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Sudan University of Science and Technology
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Isolation of Protein Concentrate from Defatted Kernels of *Balanites aegyptiaca* Seeds and Investigation of its Functional Properties

عزل مركز بروتين من أنوية بذور اللالوب منزوعة الدهن وتقصي خصائصه الوظيفية

Dissertation submitted in partial Fulfillment of the Requirements of Master Degree in Chemistry

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Dedication

I dedicate this research work to my parents, Khalifa omer and Om kalthoum Ahmed for everything they have done for me.

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First of all I would like to thank Allah for giving me inspiration, courage, energy and patience to complete this study.

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Abstract

The aim of this study was to produce protein concentrate from the defatted kernel seeds of *Balanites aegyptiaca* and investigate its functional properties. The proximate chemical composition of the kernel seeds was determined. The results showed that the moisture, ash, crude protein, oil, and total carbohydrate contents were 3.89, 3.40, 42.41, 40.24 and 10.06% respectively. Protein concentrate was isolated by using alkaline extraction method which followed by isoelectric precipitation and freeze drying. The results showed that the protein concentrate was 63.46%. Furthermore, the functional properties of *Balanites aegyptiaca* protein concentrate were examined. The results displayed that the water (WAC) and oil (OAC) absorption capacities were 2.21% and 2.68%, respectively. The minimum solubility of the protein concentrate was obtained at pH 4.5, while the maximum protein solubility was achieved at pH 9.0. In addition, the emulsion capacity (EC) and the foaming capacity (FC) were determined and the findings demonstrated that they were completely pH-dependent. The minimum values of both emulsion and foaming capacities were obtained at pH 4.5 while maximum emulsion capacity was obtained at pH 9.0 and maximum foaming capacity was found at pH 11.0.

المستخلص

في هذا البحث تم إنتاج مركز بروتين من نواة بذرة اللألوب وتمت دراسة خواصه الوظيفية. حدد التركيب التقريبى للأنوية وقد أوضحت النتائج أن محتوى الرطوبة والرماد والبروتين ومحتوى الكربوهيدرات الكلي هو على التوالي3.89 و3.40و42.41و40.24و10.06%. و من ثم تم عزل مركز البروتين باستخدام طريقة الاستخلاص القلوي والتي أتبعت بالترسيب Isoelectric precipitation و أخيرا التجفيف بالتجمد. اوضحت النتائج ان نسبة مركز بروتين اللالوب هي 63.46%. علاوة على ذلك تمت دراسة الخواص الوظيفيه لمركز البروتين. أظهرت النتائج أن سعة إمتصاص مركز بروتين اللالوب للماء والزيت 2.21% و2.68% على التوالي. وقد تم الحصول على أقل قيمة للذوبانية لمركز البروتين في الماء عند الرقم الهيدروجيني 4.5 بينما كانت أعلى قيمة للذوبانية عند الرقم الهيدروجيني 9.0. بالإضافة إلى ما سبق تم تحديد سعة الإستحلاب والقابلية لتكوين الرغوة لمركز البروتين. بينت النتائج أنهما يعتمدان على الرقم الهيدروجيني بصورة وثيقة. كما تم الحصول على الحد الأدنى لكليهما عند الرقم الهيدروجيني 4.5 وكانت أعلى قيمة لسعة الإستحلاب عند الرقم الهيدروجيني 9.0 و لسعة تكوين الرغوة عند الرقم الهيدروجيني 11.0.

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Chapter One

Introduction and literature review

1.1 Introduction

In the past few decades, the growing demand for meat together with economical and environmental factors as well as the rapid growth in population especially in the developing countries have shifted the attention of scientists and governments to look for alternative sources of proteins. Plant proteins have received great consideration as good and promising substitutes for meats or animal proteins. In addition, because of availability, risk and cost factors associated with diseases from animal protein sources which make nutritionist consider alternative plant protein sources for human nutrition and feedstock preparation. Although, most proteins from plant sources are incomplete proteins and contain smaller amounts of proteins than their animal sources counterparts. However, legumes are an exception: peas, lentils, beans, chickpeas, lima beans, soybeans and peanuts have large amounts of proteins in their seeds [1-6].

Balanites aegyptiaca belongs to the family of *Balanitaceae.* It is found in most arid to subhumid tropical savannas of Africa, all over the Sahel extending from the Atlantic coastline of Senegal to the red sea and Indian Ocean and the Arabian Peninsula [7]. In Sudan it is located on dark cracking clays of the central Sudan, often it is associated with Acacia seyal on short grass savanna [8].

A number of studies have been published in the proximate composition of *Balanites aegyptiaca* kernel seeds collected from different regions of Sudan [9-11]. The results have shown that the crude protein is ranged between 26.1 to 37%. Based on the above studies, the kernel seeds of *Balanites aegyptiaca* could be considered as a good renewable and low cost source of proteins. To the best of our knowledge there are no previous studies in the literature which reported on the isolation and characterization of protein concentrate from *balanites aegyptiaca* kernel seeds.

1.1.1 Objective

The objective of the present study is to isolate protein concentrate from defatted kernel seeds of *Balanites aegyptiaca* originated from Sudan and study its functional properties.

1.2 Protein: general consideration

Proteins are macromolecules consisting of one or more polypeptides; each polypeptide consists of a chain of amino acids linked together by peptide (amide) bonds. They are abundant components in all cells, and almost all except storage proteins are important for biological functions and cell structure. Proteins vary in molecular mass, ranging from approximately 5000 to more than a million Daltons. They are composed of elements including hydrogen, carbon, nitrogen, oxygen, and sulfur. Twenty α-amino acids are the building blocks of proteins; the amino acid residues in a protein are linked by peptide bonds. Nitrogen is the most distinguishing element present in proteins. However, nitrogen content in various food proteins ranges from 13.4 to 19.1% due to the variation in the specific amino acid composition of proteins. Generally, proteins rich in basic amino acids contain more nitrogen [12-14].

Proteins can be classified to fibrous or globular proteins. Fibrous proteins are simpler three-dimensional shapes and usually quite elongated such as α-Keratin, β-keratin and collagen. While globular proteins are more complex three-dimensional shape, in which the amino acid chain in generally tightly folded into an approximately spherical shape. Proteins are also sometimes classified as simple or conjugated. Simple proteins consist exclusively of polypeptide chain(s) with no additional chemical component being present or being required for biological activity. Conjugated proteins, in addition to their polypeptide components(s), contain one or more non poly peptide constituent known as prosthetic group(s). The most common prosthetic groups found in association with protein include carbohydrates, phosphate group, vitamin derivatives, and metal ion. Furthermore proteins can be classified according to their structures [13].

1.3 Sources of proteins

1.3.1 Animal proteins

Animal proteins are complex macromolecules that contain a number of chemically linked amino acid monomers, which together form polypeptide chains, constituting the primary structure. The helix and sheet patterns of the polypeptide chains are called secondary structures. Also it is a complete protein because has a balanced combination of all amino acids and contain the essential amino acids needed for all adult diet. It is generally associated with high fat content and, because of this, when consumed in large amounts; it leads to high risks of diseases, including high blood pressure and heart diseases. Animal products, meat,

milk, milk products, egg, poultry and fish are rich sources of protein containing a balanced level of amino acids [14,15].

1.3.2 Plant proteins

Plant proteins contain a number of side chains that are connected to the amino acid monomers. These side chains interact with each other, mainly through hydrogen and disulfide bonds, to form tertiary or quaternary structures. They often have large molecular weights, which makes them suitable for polymers and adhesives. Plant proteins are amino acid polymers derived mainly from oilseeds (i.e., soybeans) and grains (i.e. wheat and corn), and are usually produced as byproducts of processing oils and starches. Furthermore, plant (vegetable) proteins are incomplete protein; soybean protein is an exception to this. Vegetables such as (Barley, brown rice, buckwheat, millet, oatmeal, Quinoa, rye, wheat germ), legumes (Garbanzo beans, kidney beans, lentils, lima beans, navy beans, soybeans, split peas) and fruits (Apple, banana, cantaloupe, grape, Grapefruit, honeydew melon, orange, papaya, peach, and pear) are good sources of protein. Legumes have a higher content of protein than vegetables and fruits. Plant proteins are widely used as major ingredient for food, feed, pharmaceutical, nutraceuticals, paper coating, textile sizing, and adhesives [14,15].

