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# Food-derived peptides with biological activity: from research to food applications

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Many peptides that are released *in vitro* or *in vivo* from animal or plant proteins are bioactive and have regulatory functions in humans beyond normal and adequate nutrition. Different health effects have been attributed to food-derived peptides, including antimicrobial properties, blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral absorption and/or bioavailability, cyto- or immunomodulatory effects, and opioid activities. Numerous products are already on the market or under development by food companies that exploit the potential of food-derived bioactive peptides and which ascribe scientifically evidenced health claims to consumption of these functional foods.

## Addresses

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## Introduction

Proteins and peptides from food have been found to be physiologically active or bioactive, either in a direct manner through their presence in the undisturbed food itself or after their release from the respective host proteins by hydrolysis *in vivo* or *in vitro* (e.g. cheese ripening [1] and food fermentation [2,3]).

In this context, the term ‘food-derived bioactive peptides’ refers to different peptides of plant or animal origin that may have regulatory functions in the human system beyond normal and adequate nutrition.

Research in the field of bioactive peptides has intensified during the past two decades and has been extensively reviewed [4–7]. The potential for bioactive peptides to contribute to a healthier nutrition (e.g. by ingesting them with functional foods) has been widely discussed in the scientific community. Chemical and biological methods have been applied and developed to screen for bioactive

peptides that could promote different health effects; however, only some of the postulated health effects have been proven in human studies.

The present review primarily focuses on the past two years of research in the field of food-derived bioactive peptides and gives an overview of commercially available food products that are ascribed a certain health benefit.

## Food-derived bioactive peptides

Many peptides of plant and animal origin with relevant bioactive potential have been discovered, with by far the most being isolated from milk-based products. Candidate proteins containing these latent biological activities are found in milk, eggs, meat and fish as well as in different plant protein sources such as soy, wheat, and so on (Table 1).

A wide range of activities has been described (see Figure 1), including antimicrobial properties, blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral absorption/bioavailability, cyto- or immunomodulatory effects, and opioid activities. Moreover, some peptides are multifunctional and can exert more than one of the effects mentioned [8].

## Opioid peptides

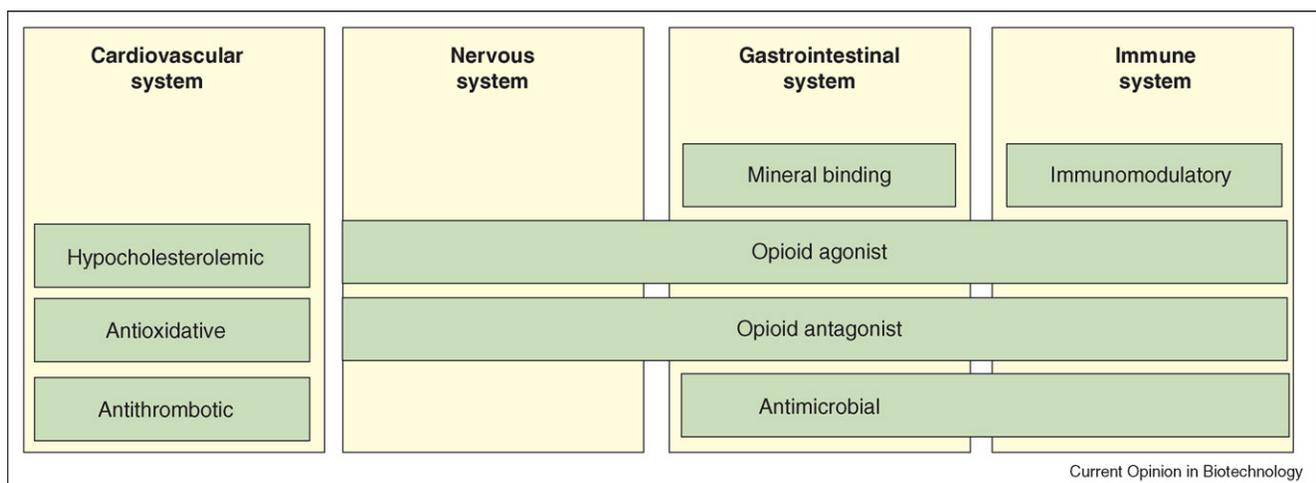
Food-derived peptides with opioid activity were first found in the late 1970s [9] and were termed ‘exorphins’ on the basis of their structural similarity to endogenous ligands (endorphins and enkephalins), which interact with opioid receptors of the  $\delta$ -,  $\mu$ - or  $\kappa$ -type. The common structural motif exhibited by both endogenous and exogenous ligands is an N-terminal tyrosine residue (except  $\alpha$ -casein opioids) and the presence of another aromatic residue in the third or fourth position from the N terminus (Phe or Tyr). Furthermore, a proline residue in the second position is crucial for the three-dimensional orientation of the tyrosine and phenylalanine sidechains. Agonistic activity is comparable to that of endogenous ligands, whereas antagonistic peptides exert inhibitory effects similar to naloxone, a potent opiate receptor antagonist applied as a drug (e.g. in the case of heroine overdoses) [10].

