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The Background and the Significance of Sesame Seed (Benni Seed) as a Healthy Complementary Food for Children (6-24 Months)

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Keywords

Benni Seed, Nutritional Value, Healthy Diet, Protein-rich Foods, Plant-based Nutrition.

Introduction

Sesame seed is one of the world's important and oldest oilseed crops known to man [1]. Sesame has different names according to the region of production, in some areas it is known as sesamum (Mexico, South and Central America), *Zhi'ma* (China) gingelly (South India, Burma), benniseed (Sierra Leone, Guinea in West Africa), sim-sim (Middle East) and till (East and North Africa). It has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein [2]. China, India, Sudan, Mexico, and Burma are the major producers of sesame seeds worldwide, contributing to approximately 60% of its total world production. In Burma, it is the major source of edible oil for local consumption [3] in Sierra Leone, West Africa is a major crop produced as mix cropping in rice farms and it is produced on a larger scale but it is underutilized as it is only use as animal feed and use as "benni cake" prepared for school going children and for the production of "ogerie" a local food prepares in local dishes [4]. Sesame is an important source of food worldwide and constitutes an inexpensive source of protein, fat, minerals in the diets of rural populations, especially children [5].

Enzymatic hydrolysates have improved functional properties such as better whipping, foaming, emulsifying and solubility [6]. Additionally, because of their fairly predictable hydrolysis properties, enzymatic hydrolysates may be tailored to yield uniform products whereas chemical hydrolyses produce heterogeneous products. Thus, enzymatic hydrolysates largely made of peptides are obtained when suitable enzymes are used as opposed to the chemical process whereby a mixture containing a high amount of free amino acids (FAAs) is the likely end-product. FAAs are a huge burden to the consumer body's osmotic balance, have poor solubility as opposed to peptides, and are poorly assimilated by the human body unlike the peptides [7].

Literature Review

Sesame

Sesame belongs to the genus *Sesamum*, one of the 16 genera in the family *pedaliaceae.* The genus *Sesamum* comprises about 35 wild species besides the only cultivated species, *Sesamum indicum L*. [8]. The name sesame comes from the Arabic word "simsim". Brdigian & Harlan [9] cited India as the origin of sesame, however, there is also a belief that the actual origin was Africa, where many wild species are found [5]. Sesame is a not too long narrow-leaf summer crop with bell-shaped flowers and opposite leaves as shown in Figure 1. It is an annual plant that can reach 1-1.8 m high. The plant prefers fertile, well-drained, and neutral to slightly alkaline pH. Sesame is cultivated in tropical areas and needs growing season of 110-150 frost free days. The best air temperature is 30-35°C and the soil temperature should not be lower than 20°C. Flowering starts about 40 days after planting the seeds and continues for almost another 40 days. The flowers then develop into seed pods (capsules), which contain 70-100 seeds each.

The color of seeds varies from white to brown, gold, gray, violet and black as shown in Figure 2. Sesame seeds are tiny and weigh 2-3.5 g/1000 seeds. Seed yield ranges from 350 to 1700 kg/ha depend on the variety used and cultivation techniques. According

to Food Agricultural Organization (FAO), the average yield is almost 500 kg/ha.

Figure 1: Sesame crop.

Figure 2: Different sesame seed colors.

Sesamum indicum is the major commercial source of sesame seeds and is primarily grown in China, India, Mexico, and Sudan. The production of sesame seed in 2008 was reported as 120 million tons worldwide, which puts sesame as the 2nd oil crop following soybean (FAOSTAT, www.fao.org). The production of sesame seeds is low in comparison with soybean with 180 million ton of production. This is mainly because of the requirement of manual harvest since the sesame plant should be harvested before the seed capsules get open on the field and the seeds are lost.

Health Benefits of Sesame Seeds

Sesame seed has been an important oil seed since ancient times [5]. It was claimed in ancient Chinese books that consumption of sesame seeds (*Zhi'ma* in Chinese) provides increased energy and prevents aging. Sesame oil (*Tila in* ancient Indian language, Sanskrit) has been used as a domestic *Ayurvedic* medicine (roughly translated to the science of life). Recently, scientific studies have attributed many of the health-promoting effects of sesame seed to its lignans especially sesamin, which is the major oil-soluble lignans [10]. Sesame has the following benefits when incorporated in diet for human beings. They are discussed below;

Reduction of Cholesterol Tendency of Sesame

Coronary heart disease (CHD) is the number one cause of morbidity and mortality in industrialized western countries. A positive relationship between high blood cholesterol concentrations, particularly low-density lipoprotein cholesterol (LDL-C), and the incidence of atherosclerosis and CHD has been clearly established. Lowering blood cholesterol by dietary intervention is one of the first measures in the prevention of CHD [11]. Sesame seeds have been investigated for their cholesterol-lowering effect. It was observed that sesame oil reduces serum cholesterol levels in rats compared to corn oil in spite of the comparable fatty acid composition of the two oils [12]. Hirose et al., [13] showed that serum and liver cholesterol were reduced in rats fed diet containing 0.5% sesamin.

