

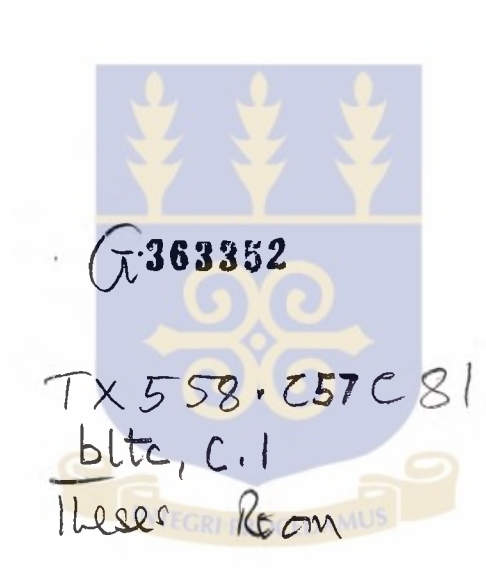
CHEMICAL MODIFICATION AND COWPEA FORTIFICATION OF MAIZE



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DECLARATION

This research was conducted by me under the supervision of Prof. S. Sefa-Dedeh of the Department of Nutrition and Food Science, University of Ghana, Legon.

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PROF. S. SEFA-DEDEH



DEDICATION

**I dedicate this work to God, the love of my life....
“...You have loved me and I have let myself be loved...”**

and

**to my parents,
I have come this far because you believed!**



ABSTRACT

The high utilization and consumption levels of maize in developing countries call for the investigation of new methods of processing to introduce variety as well as improve the functionality and nutrient quality of maize-based foods.

This study was carried out to determine the effect of nixtamalization, fermentation and cowpea fortification on maize and to identify the dominant microflora in fermented nixtamalized maize. The effect of cooking and lime concentration was determined using a 2x4 factorial experimental design, with cooking time (0, 30 mins) and lime concentration (0, 0.33, 0.5 and 1.0%). The cooking time and lime concentration, significantly influenced the moisture absorption, pH and colour of the samples. Water absorption capacity was dependent on the lime concentration. These indices increased with increasing lime concentration used. The lime did not significantly alter cooked paste viscosity, ash and protein content. A 3 x 4 factorial experiment was used to study the effect of fermentation on the characteristics of nixtamalized maize dough. Fermentation resulted in decreased pH with a corresponding increase in titratable acidity. Fermentation decreased the texture, water absorption capacity (25°C), cooked paste viscosity and colour intensity of nixtamalized maize dough. Traditional maize dough facilitated fermentation, by acting as a starter culture to produce lower pH and higher acidity in the steeped:nixtamalized maize dough blends.

The central composite rotatable design for $K = 3$ was used to study the combined effect of lime concentration (0–1%), moisture content (55–65%) and cowpea level

(0–30%) on pH, titratable acidity, water absorption, texture, protein and viscosity of nixtamalized maize dough (masa) during fermentation. The lime and cowpea influenced the titratable acidity, water absorption capacity, protein content and the cooked paste viscosity of the fermented cowpea fortified nixtamalized maize. Increasing concentration of lime, during fermentation, generally decreased titratable acidity, water absorption, work required to back extrude an amount of cooked set slurry and cooked paste viscosity while the addition of cowpea increased most of these indices.

The final pH and titratable acidity of the fermented nixtamalized maize were comparable to that of the traditional maize dough. The microbial counts obtained (aerobic mesophiles and lactic acid bacteria) for the nixtamalized maize and traditional maize dough, were comparable after 24 hours of fermentation. Yeast counts were slightly lower in the fermented nixtamalized maize dough. These results showed that nixtamalized maize dough lends itself well to traditional spontaneous fermentation with the resultant souring and development of the characteristic flavours of traditionally fermented maize. The Lactic acid bacteria identified in the fermented masa were *L. plantarum*, *L. fermentum* and *L. cellobiosus*. *Pediococcus spp.* were also identified (found in fermented masa).

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To God Almighty, who has made me who I am...I owe it all to You!!!

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1.0 **INTRODUCTION**

1.1 **THE IMPORTANCE OF MAIZE**

Maize (*Zea mays*), is the most important cereal grain in tropical Africa (Kordylas, 1990). Together with other cereals such as sorghum, millet and rice, they are vital to the survival and food security of a large segment of the population in Ghana, contributing over 55% of the total energy intake (Sefa-Dedeh and Mensah, 1989).

Maize is the most important of the cereals grown in Ghana, in terms of utilization, acreage, cultivation and production. It is a staple food for most Ghanaians, particularly those in the coastal areas and features prominently in infant weaning foods, countrywide. The importance of maize is evidenced by the variety of products based on this commodity. In a Greater-Accra regional survey of Ghanaian foods, 91% of the food products encountered were made with maize (Sefa-Dedeh and Mensah, 1989).

Typically, maize grains contain 65-84% starch, 9-10% protein, 12-15% moisture, 3-5% fibre, 3% Ash and 410 calories of food energy (Ihekoronye and Ngoddy, 1985). In Ghana, there are over ten high yielding varieties of maize being promoted for cultivation. These include, Abrotia, Laposta, Obatanpa, Dobidi, Golden Crystal and Abeleehi.

1.2 **MAJOR PROBLEMS ASSOCIATED WITH THE UTILIZATION OF MAIZE**

Maize is deficient in the vitamin niacin and contains a low amount of protein, which is also deficient in the amino acids, lysine and tryptophan. It however,

contains a fair amount of sulphur containing amino acids cystine and methionine and is high in leucine and aromatic amino acids.

The disease pellagra is prevalent among people who rely on maize as their staple food commodity (Ihekoronye and Ngoddy, 1985; FAO, 1992). Pellagra is caused by a deficiency of niacin or niacinamide. Maize contains niacin but most of it occurs in a bound form, niacytin, which is biologically unavailable and renders the maize deficient in niacin.

1.3 METHODS OF IMPROVING THE NUTRITIONAL VALUE OF MAIZE

Fermentation is one of the oldest methods of preparing and preserving food. It involves the catalytic breakdown of complex molecules by microorganisms and their enzymes, with the resultant changes in flavour, pH and nutritive value (Ashworth and Draper, 1992).

Fermentation significantly improves the protein quality and the level of lysine in maize, millet, sorghum and other cereals. Another advantage of fermentation, which has been reported extensively, is the antimicrobial properties exhibited by fermented foods. Mensah *et al.* (1991) reported that maize dough that had been fermented for three days inhibited *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) by eight hours. Work done by Hymore *et al.* (1997), also showed that maize-cowpea blends exhibited antimicrobial activity during fermentation. In their work, maize dough containing 40% cowpea inhibited *E. coli* (J 1060) after 8 hours of fermentation. There is considerable evidence that, lactic acid

fermentation inhibits the survival and multiplication of a number of bacterial pathogens (Motarjemi and Nout, 1996). The antimicrobial properties of fermented foods appear to be their most interesting quality.

The combination of reduced pH and the presence of lactic and acetic acid significantly inhibit the growth of Bacillaceae, Micrococcaceae and Enterobacteriaceae (Nout, 1993).

Cereal-legume complementation, has been suggested by many researchers, as a method of improving the protein content of traditional cereal foods (Nout, 1993; Osei and Sefa-Dedeh, 1993). The protein content of maize- cowpea blends is higher than the maize dough and increases with increasing cowpea level (Kluytse, 1995). Ampadu (1994) reported that the addition of 20% dehulled soybean flour to maize dough resulted in an 80% increase in protein content. The combination of cereals and legumes results in increased protein content of cereal-based products and produces a better amino acid balance than cereals alone. This is because legumes are a good source of essential amino acids such as lysine, which is deficient in maize.

1.4 NIXTAMALIZATION

Nixtamalization refers to the alkaline cooking of maize (*Zea mays*). The process involves the cooking and steeping of whole maize in excess water containing calcium hydroxide (lime) solution. The cooked maize (nixtamal) is removed from the cook-steep water (nejayote), washed and ground into a dough known as masa

(Serna-Saldivar, 1990). The masa is further processed into tortillas, tacos, tortilla chips, maize chips and other related products.

Several significant nutritional improvements have been reported from studies on alkaline treatment of various cereals such as maize, sorghum and millet. Some of these include;

- a. Decrease in tannin levels of high tannin grain.
- b. Increase in free calcium levels
- c. Increase in the bioavailability of iron and other minerals
- d. Faster release of amino acids
- e. Increase in free nicotinic acid
- f. Increase in availability of niacin

(Bressani *et al.*,1958; Hulse *et al.*,1980; Vivas *et al.*,1987; Bharati and Vaidehi, 1989).

The process of nixtamalization in combination with other processes, can be applied to further improve the processing and utilization of maize. Work done by Sefa-Dedeh, (1991) showed a drastic reduction in amylograph viscosity when nixtamalized maize dough (masa) was fermented. As a means of resolving the problem of low energy density of weaning foods caused by the high starch content of cereals. Cowpea fortification of traditional foods in Ghana has led to an improvement of the protein quantity and quality of these foods which are usually made from maize (Amegatse, 1995; Kluitse, 1995). A combination of cowpea

fortification, fermentation and nixtamalization may prove a means of improving functionality, protein nutrition and micro-nutrient availability in traditional foods.

Although nixtamalization and lime-cooked maize products are increasing popularity worldwide, the process with its nutritional benefits, is unknown to most Ghanaian food processors. Considering the fact that maize, with respect to the other cereals produced in Ghana has the highest production and consumption levels, the process of nixtamalization has the potential of introducing variety to the traditional food base as well as improving the food and nutrition security of Ghanaians.

1.5 OBJECTIVES

The main objectives of this study were to apply the process of nixtamalization to the processing of maize, and to determine the effects of fermentation and cowpea fortification on the product characteristics.

SPECIFIC AIMS

1. To determine the effect of nixtamalization on the chemical and functional properties of maize.
2. To study the effect of fermentation on the characteristics of nixtamalized maize dough.

3. To study the combined effect of lime concentration, moisture and cowpea content on the chemical and functional properties of fermenting maize.
4. To determine the microbial profile of fermenting nixtamalized maize.

2.0 **LITERATURE REVIEW**

2.1 **MAIZE** (*Zea mays*)

The maize kernel, a caropsis, is made up of four physical structures : the pericarp, the germ, the tip cap and the endosperm. The endosperm of the maize kernel is made up of an outer layer and inner portion, which together, constitute about 83% of its total weight (Guy, 1994). The outer layer of the endosperm is hard and vitreous, with densely packed polygonally shaped starch. The inner portion is soft and mealy and contains loosely bound starch and protein bodies with air cavities in between them. The starch granules are globular, with smooth surfaces (Guy, 1994).

The relative proportions of the two endosperm types account for the variations in starch content, structure and composition of different varieties of maize and subsequently the differences in physical and functional properties. Bedolla and Rooney (1984), reported that the texture of masa was affected by the endosperm texture and type, drying, storage and soundness of the maize kernel.

2.2 **PROCESSING METHODS APPLIED TO MAIZE IN GHANA**

Maize Processing in Ghana is based on traditional and indigenous technologies which utilize local raw materials and in most cases, local equipment. These technologies are simple, with most of them having been developed through experience in the production of products of desirable quality. Common unit operations involved include, steeping, sprouting, dehulling, milling, cooking (boiling, roasting, steaming) and fermentation (Sefa-Dedeh and Mensah, 1989).

A wide variety of foods and beverages are prepared from maize. Some of these are *koko*, *kenkey*, *apkle*, *abele* and *nmeda*. *Koko* is prepared from fermented maize dough. The dough is prepared by soaking maize in water for 24 hours, after which the maize grains are washed and milled in a disc attrition mill. The meal is then mixed with water and worked into a dough which is fermented for 24 hours. *Koko* is prepared by cooking a thin slurry of dough into a porridge. It is sweetened with sugar before eating. This porridge is a common weaning food for children in Ghana. The process may be modified during the dough preparation to include other cereals or legumes and some spices (Sefa-Dedeh and Mensah, 1989).

Kenkey is another product from maize, which is widely eaten as a main meal. Several types of *kenkey* are produced but the two main kinds are *Fante* and *Ga kenkey*. These differ in their packaging and generally *Ga kenkey* is salted while *Fante kenkey* is not. During its processing, a dough prepared as described above and fermented for 3 days is used. A portion of the dough is made into a thick slurry and cooked to form a thick paste. An equal portion of uncooked dough is added and mixed thoroughly to form a product called *afkata*. Moulds of the *afkata* which will give the desired size of *kenkey* are packaged in dried plantain (*Musa paradisiaca*) leaves (*Fante kenkey*) or maize husks (*Ga kenkey*) and boiled till cooked (Sefa-Dedeh and Mensah, 1989). *Ga kenkey* is packaged with dried maize (*Zea mays*) husks. *Kenkey* is normally eaten with vegetable stews and soups as well as pepper and fried fish.

Maize is also used in preparing *akple*, a dumpling-like product, which is also eaten as a main meal with vegetable stews or soups. The maize is washed and milled into flour. A slurry of the maize flour is added to boiling water while stirring continuously. The stirring is continued until a thick smooth paste is obtained. Salt is added to impart taste to the product. (Sefa-Dedeh and Mensah, 1989).

Abele is a snack type food, which is made from maize. The maize is cleaned, tempered for a short time and dehulled. The dehulled grains are steeped in salted water for some time and packaged in small quantities in maize husk. The product is boiled for 6-8hrs. Another snack product known as *dzowe*, is made from a mixture of maize and groundnuts. The maize and groundnuts are roasted, milled and mixed together with sugar pepper, ginger and some other local spices. The mixture is moulded into balls of desired sizes. The product combines a cereal and a legume to produce a snack type food with good nutritional quality (Sefa-Dedeh and Mensah, 1989).

Non-alcoholic beverages are also prepared from maize malt. Examples of these are *aliha*, *nmeda* and *asaana*. *Aliha* is the Ewe description of the product. A similar product is referred to as *nmeda* by Gas in the Greater Accra Region. Others refer to it as *asaana*. Processing of these beverages follow the same basic unit operations. Maize is steeped for a day and sprouted for 3-4 days in a basket. The sprouting grains are kept moist by sprinkling water on them from time to time until the end of the sprouting period. The sprouted grain is sun dried for about 3-4 days and ground into a coarse flour. For the preparation of *aliha*, a dilute slurry is prepared from the ground malt to aid extraction of desired flavour,

colour and nutrients. The mixture is then boiled for about 30-45 minutes, strained through a basket and left overnight, during which time some fermentation occurs. Caramel is added to impart a brown colour to the drink. *Nmeda* and *asaana* processing follow the same procedure, however the extraction time is relatively longer (1-2 hours) and sugar is added to impart sweetness (Sefa-Dedeh and Mensah, 1989).

2.3 TRADITIONAL PROCESSING METHODS APPLIED IN THE NUTRITIONAL IMPROVEMENT OF MAIZE

The high dependence on maize as a staple food in tropical Africa, coupled with the low nutritive value of the commodity has led to the investigation of simple traditional methods in the improvement of the nutritional quality of maize based foods. Methods such as fermentation and legume fortification have been studied by many researchers (Mensah *et al.*, 1991; Nout, 1991; Ashworth and Draper, 1992; Sefa-Dedeh and Osei, 1994). Work done by Amegatse (1995) showed that traditional fermentation improved the protein quality of maize dough. The observed improvement was even higher when the maize dough was fortified with cowpea. Fermentation has also been found to improve the nutritional quality by causing a reduction in anti-nutritional factors and non-digestible components in legumes during solid state fermentation with maize. Fermentation of maize cowpea blends caused a reduction in starchyose and glucose/galactose (Kluytse, 1995).

Ampadu (1991) incorporated soybean flour into traditional maize dough and found that increasing the level of soybean flour in the dough up to 20%, increased the

protein content from 11.74%-21.07%, almost a 100% increase in the protein content. Several traditional foods prepared from fermented and unfermented maize dough have been fortified with legumes such as cowpea and soybean in an attempt to improve their nutritional quality. In addition to the improved protein content and quality, these products have generally been acceptable to consumers. General acceptability scores of *Ga kenkey* and *akasa*, prepared from soy-fortified maize dough, indicated no significant difference between samples with and without full fat soy flour and were both acceptable to consumers (Ampadu, 1994).

2.3.1 Fermentation

Fermentation for years has been one of the economic and popular methods of preparing and preserving food. The process involves the biochemical modification of primary products brought about by the action of microorganisms and their enzymes (Motarjemi and Nout, 1996).

It has been applied to develop and enhance taste and flavour, improve and modify texture and viscosity, reduce anti-nutritional factors and to improve the shelf life and microbial safety of foods. Fermented maize and cassava products by tradition constitute an important part of the diet of people in Ghana and other West African countries (Odufa, 1985). One of the primary objectives for the traditional fermentation of maize dough in Ghana is to cause souring of the dough with its associated improvement in taste, flavour and texture (Sefa-Dedeh and Plange, 1989). The main aroma components in fermented maize doughs have been

described to be lactic, acetic, butyric and propionic acids (Banigo and Muller, 1972; Plahar and Leung, 1982). These fermentations as carried out traditionally, are spontaneous.

Studies on microbial succession of these spontaneous and largely uncontrolled fermentations have indicated a selection towards a micro-population, dominated by lactic acid bacteria and possibly yeasts (Ngaba and Lee, 1979; Okafor *et al.*, 1984; Okafor and Uzuegbu, 1987; Oyewole and Odunfa, 1990; Halm *et al.*, 1993).

During these lactic acid fermentations the combination of the reduced pH and the presence of lactic and acetic acids significantly inhibit the growth of Bacillaceae, Micrococcaceae and Enterobacteriaceae which consequently have a stabilizing effect on the microbial population of the product. The antimicrobial effect of lactic fermentations has been extensively studied in various fermented foods by several researchers.

Evidence of antimicrobial properties has been found in fermented sorghum-based porridge from Lesotho (Nout *et al.*, 1988; Sakoane and Walsh, 1988), maize flour from Kenya (Mbugua, 1988), maize dough from Ghana (Mensah *et al.*, 1991). Work done by these authors revealed that enteric pathogens were inactivated in these fermented foods.

Another important advantage of fermentation is the significant improvement in the nutritional value (protein quality) and reduction in anti-nutritional factors. Hamad (1979), reported significant improvement in the relative nutritive value (protein

quality) as well the level of lysine in maize, millet and sorghum. Work done on fermented Ghanaian maize dough has shown significant reductions in phytate. After 48 hours of fermentation, a reduction to 20% phytate concentration has been reported (Mensah *et al.*, 1991).

The significance of textural changes during fermentation cannot be over-emphasized. Findings on these changes however appear to be varied and contradictory, with significant dependence on the method of fermentation and the microorganisms responsible (Ashworth and Draper, 1992; Wanink *et al.*, 1994). Solid state fermentation of maize dough has been reported by some researchers to produce an increase in cooked paste viscosity (Anim, 1991; Osa-Mensah, 1991). Work done by Mensah *et al.* (1991) on fermentation of maize dough revealed that porridge cooked from a meal prepared from maize grain that had been soaked in water for 24 hours had a lower Brookfield viscosity than that prepared from dry maize flour. Viscosities of porridge prepared after 24, 48 and 72 hours of fermentation of the maize dough remained lower than that prepared from the dry maize flour. Decreases in viscosity have also been reported by (Mlingi, 1988) in fermented cassava-based weaning foods and (Gallat, 1989) in fermented sorghum porridge.

The presence of admixtures have been reported to decrease the viscosity of fermented maize dough. Ampadu (1991), reported that soy-flour reduced the viscosity of maize dough. Work done by Sefa-Dedeh (1991), showed a reduction in viscosity when maize dough containing lime (Ca(OH)_2), was fermented.

2.3.1.1 Important Microorganisms in Maize Dough Fermentation

A. **Lactic Acid Bacteria**

Lactic acid bacteria have been variously defined as gram-positive, non-sporing cocci, coccobacillus or rods which lack catalase and are non-aerobic but aerotolerant. They require a fermentable carbohydrate for growth and convert glucose mainly to lactic acid, or lactic acid, CO₂, ethanol and or acetic acid (Sharpe, 1979; Axelsson, 1993).

Lactic acid bacteria are involved in the production of fermented foods from various commodity groups. They have been extensively reported to be responsible for the fermentation of several indigenous African foods including cassava, maize, sorghum and millet. In spontaneous fermentation of maize meal under laboratory conditions, Fields *et al.* (1981) identified the dominating lactic acid bacteria to be heterofermentative *Lactobacillus fermentum* and *Lactobacillus cellobiosus* as well as *Pediococcus acidilacti*. Hounhouigan *et al.* (1991) found lactic acid bacteria, mainly *Lactobacillus spp*, and yeasts to be responsible for the fermentation of *mawe*, a maize product in Benin. Halm *et al.* (1993) found *Lactobacillus fermentum* to be the dominant lactic acid bacteria responsible for the fermentation of maize dough during kenkey production in Ghana.

The primary role of lactic acid bacteria, historically, has been to effect preservation by converting sugars to organic acids which cause a reduction in pH, by breaking down carbohydrates as nutrient sources and by producing antimicrobial compounds like hydrogen peroxide, bacteriocins, diacetyl and secondary reaction products.

Evidence of the antimicrobial effect of a variety of fermented foods has increased in recent times. The role of lactic acid bacteria in providing microbial safety has been demonstrated by several workers. Work done by Nout *et al.* (1988) showed that *Salmonella typhimurium* was unable to survive in lactic acid fermented sorghum based porridge during storage at 30°C for 24 hours. Significant inhibition of *Shigella flexneri*, and other Gram-negative bacteria has been demonstrated in Ghanaian fermented maize dough (Mensah *et al.*, 1988, 1990, 1991). Mbugua and Njenga (1991) showed that the numbers of *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Shigella dysenteriae* decreased when inoculated into fermenting *uji*, a Kenyan fermented cereal porridge during fermentation and storage.

Lactic acid bacteria are currently used to introduce variety in foods by altering flavour, texture and appearance of raw commodities in a desirable way. The sour aromatic flavours imparted by lactic acid fermentation are desirable and give a natural image to the product (Sefa-Dedeh and Plange, 1989; Davidson, 1993; Chassy and Murphy, 1993).

B. Yeasts and Molds

The involvement of yeasts and molds in the fermentation of maize meal has been documented in literature. Yeasts are known to produce a wide range of aromatic compounds such as organic acids, esters, alcohols and aldehydes which contribute to the development of flavour in fermented maize products (Janssens *et al.*, 1992; Jespersen *et al.*, 1994).

In inoculum studies for *fufu*, a traditional African fermented food, *Candida krusei* has been shown to have significant influence on the typical odour of the product (Oyewole, 1990). Hamad *et al.* (1992) found that fermented sorghum doughs with high numbers of *C. krusei* (10^6 cfu/g) had a more pleasant smell than dough with less yeast. Nyarko and Danso (1992) found that inoculation of maize dough with 10^6 cfu/g of *Sacharomyces cerevisiae* in pure culture or in combination with various *Candida spp.* increased the organoleptic scores of the dough significantly.

Several researchers (Akinrele, 1970; Fields *et al.*, 1981; Hounhouigan *et al.*, 1991; Halm *et al.*, 1993; Jespersen *et al.*, 1994) have reported a distinct decrease in viable mold count during the early stage of fermentation. Work done by Jespersen *et al.* (1994) on fermenting maize dough showed a reduction in the initial high counts of 10^5 cfu/g for molds to less than 10^2 cfu/g within 24 hours of fermentation. These decreases could be attributed to the antimicrobial activity of lactic acid bacteria during fermentation.

Increases in yeast numbers during maize dough fermentation has been demonstrated. Work done by Jespersen *et al.*, (1994) on fermenting maize dough revealed that a succession of yeast species took place with *C. krusei* and *S. cerevisiae* becoming the dominating species.

2.3.2 Fortification with Legumes

Cereals and legumes, particularly maize and cowpea are widely grown in developing countries of tropical and warm temperate regions where they feature

prominently in traditional diets (Dovlo *et al.*, 1976; Ali and Willis, 1983).

Cereals such as maize though widely eaten, have inadequate nutritive value due to their low protein content which is also deficient in the essential amino acids, lysine and tryptophan. Legumes on the other hand are rich sources of protein, vitamins and minerals (Kordylas, 1990; Borejszo and Khan, 1992). Cereals and legumes are both cheap and available and whilst each has its nutritional deficiencies, together, they are complementary (Akinyele *et al.*, 1988)..

