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Review Article

Fibroin and sericin-derived bioactive peptides and hydrolysates as alternative sources of food additive for promotion of human health: A review

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Abstract - Clothing, food and housing are the fundamental three items for human beings to live humanly. Sericulture has contributed to clothing and housing for more than four thousand years via the production of clothes and goods for houses, such as curtains and bed covers, but has contributed rather little to food, except entomophagy or eating larvae or pupae inside cocoons. When we consider ingestion of silk proteins, fibroin and sericin, from cocoons in the form of bioactive peptides and hydrolysates as in bioactive peptides and hydrolysates from food proteins, such as soy, fish, meat, milk, egg, wheat, broccoli and rice, which are known to be beneficial for the promotion of human health, modern sericulture should contribute to food, and therefore contribute to clothing, food and housing equally. For the preparation of bioactive peptides and hydrolysates from fibroin and sericin, enzymatic hydrolysis is a powerful tool. Based on our experience of the study of silk digestion enzyme for more than twenty years, in this review we summarize current knowledge of bioactive peptides and hydrolysates prepared from fibroin and sericin from domesticated silkworm, *Bombyx mori,* as well as from wild silkmoths, by proteases and their potency for the promotion of human health. Although the number of bioactive peptides and hydrolysates from fibroin and sericin is currently limited, we believe more products will be added in the future from fibroin and sericin and the contribution of modern sericulture to the promotion of human health from this aspect is likely to be assured. We encourage researchers related to silk proteins, fibroin and sericin, to perform further comprehensive studies on bioactive peptides and hydrolysates from fibroin and sericin from domesticated silkworm and wild silkmoths, both of which should provide fruitful resources for the welfare of human beings.

Keywords: Fibroin, sericin, bioactive peptides, *Bombyx mori*, wild silkmoths

1. Introduction

Clothing, food and housing are the fundamental three items needed for human beings to live humanly. Sericulture has contributed to clothing and housing for more than four thousand years (Good *et al*., 2009). This is because sericulture is a systematic human activity: starting from cultivating mulberry trees in the field for harvesting fresh mulberry leaves, rearing silkworm larvae on fresh mulberry leaves for harvesting cocoons, into reeling silk yarns from harvested cocoons and dyeing silk yarns for weaving silk fabrics for making clothes and goods for houses, such as curtains, covers of cushions and furniture, table cloths and bed covers. As for food, sericulture has contributed rather little except larvae or pupae inside cocoons as sources of protein nutrition (Lokeshwari and Shantibala, 2010). When we consider the possibility of ingestion of silk proteins, fibroin and sericin, from cocoons, as food additives, the potential contribution of modern sericulture to food becomes tremendous. Accordingly, sericulture should contribute equally to clothing, food and housing.

To our knowledge, the first time that a method of ingesting the silk protein, fibroin, was devised was in 1989 (Hirabayashi *et al*., 1989). In the 1980s the preparation of a food additive from fibroin that was prepared from extremely low cost silk, such as thin shell cocoons, which were no longer utilized for silk reeling, and discarded silk fabrics, was proposed. The authors solubilized solid fibroin with a calcium chloride solution to make a fibroin solution after degumming the cocoons and silk fabrics or removing

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sericin from them, studied the gelation of the fibroin solution to prepare fibroin powder and determined the effect of feeding fibroin powder to the assay subject. The research group obtained beneficial effects from the fibroin powder. Fibroin powder prepared by the above method is currently available as a health food or food additive, by the name of 'Kaya silk powder' from a company in Yosano Town (formerly Kaya Town), Yosano County, Kyoto Prefecture, Japan. Various food products containing fibroin powder are available on the market, e.g., Japanese noodle, Japanese soba noodle, bread, cake, tofu, soft drinks and cooked rice. Subsequently, four forms of fibroin (fibroin solution, fibroin gel, bubbled fibroin gel and fibroin powder) were prepared and the application of these products as food additive and bioactive peptides from fibroin on animal subjects were studied (Hirao and Igarashi, 2013), which clearly demonstrated the usefulness of fibroin products as food additives to various types of foods and of fibroin-derived bioactive peptides to hypertensive rat (Igarashi *et al*., 2006). Fibroin solution is used to make bread containing 30% Japanese willet powder, blancmange and yogurt jelly. Fibroin gel is used to cook sticky rice and for making rice cakes, steamed buns, soft adzuki-bean jelly, noodles, sponge cakes and sticky rice powder balls. Bubbled fibroin gel is used for making meringues, bubbled soft adzuki-bean jelly and sponge cakes. Fibroin gel prepared by the method of Hirao and Igarashi (2013) for food is available from Matsuoka Co. Ltd., Tsuruoka City, Yamagata Prefecture, Japan.

Fibroin-derived bioactive peptides and hydrolysates have been prepared by several methods, among which the typical ones were (1) acid hydrolysis of solid fibroin using hydrochloric acid solution or (2) solubilization of solid fibroin with calcium chloride solution, followed by enzymatic hydrolysis using protease or protein digestion enzyme. The latter method has a link to our study and is the reason why we wrote this review; we have been studying silk digestion enzyme from silk glands of insects for more than twenty years (Sumida, 2010). The silk digestion enzyme is synthesized by silk gland cells, secreted into the lumen of silk glands at each molt period in the larva (Sutthikhum *et al*., 2004a; Watanabe *et al*., 2004) and it digests the fibroin and sericin stored in the silk glands (Watanabe *et al*., 2007), and it is found in domesticated silkworm, *Bombyx mori* and in wild silkmoth, *Samia cynthia ricini* (Watanabe and Sumida, 2006; Watanabe *et al*., 2006c). This is done so the larva of the silkworm or wild silkmoth ensures that the lumen contents of the silk glands are vacant so that the next instar larva can synthesize fibroin and sericin in the cells of the silk glands and secrete them into the lumen of the silk glands. Thus, silk digestion enzyme is a good candidate to prepare bioactive peptides from fibroin and sericin. In the classification of proteinases, the silk digestion enzyme belongs to cysteine proteinase (Watanabe *et al*., 2006d). It functions optimally at an acidic pH of 4. The important implication of our study was that it gave a rational to use cysteine proteinase for the digestion of fibroin and sericin in vitro. Hydrolysates of fibroin and sericin, which can be prepared by acid hydrolysis or by the digestion of protease, are consisted to be a mixture of amino acids and oligopeptides, and they are known to have beneficial effects on animal subjects (See Table 3 in Section 8) and they could show similar beneficial effects on humans.

