

**THE APPLICATION OF KUDEME AS FERMENTABLE  
MODIFIERS IN NIXTAMALIZED MAIZE**

**BY**



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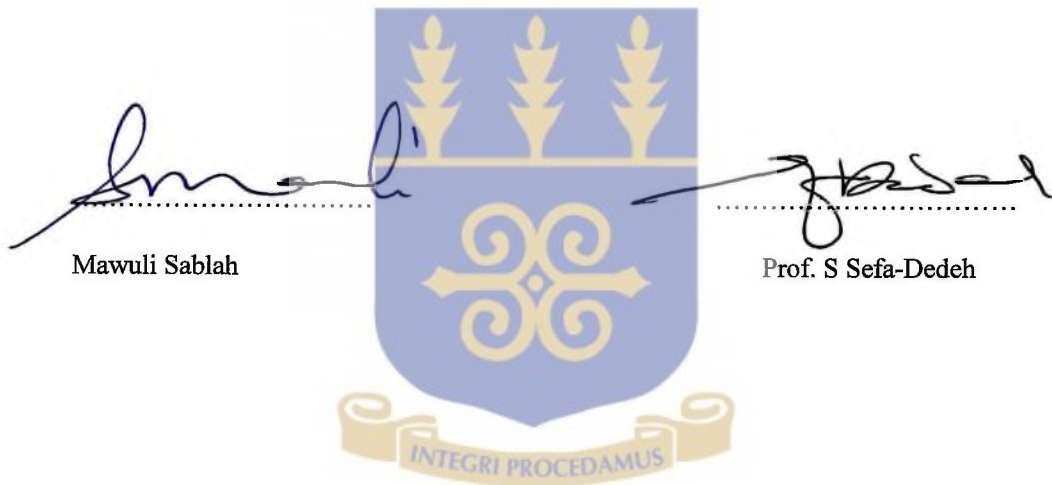
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## DECLARATION

I, Mawuli Sablah, conducted this Scientific Research, under the supervision of Professor Samuel Sefa-Dedeh of the University of Ghana, Legon. And I hereby declare the status of this thesis document as original.



Mawuli Sablah

Prof. S Sefa-Dedeh

## **DEDICATION**

To God for His purpose for my life; My Parents for their love, protection and encouragement and Siblings for the common joys and grieves we share.



## ABSTRACT

Fermentation and nixtamalization have been used to enhance the quality and nutritional value of cereals. “Kudeme” a product of microbial and biochemical disintegration of cassava was inoculated into nixtamalized maize dough and fermented, to study the influence of the addition of kudeme on the characteristics of nixtamalized maize dough. The functional and physico-chemical indices were monitored. A 4 x 5 factorial design of kudeme level by fermentation time was used in pre-testing the system and a central composite design with  $k = 3$ , then used to optimize physico-chemical and functional responses. Preliminary results indicated almost constant moisture levels of 55%, pH dropped from 8.17 to 3.90 with corresponding increase in titrable acidity from 0.0077 to 0.2160g /100gLA. There was no significant difference in water absorption at 25°C and 70°C, which ranged between 49.37 to 93.55%. Pasting temperatures ranged between 68.5 and 86.4°C and temperature at peak viscosity between 70.0 and 92.5°C, and these as well as viscosity, increased with increasing kudeme levels and fermentation time. Viscosity ranged between 80 to 470 BU.  $R^2$  values of indices; an indication of the degree to which variations in dependent variables were explained by independent variables for regression models ranged between 18.24 and 99.73% for biochemical responses, 11.04 to 90.76% for water absorption and swelling index, 23.34 to 85.18% for colour and 28.39 to 87.61% for 7 and 8% slurry viscosity and cooking temperatures. No lacks of fit for most models were observed. Response surface plots as well as two dimensional plots for various parameters indicated that kudeme level and duration in fermentation influenced trends in these parameters to varying increasing or decreasing orders. Particle size decreased with increasing kudeme levels. And this was believed to be the major factor influencing the

outcome of other parameters studied. There were high positive correlation between kudeme level and Amylograph indices studied. Blanched kudeme was not an effective modifier, compared to roasted kudeme. The modifications of quality indices, induced by kudeme on fermentation of nixtamalized maize could be exploited to promote fineness, better physico-chemical and functional properties of nixtamalized maize, making it traditionally relevant to Africans.

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And to all of you my dream shall forever remain; to laugh often and much, to win the respect of intelligent people and the affection of children, to earn the approval of honest critiques and endure the betrayal of false friends if any, to make the world a bit better, whether by a healthy child, a garden patch or a redeemed social condition and to make it possible for at least one life to breath easier because I have lived.

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## 1.0 INTRODUCTION

### 1.1 Importance of fermentation in cereal processing

Fermentation may be defined as the anaerobic breakdown of organic compounds into simpler products involving the biochemical modification of complex food molecules by microorganisms and their enzymes. The catalytic breakdown via fermentation by microorganisms and the food enzymes, results in changes in flavour, pH, nutritive value and other physico-chemical properties of foods (Ashworth and Draper, 1992).

The process of fermentation has been used over several years to effect desirable qualities in food products. According to Odunfa (1985), fermented maize and cassava products by tradition constitute an important part of the diet of people in West Africa countries, while Wood (1985), also stated that fermented foods constitute a significant component of the African diets. A wide range of food commodities in Ghana is fermented during food processing. Fermentation of starch rich substrate by microorganisms into fermented food is practiced mainly in Africa with the most important raw materials being maize and cassava (Rose, 1982). Wood (1985) reported cereal grains as the most important substrate of fermented foods in Tropical Africa. The cereal grains are milled and fermented to produce a gruel, which is known by various names in different parts of West Africa. In the Yoruba land of Nigeria, it is called “ogi” and in Ghana “koko”. “Mahewn” is a sour maize meal similar to “ogi”, consumed by the Bantus of South Africa (Wood, 1985).

Fermentation has been applied to improve the taste, flavour, texture, and reduction in anti-nutritional factors as well as in the enhancement of the microbial safety of cereal foods. Improvement in the nutritional quality of cereals during fermentation with lactic acid bacteria and yeast are related to changes in the availability of protein and B-group vitamins. Protease inhibitors, flavus producing sugars, tannins, and metal binding agents are also removed to a great extent during

fermentation. The combination of reduced pH and lactic and acetic acid bacteria significantly inhibits the growth and survival of pathogens (Nout, 1993), and according to Cornelius (1999), the anti-microbial properties of fermented foods appear to be their most interesting quality.

Traditionally, food fermentation in the informal sectors of African societies is deeply rooted in tradition and experience through the use of simple indigenous procedures (Sefa-Dedeh, 1989). Sefa-Dedeh (1989), indicated that traditional food processing such as fermentation have strong links with rural traditional environments and the processing technologies may be at a rudimentary stage using simple techniques and home-based operations, with women as the major executors. According to Sefa-Dedeh and Mensah (1989), traditional fermentation of cereals involves steeping grains, wet-milling with the addition of water to form stiff dough, which undergoes spontaneous fermentation for up to 72 hours. Sefa-Dedeh and Plange (1989), further indicated that one of the primary objectives for the traditional fermentation of maize dough in Ghana was to cause souring and associated improvement in taste, flavour and texture.

There are several disadvantages associated with indigenous fermentation and these include; high labour input, uneconomical operations, low efficiency, inefficient time utilization due to the process and lack of quality assurance.

However indigenous fermentation employs mixed cultures in spontaneous solid state fermentation and have the advantages of relying on various microorganisms to produce different enzymes to break down the substrate (Sefa-Dedeh, 1994). In Ghana, various foods are processed for consumption using fermentation and in southern Ghana; fermented cereal foods contribute a large proportion of the daily food intake.

## 1.2 The process of nixtamalization

The process of nixtamalization involves the cooking and steeping of whole corn in excess water containing commercial calcium hydroxide (lime) solution. The cooked corn (nixtamal), is removed from the “nejayote” (cook steep liquor), washed and ground into dough known as “masa” (Serna-Saldivar *et al.*, 1990). The “masa” is processed into tortilla, tacos, tortilla chips, taco shells and related products, all of which Ghanaians may not be so familiar with. Products from lime-cooked maize are popular in Central America but the process has not been fully integrated into foods of West Africa and Ghana. It is believed that, ancient Central Americans cooked maize in a leachate of wood ash or lime to produce corn tortillas, their major form of bread (Rooney and Saldivar, 1989). Conventionally however, the 3-basic ingredients for the production of a nixtamal are; corn, food grade lime and water. Thus this ancient technique having undergone little change still involves cooking and steeping whole kernel corn in a solution of lime (Katza *et al.*, 1974). The nixtamal, which is stone ground into masa, is subsequently sheeted, formed and cut for the production of tortilla and related snacks (Gomez *et al.*, 1989; Rooney and Suhendro, 1999). Alternatively the nixtamal could be coarsely ground, dehydrated and milled into instant nixtamalized masa flour (Gomez *et al.*, 1987). Food processors often prefer the convenience of using instant nixtamalized corn flour to the expense and time required to process whole kernel corn.

Considering the fact that maize has the highest production and consumption levels in relation to other cereals in Ghana, the process of nixtamalization has the potential of introducing variety to the traditional food base whilst improving the nutritional value of products from maize. Nixtamalization improves the free calcium levels of maize, increases the bio-availability of iron and other minerals, and permits the faster release of amino-acids whilst increasing the free nicotinic acid

and available niacin. The process therefore has a unique potential in improving the nutritional well being of Ghanaian maize consumers.

### **1.3 Preparation and application of kudeme in traditional food fermentation**

Sefa-Dedeh (1989), reported the use of a specially treated fermented cassava in the fermentation of “agbelima” (cassava dough), among “agbelima” processors in Ghana. The treated fermented cassava used as inoculum is locally called “kudeme” which originated from Ewe-land. The processing and application of “kudeme” as an inoculum has been handed down several generations through oral history (Adjei, 1990).

Traditionally, the preparation of “kudeme” involves the fermentation of the cassava, which is wrapped in a rag or a piece of cloth used previously in other batch fermentation of the inoculum. The process provides a suitable environment for the spontaneous inoculation of the cassava and growth of the microorganisms. According to Sefa-Dedeh, (1989), “kudeme” is prepared as follows; fresh cassava is peeled and washed and then cut into sizeable chips (8-10cm long), the cassava chips are either roasted over fire (10mins.), blanched (5mins.) or warmed in open air (30mins). Afterwards, the cassava is wrapped in a piece of cloth previously used to prepare inoculum and placed in a basket to ferment for 2-4 days into inoculum. An alternative method is to surface dry the cassava chips in the open air at ambient temperature for 6 hours and then places directly under thatch roof of a hut to ferment into inoculum.

Sefa-Dedeh (1989), observed the fine texture of “agbelima”, and speculated the involvement of microorganisms in reducing the particle size of the grated cassava. The fermentation of cassava mash with “kudeme” into the widely consumed “agbelima” in Ghana, accomplishes four main

objectives; a breakdown of the coarse texture, a souring of the dough, reduction in the content of cyanogenic glucoside and the production of volatile aromatic compounds (Amoa-Awua *et al.*, 1997). This confirmed the findings of Sefa-Dedeh (1989).

During the fermentation of cassava into “kudeme”, the moisture content of the product increases and the cassava becomes soft or porous as a result of starch hydrolysis. The softness of the kudeme is used to determine the extent of fermentation.

There is a cassava dough inoculum used in Cote d’Ivoire, which is similar in its method of preparation to that of the blanched “kudeme”. The inoculum is prepared by boiling cassava tubers for 15 to 20 minutes, which are then wrapped with a plastic sheet, jute bag or plantain leaves and allowed to ferment for 3-days until the root softens. The inoculum is used to ferment cassava dough during the preparation of the indigenous product “attieke” and “placalli”, in order to obtain smooth textured dough with a characteristic flavour. Alternatively, some traditional cassava dough processors prepare “kudeme” by tight packing of unpeeled cassava tubers in earthen-ware pot, covered with plastic sheets; which is left to ferment for about 7-days or by sun-drying for 3-5 days of peeled split cassava ( Budu, 1990; Sefa-Dedeh,1994; AmoA-Awua, 1996).

#### **1.4 Relevance of the study**

In terms of utilization, production and consumption, maize is the most important cereal in Ghana, contributing over 55% of the total energy intake of Ghanaians and 91% of the food products in the Greater Accra Region of Ghana are made from maize (Sefa-Dedeh and Mensah, 1989). Odunfa (1985b), also indicated that most cereal foods including those from maize are traditionally fermented before consumption in Africa, and this significantly affects the nutritional, organoleptic

microbiological safety and other physico-chemical qualities of African traditional cereal foods. The traditional fermentation of cereal products widely practised in Africa usually involves a spontaneous development of different lactic acid bacteria and the final microbiological status of the product is influenced by the raw material and processing method (Steinkraus, 1983).

The pH of nixtamalized maize flour is generally high due to the use of alkali during processing. Halm *et al.*, (1993), however observed that maize dough produced by spontaneous fermentation led to a decrease in pH, from 5.9 at the beginning of maize steeping to around 3.7 after 24-48hrs of dough fermentation. According to Sefa-Dedeh (1989), some cereal processors in the Ga district of Greater Accra Region of Ghana, complained of chaffiness, poor cooked paste viscosity, texture, flavour and swelling of some varieties of certain cereals. The introduction of kudeme into these cereals could greatly enhance their physico-chemical and functional properties. However, it is generally believed that during traditional nixtamalization in which whole kernel corn is cooked and steeped in lime solution, lime plays a critical role in the transformation of maize to nixtamal and “masa” (Gomez *et al.*, 1989).

“Masa” is a formed dough or flour system in which changes in starch during nixtamalization determines its mechanical properties, although only partial starch gelatinization occurs during nixtamalization and most starch granules maintain their integrity with the endosperm (Gomez *et al.*, 1989). Masa is held together by a glue-like mixture of dispersed material that consists of gelatinized starch, hydrated and hydrolyzed proteins, lipids and ions (Rooney *et al.*, 1989).

Nixtamalization in conjunction with other processes can be exploited to further improve the processing and utilization of maize. A combination of fermentation and cowpea fortification of

nixtamalized food products may prove a means of enhancing product functionality, protein nutrition and micro nutrient availability in nixtamalized products (Sefa-Dedeh *et al*, 2002b).

Preliminary work done by Sefa-Dedeh (1989), proved that milling of corn nixtamal mixed with raw soaked corn gives a product that has rheological characteristics close to that of the mixed aflata and the raw corn dough in kenkey processing. The adaptation of this process in kenkey processing may reduce the labour input in the mixing of aflata with raw corn dough. The author further reiterated that in addition to cutting out the laborious mixing process of aflatization, the nixtamal, prepared with  $\text{Ca}(\text{OH})_2$ , will introduce calcium into the product. “Fonfom” is another traditional cereal product resembling the nixtamalized modification suggested for kenkey. Sefa-Dedeh *et al.*, (2002a), investigated the effects of fermentation and cowpea addition on some chemical, functional and sensory characteristics of nixtamalized maize. This investigation led to the observation that, in nixtamalized products, fermentation reduced the hardness of fried snacks from “masa” and improved the general malleability of the products whilst non-nixtamalized products were much harder. The addition of cowpea further increased the hardness and did not change significantly with fermentation. However fermentation also enhanced the acceptability of the products. Sefa-Dedeh (1989), earlier suggested the possibility of investigating the efficiency of some of these innovative operations using modern science and technology to make the production process of these foods easier and of better sensory attributes.

Research will therefore go a long way to eliminate some of the drudgeries in traditional food processing Kenkey, though an important fermented cereal food, involves great effort and long hours of steeping and fermentation.. The total time spent for processing a batch of kenkey ranges from 100 to 146 hours. Even though the aflatization process takes a relatively short duration, it is labour intensive, requiring a lot of human energy and fuel.

Fermentation of cereal is a simple biotechnological process and the resultant dough is able to develop the physical, bio-chemical and organoleptic qualities necessary for preparing good quality products (Sefa-Dedeh, 1989). The fermentation process generally takes two to four days for non-nixtamalized dough. The longer the time of fermentation, the more sour the product. For nixtamalized dough of high alkaline pH, the period required to ferment the product may take even much longer to yield desirable product. Sefa-Dedeh (1989), however envisaged that the spontaneous fermentation of cereals without inoculant as practiced at the time might be in use for some years until the scientific basis for the process was further understood.

Sefa-Dedeh (1994), subsequently reported the characteristic fermentation of “agbelima” to be both a souring and texture degrading process important to the development of organoleptic qualities, and suggested that the use of inoculum serves as a source of microorganisms and enzymes needed to cause both souring and texture modification and emphasized that without the use of inoculum a coarse textured sour dough is obtained. The addition of a suitable inoculum to a fermenting medium ensures that specific characteristics are produced within the material over a period of time. The seed or inoculum constitutes 1-10% by volume of the final fermentation batch and inoculum should usually accelerate the process time. “Agbelima processors who do not use inoculants usually soak cassava for about 4-days and according to Okafor et al., (1984), soaking of cassava contributes to the production of some root-softening enzymes which may contribute to fine texture. According to Sefa-Dedeh (1994), cassava dough when ready is either cooked into a stiff gelatinized paste either alone or with cereal flour such as maize. The introduction of “kudeme” into nixtamalized dough could therefore effect direct and higher quality characteristics than the traditional maize/cassava blend usually prepared into “banku”; a traditional dumpling.

The microbial forms of life present in “kudeme”, responsible for the fermentation processes are mostly yeast, moulds and bacteria and their enzymes could therefore be used to induce characteristic qualities in fermenting nixtamalized maize dough. Nixtamalization increases enzyme susceptible starch values due to aqueous heat treatment. In addition to increasing calcium levels and the bio-availability of iron and niacin, nixtamalization could be combined effectively with improved inoculum fermentation to yield desirable “masa” product of refined particle size and other physico-chemical characteristics (Carter and Carpenter, 1982). The use of inoculants to aid the transformation of product characteristics is important. Inoculants, appear to be texture modifying agents, improving the functionality and physico-chemical properties of traditional foods especially requiring fine particle size. This research could therefore serve as an innovation for an alternative process in the production of fermented “masa”, since nixtamalization in combination with inoculum fermentation has the potential of introducing variety and higher qualities into the traditional cereal food base.

## **1.5 Objective**

To study the effect of “kudeme” on the biochemical, physico-chemical and functional properties of fermented nixtamalized maize;

### **Specific Objectives;**

- a) Evaluate the chemical and functional effect of “kudeme” and fermentation on nixtamalized maize.
- b) To study the influence of “kudeme” on the particle size of fermented nixtamalized maize.

- c) Study the effect of “kudeme” on the cooking quality of fermented nixtamalized maize.
  
- d) To optimize the levels of some independent variables utilized in the production of “kudeme” inoculated fermented nixtamalized maize using response surface methodology.

## 2.0 LITERATURE REVIEW

### 2.1 Maize an important cereal in food processing and nutrition

#### 2.1.1 Definition and description of maize

Maize (corn) is the shelled grain of the species *Zea mays* of the grass family gramineae (Codex Alimentarius, 1995). The kernels are often white and yellow in color, but with infrequent occurrence of a mixture of other colors, such as purple, red and black. Maize, the American and Indian word for corn, literally means, “that which sustains life”. It is, after wheat and rice, the most important cereal grain in the world, providing nutrients for humans and serving as raw material for the production of several commodities including starch, oil and protein, alcoholic beverages, food sweeteners and, generally, as food (FAO, 1992).

There are a number of grain types, distinguished by differences in the chemical compounds deposited or stored in the kernel. Sweet corn and popcorn are especially grown for food uses as well as flourey and flint maize. Flourey maize is a grain with a soft endosperm used as food in Mexico, Guatemala and the Andean countries. The most popular types of maize are grain shelled dent maize (*Zea mays indentata* L.) and shelled flint maize (*Zea mays indent* L.). The dent type of maize has vitreous, horny endosperm at the sides and back of the kernels, whilst the flint kernels, have a thick and hard vitreous endosperm surrounding a small granule starchy center (FAO, 1992). The maize kernel is known botanically as a *caryopsis*; a single grain contains the seed coat and the seed. The four major physical structure of the kernel includes; the pericarp, hull or bran; the germ or embryo; the endosperm; and the tip cap. Some Researchers had earlier described the gross anatomy and microscopic structures of these anatomical components. The endosperm, the largest structure, provides about 83% of the kernel weight while the germ averages 11% and the pericarp, 5%. The

remainder is the tip-cap, a conical structure that together with the pedicel attaches the kernel to the ear of the maize.

### 2.1.2 Consumption of maize and maize varieties in Ghana

Maize in its different forms is an important food for large numbers of people in the developing world, providing significant amounts of nutrient in particular calories and protein. Its nutritional quality is particularly important for small children. For example children in a rural area in Guatemala consume up to 64 to 120g of maize per day in the form of lime treated maize and this provides about 30% of the daily protein intake and close to 40% of the daily energy intake. Garcia and Uratia (1978), reported an intake of 226g of tortilla by weaned three-year old children, providing 47% of their calories. Although these findings may not be very encouraging, adequate supplementary foods are often not provided or are given only in insignificant amounts. Food legumes are the most readily available supplementary food in developing countries; however, the amounts are generally very small. Data for 1979 – 1981 from FAO (1984), showed that 22 to 145 countries had a maize consumption of more than 100g per person, and provides between 33% to 1,422 calories per person per day. The figures, confirm the importance of maize as a staple food in some countries; following that if maize intake is high, maize contributes significant amount of calories and protein to the daily intake of people in these countries (FAO, 1992)

The varieties of maize grown in Ghana include “*dobidi*”, “*aburotia*”, “*abeleehi*”, etc. According to Sefa-Dedeh and Mensah (1989), the bulk of the maize grown in Ghana is cultivated in the Brong Ahafo and Ashanti Regions and some part of the Northern Regions. Sefa-Dedeh (1989), identified some local varieties as “*awona-aburow*”, “*nkran-aburow*”, “*anala*” and “*abeleehi*”; whilst recognizing “*obatampa*”, “*okomasa*”, “*safita 2*”, “*golden crystal*” and “*kwanzie*” as commercial and improved varieties. A report by the National Agricultural Research Program (1992), on maize,

indicated, that an improved variety of maize; Diacol 153, showed differences in moisture absorption, steeping and milling properties, yield and aroma of kenkey. It was recognized that very little attention has been given to the utilization of other improved varieties and called for a collaborative work between breeders and Food Technologist in exploiting the potential of new maize varieties in Ghana.

### **2.1.3 Approaches to improving the nutritional/food value of maize**

Owing to the great importance of maize as a basic staple food for large population groups, particularly in developing countries, and its low nutritional value mainly with respect to protein, many efforts have been made to improve the biological utilization of the nutrients it contains. Three approaches have been tried; genetic manipulation, processing and fortification (FAO, 1992). A lot of research has been carried out to increase or vary the carbohydrate, protein, oil contents and other nutrients, by genetic manipulation. Often the processing of foodstuff stabilizes nutrients in the food but losses may occur when optimum conditions are exceeded. There are cases however, in which processing induces beneficial changes in the food. Lime cooking of maize for instance causes some losses in nutrient content, but it also induces some important nutritional changes, such as its effect on increasing calcium, amino acids, and niacin content. In addition, natural fermentation of cooked maize has been shown to result in higher B-vitamins concentration and protein quality (Wang and Fields, 1978). Pozol, a food made from lime-treated maize and allowed to ferment naturally, proved to be of higher nutritional quality than raw maize or tortilla while germination of the grain has also been reported to improve the nutritional value of maize by increasing the lysine and, to some extent, tryptophan content (Tsai, Dalby and Jones, 1975; Martinez, Gomez, Brenes and Bressani, 1980).

A third approach often used to improve the nutritive value of cereal grains is fortification, through the addition of amino acids or protein sources rich in the limiting amino acids. Cornelius (1999), studied the fortification of fermented nixtamalized maize dough fortified with cowpea. Studies on protein supplementation of lime treated maize flour have also been published by many researchers using different food sources, including milk, sorghum, cotton seed flour, fish flour, torula yeast and casein (FAO, 1992). Most of the supplements have relatively higher protein content and are good sources of lysine, with the exception of cottonseed oil and sesame oil meal, which are good sources of methionine. With the exception of casein and /or milk or fish protein concentrate, they are of vegetable origin. The improvement in quality of protein in tortilla flour is, in most cases, a synergistic response to lysine and tryptophan enhancement, and, to a higher level, of protein, both provided by the supplement. Soybean is used greatly as a supplement in tortilla flour and studies have shown that, maximum protein energy ratio (PER) is achieved upon addition of 4 to 6 percent soybean protein, whether from whole soy, soy flour (50%), soy protein concentrate or soy protein isolate (Bressani *et al.*, 1978; Bressani *et al.*, 1981). Tamalito, a product from masa, wrapped in maize husk placed over steam and prepared with native vegetables such as crotalaria and amaranthus has been shown to improve the protein quality of the dough (Bressani, 1983). These vegetables have relatively high levels of protein rich in lysine and tryptophan, in addition to mineral and vitamins such as pro-vitamin A.

As food, the whole maize grain, either mature or immature, may be used or the maize may be processed by dry milling techniques to give a relatively larger number of intermediary products such as maize grits of different particle sizes, maize meal, maize flour and flaking grits. These materials in turn have a great number of applications in a large variety of foods (FAO, 1992). Wet milling is a process applicable mainly in the industrial use of maize, although the alkaline cooking

process used in manufacturing tortillas is also a wet milling process that removes only the pericarp (Bressani, 1990).

## **2.2 The physico-chemical and functional properties of fermented maize products**

Fermentation induces textural changes in cereal products. The textural 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). Mensah *et al.*, (1991), however reported that porridge, cooked from a meal of maize grain soaked in water for 24 hours had 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 remains lower than that prepared from the dry maize flour. Mlingi (1988) also reported decreases in viscosity of fermented cassava weaning foods. The presence of admixtures has been observed to decrease viscosity of fermented maize dough. Ampadu (1991) reported that soy flour reduced the viscosity of maize dough. Sefa-Dedeh (1991), reported a reduction in viscosity when maize dough containing lime  $\text{Ca(OH)}_2$  was fermented and that processes such as soaking, size reduction and fermentation contribute to the development of flavour, color, texture and other product qualities in cereal products.

The process of alkaline cooking produces yellowish products, (Serna-Saldivar *et al.*, 1990). According to Serna Saldivar *et al.* (1990), color intensity of alkaline cooked maize is closely related to carotenoid pigments, flavanoids and pH. Cornelius (1999) found L-values (lightness) increased, while b-value (yellowness) decreased with increasing fermentation time of nixtamalized dough,

suggesting that, the process of fermentation break down the color of the blends thereby producing whiter dough. Cornelius (1999) attributed the effect of fermentation on the color intensity to the changes in pH and titrable acidity resulting from the breakdown of the complex food substances into lower molecular weight carbohydrates, including organic acids. Ghanaians are used to traditional maize dough, having an off-white color. Therefore the ability of the process of fermentation to reduce the intensity of the yellow color, which is developed as a result of nixtamalization, may facilitate consumer acceptability of fermented nixtamalized maize blends (Cornelius, 1999). Sefa-Dedeh and Plange(1989), stated that acids produced during traditional maize fermentation, reduces pH, whilst imparting sour aromatic flavors, which are desirable and give a natural image to the products. Verification of fermenting nixtamalized corn by Sefa-Dedeh (1991), revealed that the pH of lime treated corn decreased with increasing fermentation time. During cereal fermentation, there is imbibition of water and enzymatic activity is enhanced whilst starch is broken down into low molecular weight compounds. These fermentable sugars leach out and through the action of lactic acid bacteria via the Embden Meyer Hoff pathway, the resultant acid causes a decrease in the pH of the dough. *Banigo et al.*, (1972), identified eleven carboxylic acids as product of maize fermentation. Andah (1974) identified lactic, acetic and butyric acids as the most important acids in fermented Ghanaian maize meals. Watson *et al* (1955) observed an increase in lactic acid content of the steep water used during preparation of maize dough.

