ELSEVIER

Food Chemistry 69 (2000) 245–257

Food Chemistry

www.elsevier.com/locate/foodchem

Neotame: discovery, properties, utility

Claude Nofre[a,*], Jean-Marie Tinti[b]

[a]114, Cours Albert Thomas, F-69008 Lyon, France
[b]123, Cours Albert Thomas, F-69003 Lyon, France

Received 18 September 1999; received in revised form 25 October 1999; accepted 25 October 1999

Abstract

Neotame (NTM) is a new nonnutritive sweetener. NTM is a derivative of aspartame (APM). NTM has a clean sweet taste and a good flavour profile. It is a high-potency sweetener; it is 6000–10 000 times sweeter than sucrose, and 30–60 times sweeter than APM. NTM is a noncaloric, noncariogenic sweetener. NTM has an extensive shelf life in dry conditions. In aqueous food systems, it presents the same functionalities as APM in acidic medium, but it is significantly more stable in neutral medium. Consequently, NTM should be a useful sweetener in baked goods. NTM is compatible with reducing sugars and aldehyde-based flavouring agents. It has flavour-enhancing properties. Its relative cost is expected to be lower than sucrose or APM at sweetness equivalence. A petition was filed in the USA in December 1998 for its approval as a general use sweetener; other regulatory activities are underway in several countries.

1. Introduction

Neotame (NTM) is a new high-potency nonnutritive sweetener which is considered as the potential successor of aspartame (APM). As a close derivative of APM (Fig. 1), it has the intrinsic qualities of APM which were at the root of the APM commercial success, notably a very clean sweet taste, close to sucrose, with no undesirable bitter or metallic off-taste which occur in other well-known artificial sweeteners. Moreover, neotame offers additional salient advantages, such as: a status, at use levels, of a no-calorie sweetener; an increase of stability in the neutral pH range which strongly improves or widens the APM applications (e.g. in baked goods); a chemical inertness towards reducing sugars and aldehydic derivatives allowing its association with reducing sugars (glucose, fructose, high-fructose corn syrup, maltose, lactose, etc.) and flavouring agents based on aldehydic constituents (vanillin, ethyl vanillin, cinnamaldehyde, benzaldehyde, citral, etc.); an insignificant release of methanol and phenylalanine into the organism after NTM intake (with, in particular, no possible hazard for phenylketonuric subjects); a foreseeable highly competitive relative cost (cost per sucrose equivalent) as a result of its high sweetness potency. The purpose of this brief account is to answer the three questions often asked about neotame: How was it discovered? What are its properties? What is its utility?

2. Discovery

In 1991, from extensive structure–activity relationship studies in the field of high-potency sweetening agents (particularly by using our own findings in various sweetener series — Nofre & Tinti, 1983, 1986, 1989, 1990, 1992a,b, Nofre, Tinti & Ouar Chatzopoulos, 1986, 1987), we proposed, at an ECRO Symposium (see Nofre & Tinti, 1993a), that the human sweetness receptor (HSR) may contain two clearly distinct hydrophobic binding pockets located ~1 nm apart.

At that time, it was widely accepted that aspartame (APM) interacts with the HSR through one hydrophobic interaction shared between its phenyl ring and a hydrophobic binding pocket (HBP) of the HSR. On the basis of the double-HBP hypothesis, we assumed that it should be possible to reach the putative 2nd-HBP target through hydrophobic derivatives of APM (Fig. 2a). From molecular models, we estimated that the best and simplest way to touch the presumed 2nd HBP from APM was to bind hydrophobic substituents to its amino group. After different attempts, we reached the expected 2nd HBP by means of several *N*-alkyl or *N*-cycloalkyl

* Corresponding author. Fax: +33-4-37-90-50-36.
E-mail address: cnofre@worldnet.fr (C. Nofre).

0308-8146/00/$ - see front matter
PII: S0308-8146(99)00254-X

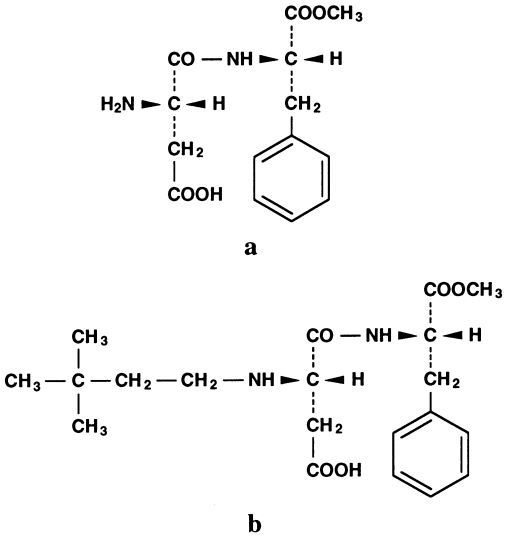


Fig. 1. Structural formulas of: (a) aspartame (APM) (Schlatter, 1966); (b) neotame (NTM) (Nofre & Tinti, 1992c; 1993b).

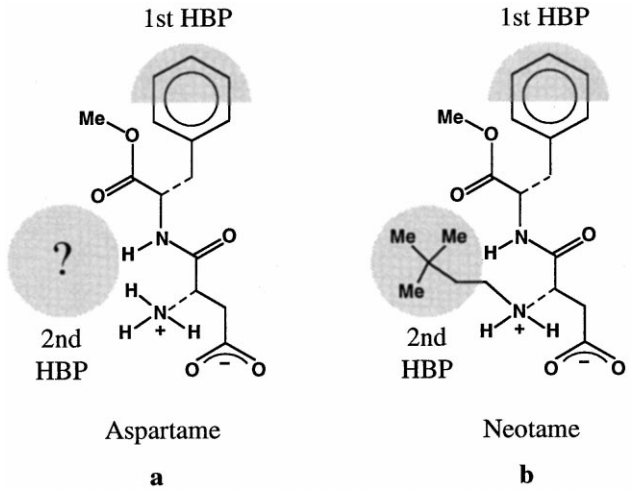
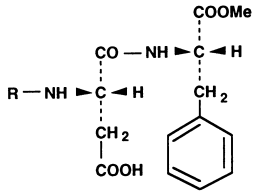


Fig. 2. (a) Searching for a second hydrophobic binding pocket (HBP) in the human sweetness receptor; (b) hitting the second hydrophobic binding pocket with neotame.

substituents (Table 1). The most efficient substituent was the 3,3-dimethylbutyl group of neotame (Fig. 2b) (Nofre & Tinti, 1992c, 1993b); with this substituent, the sweetness potency of aspartame, compared to a 2% sucrose solution, passed from ~170 × sucrose for APM to ~11 000 × for neotame on a molar basis (from ~200 × to ~10 000 × on a weight basis), which confirmed the hypothesis of the existence of two independent hydrophobic binding pockets in the human sweetness receptor.

The L-phenylalanine methyl ester moiety of neotame can be replaced by other substituents (Table 2). The two most powerful substituents are: (i) the L-hexahydrophenylalanine methyl ester group (Nofre & Tinti, 1994a, 1995a), a constituent of hexahydroneotame (~13 500 × sucrose on a molar basis, ~12 000 × on a weight basis) (Fig. 3a); (ii) the *S-tert*-butyl-L-cysteine methyl ester group (Nofre & Tinti, 1994b), a constituent of cybelame (~23 000 × sucrose on a molar basis, ~20 000 × on a weight basis) (Fig. 3b). Cybelame is the sweetest compound found in the neotame series.

Table 1
Selected structure–activity relationships in compounds of general formula



Cpd	R[a]	SP[b]	Ref.[c]
1	$(Me)_3CCH_2CH_2$	11 000	1
2	$(Et)_2CHCH_2$	2 200	1
3	c-$C_5H_9CH_2$	1 650	1
4	$(Me)_2CHCH_2CH_2$	1 400	1
5	c-C_8H_{15}	1 200	1
6	c-C_7H_{13}	1 000	1
7	c-$C_6H_{11}CH_{12}$	900	1
8	c-C_6H_{11}	900	1
9	(R,S)-$(Me)_2CHCH(Me)CH_2$	650	2
10	$(Me)_2CHCH_2$	500	1
11	$CH_3CH_2CH_2CH_2$	400	1
12	$CH_3CH_2CH_2CH_2CH_2$	100	2
13	c-$C_3H_5CH_2$	100	2
14	(R,S)-$CH_2OHCHOHCH_2$	40	2
15	CH_2OHCH_2	15	2

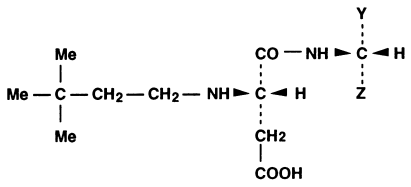
[a] Abbreviations: Me: methyl; Et: ethyl; *c*: cyclo.
[b] Sweetness potency (SP) is given on a molar basis relative to a 2% (58.4 mmol/l) sucrose solution.
[c] References: (1) Nofre and Tinti (1993b); (2) Nofre and Tinti, unpublished data.

