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(71) Applicant: THE NUTRASWEET COMPANY [US/US]; Suite 900, 200 World Trade Center, Chicago, IL 60654–1001 (US).

(72) Inventors: PAJOR, Laurie, L.; 1041 Alder Lane, Naperville, IL 60540 (US). GIBES, Kernon, M.; 13 North Wille Street, Mount Prospect, IL 60056 (US).

(74) Agents: MANDRA, Raymond, R. et al.; Fitzpatrick, Cella, Harper & Scinto, 30 Rockefeller Plaza, New York, NY 10112–3801 (US).

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(54) Title: N–[N–(3,3–DIMETHYLBUTYL)–L–α–ASPARTYL]–L–PHENYLALANINE 1–METHYL ESTER SYNERGISTIC SWEETENER BLENDS

(57) Abstract

This invention provides sweetener blends comprising N–[N–(3,3–dimethylbutyl)–L–α–aspartyl]–L–phenylalanine 1–methyl ester and another sweetener. The sweetener blends of this invention exhibit isobole synergy.

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TITLE

N-[N-(3,3-DIMETHYLBUTYL)-L-α-ASPARTYL]-L-PHENYLALANINE
1-METHYL ESTER
SYNERGISTIC SWEETENER BLENDS

BACKGROUND OF THE INVENTION

Field of the Invention

5

The present invention relates to novel sweetener blends
of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester (also known as neotame)
and another sweetener.

10

Related Background Art

N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine
1-methyl ester is a derivative of aspartame that has a

15   sweetening potency that is about 40 times that of
aspartame (and about 8,000 times that of sucrose). N-
[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-
methyl ester may be prepared from aspartame as
described in U.S. Patent No. 5,480,668, U.S. Patent No.
20   5,510,508, and U.S. Patent No. 5,728,862, all of which
are incorporated by reference herein.

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These are four possible outcomes from sensory analysis
of a blend of two physiologically active agents:
suppression < no synergy < isobole synergy < additive
synergy. It is known that certain sweetener agents
5   exhibit additive synergy when combined with each other.
For example, U.S. Patent No. 3,780,189 describes
synergistic sweetener blends of aspartame and
saccharin. However, isobole synergy in sweetener
blends has not been previously recognized. Moreover,
10   there is no disclosure or suggestion that N-[N-(3,3-
dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester sweetener blends could be isobole synergistic.

The known additive synergy exhibited by aspartame
15   sweetener blends is not a reasonable prediction of even
additive synergy of neotame sweetener blends, let alone
that such blends could be isobole synergistic.
Structurally, N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester and aspartame differ in
20   that, in N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester, a bulky neohexyl
substituent is present on the amine nitrogen.
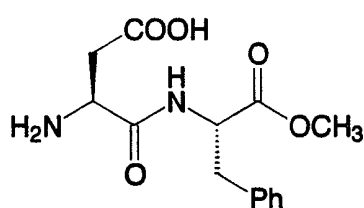
Aspartame   Neotame
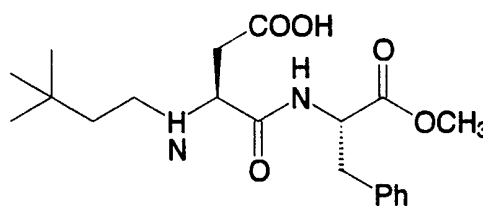
25   This structural difference results in dramatic
differences in the physical and chemical properties of
these compounds. For example, the melting point of N-
[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-
methyl ester is about 82.2°C, while that of aspartame
30   is 248°C. In addition, N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester has much

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higher solubility in organic solvents than aspartame,
and a much lower solubility in water. It is also known
that N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester has a higher stability

5   than aspartame under some pH conditions, as described
in U.S. Patent No. 5,480,688. The pronounced
difference in sweetness potency between the two
compounds is further evidence of their chemical
dissimilarity.

10

Moreover, it is also known that a primary amino group
such as the one on aspartame (pKa 7.7) generally has a
different pKa than those from a secondary amino group
such as the one on N-[N-(3,3-dimethylbutyl)-L-α-

15   aspartyl]-L-phenylalanine 1-methyl ester (pKa 8.1).
Moreover, the pKa's of an amino acid are known to have
a profound impact on food applications (Labuza, T.P.
and Basisier, M.W., 1992, "Physical Chemistry of
Foods", H.G. Schwartzber and R.W. Hartel (Eds.), Marcel

20   Dekker, Inc., New York). It is also well known that a
secondary amine group can not form Schiff base type
compounds with carbonyl compounds while a primary amine
may. Furthermore, N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester exhibits

25   physiologically different behavior than aspartame as
exemplified by the dramatic difference in sweetness.
These differences are clearly indicative that the
characteristics and properties of one can not be said
to suggest those of the other.

30

N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine
1-methyl ester may be used for sweetening a variety of
products, including drinks, foods, confectionery,
pastries, chewing gums, personal care, hygiene products

35   and toiletries, as well as cosmetic, pharmaceutical and
veterinary products. Its superior sweetening potency
makes N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-

phenylalanine 1-methyl ester an attractive alternative
to aspartame because it permits the use of N-[N-(3,3-
dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester in substantially smaller quantities than is
5   required for aspartame to achieve an equivalent
sweetening effect.