### **2.3 The effect of nixtamalization on the physical, chemical and functional characteristics of maize**

Cornelius (1999), observed that moisture content increased with increasing lime concentration, an indication that lime facilitates the absorption of water by maize grains. The moisture content of cooked grains ranged between 39.77 to 45.77%. Maize grains cooked in lime before steeping had higher moisture content at all levels of lime concentration than uncooked steeped maize. This was attributed to the gelatinization of the maize starch during cooking, making hydration of the endosperm easier and faster. In the presence of lime, there is the possibility of an osmotic potential developing in the maize grains to absorb more water till equilibrium is attained. There is therefore an inter-play of osmotic effect and gelatinization of endosperm resulting in the high moisture levels of lime cooked corn. This was a confirmation of what Chang and Hsu (1985), who earlier observed that maize cooked in lime solution, absorbs more water than that cooked in ordinary water. In the production of alkaline cooked products from maize, the moisture content of the lime-cooked grains among other factors, is a significant determinant of the quality and acceptability of the final product. The suggested control limit for soft tortillas and corn chips are 45 – 51% and 48 – 54% respectively (Snack Food Association; 1987; Serna-Saldivar *et al.*, 1990).

The pH of nixtamalized maize affects the flavor and shelf life of products from alkalized maize (Serna-Saldivar *et al.*, 1990). Cornelius (1999), recorded increasing pH of lime treated maize with increasing concentration of lime and work done by other researchers showed that the pH of alkaline cooked maize and its products are closely related to the amount of lime used and retained during cooking and steeping (Serna-Saldivar, 1990). Bedolla and Rooney (1984), reported that pH values

of nixtamalized maize flour from the commercial market ranged from 7.1 to 7.4 and Cornelius (1999), recorded pH range of 7.01 to 7.88.

Cornelius (1999), reported that the protein content of nixtamalized maize increased from that of 8.14% for raw maize to 8.88% in maize samples cooked for 30 minutes in 1% lime solution while protein content generally increased with increasing lime concentration for cooked maize, but decreased with increasing lime concentration for uncooked steeped maize. However Cornelius (1999) did not explain the small changes in protein content with increasing lime concentration for cooked nixtamal. Bressani *et al.* (1958), reported increased protein content from raw maize to nixtamal to tortilla to be 9.6, 10.3 and 10.7% respectively. Serna-Saldivar *et al.* (1987) and Gomez *et al.* (1987) also recorded similar amount of protein when alkaline cooked corn products were compared to that of the original grain. Cornelius (1999), did not observe any significant effect of cooking and lime concentration on the protein content of maize.

For ash content of nixtamalized maize, Cornelius (1999) observed that, samples cooked with lime increased in ash as lime concentration was decreased from 1% to 0.5 %. Similar trends was also observed for moisture content, where maize samples containing 0.5% lime had higher moisture levels compared with 1% lime treated maize samples. The presence of lime has always resulted in increased total ash content from maize to tortilla (FAO, 1992). Cornelius (1999) recorded the highest ash content to be 0.20% for lime treated maize samples and no significant difference was observed in ash by cooking or lime treatment on maize.

Water absorption refers to the weight of water bound per gram of dry sample and it is dependent on the availability of hydrophilic groups that bind water molecules and on gel forming capacity of macromolecules (Gomez and Aguilera, 1983).

According to Sefa-Dedeh *et al* (2002b), the water absorption capacity at room temperature, of uncooked lime treated maize increased with increasing lime concentration whilst that of the cooked samples decreased to a minimum and increased when it was cooked in 1% lime. However, a reverse trend was observed for water absorption at 70°C. Cornelius (1999), attributed such differences in water absorption to the effect of lime on gelatinization and  $\text{Ca}^{2+} + \text{Ca}(\text{OH})^+$  - starch interactions. Bryant and Hamaker (1997), indicated that the effect of gelatinization properties of corn starch is a complex, concentration dependent phenomenon. Water absorption at 25°C and 70°C, for uncooked samples at all concentrations of lime was observed to be lower than that of cooked samples. This according to Cornelius (1999), could be due to the raw nature of the starch in the uncooked sample, since gelatinized starch is more readily hydrated than non-gelatinized starch. Lime concentration and cooking had significant effect on water absorption capacities of nixtamalized maize flour samples at both 25 and 70°C (Cornelius, 1999).

Cornelius (1999) observed pasting temperatures of between 69.0 to 78.0°C for nixtamalized maize flour. Nixtamalized maize flour from maize cooked for 30minutes in 1% lime had the highest peak viscosity. The lime treated samples (cooked and uncooked) had a more distinct peak than that of raw (untreated) maize flour. In confirming the findings of Sefa-Dedeh (1991), Sefa-Dedeh *et al.*, (2002b) further observed that lime treatment resulted in a drastic reduction in cook paste viscosities, with the observed reductions being more pronounced in the cooled paste viscosities. This was attributed to saturation of the starch hydroxyl sites with  $\text{Ca}^{2+}$  and  $\text{Ca}(\text{OH})^+$  ions preventing further

association of the starch molecules on cooling. All the nixtamalized maize flour had their highest viscosities, observed after holding at 50°C for 15 minutes and this increased with increasing lime concentration; an indication that lime concentration influences set-back viscosity (Cornelius, 1999). The author also found out that, the highest viscosity values were obtained after the cooling period, for nixtamalized maize flour prepared from 1% lime. This confirmed earlier report that maize flour, heated in 1% lime solution, has highest viscosity values at the end of holding slurry at 95°C. Analyses of variance by Cornelius (1999), indicated that cooking of grains had significant effect on the pasting temperature of maize flour, but, the increasing effect of cooking and lime concentration on all other critical viscosity points apart from pasting temperature, were not significant.

#### **2.4 Optimization data survey on fortified nixtamalized maize**

Cornelius (1999), studied the effect of cowpea level, moisture content and the concentration of lime on the chemical and functional properties of nixtamalized maize, using response surface methodology, where regression models were developed to relate lime concentration, cowpea levels and nixtamalized maize moisture content on the dough characteristics of samples. The author used the lack of fit and  $R^2$  values to test for the adequacy of models. For a good fit of a model, Joklekar and May (1987), suggested an  $R^2$  value of at least 80%, but, Malcolmson *et al.* (1993), recommended an  $R^2$  of 60% for preliminary studies. Cornelius (1999) observed that the quadratic term for lime concentration was the most significant variable influencing pH. Gomez *et al.*, (1987), and Serna-Saldivar (1990), also found that the pH of nixtamalized maize was dependent on the concentration of lime used, due to lime retention. However, cowpea concentration did not significantly influence the pH of the nixtamalized maize, though this was the most important processing variable influencing the titrable acidity after fermentation. There was significant

interaction between moisture content and lime concentration with titrable acidity decreasing with increasing lime concentration at all moisture and cowpea levels, (Cornelius 1999).

The author could not explain observed variation in water absorption at 25°C, but at 70°C, the quadratic term of lime concentration and interaction between moisture and cowpea level as well as cowpea and lime concentration significantly influenced water absorption. Oosten (1982), suggested that divalent cations bind tightly with starch molecules and actually cause water holding capacity to decrease. The observation by Cornelius (1999), thus suggested a saturation and anchorage of excess  $\text{Ca}^{2+}$  and  $\text{Ca}(\text{OH})^+$  on the surface of the starch granules during fermentation and 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. Osei and Sefa-Dedeh (1993), reported that the addition of cowpea improved the water holding capacity of fermented maize dough systems. Cowpea increases protein levels, which may be primary sites of water absorption, (Sefa-Dedeh and Farkye, 1988). Sefa-Dedeh *et al.*, (2002b), further found that fermentation and cowpea fortification influenced the capacity of macromolecules (protein, starch, and fiber), involved in water absorption and that nixtamalization brought about a significant increase in water absorption at 70°C. Model developed for texture were inadequate in explaining variations in this index. However, cowpea concentration was found to be the most influential variable for protein content of fermented cowpea fortified nixtamalized maize. Akpapunam and Sefa-Dedeh (1995) and Amegatse (1995) all reported marked improvement in the protein content of cereals when fortified with legumes. Bressani *et al.* (1958), reported increased protein content in nixtamalized maize and tortilla. Peak viscosity of fermented nixtamalized maize increases to a maximum and decreased with increasing lime concentration. Hot paste viscosity also increased to a

maximum and decreased with increasing lime concentration, but the maximum hot paste viscosities were similar and did not change significantly with increasing cowpea fortification.

## **2.5 The microbial profile of fermenting nixtamalized maize in relation to pH**

Lactic acid bacteria have been found to be the main bacteria responsible for most of the biochemical changes occurring during fermentation of maize (Halm *et al.*, 1993), whilst yeast was observed to contribute to the production of aroma compounds (Jespersen *et al.*, 1994). Halm *et al.*, (1993), reported that there was decrease in pH during steeping of maize, attributed to the onset of fermentation through the proliferation of lactic acid bacteria. However Sefa-Dedeh *et al.*, (2002b), did not record any decrease in pH for steeped nixtamalized maize and were convinced that the boiling and steeping of maize in lime during nixtamalization resulted in the destruction of the microflora responsible for initiating fermentation. Sefa-Dedeh *et al.*, (2002b), recorded decrease pH levels in fermenting nixtamalized dough, indicating that during milling and kneading of dough the fairly sterile nixtamalized dough were sufficiently inoculated with appropriate bacteria species which initiated fermentation and were able to grow at the initial high pH of the nixtamalized dough samples. Initial pH of nixtamalized dough recorded by Cornelius (1999) was as high as 9.7, reducing to 4.1 after 72 hours of fermentation with acidity levels of 0.385gLA/100g. A lower pH for non-nixtamalized maize dough used as control was obtained in comparison with nixtamalized dough after 72 hours of fermentation indicating that in the higher alkaline medium, growth of lactic acid bacteria would be slower and more acid was required to neutralize the alkali present before a rise in acidity which eventually leads to lowering of the pH (Cornelius, 1999). Cornelius (1999), recorded 109 cfu/g of bacteria for both nixtamalized and non-nixtamalized dough, even though their pH and acidity levels varied, and the same amount of acid might have been produced in all dough due to the comparable bacterial numbers, indicating that the differences in pH was due to the acid

required to neutralize the alkaline dough since acidity could only be expressed in excess of neutralization. Cornelius (1999) isolated some spore formers that were able to survive on nixtamalization. The author further observed increase in the population of aerobic mesophiles during fermentation, for both nixtamalized and non-nixtamalized dough with cell morphologies resembling those of lactic acid bacteria. However, there was absence of yeast and moulds during nixtamalization but their introduction occurred during subsequent processes such as milling and kneading. Yeast counts were however constant throughout fermentation of nixtamalized dough whilst a 100-fold increase in yeast counts were recorded after 72 hours of fermenting non-nixtamalized dough (Cornelius, 1999). Five groups of lactic acid bacteria were classified. The dominant strains of lactobacillus in the fermenting nixtamalized dough were *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus cellobiosus* and *Pediococci spp.* Nixtamalized dough prepared with 1% lime concentration, had *Pediococci spp.* as the dominant lactic acid bacteria. 16 different isolates were identified at levels of  $10^8$  and  $10^9$  cfu/g in nixtamalized maize, (Cornelius, 1999). Nche *et al.* (1994), reported that *Lactobacillus plantarum* and *Pediococcus spp.* dominate the latter stages of maize dough fermentation. Christian (1970) and Halm *et al.* (1993), also reported that *L. fermentum* play a dominant role in maize dough fermentation.

According to Cornelius (1999), since *L. plantarum* was dominant in fermenting nixtamalized maize prepared with 0.5% lime and *Pediococci spp.* dominated in fermenting nixtamalized maize prepared with 1% lime, there could be some microbial succession, which may be dependent on the concentration of lime in the fermenting nixtamalized maize. Studies on microbial succession of spontaneous and largely uncontrolled fermentation 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 lactic

acid fermentation, the combination of the reduced pH and the presence of lactic and acetic acids significantly inhibit the growth of *Bacillus*, *Micrococaceae* and *Enterobacteriaceae* which consequently have a stabilizing effect on the microbial population of the product. Evidence of the anti-microbial properties has been found in fermented maize flour from Kenya (Mbuga, 1988) and maize dough from Ghana (Mensah *et al.*, 1991). These studies revealed that enteric pathogens were inactivated in these fermented foods.

## **2.6 Development and use of inoculum in food fermentation**

The origin of cultured dairy products probably dates back to the dawn of civilization. It was not until the beginning of the present century that cheese workers developed the modern practice of using carefully selected pure strains of some bacteria comprising starter cultures which were deliberately added to cheese in standard amounts, depending on the type of cheese required (Rose, 1982). Hansen (1896), pioneered the use of pure inocula and devised a yeast propagation scheme, utilizing a 10% inoculum volume at each stage in the program and employing conditions similar to those used during brewing. Reed and Pepler (1973), discussed the development of inoculum for the production of bakers yeast. The commercial productions of bakers yeast involve the development of an inoculum through a large number of stages. The main objective of inoculum development for bacterial fermentation is to produce an active inoculum, which will give a short lag phase as possible in subsequent culture. The length of the lag phase is affected by the size of the inoculum and its physiological conditions (Meyrath and Suchanek, 1972). Lincoln (1960), stressed that bacterial inoculum should be transferred in the logarithmic phase of growth, when cells are still metabolically active. The age of the inoculum is particularly important in the growth of sporulating bacteria, since sporulation is induced at the end of the logarithmic phase and the use of an inoculum containing a high percentage of spores would result in a long lag phase in a

subsequent fermentation. Keay *et al* (1972), quoted the use of a 5% inoculum of a logarithmically growing culture of a thermophilic *Bacillus* for the production of proteases while Aunstrup (1974) described a two stage inoculum development programme in the production of proteases by *Bacillus subtilis*.

Inoculum for a seed fermenter was grown for 1 to 2 days on a solid or liquid medium and then transferred to a seed vessel where the organism was allowed to grow for a further 10-generation before transfer to the production stage. The quantity of inoculum normally used is between 3 and 10% of the medium volume (Lincoln, 1960; Meyrath and Suchanek, 1972), so that, starting from a stock culture, the inoculum must be built up in a number of stages to produce sufficient biomass to inoculate the production stage fermenter. It is a common practice in the brewing industry to use the yeasts from the previous fermentation to inoculate (pitch) a fresh batch of wort. Underkofler (1976), emphasized that in the production of bacterial enzymes, for example, the lag phase in plant fermenters may almost be completely eliminated by using inoculum medium of the same composition as used in the production fermenter and employing large inocula of actively growing seed cultures.

The necessity to use an inoculum in an active physiological state is taken to its extreme in the production of vinegar. The acetic acid bacteria used in vinegar process are highly aerobic and are extremely sensitive to oxygen starvation. Therefore to avoid disturbing the system, the cells at the end of fermentation, are used as inoculum for the next batch by removing approximately 60% of the culture and restoring the original level with fresh medium (Conner and Allgeier, 1976). The advantage of a highly active inoculum apparently outweighs the disadvantage of possible strain degeneration and contaminant accumulation.

An example of the development of inoculum for an anaerobic bacterial process was provided by the clostridial acetone-butanol fermentation largely carried out in South Africa, and was a very important development in the fermentation industry during and soon after the First World War, (Bu'lock, 1975).

The development of inocula for fungal fermentation may be probably more involving than that for bacterial and yeast processes (Hockanhull, 1980). The majority of industrially important fungi are capable of asexual sporulation, so it is common practice to use a spore suspension as seed during an inoculum development program. Two basic techniques used to develop a high concentration of spores for use as an inoculum are sporulation on solid media, such as surfaces of cereal and sporulation in submerged cultures. The stage in an inoculum development program at which a large-scale spore inoculum is used varies according to the process, but what appears to be the common practice is that the penultimate stage is so inoculated. However the choice of inoculum for the production stage depends on the length of the cycle of the fermentation process and the availability and cost of labour (Lockwood, 1975).

## **2.7 The microbiological properties and use of “kudeme” as inoculants in traditional fermentation**

Pederson (1971), indicated that microorganisms seldom exists as pure cultures in foods whilst Rose (1982), reported that most categories of fermentation are mainly due to a mixture of mould and yeast or moulds followed by bacteria and yeast. Earlier, some researchers observed a two stage cassava fermentation into gari in which the first stage involves the attack of the starch by *Corynebacterium manihot* , leading to the production of various organic acids. Abioye (1980) and

Okafor *et al* (1984) confirmed this observation by isolating several microorganisms from fufu preparation and finding *Bacillus* and *Corynebacterium species* to be the starch hydrolysers. Ngaba and Lee (1979) identified *Streptococcus sp.* to be active starch hydrolysers in cassava fermentation producing significant organic acids. During fermentation of cassava the moisture content of the product increases and the cassava becomes soft or porous as a result of starch hydrolyses. The softness or porosity of the fermented cassava (“kudeme”) is used to determine the extent of fermentation. Studies by Adjei (1990), revealed that the pH of “kudeme” was 4.1. Microorganisms isolated and identified in “kudeme” (Adjei, 1990), constitute a mixed population of yeast, coliforms, lactic acid bacteria and other acid formers. Six bacteria genera of which two were found to be lactic acid bacteria and three yeast genera were encountered. *Corynebacterium sp.* and *Streptococcus sp* which were two of the bacteria isolated were found to be starch hydrolysers. The starch and sugar were broken down to produce organic acids; lactic; acetic and formic acids with accompanying percent titrable acidity of 0.325 and volatile acidity of 0.057%. Adjei (1990), further observed that cassava dough inoculated with “kudeme” caused a familiar trend of decrease in pH with an increase in acidity and concluded that many of the microorganisms isolated from “kudeme” exhibited the ability to produce acids and enzymes which can alter acidity, pH and nutrient or chemical content of the kudeme and the “agbelima”. The author suggested that the amount of kudeme used in fermenting cassava dough should exceed 3% dry weight of the dough. Microorganisms isolated by Adjei (1990) from kudeme, included bacteria (*Escherichia sp.*, *Acrobacter sp.*, *Streptococcus sp.* *Corynebacterium sp.*, *Leuconostoc sp.*, *Pediococcus sp*), yeasts (*Saccharomyces sp.*, *Candida sp.* and *Torula sp*). Budu (1990), also isolated the following microorganisms from kudeme; *Saccharomyces spp.*, *Candida spp.*, *Leuconostoc spp.*, *Corynebacterium* and *Alcaligenes*. During sensory evaluation “agbelima” samples inoculated with 3% kudeme was highly preferred to other samples, as this sample had smaller particle size

and a smoother texture whilst uninoculated “agbelima” samples was least preferred due to their very coarse texture, (Budu, 1990). Amoa-Awua *et al.* (1996), also reported that, the breakdown of cassava texture occurs through hydrolyses of cassava tuber cellulose by cellulases produced by some dominating microbial species in the “kudeme” (cassava inoculum). *Bacillus subtilis*, *B. mycooides*, *B. pumilus*, *B. amyloliquefaciens*, and *B. licheniformis* were the tissue degrading microbial agents in 3-types of “kudeme” examined and the moulds; *Penicillium nodulum*, *P. citrinum* and *Geotrichum candidum* dominated the fourth type of inoculum, (Amoa-Awua *et al.*, 1996). The souring of “agbelima” is accomplished through the production of lactic and acetic acids by *Lactobacillus plantarum* and other lactic acid bacteria including *Lactobacillus brevis* and *Leuconostoc mesenteroides*.

### 3.0 METHODOLOGY

#### 3.1 Experimental designs

##### 3.1.1 Pre-testing of maize nixtamal-kudeme fermenting system

A 4 x 5 factorial design, in which nixtamalized maize dough samples were inoculated with 0, 1, 3 and 5% levels of roasted kudeme and fermented for 0, 12, 24, 48 and 72 hours, was used in pretesting the effect of kudeme on nixtamalized maize dough fermenting system in order to determine the minimum and maximum levels for independent variables in future optimization studies of the system. The moisture content was maintained at 55% for all samples in this preliminary study. Preliminary study also involved the inoculation of nixtamalized maize with 0, 2, 5, 8 and 10% “kudeme”, fermented over various time intervals and analytical results used in plotting graph trends.

##### 3.1.2 Optimization study design for maize nixtamal - kudeme fermenting system by response surface method (RSM)

The central composite rotatable design for  $K=3$  was applied (Cochran and Cox, 1957). The independent process variables were the levels of kudeme, moisture content and fermentation time. The coded levels derived by simultaneous equations are summarized in the Table 1 below. Twenty (20) sample combinations were generated as indicated in Table 2.

**Table 1. Preset variable combinations used in central composite design ( $k = 3$ ).**

Independent variables	Variable codes/levels				
	-1.682	-1	0	1	1.682
Kudeme(%)	0.00	2.03	5.01	7.98	10.01
Moisture (%)	50.00	52.03	55.00	57.97	60.00
Fermentation (hrs)	0.00	9.73	23.99	38.26	47.99

In the design of an experiment, there is a need to verify the difference among levels of the factors. And for a quantitative data, the entire data range is used to generate a predictive model for other data ranges by interpolation. The general approach to fitting equations to data is regression analysis. Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for the modeling and analyses of problems in which a response of interest is influenced by several variables with the objective of optimizing the response. In this experiment our dependent variables, which are the chemical and functional properties, is a function of the independent variables of “kudeme” level, moisture content and fermentation time.

Response surfaces are represented by three-dimensional solid surfaces and the lines of constant response (independent variables) are usually drawn on the  $X_1$  and  $X_2$  planes with each contour corresponding to a particular height of the response surface. These plots help in studying the effect of the independent variables on the dependent variables. Since we did not know the relationship between our response and the independent variables, we first found a suitable approximation for our true functional relationship between our response and our independent variables, using a second order polynomial by the method of least squares.

According to Montgomery (1991), the objective of RSM is to determine the optimum operating conditions for the system or to determine region of the factor space in which operating specification are satisfied. In this study, response curves were fitted to some quantitative factors to relate regression equations to responses at factor levels among those actually used in the experiment leading to process optimization.

**Table 2. Design matrix and actual variable combinations used in sample preparations**

Sample no.	Level codes			Variable levels		
	Kudeme level	Moisture Content	Fermentation	Kudeme level	Moisture (%)	Ferm. time(hrs)
1	-1	-1	-1	2.03	53.03	9.73
2	-1	1	1	2.03	61.95	38.26
3	1	-1	1	7.98	53.03	38.26
4	1	1	-1	7.98	61.95	9.73
5	0	0	0	5.01	57.49	23.99
6	0	0	0	5.01	57.49	23.99
7	-1	-1	1	2.03	53.03	38.26
8	-1	1	-1	2.03	61.95	9.73
9	1	-1	-1	7.98	53.03	9.73
10	1	1	1	7.98	61.95	38.26
11	0	0	0	5.01	57.49	23.99
12	0	0	0	5.01	57.49	23.99
13	1.682	0	0	10.01	57.49	23.99
14	-1.682	0	0	0.00	57.49	23.99
15	0	1.682	0	5.01	64.99	23.99
16	0	-1.682	0	5.01	49.99	23.99
17	0	0	1.682	5.01	57.49	47.99
18	0	0	-1.682	5.01	57.49	0.00
19	0	0	0	5.01	57.49	23.99
20	0	0	0	5.01	57.49	23.99

## **3.2 Materials and sample preparation**

### **3.2.1 Sources of materials**

Maize (Obatampa), was obtained from the Greater- Accra Regional Office of The Ministry Of Agriculture, Ghana and stored at 4°C.

Food grade lime, Ca(OH)<sub>2</sub>, was obtained from Alpha Chem.; New York and manufactured by Spectrum Laboratory Chemicals Division of Spectrum Quality Products.

The “kudeme” samples obtained were prepared by roasting and blanching methods described by Sefa-Dedeh (1989),n from cassava processing women at Midie and Dobro, in the Eastern Region Of Ghana, and they were earlier identified by Sefa-Dedeh (1989). They were milled into a wet dough like substance before use.

### **3.2.2 Preparation of nixtamalized maize**

Maize nixtamalization was uniformly carried out for all experimental trials. A 1% lime solution was first prepared by dispersing a weighed amount of lime into a measured amount of water. The solution was prepared in steam-jacketed pan (UW12C, 304SS Coulter Copper And Brass Co. Ltd., Toronto, Canada), for subsequent heating. Lime solution was heated to 100°C. A weighed quantity of maize to give a maize : lime solution ratio of 1 : 3 was then poured into the preheated lime solution and boiled for 30min. Energy dissipation and boiling temperature fluctuation were kept at minimal ranges. After 30 minutes heating was discontinued and steeping time monitored for 14 hours (Ramirez-Wong *et al.*, 1994). The cook liquor (nejayote), was drained and the treated maize nixtamal was thoroughly washed with fresh clean water five times (changing the water after each wash) and then milled into a fine meal using the disc attrition mill (model 10 – 2A, New Delhi).

The moisture content of the meal was then determined and the moisture level adjusted by the addition of predetermined amounts of water to the meal of specific weights based on available dry matter, the difference in water required and the amount already present, to attain a dough of specific moisture content.

### **3.2.3 Inoculation of dough**

The kudeme obtained was milled into wet dough-like substance, using a laboratory disc attrition mill (Straub Model 4E, Straub Co. Phila PA 19020, U.S.A). Various proportions were then used to inoculate nixtamalized maize dough at predetermined rates. Various quantities of nixtamalized maize dough were inoculated with various quantities of kudeme based on dry matter.

## **3.3 Chemical analysis**

### **3.3.1 Moisture content**

Moisture content was determined by the AOAC (1975) method. Two grams of samples were completely dried at 130°C for an hour in an air oven using aluminium moisture dishes as containers. Percentage loss in weight of samples was recorded as equivalent moisture content.

### **3.3.2 Measurement of pH**

Ten grams of the samples were mixed in 100 ml of CO<sub>2</sub> free de-ionized water. The suspensions were left to stand for 15minutes, with stirring and shaking at 5 minutes intervals and filtered with a Whatman filter paper. The pHs of the filtrates were measured using a pH meter (CD 1507QI, TOA Electronic Ltd.).

### **3.3.2 Equivalent titrable acidity**

Ten milliliters of the filtrates from 3.3.2 were titrated in triplicate against 0.1N NaOH using 1% phenolphthalein as indicator. The acidity was calculated as gram lactic acid per hundred-gram dry matter sample (the equivalent weight of lactic acid; 70g, was used).

### **3.3.4 Determination of ash**

Approximately 2g of nixtamalized maize was weighed into pre-weighed crucibles and placed in preheated furnace (Gallenkamp Muffler Furnace; size 3) of 550 – 600°C overnight. After complete combustion the crucibles were removed, cooled, and weighed to determine the weight by difference of the ash content of samples. The ash content was calculated on 100g dry matter sample weight basis and in duplicates (AOAC, 1975).

### **3.3.5 Protein content determination**

The protein content of samples was determined by the method of AOAC, (1975). Factor conversion of nitrogen to protein was 6.25.