Casomorphins, for example, seem to be involved in regulating functions of the gut and enhance net water and electrolyte absorption, subsequently decelerating intestinal transit of the chyme and thus acting as an antidiarrheal agent [11].

**Table 1****Examples of bioactive peptides derived from food.**

Effect	Origin	Encrypting protein(s)	Name/remarks/sequence (in single-letter code)	Ref	
ACE inhibitory/ hypotensive	Soy	Soy protein	NWGPLV	[39]	
	Fish	Fish muscle protein	LKP, IKP, LRP (derived from sardine, bonito, tuna, squid)	[40]	
	Meat	Meat muscle protein	IKW, LKP	[41]	
	Milk		$\alpha$ -LA, $\beta$ -LG	Lactokinins (e.g. WLAHK, LRP, LKP)	[42**]
			$\alpha$ -, $\beta$ -, $\kappa$ -CN	Casokinins (e.g. FFVAP, FALPQY, VPP)	[43]
	Egg		Ovotransferrin	KVREGTTY	[44]
			Ovalbumin	Ovokinin (FRADHPPL)	[45]
				Ovokinin (2-7) (KVREGTTY)	
	Wheat		Wheat gliadin	IAP	[46]
			Broccoli	Plant protein	YPK
Immunomodulatory	Rice	Rice albumin	Oryzatesin (GYPMYPLR)	[48]	
	Egg	Ovalbumin	Peptides not specified	[49**]	
Milk		$\alpha$ -, $\beta$ -, $\kappa$ -CN, $\alpha$ -LA	Immunopeptides (e.g. $\alpha$ <sub>s1</sub> -immunocasinin) (TTMPLW)	[11]	
		Wheat	Wheat gluten	Immunopeptides	[28]
Cytomodulatory	Milk	$\alpha$ -, $\beta$ -CN	$\alpha$ -Casomorphin (HIQKED(V)), $\beta$ -casomorphin-7 (YFPFGPI)	[50]	
Opioid agonist	Wheat	Wheat gluten	Gluten-exorphins A4, A5 (GYYPT), B4, B5, and C (YPISL)	[51,52]	
	Milk	$\alpha$ -LA, $\beta$ -LG	$\alpha$ -Lactorphins, $\beta$ -lactorphins	[53]	
Opioid antagonist	Milk	Lactoferrin	Lactoferroxins	[10]	
		$\kappa$ -CN	Casoxins		
Antimicrobial	Egg	Ovotransferrin	OTAP-92 (f109-200) <sup>a</sup>	[49**]	
		Lysozyme	Peptides not specified		
Milk		Lactoferrin	Lactoferricin	[27]	
		$\alpha$ -, $\beta$ -, $\kappa$ -CN	Casecidins, isracidin, kappacin	[54]	
Antithrombotic	Milk	$\kappa$ -CN (glycomaclopeptide)	$\kappa$ -CN (f106-116) <sup>a</sup> , casoplatelin	[29]	
Mineral binding, anticariogenic	Milk	$\alpha$ -, $\beta$ -CN	Caseinophosphopeptides	[55**]	
Hypocholesterolemic	Soy	Glycinin	LPYPR	[56*]	
	Milk	$\beta$ -LG	IIAEK	[31]	
Antioxidant	Fish	Sardine muscle	MY	[57]	
	Wheat	Wheat germ protein	Peptides not specified	[58]	
	Milk	$\alpha$ -LA, $\beta$ -LG	MHIRL, YVEEL, WYSLAMAASDI	[59]	

CN, casein; LA, lactalbumin; LG, lactoglobulin.

