

BITTER PEPTIDES, OCCURRENCE AND STRUCTURE

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Abstract. The bitter taste of many protein rich foods resides in the peptide fraction. 61 bitter tasting peptides, isolated from natural systems, and 145 bitter tasting synthetic peptides are reviewed. The relationships between average hydrophobicity and bitter taste are then discussed.

1. Introduction

It has long been established that the bitter taste of protein rich foods resides in the peptide fraction, and that the hydrolysis of proteins with proteolytic enzymes is very often accompanied by formation of bitter substances. Murray and Baker (1952) were the first authors interested in the taste of enzymatic protein hydrolysates. They found a bitter taste in enzymatic hydrolysates from casein and lactalbumin, obtained with commercial proteinases, and a neutral taste in corresponding hydrolysates from gelatin. Ichikawa *et al.* (1959) hydrolysed casein, soy protein, ovalbumin and gluten with a proteinase from *Bacillus subtilis* and found the development of a bitter taste; a pronounced bitter taste was obtained with casein hydrolysates. Fujimaki *et al.* (1968b) reported that the hydrolysis of soy protein with different proteinases always results in a bitter tasting hydrolysate, due to the formation of bitter tasting peptides. Cserhati and Hollo (1974) described the formation of bitter tasting peptides, formed through enzymatic breakdown of casein by bacterial proteolytic enzymes. Petritschek *et al.* (1972) degraded proteins with five different proteolytic enzymes; a pronounced bitter taste was obtained with casein, soy protein, zein, gliadin and proteins of the lactoserum, while serum albumin, gluten and gelatin did not give a bitter note. Apparently, the substrate is of prime importance, and casein gives most bitter hydrolysates. The proteolytic enzymes applied play only a secondary rôle. Therefore the primary structure of the proteins serving as substrates for the enzymes and the amino acid composition of formed peptides are most important factors.

2. Average Hydrophobicity and Bitter Taste

Ney (1971) examined the relationships between the average hydrophobicity (Q) of many peptides and the bitter taste of these compounds, as reported in the literature. He based his studies on the data published by Tanford (1962, 1972). Tanford (1962) has shown that the hydrophobicity of amino acids, peptides and proteins can be assessed from solubility data of free amino acids. If the solubility for phenylalanine, for example, is N_{HOH} in water, and N_{EtOH} in ethyl alcohol, then the free energy of transferring one mole of phenylalanine from an aqueous solution (at a certain concentration) to an ethanolic solution (at the same concentration) is, apart from activity

coefficients:

$$\Delta F_t = -RT \ln \frac{N_{\text{EtOH}}}{N_{\text{HOH}}}, \quad (1)$$

ΔF_t , the transfer free energy, is a roughly additive function of molecular structure, that is of the different parts of the molecule:

$$\Delta F_t = \sum \Delta f_i. \quad (2)$$

For phenylalanine it can be regarded as the sum of two terms, one for the benzyl side chain and one for the dissociated backbone. The transfer free energy for the backbone must be similar to that for glycine, which is determined by the use of Equation (1) with glycine solubility data. Subtraction therefore leads to a transfer free energy for the side chain itself (Bigelow, 1967):

$$\Delta f_{tR} = \Delta F_t - \Delta f_{t\text{glycine}}, \quad (3)$$

where:

Δf_{tR} = transfer free energy for the side chain,

ΔF_t = transfer free energy for the amino acid,

$\Delta f_{t\text{glycine}}$ = transfer free energy for glycine.

Tanford (1962) and Nozaki and Tanford (1971) calculated the transfer free energies of the side chains of amino acids; the data are presented in Table I. Dunhill (1965) introduced the expression 'hydrophobicity' for these values.

Based on these data, Bigelow (1967) and Ney (1971) introduced a new parameter for peptides and proteins, the average hydrophobicity (Q), which is obtained as follows:

$$Q = \frac{\sum \Delta f_{tR}}{n} \quad (\text{cal res}^{-1}),$$

where n = number of amino acid residues.

Ney (1971) proposed the following hypothesis, which will be called 'Ney's rule' in this paper:

- all peptides having low average hydrophobicities, 1300 cal res⁻¹ or lower, are not bitter in taste;
- all peptides having high average hydrophobicities, 1400 cal res⁻¹ or higher, are bitter in taste;
- the sequences of the amino acids in the peptides have no influence on the bitter taste.

