OPTIMIZATION OF BROMATE-FREE IMPROVER FORMULATIONS FOR USE WITH A MEXICAN BREAD FLOUR AND WITH BLENDS OF CANADIAN HARD RED SPRING AND PRAIRIE SPRING WHEAT FLOURS

by

Armando Conca-Torres

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ARMANDO CONCA-TORRES

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCES

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ABSTRACT

The effect of bread improvers on the quality characteristics of white pan-bread made with a Mexican commercial wheat flour and with blends of hard red spring and prairie spring wheat flours were evaluated. The study was divided into two optimization experiments. A rotatable central composite design was used in both experiments to identify the main effects and interactions between the factors.

In the first optimization experiment, two baking tests, a liquid ferment and the Canadian Short Process, were compared for sensitivity to improver formulations containing diacetyl tartaric acid ester of monoglycerides (DATEM), ascorbic acid, and α-amylase. The liquid ferment was more sensitive to the effect of the improvers than the Canadian Short Process. Ascorbic acid and α-amylase showed fewer significant effects in the Canadian Short Process than in the liquid ferment. Using the liquid ferment, high quality loaves were possible at any given concentration of DATEM. With low DATEM, the requirements for α-amylase were high (above 80 SKB units), while at higher levels of DATEM, α-amylase requirements were as low as 40 SKB units. However, the liquid ferment doughs showed excessive stickiness after mixing. A further experiment indicated that appropriate handling properties could be attained at a reduced water absorption of FAB - 3%.

In the second optimization experiment, the liquid ferment baking test was used to evaluate the effects of ascorbic acid and α-amylase on flour blends.

DATEM was used at fixed level of 0.375%. Hard red spring (CWRS and CWES) were blended at different ratios with CPS white and red flours. CWRS performed better than CWES when blended with CPS white. Optimized combinations of ascorbic acid and α-amylase, that satisfied all the optimization quality criteria, could be identified at any level of CWRS, while optimized combinations were only identified at high levels of CWES. Ascorbic acid interacted strongly with CWRS but not with CWES. This was more evident in loaf volume where at low levels of CWRS higher loaf volumes could be attained by using higher concentrations of ascorbic acid. Alpha-amylase had a strong interaction with CWRS. Concentrations required to maximize loaf volume were lower with higher amounts of CWRS. In the CWES blends, maxima in loaf volume were achieved at 75 SKB units α-amylase. High quality loaves could be made with a high percent of CPS white flour blended with CWRS flour by using high concentrations of ascorbic acid (>119 ppm) and low to medium levels of α-amylase (16-88 SKB units).

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1. INTRODUCTION.

Wheat is the most technologically intensive annual-harvested staple in Mexico. It is grown on large farms using modern agricultural techniques. Mexico's wheat production comprises mainly hard to semi-hard wheats, although lesser amounts of soft and durum wheat are also grown (Peña, 1995). Wheat production was estimated at 3.8 million metric tonnes for the 1996-1997 period, but is not sufficient to fulfill local requirements. The country has been forced to import wheat, which comes from the USA and Canada, its main trading partners. Mexico has been a substantial market for Canadian wheat for over two decades, although imports have been erratic due to the administration of import licenses. However, with the enactment of the North America Free Trade Agreement (NAFTA) on January 1, 1994, restrictions on wheat imports are being gradually eliminated (Nichols, 1993).

Mexican bread wheats are high yielding cultivars (4.1 tons per ha in average) of low to intermediate protein content (9.0 to 12.0%) and with gluten of intermediate strength, lower in comparison to Canadian and American bread wheats, which have high gluten strength. Mills can compensate to some extent for the lower strength of gluten in weaker Mexican wheats by blending with stronger local varieties. These blends are sold to small bakeries, which are mainly located in the rural areas and produce mainly sweet-type breads with dense crumb. However, industrial bakeries demand blends with stronger properties. This strength can be obtained by blending Mexican wheat varieties

with stronger imported wheat for the production of a large variety of commercial bread products (Peña, 1995). Mexican milling and baking industry has relied on Canadian red spring wheat varieties, such as Canada Western Red Spring wheat, to "carry" or give added strength to weaker flours.

Canada Western Red Spring (CWRS) is a high-protein wheat known for its excellent milling and baking quality and is well regarded for the production of high-volume pan breads. Due to its high gluten strength, it is also extensively used either alone or in blends with weaker lower-protein wheats for the production of a diverse range of products.

Canada Western Extra Strong (CWES) is a premium quality red spring wheat used for blending where strong dough properties are required. The predominant variety in this class is Glenlea, which was developed by the Plant Science Department at the University of Manitoba. Despite its longer mixing requirements, CWES may show adequate baking performance compared to a CWRS wheat when dough is mixed until optimized development (Bushuk, 1969). It has been shown that less CWES wheat flour is needed to attain equal baking performance compared to a CWRS wheat (Bushuk, 1980).

In recent years, the use of potassium bromate in bread formulations is being phase out or banned entirely due to its possible carcinogenicity. Many countries in Latin America and the Far East still use bromate at concentrations up to 100 ppm, and in most European Community countries it has never been allowed. In the US, the use of potassium bromate is prior-sanctioned with maximum permitted levels of 50 or 75 ppm, although baking companies have

voluntarily stopped using bromate following a request from the FDA in 1991. In Canada, as of June 1994, the use of potassium bromate has been prohibited.

The changing regulations regarding the use of bromate in flour and baking products has led to continuing research focused on finding adequate bromate replacers, a task that has proved to be difficult since bromate seems to exert its effect in many aspects of dough and bread quality. Besides stressing good manufacturing practices and attention to details in the baking process to solve problems arising from removing bromate from bread formulations (Zimmerman, 1991), the baking industry has focused its attention in the use of combinations of improvers that when optimized for the conditions in each bakery may give a situation similar to using bromate in the dough (Barnard, 1992). Ascorbic acid, DATEM (diacetyl tartaric acid esters of monoglycerides), and fungal α-amylase are some of the improvers that have been extensively studied for the replacement of bromate in bread formulations, and are currently used in the Mexican baking industry.

Ascorbic acid is the most accepted additive for the improvement of bread dough structure due to the lack of health and safety risks associated with it. It is now generally agreed that the oxidized form of ascorbic acid (L-dehydroascorbic acid) is the active form of the improver which oxidizes dough sulfhydryl groups into disulphide groups by means of an enzymatic mechanism (Sandstedt and Hites, 1945, Fitchett and Frazier, 1986, Stear, 1990). However, there is also evidence that the oxidation effect of L-dehydroascorbic acid may not be the only event involved in the strengthening effect of ascorbic acid in dough. Other

mechanisms have proposed the possible involvement of free radical species formed during the oxidation of ascorbic acid, that might be also involved in the oxidation of thiolate ions into thyl radicals for the formation of disulphide bonds (Nakamura and Kurata, 1997a, 1997b).

DATEM is classified as an anionic emulsifier with good dough strengthening and medium crumb softening properties. Its dough strengthening properties are closely related to its ability to form a complex with the protein fraction of dough, leading to the formation of a strong protein network that allows for a better retention of the carbon dioxide produced during the baking process (Kamel and Ponte, 1993). DATEM can also form a complex with the helical structures in starch reducing the rate of starch crystallization and hence reducing the rate of crumb firming (Krog and Jensen, 1970, De Stefanis *et al*, 1977).

Alpha-amylases for use in bakery foods are produced from cereal, bacterial and fungal sources, each with different characteristics such as thermostability and optimal pH and temperature of activity. Besides providing yeast with fermentable sugars during the fermentation stage, the main role of amylases resides in retardation of bread staling. Alpha-amylase has been proposed to act on the amylopectin branches that protrude into the intergranular space from the starch granules, hydrolyzing these branches up to the branch points, leaving fewer, shorter or no amylopectin branches available to crosslink for development of crumb firmness (Lineback, 1984, Bowles, 1996).

Some industrial bakeries in Mexico use a liquid ferment as their breadmaking process. A liquid ferment differs from conventional sponges in that flour levels vary from 0 to 70% in the initial ferment and water level is higher in order to produce a proper consistency appropriate for mechanical transfer. The advantages of a liquid ferment over the conventional sponge-and-dough process are that it saves in plant space and labor, increases processing flexibility, and allows for improved sanitation (Kulp, 1983). The development of a liquid ferment baking test is required to evaluate the effects of improvers on the quality of pan-bread consumed in Mexico, as opposed to no-bulk fermentation, high-speed mixing baking tests, such as the Canadian Short Process, which simulate the processes used in large bakeries in Canada and the US.

Several studies have investigated the "optimization" of improvers for enhancement of quality of bakery foods. However, most of these studies have investigated the effect of a specific improver by holding the levels of the other improvers constant. Although easier to analyze, this "one-factor-at-a-time" approach does not allow to identify potential important interactions between the improvers. More a more often, the literature has cited the use of appropriate experimental designs to overcome this problem and Response Surface Methodology (RSM) techniques offer an adequate solution.

RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes, for development of new products and improvement of existing product designs. Based on RSM, response surface methods use quantitative data to build an empirical model that

describes the relationship between each factor investigated and the response or responses. This model takes into account the effects of each factor, interactions between factors, and curvature.

No studies have been published on the optimization of improving agents in bromate-free formulations using a liquid ferment process. Studies on improvers using liquid ferments have used a "one-factor-at-a-time" approach to determine the effect of improvers or processing conditions on the quality characteristics of bread (Kulp 1983, 1986, Kulp *et al*, 1985). However, although the effect of individual improvers or processing conditions has been reported, interactions between them have not been identified. Optimization of improver formulations using a liquid ferment process would be of interest to bakeries in Mexico, where this baking process is used in large commercial operations, and where the increasing expansion of large bakeries to international markets, some of which have prohibited the use of bromate, required development of bromate-free formulations.

The general objectives of this research were:

- To optimize improver combinations for use with a commercial Mexican bread wheat flour in bromate-free formulations using a liquid ferment and the Canadian Short Process.
- From the previous objective, select a baking test that is the most adequate to study the effect of improver combinations on the quality characteristics of white pan-bread.

- To optimize improver combinations for use with blends of flours of different gluten strength using the most adequate baking test from the previous objective.

2. LITERATURE REVIEW.

2.1 World wheat production

Domestication and cultivation of grains have laid the ground through history for development of civilizations around the world: wheat in the West, rice in the Orient, and maize in Pre-Colombian America. Grains belong to the *Gramineae* family except for buckwheat, which belongs to the *Polygonaceae* family but is traded internationally as a cereal. The major cereal grains such as barley, maize, millet, oats, rice, rye, sorghum, and wheat, provide the bulk in food supply in terms of calories, and also in terms of protein in most countries.

Wheat is a special case. The interdependence between wheat and man is a global one, not limited by regional or ethnic preferences. The Food and Agriculture Organization (FAO) has ranked wheat equally with rice as one of the most important grains in the world, and among the most important food staples along with maize and potatoes. Wheat is also the most important grain in world commerce. Its financial and even political importance is owed to its unique physical and physicochemical attributes, mostly conferred by its protein fraction which has such a structure that it is able to retain gas in a leavened dough and produce a light aerated loaf of bread (Williams, 1993). Many types of wheat exist, but Orth and Shellenberger (1988) consider as commercially relevant four species of the genus *Triticum: T. monococcum* (diploid), *T. turgidum* (tetraploid), *T. timopheevi* (tetraploid), and *T. aestivum* (hexaploid). By far, the most widely

grown are *T. aestivum*, which includes the common or bread wheats, and *T. turgidum*, which includes the durum wheats.

Since the Second World War production of wheat has risen continuously, reflecting improvements in yield and techniques of growing, as well as government policy. Sewell (1992) said that world wheat production tends to outrun consumption. He stated that this is so despite the fact that an extra 12 million tonnes of wheat have to be produced each year, compared to the preceding year, in order to feed the population born in the same period of time. Recent estimates from the United States Department of Agriculture (USDA), and the International Grains Council (IGC), forecast a record world wheat production, including durum wheat, for 1997-1998: USDA estimates global wheat production at 603 million metric tonnes (MMT), while IGC's estimate is 598 MMT. These figures are higher than the record of 588 MMT set in 1990-1991 (CWB, 1997).

2.2 Wheat in Canada.

In Canada most of the wheat is grown in an area that encompasses the provinces of Manitoba, Saskatchewan, Alberta and a portion of northeastern British Columbia, which have a joint average production representing around 95% of the total (Canada Grains Council, 1996). Wheat dominates the Canadian cereal grain industry largely for climatic and agronomic reasons and contributes more to farm cash than any other commodity. Wheat makes up about half of the land seeded each year and is followed by barley as the second largest crop,

while canola ranks third. Durum wheat is grown in the drier, southern prairie areas.. Canada is the second highest exporter of wheat in the world. Exports averaged 26.73 MMT a year between 1986-1995. Projections for the year 2005 say that Canadian wheat exports will exceed 1 MMT to each of the following markets: Algeria, Brazil, China, Indonesia, Iran, Japan, Mexico, South Korea, and the US. A continued shift in market focus to Latin America and the Asia-Pacific region is also forecast (CWB and CGC, 1997).

2.2.1 Western Canada wheat classes.

Western Canada produces and exports seven classes of wheat: Canada Western Red Spring (CWRS), Canada Western Amber Durum (CWAD), Canada Western Red Winter (CWRW), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Extra-Strong Red Spring (CWES) and Canada Western Soft White Spring (CWSWS). A report issued by the Canadian Wheat Board and the Canadian Grain Commission (1997) projects an increase in exports of CWRS wheat from 14 MMT, the average for the period 1989-90-1993-94, to 17 MMT by the year 2005. CWAD exports are also expected to increase from 2.8 MMT to 3.4 MMT in the same period of time. For the rest of Canadian wheat classes, the projections on exports for 2005 are: CWES, 365,000 tonnes; CPSR, 1.5 MMT; CPSW, 1.8 MMT; CWSWS, 475,000 tonnes; CWRW, 80,000 tonnes.

Canadian domestic market absorbs about one-fourth of all Canadian wheat sales (seed, feed, food use), with the remainder being exported. This high proportion of export to production has led to the establishment of a complicated structure for grain movement and grain support institutions, both governmental and non-governmental (Sewell, 1992). Canadian wheat is classified by a grading system in existence since the beginning of the century and is considered to be the key to the success of Canada's wheat marketing, due to a rigid policy regarding license or release of new varieties. Varietal quality control is established under the western Canadian grading system by means of the use of varietal standards in the official grade definitions for the top grades in the system. Varieties eligible to be included in the top grades must be of equal or superior quality to this varietal standards, such as Neepawa for the CWRS class and Hercules for the CWAD class. For the other classes of Canadian wheat, specific quality guidelines are used instead of official varietal standards. Overall, the process ensures that all wheat produced, graded and sold will meet minimum quality standards related to the end-use of the wheat and the agronomic performance needs of Canadian farmers (CWB, 1997).

2.2.1.1 Canada Western Red Spring (CWRS) wheat class.

Canada is the world's largest exporter of hard red spring wheat, which represents about 85% of its production. This high-protein wheat is well known for its excellent milling and baking quality and is well regarded for the production of

high-volume pan breads. Because of its high gluten strength, it is also used extensively either alone or in blends with weaker lower-protein wheat for the production of a diverse range of products such as hearth breads, noodles, flat breads and steam breads. CWRS wheat is marketed in three separate milling grades. Grading decisions are based on physical characteristics such as test weight, kernel soundness, the percentage of hard vitreous kernels, foreign material and the presence of other classes of wheat within the sample. The top two grades, CWRS No. 1 and CWRS No. 2, are further segregated by protein (Williams, 1993).

Canada Western Red Spring No. 1 wheat shows a high degree of uniformity from shipment to shipment and from year to year. This wheat is sound, with low α-amylase activity and correspondingly high falling number values and flour amylograph peak viscosity. The milling and baking quality of this grade is consistently very high. Protein loss during milling is minimal. Flour produced from CWRS No. 1 wheat is characterized by strong, yet mellow, gluten properties and very high water absorption potential. CWRS No. 2 is very similar in quality to CWRS No. 1. Wheat qualifying for this grade must be reasonably sound, fairly well matured and reasonably free from severely weather-damaged kernels. The α-amylase activity of this grade of wheat is quite low and the falling number values are, therefore, quite high. As with CWRS No. 1, this grade of wheat has good milling and end-use quality. CWRS No. 3 wheat is high in protein content and has fair milling and end-use quality. The content of immature

and damaged kernels and tolerances for other cereals, foreign material and other types of wheat are somewhat higher than for the top grades (CWB, 1996).

2.2.1.2 Canada Western Extra Strong Red Spring (CWES) wheat class.

Canadian Western Extra Strong Red Spring is a premium quality red spring wheat used for blending where strong dough properties are required. The extra-strong variety Glenlea was developed by the Plant Science Department, University of Manitoba in 1965 and licensed in Canada in 1972 under the Spring Utility wheat class (Evans *et al*, 1972). Initially, Glenlea did not qualify for the CWRS class due to its long mixing requirements which result in low loaf volume using baking tests that use constant mixing time. Bushuk (1969) assessed Glenlea baking performance by a baking test that extended the time of mixing until optimized dough development and found that Glenlea performed essentially the same as Manitou, a licensed variety of the CWRS class.

Effective August 1, 1993, the Canada Western Utility (CWU) wheat class was renamed to Canada Western Extra Strong Red Spring wheat class to reflect more accurately the properties of this of this wheat (CWB, 1993). CWES wheat, in blends with other wheats, can be used to produce pan breads, hearth breads, buns and similar products. It has shown promising applications in whole wheat and specialty breads. Tests have shown that the use of white flour or whole wheat flour containing flour from CWES can allow a bakery to reduce, or even eliminate, the addition of vital wheat gluten in the manufacture of these breads.

Flour from CWES wheat will display higher ash levels compared to other red spring wheat types milled at the same extraction rates, due to the higher natural mineral content in the endosperm of the wheat which does not have a negative impact on the color of the flour. In a blending study using Glenlea and CWRS wheat flours, Bushuk (1980) found that in terms of carrying ability, Glenlea performed substantially better since equal baking performance was attained with considerably less Glenlea wheat (23%) than CWRS wheat (50%) in blends with a weaker flour. Addition of CWES flours has revealed two-to-threefold increases of shelf-life expectancy of doughs with excellent results when the product is thawed, proofed and baked.

2.2.1.3 Canada Prairie Spring Red (CPSR) wheat class.

Canada Prairie Spring Red (CPSR) is a semi-hard wheat with medium-strong dough properties and protein content averaging close to 11.0 per cent (basis 13.5 per cent moisture). Commercial milling experience indicates that CPSR has very good milling quality. CPSR wheat is particularly suitable for the production of French-type hearth breads. It can also be used alone or in blends to produce various types of flat breads, noodles, steam breads, pan breads, crackers and related products.

2.2.1.4 Canada Prairie Spring White (CPSW) wheat class.

Production of Canada Prairie Spring White wheat (CPSW) is now firmly established in Western Canada. Straight grade flours from CPSW have medium-strength dough properties and can be used alone or in blends for the production of many types of noodles, flat breads and some household flours. High extraction flours produced from this white-skinned wheat are well suited for various types of flat breads and chapattis.

2.3 Wheat in Mexico.

2.3.1 Wheat production.

In Mexico, wheat is grown by large commercial farms using modern technology. Better-quality wheat-based food items have seen their presence increased and diversified in the market. These items are mainly bread products (white bread and sweet bread), which vary in their type across the different regions in the country.

Mexico's wheat production comprises mainly hard to semi-hard wheats, although lesser amounts of soft and durum wheats are also grown. In the sixteenth century, wheat cultivation began close to what is now Mexico City. It spread to the west to an area part of the central plateau named "El Bajío", which covers the states of Querétaro, Guanajuato, Jalisco, and Michoacán (Figure 2.1). This area was reported in 1988 to comprise 200,000 hectares with a total production of 1.1 MMT and an average yield of 5.5 ton/ha. In 1940 the Mexican government, supported by the Rockefeller Foundation, began the production of wheat in the Northwestern states of Baja California, Sonora, and Sinaloa, and in 1960 it becomes the main wheat-producing zone in the country. As of 1988, the latter covered an area of 650,000 hectares with a total production of 3 MMT tons and an average yield of 4.6 ton/ha, accounting for 65% of the production of the wheat harvested by that year (Calderón, 1992; Westall, 1990; Salazar, 1992).

Wheat production in Mexico for the 1996-1997 crop year was estimated at 3.8 MMT. This figure represents around 25% the production of maize, the main staple food (Presidencia de los Estados Unidos Mexicanos, 1997). Wheat production is projected to reach 4.7 MMT by the year 2000. However, the population has grown at an average rate of 2.0% in the late 80's and 1.8% in the nineties. This has been reflected in a decline in the consumption of wheat in Mexico. The growth rate of per capita wheat consumption was -1.9% per year on the period 1985-1994, mainly due to economic factors. Maize tortillas, which compete directly with "bolillo" or standard hard roll, the most common baked flour product, are still heavily subsidized by the government, encouraging low-income consumers to buy more tortillas and fewer bread products. Nevertheless, in recent years there has been a slight increase on wheat consumption (Peña, *personal comm.*).

Wheat production is not sufficient to fulfill local requirements (local consumption averaged 5 MMT between 1992-1996) and Mexico has been obliged to import wheat, mainly from the USA and Canada, its main trading partners. Mexico has been a substantial market for Canadian wheat for over two decades although imports had been erratic due to the administration of import licenses. The U.S. possessed 100% of the market from 1974 to 1977, and also in 1989. Australia occupied nearly 100% of the market in 1985. In some years, imports were less than 15 tonnes. The three partners now have an agreement that precludes the use of export subsidies for this trade. In 1995, Canada's exports of wheat to Mexico (excluding durum wheat) totaled CDN$97 million,

remaining as Canada's second most important export to Mexico, following exports of canola seed. These two commodities made up 77% of Canadian total agri-food exports to Mexico in 1995 (Agri-Food Trade Service, 1996).

Average wheat yield has been inversely related to protein quantity in wheat (Dexter, 1993). Average wheat yield in Mexico is almost two times that of Canada's since Mexican farmers have encouraged research institutions to develop high-yielding cultivars in order to fulfill the increasing local demand. Due to this, Mexican wheats are high-yielding cultivars (4.1 tons/ha average), of low to intermediate protein content (9.0-12.0%), and have gluten with intermediate strength, lower in comparison to Canadian and American wheats. However, with the enactment of the North America Free Trade Agreement (NAFTA) on January 1, 1994, a gradual increase in the import of quality wheat was foreseen which prompted scientific institutions to undergo different programs for the improvement of quality of Mexican wheats.

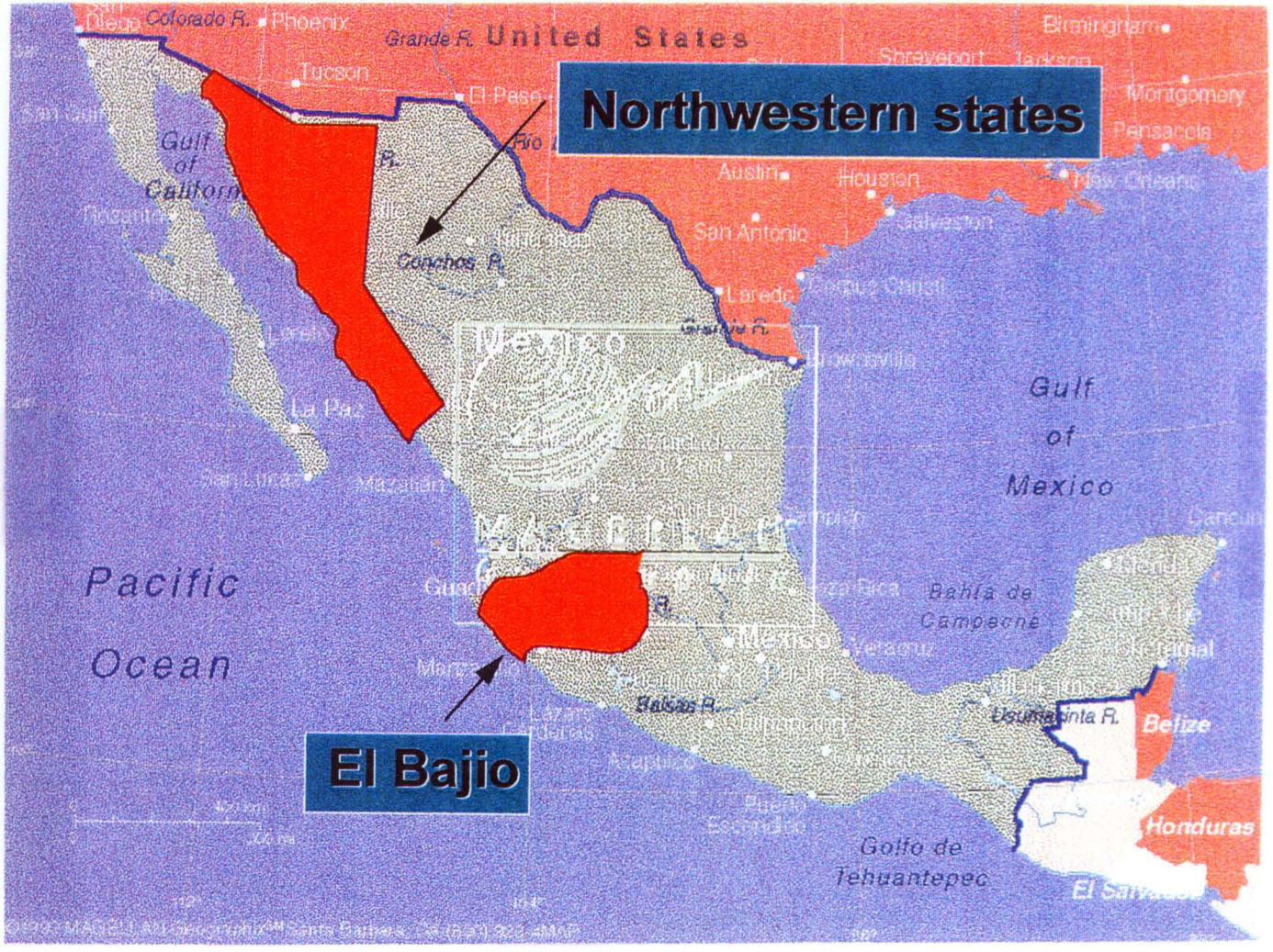


Figure 2.1 Main wheat-producing areas in Mexico.

2.3.2 Milling and baking industry.

The Mexican baking industry is very diverse. Most of small bakeries are located in the rural areas and they produce mainly sweet-type breads with dense crumb. In the urban areas, the baking industry is formed by small to large semi-mechanized bakeries that produce a large variety of breads, both regional and international. Most of the production in the baking industry is carried out by a small number of very large companies with long production runs and sophisticated national distribution networks.

Mills may compensate for the lower strength in Mexican wheats by blending with stronger local wheats, and flours thus obtained are sold to small bakeries. However, industrial bakeries are provided with blends of Mexican wheats with wheats of stronger properties, mainly Canada Western Red Spring No. 1 and No. 2 and American Hard Red Winter. Hard wheat imports represent between 5 to 15% of the total wheat used for food purposes. In the last years, preference was given to Canadian wheats due to quality consistency, although this has fluctuated due to cost issues (Peña, 1995).

Table 2.1 Mexican wheat quality groups.

Quality group	Grain Type	Gluten Strength Type	End-Use	End-Use Distribution (1992)	
				1000 tons	%
Group 1	Hard	Strong, balanced to extensible	Pan-type bread; to correct weaker wheats	615	15
Group 2	Hard to semi-hard	Medium strong	French and Spanish-type bread; light crumb and flake dough sweet breads	1,845	45
Group 3	Soft	Weak, extensible	Cookies, cakes, flour tortillas, sweet breads	1,025	25
Group 4	Hard to semi-hard	Strong to weak, tenacious	Cakes, dense-crumb breads and pastry	410	10
Group 5	Durum	Medium strong and strong	Long and short Italian pasta, noodles	205	5

*From Peña (1995).

A wheat end-use classification system has been established by the baking industry based on hardness and gluten strength. The latter is determined by the use of the alveograph. Table 2.1 shows quality groups of Mexican wheats and end-use distribution as of 1992. Most of the wheat produced goes to the production of French and Spanish-type white breads and sweet breads and the production of cookies, cakes, and flour tortillas.

2.4 Dough conditioning

At first glance, bread seems to be a very simple food item. It can easily be made from flour, water, salt, and yeast. However, for the baker this type of formulation presents problems of uniformity in the final product in terms of appearance, flavor, and texture. Minor additions to the formula are reflected in improvement in the criteria of quality mentioned above and in simplification of the baking process. Bakers may add different other ingredients such as shortening for texture and flavor, sugar as fermentation substrates and flavor improvers, emulsifiers for dough handling and shelf life improvement, oxidizers or reducers to compensate for deficiencies in the flour or mixing procedure, enzymes or enzymatic preparations to give a continuous supply of substrate for fermentation or to modify dough properties, and colours, flavors, and other substances to make the product more acceptable from the consumer's point of view (Matz, 1994).

The term conditioning has sometimes been used to describe the action of added ingredients or substances on the physical properties of dough. The term has also been used to describe the action of adjuncts that aid in the development of less tacky, more extensible doughs that are processed through machinery that result in a product of finer crumb structure, and improved volume and symmetry. In summary, dough conditioning may be reflected in: increased mixing and machining tolerance of the dough, increased tolerance to ingredient variations, diminished knockdown during handling, improvement in dough

absorption, improvement in loaf volume, structure, texture, and other quality characteristics, extension in product quality (shelf life), and facilitation of production of variety breads (Orthoefer, 1997). Part of this conditioning is the modification of the chemical environment in the dough so that hydration and cross-linking of the gluten molecules proceeds to an extent suitable for the kind of process and product under consideration. In order to understand what is involved in dough conditioning, some general aspects of dough oxidation are given in the following section.

2.4.1 Theoretical aspects of flour and dough oxidation.

Redox systems exert a significant role on the physical and chemical properties of wheat-flour dough and hence on the characteristics of the final product, due to the nature of wheat-flour macrocomponents such as thiol and disulphide bonds in proteins and peptides, linoleic and linolenic acids in lipids, and phenolic compounds, all of them prone to undergo redox reactions given the appropriate conditions.

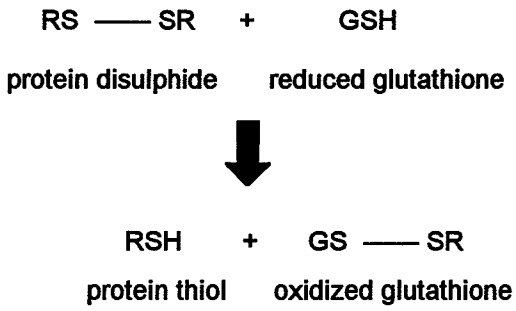
In wheat flour there exists an optimum degree of oxidation in order to get an adequate baking performance. This oxidation can be attained naturally in flour only after long storage by the oxidizing action of atmospheric oxygen. However, it is a general practice to add oxidizing agents to flour at the mill or bakery to improve its baking quality. The level of oxidizing agent to be added depends upon the inherent nature of the oxidant, the processing conditions, and

the properties of the flour. On the other hand, the oxidative response of flour is dependent upon the choice of wheat type, storage time and conditions of storage of wheat or flour, and milling procedure (Preston and Dexter, 1994). Untreated flours which lack an adequate degree of oxidation are known as "green" or under-developed flours and result in doughs which are too soft and pliable, that lack of elasticity, and have low oven-spring properties. Bread obtained from these flours have small volume, crumb with open cells, poor color, and coarse texture (Pyler, 1973).

Due to its complex nature, redox reactions in dough have not been fully explained. Diverse mechanisms proposed include:

1. oxidation of phosphatides in wheat flour (Geddes and Larmour, 1933);
2. inhibition of proteases (Jørgensen ,1936);
3. oxidation of sulfhydryl groups (Sullivan *et al*, 1936) ;
4. disulphide-sulfhydryl interchange reactions (Goldstein, 1957).

Theories No. 3 and 4 have been the most widely accepted. The disulphide-sulfhydryl interchange reaction is an exchange between the disulphide bonds in the gluten proteins (RS-SR) and the low-molecular-weight thiols, primarily glutathione (GSH reduced form). The reaction is illustrated as follows:

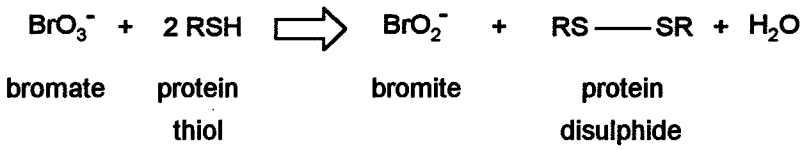


2.4.2 Potassium bromate.

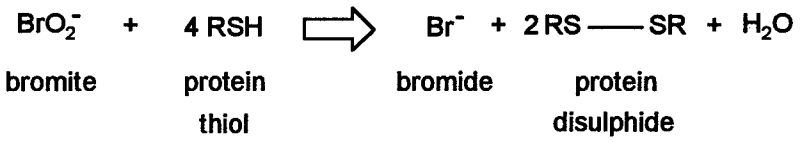
Among the diverse oxidants available in the market, potassium bromate has been by far the most widely used. Its usage in the baking industry dates back to 1915, when it was reported in a patent and sold to bakers as a mixture of breadmaking salts classified as "yeast food" (Kohman *et* al, 1915). The milling and baking industries have grown in a parallel way with bromate. It has influenced the breeding programs to develop new types of wheat and the types of processes used by bakeries. Furthermore, the usage of bromate increased as bakeries turned from bulk fermentation to larger and more automated processes.

It is agreed that potassium bromate acts on the sulfhydryl groups in gluten proteins and free thiol compounds present in dough by oxidizing these low-molecular-weight free thiols and protein thiols to corresponding disulphides. This would prevent these low-molecular-weight thiols from reducing molecular weight of gluten proteins due to this sulfhydryl-disulphide interchange reaction (Stear, 1990). Tkachuk and Hlynka (1961) proposed the following mechanism for the redox reaction of potassium bromate in wheat flour:

a) slow, rate controlling



b) fast



The first reaction (the slow reduction of bromate to bromite) was proposed to be rate-limiting, and the oxidation of flour proceeds *via* bromite in a fast stage. Unlike other oxidizers, the action of potassium bromate is slow and it occurs during the baking stage as the dough is heated. Tsen (1968) proposed that bromate requires a temperature of 40°C to be completely reduced. Other factors also influence the action of bromate. Cunningham and Hlynka (1958) observed that the presence of lipids on dough accelerated the bromate reaction and they suggested that lipids reacted with atmospheric oxygen, leaving more SH groups available to be oxidized by bromate. Bushuk and Hlynka (1960) demonstrated that a reduction on pH increases the rate of oxidation of SH groups by bromate.

2.4.2.1 Toxicological aspects of potassium bromate.

In recent years, the use of bromate has been limited by toxicological studies that indicate that it can be a possible carcinogen. Acute and subacute

toxicity studies have not yielded results that can point out that potassium bromate is of serious danger for humans at the levels used in baking goods.

2.4.2.1.1 Chronic toxicity and carcinogenicity studies.

The first chronic toxicity studies on potassium bromate were conducted in Great Britain by Fisher and colleagues (1979) who found no evidence of carcinogenicity, chronic toxicity, and retention or accumulation of covalent bromine in adipose tissue in rats fed for 104 weeks and mice for 80 weeks with bread-based diets made with untreated and treated flour with 0, 50 and 75 ppm potassium bromate alone or with other additives (Fisher et al, 1979).

Most of chronic toxicity studies have been done mainly in Japan and a comprehensive review is given by Kurokawa and colleagues (1990). In a study with male and female F344 rats fed 250 and 500 ppm bromate in drinking water over 110 weeks, the animals developed renal cell tumors (RCT; combined adenocarcinomas and adenomas) in all groups treated, and peritoneal tumors in male rats treated with 500 ppm. Incidence was significantly different from controls (Kurokawa et al, 1983).

In a dose-response study, male F344 rats were administered potassium bromate (0-500 ppm) in drinking water for 104 weeks. Survival times and growth were significantly different from controls in the 500 ppm group. Incidences of adenomas but not adenocarcinomas were significantly high in groups given 500, 250, and 125 ppm bromate. Combined incidences for follicular adenocarcinomas

and adenomas of the thyroid and for mesotheliomas of the peritoneum were significantly higher for rats given 500 ppm bromate (Kurokawa et al, 1986).

Bromate was not found to significantly induce tumorigenesis in female mice given 500 and 1000 ppm bromate in drinking water for 78 weeks, but it was reported to potentially induce tumorigenesis because spontaneous occurrence of renal cell tumors in mice is low and these were morphologically similar to those induced in rats (Kurokawa, 1986b).

Rats, mice and hamsters were given bromate by intravenous administration and the effect was studied on renal lipid peroxidation. Strong oxidative damage was found to occur in the kidneys of male rats, but was not significant in mice or hamsters. It was concluded that given the evidence of the important role of oxygen free radicals in carcinogenesis and the results reported in the study, bromate was reported to have a strong possibility to generate active oxygen radicals which induced lipid peroxidation and renal cell tumors in the kidney (Kurokawa et al, 1987).

2.4.2.2 Regulations

Based on these studies and on detectable levels of bromate in bread made from flour with 75 ppm, the joint FAO/WHO Expert Committee on Food Additives (JEFCA) reduced the recommended level of treatment of flour from 0-75 ppm to 0-60 ppm (WHO, 1989). This was further reduced by Codex Alimentarius to 0-50 ppm. In 1992 JEFCA recommended the exclusion of

bromate from flour standards and in July 1993, the Codex Committee on Food Additives and Contaminants approved the recommendation (Dupuis, 1997).

Many countries in Latin America and the Far East still use bromate at levels sometimes up to 100 ppm. Most EU countries have never allowed the use of bromate, and its use has been banned in the United Kingdom, Japan and New Zealand. In Canada, the levels of bromate allowed were up to 50 ppm at the mills and 100 ppm on the bakeries, although the baking industry had been using less than 60 ppm. As of June 1994, the use of bromate in bakery products has been prohibited (Dupuis, 1997).

2.4.2.3 Alternatives to the use of potassium bromate.

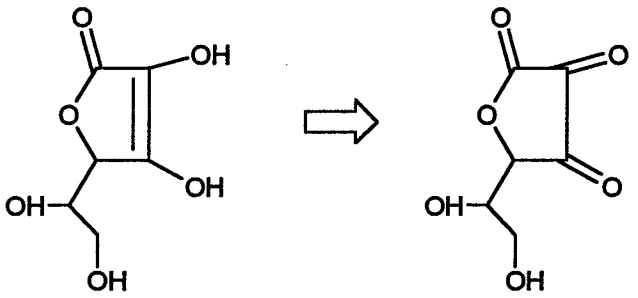
Due to the later, research has focused on finding adequate bromate replacers. This has proved to be not easy, since bromate seems to exert its effect in other aspects besides the oxidation reaction. Although it is classified as an oxidant, merely replacing it with other oxidants such as ascorbic acid or azodicarbonamide does not provide the same results in the finished product (Barnard, 1992). Zimmerman (1991) stressed good manufacturing practices and attention to details in the baking process as solutions to eliminating bromate in formulations. The baking industry has focused its attention on the later but also combined with the use of combinations of improvers that when optimized for the specific conditions in each bakery gives the baker a situation similar to using bromate in the dough (Barnard, 1992).

We can classify the different improvers in the following categories:

- redox agents: oxidizers and reducers (ascorbic acid),
- emulsifiers (DATEM),
- enzymes (amylases)

2.4.3 Ascorbic acid.

Ascorbic acid (AA) is the most accepted additive used to improve the structure of bread doughs. Listed as a vitamin (vitamin C) its improving action was recognized by Jørgensen (1935), who considered that the mechanism of action of AA involved a decrease in the activity of wheat proteases. Its rate of reaction is considered intermediate in comparison to bromate (slow) and azodicarbonamide (fast). It has four stereoisomers, the L-threo-isomer enhances the most the handling and baking characteristics of dough; the D- and L-erythro-isomers are less active and the D-threo-isomer is inactive (Kieffer *et al*, 1989).



L-Ascorbic acid L-Dehydroascorbic acid

Figure 2.2 Chemical structure of ascorbic acid and its oxidized form L-dehydroascorbic acid.

The mechanism of action of AA is quite different from that of bromate. In the absence of air, AA acts as a reducing agent and is used as such in continuous doughmaking processes. Melville and Shattcock (1938) concluded that the oxidized form of AA (L-dehydroascorbic acid, or DHAA) was the active form of the improver (Figure 2.2). Sandstedt and Hites (1945) suggested the involvement of enzymes in the conversion of AA to DHAA since structural analogues of AA were ineffective as dough improvers. It is generally accepted that the oxidation of AA is mediated by an enzyme present naturally in flour known as ascorbic acid oxidase. The reaction needs the presence of atmospheric oxygen that is incorporated in the dough by mixing. L-threo-DHAA then oxidizes the sulfhydryl groups into disulphide groups as stated previously in the mechanism of potassium bromate. This oxidative effect of L-threo-DHAA is catalyzed by another enzyme (L-threo-DHAA reductase) that yields L-threo-AA, using glutathione as the electron donor (Figure 2.3) (Fitchett and Frazier, 1986; Stear, 1990).

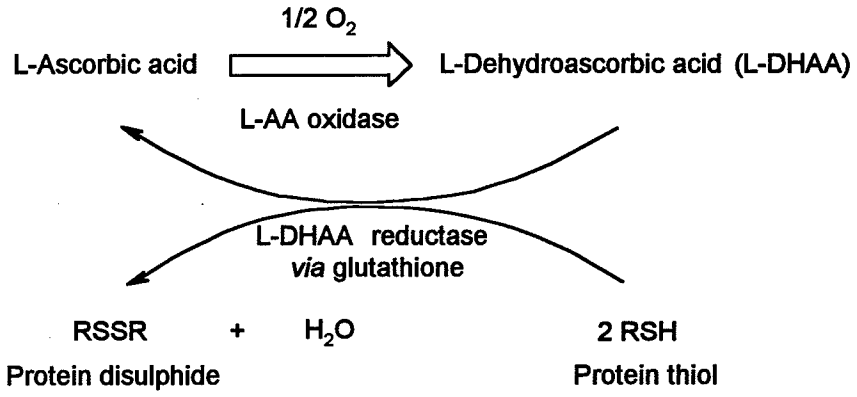


Figure 2.3 Oxidative mechanism of ascorbic acid in bread dough.

