Trends in Food Science & Technology 80 (2018) 187–198

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

Enhancing enzymatic hydrolysis of food proteins and production of bioactive peptides using high hydrostatic pressure technology

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ARTICLE INFO

Keywords:
Non-thermal processing
Enzymatic hydrolysis
Food byproduct valorization

ABSTRACT

Background: Bioactive peptides (BPs) generated by hydrolysis of food proteins exhibit a broad spectrum of biological properties (antihypertensive, hypocholesterolemic, antimicrobial, antioxidant, etc.) in both *in vitro* and *in vivo* models. Initially obtained from milk and egg products, BPs have now largely been obtained from food byproducts such as marine, animal and plant biomasses. Amongst the various strategies being developed for BPs production, enzymatic hydrolysis (EH) is the most widely preferred due to its GRAS nature. However, the main challenge of EH is to decrease the time and quantity of enzyme, and improve yield and bioactivity of BPs.

Scope and approach: Consequently, innovative and efficient food technologies have been developed to satisfy these needs. High hydrostatic pressure (HHP) processing, a non-thermal technology, initially developed to extend food shelf-life, is being considered as a promising tool to improve the efficiency of EH and generate high value-added peptide fractions from various complex biomasses.

Findings and conclusions: This innovative and emerging technology enhances EH by inducing protein unfolding/denaturation, as well as activating the enzymes used while maintaining their nutritional and functional properties. This review discusses the state of the art of HHP technique, its applications in combination with EH, and potential challenges for the production of BPs from food-derived protein sources.

1. Introduction

Over the last decade, many studies have described the role of proteins as a source of biologically active peptides and different strategies to improve their production. Bioactive peptides (BPs), generally composed of 2–20 amino acid residues and inactive in the sequence of their native protein, have been investigated extensively as they have positive effects on physiological functions, improving health (Kitts & Weiler, 2003; H; Korhonen & Pihlanto, 2003). Hence, BPs can be added to many products or ingredients and labeled as “functional foods” or “nutraceuticals” (Hartmann & Meisel, 2007). Different strategies are used to produce BPs. Microbial fermentation and enzyme-catalyzed proteolysis is the most widely studied and applied, while autolysis and acid hydrolysis are less common. Although there have been many studies on the production and optimization of the BPs production process, there are still many challenges to developing an industrial-scale production system with higher peptide yields and lower cost. Recently, the *in-silico* approach allowed researchers to predict the production of BPs from food proteins using bioinformatics and databases. Combined with classical approaches this method can determine the best BPs production parameters, such as the type of enzyme to be used (Fu, Wu, Zhu, & Xiao, 2016; Udenigwe, 2014). Other issues include demonstrating effective bioactivity and health benefits through *in vitro* and *in vivo* experiments as well as clinical trials for pharmaceutical applications (Government, 2003). Finally, safety assessments must be completed and efficient regulatory guidelines for the use of BPs must be developed. Nevertheless, a few products are already supplemented with BPs and are available in international markets (Hartmann & Meisel, 2007; H; Korhonen & Pihlanto, 2003).

Bioactive peptide production benefits from the increasing application of novel and emerging food processing technologies. In this regard, technologies such as high hydrostatic pressure (HHP), microwave, and pulsed electric field have been recognized as three of the most promising emerging technologies with growing commercial interest (Jermann, Koutchma, Margas, Leadley, & Ros-Polski, 2015). More specifically, HHP applications have attracted considerable research attention for their ability to increase food product shelf-life (J.-C. Cheftel, 1992; J. C. Cheftel, 1995) and modulate food proteins. Diverse fields

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https://doi.org/10.1016/j.tifs.2018.08.013
Received 16 February 2018; Received in revised form 5 July 2018; Accepted 19 August 2018
Available online 22 August 2018
0924-2244/

are increasingly turning to HHP processing since it is regarded as one of the most sustainable and green technologies. Functional and nutritional properties, as well as the organoleptic quality of food products, are generally maintained after HHP treatment but modifications to protein structure and conformation may be induced (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). Indeed, HHP treatment affects significantly on hydrophobic and electrostatic bonds, but a very little on covalent bonds causing the proteins to unfold or denature (Lullien-Pellerin & Balny, 2002; Vadim V; Mozhaev, Heremans, Frank, Masson, & Balny, 1996; V. V.; Mozhaev, Lange, Kudryashova, & Balny, 1996; Rivalain, Roquain, & Demazeau, 2010). Thereupon, using HHP can alter enzyme-substrate (protein) interaction and hydrolysis rate. Over the last decade, many studies have evaluated the impact of HHP on protein denaturation and aggregation. These studies have demonstrated that HHP treatment can improve enzymatic hydrolysis of a food-derived protein (from plant to dairy and meat proteins) and enhance generation of BPs by using a large spectrum of enzymes (Bamdad, Shin, Suh, Nimalaratne, & Sunwoo, 2017; Boukil, Suwal, Chamberland, Pouliot, & Doyen, 2018; Guan, Diao, Jiang, Han, & Kong, 2018; Homma, Ikeuchi, & Suzuki, 1994). This critical review discusses the potential application of HHP as an emerging technology to improve food-derived protein digestibility and generation of BPs.

2. Bioactive peptides

Bioactive peptides are composed of 2–20 amino acid residues, generated from parent proteins where their native structure is inactive. According to their structural properties, amino acid composition, charge and sequence, these low molecular weight molecules exhibit many biological properties (Hartmann & Meisel, 2007; Hannu Korhonen & Pihlanto, 2006). Bioactive peptides have been generated from a wide range of food proteins. Milk and egg proteins (Clare & Swaisgood, 2000; Mine, 2007) are the largest category of BPs precursors (Arrutia, Rubio, & Riera, 2016; da Costa, da Rocha Gontijo, & Netto, 2007; Hernández-Ledesma, Recio, & Amigo, 2008; Hannu; Korhonen & Pihlanto-Leppälä, 2004; Madureira, Tavares, Gomes, Pintado, & Malcata, 2010; Meisel, 1998; Phelan, Aherne, FitzGerald, & O'Brien, 2009; Power, Jakeman, & FitzGerald, 2013; Silva & Malcata, 2005; Urista, Fernández, Rodriguez, Cuenca, & Jurado, 2011; Wada & Lönnerdal, 2014). However, meat, marine and plant proteins have also been used as sources of high value-added ingredients (Bah, Bekhit, Carne, & McConnell, 2015; Lafarga & Hayes, 2014) due to their high protein contents. Presently, in the context of reducing food losses and waste, most studies focus on the value of food byproducts for producing BPs.

While chemical synthesis of BPs is possible, the two most common methods of BPs production are enzymatic hydrolysis and microbial fermentation. A newer bioinformatics (*in-silico*) approach is increasingly being used that predicts the potential of a protein as the precursor of BPs by using combinations of protein sequences and enzyme specificity. Whatever the methodology used, many studies have demonstrated different peptide biological properties (antimicrobial, antidiabetic, antihypertensive, antioxidant, anticancer, hypocholesterolemic and multifunctional peptides) *in vivo* and *in vitro* (Fig. 1 and Table 1) (Hancock & Sahl, 2006; E.; Huang, Mittal, & Griffiths, 2006; S. M.; Huang, Chen, Chen, Hong, & Chen, 2010; S.-K.; Kim & Wijesekara, 2010). These properties are the most studied because of their impact on the most common diseases.