Enhancement of Vitamin E level of Sesame

The tocopherol and tocotrienol vitamers that comprise the vitamin E family are considered the most important lipophilic radical-quenching antioxidants in cell membranes. While their function is most often associated with the reduction of peroxyl radicals, novel vitamer-specific roles for tocopherols in signal transduction and in the quenching of other reactive chemical species such as nitrogen dioxide and peroxynitrite are now being investigated [14]. While a-tocopherol has attracted a lot of attention, recent studies indicate that several of these important roles may be specific to -tocopherol. For example, it was shown that -tocopherol and its major metabolite,2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman(-CEHC), inhibit cyclooxygenase activity in stimulated macrophages and epithelial cells and therefore, reduces the synthesis of prostaglandin E^2(PGE) [15].

Protein Modification

Modification of a protein usually refers to physical, chemical, or enzymatic treatments changing its conformation and structure and consequently its physicochemical and functional properties. Compared to acid or alkali hydrolysis, enzymatic hydrolysis of protein, using selective proteases, provides more moderate conditions of the process and few or no undesirable side reactions on the final products. In addition, the final hydrolysate after neutralization may contain bitter and salty tastes. The functionality of the final product can be controlled by selection of specific enzymes and reaction factors [16]. Enzymatic modification of proteins using selected proteases to split specific peptide bonds is widely used. The peptides produced by proteolysis have smaller molecular sizes and less secondary structure than native proteins and may be expected to have increased solubility near the isoelectric point, decrease viscosity, and significant changes in the foaming, gelling, and emulsifying properties from those of original proteins. The peptides may be useful in various food processing operations. Moreover, apart from the nutritional change (e.g. improved digestibility and subsequently absorption of the protein by the human system); hydrolysis may result in the liberation of bioactive peptides [16].

Enzymatic Protein Hydrolysis

Enzymatic protein hydrolysis is the degradation of protein into peptides and/or amino acids by proteolytic enzymes. During protein hydrolysis amide bonds are cleaved, and after addition of a water molecule, peptides and/or free amino acids are released (Figure 3). The newly formed peptides can be new substrates for the enzyme [17].

The hydrolysis process is proposed to occur as three consecutive reactions. First, a Michaelis complex of substrate (protein) and

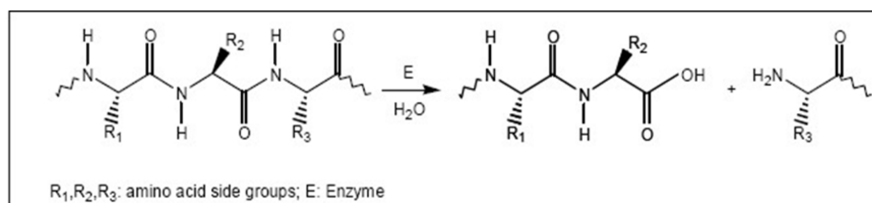

Figure 3: The mechanism of enzymatic hydrolysis. Source [17].

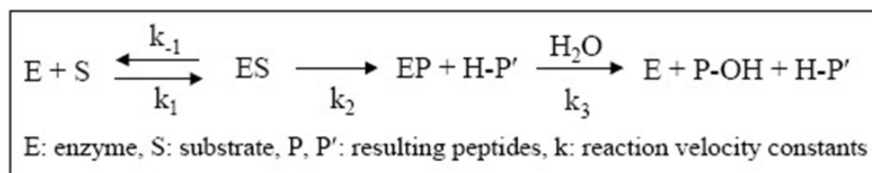

Figure 4: The three stages of hydrolysis process. Source [17].

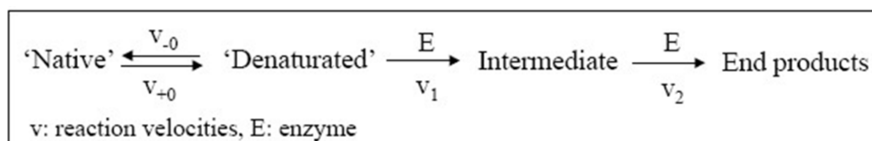

Figure 5: The operation that occurs in hydrolyzing globular proteins. Source [18].

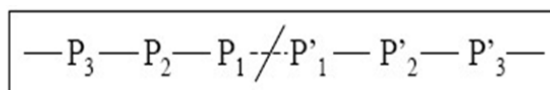

Figure 6: Denotation of amino acid residues at carboxylic (Pn) and amino (P'n) site of cleaved amide bond. Source [22]

enzyme is formed, then the peptide bond is cleaved resulting in the liberation of one peptide, and finally, the remaining peptide is cleaved off the enzyme after a nucleophilic attack by water molecule. These three steps are schematically shown in Figure 4 [17].

For protein hydrolysis, enzyme-substrate binding is essential. In case of globular proteins, most peptide bonds are located in the interior of the protein and are not accessible for the enzyme. For these globular proteins it was postulated by Adler-Nissen [17] that reversible denaturation of the protein is needed for protein breakdown, as after denaturation more peptide bonds are exposed. In solution the folded and unfolded (denaturated) states of proteins are in equilibrium. Only the unfolded molecules are susceptible to degradation by proteolytic enzymes, as schematically represented in Figure 5 [18].

