

## MINI REVIEW

### Neotame: High Intensity Low Caloric Sweetener

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Neotame is an artificial sugar substitute which is N-alkyl derivative of aspartame. It is non caloric and 7000-13,000 times sweeter than sucrose, 30-60 times sweeter than aspartame respectively. It has excellent shelf life in dry conditions. Neotame is more stable in aqueous form at neutral pH. It is heat stable and consequently used in cooking and baking. Unlike aspartame it is safe for people with phenylketonurea. It is compatible with wide range of food ingredients, so blended with other caloric sweeteners to enhance taste and flavour. It doesn't show any significant toxicity and is non-carcinogenic. Neotame is a highly potent sweetener that can be used to modify and enhance flavour of foods and beverages.

**Key Words:** Neotame, Sweetner.

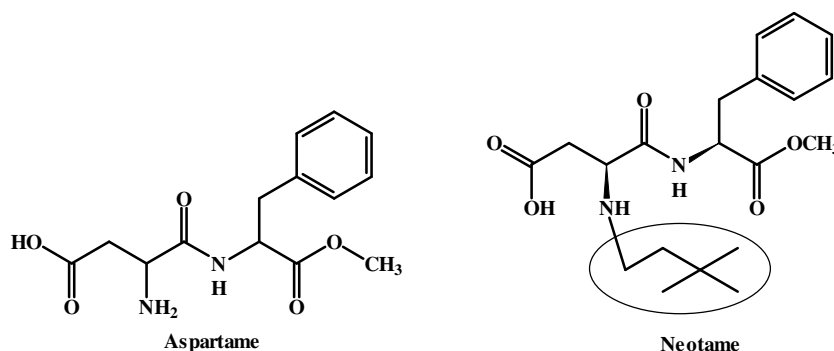
## INTRODUCTION

Sweeteners are the agents added to foods to enhance the taste. Nowadays most of people use food and beverages with sugars which are less caloric sweeteners. Naturally occurring sweeteners such as sucrose, fructose and maltose are used, but because of their toxicity and high food value, artificial sweeteners are used like sucrose, fructose, aspartame, saccharin which are sweeter and low caloric value than table sugar. Neotame is an artificial sweetener manufactured by NutraSweet Company in 1991.

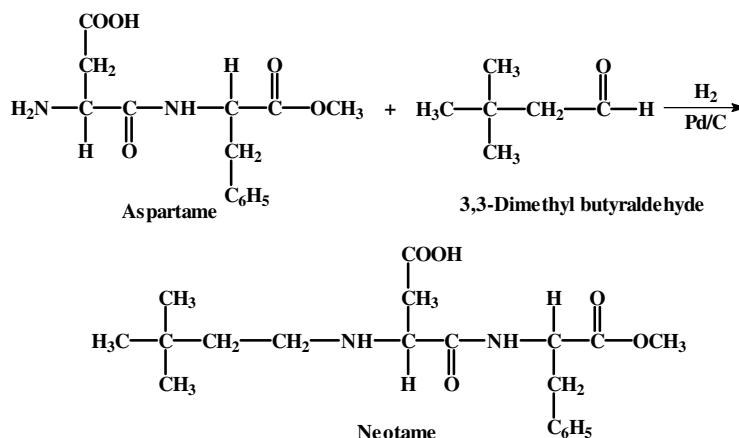
Neotame is a non-nutritive, non-caloric sweetener and 7000-13000 times and 30-60 times sweeter than regular table sugar and aspartame, respectively. Because of its high potency only very small amount is needed to sweeten food and beverages. Food and drug administration (FDA) of USA approved neotame in general food category as sweetener and flavour in 2002.

Chemically neotame is a dipeptide methyl ester derivate. Its chemical name is N-(N-(3, 3-dimethylbutyl)-L- $\alpha$ -aspartyl)-1-L-phenylalanine-1-methylester. The N-substituted dimethyl group is essential for sweetness, stability and plays an important role in metabolism of neotame<sup>1-6</sup>. Based on this structure neotame is considered as N-(3,3-dimethyl butyl) derivative of aspartame. The structure of neotame as proposed by Nofre and Tinti<sup>7</sup> is based on the mechanism of sweetness. Human tongue has sweetness receptor [human sweetness receptor (HSR)] which consists of two clearly distinct hydrophobic binding pockets (HBP) located 1 nm

apart each other. Aspartame interacts with HSR through interaction between one HBP and one phenyl hydrophobic group of aspartame. Based on this interaction between HBP of HSR and aspartame, thousands of aspartame analogues were prepared since 1965. In 1992 Nofre and Tinti proposed N-substituted derivative of aspartame, which is preferentially binding with two HBPS of HSR with improved sweetness and stability of aspartame. The N-substitute was observed as 3,3-dimethyl butyl group<sup>7</sup>. Aspartame also has one primary amino group which is responsible for undesirable milliard type reaction.