1.4 Functional properties of plant (vegetable) proteins

The functional properties of protein have been defined as those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption [16]. The physical and chemical properties that influence functional behavior of proteins in food include their size, shape, amino acid composition and sequence, net charge, charge distribution, hydrophobicity, hydrophilicity, structures (secondary, tertiary and quaternary), molecular flexibility and rigidity in response to external environment such as (pH, temperature, and salt concentration), or interaction with other food constituents [17]. It is contributed to the quality attributes, organoleptic properties, and processing yields of food [12].

The functional properties of proteins can be classified in three groups according to their action mechanism: properties related with hydration such as solubility and absorption of water, properties related to protein structure and rheological characteristics such as viscosity and gelification, properties related to protein surface such as emulsifying and foaming activities [16].

1.4.1 Functional properties related with hydration

1.4.1.1 Solubility

It is one of the most popular tests of protein functionality. Proteins usually need to be soluble under the conditions of use for optimal functionality in food systems. Many other important functional attributes of proteins are influenced by protein solubility, such as thickening (viscosity effects), foaming, emulsification, water binding, and gelation properties [12].

The solubility of protein is the thermodynamic manifestation of the equilibrium between protein-protein and protein- solvent interactions. It is related to the net free energy change arising from the interactions of hydrophobic and hydrophilic residues of the protein with surrounding aqueous solvent .The water–soluble proteins generally contains about 25-30% hydrophobic amino acid residues and high percentage of charged residues. While almost the charged and hydrophilic residues are located as the surface, most of the hydrophobic residues are buried in the interior. The solubility of proteins is affected by many factors such as pH, temperature, ionic strength, ion type, solvent polarity, and processing conditions. These factors affect the solubility of proteins principally by causing alterations in the ionic, hydrophilic, and hydrophobic interactions at the protein surface [18].

There are a numbers of studies on the effect of pH on protein solubility. The solubility profiles of defatted walnut flour (DFWF), walnut protein concentrate (WPC), and walnut protein isolate (WPI) in water at different pH (2-12) have shown U-shaped curves, with minimal solubility at isoelectric point and then increased as the sample pH increased [19]. Also the solubility of defatted cashew nut powder (DCNP), cashew nut protein concentrate (CNPC), and cashew nut protein isolate (CNPI) showed the same U-shapes at different PHs with a high solubility in both sides of the isoelectric point [20]. A similar behavior has been reported [21] for Canavalia ensiformis flour (CF), Phaseolus Iunatus protein isolate (PPI), and Canavalia ensiformis protein isolate (CPI) which exhibited good nitrogen solubility at both extremes of the pH range. One study was examined the effect of stabilization treatment on protein solubility of rice brain protein concentrates. The results showed that the freeze-dried protein concentrates for unstabilised, acid-stabilised, heat-stabilised, and parboiled rice brain exhibited similar nitrogen solubilities, while both cabinet- and roller-dried protein concentrate showed lower nitrogen solubilities compared to freeze-dried samples [22]. Furthermore Yemisi *et al.* [23] have studied the effect of extraction method on solubility of protein isolates from bambara groundnut. The results showed that micellised protein isolates had higher solubility than the corresponding isoelectric isolates. In another study, heat treatment of rice

brain protein concentrates (RPPCs) has lead to reduction of solubility at pH 6, 8, and 10, while the nitrogen solubility at pH 2 and 4 was not affected [24].

1.4.1.2 Water/Oil absorption capacity

In food systems, the water absorption capacity of a protein is the ability to hold water against gravity and form network structures with other proteins via non-covalent interactions. The capacity of retaining moisture influences the texture and mouth-feel of foodstuffs [25]. The amount of water associated to proteins is closely related with its amino acids profile and increases with the number of charged residues, conformation, hydrophobicity, pH, temperature, ionic strength and protein concentration [16]. Similarly the oil adsorption capacity (OAC) of any food compound is important for food applications because it relies mainly on its capacity to physically entrap oil by a complex capillary-attraction process. In many food applications, such as emulsion-type meat products, the ability of a food component to entrap oil is an important characteristic because fat acts as a flavor retainer, a consistency trait and an enhancer of mouth feel [26]. Abdalbasit *et al.* [27] and Gurpreet *et al.* [3] have reported that the high oil absorption capacity is essential in the formulation of food system like cake better, and sausages while high water absorption capacity help to reduce moisture less in food products during packed bakery goods, and it is required to maintain freshness and moist mouth feel. The data obtained from the study done by Jianmei *et al.* [28] were shown that roasting reduced both water holding capacities (WHC) and oil binding capacities (OBC) of peanut flour while fermentation increased WHC and OBC of both raw and roasting. The effects of drying process on fat and water absorption capacities were investigated by Prakash *et al.* The results showed that the freeze-dried rice brain protein concentrates have higher FAC ranging from 2600 to 3200 mL/kg compared to other samples while roller-dried samples showed high WAC (2400 ml/kg) for all samples [22]. Fat absorption capacity obtained from defatted walnut flour (DFWF), walnut protein concentrate (WPC), and walnut protein isolate (WPI) were significantly different, with WPI having the highest FAC (2.81 g/g) followed by WPC (2.5 g/g) and DFWF (1.87 g/g) respectively. This could be attributed to the presence of more non polar amino acid in WPC and WPI than DFWF. DFWF showed higher water absorption capacity (3.57 g/g) than WPI and WPC [19]. According to [24] the oil absorption capacity of heat-treated rice brain protein concentrates (RBPCs) was found to be between 1.64-6.89 g/g. Moreover, the results have shown an improvement in FAC when the temperature was increased from 30 to 60^0 C. The findings also demonstrated that the ability of RBPCs to bind water was 1.02-2.27 g/g which decreased during the extraction with an

increase in temperature from 30 to 45^0C and with further increase in temperature it was found to increase again.

1.4.2 Properties related to protein surface

Emulsions and foams are two phase systems, with one of the phases dispersed in an aqueous continuous one. Both are commonly found in food systems and their formation is significantly affected by protein surface activity. Emulsifiers or foaming agents decrease the interfacial tension and facilitate formation of stable oil–water and air–water interfaces. Low molecular weight surfactants (phospholipids, mono and diglycerides or monoesterarates) are more effective than high molecular weight ones (proteins and gums) in decreasing the interfacial tension. This is because the conformational characteristics of proteins with respect of proteins limit their adsorption, which generally occurs by the sequential attachment of polypeptides or non-polar residues on the surface [16].

1.4.2.1 Emulsifying properties

Food emulsion are thermodynamically unstable mixtures of immiscible liquids (water and oil), the formation and stability of emulsion is very important in food systems such as salad dressing [28]. Emulsions are formed due to the presence of hydrophobic and hydrophilic groups of proteins [3]. Emulsions can also be defined as mixtures of two or more immiscible liquids, one of which is dispersed as droplets in the other. Oil and water are the two most common immiscible liquids found in food emulsions, although many other food components are usually present. The droplets are collectively called the discontinuous or dispersed phase, whereas the liquid surrounding the droplets is the continuous phase. Energy, in the form of homogenization, blending, or shaking, is used to disperse one immiscible liquid into the other. Proteins are used as emulsifiers to lower the interfacial energy between phases to facilitate emulsion formation and to improve the stability of emulsions. Proteins migrate to the surface of a droplet during emulsion formation to form a protective layer or membrane on the surface, thus reducing interactions between the two immiscible phases [12]. According to Adebowale *et al*. [23] who explained that the emulsifying properties depends on the hydrophilic-lipophilic balance at the oil-water interface. The protein orients lipophilic to the oil phase and hydrophilic residues to aqueous phase, the net charge of lipophilic-hydrophilic interface determines the emulsifying properties. Also it was noticed that the emulsions properties are affected by many factors such as molar mass, hydrophobicity, conformation stability, charge and physico-chemical factors as pH, ionic strength and temperature [16]. Mao and Hua [19] have reported that the emulsion capacity is a pH-dependent and the alkaline pH was found to enhance the emulsion capacity more than acidic pH. The highest emulsion activity was