If the rate of denaturation (v0=v+0-v-0) is much smaller than v1, the denaturation step is the rate limiting step for hydrolysis and each denatured protein molecule will be quickly hydrolyzed to end products. The resulting hydrolysate will contain both intact proteins and end products but will be deficient in intermediate size peptides [18]. This type of reaction is designated as a 'one by one' reaction. If, otherwise protein denaturation is faster than hydrolysis (v1<v0), the protein molecules will be degraded to intermediates but are subsequently only slowly degraded to the end products. This type of reaction is called a 'zipper' reaction, resulting in a hydrolysate containing mainly intermediate sized peptides. In most proteolytic reactions, both hydrolysis mechanisms are involved [18]. If the protein is irreversibly denaturated before hydrolysis, the number of accessible peptide bonds is largely increased, and the degradation of the protein is expected to proceed. For these denaturated proteins other factors like decreased solubility might influence the initial reaction rate [18].

The result of proteolysis (the peptide composition of a hydrolysate) depends on three main factors: protein substrate, type of protease (s) used, and hydrolysis conditions [18]. These factors are discussed below;

Protein Substrate

Amino acid sequence and the three-dimensional structure of proteins affect their sensibility towards proteolytic attack and the type of peptides formed during hydrolysis. Some plant proteins are rather flexible and are, therefore, hydrolyzed fairly easily [19]. While some plant proteins that are globular are difficult to access by proteolytic enzymes. Their digestibility can be improved by heat denaturation [20]. Another important difference between proteins is their primary amino acid sequence. In plant proteins, hydrophobic and hydrophilic amino acids are randomly distributed over the peptide backbone, while most contain distinct hydrophobic and hydrophilic domains [21].

Proteases

Many food-grade proteases are available for protein hydrolysis (Table 1). These proteases can be classified based on their origin,

i.e. animal, plant, or microbial origin, their mode of catalytic action, *i.e.* endo- or exo-activity, or on the basis of their catalytic site [17]. Endo-proteases cleave amide bonds within the protein chain, contrary to exo-proteases that remove terminal amino acids from proteins or peptides, either at the C- terminus (carboxypeptidases) or at the N-terminus (aminopeptidases). The nature of the catalytic site of proteases differs according to the active group that will form the enzyme/substrate intermediate. The active group can be either an amino acid, *i.e.* serine, cysteine, or aspartic acid, or a metallo group, most often zinc [17]. The serine proteases are all endo-proteases, but metallo proteases are mostly exo-proteases [22]. The pH specificity of proteases depends on the group in the catalytic site; the cysteine and metallo proteases are active at neutral pH, and the serine and aspartic acid proteases are active at alkaline and acidic pH, respectively [17]. The amino acid residues of the substrate at both sites of the cleaved amide bond are denoted P1 and P'1 for the carboxyl and amino sites, respectively (Figure 6) [22].

The difference in enzyme specificity for proteolytic enzymes belonging to the same group is exemplified by three endo-proteases from animal origin, *i.e.* trypsin, chymotrypsin and elastase, which are all serine proteases (Table 1). These enzymes differ in their preferential specificity for the amino acid at the carboxylic side of the amide bond. Trypsin prefers either arginine or lysine residues, whereas chymotrypsin prefers aromatic amino acids (phenylalanine, tryptophan, tyrosine) and elastase prefers amino acids with small side-chains, such as alanine [22]. Commercial enzyme preparations are often mixtures of different types of proteases, such as pancreatin that contains both endo- and exo-proteases, and crude papain which is a mixture of three proteases (Table 1).

Table 1: Examples of proteases used for hydrolysis of food proteins.

Enzyme	Form pH	T (°C)		Source
Protamex	Grain	5.5-7.5	35-60	*Bacillus Subtilis*
Neutrase	Liquid/Grain	5.5-7.5	45-55	*Bacillus Subtilis*
Alcalase	Liquid/Grain	7.0-10.0	50-60	*Bacillus Licheniformis*
Flavourzyme	Powder	5.0-7.0	45-50	*Aspergillus oryzae*
Papain	Powder	4.0-9.0	65-80	*Carica Papaya*
Trypsin	Powder	7.0-9.0	45-55	*Porcine pancreas*
Corolase PP	Powder	6.0-8.5	60	*Pancreatic extract*
Corolase PN	Powder	5.0-6.0	45	*Aspergillus sojae*
Corolase 7089	Powder	6.0-8.0	55	*Bacillus substilis*
Bromelain	Powder	4.0-8.0	50-60	*Ananas comosus*
Pepsin	Powder	1.8-4.0	50-60	*Porcine*

Source: Godfrey [29].

Hydrolysis Conditions

Once a protein/protease combination is selected, including a possible pre-treatment step of the protein, the reaction conditions of the hydrolysis process should be defined. The main variables determining the result of the reaction are temperature, pH, enzyme to substrate ratio and reaction time. The first three factors determine the reaction rate and may influence the specificity of the enzyme mixture. The reaction time only determines the final extent of hydrolysis [18]. The interactive effects between the hydrolysis parameters also influence the hydrolysate composition.

