Ogori AF, J Food Sci Nutr 2023, 9: 164
DOI: 10.24966/FSN-1076/100164

HERALD

HSOA Journal of Food Science and Nutrition

Review Article

Bioactive Protein and Peptides; A Potential Pharma Ingredient For 21st Century Functional Foods

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Abstract

Bioactive peptides have upsurge advancement in food and health domains in recent years. This was driven by the understanding of amino acid structure and functions in plant and animal as bioactive peptide sources. The food functionality of plant and animal peptides bioactivities are driven by their function -structure relationships. The interplay between function -structure of protein polypeptides from native state to denatures state has opened new research waves and much needed innovative ingredient or recipes to formulate functional foods on our shelves, however, this is far more important than pharmaceutical drugs but now as décor food or pharma foods. Application of peptides ingredient for making functional foods so as to develop, utilize, manage population, family and the individual in chronic disease and health conditions were emphasized. Aligning ingestion of bioactive peptides, enveloped as functional foods with circadian rhythm and metabolic curves within appropriate time scale for enhanced nutrition could maximize absorption, utilization, and metabolic responses. Hence, the use and potential of functional foods for individual and societal interventions and the overall societal health scale up.

Keywords: Bioactive; Decor peptides; Foods; Functional; Nawadays; Nutrition

Introduction

Nutrient bioavailability in developing countries is on the decline and nutrient availability for cells in-vivo is at a dilemma, and somehow lacking, because of the rising degenerating nutritional diseases, leading to cancer, diabesity, hypertension, tissue failures and loss of biological integrity of cells as well as aging and other cellular mutagens which are fast on the rise, hence reducing human life expectancy. Human biophysical anatomy is made of largely protein micelles, these protein cellular microstructures (skeletons) are largely involved in biological phenomenon for sustaining life. Our aerosol laden chemical environment ranging from insecticides to preservatives poses health challenges from distance approach. These could alter plant natural biochemical pathway, overturning ecological niche and cycles, introducing other myriad disease initiations that could be trace to handling, malnutrition, and oxidative stresses. Oxidative stress result from free radical and reactive oxygen species through cell redox activity damaging macromolecules, homeostatic and pathological disruption [1,2]. Excess release of rennin and angiotensin converting enzymes from the liver, kidney or lungs have been traced to hypertension. Diabesity is another ravaging disease associated with impaired metabolism of proteins, fats, and carbohydrates however now not linked to only the violation of carbohydrate metabolism to glucose [3]. This disease has been projected to become pandemic in 2040 in Africa [4]. The use of pharmaceutical drugs is proven with negative hepatic issues and high cost [2,5], hence the motivation to use food bioactive as a décor for the management of these physiological disorders and these food sources are available and affordable. Health promoting foods are on the increase as derived bioactive because they can protect against disease progression [6]. Today, it is known that certain proteins meal, concentrate, isolates, hydrolysates, peptides and peptides fractions present in foods do participate in cellular metabolism in an interactive way by inherent bioactive peptides, proffering nutritional functions (nutraceuticals) that could be established from bounded native proteins, to reduce nutritional disease conditions. The utilization and success of plant derived protein as ingredient depends upon the plant beneficial qualities impact- able to food and food systems, which solely depends on their nutritional and functional properties. Amino acid as a building block food component do participate in the normal functioning of living organisms as well as contribute to food functionalities. This is because interactions of encrypted peptides with other components of the food system make it possible to exhibit better functionality. Peptides with nutritional disease alleviating potentials have been opined [7-9]. Bioactive peptides molecular stereo-functionality do help to prevent and manage chronic diseases as well as improves health. Extracted bioactive ingredient in designing functional food especially in developing countries could define better health domain, reduce stress on medical facilities and cost of healthy living in our deep recess villages and towns. This review focus on creating more awareness of the use of functional bioactive food ingredient to decorate and enrich our processed or semi processed food at any state along our food and supply chains.

