61st JECFA - Chemical and Technical Assessment (CTA), 2003

NEOTAME

Chemical and Technical Assessment (CTA)

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1 Summary

Neotame has been recommended by CCFAC for priority evaluation by JECFA. As neotame has not previously been evaluated, the information required includes all relevant information relating to toxicity, intake and specifications.

Neotame is the common name for chemical N-(N-(3,3-dimethylbutyl)-L-α-aspartyl) -1-L phenylalanine 1-methylester. It is synthesized by reductive N-alkylation of L-α-aspartyl)-1-L phenylalanine 1-methylester (aspartame). Like aspartame its intended use is as an intense sweetener and flavour enhancer.

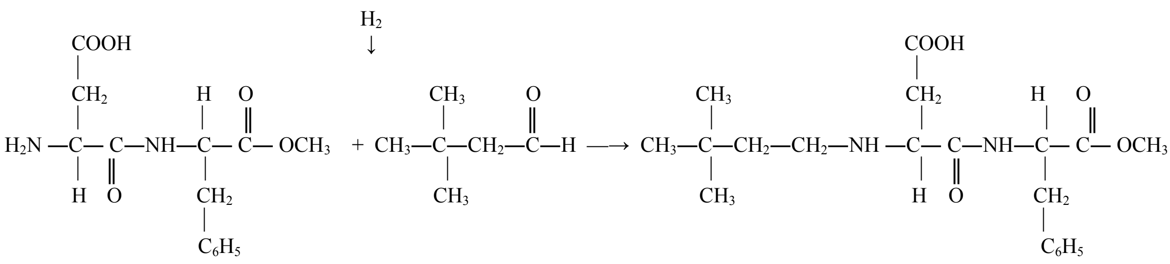
2 Description

Neotame is a white to off-white powder with an intense sweet taste. It is sparingly soluble in water but very soluble in ethanol. It has a melting range of 81o - 84o. A 0.5% aqueous solution of neotame is slightly acidic, with a pH between 5.0 and 7.0.

3 Manufacturing

3.1 Method of manufacture

Neotame is manufactured from aspartame and 3,3-dimethylbutyraldehyde. Equimolar amounts of aspartame and 3,3-dimethylbutyraldehyde are reacted with hydrogen gas in methanol.



Aspartame 3,3-dimethylbutyraldehyde Neotame

The isolation and purification of neotame is carried out by the distillation of a portion of the methanol followed by the addition of water (approximately 2.5 litres per mole aspartame). The mixture is cooled for a number of hours and neotame is then isolated by an appropriate solid/liquid separation method such as centrifugation. Additional water is used to wash the product, which is then dried in appropriate equipment (e.g. a vacuum drier).

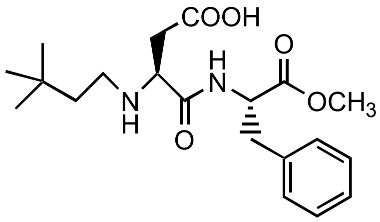
3.2 Raw materials

The raw materials used in the manufacture of neotame are aspartame, 3,3-dimethylbutyraldehyde, methanol, hydrogen, palladium/carbon catalyst, and diatomaceous earth (filtering aid). The aspartame, methanol and diatomaceous earth are food grade. The 3,3-dimethylbutyraldehyde has a purity of 95% or greater and the hydrogen and the palladium/carbon catalyst are of a quality appropriate for food use.

4 Chemical characterization

4.1 **Composition** of the food additive

The chemical structure of neotame is



4.2 Possible impurities

As neotame is manufactured in a single process, there are no isolated intermediates.

The major impurity of neotame is N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine (de-esterified neotame), which is produced by the hydrolysis of the methyl ester group of neotame. The other main impurities are present at a total concentration of less than 2%.

4.3 Analytical methods

Assay- The content of the commercial product is not less than 97.0% and not more than 102.0% on a dry basis, based on the HPLC method

Identification Tests : The product is identified by comparing the infrared absorption spectrum of a potassium bromide dispersion with that of a reference standard.

The contents of N-[N-(3,3-ethylbutyl)-α-aspartyl]-L-phenylalanine and other related substances are estimated using the HPLC method described in the Method of Assay. The total amount of other related substances is estimated by summing the peak area for all secondary peaks other than those for neotame and the degradation product N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine.

5 Functional uses

Neotame is a high potency sweetener that can be used to sweeten foods and beverages and can also modify and enhance the flavour of foods and beverages when used at non-sweetening levels. In appendix 2, representative use levels are reported.

At high concentrations, neotame is exhibits a clean sensory taste profile across a wide range of usage levels, with sweetness as the predominant sensory characteristic. Other sweetener attributes are said to be negligible. As with aspartame and other high intensity sweeteners, the sweetness potency of neotame is concentration-dependent and can vary depending on the specific food or beverage.