Characterization of Hydrolysates

Due to protein hydrolysis, molecular properties of protein change, like decreased molecular weight, increased charge, exposure of hydrophobic groups and disclosure of reactive amino acid side-chains [23]. These molecular changes can be detected with several analytical methods, which reflect one or several molecular properties (Figure 7). As a result of the molecular changes, the functional properties of proteins are affected (Figure 7). Although the term functional property is often only applied to indicate techno-functional properties of hydrolysates, it should also comprise bio-functional properties, which can be subdivided in nutritional and physiological or biological functionality [24] as in (Figure 7). Nutritional properties of hydrolysates reflect for example their increased digestibility and decreased allergenicity compared to the parental proteins. The physiological properties comprise potential bio-activities of hydrolysates, which originate from the liberation of bioactive peptides. Finally, the techno-functional properties represent technological functionality, such as solubility, foam and emulsion properties (Figure 7). Some molecular characterization methods and functional properties will be discussed in the next paragraphs.

Molecular Characterization

The most commonly used parameter describing the result of hydrolysis process is the degree of hydrolysis (DH), used as an indicator of the extent of hydrolysis. Another important parameter for protein hydrolysis is the molecular weight distribution of the peptides in hydrolysates. The molecular weight distribution is indicated by SDS-PAGE [25] or by size exclusion chromatography [26]. These techniques are most often used to compare the hydrolytic action of various proteases, or to characterize hypoallergenic hydrolysates. Finally, hydrolysates are occasionally characterized by reversed phase chromatography [27], which yields detailed information about the complexity of hydrolysates, although direct comparison of hydrolysates using reverse phase chromatography (RPC) profiles is difficult [28].

Determination of the Degree of Hydrolysis

The degree of hydrolysis represents the proportion of peptide bonds hydrolysed and is calculated according to equation 1, with h being the number of peptide bonds hydrolysed and h_{tot} being the total number of peptide bonds present in the parental protein. Both h and h_{tot} are expressed in meq/g [18].

$$DH = \frac{h}{h_{tot}} \times 100\% \quad \text{.......................................Eq.[1]}$$

The h_{tot} of a protein is calculated from the protein amino acid composition [18]. During hydrolysis, a new carboxyl and a new amino group is released for each cleaved amide bond (Figure 3). Therefore, the number of hydrolysed peptide bonds can be deduced from the determination of the number of newly formed C- and/or N-terminal groups in hydrolysates. As explained before, the amino and carboxyl groups are more or less (de)- protonated after hydrolysis, depending on the pH of the solution. If hydrolysis

is performed in a pH-stat set-up, the amount of added acid or base is used to calculate the DH directly, since the addition of acid or base is related to the amount of liberated amino and carboxyl groups [18].

However, this method is only applicable for hydrolysis at neutral/alkaline (pH>7) or acidic pH (pH< 3). At pH 5 to 6 there is no net release or uptake of protons, as the protonation and deprotonation of the acid/base groups are in equilibrium. Moreover, pK values, (which is equal to $-\log_{10} K_a$, may also be referred to as an acid dissociation constant, used to calculate the degree of dissociation of the acid/base groups) and not constant during hydrolysis since they depend on the peptide chain length and on the side chain of the terminal amino acid [18]. Because of the problems occurring with groups are also needed. determination of DH from pH-stat results, other methods to determine released amino.

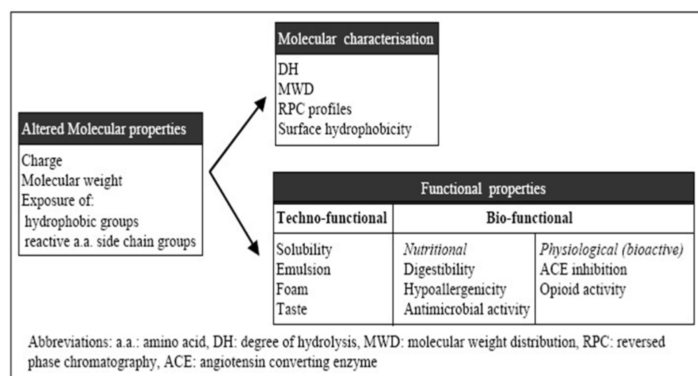

Figure 7: Changes in protein characteristics due to hydrolysis. Source: Adler-Nissen [17].

The amount of released a-amino groups can be measured using reagents that react specifically with amino groups, yielding derivatives that can be detected spectrophotometrically. Reagents that are generally used are ninhydrin, *o*-phthaldialdehyde (OPA) and trinitrobenzenesulphonic acid (TNBS). Determination of the DH with these three reagents showed that results obtained with OPA and TNBS correlate well, whereas determination with ninhydrin resulted in much lower DH values [30]. Turgeon et al., [31] preferred the OPA method over the TNBS method as it was faster and more accurate. In the present study measurement of DH was calculated by the pH-stat method as described by the work of Adler-Nissen [18].

Molecular Weight Distribution

From the degree of hydrolysis the average peptide chain length (PCL) of hydrolysates can be estimated according to equation 2, assuming that the entire hydrolysate is soluble [18].

$$PCL = \frac{100}{DH\,\%} \quad \text{Eq.(2)}$$

The peptide chain length is related to the average molecular weight of peptides in the hydrolysates. However, hydrolysates with similar PCL may have substantially different peptide molecular weight distributions. Enzyme specificity, for example, influences the peptide size distribution: a pure endoprotease will yield peptides with varying lengths, whereas an enzyme mixture that is mainly composed of exopeptidases will yield hydrolysates with mostly free amino acids combined with remaining large peptides.