3. Properties

The major characteristics (chemical, physical and biological properties) of neotame are summarized in Table 3. A comparison (similarities and differences) between the main properties of aspartame and neotame is summed up in Table 4; more detailed features on aspartame can be found in the following articles, books or general reviews: Beck (1974, 1978); Crosby & Furia (1983); DuBois (1991); Kim and DuBois (1991); Marie (1991); Mazur and Ripper (1979); Ripper, Homler and Miller (1986); Salminen and Hallikainen (1990); Schiffman and Gatlin (1993); Stegink and Filer (1984); Tschanz, Butchko, Stargel and Kotsonis (1996); Wells (1989).

Neotame (abbreviated NTM) is the generic name for *N*-[*N*-(3,3-dimethylbutyl)-L–α-aspartyl]-L-phenylalanine 1-methyl ester. Its structural formula is given in Fig.1b. Its molecular formula is $C_{20}H_{30}N_2O_5$, its molecular weight 378.47.

Table 2
Selected structure–activity relationships in compounds of general formula



Cpd	Y^a	Z^a	SP[b]	Ref.[c]
1	CO_2Me	$CH_2SC(Me)_3$	23 000	2
2	CO_2Me	$CH_2c\text{-}C_6H_{11}$	13 500	3
3	CO_2Me	CH_2Ph	11 000	1
4	Et	CONHCH(Et)Ph (S)	9500	4
5	iPr	$CONHCH(CH_2OMe)Ph$ (R)	5100	4
6	Et	$CONHCH(CH_2OMe)Ph$ (R)	4900	4
7	CO_2Me	CH_2(4-OH)Ph	4600	1
8	CO_2Me	$CH_2OC(Me)_3$	4300	2
9	iPr	CONHCH(Et)Ph (S)	3700	4
10	Et	CONHCH(Me)Ph (S)	2300	4
11	CO_2Et	CH_2Ph	2300	1
12	CO_2iPr	CH_2Ph	230	5
13	Me	CH_2Ph	100	5
14	CH_2OH	CH_2Ph	30	5
15	CO_2Me	Me	0	5

[a] Abbreviations: Me: methyl; Et: ethyl; iPr: isopropyl; Ph: phenyl; *c*: cyclo.

[b] Sweetness potency (SP) is given on a molar basis relative to a 2% (58.4 mmol/l) sucrose solution.

[c] References: (1) Nofre and Tinti (1993b); (2) Nofre and Tinti (1994b); (3) Nofre and Tinti (1994a, 1995a); (4) Nofre and Tinti (1997); (5) Nofre and Tinti, unpublished data.

NTM is conveniently prepared from APM and 3,3-dimethylbutyraldehyde, in a one-step high-yield process, by reductive *N*-alkylation (Fig. 4); the method (Nofre & Tinti, 1995b) consists in treating a methanolic solution of APM and 3,3-dimethylbutyraldehyde with hydrogen in the presence of a palladium (Pd/C) or platinum (Pt/C) hydrogenation catalyst (Nofre & Tinti, 1995b; Prakash, 1997).

NTM is an odourless white crystalline compound which may be obtained anhydrous or, more usually, as a hydrate (4.5% hydration water; empirical formula $C_{20}H_{30}N_2O_5.H_2O$; formula weight 396.48). The melting point of the NTM hydrate is 80.9–83.4°C (without decomposition of the molecule below ~200°C).

The crystal structure of NTM was obtained from single crystal X-ray diffraction analysis; the two hydrophobic groups of the NTM molecule are superposed in the crystal in a U-shaped conformation (Fig. 5).

NTM is stable under dry storage conditions; in dry conditions, its estimated shelf life is several years at ambient temperature. The monohydrated form is not hygroscopic.

The solubility in water of NTM is 12.6 g/l (10 g/l for APM) at 25°C. Its MRS 10% (i.e. the multiple of the required solubility to match the sweetness intensity of 10% sucrose) is ~740, which means that the NTM solubility is ~740 times greater than necessary to obtain a sweetness level matching a 10% sucrose solution (17 mg/l of NTM). By comparison, the MRS 10% of APM is ~19. Note that the speed of dissolution of NTM in aqueous systems can be significantly increased by using NTM in a salt form (e.g. as a phosphate salt) or a complex form (e.g. with β-cyclodextrin). In anhydrous ethanol, the NTM solubility is very high (~950 g/l at 25°C), over 250 × that of APM (3.7 g/l at 25°C).

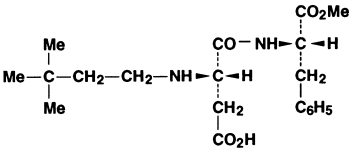
NTM is an amphoteric compound. At 25°C, its pK_1 is 3.01, its pK_2 8.02 (3.1 and 7.9, respectively, for APM). The pH of its isoelectric point (minimal charge and, in general, minimal solubility) is ~5.5 (close to that of APM).

NTM, at use levels, has negligible effects on the viscosity (< 5 mPa.s at 5 g/l), surface tension (~65 mN/m at 0.015 g/l, ~38 mN/m at 5 g/l) and pH (pH 7.01 at 0.15 g/l, 6.25 at 1 g/l, 5.8 at 5 g/l) of its aqueous solutions; its insignificant viscosity should not give rise to any mixing problems, and the negligible lowering of the surface tension and pH of its solutions should not lead to excessive foaming, e.g. in carbonated soft drinks.

In aqueous solution, the stability of NTM varies strongly with the pH and the temperature (see Fig. 6 and Table 5). Like APM, NTM is relatively stable at pH from 3 to 5.5. The optimal pH for NTM stability is ~4.5. The degradation of NTM follows a pseudo first-order kinetics. At pH 4.5, the half-life of NTM is ~30 weeks at 25°C, ~45 days at 40°C, ~40 hours at 80°C in 0.1 M phosphate buffers. At pH 3, the half-life of NTM is ~11 weeks at 25°C, ~22 days at 40°C, ~24 h at 80°C. The stability of NTM at 80°C in pH range of 3-5.5 implies that high-temperature short-time (HTST) processing is possible with food products sweetened with NTM; for example, after 30 min at 80°C, NTM percentage remaining in a pH 3 solution is ~98.6%, which indicates that there is practically no loss of NTM within 30 min at 80°C. At pH 7, the half-life of NTM is ~2 weeks at 25°C, ~3 days at 40°C and ~4 h at 80°C. NTM has approximately the same stability as APM in the acidic pH range; in the neutral pH range, NTM is significantly more stable than APM: at pH 7, e.g., the half-life of NTM is 124 days, that of APM 36 days at 5°C; at 30°C, 6.6 days for NTM, 1.5 days for APM; at 70°C, 13 h for NTM, 1 h for APM.

In aqueous systems, the major decomposition pathway of NTM is the hydrolysis of the methyl ester group into dimethylbutylaspartylphenylalanine (DMB-Asp-Phe) and methanol (MeOH), in both the acidic and the neutral pH ranges (Fig. 7). For APM (Fig. 8), two major degradation routes coexist: the first route, identical to that of NTM, is the result of the hydrolytic scission of the methyl ester group into aspartylphenylalanine (Asp-Phe) and MeOH; the second route, which is important mainly in the neutral pH range, leads, through a cyclization process, to cycloaspartylphenylalanine [cyclo(Asp-Phe)],

Table 3
Main characteristics of neotame

Generic name	Neotame
Chemical name	*N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester
Simplified chemical name	Dimethylbutylaspartylphenylalanine methyl ester
Abbreviation	NTM
CAS registry number	[165450-17-9]
Molecular formula	$C_{20}H_{30}N_2O_5$
Molecular weight	378.47
Percentage composition	C 63.47%, H 7.99%, N 7.40%, 0 21.14%
Structural formula	
Symbolic formula	DMB-Asp-Phe-OMe
Preparation	Reductive alkylation of APM with 3,3-dimethylbutyraldehyde over Pd/C
Physical state	White crystalline powder; usually obtained as a monohydrate (H_2O∼4.5%; empirical formula $C_{20}H_{30}N_2O_5.H_2O$; formula weight 396.48)
Melting point	80.9–83.4°C (from MeOH aq) without decomposition
Density, compressibility	0.33 g/cm^3 (aerated bulk powder); compressibility 47.5% (packed bulk density 0.63 g/cm^3)
Bulk chemical stability	stable under dry storage conditions; non hygroscopic
Solubility	Water (g/l): 10.6 (15°C), 12.6 (25°C), 47.5 (60°C); ethyl acetate (g/l): 43.6 (15°C), 77 (25°C), > 1000 (60°C); abs. ethanol (g/l): ∼950 (25°C)
pK_a values	$pK_1(CO_2H)$ 3.01, pK_2 (NH+) 8.02; isoelectric point: pH 5.5
Specific rotation	$[\alpha]_D^{20}$ −39.8°(c = 0.5 in water)
Viscosity	at 20 and 40°C < 5 mPa.s (5 g/l)
Surface tension	at 20°C ∼65 mN/m (0.015 g/l), ∼38 nN/m (5 g/l)
pH in aq. solution	7.01 (0.15 g/l); 6.25 (1 g/l); 5.8 (5 g/l)
Solution stability	at pH 3, in 0.1 M phosphate buffers, $t_{1/2}$ 11 weeks at 25°C, 22 days at 40°C, 24 h at 80°C; at pH 7, 2.25 weeks at 25°C, 3 days at 40°C, 4 h at 80°C
pH of maximal stability	∼4.5
Degradative pathways	In aqueous solution, slow hydrolysis of the ester into DMB-Asp-Phe and MeOH; no cyclization reaction into a diketopiperazine derivative, contrary to APM
Chemical inertness	No reaction with reducing sugars (fructose, glucose, high-fructose corn syrup, lactose, maltose, etc.) or with aldehydic flavouring agents (vanillin, ethyl vanilin, cinnamaldehyde, benzaldehyde, citral, etc.), contrary to APM
Taste quality	Clean sweet taste (close to APM) without bitter or metallic off-taste; temporal profile close to APM
Sweetness potency	On a weight basis: ∼10 000 × sucrose vs 2% sucrose, ∼9000 × vs 5% sucrose, ∼6000 vs 10% sucrose, ∼30–60 × APM
Flavour enhancement	Enhancer of certain natural flavours, such as acidic fruit flavours
Taste synergy	No significant synergy with acesulfame-K or saccharin, contrary to APM
Metabolic pathways	Approximately half the ingested dose is nonabsorbed and excreted through the faeces, and half is absorbed then excreted through the urine as de-esterified NTM (DMB-Asp-Phe)
Safety	At the estimated 90th percentile of use, the margins of safety for consumers are > 10 000 (vs rabbits), 16 000 (dogs), 20 000 (rats) and 80 000 (mice)
Utility	Nonnutritive, noncaloric, noncariogenic sweetener
Caloric value	< 1.2 kJ/g; < 0.0002 kJ per unit of sweetness
Typical use levels	Tabletop: 0.9 mg/unit; lemon tea (hot packed): 8 mg/l; yogurt: 15 mg/l; carbonated soft drinks: 17 mg/l; yellow cake: 35 mg/kg; chewing gum: 250 mg/kg
Expected relative cost	Lower than sucrose or APM at sweetness equivalence