In Africa, cowpeas have been used extensively in weaning foods where they provide abundant and high quality protein (Chan and Phillips, 1994). Work done on cereal-legume complementation has shown a marked increase in protein quantity of legume fortified cereal gruels (Svanberg, 1990; Mensah and Sefa-Dedeh, 1991; Nout, 1993; Osei and Sefa-Dedeh, 1993; Akpapunam and Sefa-Dedeh, 1995).

Sulphur containing amino acids, cysteine and methionine, which are deficient in cowpea are in sufficient amounts in cereals such as maize. Essential amino acids such as lysine and tryptophan which are deficient in cereals are abundant in cowpea (Steiner-Asiedu, 1989). The combination of cereals and legumes, though not as nutritious as animal proteins is less expensive and produces a better amino acid balance than cereals alone.

Work done by Amegatse (1995) showed that fortification of maize dough with dehulled cowpea improved the protein quality of the dough considerably. He reported high levels of isoleucine, lysine and tryptophan in cowpea fortified maize dough, further promoting the advantages of fortifying cereals with legumes.

2.4 APPLICATION OF NIXTAMALIZATION IN THE UTILIZATION OF MAIZE

The lime cooking process (nixtamalization) of maize into tortilla and related products has existed for centuries (Serna-Saldivar *et al.*, 1990; Bressani *et al.*, 1990). The technology, though particular to Mexico and Central America, has been exported into other countries, such as the United States, where it has spread rapidly. Over 4.3 billion US dollars worth of tortilla and maize chips and 1.0 billion US dollars worth of tortillas are produced each year in the United States (Rooney and Suhendro, 1999). Sales increase 8-10% each year. Today, tortilla chips and related products are becoming familiar to Asians and Europeans with increasing importance worldwide.

Nixtamalization is the process of cooking and steeping maize in lime to produce nixtamal, which is then stone ground to form a soft, moist dough called masa. Masa is the raw material from which table tortillas, maize chips, tortilla chips and other foods are made.

The wide range of foods produced from masa are consumed as snacks or as part of breakfast and main meals. In addition to the variety introduced in the utilization of maize, the important effects of nixtamalization include, increased bioavailability

of niacin, improved protein quality and increase in calcium content of masa products (Serna-Saldivar *et al.*, 1987, 1988a; Wall and Carpenter, 1988; Bressani, 1990; Apedo, 1988). Another advantage of the process is the significant reduction of aflatoxin concentration in masa products.

Work done by Sefa-Dedeh (1991) on the fermentation of nixtamalized maize dough revealed a drastic reduction in Brabender cooked paste viscosity during fermentation. From his research, the pH of nixtamalized maize dough also decreased with a corresponding increase in titratable acidity during fermentation, an indication that fermentation of nixtamalized maize dough leads to souring as observed in traditional maize dough. Annor-Wiafe (1991) also reported decreased peak viscosity and pH and increased acidity during fermentation of sorghum and millet. These findings are very important, providing a means for the improvement of cereal processing (Sefa-Dedeh, 1991).

The raw material which is popularly processed by nixtamalization is maize, which also happens to be an important staple in tropical Africa, used in many traditional diets. Though the process of nixtamalization is unknown to Ghanaian food processors, the unit operations involved are not totally new. Nixtamalization can be employed to improve the nutritive quality of maize (Sefa-Dedeh, 1991), as well as introduce and develop variety in maize processing and utilisation in Ghana.

2.4.1 **Products Made From Lime-cooked Maize**

Small changes in the nixtamalization process produce a wide range of foods. Table 1 below contains a discussion of some important Mexican foods made from nixtamalized maize.

2.4.2 **Changes Which Occur During Nixtamalization**

The process of nixtamalization involves the use of water, calcium hydroxide and heat. These acting together produce significant modifications in the physical, functional and chemical properties of the raw material, during nixtamalization.

2.4.2.1 Physical and Functional Changes

Alkaline cooking and steeping of grains causes swelling and weakening of the cell walls and fiber components. The aleurone cells remain intact and attached to the peripheral endosperm and much of the germ tissue is retained, which positively affects the protein quality of masa products (Paredes-Lopez and Saharopulos, 1982; Gomez *et al.*, 1989).

Portions of these weakened and solubilized parts of the maize grains are lost during unit operations such as washing. These losses however, depend on a number of variables such as:

- a. The type of maize (hard or soft endosperm)
- b. Kernel integrity (whole or broken kernels)
- c. Cooking procedure (traditional, steam cooking, pressure cooking)
- d. The levels of lime used

Table 1. Major Uses of Lime-Cooked Maize

Product	Process
Soft or table tortillas	Lime-cooked maize is finely ground into a smooth masa, which is shaped into disks (2mm thick and 15-20cm in diameter) and baked. The tortilla generally puffs during the final stages of cooking.
Maize chips	Lime-cooked maize is coarsely ground, extruded or laminated into masa strips, and fried.
Tortilla chips	Lime-cooked maize is coarsely ground, sheeted topopos out into small triangles or circles, baked, and fried. Restaurant-style of tortilla chips are from fine masa that is cut into thin chips.
Tamales	Lime-cooked maize is ground into masa, which is mixed with one-quarter part lard, salt, chicken broth, baking powder, and spices. A small quantity of the resulting masa is spread on soaked maize husks or cooked banana leaves (25*25 cm) and filled with spicy beans, chicken, beef, pork, fish, smashed immature maize kernels, sweets etc. The resulting tamales are steam-cooked for 60-90 min. More than 20 different types of tamales are produced in Mexico
Tacos	The most popular use of tortillas is in these foods. Tacos are filled with refried beans or shredded meats; quesadillas with cheese. With any type of filling, tacos are generally rolled or folded and fried for a few seconds.
Pozol	In southern Mexico, pozol is maize masa shaped into balls that are wrapped in banana leaves and fermented for 1-14 days. The fermented dough balls are diluted with water to obtain a thick beverage.
Atole	Lime-cooked maize is ground into masa, which is wet-sieved to remove large particles. The slurry is boiled for 15-20 min and blended with water and flavourings (milk, sugar, cinnamon, and orange leaves). Atoles are also produced from immature kernels or steeped maize.
Pincle	Raw or lime-cooked maize is roasted on a griddle for 3-15 min, dry-milled into a meal, and blended with spices (cinnamon and anise) and brown sugar. The meal mix is blended with water or milk, boiled for 4 min, and consumed like atole. It has a granular texture.

- e. Cooking time and steeping time, as well as
- f. The extent of rubbing to remove the seed-coat washing of the kernels.

When nixtamal is ground, the kernel components preconditioned by cooking and steeping are disrupted, and the attrition causes the formation of masa. Masa consists of pieces of germ, remnant pericarp and endosperm held together by a glue-like mixture of 'melted' starch granules, 'sheets' of protein matrix, and emulsified lipids (Gomez *et al.*, 1989).

During nixtamalization, $\text{Ca}(\text{OH})_2$ used reacts with the components of the grain to produce colour. Even when tortillas are produced from white kernels, a high concentration of lime leads to a yellowish end product (Serna-Saldivar *et al.*, 1990). The intensity of the colour is higher, in the cell wall i.e. pericarp. As a result, part of this colour is lost during the process of washing. The extent of washing therefore affects the colour intensity of the kernels and hence the masa which is produced.

A number of researchers have investigated the effect of various treatments on the colour of alkaline cooked maize and its products. Work done by Bazua *et al.* (1979) on extruded maize flour for tortilla preparation showed an increase in intensity of colour i.e. darker (lower L-values) with increasing concentration of $\text{Ca}(\text{OH})_2$ used for the treatment.

Johnson *et al.* (1980) reported similar trends in his work on micronised sorghum flours and maize flours. The colour of dry masa flours, ranges from white to dark yellow, depending on the maize type, alkali concentration and processing conditions (Gomez *et al.*, 1987).

The process of nixtamalization significantly affects the water absorption properties of maize. Work done by Chang and Hsu (1985), showed that, maize cooked in a lime solution absorbs more water than that cooked in water. During lime cooking, the moisture content of the grain increases from 10-12% to 40-42%. The grain absorbs water rapidly during the first 15 min of cooking (Serna-Saldivar *et al.*, 1988b). Trejo-Gonzalez *et al.* (1982) also indicated a very rapid initial water uptake during the nixtamalization of a Mexican maize variety. Steeping cooked grains further increases the moisture content by 4-7 percentage points and distributes the water more evenly throughout the kernel (Serna-Saldivar *et al.*, 1988b; 1990).

During lime-cooking the grain starches are partially gelatinized, while other grain components are hydrated and altered. These transformations affect the water absorption properties of masa products. The cooking and drying process employed in the production of dry masa flours, cause some starch gelatinisation and increases the porosity of the maize endosperm fragments resulting in high water absorption (Gomez *et al.*, 1987). Other researchers have indicated that water absorption capacity is dependent on the concentration of lime used. Bryant and Hamaker (1997) reported that water retention capacity, which is a starch gelatinization indicator, is increased in defatted maize flour by the addition of lime at levels between 0 and 0.4-0.5%, peaking at 0.2%. At levels greater than 0.4-0.5%, water absorption is retarded by the presence of lime.

Bedolla and Rooney (1984) found that the water absorption capacity of tortilla flours depends on the pH, protein content, degree of enzyme susceptible starch, and particle size.

2.4.2.2 Chemical Changes

An increase in pH due to retention of calcium hydroxide (lime) is observed in alkalized grains. The pH of masa, and its products, such as tortillas is closely related to the amount of lime used and retained during cooking and steeping, and the extent to which the nixtamal is washed (Serna-Saldivar, 1990). In most cases, tortillas have a neutral or slightly alkaline pH. The pH of rehydrated masa varies from 5.2 to 10.5 with values of 7 to 9 most common (Gomez *et al.*, 1987).

Other significant transformations involving both positive chemical transformations and nutrient losses take place during nixtamalization. When compared to the original grain tortillas contain less crude fibre and fat; comparable amounts of protein, ash and carbohydrates; and 10 to 20 times as much calcium (Cravioto, 1945; Braham and Bressani, 1966). Below is a table presenting the typical composition of raw maize and tortillas (Table 2).

The crude fibre content of maize decreases as the kernel is converted into tortillas. Lime treatment at 96°C for about 55 minutes hydrolyzes the pericarp, which is removed during washing, pulling the tip cap with it, and this would account to a large extent for fibre loss.

Table 2. Typical Composition of Raw Maize and Tortillas

Source	Product	Composition (g/100g)				
		Protein (N x 6.25)	Ether Extract	Carbohydrates	Ash	Crude Fibre
Bressani <i>et al.</i> (1958)	Raw maize	9.6	5.1	84.0	1.3	1.5
	Nixtamal	10.3	3.9	84.3	1.5	1.3
	Tortilla	10.7	3.0	84.7	1.5	1.1
Serna-Saldivar <i>et al.</i> (1987)	Raw maize	11.1	4.9		1.4	
	Nixtamal	11.1	4.6		1.5	
	Tortilla	11.2	4.4		1.6	

Using the method of Asp *et al.*(1983), Acevedo and Bressani (1990) detected a decrease in insoluble fibre from raw maize(13%), to the dough (6%) and an increase in tortillas (7%). Soluble fibre increased from 0.88% in raw maize to 1.31% in the dough, and further increased to 1.74% in tortillas. The increase in fibre from masa to tortillas has also been reported by Reinhold and Garcia (1979). These have been attributed to the formation of insoluble Maillard Browning products, as has been reported in baked wheat products (Ranhotra and Gelroth, 1988).

Masa products have been reported to contain less fat than is found in the untreated grain, suggesting losses in fat content during nixtamalization.

Pflugfelder *et al.* (1988) found losses of 1.8 to 18.1% in ether-extractable substances, in nixtamalized maize and suggested that these could be partly due to the vigorous handling of cooked maize at the industrial plant. Bedolla *et al.*(1983) found ether extract values of 5.0, 3.1 and 3.6% in raw maize, cooked

maize and tortillas respectively. This loss has not been fully explained; however, it may result from the loss of the seed-coat, the tip cap, the aleurone layer and possibly part of the germ, and also from ether extractable substances not necessarily fat. Even though ether extractable substances are lost in the process of converting maize into tortillas, the fatty acid make-up of the fat does not change in maize (Bressani *et al.*, 1990).

Most findings, in research work on alkaline cooking have shown an increase in total ash content from maize to tortillas. These increases have been attributed to the absorption of lime, which significantly increases the calcium content (Saldana and Brown, 1984; Ranhotra, 1985). The mineral contents of alkaline-cooked maize products as determined by different researchers are varied and seem to be dependent on a number of factors including the variety of maize and the method of determination (Table 3).

Table 3. Mineral Content of Raw maize and Home and Industrialized Samples of Tortillas (mg/100g)

Product	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Maize	300	325	48	108	54	4.8	1.3	1.0	4.6
Home made tortilla 1	309	273	217	123	71	7.0	2.0	1.0	5.4
Home made tortilla 2			202			2.7	0.3		3.4
Home made tortilla 3	294		104	72		3.5	1.3		4.6
Industrial tortilla 1	315		182	106		4.0	2.5	-	3.2
Industrial tortilla 2	240	142	198	60	2	1.2	0.17	0.41	1.2
Industrial tortilla 3	269	185	205	63	9	1.5	0.19	0.40	1.1

Sources: Bressani *et al.*, 1990; Krause, 1988; Ranhotra, 1985; Vargas, Munoz and Gomez, 1986

According to Pflugfelder *et al.* (1988), Calcium content in the dough is influenced by lime levels, cooking and steeping temperatures and maize characteristics. The changes that occur in other minerals are variable and may depend on the purity of the lime used as well as on the type of milling equipment.

The combined effects of water, calcium hydroxide and heat produce significant changes in the carbohydrates contained in maize during nixtamalization. Robles, Murray and Paredes-Lopez (1988) found that alkali-cooking of maize caused large increases in viscosity and that cooking time had a significant effect on pasting properties, although there was no extensive gelatinization of the starch. A decrease in sugar from 2.4% in maize to 0.343% in tortillas has also been reported (FAO, 1992). In the process of converting maize into nixtamal, enzyme-susceptible starch, which is an indicator of starch gelatinisation, increases as cooking time lengthens.

Most researchers have reported a small increase in nitrogen content, which is attributed to concentration effect (FAO, 1992). Bressani *et al.* (1958) reported an increase in protein content when maize was processed into nixtamal and further into tortillas.

The solubility of all protein fractions has been reported to decrease from raw maize to tortillas, with an increase in the insoluble fraction. Bressani and Scrimshaw (1958) extracted the nitrogen from raw maize and tortillas and reported that the protein fractions were significantly lower in tortillas. Ortega *et al.*

(1986) observed similar changes in both Common and Quality Protein Maize (QPM) maize.

Bressani and Scrimshaw (1958) carried out studies on amino acids using *in vitro* enzymatic digestion with pepsin, trypsin and pancreatin. After 60 hours of hydrolysis the percentage of enzymatically released amino acids with respect to the acid-hydrolyzed amino acids suggested a faster release from tortillas than from maize. Serna-Saldivar *et al.* (1987), on the other hand, working with ileum-cannulated pigs found that at this level in the intestinal tract, the digestibility of most of the essential amino acids was somewhat higher from water-cooked maize than from lime-cooked maize. Digestibility of the protein decreased slightly, possibly because of the heat treatment involved (Bressani *et al.*, 1990). Other researchers have suggested that during maize processing, hydrophobic interactions, protein denaturation and cross-linking of proteins are probably responsible for changes in the solubility of these components, which could affect amino acid release during enzymatic digestion.

2.4.3 Effect of Nixtamalization on Niacin Bioavailability

Pellagra, which is caused primarily by a deficiency in niacin, is one of the most common diseases in populations where maize constitutes the major part of the diet. Maize is low in tryptophan, contains bound niacin, and has an unfavourable ratio of isoleucine to leucine (Katz *et al.*, 1974). Despite these facts, pellagra has been virtually unknown in Mesoamerican countries (e.g. Mexico and Central America), because the population consumes maize in the form of tortillas or

related products. The alkaline treatment of maize has been reported to destroy its pellagragenic factor. It has been reported that lime cooking of maize releases part of the bound niacin and improves the isoleucine-leucine ratio (Bressani and Scrimshaw, 1958; Katz *et al.*, 1974).

Evidence from a large number of researchers has suggested that pellagra results from an imbalance of the essential amino acids, increasing the niacin requirement of the animal. (FAO, 1990). This point has been extensively debated between those who claim that niacin in maize is bound and not available to the animal and those who favour the theory of improved amino acid balance induced by the alkaline-cooking process, resulting in the release of the bound niacin. Work done by Wall and Carpenter, (1988), show that raw maize contains 26 μg of niacin per gram but only 0.4 μg as free nicotinic acid, whereas tortillas contain 11.7 μg of free nicotinic acid per gram even though niacin is lost during cooking and washing. Work done with experimental animals showed that pigs and rats fed tortillas without supplemented niacin performed better than their counterparts fed raw maize (Chaudhuri and Kodicek, 1950; Cravioto *et al.*, 1956).

2.4.4 **Effect of Nixtamalization on Nutrient Digestibilities and Protein Quality**

Changes in nutritional value, particularly that of protein, during the transition from raw maize to tortillas have been studied mainly in animals. Even though chemical losses in some nutrients take place upon lime-cooking of maize, protein quality is slightly but consistently better in tortillas than raw maize (FAO, 1992). The protein

efficiency ratio of the tortillas is in general somewhat higher than that of the raw maize, although some studies have reported otherwise.

In a study involving pigs, Serna-Saldivar *et al.* (1987) reported on dry matter, gross energy and nitrogen digestibilities of maize, cooked with and without lime. The pigs digested similar amounts of dry matter and derived similar amounts of energy from maize cooked with and without lime. Protein and lysine digestibilities and nitrogen retention were however slightly lower (5% points less) in pigs fed nixtamal than in their counterparts fed maize cooked in water. Similar results were found in another study with rats (Serna-Saldivar *et al.*, 1988a; Sproule *et al.*, 1988). The improvement in dry matter and energy digestibilities, likely reflects the reduction in crude fibre which is known to inhibit digestive enzymes and to be indigestible. Most findings on protein digestibilities of nixtamal and tortillas suggest that a depression in protein digestibility occurs when raw grain is nixtamalized.

3.0 **MATERIALS AND METHODS**

3.1 **MATERIALS**

Maize (*Zea mays*) was obtained from the Timber market in Accra. Cowpea (*Vigna unguiculata*) var *Bengpla* was obtained from the Crop Research Institute, Kumasi, Ghana. This was stored at cold room temperature (4°C) during the experimental period. Lime (Ca(OH)₂) Laboratory grade, was obtained from BDH Chemicals Ltd., Poole, England.

3.2 **EXPERIMENTAL METHODS**

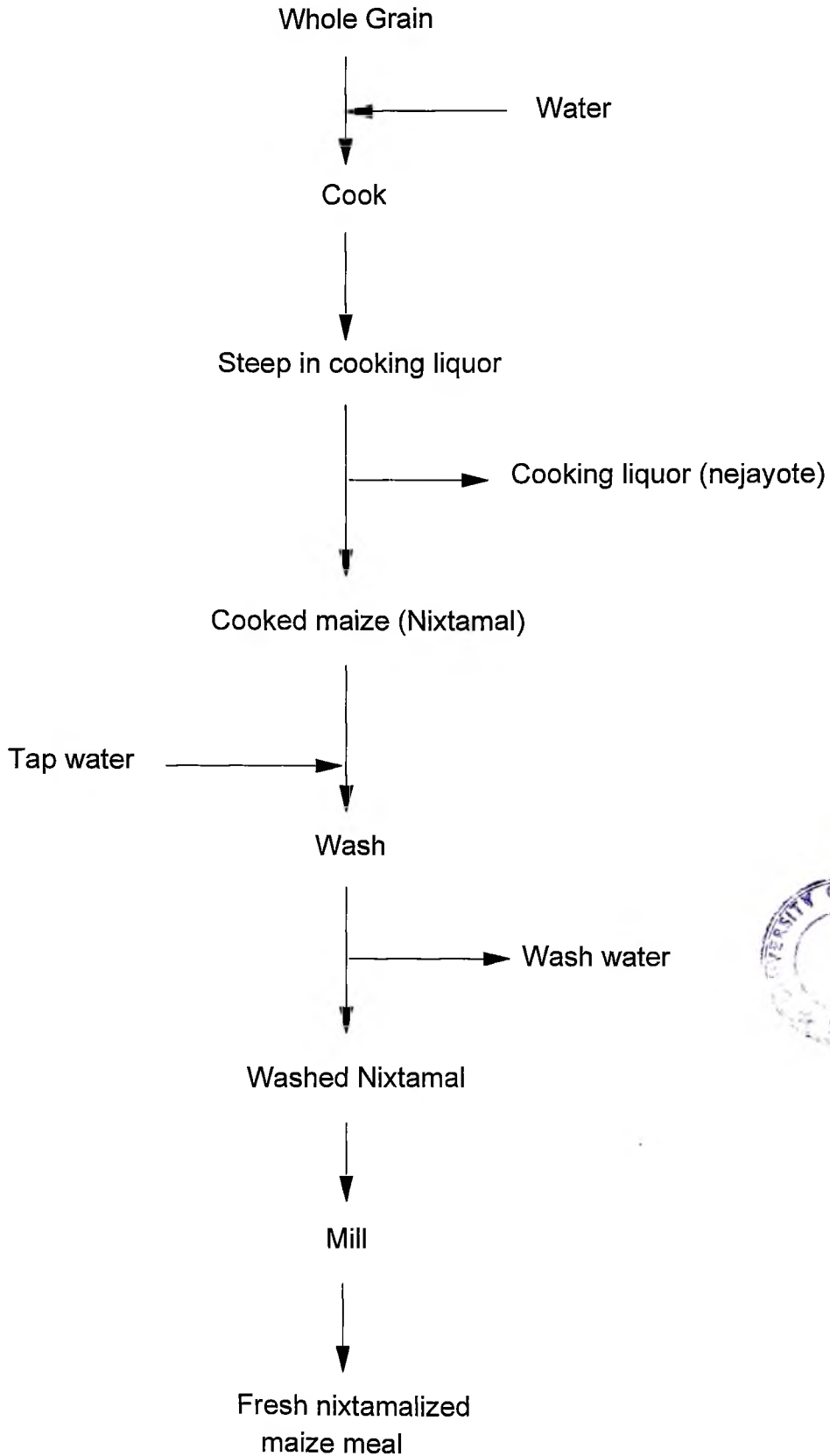
3.2.1 **Effect of Nixtamalization on the Chemical and Functional Properties of Maize**

a. **Experimental Design and Sample Preparation**

A 2 x 4 factorial experimental design with cooking time (0, 30 min) and lime concentration (0, 0.33, 0.5, 1.0 %) was used.