Sources and Extractions Protocols of Bioactive

Animal source proteins and peptides

Animal proteins do have complex macromolecules with varied polypeptide chains or structures encoded in either the primary, secondary, tertiary and quant nary structure of the amino acids. α-helix and the β -sheets are called secondary structures. With complete set of amino acid in its finite structures. Secondary amino acid structures

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Citation: Ogori AF (2023) Bioactive Protein and Peptides; A Potential Pharma Ingredient For 21st Century Functional Foods. J Food Sci Nutr 9: 164.

Received: August 17, 2023; **Accepted:** September 13, 2023; **Published:** September 20, 2023

derived from animal source do have cross linked with other biomolecules m this could trigger diseases, including high blood pressure and heart diseases [10].

Plant source of proteins and peptides

Food bioactive protein and peptides majorly comes from animal or plants. Plant food protein have been arrayed from legumes, nuts, cereals, vegetables and food waste materials [10]. However, peptides fractions from chicken muscles [11]; peptides fractions expressed from flaxseed [12]; Peptides fractions expressed from sweet potato [13] has multi-functional properties for health enhancement.

Plant proteins contain numbers of side chains of amino acid monomers. These side chains interact to form structures with large molecular weights, which makes them suitable as stretchable biopolymers and bio adhesives. The polymer string from where the peptides are attached could be folded or open via process protocols that could defined their further function -structure or conformational relationships. Major plant proteins are sourced from oilseed usually produced as byproducts of processing oils and starches. Plant (legumes) proteins are widely used as major ingredient for food, feed, pharmaceutical, nutraceuticals [10].

Preparation and Extraction Protocols of Bioactive Peptides

Biological material as plant protein involves homogenization during analysis. Plants are more difficult for protein extraction protocols due tissue richness in enzymes and artifacts [14]. Amino acids have pockets in protein bodies imbedded in cell walls of the plant. The mass disruption of plant cells is required for total solubilization and extraction. The general procedure for sample preparation strongly depends on the plant type and sources.

Techniques for Cell Mass Disruptions

Different biochemical techniques could be used to break the cell wall. The usual methods are the mechanical homogenization, temperature treatment, pH adjustment, solubilization and precipitation of mixtures.

Mechanical homogenization is commonly used method for cell disruption during protein extraction. To homogenize dry sample, usually open blade homogenizers, otherwise known as blenders could be employed. Mechanical homogenization seems to be one of the best methods for disruption of plant with strong cell walls. However, Fukuda *et al*. (2003) reported the use of colloid milling and homogenization to perform bran disruption and extract rice protein. Colloid milling and homogenization did not result in any significant denaturation of proteins. They further recommend mechanical homogenization with buffers for effective protein extraction, like those in rice (Fukuda *et al.,* 2003) and olive tree seeds (Alche *et al.,* 2006).

Temperature treatments employs for protein extraction include the use of freeze–thawing and heat treatments processes. Freeze-thawing employs ice crystal formation on the tissue during the freezing processing. Flash-freezing using liquid nitrogen at temperature that is less than -4℃ to -8℃ before mortar homogenization results in death of cells (Bader *et al.,* 2011). A lot of work has been done on protein extractions from plant cell mass, such [14] as the work of [14], extracted alfalfa leaves protein using temperature treatments of (-5℃). also an efficient cell disruption method for grape berry clusters, which were shrived, frozen with dry ice at (-4℃) using a stainless-steel blender was developed. This method however increased the yield of protein extracted with improved functional properties especially in recalcitrant plants protein materials.

Heat is common in protein processing. Heating protein solution above ambient temperature improve protein functionality (Khan *et al.,* 2011). However, Khan *et al.* (2011) suggested that the protein solutions should not be heated to the point of denaturation as this could affect the functionality of the proteins. De Mesa-Stonestreet *et al.* (2010) suggested heating of protein solution below 70℃ preserve the functionality of the proteins as observed in Sorghum grain proteins.