For instance, hypertension is the main risk factor responsible for premature cardiovascular disease while hypercholesterolemia, defined as an excessive concentration of cholesterol in the blood, increases the risk of cardiovascular disease (Nagaoka et al., 2001). Furthermore, diabetes is group of metabolic diseases characterized by high blood sugar levels due to either insufficient insulin secretion, insufficient insulin action, or both (Daliri, Oh, & Lee, 2017) and continuous consumption of our current synthetic antidiabetic medicines can cause weight gain (Thulé & Umpierrez, 2014) or gastrointestinal side effects

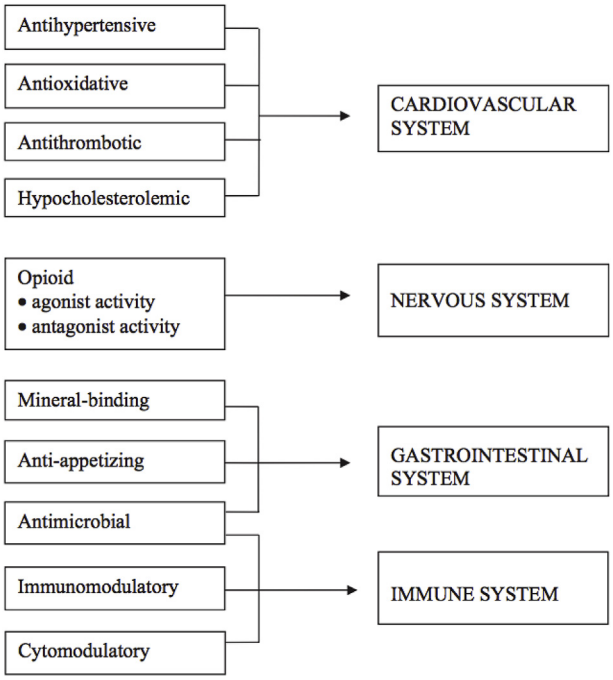

Fig. 1. Several activities from one of the main sources of BPs, milk, and their physiological targets in humans (adapted from (Hartmann & Meisel, 2007; Hannu Korhonen & Pihlanto, 2006)).

(Thong, Gupta, Blann, & Ryder, 2015). In addition, acquired immune deficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV) infection, remains a topical subject. While the efficiency of anti-HIV therapy is constantly increasing, it is essential to investigate and isolate innovative anti-HIV therapeutics from natural sources. Besides, peptides with antimicrobial, opioid or antioxidant activity are also widely studied. For instance, antimicrobial peptide are considered as better alternatives than conventional antibiotics, which have more resistance among pathogenic bacteria (Daliri et al., 2017).

Thus, interest in the commercial production of BPs is growing. However, peptide toxicity, cause and effect relationships between peptide consumption and their impact on human health, consumer acceptability and regulation of peptide products are the main challenges for commercialization. The lack of industrial-scale, viable processes also make large scale peptide production more difficult. So, food, nutraceutical and pharmaceutical industries must develop and use emerging and eco-efficient technologies to meet these ambitious challenges.

3. Production of bioactive peptides

3.1. Autolysis

Autolysis is the cleavage of proteins by endogenous proteases. In this context, the term *autolysate* is preferred to *hydrolysate*. Numerous studies have clearly demonstrated the utility of autolysis for BPs production, mostly from marine byproducts due to their high endogenous enzyme content. Depending on the protein source used, a large number of digestive enzymes were characterized, such as pepsin, trypsin, chymotrypsin or cathepsin (Song, Zhang, & Wei, 2016). For instance, autolysate (100 min–50 °C) of squid (*Ommastrephes bartrami*) viscera showed high antioxidative activities throughout the production of 19 peptides. However, several factors affect the efficiency of autolysis, such as enzyme activity, the physiological condition of the marine product, pH and temperature (Raa, Gildberg, & Olley, 1982). Nevertheless, because there are no enzyme costs for this process, autolysis is a simple and economical way to produce BPs (Shahidi & Kamil, 2001).

Table 1
Summary of peptide bioactivities, mechanisms of action and structural/functional characteristics.

Bioactivities	Mechanism	Characteristics	Commercialized product	References
Antihypertensive	Inhibition of Angiotensin I-Converting Enzyme (ACE) – blood pressure regulator	- Short chain peptides - Polar amino-acids (Proline)	Ameal S®/Calpis® (Calpis Co. Ltd., Japan) Valio Evolus® (Valio Ltd., Finland) peptACE® (Natural Factors,Canada)	(Tauzin, Miclo, & Gaillard, 2002) (H Korhonen & Pihlanto, 2003) (G.-H. Li, Le, Shi, & Shrestha, 2004) (Hartmann & Meisel, 2007) (Shahidi & Zhong, 2008) (Espejo-Carpio, De Gobba, Guadix, Guadix, & Otte, 2013) (Aluko, 2015) (Lee & Hur, 2017) (Hanafi et al., 2018)
Antimicrobial	Penetrate membrane bilayers Induce growth inhibition and death of several microorganisms	- Positively charged (naturally or using positive metal ion as a cofactor) - Low molecular weight - α-helical linear - Disulfide-bridged/open-ended cyclic - High proportions of Proline, Glycine or Histidine - Amphipathic structure - Can exhibits antioxidant activity	60 peptide drugs in the market 140 therapeutic peptides tested in clinical trials	(Borer, 2007) (Pimenta & De Lima, 2005) (Mansour, Pena, & Hancock, 2014) (Tomioka et al., 2014) (Fosgerau & Hoffmann, 2015) (Pane et al., 2017)
Hypocholesterolemic	Minimize cholesterol absorption in the human body Mechanism not well known	- Not well defined e.g. Ile-Ile-Ala-Glu-Lys (β-lg) enterostatin (soy)		(Nagaoka et al., 2001)
Antioxidant	Scavengers of free radicals Inhibitors of lipid peroxidation Metal ion chelators Inhibition of the formation of reactive oxygen species	- Aromatic amino-acids - Leucine, Proline or Histidine - Metal chelation activity = rich in Cystein, Histidine, Aspartic acid and Glutamic acid		(Hartmann & Meisel, 2007) (Shahidi & Zhong, 2008) (S.-Kim & Wijesekara, 2010) (Maestri et al., 2016)
Opioid	Defined as exorphins Binding to opioid receptors located in gastrointestinal tract Can penetrate into bloodstream	- N-terminal Tyrosine to bind receptors - Proline in position 2 to induce opioid activity - Low molecular weight		(Hannu Korhonen & Pihlanto, 2006) (Whiteley et al., 2012) (Maestri et al., 2016)
Immunodulatory	Inhibition of HIV protease Inhibition of virus proliferation Enhancement of immune activity Modulate innate immune responses			(del Mar Yust et al., 2004) (Gauthier, Pouliot, & Saint-Sauveur, 2006)
Antidiabetic	Induce insulin-stimulated glucose. Uptake Antagonize PPAR-γ activity	- Low molecular weight - Enriched in Glutamic acid, Alanine, Lysine, Arginine and Histidine		(Kwon et al., 2011) (Roblet et al., 2016) (Daliri et al., 2017)

3.2. Microbial fermentation

Some starter and non-starter bacteria already used in the food industry for large-scale production of fermented dairy products were used to generate BPs. These microorganisms, such as lactic acid bacteria (LAB) (*Lactococcus lactis*, *Lactobacillus helveticus* and *Lactobacillus delbrueckii ssp. bulgaricus*) or *Bacillus* spp., and *Bifidobacterium* are highly proteolytic since these microorganisms produce several intracellular and extracellular proteinases. Nevertheless, the microbial peptidase activity is affected by growth conditions that could help to modulate BPs production (Hannu Korhonen & Pihlanto, 2006) but could also result in non-optimal control of hydrolysis reactions. Moreover, inoculum conditions can be controlled to enhance the proteolytic potential of the microorganism used to produce BPs (Hannu Korhonen & Pihlanto, 2006). For example, CN from milk can stimulate microbial proteinase production in pH-controlled fermentations using *Lactobacillus plantarum* strain ACADC2101 and *Lactobacillus bulgaricus* strain ACADC2317 (Sakellaris & Gikas, 1991). Compared to hydrolysis reactions using commercial enzymes, microbial proteases have several advantages. It costs much less to cultivate microorganisms due to their minimal nutritional requirements and short time of maturation. The proteases of most microorganisms, mainly the LAB, are expressed on the cell-membrane which makes harvesting and purification relatively inexpensive and easy.

