Non-marine Animal Bioactive Peptides

Properties, Sources, and Applications

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Abstract

Animal proteins and their derivatives play a fundamental role in human nutrition as a source of valuable nutrients and bioactive peptides that have shown an increased interest for their application in the food, pharmaceutical, medicine, and cosmetics industry.

In the past years, animal peptides have increased their application in the pharmaceutical industry, due to their great versatility, varied pharmacological functions, high specificity, and low levels of toxicity as well as being natural compounds with minor or no adverse effects when compared to synthetic drugs. Strikingly, only some of the bioactive peptides are sold on the market as functional foods. The possible reason is that most of them do not have sufficient evidence in terms of efficacy and food safety. Additionally, different processing methods may have a certain impact on their bioavailability, affecting their application in food. Therefore, several encapsulation methodologies are being studied in order to protect bioactive peptides, enhance their bioavailability as well as to enhance their sensory and physicochemical properties.

This chapter summarizes current knowledge on peptides from meat, milk, and eggs, the main methods of obtaining peptides (enzymatic hydrolysis with commercial proteases, fermentation, and gastrointestinal digestion) and their bioactive properties (antihypertensive and cardiovascular health improvement, antioxidant, antidiabetic, antiobesity, antitumor activity, gut, and neurological health improvement). Moreover, peptide bioaccessibility and bioavailability studies will also be discussed, since these are key factors for these compounds to exert their mechanisms of action in the body. Finally, a discussion about the food applications and perspective studies for promoting animal bioactive peptides use is carried out.

In sum, the full set of reported bioactivities plus future research on the efficacy and safety through clinical studies, coupled with the efficient use of byproducts for a sustainable production of these peptides, will reveal the full scope of potential applications of these natural molecules.

Keywords

Animal peptides · By products · Biological activity · Bioavailability · Bioaccessibility

1 Introduction

Proteins of animal origin are well known for their essential role in human nutrition. These kinds of proteins present a high quality due to its amino acid pattern and digestibility. Moreover, food proteins constitute an interesting source of bioactive peptides. Bioactive peptides are specific protein fragments (in general from 2 to 20 amino acids) that exert biological activities in the body, and therefore, improve the health of consumers (Xing et al. 2021; Peighambardoust et al. 2021). The amount of peptides characterized and the interest in their study and application grows day by day among the scientific community and the general public as evidence of the beneficial impact on health of their consumption increases. There are databases such as BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep), where the different research groups report and/or consult the main characteristics of peptides such as their biological activity, sequence of amino acids, molecular weight (MW), etc.

There are several alternatives for obtaining bioactive peptides from food matrix: the use of solvents, enzymes, and fermentation are some examples (Dávalos et al. 2004; Sacchetti et al. 2008; Rebouillat and Ortega-Requena 2015; Ohata et al. 2016; Mohanty et al. 2016; Sharma et al. 2020; Yuan et al. 2020).

Meat is a valuable source of nutrients and a very important part of the human diet. The term “meat” refers to the edible part extracted from animals, in particular, livestock and wild carcass (Xing et al. 2021). Its world production is large, in 2019 Food and Agriculture Organization of the United Nations (FAO) reported a total value of approximately 330 million tons (https://www.fao.org/faostat/en/#data/QCL9 2019).

An increasing number of studies reports a variety of biological activities of bioactive peptides from meat and their by-products: antioxidant, antihypertensive, antithrombotic, and antimicrobial activity, among others (Wu et al. 2015; Borrajo et al. 2019; Xing et al. 2021). By-products of animal origin are those parts which are not intended for human consumption. This includes bones, blood, collagen, gelatin, liver, skin, etc. As previously mentioned, the possibility of obtaining bioactive peptides from this source is very interesting since they represent a high percentage of the weight of the animal (around 50%) (Borrajo et al. 2019). In this way, the extraction of compounds of high commercial value from this source can help to minimize negative aspects related to their disposal, such as the impact on the environment and the costs of pre-treatments. In meat, there are endogenous bioactive peptides that have also been extensively studied. This is the case of imidazole peptides or histidine derivatives: carnosine (β-alanyl-L-histidine), anserine (β-alanyl-N-π-methyl-L-histidine), balenin (β-alanyl-N-τ -methyl-L-histidine), and homocarnosine (ɣ-aminobutyryl-L-histidine). These are antioxidant compounds and are found exclusively in animal tissues, highly concentrated in skeletal muscle and nervous tissue, where carnosine and anserine are the majority (Preedy 2015).

They are antioxidant dipeptides and their content, especially carnosine, will depend on the genotype, gender, age, type of muscle and feeding conditions.

As to other protein sources of animal origin, milk is the most complete food, providing all the energy and nutrients necessary for the proper growth and development of the newborn.

Bovine milk and its derivatives such as cheese and yogurt are the major sources of protein and bioactive peptides of high nutritive and pharmaceutical value derived from food (Sánchez and Vázquez 2017). The protein content of normal bovine milk is approximately 3.5%. This value changes significantly during the different stages of lactation, mainly in the first day's postpartum (Mohanty et al. 2016).

Milk proteins can be divided into two different groups: proteins that precipitate at pH 4.6 (isoelectric point) at 30 °C, and those that remain soluble under these conditions. The first group of proteins are known as caseins, and they represent around 80% of the total protein in bovine milk, for example. The proteins in the second group are called whey proteins. The proportion of caseins and whey proteins present great differences among the different species, reflecting the nutritional and physiological requirements of young animals (Rebouillat and Ortega-Requena 2015). The group of caseins, the main proteins, is classified into α-caseins, β-caseins, and k-caseins. Whey proteins are composed of two main proteins: β-lactoglobulin (58% w/w of whey proteins) and α-lactalbumin (20% w/w of whey proteins). Whey also has several minor proteins in its composition that present different biological activities such as enzymes, immunoglobulins, and mineral binding (Mohanty et al. 2016). The molecular structures of caseins and whey proteins are different, caseins present a disordered flexible structure and whey proteins, on the contrary, have a compact globular structure (Rebouillat and Ortega-Requena 2015). Bioactive peptides released from milk proteins by different food processes, have been widely studied due to their health-promoting properties (Mohanty et al. 2016).

Regarding other animal protein sources, eggs are one of the most consumed foods in the world due to their commercial availability and their nutritional value as they are an excellent source of proteins, lipids, vitamins, and minerals. Also, eggs present very good functional properties such as gelling, emulsifying, and foaming, which has extended their application in the food processing and industry (Abeyrathne et al. 2013).

A whole egg contains 12% protein, which is distributed between the egg white (54%) and yolk (46%). Egg whites contain ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), ovomucin (3.5%) avidin (0.05%), cystatin (0.05%), ovomacroglobulin (0.5%), ovoflavoprotein (0.8%), ovoglycoprotein (1.0%), and ovoinhibitor (1.5%). In yolk lipovitellins (36%), livetins (38%), phosvitin (8%), and low-density lipoproteins (17%) can be found (Abeyrathne et al. 2013). Some of these proteins have bioactive properties such as antioxidant and antimicrobial properties, but it has been seen that peptides derived from them have a higher bioactive potential. These functional peptides have been obtained from isolated proteins but also from egg white or yolk (Mine et al. 2004; Liu et al. 2018).

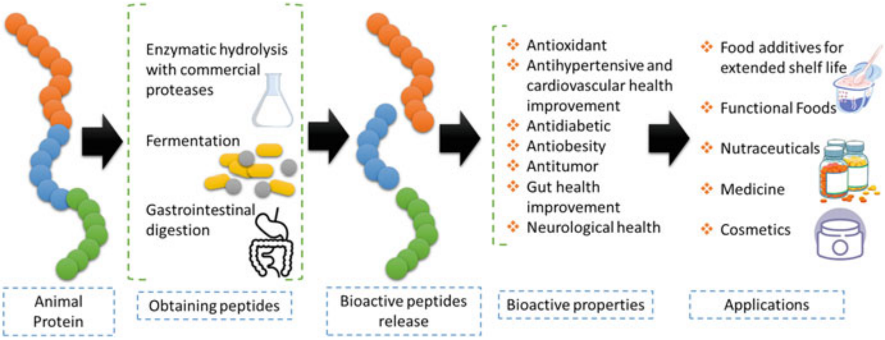

Fig. 1 Possible mechanisms for animal peptides' release, bioactive properties and applications

This chapter will focus on the methods of obtaining bioactive peptides, the studies that have been carried out on peptides from meat, milk, and eggs, as well as their biological activities (Fig. 1). Peptide bioavailability studies will also be discussed, since this is a key factor for these compounds to exert their action in the body. Finally, applications and future perspectives will be mentioned.

2 Meat

2.1 Obtaining Meat Peptides

Bioactive peptides can be obtained from different types of meat. Fresh meat, meat products, or by-products can be used. Peptides can be generated during meat ageing through the action of endogenous proteases. Exogenous proteases can also be used to release protein peptides in meat, meat products, and by-products (Onuh et al. 2021).

Distilled water is one of the most suitable solvents than organic solvents to extract endogenous peptides from fresh meat with antioxidant activity. For example, Sacchetti et al. (2008) extracted peptides from poultry meat, where the hydrophilic fraction showed a higher value of Trolox equivalent antioxidant capacity than the lipid soluble fraction in the ABTS assay. To extract peptides with antioxidant activity from cured meat products, the most suitable solvent is 0.2 mM phosphate buffer (Ohata et al. 2016). Likewise, for the extraction of anserine and carnosine from fresh meat, a simple procedure is used in which the sample is homogenized with trifluoroacetic acid (TFA) and the proteins are separated from the small peptides (Preedy 2015).