2. Cocoon filament and fibroin and sericin

A single cocoon filament from a spinneret of a matured larva of the domesticated silkworm, *B. mori*, consists of two silk proteins, fibroin and sericin (Mondal *et al*., 2007; Vepari and Kaplan, 2007; Kundu *et al*., 2008). In a cross section of a single cocoon filament, there are two columns of fibroin fiber with sericin around them. This is because a silkworm larva has a pair of silk glands and each of the silk gland connects to a single spinneret in the anterior part. Accordingly two columns of fibroin fiber come from a spinneret.

A silk gland is a tubular organ that consists of three parts: from posterior to anterior, the posterior silk gland, middle silk gland and anterior silk gland (see figure 3 of silk gland in Mondal *et al*., 2007). The cells of the posterior silk gland synthesize and secrete fibroin into the lumen. The distal part of the posterior silk gland is made of a blind tube, and fibroin secreted into the lumen of the posterior silk gland is extruded gradually in the anterior direction, i.e., to the lumen of the middle silk gland. Cells of the middle silk gland synthesize and secrete sericin into the lumen. As a result, in a cross section of the middle silk gland, a column of fibroin in the center can be seen that is surrounded by several layers of sericin. The anterior silk gland is the path of the fibroin and sericin when a mature larva spins silk thread.

The protein structure of fibroin and sericin in domesticated silkworm, *B. mori,* and other insect species is summarized in a review by Sehnal and Sutherland (2008). Fibroin is secreted from the cells of the posterior silk glands into the lumen as an assembled form of a high molecular mass elementary unit consisting of H-chain, L-chain and P25 with a 6:6:1 molar ratio (Inoue *et al*., 2000). Molecular masses of H-chain, L-chain and P25 are 350, 25 and 27 or 30 kDa due to differential glycosylation of P25 (Tanaka *et al*., 1993), respectively. Gene sequence of the H-chain has been reported (Zhou *et al*., 2000). Cloning of the cDNA of the L-chain and its primary structure has been reported (Kimura *et al*., 1985; Yamaguchi *et al*., 1989). Amino acid sequence of P25 was reported (Chevillard *et al*., 1986). Sericin mainly consists of three proteins (A, M and P) that are synthesized and secreted into the anterior, middle and posterior subparts of the middle silk gland, respectively (Takasu *et al*., 2002). The molecular masses of sericin A, M and P are 250, 400 and 150 kDa, respectively, as estimated by SDS-PAGE (Takasu *et al*., 2002). Three genes for sericin are known, namely *Ser1* (Okamoto *et al*., 1982), *Ser2* (Michaille *et al*., 1990) and *Ser3* (Takasu *et al*., 2007). The *Ser1* gene encodes sericins M and P. The *Ser2* gene encodes unique sericin proteins with molecular masses of 230 and 120 kDa, which are different from sericin M, P and A, and which are not incorporated into the cocoon silk (Kludkiewicz *et al*.,

2009). The *Ser3* gene encodes sericin A (Takasu *et al*., 2005; 2007). Four species of small molecular mass proteins have been identified in the silk of *B. mori* (Nirmala *et al*., 2001). They are seroin 1 and seroin 2, with molecular masses of 9.9 and 10.3 kDa, respectively, and Kunitz-type and somewhat unusual Kazal-type proteinase inhibitors with molecular masses of 6 and 4.7 kDa, respectively. It is assumed that seroins and proteinase inhibitors function in cocoon protection against predators and microbes. Interestingly, fibroin and sericin are different in different insect species in protein composition, protein structure and amino acid sequence (Sehnal and Sutherland, 2008). This will open an opportunity to produce a wide variety of bioactive peptides and hydrolysates from fibroin and sericin from insect species distributed worldwide.

3. Bioactive peptides derived from fibroin of domesticated silkworm, *B. mori*

Bioactive peptides derived from fibroin are summarized in Table 1. Three peptides are known, with the sequences of GY, GVGAGY and GVGY. For their functions, GY has both ACE inhibitory activity and an antihypertensive function. For GVGAGY, ACE inhibitory activity is known. For GVGY, there is an antihypertensive function. It is interesting to note that the three peptides described above are unique peptides that are derived from fibroin with antihypertensive functions, because no similar sequences of peptides have been identified in bioactive peptides from food sources (see Hong *et al*. (2008)). For the various kinds of bioactive peptides identified from food proteins, the following physiological functions on four kinds of human systems are known (Hartmann and Meisel, 2007): (1) cardiovascular system, hypocholesterolemic, antioxidative and antithrombotic functions; (2) nervous system, opioid agonist and opioid antagonist functions; (3) gastrointestinal system, mineral binding, opioid agonist, opioid antagonist and antimicrobial functions; and (4) immune system, immunomodulatory, opioid agonist, opioid antagonist and antimicrobial functions. Erdmann *et al*. (2008) added antihypertensive, hypotriglyceridemic and antiobesitic functions to those described above. Compared to these functions known in bioactive peptides derived from food proteins, in bioactive peptides derived from fibroin, only an antihypertensive function is known. The reason for this may be due to the short history of research on bioactive peptides derived from fibroin, and also due to the comparatively smaller number of researchers to study fibroin derived bioactive peptides. Taking into consideration the unique amino acid sequences in bioactive peptides from fibroin, such as GY and GVGAGY with ACE inhibitory activity and GY and GVGY with antihypertensive function, further studies on bioactive peptides from fibroin will elucidate more unique bioactive peptides with various beneficial functions on human health.

Table 1. Bioactive peptides derived from fibroin and sericin of domesticated silkworm, *Bombyx mori* and function on assay subject.

