KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
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DEPARTMENT OF FOOD SCIENCE AND BIOTECHNOLOGY

LIMITED PROTEOLYSIS OF BAMBARA GROUNDNUT PROTEINS AND EVALUATION OF THEIR SURFACE FUNCTIONAL AND SENSORY PROPERTIES IN CAKE PRODUCTS

THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE MASTER OF SCIENCE (MSc.) DEGREE OF FOOD SCIENCE AND TECHNOLOGY

BY

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DECLARATION

I, Kingsely Boamah, do hereby declare that the work presented in this book, *Limited Proteolysis Of Bambara Groundnut Proteins, And Evaluation Of Their Surface Functional Properties And Sensory Characteristics In Cake Products*, was entirely done by me under the supervision of Mr. Isaac Williams Ofosu and Professor (Mrs.) Ibok Oduro of the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi.

This dissertation has never been partially or wholly presented for any award anywhere.

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DEDICATION

This Thesis is dedicated to my wife, Mrs. Theodora Eva Korkor Boamah and my mother, Mrs. Veronica Nti Frimpong for their support and encouragement that have brought me so far in life.

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ABSTRACT

Limited proteolysis approach was used to modify Bambara groundnut protein isolates (BPIs) to find how the modified protein isolates compare to egg white by studying their surface functional properties and sensory characteristics. Proteolytic treatments were carried out for 10, 35, 60 and 120 minutes to give products labeled as BAM-10, BAM-35, BAM-60 and BAM-120 respectively. The untreated protein isolate was also labeled as *Native*. The surface functional properties were compared to egg white which served as the control at pHs 4, 7 and 9. The results showed that limited proteolysis increased the foaming properties with BAM-35 at pH 9 giving most of the desired results: foam activity and foam stability were 376 % and 78.4 % respectively; emulsion capacity also increased with the highest value of 70 % at pH 4. Emulsion activity and emulsion stability however decreased with proteolysis. Water binding capacity (WBC) and Oil absorption capacity (OAC) also increased with the highest values being 424 % and 386 % respectively for BAM-120. The optimum results obtained for the protein isolates were all comparable to those obtained for the egg white. Improved foaming properties, WBC and OAC suggest that the modified BPIs could find applications in foods that require high foaming and flavour retention. The BPIs however, showed poorer sensory characteristics compared to the egg white when applied in model cakes.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER ONE

1.0 Introduction

1.1 Background

Increasingly, proteins are used as ingredients in foods to provide specific food functional characteristics such as emulsification, foaming, gelation, viscosity and water-holding capabilities (Kinsella *et al.,* 1994; Phillips *et al.,* 1994). Proteins also play a critical role in the shapes and designs of modeled foods such as cake, ice cream and candies to make them more appealing; and the consumers desire to have satisfying mouth-feel make food functionality an important component in formulations of foods (Kinsella *et al.,* 1994).

An ingredient which has remained indispensable towards the achievements of these shapes, designs and the enjoyable mouth-feel is the proteins in egg white. Eggs are known to be laden with dangerous disease-causing *Salmonella enteritidis*, and as a food laden with artery-clogging cholesterol of about 240 mg per yolk (Potter and Hotchicks, 1996). As a result, many of the best features of egg like ease of use, good taste, functionality, and low cost have been lost in the stir (Hennessy *et al.,*1996). Although eggs are nutritious, many people do not consider them as meat and strict vegetarians also stay away from egg products. Furthermore, there are some diseases associated with poultry products such as the avian influenza which further deepens the dislike for egg products (Olsen *et al.,* 2005; Swayne and Beck, 2005). These factors, among others threaten the use of egg in food systems. Although egg consumptions are faced with such challenges, they are still used broadly to improve food functional properties because there are no known substitutes. It is however anticipated that there could be native or modified leguminous proteins, which might be used either in its native or treated form as substitute for egg.

According to Marcello and Gius (1997), plant protein products are gaining increasing interest as ingredients in food systems throughout many parts of the world and plant proteins are now regarded as versatile functional ingredients. Among plant protein

sources which have a potential to be used as an alternative to egg is the protein of *Vigna subterranean (*Bambara groundnut).

Unlike other common legumes such as cowpea, soybean and pigeon pea which functional properties have been extensively studied (Coffman and Garcia, 1977; Ahmed and Schmidt, 1979; Akobundu *et al.*, 1982; Narayana and Rao, 1982; Sathe *et al.*, 1982), Bambara groundnut is underutilized and has less commercial value. Thus, the need to modify Bambara groundnut protein with the view to improve, and study its surface functional properties and some of its applications as ingredients in food.

1.2 Statement of the problem

Bambara groundnut proteins in its native form have some disadvantages with regards to its food functional properties though it has a high quality protein. It has a number of problems associated with its use including the presence of trypsin inhibitors that slow down digestion. Furthermore, it has reduced foaming and emulsion due to poor interfacial film formation which is desirable for emulsion, and foam formation (Kato *et al.*, 1987; Kato and Yutani, 1988). The hard testa of the legume make cooking to last for several h and the consumption of native Bambara groundnut leads to excessive flatulence leading to low consumption pattern.

1.3 Justification of work

Legumes such as groundnut (*Arachis hypogaea*) and soya bean in their native forms have many applications in the food industry but Bambara groundnut in its native form has low applications because of its poor functional properties (Kato and Nakai, 1980). This project therefore seeks to make the Bambara groundnut find larger applications by extracting the proteins and modify them using limited proteolysis to improve the protein functionality. A wider use of the Bambara groundnut will also boost its production and that will go a long way to provide jobs for most rural poor communities to bring about poverty reduction. A greater application of Bambara groundnut will further improve protein consumption especially in low income areas where animal protein is expensive. Additionally, the overdependence on known legumes such as the groundnut (*Arachis*

hypogaea) and soya bean will be reduced and once Bambara groundnut gains greater acceptability, society will take the necessary steps to sustainably use it.

The following functional properties will be investigated into on the proteolytic product of the Bambara groundnut: foaming, emulsion, water binding capacity, oil binding capacity and also, the sensory properties.

1.4 Objectives

The main objective is to modify Bambara groundnut protein isolates and to evaluate their surface functional properties and their applications. The specific objectives are:

1. To establish a proteolytic procedure to modify Bambara groundnut proteins.
2. To evaluate the surface functional properties of modified Bambara groundnut proteins and their applications in cake products.

1.5 Research hypothesis

From the specific objectives, the research has been designed to answer the hypotheses below:

1. Limited proteolysis of Bambara groundnut protein isolates would give modified Bambara groundnut proteins of comparable food functional properties as that of egg proteins.

2. Cakes formulated from modified Bambara groundnut proteins would be comparable to those formulated using egg proteins.

CHAPTER TWO

2.0 Literature review

2.1 Overview of Bambara groundnut

The Bambara groundnut is widespread in Africa and was collected first in 1909 by Dalziel in Nigeria and by Ledermann in North Cameroon (Harms, 1912; Stapf. 1913). The name of this African groundnut originates from Bambara, a district on the Upper Niger near *Timbuktu* in Mali (Linnemann, 1987). According to the National Research Council (2006), the original local name for Bambara groundnut is *cokon* in the Bamanankan language spoken by the Bamanan people of Mali and it has several other names depending on the location where it is found. For instance, they are also known as *jugo* beans or in Swahili as *njugumawe*. In the Republic of Zambia, they are known as either *ntoyo* (ciBemba) or *katoyo* (kiKaonde). In Shangaan they are known as *tindluwa* and in Malagasy they are known as *voanjobory.* The groundnuts are related to cowpeas and botanically it is known as *Vigna subterranea*. There are two botanical varieties namely *V. subterranea* var. *spontanea* which includes the wild varieties and *V. subterranea* var. *subterranea* which includes the cultivated varieties (Linnemann, 1987). It is a member of the family Fabaceae and according to some authors; Bambara groundnut is called *Voandzeia subterranean* (National Research Council, 2006).

2.1.1 The importance of Bambara groundnut

Sellscope (1962) concluded in a research that Bambara groundnut was the third most important legume after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in Africa. In Ghana, Bambara groundnut has been ranked as the second most important grain legume after cowpea (Doku and Karikari, 1971). West Africa produces 45 – 50 % of the world production (Coudert, 1984), estimated at 330,000 t, although most countries, including Ghana, do not collect accurate statistics on internal agricultural production and marketing of this crop. For many years in Ghana, production and utilization was slightly ahead of cowpea (Doku, 1996). However, the introduction of high yielding varieties of cowpea and improved methods for controlling cowpea field pests led to the neglect of Bambara groundnut. Bambara groundnut produced is both for subsistence and cash, with families in the Northern sector depending on Bambara groundnut as household food stock more than those in the Southern sector. On the average, 30 % of Bambara produced is kept for household consumption (Plahar, 2002).

Brough and Azam- Ali (1992) reported that Bambara groundnut seed makes a balance food as it contains sufficient quantities of carbohydrate (63 %), protein (16.25 %) and fats (6.3 %) and relatively high proportions of lysine (6.6 %) and methionine (1.3 %) as percentage of the protein (6.6 and 1.3 % respectively). Coudert (1982) also reported that it is a rich source of protein (16-25 %). The essential amino acid content of Bambara groundnut such as lysine 6.82 g/16 g N, methoinine 1.85 g/16 g N and cysteine 1.24 g/16 g N is comparable to that of soybean (6.24 g/16 g N lysine, 1.14 g/16 g N methionine and 1.80 g/16 g N cysteine (Fetuga *et al.,* 1975). It is nutritionally superior to other legumes and it is the preferred food crop of many people in the sub- Sahara Africa (Linnemann, 1990; Brough and Azam-Ali, 1992). The crop has a number of production advantages in that it can yield on poor soils and under better conditions, it yields better. Chemical fertilizers are usually not applied to the crop as the nitrogen requirement is obtained by the natural nitrogen fixation as evidenced by several nodulation studies (Somasegaran *et al.,* 1990).

2.1.2 Commercial values and uses of Bambara groundnut

Research works by Kay (1979) and Linnemann (1988) have revealed that Bambara groundnut is mainly used for human consumption. The researchers also showed that the seeds are consumed both when it is immature or fully ripe and dry. Immature seeds are consumed fresh or grilled. They can also be boiled, either shelled or unshelled, and eaten as a meal or mixed with immature groundnuts or green maize. The ripe and dry seeds are hard and therefore difficult to grind. Usually they are either pounded to flour and boiled to a stiff porridge, or soaked and then boiled. The porridge keeps well and is traditionally used on journeys. The ripe and dry seeds are also roasted, broken into pieces, boiled, crushed and eaten as a relish with *sadza* (maize-meal porridge). They further revealed that in restaurants in Angola and Mozambique, the boiled and salted seeds are often served as appetisers. They again reported that Bambara groundnut is also fried or boiled with salt and eaten as snack or pounded into flour and used in the preparation of soup, porridge and various fried or steamed food products such as '*akara*', '*moi-moi*' and '*okpa*' in Nigeria. It also finds a use in the preparation of the local food drink '*kunu*' and such dish as '*tuwo*'.

Linnemann (1990) also reported that Bambara groundnut flour has been used in making bread in Zambia, and Brough *et al.* (1993) have also noted that the milk prepared from Bambara groundnut gave a preferred flavour to that of milks from cowpea, pigeon pea and soybean. Atiku (2000) found that in North Eastern Nigeria, Bambara groundnut is not only consumed as food, but also used for medicinal purposes. The haulm is used for livestock feed (Tanimu and Aliyu, 1997) and the leaves, which have been reported to be rich in nitrogen and phosphorus, are suitable for animal grazing.

2.1.3 Areas of cultivation and production figures of Bambara groundnut in Ghana

The *Dangme* East district of the Greater Accra, and North *Tongu* district of Volta regions have been identified as major producing areas in the Southern sector. In the Northern sector, the *Bawku* East, *Tolon-Kumbungu*,West *Gonja* districts as well as most districts in the Upper West Region were also identified as major producing areas (Plahar, 2002). According to the researcher, Bambara groundnut is generally cultivated on a smaller

scale compared to other legumes like groundnuts, cowpea and soybean. An average of 3 acres of land is cropped by each farmer, with an overall average yield of 3.5 maxi bags in a year. The most important constraints to production faced by Bambara groundnut farmers in Ghana are poor climate, lack of processing facilities, limited access to land, pest infestation and high cost of labour and inputs. Bambara groundnut is usually stored shelled or unshelled over an average period of 8 months. Traditional pre-storage treatment is more commonly used than the application of agro-chemicals. Generally, processing of Bambara groundnut is limited to the production of flours and pastes, using simple traditional techniques. Bambara groundnut plays a more important role in the diets of households in the Northern sector than in the South. The major constraints to Bambara groundnut processing and utilization identified by the researcher were long cooking periods and inadequate processing techniques (Plahar, 2002).

The producers of Bambara groundnut sell the groundnut to traders in the producing areas from whom most of the retailers in the urban markets such as *Techiman*, *Tamale* and *Bawku* buy from. The retailing takes place in most of the urban markets such as *Kpassa*, *Kasseh* junction, *Tamale*, *Bawku, Techiman* and the *Agbogbloshie* markets. Mainly market women, mostly of low educational level, do retailing in the market places (Plahar, 2002).

2.1.4 Limitations of native Bambara groundnut proteins in the food industry

Though the Bambara groundnut has global availability especially in hostile tropical environments, the African legume is underutilized (Heller *et al.*, 1997) and remains one of the crops most abandoned by science (Carlson and Tookey, 1983). This is because the untreated protein of Bambara groundnut has some drawbacks with regards to the functional properties despite the high protein content, and because of this it has not gain the desired industrial application. The usefulness in the food industry is often limited by the presence of toxic and antinutritive components, which include trypsin inhibitors (Carlson and Tookey, 1983).

Bambara groundnut protein in its untreated form also has reduced foaming and emulsion due to poor interfacial film formation, which is desirable for emulsion, and foam formation (Kato *et al.,* 1985), off flavour development during storage and thus degrading the product, the testa of the bean is very and this makes cooking to last for several hours.

According to Lásztity *et al.* (1993), consumption of the legume grains also leads to excessive flatulence causing embarrassment to consumers. They reported that the principal cause of flatulence, as with all legume grains, is the oligosaccharides in the carbohydrate portion. The best-known oligosaccharides in legume grains are raffinose and stachyose. They also added that neither home cooking nor high-temperature industrial heating processes would eliminate raffinose and stachyose since they are very heat stable. They rather require the enzyme alpha-galactosidase to be digested properly. Unfortunately, humans and other mammals do not possess the enzymes to digest the oligosaccharides. The result is that the raffinose and stachyose pass through the small intestine undigested to arrive in the large intestine where the inhabiting micro flora and fauna attack them. The digestive fermentation that takes place always results in flatus gas which causes embarrassment making people shy away from the consumption of Bambara groundnut. Nonetheless, experiential evidence and some research results suggest that it is a crop with great potential and hence there is the need to find ways to improve the functionality. To improve the functionality, some applications of proteins and protein functionality must be brought to the fore.

2.2 Some uses of proteins in the food industry

Proteins are often used in the food industry as functional ingredients to impart some desirable trait to food products. One potential application within the food industry includes gelation or the formation of three-dimensional networks resulting in solids or semi-solids. This attribute contributes to the textural properties of food, flavour release and moisture holding capacity. Other potential applications include foam formation and stability which influences texture of products such as angel food cake. Proteins are also used in emulsions (defined as a dispersion of one liquid into another liquid or solid), as stabilizers due to interfacial properties with polar groups; thus allowing for interaction

within a liquid such as water and a non-polar group that allows for interaction within a liquid such as oil (Fennema, 1996).

2.3 Proteins functionality in the food industry

Commercially available protein foods are obtained from a range of animal and plant sources and are used as functional ingredients (Periago *et al.,* 1998) and, plant protein products are also gaining interest as ingredient in food systems throughout many parts of the world (Kinsella, 1976). Besides the nutritional value, functional properties are very important characteristics of food (Kim and Kinsella, 1987).

Food functionality has been defined as any property of a food or food ingredient, except its nutritional ones, that affects its utilization (Zayas, 1997). These properties are intrinsic physico-chemical characteristics which affect the behaviour of properties in food systems during processing, manufacturing, storage and preparation (Dua *et al.,* 1996). In order for a protein to exhibit functionality, it must interact with other components of the food system. These interactions may often require that the protein be free to either move throughout the system or to alter its structure in such a way to allow interactions with other components. In some cases the simple presence of other molecules in the protein solution will allow interaction to occur, but more commonly, the interactions require an input of energy into the system to ensure adequate mixing. This energy may alter the physical nature of the molecules being mixed, and decrease the average fat globule size and also alter the conformation of the protein molecule (Graham and Philips, 1976). Functional properties vary with source of protein, composition, method of preparation/extraction, thermal history, prevailing environment i.e. pH, ionic strength, temperature, presence of salts etc (Mata and Joseph, 1977).

A great deal of research goes into studies of functional properties by the food industry in order to understand the basics so that new processes, foods, etc can be developed. Films of proteins at fluid interfaces exist in a range of situations, which are very important in biochemistry and protein technology. For proteins then, there must be a large number of functions and functional properties. Some of the most important ones to consider when

discussing proteins employed in the manufacture of food products are the flavour, water binding, viscosity, gelation, dough formation, emulsification and foam formation. Protein functionality is applied in food system and examples are shown in Table 1 below.

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Table1. Food protein requirements for application in different food products (Zayas, 1997).

Food Product	**Required functions for all products**	**Functions required for some products**
Beverages	solubility, colloidal stability	acid stability, emulsifying, water binding
Bakery	solubility, emulsifying, gelation	foaming, foam stability water binding, gluten modification
Confectionery	foaming, solubility	Gelation
Frozen desserts	emulsifying, foaming, dispersibility	solubility, water binding, fat mimetic

Imitation dairy	emulsifying, colloidal stability	solubility, foaming, foam stability
Infant formula	nutrition, solubility, emulsification, colloidal stability to heat	mimic human milk composition
Reformed meat	emulsification, water binding	salt solubility, low viscosity in solution, gelation, fat mimetic
Retortable sauces	emulsifying, colloid stability to heat	water binding, viscosity building

2.4 Protein modification

Proteins are a common food ingredients used by food technologists and modifications are often made to the proteins to further enhance their functional benefits (Akhtar and Dickinson, 2003). Various means to modify the structure of proteins have been studied and these include chemical, enzymatic and genetic methods (Kester and Richardson, 1984). Use of enzymes offers many advantages, including the ability to perform modifications under physiological conditions with great specificity and stereoselectivity without undesirable side reactions. Compared to chemical hydrolysis, enzymatic hydrolysis of protein, using selective proteases, provides more moderate conditions of the process and few or no undesirable side reactions or products. In addition, the final hydrolysate after neutralization contains less salts and the functionality of the final product can be controlled by selection of specific enzymes and reaction factors (Chiang *et al.,* 1999; Darwicz *et al.,* 2000). They also provide an aesthetic advantage because enzymes are natural products (Kester and Richardson, 1984).

2.4.1 Proteases

Proteases are a group of enzymes that catalyze the cleavage of peptide bonds in proteins, thereby causing the disassembly of protein molecules. They differ in their ability to hydrolyze various peptide bonds as each type of protease has a specific kind of peptide bond it cleaves. According to Rao *et al.* (1998), proteases can be obtained from animal, plant and microbial sources. Some examples include trypsin, chemotropism (animal pancreases), pepsin (gastric stomach), bromelain (pineapple), papain in (papaya latex), facing (fescues latex), subsidizing (*Bacillus subtilis* collagenase (clostridia), fungal proteases, viral proteases and other bacteria proteases are also known.

Besides the numerous physiological functions of proteases they have a variety of applications in the food and detergents industry (Phadatare *et al.,* 1993). Fungal neutral proteases supplement the action of plant, animal and bacterial proteases in reducing bitterness of food protein hydrolysate. Fungal alkaline proteases are also used in food protein modification (Impoolsup *et al.,* 1981). The inability of plant and animal proteases to meet current world demand has led to an increased interest in microbial proteases both for basic understanding of enzyme mechanism and industrial applications (Rao *et al.*, 1998).

2.4.2 Limited proteolysis

Limited proteolysis is a process by which peptides are produced by partial hydrolysis of proteins with enzymes to obtain smaller molecular size and less quaternary structure than the original proteins (Chamacco *et al.,* 1993). A schematic diagram of the process is as shown in Figure 1 below:

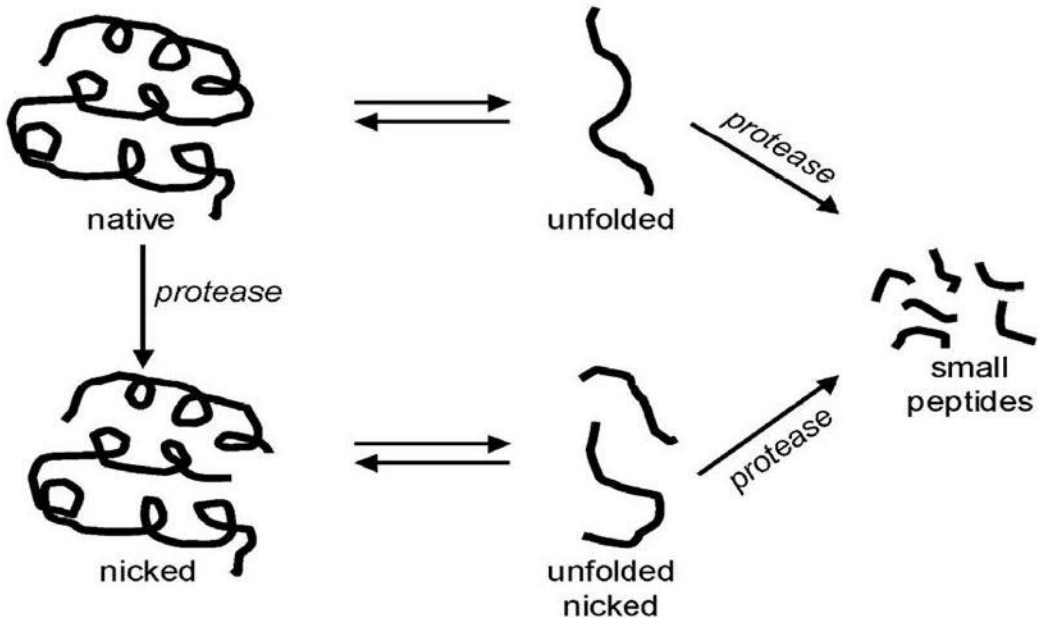


Figure 1. Schematic view of the mechanism of proteolysis of a globular protein (Fontana *et al.*, 2004).

