMATE GLENLEA	OBSERVED NORSTAR	ESTIMATE NORSTAR	OBSERVED HY320	ESTIMATE HY320	OBSERVED FIELDER	ESTIMATE FIELDER
1ST PAT. 75%	19.9	16.9	17.9	29.7	16.7	18.5	43.8	38.9	18.0	19.4
2ND PAT. 75%	94.3	83.8	117.8	109.4	76.4	78.1	109.5	103.6	130.0	109.5
1ST CLR. 75%	282.0	185.3	289.0	280.8	161.6	159.9	440.0	301.9	423.0	348.6
2ND CLR. 75%	670.0	695.5	812.0	655.4	645.6	629.7	541.0	481.3	463.0	445.2
ST.GRADE 75%	91.5	89.6	98.7	91.9	124.6	85.7	102.7	93.6	73.7	69.8
1ST PAT. 80%	44.6	33.3	29.9	29.8	19.9	19.3	31.8	26.0	31.8	27.1
2ND PAT. 80%	113.0	99.0	193.0	170.8	103.5	115.0	191.0	177.1	161.0	140.1
1ST CLR. 80%	375.0	310.6	389.0	384.9	238.8	242.9	404.0	478.6	358.0	371.1
2ND CLR. 80%	697.0	627.0	767.0	853.9	622.0	600.5	1108.0	927.2	913.0	982.6
ST.GRADE 80%	152.0	119.0	175.1	155.9	108.7	109.5	197.4	166.0	228.0	144.0
ST.GRADE 85%	199.0	431.0	246.0	437.0	191.0	284.3	248.0	337.0	238.0	243.0
CHIN.STD 85%	412.0	389.0	436.0	420.2	488.0	436.4	478.0	428.0	536.0	515.0
WHEAT	1266.0		1767.0		1011.0		1369.0		864.0	

All Values are expressed in nmoles O_2 /g /min

counterparts. The overall enzyme content however, in all the flours, was extremely low and did not exceed 3.7% of the total activity found in the ground grain.

4.03.2 2nd Patent Flours

The 75% extraction 2nd patent flours, contributing the next 22.5% of the flour yield, displayed very large increases in activity compared to their 1st patent flours. Each variety with the exception of HY320 exhibited approximately a five fold increase in enzyme activity. The HY320 2nd patent flour was distinct from its 1st patent flour although the increase was only 2.5 fold. Each variety showed good agreement between estimated and experimental activities.

The major component of the 2nd patent flour was the 4th middling stream in each variety except Fielder where the 2nd break flour was dominant. Enzyme activity was evenly divided amongst the contributing streams although as in the 1st patent flour, the largest amount of activity was not supplied by the major mass component. The best example of this phenomena was seen in Fielder, where due to its softer nature, milled distinctively different from the hard wheats. Mill specifications were established to achieve the desired yields on the basis of the hard wheat Katepwa. Logistical considerations did not allow for establishing optimum milling conditions for each variety. The lack of an extended

milling process resulted in the incorporation of the bran flour in Fielder's 2nd patent flour. The bran flour contributed approximately 15% of the 2nd patent mass yet accounted for 43 % of the flour's activity. None of the remaining varieties had any single stream supply greater than 25 % of the total activity.

Each variety's 80% extraction 2nd patent flour displayed a statistically significant, $p < 0.05$, increase in enzyme activity compared with their 75 % flours. Only Glenlea and HY320 however, revealed a meaningful increase by almost doubling their respective activity levels. In each variety the bulk of the activity was supplied by the 4th middling stream well in excess of its mass contribution

.

4.03.3 1st Clear Flours

The 75% 1st clear flours, while only achieving an additional 5.5% increase in cumulative yield, showed a large increase in enzyme activity over their corresponding 2nd patent flours. The softer wheats, Fielder and HY320, showed the greatest activities with values of 423 and 440 nmoles O_2/g/min respectively. These enzyme levels represent an increase approaching four times that found in their 2nd patent flours. In Fielder, this elevated level represented 49% of the total activity in a ground wheat sample and was considerably higher than the 32% achieved by HY320. The harder wheats, Glenlea and Katepwa, increased by 3 fold with

equivalent enzyme content's of 289 and 282 nmoles O_2/g/min Norstar's response was somewhat muted with only a doubling in activity to 162 nmoles O_2/g/min Norstar and Glenlea displayed the lowest percentage of total enzyme content having only 16% of the total activity within the ground grain sample. Katepwa, although slightly higher at 22% was still well below the percentages achieved by the softer wheats.

The varieties revealed clear distinctions within this flour, as the softer wheats had the highest activity due in large part to their milling character. Unlike the harder wheats, the bran flour was the major component in both the softer wheats 1st clear 75% extraction flour. The HY320 and Fielder bran flours had almost equal activities while accounting for 63 and 57 % of their respective pooled streams' mass. The experimental values for HY320, Fielder, and Katepwa were considerably higher than those estimated on the basis of their stream components. In each case the activity was at least 100 nmoles greater than anticipated. The estimated values for both Glenlea and Norstar however were in good agreement with the observed enzyme activities.

Only the harder wheats, Glenlea, Katepwa, and Norstar, displayed significant, $p < 0.05$, increases in this stream at the 80% extraction level. In general, these hard wheats had a 33% increase in enzyme content, yet the activities achieved were still lower than those of the softer wheats. Glenlea and Katepwa reached values of the same magnitude,

389 and 375 nmoles O_2/g/min and similar percentage of total wheat activity, 22 and 29.6% respectively. This sample of Norstar clearly showed its distinctive nature having a significantly, $p < 0.05$, lower content of 238 nmoles O_2/g/min Although numerically distinct, Norstar's content on a percentage of total activity was clearly grouped with the other hard wheats at 23.6 % . Katepwa and Norstar had over 70% of the 1st clear 80% extraction flour supplied by the 4 middling stream, yet the results were quite different. The estimated enzyme activity in this 1st clear flour for Norstar agreed with the experimentally determined values. Katepwa, however, continued the trend seen in the corresponding 75 % flour displaying the experimentally determined activity values considerably larger than anticipated based upon individual stream contributions.

4.03.4 2nd Clear Flours

The 75% extraction 2nd clear flours for all three hard wheats exhibited noticeable increases over the softer wheat varieties. The greatest activity was found in the hardest wheat, Glenlea, with 812 nmoles O_2/g/min Glenlea and Katepwa underwent a 2 fold increase in enzyme content from their 1st clear flours. Norstar, however, presented a very major 4 fold increase in total activity to fall within the same range as the other two hard wheats. The large increase observed in the Norstar sample represented 64% of the total

grain activity which was considerably higher than any other variety. This increase was very interesting in light of the fact that the bran flour component in Norstar was less than either Katepwa or Glenlea on a mass percentage basis. However, the activity found in Norstar's bran flour was the highest of all five varieties and with the exception of Katepwa, was approximately twice that of the others.

HY320 increased to a lesser extent over its 75% extraction 1st clear flour while Fielder remained relatively unchanged.

The large increases viewed in the 75% 2nd clear hard wheats from their 1st clear counterparts, with minimal change in the softer wheats, was reversed at the 80% extraction level. HY320 and Fielder double in enzyme content while the three harder wheats remained relatively unchanged. The enzyme content of HY320 represented approximately 81% of the total grain activity while Fielder displayed a content in excess of the ground grain, 106%. This was in part due to the mill being optimized for the HRS wheat Katepwa, and as such, the later streams of the softer wheats had a disproportionate amount of bran contamination and increased enzyme levels. This influence was observed in the corresponding individual 5th middling streams of Katepwa versus Fielder and HY320. The enzyme values in the softer wheats were at least twice that found in Katepwa. The nature of the pooled streams which made up the 2nd clear flour for the hard wheats had also changed. At the 75% extraction

level the shorts duster and bran flour streams were the major components. However at 80% extraction, the 5th and 6th middling streams contributed to this pooled flour. The shorts duster and bran flour increased only slightly in enzyme activity, but this was diluted by the lower enzyme levels supplied by the 5th and 6th middling streams relative to the softer wheats.

4.03.5 Straight Grade Flours

The straight grade 75% extraction flours from the five varieties segregated into three statistically significant, $p < 0.05$, groups. The highest value was found for Norstar while Fielder had the lowest enzyme level. Glenlea, HY320 and Katepwa had similar activities and were not statistically different from each other. Only Norstar had an experimental value not in good agreement with the estimated activity based upon individual stream contributions. In terms of the percentage of total grain activity, all varieties except Norstar, fell between 5.6 to 8.5 % of the total available, while Norstar achieved 12.3%.

The greatest increases were observed in the softer wheats when assaying the 80% extraction straight grade flours. Fielder displayed the highest activity, followed by HY320. These values represented a three fold increase in

activity for Fielder and an approximate two fold increase for HY320 from their 75% extraction counterparts. All of the varieties except Norstar had experimental activities in excess of the predicted levels. The softer nature of Fielder was clearly seen in this sample as expressed on a percentage of total grain activity the 80% straight grade flour has 26.4% of the total activity. This percentage is almost twice that observed for its nearest neighbor, HY320, at 14.4%. The harder wheats percentage was even lower, ranging from 9.9 to 12.0% of the total available.

At the 85% extraction rate, all varieties except Fielder displayed noticeably lower than estimated straight grade enzyme levels. Fielder's experimental value of 238 nmoles O_2/g/min was in excellent agreement with the predicted value of 242 nmoles O_2/g/min However, as a percentage of the total activity in the ground grain, this represented only a slight increase from the 80% extraction stream, at 27.5%. Glenlea and Katepwa displayed 24.9 and 32.5% of their total activities while both Norstar and HY320 segregated at the lower level of 18%.

4.03.6 Summary

Examination of the cumulative enzyme versus cumulative yield can be seen in Figs15-19. The most noticeable feature observed was that for all varieties at each extraction rate, 75-85%, the cumulative amount of enzyme was extremely low, less than 60 nmoles O2/g/min, until passing a 60% cumulative

yield. This aspect has particular relevance to the millers and their milling practices of Canadian wheats. This would allow optimization of their extraction yields to values as high as 85% yet remaining confident that their initial patent flours would be free of serious polyphenol oxidase levels and the subsequent end-products would not suffer discoloration. It would allow the miller the ability to produce flours for two different markets such as noodles, where whiteness is critical, and steam buns or chapattis, using the later high extraction streams while still maximizing mill efficiency. Above 70% cumulative yield, the enzyme's presence however increased rapidly. The rapid increase was particularly noticeable in the high extraction, 85%, millings where 400 % increases in total activity occurred within the remaining 10% yield. This rapid increase in enzyme content was indicative of the very high levels of enzyme observed earlier in the later individual mill streams of high extraction runs.

Figure 15 Cumulative PPO Levels Versus Cumulative Yield For the Three Different Extraction Rate Millings of Katepwa (HRS)

* 75% Extraction Rate

◇ 80% Extraction Rate

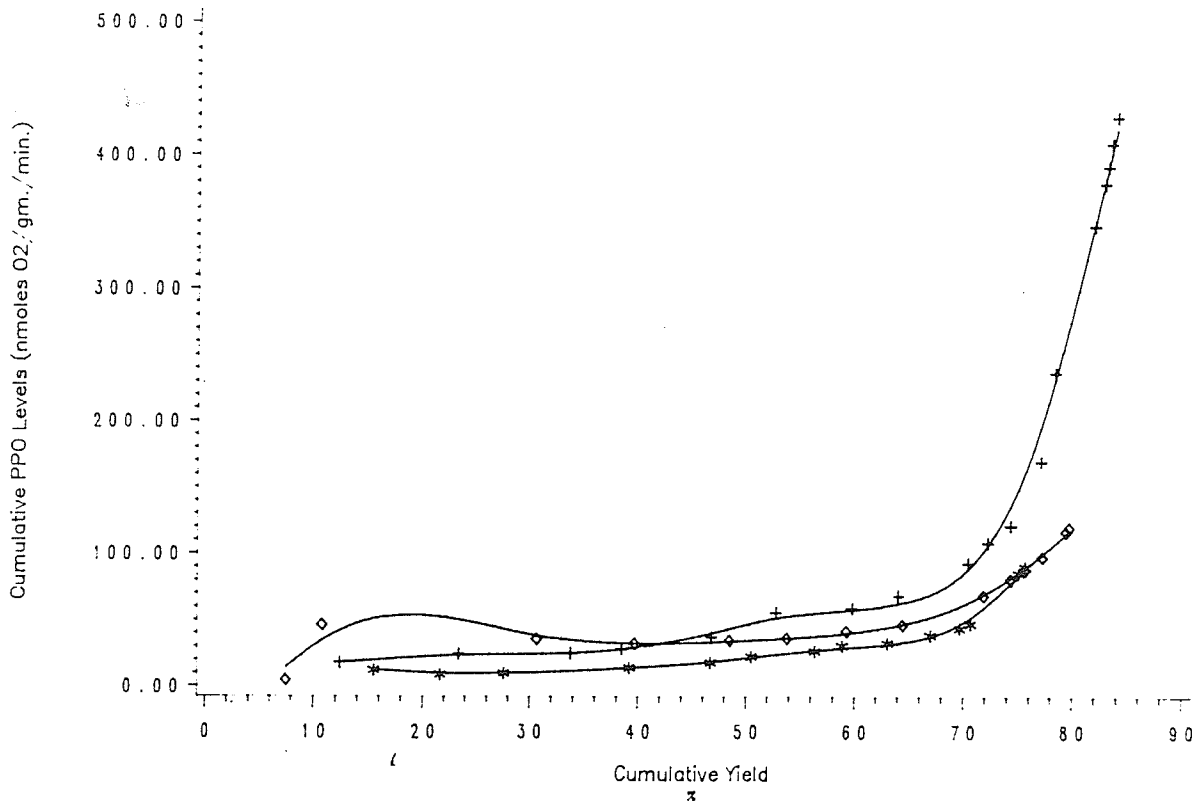
+ 85% Extraction Rate

Figure 16 Cumulative PPO Levels Versus Cumulative Yield For the Three Different Extraction Rate Millings of Glenlea (CU)

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate


500.00
400.00
300.00
200.00
100.00
0.00
Cumulative PPO Levels (nmoles O2/gm./min.)
0
10
20
30
40
50
60
70
80
90
Cumulative Yield
%

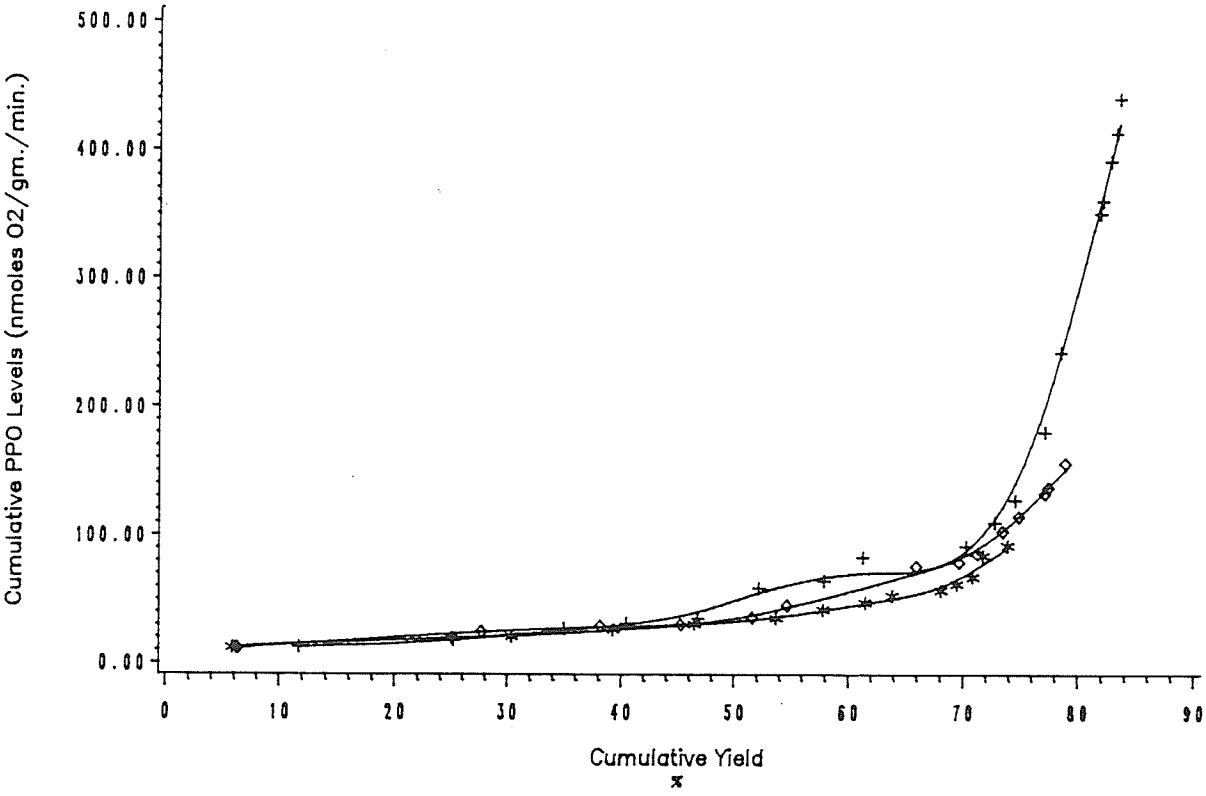

500.00
400.00
300.00
200.00
100.00
0.00
Cumulative PPO Levels (nmoles O2/gm./min.)
0
10
20
30
40
50
60
70
80
90
Cumulative Yield
%

Figure 17 Cumulative PPO Levels Versus Cumulative Yield For the Three Different Extraction Rate MIllings of Norstar (HRW)

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate

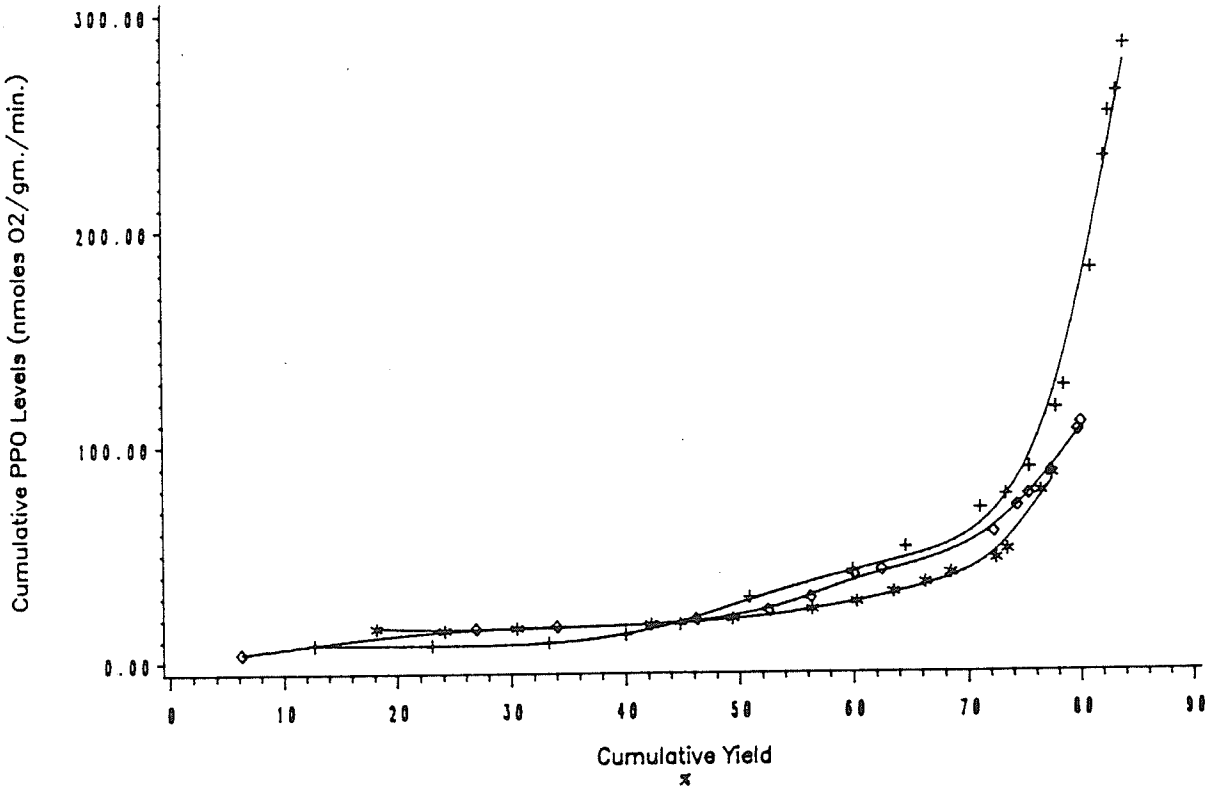

300.00
200.00
100.00
0.00
Cumulative PPO Levels (nmoles O2/gm./min.)
0
10
20
30
40
50
60
70
80
90
Cumulative Yield
%

Figure 18 Cumulative PPO Levels Versus Cumulative Yield For the Three Different Extraction Rate Millings of Fielder (SWS)

* 75% Extraction Rate

◇ 80% Extraction Rate

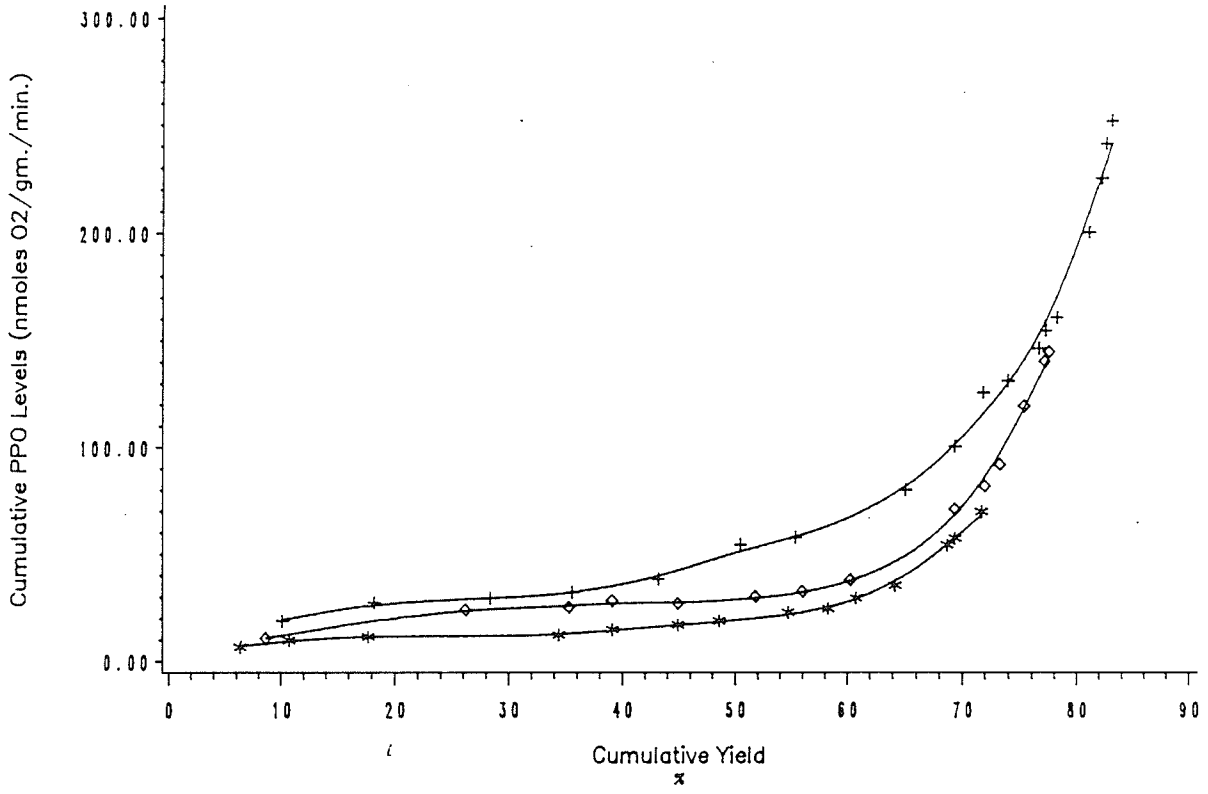
+ 85% Extraction Rate

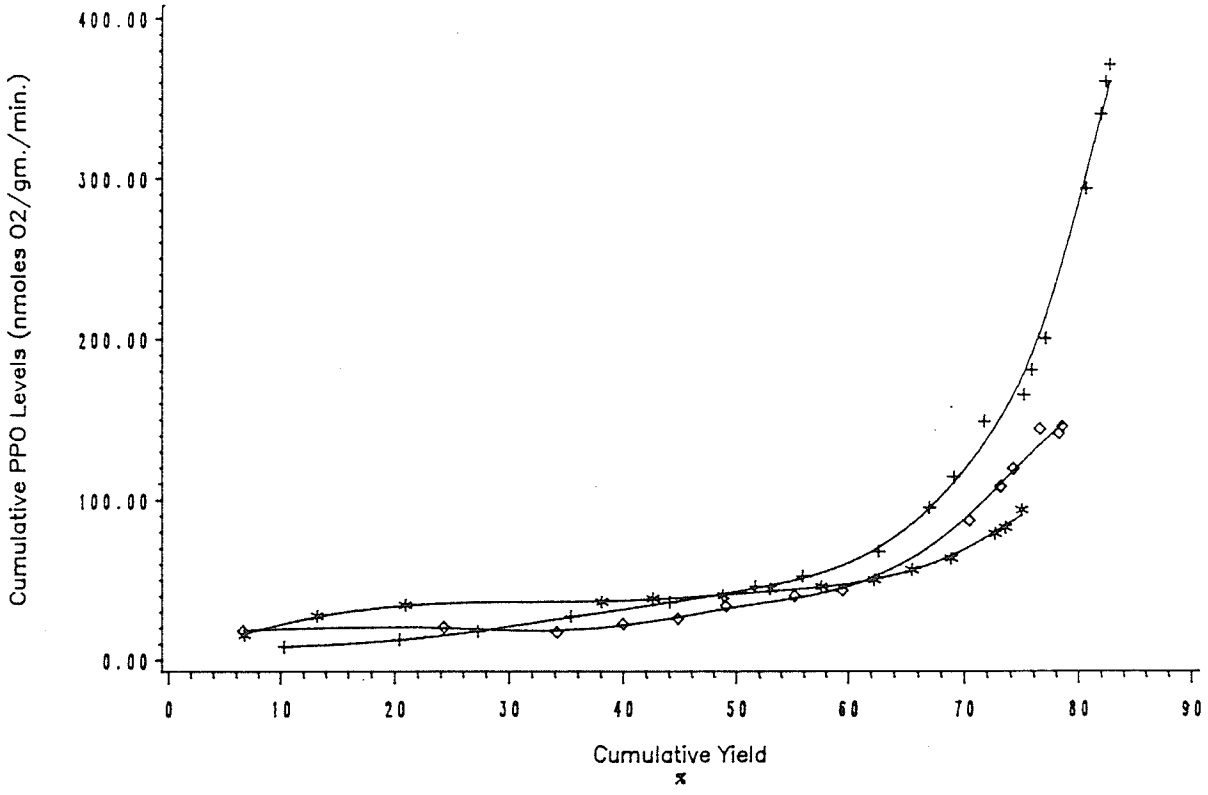
Figure 19 Cumulative PPO Levels Versus Cumulative Yield For the Three Different Extraction Rate Millings of HY320 (CPS)

* 75% Extraction Rate

◇ 80% Extraction Rate

+ 85% Extraction Rate


300.00
200.00
100.00
0.00
Cumulative PPO Levels (nmoles O2/gm./min.)
0
10
20
30
40
50
60
70
80
90
Cumulative Yield
%


400.00
300.00
200.00
100.00
0.00
Cumulative PPO Levels (nmoles O2/gm./min.)
0
10
20
30
40
50
60
70
80
90
Cumulative Yield
%

4.04 Phenolic Acid Composition of Pooled Flours

The analysis of the phenolic acids was divided into three groups; insoluble bound, soluble bound, and the free phenolic acids as outlined by Krygier *et al* (1982a).

Measurements on the reproducibility of the analysis for the individual components can be found in Table 4. As the analysis was confined to duplicate replications the coefficient of variation was averaged over each acid in its respective category. It should be noted that average values in all categories were within acceptable limits. The range of coefficient of variations was included to reflect the problem of the small sample size. It was particularly noticeable in the higher quality streams for the occasional replicate to have a large coefficient of variation as actual acid content was very low.

4.04.1 Insoluble Bound Phenolic Acids

The insoluble phenolics were the major component in all of the five varieties providing over 80% of the total phenolic content in every flour examined. Without exception, ferulic acid was the only simple phenolic component detected at measurable levels. It was surprising to observe that the amount of insoluble ferulic acid was quite similar across the five varieties examined as shown in Fig. 20 .

Table 4

AVERAGE AND RANGE OF COEFFICIENT OF VARIATION FOR HPLC ANALYSIS OF INDIVIDUAL PHENOLIC ACIDS

Phenolic Acid N=65	Free Acid (%)	Free Acid Range (min-max)	Soluble Bound Acid (%)	Soluble Bound Acid Range (min-max)	Insoluble Ferulic (%)	Insoluble Ferulic Range (min-max)
Ferulic	4.31	0.94-7.10	2.53	0.23-5.43	2.83	0.67-4.92
Vanillic	4.08*	0.00-12.4	3.74	0.13-12.2	N.D.	
Sinapic	N.D.		5.27	2.33-8.14	N.D.	
Caffeic	2.59**	0.75-7.32	2.83	0.90-5.10	N.D.	
Coumaric	4.47***	1.03-5.62	4.97	0.00-17.9	N.D.	
Syringic	6.93****	0.43-16.6	3.66	1.17-7.55	N.D.	

* N= 64
** N= 55
*** N= 46
**** N= 58
N.D. =Not Determined

Figure 20. Insoluble Bound Ferulic Acid Content of the Pooled Wheat Flours

A. Katepwa, Glenlea, and Norstar

B. Fielder and HY 320

P1_75 1st Patent Flour 75% Extraction

P2_75 2nd Patent Flour 75% Extraction

C1_75 1st Clear Flour 75% Extraction

C2_75 2nd Clear Flour 75% Extraction

SG_80 Straight Grade Flour 80% Extraction

P1_80 1st Patent Flour 80% Extraction

P2_80 2nd Patent Flour 80% Extraction

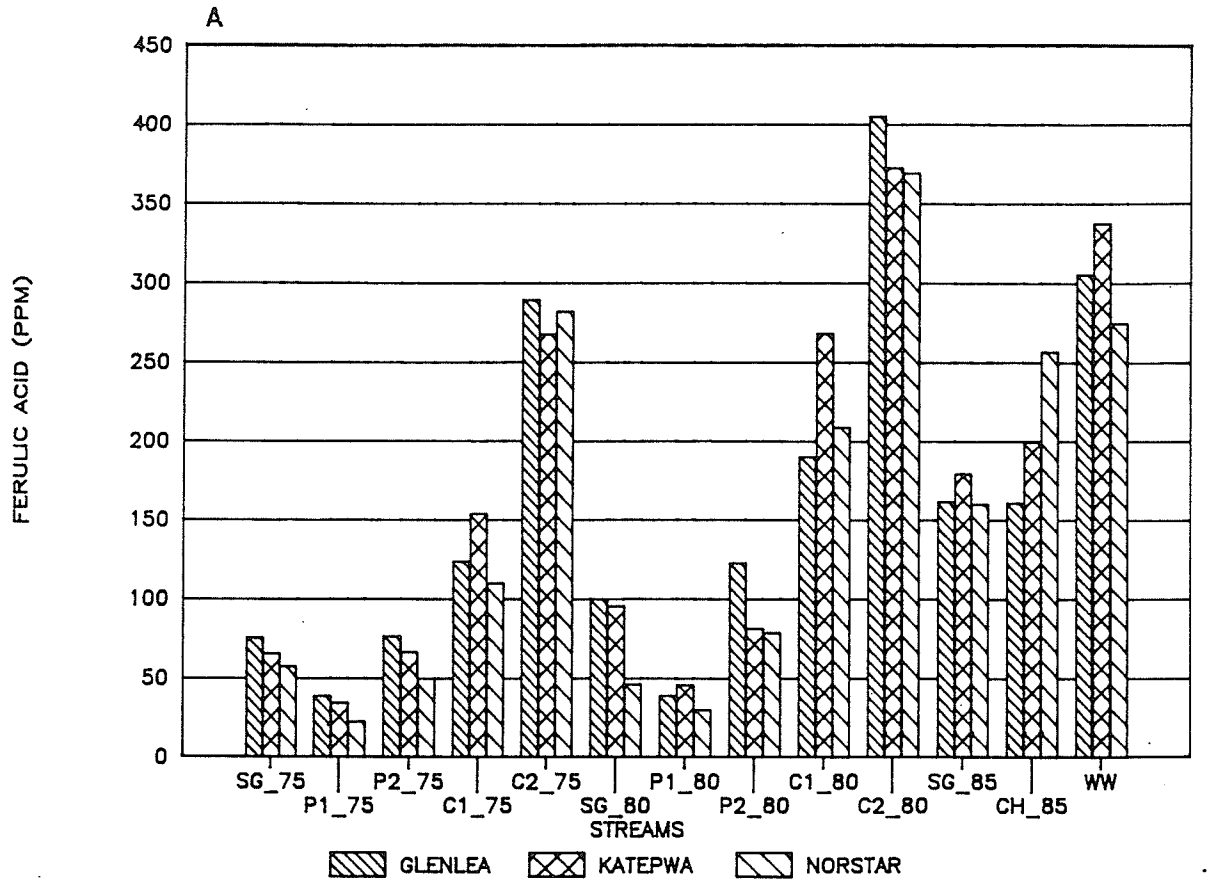
C1_80 1st Clear Flour 80% Extraction

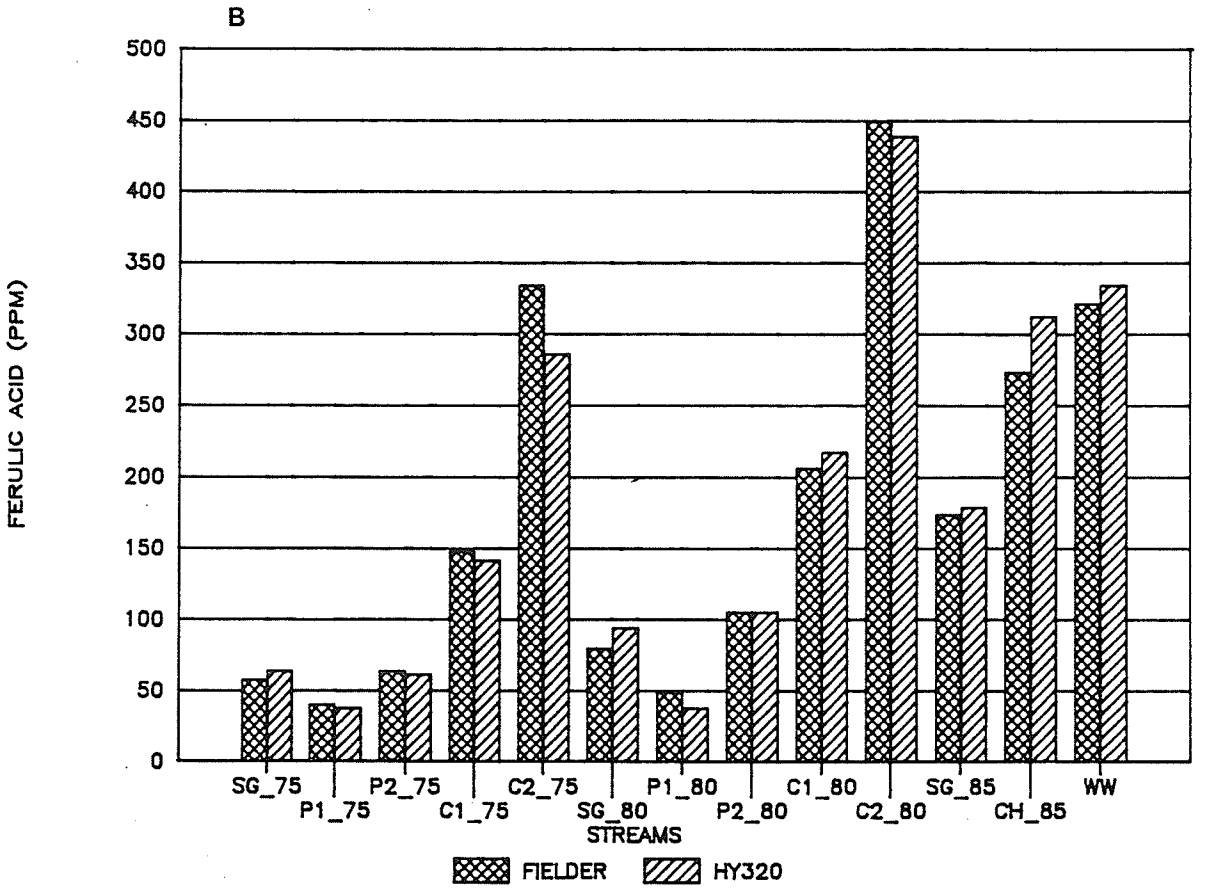
C2_80 2nd Clear Flour 80% Extraction

SG_85 Straight Grade Flour 85% Extraction

CH Chinese Standard Flour 85% Extraction

WW Ground Whole Wheat Sample


A
FERULIC ACID (PPM)
450
400
350
300
250
200
150
100
50
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH_85
WW
STREAMS
GLENLEA
KATEPWA
NORSTAR


B
FERULIC ACID (PPM)
500
450
400
350
300
250
200
150
100
50
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH_85
WW
STREAMS
FIELDER
HY320

4.04.1.1 1st Patent Flours

The 75% extraction 1st patent flour had, as anticipated, the lowest concentration of the insoluble bound ferulic acid. Except for Norstar, which had the lowest and significantly different, $p < 0.05$, concentration of 22.5 ppm., the four remaining varieties' 1st patent flours were tightly grouped between 34.4 to 40.0 ppm. The values found in this study agreed well with those reported by Jackson(1983) who found 36.1 ppm ferulic acid in his patent flour.

Examination of the 80% extraction 1st patent flour indicated that the concentration of the insoluble ferulic acid remained unchanged for both HY320 and Glenlea. Norstar, Katepwa and Fielder however did show significant, $p < 0.05$, increases in their insoluble ferulic acid content. The concentration detected in Norstar at the 80% extraction level, 30.0 ppm., was noted to be lower than all other varieties milled at the 75% extraction rate. The ferulic acid content of this flour extended from 30.0 ppm to a high of 48.8 ppm in Fielder. Conversion of these level to a percentage of the total amount present in the ground grain indicated Fielder had the largest component, 15.2% while Norstar the smallest at 10.9%. Each of the varieties displaying an increase in this phenolic acid also exhibited an increase in ash content relative to the 75% 1st patent flour.

4.04.1.2 2nd Patent Flours

Examination of the 2nd patent flours, milled at the 75% extraction rate, indicated a marked increase in levels for all five varieties. The harder wheats, Glenlea, Katepwa, and Norstar, doubled their ferulic acid content while the softer HY320 and Fielder samples increased by a smaller extent. Norstar continued to display the lowest content in absolute terms at 50.0 ppm while Glenlea had the greatest at 76.3 ppm.

Each varieties 80% 2nd patent flour however displayed a noticeable increase in their level of insoluble ferulic acid with respect to both the 80% 1st patent flour, and the 75% 2nd patent flour. Glenlea had the highest concentration at 122.5 ppm, which was significantly different, $p < 0.05$, from the next closest group of HY320 and Fielder, with 105 ppm. each. A third group composed of Katepwa and Norstar were also distinct from the others with similar values of 81.3 and 78.8 ppm respectively. Each variety, with the exception of Katepwa, showed a two fold increase in this form of ferulic acid over their 80% 1st patent values. These 80% 2nd patent flour acid levels, expressed on a relative percentage of the total available, extended from 24.1 % for Katepwa to 40% in Glenlea.

4.04.1.3 1st Clear Flours

An additional two fold increase was observed in the analysis of the 75% extraction 1st clear flours relative to their 2nd patent counterparts in each variety. The values extended from the lowest, 110 ppm, in Norstar, to a high of 153.8 ppm found in Katepwa. The insoluble ferulic acid content of the hard wheats was found to be statistically distinct, $p < 0.05$, from each other and the softer wheats.

Increases were observed in the 1st clear streams at the 80 % extraction level. Katepwa underwent a three fold increase, achieving the maximum detected, 268 ppm. This was equivalent to 79.4 % of the total insoluble ferulic acid available. Norstar, Fielder, and HY320 all doubled in content, to achieve values tightly grouped between 206-217 ppm. No statistical difference could be determined between these three varieties. Glenlea distinguished itself from the other samples by demonstrating only a modest 50% increase relative to its 2nd patent flour and having the minimum content of 190 ppm. Although considerably lower than the other varieties, this level on a relative percentage basis of total insoluble ferulic acid available, represented 62.2%, which was equivalent to that observed for both Fielder and HY320.

4.04.1.4 2nd Clear Flours

The additional minor increase in yield to form the 75% 2nd clear flours resulted in large increases in the insoluble ferulic acid content in each variety. Fielder displayed the largest change rising to 334 ppm, 103.9% of the total available, and was found to be significantly distinct, $p < 0.05$, from all other varieties. The harder wheats tended to group together with an average value for these wheats of 281.1 ppm insoluble bound ferulic acid. The very different content found in the Fielder sample was suspected to be due to the composition of this flour. Unlike the harder wheats, Fielder's 75% extraction 2nd clear flour was composed entirely of the high ash shorts duster stream while in the other samples this stream did not exceed 30% of the pooled flour.

As was the case in the 75% 2nd clear flours, Fielder's 449.4 ppm., displayed the highest concentration at the 80% extraction level. Similarly, the softer HY320 followed closely at 438.8 ppm. The harder wheats were noticeably lower with Glenlea, the highest, at just over 405 ppm. Katepwa and Norstar were even further removed at values of 369.4 and 372.5 ppm respectively. In each variety the insoluble ferulic acid content exceeded 100% of that found in the whole wheat sample.

4.04.1.5 Straight Grade Flours

Analysis of each variety's straight grade flour at the 75% extraction level revealed that the insoluble bound ferulic acid contents were statistically indistinguishable, $p < 0.05$, from their respective 2nd patent counterparts. As before , Glenlea displayed the highest concentration at 76.3 ppm. while Norstar and Fielder the least. These straight grade flour insoluble phenolic acid levels were in agreement with those reported by Sosulski *et al.*(1982) for a Neepawa wheat flour of 63.6 ppm.

The 80% straight grade flour's insoluble ferulic acid level in each variety reflected the differences observed in the ash content relative to their corresponding 2nd patent. Where the ash content was lower in the straight grade flour, there was a corresponding decrease in the ferulic acid level relative to the 2nd patent flour. This was observed in the varieties Norstar, HY320, Glenlea and Fielder. The insoluble ferulic acid contents ranged from a low of 46.3 ppm for Norstar to a high of 100.0 ppm in Glenlea. Katepwa and HY320 had values similar to Glenlea and were not found to be distinguishable from its value.

Considerably higher insoluble ferulic acid levels were observed for the 85% extraction straight grade flours ranging from 160.2 -179.0 ppm. Due to the limited range no significant difference, $p < 0.05$, could be detected amongst the varieties. These values represented a range of 53 - 58%

of the total insoluble ferulic acid found in the whole wheat sample.

4.04.1.6 Other Samples

The Chinese standard flours were prepared from selected 85% extraction streams to yield an ash content maximum of 1.20 %. This elevated ash highlighted the differences between the varieties. Since the criteria for preparing this flour was based solely on the final cumulative ash content, which was controlled by both variety and environment, direct comparison between varieties was limited due to their different stream composition. The higher ash hard wheats displayed the lowest insoluble ferulic acid as their stream composition limited the later stream additions to the pooled flour. Norstar, the lowest ash wheat was able to incorporate higher ash streams into this flour than the other hard wheats which in turn was reflected in its considerably higher ferulic content, 256.6 ppm, than both Katepwa and Glenlea. Actual contents extended from 312.4 ppm in HY320 to 161.0 ppm in Glenlea.

The whole wheat samples revealed marked similarities across varietal lines. Only Norstar, at 274 ppm, was found to be significantly different, $p < 0.05$, than the other 4 varieties which averaged 325 ppm.

4.04.1.7 Comments

The relationship between insoluble bound ferulic acid and ash content showed a very good linear agreement as can be seen if Figs. 21 - 25. The correlation coefficient ranged from 0.93 for Fielder to a high of 0.98 for Glenlea at $p < 0.05$.

4.04.2 Soluble Bound Phenolic Acids

The soluble phenolic acids detected in the five wheats fell into two broad categories; the dominant components, consisting of sinapic, ferulic and vanillic acids , and the detectable, comprised of syringic, caffeic, and p-coumaric acids. Sinapic acid was the predominant acid in all five varieties. The soluble phenolic acids analyzed represented a maximum of 17% of the overall total phenolic content of a prepared flour, reaching a maximum value of 93.8 ppm for Fielder. Figs 26-30 represent the individual acids detected within a flour for each variety.

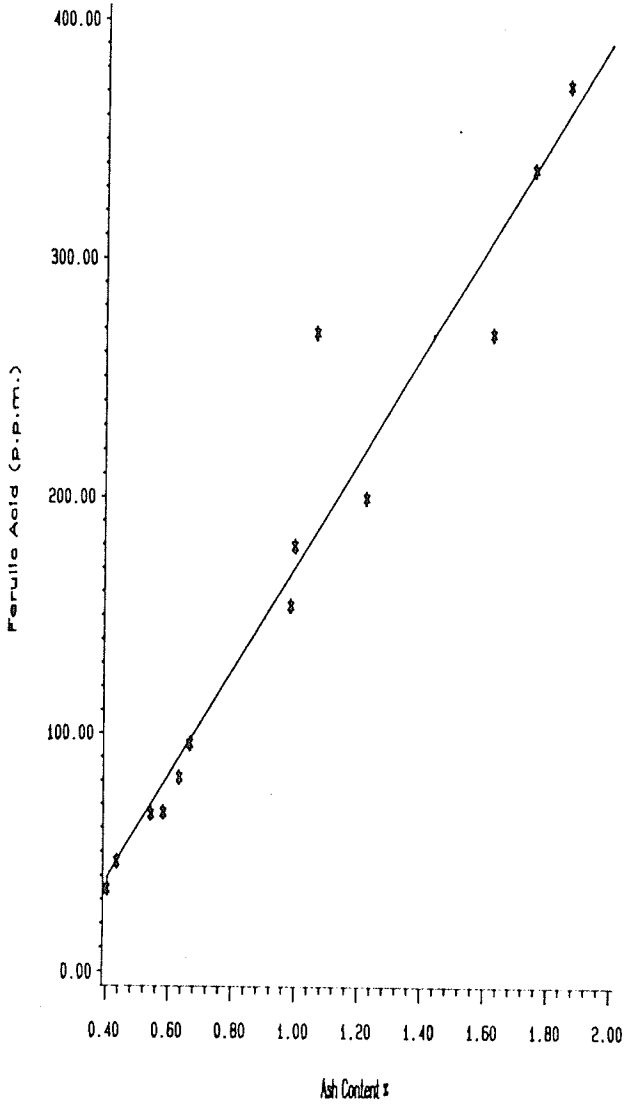
4.04.2.1 1st Patent Flour

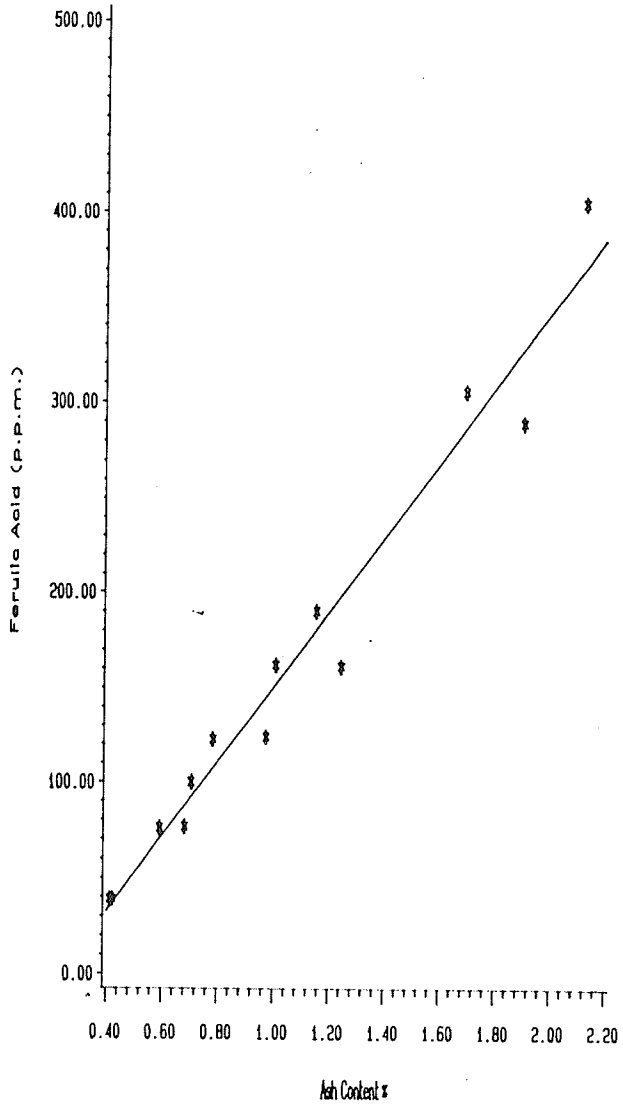
The 1st patent (75%) flours varied significantly ,$p < 0.05$, in their total soluble bound phenolic acid components. Total content varied greatly with variety extending from a low of 0.91 ppm for Norstar to a high of 4.25 ppm. in Fielder. Coumaric acid was not detected in any of the

Figure 21 Insoluble Bound Ferulic Acid Content's Relationship with Ash in the Variety Norstar

Figure 22 Insoluble Bound Ferulic Acid Content's Relationship with Ash in the Variety Glenlea

Figure 23 Insoluble Bound Ferulic Acid Content's Relationship with Ash in the Variety Katepwa


Ferulic Acid (p.p.m.)
400.00
300.00
200.00
100.00
0.00
0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00
Ash Content %


Ferulic Acid (p.p.m.)
500.00
400.00
300.00
200.00
100.00
0.00
0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20
Ash Content %

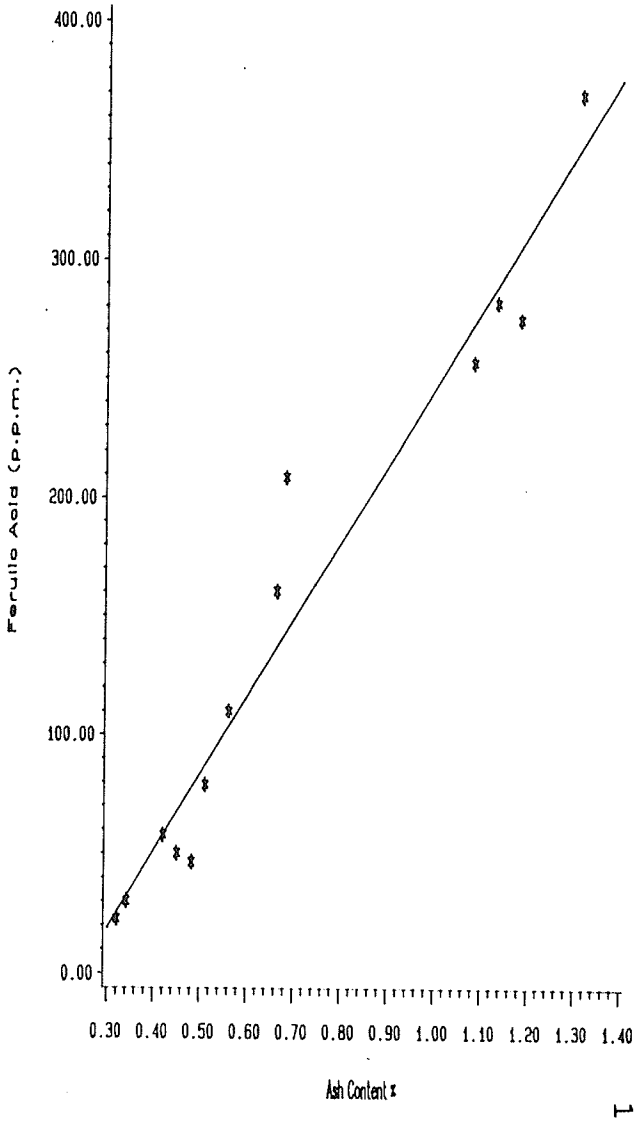
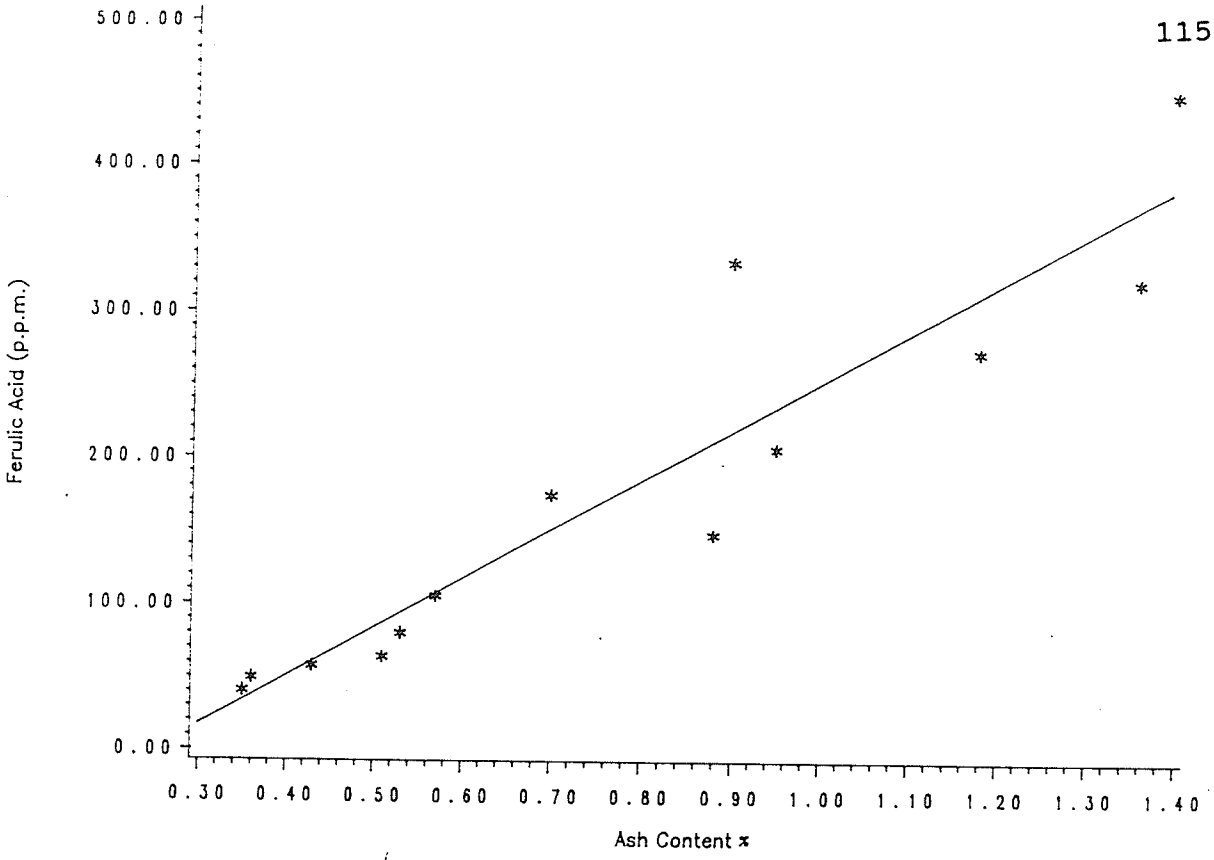

Ferulic Acid (p.p.m.)
400.00
300.00
200.00
100.00
0.00
0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40
Ash Content %

Figure 24 Insoluble Bound Ferulic Acid Content's Relationship with Ash in the Variety Fielder

Figure 25 Insoluble Bound Ferulic Acid Content's Relationship with Ash in the Variety HY320


500.00
400.00
300.00
200.00
100.00
0.00
Ferulic Acid (p.p.m.)
0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40
Ash Content %

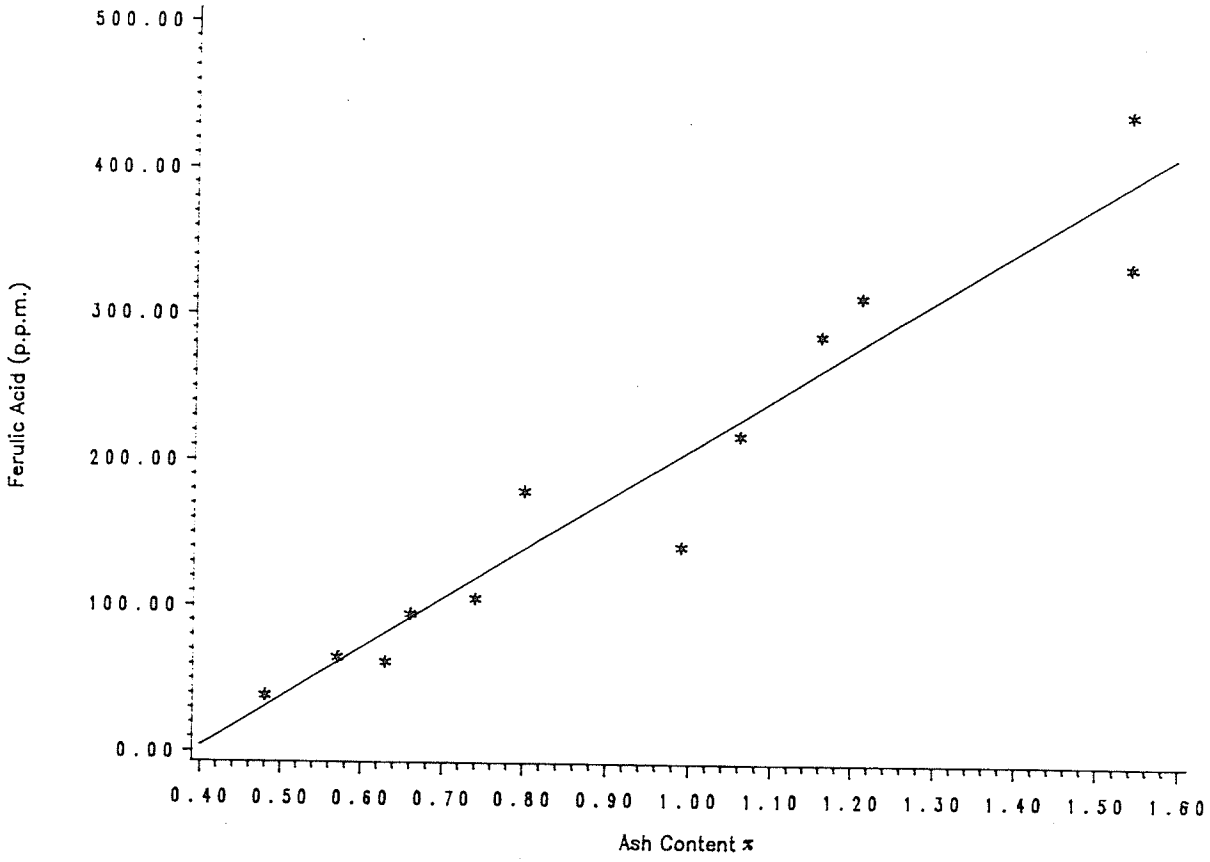

500.00
400.00
300.00
200.00
100.00
0.00
Ferulic Acid (p.p.m.)
0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60
Ash Content %

Figure 26 Major and Secondary Soluble Bound Phenolic Acids in the Variety Katepwa

SG_75 Straight Grade Flour 75% Extraction

P1_75 1st Patent Flour 75% Extraction

P2_75 2nd Patent Flour 75% Extraction

C1_75 1st Clear Flour 75% Extraction

C2_75 2nd Clear Flour 75% Extraction

SG_80 Straight Grade Flour 80% Extraction

P1_80 1st Patent Flour 80% Extraction

P2_80 2nd Patent Flour 80% Extraction

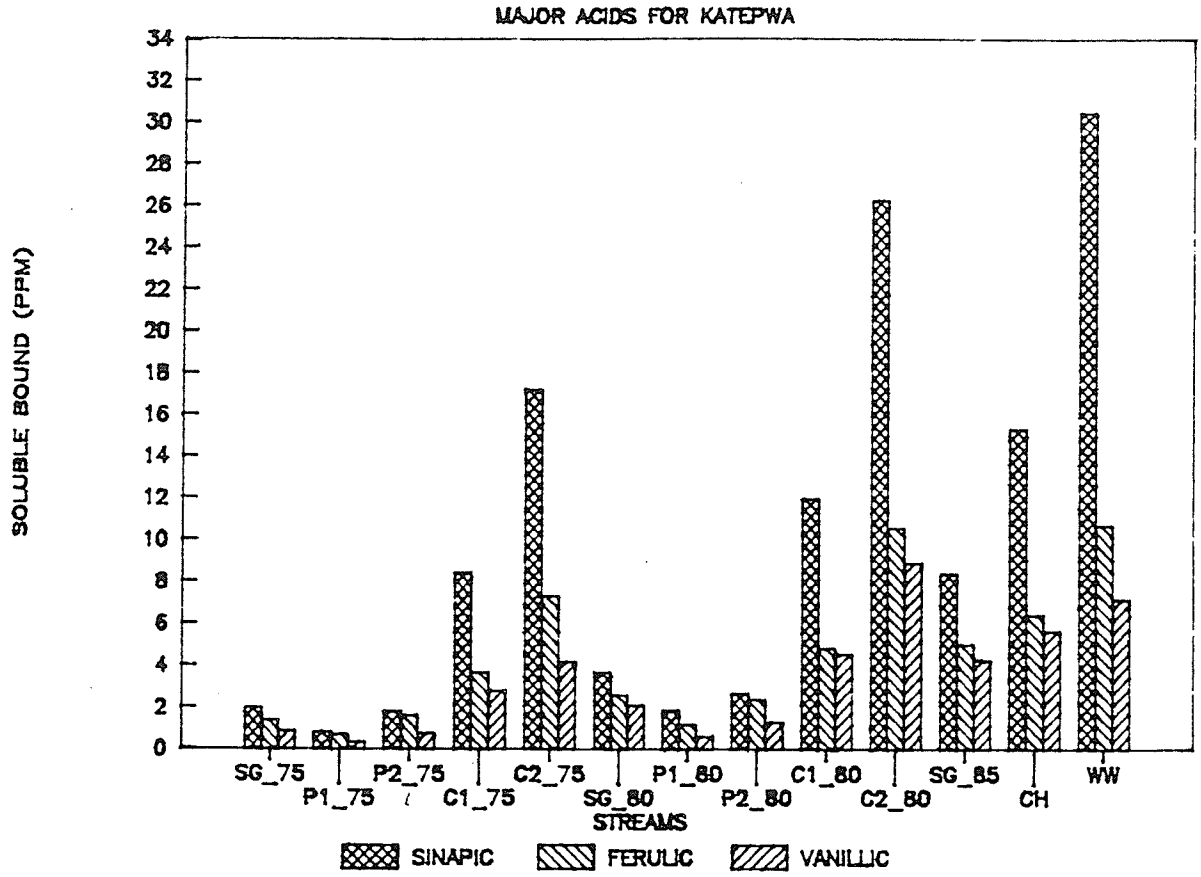
C1_80 1st Clear Flour 80% Extraction

C2_80 2nd Clear Flour 80% Extraction

SG_85 Straight Grade Flour 85% Extraction

CH Chinese Standard Flour 85% Extraction

WW Ground Whole Wheat Sample


MAJOR ACIDS FOR KATEPWA
SOLUBLE BOUND (PPM)
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
SINAPIC
FERULIC
VANILLIC

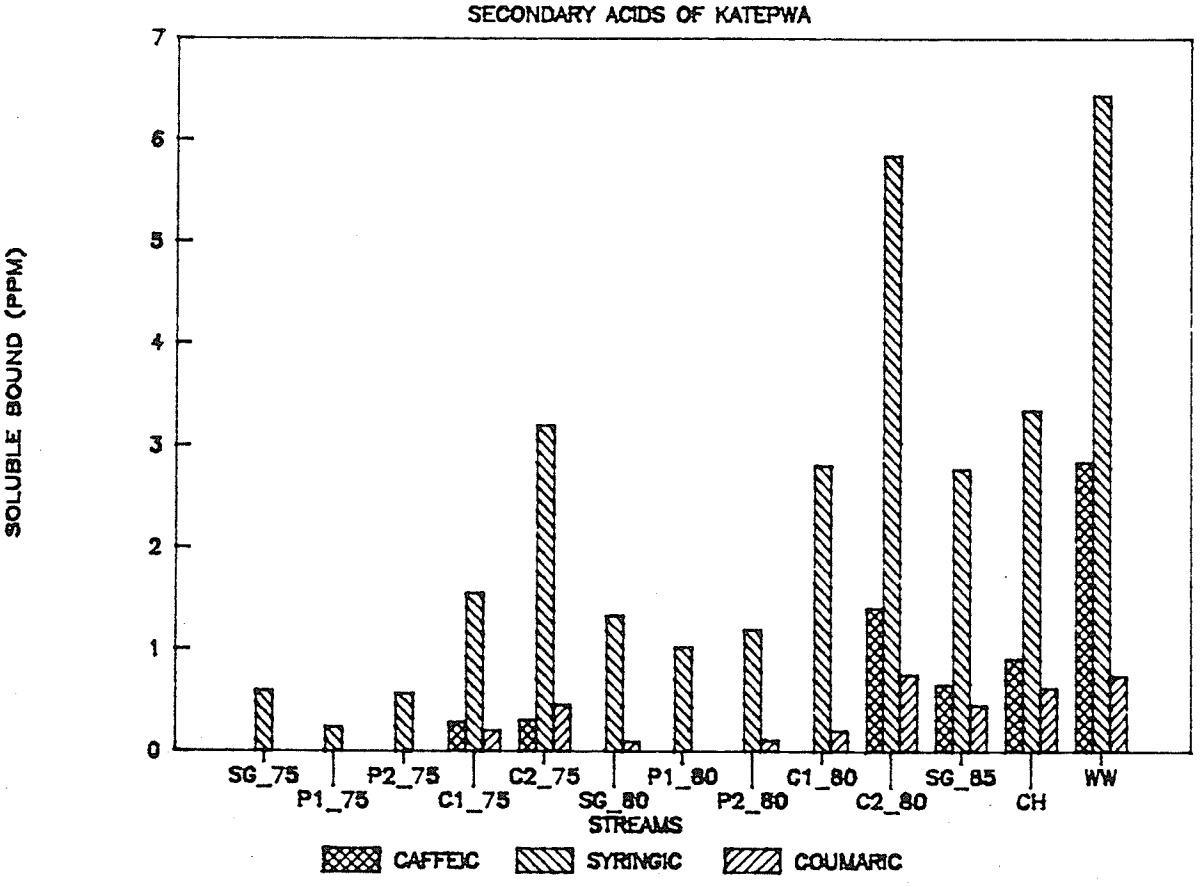

SECONDARY ACIDS OF KATEPWA
SOLUBLE BOUND (PPM)
7
6
5
4
3
2
1
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
CAFFEIC
SYRINGIC
COUMARIC

Figure 27 Major and Secondary Soluble Bound Phenolic Acids in the Variety Glenlea

SG_75 Straight Grade Flour 75% Extraction

P1_75 1st Patent Flour 75% Extraction

P2_75 2nd Patent Flour 75% Extraction

C1_75 1st Clear Flour 75% Extraction

C2_75 2nd Clear Flour 75% Extraction

SG_80 Straight Grade Flour 80% Extraction

P1_80 1st Patent Flour 80% Extraction

P2_80 2nd Patent Flour 80% Extraction

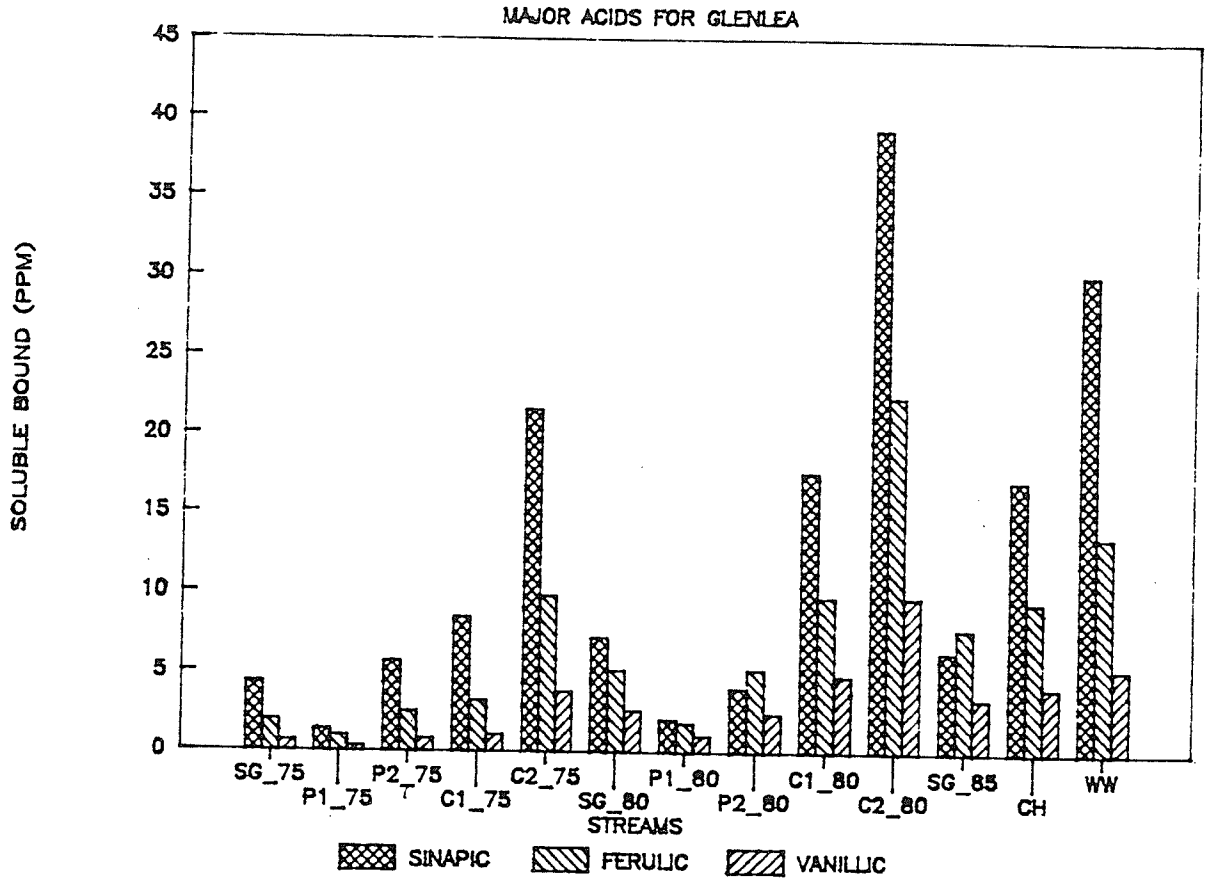
C1_80 1st Clear Flour 80% Extraction

C2_80 2nd Clear Flour 80% Extraction

SG_85 Straight Grade Flour 85% Extraction

CH Chinese Standard Flour 85% Extraction

WW Ground Whole Wheat Sample


MAJOR ACIDS FOR GLENLEA
SOLUBLE BOUND (PPM)
45
40
35
30
25
20
15
10
5
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
SINAPIC
FERULIC
VANILLIC

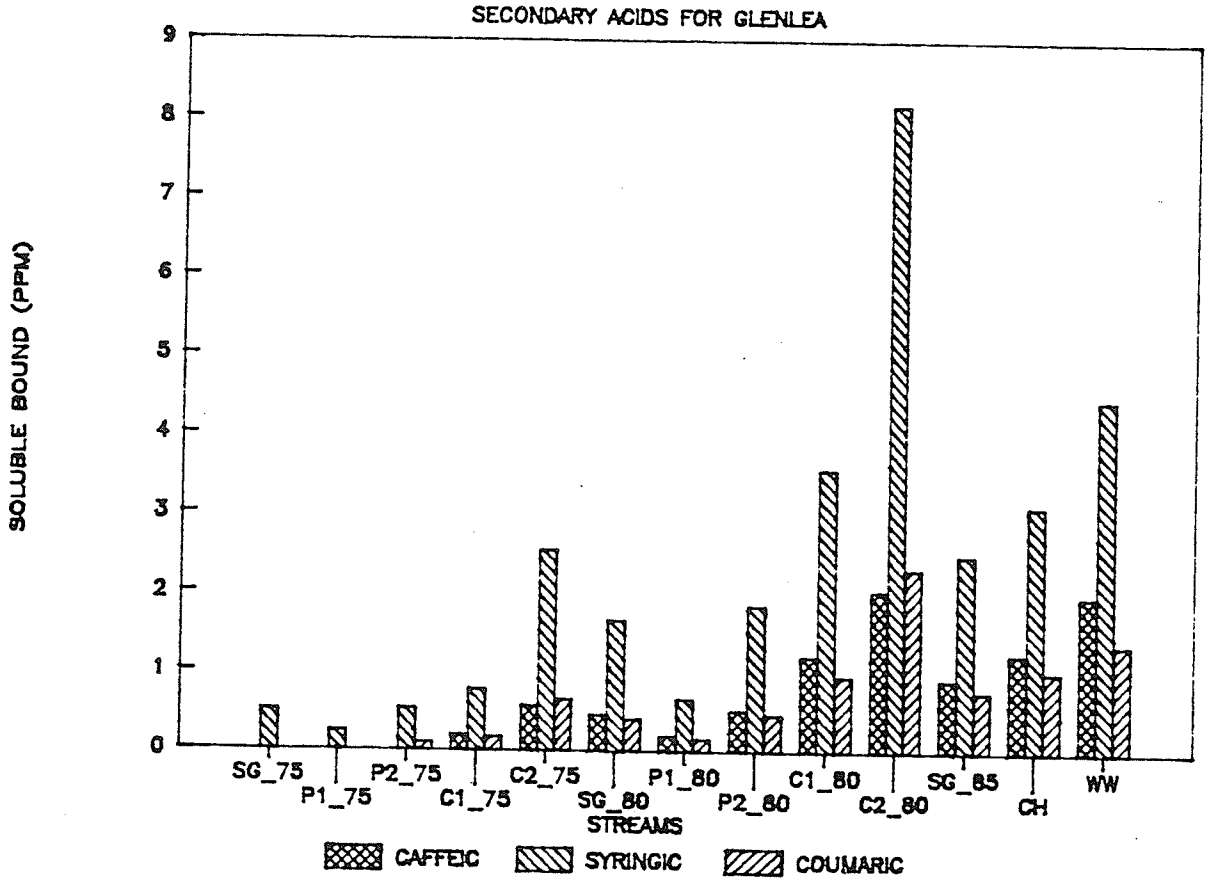

SECONDARY ACIDS FOR GLENLEA
SOLUBLE BOUND (PPM)
9
8
7
6
5
4
3
2
1
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
CAFFEIC
SYRINGIC
COUMARIC

Figure 28 Major and Secondary Soluble Bound Phenolic Acids in the Variety Norstar

SG_75 Straight Grade Flour 75% Extraction

P1_75 1st Patent Flour 75% Extraction

P2_75 2nd Patent Flour 75% Extraction

C1_75 1st Clear Flour 75% Extraction

C2_75 2nd Clear Flour 75% Extraction

SG_80 Straight Grade Flour 80% Extraction

P1_80 1st Patent Flour 80% Extraction

P2_80 2nd Patent Flour 80% Extraction

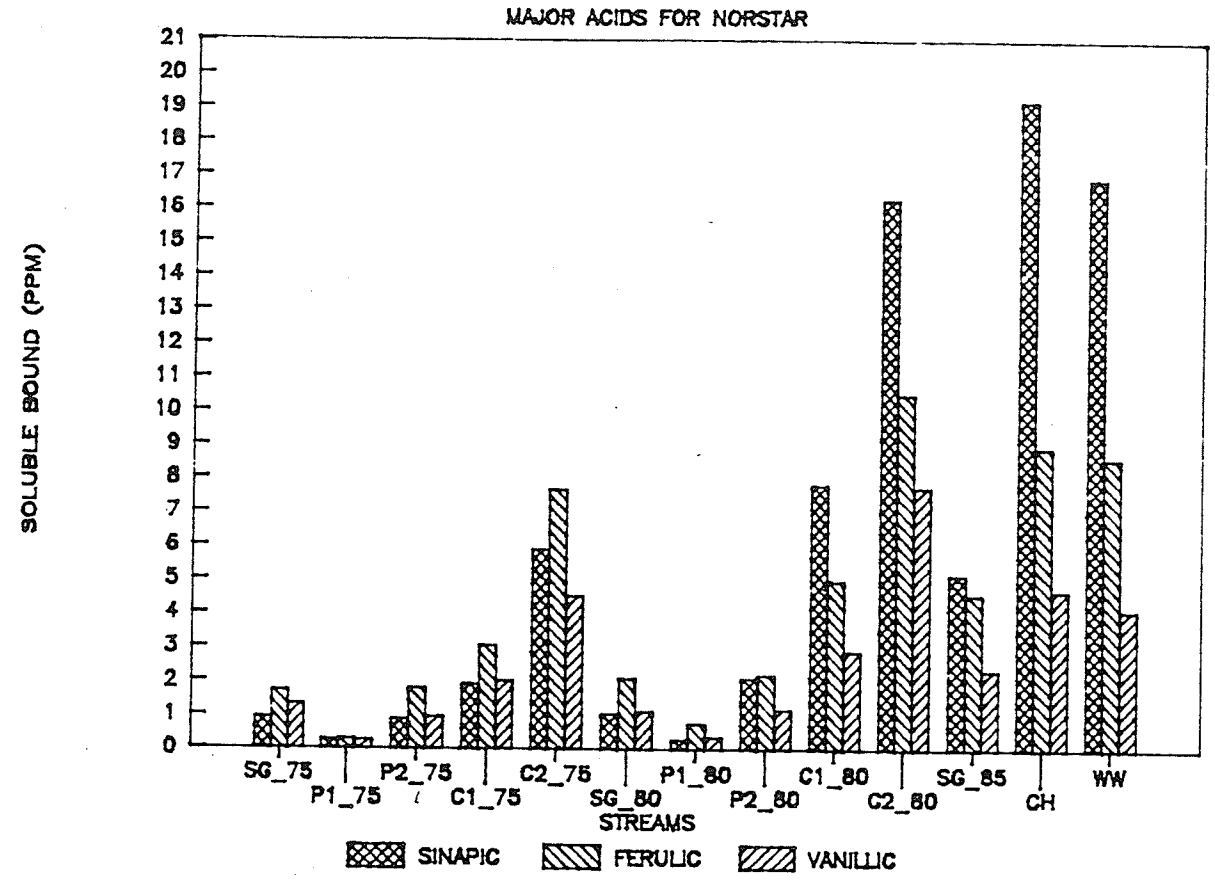
C1_80 1st Clear Flour 80% Extraction

C2_80 2nd Clear Flour 80% Extraction

SG_85 Straight Grade Flour 85% Extraction

CH Chinese Standard Flour 85% Extraction

WW Ground Whole Wheat Sample


MAJOR ACIDS FOR NORSTAR
SOLUBLE BOUND (PPM)
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
SINAPIC
FERULIC
VANILLIC

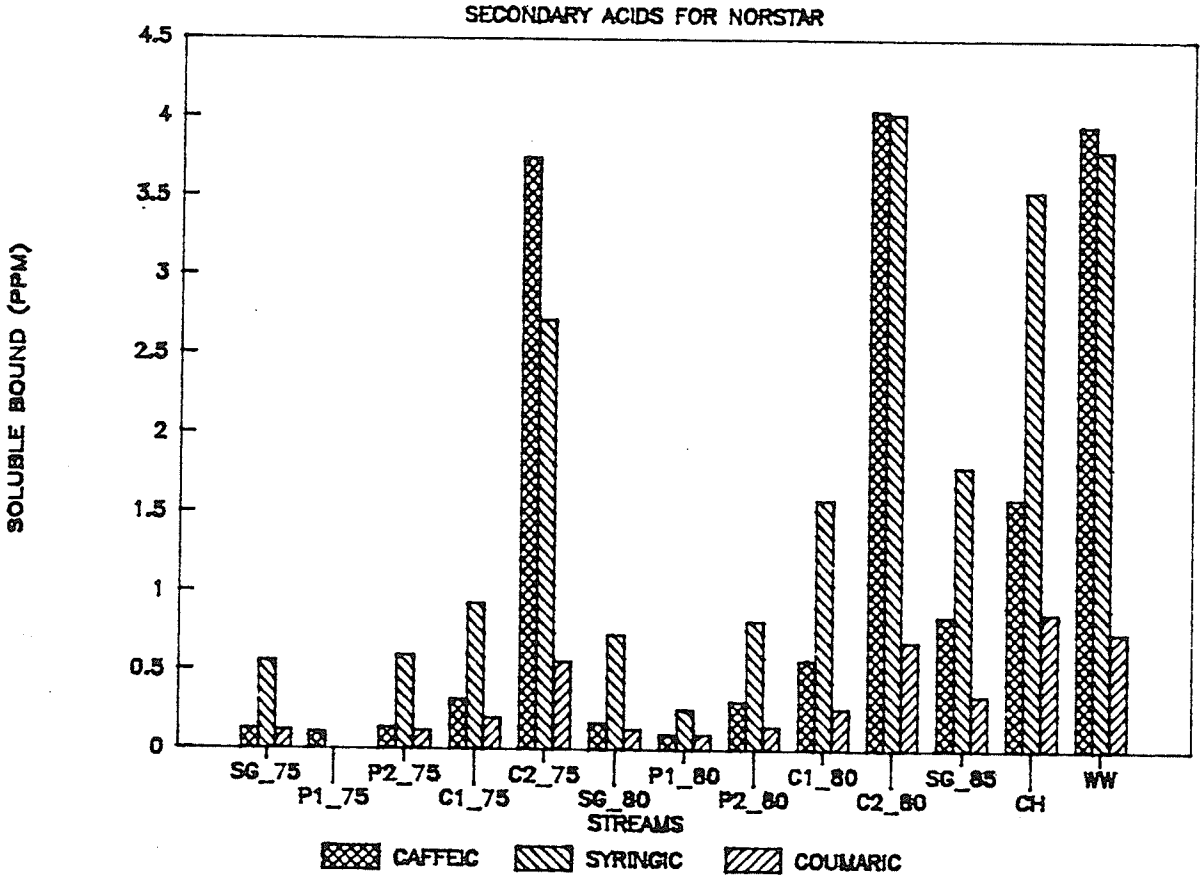

SECONDARY ACIDS FOR NORSTAR
SOLUBLE BOUND (PPM)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
CAFFEIC
SYRINGIC
COUMARIC

Figure 29 Major and Secondary Soluble Bound Phenolic Acids in the Variety HY320

P1_75 1st Patent Flour 75% Extraction

P2_75 2nd Patent Flour 75% Extraction

C1_75 1st Clear Flour 75% Extraction

C2_75 2nd Clear Flour 75% Extraction

SG_80 Straight Grade Flour 80% Extraction

P1_80 1st Patent Flour 80% Extraction

P2_80 2nd Patent Flour 80% Extraction

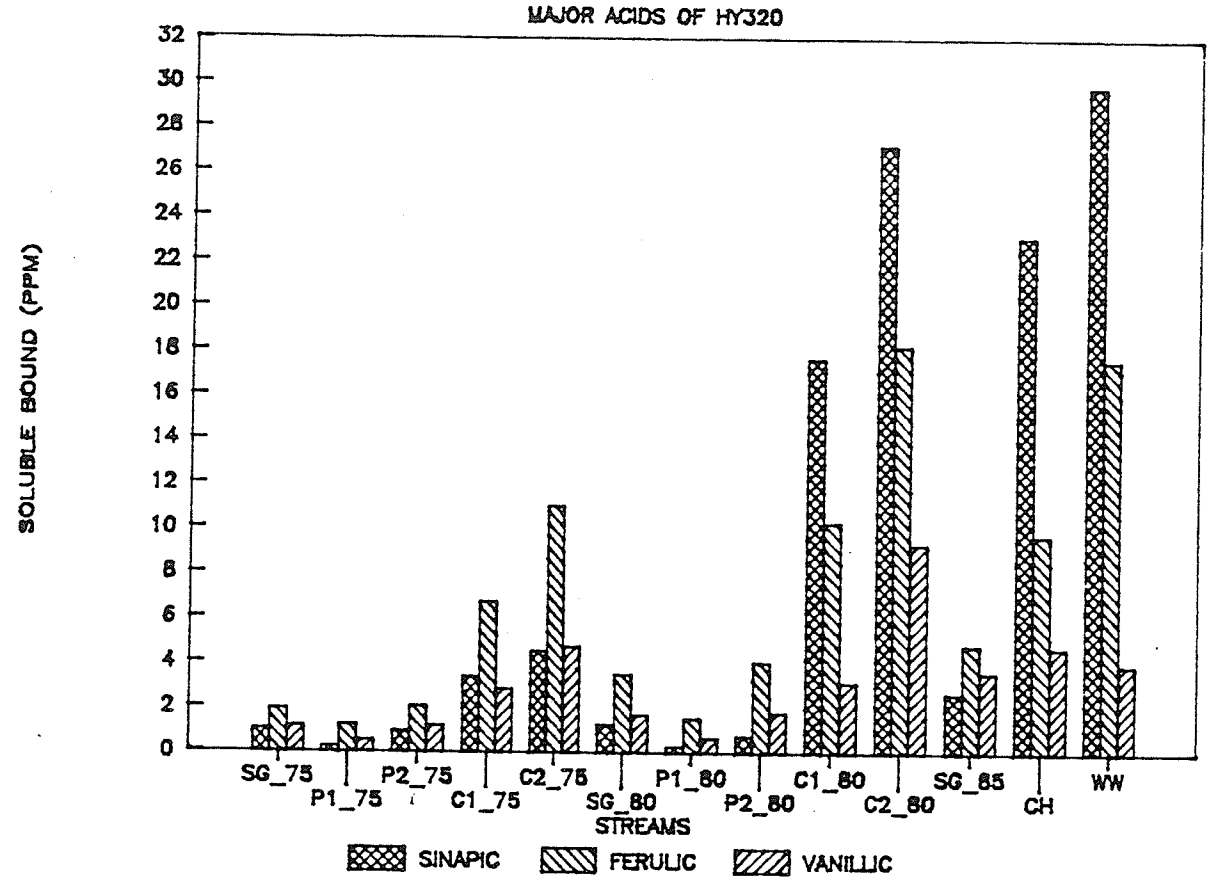
C1_80 1st Clear Flour 80% Extraction

C2_80 2nd Clear Flour 80% Extraction

SG_85 Straight Grade Flour 85% Extraction

CH Chinese Standard Flour 85% Extraction

WW Ground Whole Wheat Sample


MAJOR ACIDS OF HY320
SOLUBLE BOUND (PPM)
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
SINAPIC
FERULIC
VANILLIC

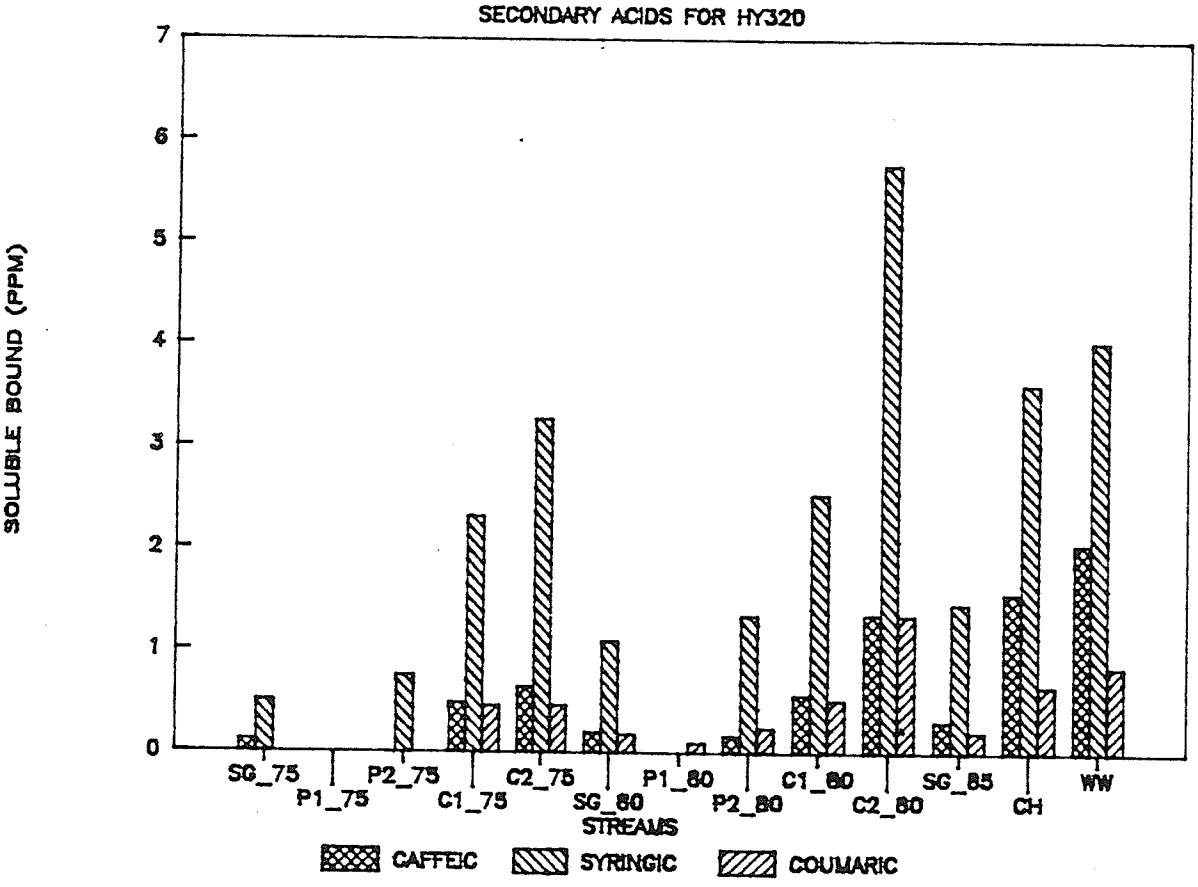

SECONDARY ACIDS FOR HY320
SOLUBLE BOUND (PPM)
7
6
5
4
3
2
1
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
CAFFEIC
SYRINGIC
COUMARIC

Figure 30 Major and Secondary Soluble Bound Phenolic Acids in the Variety Fielder

P1_75 1st Patent Flour 75% Extraction

P2_75 2nd Patent Flour 75% Extraction

C1_75 1st Clear Flour 75% Extraction

C2_75 2nd Clear Flour 75% Extraction

SG_80 Straight Grade Flour 80% Extraction

P1_80 1st Patent Flour 80% Extraction

P2_80 2nd Patent Flour 80% Extraction

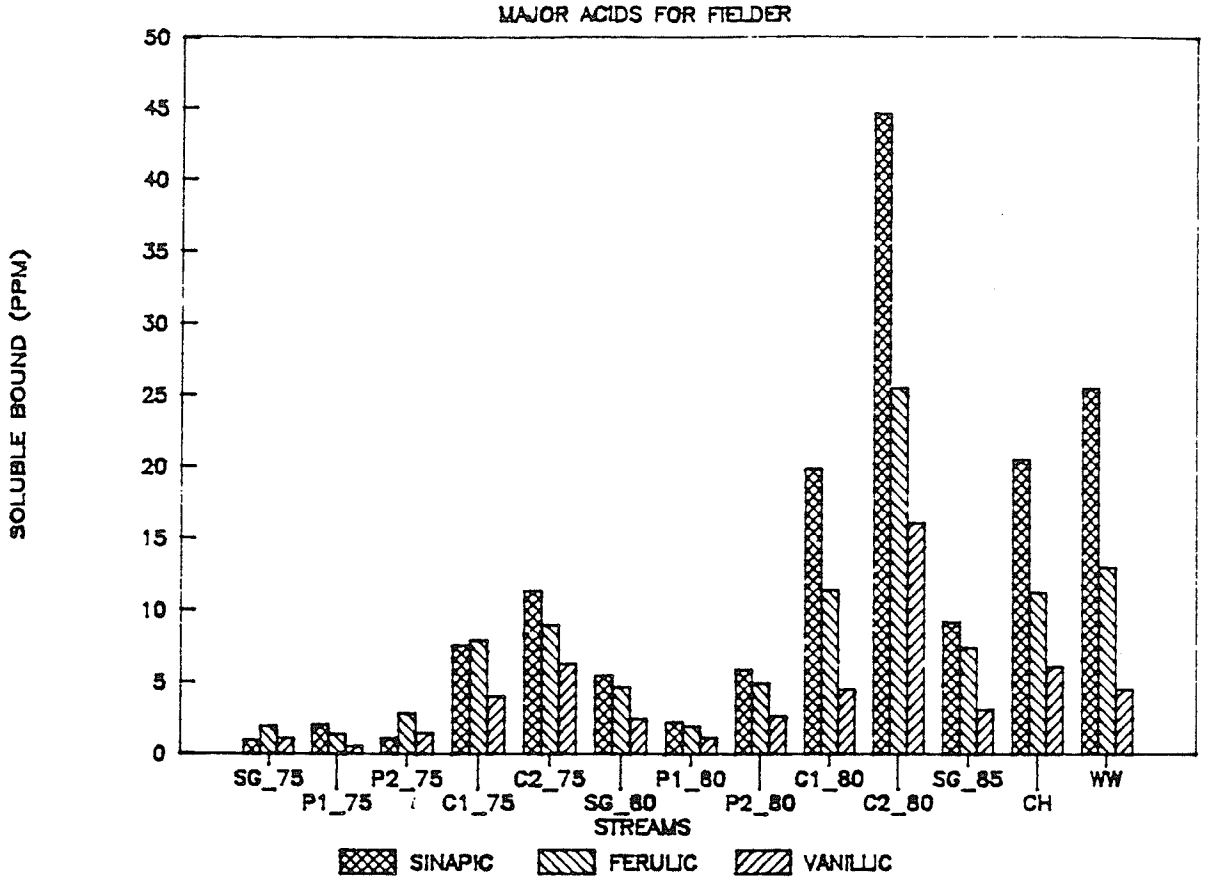
C1_80 1st Clear Flour 80% Extraction

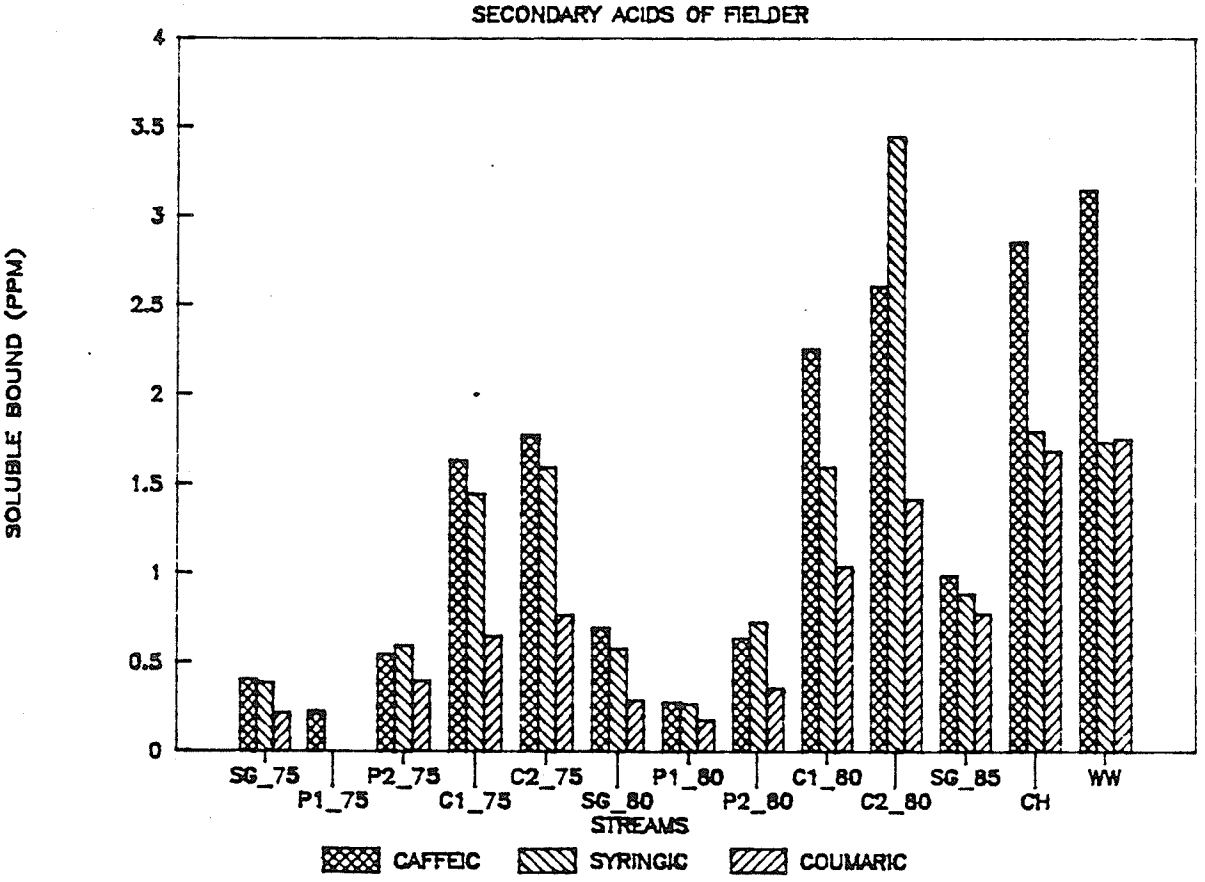
C2_80 2nd Clear Flour 80% Extraction

SG_85 Straight Grade Flour 85% Extraction

CH Chinese Standard Flour 85% Extraction

WW Ground Whole Wheat Sample


MAJOR ACIDS FOR FIELDER
SOLUBLE BOUND (PPM)
50
45
40
35
30
25
20
15
10
5
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
SINAPIC
FERULIC
VANILLIC


SECONDARY ACIDS OF FIELDER
SOLUBLE BOUND (PPM)
4
3.5
3
2.5
2
1.5
1
0.5
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
CAFFEIC
SYRINGIC
COUMARIC

varieties in this flour. Sinapic acid was the major acid in the wheat flours, accounting for over 40% of both HY320's and Fielder's composition. Ferulic acid was a major contributor to the total soluble bound acids present in each variety. This flour represented a maximum of 8.5% of the total soluble bound phenolic acids detected in the whole wheat sample. Although Fielder did display the maximum percentage, the remaining four varieties did not exceed 5.0%.

In the subsequent 80% extraction flours the soluble phenolic acid content doubled for each of the three hard wheats. The softer HY320 and Fielder increased to a lesser extent although Fielder still contained the largest total amount at 6.09 ppm. Glenlea was not discernibly different at 6.07 ppm. In both Glenlea and Fielder, although sinapic acid remained the dominant component, its relative importance was decreased. The difference was due to a noticeable increase in the role of vanillic and syringic acids. Ferulic acid, the second major component continued to contribute approximately the same percentage as in the 75% extraction flour for these two varieties. An interesting observation was that only in HY320 did ferulic acid contribute 60% of the total soluble acid content in both patent flours. Fielder again represented the variety having the largest percentage of the total available bound acids at 12.2%. Norstar and HY320 however continued to remain below 5.0% even at this higher extraction level.

4.04.2.2 2nd Patent Flour

Major increases in the soluble bound phenolic acid content were observed in all varieties moving from the 1st patent to 2nd patent flours at the 75% extraction level. All varieties displayed a minimum two fold increase with Norstar reaching a 450 % increase. It was only in Glenlea that sinapic acid accounted for a disproportionate percentage, 58.8%, of the total. Coumaric acid was still not detected in either HY320 or Katepwa in this flour. The total soluble bound phenolic acid content extended from 4.52 ppm in Norstar to 9.73 ppm in Glenlea.

At the 80% extraction level, both Fielder and Glenlea's 2nd patent flours continued to be distinct from the other varieties in terms of their total soluble bound acid content. The respective values of 15.3 and 14.6 ppm were at approximately twice that found in the remaining varieties. These acid levels represented 30.6 and 25.1% of the total soluble bound phenolic acids available in the ground grain. The other varieties relative acid content accounted for a noticeably lower percentage, 13.1-17.8%. The greatest increase in acid content on a percentage basis, 30%, from their 80% 1st patent flours was displayed by both Norstar and HY320. Only Fielder doubled its total content compared to its 75% extraction stream. Examination of the acid levels detected in the 80% 2nd patent flour relative to those in

the 75% flour indicated that Fielder doubled in content while the other varieties displayed only an approximate 50% increase.

4.04.2.3 1st Clear Flours

The 75 % extraction 1st clear flours represented the first flour in which all of the phenolic acids were detected in each variety. Large increases in the soluble bound phenolic acids relative to the 2nd patent flour were observed. Fielder continued to display the greatest quantity of these acids with ferulic and sinapic acids being found in approximately equal quantity. The ferulic acid component of Fielder, 7.96 ppm., was roughly twice that found in any of the hard wheat varieties. HY320 approached this level with a value of 6.74 ppm. The major difference between HY320 and Fielder was the significantly reduced content of sinapic acid, approximately half of that found in Fielder. The concentration of ferulic acid in the three hard wheats was essentially equivalent, ranging from 3.07 to 3.69 ppm . However they did displayed large disparities in their sinapic acid contents ranging from 1.93 to 8.48 ppm. Norstar's total soluble acid content was noticeably lower, 8.49 ppm, than any of the other varieties and this represented only 22.2 % of the total available. Fielder, at the other extreme, 23.4 ppm, had 46.9 % of its total soluble acid content found in this flour.

Like their 75% counterparts, the 80% extraction 1st clear flours also displayed noticeable increases in all varieties from their 80% 2nd patent flours. The average increase in their soluble bound acid content was greater than three fold. Compared to their 1st clear 75% extraction counterparts, each variety displayed a major increase in acid content. Although the increase for Fielder was not as great as seen in Glenlea, it still contained the largest quantity of soluble bound phenolics at 40.8 ppm while Norstar remained the lowest at 18.2 ppm. These two varieties also reflected the extremes in the percentage of total available, extending from 81.9 to 47.6% respectively. Sinapic acid accounted for over than 40% of the total acids in all varieties including HY320.

4.04.2.4 2nd Clear Flours

The 2nd clear pooled flours at the 75% extraction level represented the second highest concentration of soluble bound phenolics analyzed for all varieties except Fielder. Large increases were observed in the quantity of sinapic acid in particular. Bound vanillic acid in Fielder was noticeably higher than any of the other wheats. HY320, Katepwa, and Glenlea exhibited vanillic acid levels of equivalent magnitude while Norstar was notably reduced relative to all others. Ferulic acid displayed major increases across all varieties. Syringic acid was a

noticeable component of this flour ranging from 1.6 ppm in Fielder to 3.2 ppm in Katepwa. The distinct nature of Norstar was also revealed by its caffeic acid content. Norstar had a soluble bound caffeic acid content of 3.75 ppm and except for Fielder at 1.78 ppm, all other wheats had less than 1 ppm. The 75% extraction flours displayed a wide range in relative percentages of available soluble bound acids. The percentages extended from 42.5 % in HY320 to a high of 67.3 % for Glenlea.