Ney (1972), in applying his theory to proteins, calculates an average hydrophobicity of total protein molecules. The Q -value obtained permits an evaluation of the bitterness of the corresponding protein hydrolyzates. Average hydrophobicities (Q) for several proteins are reported in Table II.

It has not been reported in the literature that an enzymatic hydrolysis of collagen or beef muscle (meat proteins) have ever given a bitter tasting hydrolysate. On the other

TABLE I
Hydrophobicity of the side chains of amino acids

	After Tanford (1962) ^a ΔF_i (cal)	After Nozaki and Tanford (1971) ^b ΔF_i (cal)
Tryptophan	3000	3400
Isoleucine	2970	
Tyrosine	2870	2300
Phenylalanine	2650	2500
Proline	2600	
Leucine	2420	1800
Valine	1690	1500
Lysine	1500	
Methionine	1300	1300
Alanine	730	500
Arginine	730	
Histidine		500
Glutamic acid	550	
Aspartic acid	540	
Threonine	440	400
Serine	40	-300
Glycine	0	0
Asparagine	-10	
Glutamine	-100	

^a Hydrophobicity calculated from free energy of transfer from ethanol to water.

^b Hydrophobicity calculated from free energy of transfer from ethanol and dioxane solutions to water.

TABLE II
Average hydrophobicities of several
proteins after Ney (1972)

	Q (cal res ⁻¹)
casein	1605
zein	1480
soy protein	1540
meat protein	1300
collagen	1280

side there is ample evidence that all proteins giving bitter tasting enzymatic hydrolysates have a high average hydrophobicity (Ney, 1972; Petritschek *et al.*, 1972).

Bigelow (1967) has published a list with average hydrophobicities (Q) for 150 proteins; the values vary from 440 to 2020 cal res⁻¹. Belitz (1973) discussed relationships between structure, taste and hydrophobicity of bitter peptides.

3. Occurrence of Bitter Tasting Peptides

In this section the bitter tasting peptides described in the literature are listed and discussed. The average hydrophobicities (Q , cal res⁻¹) of many of these peptides were calculated according to Ney (1972), using the data from Tanford (1962), and are also listed. Peptides with an average hydrophobicity of $Q < 1300$ cal res⁻¹ having a bitter taste are marked with an asterisk (*).

3.1. BITTER PEPTIDES FROM CASEIN

Carr *et al.* (1956) studied the bitter taste of enzymatic hydrolysates obtained from α -, β - and γ -casein; they isolated a bitter peptide composed of Asp, Glu, Gly, Ala, Val, Pro, Tyr and Leu. This peptide did not react with ninhydrin; therefore a cyclic N-terminal structure was proposed with the following C-terminal sequence: -Leu-Val-Glu-Leu-OH.

Gordon and Speck (1965) isolated several bitter peptides from skim milk cultures of certain strains of lactic bacteria. One compound has been characterized by Sullivan *et al.* (1971), and has the following structure, which corresponds to the residues 194–209 of the C-terminal sequence of β -casein (Ribadeau-Dumas *et al.*, 1972).

(1) pyroglutamic acid-Gln-Pro-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH ($Q=1700$).

Matoba *et al.* (1970) described the isolation of three bitter peptides from a tryptic hydrolysate of casein. The peptides have the following structure:

(2) H-Gly-Pro-Phe-Pro-Val-Ile-OH ($Q=2085$).

(3) H-Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys-OH ($Q=1830$).

(4) H-Phe-Ala-Leu-Pro-Gln-Tyr-Leu-Lys-OH ($Q=1898$).

Peptide (2) corresponds approximately to the residues 203–209 of β -casein, and peptide (3) corresponds to the residues 23–34 of α_{s1} -casein (Mercier *et al.*, 1971). Belitz and Sparrer (1971) and Sparrer and Belitz (1975) obtained two peptides (5) and (6) from a total hydrolysate of casein with chymotrypsin, and a peptide (7) using trypsin as enzyme. The peptides have the following structure:

(5) H-Phe-Tyr-Pro-Glu-Leu-Phe-OH ($Q=2290$).

(6) H-Val-Glu-Val-Phe-Ala-Pro-Pro-Phe-OH ($Q=1895$).

