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OPTIMIZATION OF BROMATE-FREE IMPROVER SYSTEMS
FOR USE WITH
CWRS AND CWES WHEAT FLOURS

by

Constance Elaine Perron

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the University of Manitoba
in partial fulfilment of the
requirement for the degree of
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in
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CONSTANCE ELAINE PERRON

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ABSTRACT

Two flours of different breadmaking potential, CWRS and CWES wheat flour, were evaluated to determine their requirement for improving agents. Bromate-free improver systems which optimized the quality of breads made with CWRS and CWES wheat flours, both alone and in blends, were identified and the contribution of CWES wheat flour to the quality of bromate-free breads determined. For this research, fractional factorial designs were used for screening and a central composite response surface design for optimization. The research was carried out in three stages: screening, optimization and verification.

Seven improvers, ascorbic acid, azodicarbonamide, fungal α -amylase and protease, diacetyl tartaric acid esters of monoglycerides (DATEM) and sodium stearoyl-2-lactylate (SSL), and L-cysteine hydrochloride, were screened to determine their relative effects on bread quality. CWRS and CWES wheat flours differed in their requirements for the individual improvers. Average volumes of breads made with CWRS wheat flour increased by 58 cc and 34 cc when ascorbic acid and protease, respectively, were added at a high level versus a low level. Alpha-amylase and cysteine were more effective in CWES wheat flour breads: the average loaf volume increased 71 cc and 44 cc, respectively, with high level of these additives. ADA and DATEM did not improve the volume of breads prepared with either flour. The high level of SSL reduced average volume of breads made with CWRS wheat flour. Addition of cysteine (50 ppm) reduced the mix time of CWRS dough by 37% and the mix time of CWES dough by 43%.

In the second screening experiment, the five most critical improvers, ascorbic acid, protease, α -amylase, DATEM, and cysteine, were tested for their effects on bread quality. DATEM was most important to loaf volumes of breads made with both flours. Mean volume of CWRS breads were also increased by a high level of protease (30 cc) whereas mean volume of CWES breads also increased with a high level of cysteine (52 cc). The high level of α -amylase used caused a reduction in the average CWRS loaf volume of 33 cc. The external and internal loaf characteristic scores were reduced by the DATEM, both as a main effect and through its involvement in interactions with other additives. The high level of 75 ppm cysteine reduced mix times of CWRS and CWES doughs by 53% and 67%, respectively.

Ascorbic acid, α -amylase, cysteine and percent CWES wheat flour were used as variables in the optimization experiment. Loaf volumes were influenced by a strong ascorbic acid by cysteine interaction. Best volumes were obtained when a high level of one was used with a low

level of the other. This effect was evident across all CWES blends. Blends with less than 50% CWES wheat flour gave good volumes with low levels of α -amylase (20 SKB units). Blends with more than 50% CWES wheat flour had highest volumes with high α -amylase (60 SKB units), although good loaf quality scores were predicted across all α -amylase levels when the percent CWES flour in the blend was high.

CWES wheat flour had an improving effect on bread quality when blended with the CWRS wheat flour. The crumb and external appearance of the loaves improved as the percentage of CWES flour in the blend increased up to 100%. Blends with higher percent CWES tolerated cysteine levels up to 90 ppm without the deterioration of dough handling properties, whereas a maximum of 40 ppm cysteine was possible in blend with less than 50% CWES wheat flour. The extension in mix time with increasing CWES flour was reversed through the addition of cysteine.

The optimized improver combinations selected for CWRS and CWES flours and blends were tested in the verification experiment. Optimized loaves had high loaf volumes, low mix times and very good external appearance. The internal loaf characteristics scores lower than expected. Compared to breads made with a standard bromated formulation, optimized breads were of equal quality. Excellent breads were obtained using the 25% CWES wheat flour blend and 60 ppm ascorbic acid, 20 SKB units α -amylase and 20 ppm cysteine.

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Chapter 1

INTRODUCTION

Flours milled from wheats of the Canadian Western Extra Strong (CWES) class, of which Glenlea is the predominant variety, produce very strong doughs. In the past, Glenlea wheat has been used primarily for blending because its ability to "carry" weaker flours is superior to that of Canadian Western Red Spring (CWRS) bread wheats. It also has been used in frozen dough production to improve bread quality. The upward trend in CWES wheat exports in the past few years has been attributed to the worldwide growth in the frozen dough industry (Oppenheim, 1994).

Benefits of using the extra strong flours in blends or frozen doughs have been demonstrated using standard formulations. It may be possible to enhance the performance of CWES flour by using optimum combinations of improvers. Much more information on effects of improvers in formulations containing CWES flour is needed to establish the effects and levels of improvers needed for optimum performance in dough systems and bread production.

In the North American bread industry, the oxidizing agent potassium bromate has traditionally been used to bring about changes in the dough system and improve the quality of the finished product. Alone or in combination with other oxidants, potassium bromate improves gas retention and oven rise properties of doughs and produces breads with high volumes and good quality. When potassium bromate is eliminated from the flour or bread formulation the result is a reduction in loaf volume, poorer crumb characteristics and a loss in dough tolerance. However, evidence regarding the presence of carcinogenic bromate residues in the crumb of baked bread has led to a search for alternative oxidizing systems. Although there is no single additive which can effectively replace potassium bromate, a combination of improvers might provide the needed functionality. There is also a strong possibility that by using wheat flours which produce doughs with extra strong properties, some of the loss in baking quality and dough tolerance observed when working without potassium bromate may be restored.

Many different types of improvers have been developed to help the baker overcome some of the problems encountered when baking without bromate. These usually consist of a combination of oxidizing and reducing agents, and enzymes and surfactants. The most frequently used are the oxidants ascorbic acid and azodicarbonamide, fungal α -amylase and protease enzymes, surfactants such as sodium stearoyl-2-lactylate and diacetyl tartaric acid esters of monoglycerides, and the reducing agent L-cysteine hydrochloride.

Ascorbic acid and azodicarbonamide (ADA) are commonly used in bread formulations to provide oxidation. Ascorbic acid is widely favoured because of the lack of health and safety risks associated with it and because of its moderate rate of reaction. Although, ascorbic acid acts as a reducing agent or antioxidant in many applications, in bread dough, the oxidized form, dehydroascorbic acid, is an effective oxidizing agent. ADA, a very fast acting, highly reactive oxidant, is also permitted for use in bread production and its inclusion in an optimized improver system should be considered. The effect of adding oxidants is to bring about changes in the dough system which improve the volume and crumb structure of the resultant bread.

Enzymes in relatively pure form or as part of a malt supplement are included to improve fermentation and enhance bread quality. Alpha-amylase, naturally present in wheat, catalyses hydrolysis of the α -1,4 glucosidic linkages of starch in damaged starch granules, producing a series of intermediate chain length products known as dextrins. The β -amylase which is naturally abundant in wheat flour hydrolyses these dextrins, producing maltose. These steps result in a continued supply of the fermentable sugars required for yeast metabolism throughout the fermentation and proofing stages. Enzymic degradation of starch leads to changes in dough consistency and extensibility. At optimum α -amylase levels, oven-spring is enhanced, volumes increase and crumb structure improves. Excessive α -amylase activity in wheat flour, usually the result of pre-harvest sprouting, is detrimental to bread quality. To avoid this problem, millers prefer to produce flour from high quality, sound wheat with low α -amylase activity, and to supplement the flour with α -amylase derived from cereal or fungal sources.

Proteases split internal peptide bonds of gluten molecules, thereby mellowing the dough, making it less "bucky". In some cases, protease reduce the mixing requirement of doughs. These effects are of particular importance for doughs with extra strong properties which require long mixing times. Proteases also split single amino acid units from the terminal end of the gluten protein molecule. Through reaction of these amino acids with reducing sugars and other carbonyl compounds generated by yeast fermentation, crust colour and bread flavour are enhanced.

Mixing requirements of extra strong doughs can also be reduced by incorporating a reducing agent such as L-cysteine hydrochloride (or simply cysteine) into the bread formulation. Cysteine acts quickly to split the disulphide linkages in the protein network. As a result, the gluten structure is weakened and the dough becomes less elastic and more extensible. This dough weakening effect is the basis for cysteine's extensive use in Activated Dough Development (ADD), as a lower amount of mechanical energy is required to develop the dough, resulting in a considerable reduction in mixing requirement. Cysteine may prove to be highly beneficial for

long mixing, extra strong wheat flour doughs.

Surfactants, or emulsifiers, are added to wheat flour doughs for their crumb softening and/or dough strengthening effects. Beneficial effects of surfactant addition include improved gas retention and oven-spring properties, and an increased tolerance of doughs to overmixing and abuse. Breads have greater volume, finer crumb structure, strong side-walls and improved slicing characteristics. Two surfactants commonly used for dough strengthening are DATEM (diacetyl tartaric acid esters of monoglycerides) and SSL (sodium stearyl-2-lactylate). Several theories have been advanced to explain how these substances strengthen the dough. Surfactants may act to neutralize the positive charges on the surface of the gluten and promote its aggregation. Gluten structure may be strengthened through hydrophobic and/or hydrophilic bonding between the surfactants and the gluten proteins. Gas retention properties of doughs may be enhanced through the formation of a gliadin-surfactant-glutenin complex. Surfactants may also associate with the water phase which surrounds the gas bubbles in the dough, forming lamellar type structures (gel structures) in water at dough temperatures which contribute to dough elasticity.

Dough additives are usually combined in order to take advantage of additive and synergistic effects. There are some published works in which the interactions between oxidants and between oxidants and reductants have been demonstrated. Synergistic effects of emulsifiers and oxidants, fungal α -amylase and protease, and enzymes and surfactants have also been investigated. However, most studies which assess the effectiveness of different additives on dough and bread properties have been carried out by examining each improver individually. Doerry (1991) looked at various combinations of oxidants, enzymes and surfactants in a variety of bread-types, using four different bread processes and two different flours to determine whether acceptable bromate-free breads could be produced. He concluded that bromate could successfully be replaced with certain combinations of additives. However, the lack of a true experimental design may have resulted in a lack of information on the true optimum combinations. This problem could be overcome by using an appropriate experimental design such as those used in Response Surface Methodology (RSM).

RSM is a statistical technique used widely in the area of product development. The principle advantage of RSM is that a large number of variables can be assessed for their effectiveness in a product with a relatively small number of experimental trials. By analyzing the results from these trials, a predictive model can be developed and the effects of untested combinations can be projected.

The overall purpose of this study was to optimize bromate-free improver combinations for

use with CWRS and CWES wheat flours both alone and in blends.

The general objectives of the research:

To screen selected improvers in order to identify the additives most important to loaf volume, mix time, and crumb structure of breads made with both CWRS and CWES wheat flours.

To optimize improver combinations for use with CWES and CWRS wheat flours alone and in blends.

To verify that the optimized improver combinations identified produced bread of acceptable quality consistent with the projected results based on the response surface models developed.

The research was carried out in three main steps: screening, optimization and verification, each related to one of the three objectives listed above. The screening step was carried out as two separate experiments. Based on the first screening experiment the number of potential variables was reduced from seven to five. Based on the second, the variables were reduced to the three which were most important in terms of their main effects and involvement in interactions with other variables. In both screening experiments, CWRS and CWES flours were evaluated separately.

The optimization experiment was carried out as one experiment with four variables (three additives plus % CWES wheat flour in the blend). In the discussion of the results, each flour blend was considered separately in order to determine the effect that CWES wheat flour had on the requirement for improvers.

The verification experiment involved the preparation of breads using each of the five flour blends with either two or three improver combinations. The actual outcomes were compared with the predicted outcomes and multiple comparison tests were performed. Thus, the ability to produce breads using flour blends consisting of increasing proportions of CWES wheat flour of comparable or better quality to those produced using only CWRS wheat flour was determined.

Chapter 2

LITERATURE REVIEW

WHEAT FLOUR AND BREADMAKING

Wheat flour doughs are able to retain the carbon dioxide produced by yeast during fermentation to a much greater extent than those of any other cereal grain. This ability is due primarily to the protein fraction of the wheat flour (for review see Tweed, 1993). When hydrated and mixed, the wheat proteins form a continuous matrix in which starch granules are embedded. Air occluded during mixing is entrapped in this phase. This protein structure is further developed during fermentation and proofing, resulting in the formation of thin gluten lamellae between the gas cells. Thus, a fine vesicular structure is built and maintained until it is fixed in the oven by protein denaturation and starch gelatinization (Bloksma, 1971).

The breadmaking potential of a wheat flour is governed by both its protein quantity and protein quality. For a single variety of wheat, there is usually a positive linear relationship between protein content and loaf volume (Tipples and Kilborn, 1974). In such cases, protein content is often equated with the term "strength", with high protein flour (> 14% protein) being "strong", and low protein flour being "weak" (Tipples et al, 1982). However, two flours with the same protein content can give breads of different loaf volume and crumb characteristics (Bushuk et al, 1969). Thus, wheats can vary in terms of their protein quality.

The gliadin and glutenin proteins account for approximately 80% of the proteins in wheat flour. When hydrated, glutenins form a tough rubbery mass while the gliadin fraction is viscous and fluid. Together they are responsible for the viscoelastic properties of dough and determine suitability of a flour for processing into bread (Schofield, 1986; Wall, 1979). Protein quality can be defined as "the inherent quality of the flour protein for the production of bread" (Tweed, 1993) and has been linked mainly to the glutenin group of proteins (Payne et al, 1979). Glutenins can be separated into two fractions after reduction: high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits (Payne and Corfield, 1979). Wheat varieties differ in their glutenin subunit composition and this could account for the differences in breadmaking potential. In fact, the presence or absence of specific HMW subunits is the basis of the Glu-1 quality score used to predict the breadmaking quality of different wheat varieties (Payne et al, 1987).

Canadian Wheats

Much of the wheat grown worldwide is of the common hexaploid species *Triticum aestivum*. Within this species, wheats are further differentiated according to such factors as kernel hardness and vitreousness (hard/soft), growth habit (spring/winter) and physical properties of the dough (strong/weak) (Bushuk and Scanlon, 1993). In Canada, there are currently nine classes of wheat recognized by the Canadian Grain Commission. These are Canada Western Red Spring, Canada Western Red Winter, Canada Western Soft White Spring, Canada Prairie Spring (Red and White), Canada Western Extra Strong, Canada Western Amber Durum and Canada Eastern Red and White Winter wheats (Williams, 1993). Varieties of the Canadian Western Red Spring (CWRS) class of wheats have been rigorously selected for their breadmaking potential. These wheats are normally high in protein content, have strong physical dough properties and high loaf volume potential. Varieties of the Canadian Western Extra Strong (CWES) class of wheats produce flours with very strong dough characteristics and have been used primarily for blending with other flours.

CWES Wheat Class

The predominant variety in the CWES wheat class, formerly Canada Western Utility (CWU), is Glenlea (Preston et al, 1993). Developed at the University of Manitoba in 1965 and licensed in Canada in 1972, Glenlea can be distinguished from CWRS wheats by its larger and slightly harder kernel. It is resistant to test weight loss and bleaching caused by adverse weather conditions before harvesting (Czarnecki and Evans, 1986), and to infestation by a large group of stored-product insect species (Sinha et al, 1988). In a study comparing several hard red spring varieties, Glenlea consistently produced the highest grain yields (Waterer and Evans, 1985). Flour yields of Glenlea are generally good, but protein content is 1.5 - 2.0% lower than other CWRS varieties grown under the same conditions (Preston et al, 1987).

A long mixing time requirement is a distinguishing characteristic of the CWES wheat flours. It is primarily because of this trait that these varieties do not qualify for the CWRS class. Despite the very high Glu-1 quality score assigned to Glenlea wheat flour (Lukow et al, 1989), it has been reported to have inferior breadmaking quality. This assessment of the breadmaking quality of Glenlea has been attributed to the test-baking methods used (Bushuk, 1980). The GRL Remix Method (Kilborn and Tipples, 1981) commonly used to assess the baking quality of wheat flours, uses a constant mixing time of 2½ minutes. This mixing time is insufficient to properly develop the gluten in the extra-strong wheat flour and as a result, loaf volumes are lower. Bushuk

et al (1969) found that when the remix time was increased to 5 minutes, the baking performance of very strong wheat lines was made equal to that of Manitou. Finney et al (1976) and Dexter (1993) also reported higher bread scores for loaves produced from extra strong, long mixing flours when remix time was extended to ensure full dough development.

CWES wheat flours are excellent for blending with weaker flours to improve their breadmaking quality. Using the Remix Blend Procedure (Kilborn and Tipples, 1981), which measures the ability of a flour to "carry" a weaker one, the extra-strong flours performed better than the standard CWRS wheat flours, giving greater Remix Blend loaf volumes (Tipples and Kilborn, 1982) and better crumb characteristics (Dexter, 1993). Bushuk (1980) reported that a smaller percent of Glenlea (23%) than of Neepawa (50%) was required in a blend with a weaker flour, to achieve similar loaf volumes. Waterer and Evans (1985) found that although Glenlea had poorer remix loaf volumes and a lower Baking Strength Index (BSI = 93%) than several CWRS wheat varieties, when it was blended with an equal portion of low protein flour, the BSI increased to 108%. This indicated exceptional carrying power. Glenlea performed better than the other varieties in the blend remix baking test.

The most recent application of CWES wheat flours in breadmaking is in the area of frozen doughs. Inoue and Bushuk (1992) found that when Glenlea flour doughs were mixed to peak development, greater loaf volumes were obtained after freezing and freeze-thaw cycles than were obtained from high quality CWRS wheat flours. Extensive research being carried out to assess the performance of Glenlea and other wheat flours in frozen dough systems. Surprisingly little attention has been given to ways in which the performance of different wheat flours in frozen doughs can be enhanced through the addition of improving agents.

The Role of CWES Wheat in Bromate-free Breads

Potassium bromate has been used as a flour additive to improve the gas retention and stability of wheat flour doughs (Brown, 1993). When it is removed from the bread system there is a deterioration in loaf volume and crumb characteristics, the mixing requirement and water absorption changes, and there is a loss in dough tolerance (Barnard, 1992). Considerable progress has been made in industry to develop improver mixtures which effectively restore the loaf volume lost by the omission of potassium bromate. These mixtures usually consist of combinations of oxidants, enzymes and emulsifiers. Another approach suggested by Zimmerman (1991) is to use stronger flours.

CWES wheat flours have very strong dough properties according to extensigraph tests.

The extensigraph measures the force required to stretch a piece of dough (resistance to extension) and the time it takes to stretch the dough to the breaking point (extensibility) (Shuey, 1975). Extensigraph data for CWES and CWRS wheat flour composite samples from the 1993 crop year are given in Table 2.1. The exceptional strength of CWES wheat flour doughs is indicated by much greater resistance to extension than the CWRS wheat flour doughs. It is possible that by incorporating extra-strong flours into a bread system, some of the requirement for strengthening agents such as bromate can be reduced.

Use of CWES wheat flour in a bromate-free bread formulation may also help to improve dough stability. Figure 2.1 shows the farinograph curves of composite samples of CWRS and CWES wheat flour from the 1993 crop year. The farinograph is used routinely to measure dough mobility. The Mixing Tolerance Index (MTI) gives an indication of a flour's tolerance, with a lower value meaning a greater tolerance to mixing (Shuey, 1975). The curves show a much greater tolerance of CWES flour to mixing (MTI = 17.8 BU) than the CWRS wheat flour (MTI = 27.8 BU). The higher tolerance of CWES flour doughs suggests that these flours may help to replace some of the loss in dough stability which occurs without bromate.

Although there are no published studies which examine the improver requirement of CWES wheat flours, there are some reports of the oxidative requirements of other strong, long mixing flours. Finney et al (1976) reported that whereas the standard commercial composite flour (11.8% protein; 3¾ minute mix time) had a potassium bromate requirement of 25 ppm, the Red River 68 flour (11.6% protein; 7 min mix time) required no potassium bromate at all. Finney et al (1987) compared a strong and a weak flour and noted the weak flour had an oxidative requirement 3 times that of the stronger flour. They also cited previous findings which showed that 10 ppm potassium bromate plus 50 ppm ascorbic acid was required in short to medium-short mixing flours to achieve comparable results to the medium-long to long mixing flours with only 10-20 ppm ascorbic acid. An investigation of the improver requirement of CWES wheat flour is needed in light of their potential for use in both frozen and non-frozen doughs.

The ideal improver mixture for use in CWES flour breads would likely include a reducing agent such as L-cysteine hydrochloride to accelerate the dough development process and reduce the mixing time requirement, in conjunction with an optimum combination of oxidants, enzymes and emulsifiers (surfactants). In this way, it may be possible to achieve a bromate-free product of excellent quality using CWES alone or in part in the bread formulation.

Table 2.1. Summary of extensigraph data for CWRS and CWES wheat flour composite samples from the 1993 crop year^a.

	CWRS Wheat Flour	CWES Wheat Flour
Protein	11.5%	11.0%
Resistance to extension (maximum height, BU)	410	630
Extensibility (length, cm)	21	26

^a Source: Preston et al, 1993.

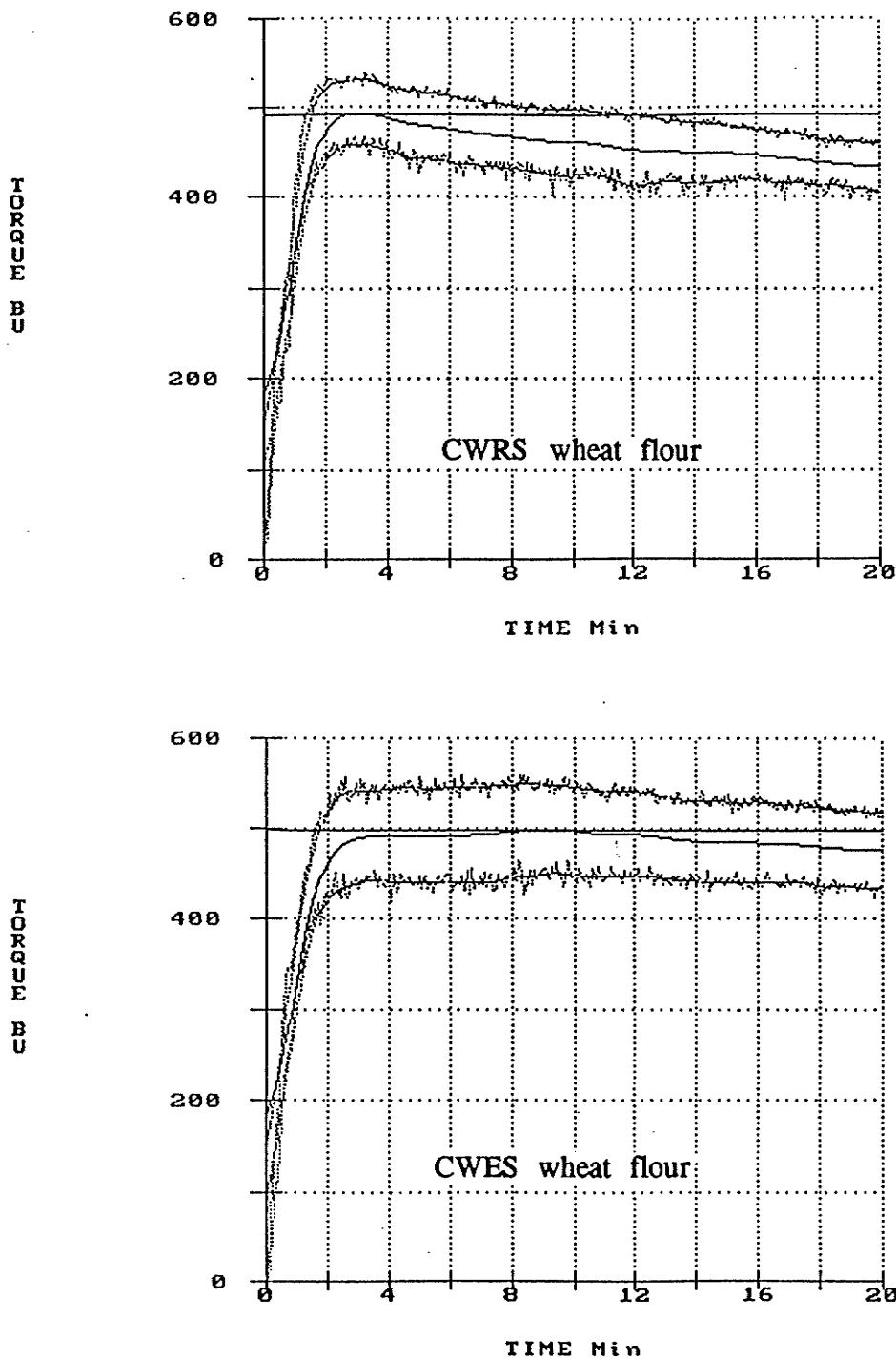


Figure 2.1 Farinograms of doughs prepared with flour milled from composite CWRS and CWES wheat samples from the 1993 crop year. Mixing Tolerance Index (MTI) values are 27.82 BU and 17.81 BU for CWRS and CWES wheat flour, respectively. (Source: L. Schlichting, 1995).

OXIDIZING AGENTS

The importance of the oxidation process to bread quality has been well recognized by the bread industry. When added in minute amounts, oxidizing agents can effect physical changes in the dough, thereby improving loaf volume and textural characteristics, a phenomenon commonly known as the "improver effect" (Tweed, 1993). In Canada and the U.S.A., potassium bromate has been the most commonly used bread improver. The removal of potassium bromate from the list of permitted bread additives in the UK has led to a heavy reliance on alternate oxidizing agents such as ascorbic acid and azodicarbonamide (ADA). Although the way in which these oxidants exert their effects is similar to that of potassium bromate, there are also some differences which must be understood if these agents are to be used in bromate-free improver systems.

Theoretical Aspects of Oxidation

Breadmaking quality of wheat flours which have aged or matured for several months improve noticeably (Fisher et al, 1937). Doughs prepared from these flours are tougher or stiffer than those prepared from freshly milled, "green" flours. During aging complex reactions, such as auto-oxidation of certain flour components, bring about these beneficial changes (Ewart, 1988b). However, natural oxidation that occurs during long-term storage is often not feasible, not only because of economic considerations but also because control of oxidative changes is difficult due to such factors as storage temperature, flour extraction rates, enzyme levels and oxygen supply (Klauri, 1985). Controlled oxidation through the use of chemical oxidizing agents is the practice followed by the majority of millers and bakers.

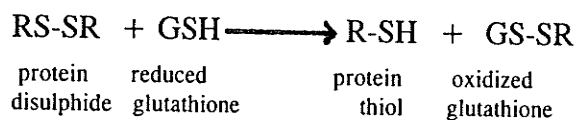
Over the years, many researchers have attempted to explain how oxidants exert their beneficial effects. As dough is an extremely complex system, it is not surprising that the mechanisms are still not fully understood. Hypotheses proposed to explain the beneficial effect have included: 1) inhibition of proteases; 2) oxidation of thiols; and 3) the thiol-disulphide interchange reactions.

In 1935, Jørgensen recognized the improving action of ascorbic acid and bromate. He hypothesized that the presence of "powerful but latent" proteolytic enzymes in the flour acted to weaken the flour and diminish its baking strength. The addition of thiol compounds to doughs enhanced this activity. Additives such as potassium bromate were thought to inhibit these enzymes, and consequently strengthen the dough (Sullivan et al, 1940). Insufficient evidence to support the existence of such enzymes prompted chemists to seek alternative explanations.

The physical properties of dough depend primarily on the composition of the gluten proteins and their state of aggregation. This aggregation is thought to be mediated primarily by disulphide bonds (Grosch, 1986). Other non-covalent bonds, such as hydrogen bonds, ionic bonds, and Van der Waals bonds, and hydrophobic bonds, also contribute to protein conformation (Pomeranz, 1987). During dough development, disulphide linkages (SS) are formed between the gluten proteins via oxidation of the thiol (SH) groups on the protein (Sullivan et al, 1940). As a result of this cross-linking, the dough becomes stiffer and less extensible. Addition of oxidants promotes the oxidation of the thiol groups, enhancing the rate at which additional cross-links are formed (Stauffer, 1983), and increasing the strength of the gluten structure (Dahle and Murthy, 1970).

Reducing agents or thiol compounds, such as glutathione or cysteine, have been observed to have the opposite effect to oxidizing agents (Sullivan et al, 1940). The dough becomes softer and more extensible, presumably as a result of disulphide bonds splitting. These findings support the involvement of sulphhydryl groups in the dough and prompted several researchers (Bushuk and Hlynka, 1962; McWatters, 1978; Sullivan et al, 1961) to examine the effects of thiol-blocking agents such as N-ethylmaleimide (NEMI), which prevent the formation of disulphide linkages, on the behaviour of doughs. Mecham (1959) suggested that blocking thiol groups with NEMI would prevent the oxidation of sulphhydryl groups by atmospheric oxygen during mixing in air and result in doughs with the same mixing behaviour as those mixed in nitrogen. The results showed that adding these agents produced a similar, yet more marked effect to that observed in the oxidized doughs. Other research produced similar results (Bushuk and Hlynka, 1962; Sullivan et al, 1961). Therefore, the improvement in rheological properties is not due solely to the formation of new disulphide bonds, but rather to the removal of thiol groups in the dough.

The thiol-disulphide (SH/SS) interchange is an exchange reaction between the disulphide bonds in the gluten proteins (RS-SR) and low molecular weight thiol compounds present in the flour, primarily glutathione (GSH - reduced form) (Fitchett and Frazier, 1986). The reaction is as follows:



As this reaction proceeds, interchain disulphide bonds are broken, resulting in

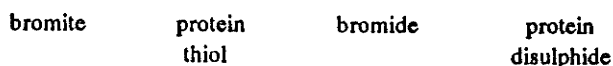
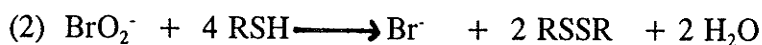
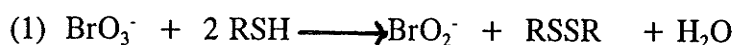
depolymerization of the gluten proteins and subsequent dough weakening. Doughs exhibit decreased resistance to extension and increased extensibility. When minute amounts of oxidizing agents are added to the system they effectively oxidize the free thiol groups, making them unavailable to participate in the SH/SS interchange reaction. Thus, according to this theory, chemical oxidants do not necessarily improve the rheological properties of the dough by increasing disulphide bond cross-linking, but rather block a normally occurring deleterious reaction from taking place (Grosch, 1986; Hoseney, 1991).

Confirmation of the role that flour thiol groups and the SH/SS interchange reaction play in the rheological properties of dough has been provided. Jones et al (1974) found that when dough was reduced by the addition of glutathione, potassium iodate, a fast-acting oxidant, reversed the effects, producing a sudden, significant increase in resistance to mixing. Elkassabany and Hoseney (1980) reported increased dough mobility when glutathione or cysteine were added to flour/water doughs, with subsequent addition of dehydro-ascorbic acid effectively reversing this effect. Bloksma (1972) also observed a stiffening of doughs as a consequence of oxidation and credited this to disulphide cross-links in the gluten phase of the dough. This effect was attributed a reduction in thiol levels as a result of oxidation and subsequent inability to participate in the SH/SS interchange reaction. Most published reviews summarizing the way in which oxidizing agents affect dough properties cite this reaction as the generally accepted explanation (for reviews, see Bloksma, 1974, 1975; Bloksma and Bushuk, 1988; Fitchett and Frazier, 1986).

Potassium Bromate

The use of potassium bromate in bread formulations dates as far back as 1915 when it was first used as a component of yeast food mixtures (Ranum, 1992a). Presently, potassium bromate can be added to flours at the mill at levels not exceeding 50 ppm. At the bakery, maximum levels of addition permitted are 100 ppm in Canada and 75 ppm in the USA (Ranum, 1992b).

It is widely accepted that the bromate reaction involves the oxidation of protein thiols in the dough. The reaction proposed by Tkachuk and Hlynka (1961) is a two stage process. Bromate is first reduced to bromite, followed by a further reduction of the bromite to bromide. At the same time, the thiol groups (RSH) are oxidized to disulphides, making them unable to participate in the deleterious SH/SS interchange reactions. The bromate reaction can be written as follows (Fitchett and Frazier, 1986):



The reaction rate of bromate is relatively slow (Dempster et al, 1956; Bushuk and Hlynka, 1960a), its effect manifested primarily at the baking stage. Tsen (1968) proposed that a minimum temperature of 40°C is required for the complete reduction of bromate in the dough. After mixing and a four hour rest, Bushuk and Hlynka (1960c) noted that half the added bromate was still present in the dough, whereas there was a complete absence of bromate in the crumb of the baked bread. Both Baker and Mize (1939a,b) and Yamada and Preston (1992) attributed the improvement in the oven-rise properties of bromate treated doughs to improved gas retention properties of the dough during baking.

Other factors have been shown to influence the bromate reaction in doughs. The effect of pH on the oxidation of SH groups by bromate has been demonstrated (Bushuk and Hlynka, 1960b; Tsen, 1968), with a decrease in pH causing the extent and rate of oxidation by bromate to increase. The presence of lipids also affects the bromate reaction. Cunningham and Hlynka (1958) noted an acceleration of the bromate reaction when lipids were present and a decrease when lipids were removed, linking this effect to oxygen consumption in the doughs. They suggested that when lipids are removed, there is less oxygen consumed by the lipids and more available for direct oxidation of SH groups. Only part of the SH groups require bromate for their oxidation and thus less bromate is required. When lipids are present, they react with a large part of the oxygen, lessening the inhibitory effect of oxygen on the bromate reaction (Bushuk and Hlynka, 1961).

More recently, Andrews et al (1995) examined the effect of heat and bromate on the free sulphydryl content of wheat flours. Similar decreases in sulphydryl content was observed with increased dough temperature for both bromated and non-bromated doughs. The authors attributed only a small fraction of sulphydryl loss to bromate action, the principle cause of oxidation being heating and mixing. These results indicate that further work is necessary if the bromate reaction in wheat flour doughs is to be fully understood.

Some serious health problems associated with the use of potassium bromate in breads concern its safety, toxicity and carcinogenicity. Potassium bromate is highly explosive when it comes into contact with organic material such as flour. Serious accidents, even deaths, have been reported as a result of improper handling of this substance. By diluting bromate in solution or using it in tablet form and by keeping millers and bakers well informed about the risks involved in improper handling, potassium bromate can be quite safe to use (Gonzalez, 1993).

Several poisonings causing death due to the accidental ingestion of potassium bromate in the form of a neutralizing solution from a permanent wave kit have been reported (Dunsky, 1947; Ranum, 1992a). Another outbreak of potassium bromate poisoning was reported in 1968 in South Africa, during which 816 people were affected, 68 of them requiring hospitalization (Stewart et al, 1969). Yet despite the grave consequences associated with accidental poisoning, there continued to be widespread acceptance of this substance in the bread industry.

The most serious problem associated with potassium bromate is its carcinogenicity. Although two studies which examined the carcinogenicity of bread made with flour treated with 50 and 70 ppm potassium bromate presented no evidence of increased incidence of tumours in the organs of either rats or mice (Ginnocchio et al, 1979; Fisher et al, 1979), Kurokawa et al, 1983 reported that when administered orally to rats through their drinking water, potassium bromate caused a high incidence of renal cell tumours (Kurokawa et al, 1983). As a result, the International Agency for Research on Cancer decided to include potassium bromate in the list of carcinogenic substances (Ranum, 1992a).

The safe usage of potassium bromate as an improving agent in bread is based on the absence of residual bromate in the baked bread. Early studies using amperometric titration techniques (Bushuk and Hlynka, 1960c) and radioactive tracer methods (Lee and Tkachuk, 1959) indicated that at levels of 0-80 ppm, potassium bromate completely disappeared from the crumb of bread during baking for 20-25 minutes. Thewlis (1974, 1977) found that at up to 50 ppm bromate, no potassium bromate was detected in the baked bread using either a long bulk fermentation method or the Chorleywood Bread Process (no bulk fermentation). It was suggested that any residual bromate in the baked bread made with up to 50 ppm potassium bromate may be too small to be detected by this method. Osborne et al (1988) used gas-liquid chromatography and thin-layer chromatography to analyze for the presence of bromide and bromate, respectively, in the crumb of bread. They found that up to 75 ppm, no potassium bromate was detectable in the bread (ie., less than 0.06 mg/kg) and all was accounted for in the form of bromide. However, methods used in these studies were not sensitive enough to detect the levels at which consumption

should not exceed, that is 10 ppb (Ranum, 1992a). Using more sensitive gas-chromatography technique, workers at the British Ministry of Agriculture, Fisheries and Food reported residual bromate in some breads at levels greater than 10 ppb. Although the validity and reproducibility of the test methods used have been questioned (Anon, 1991), these findings prompted the Ministry to remove potassium bromate from the list of additives permitted in flour and bread in the UK, effective April 1, 1990 (Anon, 1990). In anticipation of its removal from the list of permitted flour and bread additives in the USA and Canada, many bakers have voluntarily reduced or eliminated potassium bromate from their bread formulations (Tweed, 1993).

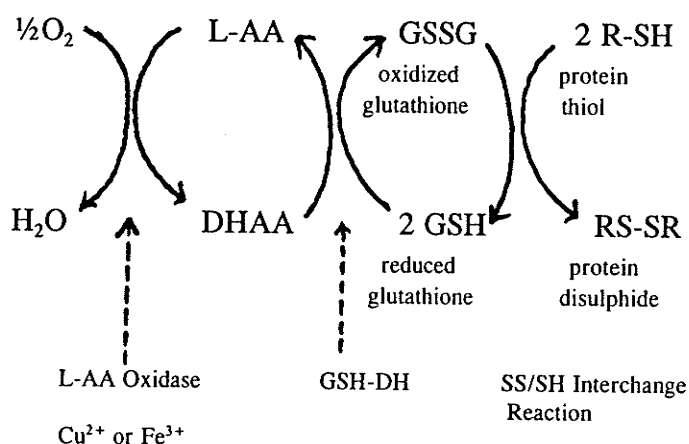
Doughs, prepared without bromate, look and process differently. They may require increased mixing times, their water absorption capacity changes (Barnard, 1993) and they are less tolerant to processing variations. A search for adequate bromate replacers to overcome the changes has been underway. Azodicarbonamide and ascorbic acid are the most likely choices, yet one cannot simply replace bromate with either of these substances as not all oxidizing agents exert their effect in the same way. Therefore, the mechanism by which these oxidants work should be clear in order to understand their behaviour in and contributions to a bromate-free improving system.

Ascorbic Acid

L-ascorbic acid is the trivial name for L-threo-2-hexano-1,4-lactone. There are four stereoisomers of ascorbic acid: L-threo-ascorbic acid, D-threo-ascorbic acid, L-erythro-ascorbic acid and D-erythro-ascorbic acid. The isomer found in food and the body is L-AA, referred to here simply as ascorbic acid. Nutritionally, it is essential in the human diet and plays an important role in the prevention of certain disorders. In the food industry, its use in a wide variety of products is based on its reducing properties (antioxidant). In bread, the maximum permitted level of ascorbic acid is 200 ppm.

The discovery that ascorbic acid could act as a bread improver dates back to findings by Jørgensen in 1935. He found that lemon juice gave similar improving effects as bromate and iodate, and that six month old lemon juice was just as potent as the fresh. Melville and Shattock (1938) looked at both ascorbic acid and its immediate oxidation product, dehydro-ascorbic acid and found that both substances gave the same improving effect as bromate, with dehydro-ascorbic acid being more effective ascorbic acid. Thus, ascorbic acid acts as an oxidizing agent in its oxidized form and some mechanism exists in the flour to effect the oxidation of ascorbic acid.

