A proteome-based design of bitter peptide digestion regime to attenuate bone soup bitterness: comparison with a rainbow trout extract-mediated bitter taste masking approach

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ABSTRACT

BACKGROUND: The fresh bones (with some meat on them; frequently discarded as a large quantity of industry garbage) of marine fish such as cod and salmon are good materials for manufacture of food additives (taste adjusters). However, such fish-bone originated additives often have apparent bitter taste and need additional debittering regime. **RESULTS:** In this study, 46 known bitter peptides in the cod proteome were targeted for specific protease digestion to eliminate bitter taste from the cod bone soup. Though the debittering effect was apparent, the bitter taste was not completely removed. However, the bitter taste can be removed by addition of trout extract to a complete extent. The strong debittering power of rainbow trout extract was further confirmed by the debittering experiments on salmon bone soup and bitter melon, both with perfect results. **CONCLUSION:** These results indicated that the cod bone soup bitterness comes not only from bitter peptide but also from other substances that can be masked by trout extract. Considering the fact that trout proteome has more potential bitter peptides than cod, trout extract shall have a strong bitter masking substance to be determined in the future.

Key words: bitter peptide; proteome; cod; trout; bitterness-masking

INTRODUCTION

Many kinds of food will form a bitter taste after the hydrolysis, seriously affecting the market prospects. The bitterness of a protein hydrolytes is largely due to the release of bitter peptides, while many bitter substances other than bitter peptides have not yet been well identified. Common bitterness in a food is often derived from cholic acid or bitter amino acids (histidine, leucine, isoleucine, methionine, etc.). Fish tissue waste (skin, tail, bones, fishbone, fins, visceral, etc.) is often mixed with bile or bile acid and other bitter substances, resulting in a taste of obvious bitterness. In principle, it is possible to eliminate the cholic acid or bitter amino acids through specific biotransformation reactions. At present, there are at least 46 known bitter peptides¹⁻¹⁰ (Table 1), but only 15 bitter peptides were listed in the BitterDB database.¹¹

Various protein hydrolysates with bitter tastes were found from 1950s.¹² Since then, researchers have proposed debittering methods such as activated-carbon adsorption, chloroform extraction, ethanol extraction, isoelectric point precipitation, hydrophobic interaction chromatography, and bitter masking.¹³⁻¹⁸ Some types of food are hard to de-bitter and need combinations of different technologies and approaches for satisfactory debittering effect, but possibly resulting in high cost of food processing.

Few reports have been seen in that the whole proteome sequences are directly employed to quantify the types of potential bitter peptides and thereby design specific enzyme cutting strategy for those potential bitter peptides. In this study, bitter peptide breakage regimes based on cod proteome sequences were set up, but it was found that such regimes were not able to well eliminate the bitter taste of cod bone soup. Trout bone soup didn't taste bitter at all though its proteome has more potential bitter peptides than cod. Salmon proteome has more potential bitter peptides than both cod and trout, but its bone soup was similarly as bitter as cod bone soup. Besides, mixing trout bone soup with either cod bone soup or salmon bone soup completely eliminated the bitter taste, suggesting that trout bone soup has some bitter-masking substance.

Surprisingly, even the bitter taste of bitter melon¹⁹ soup was also mostly or completely eliminated by trout bone soup.

MATERIALS AND METHODS

Bone soup

All kinds of fresh fish bone were provided by The YueYi Biotech Ltd, RiZhao, Shandong, China. Cod fish bone was equally mixed with water (W/W), smashed with a stirrer to get a cod bone suspension. The suspension was equally mixed with water (V/V) and then heated to the boiling state for 20 min to get the cod bone soup. Other kind of bone soup mentioned in this study was made the same way. Amino acids and fatty acids compositions in three types of bone soup were measured (averaged from three repetitions) in Merieux Nutrisciences, Sino Analytical (Qingdao) Ltd, China.