(7) H-Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys-OH ($Q=1830$).

Peptide (5) has been further degraded to (8) and (9) with the action of thermolysine:

(8) H-Phe-Tyr-Pro-Glu-OH ($Q=2167$).

(9) H-Leu-Phe-OH ($Q=2535$).

The peptides (8) and (9) are also very bitter. Peptide (7) is identical with peptide (3) described by Matoba *et al.* (1970). Peptide (5) corresponds to the residues 145–150 of α_{s1} -casein (Mercier *et al.*, 1971).

Two bitter peptides, isolated from an enzymatic hydrolysate of casein with alkaline protease from *Bacillus subtilis*, have been described by Minamiura *et al.* (1972a, 1972b):

(10) H-Arg-Gly-Pro-Pro-Phe-Phe-Ile-Val-OH ($Q=1891$).

(11) Cyclo (Trp-Leu-Trp-Leu).

The bitter taste of the peptide (10) was not influenced by a specific elimination of the terminal amino acids Arg, Val or Val+Ile. However, an elimination of Arg+Gly removed the bitterness. Shiba and Nunami (1974) showed that the peptide (11) was a diketopiperazine:

(11) Cyclo (Leu-Trp).

Peptide (10) corresponds to the C-terminal sequence of β -casein, and peptide (11) to the C-terminal sequence of α_{s1} -casein (Mercier *et al.*, 1971).

In studying the action of rennin on casein, Pélissier *et al.* (1974) isolated six bitter peptides. The structures of the peptides, assigned after comparison with the amino acid sequences of corresponding casein fractions, are as follows.

Assigned to β -casein:

(12) H-Phe-Leu-Leu-OH (residues 190–192) ($Q=2497$).

Assigned to α_{s1} -casein:

(13) H-Leu-Arg-Phe-OH (residues 21–23) ($Q=1933$).

(14) H-Val-Pro-Leu-Gly-Thr-Gln-Tyr-Thr-Asp-Ala-Pro-Ser-Phe-OH
(residues 167–179) ($Q=1309$).

(15) H-Pro-Gln-Val-Phe-OH (residues 29–32) ($Q=1735$).

(16) H-Ala-Tyr-Phe-Tyr-Pro-Glu-Leu-OH (residues 143–149) ($Q=2099$).

(17) H-Leu-Arg-Leu-OH (residues 99–101) ($Q=1857$).

Mercier *et al.* (1971), Ribadeau-Dumas *et al.* (1972) and Pelissier *et al.* (1974) isolated another series of bitter tasting peptides during studies of casein. From α_{s1} -casein they obtained the following peptides:

(18) H-Leu-Gly-Tyr-Leu-Glu-Gln-Leu-Leu-OH (residues 92–99) ($Q=1638$).

(19) H-Phe-Tyr-Pro-Glu-Leu-Phe-Arg-OH (residues 145–151) ($Q=2067$).

(20) H-Phe-Phe-Val-Ala-Pro-Phe-Pro-Gln-Val-
Phe-Gly-Lys-OH (residues 23–34) ($Q=1784$).

Studies with β -casein led to the compounds

(21) H-Ala-Pro-Lys-OH (residues 103–105) ($Q=1610$).

(22) H-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH (residues 203–209) ($Q=2211$).

The peptide (20) has also been described by Matoba *et al.* (1970) and by Sparrer and Belitz (1975). The peptide (22) resembles structurally to the peptide (2) after Matoba *et al.* (1970). The action of papain on casein liberates a bitter peptide corresponding to the residues 53–79 of β -casein, as reported by Clegg *et al.* (1974):

(23) H-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-
Ile-Pro-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-
Gln-OH ($Q=1493$).

Hill and Van Leeuwen (1974) isolated three bitter peptides from a tryptic hydrolysate of casein, having the following structures:

(24) H-Phe-Phe-Val-Ala-Pro-Phe-Pro-Gln-Val-Phe-Gly-Lys-OH ($Q=1784$).

(25) H-Tyr-Leu-Gly-Tyr-Leu-Glu-Gln-Leu-Leu-Arg-OH ($Q=1670$).

(26) H-Phe-Tyr-Pro-Glu-Leu-Phe-Arg-OH ($Q=2067$).