According to published literature (Sharma et al. 2020), common methods used to obtain bioactive peptides from food or other protein-rich sources include enzymatic proteolysis or fermentation with probiotics they can also be obtained through chemical synthesis. The most commonly used enzymes to produce proteolysis include flavorzyme, pepsin, alcalase, trypsin, and pancreatin. Regarding the fermentation processes, the most used microorganisms for the production of peptides are Bacillus subtilis and Aspergillus, which have proteolytic activity.

Enzymes act specifically on certain peptide bonds, so that the type of protease will not only influence the composition and profile of amino acids and peptides, but will also have an impact on the biological activity of the peptides generated. The resulting antioxidant activity, for example, will depend on the type of enzyme, the enzyme mixture, and the degree of hydrolysis achieved. The enzymes most used to produce peptides with antioxidant activity are alcalase, papain, and pepsin. Peptides released were smaller than those released with bromelain or flavorzyme. Biological activity is probably linked to the presence of hydrophobic and aromatic amino acids, because the amino acid content determines its biological activity (Peighambardoust et al. 2021). As previously mentioned, meat and by-products of the bovine, porcine, and poultry meat processing industry (such as blood, kidney, liver, lung, pancreas, bones, and skin) are an interesting source to produce peptides with biological activity. Enzymes such as papain, pepsin, alcalase, and collagenase are suitable for hydrolyzing meat and meat by-products, for example, skin, blood, and muscle (Lafarga et al. 2017) and chicken protein (Xing et al. 2021). Other enzymes such as flavorzyme and alcalase are used to produce commercial bioactive peptides from duck meat and porcine blood plasma.

A non-conventional hydrolysis methodology for the extraction of peptides is the use of subcritical water hydrolysis (SWH). This methodology has been used for the extraction of peptides, from meat and by-products, with an antioxidant capacity greater than that obtained by enzymatic hydrolysis (Borrajo et al. 2019).

It is well known that one way to optimize the process conditions for the release of peptides with biological activities is through the response surface methodology (RSM). On the one hand, temperature, degree of hydrolysis, proteins, and enzymatic activity can be considered as process variables, on the other hand, pH, enzyme: substrate ratio and hydrolysis time can be considered process constants.

The food fermentation process is widely used, particularly in East Asia, as a preservation method. During this process, protein degradation occurs due to the action of microorganisms and endogenous proteolytic enzymes. Microorganisms play an important role in fermented meat products, since they promote the production of special flavors. Microorganisms such as Staphylococcus carnosus and Staphylococcus xylosus have been shown to promote hydrolysis of fats and meat proteins during the preservation of meat products, improve color, and originate flavor. Esterases, proteases, and catalases produced by microorganisms have synergistic effects with endogenous enzymes in raw meat, causing changes in proteins and fats and releasing amino acids, esters, peptides, and volatile fatty acids (Wang et al. 2021).

Finally, bioactive peptides could be generated during gastrointestinal digestion of meat protein by the action of endogenous enzymes such as pepsin, trypsin, and chymotrypsin (Wu et al. 2015). Ferranti et al. (2014) evaluated the influence of gastrointestinal simulation on the proteins of the Bresaola, and he identified several peptides with biological activities such as antioxidant and antihypertensive.

Once the peptides are obtained, they can be purified, for which their physicochemical properties must be taken into account. The hydrolysates must be fractionated, sequenced, and characterized. Taking into account their molecular weights, gel filtration and/or nano and ultrafiltration methods are used (Onuh et al. 2021). Regarding their net charge, ion exchange chromatography could be used; and considering the hydrophobicity and hydrophilicity of the peptides, reverse phase liquid chromatography (RP-HPLC) is employed. There are innovative methods such as electro-membrane processes, including electrodialysis (ED), electrodialysis with bipolar membrane (EDBM), electromembrane filtration (EMF), and electrodialysis with ultrafiltration membrane (EDUF); these methods allow for better selectivity and produce less contamination compared to pressure driven membrane operations (Castro-Muñoz et al. 2021).

A combination of UF membranes (1, 3, and 10 kDa) with high performance liquid chromatography was used by Lafarga et al. (2016) for the purification of bioactive bovine hemoglobin hydrolysates with inhibitory properties of ACE-I and renin.

The identity of the peptides is essential in order to determine their health benefits. To identify peptide sequences, the methodologies used include MALDI -MS (matrix-assisted laser desorption/ionization mass spectrometry), ESI (electrospray ionization mass), and MALDI-TOF (matrix-assisted laser deionization time-of-flight) (Onuh et al. 2021).

In addition to the biological activity of the peptides obtained by the indicated methodologies, it is important to mention that it has been found that they can also contribute to food, functional properties such as water retention capacity (WHC), texture attributes, gel formation capacity, whipping and emulsifying properties (Peighambardoust et al. 2021).

2.2 Bioactivity of Meat Peptides

As mentioned in the introduction, several studies have found a variety of biological activities of bioactive peptides from meat and their by-products such as antioxidant, antihypertensive, antithrombotic and antimicrobial activity, among others (Wu et al. 2015*; Borrajo et al.* 2019*; Xing et al.* 2021*)* (Table 1).

2.2.1 Antihypertensive Activity

Hypertension is a major problem in developed countries. According to the World Health Organization, it is a serious medical disorder that can increase the risk of cardiovascular, brain, kidney, and other diseases. One of the mechanisms for regulating blood pressure in the body is through the renin-angiotensin system (RAAS). In this system, renin enzymes and angiotensin converting enzyme (ACE) are key elements to maintain blood pressure (Wu et al. 2015; Xing et al. 2021). A large number of peptides with antihypertensive activity inhibit the action of the ACE enzyme. At the moment, at least 30 ACE inhibitor peptides from meat of different species have been identified (Xing et al. 2021). Jang and Lee (2005) obtained hydrolysates of sarcoplasmic proteins from beef rump using thermolysin, proteinase

Table 1 Bioactive peptides in meat. Protein source, obtaining method, bioactivity and peptide sequence

Matrix	Protein	Obtaining method	Bioactivity	Peptide sequence	Reference
Meat	Sarcoplasmic protein of beef	Thermolysin, proteinase A, protease type XIII	ACE-inhibition	VLAQYK	Jang and Lee (2005)
	Proteins from beef	Thermolysin	ACE-inhibition	LSW/FGY/YRQ	Choe et al. (2019)
	Spanish dry-cured ham	Dry-curing/endogenous muscle enzymes	ACE-inhibition/ antihypertensive effects on SHRs	AAATP	Escudero et al. (2013)
	Bovine bone collagen	Alcalase	ACE-inhibition/ antihypertensive effects on SHRs	RGLOGL/RGMOGF	Cao et al. (2020)
	Bovine hemoglobin	Papain	ACE-inhibition	HF	Lafarga et al. (2016)
	Bovine hemoglobin	Papain	ACE-inhibition and renin-inhibition	HR/YR/HLP	Lafarga et al. (2016)
	Dry fermented sausages	*Lactobacillus plantarum* and *Staphylococcus simulans*	Antioxidant		Yu et al. (2021)
	Spanish dry-cured ham	Dry-curing/endogenous muscle enzymes	Antioxidant	SNAAC	Gallego et al. (2018)
	Chicken liver	Alcalase	Antioxidant		Chakka et al. (2015)
	Bovine bone collagen	Papain, alcalase, collagenase/ Neutrase, savinase, esperase	Antioxidant		Aubry et al. (2020)
	Tropomyosin (porcine)	Papain	Antioxidant	DAQEKLE	Saiga et al. (2003)
	Actin (porcine)	Papain	Antioxidant	DSGVT	Saiga et al. (2003)
	Tropomyosin (porcine)	Papain	Antioxidant	EELDNALN	Saiga et al. (2003)
	Myosin (porcine)	Papain	Antioxidant	VPSIDDQEELM	Saiga et al. (2003)
		Papain			

	Sarcoplasmic protein of brisket muscle		Antioxidant/ antihypertensive	Precursors or potential bioactive peptide	Di Bernardini et al. (2012)
			Antioxidant	AH (carnosine)	Aldini et al. (2005)
	Breast chicken	Water-extraction	Antioxidant		Sacchetti et al. (2008)
	Fermented meat sauce (pork)	Fermentation	Antioxidant		Ohata et al. (2016)
	Bresaola	In vitro simulation of digestion	Antihypertensive/ antioxidant	More than 70 precursors or potential bioactive peptide (according to BIOPEP)	Ferranti et al. (2014)
	Pork meat	Papain	Antithrombotic		Shimizu et al. (2009)
	Sarcoplasmic protein of beef	Commercial enzymes	Antimicrobial	GFHI/FHG/GLSDGEWQ/DFHING	Jang et al. (2008)
	Bovine hemoglobin	Pepsin	Antimicrobial	TSKYR	Przybylski et al. (2016)
	Chicken liver	Fermentation with lactic acid bacteria/hydrolysis commercial enzymes	Antimicrobial		Chakka et al. (2015)

A and protease type XIII. From these hydrolysates, they were able to purify and identify the VLAQYK peptide, which was shown to inhibit the action of ACE. Also in cattle, Choe et al. (2019) isolated ACE inhibitor peptides by injecting thermolysin into beef (*M. longissimus*). In this study, the peptides with the highest activities were identified as LSW, FGY and YRQ with IC50 values of 0.89, 2.69, and 3.09 mM, respectively. The formation of antihypertensive bioactive peptides in meat products has also been reported. During the maturation of Spanish dry-cured ham, Escudero et al. (2013) reported the generation of various antihypertensive peptides. These peptides were synthesized in order to test their hypotensive effects through the ACE enzyme inhibition assay. The peptide AAATP showed the highest activity with an IC50 of 100 uM. The researchers corroborated the in vivo bioactivity of AAATP by treating spontaneously hypertensive rats with a solution of this peptide (1 mg AAATP/kg). They noted a decrease in systolic pressure of −25.6 mmHg after 8 h of administration. Regarding by-products, Cao et al. (2020) isolated and identified ACE inhibitor peptides in hydrolyzed bovine bone collagen obtained with alcalase. The maximum inhibitors were synthesized to verify this activity: the RGLOGL and RGMOGF peptides were the most active with IC50 values of 1.44 and 10.23 μM. These peptides also exhibited a decrease in systolic pressure in spontaneously hypertensive rats of 31.3 and 38.6 mmHg with a dose of 30 mg/kg.