3.3. Enzymatic hydrolysis

The most common technique for producing BPs from parent protein molecules is through enzymatic hydrolysis (Oseguera-Toledo, de Mejia, Reynoso-Camacho, Cardador-Martínez, & Amaya-Llano, 2014) since these reactions do not result in residual organic solvents or toxic chemicals in the final products (S.-Kim & Wijesekara, 2010). Moreover, the hydrolysis conditions can be easily controlled. However, some key parameters such as pH and temperature must be measured and controlled throughout digestion for optimal hydrolysis. In addition, the duration of hydrolysis is crucial since it is directly related to the degree of hydrolysis, which influences size and amino acid composition and therefore, the bioactivities of the generated peptides (Udenigwe & Aluko, 2012). Overall, the most popular enzymes employed for BPs generation are pepsin, trypsin and chymotrypsin, as well as commercial proteases such as Alcalase™, Protamex™ and Flavourzyme™ (Shahidi & Zhong, 2008) since they release numerous BPs (Boukil et al., 2018; FitzGerald & Meisel, 2003; FitzGerald, Murray, & Walsh, 2004; Gobbetti, Minervini, & Rizzello, 2004; Yamamoto, Ejiri, & Mizuno, 2003). Different combinations of proteinases, including Alcalase®, chymotrypsin, pancreatin, pepsin and Thermolysin™ as well as enzymes from bacterial and fungal sources, have also been used to produce BPs from various proteins (Guan et al., 2018; Kilara & Panyam, 2003; Hannu; Korhonen & Pihlanto, 2006; Sánchez & Vázquez, 2017; M.; Zhang & Mu, 2017). Digestive enzymes are of particular interest since

simulated gastrointestinal enzymatic methods were developed to mimic normal human digestion of proteins to assess the possibility of releasing effective BPs after consumption of various matrices. Even though enzymatic hydrolysis is considered a safe and promising method for the generation of BPs, the reaction time and amount of enzyme(s) required are still critical for industrial implementation.

Considering the structural complexity of the food and byproduct matrices, optimum enzymatic hydrolysis is challenging to achieve due to limited interaction between enzyme and substrate, making it difficult to control and predict the reaction product. Significant research efforts have focused on developing an efficient and industrially viable process as well as pretreatment methods to improve the hydrolysis rate and minimize enzyme quantity. In recent years, various methods have been proposed to improve the hydrolysis and release of potential BPs, such as microwave, ultrasound, high voltage pulsed electric field and more recently high hydrostatic pressure (HHP)-assisted enzyme hydrolysis (HHP-EH) (Abadía-García et al., 2016; Chen et al., 2014; Kadam, Tiwari, Álvarez, & O'Donnell, 2015; Lin et al., 2011; Lin et al., 2013; Mikhaylin, Boussetta, Vorobiev, & Bazinet, 2017; Uluko et al., 2015; Wu & Majumder, 2016).

4. High hydrostatic pressure processing

4.1. Fundamental aspects

High hydrostatic pressure, also called “pascalization” or “cold pasteurization”, is a non-thermal and eco-efficient technology governed by Le Chatelier's principle, which states that any phenomenon leading to a decrease in volume is enhanced by pressure (Vadim V Mozhaev, Heremans, Frank, Masson, & Balny, 1994). While HHP was first applied at a laboratory-scale by Hite in 1989 to destroy microorganisms in milk in order to improve its shelf-life, the technology was considered an emerging process since the first industrial-scale systems were available in 1990 in Japan (Yordanov & Angelova, 2010). High hydrostatic pressure processing applies an isostatic (uniform) pressure (100–1000 MPa) transmitted instantaneously and uniformly, independent of the size and geometry of food, on flexible packaging materials filled with liquid or solid food products. The pressure transmission fluid is generally water and the process can be used with or without heat. Mostly used to improve shelf-life and preserve components of a wide range of food products, a large number of studies (Fig. 2) recently focused on innovative applications of HHP to improve the functional and bioactive properties of food products (Bermúdez-Aguirre & Barbosa-Cánovas, 2011).

4.2. Protein thermodynamics under HHP

Contrary to thermal treatments, where covalent and non-covalent bonds are affected, HHP treatment disrupts relatively weak chemical bonds (hydrogen, hydrophobic and ionic bonds, Fig. 3) (Rivalain et al., 2010). For that reason, HHP induces denaturation of native protein and modulates protein-protein and protein-solvent interactions. More specifically, pressurization of protein induces the formation of monomeric, oligomeric and aggregated species without the addition of chemicals or use of high temperature. Since covalent bonds (disulfide and peptidic, Fig. 3) are not affected by HHP treatment, the primary structure of protein is not modified. Low pressure treatment (< 400 MPa) increases the number of hydrogen bonds while higher pressure (> 400 MPa) disrupts these bonds. Therefore, HHP specifically affects protein secondary structure and the structural modifications may be reversible or irreversible depending on the pressurization parameters and the properties of the proteins.

4.3. Applications of HHP to enhance protein digestibility and improve bioactive peptide production

The impact of HHP on protein structure is of particular interest since pressurization may cause protein unfolding which could improve its susceptibility to enzymatic hydrolysis by exposing access sites for enzymes (Fig. 4). Thereby, pre-treatment of protein with HHP before enzymatic treatment or protein hydrolysis performed under HHP (simultaneous pressurization and hydrolysis) can be efficient, innovative and eco-efficient strategies for producing bioactive molecules from various sources of food proteins (Bonomi et al., 2003). The HHP parameters and their effects on bioactive peptide production from various foods and byproducts are summarized in Table 2.

4.3.1. Milk-derived peptides

Currently, milk proteins are the most important source of peptides with biological activities (H Korhonen & Pihlanto, 2003). The main protein fractions, specifically CN, α-la and β-lg, were shown to be potential sources of BPs liberated after gastrointestinal digestion, fermentation with proteolytic starter cultures or enzymatic hydrolysis (Hasler, 2000). These BPs had a range of biological properties including

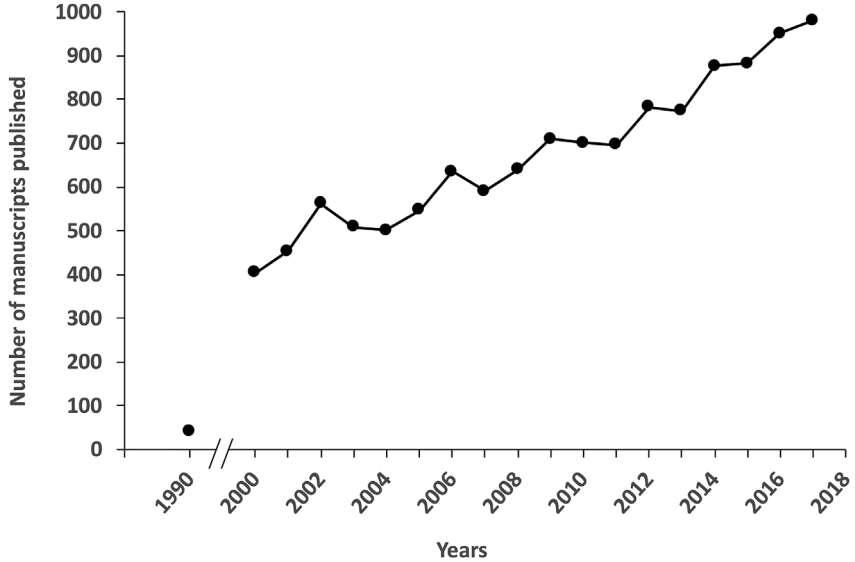

Fig. 2. Number of manuscripts published in the last decade (from 1990 to 2017) in the field of HHP. Search Engine: Web of Science with the keywords High Hydrostatic Pressure, Date: July 01, 2018.

