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Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review

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ABSTRACT

Bioactive peptides are oligopeptides that consist of 2–20 amino acids that can exert beneficial effects on human health in addition to basic nutritional effects. Food derived protein hydrolysates or peptides with immunomodulatory and anticancer activities have been reported from a variety of food protein sources such as milk, egg, fish, rice, soybean, pea, chlorella, spirulina, oyster and mussel. *In vitro* hydrolysis of food proteins using commercial proteolytic enzymes is the most commonly employed process for the production of immunomodulatory and anticancer food protein hydrolysates. The immunomodulatory and anticancer activities of food derived protein hydrolysates or peptides are related to the amino acid composition, sequence and length. Most immunomodulatory and anticancer food protein hydrolysates or peptides were tested using cell culture and animal models, while a few involved clinical trials. This review provides a comprehensive overview of immunomodulatory and anticancer food derived protein hydrolysates or peptides, their production and mechanisms of action.

1. Introduction

Protein is an important macronutrient as a source of essential amino acids and energy. In addition to basic nutrition, some food proteins can provide extra health benefits through the release of bioactive peptides encrypted in their sequences. In recent years, research on protein hydrolysates or bioactive peptides for the production of value-added food ingredients has attracted great attention of food scientists worldwide. Generally, bioactive peptides are oligopeptides that are inactive within the sequence of the protein molecule but can be released by enzymatic hydrolysis, fermentation and gastrointestinal digestion (Bhat, Sunil Kumar, & Bhat, 2015; Chalamaiah, Dinesh Kumar, Hemalatha, & Jyothirmayi, 2012; Garcia, Puchalska, Esteve, & Marina, 2013; He, Cao, Pan, Yang, & Zhang, 2015). The low molecular weight (MW) peptides are easily digestible and more bioavailable than proteins for a variety of physiological functions of the human body. They generally exhibit greater bioactivity than the parent protein.

Protein hydrolysates (peptides) derived from several food proteins have been reported to possess a wide range of bioactivities including immunomodulatory, anticancer, antihypertensive, antioxidant, anti-inflammatory, mineral binding, opiate, antilipemic, osteoprotective, and antimicrobial effects (Bhat et al., 2015; Chalamaiah et al., 2012; Garcia et al., 2013). In addition to bioactivities, food derived protein hydrolysates (peptides) possess various physicochemical properties including solubility, lipid binding, foaming, and emulsification properties depending on their composition, sequence, and length (Cho et al., 2014; Pokora et al., 2013). Hence, food derived protein hydrolysates are promising ingredients for developing functional foods (Chalamaiah et al., 2012). Additionally, the peptide preparation could help the translation of underutilized food proteins and protein by-products into valuable products, which is of great interest to food companies.

Bioactive protein hydrolysates or peptides can be obtained from different food protein sources. The most investigated sources of bioactive peptides are milk, whey, egg, fish, marine species, soybean, rice,

Abbreviations: ACE, angiotensin converting enzyme; AO, acridine orange; BAD, Bcl-2-associated death promoter; BAX, BCL2-associated X protein; B cells, B lymphocytes; Bcl-2, B-cell lymphoma 2; CCL18, chemokine (CC motif) ligand 18; CD, cluster of differentiation; CDK2, cyclin-dependent kinase 2; CO_2, carbon dioxide; Con-A, concanavalin A; COX-2, cyclooxygenases 2; Da, dalton; DH, degree of hydrolysis; DMBA, 7,12-dimethylbenz[*a*]anthracene; DNA, deoxyribonucleic acid; EB, ethidium bromide; ELISA, enzyme linked immunosorbent assay; ERK, extracellular signal–regulated kinase; EW, whole egg white; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; FPHs, food protein hydrolysates; IC_{50}, inhibitory concentration 50; IFN-γ, interferon-γ; Ig, immunoglobulin; IL, interleukin; iNOS, inducible nitric oxide synthase; kDa, kilodalton; LfcinB, lactoferricin; LPS, lipopolysaccharide; LYS, lysozyme; MAPKs, mitogen-activated protein kinases; MHC, major histocompatibility complex; MTT, 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MW, molecular weight; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cells, natural killer cells; NO, nitric oxide; OM, ovomucoid; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; PGE2, prostaglandin E2; PI, propidium iodide; PS, phosphatidylserine; S-IgA, secretory-immunoglobulin A; SRBCs, sheep red blood cells; Tc cells, T cytotoxic cells; T cells, T lymphocytes; TGF-β, transforming growth factor beta; Th cells, T helper cells; TNF-α, tumor necrosis factor-alpha; Treg cells, regulatory T cells; WHO, world health organization

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peanut, chickpea, amaranth, corn, and algae. Among food-derived protein hydrolysates or peptides, those with antioxidant, anti-hypertensive, anti-inflammatory, antimicrobial, intestine-modulatory, opioid, hypocholesterolemic, and metal chelating activities have been extensively reviewed in literature (Chalamaiah et al., 2012; Erdmann, Cheung, & Schröder, 2008; Garcia et al., 2013; Stefanucci et al., 2017), while those with immunomodulatory and anticancer activities have not been reviewed to that extend. This encouraged the authors to review recent research on the production and mechanisms of action of immunomodulatory and anticancer protein hydrolysates or peptides derived from various food sources. Additionally, the assays for evaluation of immunomodulatory and anticancer activities of food protein hydrolysates are presented.

2. Immunomodulatory food protein hydrolysates

2.1. Brief overview of the immune system

The immune system is a network of cells, tissues, and organs that acts to eliminate potentially harmful substances such as bacteria, viruses, fungi, protozoans, and prevents the growth of cancer cells. The immune system is classified into two main functional categories namely innate and adaptive immunity. Innate immunity, also called natural immunity or native immunity, is non-specific and provides the first line of defense through anatomic (skin, mucous membranes), physiologic (low pH, temperature and chemical mediators), cellular (macrophages, polymorphonuclear leukocytes, dendritic cells and natural killer (NK) cells), or inflammatory components (cytokines, interferons, complement, defensins, leukotrienes, acute phase proteins and prostaglandins). The macrophages and neutrophils of the innate immune system play an important role in phagocytosis. NK cells play a vital role against tumor cells and virus-infected cells non-specifically.

Adaptive immunity, also called specific immunity or acquired immunity, is highly specific to potentially dangerous foreign antigens. The adaptive immunity is divided into two types, i.e. cell-mediated and antibody-mediated (humoral) immunity. T lymphocytes (T cells) and B lymphocytes (B cells) are the most important cells in adaptive immunity. Humoral immunity involves B lymphocytes that are responsible for the production of antibodies upon interaction with specific antigens. The antibodies can bind to antigens of invading pathogens and label them for destruction by macrophages.

Cell-mediated immunity consists of effector T lymphocytes which secrete immune regulatory factors, such as cytokines, and mediate a cellular immune response upon interaction with antigen presenting cells (APSs). T lymphocytes are divided into three sub-groups of cells namely helper T (Th cells) cells, cytotoxic T (Tc cells) cells and regulatory T (Treg cells) cells. Tc cells express a surface receptor, cluster of differentiation (CD) 8+, and recognize endogenous antigens associated with class I major histocompatibility complex (MHC) and kill cancer cells and cells infected with viruses. Whereas Th cells display a surface marker, CD4+ and recognize exogenous antigens complexed with MHC class II. Th cells secret cytokines such as interferon-γ (IFN-γ), interleukin (IL) 2, IL-4, IL-5, IL-6, IL-10, IL-13, and IL-25 and help activate B cells, T cells and other immune cells (e.g. macrophages) to participate in immune response (Nijkamp & Parnham, 2011). Treg cells are responsible for suppression of the immune responses of other T cells and prevent autoimmune diseases by maintaining self-tolerance.

2.2. Production of immunomodulatory protein hydrolysates or peptides from food proteins

An immunomodulator is any substance that can augment, decrease or modify the immune responses by altering any part of the immune system including both innate and adaptive functional categories of the immune system. The immune system is vital for our survival and provides defense against pathogens but can be influenced by many factors including stress, unhealthy lifestyle practices, pathogens, and antigens (Segerstrom & Miller, 2004). Several drugs such as cyclosporine, tacrolimus, glucocorticoids, phytol, aristolochic acid, plumbagin and levamisole have successfully been applied to modulate the immune response in humans (Gertsch, Viveros-paredes, & Taylor, 2011). However, the toxic side effects and high cost of the allopathic drugs have restricted their usage in patients and most immunomodulatory drugs are not appropriate for chronic or preventive uses (Wang et al., 2010). Modulation of immune function through the dietary components has been reported to be an effective and efficient strategy; meanwhile the discovery of novel immune modulating peptides from food proteins could provide further advantage to the dietary treatment (Chalamaiah et al., 2014).

Immunomodulatory substances in food play significant role as health-benefiting factors that protect the body from invading pathogens. Recently, immunomodulatory protein hydrolysates or peptides have been produced from a wide range of food protein sources. These include milk (Biziulevicius, Kislukhina, Kazlauskaite, & Zukaite, 2006; Kazlauskaite et al., 2005; Masotti, Buckley, Champagne, & Green-Johnson, 2011; Pan, Wu, Liu, Cao, & Zeng, 2013), whey (Javier, Ayoa, Francisco, & Ana, 2014; Ma et al., 2014; Saint-Sauveur, Gauthier, Boutin, & Montoni, 2008; Saint-Sauveur, Gauthier, Boutin, Montoni, & Fliss, 2009), egg (Lee et al., 2009; Lozano-Ojalvo, Molina, & Lopez-Fandino, 2016; Marie et al., 2009; Nelson, Katayama, Mine, Duarte, & Matar, 2007), fish (Karnjanapratum, O'Callaghan, Benjakul, & O'Brien, 2016; Chalamaiah, Hemalatha et al., 2015, 2014; Ahn, Cho, & Je, 2015; Mallet et al., 2014; Hou, Fan, Li, Xue, & Yu, 2012), shellfish (Kim, Kim, Hwang, Kang et al., 2013), sheep (Hua, Yu-hua, Li-zhen, & Bao-hua, 2009), frog (Huang et al., 2014), Oyster (Wang et al., 2010; Cai, Pan, Wu, Wan, & Sun, 2013), clam (He, Cao, Pan, Yang, & Zhang, 2015; Lee et al., 2012), rice (Takahashi, Moriguchi, Yoshikawa, & Sasaki, 1994), wheat (Horiguchi, Horiguchi, & Suzuki, 2005), soybean (Egusa & Otani, 2009; Kong, Guo, Hua, Cao, & Zhang, 2008; Masotti et al., 2011; Vernaza, Dia, Mejia, & Chang, 2012; Yimit, Hoxur, Amat, Uchikawa, & Yamaguchi, 2012), yellow pea (Ndiaye, Vuong, Duarte, Aluko, & Matar, 2012), lupin (Millan-Linares, Bermudez, Yust, Millan, & Pedroche, 2014), flaxseed (Udenigwe, Lu, Han, Hou, & Aluko, 2009), amaranth (Montoya-Rodriguez, Gonzalez de Mejía, Dia, Reyes-Moreno, & Milan-Carrillo, 2014) and microalgae (Vo, Ryu, & Kim, 2013; Senevirathne, Ahn, & Je, 2010; Morris et al., 2007).

The various steps involved in the production of food derived immunomodulatory protein hydrolysates or peptides are shown in Fig. 1. Various recent studies have shown that protein hydrolysates or peptides with immunomodulatory activities can be produced from a variety of food protein sources by using various methods such as *in vitro* enzymatic hydrolysis, autolytic process using endogenous enzymes and microbial fermentation (Chalamaiah, Hemalatha et al., 2015; Duarte, Vinderola, Ritz, Perdigon, & Matar, 2006; Karnjanapratum, O'Callaghan, Benjakul, & O'Brien, 2016; Lozano-Ojalvo et al., 2016; Ma et al., 2014; Masotti et al., 2011; Ndiaye et al., 2012; Nesse, Nagalakshmi, Marimuthu, & Mamta Singh, 2011). Among these methods, *in vitro* hydrolysis of food proteins using commercial proteolytic enzymes is the most commonly employed process, in which peptide bond cleavage allows the release of active peptides capable of exhibiting immunomodulatory activities. Selection of a suitable proteolytic enzyme is a vital factor, due to different cleavage specificity of enzymes, that can affect the release of immunomodulatory peptides from food proteins (Chalamaiah et al., 2014; Chalamaiah, Hemalatha et al., 2015; Lozano-Ojalvo et al., 2016). Several proteolytic enzymes from plant, animal and microbial sources have been successfully used to produce immunomodulatory protein hydrolysates or peptides. These include trypsin, Alcalase, pepsin, papain, pancreatin, chymotrypsin, thermolysin, Flavourzyme, Protamex, and Neutrase (Biziulevicius et al., 2006; Chalamaiah, Hemalatha et al., 2015; Kim, Kim, Hwang, Kang et al., 2013; Kong et al., 2008; Lee et al., 2012; Lozano-Ojalvo et al., 2016; Mallet et al., 2014; Morris et al., 2007; Ndiaye et al., 2012; Saint-

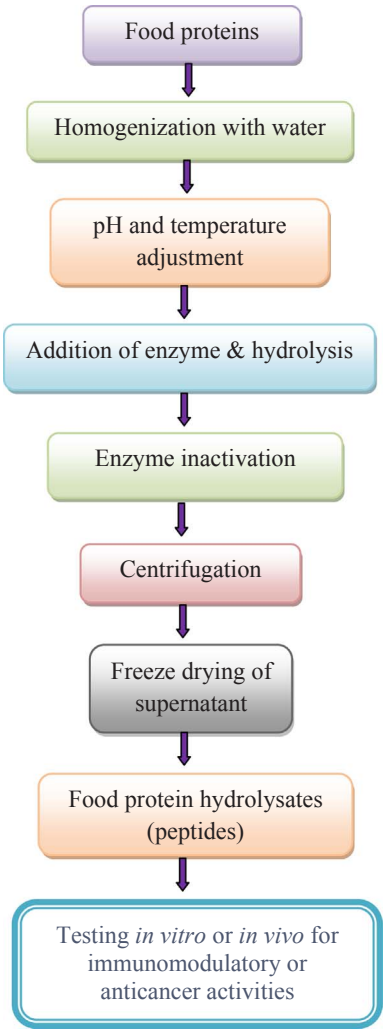

Fig. 1. Flow chart showing the various steps involved in the production of immunomodulatory or anticancer protein hydrolysates or peptides from food proteins.