The sweetness potency of neotame ranges from about 30 to 60 times that of aspartame and approximately 7,000 to 13,000 times that of sucrose. The relative potency of neotame to aspartame in water shows that neotame is about 55 sweeter than aspartame at sweetness levels common for tabletop applications, i.e. about 3 to 6% (w/v) sugar or about 2 to 4 teaspoons of sugar per 240 ml. In applications such as carbonated soft drinks, the relative potency of neotame is less.

6 Reactions and Fate in Food

The potential degradation products of neotame were predicted by the manufacturer on the basis of the comprehensive knowledge of aspartame chemistry. Exploratory forced degradation experiments were also carried out to confirm the identity of these degradation products. The results of these were consistent with predicted neotame degradation pathways. The potential degradation products are shown in Appendix 1.

As in the case of aspartame, the stability of neotame is dependent on processing and storage temperatures, pH and moisture levels. Several stability studies of dry neotame and of aqueous solutions of the substance under different conditions of temperature, pH and moisture content have been used to predict the fate of neotame in food products. In addition, several studies in specific food products were conducted. These are summarized below.

(i) The bulk chemical stability of neotame in the dry form was evaluated in several studies in which HPLC analysis was used to analyse samples for concentrations of neotame and known potential degradation products. Sealed samples of dry neotame stored in the dark at ambient humidity at 5 ° and 30 ° for up to 208 weeks, and at 40 ° for up to 156 weeks, were found to be stable, giving assay values of 95.7, 96.2 and 98.8% respectively. De-esterified neotame, (N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine), was the major degradation product, being present at levels up to 1.2% in all samples. One minor degradation product, N-fumarylphenylalanine 1-methyl ester, was also found, but only in samples stored at 40° and for 156 weeks. No unpredicted degradation products were detected at levels above 0.1%.

(ii) Additional samples of neotame were stored for 52 weeks at 35° and 80% relative humidity. There was a slight increase in the amounts of de-esterified neotame (approximately 3.0% at 52 weeks) compared with the product stored under ambient conditions, but there were no significantly increased amounts of other degradation products.

(iii) Three additional lots of neotame stored at 40° and 75% relative humidity demonstrated some degradation of neotame beginning at week 14 when levels of de-esterified neotame started to increase slightly from ≤0.4% at week 14 to 1.2 - 3.5% at week 52. However the only other degradation product detected under these conditions was N-[N-(3,3-dimethylbutyl)-L-β-aspartyl]-L-phenylalanine, and this remained at low levels (≤0.2%) throughout the 52-week storage period.

(iv) In another study, neotame degradation was evaluated in solution at a range of conditions simulating beverage formulations, in which the pH was varied from 2.8 to 4.5 at a variety of different temperatures (5, 20, 30, 35°) for 26 weeks. These conditions were designed to simulate both typical commercial and unusually extreme storage conditions.

(v) Samples were analyzed for neotame and its known degradation products and monitored for the development of unknown degradation products at scheduled intervals throughout the 26-week period. Simulated beverage samples were formulated at 200 mg/l, a concentration 12-13 times the anticipated use level. The comparison sample was formulated at 15 mg/l and pH 3.2. The results showed that the rate and extent of degradation was higher with decreasing pH and increasing temperature, with the majority of degradation occurring after extended storage. Using the relevant commercial use conditions of pH 3.2 and 20°, the study showed that 89.3% of neotame remained unchanged after storage for 8 weeks.

The major route of degradation was the hydrolysis of the methyl ester moiety to form de-esterified neotame, the only degradation product formed at >1%. The minor degradation products detected at levels approaching or above 0.5% under these conditions were N-[N-(3,3-dimethylbutyl)-L-β-aspartyl]-L-phenylalanine 1-methyl ester, N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine 1-methyl ester and N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine (formed by a β-rearrangement of neotame, cyclization of neotame, and methyl ester hydrolysis of N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine 1-methyl ester, respectively; a similar degradation pathway occurs with aspartame).

In this stability study the concentration of neotame was set at 200 mg/l in order to ensure the accurate determination of the minor degradation products. These degradation products would not be expected to be detected at actual use levels of approximately 15-17 mg/l in carbonated soft drinks, which is approximately 12-13 times less than the 200 mg/l used in the study. Two minor degradation products (N-[N-(3,3-

dimethylbutyl)-L-β-aspartyl]-L-phenylalanine and N-(3,3-dimethylbutyl)-L-aspartic acid) were detected, but only in conditions in which high concentrations of neotame were subjected to extreme conditions of pH, temperature and time, conditions that are not relevant to soft drink beverages.

