Effect of Autoclaving on Solubility and Functional Properties of Chickpea (*Cicer arietinum* L) Flour

**BY**

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Dedication

This work is dedicated to my parents, brothers and sisters for their unlimited help and support and to the soul of my brother Elfatih.
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ABSTRACT

The effect of autoclaving on certain functional properties of defatted chickpea flour as a function of pH and sodium chloride solutions (M) were investigated. Autoclaving improved both water retention capacity and bulk density from 130 to 141% and from 0.55 to 0.57 g/ml, respectively, but decreased fat absorption capacity from 140 to 130%. Both samples showed higher dispersibility at neutral and alkaline pHs than at acidic pHs. The protein solubility, emulsion capacity and foaming capacity showed minimum values at pH 4 (isoelectric region) and maximum values at alkaline pHs than acidic pH. The protein solubility of both samples showed minimum solubility at 0.2 M sodium chloride and maximum solubility at 0.4 M sodium chloride. Higher emulsion capacity was observed at 0.6 M sodium chloride and then decreased. Maximum foaming capacity of the samples was obtained at 0.2 M sodium chloride and thereafter started to decrease. The emulsifying activity was higher at pH 2 while at pH 6 emulsion stability was higher. Both emulsifying activity and emulsion stability decreased at alkaline pHs with minimum values at pH 4. They were also decreased gradually when sodium chloride was added. The foam stability for both samples was improved by addition of sodium chloride and when dispersed in distilled water at different pH values they showed maximum values at acidic pHs (pH 2) compared to alkaline pHs. The least gelation concentration for both samples at all pH values and sodium chloride solutions was 6%.
Both samples showed good wettability. Autoclaving had no marked effect on the functional properties of untreated chickpea flour except the foaming capacity, it decreased at different pH values and sodium chloride solutions.
خلاصة الأطروحة

تمت دراسة أثر التسخين الرطب على بعض الخواص الوظيفية لدقى الحمص بعد إزالة الزيت منه بواسطة الهكسين تحت تأثير محاليل ذات أس هيدروجيني مختلف ومحاليل ملح طعام مختلفة التركيز المولاري. لوحظ تحسن في سعة احتباس الدقيق للماء وكتافة الكتلة نتيجة للتسخين الرطب. إذ ارتفعت كل منهما من 130% و 0.55 جم / مل إلى 130% و 0.57 جم / مل بالترتب. كما لوحظ انخفض في سعة احتباس الدقيق للزيت من 140% - 130% كانت الإثباتين أظهرت نسبة انتشار عالية في الوسطين المعتاد و القاعدي مقارنة بالوسط الحمضي. وقد كانت كل من نسبة ثبات البروتين و سعة الاستحلاب و السعة الرغوية في كلتا العينتين متدنية عند أس هيدروجيني 4 و عالية في الوسط القاعدي مقارنة بالوسط الحمضي. وجد أيضا أن نسبة ذوبان البروتين في كلتا العينتين قد انخفضت في وسط ملح طعام تركيزه المولاري 0.2 ثم بلغت أعلى قيمة لها عند تركيز 0.4 ثم انخفضت بعد ذلك. كما وجد أن سعة الاستحلاب في كلتا العينتين تزداد بزيادة تركيز ملح الطعام المولاري حتى 0.6 مولار ثم تخففت تدريجيا. بالنسبة للسعة الرغوية لكلا العينتين فقد بلغت أعلى قيمة لها في وسط ملح طعام تركيزها المولاري 0.2 ثم انخفضت تدريجيا. وقد كانت كل من نسبة نشاط المستحلب و ثبات المستحلب في كلتا العينتين مرتفعة عند أس هيدروجيني 2 في حالة نشاط المستحلب و عند أس هيدروجيني 6 في حالة ثبات المستحلب ثم انخفضت في الوسط القاعدي و بلغت أقل قيمها عند أس هيدروجيني 4. وجد أيضا أن كل من نسبة نشاط المستحلب و ثبات المستحلب في كلتا العينتين يكون مرتفعا في الماء المقترب و ينخفض في وجود ملح الطعام. لوحظ أيضا تحسن في ثبات الرغوة في كلتا العينتين مع ازدياد التركيز المولاري لملح الطعام، بينما كان ثبات الرغوة مرتفعا في الوسط الحمضي ( أس هيدروجيني 2) مقارنة بالوسط القاعدي. تكون الهلام في كلتا العينتين عند تركيز 6% في كل المحاليل ذات كم الأس هيدروجيني و ملح الطعام المختلفة. أما مقدرة كلتا العينتين على الترطيب فقد كانت جيدة. لم يؤثر التسخين الرطب آثرا واضحا على الخواص الوظيفية لدقى الحمص عدا في السعة الرغوية حيث تسبب في انخفضها في معظم محاليل الأس الهيدروجيني و ملح الطعام المختلفة.
Chapter One

Introduction

Chickpea (*Cicer arietinum* L) which belong to the family Leguminosae is one of the important crops in World. It has been grown in Turkey since about 7000 B.C. and has been produced in semi-arid zones of India and Middle Eastern countries (Chavan *et al*, 1986). In Sudan it is cultivated in a wide range in Shindi, Aljazeera and North Sudan during winter. There are two types of chickpea seeds, one of them is Desi (Indian origin) which has a thick, colored seed coat and the other is Kabuli which is found widely in Sudan has a white seed coat and has larger seed than Desi (Salunkhe *et al*, 1985). Chickpea seeds are mainly deficient in sulfur containing amino acids and tryptophan (Kaul and Gassi, 1971 & Peruanskii, 1974), but they are rich in lysine (Chatterjee, 1977). In Sudan chickpea are consumed in several manners, like boiling (Balela) and frying (Taamia) after soaking, but it is not utilized as flour such as some countries. Like other legumes, chickpea flour improve the nutritive value of the other foods by balancing some of the deficient essential and nonessential amino acids, since it comprise a cheap source of protein compared to animal protein. Chickpea flour is a good source of protein, carbohydrates, fats and some minerals (Milán-Carrillo *et al*, 2000), but the presence of antinutritional factors reduce its nutritive value. Utilizing of autoclaving process to reduce some antinutritional factors is success obviously on decreasing trypsin inhibitor (Bansal *et al*, 1988) and hemagglutinin activities. It is also increased the *in vitro* protein digestibility of flour. The objectives of this study were to investigate the effect autoclaving on the protein solubility and functional properties of chickpea flour as a function of pH and sodium chloride.
solutions (M) and to predict the possibility to apply both raw and autoclaved chickpea flours in the food industry.

Chapter Two
Literature Review

2.1 Introduction:

Pulses play an important role in the acceptability of monotonous diets in many parts of the world. Their role as protein supplements in the diets based on cereals and millets is well recognized (Walker and Kocher, 1982). Recently, attention has also been directed towards increasing the utilization of these protein sources for food use. The ultimate success of utilizing plant proteins in food formulations depends largely upon the functional attributes (McWatters, 1980). Among legumes, chickpea (Cicer arietinum L) ranks fifth in world production (Chavan et al, 1986). Chickpeas are a good source of energy, proteins, some vitamins (thiamin, niacin and ascorbic acid) and mineral (Ca, P, Fe, Mg and K) (Reyes-Móreno et al, 2002). Like other pulses, chickpeas contain several antinutritional factors, which may limit their consumption and the nutritive utilization of their protein. Proteases and amylase inhibitors, lectins, polyphenols, and certain sugars are the main antinutritional factors of chickpea seeds (Singh, 1988). Other antinutritional factors that found in chickpea seeds are the phytic acid and indigestible carbohydrates as raffinose and starchyose. Most of these compounds inhibit the digestive enzymes or react with essential amino acids limiting the application of the whole seed in many food products. These problems could be overcome if the chickpea proteins are isolated (Sánchez-Voique et al, 1999). These antinutritional factors can also be eliminated or reduced.
by cooking or with other simple technologies (Nestares, 1993; Vidal, 1994 & Urbano, 1995). Efforts to increase the availability of protein in man's diets have encouraged use of high-protein plant materials as ingredients in a variety of foods. Such wheat-based baked goods as bread, cakes, and cookies are popular foods and provide an excellent means of improving nutritional quality through incorporation of vegetable proteins (McWatters, 1978). Since chickpea is rich in lysine (Chatterjee, 1977), it occupies important place in supplementation of cereal-rich Indian diets in alleviating protein malnutrition (Cupta et al., 1979). Amino acid analysis showed that sulfur containing amino acids and threonine are the most limiting amino acids in chickpea (Kaul and Gassi, 1971 & Peruanskii, 1974). The storage proteins of chickpea seeds include 12% albumin (water soluble), 56% globulins (salt soluble), 18.1% glutenins (acid/alkali soluble), 2.8% prolamines (alcohol soluble) and residual proteins. The globulins consisting mainly leguminin and vicilin (Chavan et al., 1986). Bansal et al (1988) reported that autoclaving of chickpea seeds for 10 min decreased trypsin inhibitor activity (TIA) in different cultivars to different extents. Moreover, autoclaving for a long time up to 20 min caused a reduction in (TIA) among cultivars.

2.2. The proximate composition of chickpea flour:

Milán-Carrillo et al (2000) found that four varieties of chickpea flour had similar chemical composition; the protein content was 22.53 % carbohydrate was 69.45% and lipids and ash contents were 5.01% and 2.98%, respectively. Sánchez-Vioque et al (1999) reported that chickpea flour contained about 8.1 % moisture, 3.7% ash, 18.8 % fibre, 24.7% protein, 1.5 % lipids and 51.3 % carbohydrates on the
Although chickpea flour was extracted using hexane, lipids were not removed completely and parts of them (1.5%) was remained in the flour and were associated with the protein isolates (Sánchez-Vioque et al, 1998). Ulloa et al (1988) observed that chickpea flour had a protein content of 26.2%, carbohydrates of 59.5%, lipids of 6 %, crude fibre of 5.5 % and ash content of 2.8%. Compared to chickpea, lupin seed flour contained 6.86 % moisture, 44.43% protein, 19.12 % crude fat and 4.92% ash as the major constituents on dry weight basis (Sathe et al, 1982). Venktesh and Prakash (1993) found that autoclaving of sunflower flour at 1 and 2 kg/cm^3 increased moisture content from 8% at the control flour to 11% and 10 %, respectively but decreased the protein from 49% to 46% and 47%, residual fats from 3.4% to 1.8% and 3.2% and carbohydrates from 13.6% to 10.4 and 10%.

