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THE OPTIMIZATION OF A SOY-ENRICHED RICE-CASSAVA YEAST BREAD USING RESPONSE SURFACE METHODOLOGY

by

Janice Vilma Patricia Archibald

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

The development of a soy-enriched rice/cassava/ yeast bread which could suitably replace a wheat flour bread in the diet of developing countries was investigated. A preliminary study of the physicochemical properties of selected starches and flours determined that cassava starch rather than cassava flour could be a successful replacement for potato starch in a rice flour based yeast bread. The gelatinization characteristics of the two starches were similar , in blends with rice flour, as well as with and without addition of the gums, carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC) and xanthan. For rice /potato and rice/cassava (80/20) blends, gelatinization temperatures (GT) were 60°C and 63°C, peak viscosities (PV) were 1140 and 1090 B.U., and cooled viscosities (CV) were 990 and 830 B.U. respectively.

A rice/potato formula with 0.9% CMC and 3.5% HPMC served as the basis for the optimization of the rice/cassava/soy bread. Response surface methodology (RSM) was used to optimize the levels of cassava starch, soy protein concentrate (SPC) and water, and to select the appropriate proof time. These variables were identified previously as critical to loaf quality. The physical and textural characteristics measured to evaluate the success of the bread were specific volume, loaf shape, Instron hardness, gumminess, cell size cell uniformity, top crust colour, crumb colour and moisture content. Cassava starch and water had highly significant effects on almost all the response variables while SPC significantly affected the majority including top crust colour which was affected only by SPC.

With SPC levels held constant at four levels (3, 5, 7 and 9%) of the total flour/starch weight in the formula, low cassava (10 - 15%) formulations were identified which met all criteria for reference standards, as well as formulae with high cassava (20 - 27%) that met all the criteria except cell uniformity. Protein content of the bread with 9% SPC was comparable to a wheat flour white bread (6.6%). The study has successfully used RSM to develop formulations for a soy-enriched rice/cassava yeast bread which had acceptable physical and textural characteristics.

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FOR MY SISTERS

Dawn and her family

Pansy and her family

Their love and belief in me kept me going through many 'rough spots'

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INTRODUCTION

The development of a protein-enriched rice/cassava yeast bread has obvious benefits for developing countries as well as for those individuals suffering from wheat allergies. The use of indigenous crops to produce a bread which could suitably replace a wheat flour bread could provide economic relief to countries which have been importing low grade wheat at high cost.

Projects to develop wheat composite and non-wheat flour breads have been conducted for almost four decades and met with varying degrees of success. The major objective of the projects had been to get developing countries to accept the challenge of using indigenous starchy crops as a replacement for wheat in breadmaking. However, this has not been achieved primarily because the non-wheat products are usually evaluated by wheat standards and are therefore found less acceptable. Asselbergs (1970) reported the suggestion that composite flour baked goods should be considered on their own merit and not be in competition with wheat flour.

In Guyana, wheat bread is an important staple and is eaten particularly for breakfast. However wheat is not grown in Guyana and the cost of imported wheat has increased. The Government of Guyana had declared its intention to utilise as many of the country's natural resources as possible in order to reduce importation costs as well as expenditure of very limited foreign exchange. It has become necessary to consider alternative types of flours which would make a bread acceptable to the average consumer. The ultimate aim is to find a replacement for wheat flour bread, and consequently a means of relieving the foreign exchange problem which the country faces.

Rice is one of the major agricultural resources of Guyana, so the Government had recommended that rice flour be considered as the major replacement for wheat. In order to implement the Government's policy of "using our natural resources", several projects were encouraged in an attempt to develop bread-like products using alternative starchy cereals such as rice, as well as other starches. Many were successful but the least acceptable has been the yeast bread which, although edible was not comparable to wheat flour yeast bread. Important quality attributes such as flavour, texture and general appearance, left much to be desired.

Internationally, several rice flour bread formulae are available but they contain ingredients which may not be readily accessible in Guyana. Further to this it has been reported from several studies (Nishita, 1973; Ylimaki, 1987) that a composite of non-wheat flour and/or starches produce a more acceptable bread than 100% rice flour formulations. In Ylimaki's formula (1987) rice flour and potato starch were used in the ratio of 4:1 (80% and 20% rice and potato starch respectively).

Cassava is abundantly grown and consumed in various regions of Guyana. Cassava flour, starch or the freshly grated pulp is used in combination with wheat in many Guyanese dishes. Presently the flour is used to supplement wheat flour in accordance with Government regulations. Several papers (Pizzinatto and Vitti, 1975; Ciacco and D'Appolonia, 1977; Chacon, 1983; Keya and Hadziyev,1985; Pertz et al, 1986) indicated the successful results achieved from the incorporation of cassava into wheat flour and composite flour bread formulae. However, with the exception of Casier et al. (1979) there have not been found (to date) any recent studies or general information on the use of non-wheat composite flours in bread formulations.

Wheat flour, the major structural component of bread, contains gluten-forming proteins which are responsible for forming a dough that will incorporate air, retain gas and produce fine cells which eventually determine the finished quality of the bread. In considering the use of non-wheat flours it is important to find some way to produce a similar structure to a wheat flour dough by binding the water required to gelatinize the starch, as well as retaining the gas produced during fermentation (De Ruiter, 1978). Several gums have been used as gluten substitutes in attempts to produce successful gluten-free breads. Some of those used with satisfactory results are sodium carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC) and xanthan gum (X). These products assist in maintaining the structure and texture of the bread.

Notwithstanding the importance of gluten in bread-making, the starch component of the flour is equally important for its structural role. In fact, the strength of wheat flour depends on the strength of the bonding between the protein and starch in the endosperm. Scanning Electron Microscopy (SEM) pictures have shown quite clearly the starch gelatinized into fibrous strands interwoven with thin protein strands to form one cohesive mass. Starch gelatinization is an important stage in the baking procedure. While the gluten achieves and maintains volume and skeletal structure, it is the starch which provides the crumb with its textural characteristics. Non-wheat starches differ in nature and behaviour from wheat starches. These differences may be determined through amylograph tests, the results of which may be used to give some indication of the outcome of the baked product.

While it might be quite possible to produce a rice/cassava bread with acceptable physical attributes, its nutritional quality would be lower than that of the average wheat bread. Since the maintenance of nutritionally well-balanced products is also a priority for Guyana, some consideration must be given to this aspect of the problem. Compared to wheat, rice has a low protein content and blending it with cassava starch would further dilute the overall protein level of the composite. A protein supplement in the formulation could improve nutritional quality significantly. Reports indicate that soy protein has been used extensively to enrich baked goods. It has been used in the form of flour, concentrate or isolate. Soya beans are cultivated in Guyana as a secondary crop and there is potential for increased production. The inclusion of a soy protein in the formulation would improve not only the protein quantity but the quality as well. It might be possible to produce a rice flour bread with a protein value equivalent to, or even better than that of an average wheat flour white bread.

To determine proportions of rice flour, cassava flour or starch, and soy protein concentrate which will produce the optimum results, Response Surface Methodology (RSM) can be used. This is a statistical technique which has been used extensively in product development. It allows results of many combinations to be projected through preparation and evaluation of a limited number of treatments.

If an acceptable rice/cassava/soy composite bread can be developed then there is the distinct possibility of the product eventually being produced commercially. In Guyana this would be beneficial to small business corporations and industries as well as to individuals, as the increased demand for the ingredients could result in increased cassava and soyabean production and processing, this in turn improving the economic situation as well as leading to more employment opportunities.

In addition such a product could be of benefit to other individuals suffering from celiac disease, wheat intolerance or allergies. Recent investigations also suggest that it may be a useful item for the diabetic diet, because the slow absorption rate of rice carbohydrate depresses the blood glucose peak.

The overall purpose of this study was therefore to optimize the formula for an acceptable soy-enriched rice/cassava yeast bread suitable for use in the Guyanese diet.

The general objectives of the present study were:

1. **To investigate the gelatinization temperatures and pasting properties of flours and starches for use in wheatless bread formulations.**

2. **To optimize the formula for an acceptable soy enriched rice/cassava yeast bread, using response surface methodology.**

The study was divided into two experiments each based on one of the previously stated objectives. The first experiment was conducted mainly to investigate the gelatinization temperatures and pasting properties of selected non-wheat flours and starches. This information was then used to predict whether they would be suitable for use in a rice/cassava/soy yeast bread. This prediction was based on other studies which found that there was a relationship between gelatinization characteristics of flours and starches, and bread quality.

The second experiment was divided into two phases. The first phase was conducted to standardise the basic formulation and methodology for a rice/cassava/soy bread. The second phase was the final optimization of the formulation through the use of response surface methodology.

The thesis has been written as three main papers , the first on experiment one, the second on phase one of experiment two, and the third on phase two of the second experiment. These papers are preceded by a general introduction and literature review and succeeded by a general summary and conclusions and recommendations for future research. The specific objectives of each experiment are stated in the relevant paper.

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Chapter 2

REVIEW OF LITERATURE

INTRODUCTION

This review begins with a discussion of non-wheat flours and starches from alternative starchy cereals and roots and tubers. These are under consideration as replacements for wheat therefore their functional characteristics are of utmost importance.

Wheatless breads and rice flour yeast breads in particular, are reviewed with special attention on protein enrichment or fortification. Very little work has been conducted on the enrichment of 100% non-wheat breads although there are a number of reports about enrichment and fortification of wheat and wheat flour composites.

In order to obtain the light, open texture which is characteristic of wheat bread there must be some film-forming material used with the wheatless gluten-free flours and starches. Gums which have been used to replace gluten will form the basis of a discussion of their properties and utilization in baked products.

These discussions will be followed by a review of some of the objective methods of wheat bread evaluation and their application to non-wheat breads. Finally there will be a discussion of Response Surface Methodology (RSM) as a statistical tool in the optimization of formulae during product development.

Non-wheat Flours and Starches

The properties of starch are very important in baked goods (Hoseney et al., 1978). Cereal grains, tubers and roots are the main sources of starches (Zobel, 1984), which are found in various parts of plants, in the form of granules. The granules vary greatly in size, shape, position of the hilum and other characteristics, depending on their source. The starch sources of primary interest for this review are rice, cassava and potato.

According to Heckman (1977) rice (Oryza sativa) has the smallest granules, between 3-8 microns. They are polygonal in shape and tend to aggregate in clusters. Cassava (tapioca, Manihot utilissima) granules range in size from 5-35 microns and are rounded and truncated at one end to form kettle drum shapes. The potato has the largest granules which range from about 15-100 microns. These granules are oval and egg shaped and have detectable striations. In contrast to the others wheat appears to have flat, rounded or elliptical granules which tend to cluster in two ranges. The small ones range from about 2-10 microns and the large ones from 20-35 microns. If any of the other starches is to be substituted for wheat starch it is probable that there would have to be some modification as the starches have different structural characteristics and will likely display different behaviour patterns.

Kulp and Lorenz (1981) indicated that chemical composition and structural features of starch polymers are important in starch performance. Starch granules are largely composed of carbohydrate but minor constituents such as lipids, protein , phosphate and ash react in a starch solution and influence the properties of the granules (Heckman, 1977). The amount of starch in cereal grains varies from 60 to 75% of the weight of the grain (Hoseney, 1986), whereas the starch content of cassava ranges from 64% to 87% depending on the maturity of the tuber (Ketiku and Oyenuga, 1972; Okezie and Kosikowski, 1982). Potato has 80% carbohydrate of which three-quarters is starch (Pyler, 1988).

Starch comprises two major components, amylose and amylopectin, in addition to which there may be 5 to 10% of intermediate material in most cereal and root starches. The average amylose content of rice ranges from 12 - 35% of the total starch (Bechtel and Pomeranz, 1980). Typical amylose values for potato and cassava are 18 - 22% and 17 - 19% respectively (Radley, 1976; Okezie and Kosikowski, 1982). Greenwood (1976) suggested that the ratio of amylose to amylopectin is usually considered the first essential characteristic of a starch. Amylose is a linear polysaccharide and is the straight chain component of naturally occurring starches, consisting of repeat units of glucose interlinked by oxygen bridge at the 1,4 position (Heckman, 1977). This has been the traditionally accepted view (Hoseney et al. 1978), but amylose is now known to contain limited branching. According to Galliard and Bowler (1987) it has been confirmed that amyloses from different sources contain on average 2-8 branch points per molecule. Amylopectin is a highly- branched configuration of linear chains with branch points formed by 1,6 linkages.

The nature of these two components and their behaviour in solution are of utmost importance to the structure and texture of cooked starch products. The branched character of amylopectin is responsible for preventing gel formation, as the chains do not associate as do the amylose chains. When starch is in solution, the linear molecules of amylose become oriented parallel to one another and approach each other closely enough to permit association through hydrogen bonding. With amylopectin, its branched nature severely limits intermolecular hydrogen bonding, thus the rigidity needed for gel formation cannot occur. From a study of seven non-waxy starches, Juliano (1980) indicated that amylopectin contributes more to gel viscosity and consistency than amylose. These results suggested that the molecular size of amylopectin is the major factor determining the gel viscosity of starches. The amylose fraction of starch, perhaps by rapid retrogradation, is responsible for setting the crumb structure of bread (Hoseney et al., 1978). This occurs during the initial cooling of the loaves. The amylopectin fraction is implicated in the further firming of bread.

Gelatinization and Pasting Properties of Starches

When starch is gelatinized in an aqueous medium, the individual granules undergo a series of physical changes which result in the granules swelling and producing a viscous paste. Atwell (1986) discussed the factors influencing viscosity: starch concentration, molecular weight and size of granules, types of associative forces between starch polymers, temperature, and time at a constant shear rate. He reported that concentration, molecular weight and size of granules correlated positively with viscosity, but negatively with gelatinization temperature. Lorenz and Kulp (1982) cited several studies which show that pasting and swelling properties of starches can be attributed to the type of associative forces present in granules. Fundamentally, the gelatinization of starch in various media is attributed to the chemical affinity of the starch molecules, particularly the hydroxyl groups (Leach, 1984). Ciacco and D'Appolonia (1977) also attributed some properties to the arrangements of the amylose and amylopectin molecules within the granule. Olkku and Rha (1978) described the structure of the starch granule as being amorphous near the surface of the granule with the amylose and amylopectin macromolecules being of a crystalline nature inside the granule. They felt that even before a slurry reaches its initial gelatinization temperature, part of the starch inside the granule is already in solution just awaiting the disruption of the amorphous in order to be exuded. Pyler (1988) explained that when a critical temperature is reached, usually at about 60°C, the energy level is high enough to dissociate the weaker bonds in the granule. Increased temperature above the gelatinization range results in a progressive disruption of hydrogen bonds, a greater penetration of water molecules into the granule where they become attached to the liberated hydroxyl groups, and a continued swelling of the granules. This continues until all surrounding free water has been absorbed by the granules, and they crowd against one another to form a viscous paste. Ghasi et al. (1982) and Hoseney (1986) reported that paste viscosity should not only be attributed to granule swelling but that a large part of the increase in viscosity should be attributed to the leached starch exudate, which becomes more concentrated as water becomes bound.

Zobel (1984) discussed some methods of examining characteristics of a starch paste. Among these are light microscopy, electron microscopy, light transmission, enzymic analysis and viscometry. With one type of viscometry the slurry is heated in an amylograph. The determination of pasting behaviour with a Brabender viscoamylograph has long been a standard analytical tool (Bhattacharya and Sowbhagya, 1978), and is used most frequently in determining alpha amylase activity in wheat flour. It has also proved useful for gelatinization determinations in rheological studies of other flours and starches (Horiuchi, 1967; Loh, 1986; Luh and Liu, 1980; Shuey and Tipples, 1980). Although there are doubts about the efficiency of extrapolating amylograph measurements from slurries of relatively low starch concentration to the highly concentrated dough in which water is limited, Loh (1986) suggested that the parameters measured do indicate the anticipated effects on the final product. According to Bernetti et al. (1990) starch gelatinization in water and the behaviour of aqueous starch paste during amylography could aptly describe most of the potential end-use properties of starch in food systems.

The amylograph works on a programmed heating and cooling cycle and the results are taken from a curve which is charted during the process. Several significant points on the curve which provide information on gelatinization of the starch, are discussed by Shuey and Tipples (1980). Possible disadvantages of this instrument may be in the time required for cleaning the bowl and readjusting the controls (Bernetti et al., 1990) but the curves are quite comparable from sample to sample and between instruments, and viscosity is usually reproducible within 10 Brabender units.

Retrogradation, which manifests itself in the firmness of the starch on cooling, is responsible for the setback in a starch paste and appears to be limited essentially to the amylose fractions. Halick and Kelly (1959) concluded that high-amylose starches would show maximal setback on cooling. According to Pyler (1973), after the formation of hydrogen bonds, the randomly coiled amylose chains straighten out and align themselves linearly. There follows a formation of hydrogen bonds between adjacent hydroxyl groups.

Rice Flour: During gelatinization, flours or starches behave differently depending on their source and their composition. Bean (1986) stated that among the various starches in the world, rice has more diverse combinations of physicochemical characteristics than any other common cereal, root or tuber starch. Rice flour is prepared from broken milled rice therefore the chemical composition is the same as the whole rice (Luh and Liu, 1980). According to these researchers, compositional differences contribute to the diversity of chemical and physical properties of various rice flours, such as viscometric properties and other characteristics. More specifically, Bechtel and Pomeranz (1980) indicated that rice grain quality was related to the amylose:amylopectin ratio which governs water absorption and volume expansion during cooking, and to the cohesiveness and tenderness of cooked rice. Nishita and Bean (1979) used the relationship between this ratio and the cohesiveness of cooked rice to determine suitability of rice flours for breadmaking. Kongseree and Juliano (1972) suggested that although most physical properties of the grain appear to be correlated with gelatinization temperature, a physical property, the physical properties correlated better with amylose content, a chemical property. They attributed this to the changes in the physical structure of the raw starch granules during cooking. Bean and Nishita (1985) stated that pasting behaviour as measured in an amylograph , is an important functional property that reflects the combined effects of amylose content, birefringence end-point temperature (BEPT), particle size distribution, pretreatment, and physical state of the starch granules after milling to flour. Juliano (1984) reported that final gelatinization or BEPT of rice starch varies widely among varieties. BEPT can also be affected by ambient temperature during grain development, whereby high temperatures increase BEPT while conversely decreasing amylose content. Rice starch pastes increase in viscosity when cooled due to the retrogradation of linear amylose chains. Horiuchi (1967) confirmed a strong positive correlation between cold paste rigidity and amylose content. In his amylograph study it was found that rice flour displayed higher gelatinization temperature than rice starch but the relation between peak viscosity and breakdown of the two were similar.

Potato and Cassava Flour/Starch: Chabot et al (1974) discussed the changes noted in cassava starch during cooking in a Brabender amylograph. In their study samples were taken from the amylograph at various temperatures. It was noted that granule swelling had begun before any rise in viscosity had been recorded on the instrument. They described cooked tapioca (cassava) starch as very fragile and easily subject to mechanical shear in amylogram deformation. No intact swollen granules were evident at peak viscosity but rather a honeycomb arrangement of sheets of nongranular starch was evident.

Rasper et al (1974) after comparing six different non-wheat starches, reported a high degree of swelling in cassava starch but very little increase in consistency on cooling. This gave evidence of its low gel-forming potential. Zobel (1984) found that potato starch granules give the highest peak viscosity, but undergo the most breakdown. In addition the pastes disintegrate and become less viscous when cooled. Cassava starches produce less viscosity and have a lesser degree of swelling than potato. The importance of high amylopectin content of cassava is seen in its unusual viscosity characteristics (Okezie and Kosikowski, 1982) which causes it to gelatinize to form a colourless, odourless and transparent soft gel when set. Kim et al., (1976) commented that since linear amylose molecules associate easily and therefore retrograde rapidly, then cassava starch resists retrogradation due to its lower amylose content. Priestley (1977) stated that starches such as cassava and potato, which are easily solubilized, are more adhesive than the less soluble cereal starches. De Ruiter (1978) identified this as the reason that the best cohesive properties were found in the bread with cassava starch.

The pasting properties of rice and cassava starches indicate that they could serve as suitable replacements for wheat in breads, when used in combination with some structure-forming substances.

Wheatless Breads

Rice Flour Breads: Most of the research work on gluten-free bread has been conducted using wheat starch (McGreer, 1967; Christianson et al., 1974), while studies on composite flour breads have usually included wheat flour as a component (Mosqueda-Suarez, 1958; Kim and De Ruiter, 1968; 1969; Gim and Lin, 1978). Prior to the work of Ylimaki (1987), only Nishita (1973), Nishita et al.(1976), Nishita and Bean (1979), Delgado (1977) and Casier and coworkers (1979) had reported major studies on rice bread development and improvement.

Several problems have been associated with the development of rice breads. Two important factors are the varietal difference in rice flours, and the lack of gluten in the flour. The main differences in rice flours are found in the varieties of rice, the grain length, protein content and the amylose content of the rice. These all relate to the behaviour of the flour, and its functionality in the baked product. Nishita and Bean (1979), reported that rice flour with a low amylose content (<20%) and low gelatinization temperature (<67°C) produced rice flour yeast breads with acceptable crumb texture. Bean et al (1983) in their study on rice flour layer cakes suggested that hydration of rice flour just before use could enhance its functionality in baked products. They had observed a noticeable improvement in bread texture when wet-milled flour was used in place of dry-milled. Even the age of rice flour has been known to affect the final baked product (Bean and Nishita, 1983). These researchers discussed the fact that the viscosity of flours which had been stored was higher than that of flours from freshly milled rice.

Nishita et al. (1976) reported the development of a yeast-leavened rice-bread formula, in which the rice flour used was from a blend of short and medium grain rice. Several factors were investigated and water level was found to have a critical effect on loaf volume and crumb texture, as well as the final shape of the baked loaf. Insufficient water produced a stiff dough with very small final volume, while excessive water caused overexpansion.

Casier and coworkers (1979) produced rice flour breads which were acceptable, although the dough was very heavy due to high water uptake. When compared to breads from pure rice starch, which required 196mL/250g of water, the final specific volume of the rice flour bread was 3.92 compared to 5.96 for the rice starch bread. They concluded that the water levels used needed to be varied depending on the type of flour used.

Ylimaki (1989) found that the flavour and texture of the rice flour yeast breads previously developed required improvement to be acceptable to consumers. In addition, reproducibility of the recipes was difficult due to the effects of varietal differences on the rice flours, which in turn affected bread quality. In preliminary standardisation experiments with three types of rice flours, Ylimaki (1987) modified the mixing and baking procedures from Nishita's formula (1977), resulting in improvements to the bread's appearance, volume, grain, flavour as well as to reproducibility of the formula.

Cassava Flour/Starch Breads: Several papers (Pizzinatto and Vitti, 1975; Ciacco and D'Appolonia, 1977, 1978; Chacon, 1983; Keya and Hadziyev, 1985; Pertz et al., 1986) have reported successful results from the incorporation of cassava into wheat flour and composite flour bread formulae. In attempting to incorporate cassava into wheat bread, cassava starch does not present a serious problem (Hudson and Ogunsa, 1976), but bread quality deteriorates with the addition of about 10% cassava flour. Ciacco and D'Appolonia (1978) investigated the functional properties of composite flours containing tuber flour or starch. They found that although breads containing cassava starch produced a somewhat soggy crumb, this was corrected by decreasing the amount of water used. Overall cassava starch performed better than cassava flour and it was concluded that the presence of fibre was responsible for the inferior baking quality of cassava flour. According to Hudson and Ogunsa (1976) the cassava fibres interfere with the formation of the network of films usually formed by the wheat proteins. De Ruiter (1978) reported that the bread containing cassava starch had the best cohesive properties because the gel produced on gelatinization of this starch possesses far greater cohesion than do the gels of grain starches and most other tubers.

Gim and Lin (1978) reported that use of cassava flour in a composite was marked by a reduction in the amylase activity. This led to lower carbon dioxide production during fermentation resulting in poor loaf volume. Winged bean flour helped to increase the amylase activity and the addition of 5% winged bean flour in a 75/20/5 (wheat/ cassava/ winged bean) flour mixture gave a protein content of 13.2% and a loaf volume comparable to that of the control (100% wheat). With up to 30% of cassava flour, the scores for the organoleptic properties were not significantly poorer than those for the control bread. Higher concentrations of cassava flour, were responsible for the unacceptable yellowish colour and the coarse texture of the crumb.

Casier et al. (1979) reported using of cassava flour or starch alone, and in combination with rice flour. They found that cassava starch produced better results than the cassava flour whether used alone or in combination with rice flour. They further reported that cassava starch/ rice flour blends normally resulted in better loaf volume and texture than cassava flour/ rice flour blends.

Nishita (1973) reported overall textural improvement from substituting 20% rice flour with potato, tapioca or wheat starch. Ylimaki (1987) used a rice flour/potato starch 80:20 combination with similar results. The main advantage of the potato starch was that it produced a softer and whiter crumb.

Satin (1988) reported satisfactory results from work on cassava flour breads in which he used pregelatinized flour in place of gums. Cassava flour breads were reported to be soggy and rubbery immediately after baking, but they improved after half a day (De Ruiter, 1978) or twenty hours (Satin, 1988) storage. The eating characteristics then resembled cake.

Protein-enrichment of Breads

There has been much research conducted on enrichment or fortification of wheat breads or wheat composite breads. Protein inadequacies are a problem facing risk population groups of the world, particularly populations in developing countries (Silaula et al., 1989). Although several attempts have been made to improve protein content of wheat bread, with maize flour and its major components (Mukulumwa, 1976), nonfat milk and wheat gluten (Delgado, 1977), fish protein (Kvitka and Chen, 1982), Algae dunaliella (Finney et al., 1984) and oat bran (Krishnan et al., 1987), more use has been made of legumes.

Legumes which have been used include winged bean (Gim and Lin, 1978), peanut flour ((Ory and Conkerton, 1983), pigeon pea (Gayle et al., 1986), faba bean (Youssef and Bushuk, 1986), cowpea (Mustafa et al., 1986) and germinated chickpea (Fernandez and Berry, 1989) have been relatively successful. At levels up to 20% these breads had good volume and texture but their beany flavour, content of digestion inhibitors and high starch content in relation to protein have been reported as causes of major problems (Youssef and Bushuk, 1986).

Several studies have carefully considered the rheological and sensory effects of the protein flours on doughs (Mukulumwa, 1976; Gim and Lin, 1978; Mustafa et al., 1986). Mukulumwa et al. (1976) reported that doughs containing very small amounts of maize protein isolate (2.4%) had increased loaf volume, but with more maize isolate there was a slight deterioration in bread quality parameters. Kvitka and Chen (1982) found that up to 5% fish protein concentrate in whole wheat bread resulted in standard quality and was consumer acceptable as well. Ory and Conkerton (1983) were able to increase nutritive value of wheat bread by adding 12% peanut flour without affecting bread loaf volume. Cowpea flour with a protein content of 25%, when added to breads at the 10% level, improved nutritional quality as well as the specific volume and crumb texture of bread (Mustafa et al., 1986). Using oat bran with a protein content of 23-24%, at levels up to 10%, increased the protein content slightly but did not improve the quality of bread in a study reported by Krishnan et al.(1987). Improvement in volume, grain and texture occurred only with the addition of potassium bromate to the dough.

The most used legume protein supplements have been of soyabean origin (Ranhotra et al., 1975; Klein et al., 1980; Chen and Rasper, 1982;, Elgedaily et al., 1982; Guy, 1984). The main reason for using the soy flour, concentrate or isolate is that the nutritive value is high in terms of both digestibility and amino acid composition (Pyler, 1988). Soy products complement the cereal proteins to give a more balanced essential amino acid pattern. Increased utilization of high-protein legumes in basic food such as breads, is a method of dealing with food shortages on a world scale (Silaula et al, 1989).

The studies reported above have dealt with wheat flour breads. There are few reports of work carried out to fortify non-wheat breads. However, if such bread can be made satisfactorily, fortification may then be essential in order to achieve good nutritive value. Rice protein content is low, although of good quality, containing higher levels of the limiting essential amino acids than wheat (Hansen et al. (1981). Eggum (1976) reported that rice protein had proportionally more lysine and threonine than wheat and a comparable tryptophan percentage. However, rice protein is still not ideally balanced, being relatively low in lysine when compared to the FAO Reference Pattern. In reviewing amino acids and their levels of indispensability to man, Waslien (1988) classified lysine and threonine as 'totally indispensable', while tryptophan, fell into the second category of less indispensable. The increase in lysine and threonine obtained with the cereal supplementation would be of great nutritional value (Eggum, 1976; Bechtel and Pomeranz, 1980).

Hansen et al. (1981) discussed a technique for producing a high-protein rice flour, by alpha-amylase digestion. Bean and Nishita (1983) discussed the possibility of some of it being included in a rice flour bread. Considering the various processes to which the flour has to be treated, it is possible that the functionality in baked goods would be hampered. So far, no positive results have been obtained for the production of a high-protein rice flour which will be able to function effectively if used at 100% in a rice flour bread.

Soy protein products have been used to increase the nutritional quality of breads. Soy flour, the simplest form of soy protein, with a typical protein content of around 50%, is produced by grinding and screening defatted bean flakes.

Soy protein isolate which is approximately 90% protein, is produced by drawing the protein out of the flake through solubilization and separation, followed by isoelectric precipitation. Soy protein concentrate is extracted from the beans by various methods, to yield a final product with a protein content of approximately 70%.

Soy protein concentrates and isolates have been less widely promoted than soy flour for use in bakery products (Elgedaily et al, 1982), although the concentrate has been used to some extent in specialty breads and high-protein cookies. Soy protein concentrates are advantageous due to their high protein content and low fat level (Onayemi and Lorenz, 1978), together with reduced soluble carbohydrates and colour components, and increased flavour quality (Bradford and Orthoefer, 1983).

Pyler (1988) in discussing the effects of soy proteins on bread quality, reported the improvement of physical qualities such as the crumb structure through a more uniform expansion of the dough. Soy also imparts a firmness to the crumb which reduces the tendency toward doughiness. Although soy flour has been most used, the upper limit is generally determined by its effect on processing and final product quality (Pyler, 1988). As low as 10% soy flour affected bread characteristics adversely, which was even more pronounced with 10% of the concentrate or isolate. French (1978) indicated that at soy flour levels > 6% it was necessary to use a surfactant such as sodium stearoyl lactylate. Onayemi and Lorenz (1978) found that wheat flour breads with soy protein were higher in volume than those with soy flour. Ranhotra (1975) reported preliminary studies with non-wheat flours which indicated that soy flour and protein concentrate could not be used at high levels without decreasing bread quality.

In order to increase the protein content of a rice/ cassava bread a soy protein concentrate has been proposed. Although soy protein concentrate is more expensive than soy flour, its use can be justified in the nutritionally improved product which would be achieved with less likelihood of quality deterioration (French, 1978). Supplementation with the concentrate would reestablish the bread protein which would be decreased with the addition of cassava flour or starch.

Collins and Temalilwa (1981) reported that the dough made from cassava and soy flours was softer and less sticky to handle than the dough with no soy. They finally concluded that up to 15% soy in the cassava dough improved the quality of the dough as well as the nutritional value.

Flavour has also been found to be a prominent factor when determining acceptability of these protein supplements (Christianson et al., 1974; Gim and Lin, 1978; Ory and Conkerton, 1983; Mustafa et al., 1986). Kalbrener et al. (1971) found that the intensity of the flavour of soy products was in some part determined by the processing method used.

Christianson et al. (1974) reported that typical soy flavours could not be detected up to the 15% level of concentrate or isolate, but the sensory scores for flavour were low because the bread did not have a conventional wheat bread flavour. Henselman (1974) was able to confirm flavour as the limiting factor in using high levels of soy as the sole protein source for fortification. Malcolmson et al. (1987) discussed the flavour problems associated with the use of non-conventional proteins in foods. This was attributed to the potential of proteins to bind flavours, thereby making it difficult to develop products with acceptable flavour.

Gim and Lin (1978) examined the rheological feasibility of incorporating flour from the winged bean as a protein supplement in a cassava-wheat bread. The high protein content of the bean (46.5%) would both increase the protein levels in the breads and complement the low-lysine of the wheat. They reported that the addition of the bean flour helped to counteract the poor effects on the dough, of the high percentage of cassava flour.

Silaula et al (1989) recognized the deleterious effects of protein fortification on the functionality of bread doughs, when the amount of legume flour used was sufficient to achieve the desired nutritional benefit. However, the use of dough strengtheners and surfactants resulted in satisfactory bread characteristics. The researchers discussed the functionality problems in relation to the tendency of the plant proteins to disrupt the well-defined protein-starch complex of the wheat flour dough. According to Fleming and Sosulski (1978) the concentrated proteins cause small pores and a ruptured cell structure in bread. The legume proteins interact with the gliadin and glutenin in the wheat and interfere with gluten formation, resulting in reduced dough elasticity (Pyler, 1988). In addition, the binding power of soy provides resistance to dough expansion. This can be overcome somewhat by adjusting the water levels in the dough and allowing a longer proofing time.