The 80% extraction 2nd clear flours also had major increases in their total soluble bound content. In each of the three hard wheats all of the dominant phenolic acids double in concentration from the 80% 1st clear flour. Each variety was distinct in terms of total concentration in this flour, ranging from 43.4 to 93.8 ppm. Sinapic acid remained the dominant component in all varieties although its contribution ranged from 37.6% in Norstar to 48.8 % in Katepwa. The unique nature of Norstar's caffeic acid content was also maintained at this higher extraction level. Every variety displayed a total soluble bound acid content in excess of that found in the whole wheat sample. This was not unexpected in light of the high ash content of this flour.

4.04.2.5 Straight Grade Flours

The 75% extraction straight grade flours were quite similar in terms of their total soluble bound phenolic acids

with the exception of Glenlea. The levels of the four other varieties ranged from 4.8-5.10 ppm. yet Glenlea recorded a significantly, $p < 0.05$, higher value of 7.6 ppm. Glenlea's higher content was attributed to the dominance of sinapic acid accounting for 58% of the total amount. The remaining phenolic acids were of the same magnitude as found in the other varieties. The detected phenolic acids represented a maximum of 13.1 % of the total soluble bound acids available in the ground grain.

At the 80% extraction level all varieties' straight grade flours, except Norstar, exhibited a greater than two fold increase in soluble bound content compared to their lower extraction millings. Norstar underwent only a minimal change in total content mirroring the smallest increase in ash content of any of the five varieties. A noticeable shift was observed for Fielder alone in terms of the sinapic acid contribution to the straight grade flour. At the lower 75% extraction rate sinapic acid accounted for 19% of the total content. However in the 80% extraction flour it accounted for 38% of the total. All other varieties had their sinapic acid content remain relatively constant when increasing the extraction rate.

The corresponding 85% extraction straight grade flours each displayed significant, $p < 0.05$, increases in their total soluble phenolic acid content although the extent of the increase was variable. Each of the hard wheats revealed a greater than two fold increase in their totals while both

HY320 and Fielder were considerably less. The soluble bound acid levels varied from 13.1-22.4 ppm. Fielder had the highest total content although both Glenlea and Katepwa values were not significantly, $p < 0.05$, different. Expressed on a percentage basis, these values represented a range of 22.4 to 45.0% of the total soluble bound acids available in a wheat sample.

An interesting observation within the whole wheat samples was the similarity of sinapic acid composition in four varieties. Except for Norstar, 44 %, all the varieties had sinapic acid contributing 51-52% of the total soluble bound acids. This high contribution may be due to the large involvement of sinapic acid in the "lignin core" of bran alluded to by Schwartz *et al* (1989).

4.04.2.6 Comments

The correlation between the various individual soluble bound phenolics and ash content was very strong for each acid within its own variety. Figs 31-33 illustrate this relationship in the variety Katepwa. Plots for the remaining varieties may be found in Appendix C. The strength of the correlations vary from 0.63 to 0.98 and are summarized in Table 5. It was found that the percentage of an individual acid to the total bound phenolics did not correlate and were not found to be significant, $p < 0.05$. The relationship between the insoluble ferulic acid and the soluble bound

ferulic acid was 0.92 to 0.98. Very significant correlations were observed between the individual soluble phenolic acids and polyphenol oxidase levels in each variety. The correlations ranged from r=0.62 to r=0.96 and are summarized in Table 6. In general, Fielder's soluble bound phenolic acids displayed the strongest correlations with the enzyme while Glenlea the least. A very significant relationship between total bound phenolics and polyphenol oxidase was also observed. The correlation was very strong for four of the five varieties with a minimum value of r=0.76 for Glenlea.

Figure 31 Soluble Bound Sinapic and Ferulic Acids Versus Ash Content in the Variety Katepwa

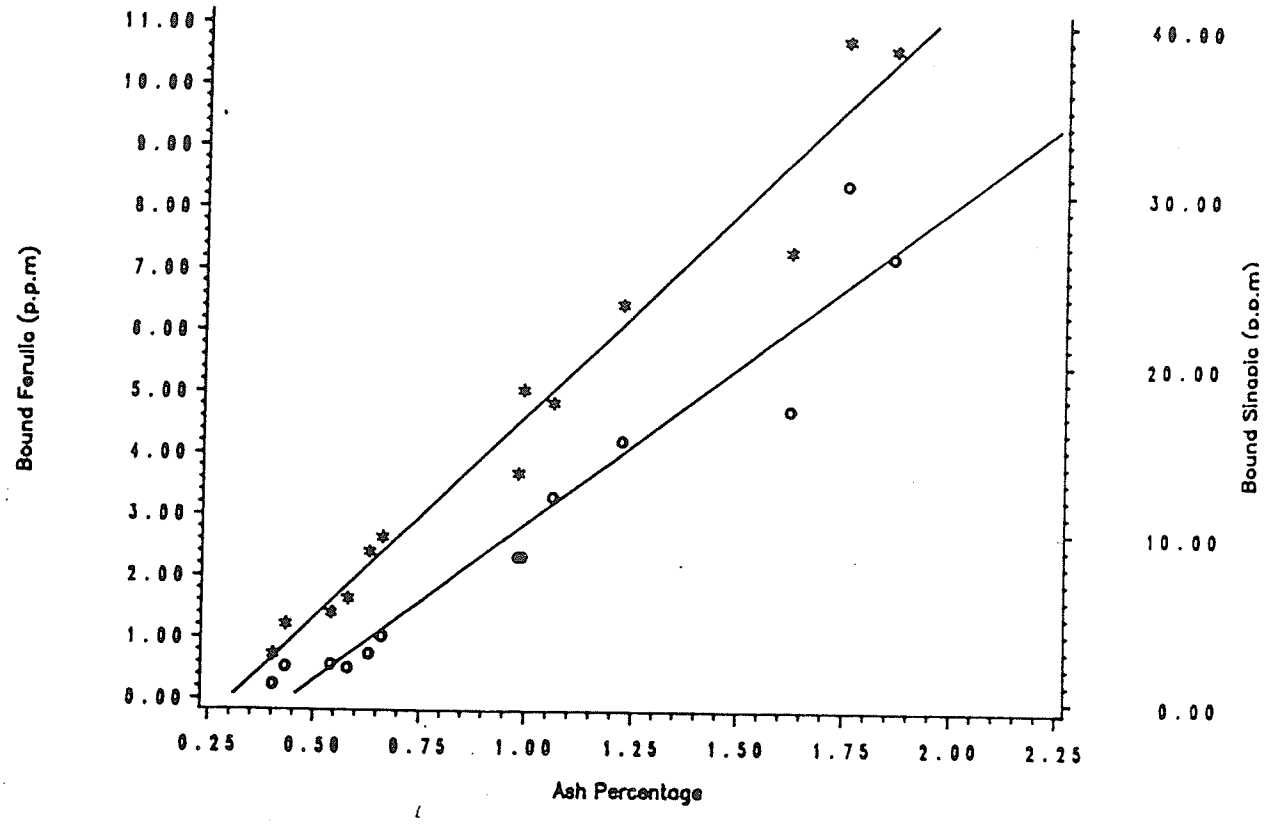
* Ferulic Acid

o Sinapic Acid

Figure 32 Soluble Bound Vanillic and Syringic Acids Versus Ash Content in the Variety Katepwa

* Vanillic Acid

o Syringic Acid


Bound Ferulic (p.p.m)
Bound Sinapic (p.p.m)
Ash Percentage
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
40.00
30.00
20.00
10.00
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25

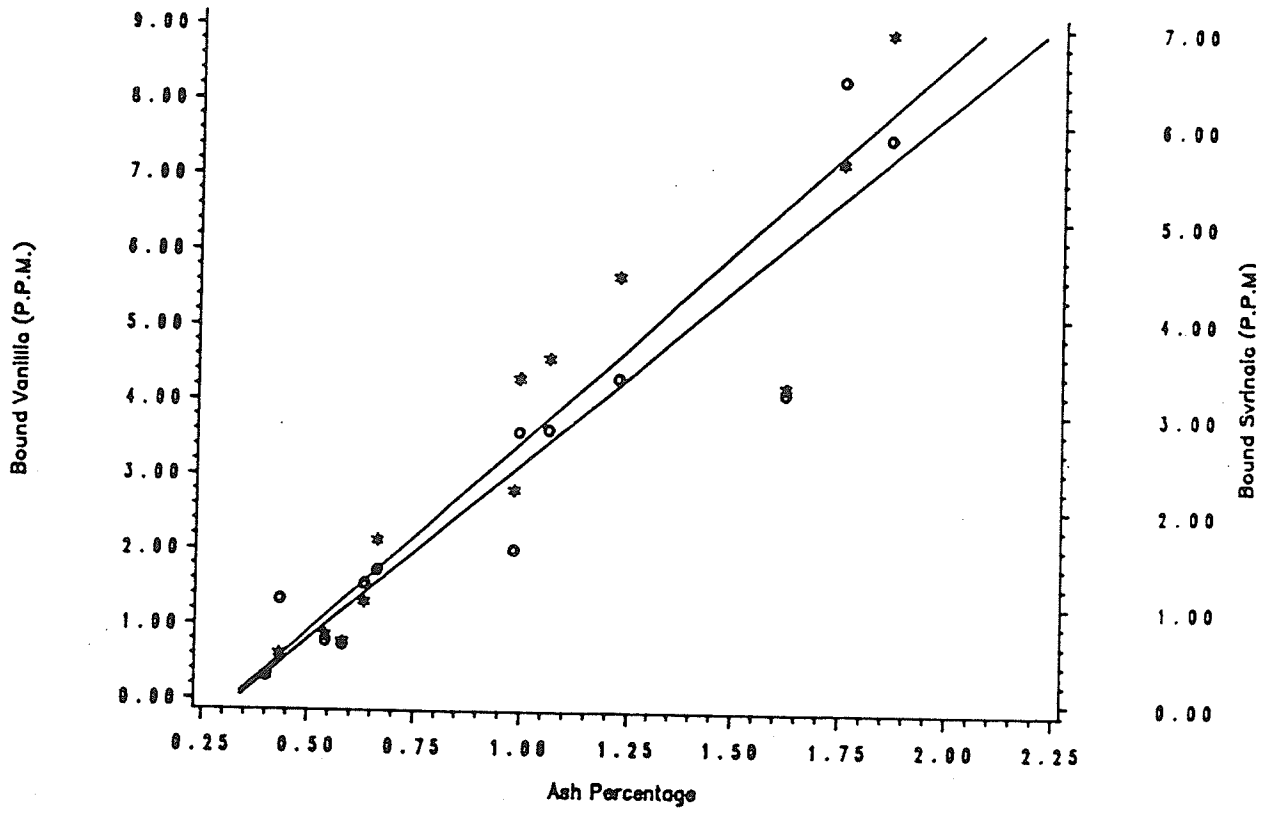

Bound Vanillic (P.P.M.)
Bound Syringic (P.P.M)
Ash Percentage
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25

Figure 33 Soluble Bound Caffeic and Coumaric Acids Versus Ash Content in the Variety Katepwa

* Caffeic Acid

o p-Coumaric Acid

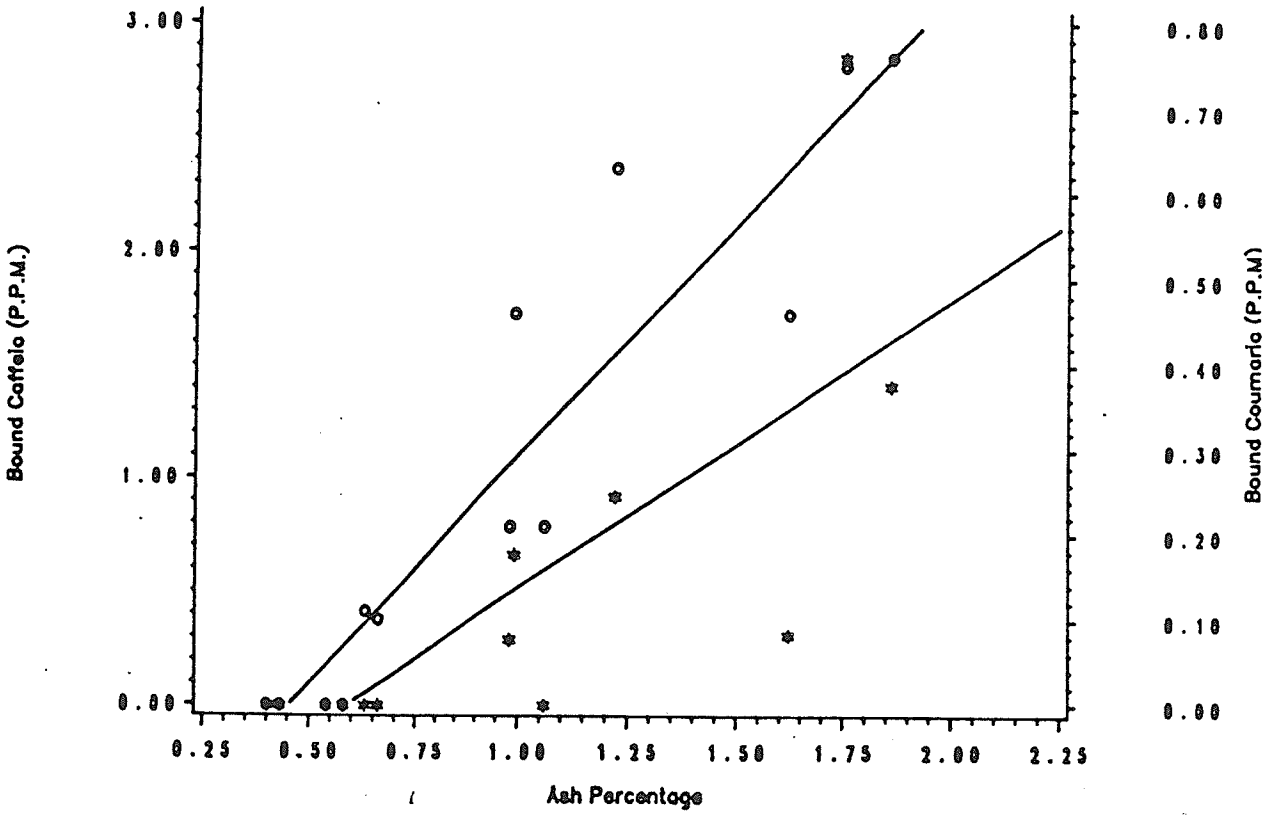

3.00
2.00
1.00
0.00
Bound Caffeic (P.P.M.)
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Bound Coumaric (P.P.M)
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
Ash Percentage

Table 5

PHENOLIC ACIDS

CORRELATION MATRIX WITH FLOUR ASH

Component	FIELDER	HY320	GLENLEA	KATEPWA	NORSTAR
BOUND PHENOLICS:					
Ferulic	0.91	0.98	0.91	0.98	0.99
Vanillic	0.80	0.87	0.87	0.94	0.95
Sinapic	0.91	0.90	0.94	0.97	0.89
Syringic	0.92	0.98	0.85	0.94	0.98
Caffeic	0.98	0.92	0.80	0.76	0.94
Coumaric	0.96	0.94	0.85	0.93	0.95
INSOLUBLE Ferulic	0.93	0.97	0.98	0.97	0.97
FREE PHENOLICS:					
Ferulic	0.92	0.91	0.87	0.92	0.96
Vanillic	0.97	0.92	0.92	0.92	0.93
Syringic	0.82	0.84	0.95	0.88	0.93
Caffeic	0.95	0.89	0.69	0.63	0.91
Coumaric	NS	0.95	0.81	0.75	0.97

Note: All values listed have $p < 0.05$
NS = Not significant

Table 6

PHENOLIC ACIDS:

CORRELATIONS WITH POLYPHENOL OXIDASE

Component	FIELDER	HY320	GLENLEA	KATEPWA	NORSTAR
BOUND PHENOLICS:					
Ferulic	0.91	0.96	0.69	0.92	0.89
Vanillic	0.91	0.88	0.81	0.96	0.83
Sinapic	0.82	0.77	0.62	0.83	0.80
Syringic	0.92	0.91	0.77	0.90	0.94
Caffeic	0.92	0.89	0.64	0.93	0.91
Coumaric	0.90	0.89	0.66	0.84	0.88
Total Bound	0.92	0.93	0.76	0.94	0.89
INSOLUBLE Ferulic	0.93	0.88	0.79	0.88	0.86
FREE PHENOLICS:					
Ferulic	0.94	0.82	0.80	0.90	0.96
Vanillic	0.97	0.80	0.79	0.96	0.97
Total Free	0.98	0.84	0.84	0.94	0.96
TOTAL PHENOLICS	0.94	0.89	0.79	0.89	0.88

4.04.3 Free Phenolic Acid Composition

The most notable observation in the analysis of the free phenolic acids was the lack of sinapic acid, the dominant soluble bound component, in any of the flour samples shown in Figs.34 - 38.

4.04.3.1 1st Patent Flours

In the 75% extraction 1st patent flours the softest wheat, Fielder, followed by HY320, had the maximum content of free phenolic acids at 2.77 and 1.30 ppm respectively while the harder wheats each displayed less than 1.0 ppm. Ferulic acid was the major component found in all five varieties. The high levels detected in Fielder represented the maximum, 25.7%, of any of the varieties total free phenolic content.

The corresponding 80% extraction flours remained relatively unchanged in terms of total free acid content for each variety. The two fold increase observed in the soluble bound acids was not reflected in the free phenolics. The three hard wheats continued to remain below 1 ppm which represented 8.0-8.3% of the total free acids detected in their respective whole wheat samples.

Figure 34 Free Phenolic Acids Detected in the Pooled Flours of Katepwa

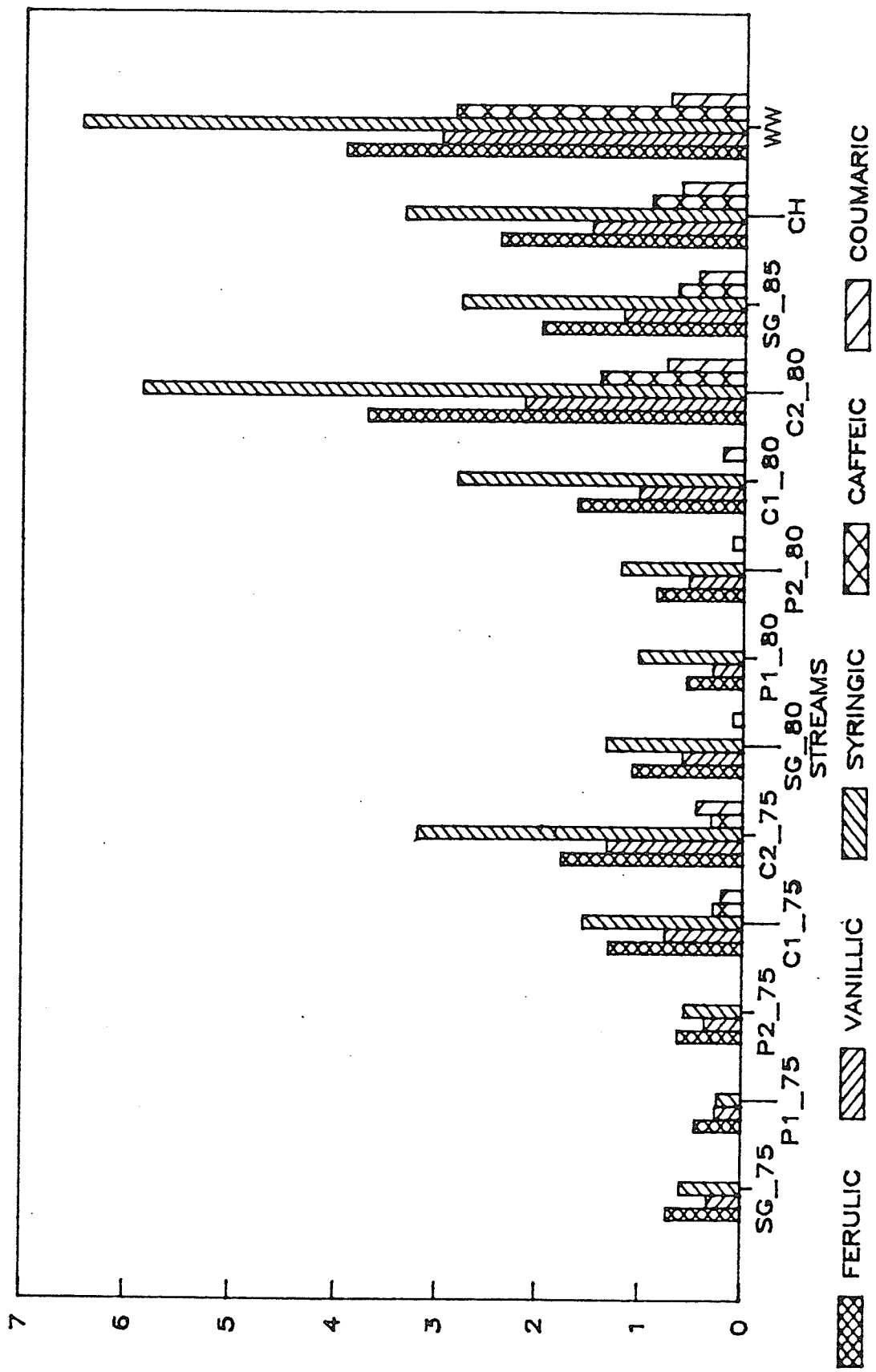

FREE ACIDS (PPM)
0
1
2
3
4
5
6
7
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
FERULIC
VANILLIC
SYRINGIC
CAFFEIC
COUMARIC

Figure 35 Free Phenolic Acids Detected in the Pooled Flours of Glenlea

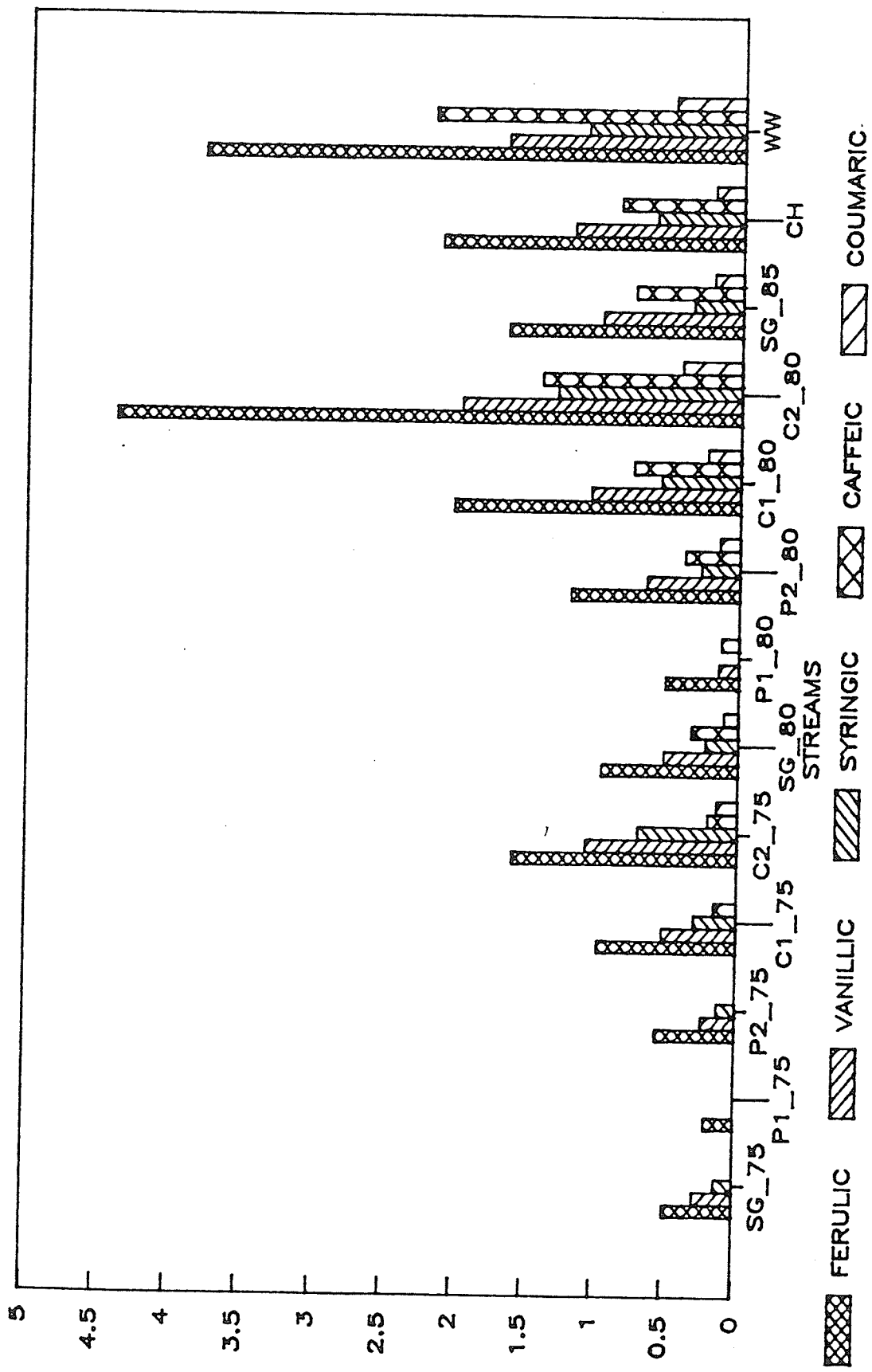

FREE ACIDS (PPM)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
FERULIC
VANILLIC
SYRINGIC
CAFFEIC
COUMARIC

Figure 36 Free Phenolic Acids Detected in the Pooled Flours of Norstar

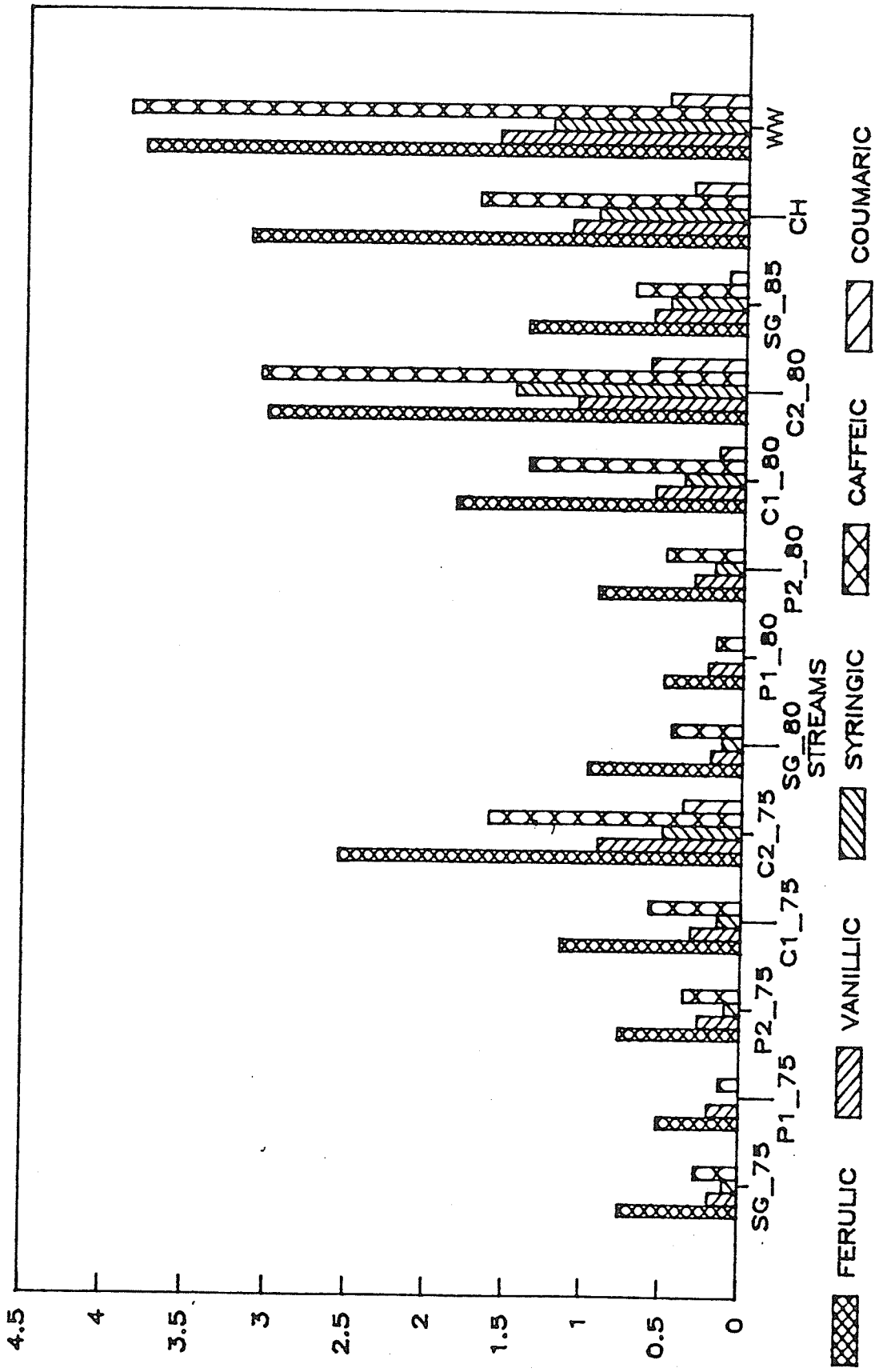

FREE ACIDS (PPM)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
FERULIC
VANILLIC
SYRINGIC
CAFFEIC
COUMARIC

Figure 37 Free Phenolic Acids Detected in the Pooled Flours of HY320

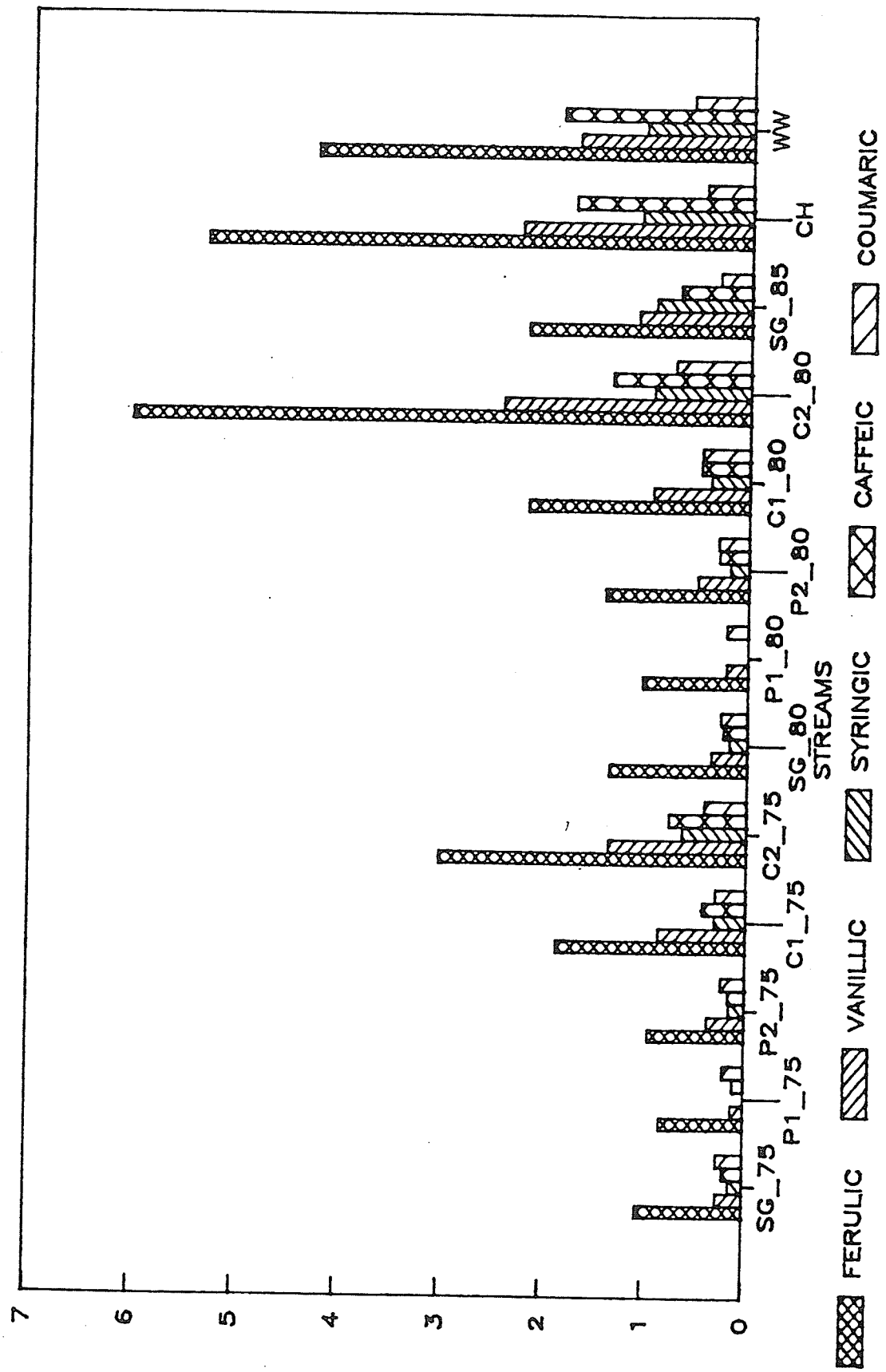
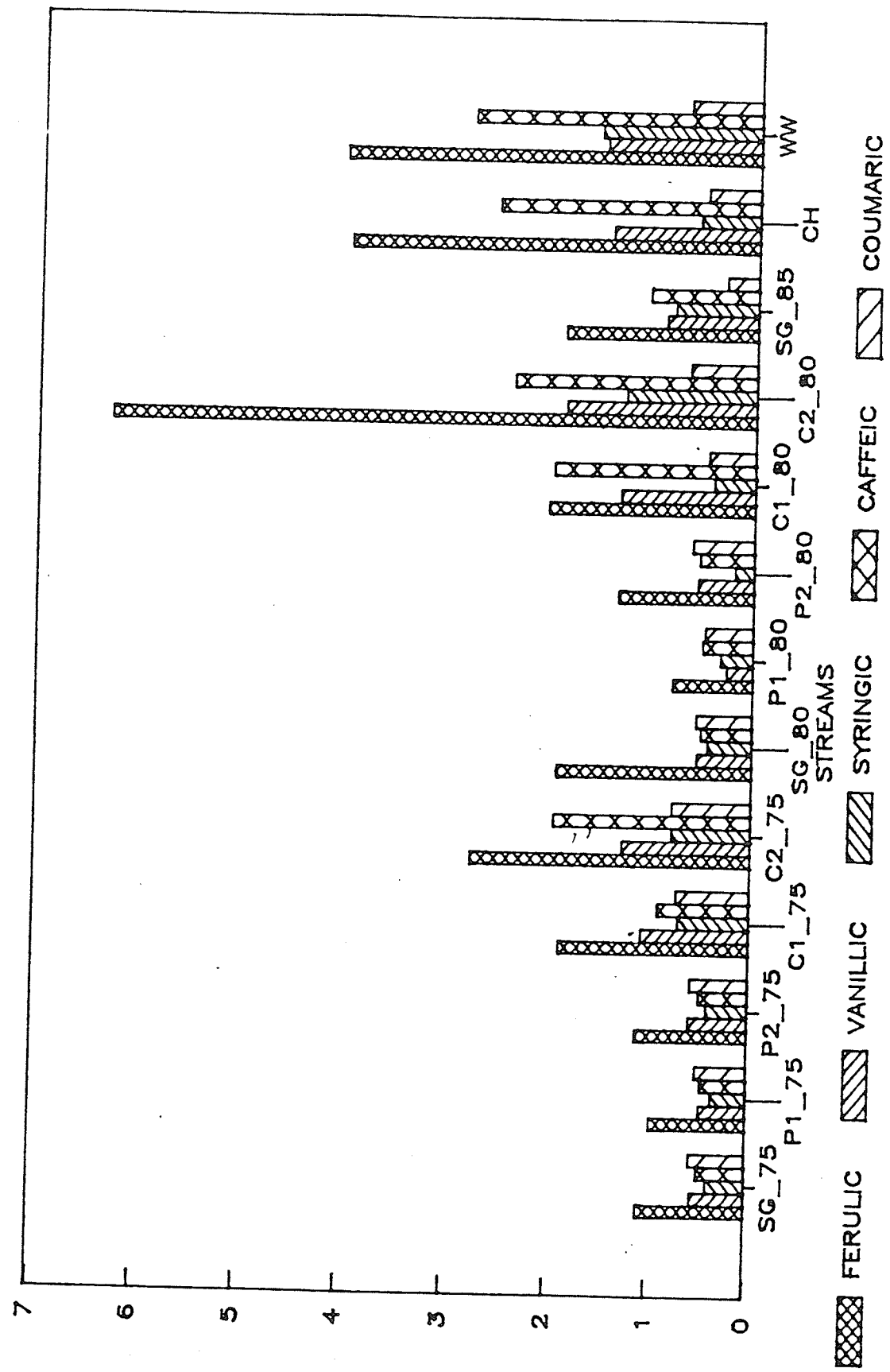

FREE ACIDS (PPM)
0
1
2
3
4
5
6
7
STREAMS
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
FERULIC
VANILLIC
SYRINGIC
CAFFEIC
COUMARIC

Figure 38 Free Phenolic Acids Detected in the Pooled Flours of Fielder


FREE ACIDS (PPM)
0
1
2
3
4
5
6
7
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
STREAMS
FERULIC
VANILLIC
SYRINGIC
CAFFEIC
COUMARIC

4.04.3.2 2nd Patent Flours

The 75% extraction 2nd patent flours did display an increase in the total free phenolic acid content as both Fielder and HY320 maintained the highest levels. Fielder's 3.21 ppm accounted for the 29.8% of the total free acids available. Unlike the soluble bound acids, Norstar had the highest free phenolic acid level of the hard wheats at 1.50 ppm, distinct from both Katepwa and Glenlea.

Noticeable increases in the free phenolic acid levels detected were observed in all varieties except Fielder in the 80% extraction flours. A largest increase, approaching three fold, was noted in the Glenlea sample.. This increase was due to elevated levels of caffeic and coumaric acids which were not detected in the 75% extraction flour. Although Fielder's 80% extraction flour free acid content remained relatively unchanged, it continued to represent the maximum percentage of the total available, 30.4 %, of any of the varieties.

4.04.3.3 1st Clear Flours

The 75% extraction 1st clear flours displayed noticeable increases in their free acids for each variety. Fielder continued to have the highest concentration of free acids at 5.41 ppm representing over 50% of the ground wheat contents. Ferulic acid remained the dominant acid in each

variety although its contribution to the total free phenolic acids varied from 35-52%. Vanillic and caffeic acids also contributed a major portion to the total composition.

Examination of the corresponding 80% extraction stream indicated that the softer wheats underwent only slight increases in total free acids. The harder wheats, in particular Norstar and Glenlea displayed a two fold increase. Katepwa had the minimum content of free phenolic acids with 3.39 ppm while Fielder displayed the maximum at almost twice the value of 6.33 ppm equivalent to 58.7% of the available free acid.

4.04.3.4 2nd Clear Flours

The 75% 2nd clear flour maintained the same pattern observed in the 1st clear flours. The softer wheats, Fielder and HY320 continued to have the highest free acid content. Norstar's levels remained the highest of the hard wheats at 5.95 ppm. This level was statistically distinct, $p < 0.001$, from both Katepwa and Glenlea. Ferulic acid remained the dominant acid in the hard wheats and was tightly grouped at 43% of the total composition. Fielder and HY320 differed on the relative importance of ferulic acid within this flour. In HY320 ferulic acid played a very dominant role accounting for almost 50% of the total content while in Fielder, the role was reversed at 36%. Analysis of the 2nd clear flour yielded significant quantities of the minor free acids,

especially syringic and caffeic acids, which together accounted for 20-35% of the free phenolics depending upon variety.

At the 80% extraction level the total free phenolic content of the 2nd clear flour approached a two fold increase over the lower 75% extraction rate in each of the five varieties. The free acid levels extended from 7.6 ppm in Katepwa to 12.6 ppm in Fielder. There remained the obvious grouping of the softer wheats maintaining the highest level of free acids, approximately 30% higher than both Norstar and Glenlea. Unlike the soluble bound acids, only Fielder, HY320, and Glenlea had free acid levels in excess of that found in their respective ground grain samples. Katepwa's content however, represented only 63 % of the total available.

4.04.3.5 Straight Grade Flours

Examination of the straight grade flours confirmed trends seen in the previous flours with Fielder having a significantly higher, $p=0.0001$, free acid content than the other varieties. The softer wheats, Fielder and HY320, had detectable quantities of all acids except sinapic whereas none of the hard wheats had detectable levels of coumaric acid and two were missing caffeic acid. As was the case in the soluble bound phenolics, the 75% straight grade free

phenolic acid content was equivalent to that found in the corresponding 2nd patent flour for each variety.

The 80% extraction flours displayed significant increases, $p < 0.05$, in total free phenolic acids over their 75% flours for every variety. Norstar displayed the minimum level detected, 1.76 ppm, while Fielder yielded the maximum of 4.02 ppm.

A noticeable increase in total free phenolic acids was also observed upon achieving the 85% extraction level. Katepwa, Glenlea, and HY320 85% straight grade flours showed over a 2 fold increase when moving to the elevated extraction level. Caffeic acid played a major role in the increases observed in these three varieties. In all cases ferulic acid remained the dominant component rising to values as high as 56% of the total free acids in both Katepwa and HY320. At this elevated extraction level, the straight grade flours free phenolic acid content extended from 29.7% in Norstar, to only 55.7% in HY320, of that available in their ground wheats.

4.04.3.6 Other Samples

Examination of the total free phenolic acid content in the whole wheat samples indicated that while Fielder had the highest free acid content in the flour samples, Katepwa had a significantly, $p < 0.05$, higher value in the total grain sample at 12.2 ppm. The suspected cause for this discrepancy

was due to the milling behavior of the softer Fielder. In the flour samples, bran contamination was much greater in the soft wheats' flours and thus elevated the free acid content. Placed in perspective, the free phenolic acids accounted for only 2-3.5% of the total phenolic acid content in the ground wheat sample.

4.04.3.7 Comments

For each variety there were good correlations between ash content and both ferulic and vanillic acids. There were corresponding high correlations between the free ferulic acid content and both the soluble bound, and insoluble bound ferulic acid levels.

4.05.0 Pigment Content

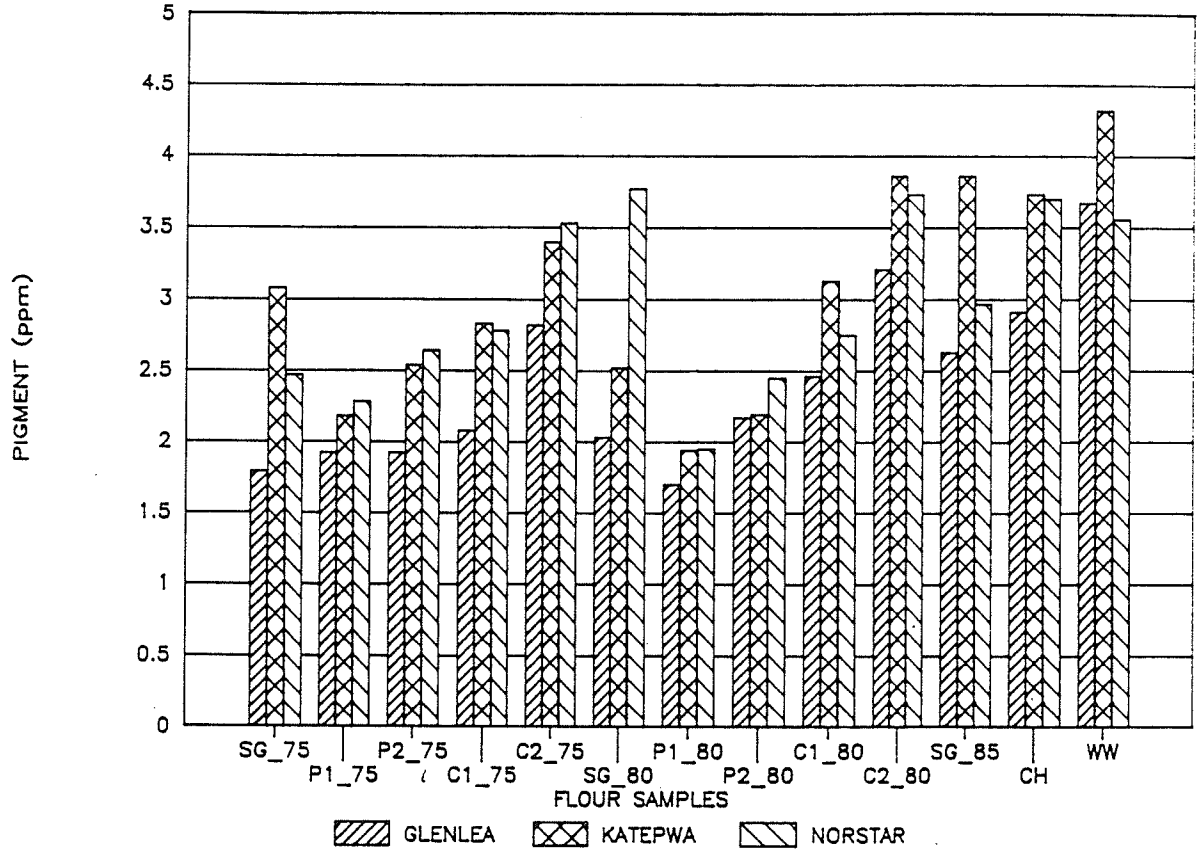
Analysis of the various flours for pigment content indicated the range of values amongst the five varieties was quite limited. Values extended from 1.49 to a maximum of 5.03 ppm. for Fielder's 80% extraction 2nd clear flour. Values for individual pooled flours, by variety, can be viewed in Figs. 39-40. The pigment content was observed to increase with increasing bran contamination for each variety. A strong relationship was observed between ash and

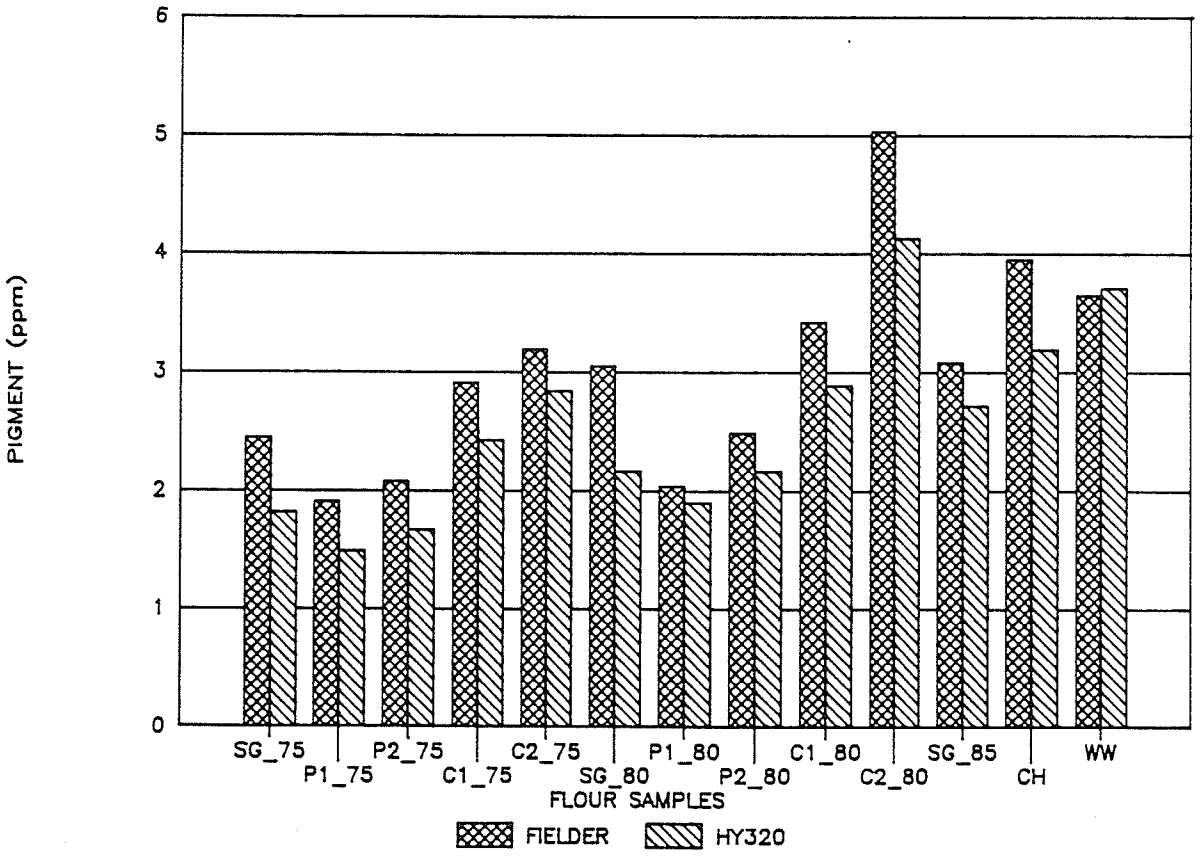
pigment content with correlation coefficients of r=0.80 to r=0.96, $p < 0.05$, respectively.

Katepwa displayed the highest pigment content, 4.32 ppm, in the ground grain sample, and was significantly different, $p < 0.05$, from the four other varieties which were tightly grouped between 3.56-3.71 ppm. Although Katepwa's grain sample contained the largest pigment concentration, it was the softer wheat, Fielder, which had the higher concentration of pigment in the poorer quality flours.

Figure 39 Pigment Content of the Pooled Flours of the Hard Wheats

Figure 40 Pigment Content of the Pooled Flours of Fielder and HY320


PIGMENT (ppm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
FLOUR SAMPLES
GLENLEA
KATEPWA
NORSTAR


PIGMENT (ppm)
6
5
4
3
2
1
0
SG_75
P1_75
P2_75
C1_75
C2_75
SG_80
P1_80
P2_80
C1_80
C2_80
SG_85
CH
WW
FLOUR SAMPLES
FIELDER
HY320

4.06 Color Evaluation

4.06.1 Hunter Lab Reproducibility

The reproducibility of the Hunter Lab slurry technique used in subsequent analysis was determined using two 75 % extraction Katepwa flours of divergent character. The flours chosen were the 1st patent and the 2nd clears. Five individual replicates were prepared from each flour and monitored over a 6 h period. The results can be seen in Table 7.

The readings for each individual sample were an average of four readings taken over a 20 sec time period while the paste was stirred. Examination of the standard deviations of both the brightness (L^*) and yellowness (b^*) values for both samples indicated excellent reproducibility as the coefficient of variation (c.v.) achieved a maximum of 1.13%. Unfortunately, due to the low red values of the 1st patent flour, the variation across samples did exert an influence on a values as the average standard deviation represented a maximum cv of 14.7%. This high variation was considerably reduced to a maximum of 5.4% in the 2nd clear sample due largely to the higher red scores.

Table 7

REPRODUCIBILITY OF SLURRY HUNTER LAB VALUES

Katepwa 75% 1st Patent

Time	L	a	b
0 hrs.	89.90 (0.08)	0.51 (0.05)	15.53 (0.16)
1	90.06 (0.16)	0.44 (0.05)	14.55 (0.13)
2	90.18 (0.17)	0.41 (0.06)	14.06 (0.17)
3	90.18 (0.05)	0.41 (0.07)	13.80 (0.13)
4	90.16 (0.08)	0.44 (0.01)	13.67 (0.05)
5	89.98 (0.16)	0.34 (0.06)	13.25 (0.23)
6	89.95 (0.05)	0.40 (0.08)	13.44 (0.15)
n=35			
Avg.Std.Dev.			
Across Samples	(0.11)	(0.05)	(0.15)
Within Samples	(0.02)	(0.02)	(0.03)

Katepwa 75% 2nd Clears

Time	L	a	b
0 hrs.	81.53 (0.08)	2.61 (0.06)	18.94 (0.10)
1	81.16 (0.17)	2.46 (0.02)	17.95 (0.04)
2	80.54 (0.14)	2.40 (0.05)	16.53 (0.07)
3	79.93 (0.10)	2.46 (0.08)	15.73 (0.09)
4	79.41 (0.15)	2.63 (0.15)	15.38 (0.06)
5	78.85 (0.16)	2.95 (0.31)	15.39 (0.11)
6	78.20 (0.14)	3.45 (0.23)	15.52 (0.10)
n=35			
Avg. Std.Dev			
Across Samples	(0.13)	(0.13)	(0.08)
Within Samples	(0.04)	(0.02)	(0.03)

L = Brightness a = Redness b = Yellowness

Other sources of error to be noted were the positioning of the Agtron cup on the colorimeter and the formation of gluten strands in the paste as a function of mixing time.

4.06.2 Colorimetric Evaluation and Major Factor Correlation

The correlations between the various colorimetric methods and the major factors in this study; ash, polyphenol oxidase , pigment, protein, and the phenolic acids, free, bound, and insoluble are addressed in Tables 8-9. The corresponding relationships utilizing the Kan Sui reagent are listed in Table 10.

4.06.2.1 Ash

All five flour paste color indexes, Hunter L^*, a^*, b^*, Kent-Jones CGF, and Agtron values displayed very significant correlations with the ash content. Graphs showing these relationships for the Hunter L^*, a^*, and b^* values can be seen in Figs 41-46. The Hunter redness index, a^*, displayed the weakest relationship with correlation coefficients ranging from 0.74 to 0.88 depending on variety.

Use of the Kan Sui reagent in the flour paste did not alter the high correlations between paste brightness and ash content. However, the strength of the correlations between both yellow and red components with ash was reduced. The red component was severely affected as 3 of the varieties had correlations below the 95% probability limit and the

Table 8

FLOUR PASTE

CORRELATION MATRIX OF HUNTER LAB COLOR COMPONENTS

	FIELDER			HY320			GLENLEA			KATEPWA			NORSTAR		
Component	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Ash	-0.97	0.88	0.95	-0.96	0.87	0.99	-0.84	0.74	0.96	-0.88	0.80	0.97	-0.91	0.79	0.98
Polyphenol Oxidase	-0.94	0.86	0.94	-0.94	0.90	0.92	-0.98	0.97	0.87	-0.97	0.95	0.95	-0.98	0.93	0.95
Total Free Phenolics	-0.92	0.82	0.94	-0.87	0.76	0.91	-0.90	0.86	0.92	-0.97	0.96	0.94	-0.97	0.90	0.98
Total Bound Phenolics	-0.84	0.69	0.93	-0.92	0.83	0.95	-0.82	0.73	0.91	-0.93	0.87	0.97	-0.91	0.80	0.96
Insoluble Ferulic	-0.86	0.74	0.90	-0.89	0.77	0.96	-0.85	0.76	0.95	-0.86	0.78	0.93	-0.87	0.72	0.95
Total Phenolics	-0.87	0.74	0.90	-0.90	0.79	0.96	-0.85	0.76	0.94	-0.88	0.80	0.94	-0.88	0.74	0.96
Pigment	-0.88	0.70	0.97	-0.92	0.82	0.96	-0.95	0.93	0.97	-0.85	0.81	0.88	-0.73	0.63	0.80
Protein	-0.63	0.53	0.56	-0.66	NS	0.79	NS	NS	0.73	NS	NS	0.56	NS	NS	0.72

Note: All values listed have $p < 0.05$

NS = Not significant

L = Brightness a = Redness b = Yellowness

Table 9

FLOUR PASTE

CORRELATION MATRIX OF AGTRON AND KENT-JONES COLOR MEASUREMENTS

	FIELDER		HY320		GLENLEA		KATEPWA		NORSTAR	
Component	AGTRON	K-J	AGTRON	K-J	AGTRON	K-J	AGTRON	K-J	AGTRON	K-J
Ash	-0.87	0.97	-0.83	0.98	-0.85	0.91	-0.87	0.93	-0.90	0.92
Polyphenol Oxidase	-0.79	0.94	-0.67	0.91	-0.67	0.92	-0.72	0.94	-0.81	0.97
Total Free Phenolics	-0.81	0.92	-0.75	0.90	-0.80	0.93	-0.75	0.96	-0.84	0.97
Total Bound Phenolics	-0.73	0.83	-0.72	0.92	-0.77	0.87	-0.82	0.95	-0.88	0.92
Insoluble Ferulic	-0.84	0.89	-0.80	0.93	-0.82	0.91	-0.89	0.93	-0.91	0.87
Total Phenolics	-0.83	0.89	-0.80	0.93	-0.82	0.91	-0.89	0.94	-0.91	0.89
Pigment	-0.78	0.85	-0.81	0.94	-0.86	0.97	-0.87	0.91	-0.77	0.76
Protein	-0.80	0.70	-0.85	0.75	-0.76	0.64	-0.59	NS	-0.76	0.59

Note: All values listed have $p < 0.05$

NS = Not significant K-J = Kent-Jones

KAN SUI FLOUR PASTE

Table 10 CORRELATION MATRIX OF HUNTER LAB COLOR COMPONENTS

	FIELDER			HY320			GLENLEA			KATEPWA			NORSTAR		
Component	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Ash	-0.96	NS	0.80	-0.98	0.61	0.86	-0.88	0.78	0.76	-0.88	NS	0.79	-0.92	NS	0.91
Polyphenol Oxidase	0.95	NS	0.80	-0.93	0.70	0.70	-0.96	0.91	0.57	-0.96	0.79	0.69	-0.97	NS	0.76
Total Free Phenolics	-0.93	NS	0.83	-0.90	NS	0.87	-0.92	0.70	0.85	-0.98	0.76	0.81	-0.97	NS	0.85
Total Bound Phenolics	-0.87	NS	0.87	-0.92	0.57	0.82	-0.85	0.69	0.81	-0.94	0.63	0.82	-0.92	NS	0.92
Insoluble Ferulic	-0.90	NS	0.84	-0.92	NS	0.93	-0.89	0.78	0.78	-0.88	NS	0.85	-0.88	NS	0.95
Total Phenolics	-0.90	NS	0.85	-0.93	NS	0.92	-0.89	0.76	0.79	-0.90	0.56	0.85	-0.89	NS	0.95
Pigment	-0.91	NS	0.94	-0.93	NS	0.90	-0.97	0.77	0.82	-0.85	0.58	0.87	-0.75	NS	0.81
Protein	-0.61	NS	NS	-0.72	NS	0.87	-0.56	NS	0.65	NS	NS	NS	NS	NS	0.78

Note: All values listed have $p < 0.05$ L = Brightness a = Redness b = Yellowness

NS = Not significant

Figure 41 Relationship Between Water Flour Paste Brightness and Ash Content in the Hard Wheats

A Norstar

B Glenlea

C Katepwa

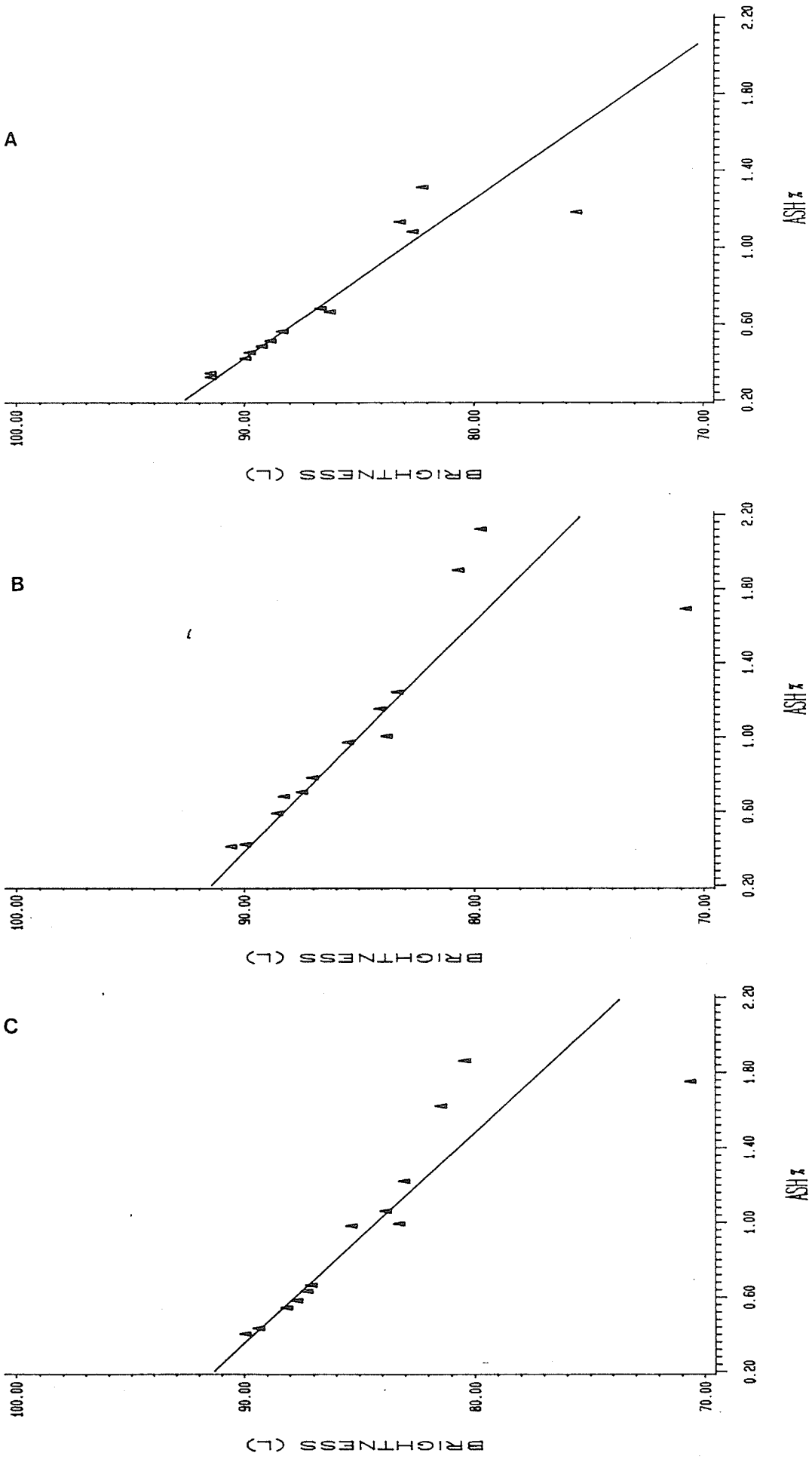
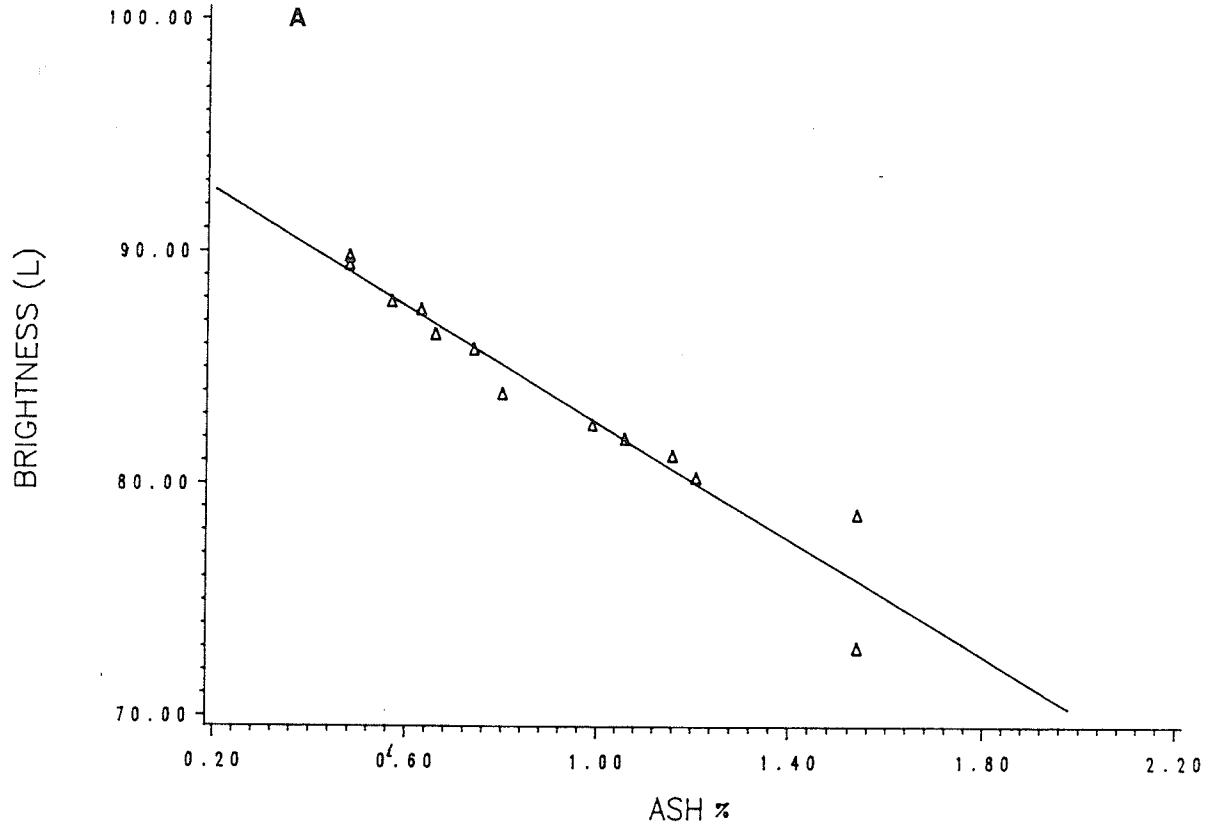
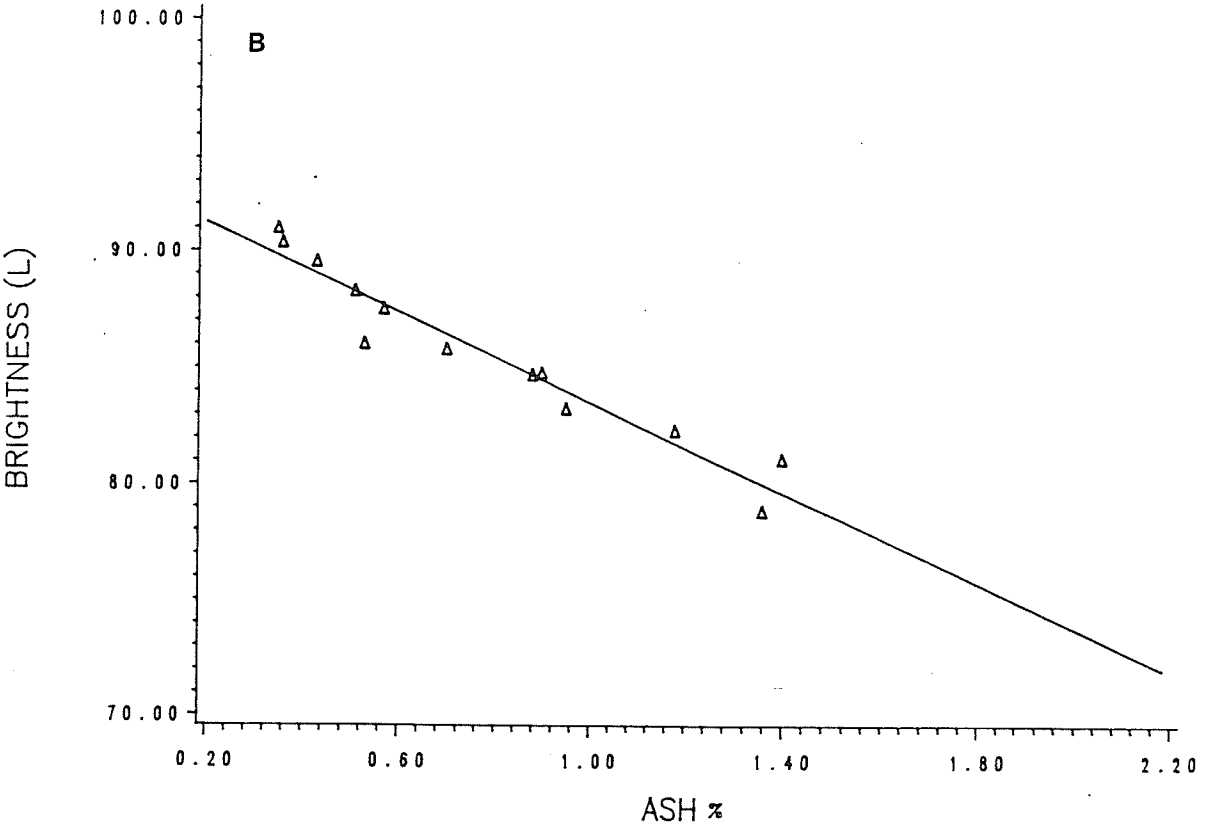

A
B
C
BRIGHTNESS (L)
100.00
90.00
80.00
70.00
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

Figure 42 Relationship Between Water Flour Paste Brightness and Ash Content in the Softer Wheats Fielder and HY320

A. HY320

B. Fielder


A
100.00
90.00
80.00
70.00
BRIGHTNESS (L)
0.20
0.60
1.00
1.40
1.80
2.20
ASH %


B
100.00
90.00
80.00
70.00
BRIGHTNESS (L)
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

A

Figure 43 Relationship Between Water Flour Paste Yellowness and Ash Content in the Hard Wheats

A. Norstar

B. Glenlea

C. Katepwa

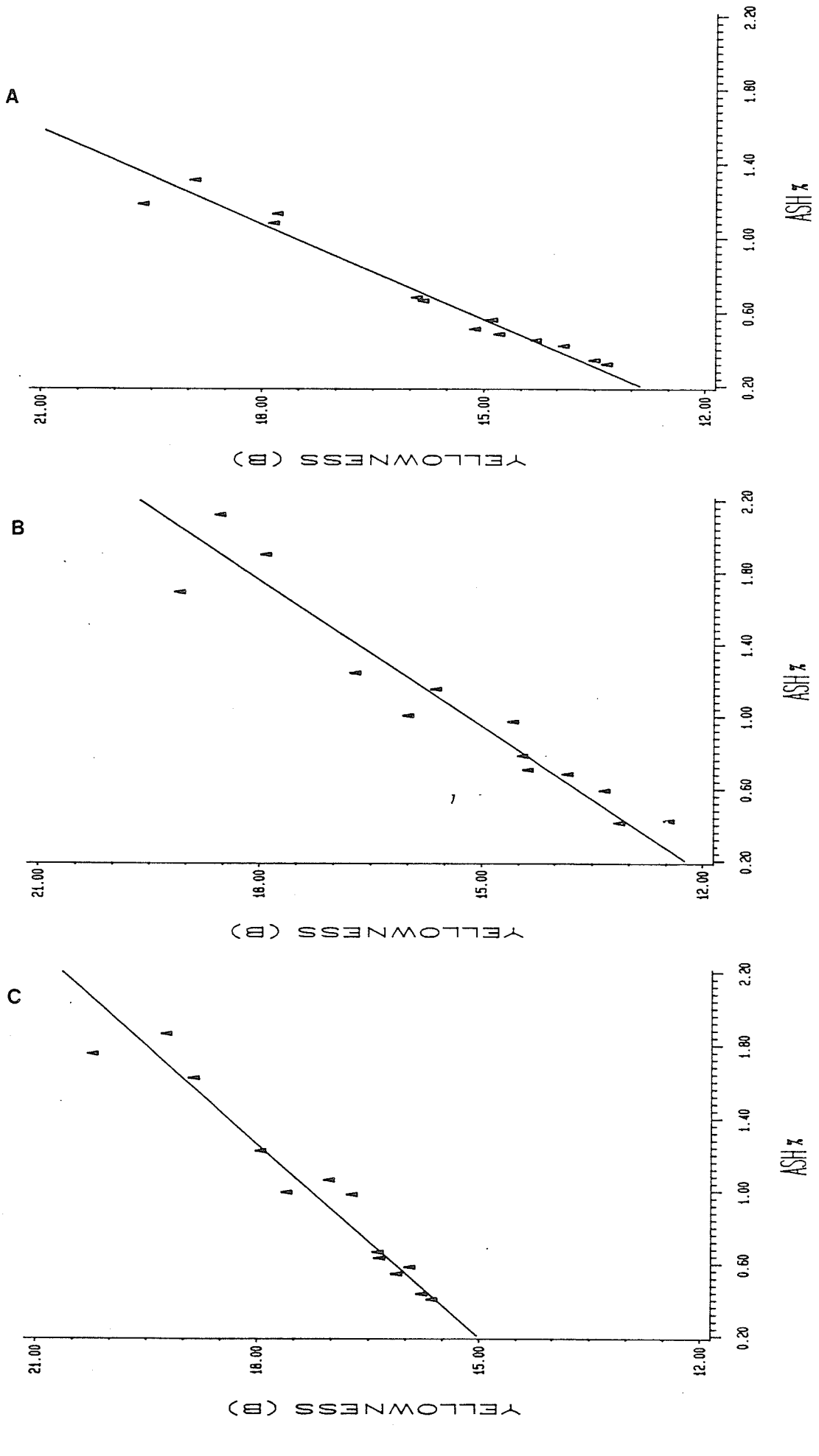
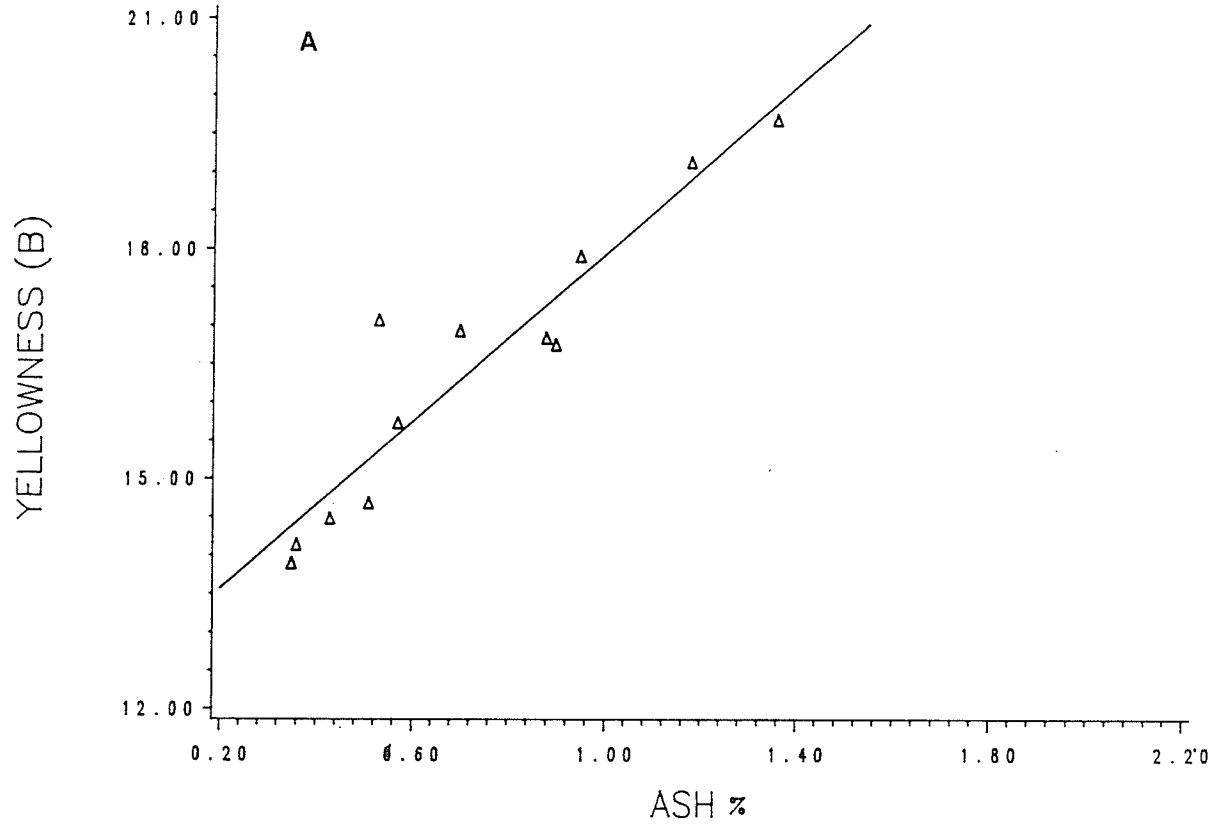

A
B
C
YELLOWNESS (B)
21.00
18.00
15.00
12.00
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

Figure 44 Relationship Between Water Flour Paste Yellowness and Ash Content in Softer Wheats Fielder and HY320

A. Fielder

B. HY320


A
21.00
18.00
15.00
12.00
YELLOWNESS (B)
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

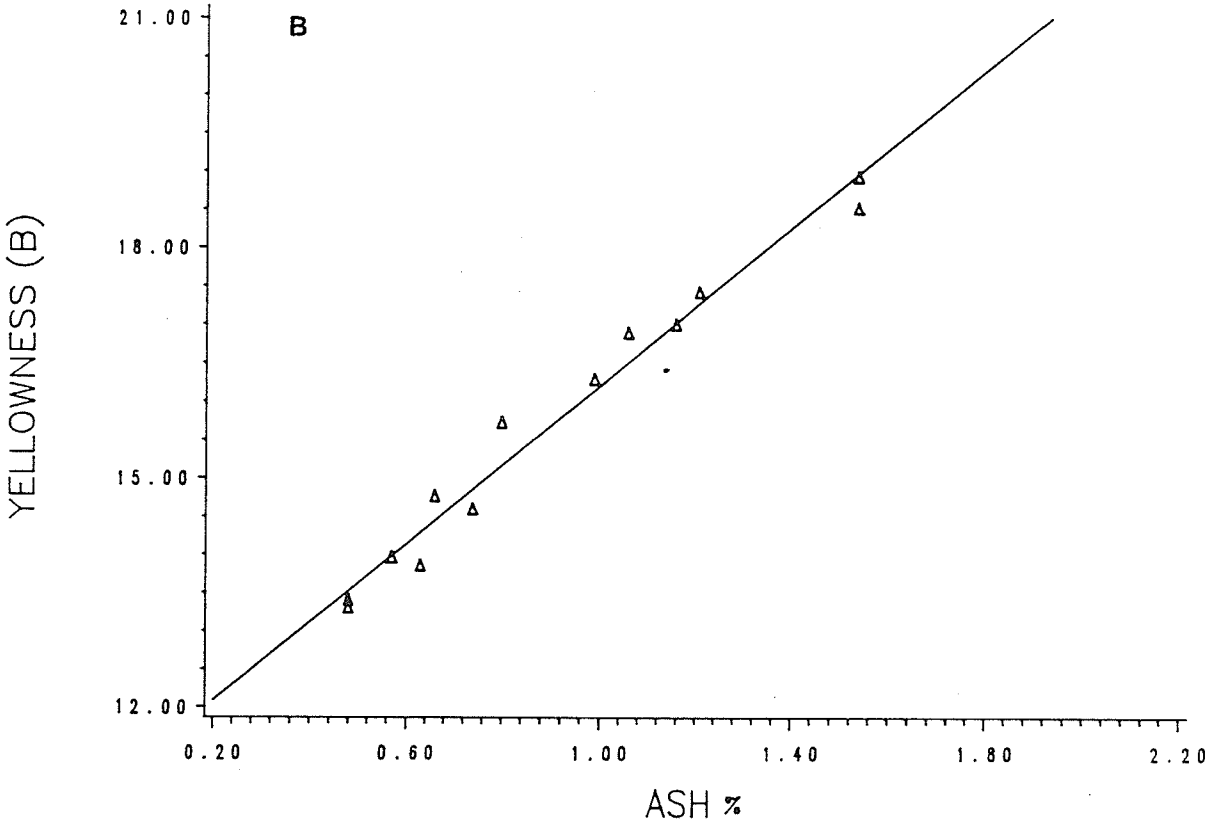

B
21.00
18.00
15.00
12.00
YELLOWNESS (B)
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

Figure 45 Relationship Between Water Flour Paste Redness and Ash Content of the Hard Wheats

A. Norstar

B. Glenlea

C. Katepwa

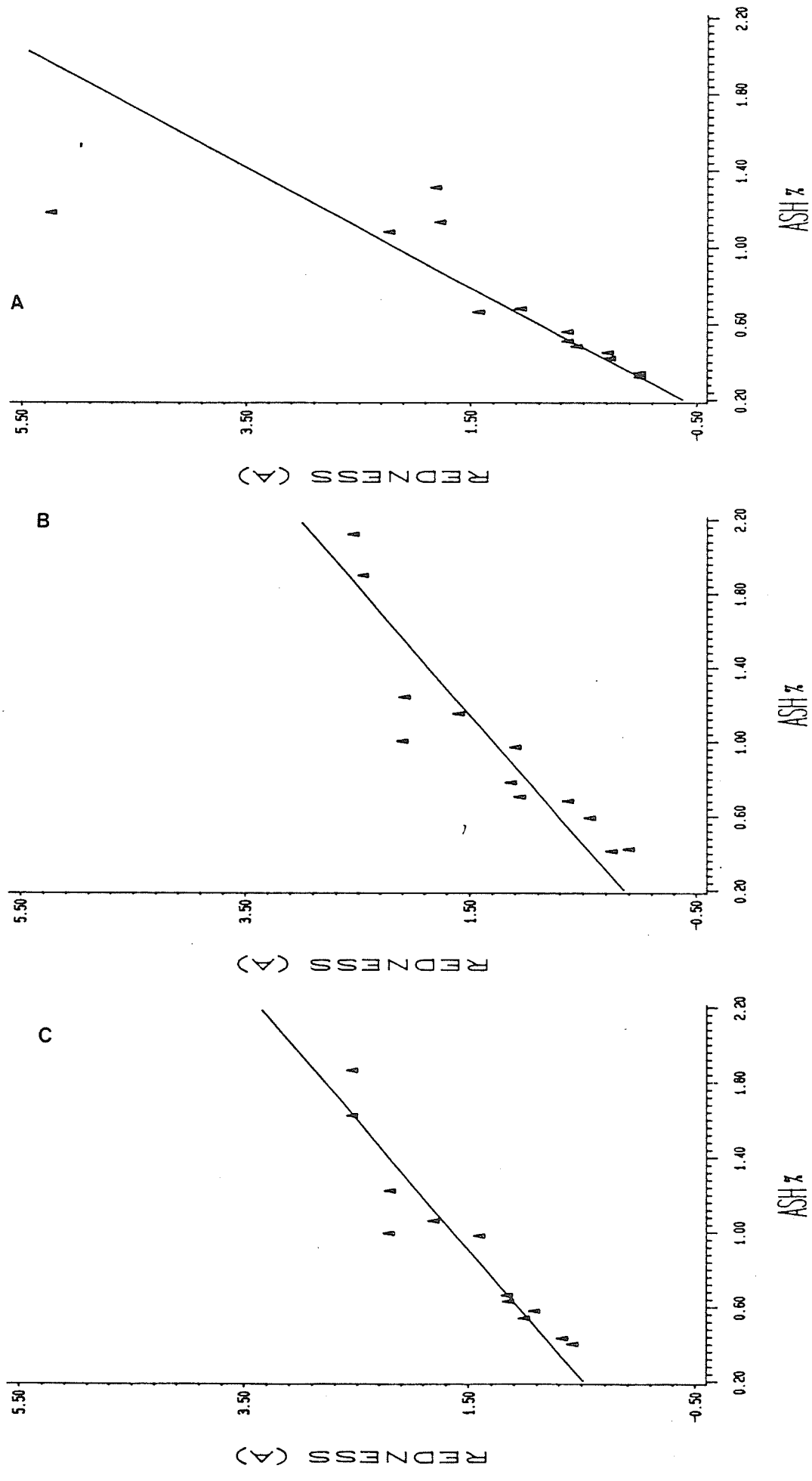
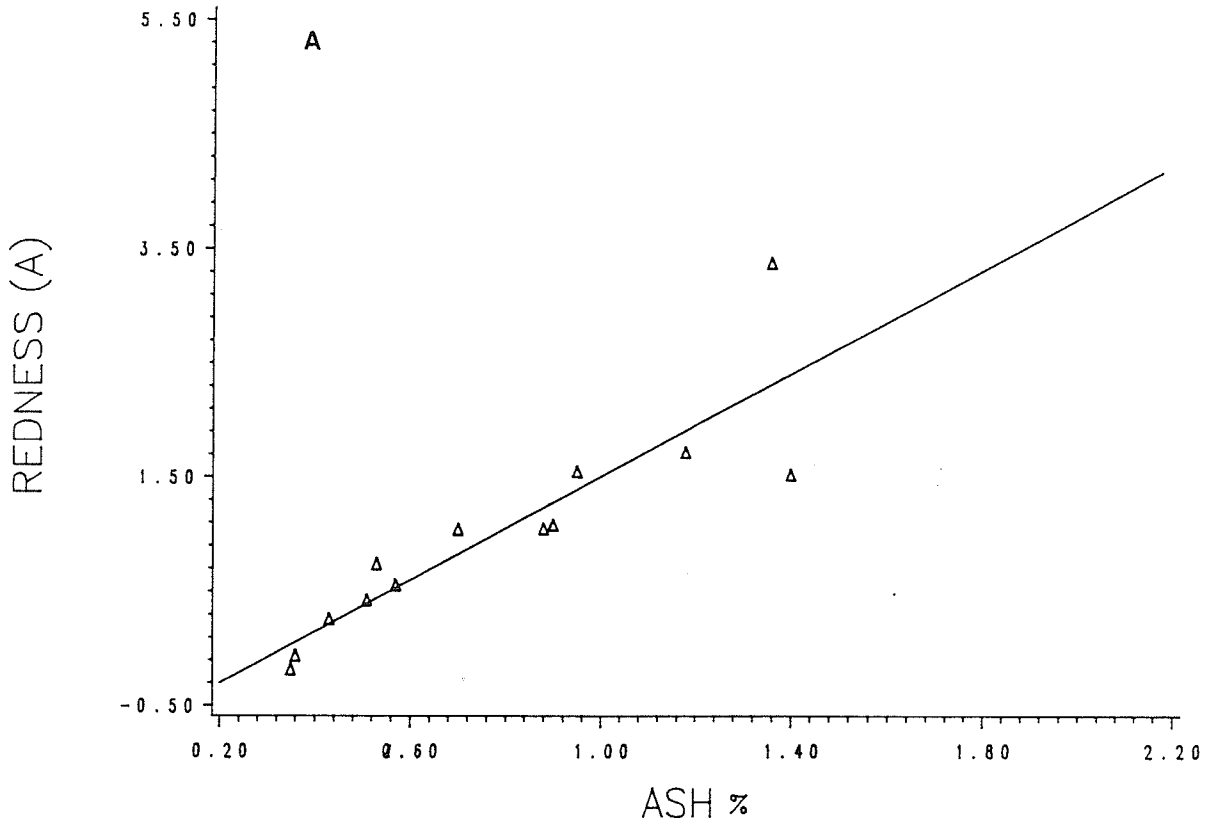
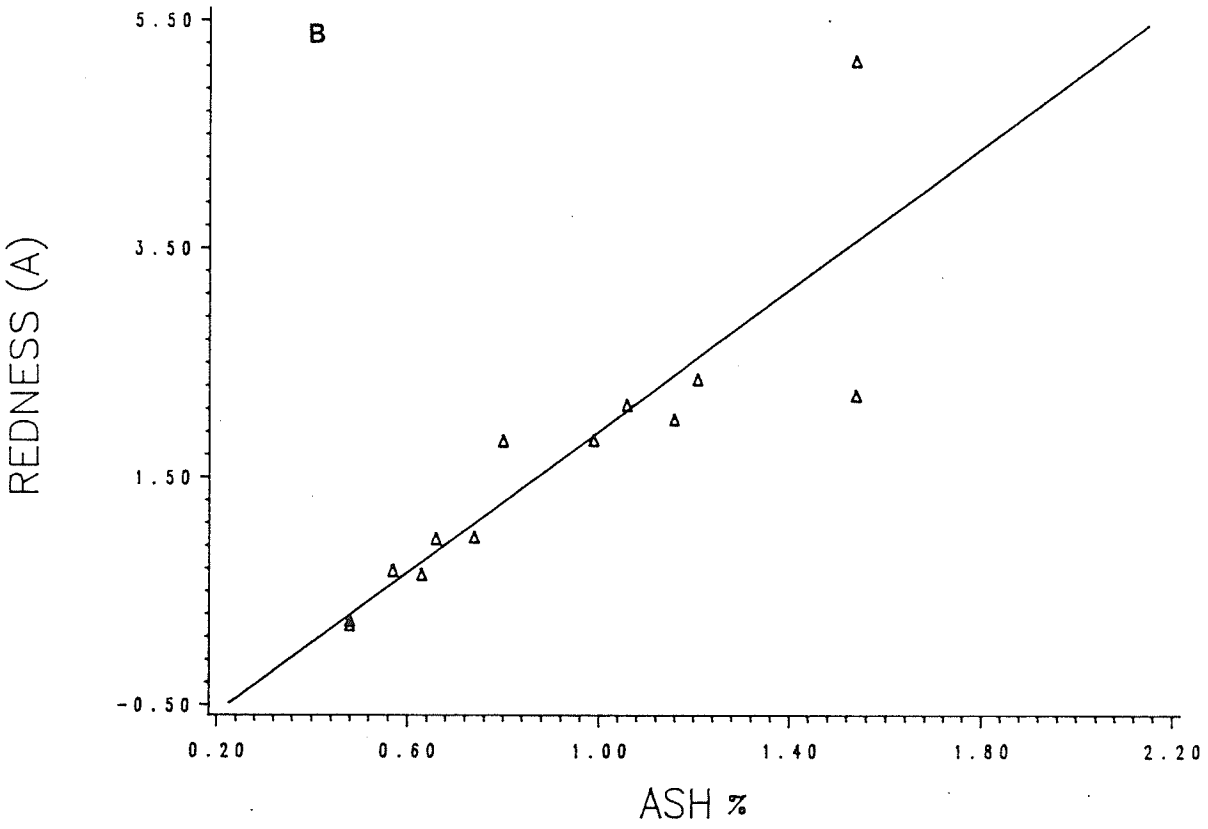

A
B
C
REDNESS (A)
5.50
3.50
1.50
-0.50
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

Figure 46 Relationship Between Water Flour Paste Redness and Ash Content in the Softer Wheats Fielder and HY320

A. Fielder

B. HY320


A
5.50
3.50
1.50
-0.50
REDNESS (A)
0.20
0.60
1.00
1.40
1.80
2.20
ASH %


B
5.50
3.50
1.50
-0.50
REDNESS (A)
0.20
0.60
1.00
1.40
1.80
2.20
ASH %

remaining cultivars were noticeably lowered. The yellow component r values underwent a slight decrease but each remained significant. As the initial brightness values in the Kan Sui reagent were basically unchanged from the water flour paste, the subsequent comments with regards to the brightness will apply to both unless indicated otherwise.

4.06.2.2 Polyphenol Oxidase

The correlation between the polyphenol oxidase levels and each of the three Hunter water flour paste color components was very strong, $r > 0.86$, for each variety as seen in Table 8. A similar relationship, $r > 0.91$, was observed between the enzyme levels and the Kent-Jones CGF values. The Agtron values, although remaining statistically significant, $p < 0.05$, had the poorest correlations with the enzyme in each of the various cultivar flours as r values ranged from -0.67 to -0.81. The influence of the alkaline reagent of the Hunter values was almost identical to that described previously. Brightness remained unaffected while the yellow and red component correlations were reduced.

4.06.2.3 Pigment

Pigment as expected showed the highest correlation with the yellow index of the Hunter lab as seen in Table 8. An unexpected observation was the weaker correlation between this variable for the variety Norstar where r=0.80. Examination of each of the colorimetric method

relationships with pigment was found to be lowest in all cases for Norstar. Brightness, Kent-Jones CGF, and Agtron values each displayed moderately strong relationships with pigment for each variety. The lowest correlations were exhibited by the Hunter a^* value. The strength of the relationship appeared to very varietal dependant ranging from r=0.63 for Norstar to r=0.93 for Glenlea.

The Kan Sui solution effect seen previously remained consistent. The low red component correlations with pigment fell below the p=0.05 level in Fielder, HY320 and Norstar as seen in Table 10.

4.06.2.4 Protein

Protein had the weakest correlations of any factor with each of the colorimetric methods as displayed in Table 8. The Hunter lab L^*, a^*, b^*,, values determined on the water flour pastes displayed the poorest correlations, with redness, a^*, having no statistical correlation, $p > 0.05$, with protein for any variety. Brightness had a questionable relationship with protein as none of the hard wheats exhibited a significant relationship. The relationship between protein and yellowness, b^*, although significant, $p < 0.05$, was weak as the correlation coefficients ranged from 0.56 to 0.79. Only the softer wheats, HY320 and Fielder displayed a significant correlation between protein and brightness with values of r= -0.66 and -0.63 respectively.

Agreement with Moss (1971) and Miskelly (1984) works would appear to conflict with these findings. However, both authors were reporting a strong correlation between brightness and protein for soft Australian wheats and in this study it was only the soft wheats which reinforced this relationship. A similar situation was observed between protein content and the Kent-Jones CGF values. The relationship was strongest in the soft wheats, decreasing in the hard wheats, with no significant relationship being detected for Katepwa. The Agtron correlation coefficients indicated only a moderate relationship with flour protein content while ranging considerably with variety

The use of the basic Kan Sui solution in the preparation of the flour paste resulted in only the yellow component, b^*,, exhibiting significant correlation coefficients, $p < 0.05$, for each variety. Brightness correlated weakly ,r=-0.61 and -0.56, for only Fielder and Glenlea respectively.

4.06.2.5.Phenolic Components

4.06.2.5.1 Total Phenolic Categories

Examination of the phenolic components of the various flours indicated that they yielded, in general, high correlations with the Hunter lab indices as shown in Table 8. Yellowness correlated best with the phenolics in all 4 total categories; free, soluble bound, insoluble bound and

overall totals, displaying a positive relationship, $r > 0.90$, for each variety. A weaker, negative relationship, $r > -0.82$, was observed between the flour paste's brightness and corresponding categories of total phenolics. The relationship between the various total phenolic groups and redness varied considerably with r values ranging from 0.69 to 0.96. The total free phenolic acid content of the flours correlated best with the redness, a^*, value while the total bound phenolic relationship showed the greatest variability.

Introduction of the Kan Sui reagent had its most profound effect on the relationship between the red color component and each of the total phenolic categories. In two of the varieties, Fielder and Norstar, no significant relationship was detected for any of the total subgroups. The influence of the basic solution on the relationship between these categories and paste yellowness was minimal as each r value declined only slightly. Contrary to the prevalent reduction in correlation coefficients between the major factors and the alkaline paste, the r values of every varieties' total phenolic subgroups either increased or remained constant with the Hunter brightness values.

Kent-Jones CGF values displayed a very strong correlation, $r > 0.83$, with each category of the total phenolics. The affiliation between Agtron readings and the various categories of total phenolics was the lowest of the colorimetric methods. An inverse relationship, $r > -0.72$, was observed for each category.

The total free phenolic acid content was found to exhibit the strongest subgroup correlations with each of the color analysis techniques when using a flour:water paste.

4.06.2.5.2 Individual Phenolic Components

Evaluation of the relationships between individual phenolic acids and the colorimetric methods are summarized in Tables 11 & 12. The strongest correlations were observed between flour paste yellowness and the individual phenolic acids for both the free and soluble bound acids. In general higher correlations were observed between the soluble bound material and yellowness than the free acids. This difference was not surprising as a number of free acids could not be detected in the better flours.

An inverse relationship was found to exist between each individual phenolic acid and flour paste brightness. Soluble bound vanillic acid exhibited the weakest correlation across each variety with values extending from $r=-0.69$ to $r=-0.79$. Individual free phenolic acids displayed correlations of similar magnitude as the soluble bound acids.

The correlations between individual phenolic acids and flour paste redness, although significant, $p < 0.05$, were in general the lowest observed.

Analysis of the relationship of both the Kent-Jones CGF values and those taken on the Agtron with the individual phenolic acids indicated a stronger correlation with the

Table 11

PHENOLIC ACIDS

CORRELATION MATRIX OF HUNTER LAB COLOR COMPONENTS

	FIELDER			HY320			GLENLEA			KATEPWA			NORSTAR		
Component	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*	L^*	a^*	b^*
BOUND PHENOLICS:															
Ferulic	-0.83	0.66	0.93	-0.94	0.85	0.96	-0.77	0.69	0.89	-0.92	0.85	0.98	-0.90	0.77	0.97
Vanillic	-0.69	NS	0.83	-0.74	0.56	0.84	-0.71	0.63	0.84	-0.84	0.75	0.91	-0.79	0.62	0.90
Sinapic	-0.84	0.71	0.93	-0.87	0.82	0.88	-0.84	0.76	0.91	-0.94	0.88	0.97	-0.88	0.80	0.90
Syringic	-0.84	0.66	0.91	-0.89	0.76	0.96	-0.71	0.64	0.83	-0.93	0.88	0.96	-0.93	0.83	0.98
Caffeic	-0.96	0.91	0.91	-0.94	0.90	0.91	-0.85	0.81	0.87	-0.93	0.94	0.87	-0.88	0.78	0.92
Coumaric	-0.94	0.91	0.91	-0.85	0.71	0.92	-0.74	0.68	0.85	-0.86	0.81	0.95	-0.91	0.82	0.94
Phenolics															
INSOLUBLE															
Ferulic	-0.86	0.74	0.89	-0.89	0.77	0.96	-0.85	0.76	0.95	-0.86	0.78	0.93	-0.87	0.72	0.95
FREE PHENOLICS:															
Ferulic	-0.84	0.71	0.93	-0.82	0.70	0.89	-0.87	0.81	0.90	-0.92	0.87	0.95	-0.97	0.90	0.98
Vanillic	-0.92	0.81	0.92	-0.84	0.72	0.91	-0.87	0.81	0.95	-0.97	0.94	0.97	-0.98	0.94	0.96
Syringic	-0.81	0.82	0.77	-0.85	0.80	0.87	-0.89	0.82	0.95	-0.96	0.93	0.94	-0.90	0.81	0.94
Caffeic	-0.91	0.88	0.87	-0.91	0.87	0.89	-0.90	0.91	0.82	-0.91	0.96	0.78	-0.95	0.91	0.95
Coumaric	NS	NS	NS	-0.87	0.75	0.92	-0.90	0.88	0.90	-0.96	0.98	0.88	-0.90	0.79	0.95

Note: All values listed have $p < 0.05$
NS = Not significant

L = Brightness a = Redness b = Yellowness

Table 12 PHENOLIC ACIDS:

CORRELATION MATRIX OF AGTRON AND KENT-JONES COLOR MEASUREMENTS

	FIELDER		HY320		GLENLEA		KATEPWA		NORSTAR	
Component	AGTRON	K-J	AGTRON	K-J	AGTRON	K-J	AGTRON	K-J	AGTRON	K-J
BOUND PHENOLICS:										
Ferulic	-0.75	0.83	-0.76	0.94	-0.78	0.84	-0.85	0.96	-0.92	0.91
Vanillic	-0.64	0.68	-0.74	0.80	-0.76	0.80	-0.88	0.92	-0.84	0.80
Sinapic	-0.69	0.83	-0.62	0.86	-0.75	0.88	-0.80	0.95	-0.81	0.89
Syringic	-0.80	0.85	-0.81	0.93	-0.73	0.79	-0.81	0.95	-0.91	0.94
Caffeic	-0.85	0.97	-0.68	0.92	-0.79	0.88	-0.59	0.87	-0.79	0.87
Coumaric	-0.85	0.95	-0.71	0.87	-0.76	0.82	-0.86	0.93	-0.89	0.93
INSOLUBLE Ferulic	-0.84	0.89	-0.80	0.93	-0.82	0.91	-0.89	0.93	-0.91	0.87
FREE PHENOLICS:										
Ferulic	-0.71	0.83	-0.70	0.86	-0.78	0.90	-0.81	0.94	-0.88	0.97
Vanillic	-0.90	0.94	-0.78	0.90	-0.89	0.94	-0.79	0.97	-0.86	0.98
Syringic	-0.69	0.81	-0.81	0.89	-0.81	0.93	-0.77	0.96	-0.83	0.90
Caffeic	-0.83	0.93	-0.71	0.91	-0.69	0.87	NS	0.83	-0.77	0.94
Coumaric	NS	NS	-0.71	0.88	-0.77	0.91	-0.66	0.91	-0.83	0.89

Note: All values listed have $p < 0.05$

K-J = Kent-Jones

NS = Not significant

Kent Jones readings. Positive correlations were found to exist between CGF and the phenolic acids while an inverse relationship occurred with the Agtron readings. The correlation coefficient were found to be approximately equal for both the soluble bound and the free acids with each of these colorimetric measurements.

4.06.3 Comparison of Colorimetric Methods

The assessment of a flour paste slurry is a complex process as a number of factors are interacting simultaneously. In order to isolate as many of these components, the pooled flour pastes were subjected to an initial reading on the Hunter Lab Colorimeter, within a time reference similar to that of the traditional Kent-Jones measurement.

4.06.3.1 Hunter Lab and Kent-Jones

Analysis of the initial L^*, a^*, and b^* values revealed excellent agreement with the traditional Kent-Jones color values. The results of the correlations are summarized by variety in Table 13. The brightness factor, L^*, maintained the highest correlation, by variety, with the Kent-Jones readings. An inverse relationship with the CGF values was observed with $r=-0.97$ being the lowest value. The correlation remained at this high level $r=-0.98$ when varietal considerations were removed.

Table 13

FLOUR PASTE
CORRELATION MATRIX BETWEEN COLORIMETRIC METHODS

VARIETY	AGTRON	K-J
FIELDER		
L	0.86	-0.99
A	-0.77	0.94
B	-0.81	0.92
HY320		
L	0.84	-0.99
A	-0.75	0.93
B	-0.88	0.99
GLENLEA		
L	0.80	-0.98
A	-0.74	0.94
B	-0.90	0.98
KATEPWA		
L	0.77	-0.97
A	-0.68	0.93
B	-0.83	0.97
NORSTAR		
L	0.86	-1.00
A	-0.75	0.95
B	-0.91	0.97

Note: All values listed have $p < 0.05$

L = Brightness a = Redness b = Yellowness

K-J = Kent-Jones

A strong positive correlation was seen for both the redness, a=*, and yellowness, b*, values with the Kent-Jones CGF. The minimum correlation for either of these color components was r=0.92 for the a* value of Fielder. However, unlike brightness, yellowness, b*, did show an influence of variety as the correlation, independent of variety, declined to r=0.87. The red color component is minimally affected by variety, maintaining a very strong relation with the CGF values as r=0.91.

4.06.3.2 HunterLab and Agtron

The relationship of the three Hunter Lab color components with those determined by the Agtron, summarized in Table 13, were not as strong as those with Kent-Jones method. Unlike Kent-Jones CGF, the Agtron readings correlated positively with brightness as r values ranged from 0.77 to 0.86. Removal of the varietal factor indicated a* continued strong relationship as r=0.81.

The a* and b* Hunter values displayed an inverse relationship with their Agtron counterparts. Yellowness exhibited the strongest correlation of the three Hunter color components. Although redness maintained a level of significance, $p < 0.05$, the correlation coefficients were found to be the weakest with r=-.68 to -0.77. Yellowness and redness were both found to maintain a significant

correlation with the Agtron colors independent of the variety.

The strong relationship between Agtron and Kent-Jones values was evident within all five varieties tested with coefficients tightly grouped between -0.89 to -0.91.

4.06.3.3 Influence of Kan Sui Reagent

Introduction of the Kan Sui reagent had negligible effect on the brightness component of the Hunter Lab. The correlations between water flour paste brightness and the Kan Sui paste brightness were in excess of r=0.99 for each variety. The minimal change in the brightness reading in the alkaline paste resulted in consistent high correlations with the Kent-Jones CGF readings while those of the Agtron improved slightly as seen in Table 14..

Under the alkaline conditions the relationship between yellowness, b^*, and the Kent-Jones color, although remaining significant, decreased considerably for each variety investigated. It was observed that the correlation with Agtron values decreased in the softer wheats but increased slightly in the hard wheats. This was particularly noticeable for Katepwa as the correlation coefficient increased from -0.83 to -0.94.