Protein solubilization is a key step in protein preparation after the cell mass has been disrupted. Solubilization is generally employed during proteomic separation. This strongly affects the quality of the final sample results and the process could determine the success of the entire experiment. Looking at the variety of protein and the huge number of interfering crosslink present in food-derived extract, however unguided solubilization of proteins is a great challenge. Each protein food material requires specific approach that could be optimized to minimize breakdown and modification of proteins (Bodzon-Kulakowska *et al*., 2007). Organic solvents for protein solubilization and precipitation from plant materials are trichloroacetic acid (TCA) or acetone. These common solvent or solvent combination for extraction of plant proteins has been used to extract proteins from different tissues of plants. The TCA chemical property that has extreme pH and negatively charged ions and the acetone being an immediate denaturation of the protein do arrest the activity of proteolytic and other modifying enzymes. Another disadvantage of TCA as precipitating solvent in proteolysis are that they are difficult to be re-dissolve (Nandakumar *et al.,* 2003) and upsurge respiratory diseases [15].

Defatted Meal Proteins

In this process, vegetable or plant seeds intended for human consumption are ground and treated with organic solvents to remove the oil. This is done under mild heat or cold conditions to retain the functionality of the proteins. The resulting defatted flour usually have protein content of about 40% and above may be further defatted to obtain a product that is much richer in protein [16].

Isolated Protein Concentrate

Soluble carbohydrates (oligosaccharides) and minerals can be extracted with cold water, with a water-ethanol mixture, or with hot water. Most vegetable proteins remain desirable insoluble, under these conditions, but the use of water acidified at the protein isoelectric pH is the best approach to minimizing, unfolding, aggregation and loss of functional properties of protein. The result is protein concentrate that contains, after drying, 60-70% protein contents. The properties of the concentrates are however influenced by the method of preparation (Cheftel *et al.,* 1985).

Protein isolates

It is a form of protein which is produced by dissolving the proteins of flours in alkaline water followed by centrifugation to remove the insoluble polysaccharides. Re-precipitation occurs at the isoelectric pH followed by centrifugation and washing of the protein sample to remove soluble carbohydrates salts. Protein isolate is obtained after lyophilization. This has about 80-90% protein contents.

Protein hydrolysates (peptides)

They are mainly products derived from the hydrolysis of protein (protein isolates or concentrate). Enzymatic proteolysis using protease enzyme is the most preferred method for acid or alkali hydrolysis. Protein hydrolysates products are oligopeptides consist two to twenty amino acids that have beneficial effects on human health outside addition to basic nutritional influences (Chalamaiah *et al.,* 2018). Peptides are hidden and inactive within the sequence of the protein molecule but could be released by enzymatic proteolysis, through fermentation and or gastrointestinal digestion. They are products derived from hydrolysis defined by peptide producing peptides with different sizes and functionality. This type of protein structure modifications by enzyme hydrolysis has an effect on peptides chemical and function properties [17]. It has been reported that, notable modifications that triggers functionality include molecular weight of the peptide chain, polar groups which increase hydrophilicity and molecular conformation (Liceaga and Hall, 2019).

The attacking species includes on peptides are organic acids, alkaline or enzymes which can disrupt peptide bonds. However, acid and alkali hydrolysis are less expensive procedures, but difficult to control and the protein yield are low with poor protein quality and functionality [18]. On the other hand, acid hydrolysis has been reported that acid hydrolysis is used in the production of flavor enhancers (Liceaga and Hall, 2019). Enzyme hydrolysis is more expensive compared to chemical hydrolysis. Enzymatic hydrolysis by proteases cleave peptide bonds at N-C terminals specific sites and hence increases degree of hydrolysis (Haard, 2001; He *et al.*, 2013).

