Transepithelial transport and cellular mechanisms of food-derived antioxidant peptides

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ABSTRACT

Considering the involvement of oxidative stress in the etiology of many non-communicable diseases, food-derived antioxidant peptides (FDAPs) are strong candidates for nutraceutical development for disease prevention and management. This paper reviews current evidence on the transepithelial transport and cellular mechanisms of antioxidant activities of FDAPs. Several FDAPs have multiple health benefits such as anti-inflammatory and anti-photoaging activities, in addition to antioxidant properties through which they protect cellular components from oxidative damage. Some FDAPs have been shown to permeate the intestinal epithelium, which could facilitate their bioavailability and physiological bioactivities. Molecular mechanisms of FDAPs include suppression of oxidative stress as evidenced by reduction in intracellular reactive oxygen species production, lipid peroxidation and apoptotic protein activation as well as increase in antioxidant defense mechanisms (enzymatic and non-enzymatic). Since many FDAPs have demonstrated promising antioxidant activity, future investigation should focus on further elucidation of molecular mechanisms and human studies to explore their practical application for the prevention and management of oxidative stress-related diseases.

1. Oxidative stress in health and disease, and the role of antioxidants

The human cells are constantly exposed to external sources of reactive oxygen species (ROS), such as air pollution, infective agents, UV radiation, and harmful chemicals including drug and alcohol overuse [1]. In addition, certain endogenous reactions (cytochrome P450 enzyme system, monoamine oxidase, xanthine oxidase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalyzed reaction) and mitochondrial oxidative phosphorylation produce ROS [2]. Furthermore, cells of the immune system also generate ROS in response to invasion by foreign bodies; however, hyperactive immune response produces excess ROS that may damage the host cells. ROS also include some free radicals with unpaired electrons, which render them unstable and reactive [3]. Hence, ROS can attack cellular components, such as lipids, proteins and nucleic acids, and abstract their electrons, thus initiating chain reactions. When the level of ROS exceeds the body's antioxidant defense capacity, it creates a systemic redox imbalance in favor of pro-oxidants, a condition known as oxidative stress [4]. Oxidative stress plays a key role in the initiation and progression of many diseases. For instance, oxidative stress has been linked to sickle cell anemia [5], skin diseases [6], malaria [7, 8], and gastrointestinal tract [9] and cardiovascular diseases [10]. In cancers, oxidation promotes mutation that induces resistance to apoptosis and initiation of tumorigenesis [11, 12, 13]. Similarly, hyperglycemia is associated with the glycation of cellular proteins to form advanced glycation end-products (AGEs), which promote oxidative stress and associated complications in type 2 diabetes [14, 15].

As a survival mechanism, living organisms, including plants and animals, have developed antioxidant systems to counteract the effects of ROS and free radicals, thus preventing cellular oxidative damages. The endogenous antioxidant system, including antioxidant enzymes (glutathione peroxidase [GPx], catalase [CAT], and superoxide dismutase [SOD]) and non-enzymatic antioxidants (e.g., reduced glutathione [GSH]), plays a major role in scavenging both external and endogenous ROS. However, to avoid systemic damage during oxidative stress, dietary antioxidants such as vitamins A, C and E, and phytochemicals are used to augment the endogenous antioxidants [16]. The role of antioxidants in the prevention and treatment of diseases cannot be overemphasized. Notably, FDAPs have gained the attention of scientists as natural...
alternatives to synthetic antioxidants for health promotion [17, 18, 19, 20]. This review provides a comprehensive discussion on the intestinal transport and molecular mechanisms of FDAPs.

2. Overview of food-derived antioxidant peptides (FDAPs)

Antioxidant peptides are inactive within their parent protein structure but become active upon release via enzymatic, chemical hydrolysis or microbial fermentation. Several factors influence the antioxidant activities of food-derived peptides, including the parent protein source, amino acid composition, sequence, chain length, hydrophobicity, and preparation method such enzymatic and microbial hydrolysis [18, 21]. Different peptides with antioxidant properties have been produced from enzymatic hydrolysis of milk and plant proteins using a variety of proteases from plant, animal and microbial sources [22, 23, 24, 25, 26]. Notably, low molecular weight peptides (containing 2–15 amino acid residues) appear to be more bioaccessible and, hence, may potentiate better practical health-promoting effects in vivo [27, 28, 29, 30]. Most peptides with strong antioxidant properties possess acidic, hydrophobic and aromatic amino acid residues. Cysteine-rich peptides, and peptides with C-terminal tryptophan, proline and tyrosine, and N-terminal isoleucine, valine, alanine, phenylalanine and lysine are also generally known to exhibit antioxidant properties [31, 32, 33, 34]. Other antioxidant peptides have been reported to be resistant to mild changes in temperature, pH, and intestinal proteases [35, 36]. Several peptides from plant, animal and marine sources have been shown to exhibit potent antioxidant activities (Table 1). Chi et al. [37] isolated low molecular weight peptides (WEGKP, GPP and GVPLT) from bluefin leatherjacket head hydrolyzed with papain, and reported that the peptides demonstrated DPPH, hydroxyl (•OH) and superoxide (•O2) scavenging activities, in addition to abrogation of ferrous-induced lino-leic acid peroxidation. Similarly, a proline-rich oligopeptide isolated from Lactococcus lactis SL6-fermented bovine skim milk demonstrated radical scavenging potential, and the C-terminal proline residue proposed to play an important role in the antioxidant activity [38]. Using •OH and •O2 scavenging assays, peptides (CTN, HC, CGN, and CSGD) isolated from mung bean meal protein hydrolysate were shown to have antioxidant activities that were comparable to the effect of reduced glutathione [39]. Cysteine-containing peptides also show high antioxidant activities [31, 33, 40]. Endogenously, cysteine is a precursor of glutathione (a known antioxidant) and its thiol (-SH) group of cysteine also donates electron to quench free radicals. Peptides with C-terminal and N-terminal cysteine residues have also been reported to exhibit strong antioxidant properties [41, 42]. However, it not clear if these positions are major structural requirements for bioactivity. Tyrosine-containing peptides exhibit anti-oxidant activity by trapping free radicals and ROS in the phenolic group of tyrosine [43, 44]. Recently, antioxidant peptides derived from the enzymatic hydrolysis of proteins from Porphyra haitanensis (DRST) [45], blue mussel (PIPYWK, TTANIEDRR, and FSVVPSPK) [46] and Arthrop- sira platensis (VTAGLVGGAGK and PTGNPLSP) [47] demonstrated potent inhibitory effects against oxidative stress in cultured HepG2 hepatic cells. Also, fermented meat-derived antioxidant peptides were recently shown to protect DNA from oxidative damage in copper sulfate-phenanthroline-ascorbic acid-hydrogen peroxide-DNA chemiluminescence system [48], thus supporting the potential for further development of the FDAPs in the prevention and treatment of oxidative stress-related diseases.

