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Review

Innovative applications of high-intensity ultrasound in the development of functional food ingredients: Production of protein hydrolysates and bioactive peptides

César Ozuna [a], Ingrid Paniagua-Martínez [b], Eduardo Castaño-Tostado [a], Lech Ozimek [c], Silvia L. Amaya-Llano [a,*]

[a] Programa de Posgrado en Alimentos del Centro de la República (PROPAC), Facultad de Química, Universidad Autónoma de Querétaro, Centro Universitario Cerro de las Campanas, s/n., QRO 76010 Queretaro, Mexico
[b] Grupo de Análisis y Simulación de Procesos Agroalimentarios, Departamento de Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera, s/n., E46022 Valencia, Spain
[c] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

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ABSTRACT

In recent years, the research on functional peptide generation for the development of functional foods has focused, among other issues, on the enhancement of enzymatic hydrolysis by means of high-intensity ultrasound (HIU) application. It has been suggested that the use of HIU in pretreatment and during the hydrolysis process can modify protein conformation by affecting hydrogen bonds and hydrophobic interactions, disrupting the quaternary and/or tertiary structure of proteins due to the effects of cavitation. Therefore, these structural modifications may expose more hydrolysis sites to be accessible by the enzyme, causing an increase in degree of hydrolysis and bioactivity. The main objective of this work was to review recent advances in food science and technology on the application of HIU for the enhancement of enzymatic hydrolysis of proteins of both animal and plant origin in order to generate novel protein hydrolysates and bioactive peptides which could be used as functional-food ingredients.

Contents

* Corresponding author at: Programa de Posgrado en Alimentos del Centro de la República (PROPAC), Universidad Autónoma de Querétaro, Apdo. Postal 184, Queretaro, QRO 76010, Mexico.
E-mail address: samayal@uaq.mx (S.L. Amaya-Llano).

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1. Development of functional-food ingredients

Nowadays, consumers prefer and choose foods that not only provide them with essential nutrients but also contain substances which may have positive long-term effects (Hernández-Carrión, Hernando, & Quiles, 2014). The concept of "functional food" originated in the early 1980s in Japan. Since then, it has been widely accepted and its popularity has spread around the world (Ohama, Ikeda, & Moriyama, 2006). A food could be considered "functional" when it shows to have beneficial effects on the general conditions of the body, such as improving health and reducing the risk of some diseases (Bigliardi & Galati, 2013). In 2013, the market of functional foods and beverages was worth US $176.7 billion, which accounts for 5% of the overall food market world-wide (Tripathi & Giri, 2014).

Functional food development implies the incorporation of specific bioactive compounds with positive physiological effects. In this sense, food protein has been studied beyond its nutritional role as a source of peptides encrypted in its primary structure (Li-Chan, 2015). These peptides could present higher biological activity than their parent protein and therefore could reduce the risk of developing a chronic disease and positively impact human health (Udenigwe & Aluko, 2012).

Over the last decade, there have been numerous investigations in order to generate new ingredients and processes that would contribute to the development of functional foods for improving the health of the general population. In this sense, food science and technology has shown increasing interest in the application of "new" or "emerging technologies" which modify functional properties of food as well as help intensify existing processes such as microwaves (Chen et al., 2014; Uluko et al., 2013; 2015), high hydrostatic pressures (Garcia-Mora, Peñas, Frias, Gomez, & Martinez-Villaluenga, 2015; Hoppe, Jung, Patnaik, & Zeece, 2013), and high-intensity ultrasound (see Table 1), among others.

2. Bioactive food peptides. Definition and production as functional-food ingredients

Bioactive food peptides (BFPs) are encrypted within the sequence of food proteins. Hence in their natural state, BFPs are "locked" but can be released during food processing (by enzymatic hydrolysis or fermentation) as well as in the digestive tract after human consumption (Hernández-Ledesma, Contreras, & Recio, 2011; López-Barrios, Gutiérrez-Uribe, & Serna-Saldívar, 2014). Numerous animal and plant food proteins have been investigated as a source of BFPs. On the one hand, several works have shown the potential of animal proteins such as milk (Korhonen, 2009; Nagpal et al., 2011), egg (Bhat, Kumar, & Bhat, 2015), meat muscle (Lafarga & Hayes, 2014; Mora, Reig, & Toldrá, 2014) and marine protein sources (Harnedy & FitzGerald, 2012). On the other hand, plant protein sources include, among others common bean (Luna-Vital, Mojica, González de Mejía, Mendoza, & Loarca-Piña, 2015), soybean (Yimit, Hoxur, Amat, Uchikawa, & Yamaguchi, 2012), amaranth (Silva-Sánchez et al., 2008), corn (Huang, Sun, He, Dong, & Li, 2011) rice and wheat proteins (Cavazos & Gonzalez de Mejia, 2013).

BFPs exhibit physiological functions relevant in human health sustenance, affecting the major human body systems (Udenigwe & Aluko, 2012). Regarding their antihypertensive activity, BFPs are able to inhibit the angiotensin I-converting enzyme (ACE) (Hernández-Ledesma et al., 2011; Korhonen & Pihlanto, 2006). ACE is an enzyme that is a part of the renin–angiotensin system where angiotensin I is converted into angiotensin II. In turn, angiotensin II causes severe contractions of blood vessels and limits their relaxation, which results in high blood pressure (Mora et al., 2014). Based on the function of ACE in the renin-angiotensin system pathway, inhibitors of this enzyme, such as BFPs, have been used as anti-hypertensive agents. IC_{50} is defined as the concentration of ACE inhibitors required to inhibit 50% of ACE activity. On the other hand, the antioxidant properties of BFPs include scavenging reactive oxygen species/free radicals and the inhibition of these radicals which might induce the oxidation of biological macromolecules such as lipids, proteins and DNA (Li-Chan, 2015; Power, Jakeman, & FitzGerald, 2013). The functions attributed to antioxidant peptides in food matrices include the reduction in lipid peroxidation and its associated products that help maintain organoleptic properties of food during storage, while in the human body, the antioxidant peptides reduce the effect of oxidative stress which is responsible for aging and various diseases (Power et al., 2013). The antioxidant peptides can be determined using methods based on hydrogen atom transfer (HAT), for example, the oxygen radical absorbance capacity (ORAC) and total radical trapping antioxidant parameters (TRAP); and methods based on electron transfer (ET) such as Trolox equivalent antioxidant capacity (TEAC), the ferric ion reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) (Pihlanto, 2006; Sarmadi & Ismail, 2010). Other activities of interest are their anti-inflammatory, antimicrobial, anticancer, lipid-lowering, immunomodulatory and opioid activity, among others (Udenigwe & Aluko, 2012).