2.2.2 Antioxidant Activity

Eukaryotes cells normally produce reactive oxygen and nitrogen species (ROS and NOS) that are highly reactive molecules. These cells have mechanisms, both enzymatic and non-enzymatic, to neutralize ROS and NOS and maintain homeostasis. When these mechanisms are not sufficient, there is an excess production of ROS and NOS that can damage biomolecules (DNA, proteins, lipids, sugars) leading to an oxidative stress, which may contribute to the development of chronic diseases such as hypertension, cancer, diabetes, neurodegenerative disorders, heart disease, heart attack, and aging (Wu et al. 2015; Xing et al. 2021). The incorporation of antioxidant compounds through the diet could help to maintain the correct oxidative balance, preventing damage to the macromolecules through mechanisms that include the capture of free radicals, the chelation of metals, and the neutralization of reactive intermediates.

There are several studies that report the antioxidant capacity of peptides obtained from meat protein and its by-products. A recent work by Yu et al. (2021) indicates an increase in the content of peptides with antioxidant capacity during the fermentation and maturation of dry fermented sausages using a mixture of starters: *Lactobacillus plantarum* and *Staphylococcus simulans*, measured by FRAP, ABTS, and DPPH assays. Another meat product that has been widely studied as a source of antioxidant peptides is the Spanish dry-cured ham already mentioned in the previous section due to the content of antihypertensive peptides. Gallego et al. (2018) described the SNAAC peptide as the most antioxidant of the peptides naturally formed during the production process of this product, with an IC50 of 75.2 uM in the DPPH assay and 205 uM in the FRAP assay. These values were similar to those obtained with 2,6-di-tert-butyl-4-methyl phenol (BHT), a commercial food additive. Therefore, SNAAC could be used as a natural antioxidant in food. With regard to the use of by-products, there are several examples of the use of slaughter wastes of different species. Chakka et al. (2015) used chicken liver to obtain, through enzymatic hydrolysis with and alcalase, protein hydrolysates with in-vitro antioxidant activity. Likewise, the antioxidant peptides isolated from duck skin show a great capacity to scavenge free radicals (Lafarga et al. 2017). Bones, along with leather and skin, are the largest by-product generated in the processing of meat, representing approximately 30% of the total by-products (Lafarga et al. 2017). With this material, Aubry et al. (2020) obtained collagen hydrolysates by purifying the smallest fraction (<3000 Da) and measuring its antioxidant capacity in vitro through the FRAP, ABTS, and DPPH tests. In addition, Aubry used these extracts in bovine ground meat, and noticed a reduction of carbonyls and T-BARS values compared to the control, demonstrating that the extracts prevent the oxidation of lipids and proteins of this system. All the previous reports suggest the high potential of by-products from the slaughter as a source of high value-added compounds. In meat, the main species studied are porcine, bovine, and poultry (chicken), obtaining antioxidant peptides mainly by enzymatic hydrolysis (Wu et al. 2015; Xing et al. 2021). For example, antioxidant peptides have been obtained from pig myofibrillar proteins using papain (Saiga et al. 2003). Among the peptides identified, *DAQEKLE* from *tropomyosin* showed the highest in vitro activity using the measurement of hydroperoxides in a peroxidation system. Other antioxidant peptides found were DSGVT from actin, EELDNALN from *tropomyosin*, and VPSIDDQEELM from *myosin*. Di Bernardini et al. (2012) obtained protein hydrolysates with antioxidant action from the treatment of sarcoplasmic proteins of the pectoralis muscle of cattle with *papain.* Several peptides were detected, such as AKHPSDFGADAQ and AKHPSDFGADAQA, which contain the DAQ segment already identified in the antioxidant peptide DAQEKLE by Saiga et al. (2003).

As mentioned in the introduction, there are endogenous antioxidant peptides from meat: carnosine and anserine, which are peptides derived from histidine. The antioxidant capacity of these peptides is related to the presence of histidine, one of the amino acids with the highest antioxidant activity. In histidine, part of this characteristic is due to its imidazole ring, which has the ability to chelate metals and capture free radicals (Preedy 2015). Aldini et al. (2005) provided evidence of the ability of carnosine to neutralize cytotoxic carbonyls such as 4-hydroxy-trans-2,3-nonenal (HNE) and acrolein (ACR), both derived from lipid peroxidation in spontaneously oxidized rat skeletal muscle, by ex vivo assays.

2.2.3 Other Bioactivities

Arterial thrombosis is a disorder in which clots are formed in the blood vessels, making it difficult for the blood to circulate. This can lead to atherosclerosis or heart disease and stroke (Wu et al. 2015). Platelets play an important role in their development, so their inactivation could reduce the appearance of these diseases. There are several publications on the antithrombotic activity of bioactive peptides derived from food, but very few of those derived specifically from meat proteins. Shimizu et al. (2009) studied the antithrombotic activity in vitro and in vivo (mouse model) of pig meat hydrolysates obtained with *papain*. The investigators found an antithrombotic activity in vivo in the purified fraction of the hydrolysate (70 mg/kg animal weight) comparable to that of the positive control, aspirin (50 mg/kg animal weight).

On the other hand, nowadays it is important to study alternative natural sources that have antimicrobial activity due to the growing resistance to conventional antibiotics. The disk diffusion methodology is the most widely used for the evaluation of antimicrobial activity. In this technique, the amplitude of the microbial growth inhibition halo is measured by the action of the peptide to be tested (Borrajo et al. 2019). The GFHI, FHG, GLSDGEWQ and DFHING peptides identified by Jang et al. (2008) in the sarcoplasmic protein hydrolysates of beef were synthesized and their antimicrobial activity was studied by challenging them against different microorganisms. GFHI inhibited the growth of both *Escherichia coli* and *Pseudomona aeruginosa* at concentrations of 200 and 400 μg/mL. The FHG inhibited the growth of *Pseudomona aeruginosa* at the same concentration, while the peptide GLSDGEWQ showed an inhibition of *Salmonella Typhimurium*, *Bacillus cereus*, *Escherichia coli*, and *Listeria monocytogenes* at a concentration of 100 ppm. Besides, Przybylski et al. (2016) studied the application as a food preservative of the TSKYR peptide, corresponding to fraction f (137–141) of bovine hemoglobin. He demonstrated that this compound inhibited microbial growth for 14 days in refrigeration and also this peptide delayed the rancidity of meat by reducing lipid oxidation. The hydrolysates obtained from chicken liver by fermentation with lactic acid bacteria (Pediococcus acidilactici NCIM5368) and by the use of commercial enzymes (alcalase, papain, and trypsin) also showed antimicrobial activity (Chakka et al. 2015). The hydrolysates from fermentation inhibited the growth of *Micrococcus luteus*, while those obtained by the action of enzymes showed antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*.

These findings are interesting to support the use of these compounds in food preservation, for example, in the development of novel-smart packaging and for human therapies application.

2.3 Bioaccessibility and Bioavailability of Meat Peptides

A bioactive peptide has to reach the site of action intact after a series of digestive processes that occur after its consumption in order to be able to exert its activity (Xing et al. 2021). A factor of great importance is the resistance to the action of different proteases that can easily hydrolyze it during gastrointestinal digestion (Xing et al. 2021). It must also resist the action of proteases present in the brush border of the intestinal membrane. Regarding this, Gallego et al. (2018) studied the stability to gastrointestinal digestion of the antioxidant peptide SNAAC formed during the production of Spanish dry-cured ham. These researchers observed a significant drop in the antioxidant activity in vitro by β-carotene bleaching assay, ABTS and ORAC assay due to the degradation of this peptide or the alteration of its structure, thus affecting its activity.

On the other hand, gastrointestinal digestion can lead to the generation of new bioactive peptides due to the action of enzymes on oligopeptides and proteins. For example, the amount of carnosine absorbed in the intestine will be determined by that originally present in the food and that which can be released from the food matrix (Xing et al. 2021). Likewise, Ferranti et al. (2014) carried out a gastrointestinal simulation to study the influence of this process on the proteins of the Bresaola, a meat product widely consumed in Italy. After simulation, he identified numerous bioactive peptides – or their precursors – reported in the BIOPEP database as antihypertensive and antioxidant peptides, derived from the hydrolysis of myosin, actin, and sarcoplasmic proteins.

The absorption of peptides can be through different pathways: paracellular, mediated by peptide transporters (for example, PepT1), transcytosis, and transcellular passive diffusion (Xing et al. 2021).

These findings suggest the need for a strategy for the protection and transport of bioactive peptides when they are used in the preparation of functional or nutraceutical foods. Xiaohong's review (2021) reports different bioactive ingredient carrier systems in order to protect them from gastrointestinal digestion and increase their absorption, including complex coacervates, cross-linked polysaccharides, self-assembled microparticles/nanoparticles, and Pickering emulsions. In addition, these carriers also allow masking unfavorable flavor of bioactive peptides, such as bitterness (Table 1).

3 Milk

3.1 Obtaining Milk Peptides

As mentioned in the Introduction, milk and dairy products currently represent the largest source of biologically active peptides. These peptides are generally inactive in the sequence of their precursor proteins and can be extracted by three main processes: enzymatic hydrolysis by commercial enzymes and enzymes derived from microorganisms and plants (in vitro), milk fermentation using proteolytic starter cultures, and enzymatic hydrolysis during gastrointestinal digestion (in vivo) (Rebouillat and Ortega-Requena 2015; Mohanty et al. 2016).