Determination of potential bitter peptide contents in a proteome

In the database BitterDB (<http://bitterdb.agri.huji.ac.il/dbbitter.php#Home>)¹¹, there are 680 bitter compounds and only 15 bitter peptides included. In this study, total 46 bitter peptides (Table 1) were included (Some other bitter peptides are not included in this study at the time of submission). They were scanned and calculated along the whole proteome using a laboratory software program designed by the authors or simply search and calculate using the office software WORD's basic function. Proteome sequences of Cod (*Gadus morhua*) were obtained from http://www.ensembl.org/Gadus_morhua/Info/Index; Proteome sequences of rainbow trout (*Oncorhynchus mykiss*) were downloaded from <https://www.ncbi.nlm.nih.gov/genome/?term=Oncorhynchus+mykiss>; and the proteome sequences of *Salmosalar* (Atlantic salmon) were obtained from <https://www.ncbi.nlm.nih.gov/genome/369>. Because proteins in the fish bone belong to part of the whole proteome, bitter peptides scanned from the whole proteome shall cover those in the fish bone soup.

Table 1 Predicted numbers of 46 bitter peptides in three types of seafood

	Bitter peptide	N(cod)	N(trout)	N(salmon)		Bitter peptide	N(cod)	N(trout)	N(salmon)
1	LL ³	98898	159946	561761	24	LQL ²	4581	7682	27881
2	GL ¹	62282	100397	383180	25	AIA ²	2272	3519	12309
3	RL ¹	58845	93040	348242	26	LLLL ⁴	1410	2201	6647
4	AA ²	56830	83641	340425	27	YFL ¹	1343	2231	6024
5	LK ¹	55506	97567	357128	28	RPK ⁷	1255	2430	9275
6	FL ¹	41172	69029	218202	29	RPF ⁷	1118	2055	6862
7	LF ¹	36253	62872	195777	30	LPW ²	679	1101	3411
8	VF ²	29197	47031	151040	31	EVLN ¹	179	305	1259
9	FV ²	23617	37917	123530	32	SKGL ¹	179	345	1504
10	LLL ³	10294	16705	54490	33	EYFL ¹	53	96	269
11	RLL ¹	6763	9805	36478	34	LAVIL ²	25	34	80
12	LVL ²	6291	9639	30963	35	VVYPW ²	6	16	16
13	LEL ²	5371	8510	31844	36	FIEGV ¹	5	9	20
14	WF ²	5075	8266	26284	37	NENLL ¹	5	5	11
15	WP ²	4766	8618	28215	38	LPFSQL ²	1	0	0
16	GAL ²	4615	6501	24786	39	FYPELF ⁸	1	3	4
17	HWPWMK ⁵	5	3	5	40	AVVLI ⁵	2	5	33
18	PFPPIV ⁶	0	0	0	41	APFPEVF ¹	0	0	0
19	GPFPIV ¹	0	0	0	42	KGPFPIV ²	0	0	0
20	FALPQYLK ¹	0	0	0	43	GPFPIV ⁹	0	0	0

21	FFVAPFPEVFGK ¹	0	0	0	44	RGPPFIV ¹⁰	0	0	0
22	LVPKYFG ¹	0	0	0	45	LPFNQL ²	0	0	0
23	VYPFPPGINH ¹	0	0	0	46	VVYPWTQRF ²	0	0	0

Note: N(cod), N(trout) and N(salmon) mean the number of a bitter peptide in the three types of seafood.

Proteases used to digest the whole proteome

The enzymatic specificity characteristics of several proteases were listed in Table 2. In order to theoretically cut apart all twenty kinds of amino acids, several combinations of proteases can be considered as follow. (1) Carboxypeptidase A, B and C can work together to cut all amino acids; (2) Bromelain and protease K are another potential combination; (3) Pepsin, trypsin and Bromelain can work as a full functional combination; (4) Pepsin, trypsin and Carboxypeptidase A are also a good combination; Apparently there are also several other different combinations capable of digesting all twenty amino acids. However, cost efficiency is an important consideration in practice. Protease K and Carboxypeptidases are much more expensive than normal proteases such as pepsin, trypsin and Bromelain. So the combination of Pepsin, trypsin and Bromelain was selected in this study. Pepsin (Cat.Num.150624, 12k U g⁻¹), Trypsin (Cat.Num.150726, titer 1:250), and Bromelain (Cat. Num.150618, 800k U g⁻¹) were purchased from Beijing HongRunBaoShun Scientific Ltd. Company, China. The general regime included (1) all cuttings were set carboxyl-terminal; (2) R and K residues were subjected to Trypsin; F, Y, W, D, E, A, V, L, I, P, M residues were subjected to pepsin; and (3) all other residues were in charged by Bromelain.