<sup>a</sup> f, fragment.**Figure 1**

Overview of the beneficial effects ascribed to bioactive peptides derived from food proteins.

### Hypotensive (ACE inhibitory) peptides

Hypertension affects about a quarter of the world's population [12] and is a major, yet controllable, risk factor in cardiovascular disease and related complications.

Angiotensin I-converting enzyme (ACE), a dipeptidyl carboxypeptidase, catalyzes the conversion of angiotensin I (decapeptide) to the potent vasoconstrictor angiotensin II (octapeptide) and plays an important physiological role in regulating blood pressure and fluid and salt balance in mammals.

Since the first discovery of exogenous ACE inhibitors in snake venom [13], a great number of ACE inhibitory peptides have been isolated from the digestion of various food proteins, being found especially in milk but also in fish and meat. ACE inhibitory peptides are generally short-chain peptides, often carrying polar amino acid residues like proline [14]. The hypotensive and immunomodulatory peptides Val-Pro-Pro and Ile-Pro-Pro, for example, can be released from precursor proteins ( $\beta$ -casein and  $\kappa$ -casein) by enzymes from *Lactobacillus helveticus* [7,15].

The  $IC_{50}$  value (inhibitor concentration leading to 50% inhibition) is used to estimate the effectiveness of different ACE inhibitory peptides. However, it is not always directly related to the *in vivo* hypotensive effect. Some peptides can be susceptible to degradation or modification in the gut, the vascular system and the liver. By contrast, hypotensive activity of a long-chain candidate peptide can be caused by peptide fragments generated by gastrointestinal enzymes [16].

### Mineral-binding peptides

Milk caseins (CN) stabilize calcium and phosphate ions. Tryptic digestion of the casein proteins yields caseinophosphopeptides (CPPs) from the N terminus polar region, which contain clusters of phosphorylated seryl residues [17]. These phosphoserine clusters have been hypothesized to be responsible for the interaction between the caseins and calcium phosphate that leads to the formation of casein micelles. CPPs retain the ability of whole casein to stabilize calcium and phosphate ions through the formation of complexes, thus enhancing their general bioavailability.

Published data on the effect of CPPs on intestinal calcium absorption in rat model systems indicate that CPPs increase passive calcium transport in the distal small intestine [18]. In humans, Chabance *et al.* [19] detected small casein phosphopeptides in the stomach and duodenum of humans following milk ingestion. In addition, the ability of CPPs to survive the passage down to the distal human ileum was confirmed for the first time when CPPs were found in the ileostomy fluid of human volunteers given milk [20]. However, results from animal and human studies are contradictory. Until now, no evidence

has been supplied for the effectiveness of CPPs in increasing passive calcium absorption in humans. On the basis of human studies conducted within the framework of an EU research project (CT98-3077, *Caseinophosphopeptides: Nutraceutical/Functional Food Ingredients for Food and Pharmaceutical Applications*), Teucher and colleagues [21,22] concluded that CPPs cannot enhance calcium absorption in the gut.

However, CPPs can prevent spontaneous precipitation of hydroxyapatite (fluorapatite) [23]. Reynolds *et al.* [24] have demonstrated that phosphopeptides from casein that stabilize and localize calcium and phosphate ions at the tooth surface promote remineralization of enamel subsurface lesions.

### Antimicrobial peptides

Antimicrobial peptides have been identified from many protein hydrolysates, especially from milk. The most well-studied are the lactoferricins, which are derived from bovine and human lactoferrin [25]. Additionally, a few antibacterial peptides have been identified from  $\alpha_{S1}$ -casein and  $\alpha_{S2}$ -casein [26,27]. Antimicrobial peptides act against different Gram-positive and Gram-negative bacteria (*Escherichia*, *Helicobacter*, *Listeria*, *Salmonella* and *Staphylococcus*), yeasts and filamentous fungi. The disruption of normal membrane permeability is at least partly responsible for the antibacterial mechanism of lactoferricins [2].