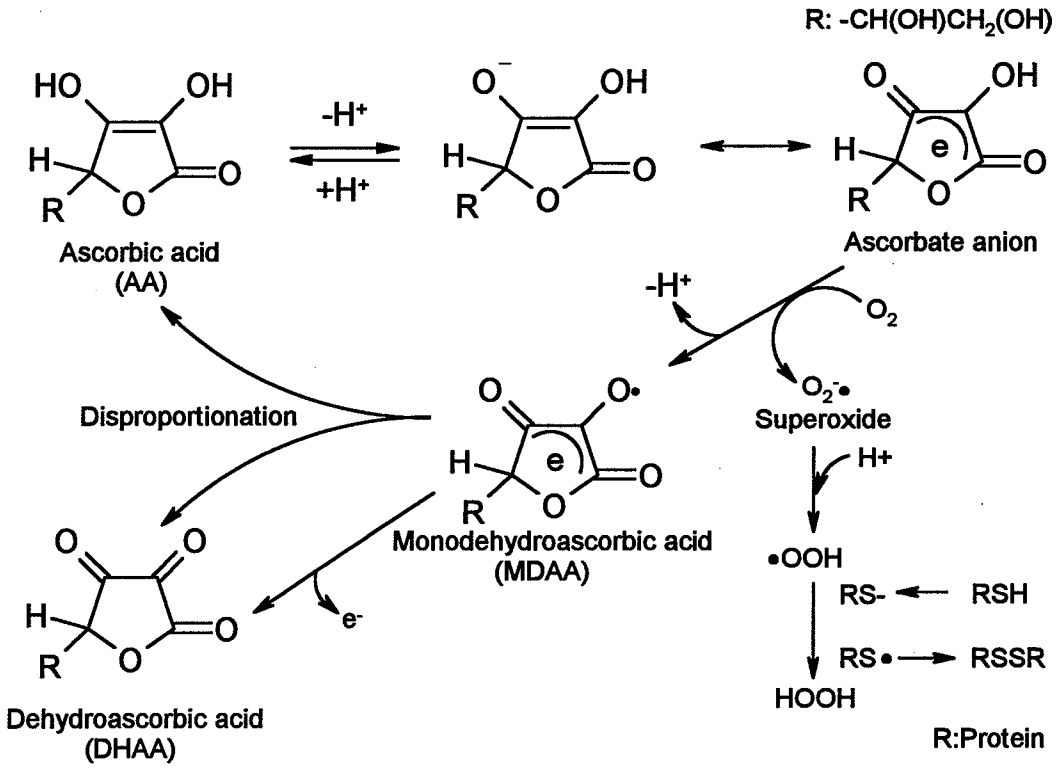


Figure 2.4 Superoxide reaction mechanism of ascorbic acid (Nakamura and Kurata 1997b, Sonntag *et al*, 1993).

However, there is evidence that the oxidation effect of L-DHAA is not the main event that is involved in the strengthening effect of AA in dough. Nakamura and Kurata (1997a, 1997b) suggested the possible involvement of free radical species formed during the oxidation of AA, such as superoxide anion radical (O_2^-). They proposed that the improvement effect of AA in dough may be due mainly to O_2^-, which would affect intra or intermolecular SH-SS interchange reaction of proteins and would form a tridimensional network. They proposed a mechanism in which L-DHAA is produced by the successive one-electron oxidation process of AA or by disproportionation of reactive

monodehydroascorbic acid (MDAA) as the first one-electron oxidation product of AA. O_2^- is generated by the transfer of an electron from AA to dioxygen with the formation of MDAA. This O_2- may be immediately reduced to H_2O_2 by dismutation reaction, along with the production of a hydroperoxyl radical (•OOH), which might oxidize thiolate ion to a thiyl radical forming an SS bond (Figure 2.4).

2.4.4 DATEM

The use of added emulsifiers in the baking industry dates back to the thirties, when super-glycerinated shortenings containing 10% mono-diglycerides were used by the baking industry as replacements of fat. In the fifties, diacetyl tartaric acid esters of monoglycerides (DATEM) were introduced in the US market as crumb softeners for white bread. However, in Europe DATEM was introduced as a dough strengthener in yeast-raised products. Later on, the introduction of other emulsifiers in the 60's, such as ethoxylated monoglycerides (EMG), soft mono-diglycerides, stearoyl lactylates (CSL, SSL), and succinylated monoglycerides (SMG) adversely affected the use of DATEM, situation now reverted due to the popularity of DATEM as both a crumb softener and a dough strengthener. The European baking industry is still using DATEM as the main dough strengthener, while in the US preference is given to stearoyl lactylates, polysorbates, and ethoxylated monoglycerides (Tamstorf *et al*, 1986).

The function of an emulsifier as a crumb softener is closely related to its ability to interact with starch in order to form a complex, particularly the linear amylose fraction, but also with amylopectin, although there is some controversy as to whether this effect is due to actual softening of bread or retardation of rate of crumb firming. As dough strengtheners, the emulsifier acts directly upon the colloidal character of the dough either through modification of its gluten, its state of oxidation or the surface tension between its aqueous and fat phase (Kamel and Ponte Jr., 1993).

Most emulsifiers consist of hydrophobic acid chains esterified to a hydrophilic group which may originate from different types of polyvalent alcohols, such as propylene glycol, glycerol, sorbitan, or sucrose (Krog, 1981). DATEMs are esters formed by the reaction between a monoglyceride (preferably distilled type) and diacetyl tartaric acid anhydride (made from 1 mole tartaric acid and 3 moles acetic anhydride) at 100-130°C (Figure 2.5). DATEMs synthesized from saturated monoglycerides have a melting point of about 45°C. DATEMs are anionic emulsifiers, more hydrophilic than mono and diglycerides (HLB 8-10), and carry a negative charge, which enables them to be markedly influenced by pH and ionic strength (Kamel and Ponte Jr., 1993).

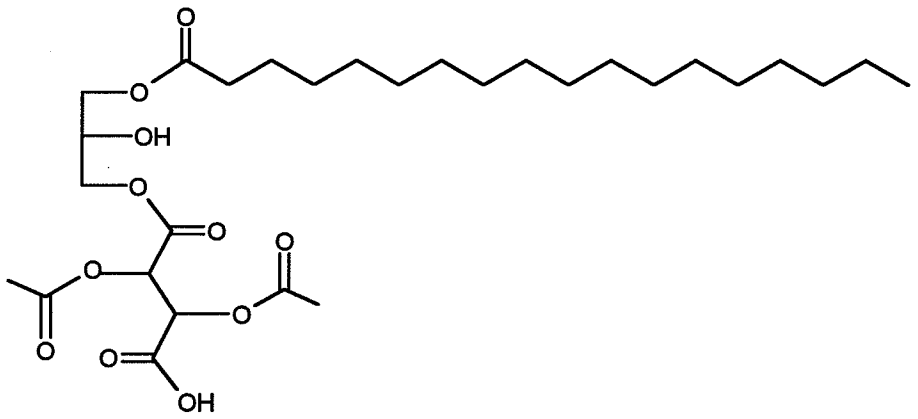


Figure 2.5 Chemical structure of DATEM.

Emulsifiers such as DATEM have different effects on dough and final product all along the breadmaking process (Schuster *et al*, 1984). :

- Mixing
  - Decrease of mixing time and mixing speed
  - Reduction of shortening levels
  - Improvement of mixing tolerance
  - Improvement of machinability
- Baking
  - Improvement of gas-retaining properties
  - Better loaf volume
  - Better texture
  - Better crumb grain
  - Better uniformity
  - Decrease of water loss
- Fermentation
  - Improvement of gas- retaining properties
  - Shorter fermentation
  - Greater shock-tolerance

- Storage
  - Improvement of crumb softness
  - Longer shelf-life

These effects on dough and final product are due to a number of different factors. During mixing they improve dough wettability, stabilization of distributed phases, and better distribution of shortening present in the formulation. On the other breadmaking stages, their effect is due to the different interactions of the emulsifier with the protein, lipid and carbohydrate fractions.

Starch and protein are amphiphilic and their structures consist in helical regions. Emulsifiers such as DATEM can form a complex with starch helical regions and retard starch crystallization in bread crumb, hence reducing crumb firming. Krog and Jensen (1970) found that there is a good correlation between the amount of emulsifier complexed with starch and the degree of crumb softening in bread. De Stefanis *et al* (1977) showed that a surfactant formed a complex with both fractions of starch (amylose and amylopectin) in bread and concluded that emulsifiers with different structures differ widely in their effect in crumb firmness. Gawrilow (1977) developed a mathematical model to predict the effect of added emulsifiers on bread quality. Pisesookbunterng and D'Appolonia (1983) reported that crumb softeners seem to reduce the water migration by complexing with starch and absorbing to its surface.

The formation of a complex between the emulsifier and the protein fraction in the dough is the basis for its dough strengthening properties. When this complex is formed a strong protein network is formed, allowing for a better

retention of the carbon dioxide produced during the fermentation and proofing stages of the breadmaking process. This is also reflected in a better texture and increased loaf volume. Anionic emulsifiers such as DATEM will bind to the hydrophobic surface of the protein, promoting aggregation of gluten protein by neutralizing the positive charges present in the surface of the protein. The latter may also contribute to the unfolding of the protein structure, promoting binding of emulsifier and strengthening the dough (Kamel and Ponte, 1993).

In Canada, the maximum permitted level of DATEM in breads is 0.6% flour weight, whereas in the USA it has a GRAS (generally recognized as safe) status.

2.4.5 Amylase

Enzyme systems in raw materials and baking additives play an important role in all stages of the breadmaking process. The objectives of the application of enzymes in baking technology are the optimization of dough properties and the quality improvement of bakery products (Kulp, 1993). Generally speaking, amylases are enzymes that exert their activity by hydrolyzing gelatinized or damaged starch granules and are generally ineffective on native starch granules.

Alpha-amylase is an endoenzyme that hydrolyzes the α-(1→4) glycosidic bonds in starch in a random manner, yielding dextrins, oligosaccharides and maltose as final products. β-amylase is an exoenzyme that also hydrolyzes α-

(1→4) glycosidic bonds, but it attacks only at the non-reducing end of the starch chain, yielding maltose units and β-limit dextrins and it stops at the α-(1→6) glycosidic bonds present in amylopectin. It has been observed that the rate of hydrolysis is higher in α-amylase than in β-amylase (Richardson and Hyslop, 1985).

Wheat grain germinates when humidity conditions are high before harvest, resulting in excessive α-amylase activity which makes flour unsuitable for breadmaking. Bread made from this flour has sticky crumb and over-colored crust. On the other hand, wheat flour from ungerminated grains contains a rather high content of β-amylase, but the low α-amylase activity reduces the production of fermentable carbohydrates in the dough and α-amylase supplementation is needed. This supplementation also results in improvement in rheological properties of the dough and crumb and crust characteristics of bread (Drapron and Godon, 1987).

Amylases used in bakery foods are produced from cereal, bacterial or fungal sources. Barley and wheat malt flours are produced by germinating these cereals, drying and subsequent milling to flour fineness. Malt syrups are obtained from these germinated grains by extraction and concentration of the enzymes (Table 2.2).

Bacterial α-amylases are usually obtained from strains of *Bacillus subtilis*. They show high heat stability and due to this their addition level is very difficult to control since active enzyme remains at the baking stage and produces excessive levels of soluble dextrins. The final product is unacceptably gummy or

sticky, with poor slicing properties. Furthermore, residual activity is present in the baked bread and gumminess, although may not be present in fresh final product, may develop during storage.

Fungal α-amylases are prepared as concentrated dried extracts from cultures of *Aspergillus oryzae*. These preparations are relatively pure, containing low levels of other enzymes such as proteases, pentosanases, cellulases, oxidases, etc. The levels of secondary activity vary with the process of preparation and the manufacturer of the enzyme. However, they show limited thermostability and are inactivated prior to the onset of starch gelatinization during baking. As a result, they have little effect on amylopectin hydrolysis and do not exhibit significant staling activity (Hebeda *et al*, 1990).

Besides providing yeast with fermentable sugars, the main role of amylases in breadmaking resides on retardation of bread staling. Bowles (1996) suggested a staling model based on Lineback's model (Lineback, 1984). α-amylases would act on the amylopectin branches that protrude into the intergranular space from the starch granules. Due to their higher susceptibility to enzymatic attack, exoamylases would hydrolyze these branches up to the branch points, leaving fewer, shorter or no amylopectin branches available to crosslink for development of crumb firmness (Figure 2.6).

The improving effect of amylases on bread characteristics such as loaf volume, crumb and crust characteristics and final product shelf-life is evident. Processors must adequate these enzymes and their dosage to their specific formulations and processes.

Table 2.2 Physical properties of amylase used in baking.

Origin	Type	pH ranges		Temperature ranges	
		Optimum	Stability	Optimum	Effective
Fungal					
A. oryzae	α-glucoamylase	4.8-5.8	5.5-8.5	45-55°C	up to 60°C
A. niger		4.0-4.5	3.5-5.5	55-60°C	up to 70°C
Bacterial					
B. subtilis	α	5.0-7.0	4.8-8.5	60-70°C	up to 90°C
Cereal					
Barley malt	β	5.0-5.5	4.5-8.0	40-50°C	up to 70°C

From Stear (1990).

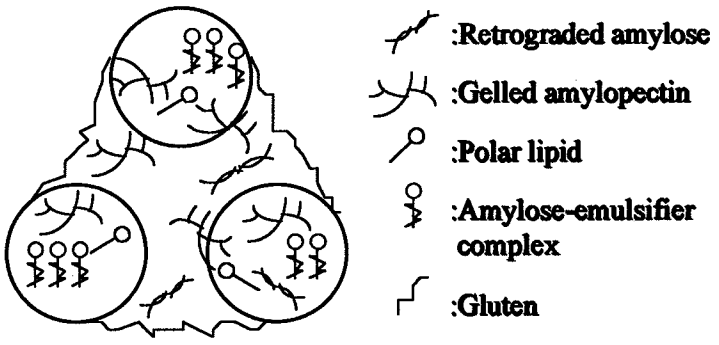


Figure 2.6 Bowles' model for bread staling.

2.5 Baking test methods.

2.5.1 GRL Canadian Short Process.

The GRL Canadian Short Process (CSP) is a baking test developed at the Grain Research Laboratory of the Canadian Grain Commission in Winnipeg, Manitoba. The CSP is a high-speed mixing, short fermentation laboratory method, modified from commercial procedures, that resembles the processing conditions and formulations of processes used in Canadian commercial bakeries.

The CSP was first reported in a study for the assessment of physical dough properties and baking characteristics of a straight flour and thirteen flour streams obtained from a No. 1 Canada Red Spring wheat (Preston *et al* 1982a). In this study, the CSP was used along with three bulk-fermentation procedures and the GRL sponge-and-dough process. CSP had the highest baking absorption of the straight grade flour and the flour streams (66%). The authors stated that this high absorption may be due to a decrease in fermentation losses as a result of a lack of a bulk fermentation. It performed equally with the GRL sponge-and-dough procedure in yielding loaves with higher crumb colour values, probably due to a richer formula and larger loaf volumes. Overall bread scores of the straight grade flour were higher with the CSP than with the other procedures.

The CSP has been used to evaluate the baking performance and oxidation requirements of Canadian bread wheat varieties in short-time baking

methods, where bulk fermentation has been partially or completely eliminated. Yamada and Preston (1992) studied the effect of individual oxidants (iodate, ADA, ascorbic acid, and bromate), on the oven rise and bread properties of Canadian Short Process bread using a straight grade Canadian red spring flour. Stepwise increases in the levels of each oxidant resulted in increased loaf volumes and improved bread score until the optimum concentration of the oxidant was attained. At these optimum levels, there were no significant differences among the oxidants in improving loaf volumes and bread scores. Higher levels of oxidants were required in the CSP in comparison to conventional bulk-fermentation processes, an observation supported by a later study (Preston and Dexter 1994).

The CSP has also been used as a reference quality test in studies evaluating the performance of Canadian wheat varieties on processing conditions used in new markets for Canadian wheat. Dexter and coworkers (1989) evaluated the performance of Canada Western Red Spring (CWRS), Canada Utility (CU), Canada Western Red Winter (CWRW), and Canada Prairie Spring (CPS) wheat classes baked by a Colombian high-fat, high-sugar short process. Compared to the CSP, the Colombian process yielded loaves with reduced proof heights and specific volumes, due to the high sugar concentration that inhibited gas production during proofing. Optimum baking absorptions were significantly lower for the Colombian process doughs than for the CSP doughs, a result of higher shortening levels in the Colombian formula, and the use of sheeting rolls for dough development. Breads made with the Colombian process

had firmer and less resilient crumb properties than those breads obtained with the CSP using corresponding flours.

2.5.2 Liquid ferments.

For many years, the most popular breadmaking methods in North America have been the straight-dough and the sponge-and-dough processes, or modifications of these. However, in the fifties some conventional sponge processes were modified from a stiff plastic dough to a liquid form which could be transferred by pumping thanks to the improvements in the technology of continuous mixing processes. At the beginning of the century, Jago and Jago (1911) described a procedure for the production of different types of liquid or semi-liquid ferments that were used by bakers in France and England during the past century.

Kulp (1983) applied the term "liquid ferment" to those sponges that could be transferred by pumps, and are also known as "liquid sponges", "brews" or "broths". The advantages of a liquid ferment process over the conventional sponge-and-dough process are: 1) savings in plant space, 2) labor savings, 3) increased processing flexibility, and 4) improved sanitation. By carrying out the fermentation in a liquid to semi-liquid medium, the baker was provided with a greater flexibility since the ferment could be cooled down readily and if necessary kept overnight without any noticeable deterioration. The nature of the initial Stable Ferment Process, introduced by the American Dry Milk Institute

(MacLaren, 1954) left enough room for modification and improvement that enabled the automation of a continuous liquid ferment system (Borthwick, 1971).

In general, liquid ferments differ from conventional sponges in the following (Kulp, 1983):

- yeast is allowed to achieve its maximum rate of activity before it is added to the dough, producing by-products that will contribute to the final flavour of the product,
- flour levels vary from 0% for water brews up to 70% for flour ferments, which is the highest level at which the ferment can still be pumped. Conventional plastic sponges contain 60-100% of the total amount of flour,
- water level is higher in liquid ferments in order to produce a proper consistency appropriate for mechanical transfer,
- buffers are generally added to the water ferments to keep the pH within an acceptable range for yeast activity,
- sugar is used in liquid ferments to support fermentation and its use is higher than in plastic sponges,
- salt is added to control fermentation and release carbon dioxide from the fermenting brew,
- slightly higher levels of dough strengtheners are required since it has been observed that higher levels of oxidation are necessary for the brew than for the sponge-and-dough process.

However, more basic biochemical information was needed to improve bread manufactured by liquid ferment processes. Kulp and coworkers (1985), using water ferments, reported that the use of a commercial buffer not only was useful for controlling pH and acidity, but also enhanced glucose and fructose utilization by yeast during the initial fermentation. Production of ethanol was observed to be depleted in the buffered system and consumption of sugar was faster in the water ferment than in a sponge-and-dough system, although higher amounts of fermentable sugars were required for the liquid ferment, mainly due to higher utilization of sucrose, that was readily cleaved into glucose and fructose. They suggested the determinations of ethanol or sugars during fermentation as more reliable indices in following the fermentation process than monitoring pH and titratable acidity. Similar results were reported in a further study (Kulp, 1986), where it was seen that firmness of loaves made with water ferments was higher than with 40% flour ferments Water ferment loaves were also scored as less fresh and less flavored compared to flour ferments. It was also reported that flour could act as a natural buffer.

Maselli (1955) showed that the maximum rate of gas production was attained at pH values between 4.0 and 5.4. Below pH 4.0 yeast activity was inhibited and this inhibition was to some extent non-reversible. This was confirmed by Bayfield and coworkers (1963), who concluded that at pH values lower than 3.0 doughs obtained were not manageable and extremely sticky. They used different acids to acidify dough and saw that the type of acid was not important in influencing bread characteristics.

In some cases, buffers may be used to control pH in the ferment in order to have suitable yeast activity, mainly in the case of water ferments. Proteins from milk, wheat flour itself, or soy flour may serve as effective buffers and additionally supply yeast nutrients. Gross and coworkers (1968) used calcium propionate as a buffering agent and reported that addition of 0.1% of calcium propionate to a water ferment along with 0.33% non-fat dry milk was effective to keep pH of ferment above 4.0 for a fermentation time as long as 3.5 hours.

Carroll and coworkers (1956) investigated the importance of pH for enzymatic activity in ferments. Using malted wheat flour and fungal α-amylase, they concluded that in buffered systems the activity of these two enzymatic preparations remained constant after 24 hours of fermentation. However, in unbuffered systems no α-amylase activity remained after 2.5 hours of fermentation. Protease activity of preparations was less affected and at least 50% of initial activity remained after 6 hours of fermentation. No difference was found if enzymatic preparations were added either to the ferment or to the dough.

2.6 Statistical designed experiments. Optimization techniques.

2.6.1 Theory of designed experiments.

One of the most important and interesting aspects of product development is the implementation of techniques which minimize cost by reducing the number of experimental formulations required to study a particular product characteristic

(Mullen and Ennis, 1979). Statistical experiments are more efficient than are "one factor at a time" or single-step experiments. This advantage is also reflected in the ability of statistical experiments to identify "true" optimums and to determine the effects of individual variables and interactions between variables. Dziezak (1990) highlights the following steps to implement designed experiments:

- Define the purpose of the study and identify the factors and the responses.
- Develop a model for each response to be evaluated. This model is an equation that will be used for prediction of response values at different factor levels after the analysis of data.
- Select the specific design to be used to test the factors in a minimum number of trials. Consider the number of factors and whether the model includes interaction and/or curvature effects. Determine number of runs minimum to test for experimental error and lack of fit.
- Conduct experiments in a randomized or blocked order where appropriate in order to minimize error.
- Analyze data to the extent outlined by the experimenter's objectives.
- Examine the data in a graphical form to discern relationships between factors and responses and to point out regions for further study.

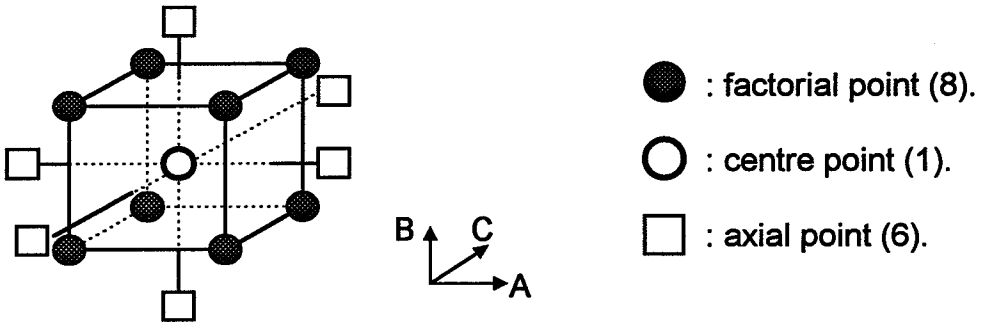


Figure 2.7 Central composite design for k= 3.

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It is also used in the development of new products and in the improvement of existing product designs. Based on RSM, response surface methods use quantitative data to build an empirical model that describes the relationship between each factor investigated and the response. This model takes into account the effects of each factor, interactions between factors, and curvature.

The central composite design (CCD), introduced by Box and Wilson (1951), is by far the most popular class of second-order designs and widely used in RSM. It involves the use of a two level factorial or fractional factorial (resolution V) combined with 2k axial or star points (k= number of factors) (Figure 2.7). The factorial points represent a variance optimal design for a first-order model or a first-order + two-factor interaction model. Centre points provide information about estimation of internal or pure error and existence of curvature in the system. If curvature is found, the axial points are used for the estimation of

the pure quadratic terms and they do not contribute to the estimation of the interaction terms (Myers and Montgomery, 1995).

In many cases, use of RSM has to deal with several dependent variables or responses that need to be optimized at the same time. Several techniques have been developed to deal with this situation. The use of overlay plots is a good approach when having few responses. Contour plots originated from individual models fitted to the dependent variables are constructed following a set of criteria. These plots are superimposed over each other and the regions in which acceptable predictions for the independent variables overlap are identified. A disadvantage from this approach is that the analysis of overlay plots becomes more complex as the number of the dependent variables increases (Myers and Montgomery 1995).

2.6.2 Applications in the baking industry.

Saguy *et al* (1984) mentioned that slowdown in the implementation of optimization techniques in food engineering was due to two main reasons: 1) the difficulties in the mathematical modeling and simulation of the behavior of foods, originated by the extreme complexity of their physicochemical characteristics, and 2) lack of training in optimization techniques that may provoke fear of entering the field due to preconceived views of its complexity. Nonetheless, adoption of optimization techniques has increased thanks to advances in optimization theory, applied mathematics, numerical analysis and to progress in

computer hardware and software that have eased the analysis required by this optimization techniques.

Several studies in the baking field have been reported where optimization procedures have been used to design more efficient processes and to elucidate the interactions between the different bread components. Mitchell *et al* (1986) cites the following: a study on breads fortified with added protein from different sources, where the independent variables were the type of protein added and its concentrations; study of the effect of oven variables on bread quality where baking temperature, height of the bread in the oven, humidity, and air circulation velocity were the independent variables. More recently, Toufeili *et al* (1994) used RSM to analyze the effects of methylcellulose, egg albumen, and gum arabic on the sensory properties of gluten-free pocket-type flat bread baked from formulas based on pregelatinized rice flour and pregelatinized corn starch with corn flour; and Perron (1995) evaluated CWRS and CWES wheat flours, alone or in blends, to determine their requirement for improving agents in bromate-free formulations using the Canadian Short Process.

However, no studies have been published on the optimization of improving agents in bromate-free formulations using a liquid ferment process. Studies on improvers using liquid ferments have used a "one-factor-at-a-time" approach to determine the effect of improvers or processing conditions on the quality characteristics of bread (Kulp 1983, 1986, Kulp *et al*, 1985). However, although the effect of individual improvers or processing conditions has been reported, interactions between them have not been identified. Optimization of

improver formulations using a liquid ferment process would be of interest to bakeries in Mexico, where this baking process is used in large commercial operations, and where the increasing expansion of large bakeries to international markets, some of which have prohibited the use of bromate, may require development of bromate-free formulations.

3. OPTIMIZATION EXPERIMENT #1: OPTIMIZATION OF IMPROVER SYSTEMS IN PAN-BREAD FORMULATIONS USING A COMMERCIAL MEXICAN WHEAT FLOUR

The beneficial use of improvers in the baking industry, for improvement of processing conditions and enhancement of the quality of dough and the final product, has been well documented in the literature. Ongoing research and product innovations in this area have been promoted by the relatively recent ban on the use of potassium bromate in pan-bread formulations In Canada and parts of the US, due to concerns with bromate's potential carcinogenicity. The purpose of this experiment was to study the effects of DATEM, ascorbic acid, and α-amylase, improvers commonly used in the Mexican baking industry, in bromate-free pan-bread formulations using a commercial Mexican bread wheat flour. Comparison of improver effects was carried out using both a liquid ferment baking test, developed in our laboratory to simulate the formulation and processing conditions used by industrial bakeries in Mexico for production of white pan-bread, and the Canadian Short Process, a testing method developed at the Grain Research Laboratory of the Canadian Grain Commission in Winnipeg for evaluating bread wheat quality. The two methods were compared for their sensitivity to the improvers listed above. Response Surface Methodology was used to identify the most critical factors and appropriate levels for enhancement of quality characteristics of pan-bread.

3.1 Objectives

1. To evaluate the effect of improver formulations containing DATEM, ascorbic acid and α-amylase on the quality of white pan-bread baked from a commercial Mexican bread wheat flour.
2. To compare two baking tests, a liquid ferment and the Canadian Short Process, for sensitivity to the effects of these improver formulations.
3. To identify levels of improvers and develop predictive models that optimize quality characteristics of pan bread, by using statistically designed optimization techniques.

3.2 Materials and Methods.

3.2.1 Flour.

A commercial Mexican bread wheat flour was obtained as a donation from Grupo Industrial Bimbo through the mill La Espiga located in Mexico City. This flour was a composite sample of Mexican wheat and a stronger imported wheat at an unknown blend ratio. The blend contained no additives and was representative of the type of flour used at an industrial baking plant for production of white pan bread. Flour had been recently milled, and so was allowed to mature naturally at room temperature in its original container for a period of 6 weeks. Then it was weighed into 200-g portions, packaged in heavy

plastic bags, and stored at 5°C. Flour was withdrawn from the refrigerator 24 hours before it was needed for a baking test .

Approved methods (AACC 1993) were used to determine moisture (method 44-15A), protein (N x 5.7) (method 46-13 and as modified by Williams (1993)), ash (method 08-01), farinograph parametres (method 54-21), and mixograph parameters (method 54-40A).

3.2.2 Ingredients and additives.

Sugar (Rogers), whey powder, and Crisco shortening (Procter & Gamble, Inc., Toronto, ON) were obtained at local supermarkets. Fresh compressed yeast (Fleischmann's Yeast, La Salle, QC) was purchased weekly from Penner Foods Bakeshop, Winnipeg. Salt and ammonium phosphate were obtained from Sigma Chemical Company (St. Louis, MO). Brew ferment buffer with no oxidizing agents was a donation from ADM Arkady (Olathe, KS) and was used both as a buffering agent and as yeast food.

Diacetyl tartaric acid esters of monoglycerides (DATEM, PANODAN® 205K), and fungal α-amylase (GRYNDAMYL™ S-250, 500 SKB u/g) were obtained from Danisco Ingredients Canada, Inc., (Rexdale, ON); L-ascorbic acid was from Sigma Chemical Co., (St. Louis, MO). All other chemicals were reagent grade.

3.2.3 Breadmaking procedures.

Two baking tests were used to produce pan bread: a liquid ferment baking test (Table 3.1), and the Canadian Short Process (Table 3.2).

3.2.3.1 Liquid ferment baking test.

A liquid ferment baking test was developed in the laboratory to simulate the formulation and process used in an industrial bakery in Mexico, based on the personal description of the formulation and process from personnel in a large commercial bakery in Mexico (Contreras, *personal comm.*). Compressed yeast, sugar-salt, and L-ascorbic acid solutions were prepared as described in Appendix I. Pup loaves were made from 100 g of flour (14% mb). A diagram of the liquid ferment baking test is presented in Figure 3.1. Farinograph water absorption (FAB) was used as the level of water added in the formulation, and the amount of water was corrected taking into account water present in solutions. Initial ferment components were mixed in a GRL mixer 200 at 140 rpm for 2 min at 30°C. The resulting ferment was placed in a bowl, covered to avoid moisture loss, and allowed to rest in a proofing cabinet (37°C, 83% rh) for 2 hours. This fermentation time was determined to be adequate based on a preliminary experiment where the criteria to assess proper ferment development were pH and titratable acidity (TTA) (Appendix II). After two hours of fermentation, the rest of the ingredients and the improvers were added (L-

ascorbic acid always as the final ingredient) and mixed at 30°C to a maximum of 10% past peak. Dough was rounded by hand, sheeted through 12.8, 6.4, and 3.2 mm gaps, rolled, and placed in a metal bread pan. It was allowed to proof to 95 mm height in the proofing cabinet. Doughs were baked at 205°C for 25 min., cooled for 30 minutes, and stored in individual plastic bags.

3.2.3.2 Canadian Short Process baking test.

For the Canadian Short Process baking test, the procedure developed at the Grain Research Laboratory of the Canadian Grain Commission in Winnipeg, Manitoba and first described by Preston *et al* (1982) was followed, with the modifications proposed by Perron (1995). This method most closely resembles the processing conditions (high speed mixing, short fermentation) and formulations used in most large Canadian commercial bakeries. A diagram of the baking test is shown in Figure 3.2. Compressed yeast, ammonium phosphate, L-ascorbic acid and the sugar-salt solutions were prepared according to Appendix I. A standard water level of farinograph water absorption plus 1% , and a mixer speed of 140 rpm were used. Doughs were also proofed to 95 mm height as was done in the liquid ferment baking test.

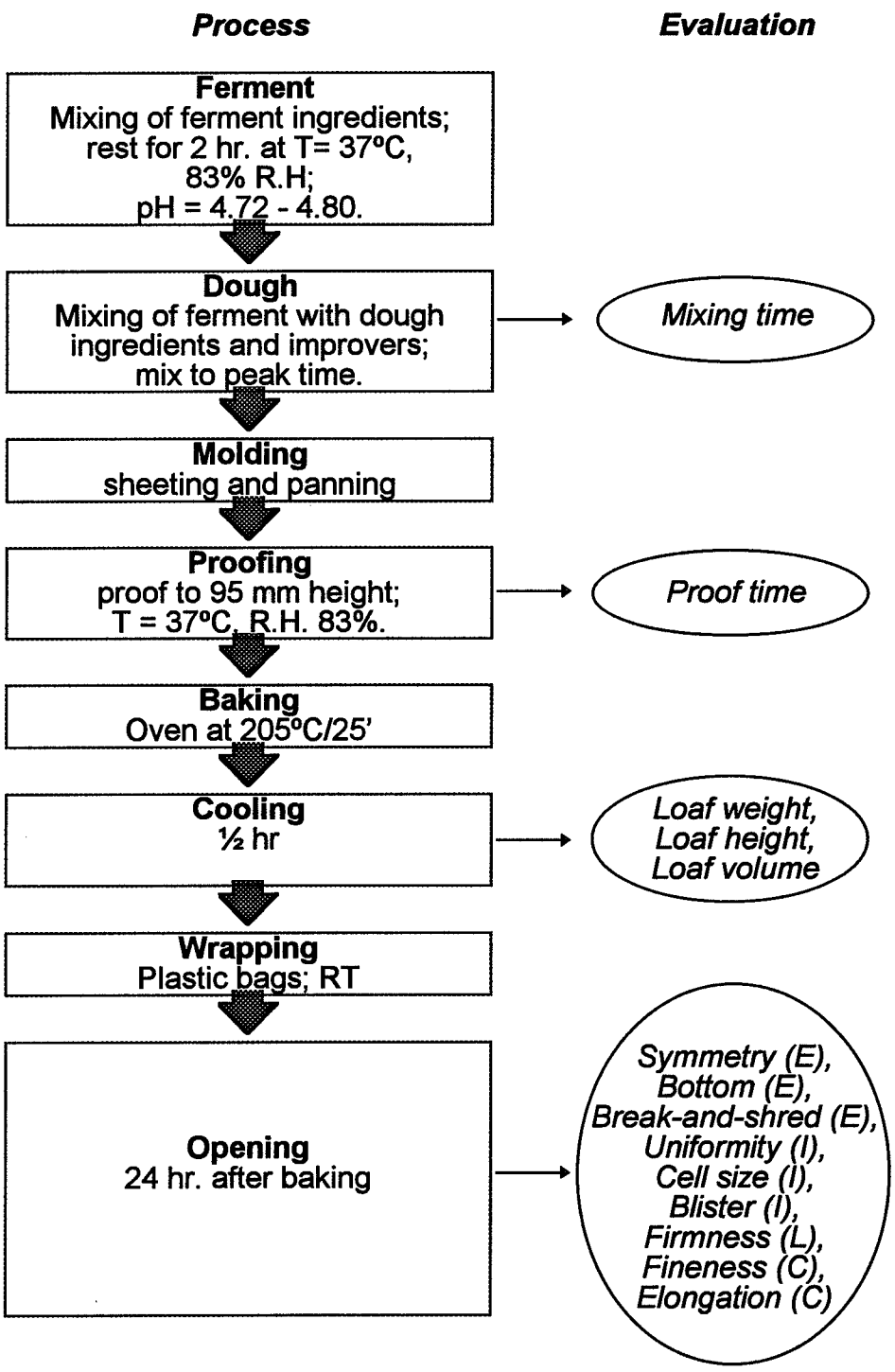
Table 3.1 Liquid Ferment baking test formulation for pan-bread.

	Ferment	Dough	Total
Flour (g; 14% mb)	56.0	44.0	100.0
Water (ml)[1]	30.6	25.1	55.7
Compressed yeast (g)	3.0	-	3.0
Sucrose (g)	2.1	6.4	8.5
Salt (g)	0.5	1.5	2.0
Shortening (g)	-	3.0	3.0
Ferment Buffer (g)	0.2	-	0.2
DATEM (g)	-	variable	
Ascorbic acid (ppm)	-	variable	
Fungal α-amylase (SKB u)	-	variable	

[1] Total amount of water based on FAB. Total amount water is distributed as follows: 55% in ferment, and 45% in dough.

Table 3.2 Canadian Short Process baking test for pan-bread.

	Total
Flour (g; 14% mb)	100.0
Water (ml)	variable
Compressed yeast (g)	3.0
Sucrose (g)	4.0
Salt (g)	2.4
Shortening (g)	3.0
Ammonium phosphate (g)	0.1
Whey (g)	4.0
DATEM (g)	variable
Ascorbic acid (ppm)	variable
Fungal α-amylase (SKB u)	variable



E: External characteristics *I*: Internal characteristics

L: Lloyd Universal Testing Machine *C*: CrumbScan® scores.

Figure 3.1 Liquid ferment baking test and responses analyzed.

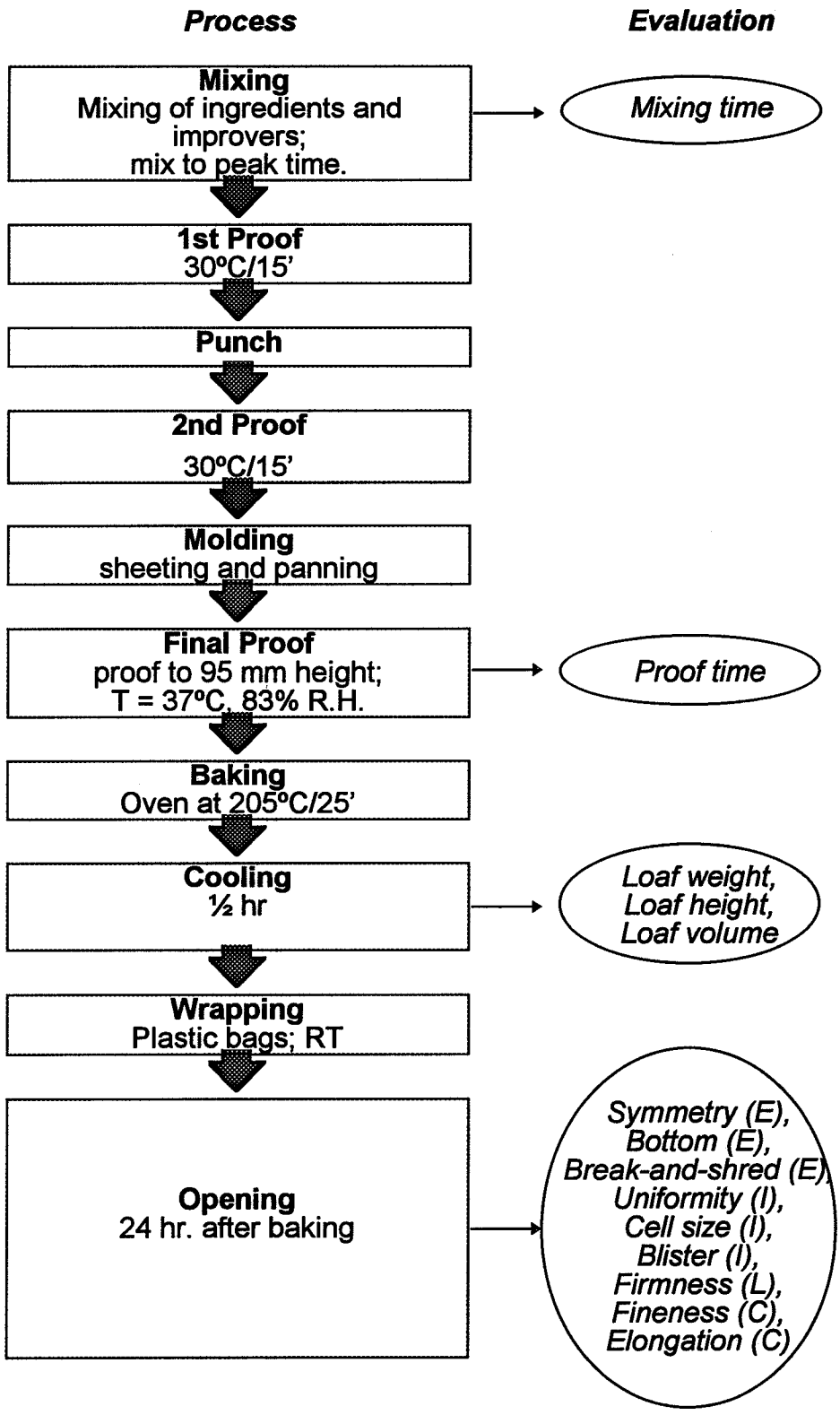


Figure 3.2 Canadian Short Process baking test and responses analyzed.

3.2.4 Evaluation.

Mixing time (second mixing when using the liquid ferment baking test) and proof time were recorded, as well as loaf weight, loaf height and loaf volume after 30 min. cooling. Loaf volume was determined by a rapeseed displacement volumeter (Manufacturing Co., Lincoln, NB). External loaf characteristics (loaf symmetry, shape of bottom, and break-and-shred), and internal loaf characteristics (crumb uniformity, cell size, and presence of blisters) were assessed 24 hours after baking by means of individual 10-point scales in a ballot as described by Perron (1995) and shown in Appendix III. Break-and-shred develops as a result of the reactions during oven spring, and is used as a parameter for evaluating flour quality. Weak flours will form poor break-and-shred, whereas too little proof will lead to a large oven spring, and thus undesirable break-and-shred (D'Appolonia 1996).

After evaluation of external and internal loaf characteristics, a 2.5-cm thick slice was taken from the center of the loaf. Crumb firmness was measured on this slice using a Lloyd Universal Testing Machine equipped with a 100 N load cell according to AACC method 74-09 (AACC, 1993). Crumb firmness was reported at 25% compression. This same slice was analyzed for crumb fineness and cell elongation using the CrumbScan® software (American Institute of Baking, Manhattan, KS). Fineness score is a measure of crumb cell uniformity and it increases as crumb is more homogeneous. Elongation score is a measure of cell average shape and it tends to 1 as crumb cells are rounder.

3.2.5 Experimental Design.

The independent variables used in the study were DATEM , ascorbic acid, and α-amylase. Levels of the factors were selected based on permissible levels of usage in Mexico as described in the official legislation for pan-bread (NOM-F-159-1983). This legislation states that the limits of additives in white pan bread are: emulsifiers such as lecithin, mono- and diglycerides and sodium stearoyl lactylate can be used up to a concentration of 5% w/w; preservatives such as sorbic acid can be used up to 0.16% w/w; oxidizers such as potassium bromate, calcium bromate, potassium iodate and calcium peroxide can be used alone or in combination at concentrations not higher than 75 ppm, and in the case of azodicarbonamide at concentrations not higher than 45 ppm.