observed at pH 12 with values 63.62%, 58.45%, and 55.12% for defatted walnut flour (DFWF), walnut protein concentrate (WPC), and walnut protein isolate (WPI) respectively. A similar study by Gurpreet and Sogi [3] was displayed that the emulsion capacity was increased when pH was shifted from acidic to neutral. Haiwen *et al.* [29] have shown that the emulsifying activity (EA) of peanut protein concentrate increased with an increase in pH. It was also noticed that as the pH increased the emulsification capacity (EC) of both acid-stabilised and untreated rice brain protein concentrate was also increased [22]. Gupta *et al.* [24] were found that the emulsion capacity of rice brain protein concentrates (RPPCs) was decreased when the temperature was increased from 30 to 75^0C under all salt and sugar concentrations. While high emulsifying capacity of RPPCs was observed in sugar based system and increased with increased in sugar content [3]. The effect of fermentation process on emulsion capacity of peanut protein isolates was studied by Jianmei *et al*. [28]. The results exhibited an improvement in EC of raw and roasted defatted peanut flour (DPF) by 41% and 96% respectively. The Enzyme treatment of protein concentrates from meals exhibited higher emulsion activity than those of respective controls. Similarly emulsion activity of protein concentrate from heat-treated seeds before pressing was higher than those of protein concentrates from cold-pressed seeds [30].

1.4.2.2 Foaming properties

The formation of foam is analogous to the formation of emulsion. The water molecules surround air droplets, and air is non polar phase. Theoretically the amphipathic character of protein makes them the good foaming agent that work at air- water interface to prevent bubble coalescence [28]. Foams are coarse dispersions of gas bubbles in a liquid or semisolid continuous phase. Like emulsions, foams require energy input during formation and are inherently unstable. Whipping, shaking, and sparging (gas injection) are three common methods of foam formation. Proteins or other large macromolecules in the continuous phase lower the surface tension between the two phases during foam formation and impart stability to films formed around the gas bubbles [12]. Foam capacity is determined by the ability of the protein to reduce the surface tension, the molecular flexibility and physico-chemical properties (hydrophobicity, net charge and charge distribution, hydrodynamic properties) and external factors such as (pH, ionic strength, and temperature). Good foaming proteins must be rapidly adsorb during whipping and bubbling, have a rapid conformational change, rearranging at the air–water interface with reduction of surface tension and form a visco-elastic cohesive film through intermolecular Interactions [16]. Gupta *et al.* [24] have noticed that the foaming capacity of rice brain protein concentrates (RPPCs) was decreased when the

extraction temperature was increased from 30 to 60°C at all pH values (5-9). Moreover, the results showed an increase in foaming capacity with an increase in salt and sugar concentrations. The RPPC was shown an improvement in foaming capacity at 0.5% salt solution while foaming diminished for all the three protein concentrates when the salt solution was increased from 0.5% to1% and decreased with further increase in salt concentration [3]. Similar studies by Prakash *et al.* and Nasri *et al.* [22,31] have shown the dependency of foaming capacity on pH as discussed previously.

1.5 Protein purification

Many protein separation techniques are available to food scientists. Separation techniques exploit the biochemical differences in protein solubility, size, charge, adsorption characteristics, and biological affinities for other molecules. These physical characteristics then are used to purify individual proteins from complex mixtures [12]. The precipitation of protein is shown below:

1.5.1 Isoelectric precipitation

The isoelectric point (pI) is defined as the pH at which a protein has no net charge in solution. Proteins aggregate and precipitate at their pI because there is no electrostatic repulsion between molecules. Proteins have different pIs; thus, they can be separated from each other by adjusting solution pH. When the pH of a solution is adjusted to the pI of a protein, the protein precipitates while proteins with different pIs remain in solution. The precipitated protein can be resolubilized in another solution of different pH [12].

A number of studies were used isoelectric precipitation to obtain protein concentrate or isolate from different plants. Gurpreet and Sogi [3] have produced protein concentrates from different species of defatted rice brains using isoelectric precipitation. The results indicated that the protein contents of Basmati 370,386 and HBC 19 were 54.08%, 58.92% and 52.46% respectively. In a similar study, Jianmei *et al.* [28] have obtained protein concentrate from raw and roasted peanut flour by using isoelectric precipitation with values 77.82% and 85.67% respectively. Furthermore, defatted walnut flour (DFWF), walnut protein concentrate (WPC) and walnut protein isolate (WPI) were extracted by alkaline extraction-isoelectric precipitation with different values equal 52.51%, 75.56%, and 90.50% respectively [19]. Maninder and Narpinder [32] were prepared protein isolates using isoelectric precipitation from Desi type chickpea (PBG-1, PDG-4, PDG-3, GL-769, and GPF-2) and Kabuli type chickpea (L-550). Soapnut protein isolate (SNPI) was extracted from defatted soapnut flour using isoelectric precipitation method with value equal 85.2% [33].

1.5.2 Solvent fractionation

Protein solubility at a fixed pH and ionic strength is a function of the dielectric constant of a solution. Thus, proteins can be separated based on solubility differences in organic solvent–water mixtures. The addition of water-miscible organic solvents, such as ethanol or acetone, decreases the dielectric constant of an aqueous solution and decreases the solubility of most proteins. Organic solvents decrease ionization of charged amino acids, resulting in protein aggregation and precipitation. The optimum quantity of organic solvent to precipitate a protein varies from 5 to 60%. Solvent fractionation is usually performed at 0◦C or below to prevent protein denaturation caused by temperature increases that occur when organic solvents are mixed with water [12]. The most common method used for the extraction of plant proteins is tricholoracetic acid (TCA) and acetone, this method has been utilized to extract proteins from cereals, legumes and fruits. The extreme pH and negative charge of TCA and addition of acetone realizes an immediate denaturation of the protein, along with precipitation [34]. Alcoholic extraction process has high efficiency of protein recovery [35]. Ogunwolu *et al.* [20] have reported the extraction of protein concentrates and isolates from cashew nut powder using methanol precipitation method.

1.5.3 Membrane Processes

Microfiltration, ultrafiltration, nanofiltration, and reverse osmosis all are processes that use a semi-permeable membrane for the separation of solutes on the basis of size under an applied pressure. These methods are similar to dialysis but are much faster and are applicable to large-scale separations. Molecules larger than the membrane cutoff are retained and become part of the retentate, while smaller molecules pass through the membrane and become part of the filtrate. These membrane processes differ mainly in the porosity of the membranes and in the operating pressure used. The porosity of the membrane sequentially decreases and the pressure used sequentially increases, respectively, for microfiltration, ultrafiltration, nanofiltration, and reverse osmosis [12]. Andre's *et al.* [30] were produced Protein concentrate from Guevina avellana pressing cakes using membrane filtration. The results showed that the cold- pressed seeds had higher protein content of the protein concentrate than the thermally treated seeds. Boye *et al.* [36] were optimized ultrafiltration technique for the extraction of protein concentrates from yellow pea, red and green lentils, desi and Kabuli chickpieas, with values equal 83.9%, 88.6%, 82.7%, 76.5%, and 68.5% respectively.

1.5.4 Other methods for protein purification

Protein isolates from two variety of Bambara groundnut (white and brown variety) were obtained using micellisation technique, the results showed that the brown variety protein isolates had higher protein content than the white variety with value 78.0% and 75.0% [23].

Gupta *et al*. [24] were produced protein concentrates from defatted rice brain at different extraction temperature (30-75^{0}C). The results indicated that the protein content of rice brain protein concentrates (RPPCs) were decreased from 79.9 to 71% as the temperature was increased from 30^0 to 75^0 C.