Raidl and Klein (1983) found that 5% and 15% soy flour quick bread loaves had bigger volumes than wheat quick breads and suggested that since gluten development was not critical for this process, the dilution of gluten would affect yeast breads more than quick breads.

Using Gums To Replace Gluten Functionality

Two characteristics which make wheat unique are its ability to form a dough when mixed with water, and the ability to retain the gas produced during fermentation to produce a leavened product (Hoseney, 1986). The protein of rice will not develop a film that is capable of holding fermentation gases, thus rice cannot be substituted directly for wheat in a yeast-leavened product (Bean and Nishita, 1985).

Since gluten is really the skeleton of a wheat dough and largely determines its physical character (Kent-Jones and

Mitchell, 1962), then there needs to be some material which could suitably perform similar functions in non-wheat flour and starch doughs. Hoseney (1986) reported that two different components, gums and surfactants, appear to enhance the ability of gluten- free systems to retain gas. Reported data have clearly shown that use of these substances result in gas retention in gluten-free systems (Ranhotra, 1975; Nishita et al., 1976; Ylimaki, 1987).

Hydrocolloids, or hydrophilic colloids, commonly referred to as gums, are important because they influence textural properties of food systems (Igoe, 1982). They impart desirable functional properties such as thickening, gelling, emulsifying, binding and oil resistance (Glicksman, 1963; Frost et al., 1984). In baked products gums have become increasingly important ingredients because of their ability to modify texture, improve water retention and maintain shelf life quality. The use of gums with gluten lacking non-wheat flours and starches is essential for maintaining structure.

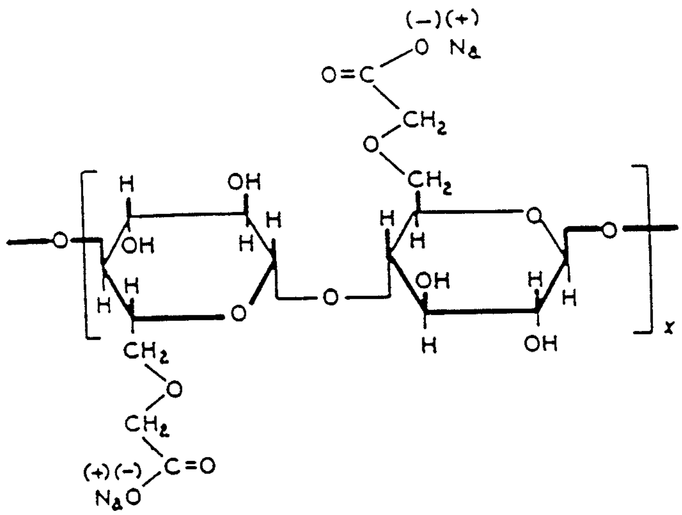
Several studies have been conducted to investigate starch-gum interactions (Igoe, 1982; Christianson et al., 1981; Sanderson, 1982; Ghasi et al., 1982). Christianson et al. (1981) described the two stages of swelling of the starch granule. They explained that the first stage is not normally detected by the amylograph. However, when CMC or other gums were added to the solution, the two stage swelling became apparent and the gelatinization temperature was recorded earlier. Further investigation of the starch interaction with other hydrocolloids during pasting and gelation may show a possible means to improve stabilization of starchy types of foods (Christianson et al., 1981).

The three gums of particular interest for this study are sodium carboxymethycellulose (CMC) and hydroxypropylmethylcellulose (HPMC),cellulose derivatives, and xanthan (X) which is obtained from microbial fermentation. These three gums have been advocated for use in non-wheat and composite flour baked products. The characteristics of each gum depend on its viscosity-producing capability when dispersed in water. The solution properties of one gum can often be modified by interactions with others.

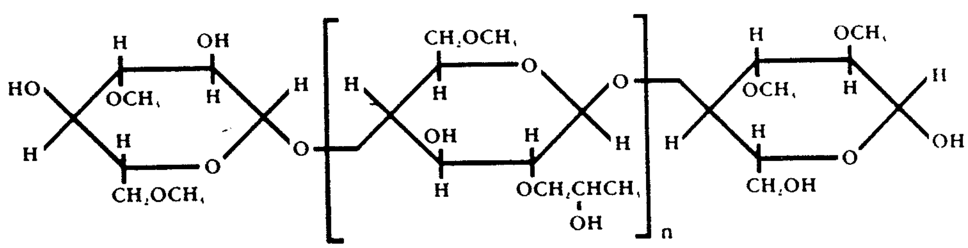
Sodium carboxymethylcellulose is an anionic, water-soluble polymer, and is widely used in the food industry (Ganz, 1977). It is by far the most important cellulose derivative for food applications (Torres and Thomas, 1981). The gum is prepared by treating cellulose with sodium hydroxide then reacting it with sodium monochloroacetate under rigidly controlled conditions (Hercules, 1984). The product is then purified to remove the salts. The structural formula is shown in Figure 2.1. Various types of CMC are available with different degrees of substitution (DS), and within these types there are several viscosity grades. The most widely used types of cellulose gum have a DS of 0.7, or an average of 7 carboxymethyl groups per 10 anhydroglucose units. The DS determines the compatibility of the gum with other soluble components. According to Igoe (1982) CMC shows a synergistic effect when blended with nonionic polymers such as HPMC.

Carboxymethylcellulose has been used in the production of breads, using wheat starch and rice flour. There has been limited success reported when CMC has been used alone. McGreer (1967) reported that a wheat starch/potato flour bread made with CMC had a relatively low volume with an even but coarse structure. This bread was not heavy, however, sliced without crumbling and remained tender over six days of storage. Kulp et al. (1974) described a wheat starch bread with CMC as structurally acceptable but with low volume and poor grain and texture. Nishita et al. (1976) reported a compact, gummy texture and low loaf volumes for breads made with CMC. These results did not differ whether the gums were hydrated or dry.

Hydroxypropylmethylcellulose (Figure 2.1) which is nonionic and water-soluble, is also widely used. This gum is prepared by reacting alkali cellulose with propylene oxide. The cellulose backbone is a basic repeating structure of anhydroglucose units (Dow Chemical Co., 1988). In each type of HPMC there are varying ratios of hydroxypropyl substitution to methoxyl substitutions, a factor which influences solubility and the thermal gel point of aqueous solutions.



A



B

Figure 2.1. Structural formulae for sodium carboxymethylcellulose (A) and hydroxypropylmethylcellulose (B)

As with CMC, the degree of substitution determines the properties of various types of HPMC.

Glicksman (1963) indicated that certain methylcelluloses, such as Methocel, 65 HG, 4000 cps (HPMC), have a gel point in the same range as the gelation temperature of starches and the coagulation temperatures of wheat proteins. He reported that they were therefore effective for use with gluten-free flours and starches. During baking, the thermal gel structure aids in gas retention, increasing volume and uniformity of texture particularly in products such as rice breads (Dow Chemical Co., 1985). De Ruiter (1978) reported that HPMC in a wheat/cassava starch bread produced a soggy and rubbery crumb, but the results of a composite containing potato starch in place of cassava gave much more acceptable crumb texture. From among several gums tested, Nishita et al (1976) found only the methylcelluloses provided the proper dough viscosity and film-forming characteristics (Bean and Nishita, 1983). These gums hold water at room temperature and release it during heating, so allowing the starch to gelatinize (Nishita et al., 1976), which is precisely the function of hydrated gluten in wheat bread. Nishita et al. (1976) reported very good volume from use of K4M (HPMC) but despite the desirable volume, other characteristics such as crumb texture, cell size and crumb cell size uniformity were less than acceptable.

Nishita et al. (1976) reported that the only gum that permitted gas retention in rice flour bread was HPMC, but

Ylimaki (1987) reported that a combination of CMC and HPMC produced a more desirable breads than either gum used singly.

Xanthan gum is a high-molecular-weight polysaccharide produced by the microorganism Xanthomonas campestris. The polymer backbone is made up of beta-(1 - 4) -linked D-glucose residues (Figure 2.2) and is therefore identical to the cellulose molecule (Kelco Division, 1985). This cellulosic backbone is rendered water-soluble by the presence of short side chains attached to every second glucose residue in the main chain (Torres and Thomas, 1981). The fact that the side chains shield the backbone could be the major reason for the extraordinary enzymatic resistance of the gum. (Kovacs and Kang, 1977). The presence of the beta-(1-4) linkages and the specific nature of the branching, is responsible for the structural rigidity of the polymer, and results in several of the unusual properties of the gum.

Xanthan is compatible with starch to give viscosity and form a gel (Kelco Division, 1980). Igoe (1982) discussed its interactions with several other gums in foods such as pie fillings and beverages, but did not mention such combinations in breads. A number of researchers have, however, used xanthan in gluten-free breads (Kulp et al., 1974; Ranhotra, 1975 ; Nishita et al, 1976). Kulp et al. (1974) reported a wheat starch and xanthan bread superior in volume, general properties and flavour to breads made with other gums.

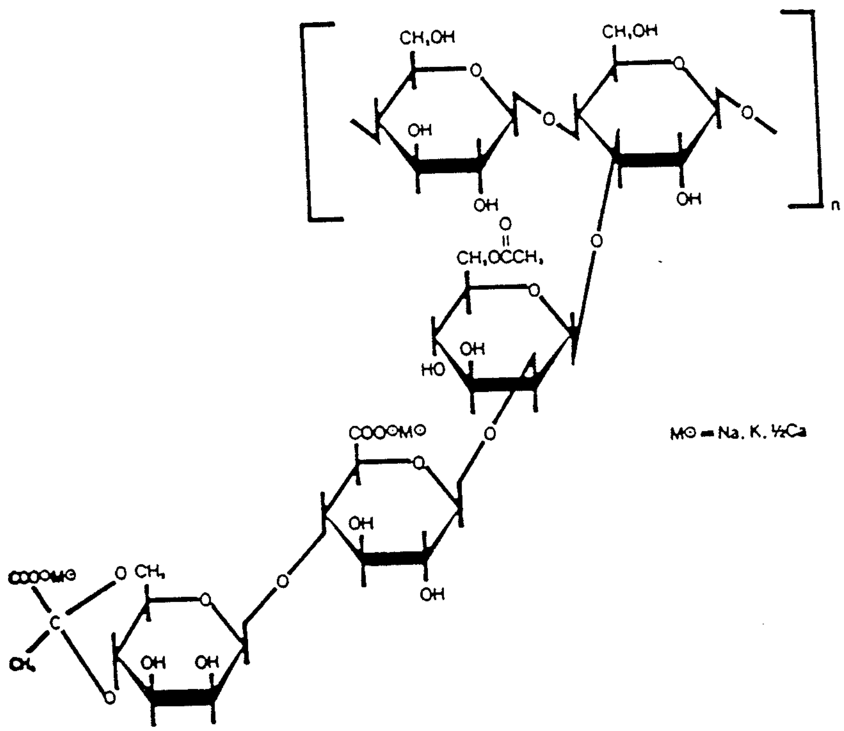


Figure 2.2 Structural formula for xanthan gum.

Nishita et al. (1976) confirmed the successful use of xanthan in wheat starch bread, but were unable to explain their inability to achieve similar results with rice flour. Satin (1988) was successful with xanthan in cassava flour breads and further reported that xanthan gum gave better results when hydrated than when dry. The product was light and airy with a less dense and compact crumb. Christianson et al (1974) indicated that xanthan was reported to improve the cohesion of starch granules and produce a bread-like structure comparable in appearance, mouthfeel, loaf volume, and staling to most commercial breads.

Objective Bread Evaluation

Objective methods of evaluation employed to determine bread quality can be chemical or instrumental. The focus here will be on instrumental measurements because the main interest is on the textural characteristics of the bread. Bread evaluation methods have been developed for wheat breads and the tendency is to evaluate non-wheat breads by wheat bread standards, although gluten-free breads generally differ considerably from these standards. Asselbergs (1970) reported that composite or non-wheat breads should be judged on their own merit and not in comparison with wheat breads. It is therefore necessary to develop a set of standards by which gluten-free breads may be evaluated. Ylimaki (1987) suggested that objective measurement of the quality characteristics of gluten-free breads, particularly texture, is important for the production of acceptable gluten-free breads.

Usually the validity and usefulness of objective measurements is dependent upon their agreement with sensory evaluation (Funk et al, 1969). This, however does not hold true particularly when the responses of the sensory evaluation are dependent upon the acceptance of a consumer group for which the product is not intended. Brady and Meyer (1985) attempted to find correlations between sensory and instrumental measures of bread texture, but suggested that further studies should be conducted to determine whether the two tests were measuring the same parameters. Betker (1990) reported that sensory cohesiveness did not correlate with Instron cohesiveness. In fact it appeared that what the Instron recorded as cohesiveness was perceived by the sensory panel as springiness. More research is necessary to understand the relation of test parameters measured by the Instron to human perception (Betker, 1990). Objective measurements offer greater precision than sensory evaluation and the measurements are repeatable (Funk et al., 1969). Those measurements most commonly used for breads include loaf volume, crumb structure and firmness , and crumb colour.

Bread loaf volume is one of the most common tests conducted to evaluate bread quality. The measurements are taken by rapeseed displacement using a volumeter. Several studies have used this technique, taking a mean of two or more measurements on each loaf (Rasper et al., 1974; Crabtree et al., 1978; Elgedaily et al, 1982; Guy, 1984; Youssef and Bushuk, 1986; Johnson, 1990). Gim and Lin (1975) used sago 'pearls' with a diameter of 2mm. Specific volume which indicates the compactness of the loaf (Gim and Lin, 1975) is usually obtained by dividing loaf volume by weight. Campbell et al. (1979) stated that it allows a comparison of products with different weights.

Cell structure may be studied from permanent recordings such as ink prints or photography (Funk et al., 1969). Early studies were reported in which ink prints and photography were successfully used to provide comparative evaluation. Cooley and Davies (1936) discussed techniques for reliable permanent records of baking studies using photography. The study of cell size and cell distribution can be greatly enhanced by photocopies. According to Campbell et al. (1979) photocopies provide a record of actual size, shape and grain of bread. Crabtree et al. (1978) used photographs for visual evaluation. The photocopies or photographs may be used in combination with the type of scoring used by Faridi and Rubenthaler (1984), where the characteristics are graded from excellent to unsatisfactory, with corresponding values.

Texture is among the most important factors in consumer acceptance of bread (Ponte and Faubion, 1985). In the determination of bread quality, firmness is considered the major factor (Hibberd and Parker, 1985; Ponte and Faubion, 1985). Firmness is defined as a measure of bread crumb resistance to deformation.

Instruments used to measure firmness include the Precision Penetrometer (Rasper et al, 1974; Kamel et al., 1986), the Baker Compressimeter (Lorenz and Dilsaver, 1982; Kamel and Rasper, 1986), the GRL Compression Tester (Kilborn et al., 1983), the Voland Stevens LFRA Texture analyzer (Persaud et al., 1990) and the Instron Universal Testing Machine (IUTM), an instrument designed for many applications (Baker and Ponte, 1986). Hibberd and Parker (1985) stated that it was not possible to select a "best set" of conditions for crumb texture measurements using the IUTM, because of the varied factors and conditions which are presented in each study. However, Baker and Ponte (1986) developed a standard method for measuring bread crumb firmness with the IUTM. This has since been published as an AACC standard method (74-09). The IUTM has been used in several studies with various attachments such as the Kramer extrusion cell (Collins and Temalilwa, 1981), the compression cage (Elgedaily et al., 1982), in compression mode with a Kramer shear-compression cell attachment (Rogers et al., 1990), and with plungers (Bashford and Hartung, 1976; Baker et a., 1986; 1988; Ylimaki, 1987). Using the IUTM additional texture parameters can be measured such as cohesiveness, gumminess and springiness. Elgedaily et al. (1982) tested all those parameters on soy-enriched wheat yeast breads.

Crumb and crust colour are also very important in evaluating bread appearance. However, these parameters are not evaluated frequently except when composite flours are being used or other additives such as protein supplements are included in the formulation. Colour has been measured instrumentally by a Gardner Colour Difference Meter (Raidl and Klein, 1983; Johnson, 1990) and by the Hunter colour Difference Meter (Onayemi and Lorenz, 1978; Chen and Rasper, 1982; Ylimaki, 1987).

Moisture content of bread crumb is not often measured unless there is a major concern with water activity in the dough. It is a measure of the water content of the dough from which the bread was baked, rather than criterion of adequate baking and proper crust formation (Czuchajowska et al., 1989). Some studies have measured moisture content using the oven-drying method which is most popular (Pulle and Ino, 1975; Nishita et al., 1976; Ylimaki, 1987; Czuchajowska et al., 1989)

Generally it seems that volume, cell structure, texture as well as crumb and crust colour can be evaluated in standardized tests to give a good indication of bread quality.

Response Surface Methodology

Response Surface Methodology (RSM) is a technique which considers several factors (ingredients) at different levels in a product, as well as the corresponding interactions among these factors and levels, simultaneously. Henika (1982) explained that the method tests several variables at a time while using special experimental designs to cut costs. Only a fraction of the treatment combinations need to be produced and examined, and statistical methods are used to predict remaining results. Theoretically, formulations can be derived from the data which optimize the response characteristics (Neville and Setser, 1986).

This type of experimental design is becoming popular in food product development. Henselman et al. (1974) demonstrated the compatability of the breadmaking process and sensory evaluation with RSM, in developing an acceptable loaf. They also suggested that confirming tests should be made and the products evaluated to ensure success. Several other studies (Giovanni, 1983; Ooraikul et al., 1983; Joglekar and May, 1987; Ylimaki, 1987; Payton et al., 1988; Rubenthaler et al., 1990) support the validity of this technique.

Joglekar and May (1987) discussed the reliability of the technique compared to the one-variable-at-a-time approach. Using the RSM design in which both factors, as well as their interactions were considered simultaneously, the benefits of this design were aptly demonstrated. An important aspect of this design is in its ability to consider interactions. Ylimaki (1987) used the technique and found several optimum combinations of HPMC, CMC and water that could result in rice breads with characteristics comparable to those of the reference wheat bread used. She concluded that RSM can be successfully applied to the development of acceptable rice bread.

The survey of literature confirms the feasibility of developing a soy-enriched rice/cassava yeast bread. Cassava starch and potato starch have been shown to have similar characteristics, and it appears that a successful substitution could be effected. Soy protein concentrate, if used within specific limits might not affect bread quality adversely, in fact might even improve the quality, and it would also be used for enrichment. The use of RSM to optimize and predict successful formulations would allow a significant reduction in the number of treatments which would be otherwise required. It seems quite probable that such a soy-enriched rice/cassava yeast bread could be developed, with due consideration being given to the problems which previous researchers have encountered.

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Chapter 3

A COMPARISON OF THE GELATINISATION TEMPERATURES AND PASTING CHARACTERISTICS OF NON-WHEAT FLOURS AND STARCHES WITH AND WITHOUT ADDED GUMS

INTRODUCTION

The development of non-wheat breads has very important implications for developing countries. Satin (1988) suggested that the economy of developing countries depended on their agriculture base, and would benefit if they could reduce their dependence on imported wheat for white bread and increase their use of locally grown crops. He also reported that several formulations had been developed for non-wheat breads, using rice, cassava and other indigenous flours and starches, with varying degrees of success.

Nishita (1973) reported the development of a 100% rice bread formulation but later (1977) recommended improvement of the bread by replacing 20% of the rice flour with potato starch. The rice/potato formula was later improved by Ylimaki (1987). Nishita and Ylimaki both developed a product with special emphasis on individuals with coeliac disease and other allergies. However, their formulae could provide a useful basis for further product development. More recently the production of other non-wheat composite flour breads using rice and cassava among others have been reported (Casier et al., 1979; Satin, 1988).

The nature of bakery products is influenced by interrelated effects of flour constituents and physical properties of water-flour mixtures (Pulle and Ino, 1975). In addition, during baking, gelatinization of starch within a loaf of bread influences the condition of the crumb (Gim and Lin, 1978). These effects were confirmed by Nishita and Bean (1979) when they reported that the behaviour of rice flour slurries during gelatinization had a direct relationship to the interior texture of breads made from the same flours. After comparing chemical and physicochemical data with quality evaluations for thirteen different types of rice, these researchers concluded that it was essential to use flours from low amylose (< 20%) varieties, which also had low gelatinization temperatures (<65°C) in order to produce bread with a soft rather than sandy texture. Nishita and Bean (1979) also suggested that low amylose, low gelatinization temperature and low amylograph paste viscosity after cooling to 50°C, might be used to predict breadmaking quality of rice flours.

The Brabender amylograph is used most frequently in gelatinization determinations (Gim and Lin, 1978; Nishita and Bean, 1979; Christianson et al, 1981; Faridi and Rubenthaler, 1984). According to Gim and Lin (1978) the amylograph simulates the effect of baking on starch. The instrument works on a programmed heating and cooling cycle and supplies information on the gelatinization temperatures and pasting properties of various starch slurries. The results are taken from a curve which is charted during the process. The significant points on the curve which provide the information required, have been discussed by Shuey and Tipples (1980).

The main problem with rice flour bread is the lack of gluten which serves to provide structure and retain the air in wheat flour breads. Gums have been used to compensate for the absence of gluten in rice breads. Nishita (1973) found hydroxypropylmethylcellulose (HPMC) to be most successful in 100% rice flour breads, while Ylimaki (1987) reported improvement in loaf shape and bread crumb texture with a combination of sodium carboxymethylcellulose (CMC) and HPMC. Satin (1988) reported success with xanthan in cassava breads although he further recommended the use of pregelatinized starches in the formulations, in order to eliminate the problems related to the importation and probable high cost of the gum. Other studies (Christianson et al., 1974; Ranhotra et al., 1975; Sanderson, 1982;) also reported the use of CMC, HPMC and xanthan with satisfactory results.

The physicochemical characteristics of flours and starches appear to have a critical influence on their performance in breads. Therefore the purpose of this study was to determine the feasibility of using cassava flour or starch to replace potato starch in a rice bread formulation, by comparing gelatinization temperatures and pasting properties of flours and starches with and without added gums. The main criteria for evaluation were the gelatinization temperature (GT), peak viscosity (PV) and viscosity when cooled to 50 °C (CV).

The specific objectives were therefore to:

1. determine and compare the gelatinization temperatures and pasting properties of rice flour, cassava flour, cassava starch and potato starch.
2. examine the effects of the addition of cassava flour, cassava starch and potato starch, on the pasting properties of rice flour.
3. examine the effects of the gums CMC, HPMC and xanthan, on the gelatinisation characteristics of rice flour and rice flour/cassava starch blends.

MATERIALS AND METHODS

Materials

Finely ground, long grain, white rice flour was obtained from Dainty Foods Inc., Dorval, QB, and potato starch (Casco Potato Flour) from Canada Starch Co. Etobicoke, ON. Cassava flour and cassava (tapioca) starch were provided by Grain Process Enterprises Ltd. Scarborough, ON. Xanthan gum (Keltrol F) was supplied by Kelco Division, Merck and Co., Inc. Rahway, N.J., U.S.A. Sodium carboxymethylcellulose (7HF) was obtained from Hercules Inc. Wilmington, Delaware, and hydroxypropylmethylcellulose (Methocel K4M) from Dow Chemical

Co.,Midland Michigan.

Analytical Methods

The standard AACC methods (AACC 1983) were used to analyze flour and starch samples for protein , ash, moisture and damaged starch. For the Kjeldahl procedure, titanium oxide was used as the catalyst as recommended by Williams (1973). Fat content was determined by extracting ground freeze-dried samples with petroleum ether for sixteen hours in a Soxhlet apparatus. Amylose was determined by iodometric titration as described by Schoch (1964). Total starch was determined by the method described by Aman and Hesselman (1984), with the following modifications: 10 ml 0.2M acetate buffer was used instead of 25 ml 0.1 M and 200 ul instead of 100 ul of thermostable alpha-amylase. Overnight incubation was at 35°C rather than 60°C. These methods are given in detail in Appendices 1-4.

Gelatinization Temperatures and Viscosity Measurements

Gelatinization temperatures and pasting characteristics of the flours and starches were determined with a C.W. Brabender VISCO/amylo/GRAPH (model 30591/66) equipped with a 700 cm.g sensitivity cartridge and a cooling coil, and using a rotation speed of 75 rpm. Twenty percent slurries were used to determine the gelatinization temperature of all the flours and starches. This was taken as the point where the viscosity curve left the base line. For pasting characteristics, the method of Halick and Kelly (1959) was used although for this study all slurries were 8% except for the potato starch slurry which was 4.6%. Slurries were initially heated to 50°C (Shuey and Tipples, 1980) instead of 30°C, followed by heating in which the temperature was increased at a rate of 1.5°C per minute, to 94°C. Pastes were then held at this temperature, with continuous stirring for 20 min. They were cooled by lowering the temperature at the same rate to 50°C. The final holding period was for 20 minutes at 50°C.

Experimental Design

Amylograph curves were run for each of the flours and starches according to the design shown in table 3.1. Graphs were used to visually evaluate the effects on gelatinisation parameters.

Table 3.1. Experimental Design

Values presented as percentages

TRIALS	RICE FLOUR	CASSAVA FLOUR	CASSAVA STARCH	POTATO STARCH
1 A	100	--	--	--
1 B	--	100	--	--
1 C	--	--	100	--
1 D	--	--	--	100
2 A	80	20	--	--
2 B	80	--	20	--
2 C	80	--	--	20
2 D	60	40	--	--
2 E	60	--	40	--
3 A - O*	80	--	20	--

*With xanthan, CMC and HPMC each at five levels (0.8, 1.2, 1.6, 2.4, 3.6). A total of 15 trials.

RESULTS AND DISCUSSION

Chemical composition, damaged starch values and gelatinization temperatures (GT) for the two flours and the two starches used in the study are given in Table 3.2. During amylose determinations, the results for the rice flour were variable but consistently lower than 20%. For reporting purposes it was estimated to be 19.2%, based on Nishita and Bean (1979). The GT was 64°C (low). Although identified by the supplier as a long grain rice flour, the flour conformed to the specifications set out by Nishita and Bean (1979). The best flour in Ylimaki's experiment (1987) had a GT of 64.5°C. The cassava flour and starch as well as the potato starch had < .15% protein.

Total starch values for the potato starch, cassava flour, cassava starch were 83% to 88%, while for the rice flour total starch was 77%. Ketiku and Oyenuga (1972) as well as Gaillard (1987) reported similar total starch values. The amylose content of the two cassava products were similar (17%) while that of the potato starch was appreciably higher (25.6%). These values correspond with those cited in Heckman (1977) and Okezie and Kosikowski (1982). Absence of damaged starch in the cassava and potato starches was not surprising since they were likely extracted by other than mechanical means. The damaged starch value for the cassava flour was very high (76 F.U.). Canadian Grain Commission Report (1989) gave starch damage values for bread flours as ranging from 19 to 31 F.U.

Table 3.2. Gelatinization temperature and compositional analysis of flours and starches[1]

SAMPLES	MOISTURE (%)	PROTEIN[2] (%)	FAT (%)	ASH (%)	TOTAL STARCH (%)	AMYLOSE (%)	STARCH DAMAGE (F.U.)[3]	GEL TEMP (° C)
Rice Flour	12.20	7.25	1.20	0.66	77.1	19.2[4]	34	64
Cassava Flour	11.40	0.14	0.16	0.06	85.4	17.5	76	59
Cassava Starch	12.60	0.06	0.02	0.04	88.0	17.1	0	62
Potato Starch	13.40	0.06	0.03	---	83.4	25.2	0	58

[1] All values recorded on "as is" basis

[2] PROTEIN = %N x 5.95 for rice flour
PROTEIN = %N x 5.70 for cassava

[3] Farrand units

[4] Estimated from Nishita and Bean (1979)

Williams and LeSeelleur (1970) stated that starch damage indicated the proportion of flour available to contribute toward gassing power and water absorption. They further reported that excessive levels resulted in over-absorption of water and, consequently, in deterioration of the crumb and loaf volume of the bread. Rapid absorption was apparent with the cassava flour slurry which had obvious granule swelling even as the slurry was being mixed. It was quite viscous before being placed in the amylograph. Chabot et al (1974) found that cassava granule swelling occurred before any rise in viscosity was recorded on the amylograph. Ciacco and D'Appolonia (1978) suggested that the high content of damaged starch present in cassava flour might restrict the amount which could be used in blends.

Gelatinization temperatures ranged from 58°C for the potato starch to 64°C for the rice flour. Potato starch reached its GT in the shortest time. Pyler (1988) reported some correlation between the time at which gelatinization began and the crumb characteristics of a finished loaf. He concluded that the longer the time that elapses before gelatinization is initiated the poorer is the loaf crumb quality.

Pasting curves of the two flours and two starches used in this study are illustrated in Figure 3.1. Eight percent slurries were used with the exception of the potato starch slurry which was 4.6%. Cassava starch and potato starch

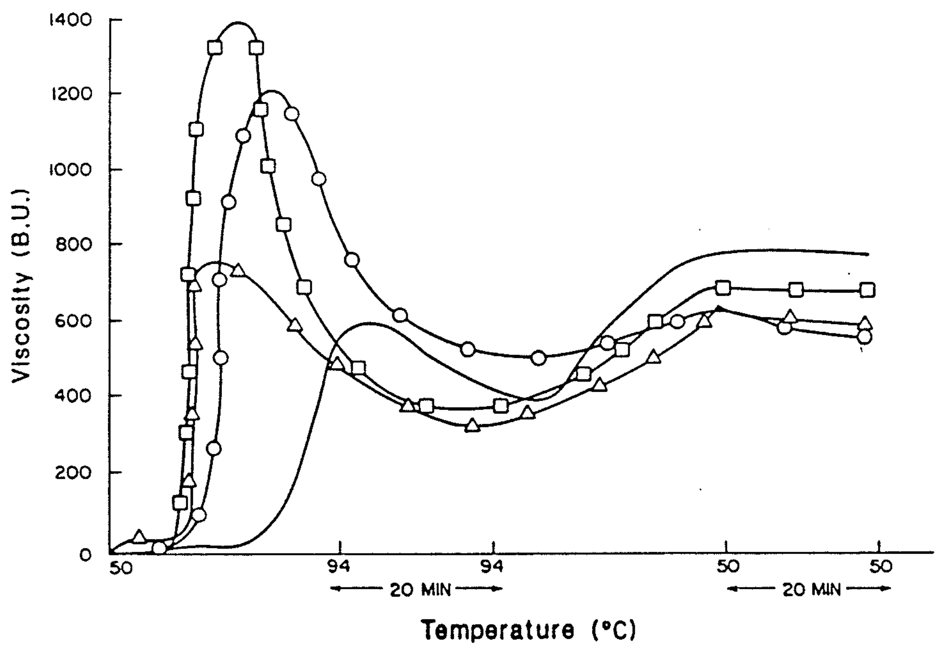


Figure 3.1. Amylograph pasting curves for 8% slurries of rice flour (———), cassava flour (—△—) and cassava starch (—□—) and for 4.6% slurry of potato starch (—○—).

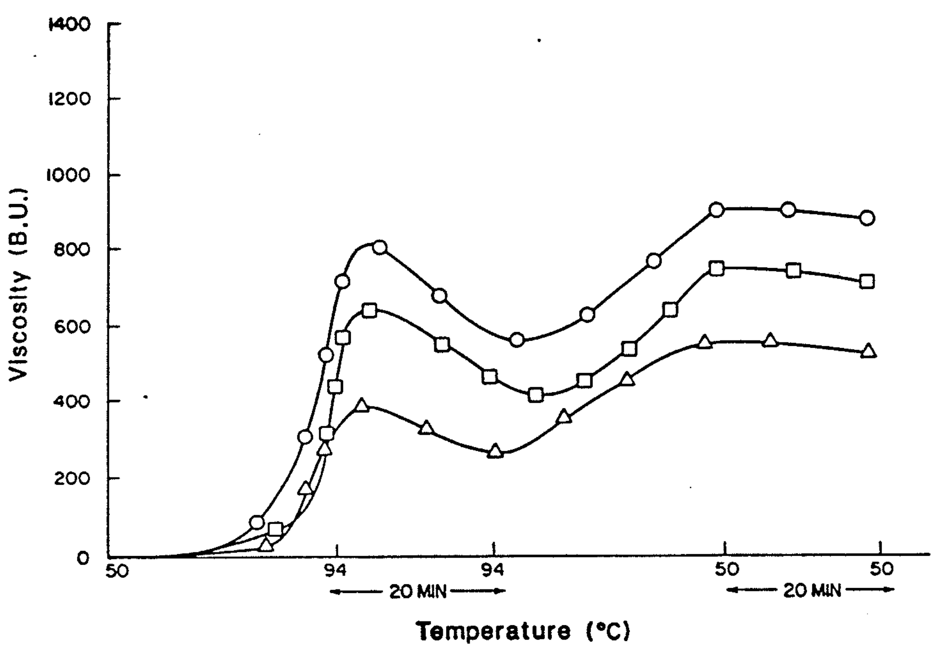


Figure 3.2. Effect of the 80/20 blends of rice flour with cassava flour (—△—), cassava starch (—□—) and potato starch (—○—) on amylograph pasting curves.