Function	Precursor protein	Peptide sequence	Origin	Reference
ACE* inhibitory	Fibroin	GY	Cocoon	Ni *et al*. (2001)
ACE inhibitory	Fibroin	GVGAGY	Cocoon	Igarashi *et al*. (2006)
Antihypertensive	Fibroin	GVGY	Cocoon	Igarashi *et al*. (2006)
Antihypertensive	Fibroin	GY	Cocoon	Zhou *et al*. (2010)
Protection from death of Sf 9 cells from serum	Sericin	SGGSSTYGYS	Chemical synthesis**	Takahashi *et al*. (2005)
deprivation	Sericin	SGGSSTWGWS	Chemical synthesis***	Takahashi *et al*. (2005)

ACE*, Angiotensin I-converting enzyme

**, chemically synthesized based on amino acid sequence of a peptide of 38 amino acids obtained from sericin hydrolysate which showed activity of cellular protection.

***, chemically synthesized based on SGGSSTYGYS by replacing Tyr (Y) with Trp (W).

4. Bioactive peptides derived from sericin of domesti cated silkworm, *B. mori*

Bioactive peptides derived from sericin are summarized also in Table 1. Two peptides of biological activity that support the survival of cultured cells are known, for which the sequences are SGGSSTYGYS and SGGSSTWGWS (Takahashi *et al*., 2005). The former peptide was chemically synthesized with reference to a characteristic amino acid sequence of sericin. The latter was chemically synthesized with reference to the former peptide and by replacing Tyr (Y) with Trp (W). In the case of sericin-derived bioactive peptides, only two peptides are known. The reason may be similar to the case of fibroin-derived bioactive peptides, i.e., due to a short history of research and smaller number of researchers. More bioactive peptides derived from sericin, which is another silk protein, will be added to the list by further studies.

5. Enzymes used for preparation of bioactive peptides and hydrolysates from fibroin and sericin

Alcalase was used in the preparation of bioactive peptides from fibroin (Ni *et al*., 2001; Igarashi *et al*., 2006; Zhou *et al*., 2010). Chymotrypsin (Chen *et al*., 1991), alcalase, trypsin and pepsin (Park *et al*., 2002) were used for the preparation of fibroin hydrolysate. Protease N "amino", protease P "amino" 6, alcalase 2.4L, neutrase 1.5MG and 1.398 neutral protease were used for preparation of sericin hydrolysate (Wu *et al*., 2008). Since the preparation of food additives from fibroin and sericin is the objective, enzymes that have a long history of use for preparation of bioactive peptides from foods and enzymes that are guaranteed for their safety to humans should be chosen, as in the production of milk-derived antioxidative peptides in which trypsin, chymotrypsin, validase, pepsin, bacterial and plant food grade enzymes, alcalase, protamex, neutrase, thermolysin and corolase PP were used (Power *et al*., 2013).

6. Silk digestion enzyme

Silk digestion enzyme or fibroinase, the name given in our initial study, was, at first, detected by its ability to digest liquid fibroin in vitro, visualized by SDS-PAGE, and it was found from degenerating silk glands from day one pupa, of the silkworm, *B. mori* (Sumida *et al*., 1993a). The question, 'Do silk glands produce silk digestion enzyme or fibroinase?' based on the experience from the enzyme study in *B. mori,* and it was tested using, at first, a homogenate of silk glands remaining in a day one pupa of *B. mori* as the enzyme source, since the silk glands remaining in the pupa become slender from day zero to day one pupa, which suggested that fibroin and sericin remaining within the pupal silk glands must be digested by some protease within the silk glands from day zero to day one pupa. The history of the study of silk digestion enzyme will be reported elsewhere as a case study (Sutthikhum and Sumida, in preparation). The literature survey, then, showed that Akai (1965) described observations by light microscopy in which the complete digestion of fibroin and sericin occurs in the lumen contents of the silk glands of *B. mori* at the fourth molt period in the fourth instar larva. This suggested the presence of silk digestion enzyme or fibroinase in the silk glands at the fourth molt period in the fourth instar larva. We assayed fibroinase activity using silk glands from the fourth molt period of the fourth instar larva as the enzyme source (Sumida *et al*., 1993b). Strong activity was detected, which is comparable to the high activity in day one pupa. The result suggested that fibroinase is involved in the physiological function in silk glands to prepare vacant lumen contents at each molt period in the larva for the next instar larva to synthesize fibroin and sericin in the cells of the silk glands and to enable the next instar larva to secrete them into the lumen of the silk glands. Subsequently, we purified fibroinase from the silk glands of *B. mori* from the fourth molt period in the fourth instar larva (Watanabe *et al*., 2004) and from day one pupa (Watanabe *et al*., 2006a) and characterized the enzymatic properties. Fibroinase was found to be a cysteine proteinase and the properties of the enzyme were similar to those of cathepsin L. When purified by the method of Takasu *et al*. (2002), sericine became available, and we tested if purified fibroinase digested sericine (Watanabe *et al*., 2007), and it was found out that fibroinase digests sericin. We re-named fibroinase as silk digestion enzyme since it digests both fibroin and sericin (Watanabe *et al*., 2006d). Accordingly, the presence of fibroinase alone in the lumen contents of silk glands is sufficient for the digestion of both the fibroin and sericin stored in the silk glands at each molt period in the larva and early pupa in *B. mori*. We demonstrated that fibroinase in the silk glands of the eri silkworm, *S. cynthia ricini,* shows extremely high activity at the end of the spinning period, some 38.3-fold higher activity per individual insect than the maximum activity of the silkworm, *B. mori,* at the fourth molt period in the fourth instar larva or day one pupa (Watanabe *et al*., 2006c), both of which show similar high activity, and the enzymatic entity of the eri silkworm is highly likely to be slightly different from that of *B. mori,* such as a slightly different N-terminal amino acid sequence of the enzyme (Watanabe and Sumida, 2006). This suggests that variation in the silk digestion enzyme among different insect species exists in the developmental expression profile as well as in the quantities produced during development and probably in the molecular structure of the enzyme itself. The high activity of the silk digestion enzyme in the silk glands of *S. cynthia ricini* at the end of spinning in the fifth instar larva opens an application for this enzyme as a source of a degumming agent as well as for an enzyme to prepare fibroin and sericin-derived bioactive peptides and hydrolysates. On the other hand, from an academic point of view, we believe that if the molecular features of the silk digestion enzyme in each insect species would be revealed, the mechanism of co-evolution of the substrate and enzyme, or between fibroin or sericin and silk digestion enzyme, will be elucidated in future studies. Anyhow, silk digestion enzyme digests both fibroin and sericin; and accordingly, silk digestion enzyme is a candidate enzyme to prepare bioactive peptides and hydorlysates from fibroin and sericine. Incidentally, another physiological function of silk digestion enzyme was found in the feeding period at each larval stage and at the spinning period in the last fifth instar larva in *B. mori* (Sutthikhum *et al*., 2004a, b). The enzyme functions as a lysosomal enzyme in these developmental periods, and functions within lysosomes in the silk gland cells to digest obsolete proteins and organelles transported into the lysosomes, such as endoplasmic reticulum and mitochondria that are no longer function to regenerate highly functional protein synthesizing machinery of the silk gland cells.