## **3.4 Functional analyses**

### **3.4.1 Swelling capacity**

Ten grams of samples were weighed into 100ml-graduated measuring cylinders and the bulk volumes noted. Hundred milliliters of distilled water (25°C and 70°C) were then used in mixing each sample into a thorough suspension and allowed to stand. The volumes of samples were then read at 15 minutes intervals for one hour.

### 3.4.2 Water absorption capacity

Five grams of samples were weighed into centrifuge tubes and 30ml of distilled water added and stirred to form suspensions. The suspensions were allowed to stand for 30 minutes and centrifuged at 3000 rpm for 15 minutes, using a Denley Centrifuge (BS 4402/D, Denley, England). The supernatants were decanted and the increases in weights noted. The water absorption capacities were expressed as percentages of initial sample weights. Each determination was done in duplicate at 25 and 70° C (Anderson *et al.*, 1969).

### 3.4.3 Colour

A Minolta CR-310 compact tristimulus chroma-meter (Minolta Camera Co. Ltd., Osaka, Japan), with 8mm diameter measuring head, diffuse illumination and a 0° viewing angle was used in the measurement of color of dry flour samples. The meter was calibrated with a standard white tile (L = 103.99, a = 0.41, b = 6.22). Psychrometric color terms, “L” (lightness), “a” (red-greenness) and “b” (yellow-blueness) and total color change were recorded.

## 3.5 Analysis of particle size of wet samples

Wet sample particle size analysis was carried out by the method of Sefa-Dedeh (1989), which was appropriately modified. Nixtamalized maize dough were prepared and inoculated with 0,1,3,5 and 10% “kudeme” levels. Dough were fermented for 48 hrs and stored frozen. Prior to analysis the dough were removed from freezing compartments, thawed and equilibrated to room temperature. A 100 g of wet dough samples were weighed over 1,000, 500, 250 and 125 micrometer (U.S No. 18,35,60 and 120) standard mesh screens arranged in decreasing screen sizes on an electromagnetic vibration sieve shaker (Vibro VS 1,000; Retsch GMBH and Co. KG, 5657 Haan, Germany C.E 93).

During the wet sieving, a splashguard hood with spray nozzle clamped over the topmost sieve was connected to a tap whilst a collecting pan with outlet tube supported the base sieve. Sieves were firmly supported over the vibrator by clamp and the equipment was operated at amplitude of 25 vibrations/sec. for 15minutes with regulated intermittent jet water sprays over sieves. The draining from the bottom sieve (125 $\mu$ m) measuring approximately 1,500ml suspension was collected into buckets. Total solids were determined by oven drying 30ml of the suspension in aluminium dishes at 105 $^{\circ}$ C for 12hrs and estimating for total volume collected. Pre-weighed dry sieves were dried again at 105 $^{\circ}$ C for 2 hrs after the shaking process and weighed again to determine the quantity of equivalent dough retained by each sieve. The percentage moisture content of dough was determined by drying 10g-wet dough at 105 $^{\circ}$ C for 12 hrs and based on percentage dry matter, the percentage particle size distributions for each sample were calculated for plotting.

### **3.6 Cooking rheology; viscosity and pasting properties**

The viscosity and pasting properties of fermented nixtamalized maize dough inoculated with “kudeme” were determined using a Brabender Viscoamylograph (Brabender Instrument Inc. Duisberg, Germany.), equipped with a 700cmg sensitivity cartridge. Different concentrations of slurry suspensions (3, 5, 7 and 8%), based on equivalent dry matter were prepared and each poured into a sample bowl. The viscosity and pasting properties were monitored as the samples went through heating, holding and cooling cycles. The samples were heated at the rate of 1.5 $^{\circ}$ C/minute from 25 $^{\circ}$ C to 95 $^{\circ}$ C, held at 95 $^{\circ}$ C for 30minutes and then cooled at the same rate to 50 $^{\circ}$ C and held at 50 $^{\circ}$ C for another 15 minutes. The Brabender Viscoamylograph indices were measured. These include; pasting temperature, peak viscosity, viscosity at 95 $^{\circ}$ C, Viscosity at 95 $^{\circ}$ C-hold, Viscosity at 50 $^{\circ}$ C and Viscosity at 50 $^{\circ}$ C-hold.

## 4.0 RESULTS AND DISCUSSION

### 4.1 Preliminary results

#### 4.1.1 Effect of kudeme concentration and fermentation time on moisture, pH and titrable acidity of nixtamalized maize

The moisture content of nixtamalized maize inoculated with kudeme remained almost constant throughout the period of fermentation. Neither the period of fermentation nor the level of kudeme had any significant effect on the moisture content of nixtamalized dough. The moisture content ranged between 53.44 to 54.84% (Table 3), after fermentation from the initial moisture content of 55%.

**Table 3** Moisture content (%) of nixtamalized maize inoculated with kudeme.

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	54.76 ± 0.17	54.61 ± 0.35	54.24 ± 0.20	54.27 ± 0.05
12	54.39 ± 0.09	54.70 ± 0.30	54.22 ± 0.21	54.60 ± 0.04
24	54.26 ± 0.62	54.70 ± 0.11	54.66 ± 0.11	54.41 ± 0.14
48	54.42 ± 0.25	54.17 ± 0.12	54.84 ± 0.16	54.38 ± 0.24
72	53.91 ± 0.38	53.44 ± 0.25	54.73 ± 0.42	54.64 ± 0.40

Several research reports have indicated a general loss in moisture content of cereal dough during fermentation (Nche *et al.*, 1994, Rose, 1982), however an almost constant moisture level was observed for fermenting nixtamalized dough inoculated with roasted kudeme. During the process of fermentation of nixtamalized dough of high alkalinity, it is believed that several compounds are produced including organic acids such as lactic, butyric and acetic acids. The hydrogen ions of these acids in reacting with excess hydroxyl ions from  $\text{Ca}(\text{OH})_2$ , produce water which contributes to the maintenance of a constant moisture level within nixtamalized dough during fermentation. The breakdown of starch into simpler sugars containing alcohol groups, plus aldehyde and ketone

groups may oxidize under alkaline condition to carboxyl groups forming aldonic acids which may sequest  $\text{Ca}^{2+}$  from  $\text{Ca}(\text{OH})_2$ , releasing the  $\text{OH}^-$  group to form water with  $\text{H}^+$  ions.

Research had also shown that, three types of reactions may taking place in alkaline solutions or suspensions of carbohydrates; isomerization, cleavage into smaller fragments or internal oxidation, reduction and rearrangements.

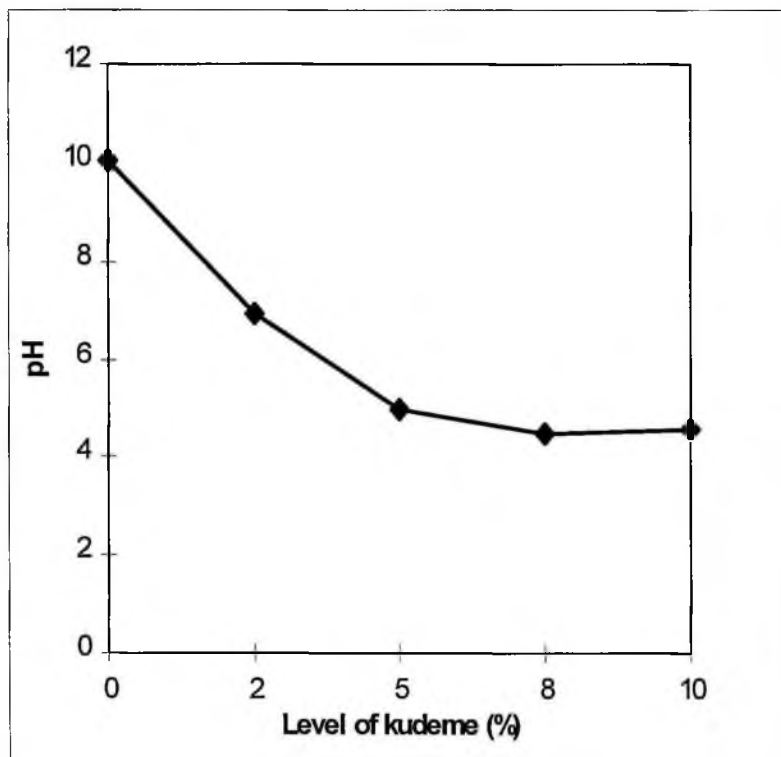
The highest pH of 8.67 was recorded for 0%-kudeme level of nixtamalized maize at 0 hr fermentation and the lowest pH of 3.90 was recorded for 5%-kudeme level of kudeme inoculated nixtamalized maize after 72 hrs of fermentation. The reversed titrable acidity trends of 0.0077g lactic acid/100g and 0.2160g lactic acid/100g (dry matter) were recorded for the same samples respectively.

**Table 4. pH of nixtamalized maize inoculated with kudeme.**

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	8.67±0.01	8.23±0.02	6.97±0.01	6.67±0.01
12	4.80±0.02	4.63±0.01	4.93±0.00	4.75±0.01
24	4.64±0.01	4.41±0.00	4.54±0.00	4.37±0.01
48	4.37±0.02	4.12±0.01	4.14±0.01	4.08±0.00
72	4.18±0.02	3.98±0.01	4.11±0.02	3.90±0.00

In general the pH of nixtamalized dough dropped continuously with increase in fermentation time as well as kudeme level (Fig. 1). The pH of the nixtamalized maize dough at each kudeme level was dependent on how long the fermentation had taken place. The drop in pH however increased with decrease in kudeme level, from a low of 2.77 for dough inoculated with 5% kudeme to 4.49 for dough without kudeme (0% kudeme).

**Figure 1: pH trend of fermented (48Hrs) nixtamalized maize inoculated with roasted kudeme**



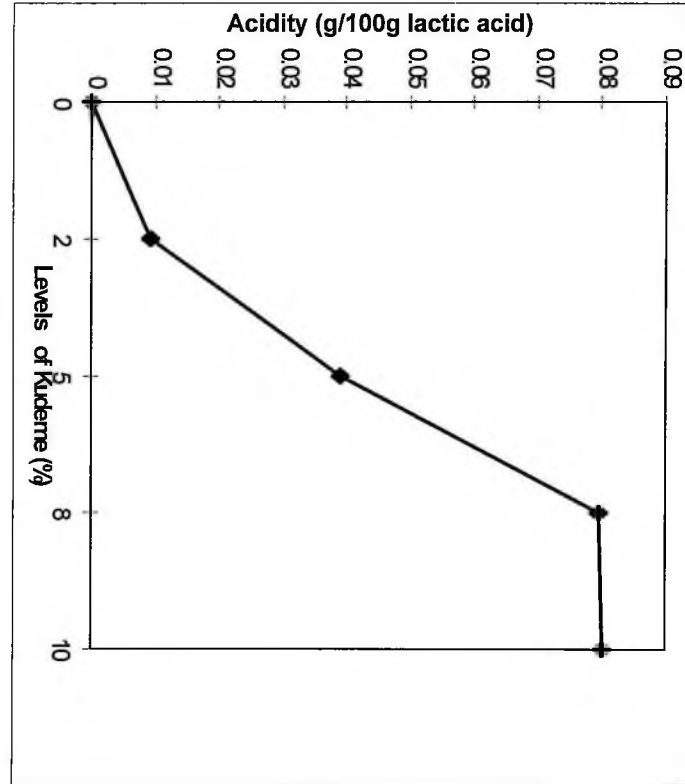
The pH of the 0% level “kudeme” however dropped with increasing level of kudeme inoculation, suggesting a possible high initial pH of the kudeme or possible rapid fermentation of dough sample during analysis of pH which increased with increasing kudeme level. Fermentation had a significant effect on dough pH, (Table 10). This confirmed the findings of Sefa-Dedeh (1991), that pH of nixtamalized maize decreased with fermentation time, suggesting the activity of lactic acid bacteria which is further enhanced in this research by the high initial microbial load of kudeme and possible high pH of kudeme.

**Table 5. Titrable acidity (gLA/100g) of nixtamalized maize inoculated with kudeme**

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	0.0077±0.0011	0.0154±0.0013	0.0153±0.0011	0.0306±0.0012
12	0.0770±0.0002	0.0770±0.0012	0.0760±0.0006	0.0930±0.0005
24	0.0770±0.0015	0.0924±0.0006	0.0930±0.0011	0.1310±0.0012
48	0.1073±0.0008	0.1373±0.0014	0.1550±0.0011	0.1760±0.0012
72	0.1367±0.0006	0.1656±0.0011	0.1700±0.0005	0.2160±0.0008

Fermentable Sugars leach out as a result of the action of lactic acid bacteria via the Embden Meyer Hoff pathway. This decreasing trend in pH with increasing fermentation and kudeme levels was further confirmed by results of titrable acidity (Fig.2), which indicated an increasing trend with concomitant increase in levels of kudeme and periods of fermentation. During lactic acid fermentation, pyruvic acid is reduced to lactic acid and the organisms inducing this conversion have NAD<sup>+</sup> linked lactic dehydrogenase enzyme and produce other enzymes, which facilitate the conversion of starch into other simpler, starch derivatives and organic molecules. These explain the trends observed for pH and titrable acidity of fermenting nixtamalized dough inoculated with kudeme.

**Figure 2: Titrable acidity trend of fermented (48Hrs) nixtamalized maize inoculated with roasted kudeme.**



The kudeme level and fermentation time contributed significantly to the pH and titrable acidity of fermenting nixtamalized maize inoculated with kudeme (Table 10). Sefa-Dedeh (1989), observed that in general the higher the level of kudeme introduced into agbelima fermentation, the lower the acid production at all fermentation times, suggesting that the inoculant decreases acid production ability or that the inoculant facilitates further breakdown of acids produced as a result of fermentation. However, the acidity of fermenting nixtamalized dough inoculated with kudeme increased with increasing level of kudeme.

#### 4.1.2 Effect of kudeme and fermentation on water absorption capacity (25°C and 70°C) on nixtamalized maize

The water absorption capacity ranged between 45.76 to 90.32% at 25°C and 49.37 and 93.55% at 70°C, as indicated in Table 6 and Table 7 respectively.

**Table 6. Water absorption capacity (25°C; %DMB) for nixtamalized maize inoculated with kudeme.**

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	86.21±0.20	78.72±0.43	52.75±0.45	50.12±0.33
12	57.55±0.11	63.87±0.23	55.83±0.52	51.85±0.43
24	56.55±0.11	54.79±0.24	67.93±0.55	63.68±0.54
48	50.55±0.23	45.76±0.31	77.21±0.56	81.48±0.64
72	52.01±0.42	59.78±0.23	88.54±0.48	90.32±0.55

Generally the water absorption increased with increasing temperature and therefore water absorption capacities at 70°C were higher than water absorption capacities at 25°C. From 0-24 hours of fermentation, the amount of water absorbed decreased for 1,3 and 5% levels of kudeme but increased from 48 to 72 hours of fermentation. There was a decrease in water absorption for 0 and

1% levels of kudeme samples with increasing fermentation; from 0 to 48 hours of fermentation period and an increase after 72 hours of fermentation at 25°C. However trend observed for samples with 3 and 5% “kudeme” levels, consistently increased in water absorption with fermentation time.

**Table 7. Water absorption capacity (70°C; %DMB) for nixtamalized maize inoculated with kudeme.**

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	65.75±0.43	51.35±0.11	56.80±0.13	55.45±0.55
12	49.37±0.53	55.33±0.23	60.16±0.12	66.58±0.43
24	60.18±0.64	52.99±0.54	90.47±0.59	93.55±0.23
48	69.23±0.55	73.75±0.28	80.93±0.47	70.96±0.23
72	58.76±0.15	69.75±0.34	68.76±0.61	70.37±0.37

The water absorption at 70°C were however inconsistent for all samples during fermentation. Since the moisture content of dough was around 55% and remained almost constant throughout the period of fermentation independent of kudeme levels, the excess water absorbed mostly resulted from  $\text{Ca}^{2+}$  ion and  $\text{Ca}(\text{OH})^+$  complexing with the negative polar end of water, which was highest at a high temperature of 70°C, than for 25°C. However, starch molecules also absorbed some amount of water, though this was not indicated by specific trends. Cornelius (1999), recorded an increase in water absorption for nixtamalized maize prepared with 1% lime at 25°C and a decrease in water absorption at 70°C for this index and attributed these differences to the effect of lime on gelatinization as well as  $\text{Ca}^{2+}$  and  $\text{Ca}(\text{OH})^+$  ion - starch interaction. The water absorption capacity of nixtamalized maize inoculated with kudeme was not significant at both 25°C and 70°C.

#### 4.1.3 Effect of kudeme and fermentation on viscoamylograph indices of nixtamalized maize inoculated with kudeme.

##### a. Pasting temperature and temperature at peak viscosity

There was an increasing trend in pasting temperature with increasing levels of kudeme (Figure 3) and values indicated slightly increasing pasting temperature with increasing fermentation time as indicated in Table 8. This could be attributed to an increase in fineness of starch granules of samples with higher levels of kudeme resulting from the breakdown of starch granules by the activity of microorganisms and their enzymes which increase with increasing levels of the inoculant; kudeme.

**Table 8: Pasting temperatures (°C) of nixtamalized maize inoculated with kudeme.**

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	68.5	73.7	86.7	86.3
12	71.8	73.8	86.4	86.2
24	73.0	73.9	84.3	86.0
48	75.8	73.5	84.3	85.9
72	78.6	74.8	84.0	84.2

Kudeme level had significant effect on pasting temperature. There was no independent significant effect resulting from fermentation time. Similar observations were made for the temperature at peak viscosity.

The gelatinization temperature determines the strength of bonding in the micellar granules on heating starch suspensions, though larger granules of the same sample usually start to swell at lower temperatures than the smaller ones. The position of the gelatinization temperature is characteristic for starches of different treatments and assists in determining the technological qualities of the

starch. Physico-chemical changes occur in starch suspensions after attaining gelatinization temperature on prolonged heating. The starch granules represent a micellar network of linear (amylose) and branch (amylopectin) molecules, the strength of which depending on the degree of molecular association and arrangement is a factor that controls the behavior of the granules in water. The limited elasticity of the micellar network results in starch granules, possessing only a limited cold water sorbing capacity and ability for reversible swelling. As the hydrogen bonds linking the molecules in the micellar network become disrupted by heat in the presence of excess water, the hydration of the network becomes encouraged and an irreversible process of swelling begins at a critical temperature known as the gelatinization or pasting temperature.

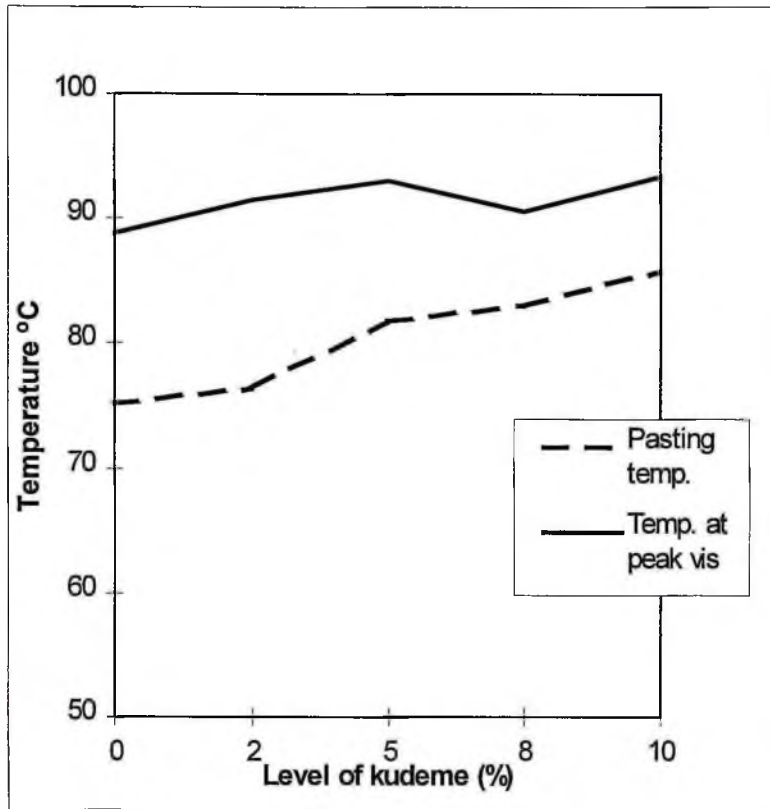
One of the practical properties of starch is its ability to swell and yield viscous paste when water suspensions of the granules are heated above the gelatinization temperature. The swelling of the granules upon prolonged heating leads to changes in viscosity of the paste. Thus continued heating in the presence of excess water of starch granules results in a complete loss of crystallinity and the point at which gel formation first appears is regarded as the gelatinization temperature. And this usually occurs over a narrow temperature range with larger granules gelatinizing first and smaller granules later. During gelatinization, granules swell extensively. The viscosity of the paste results from the flow resistance of the enlarged granules that now occupy the entire sample volume. Mild stirring can disintegrate highly swollen granules, resulting in a decrease in paste viscosity. The first highest viscosity observed prior to this decrease is known as peak viscosity. And the temperature at peak viscosity varies based on the treatment given to the starch. Viscosity at the gelatinization temperature is caused by exudates containing colloidal and molecularly dispersed starch molecules leached from the granules into the surrounding aqueous phase during the initial free swelling stage.

The temperature at peak viscosity showed similar trends with the exception of 0% and 1% levels of kudeme where the 0% level had high temperature at peak viscosity as compared to temperature at peak viscosity for 1% level of kudeme. The pasting temperatures ranged between 68.5 and 86.7°C whilst the temperature at peak viscosity ranged between 75.0 and 92.5°C (Table 8 and Table 9 respectively). The kudeme therefore raised the pasting temperature and the temperature at peak viscosity as a result of its potency in breaking down the starch molecules into finer particles. There was a high positive correlation of 98.07% between the kudeme level and pasting temperature and correlation between kudeme level and the temperature at peak viscosity was 65.49 after 48 hours of fermentation. Cornelius (1999) observed pasting temperatures of between 69.0 and 78.0° C for nixtamalized maize flour.

**Table 9. Temperature at peak viscosity (°C) of nixtamalized maize inoculated with kudeme**

Fermentation time (hrs)	Levels of kudeme (%)			
	0	1	3	5
0	76.3	75.0	88.7	90.5
12	87.2	75.0	90.7	90.4
24	87.4	75.6	92.0	90.1
48	89.9	78.5	92.5	90.5
72	89.3	79.0	92.3	90.0

**Figure 3: Pasting temperature and temperature at peak viscosity for roasted kudeme inoculated fermented (48Hrs) nixtamalized maize**

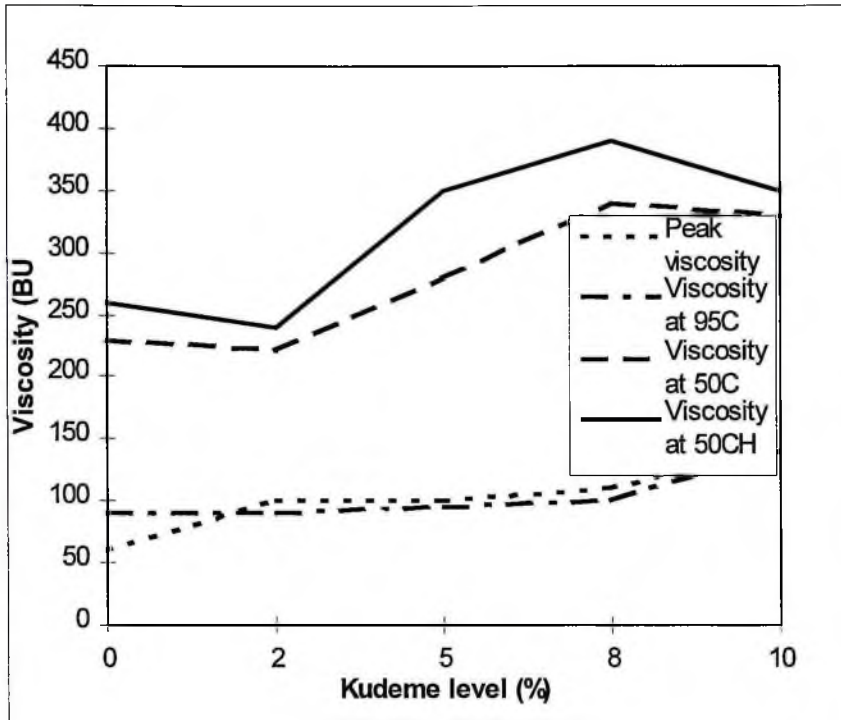


**b. Peak viscosity**

The initial peak viscosity of fermented nixtamalized maize inoculated with “kudeme” ranged between 80 to 125BU and peak viscosity increased with increasing levels of kudeme but remained slightly constant during fermentation. The peak viscosity obtained at each level of kudeme was dependent on the time of fermentation. According to Cornelius (1999), nixtamalized maize flour had more distinct peaks than raw maize flour.

Prolonged heating of the paste results in the disintegration of the granules, which leads to a decrease in paste viscosity after the initial peak viscosity. At this stage of starch cooking, two processes, occurring simultaneously but affecting the viscosity in opposite directions control the viscosity of the paste. In the first instance, swelling of the granules and release of the exudates increase the viscosity of the paste and secondly, weakening of bonding forces within the granules and their physical breakdown, decrease the viscosity of the paste. A correlation coefficient of 0.9105 was recorded between the level of “kudeme” and peak viscosity. This is due to the fact that the fineness of the samples increased with increasing levels of kudeme for a constant fermentation time.

**Figure 4: Peak viscosity, viscosity at 95°C and viscosity at both 50°C and 50°C-hold for fermented (48Hrs) nixtamalized maize inoculated with roasted kudeme.**



Starch pastes are generally considered as non-Newtonian systems since their viscosity generally depends on the rate of their shear. Starch paste are also thixotropic in nature exhibiting gel characteristics when quiescent and fluid properties when a shearing force is applied.

As starch granules swell and gelatinization temperature is attained, there is increase in viscosity as swollen granules come into contact with each other and requires more work to pass each other as they continue to swell. The process of leaching starts with dissolving molecules of low molecular weights from the surface of granules and as water penetrates the elastic meshes of granules; other molecules of low molecular weight within granules also become dissolved. As granules swell to the point where granules occupy the entire volume, the soluble portions of the exudates re-diffuse back into the swollen granules and the system becomes a gel-like continuum. At this stage granules become highly susceptible to thermal or mechanical breakdown due to both their highly swollen conditions and progressive weakening of bonding forces within the micellar lattice. The highest viscosity attained after the pasting temperature prior to this breakdown is known as the peak viscosity.

**c. Viscosity at 95°C and 95°C-hold**

However, for nixtamalized maize inoculated with kudeme, the viscosity remained almost constant after the observed peak viscosity and viscosity at 95°C, were comparable to the peak viscosity with relative increase in viscosity at 95°C for samples without kudeme and those with 1% kudeme levels. Kudeme level still had significant influence on this index with a positive correlation of 80.48%. Viscosity at 95°C-hold were higher than peak viscosity and viscosity at 95°C, but there were no significant effects by any of the variables to this index; independently or by interaction (Table 10). The viscosity ranged between 100 to 160 BU, reducing with increasing fermentation time. In solid state fermentation, Anim (1991) and Osa Mensah (1991), observed that fermentation of maize

dough increases cooked paste viscosity but Cornelius (1999), indicated that nixtamalization reduces the viscosity of fermented maize dough, with the highest influence on set-back viscosity.