Table 2 Proteases used to cut peptide bonds

Protease	Target residue	Note
Pepsin	-Ⓔ-, -Y-, -W-, -D-, -E-, -A-, -Ⓜ-, -Ⓛ-, -I-, -Ⓕ-, -M-	✓
Trypsin	K-, R-	✓
Chymotrypsin	Y-, W-, Ⓔ-	Replaceable by Pepsin and Trypsin or protease K
Papain	R-, K-, Ⓔ-, W-, Y-,	Replaceable by Pepsin and Trypsin, or protease K
Bromelain	All residues; low specificity	high activity enzyme ✓
ProteaseK	A-, Ⓜ-, Ⓛ-, I-, D-, E-, K-, R-, G-, S-, T-, C-, Ⓔ-, Y-, W-	expensive
CarboxypeptidaseA	All residues other than R,K,P	expensive
CarboxypeptidaseB	R-, K-	expensive
CarboxypeptidaseC	(penultimate)Ⓔ-	expensive

Note: -X, X-, and -X- mean amino-terminal, carboxyl-terminal or both terminal cuttings. Residues in a circle represented four most frequent ones in the bitter peptides.

Proteases used to cut the bitter peptides

The above protease combination was also applicable for bitter peptide digestion away from the proteome. The most frequent residues in a bitter peptide were L, P, F and V, and less frequent bitter-peptide residues were G, H, A, Y, R, E and R. So the pepsin-trypsin-bromelain combination shall be good for bitter peptide digestion. For any bitter peptide -NNNX₁X₂...X_{n-1}X_nNNN (X, bitter peptide residue; N, neighboring non-bitter peptide residue), a general digestion regime was undertaken as -NNNX₁ ↓ X₂ ↓ ...X_{n-1} ↓ X_n NNN, in which the cutting arrows were carboxyl for X₁ to X_{n-1} respectively.

Sensory evaluation

The bitterness of each soup sample was estimated (averaged with three repetitions) by the quinine-sulfate (purchased from Sangon, Shanghai) equivalent test. The bitterness of a sample was compared with a series of quinine-sulfate dilutions using a sensory panel composed of five people. The panelists were trained with standard quinine-sulfate solutions set at several concentrations near the threshold levels. The panelists rinsed their mouths thoroughly with water and then keep 2ml soup in the mouth for 10 sec before evaluation. The degree of bitterness was rated as not (-), slightly (+), distinctly (++), moderately

(+++), very (++++), and extremely bitter (+++++). Each degree represented a quinine-sulfate concentration of 1.6, 2.4, 3.2, 4.0, 4.8 and 5.6×10^{-5} mol L⁻¹, respectively.

RESULTS

Bitter peptide profiles in cod, salmon and rainbow trout

According to EBI genomes and the relevant specialized website for complete proteomics information, the contents of bitter peptides of the three species were obtained as follows (Table 3). Cod, salmon and trout have different sizes of genomes, so their proteomes. This is the main reason why they had different numbers of bitter peptides (Table 1). Some longer bitter peptides include several shorter bitter peptides in them, so cutting of longer bitter peptides will release shorter ones that might be more bitter. So complete enzymatic cutting was expected in order to finally reach individual amino acid residues.

Table 3 Enzymatic cutting site numbers of each protease in three proteomes

Protease	cod	salmon	trout	cod	salmon	trout
	Bitter peptide cutting			Whole proteome cutting		
Pepsin	427259	2438483	694866	5562656	39200011	9635931
(amount)	(6g)	(34g)	(9.8g)	(78g)	(550g)	(135g)
Trypsin	68160	402361	107675	1127717	8155897	1987439
(amount)	(0.2g)	(1.2g)	(0.3g)	(3.3g)	(24g)	(5.8g)
Bromelain	71858	411021	115282	3329959	25010690	6188981
(amount)	(12mg)	(69mg)	(19mg)	(556mg)	(4.2g)	(1g)
Total protease mixture (g)	6.2	35.2	10.1	81.8	578.2	141.8

Effects of bitter peptide cutting on debittering of the bone soup

Fresh cod fish soup 600ml was heated to boil for 20min, then cooled to room temperature and divided into four equal parts (150 ml each) into the same four containers. Protease mixtures were added according to Table 4 to purposely digest bitter peptides. Bitter taste was evaluated at three time intervals. Some residual bitter taste was still sensed after 24 hours. Similar results were seen for salmon soup (data not shown). Since the trout soup was found not bitter at all, so the bitter peptide cutting test was not undertaken on it.