The reversible redox system proposed by Melville and Shattock (1938) involves two systems. First, ascorbic acid is oxidized to dehydro-ascorbic acid by atmospheric oxygen in the presence of ascorbic acid oxidase (ascorbic acid oxidase) and/or an inorganic catalyst, such as ferric and cupric ions. The dehydro-ascorbic acid oxidizes endogenous thiol compounds in the flour (ie. glutathione), and is reduced back to ascorbic acid, a reaction mediated by the enzyme glutathione dehydrogenase (GSH-DH), also called dehydro-ascorbic acid reductase. The oxidation of thiol compounds prevents their participation in deleterious SH/SS interchange reactions and dough breakdown is prevented. The overall effect is to increase the dough resistance to extension, decrease its extensibility (Kuninori and Matsumoto, 1963), give larger loaf volumes and better texture (Yamada and Preston, 1992). The ascorbic acid reaction can be written as follows:



The reaction is both immediate and time dependent, its rate considered intermediate compared to bromate (slow) and iodate or azodicarbonamide (fast) (Elkassabany and Hosney, 1980; Meredith, 1965). Using a spread test to assess changes in dough properties, Lillard et al (1982) found that ascorbic acid retarded the flow of dough immediately after mixing and even more after a rest period of 1 hour at 30°C. Elkassabany and Hosney (1980) reported an immediate decrease in spread ratios following mixing with added DHAA and throughout fermentation.

Ascorbic acid requires to presence of oxygen to exert its improving effect. Meredith (1965) and Tsen (1965) found that when doughs are mixed anaerobically (in nitrogen) with added

ascorbic acid or dehydro-ascorbic acid, only the dehydro-ascorbic acid-treated doughs showed any improvement in rheological properties.

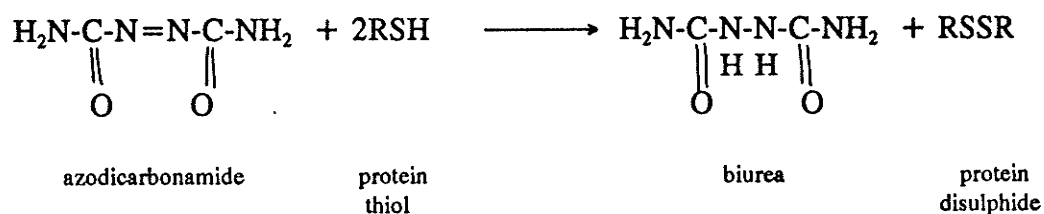
Although an earlier study showed no change in SH levels of flour extracts treated with dehydro-ascorbic acid (Kuninori and Matsumoto, 1964), there has since been evidence that SH contents decrease upon the addition of ascorbic acid. Mair and Grosch (1979) and Sarwin et al (1993) looked at the change in levels of endogenous glutathione (GSH) when flours were treated with ascorbic acid. Both found a disappearance of GSH upon mixing, attributing this to the formation of disulphides (GSSG). Addition of ascorbic acid accelerated the diminution of GSH in these doughs. The mole ratio of reactants in the dough, ie. GSH oxidized:dehydro ascorbic acid reduced, was approximately 1:2, which is in accordance with the theory that oxidizing agents act by oxidizing two SH compounds in the dough (Kuninori and Matsumoto, 1964; Tsen, 1965).

Although dehydro-ascorbic acid is the active form of this improving agent, its instability limits its use in commercial operations. The advantage of using ascorbic acid over other oxidizing agents is that there are no health or safety risks associated with it and overtreatment is not a problem. Using the Canadian Short Process, Yamada and Preston (1992) found that as ascorbic acid levels increased, so did loaf volumes and bread scores until optimum levels were reached (100 ppm for loaf volume and 50-200 ppm for bread score) after which no further improvement or decrease in quality was observed. These results indicate that very high levels of ascorbic acid, up to maximum permitted levels, were not required and did not produce breads with typical overoxidation characteristics.

Azodicarbonamide

Azodicarbonamide (ADA) was introduced into the breadmaking industry in 1962 under the tradename Maturox. It is a nonexplosive, nonflammable crystalline solid which has been shown to be safe for human consumption (Joiner, 1963). As ADA does not react with dry flour, it can be stored in a mixture with flour without its activity deteriorating (Tsen, 1963). In Canada and the U.S.A., the maximum level permitted in bread is 45 ppm.

As with potassium bromate and ascorbic acid, the improver effect is attributed to the oxidation of thiol groups in the flour via the following reaction (Fitchett and Frazier, 1986):



ADA exerts its effect very quickly, the reaction proceeding to completion within the first 2.5 minutes of mixing. Tsen (1963) found that a linear relation exists between the loss of thiol groups in the dough and the amount of ADA added. That is, as levels of ADA addition increased, the thiol content of the dough decreased to a certain point after which further addition of ADA had no effect. This was attributed to the unavailability of some of the thiol groups in the flour as they are hidden or masked in the protein network.

The impact of ADA on dough properties has been examined through various rheological tests. Extensigraph studies have shown that when ADA is added in increasing concentrations, dough extensibility decreases (Tsen, 1963, 1964). Hoseney et al (1979) reported a reduction in spread ratios of doughs during fermentation with the addition of 20 ppm ADA, indicating increased dough strength. Although mixograms have shown little effect of ADA on peak height, decreased time to peak development (Lang et al, 1992) and accelerated dough breakdown after the mixing peak was reached (Weak et al, 1977) with the addition of 30 ppm ADA has been reported. Therefore, shorter mixing times should be used with ADA than with bromate in order to maximize its performance.

The effect of ADA on the quality of the baked breads has also been examined. Joiner et al (1963) found the ADA caused an improvement in the volume, texture and appearance of the baked loaves when used at 20 ppm in both no-time and sponge-and-dough procedures. Using the sponge-and-dough procedure, Yamada and Preston (1994) also found that optimal loaf volumes and bread scores were obtained in the range of 5-20 ppm ADA addition, whereas adding more than this was detrimental to bread quality. The Canadian Short Process requires higher levels of addition, with maximum loaf volumes and bread scores obtained at 40-70 and 10-70 ppm ADA, respectively (Yamada and Preston, 1992).

There are several potential problems associated with the use of ADA in bread systems. Because of its very rapid rate of reaction, it may be used up prior to full dough development when it is needed most. It may also react with reducing substances produced by the yeast or with other oxidants in the dough formulation such as ascorbic acid thereby resulting in a rapid removal of ADA from the system and a lack of oxidation at the critical mixing stages (Ranum 1992a). Since

only very minute quantities are required for best results, overtreatment through the addition of too much ADA could result in a tight, inextensible dough with the resulting loaves having poor volume and a grey, streaky crumb. ADA also tends to cause "checking" of the crust, characterized by fine cracks across the top crust of the loaf (Fitchett and Frazier, 1986), as well as "key holing" or the tendency for the bread to shrink (Maningat et al, 1988). The advantages of ADA are therefore offset by a number of disadvantages.

ENZYMES

Enzymes are used in a wide variety of food products. In the milling and baking industry, their use stems primarily from a lack of naturally occurring enzymes in the wheat and flour (Barrett, 1975). The enzymes used most in breadmaking are α -amylase and protease. Their addition at appropriate levels can improve the dough properties and hence the quality of baked bread in terms of loaf volume, crumb and crust characteristics, flavour and shelf life (Pylar, 1988).

Amylase

Germination of the wheat grain prior to harvesting results in excessive α -amylase activity. Flour milled from this wheat tends to produce breads with low volume, high crust colour, an open crumb which is moist and sticky. However, a small amount of α -amylase activity can be beneficial to bread quality. The α -amylase exerts its beneficial effects in two ways: 1) acts on damaged starch granules to produce fermentable sugars for yeast metabolism; and 2) acts on gelatinizing starch during baking to improve oven spring.

Flour from sound wheat only has small quantities of fermentable sugars (~0.5%), a level which is insufficient for optimal yeast growth and gas production. When added in small quantities, α -amylase acts on the damaged starch granule, producing dextrins. The β -amylase which is naturally present and abundant in wheat flour further hydrolyses these dextrins into maltose, which the yeast is able to use. Thus, there is a continued supply of fermentable sugars for yeast metabolism throughout the fermentation period (Barrett, 1975).

The second function of α -amylase supplementation is to enhance the oven spring properties of the dough, thereby increasing loaf volume. During baking, starch molecules begin to gelatinize

when the dough reaches approximately 56°C (Drapon and Godon, 1987), a process in which the starch granule swells, loses its birefringence and exudes part of its amylose fraction. The α -amylase dextrinizes this leached starch, thereby helping to maintain the fluidity of the dough for a longer period of time. As a result, the setting point of the dough is delayed, oven spring is enhanced and loaf volume is increased (Van Dam and Hillie, 1992). This theory is supported by data collected by Cauvain and Chamberlain (1988), who examined the effect of fungal α -amylase on dough and bread properties using the Chorleywood Bread Process. They reported that as the level of fungal α -amylase increased the consistency of the doughs following mixing decreased. The dough piece also expanded to a greater extent and for a long time (ie. enhanced oven spring) causing loaf volumes to increase.

For optimal baking results, it is best to use flours milled from sound, ungerminated wheat with low α -amylase activity and to supplement these flours with controlled levels of α -amylase (Tweed, 1993). The α -amylases used for wheat flour supplementation are of three main types: cereal, added as malted wheat or barley flour; bacterial, from *Bacillus subtilis*; and fungal, primarily from *Aspergillus oryzae*. Studies comparing the effectiveness of α -amylase from these sources have shown bacterial α -amylase to be the most effective at releasing dextrans and decreasing the viscosity of the gelatinizing starch but had a tendency to produce breads with a sticky, gummy crumb. Fungal α -amylase was the most effective at improving bread characteristics without developing an undesirably sticky crumb (Johnson and Miller, 1948). These differences are attributed to the inactivation temperature of the α -amylase from different sources (Miller et al, 1953). Bacterial α -amylase has the greatest thermotolerance, followed by cereal α -amylase, with fungal α -amylase being the least heat stable. Bacterial and cereal α -amylase are inactivated in the range of 65 to 80°C and 70 to >100°C, respectively, whereas fungal α -amylase has a much lower inactivation temperature, starting at approximately 55°C (Drapon and Godon, 1987). Wheat starch begins to gelatinize at about 56°C and continues until 72°C, a temperature change which is achieved in about 2-3 minutes during baking (Fox and Mulvihill, 1982). During this time both cereal and bacterial α -amylase are able to hydrolyze the gelatinizing starch, causing the crumb of the baked bread to become undesirably sticky due to an overproduction of dextrans. Bacterial α -amylase may also be present and continue to have detrimental effects on crumb even after the bread is removed from the oven (Dubois, 1980). Alternately, there is only a small window of time during which fungal α -amylase is able to react with the gelatinizing starch before it is denatured by heat. Thus, there is a margin of safety against over-dextrinization and the potential for gumminess developing in the crumb of the baked bread is avoided even when fairly

high levels are used.

There are several procedures for measuring the α -amylase activity, the ideal unit of measure being dependent on the material being tested, ie, wheat, flour and liquids. For concentrated enzyme preparations, the most common unit of measure is the Sandstedt-Kneen-Blish (SKB) unit. This method of determining enzyme activity was one of the first widely used tests. It is a measure of the amount of time required, in the presence of excess β -amylase, for a given amount of available starch to be hydrolyzed by α -amylase to the point at which it no longer reacts with iodine to produce the blue/black colour (Miller and Johnston, 1955). The SKB unit is actually an inverse of the reaction time (Barrett, 1975).

The amount of α -amylase a baker should use to obtain the best improving results depends upon several factors. The level of α -amylase activity and amount of damaged starch in the flour are likely the most influential factors (Prouty, 1960). Other characteristics of the flour such as protein level and extraction rate may also be important (Drapon and Godon, 1987). The bread process used may also affect the amount of α -amylase supplementation required as problem associated with dough softening are more prevalent when long fermentation times are employed (Cauvain and Chamberlain, 1988). Generally, the amount of fungal α -amylase commonly used in pan breads is in the range of 13-26 SKB units/100 g flour. The maximum level allowed in Canada dictated by Good Manufacturing Practices, although excessively high levels would likely be deleterious to the quality of baked bread in the same manner as using flour milled from germinated wheat.

Protease

The use of fungal proteases as an enzyme supplement in bread became significant in the 1950's when fungal α -amylases gained popularity as those supplements were relatively high in protease activity (Fox and Mulvihill, 1982). Since that time, commercial enzyme preparations for bread improvement consistently include some degree of protease activity.

Proteases are enzymes which act on protein molecules. Fungal protease, derived primarily from *Aspergillus oryzae*, contains both exo- and endoenzyme activity (Dziezak, 1991). As an exoenzyme, it liberates single amino acid units from the terminal end of the gluten protein molecule. Its endoenzyme activity causes a breakage of splitting of the internal peptide bonds of the gluten molecule (Kulp, 1933). Because fungal protease is inactivated at a fairly low temperature of 65°C (Drapon and Godon, 1987), its effects are manifested at the mixing and fermentation stages of the bread process rather than during baking (Underkofler, 1961)

Protease enzymes have been shown to "mellow" the dough by increasing the extensibility, making them less "bucky" or tight, improving their machinability (Dubois, 1980). This is of particular importance when working with flours with high protein contents and strong dough characteristics. Because of the greater extensibility, the gas retention properties of the dough is improved, resulting in larger loaf volumes. Improved loaf symmetry and uniformity, better crumb grain and texture, plus some improvement in shelf life have been reported (Dubois, 1980). Also, as the disulphide bonds of the dough are not affected by proteases, doughs which are too tight as a result of oxidizing agents can be modified without compromising the desirable protein network (Fox and Mulvihill, 1982).

One of the primary applications of fungal proteases in breadmaking is as a mix time reducer. Reductions in mix time of up to 30% have been noted (Dekker, 1994). In fact, Reed and Thorn (1957) cited findings of a study which showed a reduction in mix time of up to 65%. This effect is beneficial primarily in plants which operate close mixing schedules (Pylar, 1988). The softening effect of proteases could effectively substitute a large part of the long mixing requirements which very strong flours normally require (Underkofler, 1961).

Free amino acids liberated as a result of the exoenzyme activity of fungal protease can react with reducing sugars (Maillard reaction) and enhance the colour of the crust. Better flavour may also arise out of similar Maillard reactions as well as from interactions between amino acids and carbonyl compounds generated by yeast fermentation (El-Dash and Johnson, 1967; Kulp, 1993).

Time is one of the major factors dictating the extent of the effects of protease. Proteases begin breaking down the gluten proteins immediately upon wetting of the flour. However, because it needs time to react, processes which involve a long period of fermentation will be affected by the protease to a greater extent. Thus, protease will be more effective in a sponge-and-dough and straight dough process than in the short no-time systems. Also, the effect of reducing mix time is more evident in the sponge-and-dough process, as the protease has the long fermentation time in the sponge to react with the protein and reduce the mixing requirement (Dubois, 1980). Mixograph curves have also illustrated the effect of protease on dough development time (Reed and Thorn, 1957; Woods et al, 1980).

A commonly used unit of fungal protease activity is the Hemoglobin Unit (HU). The extent of hydrolysis of hemoglobin by protease is measured by determining the amount of nitrogen which remains soluble after the addition of trichloroacetic acid (Reed and Thorn, 1957). Generally, bread dough is supplemented with approximately 100-220 HU/100 g flour. However, the

optimum level of usage will depend primarily on the breadmaking process being used.

The upper limit of usage for fungal protease is not as liberal as that of fungal α -amylase. Overtreatment results in doughs which are excessively sticky and difficult to handle. The gluten protein structure is weakened, an irreversible process, and the loaves may flatten on top during proofing. The resulting bread is of low volume with a coarse uneven grain (Kulp, 1993).

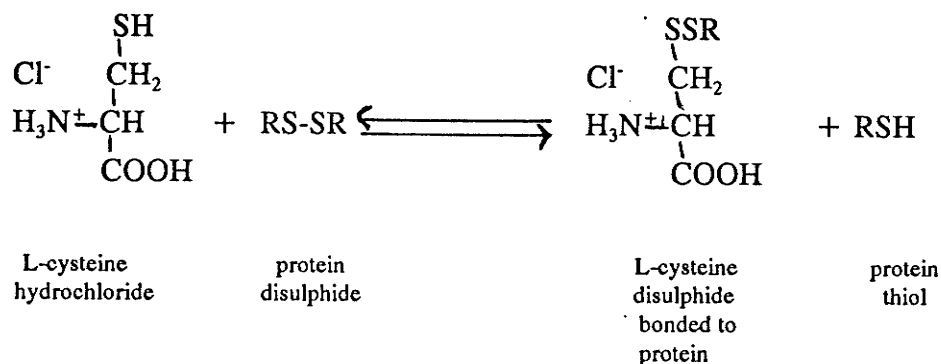
REDUCING AGENTS

The use of reducing agents in bread production stems from their ability to break the disulphide linkages in the gluten network thereby weakening the dough. This is particularly useful in chemical or activated dough development (ADD) in which the mechanical energy required to develop the dough is dramatically reduced (Fitchett and Frazier, 1986). The reducing agent used most commonly for this purpose is L-cysteine hydrochloride.

L-Cysteine Hydrochloride

The amino acid L-cysteine hydrochloride (referred to here simply as cysteine) is a reducing agent which causes changes in dough properties opposite to that induced by oxidizing agents. Although its use in the breadmaking industry has been limited primarily to chemical or activated dough development, its use as a mix time reducer in breads made with flours with extra-long mixing requirements is worth investigating.

When added to wheat flour dough, cysteine acts quickly to split the disulphide linkages in the protein network. This reaction is as follows:



The rapid splitting of the disulphide bonds facilitates the unfolding of the protein molecule. As a result, the gluten structure is weakened and less elastic (Fitchett and Frazier, 1986). Using size-exclusion high-performance liquid chromatography, Békés et al (1994) showed that there was a reduction in the amount of highest molecular weight material in reduced doughs, suggesting a lower degree of protein aggregation and dough strength.

Its use in ADD is based on cysteine's dough weakening effect resulting in lowered mechanical energy requirement to develop the dough and thus a substantial reduction in mixing requirement. Mixograph studies have shown that cysteine reduces the time to peak dough development by approximately 30% when added at a level of 20 ppm (Lang et al, 1992; Weak et al, 1977) and up to 70% reduction with higher levels of 120 ppm (Finney et al, 1971). However, in ADD, the oxidizing agents must also be included to assure the oxidation of excess thiol compounds, promoting the formation of disulphide linkages in order to avoid an overly soft dough during proofing and baking (Bloksma and Bushuk, 1988; Johnston and Mauseth, 1972).

Cysteine can be especially useful when working with over-strong flours which require excessively long mixing requirements. Finney et al (1971) examined the effect of cysteine on the properties of dough and bread made with the long mixing flour Red River 68 in a straight-dough process. At 120 ppm cysteine, mix time was reduced by almost 70%, internal loaf structure was of equal or better quality than control loaves and loaf volumes increase significantly. Kilborn and Tipples (1973) also worked with Red River 68 flour using a Chorleywood type bread process. Mix time decreased from 24 to 8 minutes when cysteine addition increased from 40 to 80 ppm. Loaf volume increased to a maximum at 160 ppm cysteine, and crumb scores were best at 80 ppm cysteine. These findings indicate that cysteine not only reduced the mixing requirement of long mixing flours, but also improves the quality of the bread made from them.

An oxidizing agent is usually included in the bread formulation when cysteine is being added to ensure that optimum physical dough properties are obtained during the proofing and baking. In Kilborn and Tipples (1973) used 75 ppm ascorbic acid and 45 ppm bromate in bread treated with cysteine. Finney et al (1971) stated that an additional 5 ppm bromate was required for each additional 40 ppm cysteine added.

The oxidant selected for use with cysteine is important. Fast acting oxidants such as potassium iodate and azodicarbonamide restore dough properties during mixing and negate the beneficial effects of reduced mix time. The slower acting oxidants bromate and ascorbic acid work better with cysteine as they are still present in the dough after peak dough development to reform the broken disulphide bonds, making the dough stronger and more elastic and able to retain

gas during baking.

In Canada, the maximum permitted level of cysteine in bread is 90 ppm. However, the level which gives maximum results in terms of mix time reduction and bread quality improvement is determined by the flour strength. Kilborn and Tipples (1973) reported that in order to reduce energy required to develop dough to 50% of control dough mix times, the long mixing flour required the lowest amount of cysteine (40 ppm) compared to a moderately strong flour (50 ppm) and a soft flour (110 ppm).

SURFACTANTS

Surfactants are used extensively in the food industry. In the bread industry, they are commonly used to improve the volume, texture and shelf-life of the baked bread (Penny, 1992). In the baking industry, surfactants (or emulsifiers) are often referred to as crumb softeners and dough conditioners or strengtheners. Although there are several surfactants available for use in breadmaking, this discussion will focus only on two surfactants used primarily for their dough strengthening abilities: diacetyl tartaric acid esters of monoglycerides (DATEM) and sodium stearoyl-2-lactylate (SSL).

Chemical Structure of Surfactants

Surfactants are amphiphilic, having both a hydrophilic and lipophilic group. In a typical O/W or W/O emulsion, the surfactant aligns itself so that the hydrophilic (polar) portion is absorbed in the water phase and the lipophilic (non-polar) portion is absorbed in the oil phase, thereby reducing the interfacial tension and promoting the stable emulsification of the two normally immiscible liquids (Krog, 1981). The functionality of surfactants in a dough system is based primarily on their chemical structure and their ability to interact with the various constituents of wheat flour dough rather, thereby stabilizing the dough structure (Cole, 1973).

Surfactants are commonly classified according to their hydrophilic-lipophilic balance (HLB). The HLB value is a ratio of the hydrophilic to lipophilic groups of the surfactant, with an HLB value of 0 being totally lipophilic and an HLB value of 20 totally hydrophilic (Penny, 1992). The lipophilic group consists of fatty acid chains of varying length (C12 to C20) and degree of saturation (Krog, 1981) which is esterified to the hydrophilic compounds originating from polyvalent alcohols such as glycerol, propylene glycol, sorbitan or sucrose. The hydrophilic group can be modified by esterification with organic acids such as lactic, acetic, tartaric and

succinic acids, or by reacting them with ethylene oxide. As a result, a variety of surfactants have been produced with a wide range of HLB values (Pyler, 1988).

Sodium Stearoyl-2-Lactylate (SSL)

SSL has excellent dough strengthening and crumb softening characteristics and is usually used as a free flowing powder (Tenney, 1991). It is the reaction product of lactic acid with stearic acid partially neutralized in the form of its sodium (SSL) salt (Tamstorf et al, 1986). It is anionic with HLB value of 10-12. The maximum permitted level of SSL in bread is 0.375% (flour wt) in Canada and 0.5% (flour wt) in the USA.

Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM)

DATEMS have excellent dough strengthening capabilities but very little crumb softening effects. They are produced by reacting monoglycerides with diacetyl tartaric acid anhydride. DATEMs are non-ionic and hydrophilic and have HLB values of 8-10. The physical properties of DATEMs depend on the amount of tartaric acid and the type of fatty acid used. DATEMS with the best dough strengthening properties are those made with fully hydrogenated fat. Their maximum permitted level in breads is 0.6% (flour wt) in Canada, but have GRAS status in the USA.

Effects of Surfactants on Dough and Bread Properties

The properties of wheat flour doughs are altered with the addition of dough strengthening surfactants. Water absorption increases (Garti et al, 1980) and the doughs are more tolerant to over-mixing (Lorenz, 1983; Tsen and Weber, 1981) and to abuse on conveyor systems (Dubois, 1979a). Thompson and Buddemeyer (1954) found that both DATEM and SSL promoted gas production thereby shortening proof times. They attributed these effects to the surfactants ability to interact with wheat flour components and enhance the doughs ability to form air cells and retain gas during expansion. Kilborn et al (1990) also attributed SSL's ability to improve oven-spring to the improved gas retention ability of the dough. The effect of DATEM was also shown to have its greatest effect at the oven-rise stage as a result of the dough's improved gas retention properties (Mettler and Seibel, 1993).

Breads treated with surfactants such as SSL and DATEM have strong sidewalls and improved slicing characteristics (Dubois, 1979a). Increased loaf volumes, higher breads scores and a brighter crumb have also been reported (Garti et al, 1980; Thompson and Buddemeyer,

1954). Junge et al (1981) proposed that the fine grain originates either from an increased incorporation of air during mixing or an increase in the number of gas cells formed during mixing. These researchers used a scanning electron microscope (SEM) to examine the distribution and size of air cells in the crumb and the density of doughs. They concluded that the fine texture of breads with added surfactants resulted not from a greater incorporation of air but from the formation of more and smaller cells in the dough during mixing.

Mechanisms of Surfactants as Dough Strengtheners

A study by Swanson and Andrews (1942) demonstrated an increase in mixing time due to the action of anionic surface active agents although no correlation was found between surface tension changes and increased mixing times. These authors postulated that surfactants had the ability to alter the configuration of the protein molecule as a result of some sort of protein denaturation. Thompson and Buddemeyer (1954) agreed, but suggested that surface activity may play a minor role in the action of surface active agents on the rheology of doughs. Since that time, several theories have been advanced to explain how these substances exert their effects.

Hoseney et al (1970) looked at the way in which lipids are bound in wheat flour doughs during mixing. They suggested that free polar lipids (glycolipids) are bound to both glutenins, via hydrophobic bonds, and gliadins, via hydrogen and electrostatic bonds. These gliadin-glycolipid-glutenin complexes may contribute to the gas retention properties of the gluten. Surfactants act in the same way as the polar lipids, binding simultaneously to both gliadin and glutenin, thereby enhancing the gas retention capacity of the dough.

Another model for gluten structure was proposed by Grosskreutz (1961). Using electron microscopy and X-ray studies, he showed that upon hydration and mixing, wheat phospholipids form bimolecular leaflets in the gluten. The protein chains, in the form of platelets, are bound to the outer edges of the phospholipid leaflet via hydrogen and electrostatic bonds. Stutz et al (1973) proposed that dough strengthening surfactants behave in the same way as the lipid bilayer of Grosskreutz's model, orienting itself on the outer edges of the gluten sheets or by cross-linking between adjacent gluten sheets, thereby strengthening the gluten.

Tu and Tsen (1978) also used SEM to examine the structural changes in glutenin during mixing. They found that glutenin fibers associate together to form sheet-like structures. Upon addition of SSL, a surfactant-glutenin complex is formed which may explain the increased dough stability observed in these doughs.

According to Krog (1981), surfactants strengthen wheat flour doughs in two ways. Firstly,

hydrophobic and/or hydrophilic bonding between surfactants and gluten proteins acts to strengthen the gluten structure. Secondly, native polar lipids are able to interact with the water phase which surrounds the gas bubbles in the dough, forming associated lipid-water structures of the lamellar type (gel structure). These contribute to the elasticity of the dough and thus the ability of the gas cells to expand. The surfactants which are most effective at strengthening dough, such as SSL and DATEM, are also able to form these lamellar mesophases in water at dough temperatures, and may act to enhance dough properties in the same way as the native polar lipids.

Although the way in which some surfactants strengthen wheat flour dough is still under investigation, the benefits derived from using them in the bread formulation have been well documented. As a result, most commercially available improver mixtures include surfactants. Generally a combination of surfactants are used to provide both crumb softening and dough strengthening. In light of their strengthening ability and contribution to dough stability, surfactants may play an important role in improving the quality of bromate-free breads.

REPLACEMENT OF POTASSIUM BROMATE

The removal of potassium bromate from the list of permitted food additives in the UK resulted in an immediate and urgent need for adequate bromate replacers. Ascorbic acid was quickly adopted and processing parameters used in the Chorleywood Bread Process have been and continue to be adjusted to enhance the reducing/oxidizing activity of this additive (Collin, 1994). In North America, much of the bread research is now being conducted with ascorbic acid rather than potassium bromate. Within commercial bakeries, the problem of eliminating bromate is being dealt with by using combinations of oxidants, enzymes and emulsifiers (Barnard, 1993). Companies which manufacture improver mixture have responded with new bromate-free products containing these additive combinations. Despite this ongoing work, there is very little in the published literature which deals directly with the optimization of alternate improving agents in bromate-free bread formulations.

A study conducted by Doerry (1991) at the American Institute of Baking investigated whether potassium bromate could be replaced by different combinations of ascorbic acid, azodicarbonamide, fungal amylase and protease, and the surfactants SSL and DATEM. He used two types of flour (hard red spring and winter), four different bread processes (straight dough, sponge-and-dough, 40% flour liquid ferment and no-flour liquid brew) and four types of bread (white, whole wheat, multigrain and high-fibre). Most of the work was carried out and data

presented on the white pan bread.

Some of the general results of the study were that both emulsifiers had significant improving effects, often equal to that contributed by the potassium bromate. The enzymes were also very effective in improving bread quality, but their effectiveness appeared dependent on the bread process used. SSL in conjunction with fungal protease worked better than DATEM, alone or with enzymes and AA was the preferred oxidant over ADA.

This study was useful in that it provided bakers with possible improver combinations to replace potassium bromate. However, it also left several questions unanswered. For example, SSL plus protease worked better than DATEM, but did SSL alone work better than DATEM or did it require the inclusion of protease to enhance its effectiveness? Another question is whether amylase and protease were ever used in combination and what is the level of amylase activity in the protease preparation? These questions could not be addressed in the study due to the lack of an appropriate experimental design. By using a carefully designed experiment, the true optimum improver formulations for each bread type could have been identified.

RESPONSE SURFACE METHODOLOGY

Response surface methodology (RSM) is a statistical technique in which the effects of two or more independent variables on the response variable(s) can be examined simultaneously (Walker and Parkhurst, 1984). The benefits of using RSM in the food industry was realized in the 1960's, and since that time RSM has been used for the development of a variety of food products (Henika, 1982). In the breadmaking industry specifically, RSM has been applied successfully to the optimization of whole wheat breads (Mettler and Seibel, 1993), high protein bread (Henselman et al, 1974), gluten-free breads (Ylimaki et al, 1988, 1991) and bread formulations for the elderly (Payton et al, 1988).

There are two approaches which have traditionally been used to determine what combinations give the best results in a product (Giovanni, 1983; Haaland, 1989). The first one is the "one variable at time" approach in which all variables are set at a fixed levels, while one variable is studied at a range of level and its effect determined. Once its best setting value is obtained, that variable level is fixed and the remaining variables are tested one at a time in the same manner. There are several problems which arise out of this type of approach (Joglekar and May, 1987). This method fails to detect non-linear effect and interactions among the variables in which level of one variable influences the effectiveness of another variable. If such interactions

are not taken into consideration, the optimum solution may be missed as the space of possible solutions will not be thoroughly explored. Another problem is that no equation is developed to describe the relationship between the variables and responses (Giovanni, 1983). These problems could be overcome by using the matrix approach (Haaland, 1989), in which all possible combinations are tested until the optimum is found. However, this would require a large number of experimental runs, especially if a large number of variables are being considered. In RSM studies, only specific variable combinations are tested. As the number of experimental runs required to study a product characteristic, costs are reduced and time saved (Mullen and Ennis, 1985).

There are three stages of experimentation in statistical design approach (Haaland, 1989), screening, optimization and verification. At the beginning of an experiment, there may be a large number of variables which may be important in affecting the response. In order to eliminate some of the less important variables and identify the most critical ones, a preliminary screening experiment can be carried out. Factorial designs are used at this stage, in which each variable is tested at two levels and their effects estimated. (Box and Draper, 1987). If the number of variables considered is large, a full factorial design in which all possible combinations are tested may be unrealistic given the large number of experimental runs required. However, by using a fractional factorial design in which only a fraction of the variable combinations are tested, the number of trials is greatly reduced while still obtaining the most important information (Dziezak, 1990; Mitchell et al, 1986). Mullen and Ennis (1985) suggest that if there are a large number of variables initially, screening should be carried out in two stages, first to reduce the number of potential variables to five or less, and a second set to take a more detailed look at these key variables, their interactions and the responses.

The experimental design used most frequently in the optimization of baked products is the central composite design, in which the critical variables are tested at five levels. The design includes factorial points, center points at which all variables are held at their midlevels, and star points, which are the very high and very low levels of the variables (Mitchell et al 1986). Data is collected and analyzed by polynomial regression analysis. A full model is initially fit to the data and can be re-evaluated and changed until tests for adequacy and lack of fit indicate it is satisfactory (Joglekar and May, 1987). This model describes the relationship between the variables and response(s) which can be visualized by creating contour plots. These plots give an indication of the optimal levels of each variable needed to achieve the best response and provided information about how a response might be affected when factor levels are changed (Dziezak,

1990). Thus, RSM allows the identification of the variable combination, based on the predictive model, which optimize the response (Cornell, 1984).

A response surface study can be concluded by carrying out a verification experiment. Because identification of the best-setting values of the each variables is based on prediction, it is important to verify that these solutions work in practice. This involves additional experimental runs at selected variable combinations. In this way, the results obtained in the optimization experiment can be confirmed (Haaland, 1989).

RSM has a major advantages over the more traditional methods of experimentation in that it greatly reduces the time and expense required to optimize a product formulation. Despite its potential economic benefits, RSM may initially appear intimidating, and many researchers tend to stick with the experimental methods they are accustomed to (Dziedzak, 1990). Hopefully, the availability of user-friendly, interactive software packages will lead to an increase in the acceptance of RSM as a viable alternative to traditional experimental techniques.

Chapter 3

SCREENING EXPERIMENT #1: IDENTIFICATION OF CRITICAL INDEPENDENT VARIABLES

INTRODUCTION

The recent "phasing-out" of potassium bromate by the bread industry has led to a search for additives that separately or together will provide a comparable improvement in dough strength and bread quality. Many additives are available which improve dough handling properties and quality of bread. An optimum combination of oxidants, emulsifiers and enzymes and the use of stronger flours has been suggested (Zimmerman, 1991). Ascorbic acid, alone or in combination with azodicarbonamide (ADA) is commonly used for oxidation of bread flour. Diacetyl tartaric acid esters of monoglycerides (DATEM) and sodium stearoyl-2-lactylate (SSL) are the emulsifiers used most extensively in North America and the U.K. for their dough strengthening capabilities. Fungal α -amylase and protease are routinely added to bread to improve loaf volume and crumb structure and L-cysteine hydrochloride is added to reduce mixing time.

Identifying optimum combinations of these improvers is the ultimate goal of this research. However, optimization of seven additives in a bread formulation would be not only time consuming but also too complex to be feasible. For optimization, the number of variables must be limited to those most critical to the responses being examined (Joglekar and May, 1987). Giovanni (1983) suggested that the number of variables to optimize should be kept to two or three in order to facilitate interpretation. Reduction of variables can be done by initially employing an appropriate experimental design, such as a fractional factorial, to assess the relative effectiveness of the seven additives. Then the variables most important to bread quality improvement can be selected and the optimization process carried out. The specific objectives of this experiment were:

1. To determine the effect of seven improvers (ascorbic acid, ADA, SSL, DATEM, α -amylase, protease and cysteine) on the mix time, loaf volume and crumb characteristics of white pan bread made with commercial CWRS and CWES wheat flours.

2. **To select the independent variables which have the greatest effect on mix time requirement and loaf characteristics for use in subsequent optimization experiments.**
3. **To select appropriate levels of the independent variables chosen for the product optimization.**

MATERIALS AND METHODS

Flour

No. 1 Canadian Western Red Spring (CWRS) and No. 1 Canadian Western Extra Strong (CWES) wheat composite samples from the 1993 crop year were milled to straight grade flour on a small scale commercial mill at the Canadian International Grains Institute (CIGI), Winnipeg. Protein determinations were made using the Kjeldahl method as modified by Williams (1973). Moisture contents were calculated using the oven-dry method (AACC Method 44-15A, 1981). Farinograph procedure followed the method outlined by Preston et al (1993). Dough development time for the CWES flour is the hydration peak time rather than the actual peak development time. Falling numbers were obtained to provide an indication of the α -amylase activity of the flours. Analysis was carried out on a 5 gram flour sample using AACC Method 56-81B (1992). Starch damage values were obtained using the spectrophotometric method (AACC Method 76-31, 1992). The damaged starch is expressed as the percentage of the flour on an "as is" basis. The flour characteristics are summarized as follows:

	CWRS	CWES
Flour Protein (%)	13.9	12.0
Moisture (%)	13.4	12.0
Farinograph		
Water Absorption	63.7	59.7
Devel. Time (min)	6.50	2.25
Mixing Tolerance	25	25
Stability	16.00	18.00
Falling Number (sec)	282	290
Starch Damage (%)	8.7	8.1

Enough flour was obtained to carry out the entire study and was stored in sealed plastic containers at approximately -20°C for the duration of the study. Flour for each trial was brought to room temperature for a minimum of 24 hours prior to baking.

Ingredients and Additives

The ingredients for the standard bread formulation were obtained from various sources. The sugar (Rogers), whey powder, and shortening (Crisco) were obtained locally. Fresh yeast (Fleishmans) was purchased weekly from the Safeway Bakeshop. Malt syrup was supplied by the Grain Research Laboratory (Canadian Grain Commission) in Winnipeg. Salt (NaCl) and ammonium sulphate was purchased from Sigma Chemical Company (St. Louis, MO).

The seven dough additives were also obtained from various suppliers. The L-ascorbic acid and cysteine hydrochloride were from Sigma Chemical Company (St. Louis, MO). The azodicarbonamide (ADA) preparation used was Maturox[®] (Pennwalt Flour Service, Oakville, Ont.). The SSL (sodium stearoyl-2-lactylate) was supplied by J.R. Short Canadian Mills Ltd. (Toronto, Ont.). Both the DATEM (Diacetyl tartaric acid esters of monoglycerides: Panodan[®] 205-K) and the fungal α -amylase (Grindamyl[®] S250 - 500 SKB units/g) preparations were from Grinsted Canada Inc. (Rexdale, Ont.). A fungal protease preparation (Fungal Protease 60,000) was provided by Solvay Enzymes, Inc. (Elkhart, IN). As is commonly the case with commercially available protease preparations there was also some amylase activity (protease activity: 60,000 HU/g; amylase activity: 3,500 SKB units/g).

The sugar, salt, malt, ammonium sulphate, potassium bromate, ascorbic acid, protease and cysteine were all made up as solutions (Appendix I) for addition to flour. The remaining ingredients were added directly to the flour prior to mixing.

Breadmaking Procedure

A modification of the Canadian Short Process (CSP) was used for bread preparation. This breadmaking method was developed by workers at the Grain Research Laboratory of the Canadian Grain Commission in Winnipeg, Manitoba and was first published in 1982 (Preston et al, 1982b). This method most closely resembles the processing conditions (high speed mixing, short fermentation) and formulations used in most Canadian plant bakeries. The control bread formulation, based on 100 g flour (14% moisture basis), is given in Table 3.1. The CSP method and equipment specifications are included in Appendix II. Work was carried out at Agriculture and Agri-Food Canada Research Centre, Winnipeg. The method was followed with the following modifications:

1. 100 g flour loaves were prepared.
2. A standard water level of farinograph water absorption plus 3% was used for all doughs.

Table 3.1. Canadian Short Process bread formulation.

Flour (14% moisture basis)	100 g
Yeast	3 g
Salt	2.4 g
Sucrose	4.0 g
Ammonium sulphate	0.1 g
Potassium Bromate ^a	30 ppm
Ascorbic Acid ^b	37.5 ppm
Malt ^a	0.6 g
Shortening	3.0 g
Whey	4.0 g
Water	variable

^a Potassium bromate and malt used only in control loaves

^b Ascorbic acid used at this level only in control loaves

3. A mixer speed of 140 rpm was used.
4. Control loaves were baked daily using the standard formula including both potassium bromate and ascorbic acid in order to provide a loaf by which yeast activity could be monitored.
5. After the rest and intermediate proof, the dough piece was sheeted an extra time at the smallest gap of 3.2 mm in order to make the interior crumb structure more even.
6. All dough pieces were proofed to 95mm and proof times were recorded in order to gain some control over the variability caused by batch-to-batch variation in yeast activity.
7. All test loaves were prepared with additives specified by the experimental design. Malt syrup and potassium bromate were omitted from these loaves, and ascorbic acid added at specified levels.

After loaves were removed from the oven, they were allowed to cool for ½ hour, were weighed and volumes determined by rapeseed displacement. After cooling a further 15 minutes, loaves were stored in a large plastic box with a fitted lid and kept for evaluation. The day following baking, loaves were sliced in half across the center of the loaf using an electric knife. Half loaves were photographed and a photocopy was taken (on photo setting) of each loaf for evaluation and to provide a record of the crumb structure (see Figure 3.1).

Test baking with the CWES wheat flour was carried out in duplicate, on four different baking days. Test baking with the CWRS wheat flour was carried out in triplicate on six different days. Originally, duplicate loaves only were to be baked with CWRS wheat flour. However, it became apparent that there was a high proportion of loaves with large blisters or air bubbles and that the loaf volume readings were unrealistic. Therefore, a third replication was baked and used in the analysis of loaf volume.

