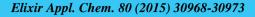
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Isolation of Bioactive Components (peptides) from Functional Foods Utilising **Bioinfomatics** approach

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ABSTRACT

Recent research has shown that the role of food has gone beyond serving as fuel, providing energy needed to perform daily functions, and to maintain the normal metabolic process, but it now provides an opportunity to improve health, reduce the risk of chronic disease and reduce health care cost. Such foods are known as Functional Foods; they provide more than simple nutrition by supplying additional physiological benefits and promoting health to the consumer. In the past decades, the traditional process of isolating bioactive compounds from food is usually very long, tedious and slow, which takes years before tangible results are obtained. The advent of bioinformatics has helped researchers to improve on the purity, yield, time and cost of their search especially in the drug industry but unfortunately not in the food industry, due to lack of specific bioinformatics coded mining tools. A combination of experimental mining and an integrated computational bioinformatics database approach is herein illustrated for the mining of novel bioactive peptides from functional foods.

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Introduction

In the last decade researchers have used different techniques for the extraction of bioactive components from natural sources. The structure and functionality of these molecules obtained are related to the method used and their natural source. The benefit of food has gone beyond the traditional form of providing energy and growth; it is now seen to provide health benefits by reducing the risk of chronic disease and enhancing the ability to manage these chronic diseases, thus improving the quality of life. Functional foods are known to promote growth and development and enhance performance [1]. Functional foods vary in forms, some may be conventional food with bioactive components capable of providing health related activities [2], while there are those fortified or enhanced with specific compounds to increase the level present in the food and for the reduction of disease risk e.g soybean protein, cranberries, calcium fortified orange juice [3].

Recent researchers have identified functional food components that may improve memory, reduce arthritis, reduce cardiovascular diseases and provide other benefits typically associated with drugs [4,5]. This development has alerted food industries to mining food with the aim of developing functional foods containing bioactive components [6]. The health benefit of foods to consumers in terms of disease control, based on their health enhancing components in the food is on the increase as compared to the nutritional benefits [7].

The Japanese have coined the phrase Food for Specific Health Use (FOSHU) in 1991, in place of functional foods. There are over 69 foods that have been approved by the Japanese authority with their ingredients shown to have enough evidence to support some health claims [8]. The Japanese system relied on this list of approved food and it has helped them to regulate both safety and health as it demands that all foods must be analyzed for the amount of effective components

Tele: E-mail addresses: sirepberezi@yahoo.com contained [9]. Foods or their ingredients that are eligible for FOSHU designation can be classified into the familiar categories of carbohydrates, proteins, fats, mineral, vitamins etc needed for the body healthy existence, as shown in Table 1.

Examples of Bioactive Components in Food and Health Benefits. The naturally occurring chemical compounds contained in or derived from a plant, animal or marine source, that exert the desired health.

Examples of Functional Foods Ingredients Probiotics and Prebiotics

The human system contains various kinds of bacteria, the ones that are injurious to the body and those that are friendly. Antibiotic therapy, stress and poor dietery choices may cause intestinal dysbiosis (a bacterial inbalance which causes over growth of bad bacteria and yeast) [12]. Probiotics are the beneficial bacteria that can be found in various foods, common strains include Lactobacillus and Bifidobacterium families of bacteria. Probiotic bacteria like lactobacilli are naturally found in fermented foods like sauerkraut and yogurt [13]. Prebiotics are non-digestible foods wellness benefit are regarded as the bioactive compounds [10], while the functional ingredients are the standardized and characterized preparations, fractions or extracts containing bioactive compound of varying purity used by manufacturer in the food [11]. That make their way through our digestive system and help good bacteria grow and flourish. Probiotics and Prebiotics can restore the balance of bacterial in our digestive tract [14].

Protein and Peptides

Protein is the long chain polymers of amino acids while peptides represent the shorter forms. Peptides from soybean protein have been shown to be capable of preventing the production of cholesterol by liver cells, which can lead to lower levels of cholesterol in the blood [15]. A wide range of activities has been described for bioactive peptides including

antimicrobial and antifungal properties, blood pressure lowering effects, cholesterol lowering ability, antithrombotic effects and enhancement of mineral absorption [16].

Carbohydrates and Fibres

Carbohydrates take the form of a wide range of sugars, oligosaccharides, starches and fibres and are one of the three major macro-nutrients which supply the body with energy. Apart from the direct benefits of carbohydrates for the body, to provide fuel for physical performance; dietary fibres, it helps keep the bowel functioning correctly [17]. There is now good evidence that at least 55% of our daily calories should come from carbohydrates. Dietary fibre is found in plant foods (fruit, vegetables and whole grains) that cannot be fully digested and is often called bulk or roughage. Soluble fibre, which can dissolve in water, is found in beans, fruits and oat products and can help to lower blood fats and maintain blood sugar. Insoluble fibre cannot dissolve in water, so passes directly through the digestive system. A high-fibre diet may reduce the risk of developing diabetes and colorectal cancer [18, 19].