**d. Viscosity at 50°C and 50°C-hold**

Continuous heating at 95°C-hold in the presence of shear due to stirring produced a paste that is a mixture of swollen granules, granule fragments and dispersed starch molecules from the granules. As the temperature subsequently decreased to 50°C, the elements present in the paste starts to associate or retrograde, then further increase in viscosity occurred. Factors believed to influence differences in viscosity at 50°C are the particle sizes of starch granules, since finer particles retrograde at a faster and easier rate than larger ones. Viscosity at 50°C for preliminary study on nixtamalized maize inoculated with “kudeme” ranged between 180 to 600 BU and at 50°C-hold the range was between 210 to 480 BU. The highest viscosity was obtained at 50°C (Fig.4). Analysis of variance for viscosity at 50°C revealed that the main effects of “kudeme” level and fermentation produced a significant F-ratio of 17.11\*\*\*, with “kudeme” level producing a significant F-ratio of 28.95\*\* and fermentation time, 8.22\*\*\* (Table 10). The viscosity recorded at 50°C at each “kudeme” level was therefore dependent on the fermentation time. The correlation coefficients calculated between kudeme level and viscosity at 50°C as well as 50°C-hold was 0.9442 and 0.8451 respectively. Thus the level of kudeme actually modifies the physical feature, such as texture of nixtamalized maize during fermentation, which subsequently affects the visco- amylograph properties of nixtamalized maize.

**Table 10. F-Ratio and significant levels for various parameters on the effect of kudeme and fermentation time on nixtamalized maize during pre-testing of nixtamalized maize – kudeme fermenting system.**

Parameter	Main effects	Kudeme level	fermentation Time
Moisture	0.45	0.35	0.51
pH	29.58***	1.69	50.50***
Titration Acidity	59.44***	13.16***	94.15***
Water Absorption 25°C	0.47	0.36	0.55
Water Absorption 70°C	2.48	2.02	2.83
Pasting Temperature	18.38***	42.53***	0.27
Temp. @ Peak vis.	16.48***	33.99***	2.69
Peak Viscosity	16.482***	37.46***	0.750
Viscosity @ 95°C	3.12*	6.50**	0.59
Viscosity @ 95°C- Hold	2.61	0.79	3.99
Viscosity @ 50°C	17.11***	28.95**	8.22***
Viscosity @ 50°C- Hold	22.09***	38.62***	9.68***

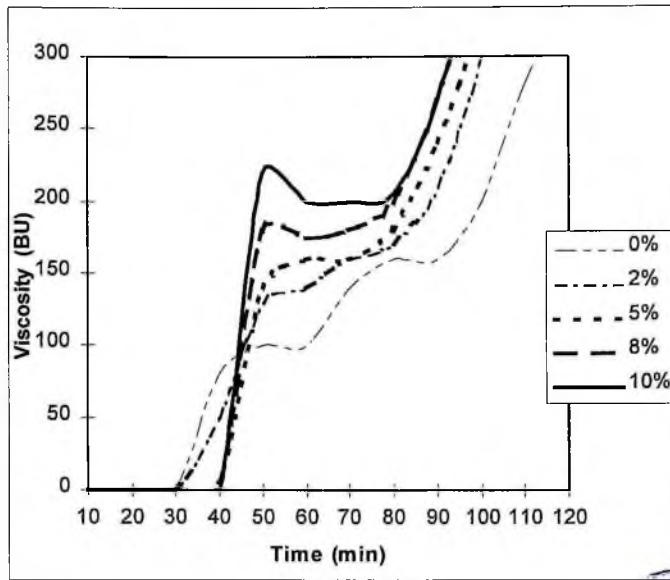
\*  $\approx p \leq 0.05$     \*\*  $\approx p = 0.01$     \*\*\*  $p = 0.001$

#### 4.1.4 Brabender viscoamylograph of nixtamalized maize during cooking at varying levels of kudeme and slurry concentrations

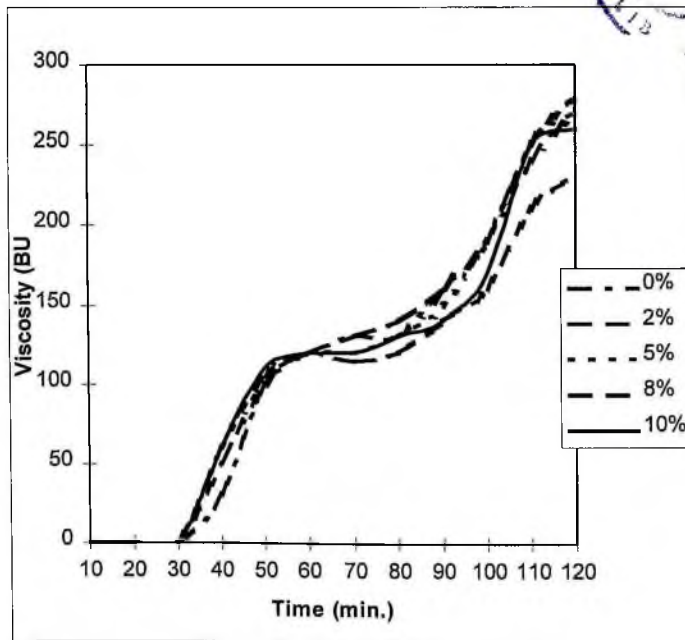
Nixtamalized maize with 0% and 2% roasted kudeme begun pasting 30min from start of cooking time, whilst nixtamalized maize with 5% to 10% kudeme level begun pasting 40min from start of cooking time (Fig. 5A). It is postulated that the granules of 0 and 2% kudeme level nixtamalized maize are bigger and therefore imbibed water at a faster rate resulting in increased granule size which led to high flow resistance earlier than was observed for nixtamalized maize with more than 5% kudeme levels, which were believed to have finer starch granules and therefore delayed initial flow resistance (initial stage of pasting). Figure 5A, also showed that viscosity generally increases

**Figure 5: Brabender amylograms of nixtamalized maize (7%) inoculated with roasted kudeme (A) and blanched kudeme (B)**

A



B



with increase in kudeme level with simultaneous increase in peak viscosity as kudeme level increases. Nixtamalized maize without kudeme recorded a peak viscosity of about 100 BU whilst those with 8 and 10% kudeme registered around 200 BU for peak viscosity (Fig. 5A). However the peak viscosity of samples with higher levels of kudeme was subsequently more distinct than those with lower or 0-level kudeme. The highest viscosity recorded at the end of 120 minutes was 600 BU for the sample with 10% kudeme level of fermented nixtamalized maize of roasted kudeme.

However in the case of the nixtamalized maize of blanched kudeme however, kudeme level did not show a clear distinct variation in the rate of viscosity and viscosity recorded was generally lower than those recorded for nixtamalized maize of roasted kudeme (Fig. 5B). Pasting, begun 30 minutes from start of cooking time for all fermented nixtamalized maize of blanched kudeme in spite of the varying levels of kudeme. In this instance, the 8% kudeme level of blanched kudeme inoculated nixtamalized maize recorded the lowest final paste viscosity after 120 min. The highest viscosity was however recorded during the cooling stages for both types of kudeme inoculated nixtamalized maize. Cornelius (1999) also recorded highest viscosity at this stage for nixtamalized maize. For effective kudeme activity on fermenting nixtamalized maize, the kudeme level should exceed 3% as suggested by earlier researchers as the amount of seed inoculant required in fermenting systems (Lincoln, 1960; Meyrath and Suchanek, 1972). From the above results, roasted kudeme could be said to produce higher levels of microbial and enzymatic activity and therefore produced better Viscoamylograph properties of kudeme inoculated nixtamalized maize.

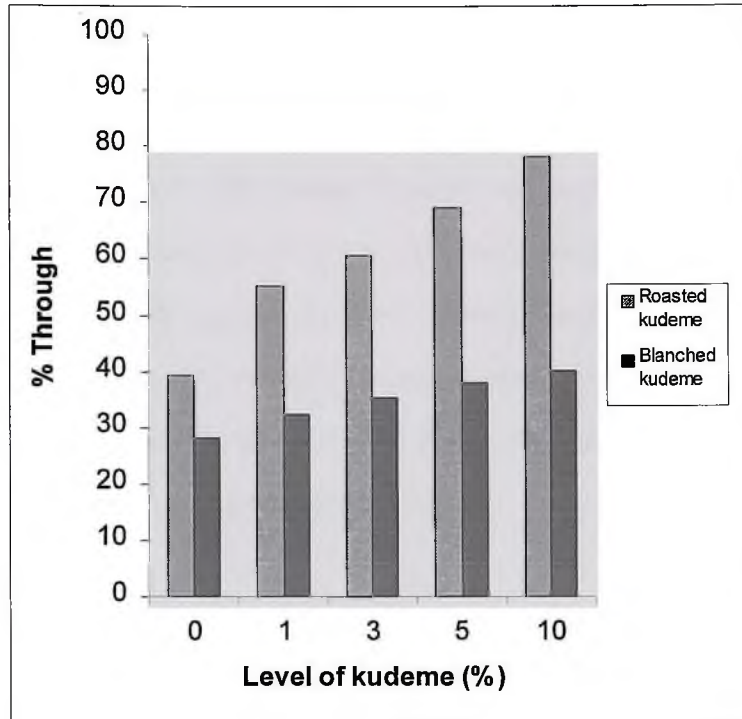
The amylograph conveniently verifies the starch liquefying properties of alpha-amylase from fungal and bacterial sources from the kudeme used in the inoculation of fermented nixtamalized maize. The diastatic activity of alpha-amylase as well as beta-amylase and other enzymes present in

“kudeme” and the susceptibility of nixtamalized maize to enzyme attack may all be dependent on the quantity of “kudeme” used. Swelling of the starch granules and their gelatinization first causes the rise in viscosity as temperature of starch slurry increases with time. The second stage involves the liquefaction of the gelatinized starch granules by the enzymes present from the “kudeme” inoculated nixtamalized maize. Amoa-Awua and Jakobsen (1995) indicated that *Bacillus* isolates from kudeme produced wide spectrum of enzymes; including amylases, cellulases and polygalacturonases. Amoa-Awua *et al.* (1997), also reported that yeast and moulds from “kudeme” were able to produce tissue-degrading enzymes which degrade cassava tissues during agbelima processing. The maximum or peak viscosity reached may be used as an index of the degree of microbial and biochemical enzyme activity within fermented nixtamalized maize inoculated with “kudeme”. The amylograph is sensitive to small levels of enzyme activity.

#### **4.1.5 Effect of kudeme on the particle size of fermented nixtamalized maize**

In this research, a wet sample particle size determination was carried out as enumerated in the methodology, on fermented nixtamalized maize inoculated with various levels of “kudeme”. Observations from Figure 6, suggested that as kudeme level increased, the quantity of “masa” going through the sieve aperture of 125µm increased; that is, the product becomes finer with increasing kudeme level as observed for pure cassava system (Sefa-Dedeh, 1989). Whereas over 50% of masa with kudeme passed through the sieve aperture of 125µm, less than 40% of masa with 0% kudeme could pass through this sieve aperture. This was the case for fermented nixtamalized maize of roasted kudeme.

**Figure 6: Percentage of nixtamalized maize inoculated with kudeme passing through 125 $\mu$ m sieve aperture**

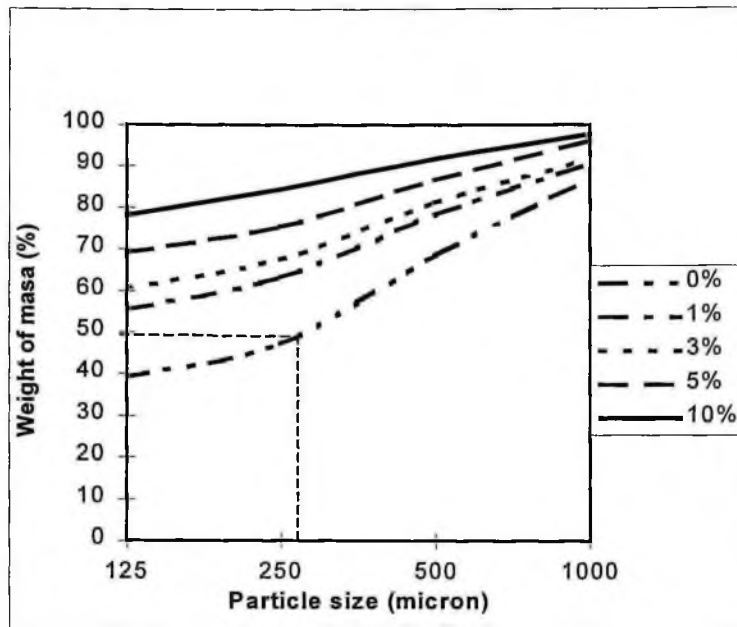


The sample containing the blanched kudeme however did not have the same degree of fineness. The blanched kudeme inoculated nixtamalized maize had less than 40% of its particle size distribution below 125 $\mu$ m (Fig. 6). The mean particle diameter ( $Sd_{50}$ ), which is the estimated sieve through which 50% of the sample would pass, increased with increasing kudeme level for blanched kudeme inoculated nixtamalized maize. The  $Sd_{50}$  for roasted kudeme inoculated nixtamalized maize were all above the 125 $\mu$ m minimum sieve aperture (Fig. 7A). Figure 7B however showed that the  $Sd_{50}$  for nixtamalized maize inoculated with blanched kudeme were mostly above 250 $\mu$ m, with the exception of samples having more than 5% kudeme. The sample containing the roasted kudeme was more effective in reducing the particle size of the fermenting nixtamalized maize and even the addition of 1% kudeme (Fig. 7A), resulted in a product where over 50% of the particles passed through a sieve size of 125 $\mu$ m. The sample with the blanched kudeme showed that with increasing kudeme level the nixtamalized maize became finer (Fig. 7B).

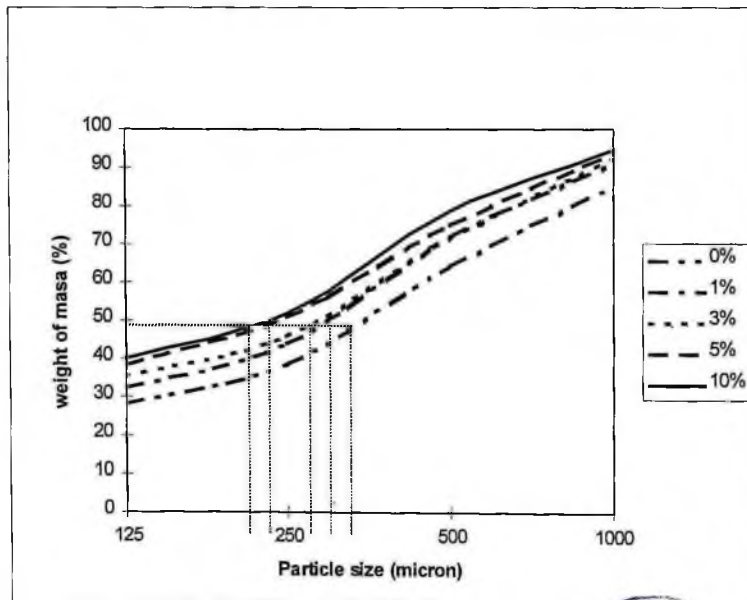
The particle size is believed to be a major factor for the observed variation in peak viscosity and viscosity in general, as these correlated positively with finer particles size giving higher peak viscosity. Differences in pasting behaviors may be due to increased concentration of free starch granules in small particles. The free starch granules were responsible for increased rapid peak viscosity during pasting in roasted kudeme inoculated nixtamalized maize whilst slower water diffusion into coarse particles and limited swelling of starch granules accounted for the low viscosity and peak viscosity in samples without kudeme and those inoculated with blanched kudeme. Sefa-Dedeh (1989), observed that particle size affected the viscosity characteristics of maize and cassava dough and concluded that the finer the dough, the higher the viscosity and indicated that the gelatinization or pasting temperature tended to increase as the particle size of samples increased.

**Figure 7: Particle size profile for nixtamalized maize inoculated with roasted (A) and blanched (B) kudeme after 48hr fermentation**

A



B



From these deductions, it was inferred that the different trends observed for viscosity and other cooking properties of nixtamalized maize inoculated with kudeme, resulted from the ability of kudeme to breakdown starch during fermentation and this was more pronounced in the roasted kudeme inoculated nixtamalized maize than the blanched kudeme inoculated nixtamalized maize.

The particle size of dough or flour of cereals is critical in determining the functional properties of the flour or dough in various foods). Sefa-Dedeh, (1989), evaluated the quality characteristics of cassava and maize dough to verify the role played by particle size in the performance of these dough in traditional African foods. In addition, due to lack of standard techniques for measuring “masa” functionality, processors and or manufacturers use “masa” flour particle size distribution and rheological characteristics in an attempt to predict its end use. Particle size distribution is therefore considered the most important criterion for “masa” flour applications, and in this regard different sieve fractions (particle sizes) are blended to obtain optimum particle size distribution suitable for different applications (Gomez *et al.*, 1991). It is generally believed that finer particles are responsible for most of the water uptake, cohesiveness, plasticity, and smoothness of “masa”, whilst coarse particles are considered responsible for crispiness and blistering products, which normally disrupt the dough network of most products (Gomez *et al.*, 1991). Pfulgfelder *et al* (1988) concluded that “masa” cannot be considered as a homogenous dough but a complex mixture of fractions whose reactions and interactions determines the behavior of the dough and that re-hydrated “masa” flour dough functionality and textural attributes are governed by properties of the constituent particle size fractions. Gomez *et al* (1991) therefore studied particle size distribution and functionality of commercial “masa” and observed differences in starch pasting, x-ray crystallinity and apparent solubility of various fractions.

## 4.2 Multiple regression and response surface optimization results on the effect of kudeme, moisture and fermentation on the chemical components of nixtamalized maize.

### 4.2.1 Moisture profile of fermenting nixtamalized maize inoculated with kudeme

The regression model developed for moisture of nixtamalized maize inoculated with roasted kudeme was;

$$Z = 46.644672 + 0.027465X_1 - 0.0661675X_2 - 0.007528X_1X_2 + 0.027764X_1X_3 - 0.000543X_1X_2X_3 + 0.014054X_1^2 + 0.014717X_2^2 + 0.000467X_3^2,$$

with an  $R^2$  of 98.05%. In foods water is not just a medium for reactions, but also an active ingredient used to control reactions, texture and general physical and biological behaviour. For this index, the most significant variable contributing to the model was the initial moisture level, which contributed as much as 97% independently to this model. ANOVA for the full regression had an F-ratio of 120.689\*\*\* as indicated in Table 11.

**Table 11. ANOVA summary for the full regression models with lack of fit for chemical properties of nixtamalized maize inoculated with roasted kudeme.**

Sources of variation	Moisture F-ratio	pH F-ratio	Acidity F-ratio	Ash F-ratio	Protein F-ratio
Model	120.6890**	12.7717**	64.9214**	1.6055	2.9730*
Lack of Fit	0.6127	1.2960	0.3040		1.2593

For nixtamalized maize inoculated with blanched kudeme, the model generated for moisture was;

$$Z = 20.685085 + 0.284359 X_2 - 0.103187X_3 + 0.03745 X_1X_3 + 0.001679 X_2X_3 - 0.000669 X_1X_2X_3 + 0.003736 X_1^2 + 0.006104 X_2^2,$$

with a high  $R^2$  of 99.73% and an insignificant lack of fit; Table 12

**Table 12. ANOVA summary for the full regression models with lack of fit for chemical properties of nixtamalized maize inoculated with blanched kudeme.**

Sources of variation	Moisture F-ratio	PH F-ratio	Ash F-ratio	Protein F-ratio
Model	996.5970**	9.0046**	1.6023	2.8818*
Lack of Fit	3.4243	1.2554	-	1.3330

Significant variables affecting this model were moisture, fermentation time and interaction between all 3-independent variables. In both instances, the initial moisture levels had the most significant effect on the final moisture level contributing over 90% to the final model. However roasted kudeme influenced the level of moisture in nixtamalized maize to a greater extent as compared with the blanched kudeme. The F-values for the contributing variables in this model is summarized in Table 13 for roasted kudeme inoculated nixtamalized maize and Table 14 for blanched kudeme inoculated nixtamalized maize respectively.

**Table 13. ANOVA summary (showing only F-values) for chemical properties of nixtamalized maize inoculated with roasted kudeme.**

Sources of Variation	Moisture	pH	Acidity	Ash	Protein
X <sub>1</sub>	4.99*	20.84**	172.95**	0.13	
X <sub>2</sub>	950.04**	1.00	0.06		1.56
X <sub>3</sub>		25.10**	-	0.14	6.66*
X <sub>1</sub> X <sub>2</sub>	2.42				0.00
X <sub>1</sub> X <sub>3</sub>	0.02	0.95	193.32**		-
X <sub>2</sub> X <sub>3</sub>			3.62	0.07	
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	-1.1477			0.9077	5.24*
X <sub>1</sub> <sup>2</sup>	0.48		16.82**	4.49*	2.69
X <sub>2</sub> <sup>2</sup>	4.75*	16.85**	60.11**	1.19	-
X <sub>3</sub> <sup>2</sup>	1.50	11.89**	7.56**	4.39*	1.70

\* sig. at p ≤ 0.05

\*\* sig. at p ≤ 0.01

**Table 14. ANOVA summary (showing only F-values) for chemical properties of nixtamalized maize inoculated with blanched kudeme.**

Sources of Variation	Moisture	PH	Ash	Protein
X <sub>1</sub>	-	5.86*	0.44	1.91
X <sub>2</sub>	6941.30**	-	0.00	1.19
X <sub>3</sub>	10.94**	40.27**	1.14	4.97*
X <sub>1</sub> X <sub>2</sub>	-	0.56	0.00	6.37*
X <sub>1</sub> X <sub>3</sub>	0.18	-	0.52	2.11
X <sub>2</sub> X <sub>3</sub>	2.58	3.33	0.25	3.95
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	14.03**	-	8.30**	1.11
X <sub>1</sub> <sup>2</sup>	1.00	1.66	-	2.89
X <sub>2</sub> <sup>2</sup>	6.16*	-	1.18	-
X <sub>3</sub> <sup>2</sup>	-	2.35	2.58	1.43

\* sig. at  $p \leq 0.05$       \*\* sig. at  $p \leq 0.01$

(X<sub>1</sub> = kudeme level; X<sub>2</sub> = moisture level; X<sub>3</sub> = fermentation time)

It is believed that organic acids produced by microbial activity and their enzymes reacted with Ca(OH)<sub>2</sub> to provide some extra water which helped in maintaining the moisture levels of nixtamalized maize throughout the period of fermentation. Sefa-Dedeh and Mensah (1989) reported that moisture content of fermented maize decrease with fermentation time. In Figure 8, it is observed that the moisture content decreased slightly with increasing level of kudeme but increased with fermentation time. Thus, as fermentation proceeds there is increase in the activity of microorganisms resulting in the production of acids which reacts with the Ca(OH)<sub>2</sub> to produce water. Roasted kudeme could be used in nixtamalized maize fermentation to induce a relatively constant moisture level. This is due to an indication that there was a much more prominent microbial activity in roasted kudeme inoculated nixtamalized maize fermenting system; maintaining higher moisture levels than in the blanched kudeme inoculated nixtamalized maize.

**Figure 8: Response surface plot for moisture content of fermented nixtamalized maize inoculated with roasted kudeme.**

Model;

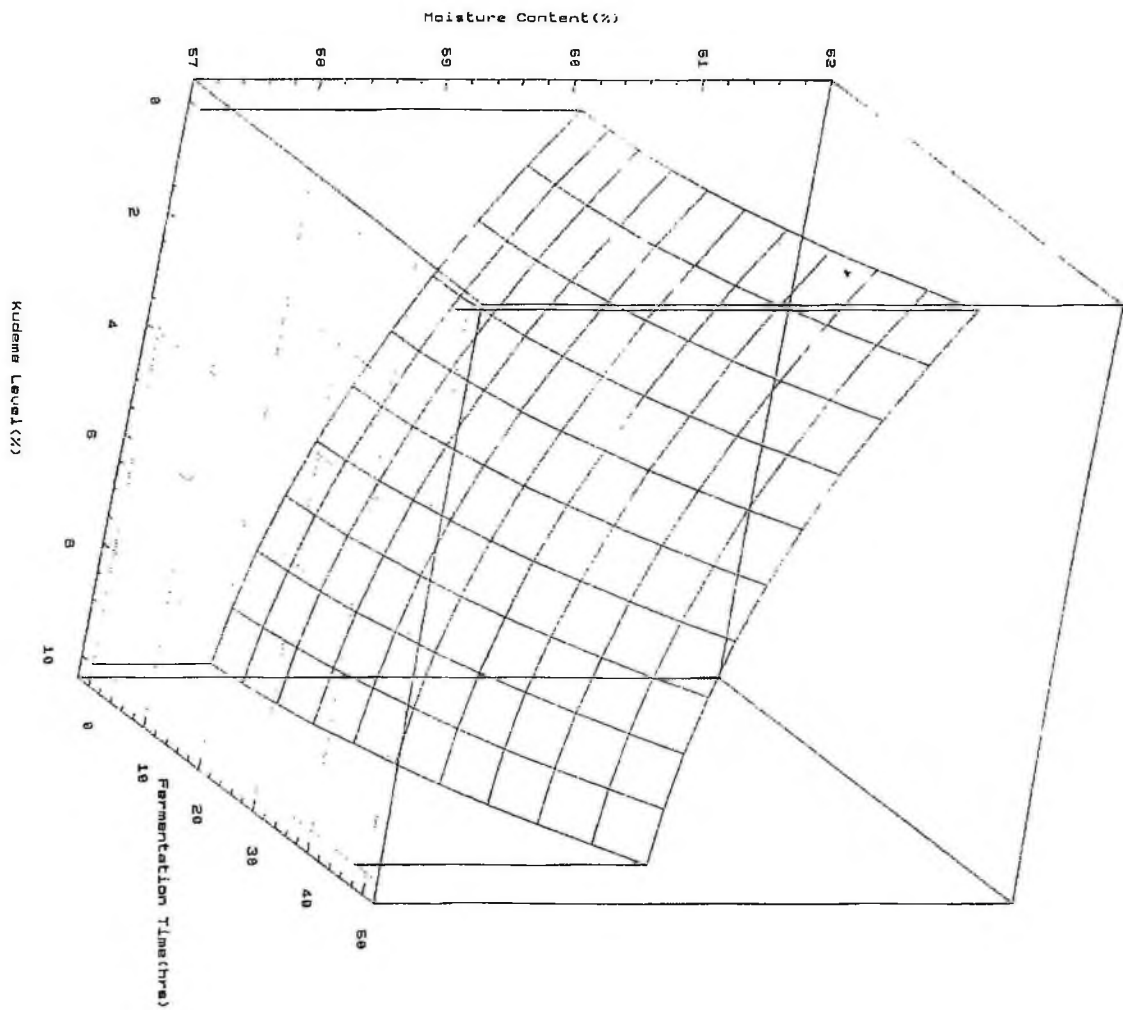
$$Z = 46.644672 + 0.027465X_1 - 0.0661675X_2 - 0.007528X_1X_2 + 0.027764X_1X_3 - 0.000543X_1X_2X_3 + 0.014054X_1^2 + 0.014717X_2^2 + 0.000467X_3^2,$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 98.05\%$



#### 4.2.2 The pH of nixtamalized maize

Fresh nixtamalized masa is a high pH product and Serna-Saldivar *et al* (1990), reported that pH of nixtamalized maize is dependent on the concentration of lime used. At the end of various fermentation times in this study, the pH ranged between 10.08 – 4.47 for nixtamalized maize inoculated with roasted kudeme and 10.17 – 8.06 for nixtamalized maize inoculated with blanched kudeme. This was a further proof that fermentation proceeded at a faster rate in roasted kudeme system than in the blanched kudeme system. The regression model with equation;

$$Z = 175.80873 - 0.531763 X_1 - 5.755638 X_2 - 0.303913 X_3 + 0.007091 X_1 X_3 + 0.050517 X_2^2 + 0.003868 X_3^2,$$

and an  $R^2$  of 78.80%, developed for fermented nixtamalized maize inoculated with roasted kudeme led to the conclusion that kudeme level influenced to a large extent the pH of fermenting nixtamalized maize inoculated with this kudeme. The pH at all levels of kudeme concentration decreased with fermentation time to a minimum, followed by increase. This suggested that there was an optimum fermentation time for the lowest pH (Fig. 9). For example, to obtain a pH of 4.0 at a moisture content of 55% and kudeme level of 5%, the nixtamalized maize dough should have been fermented for 49 hours, similarly, while a pH of 3.5 at the same level for moisture and kudeme the nixtamalized maize dough would need to be fermented for at least 53 hours. All 3-independent variables contributed to some degree to this parameter even though preliminary research on kudeme inoculated fermenting nixtamalized maize system did not reveal the significant effect of kudeme level to this index. The high amount of acid produced as kudeme level increases and as fermentation proceeds resulted in the reduction in pH.