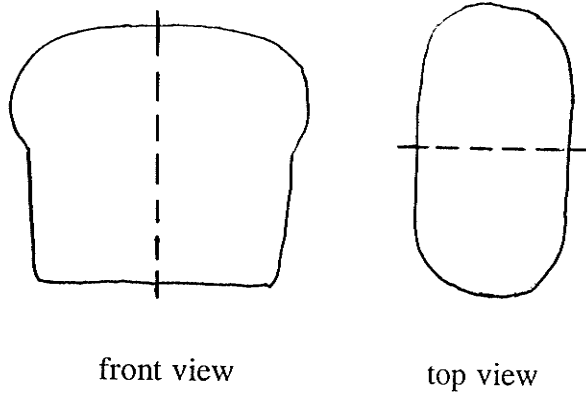
Evaluation

Loaf Volume

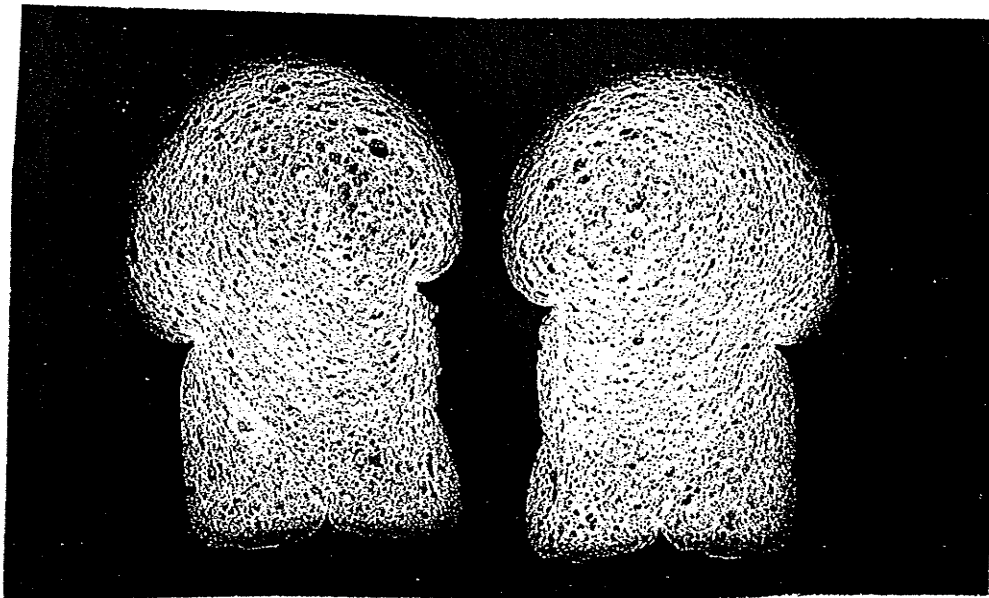
Loaf volume determinations were made using a rapeseed displacement volumeter (National Manufacturing Co., Lincoln, NB). All volumes were an average of 2 determinations. When large indentations were present in the bottom of the loaf, volumes were measured by placing the loaf in the volumeter upside-down. In many cases, large holes or bubbles were present in the top of the loaves. In order to account for these holes and get a more realistic loaf volume measurement, a puncture was made in the top of the loaf to allow the rapeseed to fill the hole.

Figure 3.1. Method of slicing and photocopying bread loaves in screening experiment #1.

Slicing loaves:



Photocopy half-loaves:



The loaf volume measurements used in the analysis were those derived from the third replication for the CWRS flour. The measurements analyzed for the CWES wheat flour were an average value of the two replications.

Crumb Characteristics

The crumb characteristics of the test loaves were evaluated by a small panel consisting of 4 members of the Department of Foods and Nutrition (including the experimenter) all of whom have had experience in the area of bread quality evaluation. Evaluations were made on the photocopied image of the bread. Two crumb characteristics were evaluated:

- 1) Predominant Cell Size (PredomCS) - to assess the openness of the bread crumb.
- 2) Cell Size Uniformity (CSUniform) - to assess the evenness or uniformity of the crumb.

Judgements were made on a 15cm line scale (ballots included in Appendix IIIa,b). A score of 0 indicated very irregular (CSUniform) or large cells (PredomCS), whereas a score of 15 indicated a uniform crumb (CSUniform) or a majority of small size cells (PredomCS). The end points of the scales were determined in preliminary baking trials. A visual reference for both characteristics was provided and is included in Appendix IV. All loaf images were evaluated in random order. Scores for each test loaf were calculated as an average of the 4 judgements. Standard deviations and coefficients of variation were also calculated for each treatment. Average scores for 3 replications for CWRS wheat flour and 2 replications for CWES wheat flour were used in the analysis.

Experimental Design

The software package DISCOVERY (Int'l Qual-Tech, Ltd.) was used for generation of screening experiment designs and all data analysis. To determine the relative effectiveness of the seven variables on mix time requirement, loaf volume and crumb characteristics, a two-level, fractional factorial design was generated by DISCOVERY. The experimental design was a 1/8 fraction of the full 2^7 (two-level, seven variables) factorial. The design is a Resolution IV design in which all main effects are clear of two factor interactions, but two factor interactions are confounded with one another. The 16 different combinations of improvers (runs) of the design plus 4 center points, and the confounding pattern, are shown in Table 3.2. The center point trial included all seven improver at a mid level and was repeated in order to provide information on the variability of the data and to indicate any non-linear effects. The two levels (high and low) of each of the seven variables used were coded as +1 and -1, with 0 indicating a midlevel. The

Table 3.2. Experimental design for screening experiment #1.

Runs	Variables ^{ab}						
	A	B	C	D	E	F	G
1	-1	-1	-1	-1	-1	-1	-1
2	+1	+1	-1	+1	-1	-1	-1
3	-1	+1	+1	-1	+1	-1	-1
4	+1	-1	+1	+1	+1	-1	-1
5	-1	+1	+1	+1	-1	+1	-1
6	+1	-1	+1	-1	-1	+1	-1
7	-1	-1	-1	+1	+1	+1	-1
8	+1	+1	-1	-1	+1	+1	-1
9	-1	-1	+1	+1	-1	-1	+1
10	+1	+1	+1	-1	-1	-1	+1
11	-1	+1	-1	+1	+1	-1	+1
12	+1	-1	-1	-1	+1	-1	+1
13	-1	+1	-1	-1	-1	+1	+1
14	+1	-1	-1	+1	-1	+1	+1
15	-1	-1	+1	-1	+1	+1	+1
16	+1	+1	+1	+1	+1	+1	+1
17	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0

Confounding Pattern: Main effects are not confounded with two factor interactions. Only one two-factor interaction from each line below can be estimated.

AE = BF = CD

AF = BE = DG

AG = BC = DF

AB = CG = EF

BD = CF = EG

AD = CE = FG

AC = BG = DE

^a Variables: A = ascorbic acid, B = ADA, C = SSL, D = DATEM, E = amylase, F = protease, G = cysteine.

^b Levels: +1 = high, -1 = low, 0 = midlevel

Table 3.3. Variables and their levels used in screening experiment #1.

Independent Variable	Low Levels	Mid Level	High Level
Coded Levels	-1	0	+1
A - Ascorbic Acid (ppm)	30	75	120
B - ADA (ppm)	2	23.5	45
C - SSL (%)	0.1	0.3	0.5
D - DATEM (%)	0.1	0.35	0.6
E - Amylase (SKB Units)	0	25	50
F - Protease (HU)	0	120	240
G - Cysteine (ppm)	0	25	50

actual high, low and mid levels used are shown in Table 3.3. The ranges of improver levels tested were chosen to reflect a wider range than what would normally be used commercially and are based on suggested levels of addition cited in the literature or by the improver manufacturer. The 20 experimental runs were baked in random order.

Statistical Analysis

The effect of each improver was calculated by subtracting the mean of the specific response (loaf volume, mix time and crumb characteristics) for all trials containing the improver at the low level from the mean response for all trials with the improver at the high level. Half-normal plots were produced to determine the statistical significance of the variable effects. However, knowledge about the variability of the data was important in determining the practical significance of the effects. Therefore, a set of criteria for each response variable was developed. A loaf volume change of 30 cc was established as the minimum to consider an effect on loaf volume to be important. For both cell size uniformity and predominant cell size scores, a change of at least 10% of the maximum score (15 points), ie. 1.5 points, was considered a requirement to identify the change as an important one. For a mix time effect to be seen as important, a 25% reduction was required. The criteria are summarized in Figure 3.2. Confounded interactions were examined using two-way tables which were constructed in order to clarify the interaction effect.

Figure 3.2. Criteria used for determining importance of improver effects in screening experiment #1.

Loaf Volume

An independent variable (additive) must cause an average loaf volume change of at least 30 cc in order for its effect to be considered important.

Predominant Cell Size and Cell Size Uniformity

An independent variable must cause a change in the average internal structure parameters (Cell Size and Cell Uniformity scores) of at least 10% of the maximum score (15 points) for its effect to be considered important, ie. 1.5 points.

Mix Time

Mix time must be reduced by at least 25% of the average mix time in order for the effect to be considered important.

RESULTS

CWRS and CWES wheat flours were tested separately to determine the effect of seven improvers on quality characteristics. The quality characteristics examined were loaf volume, mix time and crumb scores. Loaf volumes were influenced by several of the additives and responses were different for the two flours. Mix times were altered by only one additive, ie cysteine, which had a similar effect on both flours.

Crumb characteristic scores did not differentiate between treatments in breads prepared with CWRS flour, although differences were detected in the crumb scores for the CWES flour breads. Table 3.4 summarizes the data from the four center point baking trials in which all improvers were added at their mid levels. The coefficients of variation associated with the internal cell structure scores were fairly high, indicating a large degree of variability in the data. In some instances, the coefficient of variation between judges was over 100% (data not shown), suggesting that the method of evaluation and/or training was inadequate for discriminating differences among the test loaves. As a result, the criteria used to judge the improver effects in the CWRS wheat flour bread were limited to loaf volume and mix time.

The Effect of Improvers on the Quality of Breads made with CWRS Wheat Flour

The effect of the individual improvers and their interactions in CWRS wheat flour breads are summarized in Table 3.5. The differences represent the average change in the response (loaf volume, mix time, cell size and cell uniformity score) when the high level was used versus the low level. The interactions represent the influence of the level of one additive on the effectiveness of the other. The data on which this analysis was based is included in Appendix Va.

The additives most important to the improvement of volume of breads made with CWRS wheat flour were ascorbic acid and protease, while SSL had a detrimental effect on this response. Increasing ascorbic acid and protease levels from low to high resulted in increases in the mean loaf volumes of 58.13 cc and 34.38 cc, respectively. SSL had a large negative effect on loaf volume with mean volume dropping by 30.63 cc at the high level of addition. These additives were influenced by the less important improvers. Although the DATEM, α -amylase and cysteine had only small individual effects on loaf volume (<30 cc), they were involved in important interactions with the ascorbic acid, protease and SSL.

The confounded interactions AD=CE=FG which were important to loaf volume are clarified in the two-way tables included in Figure 3.3. These tables give the mean loaf volume

Table 3.4. Mean values, standard deviations and coefficients of variation for bread quality characteristics of the four loaves prepared using the seven improvers at their mid-levels (centre points) in both CWRS and CWES wheat flour breads in screening experiment #1.

	EXPERIMENTAL RUNS IN DESIGN				Mean	Standard Deviation	Coefficient of Variation (%)
	17	18	19	20			
CWRS Wheat Flour							
Loaf Volume ^a (cc)	1180	1165	1165	1175	1171	7.50	0.64
Mix Time ^b (min)	7.5	6.6	7.3	7.3	7.2	0.39	5.42
Cell Uniformity Score ^b	4.9	5.7	5.6	4.1	5.1	0.74	14.51
Cell Size Score ^b	4.2	3.8	3.9	3.0	3.7	0.51	13.78
CWES Wheat Flour^c							
Loaf Volume (cc)	1040	1040	1023	1035	1035	8.02	0.78
Mix Time (min)	17.2	18.0	19.5	20.4	18.8	1.44	7.66
Cell Uniformity Score	6.5	7.8	8.6	9.2	8.0	1.17	14.63
Cell Size Score	4.0	5.6	6.5	7.5	5.9	1.49	25.25

^a Loaf volume values for CWRS wheat flour breads are based on 3rd replication only due to blistering in replications 1 and 2.

^b Mix time and loaf quality scores for CWRS wheat flour breads are averages of 3 replications.

^c All scores for CWES wheat flour breads are averages of 2 replications.

Table 3.5. Differences in mean CWRs bread quality characteristic values when improvers were used at high versus low levels.

Variables	Volume ^a (cc)	Cell Size Score ^b	Cell Uniformity Score ^b	Mix Time ^c (min)
OVERALL MEAN	1089.68	5.26	5.68	7.80
<u>Improver</u>				
AA [A]	58.13 ^d	0.29	0.21	-0.55
ADA [B]	-5.63	-0.28	0.09	0.25
SSL [C]	-30.63 [*]	0.06	0.12	0.05
DATEM [D]	4.38	-1.11	-0.89	0.08
Amylase [E]	20.63	-1.07	-1.09	-0.08
Protease [F]	34.38 [*]	0.44	0.53	-0.23
Cysteine [G]	0.63	-0.20	-0.10	-3.60 [*]
<u>Interactions</u>				
AE=BF=CD	-18.13	0.95	1.33	-0.33
AF=BE=DG	3.13	-1.13	0.66	0.23
AG=BC=DF	11.88	-0.78	-0.01	0.35
AB=CG=EF	28.13	-0.22	0.12	-0.20
BD=CF=EF	14.38	0.64	-0.09	0.03
AD=CE=FG	-51.88 [*]	0.62	1.07	0.03
AC=BG=DE	3.13	-0.04	-1.10	0.20

^a Volume determinations based on 3rd replication only due to blistering in replications 1 and 2.

^b Mean scores for 4 judges over 3 replications.

^c Mean of 3 replications.

^d Effect marked with an * meet the criteria outlined in Figure 3.2.

Figure 3.3 Two-way tables illustrating the confounded loaf volume interactions in breads made with CWRS wheat flour.

		AA (ppm)			
		30 (-)	120 (+)		
Ascorbic Acid X DATEM		1089	1095	0.6 (+)	DATEM (%)
		1033	1143	0.1 (-)	

		SSL (%)			
		0.1 (-)	0.5 (+)		
SSL X Amylase		1141	1059	50 (+)	Amylase (SKB)
		1069	1090	0 (-)	

		Protease (HU)			
		0 (-)	240 (+)		
Protease X Cysteine		1099	1081	50 (+)	Cysteine (ppm)
		1046	1133	0 2(-)	

for all trials in which high and low levels of one additive were used in combination with both high and low levels of another variable. These tables indicate that the effect of the ascorbic acid depended on the level of DATEM used. With the high level of ascorbic acid and low DATEM, the mean volume increase was 110 cc, but was only 6 cc when DATEM was at a high level. Protease had an important positive effect on the average loaf volume (87 cc increase) when cysteine was omitted but a slightly negative effect at the high level of cysteine. SSL was not important when α -amylase was omitted but SSL decreased average loaf volume by 82 cc when the high level of α -amylase was present.

Cysteine was the one additive which affected mix time. Through the addition of 50 ppm cysteine, mix times of CWRS flour doughs were reduced by 37% (Table 3.6).

The Effect of Improvers on the Quality of Breads made with CWES Wheat Flour

The effect of the individual improvers and their interactions in CWES wheat flour breads are summarized in Table 3.7. The data on which this analysis was based is included in Appendix Vb.

Cysteine and α -amylase were most important to the improvement of loaf volume of breads made with CWES wheat flour. Average loaf volume was 43.88 cc greater at the high cysteine level than when cysteine was omitted. The high level of α -amylase caused an average loaf volume increase of 70.88 cc. However, at a high level, α -amylase reduced the beneficial effect of ascorbic acid.

The two-way tables in Figure 3.4 illustrate the confounded interaction effects. Ascorbic acid did not have an important main effect but it caused the average loaf volume to increase by 49 cc when α -amylase was not included. Ascorbic acid had a slightly negative effect on volume when α -amylase was included in the formulation. Two other interactions also had some effect on the CWES loaf volumes. ADA had a negative effect on loaf volume when protease was included, causing the average loaf volume to drop by 46 cc when its level of addition increased from low to high. SSL was beneficial to loaf volume only when DATEM was kept at the low level.

Whereas α -amylase improved the loaf volume of CWES wheat flour breads, it had a detrimental effect on the crumb structure of these loaves. When added at the high level, this additive caused a drop in scores of 1.54 and 3.32 for Predominant Cell Size and Cell Size Uniformity, respectively, indicating that the crumb became more open and coarse when α -amylase was included in the formulation.

As with CWRS wheat flour bread, cysteine was very effective at reducing the mixing

Table 3.6. The effect of cysteine on the mix time of doughs prepared with CWRS and CWES wheat flour in screening experiment #1.

Cysteine (ppm)	CWRS Wheat Flour		CWES Wheat Flour	
	Mix Time (min)	Mix Time Reduction ^a	Mix Time (min)	Mix Time Reduction
0	9.5 ^b		23.3 ^c	
25	7.2 ^d	24%	18.8 ^d	19%
50	6.0 ^d	37%	13.4 ^d	43%

^a Mix time reduction calculated as the percent reduction from mix times obtained with 0 ppm cysteine.

^b Value is the average of 8 experimental runs plus 5 control loaves prepared over the 3 replications.

^c Value is the average of 8 experimental runs plus 5 control loaves prepared over the 2 replications.

^d Values are averages over 4 and 8 experimental runs for 25 ppm and 50 ppm, respectively.

Table 3.7. Differences in mean CWES bread quality characteristic values when improvers were used at high versus low levels.

Variables	Volume ^a (cc)	Cell Size Score ^b	Cell Uniformity Score ^b	Mix Time ^a (min)
OVERALL MEAN	1012.69	6.28	6.35	18.26
Improver				
AA [A]	11.63	0.23	0.30	0.44
ADA [B]	-8.88	-0.49	0.61	-0.14
SSL [C]	7.38	0.20	0.25	1.24
DATEM [D]	18.13	-0.56	-0.94	-0.49
Amylase [E]	70.88 ^c	-1.54 [*]	-3.32 [*]	0.51
Protease [F]	12.88	-0.59	0.51	-0.46
Cysteine [G]	43.88 [*]	-0.30	-0.28	-9.54 [*]
Interaction				
AE=BF=CD	-36.88 [*]	0.97	0.56	0.24
AF=BE=DG	-0.38	0.11	-0.15	-0.04
AG=BC=DF	-27.88	-0.67	0.13	-0.71
AB=CG=EF	-8.63	0.51	-0.06	0.94
BD=CF=EG	-8.13	-0.21	-0.19	2.11
AD=CE=FG	-0.63	-0.28	-0.29	1.29
AC=BG=DE	0.13	0.91	0.20	0.86

^a Mean of 2 replications.

^b Mean scores for 4 judges over 2 replications.

^c Effect marked with an * meet the criteria outlined in Figure 3.2.

Figure 3.4. Two-way tables illustrating the confounded loaf volume interactions in breads made with CWES wheat flour.

		Ascorbic Acid (ppm)		
		30 (-)	120 (+)	
Ascorbic Acid X Amylase		1061	1036	50 (+)
		953	1002	0 (-)
				Amylase (SKB)

		ADA (ppm)		
		2 (-)	45 (+)	
ADA X Protease		1042	996	240 (+)
		992	1020	0 (-)
				Protease (HU)

		SSL (%)		
		0.1 (-)	0.5 (+)	
SSL X DATEM		1037	1007	0.6 (+)
		982	1026	0.1 (-)
				DATEM (%)

requirement of doughs made with CWES flour. A 43% reduction in mix time was observed as a result of the inclusion of 50 ppm (Table 3.6).

DISCUSSION

The results of this initial screening experiment indicate that the two flours, CWRS and CWES wheat flour, differed in their requirements for the improvers tested. Ascorbic acid and protease were most important for improving the volume of CWRS breads whereas the α -amylase and cysteine were the factors most critical to CWES loaf volumes. The benefits of using these improving agents individually has been well documented. However, the use of improver combinations, their interacting effects, and the influence of wheat type on improver requirement has not been thoroughly examined.

The volume of the CWRS wheat flour bread was enhanced with the addition of ascorbic acid levels up to 120 ppm. The CWES wheat flour bread did not give such a positive response. Perhaps the CWES flour had a lower oxidative requirement due to both the lower protein content and longer mixing requirement. Using two flours milled from the cultivar Pawnee with protein contents of 8% and 16%, Finney et al (1987) found that the lower protein flour had less requirement for oxidation. It required 75% less potassium bromate than the higher protein content flour. Finney et al (1987) also compared two flours with similar protein contents and different mixing requirements. The flour which required longer mixing time had a much lower requirement for potassium bromate. The results from the present study agree with those of Finney et al (1987), that longer mixing, lower protein CWES wheat flour required less oxidation than the CWRS wheat flour.

Surprisingly, ADA did not have any large effect on the loaf volumes of breads made with either of the two flours tested. Ranum (1992b) stated that fast acting oxidants may not work as well with cysteine as a slower acting oxidant such as potassium bromate or ascorbic acid. ADA is a very fast acting oxidizing agent which exerts its effect at the dough mixing stage. Cysteine has an opposing effect which also occurs in the mixer. Cysteine is a strong reducing agent which breaks disulphide bonds in the gluten network, thereby weakening its structure. As a result, the cysteine may be negating any positive effect that ADA might have exerted on loaf volume had cysteine not been included in the formulation.

The two flours reacted differently to the addition of α -amylase. The CWES wheat flour breads increased in volume substantially when amylase was included in the formulation, whereas the increase in CWRS bread volume was much less pronounced. The contribution of the natural α -amylase activity of the flours to this phenomenon did not provide an explanation. Both flours

had similar falling number values. Both flours also had similar starch damage levels which eliminates this factor as a possible explanation for the different responses of CWRS and CWES flour to α -amylase supplementation. Clearly, this result needs further investigation.

It was surprising to find that the SSL lowered the volumes of the CWRS breads, as numerous authors (Garti et al, 1980; Kilborn et al, 1990; Thompson and Buddemeyer, 1954) have cited evidence supporting its dough strengthening ability and subsequent benefits to loaf volume. This negative effect of SSL was related to the level of α -amylase used in the study. The SSL and α -amylase were shown to interact. When amylase was added at the high level, increasing SSL addition was detrimental to loaf volume. This interaction can also be interpreted from the perspective that the amylase improves loaf volumes only when SSL addition is kept at the low level. Asp et al (1988) had considered the possibility that since surfactants interact with and stabilize proteins, the activity of enzymes in dough may be reduced. However, the findings of these authors showed that SSL did not affect the activity of α -amylase during the breadmaking process. An alternative explanation for the SSL by α -amylase interaction involves the starch fraction of the wheat flour. SSL also acts as a crumb softening agent, complexing with both the amylose and the amylopectin molecules in the starch granule and slowing the retrogradation process (Kamel, 1993). As a result, there may be a reduced availability of starch for the α -amylase to react with, resulting in a minimal loaf volume response to α -amylase.

DATEM consistently had a greater positive influence on the volume of breads made with both flours than the SSL. In the CWRS wheat flour breads, the DATEM effect was insignificant, while the SSL effect was highly negative. When both were included at either high or low levels in the CWES breads, loaf volumes were poor. It is possible that including both surfactants at the high levels causes the doughs to become less extensible, resulting in a reduction in oven-spring and a smaller volume. Ideally, either one or the other should be used if the high level is chosen, or a combination of the two at lower levels of addition.

Dubois (1980) stated that proteases act to increase the extensibility of doughs, making them less "bucky" and tight. Reductions in mixing requirement of up to 30% have been realized through the addition of protease (Dekker, 1994), which could be highly beneficial when working with very strong, long mixing flours. Because of these possible effects on the CWES wheat flour doughs, protease was included in the experimental design. The results show that although the protease did improve the volume of breads made with CWRS flour, this effect was not seen in the CWES breads. The doughs became tacky and difficult to manage at the high protease levels. Reduced mix times were not realized. As time is one of the major factors which governs the

extent of the protease effect, most of its action likely took place during the fermentation period. In order to realize similar effects on the loaf volume of the CWES breads as was seen in the CWRS breads, it may be necessary to use protease at a higher level of addition than was used in this experiment.

Cysteine was included in the experiment for its ability to reduce mixing requirements. It was effective at lowering the mixing times of doughs made with both flours at a level of 50 ppm, the CWRS flour formed an overly sticky, slack dough. CWES doughs prepared with 50 ppm cysteine had improved handling properties, similar to those of the CWRS doughs made without cysteine. This level of cysteine addition also greatly enhanced the volumes of the breads made with CWES wheat flour but not of the CWRS breads. Finney et al (1971) obtained similar results working with the long mixing flour Red River 68. They found that adding 40 to 120 ppm cysteine significantly increased the volumes of these breads, whereas the breads made with the standard bread flour had somewhat reduced volumes. Higher levels of cysteine than were used in the study should be tested for use with the extra strong flour.

CONCLUSIONS

As an initial step in the optimization process, the importance of seven additives to the quality characteristics of breads made with CWRS and CWES wheat flour was determined. CWRS bread volumes were enhanced through the addition of ascorbic acid and protease whereas α -amylase and cysteine increased volumes of the CWES breads. The higher level of SSL reduced loaf volumes overall, and this additive was involved in some highly negative interaction effects. ADA did not enhance the volumes of breads made with either flour. ADA is a fast acting oxidant and might act to reverse the desirable mix time reducing effects of cysteine (Bekes, 1994). It was concluded that SSL and ADA should be omitted from the next screening experiment.

Ascorbic acid, protease, α -amylase, DATEM and cysteine were selected for inclusion in the second experiment. Because these additives had positive effects on loaf volumes, it was concluded that all should be evaluated at higher levels in the second screening experiment except DATEM. The high level of DATEM used was 6%, the maximum permitted in Canada.

Poor reproducibility of crumb characteristic scores between judges required that a different method of evaluating this parameter be developed. The external loaf appearance should also be evaluated as problems with the loaf shape, such as a concave bottom and lopsided appearance became evident throughout this experiment.

The occurrence of blisters or large air bubbles in the loaves was a problem in this initial screening experiment. A possible cause for this phenomenon was thought to be the level of water used in the Canadian Short Process bread formulation (3% above farinograph water absorption). Before beginning the second screening experiment, a brief study should be carried out to determine whether lower water absorption would eliminate blisters.

On the basis of this preliminary screening experiment a second screening experiment can be designed. A five variable fractional factorial design in which two factor interactions are not confounded should be selected. The five additives can be further studied in an adjusted formulation with decreased water absorption using an improved method for crumb structure evaluation.

Chapter 4

SCREENING EXPERIMENT #2 IDENTIFICATION OF CRITICAL INDEPENDENT VARIABLES

INTRODUCTION

The second screening experiment was carried out in order to reduce the number of potential variables to the three most critical to breads prepared using the CWRS and CWES wheat flours. For this study, the experimental strategy followed in the first screening experiment was altered to provide more thorough information on the effects of the improvers. The methods of evaluating crumb structure was reassessed and a new method developed and the number of independent variables was reduced from seven to five. The emulsifier SSL was eliminated because of its negative effect on the loaf volume, a result primarily of volume depressing interactions with other additives such as DATEM. DATEM was, however, retained in the design to examine its effects in the absence of SSL. The oxidizing agent ADA was also removed from the experimental plan because its consistently insignificant effect on bread quality parameters. Ascorbic acid, α -amylase, protease and cysteine had positive effects on loaf volume of either one of both of the flours tested, without negative effects on other quality characteristics. The level of these additives was increased to be ensure that the optimum level was included. The specific objectives of this experiment were:

1. To determine the effect of five improvers (ascorbic acid, DATEM, α -amylase, protease and cysteine) on mix time, loaf volume and internal and external characteristics of bread made with typical CWRS and CWES wheat flours.
2. To select the improvers which have the greatest effect on mix time and loaf quality for use in the subsequent optimization experiment.
3. To select appropriate levels of the improvers to use for the product optimization experiment.

MATERIALS AND METHODS

Materials

All material used in this screening experiment were the same as used previously in screening experiment #1. Both flours were reanalyzed for moisture content and farinograph water absorption. Results showed a slight change in these flour properties during frozen storage. The new values were as follows:

	CWRS	CWES
Moisture (%)	13.8	12.3
Farinograph Water Absorption (%)	63.6	61.4

Breadmaking Procedure

The breadmaking procedure was the same as in screening experiment #1 with these changes:

1. A standard water level of 1% above farinograph water absorption (FAB) was used for all loaves.

During the first screening experiment, a large proportion of the test loaves (including blanks and controls) had large blisters or gas bubbles (Plate 4.1). This was attributed to the stickiness of the dough. Therefore, a short experiment (Appendix VI) was carried out to determine whether reducing dough water levels would help overcome this problem. The results indicated that a water absorption level of FAB + 1% produced loaves which were less likely to have blisters. Mixing time requirement was also reduced for CWRS and CWES wheat flour doughs by 16% and 12%, respectively.

2. Doughs were sheeted three times according to the method rather than 4 times which was done previously as it was thought that this may have contributed to the "blister" problem by causing the doughs to stick and tear in the sheeter.

3. A control loaf was prepared each day using the flour being tested that day. This loaf was proofed to 95 mm and the proof time was recorded. All subsequent loaves were then proofed to the standard time derived from the control loaf. For each flour, the whole screening experiment was carried out in one day in order to reduce any day-to-day variation arising from different solution batches and room conditions.

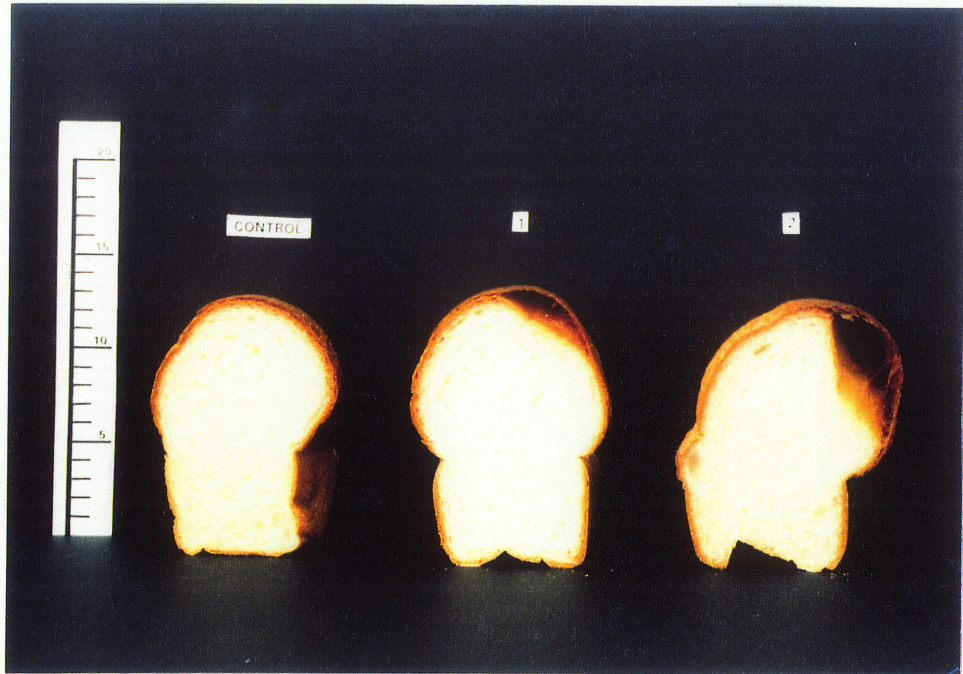


Plate 4.1. Photograph depicting the problem of blisters on the top of loaves (CWRS wheat flour: screening experiment #1).

Evaluation

Loaf Volume

Loaf volume determinations were made as per the method described in screening experiment #1.

Bread Quality Characteristics

The day following baking, whole loaves were photographed. The loaves were then sliced in half lengthwise. These loaves were photographed again and a photocopy was taken (photo setting) for evaluation (Figure 4.1). Loaves were evaluated by the baker for both external and internal characteristics according to the bread quality score card (Appendix VII) on a scale of 1 - 10, where the higher score indicates a higher quality loaf. Reference loaf images (Appendix VIIIa and VIIIb) were used for evaluation of the internal loaf characteristics.

External characteristics of the test loaves were assessed the day following baking at the time at which photographs were taken of the whole loaves. The characteristics that were assessed and scored out of 10 possible points were:

1. loaf symmetry - unsymmetrical or "lop-sided" loaves were given lower scores.
2. loaf bottom - loaves with large indentations in the bottom crust had reduced scores.
3. break and shred - loaves with greater break (measured in inches) were given higher scores.

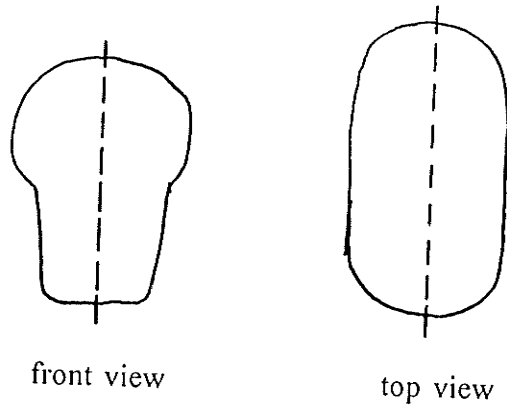
A composite score was derived by totalling the scores of the three characteristics to give a single score for the loaves' external characteristics, the maximum score attainable being 30 points.

Internal characteristics of the loaves were assessed by evaluating the photocopied images of the bread. This method was found to be highly acceptable in the first screening test as the photocopied images provide an excellent, clear record of the bread crumb. The characteristics considered and scored out of 10 points were:

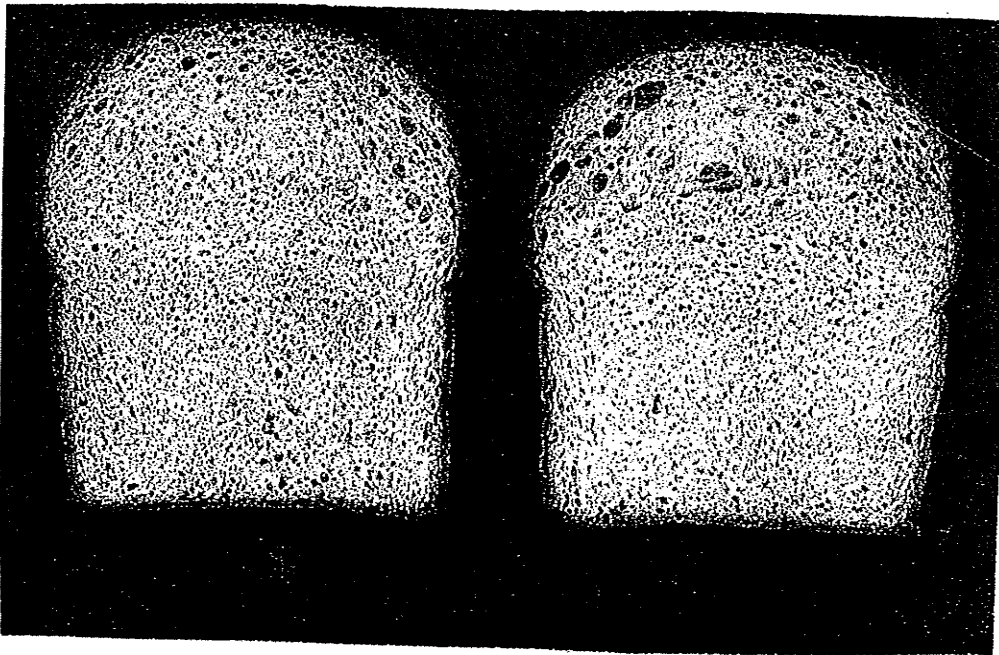
1. Cell size - breads with ideal, medium size cells were given a high score, whereas those with unusually close or large cell sizes were given lower scores.
2. Cell uniformity - high scores were given to those breads with uniform even cell size distribution, whereas those which had highly irregular cell sizes were given lower scores.
3. Blisters - the presence of large blisters or air bubbles in the tops of the loaves resulted

Figure 4.1. Method of slicing and photocopying bread loaves in screening experiment #2.

Slicing loaves:



Photocopy half-loaves:



in a loss of points, ie. large blister: score of 0.

A composite score for the internal characteristics was obtained by totalling the scores of the three characteristics to give a single score, with a maximum possible of 30 points.

Experimental Design

As in screening experiment #1, the software package DISCOVERY (Int'l Qual-Tech, Ltd.) generated the experimental design and was used for all data analysis. To determine the relative effectiveness of the seven variables on mix time requirement, loaf volume and internal and external loaf characteristics, a two-level, fractional factorial design was used. The experimental design was a $1/2$ fraction of the full 2^5 (two-level, five variable) factorial. The design was a Resolution V design in which all main effects and two factor interactions are clear (no confounding pattern). The experimental design, including 4 center points, is shown in Table 4.1. The two levels (high and low) of each of the five variables tested are coded as +1 and -1, with 0 indicating a midlevel. The actual high, low and mid-levels used are shown in Table 4.2. All baking runs were carried out in random order.

Statistical Analysis

The variables which had the greatest effect on the responses were determined by subtracting the mean of the specific response (loaf volume, mix time and internal and external loaf characteristics) for all trials containing the improver at the low level from the mean response for all trials with the improver at the high level. Two-way tables were constructed in order to clarify interactions between variables. To determine the importance of the variable effects, a set of criteria similar to that used in screening experiment #1 were used. A minimum change of 30 cc in loaf volume and a 25% reduction in mix time was required for the effect of the additives on these responses to be important. Both external and internal loaf characteristic scores required a change of at least 10% to be considered important. These criteria are outlined in Figure 4.2. On the basis of these effects, key variables were identified, less important variables eliminated and appropriate levels of addition were identified.

Table 4.1. Experimental design for screening experiment #2

Runs	Variables ^{ab}				
	A	B	C	D	E
1	-1	-1	-1	-1	+1
2	+1	-1	-1	-1	-1
3	-1	+1	-1	-1	-1
4	+1	+1	-1	-1	+1
5	-1	-1	+1	-1	-1
6	+1	-1	+1	-1	+1
7	-1	+1	+1	-1	+1
8	+1	+1	+1	-1	-1
9	-1	-1	-1	+1	-1
10	+1	-1	-1	+1	+1
11	-1	+1	-1	+1	+1
12	+1	+1	-1	+1	-1
13	-1	-1	+1	+1	+1
14	+1	-1	+1	+1	-1
15	-1	+1	+1	+1	-1
16	+1	+1	+1	+1	+1
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0

Confounding Pattern: No confounding

^a Variables: A = ascorbic acid, B = DATEM, C = amylase,
D = protease, E = cysteine.

^b Levels: +1 = high, -1 = low, 0 = midlevel

Table 4.2. Variables and their levels used in screening experiment #2.

Independent Variables	Low Level	Mid Level	High Level
Coded Levels	-1	0	+1
A - Ascorbic Acid (ppm)	60	105	150
B - DATEM (%)	0.1	0.35	0.6
C - Amylase (SKB Units)	25	50	75
D - Protease (HU)	75	187.5	300
E - Cysteine (ppm)	25	50	75

Figure 4.2 Criteria used for determining importance of improver effects in screening experiment #2.

Loaf Volume

An independent variable (additive) must cause an average loaf volume change of at least 30 cc in order for its effect to be considered important.

External and Internal Loaf Characteristics

An independent variable must cause an average change in the external and internal loaf characteristic score of at least 10% of the maximum score (30 points) for its effect to be considered important, ie. 3.0 points.

Mix Time

Mix time must be reduced by at least 25% of the average mix time in order for the effect to be considered important.

RESULTS

The effects of the five improvers on the loaf volumes, mixing times and loaf quality characteristics of breads made with CWRS and CWES wheat flour were determined separately. Differences between the two flours requirement for improvers were evident. DATEM was beneficial to the loaf volume of all breads. However, this dough strengthener interacted with many other additives, impairing their beneficial functions. As in screening experiment #1, protease remained important to CWRS loaf volumes and cysteine increased the volumes of CWES breads substantially.