Peptides are susceptible to structural changes during thermal and gastrointestinal processing, which may influence their antioxidant activities. Zhao et al. [49] reported that purple wheat bran-derived tripeptides with terminal cysteine residues (QAC and SCC) were functionally more thermostable, based on superoxide radical scavenging, than others (CGFPGHCH, RNFF and WF). This was unexpected as cysteine residue is susceptible to oxidation during thermal treatment. Previously, the other peptides (CGFPGHCH, RNFF and WF) were found to be more susceptible to degradation by pepsin, trypsin, or chymotrypsin, thereby reducing antioxidant activities [50]. Nonetheless, assessment of the structural stability of the peptides is needed to validate the proposed effect of these treatments. The presence of C-terminal proline residue is thought to increase the gastrointestinal stability of peptides, although the peptide chain length and absence of protease recognition and cleavage sites (scissile bonds) may be more important [51]. Generally, low molecular weight peptides (<1 kDa) rich in hydrophobic amino acids have been recorded to have potent antioxidant properties and are more bio-stable and bioavailable upon oral consumption [52, 53]. However, a comprehensive knowledge of the structural requirements to achieve combined biostability, bioavailability and bioactivity of FDAPs should be developed to facilitate their application as bioactive factors in nutraceuticals and functional foods.

3. Intestinal transport and potential bioavailability of FDAPs

To exhibit beneficial physiological effects, FDAPs must pass through the intestinal cell in their structurally intact form, resisting hydrolysis by gastrointestinal tract proteases and brush border peptidases. It is important to note that no mechanism of transport is specific for FDAPs; hence, FDAPs are transported using the same mechanisms as other bioactive peptides. Studies from our group and others [52, 54, 55, 56, 57, 58] have extensively discussed the current knowledge on the biostability, bioaccessibility and bioavailability of antioxidant peptides. In this section, we provide an overview of unique characteristics of peptides that favour their transepithelial transport, giving examples using FDAPs. Transepithelial transport and routes of transport of peptides vary based on physicochemical properties, including net charge, hydrophobicity, chain length and sequence of the peptide [59]. Interestingly, the in vivo bio-activities of some peptides may be directly associated with their frag- ments generated by the action of proteases and peptides during gastrointestinal digestion and intestinal transport. For more details on the effects of blood proteases and peptides on peptide bioavailability, see the review by Segura-Campos et al. [60]. For example, the anti-oxidative peptide VLPVPQK from casein hydrolysates was reported to be hydrolyzed to VLPVQ by Caco-2 cell peptides, and both the native peptide and its fragment were transported across the cells by peptide transporter (PepT1) and sodium-coupled oligopeptide transporter (SOTPT2) systems, and not by intracellular transcytosis [61]. In another transepithelial transport study in Caco-2 cells, some casein peptides were detected intact post-transport with half-lives of 14.26, 20.07 and 13.17 min, respectively for MPFPK, KEMPFPK, and KNQDKTEIPT [52]. Based on in vitro bioactivity, the transported peptides are strong candidates for further evaluation of antioxidant and LDL oxidation-inhibitory properties at the tissue levels in vivo.

Furthermore, using a Caco-2 cell monolayer co-culture with mucus-secreting goblet (HT29) cells, Xu et al. [62] proposed the mechanism of transport of antioxidant tripeptides (LKP and IQW) from egg white ovotransferrin to be mediated via PepT1 due to partial inhibition of transepithelial transport observed in the presence of a PepT1 competi- tive inhibitor. In addition to active transport via PepT1, the authors proposed a passive transport of the egg white peptides via the tight junctions. This finding is interesting because it highlights the possibility that the tripeptides permeated the mucus layer secreted by goblet cells prior to transepithelial transport. This important factor is lacking or underemphasized in most studies and may influence the bioaccessibility and bioavailability of FDAPs [51]. Generally, intestinal transport and the route of transport of FDAPs, especially via transcytosis, have been shown to depend on molecular weight, net charge and hydrophobicity of peptides [63], with small-sized, positively charged and hydrophobic peptides being generally more permeable than others. Nonetheless, there is currently no consensus on the structural requirements of FDAPs or peptides in general for transepithelial transport. This underscores the need for comprehensive structure-transport relationship studies using known bioavailable peptides and physiologically relevant intestinal models.
Table 1. Food-derived antioxidant peptides (FDAPs) from recent in vitro studies.