a diketopiperazine (DKP) derivative (3-benzyl-6-carboxymethyl-2,5-dioxopiperazine), and to MeOH (Fig. 8). The absence of a cyclization pathway in the course of the NTM degradation explains the significant increase of the NTM stability in the neutral pH range with regard to APM, which offers possibilities of new applications not directly accessible to APM (e.g. in baking applications).

In contrast to the primary amino group of APM, the secondary amino group of NTM is unable to react, through condensation reactions, with reducing sugars and aldehydic derivatives. The unreactiveness of NTM towards these compounds allows its association (1) with various reducing carbohydrates (such as glucose, fructose, high-fructose corn syrup, lactose, maltose, etc.) without the possibility of undesirable Maillard-type reactions, and (2) with various flavouring agents or aromas based on aldehydic components, such as vanillin or ethyl vanillin (vanilla), cinnamaldehyde (cinnamon), benzaldehyde (cherry and bitter almond), citral (lemon), etc. (see Fig. 9), without the possibility of Schiff base formation, contrary to APM (Fig. 10).

Table 4
Comparison between aspartame and neotame

	Aspartame	Neotame
Chemical name	L–α-Aspartyl-L-phenylalanine 1-methyl ester	*N*-[*N*-(3,3-dimethylbutyl)-L–α-aspartyl]-L-phenylalanine 1-methyl ester
Simplified chemical name	Aspartylphenylalanine methyl ester	Dimethylbutylaspartylphenylalanine methyl ester
Abbreviation	APM	NTM
CAS registry number	[22839-47-0]	[165450-17-9]
Molecular formula	$C_{14}H_{18}N_2O_5$	$C_{20}H_{30}N_2O_5$
Molecular weight	294.31	378.47
Symbolic formula	Asp-Phe-OMe	DMB-Asp-Phe-OMe
Physical state	White crystalline powder; usually obtained as a hemihydrate (H_2O∼2%; empirical formula $C_{14}H_{18}N_2O_5.1/2H_2O$; formula weight 303.32)	White crystalline powder; usually obtained as a monohydrate (H_2O∼4.5%; empirical formula $C_{20}H_{30}N_2O_5.H_2O$; formula weight 396.48)
Bulk chemical stability	Stable under dry storage conditions	Stable under dry storage conditions
Solubility (25°C)	H_2O: 10 g/l; abs. EtOH: 3.7 g/l	H_2O: 12.6 g/l; abs. EtOH: ∼950 g/l
MRS 10%[a]	19	∼740
pK_a values	pK_1 3.1, pK_2 7.9; isoelectric point: pH 5.5	pK_1, 3.01, pK_2 8.02; isoelectric point: pH 5.5
Solution stability	Approximately like NTM in the acidic pH range; less stable than NTM in the neutral pH range	Approximately like APM in the acidic pH range; more stable than APM in the neutral pH range
pH of maximal stability	∼4.2	∼4.5
Major degradants	Asp-Phe, cyclo(Asp-Phe) and MeOH (which are metabolized)	DMB-Asp-Phe (excreted in faeces) and MeOH (metabolized)
Incompatibilities	Reducing sugars (Maillard reaction), aldehydic flavouring agents (Schiff base formation)	No reaction with reducing sugars and aldehydic flavouring agents
Taste quality	Clean sweet taste	Clean sweet taste close to APM
Sweetness potency	∼180–200 × sucrose on a weight basis	∼6000–10 000 × sucrose, ∼30–60 × APM on a weight basis
Flavour enhancement	Flavour-enhancing properties	Flavour-enhancing properties
Taste synergy	Synergy with acesulfame-K or saccharin	No synergy with acesulfame-K or saccharin
Major metabolities	Asp, Phe and MeOH (which are metabolized)	DMB-Asp-Phe (excreted in the faeces and in the urine) and MeOH (metabolized)
Limit of use	Specific labelling for phenylketonurics	No specific labelling for PKU expected
Utility	Low-calorie sweetener at use level; noncariogenic	No-calorie sweetener at use level; noncariogenic
Caloric value	17 kJ/g; 0.085 kJ per unit of sweetness	< 1.2 kJ/g; < 0.0002 kJ per unit of sweetness
Typical use levels	Tabletop: 37 mg/unit; carbonated soft drinks: 525 mg/l	Tabletop: 0.9 mg/unit; carbonated soft drinks: 17 mg/l
Relative cost	Approximately equal to sucrose at sweetness equivalence	Expected relative cost should be much lower than sucrose or APM at sweetness equivalence

[a] MRS 10%: multiple of the required solubility to match a sweetness intensity of 10% sucrose.

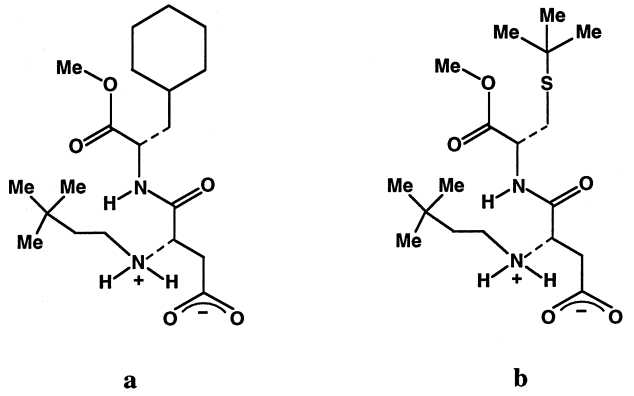


Fig. 3. The two sweetest compounds of the neotame series: (a) hexahydroneotame (Nofre & Tinti, 1994a, 1995a); (b) cybelame (Nofre & Tinti, 1994b).

NTM has a clean sweet taste close to that of APM, without the bitter or metallic notes often associated with artificial sweeteners (e.g. with acesulfame-K or saccharin). Its flavour profile does not significantly differ from APM under varied conditions (see Fig. 11 for a comparison of their flavour profiles in two different systems). Although its sweetness develops gradually like sucrose, it is perceived with a slight initial lag with regard to APM and it persists slightly longer (Fig. 12). Similarly to APM, in complex food systems these temporal effects can be modified, when desired, by association with other ingredients, such as polyols, sucrose or hydroxy acids.

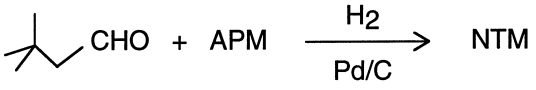


Fig. 4. Preparation of NTM by reductive *N*-alkylation with 3,3-dimethylbutyraldehyde over palladium black (Nofre & Tinti, 1995b).

The sweetness potency of NTM when compared with sucrose (see Fig. 13) is about 10 000 times that of sucrose on a weight basis (∼11 000 × on a molar basis) relative to a 2% sucrose solution, about 9000 times that of sucrose on a weight basis (∼10 000 × on a molar

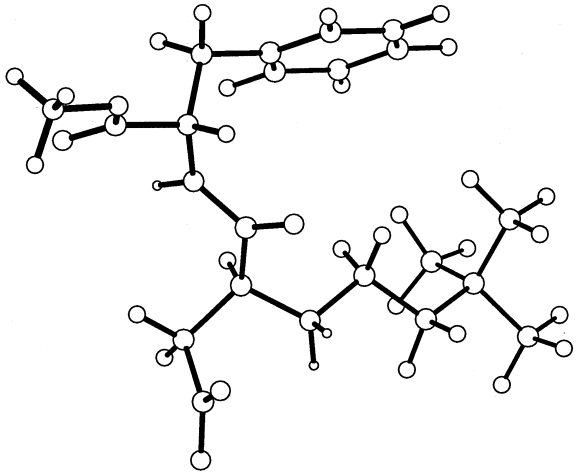


Fig. 5. The U-shaped conformation of the hydrophobic substituents of NTM in crystalline state as deduced from single crystal X-ray diffraction analysis. Crystal data: empirical formula $C_{20}H_{30}N_2O_5.H_2O$ (the water molecule of crystallization is not included in the structural model); temperature $20 \pm 2°C$; crystal system: monoclinic; space group $P2_1\text{-}C_2^2$ (No. 4) with $a = 12.7623(2)$Å, $b = 5.6017(1)$Å, $c = 15.2934(3)$Å, $\beta = 102.403(1)$, $V = 1067.83(3)$Å^3, and $Z = 2\{d_{calcd} = 1.233\ gcm^{-3}$; $\mu_a(CuK_\alpha) = 0.75\ mm^{-1}\}$.