Redness, a^*, was found to be no longer significantly correlated with the Agtron values in the Kan Sui paste for any variety. A similar trend was observed with the Kent-

Table 14

KAN SUI FLOUR PASTE
CORRELATION MATRIX BETWEEN COLORIMETRIC METHODS

VARIETY	AGTRON	K-J
FIELDER		
L	0.87	-0.98
A	NS	NS
B	-0.70	0.75
HY320		
L	0.87	-1.00
A	NS	0.67
B	-0.85	0.83
GLENLEA		
L	0.84	-0.99
A	NS	0.79
B	-0.93	0.83
KATEPWA		
L	0.80	-0.98
A	NS	0.72
B	-0.94	0.88
NORSTAR		
L	0.88	-1.00
A	NS	NS
B	-0.93	0.84

Note: All values listed have $p < 0.05$
NS = NOT SIGNIFICANT

L = Brightness a = Redness b = Yellowness K-J = Kent-Jones

Jones CGF values and redness for both Norstar and Fielder. The relationship for the remaining varieties was also found to be severely diminished.

4.06.4 HunterLab L^*, a^*, and b^* Values Over Time

Each variety had thirteen of its major pooled flours examined by the Hunter LabScan II flour paste method to monitor the changes which occurred in their respective L^*, a^*, and b^* values as a function of time. Analysis was carried out with both a water: flour and Kan Sui reagent:flour paste systems. Comparison of the individual pooled flours as well as the influence of extraction rate was limited as only to duplicate analysis was performed. However, through the use of regression analysis, best fit lines and 95% confidence intervals were established. These were employed to note trends and draw comparisons amongst samples.Representative examples can be seen in Figs 47-49 with the remaining varietal flours found in Appendix D.

4.06.4.1 Water Flour Pastes:

4.06.4.1.1 1st Patent Flours

In all varieties except Katepwa the brightness of this pooled flour at the 75% extraction level was indistinguishable from that observed at the 80% extraction rate. A similar observation was made when viewing the five

Figure 47 Water:Flour Paste Brightness of the 75% Extraction Pooled Flours of Norstar

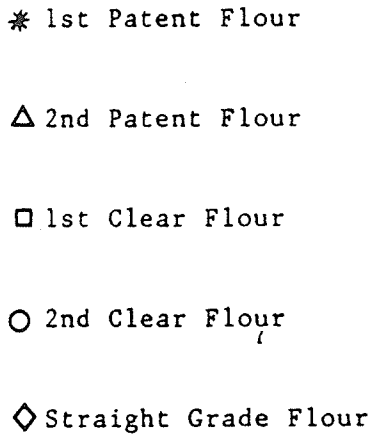
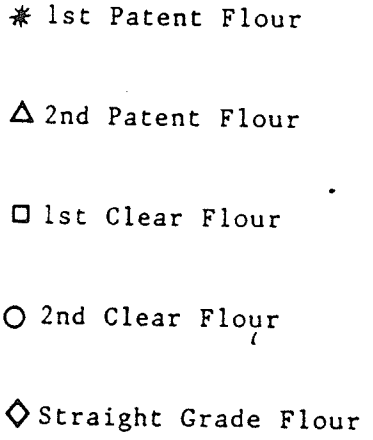
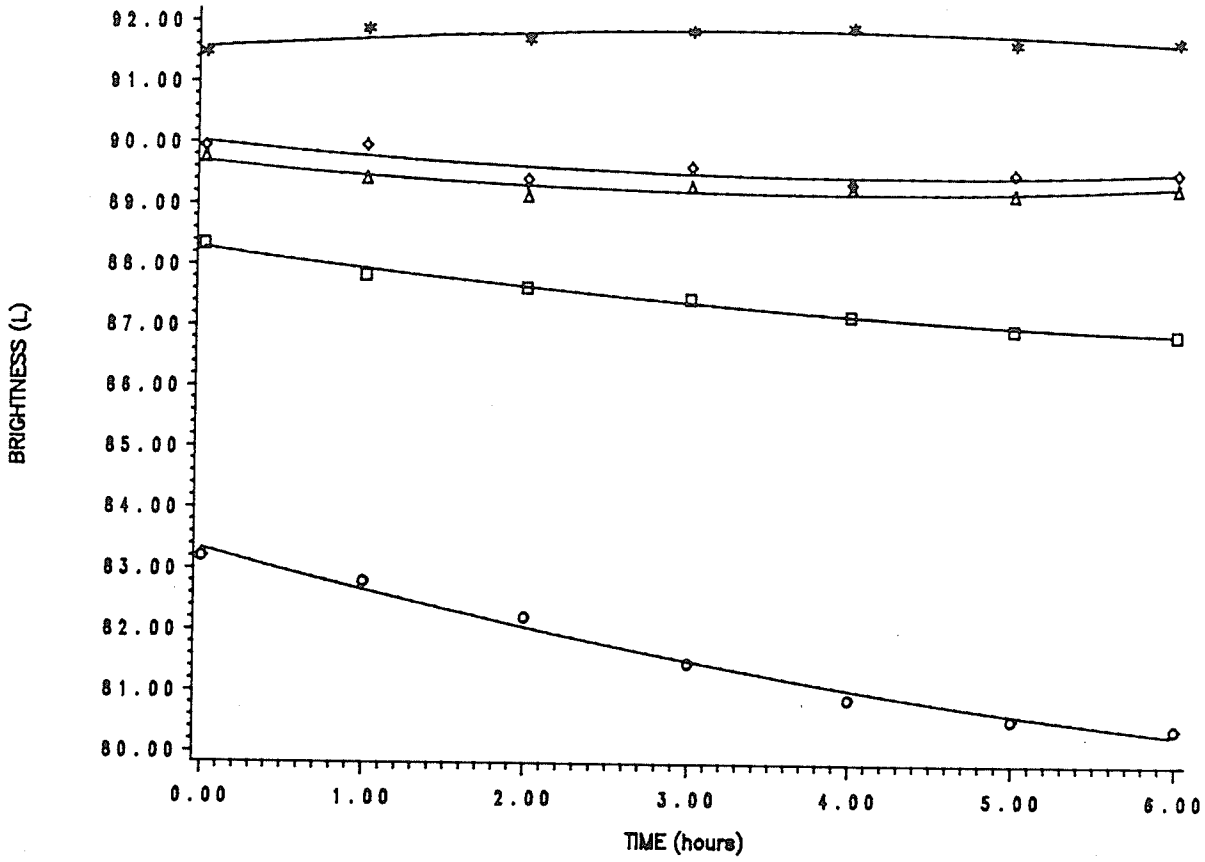


Figure 48 Water:Flour Paste Yellowness of the 75% Extraction Pooled Flours of Norstar




92.00
91.00
90.00
89.00
88.00
87.00
86.00
85.00
84.00
83.00
82.00
81.00
80.00
BRIGHTNESS (L)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

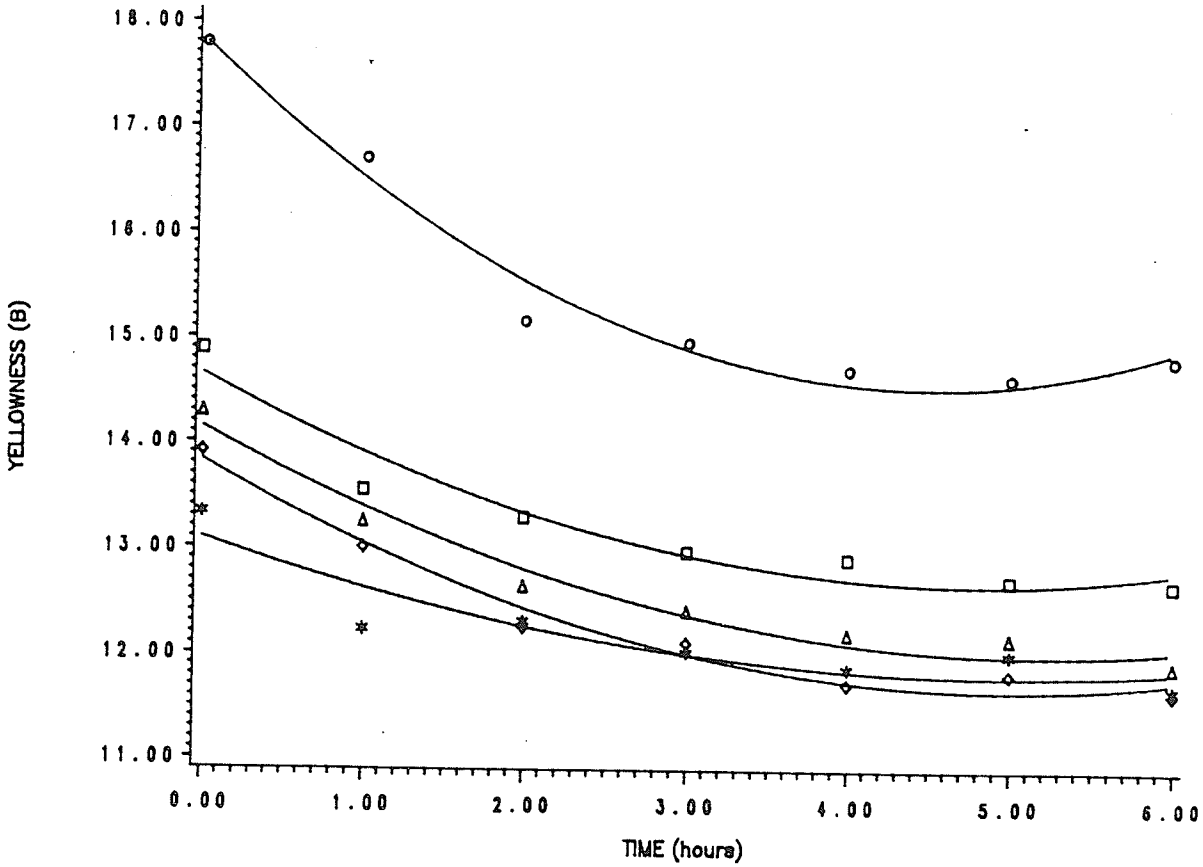
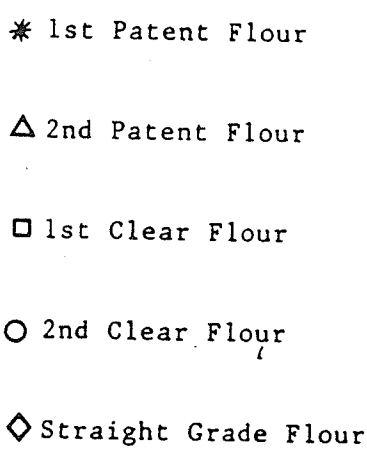
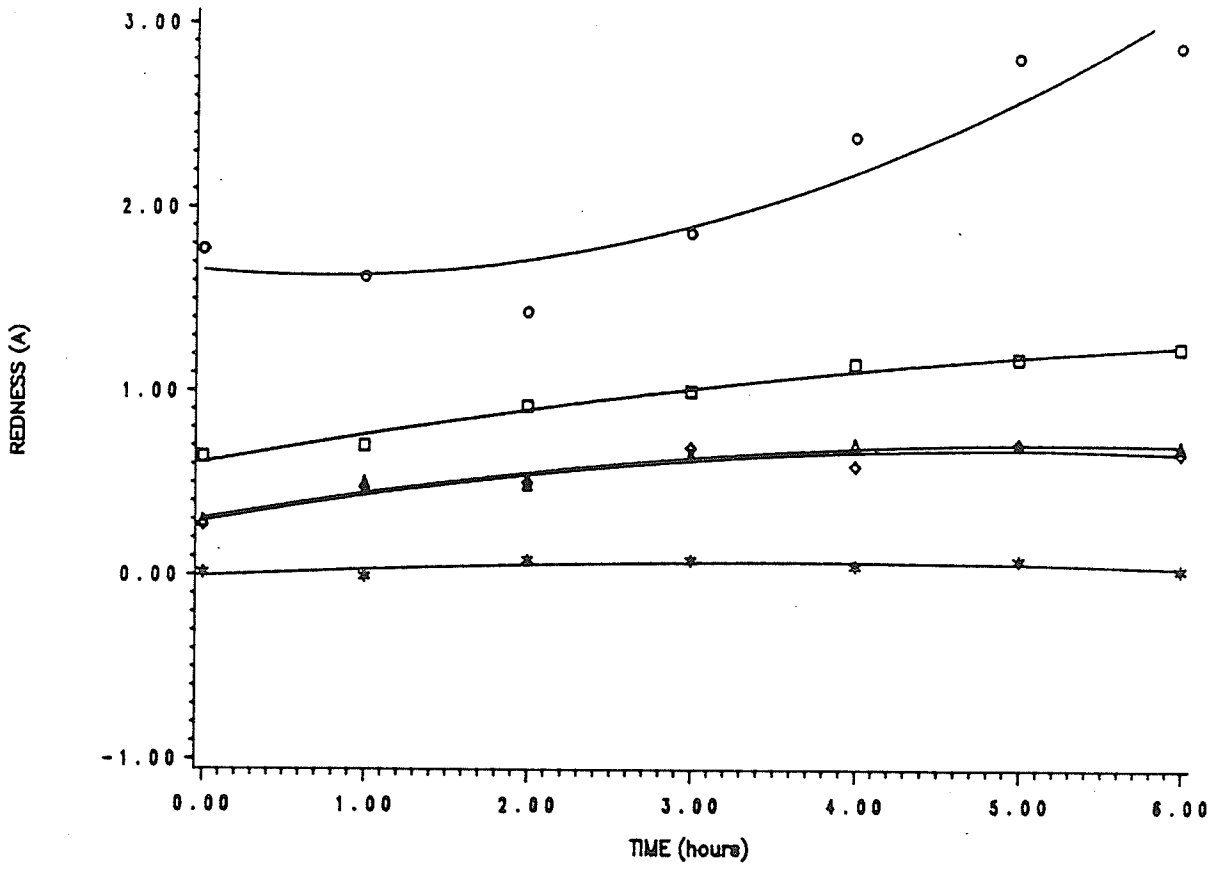

18.00
17.00
16.00
15.00
14.00
13.00
12.00
11.00
YELLOWNESS (B)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

Figure 49 Water:Flour Paste Redness of the 75% Extraction Pooled Flours of Norstar




3.00
2.00
1.00
0.00
-1.00
REDNESS (A)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

varieties' yellow component as a function of time. The inability to distinguish the flours , based upon extraction rate was also observed for the red component of the flour pastes for Glenlea, HY320, and Norstar. Only Fielder displayed an appreciable difference in the red color component between extraction levels over the 6 h time period.

The evaluation of these three color components would suggest that the color change produced by a 1st patent flour over time appeared to be independent of the extraction rate. This would confirm the earlier findings in which the polyphenol oxidase levels in this flour, at either extraction level, were minimal. A low enzyme level would therefore exert a limited influence on discoloration of an end product utilizing this flour at even the 80% extraction level.

4.06.4.1.2 2nd Patent Flour

Examination of all five varieties indicated that the 2nd patent flours were distinct from their 1st patent flours in terms of brightness at both the 75 and 80% extraction levels.

Norstar, Glenlea, and HY320 flours revealed that the 75% extraction 2nd patent flour was distinct from the higher milling rate while Katepwa and Fielder required a few hours before the disparity in the extraction rate manifested itself in a noticeable color difference.

The yellow component, b^*, of the flour pastes were however indistinguishable from the 1st patent flours at the 75% extraction level. A similar observation was made at the 80% extraction level for each variety except Norstar and HY320. Each varieties flour samples were also not able to be separated on the basis of extraction rate using this color index.

The red component exhibited the trend seen with brightness as both the 75 and 80% 2nd patent flours were distinct from their 1st patent counterparts. As was the case for brightness, Norstar, Glenlea, and HY320 also revealed a difference in red color as the milling rate was increased. Katepwa and Fielder 80% extraction flours however remained indistinguishable from the lower rate.

4.06.4.1.3 1st Clear Flours

Inspection of the decline in brightness indicated that with the exception of Glenlea, all varieties exhibited a distinct difference in brightness from the 2nd patent flours. Only the 75% Glenlea 1st clear flour overlapped the values seen in the 80% extraction 2nd patent flour. The corresponding 80% Glenlea 1st clear flour was noticeably different from each of these two pooled flours.

In each variety examined except HY320, the 75% 1st clear extraction flour was distinct in terms of brightness

from its 80% extraction flour. HY320 was similar for both extraction levels until 4 h at which time they diverged.

The overlapping of the 95% confidence intervals defining the quadratic decline of yellowness for both the 2nd patent flours and the 75% 1st clear flour was very apparent for each variety. However, at the 80% extraction level, all varieties, with the exception of Fielder displayed distinct and separate curves from either of the 2nd patent flours

In terms of separation of the 1st clear flours on the basis of extraction rate, distinguishability was variety dependant. Only Katepwa, Norstar and Fielder had discrete yellow color curves.

The red component of the 1st clear flours mirrored the characteristics displayed by their brightness component. With the exception of the Glenlea 75% flour, each was distinct from the 2nd patent flours. However, comparison within the flour at the two different milling rates indicated that only Norstar and Fielder displayed no overlap of the color curves.

4.06.4.1.4 2nd Clear Flours

Examination of the decline in brightness of this flour at both extraction levels indicated that all of the harder wheats, including HY320 had separate and distinctive values

from their 1st clear flours. In Fielder, only the 80% extraction flour did not overlap with the 1st clear flours.

The distinctive nature of this poor quality flour was noticeable as all varieties but Glenlea had discrete differences in the brightness curves due to extraction rate.

Although the flours were distinguishable from the 1st clear flours on the basis of brightness, this was not the case for the pastes' yellow component. In all varieties, except Glenlea, only the 80% extraction flour showed a distinctive trend relative to the 1st clear flours. However, only Norstar and Fielder were able to show separate curves with increasing extraction rate.

The inability to discriminate was also observed when assessing the red color component of these flours with their 1st clear counterparts. Only in Katepwa was it possible to note a clear distinction between the two different quality flours. Furthermore, it was not possible to differentiate the 2nd clear flours by extraction rate for any of the varieties.

4.06.4.1.5 Straight Grade Flours

Examination of the three different extraction rate flours, 75, 80 and 85% indicated that in all varieties, each was distinct in terms of brightness and redness. In terms of yellowness however, only Norstar and Fielder exhibited three distinct curves based upon extraction level. The remaining

varieties displayed the same general increase in yellowness with extraction rate, but the 75 and 80% flours had overlapping confidence levels. Only in HY320 was the 85% flour not distinguishable from the lower extraction flours in terms of yellowness.

It was observed in both the polyphenol oxidase levels, and the phenolic acid contents that the straight grade flours had very similar values with those observed in the corresponding 2nd patent flours. Comparison of these two flours on the basis of color indicated again the high degree of similarity between the flours. In all of the varieties, over both extraction levels and each of the three color components, there were only three isolated examples in which the two flours were distinguishable at their respective extraction rate. In each case it was the 80% straight grade flour which remained distinct.

4.06.4.2 Kan Sui Flour Pastes

Representative examples of color changes over the 6 h period utilizing the alkaline Kan Sui reagent are seen in Figs 50-52 with the remaining varietal flours found in Appendix E.

Figure 50 Kan Sui:Flour Paste Brightness of the 75% Extraction Pooled Flours of Norstar

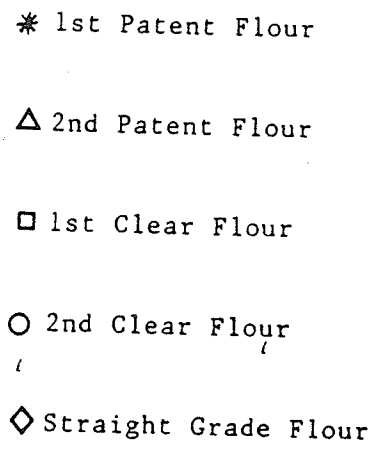
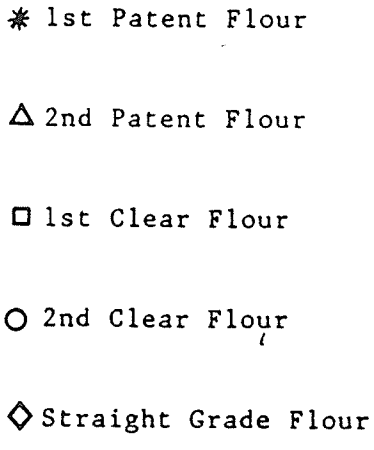
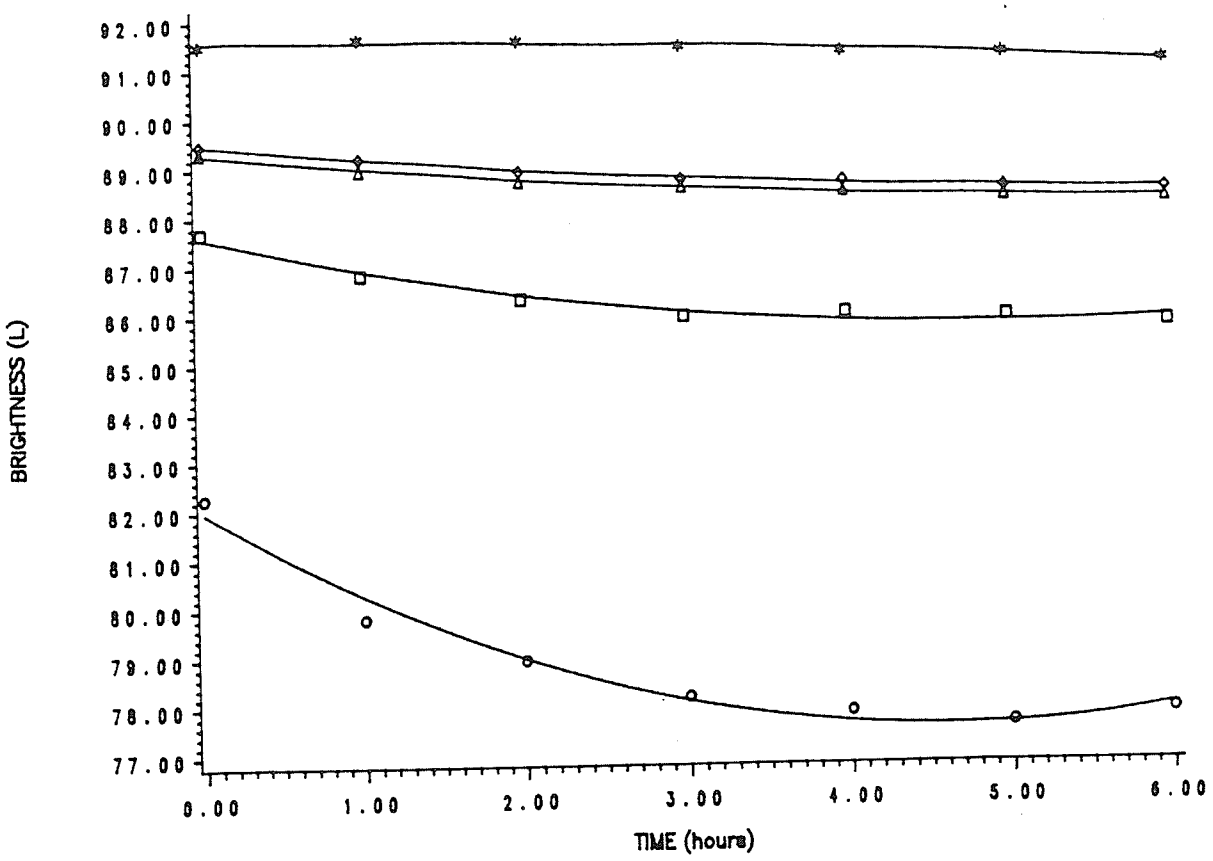


Figure 51 Kan Sui:Flour Paste Yellowness of the 75% Extraction Pooled Flours of Norstar




BRIGHTNESS (L)
92.00
91.00
90.00
89.00
88.00
87.00
86.00
85.00
84.00
83.00
82.00
81.00
80.00
79.00
78.00
77.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

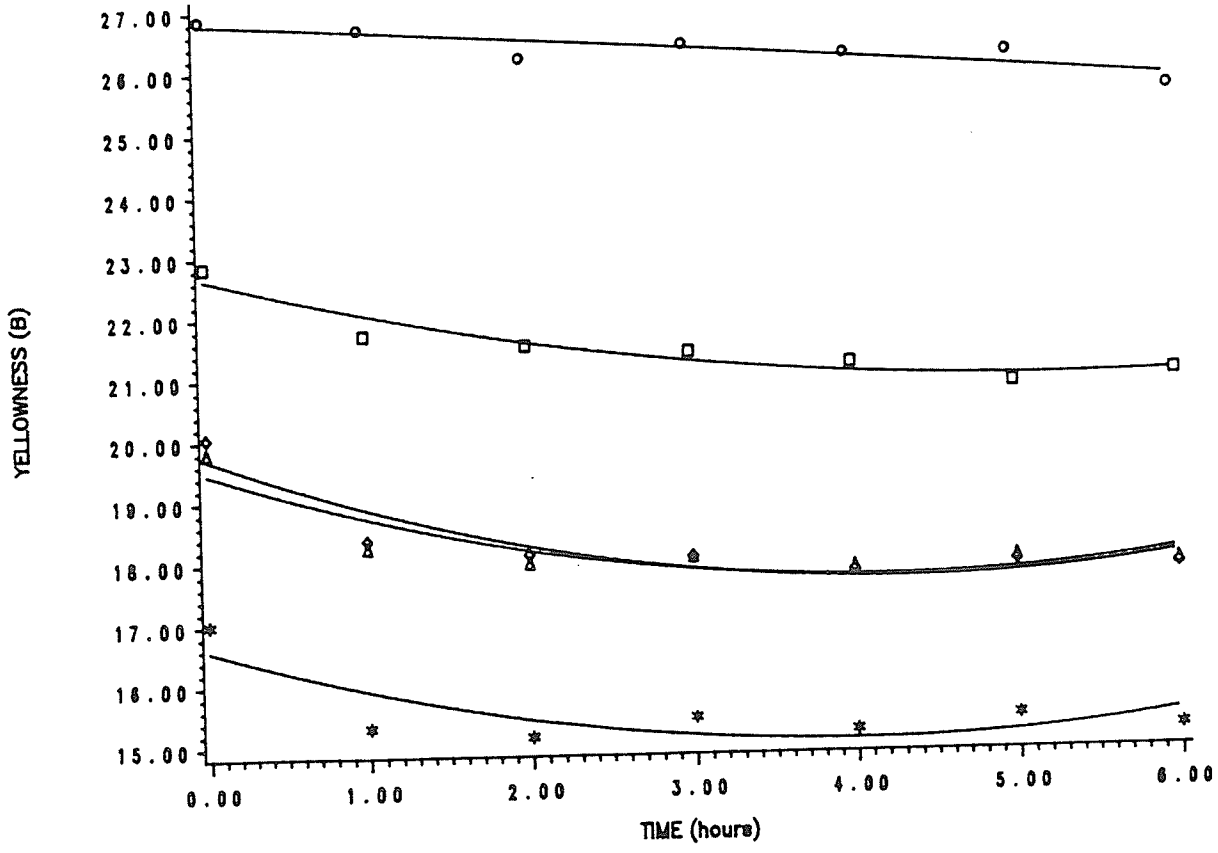

YELLOWNESS (B)
27.00
26.00
25.00
24.00
23.00
22.00
21.00
20.00
19.00
18.00
17.00
16.00
15.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

Figure 52 Kan Sui:Flour Paste Redness of the 75% Extraction Pooled Flours of Norstar

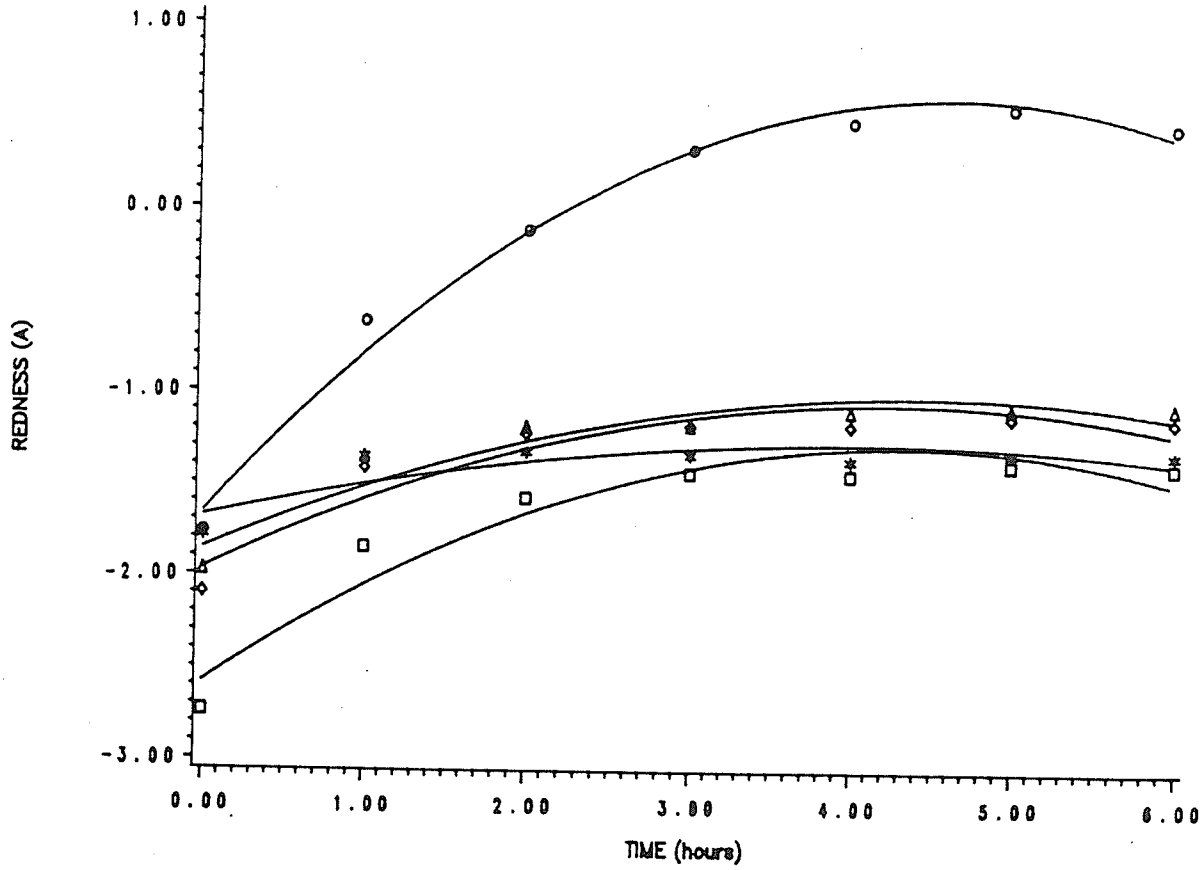
✱ 1st Patent Flour

△ 2nd Patent Flour

□ 1st Clear Flour

○ 2nd Clear Flour

◇ Straight Grade Flour


1.00
0.00
-1.00
-2.00
-3.00
REDNESS (A)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

4.06.4.2.1 1st Patent Flours

Use of the alkaline Kan Sui reagent did influence the respective color curves of these pooled flours over time. Although the pattern of decline was different for the yellow color component, the inability to distinguish between 75 and 80% flours was consistent with that observed for the water paste. This trend was also carried over into the red color range although the actual a^* values were considerably different from the water pastes. It was only in the brightness of the pastes were some differences observed on the basis of extraction for the respective varieties. Unlike the water pastes where each variety was indistinguishable Fielder and Katepwa displayed brightness curves which appeared distinct from each other at the 75 and 80% extraction level.

4.06.4.2.2 2nd Patent Flours

Under the basic conditions, only the brightness component of the 2nd patent flours were distinct from their 1st patent flours. Both the yellow and red color curves of this flour overlapped those displayed by their 1st patent flours. The only exception to this observation was seen in the red color components of the HY320 flours.

With this exception, it was also found that each variety was unable to distinguish between extraction level

for both their red and yellow time function curves. However, extraction rate did exert an influence on brightness as each variety displayed distinct curves at the two different milling levels.

4.06.4.2.3 1st Clear Flours

Brightness was the only factor which discriminated between this flour and the 2nd patent flours at either extraction level for all varieties. The red component was unable to separate the different flours while yellowness had mixed success as the 80% 2nd patent flour tended to overlap the 75% 1st clear flour.

Examination of the influence of extraction level showed that with the exception of Fielder the remaining varieties had distinct brightness curves. Furthermore, the yellow component functions were also separate for every variety which had not been seen in the previous flours. The red component however continued to be unable to resolve the flours based upon extraction rate.

4.06.4.2.4 2nd Clear Flours

The very high ash 2nd clear flours did not clearly separate themselves in their various color components from their 1st clear counterparts for most varieties. In general, the values achieved by the 2nd clear 75% extraction flour

tended to overlap those achieved by the 1st clear 80% extraction flour.

With the exception of Norstar, each variety had distinct brightness curves, and all had separate yellow curves, based upon extraction rate. As had been the case in the previous flours, no variety was able to generate non-overlapping curves for the red color component.

4.06.4.2.5 Straight Grade Flours

Examination of the three straight grade flours under alkaline conditions indicated that each was distinct in terms of its brightness component for every variety. The one exception to this was observed between the 80 and 85% extraction flours from Fielder. The extraction rate was also able to differentiate the resulting yellow curves of these flours in every case except between the 75 and 80% curves of Katepwa where there was slight overlap. Katepwa's 85% straight grade flour however was able to be isolated from the other flours. The pastes' red color component was distinguishable for the three different flours in both Norstar and HY320 but overlap was common in the remaining varieties.

4.06.4.3 Summary Comments

Inspection of each of the color components would suggest that the variety of choice for end products, especially those requiring high brightness, such as noodles, would favor the selection of Norstar. In all pooled flours examined Norstar was consistently distinct from the others while remaining the brightest. Examination of the remaining color influences, red and yellow, indicated Norstar also displayed minimal change. The decline in

yellowness across all streams was smallest in this winter wheat , being approximately half that observed in the other four classes. The red color component of the flour paste also suggests the preference towards the use of Norstar as the amount of change observed was minimal compared to the other varieties. In both patent flours at the 75 and 80% extraction level the red color component remains close to neutral, 0, over the 6 h time span. The practical significance of the limited change in color over time, exhibited by Norstar at both extraction levels, lends itself to less restrictive processing timeframe observed in non-traditional products.

Introduction of the alkaline Kan Sui reagent had a noticeable effect on the respective color curves at the varying extraction rates. The most prevalent observation dealt with the minimal change in the yellow component of the various pastes tested. In all cases, there was an obvious rapid decline in values over the first hour, followed by a general levelling off or slow increase in the b^* measurement. This trend was different from that observed in the normal water:flour paste discussed earlier.

There was also a very obvious shift in the pastes' red-green color scale. The presence of the high pH solution caused most pastes to exhibit an initial green rather than red component. However, as seen in the water paste samples, the shift towards a higher red content was clearly observed.

Brightness as a function of time displayed the smallest difference from a regular water environment. It was noted that the harder wheats demonstrated a noticeable influence of the alkaline environment. Norstar had the most pronounced difference as all flours were discretely separate and lower in brightness than their water paste counterparts. This divergence was also noted for the other two harder varieties, Katepwa and Glenlea, in both 75 % clear flours and each of the 80% extraction flours. The softer wheats, Fielder and HY320 did not distinguish themselves from their water brightness values at either extraction level.

4.06.5 Influence of Temperature on Color Production

In order to assess the influence of temperature on the flour paste color production four flour streams from the 75% extraction millings of the soft wheat Fielder, and hard wheat Katepwa, were employed. The flours were the 1st patent, 1st and 2nd clears, and the corresponding straight grade flour. These flours were chosen as they would differ widely in character allowing the temperature to have a maximum influence. The additional temperatures selected were 35 and 45°C as they would model ambient temperatures attainable in certain parts of the world. Each sample was subject to duplicate analysis with the average values being utilized.

Plots of representative individual color components, brightness, L^*, redness, a^*, and yellowness, b^*, as a

function of time over the temperatures studied are represented in Figs 53-55. Examination of the best fit lines for each sample's individual color component indicated temperature had a minimal influence on color production. Individual analysis of brightness, redness, and yellowness of the Katepwa flours revealed a high degree of overlap between each temperature's respective 95% confidence limit curve as the temperature was increased. A similar observation was noted for the Fielder samples. Although overlap of successive curves was noted, a general trend towards change was observed with increasing temperature. Brightness and yellowness of the paste both tended to decrease while redness increased with higher temperature.

Although the individual color component change appeared to be minor, examination of the overall total color change, Eab, did identify differences between the two varieties tested. Total color change, Eab, represents the sum of the range in values for each color component, from time 0 to 6 h. Figs 56-57 show the total color change observed in the different flours by variety. At 25°C Fielder displayed a larger total color change in each stream relative to Katepwa's samples. The soft wheat continued to show a considerably greater total color change than Katepwa at both elevated temperatures. However comparison of the average ratio increase of the four streams for each variety, 45°C versus 25°C, indicated there was no significant difference between the varieties. The average ratio increase in total

Figure 53 Influence of Temperature on Water:Flour Paste Brightness of Katepwa 75% Extraction 1st Clear Flour

✳ 25°C

△ 35°C

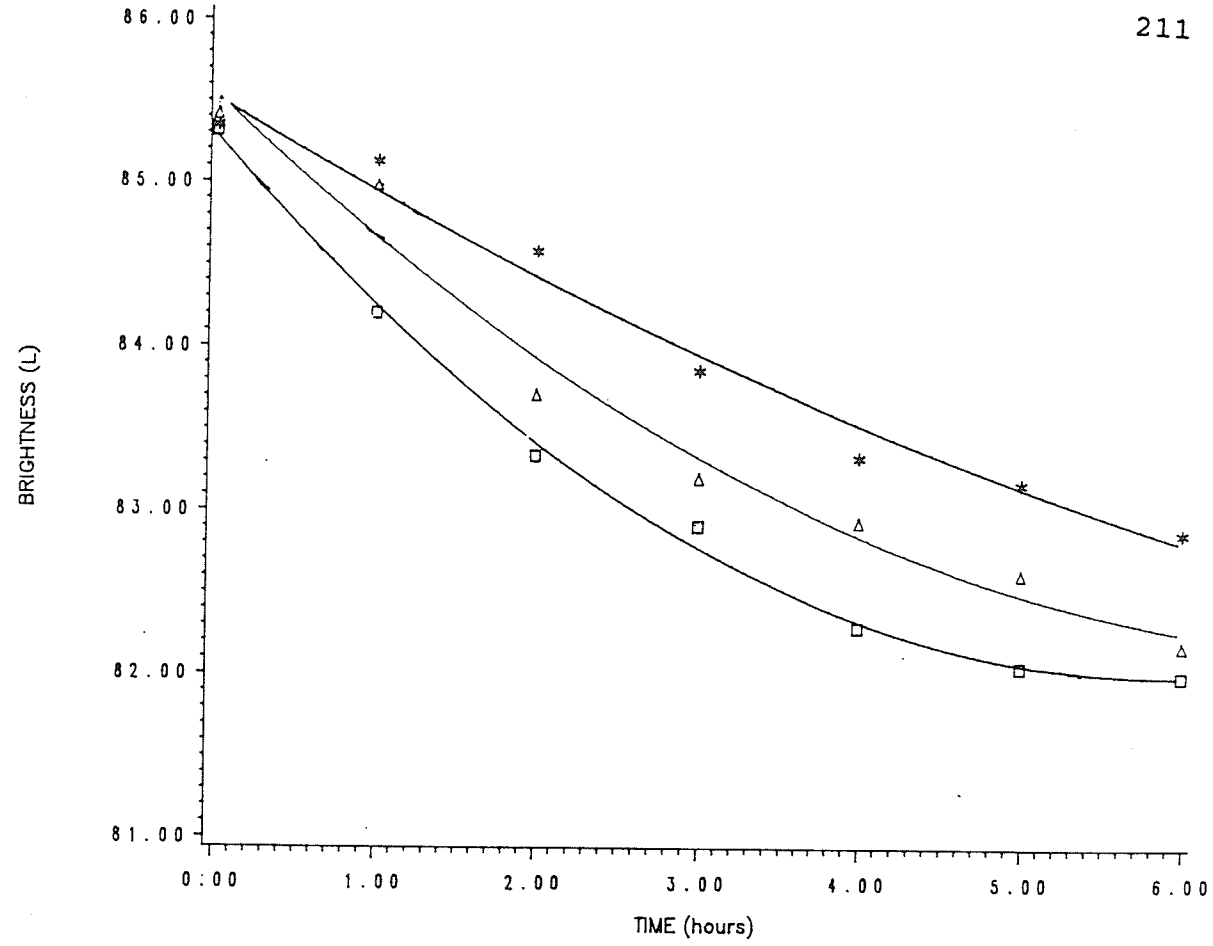
□ 45°C

Figure 54 Influence of Temperature on Water:Flour Paste Yellowness of Katepwa 75% Extraction 1st Clear Flour

✳ 25°C

△ 35°C

□ 45°C


86.00
85.00
84.00
83.00
82.00
81.00
BRIGHTNESS (L)
0:00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

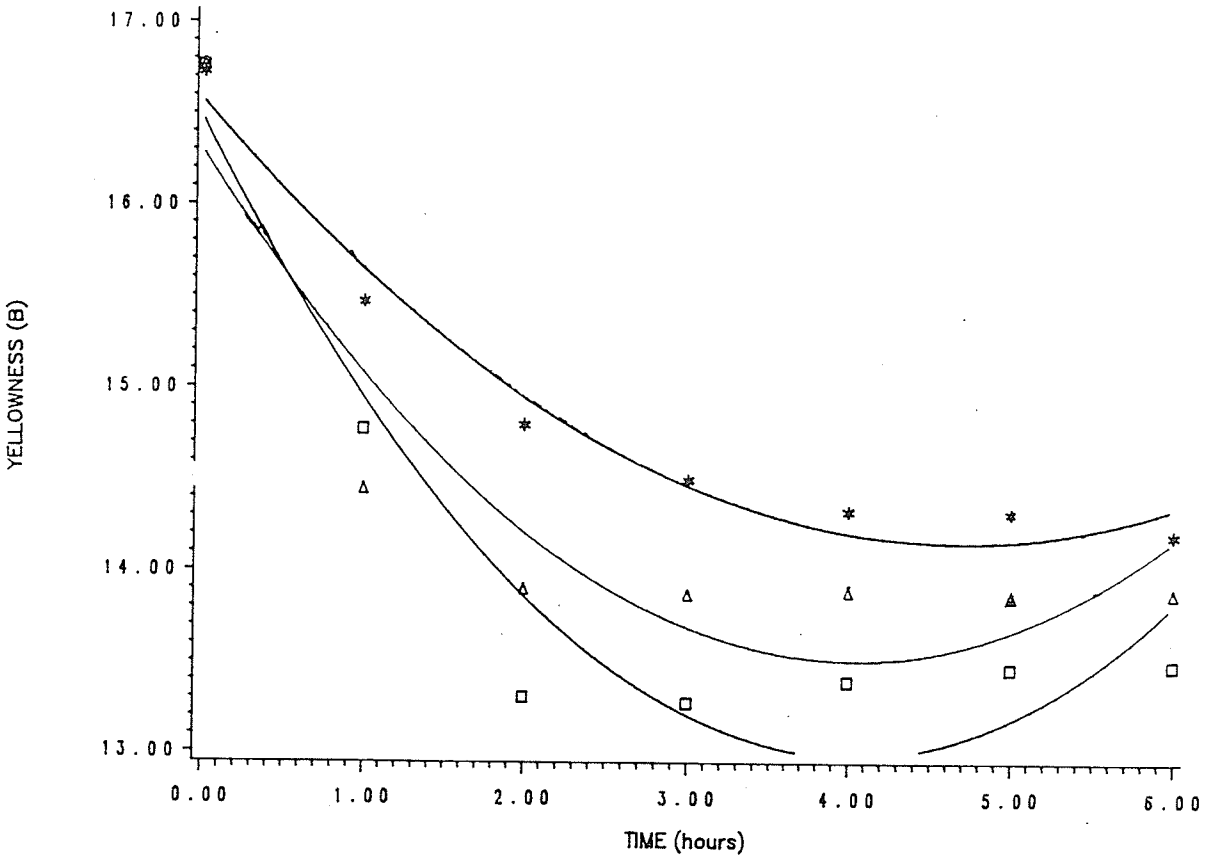

17.00
16.00
15.00
14.00
13.00
YELLOWNESS (B)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

Figure 55 Influence of Temperature on Water:Flour Paste Redness of Katepwa 75% Extraction 1st Clear Flour

✳ 25°C

△ 35°C

□ 45°C

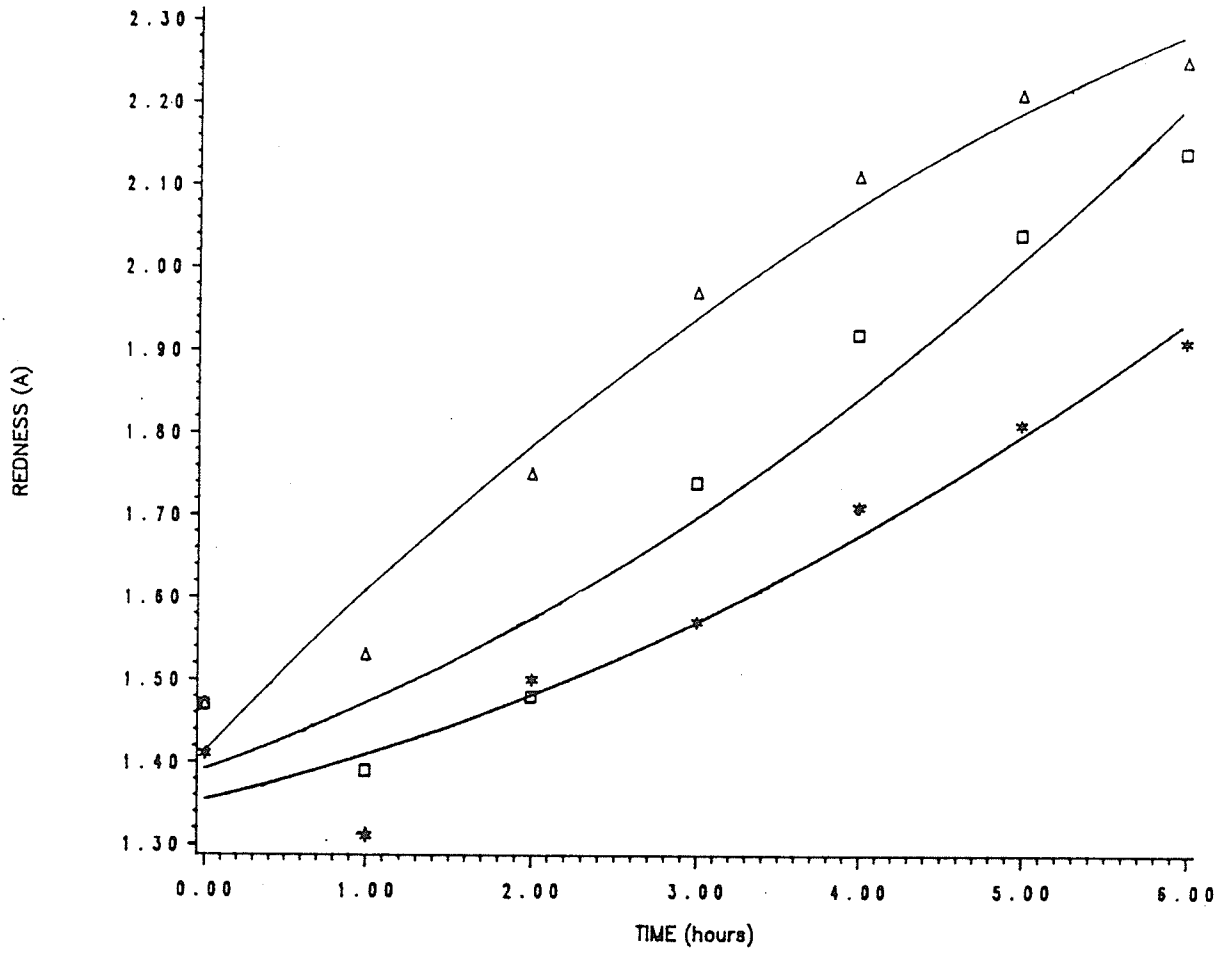

REDNESS (A)
2.30
2.20
2.10
2.00
1.90
1.80
1.70
1.60
1.50
1.40
1.30
0.00
1.00
2.00
3.00
4.00
5.00
6.00
TIME (hours)

Figure 56 The Effect of Temperature on the Total Color Change Observed in Selected 75% Extraction Flours of the Variety Katepwa

P1_75 1st Patent Flour

SG_75 Straight Grade Flour

C1_75 1st Clear Flour

C2_75 2nd Clear Flour

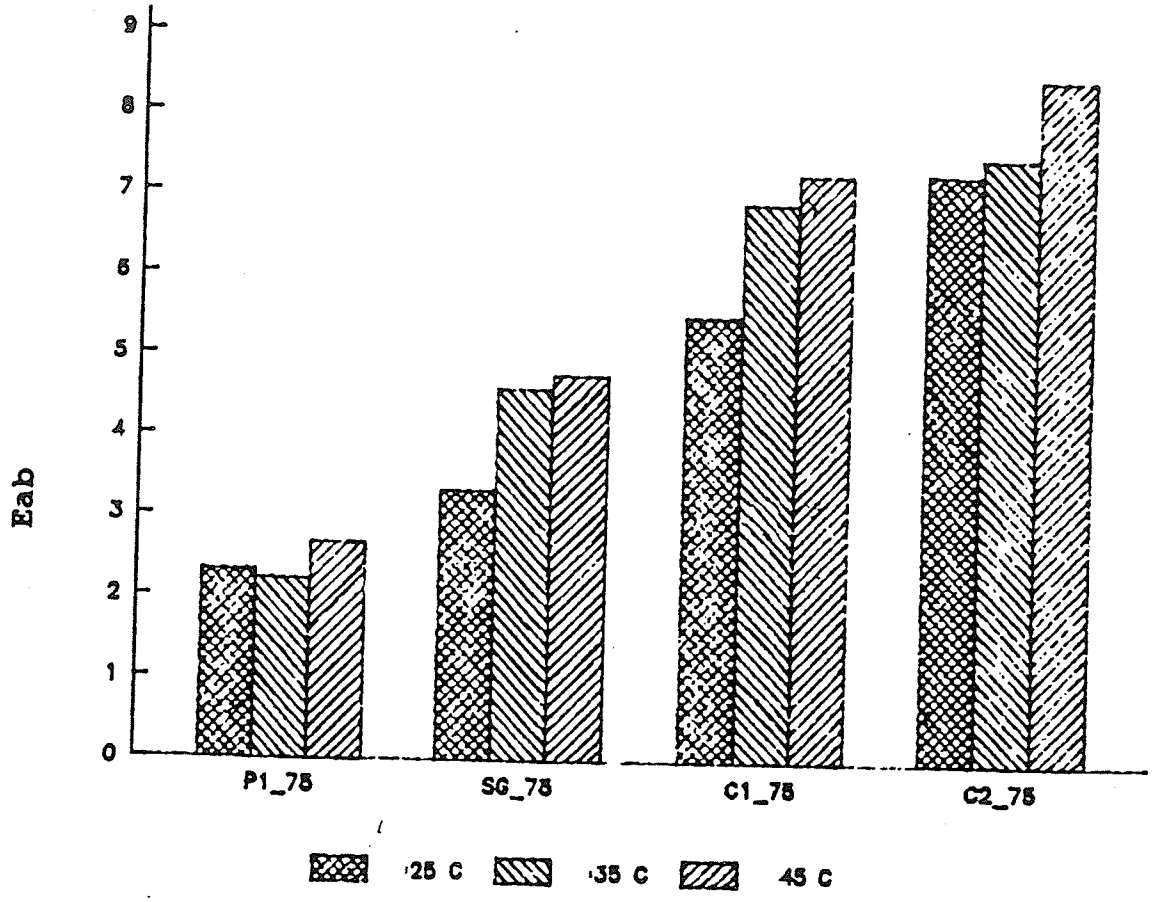
Figure 57 The Effect of Temperature on the Total Color Change Observed in Selected 75% Extraction Flours of the Variety Fielder

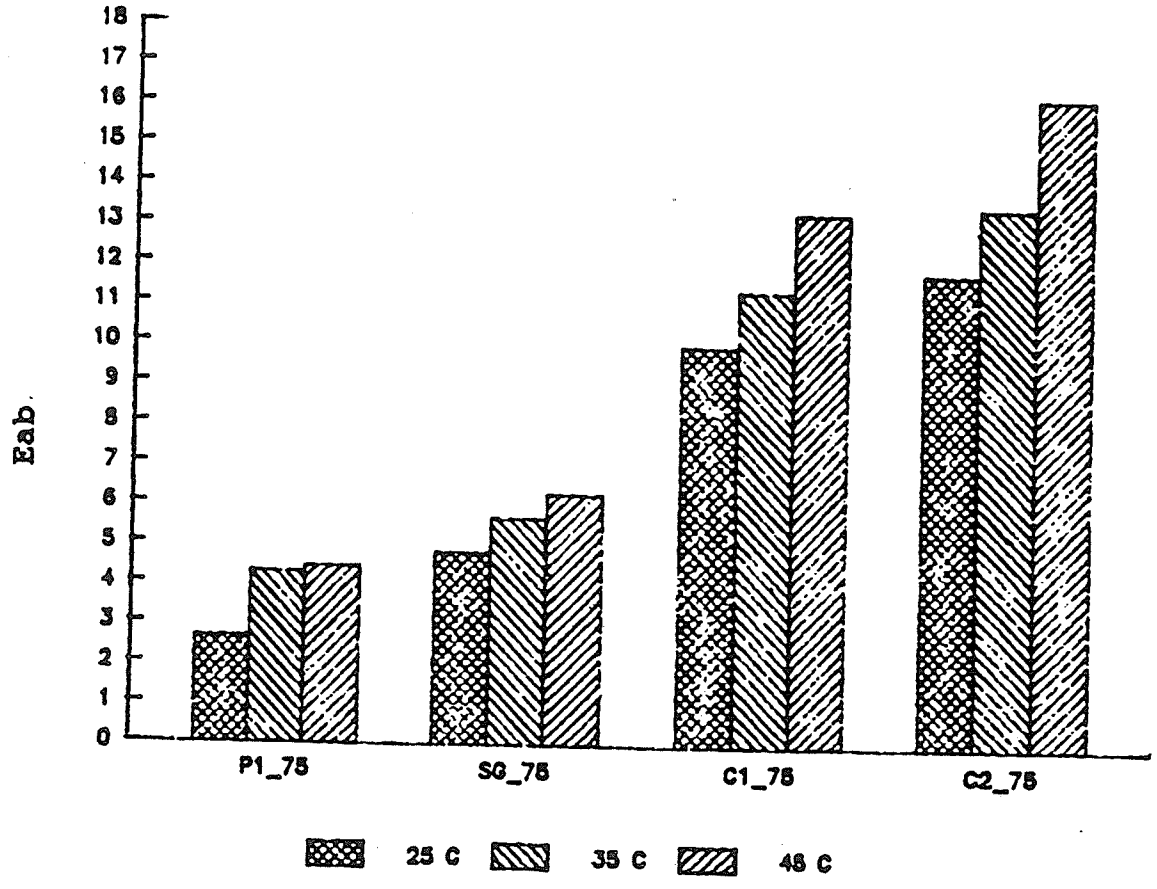
1P_75 1st Patent Flour

SG_75 Straight Grade Flour

C1_75 1st Clear Flour

C2_75 2nd Clear Flour


Eab
0
1
2
3
4
5
6
7
8
9
P1_75
SG_75
C1_75
C2_75
25 C
35 C
45 C


Eab
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
P1_75
SG_75
C1_75
C2_75
25 C
35 C
45 C

color change for each of the flours in Fielder was 1.42 (+/-0.14) while Katepwa yielded 1.30 (+/-0.12).

Review of Figs 58 & 59 which display the reflectance measurements of the 1st patent and 2nd clear flours of both Fielder and Katepwa highlighted the differences observed between varieties. The minimal total color change observed for the Katepwa 1st patent flour relative to its Fielder counterpart is focussed on the region extending from 500-700 nm. Particular attention was drawn to the 500-560 nm region where no difference was visible in the Katepwa flour at both elevated temperatures. Furthermore, there appeared to be a reversal in trends in the region of 400-500 nm. between the two varieties as a function of time. Fielder displayed a decrease in this region, corresponding to the violet-blue ends of the spectrum, while Katepwa showed a noticeable increase. The magnitude of increase in this region approaches the decline observed in the latter yellow-red portion of the spectrum.

The 2nd clear flours, Fig 59 also drew attention to the differences between the two varieties within the violet-blue portion of the spectrum. Fielder did display a significant change in this region while Katepwa did not. Maximum change in reflectance occurred in both varieties from 500 to 700 nm although the magnitude of change for Katepwa was considerably smaller than Fielder.

Examination of the total color change as a function of temperature suggests that the factors responsible are not in

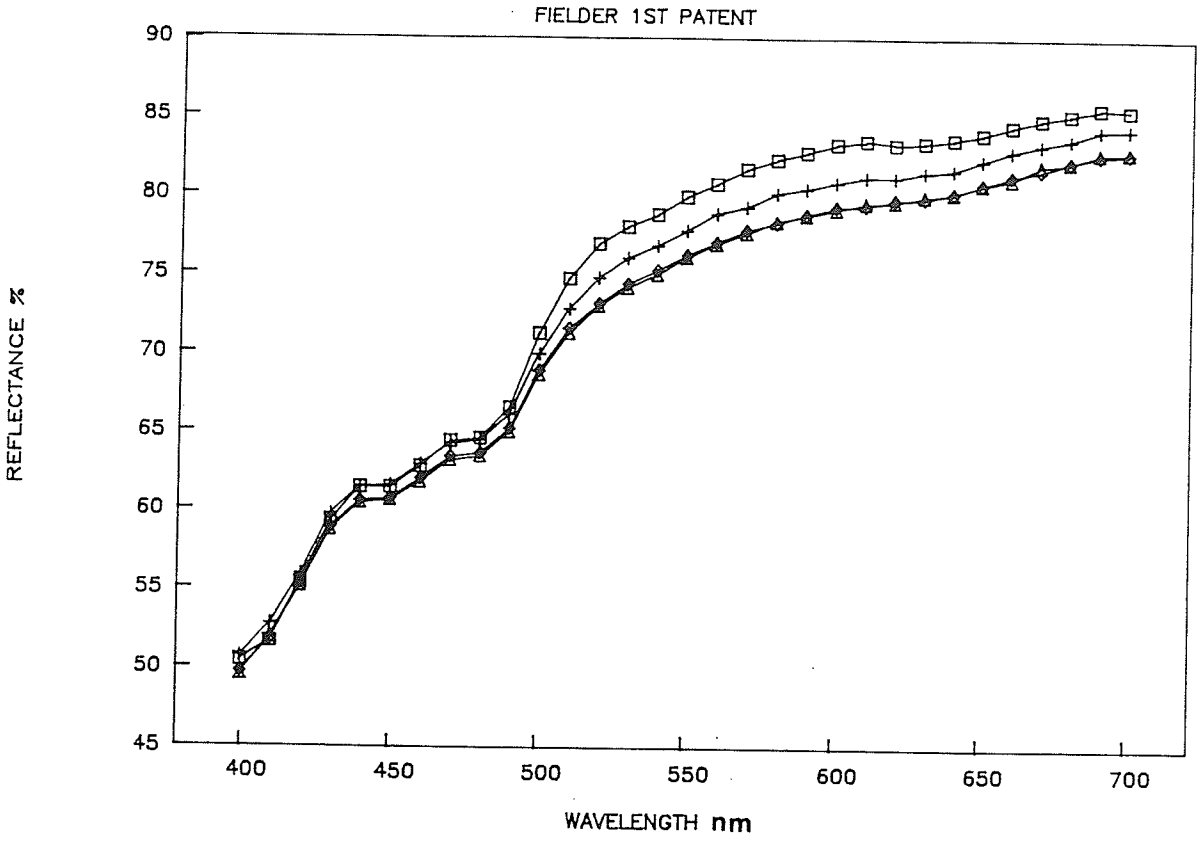
Figure 58 Temperature Influence on the Reflectance Spectrum of the 75% Extraction 1st Patent Flours of Fielder and Katepwa

□ Control Temperature 25°C 0 h

\+ Temperature 25°C 6 h

◇ Temperature 35°C 6 h

△ Temperature 45°C 6 h


FIELDER 1ST PATENT
REFLECTANCE %
90
85
80
75
70
65
60
55
50
45
400
450
500
550
600
650
700
WAVELENGTH nm

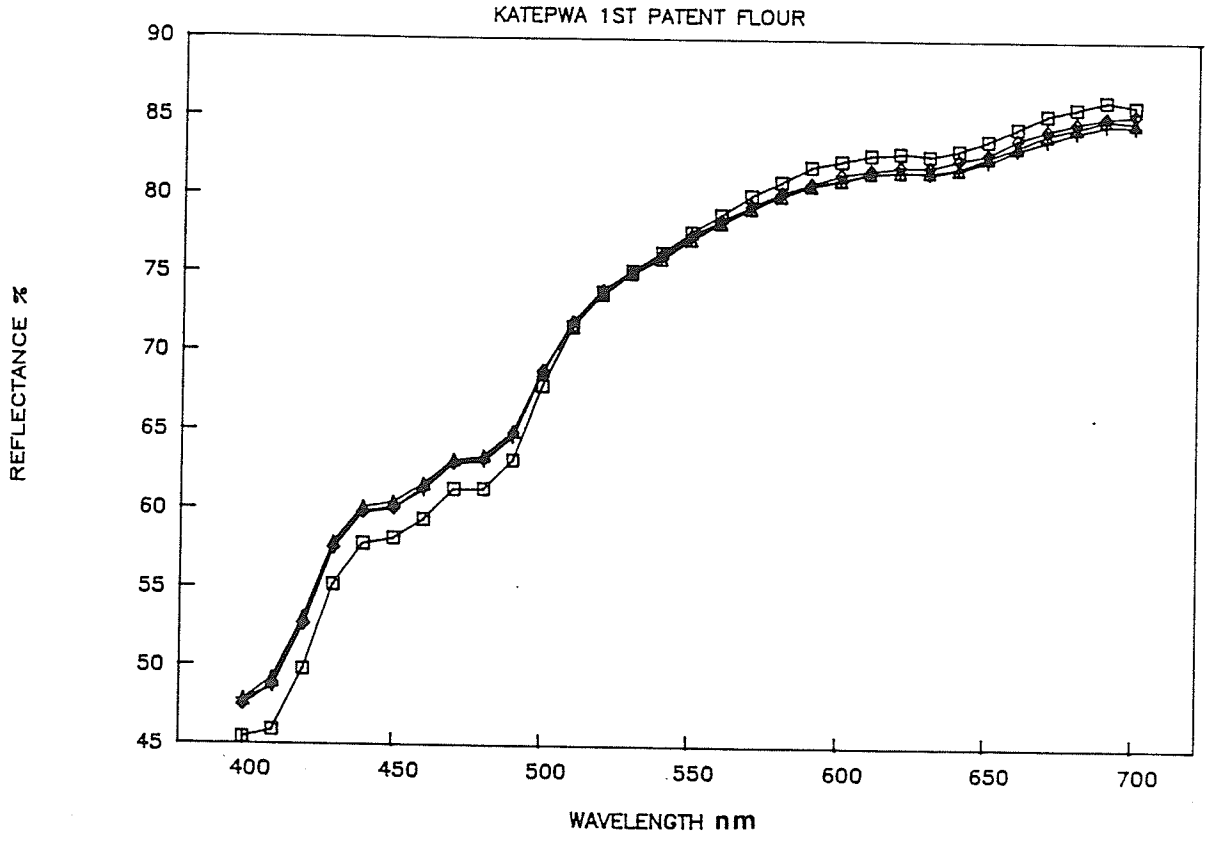

KATEPWA 1ST PATENT FLOUR
REFLECTANCE %
90
85
80
75
70
65
60
55
50
45
400
450
500
550
600
650
700
WAVELENGTH nm

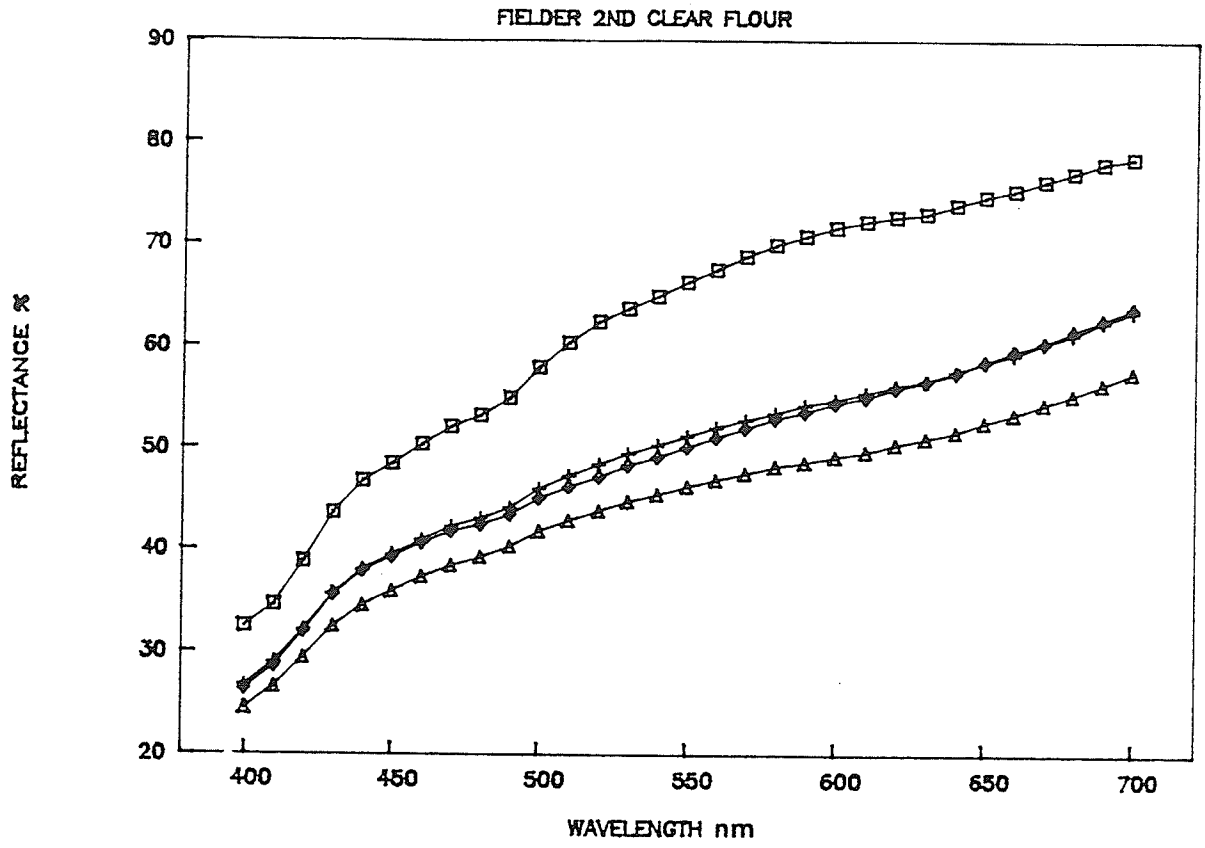
Figure 59 Temperature Effect on the Reflectance Spectrum of 75% Extraction 2nd Clear Flours of Fielder and Katepwa

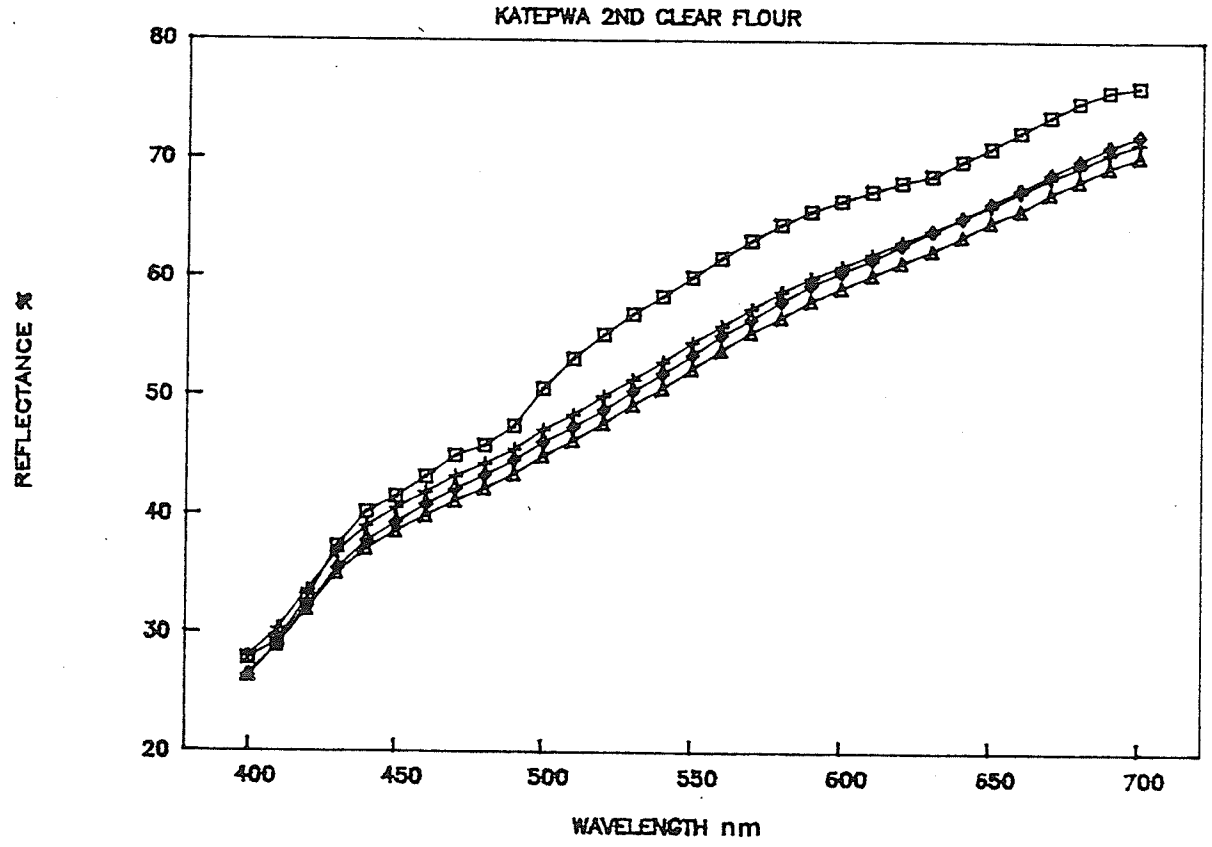
□ Control Temperature 25°C Time = 0 h

\+ Temperature 25°C Time = 6 h

◇ Temperature 35°C Time = 6 h

△ Temperature 45°C Time = 6 h


FIELDER 2ND CLEAR FLOUR
REFLECTANCE %
90
80
70
60
50
40
30
20
400
450
500
550
600
650
700
WAVELENGTH nm


KATEPWA 2ND CLEAR FLOUR
REFLECTANCE %
80
70
60
50
40
30
20
400
450
500
550
600
650
700
WAVELENGTH nm

a free suspension. If such were the case, the Q10 rule would be observed whereby the reaction rate doubles for each 10°C increase. The factor/s influencing color change appear to be consistent for both varieties as the average percentage increase from 25-45°C for each variety's flours were not significantly different. The lack of a free suspension of components is consistent with the opinion that polyphenol oxidase is a membrane bound enzyme in wheat (Marsh and Galliard,1986). Furthermore, the majority of natural phenolic acid substrates for the enzyme are also in a bound state. Under such conditions, increasing the reaction temperature would not allow the Q10 rule to be observed as physical restraint would dominate.

The concern for auto-oxidation of the phenolic acids being solely responsible for the color change is extremely small based upon two observations. The change in total color between 25°C and 35°C is considerable in Fielder's 1st patent flour. However, between 35°C and 45°C the color does not change appreciably. It would be suspected that if auto-oxidation was occurring, the Q10 rule would be detected at the higher temperatures. A similar observation was noted for Katepwa's straight grade flour. The second point for dismissing auto-oxidation as the sole source of color change was encountered when comparing the total color change, within the straight grade flours, between varieties. Examination of each variety's total color change, from 25 to 45°C, yielded an identical value of 1.44 color units.

Fielder, however, had approximately three times the free phenolic acid content of Katepwa within this flour. If auto-oxidation was solely responsible for color it would be anticipated that Fielder's total color change would be substantially larger than Katepwa.

4.06.6 Influence of Phenolic Addition on Color Production

In order to address the involvement of phenolic acids in flour paste color production a series of six phenolic acids, at 200 ppm, were added to Katepwa 1st clear 75% extraction flours. Each sample was analyzed in duplicate with the average values being used to establish the best fitting regression and the 95% confidence limits. The influence of these acids on the HunterLab color components were monitored over 4 h and the results can be seen in Figs 60-62. The most readily apparent observation was the magnified effect of caffeic acid relative to the other acids on all aspects of color. In each of the three Hunter lab color components , caffeic acids influence was noticeably different than the other acids. In each case these differences were apparent within 1 h of mixing.

Flour paste brightness appeared to be the least influenced by the addition of the 200 ppm phenolic acids. Only caffeic acid showed an immediate divergence from the control flour while both coumaric and sinapic acids displayed discrete differences after a 2 h period.

Figure 60 Influence of Phenolic Acid Addition (200 ppm) on Water:Flour Paste Brightness of Katepwa 75% Extraction 1st Clear Flour

A.

* Caffeic Acid

△ Ferulic Acid

□ Vanillic Acid

○ Control

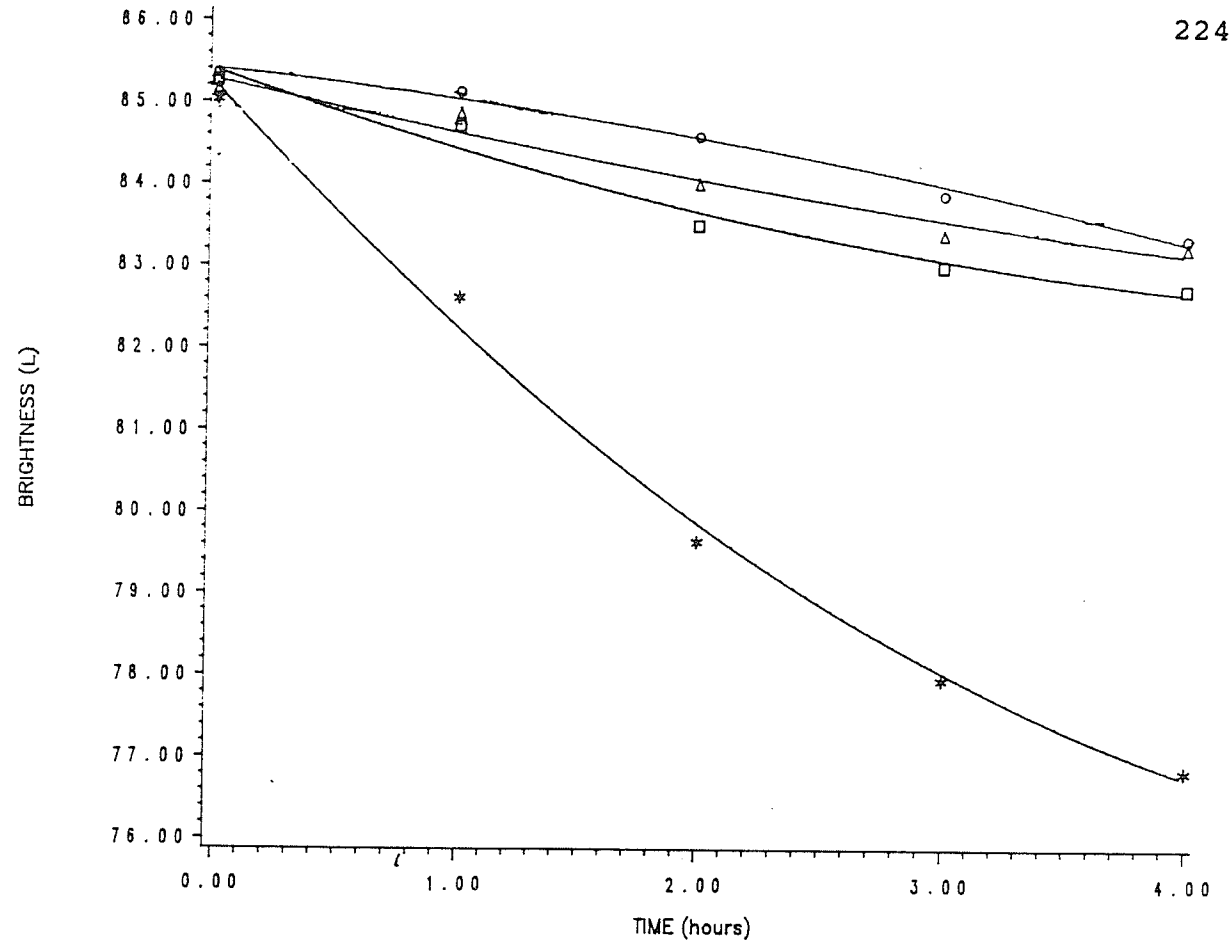
B.

* Sinapic Acid

△ Coumaric Acid

□ Syringic Acid

○ Control


86.00
85.00
84.00
83.00
82.00
81.00
80.00
79.00
78.00
77.00
76.00
BRIGHTNESS (L)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

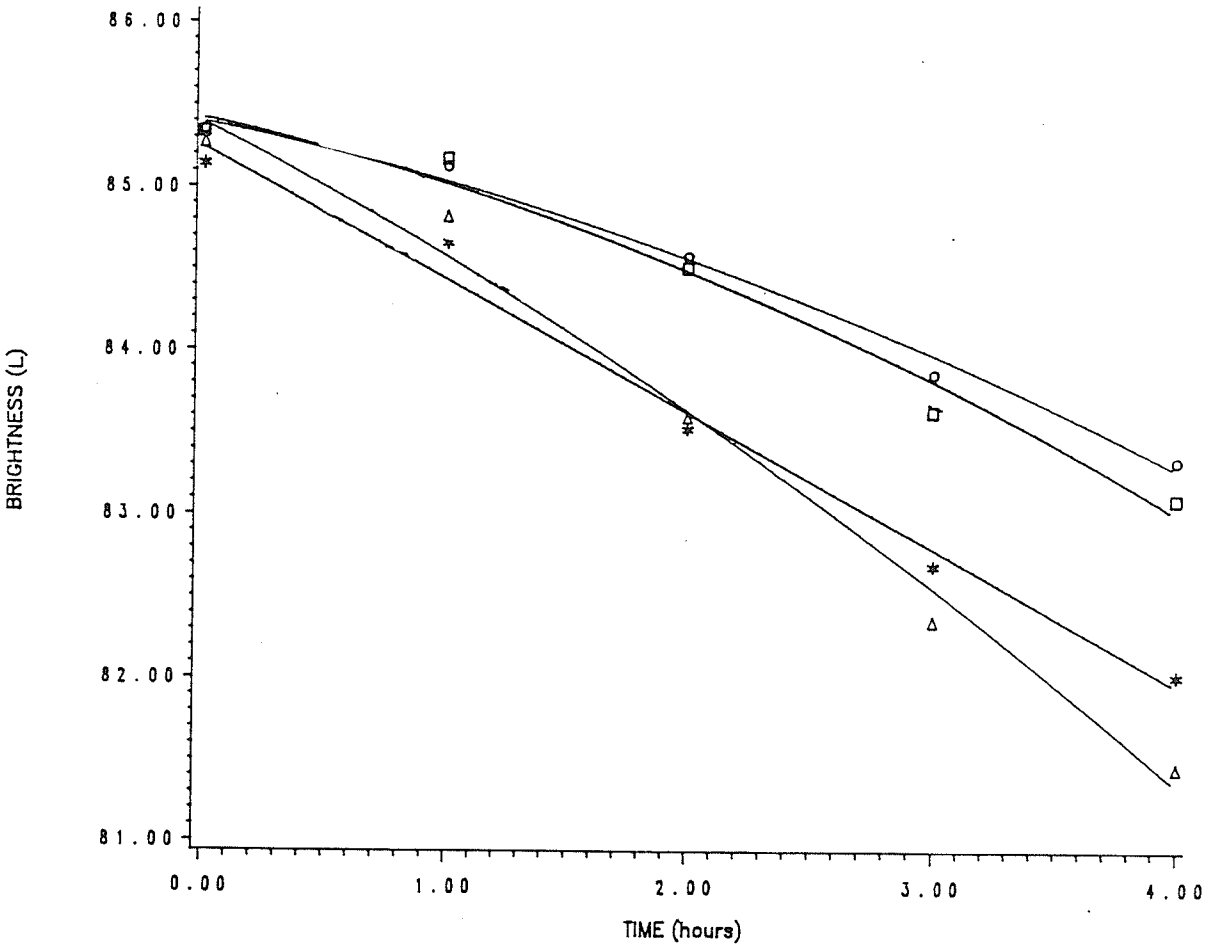

86.00
85.00
84.00
83.00
82.00
81.00
BRIGHTNESS (L)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

Figure 61 Influence of Phenolic Acid Addition (200 ppm) on Water:Flour Paste Yellowness of Katepwa 75% Extraction 1st Clear Flour

A.

* Caffeic Acid

△ Ferulic Acid

□ Vanillic Acid

○ Control

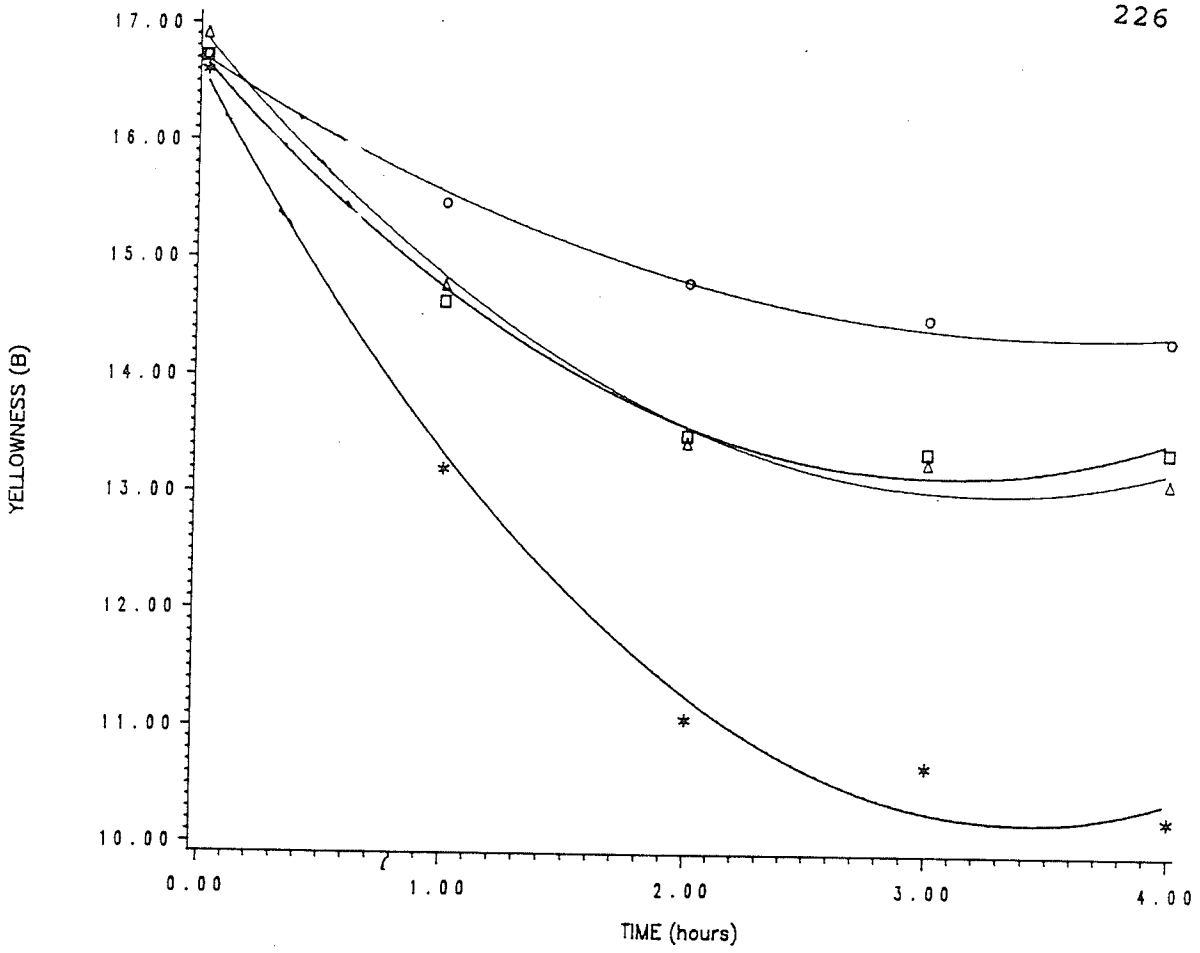
B.

* Sinapic Acid

△ Coumaric Acid

□ Syringic Acid

○ Control


17.00
16.00
15.00
14.00
13.00
12.00
11.00
10.00
YELLOWNESS (B)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

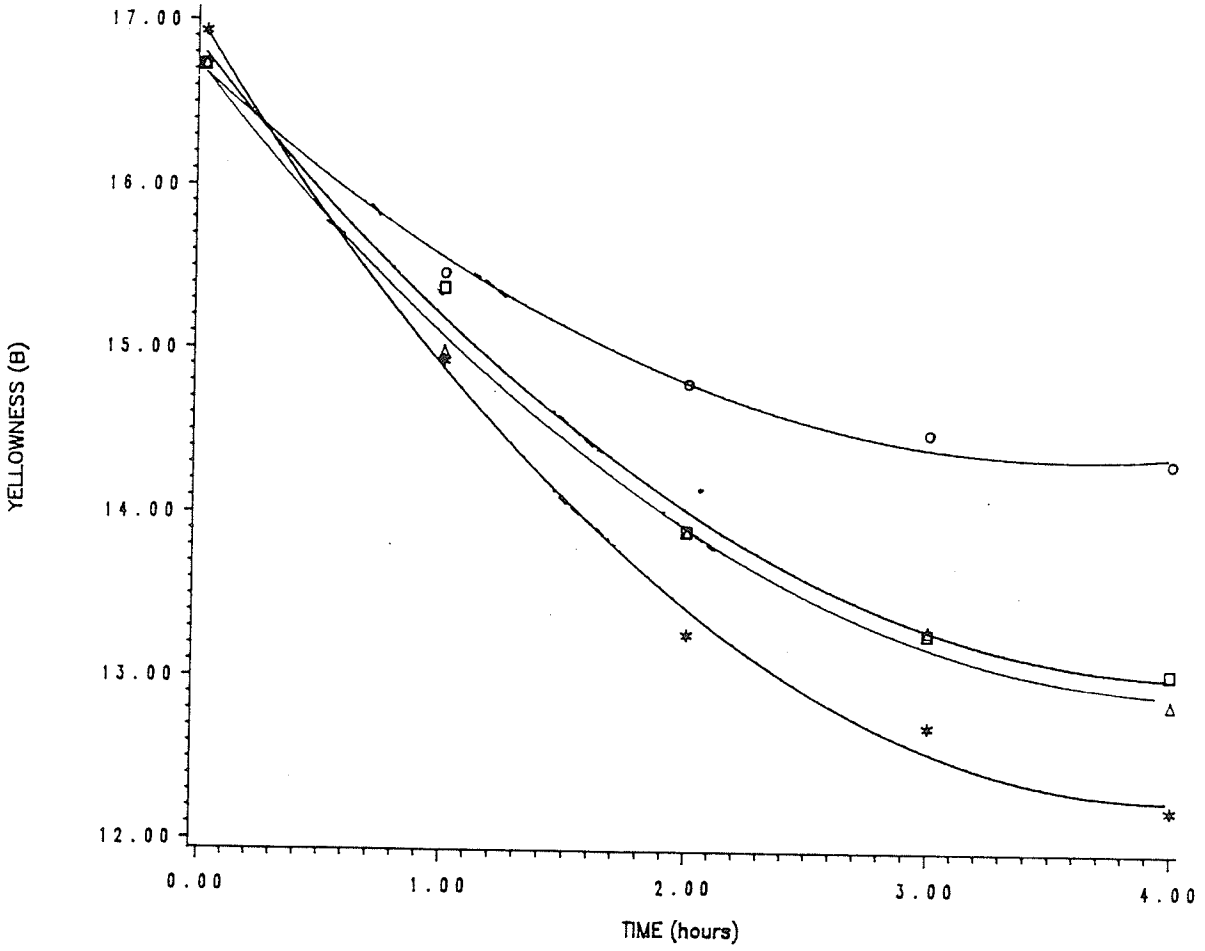

17.00
16.00
15.00
14.00
13.00
12.00
YELLOWNESS (B)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

Figure 62 Influence of Phenolic Acid Addition (200 ppm) on Water:Flour Paste Redness of Katepwa 75% Extraction 1st Clear Flour

A.

* Caffeic Acid

△ Ferulic Acid

□ Vanillic Acid

○ Control

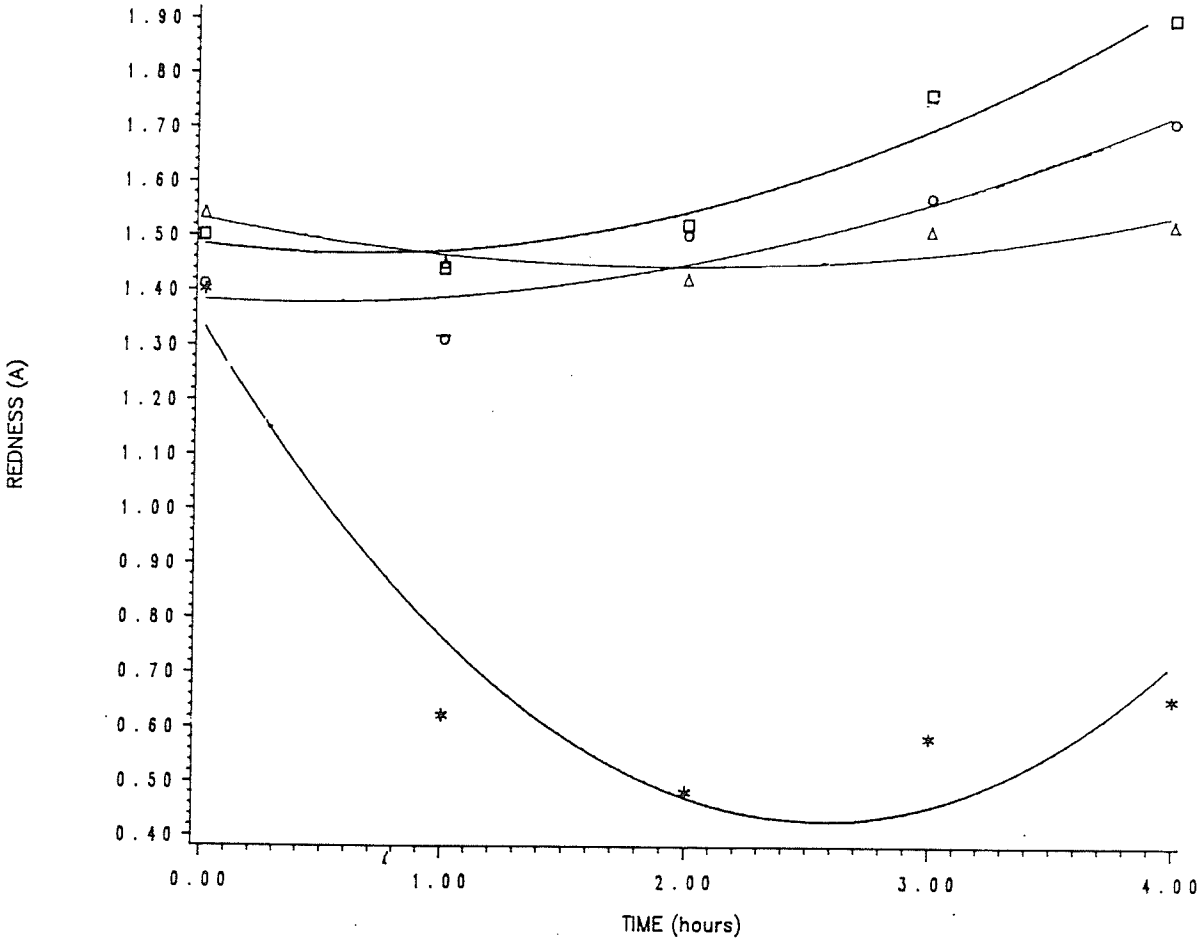
B.

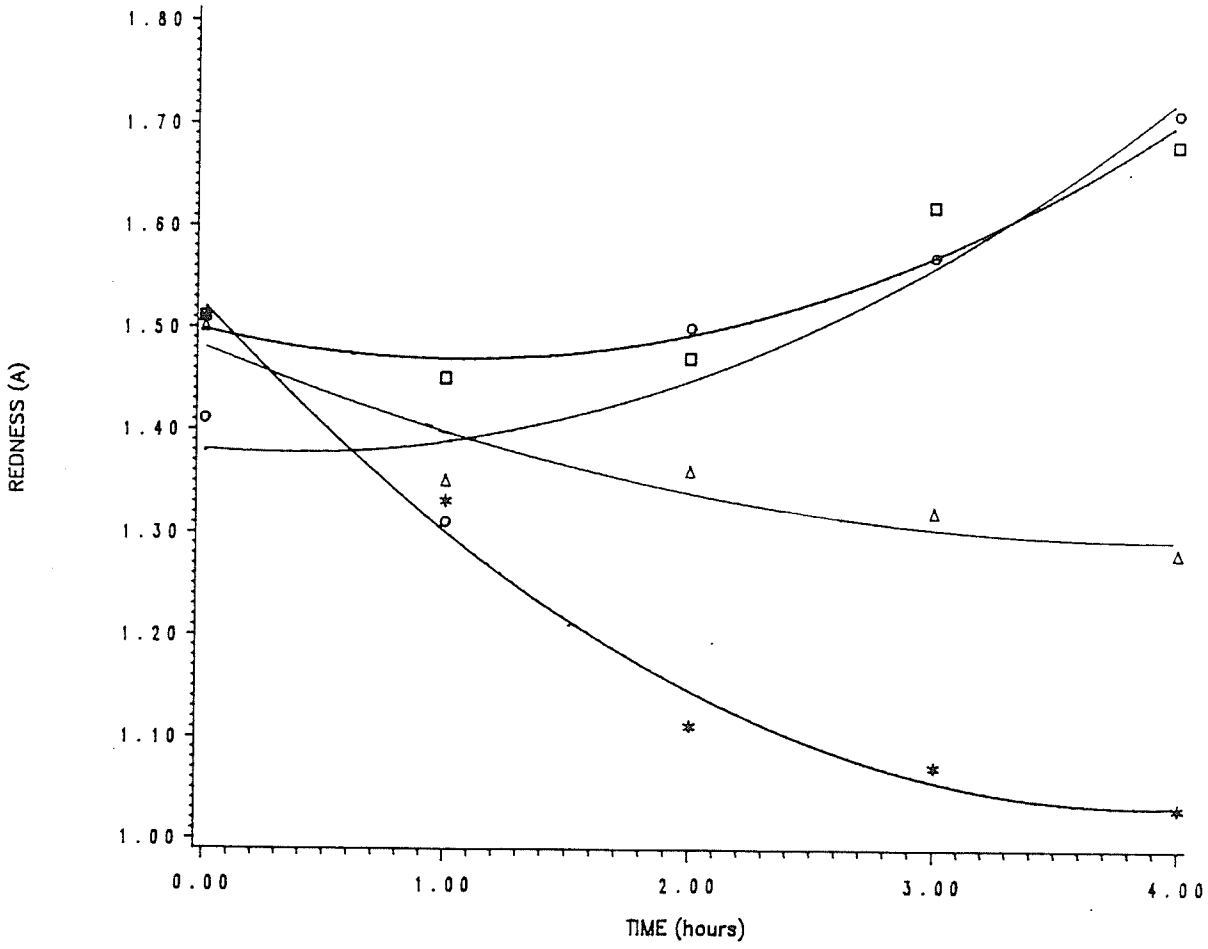
* Sinapic Acid

△ Coumaric Acid

□ Syringic Acid

○ Control


REDNESS (A)
1.90
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.00
1.00
2.00
3.00
4.00
TIME (hours)


REDNESS (A)
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.00
1.00
2.00
3.00
4.00
TIME (hours)

The remaining phenolic acids did not discretely separate from the control flours.

Paste redness highlighted certain differences between the various acids. A strong decrease was observed in the paste redness for caffeic acid within the first hour. Sinapic acid also decreased in redness, to a lesser extent than caffeic acid, over time and was different from the control flour which displayed an increase. Ferulic, syringic, and vanillic acids appeared to have minimal influence on the red color component in this flour as their respective lines overlapped those of the control flour.

Flour paste yellowness was affected by all of the phenolic acids investigated. In each case, discrete decreases in paste yellowness from the control were observed within 2 h of addition. Caffeic acid , followed by sinapic acid had the greatest decline in this color index. The remaining acids, ferulic, vanillic, syringic and coumaric all decreased in yellowness by approximately equal amounts.

Examination of selected acids' paste reflectance spectrums over the time period highlighted the differences between the various acids shown in Fig 63. Caffeic acid diverges from the control with a general decrease in reflectance along the entire length of the spectrum. Only caffeic acid was found to differ from the control in the violet to blue region of the spectrum, 400-490 nm. Sinapic acid also noticeably deviates from the control flour over

Figure 63 Influence of Phenolic Addition (200 ppm) on the Reflectance Spectrum of 75% Extraction 1st Clear Flours of Katepwa

A.

□ Control Time 0 h

\+ Control Time 4 h

◇ Ferulic Acid 4 h

△ Sinapic Acid 4 h

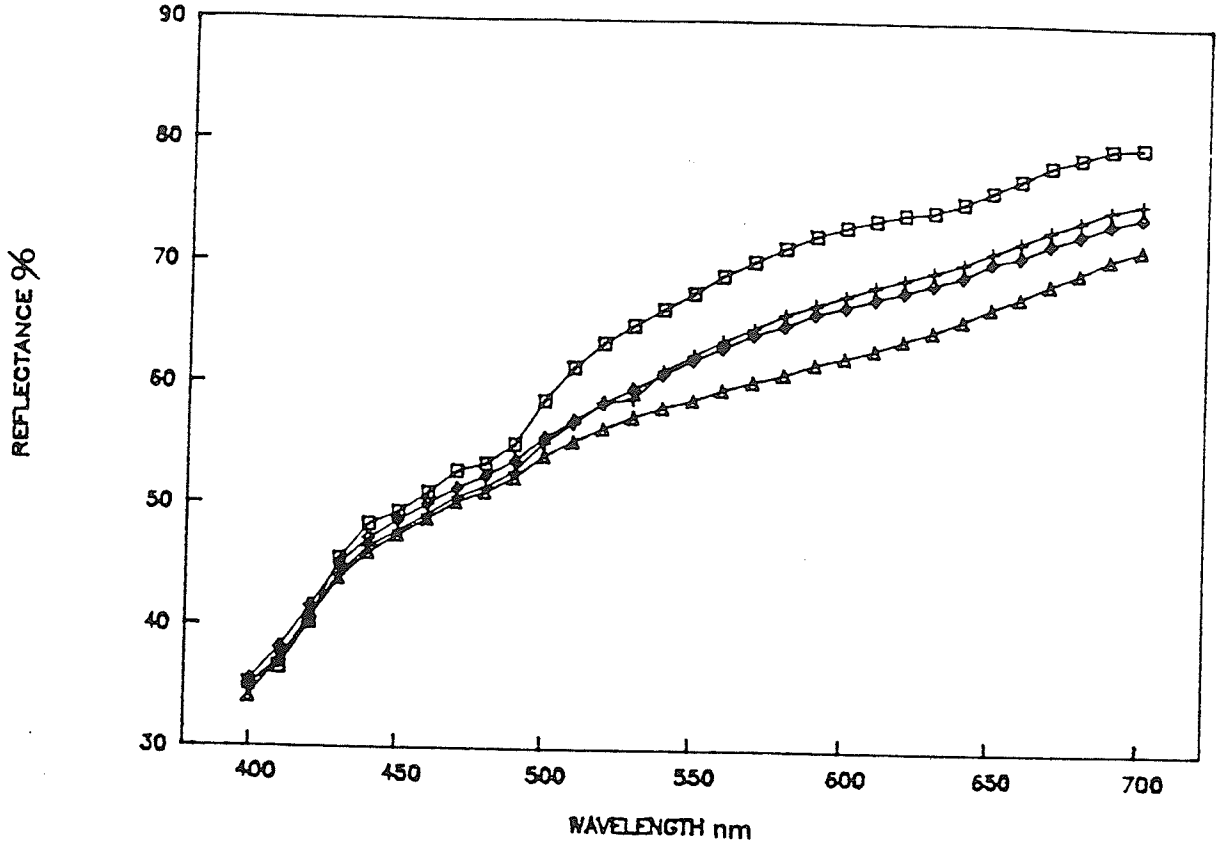
B.

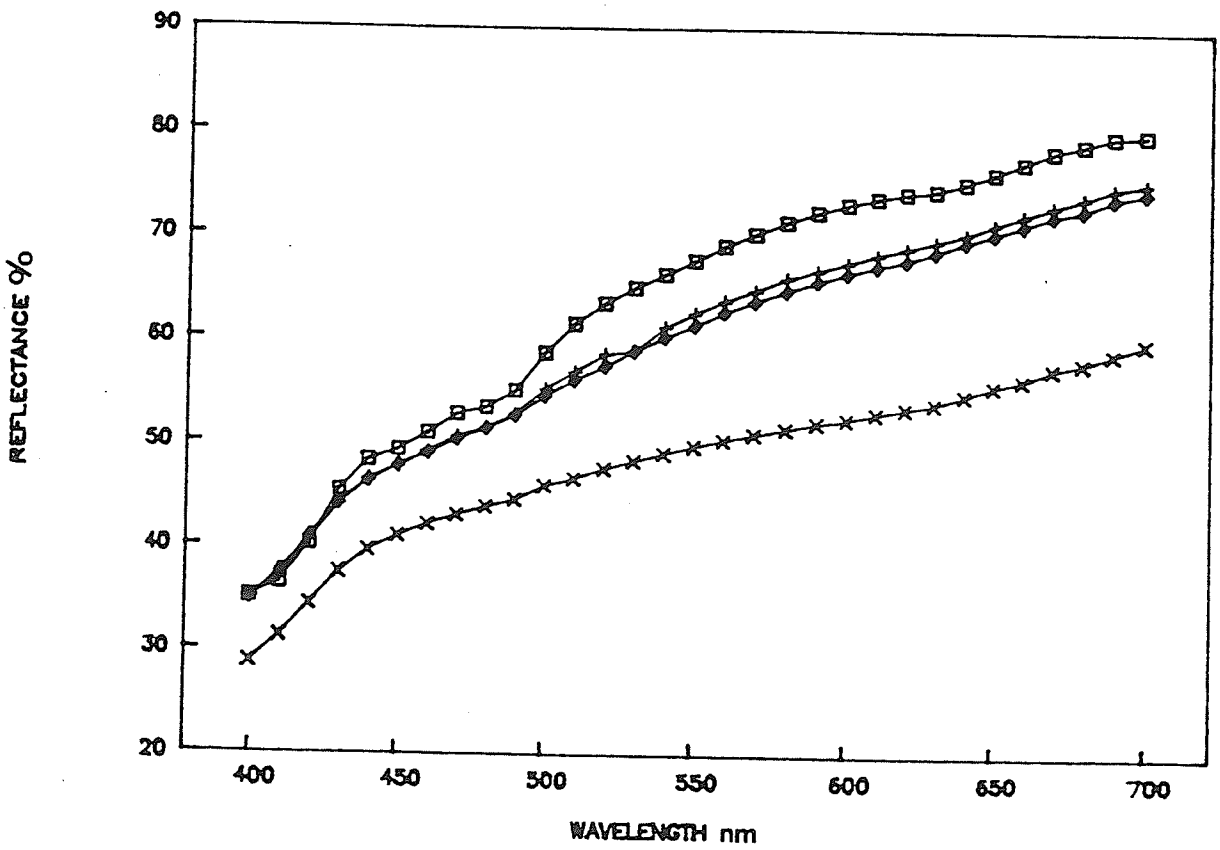
□ Control Time 0 h

\+ Control Time 4 h

◇ Vanillic Acid 4 h

x Caffeic Acid 4 h


REFLECTANCE %
90
80
70
60
50
40
30
400
450
500
550
600
650
700
WAVELENGTH nm


REFLECTANCE %
90
80
70
60
50
40
30
20
400
450
500
550
600
650
700
WAVELENGTH nm

the 4 h time period starting at the beginning of the green region, 500 nm. The largest difference for sinapic acid versus the control occurs in the portion of 580-620 nm. corresponding to the orange portion of the spectrum. Ferulic and vanillic acids display only slight declines in reflectance compared to the control above 550 nm and this difference remains relatively constant over the remaining portion of the spectrum.