There is no guarantee that we can obtain bioactive peptides from fibroin and sericin once we use silk digestion enzyme as a hydrolysis agent. There is only the fact that silk producing insects synthesize silk digestion enzyme in the cells of the silk glands themselves and utilize it for the digestion of fibroin and sericin stored in the lumen of silk glands at each molt period in the larva and early pupa as well as in the feeding period and spinning period within the lysosomes as a lysosomal enzyme. Various kinds of proteases have been utilized for production of bioactive peptides from food proteins (Power *et al.*, 2013), and they should be tried and used in addition to silk digestion

enzyme. We experienced high specificity of protease in the hydrolysis of the silk protein sericin. Hydrolysis of the sericin A fraction by the silk digestion enzyme produced a sericin peptide, s-A12, of which the sequence was XPFPKASSXF… (Watanabe *et al*., 2007). On the other hand, Takasu *et al*. (2005) obtained a peptide, 2-1, ASSSFDASSA…, as a sericin product that was hydrolyzed by lysyl endopeptidase. We speculated that XPFPKASSXF… overlapped with ASSSFDASSA… in the region of ASSXF… in our product and ASSSF in a product by Takasu *et al*. (2005), and lysyl endopeptidase cleaved the fifth amino acid, lysine, of XPFPKASSXF…. This speculation may be true. We felt that enzymatic hydrolysis of sericin was very precise. The same must be true of the hydrolysis of fibroin by proteinase. In the case of silk digestion enzyme from silk glands, the specificity is fairly high, and it cleaves preferentially the peptide bond between the Gly and Ala of the fibroin molecule (Watanabe *et al*., 2004) to produce two peptides finally from fibroin by prolonged enzymatic hydrolysis, such as AGYG and AGAGAGYG (Watanabe *et al*., 2006b).

7. Food derived bioactive peptides as references for preparation of bioactive peptides from fibroin and sericin

There are good references related to food derived bioactive peptides (Table 2). They are useful when we prepare bioactive peptides from fibroin and sericin. The food sources are diverse: they are sardine muscle, sake lees, wakame, chicken muscle, wheat germ, α-zein and α-lactalbumin, caseins, milk proteins, soy, fish, meat, eggs, broccoli, rice, β-lactoglobulin, globin, marine animals and plants, whey proteins, American lobster, mushroom, *chlorella vulgarian 87/1*, human milk, okara and bean. The diversity of foods indicates that a large variety in the amino acid sequence of food proteins provides a large number of bioactive peptides that are unique in biological functions. Related to this, we will propose the use of fibroin and sericin from a wide variety of species of wild silkmoths in addition to fibroin and sericin from domesticated silkworm, *B. mori,* in the latter section of this review. The preparation of bioactive peptides from foods is generally carried out by two methods: one is enzymatic hydrolysis and the other is fermentation. The functions of bioactive peptides from foods are diverse. They are generally classified according to the functions on each of the human systems, such as (1) cardiovascular system, (2) nervous system, (3) nutrition system or gastrointestinal system and (4) immune system. Each reference in Table 2 has special features. For example, Hartmann and Meisel (2007) provide a concise, comprehensive overview of the subject and it is especially handy to consider the functions of peptides on human health. They also provide information about commercially available functional foods or food ingredients carrying bioactive peptides. Erdmann *et al*. (2008) provided many examples of ACE inhibitory peptides with in vitro antihypertensive effects and the sequences of the peptides. They describe the structural properties of selected biofunctional peptides, which give good hints to determine the bioactive peptides from fibroin and sericin with ACE inhibitory, antioxidant, antithrombotic, hypocholesterolemic and antiobesity activities. In their comments on ACE inhibitory activities, they describe that its structural element is a Tyr or Phe as the C-terminus. This corresponds to the case of GY from fibroin (Ni *et al*., 2001) and GVGAGY also from fibroin (Igarashi *et al*., 2006) (See Table 1 in Section 3). Their remarks state that dipeptides with a C-terminal Tyr produces a higher antihypertensive effect compared to dipeptides with C-terminal Phe. It is interesting to note that GY and GVGAGY with antihypertensive effects have never been found from peptides derived from food proteins, and yet their remarks are valid for bioactive peptides from fibroin. Hong *et al*. (2008) provide a comprehensive summary of the antihypertensive effect of peptides with numerous sequences obtained from foods, such as milk, egg proteins, fish, globin hydrolysate and plants, which are a collection of data handy to find bioactive peptides from fibroin and sericin. Power *et al.* (2013) provided a comprehensive summary of the enzymatic production of bioactive peptides that specifically had antioxidative functions. They also provided a summary of the in vitro assay system of antioxidant properties. Raikos and Dassios (2014) provided a summary of biofunctional peptides derived from human and bovine milk proteins with effects on human health with the sequences of the peptides. de Castro and Sato (2015) provided a summary of the production of bioactive peptides with fermentation and proteases from food proteins, such as soy, rice, casein, okara, wheat, cow milk, bean by fermentation, soy, purified soy protein, bovine hemoglobin, bean, goby muscle, salmon and cuttlefish (*Sepia officinalis*) muscle by proteases. They also provided a comprehensive summary of the analytical methods for the purification and identification of bioactive peptides and major methods for measuring antioxidant activities of peptides in vitro and in vivo, and their respective mechanisms.