2.3. Functional properties:

Functionality of food proteins is defined as those physical and chemical properties, which affect the behavior of proteins in food systems during processing, storage, preparation and consumption (Fennema, 1996). A functional property is any nonnutritional property of a food or food additive that affects its utilization (Rhee, 1985). Many factors influence the functional properties of proteins, including moisture, temperature, pH, concentration, reaction time, enzymes, chemical additives, mechanical processing, ionic strength, and amount, sequence, rate, and time of the additives (Johnson, 1970). The range of desirable and attractive functional properties that should be looked for is almost as board as the range of foods themselves. For example, if one is considering producing a beverage, two desirable functional properties should be considered solubility and suitable viscosity. For
bread, the need is for a protein that is compatible with gluten. For various meat systems, desirable qualities would include water-binding, emulsifying properties and the ability to be formed into fibers. For other purposes, the properties of gel formation, whippability, adhesiveness and thickening might be considered beneficial (Matil, 1971).

2.3.1 Protein solubility:
For proteins and high protein foods used as functional ingredients, nitrogen solubility is one of the useful parameters for predicting water-lipid-protein interactions (Matil, 1971 & Kinsella, 1976). Thus, the amount of soluble protein can often be correlated with the amount of fat that can be emulsified or the amount of foam produced (Okaka & Potter, 1979). The solubility of the protein is the thermodynamic manifestation of the equilibrium between protein-protein and protein solvent interactions. The major interactions that influence the solubility characteristics of proteins are hydrophobic and ionic in nature. Hydrophobic interactions promote protein-protein interactions and result in decreased solubility. Whereas ionic interactions promote protein-water interactions and result in increased solubility (Fennema, 1996). Nitrogen solubility is influenced by several solution conditions, such as pH, ionic strength, temperature, and the presence of organic solvents (Fennema, 1996). Also nitrogen solubility profiles are affected by varietals differences (Conkertoz and Ory, 1976), degree of heat treatment, growing location and storage conditions (Cherry, 1975). Autoclaving had an adverse effect on the nitrogen solubility of the defatted peanut flour. The low nitrogen solubility of the autoclaved defatted peanut flour comparable to the defatted peanut flour is due to extreme protein denaturation and
coagulation induced by the heat treatment (Quinn and Beuchat, 1975). There are many factors affecting protein solubility that include:

1. Effect of pH:
Nitrogen solubility profiles over a range pH values are being used increasingly as a guide for protein functionality, since this property often correlates with important properties such as emulsification and foaming capacity (Sosulski et al, 1987). Nitrogen solubility for many legume seeds in aqueous solutions of hydrochloric acid and sodium hydroxide at different pH values was studied by many investigators (Evans and Kerr, 1943; Pusztai, 1965; Pant and Tulsiani, 1969; Hang et al, 1970; Coffman and Garcia, 1977; Fan and Sosulski, 1974 and Thompson, 1977). Their results indicated that the highest nitrogen dispersibility occurred at pH (1-2) in the acidic range and above pH 7 in the basic range with minimum solubility occurring in the pH range (4-5). Chickpea proteins had a point of minimum dispersion at pH 4 (10 % nitrogen solubility), and their solubility increased at lower and higher pH values. Maximum nitrogen solubility of 91% was obtained at pH 9.8 (Bencini, 1986). Solubility of chickpea proteins was minimum at pH 4.3 (Sánchez-Vioque et al, 1999). Raw winged bean flour had minimum nitrogen solubility of 23% around pH 4.5. On either side of this pH it increased, at pH 2.4 about 73% of nitrogen was soluble and at pH 10.5 it was about 95%. Beyond pH 10.5 there was no marked improvement in nitrogen solubility (Narayana and Narasinga Rao, 1982). The solubility behavior was similar to that of soy flour with a minimum nitrogen solubility of 10% around pH 4 and a maximum of 95% at pH 10 (Smith and Circle, 1972). Nitrogen
solubility decreased in autoclaved winged bean flour at different pH values, at pH 4.5, nitrogen solubility was 17% compared to 23% for raw flour, at pH 10.5, it was 60% compared to 95% for raw winged bean flour (Narayana and Narasinga Rao, 1982). Reduction in nitrogen solubility due to heat processing has been reported in the case of soy and peanut flours (McWatters and Holmes, 1979). Probably autoclaving denatured the proteins of winged bean flour and reduced their solubility in water at different pH values (Narayana and Narasinga Rao, 1982). The protein solubility profile of the lupin protein concentrate indicated that the minimum protein solubility was at pH 4 and increased with increasing pH (Sathe et al, 1982). These observations are in agreement with those of Ruiz and Hove (1976) who observed isoelectric pH of 4.5 for dehulled lupin seed proteins. Nitrogen solubility index of raw dehulled cowpea in deionized water was lowest at pH 4 and increased substantially as the pH decreased or increased (Okaka and Potter, 1979). Raw moth bean flour had minimum nitrogen solubility of 18% around pH 4.5. On either side of this pH, it increased. Protein solubility, however, is affected by heat treatment, which results in protein denaturation (Pawar and Ingle, 1988). The protein dispersibility of Iraqi whole mung bean flour was higher at alkaline pH than at either neutral or acid pH. A region of low nitrogen extractabilities was observed between the final pH of 3.4 and 5.7 when only 6.88 and 37.10% of nitrogen was extracted. However, the minimum extractability of nitrogenous constituents occurred at pH 4.5 (6.88%) and it remained low up to pH 5.2 when only 10.17% of nitrogen was extracted (Shehata and Thannoun, 1981). Fan and Sosulski (1974) reported minimum extractability of
nitrogenous constituents from mung bean flour to be found in the range of 4-5. Further more, Hang \textit{et al} (1970) concluded that several bean proteins, namely, mung bean, pea bean and red kidney bean, had a common point of minimum dispersion at pH 4. The low protein extractabilities at pH values of 4-6 were essentially attributed to the intermolecular attraction of protein molecules in the isoelectric zone (Molina \textit{et al}, 1974). However, part of this dispersibility could be attributed to the formation of protein-phytic acid complexes as reported for navy beans at similar pH values (Powrie, 1961). Tasneem and Subramanian (1986) reported that guar meal isolates exhibited U-shaped nitrogen solubility versus pH profiles. They showed very high solubilities of 80% - 90% at extreme acidic and alkaline regions (pH 2 or 9) and minimum of about 2% around the isoelectric point (pH 5). Raw cowpea flour gave a U-shaped curve in the pH range 2-10 with minimum solubility (26%) at pH 4 and this increased considerably at acidic (58%) and alkaline (96%) pH (Padmashree \textit{et al}, 1987). The protein solubility of the raw white and brown beans at various pH values in water showed a minimum solubility around pH 4. On both sides of this pH value the protein solubility increased up to a maximum values (90-100%), detected at the extreme values of pH range (Carbonaro \textit{et al}, 1993). The protein solubility of winged bean isolate in aqueous media over a range of pH values showed maximum solubility in the acid range of pH 2 with a minimum solubility at pH 4. Maximum solubility observed in the alkaline range was at pH 12 (Okezie and Bello, 1988).

2. Effect of sodium chloride :

According to Osborne (1924) sodium chloride is the most widely used
salt for extracting proteins from various seeds. Shehata and Thannoun (1981) reported that sodium chloride retarded nitrogen solubility of Iraqi whole mung bean at lower concentration. The increase in NaCl concentration from (0-0.05 M) decreased nitrogen extractability from 79 to 55.45%. Maximum nitrogen extractabilities were 87.78 % at 0.5 M NaCl compared to 79% in water. Hang et al (1970) reported that the amount of total nitrogen extracted from mung beans and red kidney beans by water was found to be 69.8, 68.5 and 56.3% respectively. Extraction of nitrogenous constituents of peas by different NaCl concentration evident that more nitrogen is extracted from pea beans by NaCl than from either mung beans or red kidney beans. Mung beans show a minimum dispersion at 0.05 M, whereas both red kidney beans and pea beans have a common point of minimum dispersion at 0.025M. The solubilities of the nitrogenous constituents of mung beans, pea beans and red kidney beans reach maximum at 0.75, 0.5 and 0.7 M, respectively. Sodium chloride thus disperses more nitrogenous constituents from the beans than does water (Hang et al, 1970).

2.3.2 Fat absorption capacity (FAC) :

The ability of protein to bind fat is an important functional property for food applications such as meat replacers and extenders, principally because it enhances flavour retention and reputedly improves mouth feel (Kinsella, 1976). The key role of fat in food flavouring had been demonstrated by kinsella (1975) and its capacity to improve flavour carry-over in simulated foods during processing is apparent (Wolf and Cowan, 1975). Oil absorption is mainly attributed to the physical entrapment of oil and is related to the number of nonpolar side chains
on proteins that bind hydrocarbon chains of fats (Kinsella, 1979 & Lin et al, 1974). Fat absorption is usually measured by adding excess liquid fat (oil) to a protein powder, thoroughly mixed and centrifuged. Thereafter, the amount of bound or absorbed oil will be determined (Lin et al, 1974 & Wang & Kinsella, 1976). The amount of oil, protein sample, kind of oil, holding and centrifuging conditions and units of expression have varied slightly from one investigator to another (Hutton and Campbell, 1981). The mechanism of fat absorption is not clear. However Wang and Kinsella (1976) have attributed fat absorption mostly to physical entrapment of oil. Factors affecting protein-lipid interaction include protein conformation, protein-protein interactions, and the spatial arrangement of the lipid phase resulting from the lipid-lipid interaction (Hutton and Campbell, 1981). Noncovalent bonds, such as hydrophobic, electrostatic, and hydrogen, are the forces involved in the protein-lipid interactions. Hydrogen bonding is of secondary importance in lipid protein complexes, although it is indirectly important in hydrophobic bonding (Karel, 1973), since in aqueous media the water-water by hydrogen bonding is much stronger than interaction between water and nonpolar groups, thus giving rise to hydrophophilic bonding between water and nonpolar groups, electrostatic attraction can occur between the negatively charged phosphate groups of phospholipids and positively charged protein groups (such as lysyl or guanidyl) or between a positively charged group in the phospholipids (e.g. choline) and a negatively charged amino acid side chain (e.g. aspartyl). A related mode of binding is the formation of salt bridges between a negatively charged amino acid side chain and a negatively charged phosphate.
group of phospholipids via divalent bond of calcium or other metal ions (Karel, 1973; Pomeranz, 1973 & Ryan, 1977). Hydrophobic bonding is likely to play a major role in stabilizing the interactions of both polar and nonpolar lipids with proteins (Ryan, 1977). Moreover, nonpolar dispersion or van der waals force become important when interacting groups are near (Karel, 1973) and may play a role in attraction between nonpolar groups in systems in which hydrophobic interaction is unlikely because of limited water (Pomeranz, 1973). As with the protein-protein interactions, it is not possible to attribute protein-lipid interactions to any single specific kind of molecular force (Ryan, 1977). However, according to Wall (1979) lipids bind to proteins mainly through association with hydrophobic groups. Bencini (1986) reported that raw chickpea flour showed a lower oil binding capacity than soybean flour. Narayana and Narasinga Rao (1982) reported that autoclaving increased the fat absorption capacity of winged bean flour from 1.4 to 2.2 oil/g flour. The increment of fat absorption capacity may be due to both dissociation of the heated and denatured proteins, which is expected to unmask the nonpolar residues from the interior of the protein molecule. Rahma and Mostafa (1988) found that fat absorption capacity of autoclaved peanut showed an increasing trend similar to that observed for water absorption capacity. The ability of flour to bind and retain oil increased with time of autoclaving at 121°C (15 b/in²).