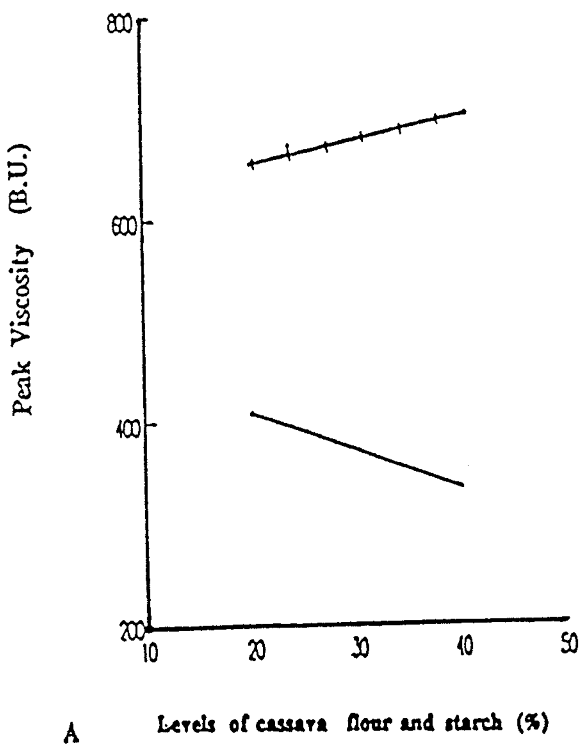
displayed sharp increases in viscosity at 81.5°C and 75°C respectively. They both became very viscous and reached peak values of 1400 and 1200 B.U. This was followed by a rapid drop in viscosity when the pastes were maintained at 94°C. Pulle and Ino (1975) demonstrated that viscosity in starch is associated primarily with the amylose. Generally for root, tubers and cereals, with higher amylose content viscosity values increased. Ciacco and D'Appolonia (1977) and Zobel (1984) reported that potato and cassava starches were readily gelatinized and were also easily ruptured by mechanical stirring. Swollen potato starch granules tend to disintegrate rapidly with agitation and the paste becomes less viscous. Low cooled viscosity may account for the softening effect of potato starch on the crumb of rice bread (Ylimaki, 1987). Cassava starch could well be a suitable replacement for the potato starch in rice breads, since with cassava starch rapid swelling also is followed by a dramatic fall in hot paste viscosity and final cooled viscosity is low.

The effects of blending rice flour with cassava flour, cassava starch and potato starch in 80:20 ratios are shown in Figure 3.2. Pasting curves for rice/cassava starch and rice/potato starch blends were similar although the values for the rice/potato were slightly but consistently higher.

The rice/cassava flour blend had the lowest viscosity values. Pyler (1988), in discussing dough testing, reported that curve height or peak viscosity as a consequence of alpha

- amylase activity, is considered an important indication of the type of crumb that can be expected in wheat flour breads. However, Horiuchi (1967) stated that there was little alpha-amylase in white rice and found no correlation between peak viscosity and alpha-amylase. Pyler (1988) claimed that a high peak viscosity indicates a dry crumb which will stale very quickly, while a low peak viscosity indicates the likelihood of a moist or even soggy crumb. Nishita and Bean (1979 and prior reports) confirmed this relationship for rice flour breads. The curve of rice flour/cassava starch blend shown in Figure 3.2 is similar to the rice/potato curve as they both show no sharp peak. This indicates the likelihood of acceptable crumb characteristics in a bread made from the rice/cassava starch blend.

Figure 3.3 shows viscosity values obtained when cassava flour or cassava starch was blended with rice flour at two levels (80/20 and 60/40). Overall the rice /cassava starch blend had much higher values for both PV and CV, than the rice/cassava flour blend. Whereas the cassava starch blend increased in peak viscosity when the level of starch increased to 40%, the opposite effect was observed for the cassava flour blend (A). However, the effect on cooled viscosity was similar for both blends (B). This suggests that cassava starch might be used at higher levels than 20% but an increase in cassava flour could have more deleterious effects than already indicated at 20%.



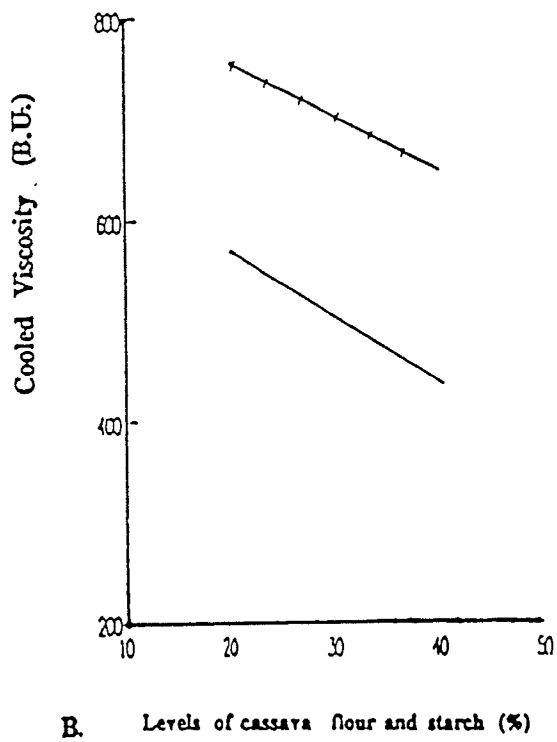


Figure 3.3 Effect of cassava flour (——) and cassava starch (++++) levels on peak viscosity (A) and viscosity when cooled to 50°C (B), of rice/cassava blends.

With the addition of gums to the slurries, there were marked differences in the pasting behaviour of the rice flour alone, and of the rice/cassava starch (80/20) blend. From Figure 3.4 it can be seen that using 0.8% CMC, the gelatinization temperature for both rice flour and the blend were similar (67°C and 66.5°C respectively). At higher levels of CMC, GT for the blend lowered to approximately 65°C while for the rice flour alone the decrease in GT was more gradual. HPMC by contrast raised the GT of the rice flour and the blend. It seemed that HPMC had a delaying effect on the gelatinization of both the rice flour and the blend, although at high levels GT of the blend was lowered significantly. With 0.8% xanthan the GT for both rice flour and the blend were similar (66.5°C and 66°C). At higher levels of xanthan , GT for the rice flour remained the same except for a slight drop at 3.6% xanthan, while for the blend, after an initial increase at 1.2% xanthan, GT decreased gradually to approximately 65°C at 3.6%.

Peak viscosities (PV) of the blends (Figure 3.5) were higher than those of the rice flour at all levels of the three gums except at 3.6% xanthan. At that xanthan level PV of the blend was lower. With each increase of CMC the rice flour alone, as well as the blends, showed a sharp increase in PV. HPMC at 1.25 and 1.6% lowered PV of the rice flour and the blends, but PV increased gradually at each higher level of the gum. Xanthan increased PV of rice flour consistently, while

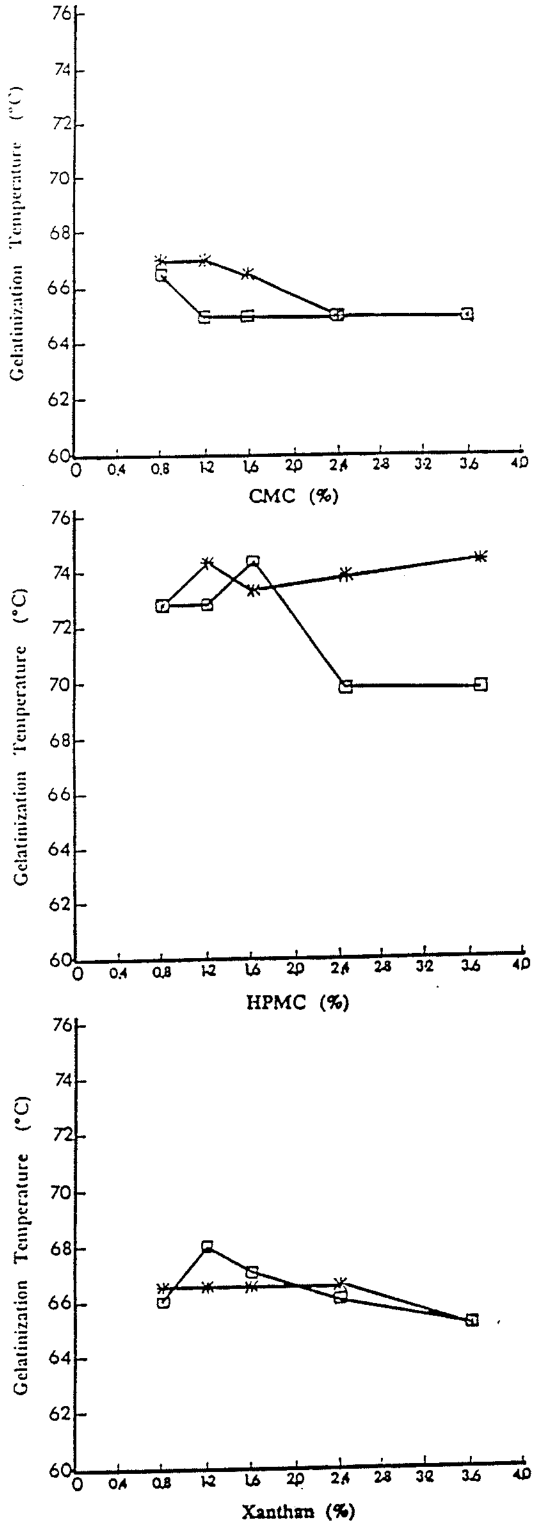


Figure 3.4. Effect of CMC, HPMC and Xanthan on the gelatinization temperature of rice flour (—✱—) and 80/20 rice flour/cassava starch blends (—□—).

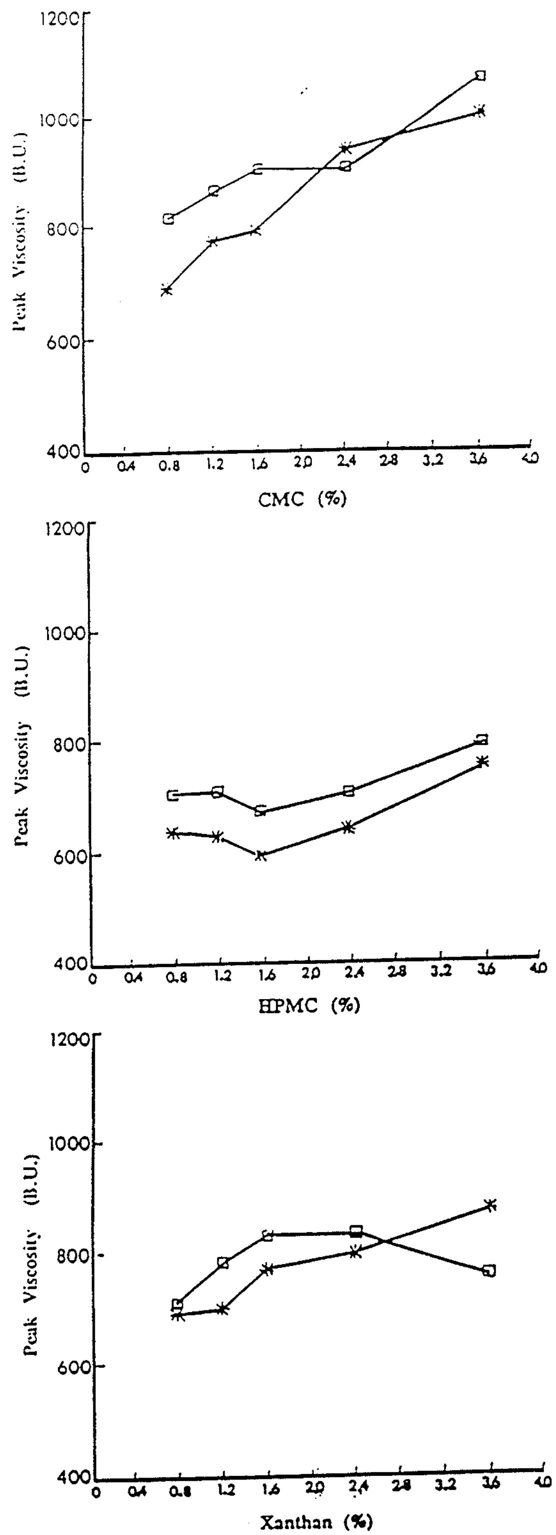


Figure 3.5. Effect of CMC, HPMC and Xanthan on the peak viscosity of rice flour (—✳—) and 80/20 rice flour/cassava starch blends (—⊟—).

for the blend there was a gradual increase with each level and a sudden decrease with the highest level. Overall it appeared that HPMC would achieve the most desirable PV if used within a range of 1.6 to 2.4%. For the cooled viscosities (CV), CMC again showed consistently higher values than the other gums, for rice flour as well as the blends (Figure 3.6). For rice flour all three gums increased CV with each increase of gum. With the blends however, CMC and HPMC increased CV with each level although with HPMC it was more gradual. Xanthan produced a sharp increase at the second level gradually decreasing over successive increased levels. Bean (1986) reported that marked viscosity increase on cooling indicated rapid retrogradation. This produces baked products with harsh dry crumbly textures within 24 hours. With all three gums the peak viscosities of the blends were higher than their cooled viscosities. Bean and Nishita (1985) suggested that this would be desirable in order to achieve a lower setback value (CV minus PV) which they reported would produce a soft crumb texture in 100% rice bread. Such an advantage in 100% rice flour bread should be increased in a blend of rice with cassava starch or potato starch as they have been reported to have a softening effect on the rice bread crumb (Ylimaki, 1987). Potato and cassava starches tend to disintegrate and become less viscous when cool therefore in a blend with rice flour would lower the CV of the rice flour. This probably explains the consistently lower values for CV

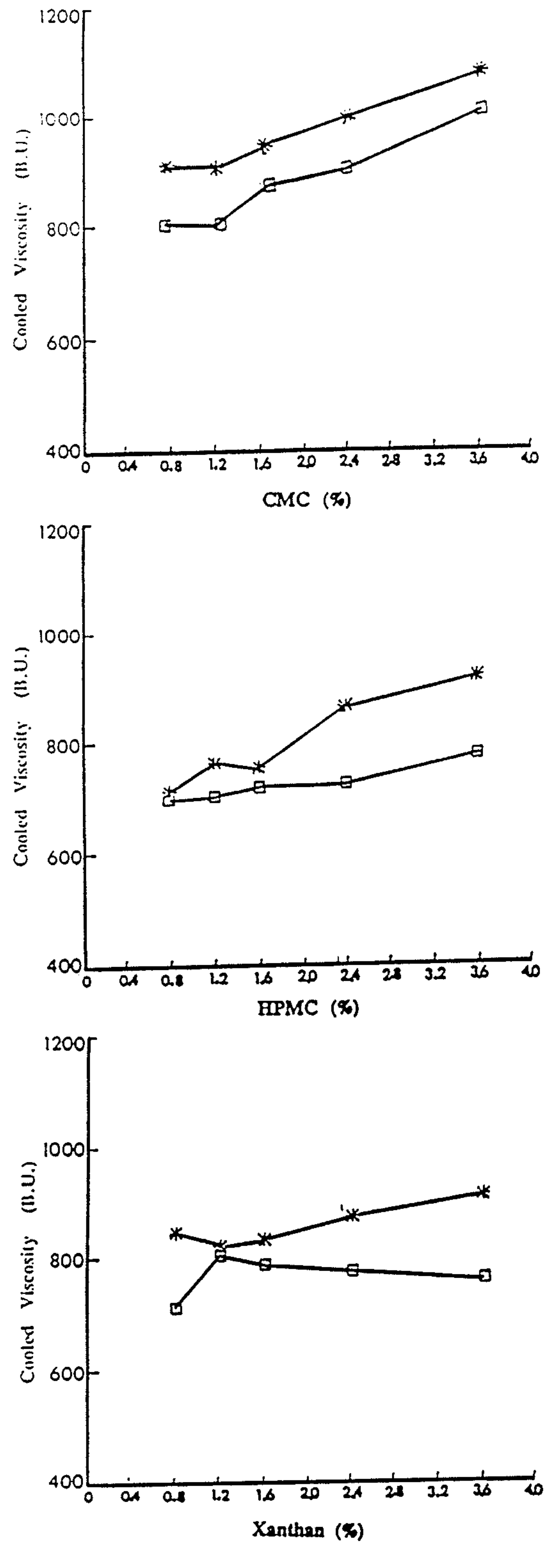


Figure 3.6. Effect of CMC, HPMC and Xanthan on the cooled viscosity of rice flour (—✱—) and 80/20 rice flour/cassava starch blends (—□—).

of the blends when compared to the rice flour alone.

Ylimaki (1987) reported that two or three gums are often more effective than any of the individual gums used alone. The use of gum combinations allows both the functional properties of the individual gums and the synergistic interactions between gums to be applied. In her study on rice flour breads it was found that a combination of CMC and HPMC produced an acceptable loaf when used in a rice/potato blend. This combination of gums was tested in this study with both the rice/potato and the rice/cassava starch blends. The results are shown in Figure 3.7. The rice/cassava starch blend had a lower CV which indicates the possibility of an - advantage for the bread crumb texture.

SUMMARY AND CONCLUSIONS

Amylograph studies were made of rice flour, singly as well as in blends with potato starch, cassava starch and cassava flour. The two cassava blends were compared to determine which was more similar in behaviour to the potato blend. The results indicated that cassava starch was a more suitable replacement for potato starch than cassava flour. The rice/cassava starch blend was then studied with three types of gums at five levels. The results indicated that while CMC was able to achieve a desirable GT, it increased peak viscosity and cooled viscosity beyond the range of

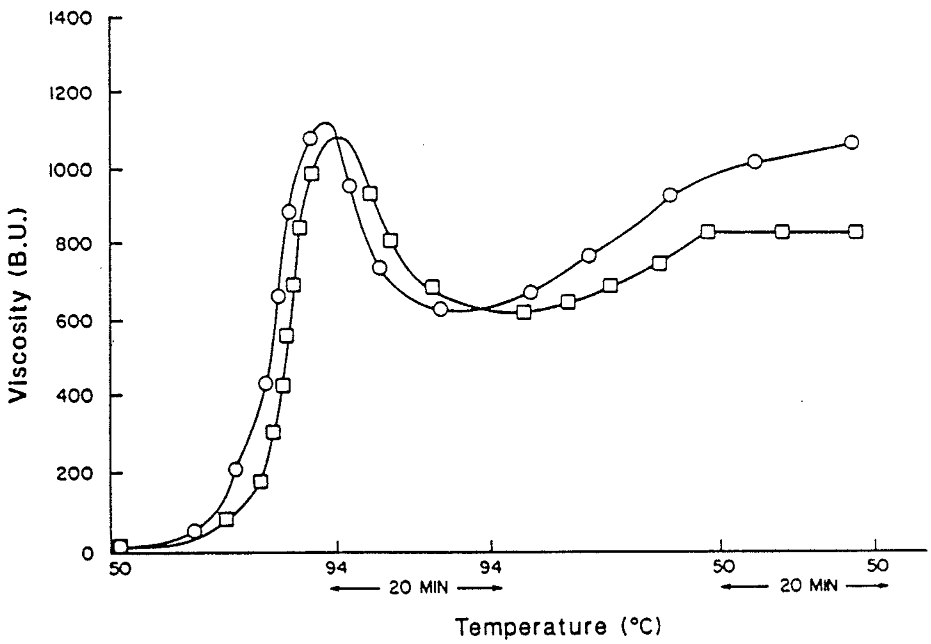


Figure 3.7. Amylograph curves for 80/20 blends of rice flour/ cassava starch (—□—)and rice flour/ potato starch (—○—), with 0.9% CMC and 3.5% HPMC.

desired values. HPMC increased GT and had an overall delaying effect on gelatinization of the rice flour alone as well as the rice/cassava starch blend, however, it produced much lower values for peak viscosity and cooled viscosity than CMC. Xanthan would need to be used at higher levels to achieve a GT of ≤ 65°C, but it also appeared that lowest level would be sufficient to achieve acceptable peak viscosity and cooled viscosity values. A further single experiment was conducted in which 80/20 blends of rice flour, first with potato starch, then with cassava starch, were combined with 0.9% CMC and 3.5% HPMC, as recommended by Ylimaki (1987). These results confirmed the similarities in the behaviour of the two blend - with and without the added gums.

It was therefore concluded from this study that cassava starch would be more suitable than cassava flour for replacement of potato starch in a rice flour based composite bread. Further to this it appeared that the combination of CMC and HPMC as used by Ylimaki (1987) would be appropriate for use in a rice/cassava bread formulation.

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Chapter 4

DEVELOPMENT AND STANDARDISATION OF A FORMULA FOR A RICE / CASSAVA / SOY YEAST BREAD

INTRODUCTION

Standardization of methodology is essential in any type of research. In this case the problem of variability in the basic ingredients had to be addressed, in addition to the establishment of a good reproducible, formula. As part of this initial work, it was also necessary to determine variables to be used in the optimization study.

The development of reliable reproducible formulae for gluten-free breads has been a problem due to the inconsistent nature of the flours in particular. Attempts to duplicate the results of reportedly successful non-wheat breads in other laboratories, have frequently been unsuccessful. Satin (1988) reported work on cassava flour breads in which he obtained satisfactory results by using pregelatinised flour in place of gums. However, there were no data presented in the article and personal communication with other researchers has indicated that the results have not, so far, proved reproducible. In further personal communication with Satin it was suggested that because of varietal differences in the cassava it would be necessary to experiment further with the formula.

Rice flour has been used in several studies and in each case factors related to the rice were suggested as the cause of the shortcomings in the final product. One such factor, the variety of rice, was shown by Nishita and Bean (1979) to be very influential in the quality of bread produced. They showed that a medium grain rice will usually exhibit favourable gelatinization characteristics, and therefore produce a satisfactory crumb. At the same time they concluded that some long grain rice varieties have desirable baking characteristics. Ylimaki (1987) stated that brands or types are often not interchangeable. Since rice flour behaviour is so influenced by variety, it would be necessary to conduct a mini preliminary test with every new batch of rice flour.

Other factors which affect the baking performance of flours / starches include the type and amount of processing of rice before milling, the type and degree of milling into flour, other ingredients in the formula and the method of bread preparation. Sharma et al (1988) studied the effects of pregelatinized rice flour in a wheat/rice composite bread and found that the pregelatinized extruded rice flour performed better than the unextruded rice flour. This was attributed to the thermal modification of the starch fraction of the rice.

Nishita and Bean (1982) found particle size to be important in the function of rice flour in breadmaking. In their study seven types of mill were used. They reported that coarser flours produced acceptable rice breads , while finer flours did not function well in 100% rice breads due to the high levels of starch damage and heating during milling.

In reporting a cassava / soy bread formula Kim and De Ruiter (1968) discussed the merits of using a remix method and second proofing period rather than a single proofing in the pan. The bread had a finer and more tender crumb texture, which was attributed to the increase in the number of gas cells when the fermented batter was remixed. Ylimaki (1987) also reported that these preparation methods gave better results for a rice/potato bread.

In our preliminary studies it was determined that a U.S. long grain rice flour (Dainty Foods Inc.) had properties similar to those of the medium grain used in Ylimaki's study. This was confirmed by amylograph studies (Chapter 3) which showed that the physicochemical characteristics were almost the same. Therefore this flour was selected for use throughout the study.

A crucial factor in making bread from non-wheat flours no matter what the base flour may be, is the need for some film-forming material which will be able to retain gas in the dough. It was found by Nishita in 1973 that fermentation time and proofed dough structure were important. Later (1976) she found water and gums to be critical. Ylimaki(1987) cited several studies in which proof time, water, gums, and the physicochemical characteristics of the rice flour were reported to be some of the reasons for differences in the rice breads produced.

Ylimaki(1987) developed a basic rice flour/potato starch bread which met approximately two-thirds of the quality criteria of a reference wheat flour white bread. The attributes of particular importance to respondents in a survey (Ylimaki et al., 1989) were flavour and texture. This agrees with Kim and De Ruiter (1968) who stated that the product will have to be palatable if it is to be accepted at all.

The ultimate aim of this research was to develop a palatable, nutritious rice/cassava/soy bread. However before any optimization could be conducted, it was necessary to standardize conditions within the laboratory, to produce the rice flour/potato starch formula which was to be used as the control throughout the study. This formula will be referred to hereafter, as Ylimaki's rice/potato formula.

The specific objectives of this preliminary study were:

1. **To test and standardize Ylimaki's rice/potato formula.**
2. **To investigate the feasibility of using cassava starch as a replacement for potato starch using Ylimaki's rice/potato formula as a basis.**
3. **To investigate the feasibility of incorporating soy protein concentrate into a rice/cassava formula.**
4. **To develop a starting point formula using rice**

flour, cassava starch and soy, which would be a basis for optimization.

5. To identify the variables most likely to have a significant effect on the physical characteristics of the loaf.

MATERIALS AND METHODS

Materials

The ingredients used for the formulations were : white rice flour (U.S. long grain, fine grind) from Dainty Foods - Inc., cassava starch from Grain Enterprise Ltd., potato starch (Casco Potato Flour) from Canada Starch Co., soy protein concentrate (Arcon F) from Archer Daniels Midland Co. Il., iodized table salt (Windsor) from Canada Salt Co., white sugar, vegetable oil (West Canola Oil) from Canbra Foods, active dry yeast (Fleischman's Traditional), sodium carboxymethylcellulose (7HF) from Hercules Inc., hydroxypropylmethylcellulose (Methocel K4M) from Dow Chemical Co., and distilled water.

Identification of Basic Formula

The formula (Table 4.1) and the procedure (Appendix 6) from Ylimaki (1987) formed the basis for this investigation. This formula was prepared and examined several times for familiarisation purposes.

Table 4.1. Original rice flour bread formula[1]

INGREDIENTS	WEIGHT (g)	FLOUR WEIGHT (%)
Rice flour	182	80.2
Potato starch	45	19.8
Granulated sugar	24	10.6
Salt	6	2.6
Yeast	7	3.1
Oil	13	5.7
carboxymethylcellulose[2]	2	0.9
hydroxypropylmethylcellulose[2]	8	3.5
Water[2]	247	108.8

[1] Ylimaki, 1987.

[2] Amounts selected from the center point of the experimental design.

Equipment

Food Mixer

The doughs were prepared using a 3-speed Braun food mixer (Model KM 32 CDN), equipped with a 5 litre plastic bowl and a whip attachment. The whip attachment was selected because it enhanced air incorporation into the dough and produced breads with large volumes (Ylimaki, 1987). If bread with a fine crumb texture is desired, the dough must ultimately contain a large number of gas cells (Kim and De Ruiter, 1968). As a general rule, the larger the number of gas cells, the thinner the walls between the cells and the softer the crumb after baking.

Proofer

A proofer (National Manufacturing Co., Lincoln, Nebraska) set at 30°C and 95% humidity was used to proof the doughs. The cabinet was equipped with three shelves each with three doors, one each for the three positions on each shelf (left, center, right). These doors enabled the efficient placing and removing of the doughs without seriously affecting the conditions within the proofing cabinet. The temperature was monitored by a thermometer hanging from a small hook over each shelf. The humidity was controlled by a Honeywell humidity control attached to the side wall of the proofer.

Oven

The loaves were baked individually in either a Caloric gas oven or a rotary oven (National Manufacturing Co., Lincoln, Nebraska) with a glass door. In the rotary oven humidity was maintained by placing two 500 ml beakers of distilled water at opposite sides of the shelf.

Bread Evaluation

After baking, the breads were cooled for 15 minutes, then removed from their pans and cooled for an additional hour at room temperature (21°C - 23°C). The loaves were then weighed and the volume determined. Volume (cc) of the loaves was measured by rapeseed displacement in a volumeter (National Manufacturing Co. Ltd., Lincoln, Nebraska). These determinations were carried out in duplicate. As recommended by Ylimaki (1987) the bread was placed in the volumeter right side up, unless the bottom surface of the loaf had a large indentation, in which case it was placed upside down. The cooled breads were then placed into plastic bags, sealed and frozen. Random bench top evaluation was conducted by the researcher and members of the Department of Foods and Nutrition. Breads were also photographed to facilitate reporting.

RESULTS AND DISCUSSION

In order to standardise Ylimaki's rice/potato formula and identify the proportions of ingredients necessary for the development of a rice/cassava/soy formula, several steps were taken. First the baking conditions were identified. Position in the proofer and oven type were selected and the oven settings were determined, using Ylimaki's rice/potato formula. The formula was then systematically adapted, first by replacement of potato starch with cassava starch, then the inclusion of soy protein concentrate in the rice/cassava formulation. The rice/cassava/soy formula was then used to adjust the amount of water, set the proofing time and the baking time. Loaf volume was the criteria used to screen out unsuitable formulae and processing conditions. Although this is an important parameter, high volume does not necessarily indicate a good loaf (Hoseney and Seib, 1978). The highest loaf volume possible consistent with a good crumb grain is most desirable.

Determining Proofer Position On Loaf Volume

Several instrumental checks in the proofing cabinet indicated that the temperature and the humidity on the bottom shelf was subject to continuous fluctuations. Whereas the temperature on the top and center shelves ranged between 30°C and 31°C, the bottom shelf thermometer recorded 35°C. A batch of baking was conducted in which one pup loaf was placed in each position on each shelf simultaneously. A visual assessment of volume was used to compare proofing positions, and it was found that the loaves on the bottom shelf were much larger than those on the other two shelves, with the exception of the loaf from the right side of the top shelf. It appeared that both the temperature and the humidity were most constant within the area of the center shelf. Since for further experimental work it would only be possible to make two loaves at a time, it was decided that only the center shelf would be used.

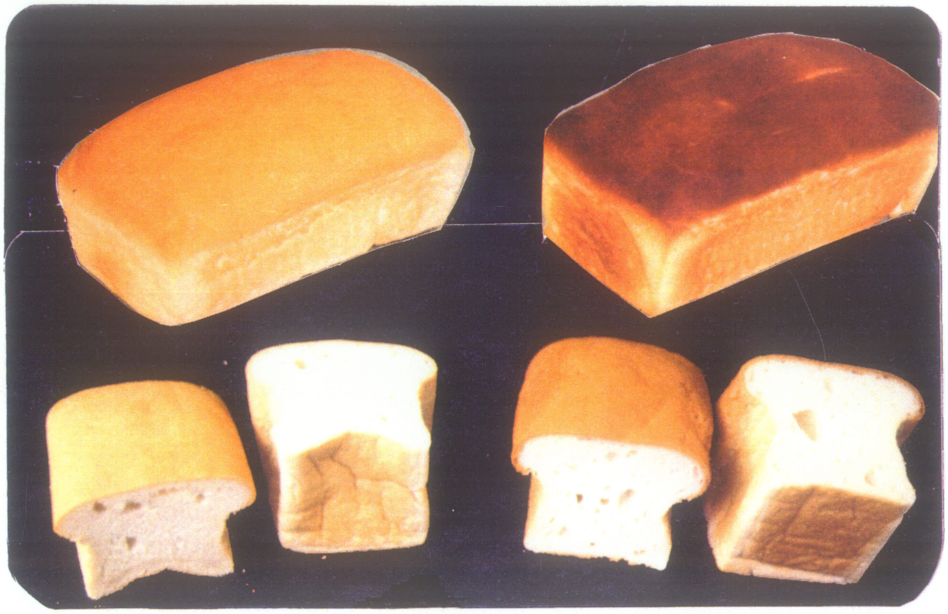
Determining Oven Type and Setting Baking Temperatures

Ovens and temperature settings were investigated using Ylimaki's rice/potato formula. Generally domestic ovens do not have an external thermometer to allow oven temperature to be monitored, therefore there was no guarantee that the actual temperature desired was obtained following the first five minutes of baking. On recommendation by Dr. G. S. Chauhan (personal communication) the rotary oven was tested instead. On the outside of the oven there was a thermometer and a light to indicate when the actual temperature inside the oven was achieved. In this oven there was one rotating shelf which enabled the bread to circulate continuously within the atmospheric conditions in the oven, thus ensuring more uniform baking.

The baking temperatures recommended by Ylimaki (1987), which had been established for a conventional household oven (Kenmore, Mark 3), were transferred, unadjusted, to the rotary oven. The differences between the two loaves produced in each oven can be seen from Plate 4.1. Overall appearance of the bread baked in the conventional oven was good although the crust was pale and very dry and the bottom of the loaf was indented. In contrast the loaf from the rotary oven had a good shape with flat bottom and straight sides and the crust was soft. The loaf was also more symmetrical but the colour was a very dark brown. When both loaves were cut two hours later, the crust from the rotary oven loaf was thicker, and the crumb felt a little more moist, than the crust and crumb of the loaf baked in the conventional oven. The thickening of the crust could be attributed to the steam generated in the oven. According to Hoseney (1986) the steam slows the rate of vaporisation thus allowing the surface of the loaf to cook for a longer time and resulting in a thicker crust. Uneven rising of the loaf was evident from the appearance of the top of the loaf baked in the conventional oven. All subsequent baking was conducted in the rotary oven.