Selected phenolic acids were also added to both Katepwa 1st patent and 2nd clear 75% extraction flours to determine their influence on different flours. In both cases, the uniqueness of caffeic acid relative to the other phenolics was highlighted. The 1st patent flour's brightness and redness was only influenced by caffeic acid. Differences in yellowness, except for caffeic acid, decreased only minimally from the control. These minor differences were clearly seen in the reflectance wavelength spectrums Figs 64-65. Investigation of the phenolic acid addition to the 2nd clear flour did highlight the influence of sinapic acid on brightness over an extended period. Other than caffeic acid, only sinapic acid produced a noticeably different decline in paste brightness by 4 h . Sinapic acid and vanillic acid also displayed a somewhat divergent influence on paste redness. While caffeic acid caused the 2nd clear flour's redness to decline relative to the control, both sinapic and vanillic produced an observable increase in the red, a^*, value after three h . Sinapic

Figure 64 Influence of Phenolic Acid Addition (200 ppm) on the Reflectance Spectrum of 75% Extraction Katepwa 1st Patent Flour

A.

□ Control Time 0 h

\+ Control Time 4 h

◇ Ferulic Acid 4 h

△ Sinapic Acid 4 h

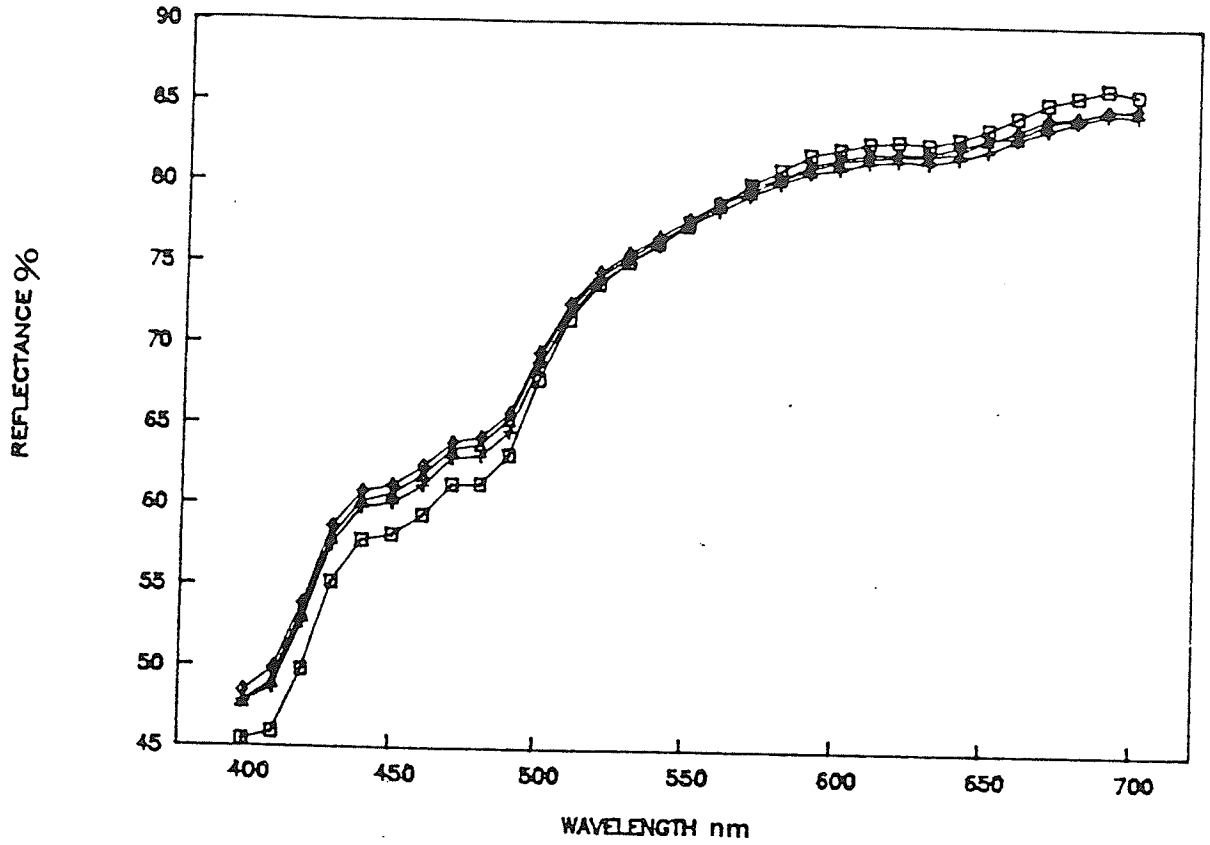
B.

□ Control Time 0 h

\+ Control Time 4 h

◇ Vanillic Acid 4 h

x Caffeic Acid 4 h


REFLECTANCE %
90
85
80
75
70
65
60
55
50
45
400
450
500
550
600
650
700
WAVELENGTH nm

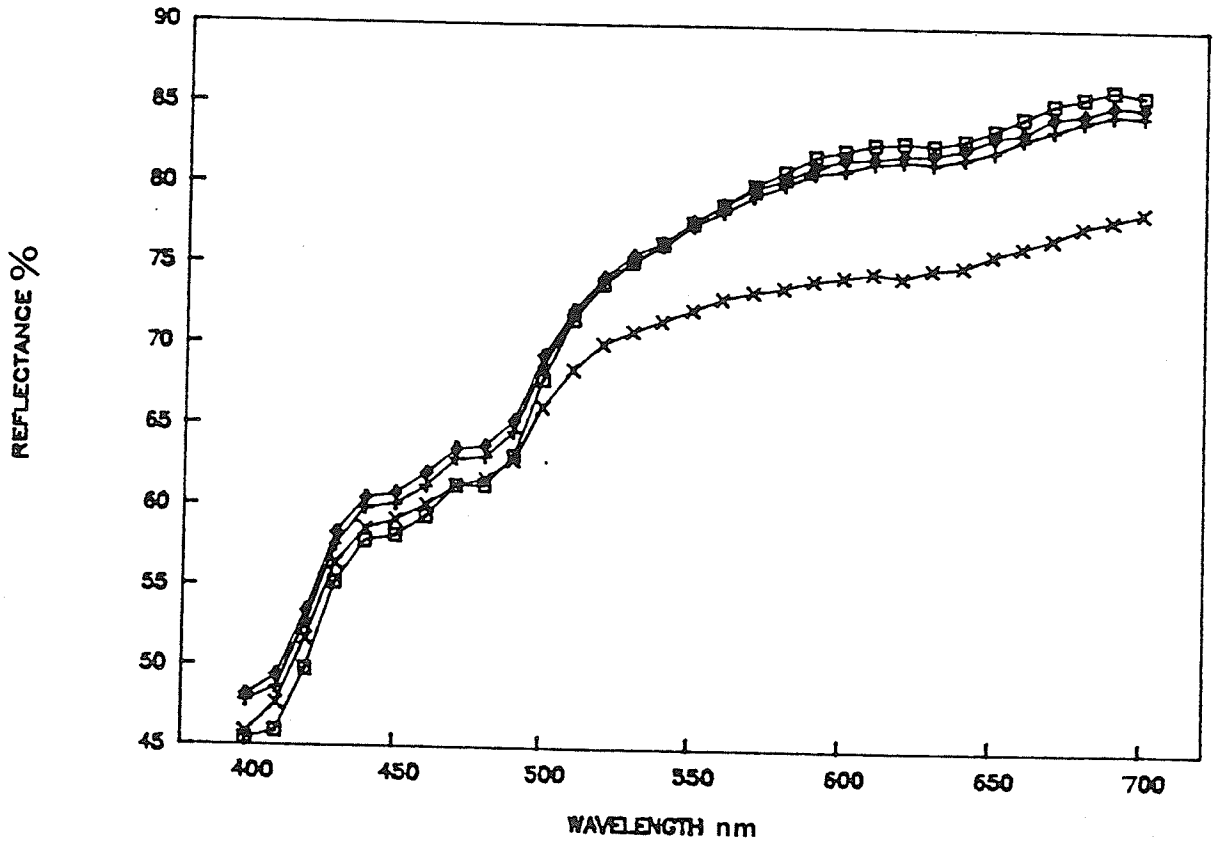

REFLECTANCE %
90
85
80
75
70
65
60
55
50
45
400
450
500
550
600
650
700
WAVELENGTH nm

Figure 65 Influence of Phenolic Acid Addition (200 ppm) on the Reflectance Spectrum of 75% Extraction Katepwa 2nd Clear Flour

A.

□ Control Time 0 h

\+ Control Time 4 h

◇ Ferulic Acid 4 h

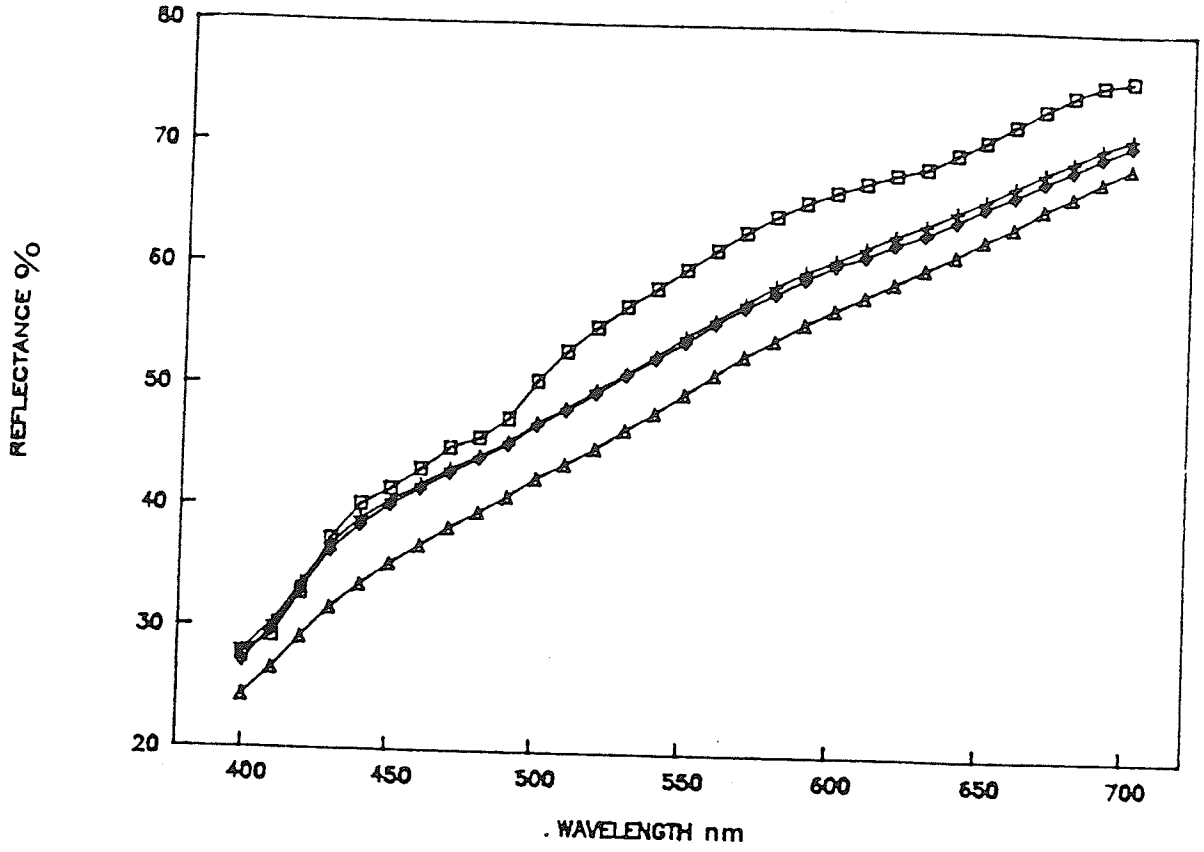
△ Sinapic Acid 4 h

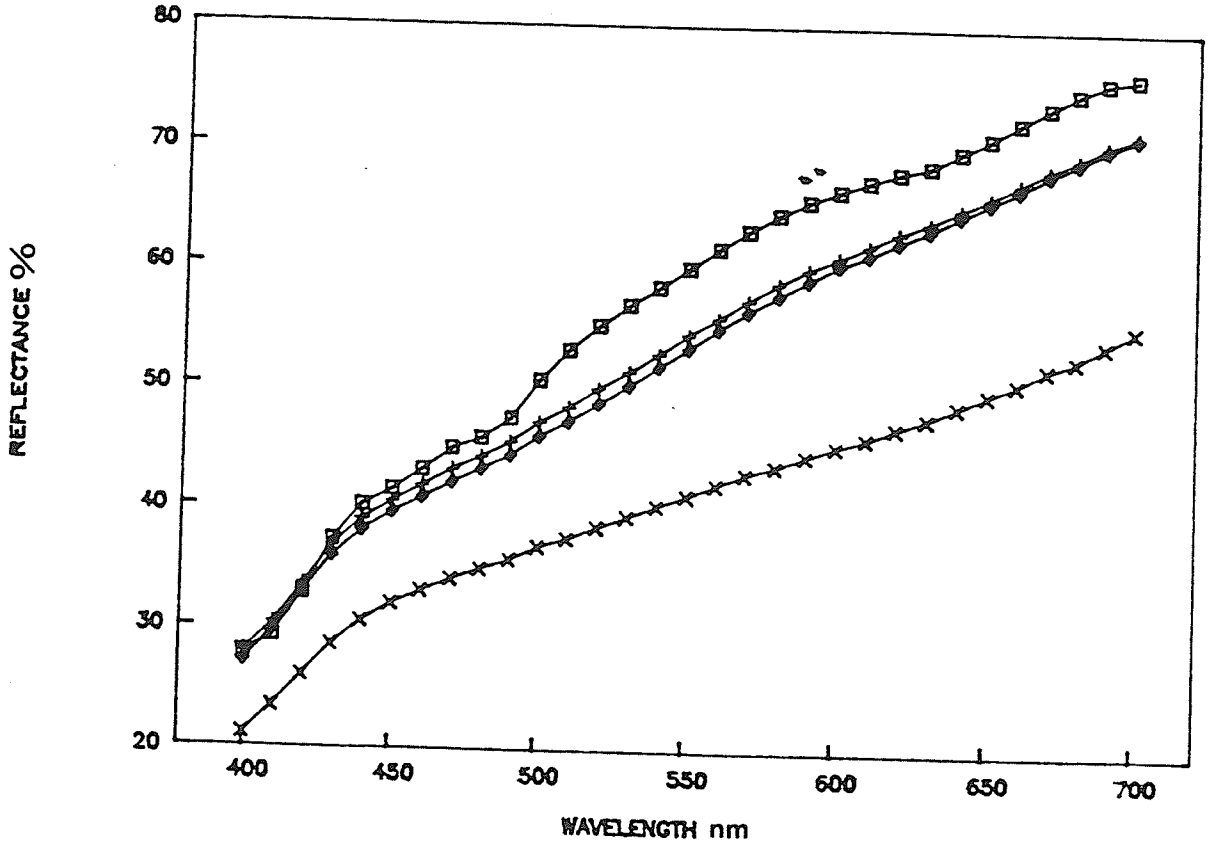
B. □ Control Time 0 h

\+ Control Time 4 h

◇ Vanillic Acid 4 h

x Caffeic Acid 4 h


REFLECTANCE %
80
70
60
50
40
30
20
400
450
500
550
600
650
700
WAVELENGTH nm


REFLECTANCE %
80
70
60
50
40
30
20
400
450
500
550
600
650
700
WAVELENGTH nm

acid's increase in red color production from 3 to 4 h was particularly abrupt. This sharp increase was observed to corresponded with a similar decline in the paste brightness. Only caffeic acid, however, invoked a response in the 2nd clear paste yellowness. This was in contrast to that observed in the 1st clear flour where all acids caused a substantial decline in yellowness.

Review of the 2nd clear flour's paste reflectance spectrum in response to the added free phenolic acids did highlight that in this flour sinapic acid as well as caffeic acid, did diverge from the control flour over the entire spectrum. The extent of this difference however was not as great as that seen for caffeic acid.

Calculation of the total color change in the respective flours upon addition of the free acids over the 4 h period can be seen in Table 15. Only caffeic acid had a dramatic influence in total color change in the 1st patent flour, a three fold increase over that seen in the control. In the 1st clear flour all of the phenolic acids displayed a greater total color change than the control. Sinapic acid, followed closely by coumaric acid, were second to caffeic acid in terms of total change. However, in the 2nd clear flour, values for sinapic, caffeic and vanillic acids are all larger than that of the control but ferulic acid was of equivalent magnitude.

Closer examination of the relative changes highlighted an important facet. The ratio of total increase from the 1st

Table 15

Influence of Free Phenolic Acid Addition on Total Color Change Within 4 Hours Using Katepwa 75% Extraction Flours

Stream	Ferulic Acid	Sinapic Acid	Caffeic Acid	Vanillic Acid	Control
1st Patent	2.78	2.77	6.91	2.32	2.08
1st Clear	5.76	8.32	15.32	6.28	4.72
2nd Clear	5.78	10.11	16.30	6.91	5.86

patent to 1st clear for both the control samples and phenolic additions were of the same magnitude. The control ratio was 2.26 while the phenolic acid ratios ranged from 2.0 to 3.0. The same was found to be true when moving from the 1st clear to the 2nd clear samples. The corresponding 2nd clear:1st clear total color ratio for the control flour was 1.24 yet the phenolic acids were slightly lower extending from 1.00 to 2.22. This would suggest that there is a factor present in each flour responsible for color production which interacts with the phenolic compounds. The magnitude of the total color change remains a function of the particular phenolic acid as the total color production for ferulic acid in the 1st clear flour is only 5.76 compared to 15.32 for caffeic acid. However on a ratio basis to the 1st patent flour, ferulic's increase of 2 fold is equivalent to that displayed by caffeic, 2.22, and the control 2.26.

Auto-oxidation of the phenolic acids being solely responsible for the color change can be eliminated based upon the following grounds. The phenolic acid analysis indicated that the 200 ppm added to the flours was well in excess of the naturally occurring soluble bound and free acids. It would therefore be expected that such acids would be the most susceptible to auto-oxidation. Subtraction of the control value from their respective 1st patent and 1st clear flour phenolic pastes yields values which were not equivalent. Caffeic acid yields a "corrected" change of

4.83 color units in the patent flour versus 10.60 units in the 1st clear flour. Sinapic acid, also has "corrected" total color changes of 0.69 and 3.60 in its corresponding flours. If auto-oxidation was solely responsible for color production the "corrected" color change in both flours would be the same utilizing the same acid. The aspect of differential oxidation of the acids was removed as a factor on the basis that the ratio between the two acids in the 1st patent flour was not maintained in the 1st clear flours. Finally, the concern that an unknown factor was involved as well in influencing color might be ruled out based on the ratio between flours across the acids being different. The ratio for total "corrected" color change between the 1st patent and 1st clear for caffeic acid was 2.19 while sinapic acid had a value of 5.22.

Support for the phenolic acid concentration being involved in color production was observed when the total color change versus phenolic acid concentration was plotted in Fig. 66 Caffeic, sinapic, vanillic and ferulic acids all displayed linear increases in their respective total color production as a function of phenolic acid concentration. Confirmation of this relationship is suggested by three of the acids having an intercept value similar to that observed for the control flour, 4.72, in Table 15.

The unique nature of caffeic acid was again noted in this series of experiments as its intercept was considerably different from that of the control flour.

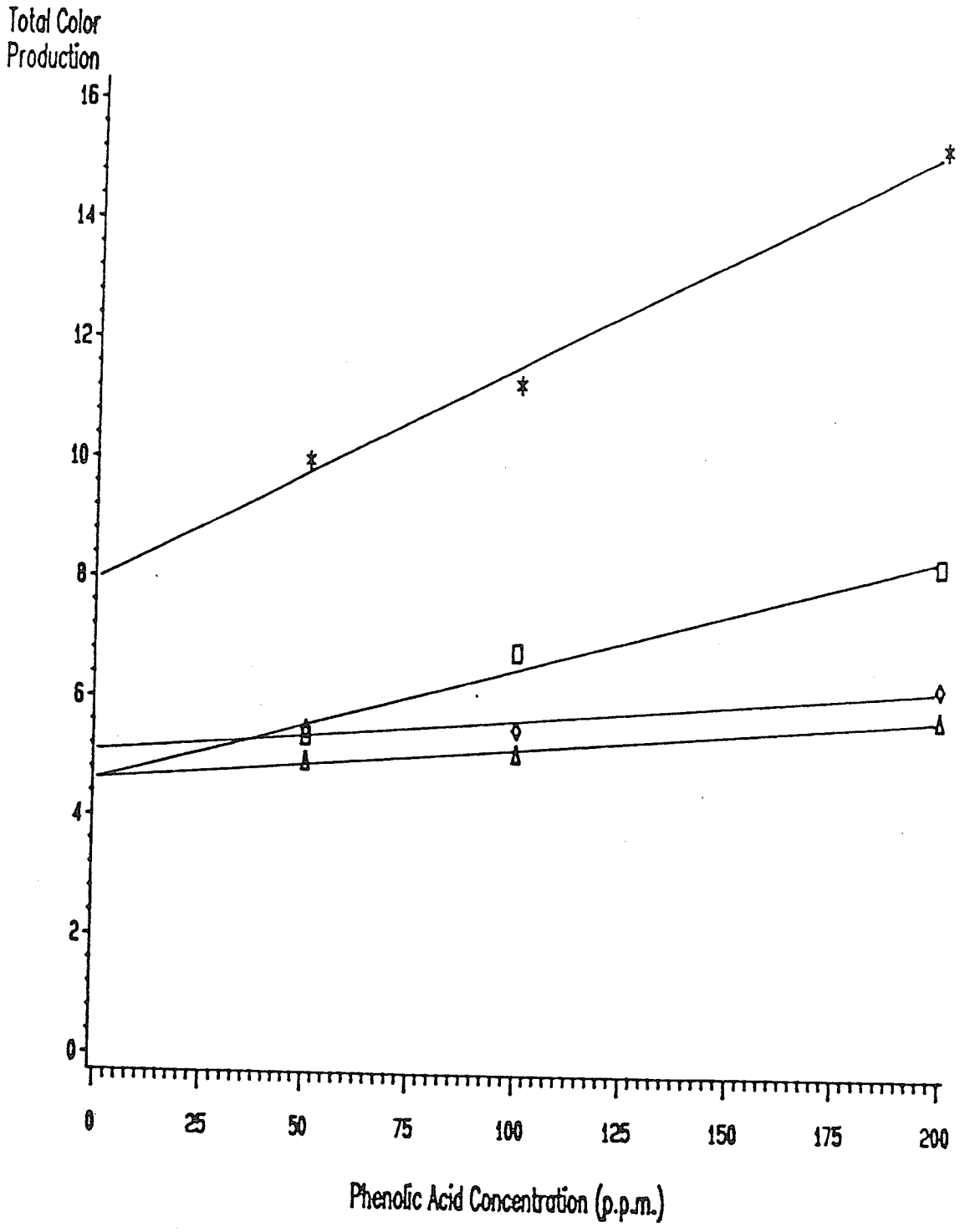
Figure 66 Phenolic Acid Concentration Effect on Total Color Change Observed in a Katepwa 75% Extraction 1st Clear Flour at 25 C

* Caffeic Acid

△ Ferulic Acid

□ Sinapic Acid

◇ Vanillic Acid


Total Color
Production
16
14
12
10
8
6
4
2
0
0
25
50
75
100
125
150
175
200
Phenolic Acid Concentration (p.p.m.)

Although the total color change appeared to be linearly related to phenolic concentration, the observed changes in L^*, a^*, and b^* values on the Hunter LabScanII colorimeter did not reflect a similar trend. Figs 67-72, highlighting both sinapic and caffeic acids indicated that the influence of the free phenolic acids on the three color components varies with the acid. Brightness for caffeic acid displayed considerable differences with the control yet the 50 and 100 ppm additions, although revealing decreasing brightness curves, were not distinct from each other. Furthermore, they did not display a difference between each other equivalent to half that observed between 100 and 200 ppm. Sinapic acid however, did display an approximate relative decrease in brightness proportional to the amount added. It should be noted that the lower concentrations, 50 and 100 ppm., did not display a discrete difference from the control flour. It was only the 200 ppm sinapic acid concentration which diverged from the control flour after 2 hrs.. Examination of the influence of phenolic acid concentration using either vanillic or ferulic acids revealed that neither acid at any concentration was distinct from the control flour's brightness.

Caffeic acid , as well as sinapic acid, continued to display their noticeably larger influence of concentration on both yellowness and redness than the other two acids examined. Sinapic acid's influence on yellowness was not, as seen previously, proportional, as the decrease between 50

Figure 67 Observed Changes in Brightness of a Katepwa 75% Extraction 1st Clear Flour as a Function of Caffeic Acid Concentration

✱ 200 ppm

△ 100 ppm

□ 50 ppm

○ Control

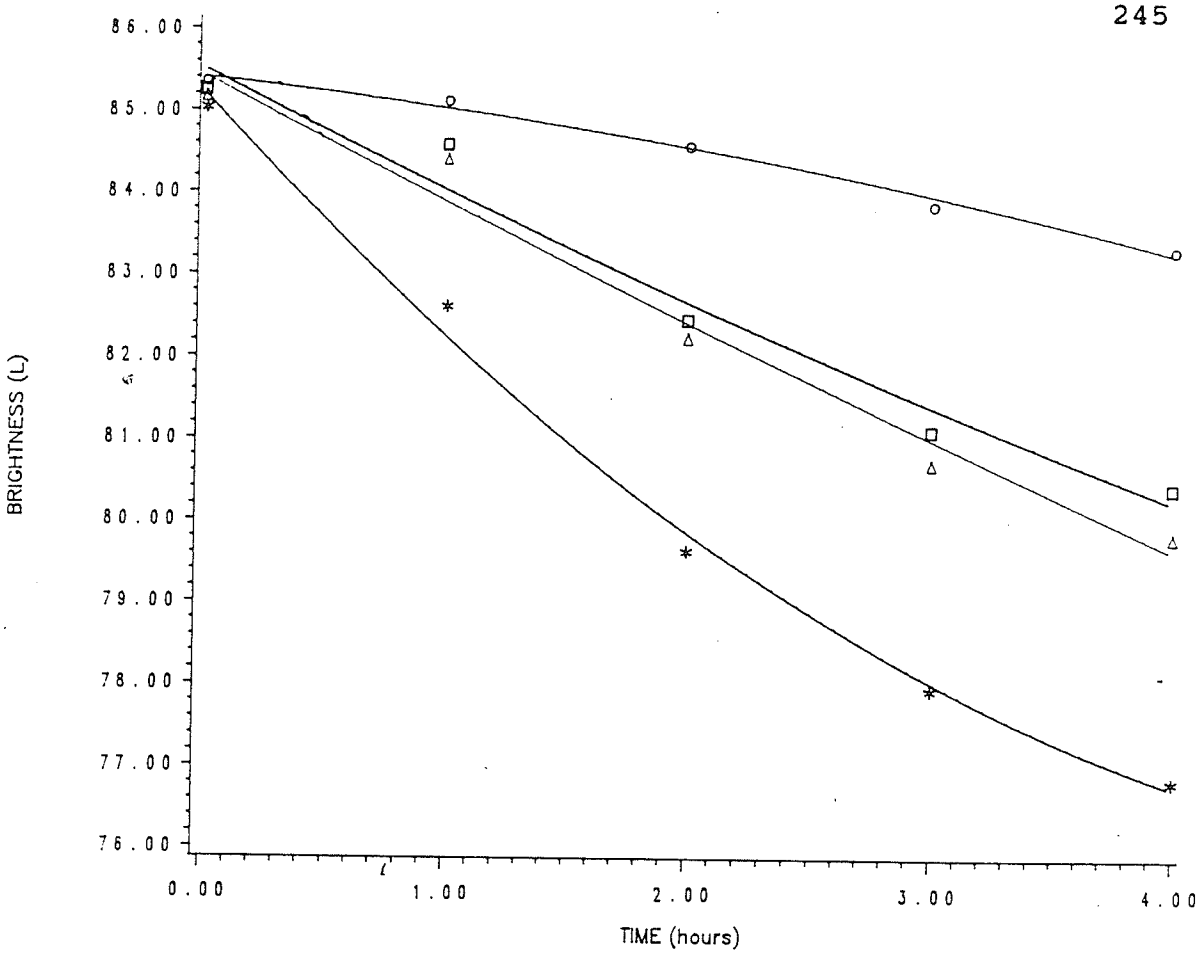
Figure 68 Observed Changes in Brightness of a Katepwa 75% Extraction 1st Clear Flour as a Function of Sinapic Acid Concentration

✱ 200 ppm

△ 100 ppm

□ 50 ppm

○ Control


86.00
85.00
84.00
83.00
82.00
81.00
80.00
79.00
78.00
77.00
76.00
BRIGHTNESS (L)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

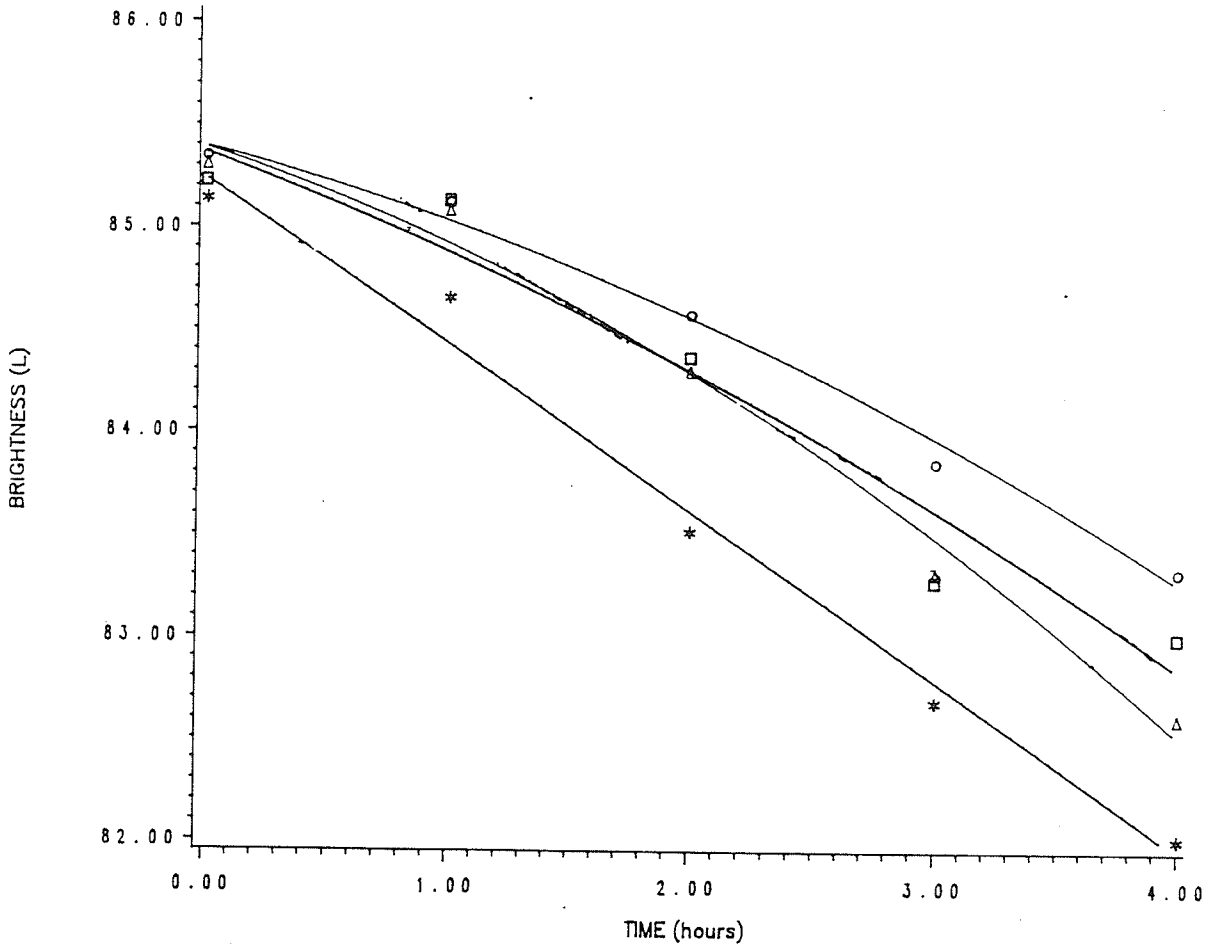

86.00
85.00
84.00
83.00
82.00
BRIGHTNESS (L)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

Figure 69 Observed Changes in Yellowness of a Katepwa 75% Extraction 1st Clear Flour as a Function of Caffeic Acid Concentration

✱ 200 ppm

△ 100 ppm

□ 50 ppm

○ Control

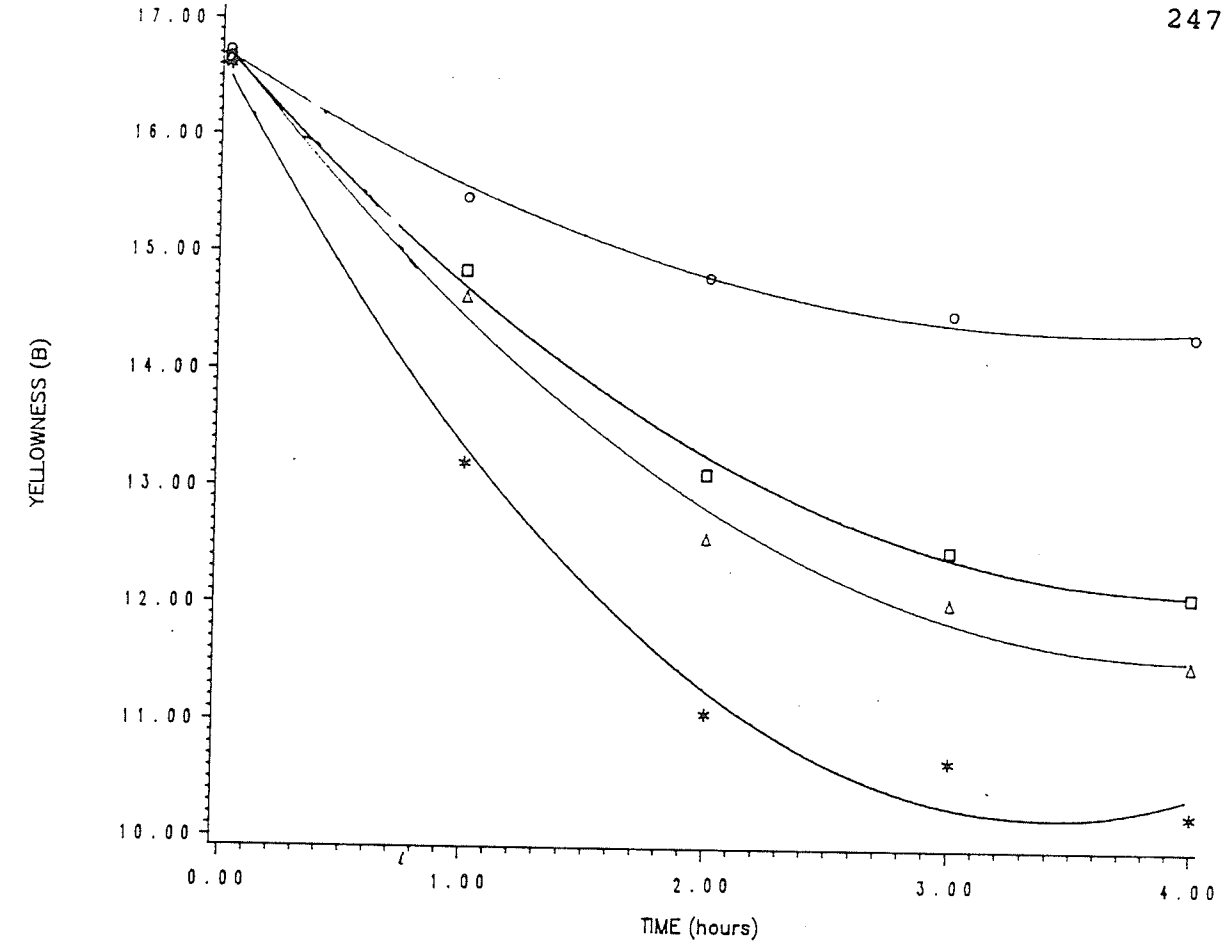
Figure 70 Observed Changes in Yellowness of a Katepwa 75% Extraction 1st Clear Flour as a Function of Sinapic Acid Concentration

✱ 200 ppm

△ 100 ppm

□ 50 ppm

○ Control


17.00
16.00
15.00
14.00
13.00
12.00
11.00
10.00
YELLOWNESS (B)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

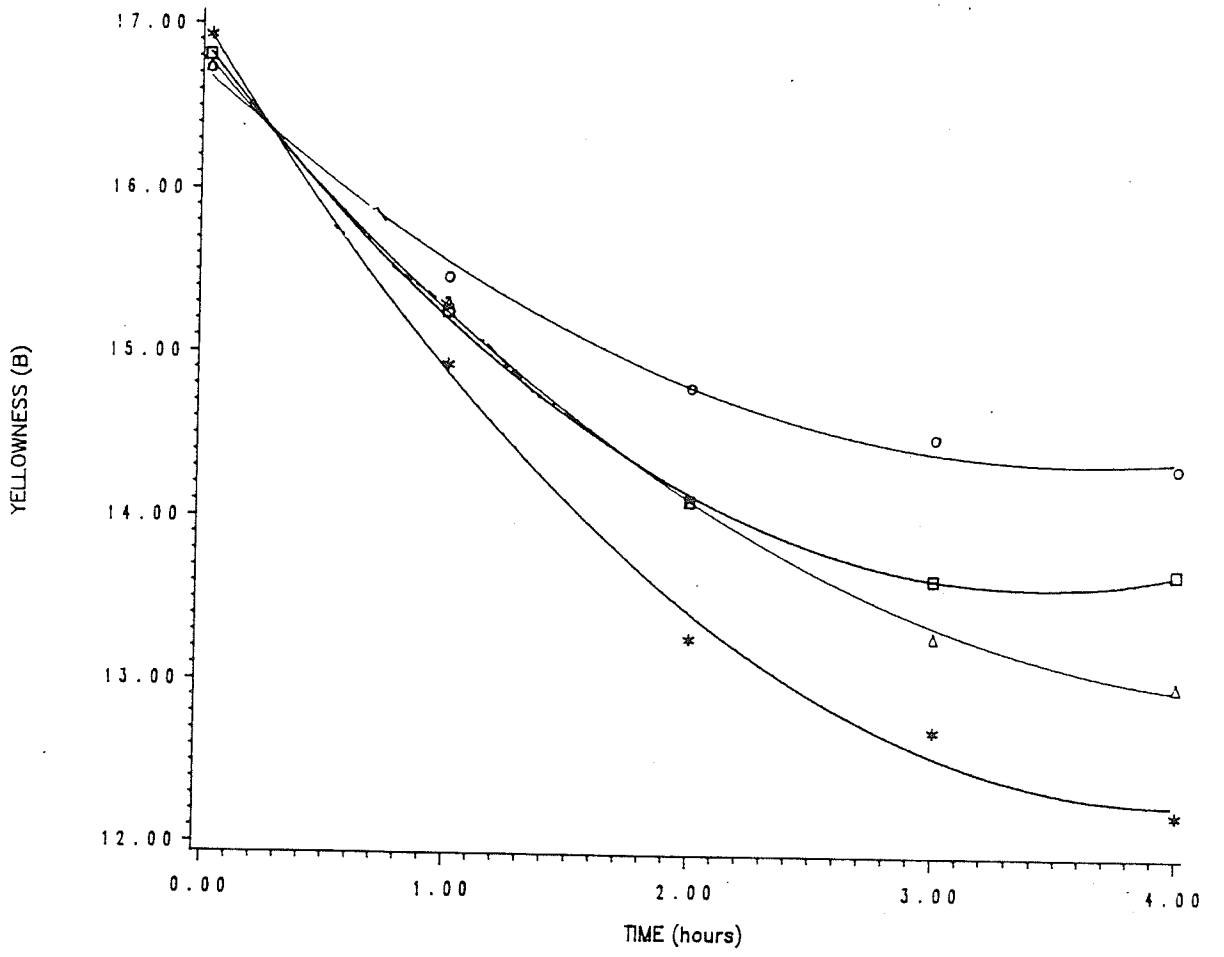

17.00
16.00
15.00
14.00
13.00
12.00
YELLOWNESS (B)
0.00
1.00
2.00
3.00
4.00
TIME (hours)

Figure 71 Observed Changes in Redness of a Katepwa 75% Extraction 1st Clear Flour as a Function of Caffeic Acid Concentration

✳ 200 ppm

△ 100 ppm

□ 50 ppm

○ Control

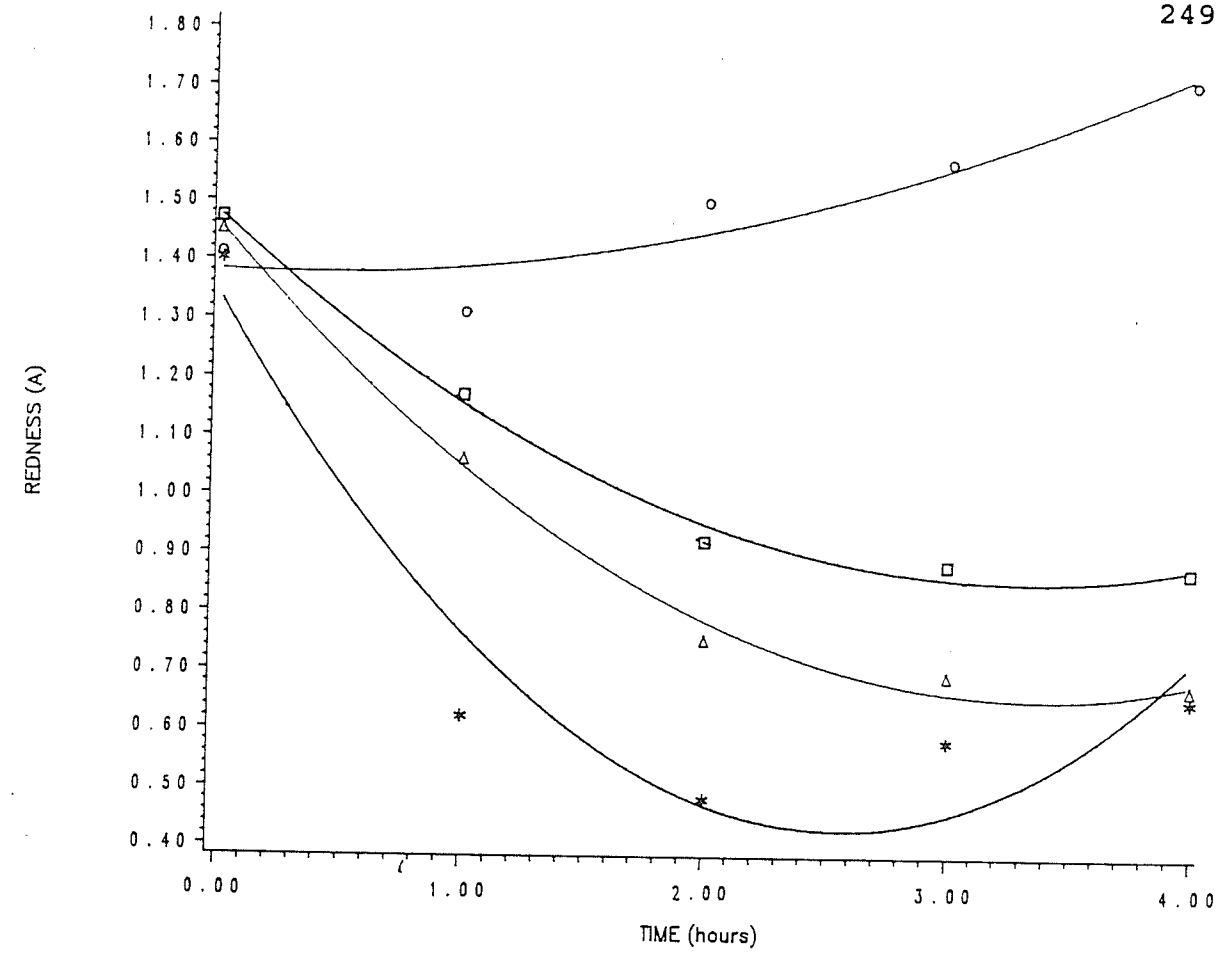
Figure 72 Observed Changes in Redness of a Katepwa 75% Extraction 1st Clear Flour as a Function of Sinapic Acid Concentration

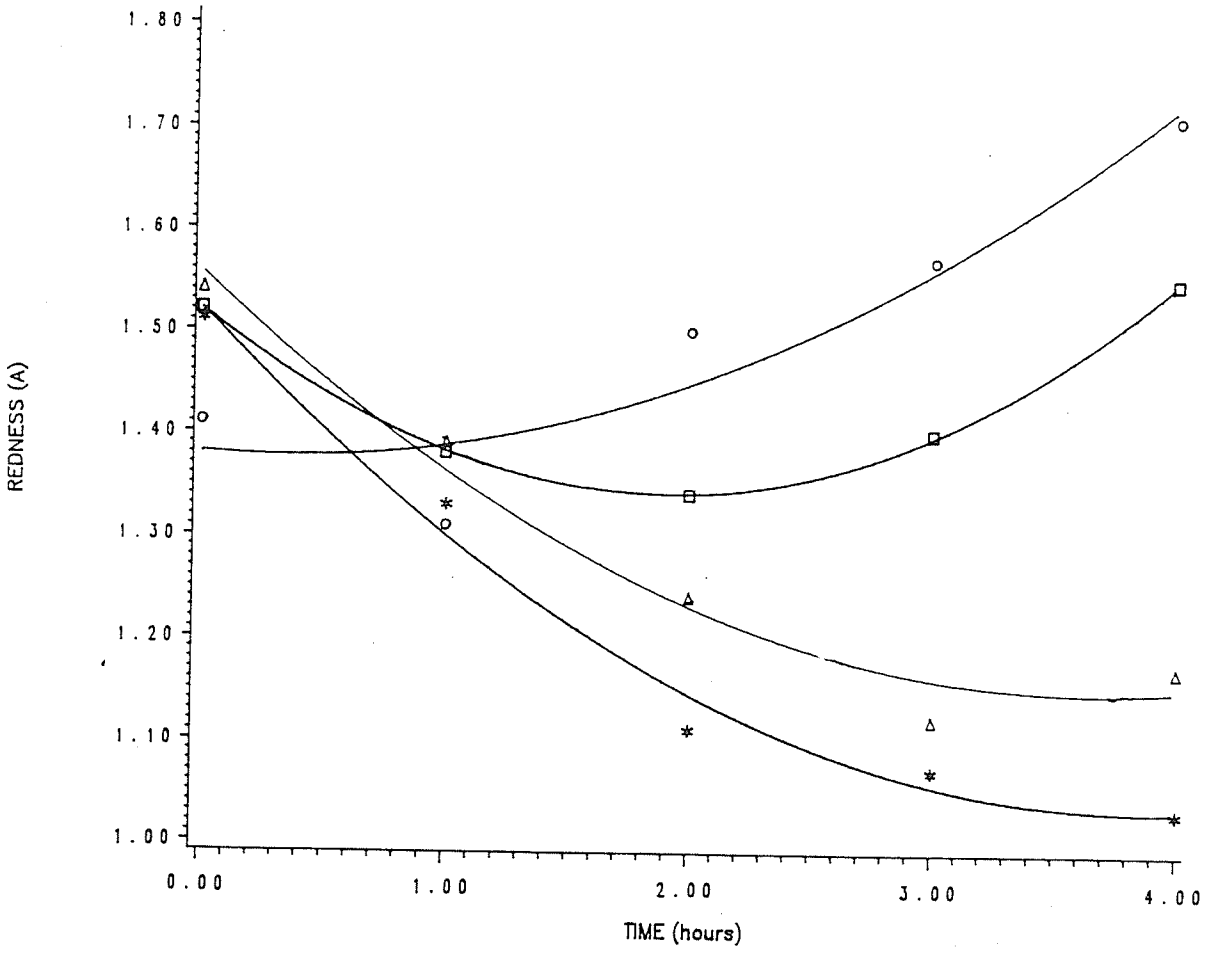
✳ 200 ppm

△ 100 ppm

□ 50 ppm

○ Control


REDNESS (A)
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.00
1.00
2.00
3.00
4.00
TIME (hours)


REDNESS (A)
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.00
1.00
2.00
3.00
4.00
TIME (hours)

and 100 ppm was roughly equivalent to that observed between 100 and 200 ppm. Although overlap existed amongst the sinapic acid pastes, each was separate from the control flour by 2 h. Similarly, each caffeic acid paste was discretely different from the control flour by 1 h of mixing. The functions describing this decline however did exhibit overlapping 95% confidence limits.

The effect of individual phenolic acids was observed to influence the degree of redness produced in the flour paste. Sinapic acid's alteration of redness varied considerably on the basis of concentration. At 50 ppm, the time curve mirrored the control displaying a slight increase in red color. However, at 100 and 200 ppm, both concentrations displayed observable differences with the control by 2 h although the discrepancy between themselves was minimal. Examination of the remaining phenolic acids indicated that only caffeic acid displayed an appreciable difference from the control flour. Each of the three acid concentration pastes were distinct from the control after 1 h of incubation.

A time function was also suggested to be present in the addition experiments. In the both the sinapic acid and caffeic acid series, a time lapse of 1 to 2 h were required before a difference could be detected between the addition curves and the control flour. This tendency would suggest that the noticeable color production at low concentrations requires a lag phase in order to develop sufficient

intensity to distinguish itself. Such a delay might suggest that a series of secondary reactions are required to generate the colored compounds in the paste. The concept of a secondary reaction agrees with the known action of polyphenol oxidase. The enzyme's oxidation of the substrate is not as important as the subsequent quinone reaction color production.

4.06.7 Predictive Modelling of Color

A predictive equation relating specific flour components to the corresponding color of the flour paste would be a highly desirable tool. Utilizing the various factors determined in this study an attempt was made to evaluate individual factor contribution to the initial paste color. The SAS statistical package (SAS Institute, Cary NC) was utilized to perform three different forms of regression modelling on each of the Hunter Lab, Kent-Jones, and Agtron assessments of flour color.

The regression models employed were the stepwise, forward, and adjusted rsquare methods. The selection process for the stepwise and forward methods was based upon maximum coefficient of determination, R^2. In the adjusted rsquare analysis, the model was limited to the best 5 factors from all present. Due to the limited number of observations per variety, n=13, the factors involved in the models were required to be limited to 11. The factors included in the

regression models were; protein, pigment, all individual soluble bound phenolic acids, insoluble ferulic acid, polyphenol oxidase levels, and the total free phenolic acid content of the flour. Ash content was not included in these models as it was felt that mineral content was indicative of the degree of bran contamination. Since each of the factors in this study reflected this phenomena, inclusion of the mineral content would interfere with the determination of the factors influencing color production.

The three regression methods were performed on every variety modelling each of the Hunter lab color components, Agtron, and Kent-Jones CGF values. Factors selected by the three models for each variety were compared across varieties and tabulated. Inclusion of a factor into the final model was based upon relative occurance and the requirement that the factor be recognized in at least four of the five variety models. Once the common factors had been determined regression equations utilizing these regressor variables were calculated for the individual variety to evaluate their ability to predict their corresponding initial color measurement.

Utilizing the flour water pastes, polyphenol oxidase was the only factor found consistently throughout each color method. Correlation coefficients for all Hunter lab and Kent-Jones individual variety models were in excess of $r=0.93$ at a 99% significance level. The correlation coefficients for the varietal Agtron models were noticeably

lower with values of r=0.82 to r=0.93 at a 95% significance level. The regression equations for individual varieties can be found in Tables 16 & 17 and representative plots of observed versus predicted values can be seen if Figs 73-86.

The regression equations were initially applied to each variety as the cultivars represent five distinct classes of wheat. Application of a variety independent regression equations, utilizing the selected factors per dependant variable, were undertaken to determine their possible applicability. The high correlations observed for the individual color components within a variety were maintained across varietal lines. The correlation coefficients for the three Hunter Lab color indexes and the Kent-Jones method remained in excess of r=0.92 at the 99.9% significance level. The lower correlations observed for the individual Agtron readings was again repeated as r= 0.84 at a 99.9% significance level.

The influence of the introduction of the Kan Sui reagent in the formation of the initial flour paste color was also analyzed in the same manner to determine if a predictive model could be derived. Examination of individual varieties' paste brightness and yellow color components indicated that significant models, $p < 0.001$, could be generated. Individual variety brightness models showed the highest correlations with r values greater than 0.97 while yellowness also displayed strong correlations with a minimum value of 0.91. Unfortunately, the paste redness in the

Table 16

REGRESSION EQUATIONS FOR WATER:FLOUR PASTES

Initial Brightness (L)

Fielder	$L = 89.02 - 1.11 \times 10^{-3}$ (PPO) - 2.34 (CAF) + 3.29×10^{-2} (VAN) + 0.596 (TOTFREE)
Glenlea	$= 89.70 - 7.19 \times 10^{-3}$ (PPO) + 0.506 (CAF) + 0.365 (VAN) - 1.05 (TOTFREE)
HY320	$= 87.90 - 5.38 \times 10^{-3}$ (PPO) - 4.24 (CAF) - 0.213 (VAN) + 0.213 (TOTFREE)
Katepwa	$= 90.71 - 6.03 \times 10^{-3}$ (PPO) + 3.35 (CAF) + 0.632 (VAN) - 2.17 (TOTFREE)
Norstar	$= 91.59 - 1.27 \times 10^{-3}$ (PPO) + 0.956 (CAF) + 0.048 (VAN) - 0.656 (TOTFREE)
All lines	$= 89.90 - 9.96 \times 10^{-3}$ (PPO) + 0.46 (CAF) + 0.23 (VAN) - 0.57 (TOTFREE)

Initial Redness (A)

Fielder	$A = 3.23 \times 10^{-3}$ (PPO) - 0.15 (VAN) + 0.686 (COUM) + 0.02
Glenlea	$= 2.96 \times 10^{-3}$ (PPO) - 0.38 (VAN) + 1.68 (COUM) + 0.55
HY320	$= 4.12 \times 10^{-3}$ (PPO) - 0.06 (VAN) - 1.13 (COUM) + 0.58
Katepwa	$= 4.03 \times 10^{-3}$ (PPO) - 0.24 (VAN) + 2.05 (COUM) + 0.73
Norstar	$= 4.71 \times 10^{-3}$ (PPO) - 0.34 (VAN) + 1.84 (COUM) + 0.12
All lines	$= 3.89 \times 10^{-3}$ (PPO) - 0.10 (VAN) - 0.02 (COUM) + 0.41

Initial Yellowness (B)

Fielder	$B = 10.97 + 5.27 \times 10^{-3}$ (PPO) - 1.29 (COUM) + 2.04 (PIG) - 0.53 (TOTFREE)
Glenlea	$= 7.29 + 1.52 \times 10^{-3}$ (PPO) - 2.44 (COUM) + 3.50 (PIG) - 0.72 (TOTFREE)
HY320	$= 10.33 + 1.30 \times 10^{-3}$ (PPO) - 0.98 (COUM) + 1.97 (PIG) + 0.05 (TOTFREE)
Katepwa	$= 15.25 + 2.98 \times 10^{-3}$ (PPO) - 2.89 (COUM) + 0.25 (PIG) - 0.17 (TOTFREE)
Norstar	$= 12.36 + 1.07 \times 10^{-3}$ (PPO) - 1.12 (COUM) + 0.46 (PIG) + 0.36 (TOTFREE)
All lines	$= 11.05 + 1.31 \times 10^{-3}$ (PPO) - 3.06 (COUM) + 1.47 (PIG) + 9.72 (TOTFREE)

Agtron Color

Fielder	$= 61.72 - 5.52 \times 10^{-3}$ (PPO) - 0.29 (INSOL) + 5.00 (TOTFREE) + 1.32 (FER)
Glenlea	$= 78.85 - 8.53 \times 10^{-3}$ (PPO) - 0.66 (INSOL) - 28.35 (TOTFREE) + 16.66 (FER)
HY320	$= 52.22 - 1.82 \times 10^{-3}$ (PPO) - 0.36 (INSOL) + 4.26 (TOTFREE) + 1.80 (FER)
Katepwa	$= 68.22 - 4.59 \times 10^{-3}$ (PPO) - 0.27 (INSOL) - 3.12 (TOTFREE) + 0.02 (FER)
Norstar	$= 78.32 - 4.36 \times 10^{-3}$ (PPO) - 0.01 (INSOL) + 7.93 (TOTFREE) - 13.50 (FER)
All lines	$= 66.26 - 1.11 \times 10^{-3}$ (PPO) - 0.25 (INSOL) + 5.38 (TOTFREE) + 5.27 (FER)

Kent-Jones Color

Fielder	$= 6.87 \times 10^{-3}$ (PPO) - 2.40 (CAF) - 0.44 (SYR) + 1.84 (COUM) - 1.57
Glenlea	$= 8.41 \times 10^{-3}$ (PPO) - 1.23 (CAF) - 2.76 (SYR) + 13.79 (COUM) + 0.42
HY320	$= 4.00 \times 10^{-3}$ (PPO) - 2.68 (CAF) + 3.75 (SYR) - 10.89 (COUM) + 0.41
Katepwa	$= 9.07 \times 10^{-3}$ (PPO) - 0.63 (CAF) + 0.19 (SYR) + 9.18 (COUM) - 0.38
Norstar	$= 1.96 \times 10^{-3}$ (PPO) - 1.95 (CAF) + 2.62 (SYR) - 2.65 (COUM) - 3.57
All lines	$= 9.11 \times 10^{-3}$ (PPO) - 0.49 (CAF) + 0.88 (SYR) + 0.96 (COUM) - 0.61

Symbol Legend

PPO	= Polyphenol Oxidase	SYR	= Soluble Bound Syringic Acid
CAF	= Soluble Bound Caffeic Acid	PIG	= Pigment
VAN	= Soluble Bound Ferulic Acid	TOTFREE	= Total Free Phenolic Acids
COUM	= Soluble Bound Coumaric Acid	INSOL	= Insoluble Bound Ferulic Acid

Table 17

REGRESSION EQUATIONS FOR KAN SUI:FLOUR PASTE COLORS

Initial Brightness

Fielder	$94.50 - 6.46 \times 10^{-3}$ (PPO) - 1.61 (CAF) + 0.12 (SIN) - 2.18 (PIG)
Glenlea	$97.26 - 5.43 \times 10^{-3}$ (PPO) + 0.07 (CAF) - 0.03 (SIN) - 4.42 (PIG)
HY320	$93.53 - 2.55 \times 10^{-3}$ (PPO) - 4.29 (CAF) + 0.15 (SIN) - 3.08 (PIG)
Katepwa	$91.86 - 9.08 \times 10^{-3}$ (PPO) - 1.62 (CAF) + 0.004 (SIN) - 1.41 (PIG)
Norstar	$92.39 - 1.59 \times 10^{-3}$ (PPO) + 0.83 (CAF) - 0.14 (SIN) - 0.68 (PIG)
All lines	$90.63 - 9.91 \times 10^{-3}$ (PPO) - 0.17 (CAF) - 0.46 (SIN) - 0.74 (PIG)

Initial Redness

Fielder	$-3.30 - 1.13 \times 10^{-3}$ (PPO) + 0.64 (CAF)*
Glenlea	$-2.43 + 3.47 \times 10^{-3}$ (PPO) - 0.78 (CAF)*
HY320	$-2.60 + 5.73 \times 10^{-3}$ (PPO) + 0.72 (CAF)*
Katepwa	$-2.57 + 1.09 \times 10^{-3}$ (PPO) + 0.50 (CAF)*
Norstar	$-2.73 + 4.64 \times 10^{-3}$ (PPO) - 0.57 (CAF)*
All lines	$-2.75 + 2.62 \times 10^{-3}$ (PPO) - 2.94 (CAF)*

Initial Yellowness

Fielder	5.52 + 8.07 (PIG) - 0.66 (FER) + 0.62 (VAN)
Glenlea	11.53 + 4.85 (PIG) - 1.62 (FER) + 3.96 (VAN
HY320	15.39 + 2.85 (PIG) - 0.23 (FER) + 1.10 (VAN)
Katepwa	15.67 + 1.90 (PIG) - 0.78 (FER) + 1.54 (VAN)
Norstar	15.58 + 1.11 (PIG) + 0.89 (FER) + 0.33 (VAN)
All lines	16.95 + 1.46 (PIG) + 0.20 (FER) + 0.66 (VAN)

Symbol Legend

PPO	= Polyphenol Oxidase
CAF	= Soluble Bound Caffeic Acid
SIN	= Soluble Bound Sinapic Acid
FER	= Soluble Bound Ferulic Acid
VAN	= Soluble Bound Ferulic Acid
PIG	= Pigment

* Not significant at 95% level.

Figure 73 An Attempt to Model The Initial Water:Flour Paste Brightness for the Variety: KATEPWA

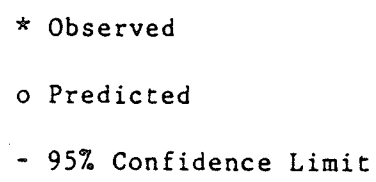
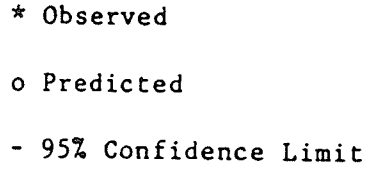
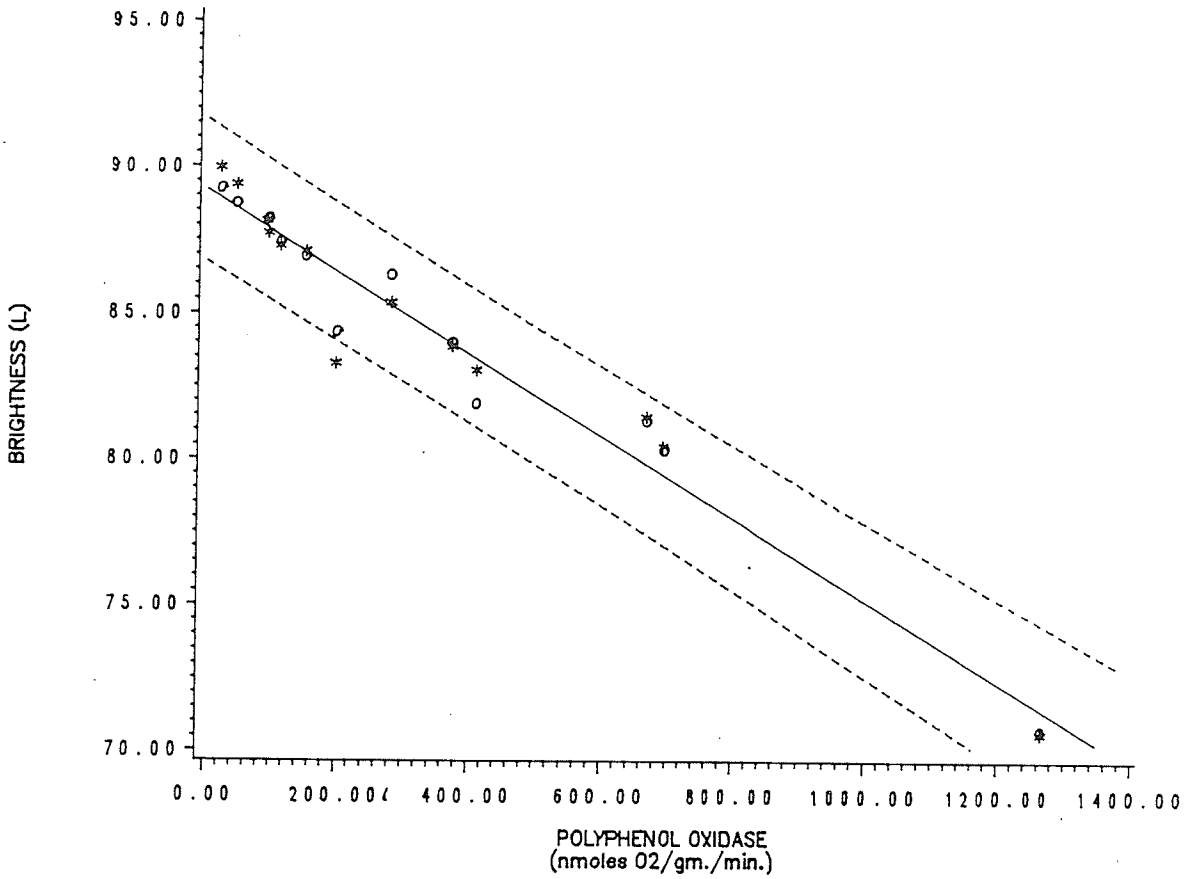


Figure 74 An Attempt to Model the Initial Water:Flour Paste Brightness Independent of Variety




95.00
90.00
85.00
80.00
75.00
70.00
BRIGHTNESS (L)
0.00
200.00
400.00
600.00
800.00
1000.00
1200.00
1400.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

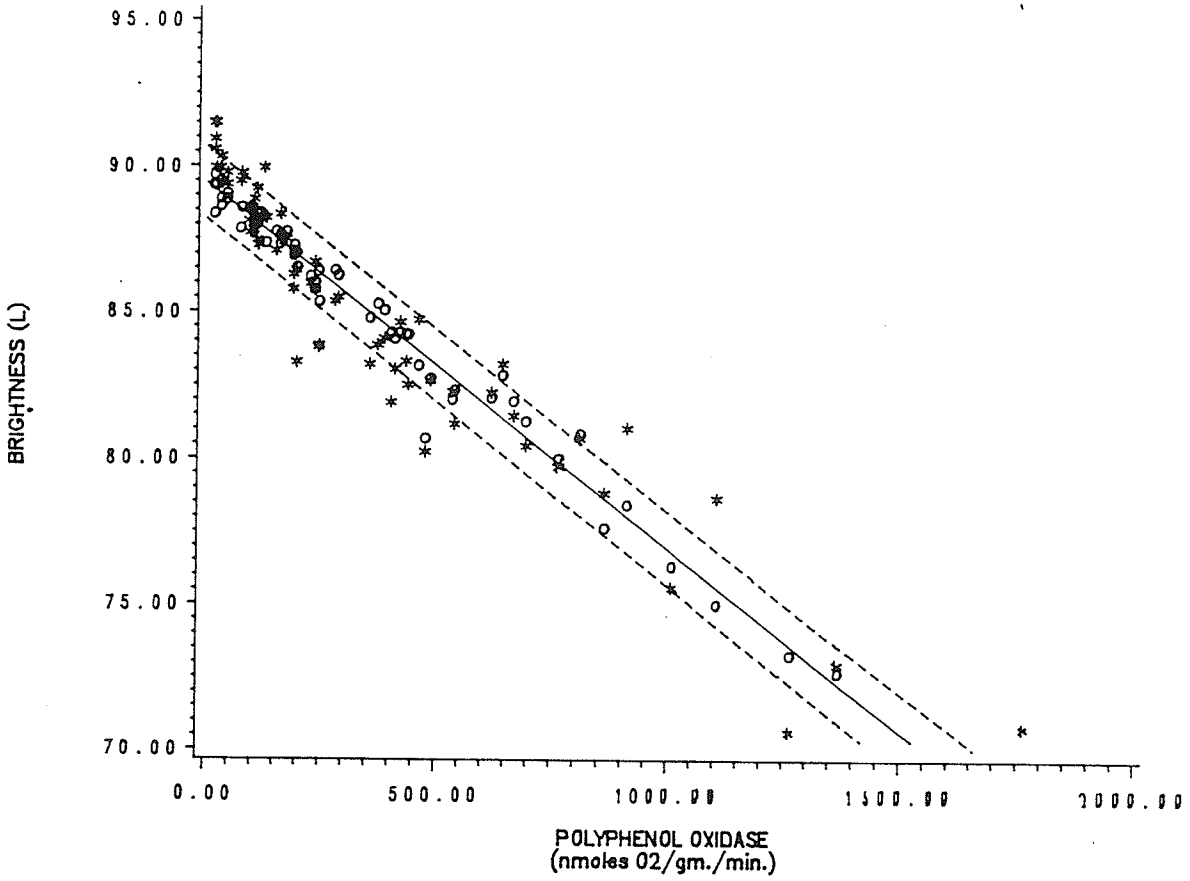

95.00
90.00
85.00
80.00
75.00
70.00
BRIGHTNESS (L)
0.00
500.00
1000.00
1500.00
2000.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 75 An Attempt to Model the Initial Water:Flour Paste Yellowness for the Variety:KATEPWA

* Observed

o Predicted

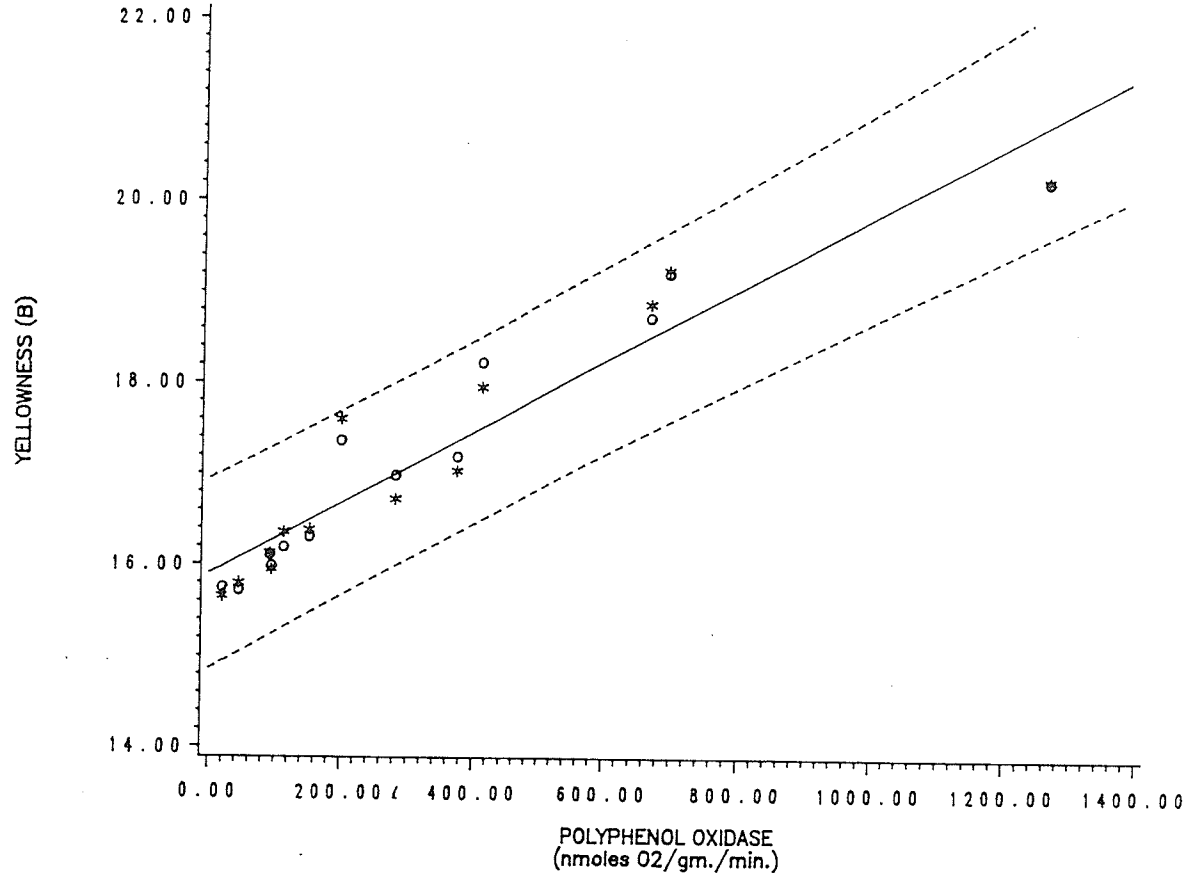
- 95% Confidence Limit

Figure 76 An Attempt to Model the Initial Water:Flour Paste Yellowness Independent of the Variety

* Observed

o Predicted

- 95% Confidence Limit


22.00
20.00
18.00
16.00
14.00
YELLOWNESS (B)
0.00
200.00
400.00
600.00
800.00
1000.00
1200.00
1400.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

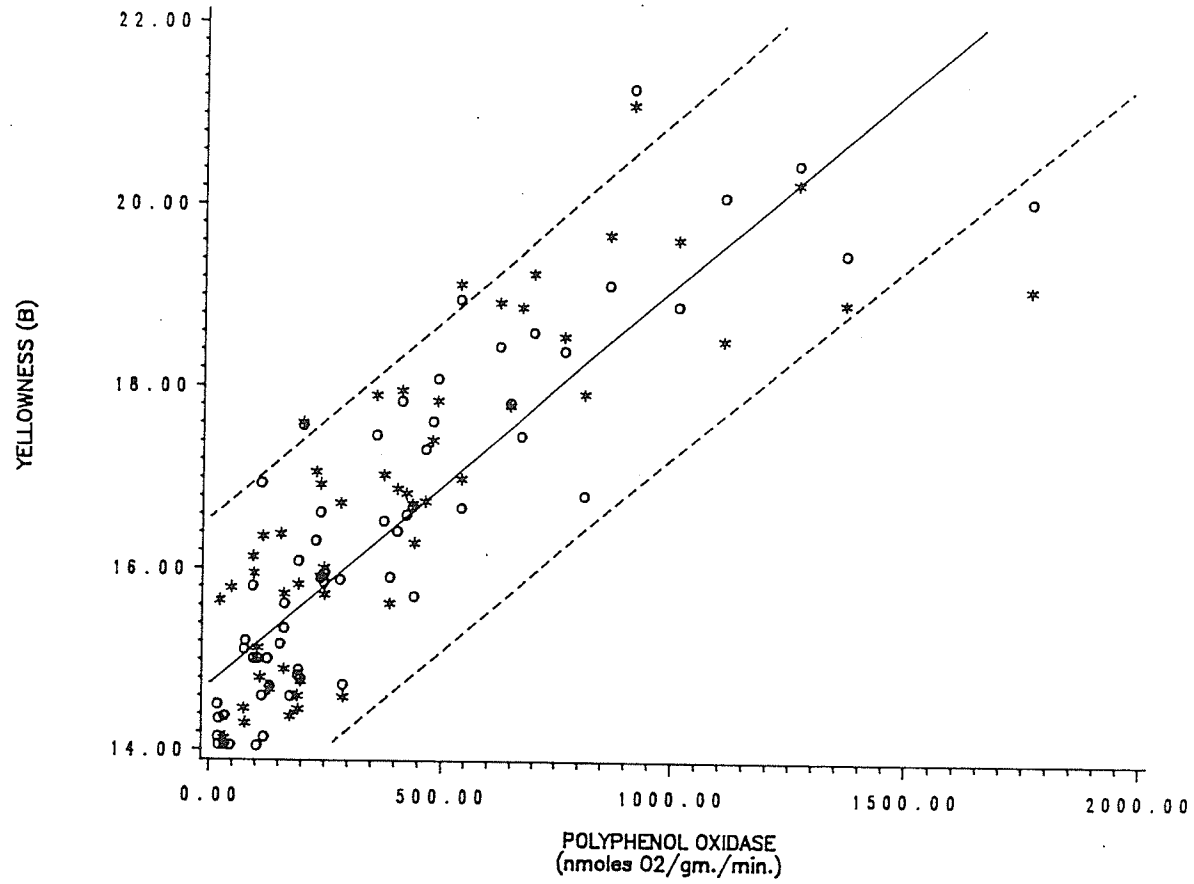

22.00
20.00
18.00
16.00
14.00
YELLOWNESS (B)
0.00
500.00
1000.00
1500.00
2000.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 77 An Attempt to Model the Initial Water:Flour Paste Redness for the Variety: FIELDER

* Observed

o Predicted

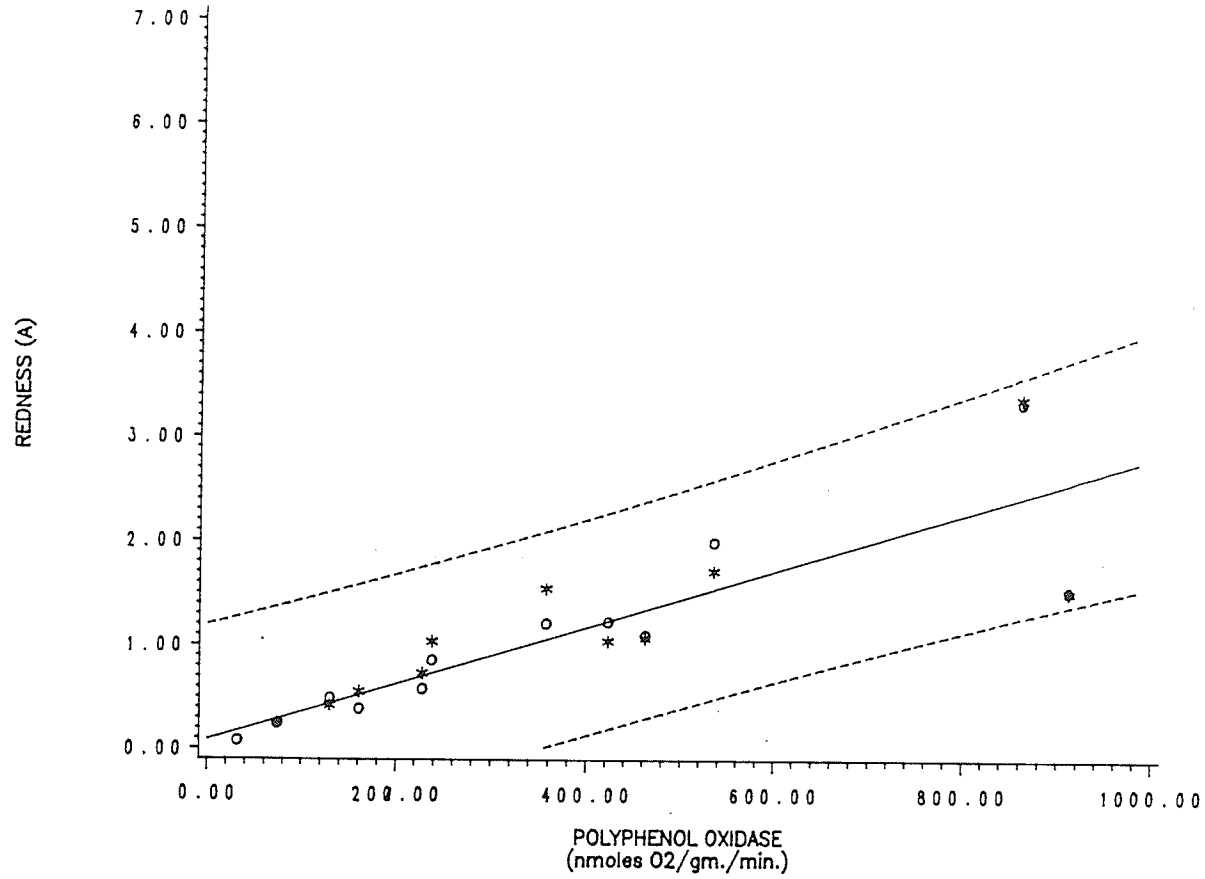
- 95% Confidence Limit

Figure 78 An Attempt to Model the Initial Water:Flour Paste Redness Independent of Variety

* Observed

o Predicted

- 95% Confidence Limit


7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
REDNESS (A)
0.00
200.00
400.00
600.00
800.00
1000.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

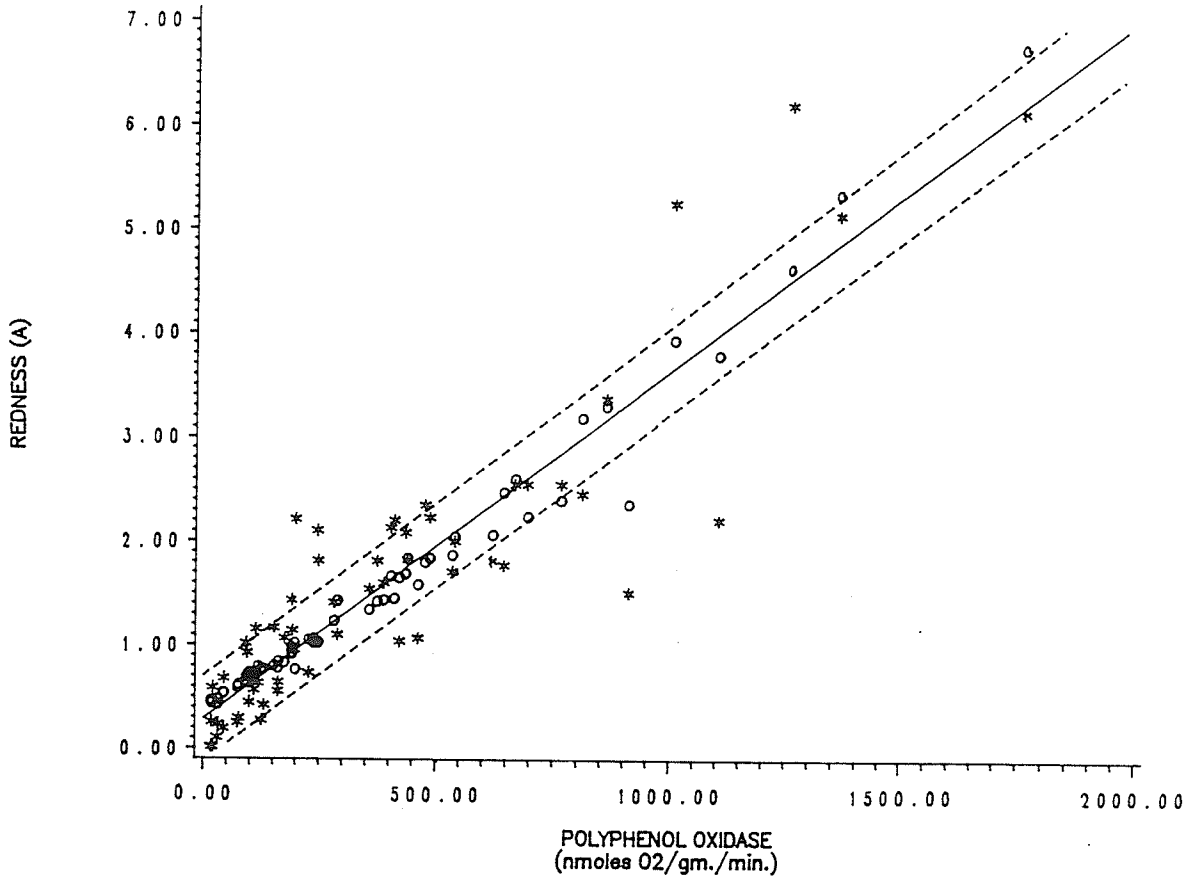

7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
REDNESS (A)
0.00
500.00
1000.00
1500.00
2000.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 79 An Attempt to Model the Initial Water:Flour Paste Kent-Jones CGF Value for the Variety: NORSTAR

* Observed

o Predicted

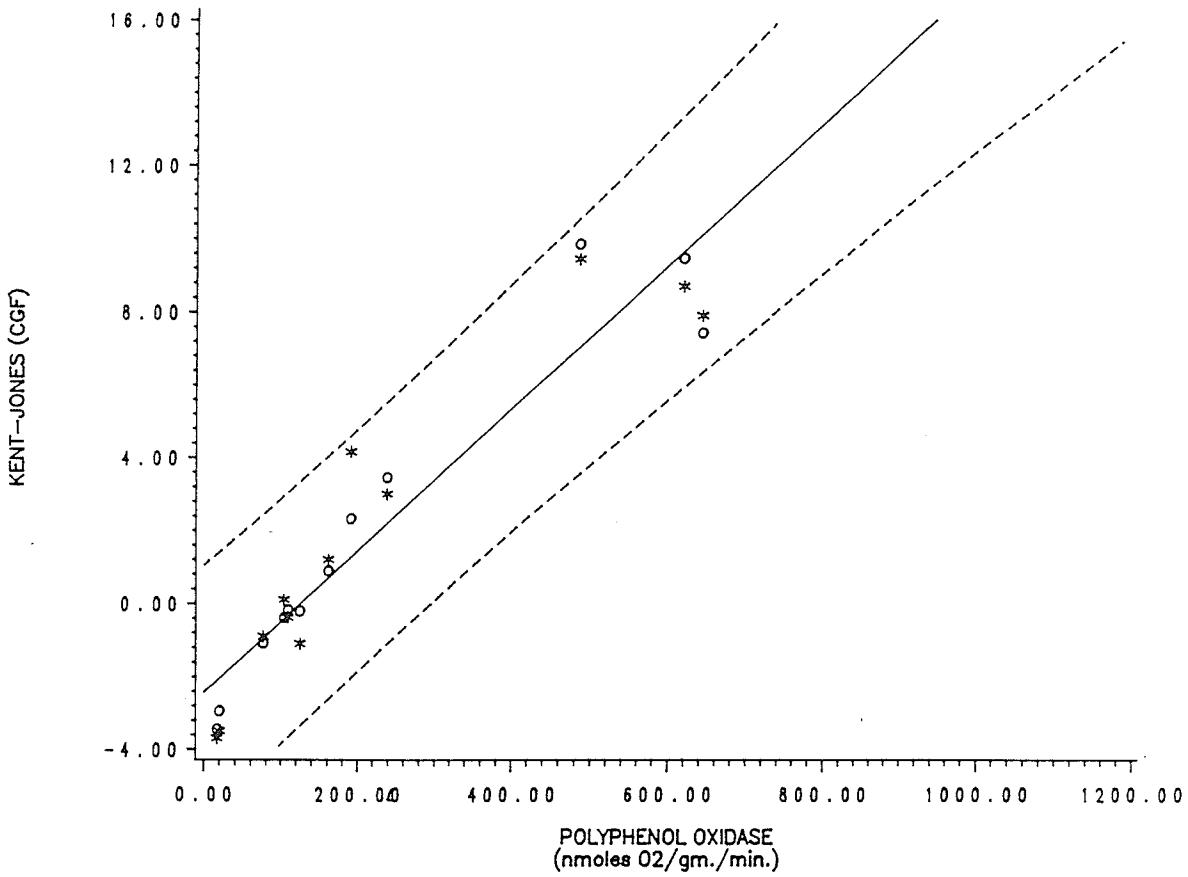
- 95% Confidence Limit

Figure 80 An Attempt to Model the Initial Water:Flour Paste Kent-Jones CGF Value Independent of Variety

* Observed

o Predicted

- 95% Confidence Limit


16.00
12.00
8.00
4.00
0.00
-4.00
KENT-JONES (CGF)
0.00
200.00
400.00
600.00
800.00
1000.00
1200.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

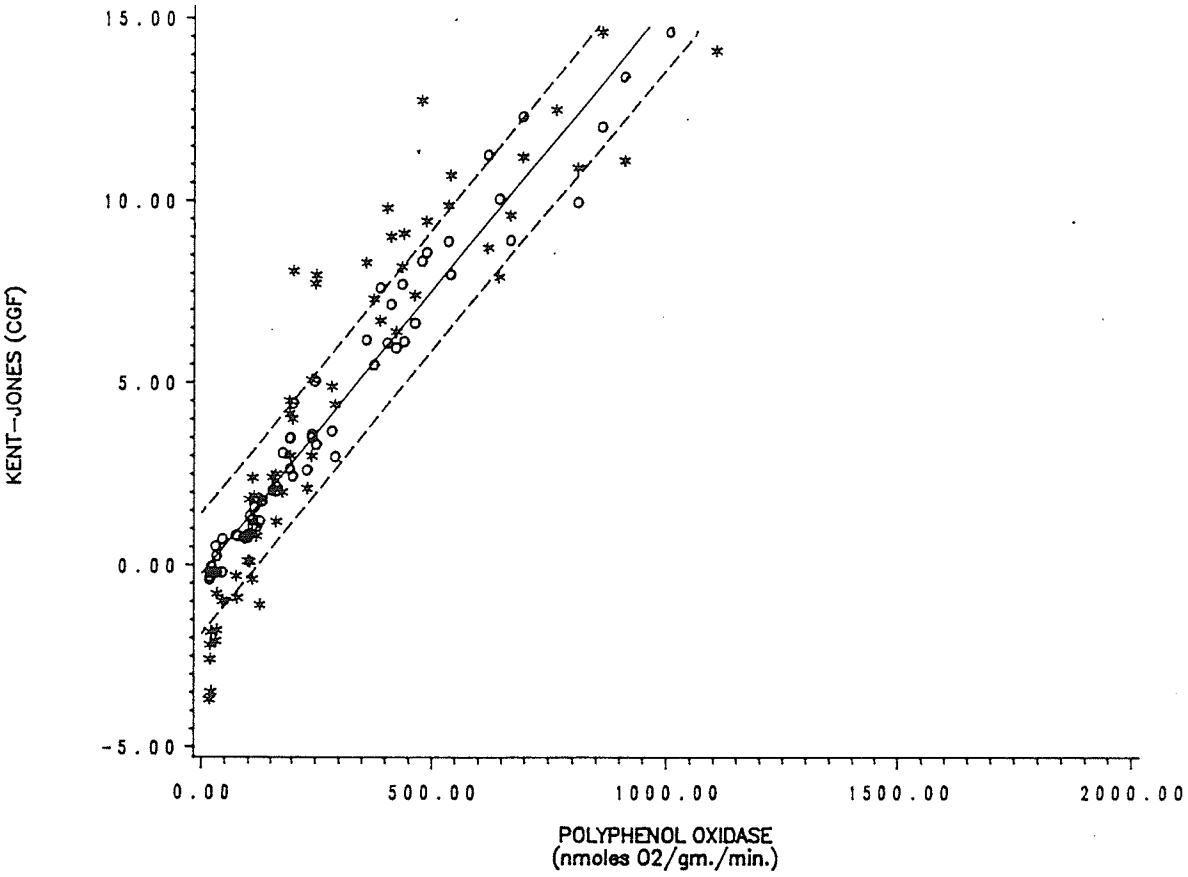

15.00
10.00
5.00
0.00
-5.00
KENT-JONES (CGF)
0.00
500.00
1000.00
1500.00
2000.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 81 An Attempt to Model the Initial Water:Flour Paste Agtron Value for the Variety: NORSTAR

* Observed

o Predicted

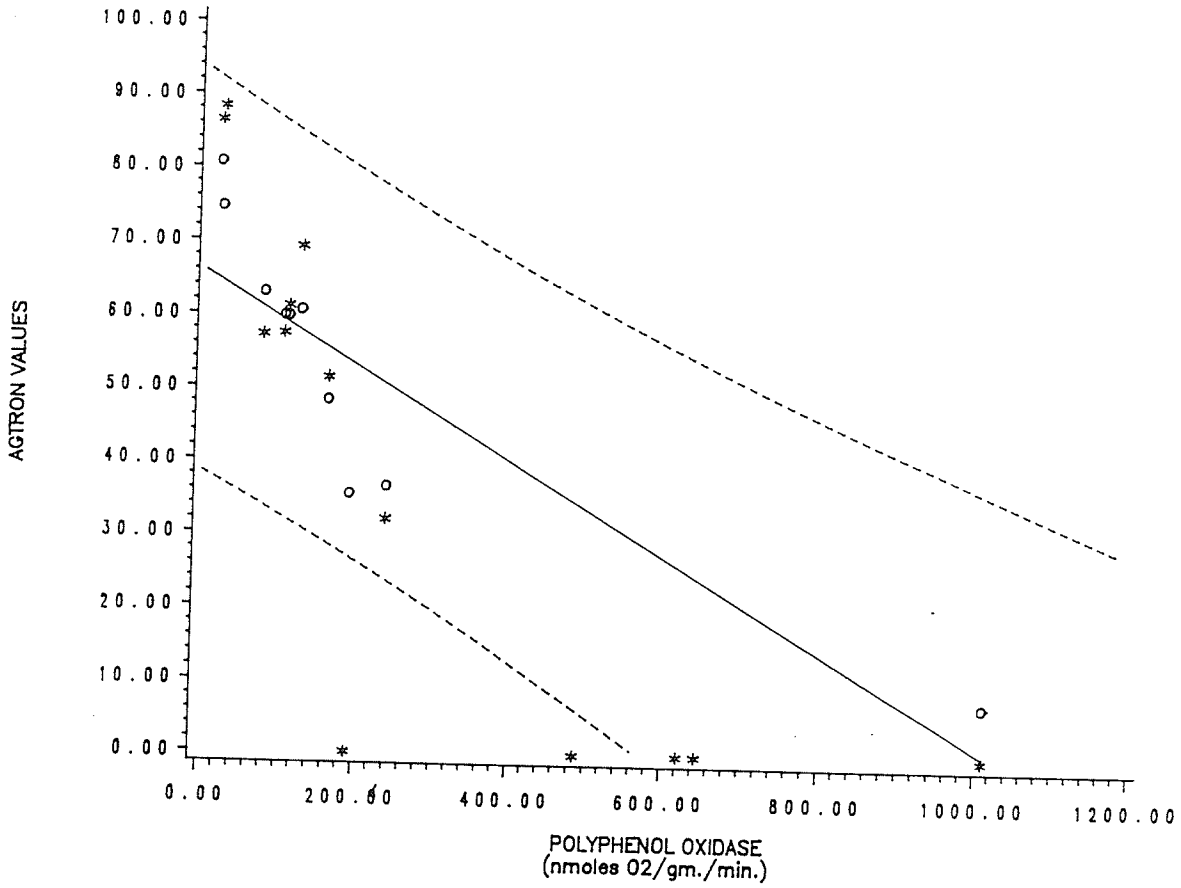
- 95% Confidence Limit

Figure 82 An Attempt to Model the Initial Water:Flour Paste Agtron Value Independent of Variety

* Observed

o Predicted

- 95% Confidence Limit


AGTRON VALUES
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
1200.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

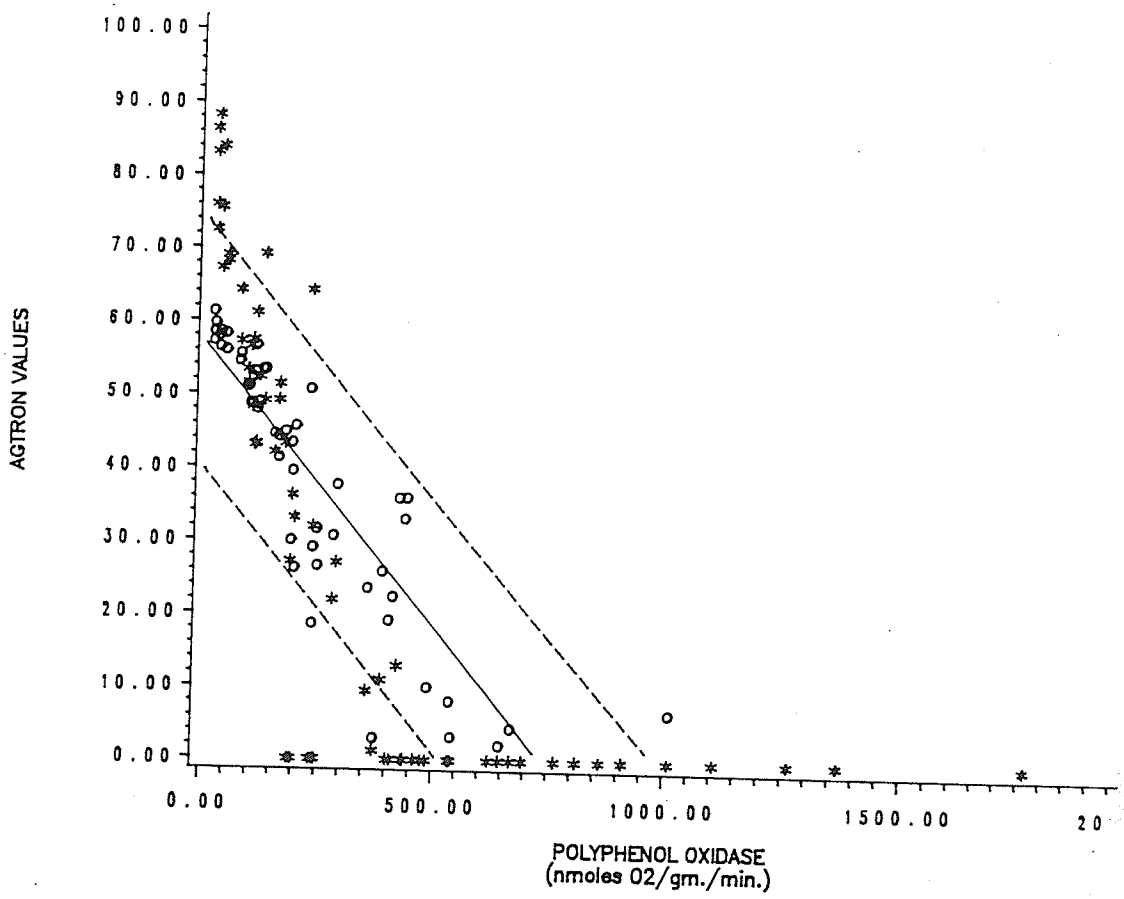

AGTRON VALUES
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
500.00
1000.00
1500.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 83 An Attempt to Model the Initial Kan Sui:Flour Paste Brightness for the Variety: NORSTAR

* Observed

o Predicted

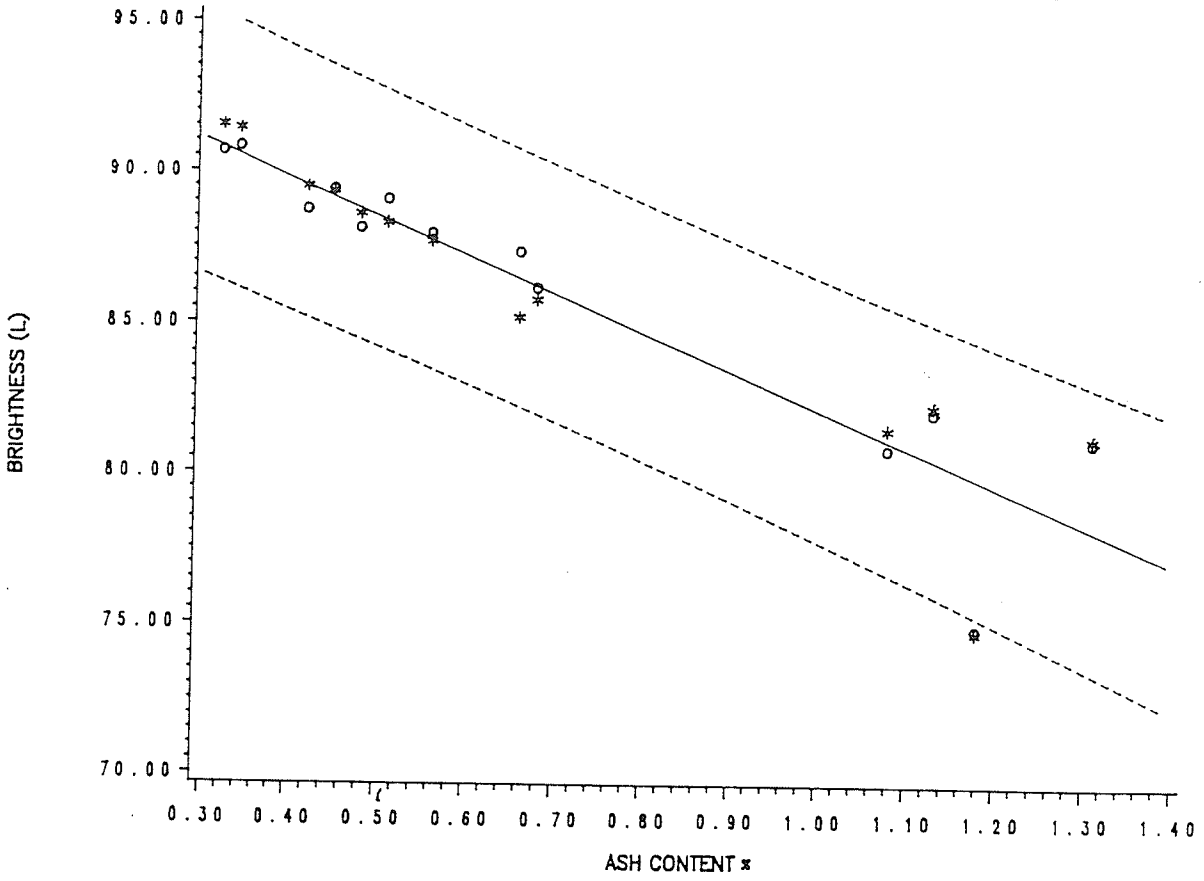
- 95% Confidence Limit

Figure 84 An Attempt to Model the Initial Kan Sui:Flour Paste Brightness Independent of Variety

* Observed

o Predicted

- 95% Confidence Limit


95.00
90.00
85.00
80.00
75.00
70.00
BRIGHTNESS (L)
0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40
ASH CONTENT %

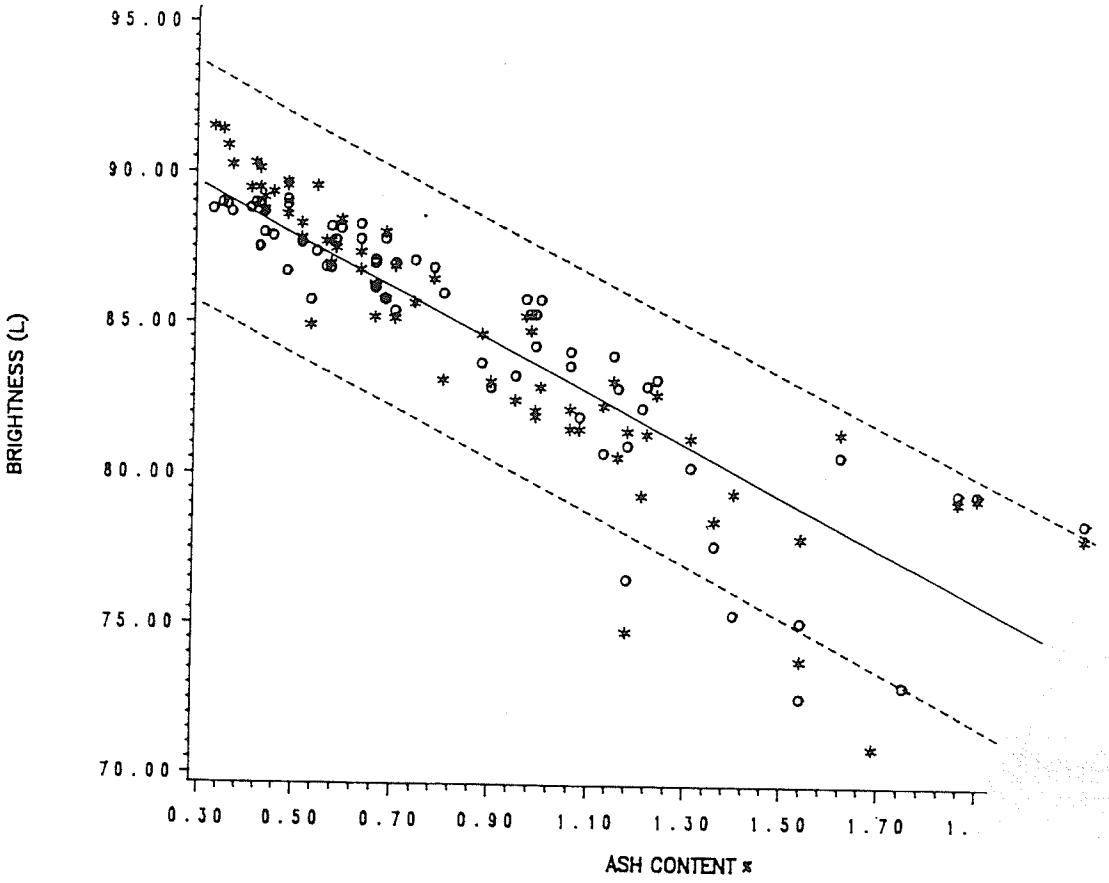

95.00
90.00
85.00
80.00
75.00
70.00
BRIGHTNESS (L)
0.30 0.50 0.70 0.90 1.10 1.30 1.50 1.70 1.
ASH CONTENT %

Figure 85 An Attempt to Model the Initial Kan Sui:Flour Paste Yellowness for the Variety: NORSTAR

* Observed

o Predicted

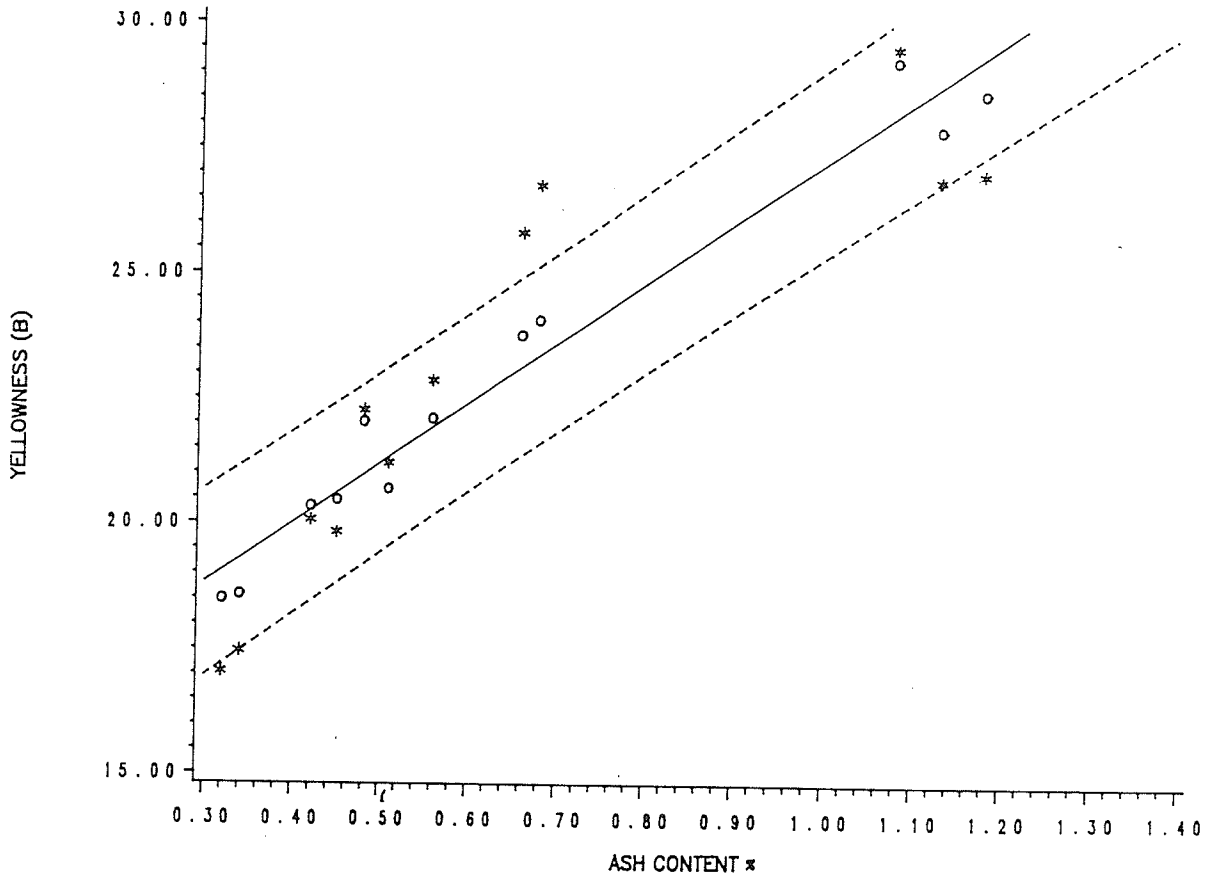
\- 95% Confidence Limit

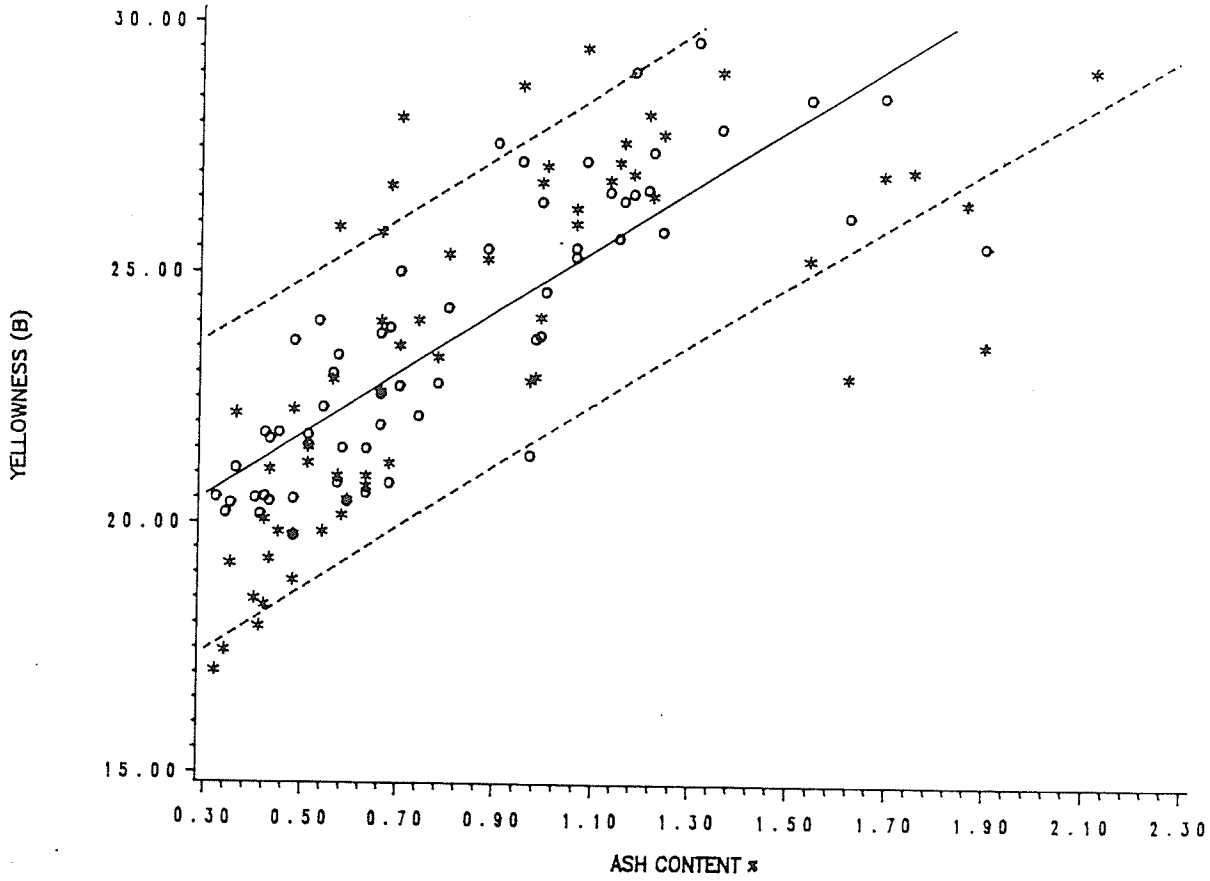
Figure 86 An Attempt to Model the Initial Kan Sui:Flour Paste Yellowness Independent of Variety

* Observed

o Predicted

\- 95% Confidence Limit


30.00
25.00
20.00
15.00
YELLOWNESS (B)
0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40
ASH CONTENT %


30.00
25.00
20.00
15.00
YELLOWNESS (B)
0.30 0.50 0.70 0.90 1.10 1.30 1.50 1.70 1.90 2.10 2.30
ASH CONTENT %

alkaline environment did not lend itself to a significant, $p < 0.05$, regression model for any variety using the regressor variables available.

