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Review on preparation methods, mechanisms and applications for antioxidant peptides in oil

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SUMMARY: Natural antioxidants, especially those used in edible oil, are safer compared to chemically synthesized antioxidants. Therefore, research on natural antioxidants has become prevelant. Antioxidant peptides derived from food protein can effectively prevent oil oxidation. Protein hydrolyzation is widely applied for the production of antioxidant peptides in industry, and bioinformatics is employed nowadays to generate the desired peptide sequence. Furthermore, the mechanism of antioxidant peptides in the oil system is still controversial, which limits the further development of antioxidant peptides as food antioxidants. This review introduces the preparation method of antioxidant peptides and their mechanisms as well as applications in the oil. It will help to comprehensively understand the function of antioxidant peptides and promote their development in the oil field.

KEYWORDS: *Antioxidant peptide; Bioinformatics; Mechanism; Oil oxidation*

RESUMEN: ***Revisión sobre métodos de preparación, mecanismos y aplicaciones de péptidos antioxidantes en aceites.*** Los antioxidantes naturales, especialmente utilizados en aceites comestibles, son más seguros en comparación con los antioxidantes sintetizados químicamente. Por lo tanto, la investigación sobre antioxidantes naturales se convierte en un punto de interés. Los péptidos antioxidantes derivados de las proteínas alimentarias pueden prevenir eficazmente la oxidación del aceite. La hidrolización de proteínas se usa ampliamente en la industria para la producción de péptidos antioxidantes y la bioinformática se emplea hoy en día para generar la secuencia de péptidos deseada. Además, el mecanismo de los péptidos antioxidantes en el sistema oleoso sigue siendo controvertido, lo que limita el desarrollo posterior de péptidos antioxidantes como antioxidantes alimentarios. Esta revisión presenta el método de preparación de péptidos antioxidantes y su mecanismo, así como las aplicaciones en aceite, lo que ayudará a comprender de manera integral la función de los péptidos antioxidantes y promoverá su desarrollo en el campo petrolero.

PALABRAS CLAVE: *Bioinformática; Mecanismo; Oxidación de aceite; Péptido antioxidante*

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1. INTRODUCTION

Oil, which is mainly derived from plants and rich in unsaturated fatty acids (UFA), can provide essential fatty acids for humans. However, the UFA can easily form free radicals at the α-C of the C=C double bond. This will initiate free radical chain reactions and oil oxidation. The oxidation products of oil will produce peculiar smells and carcinogens that can reduce the oil's nutritional value and shorten the shelf-life of the oil (Ahn *et al.*, 2012b). Therefore, it is necessary to control oil oxidation, which is of great significance to improving the level of food safety and develop the oil industry (Kiralan *et al.*, 2021; Rathod *et al.*, 2021).

In order to safely and effectively prevent or inhibit oil oxidation without changing its sense and quality, the development and research of antioxidants have been developed rapidly (Budilarto and Kamal-Eldin, 2015; Mishra *et al.*, 2020). At present, chemically synthesized antioxidants are still predominant and mainly including propyl gallate (PG), butylated hydroxy anisole (BHA), dibutyl hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). These antioxidants are phenolic antioxidants with cheap and stable performance (Tadesse and Emire, 2020). However, synthetic antioxidants have potential toxicity and carcinogenicity, so it is necessary to find safe and effective antioxidants (Taghvaei and Jafari, 2015). It is well known that in addition to vitamins and phenolic compounds, some polypeptides also have antioxidant activities. Most of these substances exist in plants and animals. (Pejin *et al.*, 2013; Im *et al.*, 2014; Tesanovic *et al.*, 2017; Karaman *et al.*, 2019; Jie *et al.*, 2019a). Nowadays, antioxidant peptides have become a focus in the development of natural antioxidants (Zarei *et al.*, 2012). Antioxidant peptides, as antioxidant components derived from protein, have the advantages of relatively simple structure, easy absorption, good stability and immunological unresponsiveness. Antioxidant peptides have efficient free radical scavenging ability, so they have a significant protective effect on oil peroxidation induced by free radicals (Yang *et al.*, 2018).

In recent years, the traditional preparation methods of antioxidant peptides have been well developed. In order to further optimize the preparation of antioxidant peptides to generate peptides with desired function and purpose, some bioinformatics have been applied to the research of antioxidant peptides (Chiozzi *et al.*, 2016; Jara *et al.*, 2018; Borawska-Dziadkiewicz *et al.*, 2021). The acquisition of purposeful antioxidant peptides from different biological resources is increasingly becoming the driving force of the oil industry (Tadesse and Emire, 2020). In order to promote the application of antioxidant peptides as food antioxidants, we discussed the preparation methods of antioxidant peptides, their different antioxidant mechanisms and applications in the oil. They are of great significance to furthering the use of antioxidants and oil storage.

2. PREPARATION OF ANTIOXIDANT PEPTIDES

The most well-known antioxidant peptides are carnosine (β-alanyl-L-histidine) and glutathione (r-glutamyl-cysteinyl-glycine). They are two kinds of natural antioxidant peptides existing in the organism. Their antioxidant functions are mainly reflected in their ability to scavenge free radicals and peroxides produced by oil oxidation. In order to find other antioxidant peptides, researchers hydrolyzed protein with different methods. The methods of hydrolyzing protein mainly include acid-base hydrolysis, microbial fermentation and enzymatic hydrolysis, which is the most common (Borrajo *et al.*, 2019).