1.6 Uses of vegetable proteins

Vegetable proteins have been widely used in food system [26,37-39]. Thompson [37] was evaluated the improvement of wheat flour bread with mung bean flour (MF) and its protein isolates (MI-I). The results indicated that the addition of 10% level from MI-I produced acceptable bread with higher protein content (41%) than the supplemented bread. While addition of 40% level MF to achieve the same supplementation as 10% MI-I was produced unacceptable bread. Canola protein was used as ingredients in different food formulations. Hydrolysates canola protein (at 7 and 14 % degree of hydrolysis (DH)) was used to replace up to 50% (w/w) of the egg yolk in a model mayonnaise preparation. The results displayed that at 7% DH, the canola protein could be used to substitute up to 20% (w/w) of the egg yolk, while at 14% DH; the maximum level of substitution was up to 50% (w/w). Also canola protein hydrolysates were studied as potential ingredient in meat. It was found to be effective in enhancing the water holding capacity and cooking yield in a meat model system. Canola protein concentrate was used as an additive to replace casein in a sausage formulation. A sensory analysis showed that the rapeseed protein sausage had improved taste, good texture and a characteristic aroma. Furthermore, the generation of meat–like flavorings from enzymatic hydrolysates of proteins for canola/rapeseed was investigated. The results indicated that the best favored products were generated at 160 ^{0}C and pH 4.0, and products with a burnt odor were produced at pH 8.0 and 180 ^{0}C [26].

Application of soy protein isolates in yogurt, whipping topping and coffee creamers were investigated. The results have shown that the soy protein isolates are most effective in improving or increasing the textural properties of yogurt which increased as the protein concentration increased compared to sodium caseinate and nonfat dry milk. Moreover coffee creamers prepared from soy protein isolate exhibited value equal to coffee creamer manufactured with sodium caseinate [38]. Soya proteins (concentrates and isolates) have been used in production of meat poultry and fish products to increased cooking yield and avoid fat

/gel separation. It is also added in coarsely comminuted (Minced) products to prevent fat losses during cooking. Soya protein concentrates and isolates are applied in whole cuts of meat to increase the amount of protein, to improve the water-holding capacity, and to produce a more tender and succulent product. Moreover, it was used as replacement for nonfat milk solids (NFMS) in bread product, which remain soft and fresh for several days longer than the conventional bread. The protein content of spaghetti and macaroni pasta type of products can be increased from 11% to 22% by addition of 20% bland soya protein concentrate. Biscuits have been developed with 13% soya protein concentrate, leading to 15% protein calories in the final biscuit. Also soy protein is used in confectionery products [39].

1.7 Balanites *aegyptiaca*

1.7.1 Taxonomy profile [40]

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Sapindales*

Family: *Balanitaceae (Zygophyllaceae)*

Genus: *Balanites Delile*

Species: Balanites aegyptiaca (L.) Delile.

Balanites aegyptiaca is called the desert date, Soapberry tree, Thorn tree, Egyptian balsam (English), Heglig (Arabic), Dattier du desert, Hagueleg, Balanite (French), Corona di Jesus (Spanish) [40].

1.7.2 Botanic Description

Semi-evergreen, spiny, small tree or shrub 5-10 m high, sometimes to 12 m. Furrowed stem or trunk up to 45 Cm diameter. The crown is roundish, yellow-green, a tangled mass of long thorny twigs whose leafless ends droop, and protrude in place from the main thicket. Branchlets are green, smooth and armed with strong green spines 8-9 cm long. Bark smooth and green with young trees and branches, brown to grey, deeply fissured and croky with the trunk of old trees .Slashed or cut bark is pale yellow. The leaves are grey-green, consist of two leaflets on short petiole and vary considerably in size, leaflets 2 to 5 long and 1 to 2.5 cm wide. Flowers are small yellowish-green, about 1cm diameter; solitary or in clusters of up to 5 and borne at base of leaves or spines. Fruits are drupes with long 3-4 or 2.5-4 cm ×1.5-2.5 cm diameter, green and densely covered with short soft hairs at first, becoming yellow to orange red and glabrous. Each contains a single, very hard stone surrounded by yellowish, bitter-sweet, sticky, fibrous edible pulp, which has a characteristic smell. The skin is thin,

sometimes wrinkled, becoming parched when ripe and easily removed from pulp. Seeds are one pointed, 5-angled stone, with a very hard and fibrous outer coat [7,41].

1.7.3 Distribution

Balanites Aegyptiaca is found in most arid, semi-aird to subhumid tropical Savannas in Africa, all over the Sahel and on many sites of the Sudan savanna common on dark cracking clays of the central Sudan, extending from the Atlantic coastline of Senegal to the red sea and India Ocean and the Arabian Peninsula. Often it is associated with Acacia seyal on short grass savanna [7,8].

Other study has reported that *Balanites aegyptiaca* is native to all dry lands of Africa, Indigenous to woodland throughout the sahel-sudan region, Egypt, East Africa, Nigeria, Niger and south to Natal. Also it is found in Israel, Jordan, Saudi Arabia and drier parts of Pakistan and India [41].

1.7.4 Propagation

Propagation is by seed or root sucker. Seeds taken from excreta of cattle germinate particularly well. To improve germination of other seeds they should be boiled for 7-10 minutes and cooled slowly or soaked overnight in warm water. The seed is sown vertically with the stalk and down. Germination is occurs in 1-4 weeks. The tree requires about 12 weeks in the nursery before planting out. Alternatively seed may be sown directly in the field. Balanites grows slowly, and protection of the seeding from fire and cattle is necessary for at least 3 years. Seeds are often attacked by borers. The fruits reach maturity during the dry season. In the Sahel flowers appear long before the rains and fruits ripen during December / January but in Zambia flowers appear at September/December and fruits ripen the following April/ August [41].

1.7.5 Chemical composition of kernels

There are a number of studies about the chemical composition of *Balanites aegyptiaca* kernel seeds. Lohlum *et al.* [42] have revealed that the crude protein, crude fibre, lipids, ash, nitrogen free extract, calcium and phosphors in the raw seed powder of balanites aegyptiaca were (31.73, 17.19, 37.11, 3.98, 9.99, 0.19 and 0.16 % respectively). While the ethanol extracted of the seed powder has shown (37.68, 21.63, 9.98, 3.81, 26.90, 0.19 and 0.20 % respectively) for crude protein, crude fibre, lipids, ash, nitrogen free extract, calcium and phosphorus.

Nour *et al.* [10] have determined the chemical composition of *Balanites Aegyptiaca L.* (lalob) fruits (pulp and kernel) grown at different regions in Sudan (kordofan and hawata). The results exhibited that the moisture was 3.2 and 3.1%, oil 45 and 46.1%, protein 34.3 and 26.1

%, and total carbohydrate 24.1 and 21.3%. Minerals content has shown that calcium was 102 and 108 mg/100g, and iron was 7.0 and 5.0 mg/100g at kordofan and hawata seeds samples respectively. In addition, they found that palmitic, stearic, oleic and linoleic were the major fatty acids in the two samples.

Mohamed *et al.* [9] have studied the chemical, physical, morphological characteristics, oil recovery and fatty acid composition of *Balanites aegyptiaca* Del. Kernels. The findings have revealed that the morphological structure of lalob kernels showed a protein matrix surrounded by oil droplets. In addition, the results have shown that the Balanites constitute of crude protein (32.4%), crude fat (49.0%), crude fibre (1.4%), moisture content (5.2%), total ash content (3.3%), calcium (246.9mg/100g), Zinc (5.8mg/100g), and Magnesium (239.3mg/100g). Furthermore, they found that the phytic acid content of the kernels was (1.2%), and sapogenin content was (1.5%).

1.7.6 Uses of *Balanites aegyptiaca*

The yellow to orange-red fleshy fruit pulp is oily, bitter-sweet and refreshing to suck. It can be pulped to make a refreshing drink which becomes alcoholic if fermented. The juice is used to flavor cereals in Nigeria. Sometimes the seed kernels are used in making a kind of bread or eaten in soups by the Shuwa Arabs of northeastern Nigeria. The bland tasteless oil is highly prized in eastern and western Sudan and is used for culinary purposes. Also the kernels are much used in Chad area and sometimes they are steeped for 3-4 days before being eaten. The boiled flowers called dobagara are eaten with couscous or added to Hausa food. The leaves are consumed as vegetable in Ethiopia [41]. A decoction of the roots is used to treat abdominal pains, intestinal worms, diarrhea and as antidote for poisoning. Gum from wood is mixed with maize meal porridge and eaten to treat chest complaints [43]. The bark is used in the treatment of syphilis, round worm infections, and as a fish poison. The fruits are used as an oral hypoglycemic and an antidiabetic, whooping cough, also in leucoderma and other skin diseases. An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice and herbal medicine. The Seed is used as expectorant, antibacterial, and antifungal. The seed oil is used to treat tumors and wounds. Also used as laxative, in treatment of hemorrhoid, stomach aches, jaundice, yellow fever, syphilis, and epilepsy [40].