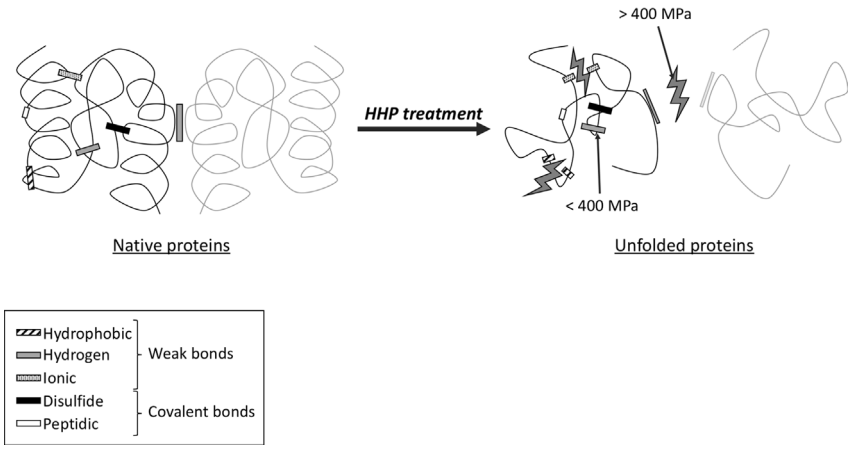

Fig. 3. Impact of high hydrostatic pressure (HHP) on weak and covalent bonds present in proteins.

anti-oxidative, anti-hypertensive, anti-microbial, immune-modulatory, mineral-carrying activity, opioid and ACE inhibitory activities (Clare & Swaisgood, 2000; Fitzgerald & Murray, 2006; Hannu; Korhonen & Pihlanto, 2006; Korhonen, 2009; Hannu; Le Maux, Nongonierma, Barre, & FitzGerald, 2016; Marcone, Belton, & Fitzgerald, 2016; Meisel & Bockelmann, 1999; Meisel, 1997, 1998; Mohanty et al., 2016; Phelan et al., 2009; Pihlanto-Leppälä, 2000; Silva & Malcata, 2005; Smacchi & Gobbetti, 2000). Numerous studies reported that HHP modified protein structure and caused protein denaturation and aggregation. These observations led to studies on the impact of hydrolysis under high pressure on milk protein digestibility and production of BPs. Other strategies use HHP as a pretreatment to destabilize milk proteins to improve their susceptibility to enzymatic hydrolysis (Boonyaratanakornkit, Park, & Clark, 2002; Boukil et al., 2018; De Maria, Ferrari, & Maresca, 2017; Funtenberger, Dumay, & Cheftel, 1997; Gaucheron et al., 1997; Heremans & Smeller, 1998; Lullien-Pellerin & Balny, 2002; López-Fandiño, 2006; Park, Namkung, Ahn, & Paik, 2004; Rivalain et al., 2010; Tedford, Kelly, Price, & Schaschke, 1999). A large number of enzymes such as Thermolysin™, pepsin, trypsin, Pronase™, chymotrypsin, pancreatin, Elastase™, Flavourzyme™ and Savinase™ have been used with dairy products to generate BPs.

Globally, HHP-EH leads to an increase in the rate of hydrolysis for pressures under 400 MPa (Bamdad et al., 2017; Beran et al., 2009; Blayo, Vidcoq, Lazennec, & Dumay, 2016; Chobert et al., 1996; De Maria et al., 2017; Dufour, Hervé, & Haertle, 1995; Izquierdo, Alli, Gómez, Ramaswamy, & Yaylayan, 2005; Knudsen, Otte, Olsen, & Skibsted, 2002; V. V.; Mozhaev, Heremans, et al., 1996; Stapelfeldt, Petersen, Kristiansen, Qvist, & Skibsted, 1996; Van Willige & Fitzgerald, 1995; Zeece, Huppertz, & Kelly, 2008), whereas, pressures above 400 MPa inactivates enzymes such as trypsin and chymotrypsin with little or no improvement in digestibility. These studies showed that applying pressures above 400 MPa (De Maria et al., 2017; Maynard, Weingand, Hau, & Jost, 1998) decreases the degree of hydrolysis. Also, in comparison to EH, pretreated protein hydrolysis and simultaneous pressurization and hydrolysis (HHP-EH) process significantly modifies the peptide profiles in the hydrolysate with the generation of new peptide species and an increase in the concentration of specific peptides (Boukil et al., 2018; Dufour et al., 1995; Knudsen et al., 2002; Maynard et al., 1998). For instance, the enzymatic hydrolysis using chymotrypsin (Knudsen et al., 2002) and trypsin (Boukil et al., 2018) generated more

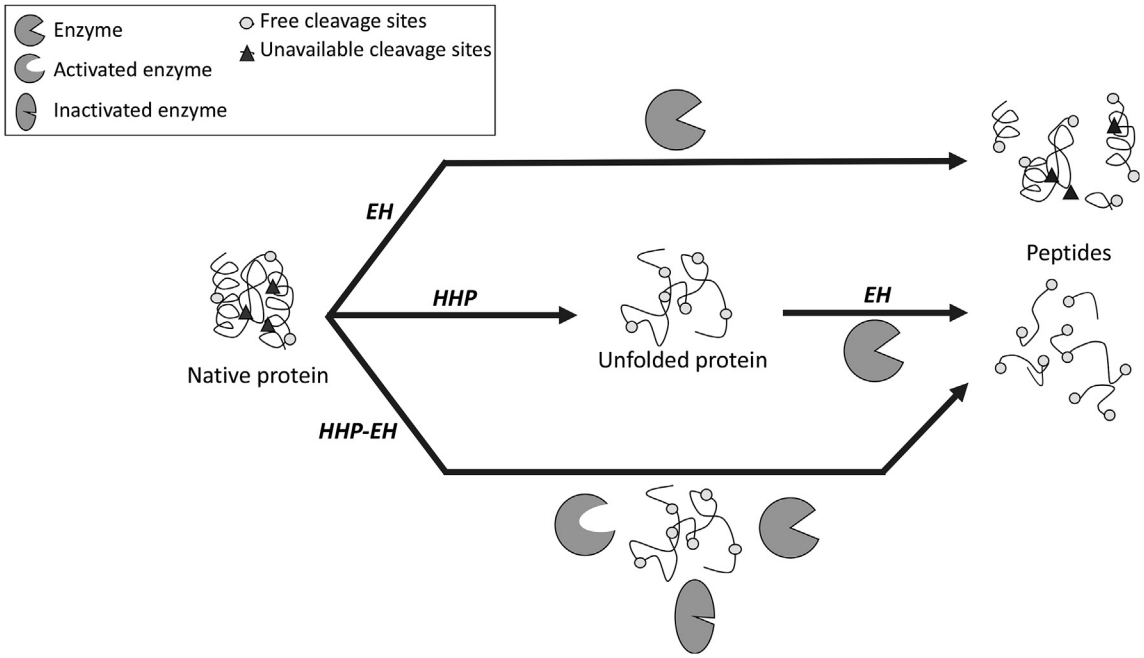

Fig. 4. Mechanisms of improvement of enzymatic hydrolysis (EH) of food proteins assisted by high hydrostatic pressure (HHP) (pre-treatment – HHP + EH, or simultaneously HHP-EH) for the production of peptides.