BFPs range in size from 2 to 20 amino acid residues and their bioactivity is based on their amino acid composition and location within the sequence of amino acids that form the peptide (Li-Chan, 2015; Mora et al., 2014). It is suggested that ACE inhibitory activity is strongly influenced by C-terminal residue, where the ACE prefers di- and tripeptide inhibitors containing hydrophobic residues such as tryptophan, tyrosine, phenylalanine or proline (Haque & Chand, 2008). Moreover, amino acids with positive charge contribute to ACE inhibition (Vermeirssen, Camp, & Verstraete, 2004). In peptides with major antioxidant activity, residues of histidine, proline, tyrosine, methionine, cysteine are present and the antioxidant properties can be intrinsic for each amino acid residue. The antioxidant properties of histidine are related to the imidazole group and as for cysteine, the thiol group seems to be the responsible for the antioxidant activity (Sarmadi & Ismail, 2010). These features suggest that the complex relationship between peptide structure and its bioactivity is not completely elucidated, and new bioinformatics tools may be useful in providing a better understanding of the topic (Zhou, Yang, Ren, et al., 2013).

The most common method of BFPs generation is enzymatic hydrolysis which is widely employed by food industries (Lafarga & Hayes, 2014; López-Barrios et al., 2014). Fig. 1 shows a diagram with the main routes of BFPs generation by enzymatic hydrolysis. The first step in the development of BFPs is the selection of protein source and enzyme. Many common peptides have been produced using gastrointestinal enzymes such as Trypsin or Pepsin (Korhonen & Pihlanto, 2006). However, as the costs associated with enzymatic reactions are relatively high, proteinases derived from cheap by-products and microorganisms have been investigated in the last years (Agyei & Danquah, 2011). In enzymatic hydrolysis, some factors to consider include enzyme-substrate ratios, hydrolysis times, enzyme pH and temperature. Usually, the hydrolysis process is performed at optimum enzyme pH and temperature (Udenigwe & Aluko, 2012). However, other factors should be considered, such as the effect of ions on enzyme activity or even the loss of enzymatic activity and product inhibition (Gonzàlez-Tello, Camacho, Jurado, Paez, & Guadix, 1994). In protein hydrolysis, the key parameter for monitoring the reaction is the degree of hydrolysis (DH). DH is an important property as it affects the functional properties of the hydrolysates including their bioactivity (Nielsen, Petersen, & Dambmann, 2001). Peptide bioactivity depends on the specificity of enzyme used, the protein source and any treatment prior to hydrolysis that modifies the native protein structure (Gauthier & Pouliot, 2003). It is considered that the substrate pretreatment is an important tool in the "design" of peptides during enzymatic hydrolysis due to intra and inter molecular rearrangement and possible protein conformational changes which could increase the accessibility of the enzyme to new cleavage sites previously hidden in a hydrophobic core (Adjonu, Doran, Torley, & Agboola, 2013). For example, thermal pretreatment

provokes a complex denaturation phenomenon (Chao, He, Jung, & Aluko, 2013; Leeb, Kulozik, & Cheison, 2011), hydrostatic pressure causes oligomer dissociation and unfolding of proteins (Garcia-Mora et al., 2015; Hoppe et al., 2013) and high-intensity ultrasound induces exposure of hydrophobic groups and hydration effects (see Table 1). These and other technologies have been used to enhance the hydrolysis process and the release of potent BFPs.

After hydrolysis, it is often necessary to fractionate and purify the mixture of hydrolysates by implementing separation techniques such as ultrafiltration, as well as to test the obtained mixture for bioactivity (Lafarga & Hayes, 2014). In this sense, ultrafiltration allows to separate hydrolysates into high and low molecular weight fractions. In several studies, low molecular weight peptides (<5 kDa) were associated with high bioactivity. However, other investigations have found that high molecular weight peptides (>5 kDa) are better antioxidants than the small fraction (Uluko et al., 2015; Zhuang, Tang, & Yuan, 2013). Once the mixture of hydrolysates is fractionated, hydrolysates which show bioactivity are purified by chromatographic techniques (ion exchange chromatography, gel filtration chromatography and reversed-phase high-performance liquid chromatography) (Pownall, Udenigwe, & Aluko, 2010; Udenigwe & Aluko, 2012). After separating hydrolysates into peptidic fractions, further studies on bioactivity are carried out. The fractions that present highest bioactivity are then characterized by means of mass spectrometry technologies. Finally, the sequenced bioactive peptides are synthesized and used in subsequent bioavailability studies. Regarding this last step, testing in vivo is required in order to determine the usefulness of BFPs. It is difficult to establish a relationship between in vitro and in vivo activity because the peptides need to be absorbed intact (or, at least, in an active form) via the different mechanisms on intestinal epithelium, however, they could be susceptible to degradation by digestive enzymes and brush border membrane peptidases. (Hernández-Ledesma et al., 2011; López-Barrios et al., 2014). On the other hand, in vitro digestion experiments offer the advantage of screening the vast amount of peptides resulting from enzyme hydrolysis of complex proteins.

The application of BFPs on food systems is a challenge due to the impact on sensory perception of these ingredients. As for hydrophobic peptides with bitter taste, the application of masking methods (Ley, Krammer, Kindel, Gatfield, & Müller, 2014; Newman, O'Riordan, Jacquier, & O'Sullivan, 2015) or encapsulation in different matrices has been applied, offering additional advantages in controlled release (Hwang, Tsai, & Hsu, 2010). Another factor to consider is the BFPs resistance to processing conditions during the manufacture of the foods. For example, thermal processing produces racemization, amino acid decomposition, glycation (Maillard reaction) and crosslinking (Hernández-Ledesma et al., 2011).

Nowadays, the interest of food technologists has focused to the use of emerging technologies such as high-intensity ultrasound, in order to modify protein structure, increase enzymatic hydrolysis and consequently obtain high-yield peptide products with potent bioactivity.

3. Ultrasound. Definition and applications in the food science and technology

Ultrasound is an acoustic wave with a frequency greater than 20 kHz, the threshold for human auditory detection, which needs a medium in order to propagate (Mason & Lorimer, 2002). The source of ultrasonic output is typically a vibrating body; the vibration of said body communicates with particles of the surrounding medium, which in turn begin to oscillate. Similarly and also by oscillation, these particles communicate the energy to other neighboring particles.

The ultrasonic power level or energy propagated to the medium can be expressed as ultrasound power (W), ultrasound intensity (W/cm^2) or acoustic energy density (W/cm^3 or W/mL). However, acoustic energy density has been widely accepted as the most appropriate measure of ultrasonic power level (Esclapez, García-Pérez, Mulet, & Cárcel, 2011). Ultrasonic intensity or acoustic energy density can be determined calorimetrically using Eqs. (1)–(3) (O'Donnell, Tiwari, Bourke, & Cullen, 2010). The absolute ultrasonic power P is given by:

$$P = mc_p\left(\frac{dT}{dt}\right) \quad (1)$$

where m is the mass, c_p is the specific heat capacity and dT/dt is the increase of temperature during the first minutes of ultrasound application.