These peptides are fragments from α_{s1} -casein (Mercier *et al.*, 1971); peptide (25)

corresponds to the residues 23–34, peptide (26) to (91)–(100) and peptide (27) to (145)–(151).

From all the above mentioned 26 peptides, 16 (eventually 17) are fragments of α_{s1} -casein, and 5 (eventually (7)) those of β -casein. Two peptides, however, cannot be located; they probably are fragments from still unknown fractions. Therefore it cannot be stated that bitter peptides are always derived from α_{s1} - and β -casein.

3.2. BITTER PEPTIDES FROM SOY PROTEIN

Most enzymatic hydrolyzates of soy protein are bitter in taste (Fujimaki *et al.*, 1971a); this is probably due to the formation of bitter tasting peptides. Fujimaki *et al.* (1968a, 1970) and Yamashita *et al.* (1969) have isolated a series of eight bitter peptides from a soy protein hydrolysate, using pepsin as enzyme:

(27) H-Gly-Leu-OH	(Q = 1210)*.
(28) H-Leu-Phe-OH	(Q = 2535).
(29) H-Ser-Lys-Gly-Leu-OH	(Q = 990).*
(30) H-Leu-Lys-OH	(Q = 1960).
(31) H-Phe-(Ile, Leu ₂)-Gln-Gly-Val-OH	(Q = 1736).
(32) H-Arg-Leu-Leu-OH	(Q = 1875).
(33) H-Arg-Leu-OH	(Q = 1575).
(34) pyroglutamic acid-Gly-Ser-Ala-Ile-Phe-Val-Leu-OH	(Q = 1313).

Peptide (34) is ninhydrin negative.

The authors underline the importance of leucine as N-terminal or C-terminal amino acid. Arai *et al.* (1970) isolated a nondialysable bitter peptide from a pepsin hydrolysate of soy protein:

(35) H-Phe-(Arg, Asp ₂ , Glu ₂ , Gly, Ile, Leu, Lys ₂ , Pro-Ser-Thr)-Trp- (Ala, Arg, Asp, Gly, Val)-Gln-Tyr-Phe-Leu-OH	(Q = 1319).
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On treatment with N-bromosuccinimide, this peptide liberates a bitter nonapeptide:

(36) H-(Ala, Arg, Asp, Gly, Val)-Gln-Tyr-Phe-Leu-OH	(Q = 1292).
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On further treatment with carboxypeptidase, the bitterness of peptide (36) decreases; therefore the authors stress the importance of the C-terminal sequence -Tyr-Phe-Leu-OH as a center for inducing bitter taste.

In order to decrease the bitter taste of enzymatic soy protein hydrolyzates, several authors propose a 'resynthesis' of larger peptides by chymotrypsin in neutral medium with the 'plastein reaction' (Fujimaki *et al.*, 1970a; Yamashita *et al.*, 1970). This treatment gives an agreeable brothy taste, probably due to the formation of peptides rich in glutamic acid. However, Arai *et al.* (1972) isolated from such a reaction mixture also a bitter peptide:

(37) H-Glu-Phe-OH	(Q = 1600).
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3.3. BITTER PEPTIDES FROM ZEIN

Several bitter peptides were isolated from a zein hydrolysate, using pepsin as enzyme (Wieser and Belitz, 1973):

(38) H-Ala-Ile-Ala-OH	(Q = 1477).
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- (39) H-Ala-Ala-Leu-OH ($Q = 1293$)*.
 (40) H-Gly-Ala-Leu-OH ($Q = 1050$)*.
 (41) H-Leu-Gln-Leu-OH ($Q = 1613$).
 (42) H-Leu-Glu-Leu-OH ($Q = 1797$).
 (43) H-Leu-Val-Leu-OH ($Q = 2177$).
 (44) H-Leu-Pro-Phe-Asn-Gln-Leu-OH ($Q = 1682$).
 (45) H-Leu-Pro-Phe-Ser-Gln-Leu-OH ($Q = 1688$).

From this series the peptide (42) was obtained after partial hydrolysis of peptide (41).