The most common way to produce bioactive peptides from milk proteins is enzymatic hydrolysis (in vitro). For the release of these peptides, commercial digestive enzymes such as *pepsin*, *trypsin*, and *pancreatin*, among others, are mainly used. In addition, different combinations of proteolytic enzymes are used such as *alcalase*, *chymotrypsin*, and *thermolysin* (Albenzio et al. 2017).

Regarding the use of enzymes derived from living microorganisms, enzymes from different bacterial and fungal sources are used. The enzymes produced by lactic acid bacteria (LAB) can be isolated, purified, and used to produce bioactive peptides from milk caseins. The generation of bioactive peptides from enzymes from commercially available microorganisms represents a safe and economically profitable alternative (Phelan et al. 2009).

Another alternative for obtaining bioactive peptides from milk proteins is fermentation through the use of different probiotic microorganisms, especially lactic acid bacteria (LAB), molds, fungi, and yeasts (Mohanty et al. 2016). In addition to these isolated microorganisms, a combination of them (co-cultures) can be used to promote hydrolysis processes.

The production, maturation, and storage of some dairy products, such as cheese and yogurt, among others, generate bioactive peptides through the fermentation process. In the case of cheese, the activities of the starter and non-starter cultures that are used to improve the organoleptic characteristics are those that determine the degree of protein hydrolysis and, consequently, the production of bioactive peptides. The selection of bacterial strains, the nature of the milk, the type of cheese, and the ripening conditions play a fundamental role in the generation of these peptides (Rafiq et al. 2021). During cheese maturation, proteolysis occurs which is a fundamental process for the generation of peptides. In this process, caseins are hydrolyzed by endogenous dairy enzymes such as plasmin, giving rise to large peptides, which are subsequently hydrolyzed, generating smaller peptides by the enzymes of the starter and non-starter cultures (Baptista and Gigante 2021). Maturation conditions such as temperature and time (determinant characteristics of the degree of hydrolysis) contribute to the production of peptides and determine their bioactivity. It has been shown that there is a positive relationship, up to a certain level, between the generation of antihypertensive peptides and the degree of proteolysis of mature cheeses (Rafiq et al. 2021).

Regarding other fermented foods, yogurt is the product obtained by fermentation of milk using a starter culture that generally contains the lactic acid bacteria *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* which are usually added in a 1: 1 ratio. These microorganisms work symbiotically and give the yogurt its typical characteristics. Other LABs can also be added to produce particular characteristic to each type of yogurt (*Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Bifidobacterium*, among others) generating various peptides with different activities (Gouda et al. 2021). During fermentation, both microorganisms cause a significant degree of hydrolysis. Milk has some free amino acids, but some such as cysteine, valine, leucine, among others, are not present in the quantities that are necessary for starters to grow. In that case, *L. delbrueckii ssp. Bulgaricus* can hydrolyze casein, releasing free amino acids and polypeptides, which in turn can be hydrolyzed by *S. thermophilus*. This proteolysis gradually decreases during storage led by complex proteolytic systems of both microorganisms, which are made up of different peptidases and exopeptidases (Mann et al. 2017).

Finally, during gastrointestinal digestion of milk and dairy products, bioactive peptides can be released. During digestion, proteins are hydrolyzed throughout the digestive tract due to the action of enzymes such as pepsin, trypsin, chymotrypsin, and brush border membrane peptidases, releasing peptides of different lengths as well as free amino acids (Sharma et al. 2020). Gastrointestinal digestion of milk and dairy products can lead to the release of new bioactive peptides and the hydrolysis of existing peptides, generating smaller active and inactive peptides. These bioaccessible peptides may exert a local action in the gastrointestinal tract or they may be bioavailable and be absorbed producing their action in different organs/ tissues. The milk matrix is decisive in the digestion process and consequently in the release of peptides, so the bioaccessibility and bioavailability of each particular case must be evaluated (Baptista and Gigante 2021).

3.2 Bioactivity of Milk/Dairy Peptides

Milk proteins have shown many health-promoting properties (antioxidant, antihypertensive, antidiabetic, antiobesity, antitumor, gut, and neurological health), mostly when the bioactive peptides encrypted in their native structure are released by different food processes, such as enzymatic hydrolysis, fermentation, and/or chemical treatment (Nongonierma and FitzGerald 2015).

3.2.1 Antihypertensive Activity

Cardiovascular diseases are one of the major causes of premature deaths worldwide. Angiotensin-converting enzyme I (ACE) converts decapeptide angiotensin I (inactive) into octapeptide angiotensin II (vasoconstrictor) increasing blood pressure that may lead to hypertension (Jakubczyk et al. 2020). Thus, inhibition of this enzyme represents a good strategy for blood pressure control.

The dairy peptides RYLGY, AYFYPEL, YQKFPQY, IPP, and VPP have shown the strongest ACE inhibitory activities (Ali et al. 2021). Particularly, casein-derived peptides IPP, VPP, SKVYP, FFVAPFPEVFGK have been reported for having ACE inhibitory activity tested in humans (Nongonierma and FitzGerald 2015). Whey protein peptides DKVGINYW, DAQSAPLRVY, and KGYGGVSLPEW from enzymatic hydrolysis have also shown ACE inhibitory activity, the latter peptide presenting the highest inhibition, but in vivo assays of the peptides have shown no effect on blood pressure modulation (Jakubczyk et al. 2020). Casein peptide NMAINPSKENLCSTFCK and whey peptides TTFHTSGY and GYDTQAIVQ have shown ACE inhibitory activity (Jakubczyk et al. 2020).

A whey protein hydrolysate short term supplementation of spontaneously hypertensive rats showed decreased systolic and diastolic blood pressure compared to control group, decreased blood pressure along with heart rate under long term supplementation, and inhibited ACE activity (Wang et al. 2012). A low molecular fraction (<1 kDa) of a whey protein isolate hydrolysate showed ACE inhibitory capacity (O’Loughlin et al. 2014).

Parmigiano Reggiano cheese presented 13 peptides which presented ACE inhibition and have shown to reduce blood pressure in vivo, as well as peptide LHLPLP which is present in Grana Padano, Parmigiano Reggiano, Gorgonzola, and Cheddar cheeses (Bouroutzika et al. 2021). Antihypertensive effects on spontaneously hypertensive rats have been found by β-casein-derived peptide KVLPVPQ from a commercial functional yogurt Grana Padano, Parmigiano Reggiano, Gorgonzola, and

Cheddar. Milk peptides have also shown antithrombotic activity (Bouroutzika et al. 2021).

3.2.2 Antioxidant Activity

Oxidative stress is related to many diseases making the use of antioxidants of especial importance to neutralize reactive oxygen species (ROS) in cells/tissues. Milk peptides have shown different levels of antioxidant capacity depending on their amino acid sequence. The type of amino acids composing the N- and C-terminal regions, the presence of aromatic and sulfur-containing amino acids are important factors for antioxidant capacity (Jakubczyk et al. 2020). Fermented dairy products possess antioxidant peptides as a result of bacterial hydrolysis of milk proteins, representing a valuable strategy for peptides release from native dairy proteins.

Particularly, β-lactoglobulin-derived peptide WYSLAMAASDI has shown similar free radical scavenging capacity to the synthetic antioxidant butylated hydroxyanisole (BHA). Moreover, whey derived peptides MHIRL and YVEEL have shown powerful antioxidant capacity (Grażyna et al. 2017).

Some casein enzymatic hydrolysates have shown to have a positive effect on glutathione (GSH) content and *catalase* activity in human cultured cells (Jurkat T cells) (Phelan et al. 2009). Whey protein concentrate enzymatic hydrolysis with trypsin (4.31 h, 41.1 °C, 0.017 enzyme/substrate ratio) also showed strong antioxidant and cytoprotective effect under menadione-induced oxidative stress conditions on epithelial cells of the rat ileum (IEC-18) (Ballatore et al. 2020). Buffalo (*Bubalus bubalis*) milk has been found to exert antioxidant effect on intestinal epithelial cells and erythrocytes (Bouroutzika et al. 2021). Peptides derived from fermented milk (NTVPAKSCQAQPTTM, EDELQDKIHPF, QGPIVLNPWDQVKR, APSFSDIPNPIGSENSE) have been reported for protecting Caco-2 cells from oxidative stress and inhibiting intracellular ROS formation (Jakubczyk et al. 2020). Sheep whey protein has shown the capacity to scavenge DPPH, ABTS+ and OH· radicals along with protecting muscle C2C12 cells under tert-butyl hydroperoxide-induced oxidation conditions, inhibiting ROS formation and improving GSH levels (Kerasioti et al. 2014).

3.2.3 Antidiabetic

Diabetes mellitus is a non-communicable chronic disease that comprises a metabolic disorder and is a pandemic of this century. One of the strategies that have been studied along the past years involve the search for natural sources of bioactive compounds with the capacity to modulate blood glucose levels by inhibiting digestion carbohydrases to avoid high postprandial glucose levels (Nongonierma and FitzGerald 2015). Other strategies involve regulating pancreatic β-cell insulin secretion through glucose-dependent insulinotropic (GIP) and glucagon-like peptide-1 (GLP-1), and inhibiting dipeptidyl peptidase-IV (DPP-IV) which degrades/inactivates incretin hormones (Nongonierma and FitzGerald 2015), among others.