### Immunomodulatory peptides

Immunomodulatory peptides can enhance immune cell functions, measured as lymphocyte proliferation, natural killer (NK) cell activity, antibody synthesis and cytokine regulation [2,28]. Moreover, immunomodulatory peptides might reduce allergic reactions in atopic humans and enhance mucosal immunity in the gastrointestinal tract [7]. Immunomodulatory peptides derived from tryptic hydrolysates of rice and soybean proteins act to stimulate superoxide anions (reactive oxygen species [ROS]), which trigger non-specific immune defense systems [25].

### Cytomodulatory peptides

Cytochemical studies have provided increasing evidence that food-derived bioactive peptides modulate viability (e.g. proliferation, differentiation and apoptosis) of different cell types. Some milk-derived peptides, for example, have been shown to trigger apoptosis, especially in malignant cells, whereas normal cells seem to be less susceptible. Together, these cytomodulatory and immunomodulatory properties might contribute to a certain protective activity of food-derived peptides in tumor development [6].

### Antithrombotic peptides

Peptides that inhibit blood platelet aggregation and fibrinogen binding ( $\gamma$ -chain) to platelet surface receptors are encrypted within the sequence of glycomacropptide,

which is released through the cleavage of  $\kappa$ -casein by rennin. These peptides can be detected in the blood plasma of newborn children after ingestion of breast milk or infant formula, although their significance remains unclear [29].

### Antioxidant peptides

Antioxidant properties that prevent enzymatic (lipoxygenase) and non-enzymatic peroxidation of essential fatty acids have also been found in peptides derived from milk proteins. Most of the peptides identified are encrypted in the sequence of  $\alpha$ -casein [2]. The addition of a leucine or proline residue to the N terminus of a His-His dipeptide, for example, can enhance antioxidant activity and facilitate further synergy with non-peptide antioxidants like BHT or BHA (butylated hydroxyanisole or toluene) [25].

### Hypocholesterolemic peptides

Hypocholesterolemic effects have been reported for casein- and whey-derived peptides, as well as for peptides from soy protein (partially bound to phospholipids) [30]. The mechanism behind these effects remains to be clarified. One possible mode of action is suggested by Nagaoka *et al.* [31] who found significantly higher levels of fecal steroid excretion in rats fed LTH (a tryptic hydrolysate of  $\beta$ -lactoglobulin) compared with rats fed casein tryptic hydrolysate. This could be due to a LTH-induced

reduction of micellar cholesterol solubility or a higher taurocholate binding capacity.

### Food applications

A large number of the bioactive peptides mentioned in this review occur naturally in traditional foods that have been consumed long before the term 'bioactive' was established. Many of these peptides are released from the encrypting host proteins by fermentation of milk, including cheese ripening [2]. Many peptides are generated by enzymatic reactions in the gut after ingestion of foods containing precursor proteins (e.g. after drinking a glass of milk) [20,32].

Bioactive peptides are fundamental constituents of many products or ingredients marketed as 'Functional Foods' or 'Nutraceuticals'. In these products the bioactive peptides are either added or enriched by modification of the usual manufacturing process (e.g. by changing process parameters or starter cultures used). Some of these products, however, are traditional foods now offered with a different marketing strategy. Table 2 lists some examples of commercially available functional foods and food ingredients that carry bioactive peptides and includes the health claim connected with the respective product.

Bioactive peptides are also included in non-food matrices to provide certain health-enhancing effects. CPPs, for

**Table 2**

#### Examples of commercially available functional foods or food ingredients carrying bioactive peptides.

Product name	Manufacturer	Type of food	Bioactive peptides	Health claim
Calpis AMEEL S (Japan) or Calpico (Europe)	Calpis Co., Japan	Sour milk	VPP, IPP from $\beta$ - and $\kappa$ -CN	Hypotensive
Evolus	Valio, Finland	Fermented milk, calcium-enriched	VPP, IPP from $\beta$ - and $\kappa$ -CN	
BioZate	Davisco, USA	$\beta$ -LG hydrolysate	Whey peptides	
C12 Peption	DMV, Netherlands	Ingredient	Casein-derived dodecapeptide FFVAPFPEVFGK	
Peptide Soup	NIPPON, Japan	Soup	Bonito-derived peptides	
Casein DP Peptio Drink	Kanebo, Japan	Soft drink	Casein-derived dodecapeptide FFVAPFPEVFGK	
BioPURE-GMP	Davisco, USA	Whey protein hydrolysate	Glycomacropeptide	Anticariogenic, antimicrobial, antithrombotic Hypocholesterolemic
CholesteBlock	Kyowa Hakko, Japan	Drink powder	Soy peptides bound to phospholipids	
CSPHP				
ProDiet F200	Ingredia, France	Milk drink, confectionery	$\alpha_{S1}$ -CN (f91–100) <sup>a</sup> : YLGYLEQLLR	Reduces stress
Capolac	Arla Foods, Denmark	Ingredient	CPP	Helps mineral absorption
Tekkotsu Inryou	Suntory, Japan	Soft drink	CPP	
Kotsu Kotsu calcium	Asahi, Japan	Soft drink	CPP	
CE90CPP	DMV, Netherlands	Ingredient	CPP (20%)	
Glutamin peptide	DMV, Netherlands	Dry milk protein hydrolysate	Glutamine-rich peptides	Immunomodulatory
WGE80GPA				
WGE80GPN				
WGE80GPU				