Table 2
Application of HHP for the production of BPs from different protein sources.

Source		HHP Parameters	Enzyme	Results	References
Milk	β-lg	50–300 and 350 MPa 150 min (S)	Thermolysin™ and pepsin	Increased rate of hydrolysis Increased sensitivity to pepsin Modification of peptide profiles	(Dufour et al., 1995)
	β-lg	100–300 MPa	Pepsin, trypsin and	Increased rate of hydrolysis	(Chobert et al., 1996)
	A and B	150 min (S) 50–300 MPa 5 min (S)	Thermolysin™	Similar rate of hydrolysis for β-lg A and B at 100 and 300 MPa and 3 times faster for β-lg A at atmospheric pressure	(Stapelfeldt et al., 1996) (Van Willige & Fitzgerald, 1995)
	β-lg	100–800 MPa 15 min (P) 100–400 MPa 100 min (S)	Trypsin	Low impact of pre-treatment on peptide profile 400 MPa induced inactivation of trypsin	(Maynard et al., 1998)
	β-lg	100–300 MPa 10–20 min (S)	Pronase™ and α-chymotrypsin	Increased rate of hydrolysis Best hydrolysis rate at 100 MPa	(Izquierdo et al., 2005)
	β-lg B	150–450 MPa 15 min (P)	Trypsin, chymotrypsin and protease from *B. licheniformis*	Increased rate of hydrolysis at 300 and 450 MPa Increased production of specific peptides	(Knudsen et al., 2002)
	β-lg	75–475 MPa (S)	Thermolysin™ and chymotrypsin	Digestibility improved at 200 and 360 MPa, respectively	(V. V. Mozhaev, Heremans, et al., 1996)
	β-lg	600 and 800 MPa 10 min (S)	Pepsin	Total hydrolysis at 1 min at 600 MPa and 800 MPa Peptides generated were < 1.5 kDa	(Zeece et al., 2008)
	β-lg	400 and 600 MPa 10 min (P)	Trypsin	Modification of peptide profile at both 400 and 600 MPa Improvement of peptide yield 400 MPa generated more bioactive peptides	(Boukil et al., 2018)
	Bovine serum albumin	500 MPa 30 min (S)	Chymotrypsin	Increased rate of hydrolysis	(Beran et al., 2009)
	Bovine serum albumin	100–500 MPa 15–25 min (S)	Trypsin and α-chymotrypsin	Decreased digestibility at > 400 MPa Increased rate of hydrolysis at 400 MPa	(De Maria et al., 2017)
	Whey protein isolate	300 MPa 15 min 25 °C (S) 550 MPa 1 min 20 °C (S)	Trypsin Pepsin-pancreatin	Increased rate of hydrolysis Higher ferric-reducing antioxidant bioactivity	(Blayo et al., 2016) (Iskandar et al., 2015)
	α_s-CN	200 and 600 MPa 5 and 15 min (S) 2*2.5 min or 3*5min (S)	Pepsin-pancreatin	Single cycle 200 and 600 MPa more efficient Antihypertensive properties at 600 MPa 5 min Antioxidant activity at 600 MPa 15 min	(Hu et al., 2017)
	Casein	25–200 MPa 15–120 min (S)	Elastase™, Flavourzyme™ Savinase™, Thermolysin™ and trypsin	Increased degree of hydrolysis, DPPH and superoxide radical scavenging capacity, and anti-inflammatory activity using Flavourzyme™ and trypsin (100 MPa–1 h)	(Bamdad et al., 2017)
	Whey proteins	400 MPa 30 min 37 °C (S)	Pepsin	Increase in < 10 kDa peptide content (50%) and total absence of allergen.	(Lozano-Ojalvo et al., 2017)
Queso Fresco cheese		200–600 MPa 5–20 min (A)	–	Highest antioxidant activity at 400 MPa 20 min	(Paul et al., 2012)
Egg	ovalbumin	200–400 MPa 60 min (S)	Chymotrypsin and trypsin	Production of antihypertensive peptide	(Quirós et al., 2007b)
	ovalbumin	400 MPa (S)	pepsin	Difference in peptide profile (specifically at acidic pH)	(López-Expósito et al., 2008)
	Egg yolk	100 MPa 37–50 °C 12–24 h	–	Increase in phosvitin-phosphopeptides (antimicrobial activity) Increased rate of hydrolysis	(Yoo, 2016) (Yoo, Bamdad, Gujral, Suh, & Sunwoo, 2017)
Plant	Soybean	100–200 MPa 15 min 37 °C (P)	Trypsin, chymotrypsin and pepsin	Increased rate of hydrolysis (more specifically at 100 MPa)	(Peñas et al., 2004)
	Soybean	200–300 MPa 15 min (P)	Alcalase™, neutrase™, corolase 7089™ corolase PNL™ Flavourzyme™	Increased rate of hydrolysis Drastically decreased immunoreactivity (Gly m1 and Gly m5)	(Peñas et al., 2004) (Peñas et al., 2006)
	Ginko seeds	200–400 MPa 20 min (S)	Papain Alcalase™, pepsin and Neutrase™	Highest proteolysis rate by papain and Alcalase® at 300 MPa and pepsin at 400 MPa Almost complete loss of allergenicity (Papain, Alcalase® and pepsin) No impact with Neutrase	(Zhou et al., 2016)
	Red kidney bean protein isolate	200–600 MPa 20 min (P)	Trypsin	No difference in digestibility at 30 min digestion time Important decrease in digestibility P > 400 MPa at 120 min digestion time Protein-protein interactions decreased trypsin digestion at 400 and 600 MPa	(Yin et al., 2008)
	Chickpea protein isolate	100–600 MPa (P) 100–300 MPa (S)	Alcalase™	Pre-treatment at P > 300 MPa increased hydrolysis rate and hydrolysis time reduced P > 400 MPa. 200 MPa 20 min (S) higher antioxidative activity HHP treatment increased the amount of low-molecular weight peptides	(T. Zhang et al., 2012)
	Isolated pea protein	200–600 MPa 5 min 24 °C (P)	Alcalase™	Best ACE-inhibitory activity at 600 MPa and low enzyme concentration HHP increased renin-inhibitory activity at low enzyme concentration 200 MPa increased the generation of low molecular weight peptide	(Chao et al., 2013)

(*continued on next page*)

Table 2 (*continued*)