8. Hydrolysate derived from fibroin of domesticated silkworm, *B. mori,* and function on assay subject

Fibroin hydrolysate from *B. mori* and its function on the assay subject are shown in Table 3 in chronological order. Fibroin hydrolysates were prepared by acid hydrolysis by HCl solution except the one listed first, in which fibroin powder was directly tested (Hirabayashi *et al*., 1989) and the one listed fourth, in which preparation of undegraded native molecular fibroin solution was described for use as a standard reference of fibroin molecule (Yamada *et al*., 2001) in the study of fibroin hydrolysis. Various effects were observed, which included lowering blood cholesterol level, antigenotoxicity, enhancement of insulin sensitivity and glucose metabolism, inhibition of adipocyte differentiation and stimulation of osteoblastic differentiation. It is likely that hydrolysate derived from fibroin contains putative bioactive peptide/peptides or it is likely that amino acids in the hydrolysate showed a synergistic function. If the former is the case, efforts towards the purification and identification of the putative peptide/peptides from fibroin hydrolysate are worthwhile.

Table 2. Bioactive peptides derived from foods.

Source	Preparation	Function	Remarks	Reference
Sardine muscle, Sake lees Wakame Chicken muscle, Wheat germ, α-zein α-lact-albumin	Enzymatic hydrolysis	Angiotensin I- converting enzyme (ACE) inhibitory	Development of a novel functional food for prevention of hypertension as well as for therapeutic purposes	Li *et al*. (2004)
Caseins	Selective enzymatic hydrolysis	Cardiovascular system Antithrombotic Antihypertensive Nervous system Opioid Agonistic Antagonistic Nutrition system Caseinophophopeptide Glycomacropeptide Immune system Immunomodulatory Antimicrobial	Biological significance, impact on human health and manufacture of novel functional food ingredients	Silva and Malcata (2005)
Milk proteins	Fermentation	Hypotensive ACE inhibitory	Peptides resistant to further degradation by gastro-intestinal and serum proteinase/ peptidase following oral ingestion	FitzGerald and Murray (2006)
Milk proteins	Enzymatic hydrolysis, fermentation	ACE inhibitory Antihypertensive Opiod Immunostimulatory Antioxidative	Preparation by (a) enzymatic hydrolysis, (b) fermentation, (c) proteolysis by microbial proteases or combination of (a) and (b) or (a) and (c)	Korhonen and Pihlanto (2006)
Soy	Enzymatic hydrolysis, fermentation	Antioxidant Hypotensive Antihypertensive Hypocholesterolemic Immunostimulating Antioxidative ACE inhibitory	Prevention of age-related chronic diseases	Wang and de Mejia (2006)
Soy Fish Meat Milk Egg Wheat Broccoli Rice	*	Cardiovascular system Hypocholesterolemic Antioxidative Antithrombotic Nervous system Opioid agonist Opioid antagonist Gastrointestinal system Mineral binding Opioid agonist Opioid antagonist	Comprehensive overview, table of commercially available functional foods or food ingredients carrying bioactive peptides	Hartmann and Meisel (2007)

Table 2. Bioactive peptides derived from foods. (Cont.)

Source	Preparation	Function	Remarks	Reference
		Antimicrobial Immune system Immunomodulatory Opioid agonist Opioid antagonistAntimicrobial		
Milk Fish Meat Egg Soy Wheat	*	Reduction of cardio-vascular disease Antihypertensive Antioxidative Antithrombotic Hypocholesterolemic Hypotriglyceridemic Antiobesitic	ACE inhibitory peptides with in vitro antihypertensive effects with sequences of peptides, table of commercially available functional foods carrying bioactive peptides, table of structural properties of selected biofunctional peptides, highly useful to find out bio-active peptides with ACE inhibitory, antioxidant, anti-thrombotic, hypocholesterolemic and antiobesitic activities	Erdmann *et al*. (2008)
β-lacto-globulin	Enzymatic hydrolysis	ACE inhibitory Antihypertensive Antioxidant Antimicrobial Immunomodulatory Opioid Hypocholesterolaemic	Structure, biological signifi-cance and mechanism of action of bioactive peptides derived from b-lactoglobulin	Hernandez-Ledesma *et al*. (2008)
Milk Fish Egg Globin Plants	*	ACE inhibitory Antihypertensive	Comprehensive summary of antihypertensive effect of peptides with numerous sequences from foods.	Hong *et al*. (2008)
Milk	Enzymatic hydrolysis, microbial fermentation	ACE inhibitory Immunostimulatory Antimicrobial Antihypertensive	Reduction of mild hypertension	Korhonen (2009)
Casein	Enzymatic hydrolysis, fermentation, proteolysis by enzymes from micro-organisms or plants	Antioxidant Cytomodulatory Immunomodulatory Antimicrobial	Selected biological effects, application in industry and safety aspects and regulations relating to the use of bioactive peptides	Phelan *et al*. (2009)
Marine animals and plants	Enzymatic hydrolysis	Antioxidant Anticoagulant Antimicrobial	Bioactive peptides in functional foods or nutraceuticals and pharmaceuticals	Kim and Wijesekara (2010)
Whey proteins	Enzymatic hydrolysis, microbial fermentation	Immune system Antimicrobial Immunomodulatory Cytomodulatory Cardiovascular system	Specific physiological effects, achieved mechanisms, stabilities of peptides in gastrointestinal route	Mandureira *et al*. (2010)

Table 2. Bioactive peptides derived from foods. (Cont.)