Information gathered from [3\*,7,60].

<sup>a</sup> f, fragment.

example, are added in combination with amorphous calcium phosphate to mouth rinse solution, toothpaste (Prospec MI Paste™, GC Tooth Mousse™) or chewing gum (Recaldent™, Trident™) [33].

Food-derived bioactive peptides also have enormous potential as ingredients of pharmaceuticals; for example, blood pressure-lowering capsules have been manufactured that contain Katsuobushi Oligopeptide LKPNM (in single-letter amino acid code) from thermolysin-treated dried bonito (a fish from tuna family), which is converted into its active form (LKP) by digestive enzymes (Vasotensin 120T™ by Metagenics, USA; PeptACE™ Peptides 90 by Natural Factors, USA).

## Conclusions

Research continues to uncover novel bioactive peptides and to reveal their possible functions and health benefits. The systematic synthesis of peptides and peptidomimetics has an important role in finding new bioactive structures and for elucidating structural information on the active conformations [34]. Furthermore, structure-activity studies based on *in silico* analysis using chemometric methods (e.g. artificial neural networks), are effective and useful for identifying bioactive sequences [16]. The application of computational chemistry will result in the creation of structure and sequence databases that will enable bioactive fragments to be searched for in the protein chain [35].

Numerous products are already on the market or under development by food companies, exploiting the potential of food-derived bioactive peptides. An important task for the production of functional foods containing bioactive peptides is to enhance their bioavailability from dairy products or to create novel foods through the addition/fortification of isolated or enriched fractions of bioactive peptides. The production of bioactive peptides during food processing, for example, by the use of specific bacterial enzymes or genetically transformed microorganisms, is of interest for future research. Moreover, genetically modified proteins will be designed to carry multiple copies of bioactive sequences [36]. Future studies should ascertain the possible involvement of cytomodulatory peptides in tumor development.

Food legislation always lags behind innovation and developments, sometimes by many years. This is particularly true in the case of functional foods in the EU, where appropriate legislation is still in '*statu nascendi*'. Current EU legislation does not yet recognize functional foods as a distinct category of foods. In contrast, Japan was the first country to adopt a legal system in relation to allowable health claims on functional foods through the introduction of the FOSHU (Foods for Specific Health Use) licensing system in 1991. Japanese companies can apply for FOSHU approval by the Japanese Ministry of Health

and Welfare. Provided that a health claim connected to a certain product has been scientifically evidenced, official permission is granted to use these claims in advertising. Since the end of 2005, 537 FOSHU products with an estimated retail value of US \$6.3 billion have already been approved to carry the FOSHU label.

There is consent over the fact that health claims must be proved in human studies. However, food components have a chronic rather than an acute effect on health. Thus, screening methods should be developed for the measurement of long term effects in order to ascertain effects of food components that are claimed to promote good health. In this context, relevant indicators or biomarkers that can predict potential benefits relating to a target function in the body have to be identified [37]. A valid biomarker must be representative of the effect brought about by the bioactive food component. Markers could be obtained *in vivo* (serum, faecal, urinary) as well as *in vitro* (cytochemical).

When assessing peptides that could provide beneficial effects, one must also take into account the possible adverse effects that might be exerted by the respective peptides themselves or their by-products that would inevitably be contained in such foods. Such safety requirements include the absence of toxicity, cytotoxicity and allergenicity [38].

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