The intensity of ultrasonic power (I) transmitted from probe tip with diameter Ø is given by:

$$I = \frac{4P}{\pi \varnothing^2}. \quad (2)$$

Acoustic energy density (D) can be calculated by dividing absolute ultrasound power with the volume (V) of the medium (cm^3 or mL)

$$D = \frac{P}{V}. \quad (3)$$

Ultrasound application in food science and technology can be classified into two major groups: low-intensity and high-intensity ultrasound. On the one hand, there are applications using high-frequency (>100 kHz) and low-power (<1 W/cm^2) ultrasound, also known as "low-intensity ultrasound." These non-invasive applications are used in product and/or process quality control (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012b) and are largely based on measurement of ultrasonic velocity, signal attenuation or frequency spectrum analysis, in order to obtain information on the medium in which the ultrasound is propagated. High-frequency ultrasound has been used to provide information on the physicochemical properties of food such as the salt and fat content in meat products (Corona et al., 2013; De Prados, García-Pérez, & Benedito, 2015) or firmness, ripeness, sugar content and acidity in fruit and vegetable products (Mizrach, 2008).

On the other hand, there are low-frequency (20–100 kHz) and high-power (>1 W/cm^2) ultrasound applications also known as "high-intensity ultrasound" or "power ultrasound" (HIU). Unlike low-intensity ultrasound, HIU is used to provoke changes in the product or in the process in which it is applied (Chemat, Huma, & Khan, 2011; Tao & Sun, 2015). When traversing a medium, HIU generates a series of effects, some of which can affect both mass and heat transport phenomena. These effects can be explained by various associated mechanisms, which vary depending on the environment in which the acoustic waves propagate (Cárcel, García-Pérez, Benedito, & Mulet, 2012).

HIU effects on liquid systems are mainly related to the cavitation phenomenon (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012a; Esclapez et al., 2011). The propagation of HIU in a liquid induces a series of compressions and decompressions, causing molecular displacement. When the acoustic power reaches a certain threshold, negative pressures that occur during the decompression cycle can overcome the attractive forces of the molecules in the liquid, especially surface tension, thus forming a gas bubble in the liquid (Soria & Villamiel, 2010). Once formed, the bubble may continue to grow by rectified diffusion mechanism, which is characterized by a net flow of gas from the liquid to the bubble (Fig. 2). In the negative pressure cycle, gas flow occurs from the liquid to the bubble which expands, increasing in size. When the pressure is positive, the flow is opposite and the bubble becomes smaller. The amount of vapor lost is always smaller than the amount of vapor gained, thus the size of the bubble increases with each cycle (Luque de Castro & Priego Capote, 2007).

Cavitation bubbles may attain a stable size with a constant size variation due to successive compressions and decompressions, generating microagitations in the liquid. This phenomenon is called "stable cavitation". However, the bubble size may also be unstable when the growth cycle of the bubble is greater than the reduction cycle, provoking a rapid growth resulting in a collapse of the bubble, and thus generating both

Table 1
Process conditions of high-intensity ultrasound application as pretreatment and during the hydrolysis process. UAS: Ultrasound Application System, F: Frequency, UPL: Ultrasonic Power Level, T: Temperature, t: time.

Protein source	Enzyme	HIU pretreatment to enzymatic hydrolysis			
		UAS	F (kHz)	UPL	T (°C)
Albumen (egg white)	Alcalase Neutrase Papain Flavourzyme	Bath	35 and 40	P = 65.7 and 21.3 W	25 ± 3.5 and 55 ± 3.5
	Alcalase	Bath	30	Non-reported	27
	Thermolysin	Probe (Ø = non-reported)	20 and 60	Non-reported	Non-reported
	Thermolysin Pepsin	Probe (Ø = non-reported)	0.060	Non-reported	Non-reported
Milk protein concentrate	Trypsin Pepsin	Probe (Ø = 13 mm)	20	P = 800 W	≤50
	Neutrase	Probe (Ø = 13 mm)	Non-reported	P = 800 W	≤50
	Neutrase	Probe (Ø = 25 mm)	20	P = 800 W	Non-reported
	Neutrase	Probe (Ø = 13 mm)	Non-reported	P = 800 W	≤50
	Alcalase Flavourzyme Neutrase Trypsin Alkaline protease	Probe (Ø = 13 mm)	Non-reported	P = 800 W	≤50
	Nanoencapsulated flavourzyme	–	–	–	–
Tilapia (*Oreochromis niloticus*)	Flavourzyme	Probe (Ø = non-reported)	20	P = 70 W	55 ± 1
	Flavourzyme	Probe (Ø = non-reported)	20	P = 10 and 70 W	55 ± 1
Defatted larva flour of *Tenebrio molitor* (L.)	Alcalase	–	–	–	–
Silkworm pupae (*B. mori*)	Alcalase	Probe (Ø = 15 mm)	20	P = 250 to 600 W	≤50
Wheat germ protein	Alcalase	Probe (Ø = 20 mm)	20	P = 0 to 1800 W	25 ± 2 (initial temperature)
	Alcalase	Bath (SFU and FFU)	SFU 24 to 68 (±2) cycle: 100 s (on time of 500 s and off time of 10 s) FFU 22 to 68	D = 0.12 W/mL I = 1.15 W/cm^2	25 ± 1
		Probe (Ø = 20 mm)	20	D = 0.12 to 1.08 W/mL I = 63.70 to 573.25 W/cm^2	25 ± 1
	Alcalase	–	–	–	–
	Alcalase	Probe (Ø = 20 mm)	20	P = 0 to 1800 W	Non-reported
Wheat gluten	Alcalase	–	–	–	–
Zein	Alcalase	Bath (SFU and FFU)	SFU 22 to 68 (±2) cycle: 500 s (on time of 10 s and off time of 3 s) FFU 22 to 68	P = 600 W	25 ± 2
Corn gluten meal	Neutrase	Probe (Ø = 25 mm)	20	D = 0 to 10 W/mL I = 0 to 407.33 W/cm^2	40 and 0 (substrate and enzyme, respectively)
Soy protein isolate	Papain	Probe (Ø = 15 mm)	25	I = 19 ± 2 to 42 ± 3 W/cm^2	2
Soy sauce lees	Alcalase	–	–	–	–
Highly denatured soybean meal	Neutrase	Bath	40	P = 200 to 500 W (output power)	45
Garlic powder	Alcalase	Bath (SFU)	24 to 68 (±2)	D = 0.1 W/mL	20 ± 1 (initial temperature)
		Probe (Ø = 20 mm)	20	P = 410 W	30 to 50
	Alcalase	Probe (Ø = 20 mm)	20	P = 410 W (output power)	45 (initial temperature)
Oat (*Avena sativa*)	Alcalase	Probe (Ø = 20 mm)	20	P = 250 to 1250 W	27 ± 1
Cowpea (*Vigna unguiculata*)	Pepsin Porcine pancreatin	Probe (Ø = non-reported)	20	25% of amplitude	Non-reported
Plum (*Prunus domestica* L.)	Alcalase Thermolysin Flavourzyme Protease P	Probe (Ø = non-reported)	20	30% of amplitude	Non-reported

Ø: probe diameter.
P: ultrasonic power.
I: intensity of ultrasonic power.
D: acoustic energy density.
SFU: sweeping frequency ultrasound.
FFU: fixed frequency ultrasound.