2.1. Preparation of antioxidant peptides by traditional hydrolysis

Hydrolyzing protein is the most common method for preparing antioxidant peptides. Acid-base hydrolysis uses acid or alkali to hydrolyze protein. Some researchers hydrolyzed ostrich egg white protein with 0.25 mol/L NaOH at 40 ºC for eight hours. They proved that the antioxidant activity of the protein was significantly enhanced after hydrolysis (Khueychai *et al.*, 2019). This method is cheap and time-saving. Nonetheless it is difficult to control the degree of hydrolysis and product quality, because the violent reaction conditions involved are destructive to amino acids (Hall and Ahmad, 1997).

Microbial fermentation can produce enzymes and enzymatic hydrolysis simultaneously. This method also can directly isolate and purify antioxidant peptides (Chai *et al.*, 2020). After fenugreek protein was fermented with *Lactococcus lactis* for 24 hours,

the free radical scavenging activities of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt (ABTS) increased by 42.9 and 40%, respectively (Setti *et al.*, 2017). However, the process of screening microorganisms is cumbersome and the safety of microbial enzyme production is uncontrollable (He *et al.*, 2012).

Enzymatic hydrolysis uses proteolytic enzymes to hydrolyze protein into small molecular polypeptides. It is characterized by mild conditions, strong operability, strong specificity and fewer by-products. The same protein hydrolyzed by different enzymes produces different antioxidant peptides (da Rosa *et al.*, 2018). A study has measured the antioxidant activity of camel colostrum protein hydrolyzed by pepsin, trypsin, pancreatin and another two enzymes. The results showed that the proteolytic peptide hydrolyzed by pancreatin had the highest antioxidant activity (Oussaief *et al.*, 2019). Hydrolysis conditions are also critical. Some researchers have optimized the hydrolysis conditions from egg white with chymotrypsin and pepsin through response surface methodology, and the antioxidant activity of the peptide was improved (Yuan *et al.*, 2020). Table 1 summarizes experiments related to the preparation of antioxidant peptides by enzymatic hydrolysis.

However, enzymatic hydrolysis usually obtains crude peptides, so it needs to be separated and purified. Usually, ultrafiltration or gel chromatography can be carried out according to the size of antioxidant peptides. Ion exchange chromatography and reverse chromatography can also be used (based on the charge and hydrophobicity of the antioxidant peptide, respectively) (Vanvi and Tsopmo, 2016). After that, tandem mass spectrometry is applied to identify the antioxidant peptides. One study extracted antioxidant peptide from duck plasma hydrolysate (DPH) and then separated and purified the antioxidant peptide by ultrafiltration, dimensional exclusion chromatography and reversed-phase high performance liquid chromatography. The researchers also identified the DPH fraction with the highest antioxidant activity by nanoscale liquid chromatography-tandem mass spectrometry (Yang *et al.*, 2020). In fact, DPPH, ABTS free-radical scavenging and oxygen-radical absorbance capacity (ORAC) tests are generally used to evaluate the effect of antioxidant peptides (Kim *et al.*, 2018). Other researchers have pre-treated the protein with ultrasound, microwave and irradiation to improve the antioxidant activity of the peptide (Wang *et al.*, 2019; Zhang *et al.*, 2019). However, the single use of enzymatic hydrolysis is time consuming when selecting hydrolytic enzymes. The process of iden-

Table 1. Preparation of antioxidant peptides by enzymatic hydrolysis

Protein source	Hydrolysis conditions	Enzyme	Application model	Reference
Zein	pH 9.0, 50 ºC, 1h	Alcalase	Myofibrillar protein oil-in-water emulsions	(Li *et al.*, 2017)
Porcine blood	pH 6.5, 50 ºC, 6h	Papain	Pork emulsion	(Verma *et al.*, 2018)
Oat brans	pH 2.0, 37 ºC, 3h	Pepsin	Not studied	(Vanvi and Tsopmo, 2016)
Common bean	pH 8.0, 50 ºC, 3h	Alcalase	Not studied	(Oseguera-Toledo *et al.*, 2011)
Cow milk	pH 3.0, 37 ºC, 3h	Porcine pepsin	Zebrafish larvae model	(Carrillo *et al.*, 2017)
Salmon byproduct	pH 7.0, 50 ºC, 8h/ pH 1.0, 37 ºC, 8h/ pH 8.0, 37 ºC, 8h	Alcalase/Flavourzyme/ Neutrase/Protamex/ Pepsin/Trypsin	Chang liver cells	(Ahn *et al.*, 2012a)
Palm kernel cake protein	pH 6.5, 65 °C/ pH 7.5, 55 °C/ pH 1.5, 37 °C/ pH 8.0, 37 °C/ pH 8.0, 55 °C/ pH 5.0, 55 °C/ pH 6.8, 50 °C, 6h	Papain/Alcalase/Pepsin/ Trypsin/Flavourzyme/Bromelain/ Chymotrypsin	Not studied	(Zarei *et al.*, 2014)
Cod protein	pH 8.1, 45 ºC, 6h	Protamex	Washed cod model	(Jonsdottir *et al.*, 2016)

Table 2. Bioinformatics databases used in antioxidant peptides

Classification	Name	Function	Reference
Protein database	UniProtKB	Provide protein sequence and function information	(Bechaux *et al.*, 2020)
	PIR	Provide protein sequence	(Panchal *et al.*, 2021)
Simulated enzymatic hydrolysis	BIOPEP-UWM	Predict the potential sites for protease cleavage of the target protein sequence	(Ibanez *et al.*, 2013)
	Peptide Cutter	Predict the potential sites for protease cleavage of the target protein sequence	(Fu *et al.*, 2016)
Antioxidant peptide database	Pep Bank	Provide peptide structure and classify	(Chiozzi *et al.*, 2016)
	NCBI	Provide peptide sequence	(Tian *et al.*, 2016)
Predict antioxidant peptide	Peptide Ranker	Evaluation of antioxidant peptides and their precursor proteins	(Ibanez *et al.*, 2013)
	BIOPEP-UWM	Predict the potential antioxidant activity of the input peptide sequence	(Su *et al.*, 2011)

tifying antioxidant peptides is complicated and the target antioxidant peptides may not be obtained (Wen *et al.*, 2020). Therefore, bioinformatics has attracted people's attention.