3.4. BITTER PEPTIDES ISOLATED FROM DIFFERENT FOODS

Many bitter peptides have been obtained from enzymatic protein hydrolysates; however, only a few have been isolated from foods rich in proteins. Raadveld (1953) isolated a bitter peptide from Gouda cheese, composed of Pro, Phe and other aliphatic amino acids. Zvyagintsev *et al.* (1972) separated and characterized several bitter peptides from different Russian cheese varieties. They all contained Leu, Val, and Phe, and a cyclic N-terminal structure was reported as an important feature. Hodges *et al.* (1972), Richardson and Creamer (1973), Hamilton *et al.* (1974) separated and characterized the following bitter peptides from Cheddar cheese:

- (46) H-Glu-Val-Leu-Asn-OH ($Q = 1165$)*.
 (47) H-Asn-Glu-Asn-Leu-Leu-OH ($Q = 1087$)*.
 (48) H-Ala-Pro-Phe-Pro-Glu-Val-Phe-OH ($Q = 1984$).
 (49) H-Gln-Asp-Lys-Ile-His-Pro-Phe-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OH ($Q = 1521$).

The peptides (46), (47), (48) and (49) correspond to the residues 14–17, 17–21 and 26–33 of α_{s1} -casein, and 46–67 of β -casein, respectively. Guigoz *et al.* (1974) isolated a bitter peptide from a Swiss mountain cheese with the following structure

- (50) H-Leu-Trp-OH ($Q = 2710$).

which corresponds to the residues 198–199, the C-terminal end of the α_{s1} -casein. Huber *et al.* (1974) isolated from Butterkäse the peptide:

- (51) H-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-OH ($Q = 1844$).

which corresponds to the residues 61–69 of β -casein.

A cyclic bitter dipeptide occurs in Japanese Saké, as reported by Takahashi *et al.* (1974):

- (52) cyclo (Pro-Leu).

Other diketopiperazines are important bitter principles in roasted cocoa, as reported by Pickenhagen (1974):

- (53) cyclo (Pro-Leu).
 (54) cyclo (Val-Phe).
 (55) cyclo (Pro-Phe).
 (56) cyclo (Pro-Gly).
 (57) cyclo (Ala-Val).
 (58) cyclo (Ala-Gly).

(59) cyclo (Ala-Phe).

(60) cyclo (Phe-Gly).

(61) cyclo (Pro-Asn).

They are formed during the roasting of cocoa and exhibit a synergistic increase in bitterness together with theobromine.

3.5. SYNTHETIC BITTER PEPTIDES

Several authors studied the bitter taste sensation of synthetic peptides in order to find a relationship between structure and taste.

Kirimura *et al.* (1969) tested the series of dipeptides listed below; they all exhibit a bitter taste in 0.2% aqueous solutions.

(62) H-Gly-Ile-OH	($Q = 1485$).	(70) H-Val-Leu-OH	($Q = 2055$).
(63) H-Gly-Met-OH	($Q = 650$)*.	(71) H-Leu-Gly-OH	($Q = 1210$)*.
(64) H-Gly-Phe-OH	($Q = 1325$).	(72) H-Leu-Leu-OH	($Q = 2420$).
(65) H-Ala-Phe-OH	($Q = 1690$).	(73) H-Leu-Tyr-OH	($Q = 2645$).
(66) H-His-His-OH		(74) H-Lys-Gly-OH	($Q = 750$)*.
(67) H-Arg-Pro-OH	($Q = 1665$).	(75) H-Lys-Ala-OH	($Q = 1115$)*.
(68) H-Val-Ala-OH	($Q = 1210$)*.	(76) H-Val-Val-Val-OH	($Q = 1690$).
(69) H-Val-Val-OH	($Q = 1690$).	(77) H-Gly-Trp-OH	($Q = 1500$).

The authors relate a bitter taste with the following structural requirements for dipeptides:

- two neutral amino acids with large alkyl groups ($C \geq 3$),
- one neutral amino acid with a large alkyl group ($C \geq 3$) and one neutral amino acid with a short alkyl group ($C \leq 3$),
- one neutral amino acid and one aromatic amino acid,
- one neutral amino acid and one basic amino acid.