A dual mechanism of protein degradation is shown, the one involving as substrate for proteolysis the (fully) unfolded protein and the other the native form of the protein. In this last case proteolysis is limited and occurs (at flexible site(s), leading to a nicked protein species that can unfold and then be degraded to small peptides.

Fontana *et al*. (1997 a, b) have reported that the basic premise of limited proteolysis is that the protease can bind to the protein substrate at sites whose conformations are complementary to its active site. Since a rigid protein structure has a specific conformation, the chances of conformational complementarily are relatively small and thus native globular proteins are usually quite resistant to proteolysis. They explained that the flexible regions of native protein imply existence in a range of local conformational isomers. This correlation was first demonstrated with a thermostable neutral metalloproteinase enzyme- thermolysin and subsequently with other proteases. As expected, the sites of limited proteolysis in proteins are sufficiently exposed to be able to bind to the protease's active site. But the exposure which is a required characteristic of the cleavage site is not sufficient to explain the specific proteolysis. Indeed, flexibility of the chain segment suffering proteolytic attack is the key parameter dictating limited proteolysis.

Research has shown that there are four groups of proteases and each cleave at certain specific sites. Pepsin cleaves protein subunits preferentially at carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine (Fujinaga *et al.,* 1995). On the other hand, collagenase degrades the helical regions in native collagen preferentially at the Y-Gly bond in the sequence Pro-Y-Pro- where Y is most frequently a neutral amino acid (Wünsch and Heinrich , 1963*).* It is also known that papain breaks peptide bonds which involves deprotonation of Cys-25 by His-159 and Asn-158 (Lopes *et al.,* 2007). Tripsin also cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either it is followed by proline (Leiros, 2004). It has been shown in several studies that there is a clear-cut correlation between sites or regions of enhanced segmental mobility and sites of limited proteolysis (Polverino de Laureto *et al.*, 1995). Proteolysis takes place at a helical segment of the proteins and the helix is likely to have been destroyed by end-effects and loss of the cooperative hydrogen bonds that stabilize it. The loss of hydrogen bonds creates charges and as a result the ends that are newly created becomes charged and if buried, might conceivably destabilize the protein core. Thus, limited proteolysis occurs preferentially at those loops which display inherent conformational flexibility, whereas the protein core remains quite rigid and thus resistant to proteolysis (Fontana *et al.*, 1986).

2.4.3 Factors influencing limited proteolysis to improve food functionality

Improving quality of protein isolate by enzymatic proteolysis is well known (Periago *et al.*, 1998) and the possibility to get peptides with desirable properties is one of benefits of limited proteolysis (Gill *et al*., 1996, Korhonen and Pihlanto, 2006). Several workers have studied limited proteolysis of soy proteins (Chamacco *et al*., 1993; Sule *et al*., 1997; Anon and Molina Ortiz (2000) and suggested that limited proteolysis contribute to increased functional properties such as solubility, foaming, gelling and emulsifying when compared to those of the native proteins.

Earlier investigations of enzyme-induced modification were conducted to remove undesirable flavour and/or their precursors as well as to mask the flavour (MacLeod and Ames, 1988). Most of these works were performed with proteases (to break the bonds

between proteins and volatile components) and some oxidases (to irreversibly oxidize aldehydes which could be easily removed). Abdo and King (1967) accomplished the earliest reported work and have been the pioneers in this area. They concluded that the extraction of defatted soy flakes with water containing a mixture of enzymes obtained from *Pestalotiopis weterdijkii* yielded a product with improved flavour. Fujimaki *et al*. (1968) also showed that 12 individual proteolytic enzymes increased beany flavour significantly and in some cases the intensity of bitterness as well. To obtain desirable functional properties of soy protein hydrolysates, hydrolysis must be carried out under strictly controlled conditions to a specified degree of hydrolysis. This is because a limited degree of hydrolysis usually improves solubility, emulsifying and foaming capacities, whereas excessive hydrolysis often causes loss of some of these functionalities (Kester and Richardson, 1984). In some cases it has been possible to correlate the degree of hydrolysis with the changes in solubility (Sule *et al*., 1997).

Anon and Molina Ortiz (2000) reported that the solubility of hydrolysates obtained with five proteases and their ability to form and stabilize foams correlated well with the structural properties. Several authors (Nakai *et al*., 1980; Nakai, 1983; Voutsinas *et al*., 1983) showed the existence of strong correlation between surface hydrophobicity and emulsifying activity of modified protein isolates. In contrast to this, Wu *et al.* (1998) detected high correlation between these properties for unmodified isolates, but for papain-obtained hydrolysates the correlation was low. They suggested that solubility and molecular size, rather than surface hydrophobicity, might be the major factors for the high emulsifying activity of the small polypeptides.

Also, the size of the peptides formed and their secondary and tertiary structures are extremely important. For example, Jost *et al*. (1982) observed improved emulsifying activity and greater emulsion stability of protein hydrolysate as compared to untreated protein using an oligopeptide fraction obtained from a tryptic digest of whey protein by ultrafiltration. The disadvantages associated with Bambara groundnut protein in its native form can be overcome by limited proteolysis to improve functionality as has been

applied to the soy and whey isolates by some earlier workers as highlighted in the above literature.

2.5 Foaming properties

Foam capacity or overrun; foam activity and foam stability are important foaming properties to the food technologist (Panyam and Kilara, 1996). Foaming is the incorporation of air into an aqueous medium by means of physical agitation and aeration. Foam is caused by the protein film to lower surface tension i.e. cohesive force of water molecules that tend to collapse a bubble, and give resistance to shearing/tearing with high level of stretching capacity. So when protein solution is whipped or stirred vigorously air is pulled down into solution, and when it tries to escape up, flexible protein surface forms a bubble (Mata and Joseph, 1997). Not all proteins foam equally well and not all of the foams are particularly stable.

Egg does not foam much, but quite stable and only gelatin provides good amount of foam that is also stable. Lyzozyme does not foam due to repulsion (Guitian and Joseph, 1997). To be a good foaming agent the protein must be capable for rapid diffusion to the air-water interface and must form a strong cohesive, elastic film by partial unfolding and the foaming properties seem to be correlated with the amount of hydrophobic amino acids that are exposed at the surface of the protein molecules (Damodaran, 1990).

2.6 Emulsifying properties

An emulsion is a dispersion of very fine droplets of one liquid in a larger volume of another liquid. There are two types: oil-in-water and water-in-oil. These two liquid are immiscible. To stop liquids separating out, emulsifying agent is needed to act as a mixer by interacting with both liquids. Milk is a natural oil-in-water emulsion with protein casein as the emulsifying agent in milk. When protein acts as emulsifying agent, it will be

due to the hydrophilic and hydrophobic amino acids on the protein which are able to interact with both water and oil in the food system (Chove *et al*., 2002). Emulsification therefore is not restricted to surface acting as a foam fine droplets are dispersed in the body of the solution. Protein interacts and is held in solution so oil droplet remains dispersed and cannot join-up with other droplets to enable separating out of the oil. Proteins are composed of charged amino acids, non charged amino acids and polar amino acids which make proteins possible emulsifiers.

The surfactant possessing both hydrophilic and hydrophobic properties is able to interact with both water and oil in food system (Chove *et al*., 2002). Related to protein structure is a degree of hydrophobicity i.e. amount of hydrophobic amino acids and ease of denaturation or unfolding to expose the hydrophobic residues which are able to interact (hydrophobic interaction) with the lipid molecules. Protein solubility and water binding capacity, though not considered as surface functional properties invariably influence the behaviour of these surface properties (Kanno, 1989).

2.7 Water binding capacity

Water binding capacity is the ability of a substance to associate with water under a limited water condition (Singh, 2001). Water molecules tend to associate with themselves through a network of hydrogen bonds. When solute molecules are placed in water, these molecules will be soluble if water- solute interactions have a lower free energy than do the separate solute-solute or water-water interactions. Proteins contain a number of amino acids that have side groups that contain electrical charges at certain pH values. The ion-dipole interactions between water and these charged groups are fairly strong with energy of about 5 Kcal/mole. With model peptides, it has been shown that from four to seven peptides, water molecules can be associated with each residue of charged amino acid (Pulski, 1975). The author again stated that proteins also contain amino acids that have polar side chains, but that do not have a charge. These polar molecules are dipoles and thus water can interact with them through dipole-dipole interactions.

Because the molecules involved all contain hydrogen as part of the dipoles, the special class of dipole-dipole interactions known as hydrogen bonds can occur. These are

typically stronger than other dipole-dipole interactions and can have energies of from 2 to 6 Kcal/mole. The non ionized polar amino acids in proteins typically have two water molecules strongly associated with them. Non polar amino acids are not soluble in water and thus, the interaction of water with these molecules is minimal. Sometimes, however, non polar groups are forced into water as a part of a specific protein structure. The intrusion of hydrophobic groups into the aqueous environment causes an ordering of the water molecules in their vicinity and thus a decrease in entropy. It has been estimated that the removal of a hydrophobic group from contact with water yields a reduction in the free energy of the protein of about 4 Kcal/mole. This is a strong driving force for the removal of these groups from the aqueous environment. Any water associated with these groups is highly ordered and has been termed hydrophobic hydration water (Pulski, 1975). In foods, the term 'water binding' is used to convey a general tendency for water to associate with hydrophilic substances including cellular materials. Increased water binding capacity results in better taste, softer crumb, delayed staling and longer keepability (Fennema, 1996).

2.8 Oil absorption capacity

Kinsella (1976) reported that oil absorption capacity is an important functional property to the food technologist since oil serves as flavour retainer and increases the mouth feel of foods. He concluded that oil absorption capacity is also related to the interaction of non-polar side chain of the proteins as well as to the conformation features of the proteins. The digestion of proteins may bring out the core of proteins non polar side chains that bind hydrocarbon moieties of oil, contributing to increased oil absorption. Proteins isolate with high water binding and oil absorption capacities are desirable for use in meat, sausages and bread (Ahmedna *et al.,* 1999).

2.9 Importance of cakes

Limited proteolysis of protein isolates usually results in improved foaming properties (Swaisgood *et al.,* 1996) and this property of proteins of the Bambara groundnut could be

explored in the preparation of cakes which high foaming is essential. This would be made possible by replacing eggs used in the preparation of cakes with modified Bambara groundnut proteins. The high foamability of the modified Bambara groundnut proteins could also add value in the model cake system since the cakes would have higher volumes compared to cakes produced from equal weight of egg protein. This in turn could make the cakes sell at a cheaper price and/or, increase profitability. Cakes are important in Ghana because they are associated with fun, happiness, enjoyment, and celebrations. They are often the dessert of choice for meals at ceremonial occasions, particularly weddings, anniversaries, and birthdays. Cakes and their decoration provide jobs that one can even start it as a home business in Ghana. Particular types of cake may be associated with particular festivals, such as *stolen* (at Christmas), *babka* and *simnel* cake (at Easter), *or moon* cake (Ayto, 2002).

CHAPTER THREE

3.0 Materials and methods

Materials: Bambara groundnuts were obtained from an out grower at *Nyankpala* in the Northern region of Ghana. Vegetable oil (*frytol*), sugar and essence (flavouring agent), as well as wheat flour from *Takoradi* Flour Mills, *Takoradi*, Ghana and *Topper* Margarine (Holland) were purchased from the Central Market, *Kumasi*, Ghana. Protease and egg white were obtained from the Department of Biochemistry and Biotechnology of the KNUST.

Chemicals: The following general-purpose reagents; ethanol, sodium hydroxide and Ninhydrin were obtained from BDH Chemicals Ltd., Poole, England whereas petroleum ether was obtained from Tema Oil Refinery, Ghana. The procedures for the preparation of reagents are shown in appendix A.

3.1 Preparation of Bambara groundnut protein isolates (BGPIs)

3.1.1 Milling, drying and defatting of Bambara groundnut

The dried seeds weighing 20 kg were milled into fine flour using grinding mills (Hunt A713, Germany). The moisture content was determined with the formula in Appendix D-1.1. The powder was dried in a solar tent dryer at a temperature of 35 to 45 ^{0}C and relative humidity of 30 to 35 % for 72 hours. Defatting of the seed meal was done using

petroleum ether in a ratio of seed flour to solvent of 1:10 w/v, in a large-scale Solvent-Liquid extractor (model E1VS, France). The defatted meal was then solar-dried for 168 h at 35 to 45 ^{0}C and relative humidity of 30 to 35 % to expel the volatile solvent. The yield of the defatted meal was calculated using the formula in Appendix D-1.2.

3.1.2 Protein extraction

Proteins were extracted from the dried defatted Bambara groundnut meal by using 0.01 M NaOH as described by the method of Gomez –Brenes *et al.* (1983). The meal to solvent ratio of 1:10 w/v was stirred in a large plastic container at 25 to 26 ^{0}C for 2 h. This mixture was allowed to stand for further 2 h and, proteins and oligosaccharides were solubilised in the supernatant while the insoluble polysaccharides and residues settled at the bottom.

The supernatant was decanted and centrifuged at 2500 rpm for 30 min in order to separate any insoluble residues. The supernatant obtained after the centrifugation was decanted and acidified to a pH range of 4.5-5.0 with 0.1 M HCl to precipitate the proteins. The supernatant was decanted and discarded. The protein suspension which was obtained after precipitation was further subjected to centrifugation at 2500 rpm for 30 min to separate proteins from soluble oligosaccharides. The precipitated proteins were washed three times with distilled water (about 2 volumes of the proteins) in order to get rid of the chemicals used for the extraction. It was subsequently freeze-dried using a freeze dryer (HETO Power Dry LL300, England). The yield of protein extract was calculated by using the procedure in Appendix D – 1.3.

3.1.3 Determination of protein to protease ratio

The Bradford (1976) method was used to determine the optimal pH for the proteolytic reaction. A stock solution of bovine serum albumin (BSA) of concentration 0.5 mg/mL was prepared with phosphate buffer of pH 5.8. Serial dilutions of 1.0, 2.5, 5.0 and 25 mL of the stock solution were then prepared. A volume of 20 mL solution of Coomassie brilliant blue dye was added to each of the prepared solutions. The solutions were

centrifuged for 5 min and the supernatant incubated at 37 ^{0}C for 10 min. The absorbances were then read at 595 nm with spectrophotometer (Helios Gamma, England). Duplicate tests were run and the mean values taken. The procedure was repeated by using phosphate buffer of pH 6.9 and 8. A graph of BSA concentration and absorbance was then plotted for each pH (Appendix B - 1.1 to 1.3.1). From the results obtained, the pH of 8 was chosen as the pH for the proteolysis because in addition to displaying the best R^2 characteristics, the protein dissolved maximally at this pH.

The protein to protease ratio was established by using the method of Braam and Reznikoff (1997) who established protein to protease ratio of 200 to 1 when working on partial proteolysis of *Tnp, Inh,* and Fusion Proteins using trypsin. The result the authors obtained was used as the basis to conduct series of experiments to establish the protein to protease ratio of brewer's yeast used for this study. Proteins to protease ratio in the range of 100:1 to 4000:1 were used in 10 % protein solution for a positive Ninhydrin test. The ratio for the most excellent Ninhydrin test determined which was 3000: 1 and was used for the treatments.

3.1.4 Determination of enzyme specific activity

The protocol of Herschlag (1988) was used with time required for reaction as the variable to determine the duration of proteolysis. From the results obtained from the determination of the protein-protease ratio, 100 mL of 10 % protein suspension was prepared into a 250 mL beaker and was heated on a water bath to 40 ^{o}C. A mass of 3.30 mg protease was dissolved in a 50 mL phosphate buffer of pH 8 and was added. The mixture was stirred immediately to start the proteolysis and a timer (Gralab 165 A, USA) started simultaneously. The proteolysis was carried out for 2 h and aliquot of 1000 µl was pipetted with a 2000 µl Fischer pipette at 5 min intervals up to 80 min, and then 100 and 120 min.

The 1000 µl aliquots were pipetted at every 5 min put into labeled clean and dry 15 mL centrifuge tubes in boiling water to stop the reaction. Three drops of Ninhydrin solution was then added immediately to develop the purple colour. The tubes containing the samples were centrifuged for 5 min at 5000 rpm and the supernatant incubated at 37 ^{o}C

for 10 min. The absorbance for each of the test sample was read at 595 nm. The procedure was repeated and the mean values obtained (Appendix C-1.0) plotted with time. The times for the proteolytic processes were then selected as 10, 35, 60 and 120 min. A graph of *absorbance* and *time* was plotted (up to 30 min) and the gradient represented the *protease activity* (Appendix B-1.4). From the protease activity, the specific *activity* was calculated as the value of the activity per unit mass protein content of the protease.

3.1.5 Protein limited proteolysis

The procedure of Khalil *et al.* (2006) was used. A volume of 2 L phosphate buffer of pH 8 was put in a 5 L glass container (with a lid). A mass of 200 g (dry basis) of the protein isolate was added to the buffer, mixed thoroughly, warmed and agitated in a Clifton thermostatic water bath (England) set at 40 ^{o}C and a homogenous solution was obtained. At 40 ^{o}C, a mass of 0.0667 g of protease was completely dissolved in a 50 mL phosphate buffer of pH 4 and was added to the protein solution as the shaking continued in order to initiate the proteolysis at protein to enzyme ratio of 3000:1. The proteolysis was stopped at 10 min by adding 4 L of broken ice cubes to the reaction medium and subsequently stored in a Sanyo deep freezer (SF-C18K (A), Japan) at -10 ^{o}C.

The procedure was repeated with three other 200 g of protein (on dry basis) while maintaining the same conditions as before except that the time for proteolysis was varied as 35, 60 and 120 min respectively. The resulting proteolysates were respectively labeled as BAM-10, BAM-35, BAM-60 and BAM-120. The unhydrolysed protein sample was also labeled as *Native* and stored in the Sanyo deep freezer (SF-C18K (A), Japan) at -10 ^{o}C.

The frozen samples were later thawed at temperatures of between 1 and 3 ^{o}C and quickly centrifuged (MSE Mistral, 300E, SG95/10/256, England) for 5 min at 3000 g to concentrate the samples after which they were put in plastic containers and stored in the freezer for 120 h and freeze dried subsequently.

3.2 Determination of foaming properties

The foam capacity, foam stability and foam activity were determined by the procedure of Coffman and Garcia (1977). Protein solution (3.4% w/v) was prepared by adding 1.7 g of powders of the respective protein isolates into 50 mL volumetric flask. A volume of 50 mL of phosphate buffer of pH 4 was added to the protein powder in the flask and was agitated to obtain a uniform suspension. A volume of 40 mL of the suspension was homogenized using Silverson homogenizer (LR4, England) at the speed of 6000 rpm for 3 min in a stainless-steel container using the Silverson homogenizer at a room temperature of 25 to 26 ^{0}C. Foam was generated in the process and the homogenate was quickly transferred into a 250 mL glass measuring cylinder . The foam settled at the top of a liquid in the cylinder. The volume of foam and that of the liquid were determined using the graduations on the glass cylinder. The solutions were allowed to stand for 30 min and the foam volume after this period was also measured. *Foam capacity* was calculated according to the equation in Appendix D-2.1 and *Foam stability* determined with the equation in Appendix D-2.2. The *foam activity* was also determined by the equation described by Johnson and Brekke (1983) as shown in Appendix D -2.3. All the tests measurements were duplicated and the procedure was repeated using phosphate buffer of pH 7 and 9.

3.3 Determination of emulsion properties

Emulsion capacity, *emulsion activity* and *emulsion stability* were determined according to the centrifugation procedures of Ahmedna *et al*. (1999). The emulsion capacity was first determined by adding 50 mL phosphate buffer of pH 4 to 1 g of the respective protein isolates in a labeled 50 mL volumetric flask. This was agitated to obtain a uniform mixture and finally poured into a stainless-steel container. A volume of 50 mL vegetable oil (frytol) was then added to obtain 50 % oil to water emulsion and homogenized at the speed of 6000 rpm for 3 min using the Silverson homogenizer at a temperature of 26 ^{0}C. A volume of 10 mL of the sample was put in a 15 mL centrifuge tube and was centrifuged at 2500 g for 20 min after which the emulsion was separated out. The volume of the emulsion was determined by using the graduations on the tube to measure the

volume of the tube occupied by the emulsion. All the tests measurements were duplicated and the procedure was repeated using phosphate buffer of pH 7 and 9. The *emulsion capacity* was calculated according to the formula in Appendix D-3.1.

The *emulsion activity* was also determined by preparing a solution of 0.1 % protein solution by adding 100 mL phosphate buffer of pH 4 into a 100 mL volumetric flask containing 0.1 g of the protein powder. The mixture was transferred into a plastic container and agitated on a mechanical shaker (Gallenkamp, UK) for one hour to form a protein suspension. The suspension was then added to vegetable oil (*frytol*) in a protein suspension to oil ratio of 4:1. The mixture was homogenized at the speed of 6000 rpm for 3 min at 26 ^{0}C. A volume of 1 mL aliquot of the homogenate was then diluted in 250 mL distilled water in a 500 mL Erlenmeyer flask. The absorbance was read at 500 nm using a spectrophotometer. A blank absorbance was earlier determined and subtracted from values obtained. All the tests measurements were duplicated and the procedure was repeated using phosphate buffer at pH 7 and 9. *Emulsion activity* was determined according to the formula in Appendix D-3.2.