Structure-Activity Relationship of Peptides Structure and Integrity

Structure-activity relationship (SAR) analysis was to elucidate specific peptide structure and their integrity to enable short period in seeking proteins with high potentials as bioactive peptides. This is based on known of the amino acid sequence and the integrity of employed enzymes to encrypt the peptide bond. Based on a for mentioned parameters, computer programs were established that could predict the number of peptides released by specific enzymes with potential to exercise different bioactivities are referred to as protein digestibility *in silico* [19].

Gu *et al.* (2011) employed proteins as precursors to ACE inhibitory peptide, with the aid of SAR *in silico* approach. This process established makes more appropriate amino acids substrates in preparation of biopeptides. These *in-silico* digestion of proteins present in foods are generated based on the specifics of the proteolytic enzymes. For instance, enzyme thermolyzing 5,709 peptides within 2 - 6 amino acid strand has been elucidated *Insilco*. However, some peptides were resistance to certain enzymes like endopeptidase and exopeptidase but other peptides on the same strands were potentially absorbable with antihypertensive potentials Gu *et al*., (2011).

Quantum and Tempo Approach of Functional Foods

The characteristics and dynamism of matter and energy at the atomic and subatomic scales is also providing frame work for the elucidation of peptides from plant or animal in relation to human disease conditions. The human body is bodied by matter and energy as well. Food component can also be quantum defined because of energy , matter and time in an atomic scale Danik and Sarah ,(2023)) .These theories emphasized optimal bioactive components , quantity or dosage in relation to bioavailability that could affect change or influence diseases condition with time in an atomic scale . The atomic scale could be likened to be the blood vessel or cellular occluded parts of the patients cell [20]**.** The application of quantum and tempo theories to functional foods and components could contributes to advancement in precision nanotechnology, delivery systems and bioavailability thereby facilitating the upsurge of innovative functional food products with improved efficacy , bioactivity in relation time [20,21]. Applying these theories, can ensured that functional foods that contain enough bioactive ingredients could be deliver at desired health target and the out benefit advertised to the consumer. Danik and Sarah, (2023) was able to show potential bioactive ingredient, squalene at high dosage at the appropriate time and quantity required to influence superoxide dismutase levels. However, cautioned that treatment using squalene for a duration exceeding eighty-four days may not be feasible.

Bioactive in the Management of Oxidative Stress

The imbalance between free radicals and the scavenging ability of endogenous anti-oxidants usually result in oxidative stress. When ROS become excessive at production within the cells, delicate organs may be damaged and other biomolecules triggers could result in the development of chronic diseases [22]. Natural peptides, in comparison to synthetic compounds are believed to be safe and of more natural antioxidants and protective agents to alleviate oxidative damage and associated diseases [22]. The antioxidant properties of bioactive peptides however depend on certain specifics such as; enzyme specificity, degree of hydrolysis, size of the peptides, molecular weight, amino acid composition and hydrophobicity of the peptides [23]. The antioxidant potentials of peptides are their ability to scavenge free radicals, abstract electron and chelate metal ions [24]. These abilities are enhanced by the presence of certain essential amino acids [25]. It has been recorded that alcalase and proteinase were more efficient proteases in releasing bioactive peptides from rapeseed with a better antioxidant properties when compared to certain endopeptidase and exopeptidases (He *et al*,. 2013). Studies have also shown some potent peptides with antioxidant property that have been released from food sources, such as cow's milk [26], eggs (Chen *et al*., 2011), soy protein [27], fish [28], wheat [29], chickpeas [30] and African yam bean [31].