Sauveur et al., 2009; Takahashi et al., 1994; Tsuruki et al., 2003) (Table 1). Among these enzymes, trypsin, Alcalase and pepsin are the most extensively studied enzymes to produce immunomodulatory peptides from a variety of food protein sources such as milk, egg, fish, rice, soybean, flaxseed, whey, microalgae (*Spirulina maxima)*, clam and frog (Chalamaiah et al., 2014; Chalamaiah, Hemalatha et al., 2015; He et al., 2015; Lozano-Ojalvo et al., 2016; Ahn et al., 2015; Ahn, Je, & Cho, 2012; Biziulevicius et al., 2006; Cai et al., 2013; Huang et al., 2014; Javier et al., 2014; Kazlauskaite et al., 2005; Kong et al., 2008; Lee et al., 2012; Nelson et al., 2007; Pan et al., 2013; Takahashi et al., 1994; Tsuruki et al., 2003; Udenigwe et al., 2009; Vo et al., 2013). Besides enzyme selection, the nature of substrate and the physicochemical conditions (e.g. temperature, pH, duration) of the hydrolysis process also affect the production of immunomodulatory peptides from food protein sources (Chalamaiah, Hemalatha et al., 2015; Hou et al., 2012; Ma et al., 2014). In addition to *in vitro* enzymatic hydrolysis, few studies have reported preparation of immunomodulatory peptides using microbial fermentation technique (Duarte et al., 2006; Masotti et al., 2011).

2.3. Assays for evaluation of immunomodulatory effects of food protein hydrolysates or peptides

Several *in vitro*, *in vivo* (animals) and clinical methods have successfully been employed to assess the immunomodulatory effects of food-derived protein hydrolysates or peptides. *In vitro* (cell lines) and *in vivo* (animal models) assays are the common methods used to evaluate the immunomodulatory effects of food-derived protein hydrolysates (Ahn et al., 2015; Cai et al., 2013; Chalamaiah, Hemalatha et al., 2015; Duarte et al., 2006; He et al., 2015; Huang et al., 2014; Karnjanapratum et al., 2016; Marie et al., 2009; Nelson et al., 2007; Pan et al., 2013). The *in vitro* immunomodulatory testing of peptides is generally performed using established cell lines. Among various cell lines, the mouse macrophage cell line i.e. RAW 264.7 has been the most widely used cell model to determine the immunomodulatory capacity of food protein hydrolysates (Ahn, Je, & Cho, 2012; Ahn et al., 2015; Chalamaiah & Wu, 2017; Huang et al., 2014; Karnjanapratum et al., 2016; Kim, Kim, Hwang, Kang et al., 2013; Senevirathne et al., 2010; Udenigwe et al., 2009; Vernaza et al., 2012). In addition to RAW 264.7 cell line, U937 (human monocytic model), THP-1 (human monocytic cell line), and jurkat T cells (human T lymphocyte model) have also been used to explore the immunomodulatory potential of food-derived peptides (Masotti et al., 2011; McCarthy et al., 2013; Millan-Linares et al., 2014; Montoya-Rodriguez et al., 2014). In cell culture experiments, the cells are treated with different concentrations (e.g. 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/ml) of the protein hydrolysates or peptides and are incubated for 6 or 12 or 24 or 48 h depending on cell type and peptide source. After peptides treatment, the immune markers are measured in the cell culture supernatant to assess the potential immunomodulatory effects of the peptides. Most of the *in vitro* cell culture studies estimated the important signaling molecules such as cytokines, including tumor necrosis factor-alpha (TNF-α), IL-1β and IL-6, and nitric oxide (NO) that play a key role in the modulation of immune response (Ahn et al., 2012; Ahn et al., 2015; Huang et al., 2014; Kim, Kim, Hwang, Kang et al., 2013; Millan-Linares et al., 2014; Montoya-Rodriguez et al., 2014; Saeleaw, O'Callaghan, Benjakul, & O'Brien, 2016; Senevirathne et al., 2010; Udenigwe et al., 2009; Vernaza et al., 2012). The concentrations of cytokines are commonly determined by using enzyme linked immunosorbent assay; while nitrite is usually quantified by Griess reagent (1% sulfanilamide, 0.1% N-1-(naphthyl)ethylenediamine- dihydrochloride in 2.5% phosphoric acid) (Kim, Kim, Hwang, Kang et al., 2013).

Several researchers have performed the animal experiments in order to demonstrate the immunomodulatory effects of food derived protein hydrolysates or peptides *in vivo*. Most of the *in vivo* immunomodulatory experiments with food derived peptides were conducted in mouse model since mouse is the animal responding most closely to that of humans (Cai et al., 2013; Chalamaiah, Hemalatha et al., 2015; Pan et al., 2013; Wang et al., 2010). In several studies the mice were orally administered with various concentrations (e.g. 0.005, 0.01, 0.05, 0.10, 0.25, 0.50, 1.0 and 1.5 g/kg/body weight) of protein hydrolysates or peptides for a specific period of time (e.g. 3 or 7 or 14 or 28 or 45 days). The treatment period and concentration of the peptides depend on the source of peptides and immune parameters under investigation. After the experimental period, the mice are sacrificed and different tissues (spleen, thymus, blood etc.) are collected for the immunological, hematological and biochemical investigations. Several *in vivo* and *ex vivo* immunological assays such as lymphocyte proliferation, peritoneal macrophage phagocytosis, NK cell activity, measurement of splenic T lymphocyte subpopulations ($CD4^+$ & $CD8^+$), determination of secretory-immunoglobulin-A (S-IgA) (mucosal immunity), measurement of serum immunoglobulins (IgA, IgM, & IgG), and cytokines (IL-2, IFN-γ, IL-5 and IL-6) are most commonly used *in vivo* methods for evaluation of immunomodulatory effects of food derived peptides (Cai et al., 2013; Chalamaiah, Hemalatha et al., 2015; Egusa & Otani, 2009; Hou, Fan, Li, Xue, & Yu, 2012; Hua et al., 2009; Kong et al., 2008; Ma et al., 2014; Mallet et al., 2014; Morris et al., 2007; Wang et al., 2010; Yang et al., 2009). Details of these assays have been reviewed elsewhere (Maestri, Marmiroli, & Marmiroli, 2016).

A few studies conducted in humans with food protein hydrolysates or peptides have measured the changes of IgM, IgG and IgA, CD4/CD8

Table 1
Immunomodulatory activities and isolated peptide sequences of various food protein hydrolysates (FPHs) produced from different food sources.

Parent protein	Enzymes used to produce FPH	Peptide sequence & molecular weight	Immunomodulatory activities showed	Reference
Clinical trials				
Wheat gluten	–	–	Significantly increased the natural killer (NK) cell activity in humans	Horiguchi et al. (2005)
Salmon fish protein	Prepared by endogenous hydrolyzing agents	Contained 60–70% di/tri peptides of < 10 kDa	Changes of IgM, IgG and IgA, & CD4/CD8 ratios were observed in malnourished Indian children	Nesse et al. (2011)
Soybean protein	Theroase, bioprase, & sumizyme FP	Peptides distributed between 120–1000 Da	Increased $CD11b^+$ and $CD56^+$ cells in humans	Yimit et al. (2012)
In vivo animal studies				
Human casein	Trypsin	Val-Glu-Pro-Ile-Pro-Tyr	Stimulated the phagocytosis of opsonized sheep red blood cells (SRBCs) by murine peritoneal macrophages and enhanced the resistance of mice to infection with *Klehsiella pneumoniae*	Parker et al. (1984)
Human and cow caseins	Trypsin	Gly-Leu-Phe from human casein and Leu-Leu-Tyr from bovine casein	Significantly enhanced the phagocytosis of SRBCs by mouse peritoneal macrophages and protected the mice against an infection with *Klehsiella pneumonia*	Berthou et al. (1987)
Soybean protein	Trypsin	His-Cys-Gln-Arg-Pro-Arg	Showed a stimulatory effect on the phagocytic ability of human polymorphonuclear leukocytes and production of tumor necrosis factor in mice	Yoshikawa et al. (1993)
Soybean protein	Pepsin	Molecular weight in the range of 300–2000 Da	Significantly enhanced the phagocytosis of alveolar macrophages and the mitogenic activity of rats	Yamauchi and Suetsuna (1993)
Casein	Trypsin	–	Enhanced phagocytosis of mouse peritoneal and blood phagocytic cells	Kazlauskaite et al. (2005)
Casein, α-lactalbumin, β-lactoglobulin, ovalbumin and serum albumin	Trypsin, α-chymotrypsin, pepsin and pancreatin	–	Enhanced the phagocytosis capacity of peritoneal macrophages of mice	Biziulevicius et al. (2006)
Pacific whiting (*Merluccius productus*) fish protein	Fermentation process for 24 h	Small peptides (< 1 kDa)	Significantly increased phagocytosis of mice peritoneal macrophages, the number of IgA + cells in the lamina propria of small intestine, content of secretory-IgA in the small intestine lumen and the number of $IL\text{-}4^+$, $IL\text{-}10^+$, $IL\text{-}6^+$, $IFN\gamma^+$ and $TNF\alpha^+$ cells	Duarte et al. (2006)
Green macroalga (*Chlorella vulgaris*) protein	Pancreatin	Three main peptides with molecular weights lower than 5000 Da	Significantly enhanced haemopoiesis, leukocyte count, peritoneal exudate cells, macrophage activity, and stimulated both humoral and cell mediated immune functions in mice	Morris et al. (2007)
Egg yolk protein	Pepsin	–	Significantly increased the phagocytosis of peritoneal macrophages, IgA^+ cells, $IL4^+$ cells and $IL10^+$ cells in mice	Nelson et al. (2007)
Whey	Trypsin and chymotrypsin	Peptides < 10 kDa	Stimulated production of serum IgA and serum interferon-gamma (IFN-γ) in mice	Saint-Sauveur et al. (2009)
Egg white protein	Aminopeptidase of *Aspergillus* sp. origin	Peptides with molecular weights lower than ~ 1.3 kDa	Significant reductions of both serum histamine and specific IgE titers and a repression of both IL-4 and IFN-γ production in cultured spleen cells of mice	Marie et al. (2009)
Oncorhynchus ketaich protein	Complex protease	Peptides distributed between 100–860 Da and rich in Glu, Asp, Lys and Leu	Significantly increased lymphocyte proliferation, NK cell cytotoxicity, IgM antibody, $CD4^+$ T cells, and the levels of IL-2, IL-5, IL-6 and IFN-γ in mice	Yang et al. (2009)
Hen egg white	Aminopeptidase (EC3.4.11.1) of Aspergillus sp.	Majority of peptides distributed between 1.3–0.07 kDa	Significantly reduced the local expression of pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IFN-γ, IL-8, and IL-17 in piglets	Lee et al. (2009)
Soybean protein	Peptidase R	–	Significantly increased the number of spleen CD11b +, CD49b +, $IL\text{-}12^+CD11b^+$, $IFN\text{-}\gamma + CD49b^+$, and $IFN\text{-}\gamma^+CD4^+$ cells in mice	Egusa and Otani (2009)
Oyster (*Crassostrea gigas*) protein	Protease from *Bacillus* sp. SM98011	Peptides below 3 kDa	Significantly enhanced spleen lymphocyte proliferation, macrophage phagocytosis and NK cell cytotoxicity in mice	Wang et al. (2010)
Shark protein	Mixture of papain and trypsin	–	Stimulated immunity in mice by increasing the weight of thymus & spleen, level of hemolysin in serum and the phagocytosis of macrophages	Pan et al. (2013)
Yellow pea seed protein	Thermolysin	Low molecular weight peptides (1000 Da)	Significantly stimulated phagocytic activity of peritoneal macrophages and the gut mucosa immune response in mice	Ndiaye et al. (2012)
Oyster protein (*Crassostrea hongkongensis*)	Bromelain, pepsin and trypsin	–	Enhanced spleen lymphocyte proliferation and the activity of natural killer (NK) cells in BALB/c mice	Cai et al. (2013)

(*continued on next page*)

Table 1 (*continued*)