| Peptide sequence | Source | Antioxidant assay | Reference |
|------------------|--------|-------------------|-----------|
| **FDAPs from plant sources** |        |                   |           |
| CG, HC, SRSA, CTN, and CSGD; LLLGI, AIVL and HADAD | Mung bean meal | DPPH, OH and O₂ radical scavenging, metal ion chelating activities, and ferric reducing antioxidant power | [39, 109] |
| TSSSLNMAVRGLTR and STTVGLGSMRSAVR | Finger millet | ABTS, DPPH and OH radical scavenging and metal ion chelating activities | [110] |
| CGFGHC, QAC, RNF, SSC, and WF | Purple wheat bran | O₂ radical scavenging and Trolox equivalent antioxidant capacity | [49] |
| GNPDEHPET, TNDRPSG, SVIKPPTDE, VIKKPTDE, GNPDEHPET, LVPQQOQ, ETIEKNQPQ, TVLNNODNDS, NSQHPEL, FEEPQQOQ | Soybean flakes | ABTS, DPPH scavenging activities, ferric reducing antioxidant power, metal ion chelating capacity, and inhibition of intracellular ROS generation | [111] |
| NL, Qi, FL, HAL, AAVL, AKTVF, and TPLTR | Wheat bran protein | Oxygen radical absorbance capacity | [24] |
| PGPYN, PFPFGPN, YVFPGPN, VYPFPGPN, VYPFPGPN, VPPPYPVPEP, EPVLGPVRGPPF, QEPVLGPVRGPPF, TPVVPPFLQPE and TQVPPVVPLQPE | β-casein | DPPH and O₂ radical scavenging capacity | [34] |
| TGFDGER, ADVNYPQ, CAGVSAIR and LTVBBQGR | Palm kernel expeller glutenin-1 hydrolysates | OH radical scavenging activity | [114] |
| PQFYW and RPEIV | Coconut cake | OH radical scavenging activity | [115] |
| SDDRLGPNNQYLPK | Pearl millet | DPPH, ABTS radical, OH radical scavenging, metal ion-chelating ability and reducing power | [116] |
| GY, PFE, YTR, FG, QY, IN, SF, SP, YFE, IY and LY | Moringa oleifera seeds | Scavenged ROO and ABTS radicals, chelates metal ions, and inhibited oxidative stress in erythrocytes | [112, 113] |
| TVFDSRGL, ADZTVNPQ, NVGLQVQ and NVTVBQGR | Chickpea sprout | DPPH radicals scavenging, FRAP and inhibition of lipid peroxidation | [118] |
| **FDAPs from terrestrial animal sources** |        |                   |           |
| YASGR | Chicken dark meat | Peroxyl radical scavenging activity | [119] |
| HYTHEVXNH and WPVLAYHF | Spotted babylon snail | ABTS and DPPH radicals scavenging activities | [120] |
| LTEQGSVPVKM | Ostrich egg white protein | DPPH, OH and O₂ radicals scavenging, ferric reducing antioxidant power, metal ion-chelating ability and inhibition of linoleic acid autoxidation | [121] |
| DLEE | Dry-cured Xuanwei ham | DPPH, OH and O₂ radicals scavenging activities | [122] |
| MVPYQR | Camel milk | ABTS radicals scavenging activity | [123] |
| AGPSIVHV, FLLPH and LLCVAV | Duck breast | DPPH, and ABTS radicals scavenging and FRAP | [124] |
| QSLVQPVMS | Hen egg-yolk | Strongly inhibited DPPH radical, moderately chelated metal ions, and strongly inhibited ACE activities | [125] |
| ITTNPYYD, IGWSPVSL, ITTNPYYDHY, and LRVAEEHPITL | Myofibrillar muscle of chicken breast | DPPH radicals scavenging, FRAP and inhibition of lipid peroxidation | [126] |
| **FDAPs from marine sources** |        |                   |           |
| GPGPAVP, GPVPA, PP and GF | Salmon gelatin | Oxygen radical absorbance capacity | [127] |
| YASV, NFWWP, FWKVV, TWKVV and IRWWW | Miniy croaker muscle | DPPH radical scavenging activities | [128] |
| ATSH | Sandfish | DPPH radical scavenging capacity | [129] |
| TVLAGGVGGAGK and PGTGNLSP | Anthroplura Platania | ABTS, and OH radical scavenging and metal ion-chelating activities | [47] |
| DKST | Porphyra haitanensis | DPPH radical scavenging capacity and inhibition of oxidative damage to HepG2 cells | [45] |
| PTVYVVWK, TTANIEDRR, and FSVVSPK | Blue mussel | DPPH radical scavenging and ORAC activities, and protected cultured cells from H₂O₂-induced hepatic damage | [46] |
| GAGLPGKRER | Pinctada fucata muscle | DPPH, OH and O₂ radicals scavenging | [130] |
| FIMGY, GPAGDY and IVAGPQ | Skate cartilage | DPPH, ABTS and OH radical scavenging, and inhibition of peroxidation of linoleic acid | [131] |
| FKGPACA and SVLGGTGC | Silkworm pupae | ABTS radical scavenging activity | [132] |
| AGD | Hippocampus abdominalis | OH and O₂ radicals scavenging activity in vitro and inhibition of cellular oxidative stress in vivo | [133] |
| ATY | Black sharkskin | ABTS radical scavenging activity | [134] |
| HGPOGE, AGPKGH and MLGYPGPS | Skipjack Tuna scales | DPPH, OH and O₂ radicals scavenging scales | [135] |
| KGYR | Round scad | DPPH, and OH radicals scavenging and FRAP | [136] |
| APLEPSSPH, IREADIDGDQVQN, PEILPDGDHD and ASDEQDSRVL | Crucian carp | DPPH radical scavenging and ferrous chelating ability | [137] |
| VENAATTYNEECZEKK and VGGGACTTGGEEGCEKK | Arca subcrenata | DPPH, and ABTS radicals scavenging and FRAP | [138] |
| GPE, GARGPQ and GPTGPPGNG | Scalloped hammerhead cartilage | DPPH, ABTS, OH and O₂ radicals scavenging, and inhibition of peroxidation of linoleic acid | [139] |