Functional Characterization

Bio-functional Properties

Protein hydrolysates retain the amino acid composition of the parental protein when the hydrolysate is not yet fractionated. However, digestibility and tolerance for proteins are improved by protein hydrolysis, which can be regarded as improved nutritional properties (Figure 5). Moreover, the physiological functionality of proteins can be altered by the release of peptides with specific physiological properties (bioactive peptides) [32].

Nutritional Properties

An important nutritional reason to use protein hydrolysates is the increased digestibility of small peptides compared to whole native proteins, which has been found to be especially beneficial for patients suffering from digestion disorders, such as cystic fibrosis, short bowel syndrome or pancreatitis [33]. A problem with the consumption of protein products is the possible occurrence of allergic reactions, especially in infants towards e.g. peanut, soybean, and whole sesame seed [34]. Hydrolysis can be applied to destroy protein epitopes responsible for allergic reactions in sensitive individuals. For hypoallergenic products, proteins are usually extensively hydrolyzed, thus resulting in products containing very low molecular weight peptides [35].

Physiological Properties

Besides nutritional aspects, proteins and peptides may exhibit specific physiological properties, like mineral binding or antimicrobial activity. Bioactive peptides may be present as such in the raw material, like growth factors in sesame hydrolysates, or can be hidden in the primary sequence of proteins. The latter peptides can be released during protein hydrolysis. Several bioactive peptides have been found in plant protein hydrolysates, for example, opioid, antihypertensive, and antithrombotic peptides [36]. The antihypertensive effect of hydrolysates is related to the inhibition of the angiotensin converting enzyme (ACE). This enzyme is involved in blood pressure regulation as iconverts the inactive peptide angiotensin I to the vasoconstrictive peptide angiotensin II. Moreover, the enzyme inactivates bradykinin, which is a vasodilatating peptide [37].

Inhibition of Angiotensin-I Converting Enzyme

The angiotensin-converting enzyme is a zinc-dependent metallopeptidase, which cleaves off C-terminal dipeptides from various oligopeptides. The enzyme has broad substrate specificity and contains two substrate binding sites, with different affinities for substrates and/or inhibitors. Hydrolysis of substrates is often chloride-dependent [22]. Peptides can act as ACE inhibitors according to different mechanisms. Firstly, peptides can bind to the active site of ACE without being hydrolysed by the enzyme. These peptides are called true inhibitors. Secondly, inhibitor peptides can be substrates of ACE. These peptides are cleaved

by ACE releasing new peptides that can either be less or more active inhibitors compared to the original peptides. The peptides yielding more effective inhibitors after cleavage by ACE are called prodrug type inhibitors, and peptides yielding less active inhibitors are called substrate type inhibitors. Examples of true inhibitor type peptides are refer to as LKP, IWH and IKP, whereas LKPNM and IWHHT are examples of prodrug type inhibitors [38].

Techno-functional Properties

Molecular changes occurring during protein hydrolysis may result in the modified techno-functional behavior of the hydrolysates compared to the intact protein such as altered solubility, viscosity, sensory properties (like taste, flavor, and appearance), emulsion, and foam properties [23]. As solubility, viscosity, gelation, sensory properties, emulsion, and foam properties were studied in more detail in the present study, these properties will be discussed to a larger extent under the various proteins extracted or hydrolyzed from defatted sesame flour.

Solubility

Generally, the solubility at the isoelectric point (pI) of proteins increases with hydrolysis, which is mainly the result of a reduction in molecular weight and the increase in the number of polar groups [23]. The effect of hydrolysis on solubility at other pH values depends on the protein studied. Some oil seed protein (Soybean and peanut) for example, are very soluble at pH values above and below the pI (pH 4-5). Consequently, at these pH values the solubility of hydrolysates is not similar to their intact protein [19-39]. While some, their hydrolyzed proteins, at the pI, are slightly less soluble than their intact protein. They show increased solubility with hydrolysis over 7-9 pH range [40].

Emulsions and Foams

Emulsions and foams are both mixtures consisting of at least two immiscible phases. Foams consist of air bubbles dispersed in a continuous phase, for example, water. Emulsions are oil-water mixtures, in which either the oil is dispersed as droplets in a continuous water phase (oil in water emulsions) or in which water is dispersed in the continuous oil phase (water in oil emulsions) [41]. Formation of these dispersed systems requires energy input and the presence of surfactants, since the two phases do not mix spontaneously. The surfactants should be soluble in the continuous phase, which implies that if (water soluble) proteins or peptides are used as surfactants, the oil phase will be dispersed in the continuous water phase [41].

Emulsion and Foam Formation

During emulsion or foam formation, three processes occur simultaneously:

i) droplets are deformed and possibly broken-down,
ii) surfactants move to and adsorb onto the newly formed interfaces, and
iii) droplets collide, possibly resulting in coalescence.