Lipid and fatty

Fish oil has the ability to reduce blood pressure and lower the risk for other cardiovascular disorder due to the presence of omega-3 [docosahexaenoic (DHA) and eicosapentaenoic acids (EPA)] and omega-6 fatty acids [20]. Other omega fatty acids such as linoleic and linolenic acids are abundant in fish oil, vegetable oil (canola, soybean and sunflower) and nuts such as peanuts and almonds [21].

Vitamins and Minerals

The clinical and epidemiological evidence is clear that select vitamins (folate, vitamins E, B6 and A) and minerals (iron, calcium, selenium and iodine) play a significant role in the maintenance of optimal health and are limiting in diets [22]. Vitamin C plays an important role in providing antioxidant protection; vitamin C supplementation is associated with a substantial reduction in risk of cataracts. Pliz et al reported that, vitamin D may play a role for cardiovascular health and in the prevention of osteoporosis [23].

Bioinformatics

The traditional methods of identifying these bioactive compounds is a very long and tedious process which may involve digestion of the food, followed by experimental analysis of the various hydrolysate to determine the bioactive set of components (peptides) responsible for the specific activity. Most often mass spectrometry is used to investigate the type of peptide present in the hydrolysate. As a result of this slow experimental route, the time and cost involved in most cases yield very small quantity for commercial products. The need for high yield and purity of these bioactive components which comes with inherent difficulties when using the traditional methods, led to the development of new advance technologies such as Bioinfomatics. It has provided innovative approaches to facilitate and ease the production of specific components that can serve as nutraceuticals.

Definition

It is conceptualising biology in terms of molecules (in the sense of physical chemistry) and applying informatics techniques to understand and organise the information associated with these molecules on a large scale [24]. In summary it is defined as the application of computational techniques to analyse the information associated with biomolecules on a large scale.

The aims of bioinformatics are subdivided into three;

• Organisation of Data. This allows the researcher to access existing information and to submit new ones as they are produced.

• Develop tools and resources that aid in analysis of collated data. E.g after sequencing a particular protein it is compared to previously characterised sequences. This requires expertise in computational theory as well as a thorough understanding of biology.

• The use of these tools to analyse the data and interpret the results in a biologically meaningful manner [25].

Bioinformatics is now taking the front burner in a wide range of subject areas including structural biology, raw DNA sequence, genomics, protein sequencing, gene expression, etc [24]. This novel technology has provided solution to researchers as they now get results in a matter of few weeks but surprisingly, very little success has been achieved using bioinformatics in mining for functional food bioactives.

Reasons have been put forward to explain the slow success of bioinformatics in food bioactive peptide mining. Some of the reasons are as follows;

1)Absence of bioinformatic mining tools specifically coded for the food research as compared to that used in the drug industry. It is only recently that the food industry is realizing that functional bioactive food is the food of the future [26]. Drug companies have always used state-of-the art technology and science in contrast to the food industry [27].

2)Lack or insufficient systematized bioinformatic tools for food companies to meet the special requirements for mining food bioactives [28].

3)Slow integration of both experimental and the in-silico food mining research. This is due to the different methods adopted from lab to lab which make it difficult to have a generic consensus or structure, hence no worldwide acceptable database in the food industry [29].Techniques previously used for Isolation of Bioactive Components. The following methods which are bioinformatic based have been used to isolate bioactive compounds from other sources but not on food. Mathed 1

Method 1

Evolutionary Conservation: Study on effect of bioactive peptides on human platelets function by Edwards et al [30], used a rational design specifically targeted at juxtamembrane regions, that were predicted to have signalling activity and could also be modulators of platelets function (Fig.1). They were able to narrow down the test samples using the evolutionary conservation method applying bioinformatics screening approach. This computational approach allowed the researchers to only test samples that most likely (conserved) carry this functionality rather than the random testing of all samples.

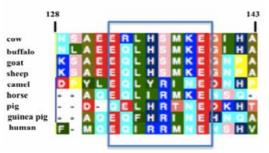


Figure 1. Alignment of the Region 128-143 of Bovine α-S1-Casein beween Related Species.