Fermentation time however had the highest influence on the response model. ANOVA for the full regression for this index was significant with an F-ratio of 12.7717\*\*\*, but there was no significant lack of fit for the model  $F = 1.2960$  (Table 11). The regression model developed for pH was therefore adequate in explaining the variation in this index. The dominant microflora of lactic acid bacteria and yeasts species as identified by Adjei (1990), to be present in kudeme and therefore present in the kudeme inoculated nixtamalized maize were mostly responsible for the production of the acids within the fermenting medium.

The regression model developed for pH of nixtamalized maize inoculated with blanched kudeme was;

$$Z = 10.55376 - 0.465087 X_1 + 0.10512 X_3 + 0.00557 X_1 X_2 - 0.001944 X_2 X_3 + 0.008772 X_1^2 - 0.000512 X_3^2,$$

and had an  $R^2$  of 71.65%. There was no significant lack of fit for this model. There was however variations in the performance of the two different types of kudeme to this response index which could be due to differences in their treatments. Whereas the roasted kudeme was prepared by roasting fresh semi-matured cassava chips over fire and fermented with an old kudeme cloth the blanched kudeme was boiled in water briefly prior to storage in the old kudeme cloth. The microbial profile could be different and there could be loss of starch molecules during the processing of the blanched type of kudeme. From the above observation the roasted kudeme could be said to have a higher microbial activity level when inoculated into nixtamalized maize as compared to its blanched counterpart.

**Figure 9: Response surface plot for pH of fermented nixtamalized maize inoculated with roasted kudeme at moisture level.**

Model;

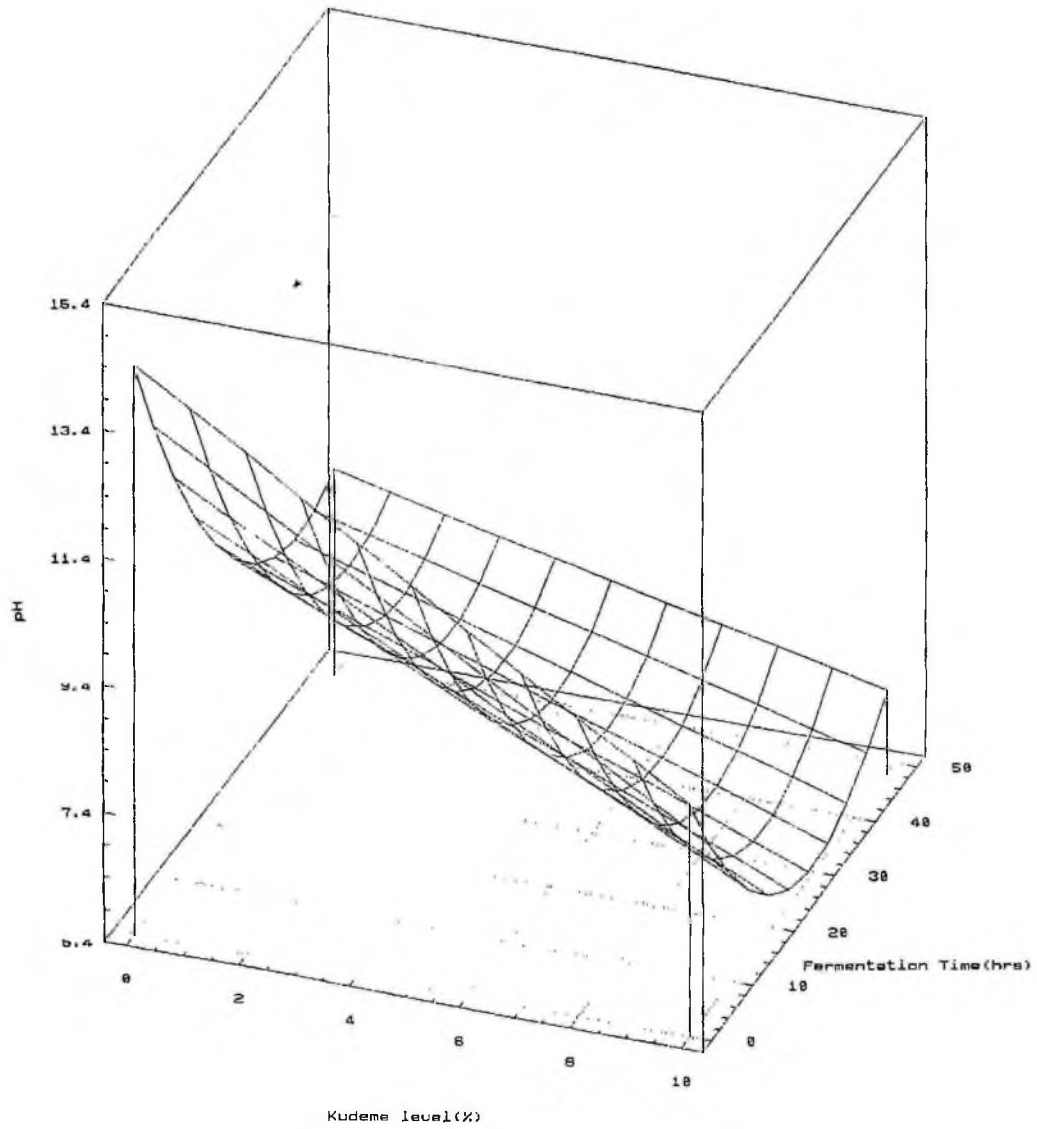
$$Z = 175.80873 - 0.531763 X_1 - 5.755638 X_2 - 0.303913 X_3 + 0.007091 X_1 X_3 + 0.050517 X_2^2 + 0.003868 X_3^2,$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 78.80\%$



The model developed for roasted kudeme inoculated nixtamalized maize could explain to a high degree;  $R^2$  of 78.80% compared to 71.65% for samples inoculated with blanched kudeme.

Cornelius (1999), observed an  $R^2$  of 71.97% for pH with a non significant F-ratio of 2.719 for lack of fit and found the quadratic term of lime concentration to be the most important process variable that influence the pH.

#### **4.2.3 Response on titrable acidity by the influence of kudeme, moisture and fermentation on nixtamalized maize.**

Cornelius (1999), reported titrable acidity, range between 0.210 to 0.560g/100gLA with model explaining 43.93% of the variation in this index for cowpea fortified nixtamalized maize with a lack of fit of 2.936.

In this study however, the model;

$$Z = -1.953974 - 0.007017X_1 + 0.068913X_2 + 0.000357X_1X_3 + 0.00000772563X_2X_3 + 0.000525 X_1^2 - 0.000602 X_2^2 - 0.00002 X_3^2,$$

developed for titrable acidity of nixtamalized maize inoculated with roasted kudeme, explained the 95.93% of the variation observed in this dependent variable by the independent variables. The most significant variables influencing titrable acidity included the interaction between kudeme and fermentation period, which contributed as much as 77.29% to the total output of this model. ANOVA for the full regression was significant with an F-ratio of 64.921\*\*\* and an insignificant lack of fit of F-ratio, 0.3040 as indicated in Table 11.

**Figure 10: Response surface plots for titrable acidity of fermented nixtamalized maize, inoculated with roasted kudeme.**

Model;

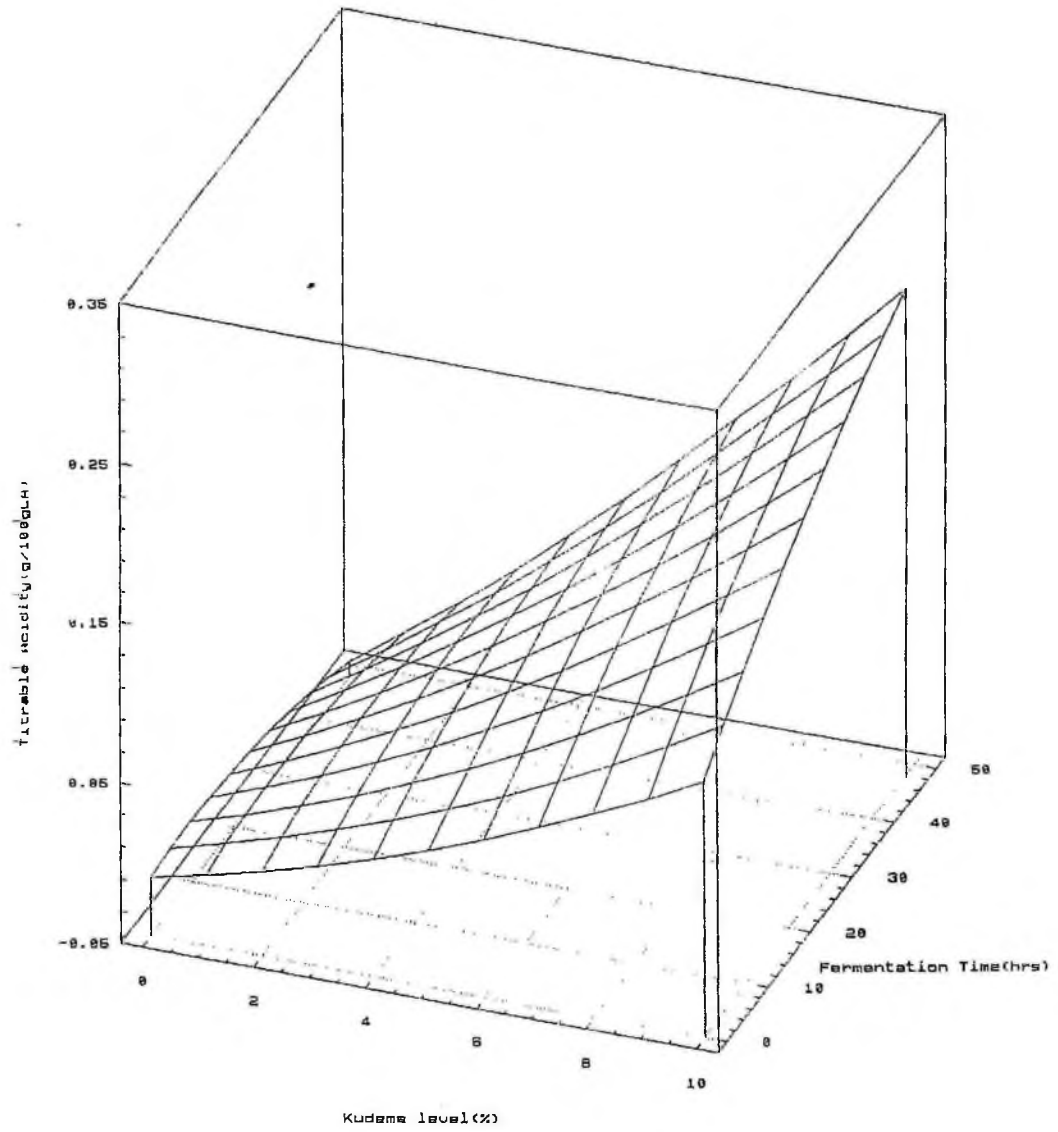
$$Z = -1.953974 - 0.007017X_1 + 0.068913X_2 + 0.000357X_1X_3 + 0.00000772563X_2X_3 + 0.000525 X_1^2 - 0.000602 X_2^2 - 0.00002 X_3^2,$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 95.93\%$



Since the pHs of all the nixtamalized maize inoculated with blanched kudeme were in the neutral region (pH above 7.0), there was no evidence of acidity on analyses and therefore no model could be developed for titrable acidity of blanched kudeme inoculated fermented nixtamalized maize. There were however slight reduction in the initial pH of nixtamalized maize after inoculation with blanched kudeme and fermentation but these were never below the pH of 7 which is the neutral pH level. This difference in the degree of acid production by the two different kudeme could have resulted from a longer and slower lag phase in the growth and activity of microorganisms present in the blanched kudeme in alkaline medium as compared to the high and shorter time span in the lag phase of microorganisms in the roasted kudeme. The acidity recorded for the roasted kudeme inoculated fermented nixtamalized maize resulted from the breakdown of starch into organic acids with lactic acid being the most predominant. The degree of fermentation was highly pronounced in this system as compared with that containing the blanched kudeme. From the response surface plot (Fig. 10), titrable acidity increased with increasing levels of kudeme and subsequently with increasing fermentation time. The acids were also believed to be reacting with the base to form other molecular substances with increase in microbial activity via increasing kudeme levels though increase in fermentation time resulted in increased production of acids irrespective of the level of kudeme.

#### **4.2.4 Total mineral and protein quality of nixtamalized maize inoculated with kudeme.**

Total mineral content of fermented nixtamalized maize inoculated with kudeme was measured in terms of total ash content. The regression model developed for fermented nixtamalized maize inoculated with roasted and blanched kudeme could explain only 18.24% and 22.20% respectively of the variation in this index, with ANOVA for both models being insignificant. These models were therefore not adequate for explaining the variations in this index and were subsequently discarded

in any further statistical evaluation. This was an indication that kudeme level and fermentation could not significantly affect the predictive levels of ash within fermented nixtamalized maize inoculated with kudeme. The quantity of kudeme used could have decreased the ash content of nixtamalized maize. The activity of microorganisms during fermentation may not result in any significant increase in total ash, though the mineral value of the fermented nixtamalized maize might have been enhanced. Research has however established that the level of calcium in the maize is also enhanced via the process of nixtamalization as well as the increased availability of other minerals.

The model developed for predicting and optimizing the protein content of fermented nixtamalized maize inoculated with kudeme could however explain 38.37 and 47.13% of the variations observed in this index for nixtamalized maize of roasted and blanched kudeme respectively.

The model;

$$Z = 8.244469 + 0.032548X_2 - 0.060765X_3 - 0.00453X_1X_2 + 0.000089X_1X_2X_3 + 0.013804X_1^2 + 0.000455X_3^2$$

developed for this index of fermented nixtamalized maize inoculated with roasted kudeme had a significant F-ratio of 2.9730\* for ANOVA of full regression model and a lack of fit with F-ratio of 1.2593 ( Table 11).

For fermented kudeme inoculated nixtamalized maize of blanched kudeme, the following model was developed;

$$Z = 19.005082 - 1.593356X_1 - 0.16329X_2 - 0.291556X_3 + 0.02418X_1X_2 + 0.026483X_1X_3 + 0.00442X_2X_3 - 0.000416X_1X_2X_3 + 0.011285X_1^2 + 0.000326X_3^2$$

From Table 12, it could be observed that the ANOVA for the full regression was significant with an F-ratio of 2.8818\* and an insignificant lack of fit of 1.3330\*.

Fermentation contributed significantly to the protein content of fermented nixtamalized maize inoculated with kudeme. Increase in the time of fermentation results in the slight increase of the protein content of nixtamalized maize.

The solubility of all protein fractions is decreased from raw maize to tortilla (product of nixtamalized maize), with an increase in the insoluble fraction. In-vitro enzymatic studies of amino acids indicated that total nitrogen was released faster from maize than from tortillas. Values for alpha-amino nitrogen released, expressed as a percentage of the total nitrogen released were higher for tortillas than raw maize after 12 hours of hydrolysis. The amino acid concentration of maize and tortilla hydrolysates reached comparable levels except for methionine (Bressani and Scrimshaw, 1958).

The process of fermentation may not have any significant effect on total protein content, but could result in the qualitative modification of proteins, often resulting in the increase of water-soluble proteins and free essential amino acids. These changes could be effected by endogenous protease,

but have also been attributed to proteolytic activity of some of the microorganism responsible for cereal fermentation (Chavan and Kadam, 1989).

#### **4.3 Impact on bulk volume of nixtamalized maize by kudeme, moisture and fermentation.**

The regression model developed for this index could explain only 36.23% of the variation observed in this index for nixtamalized maize inoculated with roasted kudeme and only 10.16% of the variation observed in this index for nixtamalized maize inoculated with blanched kudeme. In both instances only the quadratic term of kudeme level significantly contributed to this model. ANOVA for the full regression model in both instances was also not significant, indicating an insignificant variation in the contribution by independent variables to the regression model developed for bulk volume. And thus, models for this index were not suitable for explaining and predicting the variability in this index. The bulk or packed volume of flour samples were very close and this might have been the contributing factor for the inadequacy of generated models in explaining whatever variations might have been present within this index resulting from any of the independent variables.

#### **4.4 Swelling capacities at varying temperatures and times by the effect of kudeme, moisture and fermentation on nixtamalized maize.**

Swelling is the expansion accompanying the uptake of water. The water molecules enter the starch molecule structure and cause solvation of the macromolecules and further occupy the capillary and intramolecular spaces of the molecules (Sefa-Dedeh *et al.*, 1979). The imbibition of water is mostly due to proteins and is influenced by the presence of hydrophilic polysaccharides such as starch. The extent of swelling varies with the nature of macromolecules, particle size, pH and ionic strength (Kinsella, 1976). Saalia (1995) further reiterated that swelling capacity is a function of process

conditions, nature of material used with biochemical and biophysical changes. Biopolymers such as starch and proteins also contribute to the development of this characteristic. It is also temperature dependent, since at high temperatures, proteins become denatured with resultant unfolding of polypeptide chains and there is also partial breakdown and softening of starch granules. Swelling of starch granules is the first stage in the initiation of changes in hydration related properties. Undamaged starch granules are not soluble in cold water and at low temperatures, but can reversibly imbibe water and swell slightly. The percentage increase in granule diameter ranges from 9.1% for normal cornstarch to 22.7% for waxy corn. However as the temperature increases, the starch molecules vibrate more vigorously, breaking intermolecular bonds and allowing their hydrogen bonding sites to engage more water molecules. This penetration of water and the increased separation of more and longer segments of starch chains, increase randomness in the general structure and decrease the number and size of crystalline regions.

**Table 15. ANOVA summary (showing only F-values) for bulk volume and swelling index of nixtamalized maize inoculated with roasted and blanched kudeme.**

Sources of variation	Bulk vol. Roasted Kudeme	Bulk vol. blanched kudeme	Swelling capacity (1hr)			
			Roasted kudeme		Blanched kudeme	
			25°C	70°C	25°C	70°C
X <sub>1</sub>	0.54	0.19		2.45	3.24	3.08
X <sub>2</sub>	2.08	-	11.06**	9.37**	0.33	0.17
X <sub>3</sub>	0.29		2.15	0.33	3.86	6.19*
X <sub>1</sub> X <sub>2</sub>	0.49		19.61**	-		
X <sub>1</sub> X <sub>3</sub>	4.44	-	-	-		
X <sub>2</sub> X <sub>3</sub>	0.00	0.05	-	2.62	1.19	-
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	1.4047	0.78	30.93**	3.13*	-	
X <sub>1</sub> <sup>2</sup>	8.46	5.04	-	4.17*	8.98**	6.48*
X <sub>2</sub> <sup>2</sup>	1.52		5.46	4.72*	2.02	10.57**
X <sub>3</sub> <sup>2</sup>	-	1.09	8.17**	6.01*	2.46	5.89*

\* sig. at  $p \leq 0.05$

\*\* sig. at  $p \leq 0.01$

(X<sub>1</sub> = kudeme level; X<sub>2</sub> = moisture level; X<sub>3</sub> = fermentation time)

**Table 16. ANOVA summary for the full regression models with lack of fit for swelling index at 25°C and 70°C, of nixtamalized maize inoculated with roasted and blanched kudeme.**

Sources of variation	Roasted kudeme		Blanched kudeme	
	25°C	70°C	25°C	70°C
Model	12.8961**	4.1730**	3.1532*	5.3978**
Lack of Fit	0.2849	1.1346	0.8556	0.7828

The swelling index, which is given by the volume of sample after incubation per initial volume of sample was computed for nixtamalized maize, inoculated with roasted or blanched kudeme. ANOVA, for samples were significant for swelling indices computed for one-hour period of swelling. The  $R^2$  recorded for the models developed for swelling indices of roasted kudeme inoculated nixtamalized maize at 25°C and 70°C were 78.98% and 57.19% respectively. For the blanched kudeme inoculated nixtamalized maize, 44.24% and 58.14%  $R^2$  values were recorded for swelling index models at 25°C and 70°C respectively.

The model developed for the swelling index after 1hour period of swelling at 25°C for nixtamalized maize inoculated with roasted kudeme was;

$$Z = -4.922456 + 0.222059X_2 + 0.023582X_3 + 0.001419X_1X_2 - 0.000043X_1X_2X_3 - 0.001852X_2^2 - 0.0002X_3^2$$

At 70°C however the following model was obtained for the roasted kudeme inoculated nixtamalized maize;

$$Z = 9.927736 + 0.108177X_1 - 0.314572X_2 + 0.082226X_3 - 0.000965X_2X_3 - 0.00032X_1X_2X_3 - 0.00822X_1^2 + 0.003172 X_2^2 - 0.000396 X_3^2$$

The F-values of the contributing variables in these models are summarized in Table 15, whilst Table 16 shows the ANOVA for the full regression models of this index with their lack of fit. From Table 15, it is observed that the quadratic term of the kudeme contributed to these models. Figure 11 shows that at 25°C, swelling index increased with increasing kudeme level and with increasing fermentation time, but at 70°C (Fig. 12), the reversed trend was observed for swelling index.

**Figure 11: Response surface plots for swelling index at 25°C after 60min. for fermented nixtamalized maize inoculated with roasted kudeme.**

Model

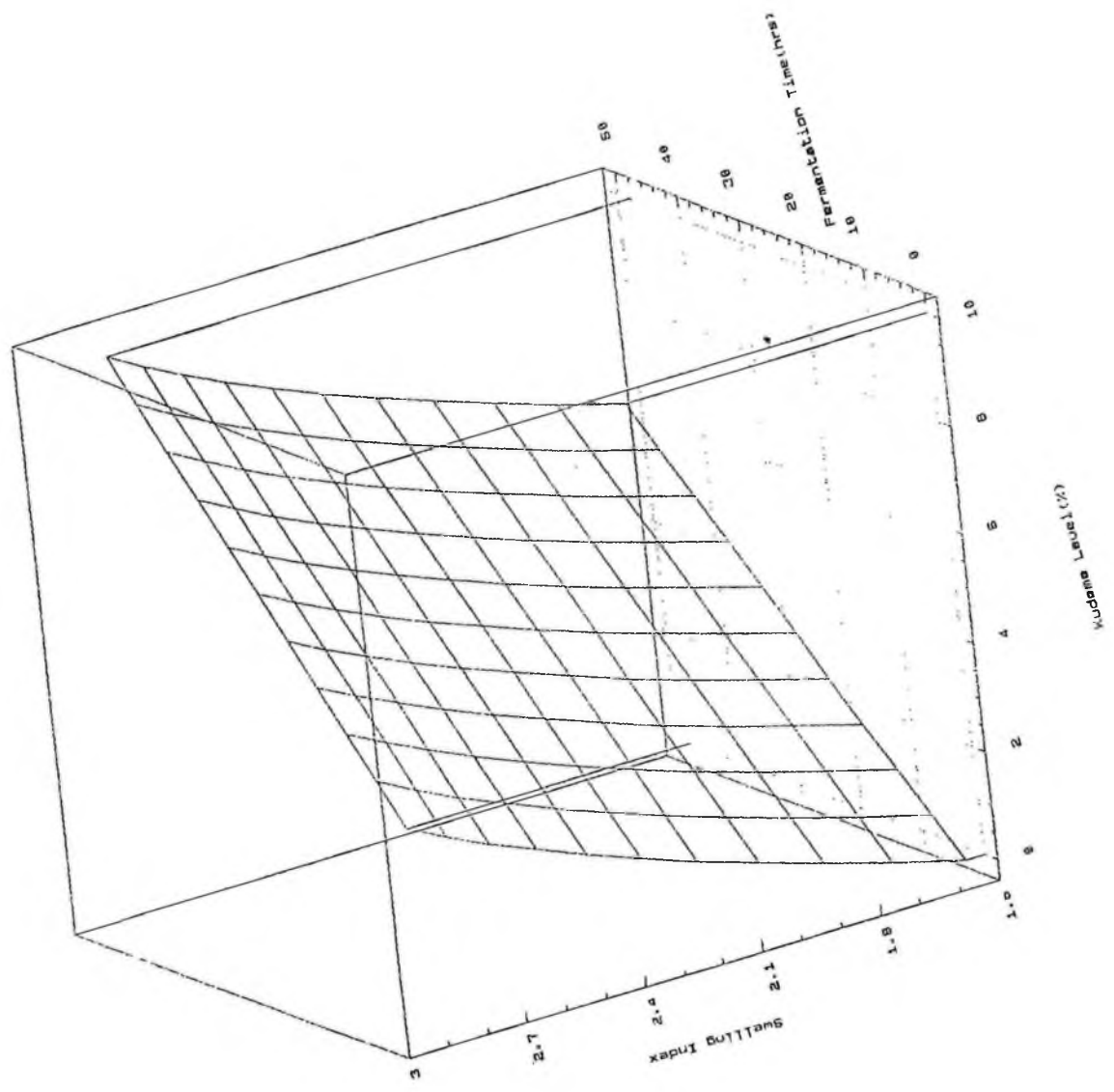
$$Z = -4.922456 + 0.222059X_2 + 0.023582X_3 + 0.001419X_1X_2 - 0.000043X_1X_2X_3 - 0.001852X_2^2 - 0.0002X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 78.98\%$



**Figure 12: Response surface plot for swelling index at 70°C after 60min. for fermented nixtamalized maize inoculated with roasted kudeme.**

Model;