Source		HHP Parameters	Enzyme	Results	References
	Isolated pea protein	200–600 MPa 5 min 24 °C (P)	Alcalase™	Best DPPH scavenging activity at 400 MPa Improvement of ORAC activity at 400 and 600 MPa	(Girgih et al., 2015)
	Lentil proteins Pinto beans hydrolysates	100–500 MPa 15 min 40 °C (S)	Alcalase™, Protamex™, Savinase™ and Corolase 7089™	300 MPa improved proteolytic efficiency for all enzymes and increase digestion rate for Alcalase®. 300 MPa – Savinase™ presents highest ACE-inhibitory and antioxidant activities	(Garcia-Mora et al., 2015) (Garcia-Mora et al., 2016)
	Sweet potato	100–300 MPa 30–60 min 57 °C (S)	Alcalase™	Increase in degree of hydrolysis, antioxidant activity and < 3 kDa peptide content	(M. Zhang & Mu, 2017)
	Pre-germinated black soybean	50–150 MPa 12–24 h 57 °C (S)	–	HHP treatment increased < 3 kDa peptides, free amino acid content and anti-inflammatory activity (150 MPa)	(M. Y. Kim et al., 2017)
	Flaxseed	600 MPa 5–20 min (P)	Trypsin Pronase™	Increase in rate of hydrolysis and antioxidant activity	(Perreault et al., 2017)
	Mushrooms foot proteins	100–500 MPa 10 min 200 MPa 2–18 min (P)	Alkaline proteases	Increased protease activity	(Zhao et al., 2017)
Meat and fish	Beef lean meat	100–500 MPa 5 min 2 °C (P)	Lysosomal enzymes	Increased cathepsin activity up to 400 MPa Increased acid phosphatase activity up to 500 MPa	(Homma et al., 1994)
	Pork	600 MPa 6 min (P)	Lysosomal enzymes	Increased cathepsin activity Change in peptide pattern Enhancement of proteolytic fragments	(Grossi et al., 2012)
	Bovine collagen	600 MPa 15 min (P)	Alcalase™, collagenase, Thermolysin™, proteinase K, pepsin and trypsin	Improvement in degree of hydrolysis for all enzymes Increased ACE-inhibitory activity with Alcalase® and collagenase	(Y. Zhang et al., 2013)
	Fish skin gelatin	100–300 MPa 15 and 30 min 50 and 37 °C (S)	Alcalase™, collagenase, trypsin and pepsin	Radical scavenging capacity with Alcalase® and collagenase	(Alemán et al., 2011)
	Hemoglobin	400 MPa 15 min 20 °C (P)	Trypsin and pepsin	Enhanced protein hydrolysis with trypsin	(Toldrà et al., 2011)

(P): pre-treated by high hydrostatic pressure and digested at atmospheric pressure, (S): simultaneous pressurization and enzymatic hydrolysis, and (A): autolysis.

hydrophobic peptides from HHP pretreated β-lg. Some studies note the increased bioactivity of HHP-EH-generated peptides (Bamdad et al., 2017; Hu et al., 2017; Iskandar et al., 2015). Recently, Bamdad et al. (2017) demonstrated that tryptic hydrolysis of casein under HHP treatment (25–200 MPa–15–125 min) could increase DPPH scavenging (antioxidant) activity up to 30% in comparison to 2% under atmospheric hydrolysis (5 mg/mL). In addition, an increase in the ACE activity by 20% and DPPH activity by 4% was observed when α_s-CN was pre-treated using HHP (200 MPa–15 min) prior to *in vitro* biphasic digestion (Hu et al., 2017). Furthermore, enhanced enzymatic hydrolysis could lead to a greater proportion of low molecular weight peptides (< 10 kDa), which is usually correlated with an increase in bioactivity (Lozano-Ojalvo, Pérez-Rodríguez, Pablos-Tanarro, López-Fandiño, & Molina, 2017; Zeece et al., 2008). In addition, HHP treatment was found to increase the autolysis on Queso Fresco cheese, thus increasing the bioactivity (Paul, Brewster, Van Hekken, & Tomasula, 2012).

4.3.2. Egg-derived bioactive peptides

High pressure has been used to treat different egg proteins to produce BPs. Hoppe, Jung, Patnaik, and Zeece (2013) and Quirós, Chichón, Recio, and López-Fandiño (2007b) investigated the applicability of the HHP technique to change the proteolytic pattern of whole egg white or isolated protein, e.g., ovalbumin. The Quirós et al. (2007b) study showed that pressurization of ovalbumin at pH 8.0 led to the formation of protein aggregates corresponding to dimers, with apparent molecular weight 90 kDa. Native ovalbumin contains one disulfide bridge and four free sulfhydryl groups whose reactivity increases on denaturation. However, the pressurization of ovalbumin at lower pH (2.5) did not give rise to protein polymers due to the low reactivity of the sulfhydryl groups under acidic conditions. Pressurization of ovalbumin during enzyme treatment greatly accelerated hydrolysis with the three enzymes (trypsin, pepsin, chymotrypsin) tested. The application of HHP (300–400 MPa, 60 min) increased the susceptibility of ovalbumin to proteolysis by chymotrypsin and trypsin. Quirós et al. (2007) showed that the proteolysis of ovalbumin under pressures of 200–400 MPa accelerated the release of antihypertensive peptides such as YAEERYPIL, FRADHPFL and RADHPFL (Quirós et al., 2007b).

Ovalbumin is one of the main proteins in hen egg and is responsible for the most common forms of food allergies in children. The application of HHP has been used as a novel technique to enhance enzymatic hydrolysis of ovalbumin and modify its immunoreactivity (López-Expósito et al., 2008). Ovalbumin was digested with pepsin under high-pressure conditions (400 MPa) and the resulting hydrolysates and peptides were characterized. Treatment of ovalbumin with pepsin at 400 MPa digested all the intact protein within a few minutes. The pressure produced differences in the peptide pattern, especially with pepsin at an acidic pH. The researchers identified the IgG and IgE binding properties of the resulting hydrolysates. These hydrolysates retained residual IgG and IgE binding properties as a result of the accumulation of large and hydrophobic peptides during the initial stages of hydrolysis (López-Expósito et al., 2008). The conformational changes of ovalbumin under pressure resulted in the production of new peptide targets but only partially facilitated the removal of allergenic epitopes (López-Expósito et al., 2008).

High pressure pretreatment has positive effects on the highly phosphorylated egg yolk protein, phosvitin, which is known to be resistant to enzymatic hydrolysis due to its extremely high negative charge. Phosvitin is the most phosphorylated protein in egg yolk and is primarily found in the granule fraction (Goulas, Triplett, & Taborsky, 1996). Some studies have shown that the structure of phosvitin remains intact after high pressures, up to 600 MPa (Castellani, Guérin-Dubiard, David-Briand, & Anton, 2004). Yoo et al. (2016) and (2017) developed and optimized HHP-EH to increase the degree of hydrolysis and yield of phosvitin-phosphopeptides (PV-P) from egg yolk. The PV-P released from phosvitin are short phosphopeptides (< 3 kDa) with greater reducing power, iron-chelating capacity, antimicrobial activity and free radical scavenging activity compared to that of peptide hydrolysate produced at atmospheric pressure. Therefore, one of the advantages of HHP could be in the production of BPs from proteins resistant to enzymatic hydrolysis.

4.3.3. Plant-derived bioactive peptides

Valorization of protein-rich by-products of food processing has led to assessment of bioactive compounds, and functional or nutritional properties, especially from the viewpoint of environmental concerns (Aluko, 2008; Girgih et al., 2015; Maestri, Marmiroli, & Marmiroli, 2016; Nakurte et al., 2013; Sanjukta & Rai, 2016; Wang et al., 2008). Over the last decade, researchers have explored the production of BPs from plant-derived food by-products under HHP or after HHP pre-treatment. Peñas, Préstamo, and Gomez (2004) observed that HHP pretreatment of soybean whey protein enhanced the degree of hydrolysis by proteases such as trypsin, chymotrypsin and pepsin. Specifically, the authors noted that HHP pre-treatment at 100 MPa improved hydrolysis of soybean whey protein. Other studies also demonstrated an increase in the rate of hydrolysis of soybean whey protein under HHP in the range of 200–300 MPa using Alcalase™, Neutrase™, Corolase 7089™, and Corolase PNL™. Zhang and Mu (2017) and Perreault, Hénaux, Bazinet, and Doyen (2017) showed that HHP could be employed to improve the degree of hydrolysis and antioxidant activity of sweet potato protein and flaxseed proteins, respectively.