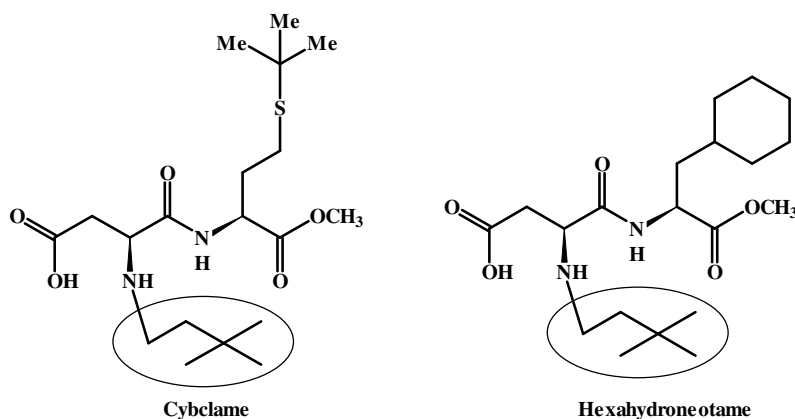
Neotame is prepared from aspartame and 3,3-dimethyl butyraldehyde by reductive alkylation in 1995 by Nofre and Tinti<sup>7</sup>.



Presently new methods are invented for synthesis of neotame by using N-3,3 dimethyl butyraldehyde precursors, instead of 3,3-dimethyl butyraldehyde as a starting material<sup>3</sup>. This method is more efficient and more economic as compared with conventional preparation of neotame. Precursors of 3,3-dimethyl butyraldehyde used include 3,3-dimethyl butyraldehyde dimethyl acetal, the bisulfite adduct of 3,3-dimethyl butyraldehyde, hydrazone, semicarbazone or oxime of 3,3-dimethyl butyraldehyde. Recent invention does not need to isolate 3,3-dimethyl butyraldehyde prior to addition of aspartame, The direct addition of aspartame to 3,3-butylaldehyde

precursors, which are hydrolyzed to release 3,3-butyraldehyde in a suitable solvent, produces neotame under suitable hydrogenation conditions with a catalyst. Other trends in the synthesis of neotame involve oxazolidinone derivatives.

On studying the structure activity relationship of neotame, by replacing the L-phenylalanine methyl ester moiety with L-hexahydro phenyl alanine methyl ester and S-*tert*-butyl-L-cysteine methyl ester moiety, it gave two powerful sweet products hexahydroneotame and cybelame, respectively. Both these two products are 12 and 23 times sweeter than sucrose, respectively on molecular basis<sup>7</sup>.



Neotame is odorless, white crystalline powder with m.p. 83.4 °C. The crystalline structure of neotame was studied by X-ray crystallography. Neotame is stable in dry form. Neotame is sparingly soluble in water *i.e.*, 12.6 g/L whereas in anhydrous alcohol it is more soluble *i.e.*, *ca.* 950 g/L at 25 °C. However the neotame solubility in aqueous system is increased by conversion into its salt from with phosphoric acid and also by complexation with  $\beta$ -cyclodextrin. The specific rotation of neotame in anhydrous form was found to be ( $[\alpha]_{20}$ ) between -40.0 and -43.3°. The other physical parameters of neotame, the density, viscosity and surface tension are 0.33 g/cm<sup>3</sup>, 5 mps and *ca.* 65 mN/m, respectively<sup>7</sup>.

As stability is concerned, several stability studies were done with both dry and aqueous form. Neotame in dry form was evaluated for 5 years in several conditions. The dry neotame was stored in dark at ambient humidity at 5 and 30 °C for up to 208 weeks and at 40° for up to 156 weeks and were found to be stable giving assay values of 95.7, 96.2 and 98.8 %, respectively. When neotame was stored at 25° C and 60 % RH virtually no loss occurred<sup>1,8</sup>. Because of this high stability, neotame in dry form is used in finished dry products such as powder soft drinks and dessert mixtures. The stability of neotame in solution depends on pH, temperature and time. Neotame was evaluated in solution form by varying pH and at variety of different temperatures. Its degradation is higher at lower pH and higher temperature. The degradation of neotame follows pseudo first order kinetics which was studied at ambient temperature. The major route for degradation of neotame is hydrolysis of the methyl ester group. Neotame is stable at pH 3.0-5.5 with optimum pH at *ca.*