(vi) A study to examine the stability of neotame during thermal processing was conducted using sample beverages at pHs of 3.2 and 6.5. These were designed to simulate non-carbonated still beverages and dairy products respectively. Each beverage was exposed to typical high temperature short time conditions of 87.8° for 30 seconds, and were cooled slowly to 4.4, 21.1 and 32.2° to simulate different plant cooling procedures. The sweetened sample beverages contained approximately 200 mg/l of neotame, which is approximately 12-13 times higher than expected use levels in most processed foods and beverages. De-esterified neotame concentrations were ≤1.0 µg/ml in all cases and was less than a 0.5% loss of neotame under all conditions.

(vii) A 3-dimensional matrix model was developed to investigate the effect on the stability of neotame of incorporating it in different foods chosen to represent extreme variations in conditions in terms of the three variables of moisture content, pH and temperature (Pariza et al., 1998). The foods chosen are shown in the table below.

Neotame Applications Representing Extremes of Matrix

Application	Physical/Chemical Conditions
Lemonade Powdered Soft Drink Mix	Low Moisture/Low Heat Exposure/Low pH
Cola-Flavoured Carbonated Soft Drink	High Moisture/Low Heat Exposure/Low pH
Pasteurized Lemon Tea	High Moisture/High Heat Exposure/Low pH
Fermented Milk Products (Strawberry Yogurt)	High Moisture/Medium-High Heat Exposure/Low-Medium pH
Baked Goods (Yellow Cake)	Moderate Moisture/ High Heat Exposure/Medium pH

Additional applications selected for assessing neotame functionality and stability included powdered tabletop preparations and chewing gum. Both applications provide pH, temperature, and moisture conditions falling within the scope of the matrix model, although they were chosen for further study because of their commercial importance in the high intensity sweetener market. Results for the product matrix model demonstrated that neotame is functional and stable in a wide range of product applications

(viii) The commonest reaction undergone by maltodextrins and/or glucose in food is the formation of Maillard reaction products (Namiki, 1988). The possible formation of Maillard reaction products as a result of the interaction of the amino acid components of neotame with sugar moieties in the food matrix has also been studied .

The extent to which the Maillard reaction occurs depends on a number of factors, including the type of amino acid, pH, sugar type, solvent state and temperature. Neotame is a secondary amine with a hydrophobic side chain and would therefore be expected to undergo the Maillard reaction only very slowly. Also at a pH of 6 or less, which is typical of most foods, the amount of unprotonated amine present is less than 0.02% and thus the degree to which the Maillard reaction proceeds would be expected to be small. It was considered, therefore, that the Maillard reaction is unlikely to occur to any significant effect with neotame.

7 References

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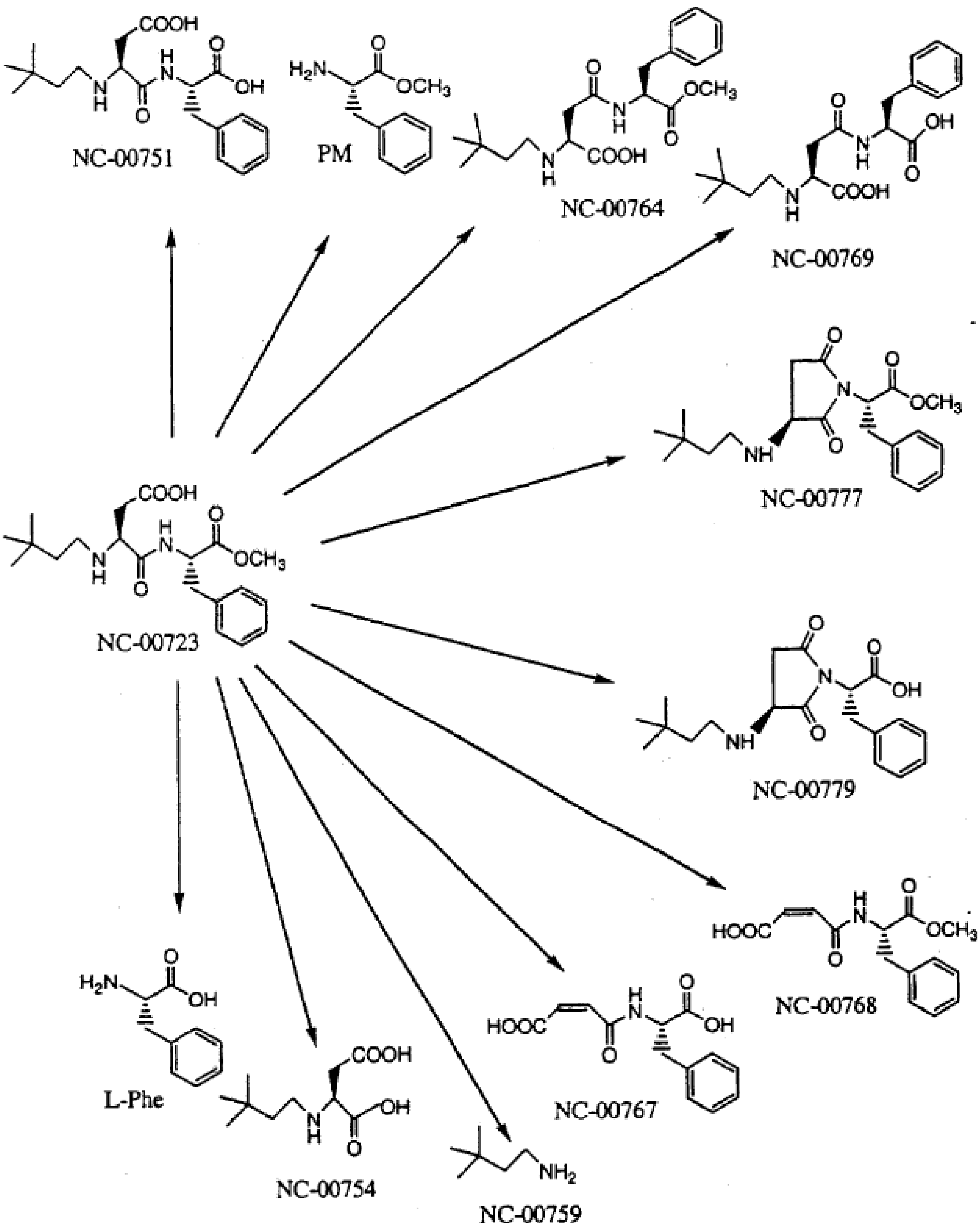
Appendix 1