Certain nutritional diseases have been proposed mediated by oxidant species, these antioxidant compounds can inhibit, or prevent radical-initiated or elucidate mechanistic activities (Krinsky, 1992). The know how of the various mechanisms by which bioactive peptides are able to play roles as an anti-oxidants in the control of oxidative stress are linked to related ailments are known. But tailored peptide and encrypted peptide sequences from desired amino acid composition could potentially scavenge, reduce reactive oxygen species and reactive nitrogen species well as inhibits lipid peroxidation [2]. Several mechanisms of antioxidant potential of derived bioactive peptides have been proposed (Dai and Mumper, 2010). The mechanistic approach prosed include:

i. Radical scavenging species. This includes ROS and KNO. Their action is to scavenge free radicals via peptide bonds and hydroxyl substituents.

ii. Suppression of reactive oxygen species is another mechanistic approach via inhibition of certain pro-oxidant enzymes and chelating of transition metal ions that are involved in catalyzing free radical production.

iii. The up regulation of the function of the antioxidant enzyme-linked defense mediated by endogenous anti-oxidants. Enzyme modulation of cellular physiological process and biochemical reactions is another up regulation activities.(Vattem et al,. 2005).

Biopeptides and sizes influence antioxidant potentials or action in hydrolysates from varieties of sources [27]. Je *et al*. (2007) was able to obtained peptides from dark muscles of varieties of tuna (*Thunnus obesus*) through the enzymatic action of several enzymes and discovered great deal of anti-oxidant potentials ingredient in the protection of functional foods. [32] was able to describe antioxidant activity of peptides derived from hydrolysates of several species of fish based on size and these authors reported that low molecular weight sized peptides have better antioxidant potentials compared to large molecular weight peptides. The work by (Mader *et al*., 2006) revealed low mw peptide when flavoenzyme was used compared to when alcalase was used to hydrolyze fish protein. But [27] reported on a number of peptides with antioxidant potentials isolated from different species of fish.

Bioactive in Diabetes Management

DM, another chronic metabolic disorder result from defective insulin production and is characterized by hyperglycemia. Prevalence of diabetes mellitus (DM) is on the increase because of aging, population growth, increasing urbanization, incidences of obesity, and more sedentary lifestyles [7]. The regulation of alpha-glucosidase and dipeptidyl peptidase IV (DPP-IV) enzyme in T2D through satiet response and regulation of incretin hormones regulations are the major mechanistic approach to reduce the activity of carbohydrate degrading digestive enzymes [33].

Bioactive as Supplementary ingredients in Functional Foods

BAPs are formed during protein hydrolysis, during normal digestive process or in controlled proteolysis *in vitro*. Presently, little is known about the quantities, bioavailability and bioactivity of the peptides that are formed in the digestive process of foods (Sgarbieri, 2017). Quantifying and measuring the activities of peptides is necessary to break the structure of the proteins, either as food component or as an isolate, extract or concentrate. The employed technology for extraction, such as hydrolysis, concentration, and purification as well as determination of the amino acid composition and sequence of the peptides are available and have been established, however very expensive. (Sgarbieri,2017) [34-37]. The cost-benefit analysis for peptide production ought to be evaluated to justify the investment, considering both economic and human health aspects. Developed countries have used functional products with application of bioactive peptides (Sgarbieri,2017). Such instance is in the fish industry. Fish hydrolysates have the potential applications as functional ingredients in different foods because hydrolysate from fish have excellent physiochemical and functional properties (Chalamaiah *et al.,* 2012). Unlike developing and underdeveloped countries , much has not been done with respect to the application of bioactive peptides and hydrolysates as food ingredients, in the formulation of specific functional consumed by the general population .The gap created by lack of applied technology for production of biopeptides in developing countries maybe due to ineffective utilization of plant and animal bioactive peptides due to the lack of harvesting protocols .Another consideration may be low or no synergy between universities, research institutes and the food industry, for the development of integrated multidisciplinary projects with research institute, industry, and funding agencies for functional food research and technology process. The discovery of new components in foods could transform human nutrition or healthy living, considering non-nutrients, but for being bioactive could contributed to improve health in some complementary aspects.