Milk-derived peptides regulate serum glucose levels by modulating metabolic enzymes such as α-amylase, α-glucosidase, and DPP-IV in addition to incretin secretagogue action and insulinotropic activity (Nongonierma and FitzGerald

2015). Particularly, camel milk peptides seem to have a positive effect on the insulin receptor enhancing its signal and to control insulin synthesis and secretion by improving pancreatic β-cells (Ayoub et al. 2018). VPV, YPI, and VPF camel whey-derived peptides presented DPP-IV inhibition (Zhou et al. 2021). Goat milk casein-derived peptides SDIPNPIGSE, NPWDQVKR, SLSSSEESITH, and QEPVLGPVRGPFP, which are also found in sheep, buffalo, and cow, have shown to significantly improve glucose metabolism in insulin-resistant HEPG-2 cells. Other goat milk-derived peptides MHQPPQPL, SPTVMFPPQSVL, VMFPPQSVL, INNQFLPYPY, and AWPQYL have shown DPP-IV inhibitory activity, as well as camel milk peptides FLQY, FQLGASPY, ILDKEGIDY, ILELA, LLQLEAIR, LPVP, LQALHQGQIV, MPVQA, and SPVVPF (Antony and Vijayan 2021). Casein-derived peptide IPPKKNQDKTE modulated the IRS-1/PI3K/Akt signaling pathway via AMPK activation in addition to inhibition of insulin resistance in HepG2 cells under high glucose-induced conditions (Zhou et al. 2021). Derived peptides from whey protein isolate and α-lactalbumin LKPTPEGDL and LKPTPEGDLEIL presented inhibition of DPP-IV (Zhou et al. 2021). β-lactoglobulin peptides, especially IPAVF fragment [β-lactoglobulin f(78–82)], have shown to inhibit DPP-IV (Kondrashina et al. 2020).

3.2.4 Gut Health Improvement

Gut health may improve by enhancing beneficial microbiota growth and unfavoring pathogenic strains growth, as well as by promoting intestinal epithelial cells health.

Dairy products such as milk, yogurt, and kefir have shown an increase in beneficial genera Lactobacillus and Bifidobacterium. Particularly, β-lactoglobulin peptides obtained after proteolytic digestion and lactoferrin hydrolysates have shown a beneficial effect on the proliferation of beneficial microbiota. In addition, yogurt has also shown a reduction of the pathogenic strain Bacteroides fragilis improving gut health (Aslam et al. 2020).

On the other hand, gut health is also promoted by the elimination of certain pathogenic strains by milk peptides such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus carnosus*, *Staphylococcus epidermidis*, *Staphylococci*, *Listeria monocytogenes*, *Clostridium perfringens*, *Klebsiella pneumoniae*, and *Candida albicans* (Aslam et al. 2020; Bouroutzika et al. 2021).

The MBCP peptide found after the simulation of digestion of Mozzarella di Bufala Campana DOP may improve intestinal epithelial health by ameliorating inflammation and hypermotility, which was tested in murine models of intestinal inflammation (Bouroutzika et al. 2021).

3.2.5 Other Bioactivities

Obesity is a result of ingesting more energy (food) than what the organism needs. This epidemic is the main risk factor for type 2 diabetes and cardiovascular diseases. Thus, controlling appetite and/or fatty acids absorption in the intestine might be essential strategies for body weight control. In this sense, dairy peptides derived from caseins such as β-casomorphin-7 (β-CM7, YPFPGPI), glycomacropeptide

(GMP, 106–169 AA), GPVRGPFPIIV (199–209 AA), casoxin C (YIPIQYVLSR), RF dipeptide, as well as some whey peptides have been related with satiety (Kondrashina et al. 2020). Peptides derived from caseins have shown to modulate satiety by increasing the release of gut hormones (CCK) and inhibiting gastric secretions (Nongonierma and FitzGerald 2015).

Another bioactivity is antitumor activity, which bovine α-, β-, and κ-casein proteins (α-casein being the most potent) have shown to reduce the migration of human breast cancer cells and murine mammary tumor cells. Bovine casomorphins (peptides derived from α- and β-caseins) have shown antiproliferative effects on some human prostatic cancer cell lines and breast cancer cells. Casein phosphopeptides have shown modulation of proliferation and apoptosis depending on intestinal tumor HT-29 cell differentiation (Leischner et al. 2021).

Regarding whey proteins, bovine lactoferrin has inhibited cell migration in a human glioblastoma model, affected tumor cell growth, inhibited colon carcinoma in rats, induced apoptosis in human oral squamous cell carcinoma cells SAS, and human myeloid leukemia cells (HL-60). Lactoferricin, α-lactalbumin (α-LA), bovine α-lactalbumin made lethal to tumor cells (BAMLET), β-lactoglobulin (β-LG) has also shown antitumor activity (Leischner et al. 2021).

Milk proteins have also shown to exert neurological health improvements. Whey peptide rich in tryptophan-tyrosine-related peptides was found to improve the cognitive performance of healthy adults with a self-awareness of cognitive decline in a randomized, double-blind, placebo-controlled design (Kita et al. 2018). A randomized, double-blind, placebo-controlled study on the supplementation with β-lactolin whey peptide [β-lactopeptide of glycine-thereonine-tryptophan-tyrosine (GTWY)] has shown to improve the cognitive performance of healthy older adults associated with the frontal cortex activity (Kita et al. 2019).

3.3 Bioaccessibility and Bioavailability of Milk Peptides

For bioactive peptides to exert their health promoting properties, they have to resist the conditions of the gastrointestinal tract, having the possibility to have an effect directly on the intestine or being absorbed at the intestine to reach the tissue of interest. The molecular size, amino acid composition, and hydrophobicity may determine the stability and bioavailability of bioactive peptides.

Casein and whey-derived peptides (β-lactoglobulin 125–135, sequence TPEVDDEALEK) after simulation of digestion have been reported for their transepithelial transport across human intestinal cells Caco-2 (Picariello et al. 2013). A casein hydrolysate-derived peptide (VLPVPQK) has been reported for hydrolyzing by the brush-border peptidases of Caco-2 cells and their transepithelial transport through PepT1 like transporters/SOPT2 (Vij et al. 2016). Casein alcalase hydrolysates fractions have been reported for being bioavailable when assayed by carrying out the simulation of digestion followed by Caco-2 cell transepithelial transport, especially for the most hydrophobic fraction (Xie et al. 2015). Stuknytė et al. (2015) showed that casein-derived peptides VPP, IPP, HLPLP, and LHLPLP were found in the bioaccessible fractions of Cheddar, Gorgonzola, Maasdam, and Grana Padano cheeses which presented inhibition of angiotensin converting enzyme.

Whey bioaccessible peptides have been recently identified (IWCKDDQNPH, KFLDDDLTDDIM, and DKFLDDDLTDDIM), among which the peptide with the sequence IWCKDDQNPH showed the highest antioxidant capacity (IC50 = 0.015 ± 0.002, 0.45 ± 0.02, and 1.30 ± 0.05 mg/mL, determined by ORAC, ABTS, and HORAC, respectively) (Báez et al. 2021).

Regarding dairy products from buffalo-milk, the ricotta cheese digest showed antioxidant capacity by DPPH• test (EC50 = 1.91 mg/mL). Moreover, a bioaccessible peptide from β-lactoglobulin of buffalo ricotta cheese (YVEELKPTPEGDL, f:60–72) showed an oxidative stress reduction on hydrogen peroxide (H2O2)-induced intestinal epithelial cells (IEC-6 cell line). This reduction was associated to the activation of Nrf2 pathway that leads to the expression of cytoprotective enzymes (Basilicata et al. 2018).

The infant simulation of digestion of goat and cow milk showed similar peptides mostly derived from casein proteins, some novel identified peptides, and some of them previously reported for having bioactive properties (Hodgkinson et al. 2019). MBCP peptide from Mozzarella di Bufala Campana DOP bioaccessible fraction has shown good bioavailability by determining the transepithelial transport through the intestinal monolayer (Bouroutzika et al. 2021) (Table 2).

4 Egg

4.1 Obtaining Egg Peptides

Egg proteins are an important source of biologically active peptides; they can be obtained by in vitro and in vivo proteolytic processes, such as enzymatic hydrolysis. But they are also generated naturally in the egg during ambient storage or during food processing.

Liu et al. (2018) reported that the content of peptides in the egg white fraction of <10 kDa and the fraction of <3 kDa increased gradually during storage at room temperature. These fractions are associated with the formation of 6 peptide fragments from ovotransferrin and 11 peptides from ovomucin. Since these proteins are related to antimicrobial activity, the degradation of these proteins during storage contributes to egg white thinning and increased susceptibility to bacterial contamination.

The main procedure used to obtain peptides has been hydrolysis with commercial food-grade proteolytic enzymes which are used singly or in combination, the most commonly used being pepsin, trypsin, chymotrypsin, alcalase, and papain.

Dávalos et al. (2004) optimized the use of pepsin for the hydrolysis of egg white, achieving an improvement in antioxidant activity in 3 h at pH 2 and 37 °C with an enzyme to substrate ratio of 1/100. In turn, there are examples in which the combined use of enzymes allows an improvement in bioactivity, obtaining better yields. In the case of the work published by Yuan et al. (2020), using combinations of pepsin,

Table 2 Bioactive peptides in milk. Protein source, obtaining method, bioactivity and peptide sequence

Matrix	Protein	Obtaining method	Bioactivity	Peptide sequence	Reference
Milk	Whey		Antioxidant	MHIRL and YVEEL	Grażyna et al. (2017)
	β-Lactoglobulin	Pepsin, pancreatic proteases and thermolysin	Antioxidant	WYSLAMAASDI	Grażyna et al. (2017)
	Casein	Enzymatic hydrolysis	Antioxidant		Phelan et al. (2009)
	Whey protein concentrate	Enzymatic hydrolysis	Antioxidant		Ballatore et al. (2020)
	"Mozzarella di Bufala Campana DOP" peptides	In vitro simulation of digestion	Antioxidant		Bouroutzika et al. (2021)
	Buffalo (Bubalus bubalis) milk		Antioxidant		Bouroutzika et al. (2021)
	Milk	Fermented milk	Antioxidant	NTVPAKSCQAQPTTM, EDELQDKIHPF, QGPIVLNPWDQVKR, APSFSDIPNPIGSENSE	Jakubczyk et al. (2020)
	Sheep whey		Antioxidant		Kerasioti et al. (2014)
	Casein	Fermented milk, cheese	Antihypertensive	IPP, VPP, SKVYP, FFVAPFPEVFGK	Nongonierma and FitzGerald (2015)
	Whey protein peptides	Ezymatic hydrolysis	Antihypertensive	DKVGINYW, DAQSAPLRVY, and KGYGGVSLPEW. TTFHTSGY and GYDTQAIVQ	Jakubczyk et al. (2020)
	Casein		Antihypertensive	NMAINPSKENLCSTFCK	Jakubczyk et al. (2020)
	Whey	Enzymatic hydrolysis	Antihypertensive		Wang et al. (2012)
	Whey	Enzymatic hydrolysis	Antihypertensive		O'Loughlin et al. (2014)