Table 1 *(continued)*

Protein source	HIU pretreatment to enzymatic hydrolysis	Enzymatic hydrolysis assisted by HIU					Reference
	t (min)	UAS	F (kHz)	UPL	T (°C)	t (min)	
Albumen (egg white)	15 to 60	–	–	–	–	–	Stefanović et al. (2014)
	15 to 180	–	–	–	–	–	Knezevic-Jugovic et al. (2012)
	0.08 to 8 (pulse duration of 30 s)	–	–	–	–	–	Lei et al. (2011)
	4 pulses (30 s for each pulse)	–	–	–	–	–	Majumder and Wu (2010), (2011)
Milk protein concentrate	10	–	–	–	–	–	Uluko et al. (2015)
	1 to 8						Uluko et al. (2014)
	4 (pulse durations of on-time 6 s and off-time 3 s)	–	–	–	–	–	Uluko et al. (2014)
	3.3 to 6.7	–	–	–	–	–	Uluko et al. (2013)
	0 to 8	–	–	–	–	–	Uluko et al. (2013)
	–	Probe (Ø = non-reported)	Non-reported	~1.5 W Amplitude: ~4 μm	Room temperature	0 to 90	Madadlou et al. (2011)
Tilapia (*Oreochromis niloticus*)	30 and 45 (pulse durations of on-time 10 s and off-time 20 s)	–	–	–	–	–	Kangsanant et al. (2015)
	15 to 45 (pulse durations of on-time 10 s and off-time 20 s)	Probe (Ø = non-reported)	20	P = 10 and 70 W	55 ± 1	60 (pulse durations of on-time 10 s and off-time 20 s)	Kangsanant et al. (2014)
Defatted larva flour of *Tenebrio molitor* (L.)	–	Bath	25	I = 0.16 W/cm^2	50 ± 2	0 to 5	Dai et al. (2011)
Silkworm pupae (*B. mori*)	24 (pulse durations of on-time 2 s and off-time 2 s)	–	–	–	–	–	Jia et al. (2015)
Wheat germ protein	0 to 60 (pulse durations of on-time and off-time, 2 s)	–	–	–	–	–	Huang et al. (2014)
	30	–	–	–	–	–	Zhou, Ma, Yu, et al. (2013)
	0 to 60 (pulse durations of on-time 2 s and off-time 2 s)						
	–	Bath (SFU)	24 ± 2 Cycle time of SFU: 18 s	P = 24 W	50	120 (pulse durations of on-time 500 s and off-time 5 s)	Qu et al. (2012)
	20 (pulse durations of on-time 2 s and off-time 2 s)	Probe (Ø = 6 mm)	20	P = 40 W	50 ± 5	210 (pulse durations of on-time 2 s and off-time 4 s)	Jia et al. (2010)
Wheat gluten	–	Bath	25 to 69	I = 0.707 W/cm^2	50	30	Zhu et al. (2011)
Zein	30	–	–	–	–	–	Ren et al. (2013)
Corn gluten meal	0 to 90 (pulse durations of on-time 2 s and off-time 1 s)	–	–	–	–	–	Zhou, Ma, Ding, et al. (2013)
Soy protein isolate	15 (pulse duration of on-time 9 s and off-time 1 s)	–	–	–	–	–	Chen et al. (2011)
Soy sauce lees	–	Bath	Non-reported	P = 120 W (output power)	50	30	Yang et al. (2011)
Highly denatured soybean meal	30	–	–	–	–	–	Xu et al. (2013)
Garlic powder	50 with cycle times of 0 to 50 ms (pulse durations of on-time 10 s and off-time 5 s)	–	–	–	–	–	Ma et al. (2015)
	40 to 80						
	72 (pulse durations of on-time 3 s and off-time 2 s)	–	–	–	–	–	Huang et al. (2015)
Oat (*Avena sativa*)	10 to 50	–	–	–	–	–	Wang et al. (2014)
Cowpea (*Vigna unguiculata*)	10	–	–	–	–	–	Quansah et al. (2013)
Plum (*Prunus domestica* L.)	1	–	–	–	–	–	González-García et al. (2014)

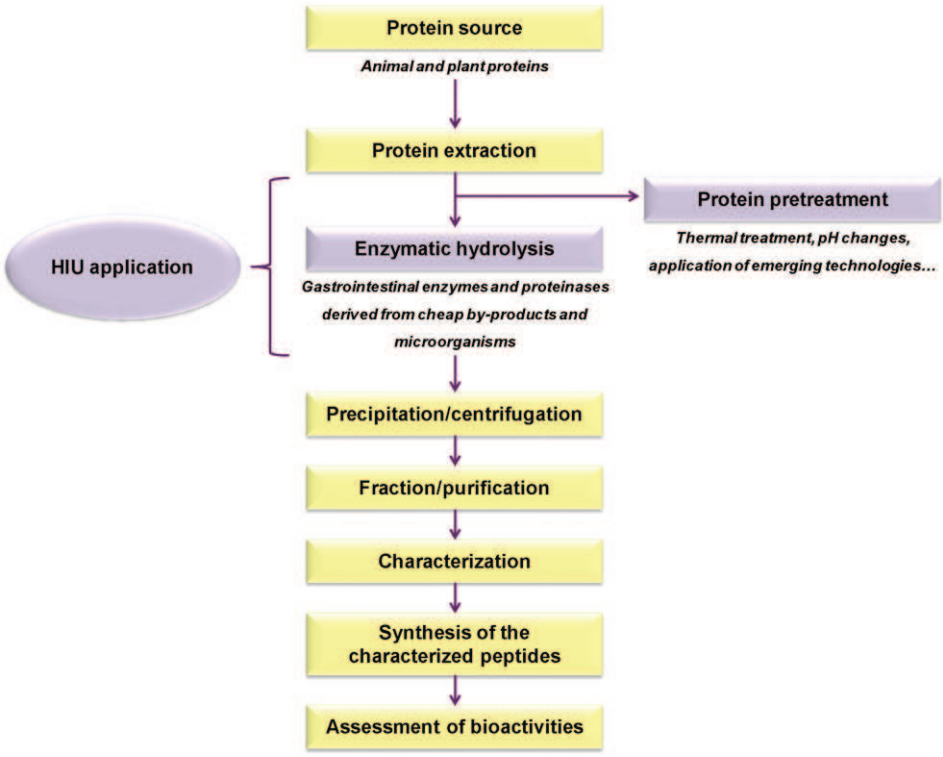

Fig. 1. Diagram for the generation of bioactive peptides through the enzymatic hydrolysis.

high temperatures (5000 K) and pressures (100 MPa) in the cavitation zone (Luque de Castro & Priego Capote, 2007) (Fig. 2). This type of cavitation, called "transient cavitation", can produce shearing forces and turbulence in the medium (Cárcel et al., 2012; Pingret, Fabiano-Tixier, & Chemat, 2013). Another important effect is that water molecules can be broken, generating highly reactive free radicals ($H_2O \rightarrow H + {}^{\bullet}OH$) that may react and consequently modify other molecules, such as proteins (Arzeni, Martínez, et al., 2012). This wide range of mechanisms involved in the HIU treatment induces physical and biochemical effects with several potential applications in the food industry.