2.3.3 Water retention (Holding) capacity (WRC):
Water retention is a basic functional property of food components such as proteins and carbohydrates (Zayas & Lin, 1989). Water retention is defined as the ability of the food material to hold water against
gravity (Hansen, 1978 & Chou & Morr, 1979). In food applications, water holding capacity of a protein preparation is more important than water binding capacity. Water holding capacity refers to the ability of the protein to imbibe water and retain it against gravitational force within a protein matrix such as protein gels or beef and fish muscle. The contribution of the physically entrapped water to water holding capacity is much larger than those of the bound and hydrodynamic water. However studies have shown that water holing capacity of proteins is positively correlated with water binding capacity (Fennema, 1996). Water holding capacity by protein is a function of several parameters including size, shape, conformational characteristics, stearic factors, hydrophilic-hydrophobic balance of amino acids in the protein molecules, lipids and carbohydrates associated with the proteins, thermodynamic properties of the system (energy of bonding, interfacial tension, etc), physico-chemical environment (pH, ionic strength, vapor pressure, temperature, presence or absence of surfactant, etc) and solubility of protein molecules (Chou and Morr, 1979). However, polar amino groups of protein molecules are the primary sites of protein-water interactions that bind different amounts of water at cationic, anionic, and nonionic sites (Kuntz, 1971). Chickpea showed the lowest water holding capacity compared to navy bean (Han and Khan, 1990)\textsuperscript{a}, due to its lipid content which is higher than navy bean (Han and Khan, 1990)\textsuperscript{b}. The mechanism of water and oil retention may be more physical than chemical as evident by the amounts of water and oil retained by the autoclaved defatted peanut flour compared to the non heated defatted peanut flour (Quinn and Beuchat, 1975). Wu and Inglett (1974) suggested that as nitrogen solubility of soy flour decreases, water
absorption first increases and then decreases with further decreasing nitrogen solubility. A similar relationship between nitrogen and water retention may be exemplified by the low nitrogen solubility of the autoclaved defatted peanut flour and its high water retention capacity (Quinn and Beuchat, 1975). Carbohydrate fraction influenced water retention capacity of food materials (Kilara, 1972). Addition of edible groundnut flour to sorghum flour increased WRC of sorghum flour from 62 to 72 g/100g. This may be due to the increase in the protein content of the blend (Ahmed and Ramanatham, 1988). The degree of water retention is considered to be useful as an indication of performance for several food formulations, especially those involving dough handling (Circle and Smith, 1972). Water retention has been used as a criteria for selection of protein additives for food systems especially meat products (Lin and Zayas, 1987). The ability of protein to entrap water is associated with juiciness and tenderness of comminuted meat products and desirable textural properties of bakery and other gel-type products (Fennema, 1996).

2.3.4 Bulk density (BD):

Bulk density depends on interrelated factors including intensity of attractive interparticle forces, particle size, number of contact points (Peleg and Bagley, 1983). It also depends on type of solvent used to extract the protein products (Wang and Kinsella, 1976) and on method of drying (Bryant, 1988). The bulk density of raw chickpea flour was found to be 0.46 g/cm³ (Milan-Carrillo et al, 2000), while the bulk density of raw cowpea flour was 0.88 g/cm³. The bulk densities of soy protein isolate (0.48 g/cm³) and winged bean flour (0.53 g/cm³) were 12 to 13 times higher than the winged bean protein isolate (0.04 g/cm³). The bulk density of cottonseed defatted flour was 0.24 g/ml
(Rahma and Narasinga, 1983), whereas the bulk density of winged bean defatted flour and soybean defatted flour were 0.45 and 0.46 g/ml, respectively (Densh, 1982). Venktesh and Prakash (1993) reported that autoclaving of defatted sunflower flour at 2 kg/cm³ decreased the bulk density of the control flour value from 0.26 g/cm³ to 0.25 g/cm³, possibly due to puffing of each particle at high temperatures (Kinsella, 1976). But autoclaving the flour at 1 kg/cm³ increased the bulk density of the control flour from 0.26 g/cm³ to 0.27 g/cm³. This increment may be due to the removal of the residual moisture in the flour, resulting in dense packing of the flour particles for the same unit volume (Venktesh and Prakash, 1993). Higher bulk density is desirable since it helps to reduce the paste thickness, which is an important factor in convalescent and child feeding (Padmashree et al., 1987).

2.3.5. Gelation:
Gelation may be defined as protein aggregation in which polymer-polymer and polymer solvent interactions as well as attractive and repulsive forces are so balanced that a tertiary network or matrix is formed. Such a matrix is capable of immobilizing or trapping large amounts of water (Schmidt, 1981). Factors that affect gelation properties include protein concentration, protein components in a complex food system, nonprotein components, pH, ionic and reducing agents and heat treatment conditions (Schmidt, 1981). Gelation involves the formation of a continuous network that exhibits order. Higher protein concentration may enhance the rate at which such a network is formed (Deshpande et al., 1982). The least gelation concentrations for the control dry moth bean was 9% (Pawar & Ingle,
while the least gelation concentration for lupin seed flour and the protein concentrate was 14% and 8% (w/v), respectively (Sathe et al, 1982). The raw chickpea flour required a minimum concentration of 16% (Bencini, 1986). Differences in the gelling ability of different species of pulses could be due to differences in protein and to the nature of proteins. Higher proportion of globular proteins could contribute to higher least gelation concentration value (Sathe et al, 1982). According to Narayana and Rajagopal (1974) globulins account for 60-80% of the total protein in chickpea seeds. This could be one of the reasons why chickpeas have higher least gelation concentration than other pulses (Bencini, 1986). Also Sosulski (1976) observed that great northern bean gel was found to be firmer compared to that of lupin flour. Variation in the gelling properties of different legume flours may be attributed to the relative ratio of different constituents (proteins, carbohydrates and lipids), such components may have a significant role in functional properties (Sathe et al, 1982). On the other hand, sorghum flour concentrations for gelation were significantly lower than those of partially defatted peanut flour. Sorghum flour contains starch, which induced gelation due to starch and/or starch-protein interactions. Partially defatted sorghum-peanut composite flour required a higher flour concentration than sorghum flour for gelation because the starch content decreased due to fortification with peanut flour (Singh & Singh, 1991). Wiseman & Price (1987) suggested that less protein was required for gelation at neutral and alkaline pH than at acidic pH.

2.3.6. Foaming properties:
Foaming action is a property desirable for whipped toppings, whipped desserts and frozen desserts (Circle and Smith, 1972a, 1972b). The foaming property of a protein refers to its ability to form a thin tenacious film at gas-liquid interfaces so that large quantities of gas bubbles can be incorporated and stabilized (Fennema, 1996). In food systems, foams are often very complex, including several phases such as a mixture of gases, liquids and multicomponent solutions of water, polymers and surfactants (Richert, 1979). The factors that affecting foaming formation and stability are environmental factors (pH, sugars, lipids and protein concentration) and molecular properties (Fennema, 1996). McWatters and Cherry (1977) observed that protein solubility was more closely related to the type of foam produced than the increase in volume. Soybean and peanut flour suspensions contained higher levels of soluble protein than those of field pea and pecan and produced foams of much thicker consistency and small air cells.

1. Foaming capacity (FC):

The foaming capacity of a protein refers to the amount of interfacial area that can be created by the protein (Fennema, 1996). There are many factors affecting foaming capacity that include:

1.1. Effect of pH:

The foam capacity versus pH profile of raw winged bean flour closely resembled in shape its nitrogen solubility versus pH profile suggesting that foaming property was also dependent on the solubilized protein. Minimum foam capacity of 76% was observed at pH 4.6 and maximum of 150% at pH 9.8 (Narayana and Narasinga Rao, 1982).
Autoclaving considerably lowered the foam capacity of winged bean flour at pH value up to 10. At pH 4.6, a volume increase of 60% was observed for autoclaved flour compared to 76% for raw flour and at pH 2.5 the foam capacity were 120% and 150% for autoclaved and raw, respectively. However, at a pH above 10 autoclaved flour had higher foam capacity than the raw flour; the reason for this is not obvious (Narayana and Narasinga Rao, 1982). Foaming properties of lupin seed protein concentrate were pH dependent and it was highest at pH 2 and least at the iso-electric region (Sathe et al, 1982). The foam capacity versus pH profile of 2% aqueous dispersion of raw moth bean flour closely resembled in shape its nitrogen solubility versus pH profile. Minimum foam capacity of moth bean flour was observed to be 31% at pH 4.5 and maximum foam capacity at both acidic and alkaline pH values (Pawar and Ingle, 1988). The foaming capacities of whole and dehulled bean flours were pH-dependent, at pH 4 which is the apparent isoelectric pH of bean proteins at which minimal foaming capacity was observed and the greatest foaming capacity was at pH 2, 6 or 10 depending on the cultivar (Deshpande et al, 1982).

1.2. Effect of sodium chloride:

Narayana and Narasinga Rao (1982) reported that the foam capacity of raw winged bean flour at a lower concentration of NaCl (0.2M) increased to a maximum value and there was no further increase beyond this concentration. The volume increase at 0.2M NaCl concentration was 150% and 174% for raw winged bean and raw soybean flour, respectively. Addition of NaCl, up to 0.2M concentration, increased the foam capacity of autoclaved winged bean flour; greater concentration of NaCl decreased it considerably
For winged bean and soybean flour the foam capacity at 1M NaCl was higher than in water, but after autoclaving winged bean flour it was reduced. Low concentrations of salt enhance protein solubility whereas high concentrations decrease it due to salting out. Since foam capacity appears to be due to solubilized protein, the differing effects of salt concentration may be explained on this basis (Narayana and Narasinga Rao, 1982). Sathe et al (1982) reported that addition of salt (NaCl) improved foaming capacity of lupin protein concentrate. Improvement was maximum at a salt concentration of 0.6% (w/v) in the slurry. The improvement may be due to increased in protein solubility (Sosulski, 1977). Also addition of salt may improve protein solubility at the interface of the colloidal suspensions during foam formation, thus improving foam capacity (Kinsella, 1976). Bera and Mukherjee (1989) reported that salts, depending upon their concentration probably affect foaming capacity of rice bran protein concentrates by enhancing solubility initially, whereas salting-out may occur when high concentrations were used. Ahmed and Ramanatham (1988) suggested that improvement in poor foaming capacity of sorghum meal by addition of salt was negligible compared to a noticeable one recorded for the composite flour with 0.4 M NaCl. Increasing salt concentration (0.8 - 1 M) resulted in less foam as observed for the composite flour (Ahmed & Ramanatham, 1988).

