FOOD MATRICES FOR THE DELIVERY OF ANTIHYPERTENSIVE PEPTIDES IN FUNCTIONAL FOODS

MATRICES ALIMENTARIAS PARA LA LIBERACIÓN DE PÉPTIDOS ANTIHIPERTENSIVOS EN ALIMENTOS FUNCIONALES

Ramírez-Torres GI[1,2], Ontiveros N[3], López-Teros V[1], Suarez-Jiménez GM[4], Cabrera-Chávez F[5,*]

[1] Departamento de Ciencias Químico Biológicas, Universidad de Sonora. Hermosillo, Sonora, 83000, México.
[2] Escuela Superior de Educación Física, Universidad Autónoma de Sinaloa. Culiacán, Sinaloa 80019, México.
[3] Departamento de Ciencias Químico Biológicas y Agropecuarias, Universidad de Sonora. Navojoa, Sonora 85880, México.
[4] Departamento de Investigación y Posgrado en Alimentos. Universidad de Sonora. Hermosillo, Sonora, 83000, México.
[5] Unidad Académica de Ciencias de la nutrición y Gastronomía. Universidad Autónoma de Sinaloa. Culiacán, Sinaloa 80019, México.

ABSTRACT

Many food-derived peptides with antihypertensive activity have been reported. However, a reduced number of studies have been conducted to prove in vivo the efficacy of most of the currently reported antihypertensive peptides. Thus, just a few of these bioactive peptides are utilized as supplements or ingredients for functional foods production. In addition to in vivo evaluations, another challenging task is the delivery of bioactive peptides in physiological conditions, but studies about this topic are scarce. Notably, some proteins are able to form gels that have different characteristics related to the pH of the environment. Bioactive peptides can be entrapped into such gels structure and be released in different physiological environments (e. g. low pH in the stomach and neutral in the intestine). Thus, the selection of macronutrients could play a critical role in the design of food matrices intended to be used as containers and releasers of antihypertensive peptides.

Keywords: Bioactive compounds; Food matrix; Bioavailability.

RESUMEN

Muchos péptidos derivados de los alimentos con actividad antihipertensiva han sido reportados. Sin embargo, un reducido número de estudios han sido conducidos para probar la eficacia de la mayoría de los péptidos que actualmente reportan actividad antihipertensiva. Así, solo unos pocos de esos péptidos bioactivos son utilizados como suplementos o ingredientes para la producción de alimentos funcionales. Además de las evaluaciones *in vivo*, otra tarea que resulta un reto es la liberación péptidos bioactivos en condiciones fisiológicas, pero los estudios sobre este tópico son escasos. Notablemente, algunas proteínas son capaces de formar geles que tienen diferentes características relacionadas con el pH del medio en el que se encuentren. Los péptidos bioactivos pueden quedar atrapados en las estructuras de esos geles y ser luego liberados en diferentes ambientes fisiológicos (e. g. bajo pH en el estómago y neutro en el intestino). Así, la selección de macronutrimentos puede jugar un papel crítico en el diseño de matrices alimentarias para ser usadas como vehículos y liberadores de péptidos antihipertensivos.

Palabras clave: Compuestos bioactivos; Matriz alimentaria; Biodisponibilidad.

INTRODUCTION

Many peptides derived from a wide range of food proteins (animal and non-animal protein sources) and with anti-hypertensive properties have been reported. From this large spectrum of peptides, just a few of them have been tested in clinical trials. For instance, in the website of the U.S. National Institutes of Health only 3 studies can be found entering the key words 'antihypertensive peptide' categorized as 'dietary supplement' and just two involving the dietary intervention with peptides into a food matrix (US NIH, 2017). Although antihypertensive peptides generally show lower antihypertensive activity than synthetic drugs, either *in vitro* or *in vivo*, they are less likely to accumulate in the body and to trigger side effects (Li-Chan, 2015). Thus, many studies have been conducted in the last two decades to discover new antihypertensive peptides with potential to be used as ingredients in functional foods.