2.2. Bioinformatics guides the preparation of antioxidant peptides by enzymatic hydrolysis

Bioinformatics based on computer technology can guide the selection of precursor proteins and simulate enzymatic hydrolysis (Zhou *et al.*, 2019). Moreover, the function of the enzymatic hydrolysis products can be predicted and identified based on the sequence, structure and other parameters of proteins and peptides by bioinformatics. It is widely used in the research of hydrolyzing protein to obtain antioxidant peptides (Tejano *et al.*, 2019). This method is often used with databases such as Peptide Ranker, BIOPEP-UWM and UniProtKB (Chen *et al.*, 2017; Pearman *et al.*, 2020). Some commonly used bioinformatic databases are listed in Table 2. These databases contain the amino acid sequences of various proteins and peptides to improve the efficiency of obtaining target peptides at a low cost (Tadesse and Emire, 2020). Darewicz *et al.* (2016) retrieved the amino acid sequence, molecular weight and chain length of *Cyprinus carpio* protein from UniProtKB. After using the ClustalW2–Multiple Sequence Alignment program to eliminate the same sequence, they selected 33 carp protein amino acid sequences with less than 90% identity for further analysis. The number of amino acid residues in the analyzed carp protein amino acid sequence ranged from 62 (light myosin, Q90335) to 1938 (myosin heavy chain, Q2HX56).

These bioinformatic methods can also simulate protease hydrolysis. In 2018, researchers used Peptide Cutter to simulate 100 tripeptides from the myosin of *Mizuhopecten yessoensis* by pepsin and trypsin (Yu *et al.*, 2018). For example, the BIOPEP-UWM database can not only simulate hydrolysis, but also predict the bioactive peptides released from protein sequences and their bioactivity and physicochemical properties (Yang *et al.*, 2017). Researchers estimated the solubility of antioxidant peptides from flaxseed proteins in water by using Innovagen Peptide Solubility Calculator Proteomic. They also predicted the release of antioxidant peptides by opening the 'search for active fragments tab of BIOPEP. Thereafter, they estimated several properties and indices of the silico-derived antioxidant peptides by using the application of the 'Peptides' package in R or ProtParam of ExPASy. These properties included amino acid composition, amino acid length, molecular weight (MW), isoelectric point (pI), Boman index, net charge and hydrophobicity index (Ji *et al.*, 2019). In another research, Salim and Gan (2020) used Peptide Cutter to simulate the digestion of egg white ovalbumin, then Peptide Ranker was used to screen the obtained peptide sequences. After that, they predicted the peptide binding sites by Pepsite2 and finally used an in vitro assay to verify the bioactivity of the peptide. The *AnOxPePred*, a method for predicting the free-radical scavenging ability and chelating properties of antioxidant peptides was developed. When constructing the predictive variable,

a standard database consisting of peptides and their free-radical scavenging capabilities and chelating properties was created (Olsen *et al.*, 2020).

Although bioinformatics is an effective method to study and predict the antioxidant peptides released by proteins and their potential activities, there are some limitations to this approach. For example, there is a lack of research on the protein sequence in simulated hydrolysis and its influence on enzymatic hydrolysis (Zhou *et al.*, 2019). Bioinformatics simulates the hydrolysis of the protein sequence completely, but it is often necessary to consider the secondary and tertiary structures of protein and other factors such as inhibitors, pH and temperature when verifying in vitro (Bechaux *et al.*, 2020). Some scientists showed that in the peptides obtained by computer simulation of a porcine actin sequence, only a few could be verified through laboratory experiments (Keska and Stadnik, 2016). This proved that bioinformatics was an auxiliary method for selecting protein families and optimal hydrolyses. Bioinformatics was still theoretical, so it needed to be verified by in vitro experiments.

In order to efficiently and economically prepare antioxidant peptides, more and more researchers combine enzymatic hydrolysis and bioinformatics. The amino acid sequence of the target protein can be searched in the database to facilitate the selection of hydrolases, and it is estimated that there are more than 8 million amino acid sequences of proteins in the database (Agyei *et al.*, 2018). Some researchers used the amino acid sequence of the 11S coffee globulin (the entry Q9ZNY2_COFAR and accession number NCBI 13443) deposited in the UniProtKB database. Subsequently, they selected enzymes (pepsin (EC 3.4.23.1), trypsin (EC 3.4.23.4) and chymotrypsin (EC 3.4.21.1)) to simulate gastrointestinal digestion. They carried out the prediction of peptides generated through simulation by using the BIOPEP-UWM "enzyme action". Based on the results for the prediction of bioactivity of the 11S globulin, a series of in vitro studies was carried out, including protein extraction, enzymatic hydrolysis, protein isolation and determination of its antioxidant capacity. Finally, they proved that the hydrolyzed peptides of roasted coffee beans have higher antioxidant activity (Ribeiro *et al.*, 2021). Computer simulation of hydrolysis can also greatly improve the screening efficiency of hydrolytic enzymes. Jie *et al*. (2020) used gluten as the bioinformatics template of *Caragana korshinskii* seed protein, then carried out virtual enzymatic hydrolysis with the assistance of the BIOPEP-UWM database. They determined that papain was the main protease to hydrolyze the *Caragana korshinskii* seed protein, which improved preparation efficiency.