The 21st Century Need of Functional Foods

The 20th Century is a great time to achieve status coque human nutrition and health due to advances in bioactives , preventive and curative medicine with respect to contagious and communicable diseases, There has been predominant increase of the elderly population and an increasing incidence of chronic and degenerative diseases with multiple cause and much more complex treatments, such as cardiovascular diseases, cerebrovascular disease, diabetes, obstructive lung disease, inflammatory diseases, obesity [38-40], and osteoporosis. Due to these diseases, satisfactory solutions are still being needed by food and nutrition. Considering the premise that the human species is still evolving, the goal of achieving an ideal diet seems unattainable and maybe striving to reach a frontier that moves by evolutionary pressure. However, with the advance in genetics and human metabolism, the concepts of nutrigenomics (genomics, proteomics, metabolomics), quantum and tempo approaches can now be applied, which enables the development of special diets for specific individuals and groups, to solve some dietary problems in the short, medium and long terms.

Bioactive, Flexibility and the Challenges for the twenty first century

According to Kremer *et al*. (2015) there is growing concern that health is not merely the absence of disease but also continuum in adaptation to environmental changes, this arose the need for functional food to with stand the evolving environmental fluctuation at molecular levels [40,41]. Another definition of health emphasizes the ability of the organism to adapt to the constant challenges of physical, social, and emotional triggers Huer *et al* .(2001). Healthy organism can maintain physiological homeostasis through changing circumstances, in the context of metabolic health, this ability to adapt has been called "phenotypic flexibility" (Schulkin 2004; Van –Ommen *et al* .2014). Excess or lack of active food components in diet induces changes in phenotypic flexibility Sgarbieri. (2017). Micronutrient and bioactive compound could play certain key roles in the maintenance of phenotypic flexibility, but high intake of glucose, sucrose and fructose or certain trans-fatty acids could cause a decline in phenotypic flexibility. (Sgarbieri. (2017). The micronutrients are involved in many metabolic processes with exclusive and interactive functions in the body, and which have been studied mainly in isolation. Human health is based on a complete network of interactions between metabolic cycles, mechanisms, processes and organs. Many of these processes must work in a constantly changing environment such as diet, infections, stress, temperature, exercise, therefore, in constant struggle to maintain internal homeostasis and adapting to these changes (Schukin, 2004). According to Kremer *et al*. (2015), progress in nutrition-related health, are characterized by complexity that exists in the interaction between the nutrients and the human biophysiochemical domains [41-44].

The agenda of global nutrition, the integrated nutritional research is needed using a system of health markers that cover relevant aspects of the domains of physical, mental and social health but chronic nutritional diseases has a stem root. Considering the much literature available on what has been developed in functional foods and nutrigenomics [45,46], as well as the new concepts of nutrition and health, in the last century to the present, it is unexpected fact that the concept of functional food at the public domain still leave much to be desired. Considering the resource at disposal for research and development are become increasingly scarce and scientific progress has become more complex in recent times. It is clear today that more surg for the need for joint action through functional foods and Molecular sciences as a pivotal way to achieve the desired objectives and progress in food and nutritional improvement is part of a larger goals.

Conclusion

Decorating food matrix with bioactive ingredients to make functional foods offer valuable properties beyond basic adequate nutrition, such as antioxidant, diabetic and hypertension in the prevention of certain diseases. Bioactive ingredients or materials, when pinched across various foods chains during processing as pharma ingredient, have the potential to reduce chronic disease and their risks involved. The definition of functional food however is a continuum and their benefits are shown through specific biomarkers, promoting overall individual health and reducing the risk of chronic diseases. Suitable dosage and timing for consuming décor functional (pharma food) food material are at a crucial stage in the application in food to make functional food products (pharma food) a drug food, this is been envisaged. Continued research into décor or functional food Science and technology are essential for advocacy and to build understanding of complex relationship between food and health, and to harness the potential of functional foods to improve overall health and manage and possibly to halt chronic diseases progression.

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