The *emulsion stability* was determined from the mixture used for the emulsion activity. The mixture was allowed to stand for 30 min and 1 mL aliquot of the solution was diluted to 250 mL with distilled water in a 500 mL Erlenmeyer flask. The absorbance was read at 500 nm using a spectrophotometer (Helios Gamma, England). A blank absorbance was also measured. The actual absorbance was taken as the absorbance of the sample minus the blank absorbance. The emulsion stability was determined according to the formula in Appendix D-3.3.

3.4 Determination of water binding capacity (WBC)

This was determined according to the official method of AACC (1981). A weight of 1 g protein powder (W_0) was put into a pre-weighed centrifuge tube. The weight of the tube and the protein was determined (W_1). A volume of 10 mL distilled water was added to the protein powder to a paste-like consistency and centrifuged at 3000 g for 20 min. The clear water was decanted after the centrifugation leaving protein sediment in the tube.

The weight of the tube and the sediment was also measured (W_2). WBC was calculated according to the formula in Appendix D-4.0.

3.5 Determination of oil absorption capacity (OAC)

This was determined by the method of Sosulski (1976). A weight of 1 g each of the protein samples were weighed into separate clean and dry 15 mL pre-weighed centrifuge tubes and 10 mL vegetable oil (*frytol*) was added to the samples in each of the tubes. This was agitated for 5 min to form a homogenous paste. The tubes were centrifuged at 5000 x g for 30 min and the free oil was decanted at an angle of 45^0 for 10 min. The tubes were then weighed to determine the weight difference between the weight of the oil pellet and the original dry product. The equation in Appendix D-5.0 was used to determine the oil absorption capacity. The above procedures were also used to find the corresponding values for egg white.

3.6 Sensory Evaluation

Evaluation of the functional properties showed increased foaming properties which are essential in cake preparation. Cakes were therefore prepared using the protein isolates in accordance with the procedure of Pateras and Rosenthal (1992) as described in Appendix E-4.0. Sensory evaluation of the cake products were then carried out. Six formulations of cake products were designed for the test proteins samples using the response surface, D-optimal design (Design Expert, 2007), which projected a quadratic model to study the variations of the two factors: test proteins (A) in formulations and panelists (B) as shown in Appendix E-1.0. A full factorial design was used to project 36 serving order (Appendix E-2.0) for the 6 cake samples prepared. Six panelists who were familiar with the products then ran a quantitative descriptive analysis on the test proteins in the sample cake products. Subsequently, a 10 cm line scale that was anchored at extreme ends and ranged between 1 and 9 (1 for very bad, and 9 for excellent) was used to measure appearance, flavour and texture on a score sheet (Appendix E-3.0).

3.6.1 Statistical methods

3.6.2 Fitting the data collected

The sensory evaluation data collected were loaded into the statistical tool (Design Expert, 2007) and fitted to models that could explain the behaviour of the sensory parameters of the cake over the design space. After fitting and ANOVA studies, the model graphs were obtained and analysed.

3.6.3 Optimisation of the sensory evaluation process

The research sought to determine the sensory properties of proteins in sample cake products. Goals were therefore set by imposing constraints in the factors studied (Table 2) such that optimum conditions would be obtained. The total vegetable proteins (TVP) and treatment types were set in range for the statistical tool to select these optima.

Table 2: Optimum conditions for maximum product acceptability of the Bambara groundnut protein isolates and egg white.

Factor	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Cake Expt.	is in range	478	702	1	1	3
Panelists	is in range	P1	P6	1	1	3
Appearance	maximize	1	9	1	1	5
Flavour	maximize	1	9	1	1	5
Texture	maximize	1	9	1	1	5

CHAPTER FOUR

4.0 Results and Discussions

4.2 Bambara groundnut protein isolates (BGPIs)

A mass of 10.0 kg of previously dried Bambara groundnut flour of moisture content 8.16 % was defatted, and 8.75 kg of defatted Bambara groundnut meal (on dry basis) was obtained. The yield was 87.5 %. Protein extraction was carried out on the 8.75 kg of defatted Bambara groundnut meal and approximately 1.43 kg (dry basis) protein was obtained. This gave a yield of 16.43 %.

At a moisture level below 10 %, respiration in most food grain will almost stop, and this increases the grain's storage life (Odogola, 1994). The moisture content of the Bambara groundnut flour was determined to be 8.16 % and this implied that respiration in the groundnuts was lower than what was obtained by Odogola (1994). This therefore implied that the Bambara groundnut used in this study had increased storage life than most foods with 10 % respiration. This also suggested that the Bambara groundnut was properly dried after harvesting and was not deteriorated due to any micro-biological activity (Odogola, 1994).

The reduction in the weight of Bambara groundnut flour after defatting from 10 kg to 8.75 kg was as a result of loss of fat during the defatting. The yield of Bambara groundnut on defatting with petroleum ether was 87.5 % and is closer to 82 % obtained by Alain *et al.* (2007) who use 60 % to defat Bambara groundnut meal. The difference in the values obtain is perhaps petroleum ether is more effective in defatting Bambara groundnut flour than 60 % ethanol.

The protein yield obtained in this study was 16.43 %. This was lower than 22.78 % that was previously obtained by Alain *et al.* (2007) who also extracted proteins of Bambara groundnut. The difference in the yields could probably be due the method of Cuq (1991) that was used by Alain *et al.* (2007) for the extraction. This also suggests that perhaps the Cuq method was more efficient than the Gomez-Brenes (1983) method in protein extraction, or probably there was excessive loss of the protein during the extraction in this study.

4.3 Protein to protease ratio and specific activity

The protein to protease ratio was determined to be 3000:1 w/w. The specific activity was also calculated to be 7.58 x 10^{-3} µmol min^{-1} $mg^{-1.}$ The optimum pH was found to be 8 (Appendix B-1.1 to B 1.3) suggesting the enzyme might be the alkaline protease (Alencar *et al.*, 2003).

The specific activity of 7.58 x 10^{-3} µmol min^{-1} mg^{-1} was lower than what was observed in most literature. For instance Yossan *et al.* (2007) purified alkaline protease enzyme from *B. Megaterium* isolated from Thai fish sauce fermentation process. The specific activity they obtained for the crude enzyme extract was 0.09 µmol min^{-1} $mg^{-1.}$. When purification fold of the enzyme extract was increased to 3, the specific activity was increased to 0.029 µmol min^{-1} mg^{-1}. A further increase in purification fold to 107 again increased the specific activity to 9.66. The lower specific activity of the brewer's yeast used in this work therefore suggests that the enzyme might be a crude extract. Specific activity gives a measurement of the activity of the enzyme (Passonneau, 1993). The specific activity of 7.58 x 10^{-3} µmol min^{-1} mg^{-1} of the brewer's yeast therefore indicates the activity of the brewer's yeast was lower compare to purified alkaline protease enzyme from *B. Megaterium* isolated from Thai fish sauce fermentation process.

4.3 Optimum proteolytic condition

The optimum proteolytic condition was obtained with 10 % protein concentration, protein to protease ratio of 3000:1, proteolysis time of 35 min and at a temperature of 40 ^{o}C. Baraniak and Swieca (2008) also modified legume flours with trypsin and obtained the following optimum proteolytic condition: 10 % protein concentration, protein to protease ratio of 10:1, temperature of 37 °C and proteolysis time of 30 min. The protein concentration used in this work was the same as the one used by these researchers. The protein to protease ratio obtained by Baraniak amd Swieca (2008) was however lower than 3000: 1 obtained in this study. This implies that the brewer's yeast used in this study

is perhaps more effective to hydrolyse leguminous proteins than trypsin, and for that matter a far lower concentration of it was required.

4.4 Foaming properties

The results (appendix C-2.0) showed a general increase in foaming properties with proteolysis at all pH levels compared to the native protein. Apart from foam activity at pH 4 which showed a steady progressive increase from 20 % for the *Native* up to 114 % for BAM 120, foaming properties for all other parameters at all pH levels showed progressive increase and subsequently, decreased with increasing time of proteolysis. The trend observed for foam capacity, foam activity and foam stability have been explained below.

4.4.1 Foam capacity

The results obtained for foam capacities for all samples at pHs 7 and 9 are shown in Figure 2. Foam capacity at pH 4 was not determined because there was insufficient foam generation at this pH.

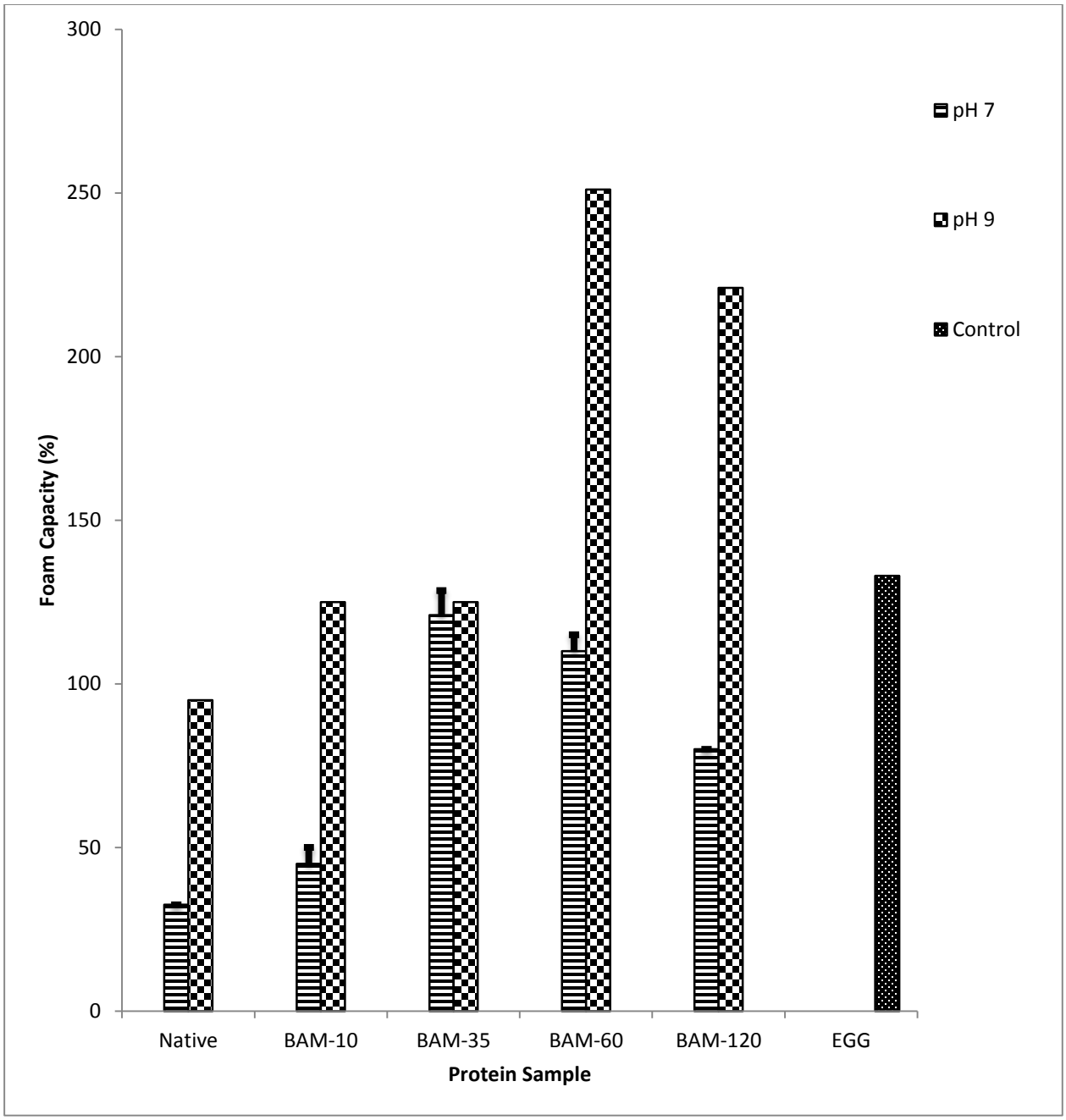


Figure 2: Foam capacities of *Native* and treated samples of Bambara groundnut protein isolates at pHs 7 and 9 compared to egg white.

Foam capacity or *overrun* is a measure of the foam volume (Panyam and Kilara, 1996) and this increased with proteolysis at all pH levels (Fig. 2). At pH 7, the foam capacity increased progressively from 32.5 % for the *Native* to 121 % for BAM- 35, and then decreased to 80 % for BAM-120. Similar trend was also observed for pH 9 as there was

increased foaming capacity from 100 % for the *Native*, to 275 % for BAM- 35, and then 221 % for BAM-120. Foaming capacity at pH 9 for all proteolysates was higher than that for respective proteolysates at pH 7. There were no determinations for pH 4 because the protein isolates could not generate substantial foam volume required to calculate the foam capacity. Basha and Pancholy (1982) observed that most proteins of plant origin show minimum solubility at their iso-electric region. The iso-electric region of the Bambara groundnut proteins during the study was observed to be in the region of pH 4.5 and 5. Basha and Pancholy (1982) further explained that within this pH range, ionic and electrostatic repulsion was minimum and this might be the reason for low solubility of the protein isolates at pH 4 and hence poor foamability.

The increase in the foaming capacity after proteolysis from 32.5 % and 95 % for the *Native* to 121 % and 275 % for BAM-35 at pHs 7 and 9 respectively were expected. This expectation was based on the fact that some specific peptide bonds were hydrolysed during the proteolysis and the peptides produced had smaller molecular weights than the natived proteins (Panyam and Kilara, 1996). It is known that the smaller the molecular weight, the faster the rate of higher their solubility and the faster the rate of protein diffusion at the air-water interface to generate foam. This was the reason for the increase in foam capacity compared to the untreated proteins. This explanation is consistent with the findings of Damodaran (1990), who reported that good protein foaming agents have the capability to rapidly diffuse to the air- water interface and form a strong cohesive and elastic film.

Proteolysis also resulted in the splitting of peptide bonds and subsequently, the exposure and increased number of the ionizable groups, together with the exposure of hydrophobic groups hitherto concealed and this further increased protein solubility (Panyam and Kilara, 1996). Townsend and Nakai (1983) reported that there was greater surface hydrophobicity after proteolysis as a result of increased in sulphydryl group of proteins which reflected a greater denaturation and unfolding of the protein. The authors reported that this phenomenon of greater surface hydrophobicity if occurs allows proteins to be extensively uncoiled at the air/water interface, and this could be the reason for the increased foaming capacity of proteins upon proteolysis. Thus increasing time of proteolysis resulted in the splitting of more peptide bonds that also increased the amount

of hydrophobic amino acids that were exposed at the surface of the protein molecules. This contributed to increased foam capacity, following a trend from *Native* to BAM-35.

Although foam capacity increased with increasing time of proteolysis, the increase was not steady from the *Native* through to BAM-120 as there was always a decrease in the foam capacity after certain times of proteolysis (Figure 2). For instance at pH 7, there was an increase from 32.5 % for the *Native* to the highest value of 121 % for BAM-35 and then reduced to 80 % for BAM-120. The results were consistent with findings of Swaisgood *et al.* (1996). In their work, they obtained the least overrun or foam capacity of 900 % for the native protein. After hydrolysis however, there was an increase to 1200 % at a degree of hydrolysis (DH) of 2.8 % which was the highest value they obtained. Upon further hydrolysis, the overrun was reduced to 700 % for DH of 4.3 %. The increase in foam capacity with proteolysis was probably because factors which promote foamability such as smaller molecular sizes and solubility were enhanced by the proteolysis to cause an increase in the foam capacity. Upon prolonged proteolysis however, molecular sizes and polypeptide chains were most likely extensively reduced beyond what was desired for foam generation and caused a decrease in foam formation as explained by Kester and Richardson (1984). These researchers explained that a limited degree of hydrolysis improved solubility and foaming capacities, whereas excessive hydrolysis reduced foamability because larger tertiary structures in the presence of smaller molecular sizes brought about stearic hindrance to foamability. Halling (1981) and Kinsella (1984) also suggested that protein, or peptide size and structure were important factors with regard to foaming characteristics because they allowed interaction between surface molecules thus providing mechanical strength to augment the foam film. They further suggested that larger peptides and unhydrolyzed proteins exerted inhibitory effect on the foaming properties of the smaller peptides either by hydrophobic interaction or by stearic hindrance at the interface. The prolonged proteolysis might have produced smaller peptides in the presence of larger peptides to cause foaming inhibitory effects.

The variation of *foam capacity* and *pH* showed the least foaming capacity at pH 4 and maximum foaming capacity at pH 9. The iso-electric point was observed to be in the region of pH 4.5 and 5 during the study. Basha and Pancholy (1982) explained that

within this pH range, ionic and electrostatic repulsion was minimum and this might be the reason for low solubility of the proteolysates at pH 4 and hence poor foamability.

The high foaming capacity at pH 9 of Bambara groundnut proteins could therefore be attributed to high solubility in alkaline region. Lin *et al.* (1974) obtained minimum foaming capacity at pH 4 for soy flour and sunflower proteins, and maximum foaming capacity at pH 10 and they attributed this observation to solubility of the polypeptides. Adebowale *et al*. (2005) also reported a similar trend of minimum foam capacity at pH 4 and maximum pH of 11 for flours of six *Mucuna* species. Aluko and Yada (1995) worked on cowpea also reported similar observation and suggested that when proteins were solubilized, they diffused more rapidly to the air- water interface to encapsulate air particles resulting in high foamability. The high foaming properties could make the treated Bambara groundnut proteins find applications in bakery products like cake (Zayas, 1997).

4.4.2 Foam activity

The results obtained for foam activities of all samples at the various pHs are shown in Figure 3.

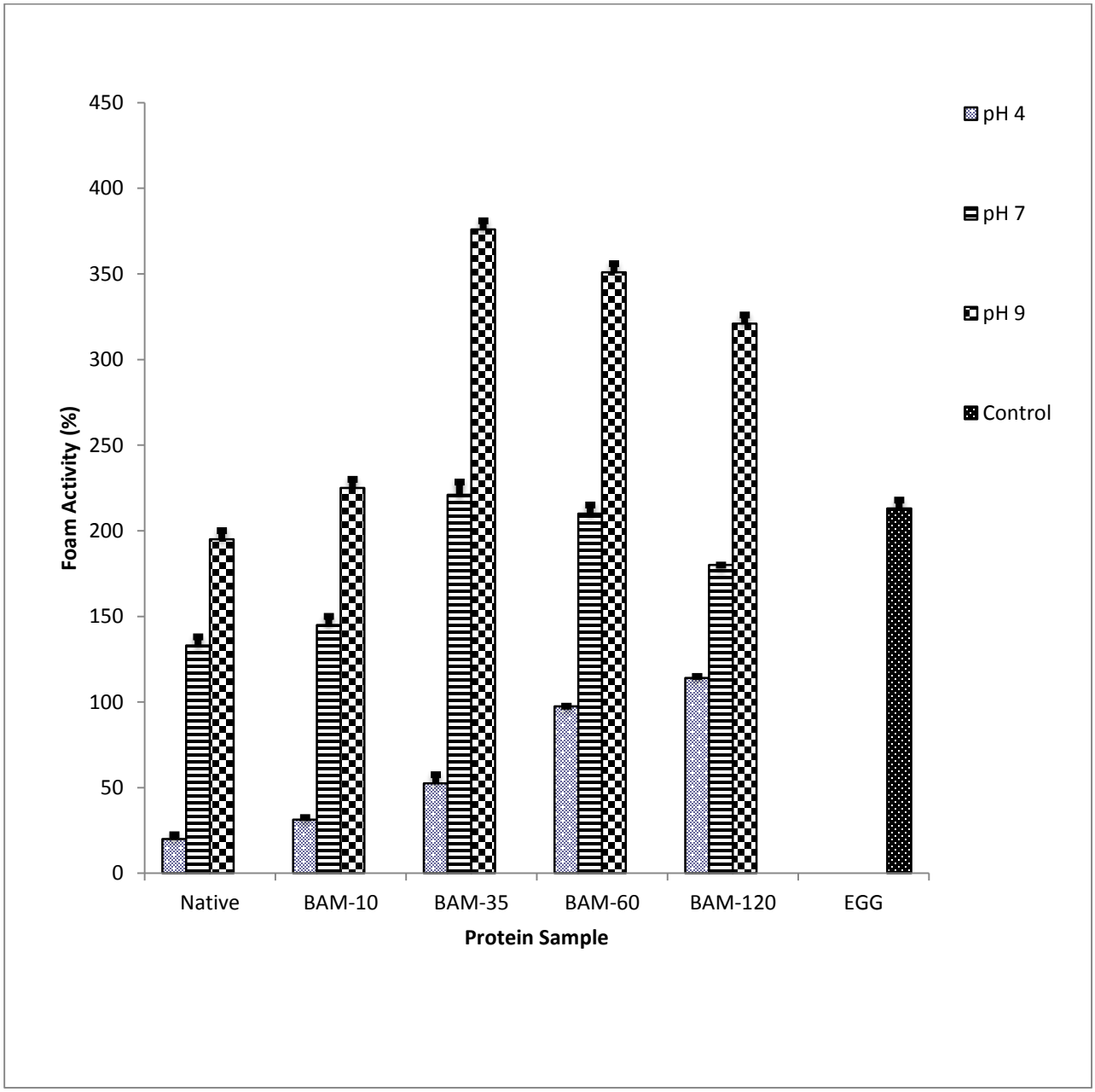


Figure 3: Foam activities of *Native* and treated samples of Bambara groundnut protein isolates at pHs 4, 7 and 9 compared to egg white.