In an attempt to eliminate the undesirable darkening and thickening of the crust, the following temperature regimen were tested: 218°C (425°F) for 30 minutes as used by Gim and Lin (1978); 230°C (446°F) for 25 minutes as used by Ciacco and D'Appolonia, 1978), and 215°C (420°F) for 5 minutes followed by 177°C (350°F) for 30 - 35 minutes. The loaf baked at 218°C for 30 minutes had a good oven spring within the first three minutes. The height was maintained during the

A



B

Plate 4.1. Effect of oven type on rice based yeast bread. A:- conventional oven; B:- rotary oven.

Plate 4.2. Effect of oven temperatures on rice based yeast bread. Top:- left: 230°C for 25 min.; center: 218°C for 30 min.; right: 215°C for 5 min. followed by 177°C for 30 min. Bottom:- left: 218°C for 30 min.; right: 215°C for 5 min. followed by 177°C for 30 min.

baking and the subsequent cooling period. There was a good brown colour and shape with straight sides. When cut 24 hours later there was a large cavity under the top crust (Plate 4.2). The loaf baked at 230°C showed a good oven spring within the first three minutes but reduced in height during baking. The final shape was very good but the colour was very dark brown. Despite the high temperature no cracking of the surface of the loaf was noted, possibly because of the humidity in the oven.

An initial high temperature appeared to be essential to the development of the "oven spring". Hoseney and Seib (1978) have explained that this phenomenon was due to the vaporization of water, heating of gases and their subsequent increase volume, carbon dioxide becoming less soluble, and the increased yeast activity. Subsequent prolonged cooking at a lower temperature allowed for the gelatinization of the starch and the setting of the crumb structure.

After these tests, the following baking conditions were established for use throughout the study: The center shelf of the proofer, the rotary oven with the beakers of water to provide humidity; the oven temperature settings of 215°C for 5 minutes, followed by a reduction to 177°C for 35 minutes.

Adapting Ylimaki's Rice/ Potato Formula

Replacing Potato Starch by Cassava Starch

In the initial test the twenty percent potato starch was replaced directly with twenty percent cassava starch with no other adjustments made to the formula. Cassava starch was used instead of cassava flour based on the findings of amylograph studies conducted prior to this investigation (Chapter 3). There it was found that the behaviour of the cassava starch alone, in blends with rice flour and with the addition of selected gums, displayed characteristics very clearly resembling potato starch. The use of cassava starch instead of cassava flour was also supported by other studies (Kim and De Ruiter, 1968; De Ruiter, 1978; Ciacco and D'Appolonia, 1978; Casier et al., 1979).

Baking trials were done to compare Ylimaki's rice/potato formula with the rice /cassava formula. It was observed that after proofing in the pans both loaves achieved approximately the same height. After 5 minutes in the oven both showed signs of a good "oven spring", although the cassava starch loaf appeared about 0.5 cm higher than the control. The difference in volume is shown in Table 4.2. After baking both loaves had a good colour and shape with straight sides and flat bottoms. Upon cooling there was a slight indentation in the sides of the cassava loaf (Plate 4.3), and after 24 hours this had increased although the loaf still maintained a good shape overall. It appeared quite feasible to replace potato starch with cassava starch.

In a cassava/soy bread Kim and De Ruiter (1968) found that with partial replacement of cassava flour by cassava

starch, it became increasingly easier to mix the dough and the final loaves had a marked improvement in volume.
De Ruiter (1978) reported that cassava starch had the best cohesive properties of a number of common starches, because the gel produced on gelatinization of this starch possesses far greater cohesion than do the gels of grain starches and most other tubers. Cassava starch performed better than cassava flour and Ciacco and D'Appolonia (1978) concluded that the presence of fibre was responsible for the inferior baking quality of cassava flour. Casier et al. (1979) found that cassava starch produced better results than the flour whether used alone or in combination. They also found that starch-flour blends normally resulted in better loaf volume and texture than flour-flour blends.

Including Soy Concentrate

An important aim of the present work was to improve the protein content of the rice/cassava bread. Rice flour contains much less protein than wheat flour, and cassava starch is basically pure starch, high in calories but with only traces of protein, ash and fat (Table 4.3). It was hypothesised that a soy supplement might be used. Soy which is an excellent complimentary protein for cereals, has been suggested for fortification of the carbohydrate-based staple foods, especially cassava (Collins and Temalilwa, 1981).

After preliminary work with varying levels of defatted

Table 4.2. Effect of replacement by cassava starch on rice flour bread

BAKING TRIAL	STARCH	VOLUME
1	Potato	1225
2	Cassava	1310

Table 4.3. Composition data on non-wheat flours and starches.

SAMPLES	MOISTURE (%)	PROTEIN (%)	FAT (%)	ASH (%)	AMYLOSE (%)
Rice Flour	12.20	7.25	1.20	0.66	19.2
Cassava Flour	11.40	0.14	0.16	0.06	19.7
Cassava Starch	12.60	0.06	0.02	0.04	17.1
Potato Starch	13.40	0.06	0.03	--	25.2
Soy Protein	6.36	59.97	0.41	6.57	--

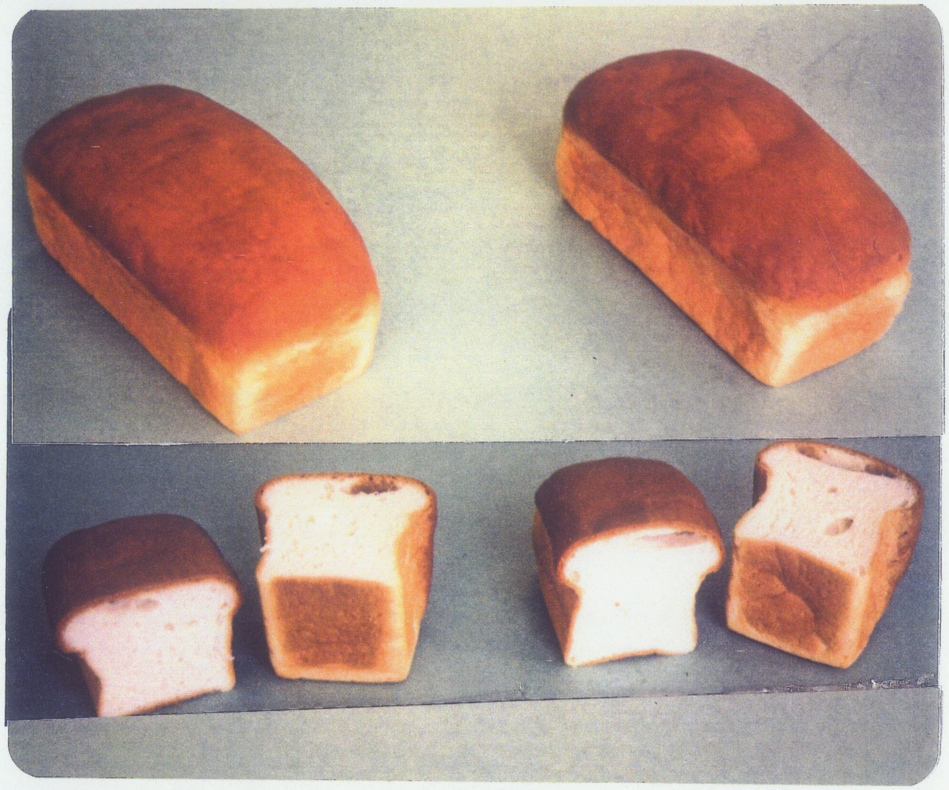


Plate 4.3. A comparison of rice based yeast breads with potato starch (left) and cassava starch (right).

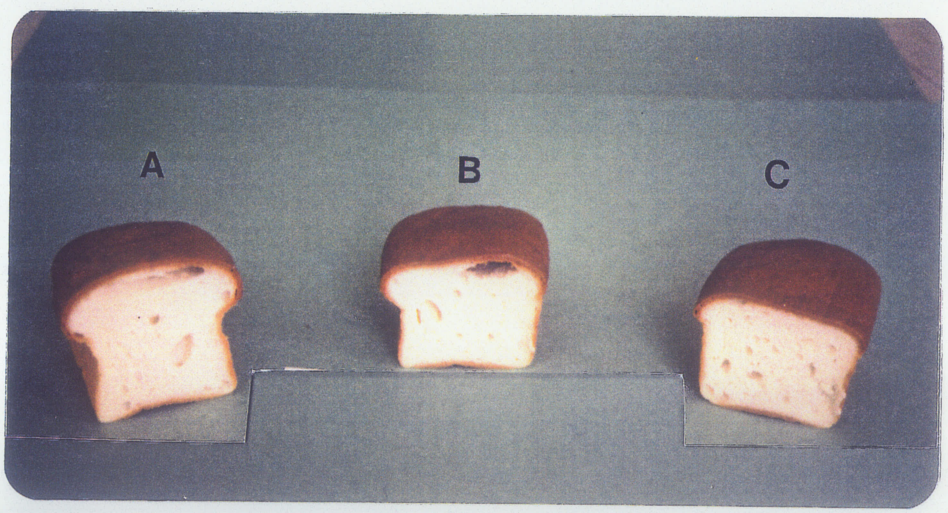


Plate 4.4. Effect of soy protein concentrate on rice/cassava/soy yeast bread [A: 80/20/0; B: 75/20/5; C: 80/15/5] %.

soyabean flour, soy protein concentrate and soy protein isolate, results indicated that soy protein concentrate was most effective in achieving the level of nutritive value required without adversely affecting the physical attributes of the product. In a comparison of soy protein concentrate with soy flour, Bradford and Orthoefer (1983) suggested that concentrates offered the advantages of increased protein (approximately 70 %), improved flavour quality and functional benefits. Reviews (FAO, 1973; De Ruiter, 1978) reported that favourable results had been obtained with as high as 20 % soy incorporated into formulae.

Calculations indicated that 5% soy concentrate would provide a loaf with a protein content equivalent to an average wheat flour white bread which is approximately 7.15 g in 100g of bread (Health and Welfare Canada, 1987). In addition the combination of rice/ cassava/soy at 80/15/5 and 75/20/5 (%) were therefore tested. Both doughs were stiffer than the dough from Ylimaki's rice/potato formula, during mixing. The second combination proofed a little higher than the first. Both obtained a good oven spring, had a good colour and shape with straight sides and flat bottoms. When cut they showed very even crumb with a few small air holes (Plate 4.4). The crumbs felt moist which was consistent with findings of Klein et al. (1980). This could be due to the fact that soy protein is effective in absorbing and retaining moisture in baked products. Both volumes were less than the loaf made without soy concentrate. Guy (1984) noted that adding high levels of soy flour to formulations depressed loaf volume.

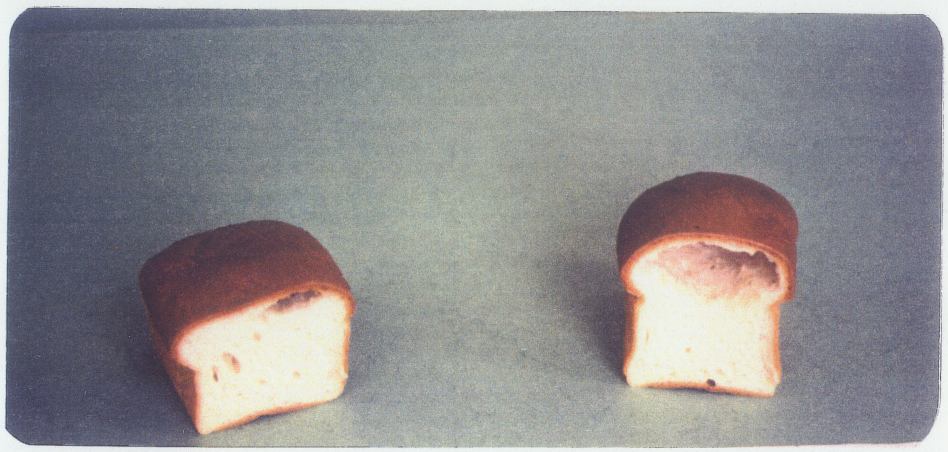
Adjusting Water Levels For Rice/Cassava/Soy Formula

Water level has been identified as a critical factor for loaf volume and crumb quality of non-wheat and composite flour breads (Kim and De Ruiter, 1968; Ranhotra et al., 1975; Nishita et al., 1976; Ylimaki, 1987; Satin, 1988). Inclusion of soy in the formula obviously caused higher absorption in the doughs causing them to be firmer than the control. Kim and De Ruiter (1968) reported that highest loaf volumes were obtained for cassava/soy breads when the greatest amounts of water were used. In the present study when water was increased from 247g to 255g , both the 80/15/5 and the 75/20/5 rice/cassava/soy combinations had increased volumes, although the formula with 75 % rice flour showed a greater volume. However when the loaves were cut both had large holes under the top crust (Plate 4.5). The remainder of the slice was good. The crumb was very tender and moist although it tended to be gummy. Further experimentation using 250 g of water indicated improvement in the slice when cut although the volume was lower.

Setting Proof Time Using The Rice/Cassava/Soy Formula

Ylimaki (1987) reported that both a remixing method and a second proofing were needed to produce rice breads with

A



B

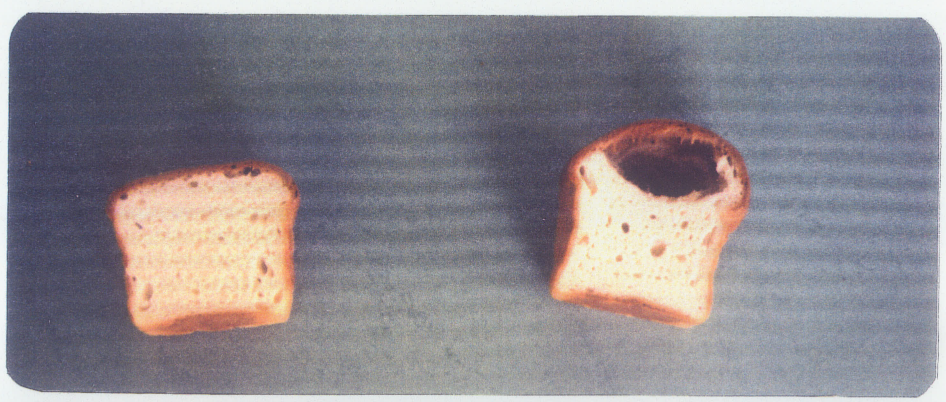


Plate 4.5. Effect of water on rice/cassava/soy yeast bread. A: 75/20/5 (left); 75/20/5 with 255g water.
B: 80/15/5 (left); 80/15/5 with 255g water.

acceptable volume, appearance and flavour. The effects of various second proofing times on the rice/cassava/soy (80/15/5) formula was investigated. In addition to the recommended 45 minutes, two trials were conducted using 30 and 60 minutes respectively.

With a 30 minutes proof time the dough was approximately 1 cm below the top of the pan. It showed a good oven spring, however in the oven it shrank from the top and the short sides of the pan, and became very brown. With 60 minutes the dough began to decrease in volume. There was evidence of several tiny air holes. After 4 minutes in the oven the loaf collapsed. After baking its sides and bottom were straight and flat despite the indented top. There were no adverse effects with the 45 minute proofing time so that time was retained.

Selecting Gum Levels For The Rice/Cassava/Soy Formula

Throughout the experimental period the adapted rice/ cassava/soy formula (80/15/5) was used with 2g CMC and 8g HPMC from Ylimaki's rice/potato formula. Although this gum combination produced a good shape and crumb texture as well as an acceptable loaf volume, it was decided to examine the feasibility of using alternative combinations with the rice/ cassava/soy formula. In this section of the study Ylimaki's formula is hereafter referred to as the reference (REF).

Nishita (1976) reported that HPMC was the only gum which produced satisfactory results in 100 % rice flour breads. It was further suggested that CMC and other gums allowed starch gelatinization during heating but did not provide the necessary gas retention properties during fermentation. Ylimaki (1987) found that a combination of HPMC and CMC produced satisfactory results in a rice/potato bread (80/20). It seemed that while HPMC was responsible for the volume, CMC had a role in the maintenance of rice bread loaf shape. To determine the effects of the gums on the rice/ cassava/soy formula, varying combinations of these two gums on loaf volume were investigated, using the 80/15/5 rice/ cassava /soy formula and 250 g water. The gum combinations and corresponding loaf volumes are reported in Table 4.4.

To confirm the effect of the HPMC the first two trials were conducted using HPMC alone. The two levels used (6.37 g and 8.00 g respectively), corresponded to the lowest and the center point levels used by Ylimaki (1987). For both treatments the volumes showed corresponding increases. With 6.37 g HPMC the dough was very light and smooth during mixing. Volume increased very much during proofing and there was a great oven spring with the height maintained during baking. There was a double-decker shape to the loaf. Even after cooling, the internal structure was so weak that the top squeezed down during handling for measurements (Plate 4.6 A [left]). With 8.00 g the behaviour of the dough as well as the final

Table 4.4. Effect of gums on loaf volume of rice/ cassava/soy yeast breads

TRIALS	CMC (g)	HPMC (g)	VOLUME (cc)
1	--	6.37	1275
2	--	9.63	560
3	0.37	6.37	1630
4	0.37	9.63	575
5	3.63	6.37	1550
6	3.63	9.63	1745
7	0.80	6.37	1475
8	0.37	8.00	1710
9	2.00	8.00	905

A

B

C

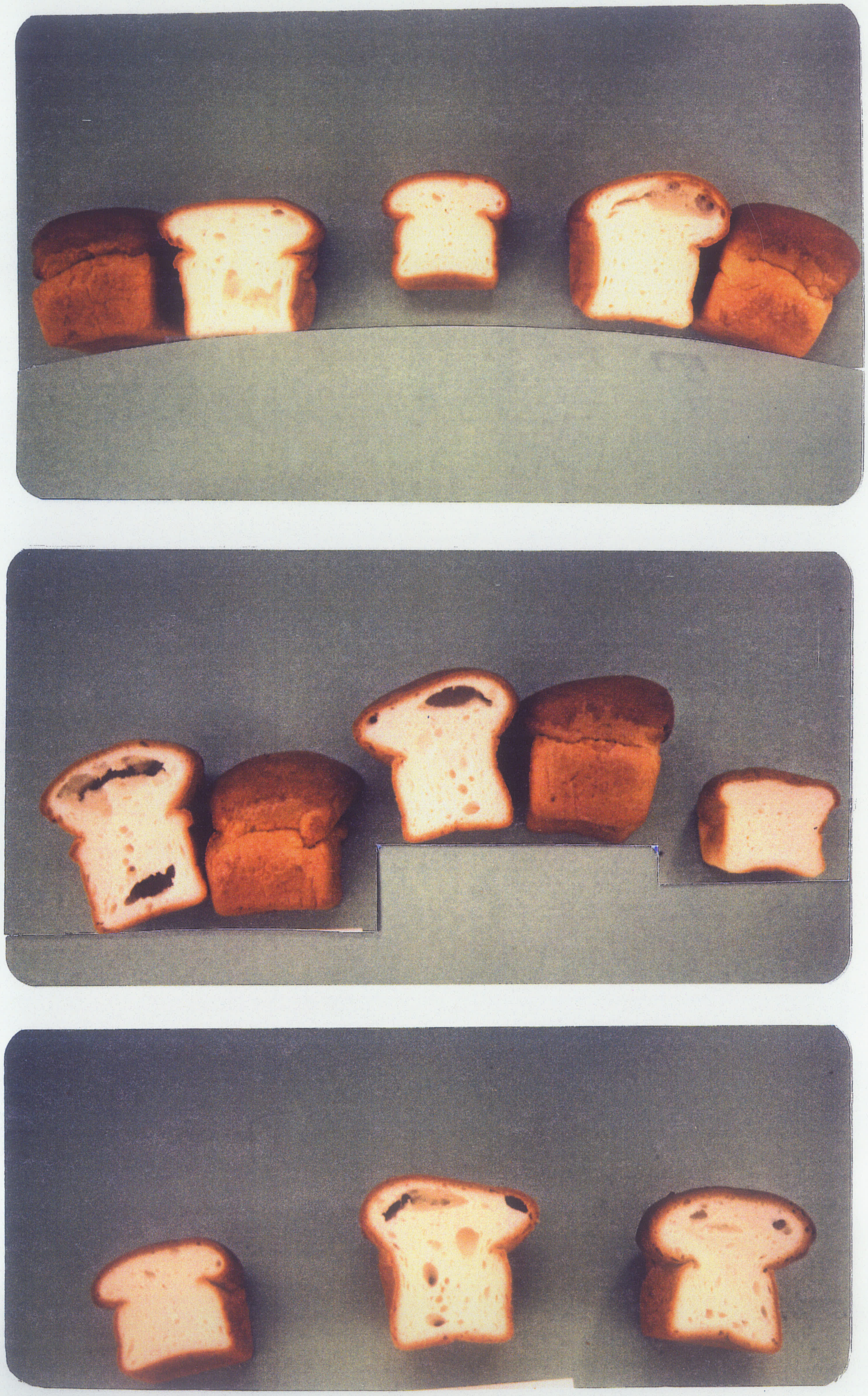


Plate 4.6. Effect of gums on rice/cassava/soy yeast bread.
A:- left: 0g CMC + 6.37g HPMC; center: 0.37g CMC + 6.37g HPMC; right: 0.8g CMC +6.37g HPMC.
B:- left: 0g CMC + 8.0g HPMC; center: 0.37g CMC + 8.0g HPMC; right: 2.0g CMC + 8.0g HPMC.
C:- left: 0.37g CMC + 6.37g HPMC; center: 0.37g CMC+ 8.0g HPMC; right: 0.37g CMC + 9.63g HPMC.

loaf were similar to the formula containing 6.37 g HPMC. The sides of the top deck were crinkled and very indented. Both had very straight sides on the lower 'deck' and flat bottoms. When cut the crumb structures were very open and there were air holes under the crust (Plate 4.6 B [left]).

In Ylimaki's work the lowest and highest levels of CMC used were 0.37 g and 3.63 g respectively, while the lowest and highest levels of HPMC used were 6.37 g and 9.63 g. Combinations of these extremes were used in trials 3 - 6 (Table 4.4), resulting in low CMC/ low HPMC (Trial 3), low CMC/ high HPMC (Trial 4), high CMC/ low HPMC (Trial 5) and high CMC/ high HPMC (Trial 6).

In Trial 3 the dough was softer than the REF. It proofed well and maintained the height during baking. The final loaf had a good volume and loaf shape. When cut the crumb was coarse and soggy but there were no air holes (Plate 4.6 A [center]). For Trial 4 with the higher HPMC, the dough felt more like the REF and proofed very high. There was a good oven spring and the height was maintained during baking. Colour was dark and shape mushroomed a little on cooling. When cut the crumb was very open textured and cell walls were weak. Top-side indentation was very pronounced (Plate 4.6 C [right]). The upper portion of the loaf had a few air holes which were elongated vertically and horizontally.

In Trial 5 using the high level of CMC the dough was stiff, proofed very little and shrank in the oven. The colour was a bright brown, almost like burnt orange. When cut the crumb was even and moist but very dense. For Trial 6 with the higher HPMC the dough was very stiff and proofed a little higher, just to the top of the pan. The loaf shrunk after 2 minutes in the oven and continued shrinking during baking. It was a small and heavy loaf.

From these six tests there is an indication that CMC was necessary for the maintenance of loaf shape, while HPMC was needed to obtain good volume. The sequences in Plates 4.6 B and 4.6 C clearly show the changes in both loaf shape and volume with increasing levels of CMC (B) and HPMC (C).

For further comparison the level suggested by Ylimaki (1987) from the formula which met the greatest number of reference standards was used. Therefore for Trial 7, 0.8 g CMC was combined with 6.37 g HPMC. This dough proofed well and had good shape but the crumb was coarse (Plate 4.6 A [right]). In order to determine whether a smaller amount of CMC than 2g would be as acceptable as the REF, the level of CMC was changed to 0.37 g with 8.00 g HPMC (Trial 8). The dough was firm, proofed very well and produced a large oven spring. The height was maintained during baking but the center of the top flattened slightly on cooling. There was also a very great indentation on the top-side on cooling (Plate 4.6 B [center]). When cut the crumb texture was very open with a cavity under part of the top crust.

The results confirmed that the synergistic interaction of the two gums was effective in producing an acceptable loaf. Although HPMC is essential for good volume it is necessary to have the CMC to control its action and produce a more acceptable shape and crumb appearance. Based on all work previously discussed, a standardised formula as a basis for further experimentation was determined (Table 4.5). Mixing and baking procedures remained the same as for Ylimaki's formula except that the rotary oven was used in place of the conventional gas oven, and the final baking time was decreased to 40 minutes.

Effects of position in proofer were examined using the standardized rice/cassava/soy formula. This formula was replicated twice using all positions on the top and center shelves. A 2 x 3 factorial design was used. Analysis of variance was computed using loaf volume as the response (Appendix 7). No effects were found for position on shelf or proofer shelf. There was however some day effect as the two replications were done on two consecutive days. The volumes on the second day were consistently lower for each loaf. This seemed to indicate the stability of the formula which suggested that it would be reproducible.

Table 4.5. Standardized formula for rice/cassava/soy bread

INGREDIENTS	WEIGHT (g)	FLOUR WEIGHT[1] (%)
Rice flour	171	75.3
Cassava starch	45	19.8
Soy protein concentrate	11	4.8
Granulated sugar	24	10.6
Salt	6	2.6
Yeast	7	3.1
Oil	13	5.7
Carboxymethylcellulose	2	0.9
Hydroxypropylmethylcellulose	8	3.5
Water	250	110.1

1 On a flour weight basis.

Conclusions

Ylimaki's formula appears to be fairly reproducible. Despite the replacement of potato starch with cassava starch, and the inclusion of soy protein concentrate, the only adjustment necessary was in the water level. With the adapted formula (80/15/5), use of the 2g CMC and 8g HPMC combination produced the best results with regard to loaf shape and crumb texture although there was a marked decrease in loaf volume compared to most of the loaves made with other gum combinations (0.37 CMC + 8.0 HPMC; 0.8 CMC + 6.37 HPMC; 0.37 CMC + 6.37 HPMC). High loaf volume is undesirable if crumb texture is unacceptable, and therefore a lower loaf volume may have to be accepted for a rice/cassava/soy bread. There was no need to adjust proof time or baking temperatures, although the type of oven and the final baking time were changed. All procedures worked satisfactorily so the adapted formula was found acceptable for use in further experimentation.

It appears that the variables most likely to affect the physical characteristics of the loaf are cassava starch, soy protein concentrate and water. Since water levels affected the consistency of the dough, it might have, in turn, influenced the rate and the amount of time needed for the dough to rise , so proof time was also considered as a variable.

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Chapter 5

OPTIMIZATION OF A SOY-ENRICHED RICE FLOUR-CASSAVA STARCH YEAST BREAD USING RESPONSE SURFACE METHODOLOGY

INTRODUCTION

The development of a soy-enriched rice/cassava yeast bread has potential benefit for developing countries as well as individuals suffering from wheat allergies. The use of rice and cassava as a replacement for wheat could reduce importation costs for wheat flour as well as improve the agro-economic conditions for those countries with potential for higher production of the alternate crops. Since the bread would need to be nutritionally well balanced, it is necessary to investigate the extent to which soy protein which can be included in the formulation without adversely affecting the physical quality of the final product. Optimization of the formula for such a product would be of great value in obtaining a bread which would be found acceptable to consumers.

Sidel and Stone (1983) defined optimization as a procedure for developing the best possible product in its class. Schultz (1983) stated that optimization approaches are ways of improving the likelihood of developing an optimum product but do not guarantee success.

The use of Response Surface Methodology (RSM) in food product development is relatively new but has been applied successfully to many baked products in the optimization of formulations. Although RSM has been used successfully for several types of products such as yoghurt (Rossi et al, 1983), cakes (Neville and Setser, 1986), noodles (Shelke et al., 1990), very few studies have reported on breads (Henselman et al., 1974; Payton et al., 1988; Rubenthaler et al., 1990), and no other has been found in the literature for gluten-free breads except Ylimaki (1987). This statistical technique allows for a reliable prediction of optimum regions within a range of ingredients, which will result in acceptable products.

In response surface methodology the important variables are known beforehand and are varied systematically (Sidel and Stone, 1983). Joglekar and May (1987) described two stages of product development at which RSM could be used. The first is at the screening stage, where the critical variables may be selected from a number of potential ones. The second stage, called optimization, is to identify the optimum setting of the critical factors which will achieve the desired quality objectives. It appears however, that despite optimum regions being determined from RSM plots, it is still necessary to experiment further within those recommended regions to produce an optimum formula.

Part of the optimization process for this study included the use of objective evaluation. Funk et al. (1969) reported that physical or objective evaluation of food products was reliable and reproducible. Prior to Ylimaki (1987) very limited information on a collective set of objective measurements of gluten-free breads has been available. Some parameters have been measured in various studies, most of which have stressed volume and specific volume (Christianson et al., 1974; Ranhotra et al., 1975; Nishita et al., 1976). Crumb firmness has been measured by Christianson and coworkers (1974) but not other parameters of the crumb such as gumminess, cell size, cell uniformity or crumb colour. Loaf shape seems to have demanded the least attention.

This study focused upon using RSM to optimize a formula for an acceptable soy-enriched rice/cassava yeast bread, by identifying the most effective combination of cassava, soy and water which would meet the required quality of the loaf. More specifically, the objectives were to:

1. To use response surface methodology to determine the importance of selected variables (cassava starch, soy protein concentrate, water and proof time) to physical characteristics (specific volume, loaf shape, crust and crumb colour, moisture content and Instron texture characteristics) of breads.

2. To produce contour plots to visually identify the most important variables, the relationship between them, and their effects on the physical

characteristics of the loaf.

3. To superimpose the contour plots of those response variables which seem to be most significantly influenced by the independent variables, in order to predict rice/cassava/ soy formulae which could meet previously established quality criteria.

4. To identify the formula which would allow inclusion of the highest proportion of soy protein concentrate while still meeting the criteria.

5. To select and recommend for use a formula or formulae which should produce an acceptable rice/cassava/soy bread with good nutritional value.

MATERIALS AND METHODS

Materials:

The ingredients used for the formulations were : White rice flour (U.S. long grain, fine grind) from Dainty Foods Inc., cassava starch from Grain Enterprise Ltd., soy protein concentrate (Arcon F) from Archer Daniels Midland Co. Il., iodized table salt (Windsor) from Canada Salt Co., white sugar, West Canola oil (Canbra Foods), active dry yeast (Fleischmann's Traditional), sodium carboxymethylcellulose

(7HF) from Hercules Inc., hydroxypropylmethylcellulose (Methocel K4M) from Dow Chemical Co., distilled water.

These ingredients were used in the quantities as described by the formula shown in Table 5.1.

Variables:

Four variables were selected for this experiment based on standardization procedures (Chapter 4). Cassava starch , soy protein concentrate and water were the ingredient independent variables. Proof time was included as one of the independent variables on the assumption that it might have been affected by changing water levels in the formulation.

Rice Bread Preparation and Baking:

The standardized formula (Table 5.1), which was developed in Chapter 3, was used. The doughs were prepared using a 3-speed Braun food mixer (Model KM 32 CDN), equipped with a 5 litre plastic bowl and a whip attachment. The preparation procedure was as follows:

1. Dissolve 4 g sugar in 50 mL water (43°C). Stir in yeast and soak for 10 minutes.
2. Mix rice flour, cassava starch, soy protein concentrate, salt, remaining sugar (20g) and methylcellulose gums at speed 1 in the food mixer for 2 minutes, using the whip attachment.

Table 5.1 Standardized formula for rice/cassava/soy bread[1]

INGREDIENTS	WEIGHT (g)	FLOUR WEIGHT (%)
Rice flour	171	75.3
Cassava starch[2]	45	19.8
Soy protein concentrate[2]	11	4.8
Granulated sugar	24	10.6
Salt	6	2.6
Yeast	7	3.1
Oil	13	5.7
Carboxymethylcellulose	2	0.9
Hydroxypropylmethylcellulose	8	3.5
Water[2]	250	110.1

[1] Formula selected as center point for the experimental design described in Table 5.2.

[2] These ingredients vary according to the levels used for each treatment as outlined in Table 5.3.