Table 4 Debittering effect of bitter peptide cutting in the cod proteome

No.	Total protease mixture (g)	Bitterness (1hr)	Bitterness (6hr)	Bitterness (24hr)
1	0	++++	++++	++++
2	0.62	++++	++	+
3	1.24	+++	+	+
4	6.2	+++	-	+

Effects of whole proteome cutting on debittering of the bone soup

Similarly as above, fresh cod fish soup 600ml was heated to boil for 20min, then cooled to room temperature and divided into four equal parts (150 ml each) into the same four containers. Protease mixtures were added according to Table 5 to purposely digest the whole proteome (Table 3). After 30 hours, there was still some residual bitter taste in the samples. Similar results were also seen for salmon soup (data not shown).

Table 5 Debittering effect of whole proteome cutting in the cod proteome

No.	Total protease mixture (g)	Bitterness (1hr)	Bitterness (6hr)	Bitterness (30hr)
1	0	++++	++++	++++
2	8.18	+++	++	++
3	16.36	+++	+	+
4	81.8	+++	+	+

Debittering effects of trout extract on cod bone soup

Freshly made 100ml cod bone soup was mixed with 100ml trout bone soup plus 200ml water. After boiled for 20 min and stayed for 1hour at the room temperature, the bitter taste was evaluated. It was found that the bitter taste was completely eliminated, with the evaluation score from (+++) or (++++) to (-). The experiment repeated three times and got the similar results.

Debittering effects of trout extract on salmon bone soup

Freshly made 100ml salmon bone soup was mixed with 100ml trout bone soup plus 200ml water. After boiled for 20 min and stayed for 1hour at the room temperature, the bitter taste was evaluated. It was found that the bitter taste was also completely eliminated, with the evaluation score from (+++) or (++) to (-). The experiment repeated three times and got the similar results, and when the ratio of salmon bone soup to trout bone soup was 1:2, the debittering effect was getting slightly better than the 1:1 ratio.

Debittering effects of trout extract on bitter melon soup

Fresh bitter melon was mixed with water at 1:1 (w/w) and then smashed into soup. Take 100ml bitter melon soup and mix with 100ml trout bone soup plus 200ml water. After boiled for 20 min and stayed for 1hour at the room temperature, the bitter taste was evaluated. It was found that the bitter taste was also almost completely eliminated, with the score from (++++) to (+) or (-). The experiment repeated three times and got the similar results, and when the ratio of bitter melon soup to trout bone soup was 1:2, the debittering effect was getting slightly better than the 1:1 ratio.

DISCUSSION

Correlation between predicted bitter peptide contents and the real bitter taste

It is hard to evaluate the contribution of a single type of bitter peptide to the total taste of a food. Meanwhile, the total numbers and types of all potential bitter peptides cannot easily get associated with the food taste (**Supplementary Table 1s and Fig1s; Fig.1**). Maybe the bitter peptide density in a proteome (the bitter peptide total numbers divided by the size of the proteome) matters. However, the bitter peptide density of the six types of food all lies within the range 34.3~43.8 per kb proteome, and apparently has no correlation with bitter taste levels. This is consistent with previous observations^{7, 20} that only those peptides with molecular weight less than 5000-6000 have a chance to be bitter. It is also a common sense that amino acids compositions of a food have no direct correlation with its bitter taste extent, though bitter melon's bitterness may be related with its relatively high ratios in several amino acids (**Supplementary Fig2s**).

Which is better, digestion of the whole proteome or only bitter peptides?

Proteome-based strategy may be inefficient without careful control of protease digestion. One of the reasons is that some bitter peptides may become bitterer after broken into pieces; Some larger bitter peptides contain a more bitter small peptide within them. This is consistent with the observation that longer time (such as 24hr) of protease digestion of cod soup would bring more bitter taste than shorter time of digestion (such as 6hr). So, no matter whether the digestion is for the whole proteome or only for the bitter peptides, the residual bitterness can not be eliminated theoretically without facilitation of other ways, such as masking by trout soup. In practice, even the specifically designed bitter peptides-digestion regime still needs facilitation by optimization and control of digestion conditions to achieve satisfactory bitterness levels.