Parent protein	Enzymes used to produce FPH	Peptide sequence & molecular weight	Immunomodulatory activities showed	Reference
Rohu (*Labeo rohita*) egg (roe) protein	Pepsin, trypsin and Alcalase	Low molecular mass peptides below 10 kDa	Significantly enhanced macrophage phagocytosis, NK cell cytotoxicity, mucosal immunity (S-IgA), splenic $CD4^+$ & $CD8^+$ T cells and the level of serum IgA in mice	Chalamaiah et al. (2014)
Shark protein	Trypsin and chymotrypsin	Small peptides below 10 kDa	Enhanced the gut barrier function via up-regulation of IgA-producing cells and intestinal cytokines production, including IL-6 and TNF-α in mice	Mallet et al. (2014)
Common carp (*Cyprinus carpio*) egg (roe) protein	Pepsin, trypsin and Alcalase	Peptides of different sizes (5–90 kDa)	Significantly enhanced the proliferation of spleen lymphocytes, NK cell cytotoxicity, mucosal immunity (S-IgA), level of serum IgA and $CD4^+$ and $CD8^+$ cells in mice	Chalamaiah et al. (2015)
Alaska Pollock protein	Trypsin	Pro-Thr-Gly-Ala-Asp-Tyr	Significantly enhanced humoral, cellular, and non-specific immunity in immunosuppressed mice	Hou, Fan, Wang, Si, and Li (2016)
Ovalbumin, lysozyme, ovomucoid and whole egg white protein	Pepsin, neutrase and Alcalase	Molecular mass lower than 10 kDa	The hydrolysates reduced Con-A stimulated mice lymphocyte proliferation and production of Th_2-biased cytokines, such as IL-13 and IL-10, and decreased the secretion of the Th_1 cytokine TNF-α	Lozano-Ojalvo et al. (2016)
Coix glutelin protein	Pepsin	Small molecular weight peptides ≤ 3 kDa	Significantly stimulated mice splenocytes and peritoneal macrophages and increased the spleen index of ICR mice	Ling-Ling et al. (2017)
In vitro and ex vivo studies				
Rice albumin	Trypsin	Gly-Tyr-Pro-Met-Tyr-Pro-Leu-Pro-Arg	Promotion of phagocytosis activity for human polymorphonuclear leukocytes and augmentation of superoxide anion production by human peripheral leukocytes	Takahashi et al. (1994)
Soybean protein	Pepsin	Ala-Glu-Ile-Asn-Met-Pro-Asp-Tyr, Ile-Gln-Gln-Gly-Asn, and Ser-Gly-Phe-Ala-Pro	Significantly stimulated the proliferation of splenocytes of mice	Chen et al. (1995)
Soybean protein	Trypsin	Tridecapeptide (MITLAIPVNKPGR)	Stimulated phagocytosis of human neutrophils	Tsuruki et al. (2003)
Soybean protein	Alcalase, flavourzyme, trypsin, papain, protease A and peptidase R	Small peptides below 1000 Da	Significantly enhanced the proliferation of lymphocytes, and phagocytosis of the peritoneal macrophages of mice	Kong et al. (2008)
Whey protein	Mixture of trypsin and chymotrypsin	Low molecular mass peptides < 2 kDa	Stimulated the proliferation of mice splenocytes and significantly increased IFN-γ secretion	Saint-Sauveur et al. (2008)
Sheep bone protein	Papain	–	Stimulated rat spleen lymphocytes	Hua et al. (2009)
Flaxseed protein	Pepsin, ficin and papain	Peptides < 1 kDa	Inhibited LPS-induced NO production in RAW 264.7 macrophages	Udenigwe et al. (2009)
Edible seaweeds (*Porphyra tenera*) protein	Protamex, neutrase, flavourzyme and Alcalase	–	Effectively inhibited LPS-induced NO production in RAW 264.7 macrophages	Senevirathne et al. (2010)
Soy and milk proteins	*Bifidobacterium longum* R0175, or *Lactobacillus helveticus* R0052 in combination with *Streptococcus thermophilus* ST5	–	Both soy and dairy ferments influenced cytokine production in human monocyte model (U937)	Masotti et al. (2011)
Alaska pollock frame protein	Trypsin	Asn-Gly-Met-Thr-Tyr (584 MW), Asn-Gly-Leu-Ala-Pro (470 MW) and Trp-Thr (305 MW)	Enhanced mice spleen lymphocyte proliferation activity	Hou, Fan, Li, Xue, and Yu (2012)
Salmon byproduct protein	Alcalase, neutrase, flavourzyme, protamex, pepsin and trypsin	Major peptides in the range of 1000–2000 Da	Inhibited TNF-α, IL-6 and IL-1β in LPS induced RAW 264.7 macrophages	Ahn et al. (2012)
Phorphyra columbina protein	Flavourzyme and fungal protease concentrate	–	Immunomodulatory effects on rat macrophages and lymphocytes, activating NF-κB and MAPK dependent pathways, and mainly inducing IL-10 production	Cian et al. (2012)
Yellow pea seed protein	Thermolysin	Low molecular weight peptides (1000 Da)	Significantly inhibited NO production, TNF-α and IL6 in RAW264.7 macrophages	Ndiaye et al. (2012)
Brazilian soybean cultivar protein	Alcalase	Low molecular mass peptides less than 1200 Da	Significantly inhibited the inflammatory markers such as NO, iNOS, PGE2, COX-2, and TNF-α in LPS-induced RAW 264.7 macrophages	Vernaza et al. (2012)
Short-necked clam (*Ruditapes philippinarum)* protein	Alcalase, papain, flavourzyme, neutrase, protamex, pepsin, trypsin, & α-chymotrypsin	Gln-Cys-Gln-Gln-Ala-Val-Gln-Ser-Ala-Val	Showed a NO inhibitory activity in LPS-stimulated RAW 264.7 macrophages	Lee et al. (2012)
Sweetfish-derived protein	Pepsin, trypsin, and α-chymotrypsin	–	Inhibited the production of NO, cytokines (TNF-α and IL-6) and PGE2 in LPS-induced RAW 264.7 macrophages	Sung et al. (2012)
Shellfish (*M. coruscus*) protein	Flavourzyme, neutrase, Alcalase, papain, pepsin, α –chymotrypsin, & trypsin	Gly-Val-Ser-Leu-Leu-Gln-Gln-Phe-Phe-Leu	Effectively inhibited LPS-induced NO production in the RAW 264.7 macrophages	Kim, Kim, Hwang, Kang et al. (2013)
Brewers' spent grain protein	Corolase PP, flavourzyme and Alcalase 2.4 L	–	Significantly decreased pro-inflammatory cytokine IFN-γ production in Con-A stimulated Jurkat T cells	McCarthy et al. (2013)

(*continued on next page*)

Table 1 (*continued*)

Parent protein	Enzymes used to produce FPH	Peptide sequence & molecular weight	Immunomodulatory activities showed	Reference
Edible microalgae *Spirulina maxima* protein	Trypsin, pepsin and α-chymotrypsin	LDAVNR (686 Da) and MMLDF (655 Da)	Inhibited histamine release and production from RBL-2H3 mast cells and IL-8 generation from EA.hy926 endothelial cells	Vo et al. (2013)
Lupine protein	Alcalase and Izyme	–	Attenuated expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and increased expression of anti-inflammatory marker CCL18 and significantly decreased NO production in THP-1 macrophages	Millan-Linares et al. (2014)
Whey protein	Alcalase	–	Significantly enhanced splenocytes proliferation activity of mice	Ma et al. (2014)
Whey β-lactoglobulin	Trypsin	–	Activated monocyte by increasing TNF-α secretion, and induced TGF-β secretion and regulatory T cell differentiation of PBMC of human	Javier et al. (2014)
Rana chensinensis protein	Papain, trypsin, neutral protease, pepsin, and alkaline protease	–	Enhanced the phagocytosis of macrophages, and increased productions of TNF-*α*, IL-1 *β*, IL-6, and NO in RAW 264.7 macrophages	Huang et al. (2014)
Amaranth protein	Pepsin and pancreatin	Low molecular mass peptides less than 2064 Da	Significantly reduced production of NO, TNF-α, PGE2 and COX2 in LPS stimulated THP-1 and RAW 264.7 cells	Montoya-Rodriguez et al. (2014)
Paphia undulata meat protein	Alcalase	Pro-His-Thr-Cys, Val-Gly-Try-Thr, Glu-Phe, Leu-Phe, and Glu-Gly-Ala-Lys, Trp-Ile or Trp-Leu	Significantly enhanced mice lymphocyte proliferation ability in *ex vivo*	He et al. (2015)
Salmon pectoral fin by-product protein	Pepsin	Pro-Ala-Tyr (349.15 Da)	Significantly inhibited the production of NO and the production of pro-inflammatory cytokines, TNF-α, IL-6 and IL-1β in RAW 264.7 macrophages	Ahn et al. (2015)
Aluterus monoceros gelatin	Glycyl endopeptidase (GE) from papaya latex	–	Significantly reduced production of IL-6, IL-1β and NO in LPS stimulated RAW 264.7 macrophages	Karnjanapratum et al. (2016)
Lates calcarifer gelatin	Alcalase	–	Significantly inhibited IL-6 and IL-1β production in LPS-induced RAW 264.7 macrophages	Sae-leaw et al. (2016)
Wheat germ globulin	Alcalase, neutrase, papain, pepsin and trypsin	Molecular weight ranged from 300–1450 Da	Enhanced lymphocyte proliferation, phagocytosis and secretion of TNF-α, IL-6 and NO production in RAW 264.7 cells	Wenjia et al. (2016)
Tilapia, casein and pea proteins	*Virgibacillus halodenitrificans* SK1-3-7 proteinase	–	Enhanced innate immunity through induction of IL-1β and COX-2 expression in THP-1 macrophages and reduced IL-1β, IL-6, IL-8, TNF-α and COX-2 production in LPS-induced THP-1 cells	Tidarat et al. (2017)
Coix glutelin	Pepsin	Small molecular weight peptides ≤ 3 kDa	The hydrolysate significantly stimulated the secretion of NO from RAW 264.7 cells in a concentration-dependent manner	Ling-Ling et al. (2017)
Egg yolk livetins	Pepsin, Alcalase, and trypsin	Low molecular weight peptides < 10 kDa	The hydrolysates significantly inhibited the Secretion of TNF-α, IL-6, IL-1β and NO in LPS-induced RAW 264.7 macrophages	Chalamaiah and Wu (2017)

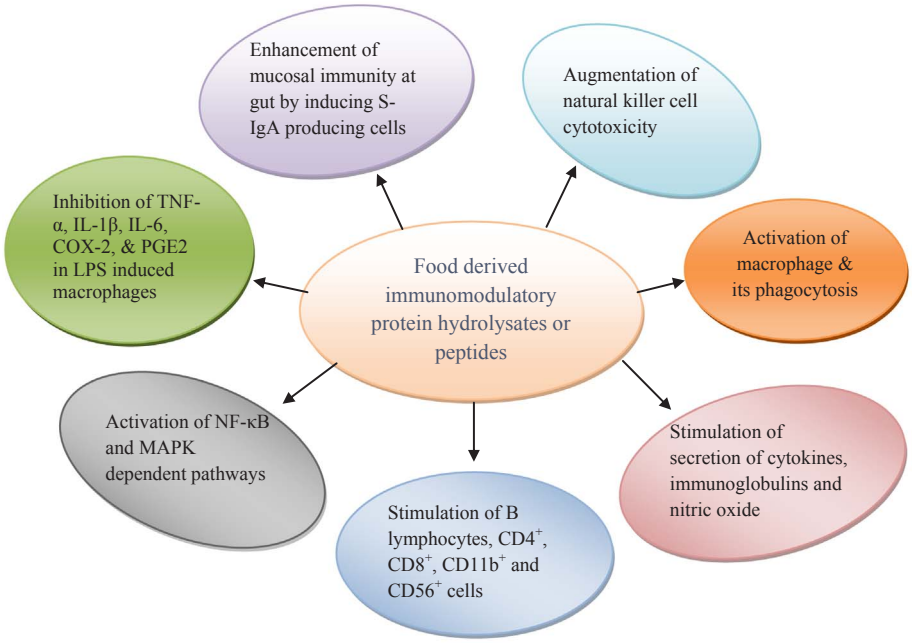

Fig. 2. Mechanisms of action of immunomodulatory protein hydrolysates or peptides derived from food proteins.

ratio, $CD11b^+$ and $CD56^+$ cells, and NK cell activity in blood in order to determine the immunomodulatory effects (Horiguchi et al., 2005; Nesse et al., 2011; Yimit et al., 2012). The cells are stained with fluorescently labelled antibodies. The CD markers of stained cells are commonly detected by fluorescence-activated cell sorting (FACS) analysis using a flow cytometer.

2.4. Immunomodulatory peptides from food proteins and their mechanism

Table 1 shows the immunomodulatory activities of food-derived protein hydrolysates or isolated peptides. Parker et al. (1984) first found immunomodulatory peptide in the enzymatic hydrolysate of human casein. Thereafter, a number of studies reported the immunomodulatory activities for various food protein hydrolysates or peptides (Cai et al., 2013; Chalamaiah, Hemalatha et al., 2015; Duarte et al., 2006; Mallet et al., 2014; Takahashi et al., 1994; Tidarat, Jurriaan, Harry, & Jirawat, 2017; Wang et al., 2010).

Recent studies have demonstrated that food-derived peptides exert various immunomodulatory effects on both innate and adaptive immune responses including induction or modulation of cytokine and antibody production, stimulation of lymphocytes to proliferate, augmentation of phagocytic ability of macrophages, enhancement of natural killer cell activity, improvement of the defensive ability of the body against invading pathogens and inhibition of proinflammatory responses of host cells to bacterial components such as lipopolysaccharide (LPS) (Chalamaiah et al., 2014; Duarte et al., 2006; Hou, Fan, Li, Xue, Yu, Zang et al., 2012; Wenjia et al., 2016; Yang et al., 2009). These effects may be mediated by direct binding of food derived peptides to receptors on the surface of immune cells. Immunomodulatory peptides do not interact directly with the pathogen but they can contribute to the host's defense response (Maestri et al., 2016).