Application of the selected variables towards modelling both brightness and yellowness, independent of the variety, was also assessed. Strong regression equations, $p < 0.0001$, were observed for both color components. Brightness displayed a correlation coefficient of 0.95 while yellowness had a value of 0.88. The red color component was also evaluated but did not yield a significant, $p < 0.05$, equation.

The ability to generate significant models for the flour pastes individual color indexes determined by the Hunter Lab suggested that the change observed over time might be modelled. The practical realization of the change in color however would be confined to the total color change observed as the human mind generates a perceived total color rather than individual color components.

Utilizing the three regression methodologies previously described, selection of regressor variables were determined. However only two variables, polyphenol oxidase and pigment content were found to be consistent across four of the five varieties. Including these variables in the model yielded significant, $p < 0.002$,regression models with correlations ranging from 0.87 for Fielder to 0.95 in Norstar. Plots of these varietal dependant total color changes are seen in Figs 87-92.

Figure 87 An Attempt to Model The Total Color Change (6 h) Observed In Katepwa's Pooled Flours

* Observed

o Predicted

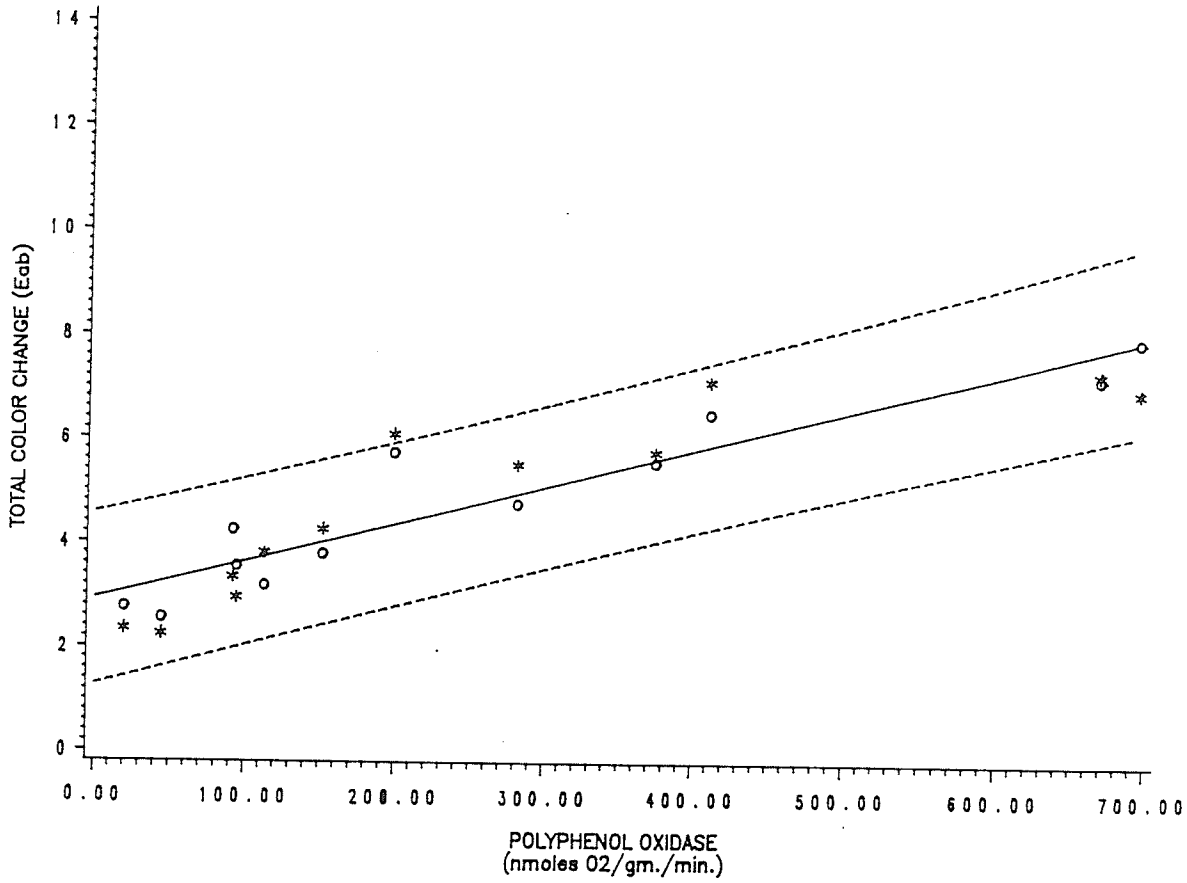
- 95% Confidence Limit

Figure 88 An Attempt To Model the Total Color Change (6 h) Observed in Glenlea's Pooled Flours

* Observed

o Predicted

- 95% Confidence Limit


TOTAL COLOR CHANGE (Eab)
14
12
10
8
6
4
2
0
0.00
100.00
200.00
300.00
400.00
500.00
600.00
700.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

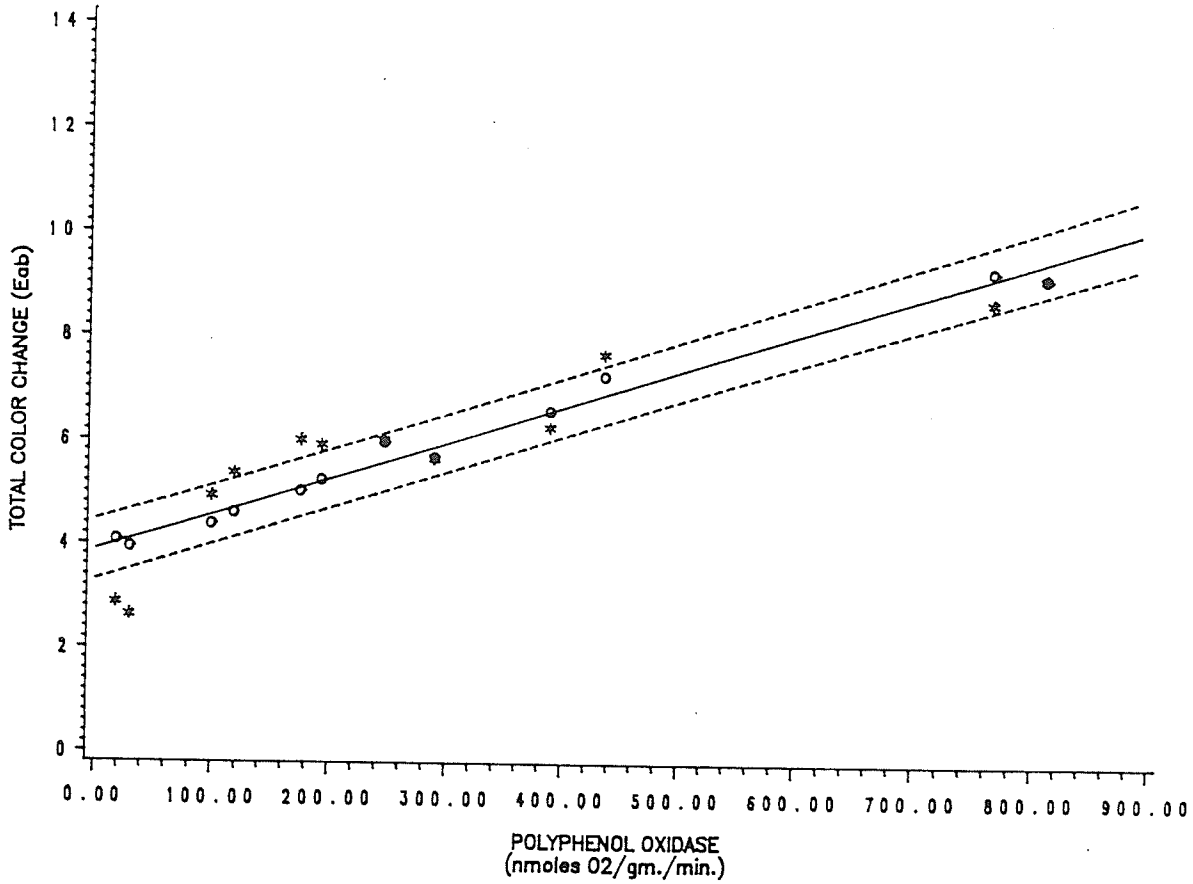

TOTAL COLOR CHANGE (Eab)
14
12
10
8
6
4
2
0
0.00
100.00
200.00
300.00
400.00
500.00
600.00
700.00
800.00
900.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 89 An Attempt to Model the Total Color Change (6 h) Observed in Norstar's Pooled Flours

* Observed

o Predicted

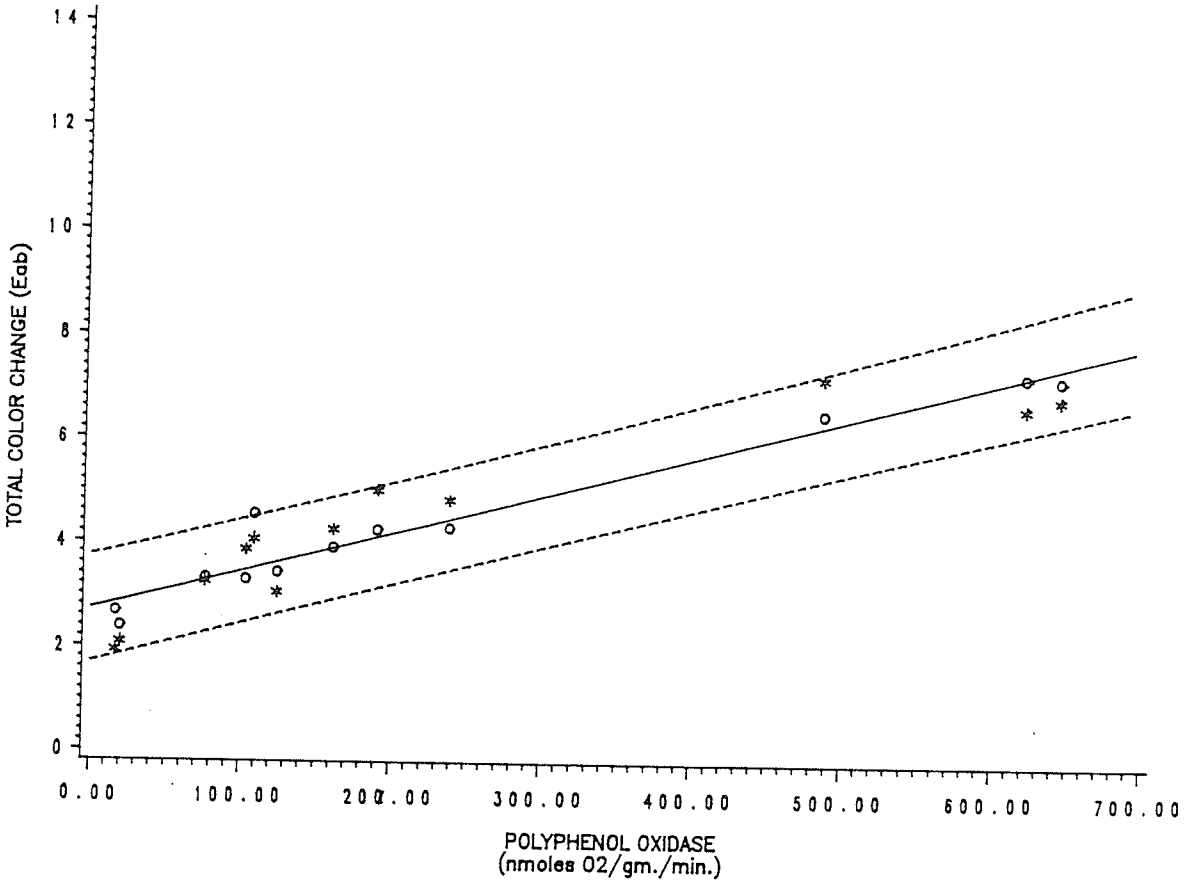
- 95% Confidence Limit

Figure 90 An Attempt to Model the Total Color Change (6 h) Observed in HY320's Pooled Flours

* Observed

o Predicted

- 95% Confidence Limit


TOTAL COLOR CHANGE (Eab)
0
2
4
6
8
10
12
14
0.00
100.00
200.00
300.00
400.00
500.00
600.00
700.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

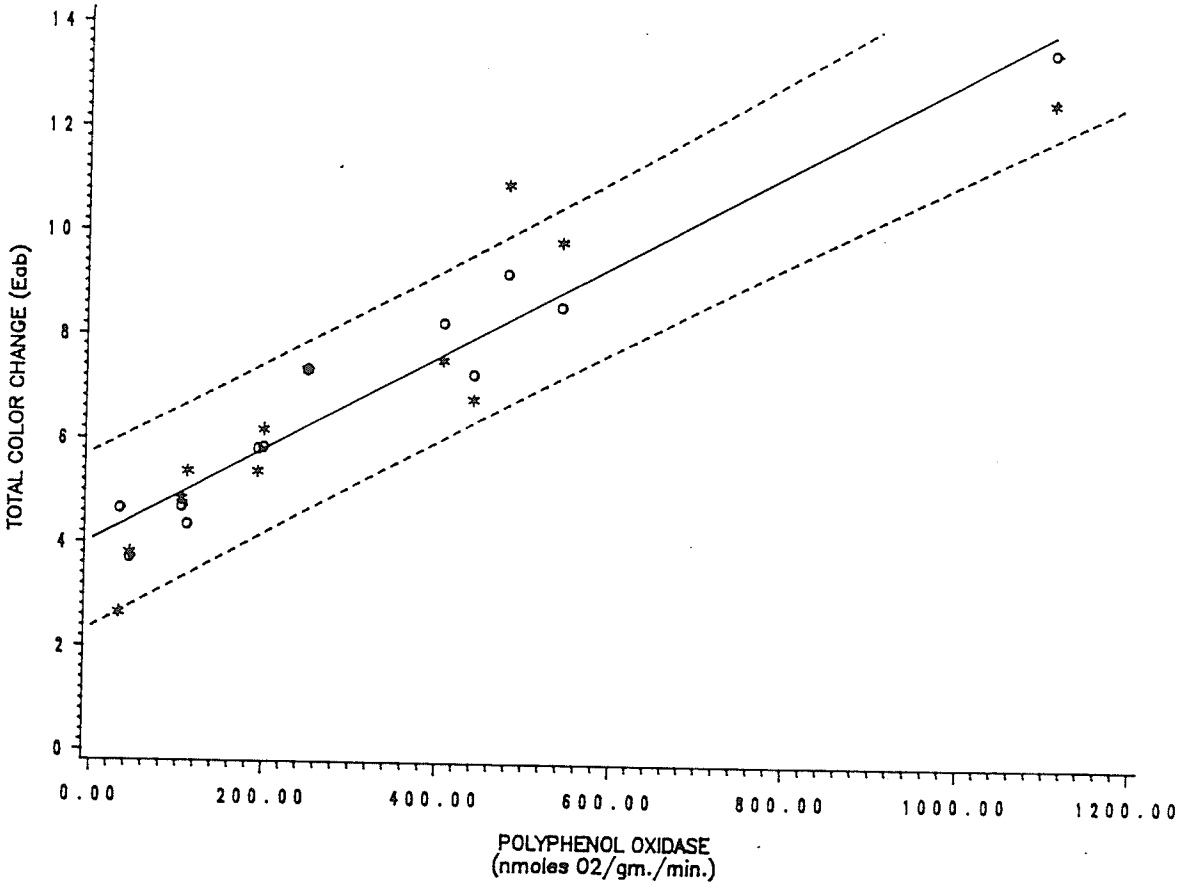

TOTAL COLOR CHANGE (Eab)
0
2
4
6
8
10
12
14
0.00
200.00
400.00
600.00
800.00
1000.00
1200.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

Figure 91 An Attempt to Model the Total Color Change (6 h) Observed in Fielder's Pooled Flours

* Observed

o Predicted

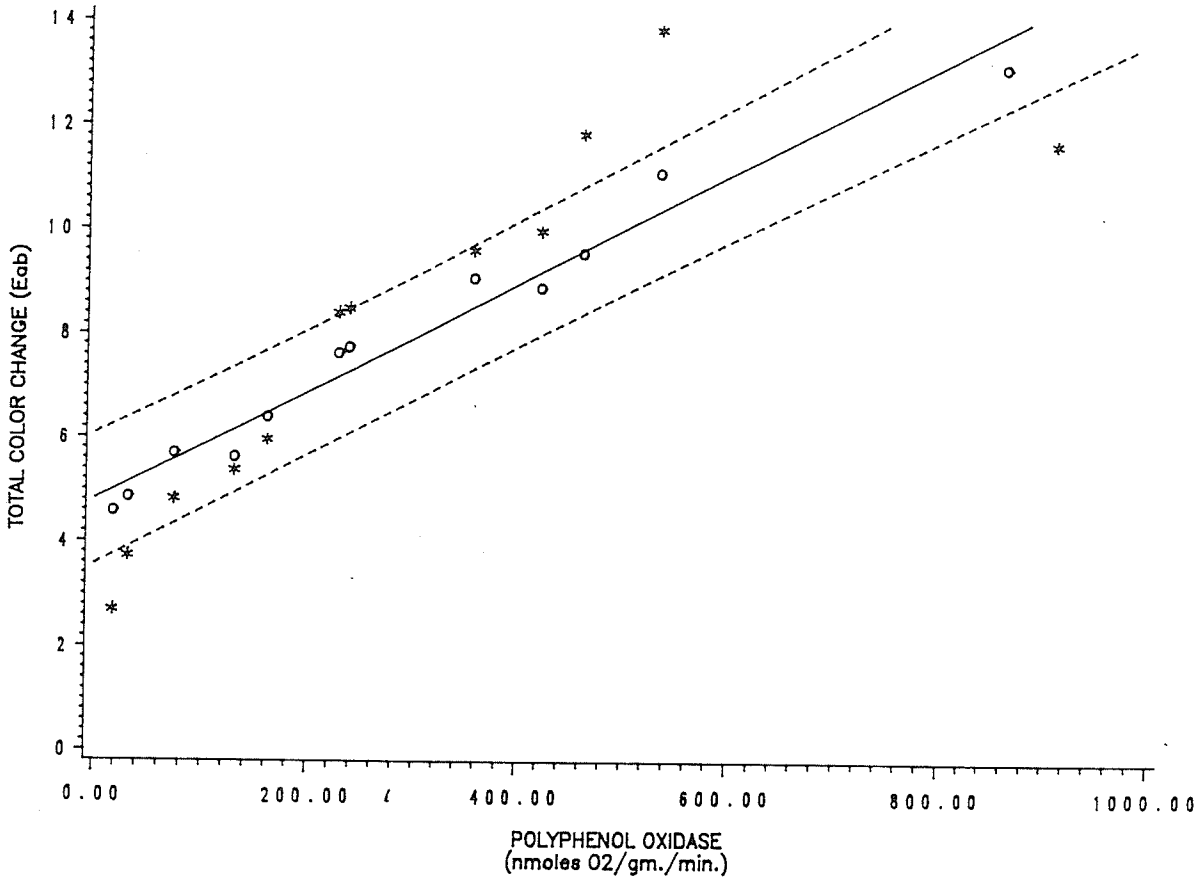
- 95% Confidence Limit

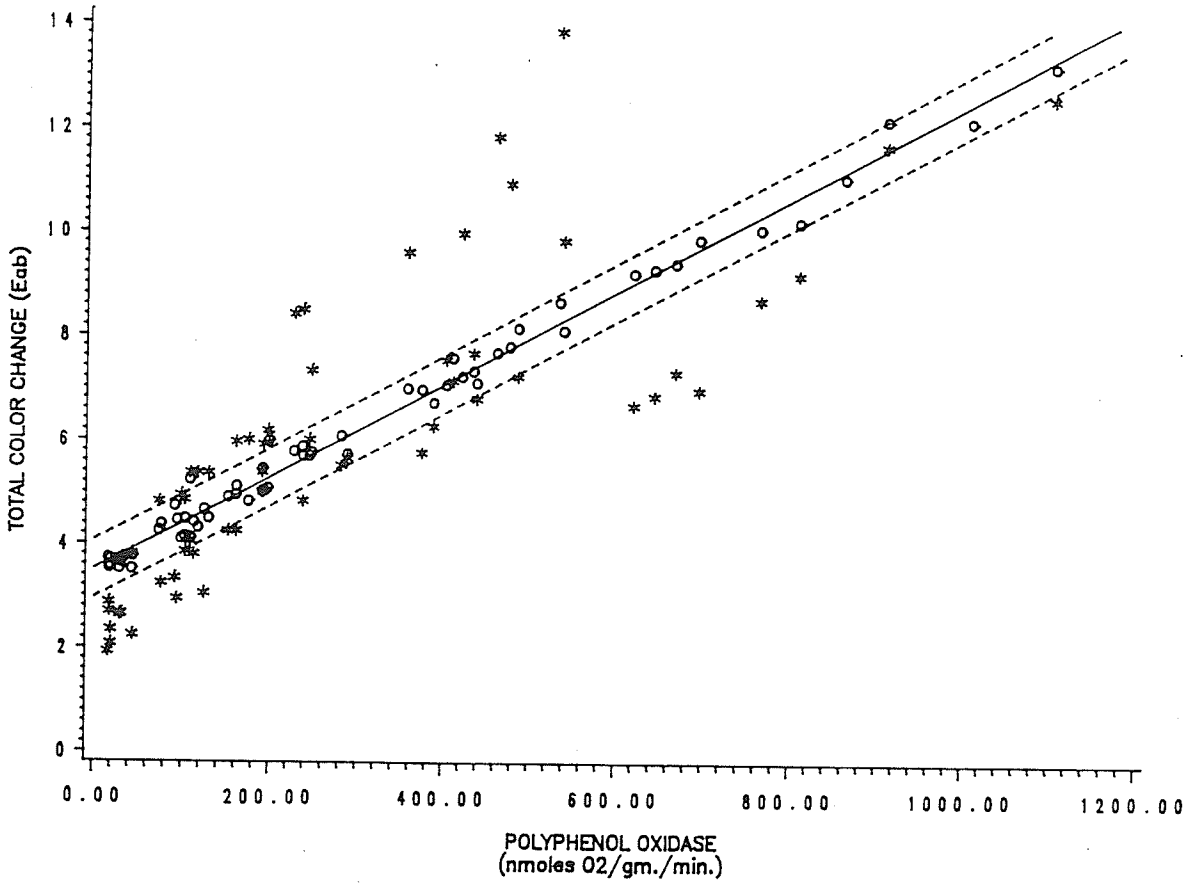
Figure 92 The Results of Attempting to Model Total Color Change (6 h) Observed in All Varieties

* Observed

o Predicted

- 95% Confidence Limit


TOTAL COLOR CHANGE (Eab)
0
2
4
6
8
10
12
14
0.00
200.00
400.00
600.00
800.00
1000.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)


TOTAL COLOR CHANGE (Eab)
0
2
4
6
8
10
12
14
0.00
200.00
400.00
600.00
800.00
1000.00
1200.00
POLYPHENOL OXIDASE
(nmoles 02/gm./min.)

The model was also calculated across varietal lines using the same two regressor variables. The significance level was found to remain high, p=0.0001, but the overall correlation coefficient declined to 0.81. The lower correlation was anticipated based upon the varied changes in individual color components by the varieties over time.

5.0 GENERAL CONCLUSIONS

The influence of polyphenol oxidase (PPO) on the final flour product appearance has been suggested by an number of authors (Abrol *et al* 1970, Abrol *et al* 1971, Singh and Sheoran 1972, Tikoo *et al* 1973). The initial objective of this research was to establish PPO levels within Canadian wheat classes milled to varying extraction rates. Previous work by Marsh and Galliard (1986) on wheat flour has indicated that the use of a variety of solvents, including detergents, was unable to solubilize more than 50% of the enzyme activity present. They suggested that in order to obtain an accurate determination of PPO levels in wheat flour, the oxygen electrode system, employing the solid sample, must be used. At the initiation of this project no studies had been undertaken to examine the individual mill stream's enzyme contribution to their flours utilizing this technique.

Investigation of all five varieties indicated that the enzyme content within a stream, regardless of the milling

extraction rate, maintained a linear relationship with ash content up to 2.5% ash. Examination of the PPO levels within the pooled flours revealed there was general agreement between the experimentally determined activity levels and those predicted on the basis of stream contribution. The combination of these two facets of wheat PPO are of particular relevance to the processing of the flour. They allow the miller the ability to estimate the enzyme level present based upon stream composition. It was observed that on a cumulative yield basis, regardless of the extraction rate, 75-85%, the cumulative PPO level remained low, less than 60 nmoles O2/g/min for each variety studied, up to a 60% cumulative yield. This value represented a range from 3%, to a maximum of only 7.0%, of the total enzyme present in the varieties assayed. These observations are of key importance to the possible use of Canadian wheats in non-traditional markets. It allows the miller the ability to maximize his flour yield on a given wheat, by milling to an elevated level, yet still providing products which can cater to different markets. It was found that across all varieties examined, less than 45 nmoles O2/g/min, were detected in the 80% extraction 1st patent flours. The enzyme levels noted would allow the miller to sell one flour to a high quality market, such as noodle production, while producing sufficient material for less stringent products such as steam buns. The choice of variety however was found to noticeably influence the PPO levels above the 60% cumulative

yield. The cumulative activities were shown to rise quickly past this point as the contributing stream's activity varied. An extreme example was Glenlea's 9th middling stream, at the 85% extraction level, being 50% greater than Katepwa's most active stream, and twice that found in the remaining varieties. Selection of a preferred variety would favor Norstar as the low enzyme levels in a large number of its streams resulted in the majority of its pooled flours at both the 75 and 80% extraction levels, as well as the 85% straight grade flour, to display the lowest enzyme levels.

It was unfortunate that no realistic relationship could be established between PPO and alpha amylase as most flours are routinely screened by users to determine amylase content. The presence of statistical correlations suggests that further investigation is warranted in this area. However, as the amylase levels in the sound samples were extremely low, practical application was extremely limited. Detection of alpha amylase is a key indicator of problems with undesirable germination. Kruger (1976) indicated that the PPO levels rise dramatically, 33 fold, in wheat after germination is initiated. Kruger's research involved liquid extracts of the grain samples and in light of Marsh and Galliard's (1986) findings, may have underestimated the full impact of germination on PPO levels. It may be possible to establish a meaningful relationship between the two enzymes, but only when there is a sufficient range in alpha amylase content within the streams.

The secondary objective of this research was to establish the phenolic acid content with representative Canadian wheat varieties' flours. Analysis of the phenolic components of various varietal flours yielded levels generally higher than reported in the literature. Unfortunately there are only a limited number of references to wheat flour phenolic levels for comparison. Agreement was noted with Sosulski *et al* (1982) and Jackson (1983) for insoluble acid content. Pussayanawin *et al* (1988) did report total ferulic acid in HRW wheat mill streams of sufficient quantity to agree in magnitude with this study's findings. However, no similarity was observed with either Sosulski's group or Jackson in terms of soluble bound or free acids. Their values were considerably lower than detected in the present study.

Ferulic acid dominated the overall phenolic composition as it was the only acid detected in the major phenolic constituent, the insoluble bound acids. This category accounted for over 80% of the total phenolics detected in any variety's flour. Sinapic acid however was found to be the premier soluble bound component in the majority of flours analyzed. Ferulic acid was consistently detected at the second highest content in all flours. The soluble bound phenolic acids accounted for a maximum of 17% of any flour's total phenolic acid composition. Analysis of the free phenolic acids revealed ferulic acid to be the major component and the complete absence of free sinapic acid in

any flour. Comparison of the whole wheats' total phenolic acid content indicated that the variety Norstar to be significantly distinct $p < 0.05$, from the remaining varieties. It was therefore not surprising to note that in the various pooled flours analyzed, Norstar consistently had the lower phenolic acid content for both insoluble and soluble bound acids.

The third and final objective of this study was to investigate the interaction between PPO and the endogenous phenolic acids in color production. In order for PPO to participate in the discoloration of a flour paste or dough there must be sufficient substrate present. The correlations between PPO and its phenolic substrates were quite evident in each variety. The vast majority of the phenolic acids measured displayed strong, significant correlations, $p > 0.05$, with both ash and PPO. Pussayanawin *et al* (1988), analyzing American hard red winter wheats' individual mill streams, prepared cumulative ferulic acid versus cumulative yield curves. Although no phenolic acid measurements were done on individual mill streams in this study, the relatively low and slowly increasing ferulic acid content displayed by Pussaywanwin and coworkers was very similar to that observed in this study's cumulative PPO versus yield figures. The similarly shaped curves supports the concept of the strong enzyme-substrate interdependence.

Examination of the interaction between PPO and phenolic acid content was also evident when changes in paste Hunter

L^*, a^*, and b^* values were monitored over time. In the 1st patent flours of each variety, where both PPO and phenolic acids were minimal, no distinction could be made between any 75 or 80% extraction flour Hunter color component over time. However, as the levels of PPO and phenolics increased, brightness was able to differentiate between pooled flours, 1st patent versus 2nd patent, and within flours, 75 versus 80% extraction rate, for each variety in over 90% of the remaining samples. Redness was able to differentiate in only 72% of the cases while paste yellowness tended to be unable to distinguish differences. Sapers *et al* (1989) also utilized changes in Hunter L^* values to establish significant relationships between brightness and both PPO activity and total phenolic content over time in six different potato cultivars. Use of the individual color components distinguished by the Hunter spectrocolorimeter confirmed the interaction of PPO and the phenolic acid constituents. The variety Norstar, seen previously to have the lowest PPO content in the flours and the minimum phenolic acid components, consistently displayed distinctly brighter flours than the other varieties . As well, Norstar exhibited the smallest change in brightness over time in the majority of its flours.

A number of authors have alluded to the complexity of color production involving PPO in non-ideal situations. Pierpoint (1966) discussed the reactions involving cholorgenic acid and the wide variety of products formed in

the presence of various reactive groups. In particular he noted the cherry red produced in the reaction with amines. He noted, as did Singleton (1987) that the initial derivatives of the reaction of gylcine and proline with the oxidized quinones underwent further oxidation producing intense colors. Singleton (1987) highlighted the continuous evolution of color and intensity of a simple oxidized benzoquinone as it underwent further polymerization. The flour pastes provided an environment in which the complex interactions and subsequent color production could be monitored. The lack of a noticeable temperature effect upon color production remained consistent with the bound nature of PPO (Marsh and Galliard 1986). The inherent poor solubility of the phenolic acids in water may also have had an influence on the results. The lack of notable increase in color production at the temperatures investigated would appear to be due to no appreciable increase in the collision frequency between components due to their limited mobility.

The complexity of the color production was apparent in the phenolic addition studies. In the present study caffeic acid addition had a distinctive impact on color levels not exhibited by the remaining phenolics. The addition of caffeic acid did result in a rapid decrease in color intensity determined by the Hunter method and the paste's reflectance spectrum. It was noted that unlike the other phenolics, caffeic acid invoked a rapid greying of the of the pastes which was evident by the decreased reflectance

across the entire spectrum but most notable in the later regions corresponding to the orange-red region. Phlobaphene, suggested by Miyamoto and Everson (1958) to be a polymer of catechin, is believed to supply the red pigment to wheat kernels. It may be possible that the reaction of the caffeic acid is having an influence on this compound. Singleton (1987) discussed the additional incorporation of nonoxidized material into subsequent polymerization without the need for direct enzymic oxidation. Such a mechanism could invoke the incorporation of previously distinct compounds altering the colored products. Furthermore, each subsequent product became increasingly reactive. An example of such a case was noted for anthocyanin involvement by Taylor and Clydesdale (1987). They reported that although pure anthocyanins will not react with PPO, due to steric reasons, they observed color production. They attributed this reaction to the indirect oxidation of these compounds by trace amounts of phenolic compounds. Singleton (1987) has suggested that in white wine, flavanoid content may play a significant role in color production. The large differences observed for caffeic acid are therefore suggested to be a combination of PPO's enhanced affinity for this phenolic acid in conjunction with possible involvement of other, more complex and indirectly oxidizable phenolics.

The color models proposed, although revealing strong correlations at significant confidence levels, are only intended to confirm the interaction of PPO and phenolic

acids in color production. Due to the limited sample size, they were developed to establish the feasibility of modelling color in the paste environment. The need to evaluate multiple samples from a variety of environmental conditions will be required to confirm these relationships.

In summation, initial polyphenol oxidase activity levels and the corresponding phenolic acid contents of flours representative of Canadian wheat classes have been determined. Subsequent interactions of these components influencing the flour paste's color have also been established.

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Appendix A: Pooled Wheat Flours Individual Stream Composition

Katepwa's Pooled Flour Stream Composition

Katepwa 75% Milling

1st Patent (45.0%)[a]= 1M (34.4%)[b] 2M (25.8%) 1S (13.3%) 2S (13.6) 3M (12.9%)

2nd Patent (22.5%)= 3M (7.6%) 4M (26.2%) 2B (16.9%) 3B (18.7%) 1B (11.1%) 6M (2.2%)

1st Clear (3.75%)= 6M (58.7%) 4B (26.7%)BF (14.6%)

2nd Clear (4.45%)= BF(86.5%) SD (13.5%)

Katepwa 80% Milling

1st Patent (45.0%)= 1M (44.2%) 2S (16.7%)1B (7.33%) 1S (20.0%) 2M (11,8%)

2nd Patent (22.5%)= 2M (15.6%) 3M (23.6%) 2B (24.4%) 3B (23.1%) 4M (13.3%)

1st Clear (6.15%)= 4M (71.5%) 5M (28.5%)

2nd Clear (6.15%)= 5M (12.2%) 4B (21.1%) 6M (26.0%) SD (4.9%) BF (35.8%)

Katepwa 85% Milling

Chinese Standard= 1B (9.6%) 3B (25.7%) 3M (22.8%) 4M (15.4%) 5M (6.3%) 6M (7.2%) 4B (10.3%) 8M (2.8%)

Note: At the 80% extraction milling the 7M was added to 4M, 8M was added to 5M, the 9M added to 6M, and the 5B added to the 4B.

1M= 1st Middling
2M= 2nd Middling
3M= 3rd Middling
4M= 4th Middling
5M= 5th Middling
6M= 6th Middling
7M= 7th Middling
8M= 8th Middling
9M= 9th Middling

1B= 1st Break
2B= 2nd Break
3B= 3rd Break
4B= 4th Break
5B= 5th Break
1S= 1st Sizings
2S= 2nd Sizings
SD= Shorts Duster
BF= Bran Flour

a= % of Cumulative Milling Yield
b= % contribution to Pooled Flour

Glenlea's Pooled Flour Stream Composition

Glenlea 75% Milling

1st Patent (45.0%)[a]=	1M (42.7%)[b] 2M (19.8%) 1S (12.0%) 2S (12.9%)
2nd Patent (22.5%)=	3M (6.7%) 4M (32.0%) 2B (10.7%) 3B (16.4%) 5M (18.2%) 6M (16.0%)
1st Clear (3.20%)=	6M (18.8%) 4B (37.5%) 1B (43.8%)
2nd Clear (3.20%)=	4B (6.3%) BF (65.6%) SD (28.1%)

Glenlea 80% Milling

1st Patent (45.0%)=	1M (47.6%) 2S (14.0%) 1S (15.1%) 2M (23.3%)
2nd Patent (22.5%)=	1S (1.3%) 4M (50.2%) 3M (28.0%) 2B (13.8%) 3B (6.7%)
1st Clear (5.75%)=	3B (38.3%) 1B (27.8%) 5M (33.9%)
2nd Clear (5.75%)=	5M (4.3%) 4B (24.3%) 6M (40.0%) SD (5.2%) BF (26.1%)

Glenlea 85% Milling

Chinese Standard=	1B (12.7%) 2B (8.2%) 3M (32.9%) 4M (20.8%) 5M (6.5%) 6M (9.2%) 4B (9.8%)

Note: At the 80% extraction milling the 7M was added to 4M, 8M was added to 5M, the 9M added to 6M, and the 5B added to the 4B.

1M= 1st Middling	1B= 1st Break
2M= 2nd Middling	2B= 2nd Break
3M= 3rd Middling	3B= 3rd Break
4M= 4th Middling	4B= 4th Break
5M= 5th Middling	5B= 5th Break
6M= 6th Middling	1S= 1st Sizings
7M= 7th Middling	2S= 2nd Sizings
8M= 8th Middling	SD= Shorts Duster
9M= 9th Middling	BF= Bran Flour

a= % of Cumulative Milling Yield
b= % contribution to Pooled Flour

Norstar's Pooled Flour Stream Composition

Norstar 75% Milling

1st Patent (45.0%)[a]=	1M (40.2%)[b] 2M (26.0%) 1S (14.2%) 2S (13.3%) 3M (6.2%)
2nd Patent (22.5%)=	3M (19.6%) 4M (31.1%) 1B (5.3%) 2B (14.2%) 3B (17.3%) 5M (12.4%)
1st Clear (5.00%)=	6M (20.0%) 1B (80.0%)
2nd Clear (5.00%)=	4B (20.0%) BF (60.0%) SD (20.0%)

Norstar 80% Milling

1st Patent (45.0%)=	1M (46.0%) 2S (13.8%) 1S (15.8%) 2M (24.4%)
2nd Patent (22.5)=	2M (5.3%) 3M (28.4%) 2B (16.4%) 3B (16.8%) B1 (10.7%) 4M (22.2%)
1st Clear (6.30%)=	4M (76.2%) 5M (23.8%)
2nd Clear (6.30%)=	5M (9.5%) 4B (15.9%) 6M (31.8%) BF (38.1%) SD (4.8%)

Norstar 85% Milling

Chinese Standard=	2B (2.7%) 1B (13.7%) 3M (18.9%) 4M (26.5%) 5M (5.9%) 4B (7.2%) 5B (2.1%) 6M (7.0%) 7M (7.0%) 8M (3.7%) 9M (1.4%) BF (2.0%) SD (2.1%)

Note: At the 80% extraction milling the 7M was added to 4M, 8M was added to 5M, the 9M added to 6M, and the 5B added to the 4B.

1M= 1st Middling	1B= 1st Break
2M= 2nd Middling	2B= 2nd Break
3M= 3rd Middling	3B= 3rd Break
4M= 4th Middling	4B= 4th Break
5M= 5th Middling	5B= 5th Break
6M= 6th Middling	1S= 1st Sizings
7M= 7th Middling	2S= 2nd Sizings
8M= 8th Middling	SD= Shorts Duster
9M= 9th Middling	BF= Bran Flour

a= % of Cumulative Milling Yield
b= % contribution to Pooled Flour

Fielder's Pooled Flour Stream Composition

Fielder 75% Milling

1st Patent (45.5%)[a]=	1M (37.1%)[b] 2M (15.6%) 1S (9.8%) 2S (14.0%) 3M (12.9%) 4M (10.7%)
2nd Patent (22.5%)=	4M (5.8%) 1B (16.0%) 2B (20.9%) 3B (16.4%) 5M (11.1%) 6M (15.1%) BF (14.7%)
1st Clear (2.10%)=	4B (33.3%) BF (57.1%) SD (9.5%)
2nd Clear (2.10%)=	SD (100%)

Fielder 80% Milling

1st Patent (45.0%)=	1M (39.1%) 2S (19.1%) 1S (8.4%) 2M (20.2%) 3M (0.2%) 2B (12.9%)
2nd Patent (22.5%)=	3M (30.2%) 3B (18.7%) 1B (19.1%) 4M 32.0%)
1st Clear (5.05%)=	4M (37.6%) 4B (10.9%) BF (51.5%)
2nd Clear (5.05%)=	5M (41,6%) 4B (14.9%) 6M (35.6%) SD (7.9%)

Fielder 85%

Chinese Standard=	1B (1.5%) 3B (14.7%) 3M (12.8%) 4M (29.4%) 5M (7.9%) 6M (6.3%) 4B (7.8%) 7M (8.6%) 8M (3.4%) 9M (1.3%) BF (2.9%)

Note: At the 80% extraction milling the 7M was added to 4M, 8M was added to 5M, the 9M added to 6M, and 5B added the 4B

1M= 1st Middling	1B= 1st Break
2M= 2nd Middling	2B= 2nd Break
3M= 3rd Middling	3B= 3rd Break
4M= 4th Middling	4B= 4th Break
5M= 5th Middling	5B= 5th Break
6M= 6th Middling	1S= 1st Sizings
7M= 7th Middling	2S= 2nd Sizings
8M= 8th Middling	SD= Shorts Duster
9M= 9th Middling	BF= Bran Flour

a= % of Cumulative Milling Yield
b= % contribution to Pooled Flour

HY 320's Pooled Flour Stream Composition

HY 320 75% Milling

1st Patent (45.0%)[a]= 1M (38.2%)[b] 2M (17.1%) 1S (14.4%) 2S (14.8%) 3M (13.8%) 2B (1.6%)

2nd Patent (22.5%)= 4M (20.9%) 2B (16.8%) 3B (20.4%) 1B (18.7%) 5M (14.2%) 6M (8.9%)

1st Clear (3.75%)= 6M (37.3%) BF (62.7%)

2nd Clear (3.75%)= 4B (24.0%) BF (38.7%) SD (37.3%)

HY 320 80% Milling

1st Patent (45.0%)= 1M (39.3%) 2S (14.7%) 1B (0.4%) 1S (12.9%) 2M (22.0%) 2B (10.7%)

2nd Patent (22.5%)= 1B (18.2%) 3M (27.1%) 3B (19.1%) 4M (35.6%)

1st Clear (5.55%)= 4M (54.1%) BF (45.9%)

2nd Clear (5.55%)= 5M (41.4%) 4B (19.8%) 6M (30.6%) BF (2.7%) SD (5.4%)

HY 320 85% Milling

Chinese Standard= 1B (5.5%) 3B (13.4%) 3M (13.7%) 4M (21.3%) 5M (10.7%) 6M (6.6%) 4B (8.3%) 7M (11.3%) 8M (3.1%) BF (4.0%)

Note: At the 80% extraction milling the 7M was added to 4M, 8M was added to 5M, the 9M added to 6M, and the 5B added to the 4B

1M= 1st Middling	1B= 1st Break
2M= 2nd Middling	2B= 2nd Break
3M= 3rd Middling	3B= 3rd Break
4M= 4th Middling	4B= 4th Break
5M= 5th Middling	5B= 5th Break
6M= 6th Middling	1S= 1st Sizings
7M= 7th Middling	2S= 2nd Sizings
8M= 8th Middling	SD= Shorts Duster
9M= 9th Middling	BF= Bran Flour

a= % of Cumulative Milling Yield
b= % of Pooled Flour

Appendix B: Polyphenol Oxidase Levels in Individual Mill Streams

KATEPWA

Polyphenol Oxidase Levels in Mill Streams of Varying Extraction Rate

(nmoles O_2/g/min)

Stream	75%	Coeff. of Var. %	80%	Coeff. of Var. %	85%	Coeff. of Var. %
B1	124.6	2.6	140.5	3.2	201.4	2.2
B2	75.6	4.8	94.3	4.1	79.6	2.6
B3	59.7	4.6	98.3	3.9	82.0	3.5
B4	277.4	6.1	497.5	5.2	1410.0	4.8
B5					3239.0	7.1
S1	13.1	3.5	21.1	4.3	31.8	4.3
S2	0.0	0.0	4.0	6.1	17.1	4.8
M1	11.9	6.0	29.1	3.6	25.1	5.1
M2	23.9	4.2	45.0	2.2	51.7	4.8
M3	38.6	4.1	50.5	2.3	336.7	3.0
M4	62.5	4.9	258.7	4.2	195.8	3.4
M5	132.5	3.6	441.0	6.6	710.0	4.3
M6	172.3	4.2	548.0	7.1	585.0	4.6
M7					2754.0	6.3
M8					4195.0	8.2
M9					3868.0	6.4
SD	689.7	7.4	914.6	4.3	3773.0	7.8
BF	696.5	7.0	784.5	4.9	5142.0	8.1

GLENLEA

Polyphenol Oxidase Levels in Mill Streams of Varying Extraction Rate
(nmoles O_2 /g/min)

Stream	75%	Coeff. of Var. %	80%	Coeff. of Var. %	85%	Coeff. of Var. %
B1	298.5	5.2	366.2	4.5	398.0	3.4
B2	188.7	4.8	201.4	4.2	265.8	4.9
B3	132.9	6.2	137.7	5.3	59.7	4.6
B4	339.1	3.6	716.4	6.4	116.2	3.8
B5					4060.0	6.6
S1	25.1	5.0	35.8	3.2	50.9	4.8
S2	11.9	8.2	11.1	6.2	11.9	5.2
M1	21.9	3.5	27.9	4.8	22.3	4.7
M2	45.0	3.6	41.0	4.1	56.9	4.2
M3	54.5	4.7	76.8	3.2	150.0	3.8
M4	66.1	6.1	22.9	4.8	116.2	2.8
M5	127.4	4.3	679.8	6.4	834.0	5.3
M6	121.8	3.8	716.4	5.2	627.0	3.4
M7					2786.0	5.2
M8					3900.0	6.4
M9					4378.0	7.2
SD	1401.0	7.6	1328.0	6.3	7960.0	9.2
BF	366.2	6.2	1130.0	5.8	3980.0	7.6

NORSTAR

Polyphenol Oxidase Levels in Mill Streams of Varying Extraction Rate

(nmoles O_2 /g/min)

Stream	75%	Coeff. of Var. %	80%	Coeff. of Var. %	85%	Coeff. of Var. %
B1	51.7	3.2	106.1	2.9	148.6	4.6
B2	33.2	3.9	18.3	4.9	67.7	3.4
B3	41.1	3.8	61.7	2.5	92.9	1.8
B4	366.0	2.8	647.0	4.8	796.0	4.7
B5					1061.0	6.2
S1	14.6	4.6	57.0	2.1	38.5	3.7
S2	6.6	5.2	10.6	5.0	18.7	4.8
M1	13.3	5.1	30.5	3.6	33.2	4.2
M2	13.9	5.0	29.0	3.4	42.6	3.6
M3	31.8	4.9	50.9	2.9	408.6	4.2
M4	54.7	4.3	290.5	2.2	206.9	2.9
M5	141.3	2.6	1071.0	5.7	573.0	4.1
M6	141.3	3.4	1014.0	4.9	318.0	3.6
M7					1273.0	2.8
M8					2070.0	3.9
M9					3502.0	5.1
SD	445.0	5.3	1008.0	4.8	1990.0	6.1
BF	323.0	4.9	371.0	5.2	684.0	5.2

HY320

Polyphenol Oxidase Levels in Mill Streams of Varying Extraction Rate

(nmoles O_2 /g/min)

Stream	75%	Coeff. of Var. %	80%	Coeff. of Var. %	85%	Coeff. of Var. %
B1	92.7	1.9	115.4	2.8	105.5	3.9
B2	55.7	2.2	54.1	3.1	71.6	3.3
B3	62.5	4.7	86.0	3.4	129.0	3.5
B4	386.0	5.6	859.7	4.9	1072.0	4.8
B5					1831.0	5.9
S1	39.8	3.4	52.9	2.6	33.8	4.6
S2	15.9	5.1	17.9	4.6	8.0	5.8
M1	38.6	4.8	21.1	3.2	17.2	5.3
M2	45.8	3.6	9.2	5.6	58.4	4.1
M3	52.9	2.8	91.5	3.8	485.0	4.8
M4	101.1	3.8	323.0	2.7	196.0	3.2
M5	175.1	2.9	945.0	4.3	515.0	3.6
M6	204.6	3.3	907.0	4.4	724.0	4.8
M7					2295.0	5.1
M8					3237.0	5.8
M9					4537.0	7.2
SD	668.6	4.8	1305.0	4.7	2547.0	5.3
BF	359.8	3.3	662.0	4.6	1604.0	4.9

FIELDER

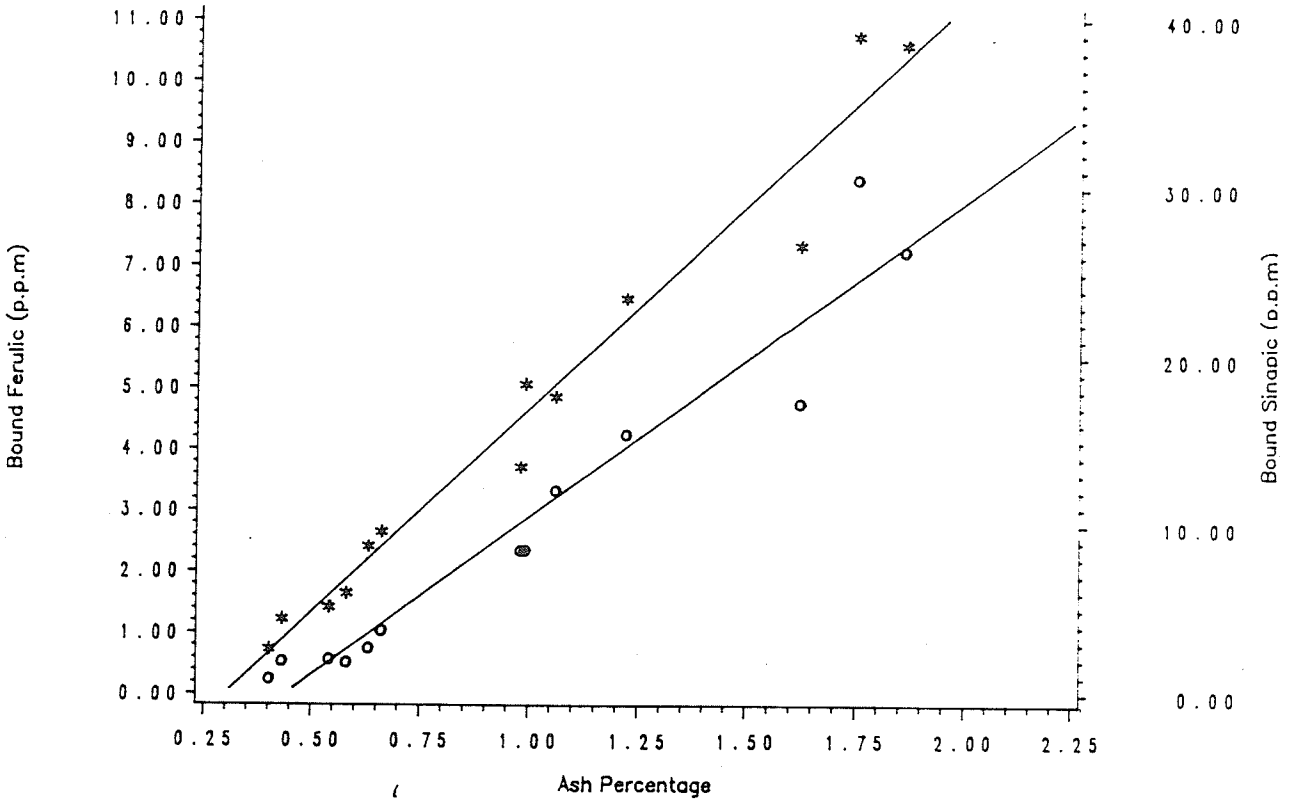
Polyphenol Oxidase Levels in Mill Streams of Varying Extraction Rate

(nmoles O_2 /g/min)

Stream	75%	Coeff. of Var. %	80%	Coeff. of Var. %	85%	Coeff. of Var. %
B1	173.9	4.3	92.7	4.6	191.0	3.4
B2	114.2	3.4	111.4	3.8	119.0	1.9
B3	76.8	5.1	197.0	4.2	55.8	2.8
B4	348.1	4.6	493.5	5.7	987.0	3.9
B5					1274.0	4.6
S1	19.9	3.6	19.9	5.1	11.9	5.7
S2	11.9	4.6	4.0	5.0	8.0	5.3
M1	15.9	5.8	19.1	4.6	8.0	4.8
M2	21.1	5.6	27.9	3.2	30.4	4.9
M3	34.6	5.3	51.7	2.4	245.8	1.6
M4	51.7	4.2	168.3	3.9	110.8	2.2
M5	133.3	4.2	481.6	4.6	544.0	4.1
M6	156.4	4.6	478.8	5.2	265.0	3.5
M7					1963.0	5.2
M8					3423.0	6.0
M9					3662.0	5.8
SD	689.3	5.6	1082.6	5.4	2896.0	6.1
BF	737.1	3.9	716.4	5.1	1393.0	5.3

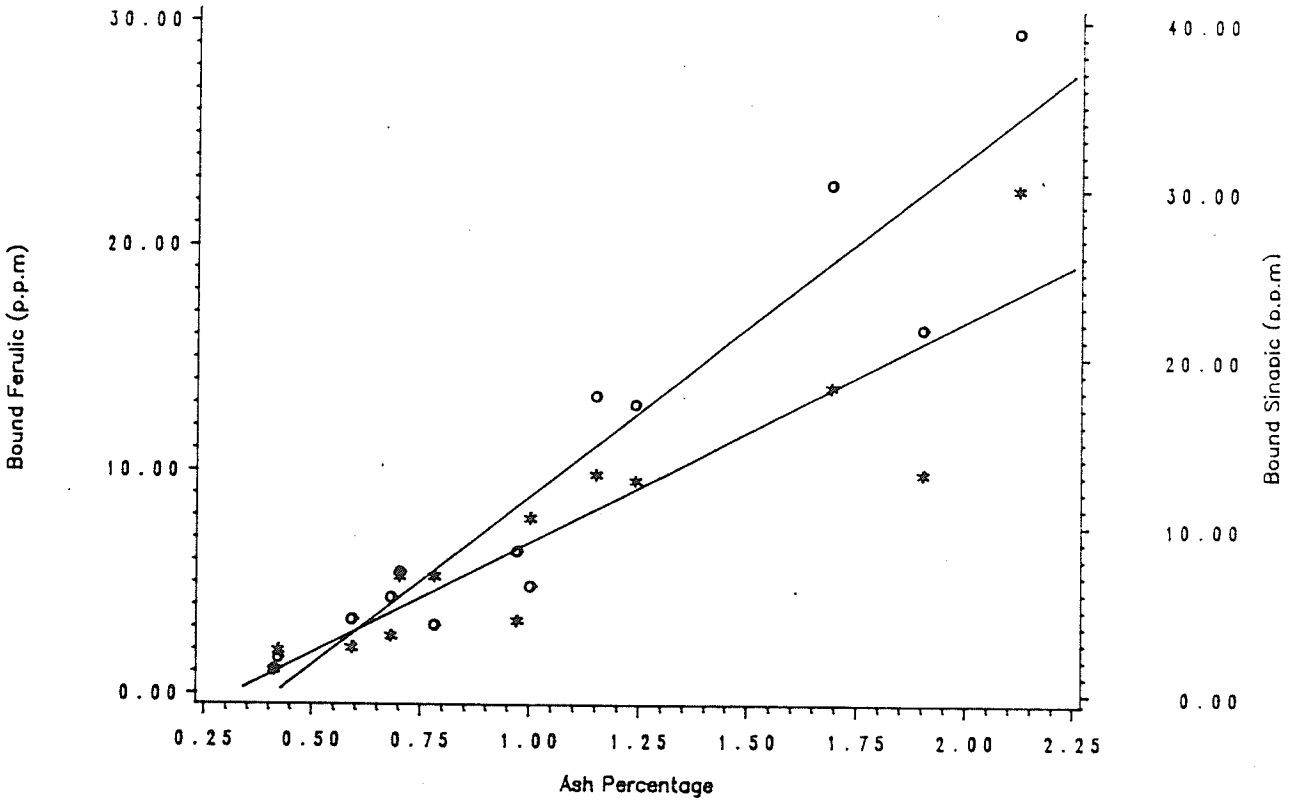
Appendix C: Soluble Bound Phenolic Acids' Relationship With Ash Content

Soluble Bound Phenolic Acid vs Ash Content
KATEPWA: Ferulic and Sinapic Acids



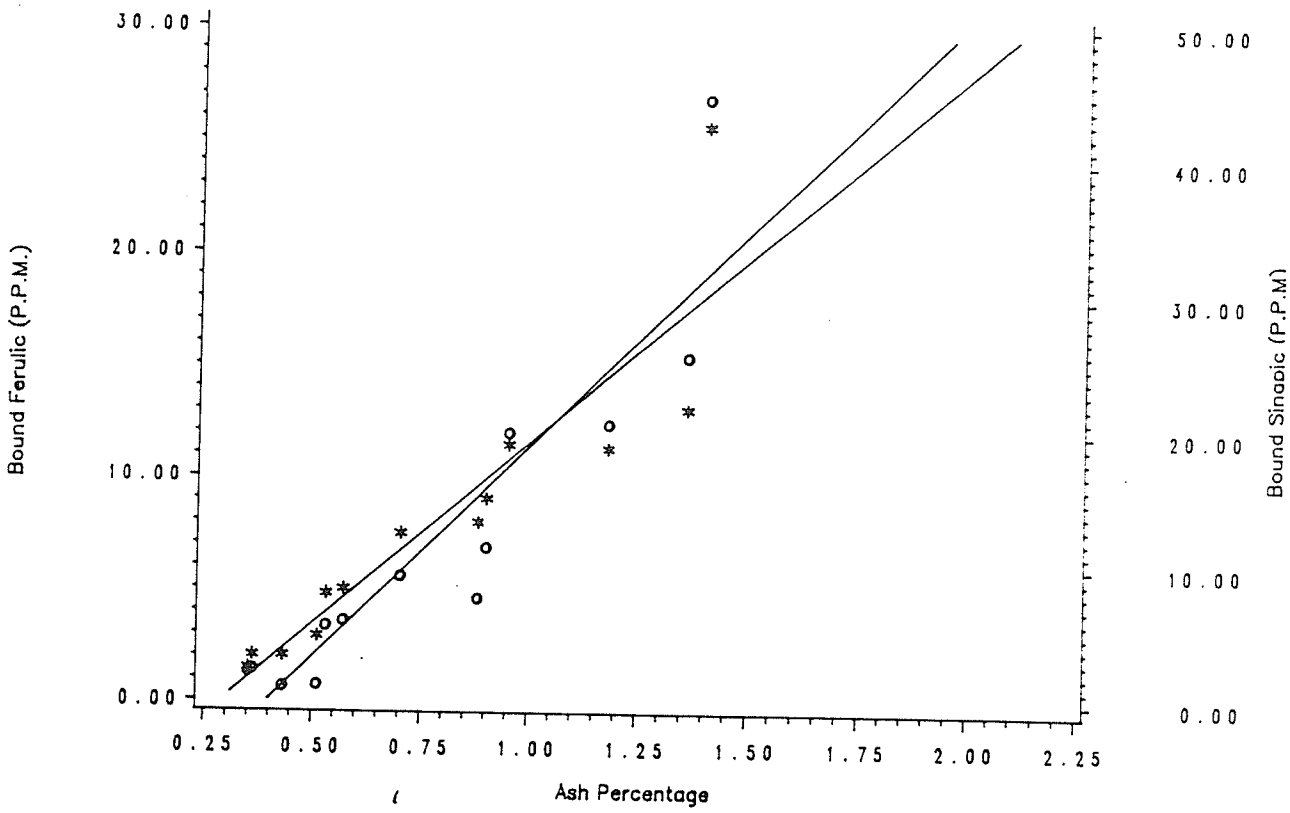
Phenolic Acids: * Ferulic o Sinapic

Soluble Bound Phenolic Acid vs Ash Content
GLENLEA: Ferulic and Sinapic Acids



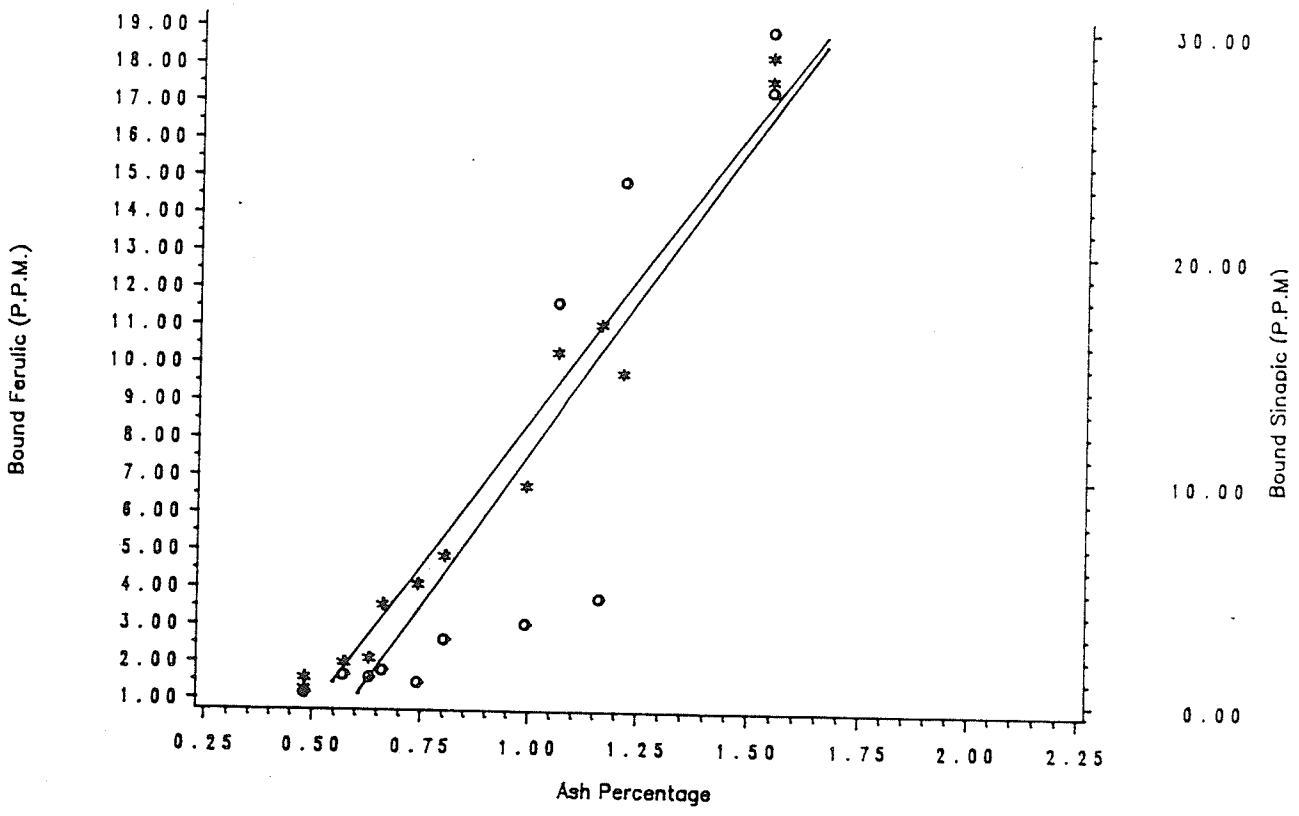
Phenolic Acids: * Ferulic o Sinapic

Soluble Bound Phenolic Acid vs Ash Content
FIELDER: Ferulic and Sinapic Acids



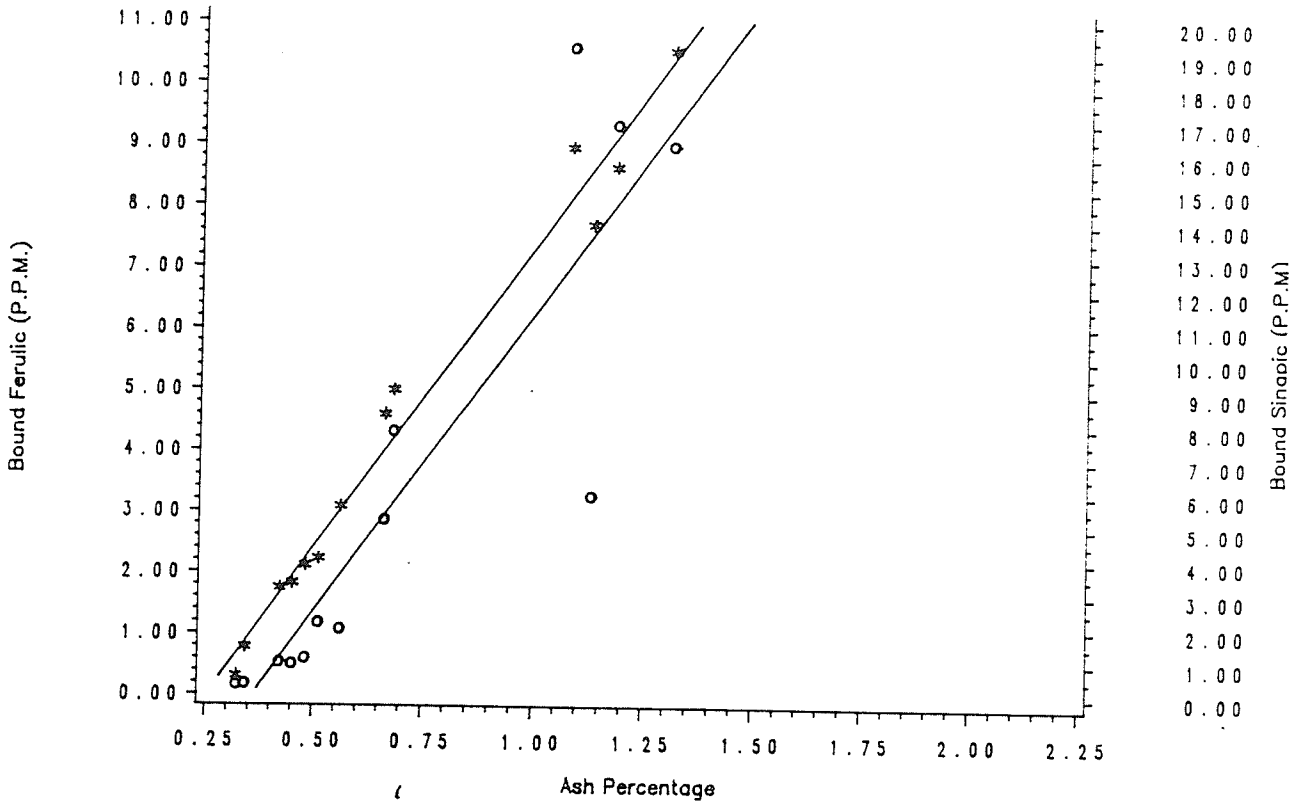
Phenolic Acids: * Ferulic o Sinapic

Soluble Bound Phenolic Acid vs Ash Content
HY320: Ferulic and Sinapic Acids



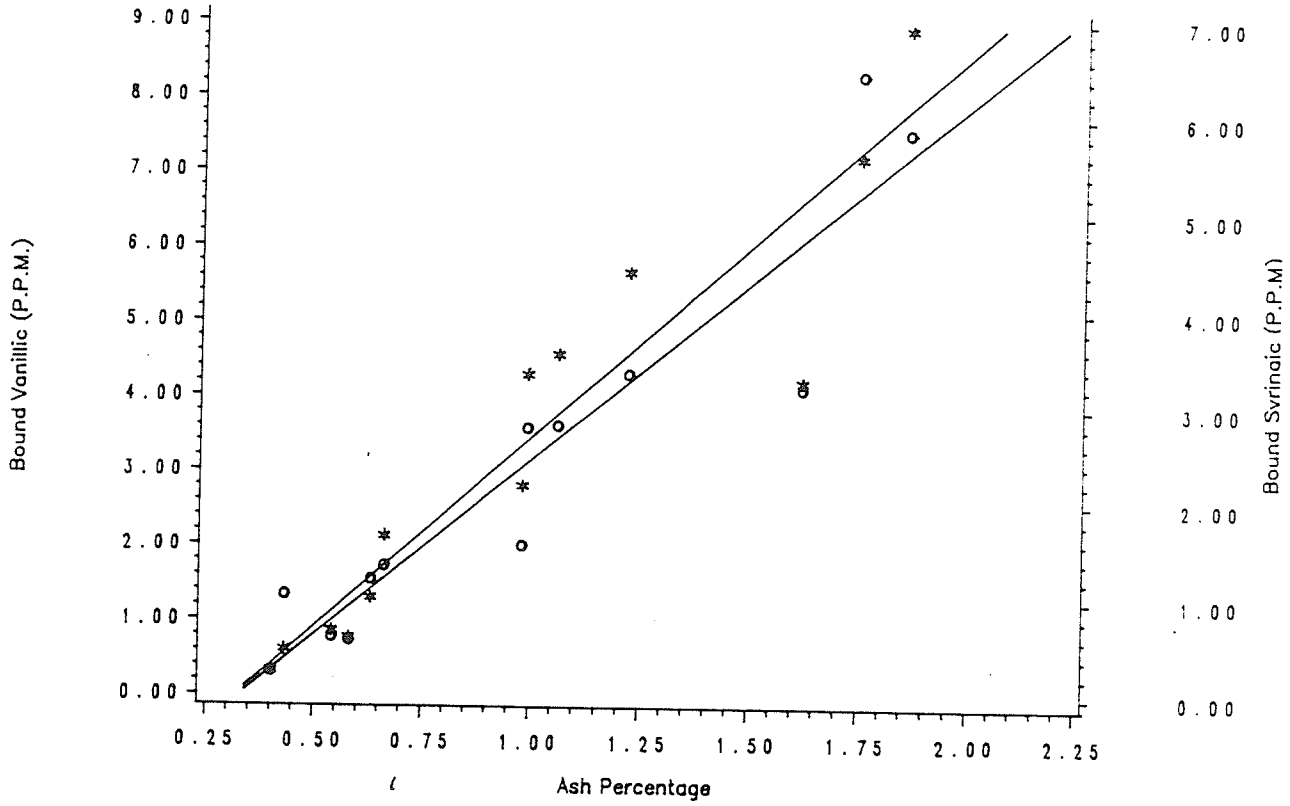
Phenolic Acids: * Ferulic o Sinapic

Soluble Bound Phenolic Acid vs Ash Content
NORSTAR: Ferulic and Sinapic Acids



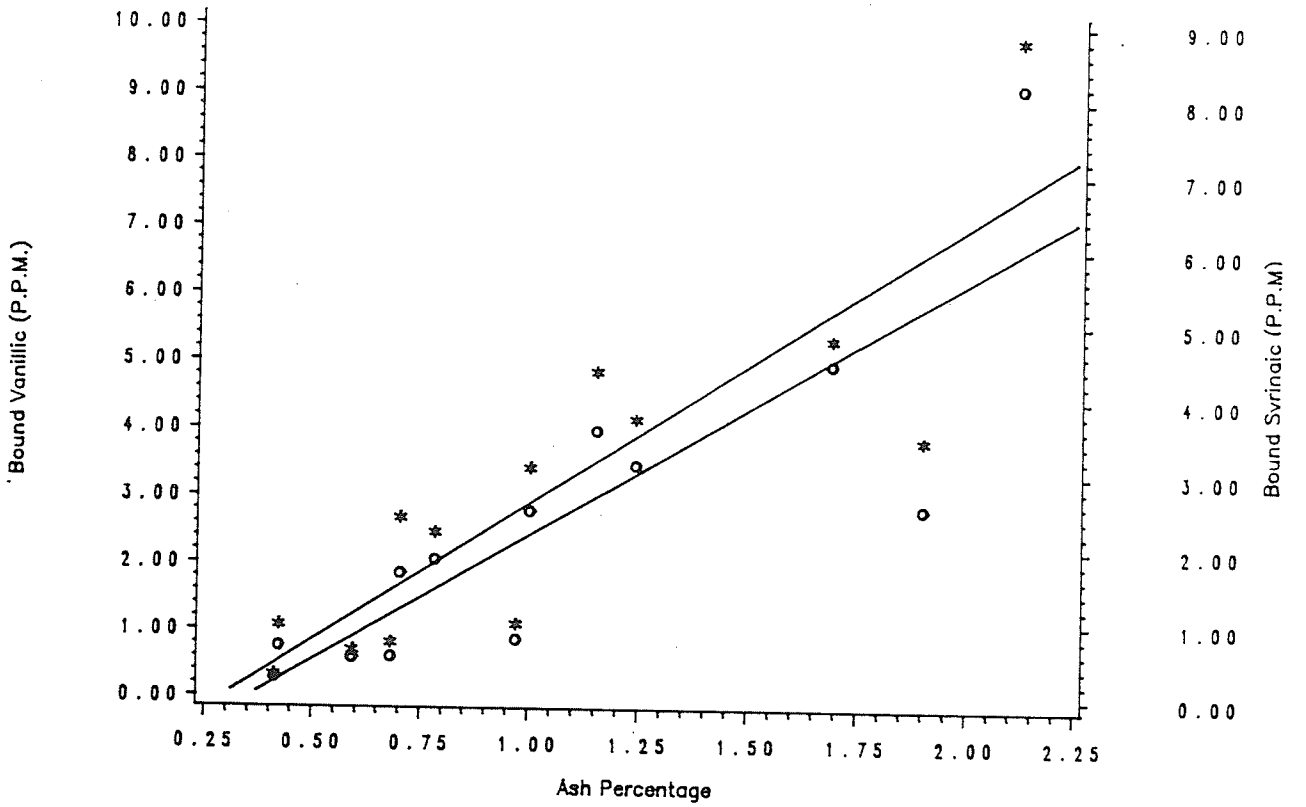
Phenolic Acids: * Ferulic o Sinapic

Soluble Bound Phenolic Acid vs Ash Content
KATEPWA: Vanillic and Syringic Acids



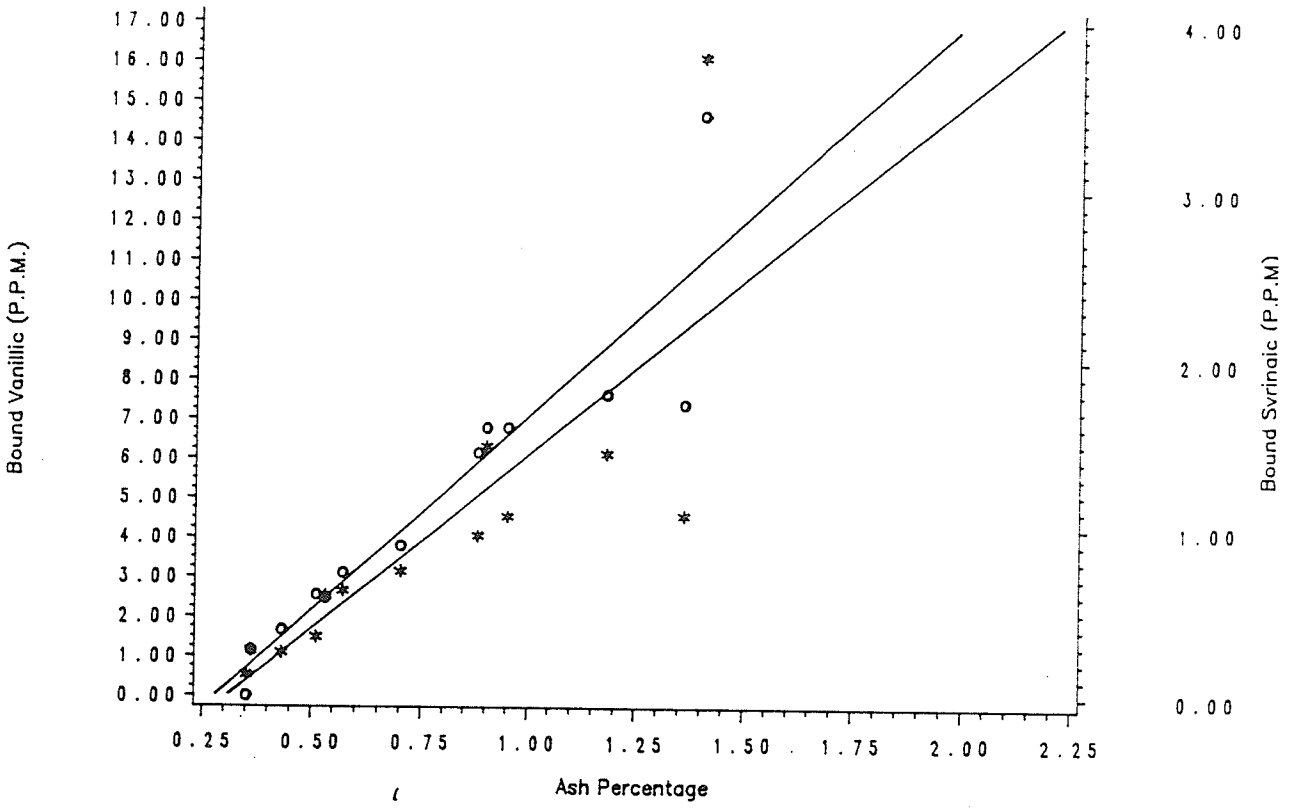
Phenolic Acids: * Vanillic o Syringic

Soluble Bound Phenolic Acid vs Ash Content
GLENLEA: Vanillic and Syringic Acids



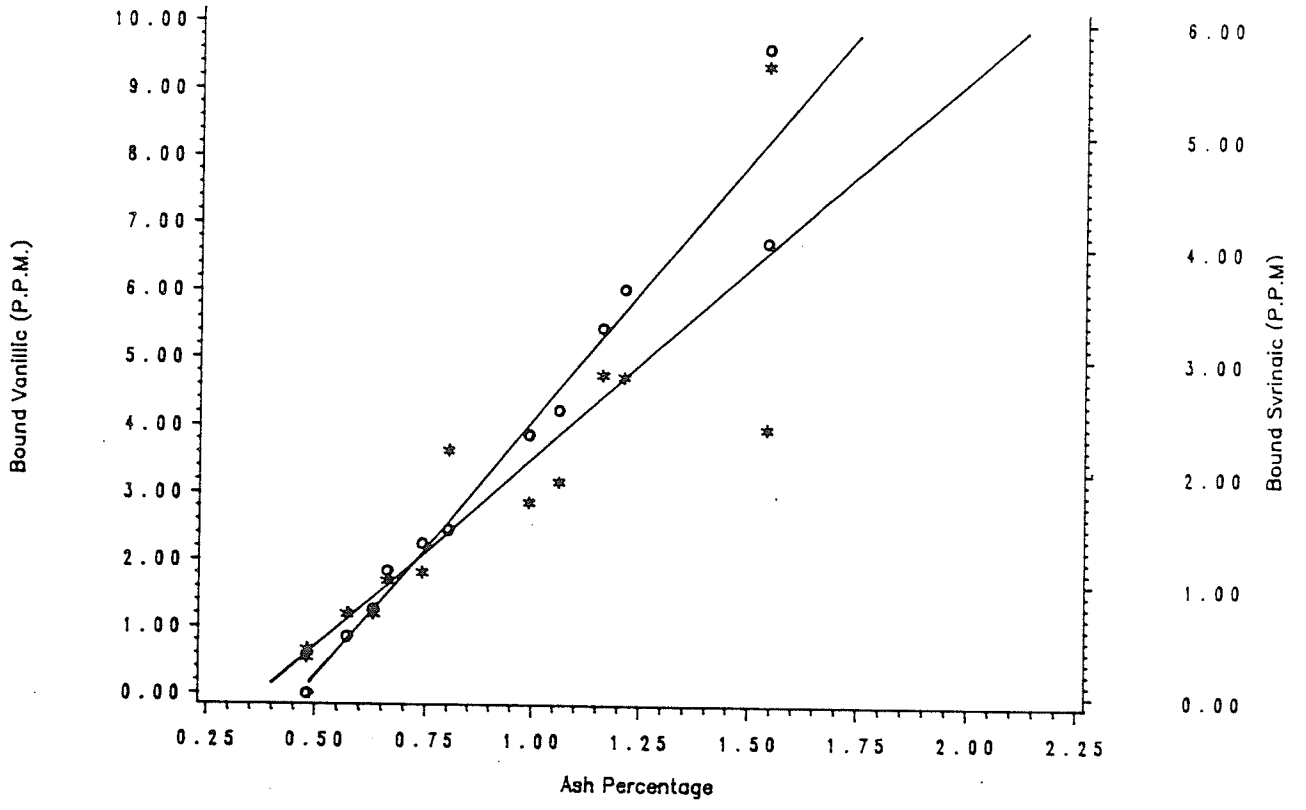
Phenolic Acids: * Vanillic o Syringic

Soluble Bound Phenolic Acid vs Ash Content
FIELDER: Vanillic and Syringic Acids



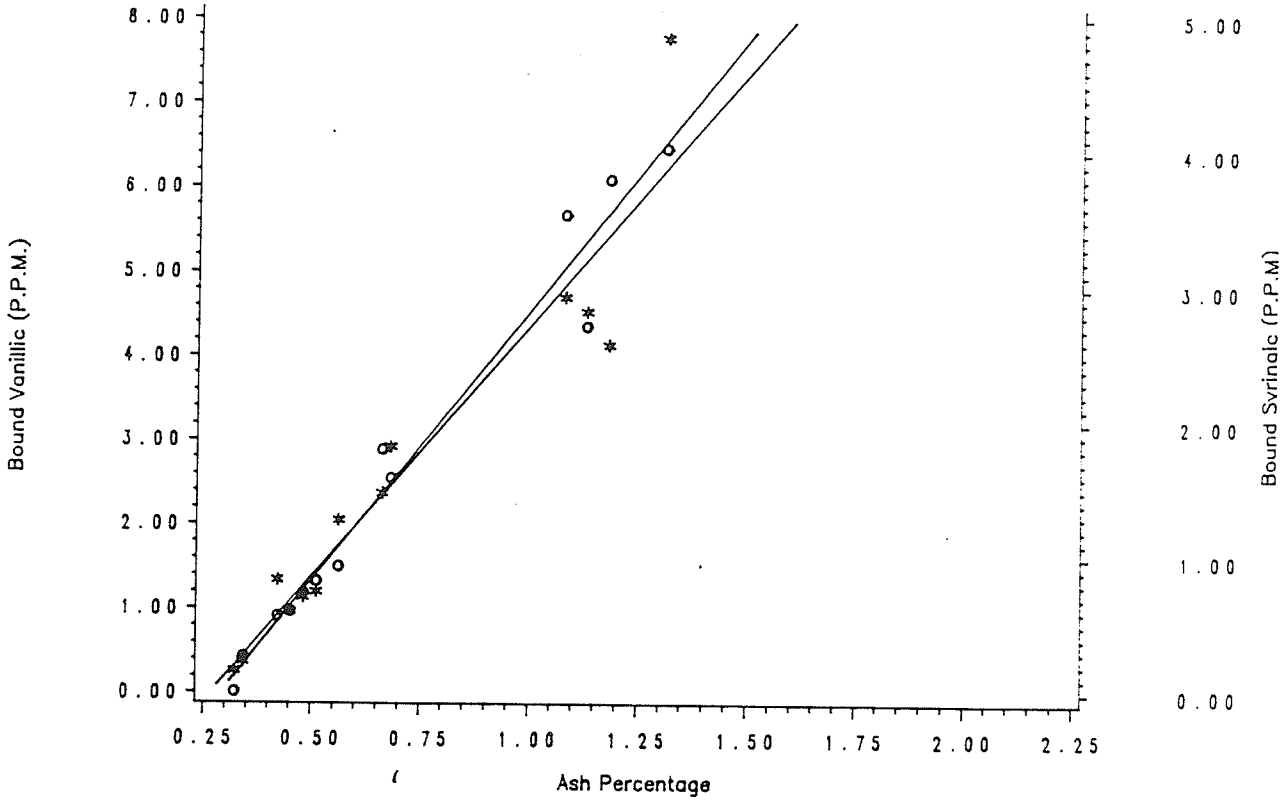
Phenolic Acids: * Vanillic o Syringic

Soluble Bound Phenolic Acid vs Ash Content
HY320: Vanillic and Syringic Acids



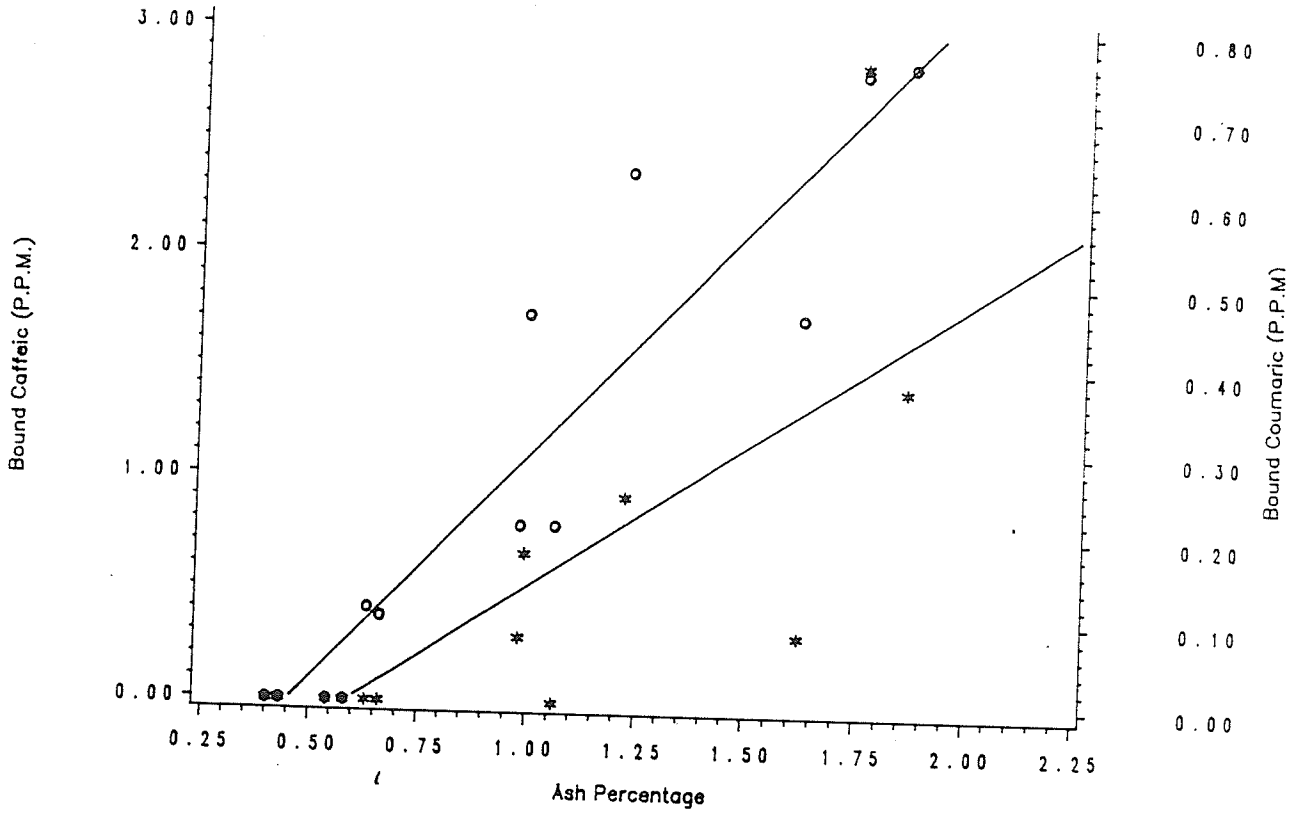
Phenolic Acids: * Vanillic o Syringic

Soluble Bound Phenolic Acid vs Ash Content
NORSTAR: Vanillic and Syringic Acids



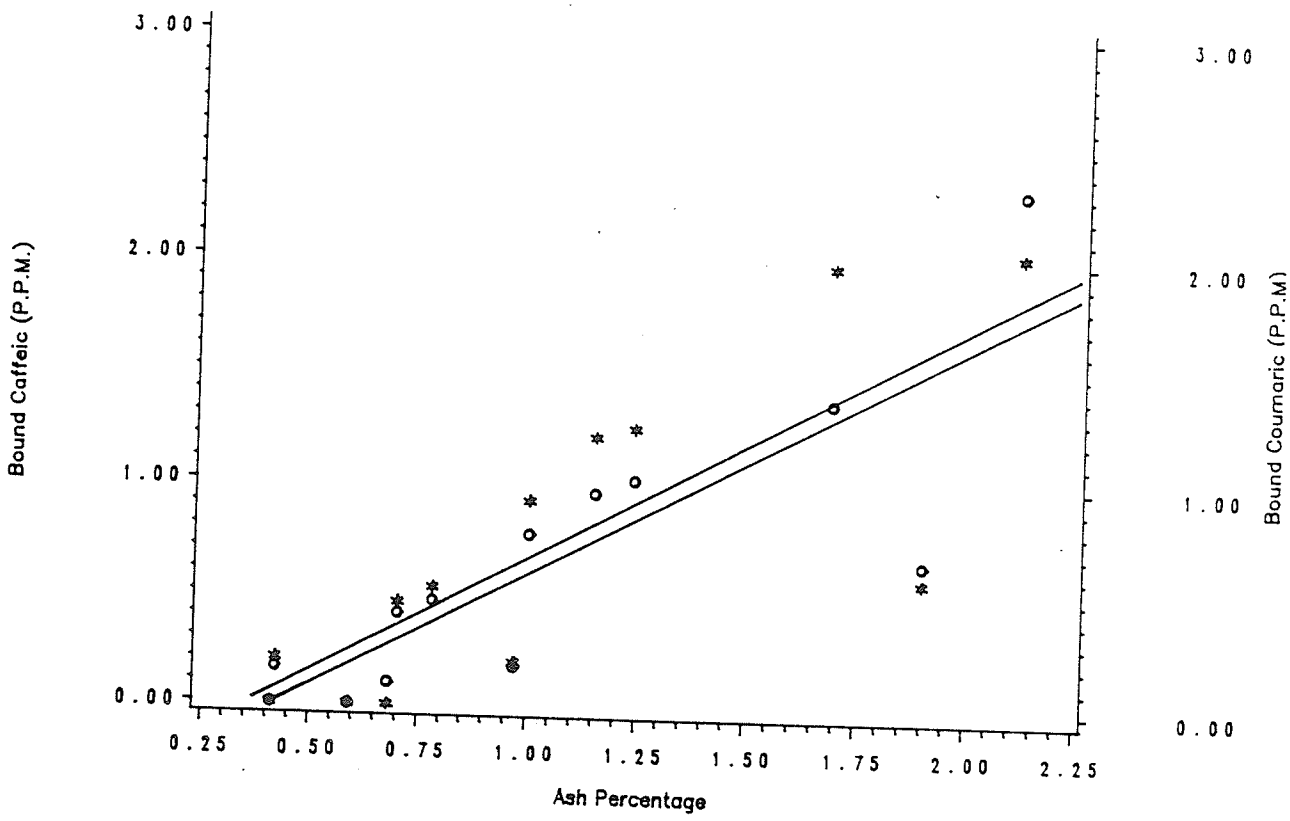
Phenolic Acids: • Vanillic o Syringic

Soluble Bound Phenolic Acid vs Ash Content
KATEPWA: Caffeic and Coumaric Acids



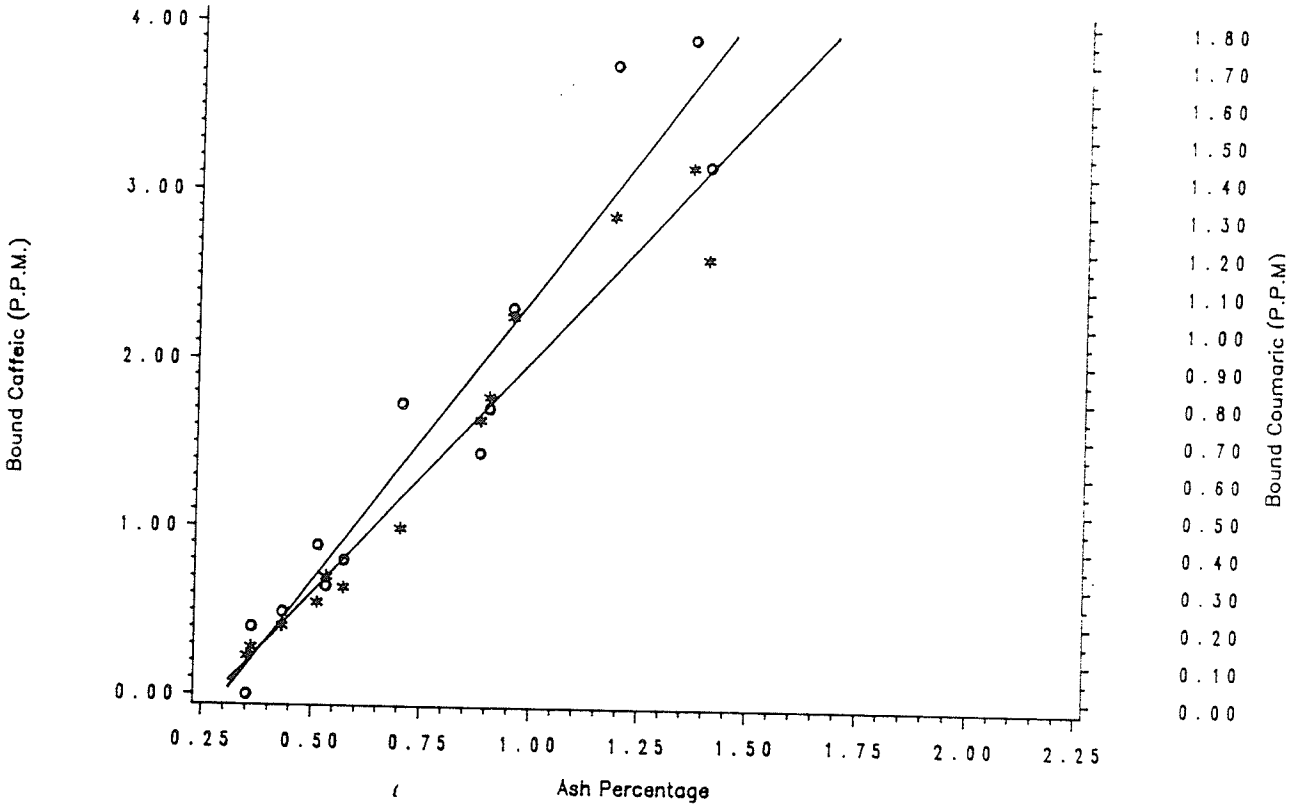
Phenolic Acids: * Caffeic o Coumaric

Soluble Bound Phenolic Acid vs Ash Content
GLENLEA: Caffeic and Coumaric Acids



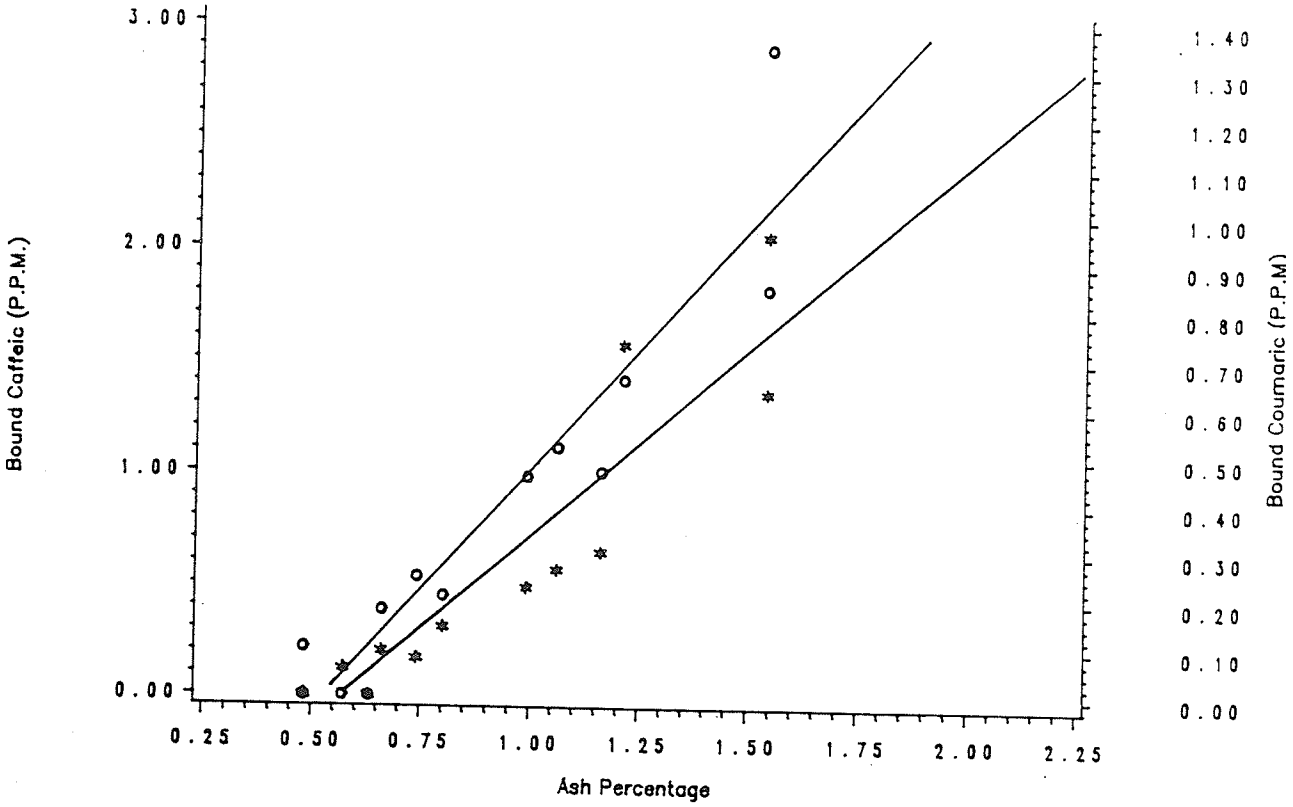
Phenolic Acids: * Caffeic o Coumaric

Soluble Bound Phenolic Acid vs Ash Content
FIELDER: Caffeic and Coumaric Acids



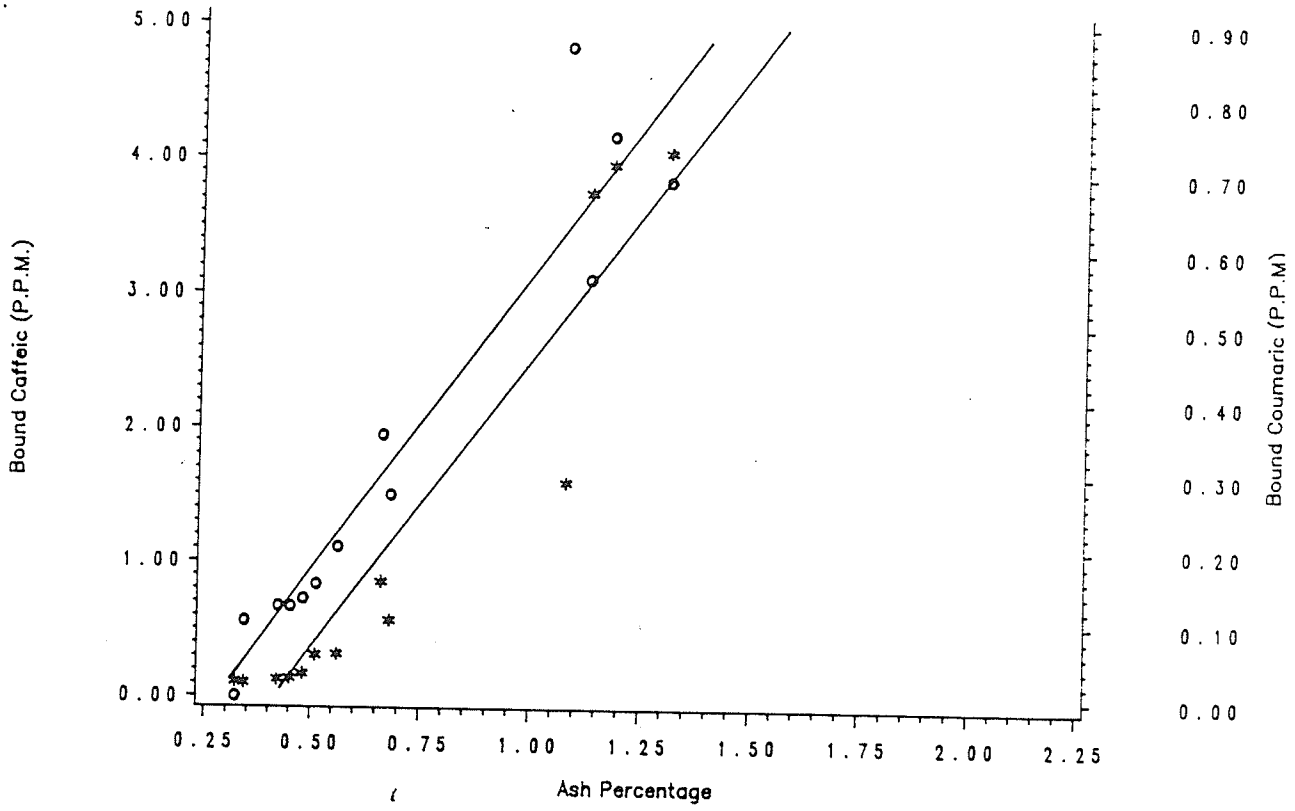
Phenolic Acids: * Caffeic o Coumaric

Soluble Bound Phenolic Acid vs Ash Content
HY320: Caffeic and Coumaric Acids



Phenolic Acids: * Caffeic o Coumaric

Soluble Bound Phenolic Acid vs Ash Content
NORSTAR: Caffeic and Coumaric Acids



Phenolic Acids: * Caffeic o Coumaric

Appendix D: Flour Paste HunterLab Values Over Time

Maximum Range in Respective Color Component
for a 95% Confidence Limit

Brightness (L^*)