Fatty acids may facilitate trout's bitter-masking capacity

The bitter taste of cod fish was previously studied using conventional methods²¹ and the authors found that Flavourzyme® did not reduce the bitterness well while the use of butanol and cholestyramine resin separately or in combination reduced the bitter taste from fish protein hydrolysates to levels hardly discernible in 1% concentration. In this study, the bitter taste of cod fish bone soup was masked completely by trout bone soup, suggesting that the latter has some substance to interact with bitter taste receptors or scavenging bitter substances themselves. There are several types of naturally occurred substances that bind

with bitter taste receptors, such as some lipoproteins, cyclodextrin and cyclofructan.²²⁻²⁴ Among them, lipoproteins may be of a particular interest for this study because trout bone soup had a much higher lipid compositions as observed than other two kinds of bone soup, though the authors were still not sure whether lipoproteins²⁵ or fatty acids²⁶ (Table 6) played important roles for trout soup to be able of masking bitter taste. Table 6 clearly indicated that the trout bone soup had higher concentrations of about 20 different fatty acids than cod and salmon soups. According to several reports, some fatty acids can be used to efficiently mask bitter taste.²⁷⁻²⁹ Tomotake et al. (1998) and Fujita et al (2004) found that fatty acid salts such as sodium stearate, palmitate, and laurate in relatively high concentrations of about 1% were able to reduce the bitter taste of a 100-ppm quinine solution significantly, while there was 2.54% palmitic acids in the trout soup (Table 6) and may contribute to mask the bitter taste. Ryousuke Homma et al³⁰ found that a 0.5mM mixture of palmitic acid, stearic acid and myristic acid significantly reduced the bitterness of quinine hydrochloride, while the concentration of the above mixture in the trout soup (99mM, 21mM and 28mM for the three types of fatty acids respectively) was about 40 times larger than that of the above mixture used for the sensory test, which shall be adequate to demonstrate the bitterness-masking effects. Though whether or how these fatty acids contribute to the bitterness-masking capacity of trout soup is still vague at this time, the literature strongly supports that fatty acids intertwine with bitterness-sensing signaling pathway³¹⁻³⁴ and likely play an important role for trout soup to mask the bitter taste.

Table 6 Fatty acid concentrations in three types of bone samples

	Fatty acid	Concentration (g kg ⁻¹)		
		cod	trout	salmon
1	Heneicosanoic acid	ND	ND	ND
2	Tricosanoic acid	ND	ND	ND
3	trans-9-Octadecenoic acid	ND	ND	ND
4	trans-trans-9,12-Octadecadienoic acid	ND	ND	ND
5	Butyric acid	ND	ND	ND
6	Caproic acid	ND	ND	ND
7	Caprylic acid	ND	ND	ND
8	Capric acid	ND	ND	ND
9	Undecanoic acid	ND	ND	ND
10	Lauric acid	ND	ND	ND
11	Tridecanoic acid	ND	ND	ND
12	Myristic acid	0.0028	0.064	0.017
13	Pentadecanoic acid	ND	0.0046	ND
14	Palmitic acid	0.015	0.254	0.034
15	Margaric acid	ND	0.0042	ND
16	Stearic acid	0.0026	0.06	0.0078
17	Arachidic acid	ND	ND	ND
18	Behenic acid	ND	ND	ND
19	Lignoceric acid	ND	ND	ND
20	cis-9-Tetradecenoic acid	ND	ND	ND
21	cis-10-Pentadecenoic acid	ND	ND	ND
22	cis-9-Hexadecenoic acid	0.0022	0.06	0.0142
23	cis-10-Heptadecenoic acid	ND	ND	ND
24	cis-11-Octadecenoic acid	ND	0.05	0.0056
25	cis-9-Octadecenoic acid	0.0146	0.668	0.034
26	cis-11-Eicosenoic acid	0.0046	0.007	0.022
27	cis-13-Docosenoic acid	ND	0.0084	0.0084
28	cis-15-Tetracosenoic acid	ND	0.0058	0.0022
29	all cis-9,12,15-Octadecatrienoioc acid	ND	0.0074	ND
30	all cis-11,14-Eicosadienoic acid	ND	0.004	ND
31	all cis-11,14,17-Eicosatrienoic acid	ND	0.042	0.024
32	all cis-8,11,14,17-Eicosatetraenoic acid	ND	ND	ND