The method of evaluating the internal and external structure of the test loaves eliminated one source of variation (judges) with all evaluation being done by the researcher. Table 4.3 summarizes the data from the four center point baking runs in which the five improvers were added at their mid levels. Compared to similar results presented in screening experiment #1, the coefficients of variation (C.V.) associated with the external and internal loaf characteristic scores were lowered for the CWES wheat flour breads. However, the C.V. associated with these parameters for the CWRS breads were higher. As they were based on only 4 baking runs, the C.V. were considered acceptable.

The Effect of Improvers on the Quality of Breads made with CWRS Wheat Flour

The effect of the individual improvers and their interactions in CWRS wheat flour breads are summarized in Table 4.4. The differences represent the average change in the response when the high level is used versus the low level. The interactions represent the influence of the level of one variable on the effectiveness of the other. The data on which this analysis was based is included in Appendix IXa.

The improvers which had the greatest effect on the loaf volumes of CWRS wheat flour breads were DATEM and protease. Increasing DATEM and protease levels from low to high resulted in increased average loaf volumes of 47.5 cc and 30.0 cc, respectively. Based on the criteria for importance of effects outlined in Figure 4.3, none of the interactions were considered important.

Analysis of the effects of the additives on the external loaf characteristics indicated that only ascorbic acid affected this response as a main effect, causing the score to drop by 3 points when the high level of 150 ppm was used. Three interactions were important to the external appearance of the loaves. All had negative effects and involved DATEM. Figure 4.3 illustrates

Table 4.3. Mean values, standard deviations and coefficients of variation for bread quality characteristics of the four loaves prepared using the five improvers at their mid-levels (centre points) in both CWRS and CWES wheat flour breads in screening experiment #2.

	EXPERIMENTAL RUNS (Centre Points)				Mean	Standard Deviation	Coefficient of Variation (%)
	17	18	19	20			
CWRS Wheat Flour							
Loaf Volume (cc)	1250	1155	1210	1165	1195	43.8	3.67
Mix Time (min)	4.5	4.4	3.5	4.4	4.2	0.47	11.19
External Loaf Characteristics	19	17	23	26	21.3	4.03	18.92
Internal Loaf Characteristics	12	23	18	24	19.3	5.50	28.50
CWES Wheat Flour							
Loaf Volume (cc)	1125	1140	1270	1250	1196	74.3	6.21
Mix Time (min)	8.8	8.4	10.2	9.0	9.1	0.8	8.79
External Loaf Characteristics	29	24	24	25	25.5	2.4	9.41
Internal Loaf Characteristics	24	24	29	24	25.3	2.5	9.88

Table 4.4. Differences in mean CWRB bread quality characteristic values when improvers were used at high versus low levels.

Variables	Volume (cc)	External Characteristics ^a	Internal Characteristics ^b	Mix Time (min)
OVERALL MEAN	1167.50	19.50	16.56	4.81
<u>Improver</u>				
AA [A]	1.25	-3.00*	-0.13	-0.20
DATEM [B]	47.50 ^c	-0.50	-5.88*	0.43
Amylase [C]	16.25	-2.25	-2.88	0.45
Protease [D]	30.00*	-1.00	-0.88	0.23
Cysteine [E]	-20.00	1.25	-4.13*	-1.33*
<u>Interactions</u>				
AB	-8.75	-4.50*	-4.13*	0.05
AC	0.00	-2.75	-3.13*	-0.08
AD	3.75	-2.00	-0.63	0.30
AE	1.25	1.25	1.63	0.45
BC	3.75	-3.25*	-3.88*	-0.06
BD	-27.5	-0.50	4.63*	-0.38
BE	-20.0	-3.25*	-4.63*	-0.43
CD	-16.25	0.25	0.13	0.50
CE	3.75	-2.50	-3.13*	0.05
DE	-10.0	-1.75	-4.13*	-0.48

^a External characteristics maximum score 30.

^b Internal characteristics maximum score 30.

^c Effects marked with an * meet the criteria outline in Figure 4.2.

Figure 4.3. Two-way tables illustrating the external loaf characteristic interactions between DATEM and three other additives in breads made with CWRS wheat flour.

		DATEM	
		0.1 (-)	0.6 (+)
Ascorbic Acid (ppm)	60 (-)	19.0	23.0
	150 (+)	20.5	15.5
Alpha-amylase (SKB)	25 (-)	19.3	22.0
	75 (+)	20.3	16.5
Cysteine (ppm)	25 (-)	18.8	21.5
	75 (+)	20.8	17.0

these interactions. Generally, DATEM improved the external shape of the loaves. However, α -amylase and cysteine were added at their high levels, increasing DATEM resulted in reduced scores for this response. Alternately, α -amylase and cysteine were not considered important to the external loaf shape given that the DATEM was incorporated at the low level. Increasing DATEM caused the α -amylase and cysteine to have detrimental effects.

DATEM was also deleterious to the internal loaf characteristics of CWRS wheat flour breads, as was the high level of cysteine. Internal scores were reduced by 5.88 and 4.13 points for DATEM and cysteine, respectively. DATEM also interacted strongly with the remaining four additives as is evident from the two-way tables included in Figure 4.4. DATEM caused internal loaf scores to drop consistently regardless of the levels of other the other additives. Conversely, cysteine, ascorbic acid and α -amylase were advantageous to the internal score only when DATEM addition remained low. When the DATEM level increased, the beneficial effects of these three improvers were no longer evident and scores were lowered considerably. Only the protease performed favourably when DATEM addition was high, yet was detrimental to the crumb structure is DATEM was added at the minimum level.

Three other interactions had important effects on the internal loaf characteristics and are illustrated in Figure 4.5. At the high cysteine level, increases in both protease and α -amylase resulted in poorer crumb structures. Cysteine itself did not affect this response given that the enzyme preparations were kept at a minimum level. However, when protease and α -amylase addition was high, increasing cysteine resulted in poor crumb structure. High α -amylase addition also caused ascorbic acid to lower the internal structure scores.

As in screening experiment #1, cysteine was the only variable which substantially reduced the mix time requirement of doughs made with CWRS wheat flour. Compared to a mix time of 8 minutes for the control formula, the average mix times of doughs prepared with 75 ppm cysteine was 4.2 minutes. This translates to a mix time reduction of 53% (Table 4.5).

The Effect of Improvers on the Quality of Breads made with CWES Wheat Flour

The effects of the five improvers and the interactions between them in breads made with CWES wheat flour are summarized in Table 4.6. The data on which this analysis was based is included in Appendix IXb.

As with the CWRS wheat flour, DATEM played an important role in the improvement of loaf volume of breads made with CWES wheat flour. An average increase of 55.63 cc was observed when its addition increased from the low to the high level. Cysteine also was important

Figure 4.4. Two-way tables illustrating the internal loaf characteristic interactions between DATEM and four other additives in breads made with CWRS wheat flour.

		DATEM	
		0.1 (-)	0.6 (+)
Protease (HU)	75 (-)	22.3	11.8
	300 (+)	16.8	15.5
Cysteine (ppm)	25 (-)	19.3	18.0
	75 (+)	19.8	9.3
Ascorbic acid (ppm)	60 (-)	17.5	15.8
	150 (+)	21.5	11.5
Alpha-amylase (SKB)	25 (-)	19.0	17.0
	75 (+)	20.0	10.3

Figure 4.5. Two-way tables illustrating the internal loaf characteristic interactions in breads prepared with CWRS wheat flour.

		Protease (HU)			
		75 (-)	300 (+)		
Protease X Cysteine		17.0	12.0	75 (+)	Cysteine (ppm)
		17.0	20.3	25 (-)	

		Ascorbic Acid (ppm)			
		60 (-)	150 (+)		
Ascorbic Acid X Amylase		16.8	13.5	75 (+)	Amylase (SKB)
		16.5	19.5	25 (-)	

		Amylase (SKB)			
		25 (-)	75 (+)		
Amylase X Cysteine		17.5	11.5	75 (+)	Cysteine (ppm)
		18.5	18.8	25 (-)	

Table 4.5. The effect of cysteine on the mix time requirement of doughs prepared with CWRS and CWES wheat flour^a in screening experiment #2.

Cysteine (ppm)	CWRS Wheat Flour		CWES Wheat Flour	
	Mix Time (min)	Mix Time Reduction ^b	Mix Time (min)	Mix Time Reduction
0 ^c	8.0		21.2	
25 ^d	5.6	30%	12.2	43%
50 ^d	4.2	53%	9.1	57%
75 ^d	4.2	53%	7.1	67%

^a All data obtained from screening experiment #2 in which a water absorption of 1% above farinograph water absorption was used.

^b Mix time reduction calculated as the percent reduction from mix times obtained with 0 ppm cysteine.

^c Mix times of doughs prepared with 0 ppm cysteine are the values of the control loaves prepared using CWRS and CWES wheat flour in screening experiment #1.

^d Values are averages over 8, 4 and 8 baking runs for 25 ppm, 50ppm and 75 ppm, respectively

Table 4.6. Differences in mean CWES bread quality characteristic values when improvers were used at high versus low levels.

Variables	Volume (cc)	External Characteristics ^a	Internal Characteristics ^b	Mix Time (min)
OVERALL MEAN	1187.81	24.88	24.63	9.59
<u>Improvers</u>				
A - AA	3.13	-0.50	-.50	0.03
B - DATEM	55.63 ^c	-3.00*	-2.25	0.95
C - Amylase	-33.13*	1.50	1.25	1.93
D - Protease	3.13	-0.50	-1.75	0.45
E - Cysteine	51.88*	-0.25	-0.50	-5.08*
<u>Interactions</u>				
AB	15.63	0.25	0.50	-0.95
AC	-43.13*	0.25	2.00	0.53
AD	18.13	0.75	2.50	0.10
AE	46.88*	-1.00	0.25	-0.73
BC	4.38	0.25	1.25	0.25
BD	3.13	0.25	-1.25	0.13
BE	16.88	1.00	-2.00	-0.75
CD	4.38	-0.25	-2.75	-0.15
CE	45.63*	-1.00	0.50	-1.48
DE	-28.13	0.50	-0.50	-0.40

^a External characteristics maximum score 30.

^b Internal characteristics maximum score 30.

^c Effects marked with an * meet the criteria outline in Figure 4.2.

to loaf volume, as was seen in screening experiment #1, causing loaf volume to increase by 51.88 cc with the high level of addition. Conversely, amylase addition at the very high level used in this experiment caused a reduction in loaf volume of 33.13 cc.

Three interactions were very important to the volume of breads made with CWES wheat flour. The two-way tables illustrating these interactions are included in Figure 4.6. It is evident that a high level of cysteine was required in order to achieve the beneficial effects of ascorbic acid and α -amylase in these breads. Also, cysteine improved the volume of CWES breads only if ascorbic acid and α -amylase were added in substantial quantities. However, when α -amylase was added at the low level, ascorbic acid no longer enhanced the volume of these breads. The relationship between these three additives appears to be extremely complex, in which their optimum levels of addition are highly interdependent.

As with the CWRS wheat flour breads, DATEM was harmful to the external loaf characteristics of CWES breads, causing a reduction in the score of 3 points when its level of addition increased from low to high. There were no important interactions between variables for this response. The internal structure was not affected to any great degree by any of the variables or interactions between them. These results suggest a high degree of tolerance of the CWES wheat flour to changes in improver addition in terms of both the external and internal structure of the baked loaves.

Cysteine alone was effective in reducing mix time requirement. The average mix time for doughs made with 75 ppm cysteine was 67% less than the standard formula CWES wheat flour doughs (Table 4.5).

Figure 4.6. Two-way tables illustrating the effects of the loaf volume interactions in breads made with CWES wheat flour.

		AA (ppm)		
		60 (-)	150 (+)	
Ascorbic Acid X Cysteine		1189	1239	75 (+)
		1184	1140	25 (-)
				Cysteine (ppm)

		Amylase (SKB)		
		25 (-)	75 (+)	
Amylase X Cysteine		1208	1220	75 (+)
		1201	1123	25 (-)
				Cysteine (ppm)

		Ascorbic Acid (ppm)		
		60 (-)	150 (+)	
Ascorbic Acid X Amylase		1191	1151	75 (+)
		1181	1228	25 (-)
				Amylase (SKB)

DISCUSSION

The second screening experiment included only five variables. Using five rather than seven additives made it easier to understand the role of each improver in the improvement of bread quality and to assess their involvement in interactions. Very complex relationships existed between some of the improvers. These were examined in greater detail in the final optimization study.

Ascorbic acid did not produce any noticeable effect on the loaf volumes of breads made with either CWRS and CWES wheat flour when the levels of addition increased to 150 ppm. In an investigation to compare the effects of individual improvers on properties of breads made using the Canadian Short Process, Yamada and Preston (1992) found that loaf volumes reached a maximum at 100 ppm ascorbic acid, with no significant change resulting from higher levels of addition. In light of these findings, the lack of loaf volume improvement by ascorbic acid in this study is probably due to the high level used. Loaf volumes were high at the low ascorbic acid level and increased until an optimal level was reached above which there was minimal change. As a result, the ascorbic acid affect was not as great as in screening experiment #1 in which a lower level of ascorbic acid was chosen as the maximum level tested.

DATEM had both a positive and a negative effect on the quality of CWRS and CWES breads. Loaf volumes of breads made with both flours were enhanced considerably through the addition of this dough strengthening agent, and the shape of the CWRS loaves was improved given that the other additives were kept at a minimum. Garti et al (1980) reported similar results, stating that DATEM and other dough strengthening surfactants increased loaf volumes by 10-15% and yielded loaves with improved symmetry. This additive was, however, detrimental to the internal structure of breads made with CWRS wheat flour. Addition of high levels of DATEM consistently lowered internal scores regardless of the level of the other improvers. This result is contrary to results published by Junge et al (1981) who found that added surfactants improved the crumb grain of breads through the formation of more and smaller cells during the mixing stage. DATEM also influenced the effectiveness of the other improvers tested. Ascorbic acid, α -amylase and cysteine were detrimental to the internal structure of the CWRS loaves only when the high level of DATEM was used. In light of these findings, it was decided that DATEM should be maintained in the formulation at a set level to improve loaf volume and external appearance. However, in order to reduce the extent of internal loaf quality deterioration, the level of addition should be reduced.

Protease was an important variable for improving the loaf volume of the CWRS breads,

as was expected from the results of screening experiment #1. However, doughs prepared with high levels of protease tended to be tacky, making the upper level of 300 HU an unrealistic level from a practical point of view. Although protease can modify very tight doughs and improve their extensibility (Fox and Mulvihill, 1982), the CWES wheat flour doughs prepared in this study did not benefit greatly from the inclusion of high levels of protease.

The high level of α -amylase used in this study was detrimental to the volume of CWES breads. These results suggest that the dough was overtreated with α -amylase, producing loaves with characteristics consistent with those obtained when baking with flours with excessive α -amylase activity. Optimum levels are likely lower than the maximum amount used here (75 SKB units). The detrimental effect of high levels of α -amylase in CWES breads was also highly dependent on the level of ascorbic acid and cysteine utilized. The effects of all three of these variables were interdependent. Fitchett and Frazier (1986) suggested that with added cysteine, doughs require greater amounts of ascorbic acid to reform the broken disulphide bonds. Thus the higher the level of cysteine, the greater the requirement for ascorbic acid. In this study, using high levels of both cysteine and ascorbic acid gave very good loaf volume for breads made with CWES wheat flour.

Both high and low α -amylase supplementation resulted in high CWES loaf volumes when cysteine was included at the high level. This enzyme also determined the ability of ascorbic acid to enhance loaf volumes in these breads, with lower α -amylase in conjunction with optimum ascorbic acid being ideal. It would be logical to assume therefore, that optimum results could be attained by using a high level of cysteine plus a lower level of α -amylase in conjunction with optimal ascorbic acid. An appropriately designed response surface study in which the interaction effects can be visualized is key to understanding how these three improvers influence each other in a bread formulation.

CONCLUSIONS

This study was successful in identifying the variables which were most important to the improvement of CWRS and CWES wheat flour breads. The DATEM had both beneficial and detrimental effects on the bread quality characteristics. It was decided to include this dough strengthening agent in the subsequent optimization experiment at a fixed level. The level chosen was the mid level used in this study of 0.35%. The protease was also important to the quality of the CWRS breads. However, it required a high level of DATEM in order for its effects to be seen. Possibly the lower level of DATEM chosen for the optimization study would not be sufficient for the protease benefits to be realized. The mellowing effect which protease has been shown to have on wheat flour doughs was not evident in this study, and mixing requirements were not reduced to any extent by its inclusion in the formulation. Therefore, protease was not selected for inclusion in the optimization study.

Ascorbic acid, α -amylase and cysteine, both alone and through interaction effects, were found to be important to the quality of breads made with both flours. Thus, they were selected as the variables to be optimized in a subsequent response surface study. It was concluded from this experiment the range of ascorbic acid levels was appropriate but a lower range of α -amylase levels should be used to avoid the reduction in loaf volume observed in CWES breads at 75 SKB units. Cysteine levels as high as 90 ppm should be used.

The blistering problem encountered in screening experiment #1 was largely solved using the reduced water absorption formulation. The method for evaluating the internal loaf characteristics gave better discrimination between loaves, and improver effects on crumb structure were apparent.

Chapter 5

OPTIMIZATION OF INDEPENDENT VARIABLES USING RESPONSE SURFACE METHODOLOGY

INTRODUCTION

As outlined in the experimental plan, the screening experiments were followed by an optimization study. The three additives identified as most beneficial to quality through the screening experiments were included in the optimization design. These were ascorbic acid, α -amylase and cysteine. DATEM was beneficial to loaf volume but did not participate in any significant interactions affecting volume. On the other hand, the high level used in the second screening experiment was detrimental to crumb characteristics. Therefore, DATEM was not included as a variable in the experiment but was kept in the base bread formula at an intermediate level of 0.35% in the expectation that this would provide the strengthening without significantly lowering internal crumb scores.

For the optimization, CWRS and CWES flours were not examined separately, but were both incorporated in the design by using a series of five flours consisting of 0 - 100% CWES flour, the remaining % as CWRS flour. The optimization design therefore had four variables: ascorbic acid, α -amylase, cysteine and % CWES. Percent CWES was included in the design in order to examine the interacting effects of the additives and the extra strong flour. The design also made it possible to examine the effects of the additives on quality of breads made from each flour or blend separately. The specific objectives of this experiment were:

1. To select best fitting models, from full response surface models, to predict the effects of four independent variables (ascorbic acid, α -amylase, cysteine and percent CWES wheat flour) on mix time, loaf volume and internal and external loaf characteristics.
2. To use the best fitting predictive models to produce contour and response surface plots in order to assess the effects of, and interactions between, three independent variables (ascorbic acid, α -amylase and cysteine) in five flour blends with increasing proportions of CWES wheat flour.

3. **To determine the effects of increased proportions of CWES wheat flour on improver requirements.**
4. **To identify at least two combinations of additives for use with each of the five flour blends which best optimize loaf volume, external and internal scores simultaneously.**
5. **To predict the response outcomes for selected optimum combinations using best fitting regression models.**

MATERIALS AND METHODS

Materials

All materials and methods used in the optimization experiment are the same as described in screening experiment #2. The moisture content and farinograph water absorption of the flour was assumed to be unchanged since the previous analysis.

Evaluation

Loaf Volume

Loaf volumes determinations were made as per the method described in screening experiment #1.

Bread Quality Characteristics

The preparation of loaves for evaluation and scoring method used was the same as described in screening experiment #2 and was carried out on the day following baking. Because large blisters on the top of the loaves were no longer present to such a large extent, this response was not included in the composite score for Internal Characteristics of the loaves. Thus, the maximum score for this response was reduced to 20.

Criteria for Response Acceptability

When evaluating the response variables, higher loaf characteristic scores and loaf volumes indicated better quality. However, the levels of improver addition which maximized one response were not necessarily ideal for another response. For example, where one improver combination enhanced loaf volumes considerably, a response such as internal loaf characteristics was adversely affected. Altering the improver combination enhanced the internal structure but gave loaf volumes which were lower yet still highly acceptable. Therefore, a minimum score for acceptability was assigned to each response variable in order to facilitate the identification of improver combinations which gave optimum results for all response variables simultaneously.

Loaf Volume

In screening experiment #1, many of the CWRS wheat flour breads which had larger volumes had more open crumb structures. Results of screening experiment #2, in which higher levels of improvers were tested, showed even greater volumes, at times in excess of 1200 cc. However, many of these had very concave loaf bottoms, sometimes exhibiting a large blister. As

such great volumes were not necessarily optimum, loaf volumes of 1100 cc were considered highly acceptable given that the internal and external characteristics rated highly. Thus, to be in the optimal region, a loaf volume ≥ 1100 cc was considered acceptable.

External Loaf Characteristics

The score for external loaf characteristics was comprised of three factors: loaf symmetry, loaf bottom (flat vs concave), and the degree of break and shred. The minimum acceptable scores for each of these factors were 8, 9 and 7, respectively. Thus, for this response, the total minimum acceptable score was 24 points

Internal Loaf Characteristics

The score for internal loaf characteristics was comprised of two factors: cell uniformity and cell size. The acceptable lower score for each of these factors was 7 points. Thus, for this response, the total minimum acceptable score was 14 points.

Experimental Design

For generation of the experimental design and all data analysis, the software package OPTIMIZATION (Int'l Qual-Tech, Ltd.) was used. To identify the settings of the three improvers which optimize the specific responses (mix time, loaf volume and internal and external loaf characteristics) in CWRS and CWES wheat flour alone and in blends, a central rotatable composite design, with four replications of the center point was used. Replication of the center point is generally done to gain information on the error associated with the response measurements (Mitchell et al, 1986). The design (Table 5.1) consisted of a four variable (ascorbic acid, α -amylase, cysteine, % CWES wheat flour), five-level pattern with 28 runs. For statistical analysis, the five levels of each of the four variables were coded as -2, -1, 0, +1 and +2. The actual levels used are shown in Table 5.2. The center points and star points of the experimental design were replicated (on a separate day) in order to assess the variability of the data and to ensure the star point values used in the analysis were truly representative. Baking runs were carried out in random order.

Statistical Analysis

Statistical analysis was performed on the data obtained for each response variable: loaf volume, mix time and external and internal loaf characteristics. Initially, a full second order

Table 5.1. Experimental design for the optimization experiment.

Runs	Variables ^{ab}			
	A	B	C	D
1	-1	-1	-1	-1
2	+1	-1	-1	-1
3	-1	+1	-1	-1
4	+1	+1	-1	-1
5	-1	-1	+1	-1
6	+1	-1	+1	-1
7	-1	+1	+1	-1
8	+1	+1	+1	-1
9	-1	-1	-1	+1
10	+1	-1	-1	+1
11	-1	+1	-1	+1
12	+1	+1	-1	+1
13	-1	-1	+1	+1
14	+1	-1	+1	+1
15	-1	+1	+1	+1
16	+1	+1	+1	+1
17	-2	0	0	0
18	+2	0	0	0
19	0	-2	0	0
20	0	+2	0	0
21	0	0	-2	0
22	0	0	+2	0
23	0	0	0	-2
24	0	0	0	+2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0

Confounding Pattern: No confounding

^a Variables: A = ascorbic acid, B = Amylase, C = Cysteine,
D = CWES wheat flour.

^b Levels: +2 = high, -2 = low (star points)
+1 = mod high, -1 = mod low (cube points)
0 = mid-level

Table 5.2. Variables and their levels used in the optimization experiment.

Response Variables	Levels				
	-2	-1	0	+1	+2
Coded Levels					
A - Ascorbic Acid (ppm)	50	75	100	125	150
B - Amylase (SKB Units)	20	30	40	50	60
C - Cysteine (ppm)	10	30	50	70	90
D - CWES wheat flour (%) ^a	0	25	50	75	100

^a Remaining %: CWRS wheat flour.

regression model was fitted which included the expected midpoint value plus all linear, quadratic and interaction terms. The full model for the 4-factor Rotatable Central Composite Design was as follows:

$$Y = b_0 + b_1A + b_2B + b_3C + b_4D + b_5A^2 + b_6B^2 + b_7C^2 + b_8D^2 + b_9AB + b_{10}AC + b_{11}AD + b_{12}BC + b_{13}BD + b_{14}CD$$

where: b_0 = center point value

b_1 to b_4 = linear coefficients

b_5 to b_8 = quadratic coefficients

b_9 to b_{14} = interaction coefficients

All model coefficients and associated percent confidences were computed. From this model, best fitting models were selected by deleting those terms with low percent confidence levels. If a linear term was not significant, but its quadratic or interaction term was, the linear term was retained in the model. The new models were then re-analyzed and new coefficients generated. The models were considered adequate when model percent confidence levels were maximized. The "goodness" of the selected model was evaluated according to several criteria (Joglekar and May, 1987):

- 1) The model percent confidence should be as high as possible, preferably > 95%.
- 2) The coefficient of multiple determination (R^2) refers to the variation accounted for by the model and should be high (> 80%).
- 3) The coefficient of variation (C.V.) is the standard error of estimate/mean X 100 and should be as low as possible (< 10%).
- 4) The percent confidence associated with the deleted terms should be very low, indicating that no important terms have been deleted from the model.

Analysis of the standardized residuals was also performed to ensure the adequacy of the fitted model.

After selection of best fitting models for each response variable, contour and response surface plots were generated to facilitate an understanding of the effects of each variable (improver) and of the interactions between the variables. From these plots, combinations of the three improvers which optimized all responses simultaneously were identified for each of the five flours (five levels of CWES wheat flour ranging from 0 to 100%).

RESULTS

The first step taken to implement the optimization experiment was to select an appropriate experimental design. A 4-factor rotatable central composite design was considered appropriate to study the effects of and interactions between three dough additives. After collecting data for each of the improver combinations specified in the design, the data for each response was fit to a full second-order regression model. Coefficients of determination (R^2), and the percent confidences associated with them, were the basis of the selection of terms to retain in the model when developing best fitting models for each response variable. Predictive equations developed from these models were used to generate contour and response surface plots for visual assessment of variable effects and interactions. In this section the focus will first be on the process followed to develop best fitting models for each response variable. The second part of the discussion will focus on analysis of contour and response surface plots to identify improver combinations which optimized all quality characteristics.

Steps in Selection of Best Fitting Models

The first step in interpreting the results of this experiment was to examine the coefficients and percent confidence associated with each in the full quadratic model. By doing this, terms which were not significant were removed from the model. A simplified model was developed which included those explanatory variables which were most critical to response variables. Table 5.3 summarizes the coefficients and percent confidence associated with them for each response variable for the full models. Table 5.4 gives the same information for the best fitting models. Model % confidence, the % confidence associated with the deleted terms (best fitting models only), the R^2 values and coefficients of variation have been included in both tables.

Selection of Best Fitting Model for Loaf Volume Optimization

In order to build a predictive model, terms with the highest % confidence were selected first for inclusion. The highest % confidence values ($> 85\%$) were for the interactions between ascorbic acid and cysteine (AC) and between α -amylase and CWES wheat flour (BD), the next highest for cysteine and the cysteine by CWES interaction. These terms and the linear terms corresponding to variables in the interaction terms were included in the best fitting model as shown in Table 5.4. Compared to the full model, the R^2 value dropped by approximately 0.08

Table 5.3. Regression equation coefficients and associated percent confidence levels for all model terms - Full Models.

Parameters	Response Variables							
	Loaf Volume		Mix Time		External Characteristics		Internal Characteristics	
	Coeff.	% Conf.	Coeff.	% Conf.	Coeff.	% Conf.	Coeff.	% Conf.
b_0 - Expected Midpoint	1154		5.93		26.50		12.25	
Linear Terms								
b_1 - Ascorbic acid [A]	-3.96	31.5	-0.01	8.0	-0.63	72.8	0.83	89.5
b_2 - α -amylase [B]	1.21	10.4	-0.02	15.9	0.00	0.0	0.33	49.2
b_3 - Cysteine [C]	1.63	13.9	-0.83	99.9	0.63	72.8	-0.25	38.2
b_4 - % CWES [D]	-4.63	36.2	0.83	99.9	0.96	90.1	1.58	99.4
Quadratic Terms								
b_5 - A ²	-6.89	51.3	-0.09	70.0	-0.19	26.5	0.04	7.0
b_6 - B ²	-1.51	13.0	-0.10	76.0	-0.38	49.0	0.79	87.8
b_7 - C ²	-11.01	73.4	0.26	99.1	-0.31	41.8	0.17	26.6
b_8 - D ²	1.62	13.9	0.05	39.6	-0.44	55.7	-0.71	83.7
Interaction Terms								
b_9 - AB	10.31	60.0	0.06	43.6	-0.06	7.6	0.25	31.9
b_{10} - AC	-19.06	87.8	0.03	0.4	-0.94	81.9	-0.50	57.9
b_{11} - AD	-6.56	41.3	-0.04	27.6	-0.19	22.1	0.88	84.0
b_{12} - BC	0.31	2.3	-0.03	19.2	-0.31	34.9	0.75	77.5
b_{13} - BD	17.81	85.4	0.04	27.6	0.44	47.0	0.38	45.7
b_{14} - CD	-11.56	64.4	-0.03	19.2	-0.44	47.0	1.13	92.4
% Confidence - MODEL	24.1%		99.9%		26.0%		90.9%	
R ²	0.42		0.95		0.43		0.70	
C.V.	4.0%		7.0%		10.1%		19.3%	

Table 5.4. Regression equation coefficients and associated percent confidence levels for all model terms - Best Fitting Models.

Parameters ^a	Response Variables							
	Loaf Volume		Mix Time		External Characteristics		Internal Characteristics	
	Coeff.	% Conf.	Coeff.	% Conf.	Coeff.	% Conf.	Coeff.	% Conf.
b_0 - Expected Midpoint	1147		5.87		25.38		12.50	
Linear Terms								
b_1 - Ascorbic acid [A]	-3.96	35.4			-0.63	83.2	0.83	93.3
b_2 - α -amylase [B]	1.21	11.9	-0.02	18.8			0.33	54.3
b_3 - Cysteine [C]	1.63	15.8	-0.83	99.9	0.63	83.2	-0.25	42.4
b_4 - % CWES [D]	-4.63	40.7	0.83	99.9	0.96	96.1	1.58	99.8
Quadratic Terms								
b_5 - A^2								
b_6 - B^2							0.75	91.9
b_7 - C^2	-9.88	78.0	0.27	99.9				
b_8 - D^2							-0.75	91.9
Interaction Terms								
b_9 - AB								
b_{10} - AC	-19.06	92.5			-0.94	90.7		
b_{11} - AD							0.88	88.8
b_{12} - BC							0.75	83.0
b_{13} - BD	17.81	90.5						
b_{14} - CD	-11.56	72.8					1.13	95.5
% Confidence - MODEL	67.3%		99.9%		95.7%		99.3%	
% Confidence - DELETED TERMS	7.2%		7.7%		1.0%		8.7%	
R^2	0.34		0.93		0.34		0.66	
C.V.	3.6%		6.2%		8.5%		16.9%	

(to 0.34). The model % confidence increased by 43%, bringing it up to 67.3%. By deleting the linear coefficients, the model % confidence could be increased to 94.8%, but when the improvers or % CWES are involved in quadratic and/or interaction effects, it is necessary to retain the linear coefficient in the model. The low R^2 value was likely a result of the variability associated with the data.

All three additives and the percent CWES flour influenced loaf volumes. The interactions between ascorbic acid and cysteine and between α -amylase and % CWES were significant (% confidence > 90%). These interactions will be discussed further using the response surface diagrams.

Selection of Best Fitting Model for Mix Time

Cysteine and % CWES in the blend were the main factors that influenced mix time (% confidence > 99%). The negative coefficient associated with the linear term for cysteine indicated a reduction in dough development time as cysteine levels increased, whereas the coefficient associated with the % CWES was positive, indicating an increase in mix time as % CWES in the blend increased. By selecting only the three significant terms, a highly simplified best fitting model was obtained. The α -amylase term was retained in this model to provide an extra axis for the contour plots. The model % confidence and the R^2 values remained very high (99% and 0.93, respectively), while the % confidence associated with the deleted terms and the coefficient of variation stayed low (Table 5.4). This indicated a highly appropriate model, and that only cysteine and the % CWES wheat flour had any significant effect on mix time requirement.

Selection of Best Fitting Model for External Loaf Characteristics Optimization

On the basis of the full model (Table 5.3) only the % CWES in the blend had a significant impact on the external loaf characteristics (> 90% confidence). Although not significant at the 90-95% confidence level, the % confidence for the interaction term between ascorbic acid and cysteine (AC) was also high (81.9%). Therefore, % CWES, ascorbic acid and cysteine were included in the best fitting model. Upon exclusion of the unnecessary terms, the % confidence associated with the % CWES coefficient increased to 96.1%, while that associated with the coefficient for the AC interaction increased to 90.7%. The model % confidence increased from 26.0% in the full model to 95.7% in the reduced model. The % confidence associated with the deleted terms was very low (1.0%) as was the coefficient of variation (8.5%). The R^2 value dropped by approximately 0.10, bringing it down to 0.34. This meant that the variation in the

data accounted for by the fitted model was only 34%, the rest of the observed variation due to experimental error. However, upon examination of several different models in which other terms were added or deleted, this model resulted in the highest R^2 value for the maximum model % confidence.

Overall, it was concluded from this analysis that improvement of the external loaf characteristics depended primarily on the % CWES wheat flour in the blend. Because the coefficient associated with this term was positive, the effect of using increased proportions of CWES flour in the blend was to improve the loaf shape, making it more symmetrical, with a flat bottom and high degree of break and shred. Ascorbic acid and cysteine interacted strongly, the effect of each on external loaf characteristics being influenced by the other.

Selection of Best Fitting Models for Internal Loaf Characteristics Optimization

The internal loaf structure was influenced primarily by the % CWES in the blend and the ascorbic acid (Table 5.3). Cysteine and % CWES flour interacted (CD), the level of one influencing the effect of the other, as did the ascorbic acid and CWES wheat flour (AD) and the α -amylase and CWES (BD). Alpha-amylase and % CWES flour also had important quadratic effects. All seven terms were included in the best fitting model as well as the linear terms corresponding to the variables in the interactions. This model had a very high model % confidence (99.3%), low % confidence associated with the deleted terms (8.7%), and an R^2 value of 0.66. The coefficient of variation at 16.9% was greater than the ideal limit of 10%. In an attempt to improve the model, the interaction terms AD and BC were deleted. This caused a dramatic increase in the % confidence associated with the deleted terms (36.0%), a reduction in the % confidence for the model and R^2 value, and an increase in the coefficient of variation. Therefore, the two interactions were retained in the best fitting model.

Internal loaf structure was primarily influenced by the % CWES, which resulted in improved crumb structure as proportions increased. The addition of ascorbic acid also increased scores for internal loaf structure. At higher levels of CWES and ascorbic acid, loaves had a fine, even crumb structure. Interactions between several improvers also contributed to the improvement of crumb structure and will be addressed in the discussion of contour and response surface plots.

Interpretation of Results Using Contour and Response Surface Plots

Contour plots were generated using the best fitting models to show effects of two variables at a time on quality characteristics. Each of the five flour blends were first considered separately and effects at that blend level explored. Because the optimum level of α -amylase for loaf volume improvement was dependent upon the proportion of CWES flour in the blend, a series of contour plots with increasing levels of α -amylase were produced for each response variable within each flour blend. From these plots, the ideal α -amylase level was set for each flour, after which optimum levels of cysteine and ascorbic acid were determined. The same strategy was used to identify improver combinations which optimized the internal loaf characteristics. For the external loaf characteristics, α -amylase was found not to be important and so was not included in the model nor the plots.

Handling properties of the doughs were not scored in this experiment but must be taken into account when assessing additive effect. A majority of the doughs had acceptable handling properties, both out of the mixer and at the dough make-up stage. However, when levels of cysteine reached 50 ppm and greater, the doughs prepared with 100% CWRS flour were sticky and hard to handle. Dough produced when 90 ppm cysteine was used in the 50% CWES flour blend was also unacceptable. Therefore, 40 ppm cysteine was the acceptable limit for use with CWRS wheat flour, rising to 80 ppm for the 50% CWES flour blend.

Effect of Improvers on Mix Time

Cysteine and % CWES wheat flour were the major determinants of mix time (Table 5.4). Alpha-amylase had much less effect but was included in the model to provide a second variable for the axis of the contour plots. Plots showing the effects of cysteine and α -amylase on mix times for the 100% CWRS flour, the 50% CWES flour blend and 100% CWES wheat flours are given in Figure 5.1. Position of the response surface for the 100% CWES flour doughs shows higher mix times at all combinations of cysteine and α -amylase compared to those for the 50% CWES flour blend and the 100% CWRS flour doughs. This extended mix time requirement for CWES wheat flour dough is one of the major deterrents for its use alone in a bread formulation.

Figure 5.1 also illustrates the reduction in mix time of both CWRS and CWES wheat flour doughs with added cysteine. Addition of up to 50 ppm cysteine causes a rapid drop in mix times. Above this level, the reduction in mix time is much less dramatic.

MIX TIME (min)

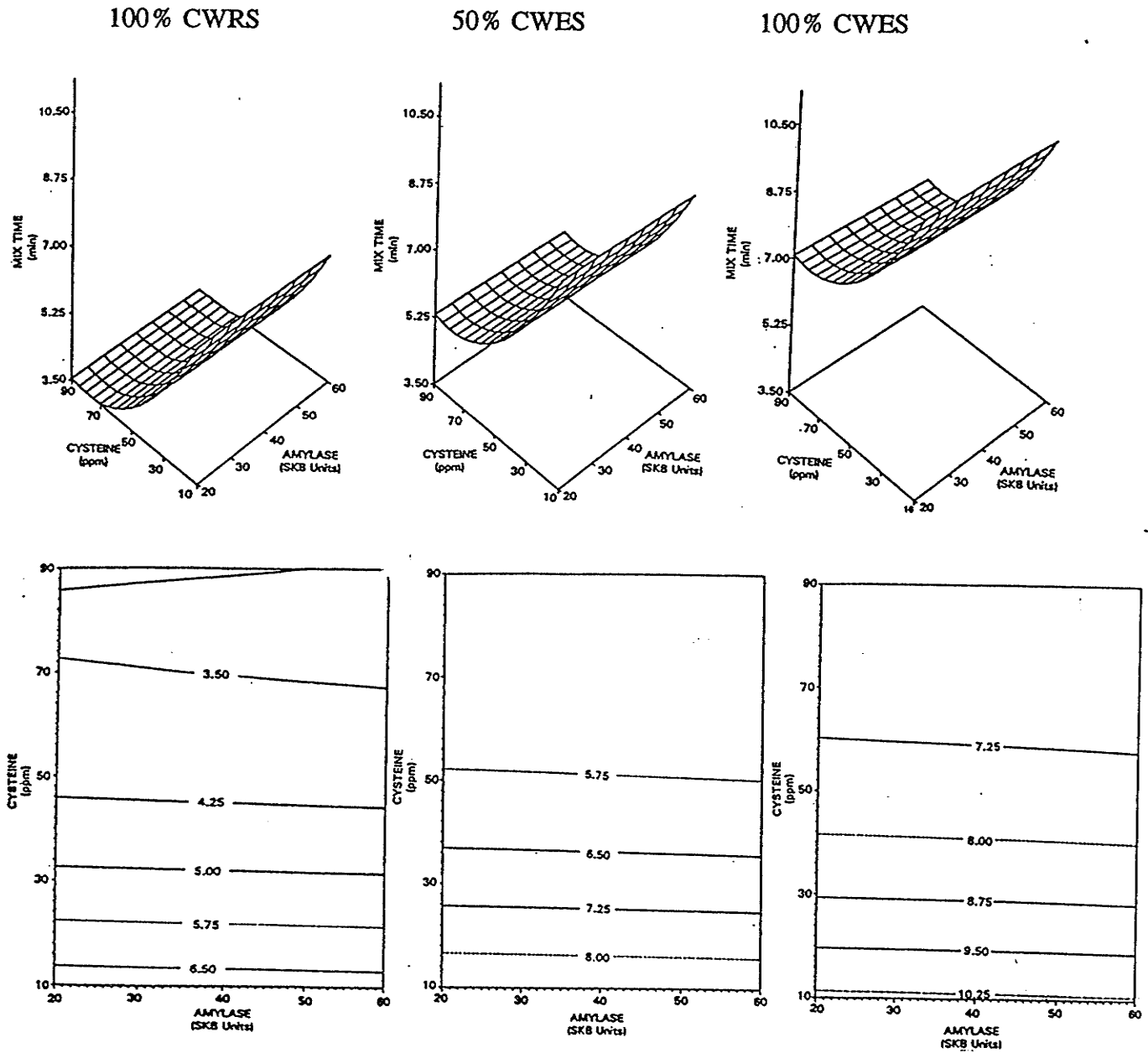


Figure 5.1. Contour and response surface plots for the effects of cysteine and α -amylase on the mix time of doughs prepared with 100% CWRs wheat flour, 50% CWES wheat flour blend and 100% CWES wheat flour.