Maize (500 g) was steeped in the lime solutions for 12 hours. Another batch of maize samples were cooked in lime (Ca(OH)₂) solution, at 0, 0.33, 0.5 and 1% concentrations respectively for 30 min. The boiled samples were then steeped in the cook liquor for 12 hours. After steeping, the maize samples were washed thoroughly to remove the excess lime. The nixtamal obtained was drained and milled with a disc attrition mill (Model 10-2A) to produce a dough known as masa. A flow diagram of the nixtamalization process used is represented in Figure 1. The masa was dried at 50°C in an air oven for 12 to 14 hours to a moisture content of 9 to 12%. The dried masa was milled into fine flour using the hammer

Figure 1 Flow Diagram for the Production of Fresh Nixtamalized Maize



mill (Christy and Norris Ltd., Chelmsford, England). The flour was analysed for the following indices: moisture content, pH, protein content, water absorption capacity at 25 and 70°C, ash, cooked paste viscosity and colour.

b. Moisture Content

The moisture content of the flours were determined using AOAC (1990) method No. 950.10 (i.e. Air oven method at 105°C).

c. pH

Ten (10) grams of the flour samples were mixed with 100 ml of CO₂-free distilled water. The mixture was allowed to stand for 15 minutes, shaken at 5 min intervals and filtered with Whatman No. 4 filter paper. The pH of the filtrate was measured using a pH meter (Model HM-30S, Tokyo, Japan).

d. Protein Content

The protein content of the dry flours were determined by the Kjeldahl method (AOAC, 1975). Factor of Conversion of nitrogen to protein was 6.25.

e. Ash

Ash content was determined using the standard AOAC Method (AOAC, 1975). Approximately 2g of sample were weighed into a known weight crucible (Pre heated in the furnace and cooled). The samples were then put in a pre-heated furnace (GallenKamp Muffler Furnace size 3) of 550-600°C overnight. After this

the crucibles were removed, cooled and weighed to determine the weight of the ash of the samples. The ash content was then calculated on 100g/sample basis. Determinations were done in duplicates.

f. Water Absorption Capacity

Duplicate analyses were done using the method of Anderson *et al.* (1969). Five grams of sample was weighed into a centrifuge tube and 30 ml of distilled water (at desired temperature i.e. 25 and 70°C in this case) was added. The solution was stirred and allowed to stand for 30 minutes and centrifuged using a Denley centrifuge (Model BS 4402/D, Denley, England), at 3000 rpm for 15 minutes. The supernatant was decanted and the increase in weight noted by weighing. The water absorption capacity was expressed as a percentage of the initial sample weight.

g. Cooked Paste Viscosity

The viscosity of the lime-treated samples was determined using AACC method NO. 22-10 (AACC, 1983) with slight modifications. The cooked paste viscosity of slurries made from concentrations of 8% (dry matter basis) flour in 500 ml water were measured using the Brabender Viscoamylograph (Brabender Duisburg, Germany), equipped with a 500 cmg sensitivity cartridge. The viscosity of the samples were continuously monitored as they were heated from 25°C at a rate of 1.5°C/min to 95°C, held for 30 min, cooled to 50° at 1.5C°/min and held at 50°C for 15 minutes. Brabender Viscoamylograph indices were measured.

3.2.2 **Effect of Fermentation on the Characteristics of Nixtamalized Maize Dough**

a. **Experimental Design and Sample Preparation**

A 3 x 5 factorial experimental design with fermentation time (0, 24, 48 hours) and level of traditional fermented maize dough (0, 25, 50, 75, 100 %) respectively was used. The traditional steeped maize was prepared by cleaning and steeping an amount of maize in water for 24 hours. The steeped maize was washed and drained.

Nixtamal was prepared by boiling whole maize in 1% lime solution for 30 minutes and steeped in the cook liquor for 14 hours. After steeping the grains were washed thoroughly with water to remove all excess lime. The resulting nixtamal was mixed with the traditional steeped maize to obtain composite mixtures of nixtamal and traditional steeped maize. The composite mixtures were milled using a Disc attrition mill (Model 10-2A) to produce blends of steeped:nixtamalized maize with the following composition; 100:0, 75:25, 50: 50, 25:75 and 0:100%.

The meals produced were mixed with water to form dough of moisture contents between 52-65%, fermented for 0, 24 and 48 hours respectively. The pH and Titratable acidity of the dough (undried) was determined at 0, 24 and 48 hours of fermentation. After 48 hours of fermentation, the dough was dried in an air oven at 50°C for 16 hours to moisture contents between 7-10%. The dried samples were milled into fine flour using the hammer mill (Christy and Norris Ltd., Chelmsford, England). The flour samples obtained were analysed for the following indices: water absorption capacity (25 and 70°C), colour, cooked paste viscosity and texture.

b. pH and Titratable Acidity

Ten (10) grams of dough (undried) was mixed with 100ml distilled water. The mixture was allowed to stand for 15 minutes, shaken at 5 min intervals and centrifuged at 3000 rpm for 15 minutes using a Denley centrifuge (Model BS4402/D, Denley, England). The supernatant was decanted and its pH was determined using a pH meter (Model HM-30S, Tokyo, Japan). Ten (10) ml aliquots (triplicate) were titrated against 0.1M NaOH using 1% phenolphthalein as indicator. Acidity was calculated as grams Lactic acid/100g sample.

c. Water Absorption Capacity

The water absorption capacity of the samples was determined by the method of Anderson *et al.* (1969), as indicated in section 3.2.1(f).

d. Cooked Paste Viscosity

The cooked paste viscosity of samples was determined by the method indicated in section 3.2.1(g).

e. Colour

Colorimetric measurements of the dry flour samples were recorded using a Minolta CR-310 tristimulus colorimeter (Minolta Camera Co. Ltd., Osaka, Japan). The instrument was calibrated with a standard white tile ($L = 97.95$, $a = -0.12$, $b = +1.64$). Surface colour differences were minimized by reporting an average of three readings of each flour sample. Psychrometric colour terms, L (Lightness), a (red-greenness) and b (yellow-blueness) were recorded.

f. **Texture**

Twelve (12) percent slurries of the dry sample flours were cooked into a porridge, which was allowed to set to room temperature (25°C) within a period of 40 min. The texture of the set slurries were determined using a TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, England), equipped with a back extrusion rig equipped with a compression disc of 45mm diameter. The work done in back extruding about 90ml of set sample slurry was determined. The test was replicated five (5) times at a crosshead speed of 5mm/s and a distance of 35mm. The force-deformation curve was plotted using the XT.RA Dimension, version 3.78 computer software (Stable Micro Systems, Surrey, England).

3.2.3 **Effect of Nixtamalization, Cowpea Fortification and Moisture Content on the Chemical and Functional Properties of Maize**

a. **Experimental Design and Statistical Analysis**

The central composite rotatable design for K=3 was used (Cochran and Cox, 1957). The independent process variables were lime concentration, cowpea level and moisture content of the nixtamalized maize. The levels are summarized in Table 4 below. Twenty (20) sample combinations were generated (Table 5).

Table 4. Process Variables used in Central Composite Rotatable Design for K=3

Independent Variables	Variable Levels				
	-1.682	-1	0	1	1.682
Lime Concentration (%)	0	0.20	0.50	0.80	1.00
Cowpea Level (%)	0	6.09	15.02	23.95	30.00
Moisture Content (%)	55.0	57.03	60.00	62.97	65.00

Table 5. Design Matrix and Variable Combinations in Experimental Samples

No.	Level Codes			Levels		
	Lime Conc.	Cowpea Level	Moisture Content	Lime Conc. (%)	Cowpea Level (%)	Moisture Content (%)
1	-1	-1	-1	0.20	6.09	57.03
2	-1	1	1	0.20	23.95	62.97
3	1	-1	1	0.80	6.09	62.97
4	1	1	-1	0.80	23.95	57.03
5	0	0	0	0.50	15.02	60.00
6	0	0	0	0.50	15.02	60.00
7	-1	-1	1	0.20	6.09	62.97
8	-1	1	-1	0.20	23.95	57.03
9	1	-1	-1	0.80	6.09	57.03
10	1	1	1	0.80	23.95	62.97
11	0	0	0	0.50	15.02	60.00
12	0	0	0	0.50	15.02	60.00
13	1.682	0	0	1.0	15.02	60.00
14	-1.682	0	0	0	15.02	60.00
15	0	1.682	0	0.50	30.00	60.00
16	0	-1.682	0	0.50	0	60.00
17	0	0	1.682	0.50	15.02	65.00
18	0	0	-1.682	0.50	15.02	55.00
19	0	0	0	0.50	15.02	60.00
20	0	0	0	0.50	15.02	60.00

b. Sample Preparation**i. Maize**

Whole maize grains were cleaned and boiled in lime solution at different concentrations as indicated in Table 5, for 30 minutes. After boiling, the grains were left to steep in the cooking liquor for 14 hours, the usual time period used (Ramirez-Wong *et al.*,1994). The nixtamal produced was washed thoroughly to remove all excess lime.

ii. Cowpea

Cowpea was soaked in water for 5 minutes and dehulled using a disc attrition mill (Model 10-2A). The dehulled cowpea was mixed with the nixtamal according to the levels indicated in Table 5. The various mixtures of dehulled cowpea and nixtamal were milled using a disc attrition mill (Model 10-2A). The moisture levels of the meals produced were adjusted by adding pre-determined amounts of water to yield the desired moisture contents indicated in Table 5. The dough produced was fermented spontaneously for 48 hours. A flow diagram of the process is presented in Figure 2. After 48 hours of fermentation, the doughs were dried in an air oven at 50°C for 16 hours. The dried samples were milled using a hammer mill (Christy and Norris Ltd., Chelmsford, England).

The moisture content, pH and titratable acidity of the dough samples were determined before and after fermentation. The dry flour samples were analysed for the following indices: water absorption capacity (25 and 70°C), protein, cooked paste viscosity and texture by the methods indicated in section 3.2.2.

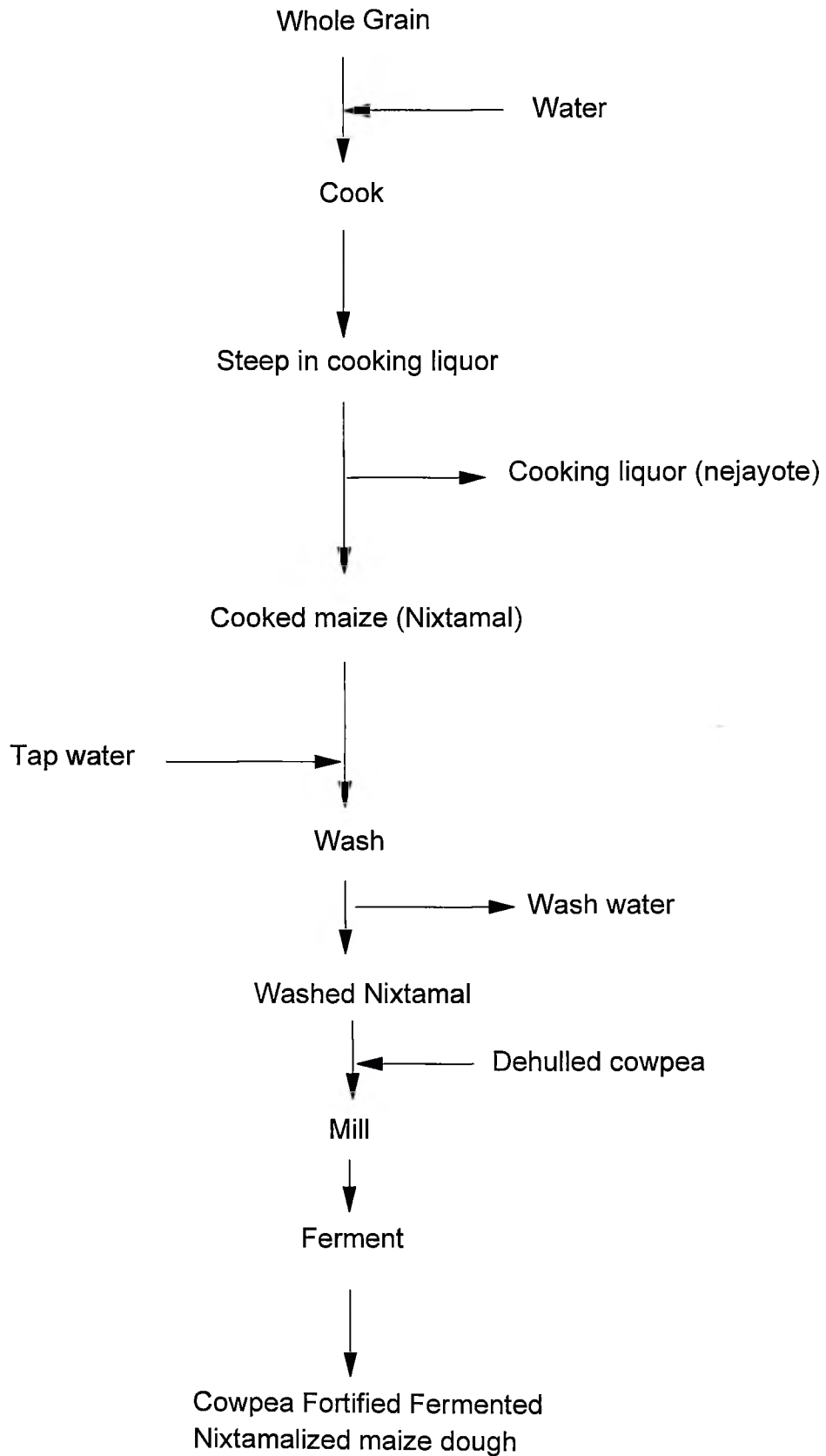
3.2.4 **Microbial Profile of Fermenting Nixtamalized Maize**

a. **Preparation of Samples**

i. Traditional Fermented Maize Dough (Control)

The control sample was prepared by the method used in preparing traditionally fermented maize dough in Ghana. Maize grains were cleaned, soaked in water for 24 h, washed thoroughly and milled with a disc attrition Mill (Model 10-2A). The meal obtained was made into a dough of about 50% moisture and fermented for a period of 72 hours.

Figure 2 Flow Diagram for the Production of Fermented Cowpea Fortified, Nixtamalized Maize Dough



ii. Fermented Nixtamalized Maize Dough

Whole maize grains were cleaned and divided into two batches of 1500g weight each. One batch of maize was boiled in 0.5% lime and the other batch in 1% lime solution, each for a period of 30 min. After boiling, the grains were steeped in the cooking liquor for 14 hours, washed thoroughly with water and milled with a disc attrition mill (Model 10-2A). The resulting meal (masa) was made into a dough of about 65% moisture by adding a pre determined amount of water. The nixtamalized maize dough was fermented for a period of 72 hours.

b. **Sampling**

I. Steep Water

Samples of 50 ml of the Steep water for both the control and nixtamalized maize were aseptically collected into sterile containers, at the beginning and the end of the steeping periods. The steep water was mixed thoroughly before collection of samples for analysis.

ii. Fermenting Dough

Samples of 10 g of the dough were aseptically collected into stomacher bags (Seward Medical, London, England) at 0, 24, 48 and 72 hours of fermentation for analysis. Fermenting dough samples were taken from within the dough after the surface layers had been removed aseptically with a sterile scalpel.

c. **Microbiological Analyses**

For the dough samples, 90 ml sterile diluent containing 0.1% peptone and 0.8% NaCl with pH adjusted to 7.2 was added to the 10 g samples in the stomacher

bags and homogenized in a Stomacher (Lab Blender, Model 4001, Seward Medical) for 30 sec at normal speed. From appropriate ten fold dilutions (up to 10^{12}) of the dough as well as the steep water samples, enumeration of aerobic mesophiles were carried out on Plate Count Agar (PCA, Merck 5463, Darmstadt, Germany) incubated at 30°C for 3 days.

Lactic acid bacteria were enumerated on MRS Agar (MRS, Merck 10660) incubated anaerobically in an anaerobic jar at 30°C for 5 days. Mold and Yeast counts were enumerated on Malt Extract Agar (MEA, Merck 5398) containing 100 mg chloramphenicol (Chloramphenicol selective supplement Oxoid) and 50 mg Chlortetracycline (Sigma # C-4881, St. Louis, MO, USA) per litre and incubated at 25°C for 5 days.

I. Isolation of Lactic Acid Bacteria from Fermented Nixtamalized Maize

All colonies, totaling 26 from the 10^9 dilution plate of the dough treated with 0.5% lime and 18 from the 10^9 dilution plate of the dough treated with 1% lime (i.e. the highest dilution MRS plates) were subcultured in MRS broth medium (MRS, Fluka 69966, Buchs, Switzerland) and streaked onto MRS agar substrate until pure cultures were obtained.

ii. Identification of Lactic Acid bacteria.

MRS isolates were examined by colony and cell morphology, Gram reaction and Catalase production. *Lactobacillus spp* were recognized as gram positive and catalase negative rods. They were further sub-grouped based on detailed examination of their cell morphology which was accomplished by comparing high

(x100) magnification fields of the cells in a microscope (Model BH-2, Olympus, Japan).

The identity of *Lactobacilli* were established at the species level by examining representative isolates for utilization of 49 carbohydrates using API 50 CHL (BioMerieux SA, Marcy-L'Etoile, France). Pure representative isolates were grown in 10 ml MRS broth at 30°C for 48 hours. These were transferred onto MRS plates. A pure colony from each of the MRS plates was transferred with a sterile loop into a tube of sterile API 50 CHL medium. The API strips were placed in an incubation tray into which distilled water had been distributed into the honey comb to maintain moist conditions. The cupules containing the dehydrated carbohydrate substrates were carefully inoculated with the API medium-bacterial suspension. The cupules were overlaid with sterile paraffin oil in order to obtain anaerobic conditions. This process was repeated for all the representative isolates. The inoculated strips were incubated anaerobically at 30°C for 72 hours. The test strips were read after 24, 48 and 72 hours of incubation. The results were recorded on results sheets and *Lactobacilli* were identified using an API 50 CHL identification table. The gram-positive, catalase-negative cocci were tentatively identified by cell morphology.

d. **Determination of pH and Titratable Acidity**

I. Steep Water

The pH of the steep water was determined at the beginning and the end of the steeping period. About a 100ml of the steep water was centrifuged at 3000 rpm for 15 minutes using a MSE. Mistral 3000i centrifuge (Model MBS 300, Sanyo,

UK). The supernatant was decanted and its pH was measured using a Lab pH meter (Model PHM 92, Radiometer, Copenhagen).

ii. Dough (Control and Nixtamalized Maize)

For the fermenting dough, ten (10) grams of sample were mixed with 100 ml of CO₂-free distilled water. The mixture was allowed to stand for 15 minutes, shaken at 5 min intervals and centrifuged at 3000 rpm for 15 min using a centrifuge (Model MSB 300). The supernatant was decanted and its pH was measured using a Lab pH meter (Model PHM 92). Ten (10) ml aliquots (triplicate) were pipetted and titrated against 0.1M NaOH using 1% phenolphthalein as an indicator. Acidity was calculated as grams Lactic acid/100g sample.

4.0 **RESULTS AND DISCUSSION**

4.1 **EFFECT OF NIXTAMALIZATION ON CHEMICAL AND FUNCTIONAL PROPERTIES OF MAIZE**

The effect of cooking and concentration of lime on the physical, chemical and functional characteristics of nixtamalized maize was studied. Analysis of variance was done on the results obtained. Multiple range tests were also done to determine the differences in the effects produced by the significant factors.

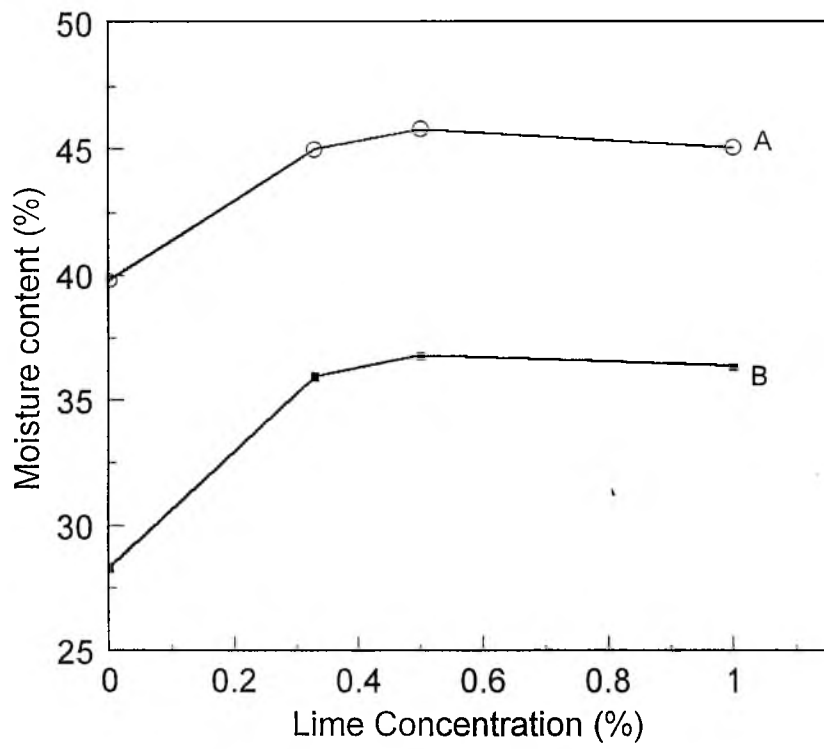
4.1.1 **Moisture Content**

The moisture content of the lime-treated maize grains followed similar patterns. The moisture contents of both the uncooked and cooked samples increased with increasing lime concentration, an indication that lime facilitates the absorption of water by the maize grains (Figure 3). The moisture content of the uncooked grains ranged between 28.27-36.75% while that for the cooked ranged between 39.77-45.77%.

The maize grains absorbed more water at concentrations between 0 - 0.33% lime, and increased slightly to its maximum moisture absorption at a lime concentration of 0.5%. The highest grain moisture absorption was obtained with 0.5% lime concentration for both treatments. Samples treated with 1% lime had a slightly less moisture content than those treated with 0.5% lime.

Maize grains cooked in lime before steeping had a higher moisture content, at all lime concentrations, than the uncooked maize. This could be due to gelatinization of the maize starch during cooking, making hydration of the endosperm easier and faster.

Figure 3 Effect of Lime Concentration on Moisture Content of Cooked (A) and Uncooked (B) Lime Treated Maize



For both cooked and uncooked maize, the lime-treated samples had higher moisture content than those without lime. In the presence of lime there is the possibility of an osmotic potential developing in the grain and this will cause the maize grains to absorb more water till equilibrium is attained. The higher moisture content of the grains treated with lime can therefore be attributed to osmotic effects. Chang and Hsu (1985) studied the effect of lime on moisture absorption, and reported that maize cooked in lime solution absorbs more water than that cooked in water.

Analysis of variance (ANOVA) of the data, showed that cooking and lime concentration significantly affected the moisture content of the nixtamal (Table 6). Multiple range analysis of the results revealed that, cooking had a significantly different effect on the moisture content of the sample.

Table 6. ANOVA Summary Table for Moisture Content of Nixtamalized Maize

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	4	253.15485	63.28871	76.367*
Cooking	1	183.64861	183.64861	221.598*
% Lime	3	69.50624	23.16875	27.956*
Residual	3	2.4862375	0.8287458	
Total correlation	7	255.64109		

* Significant; $p \leq 0.05$

Multiple range analysis to determine the effect of the lime concentration on the moisture content of the sample showed that, the effect of lime concentration on the moisture content of the samples treated with lime was significantly different from that of the maize, which did not contain lime (0% lime). The other

concentrations of lime (0.33, 0.50, 1.0%), however, had the same effect on the moisture content of the nixtamal.

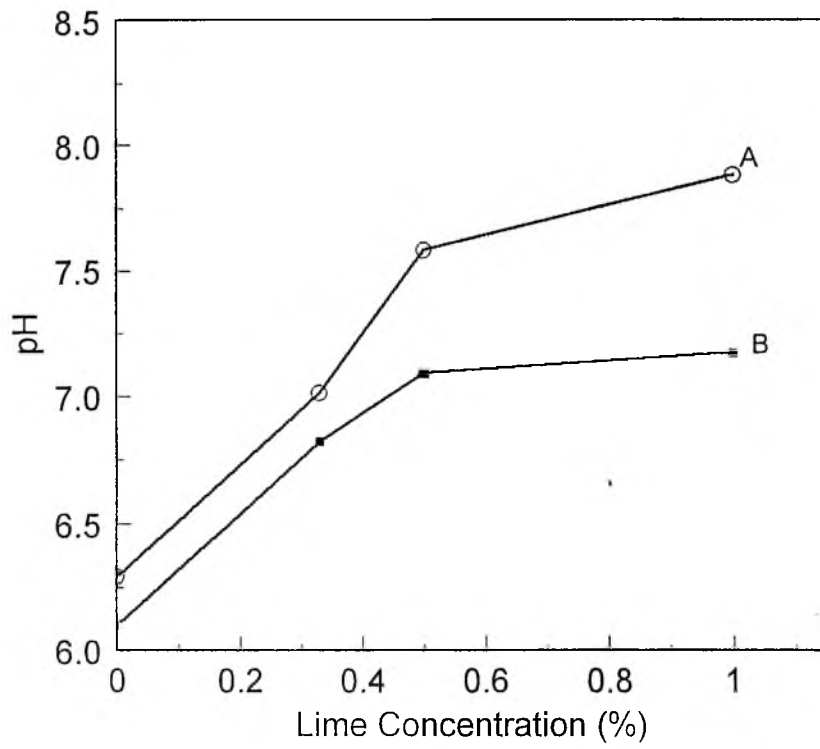
In the production of alkaline cooked products from maize, the moisture content of the lime-cooked grains (nixtamal), among other factors, is a significant determinant of the quality and acceptability of the final product. Different nixtamal moisture contents are required for different end products. The suggested control limit for soft tortillas and corn chips are 45-51% and 48-54% (Snack Food Association, 1987; Serna-Saldivar *et al.*, 1990). The results suggest that, cooking is critical in order to produce nixtamal with an acceptable moisture content.