Foam activity is a measure of the increased foam volume with respect to the initial volume (Ahmedna *et al*., 1999), and the explanations assigned for the behaviour of *foam capacity* also hold for the behaviour of *foam activity* for all protein isolates at all pH levels.

4.4.3 Foam stability

The results obtained for foam stabilities for all samples at the various pHs are shown in Figure 4.

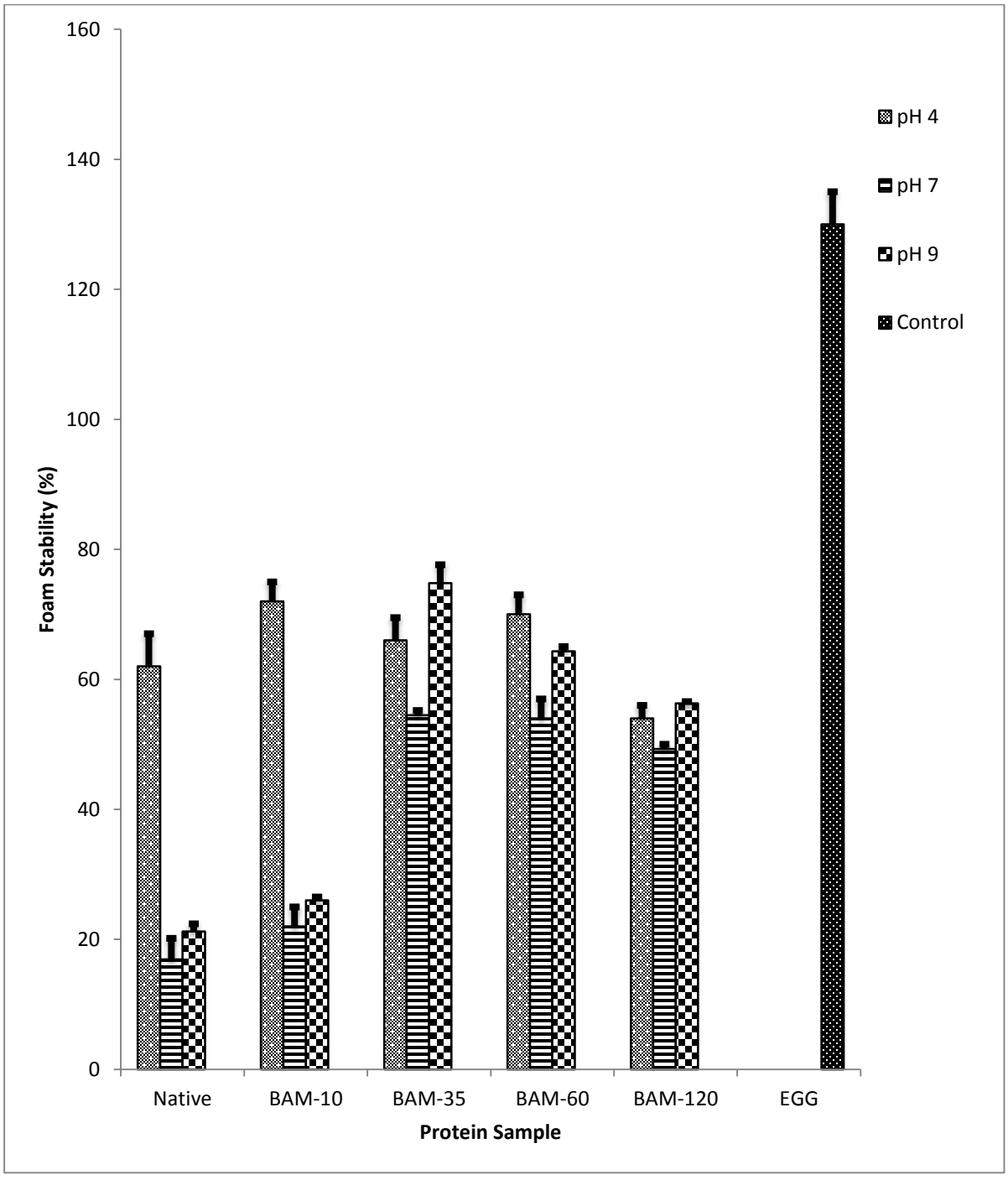


Figure 4: Foam stabilities of *Native* and treated samples of Bambara groundnut protein isolates at pHs 4, 7 and 9 compared to egg white.

Foam stability is a measure of the time taken for the collapse of foam formed (Panyam and Kilara, 1996). The pH 4 had the highest foam stability but it did not show any clear trend with increasing time of proteolysis. However, all proteolysates showed improved stability than the *Native* (Figure 4). The foam stability increased from 16.9 % and 21.1 % at pHs 7 and 9 respectively for the *Native* to 54.6 % and 74.8 % respectively in BAM -35 and, then declined to 49.3 % and 56.3 % respectively for BAM-120. The foaming stability for pH 9 was also higher than that for pH 7 for al samples.

The explanations for the increased behaviour of *foam capacity* and *foam activity* upon proteolysis probably also hold for the behaviour of foaming stability at pHs 7 and 9. The effect of proteolysis on the behaviour of foam stability at pH 4 at the moment is not properly elucidated. Although the foam activity and for that matter the foam volume at pH 4 was lower than those obtained at pHs 7 and 9 (Figure 3), the foam stability at pH 4 was however higher than those for pH 7 and 9 (Figure 4). This is probably because the Bambara groundnut proteins have the ability to form stable foams in acidic pH due to the formation of stable molecular layers in the pH range than in alkaline pH as reported by some researchers (Lin *et al.*, 1974; Sathe *et al*., 1982, Aluko and Yada, 1995; Adebowale *et al*., 2005;). These researchers studied the effects of pH on foam properties on protein isolates in Mucuna species, lupin, winged bean, sunflower and cowpea seed respectively, and attributed the greater stability in the acidic region to the formation of stable molecular layers in the pH range.

Although Bambara groundnut proteins form stable molecular layers in acidic region and hence higher foam stability was expected at pH 7 than pH 9, the contrary was observed. This was probably because of the high foam activity at pH 9 that resulted in higher foam volume, than pH of 7, and the time to monitor the foam stability trend (30 min) was not sufficient to observe the decreasing trend in stability as was the case of Adebowale *et al. (*2005) who observed the stability trend for a period of 24 h.

4.5 Emulsifying properties

There was improvement in *emulsion capacity* after proteolysis at all pH levels but it showed a decline after 35 min. *Emulsion activity* and *emulsion stability* were however lower upon proteolysis at all pH levels. Emulsifying properties are influenced by many factors among which are delicate balance of net charge and charge density, proper distribution of polar and nonpolar charges, the existence of tertiary structure and good structural flexibility (Huang *et al.,* 1996). The explanations for the trend observed for emulsion capacity, emulsion activity and emulsion stability are shown below.

4.5.1 Emulsion capacity

The results obtained for emulsion capacities of all samples at the various pH levels are shown in Figure 5.

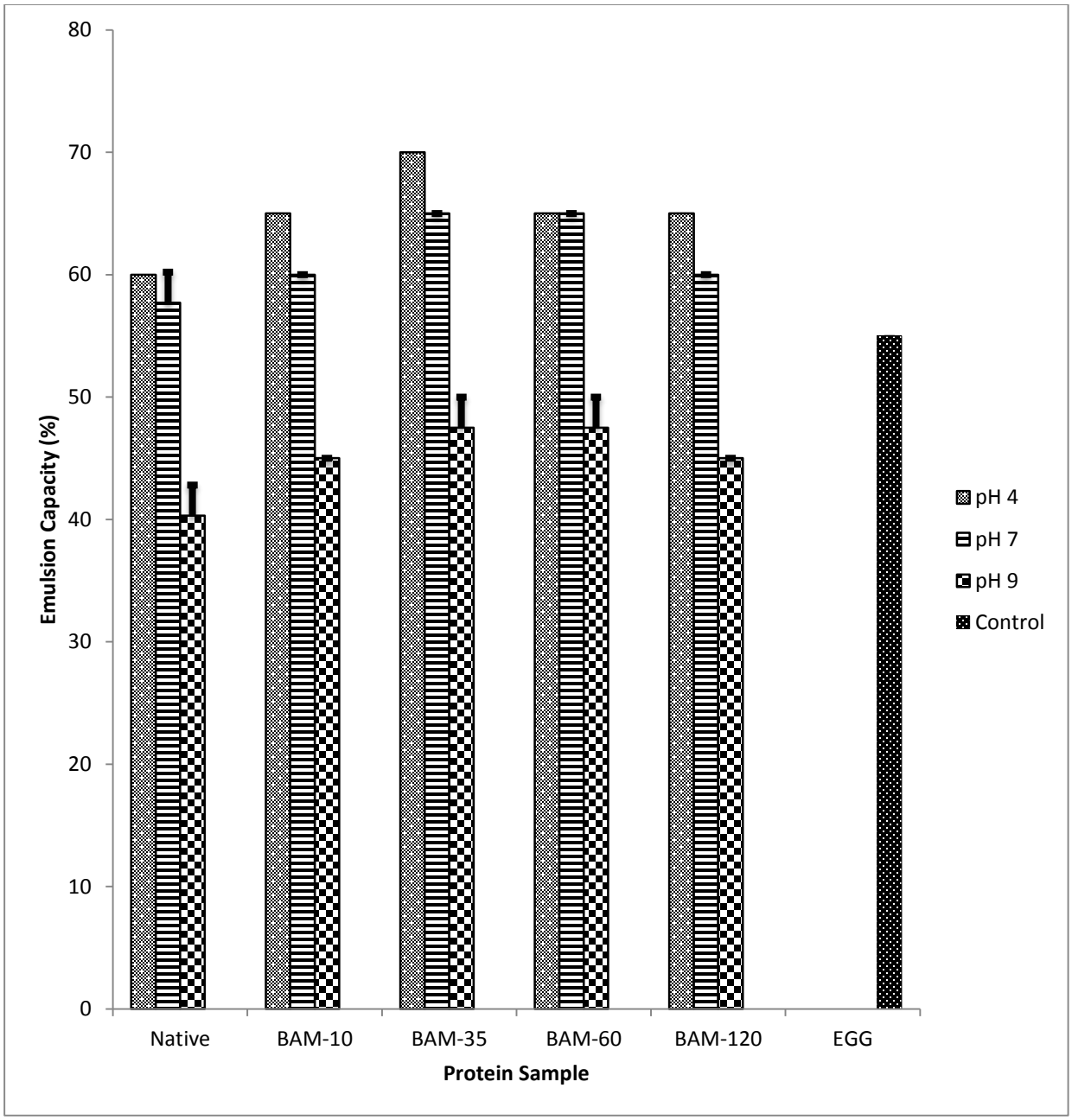


Figure 5: Emulsion capacities of *Native* and treated samples of Bambara groundnut protein isolates at pHs 4, 7 and 9 compared to egg white.

Emulsion capacity increased with proteolysis up to 35 min and then reduced up to 120 min. The increase in *emulsion capacity* was perhaps due to increased unfolding of the protein molecules into more flexible structures which promoted emulsion capacity as reported by Kester and Richardson (1984). They reported that proteolysis increased the effective hydrophobicity of globular proteins through the exposure of apolar amino acid

residues and subsequently unfolding the polypeptides chain and this probably increased the emulsion capacity from the *Native* to BAM-60 before reduction to BAM-120. The reduction in *emulsion capacity* after 35 min could be attributed to protein denaturation and extensive size reduction of peptides due to extensive proteolysis that resulted in the loss of emulsion capacity (Kuehler and Stine, 1974; Puski, 1975; Waniska *et al.*, 1981; Lakkis and Villota, 1990; Turgeon *et al.*, 1992). Friberg (1976) found that greater emulsifying properties were desired for milk-like beverages and meat analogues and the improved emulsion capacity could be employed in such applications.

Emulsion capacity was also pH dependent and it decreased with increased pH. However, Khalid *et al.*, (2003) reported that emulsion capacity of legumes increased with increased pH. Emulsion capacity is defined as the maximum amount of oil that can be emulsified under specified conditions by a unit weight of the protein (Panyam and Kilara, 1996). This implied that at pH 4, lesser amount of oil was required to form emulsion than at pHs 7 and 9. Panyam and Kilara (1996) reported that different proteins have different amino acid contents and this varied their surface activities. This was perhaps the reason why the Bambara groundnut proteins behaved differently in their emulsifying capacity from other legumes studied by Khalid *et al. (*2003). Results for water binding capacity and oil absorption capacity (Figure 8) also suggested that there were more hydrophilic groups in the respective protein isolates than hydrophobic groups and, increasing the pH might have further exposed more hydrophilic sites than hydrophobic sites and that did not promote emulsion formation at higher pH.

4.5.2 Emulsion activity and emulsion stability

The results obtained for emulsion activities of all samples at the various pH levels are shown in Figure 6.

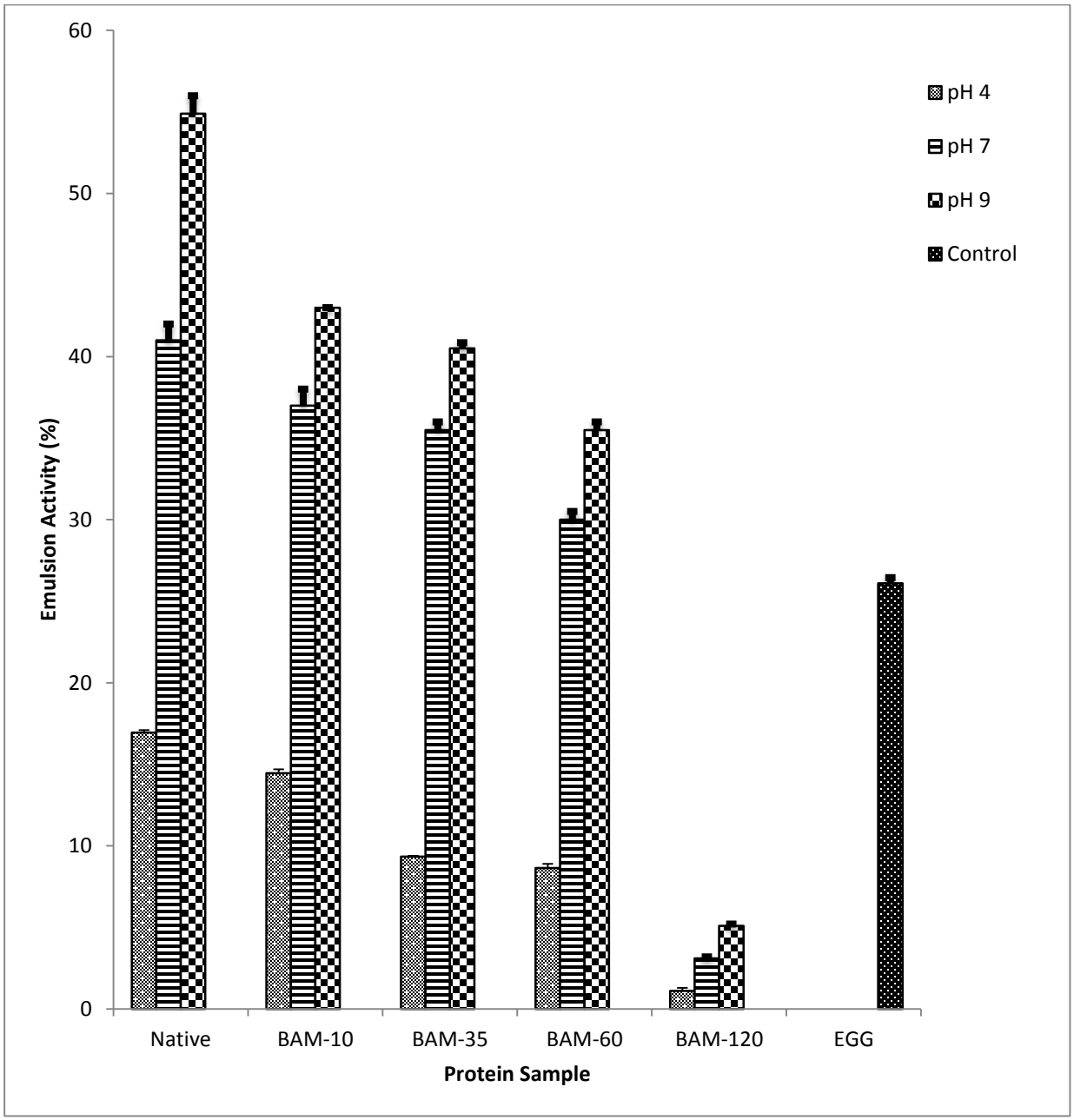


Figure 6: Emulsion activities of *Native* and treated samples of Bambara groundnut protein isolates at pHs 4, 7 and 9 compared to egg white.

The results for emulsion stabilities of all samples at the various pH levels are shown in Figure7.

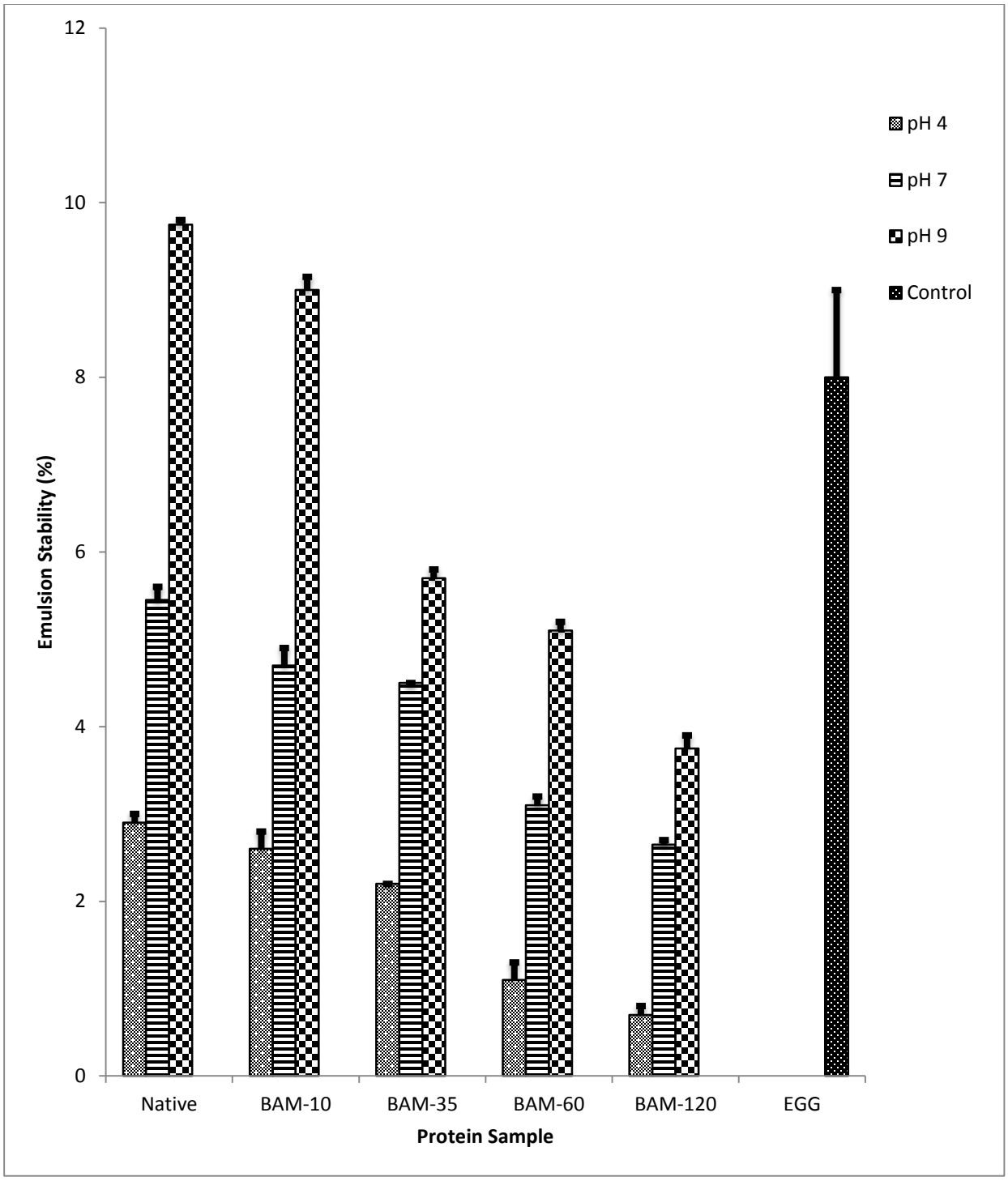


Figure 7: Emulsion stabilities of *Native a*nd treated samples of Bambara groundnut protein isolates at pHs 4, 7 and 9 compared to egg white.

Emulsion activity and *emulsion stability* decreased progressively for all proteolysates at all pH levels (Figures 6 and 7) with respect to the *Native*. Both emulsion activity and

emulsion stability were however improved with increasing pH for all the proteolysates (Figures 6 and 7).

Panyam and Kilara (1996) explained that emulsion activity is the maximum interfacial area per unit weight of protein of a stabilized solution. They also explained that emulsion stability is the ability of a protein to form an emulsion that remains unchanged during a certain period of time at a given temperature and gravitational field. The proteolysis had varied effect on all the protein isolates and this was probably due to changes in the protein's structure during the course of the proteolysis. Swaisgood (1982) reported that changes in the substrate protein functionality depended upon the protein's structure, the type of enzyme used and the conditions of the enzyme treatment. The reduction in emulsion activity and stability could therefore be due to the changes in the structure of the protein isolates that took place during the proteolysis and the type of the protease used in this study. Lqari *et al.* (2005) also worked on *Lupinus angustifolius* protein hydrolysates and explained that limited proteolysis decreased emulsifying stability of the hydrolysates obtained. Similarly, Elizalde *et al*. (1991) also reported that emulsion stability was enhanced by high protein and oil concentrations and that these factors were interrelated. This suggests that the progressive reduction in protein content during the proteolysis was detrimental to the stability of the emulsion formed and all these may have contributed to the observation in the reduction in both emulsifying activity and stability in this study. This also implied that protein concentration was critical in determining the emulsifying capacity and emulsifying stability of Bambara groundnut protein.