After simulated hydrolysis, the computer generates an amino acid sequence. By comparing the sequence with the antioxidant peptide sequences reported in databases such as Swiss-Prot, Peptide Ranker or in the literature, the activity of the antioxidant peptide is predicted, evaluated and the target peptide is selected (Tu *et al.*, 2018). For example, Sun *et al*. (2021) first prepared defatted collagen hydrolysates from yak bone, and then separated and purified the hydrolysate by ultrafiltration and reversed-phase high-performance liquid chromatography. After that, they determined the antioxidant activities of different hydrolysates and screened the best components for identification by mass spectrometry. Mass spectrometry was used to identify components with better antioxidant activity- Swiss-Prot was used to determine the amino acid sequence of the target peptide. Finally, they selected peptides with molecular weight of less than 1 kDa for synthesis according to the possible structural features of antioxidant peptides and the number of enzyme-cutting sites contained in the peptides. Other researchers isolated and purified antioxidant peptides from *Asparagus* by-products by reverse-phase chromatography, and then identified the peptides by tandem mass spectrometry. Peptide identification was carried out by using the Swiss-Prot database. PeptideRanker was used to predict the probability of the antioxidant activities of these peptides. This study only considered peptides with probability scores higher than 0.95. Therefore, only five peptides were retained and five novel peptide sequences were synthesized, and their antioxidant activity was finally evaluated in vitro (Montone *et al.*, 2019). It is also necessary to predict the toxicity of antioxidant peptides. Harvian *et al*. (2019) used ToxinPred to analyze the antioxidant peptide obtained from jack bean (*Canavalia ensiformis*) canavalin protein. ToxinPred is a *in silico* database for the prediction of the toxicity of selected peptides. The support

vector machine (SVM)-based prediction method and SVM threshold value (0.0) were used to separate toxic from non-toxic peptides. Their results showed that the antioxidant peptide was non-toxic. Of course, in vivo or in vitro tests are required to further determine the physiological activity of antioxidant peptides eventually.

In summary, the bioinformatics method can simulate protein hydrolysis, determine the most suitable hydrolase and screen out peptides with good antioxidant effects by comparing the amino acid sequences of peptides gained through experiments with those in the database for in vitro verification. The combination of the two methods will simplify the cumbersome experimental process of traditional enzymatic hydrolysis, save cost, improve preparation efficiency and provide a promising method to improve not only the availability but also the high-value utilization of antioxidant peptides (Ji *et al.*, 2019).

3. MECHANISM OF ANTIOXIDANT PEPTIDES INHIBITING OIL OXIDATION

The main ways for oil oxidation are automatic oxidation, photosensitive oxidation and enzymatic oxidation (Hammer and Schieberle, 2013; An *et al.*, 2014). Autoxidation includes three stages: chain initiation, chain transfer and chain termination (Scheme 1).

$$\text{Chain initiation: } R:H \rightarrow R\cdot + H\cdot$$

$$\text{Chain transfer: } R\cdot + O_2 \rightarrow ROO\cdot$$

$$ROO\cdot + R:H \rightarrow ROOH + R\cdot$$

$$\text{Chain termination: } R\cdot + R\cdot \rightarrow R:R$$

$$R\cdot + ROO\cdot \rightarrow ROOR$$

$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$$

Scheme 1. Mechanism of oil automatic oxidation (R· stands for free radical).

Photosensitive oxidation and enzymatic oxidation are important factors to initiate automatic oxidation in oil. Their oxidation products decompose to produce various free radicals which make the automatic oxidation chain reaction continue to circulate (Ladikos and Lougovois, 1990). The automatic oxidation of oil can damage its color, flavor and texture to reduce the nutritional value and also generate cytotoxic and genotoxic compounds (Sohaib *et al.*, 2017). These substances cause irreversible oxidative damage to humans (Qi *et al.*, 2016). Therefore, the oxidation of oil must be inhibited. Antioxidant peptides are unique to some extent. Their uniqueness is not only reflected in their small size and high utilization rate in the body, but also in that peptides are easier to metabolize than amino acids and optimize the environment in the body. (Yang *et al.*, 2021). According to current research, antioxidant peptides can inhibit oil oxidation by scavenging free radicals, suppressing the activity of catalytic metal ions and restraining the formation and reactivity of hydroperoxide, so they are potentially multifunctional antioxidants (Agyei *et al.*, 2016). The activity of antioxidant peptides is mainly related to the composition of different amino acids, the sequence of amino acids and the molecular weight of antioxidant peptides (Wu *et al.*, 2015b). This is similar to the results of another study (Chen *et al.*, 2020b).