Recently, immunomodulatory peptides have been isolated from several hydrolyzed food proteins (Table 1). The immunomodulatory capacity of peptides isolated from food protein hydrolysates is dependent on amino acid composition, sequence, length, charge, hydrophobicity and the structure of the peptide molecule (Berthou et al., 1987; Jacquot, Gauthier, Drouin, & Boutin, 2010). Food derived peptides with immunomodulatory effects are short (2–10 residues) and hydrophobic in nature (Ahn et al., 2015; He et al., 2015; Hou, Fan, Li, Xue, & Yu, 2012; Kim, Kim, Hwang, Kang et al., 2013; Lee et al., 2012; Takahashi et al., 1994; Vo et al., 2013; Yang et al., 2009). The most frequent residues of immunomodulatory peptides are hydrophobic amino acids such as glycine (Gly), valine (Val), leucine (Leu), proline (Pro), phenylalanine (Phe), negatively charged amino acid, glutamic acid (Glu), and aromatic amino acid, tyrosine (Tyr) (Table 1). It has been demonstrated that hydrophobic amino acids and one or more residues of glutamine, glutamic acid, tyrosine, tryptophan, cysteine, asparagine, and aspartic acid facilitate the immunomodulatory activities of food protein originated peptides (Chen, Suetsuna, & Yamauchi, 1995; He et al., 2015; Hou, Fan, Li, Xue, & Yu, 2012; Kim, Kim, Hwang, Kang et al., 2013; Lee et al., 2012; Vo et al., 2013). Jacquot et al. (2010) reported that longer hydrophobic peptides (from β-lactoglobulin and α-lactalbumin) bearing 2–3 positive charges have greater potential to stimulate the proliferation of murine splenocytes. Kong et al. (2008) reported that soy peptides with lower molecular weight and more positive charge were more effective in stimulating lymphocyte proliferation. Saint-Sauveur et al. (2008) suggested that acidic or neutral peptide fractions from whey protein isolate better stimulated splenocyte proliferation and the cytokine secretion. In a study, Javier et al. (2014) reported that tryptic whey β-lactoglobulin digest fraction enriched in acidic and large peptides increased the secretion of IFN-γ (a Th1 cytokine), while fractions containing short peptides enhanced TNF-α production of monocytes. Vogel et al. (2002) demonstrated that the immunomodulating and anti-inflammatory properties of lactoferricin peptide are more related to the positively charged region of the peptide. Thus, the amino acid constituents, sequence and the length of the peptides are very important for their immunomodulatory activity.

Immunomodulatory peptides have exhibited a variety of targets, including monocytes, macrophages, NK cells, mast cells, T and B lymphocytes, CD4+ and CD8+ T cells, CD49b+, $CD11b^+$ and $CD56^+$ cells (Table 1). However, at present, the exact mechanism of immunomodulatory effect of food originated peptides is not fully understood, but mechanisms of immunomodulation activity occur mainly through macrophages activation, phagocytosis stimulation, increased leukocyte count, enhanced induction of immune modulators such as cytokines, NO and immunoglobulins, NK cells stimulation, stimulatory effect on splenocytes, CD4+, CD8+, $CD11b^+$ and $CD56^+$ cells, activating transcription factor nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) dependent pathways and inhibition of pro-inflammatory mediators (see Fig. 2) (Ahn et al., 2015; Chalamaiah et al., 2014; Cian, Lopez-Posadas, Drago, Medina, & Martinez-Augustin, 2012; Duarte et al., 2006; Egusa & Otani, 2009; Huang et al., 2014;

Karnjanapratum et al., 2016; Ling-Ling, Bin, Hui-Fang, Qun, & Ling-Zhi, 2017; Lozano-Ojalvo et al., 2016; Morris et al., 2007; Pan et al., 2013; Tidarat et al., 2017; Yang et al., 2009; Yimit et al., 2012). Therefore, depending on their amino acid sequence, composition, length and structure, immunomodulatory peptides can modulate both innate and adaptive immune responses by binding to specific receptors on the surface of a target cell (Javier et al., 2014; Masotti et al., 2011).

Immunomodulatory peptides have been reported from various food protein sources. Milk is a potent source of immunomodulatory peptides. Immunomodulatory activity of peptide derived from enzymatic digestion of milk proteins was reported in the literature as early as 1984 (Parker et al., 1984). The authors isolated a hexa peptide, Val-Glu-Pro-Ile-Pro-Tyr, from human casein, which stimulated the phagocytosis of opsonized sheep red blood cells (SRBCs) by murine peritoneal macrophages. Berthou et al. (1987) identified two tripeptides, Gly-Leu-Phe from human casein and Leu-Leu-Tyr from bovine casein, which significantly enhanced phagocytosis activity of SRBCs by mouse peritoneal macrophages. In a study, Coste et al. (1992) reported that a polypeptidic fraction obtained from the hydrolysate prepared by pepsin-chymosin digestion of bovine β-caseins showed a mitogenic effect on primed lymph node cells and unprimed spleen cells of rats. In another study, whey protein hydrolysate (from a combined digestion of trypsin and chymotrypsin) and its peptide fractions exhibited immunostimulatory effects on the proliferation of splenocytes (in presence or absence of concanavalin (Con) A) and the secretion of IL-2 and IFN-γ (Saint-Sauveur et al., 2008).

In addition to milk proteins, immunomodulatory peptides have also been isolated from soybean, rice, fish and marine species. Yoshikawa et al. (1993) obtained a hexapeptide, His-Cys-Gln-Arg-Pro-Arg, from tryptic digests of soybean protein, which showed a stimulatory effect on the phagocytic ability of human polymorphonuclear leukocytes and production of TNF in mice. Chen et al. (1995) prepared enzymatic hydrolysate of soybean protein using pepsin. Three immunostimulatory peptides were isolated from the hydrolysate and were sequenced as Ala-Glu-Ile-Asn-Met-Pro-Asp-Tyr, Ile-Gln-Gln-Gly-Asn, and Ser-Gly-Phe-Ala-Pro, which significantly enhanced the proliferation of mice splenocytes. A study carried out by Takahashi et al. (1994) isolated a novel immunomodulatory peptide, Gly-Tyr-Pro-Met-Tyr-Pro-Leu-Pro-Arg, from the tryptic digest of rice soluble protein and reported that the isolated peptide showed significant enhancement of phagocytosis activity for human polymorphonuclear leukocytes. Hou, Fan, Li, Xue, Yu, Zang et al. (2012) obtained three immunomodulating peptides, Asn-Gly-Met-Thr-Tyr, Asn-Gly-Leu-Ala-Pro, and Trp-Thr, from trypsin hydrolysate of Alaska pollock frame. The three peptides showed high lymphocyte proliferation activities. Recently, He et al. (2015) reported six peptides, Pro-His-Thr-Cys,Val-Gly-Try-Thr, Glu-Phe, Leu-Phe, Glu-Gly-Ala-Lys, and Trp-Ile or Trp-Leu, from *Paphia undulate* clam, that significantly enhanced lymphocyte proliferation ability.

Apart from isolated and identified immunomodulatory peptides, numerous recent studies reported the immunomodulatory activities for crude protein hydrolysates produced from various food protein sources such as milk (Biziulevicius et al., 2006; Masotti et al., 2011; Pan et al., 2013), whey (Javier et al., 2014; Ma et al., 2014; Saint-Sauveur et al., 2008; Saint-Sauveur et al., 2009), hen egg (Lozano-Ojalvo et al., 2016; Marie et al., 2009; Nelson et al., 2007), fish (Duarte et al., 2006; Hou, Fan, Li, Xue, & Yu, 2012; Mallet et al., 2014; Nesse et al., 2011), fish egg (Chalamaiah, Hemalatha et al., 2015, 2014), soybean (Egusa & Otani, 2009; Kong et al., 2008; Yimit et al., 2012), wheat (Horiguchi et al., 2005; Wenjia et al., 2016), pea (Tidarat et al., 2017), yellow pea (Ndiaye et al., 2012), coix (Ling-Ling et al., 2017), green microalga (Morris et al., 2007), edible seaweed (Cian et al., 2012), Oyster (Cai et al., 2013; Wang et al., 2010), sheep (Hua et al., 2009), and frog (Huang et al., 2014). Table 1 shows the immunomodulatory activities of food-derived protein hydrolysates or isolated peptides.

There were several studies on the immunomodulatory activity of crude protein hydrolysates prepared from milk and whey proteins (Gauthier, Pouliot, & Saint-sauveur, 2006; Javier et al., 2014; Ma et al., 2014; Masotti et al., 2011; Pan et al., 2013; Saint-Sauveur et al., 2009; Tidarat et al., 2017). Kazlauskaite et al. (2005) conducted mouse experiments in order to investigate the immunomodulatory effects of tryptic casein hydrolysate and showed a significant enhancement of phagocytosis capacity of peritoneal macrophages and blood phagocytic cells. Pan et al. (2013) obtained full-cream milk hydrolysates by enzymatic hydrolysis with papain and trypsin and reported that milk protein hydrolysates improved immunity in mice by increasing the weight of spleen and thymus and stimulating the phagocytosis of macrophages. In a study performed by Saint-Sauveur et al. (2008) hydrolysed whey protein isolate using a mixture of trypsin:chymotrypsin and tested its peptide fractions *in vitro* in murine splenocytes. It is reported that all peptide fractions significantly enhanced the proliferation of splenocytes and the secretion of IFN-γ in the presence or absence of con-A. In another study, Ma et al. (2014) reported that micro-encapsulated whey protein hydrolysate significantly enhanced splenocyte proliferation activity. Recently, Javier et al. (2014) demonstrated diverse *ex vivo* immune effects, triggered by β-lactoglobulin tryptic-digested fractions, including increased secretion of IFN-γ from T cells and enhanced TNF-α production from monocytes.

Eggs contain a variety of biologically active peptides that can influence immune functions. Nelson et al. (2007) have demonstrated that peptidic fractions derived from egg yolk induced a humoral immune response associated with an increase of IgA + cells in lamina propria in BALB/c mice. In a recent study, Lozano-Ojalvo et al. (2016) investigated the immunomodulatory effects of pepsin, and Alcalase hydrolysates of ovalbumin (OVA), lysozyme (LYS), ovomucoid (OM) and whole egg white (EW) on cytokine production, antibody secretion, and proliferation of murine spleen and mesenteric lymph node cells stimulated with T (con-A) or B-cell mitogens (LPS). The authors reported that the Alcalase hydrolysates of OVA, LYS and EW decreased con-A stimulated lymphocyte proliferation and secretion of Th2-biased cytokines (IL-13 and IL-10) and reduced the production of the Th1 cytokine, TNF-α.

Fish and marine species-derived protein hydrolysates are another valuable natural resource of bioactive peptides. A broad spectrum of bioactivities has been reported for these peptides including immunomodulatory properties (Chalamaiah, Hemalatha et al., 2015; Senevirathne & Kim, 2012). Chalamaiah, Hemalatha et al. (2015) and Chalamaiah et al. (2014) produced protein hydrolysates from *Labeo rohita* and *Cyprinus carpio* fish eggs using Alcalase, pepsin and trypsin; these hydrolysates, when orally administered to the BALB/c mice for forty-five days, exhibited immunostimulatory effects on both innate and adaptive immune functions through modulation of spleen lymphocytes, NK cells, peritoneal macrophages, T cell subpopulations (CD4 + & CD8 +), mucosal immunity and serum IgA. In a study conducted by Mallet et al. (2014), oral administration of shark-derived protein hydrolysate enhanced the gut barrier function of mice via up-regulation of IgA-producing cells in lamina propria and cytokine (IL-4, IL-6, TNF-α, IFN-γ, IL-10, and CTLA-4) positive cells in small intestine mucosa. Oligopeptide-enriched hydrolysates from oyster have been reported to enhance spleen lymphocyte proliferation, macrophage phagocytosis and NK cell cytotoxicity in BALB/c mice after oral administration (Cai et al., 2013; Wang et al., 2010). Recently, Tidarat et al. (2017) demonstrated that tilapia mince hydrolysate has a stimulatory effect on innate immunity through induction of IL-1β and cyclooxygenase-2 (COX-2) expression in THP-1 macrophages.

A number of recent studies have reported the ability of plant derived food protein hydrolysates to modulate the immune functions *in vitro* and *in vivo*. Morris et al. (2007) reported that oral administration of a green microalga (*Chlorella vulgaris*) hydrolysate showed positive effects on the recovery of both innate and specific immune functions of undernourished BALB/c mice after a 3-day fasting. The authors demonstrated that the enzymatic hydrolysates from *Chlorella vulgaris* significantly enhanced macrophage activity, haemopoiesis, leukocyte

count (particularly the lymphocyte pool), peritoneal exudate cells, as well as stimulation on T-dependent antibody response and delayed-type hypersensitivity response. In another study, Kong et al. (2008) hydrolyzed the soy protein with several commercially available proteases (Alcalase, Flavourzyme, trypsin, papain, protease A and peptidase R); of these, the Alcalase hydrolysate showed the best immunomodulating effects *in vitro* on proliferation of murine splenic lymphocytes and phagocytic activity of peritoneal macrophages. Another study conducted by Ndiaye et al. (2012) used yellow pea seed protein to produce hydrolysates using thermolysin and reported that the hydrolysate significantly stimulated phagocytic activity of peritoneal macrophages and the number of IgA+ and cytokine positive cells in the small intestine representing gut mucosa immunity. Wenjia et al. (2016) prepared defatted wheat germ globulin hydrolysates using various proteases (Alcalase, papain, pepsin, trypsin) and reported that the hydrolysate prepared with Alcalase had the strongest immunomodulatory activity with respect to lymphocyte proliferation, phagocytosis and secretion of cytokines, IL-6 and TNF-α. Recently, Ling-Ling et al. (2017) reported that the enzymatic hydrolysate produced from coix glutelin significantly stimulated the secretion of NO from RAW 264.7 cells.