A blocked, modified, rotatable central composite design was used to evaluate the effects of the variables on the quality of white pan bread. Factor levels and level codes are shown in Table 3.3. The experiment consisted of two runs for each factorial point (8 x 2), four runs for each center point (1 x 4) and a single run for each axial point (6 x 1) (Table 3.4). The total number of runs per baking test was 26. Experiments were blocked according to baking day to account for possible effects of day-to-day variability in laboratory conditions. The liquid ferment baking test required three baking days (Table 3.5), while the Canadian Short Process baking test required two baking days (Table 3.6). Order of baking days and runs within each baking day were randomized. Data were analyzed using the statistical software Design Expert 5© version 5.0.4 (Stat

Ease Corporation, Minneapolis, MN). ANOVA, fitting of second-order models to the responses, and the production of contour and surface plots were carried out.

Table 3.3 Factor levels and level codes for the 3-factor rotatable central composite design.

Factors	Level codes				
	-2	-1	0	+1	+2
DATEM (%)	0.000	0.125	0.250	0.375	0.500
Ascorbic acid (ppm)	0	50	100	150	200
α-amylase (SKB u)	10.0	32.5	55.0	77.5	100.0

Table 3.4 Treatments for the 3-factor rotatable central composite design.

	DATEM	AA	α-amylase
• Factorial points	-1	-1	-1
(2 runs at each point)	-1	-1	+1
	-1	+1	-1
	-1	+1	+1
	+1	-1	-1
	+1	-1	+1
	+1	+1	-1
	+1	+1	+1
• Center points	0	0	0
(4 runs at each point)			
• Axial points	-2	0	0
(single run at each point)	0	-2	0
	0	0	-2
	+2	0	0
	0	+2	0
	0	0	+2

Table 3.5 Liquid ferment baking test. Allocation of treatments to baking days. Treatments were randomized within each baking day.

		DATEM	Ascorbic acid	α-amylase
DAY 1	Factorial points	-1	-1	-1
(9 runs)		-1	-1	+1
		-1	+1	-1
		+1	+1	-1
		+1	+1	+1
	Center points	0	0	0
		0	0	0
	Axial points	+2	0	0
		0	-2	0
DAY 2	Factorial points	-1	+1	+1
(9 runs)		+1	-1	-1
		+1	-1	+1
		-1	+1	-1
		-1	-1	+1
		+1	+1	-1
	Center points	0	0	0
	Axial points	-2	0	0
		0	0	+2
DAY 3	Factorial points	-1	-1	-1
(8 runs)		+1	+1	+1
		-1	+1	+1
		+1	-1	-1
		+1	-1	+1
	Center points	0	0	0
	Axial points	0	+2	0
		0	0	-2

Table 3.6 Canadian Short Process baking test. Allocation of treatments to baking days. Treatments were randomized within each baking day.

		DATEM	Ascorbic acid	?-amylase
DAY 1	Factorial points	-1	-1	-1
(13 runs)		-1	-1	+1
		-1	+1	-1
		-1	+1	+1
		+1	-1	-1
		+1	-1	+1
		+1	+1	-1
		+1	+1	+1
	Center points	0	0	0
		0	0	0
	Axial points	0	0	+2
		0	-2	0
		+2	0	0
DAY 2	Factorial points	-1	-1	-1
(13 runs)		-1	-1	+1
		-1	+1	-1
		-1	+1	+1
		+1	-1	-1
		+1	-1	+1
		+1	+1	-1
		+1	+1	+1
	Center points	0	0	0
		0	0	0
	Axial points	0	0	-2
		0	+2	0
		-2	0	0

Table 3.7 Physical and chemical characterization of commercial Mexican bread wheat flour.

• Moisture (%)	11.9
• Protein (%, nitrogen x 5.7, 14% mb)	11.9
• Ash (%)	0.6
• Farinograph	
Water absorption (%)	55.7
Stability (min)	15.0
Tolerance index (b.u.)	3.4
Arrival time (min)	1.9
Departure time (min)	17.0
• Mixograph	
Mixing development time (min)	3.2
Peak height (b.u.)	0.2

3.3 Results.

Physical and chemical properties of the commercial Mexican bread wheat flour are presented in Table 3.7. Baking absorption was slightly lower compared to Canadian hard red spring wheats, which usually have baking absorptions above 60%. Flour protein content was high and comparable to Canadian bread wheat flours. Farinograph data revealed that the Mexican wheat flour is appropriate for production of pan-bread.

The first step in the optimization experiment was the selection of an adequate experimental design. The 3-factor rotatable central composite design (CCD) chosen for this study was examined for main effects and interactions of the three independent variables (DATEM, ascorbic acid, and α-amylase). This

was performed using an ANOVA for each response variable, and data were fitted to a second-order regression model. The full second-order model for a 3-factor central composite design is:

$$Y= b_0 + b_1A + b_2B + b_3C + b_4A^2 + b_5B^2 + b_6C^2 + b_7AB + b_8AC + b_9BC$$

For this experiment, A= DATEM, B= ascorbic acid, and C= α-amylase. Adequacy of the model was judged according to the p-value of the model (preferably <0.1) and the R^2 statistic (as high as possible). After selecting the most adequate model, all the least squares coefficients were calculated along with their associated p-values. These associated p-values are interpreted as the probability of getting a coefficient as large as that observed, when the true coefficient is equal to zero. In other words, small values of p indicate significant coefficients in the model.

Fitted models were further reduced after analysis of the coefficients. A correct specification of the regression model is crucial, since leaving out important regressors introduces bias into the parameter estimates, while including unimportant variables weakens the prediction or estimation capability of the model. Deleting unimportant variables from the model improves the precision of estimation for the parameter estimates of the retained variables (Myers and Montgomery 1995). A forward stepwise regression procedure was used to select the appropriate subset of variables for the reduced regression model. This automatic search method develops a sequence of regression models by adding or deleting an independent variable *via* its partial F- or t-statistic at each step. A regressor added at an early step may later proof

redundant because of the relationship between it and regressors added later on the equation. This procedure ends with the identification of a single regression model considered to be the "best". Models are required to obey the principle of hierarchy. This principle states that the presence of higher-order terms (such as interaction and quadratic terms) requires the inclusion of all lower-order terms contained within. In other words, if a two-factor interaction term is in the model the both main effects must also be included in the model. This principle has been considered reasonable when fitting polynomials (Myers and Montgomery, 1995; Neter et al, 1990).

After selection of best fitting models for each response variable, contour and response surface plots were generated to facilitate interpretation of the effects of each independent variable in the study. A set of criteria was established to determine if the size of an effect was considered to be of practical importance, based on empirical recommendations (Table 3.8). Graphical summaries of the analysis of contour and surface plots for the different responses were produced for the liquid ferment and the Canadian Short Process baking tests to simplify the identification of factors effects on each of the responses (see Tables 3.15 and 3.20). To see the effect of a specific factor, the column corresponding to this factor should be read assuming that the levels of the other factors are kept constant. Arrows indicate whether there is an increase or decrease in the response at increasing levels of the factor under the specified conditions; a half moon symbol indicates the presence of a maximum or minimum.

Table 3.8 Criteria for determination of practical importance of the effect of the factors on the different responses.

Mixing time	≥ 0.5 min
Proof time	≥ 5 min
Loaf height	≥ 1 cm
Loaf volume	≥ 30 cc
Break-and-shred	≥ 2
Uniformity	≥ 2
Cell size	≥ 2
Firmness	-

It is worth mentioning that when reading Tables 3.10 and 3.17, interactions should be interpreted cautiously. An interaction between two factors means that the magnitude and direction of the effect of a factor depends on the level of the other factor, and viceversa. Sometimes the direction of the effect of a factor changes at different levels of the other factor, which is the case when an increase in a response at one level of a factor is reversed to a decrease at another level of the same factor. This is indicated as opposite arrows in the table. The point where the effect of the factor changes in direction is called the *stationary point* (Myers and Montgomery, 1995). This stationary point can represent a maximum in the function, a minimum, or a "saddle" point. This saddle point can be seen in the contour plots as the center of a hyperbolic series of contours, called a saddle system. When interpreting the interactions in the table, it should be considered that the size of the effect is more pronounced as the levels of the factors are set farther away from the stationary point.

3.3.1 Selection of best fitting models and interpretation of contour and surface plots.

3.3.1.1 Liquid ferment baking test.

Original data for the liquid ferment baking test are presented in Appendix IV. Probability (p) and R^2 values for the full and reduced models are shown in Table 3.9. Mixing time, proof time, loaf height, loaf volume, break-and-shred, uniformity, cell size, and firmness were responses with p-values ≤ 0.1 for the reduced models. In general these reduced models had lower R^2 values than the full models. The models fitted to mixing time and loaf volume had the highest R^2 of all the models (0.5921 and 0.8469, respectively). The rest of the models had R^2 values lower than 0.5. Coefficients of the reduced models for each of these responses and their associated p-values are shown in Table 3.10.

Table 3.9 Liquid ferment baking test. Full and reduced quadratic models[1].

Responses	Full model		Reduced model	
	p-value	R^2	p-value	R^2
Mixing Time (min.)	0.0659	0.6100	0.0039	0.5921
Proof time (min.)	0.1845	0.5197	0.0153	0.3285
Loaf Weight (g)	0.4916	0.3879	-	-
Loaf height (cm)	0.0226	0.6794	0.0024	0.3481
Loaf volume (cc)	0.0002	0.8565	< 0.0001	0.8469
Fineness	0.9251	0.1969	-	-
Elongation	0.7884	0.2735	-	-
Symmetry (E)	0.4956	0.3864	-	-
Bottom (E)	0.5155	0.3791	-	-
Break-and-shred (E)	0.1777	0.5236	0.0187	0.4476
Uniformity (I)	0.2580	0.4821	0.0042	0.4065
Cell Size (I)	0.1497	0.5407	0.0058	0.3875
Blister (I)	0.7630	0.2846	-	-
Firmness (N)	0.2797	0.4721	0.0433	0.2585

[1] A "-" symbol represents a empty model (no significant terms) after stepwise regression.

Table 3.10 Liquid ferment baking test. Reduced model coefficients in terms of coded factors.

For stepwise regression $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Mixing time		Proof time		Loaf height		Loaf volume		Break-and-shred		Uniformity	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	3.2		76.2		11.5		955.3		5.8		7.1	
b_1: % DATEM [A]	0.10	0.1130	-0.95	0.0162	0.16	0.0024	15.77	<0.0001	0.45	0.0571		
b_2: Ascorbic acid [B]	-0.06	0.2737					8.26	0.0085	0.20	0.3610		
b_3: α-amylase [C]	-0.13	0.0383	0.70	0.0673			15.89	<0.0001	0.55	0.0216	-0.24	0.4147
Quadratic terms												
b_4: A^2												
b_5: B^2							-9.51	0.0034	-0.42	0.0637		
b_6: C^2	0.14	0.0231					-12.65	0.0003			-1.04	0.0013
Interaction terms												
b_7: AB	-0.24	0.0042										
b_8: AC												
b_9: BC												
p-value	0.0039		0.0153		0.0024		<0.0001		0.0187		0.0042	
R^2	0.5921		0.3285		0.3481		0.8469		0.4476		0.4065	

Table 3.11 (cont.) Liquid ferment baking test. Reduced model coefficients in terms of coded factors.

For stepwise regression $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Cell size		Firmness	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms				
b_0: Expected midpoint	7.2		1.3	
b_1: %CWRS [A]			-0.04	0.2280
b_2: Ascorbic acid [B]				
b_3: α-amylase [C]	0.23	0.4433		
Quadratic terms				
b_4: A^2			-0.08	0.0273
b_5: B^2				
b_6: C^2	-1.04	0.0019		
Interaction terms				
b_7: AB				
b_8: AC				
b_9: BC				
p-value	0.0058		0.0433	
R^2	0.3875		0.2585	

3.3.1.1.1 Mixing time.

For mixing time, the reduced model included the main effects of DATEM, ascorbic acid, and α-amylase, the quadratic effect for α-amylase, and the two-factor interaction DATEM x ascorbic acid. The effect of DATEM on mixing time depended on the concentration of ascorbic acid, and viceversa, due to the significant two-factor interaction. The positive sign of the quadratic term for α-amylase indicates a minimum in the response, which occurs at approximately 65 SKB u α-amylase. Surface and contour plots of ascorbic acid vs α-amylase, derived from the models, are shown in Figure 3.3 at different levels of DATEM. Table 3.11 shows the predictions for mixing time at different levels of the factors.

Mixing times increase significantly at higher concentrations of DATEM when the levels of ascorbic acid are below 120 ppm. On the other hand, mixing times decrease significantly at higher concentrations of ascorbic acid when the levels of DATEM are above 0.22%. Alpha-amylase has a small effect on mixing time, but a minimum in the response occurs at 65 SKB u.

A minimum of 2.9 min in mixing time is predicted at 0.125% DATEM, 50 ppm ascorbic acid, and 55.0 - 77.5 SKB u α-amylase, whereas a maximum of 3.9 min is predicted to be at 0.375% DATEM, 50 ppm ascorbic acid, and 32.5 SKB u α-amylase.

3.3.1.1.2 Proof time.

For proof time, the reduced model included the main effects of DATEM and α-amylase. Ascorbic acid did not have a significant effect. Table 3.12 shows the predictions for proof time at different levels of DATEM and α-amylase when level of ascorbic acid is fixed at 100 ppm.

Overall, the effects of DATEM and α-amylase in proof time were small. Proof times decrease at higher concentrations of DATEM, whereas they increase at higher concentrations of α-amylase. A minimum of 74.5 min is predicted at 0.375% DATEM and 32.5 SKB u α-amylase, while a maximum of 77.8 min is at 0.125% DATEM and 77.5 SKB u α-amylase.

3.3.1.1.3 Loaf height.

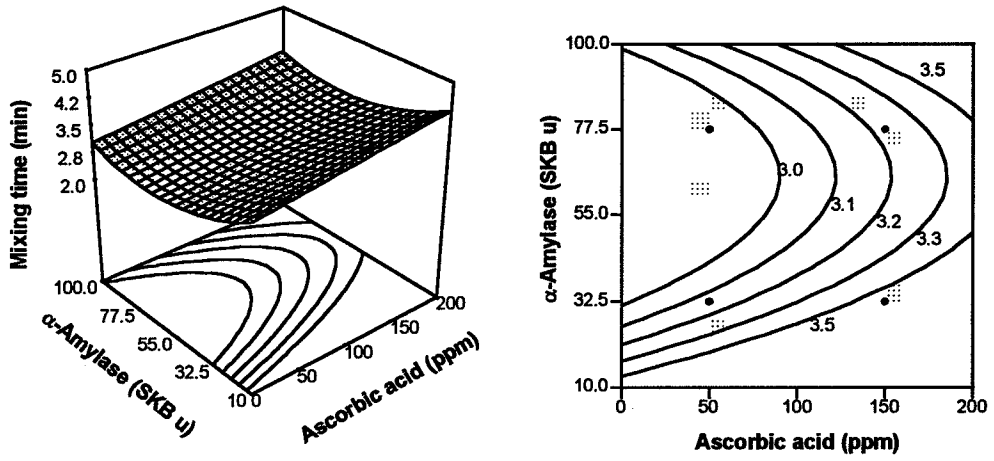
For loaf height, the reduced model included only the main effect of DATEM. The effects of ascorbic acid and α-amylase were not significant. The effect of DATEM in loaf height was small. Increasing the concentration of DATEM from 0.125% to 0.375% results in an increase of 0.3 cm in loaf height at any given concentration of the other two improvers.

Table 3.11 Liquid ferment baking test. Reduced model predictions for mixing time (min) at different levels of DATEM, ascorbic acid and α-amylase.

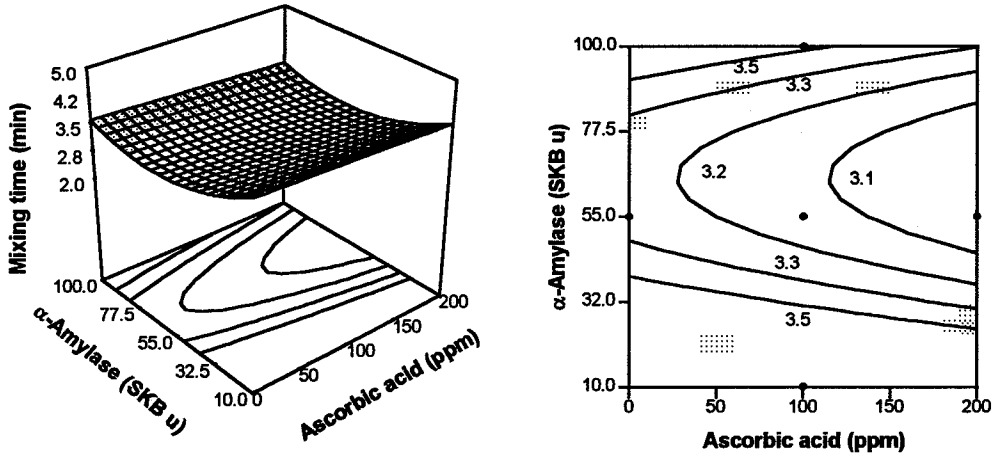
α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
	0.125% DATEM (-1)		
32.5 SKB u (-1)	3.2	3.3	3.5
55 SKB u (0)	2.9	3.1	3.3
77.5 SKB u (+1)	2.9	3.1	3.3
	0.25% DATEM (0)		
32.5 SKB u (-1)	3.5	3.4	3.4
55 SKB u (0)	3.2	3.2	3.1
77.5 SKB u (+1)	3.3	3.2	3.1
	0.375% DATEM (+1)		
32.5 SKB u (-1)	3.9	3.5	3.2
55 SKB u (0)	3.6	3.3	3.0
77.5 SKB u (+1)	3.6	3.3	3.0

Table 3.12 Liquid ferment baking test. Reduced model predictions for proof time (min) at different levels of DATEM and α-amylase. Ascorbic acid concentration fixed at 100 ppm.

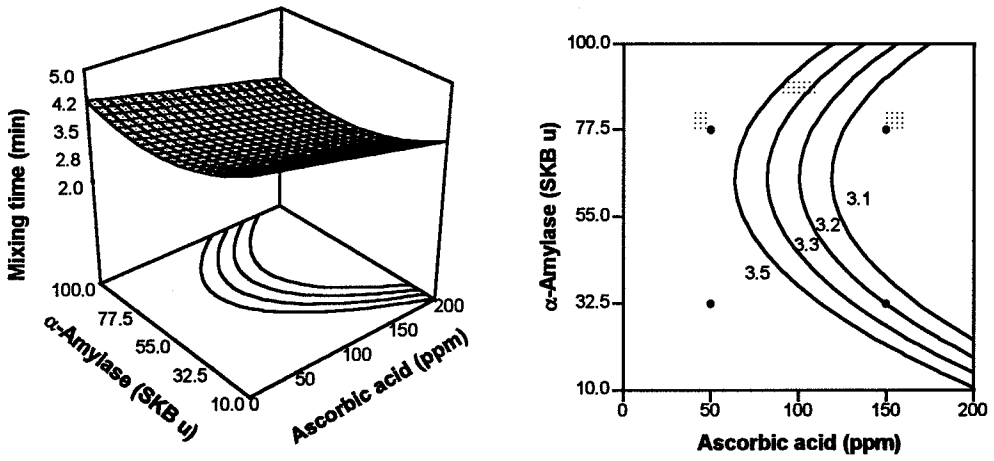
α-Amylase	**100 ppm Ascorbic acid (0)**		
	0.125% DATEM (-1)	*0.25% DATEM (-1)*	*0.375% DATEM (-1)*
32.5 SKB u (-1)	76.4	75.5	74.5
55 SKB u (0)	77.1	76.2	75.2
77.5 SKB u (+1)	77.8	76.9	75.9



0.125% DATEM



0.25% DATEM



0.375% DATEM

Figure 3.3 Liquid ferment baking test. Surface and contour plots for mixing time at different concentrations of DATEM.

3.3.1.1.4 Loaf volume.

For loaf volume, the reduced model included the main effects of DATEM, ascorbic acid and α-amylase, and the quadratic effects of ascorbic acid and α-amylase. Table 3.13 shows the predictions for loaf volume at different levels of the factors.

Loaf volumes are significantly increased at higher concentrations of DATEM. Increasing the concentration of DATEM from 0.125% to 0.375% results in an increase in loaf volume of approximately 30 ml for any given concentration of the other two improvers. Ascorbic acid has a small effect in loaf volume, but a maximum in the response is predicted at 121 ppm ascorbic acid. The effect of α-amylase was significant and a maximum in the response occurs at 68 SKB units.

The effects of the improvers are additive. A minimum of 893.2 cc is predicted to be at 0.125% DATEM, 50 ppm ascorbic acid, and 32.5 SKB u α-amylase, whereas a maximum of 977.8 cc is predicted to be at 0.375% DATEM, 121 ppm ascorbic acid and 68 SKB u α-amylase (Figure 3.4).

3.3.1.1.5 Break-and-shred.

For break-and-shred, the reduced model included the main effects of DATEM, ascorbic acid, and α-amylase, and the quadratic effect of ascorbic acid. Table 3.14 shows the predictions for break-and-shred at different levels of the factors.

Overall, the individual effects of the factors in break-and-shred scores were small. Break-and-shred scores increase at higher concentrations of DATEM and α-amylase. A maximum in the response is predicted at a concentration of ascorbic acid of 112 ppm. However, the additive effect of the improvers was significant. A minimum score of 4.2 is predicted to be 0.125% DATEM, 50 ppm ascorbic acid, and 32.5 SKB u α-amylase, whereas a maximum score of 6.8 is at 0.375% DATEM, 112 ppm ascorbic acid, and 77.5 SKB u α-amylase.

3.3.1.1.6 Uniformity

For uniformity score, a high value is desired. The reduced model included the main and quadratic effects of α-amylase. DATEM and ascorbic acid did not have a significant effect. The negative sign of the quadratic term of α-amylase predicts a maximum in the response, which occurs at 52 SKB u. The effect of α-amylase was small. Increasing the concentration of α-amylase from 32.5 to 52 SKB u results in an increase of 0.8 uniformity score units at any given concentration of the other two improvers.

Table 3.13 Liquid ferment baking test. Reduced model predictions for loaf volume (cc) at different concentrations of DATEM, ascorbic acid and α-amylase.

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
	0.125% DATEM (-1)		
32.5 SKB u (-1)	893.2	911.0	909.7
55 SKB u (0)	921.8	939.5	938.3
77.5 SKB u (+1)	925.0	942.8	941.5
	0.25% DATEM (0)		
32.5 SKB u (-1)	909.0	926.8	925.5
55 SKB u (0)	937.5	955.3	954.1
77.5 SKB u (+1)	940.8	958.6	957.3
	0.375% DATEM (+1)		
32.5 SKB u (-1)	924.8	942.5	941.3
55 SKB u (0)	953.3	971.1	969.8
77.5 SKB u (+1)	956.6	974.3	973.1

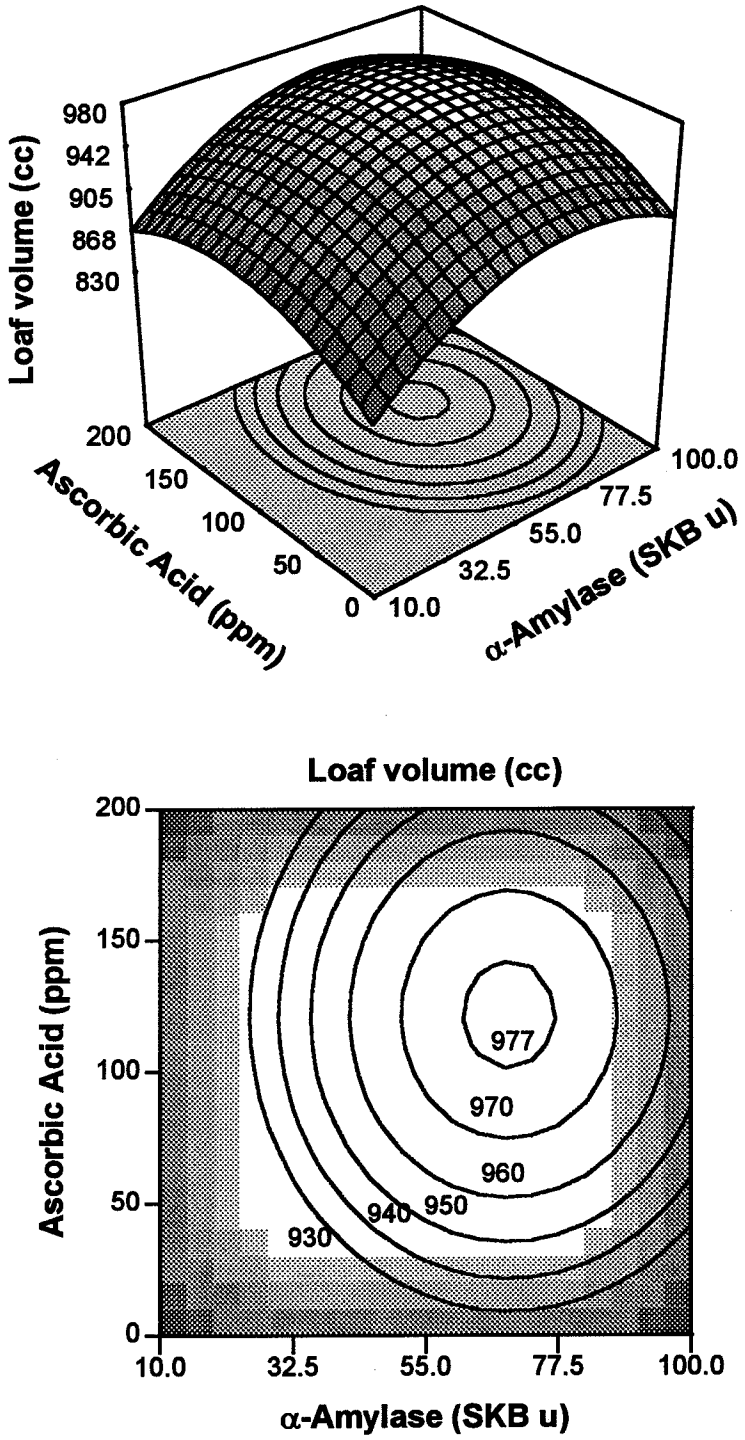


Figure 3.4 Liquid ferment baking test. Surface and contour plots for loaf volume at a fixed concentration of DATEM of 0.375%.

Table 3.14 Liquid ferment baking test. Reduced model predictions for break-and-shred at different levels of DATEM, ascorbic acid and α-amylase.[1]

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
		0.125% DATEM (-1)	
32.5 SKB u (-1)	4.2	4.8	4.6
55 SKB u (0)	4.7	5.3	5.1
77.5 SKB u (+1)	5.3	5.9	5.7
		0.25% DATEM (0)	
32.5 SKB u (-1)	4.6	5.2	5.0
55 SKB u (0)	5.2	5.8	5.6
77.5 SKB u (+1)	5.7	6.3	6.1
		0.375% DATEM (+1)	
32.5 SKB u (-1)	5.1	5.7	5.5
55 SKB u (0)	5.6	6.2	6.0
77.5 SKB u (+1)	6.2	6.8	6.6

[1] Bread and shred scores range from 0 to 10, where 0 = none and 10 = high (>2 ½").

3.3.1.1.7 Cell size.

For cell size, a high value is desired. The reduced model included the main and quadratic effects of α-amylase. DATEM and ascorbic acid did not have a significant effect. The negative sign of the quadratic term of α-amylase predicts a maximum in the response, which occurs at 57 SKB u α-amylase. The effect of α-amylase is small: increasing the concentration of α-amylase from 32.5 to 57 SKB u results in an increase of 1.3 cell-size score units at any given concentration of the other two improvers.

3.3.1.1.8 Crumb firmness.

For crumb firmness, the reduced model included the main and quadratic effects of DATEM. Ascorbic acid and α-amylase did not have a significant effect on the response. The negative sign of the quadratic term of DATEM predicts a maximum in the response, which occurs at 0.22% DATEM. The effect of DATEM on crumb firmness is small: Increasing the concentration of DATEM from 0.125 to 0.22% results in an increase of 0.04 N at any given concentration of the other two improvers.

3.3.1.1.9 Summary

DATEM has a beneficial effect on the quality characteristics of pan-bread baked using the liquid ferment baking test. This is reflected in the decrease in proof time, and increase in loaf height, loaf volume, and higher break-and-shred scores predicted. However, increasing levels of DATEM also result in increasing mixing times when ascorbic acid levels are set below 120 ppm. There results are summarized in Table 3.15.

The effect of ascorbic acid was only significant in mixing time, loaf volume and break-and-shred. The larger effects were seen in mixing times. Increasing levels of ascorbic acid result in decreased mixing times when the concentration of DATEM is set above 0.22%. Below this concentration of DATEM, the effect of ascorbic acid on mixing time was smaller.

Increasing the concentrations of α-amylase results in increases in loaf volume, uniformity and cell size scores, and a decrease in mixing time. Predicted maxima for the responses are at 68, 52, and 57 SKB u, respectively, and mixing time is at a minimum at 65 SKB u. The one detrimental effect is an increase in proof time.

Breads with low mixing time, high loaf volume and low crumb firmness can be achieved at levels of DATEM above 0.22%, ascorbic acid above 120 ppm, and α-amylase around 65 SKB u.

Table 3.15 Liquid ferment baking test. Summary of analysis of contour and surface plots.

Responses	p	R^2	Effect of A: DATEM %	Effect of B: Ascorbic acid (AA) ppm	Effect of C: α-amylase SKB u	Range of actual values Low	Range of actual values High
Mixing time (min)	0.0039	0.5921	↑ @ AA< 120 ppm AA ✓ ↓ @ AA> 120 ppm AA	↑ @ DATEM < 0.22% ↓ @ DATEM > 0.22% ✓	⌓ @ 65 SKB u	2.5	4.1
Proof time (min)	0.0153	0.3285	↓	ns	↑	73	82
Loaf height (cm)	0.0024	0.3481	↑	ns	ns	11.1	12.0
Loaf volume (cc)	<0.0001	0.8469	↑ ✓	⌒ @ 121 ppm AA	⌒ @ 68 SKB u ✓	880	985
Break-and-shred	0.0187	0.4476	↑	⌒ @ 112 ppm AA	↑	4	8
Uniformity	0.0042	0.4065	ns	ns	⌒ @ 52 SKB u ✓	2	8
Cell size	0.0058	0.3875	ns	ns	⌒ @ 57 SKB u	2	8
Firmness (N)	0.0433	0.2585	⌒ @ 0.22% DATEM	ns	ns	0.949	1.657

Key:
- ↑ Increase in response as the level of the factor is increased.
- ↓ Decrease in response as the level of the factor is increased.
- ⌒ Maximum in response.
- ⌓ Minimum in response.
- ns Effect of factor is not statistically significant.
- ✓ Significant effect of factor.

3.3.1.2 Canadian Short Process baking test.

Original data for the Canadian Short Process baking test is presented in Appendix V. Probability (p) and R^2 values for the full and reduced models are shown in Table 3.16. Responses whose reduced models had significant p-values (<0.1) were mixing time, loaf height, loaf volume, symmetry, break-and-shred, and firmness. Reduced models fitted for the Canadian Short Process baking test had relatively low R^2 values. The models fitted to mixing time and break-and-shred had the highest R^2 values, 0.4979 and 0.6723, respectively. Coefficients of the reduced models for each of these responses and their associated p-values are shown in Table 3.17.

Table 3.16 Canadian Short Process baking test. Full and reduced quadratic models.

Responses	Full model		Reduced model	
	p-value	R^2	p-value	R^2
Mixing Time (min.)	0.0603	0.5951	0.0154	0.4979
Proof time (min.)	0.6704	0.3070	-	-
Loaf Weight (g)	0.6528	0.3134	-	-
Loaf height (cm)	0.6849	0.3016	0.0209	0.2109
Loaf volume (cc)	0.5584	0.3472	0.0274	0.1943
Fineness	0.8693	0.2219	-	-
Elongation	0.9269	0.1858	-	-
Symmetry (E)	0.5432	0.3525	0.0623	0.1430
Bottom (E)	0.2830	0.4513	-	-
Break-and-shred (E)	0.0146	0.6816	0.0004	0.6723
Uniformity (I)	0.7807	0.2638	-	-
Cell Size (I)	0.7535	0.2750	-	-
Blister (I)	0.7543	0.2747	-	-
Firmness (N)	0.2962	0.4456	0.0296	0.1897

[1] A "-" symbol represents an empty model after stepwise regression.

Table 3.17 Canadian Short Process. Reduced model coefficients in terms of coded factors.

For stepwise regression $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Mixing time		Loaf height		Loaf volume		Symmetry		Break-and-shred		Firmness	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	6.7		11.6		924.2		8.8		7.6		1.2	
b_1: % DATEM [A]	0.0005	0.9500	0.14	0.0209	14.70	0.0274			0.59	0.0152	-0.06	0.0296
b_2: Ascorbic acid [B]	-0.01	0.8902							0.08	0.7161		
b_3: α-amylase [C]	-0.22	0.0139					-0.68	0.0623	0.59	0.0152		
Quadratic terms												
b_4: A^2	-0.19	0.0261										
b_5: B^2	-0.23	0.0096							-0.78	0.0014		
b_6: C^2												
Interaction terms												
b_7: AB												
b_8: AC									-0.88	0.0038		
b_9: BC												
p-value	0.0154		0.0209		0.0274		0.0623		0.0004		0.0296	
R^2	0.4979		0.2109		0.1943		0.1430		0.6723		0.1897	

3.3.1.2.1 Mixing time.

For mixing time, the reduced model included the main effects of DATEM, ascorbic acid, and α-amylase, and the quadratic terms of DATEM and ascorbic acid. Table 3.18 shows the predictions for mixing time at different levels of the factors.

Mixing times decrease significantly at higher concentrations of α-amylase. The individual effects of DATEM and ascorbic acid are small, but a maximum in the response is predicted at 0.25% and 98 ppm, respectively. The additive effect of the improvers was significant: a minimum of 6.1 min is predicted to be at high levels of α-amylase and at any combination of the extreme levels of the other two improvers; on the other hand, a maximum of 7.0 min is to be at 0.25% DATEM, 98 ppm ascorbic acid, and 32.5 SKB u α-amylase.

3.3.1.2.2 Loaf height.

For loaf height, the reduced model included only the main effect of DATEM. Ascorbic acid and α-amylase did not have a significant effect. The effect of DATEM was small: an increase in the concentration of DATEM from 0.125% to 0.375 results in an increase of 0.3 cm in loaf height at any given concentration of the other two improvers.

Table 3.18 Canadian Short Process baking test. Reduced model predictions for mixing time (min) at different levels of DATEM, ascorbic acid and α-amylase.

	Ascorbic acid		
α-Amylase	***50 ppm (-1)***	***100 ppm (0)***	***150 ppm (+1)***
	0.125% DATEM (-1)		
32.5 SKB u (-1)	6.6	6.8	6.5
55 SKB u (0)	6.4	6.6	6.3
77.5 SKB u (+1)	6.1	6.3	6.1
	0.25% DATEM (0)		
32.5 SKB u (-1)	6.8	7.0	6.7
55 SKB u (0)	6.5	6.8	6.5
77.5 SKB u (+1)	6.3	6.5	6.3
	0.375% DATEM (+1)		
32.5 SKB u (-1)	6.6	6.8	6.5
55 SKB u (0)	6.3	6.6	6.3
77.5 SKB u (+1)	6.1	6.3	6.1

3.3.1.2.3 Loaf volume.

For loaf volume, the reduced model included only the main effect of DATEM. Ascorbic acid and α-amylase did not have a significant effect. Loaf volumes are increased at higher concentrations of DATEM: an increase in the concentration of DATEM from 0.125% to 0.375% results in an increase in loaf volume from 909.5 to 938.9 cc.

3.3.1.2.4 Symmetry.

For symmetry, a high score is desired. The reduced model included only the main effect of α-amylase. DATEM and ascorbic acid did not have a significant effect. Symmetry scores decrease at higher concentrations of α-amylase: an increase in the concentration of α-amylase from 32.5 to 77.5 SKB u results in a decrease in symmetry score from 9.4 to 8.0.

3.3.1.2.5 Break-and-shred.

For break-and-shred, the reduced model included the main effects of DATEM, ascorbic acid, and α-amylase, the quadratic effect of ascorbic acid, and the interaction DATEM x α-amylase. Table 3.19 shows the predictions for break-and-shred scores at different levels of the factors.

Break-and-shred scores significantly increase at higher concentrations of DATEM when the levels of α-amylase are below 70 SKB u, or at higher concentrations of α-amylase when the levels of DATEM are below 0.33%. The negative sign of the quadratic term of ascorbic acid predicts a maximum in the response, which occurs at 102 ppm.

A minimum of 4.7 in break-and-shred score is predicted to be at 0.125% DATEM, 50 ppm ascorbic acid, and 32.5 SKB u α-amylase, whereas a maximum of 8.5 is at 0.375% DATEM, 102 ppm ascorbic acid, and 32.5 SKB u α-amylase.

Table 3.19 Canadian Short Process baking test. Reduced model predictions for break-and-shred at different levels of DATEM, ascorbic acid and α-amylase.[1]

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
		0.125% DATEM (-1)	
32.5 SKB u (-1)	4.7	5.6	4.9
55 SKB u (0)	6.2	7.1	6.4
77.5 SKB u (+1)	7.7	8.5	7.8
		0.25% DATEM (0)	
32.5 SKB u (-1)	6.2	7.1	6.4
55 SKB u (0)	6.8	7.6	6.9
77.5 SKB u (+1)	7.4	8.2	7.5
		0.375% DATEM (+1)	
32.5 SKB u (-1)	7.7	8.5	7.8
55 SKB u (0)	7.4	8.2	7.5
77.5 SKB u (+1)	7.1	7.9	7.2

[1] Bread and shred scores range from 0 to 10, where 0 = none and 10 = high (>2 ½").

3.3.1.2.6 Crumb firmness.

For crumb firmness, the reduced model included only the main effect of DATEM. Ascorbic acid and α-amylase did not have a significant effect. Crumb firmness decreases at higher concentrations of DATEM: an increase in the concentration of DATEM from 0.125% to 0.375 results in a decrease in crumb firmness from 1.31 N to 1.19 N.

3.3.1.2.7 Summary.

Models for mixing time and break-and-shred included factors that were considered significant statistically. The model fitted to loaf volume had also significant terms, although it had a lower R^2 value. For the rest of the responses, the fitted models had lower R^2 values. These result are summarized in Table 3.20.

DATEM has positive effects on the quality characteristics of pan bread when made using the Canadian Short Process baking test. Loaf height, loaf volume, and break-and-shred score (the latter when fixing the concentration of α-amylase below 70 SKB u) are increased and crumb firmness is decreased. Maximum for mixing time is predicted at around 25% DATEM.

Ascorbic acid had small effects when using the Canadian Short Process. Maximum on mixing time and break-and-shred score were predicted at 98 and 102 ppm ascorbic acid respectively.

Addition of α-amylase results in decreased in mixing times and increased break-and-shred scores (the latter when fixing DATEM at levels below 0.33%). Higher α-amylase results in small decreases in symmetry scores.

According to the above, it seems that to achieve low mixing time, high loaf volume, and high break-and-shred the levels of DATEM can be set below 0.33%, and α-amylase at around 70 SKB u.

Table 3.20 Canadian Short Process. Summary of analysis of contour and surface plots.

Responses	p	R^2	Effect of A: DATEM %	Effect of B: Ascorbic acid (AA) ppm	Effect of C: α-amylase SKB u	Range of actual values Low	Range of actual values High
Mixing time (min)	0.0154	0.4979	◠ @ 0.25% DATEM	◠ @ 98 ppm AA	↓ ✓	5.8	8.0
Loaf height (cm)	0.0209	0.2109	↑	ns	ns	11.2	12.3
Loaf volume (cc)	0.0274	0.1943	↑ ✓	ns	ns	870	985
Symmetry	0.0623	0.1430	ns	ns	↓ ✓	4	10
Break-and-shred	0.0004	0.6723	↑ 10-70 SKB u amylase ✓ ↓ 70-100 SKB u amylase	◠ @ 102 ppm AA ✓	↑ 0-0.33% DATEM ✓ ↓ 0.33-0.5% DATEM	4	8
Firmness (N)	0.0296	0.1897	↓ ✓	ns	ns	0.954	1.604

Key:
- ↑ Increase in response as the level of the factor is increased.
- ↓ Decrease in response as the level of the factor is increased.
- ◠ Maximum in response.
- ◡ Minimum in response.
- ns Effect of factor is not statistically significant.
- ✓ Significant effect of factor.

3.3.1.3 Comparison between the liquid ferment and the Canadian Short Process baking tests.

Center points of the two baking tests were compared by a t-test to detect significant differences using an α= 0.1. Means, standard deviations and coefficients of variation for the center point data are shown in Table 3.21. Baking tests differed in mixing time, proof time, and break-and-shred. Mixing time values were statistically lower for the liquid ferment baking test than for the Canadian Short Process (p= 0.0001). This was expected, since in this measurement the first 2-minute mixing for the liquid ferment was not taken into consideration, and by the second and final mixing the dough had already undergone some physical stress. Proof time values were also significantly lower for the liquid ferment than for the Canadian Short Process (p= 0.0322). Finally, break-and-shred scores for the liquid ferment baking test were lower than those for the Canadian Short Process (p= 0.0917). However, the variability within this response when using the liquid ferment was very high (CV= 27.2%). For the rest of the responses, differences between the two baking tests values were not statistically significant.

Models fitted when using the liquid ferment baking test had R^2 values higher than those for the Canadian Short Process, meaning that the models were better in explaining the data. However, R^2 values remained relatively low, except for mixing time and loaf volume in the liquid ferment baking test and break-and-shred in the Canadian Short Process.

Table 3.21 Analysis of center points for the liquid ferment and Canadian Short Process baking tests.

		Liquid ferment			*Canadian Short Process*		
Response	***Units***	***Mean***	***s***	***CV (%)***	***Mean***	***s***	***CV (%)***
Mixing time	min	3.2	0.4	12.9	6.9	0.7	9.6
Proof time	min	76.2	1.7	2.2	95.2	8.4	8.8
Loaf weight	g	121.4	1.1	0.9	122.9	0.6	0.5
Loaf height	cm	11.5	0.3	2.6	11.5	0.3	3.0
Loaf volume	cc	952.5	27.2	2.9	960.0	21.2	2.2
Fineness		775.9	30.0	3.9	891.0	60.9	6.8
Elongation		1.32	0.03	1.9	1.43	0.11	7.4
Symmetry		10.0	0.0	0.0	9.0	1.1	12.8
Bottom		9.0	1.1	12.8	9.5	1.0	10.5
Break-and-shred		6.0	1.6	27.2	8.0	0.0	0.0
Uniformity		6.5	1.0	15.4	8.0	0.0	0.0
Cell size		7.0	1.1	16.5	7.0	1.1	16.5
Blister		8.2	2.4	28.6	7.5	2.9	38.5
Firmness	N	1.23	0.11	8.5	1.14	0.14	11.9

It was also observed that when baking with the liquid ferment baking test, doughs after the second mixing were sometimes very difficult to handle due to stickiness and tended to tear when passing through the sheeter.