Often food-derived antihypertensive peptides are tested *in vitro* being the inhibition of the angiotensin-converting enzyme (ACE) (EC 3.4.15.1) the most common test (Aluko, 2015). Certainly, to be physiologically relevant *in vivo*, the peptides should remain unaltered through the gastrointestinal tract and be transported across the intestinal epithelium (Figure 1). Finally, they must preserve their bioactivity into the blood serum conditions to perform specific physiological functions (Norris and FitzGerald, 2013). This is particularly relevant when testing functional foods. Since *in vivo* the bioactive compounds must be released from the food matrix in sufficient amount to play a significant role in the organism, *in vitro* assays are considered as preliminary studies. In this mini-review we have focused on the digestion of proteins and absorption of peptides as well as on the role of food matrices characteristics in the delivery of antihypertensive peptides.

*Autor para correspondencia: Francisco Cabrera-Chávez
Correo electrónico: fcabrera@uas.edu.mx
Recibido: 04 de septiembre de 2017
Aceptado: 26 de abril de 2018

Sources of antihypertensive peptides

Antihypertensive peptides naturally occurred in foods, either from animal or non-animal sources (Table 1). Until now, the main animal sources of antihypertensive peptides are milk proteins (Hernández-Ledesma *et al*., 2011; Beltrán-Barrientos *et al*., 2016). In fact, the antihypertensive peptides commercially available and often used as nutraceutical ingredients for functional foods are derived from cow's milk casein (Ishida *et al*., 2011; Martítez-Maqueda *et al*., 2012; Cicero *et al*., 2012). Dziuba and Dziuba, (2014) showed five antihypertensive milk-derived products commercially available and just two of them were food product as it (sour milk and margarine). On the other hand, antihypertensive peptides from non-animal sources have been widely studied, but in most cases, the antihypertensive capacity of such peptides was tested *in vitro* or *in vivo* in animal models of hypertension (García *et al*., 2013). Martínez-Maqueda *et al*., (2012) reviewed the different antihypertensive peptides tested *in vivo* in murine models and their effects on the systolic hypertension. From the total of antihypertensive peptides reported in the literature, 47 were derived from milk protein, 10 from egg protein, 40 peptides from other animal protein sources, and 41 peptides were derived from non-animal sources (Martínez-Maqueda *et al*., 2012). Although some of the peptides showed antihypertensive potential *in vitro*, even better than those commercially available, there was a lack of studies proving their efficacy in human beings. This is an important issue that has hampered the use of antihypertensive peptides as nutraceutical ingredients for functional foods development.

Protein and peptides digestion and absorption

The bioactive properties of peptides are linked to their amino acid composition and sequence. However, the amino acid pattern of food-derived peptides is strongly influenced by the specific cleavage of gastrointestinal enzymes and this could limit the bioaccesibility of specific peptide sequences (Cian *et al*., 2015). Proteins and peptides digestion start at the stomach by the action of pepsin and acidic pH. At this site the acidic condition denatures/unfolds proteins increasing availability for the attack by gastric pepsin. This enzyme preferentially attacks peptide bonds involving hydrophobic aromatic amino acids i.e., phenylalanine, tryptophan. The resulting polypeptides are subjected to further hydrolysis by a set of pancreatic enzymes (trypsin, chymotrypsin, elastase, carboxypeptidases) with different peptide bonds specificities. This process generates polypeptides and amino acids at intestinal level. Further peptide bonds hydrolysis is mediated by brush border peptidases resulting in bioaccessible peptides (Kohlmeier, 2015). These peptides could become bioavailable either in an intact form or biotransformed into smaller peptides with increased or reduced bioactivity. Plasma peptidases further contribute to peptide hydrolysis limiting the plasma half-life of the peptides. Thus, to be physiologically relevant when used as nutraceuticals or functional food ingredients, food-derived bioactive peptides should

Table 1. Peptides with ACE-I Inhibition activity*.
Tabla 1. Péptidos con actividad inhibitoria de la Enzima Convertidora de Angiotensina.