3. Add yeast mixture and remaining water (40°C) and mix at speed 1 for 15 seconds.
4. Add oil and mix at speed 2 for 15 seconds, followed by 2.5 minutes at speed 3.
5. Scrape the sides of bowl and mix at speed 3 for 2.5 minutes.
6. Scrape sides of bowl, pushing dough to the bottom of the bowl and proof dough for 30 minutes in proofing cabinet (30°C/95% humidity).
7. Remix dough at speed 3 for 5 minutes.
8. Place 400 g dough in a greased pan (18.7 cm x 9.2 cm x 5.7 cm) using rubber spatulas. Press dough into the pan corners and sides, eliminating air pockets and flattening the top surface, using an oiled rubber spatula. Using the same oiled spatula, push the edges of the dough towards the center, away from the pan sides, rounding the top edges of the dough.
9. Proof panned dough for 45 minutes (30°C/95% humidity).
10. Bake in preheated oven (215°C) for 5 minutes. Lower the oven temperature to 180°C and continue baking the bread for 40 additional minutes.
11. Cool the bread at room temperature (23°C) for 15 minutes. Remove the bread from the pan. Cool bread on a rack for 1 hour before packaging.

The breads were cooled in the pan for 15 minutes, then removed and weighed. This value was used to determine percent moisture loss. The breads were cooled for an additional hour at room temperature (21°C - 23°C), after which they were weighed and the volume measurement taken. Loaf shape and top crust colour were scored within 1 - 2 hours after baking. The loaves were then placed into plastic bags, sealed and frozen. Instron firmness, gumminess, crumb colour, moisture content, cell size and cell uniformity were determined on the breads five days later.

Reference Loaves

The original formula from Ylimaki (1987) was used in the preparation of reference loaves for this experiment. This reference was selected on the basis that it had already been deemed acceptable as a gluten-free rice bread and comparable to a reference wheat flour white bread, both by objective and sensory measurements. Eight loaves were prepared, four prior to the experimental period and four following. For each objective measurement, the range of measurements of the eight loaves was taken as a reference point from which to select acceptable regions for the treatment loaves. Since cassava starch and soy protein concentrate were two critical variables it was anticipated that acceptable measurements for the treatment loaves might be different from the standard of the reference loaves. All measurement for reference loaves were taken under the same conditions as the treatment loaves.

Preparation of Samples for Objective Measurements

Loaves were removed in pairs from the freezer within half an hour of each other. They were thawed for one hour after which they were cut into ten slices, 1.25 cm thick. Samples for each type of measurement was taken on the same relative position on each loaf. The loaf was first cut exactly in half then five slices were taken from each half. The slices were allocated for measurements according to the diagram presented in Figure 5.1.

For Instron measurements, the two centre slices and the two adjacent end slices from each half were used. This provided three samples 2.5 cm thick from each loaf. Rounds 2.5 cm in diameter were cut from the center of each sample. These were wrapped in polyethylene cling film and allowed to thaw for a further hour before the measurements were taken.

The third slice from the centre of each half was used for cell evaluation as well as crumb colour testing. The remaining slice from each half was used for the determination of moisture content. Samples for crumb colour testing and moisture content determination were further cut into rounds 2.5 cm in diameter, taken from the center of each slice.

Objective Measurements

Weight, Volume and Specific Volume

Measurements were taken of bread weight when the loaves were removed from the pan 15 minutes after baking. Another

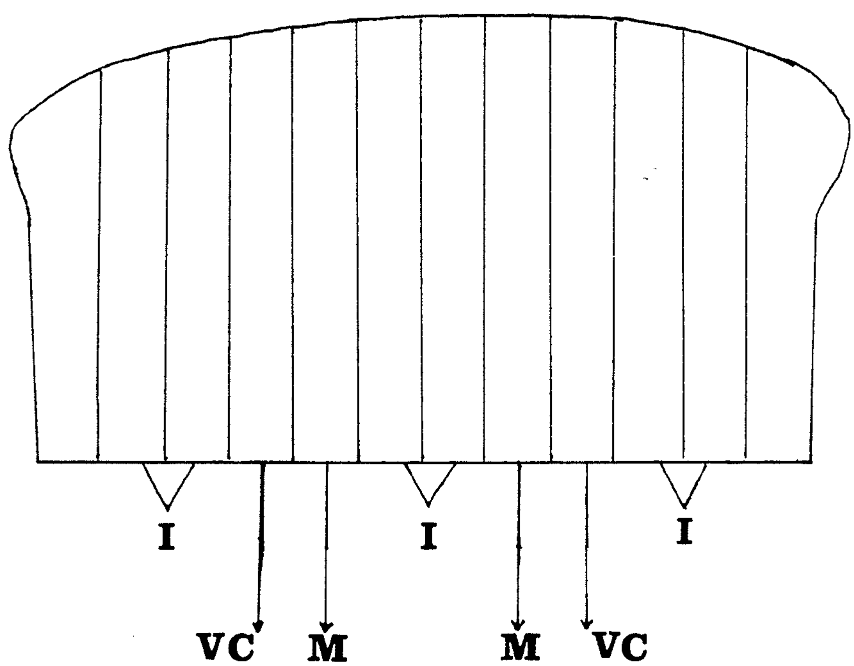


Figure 5.1. Sample allocations used for objective measurements of rice/cassava/soy yeast bread. I= Instron measurement, VC= Visual cell evaluation and crumb colour, M= Moisture measurement.

weight measurement, together with volume was taken 1 hour later (75 minutes after baking). Volume (cc) of the loaves was measured by rapeseed displacement in a volumeter (National Manufacturing Co. Ltd., Lincoln, Nebraska). These determinations were carried out in duplicate. As recommended by Ylimaki (1987) the bread was placed in the volumeter right side up, unless the bottom surface of the loaf had a large indentation, in which case it was placed upside down.

Specific volume was calculated as the volume (cc) divided by the weight (g).

Visual Scoring of Loaf Shape and Top Crust Colour

Loaf shape score and top crust colour was determined by four members of the Foods and Nutrition department, who recorded their scores on a score card (Figure 5.2), which was adapted from Ylimaki (1987). For loaf shape a 6-point category scale was used, where 6 represented the highest level of each attribute . The five attributes were symmetry and roundness of loaf top surface, size of loaf top-side indentation, straightness of loaf sides and flatness of loaf bottom surface. The maximum score was 30.

Top crust colour was evaluated using a visual reference card (Plate 5.1) showing samples of crust colours and anchor points together with the scores. Scoring was done on a 1 - 5 scale with 1 representing the lightest colour and 5 the darkest.

Cell Size and Cell Uniformity

Two slices from each loaf (one from each half) were used for this evaluation. The slices were coded with 3-digit random numbers and arranged in random order. Photographs to document visual textural characteristics as well as for use in comparative evaluation, were taken using the photo feature of the photocopying machine (Canon, Model NP - 4040F). Slices were placed on a transparency and covered with a plain piece of white paper, then photocopied. Predominant cell size and cell size uniformity were evaluated using visual reference cards showing photographs of samples with the appropriate references and anchor points together with the scores. The scoring was done using individual ballots for each sample. The ballot (Figure 5.3) used a 1 - 5 scale with 1 representing the poorest quality for the characteristic. The evaluators were presented with the reference cards, photographs of the treatment slices and a ballot. All evaluators scored the same samples.

Moisture Loss During Baking

Moisture loss during baking was calculated as the percent difference between the weight of the raw dough and the weight of the baked loaf taken after 15 minutes of cooling in the pan. This loss was considered as occurring from the time of pan proofing to the removal from the pan.

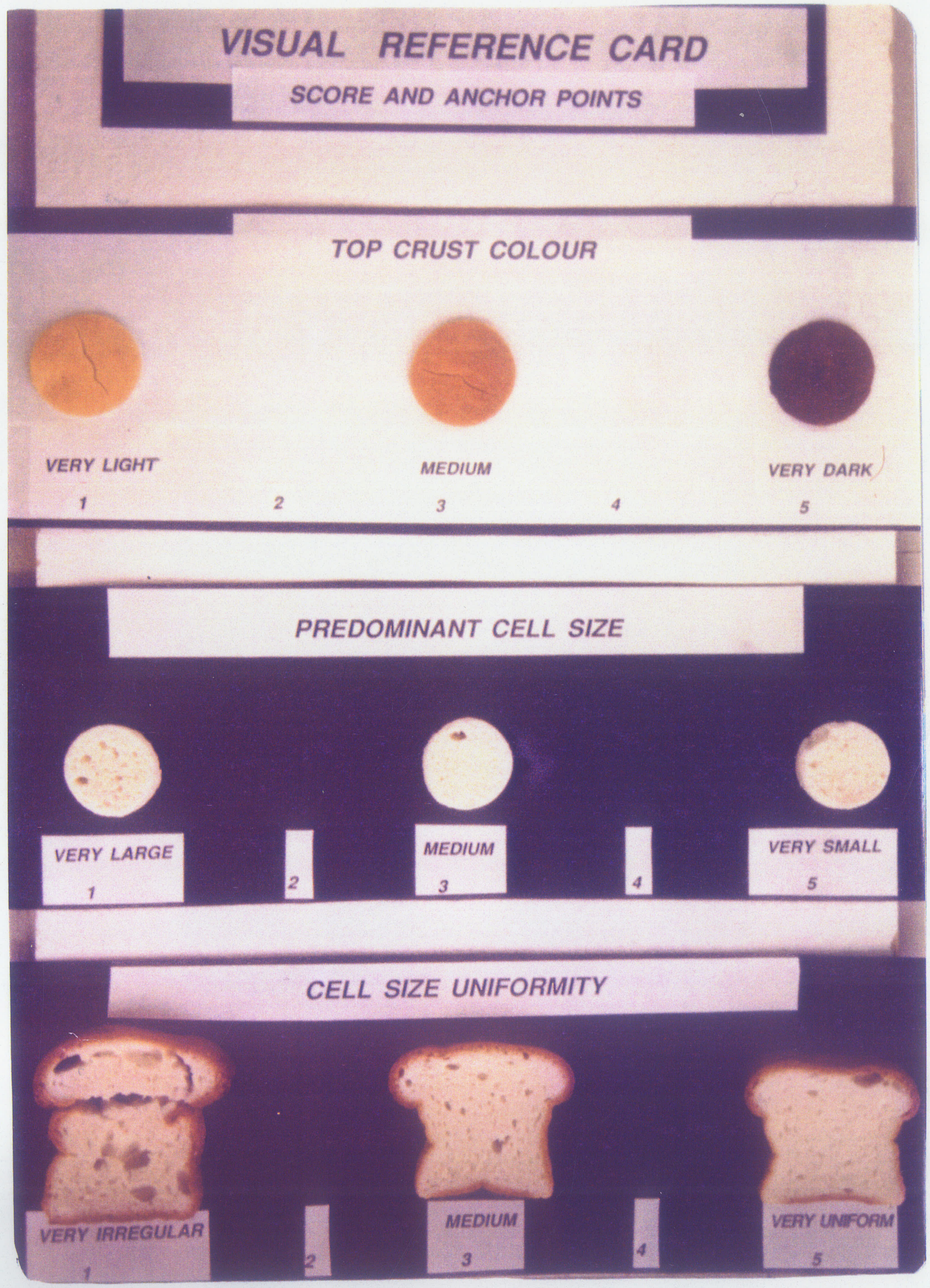


Plate 5.1. Visual reference card

SCORE CARD FOR RICE BREAD LOAF SHAPE AND TOP CRUST COLOUR

Instructions: For each characteristic listed, circle the descriptive word or phrase which best describes that attribute of the sample.

LOAF SHAPE

LOAF NUMBER: ______________ DATE: ______________

	6	5	4	3	2	1
LOAF BOTTOM SURFACE	flat	very sl concave	sl concave	concave	very concave	ext concave
LOAF SIDES	straight	very sl caved	sl caved	caved	very caved	ext caved
LOAF TOP SURFACE	ext round	very round	round	sl round	flat	caved
TOP SURFACE SYMMETRY	very symmet	symmet	sl symmet	sl unsymmet	unsymmet	very unsymmet
LOAF TOP-SIDE INDENTATION	no indent	very sl indented	sl indented	mod indented	very indented	ext indented

LOAF SHAPE SCORE ______________

TOP CRUST COLOUR

VERY LIGHT		MEDIUM		VERY DARK
1	2	3	4	5

Figure 5.2 Score card for rice bread loaf shape and top crust colour.

BALLOT FOR VISUAL CRUMB EVALUATION

Date: ________________

Instructions: Score each characteristic listed by circling the value that best describes the degree of that characteristic in the sample.

Sample #: ____________

PREDOMINANT CELL SIZE

VERY LARGE		MEDIUM		VERY SMALL
1	2	3	4	5

CELL SIZE UNIFORMITY

VERY IRREGULAR		MEDIUM		VERY UNIFORM
1	2	3	4	5

Sample #: ____________

PREDOMINANT CELL SIZE

VERY LARGE		MEDIUM		VERY SMALL
1	2	3	4	5

CELL SIZE UNIFORMITY

VERY IRREGULAR		MEDIUM		VERY UNIFORM
1	2	3	4	5

COMMENTS:

Figure 5.3. Ballot used for visual crumb evaluation

Instron Texture

Two Instron texture measurements were determined using two adjacent slices (total of 2.5 cm thick) for each measurement. The measurements were taken using the Instron Universal Testing Machine (Table model TM), attached to an Apple IIE computer. The data was recorded and analyzed by the computer using a Texture Profile Analysis program developed by Agriculture Canada (1987). The conditions for the measurements were the use of a cross-head speed of 10.0 cm/min, the plunger diameter of 35mm and 50% degree of compression. Instron hardness, taken as the peak force (N) after one compression, was recorded together with the gumminess (N). The value for gumminess was calculated by multiplying the hardness value by cohesiveness [a ratio of the two peaks on the texture profile analysis curve (Betker, 1990)].

Crumb Colour

Crumb colour was determined using a Hunterlab Tristimulus Colorimeter (Model D25M-9). The instrument was standardised using the white standard tile (L = 90.88, a = -1.25, b = 1.87). Values for the lightness (L), red - green (a) and blue - yellow (b) colouring of the samples were recorded. The two slices used for visual cell scoring were used for crumb colour evaluation. Rounds 2.5 cm in diameter were cut from the center of the slices. Each round was placed in a petri dish and covered. Quadruplicate readings were taken for each round by first rotating the sample 90° on the instrument, then sample was turned over and the process repeated. The value recorded was an average of the four readings taken for each round. The same dish was used for all samples to eliminate variations in glass colour or thickness.

Moisture Content Of Bread

The moisture content of the bread was determined by a modification of the one-stage standard AACC method 44-15A, 1983 (Appendix 8). The drying time was extended to 24 hours.

Experimental Design

A central composite design with four replications of the center point and two replications of the cube points, was used. The design (Table 5.2) consisted of a four-variable, five-level pattern with a total of forty-four test runs. The runs were conducted over seven days, with six baking tests on each of the first five days and seven each on the final two days. For the purpose of statistical analyses, the five levels of each of the four variables was coded as -2, -1, 0, +1, +2. The coded and actual values for the variables are listed on Table 5.3. The forty-four runs were all randomized using the random digit table (Steel and Torrie, 1980).

Table 5.2. Experimental Design

TREATMENT[1]	X_1 Cassava	X_2 Soy Conc.	X_3 Water	X_4 Proof Time
1 - 4[2]	0	0	0	0
5	+2	0	0	0
6	-2	0	0	0
7	0	+2	0	0
8	0	-2	0	0
9	0	0	+2	0
10	0	0	-2	0
11	0	0	0	+2
12	0	0	0	-2
13[3]	-1	-1	-1	-1
14	-1	-1	-1	+1
15	-1	-1	+1	-1
16	-1	-1	+1	+1
17	-1	+1	-1	-1
18	-1	+1	-1	+1
19	-1	+1	+1	-1
20	-1	+1	+1	+1
21	+1	-1	-1	-1
22	+1	-1	-1	+1
23	+1	-1	+1	-1
24	+1	-1	+1	+1
25	+1	+1	-1	-1
26	+1	+1	-1	+1
27	+1	+1	+1	-1
28	+1	+1	+1	+1

[1] Total of 44 treatments all randomized for the experiment.
[2] Treatments 1-4 are design center points.
[3] Treatments 13-28 are the cube points. These were replicated.

Table 5.3. Actual and coded independent variable levels.

INDEPENDENT VARIABLES		LEVELS				
Cassava	(g)	23	34	45	57	68
	(%)	10	15	20	25	30
Soy Conc.	(g)	2	7	11	16	20
	(%)	1	3	5	7	9
Water	(g)	236	243	250	257	264
	(%)	104.0	107.0	110.1	113.2	116.3
Proof Time (min)		35	40	45	50	55
Coded Levels		-2	-1	0	+1	+2

Statistical Analysis

Response Surface Regression (RSREG) analysis was conducted on each dependent variable (response), using the Statistical Analysis System (SAS, 1986). This was to determine the effects of each of the four independent variables (factors = cassava starch, soy protein concentrate, water and proof time), singly as well as for their quadratic and interaction effects. The analyses were conducted on one measurement for specific volume, two each for moisture content and crumb colour, and three for Instron firmness and gumminess. For loaf shape, top crust colour, cell size and cell size uniformity analyses were based on scores of four judges.

Contour plots were generated from the equation for the full model, using the GContour procedure of SAS. From the RSREG tables, the two variables that had the least significant effect on all the responses were identified. The two which were determined to be the most influential were used as the axes of the contour plots while the other variables were held constant at various levels.

Acceptability Criteria For Response Variables

The acceptability regions for the response variables were determined based on a range of values for the eight loaves of reference rice flour/potato starch bread, except in the case of the Instron texture measurements. These aceptability regions were estimated , based on the results of Elgedaily et al. (1982), who reported that a hardness value of 5.66 kg (12.48 N) and a gumminess value of 2.02 kg (4.45 N) were acceptable in a wheat bread fortified with soy protein isolate. In this study it was assumed that the breads might be firmer than the reference loaves due to the addition of soy protein concentrate. A region was not set for crumb colour. The parameter was included to determine whether there was a significant effect of soy protein concentrate on crumb colour. For each response variable, an area representing the formulations which met the acceptability criteria was identified. With the least significant variables held constant at various levels, the contour plots for all responses were superimposed and the rice bread formulations which met the requirements for all the responses were identified.

Bake Test

Bake tests were conducted using the acceptability regions identified for all responses on the superimposed plots. Formulations were selected at random from each set of contours at SPC levels 0, +1 and +2. No formulation was selected from the -1 level since there was no desire to use loaves with less than 5% (level 0) SPC. One batch of baking was conducted using the reference loaf formulation, the centerpoint formulation and six formulations selected from the plots.

These were all prepared under identical standardised conditions. The loaves were examined and compared visually by the researcher and other members of the Department of Foods and Nutrition.

Nutrient Analysis

A proximate analysis and amino acid determination were conducted on selected loaves in order to compare their nutritional value with wheat flour white bread.

RESULTS AND DISCUSSION

The results of the RSREG analysis were examined to determine the effects of the independent variables (factors) cassava starch, soy protein concentrate (SPC), water and proof time, on the physical characteristics (responses) of the loaf. These results are presented in Tables 5.4 to 5.8. F-values and their corresponding probabilities indicate which factors significantly affected the characteristics being measured. From Tables 5.4 and 5.5 the significant linear, quadratic and interaction effects of the full model were identified. The significant factor effects on the physical characteristics are summarized in Table 5.6. The only factor having any linear, quadratic or interaction effects on top crust colour was soy protein concentrate, while for crumb colour only cassava starch had some significance. The linear effects for all other responses were significant. For loaf shape, cell size

Table 5.4. F-values from the full model response surface regression for colour of rice flour bread.

Effects of Independent Variables	Dependent Variables			
	Top Crust Colour	Crumb Colour		
		L	a	b
LINEAR	3.39 (.0109)[1]	3.62 (.0164)	0.99 (.4265)	1.86 (.1448
x_1 (Cassava)	0.53 (.7520)	2.18 (.0842)	2.23 (.0779)	2.61 (.0455)
x_2 (Soy Conc.)	3.30 (.0075)	1.25 (.3132)	0.99 (.4428)	1.64 (.1816)
x_3 (Water)	1.38 (.2322)	1.47 (.2310)	1.18 (.3424)	2.05 (.1013)
x_4 (Proof Time)	0.71 (.6219)	3.74 (.0098)	2.04 (.1017)	2.98 (.0272)
QUADRATIC	1.00 (.4115)	1.20 (.3325)	1.90 (.1374)	2.28 (.0851)
INTERACTIONS	0.97 (.4448)	1.80 (.1335)	1.64 (.1721)	2.30 (.0615)

[1] Probabilities associated with F-values.

Full model-: $yi = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$

Table 5.5. F-values from the full model response surface regression for physical and textural rice bread measurements.

Effects of Independent Variables	Dependent Variables (yi)						
	Specific Volume	Loaf Shape	Hardness	Gumminess	Moisture Content	Cell Size	Cell Uniformity
LINEAR	33.97 (.0001)[1]	22.91 (.0001)	18.44 (.0001)	17.07 (.0001)	115.58 (.0001)	4.78 (.0011)	17.14 (.0001)
x^1 (Cassava)	13.19 (.0001)	6.02 (.0001)	11.28 (.0001)	9.41 (.0001)	0.66 (.6564)	11.18 (.0001)	16.32 (.0001)
x_2 (Soy Conc.)	5.44 (.0012)	7.41 (.0001)	4.59 (.0033)	3.75 (.0097)	1.73 (.1584)	1.22 (.3026)	3.81 (.0029)
x_3 (Water)	12.78 (.0001)	12.30 (.0001)	12.03 (.0001)	11.57 (.0001)	91.34 (.0001)	5.02 (.0003)	5.57 (.0001)
$_4$ (Proof time)	3.91 (.0078)	10.33 (.0001)	0.43 (.8248)	0.58 (.7162)	1.14 (.3612)	1.13 (.3442)	1.48 (.1985)
QUADRATIC	1.26 (.3809)	6.28 (.0001)	2.30 (.0826)	2.07 (.1100)	0.95 (.4476)	8.42 (.0001)	4.64 (.0014)
INTERACTIONS	2.88 (.0253)	3.72 (.0017)	4.98 (.0013)	4.25 (.0035)	0.72 (.6340)	3.68 (.0019)	4.38 (.0004)

[1] Probabilities associated with F-values

Full Model -: $yi = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$

Table 5.6. Summary of the significant factor effects on physical characteristics of rice/cassava/soy yeast bread

Physical Characteristics	Independent Variables			
	Cassava Starch	Soy Protein Concentrate	Water	Proof Time
Specific Volume	x	x	x	x
Loaf Shape	x	x	x	x
Top Crust Colour		x		
Crumb Colour 'L'	o			x
'a'	o			
'b'	o			x
Cell Size	x		x	
Cell Uniformity	x	x	x	
Instron Hardness	x	x	x	
Instron Gumminess	x	x	x	
Moisture Content			x	

x = very significant (< 0.05)

o = > 0.05 < 0.09

and cell uniformity, quadratic effects were very significant but the interaction effects were less important. Based on these determinations it appeared that each of the factors had a significant effect on some of the characteristics. Therefore the full model was used to generate two-dimensional contour plots for each response.

Cassava starch and water were the two factors which appeared to have the most influence on the physical characteristics of the rice/cassava/soy bread. Cassava starch had a highly significant effect on all responses except moisture content and top crust colour, and was less significant for crumb colour. Water levels were very highly significant for all responses except those related to colour. Soy protein concentrate appeared to be very highly significant for loaf shape and just a little less important to specific volume, Instron hardness and gumminess and cell uniformity. There was no significant effect of SPC level on cell size or moisture content. Proof time showed no significant effects on responses other than loaf shape and to a lesser extent, specific volume, crumb colour 'L' and 'b' values.

The F-values and associated probabilities just discussed show quite definitely that cassava starch and water were the most important of the four factors being tested. Regression coefficients, coefficients of determination (R^2), coefficients of variation (CV) and model significance are summarised in

Tables 5.7 and 5.8. Joglekar and May (1987) explained the use of each of these measures and stated that they were especially important in determining whether the model was adequate for evaluating each response. They recommended that R^2, which measures the amount (%) of variation accounted for by the model, should be at least 80%. Cornell (1984) stated that one drawback of the R^2 is that when there are no replicate observations, the value of R^2 will approach 1 even if the model is not appropriate.

The CV, which should not exceed 10%, describes the amount of variation in a population in relation to the mean. Model significance is the level of confidence that the selected model cannot be due to experimental error (Betker, 1990). According to Joglekar and May (1987) this should be at least $p< 0.05$. In this study the model significance was $p= 0.0001$ for all responses except colour, where it was significant to a lesser extent for top crust colour and crumb colour 'L' and 'b' values. It was not significant for crumb colour 'a' values.

The model explained between 78% and 94% of the variation for specific volume, hardness, gumminess and moisture content. For all others R^2 values ranged from 12% (top crust colour) to 51% (crumb colour 'L' and 'a'). For cell size and cell uniformity only 32% and 41% respectively of the variations were explained. Correspondingly, the coefficients of variation for these two responses were 43% and 21% which

Table 5.7. **Regression coefficients with probabilities, R-square and coefficients of variation for colour of rice flour bread.**

Effects of Independent Variables	Dependent Variables			
	Top Crust Colour	Crumb Colour		
		L	a	b
b_0	4.000(.0001)	70.974(.0001)	-7.325(.0003)	10.885(.0001)
LINEAR				
b_1 (Cassava)	-0.006(.8295)	0.117(.5964)	-0.621(.2738)	-0.075(.6903)
b_2 (Soy Conc.)	0.093(.0007)	-0.390(.0845)	0.669(.2393)	0.292(.1255)
b_3 (Water)	0.032(.2427)	0.297(.1852)	-0.121(.8287)	-0.165(.3800)
b_4 (Proof Time)	0.006(.8276)	-0.661(.0051)	0.620(.2745)	-0.369(.0554)
QUADRATIC				
b_{11}	-0.030(.3844)	0.495(.0861)	-1.707(.0228)	0.688(.0067)
b_{22}	-0.030(.3844)	0.408(.1537)	-0.832(.2505)	0.307(.2032)
b_{33}	-0.061(.0758)	0.139(.6224)	0.187(.7937)	0.110(.6436)
b_{44}	0.002(.9657)	0.341(.2313)	-0.825(.2547)	0.230(.3384)
INTERACTIONS				
b_{12}	0.040(.1914)	0.180(.4663)	0.182(.7725)	-0.147(.4826)
b_{13}	0.007(.8101)	0.353(.1586)	-0.695(.2732)	0.376(.0794)
b_{14}	0.008(.7822)	0.535(.0366)	-1.042(.1049)	0.157(.4536)
b_{23}	0.023(.4478)	-0.087(.7237)	-0.581(.3587)	-0.019(.9284)
b_{24}	0.039(.2030)	0.116(.6386)	-0.671(.2903)	0.387(.0719)
b_{34}	0.040(.1925)	-0.425(.0927)	1.193(.0652)	-0.503(.0215)
R^2	0.12	0.51	0.43	0.51
CV(%)	8.70	2.00	34.4	10.0
Model Significance	.0668	.0399	.1622	.0383

Table 5.8. Regression equation coefficients with probabilities, R-square and coefficients of variation for physical and textural measurements of rice bread.

Effects of Independent Variables	Dependent Variables						
	Specific Volume	Loaf Shape	Hardness	Gumminess	Moisture Content	Cell Size	Cell Uniformity
b_0	2.768 (.0001)	19.813 (.0001)	5.800 (.0001)	3.454 (.0001)	49.436 (.0001)	1.44 (.0001)	2.938 (.0001)
LINEAR							
b_1 (Cassava)	0.380 (.0001)	0.607 (.0031)	-2.241 (.0001)	-1.158 (.0001)	-0.004 (.8929)	-0.197 (.0019)	-0.347 (.0001)
b_2 (Soy Conc.)	-0.241 (.0001)	-0.805 (.0001)	1.316 (.0036)	0.638 (.0110)	-0.049 (.1418)	0.097 (.1231)	0.184 (.0003)
b_3 (Water)	0.414 (.0001)	1.293 (.0001)	-2.422 (.0001)	-1.391 (.0001)	0.687 (.0001)	-0.159 (.0117)	-0.134 (.0081)
b_4 (Proof time)	0.041 (.4417)	-1.093 (.0001)	-0.277 (.5100)	-0.282 (.2394)	0.072 (.0341)	-0.034 (.5831)	-0.009 (.8519)
QUADRATIC							
b_{11}	0.013 (.8533)	0.624 (.0159)	1.120 (.0429)	-0.522 (.0918)	-0.025 (.5507)	0.418 (.0001)	0.224 (.0006)
b_{22}	0.111 (.1076)	0.842 (.0012)	-0.217 (.6841)	-0.147 (.6264)	-0.070 (.0998)	0.004 (.9610)	-0.041 (.5181)
b_{33}	0.033 (.6313)	0.967 (.0002)	0.928 (.0899)	0.580 (.0625)	0.010 (.8051)	0.074 (.3529)	0.021 (.7419)
b_{44}	0.119 (.0868)	0.811 (.0018)	-0.344 (.5199)	-0.164 (.5890)	0.010 (.8051)	-0.082 (.3047)	-0.088 (.1692)
INTERACTIONS							
b_{12}	-0.009 (.8743)	0.446 (.0505)	-1.098 (.0248)	-0.611 (.0271)	0.056 (.1300)	-0.020 (.7802)	-0.035 (.5315)
b_{13}	-0.036 (.5491)	-0.211 (.3516)	1.854 (.0004)	0.967 (.0009)	-0.024 (.5119)	0.277 (.0001)	0.223 (.0001)
b_{14}	0.214 (.0001)	0.758 (.0010)	-0.494 (.2952)	-0.219 (.4115)	-0.008 (.8264)	-0.113 (.1069)	-0.129 (.0228)
b_{23}	0.061 (.3107)	0.538 (.0186)	-1.227 (.0130)	-0.606 (.0284)	-0.032 (.3855)	-0.098 (.1642)	-0.121 (.0323)
b_{24}	-0.091 (.1313)	-0.241 (.2885)	-0.163 (.7275)	-0.163 (.5386)	-0.021 (.5916)	0.090 (.2004)	0.012 (.8347)
b_{34}	-0.024 (.6891)	0.007 (.9770)	-0.056 (.9052)	0.069 (.7952)	-0.021 (.5567)	0.012 (.8670)	0.020 (.7280)
R^2	00.85	00.47	0.79	0.78	.94	.32	.41
CV (%)	11.0	11.0	36.0	35.0	.4	43	20
Model Significance	.0001	.0001	.0001	.0001	.0001	.0001	.0001

reflect the amount of variation among the samples relative to the mean. The CV values were below 10% for moisture content, top crust colour and crumb colour. The colour responses were not used any further for generating contour plots, since the addition of soy and cassava was expected to show considerable colour difference from the reference loaves. Overall the colour of the bread did not vary to any significant degree therefore it was no longer considered in the analysis.

Of the nine parameters measured, only moisture content and specific volume met all three criteria for determining goodness of fit. However, moisture content was affected only by water levels, in the doughs, therefore no further analysis was conducted with the response. Instron hardness and gumminess, and loaf shape met two of the criteria. Cell size and cell uniformity met the criteria for model significance alone, but they were evaluated subjectively which may in part account for the greater variability. It was felt that these two responses were important enough to be retained for further analysis.

Regression coefficients are indicative of the factor effects and describe the average change in response as the factor is changed from its low to its high level (Joglekar and May, 1987). These effects may be negative or positive which is indicated by the sign before each value. The size of the coefficients and their associated probability values indicate the relative importance of the independent variables to the prediction of the dependent variable (Betker, 1990).

From Tables 5.7 and 5.8 it was evident from the coefficient for linear effects that cassava starch and water had the greatest effects on most of the responses. However cassava starch did not influence moisture content, top crust colour and crumb colour, while water had no apparent effect on any of the colour values except for crumb colour 'L' values. It seemed that if SPC was increased there should be a decrease in specific volume, loaf shape, moisture content and crumb colour 'L', while top crust colour should be darker and the crumb of the loaf become harder and more gummy, with very small and compact cell structure. It was evident that as proof time increased there could be a deterioration in loaf shape but the bread crumb colour would become lighter.

The interaction coefficients indicated some cassava starch x proof time interaction on specific volume and loaf shape. Cassava starch also seemed to interact influentially with water on all the texture parameters (Instron hardness, gumminess, cell size and cell uniformity). However, the quadratic and interaction effects seemed better explained with the aid of contour plots.

During preliminary studies as well as the experimental period, it had been observed that some loaves had varying sizes of cavities under the crust. The incidence of cavities could not be attributed to any specific ingredient or factor. Loaves made from the identical centerpoint formulations sometimes produced different results (Figure 5.4). Ylimaki (personal communication) had suggested that large air holes in the loaves could be due to improper placing of the dough into the pans. To investigate this occurrence, RSREG was used to determine the probability of cavities occurring. The values used (1 and 0 to represent the presence and absence of cavities) were based on the results of the experiments. The F-values and corresponding probabilities indicated that water ($p = 0.013$) and cassava starch ($p = 0.022$ [Appendix 8]) had a significant effect on the occurrence of cavities. This was confirmed by the regression coefficients which showed by the linear effect that an increase in either cassava starch or water increased the probability of a cavity occurring by 17.5% and 22.5% respectively. Examination of the raw data as well as the photographs of slices suggested a relationship among cavity occurrence, specific volume and loaf shape.