Effect of Improvers on Loaf Volume

The best fitting model for loaf volume indicated that % CWES flour in the blend was a major determinant of the requirement for α -amylase. This interaction between the α -amylase and % CWES flour in the blend is depicted in the plots in Figure 5.2, in which both cysteine and ascorbic acid are held at their mid levels. When 100% CWRS wheat flour is used, best volumes can be attained using the lowest level of α -amylase, whereas breads prepared with 100% CWES wheat flour would have the greatest volume with high α -amylase addition. For the flour blends, 25% CWES breads would attained highest volumes using the low α -amylase level and the 75% CWES breads with the high level. Breads prepared with the 50% CWES flour perform best at both high and low levels of α -amylase.

The relationship between α -amylase and flour blend is also illustrated in Figure 5.3 in which % CWES and cysteine are plotted against each other at both 20 and 60 SKB units α -amylase. If the low α -amylase level is used, best volumes are obtained using a blend with a lower proportion of CWES wheat flour and if the high α -amylase level is used, best volumes occur with a higher proportion of CWES flour. In order to locate the best levels of cysteine and ascorbic acid, contour and response surface plots were generated for each flour at a set level of α -amylase considered ideal for that flour based on Figure 5.2.

In the following section, the effect of the improvers on the volume of breads made using the two flours alone (0% CWRS and 100% CWRS wheat flour) will be discussed. The improver requirements of the specific flour blends will be addressed separately.

100% CWRS Wheat Flour

Figure 5.4 shows the effect of cysteine and ascorbic acid on the volume of breads made with 100% CWRS wheat flour when α -amylase is added at a level of 20 SKB units. Greatest volumes occur at low ascorbic acid and high cysteine levels. However, cysteine levels above 50 ppm resulted in the formation of an overly sticky, slack dough and so a lower level of cysteine plus higher ascorbic acid would be the better combination. Volume may be lower but still highly acceptable and dough handling properties would be satisfactory.

100% CWES Wheat Flour

Contour and response surface plots illustrating the effects of cysteine and ascorbic acid on the volume of breads made with 100% CWES wheat flour and 60 SKB units of α -amylase are also included in Figure 5.4. Highest volumes are predicted with low cysteine and high ascorbic acid.

LOAF VOLUME (cc)

Ascorbic Acid = 100 ppm
Cysteine = 50 ppm

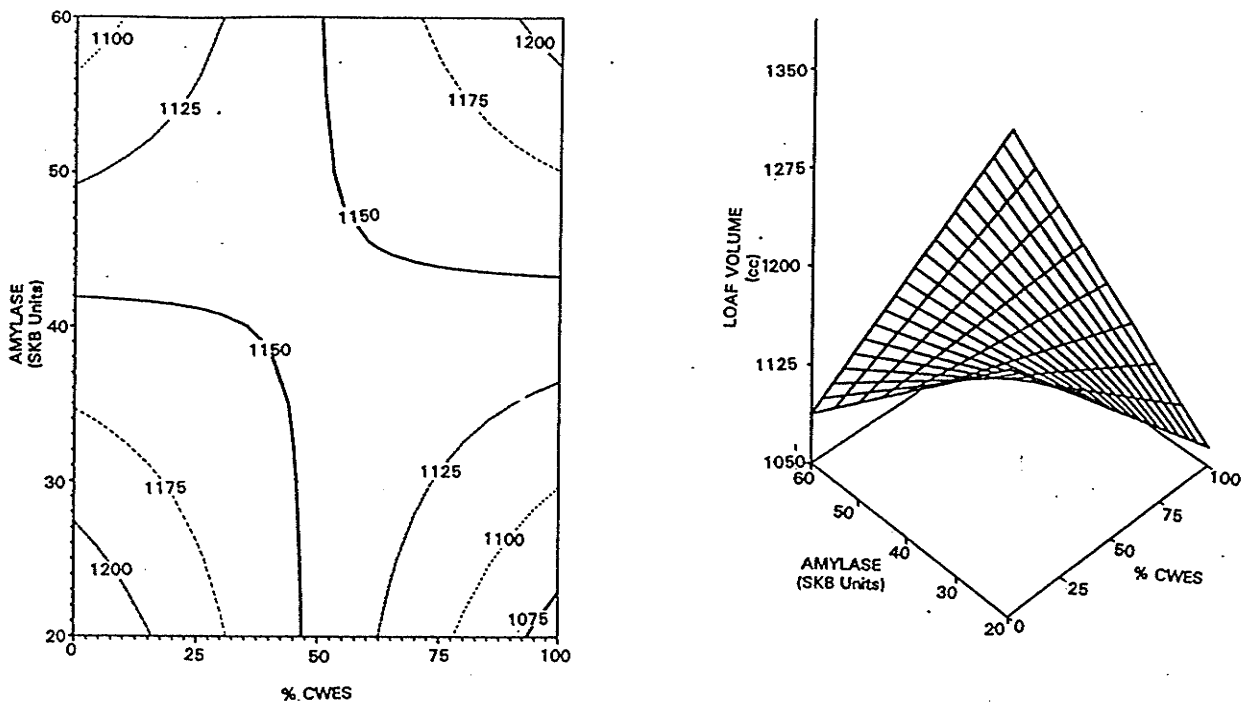
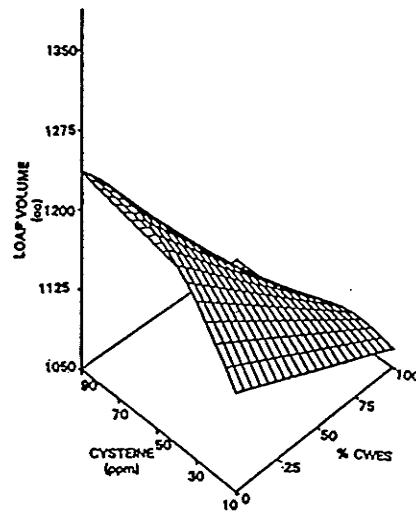
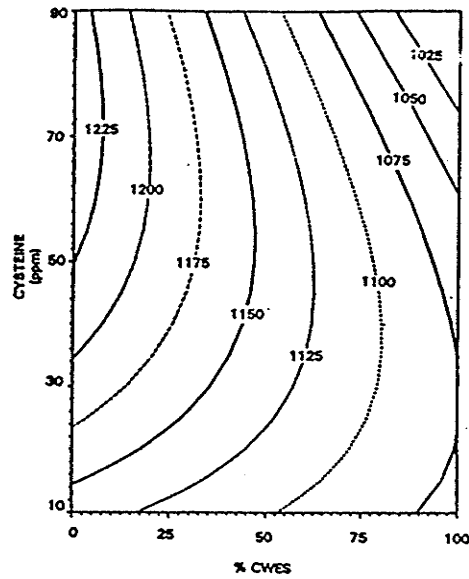


Figure 5.2. Contour and response surface plot for the effects of α -amylase and % CWES wheat flour on loaf volume. Ascorbic acid and cysteine were held constant at their mid levels.

LOAF VOLUME (cc)

Ascorbic Acid = 100 ppm
Amylase = 20 SKB Units



Ascorbic Acid = 100 ppm
Amylase = 60 SKB Units

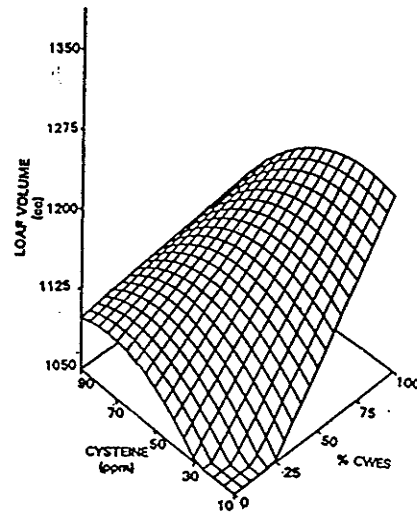
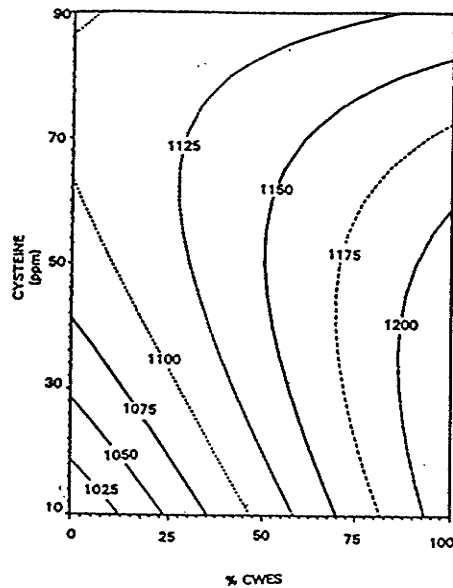
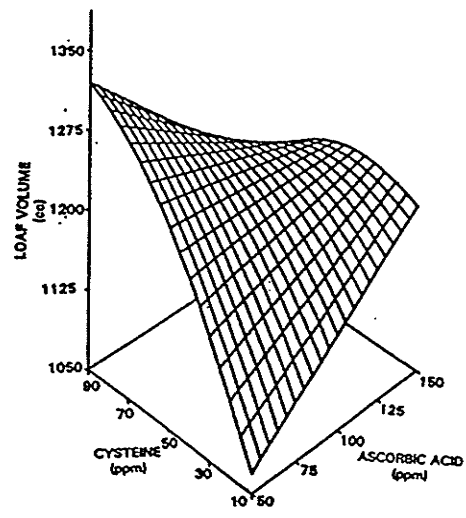
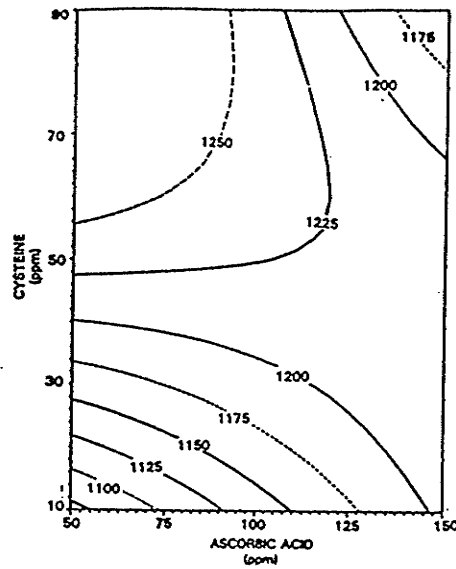


Figure 5.3. Contour and response surface plot for the effects of % CWES and cysteine on loaf volume at 20 and 60 SKB units α -amylase. Ascorbic acid was held constant at its mid level.

LOAF VOLUME (cc)

100% CWRS
Amylase = 20 SKB Units



100% CWES
Amylase = 60 SKB Units

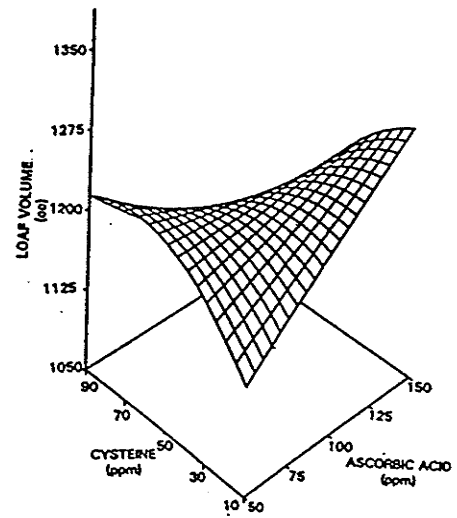
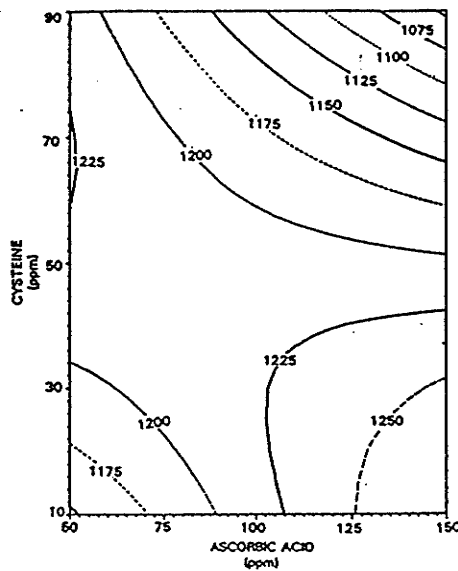


Figure 5.4. Contour and response surface plots for the effects of cysteine and ascorbic acid on loaf volume of breads prepared with 100% CWRS wheat flour plus 20 SKB units α -amylase and 100% CWES wheat flour plus 60 SKB units α -amylase.

Higher levels of cysteine and lower ascorbic acid would also give good volumes, a combination which would likely result in a substantial reduction in mix time.

Flour Blends

Figure 5.5 illustrates the interaction between cysteine and ascorbic acid in the flour blends consisting of 25%, 50% and 75% CWES wheat flour with the α -amylase levels of 20, 40 and 60 SKB units, respectively. These plots show that a combination of cysteine and ascorbic acid both at levels greater than 50 ppm should optimize the volumes of breads made using all three blends. However, as the proportion of CWES flour included in the blend increases, two things happen. First, the loaf volume potential decreases slightly, although volumes are still highly acceptable. Secondly, maximum loaf volumes are possible at a wider range of ascorbic acid/cysteine combinations. Highest volumes for the 25% CWES flour blend should occur with high cysteine and low ascorbic acid. Highest volumes for the 75% CWES flour bread are predicted with both high cysteine and low ascorbic acid and with low cysteine and high ascorbic acid combinations.

Effect of Improvers on External Loaf Characteristics

The external appearance of the loaves was dependent primarily on the level of CWES in the blend. Generally, the greater the proportion of CWES wheat flour, the better the external loaf characteristics. As α -amylase did not exhibit any significant effect on this response variable, it was not included in the model, and thus the necessity of setting its level when developing contour and response surface plots was eliminated. The interaction between the cysteine and ascorbic acid was highly significant and is illustrated in the contour and response surface plots generated for each of the flour blends.

100% CWRS Wheat Flour

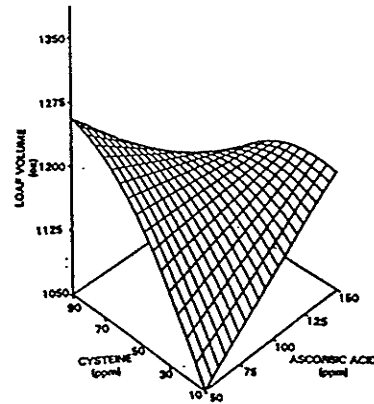
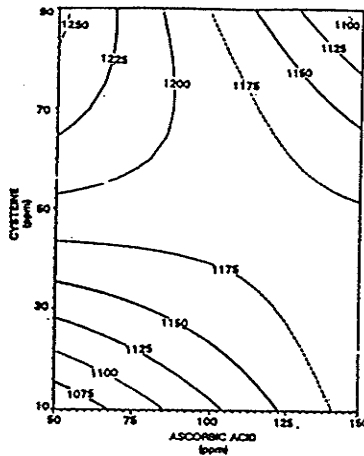
Figure 5.6 illustrates the effect of the interaction between cysteine and ascorbic acid on the external appearance of baked loaves. Best appearance is predicted when the cysteine level is high and ascorbic acid low. Acceptable external scores might also be attained with low cysteine and high ascorbic acid levels.

100% CWES Wheat Flour

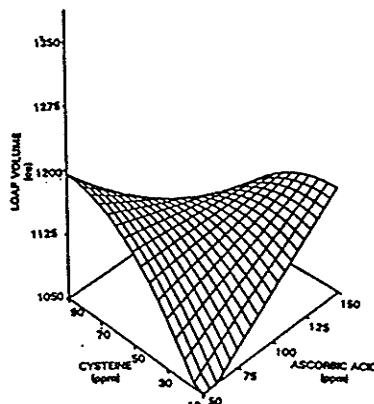
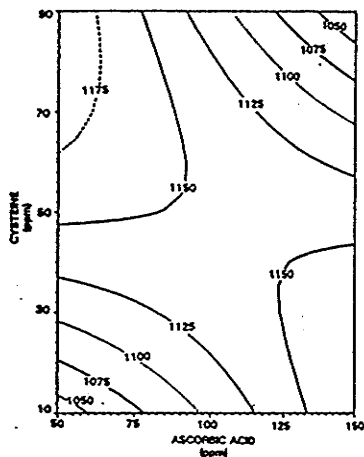
Best external scores for breads made with 100% CWES flour were also predicted to be at

LOAF VOLUME (cc)

25% CWES
Amylase = 20 SKB Units



50% CWES
Amylase = 40 SKB Units



75% CWES
Amylase = 60 SKB Units

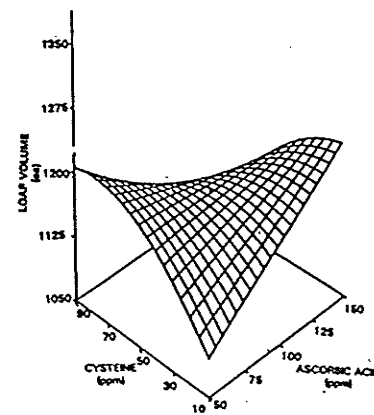
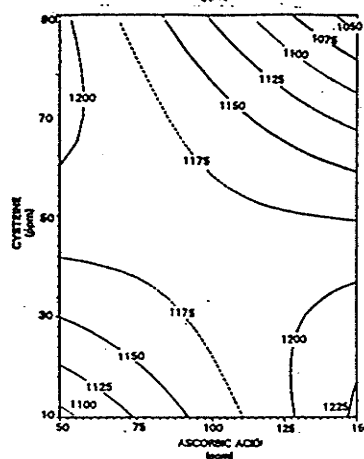
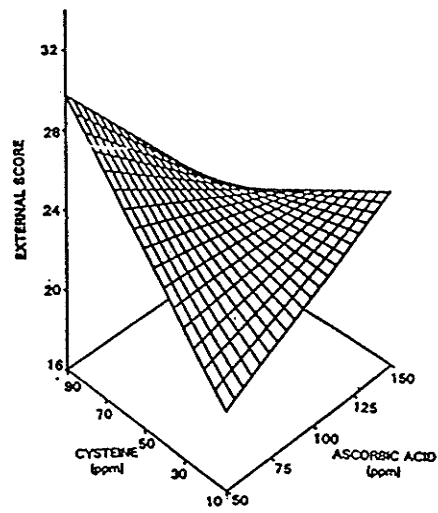
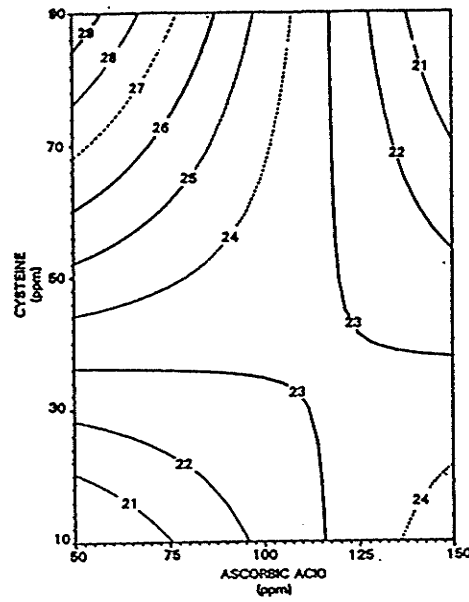


Figure 5.5. Contour and response surface plots for the effects of cysteine and ascorbic acid on loaf volume of breads prepared with 25% CWES wheat flour plus 20 SKB units α -amylase, 50% CWES wheat flour blend plus 40 SKB units α -amylase, and 75% CWES wheat flour blend plus 60 SKB units α -amylase.

EXTERNAL LOAF CHARACTERISTICS

100% CWRS



100% CWES

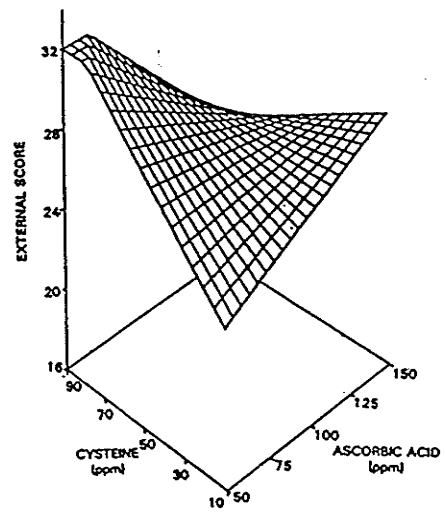
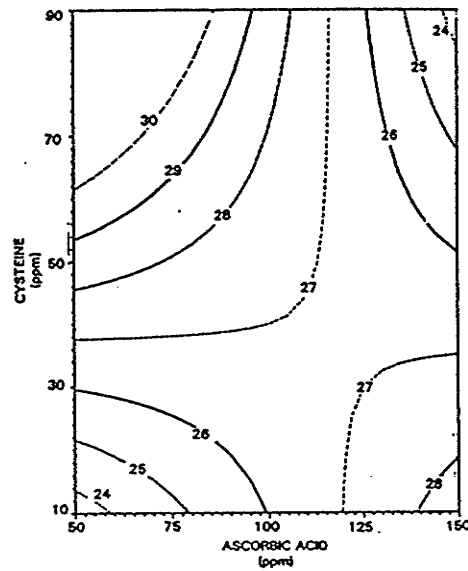


Figure 5.6. Contour and response surface plots for the effects of cysteine and ascorbic acid on external loaf characteristics of breads prepared with 100% CWRS wheat flour and 100% CWES wheat flour.

high cysteine/low ascorbic acid and low cysteine/high ascorbic acid (Figure 5.6). The improving effect of the CWES wheat flour on this response is also evident when the response surface plots of the two flours are compared. The response surface as a whole is higher for the CWES flour, indicating better predicted scores (ie. improved external appearance) overall.

Flour Blends

Predicted external scores increase generally as the proportion of CWES flour in the blend increases (Figure 5.7). The interaction between cysteine and ascorbic acid is evident across the three blends. The combination of high cysteine and low ascorbic acid would be effective for all blends. However, there is a greater range of possible optimum improver combinations when higher proportions of CWES flour are included in the blend. That is, the area of the contour plot which shows predicted results within the limits of acceptability is larger. This suggests that the extra strong flour improves the tolerance of these breads to very high and/or very low levels of the three additives in terms of the external appearance of the baked loaves.

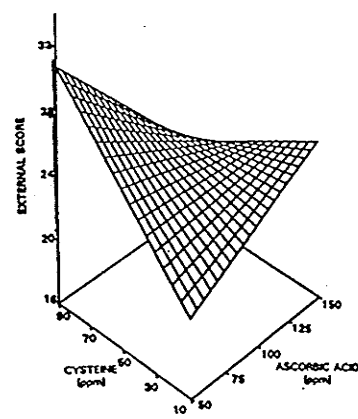
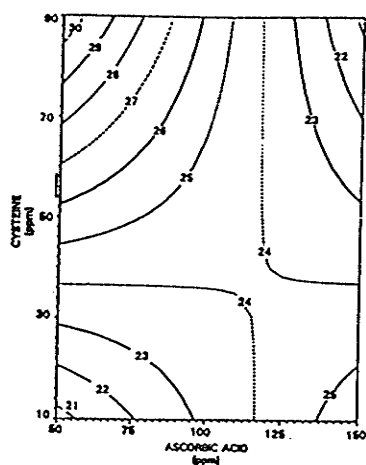
The Effect of Improvers on Internal Loaf Characteristics

The effect of the four variables on the internal loaf structure is extremely complex, as can be seen by the number of significant quadratic and interaction terms included in the response surface model (Table 5.4). The effect of α -amylase was dependent on the level of cysteine used and the effect of the cysteine was dependent upon the proportion of CWES flour in the blend. The series of contour plots included in Figure 5.8 are helpful in determining what level of α -amylase should be used for the CWRS flour, the CWES flour and the 50% CWES blend. For 100% CWRS flour, highest internal score is predicted at 20 SKB units α -amylase. For the 50% CWES blend, both 20 and 60 SKB units α -amylase give very good predicted scores. For the 100% CWES wheat flour, high scores are predicted across all α -amylase levels.

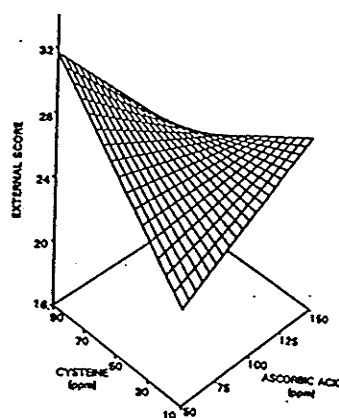
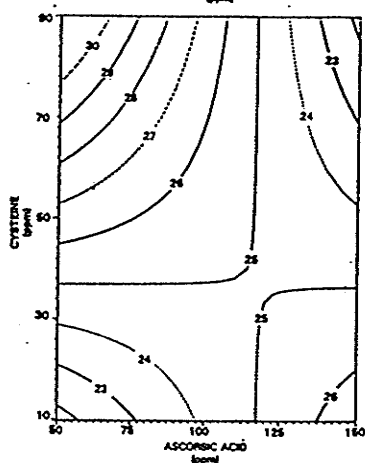
100% CWRS Wheat Flour

For breads prepared with 100% CWRS flour and 20 SKB units α -amylase, very low levels of both ascorbic acid and cysteine should be used to get high predicted internal scores (Figure 5.9). However, a wider range of ascorbic acid addition may also result in good internal loaf structure (within limits of acceptability) given cysteine addition is kept low.

25% CWES



50% CWES



75% CWES

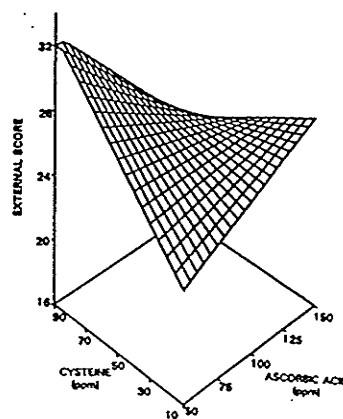
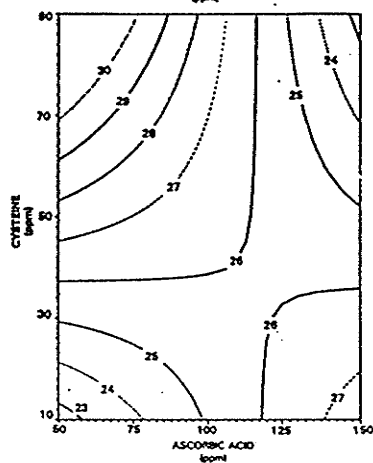


Figure 5.7. Contour and response surface plots for the effects of cysteine and ascorbic acid on external loaf characteristics of breads prepared with 25%, 50% and 75% CWES wheat flour blends.

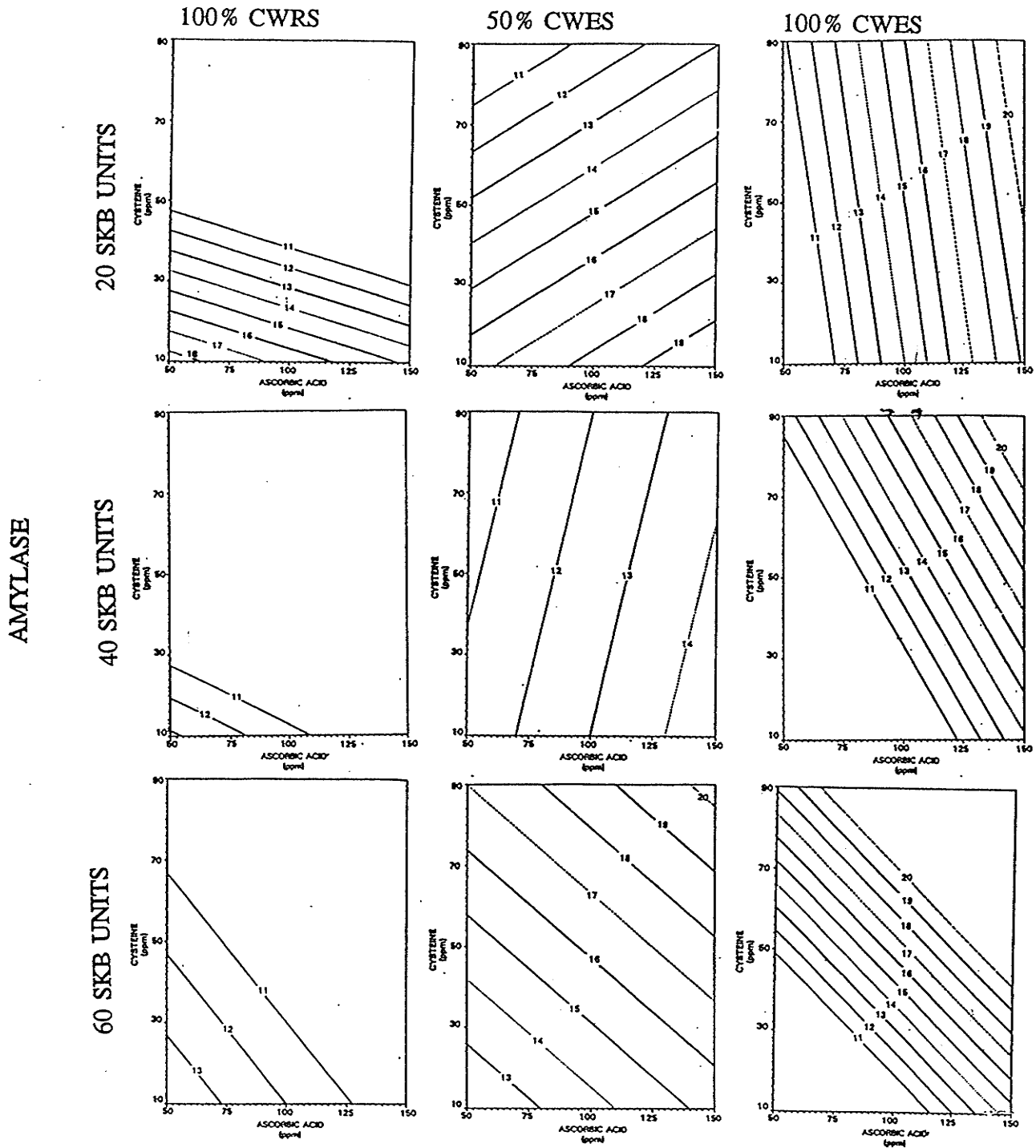
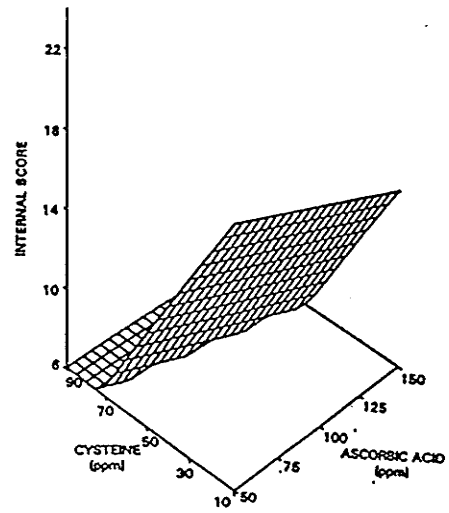
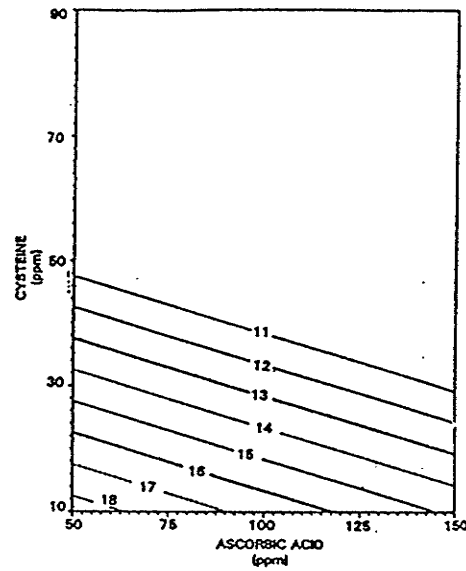


Figure 5.8. Contour plots for the effects of cysteine and ascorbic acid on internal loaf characteristics of breads prepared with 100% CWRS wheat flour, 50% CWES wheat flour and 100% CWES wheat flour at 20, 40 and 60 SKB units α -amylase.

100% CWRS



100% CWES

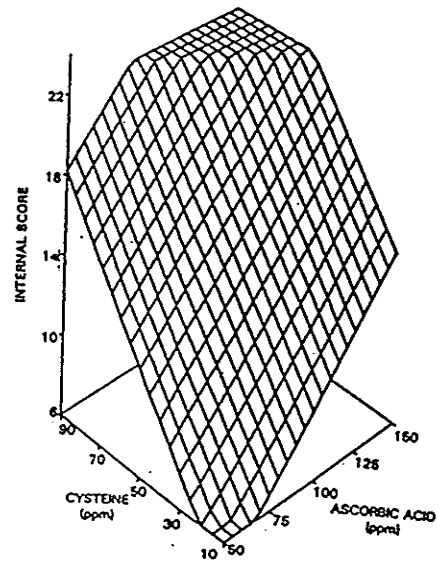
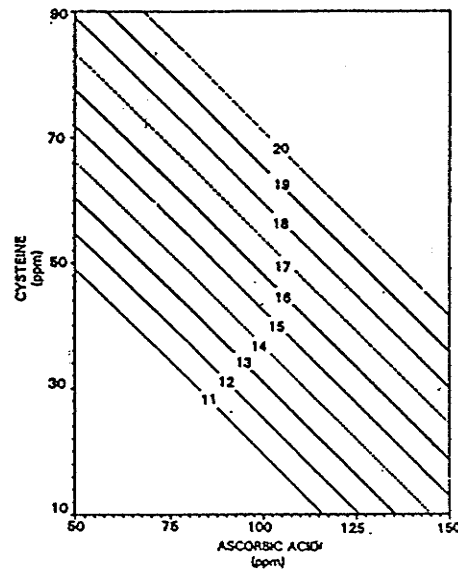


Figure 5.9. Contour and response surface plots for the effects of cysteine and ascorbic acid on internal loaf characteristics of breads prepared with 100% CWRS wheat flour plus 20 SKB units α -amylase and 100% CWES wheat flour plus 60 SKB units α -amylase.

100% CWES Wheat Flour

For breads made with 100% CWES wheat flour and 60 SKB units α -amylase, highest predicted internal scores should be achieved at cysteine levels above 50 ppm and ascorbic acid above 100 ppm. However, very high internal scores are predicted across a wide range of cysteine given an appropriate level of ascorbic acid. Similarly, the internal scores predicted across the whole range of ascorbic acid levels can be excellent depending on the cysteine level chosen.

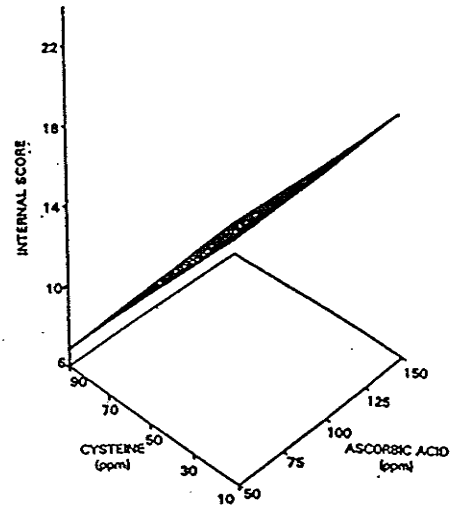
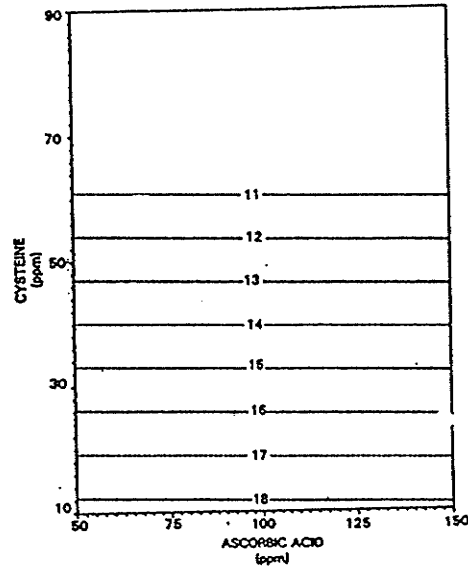
Flour Blends

Figure 5.10 depicts the effects of cysteine and ascorbic acid on the internal structure of loaves prepared with 25% and 75% CWES wheat flour blends, with α -amylase addition set at 20 and 60 SKB units, respectively. At the 25% CWES blend, cysteine but not ascorbic acid affects internal score. When the proportion of CWES flour increases to 75%, the cysteine and ascorbic acid interact, so that combinations of either high ascorbic acid and low cysteine or low ascorbic acid and high cysteine give the best predicted scores.

For the 50% CWES blend, the level of α -amylase determines the nature of the interaction between ascorbic acid and cysteine (Figure 5.11). At 20 SKB unit α -amylase, best predicted results are obtained with very low cysteine whereas a high cysteine level would be required if the 60 SKB units α -amylase were chosen. At both α -amylase levels, ascorbic acid requirement to give the best possible results remains high, although scores within the limits of acceptability are attainable across the whole range of ascorbic acid levels tested.

Optimization of the three improvers in CWRS and CWES wheat flours and their blends is a complex process. Each variable plays an important part in determining the requirement of the other. The proportion of CWES wheat flour in the blend is also an important determinant of improver requirement. Generally, CWES wheat flour improves both the internal and external structure of the bread and can yield breads of considerable volume given that an appropriate improver combination is utilized. Table 5.5 summarizes the optimum combinations of the three improvers for each of the five flours and for each of the response variables. The dough handling properties have not been taken into account in these variable combinations. As high cysteine levels were detrimental to those doughs prepared with 100% CWRS wheat flour and blends with a lower proportion of CWES wheat flour, it is important to consider which levels of cysteine would be best from a practical point of view. Therefore, in order to produce doughs with

25% CWES
Amylase = 20 SKB Units



75% CWES
Amylase = 60 SKB Units

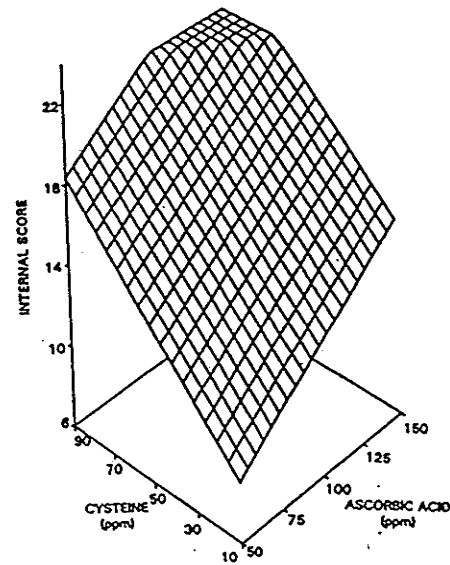
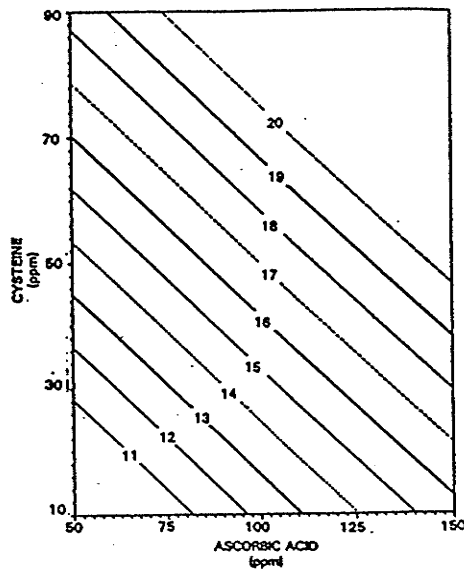
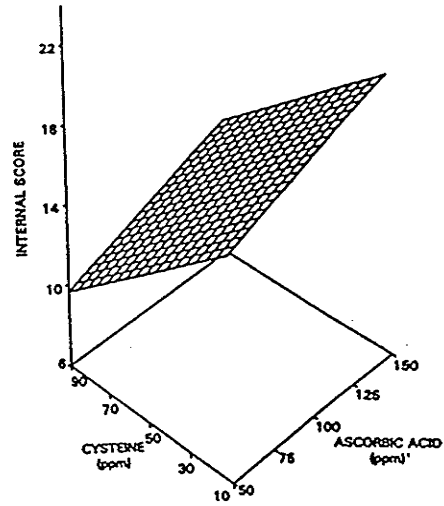
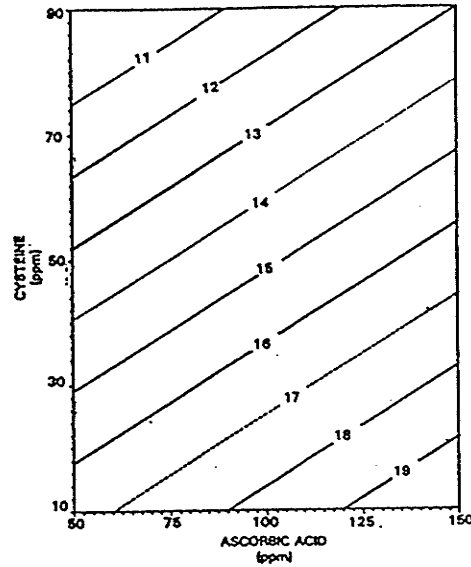


Figure 5.10. Contour and response surface plots for the effects of cysteine and ascorbic acid on internal loaf characteristics of breads prepared with 25% CWES wheat flour blend plus 20 SKB units α -amylase and 75% CWES wheat flour blend plus 60 SKB units α -amylase.