	Parmigiano Reggiano cheese		Antihypertensive	13 peptides	Bouroutzika et al. (2021)
	Grana Padano, Parmigiano Reggiano, Gorgonzola, and Cheddar cheeses		Antihypertensive	LHLPLP	Bouroutzika et al. (2021)
	β-Casein		Antihypertensive	KVLPVPQ	
	Camel milk		Antidiabetic		Ayoub et al. (2018)
	Camel whey		Antidiabetic	VPV, YPI and VPF	Zhou et al. (2021)
	Goat, sheep, buffalo, and cow milk casein		Antidiabetic	SDIPNPIGSE, NPWDQVKR, SLSSSEESITH, and QEPVLGPVRGPFP	Antony and Vijayan (2021)
	Goat milk		Antidiabetic	MHQPPQPL, SPTVMFPPQSVL, VMFPPQSVL, INNQFLPYPY and AWPQYL	Antony and Vijayan (2021)
	Camel milk		Antidiabetic	FLQY, FQLGASPY, ILDKEGIDY, ILELA, LLQLEAIR, LPVP, LQALHQGQIV, MPVQA, and SPVVPF	Antony and Vijayan (2021)
	Casein		Antidiabetic	IPPKKNQDKTE	Zhou et al. (2021)
	Whey protein isolate and α-lactalbumin		Antidiabetic	LKPTPEGDL and LKPTPEGDLEIL	Zhou et al. (2021)
	β-Lactoglobulin		Antidiabetic	IPAVF	Kondrashina et al. (2020)
	β-Lactoglobulin	Proteolytic digestion	Gut health improvement		Aslam et al. (2020)
	Lactoferrin	Enzymatic hydrolysis	Gut health improvement		Aslam et al. (2020)
	Milk		Gut health improvement		Aslam et al. (2020), Bouroutzika et al. (2021)
	Mozzarella di Bufala Campana DOP	In vitro simulation of digestion	Gut health improvement	MBCP	Bouroutzika et al. (2021)

(continued)

Table 2 (continued)

Matrix	Protein	Obtaining method	Bioactivity	Peptide sequence	Reference
	Casein		Antiobesity	β-Casomorphin-7 (β-CM7, YPFPGPI), glycomacropeptide (GMP, 106–169 AA), GPVRGPFPIIV (199–209 AA), casoxin C (YIPIQYVLSR), RF dipeptide	Kondrashina et al. (2020), Nongonierma and FitzGerald (2015)
	Casein		Antitumor	Casein proteins, bovine casomorphins and casein phosphopeptides	Leischner et al. (2021)
	Whey		Antitumor		Leischner et al. (2021)
	Whey		Neurological health improvement	Tryptophan-tyrosine-related peptides, GTWY	Kita et al. (2018, 2019)

chymotrypsin, and 2.4 L alkaline to hydrolyze egg white protein, they found that the combination of pepsin and chymotrypsin increases the cleavage sites allowing the release of a greater number of aromatic amino acid residues which present antioxidant effects.

These enzymes are usually preferred over natural ones because the site of action and optimal hydrolysis conditions are known. But new naturally occurring enzymes are also often used in order to reduce production costs and improve the sustainability of food systems using different plant or fungal matrices. Some recent studies have purified fungal proteases, such as the case of the protease purified from Aspergillus avenaceus URM 6706. This protease was used to hydrolyze egg white releasing peptides with antioxidant activity, finding a positive correlation between activity and the degree of hydrolysis, being the conditions of hydrolysis suitable for industrial application.

Another way to increase the degree of hydrolysis of egg proteins is by modifying the structure of the protein so that the binding site with the enzyme is more accessible. In the case of phosvitin protein (a phosphorylated protein, which is resistant to enzymatic hydrolysis due to its extremely high negative charge), Yoo et al. (2017) applied hydrostatic pressure combined with enzymatic hydrolysis and managed to optimize the phosvitin hydrolysis process. The authors obtained short phosvitin-phosphopeptides (<3 kDa) with greater antioxidant power and antimicrobial activity than that of the peptide hydrolysate produced at atmospheric pressure. Another example of the application of high hydrostatic pressure (HHP) was carried out on ovalbumin. Quirós et al. (2007) used three digestive enzymes for the hydrolysis of ovalbumin; the combined use with HHP caused the formation of dimers in ovalbumin, making it more accessible to enzymes, facilitating the hydrolysis process and thus shortening the incubation times.

The combination of enzymatic hydrolysis with alcalase with the application of a high intensity pulsed electric field (PEF) on egg white protein has also been used. Through this treatment, it was possible to increase the antioxidant properties of the hydrolysates compared to the systems without the application of PEFs (Zhang et al. 2021).

At laboratory level, the purification of peptides after hydrolysis is carried out by applying separation techniques such as ultrafiltration or liquid chromatography to later identify the most bioactive fractions by mass spectrometry. But, on an industrial scale, there are limitations in the commercial production of peptides. One of the separation techniques that is available once the hydrolysis has been carried out is membrane separation (Dávalos et al. 2004).

To fulfill their biological activity at the organism level, the peptides must be bioavailable at the site of action. For this, they must first resist passage through the gastrointestinal tract, due to the action of enzymes and the pH of the stomach, and then be absorbed and transported to the site of action (Remanan and Wu 2014). In some cases, it may happen that fragments that have little in vitro activity are capable of acquiring activity in vivo, thanks to the action of digestive enzymes on them; in other cases, activity is lost. In all cases, once it has been proven that bioactive peptides are bioaccessible, it is necessary to test their efficacy using different in vivo experimental models (Yu et al. 2016).

4.2 Bioactivity of Egg Peptides

These peptides have been reported for possessing health-promoting properties, recently being the focus of numerous investigations. Recent research has found that bioactive peptides obtained from egg proteins have different activities such as antihypertensive, antioxidant, anticancer, antimicrobial, and bone growth promoters (Eckert et al. 2014; Carrillo and Ramos 2018; Zheng et al. 2020).

4.2.1 Antihypertensive Activity

The antihypertensive activity has been one of the most studied, registering several peptides derived from egg proteins with said activity. The first peptide identified with bioactive properties from egg protein was a (FRADHPFL) octapeptide, which corresponded to residues 358–365 of ovalbumin. This peptide exhibited antihypertensive properties and was named ovokinin (Fujita et al. 2000).

Fujita et al. (2000) report the isolation and characterization of ovokinin. Ovokinin showed relaxing activity for a canine mesenteric artery, finding that its effect was, in part, mediated by B1 receptors, which stimulate the release of prostacyclin. In studies from the same group, they found that ovokinin showed antihypertensive effects when administered at a dose of 100 mg/kg to hypertensive rats. And this effect was enhanced when the peptide was administered emulsified in egg yolk. This effect would be associated with the fact that the phospholipids in the egg yolk protect the peptide from enzymatic digestion, improving the absorption and availability of ovokinin (Fujita et al. 2000).

Dávalos et al. (2004) studied the peptides obtained from the enzymatic hydrolysis of egg white albumin with pepsin, finding that these peptides had an effect on the angiotensin converting enzyme (ACE) -inhibitory properties. ACE controls blood pressure because it is responsible for the generation of the vasoconstrictor agent angiotensin II and for the inactivation of the vasodilator agent bradykinin and thus, ACE inhibitors are effective in the prevention and treatment of essential hypertension. Said activity was related to the peptide YAEERYPIL given the presence of amino acids Ile and Leu, associated with its vasodilating effect.

Three of the digestive enzymes most used to hydrolyze egg white proteins are pepsin, trypsin, and chymotrypsin. Hydrolysates obtained with these enzymes have angiotensin converting enzyme (ACE) – inhibitory properties. This activity was influenced by the hydrolysis time (the longer the time, the higher the activity) and by the type of enzyme, being the ones hydrolyzed with pepsin the most active. Hydrolyzing for 3 h with pepsin showed a potent ACE inhibitory activity, its IC50 value being 55 μg/mL. After ultrafiltration of this hydrolysate, a fraction with a molecular mass of less than 3000 Da was obtained, which presented an IC50 value of 34 μg/mL. In this fraction, three sequences were identified with ACE inhibitory activity YAEERYPIL, RADHPFL, and IVF which presented IC50 values equal to 4.7, 6.2, and 33.11 μM, respectively (Miguel et al. 2004).

In turn, it was found that they exhibit significant antihypertensive effects in reducing blood pressure in spontaneously hypertensive rats (SHR) due to chronic and acute administration of the egg white hydrolysate obtained by hydrolysis with pepsin. These studies in hypertensive animals made it possible to relate the antihypertensive effect with the inhibition of ACE in vivo, with a reduction in oxidative stress and in plasma levels of cholesterol and TG (Miguel et al. 2006)

Other antihypertensive peptides are released from egg white proteins by treatment with Alcalase enzymes (Liu et al. 2010; Yu et al. 2011a). Liu et al. (2010) identified the peptide RVPSL from the hydrolysis of ovotransferrin with Alcalase, which presents a high inhibitory activity of ACE (20 μmol/L). In turn, Yu et al. (2011a) isolated three peptides from egg white hydrolysate digested with Alcalase and reported that the one with the highest ACE inhibitory potential was the QIGLF peptide with an IC50 value of 75 μmol/L and low susceptibility to gastrointestinal enzymes.