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Despite their permeability across the intestinal epithelium, many bioactive peptides are not bioavailable in substantial amounts in vivo. Microbial fermented corn gluten meal containing short-chain oligopeptides were reported to suppress lipid peroxidation and enhance antioxidant enzyme activities in serum and liver tissues [64]. Although this may suggest that the FDAPs were absorbed, detection and quantification of the parent peptides in the serum and tissues are crucial to validating their biostability and bioavailability. Moreover, endogenously generated fragments of the dietary peptides could have been absorbed and contributed to the antioxidant activities. For instance, soybean-originated 43-residue peptide, lunasin, which showed chemopreventive cellular effects against oxidative stress [27], and its digestion fragment, RKQLQGVN, were reported to permeate Caco-2 cell monolayer by passive transport via paracellular tight junction permeability [28]. Based on the limited literature in this area, short chain hydrophilic peptides are hypothesized to be more resistant to hydrolysis by intestinal brush border proteases and to permeate the intestinal epithelium in their intact form [51, 63, 65]. Some peptides that are not absorbed through the intestine can also offer health benefits by protecting the intestine from oxidative damage; this topic has been extensively discussed in a recent review [66]. Aside their direct radical-scavenging properties, the peptides that are not bioavailable can stimulate the release of mucus to protect the mucosal cells of the intestine [67, 68, 69]. Unabsorbed bioactive peptides may also influence the gut microbiota population and metabolism in a way to generate a positive oxidative state. Future studies should consider establishing the role of gut microbiota on the antioxidant properties of unabsorbed FDAPs, considering that gut microbiota have been proposed to mediate other health-promoting effects of bioactive peptides [70, 71, 72].

4. Cellular mechanisms of action of FDAPs

FDAPs have been shown to elicit cellular antioxidant activities via specific proposed mechanisms, including upregulation of gene expression and activation of cytoprotective and antioxidant enzymes, and inhibition of intracellular ROS production and lipid peroxidation. These physiological events essentially follow the modulation of Keap1/Nrf2/ARE, PI3K/Akt/mTOR, NF-κB/MMPs and DAF-16 signaling pathways depending on the cells involved.

4.1. Activation of cytoprotective and antioxidant enzymes

Food protein hydrolysates and peptides have exhibited antioxidant activities in vitro and in vivo that are relevant in mitigating aberrant physiological processes and health conditions [73, 74]. For example, the aging process is linked with increased oxidative stress that damage important cellular structures [75, 76, 77]. In addition, premature aging has been associated with elevated generation of AGEs leading to increase in oxidative stress [78]. Experimentally, aging has been induced using D-galactose and muscarinic receptor blocker overload and exposure to UV radiation, leading to the formation of AGEs, inflammation and oxidative stress, which promote premature aging and memory loss [79, 80, 81]. In UV-radiation-generated mouse skin aging, Chen et al. [82] and Xu et al. [83] demonstrated that salmon skin gelatin and walnut protein hydrolysates dose-dependently abrogated UV-radiation-generated skin photoaging in rats and mice. The hydrolysates acted by inhibiting matrix metalloproteinase-1 (MMP-1) activity and lipid peroxidation and elevating the antioxidant status and immune function. Two peptides, WSREEQERE and ADHYTEEAGR, were found to retain the anti-photoaging properties of the walnut protein hydrolysates and they acted by downregulating gene expression of inflammatory cytokines (interleukins (IL)-1β and IL-6) and inhibiting MMP-1 activity and the phosphorylation of 12x and p-65 proteins, which are activators of the NF-κB/MMPs signaling pathway [83]. Similarly, pine nut-derived peptides (MQIFPK, MASVPTK, EMVELPLR and VVLIGDSGVGK) reduced D-galactose-induced premature aging in mice by suppressing lipid peroxidation and enhancing the activities of SOD and GPx in mouse serum, and heart and liver homogenates [84]. The ability to boost the activities of antioxidant enzymes (CAT, GSH, SOD and GPx) (Figure 1), inhibit lipid peroxidation (malondialdehyde [MDA]) and protein oxidation (protein carbonyl), and downregulate gene expression of MMPs in mice have been reported as the antioxidant mechanisms of other FDAPs in preventing UV radiation-induced skin photoaging [85, 86, 87]. These biological properties show that FDAPs have future application in the development of cosmeceuticals and nutraceuticals for the prevention and treatment of aging-related skin diseases.

Furthermore, FDAPs have been reported to modulate oxidant-induced elevation of the endogenous antioxidant system. For example, lunasin or its fragments showed chemopreventive effect by reducing the elevated activities of GPx and CAT, and protein carbonyl formation induced by tert-butylhydroperoxide [27]. Conversely, a dipeptide, FC, identified in the large subunit of plant and microalgae ribulose-1,5-bisphosphate carboxylase was reported to restore (at 5 μM peptide) and augment (at 10 μM peptide) the mRNA expression of SOD-1 and CAT in hydrogen peroxide-induced oxidative stress in cultured liver (HepG2) cells [88]. Interestingly, GSH levels were augmented in normal cells by the FDAPs treatments [27, 88]. These findings suggest a possible protective role of the FDAPs in normal cells or the presence of a condition/response that enhance endogenous GSH synthesis, e.g. the availability of cysteine residue in FC, which could serve as GSH precursor. As oxidants are expected to deplete endogenous antioxidants like GSH and activate the antioxidant enzymes, it is possible that the type and amount of oxidants and cells used, and duration of oxidant exposure affected the cellular responses and effect of the FDAPs. This highlights the need to establish physiologically relevant models with close simulation of the complex cellular environments under oxidative stress for specific disease and health conditions.