2. Foam stability (FS):

Foam stability refers to the ability of protein to stabilize against gravitational and mechanical stresses (Fennema, 1996). Foam stability is important since the usefulness of whipping agents depends on their ability to maintain the whip as long as possible (Lin et al, 1974). Foam
stability was determined by measuring the decrease in volume of foam as a function of time (Narayana and Narasinga Rao, 1982). Foam stability also decreased as the time of autoclaving increased. This decrease is mainly due to denaturation of the proteins, which become less soluble (Rahman and Mostafa, 1988). Factors influencing foam stability include:

2.1 Effect of pH:

The foam stability of winged bean flour was measured at several pH values. At pH values 2.5, 6.6 and 8.7 foam stability decreased markedly within 10 min and then decreased gradually. However at pH 4.5 where the initial foam capacity value was low, it decreased steadily (Narayana and Narasinga Rao, 1982). In the case of autoclaved winged bean flour, foam volume decreased markedly within 10 min at all pH values studied. At pH 2.5 the foam volume at 120 min was 45% with raw winged bean flour and 50% with autoclaved flour. At pH 4.6 the foam stability of autoclaved flour was collapsed immediately after its formation (Narayana and Narasinga Rao, 1982). It has been suggested that foam stability is related to denaturation since native protein gives higher foam stability than the denatured protein (Yatsumatsut, 1972). At pH 4.5 which is the point of minimum solubility, winged bean proteins carry no net charge and should be more stable than at other pH values. Foam stability may be expected to be maximum at pH 4.5. This explanation is also supported by the observation that the autoclaved winged bean meal not exhibited increased foam stability at pH 4.6. Thus the low foam capacity at pH 4.5 appears to be due to decreased protein solubility whereas the maximum foam stability may be due to the native conformation of the protein (Narayana and
Narasinga Rao, 1982). Maximum foam stability of lupin protein concentrate was observed at pH 4 and it progressively decreased at alkaline pH. The high stability of foams in the acid range observed, may have been due to the formation of stable molecular layers in the air-water interface, which impart texture, stability and elasticity to the foams (Sathe et al, 1982)a. Such molecular stabilizing effect in acidic pH is also reported by Richert (1979). Wang and Kinsella (1976)b found that foam stability varied with pH, being minimum in the isoelectric range (pH 3-4); being maximum at narrow pH region. At pH region above the isoelectric range the protein is slightly negatively charged and showing a rapid decrease at alkaline pH values (pH 6). The latter effect may be explained by charge repulsion between proteins with resultant lack of adhesion, and also by some solublization of the alfalfa leaf proteins in this range (Lu and Kinsella, 1972 & Betchart and Kinsella, 1973). Thereby reducing the quantity of aggregated protein necessary to stabilize the foams (Wang and Kinsella, 1975).

2.2 Effect of sodium chloride :
Kinsella (1976) reported that addition of NaCl to soybean protein suspensions gave high capacity, low stability foams, this mainly because salt improved the protein solubility at the interface of the colloidal suspensions during foam formation and it was retarded the partial denaturation of the surface polypeptides of proteins that are necessary for protein-protein interaction and stability. Bera and Mukherjee (1989) reported that foaming stability of rice bran protein concentrates was slightly improved when the salt concentration was increased from 0.1M to 1M NaCl.
2.3.7 Emulsification properties:

An emulsion is a dispersed system consisting of two immiscible or sparingly soluble liquids, termed the oil phase and the water phase, separated by a third component termed an emulsifier which may be solid, the interphase between the two phases is very large and its integrity is critical to the stability of the whole system (Friberg and Venable, 1983 & Tadros; Vincent, 1983 & Becher, 1983). Emulsification properties play a significant role in many food systems including meat products, batters and dough and salad dressings (Betschart et al., 1979). Factors that affect emulsifying properties are adsorption kinetics, interfacial load, decrease of interfacial tension, rheology of the interfacial film, and surface hydrophobicity of the interfacial film (Das and Kinsella, 1990). Many physical and chemical factors are involved in formation, rheology of the protein emulsions. Efficiency of emulsification varies with the type of protein, its concentration, pH, ionic strength, viscosity of the system, temperature and the method of preparation of emulsion (Saffle, 1968). The emulsion properties of flours cannot be solely attributed to the proteins but other food components such as carbohydrates and lipids may also contribute appreciably, possibly through protein-carbohydrate and protein-lipid interactions. Naki (1983) reported that the emulsifying properties not only depend on the protein solubility but also on hydrophilic-lipophilic balance of the particular protein.

1. Emulsion capacity (EC):

Emulsion capacity is the volume (ml) of oil that can be emulsified per
gram of protein before phase inversion occurs (Fennema, 1996). Many factors influence the emulsification capacity including equipment design, rate of oil addition, temperature, pH, protein type, solubility and concentration, kind of oil used, salt (type and concentration), sugars and water content (Saffle, 1968 & Kinsella, 1976). Autoclaving of peanut had a more marked effect in decreasing the EC from 56.5ml oil to 18 ml oil per g than dry heating (Rahma and Mostafa, 1988). McWatters and Cherry (1977) reported that poor emulsion capacity of field pea flour was related to the presence of carbohydrate when compared to soybean and peanut flours. There are many factors affecting emulsion capacity including:

1.1 Effect Of pH:

The emulsion capacity of the protein was affected markedly by the pH. The emulsion capacity of groundnut protein versus pH profile resembled a typical protein solubility curve suggesting that the emulsifying property was depend on protein solubility. The groundnut protein was more efficient in emulsifying the oil at acidic pH (3) than at alkaline pH (8). However, at pH 10 it showed highest emulsion capacity (Ramanatham et al, 1978). The effect of pH on the emulsification capacity of raw winged bean flour was determined over the pH range 2-11. At low pH (2-4) emulsification capacity of 84g oil/g protein was observed. At pH of minimum solubility, emulsification capacity was only 59g oil/g protein. As the pH increased towards the alkaline side, emulsification capacity increased to a maximum of 112g oil/g protein (pH 9.7). The emulsification capacity versus pH profile closely resembled the nitrogen solubility versus pH profile of raw winged bean flour. Autoclaving markedly decreased the emulsification
capacity of winged bean flour at all pH values studied. At pH 9.4 emulsification capacity of heat processed flour was 65 g oil/g protein compared to 84 g oil/ g protein for raw flour (Narayana and Narasinga Rao, 1982). McWatters and Holmes (1979)\(^a\) observed that soy flour and peanut flour are sensitive to moist heating and the heating time was the primary determinant in the reduction of nitrogen solubility and emulsification capacity. Emulsion capacity of lupin seed protein concentrate was pH dependent, at acidic pH the emulsion capacity was improved more than did the alkaline pH. At pH 2, emulsion capacity increased 3.55 times compared to the emulsion capacity of 2% (w/v) suspension in distilled water without pH adjustment (Sathe et al, 1982)\(^a\). Dependence of emulsion capacity on pH was expected as it is known that emulsion capacity of soluble proteins depends upon the hydrophilic-lipophilic balance (Sosulski, 1977) which is affected by pH. Emulsion capacity of 2% suspensions was highest at pH 2 but was reduced as the pH raised from 2 to 4. Between pH 4 and 7, no emulsions were formed; above pH 7, emulsion capacity increased as pH level increased, though not to be the extent exhibited at pH 2. McWatters and Holmes (1979)\(^b\) found that the emulsion capacity of soybean flour was poor for water suspensions at pH 4 (0.22 ml oil/mg protein) but was substantially improved when the pH was shifted to levels below or above pH 4.

1.2. Effect of sodium chloride:
Incorporation of NaCl at concentration up to 0.4 M had an incremental effect on the emulsification capacity of raw winged bean flour. Beyond this salt concentration emulsification capacity decreased
steadily due to salting in effect of NaCl (Narayana and Narasinga Rao, 1982). However, in the case of the autoclaved winged bean flour, addition of NaCl did not cause an observable change in the emulsification capacity. This may be due to possible denaturation of the protein by heating, moreover, solubility characteristics in water and salt solutions may change which reflect in emulsification capacity (Narayana and Narasinga Rao, 1982). Ahmed and Ramanatham (1988) reported that the emulsion capacity of sorghum meal showed an improvement with increasing NaCl morality up to 0.8 M followed by a decrease in emulsion capacity when treated with 1 M NaCl.

2. Emulsifying activity (EA):

Emulsifying activity is one of the most important functional properties of food proteins. Separate hydrophobic and hydrophilic regions are distributed in protein molecules (amphiphilic structure). This structure is required for the formation of emulsions (Kinsella, 1979). There are many factors affecting emulsifying activity that include:

2.1. Effect of pH:

Soybean isolate had lowest emulsifying activity at pH 4.5. This may be due to the increased protein-protein interaction, resulting in a low surface hydrophobicity and decreased net charge and the solubility of proteins (Yim & Lee, 2000). Soy protein assumes more rigid and staple structure which is more resistant to unfolding and film formation during the interface at the isoelectric region (Qi et al, 1997). Yim and Lee (2000) found that the emulsifying activity of soybean protein isolate was lower at pH between 4 & 5 and higher above pH 5. This could explain with respect to their higher solubility at this pH.
range. Sesame protein isolate had a minimum emulsifying activity at pH 5 with emulsifying activities increasing on either side of pH 5 (Khalid et al, 2003). Monteiro and Prakash (1994) observed that the turbidity of the total protein of peanut decreased toward pH 5 and then increased.

2.2 Effect of sodium chloride:
Monteiro and Prakash (1994) reported that the turbidity of the total protein of peanut decreased considerably at 0.3M NaCl. However, above 0.3M NaCl, turbidity increased with increase in NaCl concentration.