4.1.2 pH

The pH of nixtamalized maize and tortillas is an important quality parameter which affects the flavour and shelf life of the products made from alkalized maize (Serna-Saldivar *et al.*, 1990). The pH of the dry sample flours determined in this experiment ranged between 6.10-7.88. The maize soaked in water without lime had the lowest pH while the maize boiled in 1% lime for 30 minutes and steeped in the cooking liquor had the highest.

Similar pH trends were observed in the uncooked and cooked samples. The pH of the lime -treated maize increased with increasing lime concentration (Figure 4), and this may be due to absorption and retention of lime. Work done by other researchers show that the pH of alkaline cooked maize and its products, is closely related to the amount of lime used and retained during cooking and steeping (Serna-Saldivar *et al.*, 1990).

Figure 4 Effect of Lime Concentration on the pH of Cooked (A) and Uncooked (B) Lime Treated Maize



Bedolla and Rooney (1984) reported that the pH values of nixtamalized maize flours from the commercial market ranged from 7.1 to 7.4. In this experiment the samples boiled in alkali had a pH range of 7.01 – 7.88.

Analysis of variance of the results obtained showed that, cooking and the concentration of lime significantly changed the pH of the lime treated maize (Table 7). The pH of the lime treated maize increased with increasing lime concentration (Figure 4). Multiple range analysis on the effect of lime concentration revealed that pH of the samples treated with lime were comparable and significantly different from that treated without lime (i.e. 0% lime). The effect of treating the samples with 0.33% lime on pH was the same as that without lime.

Table 7. ANOVA Summary Table for pH of Nixtamalized Maize

Source of Variation	DF	Sum of Squares	Mean Square	F-ratio
Main effects	4	2.3978	0.59945	18.704*
Cooking	1	0.31205	0.31205	8.736*
% Lime	3	2.08575	0.69525	21.693*
Residual	3	0.09615	0.03205	
Total correlation	7	2.49395		

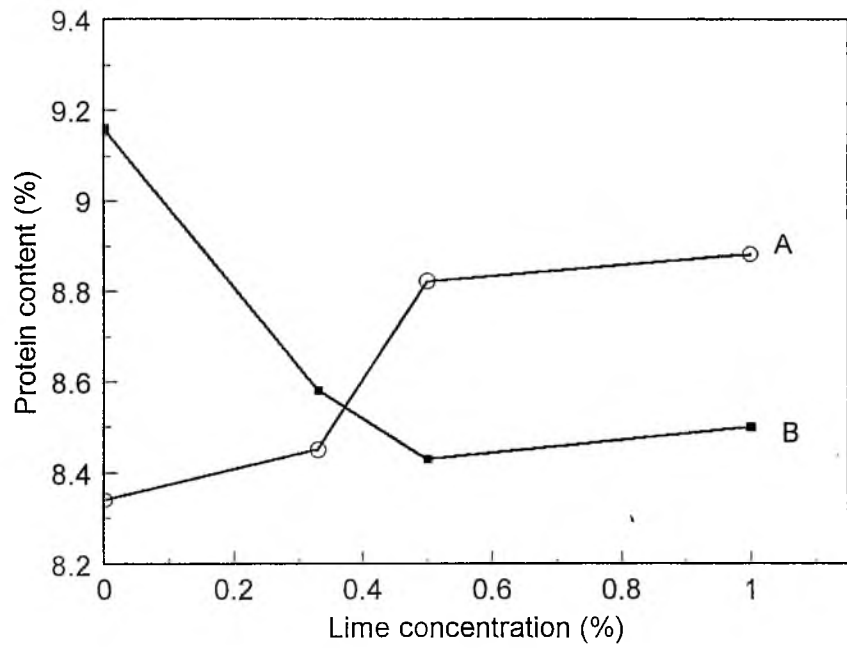
*Significant, $p \leq 0.05$

4.1.3 Protein Content

The protein content increased slightly from 8.14% in the raw maize sample to 8.88% in the sample cooked for 30 minutes in 1% lime solution. For the uncooked maize samples (steeped without cooking), the protein content generally decreased with increasing lime concentration. The opposite was observed in the cooked samples whose protein content increased with increasing lime concentration (Figure 5). These opposite trends however could not be explained.

Figure 5 Effect of Lime Concentration on the Protein Content of Cooked (A) and Uncooked (B) Lime Treated Maize

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It must also be noted that the changes in protein content observed here were not large. Most researchers have reported a small increase in nitrogen content, which has been attributed to a concentration effect. Bressani *et al.* (1958) reported increased protein content from raw maize to nixtamal to tortilla (9.6, 10.3 and 10.7) respectively.

Studies done by other researchers show comparable amounts of protein when alkaline cooked corn products are compared to the original grain (Serna-Saldivar *et al.*, 1987; Gomez *et al.*, 1987).

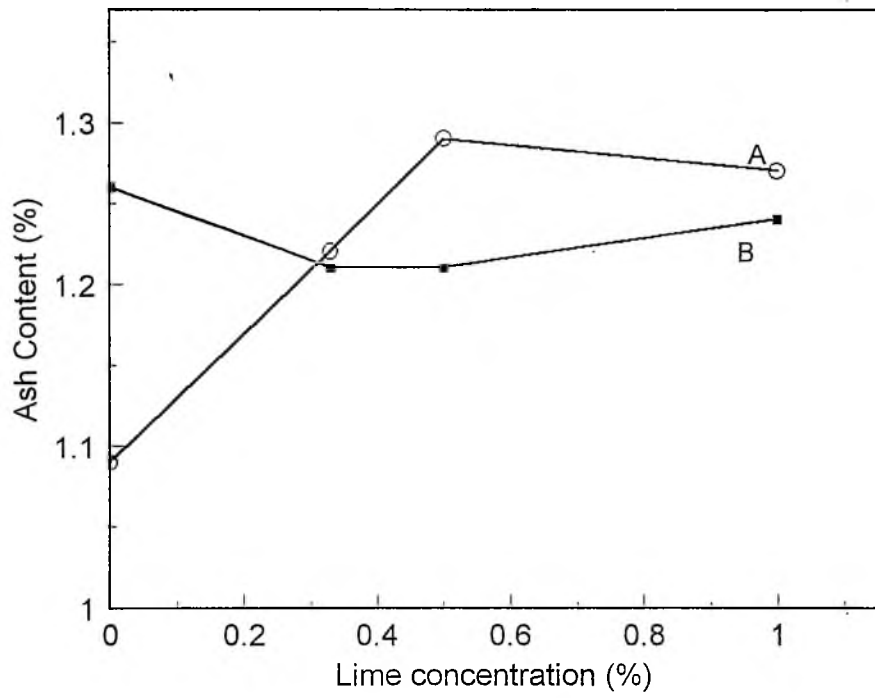
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When the data from protein measurements were subjected to analysis of variance, the results indicated that both cooking and lime concentration did not have any significant effect on the protein content of the flours produced. This could be due to the fact that the changes in protein quantity, of the lime treated samples were small. Protein values in this experiment were expressed on dry matter basis. It is possible that the increase obtained on cooking in lime was due to the procedure and the method of data expression used.

4.1.4 Ash

The ash contents of the lime treated maize ranged between 1.24 - 1.26%, (uncooked samples) and 1.09 - 1.29% (cooked samples). The ash content of the samples cooked in lime increased as the lime concentration was increased to 0.5%, and then decreased slightly when the lime concentration was increased to 1.0% (Figure 6). This trend was similar to the pattern observed in the moisture

Figure 6 Effect of Lime Concentration on the Ash Content of Cooked (A) and Uncooked (B) Lime Treated Maize



content measurements, where the samples containing 0.5% lime had the highest moisture content. Most findings in changes in ash content have shown an increase in total ash content from maize to tortillas (FAO, 1992), which may be expected because of the lime used for cooking.

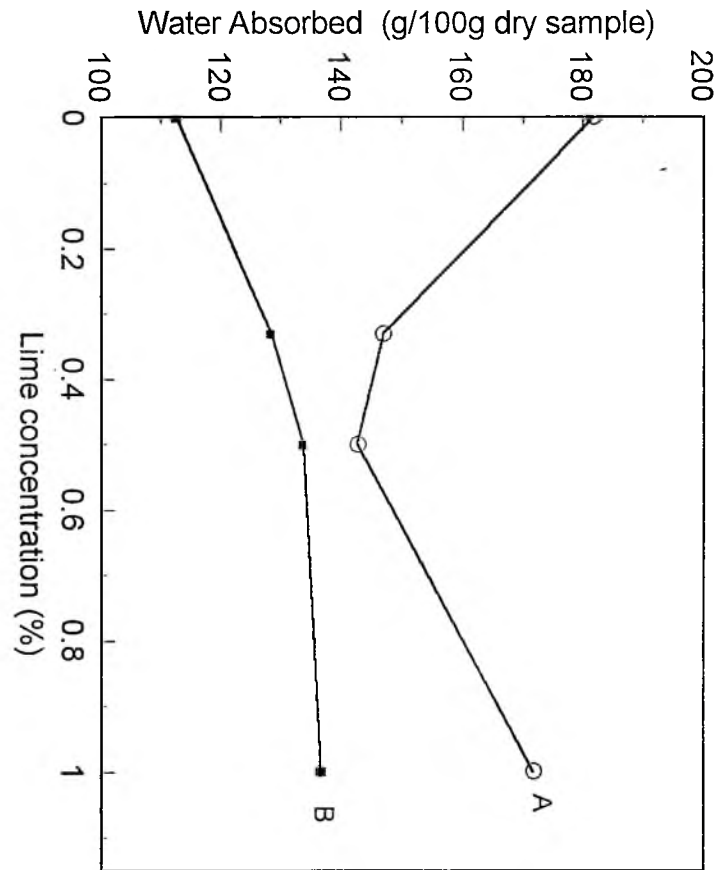
Analysis of variance of the results indicated that the effect of cooking and lime concentration on the ash content of the lime treated samples was not significant. The highest increase in ash content on lime treatment was by 0.20%, which is not a large increase and could therefore account for the lack of significance obtained.

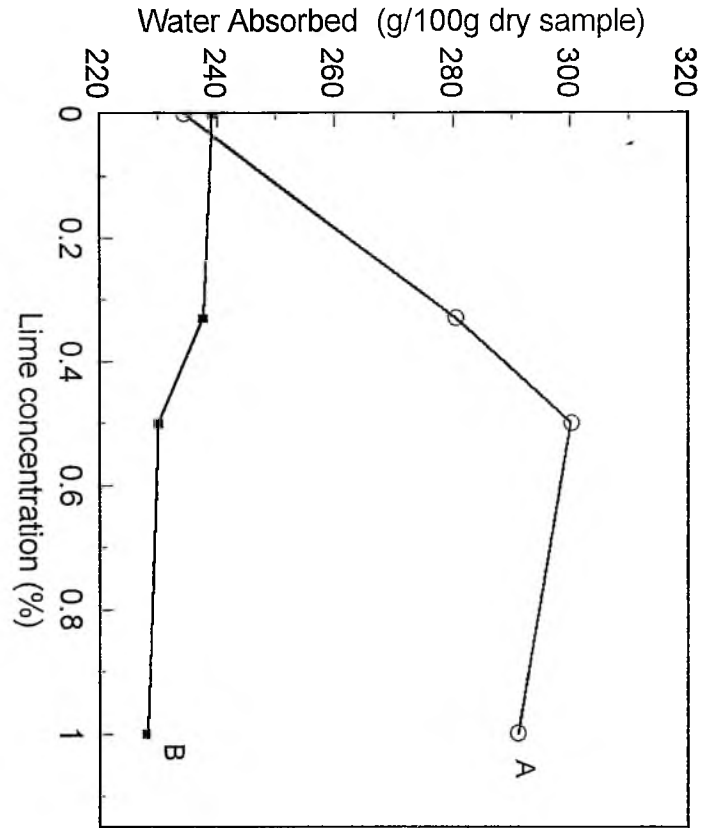
4.1.5 Water Absorption Capacity

The water absorption capacity patterns at 25°C and 70°C of the cooked and uncooked samples were different (Figure 7). At 25°C, the water absorption capacity of the uncooked samples increased with increasing lime concentration, while that of the cooked samples decreased (Figure 7) to a minimum and increased when it was cooked in 1.0% lime.

The reverse of these trends were observed when water absorption capacity was measured at 70°C. At that temperature, water absorption of the uncooked samples decreased with increasing lime concentration while that of the cooked samples increased to a maximum and decreased when the maize was cooked in 1.0% lime (Figure 7). The different trends observed in water absorption of the alkaline treated flours could be attributed to effect of lime on gelatinization, as well as Ca^{2+} and $\text{Ca}(\text{OH})^+$ - starch interactions.

Figure 7 Effect of Lime Concentration on Water Absorption Capacity at 25°C (I) and 70°C (II) of Cooked (A) and Uncooked (B) Lime Treated Maize





The increase in water absorption with concentration of lime in the cooked sample could be due to a facilitating effect of lime on gelatinization. At a 1.0% lime concentration, the starch hydroxyl sites in the maize might have been saturated, resulting in the decreased water absorption observed. Bryant and Hamaker (1997), in their work on the effect of lime on gelatinization of corn flour and starch, indicated that the effect of lime on the gelatinization properties of corn starch is a complex, concentration dependent phenomenon.

The water absorption capacities (at 25°C and 70°C) for the uncooked sample at all concentrations of lime were lower than that of the cooked samples. This could be due to the raw nature of the starch in the uncooked sample, since gelatinized starch is more readily hydrated than ungelatinized starch.

Analysis of variance of the data obtained, indicated that lime concentration and cooking significantly increased the water absorption capacities (at 25°C and 70°C) of the flour samples produced. Multiple range analysis on the effect of lime showed that, irrespective of the concentration of lime used, the lime treatment had the same effect on the water absorption capacity (25°C and 70°C) of the samples. Further analysis to determine the effect of cooking suggested that cooking enhanced the water absorption capacities of the lime treated samples.



4.1.6 Viscosity

The cooked paste viscosity characteristics of the lime treated samples were determined to find out the effect of lime. The pasting temperature, that is, the temperature of initial increase in viscosity of the samples, ranged between 69.0 to 78°C. The uncooked sample steeped in 0.5% lime had the lowest pasting temperature while the sample cooked for 30 min without lime had the highest. The latter also had the lowest peak viscosity while the sample cooked for 30 min in 1.0% lime had the highest peak viscosity (Table 8).

Table 8: Cooked Paste Viscosity (Critical Points) of Maize Treated with Lime (Ca(OH)₂)

SAMPLE	PASTING TEMP (°C)	VISCOSITY (BU)				
		Peak viscosity	95°C	95°C-Hold	50°C	50°C-Hold
Raw maize	70.0	90	90	280	960	990
Uncooked 0% lime	72.0	90	90	120	330	410
Uncooked 0.33% lime	70.3	40	30	30	90	130
Uncooked 0.5% lime	69.0	40	40	60	160	230
Uncooked 1.0% lime	69.7	50	50	50	140	220
Cooked 0% lime	78.0	30	30	70	240	340
Cooked 0.33% lime	72.6	80	80	160	320	480
Cooked 0.5% lime	72.0	90	90	200	410	680
Cooked 1.0% lime	72.9	100	100	210	380	790

The lime treated samples (cooked and uncooked) had a more distinct peak than the raw (untreated) maize sample. Lime treatment also resulted in a drastic reduction in cooked paste viscosity (Figures 8 and 9). Sefa-Dedeh (1990) also reported a drastic reduction in amylograph viscosity when maize was treated with lime. The observed reduction was more pronounced in the cooled paste viscosity, which is an indicator of the consistency at which the gruel will most likely be eaten. The reduction in viscosity, especially during the cooling period could be due to a saturation of the starch hydroxyl sites with Ca^{2+} and $\text{Ca}(\text{OH})^+$ ions preventing any further association of the starch molecules, which results in cooked paste viscosity reduction.

At all critical points on the amylogram (Table 8), the cooked paste viscosity values recorded for the cooked maize samples were higher than that of the uncooked samples which may be attributed to gelatinization of the maize starch during cooking. These viscosities however, were still much lower than that of the raw maize.

The highest viscosity values of all the samples were obtained after holding at 50°C for 15 minutes and these increased with increasing lime concentration (Figure 9), an indication that lime concentration influences set-back viscosity. The highest viscosity value obtained after the cooling period was from the sample, which was boiled in 1.0% lime. Similar trends were observed by Bryant and Hamaker (1997) who reported that the highest value recorded for the viscosity at the end of the 95°C holding period and the cooling period resulted from heating flour in 1.0% lime solution.

Figure 8 Amylograph Viscosity Characteristics of Uncooked Maize Steeped in Lime

A	Raw maize
B	0% Lime
C	0.33% Lime
D	0.5% Lime
E	1% Lime

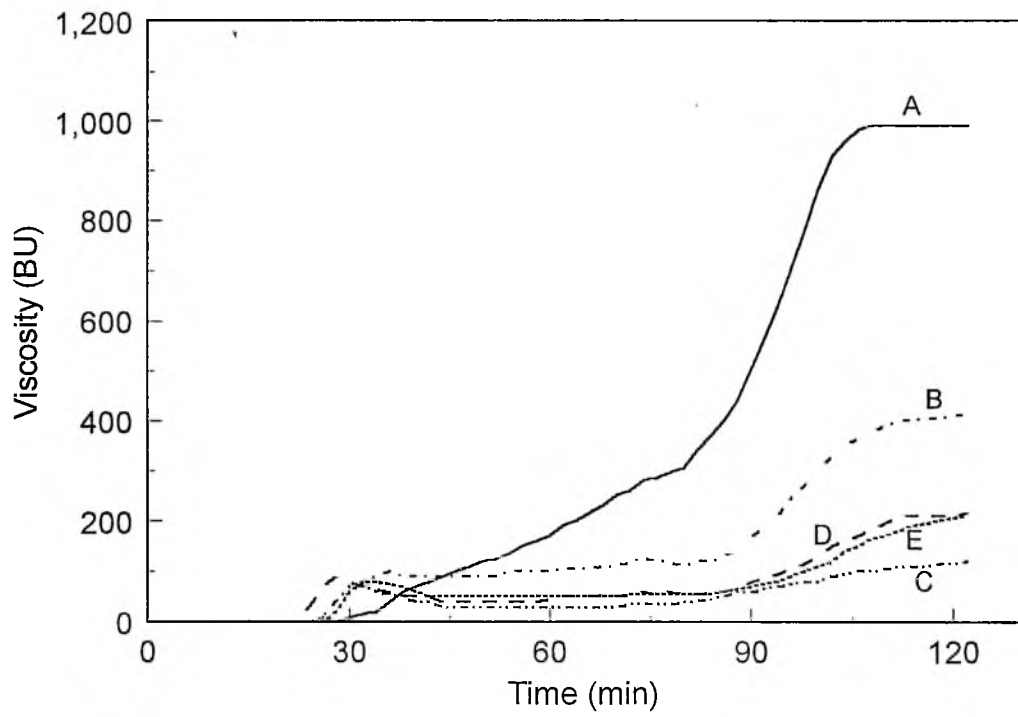
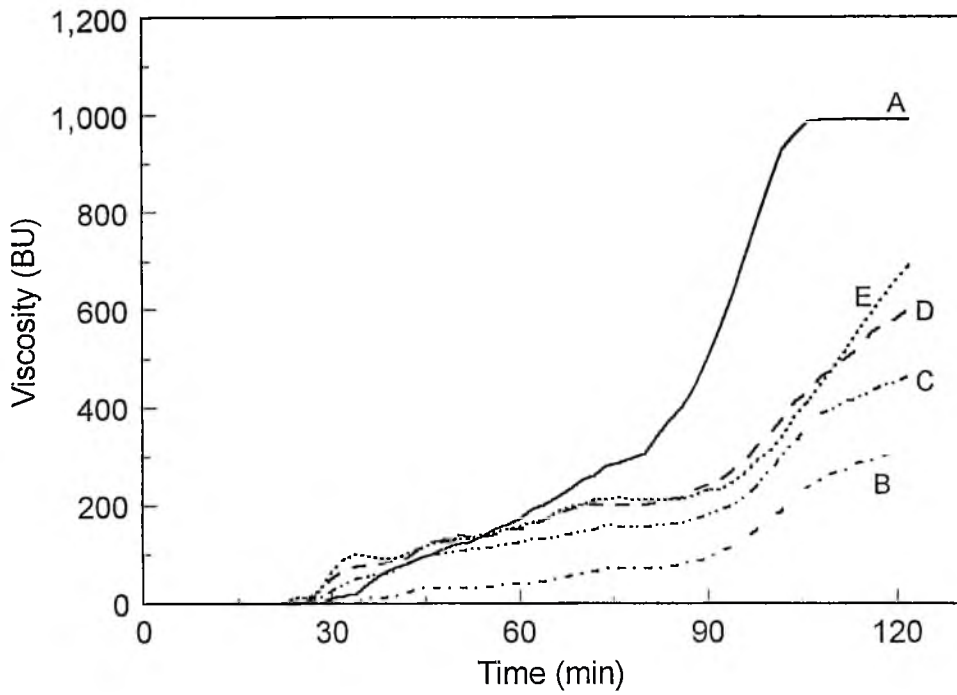


Figure 9 Amylograph Viscosity Characteristics of Nixtamalized Maize

A	Raw maize
B	0% Lime
C	0.33% Lime
D	0.5% Lime
E	1% Lime



Analysis of variance of the data indicated that cooking had a significant effect of the pasting temperature (Table 9). The increasing effect of cooking and lime concentration, on all the other critical points apart from pasting temperature, were not significant ($p > 0.05$).

Table 9: ANOVA Summary Table for Pasting Temperature of Nixtamalized Maize

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	4	50.335000	12.58375	9.476*
Cooking	1	26.281250	26.281250	19.791*
% Lime	3	24.053750	8.017917	6.038
Residual	3	3.983750	1.3279167	
Total correlation	7	54.318750		

*Significant, $p \leq 0.05$

4.1.7 Colour

The colour of alkaline cooked maize products is an important quality control parameter, which has a direct influence on the acceptability of the product. Colour development in lime treated products result from the lime used, hence the intensity of colour is closely related to the lime concentration. Even when tortillas are produced from white kernels, a high concentration of lime leads to a yellowish product. (Serna-Saldivar *et. al.*, 1990).

The L-values (i.e. lightness values) of the lime treated samples ranged between 75.24 – 83.88 for the maize cooked and steeped in 1% lime and raw maize respectively (L=100 -white). The samples which were cooked in lime before steeping, had lower L-values, and higher b-values (deeper yellow) and higher b-values (yellowness) than those which were steeped without cooking. This

observation may be due to the fact that, the process of cooking resulted in gelatinization of the maize starch allowing the cooked grains to imbibe the lime solution more readily than the uncooked grains.

The L-values of the alkaline treated maize generally decreased with increasing lime concentration. Similar work done by other researchers (Bazua *et al.*, 1979; Johnson *et al.*, 1980; Gomez *et al.*, 1987) showed that, the colour of dry nixtamalized maize flours ranges from white to dark yellow, depending on the alkali concentration, processing conditions and corn type. The samples with the darkest colour (lowest L-value) also had the highest pH (Appendix 1), confirming that the yellow colour resulted from the high amounts of lime absorbed and retained.

Analysis of variance of the results (L-values) showed that the effects of cooking and lime concentration significantly influenced the colour of the lime treated maize (Table 10). Multiple range analysis to determine the effect of cooking showed that, the colour of maize cooked in lime, was significantly different from that of the maize steeped in lime without cooking. Further multiple range analysis on the effect of lime concentration revealed that, the effect of treating maize with 1.0% lime, on the colour of the maize was significantly different from all the other samples treated with or without lime. This means that the effect of lime on the colour of alkaline treated maize, as observed is dependent on cooking.