Livestock eat fallen fruit on the ground and browse the leaves. The wood is durable and resistant to insect damage. It is easy to saw, split, polish, and turn. It is used for making tool-handles, parts of donkey or camel saddles, small furniture – stools, low tables – candlesticks, firewood, and charcoal, etc [43]

Chapter Two

Materials and methods

2.1 Sample collection and pretreatments

Balanites Aegyptiaca fruits sample was purchased from Omdurman market (Souq Omdurman), Khartoum State-Sudan. The epicarps of *Balanites Aegyptiaca* fruits were removed manually and the remaining edible flesh (mesocarp) plus the endocarps were soaked in a tap water for overnight. After complete removing of the edible flesh the endocarps were dried in sun light for two days. Finally, the endocarps were removed by hand using a hammer and the kernels were stored at 10^{o}C for the next steps.

2.2. Chemicals

n-Hexane (Assay = 95%, density= 0.656-0.665gcm^{-3}, Romil Ltd). Hydrochloric acid (Assay = 35-38 %, density = 1.18 gcm^{-3} at 20 ºC, Loba Chemie, India). Sodium hydroxide (Assay = 97 %, Central Drug House, India). Kjeldahl catalysis tablet copper (Chemicals Ltd Poole, England). Bromo-cresol green (Fisher Scientific Company, USA). Methyl Red (Hopkin and Williams Ltd.). Acetic acid (Assay = 99.7%, density = 1.048-1.050 gcm^{-3}, Oxford Lab Chem, India). Tri-chloro acetic acid (Assay= 99%, SD Fine-Chemie limited, Mumbai). Nitric acid (Assay = 69-72%, density = 1.41-1.42 gcm^{-3}, Trust Chemical Laboratories, India). Sulfuric acid (Assay = 90%, Loba Chemie, India).

2.3 Proximate analysis

2.3.1 Determination of ash content

2 g of the sample were weighed into a pre-weighed porcelain crucible. The crucible was placed in a temperature controlled furnace and the sample was ignited at 550ºC for 3 hours. Finally, the crucible was transferred directly to a desiccator, left to cool, and reweighed again. This process was repeated several times until constant weight was attained. The ash content was calculated using the following equation:

$$\text{Ash \%} = \frac{(\text{g of ash})}{(\text{g as-received sample})} \times 100 \quad \ldots\ldots (2.1)$$

2.3.2 Determination of moisture content

2 g of the sample were weighed into a pre-weighed porcelain crucible. The crucible was placed in a temperature controlled oven at 105ºC and heated for 18h. Finally, the crucible was transferred to a desiccator, left to cool, and reweighed again. This process was repeated

several times until constant weight was attained. The moisture content was calculated using the following equation:

$$\text{Moisture \%} = \frac{(\text{g as-received sample} - \text{g oven -dried sample})}{(\text{g as-received sample})} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.2)$$

2.3.3 Determination of Crude protein

1 g of the sample was weighed into a digestion flask and 5 g of Kjeldahl catalysis tablet copper plus 20 mL of sulfuric acid were added. The flask was placed on a digestion racks and heated for 3 hours, left to cool, and 200 mL of distilled water was added. Separately, 25 mL of boric acid was placed into a conical flask and 3-4 drops of methyl red/bromocresol green indicator were added. The flask was connected to a distillation apparatus and the mixture was distilled until 100 mL of distillate was collected (the distillate was turned from red/pink to green). Finally the distillate was titrated against 1N hydrochloric acid (turned from green to pink). All analysis was performed in triplicate. Crude protein was calculated using the following equation:

$$\text{N \%} = \frac{(\text{mL standard acid} \times \text{Normality acid} \times \text{dillution factor} \times 14 \times 100)}{(\text{weight of sample} \times 1000)} \quad \ldots. (2.3)$$

$$\text{Crude protein \%} = \text{N (\%)} \times 6.25 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.4)$$

Where N is the nitrogen content and 14 is the atomic weight of nitrogen

2.3.4 Determination of fibre content

2 g of the sample were weighed into a conical flask and 100 mL of acid fibre solution (Nitric acid, Acetic acid, Tri-chloro acetic acid and distilled water) was added. The mixture was refluxed for 40 min at boiling temperature and filtered. The filtrate was transferred into a porcelain crucible and placed into an oven for 18h at 105ºC. The crucible was transferred to desiccators, cooled, and reweighed. Finally, the crucible was placed into a furnace at 550ºC, heated for 3 hours, cooled, and reweighed again. All analyses were performed in a triplicate. Crude fibre was calculated using the following equation:

$$\text{Crude Fibre content \%} = \frac{w_2 - w_1}{w_0} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.5)$$

Where w_o is the weight of the dry sample, w_1 is the weight of the crucible plus ash, w_2 is weight of the crucible plus dry matter.

2.3.5 Determination of oil content

50g of the *balanites aegyptiaca* flour were weighed and transferred to an extraction thimble. The oil was extracted in a Soxhlet extractor using 500 mL of n-hexane as a solvent for 5 hours. n-Hexane was removed from the oil using a rotary evaporator and the oil was stored in a freezer for further analyses. The oil content was calculated using the following equation:

Oil content % = $\frac{\text{weight of oil}}{\text{weight of sample}} \times 100$…….. (2.6)

2.4 Protein extraction

2.4.1 Preparation of protein concentrate

50g of the defatted *balanites aegyptiaca* flour (method 3.3.5) was weighed and suspended in a distilled water at ratio 1:20(w/v). The mixture was stirred for 1h at room temperature using a magnetic stirrer while adjusting the pH at 9.0 with NaOH solution (1 M). The mixture was then centrifuged for 15 min at room temperature and the supernatant was transferred into a beaker, stirred for 30 min, and the pH adjusted to 4.5 with HCl solution (1 M). The supernatant was left undisturbed for overnight in a freezer. The supernatant was carefully siphoned off and the protein slurry was washed three times with distilled water by centrifuging for 10 min. The pellet was re-suspended in distilled water, and the pH was adjusted to 7.0. The slurry was kept overnight in a freezer then it was freezed dried. The protein concentrate was weighed.

2.5 Functional properties of protein

2.5.1 Water absorption capacity (WAC)

1 g of the sample was weighed into pre-weighed centrifuge tube. 10 mL of distilled water was added in small increments to the tube under continuous stirring with a glass rod. After being held at room temperature for 30 min, the tube was centrifuged for 20 min. The supernatant was carefully removed, and the tube was reweighed. All analyses were performed in triplicate. WAC was calculated using the following equation (expressed as grams of water per gram of sample)**:**

WAC = $\frac{w_2 - w_1}{w_0}$ …… (2.7)

Where w_0 is the weight of dry sample in gram, w_1 is weight of the tube plus the dry sample in gram, w_2 is the weight of the tube plus sediment in gram [19].

2.5.2 Fat absorption capacity (FAC)

1 g of the sample was weighed into pre-weighed centrifuge tube and thoroughly mixed with 10 mL of vegetable oil using glass rod. The sample was allowed to stand for 30 min at room temperature. The protein-oil mixture was centrifuge for 20 min. Then the supernatant was carefully removed, and the tube was reweighed. All analyses were performed in triplicate. FAC (gram of oil per gram of protein) was calculated using the following equation:

FAC = $\frac{w_2 - w_1}{w_0}$ …… (2.8)

Where w_o is the weight of the dry sample in gram, w_1 is the weight of the tube plus the dry sample in gram, w_2 is the weight of the tube plus sediment in gram [19].