Potential Neotame Degradation Products Under Forced Conditions (low pH and high temperature) Based on Identified Aspartame Degradation Products

NC-00723	N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester
NC-00751	N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine
PM	L-Phenylalanine methyl ester
NC-00764	N-[N-(3,3-dimethylbutyl)-L-β-aspartyl]-L-phenylalanine 1-methyl ester
NC-00769	N-[N-(3,3-dimethylbutyl)-L-β-aspartyl]-L-phenylalanine
NC-00777	N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine 1-methyl ester
NC-00779	N-[N-(3,3-dimethylbutyl)-L-aspartamidyl]-L-phenylalanine
NC-00768	N-Fumarylphenylalanine 1-methyl ester
NC-00767	N-Fumarylphenylalanine
NC-00759	3,3-dimethylbutylamine
NC-00754	N-(3,3-dimethylbutyl)-L-aspartic acid
L-Phe	L-Phenylalanine

Potential Degradation Products of NC-00723



Appendix 2

Representative use levels for food category[1]

Food Category	**Representative used levels**
Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, wey-based drinks)	15 mg/kg
Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drink)	15 mg/kg
Beverage whiteners	15 mg/kg
Pasteurized cream	25 mg/kg
Sterilized, UHT, whipping or whipped, and reduced fat creams	25 mg/kg
Clotted cream	25 mg/kg
Cream analogues	25 mg/kg
Milk and cream powder analogues	50 mg/kg
Cheese analogues	25 mg/kg
Dairy-based (e.g., ice milk, pudding, fruit or flavoured yoghurt)	15 mg/kg
Fat emulsions other than food category 02.2, including mixed and/or flavoured products based on fat emulsions	10 mg/kg
Fat-based desserts excluding dairy-based dessert products of food category 01.07	20 mg/kg
Edible ices, including sherbet and sorbet	20 mg/kg
Frozen fruit	100 mg/kg
Dried fruit	100 mg/kg
Fruit in vinegar, oil, or brine	100 mg/kg
Canned or bottled (pasteurized) fruit	100 mg/kg
Jams, jellies and marmalades	100 mg/kg
Fruit-based spreads (e.g., chutney) excluding products of food category 04.1.2.5	100 mg/kg
Candied fruit	100 mg/kg
Fruit preparations, including pulp, purees, fruit toppings and coconut milk	100 mg/kg
Fruit-based desserts, including fruit-flavored water-based desserts	100 mg/kg
Fermented fruit products	100 mg/kg
Fruit fillings for pastries	100 mg/kg
Coked or fried fruit	100 mg/kg
Frozen vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes ((including soybeans)), and aloe vera), seaweeds, and nuts and seeds	100 mg/kg

[1] Note: These use levels have been identified as representative and were used for intake calculations. Their publication does not imply any recommendation whether they are suitable as maximum levels for standard setting purposes, a question which is not within the mandate of JECFA.