The improvement in emulsion activity and emulsion stability with increasing pH could be attributed to the fact that most proteins of plant origin can be solubilized above iso-electric pH region which was above 5 and solubility enhanced emulsion activity and stability as observed by Fagbemi and Oshodi (1991).

4.6 Water binding capacity/ oil absorption capacity

The results obtained for water binding capacities (WBC) and oil absorption capacities (OAC) of all samples are shown in Figure 8.

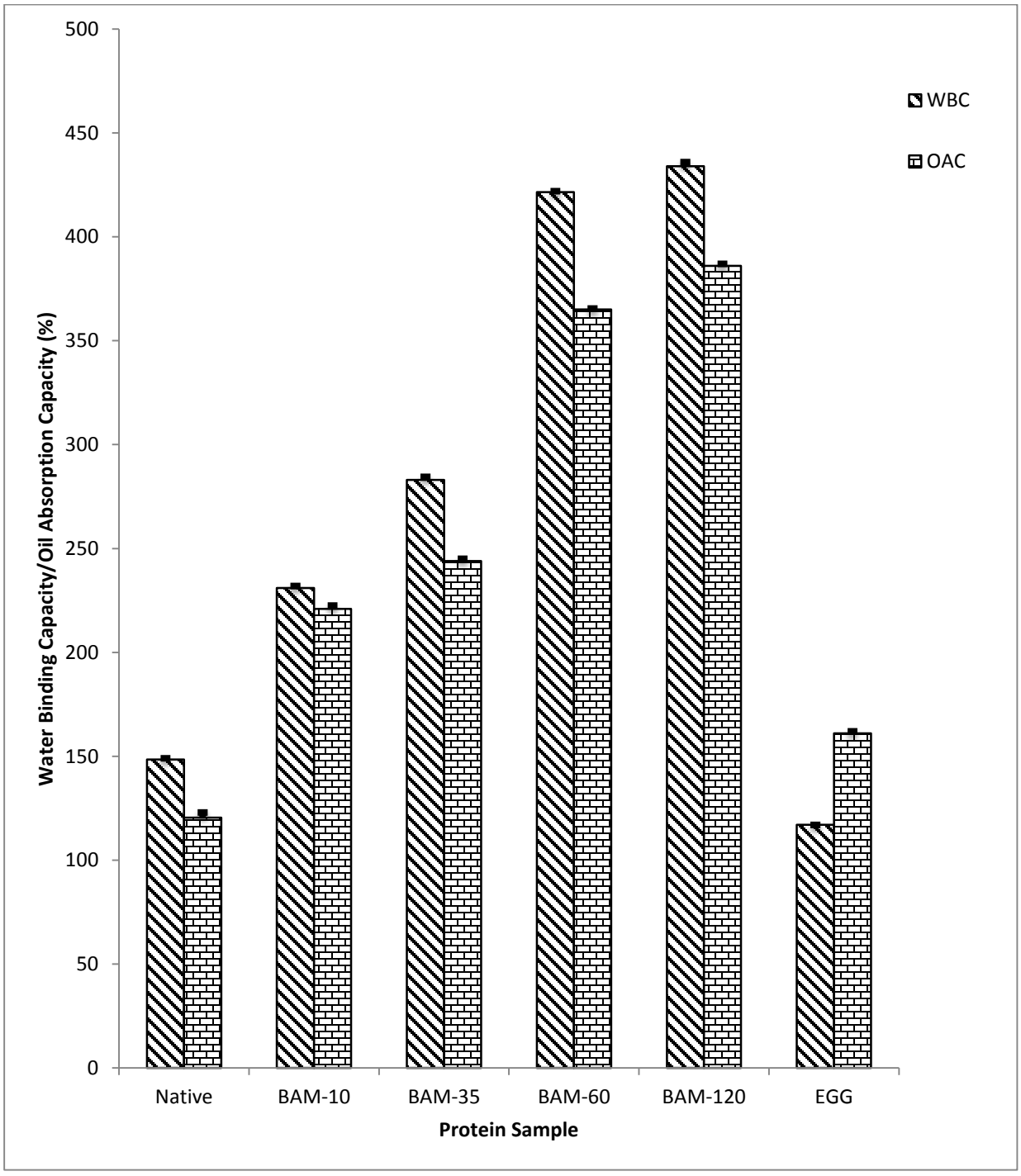


Figure 8: Water binding capacities (WBC) and oil absorption capacities (OAC) of *Native* and treated samples of Bambara groundnut protein isolates compared to egg white.

Both *water binding capacity* and *oil absorption capacity* showed progressive increase with increasing time of proteolysis. *Water binding capacity* increased from 148 % for the *Native*, to 434 % for BAM-120 which was the highest. *Oil absorption capacity* also increased from 120 % for the *Native*, to 386 % which was also the highest (Figure 8). Proteolysis brought about denaturation and also, the splitting of peptide bonds which exposed both hydrophilic and hydrophobic sites (Panyam and Kilara, 1996).

This made it possible for both water and oil to have greater and effective interactions with the proteolysis and hence the increase. Privalov (1979) also reported that increase in water binding capacity may be as a result of the coupled effect of water adsorption via existing polar binding sites distributed over protein surface, and the denaturation of proteins which resulted in molecular rearrangement leading to the exposure of more polar binding sites. Water interacts with proteins in a number of ways and a significant amount is bound by protein through hydrogen bonding. This leads to protein-water and protein-protein interactions to bring about water retention (Zayas, 1997). The greater the hydrophilic and hydrophobic sites exposed, the higher the liquid retention. Liquid retention is an index of the ability of proteins to absorb and retain oil/water. This property influences the texture and mouth feel characteristics of foods and food products like comminuted meats, extenders or analogues and baked doughs (Cheftel *et al.*, 1985; Okezie and Bello, 1988). Also, water binding capacity is a critical function of protein in various food products like soups, gravies, doughs and baked products (Sosulski *et al.*, 1976). Oil absorption capacity also plays a role in flavour retention (Kinsella, 1976).

4.7 Performance of the various protein isolates

4.7.1 Native protein

The performance of the native protein is shown in Figure 9. There was no data for foam capacity at pH 4 because the foam generated was not substantial for foam activity determination.

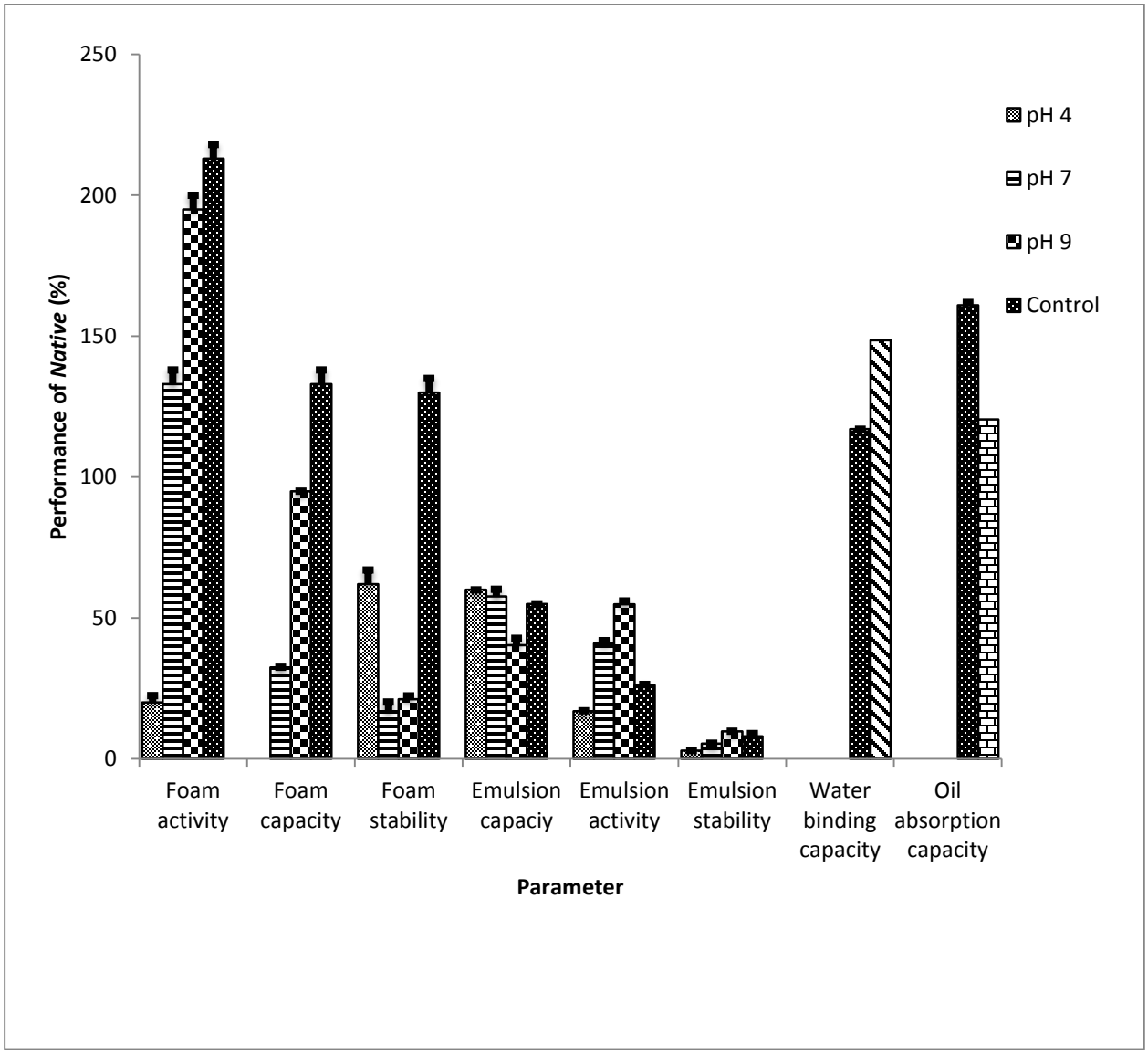


Figure 9 The performance of native proteins isolate from Bambara groundnut showing foam activity, foam stability, emulsion capacity, emulsion activity, emulsion stability at

pHs 4, 7 and 9; foam capacity at pH 7 and 9, and water binding capacity and oil absorption capacity compared to egg white.

4.7.2 BAM-10

The performance of the BAM-10 is shown in Figure 10 There was no data for foam activity at pH 4 because the foam generated was not substantial for foam activity determination.

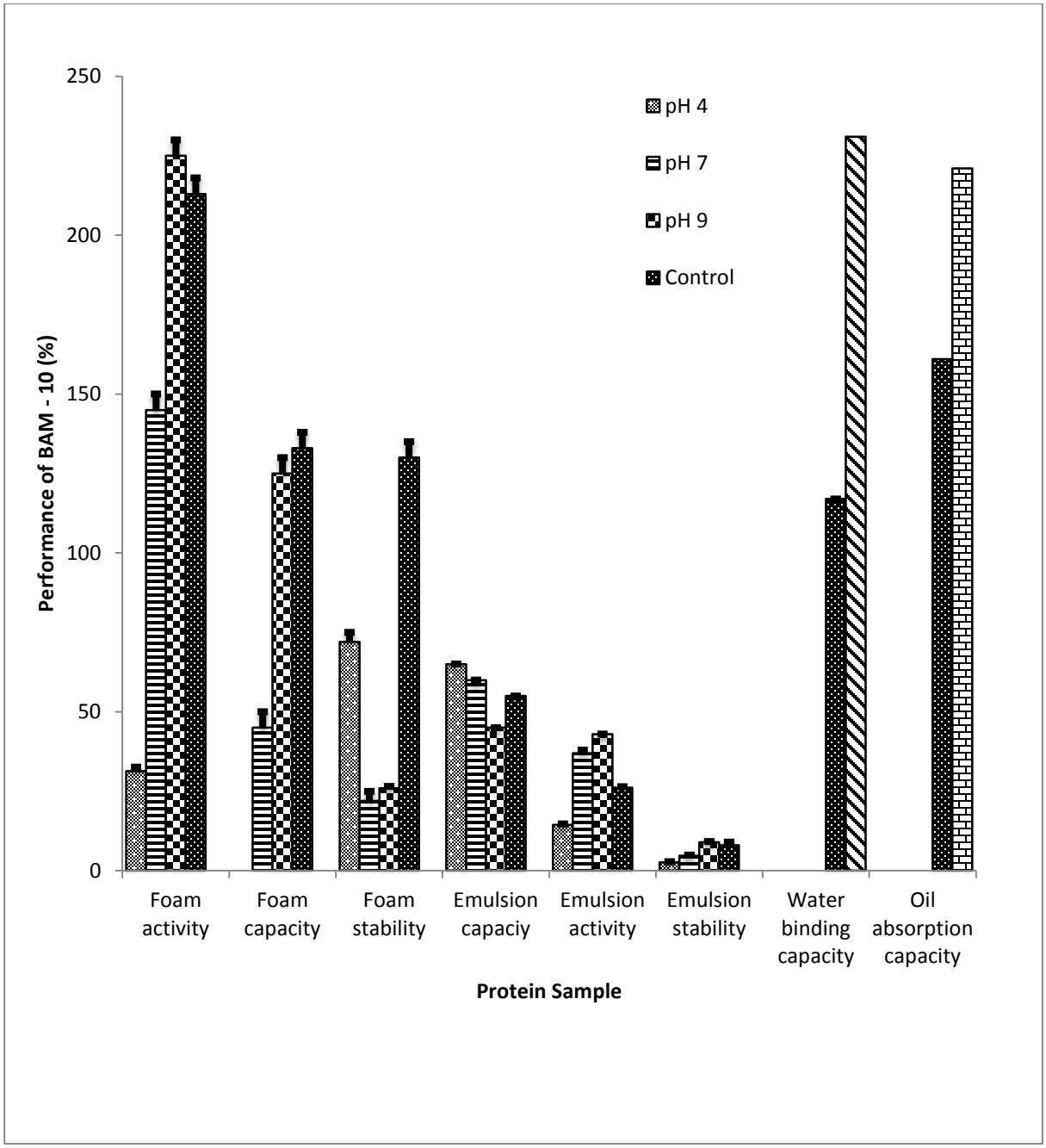


Figure10. The performance of Bambara groundnut proteins isolate treated up to ten min

(BAM-10) showing foam activity, foam stability, emulsion capacity, emulsion activity and emulsion stability at pHs 4, 7 and 9; foam capacity at pH 7 and 9; and water binding capacity and oil absorption capacity compared to egg white.

4.7.3 BAM-35

The performance of the BAM-35 is shown in Figure 11. There was no data for foam capacity at pH 4 because the foam generated was not substantial for foam activity determination.

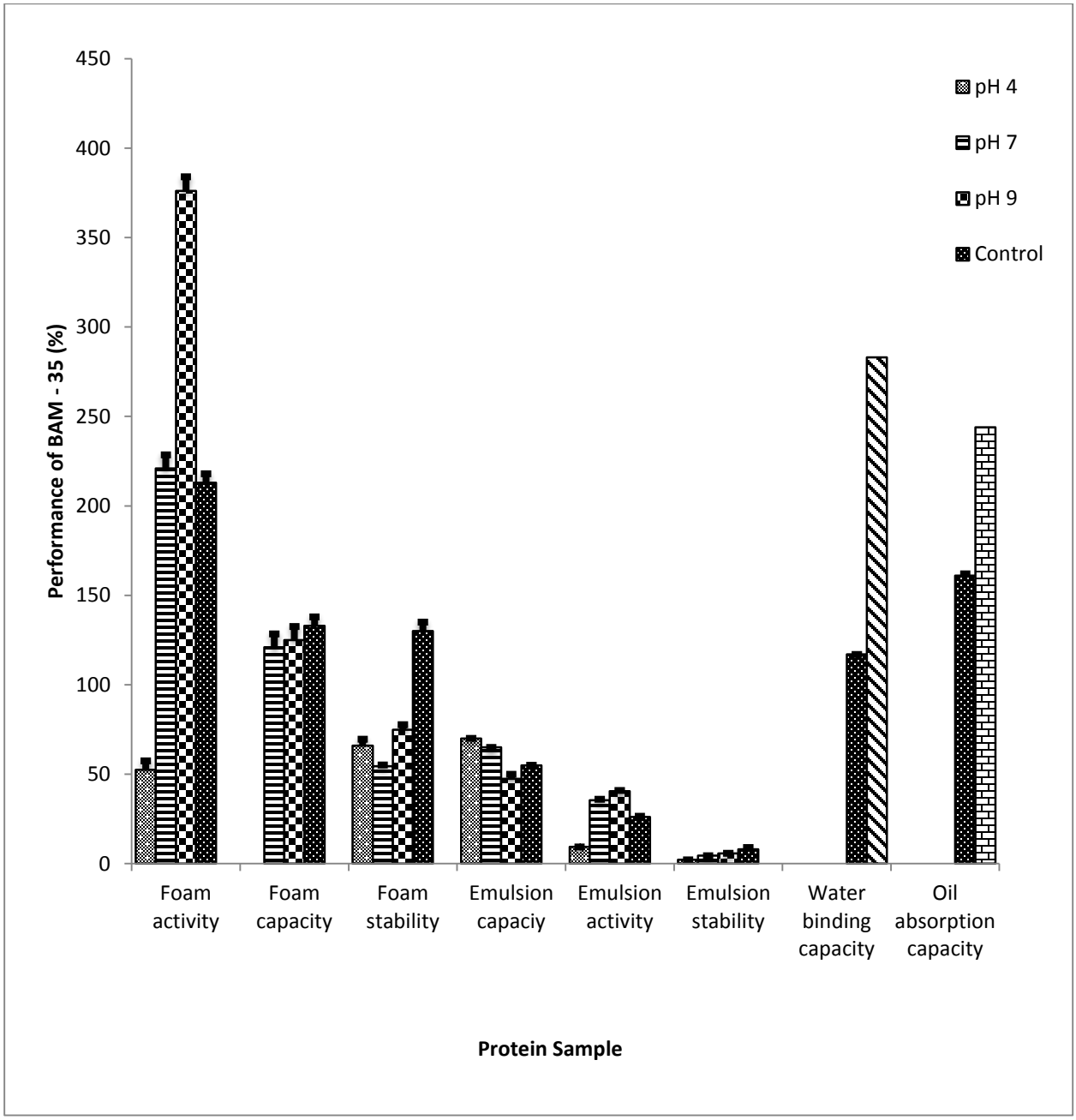


Figure 11. The performance of Bambara groundnut proteins isolate treated up to thirty five (BAM-35) showing foam activity, foam stability, emulsion capacity, emulsion activity and emulsion stability at pHs 4, 7 and 9; foam capacity at pH 7 and 9; and water binding capacity and oil absorption capacity compared to egg white.

4.7.4 BAM-60

The performance of BAM-60 is shown in Figure 12. There was no data for foam capacity at pH 4 because the foam generated was not substantial for foam activity determination.

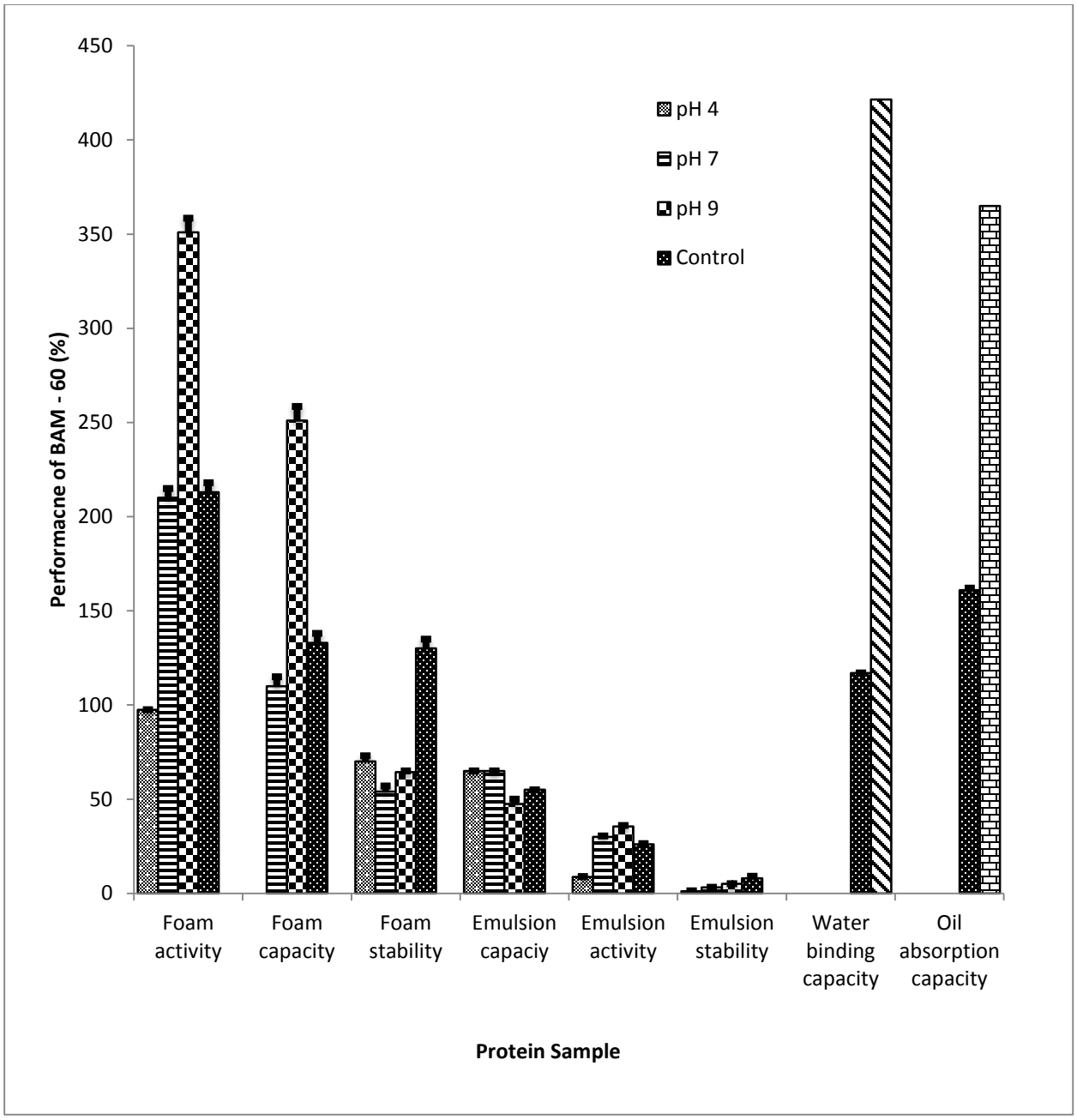


Figure 12: The performance of Bambara groundnut proteins isolate treated up to sixty min

(BAM-60) showing foam activity, foam stability, emulsion capacity, emulsion activity and emulsion stability at pHs 4, 7 and 9; foam capacity at pH 7 and 9; and water binding capacity and oil absorption capacity compared to egg white.