(ID) SEQUENCE	SOURCE	
(3361) LKL	Sardine (*Sardina pilchardus*)	Marine animals
(7678) LKPMN	Dried bonito (*katsuwonus pelamis*)	
(7638) LTF, (7811) VWIG	Tuna (*Thunnus thynnus*)	
(7751) CF, 7751 (EY)	Shark (*Carcharhinus longimanus*)	
(7506) GPL, (7747) FGASTRGA.	Alaskan pollock (*Gadus chalcogrammus*)	
(7569) LKA, (7573) RMLGNTPTK, (7575) FQKPKR.	Chicken (*Gallus gallus domesticus)*	Farm animals
(7574) VLAQYK	Cow's Beef (*Bos Taurus*)	
(7640) MNP, (7641) TNP	Pig (*Sus scrofa ssp*)	
(7648) FCF, (7686) FFGRCVSP, (7687) ERKIKVYL, (7688) FGRCVSP, (7689) NIFYCP.	Ovoalbumin (*Gallus gallus domesticus*)	
(7806) LQKW, (7807) LLF	Caprine Milk (*Capra aegagrus hircus*)	
(3374) PFPE, (3495) YIPIQYVLSR, (7801) VRYL, (7800) FFVAPFPEVFGK, (7802) NMAINPSK, (7803) IPY, (7804) ALNEINQFY, (7805) ALNEINQFYQK, (8357) RYLG, (8358) RYLGY, (8359) AYFYPEL.	Bovine Milk (*Bos Taurus*)	
(8386) RASDPLLSV, (8387) RNDDLNYIQ, (8388) LAPSLPGKPKPD, (8380) AGTTCLFTPLALPYDYSH, (8390) RADHPFL, (8391) YAEERYPIL, (8393) HLFGPPGKKDPV, (8394) VGVIKAVDKKAGGAGKVT, (8395) QIGLF.	Egg (*Gallus gallus domesticus)*	
(7560) FFL, (7681) DG, (8511) FFYY, (8512) WHP, (8513) FVP, (7555) PGTAVFK, (8507) LSW, (8508) IVF.	Soybean (*Glycine max*)	Grains
(7646) GYK, (7680) QK, (8492) KEDDEEEEQGEEE.	Pea (*Pisum sativum*)	
(7814) MDFLI, (7815) MFDL.	Chickpea (*Cicer arietinum*)	
(7819) IAP, (7820) GPP, (8413) IPALLKR, (8414) AQQLAAQLPAMCR, (8493) NPPSV.	Wheat (*Triticum spp*)	
(7821) RIY	Rapeseed (*Brassica napus*)	
(8360) LTPTSN, (8361) LVVDGEGY, (8364) LLPSY.	Olive seeds (*Olea europaea*)	
(7558) VK	Buckwheat (*Fagopyrum esculentum*)	
(8415) VNP, (8420) VWP.	Rice (*Oryza sativa*)	
(8407) RIGLF, (8408) AHEPVK.	Mushroom (*Agaricus bisporus*)	Others
(7744) GAAELPCSADWW	Bullfrog (*Rana catesbeiana*)	
(3483) GKKIATYQER	Yeast (*Saccharomyces cerevisiae*)	
(3486) VW	Sake (*Oryza sativa*)	
(7554) GEP	Tricholoma giganteum (*Macrocybe gigantea*)	
(3486) VW	Sake (*Oryza sativa*)	
(7649) LRY, (7822) IAPG, (7823) FAL.	*Algae spp*	
(7682) NY	Garlic (*Allium sativum*)	
(7651) YKY	Wakame (*Undaria pinnatifida*)	
(7556) YPLDL	Spinach (*Spinacia oleracea*)	

* Source: Biopep Database (*www.uwm.edu.pl/biochemia/index.php/pl/biopep*).
Fuente: Biopep Database

be digestion resistant or delivered in their bioactive form at specific sites of the gastrointestinal tract (Figure 1).

Peptide transport, across the small intestine, can be mediated by different mechanisms. Small hydrophilic compounds could permeate the intestinal mucosa through the paracellular route. Neutral and positively charged di- and tri-peptides are preferentially permeated trough this route. Longer peptides i.e., six amino acids length (MW around 620), are less efficiently permeated and this is independent of their net charge (Pauletti *et al*., 1997). The transcellular route is also size dependent, but it is more likely to permeate hydrophobic peptides in part due to the lipophilic nature of cell membranes (Wada and Lönerdal, 2014). The molecular weight cut-off of this passive diffusion pathway of peptides using the Caco-2 cell permeability system is estimated at MW 700 allowing the permeation of peptidic compounds such as the renin inhibitor remikiren and the HIV inhibitor saquinavir (MW > 600) (Camenisch *et al*., 1998). On the other hand, the transmembrane protein called peptide transporter 1 (PepT1), which is located in the brush border, can transport di- and tri-peptides into the enterocyte. This mechanism is thought to be independent of the peptides net charge and lypophilic or hydrophilic properties (Freeman, 2015). Overall, peptide length (size) seems more relevant than other physicochemical properties for peptide transportation through the intestinal mucosa. Thus, after oral or intragastric administration, short bioactive peptides (3-5 amino acids length) are the ones that reach systemic circulation and show antihypertensive potential.