Table 10: ANOVA Summary Table for Colour (L-values) of Nixtamalized Maize

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	4	48.112550	12.028138	5.545
Cooking	1	38.676013	38.676013	17.828*
% Lime	3	9.436538	3.145513	1.450
Residual	3	6.5081375	2.1693792	
Total correlation	7	54.620688		

*Significant, $p \leq 0.05$

4.2 EFFECT OF FERMENTATION ON THE CHARACTERISTICS OF NIXTAMALIZED MAIZE DOUGH

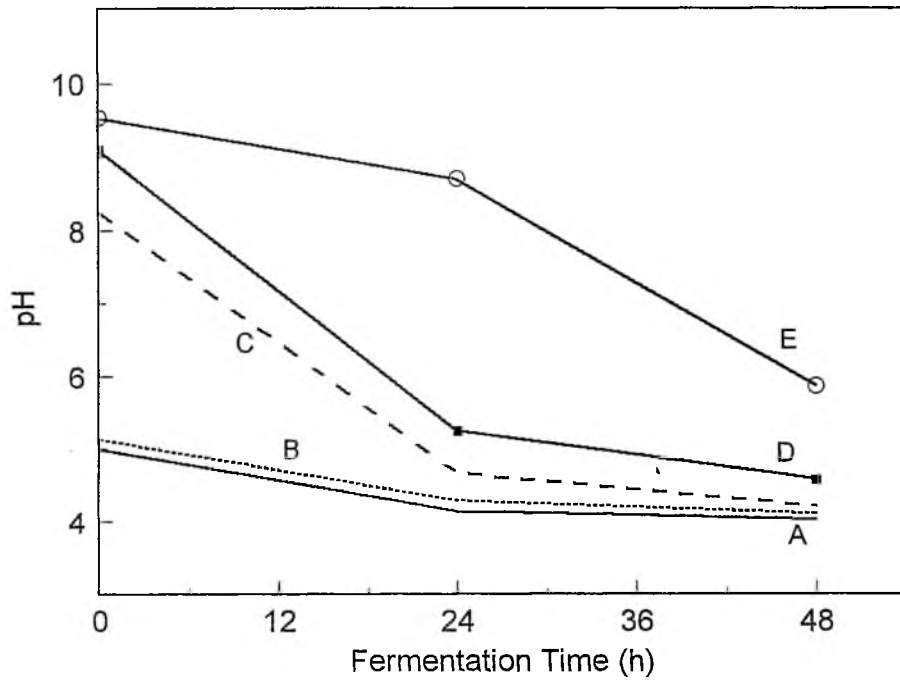
4.2.1 pH

The pH of all the samples (blends) made from a mixture of steeped (steeped in water as done traditionally) and nixtamalized maize, decreased with increasing fermentation time (Figure 10). The final pH, after 48 hours of fermentation of the samples made of 100:0, 75:25, 50:50 and 25:75 % steeped:nixtamalized maize, were close, ranging between 4.02 to 4.57. The final pH of the sample made from 100% nixtamalized maize was however 5.86. This could be due to the fact that, that sample had a higher initial pH than all the others and hence a higher final pH after fermentation. Work done by Sefa-Dedeh (1991) also showed that the pH of lime-treated maize decreased with increasing fermentation time. From his work a final pH of 4.13 was obtained after fermenting lime treated maize for 72 hours.

The decreases in pH observed during fermentation suggest the presence and activity of lactic acid bacteria during the spontaneous fermentation of the blends. Fermentation of maize in the production of traditional maize dough in Ghana has been reported to be largely lactic acid fermentation (Halm *et. al.*, 1993). The decrease in pH with increasing fermentation time is due to the hydrolysis of carbohydrates in the steeped:nixtamalized maize blends, by lactic acid bacteria, into sugars, alcohols and organic acids thereby producing a final product of low pH. The low pH obtained after fermentation of nixtamalized maize blends is important, due to the fact that most bacteria, including pathogenic organisms, do not survive in low pH environments. This is an added advantage to the use of

Figure 10. Effect of Fermentation Time on pH of Steeped: Nixtamalized Maize Blends

A	100:0 %
B	75:25 %
C	50:50 %
D	25:75 %
E	0:100 %



fermented nixtamalized maize since it can be used to provide safe foods from maize.

Analysis of variance of the data indicated that, the fermentation time and relative composition of the steeped:nixtamalized maize blends significantly affected their pH (Table 11).

Table 11: ANOVA Summary Table for pH of Fermented Nixtamalized Maize Dough

Source of Variation	DF	Sum of Squares	Mean Square	F-ratio
Main effects	6	48.093240	8.015540	7.181*
Masa level	4	26.724867	6.681217	5.985*
Fermentation time	2	21.368373	10.684187	9.571*
Residual	8	8.9302933	1.1162867	
Total correlation	14	57.023533		

*Significant, $p \leq 0.05$

Multiple range analysis on the effect of fermentation time, revealed that the pH of the steeped:nixtamalized maize blends when fermented for 24 and 48 hours was distinctly different from the pH of the unfermented blends (i.e. 0 hour fermentation). The effect of the blend composition on the pH determined by multiple range analysis showed that the effect of fermenting 100% nixtamalized maize on pH was significantly different from the resulting pH when 100% steeped maize, or a blend, made of some amount steeped, as well as nixtamalized maize, was fermented. Further, the effect of fermenting 100% steeped maize and a blend of 25:75 % steeped:nixtamalized maize on the pH was the same but significantly different from fermenting blends made up of 50:50 and 25:75 % steeped:nixtamalized maize.



4.2.2 Titratable Acidity

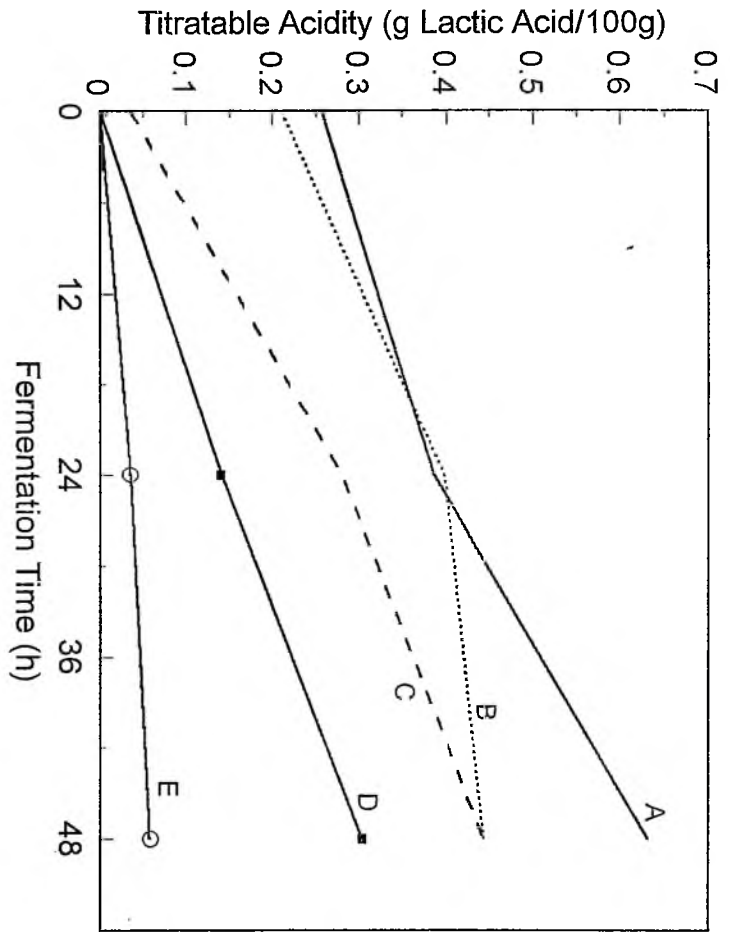
The titratable acidity of all the steeped:nixtamalized maize samples increased with increasing fermentation time (Figure 11). Apart from the 0:100, 25:75 % steeped:nixtamalized maize blends all the other blends were alkaline in nature at the beginning of fermentation and no acidity was detected in the blends made up of 25:75 and 0:100% steeped:nixtamalized maize as was expected, at 0 hour fermentation. After 48 hours of fermentation however, the titratable acidity of these samples increased from 0 to 0.303 and 0.058 gLA/100g respectively, giving evidence that the much desired souring process which goes on in traditional fermented maize dough, had taken place. Souring of maize dough during fermentation is an important and desirable quality attribute, in Ghana. The detection of acidity in fermented blends of steeped and nixtamalized maize dough therefore provides an added advantage in the acceptability of these blends.

Souring of dough has been linked to lactic acid fermentation during which lactic acid and other organic acids are produced (Plahar and Leung, 1982). Several researchers, including Nout *et al.* (1989), have reported that lactic acid fermentation exhibits antimicrobial effects on pathogenic microorganisms due to the presence of acid. Fermentation can therefore be applied to steeped:nixtamalized maize blends to produce safe, acceptable foods, which possess all the nutritional improvements nixtamalization imparts.

Apedo (1988) also reported that, titratable acidity of mixtures of alkalized and non-alkalized maize meals increased with fermentation time. Titratable acidity of the

Figure 11. Effect of Fermentation Time on the Titratable Acidity of Steeped: Nixtamalized Maize Blends

A	100:0 %
B	75:25 %
C	50:50 %
D	25:75 %
E	0:100 %



blends decreased with increasing level of nixtamalized maize in the blend (Figure 11). This observation is due to the fact that some of the acid produced will be used in neutralizing the alkali in the blends with nixtamalized maize, before the excess will be detected as acidity.

Analysis of variance of the data showed that the blend composition and the fermentation time had significant effects on the titratable acidity of the steeped: nixtamalized maize blends (Table 12). Multiple range analysis on the effect of fermentation time revealed that, each of the fermentation times (0, 24, 48 hours) had a distinctly different effect on the titratable acidity of the blends. Further analysis to determine the effect of the blend composition showed that, the effect of fermenting 100% steeped maize and a blend of 75:25 % steeped:nixtamalized maize, on titratable acidity was the same but significantly different from all the other blends. The effect the of composition of the blend on the titratable acidity of 0:100, 25:75 and 50:50% steeped: nixtamalized maize blends were also significantly different from each other. The results obtained show that the fermentation time and relative percentages of steeped and nixtamalized maize in the blend influences the titratable acidity.

Table 12: ANOVA Summary Table for Titratable Acidity of Fermented Nixtamalized Maize Dough

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	6	0.4843149	0.0807192	14.353*
Masa level	4	0.2949516	0.0737379	13.111*
Fermentation time	2	0.1893633	0.0946817	16.835*
Residual	8	0.0449920	0.0056240	
Total correlation	14	0.5293069		

*Significant, $p \leq 0.05$

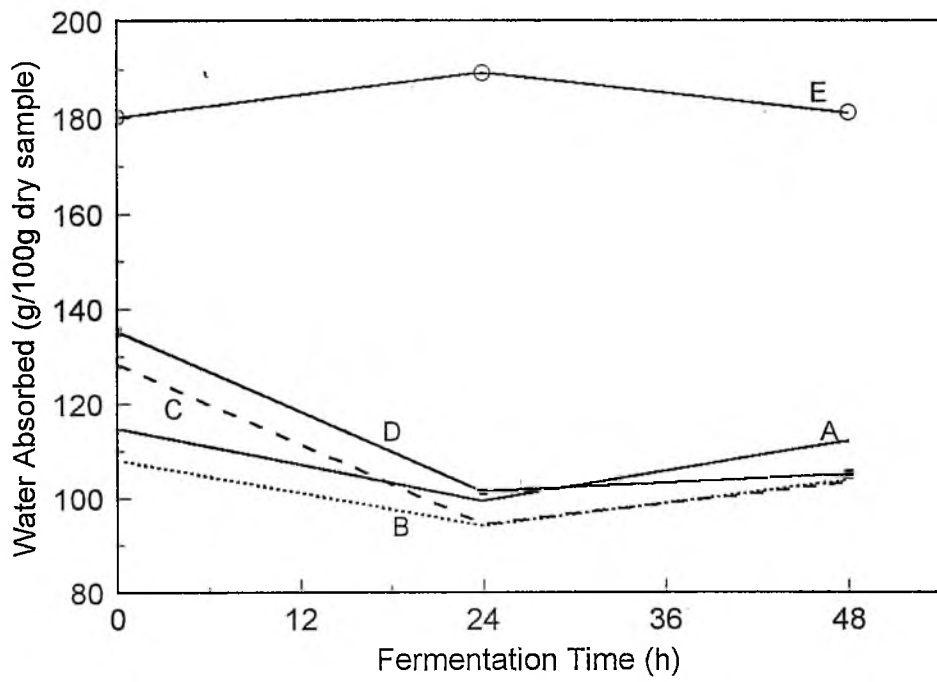
4.2.3 Water Absorption Capacity

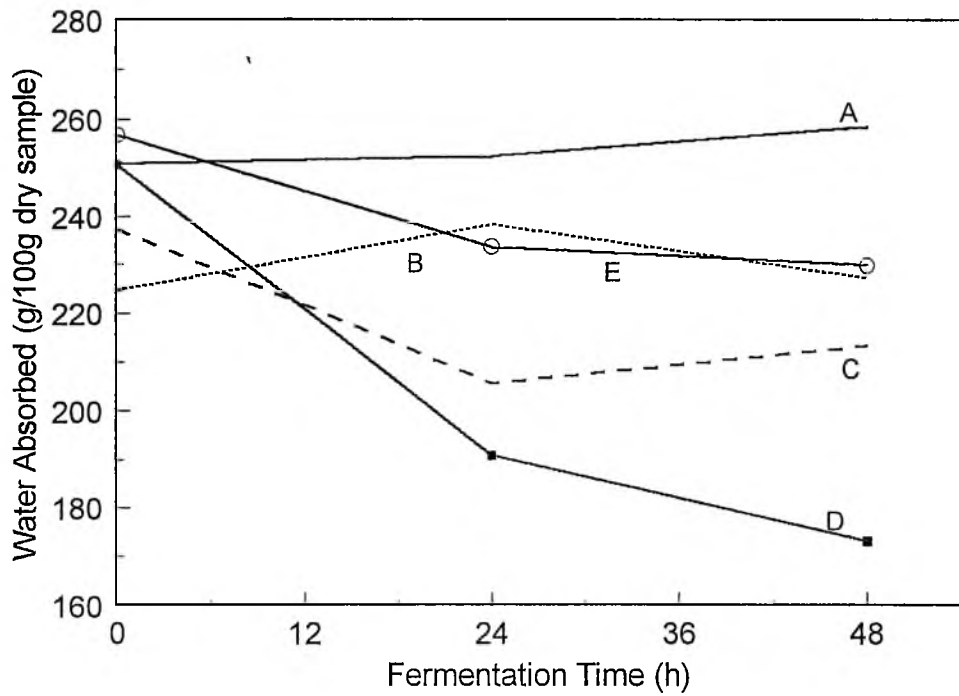
The water absorption capacity at room temperature (i.e. 25°C) of all the blends, except the 100% nixtamalized maize followed similar trends with increasing fermentation time. It decreased at 24 hours of fermentation and increased slightly at and 48 hours of fermentation (Figure 12). The water absorption capacity of the 100% nixtamalized maize on the other hand, increased slightly at 24 hours of fermentation and decreased to a value close to its initial water absorption capacity after 48 hours of fermentation.

Different trends in water absorption with fermentation time were also observed for water absorption capacity determined at 70°C. The water absorption capacity (at 70°C) for blends containing 50:50, 25:75 and 0:100%, steeped:nixtamalized maize generally decreased with increasing fermentation time while that of 100:0 and 75:25 % steeped:nixtamalized maize, increased with increasing fermentation time (Figure 12). The results obtained in this experiment suggest that fermentation of maize blends containing nixtamalized maize results in lowering of water absorption capacity and this effect seems to be produced by the presence and influence of lime. The water absorption capacity at 70°C of all the steeped:nixtamalized maize blends was higher than that at 25°C, throughout the fermentation period. This can be attributed to the hydration and swelling of food starch granules heated in excess water.

Figure 12 Effect of Fermentation Time on the Water Absorption Capacity at 25°C (I) and 70°C (II) of Steeped: Nixtamalized Maize Blends

A	100:0 %
B	75:25 %
C	50:50 %
D	25:75 %
E	0:100 %





Analysis of variance of the results showed that the fermentation time and relative composition of the blends had a significant effect on the water absorption capacity at 25 and 70°C (Table 13). Multiple range analysis to determine the effect of fermentation time on water absorption capacity showed that 24 and 48 hours of fermentation had the same effect on water absorption at 25 and 70°C, but these were significantly different from the water absorption capacity at 25 and 70°C of the unfermented (0 hour) blends. Multiple range analysis to determine the effect of the relative composition of the blends, revealed that apart from the sample containing 100% nixtamalized maize, the cold water absorption of all the other blends were similar.

Table 13: ANOVA Summary Table for Water Absorption Capacity (25°C) of Fermented Nixtamalized Maize Dough

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	6	14575.616	2429.2694	24.734*
Masa level	4	13761.705	3440.4262	35.029*
Fermentation time	2	813.911	406.9557	4.143
Residual	8	785.73335	98.216668	
Total correlation	14	15361.350		

*Significant, $p \leq 0.05$

Further analysis showed that the effect of fermenting 100% steeped and 100% nixtamalized maize on hot water absorption was significantly different from that of all the other blends which actually contained a definite amount of both steeped and nixtamalized maize. These results show that the actual presence of both steeped and nixtamalized maize result in comparable hot and cold water absorption capacities, irrespective of their relative amounts in the blends.

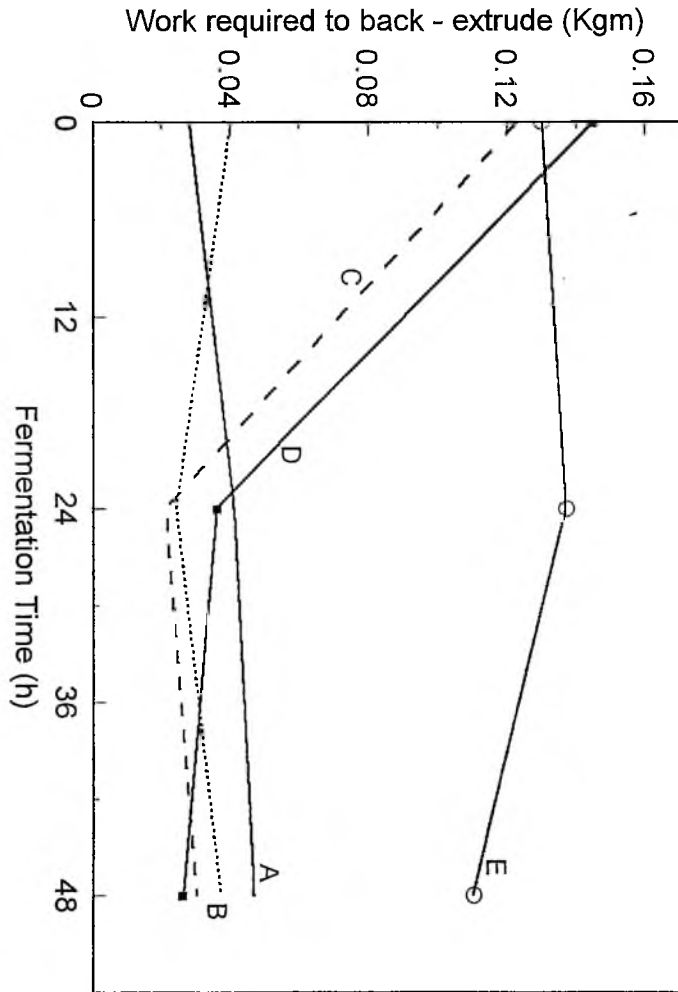
4.2.4 Texture

Nixtamalized maize texture is one of the important quality parameters used in the prediction of the quality of alkaline cooked products. A number of objective tests using various equipment, such as the mechanical stickiness device, described by Ramirez-Wong (1989), have been used for the evaluation of the texture (stickiness) of nixtamalized maize. In this experiment, texture of blends of steeped and nixtamalized maize was measured as work (Kgm) required to back-extrude a specific amount of cooked slurry of the blends that had been allowed to cool and set to room temperature (25°C). Apart from the 100:0 % steeped:nixtamalized maize, the work (Kgm) required to back-extrude the blends of steeped:nixtamalized maize generally decreased with increasing fermentation time (Figure 13). The 100:0 % steeped maize sample showed an increase in the work required to back-extrude the set sample, with increasing fermentation time, similar to its water absorption pattern at 70°C.

Though the stiffness of the cooked and set 100% steeped maize sample increased with increasing fermentation time, the work required for back-extrusion of the set slurry at the end of 48 hours of fermentation, was comparable to that of the blends containing 75:25, 50:50 and 25:75 % steeped:nixtamalized maize. The cooked and set slurry made from the 100% nixtamalized maize had the highest work values throughout the period of fermentation. This could be as a result of the degree of fermentation of this sample, being lower than the other samples. A high positive correlation ($R = 0.9396$; $p = 0.000$) was found to exist between the work required to back-extrude 90 ml of set fermented steeped:nixtamalized maize

Figure 13. Effect of Fermentation Time on the Texture of Steeped: Nixtamalized Maize Blends

A		100:0 %
B	-	75:25 %
C	-	50:50 %
D	-	25:75 %
E	-	0:100 %



slurries and the viscosity at 50°C - hold. These results show that the cooled paste viscosity and the texture of the blends follow the same trends. A high positive correlation ($R = 0.8481$; $p = 0.0001$) was also found between texture and water absorption capacity (25° C) of the blends. It may be said from these results that, the effect of fermentation and nixtamalization on the texture, water absorption capacity (25°C) and cooled paste viscosity (50°C- hold) of the blends, are similar.

Analysis of variance of the data indicated that the effect of blend composition and fermentation time on texture was not significant.

4.2.5 **Viscosity**

Viscosity is one of the important parameters measured in most starch-based foods due to its significant correlations with texture, degree of gelatinization, swelling power, etc. The viscosity of nixtamalized maize is an important quality index during the production of alkaline cooked products. Bedolla and Rooney (1984) concluded from their work on the characteristics of instant maize flours for tortilla and snack preparation that, amylograph peak viscosity was one of the objective tests which best predict the tortilla-making quality of dry nixtamalized maize flours.

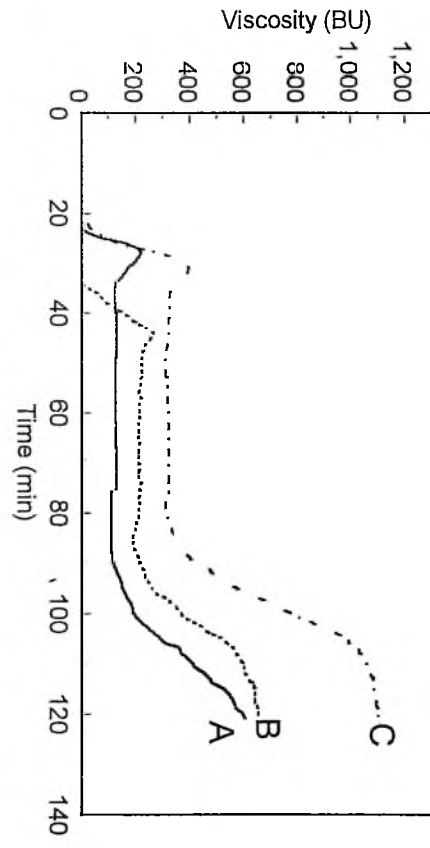
For the unfermented samples (0 hour fermentation), the general trend of viscosity at any given time along the amylogram showed that 100% nixtamalized maize had the highest viscosity followed by 25:75, 50:50, 75:25 and 100:0 % steeped: nixtamalized maize in that order (Figures 14 and 15). After fermentation for 24

hours and finally to 48 hours, the cooked paste viscosity of the 100% steeped maize and the sample containing 75% steeped and 25% nixtamalized maize, increased with increasing fermentation time (Figure 14). The samples which contained higher amounts of nixtamalized maize revealed an opposite trend. The cooked paste viscosities of the 50:50, 25:75, 0:100 % steeped:nixtamalized maize blends decreased with increasing fermentation time (Figures 14 and 15).