In relation with the bitter taste of a nondialysable peptide from soy protein hydrolysates, Arai *et al.* (1970) tested the bitter taste of the following synthetic peptides and peptide derivatives:

(78) H-Tyr-Phe-Leu-OH	(very bitter)	($Q = 2647$).
(79) H-Phe-Leu-OH	(very bitter)	($Q = 2535$).
(80) H-Tyr-Phe-OH	(bitter)	($Q = 2760$).
(81) Ac-Tyr-Phe-Leu-OH	(very bitter)	
(82) Ac-Phe-Leu-OH	(bitter)	
(83) Ac-Tyr-Phe-OH	(bitter)	
(84) H-Tyr-Phe-Leu-OEt	(not bitter)	
(85) H-Phe-Leu-OEt	(not bitter)	
(86) H-Tyr-Phe-OEt	(not bitter)	

The authors conclude that an acetylation diminishes, and an ethylation eliminates the bitter taste of dipeptides.

Arai *et al.* (1973) further studied the taste of twelve glutamyl-dipeptides. They found that the corresponding dipeptides with acid, polar and hydrophilic groups exhibit a brothy taste, while hydrophobic amino acids introduce a bitter taste; dipeptides with

intermediate structural elements have no taste. The following peptides exhibited a bitter taste:

- | | | | |
|-------------------|---------------|-------------------|---------------|
| (87) H-Glu-Ile-OH | ($Q=1760$). | (89) H-Glu-Tyr-OH | ($Q=1710$). |
| (88) H-Glu-Leu-OH | ($Q=1485$). | (90) H-Glu-Phe-OH | ($Q=1600$). |

In order to investigate the relationship between hydrophobicity and taste of peptides, the authors prepared derivatives of the tripeptide Glu-Gly-Ser and tested the taste as follows:

- | | |
|----------------------------|----------------|
| (91) H-Glu-Gly-Ser-OH | (brothy taste) |
| (92) H-Glu-Gly-O-Ac-Ser-OH | (no taste) |
| (93) H-Glu-Gly-O-Bu-Ser-OH | (bitter taste) |

They come to the conclusion that acid, hydrophilic glutamyl-oligopeptides exhibit an agreeable brothy flavor, which is diminished with increasing hydrophobicity.

Fujimaki *et al.* (1972a) observed that the dipeptide H-Glu-Tyr-OH (89) has a bitter taste below pH 10, and no bitter taste above this pH, which coincides with the dissociation of the phenolic OH-group. They attribute the loss in bitterness to the increased hydrophilic properties of the peptide. The analog compound H-Glu-Phe-OH (90) is bitter over the whole pH-range.

Shiraishi *et al.* (1973) published results on the taste of proline containing di- and tri-peptides. They differentiated between three groups, one with most bitter tasting peptides, containing proline and phenylalanine:

Group I, slightly bitter peptides:

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|-------------------|---------------|-------------------|---------------|
| (94) H-Pro-Gly-OH | ($Q=1300$). | (97) H-Ala-Pro-OH | ($Q=1665$). |
| (95) H-Gly-Pro-OH | ($Q=1300$). | (98) H-Pro-Pro-OH | ($Q=2600$). |
| (96) H-Pro-Ala-OH | ($Q=1665$). | (99) H-Val-Val-OH | ($Q=1690$). |

Group II, bitter peptides:

- | | | | |
|--------------------|---------------|--------------------|----------------|
| (100) H-Val-Pro-OH | ($Q=2145$). | (106) H-Tyr-Pro-OH | ($Q=2735$). |
| (101) H-Pro-Val-OH | ($Q=2145$). | (107) H-Pro-Tyr-OH | ($Q=2735$). |
| (102) H-Leu-Pro-OH | ($Q=2510$). | (108) H-Lys-Pro-OH | ($Q=2050$). |
| (103) H-Pro-Leu-OH | ($Q=2510$). | (109) H-Arg-Pro-OH | ($Q=1665$). |
| (104) H-Ile-Pro-OH | ($Q=2785$). | (110) H-Pro-Phe-OH | ($Q=2625$). |
| (105) H-Pro-Ile-OH | ($Q=2785$). | (111) H-Gly-Leu-OH | ($Q=1210$)*. |

Group III, very bitter peptides:

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|----------------------|---------------|------------------------|---------------|
| (112) H-Phe-Pro-OH | ($Q=2625$). | (115) H-Gly-Phe-Pro-OH | ($Q=1750$). |
| (113) H-D-Phe-Pro-OH | | (116) H-Phe-Pro-Gly-OH | ($Q=1750$). |
| (114) H-Pro-D-Phe-OH | | | |

Most interesting are differences in bitterness between these peptides due to the sequence of the amino acids. The peptide H-Pro-Phe-OH (110) is less bitter than the peptide H-Phe-Pro-OH (112); and the peptide H-Gly-Phe-Pro-OH (115) is more bitter than the peptide H-Phe-Pro-Gly-OH (116). And the two latter peptides are less bitter than the peptide H-Phe-Pro-OH (112). The peptide exhibiting the strongest bitter taste is H-D-Phe-Pro-OH (113).