2.5.3 Emulsifying properties

1g of the sample was weighed in each tube and homogenized for 1 min at room temperature in 25 mL distilled water. The pH of suspension was adjusted to 3, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12 with either 0.1 M HCl or 0.1 M NaOH. The protein solution was mixed with 25 of oil followed by homogenization for I min. Finally the emulsion was centrifuged for 5 min. All analyses were performed in triplicate. Emulsifying activity was calculated using the following equation:

$$\text{Emulsifying activity \%} = \frac{(\text{Height of emulsified layer})}{(\text{Height of the contents of the tube})} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.9)$$

2.5.4 Foaming Capacity (FC)

500 mg of sample were dispersed in a 50 mL of distilled water. The pH of protein solution was adjusted to 3, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, and 12 with either 0.1 M HCl or 0.1 M NaOH. The solutions were stirred for 2 min. The blend was immediately transferred into a 100 mL graduated cylinder. The volume was recorded before and after stirring. FC was expressed as the volume (%) increased due to stirring. All analyses were performed in triplicate. Foam capacity was calculated according to the following formula:

$$\text{FC (\%)} = \frac{(\text{volume after whipping} - \text{volume before whipping})\ \text{ml}}{(\text{volume before whipping})\text{ml}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.10)$$

2.5.5 Water Protein Solubility

200 mg of the sample were dispersed in a 20 mL distilled water and the pH of mixture was adjusted to 3, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12 with 1M HCl or 1 M NOH. The mixture was stirred at room temperature for 30 min and centrifuged for 20 min. The weight of soluble protein was determined by the difference in weight between the residue and the original sample. All analyses were performed in triplicate. Protein solubility was then calculated using the following equation:

$$\text{Solubility \%} = \frac{(\text{weight of soluble protein})}{(\text{weight of sample})} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.11)$$

Chapter Three

Results and discussion

3.1 Proximate composition

The proximate composition of *Balanites aegyptiaca* kernel seeds is shown in table 3.1. As can be seen from the table the oil and protein contents are 40.24% and 42.41% respectively. Similar results were reported for the oil content of *Balanites aegyptiaca* originated from different locations in Sudan [10,11]. Alfeel has studied the effect of location on the proximate composition of *Balanites aegyptiaca*. Three samples were collected from different regions from Sudan (Rashad, Um Abdalla and Ed Alfrissan). The results have displayed great variations in proximate composition between and within locations. The oil content was found to lie between 20% to 50%. Similar results were also reported by Nour *et al*. for *Balanites aegyptiaca* from kordofan and hawata regions. On the other hand, the protein content is observably higher than the ones reported by Alfeel and Nour *et al*. Alfeel was found that the protein content varied from 27 to 37%, while Nour *et al*. have reported that the protein content lie between 35 to 37%. These variations in protein content could also be attributed to the effect of location.

Table 3.1: **Proximate chemical composition of kernel seeds of *Balanites aegyptiaca*.**

Parameter	Value
Ash content (%)	3.40±0.01
Moisture content (%)	3.89±0.05
Oil content (%)	40.24±0.99
Crude protein (%)	42.41±0.03
Total carbohydrate content (%)	10.06
Protein concentrate (%)	63.46±1.31

The table also displayed that the ash and moisture content are 3.4 and 3.89% respectively. Analogous results were reported by Mohamed *et al*. [9] for the ash content of *Balanites aegyptiaca* collected from Al Khartoum. The results revealed that the ash content is equal 3.3%. On the other hand, the moisture content is found to be higher than that of *Balanites aegyptiaca* originated from kordofan and Hawata regions [10] with values equal 3.2 and 3.1 % but lower than the one obtained by Mohamed *et al*.[9] (5.2%). These differences could also

be attributed to the difference in locations. The table also shows that the total carbohydrate content reaches 10.06 %.

3.2 Functional properties of *Balanites aegyptiaca* protein concentrate

3.2.1 Water and fat absorption capacities (WAC and FAC)

The water and oil absorption capacities of *balanites aegyptiaca* protein concentrate are presented in table 3.2. As can be seen from the table, the fat absorption capacity (FAC) is 2.68 g/g. This value is moderately higher than many of the reported values in the literature [19,36,44]. Mao *et al*. were found that the FAC of walnut protein concentrate is 2.50g/g while Boye *et al*. have reported that the FAC of pea, chickpea, and lentil protein concentrates are ranged between 1.10 to 2.30 g/g. Aurella *et al*. were observed that the FAC of chickpea protein concentrate is 2.05g/g. It could be concluded that the obtained value indicates that the *Balanites aegyptiaca* protein concentrate has good levels of nonpolar amino acids. In addition, high protein content may also enhance the FAC of the *Balanites aegyptiaca* protein concentrate. Additionally, it has been reported that FAC is affected by size, surface area of macromolecule, charge, and hydrophobicity, extraction methods, and temperature [45]. The value of FAC of protein concentrate is in agreement with that of rice brain protein concentrate extracted at 45^0 C [24].

Table 3.2: **Water and fat absorption capacity of *Balanites aegyptiaca* protein concentrate.**

Functional property	Value
WAC (g/g)	2.21±0.05
FAC (g/g)	2.68±0.08

The table also displays the water absorption capacity (WAC) of the protein concentrate which equals 2.21 g/g. This value lies within the range of WAC 1.49-4.72 g/g but lower comparable to that of rice brain protein concentrates [3] and walnut protein concentrate [19]. This probably related to the lower content of carbohydrates (low content of hydrophilic parts such as polar or charged side chains). In addition, the low content of WAC could be due to the lower availability of polar amino acids to interact with water [46], also it is related to ability of protein to swell, dissociate and unfold [47]. Other study was found that the WAC of proteins is a function of several parameters, including shape, size, steric factors, and hydrophilic-hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with protein [45].

3.2.2 Protein solubility

The protein solubility profile of *Balanites* aegyptiaca protein concentrate at varying pH (3-11) is shown in figure 3.1. As can be seen from the figure, the protein solubility decreases as the pH increases until it reaches pH 4.5 then gradually increases up to pH 9. At pH 10 and 11 the solubility decreases again. Additionally the minimum solubility was observed at pH 4.5 with value equal 14.52%, which corresponds to the isoelectric point of balanites protein concentrates. This could be attributed to the fact that at isoelectric point there is no net charge on the protein, and the protein-protein interaction disfavor solubility [48]. The maximum water solubility was found to be 36.63% at pH 9. This may be due to the fact that at higher pH values the increased net negative charge on the protein dissociates the protein aggregates and increases solubility, but at lower pH values, the increased net positive charge contributes to the solubility [33]. Similar solubility profile has been reported for yellow pea protein concentrates which exhibited a decrease in solubility with an increase in pH until isoelectric point and then it was further increased [36].

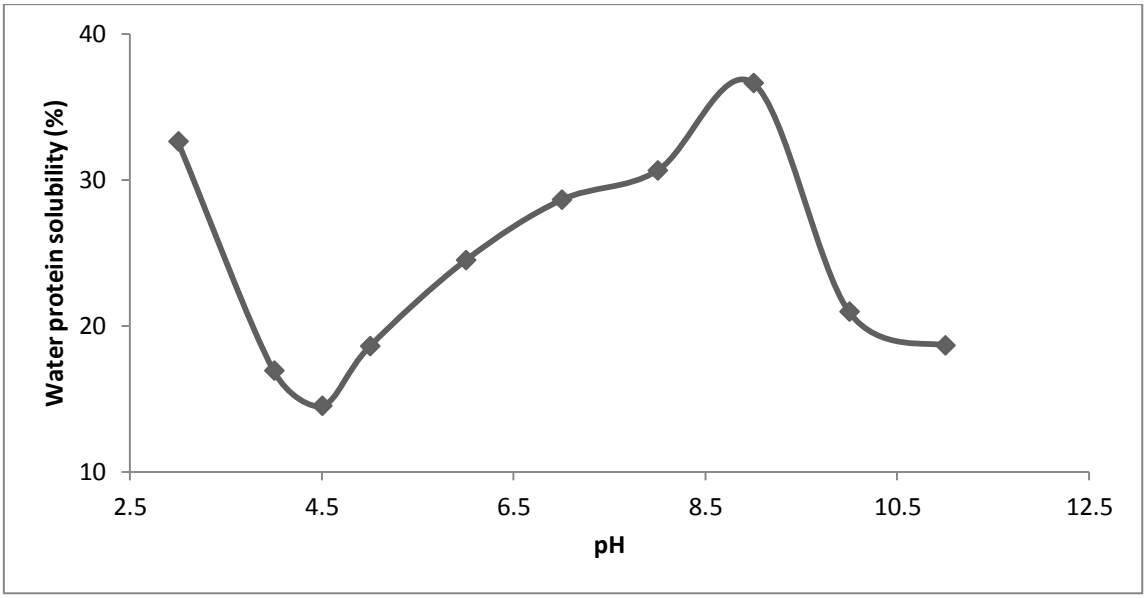

Figure 3.1: Solubility profile of *Balanites aegyptiaca* protein concentrate at different pH values.