4.5. At this pH the half life of neotame is 30 weeks at 25 °C. The stability of neotame in solution form is evaluated by exposure to high temperature short time (HTST) conditions *i.e.*, at 80 °C/30 min at pH 3.0-3.5. It is confirmed that at these conditions there is no loss of neotame, consequently neotame is used for food processing at these conditions. Neotame is also stable in acidic pH conditions, consequently it is allowed for carbonated drinks as a sweetener at pH 2.9-4.5. Neotame is more stable in neutral pH *i.e.*, at this pH half life of neotame is 124 days. For stability studies, samples were formulated at 200 mg/L rather than 15 mg/L, in order to ensure the accurate determination of the minor degradation products. These degradation products could not be detected at a concentration of 15 mg/L like in carbonated drinks<sup>1,8</sup>. Neotame was determined by using HPLC with mobile phase of 25 % acetonitrile and 75 % buffer (0.02 M heptanesulfonic acid sodium and 0.5 % v/v triethylamine). It should be not less than 97 % and not more than 102 % in anhydrous form. The neotame structure was identified by infrared spectrum of potassium iodide dispersion<sup>1</sup>.

Neotame is hydrolyzed by esterase enzyme which is distributed throughout body. Neotame undergoes hydrolysis at methyl ester group to dimethyl butyl aspartyl phenylalanine (DMB-Asp\_Phe) and methanol in both acidic and neutral pH. The amount of methanol released from neotame is small relative to the amount of methanol derived from common foods such as fruits and vegetables, juices<sup>7,8</sup>. In neutral pH, neotame doesn't form cyclazine derivative, diketopiperazine. This absence of cyclaziation derivative significantly increases the stability of neotame. Consequently this significant stability in neutral pH offers neotame applicable to baking foods<sup>7</sup>.

Neotame doesn't interact with reducing sugars and aldehyde derivative, as it has a 2° amine with hydrophobic group. Because of the inertness of the neotame towards these groups, neotame interacts with reducing sugars (glucose, fructose, sucrose and maltose) and aldehyde derivatives (vanillin) without formation of Millard-type and Schiff base reactions, respectively, but with enhanced sweetening properties<sup>7,8</sup>. Neotame is a clean sweetener, generally blends with other caloric sweeteners such as sucrose, maltose to enhance flavour and taste of foods and beverages. For example maltoneotame is non nutritive blend of 2 % neotame and maltodextrin for delivery of sugar free products. It is also used to create desirable new taste and flavour in foods and other products.

After ingestion of neotame, it is metabolized by esterase at methyl ester group to de-esterified neotame [dimethyl butyl aspartyl phenylalanine (DMB-Asp\_Phe)] and methanol. Both of these metabolites are eliminated rapidly in the urine and faeces. Besides these metabolites neotame also produces three minor derivatives such as N-[N-(3,3-dimethylbutyl)-L-asparmidyl]-L-phenylalanine 1-methylester, N-[N-(3,3-dimethyl butyl)-L-β-aspartyl]-L-phenylalanine 1-methylester and N-[N-(3,3-dimethylbutyl)-L-asparmidyl]-L-phenylalanine<sup>8</sup>.

The experimental studies of neotame on animals showed that it is non teratogenic, non-carcinogenic, without any effect on growth development of off-spring across

multiple generation. In addition, clinical studies of neotame were carried out on healthy humans and type-II diabetic patients. It revealed that neotame is safe for use in humans as sweetener. The *in vivo* and *in vitro* studies of neotame described no mutagenic effect. Neotame was tested on type-II diabetes and it couldn't show any effect on blood glucose, insulin concentration and glycemic control in diabetes with no allied adverse effects. Consequently it is recommended for type-II diabetic patients<sup>8</sup>.

Neotame is considerably safer for people with phenylketonuria, due to presence of 3,3-dimethyl group. It blocks the break down of Asp-Phen peptide linkage into amino acid phenylalanine. Consequentially it reduces the release of phenylalanine. The evaluation studies of neotame on human confirm that neotame was tolerated by healthy and diabetic patients at doses of 1.5 mg/Kg body wt. Over 100 studies confirmed the stability and functionality of neotame. The result of these studies confirmed that neotame is safe for use in children, pregnant women, lactating women and people with diabetes<sup>1,8</sup>.

Neotame can be used as a table sweetener and is also approved for use in various foods such as gelatin mixes, canned fruits, fruit juice, baked foods, syrup jelly, chewing gum and soft drinks. Neotame is currently permitted for use in the US, Australia, Newzealand, Mexico and China, Rica. In US the acceptable daily intake (ADI) of neotame is 0.3 mg/kg body weight, where as in Newzealand and Australia is 0.2 mg/kg body weight.

### Conclusion

Neotame offers versatile qualities of no caloric sweetness with stability, safety and cost. Replacing the part of the sweetener in traditional sugar free foods, neotame offers potential sweetness and flavour. Because of its stability in acidic and neutral pH, it is used for carbonated drinks and baked products respectively. It has unique flavor enhancing properties. Now-a-days, it has been used in food industries along with other sweeteners. Based on its safety concern it used for diabetic patients, children and pregnant women.

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