3. Emulsion stability:
The emulsion stability measures the tendency for the emulsion to remain unchanged. The ability of protein to stabilize an oil in water emulsion is one of the most important functional properties with respect to application in food products such as finely comminuted meats, soups, cakes and salad dressings (Jackman et al, 1989). Once formed an emulsion may break down via creaming (movement of dispersed droplets under the influence of gravity), flocculation (clustering of droplets) and/or coalescence (merging of smaller droplets into larger ones). These processes are not independent of one other (Halling, 1981 & Stainsby, 1986). Protein stabilized emulsions are often staple for days. Thus, a detectable amount of creaming or phase separation is usually not observed in a reasonable amount of time when samples are stored at atmospheric conditions. Therefore, drastic conditions, such as storage at elevated temperature or separation under centrifugal force is often used to evaluate emulsion stability.
(Fennema, 1996). Emulsion stability can be greatly increased when highly cohesive films are formed by the absorption of rigid globular protein molecules that are more resistant to mechanical deformation, e.g., lysozyme (Graham and Philips, 1980). The stability effect of proteins in emulsions results from the protective barrier they form around fat droplets, which further prevents their coalescence (Kinsella, 1979). Factors affecting emulsion stability include:

3.1. Effect of pH:

Yim and Lee (2000) reported that the effect of pH on the emulsifying stability was determined over the pH range of 3 to 8 on soybean protein isolate. The result indicated that the stability was minimal at pH 4.5, change in pH from 4.5 to 3 or 9 increased the stability. At pH 4.5, the net charges of the proteins are diminished and repulsive forces among the molecules are eliminated (Yim and Lee, 2000). The fat globules covered with soy protein are involved in strong interactions decreased the emulsion stability. However, emulsion stability increased as the pH increased. This may be due to increased protein solubility at higher pH values (Yim and Lee, 2000).

3.2 Effect of sodium chloride:

When emulsions prepared from dispersions in NaCl were compared with water, both emulsifying activity and emulsifying stability were reduced (Jane et al, 1981).

2.3.8 Wettability:

Wettability properties depend on the affinity of the protein to water and other polar solvent (Abdel kareem and Brennan, 1975). Ease of
Wettability is important in food formulations. Wettability of proteins is affected by surface polarity, topography, texture, area and by the size and microstructure of the protein particles but not necessarily by the amount of native structure (Hagerdal and Lofqvist, 1978). Although winged bean flour, winged bean protein isolate and soybean protein isolate had very poor wettability compared to reported values for single cell proteins and soybean concentrates (Okezie and Kosikowski, 1981). Winged bean flour required the least time to become completely wet. The time required to reach complete wetness was 1.5 times longer for soybean isolate than winged bean isolate. The results, therefore suggest that due to its better water absorption and retention than soy bean isolate, winged bean isolate would perform better in textured and/or comminuted meats and baked products than soy bean isolate (Okezie and Bello, 1988).

2.3.9 Dispersibility:
Ease of dispersibility is important in food formulations. The dispersibility of a mixture in water indicates its reconstitutability. The higher dispersibility the better (Kulkarni et al, 1991). Temperature, ionic composition, pH and degree of agitation of the solvent are major factors affecting dispersibility (Kinsella, 1976). Vegetable proteins solublized in water at a neutral pH or by alkaline pH give reasonably good dispersion of proteins. Isolated soy protein and casein at neutral pH (6.5-7) have good water dispersion characteristics (Johnson, 1970). It was reported that higher dispersibility enhances the emulsifying and foaming properties of proteins, which was observed during making of bread, macaroni and cookies (Kinsella, 1979). The dispersibility of sesame protein isolate was significantly higher at neutral and alkaline
pH than acidic pH (Khalid et al., 2003). The dispersibility of raw chickpea flour was 25.3% (Milan-Carrillo et al., 2000).

2.4. Application of chickpea flour:

Shehata and Fryer (1970) concluded that supplementing wheat flour with 10 to 20% chickpea flour produced a significantly higher protein efficiency ratio. These levels of supplementation had little effect on either the physical properties of the dough and bread or the palatability of the bread. In spite of the significant decrease in score for crumb colour with increasing percentages of chickpea flour, there was no significant difference in the overall acceptability scores. Shaner and Baldwin (1979) reported that substitution of meat with 30% textured soy protein or chickpea meal decreased intensity of beef aroma and flavour of beef loaves, with chickpea substitution giving the most intense bean flavour. Control loaves were the most juicy, and chickpea-substituted loaves were the least. Legume substitution resulted in significant decreases in total cooking losses and losses due to drip, with chickpea meal having a greater effect. There were no differences in mean sensory scores for loaves baked in a loaf pan or on a rack, but cooking losses trended to be greater for loaves backed on a rack. Protein content was reduced significantly by substitution with chickpea meal (Shaner and Baldwin, 1979). Juneja et al. (1980) reported that supplementation of chickpea flour with wheat & triticale flour mixture helps to improve the biological value of the mixture. Acceptability of the chapatti (unleavened bread) was slightly improved by supplementing the bengal gram flour at 20% level to the blend of the wheat & triticale flour. Finney et al. (1982) observed that flour
from whole chickpea bean produced outstanding breads when replacing 10% of baker's flour and acceptable breads when replacing 15-20% of baker's flour. Verma et al (1984) found that fresh skinless sausages were prepared in which some of the meat (mutton, pork or beef) was replaced by a protein of chickpea flour. The acceptability of mutton sausages containing chickpea flour was not affected at levels of substitution up to 40%, whereas pork and beef sausages were significantly less acceptable when substituted with more than 30%. In all the sausages incorporation of chickpea flour led to increase cooking losses and softer textures. Incorporation of chickpea flour caused discoloration of the raw sausages, which became more prominent during storage at 0º C (Verma et al, 1984).
3. Materials and Methods:

3.1 Materials:
Chickpea seeds (Cicer arimitum. (l).Shindi) which were grown at Dongla farm during the 2002/2003 seasons, obtained from The Arab Seeds Co-operation, Sudan. Refined Ground nut oil was brought from Bittar Co.ltd., Khartoum. Sudan. Unless otherwise stated all chemicals used in this study were of reagent grade.

3.2 Methods:

3.2.1 Preparation of raw defatted chickpea flour:
Chickpea seeds were first cleaned, freed from foreign matter and milled in a laboratory miller to pass through a 0.8 mm screen. To extract oil from chickpea flour, cold extraction method was used. Chickpea flour was placed in a conical flask and mixed with hexane (10:1). The mixture was stirred using a mechanical shaker for 16 hours and then filtered. The filtrate was washed again with hexane to remove traces of oil. The mixture was filtered again and the oil free flour was dried in an open air at room temperature. The dried flour was then ground to pass a 70 mesh screen and stored at 0 °C for further analysis.

3.2.2 Preparation of autoclaved defatted chickpea flour:
Appropriate amount of defatted chickpea flour was placed in one liter conical flask and autoclaved for 15 min at 121°C and stored at 0 ºC until used.

3.2.3 Proximate Analysis:
The Moisture, Total oil, Protein, Fibre and Ash contents were analyzed according to (AOAC ) 1975.

1. Protein Content:
Nitrogen content determinations were made on the defatted sample by micro -kjeldahl technique following the method of AOAC (1975). 0.2 gm of sample was weight accurately into a micor kjeldahl flask, 0.4 gm of catalyst mixture (90% potassium sulphate and 10 % cupric sulphate ) and 3.5 ml of concentrated sulphuric acid were added, the flask was placed in the digestion equipment for 3 hours. The sample placed in the distillation apparatus, 20ml of 40% NaOH were added. The ammonia evolved was received in 10 ml of 20 % Boric acid solution. The trapped ammonia is titrated against 0.02 M HCL using universal indicator (methyl red + bromocresol green).

\[
N\% = \frac{\text{volume of HCL} \times 0.02N \times 14 \times 100}{\text{Sample weight} \times 1000}
\]

Protein % = N % × 6.25

3.2.4 Determination of nitrogen solubility at various pH values:
Nitrogen solubility of both raw and autoclaved flour was determined at different pH values (2, 4, 6, 8, 10, 12) by the procedure of Hagenmaier (1972), modified by Quinn and Beuchat (1975) with a slight modification. 0.2 grams material were suspended in 10 ml distilled
water and mechanically shaken for 15 minutes before the desired pH was maintained by addition of 1N HCL or 1N NaOH. The suspension was shaken for another 45 minutes at room temperature, centrifuged at 3000 rpm for 20 minutes at room temperature, and soluble nitrogen in the supernatant was estimated by the micro-kjeldahl method. Percent protein extracted was calculated with reference to the total amount of protein in the sample.

\[
\text{Soluble protein} = \frac{T \times N \times T_v \times 14 \times 6.25 \times 100}{A \times b \times 1000}
\]

Where \( T \) = Titre reading .

\( N \) = Normality of acid. (0.02N).

\( T_v \) = Total volume of aliquot extracted.

14 = each ml of hydrochloric acid is equivalent to 14 mg nitrogen.

\( a \) = Number of ml of aliquot taken for digestion .

\( b \) = Number of (gm) sample flour extracted.

1000 = No. of mg in one gm.

6.25 = protein factor.

Percent solubility = \( \frac{\text{soluble protein} \times 100}{\text{crude protein of the sample}} \)

3.2.5 Determination of nitrogen solubility at different NaCl concentrations:

Nitrogen solubility of both raw and autoclaved flour was determined at different NaCl solutions by the procedure of Hagenmaier (1972), as described by Quinn and Beuchat (1975) with a slight modification. 0.2 grams material were dispersed in 10 ml distilled water or NaCl
solutions ranged from (0.2-2M) and mechanically shaken for 1 hour at room temperature, centrifuged at 3000 rpm for 20 minutes at room temperature, and soluble nitrogen in the supernatant was estimated by the micro-kjeldahl method. Nitrogen solubility was expressed as percent of the nitrogen content of the sample.

\[
\text{Soluble protein} = \frac{T \times N \times T_v \times 14 \times 6.25 \times 100}{A \times b \times 1000}
\]

Percent solubility = \(\frac{\text{soluble protein} \times 100}{\text{crude protein of the sample}}\)

3.2.6 Functional properties of raw and autoclaved defatted chickpea flour:

1. Water retention capacity (WRC):
   The water retention capacity (WRC) was estimated by the method of Lin et al (1974) with modification described by Quinn and Beuchat (1975). A 10% suspension (1g/10ml) was stirred in a centrifuge tube using a glass rod for 2 minutes at room temperature (26ºC). After 20 minutes equilibration the suspension was centrifuged for 20 minutes at 4400 rpm at room temperature (26ºC). The freed water was decanted into a 10 ml graduated cylinder and the volume was recorded. (WRC) was recorded as ml water retained by 100 grams materials.