Flour	Katepwa	Glenlea	Norstar	HY320	Fielder
75% Extraction					
1st Patent	0.60	1.82	0.65	0.39	3.38
2nd Patent	2.04	2.08	0.66	1.16	1.25
1st Clear	1.56	3.78	0.39	1.36	1.30
2nd Clear	2.16	1.82	0.91	1.60	1.64
St. Grade	0.96	1.30	0.85	0.54	2.73
80% Extraction					
1st Patent	1.01	1.76	0.87	1.06	1.74
2nd Patent	1.24	1.35	0.62	0.40	2.03
1st Clear	2.21	2.57	0.60	1.10	0.87
2nd Clear	3.36	5.13	1.65	2.88	1.74
St. Grade	1.92	0.41	0.42	0.66	1.45
85% Extraction					
St. Grade	1.53	1.28	0.98	3.60	0.53
Chinese	1.45	2.43	1.33	1.62	0.75
Whole Wheat	4.08	3.84	2.69	1.17	0.56

Maximum Range in Respective Color Component
for a 95% Confidence Limit

Yellowness (b^*)

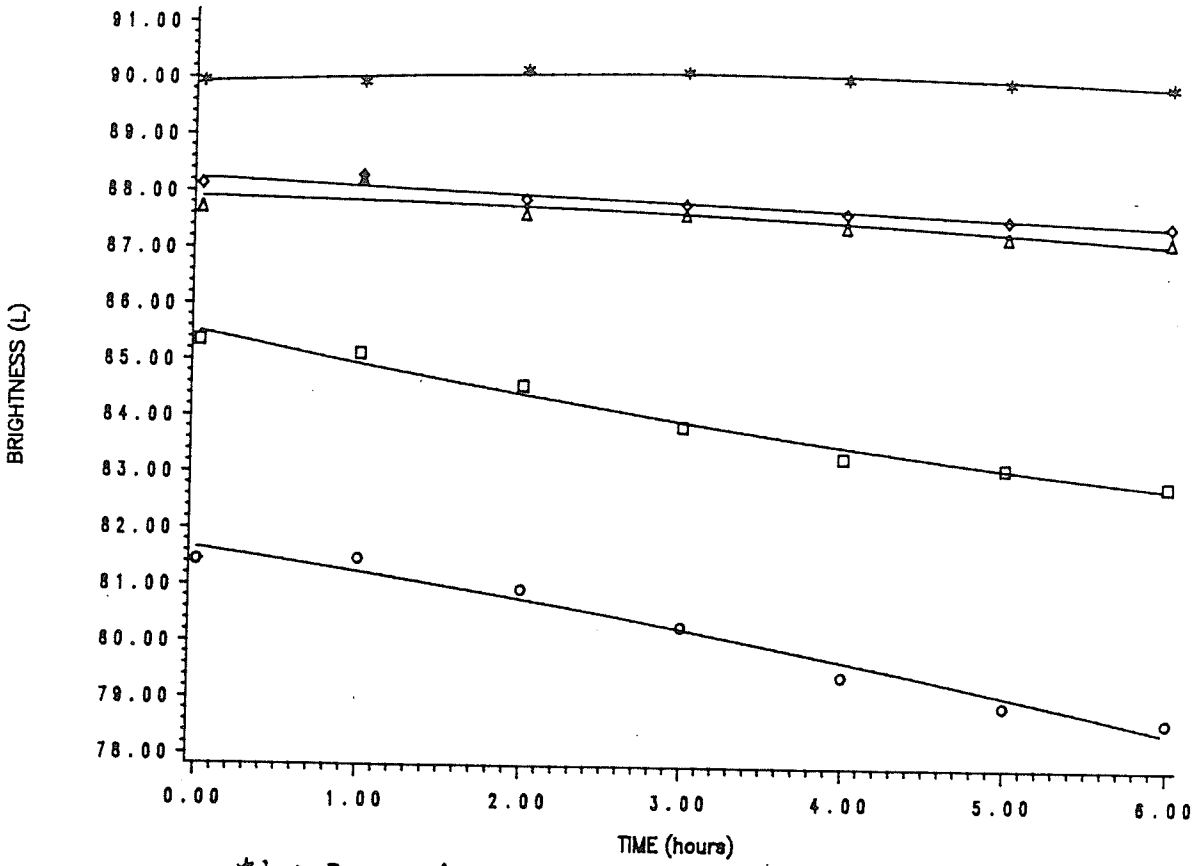
Flour	Katepwa	Glenlea	Norstar	HY320	Fielder
75% Extraction					
1st Patent	1.09	1.52	1.26	0.66	0.35
2nd Patent	0.84	1.60	0.84	1.20	0.70
1st Clear	0.84	1.00	1.12	2.16	1.20
2nd Clear	0.90	1.00	1.02	1.56	0.53
St. Grade	0.84	1.68	0.77	1.50	0.55
80% Extraction					
1st Patent	1.12	0.81	1.28	0.97	0.75
2nd Patent	0.95	2.16	1.28	1.16	0.70
1st Clear	0.77	1.62	1.22	1.89	1.22
2nd Clear	0.91	1.53	2.24	1.38	1.30
St. Grade	0.91	1.98	0.92	1.20	0.60
85% Extraction					
St. Grade	0.56	1.60	0.84	1.12	0.58
Chinese	0.81	0.59	1.86	2.25	1.80
Whole Wheat	0.35	1.12	1.58	1.12	0.75

Maximum Range in Respective Color Component for a 95% Confidence Limit

Redness (a^*)

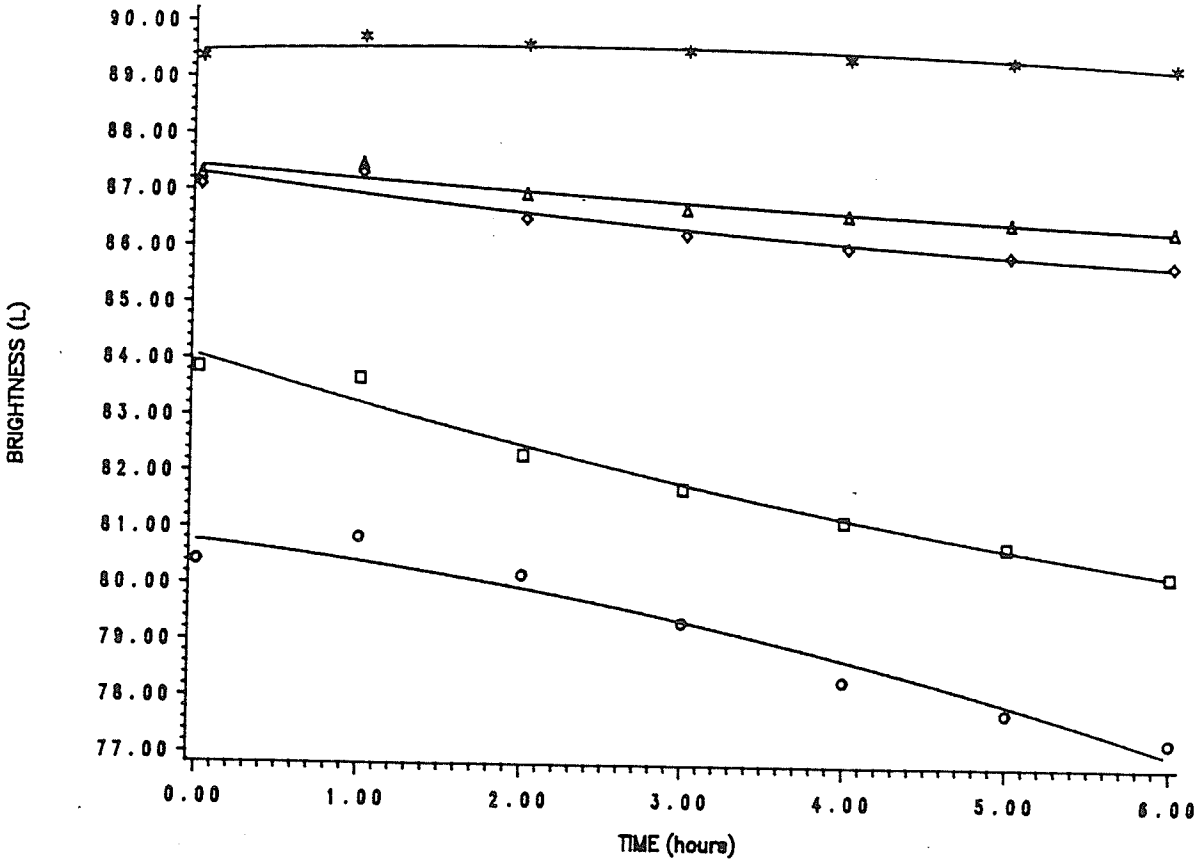
Flour	Katepwa	Glenlea	Norstar	HY320	Fielder
75% Extraction					
1st Patent	0.28	0.80	0.13	0.24	0.52
2nd Patent	0.38	0.32	0.18	0.24	0.30
1st Clear	0.28	0.70	0.20	0.82	0.06
2nd Clear	0.68	0.48	1.32	0.64	0.38
St. Grade	0.20	0.42	0.22	0.24	0.21
80% Extraction					
1st Patent	0.16	0.40	0.24	0.30	0.34
2nd Patent	0.24	0.50	0.24	0.16	0.26
1st Clear	0.22	0.95	0.24	0.62	0.46
2nd Clear	0.44	1.30	0.99	0.20	0.74
St. Grade	0.18	0.50	0.27	0.28	0.18
85% Extraction					
St. Grade	0.45	1.14	0.45	0.60	0.20
Chinese	0.24	0.51	1.00	0.52	0.54
Whole Wheat	1.20	1.38	1.50	0.28	0.40

BRIGHTNESS AS A FUNCTION OF TIME
KATEPWA 75% EXTRACTION FLOURS

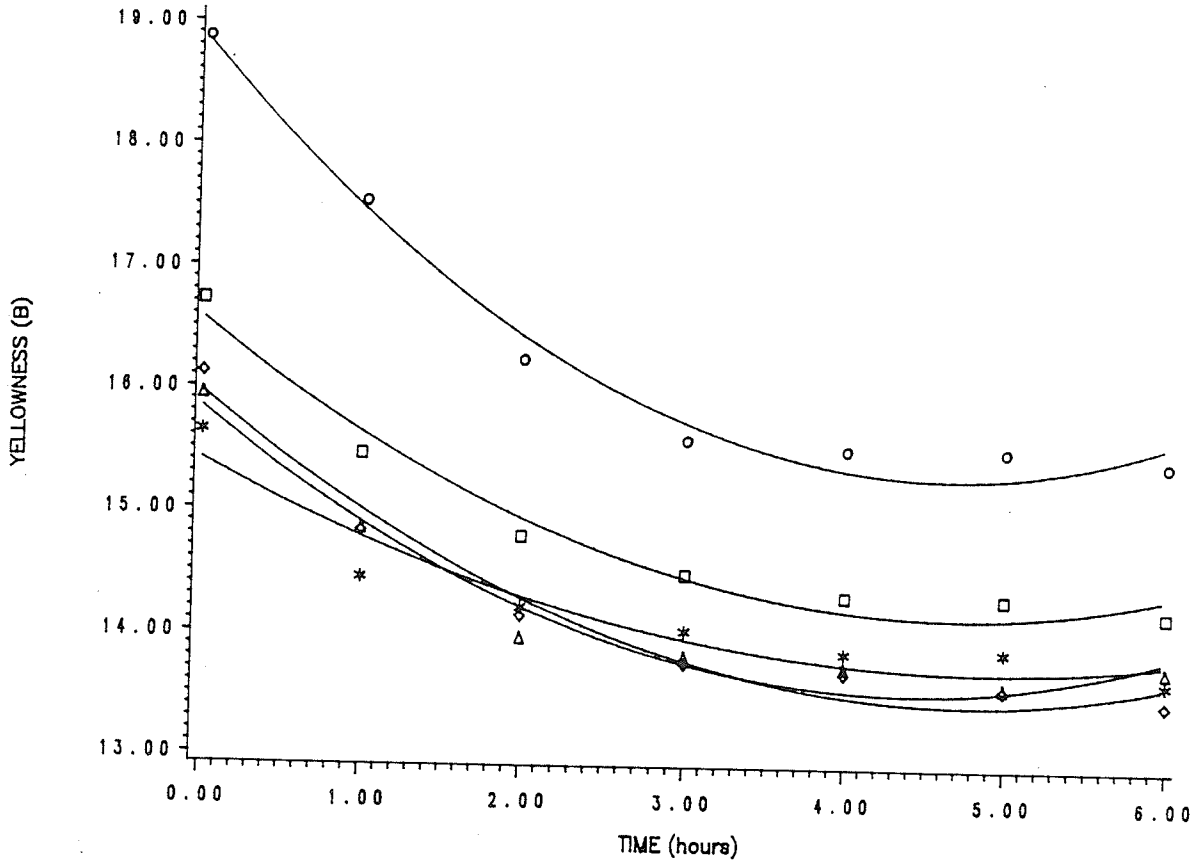


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

BRIGHTNESS AS A FUNCTION OF TIME
KATEPWA 80% EXTRACTION FLOURS

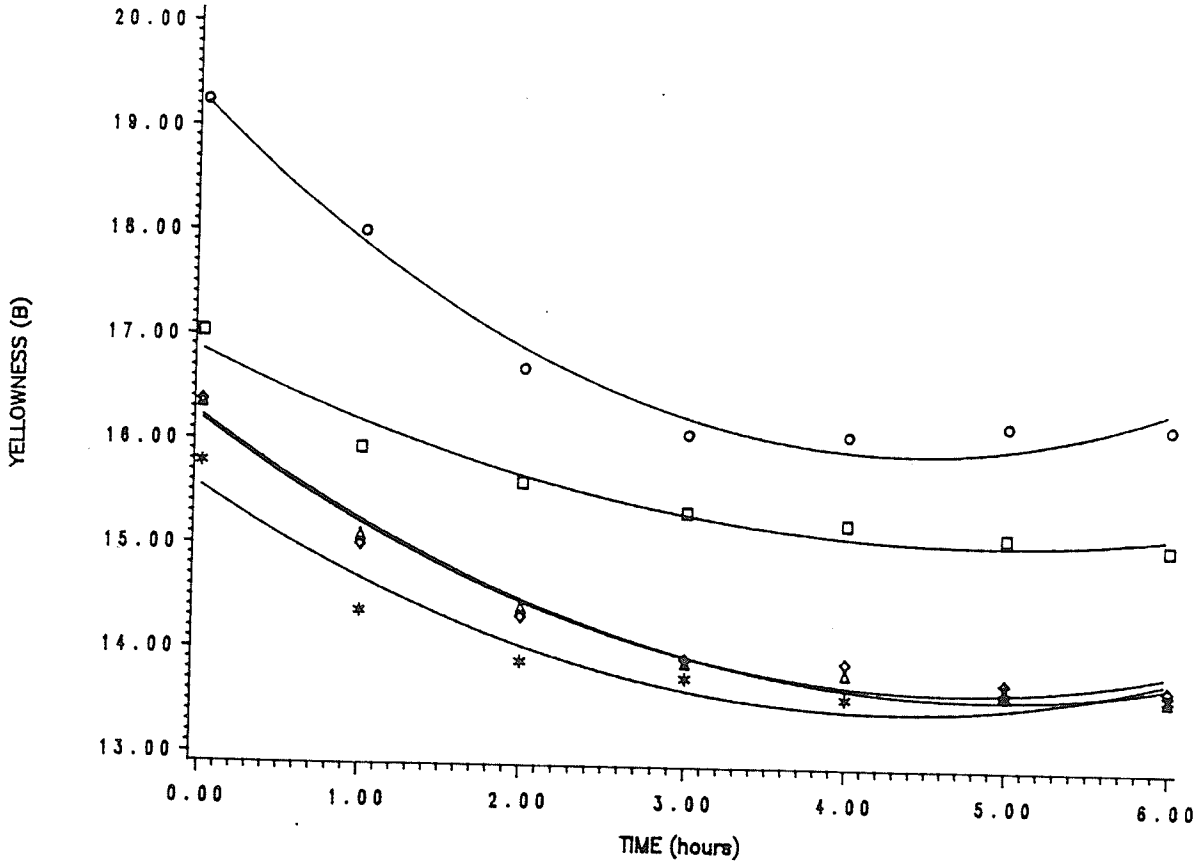


YELLOWNESS AS A FUNCTION OF TIME
KATEPWA 75% EXTRACTION FLOURS

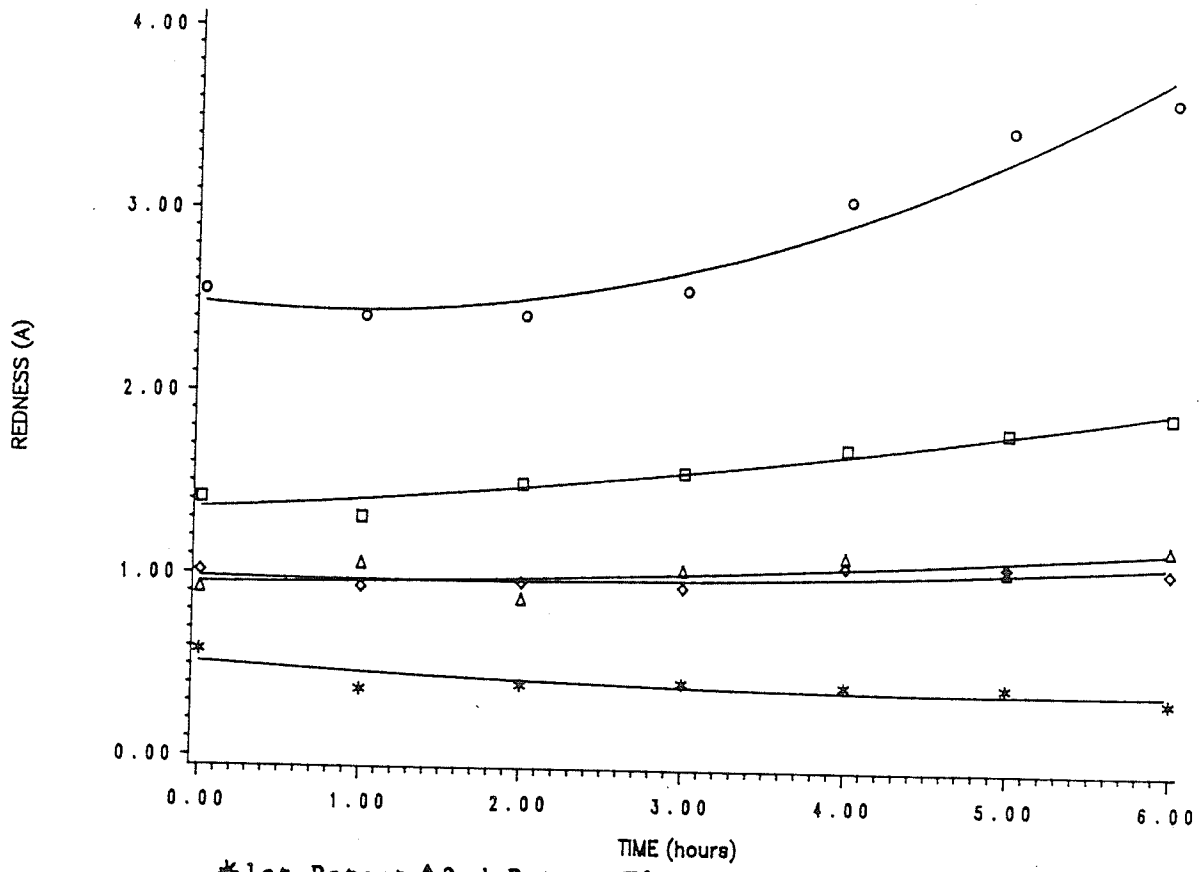


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

YELLOWNESS AS A FUNCTION OF TIME
KATEPWA 80% EXTRACTION FLOURS

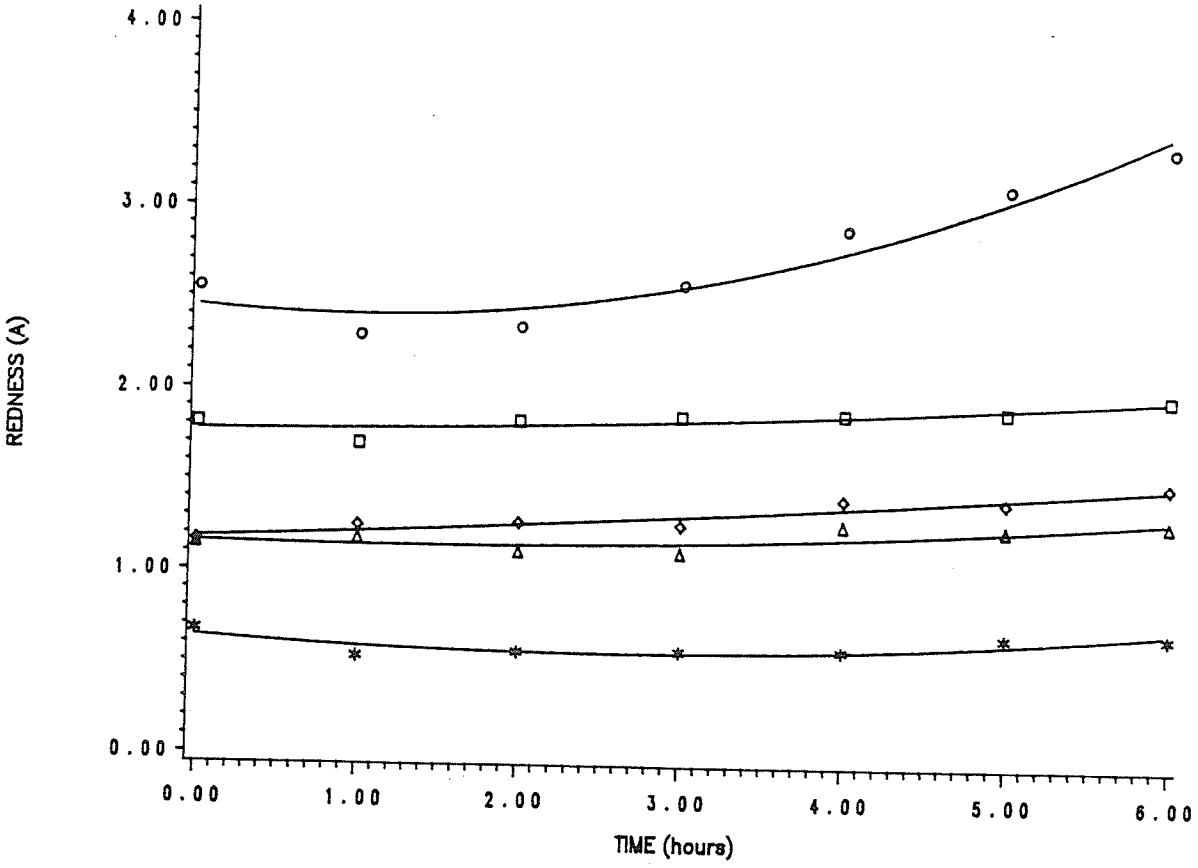


REDNESS AS A FUNCTION OF TIME
KATEPWA 75% EXTRACTION FLOURS

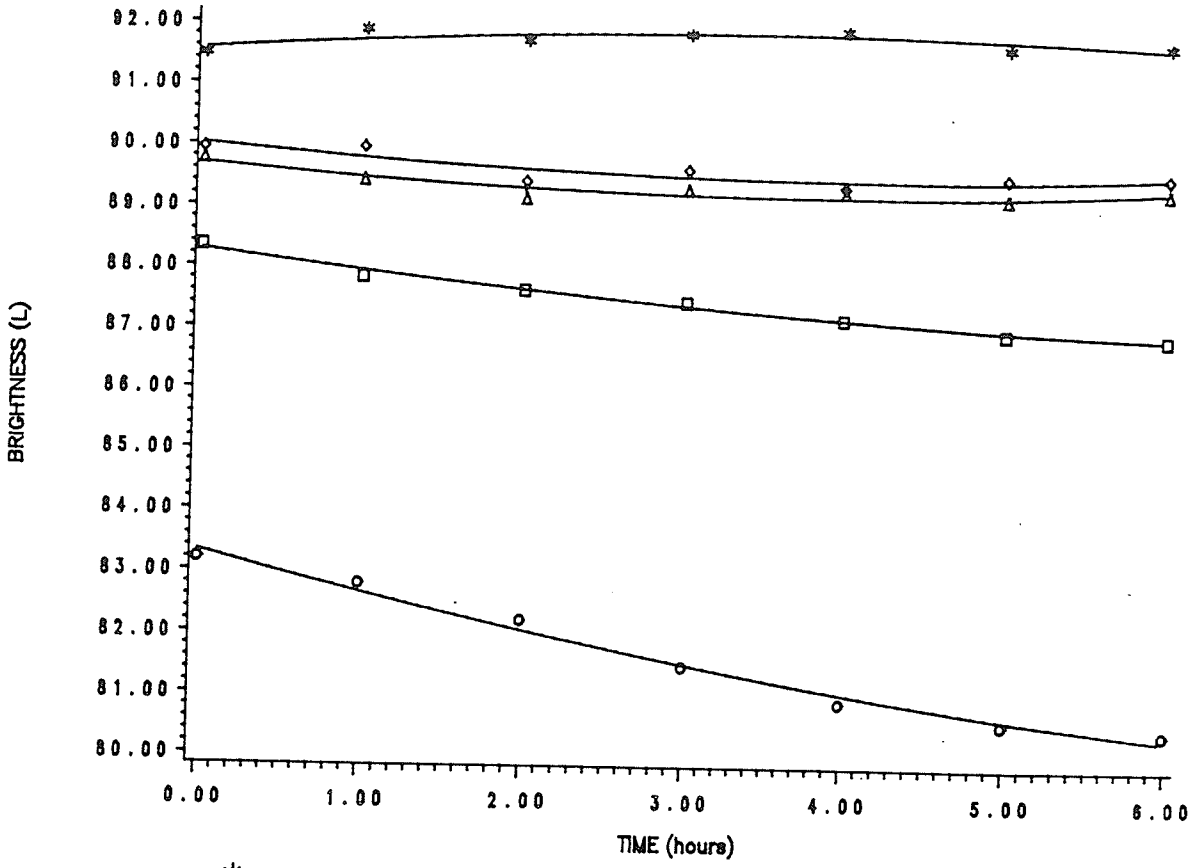


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

REDNESS AS A FUNCTION OF TIME
KATEPWA 80% EXTRACTION FLOURS

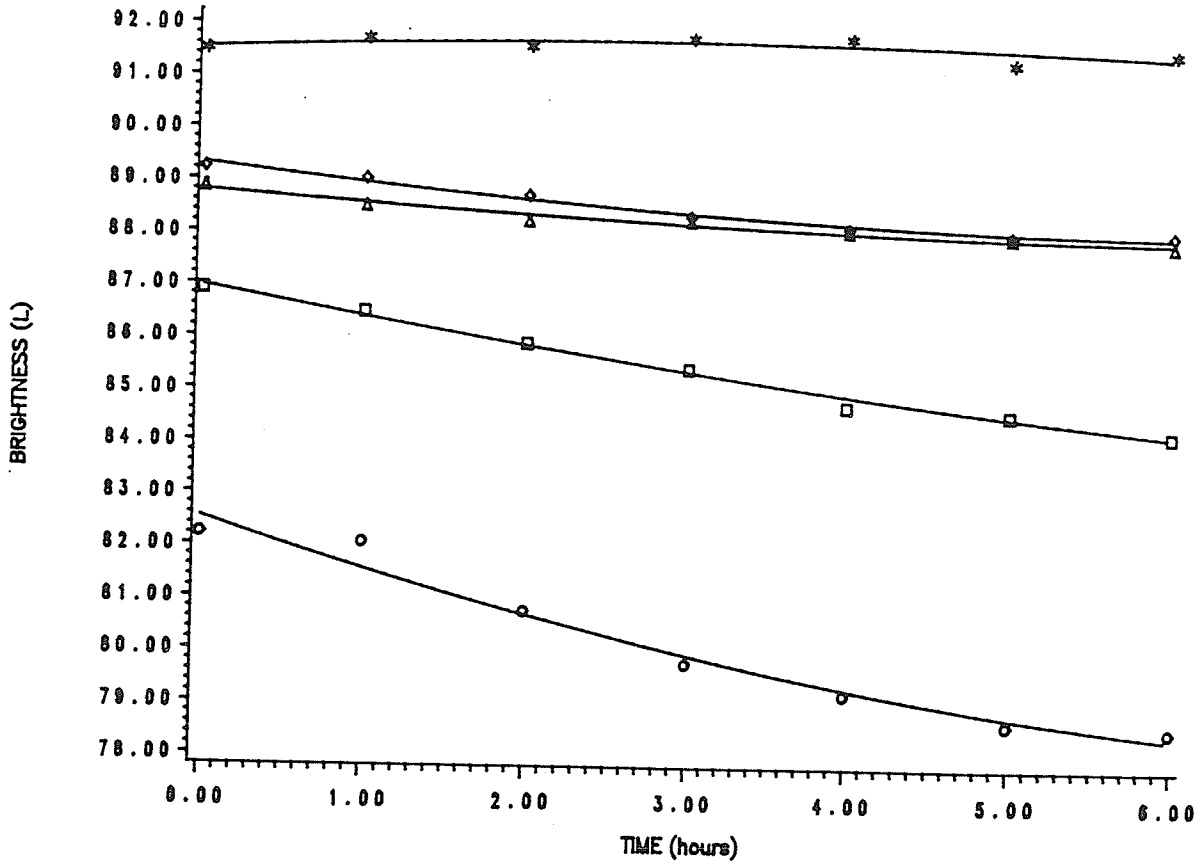


BRIGHTNESS AS A FUNCTION OF TIME
NORSTAR 75% EXTRACTION FLOURS

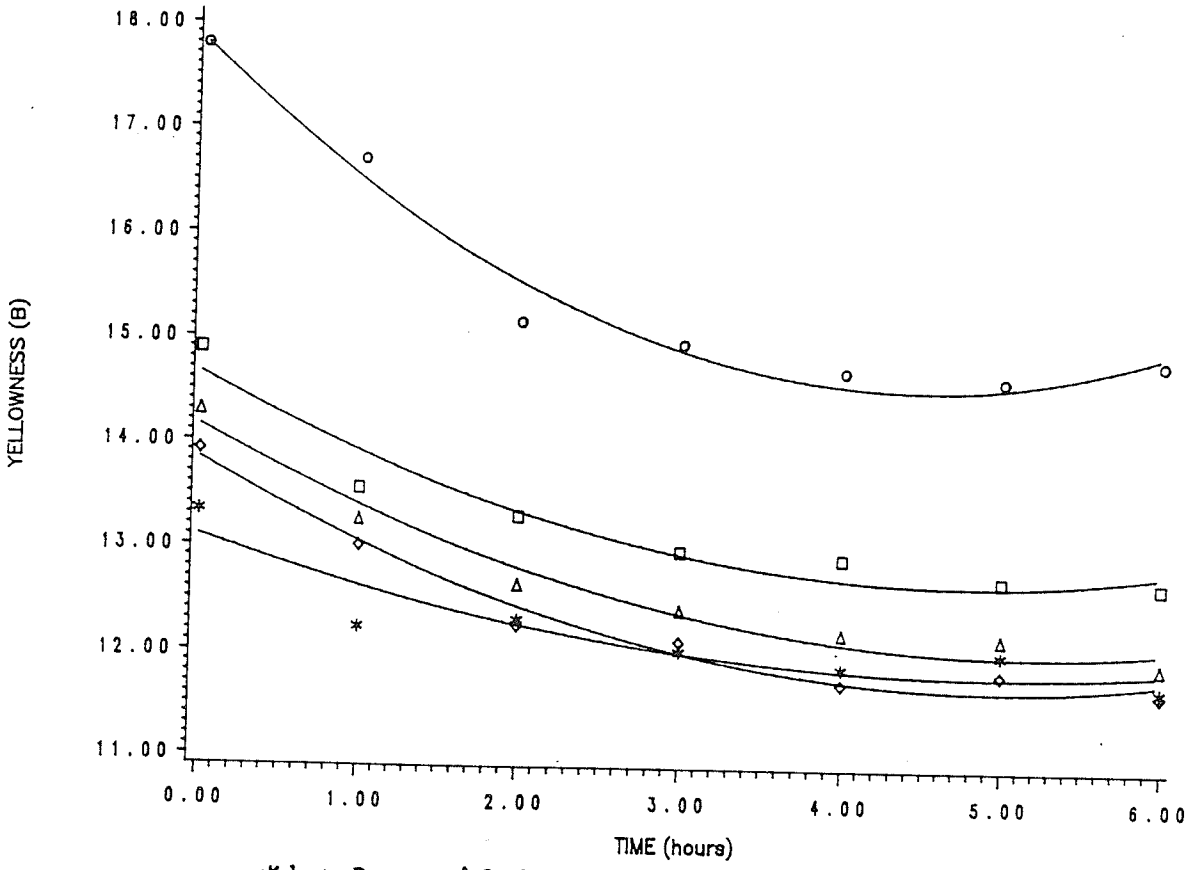


*1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

BRIGHTNESS AS A FUNCTION OF TIME
NORSTAR 80% EXTRACTION FLOURS

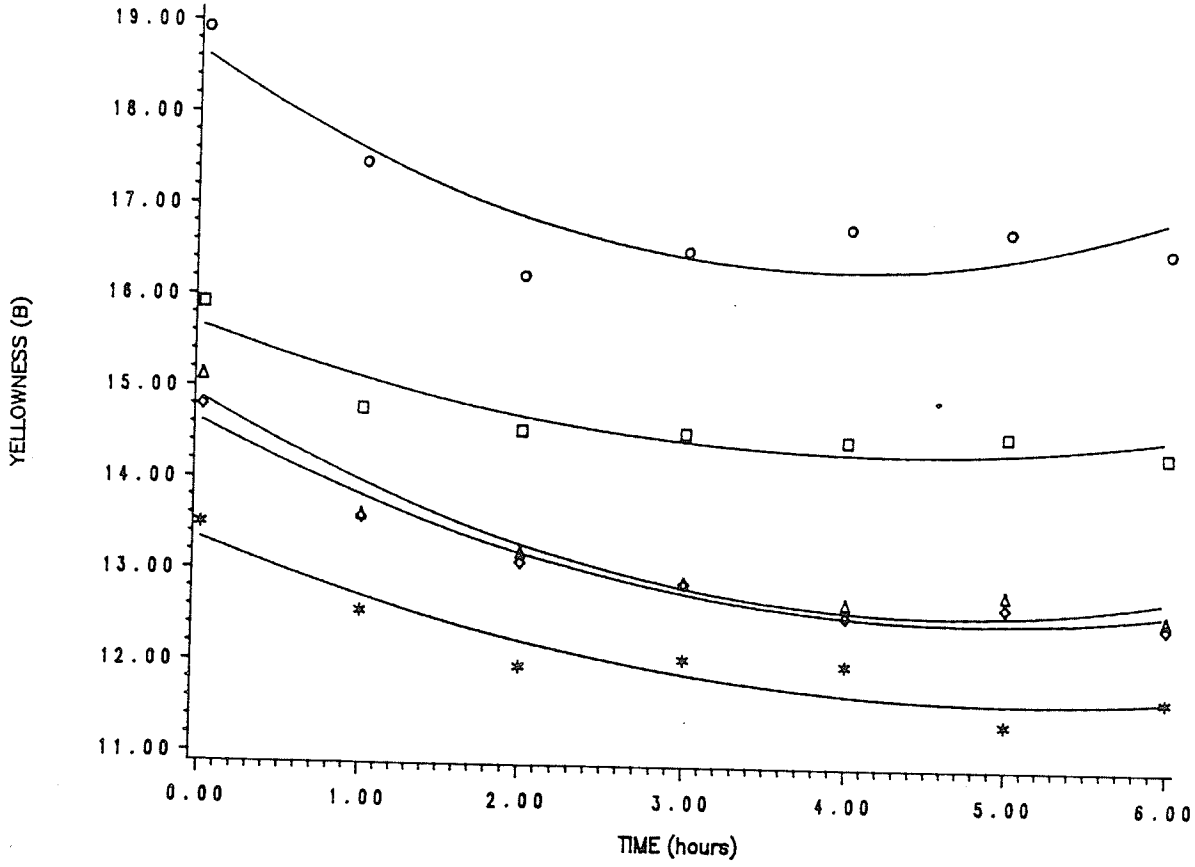


YELLOWNESS AS A FUNCTION OF TIME
NORSTAR 75% EXTRACTION FLOURS



✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

YELLOWNESS AS A FUNCTION OF TIME
NORSTAR 80% EXTRACTION FLOURS



REDNESS AS A FUNCTION OF TIME
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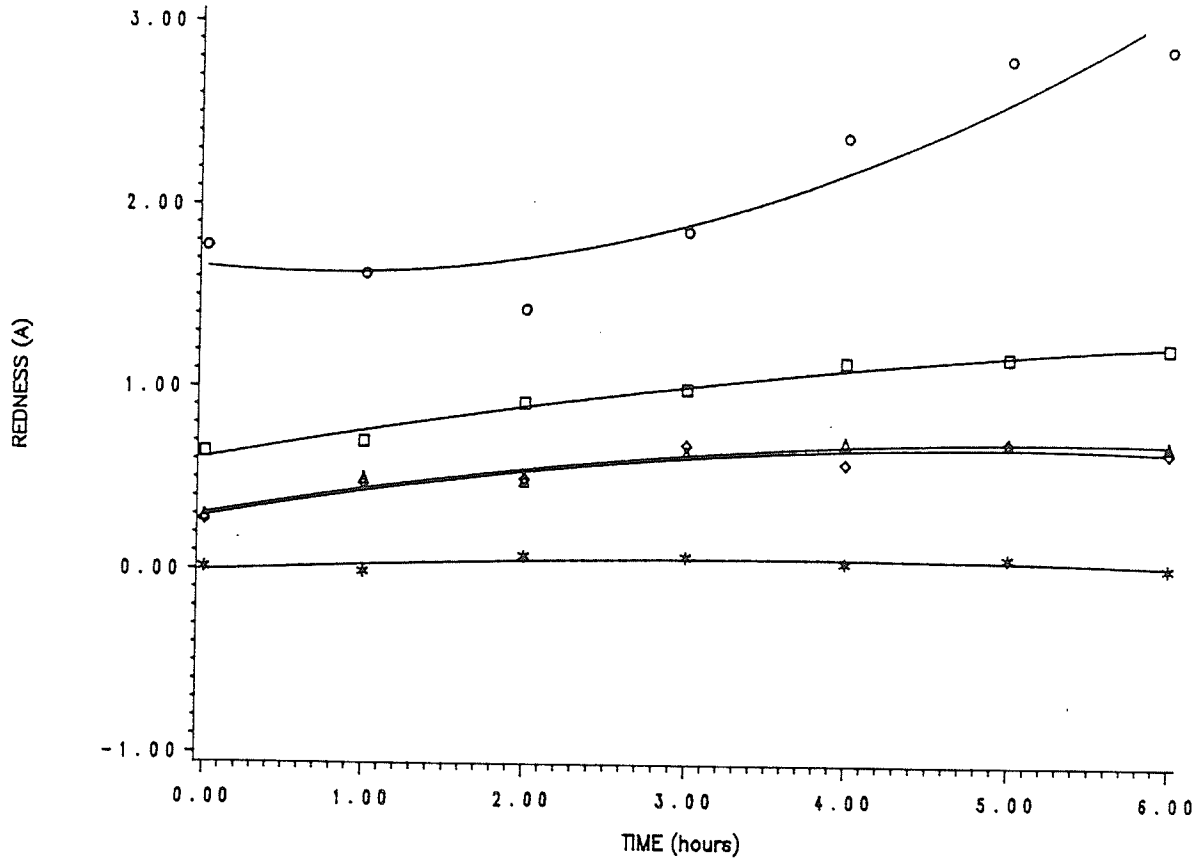

✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

REDNESS AS A FUNCTION OF TIME
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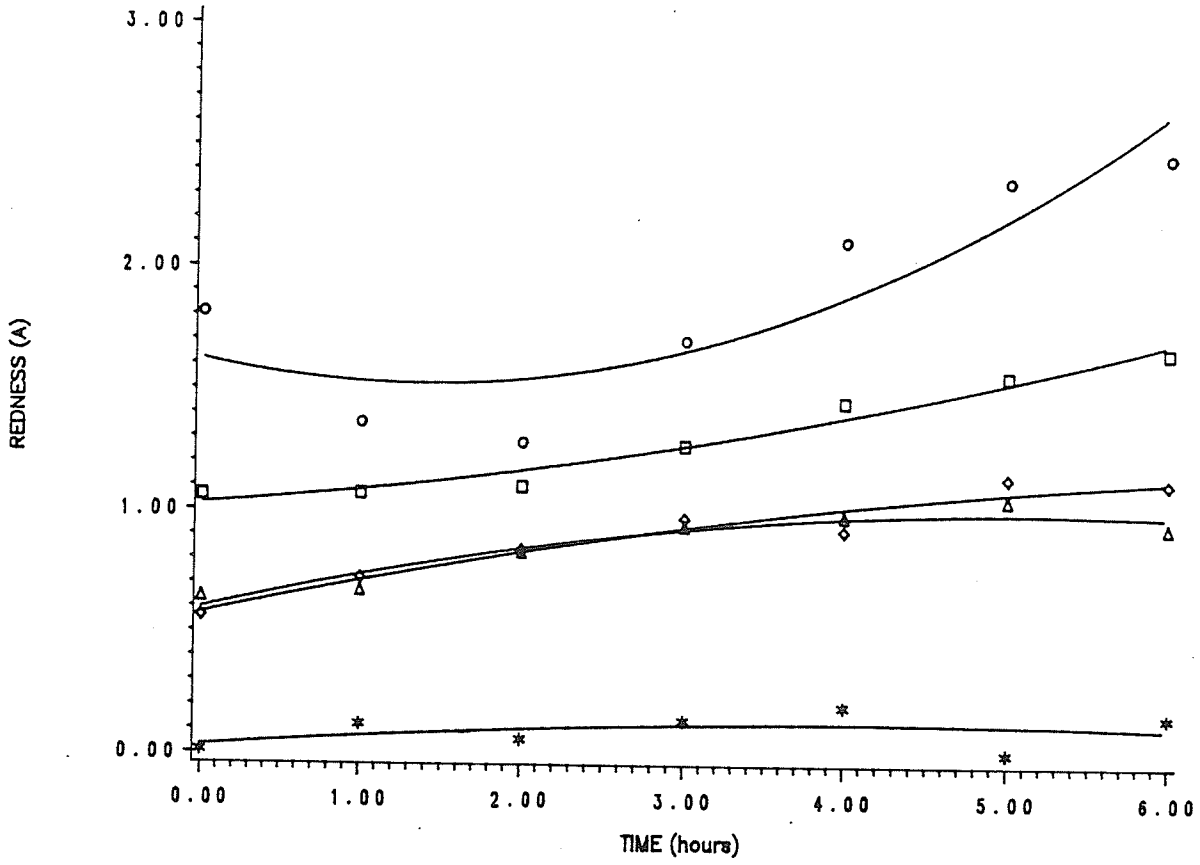

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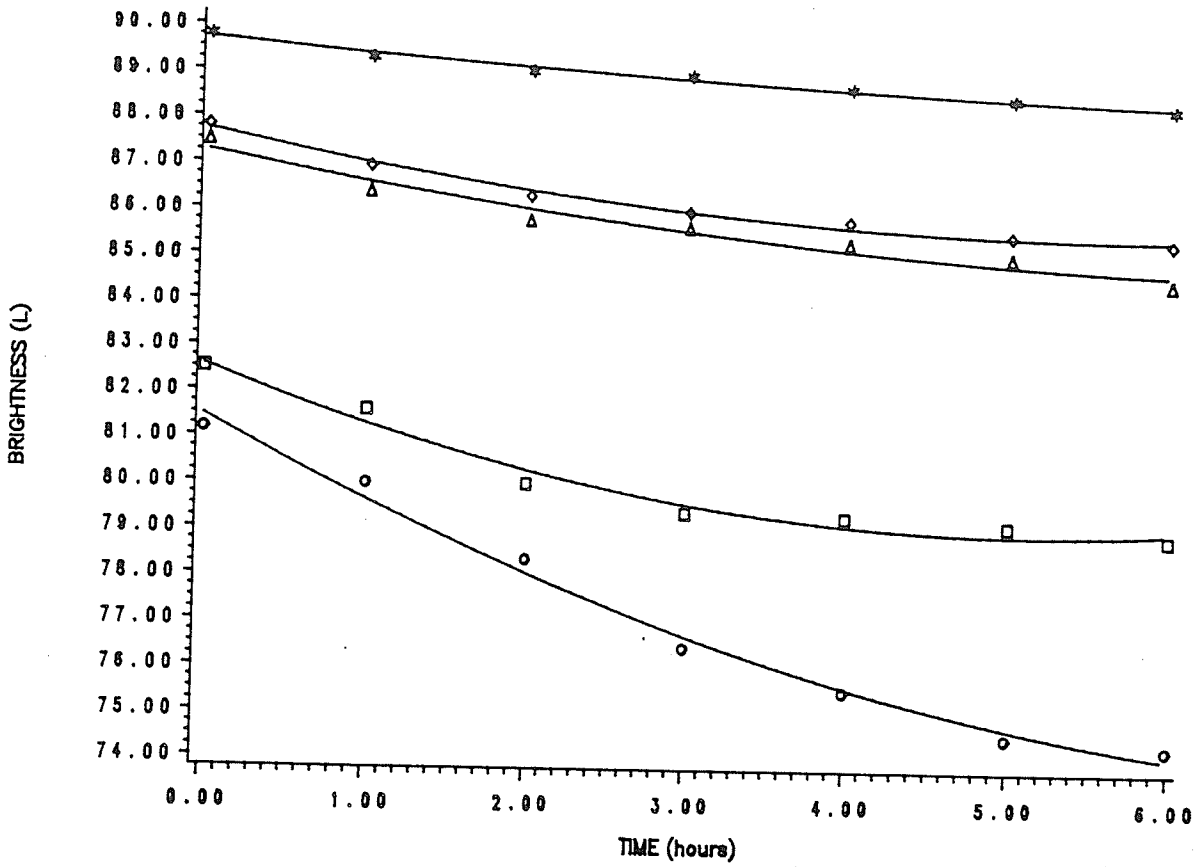

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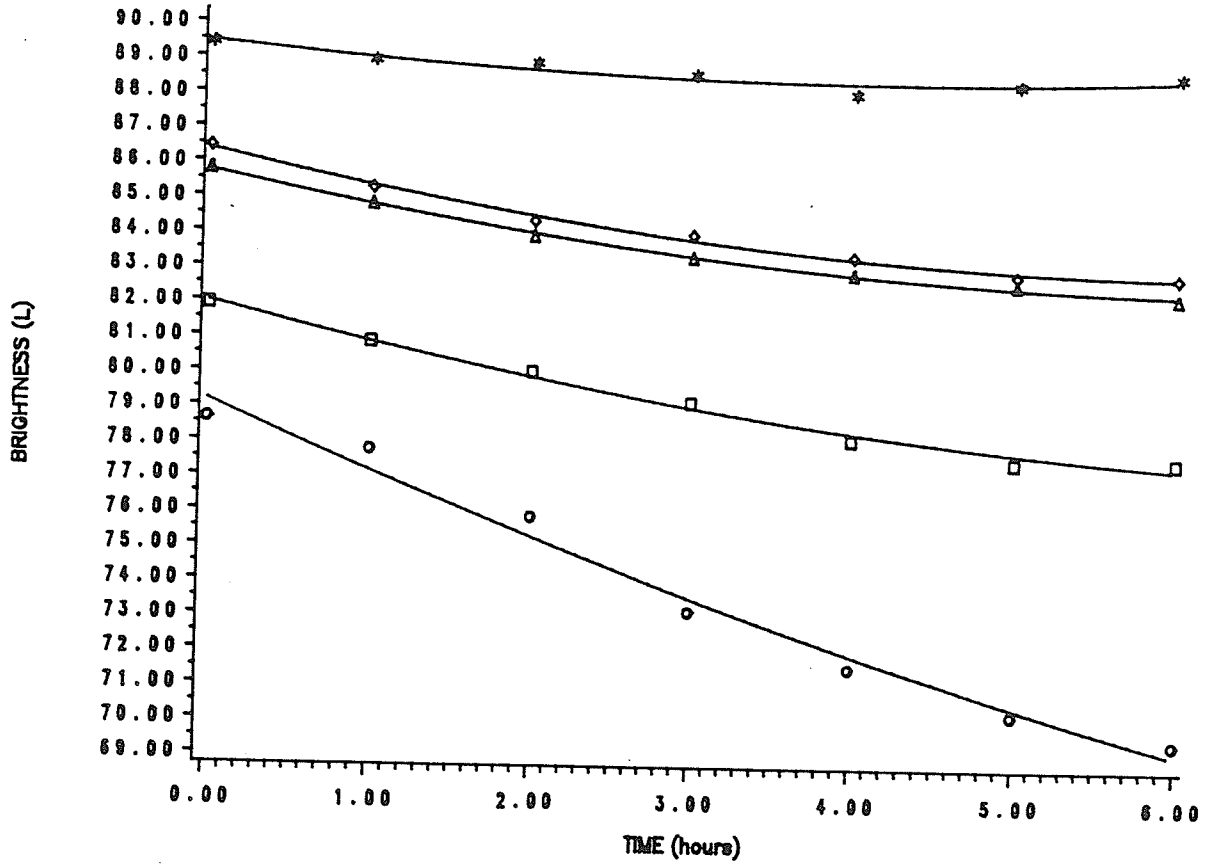

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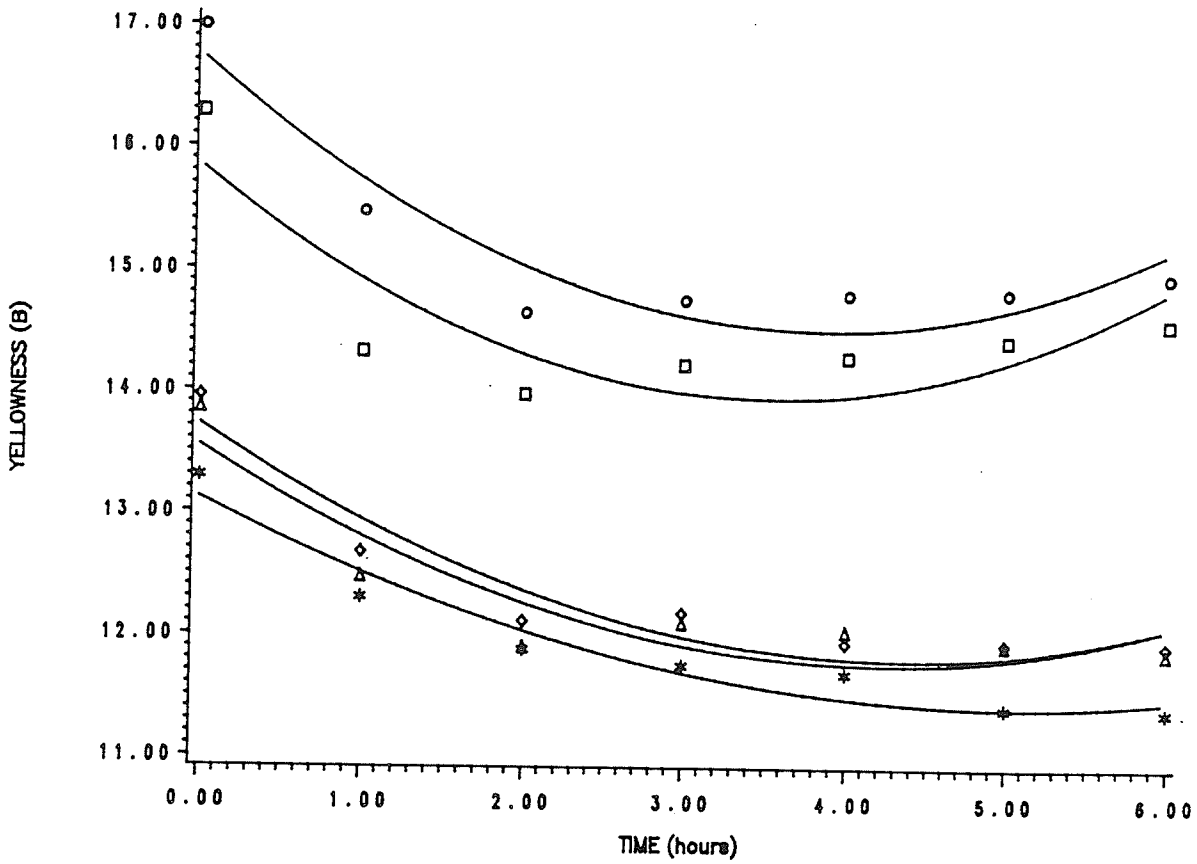

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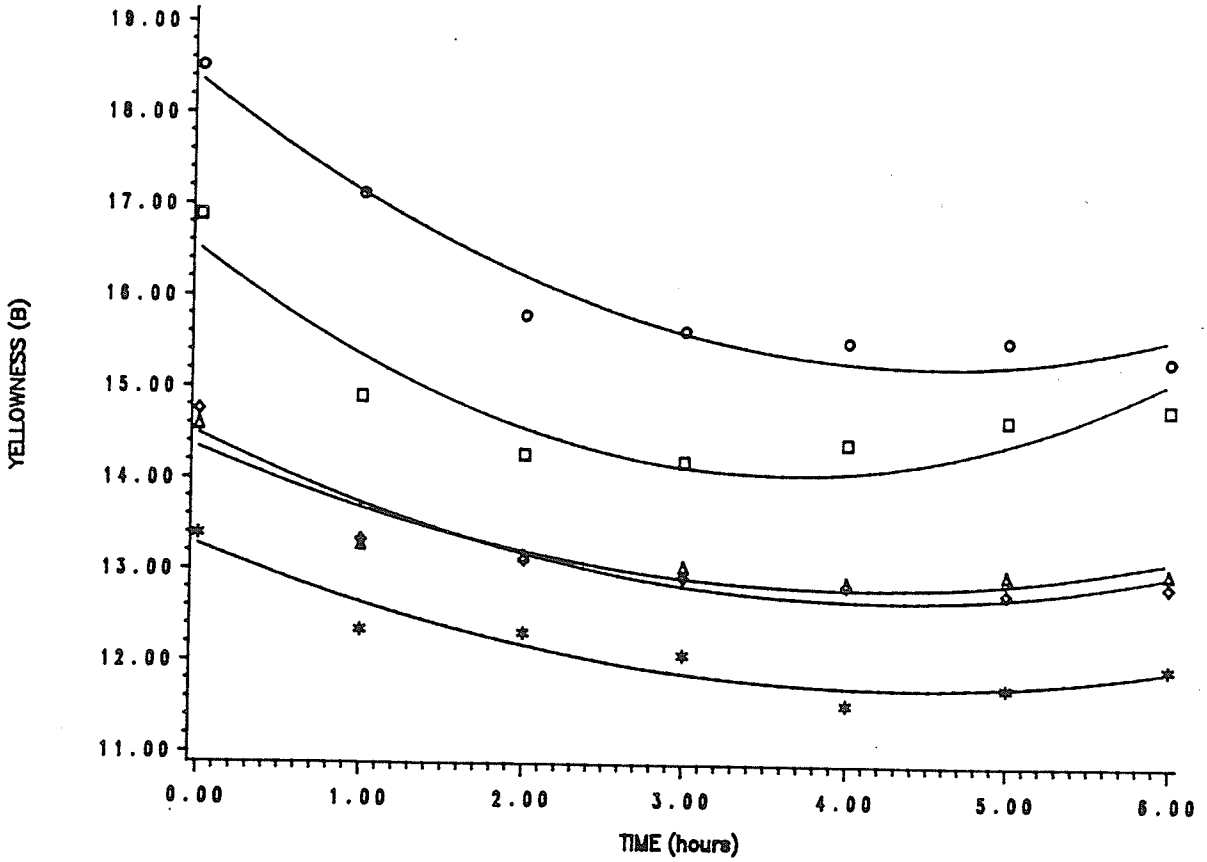

REDNESS AS A FUNCTION OF TIME
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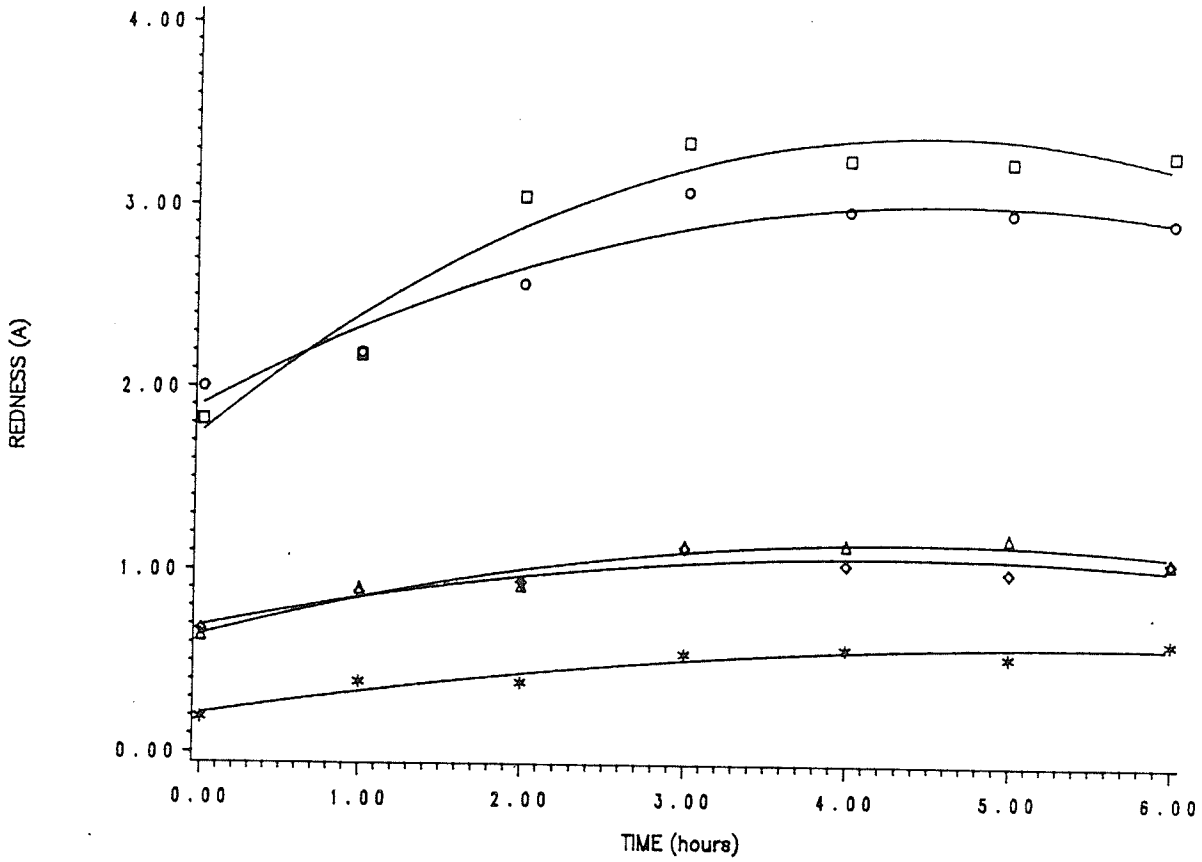

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REDNESS AS A FUNCTION OF TIME
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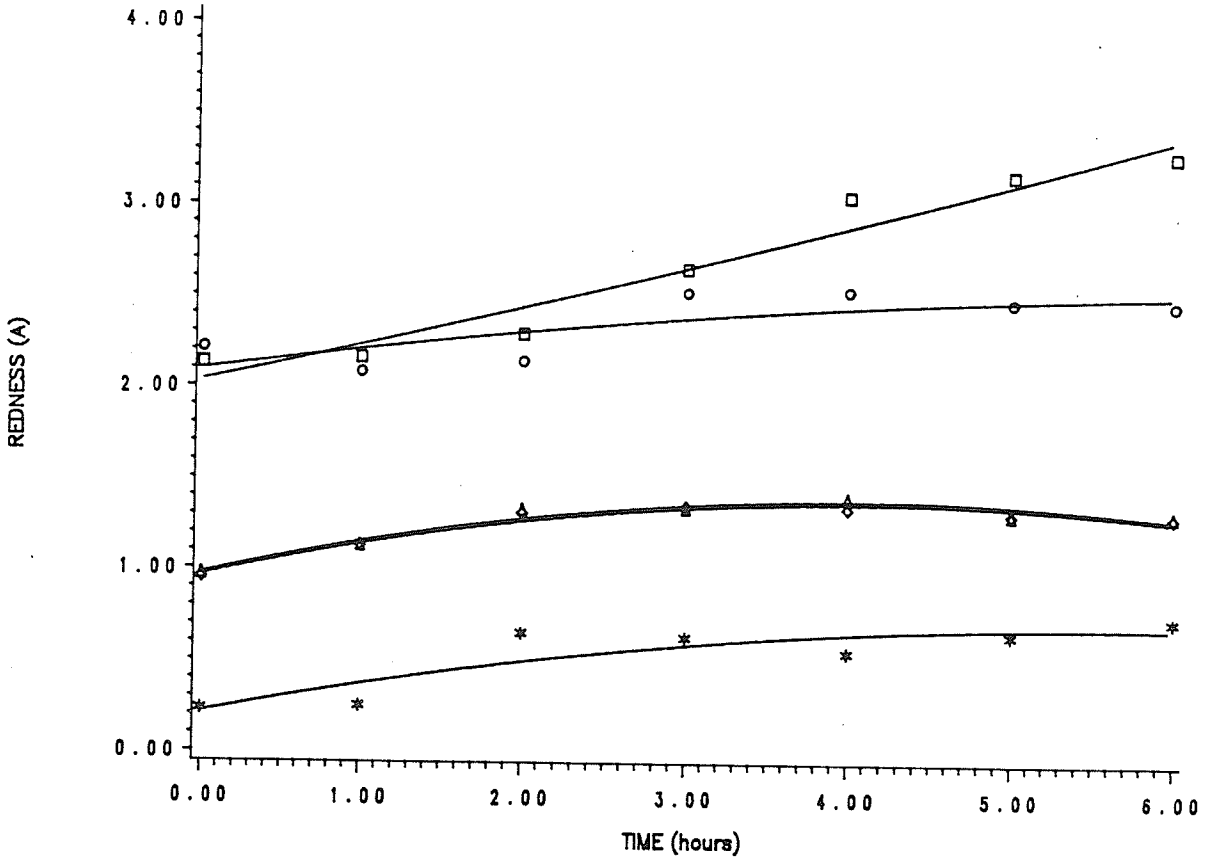
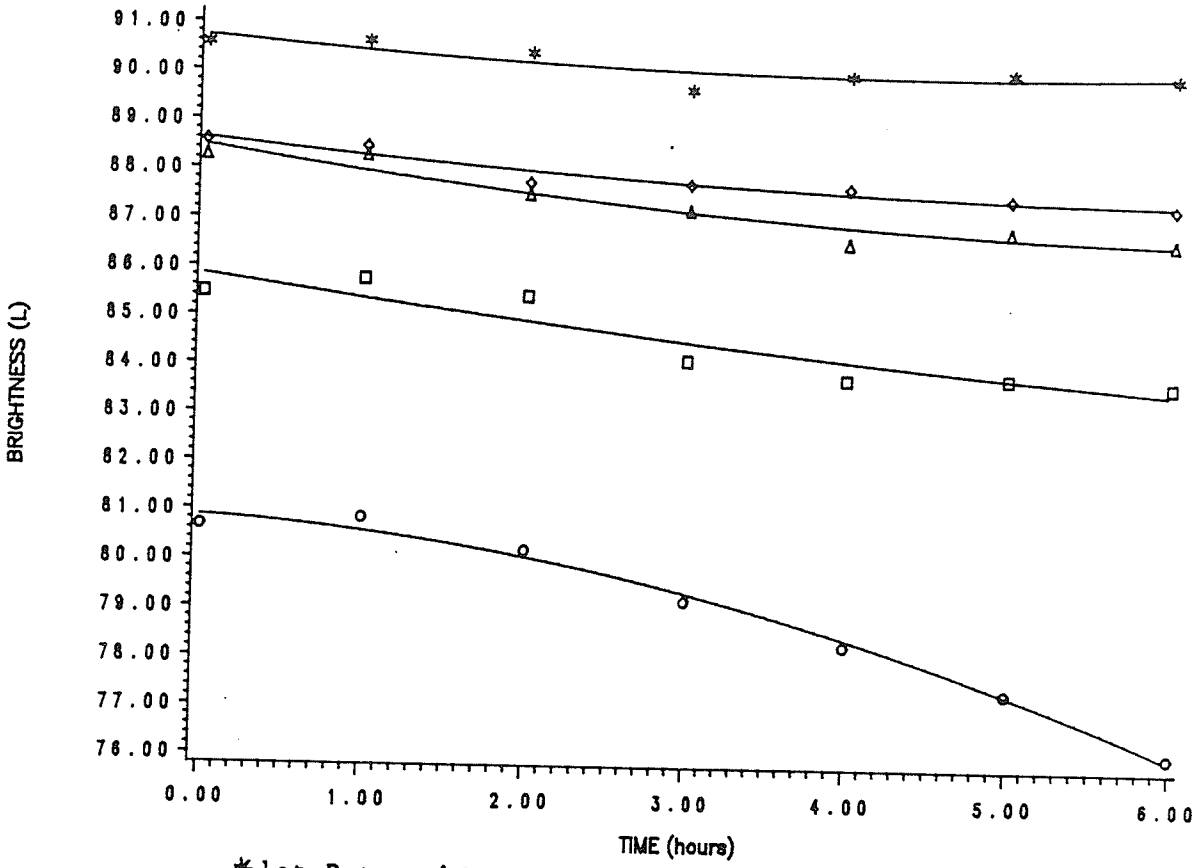
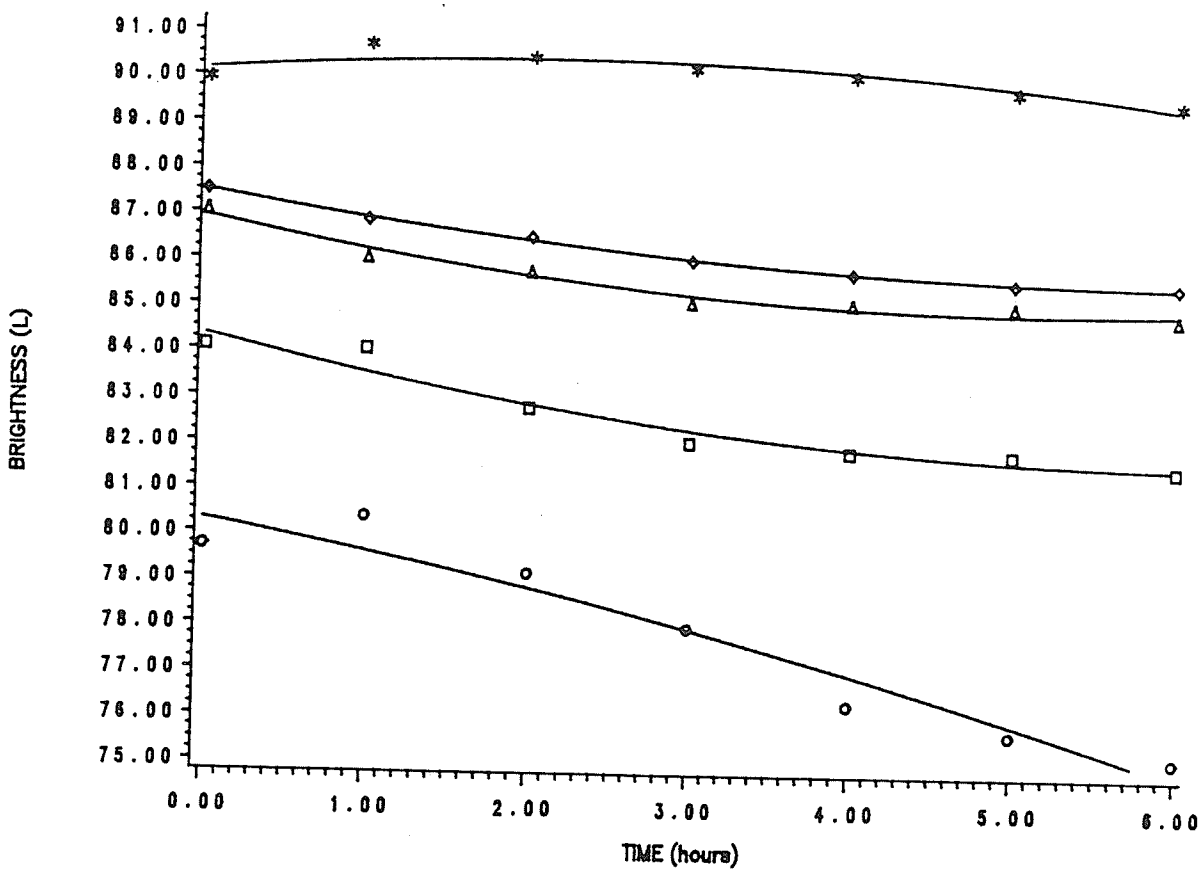


BRIGHTNESS AS A FUNCTION OF TIME
GLENLEA 75% EXTRACTION FLOURS

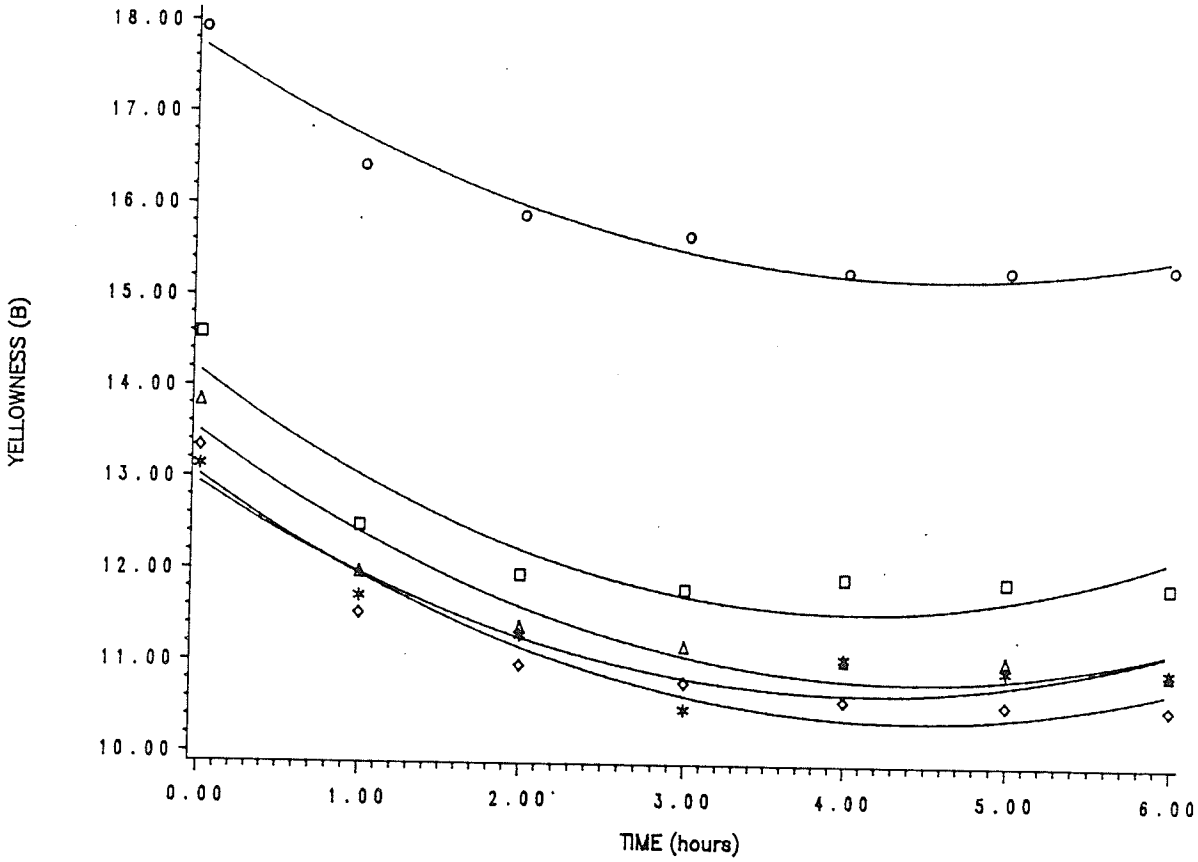


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

BRIGHTNESS AS A FUNCTION OF TIME
GLENLEA 80% EXTRACTION FLOURS

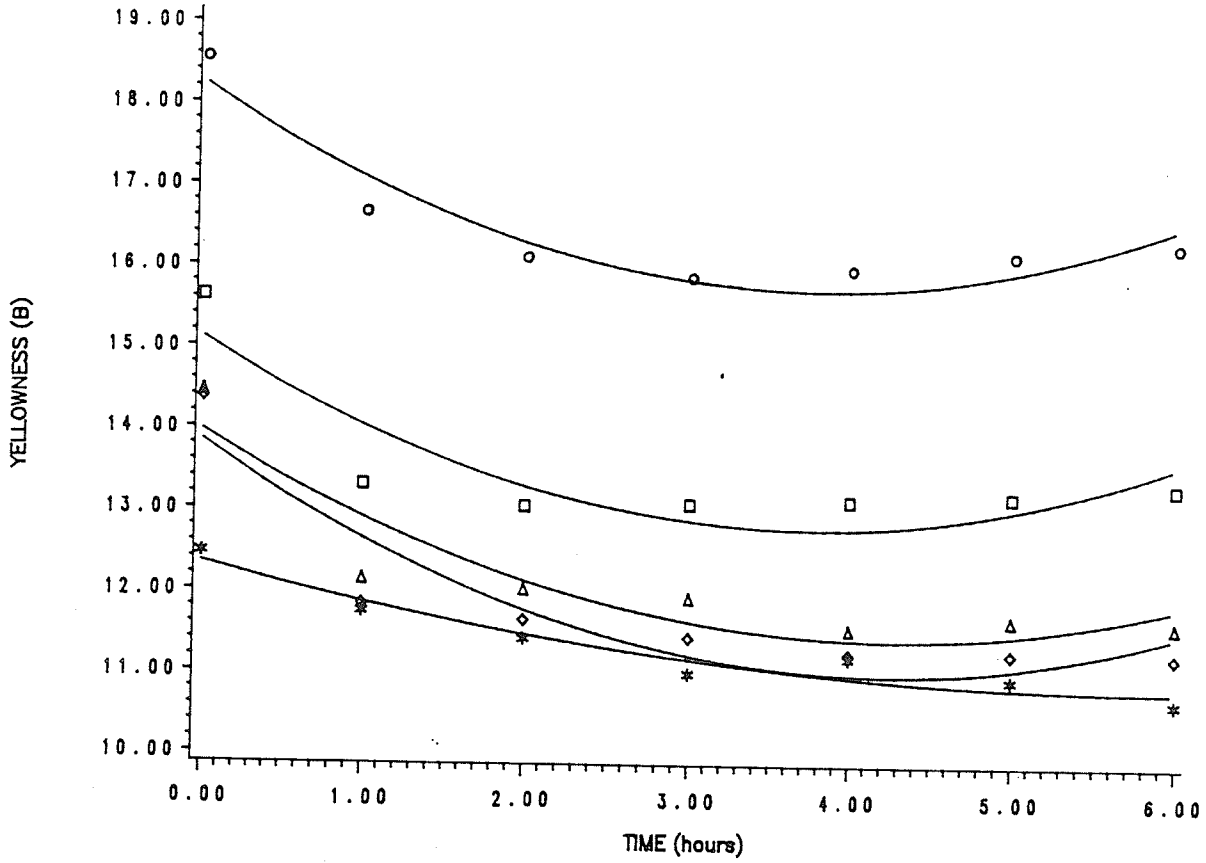


YELLOWNESS AS A FUNCTION OF TIME
GLENLEA 75% EXTRACTION FLOURS

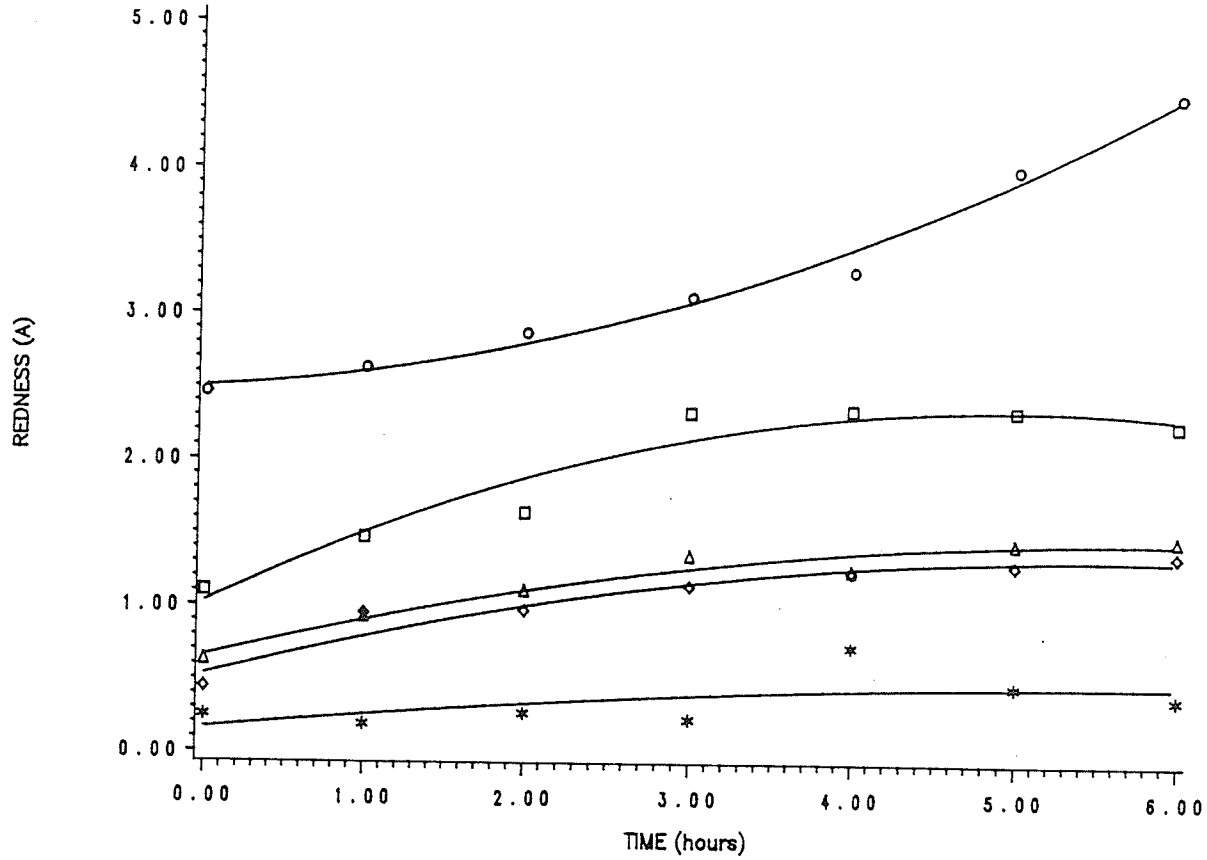


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

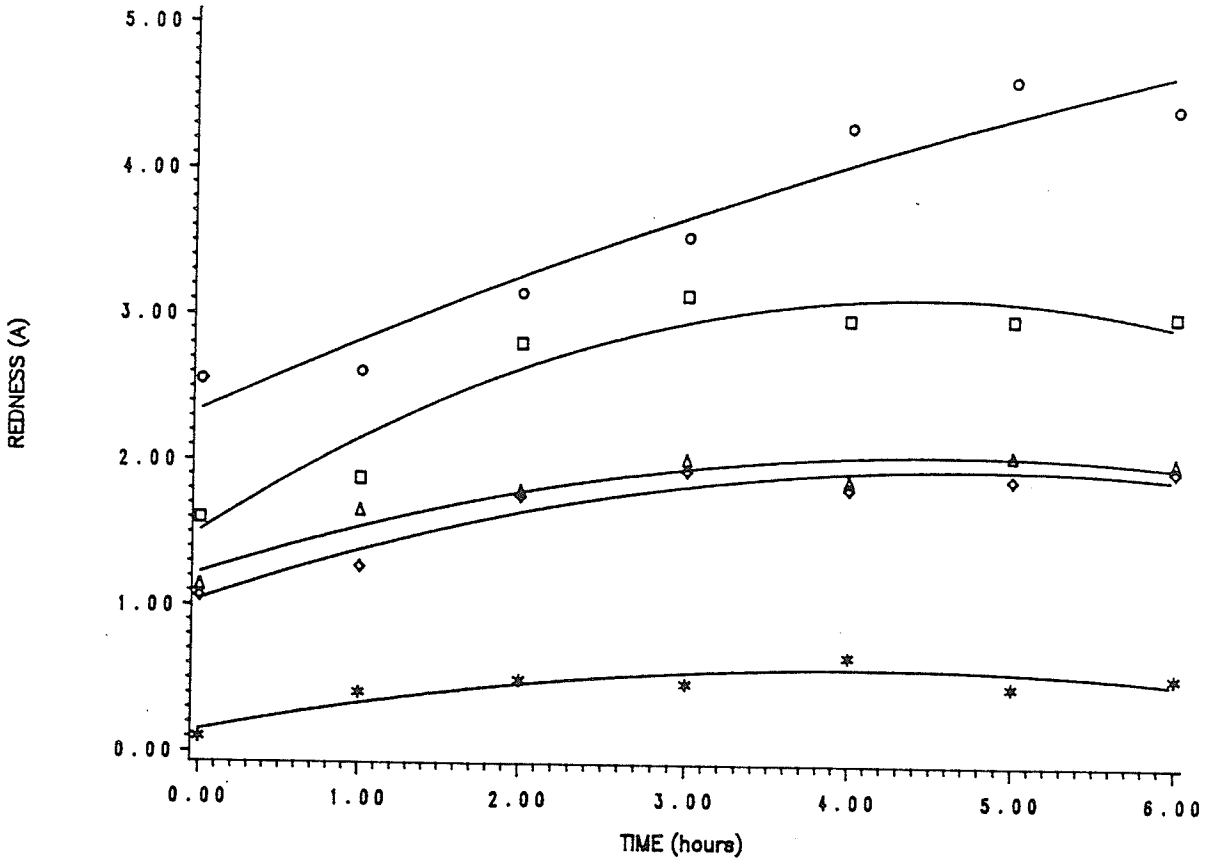
YELLOWNESS AS A FUNCTION OF TIME
GLENLEA 80% EXTRACTION FLOURS



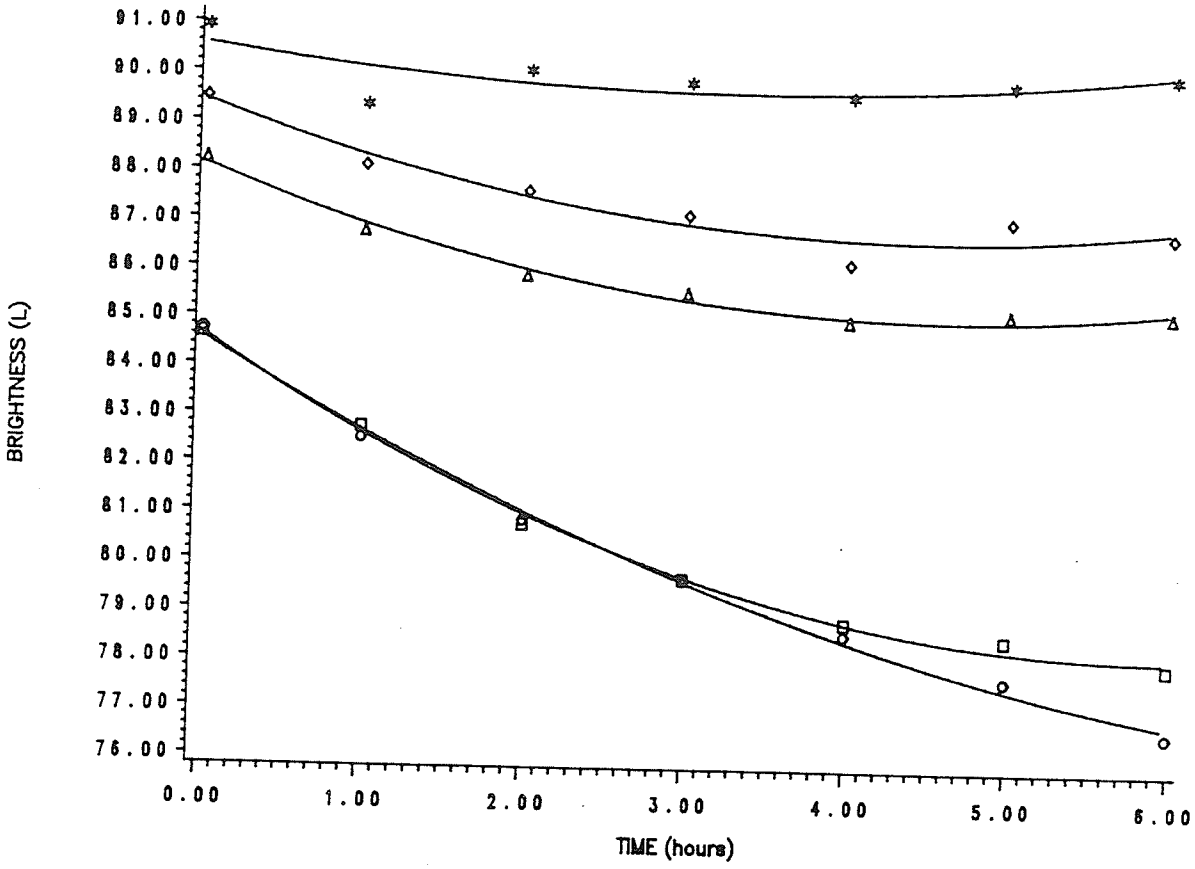
REDNESS AS A FUNCTION OF TIME
GLENLEA 75% EXTRACTION FLOURS



REDNESS AS A FUNCTION OF TIME
GLENLEA 80% EXTRACTION FLOURS

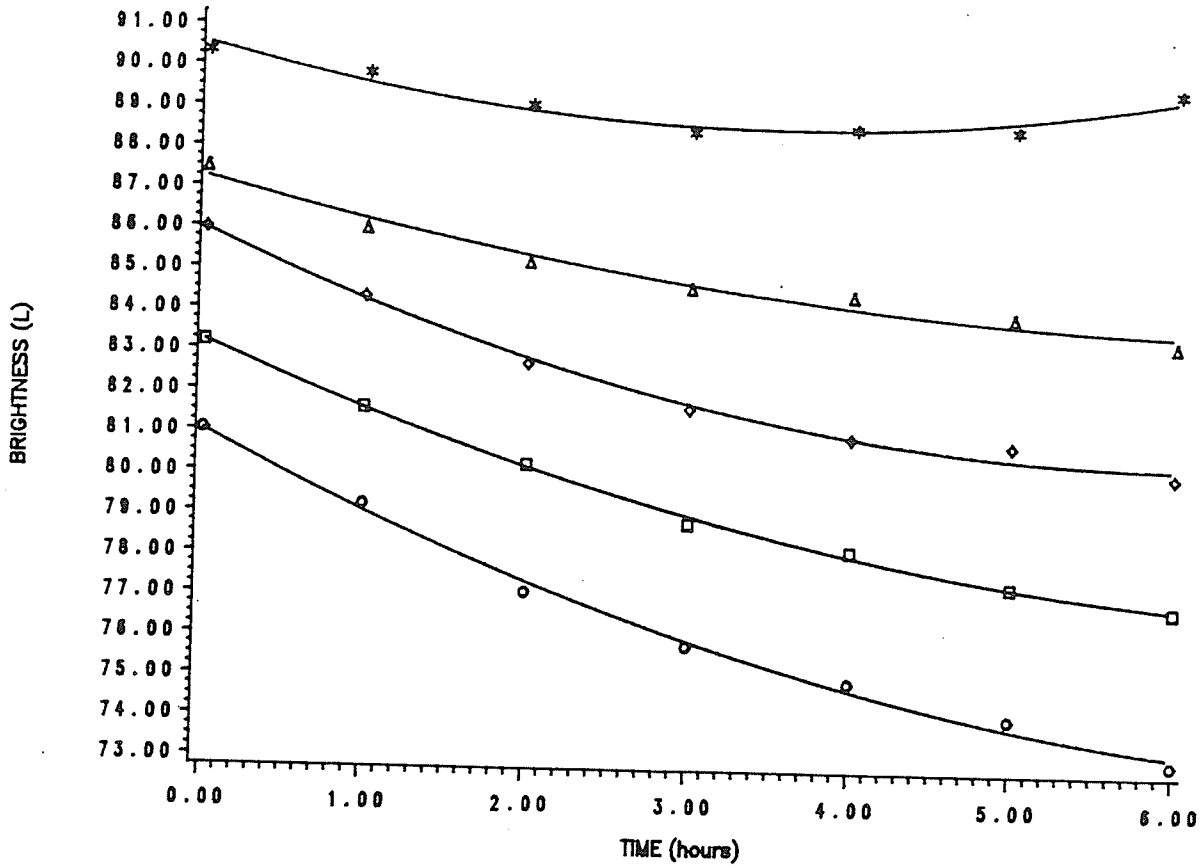


BRIGHTNESS AS A FUNCTION OF TIME
FIELDER 75% EXTRACTION FLOURS



1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

BRIGHTNESS AS A FUNCTION OF TIME
FIELDER 80% EXTRACTION FLOURS



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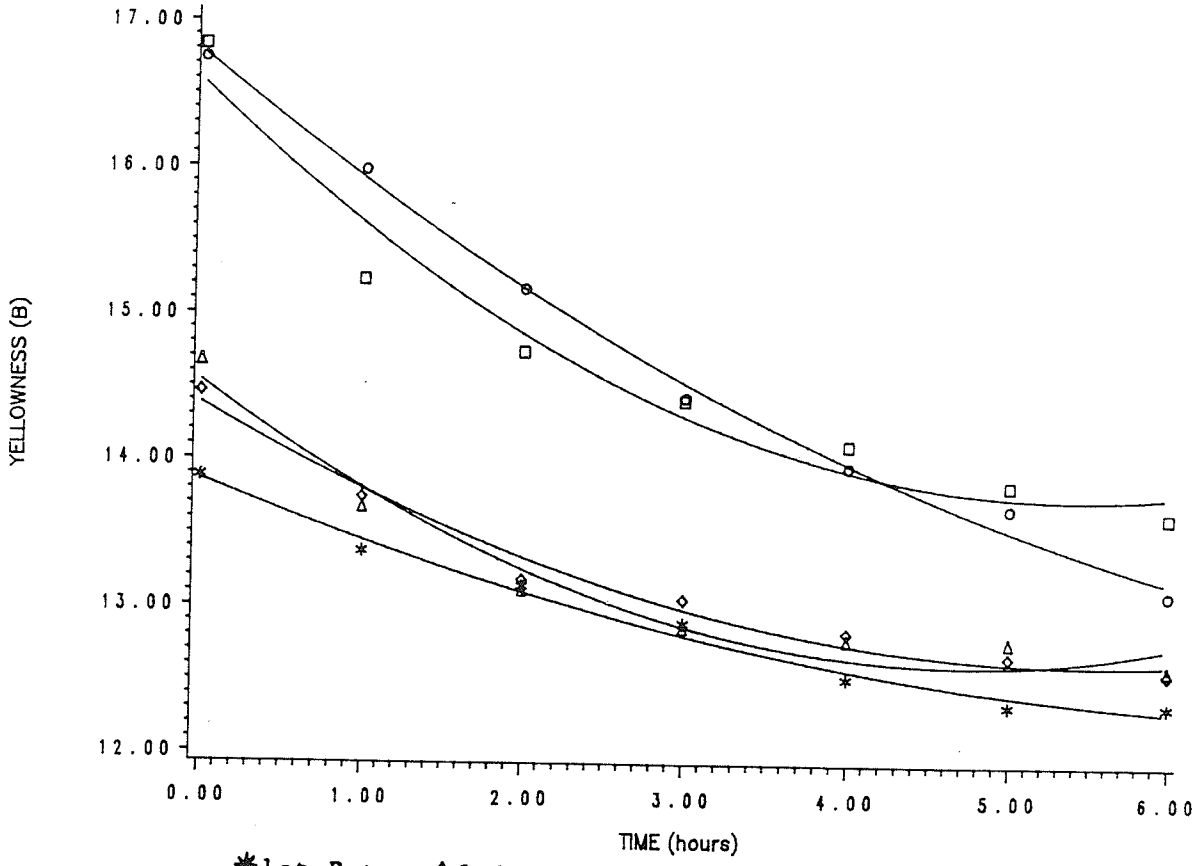

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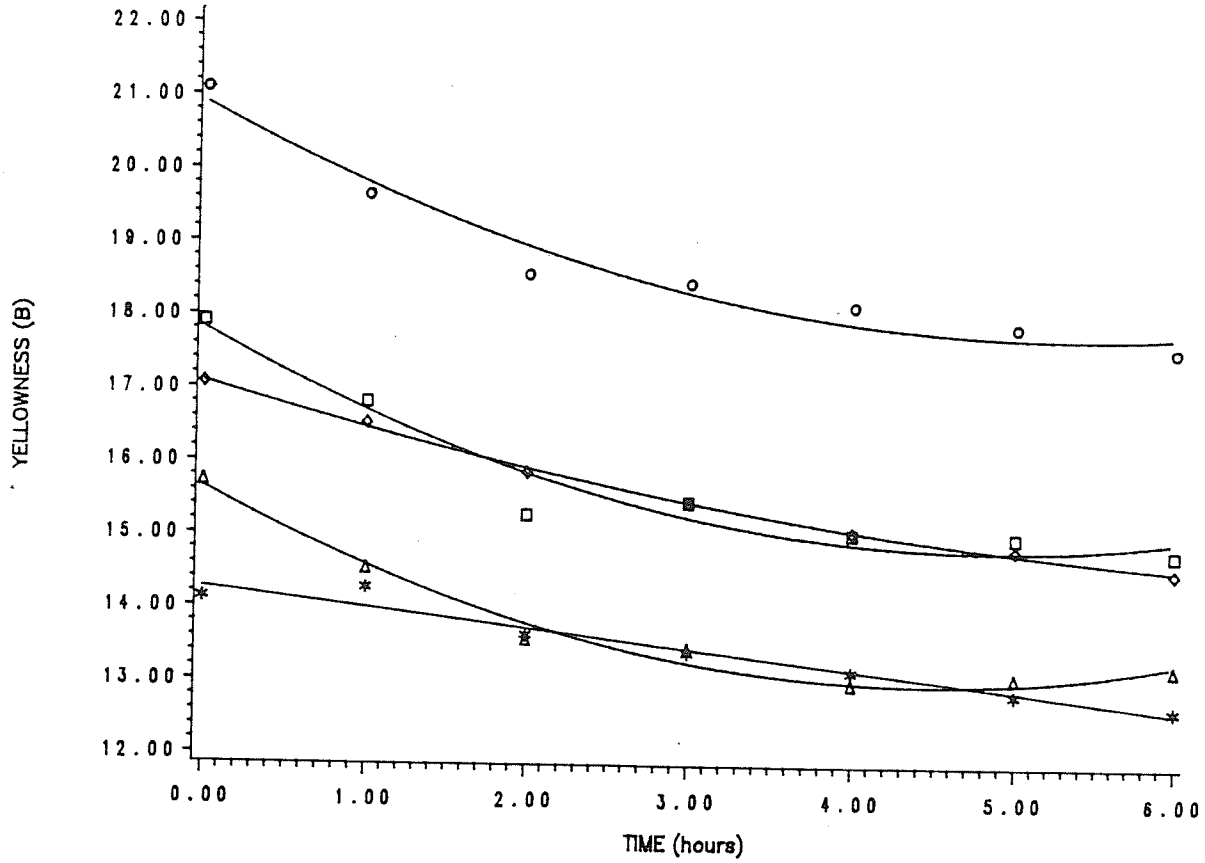
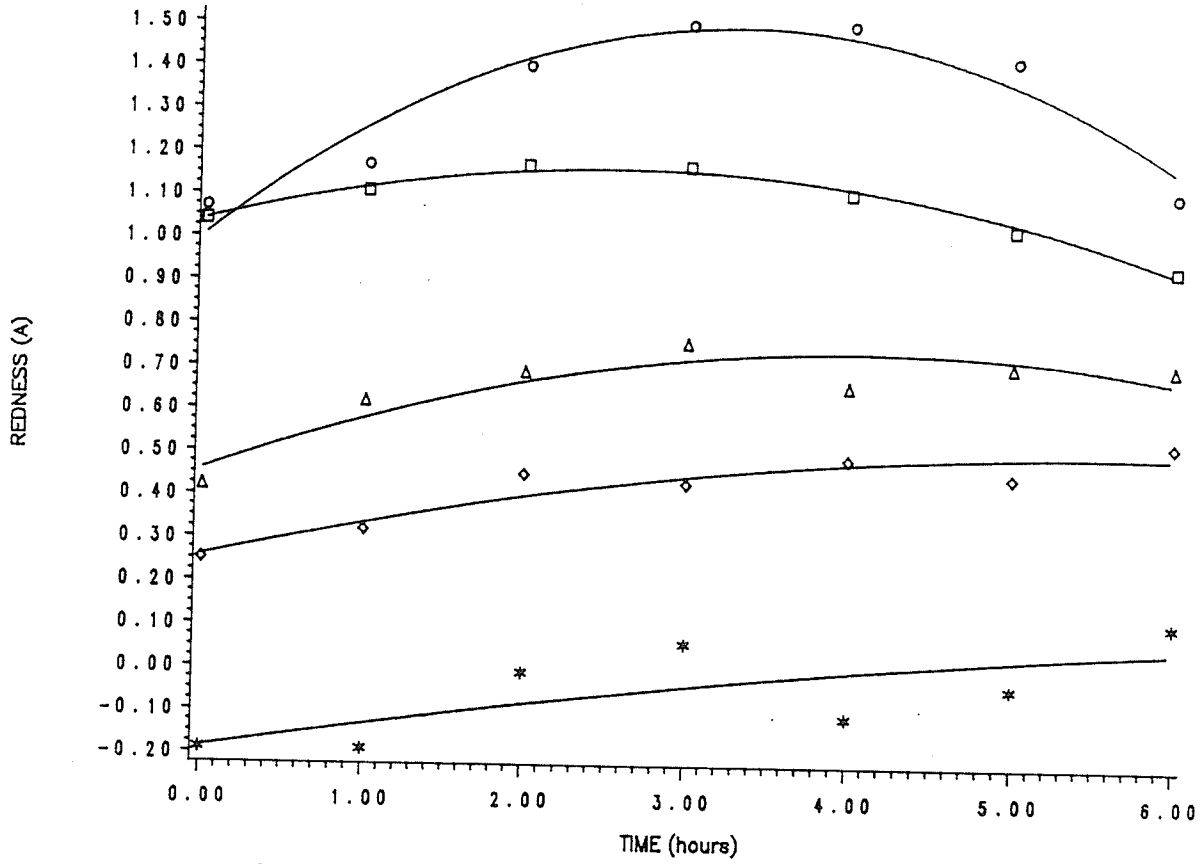
YELLOWNESS AS A FUNCTION OF TIME
FIELDER 80% EXTRACTION FLOURS