Table 5
Stability of NTM as a function of pH (0.1 M phosphate buffers) and temperature

pH	Half-life		
pH	At 25°C (weeks)	At 40°C (days)	At 80°C (hours)
2.0	3.73	9.77	14.50
3.0	11.07	22.34	24.09
4.0	22.29	44.19	39.48
4.5	29.75	44.88	39.79
5.0	21.53	37.94	34.18
5.5	16.01	24.28	21.35
6.0	8.90	12.11	11.26
7.0	2.25	2.93	4.13
8.0	0.65	0.72	1.56
9.0	0.22	0.24	0.63

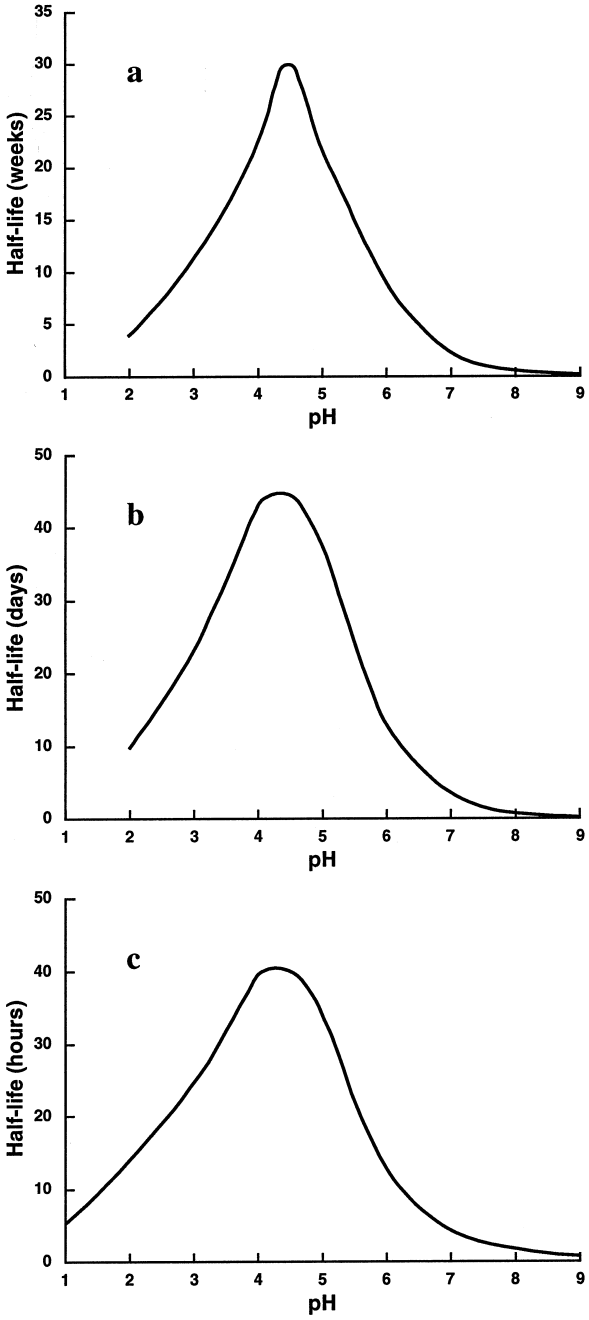


Fig 6. Stability of NTM as a function of pH (in 0.1 M phosphate buffers) and temperature: (a) at 25°C; (b) at 40°C; (c) at 80°C.

basis) relative to a 5% sucrose solution, and about 6000 times that of sucrose on a weight basis (~6600 × on a molar basis) relative to a 10% sucrose solution. Note that a sweetness potency of 10 000 on a weight basis relative to sucrose means that 1 kg of NTM is equivalent to the sweetness of 10 000 kg (10 metric tons) of sugar. As the sweetness potency of APM is estimated to be about 180–200 times that of sucrose on a weight basis, the potency of NTM is approximately 30–60 times that of APM on a weight basis in a wide range of applications. In addition, NTM is much more potent than any of the commercially available sweeteners (Table 6).

Like APM, NTM intensifies certain food and beverage flavours, particularly, acidic fruit flavours (such as orange, lemon and grapefruit) and cherry flavour. Unlike APM, there is no significant taste synergy of NTM with acesulfame-K or saccharin.

In humans, after ingestion of NTM, approximately half of the ingested dose is eliminated through the faeces as 3,3-dimethylbutylaspartylphenylalanine (DMB-Asp-Phe), and roughly half is absorbed as intact NTM, which is afterwards hydrolysed into DMB-Asp-Phe and MeOH: in the predominant process, DMB-Asp-Phe is eliminated (half-life about 2 h) in the urine without significant retention in any tissues; only a minor part is metabolized through the oxidation of the 3,3-dimethylbutyl moiety

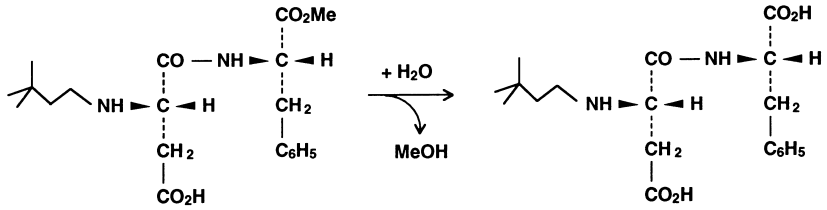


Fig 7. Hydrolytic degradation of NTM into dimethylbutylaspartylphenylalanine (DMB-Asp-Phe) and methanol (MeOH). Note that DMB-Asp-Phe is not sweet; in consequence, at an advanced stage of conversion, a diminution in sweetness may be noticed, but without development of undesirable off-tastes, the conversion products being all tasteless. It must be underlined that DMB-Asp-Phe, the major degradant, is also the major metabolite of NTM in humans.

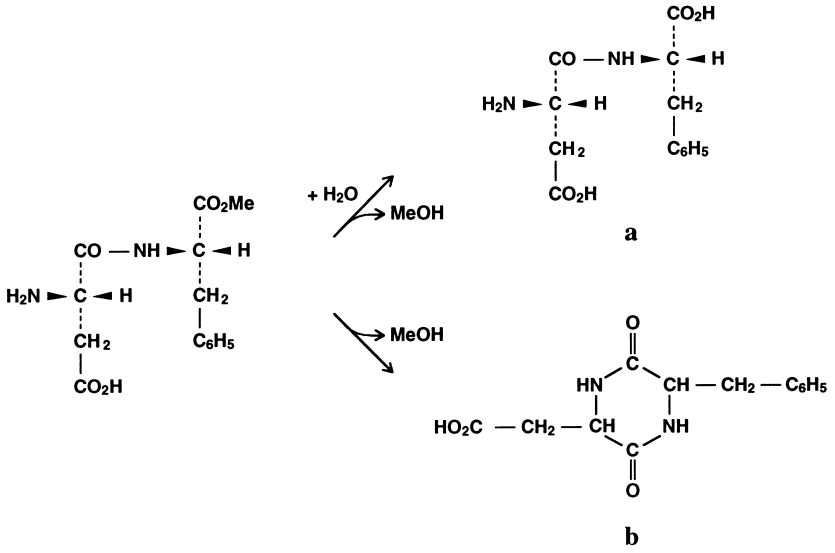


Fig. 8. The two major routes of the APM degradation in solution: (a) hydrolysis into aspartylphenylalanine (Asp-Phe) and methanol (MeOH); (b) cyclization into cycloaspartylphenylalanine (cyclo(Asp-Phe)) with a concomitant release of MeOH. Asp-Phe and cyclo(Asp-Phe) are both tasteless, which explains the sweetness decrease of aged APM solutions. Note that the cyclization process is, in solution, particularly significant under neutral or alkaline conditions (above pH 5) or under the influence of heat. In dry conditions, the cyclization reaction is also the preponderant decomposition process of APM when subjected to temperatures above 150°C (see Homler, 1984).