In vitro assessment of the bioavailability of bioactive peptides usually involves simulated digestion processes (gastric and intestinal) and absorption using the Caco-2 cell line (Ding *et al*., 2015). Until now, the antihypertensive peptides utilized in commercially available food products or sold as supplements are digestion resistant and this property could increase their bioavailability. Other food-derived antihypertensive peptides however could become more efficient or be formed after the attack of gastrointestinal enzymes. For instance, antihypertensive peptides from some ripened cheeses become more efficient after gastrointestinal digestion by human gastric or duodenal juices (Qureshi *et al*., 2012) and other peptides derived from β-casein have shown greater antihypertensive potential after *in vitro* pancreatic digestion (Maeno *et al*., 1996). Beyond the digestion resistant nature of some antihypertensive peptides, food matrices could play an important role by either increasing or reducing peptides bioaccessibility and/or bioavailability. This is particularly relevant when these compounds are intended to be used as ingredients for functional foods production.

Effect of food matrices characteristics on the bioavailability of peptides

A common definition of bioavailability of a nutrient is the amount that is absorbed from the diet and used for normal body functions (Aggett, 2010). Beyond the concept of bioavailability, some other definitions are commonly used to describe properties of nutrients. This includes bioconversion ('fraction of bioavailable nutrient that is converted into

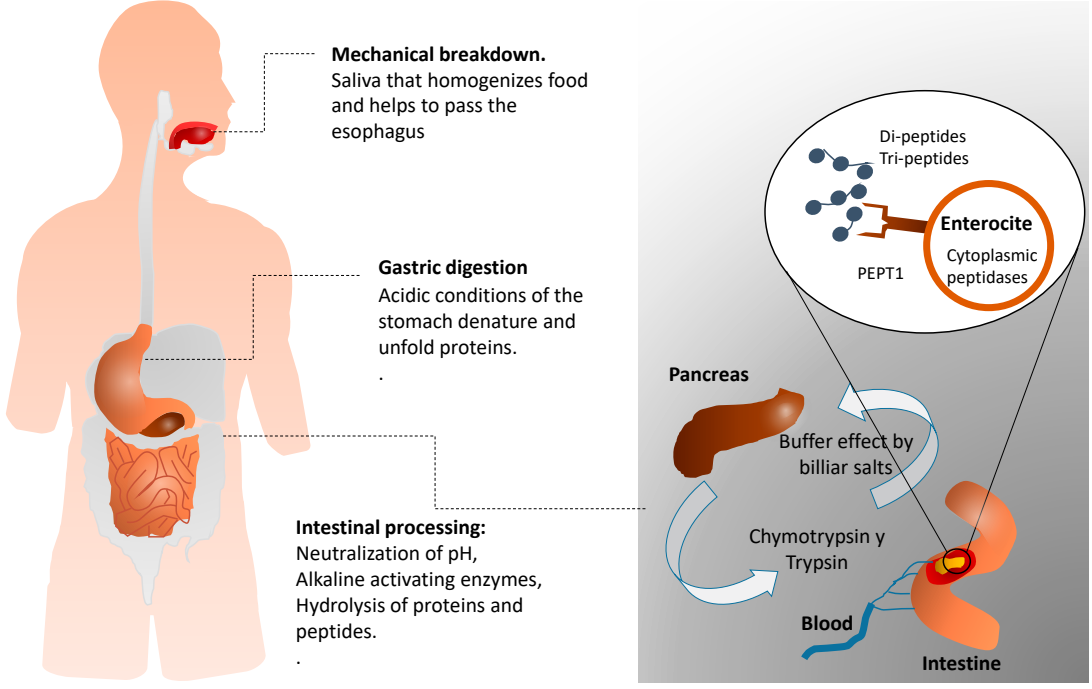

Figure 1. Physiological barriers for antihypertensive peptides.
Figura 1. Barreras fisiológicas para los péptidos antihipertensivos.

the active form'), bioefficacy ('fraction of ingested nutrient that has a nutritional effect'), bioaccessibility ('fraction that is released from food matrix and is available for intestinal absorption') and bioequivalence (Parada and Aguilera, 2007). For anti-hypertensive peptides, bioaccessibility and bioavailability are the most used terms.