The effectiveness of HIU applications in food processes largely depends on the application system efficiency. Two systems that stand out within HIU application in liquid media are ultrasonic baths and probe type systems. Ultrasonic baths consist of a stainless steel tank and a set of transducers placed in a metal base (Luque de Castro & Priego Capote, 2007). All vibrating in phase, the transducers make the entire metal base vibrate and transmit ultrasonic energy to the liquid contained within the tank. Furthermore, in probe type systems, the transducer is coupled to a probe that is responsible for transmitting the vibration to the medium. The geometry of the probe varies according to specific application (Cárcel et al., 2012). Using both HIU-generating systems, several applications have been developed in processes such as nano-emulsion preparation (Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013), inactivation of microorganisms and enzymes (Chemat et al., 2011), osmotic dehydration of vegetables (Fernandes & Rodrigues, 2008), salting and desalting of meat products (Ozuna, Puig, Garcia-Perez, & Cárcel, 2014; Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013), hot-air drying pretreatments (both osmotic and blanching) (Siucińska & Konopacka, 2014) and bioactive product extraction processes (Esclapez et al., 2011; Soria & Villamiel, 2010), among others.

4. Effects of high-intensity ultrasound on proteins

Several novel and interesting HIU applications for improving the technological properties and bioactivity of food have emerged during the last years (Ashokkumar, 2015; Soria & Villamiel, 2010). Due to the physical, chemical and mechanical effects induced mainly by cavitation, HIU has been used to provoke modifications in the structure of both animal and plant proteins (Jambrak, Mason, Lelas, & Krešić, 2010; O'Sullivan, Murray, Flynn, & Norton, 2015).

Proteins are highly functional molecules that are widely used in the chemical, pharmaceutical and food industries in processes such as emulsification, foaming, viscosity enhancement, encapsulation and gelation. The functional properties of proteins are influenced by their molecular structure that determines inter and intra molecular interactions (Foegeding & Davis, 2011). Numerous studies have shown that HIU can change the structural and/or the functional properties of food proteins by altering their molecular characteristics (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011). Nevertheless, the change induced by HIU depends on the nature of the protein and its degree of denaturation and aggregation (Arzeni, Martínez, et al., 2012). When HIU was applied to egg white protein (EWP), O'Sullivan et al. (2015) showed a reduction in EWP size with an increase in the sonication time. This effect was attributed to disruption of the hydrophobic and electrostatic interactions which maintain untreated protein aggregates produced by cavitation effects. In addition, it has observed that HIU increase the hydrophobicity and emulsion stability of EWP (Arzeni, Martínez, et al., 2012; Arzeni, Pérez, & Pilosof, 2012; O'Sullivan et al., 2015).

In case of milk proteins, Yanjun et al. (2014) found that HIU application reduced the particle size of milk protein concentrate (MPC) from 28.45 to 0.13 μm after 0.5 min of treatment which was confirmed by SEM micrographs. Jambrak et al. (2010) reported a decrease of α-lactalbumin molecular weight after HIU treatments both using a 20 kHz probe and 40 kHz bath which was consistent with the results obtained by Zisu et al. (2011) for aqueous solutions of reconstituted whey protein-concentrate and isolate powders. Regarding other effects of HIU on functional properties, Zisu, Bhaskaracharya, Kentish, and Ashokkumar (2010) observed a reduction in viscosity and an improvement in gelling properties of milk proteins by sonication in pilot scale reactors operating at a frequency of 20 kHz and varying amplitudes. On the other hand, some studies have demonstrated that sonication

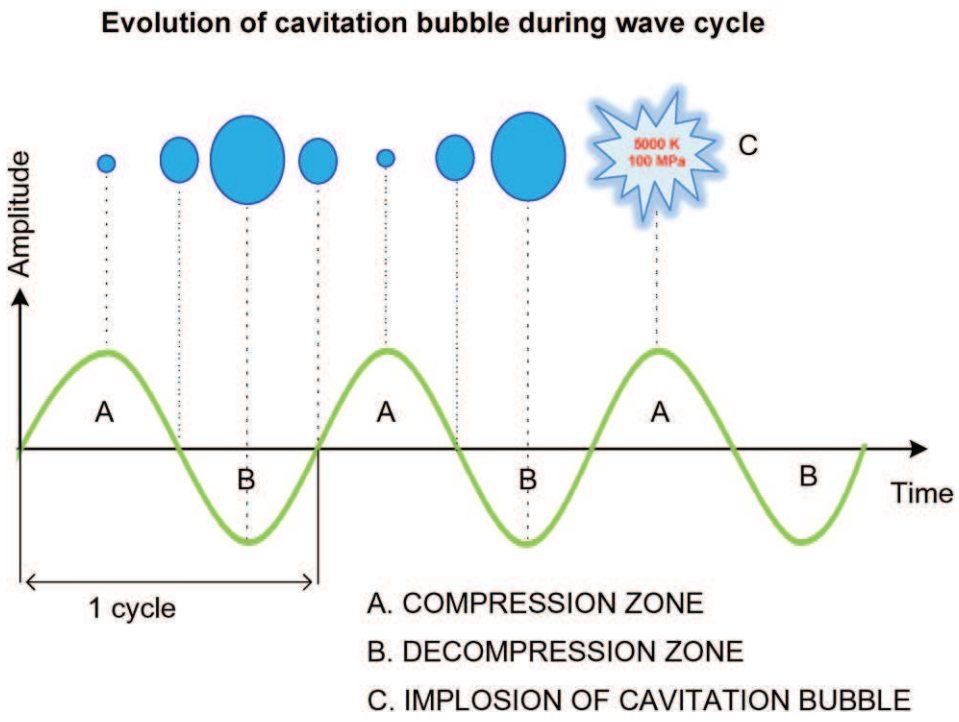

Fig. 2. Scheme of ultrasonic cavitation.

times greater than 5 min can promote the formation of milk protein aggregates (Chandrapala et al., 2011; Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014).

In plant protein sources, HIU application on soy protein isolate (SPI) resulted in partial unfolding and reduction of intermolecular interactions which was confirmed by increases in free sulfhydryl groups and surface hydrophobicity. These effects led to an improvement in solubility and fluid character of SPI dispersions (Hu et al., 2013). In the same way, Chen, Chen, Ren, and Zhao (2011) reported that HIU pretreatment (400 W, 15 min) combined with controlled papain hydrolysis could be an effective method for improving both solubility and emulsifying properties of SPI. In addition, HIU was demonstrated to be an optimal technology for enhancing the recovery of SPI from defatted soy flakes while only slightly modifying some functional properties such as solubility, emulsification and foaming capacity (Karki et al., 2009, 2010). Regarding other plant protein sources, O'Sullivan et al. (2015) studied the HIU effect (~34 W/cm^2, 2 min) on the physicochemical and emulsifying properties of pea protein isolate (PPI), rice protein isolate (RPI) and SPI. According to the results shown in this study, HIU reduced aggregated size and hydrodynamic volume of PPI and SPI, with the exception of RPI. In addition, while SPI and RPI proteins treated with HIU gave more stable emulsions, HIU provoked a reduction in PPI aggregates and an increase in their hydrophobicity which led to less stable emulsions than those prepared from untreated samples.