3.1. Scavenging free radicals

Free radicals exhibit strong reactivity, so they are initiators in oxidation reactions (Tomycz *et al.*, 2011). At present, it is generally recognized that an important cause of oil automatic oxidation is the chain reaction initiated by free radicals, such as hydroxyl radicals, peroxy free radicals and alkoxy free radicals (Sonklin *et al.*, 2018). Hydroxyl radicals can remove the hydrogen atoms in the adjacent fat chain and induce oxidation chain reactions. High-energy peroxy free radicals and alkoxy free radicals can combine hydrogen atoms from adjacent fatty chains to destroy fatty acids and deteriorate oil. Therefore, it is necessary to combine free radical scavengers with free radicals to slow down the rate of chain reactions. Many antioxidant peptides are free radical scavengers. As shown in Scheme 2, together with amino acids, they act as hydrogen donors or electron donors to terminate the chain reactions of free radicals by converting them into stable products (Yarnpakdee *et al.*, 2015).

$$\text{Hydrogen donation: } R\cdot + B:H \rightarrow R:H + B\cdot$$

$$\text{Electron donation: } R\cdot + B:H \rightarrow R^- : + B\cdot H^+ \rightarrow R:H + B\cdot$$

Scheme 2. Pathways of antioxidant peptides scavenging free radicals. B:H stands for antioxidant peptide. Hydrogen donation: the antioxidant peptide transfers H· to R· to form a stable compound R:H, while the antioxidant peptide converts to B·, which is less likely to initiate a new free radical chain reaction. Electron donation: electron transfer and proton transfer are required (Liang and Kitts, 2014).

The antioxidant capacity of peptides and their amino acid composition are closely related, and the mechanism of amino acids depends on the functional groups of their side chains (Elias *et al.*, 2008). For example, peptide sequences with hydrophobic amino acids, aromatic amino acids and sulfur-containing amino acids usually exhibit strong antioxidant activity (Wu *et al.*, 2015a). Antioxidant peptides containing hydrophobic amino acids such as alanine, valine and leucine have strong antioxidant capacity. The non-polar aliphatic hydrocarbon side chains of these amino acids can enhance the interaction between fatty acids and peptides to prevent hydrogen atoms from being attacked (as shown in Figure 1A) (Rajapakse *et al.*, 2005). Their presence can also increase the solubility of antioxidant peptides in oil, thus promoting interactions with free radicals and exhibiting higher antioxidant activity (Aguilar-Toalá and Liceaga, 2020). Some scientists proposed that hydrophobic amino acids such as valine and aspartic acid present in the peptide sequence could contribute to improving the antioxidant activity of peptides (Najafian and Babji, 2014).

Antioxidant peptides with aromatic amino acids such as tyrosine, tryptophan and phenylalanine in the sequence also have strong antioxidant properties. Aromatic amino acids can not only enhance the solubility of antioxidant peptides in oil like hydrophobic amino acids, but can also scavenge free radicals produced by oxidation by removing a H· from the phenolic hydroxyl or indole group in these amino acids. These amino acids are converted into relatively stable free radicals to terminate the chain reaction and inhibit the automatic oxidation of oil (as shown in Figure 1B) (Wang *et al.*, 2014). One study isolated two peptides containing tryptophan and tyrosine from the hydrolysate of *Sardinella aurita*, both of which have high DPPH scavenging activity (Bougatef *et al.*, 2010). A related study also showed that the antioxidant activity of the dipeptides Tyr-Leu and Phe-Tyr derived from Perilla is related to the presence of tyrosine (Yang *et al.*, 2018).

Methionine and cysteine are two kinds of sulfur-containing amino acids with active sulfhydryl in their structures. They can be used as precursors for the synthesis of glutathione. The methionine and cysteine in the antioxidant peptide sequence contribute to scavenging free radicals through the electron transfer pathway (Atmaca, 2004). The sulfhydryl group loses electrons to form free radical cations. The free radical cations then undergo a proton transfer reaction to generate stable free radicals and hydrogen ions (Scheme 3).

$$BSH + R\cdot \rightarrow BSH^{+}\cdot + R^{-}$$
$$BSH^{+}\cdot \rightarrow BS\cdot + H^{+}$$

SCHEME 3. Mechanism of scavenging free radicals by sulfur-containing amino acids.

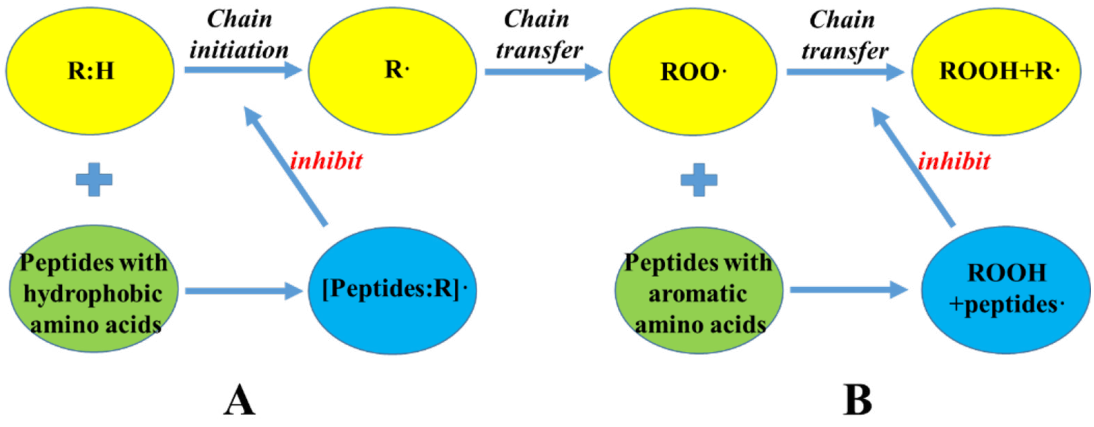

FIGURE 1. Mechanism of scavenging free radicals by hydrophobic amino acids and aromatic amino acids.