50% CWES
Amylase = 20 SKB Units



50% CWES
Amylase = 60 SKB Units

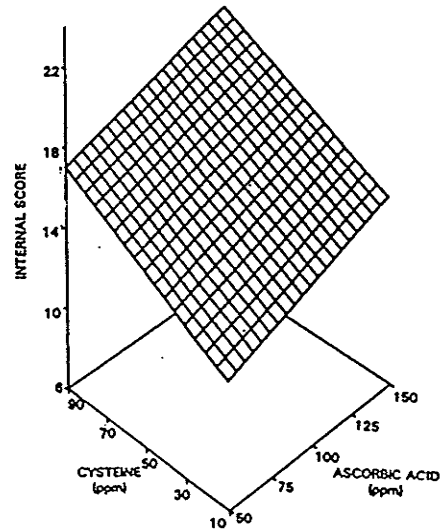
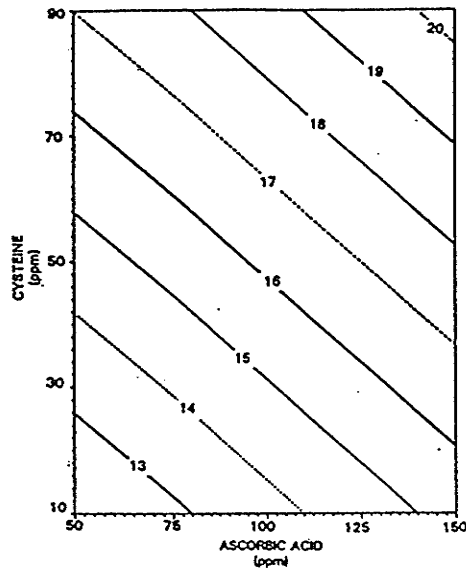


Figure 5.11. Contour and response surface plots for the effects of cysteine and ascorbic acid on internal loaf characteristics of breads prepared with 50% CWES wheat flour blend at 20 and 60 SKB units α -amylase.

Table 5.5 Best setting levels of α -amylase, ascorbic acid and cysteine which maximize the individual response variables in each of the five flour blends.

Response Variable	Flour Blend (% CWES) ^a	Independent Variables and Best Setting Levels		
		α -amylase	Ascorbic acid	Cysteine
LOAF VOLUME	0%	low	low	high
	25%	low	low	high
	50%	---	low high	high low
	75%	high	low high	high low
	100%	high	low high	high low
EXTERNAL LOAF CHARACTERISTICS	0%	---	low high	high low ^c
	25%	---	low high	high low ^c
	50%	---	low high	high low ^c
	75%	---	low high	high low ^c
	100%	---	low high	high low ^c
INTERNAL LOAF CHARACTERISTICS	0%	low	low	low
	25%	low	---	low
	50%	low high	high high	low high
	75%	low high	high high	low mid
	100%	low high	high high low	high mid-high high

^a Remaining flour in blend: CWRS wheat flour.

^b Level of addition not considered important to this response variable and therefore not included in the best fitting model.

^c Expected scores for combinations marked with an are within the regions of acceptability but are lower than expected for the alternate improver combination for that flour blend.

acceptable handling properties, it may be necessary to sacrifice some of the possible benefits of using a high level of cysteine.

Correlations Among Response Variables

Differences and similarities in the improver combinations which optimized each response variable were apparent and prompted an investigation to determine whether there were any significant correlations between the responses. Both loaf volumes and external loaf characteristics scores were maximized by using similar improver combinations. However, additive combinations which improved loaf volume also tended to lower internal scores. Therefore, it seemed pertinent to question whether the external and internal scores could be a function of increased loaf volume rather than a function of the additive combination itself.

In order to determine whether any correlations existed between the response variables within each of the five flour blends, outcomes for all possible combination of the three improvers at three levels (-2, 0, +2) ($3^3 = 27$ combinations) for each of the five flour blends were first predicted. The correlation procedure (ProcCorr) of the Statistical Analysis System (SAS) was then used to generate Pearson Correlation Coefficients between the three response variables. The correlation matrices are included in Figure 5.12.

The only significant ($p \leq 0.05$) correlation was between loaf volume and external loaf characteristics for all five flours. Coefficients ranged from 0.38 to 0.87, indicating that as loaf volume increased, so did the external score. A possible explanation for these positive correlations is that loaves with greater volume tended to have a high degree of break and shred. Since higher scores were given to those loaves with a high degree of break and shred, the external loaf score may have been quite high even if the loaf had some poorer qualities such as a concave bottom or unsymmetrical shape.

There was only one significant correlation between the internal loaf characteristics and loaf volume. For the 100% CWRS flour, a negative correlation (-0.42) between these two responses existed. This was not a surprising outcome as a tendency for some of the larger loaves to have a more open crumb structure was noted during the evaluation. When various proportions of CWES flour were added to the blend, this relationship between loaf volume and internal structure was still negative but no longer significant. These findings suggest that CWES wheat flour is capable of producing loaves of good volumes with minimal deterioration of the crumb structure.

Figure 5.12. Matrices of Pearson's Correlation Coefficients between loaf volume, external and internal loaf characteristics^a for each of the five flour blends.

100% CWRS	A	B	C
A	1.00	0.69*	-0.42
B		1.00	-0.17
C			1.00

25% CWES ^b	A	B	C
A	1.00	0.84*	-0.23
B		1.00	-0.16
C			1.00

50% CWES	A	B	C
A	1.00	0.87*	-0.06
B		1.00	-0.22
C			1.00

75% CWES	A	B	C
A	1.00	0.68*	-0.07
B		1.00	-0.14
C			1.00

100% CWES	A	B	C
A	1.00	0.38*	-0.21
B		1.00	-0.06
C			1.00

- ^a A = Loaf Volume
 B = External Loaf Characteristics
 C = Internal Loaf Characteristics
- ^b Remaining flour in blend: CWRS wheat flour.
- ^c Coefficients marked with an * are significant at $p \leq 0.05$.

Identification of Improver Combinations for Optimum Bread Quality

Improver combinations which maximized the all three quality attributes in each of the five flour blends were identified separately through the use of superimposed contour plots. Initially, criteria of acceptability were developed for each response in order to identify a range of response outcomes which would be considered acceptable. Contour plots were then produced for each response (loaf volume, internal and external loaf characteristics) for each of the five flours and the contour line representing the minimal acceptable score identified. These contour plots were generated at a set α -amylase level for each flour, using cysteine and ascorbic acid on the axes. The plots for each of the three responses in one flour were then superimposed over each other and the regions in which acceptable scores for the three response variables overlapped were identified. The lightly shaded areas of the plots represent the improver combinations which give predicted loaf volume and internal and external loaf scores within the limits of acceptability. In some cases, this region was very small, and so the area in which only loaf volume and internal loaf score were acceptable was identified and is represented by the darker shading.

100% CWRS Wheat Flour

Superimposed plots for the breads prepared with 100% CWRS wheat flour and α -amylase at 20 SKB units are included in Figure 5.13. The area of the plot in which all three responses are optimized is very small due to the opposing effect of cysteine on the internal and external loaf characteristics. A combination of ascorbic acid at a level greater than 135 ppm with less than 15 ppm cysteine should result in bread with all three quality attributes within the limits of acceptability. By sacrificing some of the external appearance, the region of improver combinations which give acceptable results for only loaf volume and internal scores is much larger. Breads with maximized scores for these two responses should be obtained by using a combination of cysteine at around 20 ppm and ascorbic acid at 50 to 100 ppm.

25% CWES Wheat Flour Blend

For the breads prepared with the 25% CWES blend, the area of the plot in which acceptable scores were predicted for all three response variable was limited (Figure 5.14). Acceptable scores were attainable at ascorbic acid greater than 120 ppm and cysteine less than 40 ppm. By considering only loaf volume and internal loaf score, a much wider range of ascorbic acid should give breads with scores within the limits of acceptability, although cysteine level should still be kept at a level of less than 40 ppm.

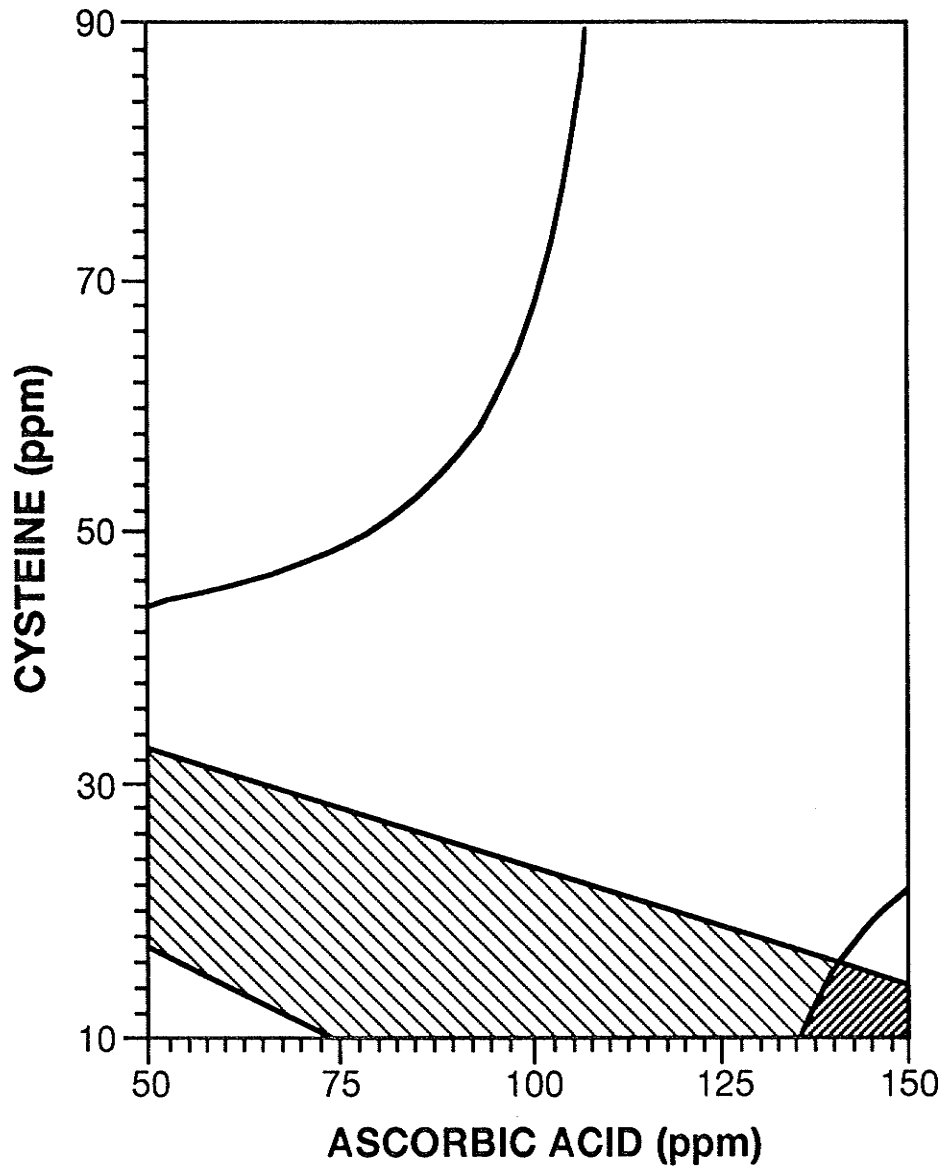


Figure 5.13. Superimposed contour plots illustrating the region of acceptability for multiple responses for breads prepared with 100% CWRS wheat flour and 20 SKB units α -amylase. The shaded region met the criteria of acceptability for loaf volume and internal loaf characteristics. The darker region met the criteria of acceptability for loaf volume and internal and external loaf characteristics.

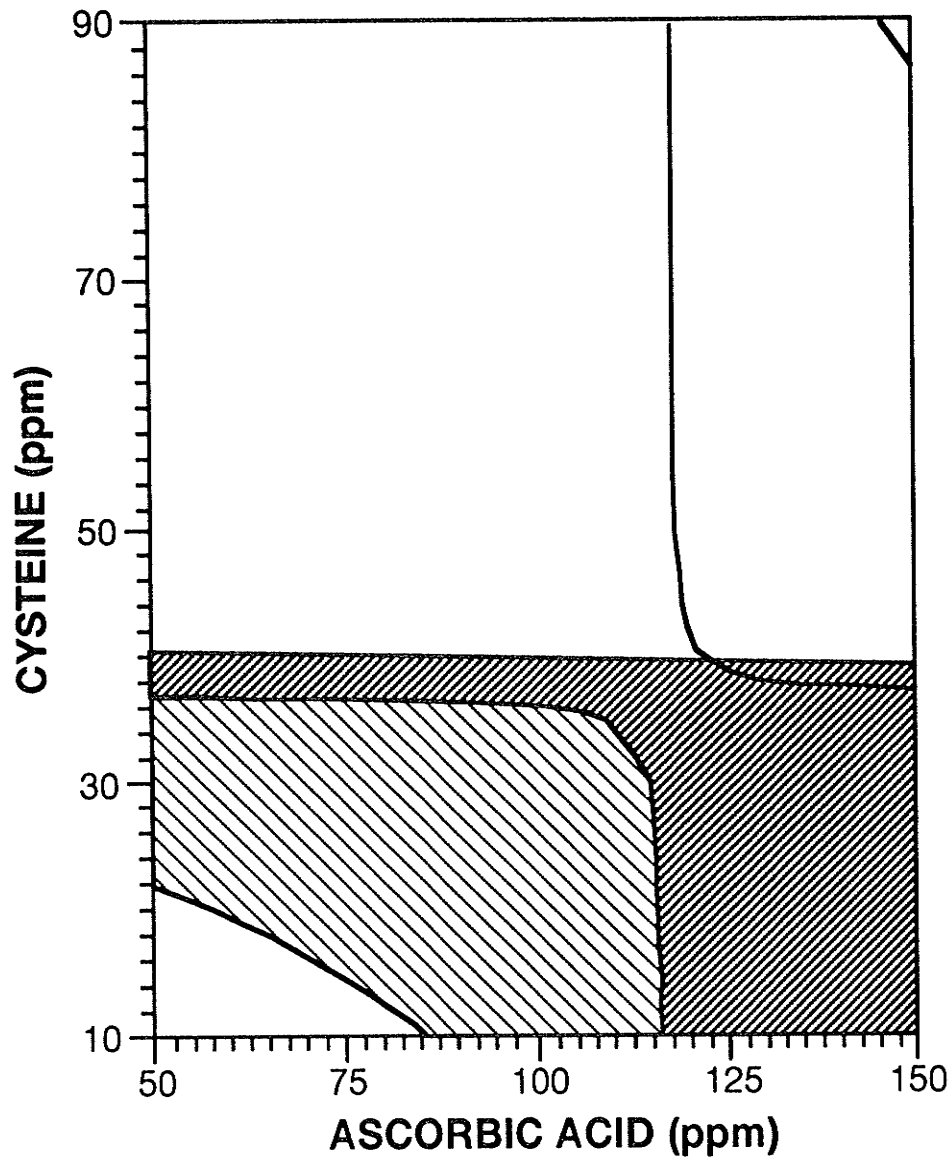


Figure 5.14. Superimposed contour plots illustrating the region of acceptability for multiple responses for breads prepared with the 25% CWES wheat flour blend and 20 SKB units α -amylase. The shaded regions met the criteria of acceptability for loaf volume and internal loaf characteristics. The darker region met the criteria of acceptability for loaf volume and internal and external loaf characteristics.

50% CWES Wheat Flour Blend

Two superimposed plots were prepared to identify optimum improver combinations for use with the 50% CWES blend, one with α -amylase at 20 SKB units and one with α -amylase at 60 SKB units (Figure 5.15). These plots illustrate the effect of the interaction between cysteine and α -amylase on internal loaf structure. When α -amylase increases from 20 to 60 SKB units, higher levels of cysteine can be used for its ability to reduce mix time and still give an acceptable product. If 20 SKB units α -amylase is used, cysteine addition less than 50 ppm plus ascorbic acid greater than 100 ppm would be expected to give bread quality results within the limits of acceptability for all three quality parameters. At 60 SKB units α -amylase, combinations of either high cysteine and low ascorbic acid or low cysteine and high ascorbic acid should give acceptable results for all three responses.

75% CWES Wheat Flour Blend

Breads made with 75% CWES blend and 60 SKB units α -amylase would be expected to have volumes and bread quality scores within the regions of acceptability across a wide range of ascorbic acid/cysteine combinations (Figure 5.16). Using either high ascorbic acid and low cysteine or low ascorbic acid and high cysteine combinations should give breads with scores for the three quality attributes within their limits of acceptability.

100% CWES Wheat Flour

The 100% CWES flour bread with 60 SKB units α -amylase should be within the regions of acceptability for all three responses across the whole range of ascorbic acid addition (Figure 5.17). A level of cysteine greater than 50 ppm should be used if a low ascorbic acid level is chosen, whereas any cysteine level up to about 70 ppm in conjunction with very high ascorbic acid would also give acceptable results for all quality attributes.

The use of superimposed plots was a useful tool in determining optimum combinations of improvers. From these plots it was evident that loaf volume alone was an insufficient indicator of bread quality as additives tested also had significant effects on the internal crumb structure and loaf appearance. Consideration of these effects through the use of superimposed plots was essential to determining the combination of improvers which optimize all three quality attributes in breads made with the two flours alone and in blends.

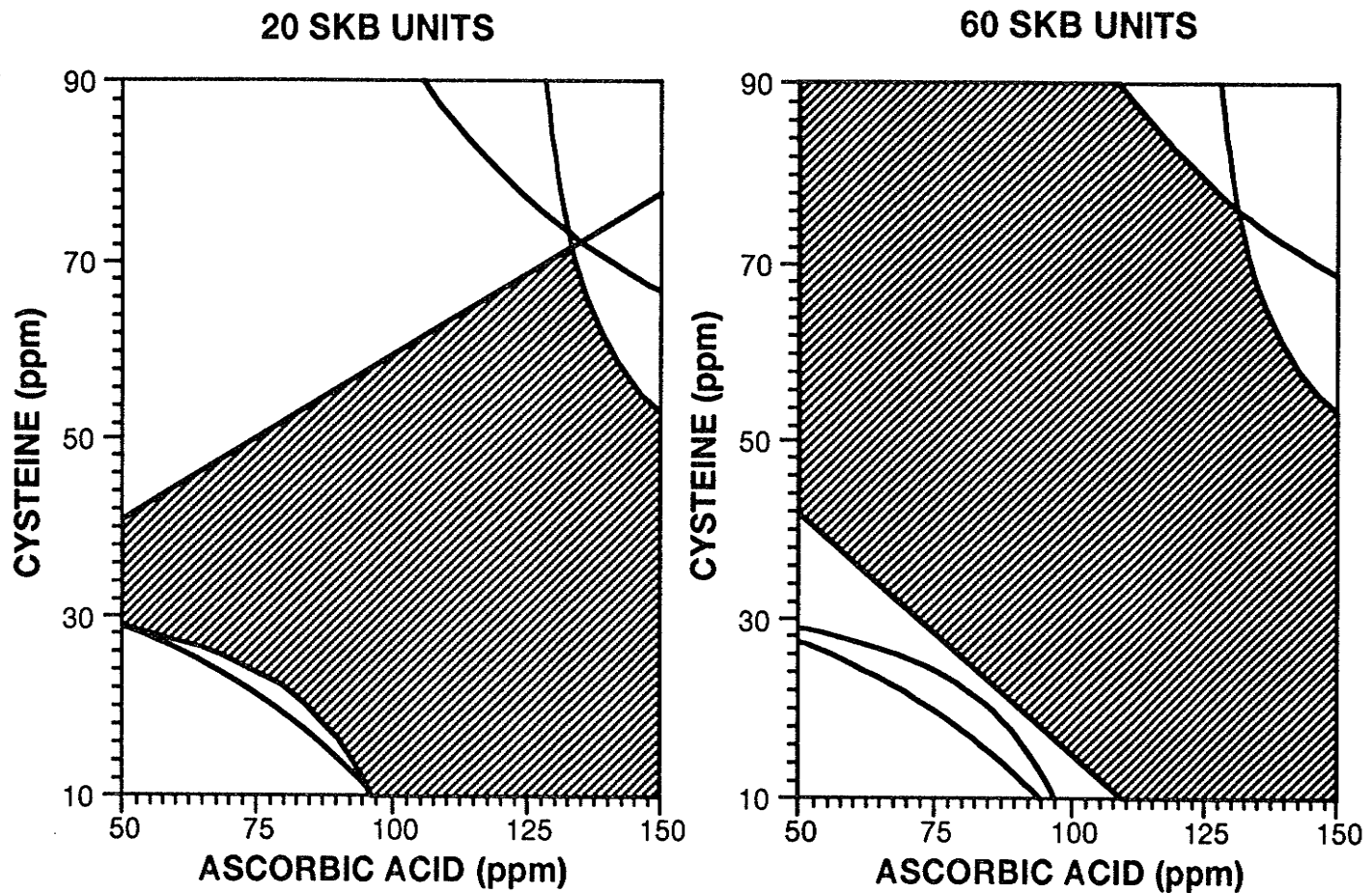


Figure 5.15. Superimposed contour plots illustrating the region of acceptability for multiple responses for breads prepared with 50% CWES wheat flour blend and 20 and 60 SKB units α -amylase. The shaded region meet the criteria of acceptability for the loaf volume, internal and external loaf characteristics.

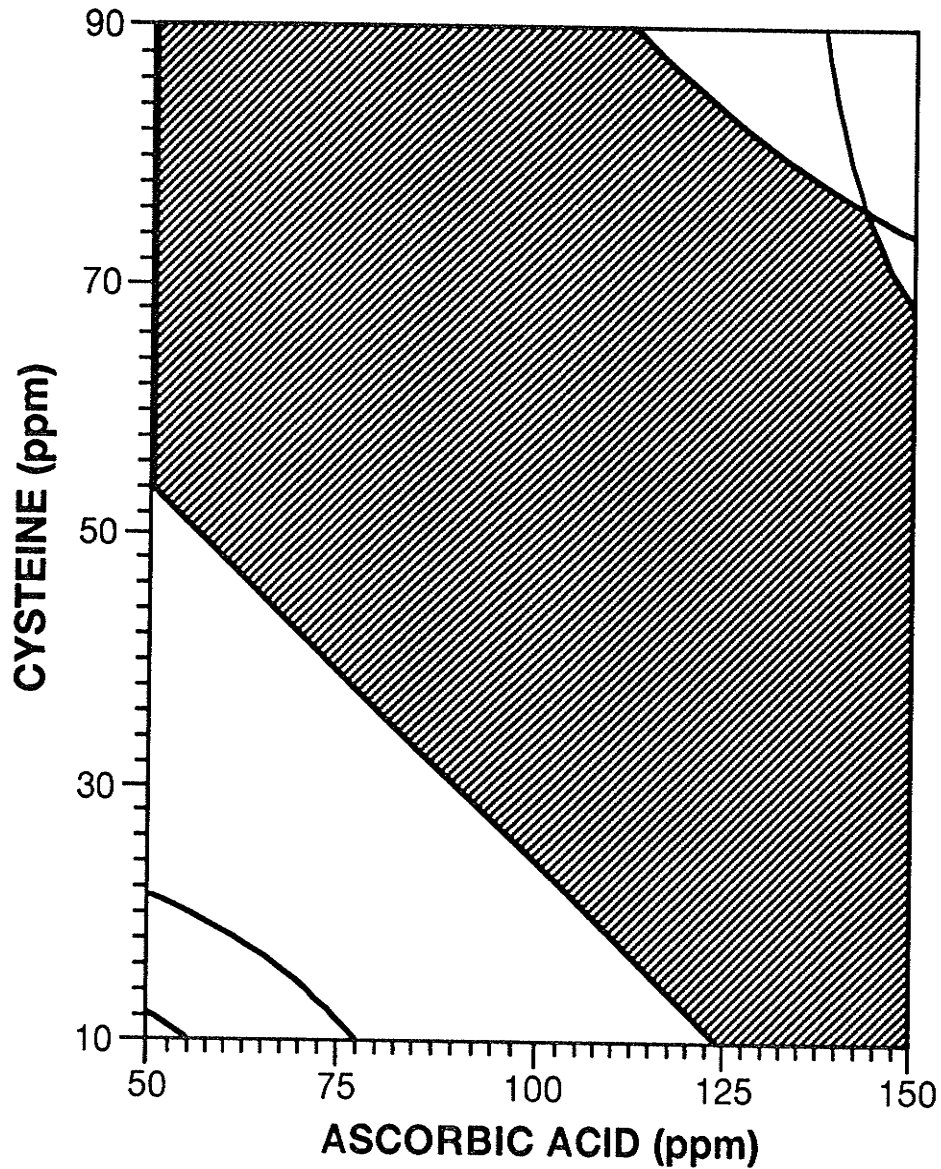


Figure 5.16. Superimposed contour plots illustrating the region of acceptability for multiple responses for breads prepared with the 75% wheat flour blend and 60 SKB units α -amylase. The shaded region met the criteria of acceptability for loaf volume, internal and external loaf characteristics.

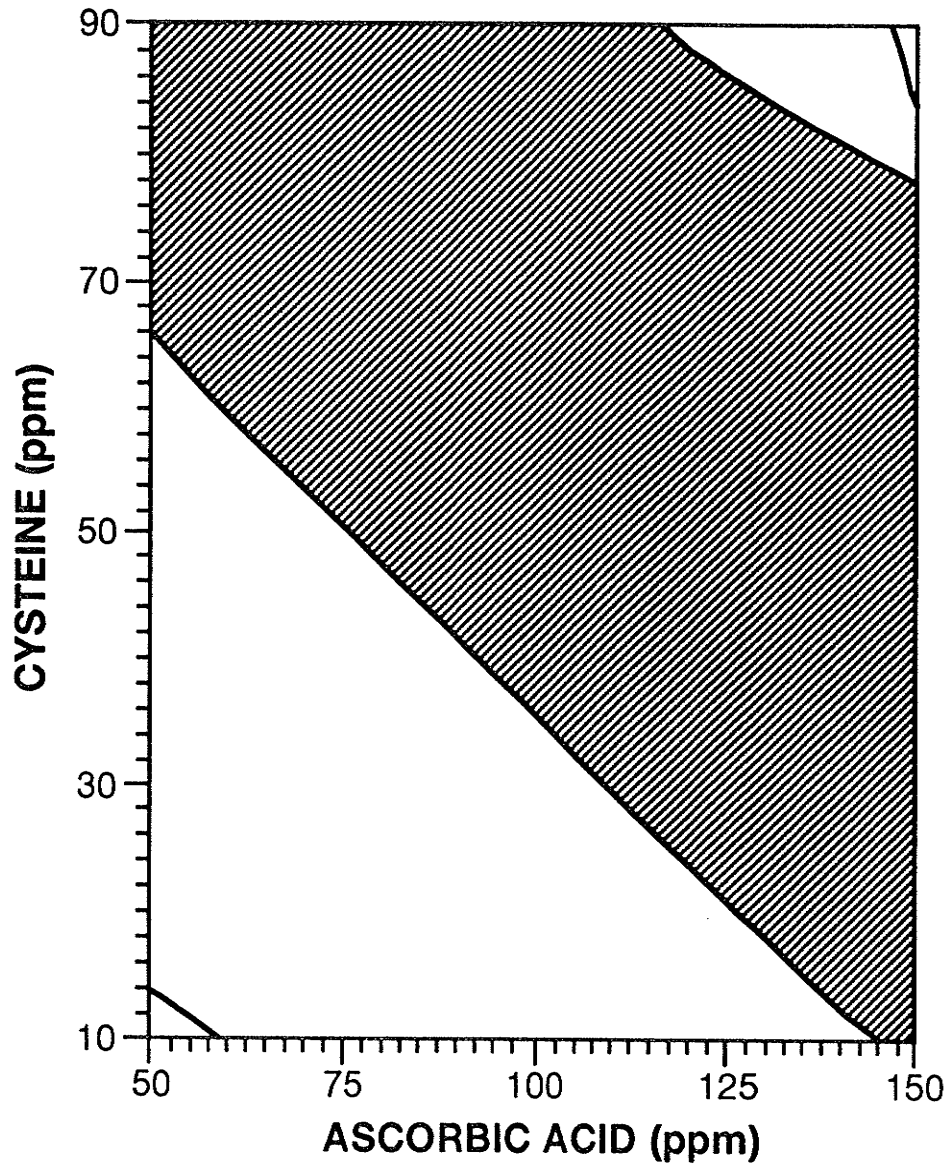


Figure 5.17. Superimposed contour plots illustrating the region of acceptability for multiple responses for breads prepared with 100% CWES wheat flour and 60 SKB units α -amylase. The shaded region met the criteria of acceptability for loaf volume, internal and external loaf characteristics.

DISCUSSION

The results of the optimization study indicated that identification of optimal improver combinations in different flour blends was a complex process. By limiting the number of variables in an optimization study to three of the most critical ones, the interpretation of variable effect is facilitated. In this experiment, only three improvers were examined in combination. However, by including the % CWES wheat flour in the design, a fourth variable was introduced and interpretation of the results became more difficult. This difficulty was overcome by exploring the improver effects for CWRS and CWES flours and three blends separately. Within each flour blend, the α -amylase requirement was identified. The requirement for cysteine and ascorbic acid and the interactions between them were then examined in each of the five flour blends.

CWES wheat flour played an important role in the improvement of internal and external loaf characteristics. According to the full model regression coefficients (Table 5.3), the % CWES in the blend was the only main effect for these responses with a percent confidence greater than 90%. Upon elimination of the less significant terms (Table 5.4), the importance of other terms to the improvement of loaf quality scores increased but the % CWES remained the most important determinant of these quality attributes. The ability of the extra strong flour to improve crumb structure and external appearance of breads is an unexpected argument for its use in blends with standard bread wheats. Most of the available information in the literature about the breadmaking performance of CWES wheat flour, usually Glenlea, has focussed on its loaf volume potential and mixing requirement rather than its ability to improve these other important bread quality characteristics (Bushuk, 1980; Bushuk et al, 1969). The contribution of these extra strong flours to the improvement of crumb grain is worth investigating further.

Cysteine significantly reduced the mixing requirement of doughs made with both CWRS and CWES flour. Cysteine splits disulphide bonds in the gluten network, thereby weakening the dough structure and lowering the energy required to develop the dough (Fitchett and Frazier, 1986). Mixograph studies have demonstrated decreased time to peak development of 30% with as little as 20 ppm cysteine (Lang et al, 1992; Weak et al, 1977). However, a strong quadratic effect of cysteine on mix time was also evident (Table 5.4). As cysteine addition increased above 50 ppm, the magnitude of its effect on mixing requirement decreases. For the CWRS flour, mix time was reduced by 40% with the first 50 ppm cysteine. Addition of a further 40 ppm resulted in a decreased mix time of only 13%. Finney et al (1971) saw similar results in which each additional 40 ppm cysteine up to a maximum of 120 ppm reduced dough development times by

1/3, 1/5 and 1/6, respectively. This quadratic effect should be considered when deciding what level of cysteine is feasible given the adverse effects it can have on the handling properties of CWRS flour doughs. Doughs with satisfactory handling properties could be obtained while receiving the maximum benefits of mix time reduction with a low level of cysteine.

A response surface experimental design was used in this study to ensure that important interactions were identified and could be interpreted clearly. Most notable was the effect of the interactions between % CWES in the blend and α -amylase on the loaf volume. Non-blended CWES flour breads gave higher predicted volumes and internal scores with a high level of α -amylase. Non-blended CWRS flour performed better in these respects with the lowest level of α -amylase. This difference in response to α -amylase of the two flours confirms the findings of Lukow and Bushuk (1984). These researchers looked at the effect of increasing α -amylase activity as a result of germination on the breadmaking properties of two different wheat cultivars, Glenlea (CWES wheat class) and Neepawa (CWRS wheat class). They found that the dough handling properties, loaf volumes and crumb and crust characteristics of breads made with the Glenlea wheat flour improved substantially with a low level of α -amylase due to germination, whereas the breads made with Neepawa wheat flour performed poorly at all levels of α -amylase activity.

The interaction between cysteine and ascorbic acid also had a pronounced effect on bread volumes. Generally, across all α -amylase levels, a combination of either low cysteine and high ascorbic acid or high cysteine and low ascorbic acid gave the best predicted loaf volumes. It has been stated that doughs with added cysteine require a higher level of oxidation to reform disulphide bonds which have been more readily broken in the presence of the cysteine (Ranum, 1992b). Finney et al (1971) suggested that an additional 5 ppm bromate should be added for every 40 ppm cysteine used. However, the need for higher ascorbic acid when cysteine was used was not observed in this experiment.

The experimental design used in this study emphasized the interactions between improvers. Studies which show increased ascorbic acid requirement with increased cysteine addition have been carried out using experimental methods in which the level of one variable is set and the ideal level of the second variable identified. This "one variable at a time" approach may give an indication of the requirement of one variable as a result of a second variable level but does not take into account interaction effects and does not identify variable combinations which optimize responses (Joglekar and May, 1987). Response surface methodology identifies combinations of variables which optimize a particular response rather than simply illustrating the effect which one variable has at a set level of another. This difference in experimental strategy is a possible

explanation for the discrepancy between the findings of this research and that of other authors.

Cysteine has been reported to have a detrimental effect on the crumb structure of wheat flour breads. Moss (1975) noted that when treated with 50 ppm cysteine, breads tended to have less uniform cell structure and thicker cell walls. Using a fairly strong hard red spring flour (variety Chinook), Kilborn and Tipples (1973) also reported decreased crumb texture score with increasing cysteine addition. However, the internal characteristics were not effected significantly by the addition of cysteine in this experiment. This may be explained in part by the interaction of cysteine with both the % CWES in the blend and the ascorbic acid.

The interaction between cysteine and CWES wheat flour indicates CWES wheat flour may moderate any negative effect of cysteine on internal loaf structure. Although there is a lack of information in the available literature on effects of cysteine in CWES wheat flour breads, work has been carried out to investigate the effect of cysteine in the wheat flour variety Red River 68, known primarily for its extremely long mixing requirements. Kilborn and Tipples (1972) found that breads prepared with this flour actually had improved crumb texture with increased cysteine addition. Finney et al (1971), who also worked with Red River 68 wheat flour, saw crumb grains of breads made with added cysteine which were of equal if not superior quality to the good control flour breads. These findings are supported by the results of this study, which show that the internal structure of breads made with CWES flour may tolerate a high level of cysteine without its apparent damaging effects.

Ascorbic acid may also have moderated the negative effect of cysteine on internal loaf structure. According to Table 5.4, the effect of ascorbic acid on the internal loaf characteristic scores was highly significant suggesting its strong involvement in the improvement of this quality attribute. Ascorbic acid has been shown to improve not only loaf volume of wheat flour breads but also their internal structure (Yamada and Preston, 1992), giving breads a finer crumb (Ranum, 1992a). This contribution to bread quality could negate to some extent the detrimental effects resulting from the addition of cysteine.

Superimposed contour plots were useful for identifying improver combinations which optimize loaf volume and internal and external loaf characteristics simultaneously. The region in which acceptable volumes and bread quality scores can be obtained was much larger when a greater proportion of CWES wheat flour was included in the blend. The extra strong flour appeared to improve the tolerance of breads to a wider range of improver combinations. By using CWES wheat flour in blends with standard bread flours, an added measure of protection against accidental overtreatment with some dough additives may be possible.

CONCLUSIONS

From this study, the effects of the three improvers on the quality of breads made with CWRS and CWES flours and blends were visually assessed through the use of contour and response surface plots. Complex interactions existed not only between the improvers but also between the improvers and the flour blends. Differences in optimum improver combinations were evident between the two flours, primarily in terms of the requirement for α -amylase. Improver combination which optimized loaf volume and internal loaf characteristics did not give good predicted results for external loaf scores.

CWES wheat flour had an improving effect on the internal and external loaf characteristics. Mix times increased significantly with increasing proportions of CWES in the blends. However, through the addition of cysteine, mix times comparable to those obtained with CWRS wheat flour were reached.

The % CWES in the blend and the level of α -amylase interacted strongly. The significance of this interaction was the basis for setting α -amylase at a set level for each flour blend when generating the contour and response surface plots. The CWRS wheat flour performed better in terms of loaf volume and internal loaf characteristics when α -amylase was added at the lowest level, whereas breads made with 100% CWES wheat flour were predicted to have greatest loaf volumes at the high level of α -amylase.

An interaction between cysteine and ascorbic acid was significant to both loaf volume and external loaf characteristics. High cysteine in conjunction with low ascorbic acid, and vice versa, gave best predicted results for these two response variables.

Overall, RSM was a useful experimental technique for understanding the way in which the three additives and the flour interact with each other and effect bread quality. Optimum improver combinations were identified for each of the five flours. However, because the optimum combinations are based on predictive models, it is essential to confirm that the results predicted for each response can be realized. To accomplish this, a final verification study should be carried out in which the quality of bread prepared with optimum improver combinations can be compared to the predicted quality scores. In such a way, the predictive power of each model can be assessed and the optimization process completed.

Chapter 6

VERIFICATION EXPERIMENT

INTRODUCTION

The final stage in the optimization process is to put the predicted solutions into practice (Haaland, 1989). This was accomplished by carrying out a verification experiment using optimized improver combinations for the CWRS and CWES wheat flours and the three blends. Two or three combinations which were predicted to result in breads with quality characteristics within the limits of acceptability were identified for each flour or blend. The specific objectives of this experiment were:

1. To compare actual and predicted responses for loaf volume, mix time requirement and external and internal loaf characteristics of breads made with optimum combinations of ascorbic acid, fungal α -amylase and cysteine.
2. To assess the ability of each response surface model to predict the outcomes for each response variable.
3. To compare outcomes for optimized loaves and control loaves (no additives) in terms of the four response variables within each flour blend.
4. To examine the effects of increasing proportions of CWES wheat flour in the blend on the quality of loaves made without additives.

MATERIALS AND METHODS

Materials

The materials used in the verification experiment were identical to those used in the optimization study. The flour characteristics were assumed to be unchanged during the short time period between the optimization experiment and this experiment.

Breadmaking Procedure

The same breadmaking procedure (Canadian Short Process) was used as described in screening experiment #2. The proof time of the test loaves was determined for each baking day based on the time required for a control loaf prepared with CWRS wheat flour to proof to 95 mm.