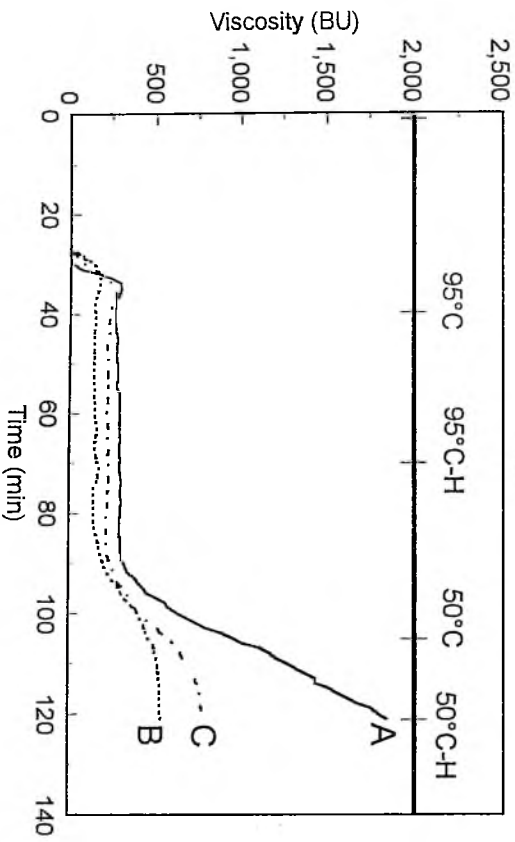
These results show that the addition of nixtamalized maize decreased the cooked paste viscosity of fermented maize dough. The viscosity reduction was more drastic in the cooled paste (viscosity at 50°C) which is the viscosity at which most gruels are eaten. As observed in the graphs, the cooked paste viscosity of 100% steeped maize increased with fermentation time. This observation is the cause of the low energy density problem of weaning foods made from fermented maize eg. koko which is common in Ghana. Akpapunam and Sefa-Dedeh (1995) reported from their work in addressing this problem, that, the addition of malted maize resulted in reduction in amylograph viscosity of maize gruels. The reduction in cooked paste viscosity when nixtamalized maize is fermented alone or with traditionally steeped maize, provides another means of producing safe, thin, energy dense fermented gruels to solve the low energy density problem of fermented weaning foods. Bryant and Hamaker (1997), concluded from their work on the effect of lime on gelatinization of corn flour starch, that the high pH of lime appears to promote starch hydroxyl group ionization thus creating opportunity for interaction between Ca^{2+} or CaOH^+ and starch molecules.

Figure 14. Amylograph Viscosity Characteristics of Steeped:Nixtamalized Maize Blends

A	0 hour fermentation
B	24 hours fermentation
C	48 hours fermentation
I	100:0 %
II	75:25 %
III	50:50 %



III



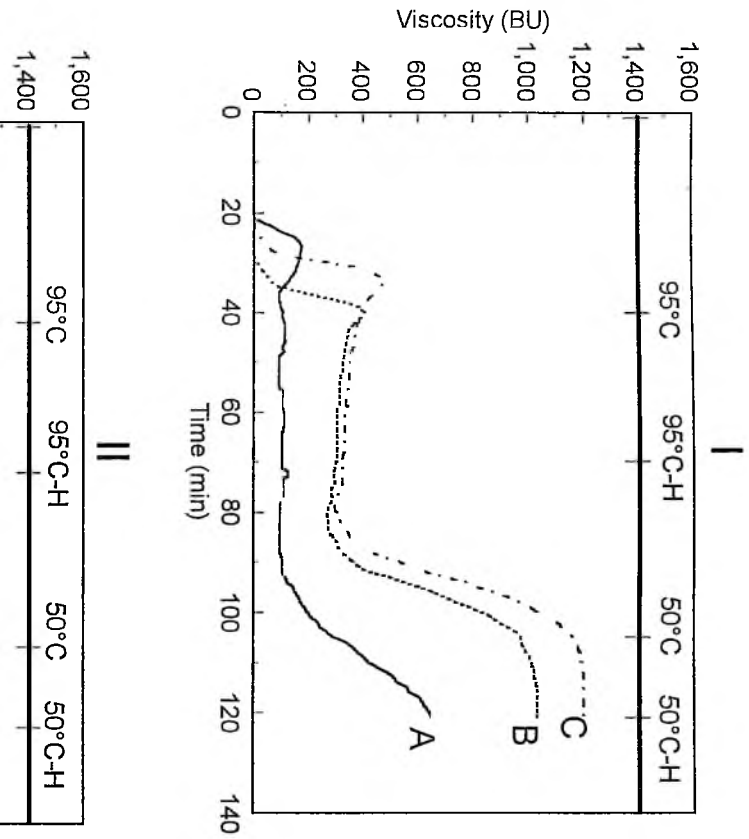
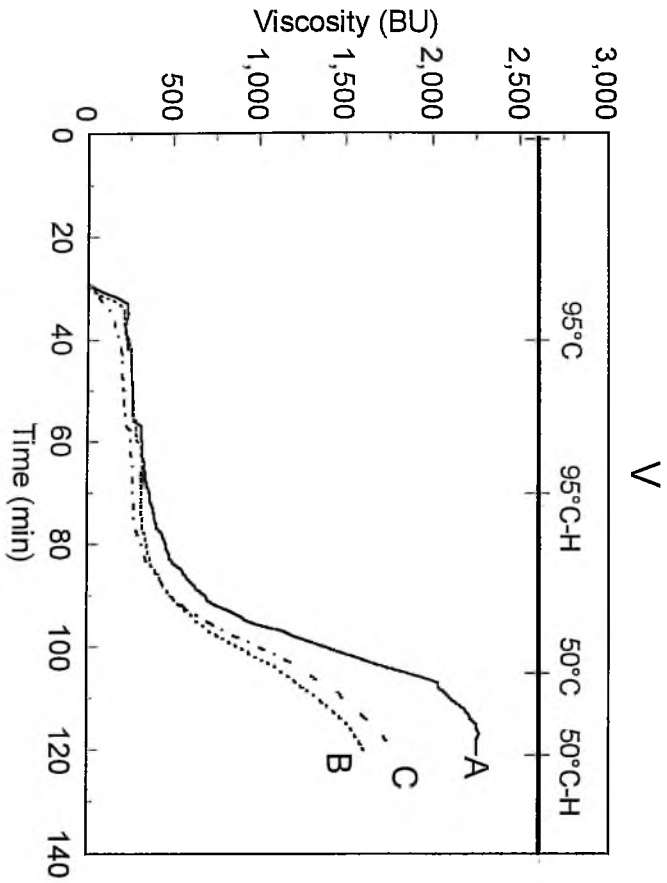
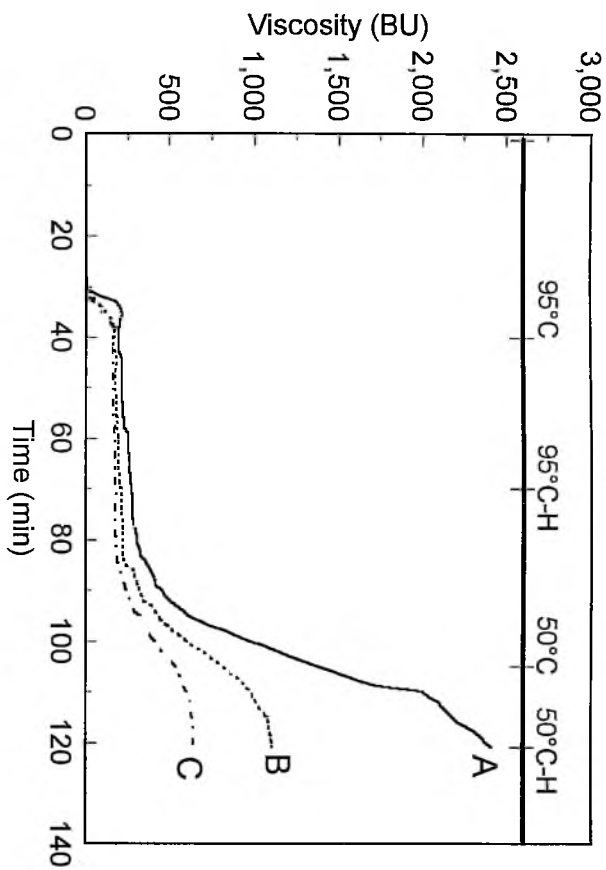


Figure 15. Amylograph Viscosity Characteristics of Steeped:Nixtamalized Maize Blends

A		0 hour fermentation
B		24 hours fermentation
C	-	48 hours fermentation
IV		25:75 %
V	-	0:100 %



IV



The viscosity reduction, which was more pronounced during the cooling period may be due to a saturation of the maize starch hydroxyl sites with Ca^{2+} and $\text{Ca}(\text{OH})^+$ ions increasing its stability and preventing the usual association of the starch molecules, during cooling, which causes high set-back viscosity.

The observed decrease in viscosity of nixtamalized maize samples has been documented in an earlier research on fermentation of nixtamalized maize dough by Sefa-Dedeh (1991). He also reported that the amylograph cooked paste viscosity of nixtamalized maize dough decreased during fermentation.

Though the viscosity of the 100% nixtamalized maize decreased with fermentation, its cooled paste viscosity after 48 hours of fermentation was higher than that of the 100% steeped maize at 48 hours of fermentation (Figures 14 and 15). The blend which contained 25:75 % steeped: nixtamalized maize on the other hand had a cooked paste viscosity of 650 BU, after 48 hours of fermentation, as against 1200 BU for the 100% steeped maize, which is the traditionally prepared maize dough, popularly used in the preparation of traditional foods including porridge for infants and children. From the results of this experiment therefore, it can be suggested that in order to obtain a high viscosity reduction, a blend of steeped: nixtamalized maize, made up of at least 25% nixtamalized maize, should be fermented, instead of 100% nixtamalized maize.

Positive correlations were found between viscosity at 50°C-hold and texture ($R=0.9396$; $P=0.0000$), and between viscosity at 50°C-hold and water absorption



capacity (25°C) ($R=0.7437$; $P=0.0015$). This gives indication that the combined effect of fermentation and of nixtamalization on the water absorption and rheological properties of the blends is similar.

Analysis of Variance of the results indicated that the composition of the blend had a significant effect on the peak viscosity of the samples (Table 14). Further analysis of the effect of blend composition on the peak viscosity, using multiple range tests revealed that, the effect of fermenting the blends which actually contained some amount of nixtamalized maize on cooked paste viscosity was comparable irrespective of the relative amounts of the components. These were however distinctly different from the effect of the blend composition on the viscosity of the 100% steeped maize.

Table 14: ANOVA Summary Table for Peak Viscosity of Fermented Nixtamalized Maize Dough

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	6	551653.33	91942.22	10.735*
Masa level	4	540440.00	135110.00	17.775*
Fermentation time	2	11213.33	5606.67	0.655
Residual	8	68520.00	8565.00	
Total correlation	14	620173.33		

*Significant, $p \leq 0.05$

Analysis of the data also showed that the blend composition and fermentation time had no significant effects on the viscosities at 95°C, 95°C - hold, 50°C and 50°C hold. However, fermentation time had a significant effect on the pasting temperature of the samples ($p \leq 0.05$).

4.2.6 Colour

One of the most important sensory quality attributes of a food is its colour. This is because no matter how nutritious, flavorful, or well textured a food, it is unlikely to be eaten unless it has the right colour. The process of alkaline cooking produces yellowish products (Serna-Saldivar, 1990), whose acceptability is dependent among other factors, on the intensity of the colour.

In the production of the steeped: nixtamalized maize blends, the samples with a higher percentage of nixtamalized maize had a deeper yellow colour (i.e. lower L-value) and higher b-values (yellowness) than those samples which had a smaller percentage of nixtamalized maize (Appendix 2).

For all the samples the L-values (lightness) increased while the b values (yellowness) decreased with increasing fermentation time. These results suggest that the process of fermentation breaks down the colour of the blends, producing whiter dough. The effect of fermentation on the colour intensity of the samples could be attributed to the changes in pH and titratable acidity resulting from the breakdown of the complex food substances into lower molecular weight carbohydrates including organic acids during fermentation. According to Serna-Saldivar *et al.* (1990), colour intensity of alkaline cooked products is closely related to carotenoid pigments, flavonoids and pH. Ghanaians are used to traditional maize dough, which generally has an off - white colour. The ability of the fermentation process to reduce the intensity of the yellow colour which is developed as a result of nixtamalization, is therefore an advantage for consumer acceptability of the steeped:nixtamalized maize blends.

Analysis of variance of the results indicated that both blend composition and fermentation time had significant effects on the L-values (lightness) of the steeped:nixtamalized maize samples (Table 15).

Table 15: ANOVA Summary Table for Colour (L-values) of Fermented Nixtamalized Maize Dough

Source of Variation	D.F.	Sum of Squares	Mean Square	F-ratio
Main effects	6	418.88101	69.813502	9.041*
Masa level	4	335.14244	83.785610	10.851*
Fermentation time	2	83.73857	41.869287	5.422*
Residual	8	61.774560	7.7218200	
Total correlation	14	480.65557		

*Significant, $p \leq 0.05$

Further analysis of the effect of blend composition on colour using multiple range tests showed that the effect on the colour of the 100% nixtamalized maize sample was significantly different from all the other blends. Apart from the 100% nixtamalized maize sample, the effect of the blend composition on the colour of all the other blends were comparable. The actual presence of steeped maize seemed to even out any differences in colour that might have resulted upon fermentation. It is possible that the steeped maize contained some lactic acid bacteria, which facilitated the fermentation of blends which had both steeped and nixtamalized maize. The effect of blend composition on 25:75 and 0:100% steeped: nixtamalized maize on the colour of the blends were also comparable.

4.3 EFFECT OF NIXTAMALIZATION, COWPEA FORTIFICATION AND MOISTURE CONTENT ON THE CHEMICAL AND FUNCTIONAL PROPERTIES OF MAIZE

The effects of cowpea level, moisture content and the concentration of lime on the chemical and functional properties of nixtamalized maize were studied, using the Response Surface Methodology. Data obtained were analyzed using Stepwise Multiple Regression procedures. Models were developed to relate lime concentration, cowpea level and nixtamalized maize moisture content on the nixtamalized maize characteristics.

Tables 16 and 17 show the coefficients of the variables in the models and their contribution to the model's variation. A test for the lack of fit and the R^2 values were used to judge the adequacy of the models. The R^2 of a model refers to the proportion of variation in the response attributed to the model rather than random error. For a good fit of a model, an R^2 of at least 80% has been suggested (Joglekar and May, 1987). Malcolmson *et al.* (1993), however, commented that 80% appeared to be excessive for a preliminary study and therefore recommended that an R^2 of 60% can also be used.

4.3.1 pH

In the production of dry nixtamalized maize flours, pH is one of the critical quality characteristics taken into consideration. This is because it has important implications on flavour, colour and shelf life of alkaline cooked products (Serna-Saldivar, 1990).

Table 16. Coefficients of Variables in the Model and their corresponding R^2

Table 17. Analysis of Variance for the Full Regression of the models

Table 16. Coefficients of Variables in the Model and their corresponding R²

Variable	pH	Titratable Acidity	Water Absorption Capacity		Texture	Protein	Peak Viscosity	Hot Paste Viscosity
			25°C	70°C				
Constant	-5.391332	0.351597	-2.451026E5	267.109968	0.030477	15.129036	78.154375	109.06869
X ₁	1.587566	-	-7.696937E4	-	-	-	2149.658936	316.455536
X ₂	0.32393	-	-	-	-	-	-	-
X ₃	-0.0033	0.005589	-2555.9307	-	0.014224	-0.507976	-	6.011767
X ₁ ²	-0.880189	-	-	-	-0.218681	-	-395.277968	-378.443382
X ₂ ²	-0.855868	-	75.244571	95.972564	-	-0.00177	-	-
X ₃ ²	-0.002804	-	-	-0.162333	-0.00024	-	-0.186898	-0.174726
X ₁ X ₂		-0.001721	-	-4.642991	0.002599	-	-28.790289	-
X ₁ X ₃		-	1.130906E5	0.052436	-	-	-59.412042	3.9944
X ₂ X ₃		-	-	6.978043	-0.000199	0.010358	0.142512	
X ₁ X ₂ X ₃		-	1753.384881	-	0.000039	0.000605	0.954824	
R ²	0.7071	0.4393	0.6506	0.6532	0.6003	0.8638	0.7057	0.6245

X₁ = Nixtamalized maize moisture content;X₂ = lime concentration;X₃ = Cowpea concentration**Table 17. Analysis of Variance for the Full Regression of the models**

Source of Variation	pH	Titratable acidity	Water Absorption Capacity at		Texture	Protein	Peak Viscosity	Hot Paste Viscosity
			25°C	70°C				
	F-ratio							
Model	10.1716	8.4417	8.0773	8.1585	5.7554	25.1047	7.5095	7.32047
Lack of Fit	2.7178	2.9363	6.2751*	1.4095	10.7483*	0.7802	3.7018	8.0811

* Significant at 95% confidence level

The process of fermentation employed during the preparation of traditional maize dough results in the production of acids, which leads to a reduction in pH of the product. The acids produced impart sour aromatic flavours, which are desirable and give a natural image to the product (Sefa-Dedeh and Plange, 1989).

The pH of the cowpea fortified nixtamalized maize dough, before fermentation, ranged between 5.11 and 9.29. After 48 hours of fermentation this range reduced to 4.04 - 4.78 respectively. An R^2 of 0.7197 was obtained for the model with a non-significant F-ratio value (2.718) for lack of fit (Tables 16 and 17). The lime concentration and its quadratic term were found to be the most important process variable influencing the pH. The response surface graph (Figure 16) showed that the pH of the fermented, cowpea fortified nixtamalized maize dough, increased with increasing lime concentration, at all moisture contents. At all levels of lime (0-1.0%), pH did not change appreciably with increasing moisture content. These same trends were observed at all levels of cowpea fortification (10-30%), an implication that increasing cowpea concentration did not significantly influence the pH of the samples. The results obtained show that the pH of nixtamalized maize is dependent on the concentration of lime used. Similar findings have been reported by Gomez *et al.*, 1987; Serna-Saldivar, 1990). The pH of nixtamalized maize increases during nixtamalization because lime is retained. An increase in lime concentration results in increased hydroxyl - ion concentration, which results in increased pH.

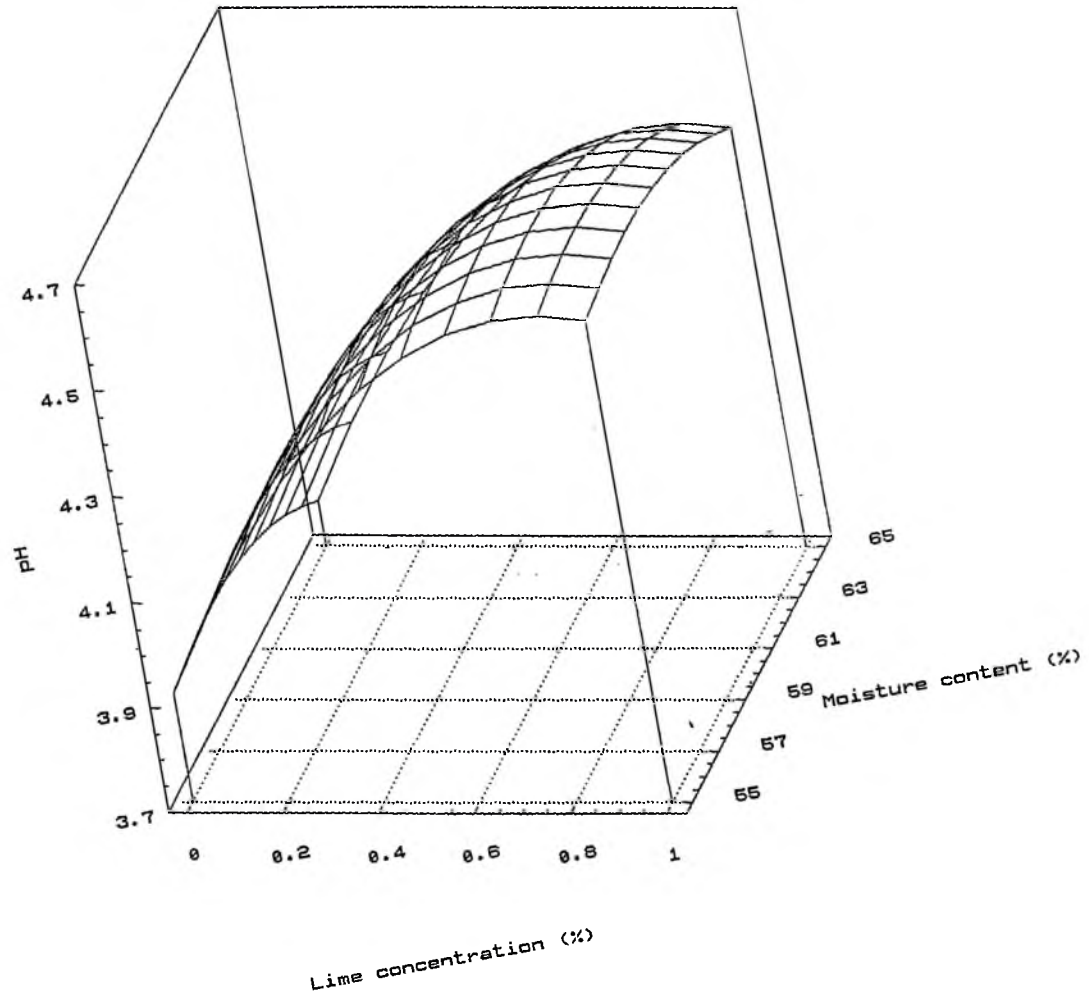
Figure 16 Response Surface Plots for pH of Cowpea Fortified Nixtamalized Maize, after 48 hours Fermentation at 10% Cowpea Level

Model

$$Y = -5.391332 + 1.587566X_1 + 0.32393X_2 - 0.880189X_1^2 - 0.002804X_2^2$$

X_1 Lime Concentration
 X_2 Moisture Content
 X_3 Cowpea Concentration

R^2 70.71%



4.3.2 Titratable Acidity

Titrateable acidity is a measure of the total acid (dissociated and undissociated) produced in the fermented product. It is an important quality attribute, which has a direct influence on the acceptability of the product. Sefa-Dedeh (1991) reported that operations such as soaking, size reduction and fermentation contribute to the development of flavour, colour, texture and other product qualities in cereal products.

The titrateable acidity values obtained for the fortified nixtamalized maize samples after 48 hours of fermentation ranged between 0.210 – 0.560 gLA/100g. The multiple regression model for predicting titrateable acidity after fermentation could explain only 43.93% of the variations in this index. The test of lack of fit however showed a non-significant F-ratio value of 2.936. The cowpea concentration was found to be the most important processing variable influencing the titrateable acidity after fermentation ($P= 0.0028$). There were also significant interactions between nixtamalized maize moisture content and lime concentration. From the response surface plots (Figure 17) obtained from the model, titrateable acidity decreased with increasing lime concentration at all moisture levels. This trend was the same at all levels of cowpea fortification (10, 20 & 30%). It was also observed that, at high levels of lime concentration, increasing moisture content led to a slight decrease in titrateable acidity. Though the trends from the graphs did not change with increasing cowpea level, the highest level of acidity obtained after fermentation increased as cowpea concentration was increased from 10 to 30%.