The break-down of droplets into smaller ones is counteracted by the Laplace pressure (P_l), which is a function of the radius of the droplet (R) and the interfacial tension [42].

$$P_l = \frac{2\gamma}{R} (Pa) \quad \text{(equation 3)}.$$

As can be seen from equation 3, the Laplace pressure increases with decreased droplet radius, thus hindering droplet break-down of small droplets. The addition of a surfactant lowers the interfacial tension, thus facilitating droplet break-down [42].

Emulsion and Foam Stability

Once an emulsion or foam is formed, it is subject to several instability processes. For both foams and emulsions, three important causes of instability can be distinguished. These three causes result in three different instability phenomena, of which two occur in instability phenomenon.

Table 2: Instability factors of dispersions and the effect on emulsions and foams.

Cause of instability	Emulsion	Foam
Density difference Between dispersed and continuous phase	Creaming	Creaming drainage
Attraction between surfactants on interfaces of different droplets	Aggregation	
Film rupture	Coalescence	Coalescence
Laplace pressure	-	Ostwald ripening

Source [43].

This may result in the separation of the dispersion in an upper layer containing a high concentration of dispersed particles and a lower layer with a continuous phase.

This process is referred to as creaming at low disperse phase volume fraction (Ψ) and drainage at high [43]. Creaming velocity, indicated by Stokes' law (equation 4), depends on the particle diameter (d), on the density difference between dispersed and continuous phases (Ψp), on the acceleration due to gravity (g) and on the continuous phase viscosity
($\eta 0$)[43].

$$Velocity = \frac{g\Delta_2}{pd} \text{ (m /s)}. \quad \text{Equation (4)}$$

Emulsions are stable towards creaming if the emulsion droplets are sufficiently small, circa 1µm, since the creaming is then opposed by the Brownian movement. Creaming of foams occurs rapidly after beating stops, due to the large density difference between air and the continuous phase and due to the relatively large bubble diameter. After creaming, the liquid starts to drain out of the foam layer [43].

Viscosity

Viscosity which can also be referred to as rheological properties as related to flow and deformation are important functional attributes of proteins. The viscosity of the aqueous protein phase

can influence texture and stability, as well as the handling of food. Proteins are charge polymers capable of binding water and causing fiber swelling by the uptake of water and loosening of the polypeptide matrix [44].

Gelation

Gelation of proteins is a thermodynamic process that occurs widely in food processing structurally, a gel is a form of matter intermediate between a solid and a liquid, consisting of strands or chains cross-linked to create a continuous network immersed in a liquid medium [45]. Rheologically, a gel is a substantially diluted system that exhibits no steady flow [46]. Thus, collectively, a gel has been referred to as a continuous network flow [45]. Despite the difficulty in precisely predicting gel properties from the protein native structure, many of the gel properties nevertheless can be related to the structure of the "intermediates," i.e., denatured proteins and their polymers or aggregates. This is because protein gelation is a multistage process involving the flowing steps as illustrated by Ferry [46].

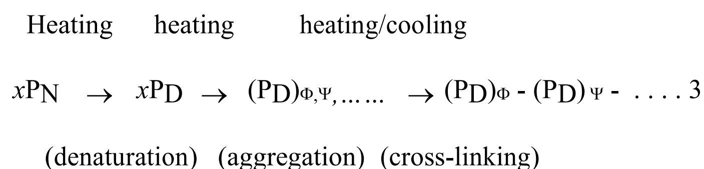

Figure 8: Steps in gelation process. Source [46].

Where x is the total number of protein molecules, and ($\Psi + \Phi + \Psi . . . = x$) is the number of molecules aggregated at a certain point of the gelation process, P_N is the native protein, and P_D is the denatured protein. The initial denaturation of protein into uncoiled polypeptides is followed by association to form aggregates or strands. When aggregation reaches a certain critical point, a gel with infinite interpeptide cross-linkages and a three-dimensional network structure is created.

Bitterness

An important negative side effect of protein hydrolysis is the liberation of bitter-tasting peptides from the protein (18). For bitterness of pure peptides, it has been shown that the presence and position of hydrophobic amino acids are related to bitterness [47,48]. Bitterness of hydrolysates is more complex and is considered to be mainly caused by small hydrophobic peptides in the hydrolysate (18). Development of bitterness in hydrolysates depend on the protein source and enzyme specificity but now that aspect can be corrected [47].

Qualitative Analysis

Separation and Identification of Peptides and Amino Acids

The separation and identification of peptides and amino acids have to be studied in order to obtain better knowledge about the composition of hydrolysates. Several methods may be used for this purpose. The high-performance liquid chromatography (HPLC), especially at reverse phase high-performance liquid chromatography (RP-HPLC), has been shown to be efficient in separating peptides from protein hydrolysates and also to give some indications about their hydrophilicity and hydrophobicity [49]. Also, the RP-HPLC technique has been shown useful in separating active peptides from protein hydrolysates. Another utilization of RP-HPLC technique in protein hydrolysate analysis is related to the identification of the specificity of different proteases. The isolation of bitter peptides from enzymatic hydrolysates is generally carried out by RP-HPLC. However, despite its high-resolution capacity, the RP-HPLC gives only a partial characterization of protein hydrolysate [49]. In fact, the separation criteria of these phases, based on the hydrophobicity or on the charges of peptides, are not good for characterizing protein hydrolysates from a nutritional point of view, where their quality is associated with the peptide size [49].