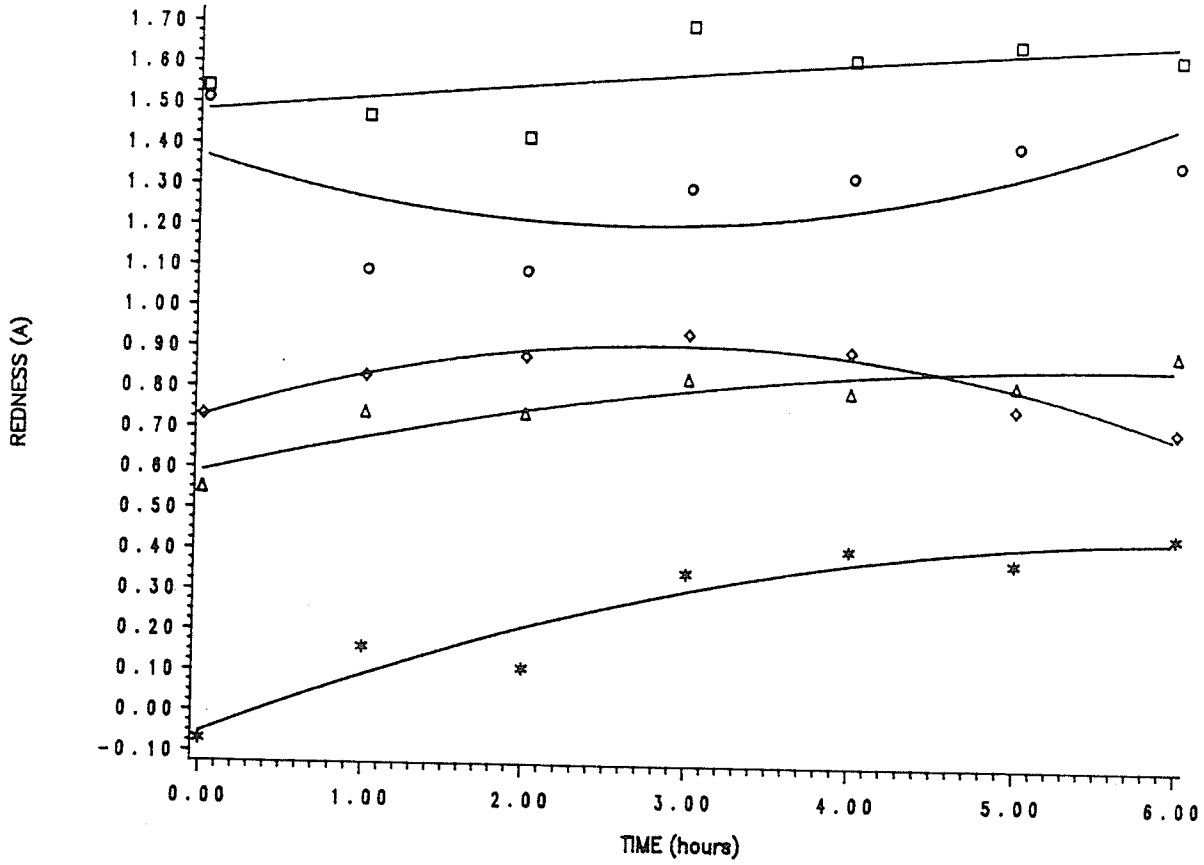


REDNESS AS A FUNCTION OF TIME
FIELDER 75% EXTRACTION FLOURS

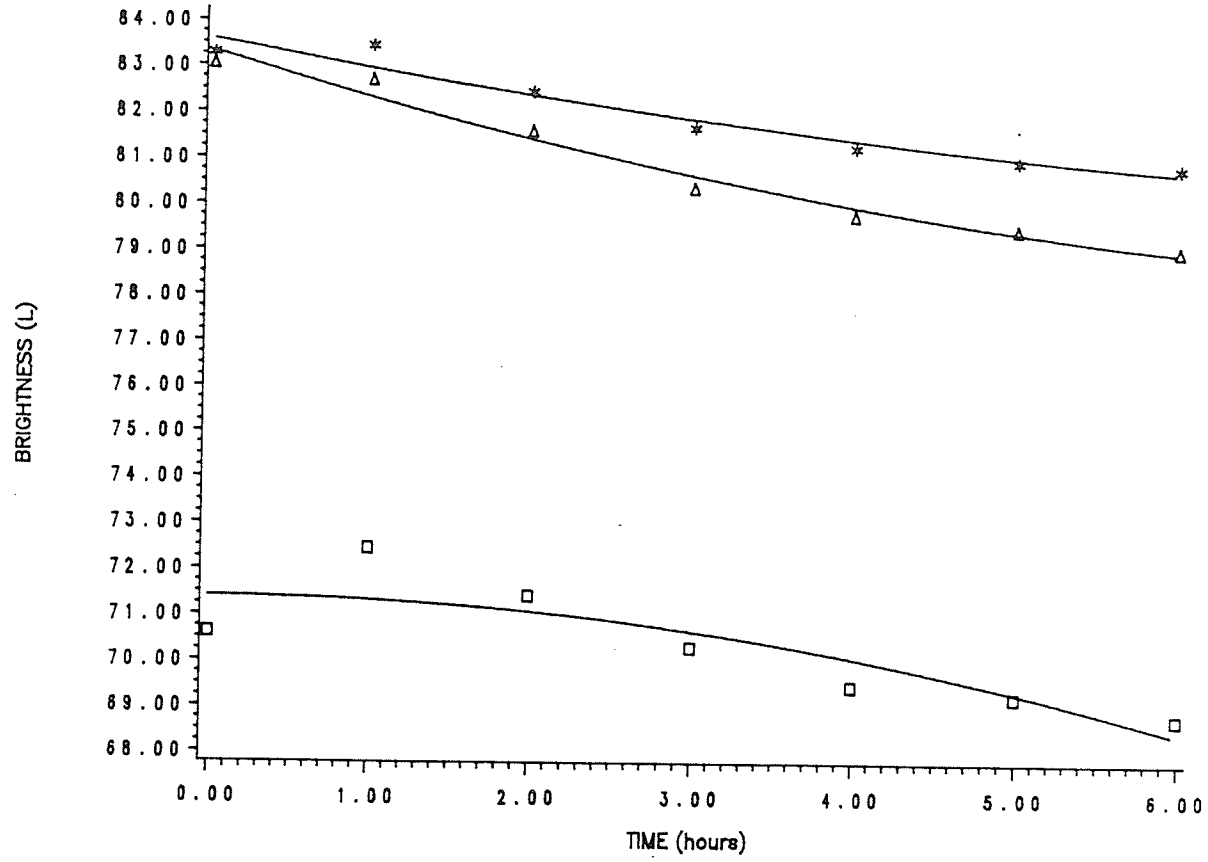


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

REDNESS AS A FUNCTION OF TIME
FIELDER 80% EXTRACTION FLOURS

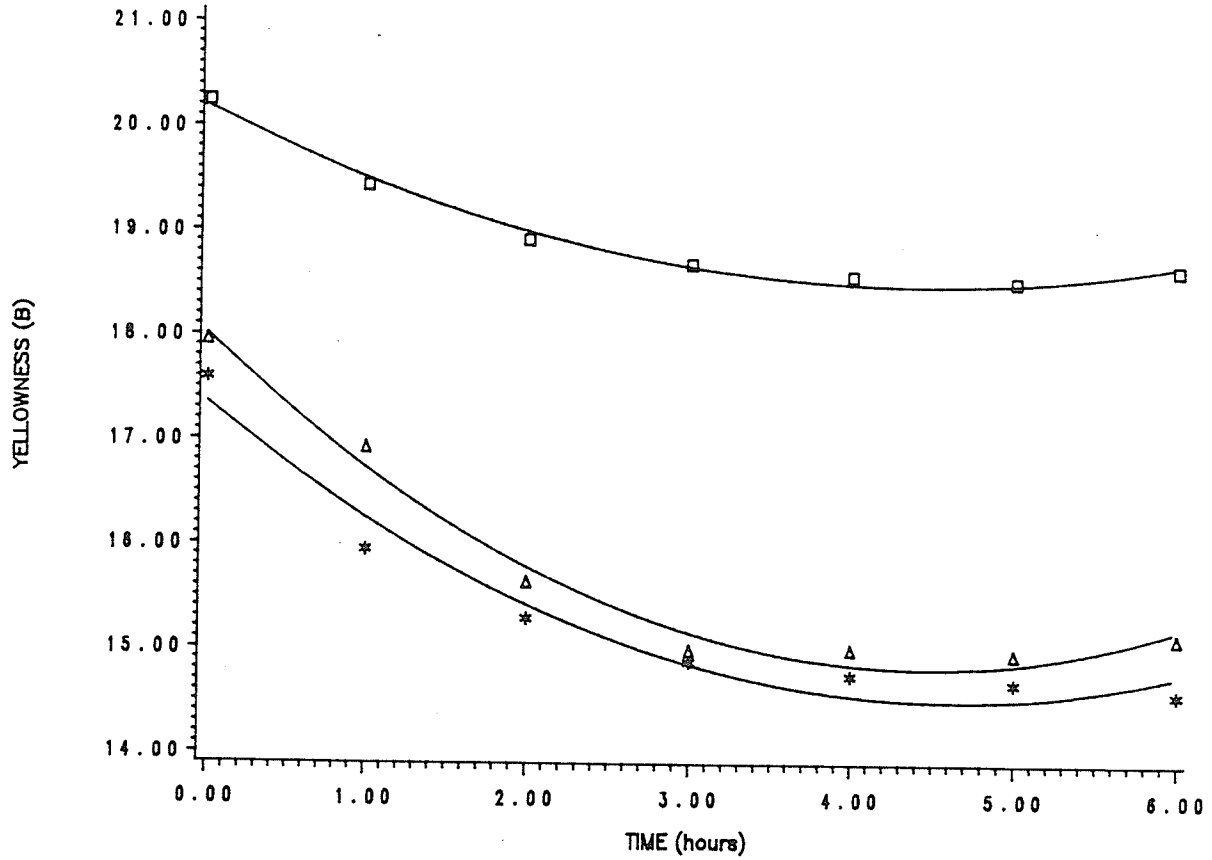


BRIGHTNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS

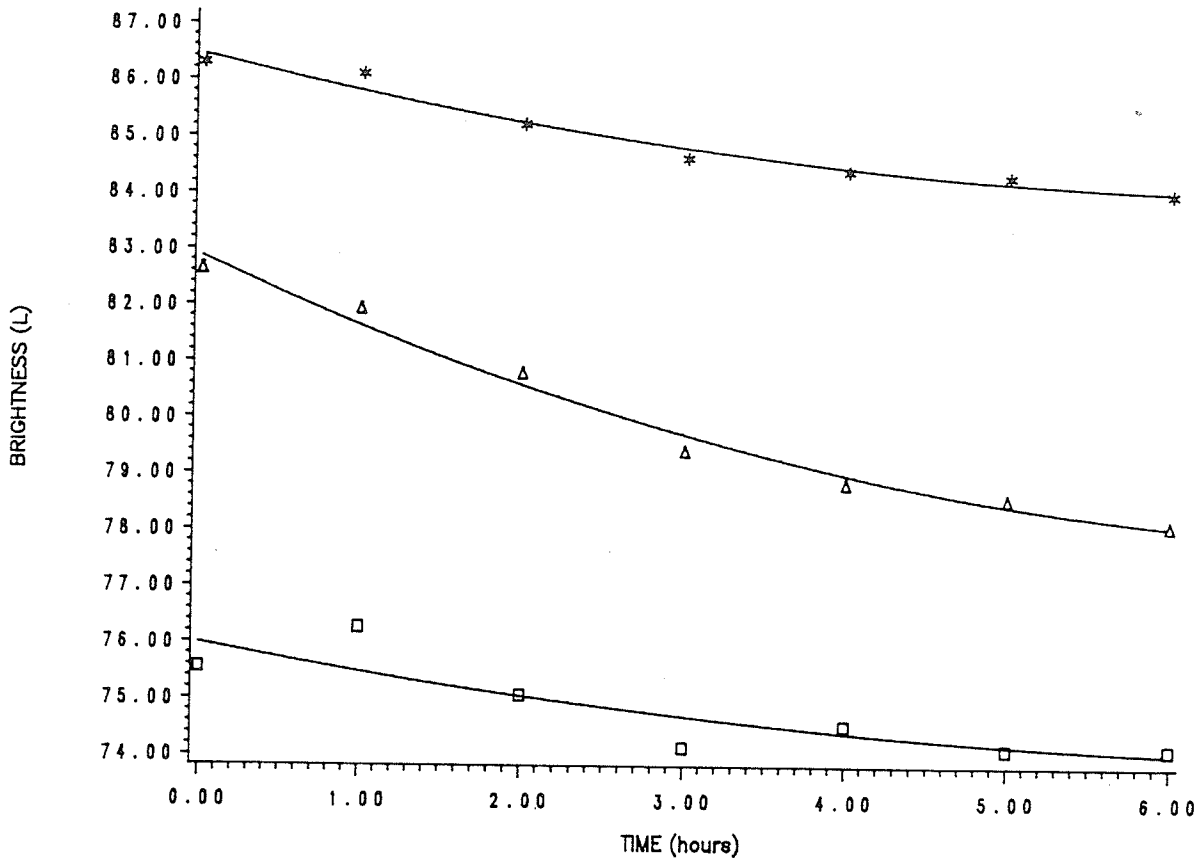


✱ St.Grade 85% △ Chinese Standard 85% □ Whole Wheat

YELLOWNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS

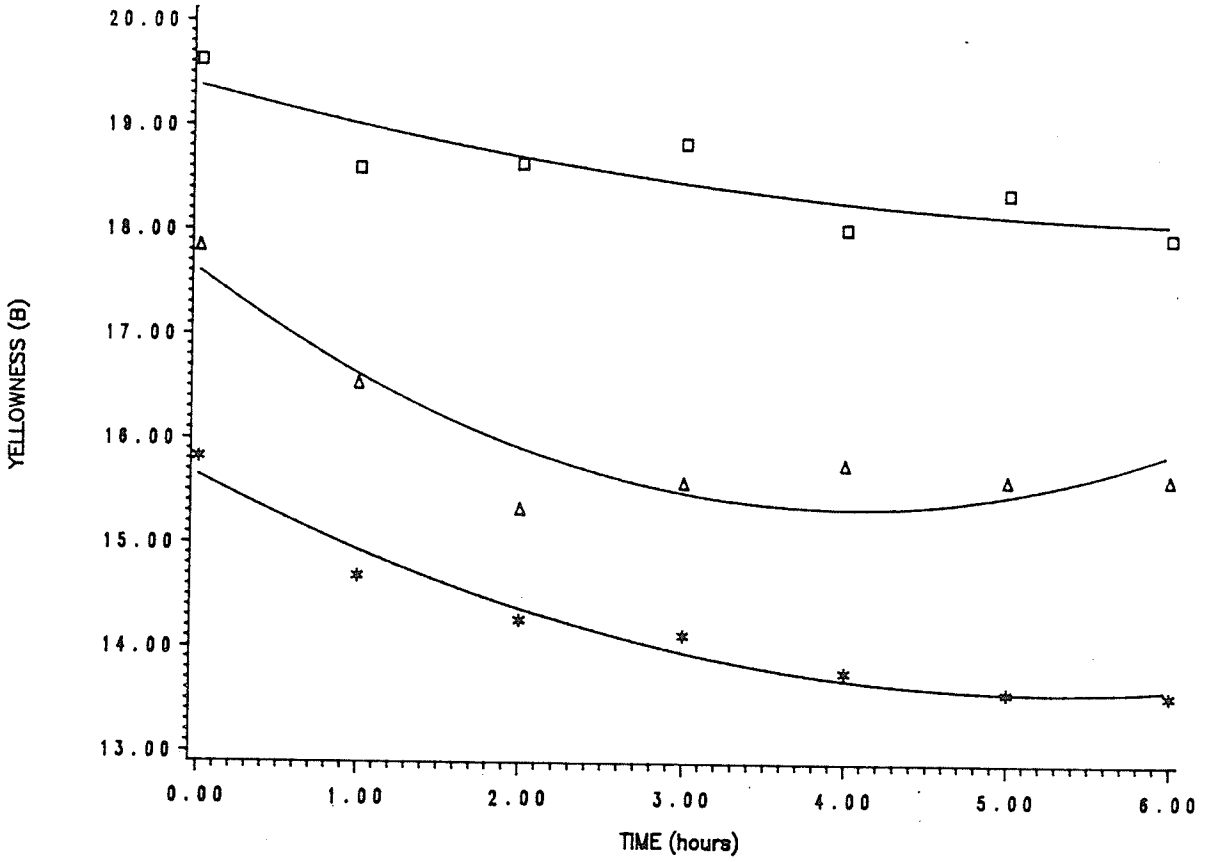


BRIGHTNESS AS A FUNCTION OF TIME
NORSTAR HIGH EXTRACTION FLOURS

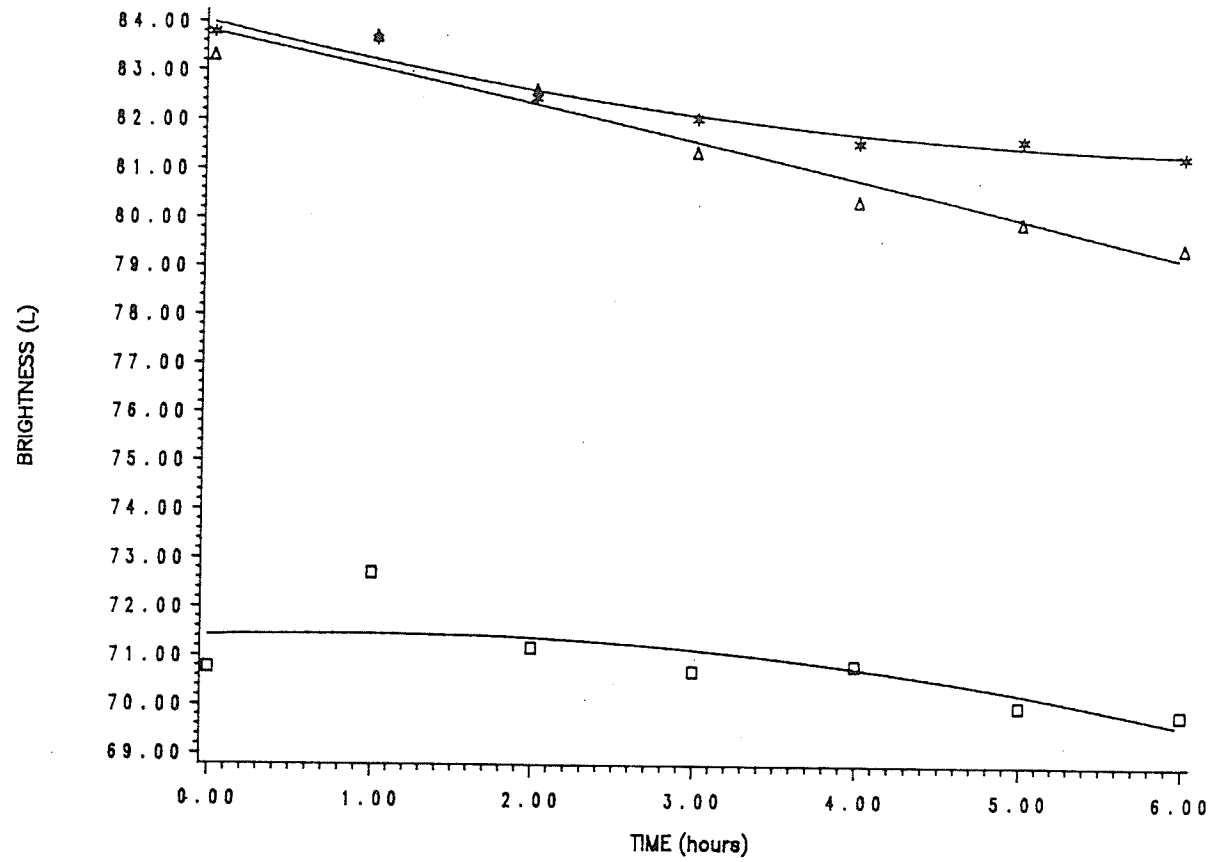


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

YELLOWNESS AS A FUNCTION OF TIME
NORSTAR HIGH EXTRACTION FLOURS

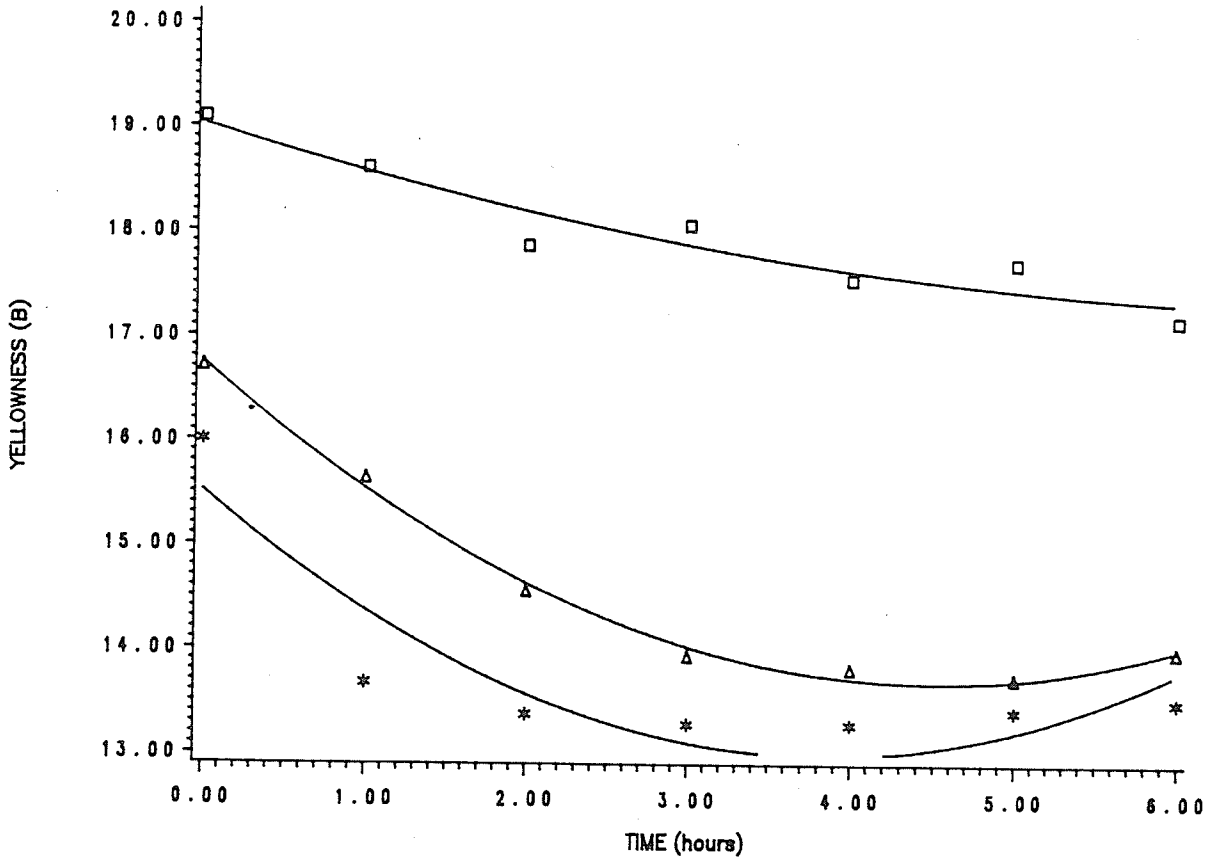


BRIGHTNESS AS A FUNCTION OF TIME
GLENLEA HIGH EXTRACTION FLOURS

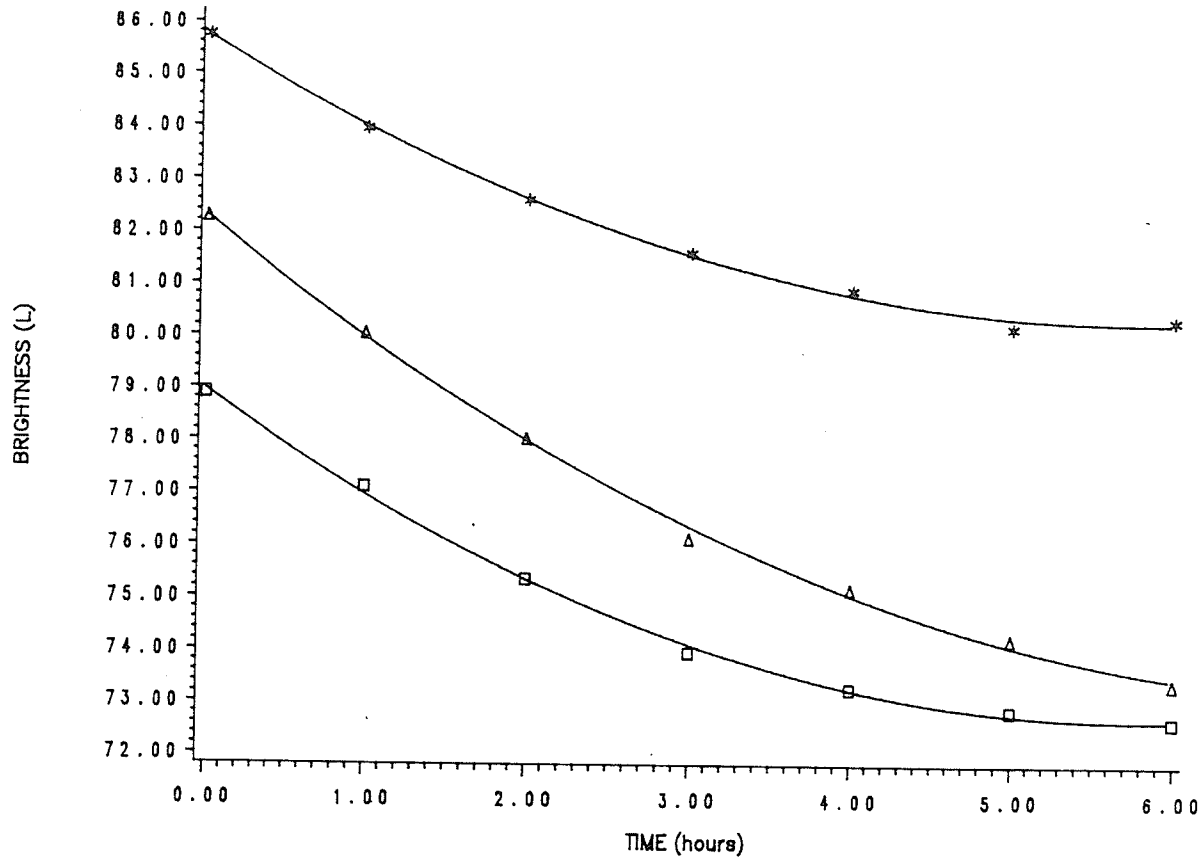


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

YELLOWNESS AS A FUNCTION OF TIME
GLENLEA HIGH EXTRACTION FLOURS

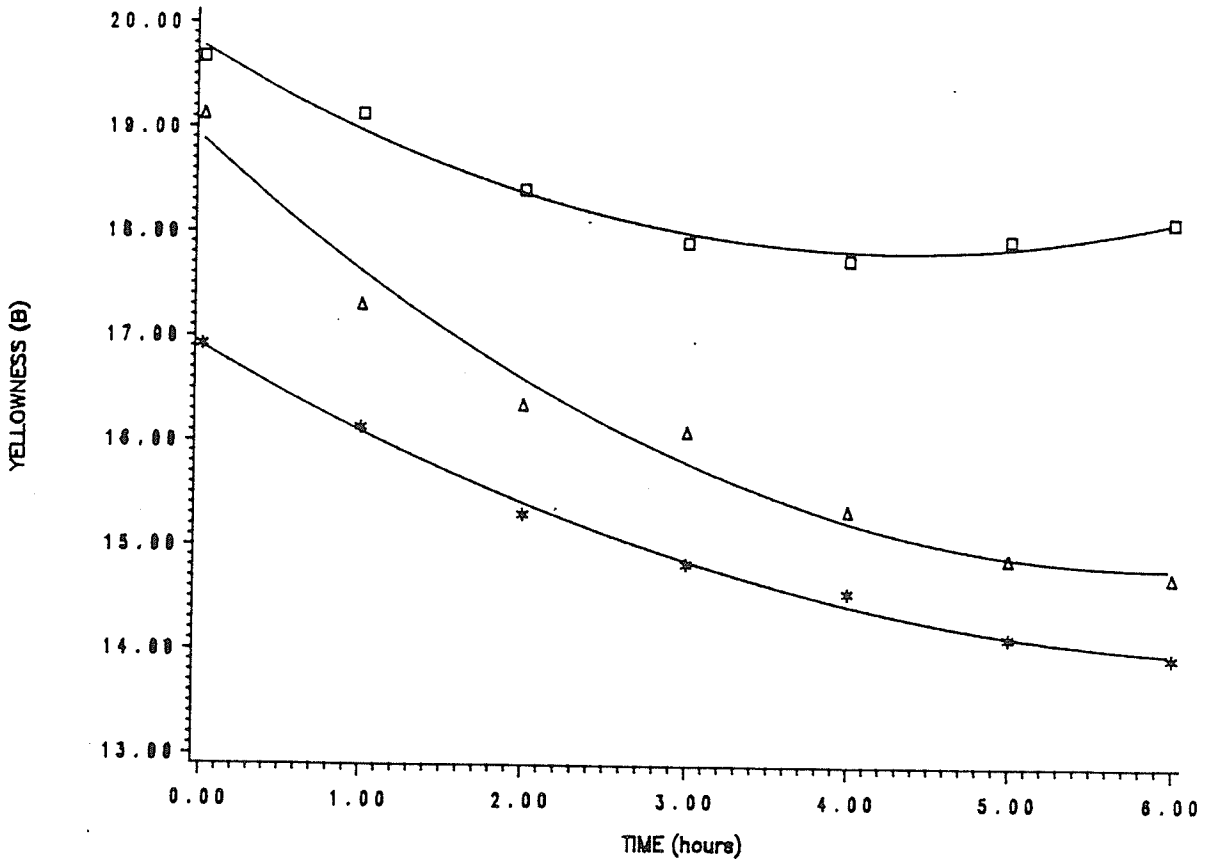


BRIGHTNESS AS A FUNCTION OF TIME
FIELDER HIGH EXTRACTION FLOURS

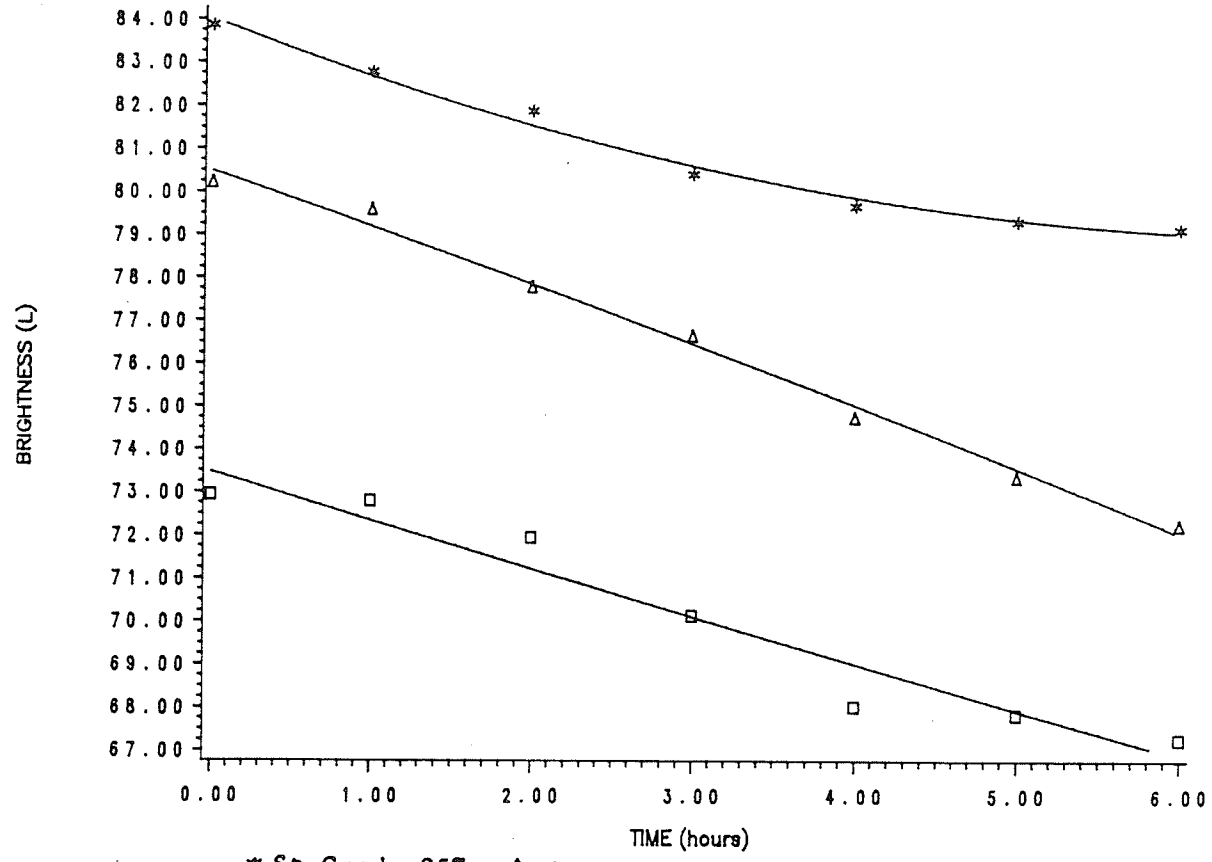


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

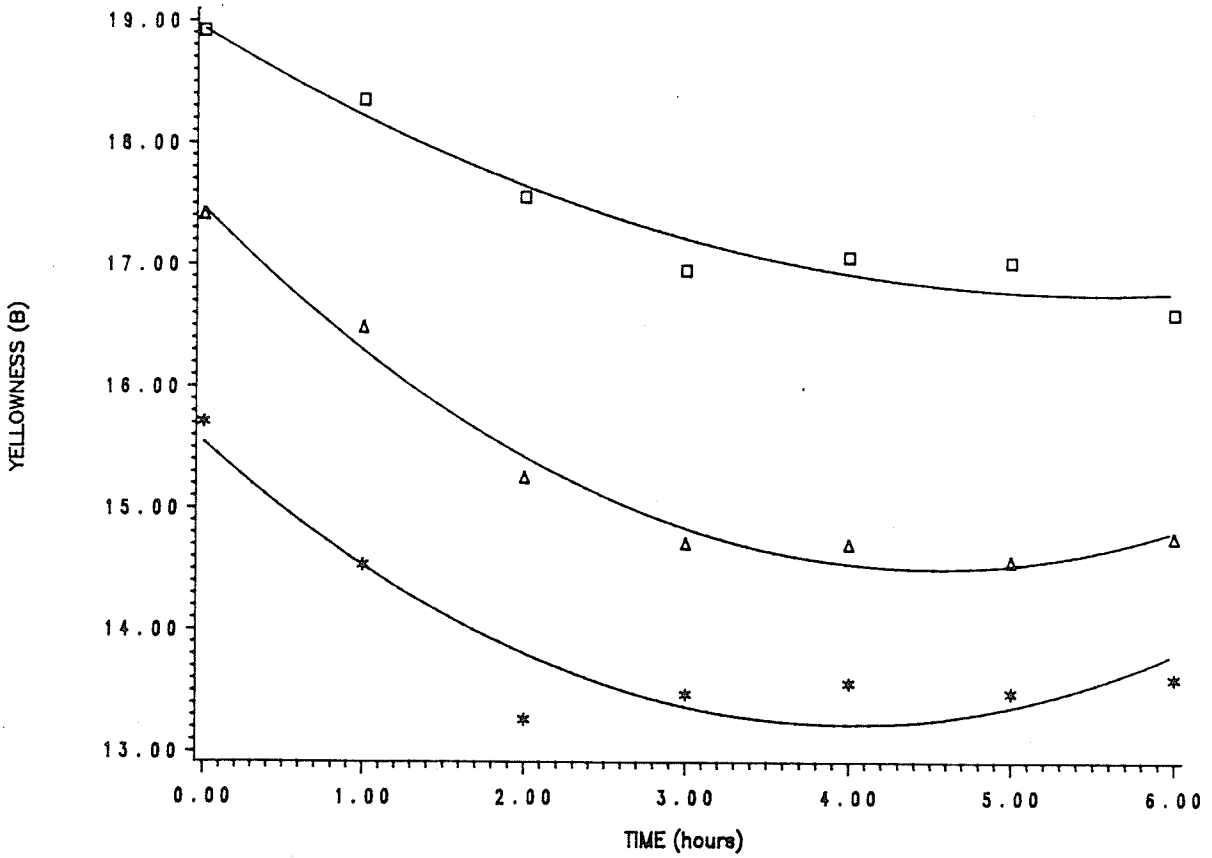
YELLOWNESS AS A FUNCTION OF TIME
FIELDER HIGH EXTRACTION FLOURS



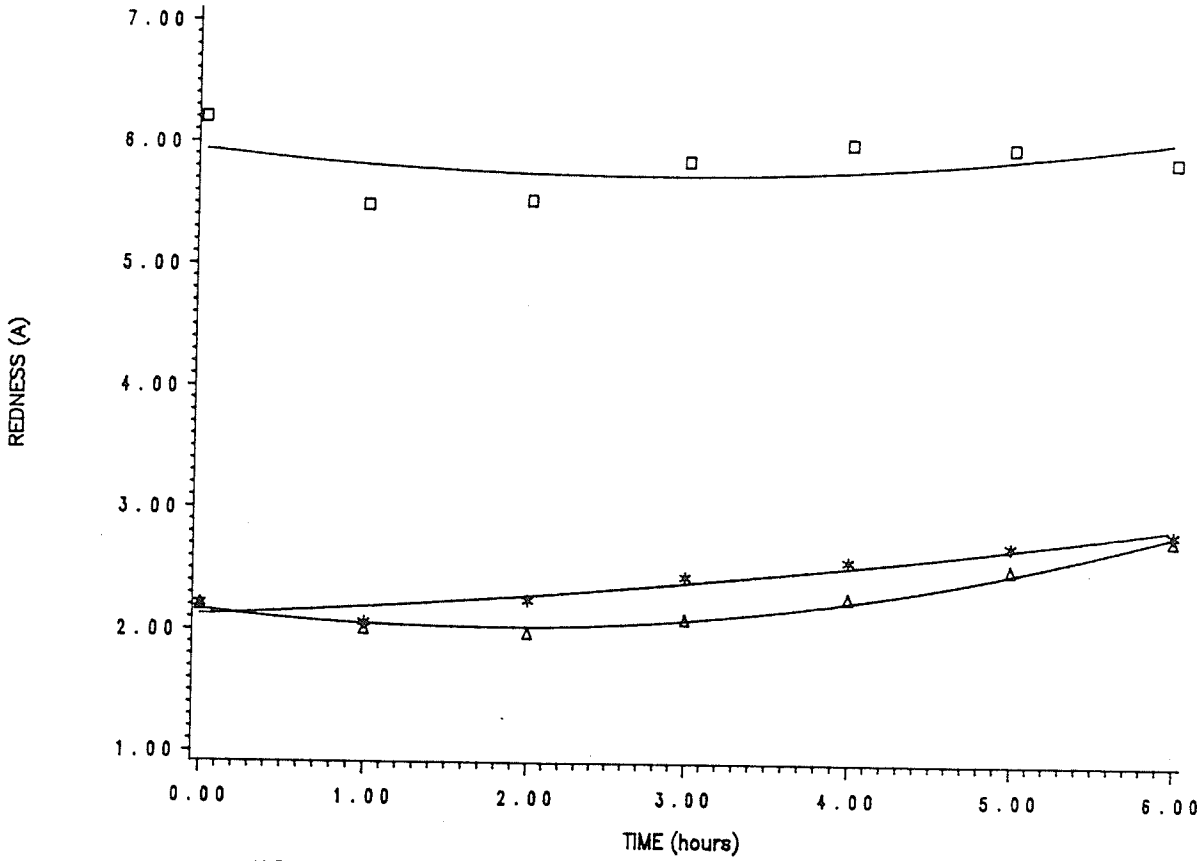
BRIGHTNESS AS A FUNCTION OF TIME
HY 320 HIGH EXTRACTION FLOURS



YELLOWNESS AS A FUNCTION OF TIME
HY 320 HIGH EXTRACTION FLOURS

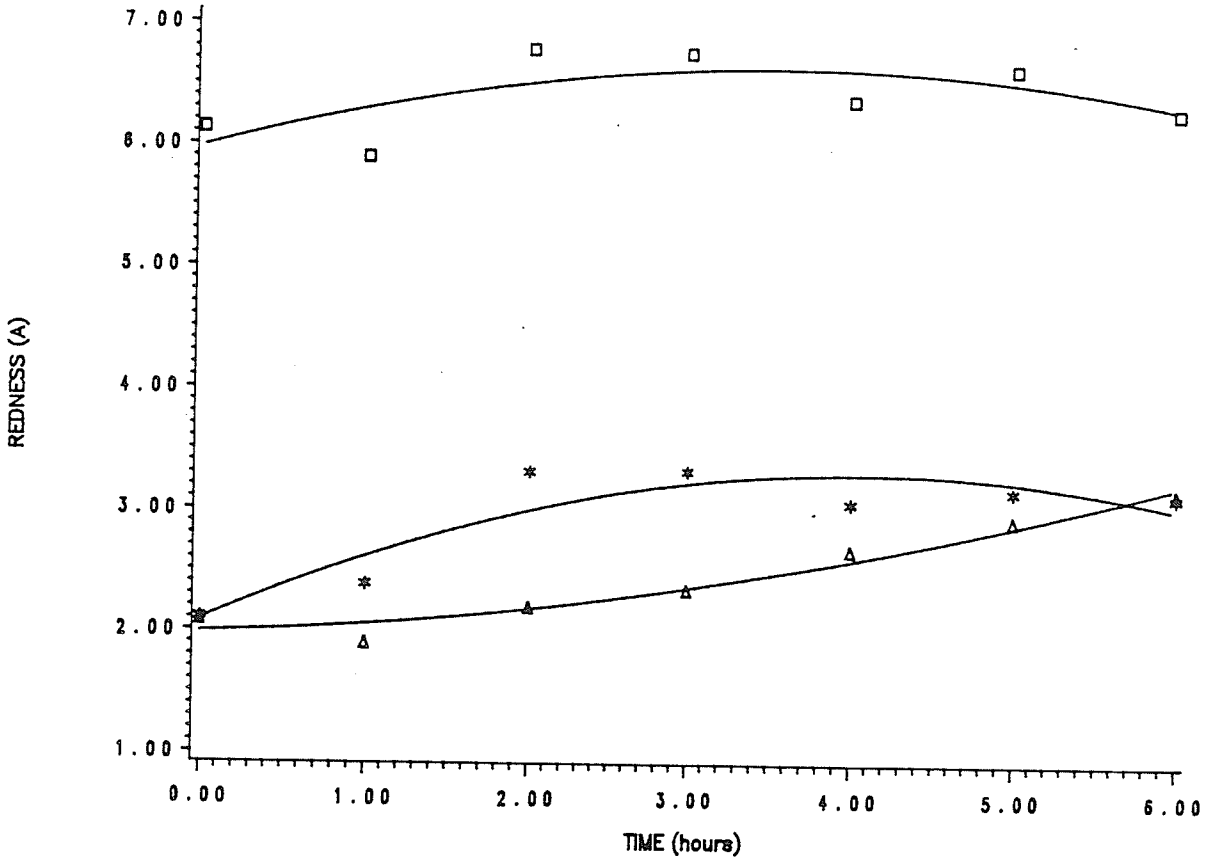


REDNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS

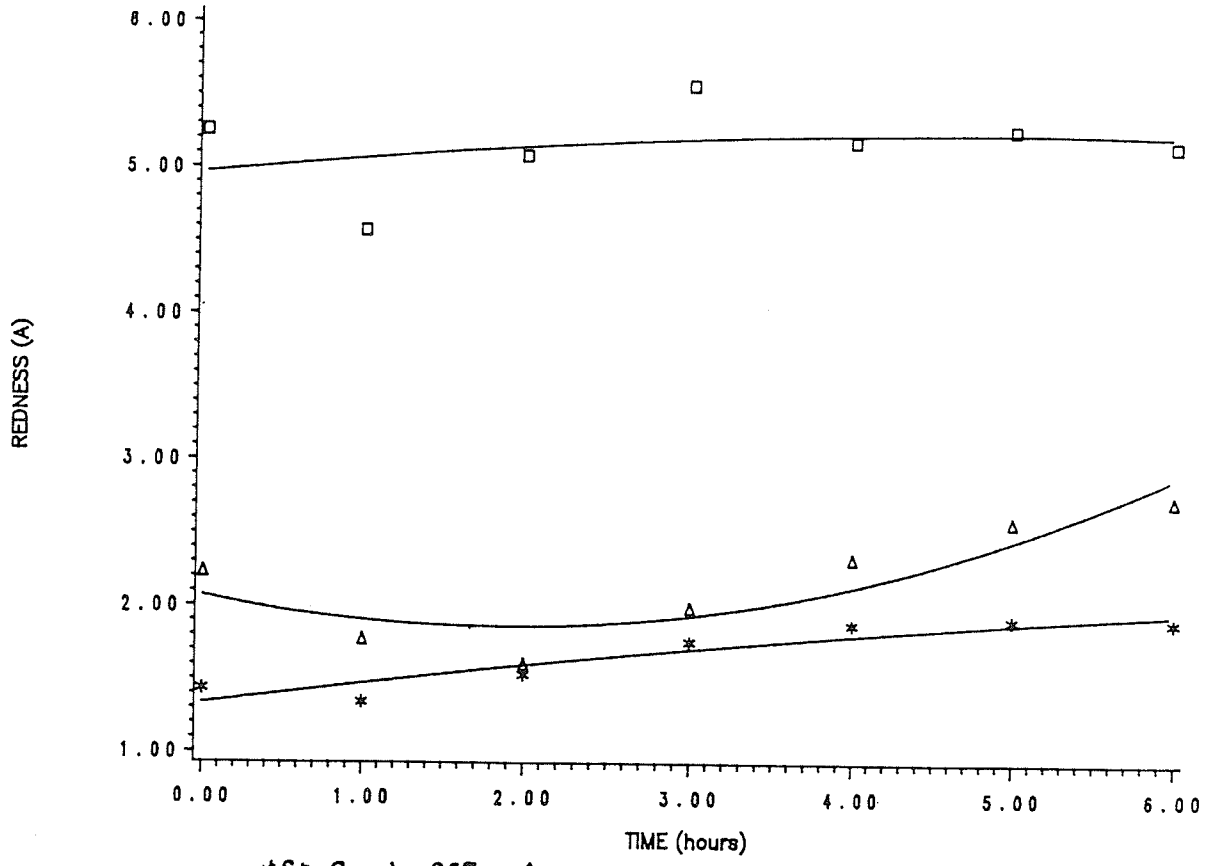


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

REDNESS AS A FUNCTION OF TIME
GLENLEA HIGH EXTRACTION FLOURS

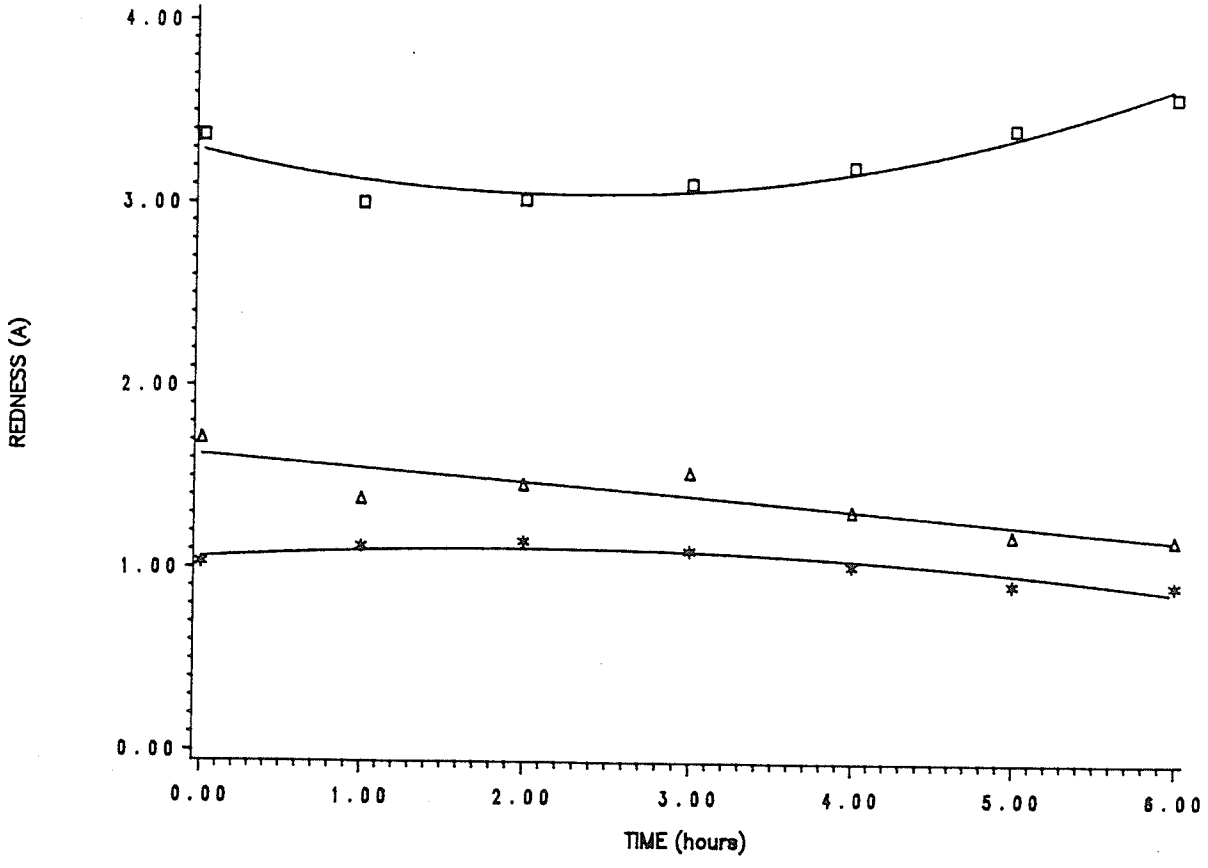


REDNESS AS A FUNCTION OF TIME
NORSTAR HIGH EXTRACTION FLOURS

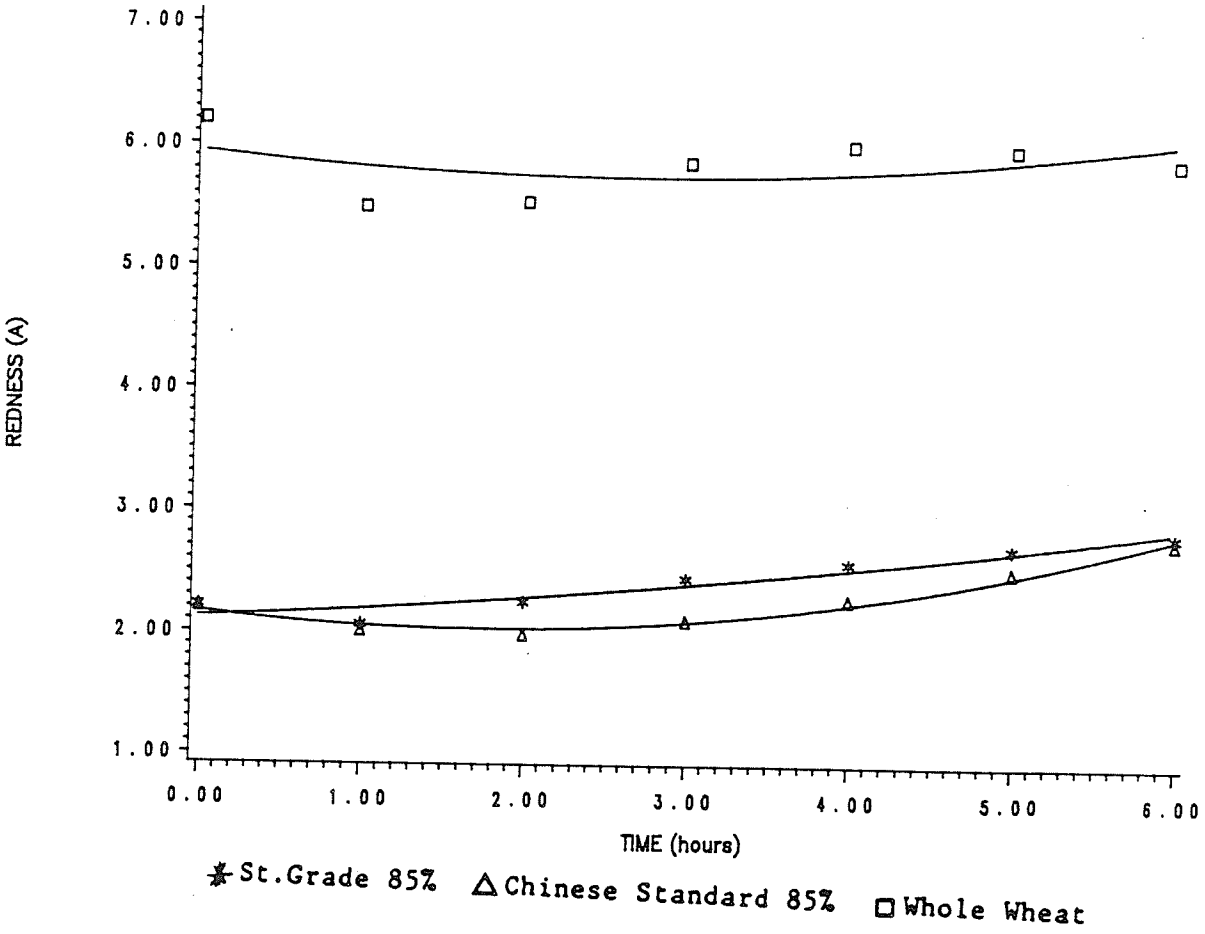


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

REDNESS AS A FUNCTION OF TIME
FIELDER HIGH EXTRACTION FLOURS



REDNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS



Appendix E: Kan Sui Flour Paste HunterLab Values Over Time

Maximum Range in Respective Kan Sui Color Component for a 95% Confidence Limit

Brightness (L^*)

Flour	Katepwa	Glenlea	Norstar	HY320	Fielder
75% Extraction					
1st Patent	0.58	0.26	0.23	0.23	0.39
2nd Patent	1.31	0.17	0.16	0.39	1.17
1st Clear	0.58	0.69	0.70	1.60	3.28
2nd Clear	1.90	1.38	1.48	1.88	1.88
St. Grade	1.45	0.17	0.16	0.48	0.63
80% Extraction					
1st Patent	0.63	0.56	0.34	0.30	0.19
2nd Patent	0.31	0.38	0.43	2.00	0.47
1st Clear	1.09	0.75	2.58	2.00	1.50
2nd Clear	1.25	2.06	2.75	1.39	0.56
St. Grade	0.63	0.38	0.43	0.79	2.63
85% Extraction					
St. Grade	0.63	0.88	0.63	1.38	0.78
Chinese	1.68	1.75	1.57	1.68	1.88
Whole Wheat	2.47	3.28	3.33	3.76	3.52

Maximum Range in Respective Kan Sui Color Component for a 95% Confidence Limit

Yellowness (b^*)

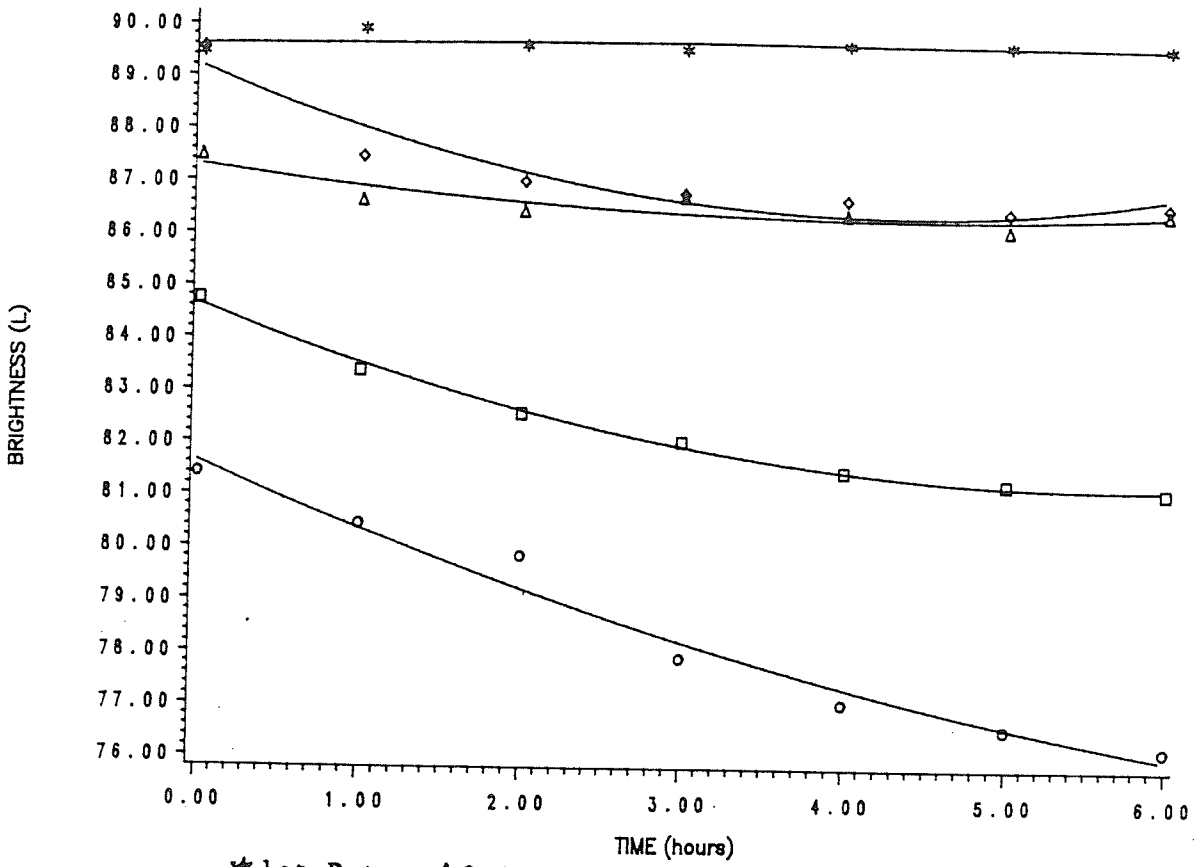
Flour	Katepwa	Glenlea	Norstar	HY320	Fielder
75% Extraction					
1st Patent	2.21	1.68	1.44	1.62	0.51
2nd Patent	1.77	1.95	1.32	1.20	1.31
1st Clear	1.77	2.04	1.08	1.80	1.75
2nd Clear	1.95	2.04	0.96	0.54	2.62
St. Grade	1.86	1.77	1.44	1.08	0.73
80% Extraction					
1st Patent	2.63	1.33	1.51	1.48	0.94
2nd Patent	2.99	1.88	1.77	1.25	0.83
1st Clear	3.47	1.72	0.88	1.17	0.73
2nd Clear	2.52	4.22	0.71	0.70	2.29
St. Grade	3.23	1.88	1.59	1.25	1.15
85% Extraction					
St. Grade	1.25	1.04	1.53	0.80	0.58
Chinese	1.35	2.17	0.73	1.39	0.66
Whole Wheat	1.46	1.08	1.75	2.77	3.06

Maximum Range in Respective Kan Sui Color Component for a 95% Confidence Limit

Redness (a^*)

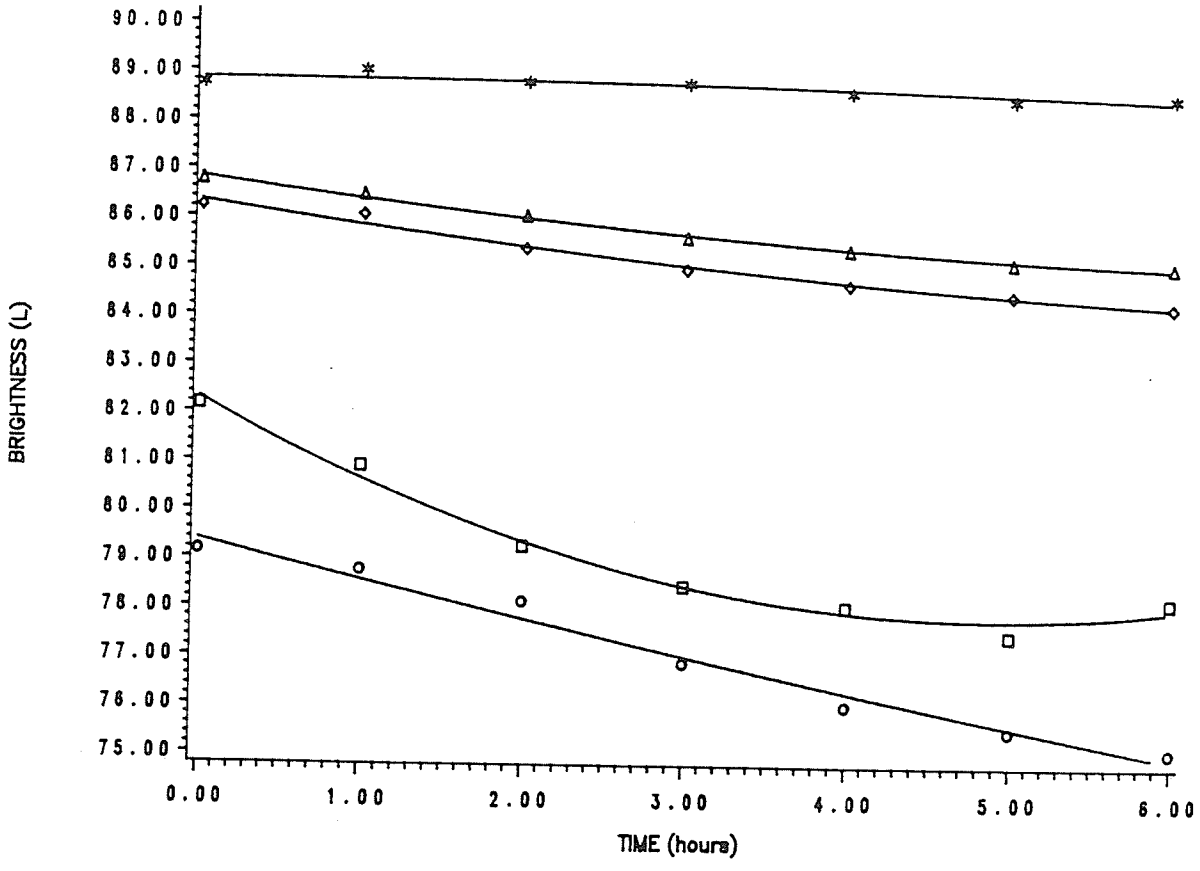
Flour	Katepwa	Glenlea	Norstar	HY320	Fielder
75% Extraction					
1st Patent	0.16	0.36	0.50	0.28	0.63
2nd Patent	0.16	0.41	0.42	0.25	0.68
1st Clear	0.22	0.31	0.63	0.34	0.75
2nd Clear	0.69	0.63	0.50	0.28	0.68
St. Grade	0.19	0.36	0.54	0.25	0.60
80% Extraction					
1st Patent	0.16	0.58	0.58	0.38	0.50
2nd Patent	0.22	0.38	0.58	0.33	0.50
1st Clear	0.22	0.50	0.50	0.25	0.63
2nd Clear	1.09	1.21	0.75	0.92	1.13
St. Grade	0.09	0.29	0.67	0.33	0.50
85% Extraction					
St. Grade	0.13	0.60	0.66	0.25	0.83
Chinese	0.50	0.67	1.13	0.67	0.92
Whole Wheat	0.63	1.33	1.59	0.75	1.83

KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
KATEPWA 75% EXTRACTION FLOURS

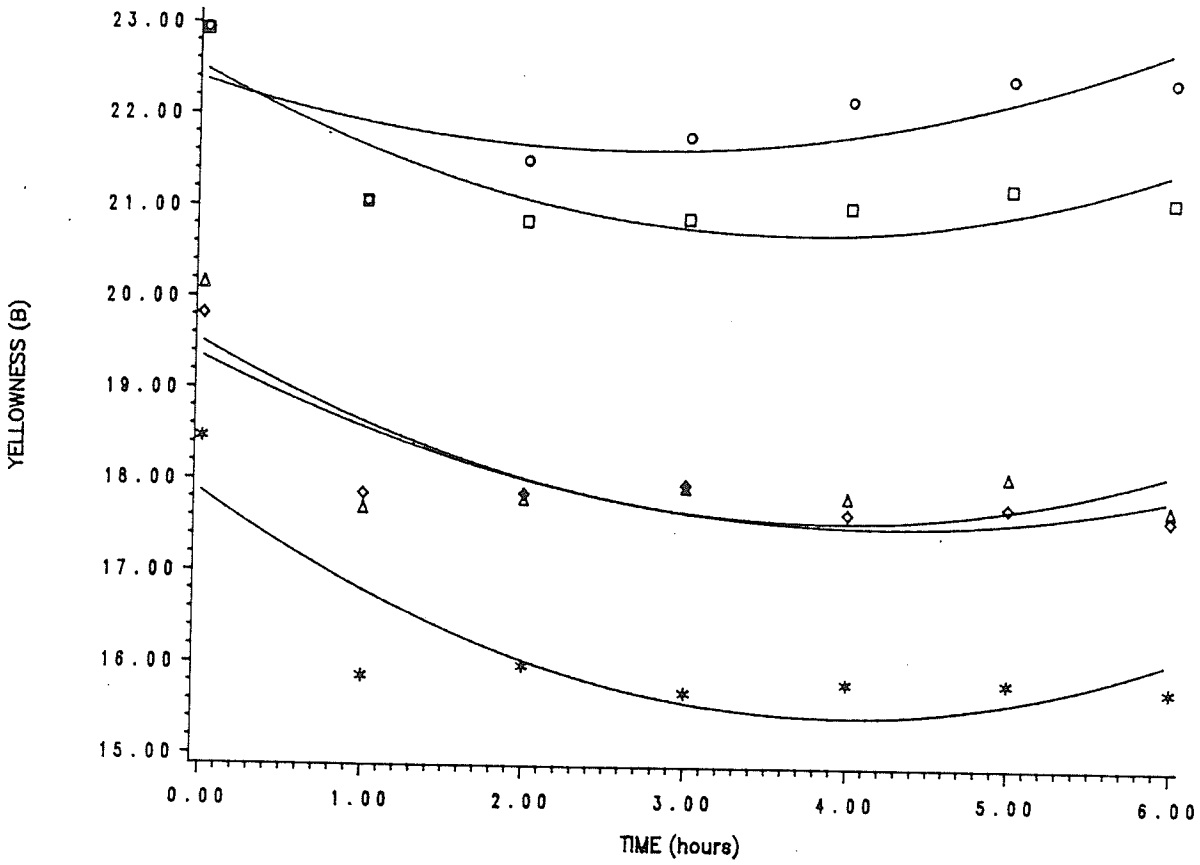


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St. Grade

KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
KATEPWA 80% EXTRACTION FLOURS

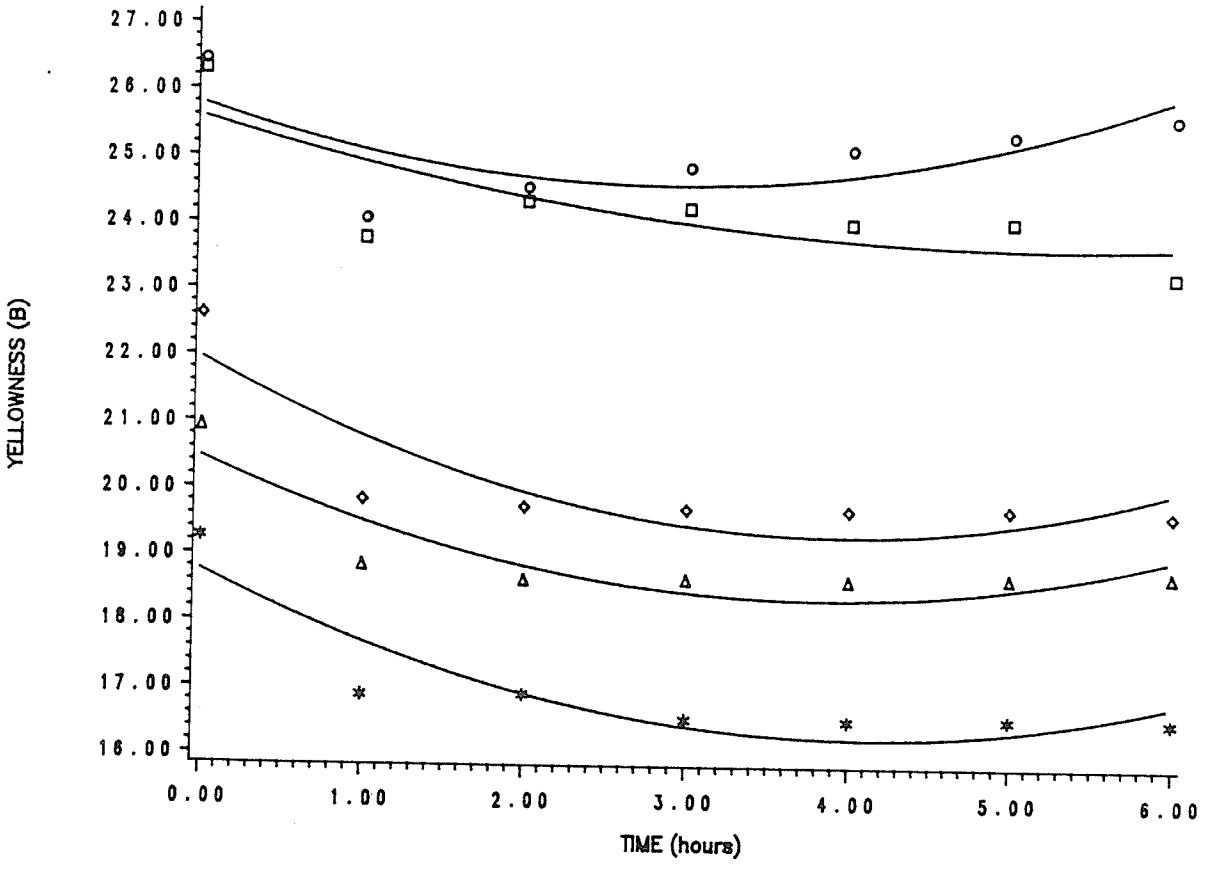


KAN SUI YELLOWNESS AS A FUNCTION OF TIME
KATEPWA 75% EXTRACTION FLOURS

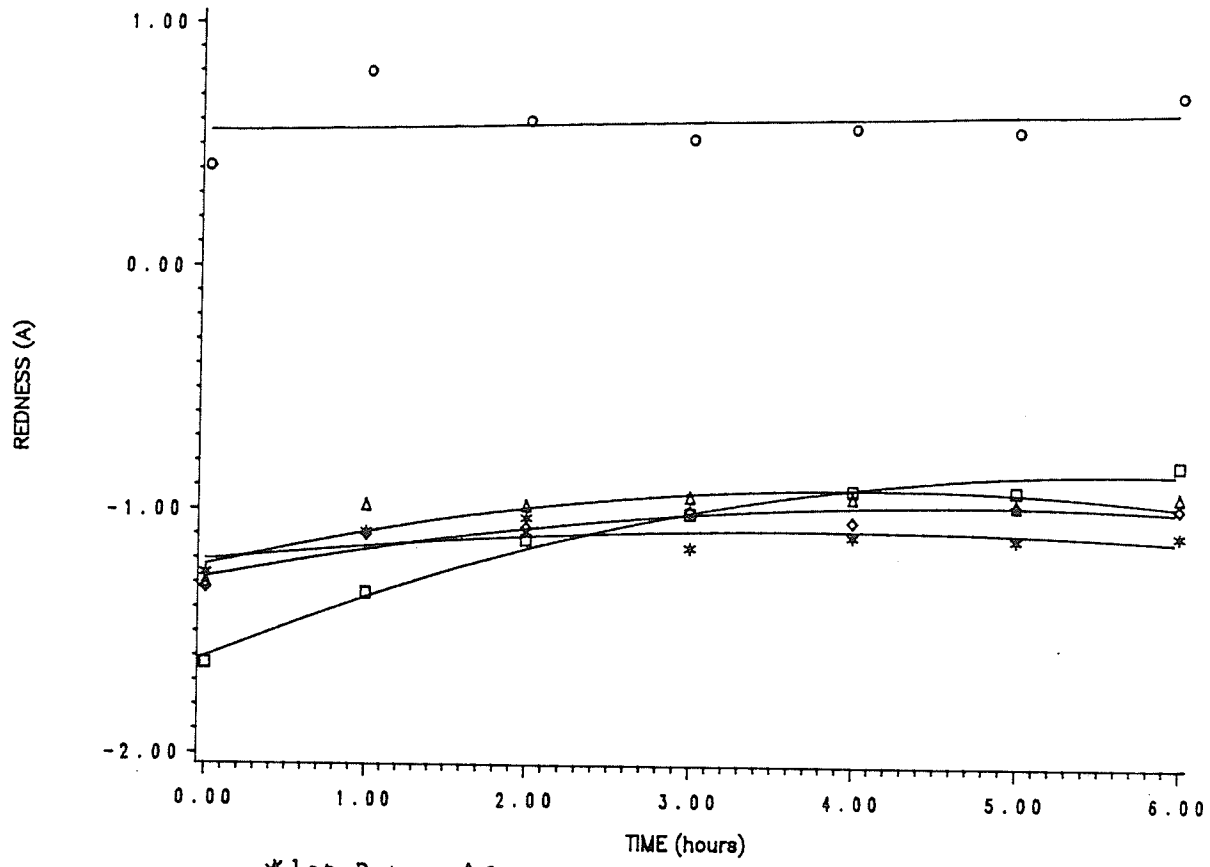


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
KATEPWA 80% EXTRACTION FLOURS

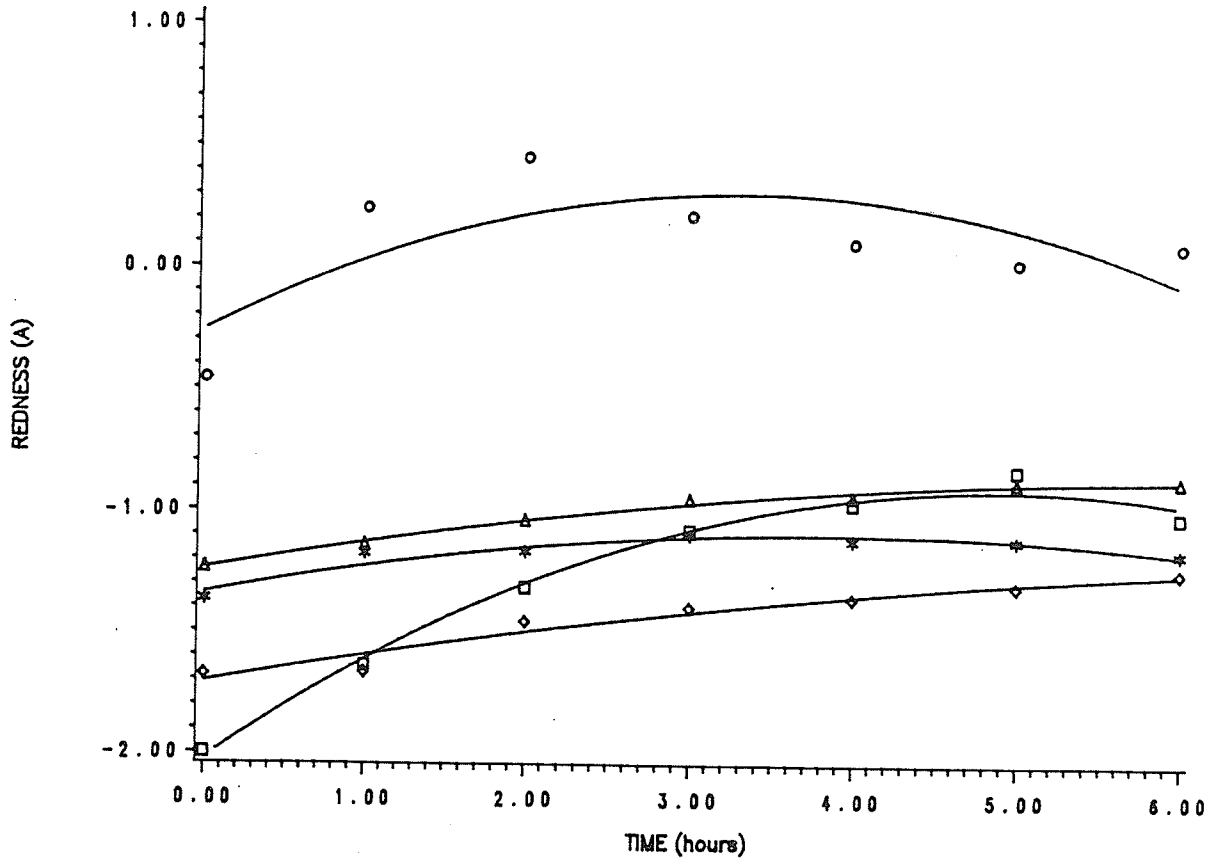


KAN SUI REDNESS AS A FUNCTION OF TIME
KATEPWA 75% EXTRACTION FLOURS

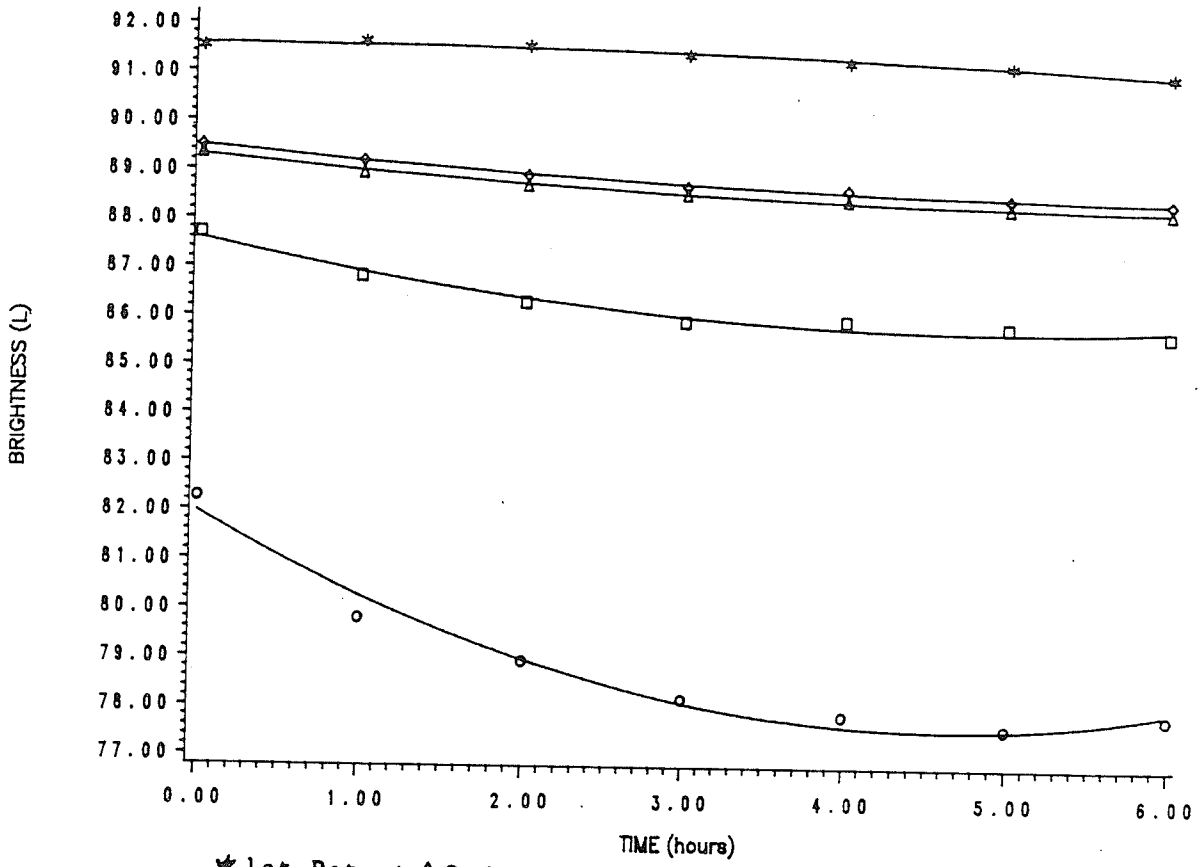


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

KAN SUI REDNESS AS A FUNCTION OF TIME
KATEPWA 80% EXTRACTION FLOURS

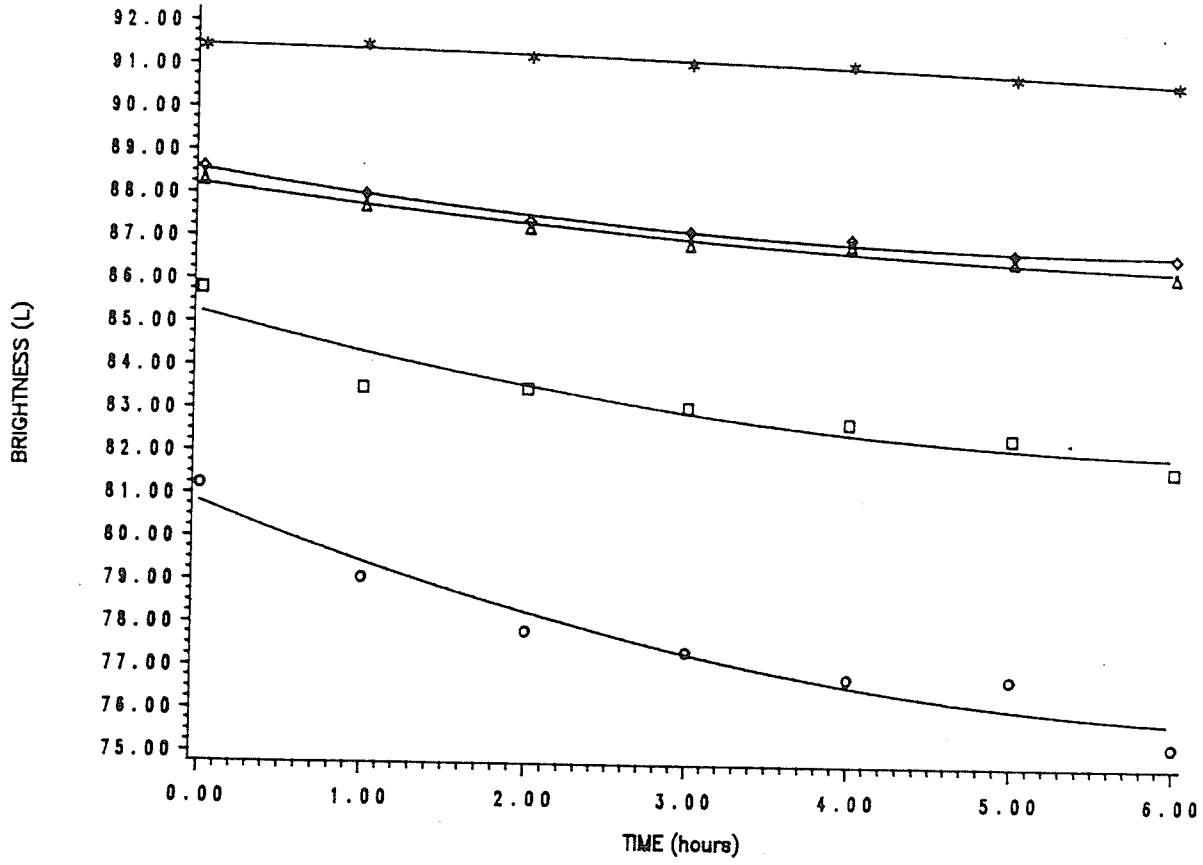


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
NORSTAR 75% EXTRACTION FLOURS

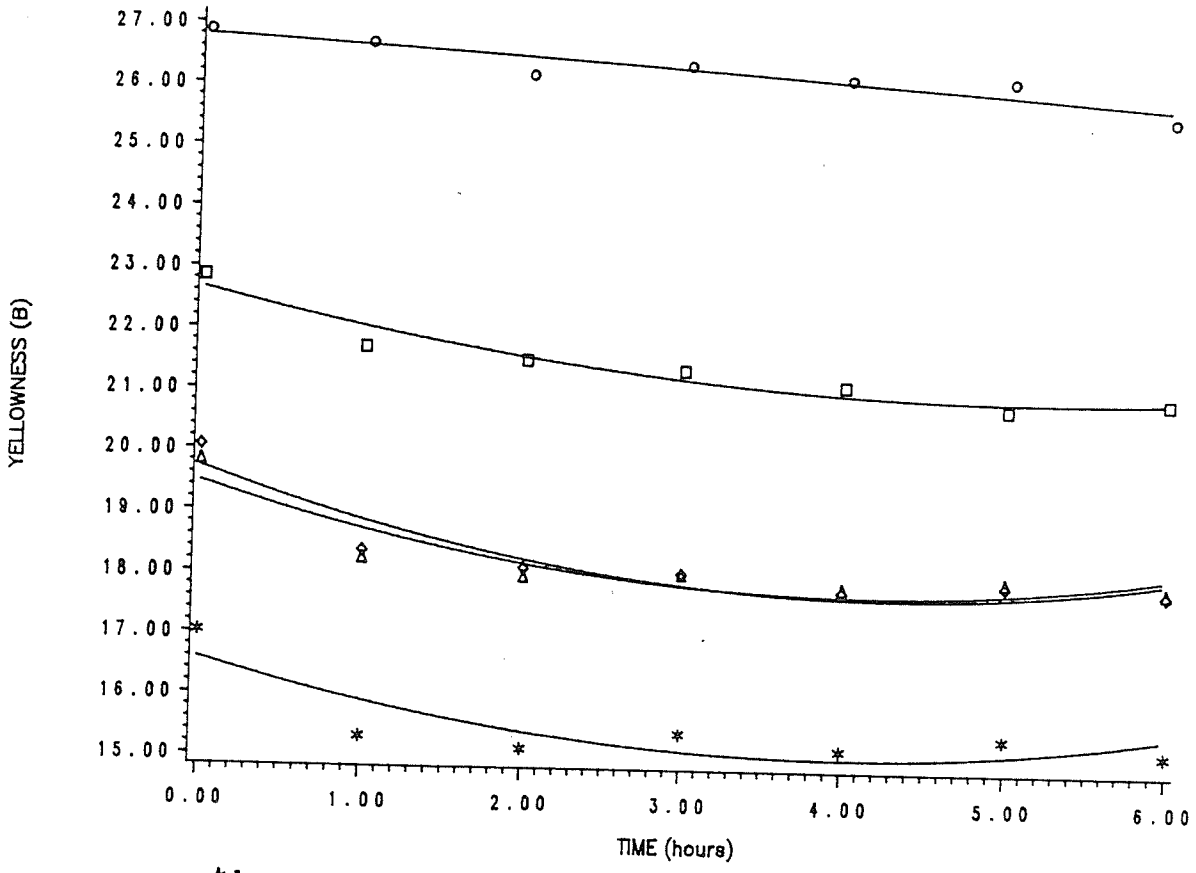


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St. Grade

KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
NORSTAR 80% EXTRACTION FLOURS

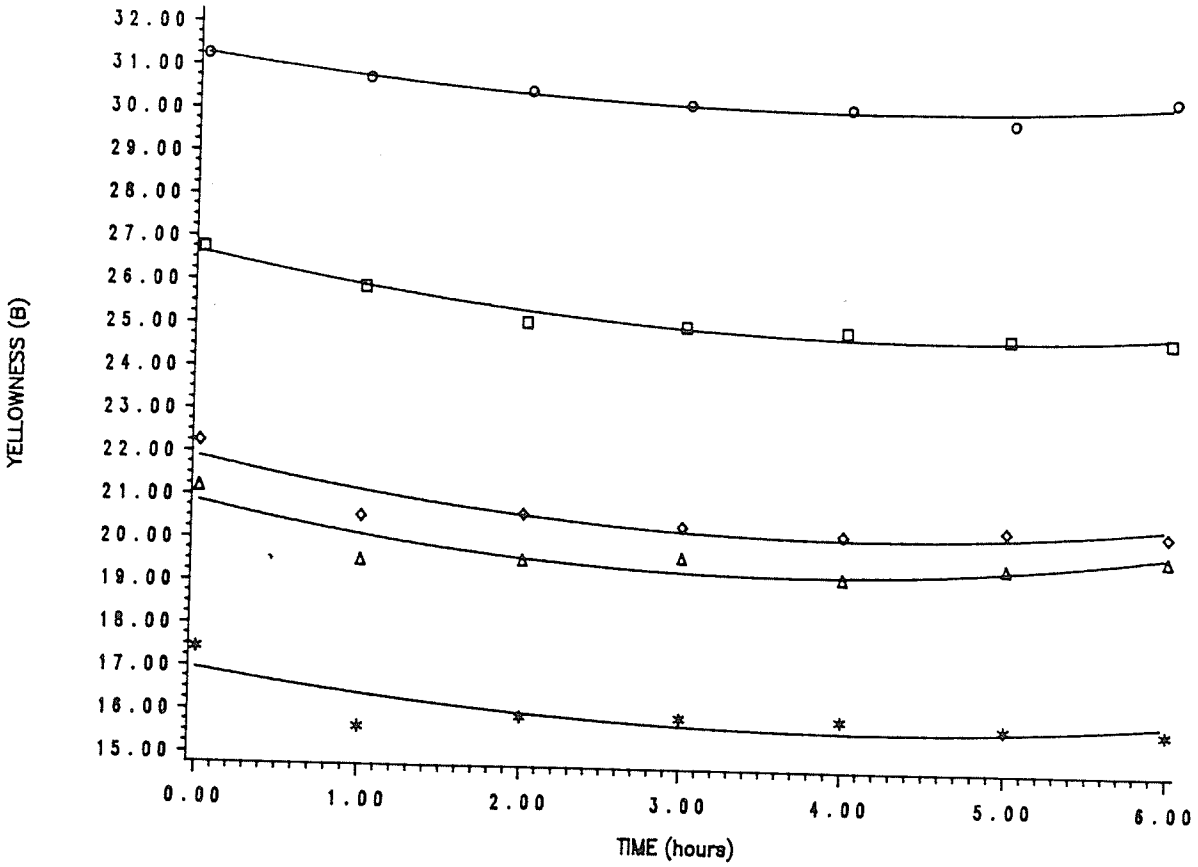


KAN SUI YELLOWNESS AS A FUNCTION OF TIME
NORSTAR 75% EXTRACTION FLOURS

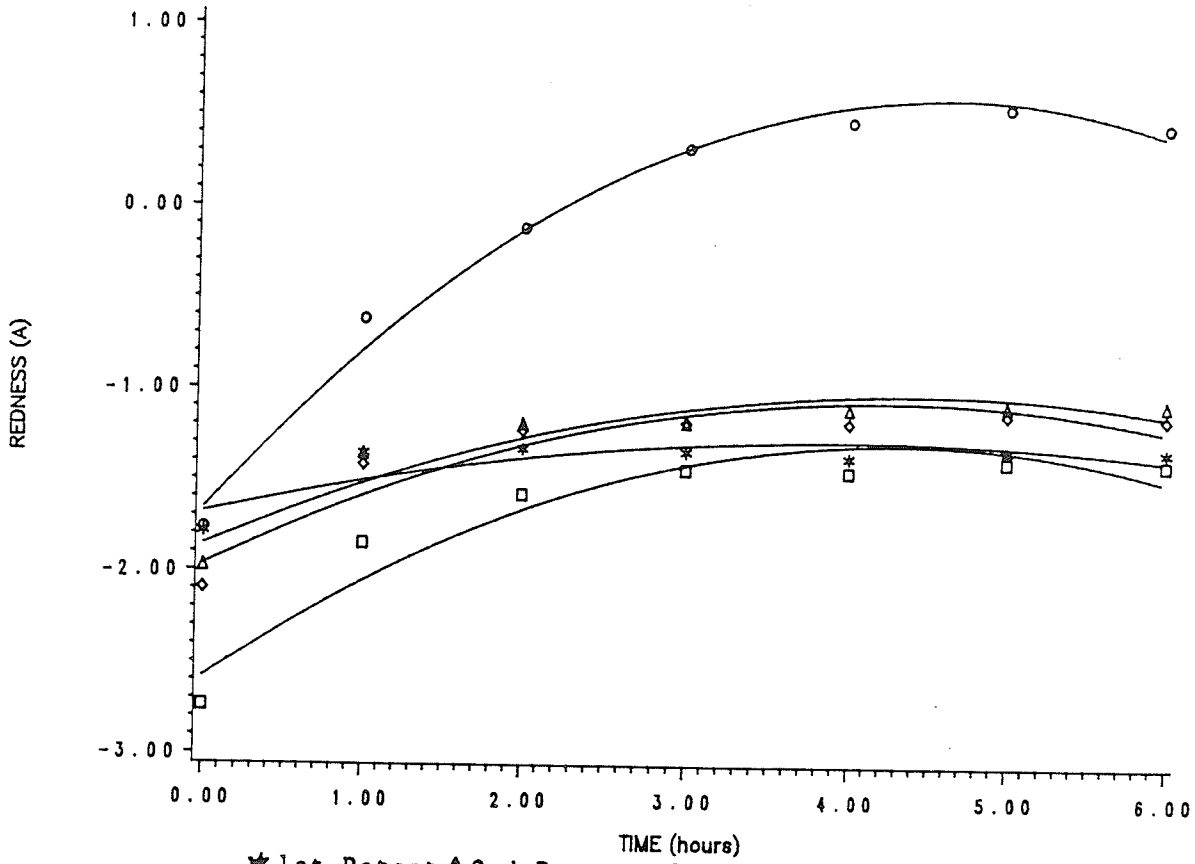


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
NORSTAR 80% EXTRACTION FLOURS

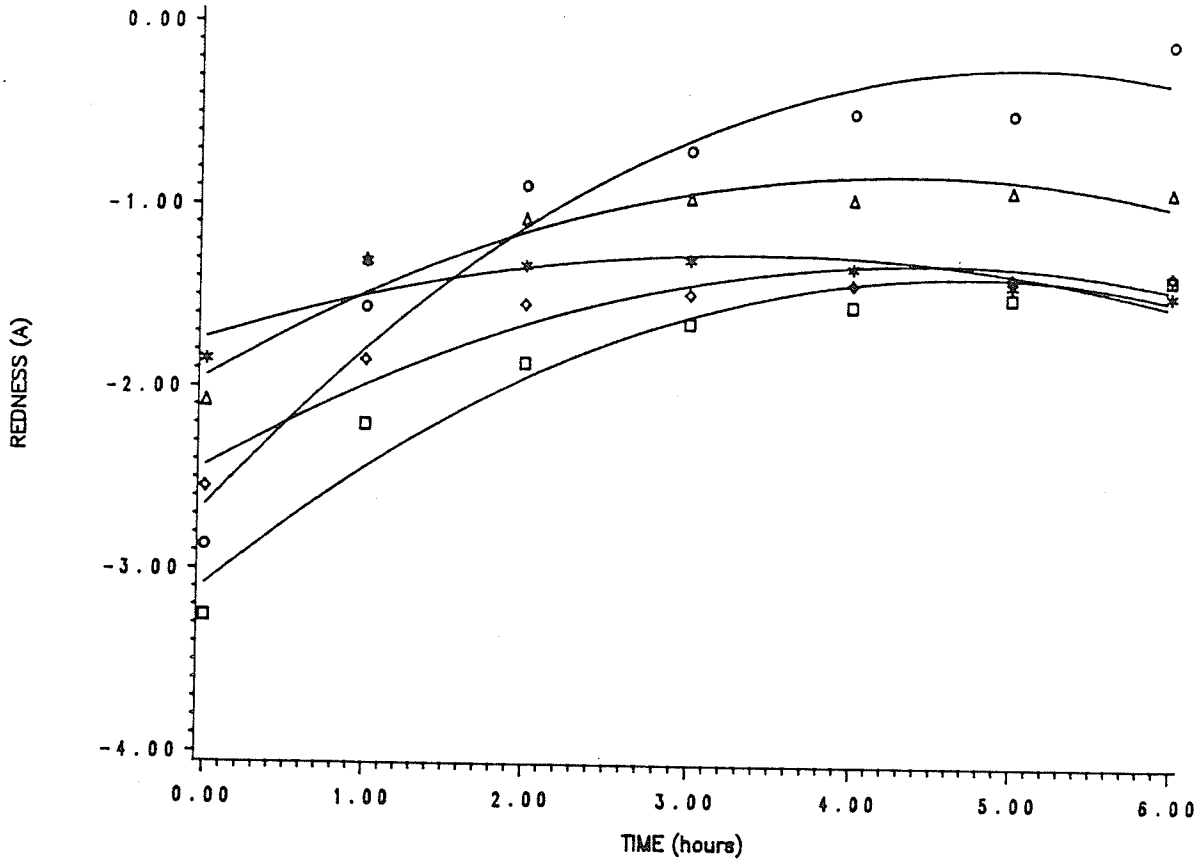


KAN SUI REDNESS AS A FUNCTION OF TIME
NORSTAR 75% EXTRACTION FLOURS

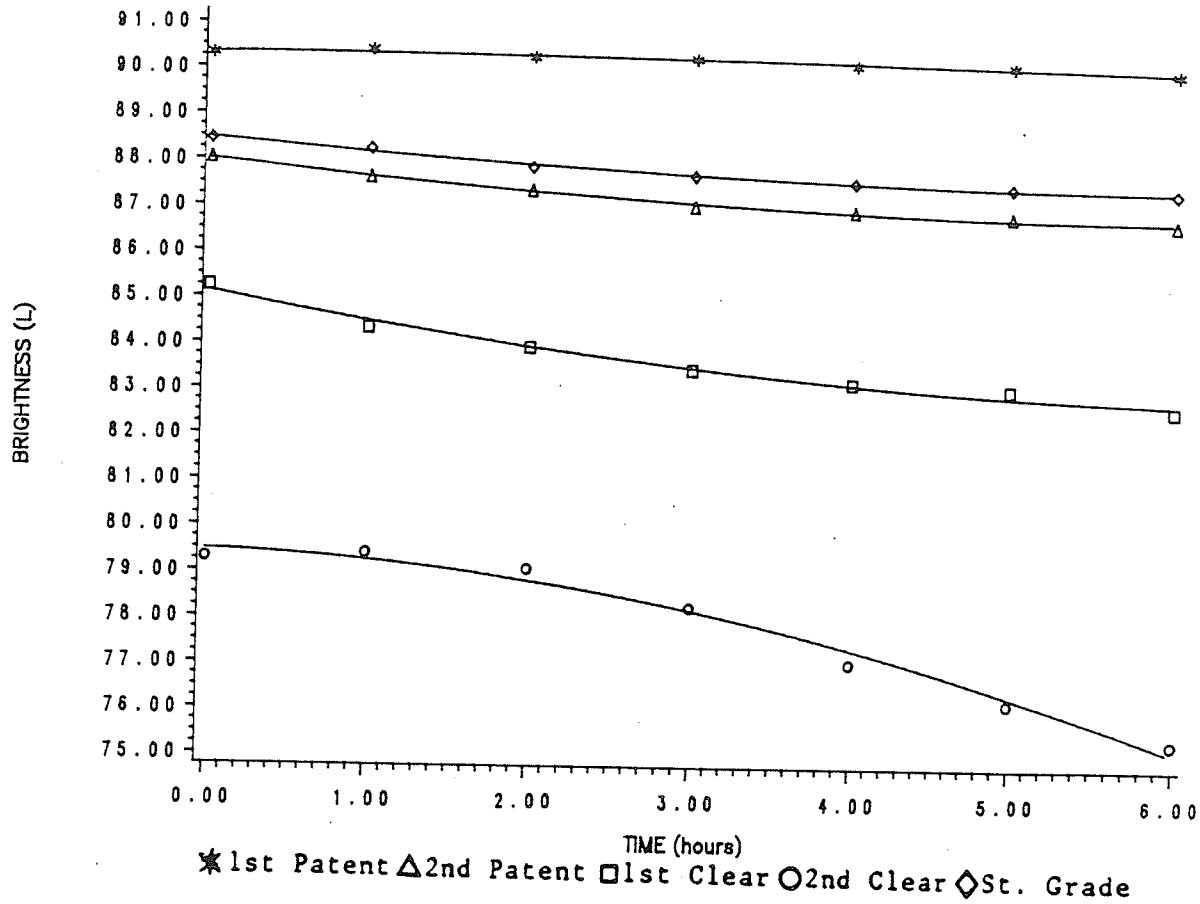


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St. Grade

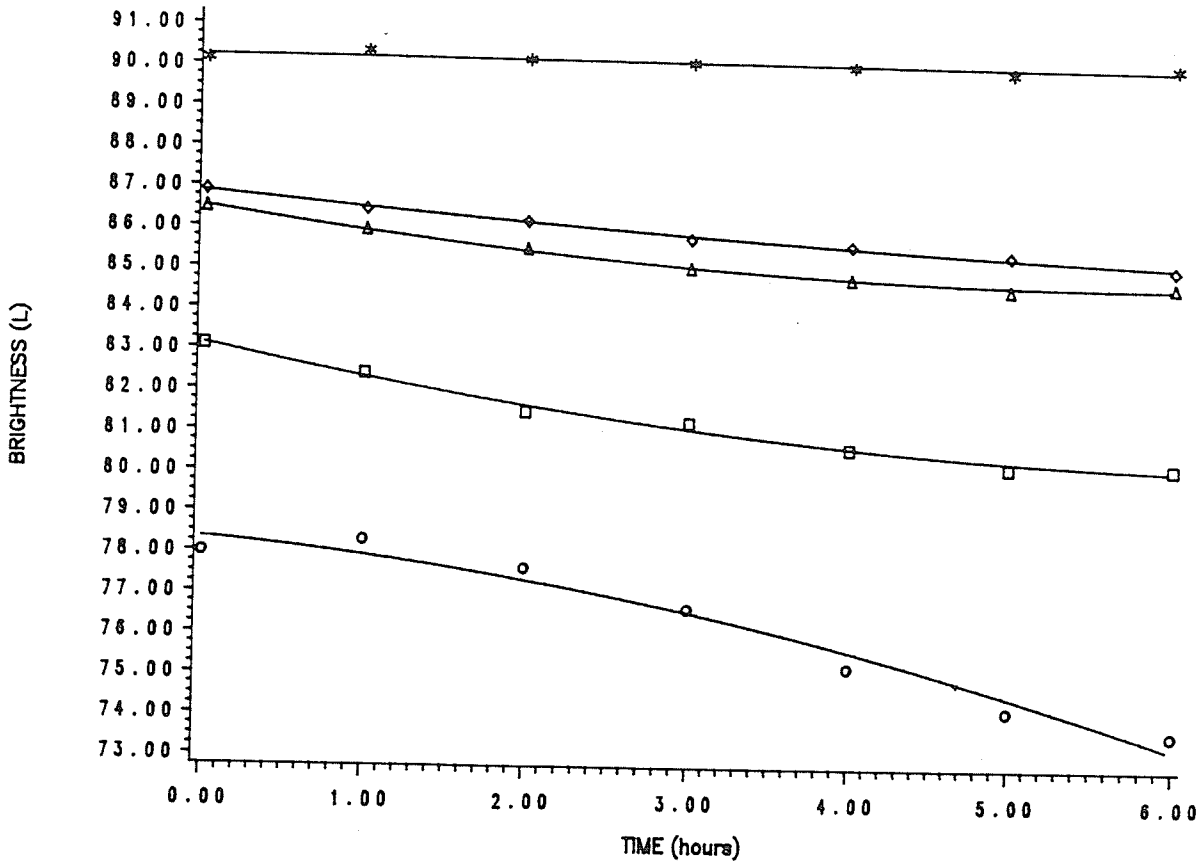
KAN SUI REDNESS AS A FUNCTION OF TIME
NORSTAR 80% EXTRACTION FLOURS



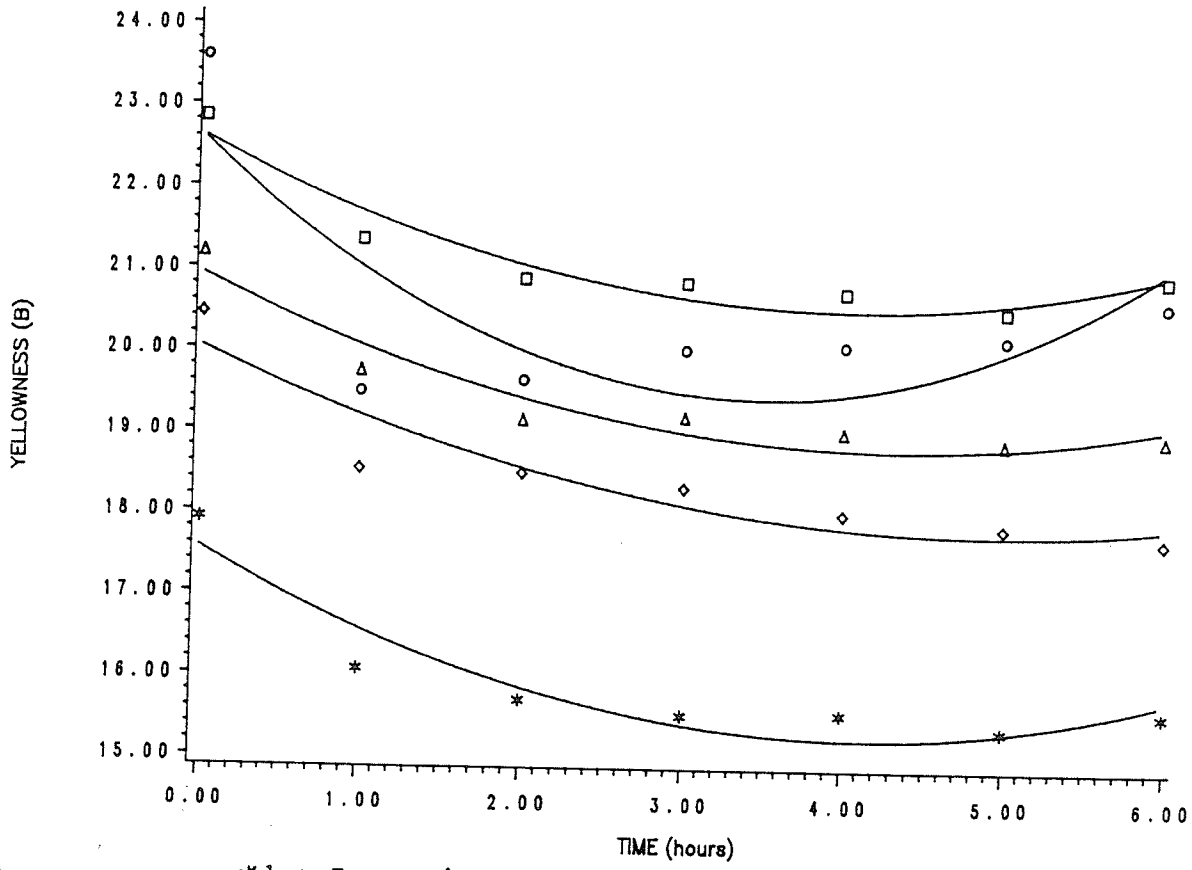
KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
GLENLEA 75% EXTRACTION FLOURS



KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
GLENLEA 80% EXTRACTION FLOURS

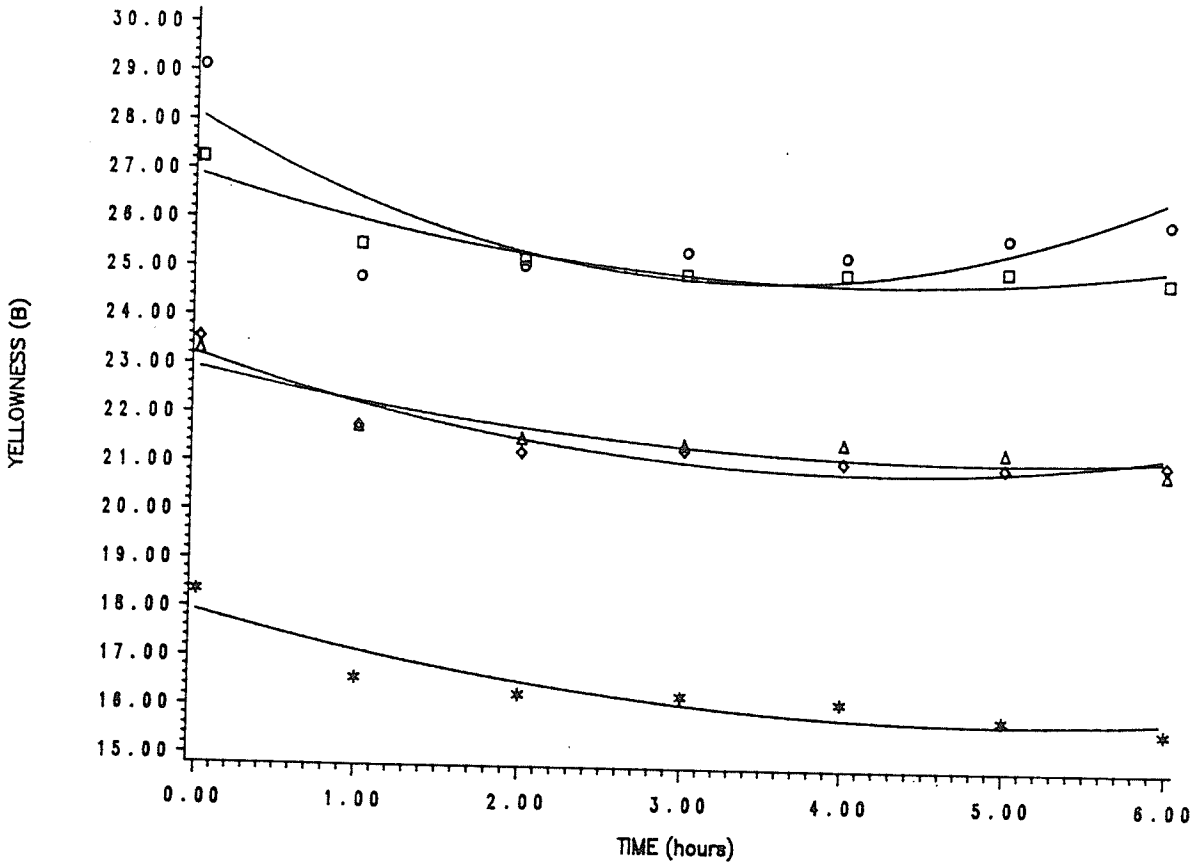


KAN SUI YELLOWNESS AS A FUNCTION OF TIME
GLENLEA 75% EXTRACTION FLOURS

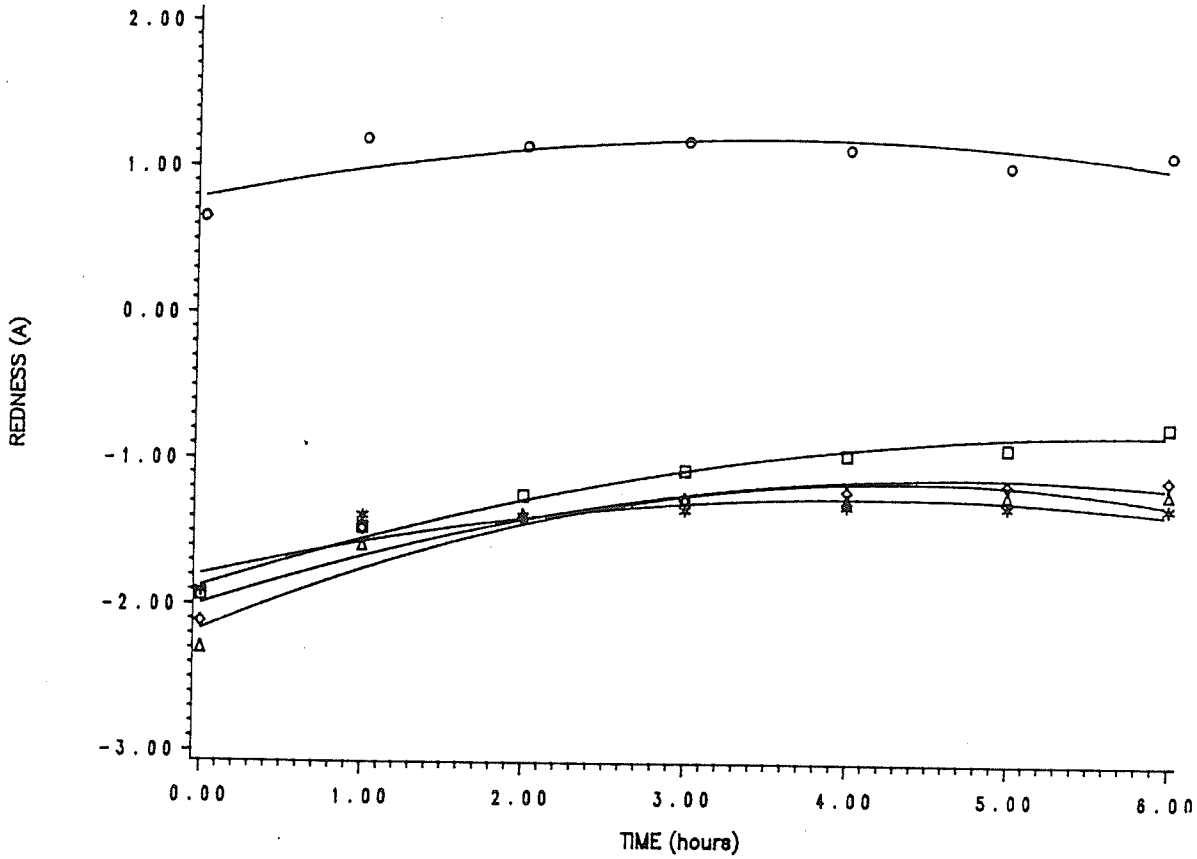


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
GLENLEA 80% EXTRACTION FLOURS

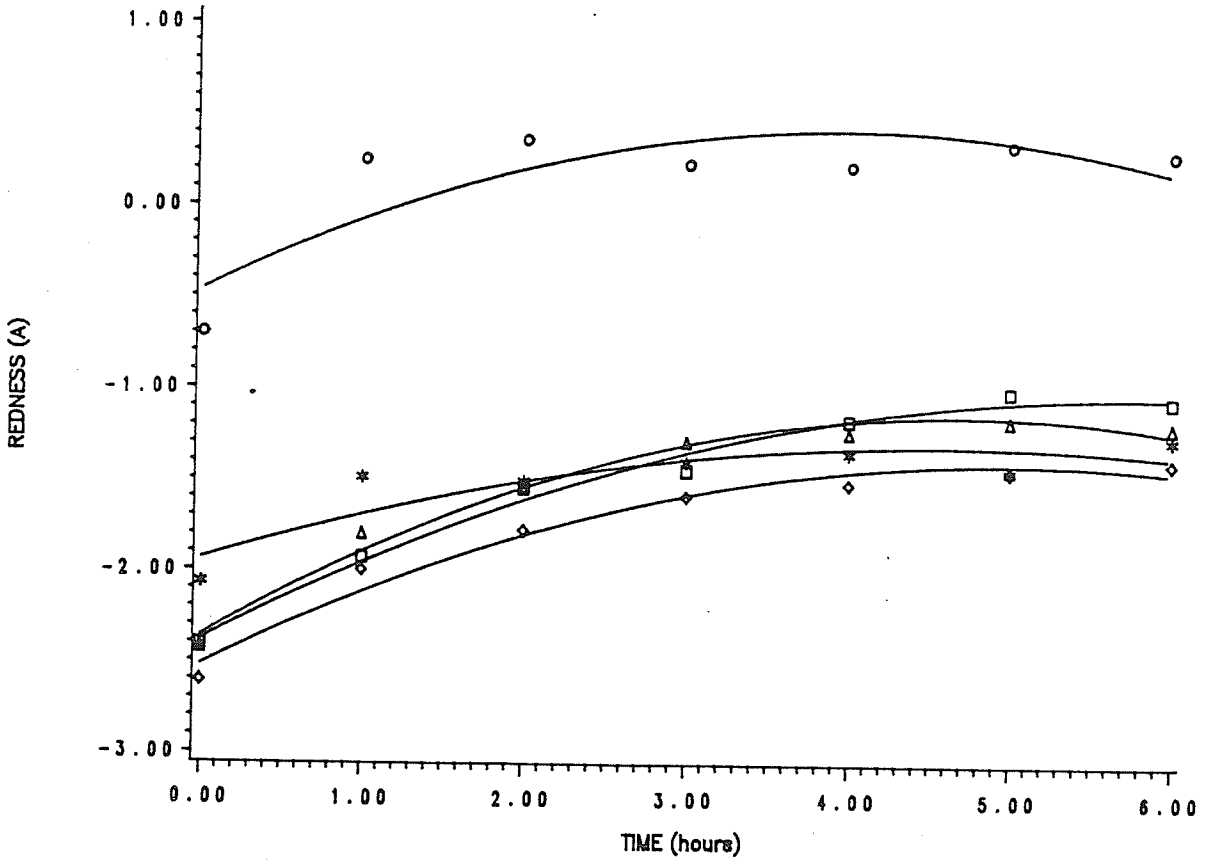


KAN SUI REDNESS AS A FUNCTION OF TIME
GLENLEA 75% EXTRACTION FLOURS

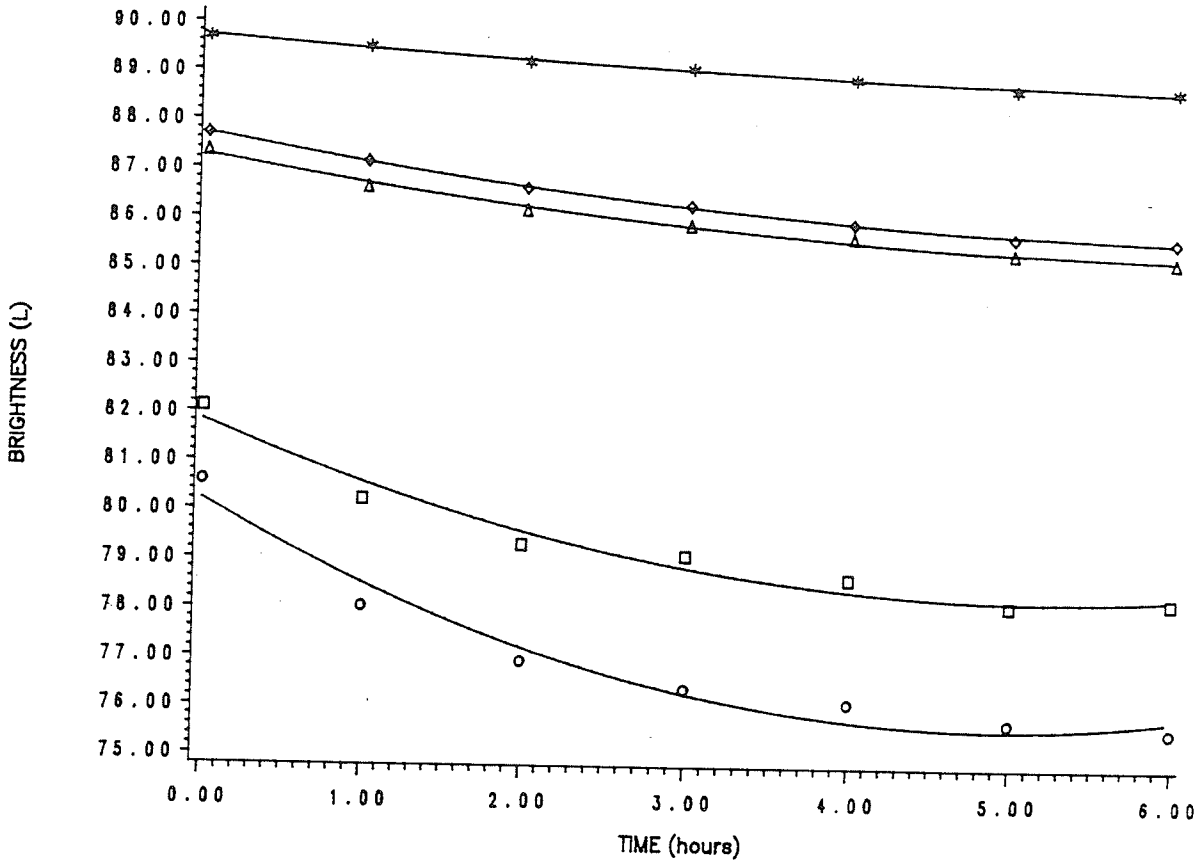


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

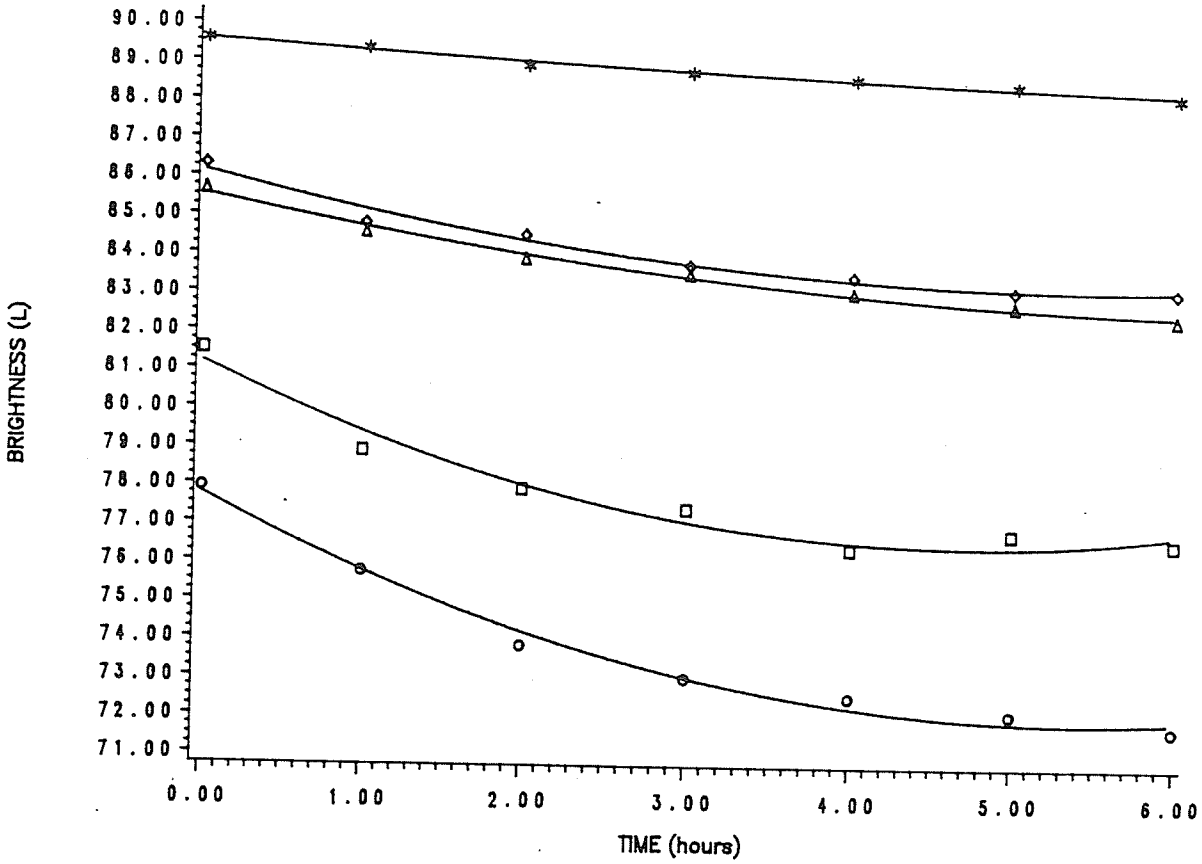
KAN SUI REDNESS AS A FUNCTION OF TIME
GLENLEA 80% EXTRACTION FLOURS



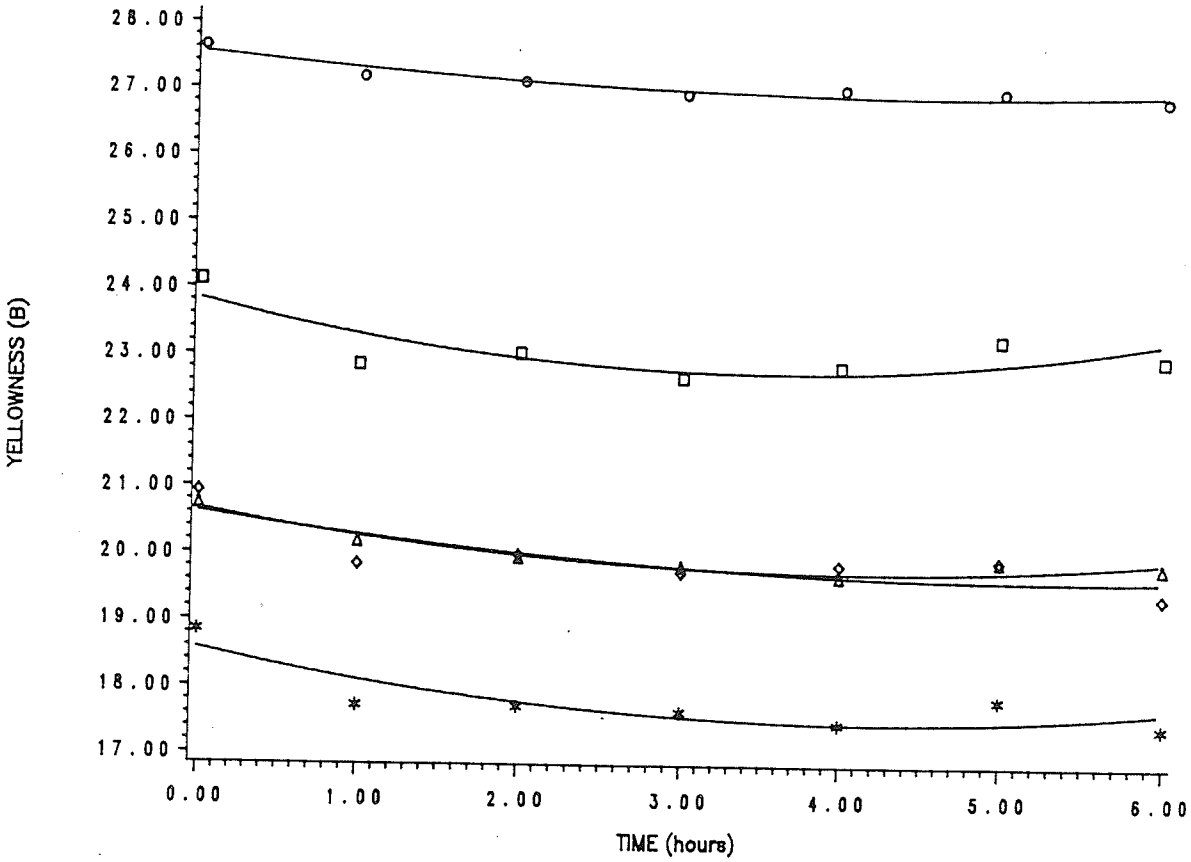
KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
HY 320 75% EXTRACTION FLOURS



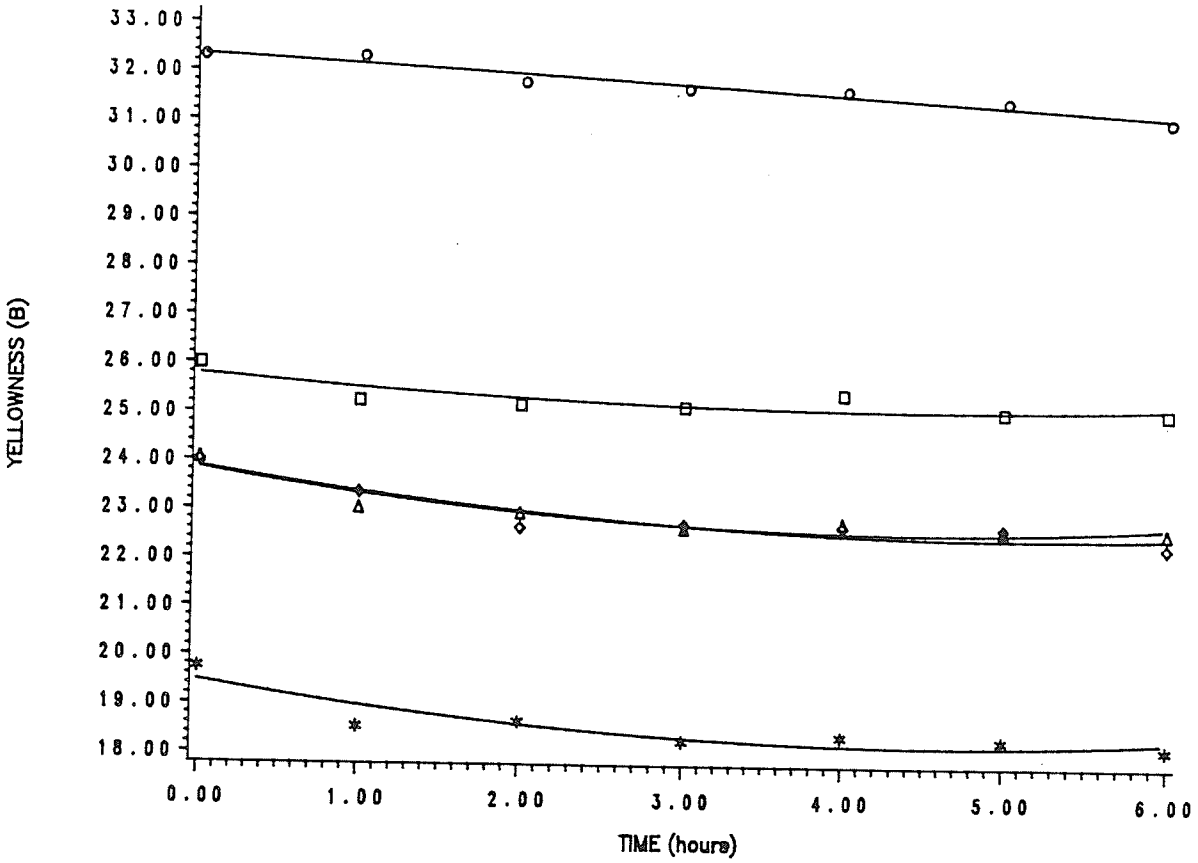
KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
HY 320 80% EXTRACTION FLOURS



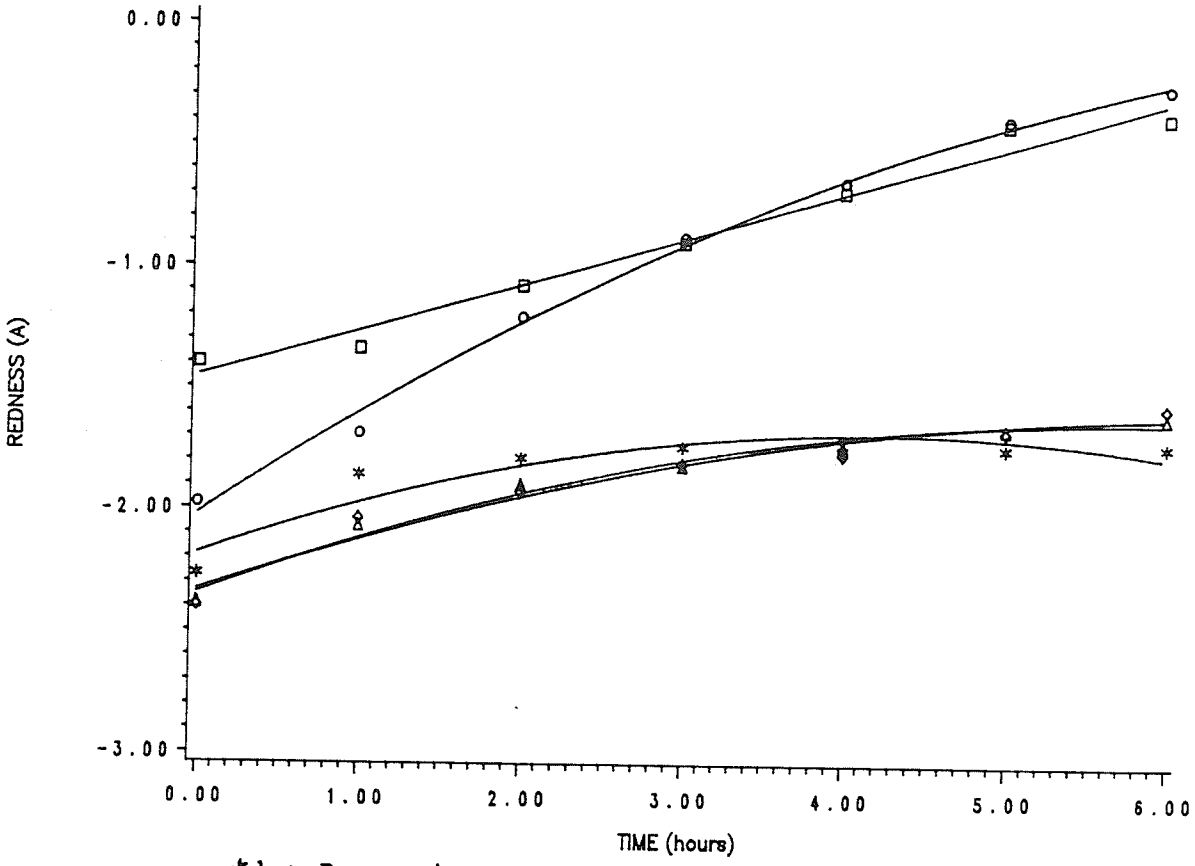
KAN SUI YELLOWNESS AS A FUNCTION OF TIME
HY 320 75% EXTRACTION FLOURS



KAN SUI YELLOWNESS AS A FUNCTION OF TIME
HY 320 80% EXTRACTION FLOURS

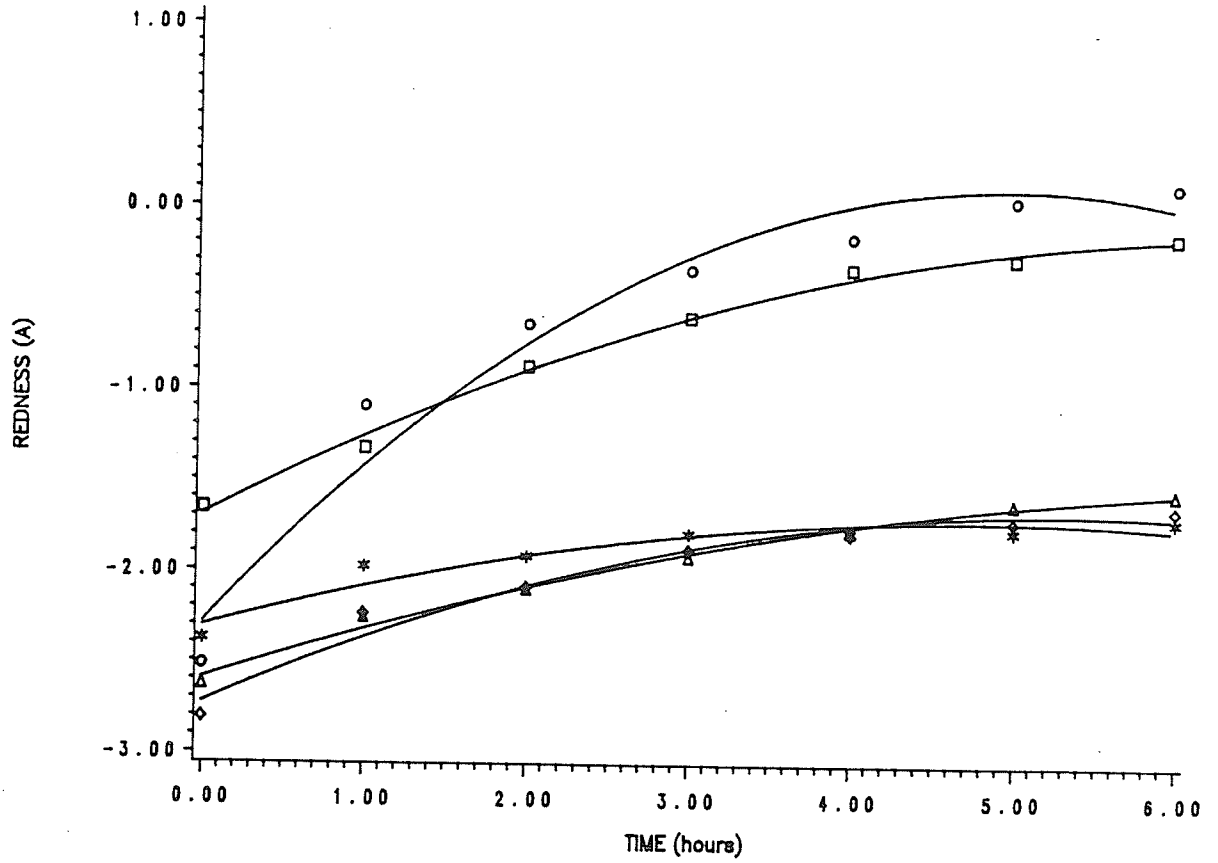


KAN SUI REDNESS AS A FUNCTION OF TIME
HY 320 75% EXTRACTION FLOURS

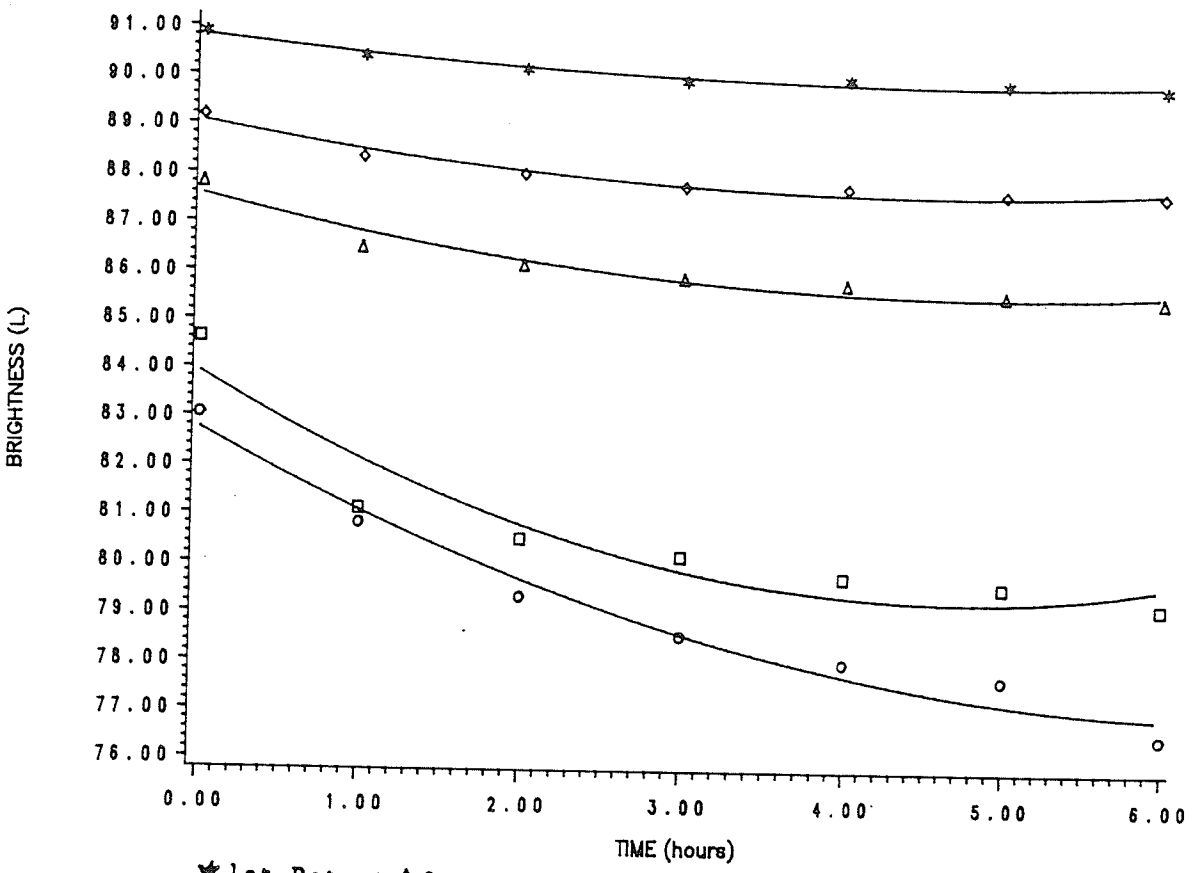


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

KAN SUI REDNESS AS A FUNCTION OF TIME
HY 320 80% EXTRACTION FLOURS

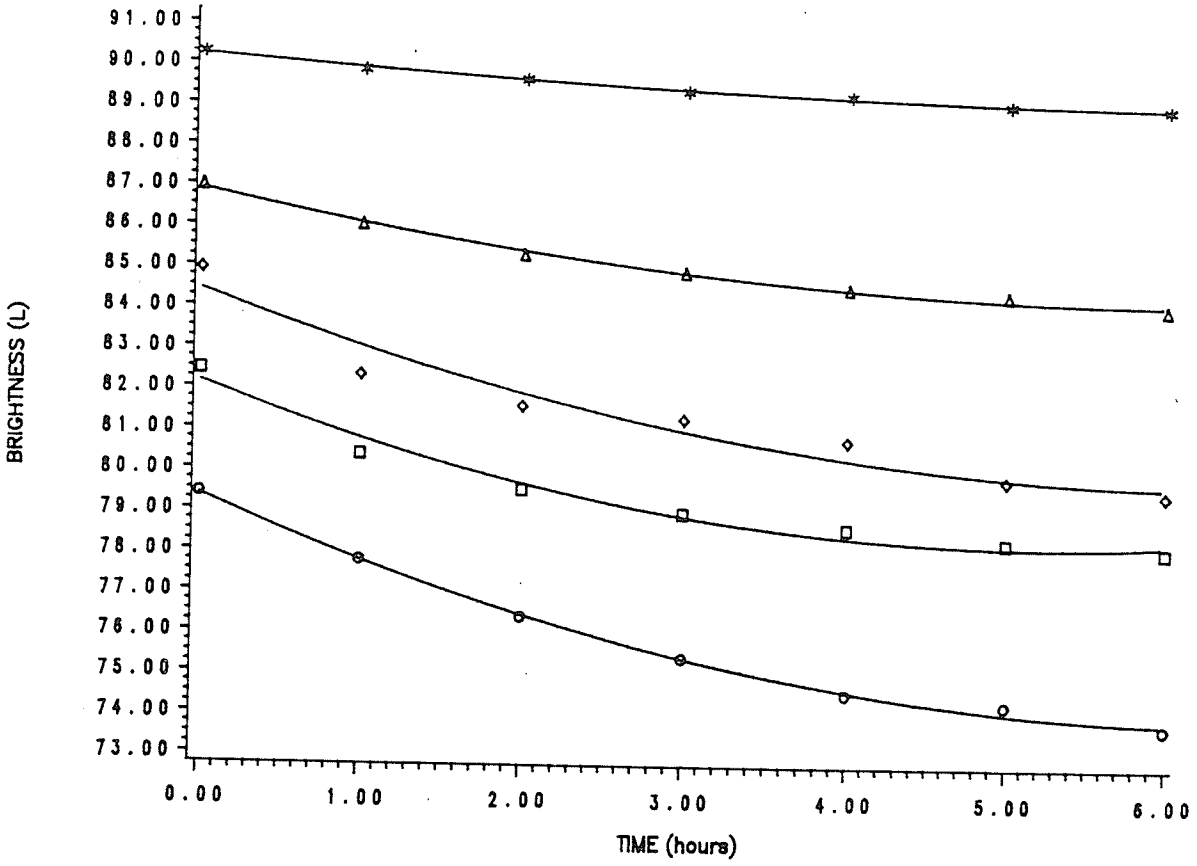


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
FIELDER 75% EXTRACTION FLOURS

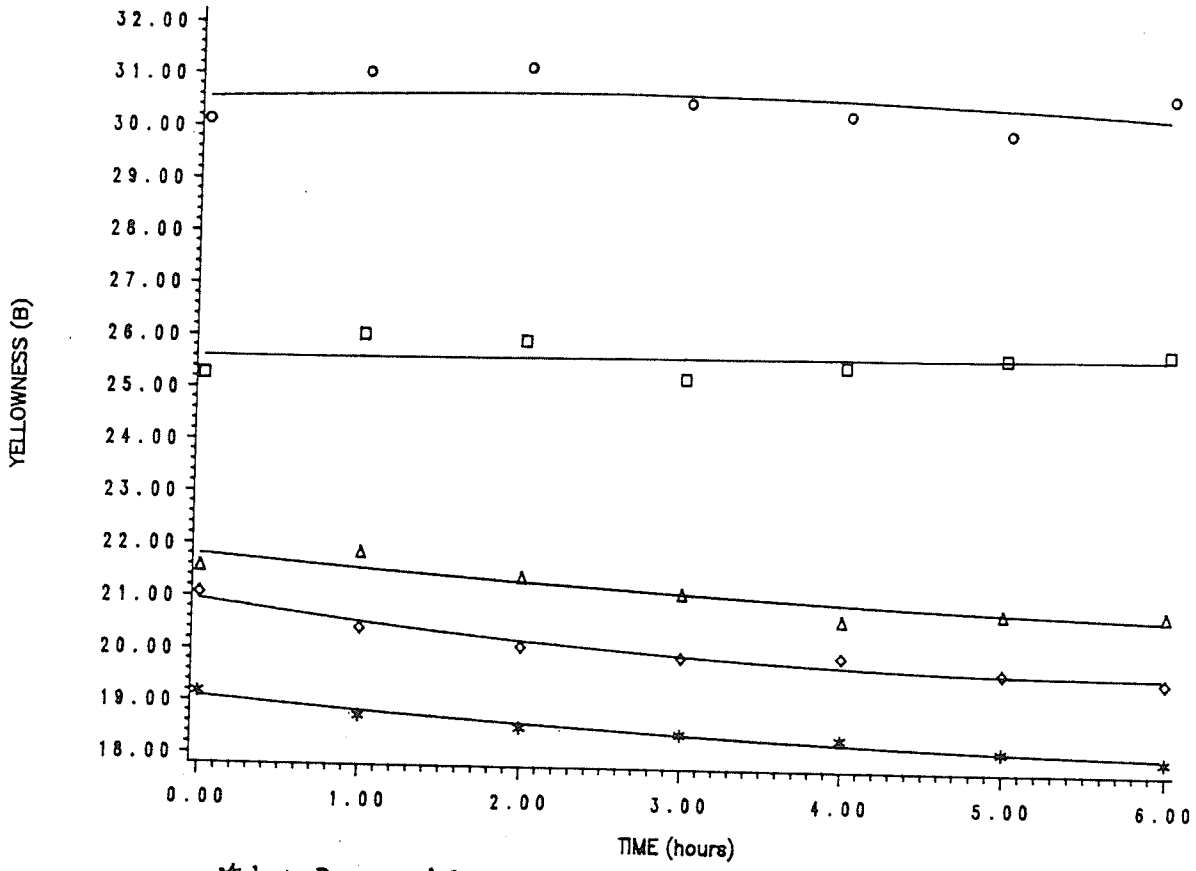


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St. Grade

KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
FIELDER 80% EXTRACTION FLOURS

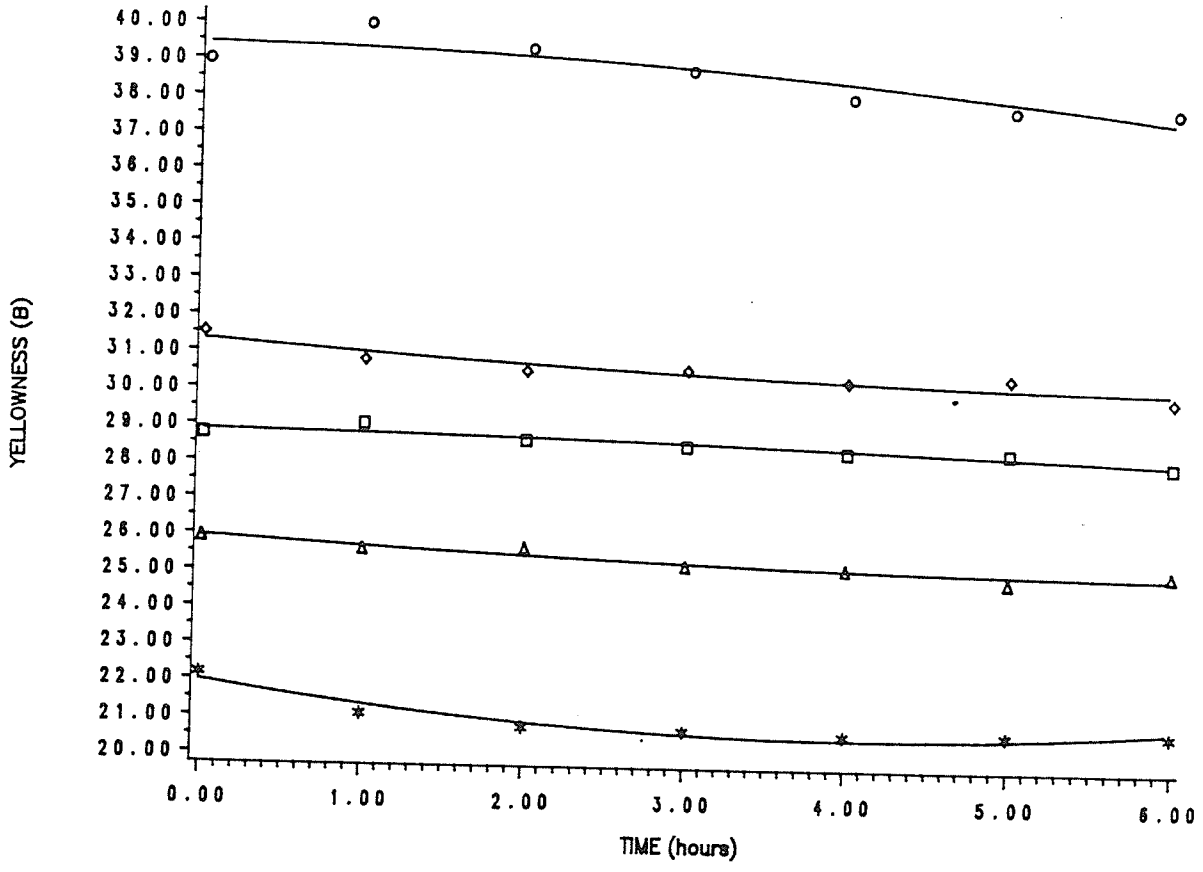


KAN SUI YELLOWNESS AS A FUNCTION OF TIME
FIELDER 75% EXTRACTION FLOURS

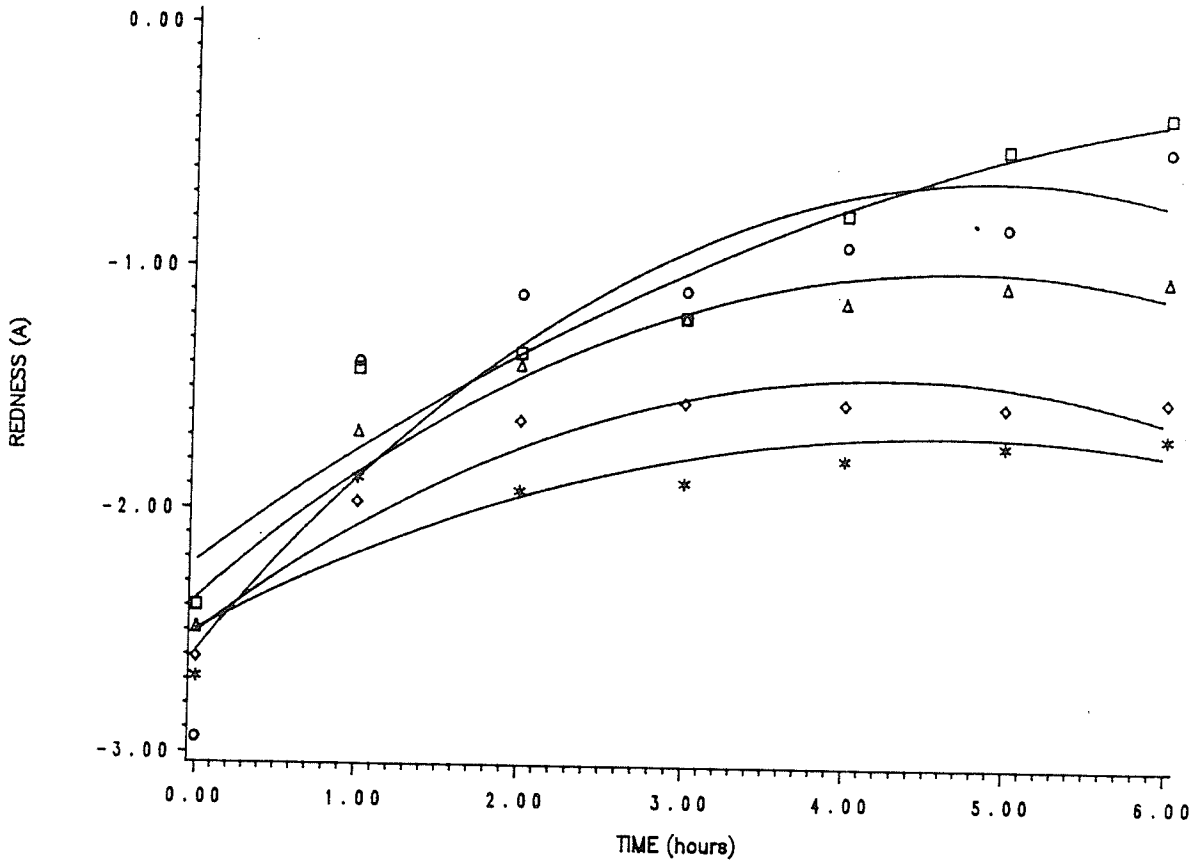


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St. Grade

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
FIELDER 80% EXTRACTION FLOURS

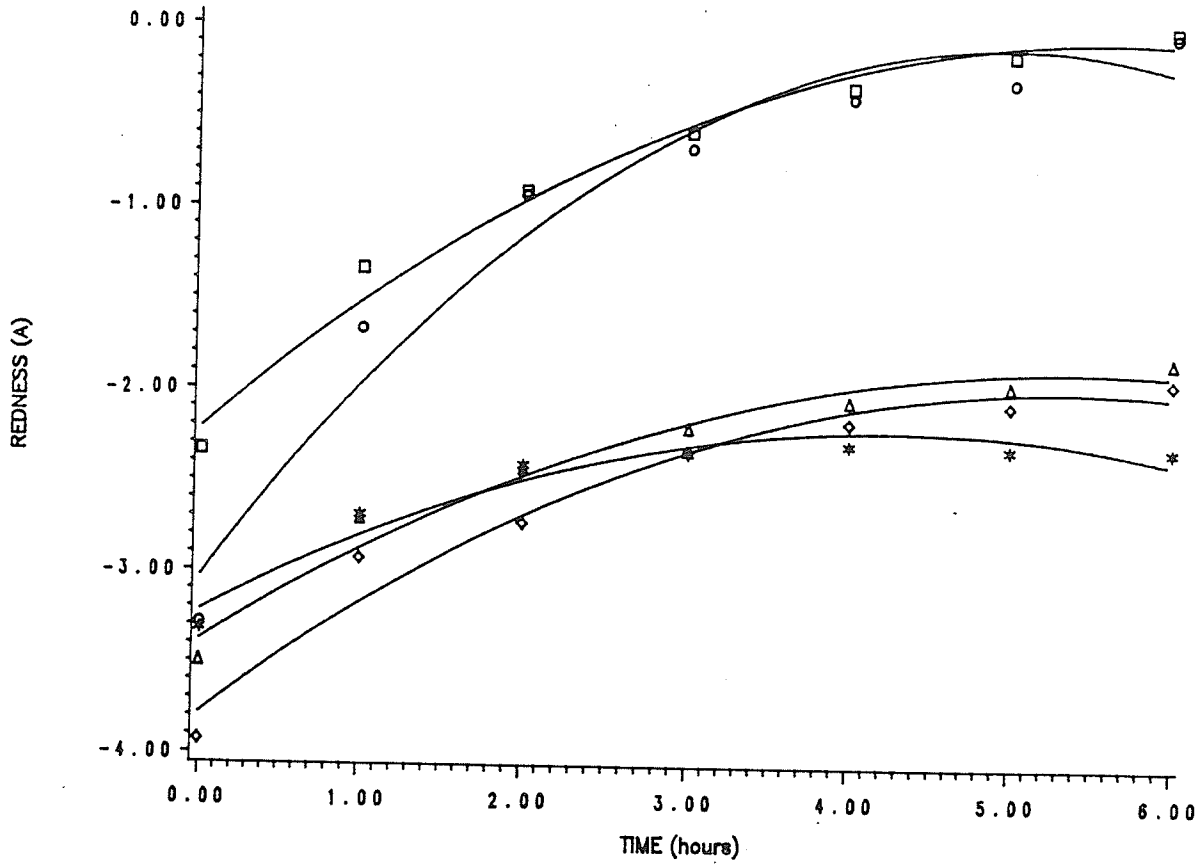


KAN SUI REDNESS AS A FUNCTION OF TIME
FIELDER 75% EXTRACTION FLOURS

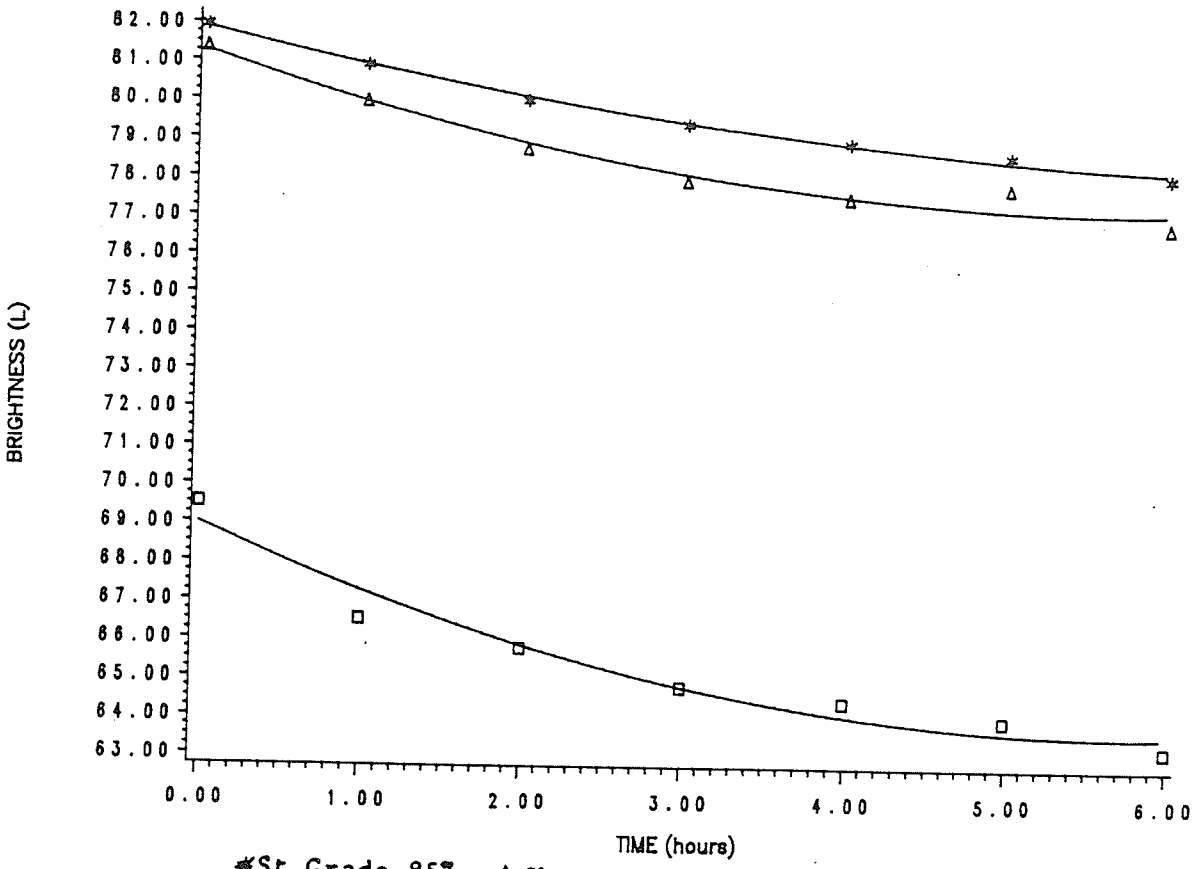


✱1st Patent △2nd Patent □1st Clear ○2nd Clear ◇St.Grade

KAN SUI REDNESS AS A FUNCTION OF TIME
FIELDER 80% EXTRACTION FLOURS

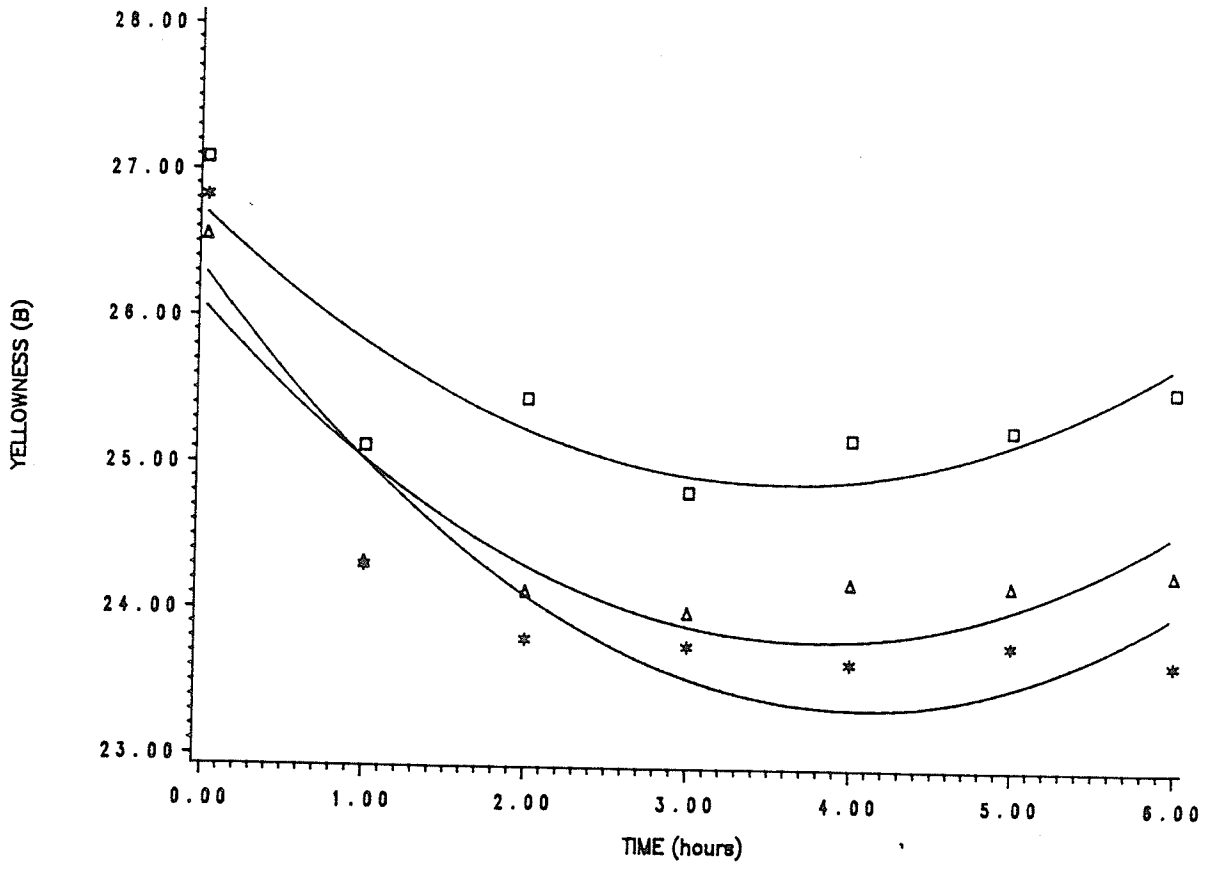


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS

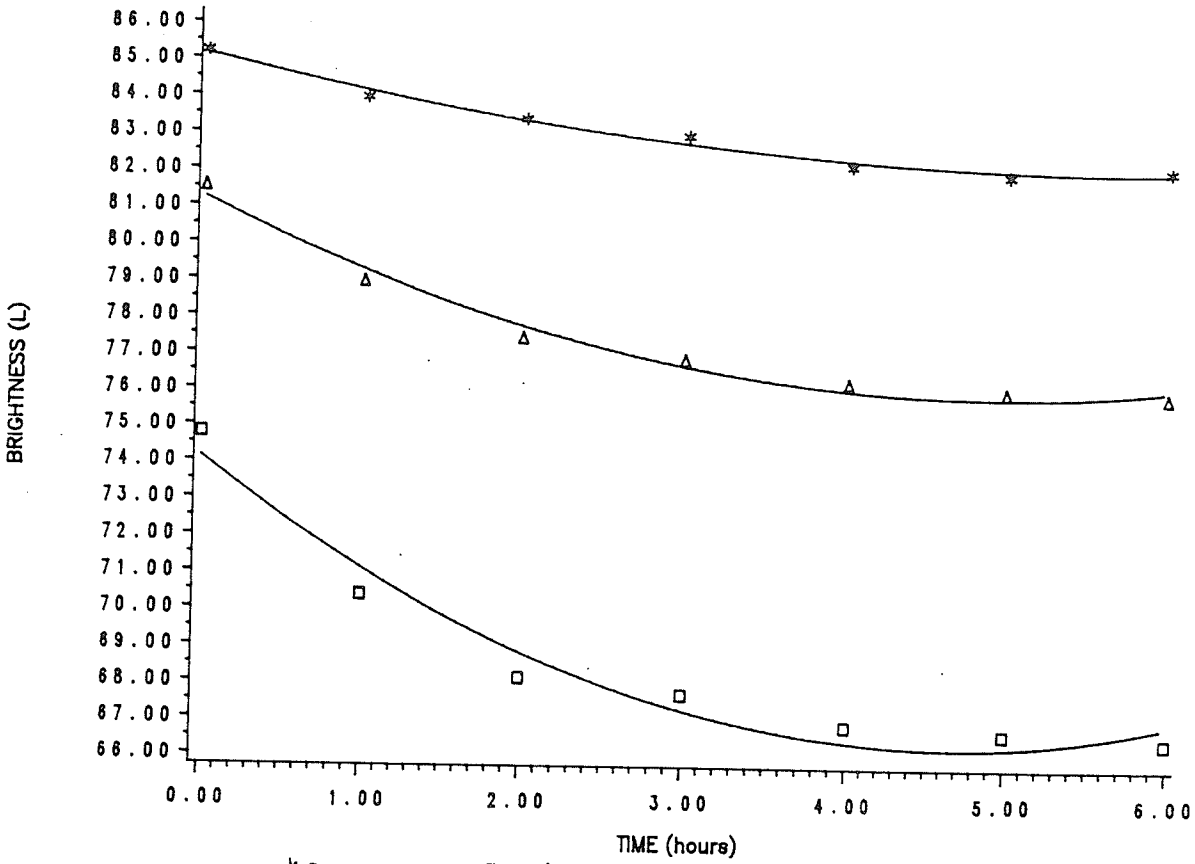


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS

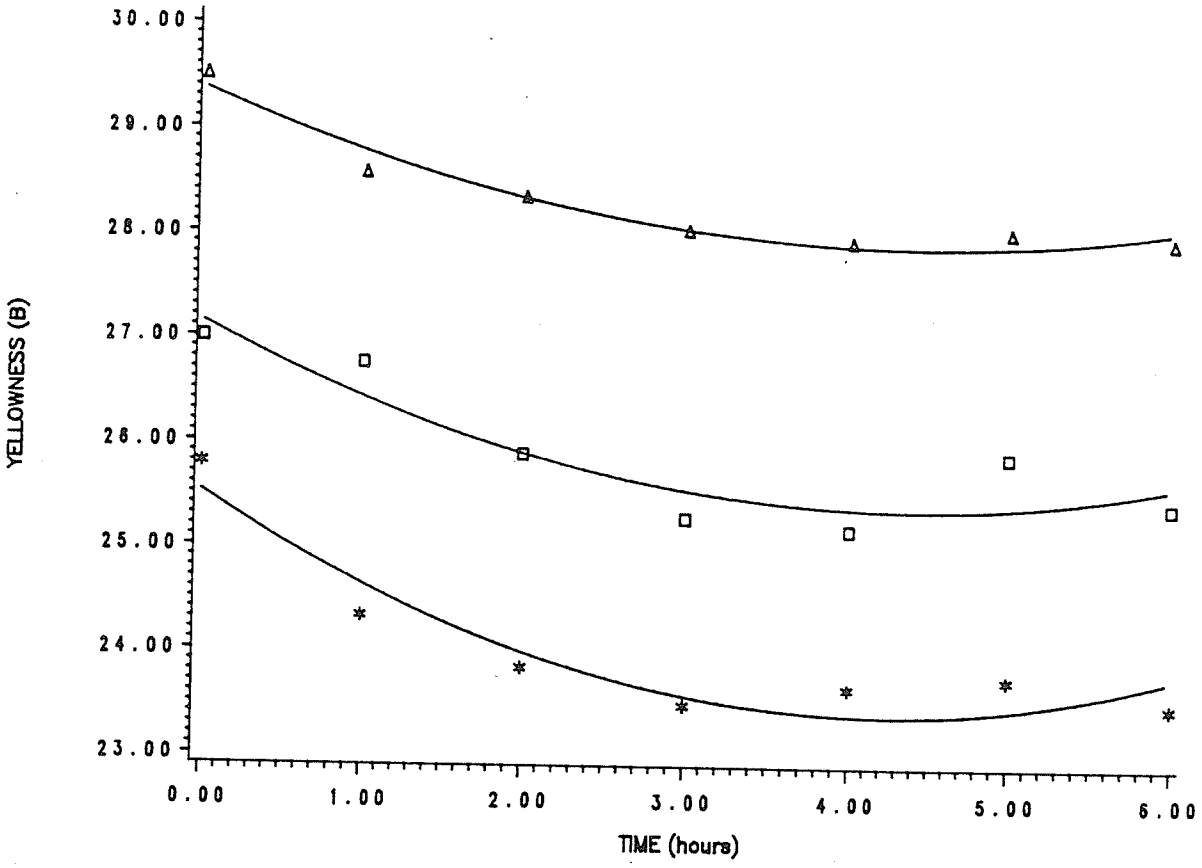


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
NORSTAR HIGH EXTRACTION FLOURS

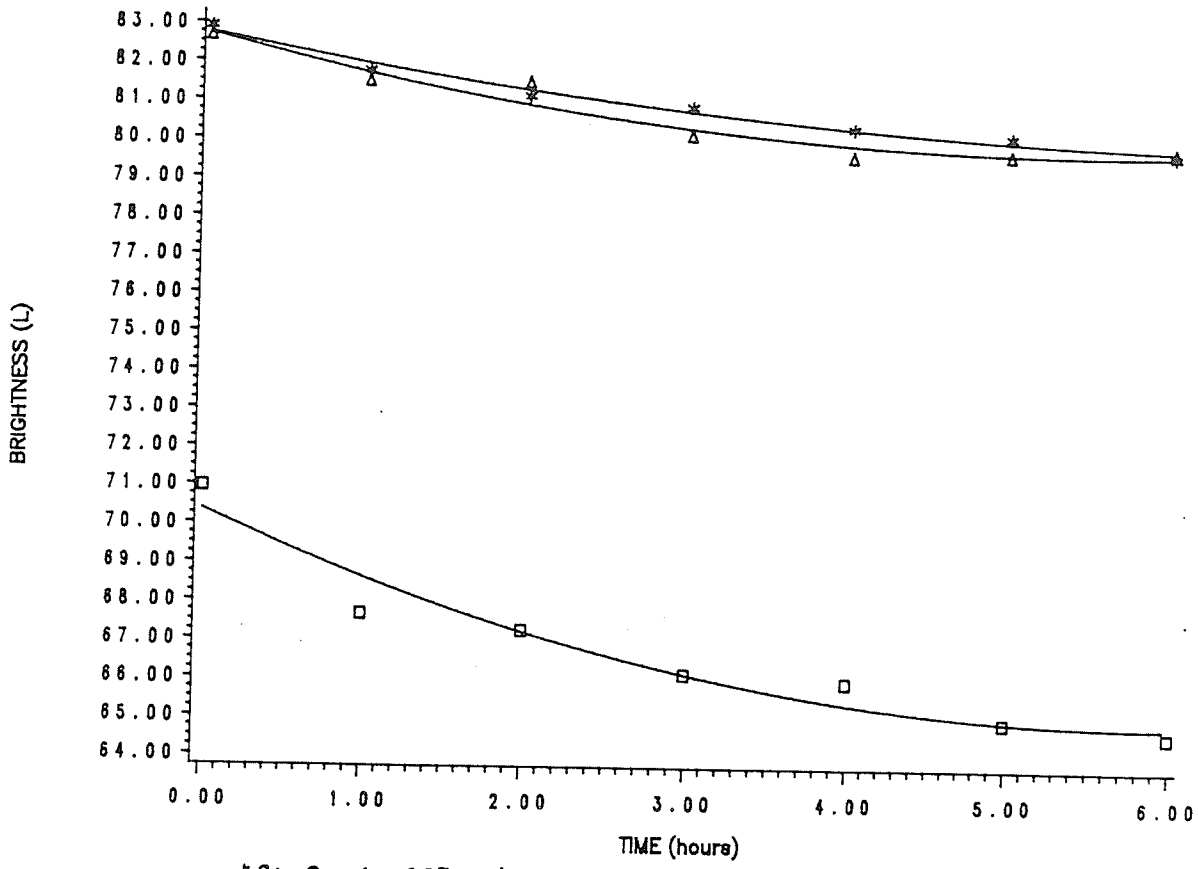


*St.Grade 85% △Chinese Standard 85% □Whole Wheat

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
NORSTAR HIGH EXTRACTION FLOURS

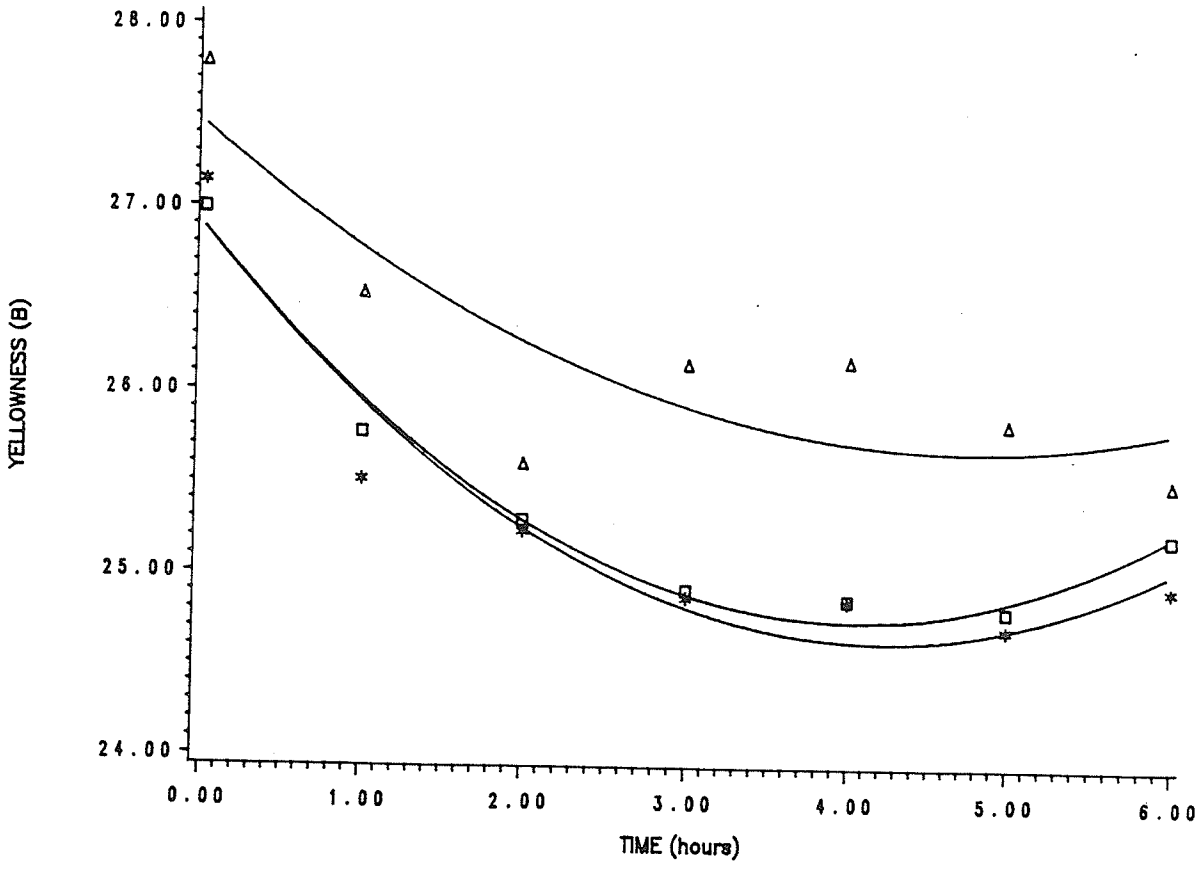


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
GLENLEA HIGH EXTRACTION FLOURS

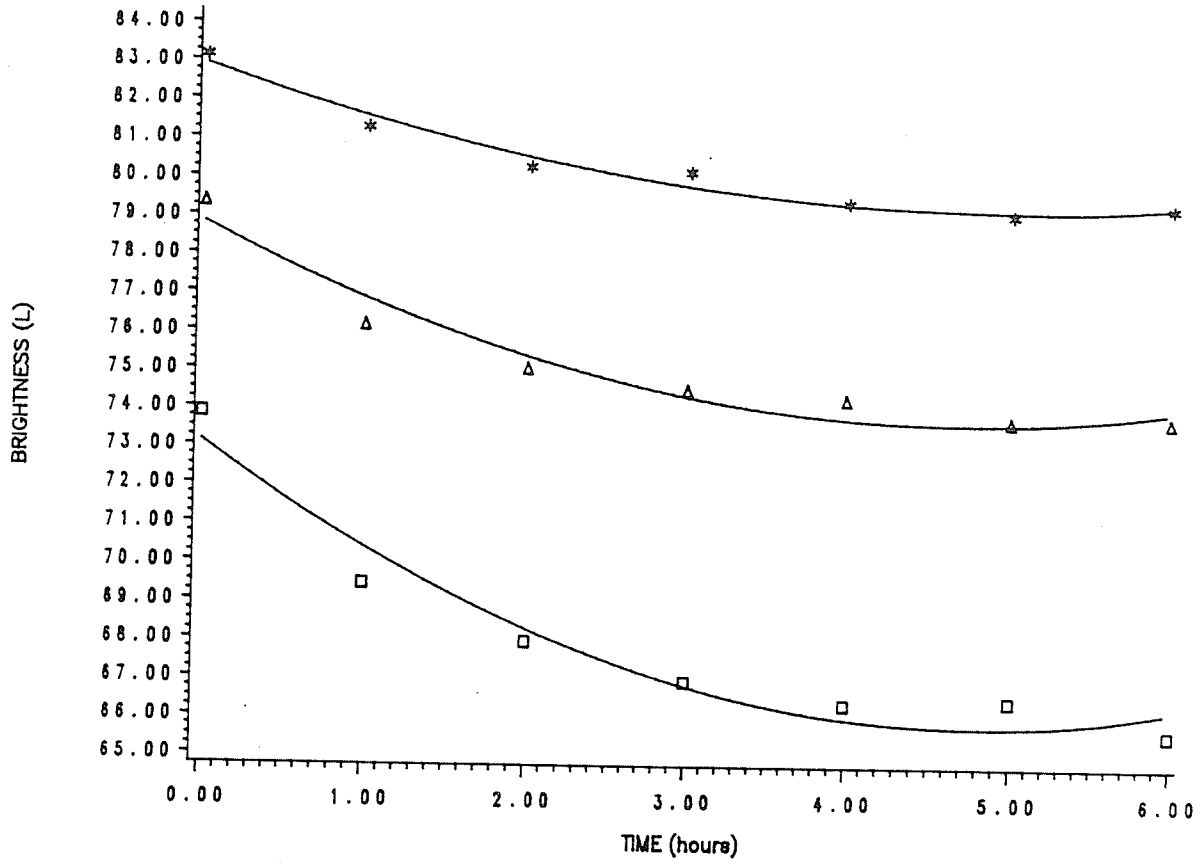


*St.Grade 85% △Chinese Standard 85% □Whole Wheat

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
GLENLEA HIGH EXTRACTION FLOURS

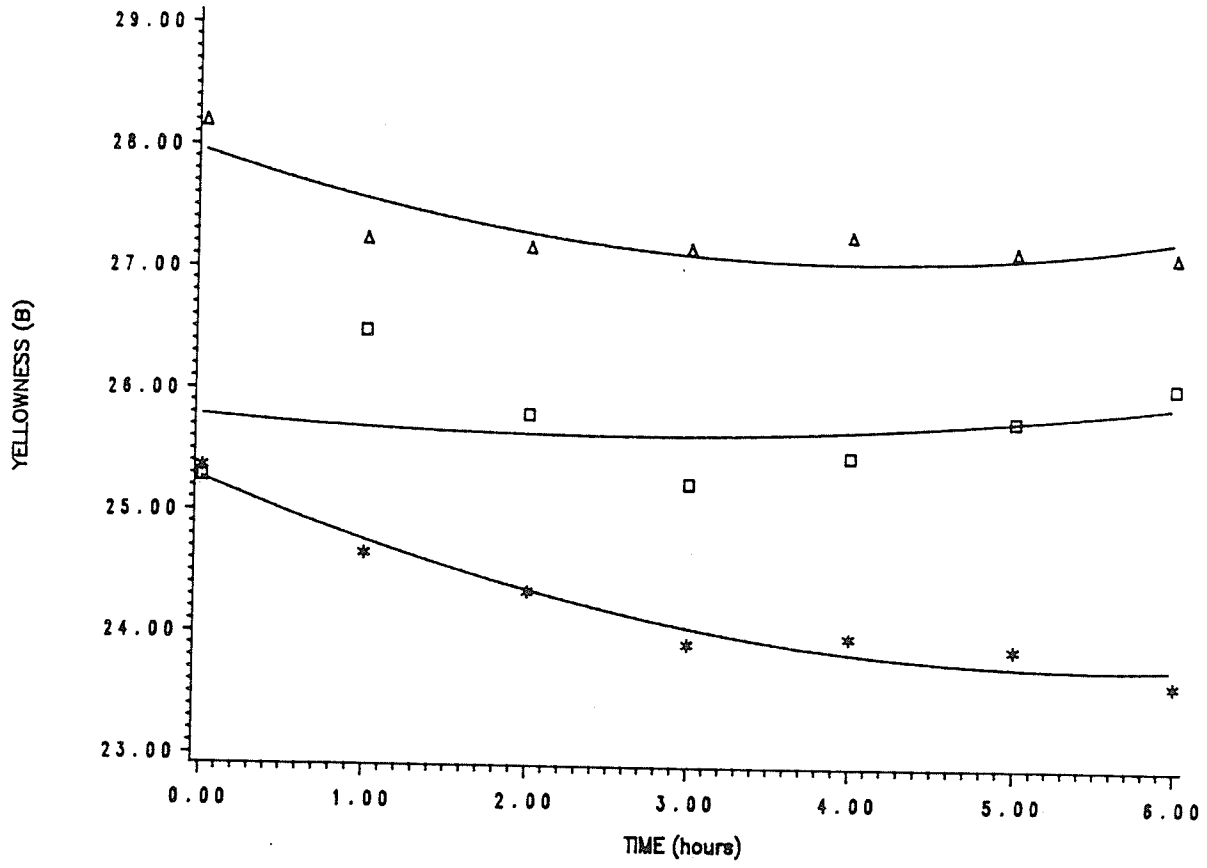


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
HY 320 HIGH EXTRACTION FLOURS

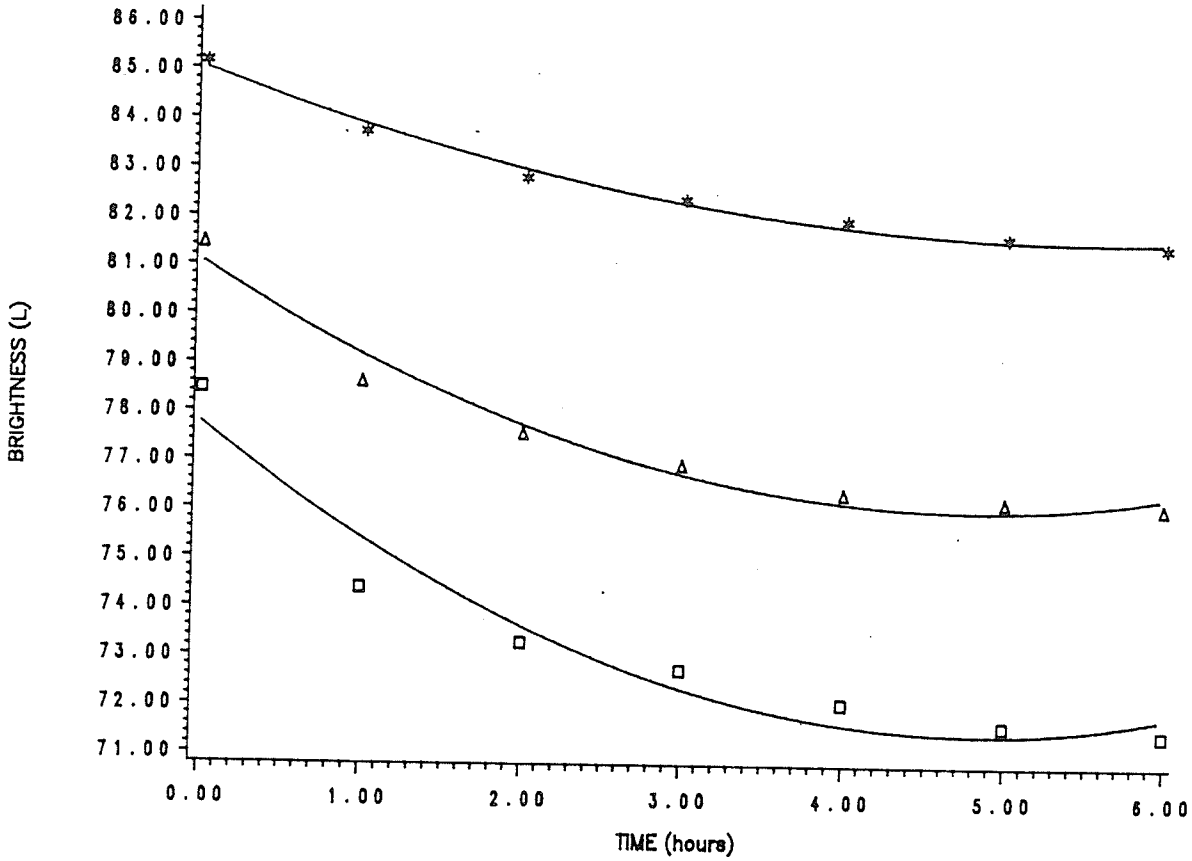


✱ St.Grade 85% △ Chinese Standard 85% □ Whole Wheat

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
HY 320 HIGH EXTRACTION FLOURS

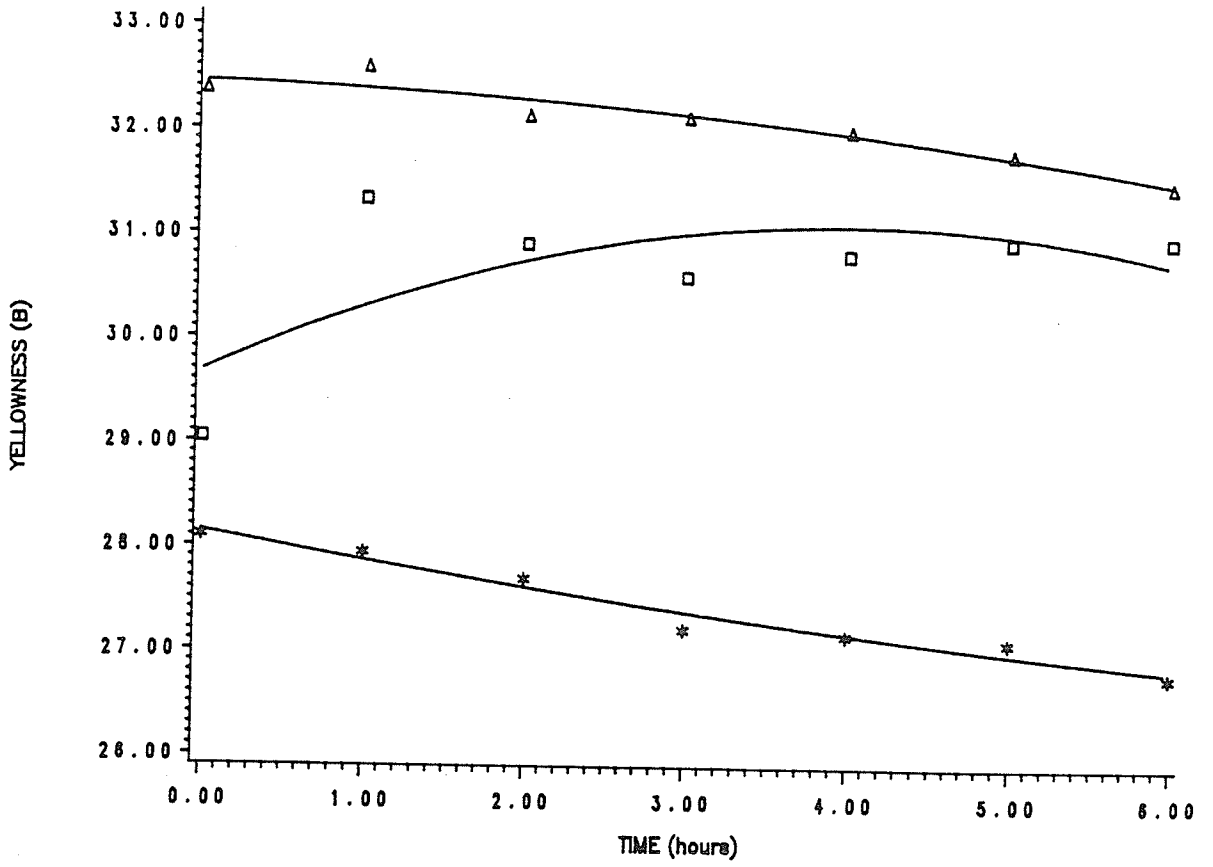


KAN SUI BRIGHTNESS AS A FUNCTION OF TIME
FIELDER HIGH EXTRACTION FLOURS

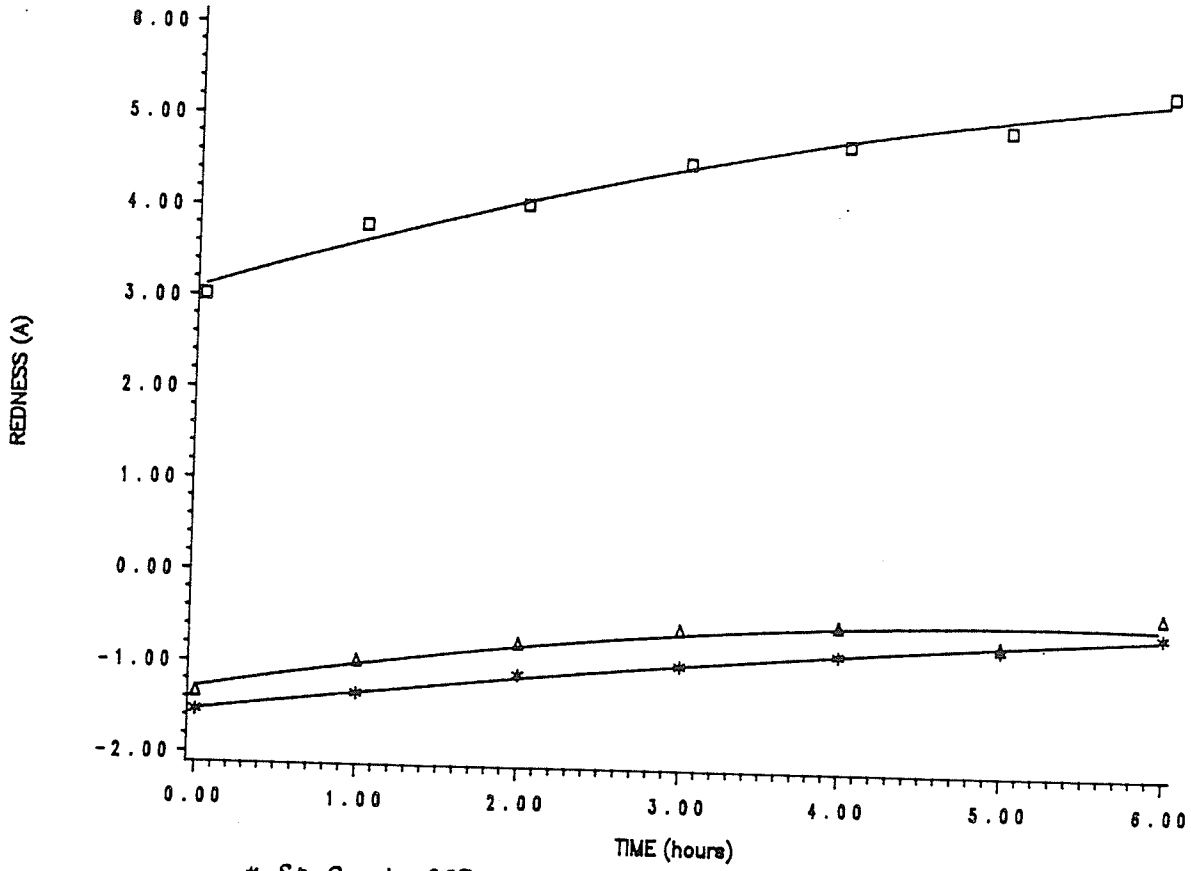


✱St.Grade 85% △Chinese Standard 85% □Whole Wheat

KAN SUI YELLOWNESS AS A FUNCTION OF TIME
FIELDER HIGH EXTRACTION FLOURS

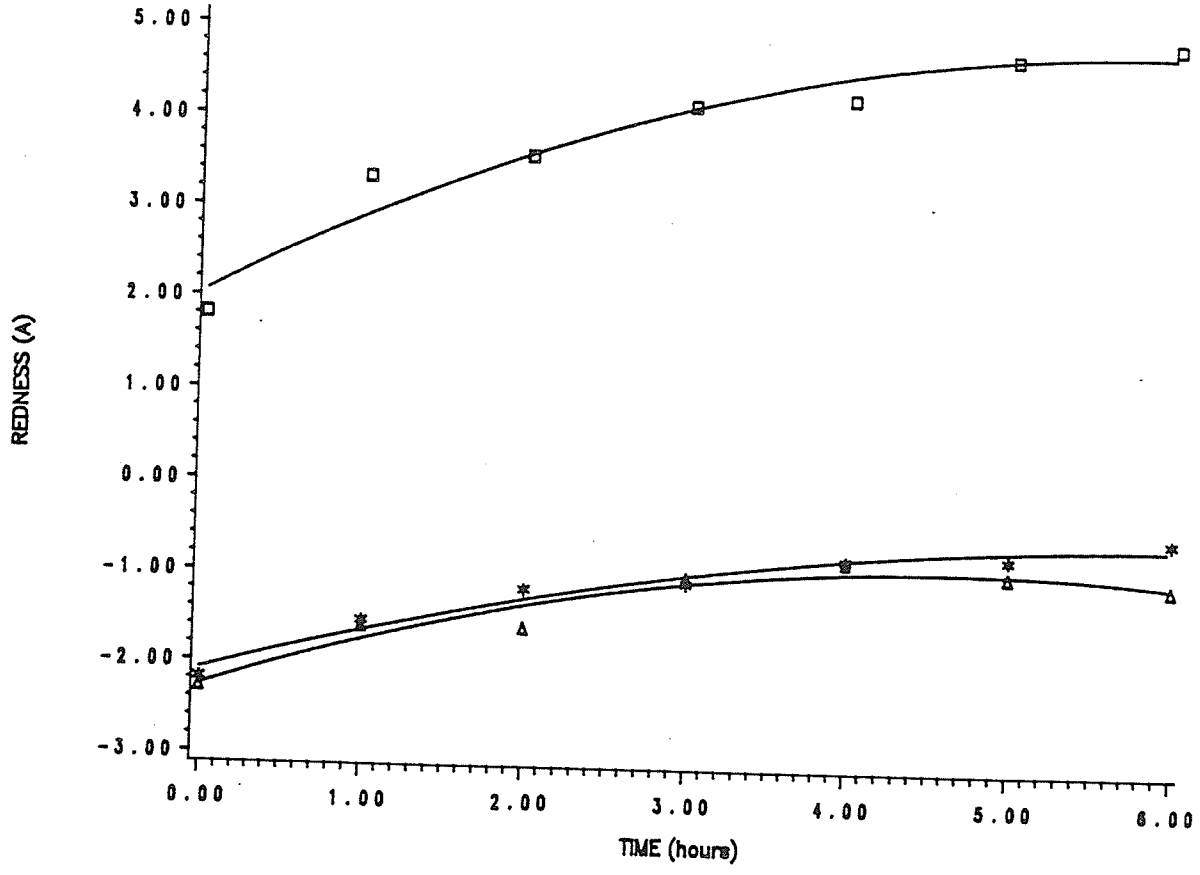


KAN SUI REDNESS AS A FUNCTION OF TIME
KATEPWA HIGH EXTRACTION FLOURS

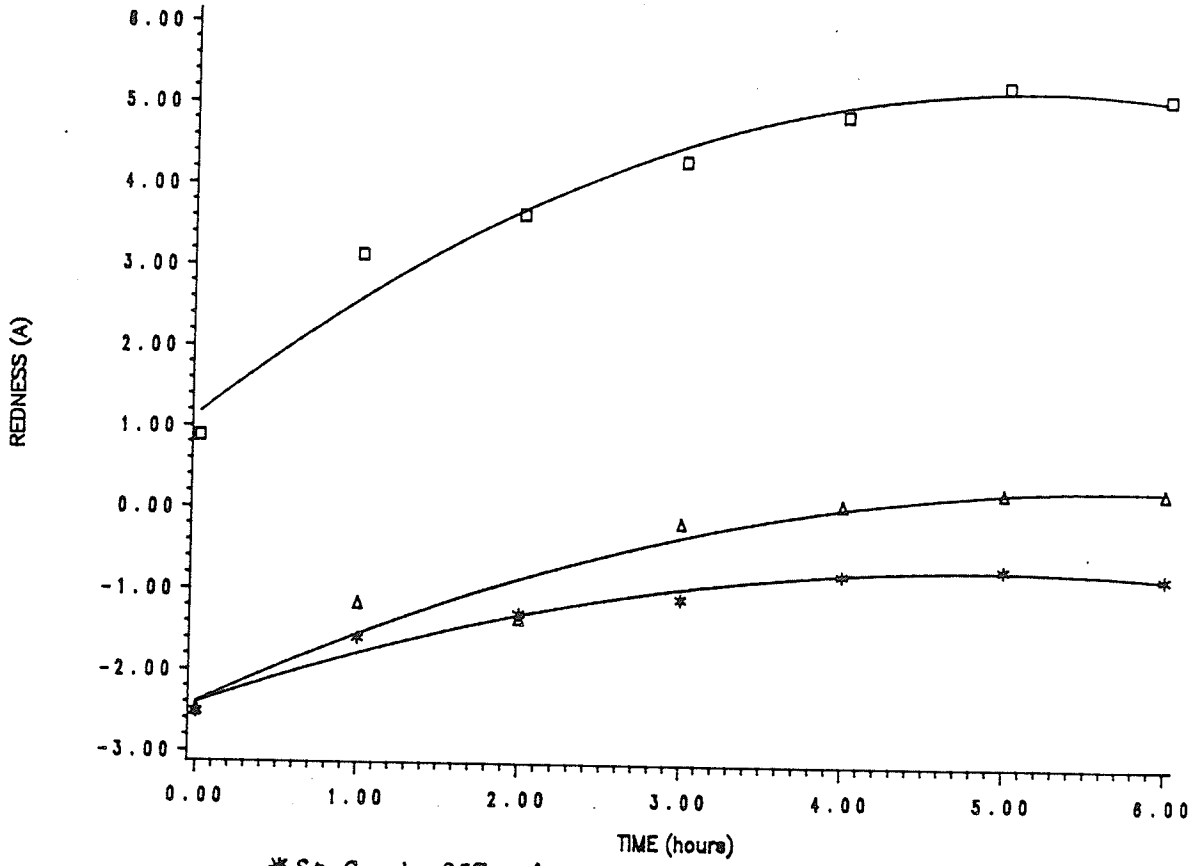


✱ St.Grade 85% △Chinese Standard 85% □Whole Wheat

KAN SUI REDNESS AS A FUNCTION OF TIME
GLENLEA HIGH EXTRACTION FLOURS

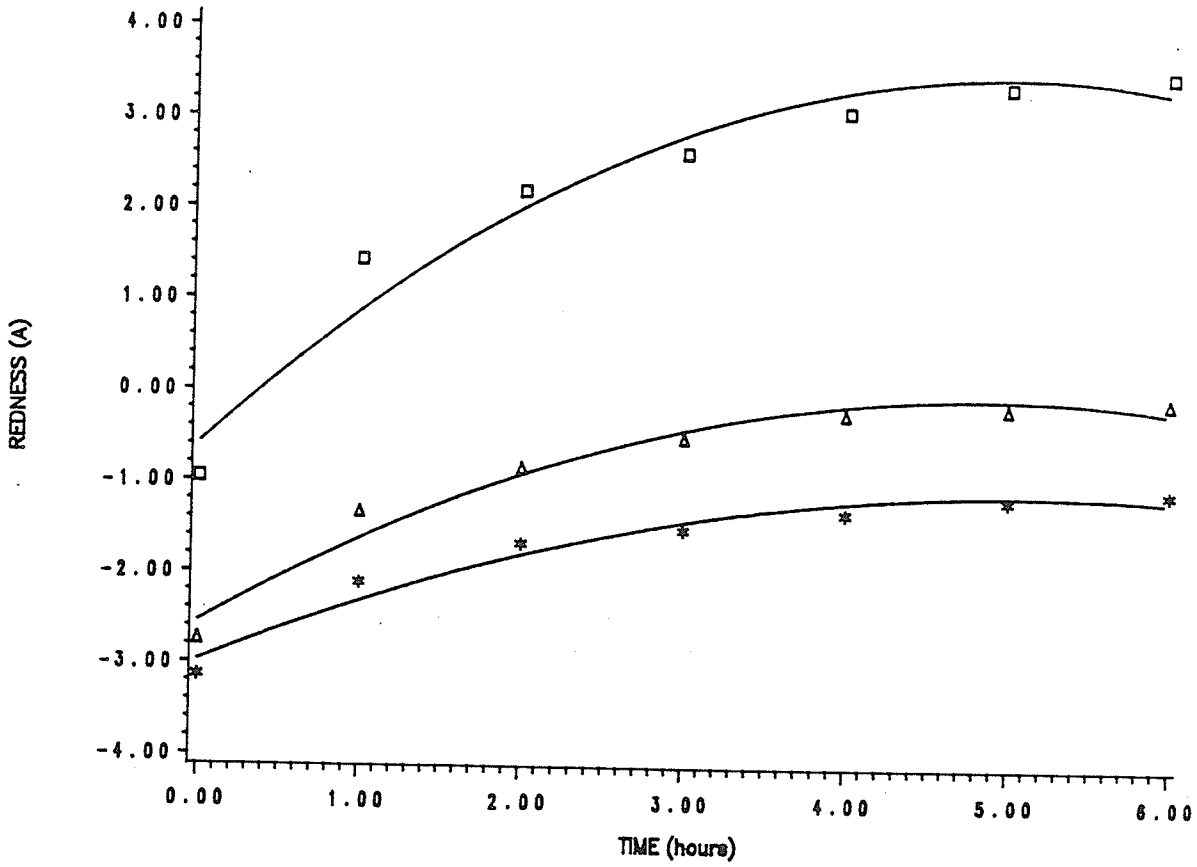


KAN SUI REDNESS AS A FUNCTION OF TIME
NORSTAR HIGH EXTRACTION FLOURS



✱ St.Grade 85% △ Chinese Standard 85% □ Whole Wheat

KAN SUI REDNESS AS A FUNCTION OF TIME
FIELDER HIGH EXTRACTION FLOURS



KAN SUI REDNESS AS A FUNCTION OF TIME
HY 320 HIGH EXTRACTION FLOURS