4.2. Inhibition of intracellular ROS production

Endotoxin or lipopolysaccharide (LPS) induces inflammation and oxidative stress by enhancing the activation of nuclear factor-kappa B (NF-κB), which translocates to the nucleus to activate INOS gene and protein expression. Activation of NF-κB signaling pathway also leads to redox imbalance in favor of oxidative stress because it causes mitochondrial dysfunction leading to the alteration of cellular energy metabolism and increase in ROS production [89]. These processes are implicated in the pathogenesis of many diseases. FDAPs have shown the ability to inhibit
intracellular production of ROS. For example, soybean-derived antioxidant peptide fractions were reported to suppress intracellular ROS production, and reduced IL-1β, IL-6 and prostaglandins E2 levels in LPS-activated macrophage (RAW 264.7) cells via blocking of the NF-κB and iNOS pathways [90]. In tert-butyl hydroperoxide (TBH)- and LPS-induced injury and microglial activation in HMC3 microglial cells, Salvia hispanica protein hydrolysates produced with pepsin-pancreatin protected HMC3 microglial cells from oxidative damage by suppressing pro-inflammatory cytokines (TNF-α and IL-6) and intracellular ROS production [91] (Figure 1). Despite the promising bioactivity, there is no information on the specific cellular target that mediates the inhibition of ROS production by FDAPs. It is also not clear if these FDAPs acted by binding extracellular receptors to trigger the intracellular effects or after cellular uptake to interact directly with components of the ROS production pathways. Furthermore, the direct reducing capacity of the sulphydryl group of dipeptide, FC, was proposed to play a role in its inhibitory effect against intracellular ROS [88]. Therefore, two mechanisms may be at play for the overall effect of FDAPs on ROS production, viz. direct antioxidant effect and regulation of intracellular antioxidant pathways. Some studies have reported physiological evidence to support the effects on ROS production. In mice treated with D-galactose, two FDAPs (FYY and DW) from fish protein hydrolysates suppressed lipid peroxidation in the brain, in addition to beneficial gene regulatory effects [92]. While these bioactivities make the FDAPs strong candidates for use as nutraceuticals, much is unknown about their intracellular targets, mechanisms of function and biological fate in tissues post-activity.

4.3. Activation of antioxidant system via oxidative signaling pathways

On oxidative stress, cells activate their antioxidant protection system, such as the activation of Keap1/Nrf2/ARE signaling pathway, leading to the upregulation of gene expression of antioxidant enzymes (NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), glutamate-cysteine ligase (GCL) and glutathione-S-transferases (GSTs)) to protect the cells from oxidative damage and apoptosis [93]. Similar to oxidant-inducible mammalian Nrf2 transcription factors, DAF-16 and SKN-1 are two transcription regulators in Caenorhabditis elegans that mediate the expression of target genes, such as superoxide dismutase-3 (sod-3), heat shock proteins (hsp-6 and hsp-16.2) for DAF-16 and GSTs (gst-4 and gst-10) for SKN-1 [94, 95, 96]. The downstream effects of these proteins result in detoxification of oxidant species and cellular protection against oxidative damage. Several compounds target the oxidative signaling pathways as a mechanism for their antioxidant effects. Recently, antioxidant peptides (DVEDLEAGLAK and EITSLAPSTM) isolated from golden cuttlefish hydrolysates were reported to exhibit their cytoprotective effects against oxidant-induced cellular injury by inhibiting ROS formation and lipid peroxidation in high-fat C. elegans via upregulation of mRNA expression of sod-3, cat-1 and ctl-1 genes [54]. This result suggests that the peptides attenuated oxidative stress by activating the DAF-16 signaling pathway. Similarly, Zhao et al. [97] reported that two peptides AAVPSGASTGIYEALELR and NPLLEAFGNAK derived from purple sea urchin gonad hydrolysates alleviated paraquat-induced oxidative stress in C. elegans via the DAF-16 signaling pathway, by upregulating gene expression of sod-3 and hsp-16.2.

Furthermore, two hexapeptides (EAMAPK and AVPPYQ) derived from β-casein were shown to exhibit cellular antioxidant properties in H2O2-treated intestinal epithelial cells (IEC-6) via activation of Nrf2/ARE signaling pathway, leading to the upregulation of sod-3 mRNA expression [98]. When activated in response to oxidative challenge, the transcription factor Nrf2 binds to antioxidant response element (ARE) to upregulate the expression of sod-3 and other antioxidant enzymes.

Table 2. Recent studies on the cellular antioxidant activities and mechanisms of antioxidant protein hydrolysates.

| Peptide/protein hydrolysate | Oxidative stress model | Cellular mechanism of action | Reference |
|----------------------------|------------------------|-----------------------------|-----------|
| Salmon skin gelatin hydrolysates | UV-radiation induced aging in mouse skin | Inhibited matrix metalloproteinase-1 (MMP-1) activity, improved antioxidant status (SOD, CAT and GPx activities) and reduced lipid peroxidation (malondialdehyde level) and activated collagen synthesis | [82] |
| Walnut protein hydrolysates | UV-radiation induced aging in mouse skin | Inhibited MMP-1 activity, increased SOD, CAT and GPx activities and reduced malondialdehyde level and activated collagen synthesis (elevated procollagen type 1 level) | [83] |
| GAGLPKGRER from Pinctada fucata protein hydrolysates | UV-radiation-induced mouse skin aging | Inhibited CAT, glutathione system and SOD activity, halted lipid peroxidation and suppressed UV-radiation-induced skin aging | [86, 130] |
| Silver carp skin hydrolysates | UV-radiation-induced mouse skin aging | Boosted antioxidant status, prevented protein oxidation and downregulating the gene expression of MMPs and halted lipid peroxidation | [142] |
| Whey protein hydrolysates | Normal Juvenile Arctic char | Improvement in growth performance such as weight gain and feed consumption but a reduction in lipid peroxidation | [143] |
| Lunasin hydrolysates from soybean | Oxidative stress-induced vascular endothelial cell (VEC) injury and in Apol deficient mice as well as azoxymethane and dextran sodium sulphate | Inhibited mitochondrial-dependent oxidative damage and endothelial apoptosis induced by H2O2 via suppression of Bax gene expression, release of cytochrome c and activities of caspases-3 and 9 while up-regulating Bcl-2 and HO-1 gene expression via PI3K/Akt/Nrf2/ARE pathway, and attenuated endothelial oxidative damage and atherogenesis in high fat | [101, 144] |