2. Fat absorption capacity (FAC):
   The fat absorption capacity (FAC) of the sample was measured by a modified method of Lin et al (1974). Four grams of the sample was treated with 20 ml of refined groundnut oil in a 50 ml centrifuge tube. The suspension was stirred with a glass rod for 5 minutes and the contents were allowed to equilibrate for a further 25 minutes at room temperature (26ºC). The suspension was centrifuged for 20 minutes at
5000 rpm at room temperature (26°C). The freed fat was decanted into a 10 ml graduated cylinder and the volume was recorded. (FAC) was expressed as ml oil pound by 100 grams dry matter.

3. Bulk density (BD):

The bulk density was determined by the method of Wang and Kinsella (1976)\(^a\). About 3 grams of material were placed in a 25ml graduated cylinder and gently packed by tapping the cylinder on the bench (10) times to a reasonable height (approximately 5-8cm). The volume of the sample was recorded. Bulk density was calculated as gram per milliliters of material.

4. Emulsification properties:

4.1 Emulsification capacity (EC):

The Emulsification Capacity (EC) of the sample was estimated by the method of Beuchat et al (1975). One gm material was blended with 50 ml of distilled water or NaCl solutions ranged from (0.2-2 M) for 30 sec. in a Braun electric blender; after complete dispersion, refined groundnut oil was added cautiously (0.4 ml/sec) from a burette and blending continued until there was a phase separation (visual observation/change in shaft sound). EC was expressed as milliliters of oil emulsified by one gram material. EC was also determined as a function of different pHs (2,4,6.8.10.12). The pH was adjusted to the desired value with either 1N HCl or 1N NaOH prior to emulsion preparation.

4.2 Emulsification activity (EA) and emulsion stability (ES):

The emulsification activity (EA) was measured by the procedure of
Yasumatsu et al (1972) with a slight modification. About 0.7 gm of material was added to 10 ml of distilled water or 10 ml of NaCl solutions ranged from 0.2 to 2M and mixed well before adding to it 10 ml of refined groundnut oil. The mixture was blended in Brown electric blender for 5 minutes, poured into centrifuge tubes and centrifuged at 2000 r.p.m for 5 minutes then poured into 50 ml measuring cylinders and stay a few minutes until the emulsified layer was stable. EA was expressed as:

\[
EA = \frac{\text{Height of emulsified layer}}{\text{Height of total content in the tube}} \times 100
\]

EA was also determined as a function of selected pH values (2, 4, 6, 8, 10, 12).

Emulsion stability (ES) was measured by recentrifugation followed by heating at 80°C for 30 minutes. And subsequently cooled to 15°C. After centrifugation the emulsion poured into 50 ml measuring cylinders and stays a few minutes until the emulsified layer was stable. ES was expressed as the percent of the total volume remaining emulsified after heating.

\[
ES = \frac{\text{Height of emulsified layer after heating}}{\text{Height of total content in the tube}} \times 100
\]

ES was also determined as a function of selected pH values (2, 4, 6, 8, 10, 12).

5. Foaming properties:
5.1 Foaming capacity (FC):
Foaming capacity of the sample was determined by following the procedure described by Lawhon et al (1972). 2 grams of the sample were blended with 100 ml distilled water or 100 ml NaCl solutions
ranged from (0.2-2M) in a moulinex blender at "hi" speed for 2 minutes. The mixture was poured into a 250 ml measuring cylinder and the foam volume was recorded after 30 sec.

\[
FC = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100
\]

FC was also determined as a function of different pH values (2, 4, 6, 8, 10, 12).

5.2 Foam stability (FS):

The foam stability (FS) was conducted according to Ahmed and Schmidt (1979). The FS was recorded at 15 minutes interval for 2.30 hours after pouring the material in a cylinder.

\[
FS = \frac{\text{Foam volume after time (t)}}{\text{Initial foam volume}} \times 100
\]

FS was also determined as a function of selected pHs (2, 4, 6, 8, 10, 12).

6. Gelation:

Least gelation concentration of the sample was measured by the method of Coffman and Garcia (1977) with a slight modification. Appropriate sample suspensions of (2, 4, 6, 8 and 10%) were prepared in 10 ml of distilled water or 10 ml NaCl solutions ranged from (0.2-2). The test tubes containing these suspensions were then heated for one hour in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were further cooled for 3 hours at (4ºC). The least gelling concentration was determined as that concentration did not fall down or slip when the test tube was inverted.

Least gelation concentration of the same concentration in distilled water was also determined as function of selected pH values (3, 7, and
7. Dispersibility:
The dispersibility of flour at selected pH levels (3, 7, 10) was measured according to the method of Kulcarni, Kulcarni and Ingle (1991). 3 grams of the flour was dispersed in distilled water in a 50 ml stoppered measuring cylinder and the desired pH was adjusted by addition of drops of dilute HCl and NaOH solutions. Then distilled water was added to reach a volume of 30 ml, The mixture was stirred vigorously and allowed to settle for three hours, the volume of settled particles was subtracted from 30 and multiplied by 100 and reported as percentage dispersibility.

8. Wettability:
The Wettability was estimated to both untreated and treated samples according to the method of Regenstein and Regenstin, (1984). Two grams of the sample were weight in a sieve and transferred to a beaker containing 80 ml distilled water and a magnetic without stirring the water. The behavior of the powder was observed on the water surface immediately after adding the sample. After 30-min. observation the material was stirred on the magnetic stirrer sufficiently fast to form a vortex which reached the bottom of the beaker. The stirring continued for one min. after which the grade describing Wettability was recorded as excellent, good, fair or poor according to the time and behavior of the dispersion (see Chart 1).

3.2. Statistical analysis:
Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the
analysis of variance (ANOVA) (Snedecor & Cochran, 1987). Duncan's multiple range test was used to separate means. Significance was accepted at $P \leq 0.05$ (Duncan, 1955).

Chart 1

| Characteristic of wet sample                                                                 |
|---------------------------------------------------------------------------------------------|---|
| * Powder wet as soon as it contacts water, even with stirring. After one half hour, the sample is completely dispersed. | Excellent |
| * Powder only wets slightly when it comes into contact with water. After one half hour the sample is wet and powder had sunk to the bottom. Stirring disperse the sample | Good |
| * Powder wets very slightly on initial contact and tend to clump and remain at the surface. After one half hour the sample still at the surface although some of the sample has disperse. After stirring there are still a few clumps left. | Fair |
| * Powder hardly wets when it initially comes contact with the water. It also clump. After one half hour the solution is slightly cloudy and most of the sample is still in clumps at the surface. After stopping the | Poor |
| stirring most of the sample still floats and clumps. |
4.1. Proximate composition of treated and untreated chickpea flour: 

The proximate composition of both treated and untreated chickpea samples are illustrated in Table 1. The moisture content of defatted untreated samples (7.83%) was approximately similar to that of untreated ones (8.1%) as reported by Sánchez-Voique et al (1999). The protein content was found to be 20.15% which is lower than that of chickpea flour reported by Sánchez-Voique et al (1999) and Milán-Carillo et al (2000). This difference may be due to variation between seeds and growing location of the cultivars. Ash content was found to be 2.9%, which is similar to that obtained by Milán-Carillo et al (2000). Fiber content was found to be 0.5% which is lower than that of chickpea (8.8%) reported by Sánchez-Voique et al (1999), this possibly due to variation between varieties, growing location and preparation conditions. Oil content was found to be 0.7%. Although chickpea flour was extracted with hexane, lipids were not removed completely and parts of them were remained in the flour and were associated with the protein isolates. Similar explanation reported by Sánchez-Vioque et al (1998). Autoclaving slightly reduced the protein, fiber, moisture, and ash contents of untreated samples. This findings agreed with the observation of Venktesh and Prakash (1993) who found that autoclaving of sunflower flour at 1 kg/cm$^3$ increased moisture content from 8% to 11% , but decreased the protein from 49% to 46%, residual fats from 3.4% to 1.8% and carbohydrates from 13.6% to 10.4%. This difference probably due to variations between cultivars
and preparation conditions.
4.2 Effect of pH on protein solubility of treated and untreated chickpea flour:

Protein solubility of both treated and untreated flour at various pH values is presented in Fig. 1. Untreated flour had a minimum protein solubility of 11% at pH 4 (the isoelectric region). On either side of this pH it increased. Alkaline pHs extracted more protein than acidic pHs and the highest protein solubility observed at pH 12 (88.28%). The occurrence of minimum solubility near the isoelectric pH is due primarily to the lack of electrostatic repulsion, which promotes aggregation and precipitation via hydrophobic interactions (Fennema, 1996). The lower protein solubility in the acid range may be due to the occurrence of phytates in chickpea flour which are water soluble and can readily form complexes with cationic protein which predominate in the acidic pH range and cause insolublization of protein. Similar phenomenon had been reported in the case of the rice bran protein (Bera and Mukherjee, 1989). Also similar results were obtained by Bencini (1986), Okezie and Bello (1988), Pawar and Ingle (1988) and Sathe et al (1982) on chickpea flour, cowpea flour, moth bean flour and lupin protein, respectively. Autoclaving had no marked effects on the protein solubility of untreated flour, except at pH 2, the protein solubility of autoclaved samples (46.49%) was higher than untreated samples (43.85%). At pH 4 minimum solubility of 10.95% was observed compared to 11% of untreated flour. These results weren’t in agreement with those of Naryana and Narasinga Rao (1982) who observed reduction on the protein solubility of raw winged bean flour due to autoclaving. This is possibly due to differences between seed type, growing location and preparation, storage conditions.
4.3 Effect of NaCl concentration on the protein solubility of treated and untreated chickpea flour:

As shown in Fig. 2. Untreated samples showed lower extractability of protein at 0.2 M compared to that treated with distilled water. Highest extractability was observed at 0.4M due to salting in effect of NaCl. Thereafter it decreased due to salting out effect of NaCl. There was no significant difference in protein solubility by dissolving the flour in 1 & 1.8M NaCl. At 2M NaCl solubility decreased slightly. Similar trends was observed by Shehata and Tahnnoun (1981) who reported that sodium chloride retarded protein solubility of Iragi whole mung bean at lower concentration. Increase in NaCl concentration from (0-0.05) caused nitrogen extractability to drop from 79 % to 55.45%. Maximum nitrogen extractabilities were 78.78% at 0.5N NaCl compared to 79% with water alone. Similar results also were reported by Hang et al (1970) on mung beans, red kidney beans and pea beans. Lower protein solubility at 0.2 M NaCl possibly due to difference on the pH of the suspension. No apparent variation observed between untreated samples and autoclaved samples.