Table 1: FUSHU foods and Ingredients

FOSHU Foods / Ingredients				
CARBOHYDRATES	PROTEINS			
Polydextrose	Casein phospho peptide			
Indigestible dextrin	Casein dodeca peptide			
Galacto oligosaccharides	Soy protein			
Lactulose				
Lactosucrose	MINERALS			
Isomalto oligosaccharides	Phosphorus			
Maltitol	Calcium as citrate malate			
Palatinose	Heme iron			
Soybean oligosaccharides				
Fructo oligosaccharides	OTHER			
Xylo oligosaccharides	Rice globulin			
Wheat bran	Eucommia leaf glycoside			
	Lactobacillus GG			

Table 2

Functional components	Source	Potential benefits
Source: International Food Informa	tion Council	
(http://www.sourcewatch.org/index	.php?title=International_Food_Information_Council))
Carotenoids		
Alpha-carotene		
Beta-carotene	Carrots, Fruits, Vegetables	Neutralize free radicals, which may cause damage to cells
Lutein	Green vegetables	Reduce the risk of macular degeneration
Lycopene	Tomato products (ketchup, sauces)	Reduce the risk of prostate cancer
Dietary Fibre		
Insoluble Fibre	Wheat Bran	Reduce risk of breast or colon cancer
Beta-Glucan	Oats, barley	Reduce risk of cardiovascular disease. Protect against heart disease
		and some cancers; lower LDL and total cholesterol
Soluble Fibre	Psyllium	
Fatty Acids		
Long chain omega-3		
Fatty Acids-DHA/EPA	Salmon and other fish oils	Reduce risk of cardiovascular disease. Improve mental, visual functions
Conjugated Linoleic Acid (CLA)	Cheese, meat products	Improve body composition. Decrease risk of certain cancers
Phenolics		
Anthocyanidins	Fruits	Neutralize free radicals; reduce risk of cancer
Catechins	Tea	
Flavonones	Citrus	
Flavones	Fruits/vegetables	
Lignans	Flax, rye, vegetables	Prevention of cancer, renal failure
Tannins (proanthocyanidines)	Cranberries, cranberry products, cocoa, chocolate	Improve urinary tract health. Reduce risk of cardiovascular disease
Plant Sterols		
Stanol ester	Corn, soy, wheat, wood oils	Lower blood cholesterol levels by inhibiting cholesterol absorption
Prebiotics/Probiotics		
Fructo-oligosaccharides (FOS)	Jerusalem artichokes, shallots, onion powder	Improve quality of intestinal microflora; gastrointestinal health
Lactobacillus	Yogurt, Other dairy	
Soy Phytoestrogens		
Isoflavones:	Soybeans and soy-based foods	Menopause symptoms, such as hot flashes Protect against heart disease
Daidzein		and some cancers; lower LDL and total cholesterol
Genistein		

Method 2

Amino Acid Composition and Preference

This method can be used to define some disease and for the discovery of new food bioactive peptides. Most of these bioactive peptide perform a given function because of their structure and amino acid composition (Fig.2). A study examined the quantitative structure-activity relationships of angiotensin-1-converting enzyme (ACE) inhibitory peptides. These peptides are known to have an effect of reducing blood pressure; as such they are very useful as cardiovascular functional food [31]. The researchers' mined pea, bovine milk and soybean proteins, they were able to predict seven (7) bioactive peptides that are carrying an angiotensin-1- converting enzyme (ACE) inhibition effect.

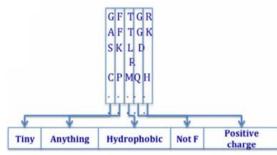


Figure 2. An illustration of where patterns of amino acids are established based on amino acid preferences at certain positions with all peptides carrying similar functionality Method 3

Mimicking Approach: This approach had been very effective in the drug industry. A study by Aitken et al and Mochly- Rosen et al on protein kinase C (PKC), a family of serine / threonine Kinase enzymes which are associated with several diseases including diabetes[32]; stroke [33]; heart failure[34, 35]; and cancer[36] showed that two unrelated proteins share a common region of binding with PKC. The results revealed that one of the protein after binding prevented access of the active enzyme to its substrate, leading to inhibition (Fig.3). This competitive inhibition of PKC by a region that mimics its interaction with other proteins is potentially a good method to indentifying bioactive peptides.



Figure 3. Mimicking a region of interaction between two proteins, the new peptides can inhibit the interaction of both proteins.