Examination of Contour Plots

In order to examine the linear, quadratic and interaction effects of the critical factors, contour plots were generated for cavities, specific volume, loaf shape, Instron hardness, gumminess, cell size and cell uniformity. The two most important variables, cassava starch and water were chosen as the axes for all the contour plots. Of the ingredient factors, soy protein concentrate had the least effect so it was held at the four levels (-1, 0, 1 and 2) in order to

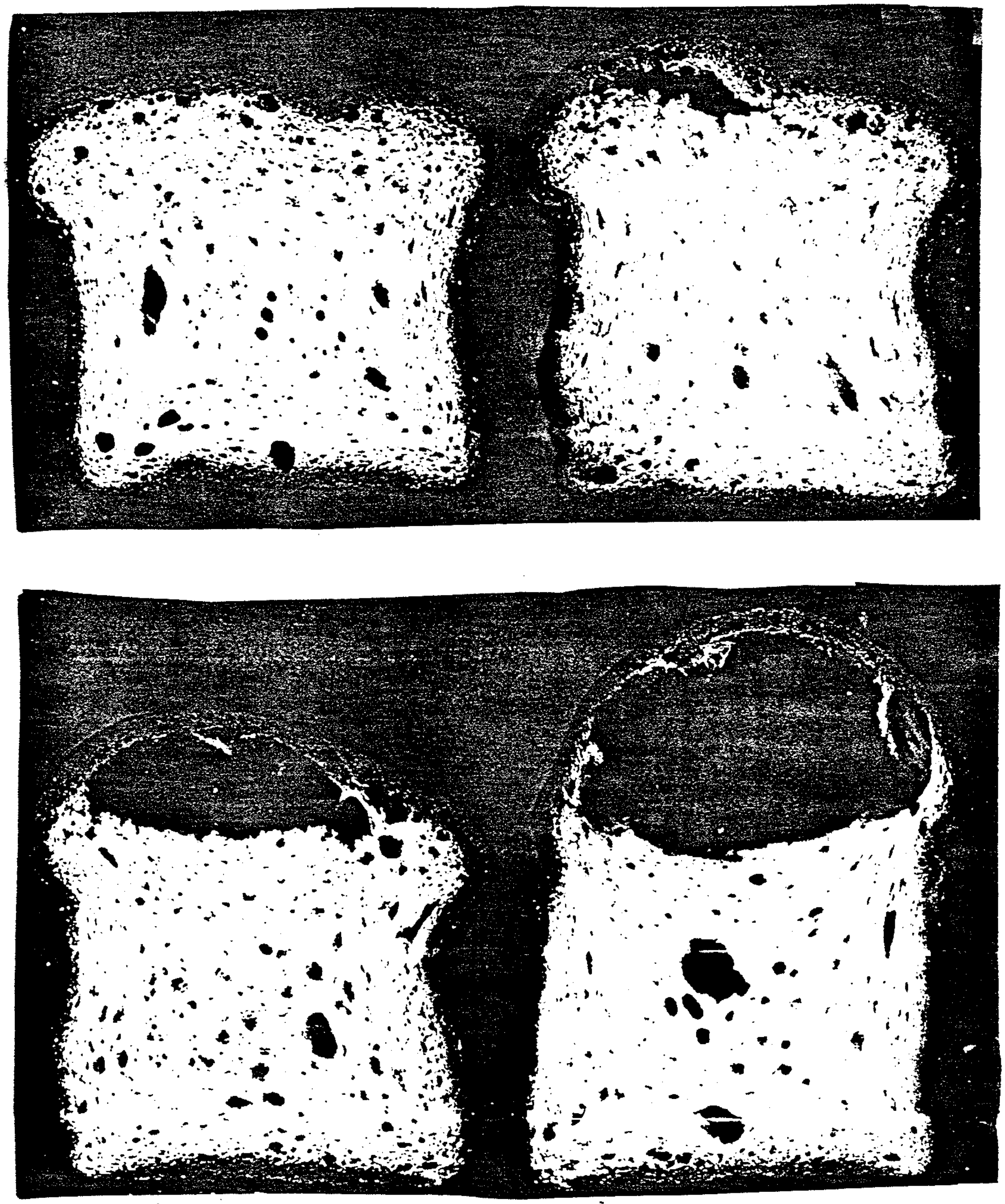


Figure 5.4. Slices indicating the incidence of cavities in loaves prepared from the same formula and under identical conditions.

evaluate its effects. Soy protein concentrate was not considered at level -2 because it was calculated that the amount of soy (2 g) would not have been sufficient to enrich the bread. For all plots proof time was held constant at the centerpoint level (0).

Proof time was included as an independent variable in the experimental design because effects of proof time might have affected conclusions regarding the effects of the other variables. To evaluate the effects of proof time, specific volume was used as the response variable. Nine plots were generated using cassava starch and water as the axes, to show the effects these factors at three proof times and three levels of SPC. These plots have been compiled in Figure 5.5.

The indication is that across each level of SPC there was a very slight increase in specific volume for each higher level of proof time, while for each proof time, it could be seen that with the higher levels of SPC, specific volumes decreased slightly. A comparison of the effects of three different levels of SPC on the cassava starch*water plots showed very little differences among the proof times. The overall picture indicated that the effects of the other factors could be interpreted effectively using a single proof time, therefore it was decided to examine the contour plots holding proof time constant at level 0 (45 minutes).

After each plot was examined to determine the effects of

SPECIFIC VOLUME

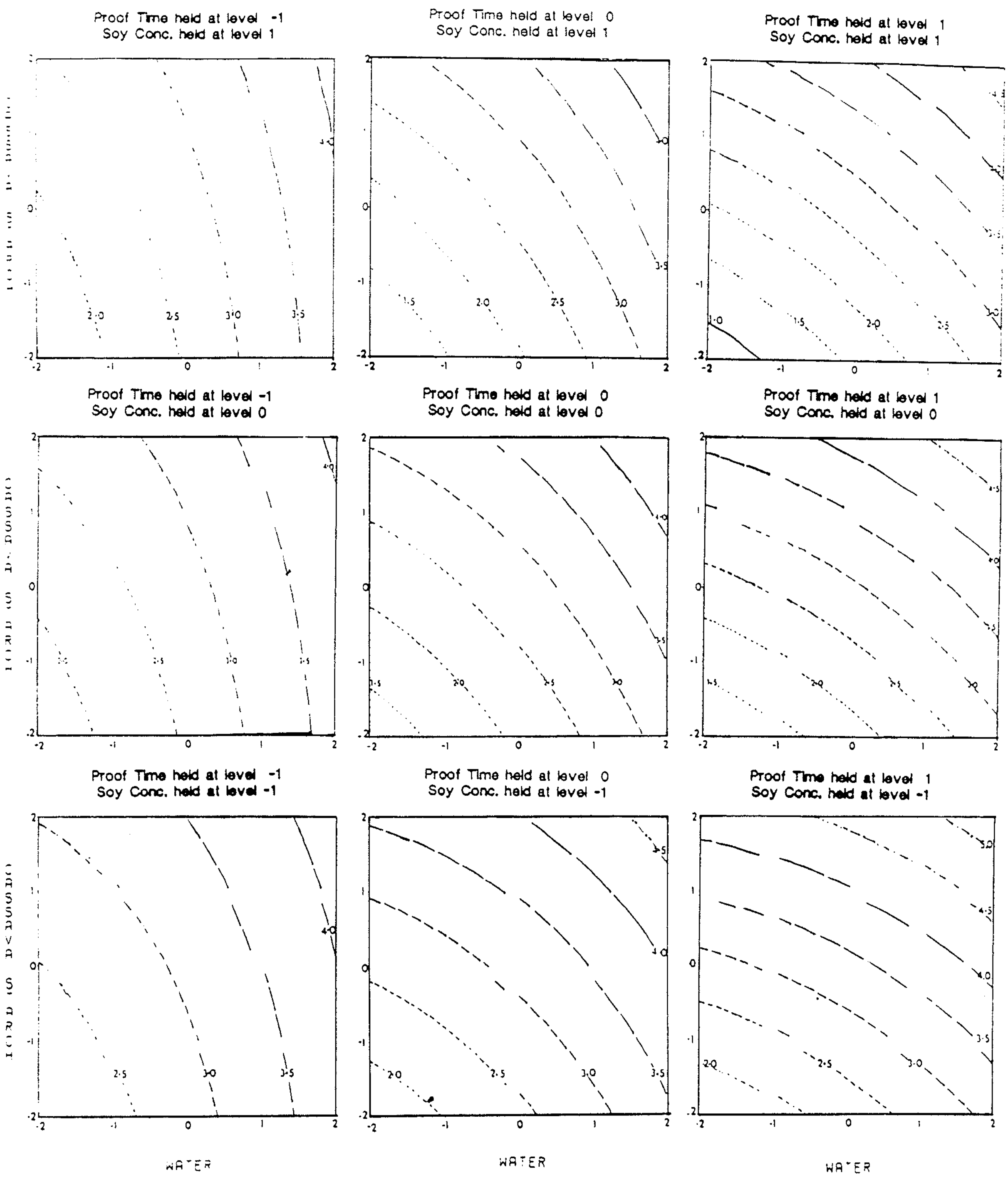


Figure 5.5. Contour plots for specific volume generated to further determine the effects of proof time.

each factor, then the areas meeting reference standards (Table 5.9) were highlighted. In the figures presented in the following sections levels of independent variables are given as coded values, while the values for the responses are the actual measurements. Amounts of cassava starch, water and soy protein concentrate that compare to the coded values are given in Table 5.2.

Investigation of the Relationship Among Cavities, Specific Volume and Loaf Shape.

Effect of cassava starch, water and soy protein concentrate on the occurrence of cavities.

Figure 5.6 presents the probability of cavities as a function of cassava starch and water, with SPC at -1, 0, +1 and +2 levels and with proof time held constant at 0 level. On these plots the highlighted regions represent the probability of 0 to 0.1 (0 to 10%) occurrence of cavities, the range that was considered acceptable for this response. The acceptability region was small at the low (-1) level of SPC and increased with each increase in SPC up to level +1. At level +2 SPC, there was a slight decrease in the size of the region. It also appeared that with the highest amount of SPC, for a given water level, there needed to be less cassava starch in order to increase the probability of no cavities. With the lowest level of SPC (-1), with low levels of water

Table 5.9. Range of values for reference loaves and acceptability regions for experimental loaves.

RESPONSE	RANGE	ACCEPTABLE REGION
Specific volume,cc/g	3.04 - 4.01	≥ 2.5
Loaf shape[1]	19.5 - 27.5	≥ 20
Top crust colour[2]	3.25 - 3.75	3.25 - 4.00
Cell[3]: size	1.50 - 2.38	1.5 - 3.0
uniformity	1.88 - 3.63	≥ 3.5
Texture: Hardness (N)	2.92 - 5.39	3.5 - 12.5
Gumminess (N)	1.87 - 2.81	2 - 5
Moisture content	48.52 - 50.00	48 - 50

[1] Maximum score = 30: 5 characteristics with a score range of 1 to 6, and 6 being the score for the highest degree of desirability.

[2] Maximum score = 5 (very dark)

[3] Maximum score = 5 (very small and very uniform)

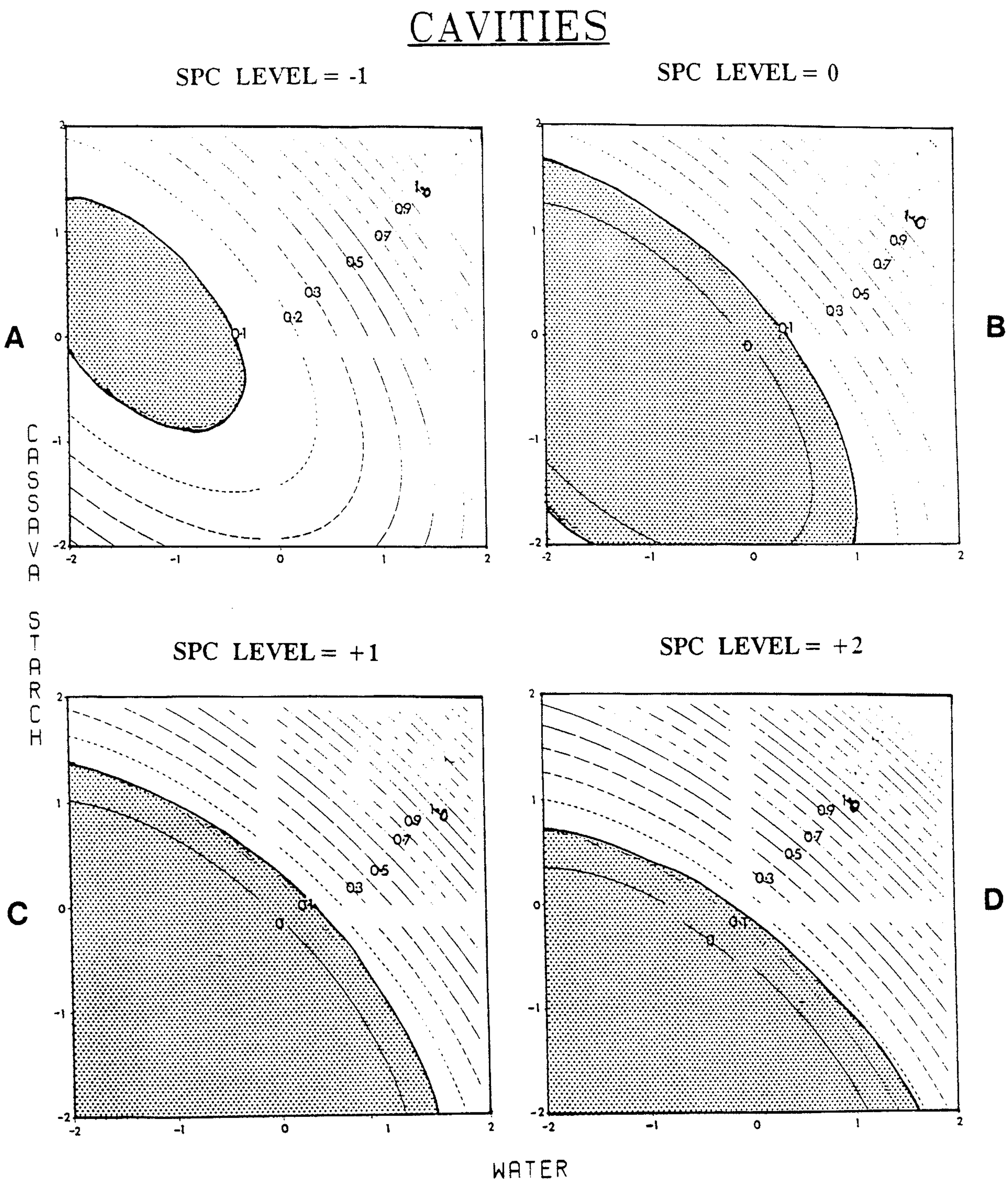


Figure 5.6. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on the incidence of cavities. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. Values for the response are probabilities values.

(-2 and -1), cassava starch needed to be between levels 0 and +1.5 to achieve a high probability of no cavities. As water levels increased, the probability of cavities increased, regardless of the level of cassava starch, indicating that cassava starch had less effect than water. As can be seen in Figures 5.6C and 5.6D water and cassava starch seem to interact inversely, as when levels of cassava starch were higher, decreased water resulted in reduced probabilities of cavities. At high levels of cassava starch and water, (eg. +1 and +1) the probability of cavities was > 0.05 for all levels of SPC.

Effect of cassava starch, water and soy protein concentrate on loaf shape

Loaf shape score represented the five specific attributes (loaf bottom surface, loaf side, loaf top surface, top surface symmetry and loaf top-side indentation), for which scores were accumulated to give one overall value. Ylimaki (1987) had indicated that whereas for wheat bread a large volume was a good indicator of bread quality, this was not so for rice flour bread. Her results indicated that together with a large volume, a good loaf shape, with a rounded top, straight sides and a flat bottom, were desirable.

From the plots in Figure 5.7. it can be seen that at lower (-1, 0) levels of SPC, with low cassava starch , loaf shape scores decreased with increasing water levels from -2

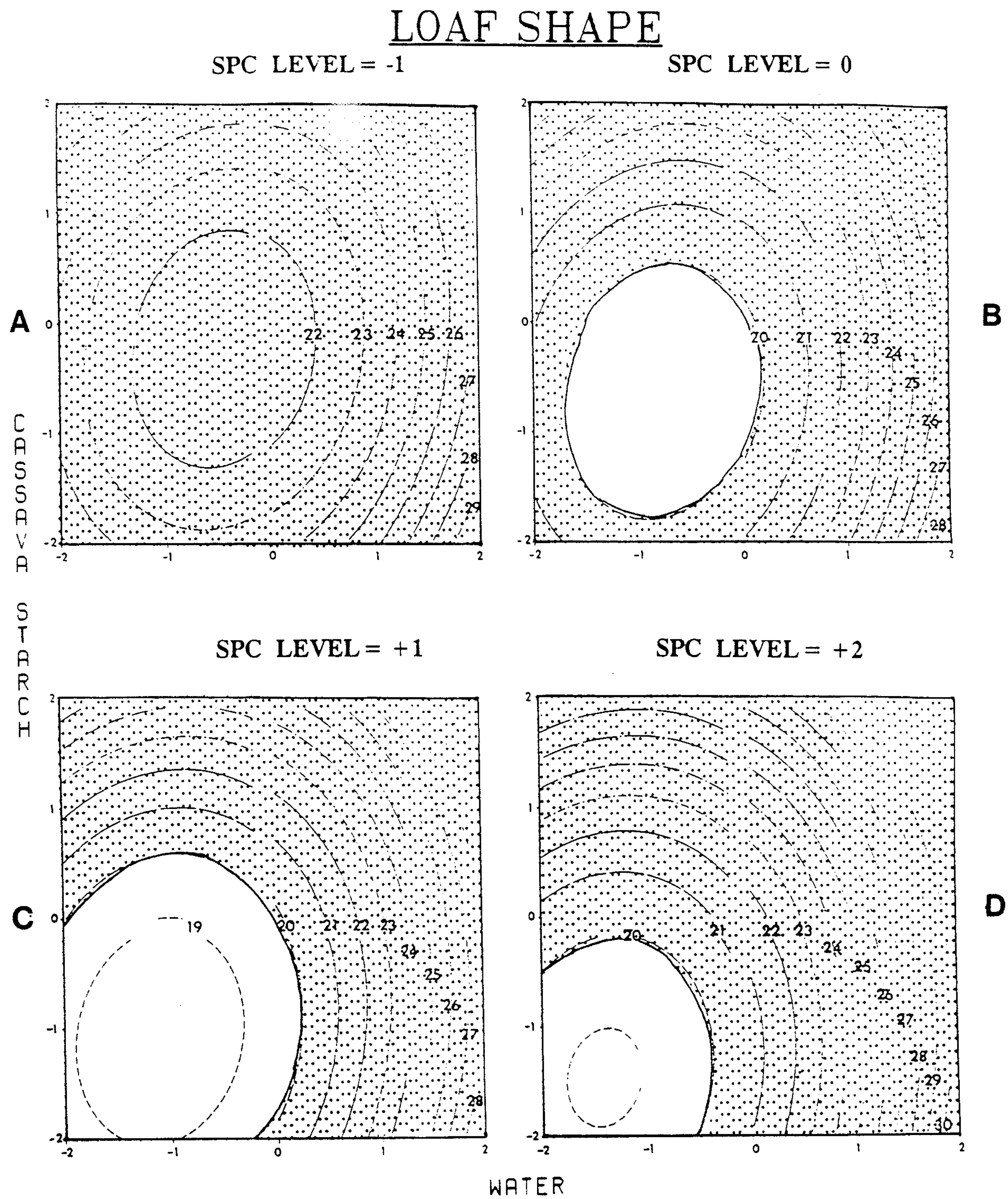


Figure 5.7. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on loaf shape. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. Values for the response are loaf shape scores.

up to centerpoint (0) then increased consistently at which point water appeared to have more effect on loaf shape than cassava starch. With SPC at +1 and +2 levels, the scores increased consistently with increasing levels of water and cassava starch, although water seemed to have the stronger influence.

From Figures 5.7C and 5.7D it could be seen that, for any specific combination of cassava starch and water (eg. 0 and +1) SPC level +2 gave a higher loaf shape score than SPC level +1 (+2 = 25 and +1 = 23).

Effect of cassava starch, water and soy protein concentrate on specific volume

Specific volume is considered an effective way of comparing loaves with different volumes and weights. It provides an indication of the density of the crumb in the loaf. For wheat breads, specific volumes ranging from 4.5 - 6 are acceptable. Ylimaki (1987) reported specific volumes that were much lower. The specific volumes for the references used in this study ranged from 3.04 to 4.01.

Figure 5.8 illustrates the effects of the ingredient factors on specific volume. At SPC level -1, cassava starch levels from as low as -0.8 and water levels ranging from +0.3 upward achieved an acceptable specific volume. With each higher level of SPC (0 and +1), cassava starch and water needed to be increased to maintain the desired specific

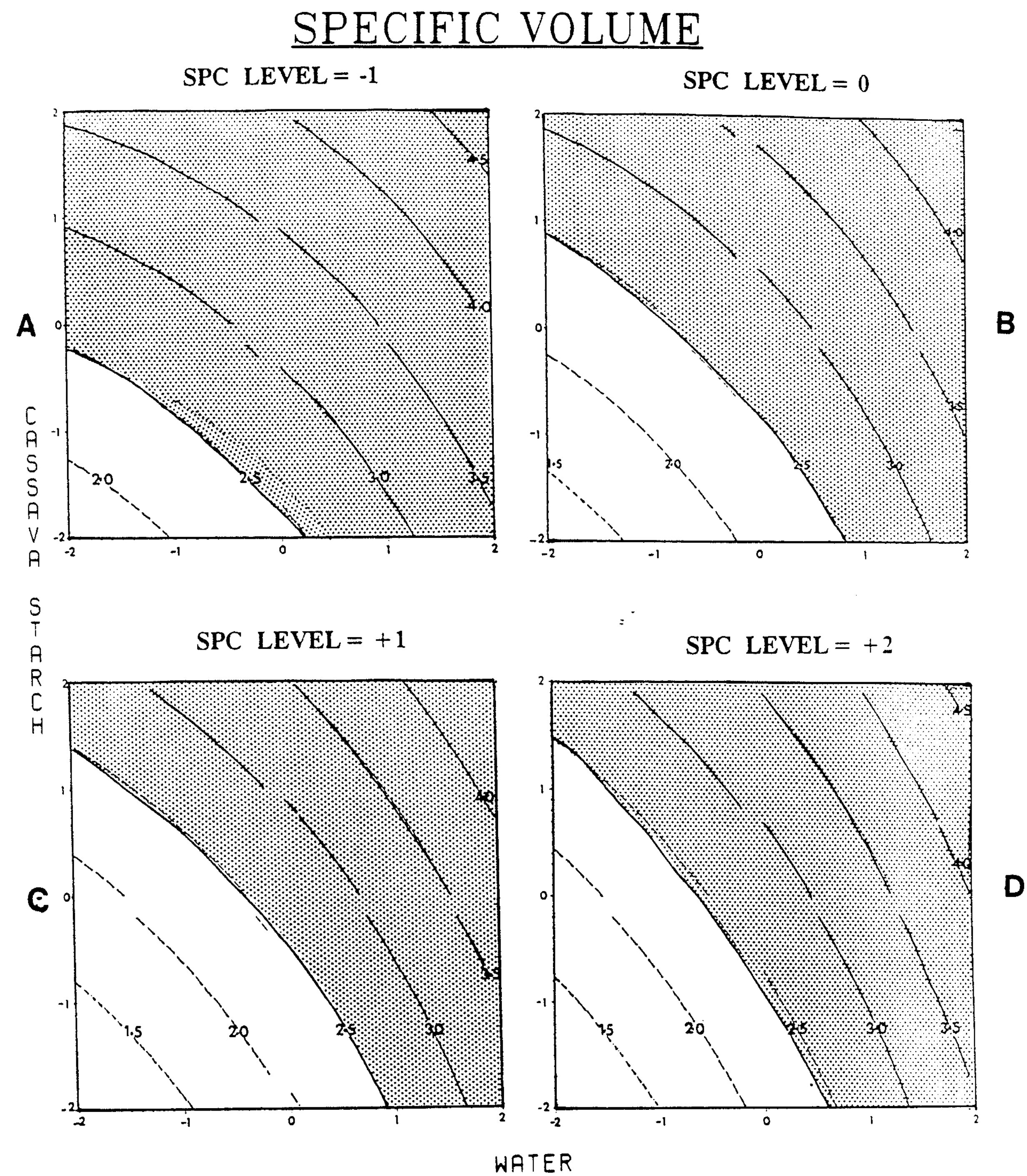


Figure 5.8. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on specific volume. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. Values for the response are specific volume measurements.

volume, although cassava starch more so than water. At SPC level +2, there was a slight decrease in water requirement. It was noticeable that whereas at the lower levels of SPC (-1 and 0) the required increase in cassava starch and water appeared to be proportional, with the higher levels of SPC (+1 and +2) changes in water levels seemed to have more effect than increased cassava starch on specific volume ($\geq$ 2.5). The large acceptability region indicated that a range of casava starch x water combinations should produce acceptable specific volume.

When photographs and raw data had been carefully examined it appeared that the higher the specific volume and loaf shape score, the greater was the tendency for cavities to occur. The contour plots for cavities, specific volume and loaf shape were therefore superimposed at the four levels of SPC, in order to identify regions that would be acceptable for all three characteristics. These results (Figure 5.9) indicated that at -1 level SPC, the formulations would need to have cassava starch at levels between -0.2 and +1.25, and water levels below -1.3 to have a probability of no cavities and still maintain a good shape and specific volume. With each increase in SPC levels (+1 and +2) the trend appeared to be that water levels could be increased with a slight decrease of cassava starch and still the desired qualities would be maintained.

For all levels of SPC it appeared that an increase in

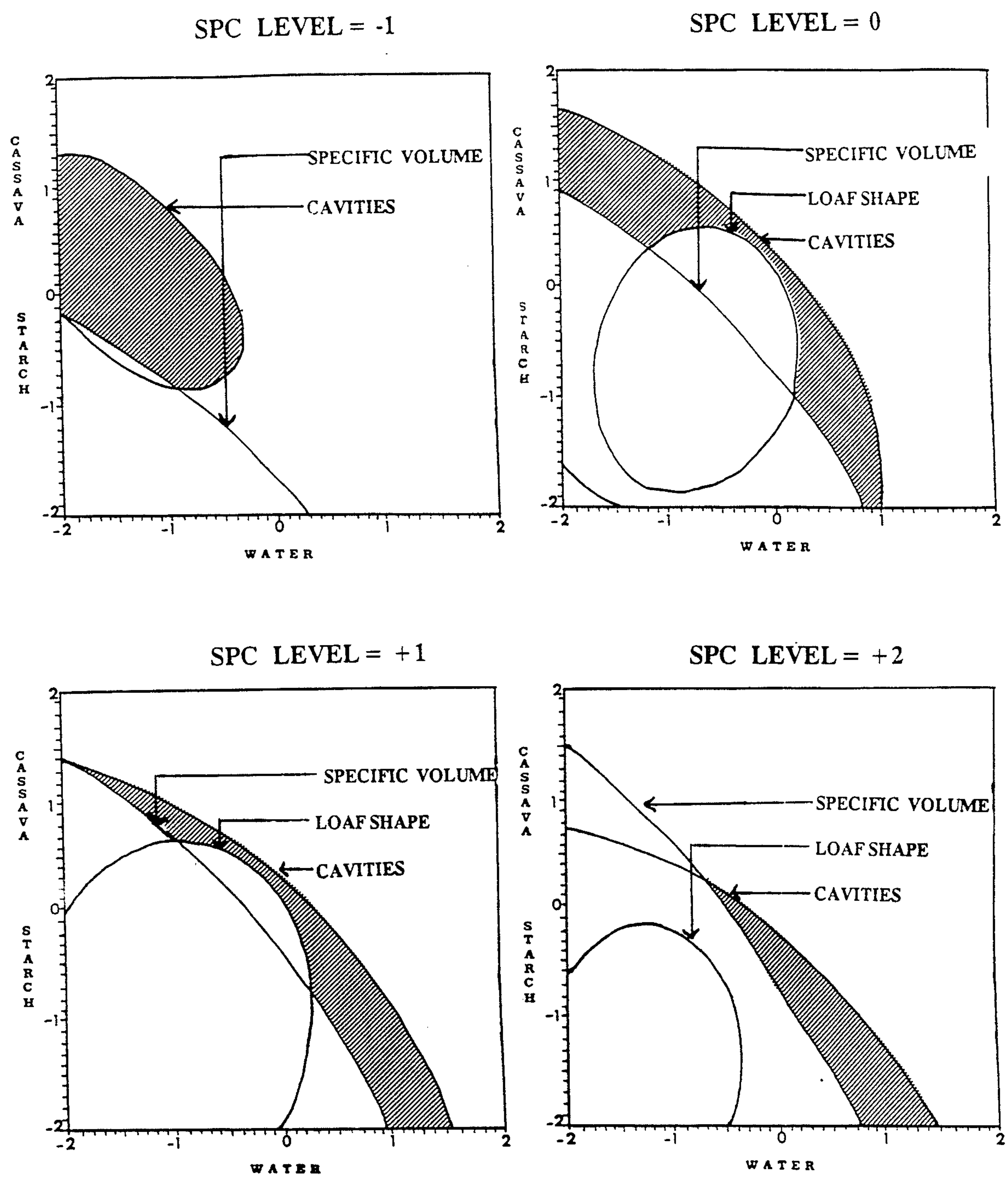


Figure 5.9. Superimposed contour plots demonstrating the relationship among specific volume, loaf shape and the incidence of cavities. The shaded area indicates the acceptability region when the three responses are examined together.

cassava starch and water levels improved specific volume and loaf shape score, but the prediction was for an increase in the occurrence of cavities. There is , however, an area in which specific volume and loaf shape are acceptable while the probability of cavities is still very low. In order to further reduce the probability of the occurrence of cavities, it might be necessary to accept a smaller specific volume and loaf shape score.

Evaluation of Top Crust Colour and Crumb Colour

Generally, top crust colour and crumb colour were the responses least affected by any of the independent variables. There was however, a significant effect of soy protein concentrate level on top crust colour, and of proof time on crumb colour 'L' (lightness) value. Quadratic and interaction effects for these two responses were not significant. Soy protein concentrate did not affect any aspects of crumb colour. The top crust colour was evaluated subjectively and the raw data showed that the crusts which had the darker colour, were in fact from the loaves with higher levels of soy. This darker coloured crust could be attributed to the presence of increased amounts of reducing sugars, especially fructose and glucose, from the soybean concentrate, or to more lysine especially when the soy is increased. These contribute to the Maillard-type browning of the crust (Hoseney, 1986). Collins and Temalilwa (1981) reported changes in the colour of dough when soyflour was added to cassava flour. However this was reflected mainly in the Hunter'a' (redness) and 'b' (yellow) values but was not perceived by visual observation. It is quite possible that the small but significant effect of proof time on crumb colour 'L' values may not have been related so much to the colour of the crumb but to differences in the density of the crumb.

Figure 5.10 illustrates the effects on top crust colour. With proof time and water held at level 0 (centerpoint) the effect of cassava starch and SPC are seen. It appeared that at lower levels (-2) cassava starch had very little effect but with increasing SPC the top crust colour increased at higher levels of cassava starch.

Evaluation of Texture Characteristics

In addition to those characteristics previously discussed, the texture characteristics were also of great importance to bread quality. Therefore the contour plots for Instron hardness, gumminess, cell size and cell uniformity were examined in order to further investigate the quadratic and interaction effects. The acceptability regions were also shaded.

Effect of cassava starch, water and soy protein concentrate on Instron hardness and gumminess.