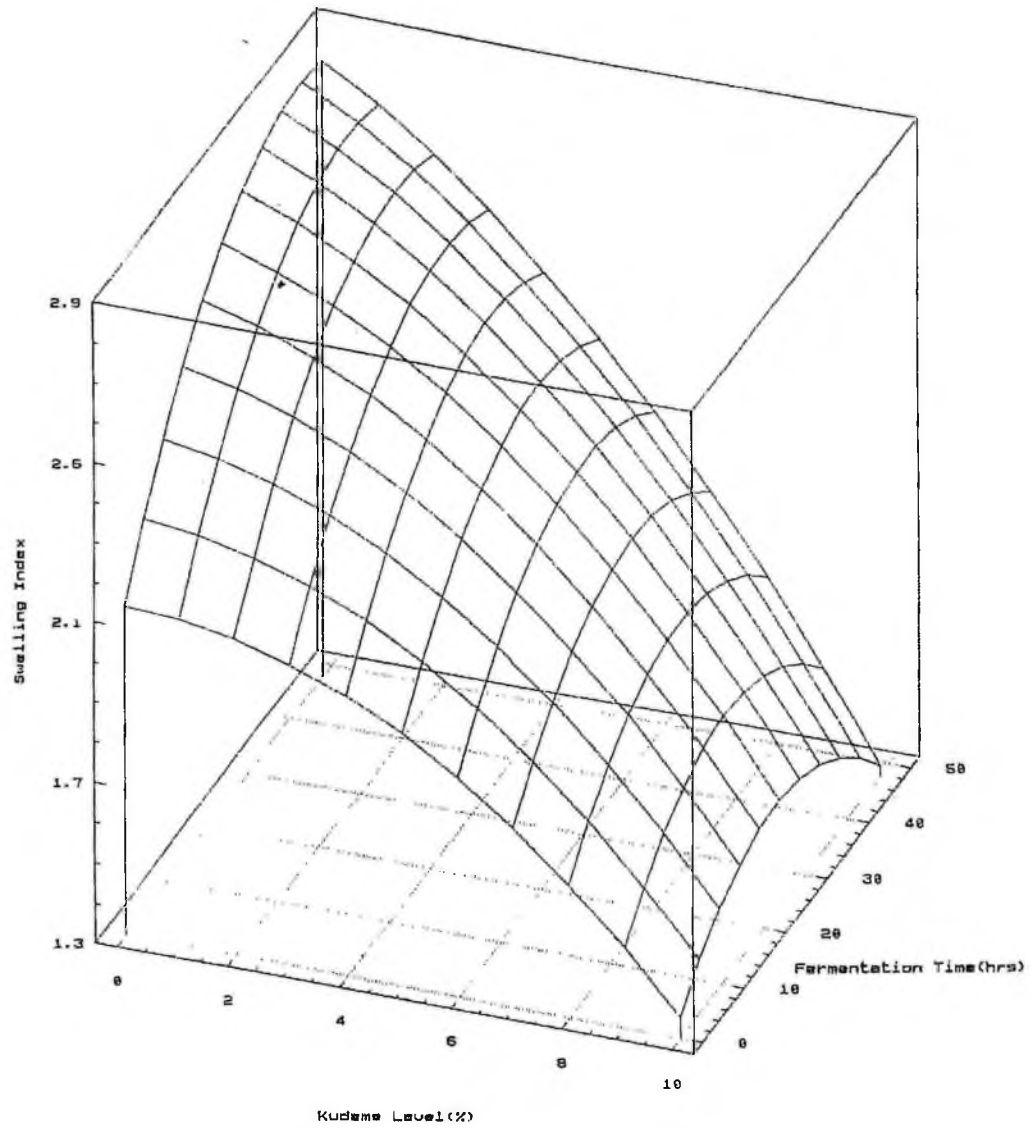
$$Z = 9.927736 + 0.108177X_1 + 0.314572X_2 + 0.082226X_3 - 0.000965X_2X_3 - 0.00032X_1X_2X_3 - 0.00822X_1^2 + 0.003172X_2^2 - 0.000396X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 57.19\%$



#### 4.5 The influence of kudeme, moisture and fermentation on water absorption capacities of nixtamalized maize

Attraction of water to carbohydrates is one of their basic and most useful physical properties. Hydrophilicity is expected in starch or carbohydrate systems because of their numerous hydroxyl groups. Hydroxyl groups interact with water molecules by hydrogen bonding, and lead to solvation of their polymers, and the structure of the carbohydrate and/or the treatment of the starch affect the rate of water binding and the amounts of water bound. The ability to bind and control water activity in foods is one of the most important properties of carbohydrates. The model developed for predicting water absorption at 25°C for nixtamalized maize inoculated with roasted kudeme was;

$$Z = 465.68931 - 11.381381X_2 + 0.143054X_1X_2 - 0.007632X_2X_3 - 0.739008X_1^2 + 0.067143X_2^2 - 0.010422X_3^2.$$

The multiple regression model developed for predicting water absorption at 25°C had R<sup>2</sup> values of 75.74% and 75.71% for nixtamalized maize inoculated with roasted and blanched kudeme respectively, with insignificant lacks of fit suggesting that models were adequate for predicting the outcome of this index at 25°C. However preliminary results on the effect of kudeme and fermentation on this index for nixtamalized maize inoculated with roasted kudeme did not show any significant effect at both 25°C and 70°C.

The quadratic term of kudeme level contributed 19.36% to this model whilst the moisture content contributed as much as 57.11%. In this index therefore, the moisture level seem to have the highest influence on the outcome of the model. From the response surface plot (Fig. 13), the water absorption capacity decreases with increasing levels of kudeme and duration of fermentation time at 25°C, an indication that the increasing levels in moisture during fermentation and increasing

kudeme level resulted in lower water absorption capacity irrespective of the increase in fineness of the dough with increasing levels of kudeme on fermentation. This could be further attributed to the nearly constant moisture levels of the dough throughout the period of fermentation as a result of acids reacting with the major base  $\text{Ca}(\text{OH})_2$  to produce water within the fermenting medium, and the increased activity of microorganisms which further boost the water availability as starch gets broken down into smaller molecules. The F-values of the variables within the model for water absorption capacities are indicated in Table 17.

For nixtamalized maize with blanched kudeme, the model generated was;

$$Z = -477.307214 - 83.739337X_1 + 23.348759X_2 + 1.442346X_1X_2 + 2.139008X_1X_3 - 0.03743X_1X_2X_3 + 0.057694X_1^2 - 0.2389X_2^2 - 0.001435X_3^2$$

**Table 17. ANOVA summary (showing only F-values) for water absorption capacity at 25°C and 70°C, of nixtamalized maize inoculated with roasted and blanched kudeme.**

Sources of variation	Roasted kudeme		Blanched kudeme	
	25°C	70°C	25°C	70°C
X <sub>1</sub>	-	8.87**	0.84	-
X <sub>2</sub>	46.50**	19.29**	12.20**	157.08**
X <sub>3</sub>	-	1.94	-	0.63
X <sub>1</sub> X <sub>2</sub>	1.99	4.52*	9.63**	-
X <sub>1</sub> X <sub>3</sub>	0.22	-	1.14	-
X <sub>2</sub> X <sub>3</sub>	-	8.70**	-	3.29
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	-	-	35.51**	-
X <sub>1</sub> <sup>2</sup>	14.32**	18.53**	0.32	0.67
X <sub>2</sub> <sup>2</sup>	0.78	1.91	7.49**	21.61**
X <sub>3</sub> <sup>2</sup>	1.51	3.03	0.08	9.23**

\* sig. at  $p \leq 0.05$       \*\* sig. at  $p \leq 0.01$

(X<sub>1</sub> = kudeme level; X<sub>2</sub> = moisture level; X<sub>3</sub> = fermentation time)

ANOVA for this model was significant with an F-ratio of 8.4018\*\*\* and an insignificant lack of fit as shown in Table 18. The moisture level contributed to the water absorption at 25°C for nixtamalized maize inoculated with kudeme whilst fermentation independently and interactively contributed to a lower extent.

**Table 18. ANOVA summary for the full regression models with lack of fit for water absorption capacity at 25°C and 70°C, of nixtamalized maize inoculated with roasted and blanched kudeme.**

Sources of variation	Roasted kudeme		Blanched kudeme	
	25°C	70°C	25°C	70°C
Model	10.8868**	8.2850**	8.4018**	32.0864**
Lack of Fit	1.1317	1.2095	1.0986	0.8484

**Figure 13: Response surface plot for water absorption at 25°C for nixtamalized maize fermented with roasted kudeme**

Model;

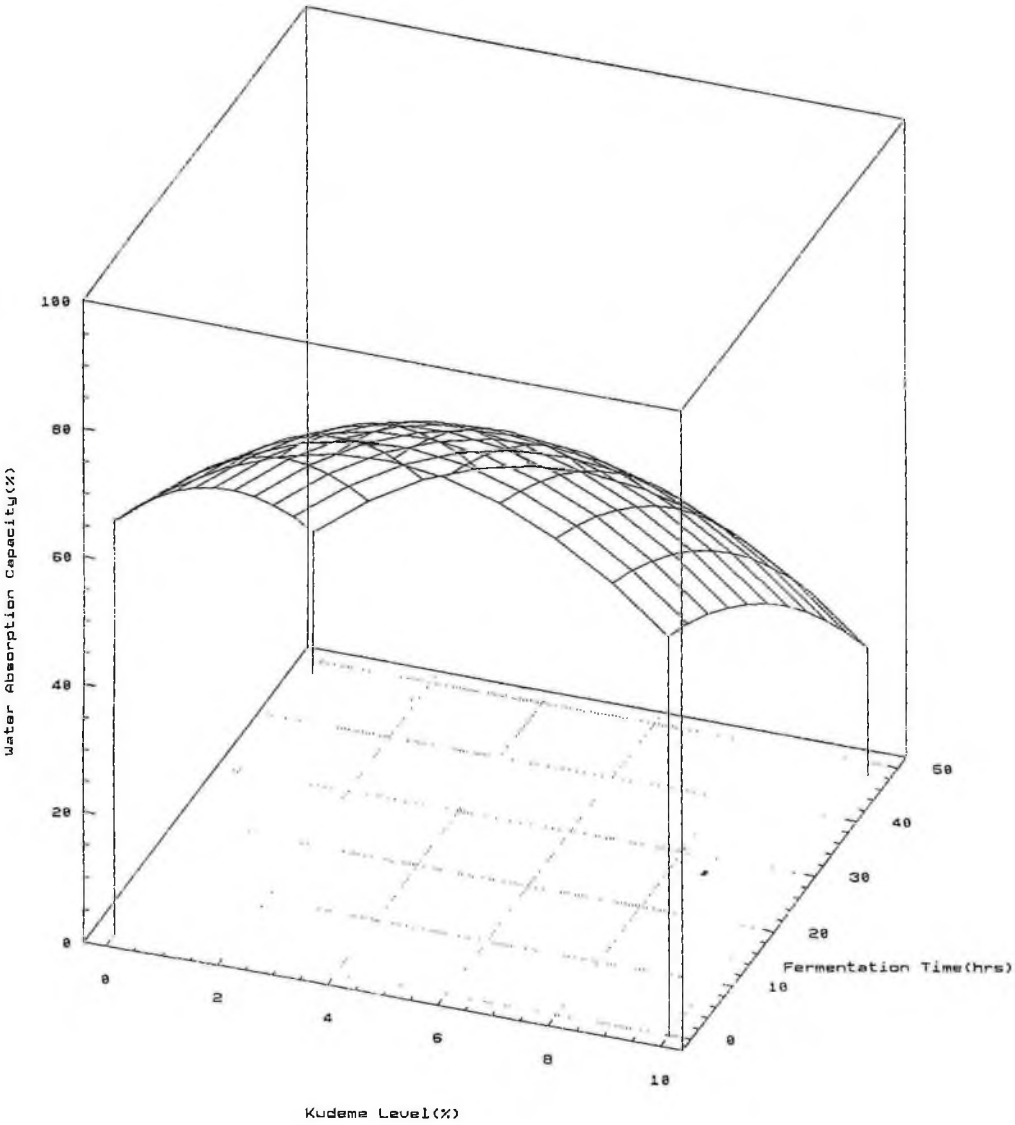
$$Z = 465.68931 - 11.381381X_2 + 0.143054X_1X_2 - 0.007632X_2X_3 - 0.739008X_1^2 + 0.067143X_2^2 - 0.010422X_3^2.$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 75.74\%$



Water absorption at 70°C was not so different in terms of contributing variables to the models generated for blanched and roasted kudeme inoculated nixtamalized maize. For roasted kudeme, the quadratic term of kudeme contributed 21.22% to the output of this model. However the model for blanched kudeme inoculated nixtamalized maize had a very high R<sup>2</sup> value of 90.76% and the quadratic term of moisture contributed as high as 78.58% to the model; being the only major contributing variable in this case. The lack of fit for this model was insignificant with an F-ratio of 0.8484; Table 18.

The model for the blanched sample was;

$$Z = -673.981424 + 28.1992X_2 + 3.066219X_3 - 0.041087X_1X_3 - 0.036634X_1^2 - 0.263632X_2^2 - 0.015953X_3^2$$

For the nixtamalized maize inoculated with roasted kudeme, the model generated for water absorption at 70°C was;

$$Z = -291.087735 + 32.742137X_1 + 14.90041X_2 - 6.081058X_3 - 0.426166X_1X_2 + 0.123384X_2X_3 - 1.021434 X_1^2 - 0.15422 X_2^2 - 0.016947 X_3^2$$

having an R<sup>2</sup> of 75.41%.



**Figure 14: Response surface plots for water absorption at 70°C for nixtamalized maize fermented with roasted kudeme**

Model;

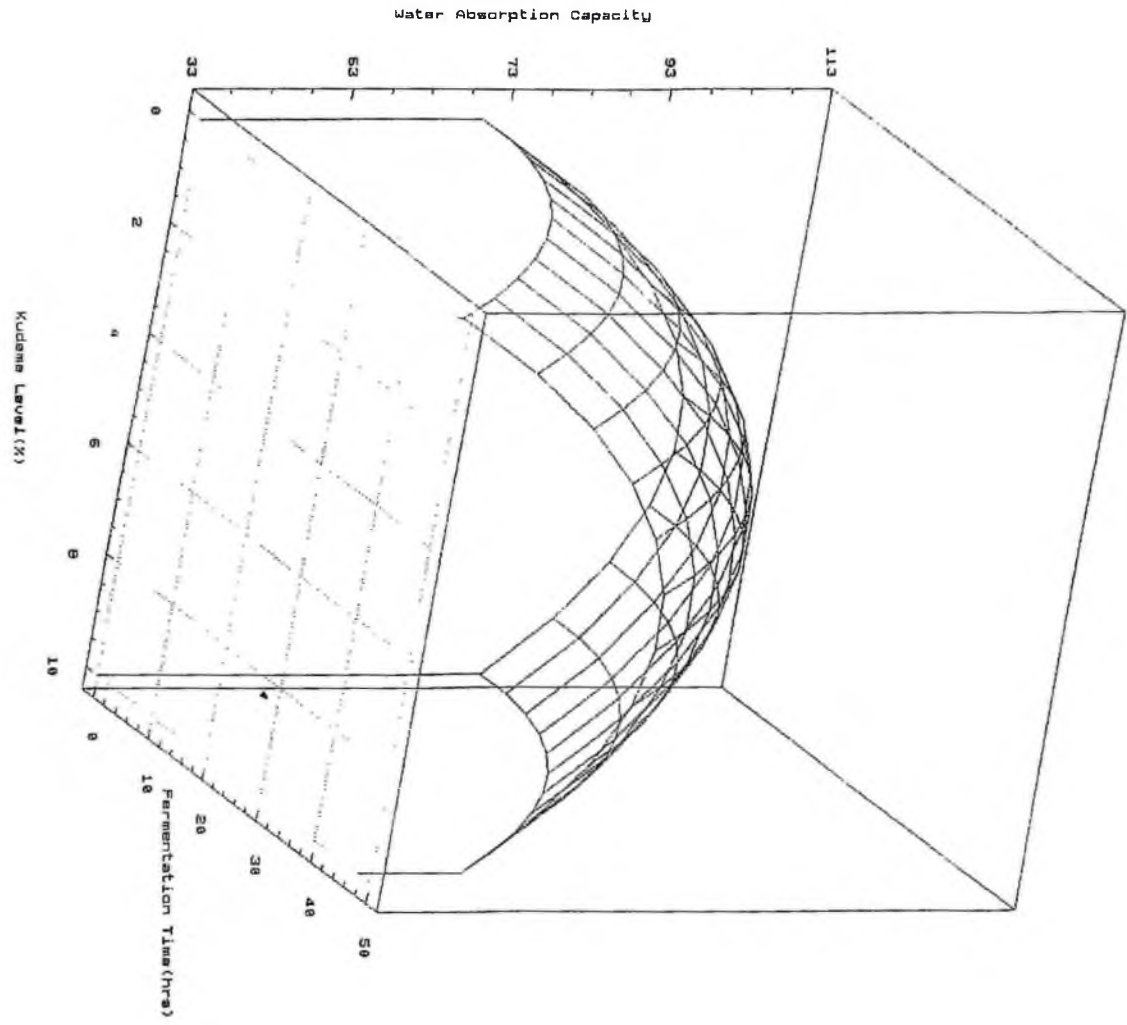
$$Z = -291.087735 + 32.742137X_1 + 14.90041X_2 - 6.081058X_3 - 0.426166X_1X_2 + 0.123384X_2X_3 - 1.021434 X_1^2 - 0.15422 X_2^2 - 0.016947 X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 75.41\%$



The contribution of kudeme to water absorption by fermented nixtamalized maize inoculated with kudeme was more obvious for roasted kudeme than blanched kudeme and with better predictive models at 70°C than at 25°C water absorption temperatures. Cornelius (1999), observed that water absorption at 70°C of cowpea fortified nixtamalized maize did not only depend on cowpea and lime concentration whilst Sefa-Dedeh (1989) also indicated that proteins were the primary sites for water absorption, but also on the moisture content, that influenced the degree and speed of fermentation. In this study, the response surface plot for water absorption at 70°C (Fig. 14), showed that water absorption increased with increasing kudeme level at 70°C, but decreased with increasing fermentation time. Whereas at 25°C, (room temperature), water absorption decreased due to increased moisture content during fermentation, at 70°C, some amount of water was absorbed, this increases with increase in temperature.

#### **4.6 Color profile of fermented nixtamalized maize inoculated with kudeme.**

Colour as perceived has 3-dimensions; hue, chroma and lightness. Chromaticity includes hue and chroma (saturation), specified by two chromaticity co-ordinates, but since the two coordinates cannot describe a colour completely; a lightness factor was included, to identify a specimen precisely. The hunter lab colour system was developed in 1966 so that the degree of difference in measured values would closely match the degree of perceived colour difference. "L" is the lightness variable "a" and "b" are the chromaticity coordinates with the total colour difference being given by  $(L^2 + a^2 + b^2)^{1/2}$ . According to Cornelius (1999), 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 results from the lime used and therefore the intensity of colour is closely related to the lime concentration and even when tortillas are produced from white kernels, a high concentration of lime leads to a yellowish product (Serna-Saldivar et al., 1990).

**Table 19. ANOVA summary (showing only F-values) for colour profile of nixtamalized maize inoculated with roasted and blanched kudeme.**

Sources of variation	Roasted kudeme			Blanched kudeme		
	L-lightness	b-yellowness	a-redness	L-lightness	b-yellowness	a-redness
X <sub>1</sub>	2.12	2.93	1.41	0.17	7.75**	7.98**
X <sub>2</sub>	-	0.13	0.01	0.53	15.73**	0.52
X <sub>3</sub>	-	2.25	0.39	0.40	47.34**	0.71
X <sub>1</sub> X <sub>2</sub>	-	-	-	-	-	-
X <sub>1</sub> X <sub>3</sub>	-	3.90	0.48	6.79*	-	-
X <sub>2</sub> X <sub>3</sub>	1.07	-	-	2.39	2.94	-
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	-	-	-	0.99	-	4.28
X <sub>1</sub> <sup>2</sup>	5.85*	1.60	6.89*	2.12	-	0.04
X <sub>2</sub> <sup>2</sup>	-	6.85*	14.05**	11.09**	28.88**	6.21*
X <sub>3</sub> <sup>2</sup>	0.72	-	-	3.99	12.57**	15.02**

\* sig. at  $p \leq 0.05$       \*\* sig. at  $p \leq 0.01$

(X<sub>1</sub> = kudeme level; X<sub>2</sub> = moisture level; X<sub>3</sub> = fermentation time)

The L-values (lightness), for nixtamalized maize inoculated with roasted kudeme ranged between 77.95 – 93.47 and that for blanched kudeme inoculated nixtamalized maize ranged from 82.92 to 85.44. Cornelius (1999), however recorded a range of 75.24 – 83.88 L-values for maize cooked and steeped in 1% lime and raw maize respectively. The b-values (yellowness) ranged from 15.05 – 22.90 and 18.32 – 24.16 for nixtamalized maize inoculated with roasted and blanched kudeme respectively, while the a-values (redness), ranged between a low negative value of -0.31 to 2.12 and 0.85 to 1.59, respectively for nixtamalized maize inoculated with roasted and blanched kudeme. The total colour therefore ranged from 13.75 to 26.97 and 22.52 to 27.52 for roasted and blanched kudeme inoculated nixtamalized maize respectively. The model developed for lightness of roasted kudeme inoculated nixtamalized maize had an R<sup>2</sup> value of 66.04%, while the model for this index for the blanched kudeme inoculated nixtamalized maize had an R<sup>2</sup> of 50.62%. Table 19; provide a summary ANOVA on the F-values of contributing variables to the color profile of nixtamalized maize inoculated with kudeme.

The degree to which variation in redness was explained by models developed ( $R^2$ ) for this index was 47.54% and 59.28% for roasted and blanched kudeme inoculated nixtamalized maize respectively. For yellowness, however, the  $R^2$  – values recorded were 38.03% and 85.18% for roasted and blanched kudeme inoculated nixtamalized maize respectively.

The models developed for total colour had ideal  $R^2$  values providing the extent to which variation are explained by independent variables in this index.

**Table 20. ANOVA summary showing F-ratios for the full regression models with lack of fit for colour profile of nixtamalized maize inoculated with roasted kudeme.**

Sources of variation	Lightness F-ratio	Yellowness F-ratio	Redness F-ratio	Total-color F-ratio
Model	6.2776**	2.9436**	3.8702**	7.6027**
Lack of fit	1.2573	1.1688	1.1002	1.2231

**Table 21. ANOVA summary showing F-ratios for the full regression models with lack of fit for colour profile of nixtamalized maize inoculated with blanched kudeme.**

Sources of variation	Lightness F-ratio	Yellowness F-ratio	Redness F-ratio	Total-color F-ratio
Model	3.1642*	19.2016**	4.9517**	6.2331**
Lack of fit	1.4158	0.7006	1.2022	0.9113

The model for total colour for nixtamalized maize inoculated with roasted kudeme was;

$$Z = 467.774416 + 1.429044 X_1 - 15.648183 X_2 - 0.190798 X_1^2 + 0.136599 X_2^2 - 0.001543 X_3^2$$

and an  $R^2$  of 63.47% was deduced for this model with the quadratic term of moisture and kudeme level having F-ratio of 20.01 and 9.72\*\* respectively. ANOVA for this model was significant with an F-ratio of 7.6027\*\*\*

Figure 15, shows an initial increase followed by decreasing trends in total color with increasing kudeme level and fermentation time. This trend seem to suggest a breakdown in color resulting from possible breakdown of particle size of starch molecules by microorganisms present in kudeme which becomes increasingly prominent with increasing fermentation time. There was a bleaching effect due to decreasing pH during fermentation and this increased with increase in kudeme level and fermentation time, resulting in the decreasing trend in total color of product after fermentation.

The modeled equation for total color of nixtamalized maize inoculated with blanched kudeme was;

$$Z = -153.635935 + 0.599199X_1 + 6.228885X_2 + 0.159213X_3 - 0.000232X_1X_2 - 0.034474X_1^2 - 0.05444X_2^2 - 0.003114X_3^2$$

with an  $R^2$  of 0.6585.

**Figure 15: Response surface plot for total colour for roasted kudeme inoculated fermented nixtamalized maize**

Model;

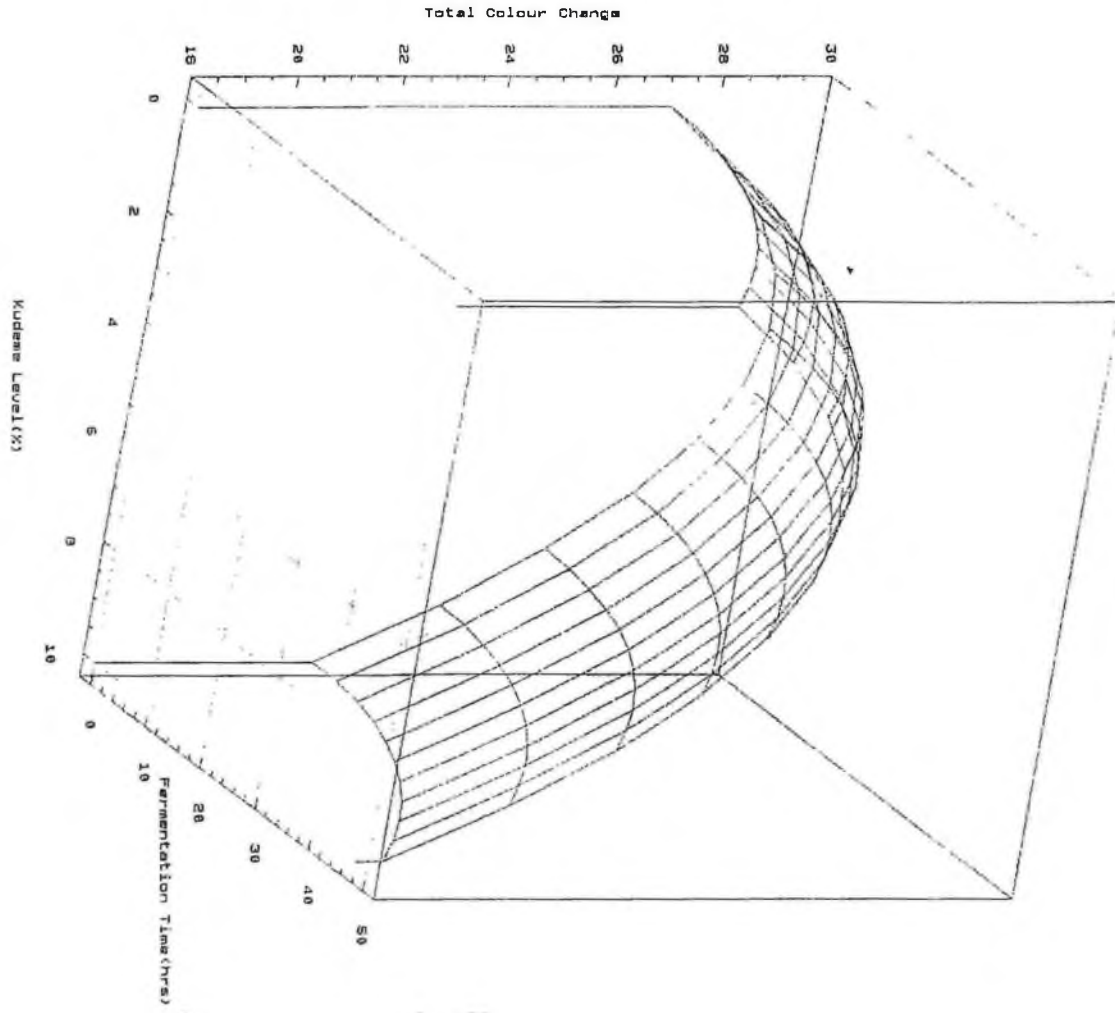
$$Z = 467.774416 + 1.429044 X_1 - 15.648183 X_2 - 0.190798 X_1^2 + 0.136599 X_2^2 - 0.001543 X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 63.47\%$



In this instance the moisture contributed as high as 80.39% to the predictive outcome of the model. The evidence that the microbial effect on the parameters of nixtamalized maize inoculated with kudeme as being more efficient in the roasted kudeme inoculated system as compared to the blanched kudeme system was once again obvious in this index. Thus as far as colour was concerned, roasted kudeme could influence this variable at the point of total colour, with moisture appearing as the most significant variable influencing hue and chromaticity in the blanched kudeme inoculated system. Alkaline cooking produces yellowish products and colour intensity, and alkaline cooked maize closely relates to carotenoid pigments, flavonoids and pH (Serna-Saldivar *et al.*, 1990). Yellowness (b-value) decreased with increase in fermentation time and kudeme level. L-values (lightness) increased to the degree to which complex starch molecules and substances were broken down into lower molecular weight carbohydrates, and this affected the colour intensity of fermented nixtamalized maize with changing pH and titrable acidity. These results are in conformity with the findings of Cornelius (1999), that fermentation breaks down the colour of nixtamalized maize resulting in increase in lightness and a reduction in yellowness of fermented nixtamalized maize. This was attributed to changes in pH and titrable acidity as a result of the breakdown of complex substances into lower molecular weight carbohydrates and organic acids.