Evaluation

Loaf volumes were determined as outlined in screening experiment #1. The preparation of loaves for evaluation, and the scoring method used, was the same as described in the optimization experiment. Crumb color and brightness were not considered in the evaluation due to the unavailability of appropriate lighting equipment. Therefore, Hunterlab Tristimulus Colorimeter data (L and b values) was obtained for each of the five flours and all test breads in order to evaluate the yellowness and brightness of both the flour and the bread crumb. For the flour itself, a petri dish was filled with the flour and leveled with a knife. For the test breads, a 1/2 inch slice was taken from the center of the loaf and cut into a circle which fit into the petri dish. For both flour and bread samples, a first reading was taken, the petri dish rotated a quarter turn, and a second reading taken. L and b values were an average of the two readings.

Experimental Design

Based on the overlay plots generated in the optimization experiment, 2 improver combinations (treatments #1 and #2), predicted to produce loaves with quality characteristics within the limits of acceptability for each response, were selected for each of the five flours (100% CWRS wheat flour, 100% CWES wheat flour and 25%, 50% and 75% CWES blends). In some cases, where this region of acceptability was limited to a very small area of the plot, an improver combination within the region of acceptability for only two of the response variables, which gave better predicted scores for the other loaf quality characteristics, was chosen as treatment #2.

Upon evaluation of the 100% CWRS flour breads and the 25% and 50% CWES flour breads prepared during the first of the two baking days, it became evident that the interior loaf characteristics were not as ideal as had been predicted. Therefore, a third improver combination (treatment #3) expected to give improved internal characteristics, was tested. The selected combinations of ascorbic acid, α -amylase and cysteine for each of the five flours are summarized in Table 6.1. An improver-free loaf was also prepared for each flour (ascorbic acid, potassium bromate and malt syrup omitted from the formulation).

All baking runs were repeated. The first set was carried out on the first baking day, the third set on the second baking day and the second set was split between the two days. Within each baking day, baking order was randomized. The third improver combination selected for the 100% CWRS breads and the 25% and 50% CWES blend breads were prepared in triplicate on the second baking day.

Statistical Analysis

Analysis of variance was performed on all data using the GLM procedure in SAS (Statistical Analysis Systems). Significant differences in mix time, loaf volume and internal and external loaf characteristics between the optimized improver combinations within each flour blend and across all flour blends were determined. A paired t-test was also performed on replicates baked on different days to ascertain whether there were any significant "baking day" effects.

Table 6.1. Selected combinations of the three independent variables for use with CWRS and CWES alone and in blends which give predicted values within the acceptable range for at least two of the response variables loaf volume and external and internal loaf characteristics.

Flour	Treatment	Independent Variables		
		Ascorbic acid (ppm)	Alpha-amylase (SKB Units)	Cysteine (ppm)
100% CWRS	#1	150	20	10
	#2	100	20	15
	#3	60	20	15
25% CWES ^a	#1	150	20	20
	#2	120	20	10
	#3	60	20	20
50% CWES	#1	130	20	30
	#2	60	60	75
	#3	130	40	10
75% CWES	#1	60	60	70
	#2	150	60	30
100% CWES	#1	60	60	80
	#2	140	60	30

^a Remaining flour: CWRS wheat flour.

RESULTS

Breads were prepared using CWRS and CWES wheat flour alone, and in blends, with optimized combinations of ascorbic acid, α -amylase and cysteine. All test loaves had good volume and external appearance, and had dough mix times well within an acceptable range. However, internal loaf scores were much lower than anticipated. This may have resulted from temperature fluctuations in the lab which occurred as external temperatures reached a high 30°C. The mean loaf volumes, mix times and scores for external and internal loaf characteristics are included in Appendix XI. The standard deviations (STD) and coefficients of variation (C.V.) are also included.

In the following discussion, treatment numbers are followed by an abbreviated indication of the improver levels used (ascorbic acid/ α -amylase/cysteine). For example, treatment #2 (100/20/15) indicates that an improver mixture consisting of 100 ppm ascorbic acid, 20 SKB units α -amylase and 15 ppm cysteine was used.

Comparison of Predicted and Actual Response Outcomes

Table 6.2 summarizes the predicted effects and actual effects of outcomes of each optimum improver combination on loaf volume, mix time and external and internal loaf characteristics. All optimized improver formulations were predicted to meet the criteria of acceptability for loaf volume (1100 cc) and internal loaf score (14 points) (see Chapter 5). The predicted external loaf characteristic scores were lower than the minimum acceptable score (24 points) for treatments #2 (100/20/15) and #3 (60/20/15) of the 100% CWRS breads and treatment #3 (60/20/20) of the 25% CWES blend bread. The region of the overlay plots in which all three responses were within the acceptable range was very small. Therefore, at the risk of sacrificing some external loaf quality, a second improver combination was chosen outside the limit of acceptability for that quality parameter. Predicted mix times of all the test doughs were considered excellent, especially in flour blends with a higher proportion of CWES wheat flour.

Although the actual volumes of the optimized loaves did not match precisely the predicted volumes, most of the volumes were greater than 1100 cc, the lower limit of acceptability. Treatment #2 (150/60/30) of the 75% CWES blend and treatment #1 (60/60/80) of the 100% CWES bread were slightly below the acceptable limit. Two treatments gave external scores lower than expected (100% CWRS treatment #1 (150/20/10) and 75% CWES treatment #1 (60/60/70)) while the remaining loaves had scores greater than the acceptable limit of 24 points. Actual mix

Table 6.2.

Predicted and actual effects of the selected improver combinations on independent variables on the response variables loaf volume, mix time and external and internal loaf characteristics of breads made with CWRS and CWES wheat flour alone and in blends.

Flour Blend	Treatment	Predicted Outcomes ^a				Actual Outcomes ^b			
		Loaf Volume (cc)	Mix Time (min)	External Score	Internal Score	Loaf Volume (cc)	Mix Time (min)	External Score	Internal Score
100% CWRS	#1	1204	6.8	24.7	14.8	1155	5.8	22.0	9.3
	#2	1152	6.4	22.4	15.7	1218	5.5	26.5	11.5
	#3	1104	6.4	20.7	17.1	1125	5.7	26.5	13.0
25% CWES	#1	1192	6.8	25.0	16.7	1183	6.1	25.7	8.3
	#2	1147	7.7	24.2	18.2	1185	7.1	24.7	9.7
	#3	1103	6.8	22.2	16.9	1145	5.9	29.0	16.0
50% CWES	#1	1151	6.9	25.1	17.6	1143	6.8	25.7	10.0
	#2	1181	5.1	29.0	16.4	1145	4.5	25.3	12.7
	#3	1145	8.6	25.6	14.0	1145	8.1	27.5	13.5
75% CWES	#1	1198	6.1	29.5	16.6	1168	5.5	22.7	12.7
	#2	1210	7.7	26.3	18.0	1088	8.1	27.3	11.3
100% CWES	#1	1209	6.9	31.5	17.5	1082	6.1	27.3	14.0
	#2	1247	8.6	27.2	17.0	1125	9.5	27.3	15.0

^a Predicted outcomes based on the response surface equation generated for each response variable in the optimization study.

^b Actual outcomes are means of 2 or 3 replications.

times were close to the expected times. For the internal loaf characteristics, the response surface model developed was not successful at predicting actual outcomes in most cases. Both breads prepared with 100% CWES flour and one with the 25% CWES blend had internal scores above the lower acceptable limit. Some of the loaves, particularly those with 50% or less CWES flour in the blend, performed very poorly in terms of this characteristics, receiving scores of 10 points or less.

Effect of CWES Wheat Flour on Quality of Improver-free Bread

Table 6.3 summarizes the effect of CWES wheat flour on the quality of breads prepared without additives. Plate 6.1 depicts the first replication of the improver-free loaves made with the five different flour blends. There was a general tendency for loaf volume to decrease when % CWES increased above 50%. No significant ($p \leq 0.05$) differences in loaf volume were detected in breads when the % CWES in the blend was 50% and lower. The 75% and 100% CWES loaves were significantly smaller than the 25% CWES blend breads. Overall, the largest volumes were obtained when 25% CWES wheat flour was used in the blend.

Mixing times did not increase significantly ($p \leq 0.05$) until the bread contained more than 50% CWES flour. Over 50% CWES flour, the mixing requirement increased significantly.

The external loaf characteristics were similar across all flour blends, with no significant ($p \leq 0.05$) differences detected. Surprisingly, the same results were found for the internal loaf characteristics. The 100% CWRS wheat flour breads received an average score of 12.0 points, whereas the breads prepared with 50% and 75% CWES wheat flour had average scores greater than 16.0 points. However, the variability in the scores for this response variable was very high for both treatment and improver-free loaves (see Appendix XI). For the test loaves, the coefficients of variation across the replications was at times in excess of 30%. Possible sources of this variation will be discussed in a later section.

Comparison of Optimized and Improver-free Loaves within Flour and Blends

Loaf volumes, mix times, external and internal scores for optimized and improver-free breads are given in Table 6.4.

Breads made with 100% CWRS flour had similar results for all four responses measured. Compared to improver-free breads, those with added improver mixtures had substantially reduced mix times. Improver-free breads did not differ from test loaves in terms of external and internal loaf characteristics, although treatment #2 (100/20/15) gave significantly larger loaf volumes. The

Table 6.3. The effect of percent CWES wheat flour in the blend on mean^a loaf volume, mix time, external and internal characteristic scores of breads prepared without improvers.

Flour Blend	Loaf Volume (cc)	Mix Time (min)	External Characteristics	Internal Characteristics
100% CWRS	1033ab ^b	7.7a	26.5a	12.0a
25% CWES	1068a	8.3a	27.7a	11.7a
50% CWES	1013ab	9.2a	27.7a	16.3a
75% CWES	967b	11.5b	27.3a	16.7a
100% CWES	953b	13.9c	27.0a	14.7a

^a All values are means of three replications.

^b Values with the same letters are not significantly different at $p \leq 0.05$.

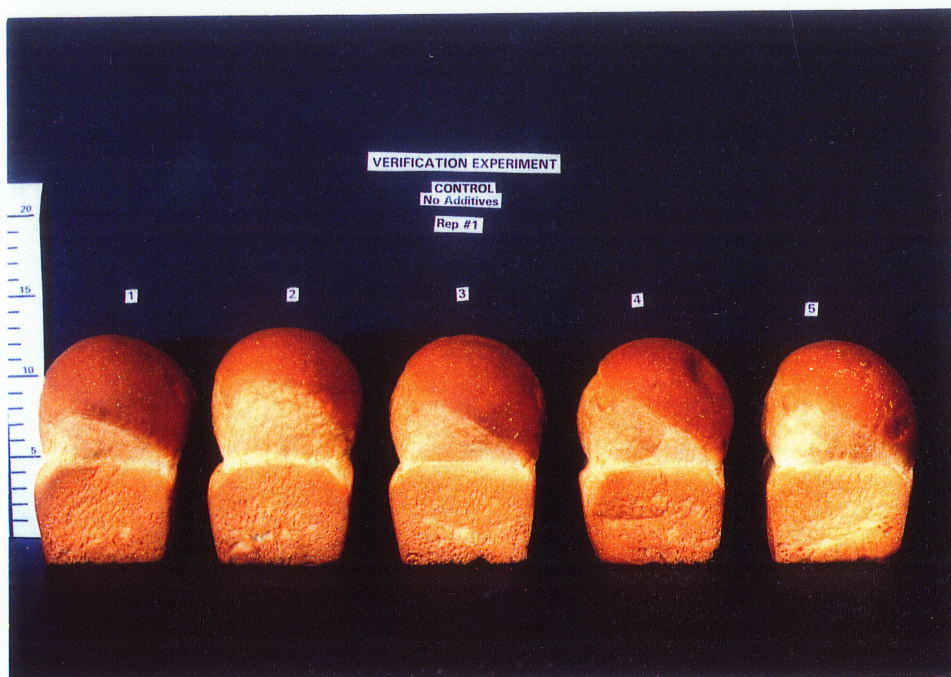


Plate 6.1 Breads prepared without improvers from: 1. 100% CWRS flour; 2. 25% CWES blend; 3. 50% CWES blend; 4. 75% CWES blend; and 5. 100% CWES flour.

Table 6.4. Mean^a loaf volumes, mix times, external and internal characteristic scores of breads made with selected improver combinations and without improvers in CWRS and CWES wheat flour alone and in blends.

100% CWRS				
Treatment	Loaf Volume (cc)	Mix Time (min)	External Character	Internal Character
1	1155ab ^b	5.8a	22.0a	9.3a
2	1218a	5.5a	26.5a	11.5a
3	1125ab	5.7a	26.5a	13.0a
Improver-free	1033b	7.7b	26.5a	12.0a
25% CWES				
Treatment	Loaf Volume (cc)	Mix Time (min)	External Characteristics	Internal Characteristics
1	1183a ^b	6.1a	25.7a	8.3a
2	1185a	7.1b	24.7a	9.7ab
3	1145a	5.9a	29.0a	16.0b
Improver-free	1065b	8.3c	27.7a	11.7ab
50% CWES				
Treatment	Loaf Volume (cc)	Mix Time (min)	External Characteristics	Internal Characteristics
1	1143a	6.8a	25.7a	10.0a
2	1145a	4.5b	25.3a	12.7a
3	1145a	8.1ac	27.5a	13.5a
Improver-free	1013b	9.2c	27.7a	16.3a
75% CWES				
Treatment	Loaf Volume (cc)	Mix Time (min)	External Characteristics	Internal Characteristics
1	1168a	5.5a	22.7a	12.7a
2	1088ab	8.1b	27.3b	11.3a
Improver-free	967b	11.5c	27.3b	16.7a
100% CWES				
Treatment	Loaf Volume (cc)	Mix Time (min)	External Characteristics	Internal Characteristics
1	1082ab	6.1a	27.3a	14.0a
2	1125a	9.5b	27.3a	15.0a
Improver-free	953b	13.9c	27.0a	14.7a

^a All values are means of three replications.

^b Within each flour, values with the same letters are not significantly different at $p \leq 0.05$.

improver mixture used in this treatment had a midlevel of ascorbic acid rather than the high or low levels used in the other two treatments.

With the 25% CWES wheat flour breads, no significant difference was detected in the external and internal loaf characteristics between the treatment and improver-free loaves. Although the improver-added loaves had similar volumes, all three improver mixtures successfully increased loaf volumes and reduced mix time requirements compared to improver-free loaves. Treatments #1 (150/20/20) and #3 (60/20/20) had the lowest mix times because of the higher level of cysteine used.

External and internal loaf characteristics did not differ significantly between treatments or between treatments and improver-free in breads made with 50% CWES flour blend. Similar loaf volumes were achieved for all three treatments, showing a significant improvement over control loaves. Cysteine at a level of 75 ppm in treatment #2 (60/60/75) resulted in a reduction in mix time of approximately 50% without any detrimental effects on loaf quality. However, at this level, these doughs tended to be slightly sticky and difficult to handle.

At 75% CWES flour, only treatment #1 (60/60/70) significantly improved loaf volumes over those achieved for improver-free loaves. This treatment included a higher level of cysteine (70 ppm) and therefore significantly reduced mix time requirements compared to treatment #2 (150/60/30). However, the external loaf characteristics were not as acceptable for these loaves. Similar internal loaf scores were obtained for the two treatments and although not significant at $p \leq 0.05$, the improver-free breads seemed to have better crumb structure.

The external and internal loaf characteristics were similar for treatment and control loaves made with 100% CWES flour. Both treatments successfully reduced mix time, with the higher level of cysteine (80 ppm) reducing the mix time back by more than 50%. However, this treatment in which 80 ppm cysteine and 60 ppm ascorbic acid were used did not result in a significant improvement in loaf volume compared to the improver-free breads. The second treatment (140/60/30), in which 140 ppm ascorbic acid and 30 ppm cysteine was included gave much better loaf volume results.

Comparison of Optimized Loaves across Flour Blends

The data obtained from all optimized loaves for all flour blends was combined and analyzed to see whether breads of equal quality could be obtained regardless of the amount of CWES wheat flour included in the blend when an optimized improving system was used. The results from the analysis are included in Table 6.5. According to these results, differences in loaf

Table 6.5. Mean^a loaf volumes, mix times, external and internal characteristic scores for breads prepared with selected optimum improver combinations for CWRS and CWES wheat flour alone and in blends.

Flour Blend	Treatment	Loaf Volume (cc)	Mix Time (min)	External Characteristics	Internal Characteristics
100% CWRS	#1	1155a ^b	5.8cd	22.0a	9.3ab
	#2	1218a	5.5cd	26.5a	11.5ab
	#3	1125a	5.7cd	26.5a	13.0ab
25% CWES	#1	1183a	6.1cd	25.7a	8.3b
	#2	1185a	7.1bc	24.7a	9.7ab
	#3	1145a	5.9cd	29.0a	16.0a
50% CWES	#1	1143a	6.8bc	25.7a	10.0ab
	#2	1145a	4.5d	25.3a	12.7ab
	#3	1145a	8.1ab	27.5a	13.5ab
75% CWES	#1	1168a	5.5cd	22.7a	12.7ab
	#2	1088a	8.1ab	27.3a	11.3ab
100% CWES	#1	1082a	6.1cd	27.3a	14.0ab
	#2	1125a	9.5a	27.3a	15.0ab

^a Means are averages over two or three replications.

^b Values with the same letter are not significantly different at $p \leq 0.05$.

volume and external loaf characteristics were not considered statistically significant, likely because of the high degree of variability observed. Only two loaves differed in their internal scores. The 25% CWES blend bread had a very good internal score of 16 points when a combination of 60 ppm ascorbic acid, 20 SKB units α -amylase and 20 ppm cysteine was used. Very good loaf volumes and external scores were also achieved with this improver mixture, and mix times were highly acceptable. Using the same flour blend of 25% CWES flour with a combination of 150 ppm ascorbic acid, 20 SKB units α -amylase and 20 ppm cysteine resulted in breads with very poor internal scores. The high level of ascorbic acid seemed to be responsible for this noticeable difference in bread quality.

Evaluation of Flour and Bread using the Hunterlab Tristimulus Colorimeter

The Hunterlab Tristimulus Colorimeter was used to gain information about the color and brightness of the two different flours and breads and to see whether the additives in any way affected these parameters. In the case of bread and flour, it is necessary only to consider the L and b values. The L value gives an idea of the lightness of the bread crumb, with the higher number indicating a lighter crumb. The b value, when positive (+), indicates the yellowness, with a higher value meaning a greater degree of yellow in the test sample. Table 6.6 summarizes the L and b values for both the flours and the baked loaves.

Only a very small difference existed between the CWRS and CWES wheat flour, with the latter being slightly whiter (lighter). The two flours differed more in the degree of yellowness, which was noticeable simply by visually comparing the flours. The CWRS wheat flour was more yellow than the CWES wheat flour, the b values being 10.68 and 8.87 for CWRS and CWES, respectively. It is likely that this difference would manifest itself in the crumb of the baked bread resulting in the CWES wheat flour breads having a whiter crumb.

The whiteness of the control loaves increased slightly (higher L value) as greater amounts of CWES wheat flour were included in the blend while the b values dropped. The degree of change in values between 100% CWRS and 100% CWES control breads was comparable to the degree of change in the straight flour. No great differences existed between the test loaves within each flour for either the L value or the b value, suggesting that the improver combinations chosen did not affect the color and brightness of the bread crumb.

Paired-Comparison T-Test

In order to determine whether the variability in the data was a result of room conditions

Table 6.6. Mean Hunterlab Tristimulus Colorimeter values^a for CWRS and CWES wheat flours alone and in blends. Values are for flours and for breads prepared with selected improver combinations and without improvers.

Flour	Treatment	L Value ^b	Value ^c
100% CWRS	Flour ^d	91.51	10.68
	#1	78.16	16.21
	#2	78.73	15.41
	#3	79.47	16.61
	Improver-free	77.70	16.25
25% CWES ^e	Flour	91.63	10.36
	#1	78.72	15.64
	#2	78.78	15.34
	#3	79.16	16.35
	Improver-free	78.92	15.92
50% CWES	Flour	91.79	9.80
	#1	79.01	14.98
	#2	79.53	15.86
	#3	80.60	15.77
	Improver-free	79.61	15.09
75% CWES	Flour	91.93	9.37
	#1	79.45	15.70
	#2	80.04	15.09
	Improver-free	81.14	14.15
100% CWES	Flour	92.15	8.87
	#1	80.70	15.35
	#2	80.18	14.67
	Improver-free	80.14	14.11

^a Values are means over 3 replications with two readings on each replication.

^b L = lightness values, where 0=black and 100=white.

^c b = blue-yellow, where (-) indicates blue, (+) indicates yellow.

^d Measurement taken on flour sample.

^e Remaining flour: CWRS wheat flour.

on the two different baking days, a paired-comparison T-test was carried out. This test indicated whether the mean difference between replicates baked on the two days is significantly different than zero. The results of the test showed that no differences existed in mix times and external and internal loaf scores between replicates baked on separate days. However, loaf volumes of breads prepared on the second baking days were significantly smaller than the same formula breads prepared on the first baking day. Thus, the variability associated with the loaf volumes of same treatment breads may have been a result of this baking day effect.

DISCUSSION

The verification experiment was essential for assessing the ability of the response surface models to predict accurately the bread quality characteristics at specific improver combinations. This study confirmed that the models developed in the optimization experiment were fairly accurate in predicting mix time, loaf volume and external loaf characteristic score when the optimized improver combinations were used. A majority of the breads produced had scores well within the limits of acceptability for these response variables. However, the internal scores of the optimized breads were much below the values predicted by the response surface equation. This may have in part been due to the high degree of variability encountered between replicates for this response variable.

Test loaves baked on the second baking day were significantly smaller than those baked on the first. The temperature in the baking laboratory were not controlled and the extreme temperature conditions of the time in which the verification experiment was carried out may have contributed to these differences. The room temperature was greater than 36°C on the first baking day and dropped slightly on the second baking day. As the rheological properties of bread dough are very sensitive to temperature, it was not surprising to find that some significant differences did exist in loaf volumes of same formula breads between the two baking days. This excessive temperature may have in part been responsible for the variability in the internal loaf scores and for the poor internal loaf structure of test loaves produced in this experiment.

By preparing breads without any improving agents, it was possible to examine the effects which CWES wheat flour had on bread quality. In the screening and optimization experiments, breads made with CWES wheat flour generally received better internal and external scores than the CWRS flour breads, with only marginally smaller loaf volumes. The verification experiment confirmed that loaf volumes of breads made with CWES flour were significantly smaller than the CWRS flour breads. However, the differences in bread quality scores were no longer significant when improvers were excluded from the formulation. The difference in bread quality observed as a result of CWES wheat flour in the screening optimization experiments may have been due to the improvers added in the standard (control) bread formulation, ie. 37.5 ppm ascorbic acid and 30 ppm potassium bromate. This improver combination may have been detrimental to the quality of the CWRS breads, while the CWES breads tolerated the additives with minimal effect on loaf quality scores.

Overall, for the optimized loaves prepared in this experiment, differences in loaf volume

and bread quality scores were not considered significant. By including cysteine in the improver mixtures, mix times of doughs prepared with higher proportions of CWES wheat flour were comparable to those of the CWRS flour doughs. Thus, it was possible to produce breads from CWES wheat flour of very good quality, high loaf volume and acceptable mix times. Although the breads often had lower than ideal internal structures with the addition of additives, this was a sacrifice which was made in order to attain acceptable volumes.

The blend of 25% CWES/75% CWRS in conjunction with 60 ppm ascorbic acid, 20 SKB units α -amylase and 20 ppm cysteine produced very good breads. The average mix time of this dough formulation was 5.9 minutes. Average loaf volume was 1145 cc and the scores for external and internal loaf quality were 29.0 and 16.0, respectively. Generally, CWES wheat flour is used in blends for its ability to carry weaker flours at a level of about 30% (Preston, 1994). The excellent results obtained at the 25% CWES flour level in this study coincides well with the blending level used in industry.

The breads made with the improver mixtures optimized here can be of the same quality as bread made with a standard, bromated formula. The standard CSP formulation used to produce control loaves in this series of experiments included 37.5 ppm ascorbic acid plus 30 ppm bromate. The control loaves produced in screening experiment #2 were prepared with a water absorption level of FAB + 1% as were the optimized breads produced in the verification experiment. A comparison of the mix times, loaf volumes and bread quality scores of control loaves produced in screening experiment #2 with the best optimized breads made with the two flours alone are included in Table 6.7. Using CWRS wheat flour, breads of equal quality to that obtained using a standard bromated formula were produced. For the CWES wheat flour breads, the optimized formula used improved loaf volume and gave internal and external scores similar to that achieved with the bromated formulation. However, the mix times were drastically reduced to a much more acceptable time of 9.5 minutes. Thus, by using an improver mixture which has been optimized for use with a particular flour, results of equal quality to a standard bromated formulation can be achieved.

Table 6.7. Loaf volumes, mix times and internal and external loaf scores of control breads prepared with CWRS and CWES wheat flours using the standard CSP^a bread formulation^b and the optimized improver mixtures which gave the best overall results for these flours in the verification experiment.

Response	CWRS Wheat Flour		CWES Wheat Flour	
	Standard Formula	Optimized Formula	Standard Formula	Optimized Formula
Loaf Volume (cc)	1155	1125	1035	1125
Mix Time (min)	8.0	5.7	21.2	9.5
External Loaf Characteristics	28.0	26.5	27	27.3
Internal Loaf Characteristics	12.0	13.0	17	15.0

^a Canadian Short Process.

^b Standard CSP formulation included 37.5 ppm ascorbic acid plus 30 ppm potassium bromate.

CONCLUSION

The verification step was a very important part of an optimization study. Before any recommendations can be made based on the results of the optimization study, it is important to first ensure that the optimized improver mixtures work in practice. The results of this verification study indicated that the response surface models developed in the optimization study enabled the production of breads with quality characteristics within the limits for all quality parameters except internal loaf scores. The extreme temperature conditions in the laboratory may explain in part the poor internal scores obtained for most of the breads prepared.

The improver mixtures tested did not affect the brightness or degree of yellowness of the bread crumb. However, the CWES flour was not as yellow as the CWRS wheat flour, a characteristic which was also manifested in the crumb of the bread.

Breads of equal quality were produced across all flour blends by using specific improver combinations. The CWES loaf quality scores did not improve substantially over those obtained when no improvers were added. However, significant increases in loaf volume were achieved and mix times were reduced substantially. The loaf volumes of the CWRS breads also improved with the optimized improvers, but had lower scores for the external and internal loaf characteristics than when no improvers were added at all. Breads with quality characteristics equal to those obtained using a standard bromated formulation were attained for both CWRS and CWES wheat flours.

In this experiment, excellent breads were made using a blend of 25% CWES and 75% CWRS wheat flours with an improver mixture consisting of 60 ppm ascorbic acid, 20 SKB units α -amylase and 20 ppm cysteine. These breads had very high volumes (1145 cc), excellent external (29.0 points) and internal (16.0 points) scores and very low mix times (5.9 minutes).

The 100% CWRS flour performed best with a combination of 60 ppm ascorbic acid, 20 SKB units α -amylase and 15 ppm cysteine. For the 100% CWES flour bread, best results were achieved using 140 ppm ascorbic acid, 60 SKB units α -amylase and 30 ppm cysteine.

Chapter 7

GENERAL DISCUSSION

The primary objective of this study was to develop bromate-free improving system for use with CWRS and CWES wheat flours, both alone and in blends. In order to meet this objective, an experimental procedure particularly useful for product optimization was utilized. Response surface methodology (RSM) proved to be an appropriate and efficient technique for the identification of improver combination which optimize several responses simultaneously. The optimization process was carried out in three stages: screening, optimization and verification. Each of these stages were dealt with as separate experiments, and are included in the individual chapters.

From the review of literature it was evident that many additives improve bread quality. However, when used alone, none have been proven to be effective bromate replacers. Rather combinations of additives such as ascorbic acid, enzymes and surfactants are more effective at restoring the loss in bread quality observed when bromate is removed from the bread system. Another approach which has not been addressed in the literature is the use of flours from the extra strong wheat class (CWES wheat flours) for the improvement of dough handling properties, dough tolerance and quality of breads made without bromate.

In the initial screening experiments, in which both flours were baked as control loaves using the standard CSP bread formulation, differences in the handling properties of doughs prepared with CWRS and CWES wheat flour were evident. CWES wheat flour doughs were very elastic and tight whereas the CWRS flour doughs were much more extensible. This was as expected given the available extensigraph data for this wheat flour which show very much higher extensigraph heights (resistance to extension) and larger extensigraph areas for CWES doughs compared to CWRS doughs (Preston et al, 1993). The greater extensibility of CWRS doughs may have contributed to the larger loaf volumes obtained for these loaves compared to volumes of loaves made with CWES wheat flour. It is generally thought that the viscoelastic nature of wheat flour dough is the main factor determining breadmaking (Schofield, 1986). The balance between the elastic and viscous properties as governed by the glutenin and gliadin protein fractions may be the basis for differences in loaf volume potential of CWRS and CWES doughs.

The contribution of CWES flour in blends to improved dough properties was evident in the optimization experiment. Whereas CWRS flour doughs tended to be very extensible and

sticky, those in which part of the CWES flour was replaced with CWES were smooth and elastic with a reduced tendency to stick in the sheeter at dough make-up.

CWES wheat flour played an important role in the improvement of internal and external loaf characteristics. The results of screening experiment #1 summarized in Tables 3.5 and 3.7 show that the overall means for crumb scores were lower for the CWRS breads than for the CWES breads. Similarly, Tables 4.4 and 4.6 show considerable differences in both external and internal characteristic scores between breads made with the two flours. Overall, the CWES flour produced breads with better symmetry and finer crumb structures than the CWRS breads, with only slightly lower average loaf volumes. In the optimization study, the regression equation coefficients summarized in Tables 5.3 and 5.4 also show that CWES flour significantly improved both the external appearance and internal structure of breads. In the past, the emphasis has generally been on the contribution of CWES flour to frozen dough production and on the ability of CWES flour to carry weaker flours in blends. However, the contribution of CWES flour to the improvement of bread quality, when used alone or in blends with other flours, should also be stressed.

CWRS and CWES wheat flours responded differently to α -amylase activity. Both screening experiments showed that α -amylase had important effects on the volume of CWES breads but not CWRS breads. The levels used initially improved CWES loaf volumes, a finding which supports the results of a study carried out by Lukow and Bushuk (1984). These researchers found that flour milled from germinated Glenlea wheat, which had a low level of α -amylase, performed better in baking tests than the flour milled from sound Glenlea wheat. The α -amylase level used in screening experiment #2 substantially decreased loaf volumes, suggesting that the level tested was too high and was detrimental to loaf volume. In both these experiments, the changes in CWRS bread loaf volumes as a result of increased α -amylase addition was not considered important. The CWRS wheat flour performed better at very low α -amylase levels, whereas the CWES bread performed well across all α -levels tested. Thus, CWES flour exhibited greater tolerance to α -amylase activity compared to CWRS flour. In order to find an explanation for this difference in α -amylase requirement, it may be necessary to consider differences in the starch component of the two flours. Starch is a very important part of the bread system and may contribute in part to the differences in baking performance found between different wheat cultivars.

Doughs prepared with CWES flour alone or in blends were also able to tolerate a greater amount of cysteine than CWRS flour doughs. At 50 ppm cysteine, the 100% CWRS wheat flour

doughs were sticky and difficult to handle. Conversely, doughs prepared with 100% CWES flour plus 50 to 90 ppm cysteine had highly acceptable handling properties. As the use of cysteine as a mix time reducer may be necessary for preparing doughs with CWES wheat flour and blends, its tolerance to levels as high as 50 ppm is crucial.

Of major concern during the experimental period was the ability of the proofing cabinet to maintain a constant temperature and humidity level. During any given baking day, changes in the humidity level in the proofer were evident by periods of condensation on the proofer door windows, followed by times of no condensation whatsoever. This may have been one of the causes of variability in the data observed throughout the study. However, in screening experiment #2, the coefficients of variation associated with external and internal loaf characteristic scores differed markedly for the two flours. The scores for the external and internal loaf characteristics ranged from 17 to 26 points and 12 to 24 points, respectively, for the four CWRS flour breads prepared with mid levels of all additives (Table 4.3). Such variability was not evident in the four centre point formulation CWES flour breads. Perhaps the bread doughs made with the extra strong flour were more tolerant to temperature changes in the proofing cabinet. The use of CWES wheat flour in blends with other breads flours could improve tolerance of doughs made without bromate.

The experimental design used in this research did not lend itself to the determination of individual improver effects. The optimization experiment showed that ascorbic acid had little effect on the volume of test loaves although it has been reported that this oxidant improves loaf volume. The lack of effect seen in the experiment likely occurred because of the very strong interaction between ascorbic acid and cysteine. To assess the effect of ascorbic acid on loaf volume, it was necessary to hold both α -amylase and cysteine at their mid-levels. As a result, cysteine level would not have been optimum for the given ascorbic acid level, and thus the effect of the ascorbic acid under optimal conditions was not predicted. To determine the oxidative requirement of a flour, a simple experimental design which includes only the oxidant tested with all other factors controlled would be more appropriate.

The verification experiment revealed some interesting findings regarding the effects of potassium bromate, ascorbic acid and possibly malt syrup on the mixing requirement of CWES wheat flour doughs. The mix times of CWRS and CWES wheat flour control doughs prepared in screening experiment #2 (water absorption: FAB + 1%) were 7.5 and 21.2 minutes, respectively. In the verification experiment, the mix times of the CWRS and CWES wheat flour doughs prepared without any additive (no potassium bromate, ascorbic acid or malt syrup) were

7.7 and 13.9 minutes, respectively. Although there was little difference in mix times between the two formulations in the CWRS doughs, mix times of the CWES doughs were reduced by approximately 33% through the elimination of dough additives. Mixograph data published by Lang et al (1992) show that potassium bromate and malt did not affect time to peak of doughs prepared with a hard red winter bread wheat flour but ascorbic acid increased this time slightly when levels of addition increased. However, these researchers did not include flours milled from different wheat varieties known to have extended mixing requirements. Further research into this phenomenon would be justified given the consistent use of high levels of oxidation in baking procedures used to screen wheat varieties for their breadmaking potential.

SUMMARY AND CONCLUSIONS

RSM was an effective experimental technique with which to examine the effects and interactions of several additives in a bread formulation. Initially, screening experiments were carried out to reduce the number of potential improvers to a number which could easily be optimized. From the first of the screening experiment, in which seven improvers were assessed for their relative effectiveness on bread quality, it was found that ascorbic acid and protease were important to the improvement of CWRS wheat flour bread volume whereas α -amylase and cysteine were more important for CWES flour bread quality. ADA did not enhance the volume of breads made with either flour and SSL had a negative effect on bread quality because of its involvement in highly negative interaction effects with other additives.

The second screening experiment was carried out using five additives. Ascorbic acid, α -amylase and cysteine had important effects on the quality of breads made with both CWRS and CWES wheat flour, both as main effects and as interaction effects. Protease was important to the CWRS flour bread volume, but did not give the desired effect of reduced mix time, improved dough handling properties and increased loaf volume when used in the CWES breads. DATEM improved the volumes of breads made with both flours, but was detrimental to the internal and external loaf characteristics and was involved in many interactions which reduced quality scores.

The optimization study was carried out to identify the combinations of ascorbic acid, α -amylase and cysteine which optimized loaf volume and internal and external loaf scores in breads made with CWRS and CWES wheat flours alone and in blends. The study led to some interesting conclusions:

1. CWES flour had a pronounced improving effect on the internal and external loaf characteristics of breads. Therefore, CWES flour has the potential to reduce the requirement for additive used to improve these quality attributes.
2. CWES breads without improvers had reduced volumes and extended mix times. A suitable combination of ascorbic acid, α -amylase and cysteine enabled loaf volumes as high as CWRS breads to be obtained and mixing times to be reduced to less than the CWRS controls.
3. The CWRS and CWES wheat flours differed in their requirement for α -amylase. The CWRS wheat flour gave the best predicted loaf volumes at the low level of α -amylase addition (20 SKB units) whereas CWES wheat flour had best predicted loaf volumes at the highest α -amylase level (60 SKB units) tested.
4. A strong interaction was evident between cysteine and ascorbic acid. Highest loaf volume

and external scores were obtained with low cysteine/high ascorbic acid and vice versa.

5. The CWES wheat flour tolerated a higher level of cysteine than the CWRS wheat flour. One hundred percent CWES flour doughs had excellent handling properties at the high level of cysteine (90 ppm), whereas the CWRS flour doughs had unacceptable handling properties at 50 ppm cysteine.

6. Increasing cysteine addition from 0 to 50 ppm reduced mix times of all doughs by approximately 50%. Above 50 ppm, the reduction in mix time was much less pronounced.

The verification experiment confirmed that the optimized improver combinations identified for each flour gave very good results (within the regions of acceptability) for both loaf volume and external loaf characteristics. Bread with quality characteristics as good as those achieved with the standard bromated formulation were obtained. Bread prepared with the 25% CWES wheat flour blend gave very good results, with excellent loaf volume, external and internal loaf scores and highly acceptable mix times.

The benefits of using CWES wheat flour for improvement of bread appearance and crumb structure has not been addressed in the literature. During this research it became evident that breads made with CWES wheat flour had excellent crumb structure and loaf appearance. The use of this flour alone appeared to impart some improvement to loaf quality. Doughs made with CWES wheat flour were more tolerant of humidity and temperature changes in the proofer. They also tolerated high levels of both cysteine and α -amylase. Based on the response surface models, very good bread quality results could be attained using a wide range of improver combinations and levels. Therefore, not only should the focus be on the ability of CWES to carry weaker flours in blends and its contribution to frozen dough production, but consideration should be given to the possible increased dough tolerance it could impart, thus helping to overcome one of the major disadvantages to baking without bromate. By using appropriate levels of cysteine, CWES wheat flour could be used on its own as a bread wheat, yielding bread of excellent quality with greatly reduced mix times.

RECOMMENDATIONS FOR FUTURE RESEARCH

This research has illuminated many possible directions in which research can continue. The behaviour of CWES wheat flours, its contribution to bread quality and its effect on improver requirement all have appeared to differ from that found with CWRS wheat flour. Findings in this study which do not coincide with those in the published literature should also be investigated.

The oxidative requirement of CWES wheat flours is thought to be lower than that of CWRS bread wheats (Tweed, 1995). This may be a result of both its generally lower protein contents and its extra long mixing requirements. There is little available data confirming this in the literature, most of the work being done on other long mixing varieties other than those in the CWES class of wheats. A systematic investigation into the oxidative requirement of CWRS versus CWES wheat flour is required. RSM may be appropriate in determining the oxidative requirement of CWES wheat flour both alone and in blends with other flours.

An increased requirement for oxidation when cysteine is included in the formulation has been suggested. However, the optimization experiments showed an opposite effect in which the use of increasing cysteine levels required only low levels of ascorbic acid. It is possible that the experimental design used in this research was more effective at illustrating the interactive effects of additives. The relationship between cysteine and ascorbic acid should be examined further, especially given the benefits of cysteine use in the production of breads made with CWES wheat flour.

The performance of flours milled from different wheat varieties in frozen dough production has received attention in the literature (Inoue and Bushuk, 1992). However, the behaviour of different flours may be enhanced by using optimum combinations of dough strengtheners and oxidants. A response surface design similar to the that presented in this research may provide some practical information for improving the quality bread made from frozen dough.

There was a noticeable effect of the oxidants potassium bromate and ascorbic acid on the mixing requirement of CWES wheat flour dough which was not evident with the CWRS flour doughs. A previous study (Lang et al, 1992) dealt with this effect of dough additives on mix time, but did not investigate the effects in a long mixing flour such as those in the extra strong wheat class. The Canadian Short Process is now used as a baking test with which to screen bread wheats for their breadmaking potential. The high level of oxidation used in this method may extend the mixing requirement of some wheat flours. Flours which perform well in other quality tests may be rejected on the basis of the mixing requirement. This phenomenon should be further examined.

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For 30 ppm potassium bromate: use 1 mL solution
considered 1 mL dough water

ASCORBIC ACID:

0.75 g ascorbic acid was weighed into a 100 cylinder and distilled water was added up to 100 mL. The mixture was shaken until dissolved and placed in a flask which was covered in brown paper. This solution was made up daily.