In addition to the innate and adaptive immunomodulatory capacity of food originated peptides or protein hydrolysates, the inflammation-related immunomodulatory potential of food-derived peptides has become an active area of research since inflammation has long been associated with the pathophysiology of many diseases such as cancer, atherosclerosis, rheumatoid arthritis, ulcerative colitis, asthama, diabetes etc. It has been shown that several peptides or protein hydrolysates derived from food proteins have inflammation-related immunomodulatory potential by inhibiting the production of NO, prostaglandin E2 (PGE2) and cytokines (IL-1β, IL-6, IL-8 and TNF-α) in LPS-induced *in vitro* cell culture models (Ahn et al., 2015; Karnjanapratum et al., 2016; Masotti et al., 2011; Montoya-Rodriguez et al., 2014; Ndiaye et al., 2012; Sae-leaw et al., 2016; Sung et al., 2012; Udenigwe et al., 2009; Vernaza et al., 2012). A well established model for investigating inflammation modulatory (anti-inflammatory) potential of food derived peptides *in vitro* is the use of macrophages that can be activated by Toll-like receptor ligands such as bacterial LPS. Activated macrophages release inflammatory mediators such as the transcription factor NF-κB, IL-1β, IL-6, IL-8, TNF-α, NO, and COX-2 that participate in the regulation of immune/inflammatory responses. Among all, RAW 264.7 is the most commonly used murine macrophage cell line (Ahn et al., 2015; Chalamaiah & Wu, 2017; Karnjanapratum et al., 2016; Lee et al., 2012; Sae-leaw et al., 2016; Sung et al., 2012), while the human monocytic cell line THP-1 was also used in study of food derived anti-inflammatory peptides (Millan-Linares et al., 2014; Montoya-Rodriguez et al., 2014; Tidarat et al., 2017). Numerous food derived peptides have been shown to modulate the inflammation-related immune response *in vitro* through distinct molecular mechanisms. These include inhibition of cytokines (IL-1β, IL-6, IL-8, and TNF-α), inhibition of cyclooxygenases and inducible nitric oxide synthase (iNOS), inhibition of MAPKs, and inhibition of transcription factor, NF-κB (Ahn et al., 2015; Karnjanapratum et al., 2016; Millan-Linares et al., 2014; Montoya-Rodriguez et al., 2014; Sung et al., 2012; Tidarat et al., 2017; Vernaza et al., 2012).

Several inflammation modulatory (anti-inflammatory) peptides were isolated and identified from various food proteins. Lee et al. (2012) reported the inhibitory effect of a purified peptide, Gln-Cys-Gln-Gln-Ala-Val-Gln-Ser-Ala-Val, from Alcalase hydrolysate of short-necked clam (*Ruditapes philippinarum*) on NO production in LPS-stimulated RAW 264.7 cells. Kim, Kim, Hwang, Kang et al. (2013) reported a novel anti-inflammatory deca-peptide from *Mytilus coruscus,* Gly-Val-Ser-Leu-Leu-Gln-Gln-Phe-Phe-Leu, that showed a potent NO inhibitory (62.16%) activity in LPS-stimulated RAW 264.7 cells. In another study, Vo et al. (2013) isolated two anti-inflammatory peptides, Leu-Asp-Ala-Val-Asn-Arg (686 Da) and Met-Met-Leu-Asp-Phe (655 Da), from enzymatic hydrolysate of the edible microalgae (*Spirulina maxima*) using gastrointestinal endopeptidases (trypsin, α-chymotrypsin and pepsin). It was reported that the two peptides exhibited significant inhibition on the production of interleukin-8 in histamine-stimulated EA.hy926 endothelial cells. Recently, Ahn et al. (2015) identified a tri peptide, Pro-Ala-Tyr (349.15 Da), from peptic hydrolysate of salmon fish protein, which inhibited NO/iNOS and PGE2/COX-2 pathways as well as production of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in LPS-stimulated RAW 264.7 cells.

In addition to isolated peptides, several other studies have also indicated the inflammation-modulatory potential of crude protein hydrolysates derived from yak milk, egg white, egg yolk, fish, soybean, pea, lupine, amaranth and seaweed (Chalamaiah & Wu, 2017; Karnjanapratum et al., 2016; Lee et al., 2009; Mao, Cheng, Wang, & Wu, 2011; Millan-Linares et al., 2014; Montoya-Rodriguez et al., 2014; Sung et al., 2012; Tidarat et al., 2017; Vernaza et al., 2012). Senevirathne et al. (2010) reported that the edible seaweed (*Porphyra tenera*) protein hydrolysate effectively inhibited LPS-induced NO production in RAW 264.7 macrophages. Mao et al. (2011) demonstrated that casein hydrolysate from yak milk produced by Alcalase substantially decreased the production of NO and the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in LPS-stimulated mice peritoneal macrophages. Sung et al. (2012) produced protein hydrolysates from sweetfish using trypsin and α-chymotrypsin, and reported that the protein hydrolysates effectively reduced the expression of LPS-induced inflammation-related factors such as NO, PGE2, and proinflammatory cytokines. The authors also revealed that the anti-inflammatory properties of sweetfish hydrolysates were mediated by the downregulation of COX-2, iNOS, extracellular signal–regulated kinase (ERK) 1/2, and NF-κB. In another study, Ndiaye et al. (2012) reported that protein hydrolysates derived from yellow pea seed showed anti-inflammatory activity by inhibiting NO production, TNF-α and IL-6 in activated macrophages. Recently, protein hydrolysates prepared from soybean and amaranth significantly attenuated the production of inflammatory markers such as NO, iNOS, PGE2, COX-2, and TNF-α in LPS-induced RAW 264.7 macrophages (Montoya-Rodriguez et al., 2014; Vernaza et al., 2012). A lupine derived protein hydrolysate was found to attenuate the expression of proinflammatory cytokines, IL-1β, IL-6, TNF-α, and NO production in THP-1 macrophages (Millan-Linares et al., 2014). In a recent study, Tidarat et al. (2017) reported that casein and pea protein hydrolysates exerted anti-inflammatory activity by attenuating LPS induced pro inflammatory gene expression in THP-1 macrophages. The authors reported that casein hydrolysate suppressed IL-1β, IL-6, IL-8, TNF-α and COX-2, and pea protein hydrolysate reduced LPS-induced IL-6 and TNF-α responses. In another recent study, Chalamaiah and Wu (2017) showed that hen egg yolk livetins fraction and its enzymatic hydrolysates inhibited the pro-inflammatory markers such as IL-1β, IL-6, TNF-α, and NO production in LPS-stimulated RAW 264.7 macrophages.

The literature review indicates that numerous *in vitro* and *in vivo* (animal) studies have been performed by many researchers to assess the immunomodulatory effects of food derived peptides. However, only few studies have evaluated the immunomodulatory effects of food derived protein hydrolysates or peptides in humans. Horiguchi et al. (2005) examined the effects of the administration of wheat gluten hydrolysate on the immune function of healthy human volunteers and reported that NK cell activity increased significantly after intervention (6 days) without severe side effects. Nesse et al. (2011) studied the immunomodulatory effect of protein hydrolysate prepared from salmon fish protein on a group of 438 Indian malnourished children (6–8 years) with respect to CD4/CD8 ratios and immunoglobulins (IgG, IgM and IgA) but found no significant effect of the hydrolysate. In another study, Yimit et al. (2012) found that soybean peptides produced by Theroase, bioprase, and sumizyme FP significantly increased the numbers of $CD11b^+$ (cell-surface antigen of macrophages and dendritic cells) and $CD56^+$ (cell-surface antigen of NK cells) cells in serum of peripheral blood from healthy human volunteers after a single oral administration of 8 g of soybean peptides.

Food-derived peptides have been greatly explored for *in vitro* and *in vivo* immunomodulatory properties tested in various cell and animal models. However, current studies have certain limitations. First, there is a lack of subsequent investigations for the reported immunomodulatory peptides and hydrolysates. For example, with the majority of peptides and hydrolysates tested *in vitro* immunomodulatory activities, only a few have been further studied *in vivo*; of the peptides and hydrolysates reported to be active in healthy animals, only a few have been verified for potential health benefits in a diseased model; compared to a large number of food-derived hydrolysates reported to exert immunomodulatory properties, limited number of active peptide sequences have been identified. To date, there are limited reports on regulatory mechanisms and structure-function relationship of immunomodulatory peptides. Second, the effects of peptide treatment on cell viability or animal health (food intake, body weight, hematology and biochemical parameters) were missing in many studies, which should be carefully examined when evaluating the benefits. For example, when testing the *in vitro* anti-inflammatory activity it is very important to provide viability data. And in animal studies, although these peptides are derived from food source, there is a need to investigate the toxicity of these peptides.

Considering animal experiments, there can be several factors such as gender, age and diet that influence the results. While performing the animal experiments with peptides, it is suggested to include both the genders because hormonal changes between male and female animals will significantly impact the results (Verthelyi, 2006). For example, female animals are more resistant and exhibit stronger immune responses to antigenic challenges than male animals (Anna & Reginald, 2013). Moreover, diet used in animal studies should be carefully designed to produce consistent results. For example, the test diets should be balanced to be isocaloric and isonitrogenous; and a semi-purified diet has the advantage in reducing batch-to-batch variation when compared to the commonly used chow diet. Additionally, it seems a "gold standard" peptide to compare results from different studies is not established so far in this area of research. Usually a hydrolysate is prepared from certain food proteins and tested in various cell or animal models using different doses, and it is hard to interpret the results without a "standard" peptide. Therefore, it is useful for the advancement of this field of research.

3. Anticancer activity of food protein hydrolysates

Cancer is among the leading causes of morbidity and mortality worldwide, with 14.1 million new cases and 8.2 million cancer-related deaths worldwide in 2012 (Torre et al., 2015). The number of new cancer cases is expected to rise from 14 million in 2012 to 22 million within the next two decades (WHO, 2017). Cancer (malignant tumor) is an abnormal growth and proliferation of cells in the body. In all types of cancer, abnormal cells begin to grow uncontrollably and spread into surrounding tissues. Therefore, inhibition of deregulated cell proliferation is an important strategy for treating tumors (Chi, Hu, Wang, Li, & Ding, 2015). Currently, chemoprevention is the most promising anticancer approach used to reduce the morbidity and mortality of cancer by slowing the progression of carcinogenesis (Pan, Zhao, Hu, Chi, & Wang, 2016; Sheih, Fang, Wu, & Lin, 2010). However, chemotherapy is costly and many unintended side effects of anticancer drugs can cause damage to normal cells (Sah, Vasiljevic, McKechnie, & Donkor, 2015). Therefore, discovery of new anticancer agents from natural sources like food proteins can provide better alternatives for cancer prevention and management.

Recently, significant progress has been made regarding our understanding of the association between diet and cancer. The use of bioactive nutrients for cancer therapy has achieved considerable success in recent years. Numerous researchers have identified specific cancer-preventive bioactive nutrients. Some promising biologically active food components for cancer prevention are soy isoflavones, lycopene, resveratrol, omega-3-fatty acids, pomegranate, curcumin and antioxidants such as selenium and vitamin-E, which were found to be beneficial in different types of cancers (Sharma et al., 2016; Umar, Dunn, & Greenwald, 2012). Over the last two decades, food derived protein hydrolysates or peptides have received great attention for their applications in food, nutraceutical and pharmaceutical industries. Recently, considerable research has been conducted to develop anticancer protein hydrolysates or peptides from a variety of food protein sources. The potential of food derived protein hydrolysates to act as anticancer agents of natural origin has been widely reported on the basis of *in vitro* and *in vivo* (animal) trials. Multiple studies have demonstrated the anticancer activities of protein hydrolysates or peptides derived from a wide range of food protein sources, such as milk, egg, fish, crabs, shrimp, sea cucumber, Oyster, mussel, chlorella (algae), spirulina, rice, soybean, corn, common bean, chickpea and rapeseed (Beaulieu, Thibodeau, Bonnet, Bryl, & Carbonneau, 2013; Kannan, Hettiarachchya, Lay, & Liyanage, 2010; Kim et al., 2000; Otani & Suzuki, 2003; Picot et al., 2006; Sheih et al., 2010; Wang & Zhang, 2017; Wang et al., 2010; Wang et al., 2016; Watanabe, Tsuge, Shimoyamada, Ogama, & Ebina, 1998; Xue, Yu, Wu, & Wang, 2009; Xue et al., 2012; Yamaguchi, Takeuchi, & Ebihara, 1997).