#### **4.7 Pasting temperatures of nixtamalized maize inoculated with kudeme;**

For the different sample combinations, studied, the pasting temperature ranged from 66.5°C to 84.1°C for 8% slurry nixtamalized maize inoculated with roasted kudeme and between 69.0 to 85.8°C for 7% slurry. The pasting temperature for 7% slurry of nixtamalized maize inoculated with blanched kudeme, however ranged between 74.7°C to 77.8°C. Generally the pasting temperature increased with decreasing slurry concentrations for all samples of roasted kudeme inoculated

fermented nixtamalized maize; as observed for 8, 7, 5 and 3% slurry concentrations of samples. Some samples did not show any sign of pasting at 3% slurry concentrations during cooking.

**Table 22. ANOVA summary (Showing only F-values) for viscoamylograph indices of nixtamalized maize inoculated with roasted kudeme.**

Sources of variation	Pasting temp.	Peak viscosity	Temp. at peak viscosity	Viscosity at 95°C	Viscosity at 50°C
X <sub>1</sub>	-	-	5.65*	10.85**	17.19**
X <sub>2</sub>	-	7.58**	-	5.16*	-
X <sub>3</sub>	6.84*	-	2.46	-	-
X <sub>1</sub> X <sub>2</sub>	104.05**	-	-	2.57	6.40*
X <sub>1</sub> X <sub>3</sub>	2.04	-	0.25	0.14	1.31
X <sub>2</sub> X <sub>3</sub>	-	-	-	-	1.63
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	0.00	-	-	2.03	-
X <sub>1</sub> <sup>2</sup>	24.57**	15.73**	5.27*	6.86*	-
X <sub>2</sub> <sup>2</sup>	-	11.36**	1.18	24.92**	-
X <sub>3</sub> <sup>2</sup>	2.86	1.75	-	4.80*	0.38

\* sig. at  $p \leq 0.05$       \*\* sig. at  $p \leq 0.01$

(X<sub>1</sub> = kudeme level; X<sub>2</sub> = moisture level; X<sub>3</sub> = fermentation time)

Starch swells when heated in the presence of excess water. The beginning of flow resistance due to swelling is indicated by the pasting or gelatinization temperature, which may increase with increase in fermentation time and degree of fineness of starch granules. Cereal fermentation apart from flavour development also contributes to improving the rate and degree of starch gelatinization and therefore promotes ease of cooking (Sefa-Dedeh, 1989). The temperature at which the first detectable viscosity is measured in the amylogram is the pasting temperature, which is a reflection of the swelling potential of the starch paste and which is affected by the starch concentration and treatment given to the starch. Generally a high starch concentration leads to a low pasting temperature. The presence of monosaccharides and oligosaccharides has been reported to lead to a

shift upward in pasting temperature. Fermentation of cereal dough causes an increase in their pasting temperatures (Akpapunam and Sefa-Dedeh, 1995).

The model developed for pasting temperature at 8% slurry concentration for nixtamalized maize inoculated with roasted kudeme had an  $R^2$  value of 77.58%. Kudeme level and its quadratic term and the combined effect of kudeme and fermentation were significant variable contributors to this model. Model developed for pasting temperature of 7% slurry concentration of roasted kudeme inoculated nixtamalized maize was;

$$Z = 69.231758 + 0.124164X_3 + 0.049349X_1X_2 + 0.083478X_1X_3 - 0.001159X_1X_2X_3 - 0.199755 X_1^2 - 0.002947 X_3^2$$

with 87.61% of the variation in this index being explained adequately by independent variables within this model. The combined effect of kudeme and moisture contributed 66.06% to the variation in this model, whilst the quadratic term of kudeme contributed 12.16%, for nixtamalized maize inoculated with roasted kudeme. ANOVA for this model was significant with an F-ratio of 23.3925\*\*\* and an insignificant lack of fit (Table 23).

**Table 23. ANOVA summary for the full regression models with Lack Of Fit for Viscoamylograph indices of nixtamalized maize inoculated with roasted kudeme.**

Sources of variation	Pasting temp.	Peak viscosity	Temp. at peak viscosity	Viscosity at 95°C	Viscosity at 50°C
Model	23.3925**	9.1043**	3.0517*	7.1681**	5.3850**
Lack Of Fit	1.1346	1.1315	1.2413	1.1601	1.3330

The pasting temperature for 7% slurry concentration for this model was observed to increase with increasing levels of kudeme and fermentation time (Figure 16). This further proves the fact that the finer the particle sizes of the dough are, the higher the pasting temperature. Kudeme inoculation in combination with fermentation therefore aids in the breakdown of the texture of nixtamalized maize dough. The model generated for 5% slurry was not adequate in explaining the variation in pasting temperature,  $R^2$  value of 0.3690 with no variables demonstrating any significant contribution to the model. ANOVA for the model was also insignificant. No pasting was observed for some 3% slurry concentrations.

**Figure 16: Response surface plot for pasting temperature of 7% slurry concentration for roasted kudeme inoculated fermented nixtamalized maize**

Model;

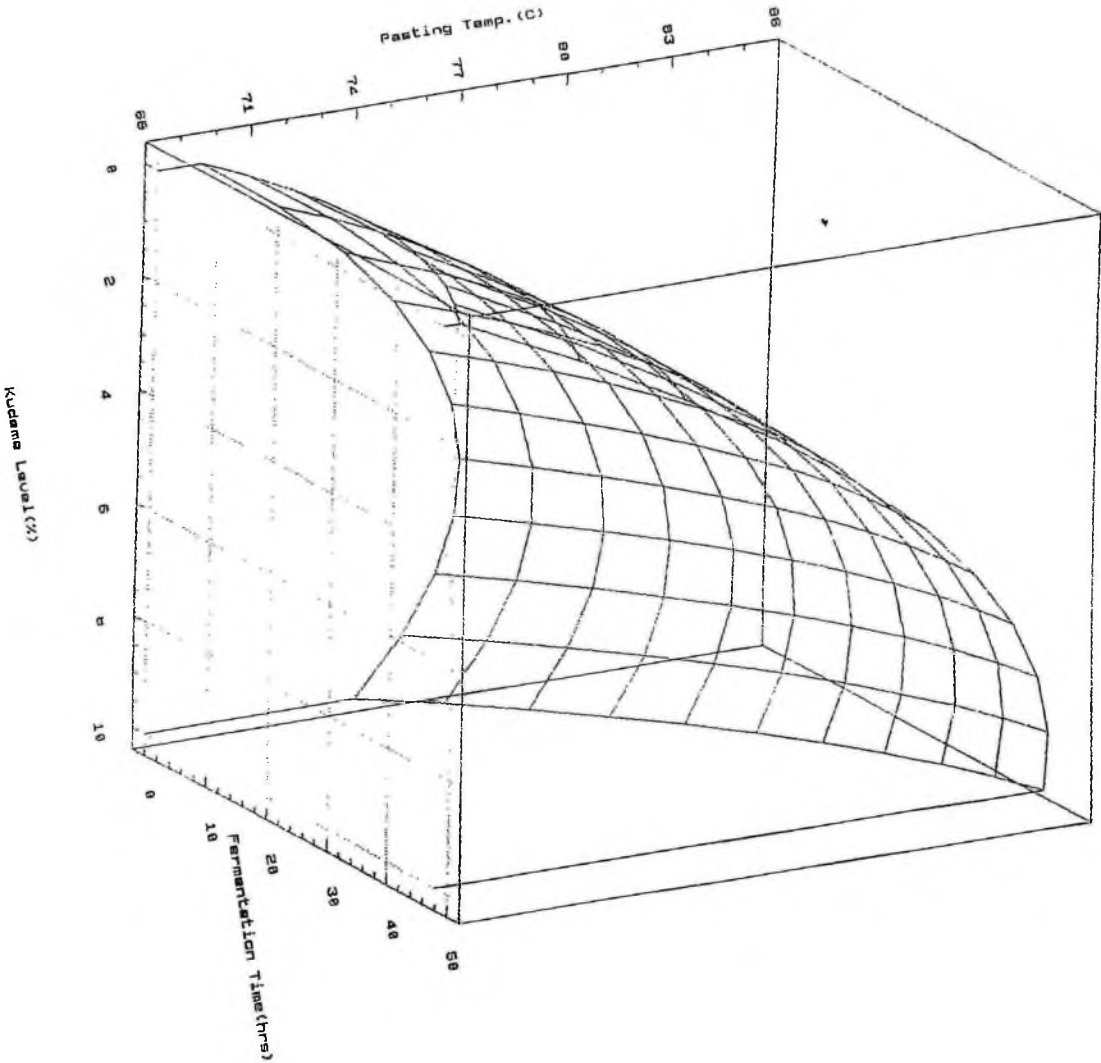
$$Z = 69.231758 + 0.124164X_3 + 0.049349X_1X_2 + 0.083478X_1X_3 - 0.001159X_1X_2X_3 - 0.199755 X_1^2 - 0.002947 X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 87.61\%$



For 7% slurry concentration of blanched kudeme inoculated fermented nixtamalized maize, an  $R^2$  of 0.7463 was observed for the following model;

$$Z = -1.731916 + 2.648787X_2 - 0.194488X_3 + 0.063867X_1X_3 + 0.002718X_2X_3 - 0.000936X_1X_2X_3 - 0.012253X_1^2 - 0.022329X_2^2$$

ANOVA for this model was significant with an F-ratio of 8.9847\*\* and an insignificant lack of fit; Table 25.

**Table 24. ANOVA summary (showing only F-values) for viscoamylograph indices of nixtamalized maize inoculated with blanched kudeme.**

Sources of variation	Pasting temp.	Peak viscosity	Temp. at peak viscosity	Viscosity at 95°C	Viscosity at 50°C
$X_1$	-	13.96**	0.16	9.72**	0.02
$X_2$	2.51	16.57**	-		0.83
$X_3$	3.28	-	0.43	0.09	
$X_1X_2$	-	17.50**	0.78	10.31**	1.05
$X_1X_3$	2.89	1.48	1.85	2.89	9.14**
$X_2X_3$	22.87**	-	3.49	0.02	
$X_1X_2X_3$	7.62**	18.44**	2.64	0.78	74.37**
$X_1^2$	2.89		3.46	0.65	4.20
$X_2^2$	22.87**	89.73**	13.16**	-	17.70**
$X_3^2$	-	4.56**	7.77**	-	27.30**

\* sig. at  $p \leq 0.05$       \*\* sig. at  $p \leq 0.01$

( $X_1$  = kudeme level;  $X_2$  = moisture level;  $X_3$  = fermentation time)

**Table 25. ANOVA summary for the full regression models with lack of fit for viscoamylograph indices of nixtamalized maize inoculated with blanched kudeme**

Sources of variation	Pasting temp.	Peak viscosity	Temp. at peak viscosity	Viscosity at 95°C	Viscosity at 50°C
Model	8.9847**	23.1809**	3.7477*	3.4950*	16.8257**
Lack Of Fit	0.6729	0.8669	1.1285	0.4796	0.5864

#### 4.8 Peak viscosity and temperatures at peak viscosity of fermented nixtamalized maize inoculated with kudeme.

Peak viscosity indicates the maximum or highest viscosity encountered during cooking of starch paste. At the second level of the study, the peak viscosity for various combinations of independent variables in study design, ranged from 80 to 220 BU for 8% slurry, from 20 to 140 BU for 7% slurry and a maximum of 40 BU recorded for 5% slurry for nixtamalized maize inoculated with roasted kudeme. The peak viscosity for 3% slurry was not distinct and absent for most of the sample combinations used in the response surface study.

The peak viscosity for the 7% slurry of the blanched kudeme inoculated nixtamalized maize was between 60 to 100 BU. Models developed for peak viscosity indicated that over 60% of the variation in this index, could be explained by independent variables at slurry concentrations of 8, 7 and 5% except for the 3% slurry concentration which registered 11.04% for coefficient of determination. The regression model developed for peak viscosity at 8% slurry concentration for fermented nixtamalized maize inoculated with roasted kudeme was;

$$Z = -782.716914 + 33.579133X_2 - 1.563148X_3 + 0.94371X_1^2 - 0.307879X_2^2 + 0.03938X_3^2$$

and had an  $R^2$  of 69.42%. The quadratic term of the kudeme level contributed significantly to this model. Cornelius (1999), recorded an  $R^2$  of 70.57 for regression model developed for peak viscosity of fermented cowpea fortified nixtamalized maize with an insignificant F-ratio of 3.702 for lack of fit. For models for 7% slurry concentration, the roasted kudeme inoculated fermented nixtamalized maize had an  $R^2$  of 63.05% and the blanched kudeme inoculated sample had an  $R^2$ -value of 89.10%. However all the variables with their quadratic and interactive terms in the model for blanched kudeme inoculated fermented nixtamalized maize contributed significantly to the model output with the exception of the interactive term between fermentation time and kudeme. The quadratic term of moisture had the highest F-ratio of 89.73\*\*\* and the quadratic term of fermentation had a low degree contribution with an F-ratio of 4.56\*. In the roasted kudeme inoculated fermented nixtamalized maize model however, the quadratic term of kudeme level and moisture level with F-ratios of 15.73\*\*\* and 11.36\*\*\* respectively contributed to this index. ANOVA in both instances were significant for models. The model for the roasted kudeme inoculated fermented nixtamalized maize for 7% slurry concentration was;

$$Z = -1877.797578 + 70.688886 X_2 + 0.490236 X_1^2 - 0.635886 X_2^2 + 0.007265 X_3^2$$

Response surface plot for this index at 7% slurry concentration for roasted kudeme inoculated nixtamalized maize after 48hr fermentation (Fig. 17), further confirms preliminary findings in Figure 4, that peak viscosity increases with increasing kudeme level. It was also observed to increase with increasing fermentation time. The model for peak viscosity at 5% slurry concentration for roasted kudeme inoculated fermented nixtamalized maize had an  $R^2$  of 69.46%, with kudeme level interaction among all 3-independent variables and that between kudeme and fermentation affecting model outcome significantly.

**Figure 17: Response surface plots for peak viscosity of 7% slurry concentration for nixtamalized maize inoculated with roasted kudeme .**

Model;

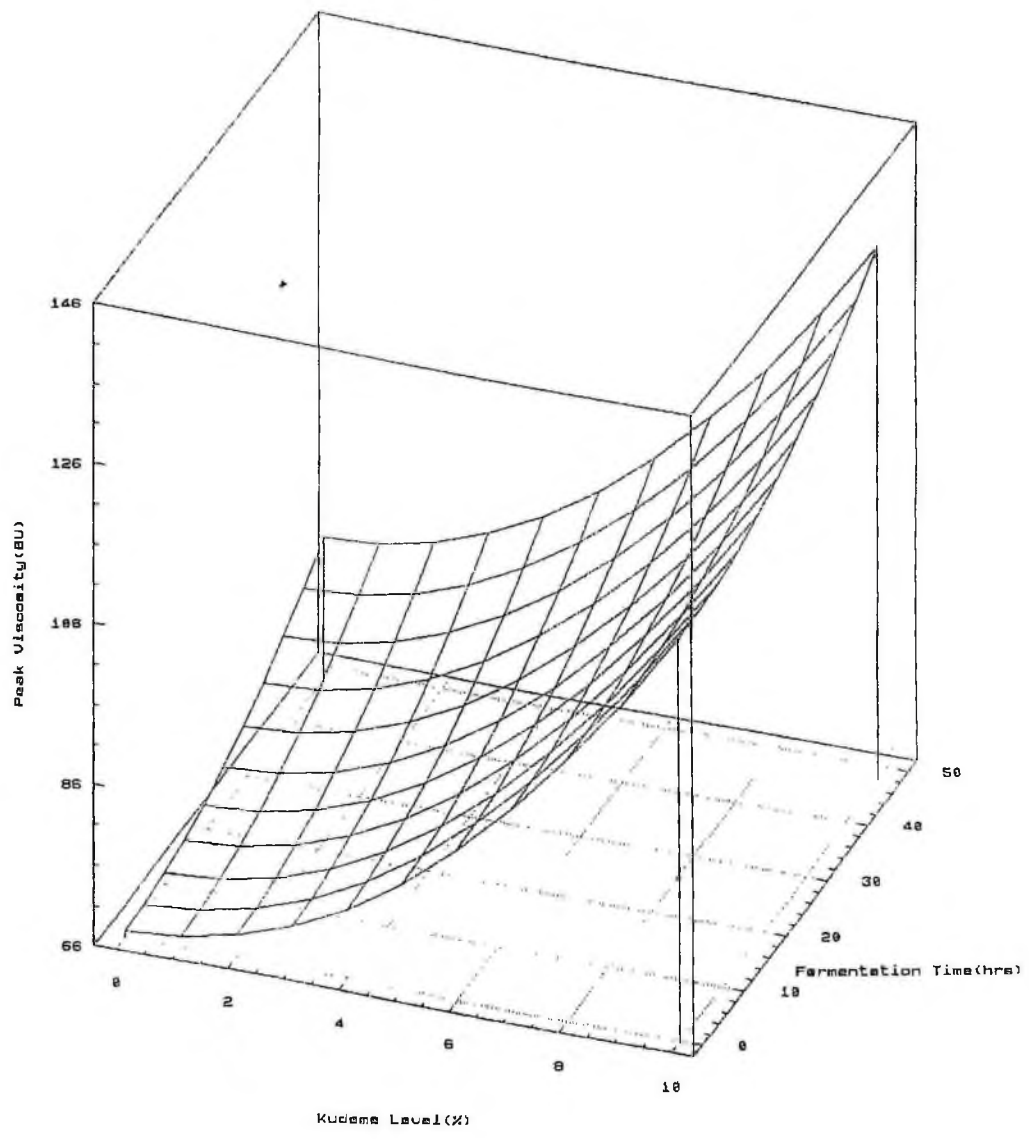
$$Z = -1877.797578 + 70.688886 X_2 + 0.490236 X_1^2 - 0.635886 X_2^2 + 0.007265 X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 63.05\%$



Temperature at peak viscosity was obvious for 8 and 7% slurries only. For 8% slurry, the temperature at peak viscosity was used to generate an adequate model for predicting the variation in this index. ANOVA for the regression model generated for temperature at peak viscosity registered an insignificance lack of fit. For 7% slurry concentration, an  $R^2$  of 35.06 was deduced for temperature at peak viscosity for fermented nixtamalized maize inoculated with roasted kudeme and 56.55% for the one inoculated with blanched kudeme. The blanched kudeme inoculated fermented nixtamalized maize had a higher  $R^2$  value but only the quadratic term of the moisture and fermentation contributed to the output of the temperature at peak viscosity. The roasted kudeme inoculated fermented nixtamalized had the kudeme level and the quadratic term of this independent variable contributing significantly to the output of the model for temperature at peak viscosity. This is an indication that the roasted kudeme was a better inoculant than the blanched kudeme for the observation influenced by inoculants in the modification of temperature at peak viscosity of fermenting nixtamalized maize inoculated with kudeme.

#### **4.9 Viscosity at 95°C and 95°C-hold of nixtamalized maize inoculated with kudeme.**

The hot paste viscosity ranged from 40 to 210 BU for 8% slurry, 10 to 170 BU for 7% slurry, 0 to 60BU for 5% slurry and 0 to 15 BU for 3% slurry of roasted kudeme inoculated fermented nixtamalized maize while a range from 90 to 160 BU for 7% slurry was obtained for blanched kudeme inoculated fermented nixtamalized maize. There was a wider range in the variations in hot paste viscosity of roasted kudeme inoculated fermented nixtamalized maize than the blanched kudeme inoculated fermented nixtamalized maize, in comparing the 7% slurry concentrations of both types of kudeme inoculated fermented nixtamalized maize. The model developed for predicting the hot paste viscosity for roasted kudeme inoculated nixtamalized registered  $R^2$  values of 51.95%, 72.20%, 82.65% and 7.96% respectively for 8,7,5 and 3% slurry concentrations for

viscosity at 95°C. For viscosity at 95°C-hold, the model registered 46.76%, 40.85%, 62.47%, and 27.04% respectively for 8,7,5 and 3% slurry concentrations respectively. From Figure 18, it was observed that the viscosity at 95°C increased with increasing kudeme level and with increasing fermentation time. This further confirms the findings that finer particle sizes results from higher levels of kudeme and therefore higher viscosities at all temperatures with increasing kudeme level. For blanched kudeme inoculated fermented nixtamalized maize, an R<sup>2</sup> values of 47.89% and 52.12% were, respectively, recorded for viscosity at 95°C and 95°C-hold for 7% slurry concentration.

Fermentation results in ease of cooking cereals, as indicated by Akpapunam and Sefa-Dedeh (1995) with the observation that viscosity at 95°C were relatively higher in fermented maize than unfermented maize, but on holding for 30minutes at this temperature, unfermented maize tended to have higher viscosity than fermented maize. The viscosity of starch paste at 95°C reflects the ease of cooking and the viscosity at 95°C-hold determines the stability or breakdown tendencies of the paste. Starch pastes with higher viscosity at 95°C are considered easier to cook. Alkaline cooking had however been shown to decrease cooked paste viscosity. Fermentation usually increase viscosity of cereal dough, but Mling (1988), observed a decrease in viscosity in fermented cassava based weaning foods, while Cornelius (1999), also observed drastic reduction in viscosity of nixtamalized maize.

The contributing variables to models generated for roasted kudeme inoculated fermented nixtamalized maize, were mostly kudeme level and the quadratic term of the kudeme level with interactions among kudeme and fermentation as well as moisture at varying degrees. In the case of the blanched kudeme inoculated nixtamalized maize however, the kudeme level and interaction

between kudeme and moisture contributed significantly to viscosity at 95°C, whilst the quadratic term of fermentation with interaction among all 3-independent variables also contributed to the viscosity at 95°C-hold.

**Figure 18: Response surface plot for viscosity at 95°C of 7% slurry concentration for roasted kudeme inoculated nixtamalized maize.**

Model;

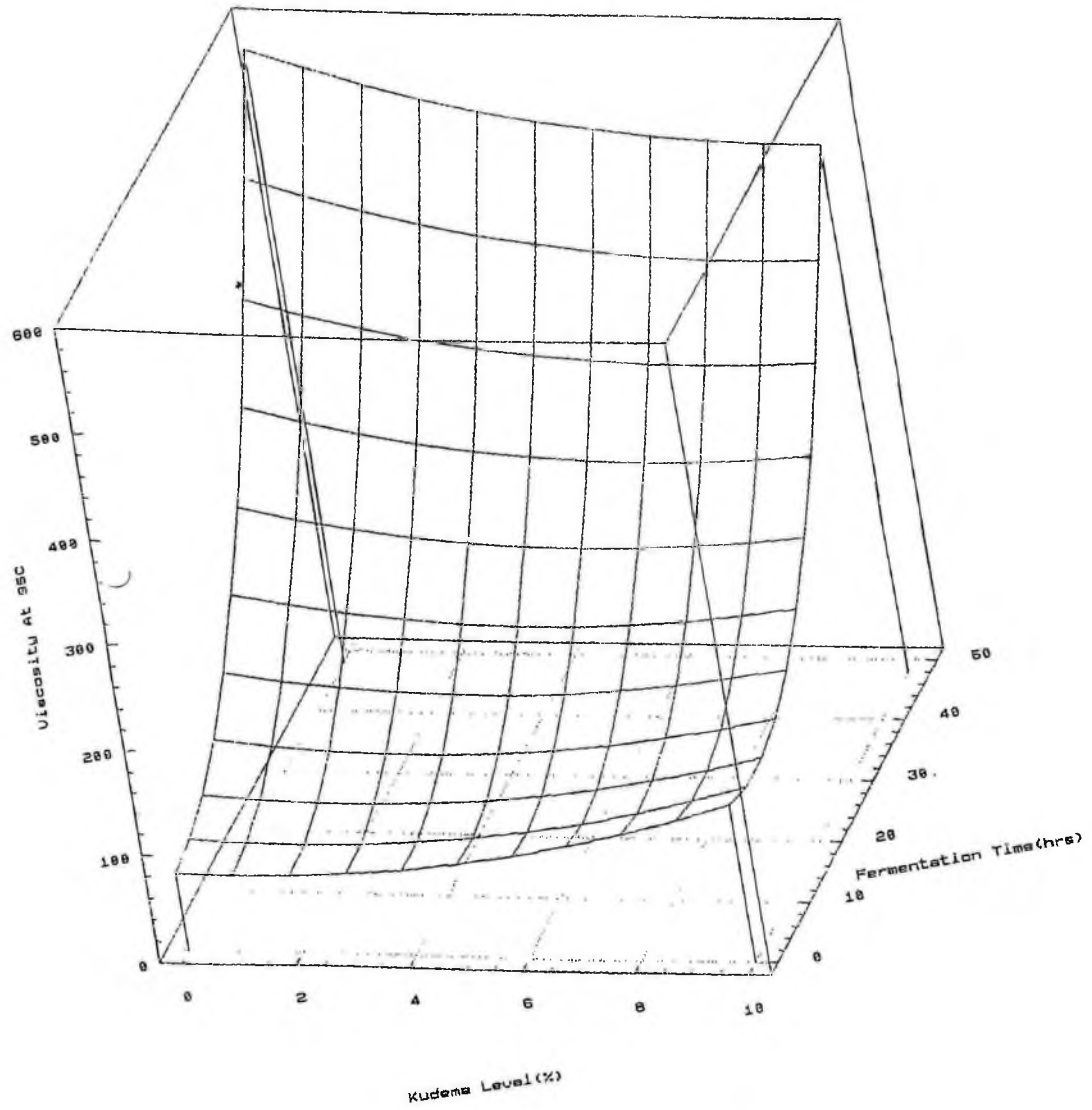
$$Z = -2445.28535 - 8.013219X_1 + 92.672643X_2 + 0.133491X_1X_2 - 1.226361X_1X_3 + 0.017957X_1X_2X_3 + 0.894332X_1^2 - 0.846401X_2^2 + 0.22244X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 72.20\%$



The model developed for roasted kudeme inoculated fermented nixtamalized maize of 7% slurry for viscosity at 95°C was;

$$Z = -2445.28535 - 8.013219X_1 + 92.672643X_2 + 0.133491X_1X_2 - 1.226361X_1X_3 + 0.017957X_1X_2X_3 + 0.894332X_1^2 - 0.846401X_2^2 + 0.22244X_3^2$$

The  $R^2$  was 72.20%. With the exception of the model for 3% slurry concentrations, all the other models did not have significant lack of fit for viscosity at 95°C and 95°C-hold for fermented nixtamalized maize inoculated with kudeme. The 3% slurry did not actually have significant ANOVA for its models and had very low  $R^2$  values for both indices. The model developed by Cornelius (1999), for predicting the hot paste viscosity of cowpea fortified nixtamalized maize had an  $R^2$  -value of 62.45% with the quadratic terms of lime concentration and cowpea level as the significant variable contributors. The response surface trends of viscosity at 95°C were similar to those of peak viscosity (Fig. 16).