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Table 2 (continued)

| Peptide/protein hydrolysate | Oxidative stress model | Cellular mechanism of action | Reference |
|-----------------------------|------------------------|----------------------------|-----------|
| Soybean protein hydrolysates | H₂O₂-induced oxidative injury in mice | Fed ApoE-deficient mice. Inhibited chemically-induced oxidative damage on the hepatocytes of the mice and prevented histological changes | [145] |
| Potato protein hydrolysates | H₂O₂-induced oxidative stress in C2C12 cells | Protected the cells from oxidative damage and increased the viability of the cells, by improving the antioxidant status | [146] |
| Casein and caseinophosphopeptide hydrolysates | AAPH and Fe²⁺-induced cytotoxicity in human Caco-2, HT-29 and Saos-2 cells | Protected Caco-2/HT-29 cells in co-culture from AAPH and Fe²⁺-induced cytotoxicity via metal chelating and antioxidant mechanisms and exhibited antioxidant effects by protecting human osteoblast (Saos-2) cells from AAPH-generated cytotoxicity | [22] |
| Coix seed protein hydrolysates | Alcohol-induced hepatic injury in ICR male mice | Increased alcohol metabolism by activating alcohol metabolic pathway and increased the activities of liver marker enzymes (AST and ALT) and SOD, attenuated inflammatory processes by suppressing serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and abrogated lipid peroxidation as evidenced by reduction in MDA level. | [147] |
| Soybean protein hydrolysates | H₂O₂-induced oxidative stress in HepG2 cells | Salvaged cellular oxidative damage, inhibited ROS production, and reduced MDA level and oxidation of GSH, improved cellular ROS detoxification system by upregulating gene expression and protein level of Nrf2-ARE signal-responsive enzymes (SOD, CAT and GPx) and inhibited lipid peroxidation by reducing MDA level | [148] |
| Duck embryo eggs protein hydrolysates | H₂O₂-induced oxidative injury in HepG2 | Protected the cells from oxidative assaults, prevented ROS production and lipid peroxidation and elevated antioxidant enzymes such as SOD, CAT and GPx | [149] |
| Blue mussel protein hydrolysates | H₂O₂-induced damage on Human umbilical vein endothelial cells (HUVECs) | Improved the viability of the cells and suppressed ROS release and lipid peroxidation, and elevated intracellular antioxidant status by increasing GSH, SOD, CAT and GPx levels in stressed cells via inhibition of caspase-3 activation and suppressed mRNA expression of apoptotic p63 and caspase-3 genes and reduced Bax/Bcl-2 ratio, suggesting the involvement of Nrf2-ARE and p53-Caspase-3 signaling pathways | [150] |
| Seahorse protein hydrolysates | H₂O₂-induced damage on Human umbilical vein endothelial cells (HUVECs) | Improved the viability of the cells and suppressed ROS release and lipid peroxidation, and elevated intracellular antioxidant status by increasing GSH, SOD, CAT and GPx levels in stressed cells via inhibition of caspase-3 activation and suppressed mRNA expression of apoptotic p63 and caspase-3 genes and reduced Bax/Bcl-2 ratio, suggesting the involvement of both Nrf2-ARE and p53-Caspase-3 signaling pathways | [151] |
| Krill protein hydrolysates | Alcohol-induced hepatic injury in mice | Increased body weight gain and survival rate, and antioxidant gene expression (SOD, CAT and GPx in hepatic tissues), and expression of transcription factors that promote antioxidant defense (Nrf2 and HO-1) while suppressing serum AST and ALT, and levels of hepatic cholesterol and gene expression of apoptotic proteins (caspase-3 and p53). The hydrolysates also attenuated hepatic tissue injury as evidenced by histological examination of alcohol injured mice liver treated with hydrolysates. | [152] |
| Whey protein hydrolysates | H₂O₂-induced oxidative stress in PC12 cells | Protected the cells from oxidative damage and increased the viability of the cells, by improving the antioxidant status | [146] |
| Nile tilapia skin gelatin hydrolysates | H₂O₂-induced oxidative injury in IPEC-J2 cells | Improved cellular viability and strengthened the cell membrane of IPEC-J2 cells but suppressed intracellular ROS formation, induced Nrf2 translocation from cytosol to nucleus and increased the expression and protein level of γ-glutamylcysteine ligase, and glutathione. Silencing of Pept1, Nrf2 or p62 diminished the cellular protective roles against oxidative damages, indicating that the mechanism of action is via activation of glutathione production and Pept1-p62-Nrf2 signaling pathway. | [154] |
Table 3. Recent studies on the cellular mechanisms of action of identified FDAPs.