4.7.5 BAM-120

The performance of BAM-120 is shown in Figure 13 .There was no data for foam capacity at pH 4 because the foam generated was not substantial for foam activity determination.

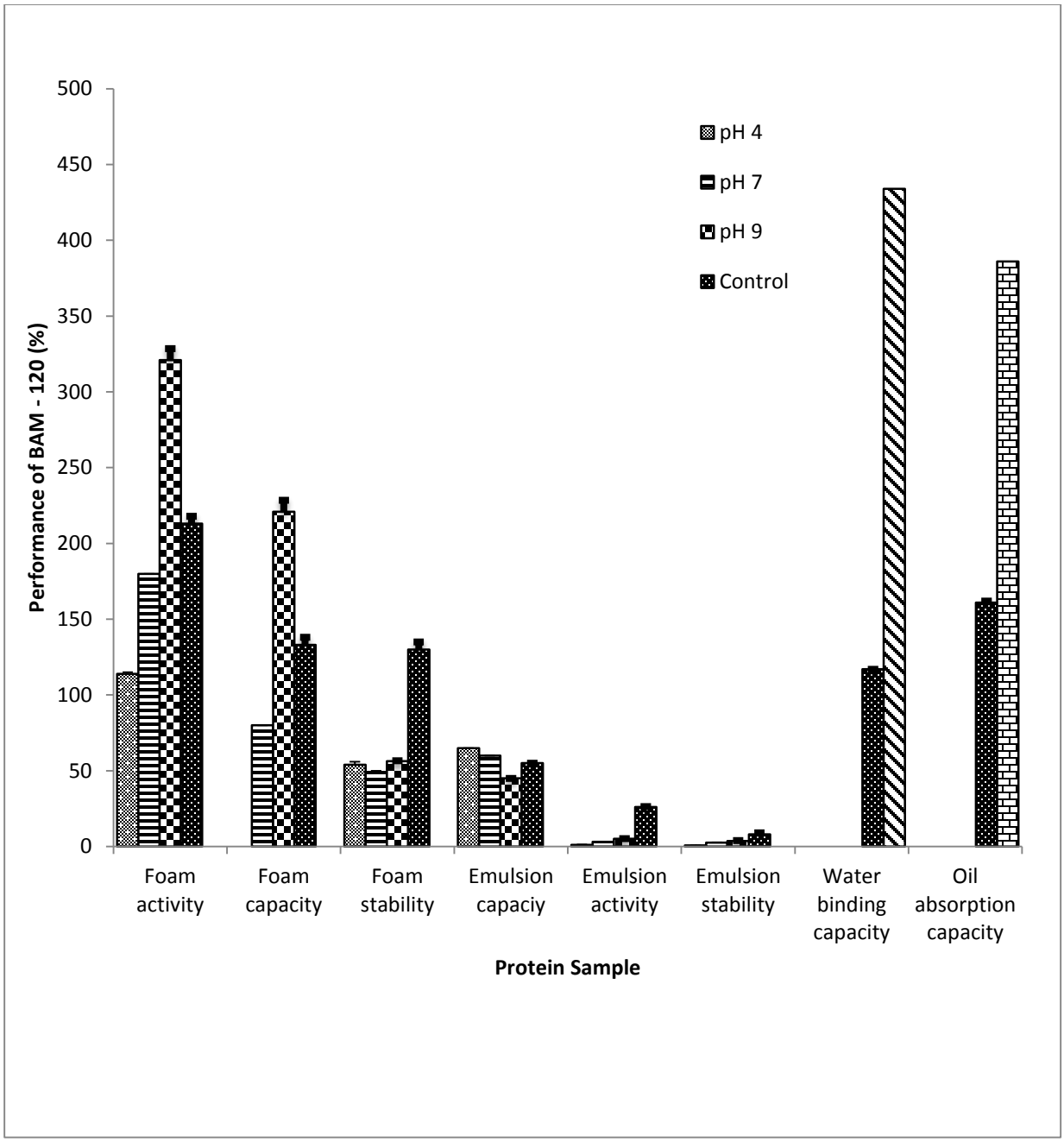


Figure 13: The performance of Bambara groundnut proteins isolate treated up to one hundred

and twenty min (BAM-120) showing foam activity, foam stability, emulsion capacity, emulsion activity and emulsion stability at pHs 4, 7 and 9; foam capacity at pH 7 and 9; and water binding capacity and oil absorption capacity compared to egg white.

The trend observed for the performance of native Bambara groundnut protein isolate (BGPI) on the surface functional properties at all pHs was generally similar to those observed for all the treated BGPIs for all the parameters measured: foam activity, foam capacity, foam stability, emulsion capacity, emulsion activity and emulsion stability. The foam activity values were the highest for all protein isolates, followed by foam capacity, foam stability, emulsion capacity, emulsion activity and emulsion stability. Foam capacity at pH 4 could not be determined for both native and all treated BGPIs. This is because the protein isolates did not produce substantial foam volumes at this pH for the determination of the foam capacity due to the proteins' poor solubility at this pH (Basha and and Pancholy, 1982).

Water binding capacity and oil absorption capacity values obtained for the native BGPIs, with respect to values obtained for the surface functional properties were very high. Similar trend was also observed for all the treated Bambara groundnut protein isolates.

Of all the protein isolates tested, BAM-35 had the highest foam capacity, foam stability and emulsion capacity (Figure 11). It was followed by BAM-60 which also had the same foam capacity value as that of BAM-35 (Figure 12). Emulsion activity and emulsion stability for the *Native* and all the treated BGPIs decreased with proteolysis. The results obtained for all the treated protein isolates were lower than those obtained for the *Native* (Appendix C-2.0). The results also showed that BAM-35 had greater foam capacity and emulsion capacity at pHs 9 and 4 respectively than the control (egg white). The foam activity value for BAM-60 at pH 9 was also higher than that for the control. The emulsion activity and emulsion stability for the *Native* were also higher than those of the control.

The values obtained for foaming properties of all the BGPIs at the various pHs were also higher than those obtained for emulsion properties at the respective pHs. Anon and Molina Ortiz (2000, and Thiele *et al*. (2002) have reported that limited proteolysis lead to breakdown of protein network and reduction of intermolecular disulphide bonds which helped to solubilise proteins. The increased in solubility also contributed to increase in functional properties such as foaming and emulsifying when compared to those of the

Native. Thus, the high values obtained for foaming properties were expected and could be the direct result of increased solubility during the proteolysis. Molina and Wagner (2002) investigated the effect of bromelain hydrolysis on protein solubility and its effect on foaming properties of native and thermally denatured isolates and, they concluded that solubility increased the foaming properties of both types of protein isolates.

Although the increase in solubility was also expected to increase emulsion values as those obtained for foaming, emulsion activity and emulsion stability however decreased with increasing time of proteolysis. This therefore suggests that in addition to solubility, there are other factors which influence emulsion properties. The trend observed in this study was however in agreement with what was previously obtained by Lqari *et al.* (2005) who worked on *Lupinus angustifolius* protein hydrolysates. The researchers explained that limited proteolysis decreased emulsifying stability of the hydrolysates obtained. Swaisgood (1982) have reported that changes in protein functionality during proteolysis depend upon the protein's structure, the type of enzyme used and the conditions of the enzyme treatment. Elizalde *et al*. (1991) also reported that emulsion stability was enhanced by high protein content and the proteolysis probably resulted in a reduction in protein content. The lower values for emulsion properties are therefore probably due to the influence of lower protein content which overrided solubility on emulsion formation. Other possible reasons could also be due to the nature of the Bambara groundnut isolates and the nature of the protease used which did not enhance emulsion activity and stability (Elizalde *et al*., 1991).

The values obtained for Water Binding Capacity and Oil Absorption Capacity were both very high compared to egg protein. The higher values therefore presented Bambara groundnut proteins as a better substitute for egg protein used in foods requiring high oil/water retention to influence texture and mouth feel characteristics, and also flavour retention (Okezie and Bello, 1988).

The values obtained for foam activity and foam capacity of the native proteins were all higher at pH 9 followed by pH 7, and then pH 4. This was because the iso-electric point

was observed to be in the region of pH 4.5 and 5. Basha and and Pancholy (1982) explained that within this pH range, ionic and electrostatic repulsion are minimum. This may be the reason for low solubility of the proteolysates at pH 4 and hence poor foamability. The high foaming capacity at pH 9 of Bambara groundnut proteins could therefore be attributed to high solubility in alkaline region than in the acidic region. High solubility could also be the reason for high emulsion activity and emulsion stability at pH 9 than at pH 7 and 4. Similar reason could also be used to explain the high emulsion activity and emulsion stability at pH 9 than at pH 7 and 4 (Fagbemi and Oshodi, 1991).

Emulsion capacity was also pH dependent but unlike emulsion activity and emulsion stability, Emulsion capacity decreased with increased pH and this is at variance to the findings of some workers such as such as Khalid *et al.* (2003), who reported that emulsion capacity of legumes increased with increased pH. Panyam and Kilara (1996) however reported that different proteins had different amino acid contents and this varied their surface activity. This was perhaps the reason why the Bambara groundnut proteins behaved differently in their emulsifying capacity from other legumes. Results for water binding capacity and oil absorption capacity (Figure 8) also suggested that there were more hydrophilic groups in the respective proteolysates than hydrophobic groups and, increasing the pH might have further exposed more hydrophilic sites than hydrophobic sites and that did not promote higher emulsion formation at higher pH.

4. 8 Sensory evaluation

Results for sensory evaluation of cake products using quantitative descriptive analysis (QDA) is shown in appendix E- 5.0.

4.8.1 Model samples cake studies for appearance, flavour and texture

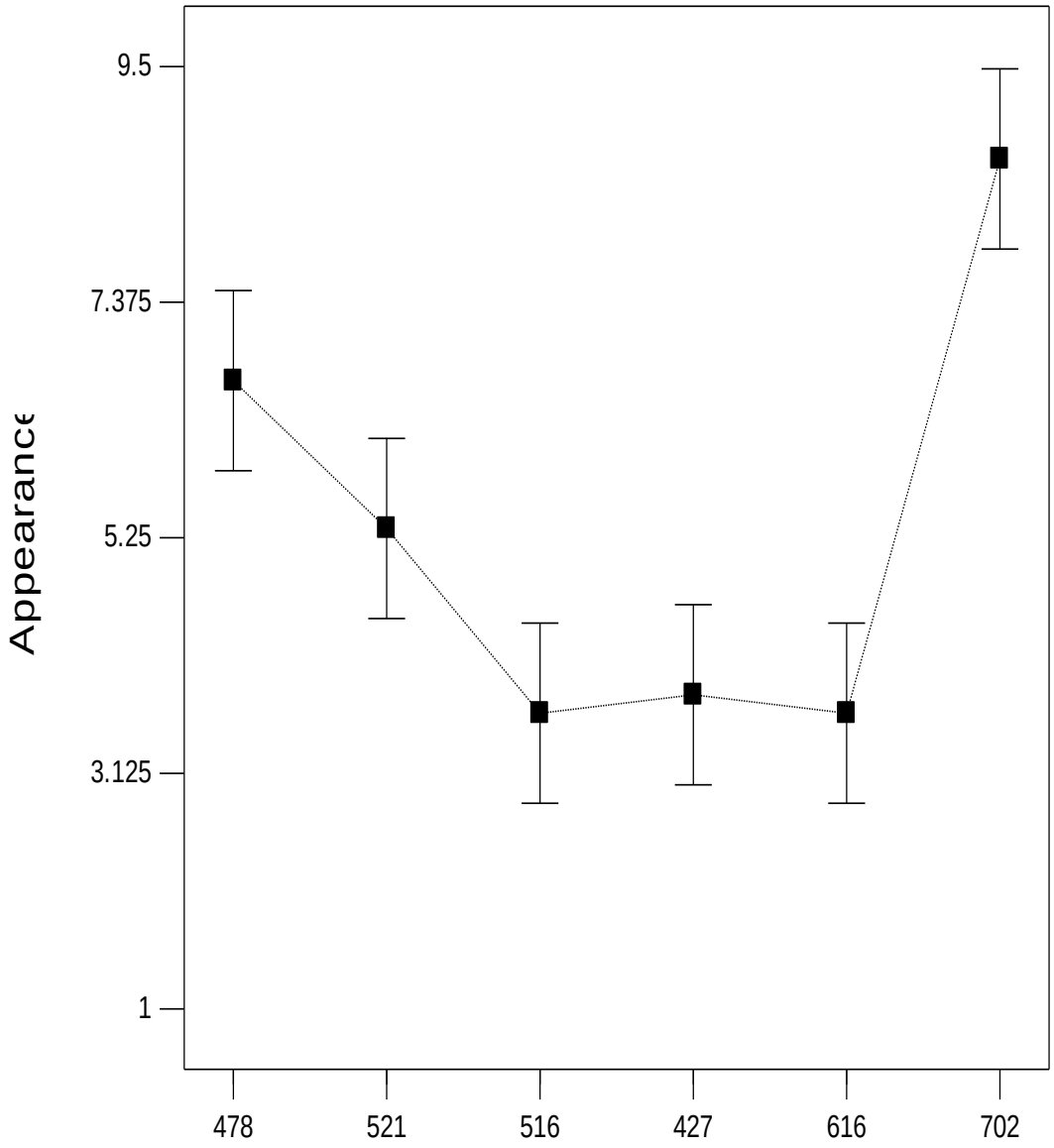
Model studies to determine the effect of proteolysis on sensory properties (appearance, texture, and flavour) of native and treated Bambara groundnut protein isolates, as well as egg protein revealed that the proteolysis had detrimental effects on the sensory properties of the treated Bambara groundnut proteins isolates generally (Figures 14, 15 and 16).

There was a reduction in appearance, flavour and texture from the *Native* to BAM-35 (516), and there were no further significant ($p > 0.05$) changes in intensity of appearance and flavour from BAM-35 (516) to BAM-120 (616). There was however an improvement in flavour from BAM-35 (516) to BAM-120 (616). The egg protein had greater sensory attributes on all the parameters measured (appearance, texture, and flavour), followed by the *Native* and then the treated Bambara groundnut protein isolates.

Limited proteolysis resulted in the breakdown of protein network and reduction of intermolecular disulphide bonds which helped to solubilise proteins (Thiele *et al*., 2002) and this brought about texture softening. The observations made on appearance and texture in this study agreed with what was previously obtained by (Kim *et al*., 1990). The researchers reported that pepsin, papain and trypsin caused enhanced solubility of soybean protein isolates with detrimental effects on texture. Lawrence *et al*. (2004) also reported that proteolysis ultimately resulted in texture softening and for that matter, appearance deterioration due to large reduction of elasticity and firmness and these might be the reason for texture and appearance reduction. The general stabililsation of appearance and texture intensities from BAM-35 (516) to BAM-120 (616) was probably due to enzyme depletion.

It has been reported by Banks *et al*. (1993), who studied the effects of proteolysis on low fat cheese that increased level of proteolysis improved flavour intensity and it was expected that the proteolysis would result in a steady increase in flavour intensity from native to BAM-120 (616). There was however a decline from the native to BAM-35 (516) and the reason for this observation was not fully elucidated yet. The expected increase was however observed from BAM-35 (516) to BAM-120 (616). The increased flavour intensity could be linked to flavour development during proteolysis as reported by Vogt (1994). Model graphs on the parameters studies are shown below:

Appearance



A: Experimental cakes

Figure 14: Model graph showing the performance of cakes formulated from proteins isolates from Bambara groundnut: 478 (*Native*), 521 (BAM-10), 516 (BAM-35), 427 (BAM-60) and 616 (BAM-120) on appearance compared to cake formulated from egg proteins (702).

Results from the analysis of variance table for appearance (Appendix E-6.1) showed there was a significant ($p < 0.05$) difference for the means of cake products on appearance. The model graph for appearance (Figure 14) further shows there was a difference between the native protein and all the treated proteins except for BAM 10; also the egg white cake differed from all the cake products made from Bambara protein isolates but there was no significant ($p > 0.05$) difference between BAM -35, BAM-60 and BAM-120. The egg white had the highest appearance characteristics and was followed by the *Native*, BAM-10, BAM -35 and BAM-60, and BAM-120.

Flavour

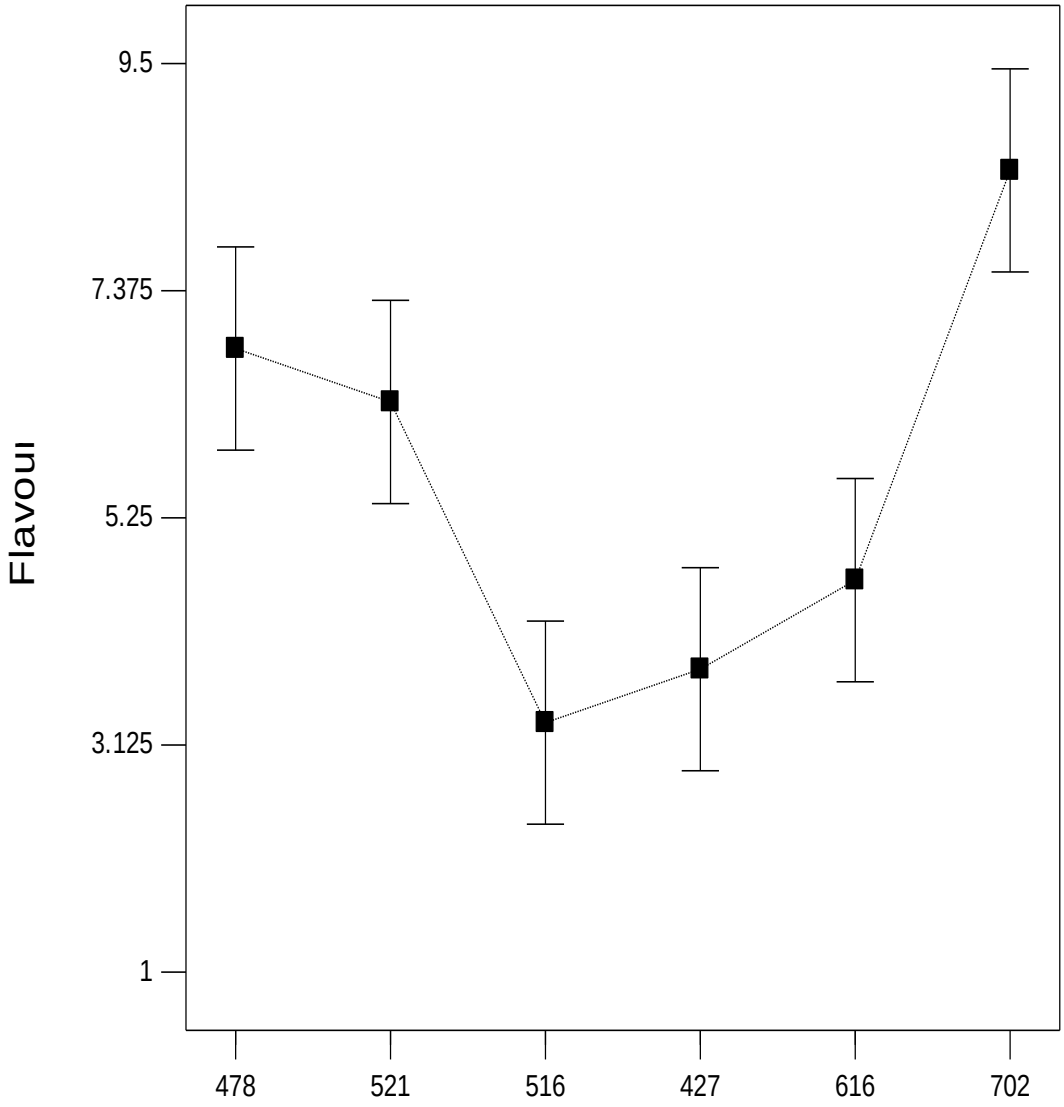


Figure 15: Model graph showing the performance of cakes formulated from proteins isolates from Bambara groundnut: 478 (*Native*), 521 (BAM-10), 516 (BAM-35), 427 (BAM-60) and 616 (BAM-120) on flavour compared to cake formulated from egg proteins (702).

The results from the analysis of variance table [classical sum of squares type II] for flavour (Appendix E-6.2) show there was significant ($p < 0.05$) difference for the cake products on flavour. The model graph for flavour (Figure 15) also shows that there was no difference between the *Native* and BAM 10; but flavour characteristics of both the *Native* and the BAM-10 significantly differed from all the other treated protein samples as well as the egg white. There was however a significant ($p < 0.05$) difference between the means of all the products of the modified proteins and the egg white. The egg white had the highest flavour characteristics and was followed by the *Native*, BAM-10, BAM -120, BAM-60, and BAM-35.