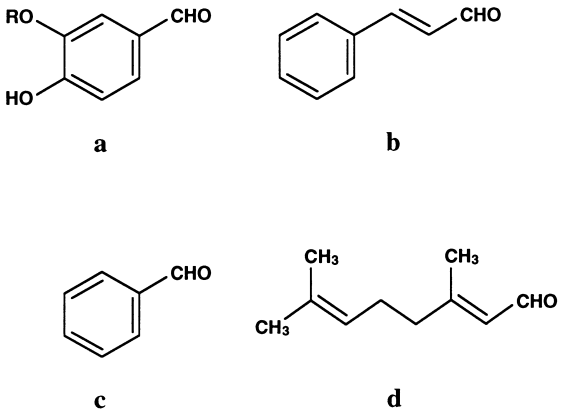


Fig. 9. Main aldehydic components of some common flavouring agents: (a) vanillin (R = CH_3) and ethyl vanillin (R = C_2H_5) (vanilla); (b) cinnamaldehyde (cinnamon); (c) benzaldehyde (cherry and bitter almond); (d) citral (lemon).

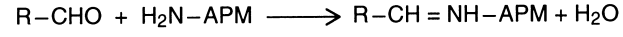


Fig. 10. Undesirable imine (Schiff base) formation by reaction between an aldehydic compound and the unsubstituted amino group of APM; this reaction is not possible with the *N*-substituted amino group of NTM.

into 3,3-dimethylbutyric acid, which is eliminated in the urine as a carnitine ester in humans (less than 5% of the NTM intake) (Fig. 14). Note that the metabolic degradation of APM is quite different from that of NTM (Fig. 15): APM is in fact broken down in the gut (in the intestinal lumen or mucosal cells) into its three components — aspartic acid (Asp), phenylalanine (Phe) and MeOH — which enter the portal circulation and are metabolized according to the usual biochemical pathways. In brief, NTM is, for the most part (over

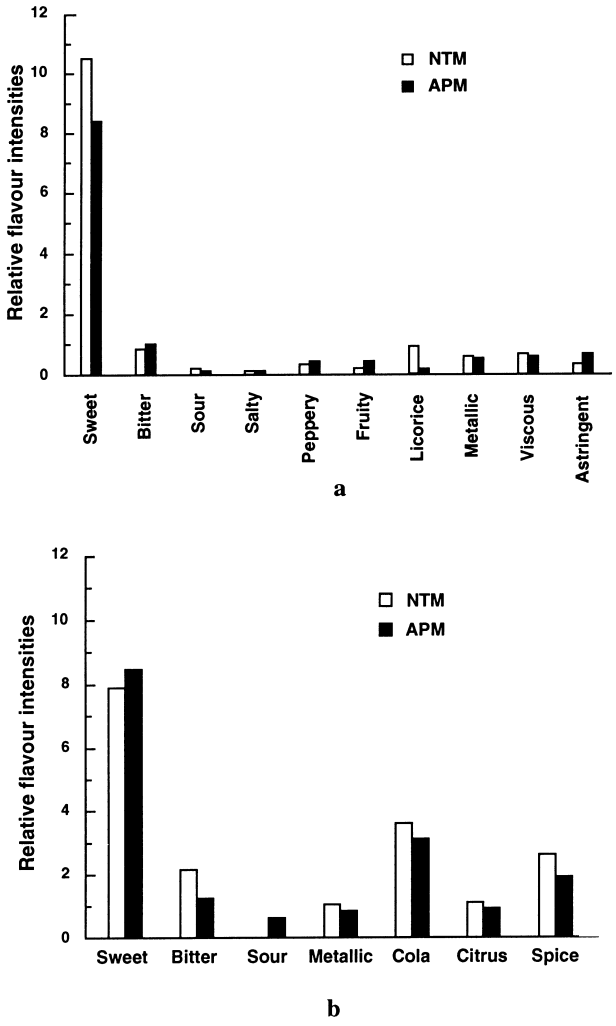


Fig. 11. Similarities between the flavour profiles of NTM- and APM-sweetened beverages: (a) in aqueous solutions (NTM 20 mg/l, APM 560 mg/l); (b) in cola soft drinks (NTM 16 mg/l, APM 525 mg/l).

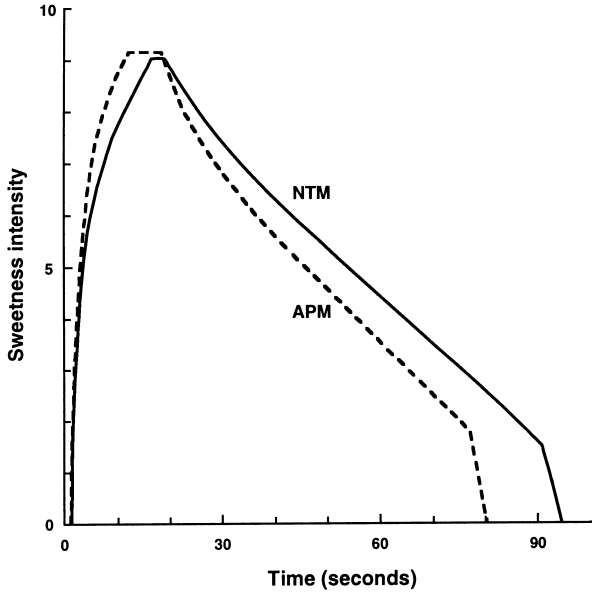


Fig. 12. Compared sweetness temporal profiles in water of NTM (17 mg/l) and APM (525 mg/l) based on the Liu and MacFie method (1990). The main parameters of the time-intensity curves are: I_{max} (maximum intensity): 9.2 for APM, 9.0 for NTM; t_{lag} (lag time, i.e. time taken for intensity to rise above baseline): 1.3 for APM, 1.5 for NTM; t_{max} (time to reach maximum intensity): 12.3 for APM, 16.6 for NTM; $t_{plateau}$ (plateau time): 6.1 for APM, 2.8 for NTM; t_{end} (finish time, i.e. time when intensity returns to baseline): 80.5 for APM, 94.8 for NTM; AUC_{tot} (total area under the curve): 425 for APM, 491 for NTM. I_{max} values represent mean intensity units (0–15); t_{lag}, t_{max}, $t_{plateau}$ and t_{end} are expressed in seconds; AUC_{tot} is expressed as intensity units × time. For a comparison of the time-sweetness intensity curve of APM with those of sucrose and of several other sweeteners, see Ott, Edwards and Palmer (1991), Ketelsen, Keay and Wiet (1993).

Table 6
Sweetness potency of some selected sweeteners relative to a 2% sucrose solution on a weight basis

Sweeteners[a]	Sweetness potency
Sucrose	1
Na cyclamate	30
Aspartame	200
Acesulfame-K	250
Na saccharin	400
Sucralose	750
Neotame	10 000

[a] Among the potently sweet artificial sweeteners mentioned above, four are approved in the United States to date, namely, saccharin, aspartame, acesulfame-K, and sucralose.

90%), eliminated from the body through the excreta, while APM is completely metabolized.

As mentioned above, among the major metabolites and degradants of NTM and APM, methanol is the only one which is common to NTM and APM. It has been abundantly shown with APM that MeOH, which is a common dietary component, is not a safety concern at usual intake doses of APM. For example, beverages sweetened with APM to a sweetness level matching 10% sucrose solution (~525 mg/l) contain the equivalent of about 56 mg/l of MeOH, which is substantially less than the MeOH content commonly found in fruit juices or in vegetable juices (see Fig. 16). With NTM, the potential MeOH content of NTM-sweetened foods or beverages will be still lower (about 40 times less) than with APM. For example, since the NTM hydrate is potentially able to release, through metabolic or degradative hydrolytic processes, 8.08% of MeOH on a weight basis, the potential MeOH content of a beverage formulated with 17 mg/l of NTM (which corresponds approximately to the sweetness of a 10% sucrose solution) is 1.37 mg/l; this amount is insignificant, since it is, e.g., ~46 times lower than the average MeOH content of one litre of orange juice, or ~220 times lower than that of 1 l of tomato juice (Fig. 16).