In recent years, different silica gels chemically bonded with hydrophilic compounds have been commercially found, and have been used for steric size-exclusion chromatography (SSE-HPLC), but for this work, HPLC was used to characterize the peptides and amino acids.

Estimation of the Molecular Weight Distribution

In some cases, the interest in characterizing protein hydrolysates is not related with the separation and identification of peptides, the determination of their molecular weight (M_W) distribution may be useful also. In fact, the peptide size profile has been correlated with the antigenic and functional properties of different protein hydrolysates and used for characterizing protein cleavage using various proteinases and for showing differences in pretreatments of the substrate [18].

The method of choice for M_W determination, because of its simplicity and rapidity, has been most frequently SDS-gel electrophoresis. However, recent developments in support for HPLC have made more rapid separations possible, and thus gel permeation or size-exclusion chromatography (SE-HPLC) has become a widely used technique that allows easier quantification, better recovery, and resolution than that achieved by gel filtration with conventional materials [32].

Quantitative Analysis

Evaluation of Peptide and Amino Acid Contents

The techniques used for the quantitative analysis of protein hydrolysates are based on two essential principles: the direct quantification or the preliminary fractionation of the different components followed by a quantifying method. However, the best characterization is obtained when the quantification procedure follows a fractionation step [30]. Among the different methods described for quantifying the components in chromatographic fractions of protein hydrolysates; the ultraviolet (UV) measurement has largely been used. As a rapid quantifying technique exists, the improvement of the analytical process for characterizing protein hydrolysates, especially when the interest is related to the small peptide contents, depends on the availability of a chromatographic support completely free of secondary interaction, where only

separation based on size occur. In this work, the quantification of the amino acid components was done to ascertain the amount of hydrophobic and as well as hydrophilic amino acids when bitterness was investigated in the hydrolysates studied.

Correlation Between Various Properties of Hydrolysates

The functional properties of hydrolysates will differ from that of the original protein and will depend on the peptide compositions of hydrolysates. The interactions between protein source, hydrolysis conditions, and hydrolysate characteristics are schematically depicted in Figure 9 [18]. Little is known about the precise correlations between the hydrolysis conditions and the hydrolysate properties, or the correlations between molecular and functional properties. In the present study, three multivariate data analysis techniques were used, which are briefly discussed in the next paragraph.

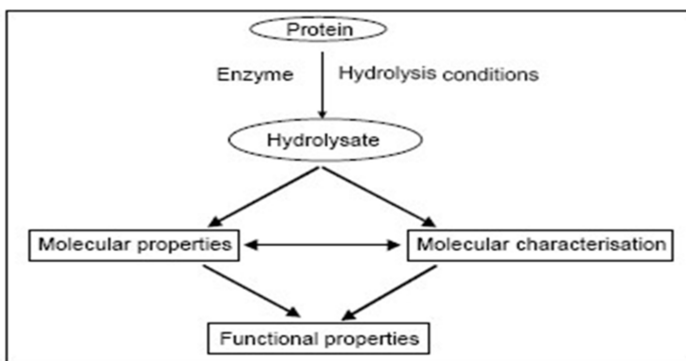

Figure 9: Relation between hydrolysis process, hydrolysis characteristics, and detection methods. Source [18]

Multivariate Data Analysis

Multivariate data analysis techniques are statistical methods applied to extract meaningful information from large data sets [50]. Three multivariate data analysis techniques were applied in the present study:

- Principal Component Analysis (PCA), to study similarities and differences between samples.
- Regression analysis with highly correlated data, using the partial least squares (PLS) method to study correlations between diverse molecular characterization methods and between molecular characteristics and functional properties.
- Regression analysis with independent regression variables to optimize hydrolysis process conditions. The settings for the studied hydrolysis parameters were defined using experimental design and results were analyzed with Response Surface Methodology (RSM).

Principal Component Analysis (PCA)

As described before this system studies the similarities and differences between samples. Hydrolysates can be characterized by various biochemical and functional properties (hydrolysate parameters) and analyzed for it similarities and differences. Each hydrolysate is described by a unique set of values for all measured parameters, which allows for discrimination between hydrolysates. The measured parameters may be correlated to each other, implying that they contain partly overlapping information. Therefore, the differences between samples (hydrolysates) are described more efficiently (*i.e.* with fewer parameters) if only independent variables are used, each containing unique information.

Regression Analysis using Partial Least Squares (PLS)

Regression analysis is used for the determination of a relation between a set of, for example easy easy-to-measure, predictive variables (regressors) and a response variable of interest. With regression models responses for new variable settings can be predicted and insight about relations between regressors and response variables can be obtained. If the data set is first decomposed to a set of new principal component's (PC's) and these PC's are used for regression, the method is called Principal Component Regression (PCR) [51]. In this method the response parameter is not considered for the calculation of the new PC's. In this regression model, during the calculation of the new vectors (PC's), the response parameter is taken into account [51]. This results in a model where the first PC (usually) explains the most variation in the response parameter. In the present study, this method was used for regression analysis with correlated data since RSM was used to optimize the protein recovery.