In other work the hydrolysis of HHP pre-treated (100–600 MPa) or high pressure treated (100–300 MPa) chickpea protein isolate was studied by Zhang, Jiang, Miao, Mu, and Li (2012). These authors found that HHP pre-treatment at pressure > 300 MPa largely increased both the hydrolysis rate by Alcalase™ and the yield of antioxidant peptides, whereas hydrolysis under HHP generated increased hydrolysis and antioxidative activity at 200 MPa. Generally, HHP (pre-treatment or simultaneous) increases the amount of low-molecular-weight peptides (T. Zhang et al., 2012). Using similar raw material, Chao, He, Jung, and Aluko (2013) and Girgih et al. (2015) evaluated the ability of HHP pre-treatment to improve hydrolysis and bioactivity of isolated pea protein by Alcalase™. The authors compared the hydrolysis at atmospheric pressure with HHP at 200 to 600 MPa and found a considerable increase in low molecular weight peptides at 200 MPa without any impact on bioactivity whereas at 400 and 600 MPa the ACE-inhibitory and antioxidant (DPPH and ORAC) activities were improved significantly in comparison to control hydrolysates. These results suggest that pressures above optimum could have negative effects, as aggregation is favored over hydrolysis after denaturation (Chao et al., 2013). Yin, Tang, Wen, Yang, and Li (2008) noticed that a high level of protein-protein interaction may decrease the susceptibility of pea proteins to enzymes, e.g., Alcalase™, over 400 MPa. Similarly, Garcia-Mora, Penas, Frias, Gomez, and Martinez-Villaluenga (2015) studied the effect of HHP on proteolysis of lentil proteins by using several enzymes (Alcalase™, Protamex™, Savinase™ and Corolase 7089™) to generate BPs. Treatment with HHP improved the proteolytic efficiency of the four proteases, leading to higher digestion rates of the major lentil storage protein. This treatment improved the production of peptides with molecular weight < 3 kDa and increased ACE inhibitory activity of the peptide fractions. Of the tested proteases, Savinase™ produced the highest ACE inhibitory and antioxidant activities of lentil hydrolysates (Garcia-Mora et al., 2015). These results were further confirmed by Garcia-Mora et al. (2016) where higher ACE inhibitory and antioxidant activities of pinto bean hydrolysates by Alcalase™ and Savinase™ were observed. Moreover, HHP was used to extract peptides with molecular weight cutoff < 3 kDa from mushroom foot protein with alcohol and aldehyde dehydrogenase activities (Zhao, Huo, Qian, Ren, & Lu, 2017).

4.3.4. Meat and fish-derived bioactive peptides

Compared to other biomass types, very few studies have examined the production of BPs by HHP-EH from meat and fish products. The use of HHP for the enzymatic hydrolysis and tenderization of meat was studied by Homma et al. (1994). Later, HHP was applied to improve the production of ACE inhibitory peptide from meat such as pork (Grossi, Gkarane, Otte, Ertbjerg, & Orlien, 2012; Homma et al., 1994; Y.; Zhang, Olsen, Grossi, & Otte, 2013). Alemán, Giménez, Gómez-Guillén, and Montero (2011) used HHP treatment for hydrolysis of fish skin gelatin using pressures of 100–300 MPa with Alcalase™, collagenase, trypsin and pepsin. These authors reported that the radical scavenging capacity of the pressured hydrolysates were only significantly enhanced when Alcalase™ or collagenase was used. Toldrà, Parés, Saguer, and Carretero (2011) performed tryptic and peptic hydrolysis of hemoglobin under HHP (400 MPa) to evaluate the impact of pressure on digestion efficiency. Their results showed that pressurization enhanced enzymatic hydrolysis of the fish protein but only when trypsin was used. However, no bioactivity tests were done on control and pressure-treated hydrolysates.

4.4. Effect of HHP on proteolytic enzymes

An important characteristic of BPs derived from enzymatic hydrolysis coupled to HHP treatment may be related to modification of enzyme activity under such high pressure. Most studies have correlated the increased degree of hydrolysis with protein denaturation (unfolding) and exposure of enzyme cleavage sites under HHP (Belloque, Chicón, & López-Fandiño, 2007). High pressure treatment has also been found to increase the surface hydrophobicity and solvent exposure of aromatic amino acids of egg white proteins (Van der Plancken, Van Loey, & Hendrickx, 2005). The hydrolytic enzymes, being protein, may aso be affected (activated or inactivated) by high pressure treatment. However, very few studies have discussed the role of HHP in enzyme activation/inactivation and enzyme substrate interaction during bioactive peptide production. The activation of enzyme is highly desirable as it can considerably reduce the amount of enzyme needed and, therefore, the cost for industrial production of bioactive peptides (Tribst, Ribeiro, & Cristianini, 2017).

Mozhaev, Heremans, et al. (1996) and Mozhaev, Lange, et al. (1996) demonstrated that high pressure treatment of α-chymotrypsin increased its degree of hydrolysis and thermal stability up to 350 MPa, above which these characteristics decreased significantly. Similarly, lysozyme (muramidase) activity was significantly improved when this enzyme was treated with HHP between 300 and 400 MPa (Tribst et al., 2017). Conversely, the conformation of Thermolysin™ changed irreversibly (inactivation under HHP above 300 MPa) whereas pepsin was resistant up to 400 MPa (Dufour et al., 1995). Maynard et al. (1998) found that HHP treatment of trypsin, alone, leads to inactivation even at 100 MPa. However, its susceptibility to HHP decreased in the presence of substrate (e.g., β-lg) but was found to be inactivated above 400 MPa.

The effect of HHP on enzyme conformation and activity depends mainly on the structure, chemical bonds, and interactions involved in the formation of active sites (Tribst et al., 2017). Moreover, pressurization conditions, such as pressure, temperature and time, and matrix/solvent properties, such as pH, ionic strength (salt concentration) and substrate (protein concentration), play important roles in the fate of enzyme during HHP (V. V. Mozhaev, Heremans, et al., 1996; Quirós, Chichón, Recio, & López-Fandiño, 2007a; Van der Plancken et al., 2005). Therefore, HHP treatment during or prior to enzymatic hydrolysis should be carefully optimized, taking into account the sensitivity and stability of enzyme and protein so that the active protease is present at the moment when the substrate (protein) is unfolded and active sites are exposed to the enzymes in the protein structure (Maynard et al., 1998).

4.5. Application of HHP-EH on allergenicity of hydrolysate

Many studies have shown that high pressure treated protein hydrolysates exhibit lower antigenicity or allergenicity than native proteins by exposing allergen epitopes to proteinases (V. M. Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015). High hydrostatic pressure assisted enzymatic hydrolysis drastically decreased the immunoreactivity of soybean whey proteins caused by the allergen Gly m1 (Peñas, Prestamo, Polo, & Gomez, 2006). Similarly, a recent

study led to a decrease in Gly m5 immunoreactivity for soybean protein hydrolysate treated prior to or during enzymatic hydrolysis, confirming the potential of HHP treatment as an allergenicity-reducing tool (Meinlschmidt et al., 2017). These effects were also observed on the allergenicity of ginkgo seed protein. Indeed, HHP-EH resulted in an almost complete loss of allergenicity, depending on the type of enzymes used (Zhou et al., 2016). Even though HHP treatment has produced positive effects in reducing the allergenicity of protein hydrolysates, many studies have shown that HHP treatment, alone, does not completely eliminate the risk of allergy (V. Balasubramaniam, Barbosa-Cánovas, & Lelieveld, 2016; Rosa Chicón, Belloque, Alonso, & López-Fandiño, 2008, 2009; R. Chicón, López-Fandiño, Alonso, & Belloque, 2008; Houska et al., 2009; Jin et al., 2015; Y. Li et al., 2013; Liu & Xue, 2010; Long, Yang, Wang, Hu, & Chen, 2015).