Several strategies have been used to produce the anticancer protein hydrolysates or peptides from food proteins. These include *in vitro* hydrolysis using commercial proteases, fermentation with bacterial strains and gastrointestinal digestion (Chalamaiah, Jyothirmayi, Diwan, & Dinesh Kumar, 2015; Huang et al., 2012; Wang et al., 2016). Enzymatic hydrolysis of food proteins is the most frequently used process for producing anticancer protein hydrolysates or peptides (Huang et al., 2012; Pan et al., 2016; Perez-Vega, Olivera-Castillo, Gomez-Ruiz, & Hernandez-Ledesma, 2013; Vital, Mejia, Dia, & Loarca-Pina, 2014; Wang & Zhang, 2017; Wang et al., 2016). A number of commercial proteases have been used successfully for the production of anticancer protein hydrolysates or peptides, including pepsin, trypsin, Alcalase, pancreatin, flavourzyme, protamex, Neutrase, chymotrypsin, pronase, protease N, thermoase, cryotin and papain (Aleman et al., 2011; Chalamaiah, Jyothirmayi et al., 2015; Kannan, Hettiarachchy, Marshall, Raghavan, & Kristinsson, 2011; Kim et al., 2000; Pan et al., 2016; Picot et al., 2006; Wang, Bringe, Berhow, & Mejia, 2008; Wang & Zhang, 2017; Watanabe et al., 1998; Xue et al., 2009; Xue et al., 2012). Among the enzymes, pepsin has been shown to be one of the most efficient in the production of anticancer peptides from food proteins (Table 2). Pepsin hydrolyzes only peptide bonds; preferentially those containing hydrophobic amino acids, especially aromatic amino acid residues such as phenylalanine, tryptophan and tyrosine. The possible mechanism is that during hydrolysis, peptide bond cleavage releases the bioactive hydrophobic peptides, which are hidden in the inner core of the parent proteins, capable of inhibiting growth of cancer cell lines, inducing apoptosis and inhibiting cell cycle. A flow chart for the production of food derived anticancer protein hydrolysates is shown in Fig. 1.

3.1. Assays used for evaluation of anticancer activity of food derived protein hydrolysates or peptides

To investigate the potential anticancer properties of food derived protein hydrolysates, many researchers examined the effects of the protein hydrolysates on different tumor-derived and *in vitro*-transformed cell lines. Various cancer cell lines, such as MCF-7, MDA-MB-231 and BT549 (human breast carcinoma); Ca9-22 and CAL 27 (human oral cancer); Hep G2 (human liver cancer); HT-29, RKO, KM12L4, DLD-1 and HCT15 (human colon carcinoma); Caco-2, TC7 and HCT-116 (human colorectal carcinoma); U87 (human glioma); PC3, LNCaP and DU-145 (human prostate cancer); THP-1 (human monocytic leukemia); Jurkat T cells (human T cell leukemia); AGS (human gastric cancer); A549 and H-1299 (human lung cancer); HeLa (human cervical cancer); 109 (human esophagus cancer); hFOB1.19 (human fetal osteoblast

Table 2
Anticancer activities and isolated peptide sequences of various food protein hydrolysates (FPHs) prepared from different food sources.

Protein hydrolysates particulars	Enzymes used to produce FPH	Peptide sequence & molecular weight	Animals or cell lines used for investigation	Anticancer activities showed	Reference
In vivo animal studies					
Corn gluten peptides	Alkaline protease	Peptides of 2000 Da (mostly dipeptides and tripeptides)	Female sprague–dawley rats with DMBA-induced mammary tumor	Tumor incidence was significantly lower in the peptides group	Yamaguchi et al. (1997)
Hen egg white ovomucin protein hydrolysates	Pronase	220 and 120 kDa peptides	BALB/c mice with inoculations of Meth-A fibrosarcoma cells	Cured directly and entirely the treated tumor, and inhibited indirectly and slightly the growth of the distant tumors in mice	Watanabe et al. (1998)
Rapeseed protein hydrolysates	Alcalase and flavourzyme	–	Female kunming nude mice with transplanted sarcoma S180 cells	Significantly decreased the weight of the tumor growth and increased the phagocytosis of coeliac macrophages	Xue et al. (2009)
Oyster (*Crassostrea gigas*) protein hydrolysates	Protease from *Bacillus* sp. SM98011	Peptides below 3 kDa	Female BALB/c mice with transplanted sarcoma S180 cells	Significantly inhibited the growth of transplanted sarcoma S180 cells in mice	Wang et al. (2010)
Peptides from bovine β-casein	Trypsin	INKKI	B16F10 melanoma tumor-bearing C57BL/6J mice	Showed dose-response cytotoxicity and induced apoptosis by increasing cleavage of caspase-3	Azevedo et al. (2012)
Chickpea albumin hydrolysate	Alcalase and flavourzyme	–	Kunming male mice bearing H-22 tumor	Significantly increased the tumor inhibition rate and decreased tumor volume	Xue et al. (2012)
Peptides from corn protein	Alcalase	–	BALB/c male mice with H22-tumor transplant and HepG2 cells	Effectively suppressed the tumor growth in vivo, and induced S cell-cycle arrest and caused apoptotic death in HepG2 cells	Li et al. (2013)
In vitro cell culture studies					
Soybean peptides	Thermoase	X-Met-Leu-Pro-Ser-Tye-Ser-Pro-Tyr (1157 Da)	Mouse monocyte macrophage (P388D1) cell line	Cell growth inhibition and significantly affected cell cycle progression by arresting P388D1 at G2/M phases	Kim et al. (2000)
Bovine lactoferrin hydrolysates	Pepsin	FKCRRWQWRMKKLGAPSITCVR (2753.88 Da)	Human acute myeloid leukemia (HL-60) cell line	Showed cell proliferation inhibition due to induction of apoptosis	Roy et al. (2002)
Bovine casein peptides	Trypsin	Tyr-Lys, Leu-Lys-Lys and Arg-Pro-Lys	Human leukemic T and B cell lines	Showed cytotoxicity due to necrosis	Otani and Suzuki (2003)
Lactoferricin (LfcinB) peptide from bovine lactoferrin	Pepsin	FKCRRWQWRMKKLGAPSITCVRRAF	Jurkat T leukemia cells	Exhibited cytotoxic activity due to mitochondria dependent apoptosis	Mader et al. (2005) and Mader et al. (2007)
Peptides from bovine lactoferrin	Pepsin	–	Neuroblastoma cells Kelly, SK-N-DZ and IMR-32	Showed cytotoxic activity induced by cleavage of caspase-6, -7 and 9	Eliassen et al. (2006)
Protein hydrolysates from blue whiting, cod, plaice and salmon	Alcalase and protamex	Free amino acids, peptides with various sizes ranging up to 7 kDa	Two human breast carcinoma cell lines, MCF-7/6 and MDA-MB-231	Exhibited cell growth inhibition	Picot et al. (2006)
Rice bran protein hydrolysates	Alcalase, pepsin and pancreatin	Peptides of > 50, 10–50, 5–10, and < 5 kDa. Glu-Gln-Arg-Pro-Arg (685.37 Da)	Caco-2, HCT-116, MCF-7, MDA-MB-231, and HepG2 cancer cell lines	Showed cell growth inhibition	Kannan et al. (2008, 2010)
Soybean protein hydrolysates	Pepsin and pancreatin	–	L1210 leukemia cell line	Showed cytotoxicity on L1210 cells	Wang et al. (2008)
Algae (*Chlorella vulgaris*) protein hydrolysate	Pepsin	VECYGPNRPQF	Human gastric cancer cell line (AGS)	Showed antiproliferative activity and induced a post-G1 cell cycle arrest in AGS cells	Sheih et al. (2010)
Chickpea protein hydrolysates	Pepsin and pancreatin	–	Caco-2 and THP-1 cells	Inhibited the growth of the cells	Giron-Calle et al. (2010)
Muscle protein hydrolysates of *Nemipterus japonicus* and *Exocoetus volitans*	Trypsin	–	Vero and HepG2 cancer cell lines	Exhibited antiproliferative effect on Hep G2 cell lines	Naqash and Nazeer (2010)
Gelatin hydrolysates from *Dosidicus gigas*	Protamex, neutrase, trypsin, savinase, esperase and Alcalase	Peptides with molecular weights ranging from 500 to 1400 Da	MCF-7 (human breast carcinoma) and U87 (glioma) cell lines	Showed cytotoxic and antiproliferative activities	Aleman et al. (2011)
Snow crab by-product hydrolysates	Protamex	Peptides of different molecular weight	A549 (lung), HCT15 (colon), BT549 (breast), and PC3 (prostate) cells	Inhibited the cell growth	Doyen et al. (2011)

(*continued on next page*)

Table 2 (*continued*)

Protein hydrolysates particulars	Enzymes used to produce FPH	Peptide sequence & molecular weight	Animals or cell lines used for investigation	Anticancer activities showed	Reference
Solitary tunicate (*Styela clava*) protein hydrolysates	Alcalase, pepsin and thermoase	–	Human colon (DLD-1), stomach (AGS), and cervical (HeLa) cancer cell lines	Exhibited cell growth inhibitory effects	Jumeri and Kim (2011)
Tuna dark muscle by-product protein hydrolysates	Papain and protease XXIII	Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr (1206 Da) and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr (1124 Da)	Human breast cancer (MCF-7) cell line	Showed the dose dependent inhibition on MCF-7 cells	Hsu et al. (2011)
Shrimp shell hydrolysates	Cryotin and pepsin	Peptides of different sizes (< 10, 10–30, > 30 kDa)	Human colon (Caco-2) and liver (HepG2) cancer cell lines	Showed Antiproliferative activity	Kannan et al. (2011)
Misgurnus anguillicaudatus muscle peptides	Papain	Peptides of different sizes (> 10 kDa; 5–10 kDa; 3–5 kDa, and < 3 kDa)	Human liver (Hep G2), breast (MCF-7), and colon (Caco-2) cancer cell lines	Exhibited antiproliferative activity	You et al. (2011)
Protein hydrolysate from *Nemipterus japonicus* backbone	Trypsin	–	Hep G2 cell line	Showed cytotoxic activity	Naqash and Nazeer (2012)
Oligopeptide from Sepia ink	Trypsin	Gln-Pro-Lys	Prostate cancer cell lines DU-145, PC-3 and LNCaP	Inhibited the cell proliferation, induced cell cycle arrest, increased apoptosis and Bax/Bcl-2 expression ratio	Huang et al. (2012)
A peptide from *Ruditapes philippinarum*	Chymotrypsin	Ala-Val-Leu-Val-Asp-Lys-Gln-Cys-Pro-Asp (1950 Da)	PC-3, A549 and MDA-MB-231 cell lines	Showed cytotoxicity activity and induced apoptosis	Kim, Kim, Hwang, Lee et al. (2013)
Blue mussel (*Mytilus edulis*) by-product hydrolysate	Protamex	Peptides of 50 kDa, 1 kDa and 200 Da	A549, BT549, HCT15, and PC3 cell lines	Inhibited cell growth	Beaulieu et al. (2013)
Peptides from sea cucumber (*Isostic hopus badionotus*)	Pepsin and corolase	Peptides of > 3, and < 3 kDa	HT-29 colorectal cancer cells	Inhibited the cell proliferation	Perez-Vega et al. (2013)
Protein hydrolysates from tuna cooking juice	Protease XXIII	KPEGMDPPLSEPEDRRDGAAGPK (2449.2 Da) and KLPPLLLAKLLM SGKLLAEPCTGR (2562.4 Da)	MCF-7 cell line	Exhibited antiproliferative activity and induced cell cycle arrest in S phase	Hung et al. (2014)
Half-fin anchovy (*Setipinna taty*) protein hydrolysates	Pepsin	YALPAH (670.35 Da)	Human prostate cancer (PC-3) cell line	Induced apoptosis of PC-3 cells	Song et al. (2014)
Common bean (*Phaseolus vulgaris*) protein hydrolysates	Pepsin and pancreatin	GLTSK (505.48 Da), LSGNK (518.29 Da), GEGSGA (521.22 Da), MPACGSS (656.01 Da) and MTEEY (671.98 Da)	Human colon cancer cell lines (HCT-116, RKO and KM12L4)	Cell growth inhibition, and modified the expression of cell cycle regulatory proteins p53, p21, cyclin B1, BAD, cytC, c-casp3, Survivin, and BIRC7	Vital et al. (2014)
Oyster (*Saccostrea cucullata*) protein hydrolysate	Protease from *B. cereus* SU12	Leu-Ala-Asn-Ala-Lys (515.29 Da)	Human colon carcinoma (HT-29) cell lines	Induced cell growth inhibition, apoptotic changes and oxidative DNA damage	Umayaparvathi et al. (2014)
Roe (egg) protein hydrolysates from rohu (*Labeo rohita*)	Pepsin	Low molecular mass peptides mostly < 10 kDa	Human colon (Caco-2) cancer cell line	Exhibited antiproliferative activity	Chalamaiah et al. (2015)
Blood clam (*Tegillarca granosa*) muscle protein hydrolysate	Neutrase	Trp-Pro-Pro (398.44 Da)	PC-3, DU-145, H-1299 and HeLa cell lines	Showed cytotoxicity and changed the morphologies of the PC-3 cells, and increased the apoptotic PC-3 cells	Chi et al. (2015)
Roe protein hydrolysates from giant grouper (*Epinephelus lanceolatus*)	Protease N	–	Oral cancer cell lines (Ca9-22 and CAL 27)	Reduced cell viability of Ca9-22 and CAL 27 cells and induced apoptosis of Ca9-22 cells	Yang et al. (2016)
Gelatin hydrolysates from *Lates calcarifer*	Alcalase	–	Caco-2 (human colon) and Hep G2 (liver) cancer cell lines	Showed antiproliferative activity	Sae-leaw et al. (2016)
Rapeseed (*Brassica campestris*) peptides	A neutral protease, *B. subtilis and A. elegans*	Trp-Thr-Pro (408.2 Da)	Hep G2 cell line	Inhibited proliferation, and significantly changed the morphology of the HepG2 cells and induced apoptosis	Wang et al. (2016)
Skate (*Raja porosa*) cartilage protein hydrolysate	Alcalase and trypsin	Phe-Ile-Met-Gly-Pro-Tyr (FIMGPY) (726.9 Da)	HeLa cell line	Exhibited anti-proliferation activity by inducing apoptosis	Pan et al. (2016)
Peptides from *Spirulina platensis*	Pepsin, trypsin, and chymotrypsin	HVLSRAPR	MCF-7, HepG-2 and SGC-7901 cell lines	Showed strong anti-proliferation activity on three cancer cells	Wang and Zhang (2017)