Regression analysis using experimental design and response surface methodology (RSM)

The above-described methods are generally used with arbitrarily chosen samples and variables. To describe samples, a large set of variables is measured and subsequently, correlations are studied. If all variables have to be optimized consecutively many experiments are needed, especially when variables have interactive effects. Therefore, experimental designs are developed for efficient experimentation. One example of an experimental design is the central composite rotatable design (CCRD) as described by Cochran & Cox [52] and Box-Behnken [53] factorial design which were used in the present study. In those designs, different levels are always defined either five or so levels are defined for each variable coded as 0,1,-1, a, and -a. The value of a depends on the number of variables studied. For three variables the experimental space is represented in Figure 10 [54]. The central composite rotatable design experiment, with all variables set at level 0, cube experiments, which are combinations of –1 and 1 settings, and star experiments, in which one variable is set at an extreme level (a or -a) and the other variables are set at level 0. The advantage of a rotatable design is the constant variance of a response variable at a fixed distance from the center point. The central composite design is one of the most important designs used for the study of quadratic response surfaces [54]. From the performed experiments a response surface model can be calculated in which the output variables are a function of the x-variables: $y = f(x_1, x_2, x_3, \ldots x_k) + e$. Most commonly, quadratic models are used, considering linear as well as quadratic and two-factor interaction effects. With the regression parameters two-dimensional response surfaces, reflecting the effect of variation in two process conditions on one response variable, can be constructed. With these response

surfaces optimal variable conditions can be estimated [54].

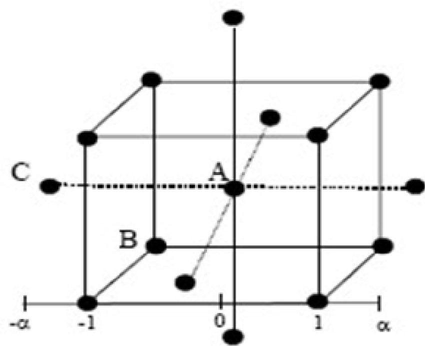

Figure 10: Schematic representation of a central composite rotatable design with five levels. Source [54].

Amino Acids Nutritional Importance

Amino Acids are the chemical units or "building blocks" of the body that make up proteins. Protein substances make up the muscles, tendons, organs, glands, nails, and hair. Growth, repair and maintenance of all cells are dependent upon them. Next to water, protein makes up the greatest portion of our body weight [55]. Amino acids (AAs) that must be obtained from the diet are called essential amino acids (EAAs) other AAs that the body can manufacture from other sources are called non essential amino acids (nEAAs).

Humans can produce 10 of the 20 amino acids. The others must be supplied in the food. Failure to obtain enough of even 1 of the 10 essential amino acids, those that we cannot make, results in degradation of the body's proteins, muscle and so forth to obtain the one amino acid that is needed [56]. The essential amino acids are arginine (required for the young, but not for adults), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, trypto-phan, and valine [57]. These amino acids are required in the diet.

Research work status in the world and current trends in the field of sesame protein: Justification for the research

Numerous researches have been conducted and reported on the enzymatic hydrolysis of some oil seed like peanut, soybean, faba bean, cowpea, sunflower [19,20,34,58]. While generally few research have been conducted in the area of defatted sesame protein for it end product to be utilized as protein supplements for infant formulation as sesame is produced in many countries in Africa and Sierra Leone been among those countries that produces sesame in larger quantity it was hope to be utilized as a new source of protein supplement for infant complementary food formulation and even protein supplement in breakfast meals for the aged. This work investigated on the right medium and conditions that allowed the isolation of the protein from defatted sesame flour with the right amino acid for infant consumption and even adult as food supplements.

Generally, few enzymatic studies have been conducted in sesame and reported [58-60], but no report is available on the characterization of the protein extracted by water, enzymatic hydrolysis and compare the two protein extracted for their functional attributes, while most of the research work conducted in sesame is more related to its oil [61-66]. There has been no publication on any systematic study of the effects of enzyme treatment on the various functional properties of proteins hydrolyzed by different proteases in different hydrolytic conditions for sesame and utilized the end product as protein supplement.

The Aim of the Research

The aim of this research was to isolate and characterize the protein from sesame seed, compare the functional properties of its native protein and hydrolysates, and the application of the hydrolysates as protein supplements in infant food formulation.

Research Objectives

The present study differs from reported studies by its holistic approach. Analytical characteristics and functional properties of a large set of hydrolysates are determined and subsequently analyzed with different parameter data analysis. This approach was initiated to improve the understanding of correlations between molecular and functional properties of hydrolysates from defatted sesame flour. Thus, the objectives are to:

1. Investigate the biochemical properties of white and black sesame seeds
2. Isolate proteins from defatted sesame flour with water only,
3. Study different enzymes with different enzymatic conditions to come up with the best enzyme with the highest protein recovery,
4. Characterize and compare the functional properties of the protein isolate from defatted sesame flour with protein hydrolysates obtained from objective 2,
5. Investigate the use of simple and less expensive methods to debitter and desalt the hydrolysates,
6. Formulate and carry out experiments related to the application of the debittered and desalted sesame protein hydrolysates as a protein supplement to infant food formulation.

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