Source	Preparation	Function	Remarks	Reference
		Antihypertensive Antioxidative Antithrombotic Hypocholesterolemic Nervous system Opioid agonist Opioid antagonist Gastrointestinal system Mineral-binding Anti-appetizing		
Chicken muscle, a-zein American lobster, Soy hydrolysates, Bovine milk Mushroom Egg Rice Chlorella vulgarian 87/1, Human milk	Enzymatic hydrolysis	ACE inhibitory Antimicrobial Antiviral Immunomodulatory Opioid antagonist Antithrombotic Hypocholesterolemic	Industrial-scale manufacturing of pharmaceutical grade peptides	Agyei and Danquah (2011)
Muscle proteins from meat and fish	Enzymatic hydrolysis	Antihypertensive Antioxidant Antimicrobial Antiproleferative	Incorporation of bioactive peptides into functional foods and neutraceuticals	Ryan *et al*. (2011)
Milk Mushroom, Soy beans Egg Wheat Chlorella vulgarian 87/1	Enzymatic hydrolysis	Antimicrobial Immunomodulatory	Further insightful research needed on the pharmaco-kinetics of immunomodulatory peptides in vivo, clinical studies needed	Agyei and Danquah (2012)
Marine proteins	Protease hydrolysis	Antihypertensive Antioxidant Hypolipidemic Hypocholesterolemic Anticancer Immunomodulatory Anti-inflammatory Multifunctional	Strategic production and processing methods, elucidation of in vivo molecular mechanisms of action, safety at various doses and pharmacological activity in maintaining homeostasis during aberrant health conditions in human subjects	Udenigwe and Aluko (2012)
Milk	Enzymatic hydrolysis	Antioxidative	Comprehensive enzymatic production of bioactive peptides specifically antioxidative	Power *et al*. (2013)

Table 2. Bioactive peptides derived from foods. (Cont.)

Source	Preparation	Function	Remarks	Reference
			function, table of in vitro assay to evaluate food protein/peptide with antioxidant properties	
Human milk Bovine milk	*	Antihypertensive Antioxidant Antithrombotic Opiod Immunomodulatory Mineral binding Antimicrobial	Peptides with effects on human health with sequences, table of in vitro assay system of antioxidant properties	Raikos and Dassios (2014)
Soy Rice Casein Okara Wheat Cow's milk Bean Salmon	Fermentation, enzymatic hydrolysis	Antihypertensive Antioxidant Immune-regulatory Immunomodulatory Antiadipogenesis Antimicrobial Anti-inflammatory Anticoagulant	Production of bioactivepeptides with fermentation and protease from food proteins, table of analytical methods of purification and identification of bio-active peptides, and methods for measuring various activities	de Castro and Sato (2015)

*, not specifically mentioned.

Table 3. Fibroin and sericin hydrolysates from cocoons of domesticated silkworm, *Bombyx mori* and function on assay subject.

Function	Assay subject	Preparation	Results or potencies	Reference
Fibroin hydrolysates				
Enhanced alcohol metabolism	Rat	0.5 g fibroin powder in 2 ml PBS + 3 ml 50% ethanol in PBS	Light coma but full recovery, no dead rats	Hirabayashi *et al*. (1989)
Digestibility of fibroin	Rat	Acid (2N HCl for 48 h), Chymotrypsin	90% digestibility 70% digestibility, higher digestibility in acid hydrolysed fibroin	Chen *et al*. (1991)
Lowering blood cholesterol level	Rat	Acid (3N HCl for 48 h)	Prevention of cardiovascular disease and brain blood vessels disease	Chen *et al*. (1993)
Undegraded native molecular fibroin solution		Ajisawa's method, LiSCN method	Standard methods to prepare native fibroin solution from silkworm cocoons	Yamada *et al*. (2001)
Antigenotoxicity	Mouse Embryo 3T3 cells	Acid (2NHCl for 4 h) Enzymatic hydrolysis (Alcalase, trypsin, pepsin)	Chemopreventive functional peptides	Park *et al*. (2002)
Enhancement of insulin sensitivity and glucose metabolism	Adipocyte 3T3-L1 cells	Acid (2M HCl for 4 h)	Improvement of diabetic hyperglycemia	Hyun *et al*. (2004)

Table 3. Fibroin and sericin hydrolysates from cocoons of domesticated silkworm, *Bombyx mori* and function on assay subjoet. (Cont.)

Function	Assay subject	Preparation	Results or potencies	Reference
Inhibition of adipocyte differentiation	Adipocyte 3T3-L1 cells	Acid (6M HCl for 5 h)	Inhibition of Notch signaling pathway, treatment of obesity and related metabolic diseases	Jung *et al*. (2011)
Stimulation of osteoblastic differentiation	Osteoblast cell C3H10T1/2 M2-1084	Acid (6M HCl for 5 h)	Suppression of Notch signaling pathway, promotion of bone healing, therapeutic intervention for bone fractures and osteoporosity	Jung *et al*. (2013)
Sericin hydrolysate				
Antioxidant, Tyrosinase-inhibitory	Rat	Enzymatic hydrolysis (Protease N "amino", protease P "amino" 6, alcalase 2.4 L, neutrase 1.5 MG, 1.398 neutral protease)	Valuable ingredients in the food, cosmetic and medicine industries	Wu *et al*. (2008)

9. Hydrolysate derived from sericin of domesticated silkworm, *B. mori,* and function on assay subject

Sericin hydrolysate from *B. mori* and its function on the assay subject are shown also in Table 3. Sericin hydrolysate was prepared by enzymatic hydrolysis using protease N "amino", protease P "amino" 6, alcalase 2.4L, neutrase 1.5MG and 1.398 neutral protease. Antioxidant activity and tyrosinase-inhibitory activity were observed. Similar mechanism of action on the assay subject as with fibroin hydrolysate are likely in the case of sericin hydrolysate, i.e., (1) presence of bioactive peptide/peptides in sericin hydrolysate or (2) synergistic function of amino acids in sericin hydrolysate.