Jiang *et al*. (2014) obtained His-Asp-His-Pro-Val-Cys and His-Glu-Lys-Val-Cys from *Decapterus maruadsi*. The DPPH scavenging activity of the former was similar to the glutathione (Jiang *et al.*, 2014). In addition, the CFCTKPC synthesized by Huang *et al*. (2012) contained three cysteines, and showed strong free radical scavenging ability and inhibited linoleic acid oxidation.

Histidine is one of the most common amino acids in antioxidant peptides. Its imidazole group can act as hydrogen donors to react with free radicals, especially superoxide anion-free radicals (Jie *et al.*, 2019b). Kim *et al*. (2020) obtained histidine-containing low-molecular-weight (LMW) peptides from tuna waste meats. The histidine-containing LMW peptides exhibited a high DPPH radical scavenging effect in a dose-dependent manner.

3.2. Inhibiting the activity of catalytic metal ions

Catalytic metal can accelerate the decomposition of oil oxidation primary products to generate free radicals as shown in Scheme 4 (Muhr *et al.*, 2020).

$$M^{n+} + ROOH \rightarrow M^{(n+1)+} + OH^- + RO\cdot$$
$$M^{n+} + ROOH \rightarrow M^{(n-1)+} + OH^- + ROO\cdot$$

SCHEME 4. Transition metal ions accelerate the decomposition of oil oxidation primary products (M^{n+} represents transition metal ions).

The catalysis of catalytic metal depends on their ability to react with unoxidized oil to form hydroperoxides (Juita *et al.*, 2011). For example, Fe^{2+} catalyzes hydrogen peroxide to generate hydroxyl radicals with high activity through the Fenton reaction, thus promoting oil oxidation (Walters *et al.*, 2018). Another theory was that the presence of transition metal would significantly reduce the content of tocopherols, thereby affecting the oxidation stability of the oil (Fomuso *et al.*, 2002).

Antioxidant peptides can reduce the chemical reactivity of metals by chelating with metal ions to form stable complexes. Antioxidant peptides can also block the interaction between metals and oil in space. For example, changing the physical position of metal ions to reduce the rate of free radical reactions indirectly achieves the antioxidant effect (Feng *et al.*, 2016). Regarding antioxidant peptides, suitable amino acids and their correct position in the sequence play a crucial role in their chelating activity (Walters *et al.*, 2018). The imidazole group of histidine in the antioxidant peptide sequence can chelate with metal ions, thus inhibiting the generation of free radicals catalyzed by them (Burkitt, 2001). Basic amino acids such as glutamic acid and aspartic acid (with carboxylic acid groups) can also inhibit the catalytic oxidation of transition metal ions (Sonklin *et al.*, 2018). Besides, amino acids such as arginine and lysine with amino groups, cysteine with thiol groups and serine and threonine with hydroxyl may form complexes with antioxidant peptides by electrostatic interactions or H-bond coordination (Canabady-Rochelle *et al.*, 2018; Walters *et al.*, 2018). It has also been reported that casein-hydrolyzed peptides have the ability to inhibit the oxidation of oil. The peptides can oxidize Fe^{2+} into less reactive Fe^{3+} to delay oil oxidation (Diaz *et al.*, 2003). Some antioxidant peptides with metal chelating properties can act as potential antioxidants because of their charged amino acids in their sequences. The charged amino acids can be electrostatically attracted to metal ions to inhibit their catalytic effect. For example, the positive charge of histidine can attract negative free radicals, which is consistent with the results of Saiga *et al.* (2013). It is worth noting that antioxidant peptides with molecular weight less than 3 kDa have less chelating force on Fe^{2+} than the peptides with the formula weight of 5-10 kDa (He *et al.*, 2013). Therefore, antioxidant peptides with large molecular weight have a better effect on inhibiting oil oxidation.

Moreover, it must be noted that even though a peptide chelates metal, that does not mean it inhibits metal-promoted oil oxidation. Some chelators bind iron but do not decrease its reactivity (Durand *et al.*, 2021). Therefore, additional assays should be conducted to better assess the ability of these peptides to inhibit oil oxidation induced by prooxidant metals.

3.3. Restraining the formation and reactivity of hydroperoxide

Hydroperoxide is the primary product of oil oxidation. It can react with the ingredients in food, and reduce its quality. With the development of oil oxidation, hydroperoxide eventually decomposes into small molecules such as aldehydes

and ketones (Chen *et al.*, 2020a). Hydroperoxides may even decompose into toxic substances such as malonaldehyde (MDA) and 4-hydroxynonenal (HNE). During the chain transfer period, the free radicals generated by the decomposition of the hydroperoxides can react with unoxidized oil, continuously creating new free radicals and hydroperoxides to accelerate the oxidation of oil (Krugovov *et al.*, 2014).