#### **4.10 Viscosity at 50°C and 50°C-hold for fermented nixtamalized maize inoculated with kudeme.**

Viscosity at 50°C and 50°C-hold, ranged from 250 to 680 BU, 70 to 520 BU, 10 to 140 BU and 0 to 20 BU for 8, 7, 5 and 3% slurry concentrations respectively for roasted kudeme inoculated fermented nixtamalized maize. The 7% slurry of blanched kudeme inoculated fermented nixtamalized maize had a range of between 200 to 320 BU.

Amylose molecules have the capacity to segregate but when temperature drops below 65°C, retrogradation becomes very severe and molecules come together. The setback on cooling, which is the viscosity after cooling paste from 95°C to 50°C, increases viscosity and reflects the retrogradation

tendency of the starch granules, whereas viscosity on holding paste at 50°C for 30 min indicates the stability of the cooked paste as might be practically used. Increase in viscosity during the cooling period demonstrates the tendency of the element in hot paste (swollen granule, fragments of swollen granules, colloidal and molecularly dispersed starch molecules), to associate or retrograde as the temperature of the paste decreases. This retrogradation is demonstrated by the difference between the viscosities of the paste on attaining 50°C and that at the beginning of the cooling period, whilst the viscosity at 50°C-hold, indicates the stability of the paste in a form in which the paste will most likely be used.

The models developed for viscosity at 50°C had  $R^2$  -values of 65.55%, 53.57%, 53.44% for 8, 7 and 5% slurry concentrations respectively for roasted kudeme inoculated fermented nixtamalized maize, while  $R^2$ -values in the same order for viscosity at 50°C-hold for roasted kudeme inoculated nixtamalized maize were; 50.38, 60.09, and 40.42%. The 3% slurry registered an  $R^2$  value of 0 for viscosity at 50°C and 23.24% for viscosity at 50°C-hold, with an insignificant ANOVA for models at this slurry concentration. The blanched kudeme inoculated fermented nixtamalized maize models however had  $R^2$  values of 86.95% for viscosity at 50°C and 81.50% for viscosity at 50°C-hold for 7% slurry concentration.

For the roasted kudeme inoculated fermented nixtamalized maize, the kudeme and its quadratic term were significant variable contributors, whilst interaction between the kudeme and other independent variables contributed at lower degrees to these models.

The model developed for the 7% slurry concentration of roasted kudeme inoculated fermented nixtamalized maize for viscosity at 50°C-hold was;

$$Z = -6357.245487 + 79.301326X_1 + 232.545635X_2 - 0.773126X_1X_2 - 0.009085X_1X_2X_3 - 2.061659X_2^2 + 0.047796X_3^2$$

with an  $R^2$  of 60.09% as reported earlier and a significant ANOVA for the full regression model with an insignificant lack of fit. The significant variable contributors to this model were the roasted kudeme level, moisture and its quadratic term. The viscosity at 50°C-hold as observed in Figure 19, increases with increasing level of kudeme and fermentation time. The finer particle resulting from the increased level of kudeme allows the faster aggregation of particles during cooling which produces the high viscosities observed for samples with higher kudeme levels.

**Figure 19: Response surface plot for viscosity at 50°C-hold for 7% slurry concentration of roasted kudeme inoculated nixtamalized maize .**

Model;

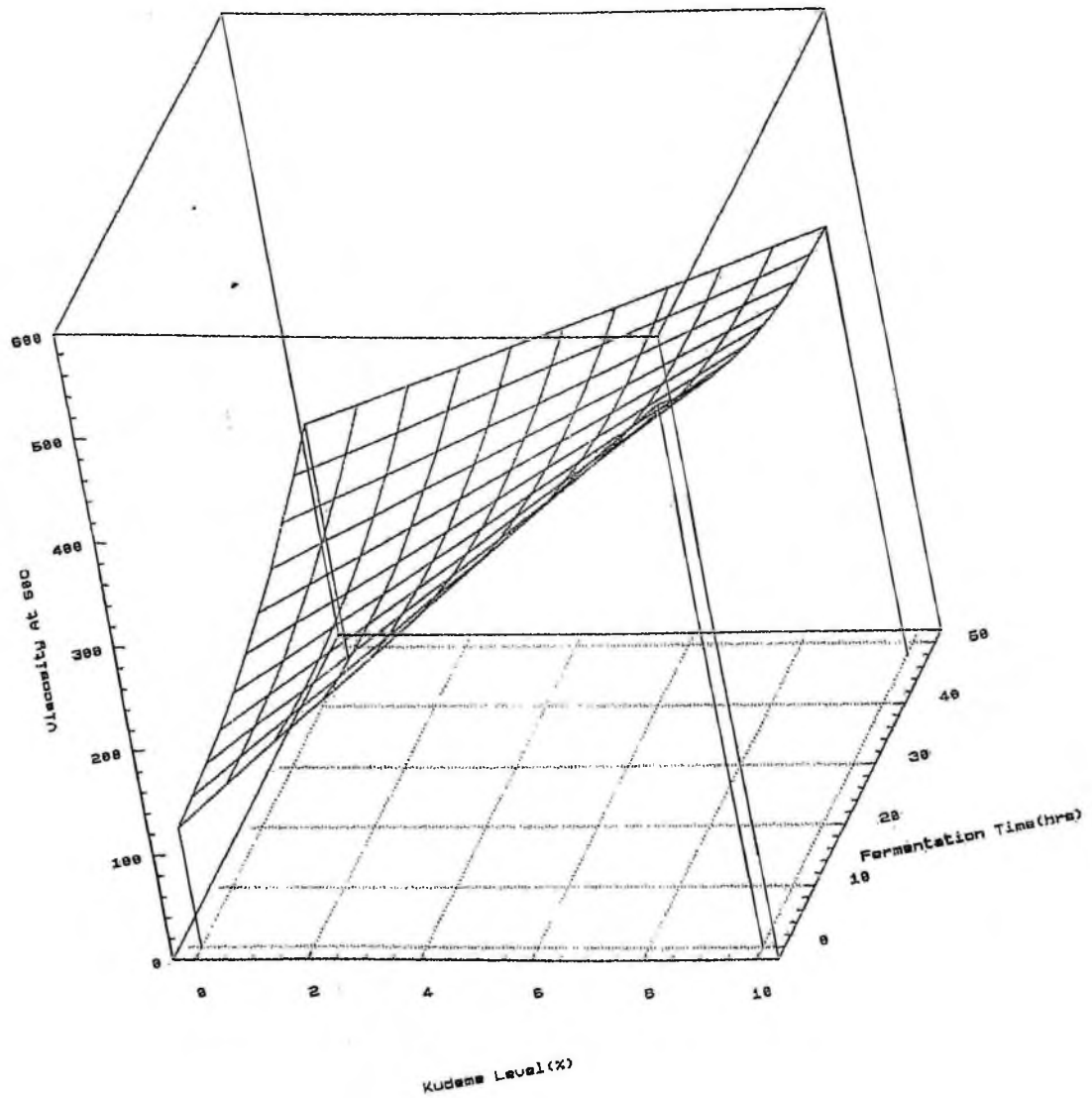
$$Z = -6357.245487 + 79.301326X_1 + 232.545635X_2 - 0.773126X_1X_2 - 0.009085X_1X_2X_3 - 2.061659X_2^2 + 0.047796X_3^2$$

$X_1$  = Kudeme Level

$X_2$  = Moisture Content

$X_3$  = Fermentation time

$R^2 = 60.09\%$



The model for the blanched kudeme inoculated fermented nixtamalized maize had a high  $R^2$  value of 81.51% for model developed for viscosity at 50°C-hold. The contributing variables to this model were the moisture level, the fermentation time, the quadratic terms of the moisture and fermentation time and the interaction between kudeme and fermentation time. Most of the independent variables, their quadratic and interactive terms were present in this model. ANOVA for this latter model was significant with an insignificant lack of fit.

## 5.0 CONCLUSIONS

1. Roasted kudeme had a much more influential impact on the physico-chemical and functional properties of fermented nixtamalized maize than the blanched kudeme. Preliminary results deduced by ANOVA indicated that kudeme in combination with solid substrate fermentation significantly influenced the outcome of pH, titrable acidity, pasting temperature, temperature at peak viscosity and viscosity at 95°C, 50°C and 50°C-hold. These chemical and functional effects of kudeme and fermentation on nixtamalized maize occurred at varying significant levels. Decreasing trends in pH, and increasing trends in titrable acidity and rate of viscosity were also observed on increasing levels of kudeme.
2. Kudeme made the particle size of nixtamalized maize finer during fermentation, and this was more pronounced for Roasted kudeme than for blanched kudeme. Roasted kudeme, is therefore ideal for the modification of nixtamalized maize during fermentation through the breaking down of the textural component of the nixtamalized maize dough, making the commodity potentially acceptable and appealing to Africans.
3. Trends observed in Brabender viscosity were not distinct for blanched kudeme as much as for roasted kudeme. Cooking properties such as pasting temperature and temperature at peak viscosity as well as peak viscosity, viscosity at 95°C, 50°C and 50°C-hold, generally increased with increasing levels of kudeme. The microbial activity and their by product of enzyme, in kudeme were believed to be the major factors inducing the desirable modifications

- 4 In most instances, roasted kudeme was found to largely influence the response parameters studied with adequate models explaining the variations observed in most response. Roasted kudeme played a significant role in the outcome of the predictive power of the overall models. Response surface plots demonstrated that most indices increased with increasing levels of kudeme. Fermentation also enhanced the outcome of some response parameters. The optimum operating condition to obtain pH of 3.5 or 4.0 at a kudeme level of 5% and a moisture level of 55%, are fermentation times of 53 and 49 hours respectively. The ability of kudeme to breakdown the texture and produce various acids were influential in the trends observed.

Based on findings in this research, it is recommended that further research be carried out in the following areas;

- a) The organoleptic and sensory qualities of fermented nixtamalized maize inoculated with roasted kudeme.
- b) The potential use of kudeme in other traditional fermenting systems such as aflata fermentation for the production of kenkey and masa and possible formulation of various products from fermented nixtamalized maize inoculated with kudeme; for example instant aflata powder for domestic home-based kenkey and masa production.
- c) The characterization of the microflora of blanched and roasted kudeme inoculated nixtamalized maize as well as all enzymatic characteristics of these organisms and how they perform in alkaline and acidic mediums.

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## 7.0 APPENDICES

Coefficients Of Independent Variables Within Models Developed For Various Chemical Responses And Their  $R^2$ , lack Of Fit And F-ratio For Anova Of The Full Regression Models: Nixta-kudemelized Maize Of Roasted And Blanched kudeme.

Variable	Coefficients/ Roasted Kudeme					Coefficients/ Blanched Kudeme				
	Moisture	PH	T.Acidity	Ash	Protein	Moisture	PH	T.Acidity	Ash	Protein
Constant	46.6447	175.8087	-1.9540	3.5472	8.2447	20.6851	10.55376	-	-5.7122	19.0051
$X_1$	0.0275*	-0.5318***	-0.0070***	0.1718			-0.4651*	-	0.7490	-1.5934
$X_2$	-0.6617***	-5.7556	0.0689		0.0325	0.2844***		-	0.9161	-0.1633
$X_3$		-0.3039***		-0.1886	-0.0608	-0.1032***	0.1051***	-	0.1314	-0.2916*
$X_1X_2$	-0.0075				-0.0045*		0.0056	-	-0.0127	-0.2422*
$X_1X_3$	0.0278	0.0071	0.0004***			0.0375		-	-0.0310	0.0265
$X_2X_3$			0.000007	0.0022		0.0017	-0.0019	-	0.0024	0.0044
$X_1X_2X_3$	-0.0005			0.00005	0.00009*	-0.0007***		-	0.0005**	-0.0004
$X_1^2$	0.0141		0.0005**	-0.0231*	0.0138	0.0037	0.0088	-		0.0113
$X_2^2$	0.0147*	0.0505**	-0.0006**	-0.0005		0.0061*		-	-0.0012	
$X_3^2$	0.0005	0.0039**	-0.00002**	0.0011*	0.0005		-0.0005	-	0.002	0.0003
$R^2$	0.9805	0.7880	0.9593	0.1824	0.3839	0.9973	0.07165	-	0.2220	0.4713
F-ratio/Reg	120.69***	12.77***	64.92***	Insig.	2.97*	996.60***	9.00***	-	Insig.	2.88*

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001

Coefficients Of Independent Variables Within Models Developed For Bulk Volume And Swelling Capacity Responses At Different Temperatures And Durations And Their  $R^2$ , lack Of Fit And F-ratio For Anova OF The Full Regression Models: Nixta-kudemelized Maize Of Roasted Kudeme.

Variable	Bulk Vol.	Coefficients/25°C Swelling				Coefficients/70°C Swelling			
		15min.	30min.	45min.	60min.	15min.	30min.	45min.	60min.
Constant	-20.2058	26.6969	-91.1271	28.3237	27.7927	36.7280	-7.4117	19.7796	59.1798
X <sub>1</sub>	1.7033	-1.0125	-0.7076	-0.1488*		-2.7184	1.6493*		
X <sub>2</sub>	1.1032	0.0822	4.1908			0.1269	0.7982		-1.3796**
X <sub>3</sub>	0.3409	0.1786	0.1578			0.2815	2.0383	1.2676	1.2182
X <sub>1</sub> X <sub>2</sub>	-0.0411	-0.0236					-0.0445		-0.0015**
X <sub>1</sub> X <sub>3</sub>	-0.0671	-0.0132	-0.0132				-0.1636	-0.0078**	-0.0111
X <sub>2</sub> X <sub>3</sub>	-0.0066			0.0031	0.0036		-0.0289	-0.0156	-0.0157*
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	0.0013			-0.0003	-0.0003		0.0026		
X <sub>1</sub> <sup>2</sup>	0.0415**	0.0274***	0.1278**	0.0778*	0.0660***	0.2152	0.0767	-0.0178	
X <sub>2</sub> <sup>2</sup>	-0.0082		-0.0365					0.0053**	0.0174
X <sub>3</sub> <sup>2</sup>		0.0011	-0.001	-0.0015	-0.0019	-0.0054	-0.0062*	-0.0068**	-0.0053**
R <sup>2</sup>	0.3623	0.2086	0.3376	0.3199	0.4256	0.1065	0.4368	0.6783	0.6252
F-ratio/Reg	Insig.	Insig.	Insig.	2.79*	4.52**	Insig.	Insig	7.68***	5.53***

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001

Coefficients Of Independent Variables Within Models Developed For Bulk Volume And Swelling Capacity Responses At Different Temperatures And Durations And Their  $R^2$ , lack Of Fit And F-ratio For Anova Of The Full Regression Models: Nixta-kudemelized Maize Of Blanched Kudeme.

Variable	Bulk vol.	Coefficients/ 25°C Swelling				Coefficients/70°C Swelling			
		15min.	30min.	45min.	60min.	15min.	30min.	45min.	60min.
Constant	15.4822	29.2424	29.8470	30.0822	30.4136	120.0934	38.4476	101.4461	135.0571
$X_1$	-0.4895	-0.9008				-7.5856		-7.0495**	-7.0910**
$X_2$						-2.2004	0.0084	-1.5815	-2.7468
$X_3$		-0.3638**	-0.0796			-1.6836	-0.7249	-1.6999	-1.6971
$X_1X_2$		0.0130	-0.0014	0.0028		0.1268		0.1143*	0.1143
$X_1X_3$		0.0724				0.3155	0.1507	0.3564**	0.3564**
$X_2X_3$	-0.0008	0.0027	-0.0018	-0.0018	-0.0013	0.0264	0.0102	0.0278	0.0278
$X_1X_2X_3$	0.00007	-0.0012				-0.0053**	-0.0024**	-0.0059**	-0.0059***
$X_1^2$	0.0373*			-0.0231	-0.0117		-0.0336**		
$X_2^2$						0.0134		0.0091	0.0192*
$X_3^2$	0.0007	0.0044	0.0041**	0.0025**	0.0019*	0.0022	0.0019	0.0009	0.0010
$R^2$	0.1016	0.4029	0.3991	0.4784	0.2256	0.4876	0.3101	0.8091	0.7569
F-ratio/Reg	Insig.	2.83*	4.16**	5.36**	Insig.	3.01*	Insig.	9.95**	7.57**

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001

Coefficients Of Independent Variables Within Models Developed For Colour Responses And Their  $R^2$ , lack Of Fit And F-ratio For Anova OF The Full Regression Models: Nixta-kudemelized Maize Of Roasted And Blanched Kudeme.

Variable	Coefficients/Roasted Kudeme				Coefficients/Blanched Kudeme			
	L	a	b	Total colour	L	A	b	Total colour
Constant	88.4381	64.0066	192.7143	467.7744	170.5864	-19.5202	-149.7273	-153.6359
$X_1$	-1.8937	0.1658	-0.3740	1.4290*	-0.6594	0.1434*	-0.1792**	0.5992
$X_2$		-2.1987	-5.9658	-15.6482	-3.0105	0.6942	6.0727**	6.2289
$X_3$		-0.0158	-0.1677		0.3035	0.0544	0.4501***	0.1592**
$X_1X_2$								-0.0002
$X_1X_3$		0.0022	0.0249		-0.0191*			
$X_2X_3$	-0.0015				-0.0078		-0.0067	
$X_1X_2X_3$					0.0006	-0.0005		
$X_1^2$	0.2308*	-0.0261*	-0.0458	-0.1908**	0.0335	-0.0038		-0.0345
$X_2^2$		0.0191**	0.0522*	0.1366***	0.0270**	-0.0060*	-0.0529**	-0.0544**
$X_3^2$	0.0031			-0.00154	0.0015	-0.0008**	-0.0032**	-0.0031*
$R^2$	0.2334	0.4754	0.3803	0.6347	0.5062	0.5928	0.8518	0.6585
F-ratio/Reg	Insig.	3.87**	2.94*	7.60**	3.16*	4.95**	19.20**	6.23**

\* .  $p \#0.05$  \*\* .  $p = 0.01$  \*\*\*  $p = 0.001$

Coefficients Of Independent Variables Within Models Developed For Cooking Temperatures For Different Concentrations And Their  $R^2$ , lack Of Fit And F-ratio For Anova Of The Full Regression Models: Nixta-kudemelized Maize Of Roasted (8,7,5 and 3% slurries) And Blanched Kudeme (7% Slurry).

Variable	Coefficients/Pasting Temperature					Coefficients/Temperature At Peak Viscosity		
	8%R	7%R	5%R	3%R	7%B	8%R	7%R	7%B
Constant	-60.9332	69.2318	162.3832	101.5365	-1.7319	-203.2604	80.8049	43.3809
$X_1$	-4.9875***		4.2752			3.6147*	2.5044*	20.2729
$X_2$	4.7341		-3.2025		2.6488	9.9154		
$X_3$		0.1246*			-0.1945		0.2235	2.6613
$X_1X_2$	0.1280	0.04933***	-0.0476	-0.0904***				-0.3776
$X_1X_3$	0.2115*	0.0835			0.0639***		0.0124	-0.6459
$X_2X_3$				0.0028***	0.0027			-0.0525
$X_1X_2X_3$	-0.0033	0.0012	0.0005	0.0004	-0.0009**			0.0116
$X_1^2$	-0.1673**	0.1997***	-0.1695	0.4489***	-0.0123	-0.2581	-0.2163*	0.1051
$X_2^2$	-0.0426		0.0312		-0.0223***	-0.0874		0.0154***
$X_3^2$	0.0008	0.0029	-0.0010	-0.0044***		0.0014	-0.0041	0.0059**
$R^2$	0.7758	0.8761	0.3690	0.9983	0.7463	0.2839	0.3506	0.5655
F-ratio/Reg	9.22**	23.39**	Insig.	1329.10**	8.98**	Insig.	3.05*	3.75*

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001

R = Roasted kudeme

B = Blanched kudeme



Coefficients Of Independent Variables Within Models Developed For Viscosity At 95°C And 95°C-Hold For Different Concentrations And Their R<sup>2</sup>, lack Of Fit And F-ratio For Anova OF The Full Regression Models: Nixta-kudemelized Maize Of Roasted (8,7,5 and 3% slurries) And Blanched Kudeme (7% Slurry).

Variable	Coefficients/Viscosity At 95°C					Coefficients/Viscosity At 95°C-Hold				
	8%R	7%R	5%R	3%R	7%B	8%R	7%R	5%R	3%R	7%B
Constant	-1936.6819	-2445.2895	-592.8967	119.6190	88.9134	211.4548	-2250.7927	22.9100	391.5486	129.4754
X <sub>1</sub>		-8.0132***	-10.8895***		-4.3093**		3.8288		12.9558	14.2075
X <sub>2</sub>	74.9142	92.6726*	20.8450	-4.3102			85.9375*		12.8781	
X <sub>3</sub>				-0.1150	1.2683	1.6528*			-0.7399	3.5020
X <sub>1</sub> X <sub>2</sub>	0.0537***	0.1335	0.2584		0.1168**				-0.1885	-0.1177
X <sub>1</sub> X <sub>3</sub>	-1.6361	-1.2264	0.5787***		-0.4813	-0.8707*				-1.7041
X <sub>2</sub> X <sub>3</sub>					-0.0150			-0.0110	0.0197	0.0835
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	0.0213	0.0180	-0.0127**	-0.0039	0.0068	0.1180		-0.0025*		0.0273*
X <sub>1</sub> <sup>2</sup>	1.3302	0.8943*	0.1898	0.8335	0.1486	0.9218***		0.5700***	-0.2360	-0.3799
X <sub>2</sub> <sup>2</sup>	-0.6853*	-0.8464***	-0.1824**	0.0395		-0.0226	-0.7793*		-0.1050	
X <sub>3</sub> <sup>2</sup>	0.0454*	0.0222*	0.0100**	0.0038			0.0117	0.0249***	-0.0103	0.04426***
R <sup>2</sup>	0.5195	0.7220	0.8285	0.0796	0.4789	0.4679	0.4085	0.6247	0.2704	0.5212
F-ratio/Reg	3.93**	7.17***	12.47***	Insig.	3.50	4.34**	4.28**	8.91***	Insig.	3.59*

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001

R = Roasted kudeme

B = Blanched kudeme

Coefficients Of Independent Variables Within Models Developed For Viscosity At 50°C And 50°C-Hold For Different Concentrations And Their R<sup>2</sup>, lack Of Fit And F-ratio For Anova OF The Full Regression Models: Nixta-kudemelized Maize Of Roasted (8,7,5 and 3% slurries) And Blanched Kudeme (7% Slurry).

Variable	Coefficients/Viscosity At 50°C					Coefficients/Viscosity At 50°C-Hold				
	8%R	7%R	5%R	3%R	7%B	8%R	7%R	5%R	3%R	7%B
Constant	-6755.9013	92.3047	-1561.1774	-255.9730	1606.5778	-2387.4248	-6357.2455	-2010.8640	-442.5890	2043.7618
X <sub>1</sub>		143.3071***		1.6189	77.1713	-114.7019***	79.3013***		1.9608	14.4097
X <sub>2</sub>	245.5778		53.2520	8.5599	-47.7626	115.5792	233.5456**	67.6891	15.4505	-57.5106
X <sub>3</sub>	12.3924		14.8793	0.5280				11.6486		-11.4834
X <sub>1</sub> X <sub>2</sub>	1.3944***	-1.7595*			1.1739	2.6374	-0.7731			-0.0350
X <sub>1</sub> X <sub>3</sub>		-0.8179	-1.1036*	0.0589	-3.5548**	-0.9761				-1.1422***
X <sub>2</sub> X <sub>3</sub>		0.0231	-0.2324			0.1373			0.0088	0.1893***
X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	-0.02040*		0.0150		0.0568***		0.0091	0.0039		0.0132
X <sub>1</sub> <sup>2</sup>	-2.5234		1.1186***		0.2757	1.2808		1.0341**	-0.1999	-0.3702
X <sub>2</sub> <sup>2</sup>	-2.2040		-0.4452	-0.0716	0.4105***	-1.2089	-2.0617**	-0.5564	-0.1333	0.4574***
X <sub>3</sub> <sup>2</sup>	-0.1285	0.0455		-0.0070	0.0277***	-0.0559	0.0478		-0.0127	0.0490***
R <sup>2</sup>	0.6555	0.5357	0.5344	0.0000	0.8695	0.5038	0.6009	0.4042	0.0000	0.8151
F-ratio/Reg	6.16***	5.38***	4.11**	Insig.	16.83***	4.3311	5.77***	3.15*	Insig.	9.3766***

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001

R = Roasted kudeme

B = Blanched kudeme

Coefficients Of Independent Variables Within Models Developed For Peak Viscosity For Different Concentrations And Their  $R^2$ , lack Of Fit And F-ratio For Anova OF The Full Regression Models: Nixta-kudemelized Maize Of Roasted (8,7,5 and 3% slurries) And Blanched Kudeme (7% Slurry).

Variables	Coefficients/Peak Viscosity				
	8%R	7%R	5%R	3%R	7%B
Constant	-782.7169	-1877.7976	-261.3152	104.9477	1883.6978
$X_1$			-22.0580***	7.2090	-48.6541**
$X_2$	33.5791	70.6889	10.1460	-4.3857	-62.4815**
$X_3$	-1.5631			-0.2946	
$X_1X_2$			0.4267	-0.1414	0.8868**
$X_1X_3$			0.7032*		0.9587
$X_2X_3$					
$X_1X_2X_3$			-0.0139***		-0.0173**
$X_1^2$	0.9437***	0.4902***	0.1847	0.1042	
$X_2^2$	-0.3079	-0.6359**	-0.0957	0.0464	0.5320***
$X_3^2$	0.0394	0.0073	0.0068	0.0054	0.0069*
$R^2$	0.6942	0.6305	0.6946	0.1104	0.8910
F-ratio/Reg	9.63***	9.10***	6.40***	Insig.	23.18***

\* . p #0.05    \*\* . p = 0.01    \*\*\* p = 0.001    R = Roasted    B = Blanched

Coefficients Of Independent Variables Within Models Developed For Water Absorption Capacity At 25oC And 70oC And Their  $R^2$ , lack Of Fit And F-ratio For Anova OF The Full Regression Models: Nixta-kudemelized Maize Of Roasted And Blanched Kudeme.

Variables	Coefficients/ Water Absorption Capacities			
	Roasted kudeme Samples		Blanched kudeme samples	
	25°C	70°C	25°C	70°C
Constant	465.6893	-291.0877	-477.3072	-673.9814
$X_1$		32.7421***	-83.7393	28.1992
$X_2$	-11.3814***	14.9004***	23.3488***	3.0662
$X_3$		-6.0811		
$X_1X_2$	0.1431	-0.4262*	1.4423**	
$X_1X_3$			2.1390	-0.0411
$X_2X_3$	0.0076	0.1234**		
$X_1X_2X_3$			-0.0374***	
$X_1^2$	-0.7390***	-1.0214***	0.0577	0.0366
$X_2^2$	0.0671	-0.1542	-0.2389**	-0.2636
$X_3^2$	-0.0104	-0.0169	-0.0014	-0.0160
$R^2$	0.7574	0.7541	0.7571	0.9076
F-ratio/Reg	10.88***	8.29***	8.40***	32.09***

\* . p #0.05    \*\*    p = 0.01    \*\*\* p = 0.001