3.3.2 Graphical optimization.

Mathematical models have been obtained to analyze the effects of improver formulations containing DATEM, ascorbic acid, and α-amylase on the quality of pan-bread. The effects of these improvers in each response have been evaluated, and the two baking tests have been compared for their sensitivity to the effects of these improvers. The following step was to identify optimum combinations of the factors that predict the production of a high-quality loaf of bread, taking into consideration all responses at once. Through the analysis of the models, it was observed that the effect of an improver was beneficial for some responses, but at the same time it was detrimental for others. It is clear that a certain compromise must be achieved between the different effects of the factors on the responses that will allow to obtain appropriate results. Graphical optimization, through the use of overlay plots, was used to identify optimized combinations of the factors, following a set of criteria of acceptability for the responses. Contours representing the minimum and maximum according to the criteria were identified. The contours for each of the responses were superimposed over each other, and the regions in which the acceptable values of the responses overlapped were identified. To simplify the analysis, the overlay plots were produced at the five levels of DATEM used.

3.3.2.1 Liquid ferment baking test.

The criteria for graphical optimization are shown in Table 3.22. Range of values in these criteria were selected based on the range of response values obtained during the experiment, and in order to obtain optimized combinations of the factors that were within the experimental space set by the levels of the factors in the experimental design. Overlay plots were produced at the five different levels of DATEM used in the experimental design (Figure 3.5).

At 0% DATEM, no zone of optimized combinations could be detected that satisfied the criteria set. A closer analysis of the overlay plot revealed that the criteria were met for mixing time, loaf volume, and crumb firmness, but the predicted break-and-shred scores are below the minimum for acceptability (6.0). The lower boundary for break-and-shred was reduced to 4. This time an optimized zone was detected, and appropriate results can be obtained at adequate combinations of ascorbic acid and α-amylase between 68 and 200 ppm and 35 and 100 SKB u, respectively. This means that a high quality loaf of bread can be obtained at 0% DATEM but with a predicted low break-and-shred score (~4.0). At higher concentrations of DATEM, criteria for responses are met, and ranges of optimized levels of ascorbic acid and α-amylase are presented in Table 3.23.

At increasing concentrations of DATEM in the formulation, the zone of optimized combinations of ascorbic acid and α-amylase increases. The range of concentrations of ascorbic acid that can be used increases and becomes less

critical, whereas optimized concentrations of α-amylase are located at lower concentrations.

Loaf volume contours were superimposed to the overlay plots at corresponding concentrations of DATEM, in order to identify conditions at which loaf volume can be maximized within these areas of optimized combinations (Figure 3.6). These plots were produced at 0.125, 0.25, and 0.375% DATEM, considering that the models are more accurate at these points since they are part of the factorial fraction of the experimental design.

At 0.125% DATEM, loaf volumes within the area of optimized combinations range between 920 and 940 cc. Loaf volume can be maximized at a level of ascorbic acid of 121 ppm and at reduced levels of α-amylase within the range 80-100 SKB u. At 0.25% DATEM, predicted loaf volumes increase as a result of the effect of increasing the concentration of DATEM. Predicted loaf volumes range between 920 and 960 cc and they can be maximized at a level of ascorbic acid of 121 ppm and reduced levels of α-amylase within the range 62-100, attaining a maximum at 68 SKB u. At 0.375% DATEM, further increase in predicted loaf volumes is observed. Predicted loaf volumes range between 930 and 970 cc and they can be maximized at 121 ppm ascorbic acid and 68 SKB u α-amylase.

Table 3.22 Liquid ferment baking test. Criteria for graphical optimization.

Response	*Goal*	*Constraints*
Mixing time	In range	2.5 to 4.1 min
Loaf volume	In range	900 to 1000 cc
Break-and-shred	In range	6 to 10
Firmness	In range	0.76 to 1.40 N

Table 3.23 Liquid ferment baking test. Graphical optimization. Zones of optimized combinations of ascorbic acid and α-amylase at different concentrations of DATEM.

DATEM (%)	*Ascorbic acid (ppm)*	*α-amylase (SKB u)*	*Achievement of criteria*
0.000	68-200	35-100	Break-and-shred = 4
0.125	60-162	80-100	✓
0.250	38-185	62-100	✓
0.375	22-200	44-100	✓
0.500	31-200	26-100	✓

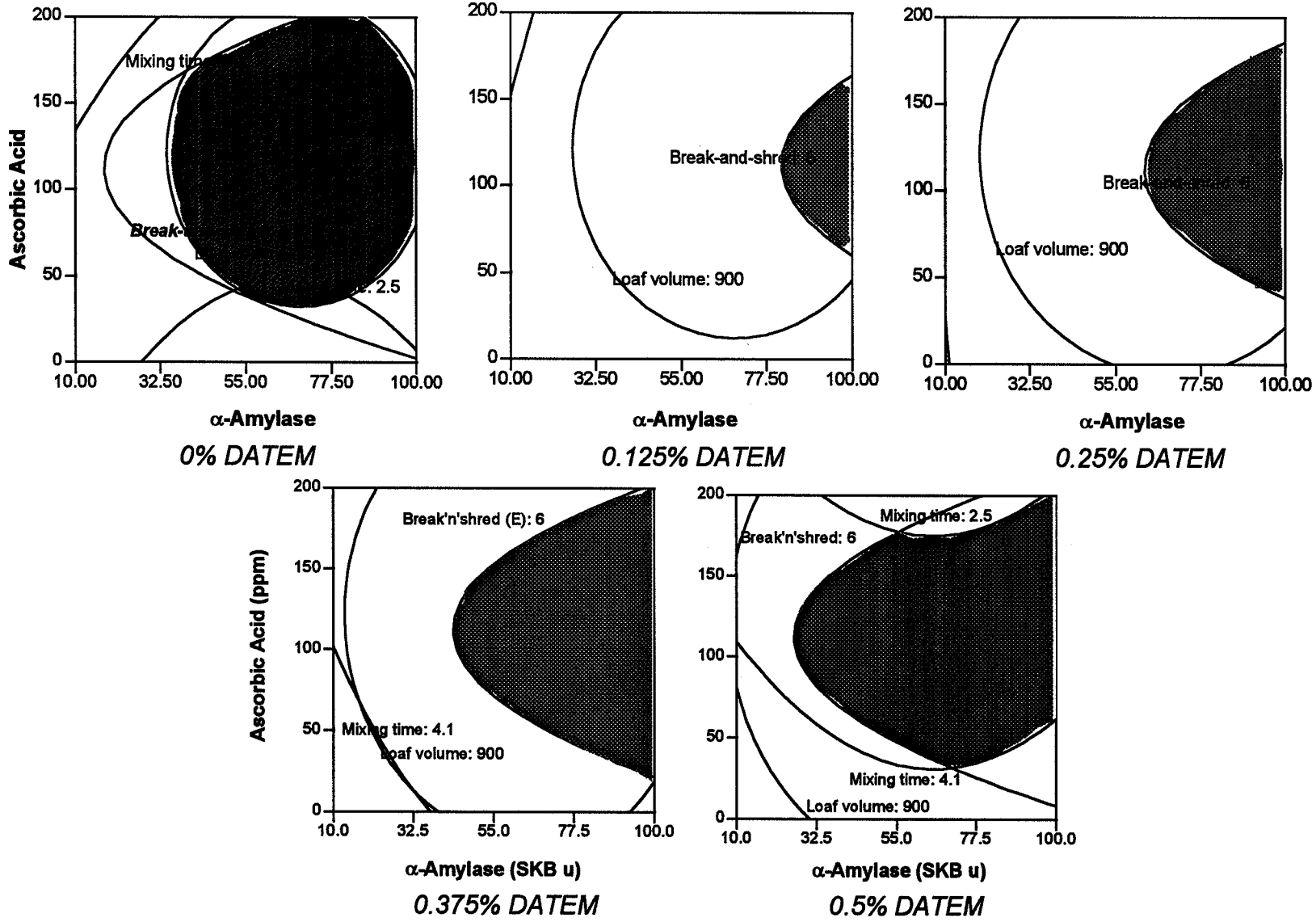
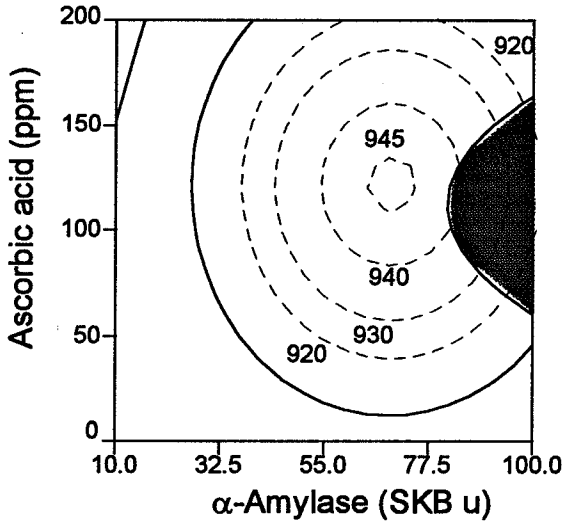
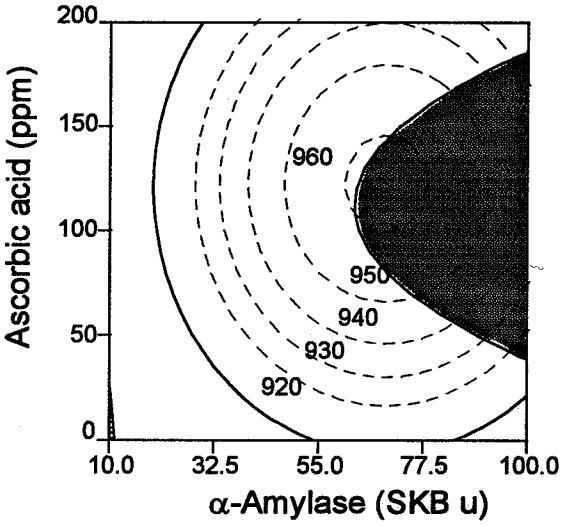


Figure 3.5 Liquid ferment baking test. Overlay plots at different concentrations of DATEM. A striped pattern at 0% DATEM indicates that predicted values for break-and-shred were below the minimum of 6 for acceptability. A dark shading indicates complete fulfillment of criteria for acceptability.

0.125% DATEM



0.25% DATEM



0.375% DATEM

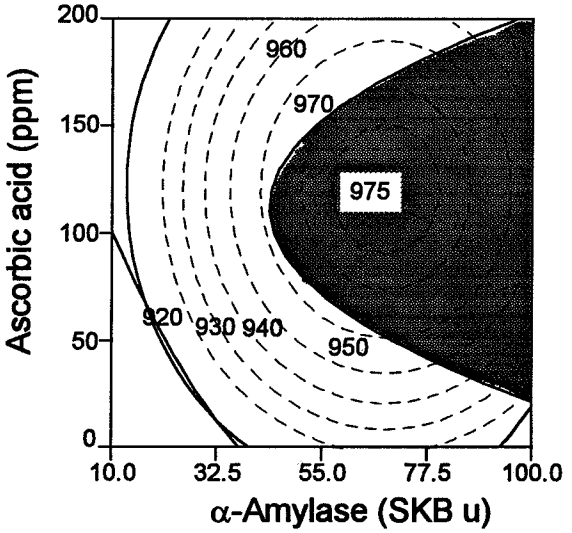


Figure 3.6 Liquid ferment baking test. Superimposed plots of zones of optimized combinations of ascorbic acid and α-amylase and loaf volume contours at different concentrations of DATEM.

3.3.2.2 Canadian Short Process.

The criteria for graphical optimization are shown in Table 3.24. The rationale for the selection of the range of values is the same as described before for the liquid ferment baking test. Criteria are the same as in the liquid ferment, except for mixing times, which were higher in the Canadian Short Process. Overlay plots were produced at the five different levels of DATEM used in the experimental design (Table 3.7).

At 0% DATEM, no zone of optimized combinations could be detected that satisfied the criteria set. A closer look at the overlay plot at this concentration of DATEM revealed that criteria were met for all the responses except for loaf volume, which was predicted to be around 890 cc, slightly below the minimum for acceptability (900 cc). Lowering the boundary further to 850 cc, a zone of optimized combinations was detected at middle levels of both improvers, between 56 and 143 ppm ascorbic acid and between 50 and 73 SKB u α-amylase. For the other four levels of DATEM, the criteria were met at adequate combinations of ascorbic acid and α-amylase as indicated in Table 3.25.

The identification of optimized levels of ascorbic acid is not critical in the Canadian Short Process to identify optimized levels of the improvers. The range of optimized levels of ascorbic acid cover almost the entire range of ascorbic acid concentrations used in this experiment at any given concentration of DATEM, except at 0% DATEM. This is also applicable to α-amylase at 0.25 and 0.375% DATEM. At low levels of DATEM, α-amylase requirements are above 38

SKB u, and at 0.5% DATEM these are located at levels below 72 SKB u. The large areas of optimized combinations of ascorbic acid and α-amylase at 0.25 and 0.375% DATEM may be an indication that the Canadian Short Process baking test is less sensitive to the effects of the improvers than the liquid ferment baking test at similar concentrations of DATEM in the formulation.

Superimposed plots of overlays and loaf volume contours could not be produced since loaf volume was only affected by the concentration of DATEM. Predicted loaf volumes increase at increasing concentrations of DATEM and the average loaf volumes at different concentrations of DATEM are: 909 cc at 0.125% DATEM, 924 cc at 0.25% DATEM, and 939 cc at 0.375% DATEM.

Table 3.24 Canadian Short Process. Criteria for graphical optimization.

Response	*Goal*	*Constraints*
Mixing time	In range	5.8 to 8.0 min
Loaf volume	In range	900 to 1000 cc
Break-and-shred	In range	6 to 10
Firmness	In range	0.76 to 1.40 N

Table 3.25 Canadian Short Process. Graphical optimization. Zones of optimized combinations of ascorbic acid and α-amylase at different concentrations of DATEM.

DATEM (%)	*Ascorbic acid (ppm)*	*α-amylase (SKB u)*	*Achievement of criteria*
0.000	56-143	50-73	Loaf volume = 890 cc
0.125	19-179	39-100	✓
0.250	15-188	10-100	✓
0.375	9-195	10-100	✓
0.500	18-179	10-72	✓

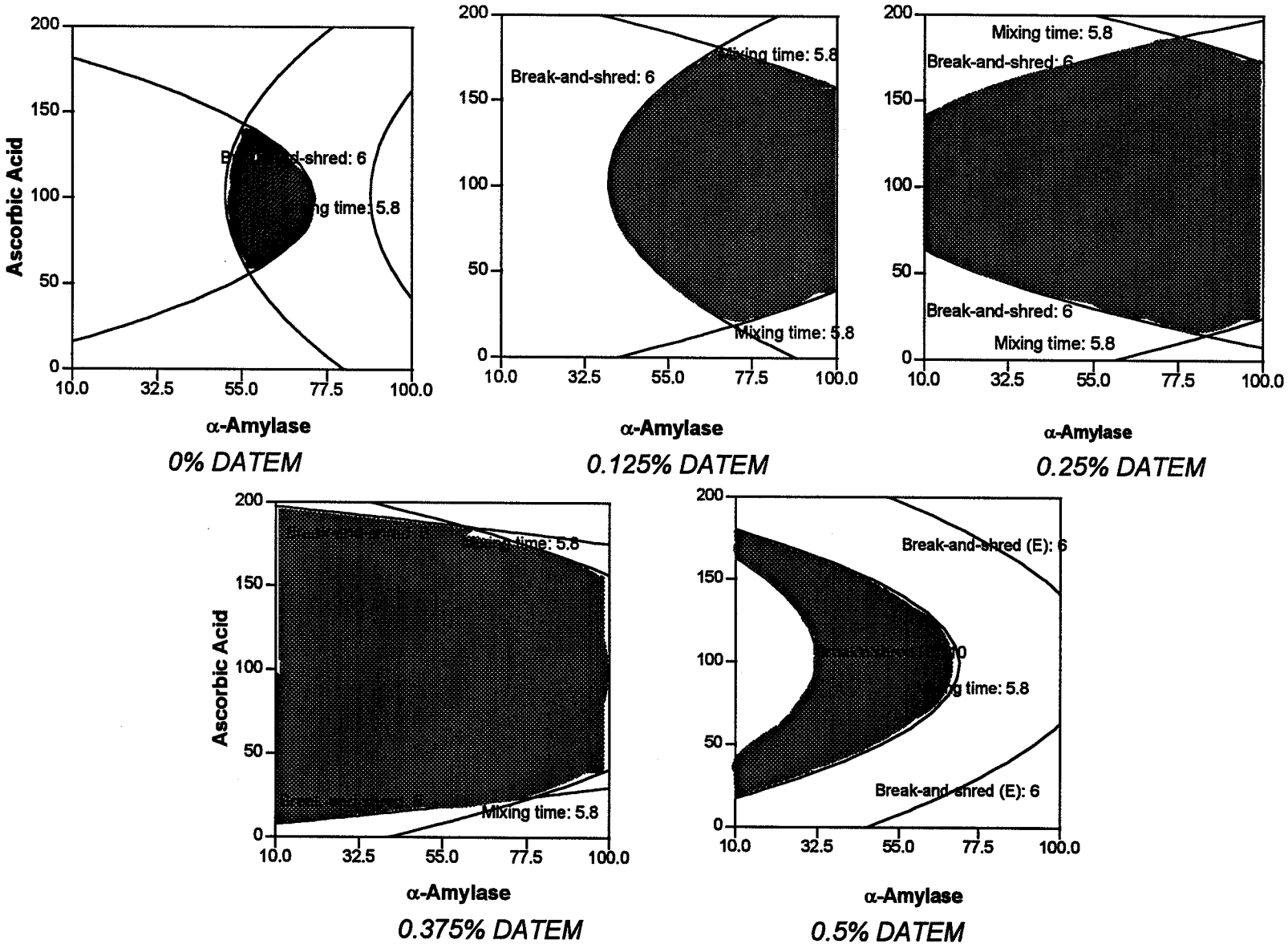


Figure 3.7 Canadian Short Process. Overlay plots at different concentrations of DATEM. A striped pattern at 0% DATEM indicates that predicted values for break-and-shred were below the minimum of 6 for acceptability. A dark shading indicates complete fulfillment of criteria for acceptability.

3.4 Discussion.

During the optimization experiment it became clear that the understanding the effects of improvers on the quality of pan-bread was not a simple process. As with many foods, there are complex interactions taking place in the dough and bread that cannot be easily explained. The statistical design used made it possible to obtain mathematical models for the analysis of not only the many effects of the improvers *per se*, but also of the interactions between them. However, these mathematical models are only approximations. They are subject to variability and only applicable within the boundaries of the experimental space set by the levels of the factors in this study.

Differences between the liquid ferment baking test (LF) and the Canadian Short Process (CSP) are mainly in the mixing and fermentation phases: the former requires separate dough mixing steps and a long fermentation, while the latter requires only one mixing step and lacks a fermentation step. These variations in the processes may account for the differences in improver requirements observed for the two tests. Baker *et al* (1988) mentioned that no-time doughs (such as those obtained in the CSP) require increased yeast level, dough temperature, and dough water absorption, as well as the addition of yeast nutrients and oxidants. However, the two baking methods were carried out using constant yeast levels, dough temperatures, and dough water absorptions to limit the analysis to the effects of the improvers. Further studies could be done to evaluate the effect of these other factors on pan-bread made with these two

baking tests.

Problems of stickiness were observed in the dough after second mixing in the LF, which was not observed in the CSP. In the LF, improvers were added in the dough stage, thus this difference in the rheological properties of the dough are attributable to reactions occurring in the long fermentation step. Yeast activity can have a very important influence on the rheological properties of dough. Yeast activity during fermentation results in lowering of the pH, changes in the interfacial tension of dough phases due to alcohol formation, physical working of the dough caused by expansion of generated CO_2, and the weakening effects of reducing reactions catalyzed by yeast enzymes (Pyler, 1973).

Stickiness may also be due to excessive endogenous amylase activity. It has been reported that in long fermentation systems, stickiness occurs as a result of release of water during the breakdown of starch (Cauvain and Chamberlain, 1988). The starch fraction in the flour used could have been overdamaged, and so more prone to the action of amylases. This softening effect may be compensated for by a reduction of water in the recipe, but this results in loss of bread yield (Sapru, 1998, personal communication). Furthermore, through mixograph studies on liquid ferments, Kulp (1986) reported that flour in liquid ferments lost some of its water-absorption capacity and produced softer doughs, and that these effects increased with the level of flour in the ferments.

Shorter mixing times were obtained in the LF than in the CSP. In the LF, the dough has experienced physical working through the short first mixing of ferment ingredients and the expansion of CO_2 during the fermentation. Another factor could be the lowering of pH due to yeast activity. Hoseney and Brown (1983) reported on the effect of pH on the mixing properties of dough. They concluded that at low pH (pH = 4.24), dough proteins have more positive charges that tend to repel each other and so diminish protein interactions, making the dough less stable. This positive charge could also cause the protein to hydrate faster and require shorter mixing time.

Water levels when using the LF need to be adjusted. The determination of the appropriate amount of water added to the dough is essential, since it has a strong influence on the rheological properties. Insufficient water results in a poorly hydrated dough and the elastic nature of the dough does not fully develop. On the other hand, an excessive level of water in dough results in an increased viscous component of the dough, decreased resistance to extension, increased extensibility, and the development of a sticky dough (Spies, 1990).

The dough strengthening effect of DATEM and ascorbic acid was evident in the mixing times in both baking tests. In the liquid ferment, a significant interaction was observed between the two improvers, and longer mixing times are predicted at higher levels of both improvers. However, mixing times decrease above certain concentrations of both improvers. It has been reported that binding of an anionic surfactant to a protein can lead to an increased negative charge of the protein fraction. These charges may alter protein stability

by increasing electrostatic repulsion and this may provoke unfolding of the protein (Blomberg, 1992). This could explain shorter mixing times at high concentrations of DATEM. The strengthening action of ascorbic acid could compensate for this effect at low levels of DATEM, but this effect may be overcome at high concentrations of the surfactant.

Levels of DATEM and α-amylase up to 0.33% and 70 SKB u, respectively, increase the break-and-shred scores of loaves made by the Canadian Short Process. However, due to the significant interaction identified between the two improvers, break-and-shred scores begin to diminish at higher levels. Break-and-shred is intimately related to the degree of oven spring occurring in the dough during baking (D'Appolonia, 1996). Protein stability in dough may be reduced as a result of high concentrations of DATEM as mentioned above. Furthermore, an excess of α-amylase can result in a dough that is too soft (Cauvain and Chamberlain, 1988). In conjunction, these effects may adversely affect the oven spring properties of the dough and hence result in lower break-and-shred scores.

The effect of ascorbic acid was more significant in the liquid ferment baking test than in the Canadian Short Process, as evidenced by fewer significant terms fitted to the responses when using the Canadian Short Process. It seems that the dough strengthening effect of DATEM was sufficient for this baking test. The oxidative requirements of the Canadian Short Process, a baking test without bulk fermentation, has been reported to be higher than conventional baking procedures where a long fermentation is required (Yamada and Preston

1992, Preston and Dexter 1994). Our results do not support these observations.

The anti-firming effect of amylase reported extensively in the literature was not observed. This may due to the fact that measurements on crumb firmness were only done one day after baking. The anti-firming effect of the enzyme could be more evident at longer periods of time after baking. A longer study could be carried out in order to study these effects.

In the Canadian Short Process, the effect of the improvers were less evident, as compared to the liquid ferment baking test. Large areas of optimized combinations of ascorbic acid and α-amylase, at any given concentration of DATEM, indicated that the Canadian Short Process may be less sensitive to the effect of the improvers. Changes occurring in the long fermentation step in the liquid ferment may influence the properties of dough in such a way that small variations in the levels of the improvers result in significant changes in the quality characteristics of bread made with this method.

3.5 Conclusions.

The liquid ferment baking test and the Canadian Short Process differed in their requirements for improvers. Requirements for dough strengtheners (both DATEM and ascorbic acid) were more critical for the liquid ferment than for the Canadian Short Process. When using the Canadian Short Process, ascorbic acid showed fewer significant effects and α-amylase requirements were less evident. In the liquid ferment baking test, α-amylase levels depended on the concentration of DATEM.

Using the liquid ferment baking test, high-quality loaves of bread can be produced at any given concentration of DATEM. With low DATEM, the requirements for α-amylase are high (above 80 SKB u), while at higher levels of DATEM, α-amylase requirements are as low as 40 SKB u. Loaf volumes are increased at increasing concentrations of DATEM and are maximized at 0.375% DATEM, 121 ppm ascorbic acid, and 68 SKB u α-amylase.

In the Canadian Short Process, the effects of the improvers were less apparent, as evidenced by the wide range of concentrations of ascorbic acid and α-amylase that could be used at any given concentration of DATEM. Areas of optimized combinations of ascorbic acid and α-amylase were very large and the concentrations less critical than in the liquid ferment baking test.

Doughs were less elastic and more extensible in the liquid ferment than in the Canadian Short Process, which was reflected in excessive dough stickiness after mixing. This problem is related to several factors, one of them being a loss

of water absorption capacity of the flour during the fermentation. An experiment should be carried out to identify the appropriate water level added to the dough in the liquid ferment method.

The liquid ferment baking test was more sensitive than the Canadian Short Process to the effects of the improvers used in this study. The liquid ferment method developed in the laboratory simulates a type of baking process used in an industrial bakery in Mexico. It proved to be an adequate model to study the effects of bromate-free improvers at concentrations normally used in Mexico. Blending of flours of different gluten strength is a widespread practice in Mexico. Further studies may contemplate the introduction of a variable related to the blend of hard bread wheat flours and weaker wheat flours. The use of the liquid ferment baking test developed in this study seems appropriate to identify optimized combinations of improvers that enhance the quality characteristics of white pan bread.

4. OPTIMIZATION EXPERIMENT #2: OPTIMIZATION OF IMPROVER SYSTEMS IN PAN-BREAD FORMULATIONS USING BLENDS OF HARD RED SPRING AND PRAIRIE SPRING WHEAT FLOURS.

Based on the previous optimization experiment, the liquid ferment was selected as the appropriate baking test to study the effect of bromate-free improver formulations on the quality of pan-bread, made with blends of CWRS and CWES with lower-protein flours. The advantage of the liquid ferment baking test when compared to the Canadian Short Process is the higher response of the former test to the effects of the improvers. Blending of strong imported wheats with weaker local varieties is a widespread practice in Mexican mills and commercial bakeries for the production of high-quality bread flours. The purpose of the study was to investigate the effects of improvers and of wheat blends on bread quality.

In this experiment, CWRS and CWES bread flours were blended at different ratios with CPS white and CPS red flours. Pup loaves of 100-g of flour were baked with the liquid ferment baking test using an adjusted water absorption. Mixing time, proof time, loaf volume, and break-and-shred were measured. A panning score was introduced in the evaluation to assess the handling properties of the dough after mixing. Response Surface Methodology was used to identify optimised flour blends and ascorbic acid and enzyme combinations that produce high quality pan-bread.

4.1 Objectives

1. To determine the effect of Hard Red Spring, CWRS and CWES, wheat flours in blends with Prairie Spring white and red flours, on the quality of white pan-bread made using the liquid ferment method.
2. To determine the effect of the improvers ascorbic acid and α-amylase in the quality of white pan-bread made from blends of Hard Red Spring and Prairie Spring wheat flours.
3. To identify optimised levels of ascorbic acid, α-amylase and blends of flours by Response Surface Methodology techniques to enhance quality characteristics of pan-bread.

4.2 Materials and methods.

4.2.1 Flours.

Canada Western Red Spring (CWRS), Canada Western Extra Strong Red Spring (CWES), and Canada Prairie Spring (CPS) white and red flours were obtained from the Canadian Grain Commission (CGC), Winnipeg. These flours were milled from composite wheat samples from the 1996 crop year, and were representative of cargo shipments. Canada Prairie Spring (CPS) flours were used to simulate the characteristics of a Mexican bread wheat flour in terms of strength and farinograph properties. It was not possible to import Mexican bread wheat flours into Canada at the time of the experiment because

of Canadian government restrictions, imposed as a result of karnal bunt problems. A Mexican bread wheat, appropriate for a commercial baking procedure, has a protein content between 10 and 12%, with a mixing development time in the mixograph of 2.0-2.5 min (Peña 1997, personal communication).

Flours were milled to produce straight-grade flour. The flours were allowed to mature naturally at room temperature in bulk containers for a period of 6 weeks. Flours were then weighed into 200-g portions, packaged in sealed heavy plastic bags, and stored at 5°C. Flours were withdrawn from the refrigerator 24 hours before they were needed for baking.

Approved methods (AACC 1993) were used to determine moisture (method 44-15A), protein (N x 5.7) (method 46-13, and as modified by Williams (1993)), ash (method 08-01), and farinograph parameters (method 54-21).

Ingredients and additives were the same as in Optimization Experiment #1, except for L-ascorbic acid, which was a donation from ADM Arkady (Olathe, KS). The manufacturer's specifications indicated that by adding 1 tablet to 100 pounds the concentration of ascorbic acid would be 120 ppm. Ascorbic acid content of the tablets was reported to be 77.86% w/w. For a 1% w/v solution, 1.2844 g of a ground tablet was dissolved in 100 ml of distilled water.

The Mexican bread wheat flour from Optimization Experiment #1 was used to bake controls each baking day. Moisture content and farinograph water absorption were as reported previously.

4.2.2 Breadmaking procedure.

The liquid ferment baking test developed in Optimization Experiment #1 was used to produce 100-g pup loaves. The formulation and flow diagram were as described in the Optimization Experiment #1, except for the following modifications:

- Fermentation time was increased from 2 to 3 hours in order to accommodate adequate number of runs per baking day.
- In the previous experiment, the use of FAB resulted in doughs that were very sticky and sometimes unmanageable during panning. An absorption of FAB-3% was selected after carrying out an experiment varying the amount of water added to dough in the four blend systems to be studied in this experiment (CWRS/CPS white, CWRS/CPS red, CWES/CPS white, and CWES/CPS red) (Appendix VI).
- Potassium sorbate (ADM Arkady, Olathe, KS) was used as an antimycotic agent at a concentration of 0.075%. Manufacturer's recommended dosage is 0.025-0.125%.
- DATEM was fixed at a concentration of 0.375%, based on the results of the previous experiment.

Water absorptions for all blends were determined by means of a farinograph. Moisture of the different blends was calculated from the moisture of the original flours.

4.2.3 Evaluation.

Some modifications on the responses selected for evaluation were made with respect to the Optimization Experiment #1. Mixing time (second mixing in the liquid ferment baking test) and proof time were recorded. A panning score was introduced to have a measurement of the handling properties of dough when it was placed in the metal pan before proofing. A 10-point scale for panning score was attached to the bread evaluation score card shown in Appendix III, and included the following categories: satisfactory (10 points), slightly bucky or slack (8), bucky or slack (6), very bucky or very slack (4), and unmanageable (0). Loaf volume was determined by a rapeseed displacement voltmeter (National Manufacturing Co., Lincoln, NB). Break-and-shred was assessed by means of an individual 10-point scale using the criteria described in Appendix III. Crumb structure was analyzed using the CrumbScan® software (American Institute of Baking, KS) on a 2.5-cm thick slice from the centre of the loaf. A fineness score was obtained, which is a measure of crumb cell uniformity. The score increases as crumb is more homogeneous.

Table 4.1 Factor levels and level codes for modified central composite design.

Factors	Level codes				
	-2	-1	0	+1	+2
Strong flour (%, 14% mb)	10	25	40	55	70
Ascorbic acid (ppm)	0	50	100	150	200
α-amylase (SKB u)	0	30	60	90	120

4.2.4 Experimental Design.

The independent variables used in this study were concentration of CWRS or CWES in the blend, ascorbic acid, and α-amylase. Four blend systems were studied: CWRS/CPS white, CWES/CPS white, CWRS/CPS red, and CWES/CPS red. A blocked modified rotatable central composite design was used for each blend system. Factor levels and level codes are shown in Table 4.1. The experiment consisted on two runs for each factorial point (8 x 2), two runs for each centre point (1 x 2), and a single run for each axial point (6 x 1). A control was baked at each baking day containing 100% of the Mexican bread wheat flour, 100 ppm ascorbic acid (middle level), and 60 SKB u α-amylase (middle level). This control was used to have an idea about the variability in between baking days due to variations in yeast activity or in equipment. The total number of runs per each blend system totalled 26. Experiments were blocked into two blocks which corresponded to the number of baking days per blend system. Order of runs within each baking day were randomized, as well as the order of baking days, and the order of baking the different blends (Table 4.2).

Data were analysed using the statistical software Design Expert 5©, version 5.0.4 (Stat Ease Corporation, Minneapolis, MN) for ANOVA, fitting of second-order models to the responses, as well as for the production of contour and surface plots and for numerical and graphical optimization.

Table 4.2 Allocation of treatments for each baking day. Treatments were randomised within each baking day.

		Hard Red Spring Flour (%)	Ascorbic acid (ppm)	α-amylase (SKB units)
DAY 1 (13 runs)	Factorial points	-1	-1	-1
		-1	-1	+1
		-1	+1	-1
		-1	+1	+1
		+1	-1	-1
		+1	-1	+1
		+1	+1	-1
		+1	+1	+1
	Centre point	0	0	0
	Axial points	0	0	+2
		0	-2	0
		+2	0	0
		Control*		
DAY 2 (13 runs)	Factorial points	-1	-1	-1
		-1	-1	+1
		-1	+1	-1
		-1	+1	+1
		+1	-1	-1
		+1	-1	+1
		+1	+1	-1
		+1	+1	+1
	Centre points	0	0	0
	Axial points	0	0	-2
		0	+2	0
		-2	0	0
		Control*		

*See Experimental Design section for improver levels and control settings.

Table 4.3 Chemical and farinograph properties of CWRS, CWES, and Prairie Spring flours. Flours courtesy of the Canadian Grain Commission (CGC), Winnipeg. Crop year 1996.

	CWRS	*CWES*	*CPS red*	*CPS white*
• Moisture (%)	14.3	14.2	14.3	13.7
• Protein (%; N x 5.7; 14% mb)	11.5	11.5	10.0	9.9
• Ash (%)	0.61	0.56	0.51	0.56
• Farinograph				
FAB (%)	64.0	62.6	60.6	57.8
Stability (min.)	11.2	>20.0	17.2	6.1
Tolerance index (b.u.)	17.0	5.0	5.0	40.0
Arrival time (min.)	0.8	0.7	0.7	1.4
Departure time (min.)	12.0	>25.0	18.0	7.5

4.3 Results.

Chemical and farinograph properties of the flours are given in Table 4.3. Protein content of CWRS and CWES bread wheat flours was identical (11.5%), and higher than the Prairie Spring flours. CPS white had the lowest protein content (9.9%). Moisture contents of flours were very similar, except for the CPS white flour, which had a slightly lower moisture content. CWRS had the highest baking absorption, followed by CWES, CPS red, and CPS white, respectively. As expected, the strong properties of the CWES flour were reflected in long stability and departure time in the farinograph, whereas the weak gluten properties of CPS white were reflected in low stability, long arrival time, and short departure time. CPS red had similar tolerance index and arrival time

compared to CWES, but lower stability and departure time. CPS red also had higher a stability value compared to CWRS. Thus, CPS red and the Hard Red Spring bread wheats were very similar except for a lower protein content in the CPS red flour. Differences in baking absorption of the flours were also evident in the baking absorptions of the blends (Table 4.4). Baking absorptions increased as a result of higher water absorption of the Hard Red Spring flours.

Data were analysed following the same procedure as described in the Optimization Experiment #1, that is:

- fitting of full second-order model to the responses;
- reduction of these models by means of stepwise regression procedure using $\alpha_{\text{to enter}} = \alpha_{\text{to exit}} = 0.1$;
- analysis of contour and surface plots to identify the effects of the factors in each of the responses and to identify significant interactions;
- graphical and numerical optimization.

Table 4.4 Water absorptions for different blends of hard red spring flours CWRS and CWES, and Prairie Spring flours CPS white and CPS red.

Hard red spring flour			
Coded level	**Actual level (% in blend)**	**Moisture (%, calculated)**	**FAB (%, 14% mb)**
	Blend: CWRS and CPS red		
-2	10	14.27	61.97
-1	25	14.27	62.97
0	40	14.26	63.28
+1	55	14.28	64.38
+2	70	14.29	65.09
	Blend: CWRS and CPS white		
-2	10	13.74	61.54
-1	25	13.83	62.73
0	40	13.93	63.23
+1	55	14.02	64.32
+2	70	14.11	65.11
	Blend: CWES and CPS red		
-2	10	14.26	62.16
-1	25	14.26	62.66
0	40	14.26	62.96
+1	55	14.25	63.25
+2	70	14.25	63.75
	Blend: CWES and CPS white		
-2	10	13.73	61.53
-1	25	13.82	62.22
0	40	13.90	63.10
+1	55	13.99	63.59
+2	70	14.07	63.87

Table 4.5 Criteria for determination of practical importance of the effects of the factors on the different responses.

Mixing time	≥ 0.5 min
Panning	≥ 2
Proof time	≥ 5 min
Loaf volume	≥ 30 cc
Fineness	not applicable
Break-and-shred	≥ 2

Graphical charts of the analysis of contour and surface plots for the different responses were produced for each blend system in order to summarize the effects of the factors on each of the responses. When reading this chart, each factor column must be read assuming that the levels of the other factors are kept constant. Arrows indicate whether there is an increase or decrease in the response with increasing levels of the factor under the specified conditions. A half-moon symbol indicates the presence of a maximum or a minimum. In the chart, statistical significance of an effect is indicated. The set of criteria used to determine whether the size of an effect was of practical importance was based on empirical recommendations as shown in Table 4.5.

4.3.1 CWRS/CPS white blend system.

4.3.1.1 Selection of best fitting models and interpretation of contour and surface plots.

Original data for the CWRS/CPS white blend system are shown on Appendix VII. Full models fitted to the different responses in terms of coded factors with their coefficients and their associated p-values are shown in Table 4.6. Terms were selected for inclusion in the predictive model based on their associated p-value. A p-value <0.1 was considered significant. Reduced models are shown in Table 4.7.

Table 4.6 CWRS/CPS white blend system. Full model coefficients in terms of coded factors.

	Mixing time		Panning		Proof time		Loaf volume		Fineness		Break-and-shred	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	2.1		7.1		94.7		961.2		732.1		6.8	
b_1: %CWRS [A]	-0.07	0.5975	0.37	0.4052	-4.85	0.0005	-1.15	0.8736	27.87	0.0525	0.79	0.1320
b_2: Ascorbic acid [B]	0.24	0.0945	-0.21	0.6415	0.77	0.4843	11.98	0.1145	7.57	0.5715	0.04	0.9360
b_3: α-amylase [C]	-0.26	0.0677	-0.38	0.4021	1.48	0.1878	31.77	0.0006	14.55	0.2849	0.29	0.5629
Quadratic terms												
b_4: A^2	-0.10	0.5411	-0.25	0.6413	2.38	0.0878	-11.25	0.2108	-14.93	0.3602	-0.75	0.2299
b_5: B^2	0.11	0.4926	0.00	1.0000	1.50	0.2646	-5.00	0.5685	-3.18	0.8429	-0.50	0.4161
b_6: C^2	0.18	0.2919	-0.50	0.3575	4.88	0.0023	-20.00	0.0359	-28.36	0.0948	-1.00	0.1168
Interaction terms												
b_7: AB	0.29	0.0881	-0.94	0.0970	0.88	0.5084	-26.87	0.0077	-36.60	0.0368	-1.00	0.1168
b_8: AC	-0.04	0.7879	-0.44	0.4190	-0.37	0.7753	-20.00	0.0359	-4.78	0.7662	0.00	1.0000
b_9: BC	0.13	0.4249	0.44	0.4190	-0.37	0.7753	0.00	1.0000	-8.06	0.6171	0.00	1.0000
p-value	0.1989		0.5905		0.0086		0.0047		0.1701		0.4984	
R^2	0.5335		0.3691		0.7508		0.7758		0.5501		0.4040	

Table 4.7 CWRS/CPS white blend system. Reduced model coefficients in terms of coded factors. For stepwise regression, $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Mixing time		Panning		Proof time		Loaf volume		Fineness		Break-and-shred	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	2.3		6.4		99.9		939.6		708.0		4.6	
b_1: %CWRS [A]	-0.07	0.5873	0.38	0.3521	-4.83	0.0002	-1.15	0.8679	27.83	0.0329	0.78	0.1036
b_2: Ascorbic acid [B]	0.24	0.0820	-0.22	0.5949			11.98	0.0970	7.57	0.5359	0.05	0.9160
b_3: α-amylase [C]	-0.26	0.0568			1.50	0.1592	31.77	0.0003	14.55	0.2414		
Quadratic terms												
b_4: A^2												
b_5: B^2												
b_6: C^2					3.58	0.0022	-14.58	0.0448	-22.32	0.0759		
Interaction terms												
b_7: AB	0.29	0.0760	-0.94	0.0693			-26.87	0.0047	-36.60	0.0215	-1.00	0.0868
b_8: AC							-20.00	0.0268				
b_9: BC												
p-value	0.0594		0.2164		0.0001		0.0005		0.0248		0.1382	
R^2	0.3806		0.2044		0.6619		0.7458		0.5033		0.2463	

4.3.1.1.1 Mixing time.

For mixing time, the reduced model included the main effects of CWRS, ascorbic acid and α-amylase, and the interaction CWRS x ascorbic acid. Table 4.8 shows the predictions for mixing time at different levels of the factors.

Mixing times decrease significantly at higher concentrations of DATEM when the levels of ascorbic acid are fixed below 112 ppm, and at higher concentrations of α-amylase. On the other hand, mixing times increase significantly at higher concentrations of DATEM when the levels of ascorbic acid are fixed above 112 ppm, or at higher concentrations of ascorbic acid when the levels of DATEM are fixed above 28%.

A minimum of 1.4 min is predicted to be at 55% CWRS, 50 ppm ascorbic acid, and 90 SKB u α-amylase, while a maximum of 3.0 min is at 55% CWRS, 150 ppm ascorbic acid, and 30 SKB u α-amylase.

4.3.1.1.2 Panning.

For panning score, the reduced model included the main effects of CWRS and ascorbic acid, and the interaction CWRS x ascorbic acid. The high probability (p) and low R^2 values of the model indicated that this response was not significantly affected by the factors.

Table 4.8 CWRS/CPS white blend system. Predictions for mixing time (min) at different levels of CWRS, ascorbic acid and α-amylase.