33	all cis-5,8,11,14,17-Eicosapentaenoic acid	ND	ND	0.0036
34	all cis-7,10,13,16,19-Docosapentaenoic acid	ND	0.0068	ND
35	all cis-4,7,10,13,16,19-Docosaheptaenoic acid	ND	0.0032	0.0022
36	cis,cis-9,12-Octadecadienoic acid	ND	0.062	0.0028
37	all cis-6,9,12-Octadecatrienoic acid	ND	0.0062	ND
38	all cis-8,11,14-Eicosatrienoic acid	ND	0.003	ND
39	all cis-5,8,11,14-Eicosatetraenoic acid	ND	ND	ND
40	all cis-13,16-Docosadienoic acid	ND	0.0024	ND
41	Total fat	0.06	1.54	0.22

Note: ND: not detected

CONCLUSION

In this study, 46 known bitter peptides in the cod proteome were targeted for specific protease digestion to eliminate bitter taste from the cod bone soup. Though the debittering effect was apparent, the bitter taste was not completely removed. However, the total bitter taste can be surprisingly removed simply by addition of trout extract. The strong debittering power of trout extract was further confirmed by the debittering tests on salmon bone soup and bitter melon, both with perfect results.³⁵ These results indicated that the cod bone soup bitterness comes not only from bitter peptide but also from other substances that can be masked by trout extract. Considering the fact that trout bone soup had much higher lipid content than cod and salmon bone soups, fatty acids are probable substances that mediate the bitterness-masking and remain worthy to be determined in the future.

References

- 1 Yang L and Bai Y. Characteristics and the debittering methods of bitter peptides in protein hydrolysates. *Guangzhou Food Sci. Technol.* **18**, 22-25 (2002).
- 2 Zhou XS. Study on Forming Mechanism and Control Methods of Bitter Taste from Protein Hydrolysates. *J. Cereal. Oil.* **8**, 20-24 (2004).
- 3 Teruyoshi M and Tadao H. Relationship between bitterness of peptides and their chemical structures. *Agr. Biol. Chem.* **36**, 1423-1431 (1972).
- 4 Ishibashi N, Arita Y, Kanehisa H, Kouge K, Okai H and Fukui S. Bitterness of leucine-containing peptides. *Agr. Biol. Chem.* **51**, 2389-2394 (1987).
- 5 Xie M, Hou H, Zhang SM, Bu L, Chang YF, Zhang ZH and Li BF. Purification and identification of bitter peptides from codfish hydrolysate. *Mod. Food Sci. Technol.* **32**, 190-195 (2016).
- 6 Huber Land Klostermeyer H. Isolation and identification of a bitter peptide from Butterkaese cheese II. *Milchwissen—schaft* **29**, 449-455 (1974).
- 7 Cho MJ. Characterization of bitter peptides from soy protein hydmlsates[D]. University of Missouri-Columbia (2000).
- 8 He H, Wang J, Pei F, Hou FL and Shi YL. Review on the relationship of structure to bitter taste of protein hydrolysate and debittering. *Food Sci.* **27**, 571-574 (2006).
- 9 Wu YW and Ouyang J. Tasting functions of amino acids and peptides. *China Condiment* **1**, 21-24 (2001).
- 10 Pedersen B. Removing bitterness from protein hydrolysates. *Food Technol.* **10**, 96-98 (1994).
- 11 Wiener A, Shudler M, Levit A and Niv MY. BitterDB: a database of bitter compounds. *Nucleic Acids Res.* **40**, 413-9 (2012).
- 12 Murray T and Baker BE. Studies on protein hydrolysis. I. Preliminary observations and the taste of enzymic protein hydrolysates. *J. Sci. Food Agric.* **3**, 470-475 (1952).
- 13 Grohmann K, Manthey JA, Cameron RG and Buslig BS. Purification of citrus peel juice and molasses. *J. Agric. Food Chem.* **47**, 4859-4867 (1999).
- 14 O'Cuinn G, FitzGerald R, Bouchier P and McDonnell M. Generation of non-bitter casein hydrolysates by using combinations of a proteinase and aminopeptidases. *Biochem. Soc. Trans.* **27**, 730-734 (1999).
- 15 Cho MJ, Unklesbay N, Hsieh FH and Clarke AD. Hydrophobicity of bitter peptides from soy protein hydrolysates. *Agric. Food Chem.* **52**, 5895-5901 (2004).
- 16 Lalasidis G. Four new methods of debittering protein hydrolysates and a fraction of hydrolysates with high content of essential amino acids. *Ann. Nutr. Aliment.* **32**, 709-723 (1978).
- 17 Roy GM. The applications and future implications of bitterness reduction and inhibition in food products. *Crit. Rev.*