4.6. Challenges of HHP treatment

High hydrostatic pressure treatment is considered one of the promising non-thermal technologies in the food and nutraceutical field because it enhances the degree of hydrolysis and the production of bioactive peptides. However, a degree of care is recommended in the choice of HHP parameters depending on the nature and properties of substrate and enzyme used. Due to the complexity of chemical reactions (denaturation and renaturation) involved in the pressurization and decompression processes, reactions leading to protein aggregation may not produce the desired hydrolysis. Hydrolysis should be carried out immediately after the HHP treatment as a continuous loss of susceptibility of HHP-treated protein to proteolysis has been observed with time (Rosa Chicón et al., 2008). As proteins unfolded during HHP can rapidly regain their native structure (folded) after the decompression stage, the effect of HHP on protein hydrolysis may not be significant. Belloque et al. (2007) observed that β-lg treated at relatively mild pressure (200 MPa) unfolded faster relative to β-lg treated at 400 MPa, limiting the accessibility of cleavage sites and reducing the degree of EH. Similar observations were made by Yin et al. (2008) who studied the digestibility of red kidney bean protein isolate after HHP treatment. They found an important decrease in digestibility at HHP > 400 MPa. Similarly, pressurization may favor protein-protein aggregation, impeding enzymatic hydrolysis. Perreault et al. (2017) found that HHP led to the formation of aggregates of flaxseed protein from isolates. Therefore, over-pressurization may negatively affect the rate of hydrolysis and yield of BPs due to protein aggregation.

In addition, finding the optimum HHP parameters for substrate unfolding and enzyme activation is difficult especially when simultaneous pressurization and hydrolysis is desired. In such cases, the optimum conditions for pressure/hydrolysis are generally a compromise between the pressure values sufficient for destabilizing (unfolding) the substrate protein and maintaining the stability of the protease (Maynard et al., 1998). Therefore, the development of kinetic models to describe changes in physicochemical properties due to pressure treatment might be useful for predicting the impact of applied pressure, allowing suitable processes to be designed depending on the desired product properties (Van der Plancken, Van Loey, & Hendrickx, 2007).

Even though protein unfolding is desirable, it is noteworthy that severe HHP treatment could lead to protein-protein or peptide-peptide interaction and aggregation. Most studies focus on digestibility of HHP-treated protein and bioactivity of the generated peptide fractions without considering subsequent processing. A significant difference in hydrolysis pattern and peptide profile was observed when the protein was hydrolyzed simultaneously during HHP and after HHP (Rosa Chicón, Belloque, Recio, & López-Fandiño, 2006). No protein aggregates were observed when the hydrolysis was performed simultaneously with HHP, compared to proteolysis at atmospheric pressure of HHP pre-treated protein. The downstream processing of HHP assisted peptide fractions may be challenging as many studies have shown that HHP treatment can result in the protein-protein interactions generating larger soluble and insoluble aggregates, and modifications in physicochemical properties, such as surface hydrophobicity (Van der Plancken et al., 2005). These modifications of the properties of the solution could have severe consequences during subsequent processes, such as fractionation/concentration by membrane filtration and drying. Generation of protein/peptide aggregates and increases in hydrophobicity of peptide fractions may lead to membrane fouling. For example, Boukil et al. (2018) studied the effect of HHP pretreated β-lg hydrolysate on the fractionation of peptides using ultrafiltration. It was observed that HHP at 400 MPa–10 min (compared to control and 600 MPa) increased the production of hydrophobic peptides (including BPs) that resulted in membrane fouling and significantly decreased the filtration performance. Consequently, there has been some research on reducing protein/peptide aggregation during and/or after HHP treatment by adding protectants such as salt and sugar or changing the pH of the protein solution. Further studies are necessary to gain clarity on the effects of HHP on downstream processing (Iametti et al., 1999; Quirós et al., 2007a; Van der Plancken et al., 2007).

4.7. Economical aspect of HHP

Energy consumption of HHP system relies mainly on running parameters (holding pressure, pressure medium compressibility, equipment scale and vessel filling efficiency). According to a recent study the holding time carries a very negligible impact on the input cost of HHP processing (Atuonwu & Tassou, 2018). This is a quite an interesting finding for simultaneous pressurization and enzymatic treatment conditions when a longer hydrolysis is period desired. Moreover, the optimization of HHP parameters based on peptide productivity is critical to decrease the energy requirement to its minimum. The HHP is a batch process whose operating cost represents only about a quarter of the total cost (Rodriguez-Gonzalez, Buckow, Koutchma, & Balasubramaniam, 2015). Furthermore, many strategies are being developed to minimize the operating cost and energy consumption mainly in the pressurization phase. For example, the development of twin-vessel system could help to recover up to half of the decompression energy, which are generally lost, and be sued for the compression of the second. The study of Balasubramaniam et al. (2016) showed that the processing cost to be about 0.117 € per kilogram of the product treated for 3 min at 600 MPa using the Hiperbaric HHP system of 135 L capacity. The use of HHP to modulate protein structure and enhance the enzymatic hydrolysis is undeniably an environmentally sustainable approach for the production of BPs (Rodriguez-Gonzalez et al., 2015). However, a detailed economic analysis for the production of BPs using HHP technology as compared to other processes and conventional methods are needed.

5. Conclusions

Bioactive peptides have been largely recognized as high value-added compounds for the nutraceutical and pharmaceutical industries. Food-derived BPs are commonly produced by enzymatic hydrolysis even though new strategies, such as in silico approaches, are being developed. Producing BPs at a commercial scale, however, involves many challenges. Optimization of hydrolysis parameters to generate and recover the maximum amount of BPs from a complex food matrix with use of minimum enzyme is crucial to accelerating the development of BP-based food products. The application of HHP as a pretreatment has been explored extensively in recent years. Many studies have demonstrated that HHP treatment followed by enzymatic hydrolysis can efficiently improve protein digestibility (degree of hydrolysis) and, subsequently, BPs production. However, the efficiency of HHP on BPs production depends on the pressurization parameters (level of pressure, temperature, and time) and the enzyme used for hydrolysis. Simultaneous pressurization and enzymatic hydrolysis could be a useful novel approach. However, maintaining optimal conditions, such as pH

of enzymatic hydrolysis during pressurization, is a difficult batch process. Moreover, HHP treatment can affect the structure of proteins, leading to protein-protein and/or peptide-peptide interactions, forming larger aggregates that negatively impact the hydrolysis process, concentration of BPs, fractionation, and bioavailability of certain proteins and BPs. Furthermore, in the case of simultaneous pressurization and hydrolysis, the effect of high pressure on the enzyme, itself, must be thoroughly studied as some proteases are susceptible to high pressure. Therefore, future research should focus on the effects of HHP on whole process of generating BPs, from their production to sensorial qualities as well as bioavailability. Thus, it is necessary to expand these studies to include a large number of protein sources to fully understand the impact of pressurization on food protein structure and digestibility, and to encourage full development of this emerging and promising food processing technology.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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