As it can be seen in the previously discussed studies, HIU application in modification of physical and functional properties of proteins has been a subject of extensive research in food science and technology. Due to the potential of this emerging technology, an interest in HIU application has been shown in enhancing the efficiency of enzymatic hydrolysis of protein in order to generate novel peptides which could be used as powerful functional ingredients.

5. Application of high-intensity ultrasound in the production of protein hydrolysates and bioactive peptides as functional food ingredients

In the last five years, the HIU application to enhance the enzymatic hydrolysis in order to generate peptides with functional properties has been a subject of research (see Table 1). It has been suggested that the use of HIU as pretreatment and during the hydrolysis process can modify protein conformation by affecting hydrogen bonds and hydrophobic interactions, disrupting the quaternary and/or tertiary structure of proteins due to the effects of cavitation. Therefore, these structural modifications may expose more hydrolytic sites to be accessible by the enzyme, causing an increase in degree of hydrolysis and bioactivity. On the other hand, HIU application during enzymatic process may adversely affect the enzyme conformation and consequently its effectiveness during hydrolysis. Thus, Yu, Zeng, Zhang, Liao, and Shi (2014) found that the activity of α-amylase and papain was inhibited by HIU application (278.8 ± 7.4 W, 40 kHz) during 30–60 min under a controlled temperature of 0–4 °C. In contrast, using the same process conditions, the activity of pepsin was activated. These effects were attributed to changes on their secondary and tertiary structure. However, the sonochemical and physical mechanisms induced by HIU on the enzymes are not well-known.

The following sections comprise an extensive literature review of recently (2010 to 2015) published papers that focus on the application of HIU for the enhancement of enzymatic hydrolysis of proteins of both animal and plant origin, in order to generate novel protein hydrolysates and bioactive peptides mentioned above.

5.1. Animal protein sources

5.1.1. Albumen (egg white)

Structural changes of egg white proteins (EWP) caused by HIU pretreatment are considered beneficial to hydrolysis promotion. Stefanović et al. (2014) found that HIU pretreatment (21.3 W and 40 kHz) of egg white proteins during 15 min at 25 °C resulted in increased initial rate and equilibrium degree of Alcalase hydrolysis by about 140 and 14% respectively. However, prolonged exposure to HIU of 60 min seemed to have a negative effect. This could be due to the formation of protein aggregates causing lower accessibility of the unfolded and aggregated proteins to the appropriate protease. Knezevic-Jugovic et al. (2012) compared the effect of HIU and high-pressure carbon dioxide processing on proteolytic hydrolysis of EWP and antioxidant activity of the obtained hydrolysates. According to the results obtained by these

authors, the combination of HIU pretreatment (1 h, 30 kHz, pH 8.3) and a subsequent enzymatic hydrolysis with Alcalase (50 °C and pH 8.0) enhanced the antioxidant activity of the obtained hydrolysates. In contrast to these works, Lei, Majumder, Shen, and Wu (2011)did not find an HIU effect (20 and 60 kHz) on the overall degree of proteolytic hydrolysis in ovotransferrin pretreated from 5 to 480 s. However, the reactive sulfhydryl groups increased over 80% for the samples with longer sonication times. ACE inhibitory activity of the hydrolysates was improved when ultrasonic time was increased from 60 to 480 s. In this sense, these authors suggested that HIU could produce an unfolding of protein chains and thus release possible peptides that are responsible for ACE-inhibitory activity.

5.1.2. Dairy proteins

As for this protein source, researcher Hankie Uluko and co-workers have conducted an extensive research about the application of HIU in the production of bioactive peptides from milk protein concentrate (MPC). In a first study, Uluko et al. (2013) compared the effect of microwave and HIU pretreatment (800 W, 1 to 8 min, 50 °C) prior to enzymatic hydrolysis (3 h) using five different enzymes (see Table 1) on kinetics and degree of hydrolysis, protein solubility, bitterness and ACE inhibitory activity. Pretreatments increased the DH and stabilized the solubility of the hydrolysates but could not significantly reduce hydrolysate bitterness depending on the choice of enzyme and pretreatment time. The ACE inhibitory activity of the hydrolysates was higher with 5-min HIU-pretreated Neutrase hydrolysates giving IC_{50} value of 0.23 mg/mL. However, after 5 min of pretreatment, there was a decline in the activity for all pretreatments, indicating that pretreatment time is a key factor in the production of potent ACE inhibitors from MPC. Based on the results obtained in this study, Uluko et al. (2013) evaluated the effect of three independent variables, HIU pretreatment time (3.3 to 6.6 min), hydrolysis time (1.7 to 3.3 h) and Neutrase-to-substrate ratio (0 to 2.51%), on ACE inhibitory activity of the obtained MPC hydrolysates. According to the response surface analysis carried out by these authors, the optimum conditions for producing hydrolysates with the highest ACE inhibition rate (IC_{50} = 0.044 mg/mL) were HIU pretreatment time, hydrolysis time and enzyme-to-substrate ratio of 4.11 min, 2.32 h and 2.33%, respectively. In a subsequent study, Uluko et al. (2014) studied the effect of HIU (800 W, 1 to 8 min) on peptidic profiles and ACE inhibition of MCP hydrolysates. Regarding the peptide profiles, these authors found an increase in number of peptides in the molecular mass range of 100–2000 Da when HIU pretreatment was applied up to 5 min. Thus, ACE inhibitory activity increased due to the formation of new small peptides in HIU pretreated samples which were not present in the control samples. Finally, Uluko et al. (2014) designed a pilot-scale process to increase the antioxidant capacity of MPC hydrolysates which involved a HIU pretreatment prior to Neutrase hydrolysis (see Table 1), membrane filtration (ultrafiltration and nanofiltration) and drying (spray drying or freeze drying). According to the results, the pilot scale membrane fractionation used in this work improved the separation of ACE inhibitory and antioxidant peptides in complex protein hydrolysates. Fractions from the hydrolysates varied in bioactivity with 0.2–3.5 kDa fraction showing the highest ACE inhibitory activity while the 3.5–8 kDa fraction showed an improved antioxidant capacity. On the other hand, the drying method had no significant effect on the bioactivities of the hydrolysates and the peptide fractions.

Recently, Uluko et al. (2015) compared the effect of HIU (800 W and 20 kHz), microwave and heat (90 °C) pretreatment (10 min) or their combination on the antioxidant capacity of MPC hydrolysates. According to the results of this work, HIU produced the highest increase in DH (32.11%) in comparison to the other pretreatments studied. The MPC hydrolysates of HIU pretreated samples had the highest radical scavenging activity (EC_{50} = 0.238 mg/mL) compared to another pretreatments, indicating that it is the most effective pretreatment.

Regarding other milk proteins, Madadlou, Sheehan, Emam-Djomeh, and Mousavi (2011) studied the generation of ACE inhibitory peptides from casein by using encapsulated fungal protease from *Aspergillus oryzae* in the absence or presence of HIU. According to the results obtained in this work, the application of HIU did not affect maximum ACE inhibitory activity but shortened the process from 180 min to 30 min.