- Food SciNutr***29**, 59-71 (1990).
- 18 Sonawane VM, Saiffee M, Shinde NY, Hawaldar AH and Pawar NA. An update of taste masking methods and evaluation techniques. *Der. Pharmacia Lettre***2**,1-15 (2010).
 - 19 Joseph B, Jini D. Antidiabetic effects of Momordicacharantia (bitter melon) and its medicinal potency. *Asian Pac. J.Trop. Dis.***3**, 93-102 (2013).
 - 20 Deng Y and Feng XW. Determination of distribution of molecular weight and bitterness of soybean peptides. *J. China Agr. Univ.* **6**, 98-102 (2001).
 - 21 Dauksas E, Slizyte R, Rustad T and Storro I. Bitterness in fish protein hydrolysates and methods for removal. *J. Aquat. Food Prod. T.***13**, 101-114 (2004).
 - 22 Mori H, Nishioka M and Nanjo F. Cyclodextrin and cyclofructan for inhibition of bitterness and astringency of foods and beverages. *Japan Patent*2006067895 (2006).
 - 23 Binello A, Cravotto G, Nano G. Synthesis of chitosan-cyclodextrin adducts and evaluation of their bitter masking properties. *Flavour Frag. J.***19**, 394-400 (2004).
 - 24 Jakob, P. L. Masking bitter taste by molecules. *Chemosens.percept.***1**, 58-77 (2008).
 - 25 Katsuragi Y and Yasumasu T. Lipoprotein that selectively inhibits taste nerve responses to bitter substances. *Brain Res.***713**, 240-245 (1996).
 - 26 Pouru A, Kuusisto P, Lahtinen R and Koponen L. Edible product with masked bitter, sour and /or astringent taste. *I. W O Patent*2004093571A1 (2004).
 - 27 Tomotake M, Sugiura Y, Umeda T and Katsuragi Y. Agent for reducing bitter taste for medicines and foods, etc. contains optionally unsaturated fatty acid/or their metal salts. *Japan Patent* 10,025,253 (1998).
 - 28 Fujita H and Kuroki A. Pharmaceutical formulations comprising sodium laurylsulfate as bitterness masking agent. *EP* 1,452,177 (2004).
 - 29 Koriyama T, Wongso S, Watanabe K and Abe H. Fatty acid compositions of oil species affect the 5 basic taste perceptions. *J. Food Sci.***67**, 868–873 (2002).
 - 30 Ryousuke H,Haruyuki Y, Junko F, Reiko U, Takanobu S, Yoshiro I, Keiko A and Tomiko A. Identification of bitterness-masking compounds from cheese.*J. Agric. Food Chem.* **60**, 4492–4499 (2012).
 - 31 Masek P and Keene AC. Drosophila fatty acid taste signals through the PLC pathway in sugar-sensing neurons. *PLoS Genet.* **9**, e1003710 (2013).
 - 32 Pittman DW, Labban CE, Anderson AA and O'Connor HE. Linoleic and oleic acids alter the licking responses to sweet, salt, sour, and bitter tastants in rats. *Chem. Senses***31**, 835-43 (2006).
 - 33 Mizushige T, Inoue K and Fushiki T. Why is fat so tasty? Chemical reception of fatty acid on the tongue. *J. Nutr. Sci. Vitaminol. (Tokyo)*. **53**, 1-4 (2007).
 - 34 Mattes RD. Effects of linoleic acid on sweet, sour, salty, and bitter taste thresholds and intensity ratings of adults. *Am. J. Physiol. Gastrointest. Liver Physiol.***292**, G1243-G1248 (2007).
 - 35 Zhang ZZ, Han Y, Jin F and Zhang Y. A method to mask food bitterness by trout extraction. *China Patent* No.201510970526.7 (2015).