Treatment of ovalbumin at high hydrostatic pressure (300–400 MPa, 60 min) increased the susceptibility of ovalbumin to proteolysis by chymotrypsin and trypsin, accelerating the release of peptides in studies. However, the ACE inhibitory activity does not improve with respect to atmospheric pressure conditions (Quirós et al. 2007).

Eckert et al. (2014) obtained a yolk hydrolysate using a protease of plant origin (extracted from Cucurbita ficifolia) for 4 h that showed ACE activity in vitro. From this hydrolysate, two peptides that showed a much higher ACE inhibitory activity were isolated and identified: LAPSLPGKPKPD (IC50 = 1.97 μmol/L) and ITMIAPSAF (IC50 = 3.24 μmol/L).

Zambrowicz et al. (2013) obtained a hydrolysate of the protein fraction of the egg yolk with pepsin for 2 h that also showed ACE inhibitory activity as well as other properties. Between the four peptides isolated and identified in this study, the YINQMPQKSRE sequence demonstrated ACEI activity (IC50 = 10.1 μg/mL).

4.2.2 Antioxidant Activity

Antioxidant activity is another of the bioactive properties studied on egg protein hydrolysates. As in the previous cases, the egg antioxidant peptides play an important role in the quality and shelf life of foods and in their effect on human health by inhibiting oxidative stress.

Antioxidant peptides obtained from hydrolysis of egg white have been identified by several authors. The first peptides have been identified with antioxidant activity by a variety of assays with different mechanisms, including H-atom transfer (HAT) or e – transfer (ET) and lipid peroxidation assays. Dávalos et al. (2004) identified four peptides of the hydrolysates of egg white with pepsin. These peptides present higher radical scavenging activity than that of Trolox and with lipid peroxidation inhibition ability. Another study with hydrolysates obtained with pepsin presented hydroxyl and superoxide anion radical-scavenging activities; those activities depend on the molecular weight of the generated peptides, showing the fraction with

2–5 kDa more activity. In turn, antioxidant activity has also been correlated in egg peptides with the presence of certain amino acids such as tyrosine, methionine, leucine, aspartic acid, serine, glutamic acid, and lysine (Liu et al. 2018).

In order to understand the behavior of egg antioxidant peptides at the molecular level, chemical studies have been complemented with cellular studies. Zhang et al. (2019) evaluated the antioxidant activity of the peptide VYLPR obtained from the hydrolysates of egg-white protein with alcalase and flavorzyme. This peptide exhibited the strongest protective effect on HEK-293 cells; it could inhibit lipid peroxidation process, maintain cell membrane integrity, inhibit intracellular LDH activity, reduce MDA content, and improve the activity of antioxidant enzyme T-SOD and GSH-Px. Liu et al. (2014) studied the anti-oxidative and anti-apoptosis effects of WNWAD derived from egg white ovomucin pepsin hydrolysates. This peptide presented oxygen radical absorption capacity (ORAC). At the cellular level, it inhibited H2O2-induced cellular oxidative stress and reduced intracellular ROS accumulation in HEK-293 cells (Yuan et al. 2020).

Some egg yolk proteins, such as fosvitin, are characterized by their high antioxidant and metal chelating activity (Chay Pak Ting et al. 2011). This has led to a search for these properties in hydrolysates. For example, the combined action of the enzymes Alcalase and protease N of delipidated egg yolk proteins was used, generating peptides to exhibit antioxidative stress properties. The egg yolk phosphopeptides supplementation was studied in an in vitro hydrogen peroxide-induced Caco-2 intestinal cell culture, observing a significant reduction in the pro-inflammatory IL-8 cytokine. In an animal model of intestinal oxidative stress, egg yolk phosphopeptides induced glutathione synthesis and gamma-glutamylcysteine synthetase mRNA expression and activity, increased antioxidative enzymes activities stress, in particular catalase and glutathione S-transferase activities, and reduced lipid and protein oxidation in the duodenum, jejunum, ileum, and colon (Young et al. 2010).

Ishikawa et al. (2004) found that fosvitine and hydrolysates protect against the formation of iron-catalyzed hydroxyl radicals and protect DNA against oxidative damage induced by Fe (II) and peroxide, suggesting that fosvitine may be useful for prevention of iron-mediated oxidative stress related to non-communicable diseases such as cancer. The iron binding capacity is associated with the presence of numerous phosphoserine residues, grouped in the amino acid sequence of fosvitine. The antioxidant activity of fosvitine and the phosvitin-galactomannan conjugate was evaluated in a powdered oil model system. Nakamura et al. (1998) produced a new macromolecular antioxidant by conjugating phosvitin with galactomannan, which could withstand a sterilization treatment at 121 °C for 15 min. Xu et al. (2007) reported that fosvitin peptides have stronger antioxidant activity in a linoleic acid system compared to fosvitin, which could be attributed to changes in phosphorus content and amino acid sequence.

In turn, the presence of peptides with antioxidant properties had also been identified during egg storage. Zheng et al. (2020) identified six peptides with antioxidant properties in egg white for reduced formation of superoxide and increased levels of superoxide dismutase (SOD) and catalase (CAT) in L6 skeletal muscle cells.

The digestive simulation study is important as an intermediate stage prior to in vivo studies to evaluate the bioavailability of peptides. Remanan and Wu (2014) determined the antioxidant activity in cooked and simulated digested egg; they found that although cooking the eggs reduced their antioxidant activity, peptides derived from ovalbumin (DSTRTQ, DVYSF and ESKPV) were generated, which was associated with an increase in antioxidant activity. Jahandideh et al. (2016) observed a reduction in tissue oxidative stress in spontaneously hypertensive rats after the administration of cooked and digested egg white by a digestive simulation study.

4.2.3 Antimicrobial Activity

Lysozyme, avidin, and ovotransferrin are the more studied antimicrobial proteins in egg white. Lysozyme is a bacteriolytic enzyme, avidin binds biotin and a protein that combines with riboflavin reducing the availability of these factors to microorganisms, and ovotransferrin chelating iron depriving necessary for bacterial growth. Antimicrobial activity was also reported for ovoinhibitor, ovomucoid, ovostatin, ovomacroglobulin, and cystatin (Carrillo and Ramos 2018).

But although integral proteins show activity, it has been seen that it increases during the formation of hydrolysates. Mine et al. (2004) obtained peptides with antimicrobial activity from lysozyme hydrolysates from egg white. The peptide (IVSDGDGMNAW), corresponded to amino acid residues 98–108 of lysozyme, inhibited the Gram-negative bacterium (Escherichia coli K-12) and the other peptide having the sequence (HGLDNYR), corresponding to amino acid residues 15–21 of lysozyme, inhibited the Gram-positive bacterium (*Staphylococcus aureus* 23–394). These peptides amplify the antimicrobial activity of lysozyme, which is why it is indicated that lysozyme has non-enzymatic bacteriostatic domains in its primary sequence and are released by proteolytic hydrolysis. Carrillo and Ramos (2018) studied egg white lysozyme hydrolysates with pepsin and identified 23 antibacterial peptides, with antibacterial activity against *Escherichia coli* and *Staphylococcus carnosus*.

In July 2018, the European Union (EU) EU 2018/991 approved the use of lysozyme hydrolysates from egg white for use in food supplements for the adult population. Additionally, the Food and Drug Administration (FDA) recognized lysozyme hydrolysate from chicken egg whites as food (GRAS), allowing its use as an ingredient in supplements.

4.2.4 Other Bioactivities

With age, the population tends to develop different bone diseases and it has been seen that calcium supplements can prevent their appearance. It has been observed that the formation of bioactive egg peptides bound to Calcium shows good solubility, absorbability, and high bioavailability of calcium. The formation of egg white-calcium chelate peptides (EWPs-Ca) was studied, obtaining, under the optimal conditions, a Calcium content of 44.1 mg/kg (peptide: calcium mass ratio of 4: 1 in 53 °C and pH 8.2 for 30 min). Using ultraviolet-visible (UV) absorption spectroscopy and Fourier transform infrared spectroscopy (FTIR), they indicated that the carboxyl oxygen and amino nitrogen atoms of egg white peptides (EWP) can be chelated with calcium during chelation. And from the amino acid analysis, it was found that glutamate, aspartic acid, cysteine, threonine, glycine, and lysine are essential during chelation. Absorption over time was studied using Caco-2 cell monolayers, going from 46.18 ± 2.12 to 123.61 ± 1.59 μg/mL from 30 to 180 min. In turn, it was observed that the proliferation rate of human fetal osteoblast cells and the activity of alkaline phosphatase (ALP) improve compared to the target (Walters et al. 2018).

It has been recognized that peptides obtained from eggs have a potential action on diabetes, participating in the regulation of postprandial hyperglycemia that can be controlled by inhibition of the dipeptidyl peptidase IV (DPP-IV) enzyme. Zhao et al. (2020) identified three new peptidase IV (DPP-IV) inhibitor peptides ADF, MIR, and FGR, from myosin and lysozyme of hen eggs. Molecular docking simulation exhibits that tripeptide activities may be due to the hydrogen bond interactions. Peptides from egg white have also been found to show potential anti-diabetic with α-glucosidase and α-amylase inhibitory activities to inhibit carbohydrate cleavages in the small intestine. Yu et al. (2011b) identified the RVPSLM peptide with inhibitory effect of α-glucosidase potential of using egg white protein hydrolysates as a functional food product with the anti-diabetic activity.

Another example of a combined potency effect was published by Zhao et al. (2020) who identified three peptides with an effect on Angiotensin converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) being important in the therapeutic target for the treatment of hypertension and diabetes (Table 3).