Figure 17 Response Surface Plots for Titratable Acidity of Cowpea Fortified Nixtamalized Maize after 48 hours Fermentation at 10% Cowpea Level

Model

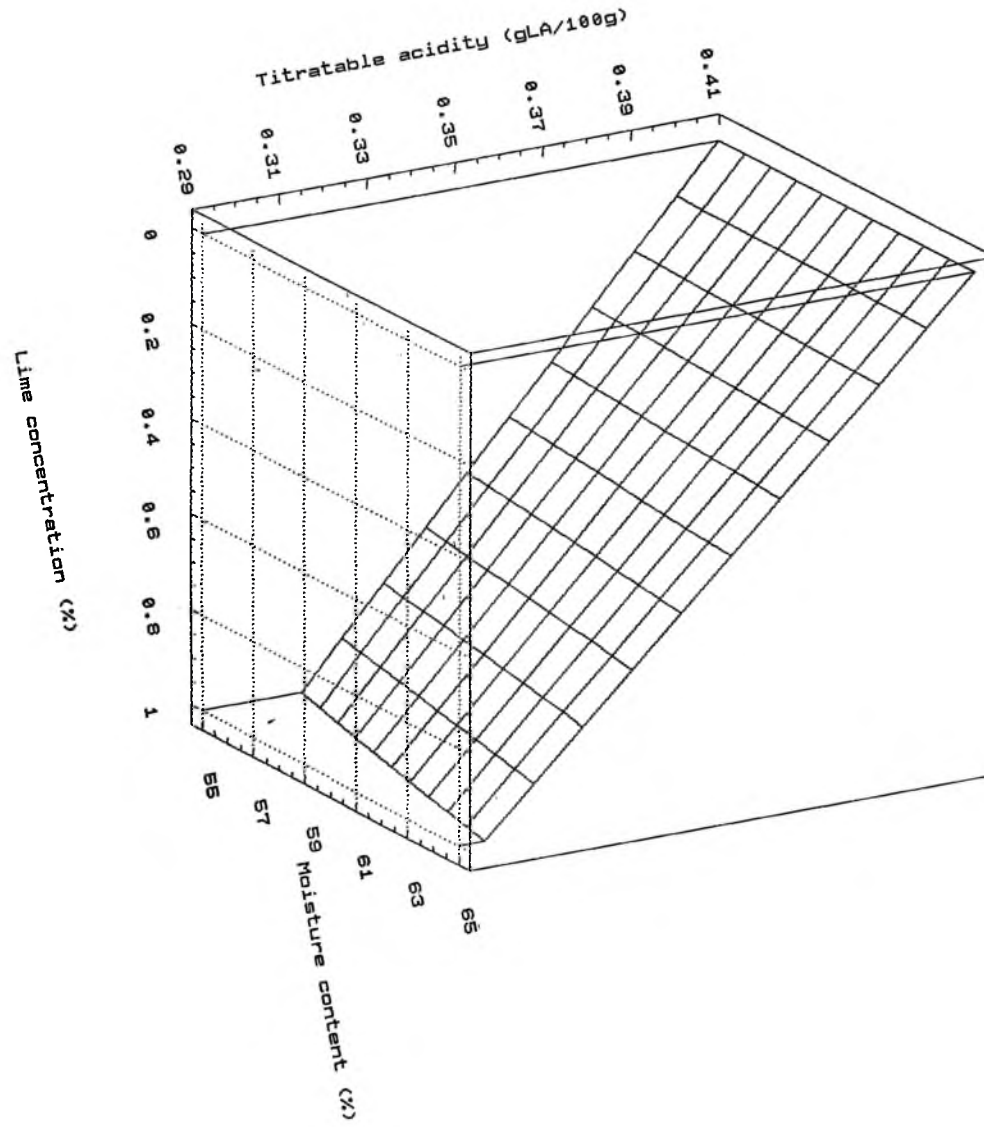
$$Y = 0.351597 + 0.005589X_3 - 0.001721X_1X_2$$

X_1 Lime Concentration

X_2 Moisture Content

X_3 Cowpea Concentration

R^2 43.93%



4.3.3 Water Absorption Capacity

Water absorption capacity which refers to the weight of water bound per gram of dry sample has been reported to be dependent on the availability of hydrophilic groups which bind water molecules and on the gel forming capacity of macromolecules (Gomez and Aguilera, 1983). In this experiment water absorption capacity of the fermented, cowpea fortified nixtamalized maize dough were determined at two temperatures, 25°C and 70°C.

The multiple regression model for predicting water absorption capacity at 25°C had an R^2 of 65.06% and a significant F-ratio of 6.2751 for lack of fit, suggesting that it might not be adequate in explaining the variations observed in this index. This model was therefore not used for any further analysis.

The model developed for predicting water absorption capacity at 70°C could explain 65.32% of the variations observed in this index and had a non-significant F-ratio value (1.410) for lack of fit (Tables 16 and 17). The variables which significantly influenced the water absorption capacity at 70°C were the quadratic term of lime concentration and interactions between moisture content and lime concentration. Significant interactions were also found between moisture content and cowpea concentration as well as between cowpea and lime concentrations. These results give indication that water absorption capacity (70°C) of the fermented fortified nixtamalized maize dough is dependent on the level of cowpea fortification, lime concentration and moisture content.

The response surface plots (Figure 18) showed different trends as the cowpea concentration was increased from 10% to 30%. At a fortification level of 10% cowpea, (Figure 18 A) the water absorption capacity at 70°C decreased with increasing lime concentration at all moisture levels. At low moisture content (55%), the water absorption leveled off at high lime concentration. There was also a slight decrease in water absorption with increasing moisture content at high lime concentration. At a cowpea concentration of 20% (Figure 18 B), water absorption decreased to a minimum and increased with increasing lime concentration at low moisture content. At high moisture content, where the degree of fermentation is expected to be high due to high water activity, the water absorbed decreased steadily to a minimum and leveled off as lime concentration was increased. At high lime concentration the water absorption decreased with increasing moisture content. These same trends were observed at cowpea fortification level of 30% (Figure 18 C), where they were even more pronounced. The results obtained show that the presence of lime in the dough during fermentation results in a depression in water absorption capacity, while the presence of cowpea produces an increase. At high moisture content when the degree of fermentation is expected to be high, the depression in water absorption as a result of lime is more prominent than at low moisture content. The increasing effect of cowpea on water absorption was also more prominent at low moisture content.

The initial decrease in the water absorption capacity at 70°C of the fortified nixtamalized maize dough could be due to a reduction in the available hydrophilic groups, which bind water.

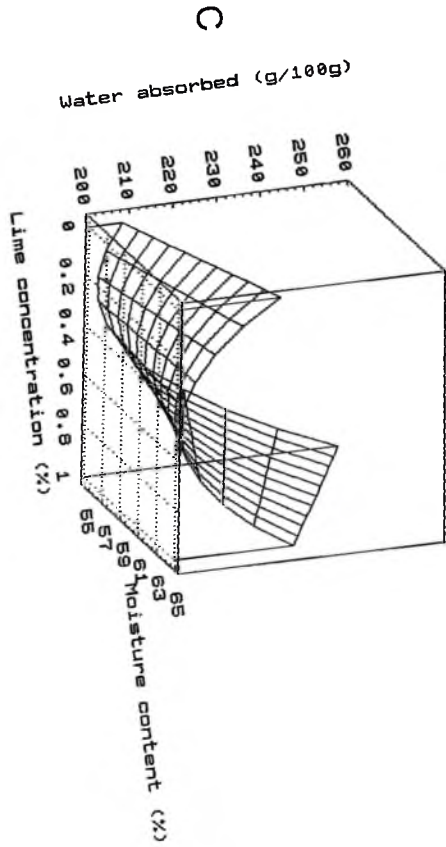
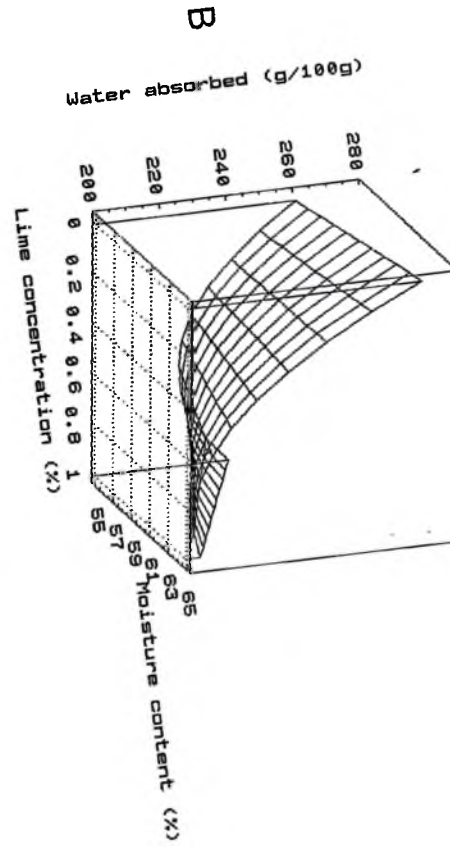
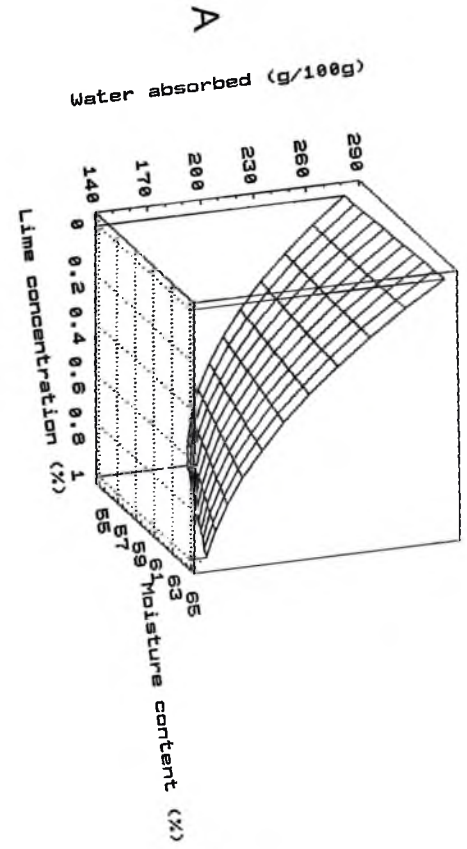
Figure 18 Response Surface Plots for Water Absorption Capacity at 70°C of Fermented, Cowpea Fortified Nixtamalized Maize at 10% Cowpea Level (A), 20% Cowpea Level (B) and 30% Cowpea Level (C)

Model

$$Y = 267.109968 + 95.972564X_1^2 + 0.162333X_3^2 + 4.642991X_1X_2 + 0.052436X_2X_3 + 6.978043X_1X_3$$

X_1 Lime Concentration
 X_2 Moisture Content
 X_3 Cowpea Concentration

R^2 65.32%



Oosten (1982) suggested that divalent cations bind tightly with starch molecules actually causing water holding capacity to decrease. The results obtained in this experiment suggest a saturation and anchorage of excess Ca^{2+} and $\text{Ca}(\text{OH})^+$ on the surface of the starch granules during fermentation.

The increases in water absorption capacity beyond lime concentrations of 0.4 and 0.2% for the 20% and 30% fermented, cowpea fortified nixtamalized maize dough, respectively, can be attributed to the increasing protein content as a result of increased cowpea levels, since proteins are the primary sites of water absorption (Sefa-Dedeh and Farkye, 1988). These results further confirm the findings of Sefa-Dedeh and Osei (1994) who reported that the addition of cowpea improved the water absorption capacity of fermented maize dough systems.

4.3.4 Texture

The texture of alkaline cooked products, among other quality characteristics has significant influence on their acceptability. Several methods have been tested for evaluating the texture of maize tortillas (Suhendro *et al.*, 1998; Ramirez-Wong *et al.*, 1996). Objective tests which measure the force and work required to roll a tortilla are highly correlated with subjective tests of rollability and flexibility (Rooney and Suhendro, 1999).

In this experiment, the texture of cooked and set slurries of the fermented, cowpea fortified nixtamalized maize flours were determined and expressed as work (Kgm) required to back-extrude 90 ml of cooked slurry which had been allowed to set and cool to room temperature (25°C).

The model developed had an R^2 of 60.03% and a significant F-ratio value of 10.7483 (Table 17). The model was therefore not used for any further analysis due to its inadequacy to explain the variations observed.

4.3.5 Protein Content

The model developed for predicting protein content, could explain 86.38% of the variation observed in this index and had an insignificant F-ratio value of 0.7802. The most important variable which influenced the protein content of the fermented, fortified nixtamalized maize, was cowpea concentration. Significant interactions were also found between cowpea and lime concentration as well as between cowpea concentration and moisture content.

Figure 19 illustrates the influence of the process variables on the protein content. At 10% cowpea fortification level (Figure 19A) the protein content increased slightly with increasing lime concentration at all levels of moisture content. Similar trends were observed when the nixtamalized maize was fortified with 20% and 30% cowpea. The increase in protein content in these samples however were higher than was observed for the fermented nixtamalized maize fortified with 10% cowpea. Alkaline cooking therefore improves the protein content of maize products and these improvements are even higher when nixtamalized maize products are fortified with cowpea.

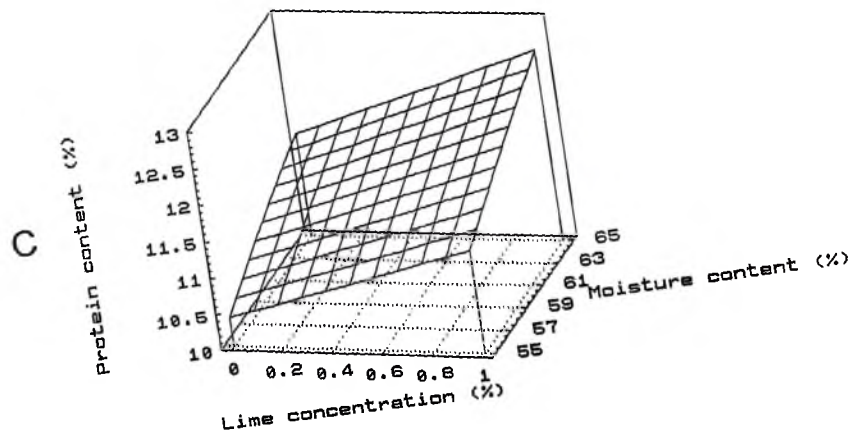
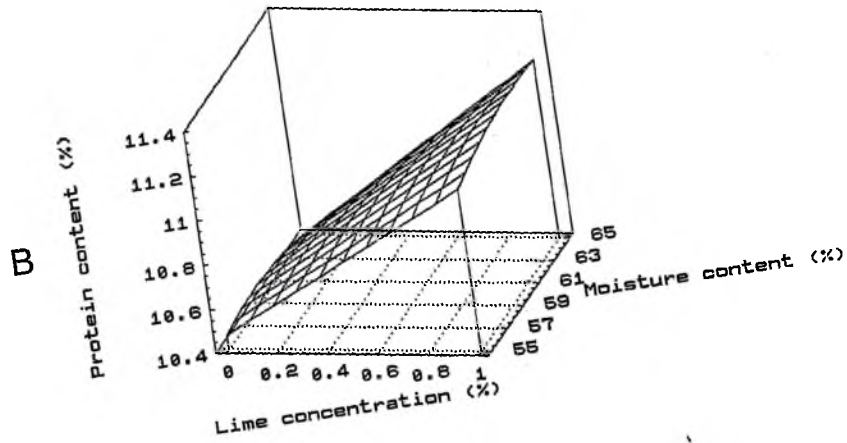
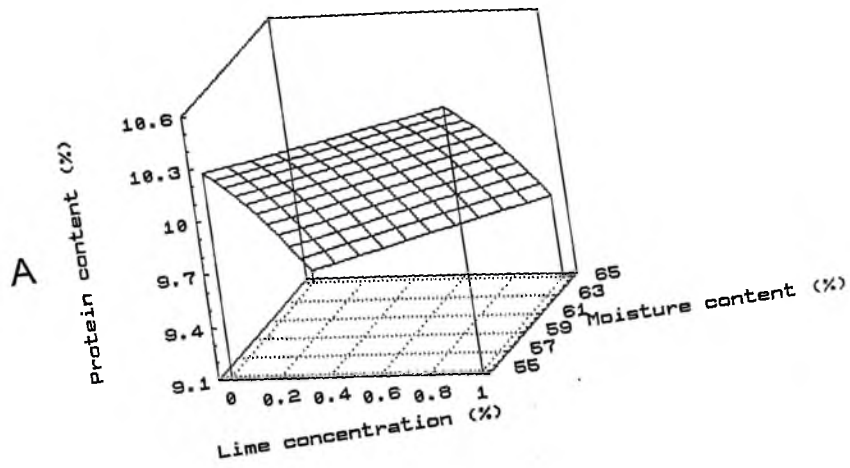
Figure 19 Response Surface Plots for Protein Content of Fermented, Cowpea Fortified Nixtamalized Maize at 10% Cowpea Level (A), 20% Cowpea Level (B) and 30% Cowpea Level (C)

Model

$$Y = 15.129036 - 0.507976X_3 - 0.00177X_2^2 - 0.001361X_3^2 + 0.010358X_2X_3 + 0.00060X_1X_2X_3$$

X_1 Lime Concentration
 X_2 Moisture Content
 X_3 Cowpea Concentration

R^2 86.38%



Several workers have also reported marked improvements in the protein content of cereals when they are fortified with legumes (Akpapunam and Sefa-Dedeh 1995; Osei and Sefa-Dedeh, 1993; Amegatse, 1995). These have been the basis of advocating cereal-legume complementation as a method of improving the protein content of traditional cereal foods.

Most researchers report a small increase in nitrogen content during nixtamalization which has been attributed to a concentration effect. Bressani *et al.* (1958) reported increased protein content in nixtamal and tortillas. Alkaline cooking and cowpea fortification, and fermentation can therefore be employed in the improvement of the nutritional quality of traditional foods made from maize.

4.3.6 Viscosity

Brabender viscoamylograms present useful information on the hot and cold paste viscosity characteristics of starch based foods. The viscosity patterns of the starch are determined primarily by the extent of swelling of the starch granules and the resistance of the swollen granules to dissolution by heat fragmentation and by shear.



The multiple regression model developed for predicting the peak viscosity of the fermented, cowpea fortified nixtamalized maize had an R^2 of 70.57% with a non-significant F-ratio value of 3.702 for lack of fit. This indicates the adequacy of the selected model in explaining the variations in the peak viscosity. The significant variable affecting the peak viscosity was the quadratic term of the lime

concentration. Significant interactions were found between the moisture content and the concentration of cowpea as well as between lime concentration and moisture content of the fermented, cowpea fortified nixtamalized maize.

The response surface plots (Figure 20), showed that the peak viscosity of the fermented nixtamalized maize samples at all moisture levels, increased to a maximum and decreased with increasing lime concentration. Work done by Sefa-Dedeh (1991) also revealed a drastic reduction in Brabender cooked paste viscosity when nixtamalized maize dough was fermented.

The peak viscosity of the fermented, nixtamalized maize dough fortified with 10 and 20% cowpea also decreased with increasing moisture content at high lime concentration. For these samples, the reverse was observed at low lime concentrations. These results further confirm the observation that fermentation, in the absence of lime leads to an increase in cooked paste viscosity, while fermentation of alkaline treated maize results in decreased cooked paste viscosity. At 30% cowpea level, however, the peak viscosity of the samples increased with increasing moisture content at all lime concentrations, which is a deviation from the trends in the preceding response surface plots.

Trends in the hot paste viscosity (viscosity at 95°C) of the fermented, cowpea-fortified nixtamalized maize were similar to that of the peak viscosity. From the response surface graphs (Figure 21), the hot paste viscosity increased to a maximum and decreased with increasing lime concentration at all lime levels. The

Figure 20 Response Surface Plots for Peak Viscosity of Fermented, Cowpea Fortified Nixtamalized Maize at 10% Cowpea Level (A), 20% Cowpea Level (B) and 30% Cowpea Level (C)

Model

$$Y = 78.154375 + 214.658936X_1 + 395.27768X_1^2 + 0.186898X_3^2 + 28.79028X_1X_2 + 59.412042X_1X_3 + 0.142512X_2X_3 + 0.954824X_1X_2X_3$$

X_1 Lime Concentration
 X_2 Moisture Content
 X_3 Cowpea Concentration

R^2 70.57%

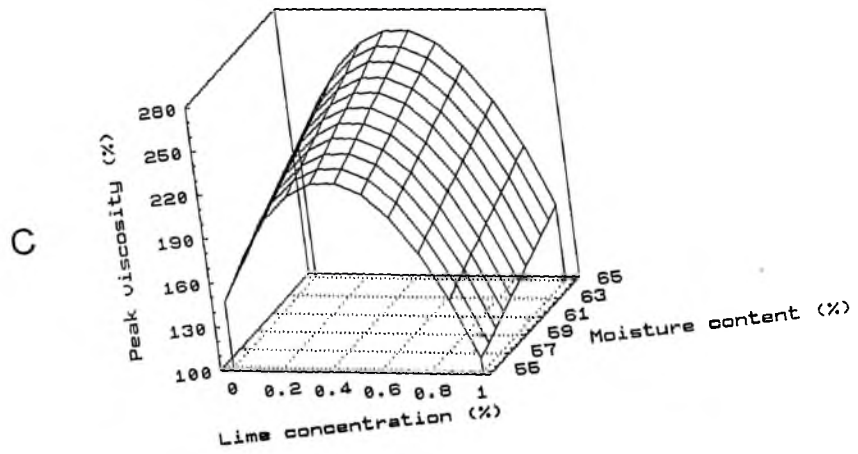
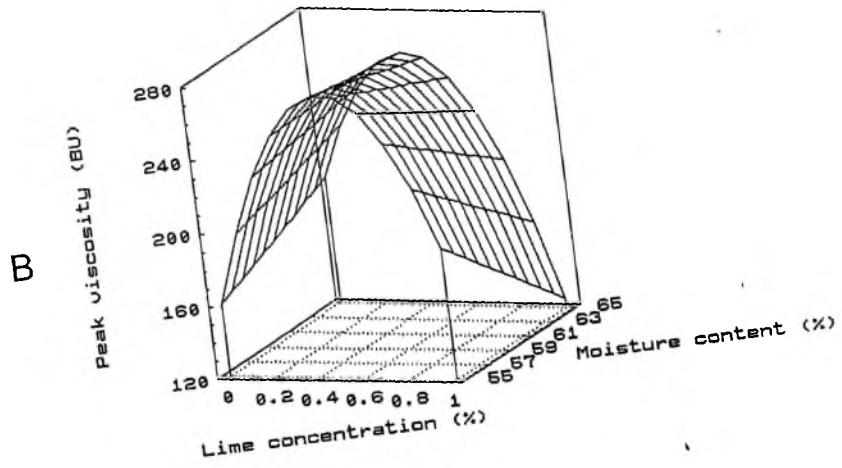
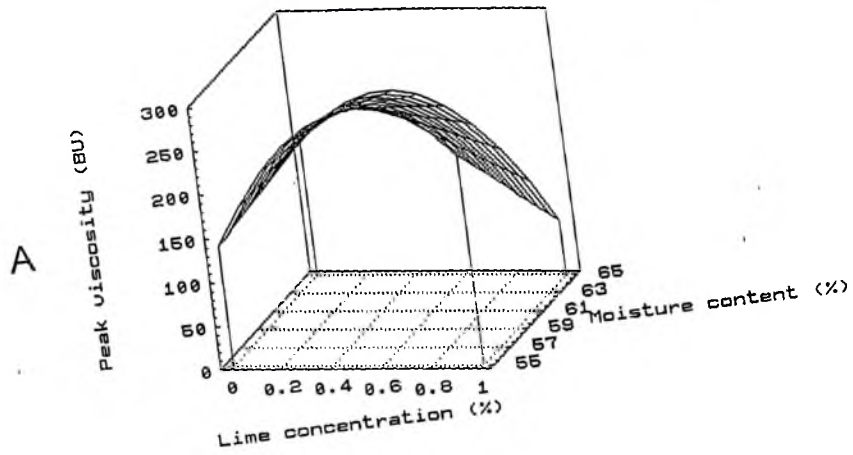


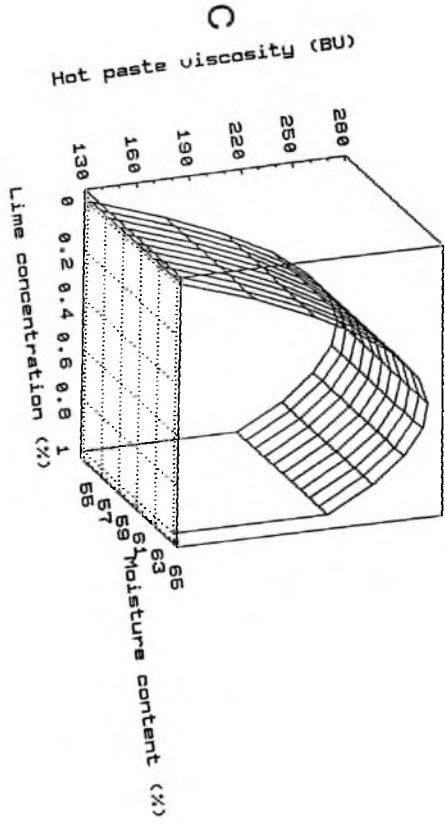
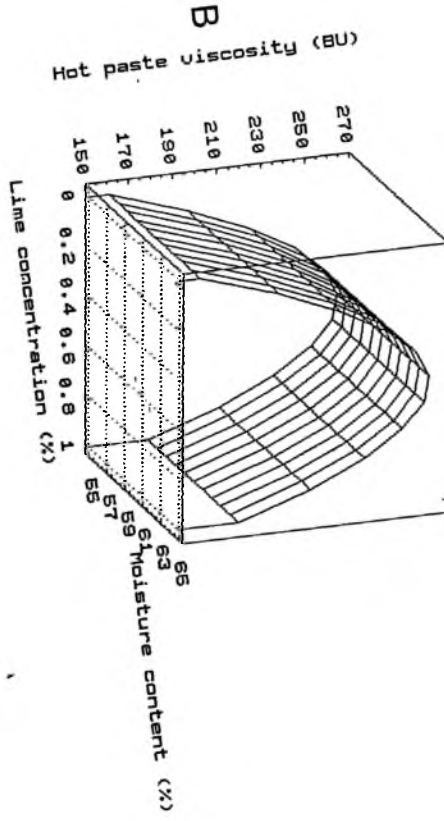
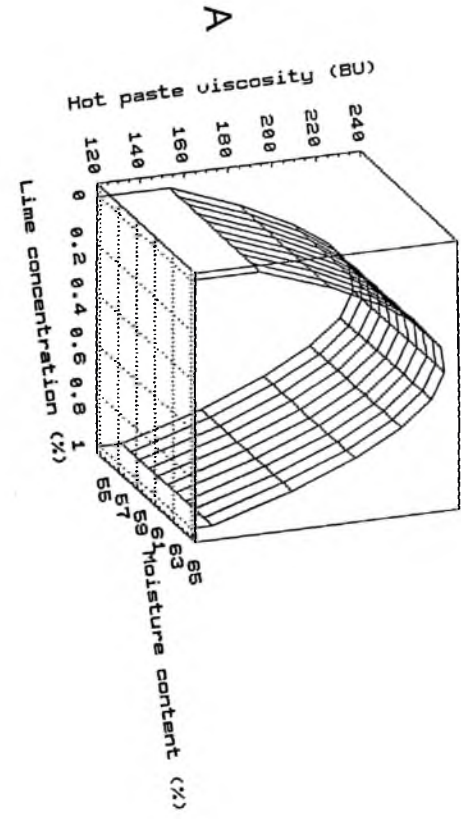
Figure 21 Response Surface Plots for Hot Paste Viscosity of Fermented Cowpea Fortified Nixtamalized Maize at 10% Cowpea Level (A), 20% Cowpea Level (B) and 30% Cowpea Level (C)

Model

$$Y = 109.06869 + 316.45563X_1 + 6.011767X_3 + 378.443382X_1^2 - 0.174726X_3^2 + 3.49944X_1X_3$$

X_1 Lime Concentration
 X_2 Moisture Content
 X_3 Cowpea Concentration

R^2 62.45%



maximum hot paste viscosity attained were similar and did not change significantly with increasing cowpea fortification level. The model developed for predicting the hot paste viscosity could explain 62.45% of the variations in the index. The important variables which influenced the hot paste viscosity of the samples were cowpea level and the quadratic term of the lime concentration.