Figure legends

Figure 1. Predicted values of bitter peptide density for six different types of food that had big difference in the bitter taste. From level 1 to level 5, the bitterness increases from (-), (+), (++) to (+++). The mussel, oyster and mellow (bitter mellow) represented another three kinds of food locally collected.

Supplementary Fig1s. Predicted numbers of 46 bitter peptides in the six proteomes. The sequences of 46 bitter peptides can be found in Table 1.

Supplementary Fig2s. Amino acids compositions (percentage of each amino acid to the whole amino acids) of six types of food. Cod, trout and salmon represented three different bone samples. Oyster and mussel represented the whole body samples except for the shells. The values were the average of three repetitions for the above five types of sample. Mellon [a,b,c] represented three cases of amino acids concentration measurement of bitter mellon as the following literature: [a] Effects of heat drying process on amino acid content of *Momordica charantia* L. *Amino Acids & Biotic Resource*.2010, 32(2):14-16.[b] Quantitative analysis of protein, amino acids and inorganic elements in *Momordica charantia*. *Bullet. Hunan Univ.* 1996,21(5): 305-307.[c] Effect of microwave treatment on quality of *Momordica charantia* L. *Mod. Food Sci. Technol.*2009, 25(11): 1250-1253.

Supplementary Table 1S

Predicted numbers of 46 bitter peptides in mussel, oyster and bitter melon in their proteomes

	Bitter peptide	N(mussel)	N(oyster)	N(melon)		Bitter peptide	N(mussel)	N(oyster)	N(melon)
1	LL ^{[3][4]}	12505	195057	123035	24	LQL ^[2]	288	7885	5358
2	GL ^[1]	6322	122248	80129	25	AIA ^[2]	549	5666	4532
3	RL ^[1]	4534	120309	68261	26	LLLL ^[4]	84	1445	1396
4	AA ^[2]	5484	100129	77300	27	YFL ^[1]	134	3075	1669
5	LK ^[1]	6124	170750	83629	28	RPK ^[7]	76	4722	1730
6	FL ^[1]	3577	99717	57344	29	RPF ^[7]	48	2358	1263
7	LF ^[1]	4064	88215	51638	30	LPW ^[2]	57	1230	830
8	VF ^[2]	2736	74046	35209	31	EVLN ^[1]	30	456	308
9	FV ^[2]	2338	69192	36162	32	SKGL ^[1]	46	573	361
10	LLL ^[3]	837	16686	11805	33	EYFL ^[1]	9	186	82
11	RLL ^[1]	454	10923	7760	34	LAVIL ^[2]	7	64	27
12	LVL ^[2]	537	11185	7208	35	VVYPW ^[2]	0	0	2
13	LEL ^[2]	502	10147	7501	36	FIEGV ^[1]	0	9	1
14	WF ^[2]	492	11659	6929	37	NENL ^[1]	1	39	20
15	WP ^[2]	3868	10298	6726	38	LPFSQL ^[2]	0	1	0
16	GAL ^[2]	368	7150	5298	39	FYPELF ^[8]	0	0	0
17	HWPWMK ^[5]	0	0	0	40	AVVLII ^[5]	0	17	1
18	PFPGPPIPN ^[6]	0	0	0	41	APFPEVF ^[1]	0	0	0
19	GPPPIV ^[1]	0	0	0	42	KGPPPIIV ^[2]	0	0	0
20	FALPQYLK ^{[1][2]}	0	0	0	43	GPPFVI ^{[9][2]}	0	0	0
21	FFVAPFPEVFGK ^{[1][2]}	0	0	0	44	RGPPFIV ^{[10][1]}	0	0	0
22	LVPKYFG ^[1]	0	0	0	45	LPFNQL ^[2]	0	0	0
23	VYPFPPGINH ^[1]	0	0	0	46	VVYPWTQRF ^[2]	0	0	0