cancer); HL-60 (human acute myeloid leukemia); Kelly, SK-N-DZ and IMR-32 (human neuroblastoma); L1210 (mouse lymphocytic leukemia); P388D1 (mouse monocyte cell line), MC3T3E1 (mouse osteoblastic), UMR106 (rat osteosarcoma) and vero (kidney carcinoma cells from monkey), have been extensively used as model cell culture systems for investigating the anticancer activities of protein hydrolysates prepared from a variety of food protein sources (Table 2) (Beaulieu et al., 2013; Chalamaiah et al., 2015; Chi et al., 2015; Doyen, Beaulieu, Saucier, Pouliot, & Bazinet, 2011; Eliassen et al., 2006; Jumeri & Kim, 2011; Kannan, Hettiarachchy, Johnson, & Nannapaneni, 2008; Roy, Kuwabara, Hara, Watanabe, & Tamai, 2002; Sae-leaw et al., 2016; Vital et al., 2014; Yang et al., 2016; You, Zhao, Liu, & Regenstein, 2011). Several researchers used different cell lines since each cell line might display various sensitivities to a given antitumor peptide and also each cell line has a different origin and have separate morphology and tumor characteristics (Daniel & Anon, 2010).

The cells are incubated with various concentrations (e.g. 0.5, 1, 2, 4, 8, 10, 15, 20, 30 and 40 mg/ml) of the protein hydrolysates or peptides for 12 or 24 or 48 or 72 h depending on cell type, peptide source and parameters under investigation. At the end of the treatment period, a wide range of assays can be used to determine the anticancer activity of food derived protein hydrolysates or peptides. Cytotoxicity is one of the chemotherapeutic targets of antitumor activity. The antiproliferative/cytotoxic effects of the food derived peptides are generally evaluated by *in vitro* microplate assay using the 3-(4,5 dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) method based on the detection of mitochondrial dehydrogenase activity in living cells. In addition to cytotoxic/antiproliferative activities, many research studies investigated the apoptosis, necrosis, and cell cycle perturbations in order to elucidate the mechanism of cell death induced by the food derived protein hydrolysates or peptides. Flow cytometry is generally used to analyze the apoptosis, necrosis and the cell cycle progression after treatment with food derived protein hydrolysates or peptides. Apoptosis is an important homeostatic mechanism that balances cell death with survival of normal cells and plays key roles in ultimate decisions of cancer cell fate (Ouyang et al., 2012). Cell apoptosis is commonly assessed by FACS analysis using a flow cytometer. The cells are stained with annexin V (conjugated to green-fluorescent fluorescein isothiocyanate (FITC) dye) and propidium iodide (PI) (red fluorescent dye) to determine if cells are viable, apoptotic, or necrotic through differences in plasma membrane integrity and permeability (Huang et al., 2012; Song, Wei, Luo, & Yang, 2014). The exposure of phosphatidylserine (PS) on the extracellular side of the cell membrane is quantified by annexin V, which indicates early stages of apoptosis. Propidium iodide binds and stains deoxyribonucleic acid (DNA) of late apoptotic and necrotic cells but does not stain live or early apoptotic cells with intact plasma membranes (Rieger, Nelson, Konowalchuk, & Barreda, 2011).

Cell cycle analysis is generally carried out using flow cytometry to distinguish cells in different phases of the cell cycle (G1 vs S vs G2/M) (Hung, Yang, Kuo, & Hsu, 2014). Propidium iodide, as a commonly used fluorescent dye that stains DNA quantitatively, can also be used to determine cell cycle phases (G1 vs S vs G2/M) based on DNA content (Fu & Zhao, 2013; Sheih et al., 2010). Additionally, apoptosis is characterised by a series of typical morphological features, such as blebbing, shrinkage, chromatin condensation, nuclear fragmentation, and the fragmentation of the cells into membrane-bound apoptotic bodies (Elmore, 2007). Several researchers investigated the effects of the protein hydrolysates on cell morphology and cytoskeletal architecture. Fluorescence microscopy and acridine orange/ethidium bromide (AO/EB) staining methods are used to observe the morphological changes to distinguish between apoptotic and normal cells (Huang et al., 2012; Pan et al., 2016). Furthermore, the ratio of B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated X (Bax) protein expression is used as an index of apoptosis. The changes in the expression of the apoptogenic (Bax, cleaved caspase-3, cytochrome c, p53, Bcl-2 associated death promoter (BAD) protein), anti-apoptotic (Bcl-2, caspase-9) and cell cycle-regulating proteins (cyclin A, cyclin E, cyclin-dependent kinase 2 (cdk2), p21 and p27) are usually investigated by western blot analysis using -selective antibodies (Huang et al., 2012; Hung, Yang, Kuo, & Hsu, 2014; Pan et al., 2016; Vital et al., 2014; Wang et al., 2016).

In addition to *in vitro* assays, antitumor activities of food derived protein hydrolysates have also been performed in animal models with significant positive results. The *in vivo* antitumor effects of food protein hydrolysates or peptides are generally assessed by using tumor bearing mice as the animal model (Azevedo et al., 2012; Li et al., 2013; Watanabe et al. 1998; Xue et al., 2009). Generally, the mice are inoculated with cancer cells through subcutaneous injection to establish a tumor model. After 24 h of tumor implantation, the protein hydrolysates or peptides are injected intraperitoneally or orally daily over a specific period of time depending on tumor type and peptide source. After experimental period, the mice are sacrificed and tumors are excised to measure the tumor sizes, tumor growth delay, tumor doubling time and tumor inhibition rate. And the tumor tissues are collected for pathological sectioning with hematoxylin–eosin (HE) stain (Azevedo et al., 2012; Li et al., 2013; Xue et al., 2009).

3.2. Isolated anticancer peptides from food proteins and their mechanism of action

The anticancer protein hydrolysates or peptides produced from food proteins are summarized in Table 2. The mechanism of how food derived peptides inhibits the growth of cancer cell lines has been widely investigated. Several researchers isolated the peptides from food sources with anti-proliferative/cytotoxic activities against several human cancer cells. Most of the food derived anticancer peptides contain short amino acid sequences ranging from 3 to 25 residues (Hsu, Li-Chan, & Jao, 2011; Huang et al., 2012; Hung et al., 2014; Mader, Salsman, Conrad, & Hoskin, 2005; Mader et al., 2007; Pan et al., 2016; Wang & Zhang, 2017; Wang et al., 2013). The anticancer activity of food derived peptides is based on their structural characteristics, such as amino acid composition, sequence, length, overall charge/hydrophobicity, etc. The predominant amino acids of food protein derived anticancer peptides are hydrophobic amino acids such as proline, leucine, glycine, alanine, and one or more residues of lysine, arginine, serine, glutamic acid, threonine and tyrosine (Chi et al., 2015; Hsu et al., 2011; Hung et al., 2014; Kim, Kim, Hwang, Lee et al., 2013; Vital et al., 2014; Wang & Zhang, 2017).

The hydrophobic amino acids can enhance interactions between anticancer peptides and the outer leaflets of tumor cell membrane bilayers, and thereby exert selective and stronger cytotoxic activity against cancer cells (Chi et al., 2015; Pan et al., 2016). Research has shown that the compositions of cell membrane bilayers and the distribution of phospholipids could determine the cell selectivity and susceptibility of the cell to lysis by the peptides (Wang, Rupasinghe, Schuler, & De Mejia, 2008a; Chi et al., 2015). It has been shown that the presence of charged (glutamic acid) and heterocyclic amino acid (proline) in the sequence could contribute to anti-cancer properties of the peptides (Chi et al., 2015; Kannan et al., 2010). Song, Wei, Luo, and Yang (2014) identified an antiproliferative peptide composed of 6 amino acid residues, Tyr-Ala-Leu-Pro-Ala-His (670.35 Da), from peptic hydrolysates of half-fin anchovy. The hydrophobic ratio of this peptide was reported to be 50%, which might contribute to its antiproliferative activity. In another study, Otani and Suzuki (2003) isolated three cytotoxic peptides, Tyr-Lys, Leu-Lys-Lys and Arg-Pro-Lys from a trypsin digest of bovine αs1-casein, which induced necrosis towards mouse spleen T and B cells and human leukemic T and B cell lines. The authors suggested that the strength of the positive charge in the peptides may be correlated with the cytotoxic activity. It is reported that lower molecular weight peptides with higher hydrophobic amino acids can exert higher anticancer activities (Hung et al., 2014; Jumeri & Kim, 2011). The shorter peptides with greater molecular mobility and diffusivity

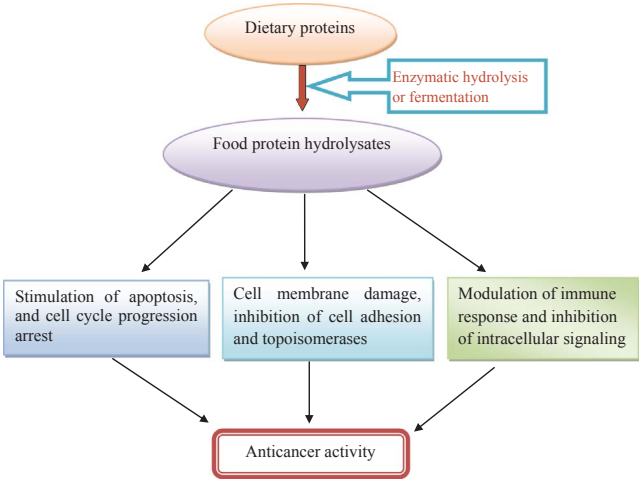

Fig. 3. Mechanisms of action of food derived anticancer protein hydrolysates or peptides.

can better interact with cancer cell components and thus show stronger anticancer activity. Therefore, the differences of molecular weight of food derived peptides could result in their different cytotoxic activities (Jumeri & Kim, 2011; Sheih et al., 2010; Song et al., 2014). Hence, the structural characteristics of peptides are important for the anticancer activity.

A large body of evidence suggests that several food derived protein hydrolysates or isolated peptides possess anticancer activities in cultured cancer cells, and also in animal models of cancer through several molecular mechanisms including stimulation of apoptosis, cell cycle progression arrest, cell membrane damage, inhibition of cell adhesion, topoisomerases, modulation of immune response, and inhibition of intracellular signaling (see Fig. 3) (Daniel & Anon, 2010; Kim et al., 2000; Li et al., 2013; Mader et al., 2007; Pan et al., 2016; Umayaparvathi et al., 2014; Vital et al., 2014; Wang, Rupasinghe, Schuler, & De Mejia, 2008b; Xue et al., 2009). However, the exact mechanism of anticancer effects of food derived protein hydrolysates or peptides remain unclear, but some amino acids such as Pro, Leu, Gly, Ala, Lys, Arg, Ser, Glu, Thr, Tyr have been reported to play an essential role in the anticancer activities of peptides or protein hydrolysates produced from food proteins (Chi et al., 2015; Hung et al., 2014; Vital et al., 2014; Wang & Zhang, 2017).

A number of peptides with anticancer activities have been reported from plant sources. Kim et al. (2000) identified a peptide, Met-Leu-Pro-Ser-Tyr-Ser-Pro-Tyr, from soybean protein hydrolyzed with thermoase and showed cytotoxic effect on P388D1 cell line. The authors demonstrated that the peptide fraction from soy protein hydrolysates affected cell cycle progression by arresting P388D1 cells at G2/M phase. Wang et al. (2008b) subjected soy protein isolates to simulated gastrointestinal digestion with pepsin and pancreatin, and identified three peptides, Phe-Glu-Ile-Thr-Pro-Glu-Lys-Asn-Pro-Gln, Ile-Glu-Thr-Trp-Asn-Pro-Asn-Asn-Lys-Pro and Val-Phe-Asp-Gly-Glu-Leu, with inhibitory effects on the human topoisomerase II, which is the target of several anticancer agents because its inhibition impedes the processes of cell proliferation and differentiation in carcinogenesis. In another investigation, Kannan et al. (2010) purified and isolated anticancer pentapeptide, Glu-Gln-Arg-Pro-Arg, from rice bran, which caused 84% inhibition to colon cancer cells (Caco-2, HCT-116) growth, 80% to breast cancer cells (MCF-7, MDA-MB-231) growth and 84% to liver cancer cells (HepG-2) growth at 600–700 μg/ml dose. In a study conducted by Vital et al. (2014), the isolation of five anticancer peptides was reported from common bean non-digestible fractions produced by *in vitro* simulated gastrointestinal digestion. These five peptides comprised 5–7 amino acid sequences, and the structures of the peptides were Gly-Leu-Thr-Ser-Lys (505.48 Da), Leu-Ser-Gly-Asn-Lys (518.29 Da), Gly-Glu-Gly-Ser-Gly-Ala (521.22 Da), Met-Pro-Ala-Cys-Gly-Ser-Ser (656.01 Da) and Met-Thr-Glu-Glu-Tyr (671.98 Da). The authors reported that the peptides contributed to the antiproliferative effect on HCT116 and RKO human colorectal cancer cells by cell cycle arrest through increasing the expression of p-p53 Ser392 and p21, and decreasing the expression of cyclin-B1. Recently, an antitumor tri peptide, Trp-Thr-Pro (408.2 Da), obtained from rapeseed by enzymatic hydrolysis and fermentation, exhibited anticancer activity on HepG2 cells through up-regulation of p53 and Bax and down-regulation of Bcl-2 (Wang et al., 2016).