10. Fibroin, sericin and other silk proteins from wild silkmoths as another source of wide variety of bioactive peptides and hydrolysates

Table 4 shows references for the genes of fibroin and other silk proteins from a wide variety of species of wild silkmoths in chronological order, except *Gonometa postica* (Mhuka *et al*., 2013), which is from a paper on fibroin protein. It is included here for convenience to show a list of the variety of species of wild silkmoths that produce silk proteins. The table shows that genes for fibroin and other silk proteins from wild silkmoths are vastly diverged. For example, the paper listed fourth from the top (Tanaka and Mizuno, 2001) showed that in *D. spectabilis* and *P. xuthus*, homologues of L-fibroin and P25 of *B. mori* were clearly identified as in *G. mellonella* in the papers listed first and second in Table 4, but in *A. yamamai*, homologues of L-fibroin and P25 of *B. mori* were not identified. In *A. yamamai*, fibroin was consisted of H-fibroin alone (Tanaka and Mizuno, 2001). Also in Caddisfly listed 12th from the top (Yonemura *et al*., 2006) fibroin is devoid of P25. In *S. cynthia ricini* listed at the bottom of Table 4 (Sezutsu *et al*., 2014), fibroin gene contains repetitive polyalanine block, which is devoid in fibroin in *B. mori*. (See also Craig and Riekel (2002) listed sixth from the top of Table 4 and Fedic *et al*., (2002) listed seventh from the top of Table 4.) Therefore the fibroin proteins seemed to be vastly diverged among wild silkmoths. These facts show that bioactive peptides and hydrolysates produced from fibroin, sericin and other silk proteins of wild silkmoths are highly likely to be unique. To our knowledge, no information is currently available on the sericin gene from wild silkmoths.

Fibroin isolated from wild silkmoths is shown in Table 5. Considerable differences among the fibroins from wild silkmoths are now known in the structural assembly of the fibroin subunits, amino acid sequence and molecular masses. For example, in *P. ricini* listed fourth from the top of Table 5 (Ahmad *et al*., 2004) and in *A. assama* listed fifth from the top of Table 5 (Ahmad *et al*., 2004) fibroin is consisted of H-chain and L-chain but the molecular mass of each subunit was different, H-chain in *P. ricini*, 97-kDa, H-chain in *A. assama*, 220-kDa, L-chain in *P. ricini*, 45-kDa, L-chain in *A. assama*, 20-kDa. In *A. mylitta* listed second from the top of Table 5 (Datta *et al*., 2001) and listed second from the bottom of Table 5 (Mandal and Kundu, 2008), although the molecular mass is not fixed yet, fibroin seemed to be consisted of dimeric fibroin molecules, which is different from the composition of fibroin of *B. mori*, which is consisted of H-chain , L-chain and P25, with a 6:6:1 molar ratio (Inoue *et al*., 2000).

Table 4. Genes for fibroin and other silk proteins from wild silkmoths.

Species	Fibroin	Characteristics	Reference
Galleria mellonella	Light chain fibroin	L-fibroin (25 kDa) occurs in two isoforms, shorter one lacking the Ala-Pro dipeptide residue at its N-terminus	Zurovec *et al*. (1995)
Galleria mellonella	P25 gene	29-and 30-kDa proteins from a single gene of P25 with post-trans-lational modifi-cation	Zurovec *et al*. (1998)
Antheraea pernyi	Fibroin gene	characterization of full length gene of fibroin, 80 tandemly arranged polyalanine-units	Sezutsu and Yukuhiro (2000)
Dendrolimus spectabilis *Papilio xuthus* *Antheraea yamamai*	L-fibroin P25	homologues of L-fibroin and P25 of *B. mori* in *D.spectabilis, P. xuthus* but not in *A. yamamai*	Tanaka and Mizuno (2001)
Galleria mellonella	H-fibroin	500 kDa H-fibroin, over 95% of the protein consists of highly ordered repetitive structures, unmatched in other species	Zurovec and Sehnal (2002)
Spiders *Bombyx mori* *Antheraea pernyi* *Chronomus tentans*	*MA* and *Flag* silk proteins, H-fibroin, sericin protein, Balbiani Ring gene proteins	comparative architecture of silk, fibrous proteins and their encoding genes	Craig and Riekel (2002)
Lepidopteran insects	H-fibroin, L-fibroin, P25, sericin, seroin, protease inhibitor	overview of the silk of Lepidoptera	Fedic *et al*. (2002)
Galleria mellonella *Ephestia kuehniella* *Plodia interpunctella*	H-fibroin gene	correlation between fibroin amino acid sequence and physical silk properties	Fedic *et al*. (2003)
Bombyx mori *Bombyx mandarina*	H-fibroin intron gene	functional significance on the regulation of transcription	Martinez *et al*. (2004)
Gelleria melonella	H-fibroin, L-fibroin, P25	construction of silk fiber core	Sehnal and Zurovec (2004)
Yponomenta evonymella	H-fibroin	design of silk fiber composition conserved for more than 150 million years	Yonemura and Sehnal (2006)
Caddisfly	H-fibroin, L-fibroin	H-fibroin (>500 kDa), L-fibroin (25 kDa), no P25	Yonemura *et al*. (2006)
Vespa simillima xanthoptera Cameron	Four major hornet silk genes	complex of alanine-rich and serine-rich sequences	Sezutsu *et al*. (2007)
Various insect species	Fibroin and sericin genes, proteins	silks produced by insect labial glands	Sehnal and Sutherland (2008)
Rhodinia fugax	Fibroin gene	Leucine-rich fibroin	Sezutsu *et al.* (2008)
Bombyx mori Antheraea yamamai	H-fibroin gene pro-moter	fibroin gene promoter conserved	Sezutsu *et al*. (2009)

Table 4. Genes for fibroin and other silk proteins from wild silkmoths (Cont.).