4.4. Effect of pH on the foaming capacity (FC) of treated and untreated chickpea flour:

As presented in Fig. 3 untreated flour had a minimum FC at pH 4 (23%) and maximum of 83% at pH 12. At pH 2 the FC was slightly higher than other pH values. The present finding agreed with Sathe et al (1982) who observed that the FC of the lupin protein concentrate was higher at pH 2 and least at pH 4. However this observation was
disagree with the findings of Narayana and Narasinga Rao (1982) who found that the FC of raw winged bean flour was minimum at pH 4.6 (76%) and maximum at pH 9.8 (150%). The behavior of FC versus pH values was not completely
agreed with the behavior of protein solubility versus pH values except at pH 12, which showed maximum protein solubility and FC. Autoclaving didn’t change the FC of untreated flour significantly except at pH 8, at which FC was decreased from 58.33% to 54.17%. The effect of autoclaving on chickpea flour in this study disagree with the observation reported by Narayana and Narasinga Rao (1982) who found that autoclaving considerably lowered the FC of winged bean flour at pH values up to 10. However, at a pH above 10 autoclaved flour had higher FC than the raw one. These differences might be due to the variation on the protein content between the two seeds and treatment conditions.

4.5. Effect of NaCl concentration on foaming capacity (FC) of treated and untreated chickpea flour:

As shown in Fig. 5 addition of NaCl improved the FC of untreated chickpea samples. The maximum improvement was observed at 0.2M NaCl and then decreased gradually due to salting out effect of NaCl. Higher FC at low salt concentration may be due to improvement of protein solubility at the interface of the colloidal suspensions during foam formation, thus improving foaming capacity (Kinsella, 1976).

The present findings supported the findings of Narayana and Narasinga Rao (1982) who observed that the FC of both raw winged bean flour and soy bean flour at 0.2M NaCl increased to a maximum
and then dropped beyond this salt concentration. Similar results were observed by Sathe et al (1982) on lupin seed protein. Autoclaving significantly ($p \leq 0.05$) reduced the FC of untreated samples at different NaCl concentrations. However, dissolving of the flour in distilled water increased the FC. There was no significant difference between flour treated at 1.8 and 2 M NaCl. This observation was similar to the findings of Narayana and Narasinga Rao (1982) who
reported that addition of NaCl up to 0.2M concentration increased the FC of autoclaved winged bean flour. Higher concentration of NaCl decreased it considerably. Foam capacity of winged bean and soybean flour at 1M NaCl was higher than in water, but after winged bean flour was autoclaved it was decreased.

4.6 Effect of pH on foam stability (FS) of treated and untreated chickpea flour:
The effect of pH on foam stability (FS) of untreated and treated chickpea samples is shown in Tables 2 and 3, respectively. FS of untreated chickpea flour at a given pH value significantly (p ≤ 0.05) decreased with time (Table 2). After standing for 150 min at room temperature the FS was decreased at pH 2 from 100 to 60.66% while at pH 12 it decreased to 18.52%. The FS at pH 8 was lower than that at pH 10 and 12. Higher FS was observed at acidic pHs compared to alkaline pHs. The high stability of foams at acidic pH may be due to the formation of stable molecular layers in the air-water interface, which impart texture, stability and elasticity to the foams. Nearly such molecular stabilizing effect in acidic range is also observed by Sathe et al (1982)a who found that the FS of lupin protein concentrates was maximum at pH 4 and it progressively decreased at alkaline pH. Wang and Kinsella (1976)b found that foam stability varied with pH
being minimum in the isoelectric range (pH 3-4) and being maximum in narrow pH regions above the isoelectric range where protein is slightly negatively charged and showing a rapid decrease at alkaline pH values (pH 6). The latter effect may be explained by charge repulsion between proteins with resultant lack of adhesion and also by some solubilization of the alfalfa leaf proteins in this range (Lu and Kinsella, 1972 & Betchart and Kinsella, 1973) thereby reducing the quantity of aggregated
protein necessary to stabilize the foams (Wang and Kinsella, 1975). Autoclaving (Table 3) decreased the FS of untreated samples at pH 2 from 60.66% to 48.36% and slightly increased the FS at pH 4 from 35.83% to 38.83% and at pH 12 from 18.52% to 22.16%. The present finding disagreed with the observation of Narayana and Narasinga Rao (1982) who reported that the FS of raw winged bean flour at pH values 2.5, 6.6 and 8.7 decreased markedly within 10 min and then decreased gradually. However at pH 4.5 where the initial foam capacity value was low, it decreased steadily. In the case of autoclaved winged bean flour, foam volume decreased markedly within 10 min at all pH values studied. At pH 2.5 the foam volume at 120 min was 45% with raw winged bean flour and 50% with autoclaved flour. At pH 4.6 the foam stability of autoclaved flour was collapsed immediately after its formation. This difference possibly due to variation between seed type and experimental conditions.

4.7 Effect of NaCl concentration on foam stability (FS) of treated and untreated chickpea flour (FS):

The effect of NaCl concentration on the foam stability (FS) of untreated and treated samples are shown in Tables 4 and 5,
respectively. The lowest FS of untreated samples observed at 0.2 M NaCl (Table 4). As the salt concentration increased the FS significantly improved at a given time. The highest FS was observed at 1.8 M NaCl. The lower stability at 0.2 M NaCl possibly because salt improved the protein solubility at the interface of the colloidal suspensions during foam formation, it retarded the partial denaturation of the surface polypeptides of proteins that are necessary for protein-protein interaction and stability (Kinsella, 1976). The improvement of FS by increasing NaCl concentrations was nearly similar to the finding
of Bera and Mukherjee (1989) who reported that foaming stability of rice bran protein concentrates was slightly improved when salt concentration was increased from 0.1M to 1M NaCl. The improvement of FS by increase in NaCl concentrations might be due to cross linking of protein molecules and creation of films with better viscoelastic properties. Similar explanation was reported by Fennema (1996) who mentioned that the FS of a protein improved by addition of divalent cations, such as Ca and Mg. Autoclaved samples (Table 5) followed similar trend of untreated samples. FS significantly increased with NaCl concentration at a given time. At 1M and 1.2M NaCl it is slightly decreased then it increased.

4.8 Effect of pH on emulsion capacity (EC) of treated and untreated chickpea flour:
The emulsion capacity of untreated and treated chickpea flour are shown in Fig. 5. Emulsion capacity of untreated samples was affected markedly by pH. The EC was poor at pH 4 (42 ml/g), but was
significantly improved when the pH was shifted below or above this value. It was more efficient in emulsifying the oil at alkaline pHs than at acidic pHs. The behavior of EC versus pH values closely resembled the behavior of the protein solubility versus pH values. This is suggested that EC was greatly affected by protein solubility. In case of soybean flour (McWatters and Holmes, 1979), raw moth bean flour (Pawar and Ingle, 1988), groundnut protein (Ramantham et al, 1978), winged bean flour (Narayana and Narasinga Rao, 1982) similar relationship between EC, pH and protein solubility has been reported. Autoclaved flour showed a slight decrease on EC at all pHs studied. The present findings agreed with the results reported by Narayana and Narasinga rao (1982) who found that autoclaving had an adverse effect on EC. It decreased the EC significantly at all pH values. The EC was
greatly affected by the protein solubility and there was no apparent differences observed between the treated and untreated flour.

4.9 Effect of NaCl concentration on emulsion capacity (EC) of treated and untreated chickpea flour:
As shown in Fig. 6. The emulsion capacity of untreated flour was higher at 0.6M NaCl (65 ml oil /g flour) and then decreased up to 1.2M NaCl. Beyond that it remained constant. Narayana and Narasiga Rao (1982) found that incorporation of NaCl at concentration up to 0.4M had an incremental effect on the EC of raw winged bean flour. Beyond this concentration EC decreased steadily, due to salting out effects of NaCl. The effect of NaCl on EC of untreated flour did not resemble the effect of NaCl on the protein solubility. This is possibly due to the
fact that emulsion properties of flour cannot be solely attributed to the proteins but other food components such as carbohydrates and lipids may also contribute appreciably, possibly through protein-carbohydrate and protein-lipid interactions. Or due to that the emulsifying property not only depend on the protein solubility but also on hydrophilic-lipophilic balance of a particular protein (Naki, 1983). McWatters & Holmes (1979) showed that large concentrations of soluble nitrogen were not necessarily related to maximum emulsifying capacity. Naki (1983) reported that solubility, surface hydrophobicity and molecular flexibility influence emulsifying behavior of globular proteins such as pea proteins that have extensive quaternary structure. The EC of autoclaved flour slightly decreased up to 0.6 M NaCl and beyond that it was slightly increased at 1.4 M NaCl compared to untreated flour (Fig. 6). As the concentration increased both samples showed similar emulsion capacity. A slight variation between the sample may be due to autoclaving. The results were not correlate with
earlier findings of Narayana and Narasiga Rao (1982) who reported that addition of NaCl to autoclaved winged bean flour did not cause an observable change in the EC and explained that it was due to possible denaturation of the protein by heating, the solubility characteristics in water and salt solutions may be changed and reflected on emulsion capacity behavior.

4.10 Effect of pH on emulsifying activity (EA) of treated and untreated chickpea flour:

The effect of pH on the emulsifying activity of treated and untreated chickpea samples are shown in Fig. 7. Untreated samples showed minimum EA of 7.47% at pH 4. This is might be due to increased
protein-protein interaction, which lowering the surface hydrophobicity and decreased the net charge and solubility of proteins. EA improved above and below pH 4. The EA was higher at acidic pHs than at alkaline pHs. EA at pH 12 was lower than that at pH values 6, 8 and 10. This observation is against the findings of Yim and Lee (2000), Khalid et al (2003) and Monteriro and Prakash (1994) who observed higher EA at alkaline pHs than at acidic pHs on soybean protein, sesame protein and peanut protein, respectively. This variation possibly due to varietal variation and methods applied to estimate EA. Autoclaving had no apparent adverse effect on the EA of untreated chickpea flour. There was a minor difference between the treated and untreated flour.