The acidic amino acids in the antioxidant peptide sequence form hydrogen bonds with unsaturated fatty oil to protect the double bonds of fatty acids and decrease the cleavage of C-H bonds, thereby reducing the generation of hydroperoxides (Jie *et al.*, 2019b). A new antioxidant peptide was isolated from the *Caragana ambiguas* seed protein. The glutamine in the *Caragana ambigua* seed peptide (CSP) can form hydrogen bonds with unsaturated oil to protect the double bond of fatty acids, thus reducing the generation of hydroperoxides. In addition, CSP can delay the autoxidation of oil by protecting the phenolic compounds (Jie *et al.*, 2019a). Antioxidant peptides can also reduce the reactivity of hydroperoxides by proton or electron transfer, thus inhibiting the chain transfer period of oil oxidation (Li and Yu, 2015; Lacou *et al.*, 2016). Some researchers prepared an antioxidant peptide (APHPH) with the molecular weight of 1801 Da. The hydrophobic amino acids in its sequence can provide protons to inhibit the reaction between hydroperoxides and free radicals, and effectively inhibit oil oxidation in the linoleic acid emulsion system (Kim *et al.*, 2007). An antioxidant peptide was prepared by using Alcalase alkaline protease and ultrafiltration technology from silkworm sericin. Silk sericin peptide has an inhibitory effect on the hydroperoxides induced by 4NQO (an oral carcinogen) in liver tissue (Fan *et al.*, 2016). Other researchers believe that antioxidant peptides can also reduce hydroperoxides to relatively inactive hydroxides through non-radical reactions. The reaction mechanism is to generate sulfenic acid and sulfoxide derivatives through double electron transfer from the sulfur of cysteine or methionine (Esfandi *et al.*, 2019).

In summary, antioxidant peptides can inhibit oil oxidation by scavenging free radicals, chelating metal ions that promote oxidation and reducing hydroperoxides generation. In addition to these common mechanisms, it has been proposed that antioxidant peptides may interact with secondary oxidation products of oil, such as aldehydes. These reactions may interfere with oil oxidation and alter the rate and pathway of oil rancidity (Berton-Carabin *et al.*, 2014). However, since the secondary oxidation products of oil are bound to the antioxidant peptides and not available for determination (e.g., by GC headspace analysis), the mechanism remains to be further explored (McClements and Decker, 2018).

4. APPLICATION OF ANTIOXIDANT PEPTODES IN THE OIL INDUSTRY

The antioxidant peptides from the enzymatic hydrolysis of proteins are effective substitutes for chemically synthesized antioxidants. Antioxidant peptides can theoretically be used as food additives to delay the oxidative rancidity of oil and maintain food quality without threatening human health. As antioxidant peptides are more accessible to free radicals and have high efficiency, only a small amount of them (0.001-0.02%) can be added to food to exert a powerful antioxidant effect (Sila and Bougatef, 2016). Besides, the antioxidant peptides incorporated into food can also provide nutrition in the form of amino acids when consumed, which is also one of the advantages of antioxidant peptides (Chai *et al.*, 2017). Although antioxidant peptides have shown their potential as food antioxidants in small-scale experiments, only their real application in food or oil can support their large-scale industrial development as food additives. However, the practical application of antioxidant peptides is mainly concentrated in the fat of meat and oil at present and there are few studies on other food stuffs.

Meat is prone to fat oxidation during storage and will lead to quality degradation, so special protection for substances is needed. Adding antioxidant peptides is one of the most effective ways to prevent fat oxidation in meat and its products. Edible vegetable oil generally contains unsaturated fatty acids, so it is prone to oxidation, which leads to rancidity (Lorenzo *et al.*, 2018). Compared to other natural antioxidants, antioxidant peptides not only show good solubility and a wide range of pH values in oily or oil-rich food, but also have nutritional and functional properties (Decher

Table 3. Applications of antioxidant peptides in meat and oil

Raw material	Enzyme	Application product	Influence on product	Reference
Bovine hemoglobin or bovine cruor	Pepsin	Ground beef	The peptide reduced fat oxidation by about 60% with a concentration of 0.5% (w/w).	(Przybylski *et al.*, 2016)
Amur sturgeon skin gelatin	Alcalase	Japanese sea bass	The PAGT (Pro–Ala–Gly–Tyr) at 12.5 ppm showed the greatest effect in lowering fat oxidation.	(Nikoo *et al.*, 2015)
Rice protein	Validase® FP/Alkaline protease/ Neutral protease	Ground beef	The fat oxidation rate was decreased by 19 and 15% respectively after one or two weeks of storage	(Zhou *et al.*, 2013)
Rahu fish	Alcalase	Broiler breast meat	Dietary interventions of peptides can increase the antioxidant and shelf stability of broiler breast meat.	(Aslam *et al.*, 2020)
Amaranth protein	Alcalase	Sunflower oil and canola oil	The hydrolysate showed inhibition of the decomposition of primary to secondary oxidation products	(Tironi and Anon, 2014)
Sheep visceral	Alcalase	Soybean oil	The peroxide value of soybean oil increased slowly.	(Meshginfar *et al.*, 2017)
Bovine visceral	Alcalase	Soybean oil	The peptide at 500 and 1000 mg/kg showed the best oxidation prevention activity in soybean oil.	(Taghvaei *et al.*, 2014)
Mushroom *Ganoderma lucidum*	\	Soybean oil	The peroxide levels in soybean oil were significantly reduced.	(Sun *et al.*, 2004)
Bovine hair	\	Peanut oil	The POV value of peanut oil was significantly reduced	(Zeng *et al.*, 2013)

et al., 2005; Harnedy and FitzGerald, 2012). Table 3 lists some applications of antioxidant peptides used in meat and oil.