3.2.3 Foaming capacity (FC)

The foaming capacity of balanites protein concentrate is displayed in figure 3.2 under various pH (3.0-12.0). As can be seen from the figure the foaming capacity lies in the range between 31.46 to 63.68%. The lowest foaming capacity was 31.46% at pH 4.5. This may be related to the isoelectric point of protein and therefore exhibited lowest value. In addition, the highest

FC was observed at pH 11.0 with value equals 63.68%. Also the figure showed an improvement in foaming capacity when the pH was shifted from acidic to alkaline pHs. This were likely due to an increase in the net charge of the protein which weakens hydrophobic interaction and increases protein solubility and flexibility, allowing the protein to spread to the air-water interface more quickly encapsulating air particles and thus increasing foam formation, as reported by Lawal *et al.* [49]. A similar trend has been reported by Mao *et al.* [19] who stated that the walnut protein concentrate has lowest foaming capacity (FC) at pH 4.5, while the highest at pH 9 and alkaline pHs was found to enhance the foaming capacity greater than the acidic pHs. Furthermore Gupta *et al*. [24] have demonstrated that as the pH shifts from acidic (pH5.0) to alkaline (pH 9.0), the foaming capacity of the brain protein concentrate increases.

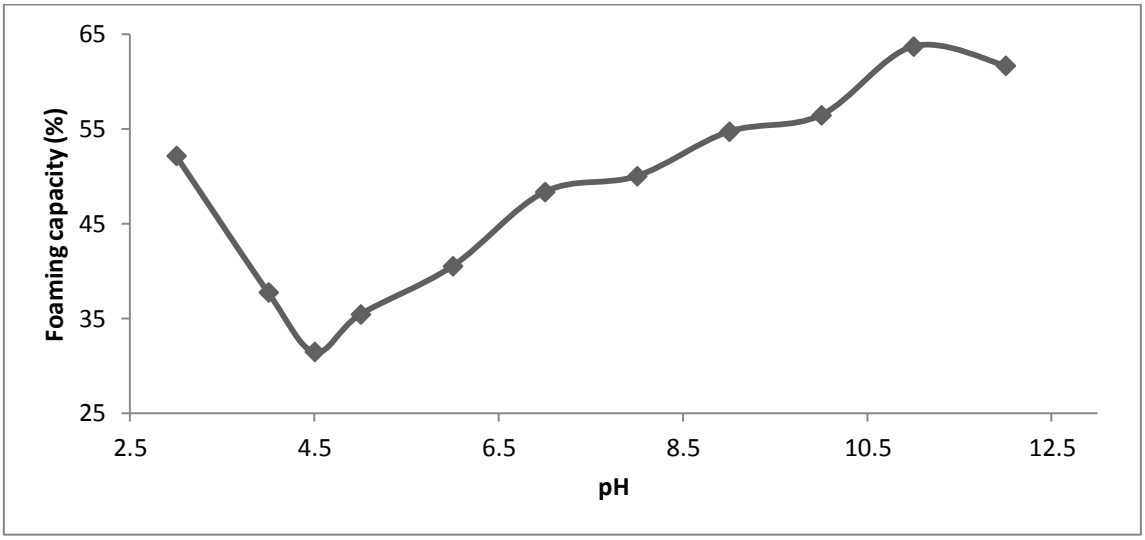

Figure 3.2: Effect of pH on the foaming capacity of *Balanites aegyptiaca* protein concentrate

3.2.4 Emulsifying capacity

The effects of pH on the emulsifying capacity of balanites protein concentrate are presented in figure 3.3. As can be seen from the figure, the minimum emulsion capacity (41.52%) was obtained at pH 4.5 (isoelectric point), where protein tend to precipitate, leading to reduction in emulsion formation. According to Damodaran [17] this is because most food proteins are sparingly soluble at their isoelectric pH, poorly hydrated, and lack electrostatic repulsive force, they are generally poor emulsifiers at this pH. It could be observed from the figure that a higher emulsifying capacity is noticed on the both sides of the isoelectric point. Additionally, the maximum emulsion capacity was 62.5% at pH 9.0. Aoki *et al*. [50] have

shown that the pH-emulsifying property of various proteins including soya protein resembles the pH-solubility profile. Similarly, the defatted walnut flour DFWF, walnut protein concentrate WPC, and walnut protein isolate WPI were shown minimum emulsifying capacity at pH 4.5 with values equal 33.28%, 28.56% and 18.91%, respectively, and the maximum emulsifying capacity at pH 12.0 [19]. However, at a pH range of 5.0 to 9.0 where protein solubility increases this enhances interaction between the oil phase and the aqueous phase. Furthermore, the figure displays that the emulsion capacity is a pH-dependent, and the alkaline pH was found to improve the emulsion capacity more than the acidic pH. The pH has pronounced effects on the emulsifying activity because soluble proteins emulsifying activity depend upon the hydrophilic and lipophilic balance [29].

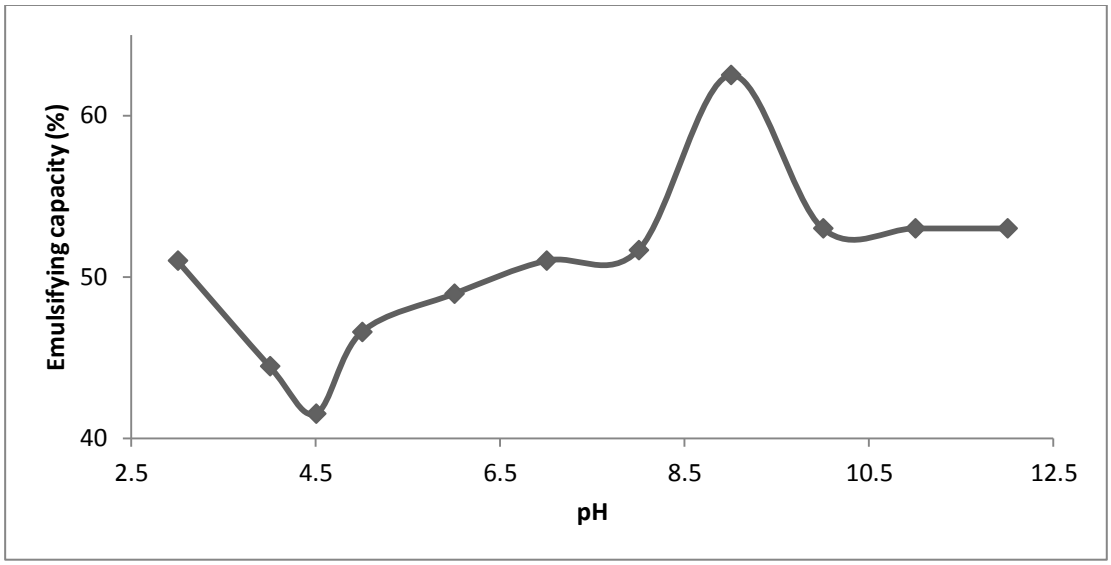

Figure 3.3: Effects of pH on the emulsifying capacity of *Balanites aegyptiaca* protein concentrate

Conclusion

The isolation and characterization of protein concentrate from defatted kernel seeds of *Balanites aegyptiaca* were investigated in this study. The results revealed that the kernel seeds are good and cheap source of proteins which can be used for human nutrition and feedstock preparation. The functionality of the protein concentrate is found to be strongly dependent on pH. The higher solubility, foaming and emulsion capacities of protein concentrate were observed at alkaline pHs more than the acidic ones.