4.11. Effect of NaCl concentration on the emulsifying activity (EA) of treated and untreated chickpea flour:

Figure 8 showed the emulsifying activity of treated and untreated flour. The EA of untreated flour was higher in distilled water then it decreased at
0.2M NaCl and then increased at 0.4 M NaCl. At 0.6 M it decreased and no obvious reduction on EA was observed after that salt concentration. Similar results were observed by Monteiro and Prakash (1994) who reported that the EA of the peanut protein isolate decreased considerably at 0.3 M NaCl. However, after 0.3 M NaCl concentration, EA increased with increase in NaCl concentration. Jane et al (1981) found that when emulsions prepared in NaCl were compared with water, emulsifying activity was reduced. No obvious variation observed between untreated and autoclaved samples except
at 2 M NaCl (Fig. 8) there was a considerable reduction in EA from 60.03% to 54.99%. Autoclaving also decreased the EA of untreated samples in distilled water from 67.15% to 65.71%. This is agreed with the findings of Pawar and Ingle (1988)\(^a\) who found that the EA of cooked moth bean flour (17%) was lower than uncooked flour (20%). Venktesh and Prakash, (1993)\(^b\) reported that autoclaving of defatted sunflower flour at 1 kg/cm\(^2\) increased the EA from 15.5 (absorbance at 500 nm) to 20.2.

4.12. Effect of pH on the emulsion stability (ES) of treated and untreated chickpea flour:

As shown in Fig. 9 untreated samples had minimum emulsion stability (ES) at pH 4. ES was higher at pH 6 (93.09%) and pH 2 (83.83%) and it decreased at alkaline pH. The lower ES at pH 4 possibly due to colloidal particles (e.g., protein) which carry an electrical charge that promotes the stability of the colloid itself as well as in emulsions formed by causing particles of similar charge to repel each other, thereby preventing precipitation (Paul and Polmer, 1972). Neutralization of the charge, which occurs at a protein's isoelectric point, causes colloidal particles to become
both unstable and less soluble (Mcwatters and Holmes, 1979). Similar
trends were obtained by Khalid (1994) who found that the ES of
sesame protein isolate at pH 2 (75%) was higher than the ES at pH 7
(70%) and pH 10 (62%) with a minimum ES at pH 4.9 (37.8%). No
apparent differences were observed between untreated flour and
autoclaved one in ES values.
4.13 Effect of NaCl concentration on emulsion stability (ES) of treated and untreated chickpea flour:

As shown in Fig. 10 addition of NaCl significantly decreased the ES of untreated flour. Higher ES value was observed when distilled water was used without addition of NaCl (92.67%) and then decreased considerably to 42.05 and 33.48% at 0.2 M and 0.8 M NaCl, respectively. Thereafter, there were no apparent decreases observed. Similar observation was reported by Jane et al. (1981) who found that when emulsions were prepared in NaCl and compared with water the emulsion stability significantly reduced. The higher emulsion stability in distilled water might be due to the globular nature of the major proteins of chickpea samples. Similar explanation reported by Sathe et al. (1982) on winged bean. Autoclaving had no adverse effects on the ES of untreated chickpea samples. Similar results were obtained by Pawar and Ingle (1988) who found that cooking of the moth bean flour for 15 min slightly decreased the ES from 12 to 11%.

4.14 Fat absorption capacity (FAC) of treated and untreated chickpea flour:

As illustrated in Table 6 FAC of untreated flour (141.25ml oil/100 g flour) was approximately similar to that of raw chickpea flour reported by Bencini (1986) and higher than that of defatted chickpea flour observed by Sánchez-Vioque et al. (1999) and lower than that of moth bean flour.
reported by Pawar and Ingle (1988). These differences may be due to variation between seed type, protein content and growing location. Autoclaving slightly affected FAC of chickpea samples; it decreased from (141.25 ml/100g) to (135 ml/100g). The decrease in FAC by
autoclaving could be due to aggregation of the flour proteins. Similar results were reported by Venktesh & Prakash (1993) on sunflower protein. This finding was also similar to the observation of Tasneem & Suberamanium (1986) who reported that the FAC of the protein isolate obtained from autoclaved guar meal (105ml/100g) was lower than the isolate from defatted guar meal (108ml/100g). Rahma & Mostafa (1988) found that autoclaving increased FAC. Oil absorption capacity may determine whether the protein material will perform well as meat extenders and analoge (Circle, 1972).

4.15 Water retention capacity (WRC) of treated and untreated chickpea flour:

WRC of treated and untreated chickpea samples are shown in Table 6. Untreated flour had a WRC of 130 ml/100g which is higher than WRC of raw chickpea flour and navy bean and lower than WRC of pinto bean reported by Han and Khan (1990). This is possibly due to low lipid content and differences in carbohydrate content of untreated flour. Autoclaved flour had higher WRC 140 ml/100g compared to untreated flour 130 ml/100g. These observations agreed with those of Quinn and Beuchat (1975) who found that WRC of autoclaved flour was higher than the defatted peanut flour which justify by the fact that the protein solubility of autoclaved flour decreases and accordingly WRC increases. The roasted bean fractions showed higher values of WRC than unroasted ones (Han and Khan, 1990). Generally, the increase in WHC in roasted beans could be caused by the dissociation of proteins that might occur as a result of heating and denaturation and also could be minimized by short-period treatment, which would
unmask the nonpolar residues from the interior of the protein molecules (Abbey and Ibeh, 1987). The degree of WRC is considered to be useful as an indication of performance in several food formulations, especially those involving dough handling (Circle and Smith, 1972)\(^a\).

4.16 Bulk density (BD) of treated and untreated chickpea flour:
As shown in Table 6 the BD of untreated flour showed about 0.55g/cm\(^3\) which is higher than the BD of raw chickpea flour observed by Milán-Carillo \textit{et al} (2000) and lower than the BD of row cowpea flour reported by Padmashree \textit{et al} (1987). This is probably due to the variation in particle size, preparation methods and seed type. Autoclaving increased the BD of chickpea samples slightly from 0.55g/cm\(^3\) to 0.57g/cm\(^3\). This finding nearly agree with those of Venktesh and Prakash (1993)\(^b\) who reported that autoclaving of defatted sunflower flour at 1Kg/cm\(^3\) increased the BD of the control flour from 0.26 g/cm\(^3\) to 0.27 g/cm\(^3\) and suggested it could be due to the removal of the residual moisture of the flour, resulting in dense packing of the flour particles for the same unit volume. Higher bulk density is desirable since it helps to reduce the paste thickness. This is an important factor in convalescent and child feeding (Padmashree \textit{et al}, 1987).

4.17 Effect of pH on dispersibility of treated and untreated chickpea flour:
As shown in Tale 7 untreated samples had a higher dispersibility at pH 7 (73.83\%) and 10 (71.67\%) compared to pH 3 (48.67\%). These values agreed with the findings of Khalid \textit{et al} (2003) who reported that
dispersibility of sesame protein isolate was significantly higher at neutral and alkaline pHs than acidic pHs. No significant (P ≤ 0.05) differences observed between untreated flour and autoclaved one in this study. It was reported that higher dispersibility enhances the emulsifying and foaming properties of proteins, which was observed during making of bread, macaroni and cookies (Kinsella, 1979).

4.18 Effect of pH on the least gelation concentration of treated and untreated chickpea flour:

The least gelation concentration of treated and untreated chickpea flour is shown in Table 8. Untreated flour was formed a weak gel at 6% (w/ml) for all pH values. A strong gel was formed at 8% and very strong one at 10%. No gel was formed at 2% and 4% concentrations.

The pH had no any effect on the least gelation concentration of untreated flour. This observation disagree with the findings of Wiseman and Price (1987) who observed that, the protein concentrates from pressed jojoba meal showed that less protein was required for gelation at neutral and alkaline pHs than at acidic pHs. This differences might be due to higher content of starch of untreated flour which induced gelation due to starch or starch-protein interactions.

The pH of the medium had no effect on the least gelation concentration of treated and untreated samples.

4.19 Effect of NaCl concentration on the least gelation concentration of treated and untreated chickpea flour:

The effect of sodium chloride concentration on the least gelation concentration of treated and untreated chickpea flour is shown in
Table 9. No gel was obtained at 2% and 4% flour in distilled water and all NaCl
solutions. This is might be due to a certain degree of protein solubility which is necessary for protein gelation reported by Balmaceda et al (1976). At 6% flour untreated flour also formed a weak gel in NaCl solution and formed a strong gel at 0.8 M. Beyond that salt concentration it formed again a weak gel. A hard gel obtained at 0.8 M NaCl might be due to globulin which account for 60%-80% of the total protein in chickpea seeds as reported by Narayana and Rajagopal (1974). Or possibly because of charge neutralization by NaCl, which promote hydrophobic aggregation upon heating (Fennma, 1996). The decrease in hardness after 0.8 M NaCl might be due to the differences in boiling temperature of the solutions. Possibly the concentration up 0.8 M NaCl have a high boiling temperature than lower concentration or might be resulted from the presence of too many ions, which interfere with the formation of protein-protein bonds, as reported by Wiseman & Price (1987). No difference was observed on the effect of NaCl on the least gelation concentration of treated and untreated flour.

4.20 Wettability of treated and untreated chickpea flour:
The wettability grade of untreated flour was good since it wet slightly when it comes into contact with water and after 30 min the sample was wet and powder has sunk to the bottom. Stirring dispersed the sample (Chart 1). Similar results obtained by Hassan (1994) who reported that wettability of the watermelon protein isolate was given the grade good, since it took the sample 30 min for complete wetness after which
it sank to the bottom of the beaker. Autoclaved flour also gave the grade good.
Chapter Five

Conclusions & Recommendations

5.1. Conclusions:

In conclusion, the data shown in the present study indicated that autoclaving had no marked effect on the functional properties of defatted chickpea flour except that it lowered the foaming capacity at different pH values and NaCl concentrations. Autoclaved flour can potentially be used in supplementary food formulations because of its high nutritive value compared to the raw flour, since it decreased both trypsin inhibitor and hemagglutinin activities and it increased the \textit{in vitro} protein digestibility as well as the role of autoclaving in destroying all bacteria and other pathogens. The high protein content of both raw and autoclaved chickpea flours indicated that they could be a valuable protein supplement in food products, (such as an extender in meat products, infant foods and cereal based foods). The high solubility of both flours at alkaline pH make them suitable in liquid foods and beverages. The good foaming capacity of both flours appeared to be promising sources of protein for use in whipped food products. With such comparatively high emulsion properties, both flours can be used in meat analogs, ice cream, baking and textured high protein food industries. The good wettability of both flours makes them suitable for use in textured and/or comminuted meats and baked products. The high bulk density of both flours is important in retarding its packing. The fat absorption capacities of both flours allow their use in sausages where they can be as good alternatives to casein.
5.2. Recommendations:

1. The problems that are facing utilization of both flours (raw-autoclaved) in food products are their beany flavor and flatulence-producing carbohydrates. Research is therefore needed to solve these problems.

2. The results of this investigation further helped to establish the potential food use of both flours. However, further research is needed to relate potential functionality of raw and autoclaved chickpea flours to their performance in specific food systems, including sensory evaluation to determine acceptability of these products.
Chapter Six

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