| Peptide/protein hydrolysate | Oxidative stress model | Cellular mechanism of action | Ref |
|-----------------------------|------------------------|----------------------------|-----|
| WSREEQERE and ADYTEEAGRR from walnut protein | UV-radiation-induced mouse skin aging | Suppressed NF-κB signaling pathway by preventing the activation ofIkB and p65 proteins, downregulated gene expression of interleukins (IL-1) and IL-6, inhibited MMP-1 activity and enhanced the expression of TGF-β and procollagen type I. | [155] |
| GAGLPGKRER from Pinctada fucata protein hydrolysates | UV-radiation-induced mouse skin aging | Inhibited CAT, glutathione system and SOD activities, halted lipid peroxidation and suppressed UV-radiation-induced skin aging | [86, 130] |
| PELDW, WPDDHW, FGYDWW, and YLHFW isolated from Spanish Mackarel muscle | H2O2-generated plasmid DNA damage | Inhibited DNA damage by boosting the antioxidant status and preventing uncoiling and strand break | [156] |
| MQIFVK, MASVPTK, EMVELPLR and VVLGDGSGVKG derived from pine nut | H2O2-challenged HepG2 cells and D-galactose-induced premature aging in mouse | Improved the viability of HepG2 cells, and suppressed lipid peroxidation and enhanced antioxidant status by increasing SOD and GPs activity in mouse | [84] |
| LEPVIGT derived from porcine plasma | H2O2-induced oxidative stress in HepG2 cells | Protected HepG2 cells via Keap1-Nrf2-ARE signaling pathway | [103] |
| FYY and DW derived from lantern fish protein | D-galactose-induced aging | Suppressed lipid peroxidation level and expression of endothelial nitric oxide synthase, and improved memory impairment by elevating brain-derived neurotrophic factor | [92] |
| DVEDLEAGL and EITSLAPSTM from golden cuttlefish | High fat Caenorhabditis elegans | Prevented oxidative damage via upregulation of mRNA expression of DAF-16 signaling pathway-regulated genes (sod-3, catalase (cat-1) and clot-1) | [54] |
| AYI, AYL, DREI and DREL from Jiuzao | 2,2′-Azobis(2-methylpropionamididine)-stressed HepG2 cells | Inhibited ROS generation, elevated SOD, CAT and GPs gene expression and improved the viability of HepG2 cells via Keap1-Nrf2-ARE signaling pathway | [102] |
| QDHGH from pine nut | Pulsed electric field-stressed and H2O2-induced oxidative stress in HepG2 cells | Improved antioxidant status (SOD, CAT, GPs and GSH-Rx), stabilized mitochondrial membrane potential and reduced lactate dehydrogenase (LDH) and malonaldehyde (MDA) levels as well inhibition of apoptosis by downregulating the gene expression of caspase-3 | [84, 157] |
| VNP and YGD from Jiupel | 1,1-Diphenyl-2-picrylhydrazyl, and 2,2′-azobis (2- methylpropionamididine)-dihydrochloride-activated HepG2 cells | Improved intracellular enzymatic and nonenzymatic antioxidant defense system | [158] |
| FC from RutilisCO large subunit | H2O2-induced oxidative stress in Chang human hepatocytes | Prevented intracellular ROS generation and lipid peroxidation and increased intracellular GSH, CAT and SOD levels | [88] |
| ILGATIDNSK from defatted round scad | Heat shock and parquat-generated oxidative stress and aging in C. elegans | Increased lifespan and activities of SOD and CAT, and reduced intracellular ROS level | [159] |
| Stable and resistant peptides (VENAACTTNEECCEKK and VEGGAACGGGEEGGCEKK) from Arca subcrenata | Paraquat-generated oxidative stress and senescence in C. elegans | Extended the lifespan, improved age-related physiological decline in the nematode, and reduced fat and lipofuscin levels that accumulate in the nematode with age. The peptides also inhibited ROS generation and down-regulated age-1 gene while upregulating oxidative stress-dependent genes (mtl-1, sod-3, clt-1 and hsp16.2) via DAF-16 signaling pathway | [138] |
| GLVYIL, YHNAAGLYYL and DVNNANQLQEPR from oat protein | AAPH-generated oxidative stress in HepG2 cells | Protected cells by increasing cellular viability by antioxidant mechanisms, including inhibition of lipid peroxidation and elevation in SOD, CAT, GPs and GSH levels | [160] |
| SF and QY from Morėngō oleifera seeds | H2O2-induced oxidative stress in Chang human hepatocytes | Protected Chang liver cells by reducing the levels of liver function enzymes such as aspartate aminotransferase (ALT) and alanine aminotransferase (AST) and lipid peroxidation marker, malondialdehyde (MDA) as well as boosting of levels of antioxidant enzymes (SOD and CAT), and preventing apoptosis of the cells. | [117] |
| ARHPHPHLSFM, AVPYPQR, NPYVPR and KVLVPVEK from milk | TBH-induced lipid peroxidation and H2O2-induced oxidative stress in Caco-2 cells | Scavenged ABTS radical and inhibited TBH-induced lipid peroxidation in Caco-2 cells. The peptides induced the translocation of Nrf2 from cytosol to nucleus, and prevented the interaction of Keap1 to Nrf2, allowing Nrf2 to bind to ARE, and activate the expression of genes that code for antioxidant and oxidants-detoxification enzymes (Txl1, TxlR1, GR, NQO1 and SOD1), suggesting that the mechanism of antioxidant effects of the peptides is via Keap1/Nrf2 signaling pathway. | [161, 162] |
| WGN, AW, RGWYE and GVPFW from mung bean | H2O2-induced oxidative stress in HepG2 cells | The peptides protected HepG2 cells from oxidative stress by increasing cellular viability via elevation in antioxidant status (CAT and GSH system) and inhibition of lipid peroxidation (MDA) | [163, 164] |
| ITTNPYDY and IGWSPLGLS from Myofofillar protein of chicken breast | H2O2-induced oxidative stress in NIH-3T3 cells | Increased the viability of NIH-3T3 cells, inhibited intracellular ROS and lipid peroxidation in H2O2-induced oxidative stress in NIH-3T3 cells and improved intracellular glutathione concentration and SOD, CAT, GPs and GSH-Tx activities, and prevented apoptosis via | [126] |