α-Amylase	Ascorbic acid		
	50 ppm (-1)	100 ppm (0)	150 ppm (+1)
25% CWRS (-1)			
30 SKB u (-1)	2.7	2.6	2.6
60 SKB u (0)	2.4	2.4	2.3
90 SKB u (+1)	2.2	2.1	2.1
40% CWRS (0)			
30 SKB u (-1)	2.3	2.6	2.8
60 SKB u (0)	2.1	2.3	2.6
90 SKB u (+1)	1.8	2.1	2.3
55% CWRS (+1)			
30 SKB u (-1)	2.0	2.5	3.0
60 SKB u (0)	1.7	2.2	2.8
90 SKB u (+1)	1.4	2.0	2.5

4.3.1.1.3 Proof time.

For proof time, the reduced model included the main effects of CWRS and α-amylase, and the quadratic term of α-amylase. Ascorbic acid did not have a significant effect. Table 4.9 shows the predictions for proof time at different levels of the factors.

Proof times decrease significantly at higher concentrations of DATEM and at higher concentrations of α-amylase up to 50 SKB u, where a minimum in the response occurs. A minimum of 95.1 min is predicted to be at 55% CWRS and

60 SKB u α-amylase, whereas a maximum of 109.8 min is at 25% CWRS and 90 SKB u α-amylase, both independently of the concentration of ascorbic acid.

4.3.1.1.4 Loaf volume.

For loaf volume, the reduced model included the main effects of CWRS, ascorbic acid, and α-amylase, the quadratic effect of α-amylase, and the two-factor interactions CWRS x ascorbic acid and CWRS x α-amylase. Table 4.10 shows the predictions for loaf volume at different levels of the factors. The significant two-factor interactions make the interpretation of the model more complex. Surface and contour plots of ascorbic acid vs α-amylase, at different levels of CWRS, are shown in Figure 4.1.

Loaf volumes increase significantly at higher concentrations of CWRS when the levels of ascorbic acid and α-amylase are below 100 ppm and 60 SKB u, respectively. On the other hand, loaf volumes decrease significantly at higher concentrations of CWRS when the levels of ascorbic acid and α-amylase are above 100 ppm and 60 SKB u, respectively. At 25% CWRS, loaf volumes increase significantly at higher concentrations of ascorbic acid and α-amylase. At 40% CWRS, loaf volumes increase at higher concentrations of ascorbic acid and α-amylase, but these effects are smaller than at 25% CWRS. At 55% CWRS, the effect of α-amylase is further reduced, while loaf volumes decrease at higher concentrations of ascorbic acid. Higher loaf volumes are predicted at low levels of CWRS and high levels of ascorbic acid and α-amylase.

Table 4.9 CWRS/CPS white blend. Predictions for proof time (min) at different levels of CWRS, ascorbic acid and α-amylase.

α-Amylase	Ascorbic acid		
	50 ppm (-1)	100 ppm (0)	150 ppm (+1)
25% CWRS (-1)			
30 SKB u (-1)	106.8	106.8	106.8
60 SKB u (0)	104.7	104.7	104.7
90 SKB u (+1)	109.8	109.8	109.8
40% CWRS (0)			
30 SKB u (-1)	102.0	102.0	102.0
60 SKB u (0)	99.9	99.9	99.9
90 SKB u (+1)	105.0	105.0	105.0
55% CWRS (+1)			
30 SKB u (-1)	97.2	97.2	97.2
60 SKB u (0)	95.1	95.1	95.1
90 SKB u (+1)	100.2	100.2	100.2

A minimum of 835.5 cc is predicted to be at 25% CWRS, 50 ppm ascorbic acid, and 30 SKB u α-amylase, while a maximum of 1016.8 cc is at 25% CWRS, 150 ppm ascorbic acid, and 90 SKB u α-amylase.

Table 4.10 CWRS/CPS white blend. Predictions for loaf volume (cc) at different levels of CWRS, ascorbic acid and α-amylase.

α-Amylase	Ascorbic acid		
	50 ppm (-1)	100 ppm (0)	150 ppm (+1)
25% CWRS (-1)			
30 SKB u (-1)	835.5	874.4	913.2
60 SKB u (0)	901.9	940.7	979.6
90 SKB u (+1)	939.1	977.9	1016.8
40% CWRS (0)			
30 SKB u (-1)	881.2	893.2	905.2
60 SKB u (0)	927.6	939.6	951.6
90 SKB u (+1)	944.8	956.8	968.7
55% CWRS (+1)			
30 SKB u (-1)	927.0	912.1	897.2
60 SKB u (0)	953.3	938.4	923.5
90 SKB u (+1)	950.1	935.6	920.7

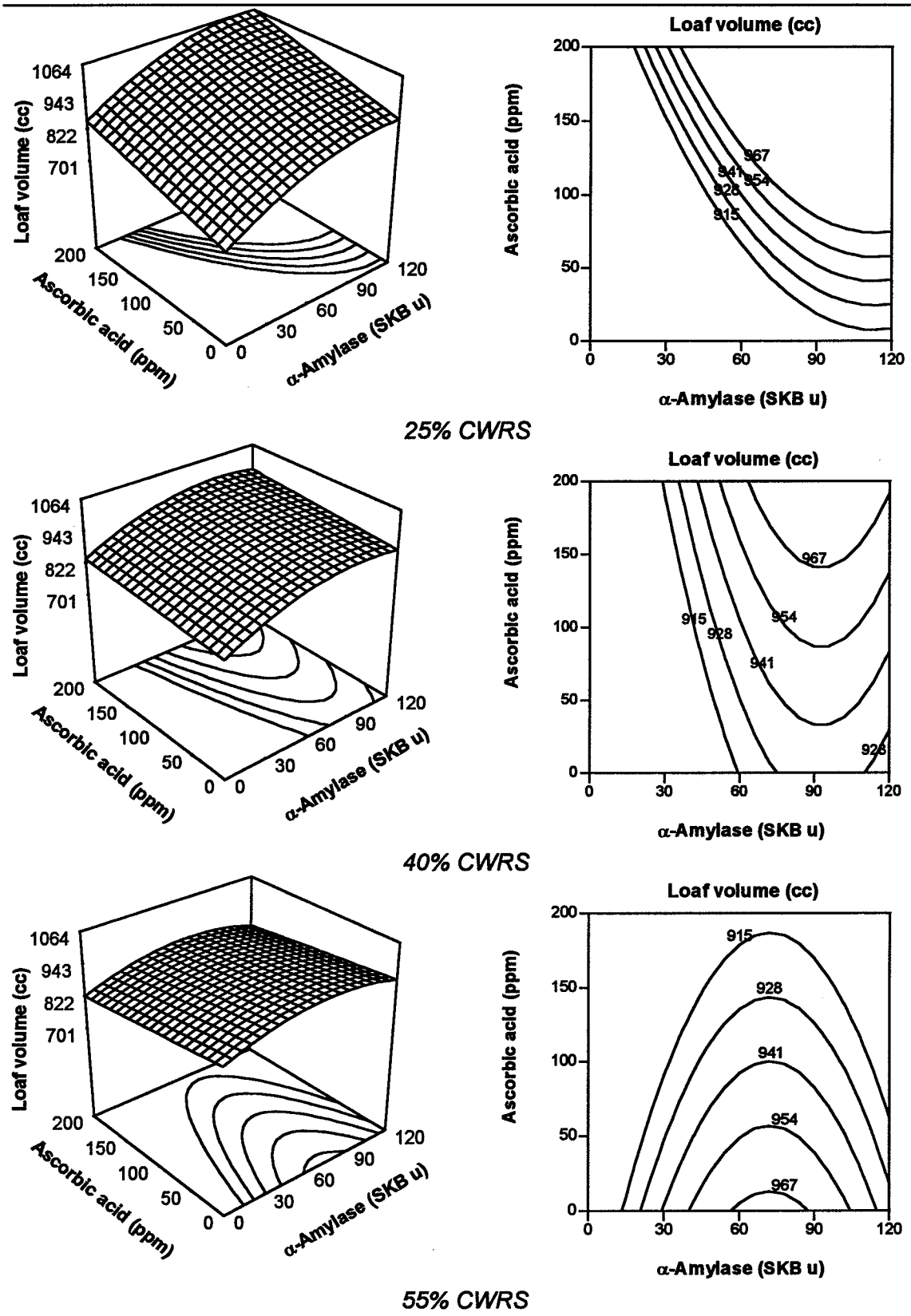


Figure 4.1 CWRS/CPS white blend. Surface and contour plots for loaf volume at different concentrations of CWRS.

4.3.1.1.5 Fineness and break-and-shred.

For fineness and break-and-shred scores, the reduced models included the main effects of CWRS, ascorbic acid, and α-amylase, and the two-factor interaction CWRS x ascorbic acid. The quadratic term of α-amylase was included in the model for fineness. Alpha-amylase did not have a significant effect in break-and-shred. Table 4.11 shows the predictions for fineness, while Table 4.12 shows the predictions for break-and-shred scores.

Fineness and break-and-shred scores were significantly correlated (Pearson's correlation coefficient = 0.8082). Both scores increase significantly at higher concentrations of CWRS when the levels of ascorbic acid are below 140 ppm, or at higher concentrations of ascorbic acid when the levels of CWRS are below 40%. The negative coefficient of the quadratic term of α-amylase indicates the presence of a maximum in fineness, which occurs at around 70 SKB u α-amylase, independently of the levels of the other two improvers. On the other hand, fineness and break-and-shred scores decrease at higher concentrations of ascorbic acid when the levels of CWRS are above 40%.

A minimum of 599.1 in fineness score and of 2.7 in break-and-shred are predicted to be at 25% CWRS, 50 ppm ascorbic acid, and 30 SKB u α-amylase. A maximum of 767.2 in fineness score (not shown) and a maximum of 6.3 in break-and-shred are at 55% CWRS, 50 ppm ascorbic acid, and 70 SKB u α-amylase.

Table 4.11 CWRS/CPS white blend. Predictions for fineness at different levels of CWRS, ascorbic acid and α-amylase.[1]

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
	25% CWRS (-1)		
30 SKB u (-1)	599.1	643.3	687.4
60 SKB u (0)	636.0	680.1	724.3
90 SKB u (+1)	628.2	672.4	716.5
	40% CWRS (0)		
30 SKB u (-1)	663.5	671.1	678.7
60 SKB u (0)	700.4	708.0	715.5
90 SKB u (+1)	692.6	700.2	707.8
	55% CWRS (+1)		
30 SKB u (-1)	728.0	698.9	669.9
60 SKB u (0)	764.8	735.8	706.8
90 SKB u (+1)	757.1	728.0	699.0

[1] Fineness scores without units.

Table 4.12 CWRS/CPS white blend. Predictions for break-and-shred at different levels of CWRS, ascorbic acid and α-amylase.[1]

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
		25% CWRS (-1)	
30 SKB u (-1)	2.7	3.8	4.9
60 SKB u (0)	2.8	3.8	4.6
90 SKB u (+1)	2.8	3.8	4.9
		40% CWRS (0)	
30 SKB u (-1)	4.5	4.6	4.6
60 SKB u (0)	4.5	4.6	4.6
90 SKB u (+1)	4.5	4.6	4.6
		55% CWRS (+1)	
30 SKB u (-1)	6.3	5.4	4.4
60 SKB u (0)	6.3	5.4	4.4
90 SKB u (+1)	6.3	5.4	4.4

[1] Break-and-shred scores from 0 to 10, where 0 = none, and 10 = high (>2 ½").

4.3.1.2 Graphical optimization.

Quality characteristics of controls, which were baked using the commercial Mexican bread wheat flour from Optimization Experiment #1, were taken as the standards of comparison for the optimization procedure. Original data for the controls are presented in Table 4.13. The goal of the optimization procedure was to obtain optimized combinations of the factors for the production of pan-bread of equal or better quality than the controls. In other words, breads were expected to have lower mixing time, lower proof time, higher loaf volume, and high panning, fineness, and break-and-shred scores, compared to the controls. Ranges of acceptable response values were established based on the experimental data obtained from the controls (Table 4.14). These ranges were also selected so that the predicted response values would be within the ranges of the actual values obtained during the experimentation. The same criteria were used for the four blend systems in order to make comparisons between the blend systems possible.

Optimized factor combinations were identified by means of overlay plots. Using the criteria of acceptability shown in Table 4.14, the Design Expert 5 © software produced the respective contour plots for each response and for each blend. Contours representing the minimum and the maximum according to the criteria were identified at the five concentrations of strong flour used in the blends. The contours for each of the responses at a fixed concentration of strong flour in the blend were superimposed over each other and the regions in which

the acceptable values of the responses overlapped were identified. The dark shaded areas of the plots represent those areas where the combinations of ascorbic acid and α-amylase give predicted mixing time, panning score, proof time, loaf volume, fineness and break-and-shred scores within the limits of acceptability. When the criteria for at least one of the responses were not met, the area is identified by a striped pattern.

Once the areas of the optimized combinations of ascorbic acid and α-amylase were identified, it was our interest to see how the model for loaf volume behaved within these areas, considering that loaf volume is one of the most important quality parameters for a baker. Thus, the overlay plot was superimposed to the contour plot corresponding to loaf volume at the same concentration of hard red spring flour. Three concentrations of the hard red spring flour were selected: 25, 40, and 55% considering that these concentrations are part of the factorial fraction of the experimental design and that the model is most accurate at these points.

Table 4.13 Graphical optimization. Original data for controls.

Mexican flour (%)	***Ascorbic acid (ppm)***	***α-amylase (SKB u)***	***Mixing time (min)***	***Panning***	***Proof time (min)***	***Loaf volume (cc)***
100	100 (0)	60(0)	1.8	8	96	910
100	100 (0)	60(0)	3.1	10	105	850
100	100 (0)	60(0)	4.1	8	111	800
100	100 (0)	60(0)	3.1	10	108	840
100	100 (0)	60(0)	3.4	10	127	820
100	100 (0)	60(0)	3.1	10	134	820
100	100 (0)	60(0)	3.2	10	125	800
Mean			3.1	9.4	115.1	834.3
s			0.6	0.9	12.7	35.4
CV (%)			20.4	9.6	11.0	4.2

Table 4.14 Graphical optimization. Criteria for responses.

	Goal	***Constraints***
Mixing time (min)	In range	1.5 to 3.1
Panning	In range	6 to 10
Proof time (min)	In range	90 to 115
Loaf volume (cc)	In range	835 to 1000
Fineness	In range	700 to 821
Break-and-shred	In range	6 to 10

Overlay plots at the five levels of CWRS used in the blends with CPS white are shown in Figure 4.2. At 25 and 40% CWRS, a zone of optimized combinations of ascorbic acid and α-amylase could not be identified. A further analysis of these overlay plots indicated that all the criteria specified for the responses were met except for break-and-shred. At these concentrations of CWRS, the model predicts values for break-and-shred below 6, which was the minimum specified for acceptability. In order to identify a zone of optimized combinations, the minimum for break-and-shred in the criteria was reset at a value of 4. With this change, the zones of optimized combinations of ascorbic acid and α-amylase were as indicated by a striped pattern. For the rest of the concentrations of CWRS (10, 55, and 70%) all the criteria specified for the responses were met and the zones of optimized combinations are indicated by a dark pattern. Boundaries of the zones of optimized combinations of ascorbic acid and α-amylase are shown in Table 4.15.

The reason why the criteria are met at 10, 55 and 70% CWRS and not at 25 and 40% CWRS is due to the significant interaction CWRS x ascorbic acid in the model for break-and-shred, according to a closer analysis of the contour and surface plots of the model for break-and-shred score. The effect of ascorbic acid on the break-and-shred score changes at different concentrations of CWRS in the blend. At low levels of CWRS (10%), acceptable break-and-shred scores (> 6.0) can be attained at high levels of ascorbic acid (>170 ppm) and the response increases at increasing levels of ascorbic acid from there on. However, at 25 and 40% CWRS, the effect of ascorbic acid on break-and-shred becomes less

significant. At 25% CWRS break-and-shred scores are below the minimum set for acceptability and range between 2.0 (~14 ppm ascorbic acid) and 5.0 (~157 ppm), with an increase in 1 point in the break-and-shred score for every 48 ppm increase in ascorbic acid. At 40% CWRS, the effect of ascorbic acid on the break-and-shred score is negligible and the mean for break-and-shred score in the experimental space is 4.5 approximately. At higher levels of CWRS (55 and 70%), acceptable break-and-shred scores can be attained at levels of ascorbic acid below 120 ppm and increased break-and-shred scores can be obtained by reducing the concentration of ascorbic acid.

At 25% CWRS, models predict the production of an acceptable loaf bread, but with a slightly low break-and-shred score (4.0 to 5.0) at adequate combinations of ascorbic acid and α-amylase between 119 and 200 ppm and 16 and 88 SKB u, respectively within the area indicated in Figure 4.3. Loaf volume and fineness are critical for determining the area of optimized combinations of ascorbic acid and α-amylase, since this area is delimited by the upper limit of the criteria for loaf volume (1000 cc) and the lower limit of the criteria for fineness (700). Oxidative requirements of the blend are relatively high as indicated by the high levels of ascorbic acid required for the optimized combinations. Within this area, loaf volume ranges between 920 and 1000 cc (Figure 4.3). Loaf volume can be maximized within this area using higher levels of ascorbic acid and higher levels of α-amylase. For example, the following are predictions that use low concentration of ascorbic acid: 920 cc (179 ppm ascorbic acid, 25 SKB u α-amylase), 950 cc (127 ppm ascorbic acid, 52 SKB u α-amylase), 980 cc (120

ppm ascorbic acid, 76 SKB u α-amylase), and 1000 cc (128 ppm ascorbic acid, 88 SKB u α-amylase). A reduction on the concentration of α-amylase results in reduced loaf volumes, which can be compensated for by an increase in the concentration of ascorbic acid.

At 40% CWRS, predictions for break-and-shred scores remain below the minimum set for acceptability. The area of optimized combinations of ascorbic acid and α-amylase is larger than at 25% CWRS. Panning score and fineness are critical for determining the area of optimized combinations of ascorbic acid and α-amylase. This area is delimited by the lower limit of the criteria for panning score (6.0) and the lower limit of the criteria for fineness (700). Oxidative requirements of the blend are lower than at 25% CWRS and ascorbic acid can be used as low as 33 ppm. Acceptable loaves of bread can be produced at adequate combinations of ascorbic acid and α-amylase between 33-186 ppm and 39-100 SKB u, respectively within the area indicated in Figure 4.3. Slightly lower loaf volumes are predicted at this concentration of CWRS than at 25% CWRS and they range from 930 to 970 cc. High loaf volumes can be attained at high levels of both improvers. For example, the following are predictions that use low concentration of ascorbic acid: 930 cc (35 ppm ascorbic acid, 67 SKB u α-amylase), 950 cc (76 ppm ascorbic acid, 86 SKB u α-amylase), and 970 cc (158 ppm ascorbic acid, 97 SKB u α-amylase). A reduction on the concentration of α-amylase results in reduced loaf volumes, which can be compensated for by an increase in the concentration of ascorbic acid.

At 55% CWRS, acceptable loaves of bread, which meet all the criteria of acceptability, are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 0-67 ppm and 7-103 SKB u respectively and within the area indicated in Figure 4.3. Break-and-shred score and mixing time are critical for determining the area of optimized combinations of ascorbic acid and α-amylase. This area is delimited by the lower limit of the criteria for break-and-shred (6.0) and the lower limit of the criteria for mixing time (1.5 min). Oxidative requirements of the blend are relatively low compared to the requirements at 25 and 40% CWRS. Slightly lower loaf volumes are predicted at this concentration of CWRS than at 25 and 40% CWRS and they range from 920 to 960 cc. The effect of ascorbic acid is reversed with respect to the effect observed at 25 and 40% CWRS; in other words, increased concentrations of ascorbic acid result in reduced loaf volumes. Loaf volume can be maximized at lower levels of ascorbic acid and higher levels of α-amylase. A reduction in the concentration of α-amylase results in reduced loaf volumes, which can be compensated for by a reduction in the concentration of ascorbic acid.

At increasing concentrations of CWRS in the blend the oxidative requirements were lower as can be seen when comparing optimized combinations predicting the same loaf volume. A predicted loaf volume of 950 cc can be achieved at 25% CWRS using 127 ppm ascorbic acid and 52 SKB u α-amylase, at 40% CWRS using 76 ppm ascorbic acid and 86 SKB u α-amylase, and at 55% CWRS using 15 ppm ascorbic acid and 41 SKB u α-amylase. No tendency was evident with respect to the requirements for α-amylase.

Table 4.15 CWRS/CPS white blend system. Graphical optimization. Zones of optimised solutions at different concentrations of CWRS.

CWRS (%)	*Ascorbic acid (ppm)*	*α-Amylase (SKB u)*	*Achievement of criteria*
10	172-200	16-45	✓
25	119-200	16-88	Break-and-shred = 4
40	33-186	39-100	Break-and-shred = 4
55	0-67	7-103	✓
70	31-103	0-120	✓

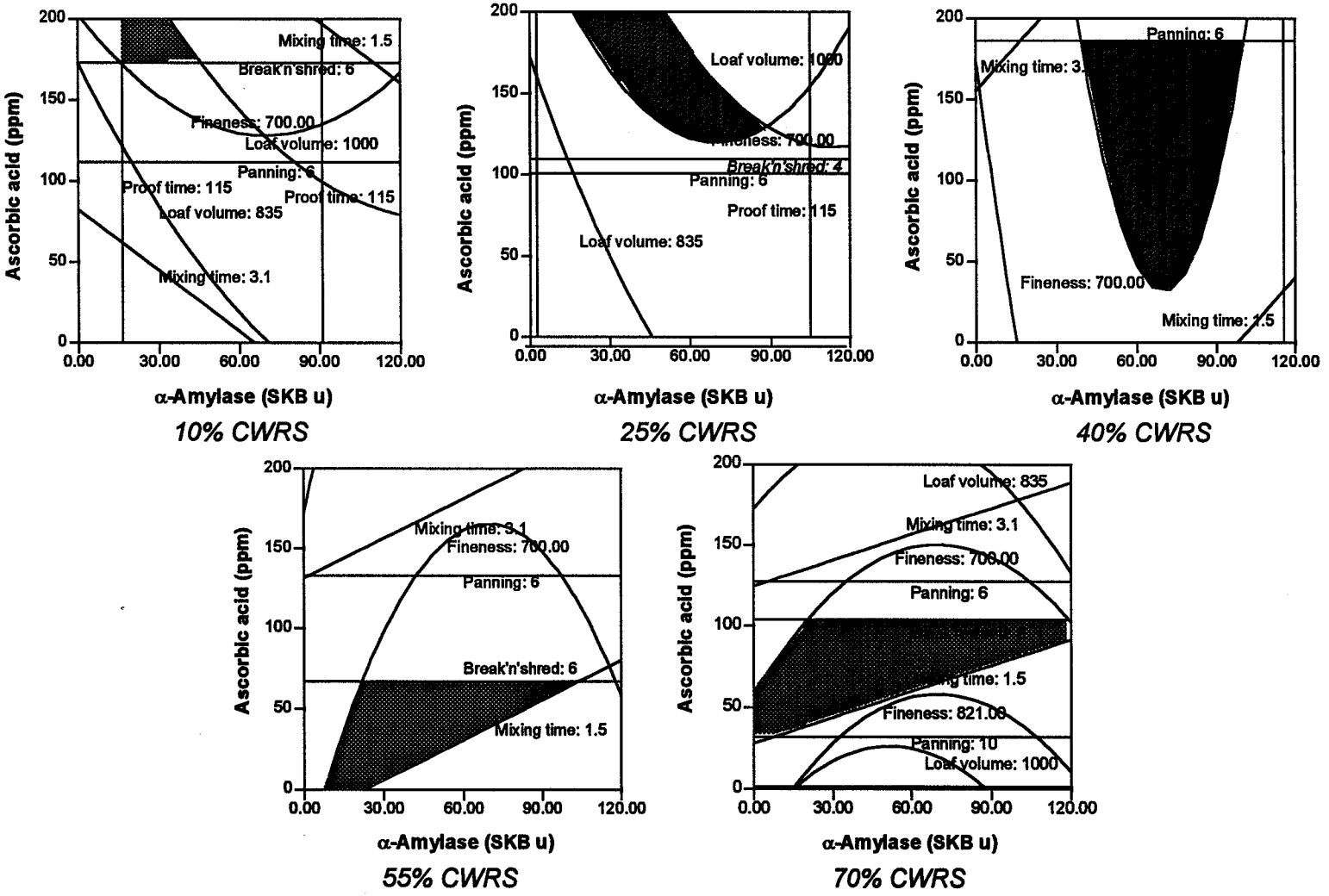
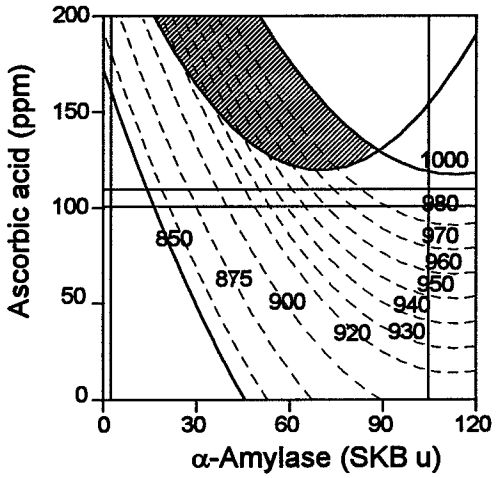
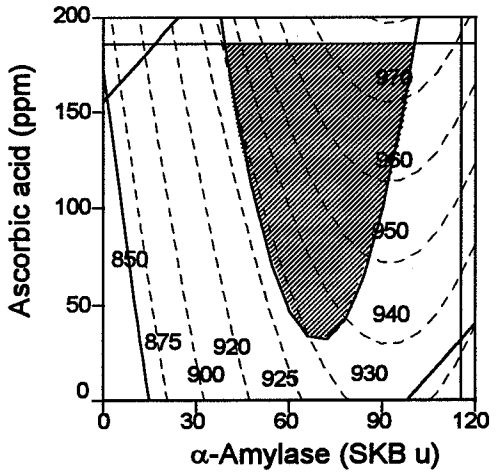


Figure 4.2 CWRS/CPS white blend system. Graphical optimization. Overlay plots at different concentration of CWRS. A striped pattern indicates predicted break-and-shred scores below the minimum for acceptability. A dark pattern indicates complete fulfilment of criteria for acceptability.

25% CWRS



40% CWRS



55% CWRS

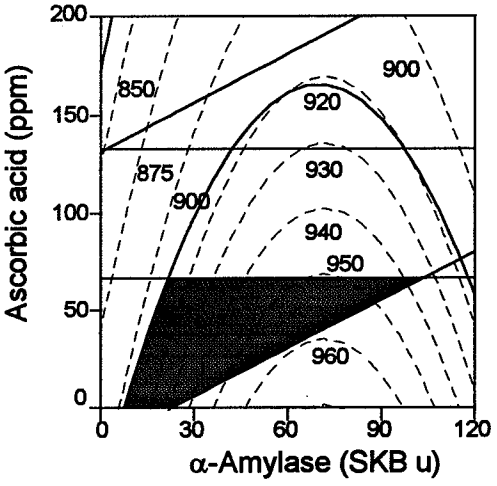


Figure 4.3 CWRS/CPS white blend system. Superimposed plots of zones of optimized combinations of ascorbic acid and α-amylase and loaf volume contours at different concentrations of CWRS. A striped pattern at 25 and 40% CWRS indicates predicted break-and-shred scores below the minimum for acceptability. Dark pattern indicates complete fulfilment of criteria for acceptability.

4.3.1.3 Summary.

The amount of CWRS, in blends with CPS white wheat, has a significant effect on mixing time, proof time, loaf volume, and fineness and break-and-shred scores, while ascorbic acid levels affect mixing time, loaf volume, and fineness and break-and-shred scores. Alpha-amylase levels influence mixing time, proof time, loaf volume, and fineness scores. None of the factors included in this experiment have significant effects on panning scores. These results are summarized in Table 4.16.

Higher concentrations of CWRS result in shorter mixing times and increased fineness and break-and-shred scores when the levels of ascorbic acid are below 112 ppm. A higher amount of CWRS in the blend also has the advantage of reducing proof times. A ten-minute reduction in proof time is predicted when the level of CWRS is raised from 25% to 55%. The effect of higher concentrations of CWRS on loaf volume depends on the levels of ascorbic acid and α-amylase, as a result of significant CWRS x ascorbic acid and CWRS x α-amylase interactions. As indicated in Table 4.16, there is a consistent increase in loaf volume with increased amounts of CWRS when the levels of ascorbic acid are as low as 50 ppm, or as high as 100 ppm with levels of α-amylase below 60 SKB u. However, loaf volumes decrease at 100 ppm ascorbic acid when the levels of α-amylase are above 60 SKB u, or at higher levels of ascorbic acid at any concentration of α-amylase.

The effect of ascorbic acid depends on the levels of CWRS in the blend. Shorter mixing times, higher loaf volumes and higher fineness and break-and-shred scores occur with increasing concentrations of ascorbic acid when CWRS levels are as low as 28%. However, these effects are reversed when the levels of CWRS are above 40%.

Alpha-amylase shortens mixing and proof times, and increases loaf volumes and fineness scores for all blends, but the effect is more important for the low concentrations of CWRS.

Graphical optimization was carried out to identify combinations of factor levels predicted to give loaves with characteristics equal or better than the controls. Loaves that met all of the criteria are formulated at 10%, 55%, and 70% CWRS. At 25 and 40% CWRS, slightly low break-and-shred scores are predicted, with the rest of the criteria being met.

A range of optimized formulations that met all of the criteria except for break-and-shred, and that predicted loaf volumes of 950 cc or higher, were identified using 25 and 40% CWRS in the blend. The 25% blend requires a minmim of 120 ppm ascorbic acid with 60 SKB u α-amylase, while the 40% blend requires a minimum of 75 ppm ascorbic acid with 85 SKB u α-amylase. With the 55% blend all the criteria can be met and a loaf volume of 950 ml achieved by adding 15 ppm ascorbic acid and 40 SKB u α-amylase.

Table 4.16 CWRS/CPS white blend system. Summary of analysis of contour and surface plots.

Responses	p	R^2	Effects of			Range of actual values	
			CWRS (%)	Ascorbic acid (AA) (ppm)	α-amylase (SKB u)	Low	High
Mixing time (min)	0.0594	0.3806	↓ @ AA < 112 ppm ✓ ↑ @ AA > 112 ppm ✓	↓ @ CWRS < 28% ↑ @ CWRS > 28% ✓	↓ ✓	0.9	3.5
Panning	0.2164	0.2044	ns	ns	ns	2	9
Proof time (min)	0.0001	0.6619	↓ ✓	ns	◡ @ 50 SKB u	89	125
Loaf volume (cc)	0.0005	0.7458	↑ @ AA < 100 ppm & amylase < 60 SKB u ✓ ↓ @ AA > 100 ppm & amylase > 60 SKB u ✓	↑ @ 25% CWRS ✓ ↑ @ 40% CWRS ↓ @ 55% CWRS ✓	↑ @ 25% CWRS ✓ ↑ @ 40% CWRS ✓ ↑ @ 55% CWRS	800	1040
Fineness	0.0248	0.5033	↑ @ AA < 140 ppm ✓ ↓ @ AA > 140 ppm ✓	↑ @ CWRS < 40% ✓ ↓ @ CWRS > 40% ✓	◠ @ 70 SKB u ✓	560.2	821.4
Break-and-shred	0.1382	0.2463	↑ @ AA < 140 ppm ✓ ↓ @ AA > 140 ppm	↑ @ CWRS < 40% ✓ ↓ @ CWRS > 40% ✓	ns	0	8

Key: ↑ Increase in response as the level of the factor is increased.

↓ Decrease in response as the level of the factor is increased.

◠ Maximum in response.

◡ Minimum in response.

ns Effect of factor is not statistically significant.

✓ Effect of factor is significant in practice.

4.3.2 CWES/CPS white blend system.

4.3.2.1 Selection of best fitting models and interpretation of contour and surface plots.

Original data for the CWES/CPS white blend system are shown on Appendix VIII. Full models fitted to the different responses in terms of coded factors with their coefficients and their associated p-values are shown in Table 4.17. Terms were selected for inclusion in the predictive model based on their associated p-value. A p-value <0.1 was considered adequate. Reduced models are shown in Table 4.18.

Table 4.17 CWES/CPS white blend system. Full model coefficients in terms of coded factors.

	Mixing time[1]		Panning		Proof time		Loaf volume		Fineness		Break-and-shred[2]	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	0.7		5.6		98.2		894.4		725.0		31.4	
b_1: %CWES [A]	-0.003	0.8886	1.63	0.0020	-6.29	0.0060	-0.49	0.9691	34.15	0.0979	11.91	0.0173
b_2: Ascorbic acid [B]	-0.04	0.0781	-0.13	0.7692	-0.62	0.7076	18.68	0.1019	19.84	0.2476	3.46	0.3688
b_3: α-amylase [C]	0.09	0.0008	-0.21	0.6314	-0.13	0.9393	28.82	0.0182	15.94	0.3481	5.21	0.1846
Quadratic terms												
b_4: A^2	-0.01	0.5985	-0.25	0.6312	1.37	0.5927	10.21	0.5412	0.31	0.9905	5.95	0.3169
b_5: B^2	-0.02	0.3314	0.00	1.0000	2.75	0.1854	-3.75	0.7731	-19.94	0.3311	1.00	0.8265
b_6: C^2	0.03	0.1799	0.00	1.0000	0.75	0.7083	-26.25	0.0613	-23.75	0.2509	-5.00	0.2845
Interaction terms												
b_7: AB	-0.007	0.7947	0.38	0.4740	-0.12	0.9501	16.88	0.2092	30.85	0.1431	0.50	0.9126
b_8: AC	0.04	0.1330	0.63	0.2408	-2.25	0.2727	-3.12	0.8100	-17.32	0.3962	4.50	0.3332
b_9: BC	-0.06	0.0301	-0.62	0.2408	-0.50	0.8027	6.88	0.5986	0.03	0.9989	-2.50	0.5857
p-value	0.0105		0.1037		0.1707		0.0972		0.2867		0.0905	
R^2	0.7413		0.5966		0.5737		0.6265		0.5127		0.6324	

[1] Mixing time was transformed into 1/sqrt(mixing time).

[2] Break and shred was transformed into (break and shred + 1)^2.

Table 4.18 CWES/CPS white blend system. Reduced model coefficients in terms of coded factors. For stepwise regression $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Mixing time[1]		Panning		Proof time		Loaf volume		Fineness		Break-and-shred[2]	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	0.6		5.3		103.0		899.9		679.2		39.6	
b_1: %CWES [A]	-0.003	0.8810	1.63	0.0003	-6.24	0.0007			38.75	0.0406	13.53	0.0020
b_2: Ascorbic acid [B]	-0.04	0.0584					18.94	0.0640				
b_3: α-amylase [C]	0.09	0.0003					28.56	0.0081			5.22	0.1446
Quadratic terms												
b_4: A^2												
b_5: B^2												
b_6: C^2	0.05	0.0226					-26.82	0.0126			-6.51	0.0765
Interaction terms												
b_7: AB												
b_8: AC	0.04	0.1070										
b_9: BC	-0.06	0.0196										
p-value	0.0010		0.0003		0.0007		0.0027		0.0460		0.0031	
R^2	0.7196		0.4641		0.4435		0.5355		0.1939		0.5284	

[1] Mixing time was transformed into 1/sqrt(mixing time).

[2] Break and shred was transformed into (break and shred + 1)^2

4.3.2.1.1 Mixing time.

Original data were transformed using y' = 1/sqrt(mixing time) to comply with the ANOVA principles of normality of error terms and equality of variance. Predictions were transformed back to the original metric. The reduced model included the main effects of CWES, ascorbic acid and α-amylase, the quadratic term of α-amylase, and the interactions CWES x α-amylase and ascorbic acid x α-amylase. Table 4.19 shows the predictions for mixing time at different levels of the factors. The significant two-factor interactions make the interpretation of the model more complex. Surface and contour plots of ascorbic acid vs α-amylase, at different levels of CWES, are shown in Figure 4.4.

Mixing times increase significantly at higher concentrations of CWES when the levels of α-amylase are below 60 SKB u, or at higher concentrations of ascorbic acid when the levels of α-amylase are above 40 SKB u. On the other hand, mixing times decrease significantly at higher concentrations of CWES when the levels of α-amylase are above 60 SKB u, or at higher concentrations of α-amylase when the levels of CWES are above 40%. At 25% CWES, mixing times decrease significantly at higher concentrations of α-amylase when the levels of ascorbic acid are below 88 ppm.

A minimum of 1.2 min is predicted to be at 55% CWES, 50 ppm ascorbic acid, and 90 SKB u α-amylase, while a maximum of 3.6 min is at 55% CWES, 50 ppm ascorbic acid, and 30 SKB u α-amylase.

Table 4.19 CWES/CPS white blend. Predictions for mixing time (min) at different levels of CWES, ascorbic acid and α-amylase.

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
		25% CWES (-1)	
30 SKB u (-1)	2.7	2.5	2.3
60 SKB u (0)	2.2	2.5	2.8
90 SKB u (+1)	1.4	1.9	2.5
		40% CWES (0)	
30 SKB u (-1)	3.1	2.9	2.7
60 SKB u (0)	2.2	2.5	2.8
90 SKB u (+1)	1.3	1.7	2.2
		55% CWES (+1)	
30 SKB u (-1)	3.6	3.3	3.1
60 SKB u (0)	2.2	2.5	2.9
90 SKB u (+1)	1.2	1.5	2.0

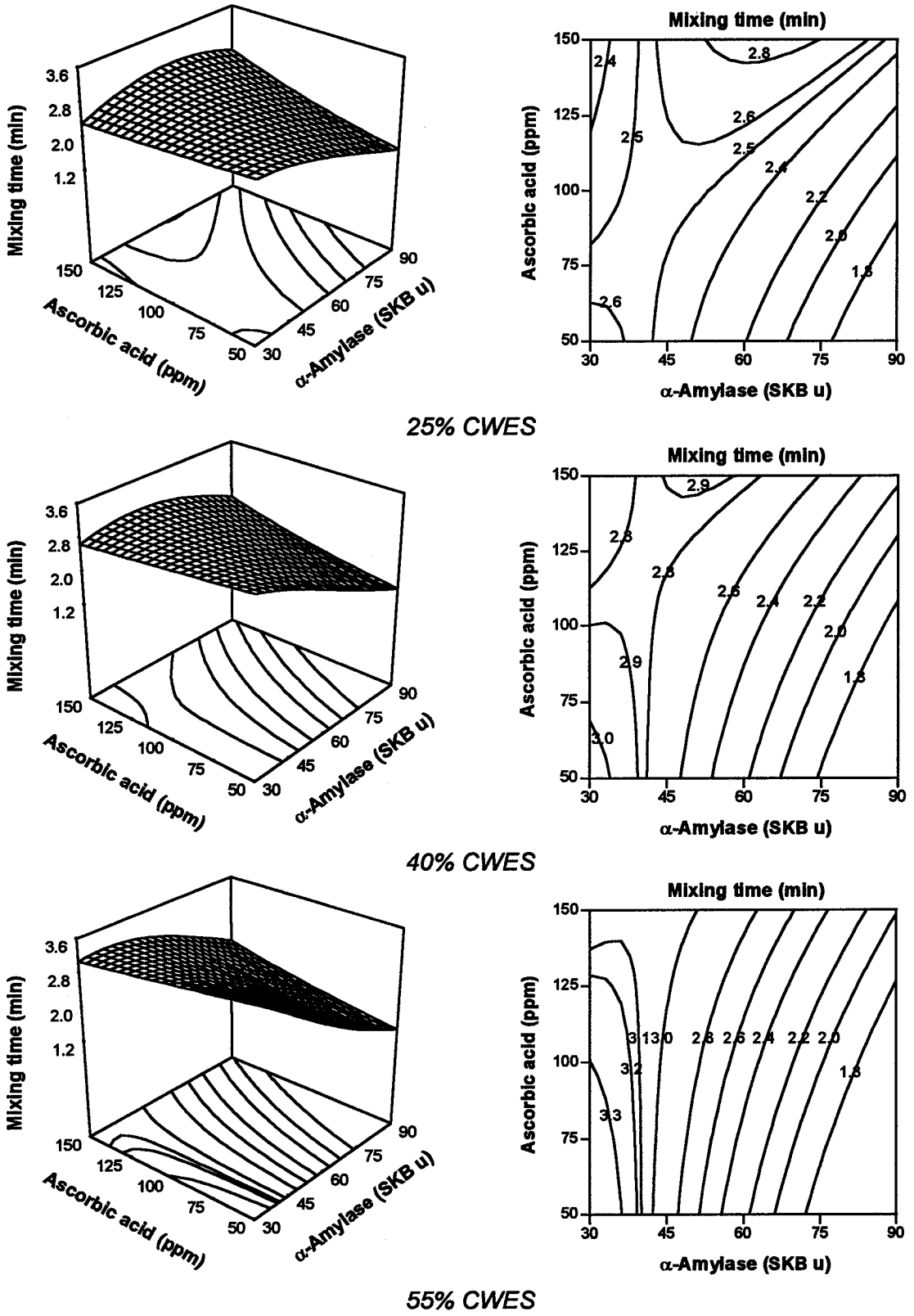


Figure 4.4 CWES/CPS white blend. Surface and contour plots for mixing time at different concentrations of CWRS.

4.3.2.1.2 Panning and proof time.

For panning and proof time, the reduced models included only the main effect of CWES. Ascorbic acid and α-amylase did not have a significant effect on both responses. Panning scores increase and proof times decrease significantly at higher concentrations of CWES: increasing the concentration of CWES from 25% to 55% results in an increase in panning score from 3.7 to 7.9, and a decrease in proof time from 109.2 to 96.8.

4.3.2.1.3 Loaf volume.

For loaf volume, the reduced model included the main effects of ascorbic acid and α-amylase, and the quadratic term of α-amylase. CWES did not have a significant effect. Table 4.20 shows the predictions for loaf volume at different levels of ascorbic acid and α-amylase when the level of CWES is fixed at 40%.

Loaf volumes increase significantly at higher concentrations of ascorbic acid and at higher concentrations of α-amylase up to 75 SKB u, where a maximum in the response occurs. A minimum of 825.6 cc is predicted to be at 50 ppm ascorbic acid and 30 SKB u α-amylase, while a maximum of 926.3 cc is at 150 ppm ascorbic acid and 70 SKB u α-amylase (Figure 4.5).

Table 4.20 CWES/CPS white blend. Predictions for loaf volume (cc)at different levels of ascorbic acid and α-amylase. Level of CWES was fixed at 40%.