Fish and marine products are great sources of anticancer peptides. Recently, anticancer activities have been reported for peptide sequences isolated from several fish and marine sources. Hsu et al. (2011) identified two antiproliferative peptides, Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr (1206 Da) and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr (1124 Da), from tuna dark muscle byproduct protein hydrolysates prepared by two commercial enzymes, papain and Protease XXIII, and reported the dose-dependent inhibition effect on human breast cancer cell line MCF-7 with IC_{50} values of 8.1 and 8.8 μM, respectively. Huang et al. (2012) identified a peptide, Gln-Pro-Lys, from trypsin digested sepia ink protein hydrolysate, which significantly inhibited the proliferation of DU-145, PC-3, and LNCaP cells in a time and dose-dependent manner. The authors reported that the peptide treatment decreased the expression of the anti-apoptotic protein Bcl-2 and increased the expression of the apoptotic protein Bax, therefore induced apoptosis in cells. Kim, Kim, Hwang, Lee et al. (2013) reported that α-cymotrypsin hydrolysate of *Ruditapes philippinarum* proteins had strong anticancer activity, from which a purified decapeptide, Ala-Val-Leu-Val-Asp-Lys-Gln-Cys-Pro-Asp, showed cytotoxic activity against PC-3 (prostate), A549 (lung) and MDA-MB-231 (breast) cancer cells, with lethal concentration 50 (LC_{50}) values of 1.29, 1.35 and 1.58 mg/ml respectively; but did not show cytotoxicity on normal liver cells. In another study, Hung et al. (2014) reported an inhibitory effect of > 2.5 kDa fraction from a tuna cooking juice protein hydrolysate on human breast cancer cell line MCF-7. Two peptides were identified in the peptide fraction as Lys-Pro-Glu-Gly-Met-Asp-Pro-Pro-Leu-Ser-Glu-Pro-Glu-Asp-Arg-Arg-Asp-Gly-Ala-Ala-Gly-Pro-Lys and Lys-Leu-Pro-Pro-Leu-Leu-Leu-Ala-Lys-Leu-Leu-Met-Ser-Gly-Lys-Leu-Leu-Ala-Glu-Pro-Cys-Thr-Gly-Arg. The authors reported that the peptide fraction induced cell cycle arrest in S phase, through the increases of p21 and p27 and decrease of cyclin A expression, and stimulated apoptosis of MCF-7 cells by down regulation of the expression of Bcl-2, poly (ADP-ribose) polymerase (PARP) and caspase 9, and upregulation of the expression of p53, Bax and cleaved caspase 3. Recently, a peptide, Leu-Ala-Asn-Ala-Lys, with MW of 515.29 Da isolated from oyster protein hydrolysates has been shown to inhibit cancer cell growth by increasing DNA damage and apoptosis in the HT-29 colon cancer cell line (Umayaparvathi et al., 2014). In a study performed by Chi et al. (2015), an anticancer peptide, Trp-Pro-Pro, was isolated from blood clam (*Tegillarca granosa*) muscle protein hydrolysate produced by Neutrase. This peptide exhibited strong cytotoxicity toward PC-3, DU-145, H-1299 and HeLa cell lines in a dose-dependent manner and significantly altered the morphologies of the PC-3 cells. In a recent study, an anticancer hexapeptide, Phe-Ile-Met-Gly-Pro-Tyr, isolated from protein hydrolysate of skate (*Raja porosa*) cartilage showed a dose-dependent anti-proliferation activity in HeLa cells with an IC_{50} of 4.81 mg/ml. Similarly, the induced apoptosis was attributed to the upregulated Bax/Bcl-2 ratio and caspase-3 activation (Pan et al., 2016). In another study, a peptide, Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe (1309 Da), derived from pepsin hydrolysate of algae protein waste, inhibited human gastric cancer cell lines AGS, with an IC_{50} value of 256.4 μM, by inducing a post-G1 cell cycle arrest (Sheih et al., 2010). Recently, Wang and Zhang (2017) isolated an antiproliferative peptide, His-Val-Leu-Ser-Arg-Ala-Pro-Arg, from *Spirulina platensis* proteins hydrolysate (prepared using gastrointestinal endopeptidases), which exhibited strong inhibition on HT-29 cancer cells with an IC_{50} value of 99.88 μg/ml.

Additionally, milk proteins are an important source of anticancer

peptides. Mader et al. (2005) reported that bovine lactoferricin (LfcinB), a peptide fragment produced by acid-pepsin hydrolysis of lactoferrin obtained from cow's milk, exhibited cytotoxic activity against Jurkat T leukemia cells through caspase-2-induced dissipation of mitochondrial transmembrane potential and subsequent activation of caspase-9 and caspase-3. In an animal model of B16F10 melanoma tumor-bearing C57BL/6J mice, a penta-peptide, Ile-Asn-Lys-Lys-Ile, isolated from bovine β-casein after tryptic hydrolysis exhibited anticancer effects, including a significant reduction in tumor volume of 72.62% and decreased metastasis number loci, in addition to significant delay in tumor growth and tumor doubling time (Azevedo et al., 2012).

Apart from isolated anticancer peptides, several crude protein hydrolysates or peptide fractions derived from various animal and plant food protein sources have been shown to possess anticancer activities *in vitro* (cell lines) and *in vivo* (animal models) (Picot et al., 2006; Wang et al., 2008; Watanabe et al., 1998; Xue et al., 2009, 2012; Giron-Calle, Alaiz, & Vioque, 2010; Naqash & Nazeer, 2010; Jumeri & Kim, 2011; Beaulieu et al., 2013; Chalamaiah et al., 2015; Li et al., 2013; Yang et al., 2016). Table 2 lists the anticancer activities reported for food protein hydrolysates and enzymes used for their production.

Watanabe et al. (1998) investigated the antitumor effects of peptide fragments (220 and 120 kDa) prepared from hen egg white ovomucin by Pronase in double grafted BALB/c mice transplanted with Meth-A fibrosarcoma cells. The authors reported that both peptide fragments cured directly and entirely the right (treated) tumor and inhibited indirectly and slightly the growth of the left (distant) one. Recently, several crude protein hydrolysates or peptide fractions derived from plant protein sources, such as rice, corn, chickpea, rapeseed and amaranth, have been shown to possess anticancer activities. Kannan et al. (2008) fractionated rice bran peptide hydrolysates into > 50, 10–50, 5–10, and < 5 kDa sizes and reported that the < 5 and 5–10 kDa sized peptide fractions inhibited growth of Caco-2 cells by 80%, and the < 5 kDa fraction inhibited growth of HepG2 cells by ~50% compared to controls. In another study, Xue et al. (2009) evaluated the antitumor activities of a rapeseed protein hydrolysate by using an *in vivo* S180 tumor-bearing Kunming mice model and reported that the rapeseed protein hydrolysate significantly decreased the tumor weight by 44% and 53% in the 100 and 150 mg/kg/day groups, respectively. Daniel and Anon (2010) studied the inhibitory effect of amaranth protein hydrolysates on cell proliferation of MC3T3E1, UMR106, Caco-2 and TC7 and reported that the degree of hydrolysis (DH) augmented the inhibitory capacity of the protein hydrolysates. Giron-Calle et al. (2010) reported that chickpea protein hydrolysates produced with the physiological enzymes pepsin and pancreatin can inhibit the proliferation of THP-1 cells up to 78% depending on DH and culture conditions. In another study, Xue, Liu, Wub, Zhuanga, and Yu (2010) showed that rapeseed peptides obtained from Alcalase and flavourzyme digestion exerted antiproliferative effect on human cervical cancer cell line (HeLa) through induction of apoptosis, DNA damage and cycle arrest in the S phase. Recently, Xue et al. (2012) investigated the antitumor effects of chickpea albumin hydrolysate in H-22 tumor-bearing mice and showed that the chickpea hydrolysate administration significantly increased the tumor inhibition rate and decreased tumor sizes. Corn derived peptides have also exhibited anti-proliferative activity in HepG2 cells through the cell cycle arrest and activation of cleaved-caspase-3 and p53 (Li et al., 2013).

In addition to plant derived anticancer protein hydrolysates, several studies have reported the anticancer activities for crude protein hydrolysates produced from various fish and marine sources. Picot et al. (2006) reported the antiproliferative activity of eighteen fish protein hydrolysates and demonstrated the highest effect (40%) on MCF-7 cell line for a cod hydrolysate obtained with Protamex and Alcalase. Wang et al. (2010) produced protein hydrolysates from Oyster with crude protease SM98011 and investigated the anticancer activity in BALB/c mice bearing transplantable sarcoma-S180 cells. The authors reported that the mice receiving oyster hydrolysates at 0.25, 0.5 and 1 mg/g of body weight by oral gavage showed 6.8%, 30.6% and 48% less tumor growth, respectively. In a study performed by You et al. (2011), the preparation of different peptide fractions (MW > 10 kDa, 5–10 kDa, 3–5 kDa, < 3 kDa) was reported from hydrolysates of loach proteins using papain digestion, and demonstrated the antiproliferative activity of peptide fractions on several human liver (HepG2), breast (MCF-7), and colon (Caco-2) cancer cell lines in a dose dependent manner. In another investigation, Kannan et al. (2011) showed that peptide fractions (< 10 and 10–30 kDa) obtained from shrimp shell whites and langostino shells can inhibit the growth of both colon (Caco-2) and liver (HepG2) cancer cells by 60%. A study conducted by Aleman et al. (2011) reported a strong cytotoxic effect of a gelatin hydrolysate from giant squid on human breast carcinoma (MCF-7) and glioma (U87) cells with IC_{50} values of 0.13 and 0.10 mg/ml, respectively. Naqash and Nazeer (2012) purified a peptide fraction from protein hydrolysates of *Nemipterus Japonicus* backbone, which showed a dose dependent cytotoxic effect against HepG2 cell line with an IC_{50} value of 61.1 μg/ml. Sea cucumber peptide fraction (< 3 kDa) produced with gastrointestinal digestion had an IC_{50} value of 1.45 mg/ml for cytotoxic effect against HT-29 cells (Perez-Vega et al., 2013). Beaulieu et al. (2013) isolated a 50 kDa fraction from enzymatic hydrolysates of blue mussel and reported the anti-proliferative activity on four cancerous cell lines: A549, BT549, HCT15 and PC3. This 50 kDa fraction induced a mortality of 90% for PC3, 89% for A549, 85% for HCT15 and 81% for BT549 cell lines at a protein concentration of 44 μg/ml. In another study, Chalamaiah, Jyothirmayi et al. (2015) demonstrated that pepsin protein hydrolysate derived from rohu egg (roe) exhibited 65% inhibitory effect on human colon cancer cell line Caco-2. Recently, Yang et al. (2016) prepared protein hydrolysates from roe of *Epinephelus lanceolatus* using protease N, which showed antiproliferative activity on two human oral cancer cell lines Ca9-22 and CAL27 by stimulation of apoptosis and sub-G1 cell cycle arrest.

Scientific evidence mentioned above indicates that peptides derived from food proteins exhibit strong anticancer activities. At present, there are several limitations in the current research of anticancer peptides to advance for human use. First of all, many studies used various types of cancerous cells and showed the inhibition of cell growth *in vitro*. In many of these studies the effects of the peptides on normal (non-cancerous) control cells were not investigated, which is very important for use of these peptides in treating tumors without damaging normal cells. Second, the molecular mechanisms of food derived anticancer peptides are still limited. Most studies focused on apoptosis and necrosis mechanisms while there are many other aspects of the peptides that should be investigated to fully elucidate the mode of action, for example, membrane receptor involvement, mediated immunity and anti-angiogenesis activities. In addition, there is not much evidence available from animal and clinical studies showing the benefits of the peptides. In animal studies, lack of safety/toxicology evaluations is major hurdle to move forward, and bioavailability of these peptides remains to be established. In the near future, it is expected that more reports of peptide identification work, animal experiments and clinical trials, and investigation on mechanism of action will emerge to provide solid understanding into this hot research area.

4. Conclusions

Food derived bioactive protein hydrolysates or peptides have been widely investigated by many researchers because of their abundant nature and various biological activities. Numerous protein hydrolysates or peptides with immunomodulatory or anticancer activities have been identified from diverse food sources. These food derived protein hydrolysates or peptides could be utilized as new materials to develop functional foods with immunomodulatory or anticancer activities. The immunomodulatory and anticancer activities of food protein hydrolysates or peptides have been demonstrated mostly *in vitro* (cell culture) or in animal models. Therefore, more clinical studies are needed to

better understand the gastrointestinal stability, bioavailability and safety of these protein hydrolysates or peptides before exploitation for human nutrition as functional foods.

Conflict of interest

The authors declare no conflicts of interest.

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