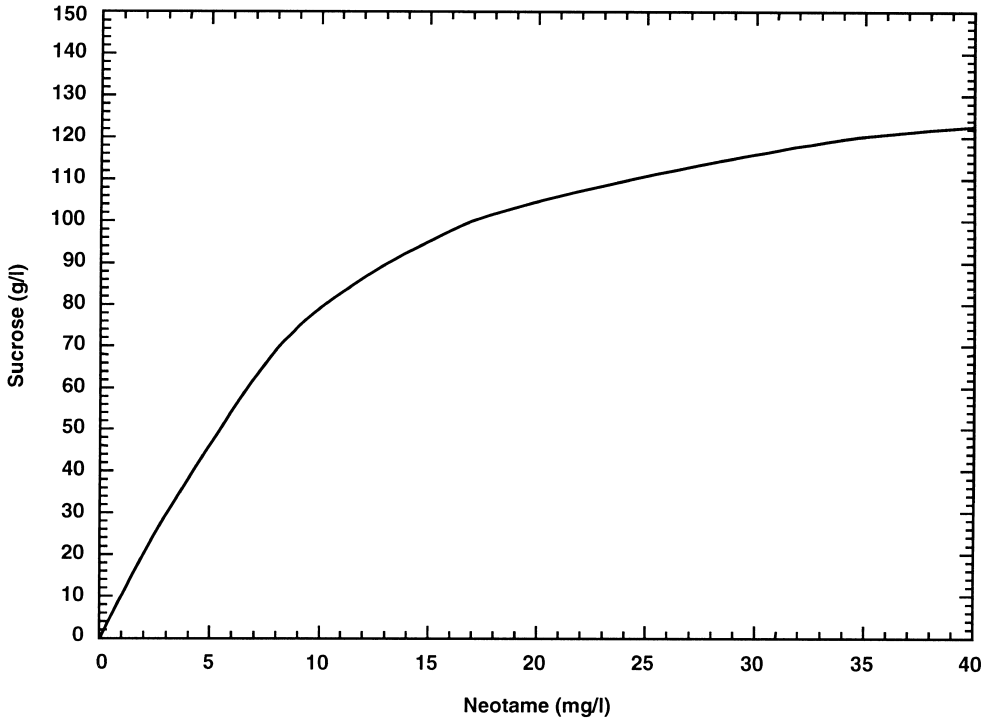


Fig. 13. Approximate sweetness equivalences between sucrose and NTM on a weight basis in water. Note that the relative sweetness potency of NTM, like that of APM or other intense sweeteners, varies with the concentration: e.g. from this graph, one can estimate that the sweetness of a 2 mg/l NTM solution is approximately equivalent to that of a 20 g/l (2%) sucrose solution (potency ~10 000 × sucrose on a weight basis), that of a 5.5 mg/l NTM solution to that of a 50 g/l (5%) sucrose solution (potency ~9000 × sucrose on a weight basis), and that of a 17 mg/l NTM solution to that of a 100 g/l (10%) sucrose solution (potency ~6000 × sucrose on a weight basis).

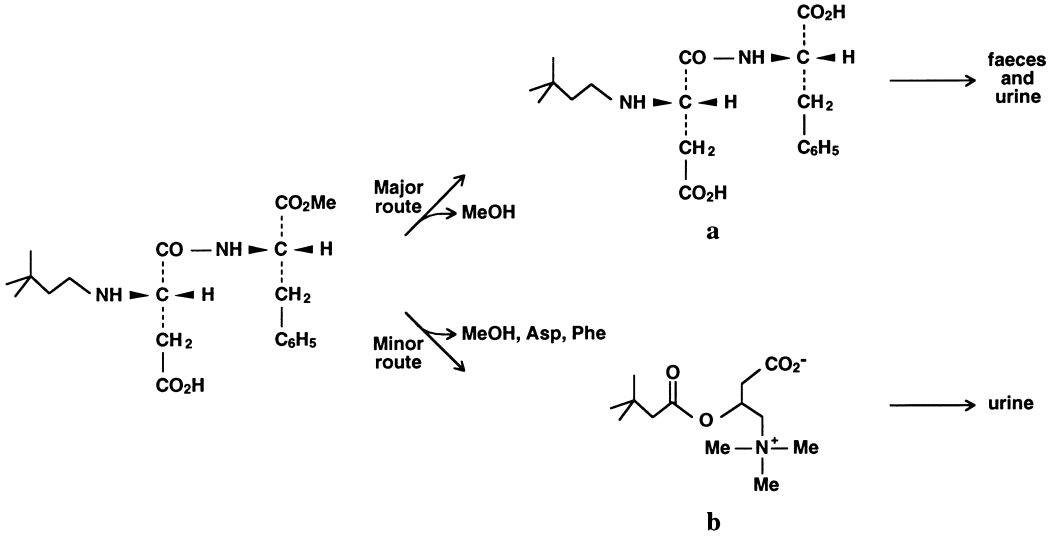


Fig. 14. Metabolic conversion of NTM: (a) in the major metabolic process (more than 90%), NTM is converted to its de-esterified derivative, DMB-Asp-Phe, which is excreted in the faeces and in the urine; (b) in a minor process (less than 10%), the 3,3-dimethylbutyl portion of DMB-Asp-Phe is oxidized to 3,3-dimethylbutyric acid which is excreted in the urine as a carnitine ester (< 5% of the ingested dose).

During its metabolism, APM releases approximately 55% by weight of phenylalanine (Phe) which is normally metabolized according to the usual metabolic pathways, except for people suffering from phenylketonuria (PKU), a rare genetic disease (one person in 10 000 approximately) characterized by a deficiency in the ability to metabolize Phe. As a consequence, excess consumptions of Phe by PKU sufferers induce higher plasma levels of this amino acid, altered synthesis of monoamine neurotransmitters, and other adverse effects; such patients must, therefore, carefully control and limit their consumption of Phe from all dietary

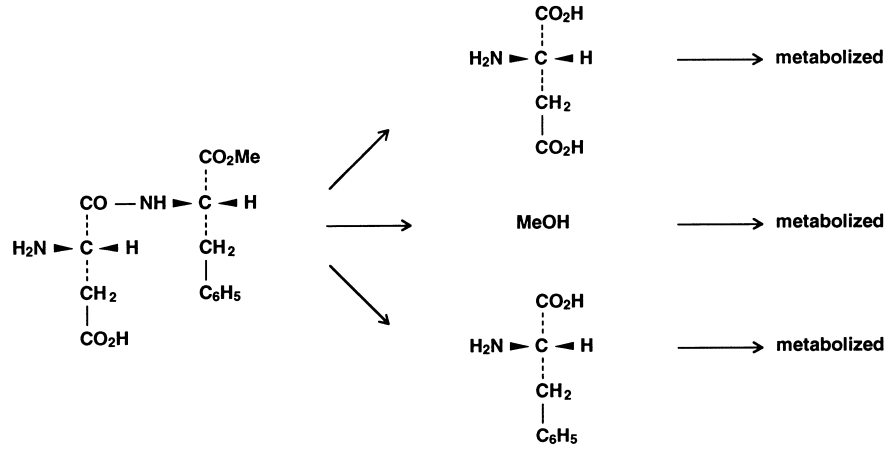


Fig. 15. Metabolic conversion of APM: in the gastrointestinal tract, APM is converted into aspartic acid (Asp), phenylalanine (Phe) and methanol (MeOH) which are absorbed and further metabolized according to the usual pathways.

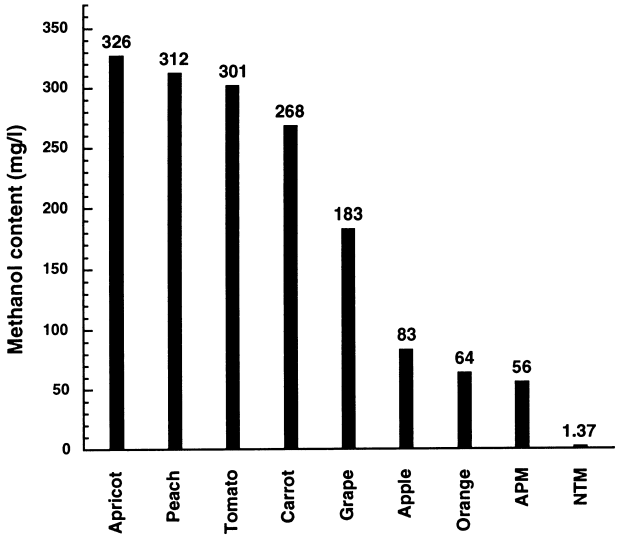


Fig. 16. Total (potential + actual) methanol content of various vegetable or fruit juices (Wucherpfennig, Dietrich & Bechtel, 1983) compared to that of beverages formulated with APM (525 mg/l) or NTM (17 mg/l). See also Lindinger, Taucher, Jordan, Hansel and Vogel (1997) for the endogenous production of methanol after the consumption of fruit.

Table 7
Phenylalanine (Phe) content of various foods and beverages (Vervack et al., 1981) compared to the Phe content of beverages sweetened with APM (525 mg/l) or NTM (17 mg/l)

Foods or beverages	Phe content (mg/l or kg)
Meat	7000–10 000
Dairy products and milks	1300–10 000
Breads and cereals	3000–6000
Vegetables	100–13 000
Fruits	64–4000
APM (525 mg/l)	290
NTM (17 mg/l)	7.08[a]

[a] 7.08 mg/l corresponds to the Phe amount (41.6%) theoretically contained in 17 mg of NTM monohydrate. Since over 90% of Phe is excreted in the faeces and in the urine as DMB-Asp-Phe, below 10% of the Phe amount (i.e. less than 0.7 mg) is in fact released into the body (i.e. lower than 4.16% of the NTM initial administered dose).

sources. Despite the low phenylalanine content of APM-based products compared to meat, milk or other protein foods (see Table 7), it has been required in many countries that APM-formulated goods carry a label on their packaging to inform phenylketonurics that the product contains phenylalanine. As NTM is formulated at concentrations approximately 40 times lower than those of APM, the Phe content of NTM-sweetened goods is insignificant (7.08 mg/l for 17 mg/l of the NTM hydrate), much lower than the Phe content of fruit juices for example (see Fig. 17); furthermore, the amount of Phe effectively released into the body from 17 mg of NTM through metabolic pathways is still lower, below 0.7 mg, i.e. ~400 times less than with APM. Consequently, with such negligible amounts, labelling for phenylketonurics should not be necessary for products containing NTM.