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
	40% CWES (0)		
30 SKB u (-1)	825.6	844.5	863.5
60 SKB u (0)	880.9	899.9	918.8
90 SKB u (+1)	882.7	901.6	920.6

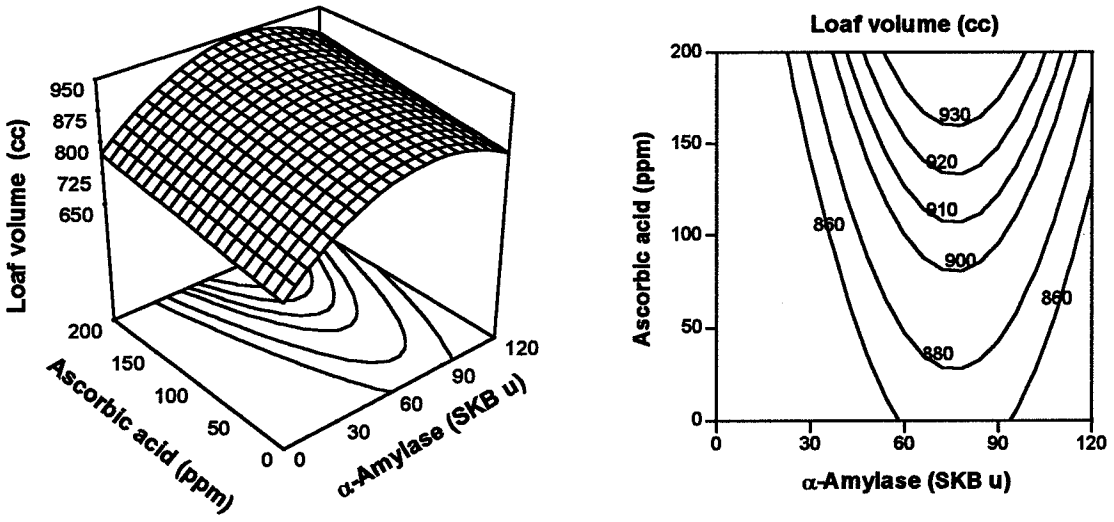


Figure 4.5 CWES/CPS white blend. Surface and contour plots for loaf volume at a fixed concentration of CWES of 40%.

4.3.2.1.4 Fineness.

For fineness, the reduced model included only the main effect of CWES. Ascorbic acid and α-amylase did not have a significant effect. Fineness scores increase at higher concentrations of CWES: increasing the concentration of CWES from 25% to 55% results in an increase in fineness score from 640.5 to 718.0, meaning that the crumb structure is more homogeneous.

4.3.2.1.5 Break-and-shred.

Original data were transformed using $y' = (\text{break and shred} + 1)^2$ to comply with the ANOVA principles of normality of error terms and equality of variance. Predictions were transformed back to the original metric. The reduced model included the main effects of CWES and α-amylase, and the quadratic term of α-amylase. Ascorbic acid did not have a significant effect on the response. Table 4.21 shows the predictions for break-and-shred scores at different levels of CWES and α-amylase when the level of ascorbic acid is fixed at 100 ppm.

Break-and-shred scores increase significantly at higher concentrations of CWES and at higher concentrations of α-amylase up to 70 SKB u, where a maximum in the response occurs. The individual effect of α-amylase is small, but the additive effect of both factors is significant: a minimum of 2.7 is predicted to be at 25% CWES and 30 SKB u α-amylase, while a maximum of 6.3 is at 55% CWES, and 70 SKB u α-amylase.

Table 4.21 CWES/CPS white blend. Predictions for break-and-shred at different concentrations of CWES and α-amylase. Level of ascorbic acid was fixed at 100 ppm.[1]

α-Amylase	*100 ppm ascorbic acid (0)*		
	25% CWES (-1)	*40% CWES (0)*	*55% CWES (+1)*
30 SKB u (-1)	2.7	4.2	5.4
60 SKB u (0)	4.0	5.3	6.2
90 SKB u (+1)	3.9	5.1	6.2

[1] Break-and-shred scores from 0 to 10, where 0 = none, and 10 = high (>2 ½").

4.3.2.2 Graphical optimization.

The optimization procedure followed was the same as described in the optimization section for the CWRS/CPS white blend (section 4.3.1.2). The goal of the optimization procedure was to obtain optimized combinations of the factors for the production of pan-bread of equal or better quality than the controls (Table 4.13). Zones of optimized factor combinations were identified at the five concentrations of CWES used in the blends with CPS white by means of overlay plots using the criteria of acceptability shown in Table 4.14. The dark-shaded areas in these plots indicate those areas where the combinations of ascorbic acid and α-amylase give predicted values for the responses within the limits of acceptability. When the criteria for at least one of the responses were not met, the area is identified by a striped pattern. Furthermore, the overlay plot was superimposed to the contour plot corresponding to loaf volume at the same concentration of CWES in the blend. Three concentrations of CWES were

selected for this step: 25, 40 and 55% considering that these concentrations are part of the factorial fraction of the experimental design.

Overlay plots at the five levels of CWES used are shown in Figure 4.6. At 10, 25 and 40% CWES, a zone of optimized combinations of ascorbic acid and α-amylase could not be identified. A further analysis of these overlay plots indicated that all the criteria specified for the responses were met except for panning, fineness, and break-and-shred scores. At these concentrations of CWES, the models predict values for panning below 6.0, for fineness below 700, and for break-and-shred below 6.0, which were the minima specified for acceptability. In order to identify a zone of optimized combinations, the minimum for these responses in the criteria were reset at lower values at each concentration of CWES. With these changes, zones of optimized combinations of ascorbic acid and α-amylase were identified and are indicated by a striped pattern. For the other two concentrations of CWES (55 and 70%), all the criteria specified for the responses were met and the zones of optimized combinations are indicated by a dark pattern. Boundaries of the zones of optimized combinations of ascorbic acid and α-amylase are shown in Table 4.22.

At 25% CWES, average predictions for panning score, fineness and break-and-shred are 3.7, 640.5, and 3.8 respectively, all of them below the minimum for acceptability. Criteria for mixing time, proof time, and loaf volume are satisfied. Thus, at this concentration of CWES the models predict the production of a dough with acceptable mixing time and proof time but with poor handling properties, resulting in a loaf of bread with acceptable loaf volume but

with low break-and-shred and low crumb uniformity. To identify areas of optimized combinations of ascorbic acid and α-amylase, the lower limits of the criteria for panning score, fineness and break-and-shred were reset at 3.0, 640, and 3.0, respectively. In this way, loaves of bread with the characteristics described above can be produced at adequate combinations of ascorbic acid and α-amylase between 0-200 ppm and 34-110 SKB u, respectively and within the area indicated in Figure 4.7. Mixing time, loaf volume and break-and-shred are critical for determining the area of optimized combinations of ascorbic acid and α-amylase, since this area is delimited by the upper and lower limits of the criteria for mixing time (1.5 and 3.1 min, in transformed scale in Figure 4.7), the lower limit of the criteria for break-and-shred (3.0), and the lower limit of the criteria for loaf volume (835 cc).

At 40% CWES, predictions for panning score, fineness and break-and-shred improve with respect to the predictions obtained at 25% CWES; however, the predictions remain slightly below the minimum for acceptability. Average predictions are 5.3, 679.2, and 5.7 for panning score, fineness and break-and-shred respectively. The lower limits of the criteria for panning score, fineness and break-and-shred were reset at 5.0, 670, and 5.0, respectively, to identify the area of optimized combinations of ascorbic acid and α-amylase. In this way, loaves of bread with slightly low scores for panning, crumb fineness and break-and-shred can be produced at adequate combinations of ascorbic acid and α-amylase between 0-200 ppm and 48-96 SKB u, respectively, and within the area indicated in Figure 4.7. As at 25% CWES, mixing time, loaf volume and break-

and-shred are critical for determining the area of optimized combinations of ascorbic acid and α-amylase, since this area is delimited by the upper and lower limits of the criteria for mixing time (1.5 and 3.1 min, in transformed scale in Figure 4.7.), the lower limit of the criteria for break-and-shred (5.0), and the lower limit of the criteria for loaf volume (835 cc).

At 55% CWES, predictions for panning score, fineness and break-and-shred further improve with respect to the predictions obtained at 25 and 40% CWES and acceptable loaves of bread, which meet all the criteria of acceptability, are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 0-200 ppm and 47-97 SKB u, respectively, and within the area indicated in Figure 4.7. Mixing time and break-and-shred proved to be critical for determining the area of combinations of ascorbic acid and α-amylase, since this area is delimited by the upper and lower limits of the criteria for mixing time (1.5 and 3.1 min, in transformed scale at Figure 4.7) and the lower limit of the criteria for break-and-shred (6.0).

The concentration of ascorbic acid is not critical at any given concentration of CWES in the blend to obtain optimized combinations, due to the large range of values that can be used. On the other hand, CWES did not have a significant effect on loaf volume and the contours for loaf volume are the same at any given concentration of CWES. Loaf volumes range from 870 to 930 cc. Loaf volume can be maximized using increased levels of ascorbic acid and increased levels of α-amylase up to 70 SKB u where volume reaches a maximum. Decreased concentrations of a-amylase results in decreased loaf

volumes, which can be compensated for by increasing the concentration of α-amylase.

Table 4.22 CWES/CPS white blend system. Graphical optimization. Zones of optimised solutions at different concentrations of CWES.

CWRS (%)	*Ascorbic acid (ppm)*	*α-Amylase (SKB u)*	*Achievement of criteria*
10	0-200	48-95	Panning = 2.1 Fineness = 601.7 Break-and-shred = 2.2
25	0-200	34-110	Panning = 3.7 Fineness = 640.5 Break-and-shred = 3.8
40	0-200	48-96	Panning = 5.3 Fineness = 679.2 Break-and-shred = 5.27
55	0-200	47-97	✓
70	0-200	44-117	✓

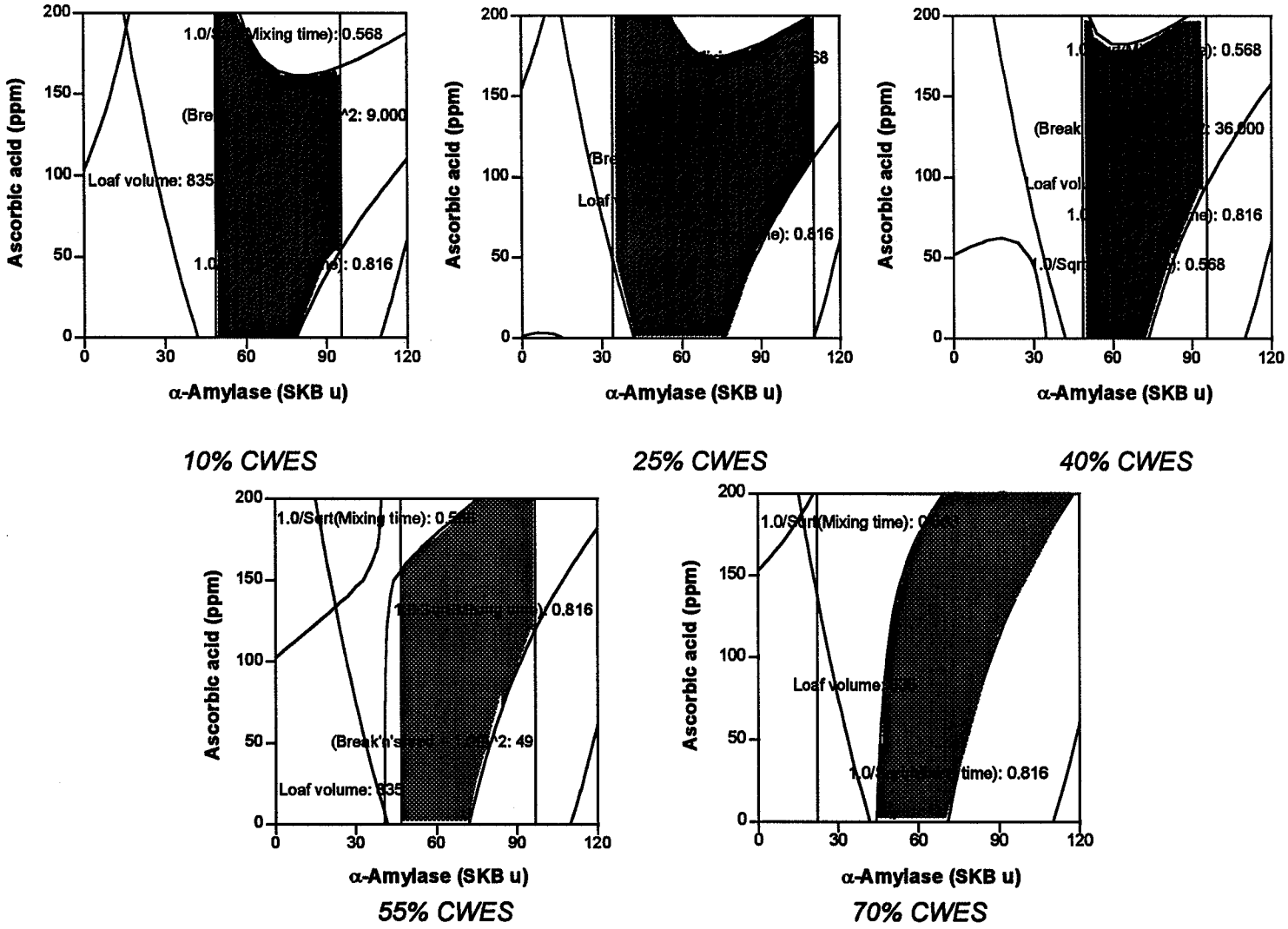


Figure 4.6 CWES/CPS white blend system. Graphical optimization. Overlay plots at different concentration of CWES. A striped pattern indicates predicted panning, fineness, and break-and-shred scores below the minimum for acceptability. A dark pattern indicates complete fulfilment of criteria for acceptability.

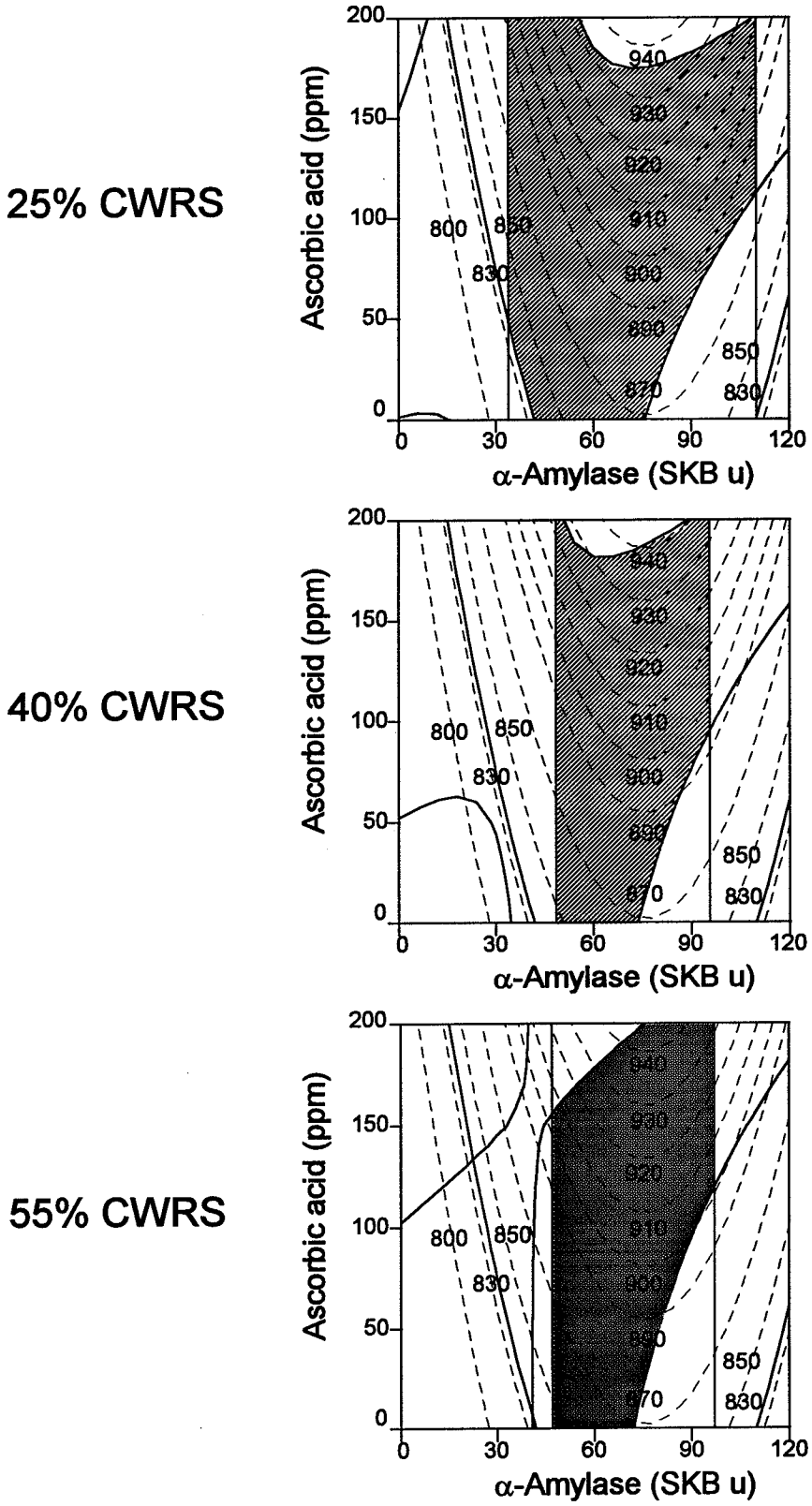


Figure 4.7 CWES/CPS white blend system. Superimposed plots of zones of optimized combinations of ascorbic acid and α-amylase and loaf volume contours at different concentrations of CWES. A striped pattern at 25 and 40% CWES indicates predicted panning score, fineness, and break-and-shred scores below the minimum for acceptability. Dark shading indicates complete fulfilment of criteria for acceptability.

4.3.2.3 Summary

The concentration of CWES, in blends with CPS white wheat, has a significant effect on mixing time, proof time, and panning, fineness and break-and-shred scores. Ascorbic acid levels has significant effects on mixing time and loaf volume, while α-amylase levels have a strong influence on mixing time, loaf volume and break-and-shred scores. These results are summarized in Table 4.23.

Higher concentrations of CWES result in increased panning, fineness and break-and-shred scores and shorter proof times. A ten-minute reduction in proof time is predicted when the level of CWES is raised from 25 to 55%. The effect of increasing concentrations of CWES on mixing time depends on the level of α-amylase, a result of the significant CWES x α-amylase interaction. There is a decrease in mixing time at higher concentrations of CWES when the levels of α-amylase are above 60 SKB u. However, mixing times increase with increasing amounts of CWES when the levels of α-amylase are below 60 SKB u.

Higher concentrations of ascorbic acid result in increased loaf volumes. The effect of increasing amounts of ascorbic acid on mixing time depends on the level of α-amylase. Mixing times decrease at higher concentrations of ascorbic acid when the levels of α-amylase are below 40 SKB u. However, this effect is reversed when the levels of α-amylase are above 40 SKB u.

Higher concentrations of α-amylase result in increased loaf volumes and break-and-shred scores up to concentrations between 70 and 75 SKB u, where a maximum in these responses is predicted. The effect of α-amylase on mixing time

depends on the levels of ascorbic acid and CWES in the blend. At low levels of CWES (25%), shorter mixing times can be achieved at increasing concentrations of α-amylase when the levels of ascorbic acid are less than 80 ppm. At higher levels of CWES, increasing amounts of α-amylase result in shorter mixing times at any level of ascorbic acid.

Using graphical optimization, a range of optimized formulations that met all the criteria, except for panning, fineness and break-and-shred scores, were identified in the blends containing 10%, 25% and 40% CWES. With the 55% and 70% blends, all the criteria can be met. Loaf volumes range between 870 cc and 940 cc for all blends and can be maximized at higher concentrations of ascorbic acid and at 75 SKB u α-amylase.

Table 4.23 CWES/CPS white blend system. Summary of analysis of contour and surface plots.

Responses	p	R^2	Effect of CWES (%)	Effect of Ascorbic acid (AA) (ppm)	Effect of α-amylase (SKB u)	Range of actual values Low	Range of actual values High
Mixing time (min)	0.0010	0.7196	↑ @ amylase < 60 SKB u ✓ ↓ @ amylase > 60 SKB u ✓	↓ @ amylase < 40 SKB u ↑ @ amylase > 40 SKB u ✓	↓ @ AA < 88 ppm & 25% CWES ✓ ↑ @ AA > 88 ppm & 25% CWES ↓ @ 40% & 55% CWES ✓	1.0	3.6
Panning	0.0003	0.4641	↕ ✓	ns	ns	0	10
Proof time (min)	0.0007	0.4435	↕ ✓	ns	ns	88	122
Loaf volume (cc)	0.0027	0.5355	ns	↑ ✓	◠ @ 75 SKB ✓	680	1000
Fineness	0.0460	0.1939	↕ ✓	ns	ns	555.7	832.4
Break-and-shred	0.0031	0.5284	↕ ✓	ns	◡ @ 70 SKB ✓	0	8

Key:
- ↑ Increase in response as the level of the factor is increased.
- ↓ Decrease in response as the level of the factor is increased.
- ◠ Maximum in response.
- ◡ Minimum in response.
- ns Effect of factor is not statistically significant.
- ✓ Significant effect of factor.

4.3.3 CWRS/CPS red blend system.

4.3.3.1 Selection of best fitting models and interpretation of contour and surface plots.

Original data for the CWRS/CPS red blend system are shown on Appendix IX. A graphical chart, summarizing the results of the contour and surface plots for the different responses, is presented in Table 4.30. Full models fitted to the different responses in terms of coded factors with their coefficients and their associated p-values are shown in Table 4.24. Reduced models are shown in Table 4.25.

Table 4.24 CWRS/CPS red blend system. Full model coefficients in terms of coded factors.

	Mixing time		Panning		Proof time		Loaf volume		Fineness		Break-and-shred	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	2.5		7.7		106.9		929.6		771.8		7.4	
b_1: %CWRS [A]	-0.23	0.2967	0.52	0.3726	-2.16	0.1969	-5.68	0.7081	-9.91	0.4353	0.83	0.0817
b_2: Ascorbic acid [B]	-0.26	0.2377	-0.68	0.2433	-0.84	0.6052	3.18	0.8336	17.73	0.1735	0.17	0.7120
b_3: α-amylase [C]	-0.34	0.1232	-1.65	0.0110	2.34	0.1641	-3.18	0.8336	-8.39	0.5075	0.33	0.4639
Quadratic terms												
b_4: A^2	0.06	0.8071	-0.50	0.4708	0.25	0.8981	-1.25	0.9454	-0.50	0.9737	-0.75	0.1828
b_5: B^2	0.23	0.3861	0.00	1.0000	0.62	0.7494	7.50	0.6823	-1.56	0.9181	-0.25	0.6468
b_6: C^2	0.30	0.2531	-0.75	0.2853	0.25	0.8981	-18.75	0.3144	-26.40	0.0989	-0.75	0.1828
Interaction terms												
b_7: AB	-0.04	0.8835	-0.62	0.3700	2.13	0.2873	-16.87	0.3634	-25.19	0.1137	-0.87	0.1246
b_8: AC	-0.51	0.0619	0.38	0.5869	-3.00	0.1413	-1.87	0.9182	12.99	0.3976	0.13	0.8182
b_9: BC	0.13	0.6266	-0.12	0.8555	-0.87	0.6553	-5.62	0.7585	-17.28	0.2655	0.38	0.4941
p-value	0.2702		0.2104		0.5268		0.9178		0.2842		0.3899	
R^2	0.4975		0.5272		0.3933		0.2136		0.4910		0.4461	

Table 4.25 CWRS/CPS red blend system. Reduced model coefficients in terms of coded factors. For stepwise regression $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Mixing time		Panning		Proof time[1]		Loaf volume[1]		Fineness		Break-and-shred	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	3.0		6.5						769.0		5.7	
b_1: %CWRS [A]	-0.23	0.2586							-9.91	0.4054	0.82	0.0640
b_2: Ascorbic acid [B]									17.73	0.1453	0.18	0.6782
b_3: α-amylase [C]	-0.35	0.0969	-1.69	0.0040					-8.39	0.4799		
Quadratic terms												
b_4: A^2												
b_5: B^2												
b_6: C^2									-25.71	0.0382		
Interaction terms												
b_7: AB									-25.19	0.0900	-0.87	0.0996
b_8: AC	-0.51	0.0477										
b_9: BC												
p-value	0.0603		0.0040						0.0865		0.1033	
R^2	0.3159		0.3326						0.4076		0.2718	

[1]Stepwise regression yielded an empty model for this response.

4.3.3.1.1 Mixing time.

For mixing time, the reduced model included the main effects of CWRS and α-amylase, and the interaction CWRS x α-amylase. Ascorbic acid did not have a significant effect. Table 4.26 shows the predictions for mixing time at different levels of CWRS and α-amylase when the level of ascorbic acid is fixed at 100 ppm.

Mixing times decrease significantly at higher concentrations of CWRS when the levels of α-amylase are above 46 SKB u, or at higher concentrations of α-amylase when the levels of CWRS are above 30%. On the other hand, small increases in mixing time are predicted at higher concentrations of CWRS when the levels of α-amylase are below 46 SKB u, or at higher concentrations of α-amylase when the levels of CWRS are below 30%.

A minimum of 1.0 min is predicted to be at 55% CWRS and 90 SKB u α-amylase, while a maximum of 3.6 min is at 55% CWRS and 30 SKB u α-amylase.

Table 4.26 CWRS/CPS red blend. Predictions for mixing time (min) at different levels of CWRS and α-amylase. Level of ascorbic acid was fixed at 100 ppm.

α-Amylase	*100 ppm ascorbic acid (0)*		
	25% CWRS (-1)	*40% CWRS (0)*	*55% CWRS (+1)*
30 SKB u (-1)	3.1	3.3	3.6
60 SKB u (0)	3.2	3.0	2.8
90 SKB u (+1)	3.4	2.6	1.9

4.3.3.1.2 Panning

For panning score, the reduced model included only the main effect of α-amylase. CWRS and ascorbic acid did not have a significant effect. Panning scores decrease significantly at higher concentrations of α-amylase: increasing the concentration of α-amylase from 30 to 90 SKB u results in a decrease in the panning score from 8.2 to 4.8, meaning that the dough turns less manageable.

4.3.3.1.3 Proof time

None of the factors had a significant effect on proof time. So, the stepwise regression procedure yielded an empty model.

4.3.3.1.4 Loaf volume.

None of the factors had a significant effect on loaf volume. So, the stepwise regression procedure yielded an empty model.

4.3.3.1.5 Fineness.

For fineness, the reduced model included the main effects of CWRS, ascorbic acid and α-amylase, the quadratic term of α-amylase, and the interaction CWRS x ascorbic acid. Table 4.27 shows the predictions for fineness score at different levels of the factors.

Fineness scores increase significantly at higher concentrations of ascorbic acid when the levels of CWRS are below 50%. On the other hand, fineness scores decrease significantly at higher concentrations of CWRS when the levels of ascorbic acid are above 80 ppm. Smaller increases in fineness scores are predicted at higher concentrations of α-amylase up to 55 SKB u, where a maximum in the response occurs.

A minimum of 702.0 is predicted at 25% CWRS, 50 ppm ascorbic acid, and 90 SKB u α-amylase, while a maximum of 821.9 is at 25% CWRS, 150 ppm ascorbic acid and 60 SKB u α-amylase.

Table 4.27 CWRS/CPS red blend. Predictions for fineness score at different levels of the factors.[1]

α-Amylase	*Ascorbic acid*		
	50 ppm (-1)	*100 ppm (0)*	*150 ppm (+1)*
	25% CWES (-1)		
30 SKB u (-1)	718.7	761.6	804.6
60 SKB u (0)	736.1	779.0	821.9
90 SKB u (+1)	702.0	744.9	787.8
	40% CWES (0)		
30 SKB u (-1)	734.0	751.7	769.5
60 SKB u (0)	751.3	769.1	786.8
90 SKB u (+1)	717.2	734.9	752.7
	55% CWES (+1)		
30 SKB u (-1)	749.3	741.8	734.4
60 SKB u (0)	766.6	759.1	751.7
90 SKB u (+1)	732.5	725.1	717.6

[1] Fineness scores without units.

4.3.3.1.6 Break-and-shred.

For break-and-shred score, the reduced model included the main effects of CWRS and ascorbic acid, and the interaction CWRS x ascorbic acid. Alpha-amylase did not have a significant effect. Table 4.28 shows the predictions for break-and-shred at different levels of CWRS and ascorbic acid when the levels of α-amylase is fixed at 60 SKB u.

Table 4.28 CWRS/CPS red blend. Predictions for break-and-shred at different levels of CWRS and ascorbic acid. Level of α-amylase was fixed at 60 SKB u.[1]

	60 SKB u α-Amylase (0)		
Ascorbic acid	***25% CWRS (-1)***	***40% CWRS (0)***	***55% CWRS (+1)***
50 ppm (-1)	3.8	5.5	7.2
100 ppm (0)	4.8	5.7	6.5
150 ppm (+1)	5.9	5.8	5.8

[1] Break-and-shred scores from 0 to 10, where 0 = none, and 10 = high (>2 ½").

Break-and-shred scores increase significantly at higher concentrations of CWRS when the levels of ascorbic acid are below 146 ppm, or at higher concentrations of ascorbic acid when the levels of CWRS are below 43%. On other hand, small decreases in break-and-shred scores are predicted at higher concentrations of CWRS when the levels of ascorbic acid are above 146 ppm, or at higher concentrations of ascorbic acid when the levels of CWRS are above 43%.

A minimum of 3.8 is predicted to be at 25% CWRS and 50 ppm ascorbic acid, while a maximum of 7.2 is at 55% CWRS and 50 ppm ascorbic acid.

4.3.3.2 Graphical optimization

The optimization procedure, goals, criteria, and considerations were the same as described in the previous blends (see section 4.3.1.2). Models corresponding to proof time and loaf volume were not included in the optimization procedure since no model could be fitted to this data. Therefore, the optimization was performed on mixing time, panning score, fineness, and break-and-shred. Overlay plots at the five levels of CWRS used are shown in Figure 4.8. Zones of optimized combinations of ascorbic acid and α-amylase were identified at any given concentration of CWRS except at 40%. A further analysis of the overlay plots at this concentration of CWRS revealed that the criteria for break-and-shred were not met. Predicted value for break-and-shred was slightly below the minimum of 6.0 set for acceptability. In order to identify a zone of optimized combinations , the minimum for break-and-shred in the criteria was reset at a lower level at this concentration of CWRS. Boundaries of the zones of the optimised solutions are presented in Table 4.29.

At 25% CWRS, acceptable loaves of bread, which meet all the criteria for acceptability (except for proof time and loaf volume, as indicated above), are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 155-200 ppm and 0-38 SKB u, respectively, and within the area indicated in Figure 4.8. Oxidative requirements of the blend are relatively high at this concentration of CWRS, while α-amylase requirements are low. Mixing time, fineness and break-and-shred proved to be critical in determining

the area of optimized combinations of ascorbic acid and α-amylase, since the area was delimited by the upper limit of mixing time (3.1 min), the upper limit of fineness (821), and the lower limit of break-and-shred (6.0).

At 40% CWRS, average predictions for break-and-shred are below 6.0, which is the minimum set for acceptability. To identify a zone of optimized combinations the lower limit of the criteria for break-and-shred was reset at 5.0. In this way, acceptable loaves of bread with a slightly low break-and-shred score (between 5.3 and 6.0), with the limitations indicated above for proof time and loaf volume, are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 0-200 ppm and 50-70 SKB u, respectively. At this concentration of CWRS, the concentration of ascorbic acid is not critical to obtain optimized combinations of the improvers. Mixing time and panning score proved to be critical for determining the area of optimized combinations of ascorbic acid and α-amylase, since the area was delimited by the upper limit of the criteria for mixing time (3.1) and the lower limit of the criteria for panning (6.0).

At 55% CWRS, acceptable loaves of bread, which meet all the criteria for acceptability (except for proof time and loaf volume, as indicated above), are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 0-135 ppm and 47-69 SKB u, respectively. Again, at this concentration of CWRS, the concentration of ascorbic acid is not critical to obtain optimized combinations, as compared with the high ascorbic acid requirements at 25% CWRS. Mixing time, panning score, and break-and-shred

proved to be critical for determining the area of optimized combinations of ascorbic acid and α-amylase, since the area was delimited by the upper limit of the criteria for mixing time (3.1 min), the lower limit of the criteria for panning (6.0), and the lower limit of the criteria for break-and-shred (6.0).

A closer look at the original data for the CWRS/CPS red blend (Appendix IX) revealed that the lack of fit of an adequate model for proof time could have been due to large variations within a treatment (point in the experimental design). Proof times at baking day 1 were consistently lower than those at baking day 2. This could have raised from variations in the conditions of the proofing cabinet. At baking day 1, temperature in the proofing cabinet was 37°C, while at baking day 2 the temperature was 34°C. Since proof time is inversely related to yeast activity, a lower temperature in the proofing cabinet at baking day 2 could have slowed down yeast activity and therefore increased proof time with respect to baking day 1.

Table 4.29 CWRS/CPS red blend system. Graphical optimization. Zones of optimised solutions at different concentrations of CWRS.

CWRS (%)	*Ascorbic acid (ppm)*	*α-Amylase (SKB u)*	*Achievement of criteria*
10	151-186	0-18	✓
25	155-200	0-38	✓
40	0-200	50-70	Break-and-shred = 5.5
55	0-135	47-69	✓
70	15-142	48-69	✓

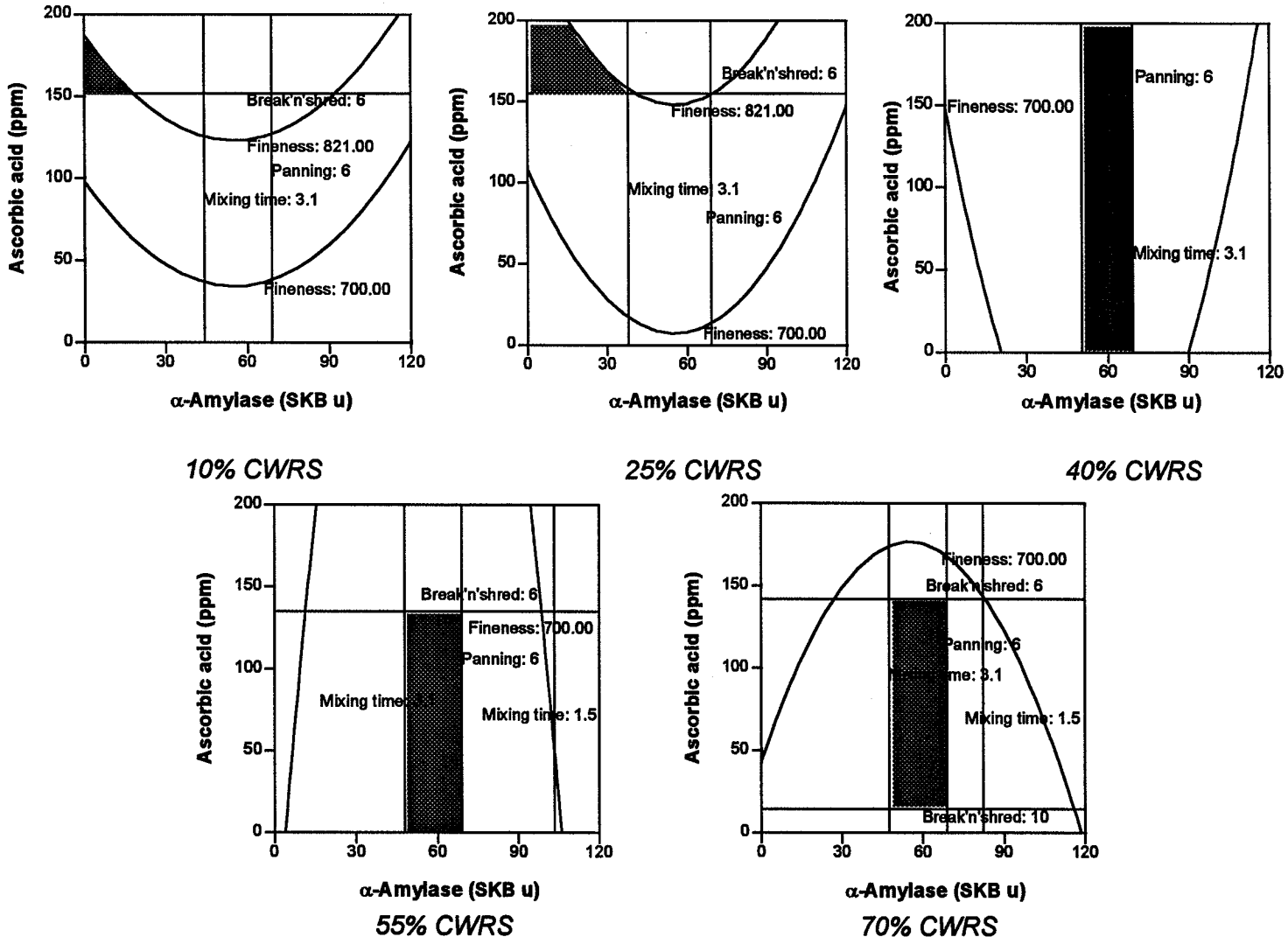


Figure 4.8 CWRS/CPS red blend system. Graphical optimization. Overlay plots at different concentration of CWRS. At striped pattern indicates predictions for break-and-shred scores below the minimum for acceptability. A dark pattern indicates complete fulfilment of criteria for acceptability.

4.3.3.3 Summary.

The concentration of CWRS, in blends with CPS red, has a significant effect on mixing time, fineness, and break-and-shred scores. Ascorbic acid levels affect fineness and break-and-shred scores, while α-amylase levels influence mixing time, panning and fineness scores. None of the factors included in this experiment have significant effects on proof time and loaf volume. These results are summarized in Table 4.30.

Higher concentrations of CWRS result in shorter mixing times when the levels of α-amylase are above 46 SKB u. The effect of increasing amounts of CWRS on fineness and break-and-shred scores depends on the level of ascorbic acid. Fineness and break-and-shred scores increase at higher concentrations of CWRS when ascorbic acid is less than 80 ppm.

The effect of ascorbic acid depends on the level of CWRS in the blend. Fineness and break-and-shred scores increase at higher amounts of ascorbic acid when the level of CWRS is below 43%. However, this effect is reversed at % CWRS greater than 43%.

Higher concentrations of α-amylase result in shorter mixing times when the levels of CWRS are above 30%. Fineness scores are also increased at higher amounts of α-amylase up to 55 SKB u, but this is also reflected in decreased panning scores.

Using graphical optimization, loaves that met all of the criteria can be formulated at 10%, 25%, 55% and 70% CWRS. At 40% slightly low break-and-shred scores are predicted with the rest of the criteria being met.

The 25% blend requires a minimum of 155 ppm ascorbic acid, while α-amylase is required at levels as high as 38 SKB u. In the 40% blend, the requirements for ascorbic acid are not critical, while those for α-amylase are located between 50 and 70 SKB u. The 55% blend requires ascorbic acid as high as 135 ppm, while α-amylase is required at levels between 47 and 69 SKB.

Variability in proofing conditions between baking days may account for the lack of significant effects on proof time and loaf volume.

Table 4.30 CWRS/CPS red blend system. Summary of analysis of contour and surface plots.

Responses	p	R^2	Effect of: CWRS (%)	Effect of: Ascorbic acid (AA) (ppm)	Effect of: α-amylase (SKB u)	Range of actual values: Low	Range of actual values: High
Mixing time (min)	0.0603	0.3159	↑ @ amylase < 46 SKB u ↓ @ amylase > 46 SKB u ✓	ns	↑ @ CWRS < 30% ↓ @ CWRS > 30% ✓	1.0	4.7
Panning	0.0040	0.3326	ns	ns	↓ ✓	0	10
Proof time (min)	-	-	ns	ns	ns	87	145
Loaf volume (cc)	-	-	ns	ns	ns	780	1030
Fineness	0.0865	0.4076	↑ @ AA < 80 ppm ✓ ↓ @ AA > 80 ppm AA ✓	↑ @ CWRS < 50% ✓ ↓ @ CWRS > 50%	◠ @ 55 SKB u	582.9	905.8
Break-and-shred	0.1033	0.2718	↑ @ AA < 146 ppm ✓ ↓ @ AA > 146 ppm	↑ @ CWRS < 43% ✓ ↓ @ CWRS > 43	ns	0	8

Key:
- ↑ Increase in response as the level of the factor is increased.
- ↓ Decrease in response as the level of the factor is increased.
- ◠ Maximum in response.
- ◡ Minimum in response.
- ns Effect of factor is not statistically significant.
- ✓ Significant effect of factor.

4.3.4 CWES/CPS red blend system.

4.3.4.1 Selection of best fitting models and interpretation of contour and surface plots.

Original data for the CWRS/CPS red blend system are shown on Appendix X. Full models fitted to the responses in terms of coded factors with their coefficients and their associated p-values are shown in Table 4.31. Reduced models are shown in Table 4.32.

4.3.4.1.1 Mixing time.

None of factors had a significant effect on mixing time. So, the stepwise regression procedure yielded an empty model.

4.3.4.1.2 Panning

For panning, the reduced model included only the main effect of α-amylase. CWES and ascorbic acid did not have a significant effect. Panning scores decrease at higher concentrations of α-amylase: increasing the concentration of α-amylase from 30 to 90 SKB u results in an decrease in the panning score from 9.4 to 7.5, but the dough still has appropriate manageability.

Table 4.31 CWES/CPS red blend system. Full model coefficients in terms of coded factors.

	Mixing time		Panning		Proof time		Loaf volume		Fineness		Break-and-shred[1]	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint	2.8		8.7		115.4		933.8		726.3		2.1	
b_1: %CWES [A]	0.06	0.7733	0.39	0.2106	-2.27	0.0121	6.16	0.5943	-1.35	0.8985	0.09	0.0763
b_2: Ascorbic acid [B]	0.11	0.6176	-0.23	0.4609	-0.82	0.3116	11.34	0.3333	9.00	0.4026	0.05	0.2691
b_3: α-amylase [C]	-0.24	0.2685	-0.94	0.0078	0.90	0.2669	19.49	0.1097	13.67	0.2115	0.09	0.0763
Quadratic terms												
b_4: A^2	0.18	0.4956	0.00	1.0000	0.25	0.7940	15.00	0.2906	-3.51	0.7839	-0.09	0.1368
b_5: B^2	0.16	0.5264	0.00	1.0000	-1.25	0.2054	3.75	0.7873	-11.83	0.3633	-0.04	0.5220
b_6: C^2	0.15	0.5582	-0.25	0.5007	-1.13	0.2517	-13.75	0.3310	-1.83	0.8865	-0.09	0.1368
Interaction terms												
b_7: AB	0.09	0.7316	0.00	1.0000	-1.06	0.2777	6.88	0.6220	20.69	0.1233	-0.007	0.8949
b_8: AC	0.00	1.0000	-0.50	0.1892	0.31	0.7443	-10.62	0.4491	-8.89	0.4912	-0.08	0.1705
b_9: BC	-0.01	0.9608	-0.25	0.5007	0.31	0.7443	-6.87	0.6220	-5.24	0.6834	-0.007	0.8949
p-value	0.9714		0.1770		0.1489		0.4155		0.6407		0.2091	
R^2	0.1587		0.5460		0.5634		0.4359		0.3499		0.5280	

[1] Break and shred response was transformed into ln(break and shred).