The bioavailability of peptides could decrease depending on the interaction with macromolecules and/or the food matrix. These interactions vary since the magnitude of the distance into the structure of the food matrix also varies. For instance, the interaction between bioactive molecules and macromolecules (e. g. iron-protein hydrogels interactions in rice flours; polyphenol-protein (Moretti *et al*., 2006; Foegeding *et al*., 2017), in food matrix structures range from 10 to 100 nanometers (Parada and Aguilera, 2007), while the interaction between bioactive compounds and the whole food matrix (e. g. phenolic and components of food matrix components (Balasundram *et al.,* 2006)) structure is ~100 micrometers (Parada and Aguilera, 2007). Thus, the design of these complexes could be used to both to entrap and to release the peptides at physiological level. Since proteins have the ability to form stable gels and emulsions at different conditions, they are a good material for controlling the release of bioactive compounds. In fact, the physicochemical modification of macromolecules could confer them novel functions and make them more suitable to develop oral peptide delivery systems (Peppas and Kavimandan, 2006).

Food gels can be considered as three-dimensional continuous polymeric networks holding large quantities of aqueous solution that shows mechanical rigidity during the time (Foegeding, 2006). Gels from proteins have been classified as globular gels and particulate gels according to their stabilization forces and shape at microstructure level. Among the globular protein gels, the fine-stranded gels, maintained by hydrophobic interactions, are composed by flexible linear strands with elastic properties and they resist the rupture forces. On the other hand, particulate gels, stabilized by van der Waals forces, are formed by large and spherical aggregates which have low elasticity and low rupture resistance (Zuñiga and Troncoso, 2012). According to Norris and Fitzgerald (2013), peptide sequences with potential to inhibit ACE have commonly limited number of amino acids (2-12) and hydrophobic residues at the C-terminal region (e. g. Proline). This last characteristic should be taken into account in the design of food matrices intended to contain anti-hypertensive peptides with potential to inhibit ACE activity. Thus, due to the hydrophobic characteristic of Proline, this amino acid will tend to interact with the hydrophobic residues of the fine-stranded globular protein gels, and this could happen independently of the extension of the hydrophilic area of the protein matrix (Schobert and Tschesche, 1978).

The gels behavior at low (gastric) or neutral (intestinal) pH influences the rate of peptide release (Flores and Kong *et al.,* 2017). This process take place essentially by decreasing protein-protein interactions and up taking water, which promotes the diffusion of peptides outside the gel (Peppas and Kavimandan, 2006).Thus, antihypertensive peptides in food matrices can be entrapped into protein or polysaccharides gels with the required properties to be released in the adequate place throughout the human gastrointestinal tract.

Ten Have *et al*., (2015) reported that the portal bioavailability of the antihypertensive peptides XPP, is prolonged if they are embedded in a protein matrix, while in a whole-macronutrients matrix the peptides are more available but at a low percentage. The sequence XPP have been reported for the antihypertensive peptides IPP and VPP (Gleeson *et al*., 2017; Rutella *et al*., 2016). Ten Have *et al*., (2015) claim that the protein matrix improves the bioavailability by prolonging gut absorption due to a delayed stomach emptying. However, other factors could decrease the release of peptides from the food matrix during gastric digestion *i.e.,* hydrophobic interactions (Mandalari *et al*., 2014). Thus, we consider that these findings highlights that antihypertensive peptides can be bioaccessible when they are ingested in food matrices as part of the dietary treatment and this encourages the design of food matrices for functional foods with antihypertensive properties.

CONCLUSIONS

Antihypertensive peptides have not been capitalized as an adjunct in the treatment of hypertension. The design of food matrices with the capability to deliver antihypertensive peptides at intestinal level is an essential step to develop functional food products that ensures the bioaccessibility and/or bioavailability of these functional compounds. Moreover, when food matrices are designed, it is encouraged to test them in order to verify their efficacy *in vivo*, preferentially in well-designed clinical trials. Finally, we believe that independently of the food technology approaches used to design food matrices for the delivery of antihypertensive peptides in functional foods, the approaches must take into account the available food producers' technologies, scientific knowledge, and mainly, the food preferences of the target population (patients).

ACKNOWLEDGEMENTS

This manuscript was done as a part of a project financially supported by the grant PROFAPI 2014/033.

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