5.1.3. Tilapia (Oreochromis niloticus)

Many investigations have reported that peptides form fish protein hydrolysate have biological activities, such as antioxidant and anti-inflammatory activity. Kangsanant, Murkovic, and Thongraung (2014) studied the application of HIU both in pretreatment and during Flavourzyme hydrolysis (see Table 1) of tilapia muscle protein and its effect on the bioactivity of the obtained hydrolysates, such as their antioxidant and anti-inflammatory activity. According to the results reported by these authors, the HIU-assisted hydrolysis provoked a decrease in DH (ranging from 23 to 35%) in comparison to the conventional process. On the other hand, the HIU pretreatment (70 W) at 30 and 45 min combined with conventional hydrolysis is the optimal condition for producing tilapia hydrolysates with in vitro nitric oxide inhibitory and antioxidant activity. In a subsequent work, Kangsanant, Thongraung, Jansakul, Murkovic, and Seechamnanturakit (2015) purified and characterized antioxidant and nitric oxide (NO) inhibitory peptides from tilapia protein hydrolysate with the optimal conditions of HIU pretreatment and Flavourzyme hydrolysis reported by Kangsanant et al. (2014). The antioxidant peptide (mass weight: 6334.49 Da) and NO-inhibitory peptide (mass weight: 6309.49 Da) produced no cytotoxity in RAW264.7 macrophage cell lines at concentration of 100 μg/mL. The purified peptides at the same concentration exhibited antioxidant and NO-inhibitory activity of 83 ± 1.1 and 40.9 ± 0.2, respectively, which were about 100 times those of the crude hydrolysates. This phenomenon can be attributed to the presence of a mixture of active and inactive peptides which, being purified by techniques such as ultrafiltration or gel chromatography, can yield a peptide fraction with higher bioactivity (Harnedy, O'Keeffe, & FitzGerald, 2015; Nimalaratne, Bandara, & Wu, 2015; Shi et al., 2014).

In summary, the peptides obtained from tilapia under the conditions studied by Kangsanant et al. (2015) could be an interesting application as bioactive food ingredients. However, antioxidant and NO-inhibitory activities in vivo and stability of these peptides are needed to determine their usefulness in development of functional foods.

5.1.4. Larvae and pupae

Dai, Ma, Gu, and Tang (2011) studied the HIU effects during Alcalase hydrolysis of defatted larva flour of *Tenebrio molitor* (L.). According to the results showed by these authors, a short-time (0–5 min) HIU treatment enhanced Alcalase activity while from 5 min onward, the enzyme activity decreased drastically. Ultraviolet absorption and fluorescence emission spectra showed changes on the tertiary structure of Alcalase induced by acoustic energy. Finally, HIU did not affect the amino acid composition of hydrolysates of defatted larva flour of *T. molitor* (L.) (Asp, Glu, Ser, His, Gly, Thr, Arg, Ala, Tyr, Cys-s, Val, Met, Phe, Ile, Leu, Lys, Pro) with DH 8%. Recently, Jia, Wu, Yan, and Gui (2015) studied the effect of ultrasonic power (250 to 600 W, 20 kHz) and time (8 to 50 min) of HIU-pretreatment to Alcalase hydrolysis on the ACE inhibitory activity of silkworm pupae (*Bombyx mori*) protein hydrolysates. ACE inhibitory activity of hydrolysates was higher with increasing ultrasonic power and ultrasonic time up to a point beyond which it decreased with the increase of these parameters. So, the hydrolysate with the highest activity was obtained at hydrolysis of 50 min when the substrate was pretreated at power of 410 W for 32 min. A novel tripeptide with a potent ACE inhibitory activity was identified in this work, Lysine-Histidine-Valine (IC_{50} = 12.82 μM), and it was stable against the gastrointestinal proteases. In addition, molecular docking simulation indicated that this peptide could effectively interact with the active site of ACE.

5.2. Plant proteins sources

5.2.1. Wheat germ protein

In wheat germ proteins, HIU technology has demonstrated to be an efficient method for producing ACE inhibitory and antioxidant peptides from wheat germ protein. However, its effects are dependent on process variables and whether HIU application has been performed as pretreatment or during the hydrolysis process. In this sense, Jia et al. (2010) studied the effect of HIU application both in pretreatment and during Alcalase hydrolysis of defatted wheat germ protein (DWGP) on ACE inhibitory activity of the hydrolysate. These authors found that HIU application during hydrolysis increased initial hydrolysis rate about 14.3–33.3%. However, HIU pretreatment produced an increase (21–40%) in ACE inhibitory activity of the hydrolysate obtained. These results agree with those obtained by Huang, Liu, Ma, and Zhang (2014) who reported an increase (32.14%) of ACE inhibitory activity of the hydrolysate obtained when wheat germ protein was pretreated with HIU at 600 W for 10 min. According to ANS (1-anilino-8-naphtalene-sulfonate) fluorescence spectra and scanning electron micrographs, Jia et al. (2010) observed an increase of hydrophobic surface of DWGP and loosening of protein tissue, which facilitated the release of ACE inhibitory peptides during enzymatic hydrolysis. Zhou, Ma, Yu, et al. (2013) compared the HIU-pretreatment effects, using an ultrasonic probe or bath reactors, on the production of ACE inhibitory peptides from DWGP. The ultrasonic probe (191.1 W/cm^2, 10 min) resulted in a significant increase in ACE-inhibitory activity compared with ultrasonic bath operating at single or dual fixed and sweep frequencies. However, in bath systems, the fixed frequency of 33 kHz and the sweep frequency of 40 ± 2 kHz increased the ACE inhibitory activity by 92 and 132%, respectively, over the control. On the other hand, Qu et al. (2012) investigated the process variable effects of sweep frequency and pulsed (SFP) ultrasound-assisted enzymolysis on the production of ACE inhibitory peptides from wheat germ protein. Taking into account the activity of the obtained ACE inhibitory peptide and operation costs, these authors reported that the optimal conditions of SPF ultrasound-assisted enzymolysis are an enzymolysis time of 120 min and substrate concentration of 24 g/L, which gave the conversion rate of the protein and ACE inhibitory activity of the peptide as 60.7 and 65.9%, respectively. Regarding the effect of HIU on antioxidant capacity of wheat germ protein hydrolysates, Zhu, Su, Guo, Peng, and Zhou (2011) evaluated the effect of Alcalase hydrolysis assisted by HIU at different ultrasonic frequencies (see Table 1) on antioxidant activity of the hydrolysate generated. According to the results showed in this work, wheat germ hydrolysates obtained at 25 kHz exhibited the strongest antioxidant activity, with an EC_{50} value of 0.513 mg/mL for ferrous ion-chelating activity and the highest proportion of peptide fractions of molecular weight between 3000 and 500 Da.