Table 4.32 CWES/CPS red blend system. Reduced model coefficients in terms of coded factors. For stepwise regression $\alpha_{to\ enter} = \alpha_{to\ exit} = 0.1$.

	Mixing time[1]		Panning		Proof time		Loaf volume		Fineness		Break-and-shred[2]	
Parameters	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
Linear terms												
b_0: Expected midpoint			8.4		113.2		958.7		709.2		1.9	
b_1: %CWES [A]					-2.31	0.0070			-1.76	0.8579	0.089	0.0644
b_2: Ascorbic acid [B]									9.40	0.3433		
b_3: α-amylase [C]			-0.96	0.0026			19.64	0.0716			0.089	0.0644
Quadratic terms												
b_4: A^2												
b_5: B^2												
b_6: C^2							-20.00	0.0635				
Interaction terms												
b_7: AB									20.69	0.0925		
b_8: AC												
b_9: BC												
p-value	-		0.0026		0.0070		0.0417		0.2825		0.0421	
R^2	-		0.3583		0.2983		0.2721		0.1777		0.2714	

[1] Stepwise regression yielded an empty model for this response.

[2] Break and shred response was transformed into ln(break and shred).

4.3.4.1.3 Proof time.

For proof time, the reduced model included only the main effect of CWES. Ascorbic acid and α-amylase did not have a significant effect. Proof times decrease at higher concentrations of CWES: increasing the concentration of CWES from 25 to 55% results in a decrease in proof time from 115.6 to 110.9.

4.3.4.1.4 Loaf volume.

For loaf volume, the reduced model included the main and quadratic terms of α-amylase. CWES and ascorbic acid did not have a significant effect. Loaf volumes increase significantly at higher concentrations of α-amylase up to 75 SKB u, where a maximum of 963 cc occurs (Figure 4.9).

4.3.4.1.5 Fineness.

For fineness, the reduced model included the main effects of CWES and ascorbic acid, and the interaction CWES x ascorbic acid. Alpha-amylase did not have a significant effect. Table 4.33 shows the predictions for fineness score at different levels of CWES and ascorbic acid when the level of α-amylase is fixed at 60 SKB u.

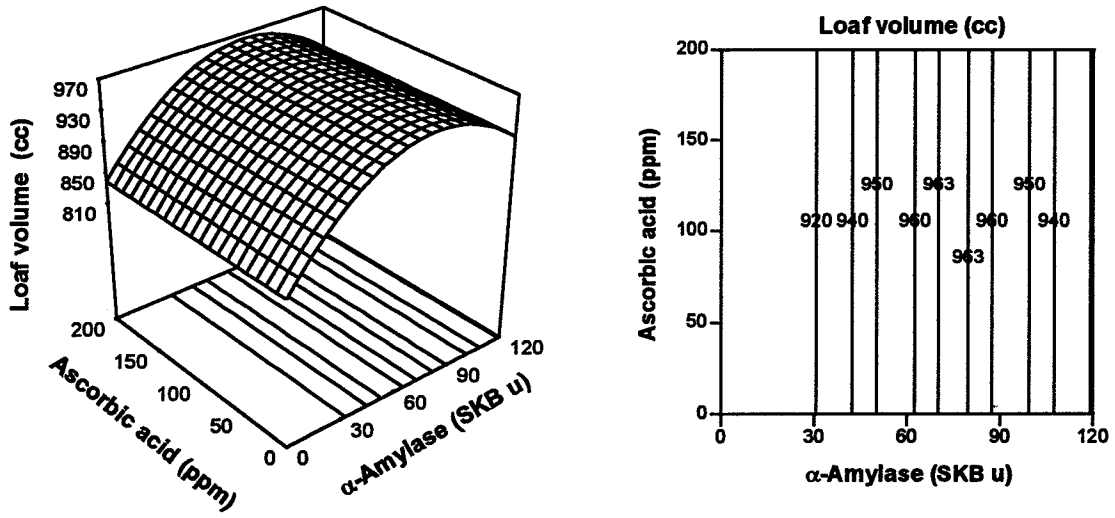


Figure 4.9 CWES/CPS red blend. Surface and contour plots for loaf volume.

Fineness scores increase significantly at higher concentrations of ascorbic acid when the levels of CWES are above 32%. On the other hand, fineness scores decrease significantly at higher concentrations of CWES when the levels of ascorbic acid are below 103 ppm. Smaller increases in the fineness scores are predicted at higher concentrations of CWES when the levels of ascorbic acid are above 103 ppm. Also, smaller decreases are predicted at higher concentrations of ascorbic acid when the levels of CWES are below 33%.

A minimum of 677.3 is predicted to be at 55% CWES and 50 ppm ascorbic acid, while a maximum of 737.5 is at 55% CWES and 150 ppm ascorbic acid.

4.3.4.1.6 Break-and-shred.

Original data were transformed using y' = ln(break and shred) to comply with the ANOVA principles of normality of error terms and equality of variance. The reduced model included the main effects of CWES and α-amylase. Ascorbic acid did not have a significant effect. Table 4.34 shows the predictions for break-and-shred scores at different levels of CWES and α-amylase when the level of ascorbic acid is fixed at 100 ppm.

The individual effects of CWES and α-amylase were small, but the additive effect was significant. Break-and-shred scores increase significantly at higher concentrations of CWES and α-amylase. A minimum of 5.6 is predicted to be at 25% CWES and 30 SKB u α-amylase, while a maximum of 8.1 is at 55% CWES and 90 SKB u α-amylase.

Table 4.33 CWES/CPS red blend. Predictions for fineness score at different levels of CWES and ascorbic acid. Level of α-amylase was fixed at 60 SKB u.[1]

Ascorbic acid	*60 SKB u α-Amylase (0)*		
	25% CWES (-1)	*40% CWES (0)*	*55% CWES (+1)*
50 ppm (-1)	722.2	699.8	677.3
100 ppm (0)	710.9	709.2	707.4
150 ppm (+1)	699.6	718.6	737.5

[1] Fineness scores without units.

Table 4.34 CWES/CPS red blend. Predictions for break-and-shred at different levels of CWRS and α-amylase. Level of ascorbic acid was fixed at 100 ppm.[1]

α-Amylase	*100 ppm ascorbic acid (0)*		
	25% CWRS (-1)	*40% CWRS (0)*	*55% CWRS (+1)*
30 SKB u (-1)	5.6	6.2	6.7
60 SKB u (0)	6.2	6.8	7.4
90 SKB u (+1)	6.7	7.4	8.1

[1] Break-and-shred scores from 0 to 10, where 0 = none, and 10 = high (>2 ½").

4.3.4.2 Graphical optimization.

The optimization procedure, goals, criteria, and considerations were the same as described in the previous blends (see section 4.3.1.2). The model corresponding to mixing time was not included into the optimization procedure since no model could be fitted to these data. Overlay plots at the five levels of CWES used are shown on Figure 4.10. At 10 and 25% CWES, no zone of optimised solutions could be identified. A further analysis of the overlay plots revealed that the criteria for proof time were not met. Predicted values for proof time were slightly higher than the maximum of 115 min set for acceptability (between 115 and 116 min). In order to identify a zone of optimized combinations of ascorbic acid and α-amylase, the maximum in the criteria for proof time was reset at 120 min at these concentrations of CWES. Boundaries of the zones are presented in Table 4.35.

Fineness and break-and-shred proved to be critical for determining the area of optimized combinations of ascorbic acid and α-amylase, since the area was delimited by the lower limit of the criteria for fineness (700) and the lower limit of the criteria for break-and-shred (6.0). At 25% CWES, acceptable loaves of bread, which meet all the criteria for acceptability (except for mixing time, as indicated above), are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 0-120 ppm and 80-120 SKB u, respectively. At 40% CWES, acceptable loaves of bread, which meet all the criteria for acceptability, are predicted to be produced at adequate combinations

of ascorbic acid and α-amylase between 51-200 ppm and 20-120 SKB u, respectively. At 55% CWES, acceptable loaves of bread, which meet all the criteria for acceptability, are predicted to be produced at adequate combinations of ascorbic acid and α-amylase between 90-200 ppm and 10-120 SKB u, respectively.

CWES and ascorbic acid did not have a significant effect on loaf volume and α-amylase had a significant quadratic effect on this response. So, at any concentration of CWES in the blend, loaf volumes range between 880 and 963 cc and it can be maximized at increased concentrations of α-amylase up to 75 SKB u, where it reaches a maximum.

Table 4.35 CWES/CPS red blend system. Graphical optimization. Zones of optimised solutions at different concentrations of CWES.

CWRS (%)	*Ascorbic acid (ppm)*	*α-Amylase (SKB u)*	*Achievement of criteria*
10	0-120	80-120	Proof time = 115-116
25	0-146	50-120	Proof time = 115-116
40	51-200	20-120	✓
55	90-200	10-120	✓
70	95-200	10-120	✓

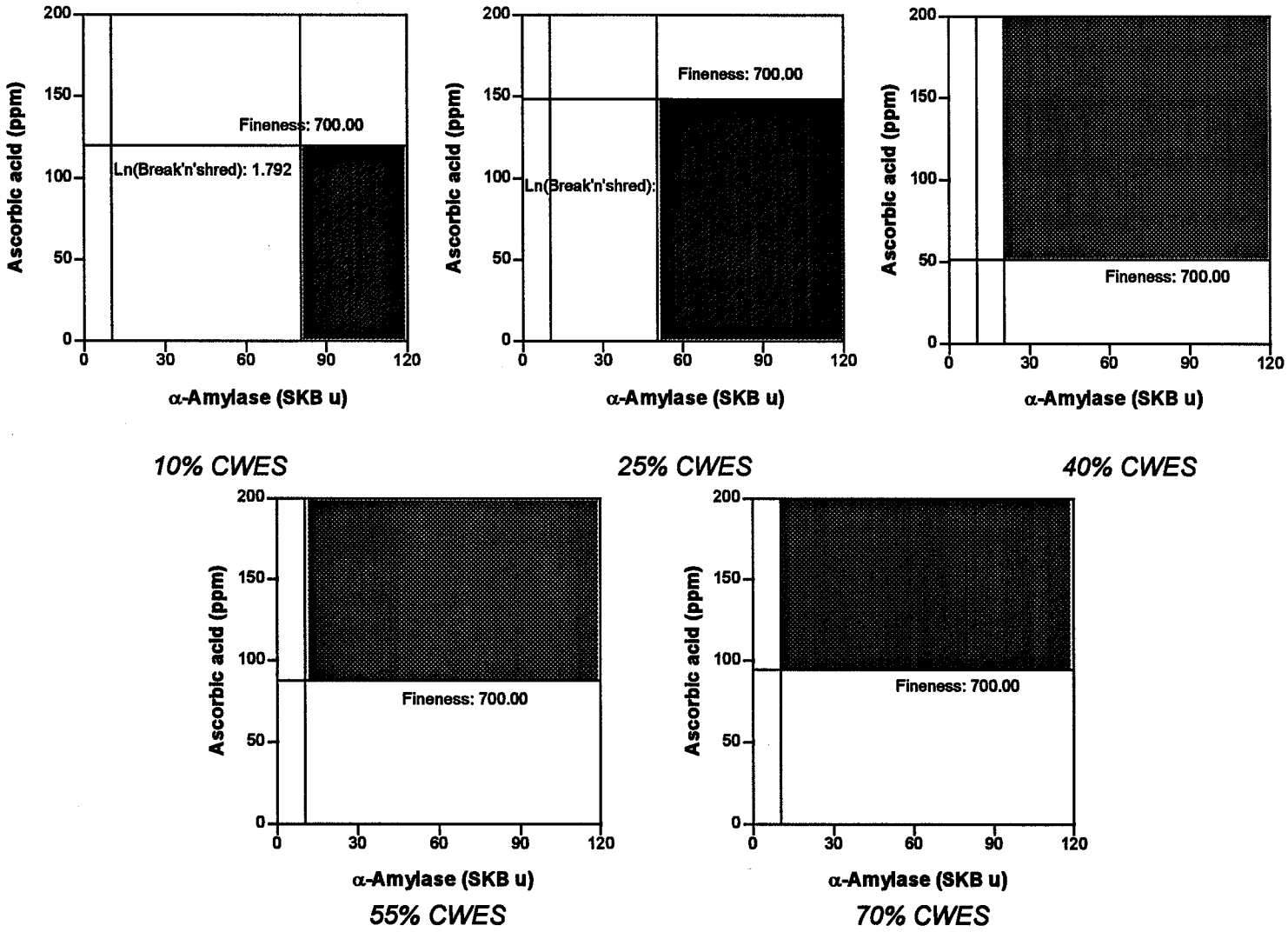


Figure 4.10 CWES/CPS white blend system. Graphical optimization. Overlay plots at different concentration of CWES. A striped pattern indicates predicted proof times above the maximum for acceptability. A dark pattern indicates complete fulfilment of criteria for acceptability.

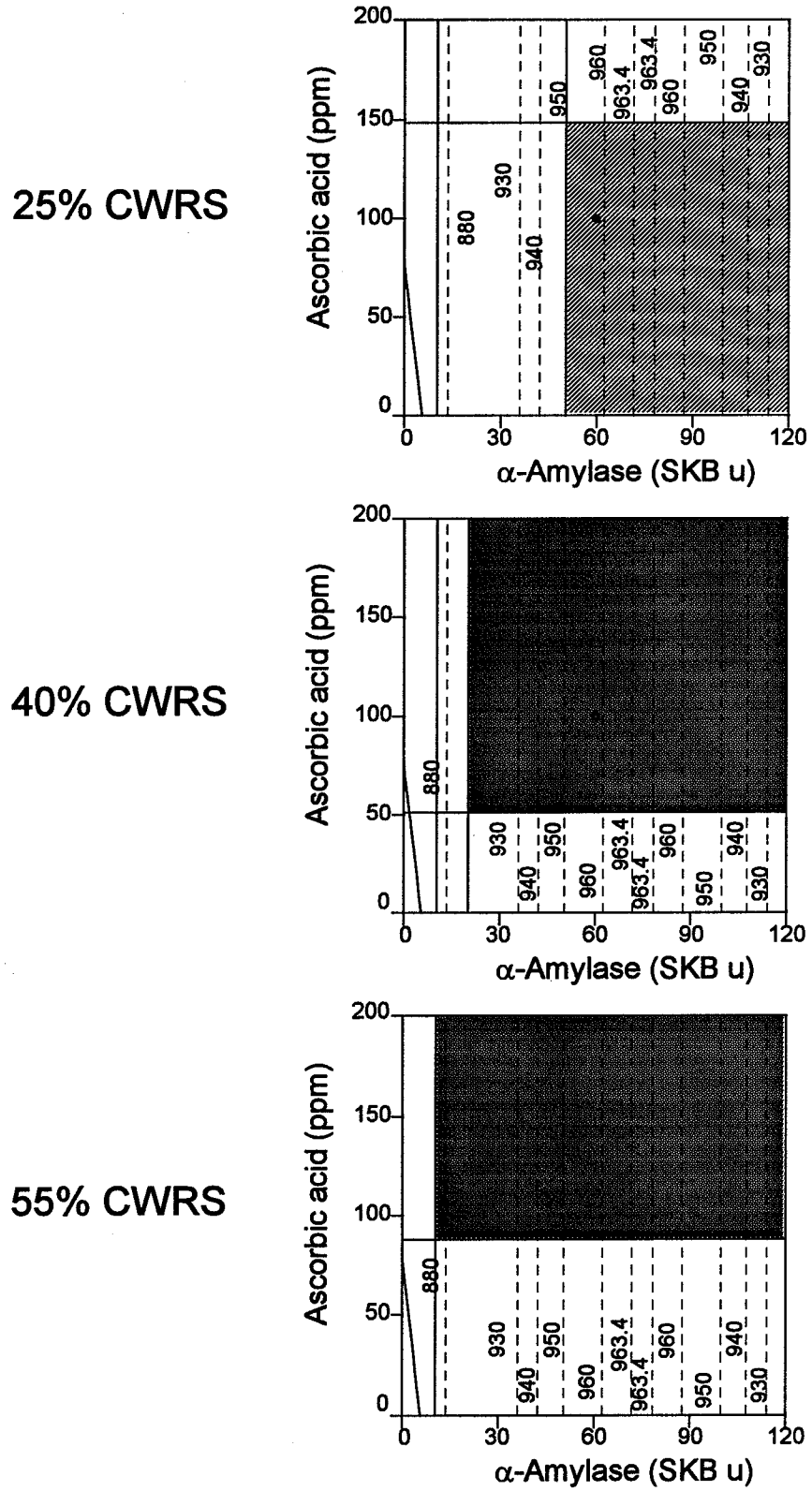


Figure 4.11 CWES/CPS red blend system. Superimposed plots of zones of optimized combinations of ascorbic acid and α-amylase and loaf volume contours at different concentrations of CWES. A striped pattern at 25% CWES indicates predicted proof time above the maximum for acceptability. Dark shading indicates complete fulfilment of criteria for acceptability.

4.3.4.3 Summary.

The amount of CWES, in blends with CPS red wheat, has a significant effect on proof time, fineness and break-and-shred scores. Ascorbic acid levels have significant effects only on fineness scores, while a-amylase levels influence panning, loaf volume, and break-and-shred scores. None of the factors included in this experiment have significant effects on mixing time. These results are summarized in Table 4.36.

Higher concentrations of CWES result in decreased proof times and increased break-and-shred scores. A five-minute reduction in proof time is predicted when the levels of CWES are raised from 25% to 55%. The effect of increasing amounts of CWES on fineness scores depends on the level of ascorbic acid, a result of the significant interaction CWES x ascorbic acid. Fineness scores increase at higher concentrations of CWES when the levels of ascorbic acid are above 103 ppm, but this effect is reversed when ascorbic acid is less than 103 ppm.

The effect of ascorbic acid depends on the levels of CWES. Higher concentrations of ascorbic acid result in increased fineness scores when the levels of CWES are above 33%, an effect that is reversed when CWES is less than 33% of the blend.

Alpha-amylase increases break-and-shred scores, but also decreases panning scores for all blends. Loaf volumes increase at higher concentrations of α-amylase up to 75 SKB u.

Graphical optimization revealed that loaves that met all the criteria except those for proof time, can be formulated for all blends, except at 10% and 25% CWES, where proof times higher than the criteria are predicted. Loaf volumes range between 880 and 963 cc for all blends and can be maximized at 75 SKB u α-amylase.

Table 4.36 CWES/CPS red blend system. Summary of analysis of contour and surface plots.

Responses	p	R^2	Effect of CWES (%)	Effect of AA (ppm)	Effect of α-amylase (SKB u)	Range of actual values Low	Range of actual values High
Mixing time (min)	-	-	ns	ns	ns	1.3	4.2
Panning	0.0026	0.3583	ns	ns	↓ ✓	6	10
Proof time (min)	0.0070	0.2983	↓ ✓	ns	ns	106	122
Loaf volume (cc)	0.0417	0.2721	ns	ns	⌒ @ 75 SKB ✓	780	1020
Fineness	0.2825	0.1777	↓ @ AA < 103 ppm ✓ ↑ @ AA > 103 ppm ✓	↓ @ CWES < 33% ✓ ↑ @ CWES > 33% ✓	ns	614.4	835.7
Break-and-shred	0.0421	0.2714	↑ ✓	ns	↑ ✓	4	8

Key:
- ↑ Increase in response as the level of the factor is increased.
- ↓ Decrease in response as the level of the factor is increased.
- ⌒ Maximum in response.
- ⌓ Minimum in response.
- ns Effect of factor is not statistically significant.
- ✓ Significant effect of the factor.

4.4 Discussion.

Statistical models were selected according to their p-value and R^2 statistic. However, removal of non-significant terms from the full model by a stepwise regression procedure not only resulted in an improvement in the p-value of the model, but also in a reduction of the R^2 statistic. This may have been due to the variability in the data not accounted for in the experimental design. Randomization and blocking were performed to reduce this variability, but factors such as temperature and relative humidity of the proofing cabinet, oven, and the baking room itself could have contributed to the error term. The reduced models were considered to have fairly good predictability and they can be verified by further experiments.

Complex interactions between the two improvers and between the improvers and the flours were identified. These interactions were more significant in the CWRS/CPS white blend system than in the CWES/CPS white blend system, as can be seen in Tables 4.16 and 4.23, respectively. Mixing times decrease as levels of both CWRS and ascorbic acid increase up to 28% and 112 ppm, respectively. This is an unexpected result since higher amounts of CWRS and ascorbic acid result in strengthening of the protein of the blend, and this should be reflected in increased mixing times. It seems that the blends with low content of CWRS need a certain degree of oxidation before the effects of increasing levels of CWRS and ascorbic acid can be observed. Flours were matured naturally for a period of 6 weeks prior to baking. It is widely recognized

that flour performance improves with age, and is related to oxidation of the protein fraction in flour, which results in reduced dough extensibility, and increased dough resistance to extension (Mailhot and Patton, 1988). The latter could be reflected in longer mixing times. However, natural aging occurs in an irregular fashion (Pyler, 1973).

Another important interaction between the improvers and CWRS was observed in the loaf volume response. Higher loaf volumes are achieved at higher levels of CWRS, ascorbic acid, and α-amylase, but the effect is less significant at high levels. Dough could be overly strengthened at high levels of CWRS and ascorbic acid in such a way that extensibility is lessened. Increasing concentrations of ascorbic acid have been reported to result in non-significant changes in loaf volumes after the optimum is achieved (Yamada and Preston, 1992). Furthermore, the production of dextrins by the hydrolytic action of α-amylase can alter the rheological properties of the dough, making it stickier, and resulting in lower loaf volumes (Drapron and Godon, 1987).

CWES played an important role on the quality characteristics of bread when blended with CPS white wheat flour. Proof time was reduced and panning, break-and-shred, and fineness scores were improved upon addition of CWES. Most of the studies published have focused on the loaf volume potential and mixing requirement of CWES wheat rather than on its ability to improve internal and external loaf characteristics (Bushuk, 1980). Perron (1995), working on bromate-free improver formulations with CWRS and CWES wheat flours, alone or in blends, reported that CWES was a major determinant on the internal and

external characteristics of breads made using the Canadian Short Process. This study supports her observations that CWES contributes significantly to the improvement of crumb and crust characteristics and that this attribute of CWES should be further investigated.

Loaf volume was not affected by the concentration of CWES in the blend, but it was influenced by the concentrations of ascorbic acid and α-amylase. Loaf volume could be maximized at high levels of ascorbic acid and medium to high levels of α-amylase: at 40% CWES (middle level), 150 ppm ascorbic acid and 70 SKB u α-amylase the model predicts a loaf volume of 920.6 cc. This level of ascorbic acid is relatively high, although it is known that a characteristic feature of ascorbic acid is that its addition level is not always critical and there is not a risk of over-treating bread doughs, as can happen with other oxidants (Fitchett and Frazier, 1986). Yamada and Preston (1992) supported this observation in a study on the effects of different oxidants on oven rise and bread properties of bread made with the Canadian Short Process. They observed that a wide range of concentration of ascorbic acid could be used in doughs without affecting bread quality. In their study, loaf volume reached an optimum value at 100 ppm and a further increase in the concentration of ascorbic acid did not result in a significant change in loaf volume. In this study, α-amylase promotes further increase in loaf volume.

Levels of ascorbic acid and α-amylase that produced high-quality loaves were identified by using optimization techniques. The most remarkable

difference between the CWRS/CPS white and CWES/CPS white blend systems was in the ascorbic acid requirements.

In the CWRS/CPS white blend, the concentration of ascorbic acid is critical and depended on the concentration of CWRS. At low CWRS (25%), requirements for ascorbic acid are above 119 ppm, while at higher levels of CWRS in the blend the requirements for ascorbic acid diminish. Finney *et al* (1987) reported that a bread flour with mellow physical dough properties required nearly three times as much oxidation as a bread flour with medium-strong physical dough properties. Thus, it would be expected that as the concentration of a strong wheat flour, such as CWRS, in blend with a weaker flour increases, the oxidative requirements of the blend would decrease.

At comparable concentrations of CWES in the blend, the requirements for ascorbic acid were between 0 and 200 ppm, covering all the range of concentrations of ascorbic acid used throughout the experiment. In other words, the concentration of ascorbic acid was not critical in the CWES/CPS white blend to get optimized combinations of the factors, although loaf volumes increased with higher concentrations of ascorbic acid. Furthermore, at concentrations of CWES below 55%, predictions for panning score, break-and-shred and fineness were low. Even though they improve at higher concentrations of CWES, these scores remain below the minimum set in the criteria for acceptability. The low predicted scores for panning are an unexpected result, since the addition of CWES would be expected to yield higher panning scores than CWRS at similar concentrations in the blend, due to its stronger gluten. In a study comparing the

carrying ability of Glenlea (a extra strong wheat variety) and CWRS, Bushuk (1980) reported that less Glenlea flour is required to attain the same baking performance. The results do not support that observation.

High-quality loaves of bread can be produced with low amounts of CWRS blended with CPS white, by using high ascorbic acid and low to medium α-amylase levels. This result should be of interest to millers. Wheat blending is performed in the mill to provide desirable quality attributes that are lacking in the individual wheats, and to reduce cost (Sarkar, 1993). Cost of the wheat is the single biggest factor in cost of flour for marketing. An optimized combination like the one mentioned above would allow quality requirements to be met at reduced cost.

In the blend systems where CPS red flour was used, the effects of the factors were less significant than on those models where CPS white flour was used. This could be a result of variability in the original data due to uncontrolable factors related to the baking equipment. The temperature in the proofing cabinet was not constant between baking days, affecting proof times considerably, and could be the reason for the lack of adequate models to explain differences in proof times and loaf volumes in the CWRS/CPS red blend and in mixing times in the CWES/CPS red blend.

Nevertheless, for the CWRS/CPS red blend, trends in the requirements for ascorbic acid were similar to those for the CWRS/CPS white blends. High levels of ascorbic acid are required at low levels of CWRS and diminish at higher amounts of CWRS. Therefore, it seems that the characteristics of CWRS

predominate over those of the prairie spring flours. It is also worth noticing that varieties with similar rheological properties behave additively when blended, whereas varieties with very different rheological properties have synergistic effects on the quality characteristics of the blend (Bolling, 1980).

The liquid ferment proved to be an appropriate baking test to evaluate the effects of improvers and blends of flours on the quality characteristics of pan-bread. The blend model used in this experiment could be applied to the Mexican flours used in commercial baking. It must be kept in mind, however, that the results of this study were obtained under laboratory conditions, which may be very different to those of a commercial operation. In commercial processing, dough undergoes harsh handling operations that greatly influence its stability. Other factors such as intermediate proofing stability, mixing efficiency, flour characteristics, processing conditions and interactions with other ingredients must also be taken into account (Yamada and Preston, 1992).

In addition, application of the blend model used in this study needs to take into consideration not only the genetic background of Mexican wheats, but also adjustments in the breadmaking methods used in modern Mexican bakeries. In order to increase resistance to several important wheat diseases, national breeding programs in Mexico have used as progenitors wheat varieties with the 1B/1R translocation, which had also been observed to result in higher yields (Rajaram *et al* 1992). In 1B/1R translocated wheats, the short arm of chromosome 1B of wheat has been replaced by the short branch of chromosome 1R or rye. However, flours from 1B/1R translocated wheats have been reported

to produce dough with increased stickiness, reduced strength, and intolerance to overmixing (Chen and Hoseney, 1995). These characteristics have been observed in Mexican bread wheat flours (Paredes-Lopez, 1985). However, in a study with advanced lines from CIMMYT's International Bread Wheat Screening Nursery, Peña *et al* (1990) reported that the presence of the 1R rye chromosome could not be associated with variations in dough viscoelasticity, mixing and baking properties. Although weak to medium-strong gluten types predominated in the wheats evaluated, the authors explained that this was due to the grain yield-protein content relationship, and they also attributed the dough stickiness problem to the high speed mixing conditions encountered in modern breadmaking methods.

The use of a statistically designed experiment enabled predictive models for the enhancement of quality characteristics of white pan-bread made using blends of hard red spring and prairie spring wheat flours to be identified. Identification of optimized settings of the factors was possible through the use of contour and surface plots for each blend. The predictive power of these models can be verified by further experimentation. In order to apply this methodology to a full-scale production process, factors such as size of production, process equipment, raw materials, environmental changes and operating personnel should be taken into consideration. A method known as Evolutionary Operation has been proposed to perform continuous monitoring and improvement of a full-scale process (Myers and Montgomery, 1995). It consists in the introduction of small changes in the levels of the process variables under consideration using a

relatively simple design (usually a 2^k design). Actual process settings are located in the center of the design. Changes in the variables are relatively small so an unacceptable product is not obtained, but at the same time changes are big enough to detect potential improvements in process performance. Eventually, the effect of one of more process variables may appear to have a significant effect on the response. At this point, the actual process settings can be modified in the direction of the improvement identified. Main objections to the implementation of these techniques have been its relative complexity and the lack of trained personnel, but an adequate training program on basic principles of Response Surface Methodology and its potential for improvement of product design and process can make a difference for processors who want to go ahead their competition.

4.5 Conclusions.

In this study, the effects of improvers on the quality of pan bread made with blends of flours of different classes were assessed through the use of statistically designed experiments. The use of a central composite design enabled important interactions between the factors to be identified. Complex interactions existed between the improvers, but also between the improvers and the flours. Differences in improver requirements of the two hard red spring wheat flours were evident.

Effects of the two hard red spring flours used, CWRS and CWES, can be attributed to the quality of their protein fractions since both flours had protein contents of 11.5% (on a 14% mb). CWRS performed better than CWES when blended with CPS white flour. Ascorbic acid and α-amylase combinations, that satisfied all the optimization quality criteria, could be identified at all concentrations of CWRS. In the blends containing CWES, optimized combinations were only identified at high CWES levels.

Ascorbic acid requirements for the two hard red spring flours were different. Identification of the optimized levels of ascorbic acid was more critical in the blends containing CWRS. Low concentrations of CWRS require levels of ascorbic acid higher than 120 ppm to formulate high quality bread, while at higher concentrations of CWRS, ascorbic acid requirements diminish. In the blends containing CWES, ascorbic acid can be used over the whole range of concentrations used in this study.

The effect of ascorbic acid on loaf volume differed between the two hard red spring flours. At low and middle levels of CWRS, loaf volumes increase at higher levels of ascorbic acid. This effect changes at high levels of CWRS, where loaf volumes increase at lower levels of ascorbic acid. At any given concentration of CWES, higher loaf volumes are obtained at higher levels of the oxidant.

The effect of α-amylase was different for the two hard red spring flours, and this was especially evident for loaf volume. In the CWRS blends, the concentrations at which loaf volume can be maximized move to lower levels at increasing concentrations of CWRS. In the CWES blends, the effect of α-amylase is the same at any concentration of CWES and loaf volumes can be maximized at 75 SKB u.

Variations on the experimental conditions of the baking equipment had a great influence on the quality characteristics of breads made with CPS red flour. Optimized combinations of the factors for the CWRS and CWES blends were identified, although these combinations should be taken with reservation due to the low significance of the models fitted to the responses.

The use of Response Surface Methodology enabled formulation of predictive models for the enhancement of quality characteristics of white pan-bread made using blends of hard red spring and prairie spring wheat flours. Identification of optimized settings of the factors was possible through the use of contour and surface plots for each blend. The predictive power of these models can be verified by further experimentation.

5. GENERAL CONCLUSIONS.

The primary objectives of this study were to develop bromate-free improver formulations for use with a Mexican bread flour and with blends of hard red spring and prairie spring flours. Response Surface Methodology was an effective technique for the examination of the effects of the improvers and the interactions between them.

In the first optimization experiment, two baking tests were compared for sensitivity to improver formulations containing DATEM, ascorbic acid and α-amylase. The liquid ferment baking test was more sensitive to the effects of the improvers than the Canadian Short Process. Requirements for DATEM and ascorbic acid were higher, while identification of requirements for α-amylase were more critical. However, doughs after mixing were very sticky and difficult to handle, an effect maybe due to a loss of water absorption capacity of flour during fermentation. A further experiment indicated that appropriate handling properties in the liquid ferment could be attained at a reduced water absorption of FAB - 3%.

The second optimization experiment was carried out with ascorbic acid and α-amylase as improvers, with the DATEM level set at 0.375% and using the modified liquid ferment method as the baking test. CWRS performed better than CWES in blends with CPS flours. The study led to some interesting conclusions:

- CWRS performed better than CWES. Optimized combinations of ascorbic acid and α-amylase that meet all the quality criteria could be

identified at any level of CWRS, while these combinations were only identified at high concentrations of CWES.

- Identification of ascorbic acid requirements in the CWRS-based blends were more critical than in the blends containing CWES, where the improver can be used all over the range of concentrations used in the study.
- Ascorbic acid interacted strongly with CWRS but not with CWES. This was more evident in loaf volume, where at low levels of CWRS higher loaf volumes are attained at increasing concentrations of ascorbic acid. At higher levels of CWRS this effect is reversed.
- Alpha-amylase also had a strong interaction with CWRS. Concentrations at which loaf volume can be maximized move to lower levels at higher amounts of CWRS. The effect of α-amylase on loaf volume on CWES is the same at any concentration of CWES.
- The superior carrying ability of CWES was not seen in this experiment. Predictions for panning, fineness and break-and-shred scores are below the criteria for acceptability at low concentrations of CWES, but increasing amounts of CWES improve these characteristics.
- High quality loaves of bread are predicted to be produced at low levels of CWRS, by using high concentrations of ascorbic acid and low concentrations of α-amylase.

The liquid ferment proved to be an adequate baking test to study the effect of bread improvers at concentrations normally used in Mexico and to identify optimized blends of flours of different strength. The blend model used in this experiment could be applied to the Mexican flours used in commercial baking, which like the CPS wheat flours used in this study, have lower protein contents and different protein quality when compared to hard red spring wheats. CPS flours provided an effective model for the Mexican flours, however, the two types of flours have very different genetic backgrounds. In addition, the quality of Mexican wheats is strongly affected by growing location and by genetic x environment interactions.

6. RECOMMENDATIONS FOR FUTURE RESEARCH.

This research led to some interesting questions that could be addressed in future studies. Problems of stickiness arose in the dough when using the liquid ferment. This was solved by a reduction of water added to dough, although this practice in commercial baking is undesirable since it results in loss of bread yield. Future studies on the liquid ferment should study the use of other improvers to solve the stickiness problem. Some lipases and oxidases, alone or in combination, have been reported to improve dough rheological properties by increasing gluten strength (Si, 1997). The use of these new enzyme preparations and a more objective measurement of stickiness using new methodologies (Wang *et al*, 1996) should be carried out.

CWRS yielded high-quality loaves of bread at low concentrations in blend with CPS white, using adequate combinations of ascorbic acid and α-amylase. This result should be further explored since its confirmation could represent in reduced wheat blend costs for millers. Wheat cost constitutes the single highest cost in a flour ready for marketing (Sarkar, 1993). An optimized combination like the one mentioned above would allow to meet quality requirements at reduced costs.

The liquid ferment developed proved to be adequate to study the effects of improvers on the quality of the type of pan-bread consumed in Mexico. Future studies should contemplate the optimization of improvers using the liquid ferment for use with Mexican wheat flours of diverse quality characteristics.

Furthermore, the liquid ferment should be verified as a reliable and consistent baking test to be used for screening of Mexican bread wheats. In order to apply the methodology used in this research to the conditions used in Mexican commercial bakeries, some adjustments should be considered. The baking results were obtained in the laboratory and testing for robustness should likely be included in the optimization methodology for reducing variation in products and processes. It should be possible to apply the results of the liquid ferment baking test to situations encountered in the commercial baking industry.

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APPENDIX I. PREPARATION OF ADDITIVE SOLUTIONS.

YEAST:

132 g fresh yeast (not more than 1 week old) was placed in a blender container, 500 ml of distilled water were added, and the blender was turned on at medium speed for 2 minutes. The remaining 440 ml of water (for a total of 990 ml water) was used to rinse out the blender container and added to the sealer jar. The jar was placed in a 30°C water bath for the whole duration of the baking day at constant agitation. Twenty-five ml of the solution makes for 3 g fresh yeast, and 22.5 ml of dough water on a 100g flour basis, which must be accounted for in further calculations.

SALT/SUCROSE:

Liquid ferment baking test.

A solution of 10% salt and 42.5% sucrose (w/v) was prepared. Fifty g of salt were slowly dissolved in 300 ml of distilled water and 212.5 g of sucrose slowly added and dissolved in the solution. This was transferred to a 500-ml flask, topped to the mark with distilled water, and transferred to a sealer jar. Solution was prepared one day before the baking day and held overnight in the warming cabinet. Next day the solution was shaken to ensure sugar and salt were dissolved and it was placed in a 30°C water bath for the whole duration of the baking day. Five ml of solution were added in the ferment stage and 15 ml in

the dough stage. Five ml of solution make for 0.5 g salt and 2.1g sucrose on a 100 g flour basis. Consider 3.53 ml dough water /5 ml solution.

Canadian Short Process baking test.

105.6 g of salt and 176 g of sucrose were dissolved in 941 ml of distilled water as described above. The solution was prepared one day before the baking day and held overnight in the warming cabinet. Next day the solution was shaken to ensure sugar and salt were dissolved and it was placed in a 30°C water bath for the whole duration of the baking day. Twenty-five ml of solution make for 2.4 g salt and 4.0 g sucrose on a 100 g flour basis. Consider 21.4 ml of dough water.

AMMONIUM PHOSPHATE:

Ten g ammonium phosphate were dissolved in a 100 ml flask and distilled water was added up to the mark. Solution was stored at room temperature for the duration of the baking day. One ml of solution makes for 1g ammonium phosphate on a 100 g flour basis. Consider 1 ml of dough water.

L-ASCORBIC ACID:

A 1% w/v solution of L-ascorbic acid was prepared on the baking day using 0.5 g of ascorbic acid dissolved in 30 ml of distilled water. This was stirred until complete dissolution, transferred to a 50-ml flask, and topped to the mark with distilled water. The solution was kept in a flask protected from light with

brown paper. One ml is equivalent to 100 ppm on a 100 g flour basis. Consider 1 ml of dough water.

APPENDIX II .DETERMINATION OF pH AND TITRATABLE ACIDITY (TTA) IN FERMENT.

Before carrying out the baking experiment using the liquid ferment baking test, a small experiment was performed trying to obtain a profile of reduction of pH and increase in TTA in the ferment with respect to time.

Objective:

- To obtain a profile of reduction of pH and increase in titratable acidity in the dough ferment using the liquid ferment baking test in order to determine the adequate ferment time.
- To compare the buffering effect of a brew buffer and the buffering provided by the flour in the ferment.

Methods:

The ferment was prepared as indicated in the Methods section of *Optimization Experiment #1* using the Mexican bread wheat flour and the dough additive solutions as indicated in Appendix I. Buffered and unbuffered ferments were prepared. Initial ferment components were mixed in a GRL mixer 200 at 140 rpm for 2 min. at 30°C. The resulting ferment was placed in a bowl, covered, and allowed to rest in a proof cabinet (37°C, 83% rh) for 3 hours. At time=0 and at 30-minute intervals, 8-g samples (approximately) were taken from the ferment and pH and TTA were determined in them. pH was determined according to

AACC method 02-52. TTA acidity was determined according to AACC method 02-31 and it was reported as grams of lactic acid / 100 g sample. Experiment was replicated in two different days.

Results:

Results are summarized in table A-1 and shown in Figure A-1. pH was reduced rapidly in the first hour of fermentation. This rate of reduction of pH was then lowered and reached a plateau for the rest of the time of fermentation. As can be observed in Figure A-1 there was not any difference on the pH profile of the ferment when the buffer was added. The two pH curves reached a plateau at a pH value between 4.7 and 5. These range of pH value in the ferment is in accordance with the results reported by Maselli (1955) and Bayfield and coworkers (1963) who reported that maximum rate of gas production was obtained when the pH of the ferment was between 4.0 and 5.4. The reason why the addition of buffer did not seem to make any difference in pH may be explained in terms of the flour itself. Flour in the ferment has been reported to act as a natural buffer in this type of systems (Kulp, 1983). The amount of flour in the ferment was 56% of the total flour in the liquid ferment formulation.

In liquid ferments, monitoring of TTA is also considered as a criteria for determining optimum development of the ferment or brew. TTA values increased rapidly during the first hour of fermentation and reached a plateau at TTA values around 0.6 g lactic acid/ 100 g ferment 1 hour of fermentation. During the first of fermentation, unbuffered ferment had a lower increase in TTA with

respect to the buffered ferment, but after 1 hour both curved tended to be very similar. It is very well known that acidity of fermenting dough is deeply affected by the presence of ammonium salts in yeast foods. The buffer used has among its different ingredients ammonium chloride. Yeast assimilates ammonia from this salt and liberates the corresponding acid in its ionized form. HCl is a strong acid which almost completely ionizes in water solution and has a strong influence on dough acidity (Pyler, 1973). This may explain the higher rate of increase in TTA in the buffered ferment. However, it seems that at later stages of the fermentation he buffering effect of the flour in the formulation overcomes this effect. Also, according to the same graph, the addition of buffer to the ferment also does not seem necessary.

Conclusion:

After 1 hour, ferment does have small changes in pH and TTA rates. However, authors recommended allowing some more time for complete development of ferment despite small changes in pH and TTA. A time of 2 hours of fermentation was considered to be adequate. Although not necessary since the flour in the formulation seemed to act as a natural buffer, it was decided to add buffer as yeast food in the formulation.

Table A-1. Liquid ferment baking test. pH and titratable acidity (TTA) in buffered and not-buffered ferment.