Finally, from an extensive safety programme, it has been demonstrated that both NTM and its major metabolite (DMB-Asp-Phe) are safe for the general population. NTM is not mutagenic, clastogenic, teratogenic or carcinogenic, and does not produce reproductive or target organ toxicity. The very low exposure to NTM, combined with the highly-favourable pharmacokinetic profiles of its molecule and of its major metabolite, leads to extremely high margins of safety for consumers exposed to NTM. Based on a projected NTM intake of ~50 μg/kg/day (inferred from the APM consumption, at the 90th percentile of use), and on a no-observable-adverse-effect level (NOAEL) of 500 mg/kg/day in rabbits, 800 in dogs, 1000 in rats and 4000 in mice, the margins of safety for consumers are greater than 10 000, 16 000, 20 000 and 80 000, respectively, with regard to these species. Moreover, NTM is perfectly tolerated in humans following studies with very high oral doses of up to 40 times the estimated 90th

percentile intake. This comprehensive database demonstrates the safety of NTM as a general use sweetener.

4. Utility

NTM is a nonnutritive, noncaloric sweetening agent. Because the caloric value of APM is estimated, as a dipeptide, to be 17 kJ/g (4 kcal/g), and as NTM is made up of ~75% by weight of APM, the inferred caloric value of NTM should be ~12 kJ/g (3 kcal/g); in fact, since less than 10% by weight of NTM is really metabolized in the body via a minor metabolic route (see Fig. 14), it follows that the effective caloric value of NTM should be less than 1.2 kJ/g (< 0.3 kcal/g). As APM is ~200 times as sweet as sucrose on a weight basis, its caloric value per unit of sweetness is 0.085 kJ (0.02 kcal); as NTM is ~6000 times sweeter than sucrose relative to a 10% sucrose solution, its caloric value per unit of sweetness should be less than 0.0002 kJ (< 0.00005 kcal), which is insignificant compared with the caloric value of sucrose (17 kJ/g). From these caloric values (17 kJ/g for sucrose, 17 kJ/g for APM, and < 1.2 kJ/g for NTM), it can be easily calculated that the energy content of a beverage formulated with 100 g/l of sucrose is 1700 kJ/l; with 525 mg/l of APM, it is 8.92 kJ/l; with 17 mg/l of NTM, it is < 0.02 kJ/l. In other words, a drink prepared with APM has an energy content ~190 × lower than a sucrose-containing drink, whereas a drink prepared with NTM has an energy content at least 446 × lower than a drink prepared with APM and at least 85 000 × lower than a drink prepared with sucrose. As a result, NTM can be considered as a no-calorie sweetener at use level.

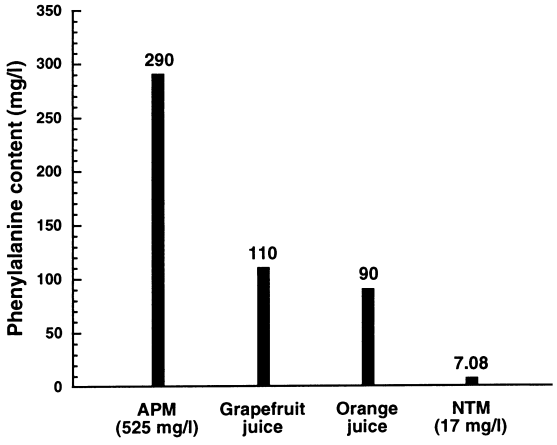


Fig. 17. Comparison between the phenylalanine (Phe) content (i) of a beverage formulated with 525 mg/l of APM, (ii) of two typical fruit juices (values from McCarthy, Orr & Watt, 1968), (iii) of a beverage formulated with 17 mg/l of NTM (to match the sweetness intensity of a 10% sucrose solution). Note that the amount of 7.08 mg for the Phe content of a beverage sweetened with 17 mg/l of NTM corresponds to the theoretical value, but that the Phe amount effectively liberated into the body is in fact inferior to 0.7 mg (see footnote of Table 7).

As micro-organisms in the mouth are not able to metabolize NTM and produce tooth-decaying acids, NTM is noncariogenic, like APM.

In Table 8, some characteristic use levels of NTM in typical applications are reported. As NTM is at least as stable as APM in the acidic pH range, all the applications previously proposed for APM in acidic medium are allowed with NTM (in carbonated soft drinks for example), either as sole sweetener or combined with one (or more) sweetener(s) (e.g. with acesulfame-K); as an example, note the excellent stability of NTM in yogurts (Fig. 18), which are in the pH range of the maximal stability of NTM. In neutral pH foodstuffs, NTM is significantly more stable than APM, which allows its application in domains not easily accessible to APM, e.g. in baked goods; note, for example, the excellent behaviour of NTM in yellow cake, a representative bakery product (Fig. 19).

As mentioned before, NTM, unlike APM, is compatible with reducing sugars (such as glucose, fructose, high-fructose corn syrup, lactose, maltose) and aldehyde-based

Table 8
Suggested neotame use levels in some typical applications

Application	Use level
Tabletop sweetener	0.9 mg/unit[a]
Lemon tea (hot packed)	8 mg/l
Yogurt	15 mg/l
Powdered soft drink	16 mg/l[b]
Carbonated soft drink	17 mg/l
Cake (yellow cake)	35 mg/kg
Chewing gum	250 mg/kg

[a] Equivalent to the sweetness of 2 teaspoons of sugar.
[b] Final concentration in the reconstituted beverage.

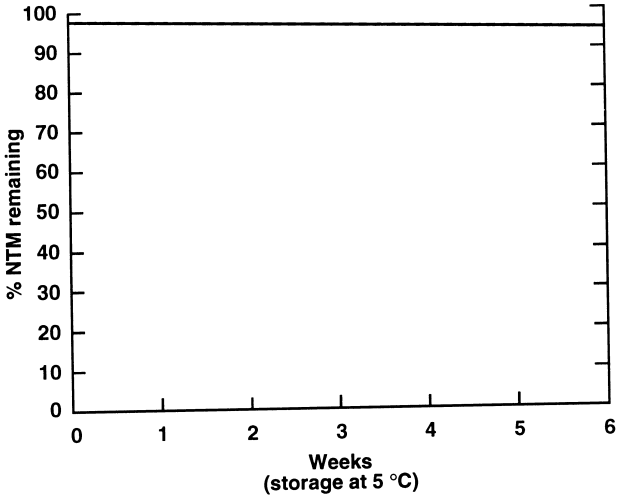


Fig. 18. NTM stability in strawberry yogurt.

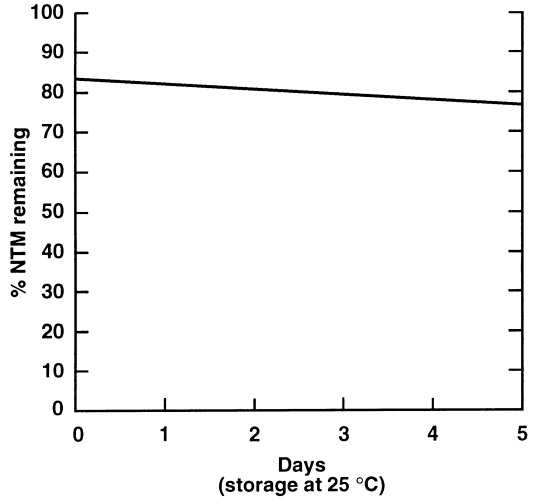


Fig. 19. NTM stability in yellow cake.

flavouring agents (such as vanilla, cinnamon, cherry, bitter almond, lemon), which considerably widens the potential utilities of NTM compared to APM.

On account of the reduced-cost preparation of NTM from APM (in a one-step, high-yield reaction) and of its highly-potent sweetness (~6000 × with regard to a 10% sucrose solution), it is anticipated that the relative cost of NTM (i.e. the quotient of its wholesale price and its sweetness potency relative to sucrose on a weight basis) should be highly attractive, and competitive with the other sweeteners on the market, including APM and sucrose.

NTM is worldwide patented (79 countries). In December 1998, a food additive petition was submitted to the US Food and Drug Administration (FDA) for approval of NTM as a general purpose sweetener; this request is currently undergoing review and is awaiting FDA approval. Additional filings are in progress in several countries outside of the USA.

5. Conclusions

NTM outstandingly meets the five basic criteria requisite for commercial viability of a nonnutritive sweetener, namely, taste, solubility, stability, safety, and cost.

NTM provides a clean sweet taste, with a good flavour profile and consumer acceptance; it is a highly potent sweetener, with a potency, on a weight basis, 30 to 60 times that of APM, and 6000 to 10 000 times that of sucrose. In aqueous systems, its solubility is several hundreds of times higher than necessary to match a 10% sucrose solution. NTM has an extensive shelf life in dry conditions. In aqueous systems, NTM is approximately as stable as APM in the acidic pH range, and has, as a result, the same functionalities as APM in such conditions; NTM is significantly more stable than APM in the neutral pH range or at transient high temperatures, which considerably widens the potential applications of NTM (e.g. in baking preparations and baked products). As a consequence of its very low usage and of its highly favourable pharmacokinetic profile, its margins of safety are considerable. Owing to the simplicity of its preparation and to its amazingly high sweetness potency, it is anticipated that the cost of NTM will be highly competitive and markedly lower than that of APM or sucrose at sweetness equivalence.

NTM offers other salient qualities. For example, it can be considered as a no-calorie sweetener at normal use levels. It is compatible with a broad range of foods and food ingredients; for example, unlike APM, it does not present any problems of interaction with reducing sugars (such as glucose, fructose, lactose, maltose) or with aldehyde-based flavouring agents (such as vanilla, cinnamon, cherry, bitter almond, lemon). It has unique flavour-enhancing properties. It is noncariogenic.