5.2.2. Corn

Ren, Ma, Mao, and Zhou (2013) studied the effects of sweeping frequency ultrasound (SFU) and fixed frequency ultrasound (FFU) on the enzymatic hydrolysis process of zein protein and ACE-inhibitory activities of its hydrolysates. This work demonstrated that HIU pretreatments (SFU and FFU) increased the DH of zein by approximately 11.5% over control samples. However, no significant differences in DH were observed between the two ultrasonic pretreatments. SFU and FFU increased ACE-inhibitory activity of zein hydrolysates by 12.3–116.7%. Regarding the changes of protein structure induced by HIU, the fluorescence intensity of SFU- and FFU-treated zein showed a weaker structure than untreated zein, indicating that more Phenylalanine, Tryptophan and Tyrosine residues were exposed outside the polypeptide chains. After zein was pretreated with SFU, α-helical content increased by 3.4% and β-sheets, β-turns and random coils content by 24.4%. Finally, microstructural analysis showed a rupture of the fine meshwork structure of zein resulting in the appearance of several micro-holes caused by HIU pretreatment. Zhou, Ma, Ding, et al. (2013) studied the effect of ultrasonic pretreatment of corn gluten meal proteins and enzyme (Neutrase) on protein conformation and the ACE inhibitory activity of hydrolysate. The results of this study indicated that the highest ACE inhibitory activity was obtained at 10 min hydrolysis when corn gluten meal was pretreated at 1000 W and 20 kHz for 10–30 min. The activities of Neutrase and ACE inhibitory peptides were highest at 250 W during 15 min of pretreatment. Furthermore, the fluorescence spectrum and UV differential spectra of the proteins of corn gluten meal and Neutrase reflected that HIU induced molecular unfolding.

5.2.3. Soy

Several studies have found that soy proteins are generally resistant to enzymatic hydrolysis due to their compact quaternary and tertiary structures that protect many of the peptide bonds (Chen et al., 2011). Therefore, a combination of HIU pretreatment and controlled enzymatic hydrolysis might be a promising way to enhance the functionality of soy proteins. Xu, Zhao, Qu, and Ye (2013) showed that HIU pretreatment at 400 W improved the antioxidant activity of highly denatured soybean meal hydrolysate prepared using Neutrase. In this investigation, the in vitro antioxidant activity and in vivo anti-fatigue effect (measured in mice after exercise) increased with decreasing molecular weight of the soybean meal hydrolysate. Both parameters of the hydrolysate peaked when the molecular weight fraction was less than 5 kDa. In contrast, Yang, Yang, Li, Li, and Jiang (2011) reported that HIU treatment produced an inhibition on the Alcalase hydrolysis of soy sauce lees proteins.

5.2.4. Garlic

Garlic is other plant protein source, which has attracted attention in functional food sector due to its health-promoting properties such as anticancer, antimicrobial and antioxidant activity. Huang, Ma, Peng, Wang, and Yang (2015)suggested that HIU pretreatment might break chemical bonds between polysaccharides and proteins in garlic cell walls. This breakage could increase the surface hydrophobicity and loosen the proteins tissue of garlic, which could facilitate the release of bioactive peptides with ACE inhibitory activity from garlic powder during enzymatic hydrolysis. Recently, Ma, Huang, Peng, Wang, and Yang (2015)studied the SFU and single frequency countercurrent ultrasound (SFCU) pretreatment on production of ACE inhibitory peptides from garlic hydrolysates. On the basis of ACE inhibitory activity of garlic hydrolysates, the ultrasonic time of 72 min, initial temperature of 45 °C and pH of 8.2 in the SFCU reactor were considered as the optimal operating conditions. For the SFU reactor, the most favorable operating conditions were total ultrasonic time 1.5 h, on-time of pulse 18 s and off-time of pulse 3 s. However, there were no significant differences in the ACE inhibitory activities and IC_{50} values obtained between both HIU pretreatments under optimum conditions.

5.2.5. Other plant sources

In addition to the plant protein sources discussed in previous sections, HIU has been used satisfactorily for extracting protein or enhancing the hydrolysis process in other products such as oat (Wang et al., 2014), cowpea (Quansah, Udenigwe, Saalia, & Yada, 2013) and plum (González-García, Marina, & García, 2014). Wang et al. (2014) reported that the pretreatment of oat protein with HIU (750 W and 20 min) followed by 60 min of enzymolysis were the best conditions for hydrolysis and ACE inhibitory activities of the peptides. After the HIU pretreatment, the hydrolysis rate and the ACE inhibitory activities of peptides were significantly ($p < 0.001$) increased by 32.1 and 53.8%, respectively compared to unpretreated samples. Quansah et al. (2013) found that both thermal and HIU pretreatment of cow protein resulted in statistically significant enhancement of ORAC (Oxygen Radical Absorbance Capacity) values and reduction of FRAP (Ferric Reducing Antioxidant Power) and SRSA (Superoxide Radical Scavenging Assay) of the obtained hydrolysates from in vitro gastrointestinal digestion of cowpea proteins. According to the amino acid analysis carried out in

this study, cow pea processing decreased total sulfur-containing amino acids in the hydrolysates, particularly cysteine. Therefore, Quansah et al. (2013) concluded that thermal and HIU pretreatment of cow pea can diminish the radical scavenging and reducing the potential of enzymatic hydrolysates possibly due to the decrease amounts of cysteine.

Regarding another plant protein source, González-García et al. (2014) developed a method for the extraction of plum (*Prunus domestica* L.) seed proteins assisted by HIU which enabled plum seed protein isolate preparation with a protein content of about 40% in less than 1 h. Furthermore, the analysis of Alcalase hydrolysate by RP-HPLC-MS/MS enabled the identification of 13 peptides with typical features of antioxidant and antihypertensive activity.

6. Future trends

Despite the amount of investigation carried out on this topic and the promising results that have been obtained, much remains to be elucidated. In this regard, it would be interesting to further investigate the HIU-induced mechanisms during application as a pretreatment or during enzymatic hydrolysis and the effects of this technology on the enzymatic process in response to the type of protein used. On the one hand, when HIU is applied as pretreatment to hydrolysis, it is necessary to deepen our knowledge of the conformational changes in proteins provoked by HIU with the aim of gaining a better understanding of the effectiveness of this emerging technology in the production of bioactive peptides. In the case of HIU application during enzymatic hydrolysis, it would be interesting to study the interaction between acoustic energy and the enzymes used during the process. Most importantly, further in vivo studies are necessary in order to identify the bioavailability of the peptides obtained by HIU application, since most studies reviewed herein have only assessed their in vitro bioactivity.

In addition, further research should determine whether the HIU application in the production of protein hydrolysates and bioactive peptides is coupled to a process profitability with the purpose of bringing this technology to industrial scale.

7. Conclusions

In this overview, the potential of HIU technology in the production of protein hydrolysates and bioactive peptides has been highlighted. The differences found in these studies could be due to various factors, reviewed in the present work. The effectiveness of HIU application during pretreatment or enzymatic hydrolysis is highly dependent on protein source, enzyme type, equipment design, ultrasonic power and conditions used. Therefore, in this literature review, there have presented recently published papers that focus on the application of HIU for the enhancement of enzymatic hydrolysis of proteins of both animal and plant origin in order to contribute to a better understanding of the effect that process variables have during hydrolysis. In this manner, researchers working in this area can choose the conditions that best suit the specific processes they are designing. Additionally, it is important to take into account the importance of the characterization of acoustic field in HIU application systems with the aim of having a more accurate measurement of the acoustic energy available in the hydrolysis process. This, in turn, would allow to obtain reproducible results in different devices using HIU technology.

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