A very systematic study on relationships between bitter taste and structure has been

published by Matoba *et al.* (1972). They propose three groups of bitter tasting peptides and derivatives, namely:

Group I, bitter peptides containing glycine:

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|------------------------|----------------|------------------------|---------------|
| (117) H-Gly-Ile-OH | ($Q=1485$). | (126) H-Gly-Pro-OH | ($Q=1300$). |
| (118) H-Ile-Gly-OH | ($Q=1485$). | (127) H-Pro-Gly-OH | ($Q=1300$). |
| (119) H-Gly-Ile-Gly-OH | ($Q=990$)*. | (128) H-Gly-Pro-Gly-OH | ($Q=867$)*. |
| (120) H-Gly-Leu-OH | ($Q=1210$)*. | (129) H-Gly-Tyr-OH | ($Q=1435$). |
| (121) H-Leu-Gly-OH | ($Q=1210$)*. | (130) H-Tyr-Gly-OH | ($Q=1435$). |
| (122) H-Gly-Leu-Gly-OH | ($Q=807$)*. | (131) H-Gly-Tyr-Gly-OH | ($Q=957$)*. |
| (123) H-Gly-Phe-OH | ($Q=1325$). | (132) H-Gly-Val-OH | ($Q=845$)*. |
| (124) H-Phe-Gly-OH | ($Q=1325$). | (133) H-Val-Gly-OH | ($Q=845$)*. |
| (125) H-Gly-Phe-Gly-OH | ($Q=883$)*. | (134) H-Gly-Val-Gly-OH | ($Q=563$)*. |

Group II, derivatives of amino acids with bitter taste

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|-------------------|--------------------|
| (135) Ac-Leu-OH | (142) H-Tyr-OMe |
| (136) H-Leu-OMe | (143) N-Ac-Tyr-OMe |
| (137) Ac-Leu-OMe | (144) Ac-Phe-OH |
| (138) Ac-Ile-OH | (145) H-Phe-OMe |
| (139) H-Ile-OMe | (146) Ac-Phe-OMe |
| (140) Ac-Ile-OMe | (147) Ac-Val-OMe |
| (141) N-Ac-Tyr-OH | |

Group III, bitter peptides containing leucine and phenylalanine:

- | | |
|----------------------|--------------------|
| (148) H-Leu-Leu-OH | (151) H-Phe-Leu-OH |
| (149) H-Leu-Phe-OH | (152) H-Phe-Phe-OH |
| (150) H-D-Phe-Leu-OH | |

Further work with derivatives of dipeptides of group III gave the following results. An acetylation decreases the bitterness; a methylation, a methylation and acetylation, and a cyclization all increase the bitterness. Diketopiperazines were the most bitter forms of all dipeptides. The results were then summarized as follows:

- the bitterness of amino acids is increased, if the $-NH_2$ and $-COOH$ groups are present as derivatives,
- peptides containing hydrophobic amino acids are bitter,
- sequence and configuration of amino acids have no influence on the bitterness of a peptide.

Ney (1971) tested large series of synthetic peptides and published the following list of bitter tasting compounds:

- | | | | |
|--------------------|---------------|--------------------|---------------|
| (153) H-Leu-Met-OH | ($Q=1860$). | (161) H-Gly-Ile-OH | ($Q=1485$). |
| (154) H-Met-Leu-OH | ($Q=1860$). | (162) H-Gly-Phe-OH | ($Q=1325$). |
| (155) H-Leu-Tyr-OH | ($Q=2645$). | (163) H-Gly-Trp-OH | ($Q=1500$). |
| (156) H-Leu-Leu-OH | ($Q=2420$). | (164) H-Ala-Phe-OH | ($Q=1690$). |
| (157) H-Arg-Pro-OH | ($Q=1665$). | (165) H-Val-Val-OH | ($Q=1690$). |
| (158) H-Asp-Phe-OH | ($Q=1595$). | (166) H-Glu-Phe-OH | ($Q=1600$). |
| (159) H-Asp-Tyr-OH | ($Q=1705$). | (167) H-Glu-Tyr-OH | ($Q=1710$). |
| (160) H-Val-Leu-OH | ($Q=2055$). | (168) H-Ala-Leu-OH | ($Q=1575$). |

(169) H-Ile-Ala-Met-Glu-Lys-OH ($Q = 1410$).