Species	Fibroin	Characteristics	Reference
Corcyra cephalonica	L-fibroin gene, P25 gene	20E regulates expression of L-fibroin gene and P25gene	Chaitanya and Dutta-Gupta (2010)
Hepialus californicus	L-fibroin, H-fibroin	L-fibroin, a pro-missing molecular marker for the study of evolutionary processes	Collin *et al*. (2010)
Antheraea mylitta	Seroin 1 gene Seroin 2 gene	transcript analysis of whole bodies and silk glands	Maity *et al*. (2010)
Caddisfly	H-fibroin	H-fibroin serines phos-phorylated	Stewart and Wang (2010)
Stenopsyche marmorata	H-fibroin	characterization of unique H-fibroin	Wang *et al*. (2010)
Cricula trifenestrata	Fibroin gene	characterization of partial coding region	Suriana *et al*. (2011)
Gonometa postica *Gonometa rufobrunnea*	Fibroin protein	chemical, structural and thermal proper-ties of fibroin	Mhuka *et al*. (2013)
Samia cynthia ricini	Fibroin gene	complete nucleotide sequence, a large region of repetitive arrays, common poly-alanine block and variable nonpoly-alanine block, fibroin dimers	Sezutsu *et al*., (2014)

Table 5. Fibroin from wild silkmoths.

Species	Fibroin	Preparation	Potencies	Reference
Antheraea pernyi	Fibroin from cocoons	4 N HCl for 48 h	Preparation of fibroin powder, soluble in water, with characteristic amino acid composition	Lu *et al*. (1996)
Antheraea mylitta	Fibroin from larval silk glands	1% lithium LDS	Homodimeric protein of two similar sized polypeptides of 197 kDa	Datta *et al*. (2001)
Cricula trifene-strata			Yellow pigment is mainly present in fibroin core of cocoon thread	Yamada *et al*. (2001)
Philosamia ricini		60% LiSCN in water for 2 h H-fibroin	97-kDa (H-chain) and 45-kDa (L-chain) constituted fribroin protein	Ahmad *et al*. (2004)
Antheraea assama	H-fibroin L-fibroin	60% LiSCN in water for 2 hr	220-kDa (H-chain) and 20-kDa (L-chain)	Ahmad *et al*. (2004)
Antheraea mylitta	fibroin	1% SDS	395 kDa and 197 kDa	Mandal and Kundu (2008)
Antheraea assama	fibroin	*	Silk-based nonwoven fibroin scafflold for application in tissue engineering and regenerative medicine	Kasoju *et al*. (2009)

*, Data are not available.

Sericin isolated from wild silkmoths is shown in Table 6. Differences among the sericins from wild silkmoths are now known in the amino acid sequence and molecular masses. Information about fibroin, sericin and other silk proteins from wild silkmoths indicates that the vast diversity in fibroin, sericin and other silk proteins from wild silkmoths should provide an indispensable resource for the preparation of bioactive peptides and hydrolysates with unique biological functions.

Useful information that is available for the preparation of bioactive peptides and hydrolysates from fibroin and sericin of wild silkmoths is as follows. Gheysens *et al*. (2011) demonstrated that demineralization enables the reeling of the cocoons of wild silkmoth, *Gonometa postica*, which is very useful and significant for obtaining intact yarns from this species as well as for obtaining undamaged biopeptides and hydrolysates after obtaining intact yarns. Rajasekhar *et al*. (2011) discussed a thermostable bacterial protease as a new way for obtaining quality silk production. This is useful for enzymatic production of bioactive peptides and hydrolysates from *B. mori* and from wild silkmoths, and the thermostability of protease is actually important in industrial processing of fibroin and sericin. Prasad *et al*. (2012) studied *Antheraea mylitta* cocoonase for its use in cocoonase cooking. Their idea was to utilize natural protease cocoonase for degumming of silk, and we appreciate it as a good proposal. Cocoonase is synthesized in the galea of the pupa and extruded onto the surface of the galea to form protein crystals of pure cocoonase. When a wild silkmoth adult ecdysed from the pupa in the cocoon, it secretes saliva from the mouth and dissolves the cocoonase crystal to make a cocoonase solution. The adult moth puts the solution onto the inner surface of the cocoon, and the sericin is hydrolyzed but the thin filament of fibroin of the cocoon thread remains. The adult moth pushes its head to the fibroin filaments to make a hole, and the adult moth emerges from this hole. Cocoonase belongs to the serine proteinases and functions at a weak alkaline pH and hydrolyzes sericin only. In this sense, cocoonase is a good degumming enzyme (see cocoonase section in Law (2015)). There is no danger of cocoonase hydrolyzing the fibroin of the silk thread.

Table 6. Sericin from wild silkmoths.

Species	Sericin	Preparation	Results or potencies	Reference
Antheraea assama	from cocoon	*	66 kDa	Ahmad *et al*. (2004)
Philosamia ricini	from cocoon	*	66 kDa	Ahmad *et al*. (2004)
Antheraea mylitta	from cocoon peduncles	8 M urea, 2% β-mercapto-ethanol, 2% SDS, 80ºC for 5 min	200 kDa sericin in the peduncle, different from sericin of *B. mori* in amino acid composition	Dash *et al*. (2006)
Antheraea mylitta	70 kDa sericin	8 M urea,2% β-mercapto-ethanol, 2% SDS, 80ºC for 5 min or 1% NaOH overnight at room temperature followed by anion exchange chromatography	Improved understanding of role of sericins in forming stable fibroin fiber-sericin composite	Dash *et al*. (2007)
Antheraea mylitta	from peduncle	*	200 kDa	Kundu *et al*. (2008)
Antheraea assama	from cocoon	*	66 kDa	Kundu *et al*. (2011)
Philosamia ricini	from cocoon	*	66 kDa	Kundu *et al*. (2011)

*, Data are not available.

11. Conclusion

Modern sericulture deals with products from domesticated silkworm, *B. mori,* as well as from a wide variety of species of wild silkmoths that include typically eri silkworm, *S. cynthia ricini,* for the preparation of a wide variety of fibroin and sericin products that include bioactive peptides and hydrolysates for the promotion of human health. This is undoubtedly one of the most important objectives to be achieved in sericulture. In this sense, modern sericulture should contribute to clothing, food and housing: all the more so because aspects of the preparation of functional foods from silk proteins have been neglected in sericulture for a very long time.

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