From the F-values and regression coefficients, it

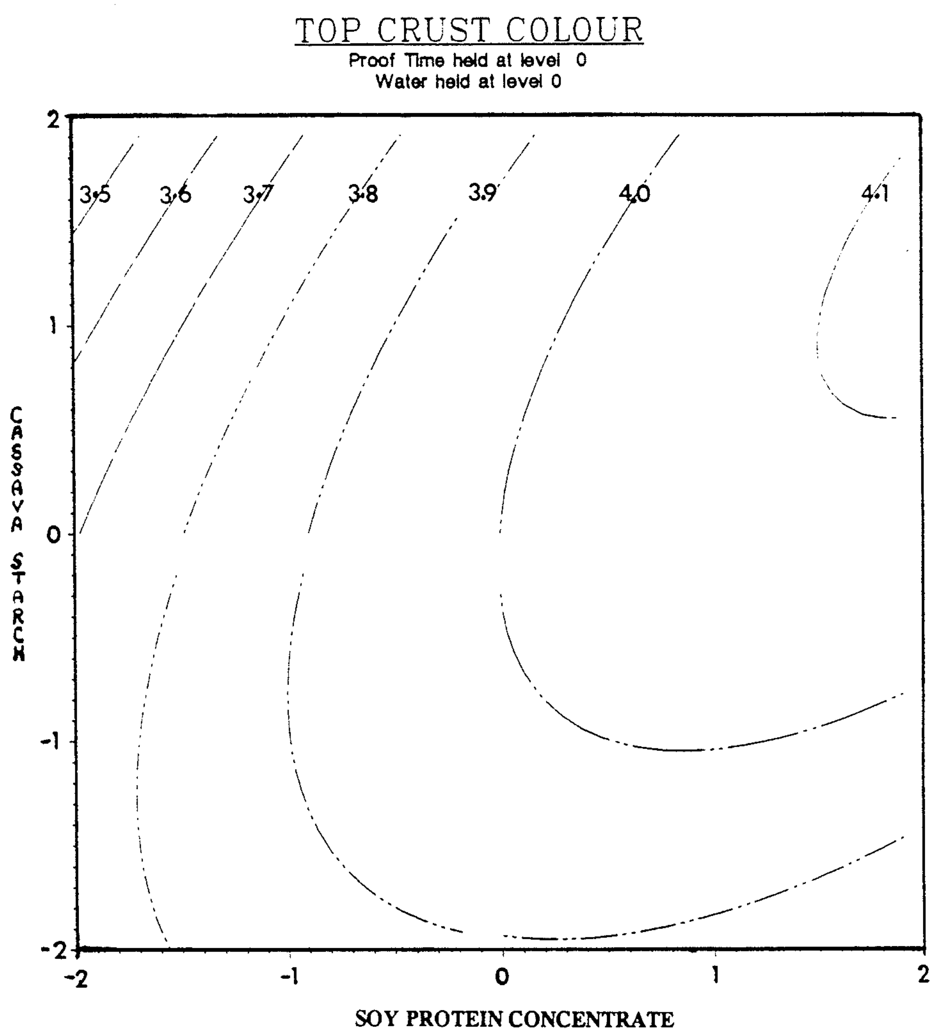


Figure 5.10. Contour plots for top crust colour with proof time and water levels held constant at 0. The levels for cassava starch, water and soy protein concentrate are coded values. Values for colour are actual scores.

appeared that the two Instron texture measurements were affected by the independent variables (factors) in the same manner therefore the two sets of plots (Figures 5.11 and 5.12) were examined simultaneously.

At low water levels, with low levels of cassava, the bread was firmer and much more gummy than the reference. As water and cassava increased, Instron hardness and gumminess values first became lower then rose again. This was the pattern of the contour plots at all the levels of SPC, but at each higher level of SPC the same level of softening and low gumminess could only be achieved by increasing both cassava starch and water. At SPC level -1, 0 and +1, acceptability regions for hardness and gumminess were large, however, the predicted values were closer to the value of the reference standards when cassava starch and water levels approached +1, as can be seen quite clearly from the plot for SPC at level +1. At level +2 SPC the water level necessary to maintain the lower point of the acceptability for hardness (3.5N), ranged from level +1 upward and beyond the boundaries of the experimental design. Similarly cassava starch levels might be from as low as -0.6 upward. This seems to indicate that very high levels of cassava starch and water may be used with the highest level of SPC in order to achieve the desired hardness and gumminess values. Therefore higher soy levels would allow use of more cassava starch and water.

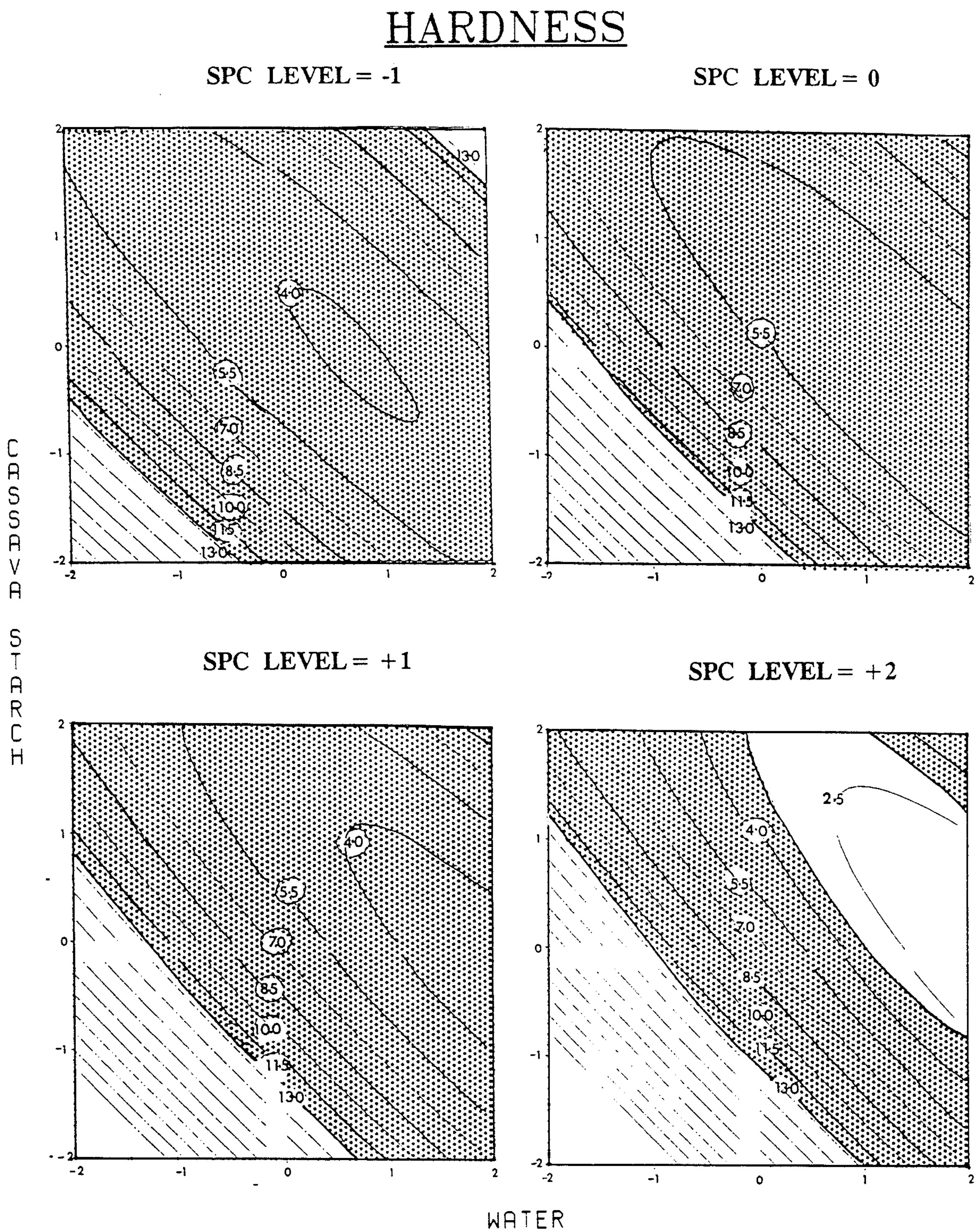


Figure 5.11. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on hardness. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. The response values are actual hardness measurements (N).

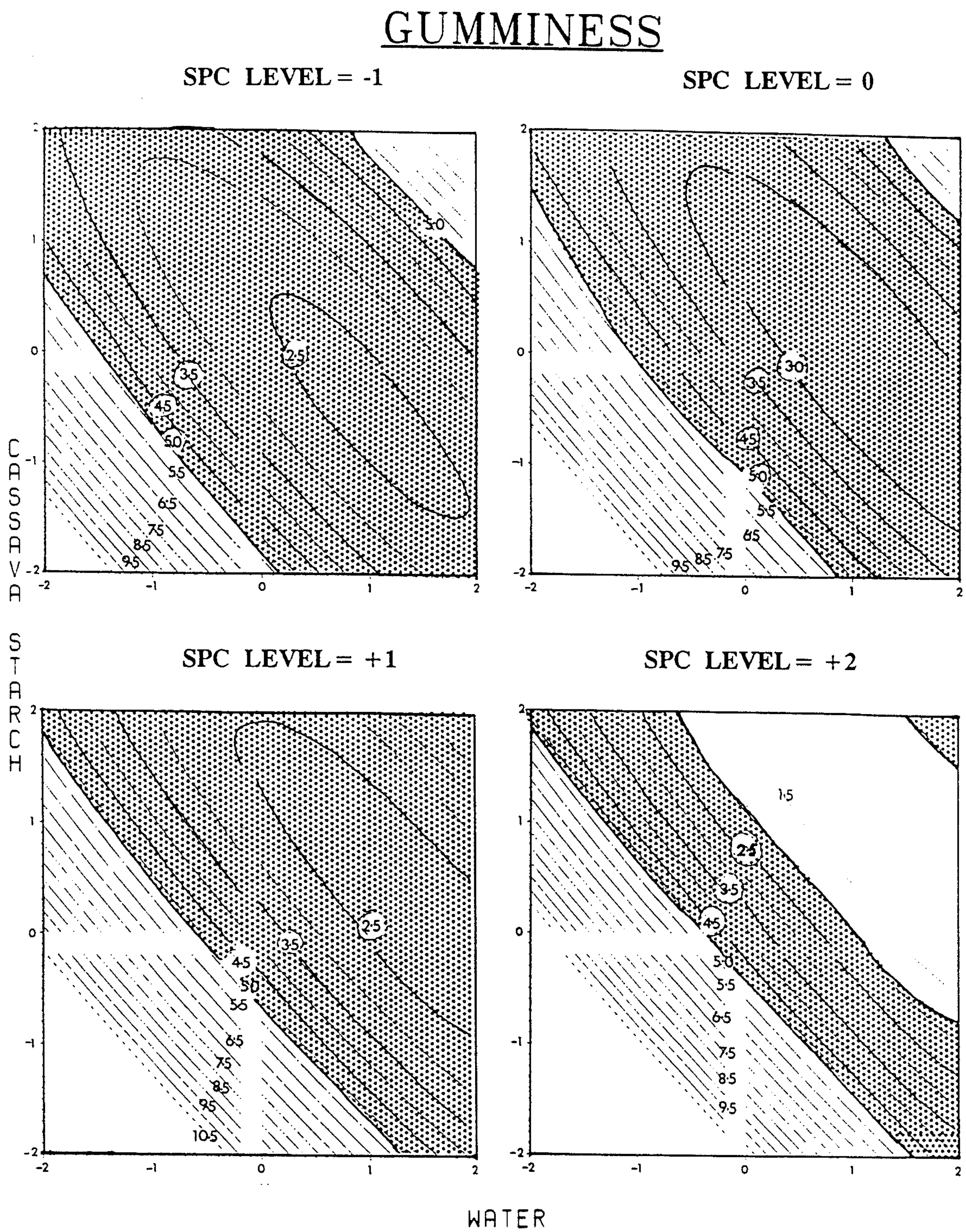


Figure 5.12. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on gumminess. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. The response values are actual gumminess measurements (N).

Effect of cassava starch, water and soy protein concentrate on cell size and cell uniformity

Overall the plots for cell size (Figure 5.13) indicated that levels of cassava starch had a stronger influence than water especially at levels -1 and 0 of SPC. At higher SPC levels (+1 and +2) the water interaction was slightly more noticeable. Generally it appears that SPC very slightly influenced the cassava starch x water interaction on cell size. The acceptability regions at all levels of SPC are large enough to permit several combinations ranging from low cassava starch and 0 level water, to high cassava starch and high water levels.

The effect of SPC on cell uniformity was different from cell size. The plots (Figure 5.14) showed that at higher SPC levels, there was a greater acceptability region. With lower levels of SPC (-1 and 0), at low levels of water it would be necessary to have low levels of cassava starch as well. With increasing water up to level +1.7, the amount of cassava starch which might be used to maintain the acceptable cell uniformity could not exceed -0.7 for soy at -1 level and - 0.3 for soy at 0 level. At the other extreme there is an indication that the highest levels of both cassava starch and water might result in improved cell uniformity. At levels +1 and +2 of SPC, cassava starch requirements increased while water levels remained the same. This suggests that cassava starch had a more significant effect than water on cell

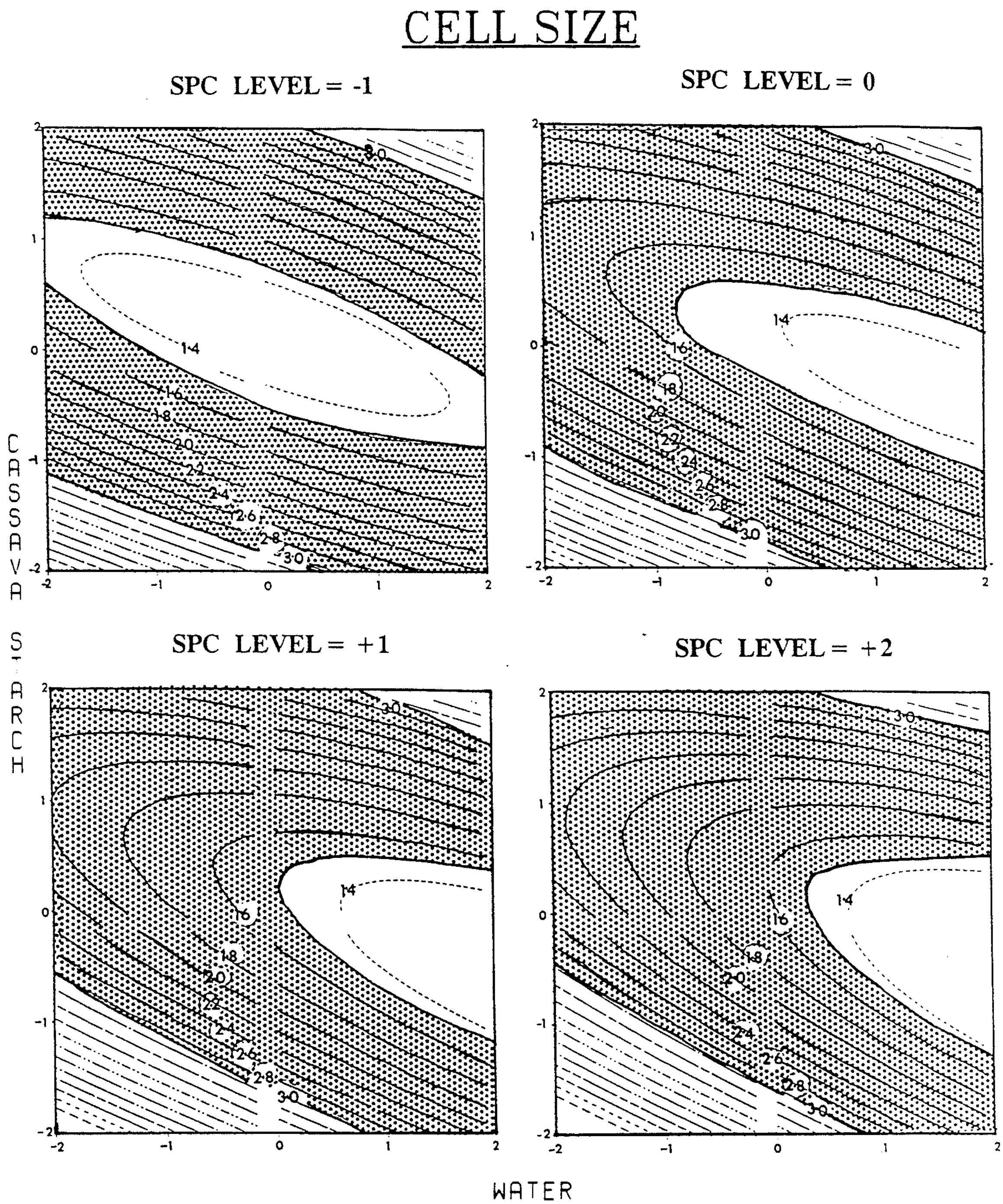


Figure 5.13. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on cell size. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. Values for the responses are actual scores.

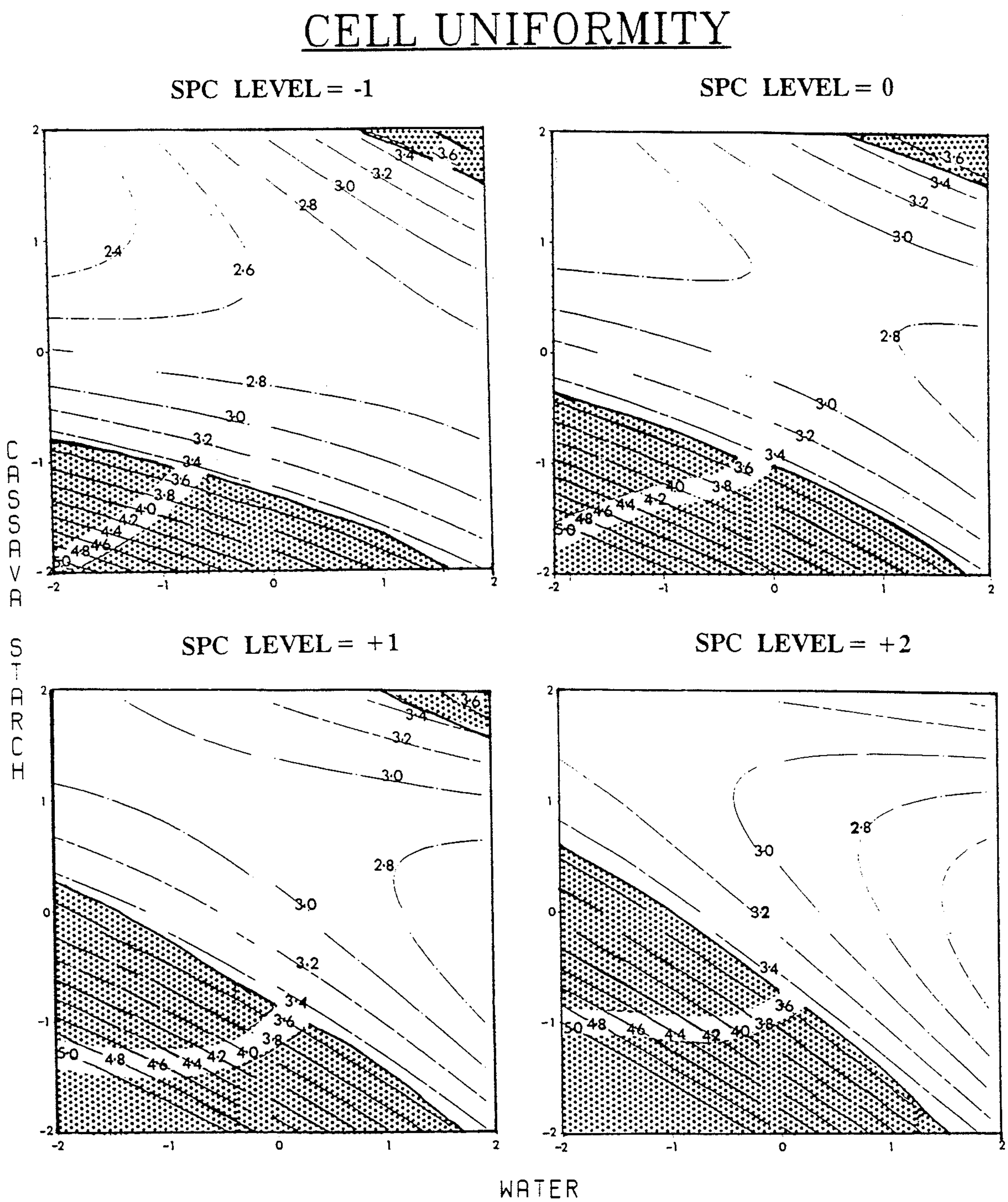


Figure 5.14. Contour plots showing the effects of cassava starch, water and soy protein concentrate levels on cell uniformity. Shaded areas represent regions of acceptability. The levels for the ingredient factors are coded values. values for the responses are actual scores.

uniformity.

The plots for Instron hardness and gumminess, cell size and cell uniformity were superimposed (Figure 5.15 A and B). The level of soy did not effect much change in the water levels required for acceptability of these characteristics. The areas of acceptability for all four characteristics , shown as the darker region in the figure, were very small. This
was due to the difference in the region for cell uniformity, which was concentrated in the lower region of the plot. This region indicated that for all characteristics to meet reference standards, the formulations at each level of SPC would require very low levels of cassava starch (-0.9 to -2) with water levels ranging from +0.3 to +2.

High levels of cassava starch seemed to interfere with the uniformity of the cell structure. Although uniformity is considered desirable, it is the criteria least likely to be influential in determining actual acceptability of the bread by consumers. Therefore it was decided that failure to meet this particular criteria should not rule out the use of the higher cassava starch formulations. With the exclusion of cell uniformity, the acceptability regions for Instron hardness, gumminess and cell size were very large in each plot and indicated that low to high levels of cassava starch might be used.

At SPC levels -1 and 0, the acceptability regions were

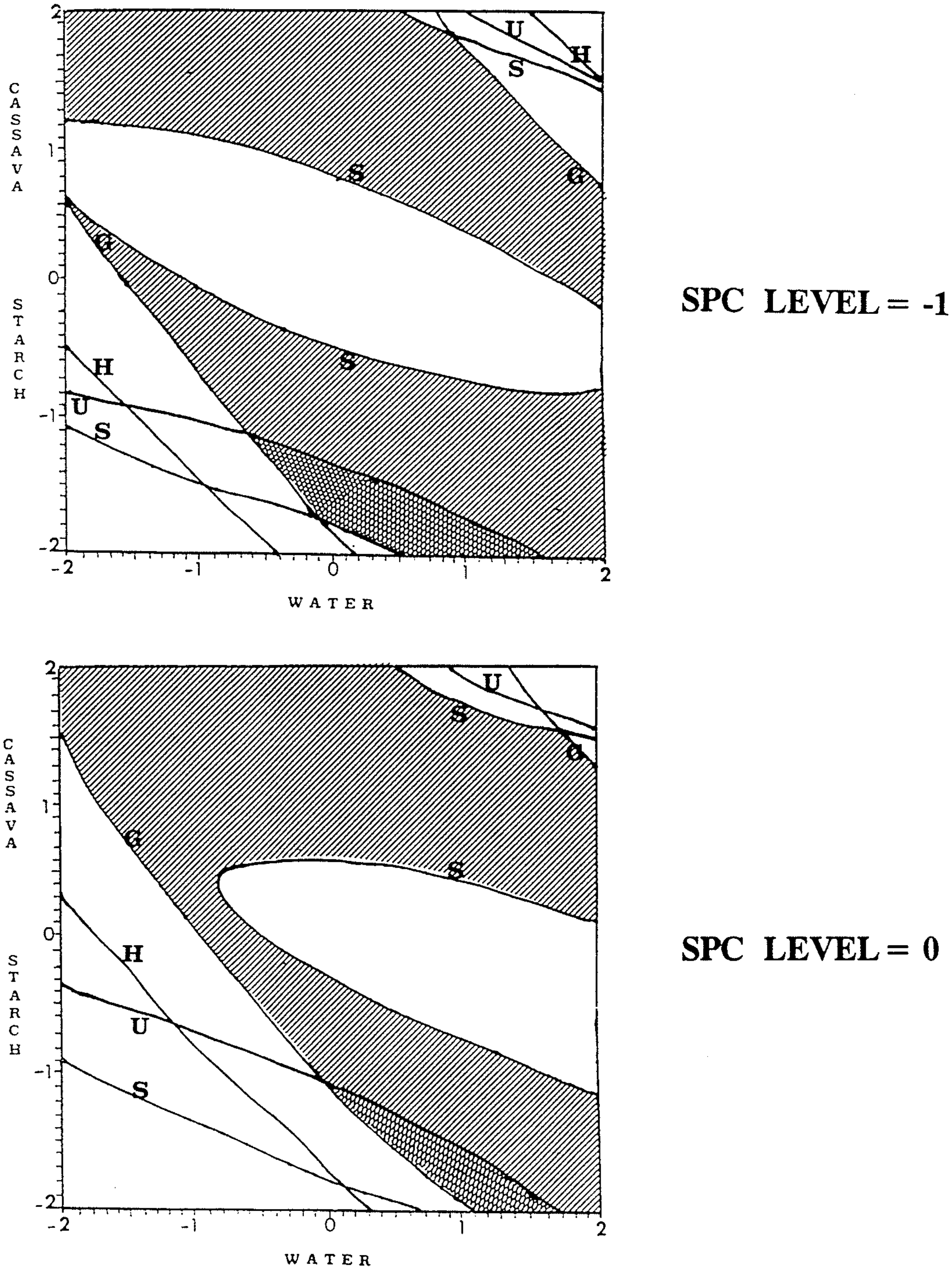
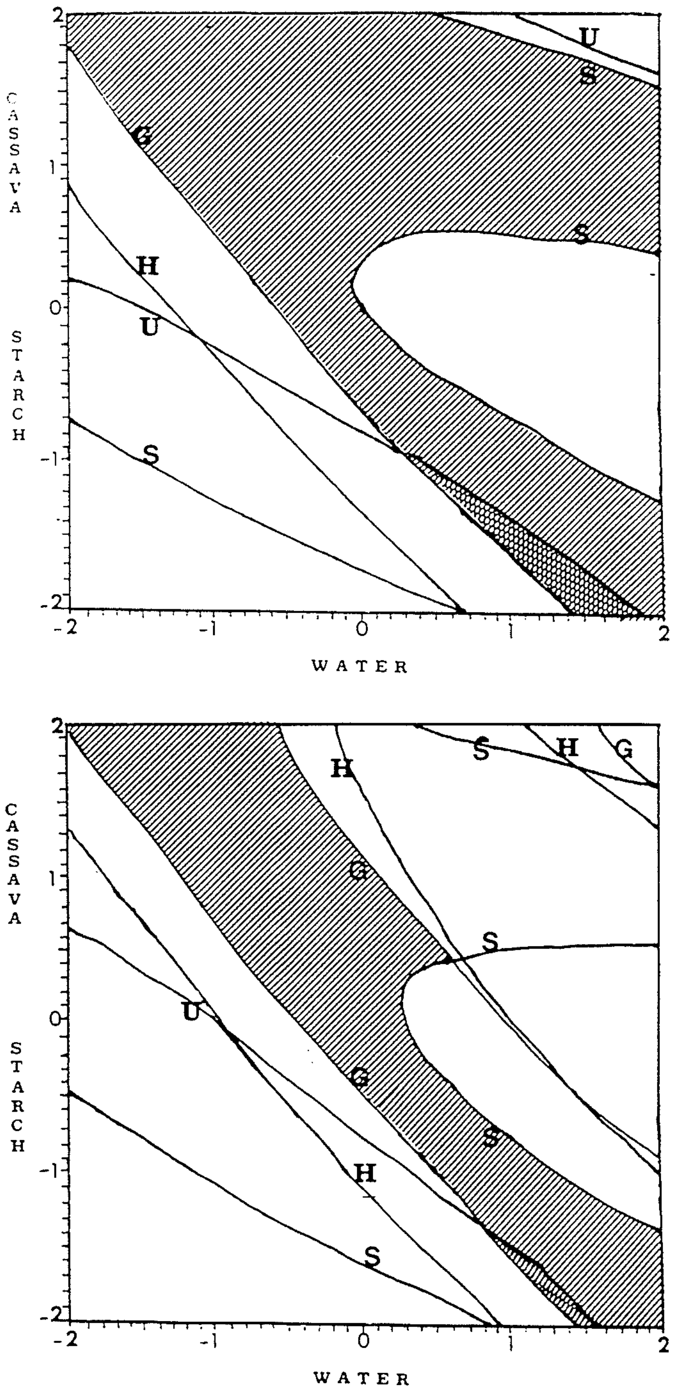


Figure 5.15A. SPC at levels -1 and 0: Superimposed contour plots illustrating the contour lines and regions of acceptability for the texture characteristics of the rice/cassava/soy yeast bread. The striped region met reference standards for Instron hardness (H) gumminess (G) and cell size (S). The darker region includes cell uniformity (U).



SPC LEVEL= +1

SPC LEVEL= +2

Figure 5.15B. SPC at levels +1 and +2: Superimposed contour plots illustrating the contour lines and regions of acceptability for the texture characteristics of the rice/cassava/soy yeast bread. The striped region met reference standards for Instron hardness (H) gumminess (G) and cell size (S). The darker region includes cell uniformity (U).

in two sections of the plot. It appeared that higher levels of cassava starch could be used without much difference in the range of water levels. A similar pattern was seen at the lower cassava starch region, except that, as the cassava starch level decreased there seemed to be a need for higher levels of water. However, at each higher level of SPC (+1 and +2), it seems it would be possible to use higher levels of cassava starch with almost the same levels of water. The overlays suggest that water levels between +0.6 and +2 would be adequate with any level of soy, to achieve optimum texture characteristics. However cassava starch requirements varied and ranged from level -2 for all to +0.7, +1.5, +1.8 and +2 for levels of SPC at -1, 0, +1 and +2 respectively. The overall indication was that as higher levels of cassava starch were used there would be the need for a decrease in water levels in order to maintain these texture attributes.

Identification of Variable Combinations That Meet All the Criteria for Acceptability

Plots were overlaid to identify regions that would meet the acceptability criteria for both whole loaf and internal characteristics of the bread. Plots for the four levels of SPC are shown in Figure 5.16. The regions which met the reference standards for probability of cavities, specific volume, loaf shape, Instron hardness and gumminess, and cell size are highlighted in the stripes. The darker area

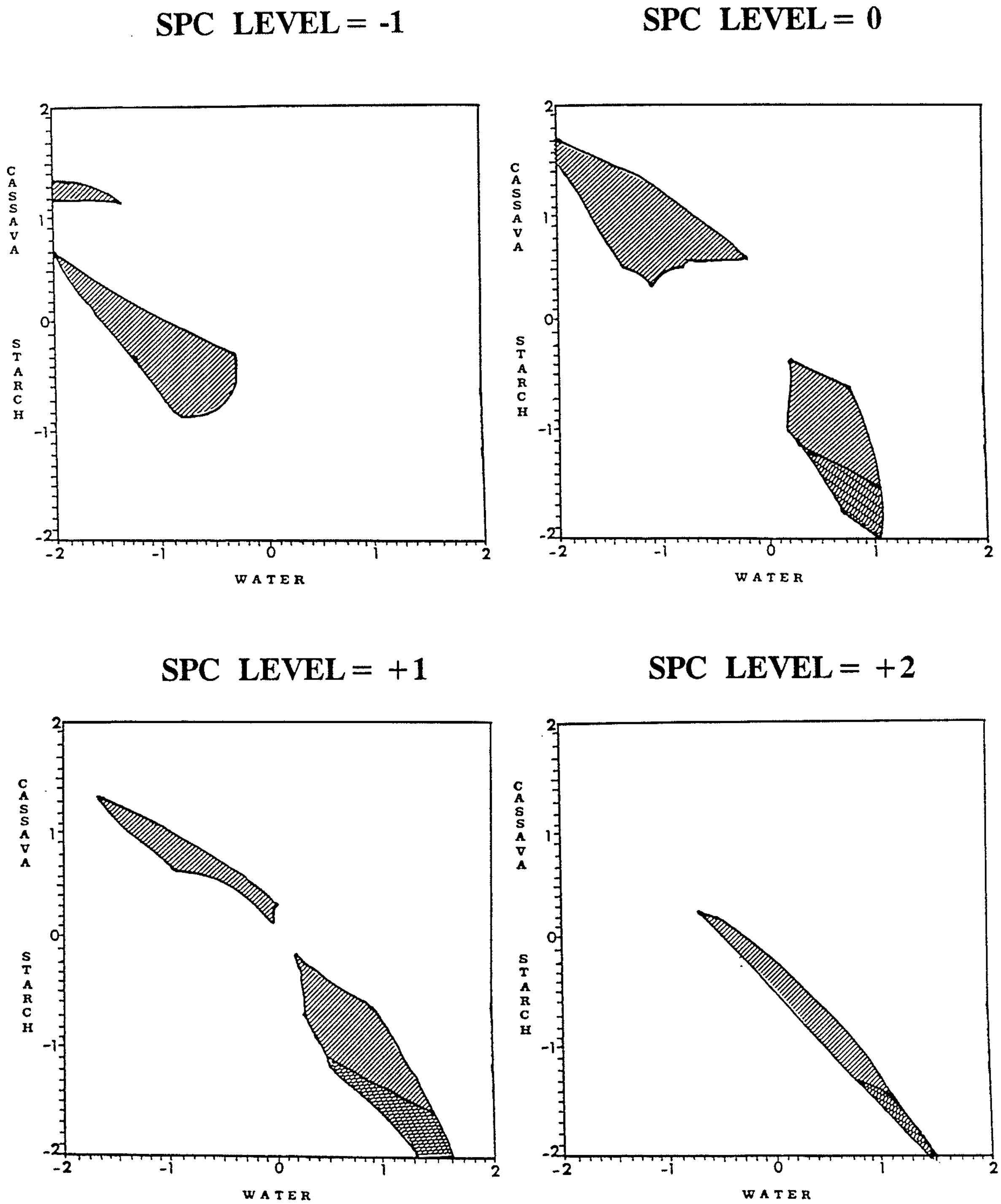


Figure 5.16. Superimposed contour plots illustrating the region of acceptability for all objective measurements for the rice/cassava/soy yeast bread. The lighter shaded region met reference standards for Instron hardness and gumminess, cell size, loaf shape, specific volume and the incidence of cavities. The darker region includes cell uniformity.

represents the regions that also met cell uniformity standards.