Texture

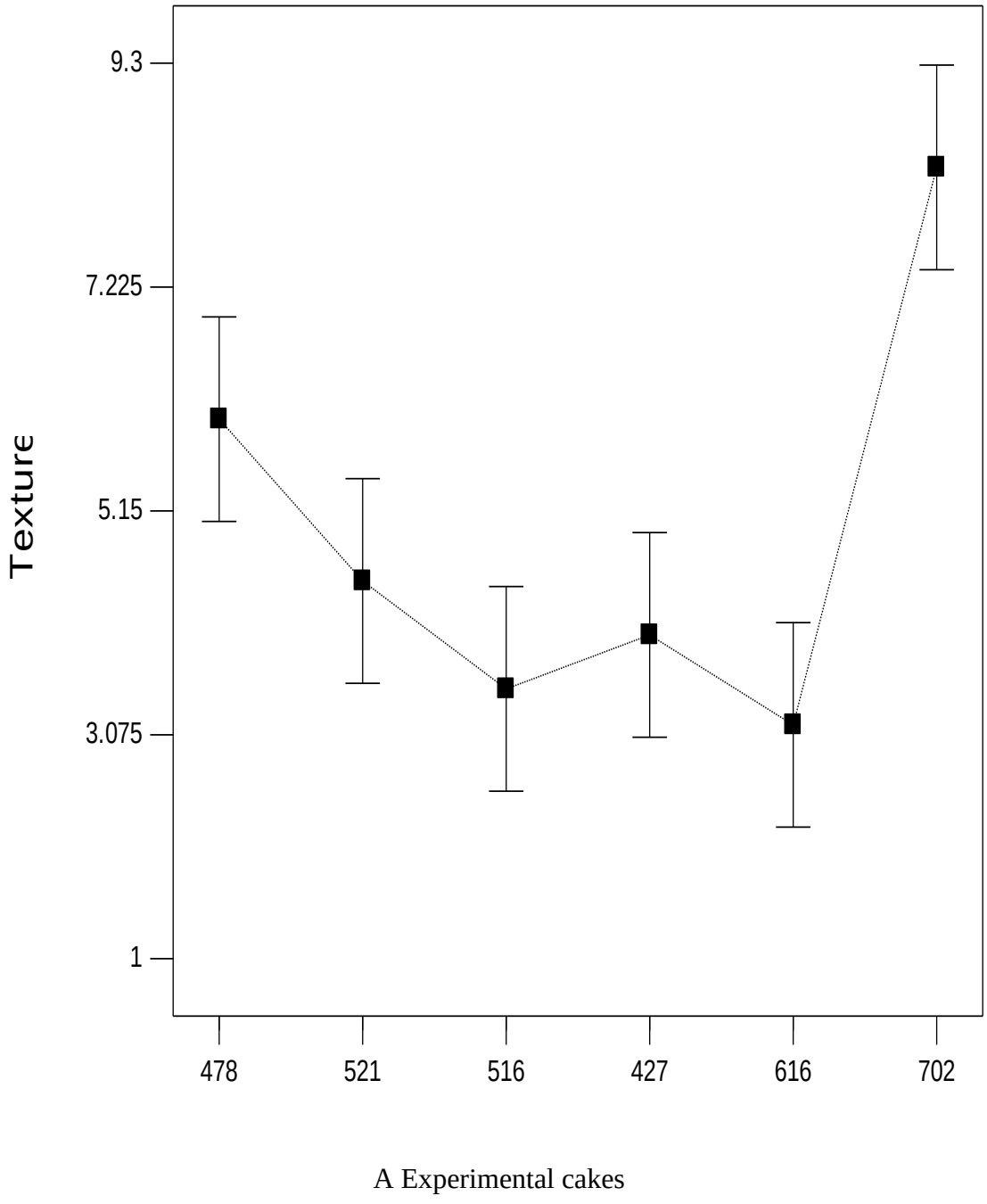


Figure16: Model graph showing the performance of cakes formulated from proteins isolates from Bambara groundnut: 478 (*Native*), 521 (BAM-10), 516 (BAM-35), 427

(BAM-60) and 616 (BAM-120) on texture compared to cake formulated from egg proteins (702).

The analysis of variance table for texture (Appendix E-6.3) showed there was a significant ($p < 0.05$) difference for the means of cake products on texture. The model graph for texture (Figure 16) further shows there was no significant ($p > 0.05$) difference between the means of the *Native* protein and BAM 10; and there was no significant ($p > 0.05$) difference for all the treated proteins. There was however a significant ($p < 0.05$) difference between the products of the egg protein and the BPIs. The egg white had the highest texture characteristics followed by the *Native*, BAM-10, BAM -60, BAM-35, and BAM-120.

4.8.2: **Optimisation of the sensory evaluation process**

Table 3: Preference order of scores under optimum condition for product sensory characteristics

Number	CakeExpt	Appearance	Flavour	Texture
1	702	11.36	9.92	10.08
2	702	8.69	9.08	8.08
3	702	8.69	8.41	8.58
4	478	9.36	8.25	7.75
5	702	8.69	7.41	8.58
6	702	7.19	8.08	7.75
7	702	7.36	8.08	6.91
8	521	8.02	7.75	6.25
9	478	6.69	7.41	5.75
10	478	6.69	6.75	6.25

Optimisation of the sensory evaluation process (Table 3) revealed that the cake product prepared from egg protein (702) ranked 1 and was the most preferred by the panelist. This was followed by cake prepared from the *Native* (478) which ranked 4, and then BAM-10 (521) which ranked 8. The proteolysis adversely affected the sensory characteristics of the modified Bambara groundnut protein isolates: the protein isolate with the highest sensory characteristics was the *Native* (sample 478), followed by BAM-10 (sample 521).

The higher foaming properties of the modified Bambara groundnut protein isolates over the *Native* and egg white however did not reflect in similar trend in their sensory characteristics. This was because the results from the model graphs (Figures 14, 15 and 16), and the overall acceptability (Appendix E- 7.0) showed that the sensory characteristics of the modified Bambara protein isolates were poorer in terms of appearance, flavour and texture than those of the *Native*, and egg white. This could be explained on the account that the proteolysis lead to denaturation and disruption of the rigid protein molecule, introduction of flexible structures and also, increased soluble peptides that lead to loss of appearance and texture characteristics (Yamaguchi *et al.*, 1996; Thiele *et al*., 2002). This might have largely contributed to the general reduction of the sensory characteristics after proteolysis.

CHAPTER FIVE

5.0 CONCLUSION RECOMMENDATIONS

5.1 Conclusion

The optimum limited proteolytic procedure was obtained with protein concentration of 10 %, protein to protease ratio of 3000:1, proteolysis time of 35 min and a temperature of 40 ^{o}C. The proteolysis improved foam activity to a maximum of 376 % at pH 9 for BAM-35. It increased foam capacity to 251 % at pH 9 for BAM-120 and also, increased emulsion capacity to 70 % at pH 4 for BAM-35. The proteolysis however decreased emulsion activity from a maximum of 54.9 % for the *Native* at pH 9, to 4.65 % at pH 4 for BAM-120. It also decreased emulsion stability of from 62 % obtained for the *Native* at pH 4, to 0.7 % at the same pH for BAM 120. Water binding and oil absorption capacities peaked at 434 % and 386 % respectively for BAM- 120.

The proteolysis resulted in better foam capacity, foam activity, emulsion capacity, water binding and oil absorption capacities with respect to the control (egg white). Foam stability, emulsion activity and emulsion stability of both native and modified protein isolates were however lower than the control. The improvement in foaming properties could make the modified Bambara groundnut protein isolates find applications in foods requiring high foam retention such as in cake to increase the volume. The high water binding and oil absorbing capacities could also make the modified proteins be used as additive in various food products requiring high liquid retention like soups, gravies and doughs. The protein isolates may however reduce the sensory appeal in baked products since the proteolysis was detrimental to the sensory properties.

5.2 Recommendations

1. Electrophoresis could not be carried out on the protein isolates to determine their molecular weights due to the unavailability of the test kit for their determinations. Future studies should consider the molecular weights determinations.

2. The temperature of 150 ^{0}C and time of 25 min for baking cakes formulated with eggs was what was used to bake the cakes formulated with protein isolates. Probably the temperature and time for baking cakes formulated with protein isolates have different baking conditions and this perhaps contributed to the poor sensory characteristics of cakes formulated using the protein isolates. Future studies should consider varying temperatures and times to establish the optimum temperature and time for baking cakes formulated with Bambara protein isolates. This may probably improve their sensory properties.

3. The high foaming properties of the modified Bambara groundnut protein isolates could be explored so as to be used in place of eggs in foods requiring high foaming like cakes, ice creams and bread.

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APPENDICES

APPENDIX A: PREPARATION OF SOLUTIONS

A: 1.1 Preparation of 0.01 M sodium hydroxide

The solution was prepared by dissolving 0.04 g of the sodium hydroxide pellets in distilled water in a 1000 mL volumetric flask to produce 1000 mL of solution.

A: 1.2 Preparation of 1 M hydrochloric acid

A volume of 86mL of the concentrated acid solution was added to a 1000 mL volumetric flask half filled with distilled water a little at a time. The flask stood in cold water with a constant gentle swirling. The volume was then made to the mark with distilled water.

A: 1.3 Preparation of the Bradford reagent

Fifty milligram of Coomassie brilliant blue dye G250 was dissolved in 50 mL methanol. A 100 mL of 85% phosphoric acid was added to the solution and this solution prepared was added to 500mL distilled water and was mixed thoroughly. It was filtered to remove precipitate and additional 350 mL of distilled water was added. It was stored in a refrigerator at 4 ^{0}C when not in use.

A: 1.4 Preparation of the Ninhydrin solution

Twenty–five grams of Ninhydrin crystals were dissolved in 100 mL ethanol. Fifty milliliters of glacial acetic acid was added slowly while mixing. The solution was filtered and stored in a dark bottle.

A: 1.5 Preparation of buffer solutions

Volumes of KH_2PO4 and NaOH used for the preparations were read from a standard table developed by *Dhanlal De Lloyd, Chem. Dept, The University of The West Indies, St. Augustine campus, 'The Republic of Trinidad and Tobago.*

A: 1.5.1 Preparation of phosphate buffer pH 4.0 determine

A 100 mL 0.1 M KH_2PO4 was added to 3.2 mL of 0.1 M NaOH and was mixed thoroughly. The pH was verified with a pH meter. Any deviation from pH 5.8 was corrected with small volumes of 0.1 M KH_2PO4 or 0.1 M NaOH to pH 5.8.

A: 1.5.2 Preparation of phosphate buffer pH 5.8

A 100 mL 0.1 M KH_2PO4 was added to 7.2 mL of 0.1 M NaOH and was mixed thoroughly. The pH was verified with a pH meter. Any deviation from pH 5.8 was corrected with small volumes of 0.1 M KH_2PO4 or 0.1 M NaOH to pH 5.8.

A: 1.5.3 Preparation of phosphate buffer pH 6.9

A 100 mL 0.1 M KH_2PO4 was added to 51.8 mL of 0.1 M NaOH and was mixed thoroughly. The pH was verified with a pH meter. Any deviation from pH 6.9 was corrected with small volumes of 0.1 M KH_2PO4 or 0.1 M NaOH to pH 6.9.

A: 1.5.4 Preparation of phosphate buffer pH 7.0

A 100 mL 0.1 M KH_2PO4 was added to 58.2 mL of 0.1 M NaOH and was mixed thoroughly. The pH was verified with a pH meter. Any deviation from pH 7.0 was corrected with small volumes of 0.1 M KH_2PO4 or 0.1 M NaOH to pH 7.0.

A: 1.5.5 Preparation of phosphate buffer pH 8.0

A 100 mL 0.1 M KH_2PO4 was added to 93.4 mL of 0.1 M NaOH and was mixed thoroughly. The pH was verified with a pH meter. Any deviation from pH 8.0 was corrected with small volumes of 0.1 M KH_2PO4 or 0.1 M NaOH to pH 8.0.

A: 1.5.6 Preparation of phosphate buffer pH 9.0

A 100 mL 0.1 M KH_2PO4 was added to 98 mL of 0.1 M NaOH and was mixed thoroughly. The pH was verified with a pH meter. Any deviation from pH 9.0 was corrected with small volumes of 0.1 M KH_2PO4 or 0.1 M NaOH to pH 9.0.

APENDIX B: STANDARD CURVES

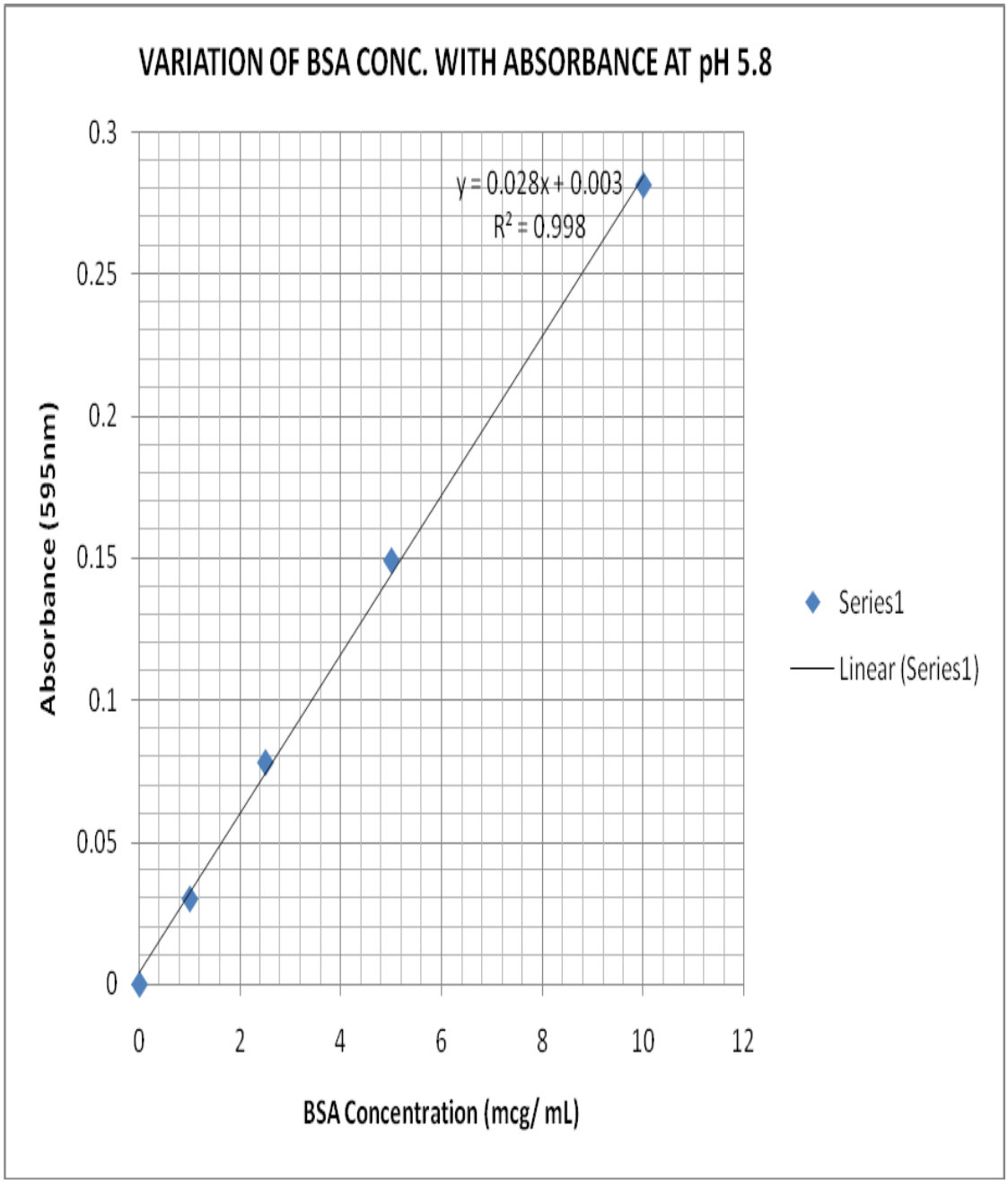
Procedure for preparation of standard curve of Bradford BSA for protein concentrations measurements

B: 1.1 Procedure for preparation of standard curve for Bradford BSA at pH 5.8

BSA Standard – mcg/mL	Volume BSA working solution (0.5 mg/mL)	Volume water/ mcL	Volume Bradford reagent/ mL	Absorbance (595 nm)		
				X_1	X_2	$X_{average}$
Blank	0	800	200	N/A	N/A	N/A
1.0	2	798	200	0.003	0.002	0.003
2.5	5	795	200	0.080	0.076	0.078
5.0	10	790	200	0.153	0.144	0.149
10.0	20	780	200	0.282	0.280	0.281

$X_{1,}$ $X_{2\ and}$ $X_{\ average}$ represent the initial, duplicate and the average absorbances respectively

B: 1.1.1 A graph of BSA concentration with absorbance at pH 5.8

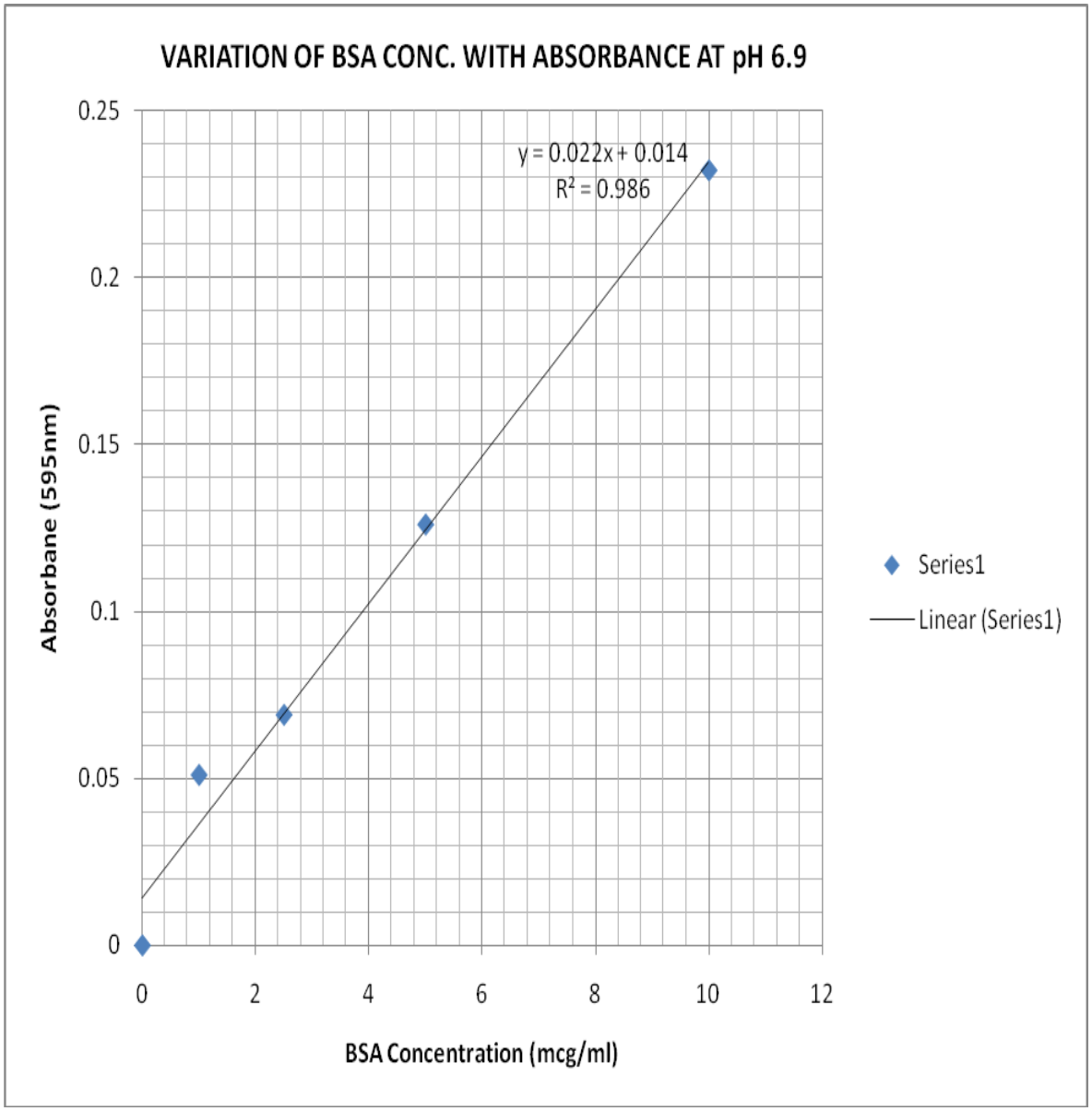

VARIATION OF BSA CONC. WITH ABSORBANCE AT pH 5.8
y = 0.028x + 0.003
R² = 0.998
Absorbance (595nm)
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
12
BSA Concentration (mcg/ mL)
Series1
Linear (Series1)

B: 1.2 Procedure for preparation of standard curve for Bradford BSA at pH 6.9

BSA Standard – mcg/mL	Volume BSA working solution (0.5 mg/mL)	Volume water/ mcL	Volume Bradford reagent/ mL	Absorbance (595 nm)		
				X_1	X_2	$X_{average}$
Blank	0	800	200	N/A	N/A	N/A
1.0	2	798	200	0.050	0.051	0.051
2.5	5	795	200	0.067	0.071	0.069
5.0	10	790	200	0.123	0.129	0.126.
10.0	20	780	200	0.230	0.234	0.232

$X_{1,}$ $X_{2\ and}$ $X_{\ average}$ represent the initial, duplicate and the average absorbances