(continued on next page)
Table 3 (continued)

| Peptide/protein hydrolysate | Oxidative stress model | Cellular mechanism of action | Ref |
|----------------------------|------------------------|----------------------------|-----|
| GLLLPH from corn gluten meal | H2O2-induced oxidative stress in HepG2 cells | Enhanced cell viability, inhibited intracellular ROS generation and lipid peroxidation and improved the expression of antioxidant status (SOD, CAT, GSH-Rx, GSH) | [165] |
| EKAP, EDPIPP, EARPPHIPP, and EARPPHPFs | H2O2-induced damage on SH-SY5Y cells | Increased viability of cells, restored altered mitochondrial membrane permeability, suppressed ROS generation, NO production and lipid peroxidation, all of which suggest antioxidant protection against oxidative stress | [166] |
| GPA from fish skin gelatin protein hydrolysate | H2O2-induced oxidative injury in IPEC-J2 cells | Enhanced ARE-luciferase activity to increase the gene expression and protein level of ARE-dependent antioxidant enzymes as well as reducing cellular ROS generation | [154] |
| YPLPSY from Allium tuberosum | H2O2-induced oxidative injury in HEK293T cells | Halted intracellular ROS generation, promoted stressed-cell survival and elevated the gene expression of PI3K, Akt, phospho-Akt, mTOR, phospho-mTOR indicating that the cellular protective mechanism is mediated via PI3K/Akt/mTOR signaling pathway | [167] |
| VRP, LKX, VRY and VVHPKES from Spent hens | Tumor necrosis factor alpha (TNFα) and angiotensin (Ang) II-induced oxidative stress in vascular smooth muscle A7r5 cells (VSMCs) and endothelial EA.hy926 cells (ECs) | Ameliorated oxidative stress by increasing the expression of antioxidant enzymes (GPx4 and SOD2) and suppressing in ROS generation | [168] |

biliverdin/bilirubin [100]. In a follow-up study, Gu et al. [101] showed that lunasin protected EA.hy926 human umbilical vein endothelial cells against H2O2-induced intracellular cellular oxidative injury and mitochondria-associated endothelial apoptosis via suppression of Bax gene expression, release of cytochrome c and activation of apoptotic proteins (caspases-3 and 9). The peptide also up-regulated gene expression of tumor suppressor gene (Bcl-2) and HO-1 via the PI3K/Akt/mTOR/Nrf2/ARE pathway, and reduced oxidant-mediated ROS production in endothelial cells, leading to the amelioration of endothelial oxidative damage and atherogenesis in high fat fed ApoE-deficient mice (Figure 1). Several other FDAPs (AYI, AYL, DREI, DREL, MQIQVK, MASVPTK, EMVLERLR, VVLLQDSVYGK and LEPVIIGT) were recently demonstrated to protect HepG2 cells via the Keap1/Nrf2/ARE signaling pathway, leading to the upregulation of gene expression of antioxidant enzymes and ROS detoxification pathways [84, 102, 103]. Similar mechanisms were demonstrated by a dipeptide (IF) from potato protein hydrolysates in hypertension-generated ROS-induced renal damage in rats [104]. Tables 2 and 3 show other food-derived protein hydrolysates and peptides, respectively, that elicit their cellular antioxidant action via oxidant signaling pathways, and Figure 1 summarizes the cellular mechanisms of FDAPs.

5. Commercialization of FDAPs

Majority of the commercially available food protein and peptide-based products, sold as nutraceuticals or supplements, target mostly hypertension, obesity, and diabetes, with little attention given to oxidative stress. Examples of the products include Nutripeptin®, Fortidium®, Peptibial®, Stabilium®, Protizen®, Seacure®, Vasotensin®, Lapis support/Valtyron®, PeptACE®, and Levenorm® from marine sources; Calpis, Biozate, Lactium®, Evolus, and BiopureGMP from dairy sources; and LunaRich® X and PeptAlde from plant sources (see reviews [105, 106] for more information). Notably, LunaRich® X, a concentrated form of lunasin, is a FDA-approved supplement for lowering cholesterol [105]. Despite the large body of evidence on the antioxidant properties of peptides from vegetal sources, plant-derived antioxidant peptides are the least commercialized. This may be associated with high cost of production, and other general limitations in translating research on bioactive peptides such as lack significant health benefits from clinical studies. Additional limitations could be attributed to poor bioavailability and lack of in-depth knowledge of the pharmacokinetics of bioactive peptides, which are needed for approval prior to commercialization, as discussed in previous reviews [106, 107]. Furthermore, FDAPs have other potential applications, e.g. as cosmeceuticals and food preservatives against oxidative deterioration of food products; see the review by Sohaib et al. [108] for more details.

6. Conclusion and future directions

Due to safety concerns associated with synthetic antioxidants, natural compounds such as FDAPs are increasingly explored as safer and sustainable alternatives. Several studies have shown that food protein-derived peptides from animal, plant, microbial and marine sources have antioxidant properties. Thus, FDAPs have promising application against oxidation-induced cellular damage and the initiation and progression of associated diseases, such as cancer, atherosclerosis, neurodegenerative diseases and diabetes. FDAPs have demonstrated their antioxidant properties via three distinct mechanisms: (1) activation of antioxidant enzymes, (2) inhibition of intracellular ROS production, and (3) activation of antioxidant system via oxidative signaling pathways. Based on cellular studies, some peptides permeate the intestinal epithelium in their intact form, which could facilitate circulation, interaction with their target cells and elicitation of their antioxidant activities. However, future studies are needed to fill some important gaps. First, there is a need for strong physiological evidence on the bioavailability of dietary FDAPs in target tissues, beyond the cellular uptake studies. Second, the specific molecular targets of FDAPs need to be identified for better understanding of their structure-function relationships. Absorption and bioavailability of dietary FDAPs, especially those with multiple antioxidant mechanisms, are crucial in building evidence for future human studies and for application of FDAPs as nutraceuticals for improving human health and wellbeing.

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Author contribution statement

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