At SPC level -1, although no region included cell uniformity, formulations that met the remaining criteria could be developed using high cassava starch levels (+0.5 to +1.5) and water levels from -2 to -0.25. At SPC level 0 there were two regions that met the six criteria, one in which cassava starch levels were high with low water levels, and the other in which cassava levels were low with high water levels. The center point formulation did not meet all the criteria because the cell size was too small, therefore it was not predicted to be acceptable. A very small region met cell uniformity criteria. For SPC levels +1 and +2, the regions identified again reflect this interaction of cassava starch and water. With SPC level at +1 an acceptable bread could be made with cassava starch at level +1 and water at level --1. Formulations with lower cassava starch (-1) and higher water (+1) should also give acceptable characteristics.

Post Evaluation

Bake Test

One batch of baking was conducted using the reference loaf formulation, the centerpoint formulation and six formulations selected from the final superimposed plots with SPC at levels 0, +1 and +2. All loaves had very good shapes with well rounded tops, straight flat sides and bottoms.

When cut the slices had acceptable cell size and cell uniformity. Some of the loaves are shown in Plate 5.2. Specific volumes for all loaves were acceptable ranging from 3.36 to 3.95 (cc/g). However the reference loaf, one of the centerpoint loaves and three of the selected loaves had varying sizes of cavities. From the six selected formulations, two loaves had no cavities. These were the loaves with 7% and 9% SPC. The formulations for these are shown in Table 5.10. The other four selected loaves included two with 7% SPC and higher levels of cassava starch (0 and +1), and two with 5% SPC and cassava starch levels at -0.5 and +1.75. The occurrence of cavities, which are found specifically under the top crust of the loaves is still not fully explained and is worthy of further investigation. The informal taste panel found the breads to be relatively bland with no pronounced flavour problems.

Nutrient Analysis

The proximate analysis was conducted on three loaves, one at each level of SPC 0 (5%), +1 (7%) and +2 (9%).

From the results in Table 5.11. it can be seen that ash and fat content did not vary significantly among the three rice breads, but increased slightly at each higher level of soy. The difference in protein content was significant. The protein content of the bread with 9% SPC was slightly lower (6.66%) than that of the white bread (7.14%). However the

A

B

Plate 5.2. Rice/cassava breads which met all the reference standards, except cell uniformity. A (+1) = 7% and B (+2) = 9% SPC.

Table 5.10. Optimized formulae for soy-enriched rice/cassava yeast bread[1]

INGREDIENTS	SPC(+1)[2]	SPC(+2)[2]
Rice flour	177	169
Cassava starch	34	38
Soy protein concentrate[3]	16	20
Granulated sugar	24	24
Salt	6	6
Yeast	7	7
Oil	13	13
Carboxymethylcellulose	2	2
Hydroxypropylmethylcellulose	8	8
Water	257	251

[1] Adapted from Ylimaki, 1987.

[2] Amounts in grams

[3] SPC (+1) - 7% soy protein concentrate
SPC (+2) - 9% soy protein concentrate

Table 5.11. Nutritional comparison of wheat flour white bread with soy-enriched rice/cassava bread

	White Bread	Rice Bread		
		5 %	7 %	9 %
Moisture	36.00	38.90	39.00	39.00
Calories	271.40	247.70	246.50	247.50
protein	7.14	5.21	5.93	6.66
Carbohydrate	50.00	52.00	51.18	50.30
Fat	tr	2.19	2.13	2.33
Ash	--	1.94	2.01	2.07

protein quality of the rice bread could be significantly superior particularly with regard to lysine and methionine content.

SUMMARY AND CONCLUSIONS

From this study contour plots generated by RSREG assisted in visually identifying any relationships among cassava starch, soy protein concentrate, water and proof time, as well as the interactions of these factors and their effects on each response. It was also possible to identify areas which met the acceptability regions, for each response, which had been set prior to the experiment.

It was determined that cassava starch and water were the factors with the most significant effect on all the physical characteristics of the bread, except those related to colour. Soy protein concentrate was effective to a slightly lesser extent, and was the only factor which affected top crust colour. Incidence of cavities seemed precipitated by high water levels with high cassava starch . At the highest SPC (+2), cassava starch levels from -2 to +0.7 with water levels from +1.4 to -2, predicted no cavities. At SPC level +1 up to level +1.4 cassava starch could be used with the same levels of water as with SPC at level +2.

Loaf shape improved with high levels of water and cassava starch, at higher levels of SPC (+1 and +2). For specific volume, water had a greater influence than cassava starch.

The large area of acceptability suggests that many combinations are possible but, also if it is desired to use very high levels of cassava starch, then the water levels would need to be lowered to maintain good specific volume.

Acceptability regions for the texture parameters were very large especially for hardness, gumminess and cell size. For these three characteristics the predictions were similar. The overall indication was that there was no soy x water interaction on the texture characteristics. It appeared that the achievement of the desired characteristics depended on almost the same level of water at each level of SPC. However the strong soy*cassava starch interaction was obvious from the differences in the required levels of cassava starch at each level of SPC. This suggested that the amount of cassava starch selected would be influenced by the amount of soy protein concentrate in the formulation.

The bake test confirmed the predictions from the plots. Ten loaves were baked which included two reference loaves, two centerpoint loaves and six loaves based on formulations selected from the acceptability regions of the final contour plots. Generally all the loaves had very good shapes and acceptable specific volumes (3.36 to 3.95 cc/g). Some of the loaves with higher cassava starch developed cavities but these were not very large and the loaves had very good cell structure in terms of cell size and cell uniformity. The breads without cavities from each level of soy protein concentrate (+1 and +2) contained levels of cassava starch at the corresponding levels of -1 and -0.67 with water at +1 and +0.2. (Table 5.11.).

It appears that an area has been achieved where the tendency for cavities is reduced. However as the baking test showed, there must be factors other than the formulations which are influencing the probability of cavities. These might be controllable, and if higher levels of cassava starch need to be used, further experimentation may be necessary in order to obtain a formulation which would produce a loaf without cavities but with all other acceptable physical attributes.

Protein improvement being one of the major objectives of this study, it seems that the formulation with the highest level of soy protein concentrate should be further examined. This would provide an adequate amount of high quality protein as shown in table 5.11.

In this study response surface methodology was successfully used to determine the effects of various ingredients on the physical characteristics of a soy- enriched rice/cassava yeast bread. It was also possible to select formulations which could produce a nutritionally adequate bread with acceptable physical attributes.

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Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

The purpose of this study was to develop a soy-enriched rice/cassava yeast bread which would be consumer acceptable in developing countries. From the review of literature it was suggested that the use of non-wheat starches and flours, and more specifically rice flour and cassava flour or starch, was quite feasible as a replacement for wheat flour, providing a gum was used to maintain the shape and cell structure of the bread. Research indicated that soy had been used successfully in wheat and wheat composite yeast breads and should therefore be quite appropriate in a rice/cassava based yeast bread. In fact it appeared that it might improve the physical and textural characteristics as well as the nutritive value.

A preliminary study was conducted to examine the gelatinization temperature (GT) and pasting properties of rice flour, cassava flour, cassava starch and potato starch. The rice flour had properties (Gt of 64°C and amylose content of 19.2%) similar to the specifications set out by Nishita and Bean (1979) who had reported a relationship between these parameters of rice flour and the textural quality of rice bread. Of the two cassava products, cassava starch displayed similar characteristics to potato starch when tested alone, in blends with rice flour, and with or without the addition of the gums, carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC) or xanthan. Therefore it was determined that cassava starch was better than cassava flour as a replacement for potato starch in a rice flour based yeast bread. Xanthan produced results in the rice/ cassava starch blends which predicted undesirable textural attributes for the rice bread. Carboxymethylcellulose and HPMC resulted in opposing effects on gelatinization temperature, peak viscosity and cooled viscosity of the blends, so from further tests it was determined that a combination of 0.9% CMC and 3.5% HPMC as used by Ylimaki (1987) would be appropriate.

Using Ylimaki's rice flour/potato starch formula as a basis, a series of tests were conducted to standardize a rice/cassava/soy formula which could then be used as a basis for optimization. These tests were conducted by systematically replacing and/or including ingredients at consecutive stages of the process. Loaf volume was the criteria used for evaluation. The rice/potato formula was used to establish conditions for the preparation and baking, and it was decided that the center shelf of the proofer seemed to provide the least variation in temperature and humidity. In addition the rotary oven was found to provide a more reliable and constant atmosphere than the conventional oven, and did not require any change in the baking temperatures used in the original formula. However, the final baking time was reduced by 5 minutes.

By sequentially adapting the rice/potato formula, it was found that total replacement of potato starch by cassava starch produced a marked improvement in loaf volume, although the crumb was softer and there was a more pronounced top-side loaf indentation in the rice/cassava loaf. Five percent soy protein concentrate was added to two rice /cassava combinations of 80/15 and 75/20, which resulted in loaf volumes both of which were less than that of the formula with no soy. However the one with 75% rice flour had a larger volume .

High levels of water produced loaves with good volume but with a huge cavity under the top crust and a moist gummy crumb. When a very small adjustment (1.2%) was made to the original amount of water, an acceptable volume was achieved. Different combinations of gums were tested to determine whether it was possible to reduce the amount of gum and still maintain an acceptable loaf. The tests confirmed that while CMC influenced crumb quality, HPMC provided volume therefore it was decided to retain the combination of 0.9% CMC and 3.5% HPMC as had been specified by Ylimaki (1987). The amount of all other ingredients, yeast, sugar and oil were retained in the formula since they did not seem to affect the quality of the bread.

Overall it was determined from these tests that the ingredients most likely to affect the quality of the bread were cassava starch, soy protein concentrate (SPC) and water. It was also anticipated that the variation in the amounts of water might affect proof time therefore proof time was considered as one of the variables.

In the final experiment optimization was done using a response surface methodology design with four variables at five levels. The design included a replication of the 16 cube points, making a total of 44 loaves. The three ingredient factors and proof time were used as independent variables to optimize formulations for a rice/cassava/soy yeast bread. The response variables, which were measured objectively, were specific volume, loaf shape, Instron hardness and gumminess, cell size, cell uniformity, top crust colour, crumb colour and moisture content.

From discussions with Ylimaki as well as during preliminary baking trials it was found that cavities occurred under the top crust without any apparent explanation. For this study it was therefore decided to examine the baked loaves from the experiment and place a value of 0 and 1 (absence and presence of cavities) for each loaf. This data was used to generate a RSREG analysis to determine the probability of cavities occurring. Results indicated that for the rice/cassava/soy loaves, cassava starch and water very strongly influenced the probability of cavities occurring, and if formulations were kept within a particular region there was less likelihood of the incidence of cavities. Very high specific volume and loaf shape scores indicated a high probability of cavities. However, formulations were identified which resulted in acceptable specific volumes and loaf shapes while the probability of cavities was low (p=0.1).

Generally, of the four variables, cassava starch and water were the ones which had the most significant effects, with soy being only slightly less significant. Proof time had a significant but small effect on some of the response variables. It was evident that the influence of the other independent variables could be evaluated by considering results at one proof time only. Therefore contour plots were generated for the centerpoint proof time (45 minutes), but not for the other proof times. For these plots, soy protein concentrate was held at four levels (-1 =3%, 0 =5%, +1 =7% and +2 =9%), with cassava starch and water forming the axes.

Moisture content and top crust colour met reference standards for all loaves. Top crust colour was affected only by SPC levels and generally was a very pleasing bright brown. Crumb colour was not strongly influenced by any of the ingredient variables.

Formulations were selected from each level of SPC for breads which met the reference criteria for specific volume, loaf shape, hardness, gumminess, cell size, cell uniformity and the probability of cavities. None of the formulations with 3% SPC met the criteria for cell uniformity. For the higher levels of SPC, the regions which met the cell uniformity criteria were very small and included only formulations using low levels of cassava starch and high levels of water.

Since for this type of non-wheat bread cell uniformity can be considered a minor quality factor, it was decided to consider formulations that met all other criteria as potentially acceptable. When the cell uniformity criteria was excluded, the acceptability region for each level of SPC was much larger and included higher levels of cassava starch with low levels of water. The centerpoint formulation for SPC at level 0 was not predicted to produce an acceptable bread. An overlay of all contours (Figure 6.1) showed that in addition to cell uniformity, the criteria of cell size was not met. This formulation had been selected as the centerpoint for the experimental design because it had initially produced a loaf with good volume and loaf shape, but in the preliminary study the crumb characteristics were not evaluated.

In order to confirm the validity of the predictions formulations selected from the acceptability regions were baked and examined. The results indicated a successful prediction as the formulations with 7% and 9% SPC and low cassava starch and produced loaves that met all reference standards. Those with high cassava starch levels were acceptable for all characteristics other than the cavities which, although not very large, did occur. Although these loaves had some tendency to cavities, they had a softer crumb. This desirable feature can be attributed to high levels of

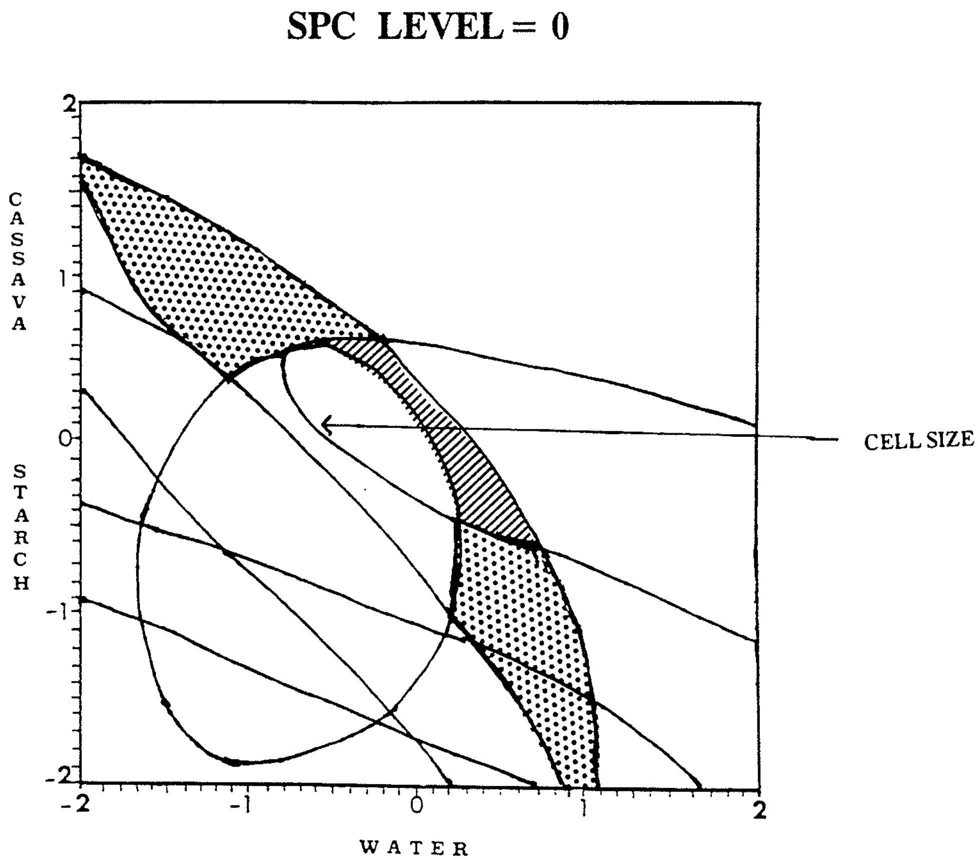

Figure 6.1. Superimposed contour plots with contour lines illustrating how the cell size criteria was not met. Striped area represents the region that did not meet cell size.

cassava starch. Perhaps if further experimentation were done by lowering the water levels or increasing the amount of SPC in the formulation, then the tendency for cavities could be reduced.

A proximate analysis of the loaf containing 9% SPC indicated that the protein content was comparable to a wheat flour white bread (6.6%).

The independent variables, cassava starch, soy protein concentrate and water proved to be very important to the quality of the rice/cassava/soy bread. Cassava starch interacted quite well with the rice flour and provided more moistness and softness to the crumb. This was evident from the hardness plots where higher levels of cassava starch with high levels of water caused softening of the loaves. The use of higher levels of cassava starch in the bread could increase the demand for the product. In some areas where cassava is a major crop this could lead to increased production and reduce wheat importation costs.

Soy protein concentrate definitely influenced the cassava starch x water interaction for most of the responses. There was also a SPC x cassava starch interaction on cell size and cell uniformity. With each higher level of soy the acceptability region for cell size and cell uniformity increased.

For specific volume, hardness, gumminess and cell uniformity, with each increased level of SPC, it was shown that more cassava starch could be used and still the characteristics met the reference standards. It appears that there is a cassava starch x SPC interaction whereby the SPC modifies the cassava starch x water interaction to prevent the event of too large volume and excessive softness which could result in cavities developing due to overexpansion of the dough and the subsequent collapse of weak structure of the crumb from the top crust. In other words the SPC absorbs more water therefore reduces water available to the cassava starch. Perhaps if higher levels of SPC were used, then it would be possible to use higher levels of cassava starch with low to medium levels of water.

The highest amount of SPC (9%) was used in this study without adversely affecting bread characteristics. Overall there was a complementary action between the cassava starch and soy protein concentrate. In a rice flour based bread this is an important feature because the higher levels of soy enabled the use of higher levels of cassava starch which in turn, caused the softening effect of the crumb. Simultaneously, the SPC reduced the tendency toward gumminess by binding some of the water. With low levels of cassava starch, the effects would not be the same since the higher proportions ofrice flour and soy protein concentrate combined produced a much firmer crumb, except when excessive amounts of water were used.

Water levels were important to most of the responses but more particularly for loaf shape, specific volume and the incidence of cavities. The cassava starch x water interaction was apparent for hardness and gumminess measurements, where it was evident that the cassava starch and water were working synergistically to affect those characteristics. There was also some SPC x water interaction where with higher levels of SPC it was possible to use higher levels of water and consequently higher levels of cassava starch.

In conclusion, RSM was used effectively for the development of a rice/cassava/soy yeast bread. This technique was quite efficient in identifying the important factors, their effects as well as their interactions. The use of contours plots helped to clarify the factor effects and facilitated the selection of formulations which proved to be reliable as shown in the subsequent bake test. A soy-enriched rice/cassava yeast bread was developed which was comparable to the reference rice/potato yeast bread that had been found consumer acceptable. These breads which were tasted by an informal group consisting of the researcher and members of the Department of Foods and Nutrition, were found to be relatively bland with no pronounced flavour problems.

RECOMMENDATIONS FOR FUTURE RESEARCH

This study indicates the need for further research in many aspects of the production of a rice/cassava/soy bread. The bread has been developed and optimized based on instrumental and subjective visual evaluation. It would be useful to conduct a complete sensory evaluation of the product to assess its acceptability to consumers. This would need to take into consideration the target populations since different groups of consumers are likely to have different criteria of acceptability. It might also be useful to conduct flavour and texture profiles of the breads to identify the profiles that are preferred by consumers.

Since it has been shown that high levels of soy protein concentrate might enable the use of high levels of cassava starch without adversely affecting bread characteristics, further studies should be conducted using higher levels of soy protein concentrate with the aim of producing a high-protein rice/cassava yeast bread.

Shelf life of these breads is also of great importance. Studies on this should be conducted to identify the storage conditions most suitable for maintaining desirable eating qualities. Other studies (Kim and De Ruiter, 1969; Satin, 1988) have reported breads containing cassava to become more acceptable (less gummy) 20 - 24 hours after baking, but after that time the breads stale rapidly. Perhaps emulsifiers or enzymes could be used to keep the crumb soft but not gummy and also delay the staling process. The effects of several types of enzyme systems on the shelf life of bread, have been reviewed by Novo Laboratories Inc. (1989), and ter Haseborg and Himmelstein (1988) reported the retardation of staling, evidenced by lower Compressimeter scores, with the use of a hemicellulase enzyme.

Should any of these formulations be considered for commercial use it would be necessary to upscale them first, at a pilot plant level, then for a commercial bakery. One of the problems anticipated would be with the size of the loaf. So far the loaf produced has been from 227g of flour. For the larger loaves the upscaling of ingredients would necessitate a new standardization test with particular attention paid to the proportions of gums. Some attention will also need to be paid to handling large amounts of the dough (batter) since the procedures are somewhat different from those of the conventional breadmaking process.

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Appendix 1: Protein Determination

Kjeldahl Procedure

1. Weigh sample on weighing paper and then fold paper and place in kjeldahl flask.

2. Add 10.5 gm of titanium dioxide kjeldahl mixture.

3. Add 20 mL conc. sulfuric acid and place on digestion rack.

4. MAKE SURE THAT THE FUMEHOODS ARE ON AND THAT THE WATER ASPIRATOR IS RUNNING.

5. Turn on heaters and digest sample for 60 min. rotating the flasks every 15 minutes or so.

6. Turn off heaters, remove flasks and immediately stopper. Allow to cool for about 12 minutes.

7. Add 50 mL tap water and swirl to dissolve salts. Then add an additional 240 mL tap water and swirl. (Samples may be stoppered and left overnight at this point.)

8. Put 50 mL of boric acid solution and indicator into a 250 mL erlenmeyer and place under condenser tips. Make sure the condenser tip is covered with liquid.

9. TURN ON CONDENSER WATER.

10. To the kjeldahl flask, slowly and without mixing add 60 mL of conc. sodium hydroxide solution. DO NOT MIX.

11. Attach rubber stopper from condenser apparatus to kjeldahl flask and while holding stopper securely in place swirl the flask to mix.

12. When all flasks are in place turn on heaters and set a timer for 40 minutes. None of the samples should be ready before this time.

13. When the level of the liquid in the erlenmeyer reaches 200 mL turn off the heater, lower the erlenmeyer to the next level, and use distilled water to rinse the condenser tip into the erlenmeyer.

14. Leave the samples for 10 minutes before titrating and then titrate with standardized hydrochloric acid

to a purple end-point.

Reference:

AACC Method 46-12, 19

Appendix 2: Ash Determination

Procedure

1. Turn muffle furnace on by turning dial A to 100 and dial B to MEDIUM.

   BE SURE THAT THERE IS NOTHING FLAMMABLE ON TOP OF OR AGAINST THE MUFFLE FURNACE.
   After 75 min. the muffle furnace will be between 650-700°C. Turn dial A to 60 and the temperature should stabilize at about 600°C.

2. TURN BOTH DIALS A AND B ON THE MUFFLE FURNACE TO THE OFF POSITION (the muffle furnace must always be turned completely off when putting samples in and out or ELECTROCUTION may occur).

3. Place empty ashing dishes in the oven. Close door and set dial A to 60 and B to medium.

4. After a minimum of 30 minutes transfer crucibles to a desiccator but first be sure to turn off muffle furnace completely and then turn it back on again once crucibles have been removed.

5. Cool crucibles in desiccator for 30-45 minutes. Record weight of crucibles and then add approximately 2 gm of sample and record weight to 4 decimal places.

6. Turn both dials A and B on the muffle furnace to the off position.

7. Immediately place ashing dishes in the oven using long handled, taped tongs, and leave the door open. Holding a match in the tongs ignite the samples if they do not ignite by themselves after about 20 seconds. They will burn for about 20 or 30 seconds and then go out. Close the door and reset dials A and B to 60 and MEDIUM respectively.

8. Char samples for exactly 2 hours. Be sure to turn off muffle furnace completely before removing the samples.

9. Remove samples and place directly in a desiccator.

10. Cool 30-45 min. and weigh. Be sure to release the pressure in the desiccator slowly when opening it or the ash may blow out of the dishes.

Reference: AACC Method 08-03, 1983

Appendix 3: Determining Moisture Content of Flour

Procedure

1. Heat drying oven to 130°C and dry numbered moisture dishes and lids for 1 hour. (place lids underneath dishes, not on top).

2. Set moisture dishes and lids in desiccator to cool (30 - 45 min)
   * Place desiccator on a cart and place it beside the oven so samples go immediately into the desiccator.

3. Weigh empty moisture dishes on analytical balance and record weight to four decimal places.

4. Add 2-2.5 gm of flour and record exact weight.

5. Dry samples and dishes in oven at 130°C for 1 hour. Sit sample containers on top of the lids. (Heat for exactly 60 min. after the oven has recovered its temperature).

6. Cover the samples, remove from oven and immediately place in a desiccator.

7. Allow to cool to room temperature (about 45 min.) and record final weight.

<u>Reference:</u>

AACC Method 44-15A, 1983

Appendix 4: Determination of Total Starch

Procedure

1. Accurately weigh 0.50g samples into centrifuge tubes (marked at 30ml level), and add 5ml 70% ethanol. Place sealed tubes in a 70°C bath for 5 minutes.

2. Allow to cool to room temperature and then centrifuge 10k 10 min. at 17°C. Withdraw supernatant and discard.

3. Add, to the sample, 10ml 0.20M Na Acetate 1mM $CaCl_2$ pH 5.50 and 200 ul thermostable alpha-amylase. Place a cap on the test tube, vortex, and incubate at 100°C in a shaking bath for 30 min. (If a shaking bath is not available, mix, with vortex mixer, three times during 30 min. incubation).

4. Allow the tubes to cool, then add 100 ul amyloglucosidase and incubate overnight at 35°C in a shaking bath.

5. Bring volume up to 30 ml. with H_2O and centrifuge 10k 10 min at 17 °C.

6. Assay for glucose using the hexokinase reagent as follows:
   i) Add 1 ml of the digested starch sample to 4 ml of the hexokinase reagent and mix well. Add 1 ml glucose standard solutions (20-150ug/ml) to 4 ml of the hexokinase solution.
   ii) Allow all samples to sit at room temperature for 15 min., then read at 340 nm.

   Note: It may be necessary to dilute the starch digests to fall into the range of the glucose standards. Typically, 50x and 100x dilutions are used. For the present study 250x and 300x dilutions were used. A glucose sample should also be run parallel to the starch samples in order to indicate any experimental error that may occur. (Typically, glucose must be diluted 150x, 300x).

REAGENTS

1. Thermostable alpha-amylase is Termamyl alpha amylase.

2. Amyloglucosidase fr. Aspergillus niger (suspension) is from Boehringer Mannheim(102 857).

3. Hexokinase reagent is from Boehringer Mannheim Gluco-Quant Glucose assay kit.
1 vial of each of vials A and B are dissolved in H_2O and made up to 400 mls.

Appendix 5: Amylograph Method

The Standard Model Amylograph (Brabender Corp., Rochelle Park, N.J.) was used in these studies.

For the complete curve, a slurry was prepared by mixing 50 g of rice flour and 450 mL of distilled water in a Waring Blender for 1.5 minutes. The slurry was then placed in the amylograph cup and heated to 30°C. At this point the chart was adjusted to a zero-minute marking, and the slurry was heated to a temperature of 94°C at a rate of 1.5°C per minute.

The starch paste was maintained at this temperature for 20 minutes and then cooled at the rate of 1.5°C per minute. The setback on cooling was evaluated at 50°C.

To accentuate the gelatinization temperature, a separate determination was made on a slurry of 100 g of ground rice and 400 mL of water. The gelatinization temperature was taken as the point of initial increase in viscosity.

Reference:

Halick, J.V. and Kelly, V.J. 1959. Gelatinization and pasting characteristics of rice varieties as related to cooking behaviour. Cereal Chem. 36:91.

Appendix 6: Standard Formulation Procedure

The preparation procedure is as follows:

1. Dissolve 4 g sugar in 50 mL water (43°C). Stir in yeast and soak for 10 minutes.

2. Mix rice flour, potato flour, salt, remaining sugar (20 g) and methylcellulose at speed 1 (Hobart Kitchen Aid Model K45SS) for 2 minutes, using the whip beater attachment.

3. Add yeast mixture and remaining water (40°C) and mix at speed 2 for 15 seconds.

4. Add oil and mix at speed 4 for 15 seconds, followed by 2.5 minutes at speed 6.

5. Scrape the sides of bowl and mix at speed 6 for 2.5 minutes.

6. Scrape sides of bowl, pushing dough to the bottom of the bowl and proof dough for 30 minutes in proofing cabinet (30°C/95% humidity).

7. Remix dough at speed 6 for 5 minutes.

8. Place 400 g dough in a greased pan (18.7 cm x 9.2 cm x 5.7 cm) using metal and rubber spatulas. Press dough into the pan corners and sides, eliminating air pockets and flattening the top surface, using an oiled rubber spatula. Using the same oiled spatula, push the edges of the dough towards the center, away from the pan sides, rounding the top edges of the dough.

9. Proof panned dough for 50 minutes (30°C/95% humidity).

10. Bake in preheated oven (215°C) for 5 minutes. Lower the oven temperature to 180°C and continue baking the bread for 40 additional minutes.

11. Cool the bread at room temperature (23°C) for 15 minutes. Remove the bread from the pan. Cool bread on a rack for 1 hour before packaging.

    When the rice flour doughs are panned, it is important that the dough is pressed well into the bottom of the pan and all air spaces are removed. Rounding the top edges produced a loaf of bread with a rounder top surface.

Reference: Ylimaki, G.L. 1987.

Appendix 7. Analysis of Variance Table: Dependent Variable: Loaf Volume.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Values	Probabilities
Day	1	44408.33	44408.33	27.78	0.0033
Shelf	1	3008.33	3008.33	1.88	0.2284
Position on Shelf	2	6329.17	3164.59	1.98	0.2326
Shelf* Position	2	3904.17	1952.09	1.22	0.3699
Error	5	7991.67	1598.33		
TOTAL	11	65641.67			

$R^2 = 0.878253$

Appendix 8: Response surface regression analysis results for the probability of cavities

CAVITIES

RESPONSE SURFACE FOR VARIABLE CAVI

RESPONSE MEAN	0.2954545
ROOT MSE	0.37772
R-SQUARE	0.548263
COEF OF VARIATION	1.278437

REGRESSION	DF	TYPE I SS	R-SQUARE	F-RATIO	PROB
LINEAR	4	3.70000000	0.4040	6.48	0.0007
QUADRATIC	4	0.38409091	0.0419	0.67	0.6161
CROSSPRODUCT	6	0.93750000	0.1024	1.10	0.3888
TOTAL REGRESS	14	5.02159091	0.5483	2.51	0.0175

RESIDUAL	DF	SS	MEAN SQUARE	F-RATIO	PROB
LACK OF FIT	10	3.63750000	0.36375000	13.823	0.0001
PURE ERROR	19	0.50000000	0.02631579		
TOTAL ERROR	29	4.13750000	0.14267241		

PARAMETER	DF	ESTIMATE	STD DEV	T-RATIO	PROB
INTERCEPT	1	2.22045E-16	0.18886001	0.00	1.0000
CS	1	0.17500000	0.05972278	2.93	0.0065
SY	1	-0.07500000	0.05972278	-1.26	0.2192
WT	1	0.22500000	0.05972278	3.77	0.0008
PF	1	-0.07500000	0.05972278	-1.26	0.2192
CS*CS	1	0.08125000	0.07613190	1.07	0.2947
SY*CS	1	0.09375000	0.06677210	1.40	0.1709
SY*SY	1	0.08125000	0.07613190	1.07	0.2947
WT*CS	1	0.09375000	0.06677210	1.40	0.1709
WT*SY	1	0.03125000	0.06677210	0.47	0.6433
WT*WT	1	0.08125000	0.07613190	1.07	0.2947
PF*CS	1	0.09375000	0.06677210	1.40	0.1709
PF*SY	1	0.03125000	0.06677210	0.47	0.6433
PF*WT	1	0.03125000	0.06677210	0.47	0.6433
PF*PF	1	0.08125000	0.07613190	1.07	0.2947

FACTOR	DF	SS	MEAN SQUARE	F-RATIO	PROB
CS	5	2.23125	0.44625	3.13	0.0223
SY	5	0.73125	0.14625	1.03	0.4213
WT	5	2.53125	0.50625	3.55	0.0126
PF	5	0.73125	0.14625	1.03	0.4213