Owing to its versatile qualities, NTM should provide a uniquely useful sweetener. It presents a rare combination of features. These features suggest that, upon approval, this ingredient should become the sweetener of choice in the food industry, as did APM two decades ago.

Acknowledgements

This work was supported by The NutraSweet Company, Chicago, IL 60654, USA. Data presented in this review represent the efforts of many individuals of The NutraSweet Company whom we thank for their essential contribution to this work. The authors are particularly grateful to Jerry Hjelle, Jeff Hoster, Frank Kotsonis, Po Lui, Waine Stargel, Etienne Veber and John Witt, for their major role in the development of neotame.

References

Beck, C. I. (1974). Sweetness, character, and applications of aspartic acid-based sweeteners. In G. E. Inglett (Ed.), *Symposium: sweeteners* (pp. 164–181). Westport, CT: Avi Publishing Company.

Beck, C. I. (1978). Application potential for aspartame in low calorie and dietetic foods. In B. K. Dwivedi (Ed.), *Low calorie and special dietary foods* (pp. 59–114). West Palm Beach, FL: CRC Press.

Crosby, G. A., & Furia, T. E. (1983). New sweeteners. In T. E. Furia (Ed.), *Handbook of food additives 2nd Edition,* Boca Raton, FL: CRC Press, Vol. 2, pp. 187-227.

DuBois, G. E. (1991). Sweeteners, nonnutritive. In Y. H. Hui (Ed.), *Encyclopedia of food science and technology*, New York: John Wiley, Vol. 4, pp. 2470–2487.

Homler, B. E. (1984). Aspartame: Implications for the food scientist. In L. D. Stegink, & L. J. Filer Jr. (Eds), *Aspartame: physiology and biochemistry*, New York: Marcel Dekker, pp. 247–262.

Ketelsen, S. M., Keay, C. L., & Wiet, S. G. (1993). Time-intensity parameters of selected carbohydrates and high potency sweeteners. *Journal of Food Science, 58*, 1418–1421.

Kim, S.-H., & DuBois, G. E. (1991). Natural high potency sweeteners. In S. Marie, & Piggott, J. R. (Eds), *Handbook of sweeteners* (pp. 116–185). Glasgow: Blackie.

Lindinger, W., Taucher, J., Jordan, A., Hansel, A., & Vogel, W. (1997). Endogenous production of methanol after the consumption of fruit. *Alcoholism Clinical and Experimental Research, 21*, 939–943.

Liu, Y.-H., & MacFie, H. J. H. (1990). Methods for averaging time-intensity curves. *Chemical Senses, 15*, 471–484.

Marie, S. (1991). Sweeteners. In J. Smith (Ed.), *Food additive user's handbook*, Glasgow: Blackie, pp. 47–74.

Mazur, R. H., & Ripper, A. (1979). Peptide-based sweeteners. In C. A. M. Hough, K. J. Parker, & A. J. Vlitos (Eds), *Developments in sweeteners* (pp. 125–134). London: Applied Science Publishers.

McCarthy, M. A., Orr, M. A., & Watt, B. K. (1968). Phenylalanine and tyrosine in vegetables and fruits. *Journal of American Dietetic Association, 52*, 130–134.

Nofre, C., & Tinti, J.-M. (1983). Sweetening agents. US Patent 4,645,678 (filed September 15, 1983; granted February 24, 1987).

Nofre, C., & Tinti, J.-M. (1986). Novel sweetening agents, process for sweetening various products and compositions containing such sweetening agents. US Patent 4,673,582 (filed March 5, 1986; granted June 16, 1987).

Nofre, C., & Tinti, J.-M. (1989). Sweetening agents derived from *N*-hydrocarbon-substituted L-aspartic and L-glutamic acids. US Patent 4,935,517 (filed April 21, 1989; granted June 19, 1990).

Nofre, C., & Tinti, J.-M. (1990). Sweetening agent derived from L-aspartic or L-glutamic acid. US Patent 5,196,540 (filed October 23, 1990; granted March 23, 1993).

Nofre, C., & Tinti, J.-M. (1992a). Sweetening agents derived from L-glutamic acid. US Patent 5,272,272 (filed April 21, 1992; granted December 21, 1993).

Nofre, C., & Tinti, J.-M. (1992b). Agent édulcorant dérivant de l'acide L-aspartique et son procédé de préparation. French Patent 92 04956 (filed April 22, 1992; granted July 22, 1994).

Nofre, C., & Tinti, J.-M. (1992c). Nouveaux composés dérivés de dipeptides ou d'analogues dipeptidiques utiles comme agents édulcorants, leur procédé de préparation. French Patent 92 13615 (filed November 12, 1992; granted January 27, 1995).

Nofre, C., & Tinti, J.-M. (1993a). In quest of hyperpotent sweeteners. In M. Mathlouthi, J. A. Kanters & G. G. Birch (Eds),*Sweet-taste chemoreception*, Elsevier, London, pp. 205–236 (from a Symposium held by the European Chemoreception Research Organization in Reims, France, on 2–6 September 1991).

Nofre, C., & Tinti, J.-M. (1993b). *N*-Substituted derivatives of aspartame useful as sweetening agents. US Patent 5,480,668 (filed November 9, 1993; granted January 2, 1996).

Nofre, C., & Tinti, J.-M. (1994a). Nouveau composé utile comme agent édulcorant, son procédé de préparation. French Patent 94 05675 (filed May 9, 1994; granted July 26, 1996).

Nofre, C., & Tinti, J.-M. (1994b). Nouveaux dérivés dipeptidiques utiles comme agents édulcorants. French Patent 94 05676 (filed May 9, 1994; granted July 26, 1996).

Nofre, C., & Tinti, J.-M. (1995a). *N*-[*N*-(3,3-Dimethylbutyl)-L–α-aspartyl]-L-hexahydrophenylalanine 1-methyl ester useful as a sweetening agent, its method of preparation. US Patent 5,773,640 (filed May 5, 1995; granted June 30, 1998).

Nofre, C., & Tinti, J.-M. (1995b). Method of preparing a compound derived from aspartame, useful as a sweetening agent. US Patent 5,510,508 (filed May 8, 1995; granted April 23, 1996).

Nofre, C., & Tinti, J.-M. (1997). *N*-(3,3-Dimethylbutyl)-L-aspartyl-D–α-aminoalkanoic acid *N*-(*S*)-1-phenyl-1-alkanamide useful as a sweetening agent. US Patent 5,777,159 (filed February 5, 1997; granted July 7, 1998).

Nofre, C., Tinti, J.-M., & Ouar Chatzopoulos F. (1986). Glycine and β-alanine derivatives as sweetening agents. US Patent 4,877,895 (filed March 4, 1986; granted October 31, 1989).

Nofre, C., Tinti, J.-M., & Ouar Chatzopoulos F. (1987). Sweetening agents. US Patent 4,921,939 (filed March 3, 1987; granted May 1, 1990).

Ott, D. B., Edwards, C. L., & Palmer, S. J. (1991). Perceived taste intensity and duration of nutritive and non-nutritive sweeteners in water using time-intensity (T-I) evaluations. *Journal of Food Science, 56*, 535–542.

Prakash, I. (1997). Method for preparing and purifying an *N*-alkylated aspartame derivative. US Patent 5,728,862 (filed January 29, 1997; granted March 17, 1998).

Ripper, A., Homler, B. E., & Miller, G. A. (1986). Aspartame. In L. O'Brien Nabors, & R. C. Gelardi (Eds), *Alternative Sweeteners* (pp. 43–70). New York: Marcel Dekker.

Salminen, S., & Hallikainen, A. (1990). Sweeteners. In A. L. Branen, P. M. Davidson, & S. Salminen (Eds), *Food Additives* (pp. 297–326). New York: Marcel Dekker.

Schiffman, S. S., & Gatlin, C. A. (1993). Sweeteners: state of knowledge review. *Neuroscience and Biobehavioral Reviews, 17*, 313–345.

Schlatter, J. M. (1966). Peptide sweetening agents. US Patent 3,492,131 (filed April 18, 1966; granted January 27, 1970).

Stegink, L. D., & Filer, L. J. Jr. (Eds) (1984). *Aspartame: Physiology and Biochemistry*, Marcel Dekker, New York, 670 pp.

Tschanz, C., Butchko, H. H., Stargel, W. W., & Kotsonis, F. N. (Eds) (1996). *The Clinical Evaluation of a Food Additive: Assessment of Aspartame*, CRC Press, Boca Raton, FL, 308 pp.

Vervack, W., Foulon, M., Moreau, I., & Vanbelle, M. (1981). Composition en acides aminés de différents aliments destinés à la nutrition humaine. *Revue des Fermentations et des Industries Alimentaires, 36*, 104–121.

Wells, A. G. (1989). The use of intense sweeteners in soft drinks. In T. H. Grenby (Ed.), *Progress in Sweeteners* (pp. 169–214). London: Elsevier.

Wucherpfennig, K., Dietrich, H., & Bechtel, J. (1983). Vorhandener, gesamter und potentieller methylalkoholgehalt von fruchtsäften. *Flüssiges Obst., 8*, 348–354.