For 30 ppm ascorbic acid: use 0.4 mL solution
considered 0.4 mL dough water

For 37.5 ppm ascorbic acid: use 0.5 mL solution
considered 0.5 mL dough water

For 75 ppm ascorbic acid: use 1 mL solution
considered 1 mL dough water

For 120 ppm ascorbic acid: use 1.6 mL solution
considered 1.6 mL dough water

For 150 ppm ascorbic acid: use 2 mL solution
considered 2 mL dough water

PROTEASE:

0.20 g fungal protease preparation was weighed into a 100 mL cylinder and distilled water added up to 100 mL. The mixture was shaken until dissolved and placed in a flask. This solution was made up daily.

For 120 HU protease activity: use 1 mL solution
considered 1 mL dough water

For 240 HU protease activity: use 2 mL solution
consider 2 mL dough water

CYSTEINE:

0.25 g cysteine hydrochloride was weighed into a 50 mL flask and distilled water added up to 50 mL. The mixture was shaken until dissolved and placed in a flask. This solution was made up daily.

For 25 ppm cysteine: use 0.5 mL solution
considered 0.5 mL dough water

For 50 ppm cysteine: use 1 mL solution considered
1 ml dough water

CANADIAN TEST BAKING PROCEDURES.

III. GRL-CANADIAN SHORT PROCESS METHOD

ABSTRACT

Details of the Grain Research Laboratory Canadian Short Process Baking Procedure are given in the ISO format. The method is applicable for untreated flour experimentally or commercially milled from wheat for the production of yeast raised breads. It provides a test of the baking performance of flours under conditions of high-speed mixing and short fermentation. Mixing characteristics and the energy consumed during dough mixing are monitored.

A detailed description of the Canadian Short Process is warranted because it has become one of the primary tests used to evaluate bread wheat cultivars for baking quality. It also gives the lab a baking method that commercial bakers can relate to.

The purpose of this article is to provide details of the procedure used for the test and to give specifications of the equipment used.

1. TITLE

GRL Canadian Short Process.

2. SCOPE

The method is applicable for untreated flour experimentally or

commercially milled from wheat for the production of yeast raised bread. It provides a test of the baking performance of flours under the conditions of high speed mixing, short fermentation and typical formulation used by Canadian plant bakeries. Mixing characteristics and energy consumed during dough mixing are monitored. Force measurements at sheeting are also obtained.

3. PRINCIPLE

The method calls for high-speed mixing in a recording dough mixer. A dough is made from flour, water, sucrose, salt, yeast, shortening, potassium bromate, ammonium phosphate, malt syrup, whey and ascorbic acid under specified conditions of temperature, mixing speed and degree of dough development as judged from the mixing curve. Doughs are rested for 15 min. lightly punched 7X, rested 15 min. and molded. They are then assessed for absorption and handling properties, placed in baking pans, proofed for 70 min. and baked for 30 min..

Loaf volume is measured after 30 min. of cooling; loaves are evaluated the following day for appearance, crust color, crumb structure and crumb color.

4. FORMULA AND INGREDIENTS

4.1 FORMULA

Flour(14.0% moisture basis)	200.0g
Yeast	3.0%
Salt	2.4%
Sucrose	4.0%
Ammonium Phosphate	0.01%
Potassium Bromate	30 ppm
Ascorbic Acid	37.5 ppm
Malt	0.6%
Shortening	3.0%
Whey	4.0%
Water	var. (maximum consistent with machinability of dough)

4.2 INGREDIENTS

4.2.1 Yeast, salt, sucrose, potassium bromate, ammonium phosphate, malt syrup and water as for the remix method (section 4.2)

4.2.2 Shortening, pure vegetable, GRL uses "Crisco"

4.2.3 Ascorbic acid, reagent grade; stored in refrigerator

4.2.4 Whey, commercial grade. GRL uses a product called "Fedeco"

5. EQUIPMENT AND APPARATUS

5.1 Baking room, refrigerator, balance, analytical balance, warming cabinet, blender, fermentation bowls, sheeting rolls, molder, baking oven, cooling rack, apparatus for loaf volume determination and bread storage cabinet are as for remix method (section 5).

5.2 Mixer, GRL 200, having a pin speed of 165 ± 2 rpm (using a 6 sec. time base) (Hlynka and Anderson, 1955)

5.3 Thermostatically controlled bath, used to control the temperature of the water jacketed mixing bowl to produce a dough temperature of $30 \text{ C.} \pm 0.5 \text{ C.}$ at the end of mixing. (Generally a thermostat setting of 27 C. will achieve this.)

5.4 Solution bath, maintained at $30 \text{ C.} \pm 1 \text{ C.}$ for tempering of the yeast suspension and the sugar-salt solution. Provision is made for continuous stirring of the yeast suspension (Kilborn and Aitken 1961).

5.5 GRL Direct Reading Energy Input Meter, measures the power and energy used by the mixer motor and has provision to dial in

mechanical efficiency and dough weight (Kilborn 1979, Kilborn and Tipples 1973a), so that power curves and energy measurements are net values expressed as watts per kilogram (or watt hours per kilogram accumulated on a counter) of dough (Fig.1).

5.6 Recorder, having a full scale response of 100 mV and a chart speed of 600 mm/hr. The recorder in use at the GRL is a Riken Denshi SP-G5V.

5.7 Proofing cabinet, controlled to maintain a temperature of 37.5 ± 1 C. and a relative humidity of $83 \pm 2\%$. Circulating air flow should be balanced so that the dough surface becomes neither too wet nor too dry while in the cabinet.

6 INGREDIENT SOLUTIONS

6.1 Yeast suspension

Prepare as for remix method (section 6.1). Use 50 ml (containing 6.0g of yeast and 45.0ml of water) per 200g of flour.

6.2 Sugar-salt solution

Weigh 105.6 ± 0.05 g of salt and 176 ± 0.05 g of sugar into the milkshake container. Add 400ml of the total water (941ml at 30 ± 0.5 C.) to the container and blend at slow speed for 1.5 min.. Pour into a 2 qt. sealer. Rinse the milk shake container and stirrer with the remaining water and add to the sealer. Shake

well and place the sealer in the solution bath. Prepare fresh daily. Use 50ml of solution (containing 4.8g salt, 8.0g of sugar and 42.8ml of water) per 200g of flour.

6.3 Malt syrup

Prepare as for remix method (section 6.3). Combine 20ml of regular solution and 60ml of water. Use 2ml per 200g flour and consider that as 2ml in dough water calculations.

6.4 Potassium Bromate

Add 3 +/- 0.001g of Potassium Bromate into a 1000ml volumetric flask and make up to volume with water. Store the solution in a stoppered bottle at room temperature (Use 2ml, equivalent to 30ppm Bromate based on flour weight) per 200g of flour, and consider that as 2ml for dough water calculations.

6.5 Ammonium Phosphate

Add 100 +/- 0.01g of Ammonium Phosphate into a 1000ml volumetric flask and make up to volume with water. Store the solution in a stoppered bottle at room temperature. (Use 2ml, equivalent to 0.1% Ammonium Phosphate based on flour weight) per 200g of flour, and consider that as 2ml for dough water calculations.

6.6 Dough Water

Amount dependent on the flour moisture, farinograph absorption, and handling properties of the dough at the time of

panning. See details for determining baking absorption and dough water. (7.1.1,7.1.2)

7 PROCEDURE

7.1 The day before test baking.

Baking absorption and net dough water should be determined in advance and written on the appropriate baking card along with the flour weight and list of ingredients. Preparing flour samples and most solutions ahead of time is most practical. Weigh flour samples into numbered tins with tightly fitting lids and place them in the warming cabinet with the yeast water and the sugar-salt solution (which is transferred to the solution bath the following morning). Temperature controlled equipment must be switched on (either manually or through time switches) well in advance of the baking tests.

7.1.1 Determination of baking absorption

The basis of the baking absorption for the Canadian Short Process procedure is 4% higher than the absorption assessed for the remix procedure. When this is not available, the absorption used for the first baking test is obtained by adding 3% to the remix absorption and rounding off to the nearest full percentage value; eg, for a farinograph absorption of 63.3%: $63.3 + 3 = 66.3 = 66.0\%$ initial absorption.

The final (reported) baking absorption is determined from

the machining and handling properties of the dough at the time of panning by the operator. If sticky or unusually slack doughs are encountered at panning, the absorption is reduced for the second bake (usually the following day). Similarly, if the dough appears capable of carrying more water, a higher absorption is used for the second bake.

7.1.2 Calculation of dough water

The calculation of dough water takes into account the flour moisture and the displacement of ingredients added in the form of solutions.

7.1.2.1 Gross dough water = $200 + (\text{absorption} * 2) - \text{flour weight}$.

7.1.2.2 Net dough water = (gross dough water) - (water added in solutions).

7.1.2.3 Example of dough water calculation

Given flour moisture = 14.2%

Then flour weight = 200.4g.

If absorption = 64.05

Then gross dough water = $200.0 + (2 * 64.0) - (200.4) = 127.6$.

Water contained in form of solutions = 98.2ml.

Therefore, net dough water = $127.6 - 98.2 = 29.4\text{ml}$.

7.2 Morning of test baking

Service equipment (wet socks, check temperatures and humidities).
Make up yeast suspension (6.1).

7.3 Baking schedule

See the baking schedule (Table 1). Mixes are 10 min. apart; therefore subsequent operations such as panning and loaf transfer to and from oven, are also 10 min. apart. Times denote completion of the operation +/- 1 min.. Full oven conditions are required for all test loaves. Therefore sufficient non test dough must be prepared to supply 4 loaves. Two loaves precede the first test sample and 2 follow the last test sample, with 10 min. intervals between blanks as in the test loaf schedule. When samples having very long mixing requirements are encountered, the 10 min. interval between the panning of samples is maintained by inserting additional blank doughs where necessary. Notations of changes are made in the "Loaf #" and "Completion of Mix" columns.

7.4 Calibration of equipment

After mixing the blanks, check the calibration of the mixing equipment as instructed in "Operation of GRL Energy Input Meter" (Appendix).

7.5 Mixing

With the mixer running empty, zero out the recording mixer. This should be repeated before each mix. Pipette into a beaker all liquid ingredients except the yeast suspension and ascorbic acid. When ready to mix, add the solutions from the beaker and the yeast suspension and ascorbic acid solution by pipette

directly to the flour in the mixing bowl. Start the mixer and mix the dough to slightly past peak consistency, as indicated by the mixing curve obtained with the recording system. As a general rule, mixing is continued to a stage corresponding to 10% more time or energy than that required to achieve peak consistency. This is normally sufficient to verify, from the shape of the mixing curve, that peak consistency was indeed reached. Note the total energy (watt hours per kilogram) and mixing time used, and determine the energy and mixing time corresponding to peak consistency. During the mixing period, measure liquid ingredients for the next test sample. Remove the dough from the mixing bowl after completion of the mix.

Continue mixing operations as above, maintaining the time schedule for the remaining samples.

7.6 Rounding and intermediate proof.

Round the doughs lightly seven times by hand; check the temperature and place the dough in lightly greased sequentially numbered fermentation bowls into the warming cabinet set at 30 C.

7.7 Sheeting and molding

Perform as for remix method (section 7.8).

7.8 Proofing

Proof for 70 +/- 5 minutes, according to proofing rate of control sample, @ 37.5 C., R.H. 83%.

7.9 Baking

Bake for 30 minutes @ 204 C..

8. EVALUATION OF LOAVES

Same as remix method (section 8).

8.1 Lighting for bread scoring

Same as remix method (section 8.1).

APPENDIX IIIa

**Ballot for Evaluation of Bread Crumb:
Cell Size Uniformity**

Please evaluate the cell size uniformity of the bread images making a vertical line at the point on the scale where you think that sample fits. Evaluate the images in the order indicated using the visual reference provided.

No. ____

Irregular	Medium	Uniform
-----------	--------	---------

No. ____

Irregular	Medium	Uniform
-----------	--------	---------

No. ____

Irregular	Medium	Uniform
-----------	--------	---------

No. ____

Irregular	Medium	Uniform
-----------	--------	---------

No. ____

Irregular	Medium	Uniform
-----------	--------	---------

No. ____

Irregular	Medium	Uniform
-----------	--------	---------

APPENDIX IIIb

**Ballot for Evaluation of Bread Crumb:
Predominant Cell Size**

Please evaluate the predominant cell size of the bread images making a vertical line at the point on the scale where you think that sample fits. Evaluate the images in the order indicated using the visual reference provided.

No. _____

Large	Medium	Small
-------	--------	-------

No. _____

Large	Medium	Small
-------	--------	-------

No. _____

Large	Medium	Small
-------	--------	-------

No. _____

Large	Medium	Small
-------	--------	-------

No. _____

Large	Medium	Small
-------	--------	-------

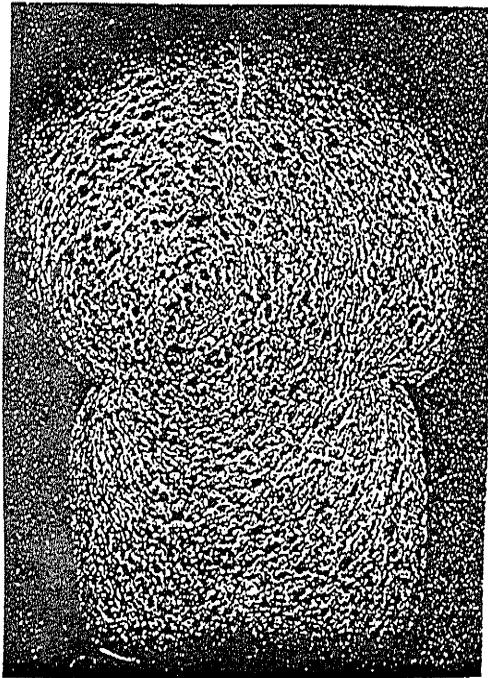
No. _____

Large	Medium	Small
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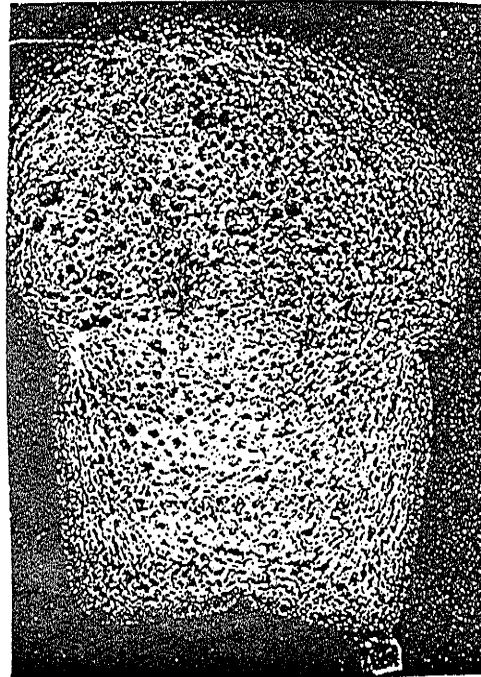
APPENDIX IVa

VISUAL REFERENCE FOR EVALUATION OF BREAD CRUMB:
SCREENING EXPERIMENT #1

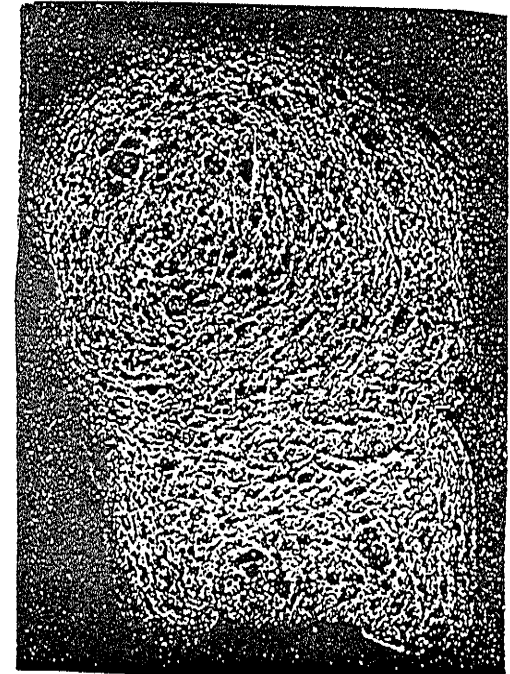
CELL SIZE UNIFORMITY



Uniform



Medium

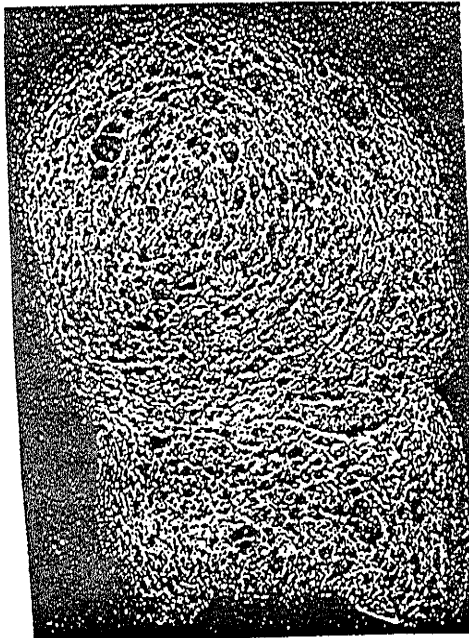


Irregular

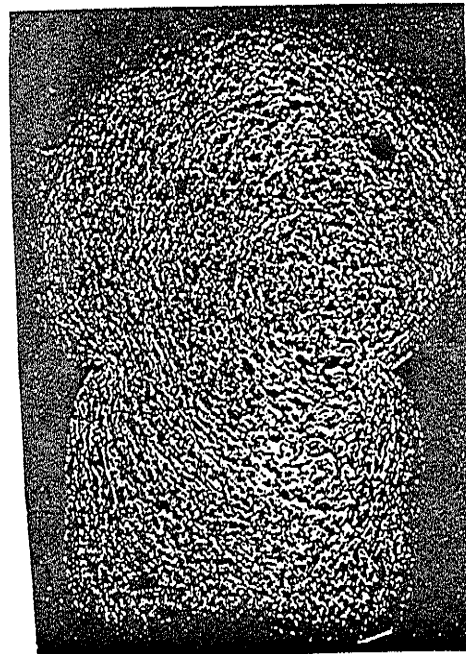
APPENDIX IVb

VISUAL REFERENCE FOR EVALUATION OF BREAD CRUMB:
SCREENING EXPERIMENT #1

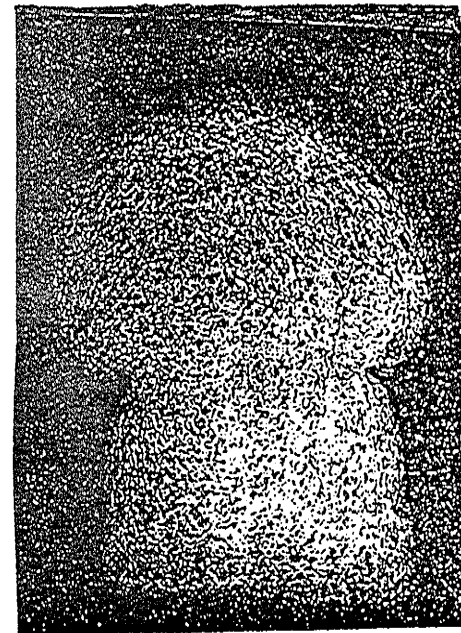
PREDOMINANT CELL SIZE



Large



Medium



Small

APPENDIX Va

Mean Results for Loaf Volume, Mix Time, and
Crumb Characteristics from Screening Experiment #1:
CWRS Wheat Flour

Response	Baking Runs (standard order)									
	1	2	3	4	5	6	7	8	9	10
Loaf Volume ^a (cc)	1035	1110	1010	1030	1055	1145	1165	1165	1050	1110
Mix Time ^b (min)	10.2	9.1	10.3	9.3	10.1	9.2	9.6	9.0	5.7	6.4
Cell Size Uniformity ^c	7.55	5.44	5.78	5.24	6.65	5.26	2.49	7.42	6.18	4.90
Predominant Cell Size ^c	6.22	6.11	3.59	5.88	6.09	6.43	3.39	5.15	4.52	5.00

Response	11	12	13	14	15	16	17	18	19	20
Loaf Volume (cc)	1085	1150	1025	1105	1060	1135	1180	1165	1165	1175
Mix Time (min)	7.0	5.3	5.8	6.2	5.9	5.7	7.5	6.6	7.3	7.3
Cell Size Uniformity	3.06	5.18	6.76	7.04	6.13	5.79	4.92	5.69	5.59	4.06
Predominant Cell Size	2.97	6.00	7.66	4.29	6.45	4.34	4.16	3.80	3.92	2.98

^a Volume determinations based on 3rd replication only.

^b Mix time values are an average over 3 replications.

^c Values for crumb characteristics based on average score of 4 judges over 3 replications. Maximum score attainable = 15 points.

APPENDIX Vb

Mean Results for Loaf Volume, Mix Time and
Crumb Characteristics from Screening Experiment #1:
CWES Wheat Flour.

Response	Baking Runs (standard order)									
	1	2	3	4	5	6	7	8	9	10
Loaf Volume (cc)	895	978	1015	1048	934	998	1040	1018	998	995
Mix Time (min)	25.0	24.1	22.1	24.4	22.1	24.1	20.6	21.8	12.3	13.4
Cell Size Uniformity ^b	8.17	7.34	4.64	4.22	8.39	8.33	4.42	6.41	6.79	8.61
Predominant Cell Size ^b	8.38	6.42	4.54	6.97	5.70	7.87	5.27	6.24	8.13	7.43
Response	11	12	13	14	15	16	17	18	19	20
Loaf Volume (cc)	1093	1028	985	1035	1095	1048	1040	1040	1023	1035
Mix Time (min)	13.6	13.0	12.2	10.8	16.4	16.2	17.2	18.0	19.5	20.4
Cell Size Uniformity	3.88	5.11	9.22	7.24	4.08	4.74	6.50	7.79	8.61	9.19
Predominant Cell Size	4.97	5.69	7.44	4.99	4.84	5.50	3.98	5.57	6.54	7.49

^a Values of all responses are averages of 2 replications.

^b Maximum score for Cell Size Uniform and Predominant Cell Size = 15.

APPENDIX VI

The Effect of Water Absorption on the Presence of Blisters in CWRS and CWES Wheat Flour Breads.

Objective:

To determine if the occurrence of large blisters in loaves can be overcome by using a lower water absorption level.

Methods:

CWRS and CWES wheat flour doughs were prepared using a series of water absorption levels, starting at farinograph water absorption (FAB) plus 4% and decreasing by 1% for each dough, down to FAB -3%. A total of 8 doughs were mixed and baked for each flour type. The Canadian Short Process standard (control) bread formulation was used (see Table 3.1) and doughs were mixed to 10% past peak development. Mixing times to peak dough development were recorded and the dough handling properties were considered. Volume determinations were made on the baked loaves and the presence or absence of blisters was noted.

Results:

Tables A-1 and A-2 summarize the results for CWRS and CWES wheat flour doughs and breads. Mix times were reduced by approximately 30% and 50% for the CWRS and CWES wheat flour doughs, respectively, when water absorption was lowered from FAB + 4% to FAB - 3%. Loaf volumes did not decline to any great extent when water addition was decreased as all doughs were mixed to optimum development. The blister problem occurred in two of the loaves for each flour. The breads prepared with the high water level (FAB + 4%) had the large hole in the top of the loaves. Although the breads prepared with FAB - 2% for both flours had a large blister, this blistering problem generally became less prevalent at the lower water addition was lowered. The handling properties of the CWRS wheat flour doughs also improved at lower water absorption, the doughs being less sticky and less likely to stick and tear in the sheeter.

Conclusion:

Although this short investigation did not give absolute proof that the blistering problem was due to the water absorption level used (FAB + 3%), the handling properties and mixing requirement of both CWRS and CWES wheat flour doughs were improved when less water was added. A water absorption level of FAB +1% was found to be ideal, reducing mixing times by approximately 16% and 12% for CWRS and CWES wheat flour, respectively. The dough was more manageable, loaf volumes were not adversely affected and the problem with blistering was less prevalent. Therefore, a water absorption level of FAB + 1% was chosen for use in screening experiment #2 and the optimization experiment.

Table A-1. The effect of water absorption on the mix time, loaf volume and occurrence of blisters in breads made with CWRS wheat flour.

Changes to Farinograph Water Absorption	Mix Time (min)	Loaf Volume (cc)	Blister
+ 4%	9.5	1265 (1205 ^a)	yes
+ 3%	9.5	1260	no
+ 2%	8.7	-	no
+ 1%	8.0	1240	no
+/- 0	7.5	1280	no
- 1%	6.8	1250	no
- 2%	6.9	1220 (1160 ^a)	yes
- 3%	6.1	1230	no

^a Loaf volumes measured by puncturing top of loaf to ensure the blister filled with rapeseed to get a more accurate loaf volume measurement.

Table A-2. The effect of water absorption on the mix time, loaf volume and occurrence of blisters in breads made with CWES wheat flour.

Changes to Farinograph Water Absorption	Mix Time (min)	Loaf Volume (cc)	Blister
+ 4%	24.0	1055	yes
+ 3%	23.8	1055	no
+ 2%	19.3	1035	no
+ 1%	21.1	1070	no
+/- 0	16.5	1040	no
- 1%	14.5	910	no
- 2%	13.0	1015	yes
- 3%	12.3	995	no

APPENDIX VII

Bread Evaluation Score Card: Screening Experiment #2.

A. DOUGH QUALITY

		Score
1.) Dough out of mixer (10)	a) Normal for method	10
	b) Slightly tight or sticky/tacky	8
	c) Tight or sticky	6
	d) Very tight or very sticky	4
	e) Unmanageable	0
2.) Dough at panning (10)	a) Satisfactory	10
	b) Slightly bucky or slack	8
	c) Bucky or slack	6
	d) Very bucky or very slack	4
	e) Unmanageable	0

B. LOAF EXTERNAL QUALITIES

1.) Loaf symmetry (10)	a) Very symmetrical with round top	10
	b) Slightly unsymmetrical	8
	c) Moderately unsymmetrical	6
	d) Unsymmetrical or with slightly flat top	4
	e) Very unsymmetrical	2
	f) Unacceptable	0
2.) Loaf Bottom (10)	a) Flat bottom, no indent	10
	b) Slightly concave	8
	c) Moderately concave	5
	d) Very concave	2
	e) Extremely concave	0
3.) Break and Shred (10)	a) High (> 2 1/2")	10
	b) Very good (1 1/2-2 1/2")	8
	c) Moderate (1-1 1/2")	6
	d) Low (1/2-1")	4
	e) Insufficient (< 1/2")	2
	f) None	0

C. LOAF INTERNAL QUALITIES

1.) Cell Uniformity (10)	a) Very even and uniform	10
	b) Slightly uneven	8
	c) Moderately uneven	6
	d) Very uneven	4
	e) Extremely uneven	2
	f) Unacceptable	0
2.) Cell Size (10)	a) Ideal, medium size cells	10
	b) Slightly open or close cells	8
	c) Moderately open or close cells	6
	d) Very open or close cells	4
	e) Extremely open or close cells	2
	f) Unacceptable	0
3.) Blisters (Air Bubbles) (10)	a) None	10
	b) Moderate hole	5
	c) Large hole	0

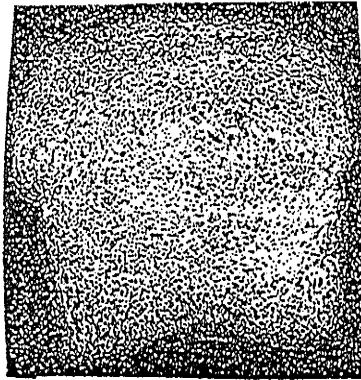
D. FLAVOR (10)

a) Normal (no off flavors)	10
b) Foreign	0

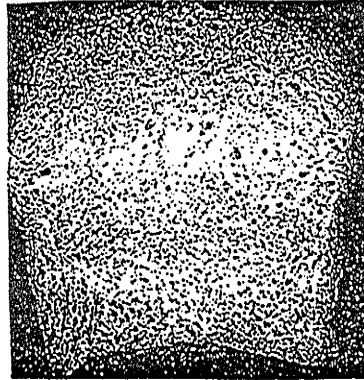
APPENDIX VIIIa

VISUAL REFERENCE FOR EVALUATION OF BREAD CRUMB:
SCREENING EXPERIMENT #2

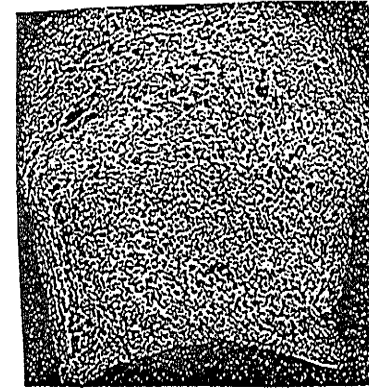
CELL SIZE



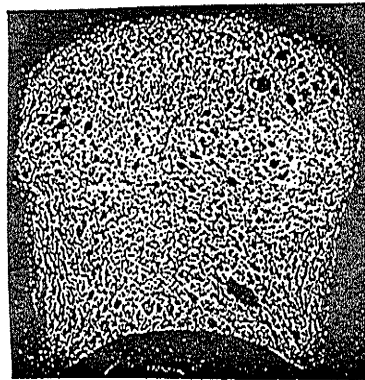
Ideal, Medium Size



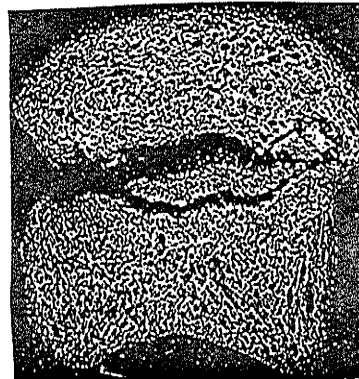
Slightly Open



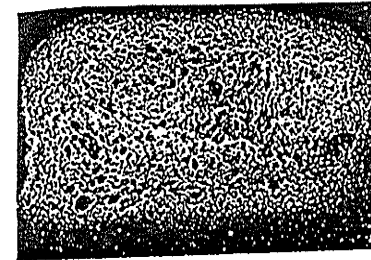
Moderately Open



Very Open



Extremely Open

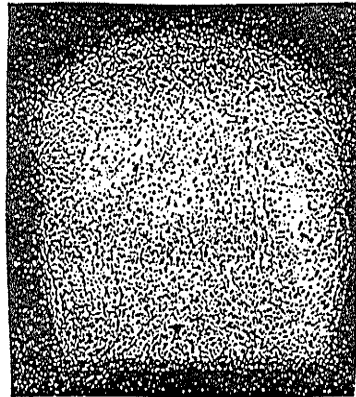


Unacceptable

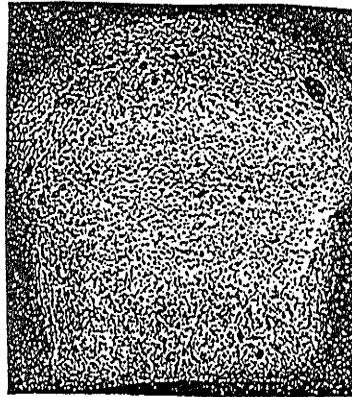
APPENDIX VIIIb

VISUAL REFERENCE FOR EVALUATION OF BREAD CRUMB:
SCREENING EXPERIMENT #2

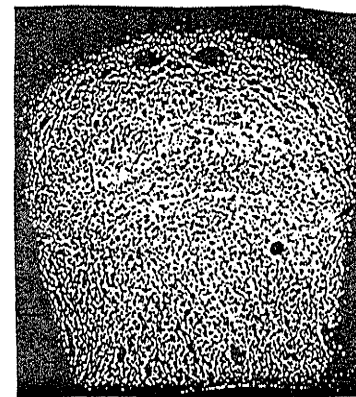
CELL UNIFORMITY



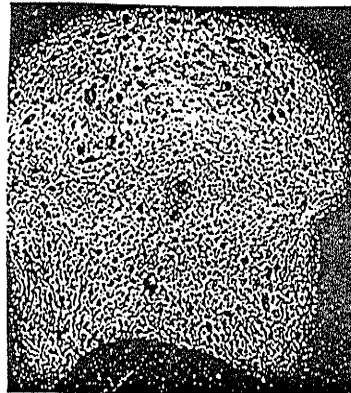
Very Even and Uniform



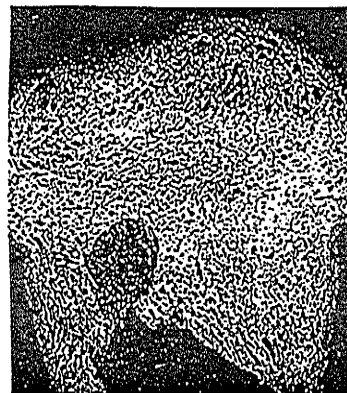
Slightly Uneven



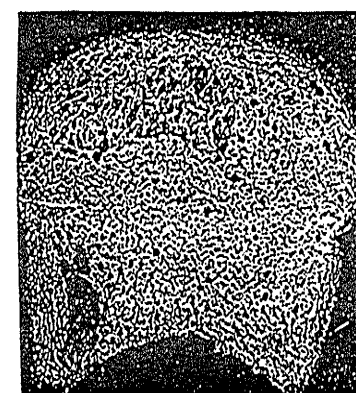
Moderately Uneven



Very Uneven



Extremely Uneven



Unacceptable

APPENDIX IXa

Results for Loaf Volume, Mix Time, and Internal and
External Loaf Characteristics from Screening Experiment #2:
CWRS Wheat Flour

Response	Baking Runs (standard order)									
	1	2	3	4	5	6	7	8	9	10
Loaf Volume (cc)	1100	1170	1195	1150	1120	1140	1200	1215	1175	1175
Mix Time (min)	4.1	4.9	6.2	4.5	5.0	4.0	4.5	5.2	5.3	4.0
External Loaf Characteristics ^a	19	21	25	22	20	22	18	15	17	22
Internal Loaf Characteristics ^b	21	22	17	15	22	25	7	8	14	20

Response	11	12	13	14	15	16	17	18	19	20
Loaf Volume (cc)	1165	1215	1160	1180	1220	1170	1250	1155	1210	1165
Mix Time (min)	3.0	5.5	4.5	5.8	6.7	4.6	4.5	4.4	3.5	4.5
External Loaf Characteristics	22	19	20	19	27	6	19	17	23	26
Internal Loaf Characteristics	14	22	13	20	25	1	12	23	18	24

^a External loaf characteristics maximum score = 30 (total of scores for loaf symmetry, loaf bottom and break and shred).

^b Internal loaf characteristics maximum score = 30 (total of scores for cell uniformity, cell size and blister).

APPENDIX IXb

Results for Loaf Volume, Mix Time and Internal and External Loaf Characteristics from Screening Experiment #2: CWES Wheat Flour.

Response	Baking Runs (standard order)									
	1	2	3	4	5	6	7	8	9	10
Loaf Volume (cc)	1160	1155	1205	1300	1145	1180	1265	1080	1195	1205
Mix Time (min)	7.0	9.8	11.0	5.5	11.5	7.5	8.1	14.5	9.5	6.8
External Loaf Characteristics ^b	27	26	22	22	29	25	25	25	25	25
Internal Loaf Characteristics ^b	28	21	26	19	27	28	27	28	27	27

Response	11	12	13	14	15	16	17	18	19	20
Loaf Volume (cc)	1165	1250	1165	1075	1190	1270	1125	1140	1270	1250
Mix Time (min)	8.0	11.4	6.5	14.3	15.0	7.0	8.8	8.4	10.2	9.0
External Loaf Characteristics	24	22	26	28	23	24	29	24	24	25
Internal Loaf Characteristics	20	24	23	25	21	23	24	24	29	24

^a External loaf characteristics maximum score = 30 (total of scores for loaf symmetry, loaf bottom and break and shred).

^b Internal loaf characteristics maximum score = 30 (total of scores for cell uniformity, cell size and blister).

APPENDIX X

Results for Loaf Volume, Mix Time and Internal and External Loaf Characteristics from the Optimization Experiment:
CWRS and CWES Wheat Flours and Blends.

	Baking Runs (standard order) ^a													
Response	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Loaf Volume (cc)	1130	1135	1050	1150	1150	1150	1165	1120	1150	1100	1135	1240	1135	1105
Mix Time (min)	5.8	6.2	5.5	6.0	4.4	4.3	4.8	4.4	7.8	6.9	7.8	7.9	6.1	6.3
External Loaf Characteristics ^b	23	21	22	26	27	27	28	20	25	27	28	24	29	22
Internal Loaf Characteristics ^c	12	12	10	15	8	9	9	8	11	18	11	15	11	13
Response	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Loaf Volume (cc)	1195	1125	1145	1090	1165	1117	1105	1100	1215	1092	1100	1125	1170	1215
Mix Time (min)	5.3	6.2	5.7	5.5	5.8	5.3	10.3	5.1	3.8	8.8	6.8	5.8	5.6	5.5
External Loaf Characteristics	28	28	25.5	25.5	25.5	24	24.5	25.5	23	26	26.5	27	23	29.5
Internal Loaf Characteristics	14	20	14	12	16	16	12	15	18	12	12	14	11	12

^a Values of runs 17-25 are averages of 2 replications.

^b External loaf characteristics maximum score = 30 (total of scores for loaf symmetry, loaf bottom and break and shred).

^c Internal loaf characteristics maximum score = 20 (total of scores for cell uniformity and cell size).

APPENDIX XI

Means, Standard Deviations (STD) and Coefficients of Variation (C.V.) for Loaf Volume, Mix Time, External and Internal Loaf Characteristics of Breads made with Selected Improver Combinations and Improver-free Breads prepared in the Verification Experiment

Flour	Treatment	LOAF VOLUME (cc)			Mix Time (min)			External Loaf Characteristics			Internal Loaf Characteristics		
		Mean	STD	C.V. (%)	Mean	STD	C.V. (%)	Mean	STD	C.V. (%)	Mean	STD	C.V. (%)
100% CWRS	#1	1155	47.70	4.13	5.8	0.15	2.62	22.0	5.20	23.62	9.3	2.08	22.30
	#2	1218	45.96	3.78	5.5	0.07	1.30	26.5	2.12	8.00	11.5	4.95	43.04
	#3	1125	0.00	0.00	5.7	0.14	2.48	26.5	0.71	2.67	13.0	1.41	10.88
	Imp-free	1033	10.61	1.03	7.7	0.21	2.77	26.5	0.71	2.67	12.0	2.83	23.57
25% CWES	#1	1183	45.37	3.83	6.1	0.42	6.79	25.7	2.08	8.11	8.3	2.08	24.98
	#2	1185	0.00	0.00	7.1	0.30	4.23	24.7	2.08	8.44	9.7	2.08	21.53
	#3	1145	7.07	0.62	5.9	0.14	2.40	29.0	0.00	0.00	16.0	2.83	17.68
	Imp-free	1068	7.64	0.72	8.3	0.28	3.41	27.7	0.58	2.09	11.7	3.79	32.48
50% CWES	#1	1142	38.80	3.23	6.8	0.25	3.72	25.7	2.31	9.00	10.0	1.73	17.32
	#2	1145	52.68	4.60	4.5	0.00	0.00	25.3	1.53	6.83	12.7	2.08	16.43
	#3	1145	49.50	4.32	8.1	0.57	6.98	27.5	0.71	2.57	13.5	3.54	26.19
	Imp-free	1013	30.55	3.01	9.2	1.04	11.30	27.7	0.58	2.09	16.3	3.21	19.68
75% CWES	#1	1168	60.28	5.16	5.5	0.50	9.10	23.7	0.58	2.55	12.7	3.06	24.12
	#2	1088	49.33	4.53	8.1	0.67	8.25	27.3	3.05	11.17	11.3	1.15	10.19
	Imp-free	967	36.17	3.74	11.5	1.00	8.70	27.3	1.15	4.22	16.7	4.93	29.60
100% CWES	#1	1082	70.06	6.48	6.1	0.44	7.15	27.3	2.08	7.62	14.0	1.73	12.37
	#2	1125	56.79	5.05	9.5	1.50	15.79	27.3	1.53	5.59	15.0	3.61	24.04
	Imp-free	953	28.43	2.98	13.9	0.36	2.59	27.0	1.73	6.42	14.7	1.15	7.87