Method 4

Positive Selection: Khaldi and Shields[37] working on the significant shift in charge and isoelectric point of milk protein between different animals, used the positive selection method, they were able to predict bioactive peptides and many of their residues due to the differences between amino acids composition at certain positions in different species milk protein. They concluded that positive selection seems to be an indicator for the presence of bioactive peptides in a protein sequence and that the peptides that contain these selected residues must be tested. **Method 5**

Molecular Recognition Features (MoRFs) and Short Linear Motif (SLiMs): These are other methods employed for the prediction of functional regions in protein sequences. Studies showed that both methods have short comings in predicting commercially viable bioactive peptides from functional foods. An explanation for the SLiMs failure is attributed to the fact that they are found in disordered regions of a protein. Disordered region are the post-translationally modified regions, phosphorylated and glycolsylated. This makes it difficult for enzymes to reach and cleave potential peptides in these regions {38, 39].

Database integration: The availability and ease to access information assists researchers in developing, discovering and breaking new grounds. This information makes analysis less cumbersome and predictions to be more defined, hence the need for a centralized database for bioinformatics information on bioactive peptides that have been tested on an assay. This database will contain both published and unpublished data so that users of the information will only select the assay they need, that conforms to known and widely acceptable [6].

The database will integrate with other food related bioactive peptide to enrich the set of peptides for each disease, thereby increasing the prediction power of new bioactive peptide, as shown in Fig.4.

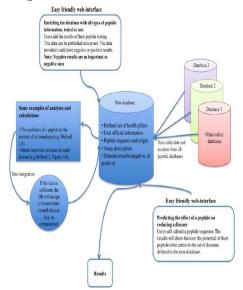


Figure 4: Schematic representation of a centralized database. Culled from Khaldi Nora (2012).

The data in the central database will constantly be updated to keep up with the latest testing bioinformatic methods.

Mining for New Bioactive Peptides

In the search of a new bioactive peptide it is recommended that the combination of experimental works at various levels and bioinformatics approaches will enhance the isolation of a bioactive peptide from its hydrolysate. In all methods used, it will start by experimentally generating the hydrolysate by digesting the functional food with different enzymes or different bacterial organisms under different conditions. The hydrolysates so produced are then experimentally tested in different assays for different diseases. It is at this point that bioinformatic computational approach is applied to organise, manage and analyse this large amount of data and also used in predicting the fragment that are most likely positive as bioactive peptides. In this study, three approaches will be described.

Selection of Functional Peptides Common between hydrolysates

In this approach, the selection of those hydrolysates that show positive action towards a disease is extracted out from the many hydrolysates tested. This step is to reduce the number of candidate peptides to a smaller fraction, which can then be tested individually to show the true bioactive peptide(s). Mass spectrometry test on each fraction can also be performed to further confirm the selected peptides [41, 42, and 43].

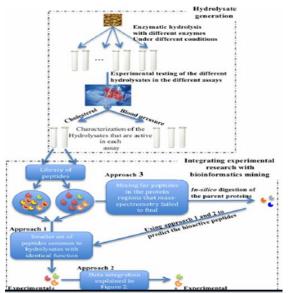


Figure 5. Intergrating experimental results with bioinformatics

Application of Bioinformatics.

The most profitable research in bioinformatics often results from integration of multiple sources of data (Gerstein, 2000). This method is employed to further reduce the set of candidate peptides obtained from the first approach. Here specific characteristics are searched for, based on the aim of the research to further narrow down the bioactive peptide. In most cases the in-silico prediction of peptides is run parallel to any hydrolysate testing and mining. The different methods and the more information that can be integrated together will assist to give a better predictive power for bioactivity [6, 26, and 44].

Filling the Gaps.

In most of the methods used, it is only a few bioactive peptides visible to mass spectrometry that are captured, which had earlier provided information of the active fragments present in the hydrolysate. Therefore filling this gap becomes very necessary: this is achieved by computationally digesting the proteins of the original functional food of interest (Fig. 5). Knowing the enzyme that was used to obtain the hydrolysate and applying computational bioinformatics, one can easily predict the areas where the enzyme will cleave which were hidden from mass spectrometry. The peptides found in this approach can be put through approach 1 and 2 to get a final small group of peptides that can be validated experimentally [41, 44 and 45]. Conclusion

Development of functional food is an expensive process for many food industries. These companies are used to the traditional methods in the research for new food product formulation. The integration of bioinformatics with food mining especially with functional foods had been very slow. Some reasons adduced are; the lack of bioinformatics mining tools, specifically coded for food research, the inadequate systemization of experimental assay testing for bioactive compounds. Lack of centralized database that bioinformatics can mine.

This work has described a method that can be used to mine for new bioactive peptides. The using of multiple approaches and integration of bioinformatics from a centralized database of previous and on-going researches can help to increase the predictive accuracy of bioinformatics in food mining.

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