The model developed for predicting the cooled paste viscosity (viscosity at 50°C), could explain only 27.71% of the variations in this index though it had a non-significant F-ratio of 0.050 for lack of fit. This means that the selected model may not be adequate in explaining the variations in this index.

The reduction in cooked paste viscosity of nixtamalized maize dough during fermentation provides another means of solving the low energy density problem of weaning foods made from fermented maize dough. The combination of nixtamalization, fermentation and cowpea fortification can be used to advantage in the formulation of high energy protein foods.

4.4 MICROBIAL PROFILE OF NIXTAMALIZED MAIZE

4.4.1 Acid Production During Steeping and Fermentation of Nixtamalized Maize and Dough

No decreases in pH were observed in nixtamalized maize samples during steeping (i.e. samples boiled and steeped in 0.5% and 1% lime) as occurred in the control sample which was steeped in water (Table 18). Decreases in pH during the steeping of maize has been reported by Halm *et al.* (1993) and is attributed to the onset of fermentation due to the proliferation of lactic acid bacteria. Thus the boiling and steeping of maize in lime during nixtamalization resulted in the destruction of the microflora responsible for initiating fermentation. In the control sample, the pH decreased from about 6.7 to 4.6 within 24 hours of steeping.

Table 18. pH of the Steep Water and Nixtamalized Maize Dough During Fermentation

SAMPLE	STEEP WATER		DOUGH			
	Steeping Time (h)		Fermentation Time (h)			
	0	24	0	24	48	72
Control	6.68	4.63	4.98	3.72	3.62	3.54
0.5% Lime	11.67	11.52	8.04	4.35	3.87	3.73
1% Lime	11.81	11.95	9.74	5.04	4.32	4.10

During the dough fermentation, the pH of both the control sample and doughs prepared from both types of nixtamalized maize decreased to levels of 4.0 or less in 72 hours showing that fermentation had occurred in all samples. Thus during milling and kneading of dough, the otherwise fairly 'sterile' nixtamalized maize were sufficiently inoculated with the appropriate bacterial species to initiate

fermentation. Furthermore, the bacterial species were able to grow at the initial high pHs of the nixtamalized doughs, 8.04 and 9.7 to cause a reduction to pHs of 3.73 and 4.1, with final acidity levels of 0.285 and 0.385 gLA/100g, for the 0.5% and 1% lime treated doughs respectively (Figures 22 and 23).

Though the final pH obtained after 72 hours of fermentation of the control sample was lower than that of the nixtamalized maize dough, the difference in pH between 0 and 72 hours of fermentation was higher in the nixtamalized maize dough. This could be explained by the fact that in the higher alkaline medium, growth of lactic acid bacteria would be slower and also more acid would be required to neutralize the alkali present before a rise in acidity and lowering of pH could be observed.

4.4.2 Microbial Population of Fermenting Dough

The population of lactic acid bacteria were enumerated as Gram positive and Catalase negative rods and cocci which grew anaerobically on MRS as shown in Table 18.

No growth was observed on MRS plates from maize samples boiled and steeped in alkali either before or after 14 hours of steeping. It is possible that the heat applied in the boiling process destroyed all resident lactic acid bacteria present on the maize and hence the lack of growth during steeping. Counts of 10^2 cfu/g were observed for the control (non-nixtamalized) maize at the start of steeping. In the control sample, fermentation of the maize was also initiated during steeping and a final count in the order of 10^7 cfu/g was obtained for the lactic acid bacteria at the

Figure 22 Effect of Fermentation on the pH of Nixtamalized Maize Dough

- A Traditional Maize dough
- B 0.5% Lime
- C 1% Lime

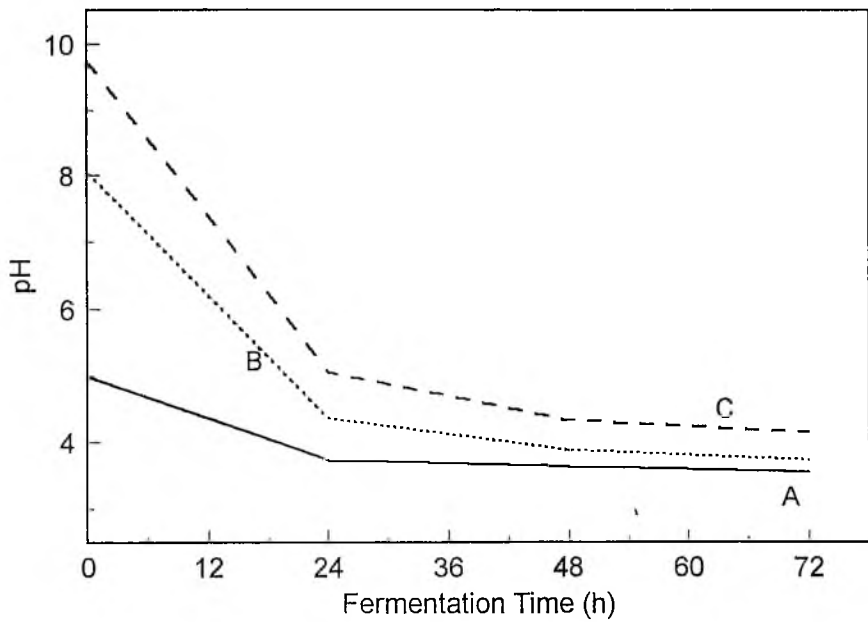
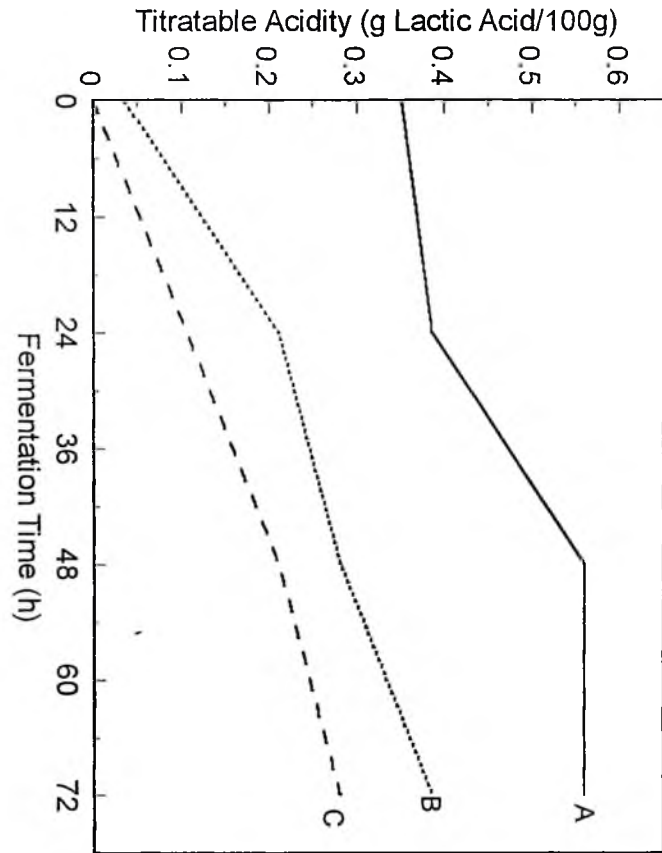


Figure 23 Effect of fermentation on Titratable Acidity of Nixtamalized Maize Dough

- | | |
|---|-------------------------|
| A | Traditional Maize dough |
| B | 0.5% Lime |
| C | 1% Lime |



end of steeping. No such fermentation occurred in the nixtamalized maize during steeping.

Immediately after dough preparation, the population of lactic acid bacteria in the dough made from 0.5% and 1% lime treated samples were about the same, 10^6 cfu/g and were ten fold lower than the population in the control sample. Since these samples after steeping did not produce growth on MRS agar, it is possible that substantial numbers of lactic acid bacteria were introduced into the samples from the utensils used as well as the process of milling and kneading. Even though both the 0.5% and 1% lime treated doughs had the same level of lactic acid bacteria at the start of fermentation, proliferation of the lactic acid bacteria in the 0.5% lime treated dough was faster than in the 1% lime treated dough (Table 19) indicating that growth of the lactic acid bacteria was slower at the higher pH.

Table 19. Population of Lactic Acid Bacteria* in cfu/g

SAMPLE	STEEP WATER		DOUGH			
	Steeping Time (h)		Fermentation Time (h)			
	0	24	0	24	48	72
Control	5.2×10^2	4.5×10^7	3.6×10^7	4.1×10^9	1.3×10^9	1.6×10^9
0.5% Lime	0.0	0.0	1.3×10^6	4.8×10^9	1.6×10^{10}	2.3×10^9
1% Lime	0.0	0.0	1.2×10^6	2.1×10^{10}	3.9×10^9	1.8×10^9

* Enumerated on MRS Agar, representing, Gram-positive, Catalase-negative rods and cocci.

The final counts of lactic acid bacteria attained in both types of nixtamalized dough as well as the control sample were the same, 10^9 cfu/g, though the pHs and acidities attained in all three samples were different. Cumulatively, the same amounts of acid might have been produced in all doughs since they had

comparable bacterial numbers. In such a situation the differences in final pH of the dough could be due to the different amounts of acid which would be required to neutralize the alkaline doughs since acidity would be expressed only in excess of neutralization.

Results of the enumeration of aerobic mesophiles on Plate Count Agar (PCA) show that a few bacteria were able to withstand the process of nixtamalization (Table 20). These counts were very low and also the surviving aerobic mesophiles did not appear to have been able to multiply significantly during steeping in lime. It is suspected that bacteria which were able to survive nixtamalization were spore formers but no further work was carried out on these colonies.

Table 20. Population of Aerobic Mesophiles* in cfu/g

SAMPLE	STEEP WATER		DOUGH			
	Steeping Time (h)		Fermentation Time (h)			
	0	24	0	24	48	72
Control	4.0×10^4	8.7×10^7	7.9×10^7	5.0×10^9	1.2×10^9	1.4×10^9
0.5% Lime	11	2.0×10^3	1.8×10^6	5.4×10^9	1.6×10^{10}	2.4×10^9
1% Lime	1.2×10^2	6.3×10^2	8.7×10^5	2.5×10^9	2.2×10^9	2.4×10^9

* Enumerated on Plate Count Agar, including both Gram-positive and Gram-negative bacteria

Increases in the population of aerobic mesophiles were observed during the fermentation of both the nixtamalized and non-nixtamalized doughs. Examination of their colony and cell morphologies showed that substantial proportions of these were similar to the lactic acid bacteria enumerated on MRS. Lactic acid bacteria are often micro aerophilic and able to grow on PCA.

Yeasts and molds were not able to survive the process of nixtamalization as seen from Table 21. However as in the case of the lactic acid bacteria, substantial contamination of the nixtamalized maize with yeasts and molds occurred during the subsequent operations of milling and kneading before the dough fermentation. Thus, where as no growths were obtained on Malt Agar plates of the 0.5% and 1% nixtamalized maize during steeping, counts of about 10^5 cfu/g were obtained in the nixtamalized doughs at the start of dough fermentation.

Table 21. Population of Yeasts* and Molds* in cfu/g

SAMPLE	STEEP WATER		DOUGH			
	Steeping Time (h)		Fermentation Time (h)			
	0	24	0	24	48	72
Control	9.0×10^3	1.7×10^7	4.6×10^5	3.2×10^6	2.0×10^7	8.0×10^7
0.5% Lime	0.0	0.0	1.7×10^5	4.4×10^6	1.5×10^5	5.0×10^5
1% Lime	3.0	0.0	6.0×10^4	5.0×10^4	1.4×10^5	1.7×10^5

* Enumerated on Malt Extract Agar, supplemented with Chloramphenicol and Chlor-tetracycline.

In the control (non-nixtamalized) dough a hundred fold increase in yeast counts were observed during 72 hours of fermentation, however in the nixtamalized doughs the yeasts counts remained constant throughout the period of fermentation. Yeasts are known to produce aroma compounds in maize dough during fermentation. The results obtained indicate that fermented nixtamalized maize develops the sour aromatic flavour of fermented maize dough and hence will be acceptable to consumers of traditional fermented maize dough.

4.4.3 **Characterization and identification of the species of the dominant lactic acid bacteria**

Since lactic acid bacteria have been reported by several workers (Halm *et al.*, 1993) to be the main bacteria responsible for most of the biochemical changes which, occur during the fermentation of maize, characterization and identification of microbial species in the present work was limited to the lactic acid bacteria. Yeasts are considered by Jespersen *et al.* (1994) to contribute to the production of aroma compounds in maize during fermentation, but in the present work no increases were observed in yeasts numbers during the fermentation of the nixtamalized doughs.

Examination of the cell morphology of the final lactic acid bacteria population of both the 0.5 and 1% lime treated doughs showed five groups of lactic acid bacteria. The first group consisted of rods occurring in pairs and chains of varying lengths. A lot of the pairs were joined at an angle with light shimmering around them as seen under the phase contrast microscope. The second group consisted of short rods in singles, pairs and chains of varying lengths. The third group were made up of long rods with few occurring in singles and a lot in pairs and chains of varying lengths. All the first three groups consisting of Gram positive, Catalase negative rods were tentatively identified as *Lactobacillus* species. The fourth group were cocci which occurred in singles, pairs and chains of varying lengths. The fifth and last group were cocci occurring mostly in pairs and fours with a few as single or aggregate cells and were tentatively identified as *Pediococcus* species.

The dominant strain of *Lactobacillus* present in the fermenting nixtamalized maize (0.5 and 1% lime) utilized L-Arabinose, Ribose, Galactose, D-Glucose, D-Fructose, D-Mannose, Mannitol, Sorbitol, D-Mannoside, N Acetyl glucosamine, Amygdaline, Arbutine, Salicine, Cellobiose, Maltose, Lactose, Melibiose, Saccharose, Trehalose, Melezitose, D-Raffinose, β -Gentiobiose, D-Turanose, and Gluconate and was identified as *Lactobacillus plantarum* (Tables 22 and 23). From the nixtamalized maize prepared with 0.5% lime, a total of 26 cultures isolated from the dough were identified. Fourteen (14) isolates representing 54% were *Lactobacillus plantarum*.

The second strain of *Lactobacillus spp.* found in relatively high amounts in the fermenting nixtamalized maize utilized, L-Arabinose, D-Xylose, Galactose, D-Glucose, D-Fructose, Maltose, Melibiose, Saccharose and D-Raffinose (Tables 22 and 23). This was identified to be *Lactobacillus fermentum*, and was found only in the nixtamalized maize prepared from 0.5% lime. Four (4) isolates of these, representing 15% of the total number of cultures were found.

Lactobacillus cellobiosus was the third of lactic acid bacteria identified in the nixtamalized maize produced with 0.5% lime. It utilized L-Arabinose, Ribose, D-Xylose, Galactose, D-Glucose, D-Fructose, D-Mannose, Salicine, Cellobiose, Maltose, Lactose, Melibiose, Saccharose, Trehalose, D-Raffinose, and β -Gentiobiose and was identified as *Lactobacillus cellobiosus* (Tables 22 and 23). Two (2) isolates of this strain, representing 8% were found. The fourth group were Gram positive Catalase negative cocci which represented 4% of the cultures

Table 22. Pattern of Carbohydrate Utilization of the Dominant Lactic Acid Bacteria from Fermented Nixtamalized Maize

Carbohydrate	<i>Lactobacillus plantarum</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus cellobiosus</i>
Glycerol	0	0	0
Erythritol	0	0	0
D-Arabinose	0	0	0
L-Arabinose	100	100	100
Ribose	100	40	100
D-Xylose	0	40	100
L-Xylose	0	0	0
Adonitol	0	0	0
β -Methyl-Xyloside	0	0	0
Galactose	100	80	80
D-Glucose	100	100	100
D-Fructose	100	60	60
D-Mannose	100	20	100
L-Sorbose	0	0	0
Rhamnose	0	0	0
Dulcitol	0	0	0
Inositol	0	0	0
Mannitol	100	0	0
Sorbitol	100	0	0
α -Methyl-D-Mannoside	100	0	0
α -Methyl-D-Glucoside	0	0	20
N-Acetyl Glucosamine	80	0	0
Amygdaline	80	0	0
Arbutine	80	0	0
Esculine	0	0	0

Table 23. Pattern of Carbohydrate Utilisation of the Dominant Lactic Acid Bacteria from Fermented Nixtamalized Maize

Carbohydrate	<i>Lactobacillus plantarum</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus cellobiosus</i>
Salicine	80	20	80
Cellobiose	100	40	100
Maltose	100	100	100
Lactose	100	0	60
Melibiose	100	100	100
Saccharose	100	100	100
Trehalose	100	0	100
Inuline	0	0	0
Melezitose	100	0	0
D-Raffinose	100	10	100
Amidon	20	0	0
Glycogene	0	0	0
Xylitol	0	0	0
β -Gentiobiose	80	0	60
D-Turanose	100	0	0
D-Lyxose	0	0	0
D-Tagatose	0	0	0
D-Fucose	0	0	0
L-Fucose	0	0	0
D-Arabitol	40	0	0
L-Arabitol	0	0	0
Gluconate	60	20	40
2 ceto-gluconate	0	0	0
5 ceto-gluconate	0	40	40

isolated. No further identification of these, were done. The last group isolates found in the fermenting nixtamalized maize prepared with 0.5% lime were *Pediococci spp* which were identified by tetrad formation. These represented 19% of the isolates identified.

The dominant Lactic acid bacteria isolated from the fermented nixtamalized maize prepared with 1% lime, were identified to be *Pediococci spp*. There were sixteen (16) isolates of these, representing 88% of the microflora present. One (1) isolate of *Lactobacillus plantarum* representing 6% of the total number was identified. The other isolate representing 6% of the microflora present were cocci (unidentified). These were identified at levels of 10^8 cfu/g. *L. plantarum* and *Pediococci spp*. were detected at levels of 10^8 to 10^9 cfu/g in both nixtamalized maize (i.e. 0.5% and 1% lime), and were the dominating microorganisms found during the fermentation of nixtamalized maize. These results tally with that of other researchers on fermenting maize dough. Nche *et al.* (1993) reported that *Lactobacillus plantarum* and *Pediococcus spp.* dominate the latter stages of maize dough fermentation. *L. fermentum* has also been reported to play a dominant role in fermented maize dough (Christian, 1970; Halm *et al.*, 1993). Their presence in the fermenting nixtamalized maize is therefore not unusual.

L. plantarum and *Pediococci spp.* were present in both nixtamalized maize, however, *L. plantarum* was the dominant microorganism in the nixtamalized maize prepared with 0.5% lime while the *Pediococci spp.* dominated the nixtamalized maize prepared with 1% lime. These observations suggest the

possibility of some microbial succession which is dependent on the concentration of lime in the fermenting nixtamalized maize. Further work on fermentation of nixtamalized maize will have to be done to ascertain this.

5.0 CONCLUSIONS

1. Masa with appropriate moisture and texture for the production of snack products can be prepared from maize, by cooking in lime solution for 30 minutes and steeping in the cooking liquor for 14 hours. The application of lime and cooking increased the pH, moisture content, water absorption capacity and produced a yellow colour in maize.
2. Fermentation of nixtamalized maize dough resulted in decreased pH, water absorption capacity, cooked paste viscosity and colour intensity (L-values). As a result of the drastic reduction in cooked paste viscosity of the blends, the process of fermentation can be applied to nixtamalized maize to produce energy dense foods to solve the low energy density problem of maize weaning foods.
3. Lime and cowpea concentration significantly influenced the titratable acidity, water absorption capacity, protein content and cooked paste viscosity of the fermented cowpea fortified nixtamalized maize. Cowpea fortification in combination with fermentation can be employed to further improve the functionality, protein quantity and quality of energy dense nixtamalized maize products.
4. The significant yeast growth, low pH and high lactic acid bacteria counts in the fermented nixtamalized maize dough suggests that fermented nixtamalized maize possesses the sour aromatic flavours and antimicrobial

properties exhibited in traditionally fermented maize dough. The dominant lactic acid bacteria identified in the fermenting nixtamalized maize dough were *Lactobacillus plantarum*, *L. fermentum*, *L. cellobiosus* and *Pediococcus spp.*, all of which play dominant roles in the fermentation of traditional maize dough.

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7.0 **APPENDICES****Appendix 1. Colour Measurements of Dry Flours Produced from Lime Treated Maize**

SAMPLE	Hunter values		
	L	a	b
Raw maize	83.88	-0.05	10.36
Uncooked 0% lime	83.02	-0.18	11.19
Uncooked 0.33% lime	83.33	-0.55	10.32
Uncooked 0.5% lime	82.64	-0.61	10.60
Uncooked 1% lime	82.52	-0.63	11.26
Cooked 0% lime	78.55	-0.50	11.99
Cooked 0.33% lime	80.00	-0.86	11.79
Cooked 0.5% lime	80.13	-1.07	13.99
Cooked 1% lime	75.24	-0.78	13.47

Appendix 2. Colour measurements of steeped:nixtamalized maize blends

BLENDS (%)	Fermentation time (h)	Hunter Values		
		L	a	b
100:0	0	82.57	-0.12	11.59
75:25	0	82.73	-0.67	10.05
50:50	0	79.03	-0.05	13.02
75:25	0	71.90	0.83	15.35
0:100	0	74.57	-0.43	18.94
100:0	24	86.55	-0.72	10.07
75:25	24	87.33	-0.78	10.38
50:50	24	86.43	-0.89	11.23
75:25	24	81.24	-0.61	11.41
0:100	24	71.57	0.63	15.80
100:0	48	86.35	-0.58	9.52
75:25	48	87.41	-0.64	10.00
50:50	48	86.50	-0.76	11.38
75:25	48	83.90	-0.63	12.05
0:100	48	73.75	-0.11	12.46