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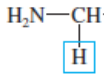
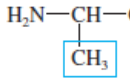
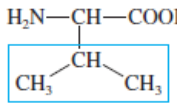
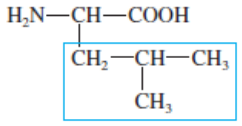
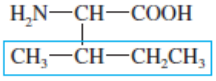
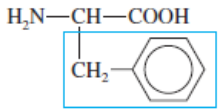
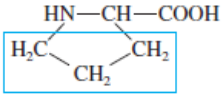
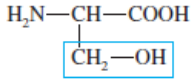
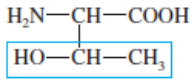
Appendix

Amino Acids Structures

The Standard Amino Acids

Name	Symbol	Abbreviation	Structure	Functional Group in Side Chain	Isoelectric Point
side chain is nonpolar, H or alkyl					
glycine	G	Gly		none	6.0
alanine	A	Ala		alkyl group	6.0
*valine	V	Val		alkyl group	6.0
*leucine	L	Leu		alkyl group	6.0
*isoleucine	I	Ile		alkyl group	6.0
*phenylalanine	F	Phe		aromatic group	5.5
proline	P	Pro		rigid cyclic structure	6.3
side chain contains an —OH					
serine	S	Ser		hydroxyl group	5.7
*threonine	T	Thr		hydroxyl group	5.6

The Standard Amino Acids (*continued*)

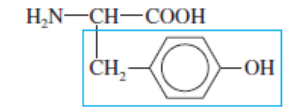
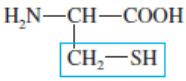
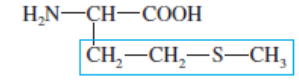
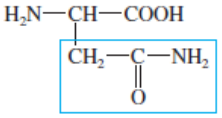
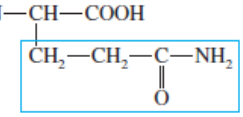
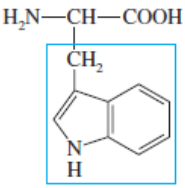
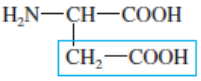
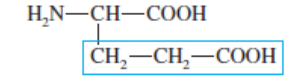
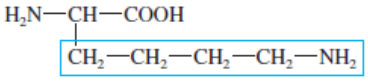
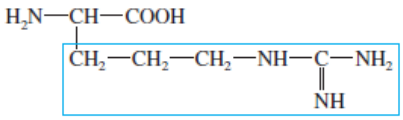
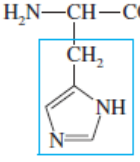
Name	Symbol	Abbreviation	Structure	Functional Group in Side Chain	Isoelectric Point
tyrosine	Y	Tyr		phenolic —OH group	5.7
side chain contains sulfur					
cysteine	C	Cys		thiol	5.0
*methionine	M	Met		sulfide	5.7
side chain contains nonbasic nitrogen					
asparagine	N	Asn		amide	5.4
glutamine	Q	Gln		amide	5.7
*tryptophan	W	Trp		indole	5.9
side chain is acidic					
aspartic acid	D	Asp		carboxylic acid	2.8
glutamic acid	E	Glu		carboxylic acid	3.2
side chain is basic					
*lysine	K	Lys		amino group	9.7
*arginine	R	Arg		guanidino group	10.8
*histidine	H	His		imidazole ring	7.6

Figure A.1: *Balanites aegyptiaca* tree (the photo was taken from Omdurman-Alshati.

Figure A.2: kernels of *Balanites aegyptiaca*

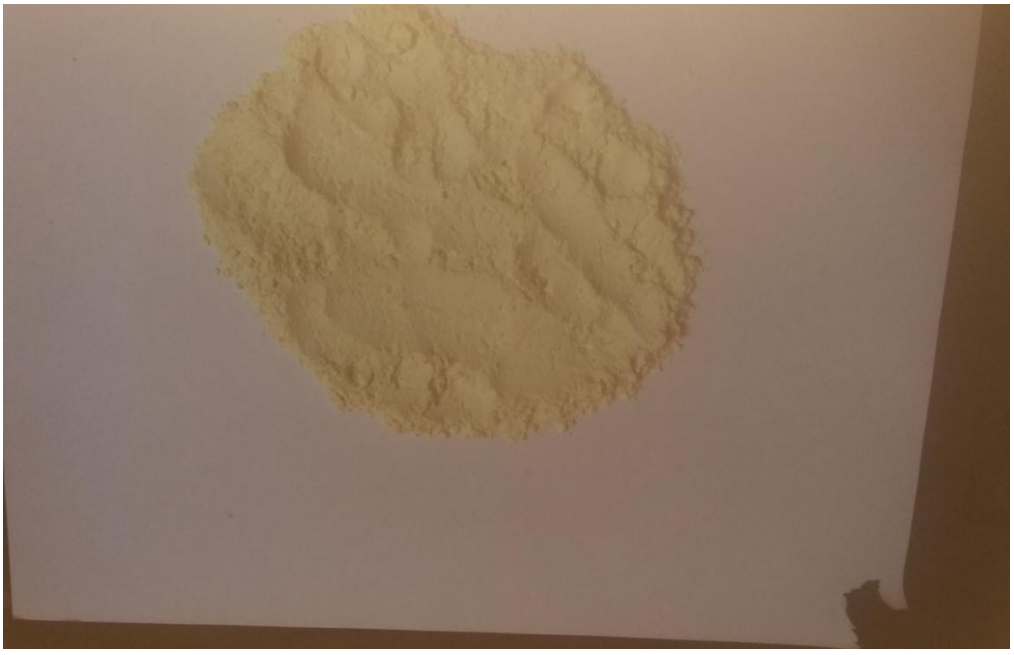


Figure A.3: defatted *Balanites aegyptiaca* flour

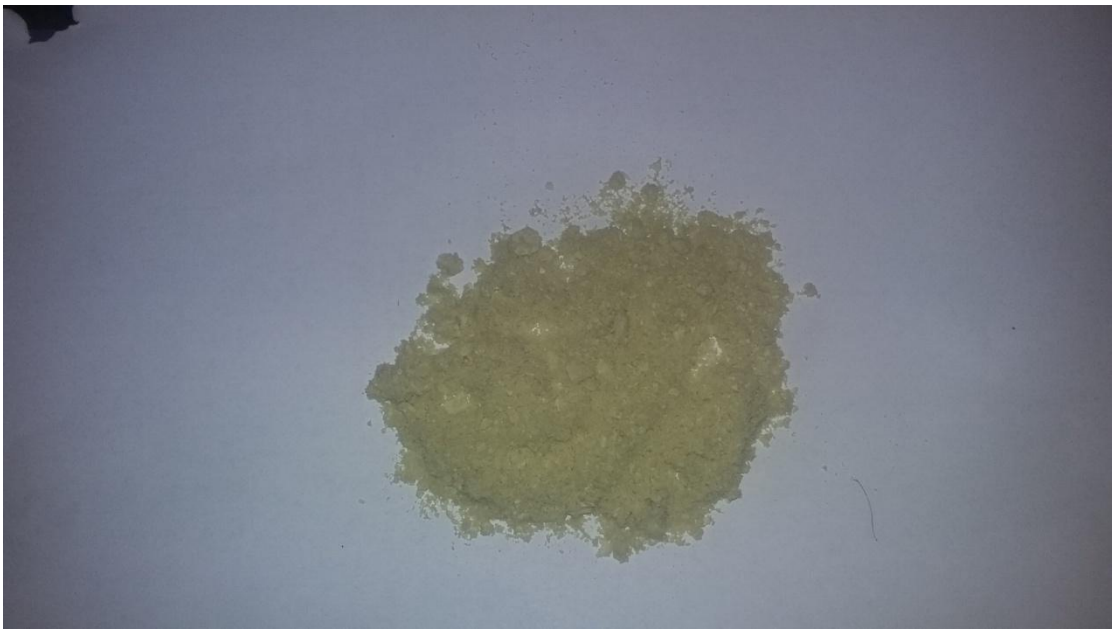


Figure A.4: *Balanites aegyptiaca* protein concentrate