B: 1.2.1 A graph of BSA concentration with absorbance at pH 6.9

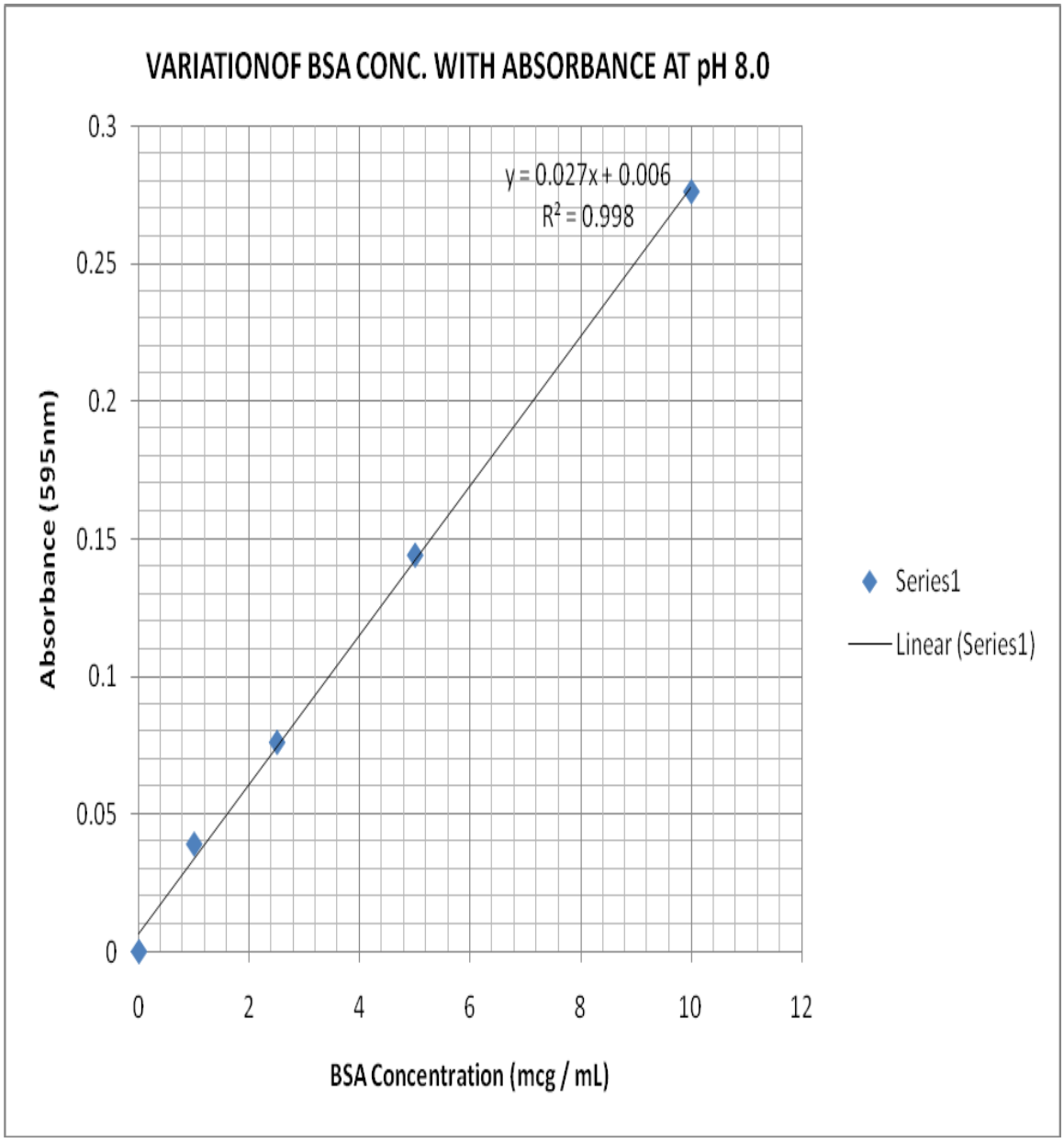


B: 1.3 Procedure for preparation of standard curve for Bradford BSA at pH 8.0

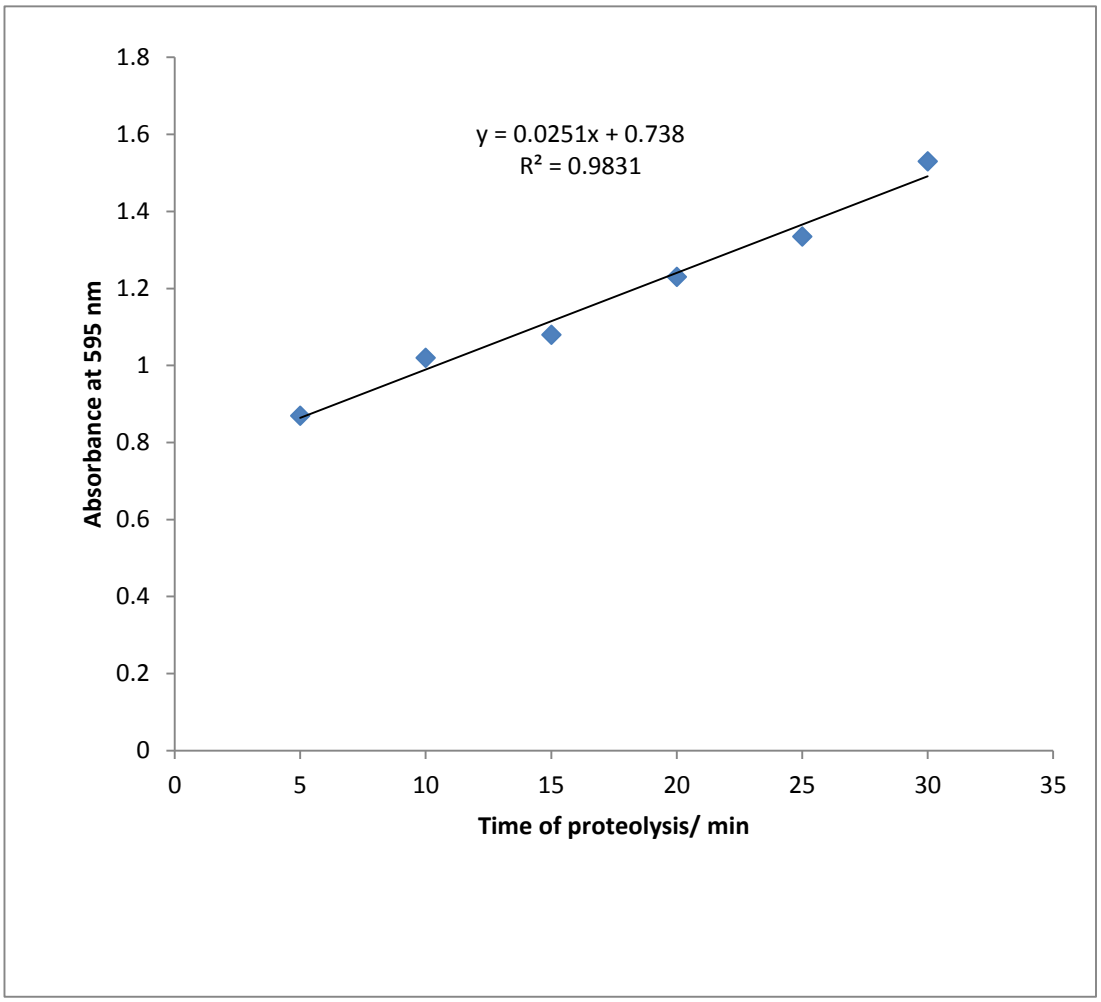
BSA Standard – mcg/mL	Volume BSA working solution (0.5 mg/mL)	Volume water/ mcL	Volume Bradford reagent/ mL	Absorbance (595 nm)		
				X_1	X_2	$X_{average}$
Blank	0	800	200	N/A	N/A	N/A
1.0	2	798	200	0.040	0.038	0.039
2.5	5	795	200	0.078	0.074	0.076
5.0	10	790	200	0.144	0.143	0.144
10.0	20	780	200	0.273	0.279	0.276

X_1, X_2 and $X_{average}$ represent the initial, duplicate and the average absorbances

B: 1.3.1 A graph of BSA concentration with absorbance at pH 8.0



B: 1.4 Determination of specific activity



The slope of the graph of absorbance and time (above) represents the *protease activity* and was determined from the graph to be **0.025 mol min^{-1}**.

Specific activity = protease activity/micrograms of protein used

Protease activity = 0.025 mol min^{-1}

Milligrams of protein used =3.3 mg

Specific activity = 0.025 mol min^{-1} /3.3 mg

= **7.58 x 10^{-3} µmol min^{-1} mg^{-1}**

APPENDIX C: RAW DATA

C: 1.0: Table of results of proteolysis for selecting times for proteolysis

Time/min	5	10	15	20	25	30	35	40	45	50	55
X_1	0.88	0.94	1.11	1.25	1.35	1.46	1.65	1.84	2.04	2.06	2.13
X_2	0.86	0.98	1.11	1.24	1.35	1.45	1.65	1.85	2.04	2.05	2.13
X average	0.87	0.96	1.11	1.25	1.35	1.46	1.65	1.85	2.04	2.06	2.13

60	65	70	75	80	100	120
2.21	2.24	2.3	2.35	2.31	2.31	2.32
2.2	2.23	2.29	2.31	2.31	2.31	2.33
2.21	2.24	2.3	2.36	2.31	2.31	2.33

X_1	represents initial absorbance
X_2	represents duplicate absorbance
X average	represents average absorbance

C: 2.0 Summary of results

FOAM ACTIVITY

Parameter	Native			BAM-10			BAM-35			BAM-60			BAM-120			EGG
pH	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	
X_1	17.5	128	200	30	140	220	47.5	228	367	97.5	205	343	115	180	328	208
X_2	22.5	138	190	32.5	150	230	57.5	213	383	97.5	215	358	113	180	313	218
X average	20	133	195	31.3	145	225	52.5	221	376	97.5	210	351	114	180	321	213

FOAM CAPACITY

Parameter	Native			BAM-10			BAM-35			BAM-60			BAM-120			EGG
pH		pH 7	pH 9		pH 7	pH 9		pH 7	pH 9		pH 7	pH 9		pH 7	pH 9	
X_1		37.5	100		40	120		128	268		105	243		80	228	108
X_2		37.5	100		50	130		113	283		115	258		80	213	118
X average		32.5	95		45	125		121	125		110	251		80	221	113

FOAM STABILITY

Parameter	Native			BAM-10			BAM-35			BAM-60			BAM-120			EGG
pH	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	
X_1	57	13.5	20	75	19	26	69	53.9	77.6	67	51	63.5	52	50	56.565	125
X_2	67	20	22.4	69	25	27	62	55.3	71.9	73	57	65	56	48.6	56	135
X average	62	16.9	21.2	72	22	26	66	54.5	74.8	70	54	64.3	54	49.3	56.3	130

EMULSION CAPACIY

Parameter	Native			BAM-10			BAM-35			BAM-60			BAM-120			EGG
pH	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	
X_1	60	60	40	65	60	45	70	65	50	65	65	50	65	60	45	55
X_2	60	55	45	65	60	45	70	65	45	65	65	45	65	60	45	55
X average	60	57.7	40.3	65	60	45	70	65	47.5	65	65	47.5	65	60	45	55

EMULSION ACTIVITY

Parameter	Native			BAM-10			BAM-35			BAM-60			BAM-120			EGG
pH	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	
X_1	17.1	40	57.8	14.2	36	43	9.3	35	40.8	8.9	31	36	1.3	3	5	25.6
X_2	16.8	42	60	14.7	38	43	9.4	36	40.1	8.4	30	35	0.9	3.2	5.2	26.3
X average	16.95	41	54.9	14.45	37	43	9.35	35.5	40.5	8.65	30	35.5	1.1	3.1	5.1	26.1

EMULSION STABILITY

Parameter	Native			BAM-10			BAM-35			BAM-60			BAM-120			EGG
pH	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	
X_1	2.8	5.3	9.7	2.4	4.5	8.8	2.2	4.5	5.6	1.3	3	5	0.8	2.7	3.6	9
X_2	3	5.6	9.8	2.8	4.9	9.1	2.2	4.5	5.8	0.9	3.2	5.2	0.6	2.6	3.9	7
X average	2.9	505	9.75	2.6	4.7	9	2.2	4.5	5.7	1.1	3.1	5.1	0.7	2.65	3.75	8

WATER BINDING CAPACITY

Parameter	Native	BAM-10	BAM-35	BAM-60	BAM-120	EGG
X_1	148	230	282	422	436	117
X_2	149	232	285	421	432	117
X average	148.5	231	283	421.5	434	117

OIL ABSORPTION CAPACITY

Parameter	Native	BAM-10	BAM-35	BAM-60	BAM-120	EGG
X_1	118	219	243	365	385	160
X_2	123	222	245	364	387	162
X average	120.5	221	244	365	386	161

APPENDIX D: FORMULAE USED FOR CALCULATIONS

D: 1.0 Yields

D: 1.1 Calculation of moisture content (MC)

MC= [Weight of dried Bambara flour / Weight of fresh Bambara flour] X 100 %

D: 1.2 Calculation of yield of defatted Bambara meal (YOD)

YOD= [Weight of defatted meal / Weight of original dried flour] X 100 %

D: 1.3 Calculation protein yield on extraction (YOE)

YOE= [Weight of protein extract / Weight of original defatted flour] X 100 %

D: 2.0 Foaming properties

D: 2.1 Foam capacity (FC)

$$FC\ (\%) = \frac{\text{Volume after homogenization} - \text{Volume before homogenization}}{\text{Volume before homogenization}} \times \frac{100}{1}$$

D: 2.2 Foam Stability (FS)

$$FS\ (\%) = \frac{\text{Foam volume after 30 min}}{\text{Initial foam volume}} \times \frac{100}{1}$$

D: 2.3 Foam Activity (FA)

$$FA\ (\%) = \frac{\text{Volume increase}}{\text{Original volume}}$$

D: 3.0 Emulsion properties

D: 3.1 Emulsion capacity (EC)

$EC\ (\%) = (V_{f1}/V_t) \times 100,$

Where V_{f1} is the volume of emulsified fraction after centrifugation and V_t is the volume of initial emulsion

D: 3.2: Emulsion activity (EA)

EA (%) = actual absorbance measured X 100

D: 3.3: Emulsion stability (ES)

ES (%) = actual absorbance measured X 100

D: 4.0 Water Binding Capacity (WBC)

WBC (g of water/g of protein) (%) = $(W_2 - W_1)/W_0$ X 100

Where W_0 is the weight of dry protein powder, W_1 is the weight of tube plus dry protein sample and W_2 is the weight of tube plus the sediment.

D: 5.0 Oil Absorption Capacity (OAC)

OAC (g of oil/g of protein) (%) = $(W_2 - W_1)/W_0$ X 100

Where W_0 is the weight of dry protein powder, W_1 is the weight of tube plus dry protein sample and W_2 is the weight of tube plus the sediment.

APPENDIX E: SENSORY EVALUATION

E: 1.0: Table for sensory analysis

	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4
Run	A:CakeExpt	B:Panelists	Appearance	Flavour	Texture	Overall acceptance
1	478	P1				
2	521	P1				
3	516	P1				
4	472	P1				
5	616	P1				
6	702	P1				
7	478	P2				
8	521	P2				
9	516	P2				
10	472	P2				
11	616	P2				
12	702	P2				
13	478	P3				
14	521	P3				
15	516	P3				
16	472	P3				
17	616	P3				
18	702	P3				
19	478	P4				
20	521	P4				
21	516	P4				
22	472	P4				
23	616	P4				
24	702	P4				
25	478	P5				
26	521	P5				
27	516	P5				
28	472	P5				
29	616	P5				
30	702	P5				
31	478	P6				
32	521	P6				
33	516	P6				
34	472	P6				
35	616	P6				
36	702	P6				

NB: Sample 472 is the Untreated or the unmodified protein isolate, 521,516, 472, 616 and 702 are BAM-10, BAM-35, BAM-60, BAM-120 and the egg albumin (control) respectively.

E: 2.0: Serving order for subjects for cake sensory evaluation

BALANCE BLOCK DESIGN FOR A SIX-PRODUCT TEST

Order of presentation within and actross sessions

Subject	**First session**		**Second session**		**Third session**	
1	4	6	5	1	2	3
2	1	4	2	3	6	5
3	6	1	3	2	5	4
4	2	5	6	4	3	1
5	3	2	1	5	4	6
6	5	3	4	6	1	2

E: 3.0 SCORE SHEET USED IN THE SENSORY EVALUATION OF BAMBARA GROUNDNUT CAKE

Date: ……

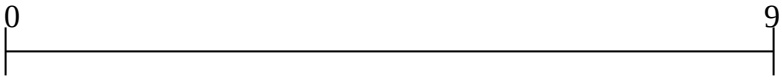
Panelist:

Sex:

You have been provided with a coded sample of Bambara groundnut cake product. Please carefully evaluate the sample and rate the appearance, the intensity of flavour, and texture by placing a vertical line to form a perpendicular on the horizontal line provided at a place that best reflects your like or dislike for the product.

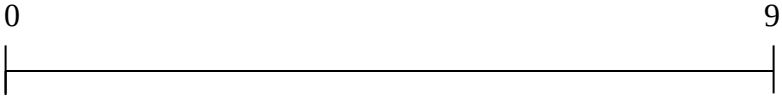
Sample 478

Appearance



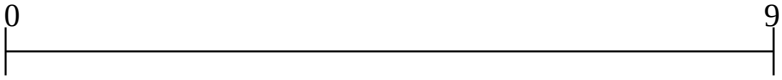
Extremely poor Extremely good

Flavour



Extremely poor Extremely good

Texture



Extremely poor Extremely good

Comments:

E: 4.0 Cake preparation

The cake was prepared by adding 60 g of granulated sugar to 40 g of cream butter and was thoroughly mixed. Fresh egg albumin of weight 250g was also stirred thoroughly and was added to the creamed butter and sugar mixture. Five drops Essence (flavouring agent) was also added and stirred to obtain a uniform consistency. 100 g flour each of the BPIs was also mixed with 150 mL of water and was used in place of the egg albumin. Each preparation was poured into metallic molds (pans) and placed in an oven at temperature of about 150 ^{0}C for 25 min. They were immediately inverted from the molds, packaged in labeled transparent polyethylene bag after cooling.

E: 5.0 Results for sensory evaluation of cake products using QDA

Run	Factor 1 A: Cake Expt.	Factor 2 B: Panelists	Response 1 Appearance	Response 2 Flavour	Response 3 Texture
1	478	P1	5.9	5.7	6.1
2	521	P1	6	3.8	2.2
3	516	P1	4.1	4.4	5.8
4	472	P1	1.8	2	5
5	616	P1	1.3	5.2	3.4
6	702	P1	8.8	8	9
7	478	P2	8.2	7.5	8.3
8	521	P2	7.3	6	4.2
9	516	P2	6.1	2.1	1.2
10	472	P2	4. 7	6.5	4.3
11	616	P2	1.2	2.3	2.2
12	702	P2	8.9	9	9.1
13	478	P3	4.8	6.7	6.3
14	521	P3	3.2	8.1	6.8
15	516	P3	3.1	4.2	2
16	472	P3	2	1.1	1.9
17	616	P3	1.9	2	1
18	702	P3	9.1	9.3	8
19	478	P4	6.3	6.2	4.7
20	521	P4	5.8	4.8	5.7
21	516	P4	5.5	3	3.1
22	472	P4	5.3	4.1	4
23	616	P4	4.2	3	5.2
24	702	P4	7.8	8.8	7.9
25	478	P5	8	7.2	6.3
26	521	P5	7.5	7.6	6.4
27	516	P5	7.7	6.8	6.9
28	472	P5	7.4	6.7	6.7
29	616	P5	6.6	6	6
30	702	P5	9.2	8.1	8.2
31	478	P6	7.2	7.8	5.1
32	521	P6	3.3	8.5	2.7
33	516	P6	2.2	2.2	2.1
34	472	P6	2	2.2	2.1
35	616	P6	2.2	2.4	1.7
36	702	P6	7.9	8.3	8.2

The QDA results shows that the subjects were independent and unbiased in the product evaluation and thus rendered the results credible for further analysis

E: 6.0 Analysis of variance table [Classical sum of squares - Type II]

6.1 Appearance

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	190.94	10	19.09	10.22	< 0.0001	Significant
A-CakeExpt	124.13	5	24.82	13.29	< 0.0001	
B-Panelists	66.80	5	13.36	7.153	0.0003	
Residual	46.69	25	1.86			
Cor Total	237.63	35				

6.2 Flavour

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	140.83	10	14.08	5.50	0.0003	Significant
A-CakeExpt	117.58	5	23.51	9.19	< 0.0001	
B-Panelists	23.25	5	4.65	1.81	0.1456	
Residual	63.91	25	2.55			
Cor Total	204.75	35				

6.3 Texture

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	147.16	10	14.7166	5.78	0.0002	Significant
A-CakeExpt	113.58	5	22.71	8.93	< 0.0001	
B-Panelists	33.58	5	6.71	2.64	0.0475	
Residual	63.58	25	2.54			
Cor Total	210.75	35				

E: 7.0: Performance of cake products from the various protein samples

Cake product	Total product score by panelist						Overall acceptability	
	P1	P2	P3	P4	P5	P6	Total product score	Average product score
478	17.7	24	17.8	17.2	21.5	20.1	118.3	19.72
521	12	17.5	18.1	16.3	21.5	14.5	99.9	16.53
516	14.3	9.4	9.3	11.6	20.4	6.5	71.5	11.9
472	8.8	10.8	5	13.4	21.6	6.3	65.9	10.99
616	9.9	5.7	4.9	12.4	18.6	6.3	57.8	9.6
702	25.8	27	26.4	24.5	25.5	24.4	153.6	26.5