Time (hr)	Buffered ferment		Not-buffered ferment	
	pH	TTA (g lactic acid/100 g ferment)	pH	TTA (g lactic acid/100 g ferment)
0.0	5.64	0.4752	5.65	0.4250
0.5	5.16	0.5564	5.13	0.4971
1.0	4.90	0.5965	4.90	0.5895
1.5	4.66	0.6203	4.76	0.5863
2.0	4.72	0.6017	4.78	0.5646
2.5	4.80	0.6742	4.64	0.5808
3.0	4.75	0.5935	4.99	0.5731

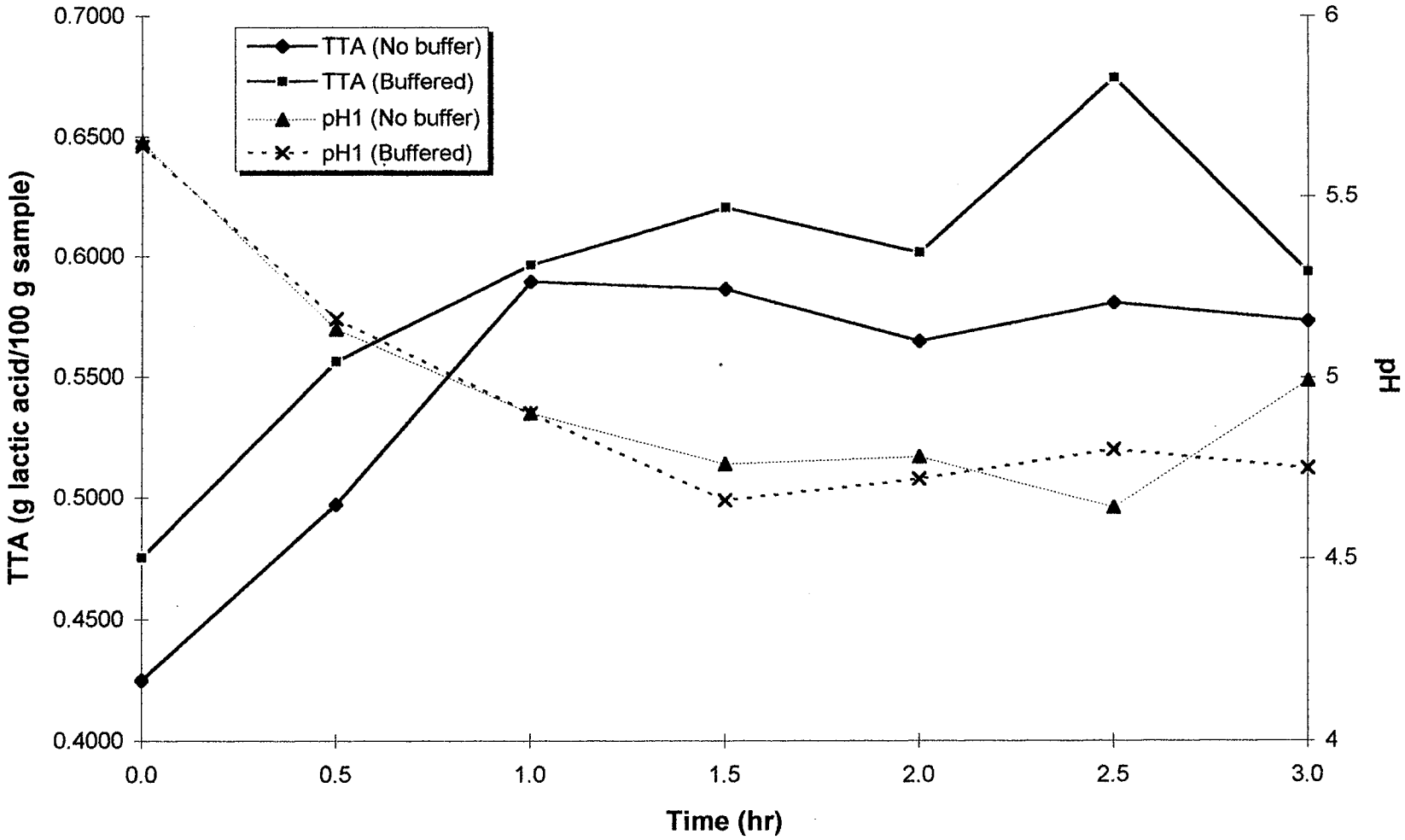

Figure A-1. Liquid ferment baking test. pH and TTA in buffered and unbuffered ferment

APPENDIX III. BREAD EVALUATION SCORE CARD.

A. DOUGH QUALITY

		Score
• Dough out of mixer	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

B. LOAF EXTERNAL CHARACTERISTICS

• Loaf symmetry	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
• Loaf bottom	a) Flat bottom, no indent	10
	b) Slightly concave	8
	c) Moderately concave	5
	d) Very concave	2
	e) Extremely concave	0
• Break and shred	a) High (>2½")	10
	b) Very good (1½-2½")	8
	c) Moderate (1-1½")	6
	d) Low (½-1")	4
	e) Insufficient (<½")	2
	f) None	0

C. LOAF INTERNAL CHARACTERISTICS

• Cell uniformity	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
• Cell size	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
• Blisters (Air bubbles)	a) None	10
	b) Moderate hole	5
	c) Large hole	0

APPENDIX IV OPTIMIZATION EXPERIMENT #1. LIQUID FERMENT BAKING TEST. ORIGINAL DATA.

			Responses													
			Time (min)		Loaf (g, cm, cc)			CrumbScan		External Characteristics			Internal Characteristics			
A	B	C	Mixing	Proof	Weight	Height	Volume	Fineness	Elongation	Symmetry	Bottom	Break-and-shred	Uniformity	Cell size	Blister	Firmness (N)
-1	-1	-1	3.1	76	121.5	11.3	885	832.1	1.3327	10	8	4	6	6	5	1.1319
-1	-1	-1	2.9	74	121.2	11.2	880	736.7	1.3285	10	10	4	8	6	10	1.4077
-1	-1	1	3.4	82	120.6	11.1	905	757.2	1.3184	10	10	4	6	8	10	1.3104
-1	-1	1	2.9	76	120.4	11.1	935	813.6	1.3417	10	10	4	8	8	10	1.1025
-1	1	-1	3.5	82	120.3	11.1	880	794.7	1.3156	10	10	4	8	8	10	1.3676
-1	1	-1	3.8	74	122.5	11.4	925	803.3	1.2300	10	10	4	6	4	10	1.2303
-1	1	1	3.3	79	121.9	11.6	935	718.8	1.2853	10	10	6	4	6	10	1.3627
-1	1	1	3.2	75	120.8	11.6	965	808.3	1.2955	10	8	6	6	6	10	1.1998
1	-1	-1	3.4	76	121.1	11.6	925	797.0	1.2924	10	8	6	8	8	5	1.1616
1	-1	-1	4.1	74	119.7	11.6	935	800.5	1.3225	10	10	6	4	4	10	1.2570
1	-1	1	3.4	75	120.4	11.8	970	824.1	1.2990	10	10	6	6	8	0	1.0224
1	-1	1	3.3	73	121.2	11.6	960	810.1	1.3575	10	10	6	6	6	10	1.2761
1	1	-1	3.8	77	121.1	11.5	930	795.8	1.3450	10	10	6	6	6	10	1.0052
1	1	-1	2.9	73	120.7	11.8	955	779.7	1.2654	10	10	6	8	8	0	1.3199
1	1	1	3.5	80	118.2	12.0	975	778.9	1.2674	10	10	6	8	8	10	1.4229
1	1	1	3.0	77	119.8	11.9	985	821.7	1.2958	10	10	8	4	4	5	1.0072
0	0	0	3.3	76	121.8	11.6	960	747.3	1.3363	10	10	6	6	6	10	1.3698
0	0	0	3.7	78	121.1	11.1	915	817.8	1.3266	10	10	4	8	8	8	1.2646
0	0	0	2.9	77	122.7	11.8	955	765.3	1.3320	10	8	8	6	8	5	1.1712
0	0	0	2.8	74	120.0	11.6	980	773.1	1.2819	10	8	6	6	6	10	1.1349
-2	0	0	2.9	76	121.4	11.5	950	751.1	1.2108	8	8	6	6	6	10	1.1674
0	-2	0	3.8	76	120.1	11.2	895	724.6	1.2837	10	10	4	8	6	10	1.6575
0	0	-2	4.0	76	120.7	11.1	885	769.2	1.2786	10	8	4	2	2	5	1.1120
2	0	0	3.6	74	122.2	11.6	955	777.5	1.3382	10	10	4	8	8	10	0.9488
0	2	0	2.5	74	120.7	11.3	925	764.9	1.3011	10	10	4	6	6	10	1.2113
0	0	2	3.0	76	120.7	11.5	940	701.2	1.2661	8	10	8	2	2	10	1.6480

A: DATEM (%); B: ascorbic acid (ppm); C: α-amylase (SKB u)

APPENDIX V OPTIMIZATION EXPERIMENT #1. CANADIAN SHORT PROCESS. ORIGINAL DATA.

			Responses													
			Time (min)		Loaf (g, cm, cc)			CrumbScan		External Characteristics			Internal Characteristics			
A	B	C	Mixing	Proof	Weight	Height	Volume	Fineness	Elongation	Symmetry	Bottom	Break-and-shred	Uniformity	Cell size	Blister	Firmness (N)
-1	-1	-1	6.9	99	122.1	11.2	880	992.8	1.6662	10	10	4	10	8	10	1.0777
-1	-1	-1	6.2	86	124.8	11.5	875	911.9	1.4645	10	10	4	6	6	10	1.3962
-1	-1	1	6.2	92	124.3	11.2	875	917.9	1.5097	6	10	8	10	8	10	1.1178
-1	-1	1	6.6	89	123.9	11.5	945	892.1	1.4667	4	10	6	6	6	0	1.4592
-1	1	-1	7.0	95	123.7	11.8	900	889.6	1.4758	10	10	4	6	6	5	1.3219
-1	1	-1	6.3	84	122.1	11.5	895	935.9	1.4703	10	10	4	8	8	10	1.6041
-1	1	1	6.2	91	123.4	11.6	890	949.8	1.5179	8	8	8	6	8	10	1.2360
-1	1	1	5.9	85	123.2	11.2	910	958.5	1.5029	10	8	8	8	6	10	1.2616
1	-1	-1	5.8	88	125.3	11.9	955	929.2	1.5313	10	10	6	8	8	10	1.0415
1	-1	-1	6.3	82	121.1	12.1	935	862.2	1.3411	8	10	8	8	8	10	1.4496
1	-1	1	6.4	87	124.2	11.6	875	910.9	1.4733	4	10	6	8	6	10	1.0529
1	-1	1	6.1	87	121.1	11.6	930	843.7	1.4222	10	10	8	10	8	10	1.3790
1	1	-1	6.4	90	121.7	11.8	915	900.3	1.3691	10	10	8	8	8	10	1.2894
1	1	-1	6.6	81	123.2	12.3	980	916.3	1.4940	10	10	8	6	4	10	1.1884
1	1	1	6.2	97	122.6	11.7	945	889.9	1.3953	8	10	8	8	8	10	1.0186
1	1	1	6.0	83	121.6	12.2	985	866.7	1.3965	10	10	8	8	8	10	1.2116
0	0	0	6.8	102	123.4	11.3	930	954.1	1.5847	8	10	8	8	6	10	0.9537
0	0	0	7.4	98	123.1	11.3	975	838.8	1.3597	10	10	8	8	8	5	1.2418
0	0	0	6.4	86	121.6	11.7	910	949.9	1.5411	10	10	8	8	6	10	1.4592
0	0	0	6.0	83	122.1	12.0	960	932.1	1.4236	8	8	8	8	6	10	1.1273
-2	0	0	5.8	98	123.7	11.8	925	868.7	1.3648	8	10	8	10	8	10	1.2970
0	-2	0	5.8	92	121.1	11.8	950	864.2	1.4873	10	8	6	10	8	5	1.3222
0	0	-2	8.0	103	122.3	11.4	930	878.4	1.3717	8	10	8	8	8	5	1.0815
2	0	0	6.2	87	122.2	11.8	945	895.1	1.4627	10	10	8	6	6	10	1.2112
0	2	0	5.9	89	120.9	11.2	870	924.3	1.4278	8	10	4	10	8	10	1.3161
0	0	2	6.0	84	122.7	11.9	945	893.9	1.3932	10	10	8	10	8	10	1.3031

A: DATEM (%); B: ascorbic acid (ppm); C: α-amylase (SKB u)

APPENDIX VI. LIQUID FERMENT BAKING TEST. REDUCTION OF WATER ADDED TO DOUGH.

It was observed that when using the liquid ferment baking test to produce white pan bread, there were problems of stickiness and manageability of the dough that provoked the dough pieces to stick and tear in the sheeter. Considering this may be due to an excess of water in the dough, a small experiment was undertaken to find the adequate amount of water to have a not-sticky and manageable dough.

Objective:

- To obtain the adequate addition of water to dough using the liquid ferment baking test in the different blending systems under study.

Methods:

Pan bread was produced by the breadmaking procedure described in the Optimization Experiment #1. Strong flour, ascorbic acid, and α-amylase were set at their middle levels, i.e. 40% hard red spring flour (CWRS or CWES), 100 ppm ascorbic acid, and 60 SKB units α-amylase. A control was baked at the beginning of each baking day with 100% Mexican bread wheat flour, 100 ppm ascorbic acid, and 60 SKB u α-amylase. Proof time was recorded for this control and the rest of the loaves were proofed to this time. A fermentation time of 3

hours was used. Water absorptions of FAB to FAB-5% were used. Mixing time, panning score, loaf volume, and fineness were assessed. Two loaves were baked for each water absorption and their results were averaged. Treatments were randomized within each baking day. The four blending systems (CWRS/CPS white, CWRS/CPS red, CWES/CPS white, and CWES/CPS red) were baked in a baking day each. Data was analyzed using the Minitab 8.0 statistical software.

Results:

Data is summarized in Tables A-2 (CWRS/CPS white), A-3 (CWRS/CPS red), A-4 (CWES/CPS white), and A-5 (CWES/CPS red). Results of analysis of variance are presented in Table A-6. Hard red spring flour, prairie spring flour, and water absorption were used as factors for the analysis. Means for each factor are presented in Table A-7. Variation in the water absorption had a significant effect in all of the responses except for fineness. Minimum mixing time was attained when water absorption was FAB-4, and maximum was obtained at FAB-2. With respect to the panning score, acceptable doughs (panning score >6.0) were obtained from FAB-2 and down. Loaf volume tended to increase from FAB to FAB-2, where it reached a maximum and eventually decreased when absorption was further reduced. Although not significant, fineness scores increased as absorption was reduced (meaning a more uniform crumb structure) and reached a maximum at FAB-3.

Plates A-1 to A-4 depict crumb structure and loaf symmetry of the four different blends at different water absorptions. Differences in crumb structure and loaf symmetry were more evident in those blends containing CPS red. Loaves with acceptable symmetry were obtained at FAB, FAB-1% and FAB-2% in the CWRS/CPS white and the CWES/CPS white blends, but they tended to have big cells and thus a non-uniform crumb structure. At FAB-3% a loaf with acceptable crumb structure and symmetry was obtained. Loaves with poor symmetry and poor crumb structure were obtained at levels of water between FAB and FAB-2% for the CWRS/CPS red and the CWES/CPs red blends. Loaf symmetry improved at FAB-3% for both blends, but problems with crumb structure were still perceived, although to a lesser degree. At lower levels of water absorption symmetry improved but crumb structure showed further defects.

The main problem arising from incorrect water absorption was dough manageability, assessed with the panning score, so this response was given added weight in trying to choose an adequate water absorption in the ferment. Loaf volume was considered the next important characteristic, since it was observed that at the lowest levels of water absorption dough began to get tight and volumes decreased. With this into account, it seems that a water absorption between FAB-2 and FAB-3 is reasonable. It has been reported that for long fermentation doughs a water absorption of FAB-3% is commonly used, so the final decision was to choose a water absorption of FAB-3% as the most adequate for the type of ferment used in this study.

It is also worth noticing the effect of the other two factors. The effect of use of strong flour in the blend was only significant in loaf volume. In average, use of CWRS results in an increase in loaf volume of around 80 cc compared when using CWES. Part of this difference may be explained in terms of proofing time. All loaves were proofed at a constant time and CWES doughs may need more time for optimum development due to its characteristic strength.

On the other hand, use of the different weak flours had a significant effect on all the responses. Use of CPS white resulted in lower mixing time, but slightly lower panning score, lower loaf volume (100 cc) and lower fineness score compared to CPS red. Although they are nor bread wheats, this may be explained due to the stronger gluten properties of CPS red in comparison to CPS white. CPS white may need further fortification with a strong flour than that attained in this experiment.

Conclusion:

A water absorption of FAB-3% was considered to be adequate for the blending systems used in this study. Better baking and loaf characteristics were obtained when using CWRS compared to CWES and CPS red compared to CPS white.

Table A-2. Water absorption experiment. Baking data for CWRS/CPS white.blend system Each value is an average of two replications.

Water absorption (%)	Mixing time (min.)	Panning	Loaf volume (cc)	Fineness
FAB	3.1	6.0	1060.0	824.6
FAB-1	3.3	6.0	962.5	847.3
FAB-2	3.1	7.5	1052.5	822.5
FAB-3	2.7	7.5	1000.0	852.8
FAB-4	2.9	7.5	990.0	843.7
FAB-5	3.0	9.0	952.5	803.6

Table A-3. Water absorption experiment. Baking data for CWRS/CPS red blend system. Each value is an average of two replications.

Water absorption (%)	Mixing time (min.)	Panning	Loaf volume (cc)	Fineness
FAB	5.1	6.5	1155.0	846.5
FAB-1	4.3	6.5	1095.0	799.8
FAB-2	4.9	7.5	1207.5	881.0
FAB-3	3.9	7.5	1192.5	905.5
FAB-4	4.2	7.5	1095.0	853.2
FAB-5	4.0	8.5	1102.5	896.0

Table A-4. Water absorption experiment. Baking data for CWES/CPS white blend system. Each value is an average of two replications.

Water absorption (%)	Mixing time (min.)	Panning	Loaf volume (cc)	Fineness
FAB	3.0	6.5	927.5	753.3
FAB-1	3.5	7.0	1000.0	796.1
FAB-2	3.6	6.5	1007.5	840.1
FAB-3	3.3	7.5	945.0	844.5
FAB-4	3.0	8.0	1000.0	846.9
FAB-5	3.1	7.0	950.0	783.3

Table A-5. Water absorption experiment. Baking data for CWES/CPS red blend system. Each value is an average of two replications.

Water absorption (%)	Mixing time (min.)	Panning	Loaf volume (cc)	Fineness
FAB	4.4	8.5	1065.0	911.7
FAB-1	4.3	6.5	1015.0	836.2
FAB-2	4.6	8.5	1080.0	897.3
FAB-3	4.3	8.5	1007.5	896.2
FAB-4	4.1	9.0	1010.0	863.2
FAB-5	3.4	9.5	985.0	884.3

Table A-5. ANOVA display for the different blend systems. Hard red spring flour, prairie spring flour, and water absorption were used as factors.

ANOVA for Mixing time

Source	df	SS	MS	F	p
Strong flour	1	0.0833	0.0833	0.74	0.395
Weak flour	1	18.7500	18.7500	166.14	0.000
Water absorption	5	1.7450	0.3490	3.09	0.019
Error	40	4.5142	0.1129		
Total	47	25.0925			

ANOVA for Panning

Source	df	SS	MS	F	p
Strong flour	1	0.333	0.333	0.20	0.655
Weak flour	1	12.000	12.000	7.28	0.010
Water absorption	5	41.000	8.200	4.98	0.001
Error	40	65.917	1.648		
Total	47	119.250			

ANOVA for Loaf volume

Source	df	SS	MS	F	p
Strong flour	1	63438	63438	29.67	0.000
Weak flour	1	112617	112617	52.68	0.000
Water absorption	5	38334	7667	3.59	0.009
Error	40	85510	2138		
Total	47	299899			

ANOVA for Fineness

Source	df	SS	MS	F	p
Strong flour	1	126	126	0.08	0.779
Weak flour	1	30020	30020	18.93	0.000
Water absorption	5	15324	3065	1.93	0.110
Error	40	63436	1586		
Total	47	108906			

Table A-7. Water absorption experiment. Means for factors hard red spring flour, prairie spring flour, and water absorption.

Factor	N	Mixing (min.)	Panning	Loaf volume (cc)	Fineness
			Strong flour		
CWRS	24	3.7	7.3	1072.1	849.16
CWES	24	3.8	7.5	999.4	845.92
			Weak flour		
CPS white	24	3.1	6.9	987.3	822.53
CPS red	24	4.4	7.9	1084.2	872.55
			Water absorption		
FAB	8	3.9	6.9	1051.9	834
FAB-1	8	3.9	5.6	1018.1	819.8
FAB-2	8	4.1	7.5	1086.9	860.2
FAB-3	8	3.6	7.7	1036.2	874.2
FAB-4	8	3.5	8.0	1023.8	855.4
FAB-5	8	3.6	8.5	997.5	841.8

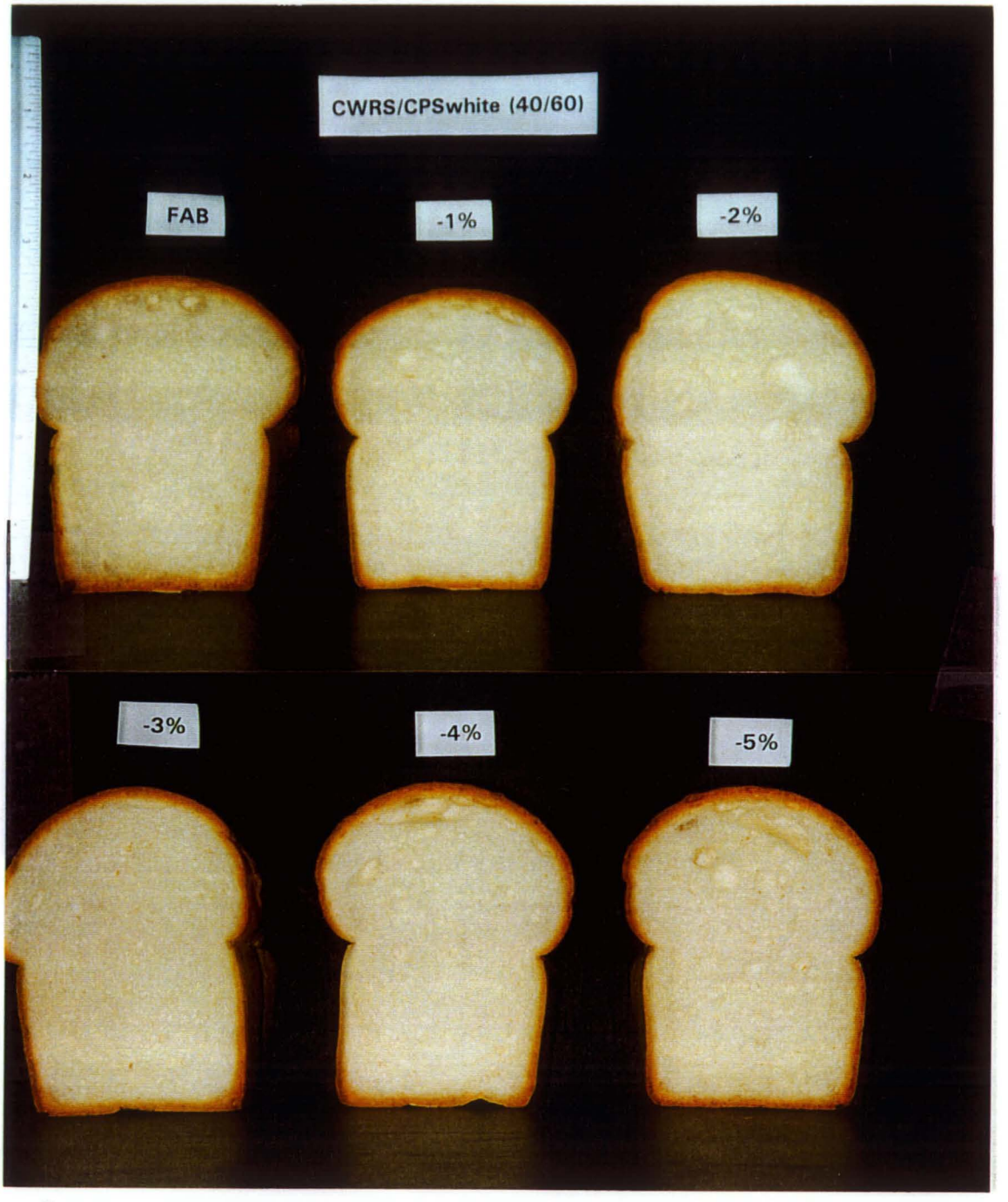

Plate A- 1 Liquid ferment baking test. Reduction of water added to dough. CWRS/CPS white blend. Breads prepared at different water absorption. From left to right: FAB, FAB-1%, FAB-2%, FAB-3%, FAB-4%, and FAB-5%.

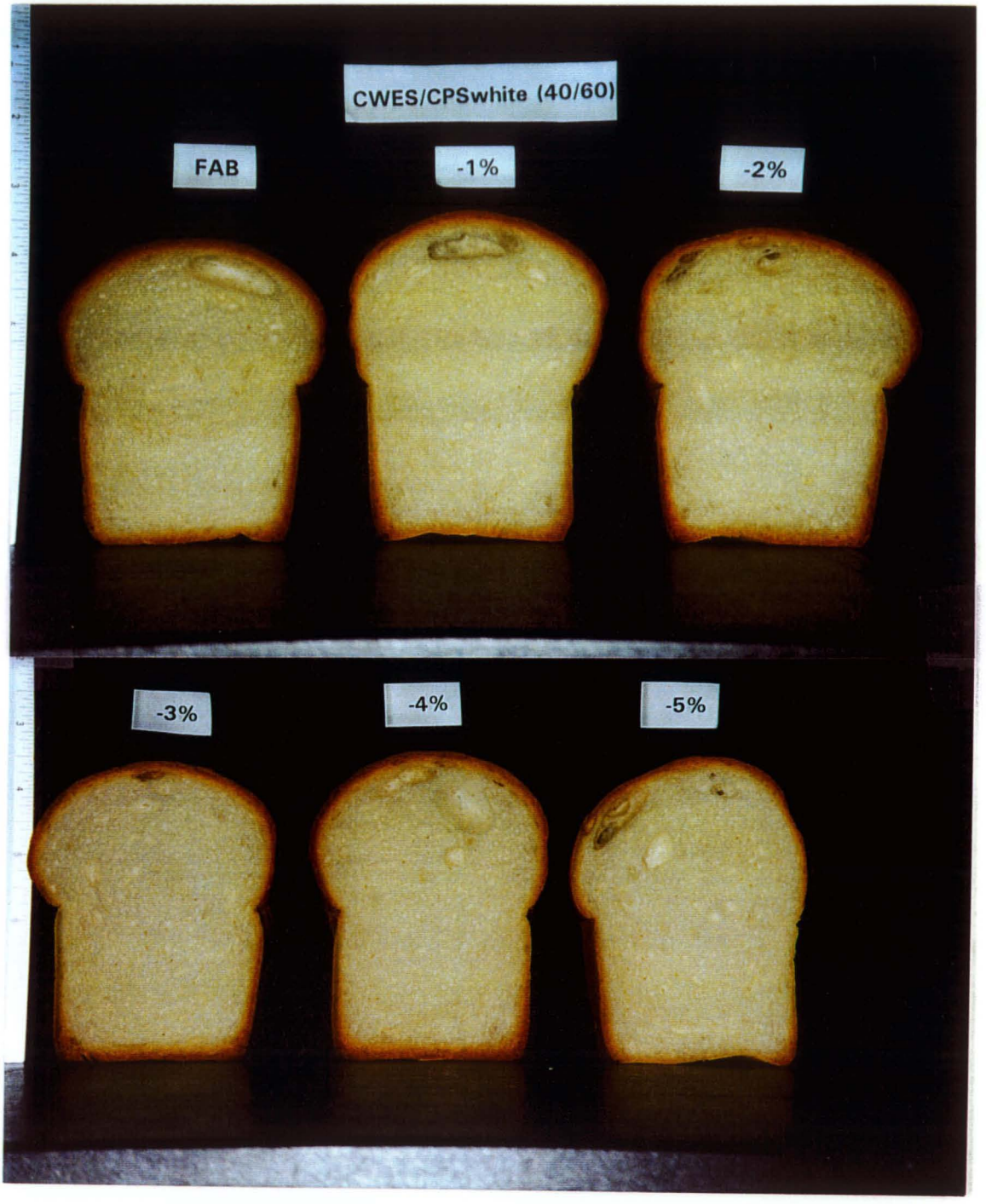

Plate A- 2 Liquid ferment baking test. Reduction of water added to dough. CWES/CPS white blend. Breads prepared at different water absorption. From left to right: FAB, FAB-1%, FAB-2%, FAB-3%, FAB-4%, and FAB-5%.

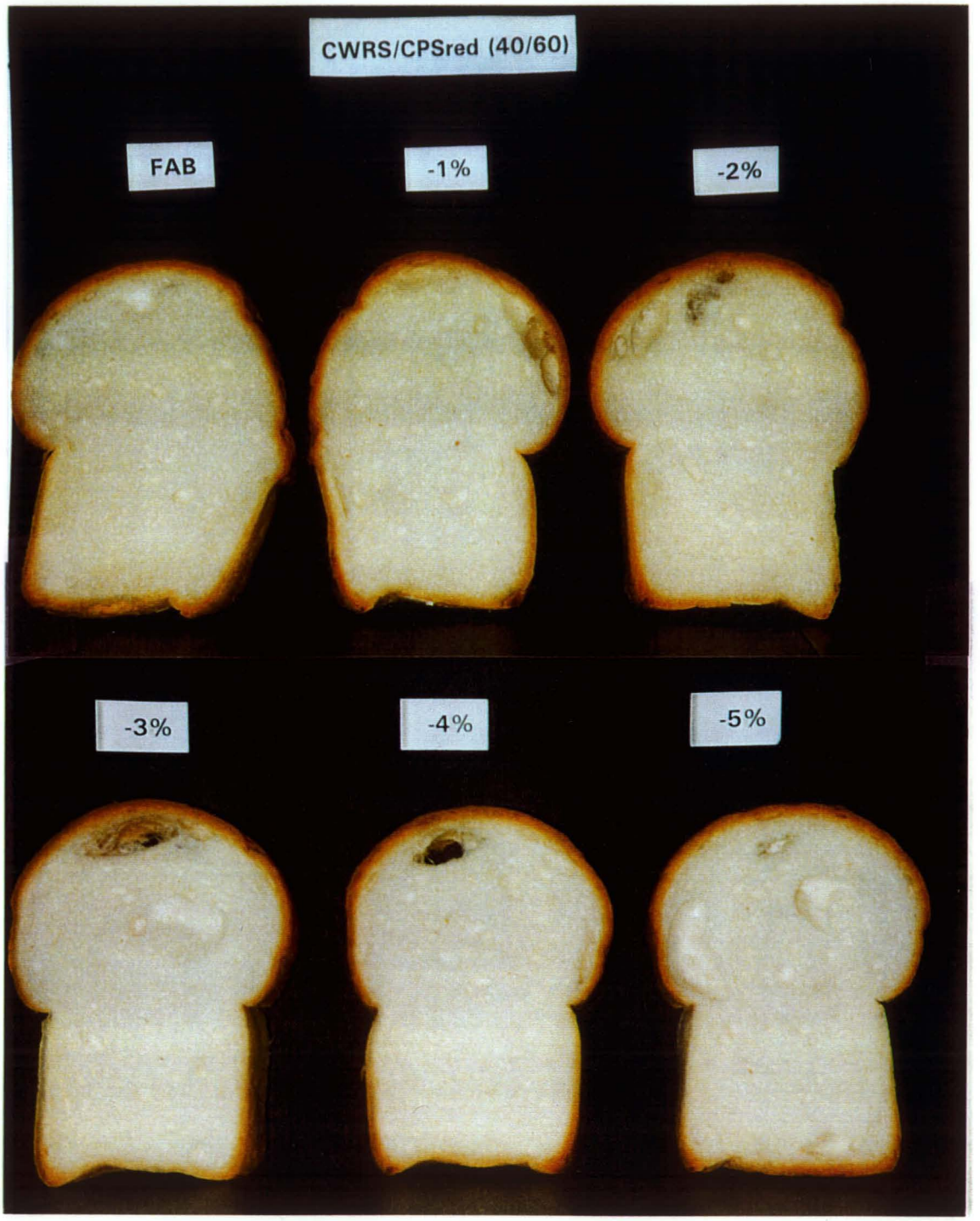

Plate A- 3 Liquid ferment baking test. Reduction of water added to dough. CWRS/CPS red blend. Breads prepared at different water absorption. From left to right: FAB, FAB-1%, FAB-2%, FAB-3%, FAB-4%, and FAB-5%.

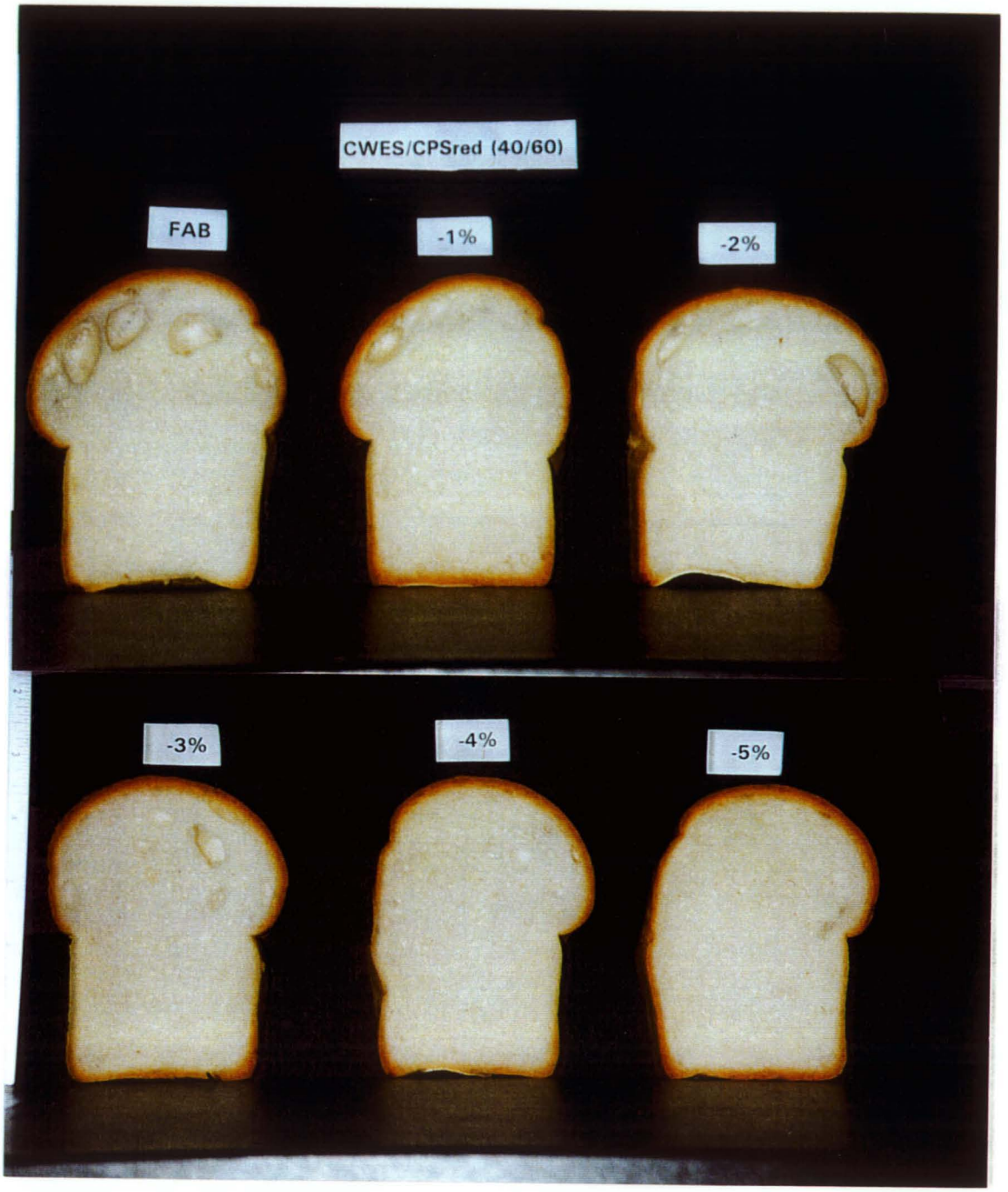

Plate A- 4 Liquid ferment baking test. Reduction of water added to dough. CWES/CPS red blend. Breads prepared at different water absorption. From left to right: FAB, FAB-1%, FAB-2%, FAB-3%, FAB-4%, and FAB-5%.

APPENDIX VII OPTIMIZATION EXPERIMENT #2. CWRS/CPS white BLEND SYSTEM. ORIGINAL DATA.

			Response					
A	*B*	*C*	*Mixing time (min)*	*Panning*	*Proof time (min)*	*Loaf Volume (cc)*	*Fineness*	*Break-and-shred*
-1	-1	-1	2.6	8	106	850	636.0	2
-1	-1	-1	2.7	4	111	850	574.3	2
-1	-1	1	2.3	8	108	1005	674.0	4
-1	-1	1	1.1	2	104	940	706.3	6
-1	1	-1	2.8	6	108	950	691.7	4
-1	1	-1	1.2	6	100	900	754.3	8
-1	1	1	2.2	8	112	1040	716.0	6
-1	1	1	1.8	8	106	1020	739.3	6
1	-1	-1	2.9	9	99	970	691.5	4
1	-1	-1	1.6	8	93	900	821.4	8
1	-1	1	2.2	8	102	1000	820.3	8
1	-1	1	0.9	6	100	920	728.1	4
1	1	-1	3.2	8	109	900	677.3	2
1	1	-1	3.2	4	99	870	682.3	4
1	1	1	3.5	2	102	925	716.3	4
1	1	1	1.7	8	95	930	710.6	8
0	0	0	3.1	6	101	950	636.1	4
0	0	0	1.4	8	89	930	735.7	8
-2	0	0	2.5	4	117	850	560.2	0
0	-2	0	2.5	8	101	890	624.7	4
0	0	-2	3.0	6	104	800	567.7	4
2	0	0	1.2	8	92	940	692.3	6
0	2	0	2.9	6	101	950	721.7	4
0	0	2	2.9	4	125	920	577.3	0

A: CWRS (%); B: ascorbic acid (ppm); C: α-amylase (SKB u)

APPENDIX VIII OPTIMIZATION EXPERIMENT #2. CWES/CPS white BLEND SYSTEM. ORIGINAL DATA.

			Response					
A	*B*	*C*	*Mixing time (min)*	*Panning*	*Proof time (min)*	*Loaf Volume (cc)*	*Fineness*	*Break-and-shred*
-1	-1	-1	2.3	8	104	850	555.7	2
-1	-1	-1	2.8	2	107	800	677.8	4
-1	-1	1	2.6	2	122	940	574.0	2
-1	-1	1	1.0	6	98	910	832.4	6
-1	1	-1	2.4	6	112	940	632.9	4
-1	1	-1	2.8	4	101	790	643.0	4
-1	1	1	2.6	2	114	940	602.1	2
-1	1	1	2.6	0	116	810	644.6	2
1	-1	-1	3.2	6	101	920	700.6	4
1	-1	-1	3.3	6	88	780	707.9	6
1	-1	1	1.1	8	98	890	623.7	6
1	-1	1	1.1	6	94	750	616.8	6
1	1	-1	3.6	6	108	840	666.2	2
1	1	-1	2.5	8	94	840	829.2	6
1	1	1	1.9	6	90	970	749.9	6
1	1	1	2.5	6	99	940	780.0	6
0	0	0	1.7	8	98	960	799.1	4
0	0	0	2.8	4	99	850	668.2	6
-2	0	0	2.0	0	-	-	-	-
0	-2	0	3.0	6	122	880	578.2	0
0	0	-2	2.4	4	103	680	567.0	2
2	0	0	3.3	10	95	1000	790.4	8
0	2	0	2.9	6	97	900	729.6	8
0	0	2	1.0	8	100	920	710.3	4

A: CWRS (%); B: ascorbic acid (ppm); C: α-amylase (SKB u)

APPENDIX IX OPTIMIZATION EXPERIMENT #2. CWRS/CPS red BLEND SYSTEM. ORIGINAL DATA.

			Response					
A	B	C	Mixing time (min)	Panning	Proof time (min)	Loaf Volume (cc)	Fineness	Break-and-shred
-1	-1	-1	4.7	8	99	950	780.7	4
-1	-1	-1	3.3	8	111	930	702.4	6
-1	-1	1	2.0	8	100	910	813.1	4
-1	-1	1	3.9	0	145	800	582.9	0
-1	1	-1	1.2	6	87	870	905.8	4
-1	1	-1	3.3	8	121	990	739.0	6
-1	1	1	3.9	2	105	880	728.3	4
-1	1	1	2.8	6	117	980	736.1	8
1	-1	-1	4.1	8	102	920	768.1	6
1	-1	-1	3.9	10	108	940	703.1	8
1	-1	1	3.4	10	95	970	766.2	6
1	-1	1	1.7	6	112	920	766.9	8
1	1	-1	3.9	6	95	890	733.7	4
1	1	-1	3.6	8	116	1030	742.7	8
1	1	1	1.3	0	104	780	727.1	4
1	1	1	1.0	8	111	910	627.8	6
0	0	0	1.8	8	100	900	791.0	8
0	0	0	3.2	8	114	960	770.0	8
-2	0	0	3.1	6	120	990	765.2	4
0	-2	0	3.4	8	101	920	716.8	6
0	0	-2	3.8	10	116	800	608.8	2
2	0	0	1.4	6	96	860	791.8	6
0	2	0	3.4	8	118	1000	831.7	8
0	0	2	3.6	0	100	910	741.0	8

A: CWRS (%); B: ascorbic acid (ppm); C: α-amylase (SKB u)

APPENDIX X OPTIMIZATION EXPERIMENT #2. CWES/CPS red BLEND SYSTEM. ORIGINAL DATA.

A	B	C	Response					
			Mixing time (min)	Panning	Proof time (min)	Loaf Volume (cc)	Fineness	Break-and-shred
-1	-1	-1	3.4	6	122	880	649.2	4
-1	-1	-1	3.5	10	110	920	728.3	8
-1	-1	1	2.0	6	111	910	835.7	6
-1	-1	1	3.3	10	117	1010	721.4	8
-1	1	-1	3.4	8	117	910	695.7	6
-1	1	-1	3.5	10	112	960	722.5	6
-1	1	1	1.8	6	114	910	707.9	8
-1	1	1	3.8	8	120	970	670.4	8
1	-1	-1	2.6	10	112	930	738.9	8
1	-1	-1	4.1	10	110	930	687.8	6
1	-1	1	4.2	8	112	990	664.0	8
1	-1	1	1.3	6	115	850	693.4	6
1	1	-1	4.1	10	110	950	679.0	8
1	1	-1	3.7	10	107	980	776.2	8
1	1	1	4.1	8	112	1020	754.1	6
1	1	1	2.0	6	106	890	767.7	8
0	0	0	3.7	8	114	930	689.0	8
0	0	0	2.6	10	118	940	727.3	8
-2	0	0	3.7	8	120	970	715.9	4
0	-2	0	3.8	10	114	930	614.4	6
0	0	-2	3.6	10	107	780	659.3	4
2	0	0	4.0	10	114	1020	672.3	8
0	2	0	3.8	8	108	970	707.3	8
0	0	2	3.9	6	116	980	742.4	8

A: CWES (%); B: ascorbic acid (ppm); C: α-amylase (SKB u)