(170) H-Ser-Pro-Pro-Pro-Gly-OH ($Q = 2028$).

Shiba *et al.* (1974) was interested in diketopiperazines, containing leucine or tryptophane as a component. The compounds are listed in the order of decreasing bitterness:

(171) cyclo (Leu-Trp). (174) cyclo (Leu-Leu).

(172) cyclo (Trp-Trp). (175) cyclo (Leu-Gly).

(173) cyclo (Trp-Gly).

In connection with their work on the sweet tasting compound H-Asp-Phe-OMe, Mazur *et al.* (1969) investigated the taste characteristics of numerous peptides and their derivatives, and found interesting relationships, which, however, cannot be treated in detail in this paper. The following list is just a summary of tested compounds having a bitter taste:

(176) H-Asp-Phe-OH ($Q = 1595$).

(177) H-Ala-Phe-OMe

(178) H-His-Phe-OMe

(179) H-Asp-Tyr-OH ($Q = 1705$).

(180) H-Ile-Phe-OMe

(181) H-Leu-Phe-OMe

(182) H-Lys-Phe-OMe

(183) H-Phe-Phe-OMe

(184) H-Pro-Phe-OMe

(185) H-Ser-Phe-OMe

(186) H-Thr-Phe-OMe

(187) H-Trp-Phe-OMe

(188) H-Tyr-Phe-OMe

(189) H-Val-Phe-OMe

(190) H-Asp-Ala-OMe

(191) H-Asp-Gly-OMe

(192) H-Asp-Ile-OMe

(193) H-Asp-Leu-OMe

(194) H-Asp-Trp-OMe

(195) H-Asp-D-Trp-OMe

(196) H-Asp-Val-OMe

(197) H-Glu-Tyr-OMe

(198) H-Asp-Phe-NHMe

(199) H-L-Asp-D-Phe-OMe

(200) H-Asp-Phe-NMe₂

(201) H-Asp-Phe-NHNH₂

(202) H-D-Asp-D-Phe-OMe

(203) H-Asp-Tyr-NHMe

(204) H-Asp-Phe-NHNMe₂

(205) H-D-Asp-L-Phe-OMe

(206) H-Asp-Tyr-NMe₂

4. Discussion

It can be seen from this compilation that 'Ney's rule' can be applied to the majority of bitter peptides known. Only several peptides containing glycine residues do not comply with this rule. However, if glycine is not considered to be a residue and is not accounted for in the calculations, then most of the peptides have average hydrophobicities ranging higher than 1300 cal res⁻¹. Leaving aside peptides with one or several glycine residues, there are only three peptides, which do not fulfill Ney's rule, namely: H-Val-Ala-OH (68), H-Ala-Ala-Leu-OH (39) and H-Lys-Ala-OH (75). These peptides have a bitter taste, but their Q -values are below 1300 cal res⁻¹.

Two other peptides, namely (46) and (47), cannot be taken into consideration because their bitter taste qualities were not determined in pure solutions.

However, inconsistent behavior in the peptides considered seems to be rare. Therefore the average hydrophobicity should be a useful assessment of the relationships

between amino acid composition and bitter taste of peptides and protein hydrolysates. Although there is some evidence that bitterness increases with hydrophobicity, it would be an interesting topic for further research to investigate this point in some detail.

Finally it should be emphasized that this review is a restricted discussion of one aspect of a broad topic. It reports experimental results of chemical analyses of bitter tasting peptides. But the bitter taste is just one taste quality of peptides. Many peptides are described in the literature having an acid taste, a sweet taste, a brothy taste etc. And these peptides certainly differ in structure and in average hydrophobicity over a wide range. Information on these problems can be found elsewhere (Kirimura *et al.*, 1969; Arai *et al.*, 1973; Mazur *et al.*, 1969; Noguchi *et al.*, 1975).

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