5. CONCLUSION AND FUTURE PROSPECTS

With more and more research on antioxidant peptides, their preparation methods are gradually being diversified. Enzymatic hydrolysis is the most common preparation method to prepare antioxidant peptides, but the selection of the optimal enzyme and the tedious enzymatic hydrolysis process make the traditional method inefficient and aimless. Therefore, bioinformatics is combined with enzymatic hydrolysis. Bioinformatics technology improves the traditional research methods for antioxidant peptides to a certain extent. It can search the target protein information on demand and prepare antioxidant peptides purposefully through virtual hydrolysis. Bioinformatics can also use the database to evaluate and predict the antioxidant peptides' function to screen out the peptides with the highest antioxidant activity. The purpose of improving the peptides' properties and the possibility of antioxidant peptides' industrial production has been achieved. However, there are still many scientific and technical problems to be solved for the combined preparation of the two methods. At present, the information on the antioxidant peptide database is still incomplete, so the preparation of some antioxidant peptides cannot be fully realized. Secondly, no unified standards and methods have been established for the assessment of antioxidant peptides' capacity, so the activity prediction system is not perfect.

Antioxidant peptides can prevent or delay oil oxidation mainly through scavenging free radicals. The non-polar aliphatic hydrocarbon side chains of hydrophobic amino acids can protect the hydrogen atoms of fatty acids in the oil and enhance the interaction between antioxidant peptides and oil. Aromatic amino acids can react with free radicals as hydrogen donors to shape into stable compounds. Sulfhydryl-containing amino acids can scavenge free radicals through the electron transfer pathway

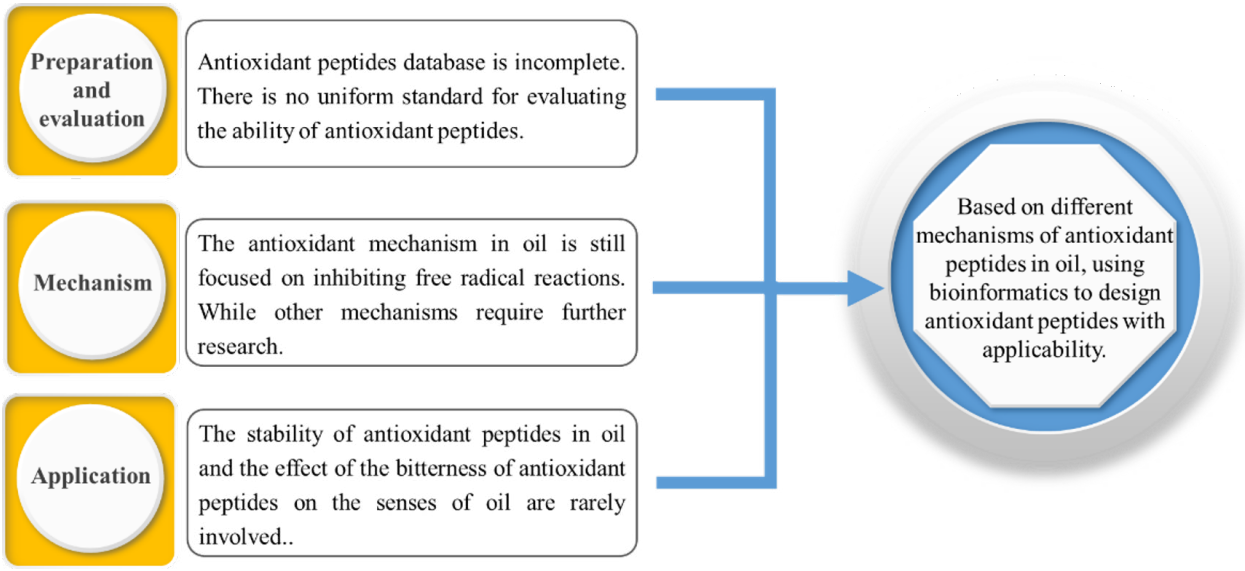

FIGURE 2. The existing problems and prospects of antioxidant peptides applied in oil.

to delay the oxidation of the oil. Antioxidant peptides can also make metal ions lose their ability to catalyze oil oxidation by chelating with metal ions, changing their valence state and electrostatic interaction. Reducing the generation of hydroperoxides and inhibiting decomposition to form new free radicals which participate in the chain reaction are also ways to delay oil oxidation. However, the current research on the antioxidant mechanism of antioxidant peptides in oil is still focused on inhibiting free radical reactions, while other mechanisms have not been fully elucidated and require further research. Moreover, although there have been relevant studies on the stability of antioxidant peptides themselves and their effect on oxidative stability in food, the stability of antioxidant peptides in oil or other food has rarely been studied at present (Jang *et al.*, 2016; Garcia-Moreno *et al.*, 2020). This is a factor which limits the further application of antioxidant peptides. In addition, although there are methods to reduce the bitterness of proteolytic peptides, there are few studies on whether the bitterness of antioxidant peptides will affect the texture of oil (Slizyte *et al.*, 2014; Tong *et al.*, 2020). Therefore, the application of antioxidant peptides in the food industry, especially in the oil industry, is limited.

It is believed that these problems will be solved with the continuous update of modern technology and in-depth research. The database on antioxidant peptides and the standard evaluation system of their activities will be gradually be improved to facilitate the screening of target protein and sequences. Moreover, the mechanism of action and safety evaluation of antioxidant peptides in oil and in vivo will be further clarified to accelerate the utilization of antioxidant peptides. Based on the different antioxidant mechanisms of antioxidant peptides in oil, it is hoped that some researchers can use bioinformatics to design antioxidant peptides with high applicability (Figure 2), so that they can exert a stable antioxidant effect and open a broader scope for the development and application of antioxidant peptides.

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