5 Application of Bioactive Peptides

Animal bioactive peptides are used in different products such as food, functional food, nutritional products, medicine, animal feed, and cosmetics. One of the technological applications of peptides is related to antioxidant and antimicrobial properties, which make peptides a desirable option to extend the shelf life of foods as they are a natural food additive.

On the other hand, bioactive peptides derived from food are very important in medicine due to their biological activity. Most of the drugs approved in the USA in recent years have peptides in their formulations, due to their great versatility, varied pharmacological functions, high specificity, and low levels of toxicity. Currently, there are more than 100 peptide drugs on the world market (Jia et al. 2021).

At present, only some of the bioactive peptides are functional foods on the market. The possible reason is that most of them do not have sufficient evidence in terms of efficacy and safety. Bioactive peptides are currently used mainly in food, beverages, tablets, capsules, powders, or liquids (Du et al. 2019). Additionally, different processing methods have a certain impact on its bioavailability, which affects its application in food (Chalamaiah et al. 2019).

Although some biologically active peptides show strong physiological functions in vitro, their application in vivo often has low bioavailability due to peptidase

Table 3 Bioactive peptides in egg. Protein source, obtaining method, bioactivity and peptide sequence

Matrix	Protein	Obtaining method	Bioactivity	Peptide sequence	Reference
Egg	Egg white	Pepsin/corolase	Antihypertensive	RADHP	Miguel et al. (2006)
	Egg white	Alcalase	Antihypertensive	RVPSL	Liu et al. (2010)
	Egg white	Pepsin	Antihypertensive	IRW, IQW, LK	Jahandideh et al. (2016)
	Egg white		Antihypertensive	RADHPFL, FRADHPFL, YAEERYPIL	Miguel et al. (2004)
	Egg white	Alcalase	Antihypertensive	QIGLF	Yu et al. (2011a)
	Egg white	Pepsin	Antihypertensive	YINQMPQKSRE	Zambrowicz et al. (2013)
	Egg white	Pepsin	Antihypertensive	YAEERYPIL, RADHPFL, IVF	Miguel et al. (2004)
	Egg white	Alcalase and flavorzyme	Antioxidant	VYLPR	Zhang et al. (2019)
	Egg white	Chymotrypsin and pepsin	Antioxidant		Yuan et al. (2020)
	Egg white	Enzymatic hydrolysis	Antioxidant	STDVPRDPWVWGSAHPQAQHTR, GDPSAWSWGAEAHS, and ALGEDIVDLDSFSEQH	Zheng et al. (2020)
	Egg white	Alcalase	Antioxidant	FFGFN, MPDAHL, DHTKE	Liu et al. (2018)
	Egg white	Pepsin, trypsin, and chymotrypsin	Antioxidant/ antihipertensive	YAEERYPIL/YQIGL/YRGGLEPING/ YPI	Dávalos et al. (2004)
	Egg white	Alcalase	Antihypertensive/ antioxidant/anticoagulation	RVPSLM	Yu et al. (2011b)
	Egg white	Alcalase	Anxiolytic/antihypertensive	TNGIIR	Yu et al. (2016)
	Egg white ovoalbumin	Pepsin	Antihypertensive	FRADHPFL	Fujita et al. (2000)

(continued)

Table 3 (continued)

Matrix	Protein	Obtaining method	Bioactivity	Peptide sequence	Reference
	Egg white ovoalbumin	In vitro simulation of digestion	Antioxidant	DSTRTQ, DVYSF and ESKPV	Remanan and Wu (2014)
	Egg white ovoalbumin	Pepsin	Antihypertensive	LW	Fujita et al. (2000)
	Egg white ovomucin	Pepsin	Antioxidant/antitumor	WNWAD	Liu et al. (2014); Yuan et al. 2020
	Egg white lysozyme		Antibacterial	AWIRGCRL, WIRGCRL, IRGCRL, RAWVAWRNR	Carrillo and Ramos (2018)
	Egg white lysozyme	Pepsin	Antimicrobial	IVSDGDGMNAW, HGLDNYR	Mine et al. (2004)
	Egg white myosin and lysozyme	Pepsin and trypsin	Antidiabetic/ antihypertensive	ADF, MIR, and FGR	Zhao et al. (2020)
	Egg yolk phosvitin	Tripsin	Antioxidant		Xu et al. (2007)
	Egg yolk phosvitin	Commercial enzymes	Antioxidant		Chay Pak Ting et al. (2011)
	Egg yolk peptides	Alcalase and protease	Antioxidant		Young et al. (2010)

activity in the human intestine and plasma, which limits their applications (Xu et al. 2019).

Furthermore, bioactive peptides often have a bitter taste and hygroscopicity. These challenges prevent the applications of bioactive peptides as functional foods and nutraceuticals (Sun et al. 2021).

Hence, it is essential to overcome these limitations for bioactive food-derived peptides to have applications without compromising bioavailability. There are some technological tools to improve the bioavailability of bioactive peptides and increase their application in food. Some of these techniques are enzyme inhibitors, penetration enhancers, nanoparticles, lipid-based nanocarriers, microparticles, microcapsules, liposomes, emulsions, hydrogels, viscose systems, cyclodextrin, site-specific delivery, chemical modification, prodrug derivatization, membrane transporter targeting, and cell-penetrating peptides (CPPs) (Xu et al. 2019). Encapsulation is a promising technology for the protection, release, and delivery of bioactive peptides to enhance their physiological effects, as well as to enhance their sensory and physicochemical properties (Sun et al. 2021).

Generally, food peptides require drying to powder for subsequent applications. The high cost of production, poor solubility of purified powdered peptides, and consumer acceptance are among some of the challenges for commercializing food-derived bioactive peptides. It is necessary to consider the effect of drying on functional properties such as bioactivity and bioavailability. It is also necessary to take into account the state of the peptide particles after drying, including moisture content, shelf-life, solubility, ease of use, taste/odor control, and others (Sun et al. 2021).

The drying processes found in the literature are divided. On the one hand, into direct drying processes of the peptides or together with the use of adjuvants (maltodextrins or starch) (Ma et al. 2014) and, on the other hand, in microencapsulation processes (Sun et al. 2021). The main objective of the first two categories is to manufacture peptide-rich powders for use as food or nutraceutical ingredients, while the main objective of the last category is to maintain bioactivity or mask the smell/taste of the peptide.

An example of the use of these technologies is chicken meat after being subjected to a hydrolysis and spray drying process. By modifying the process variables, bioactive peptides with optimal functional characteristics and good yields can be obtained. Higher inlet temperature in spray drying results in lower moisture and bulk density of the powder, as well as a spherical shape of the hydrolyzed powder (Kurozawa et al. 2009). In addition, the high inlet air temperature (170–200 °C) in spray drying can also bring advantages such as higher antioxidant activity of chicken meat hydrolysate powder (Petruczynik and Waksmundzka-Hajnos 2013). On the contrary, a lower drying temperature (120–160 °C) combined with a low feed flow rate could minimize the difference between the inlet and outlet temperature in spray drying, in order to achieve high recovery for chicken peptides. Although freeze-dried chicken skin hydrolysate showed higher water solubility, emulsion stability, and water retention capacity than spray-dried powders, spray-drying is still preferable due to its high production efficiency (Abou-Diab et al. 2021).

Another application that has been reported of the use of peptides is the possibility to create edible colloidal nanoparticles via the "bottom-up" self-assembly approach. These nanoparticles allow the formation of the so-called pickering foams and emulsion have many advantages in the stabilization. An example of application of these nanoparticles can be found in the work carried out by Xu et al. (2019). They used egg yolk peptides (EYPs) to make edible nanoparticles that acted as foam stabilizers that can have sustainable applications in food, cosmetics, and personal care products.

In summary, the complete set of reported bioactivities plus future research on bioavailability efficacy and safety through clinical studies together with the efficient and sustainable production of these peptides will reveal the full scope of potential applications of these natural molecules.

6 Conclusion

The sequences of several animal bioactive peptides have been identified and their properties have been evaluated through in vitro chemical assays, cell cultures, and in vivo. In the last years, the use of in silico methods has been increased because of representing a useful tool for simulating the proteins cleavage and so predict their potential bioactivity, solubility, absorption, distribution, metabolism, excretion, and toxicity of the peptides. Although these studies need complementary studies with in vivo experiments, in the future their use will keep increasing as an alternative option to investigate the new bioactive peptides, reducing costs and time.

Clinical studies with reduced groups have concluded that non-marine animal bioactive peptides (dairy, meat, and egg) present potential effects in prevention of non-communicable diseases. However, there are few bioavailability studies and further clinical studies should be carried out with longer intervention periods to confirm their efficiency. Clinical trials to be able to recommend the consumption of peptides for preventive and therapeutic treatments are suggested. In turn, these recommendations should be accompanied by studies on the safety and quality of foods that contain bioactive peptides.

Although there are studies on the potential effect of peptides on health, there are still no commercial products. This is attributed to the scarcity of clinical or toxicological tests to confirm bioactivity, safety, and efficacy, but also to the high cost of production, and problems in sensory quality such as the taste and color of the products with incorporated peptides. Therefore, it is necessary to continue studying the behavior of food with incorporated peptides by evaluating the techno-functional properties. Additionally, it is fundamental to study encapsulation strategies of peptides in order to improve sensory quality and bioavailability when incorporating peptides in different food matrices, in free or encapsulated forms.

It is essential to choose the source and to obtain the bioactive peptides in an appropriate way so that, after purifying and characterizing them in vitro, they exert the same activity in an in vivo system. Likewise, it is necessary to deepen the study of the various techniques for its purification and application at an industrial level.

Therefore, we must continue investigating even more about the animal peptides efficacy and safety through clinical studies, as well as their efficient production by using byproducts for their future sustainable application.

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