Synergy between two sweeteners can be defined as
occurring when the sweetness intensity of a mixture of
10   the sweeteners is greater than what would have been
expected from their individual concentration response
relationships. One explanation for the potential
existence of synergy between sweeteners is the
possibility of multiple sweetness receptor sites.
15   Synergy is important in the use of sweeteners in food
products because it offers both cost savings and flavor
improvement possibilities. For example, a synergistic
combination of aspartame and acesulfame-K is widely
used to sweeten beverages in Europe. *Fry, J., "Trends*
20   *and Innovations in Low-Calorie Sweeteners", The World*
of Ingredients p. 16-18 (March/April 1998).
Synergistic combinations are advantageous because they
permit the use of smaller quantities of each of the
individual agents than would be required otherwise.
25   Thus, the use of synergistic combinations may offer a
more cost effective way to achieve a desired
physiological effect.

It would be highly desirable to provide synergistic
30   blends of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester with other sweeteners.

SUMMARY OF THE INVENTION

35   This invention is related to sweetener blends
comprising N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester and another sweetener.

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The sweetener blends of this invention are isobole synergistic.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that synergy between two or more sweeteners may be classified as isobole synergy. A combination of sweeteners is said to display isobole synergy when the sweetness produced by the combination exceeds that which would be expected if the sweeteners did not interact. In comparison, a combination of sweeteners is said to display additive synergy when the sweetness produced by the combination exceeds that which would be expected if the individual sweetness of the sweeteners were summed.

In particular, it has been discovered that blends of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester with various other sweeteners exhibit at least isobole synergy. Some blends exhibit additive synergy as well. Sweeteners which may be combined with N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester in the synergistic blends of the present invention include, for example, saccharin, acesulfame-K, aspartame, sucrose and mixtures thereof. Of course the blend may comprise more than two sweeteners if desired. Isobole synergy may also be achieved with other blends of high intensity and natural sweeteners such as sucralose, alitame, cyclamates, stevia derivatives, thaumatin, high fructose corn syrup, crystalline fructose, high conversion corn syrup, crystalline fructose, high conversion corn syrup, glucose (dextrose), polyol sugar alcohols, invert sugar and mixtures thereof.

As noted previously, the interactions between physiological active agents may result in one of four

possibilities: suppression; no synergy; isobole
synergy or additive synergy. Suppression occurs if the
measured intensity of a mixture of agents is less
intense than what would have been expected if they did
5   not interact. No synergy is indicated when the
measured intensity of a mixture equals the predicted
intensity from a non-interactive linear curve. Isobole
synergy describes the type of synergy that occurs when
the measured intensity of a mixture is more intense
10 than what would be expected if the mixture components
did not interact together. Additive synergy occurs
when the measured intensity of a mixture is more
intense than what would be expected if the measured
intensities of the individual components of the mixture
15 were summed.

There are several methods reported in the
pharmaceutical industry for analyzing interactions
between physiologically active agents. One of these
20 methods is known as the isobole method. This method
involves constructing iso-effective curves (i.e.,
isoboles) in which a combination of agents (d_a, d_b) is
represented by a point on a graph the axes of which are
the dose-axes of the individual agents (a and b). If
25 the agents do not interact, the curve joining the point
representing the combination (d_a, d_b) to those on the
dose-axes representing doses iso-effective with the
combination (D_a along the "a" axis and D_b along the "b"
axis) will be a straight line.
30

The equation for the zero interaction line for two
agents is

$$\frac{d_a}{D_a} + \frac{d_b}{D_b} = 1 \qquad\qquad (1)$$

where D_a is the amount of agent "a" needed to produce a
35 given physiological effect; D_b is the amount of agent

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"b" needed to produce a physiological effect equal to the effect produced by D_a; d_a and d_b represent the respective amounts of agents "a" and "b" that must be used in the combination (d_a, d_b) to produce an effect

5   equal to the effect produced by D_a (or D_b). Thus, combination (d_a, d_b), D_a and D_b are iso-effective with respect to each other.

When agents in combination are more effective than

10   expected based upon their dose-response curves (i.e, synergistic), smaller amounts are needed to produce the desired effect. Thus, d_a and/or d_b are reduced, while D_a and D_b are unchanged. Consequently, the equation for a synergistic combination is

$$\frac{d_a}{D_a} + \frac{d_b}{D_b} < 1 \qquad (2)$$

15

Conversely, the equation for an antagonistic combination is

$$\frac{d_a}{D_a} + \frac{d_b}{D_b} > 1 \qquad (3)$$

Using equation 1, it is possible to calculate the

20   effect that would be expected for a non-interactive combination of agents. This is termed the "isobole expected effect." If the "actual effect" of the combination exceeds its "isobole expected effect", the combination is synergistic. Conversely, if the

25   "isobole expected effect" exceeds the "actual effect", the combination is antagonistic.

Heretofore the application of isobole synergy to sweetener blends was not recognized or appreciated. In

30   the present invention, it was discovered that the isobole methodology used by the pharmaceutical industry

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could be applied to the food and/or sweetener industry.
Instead of "agents", "physiological effect", "iso-
effective", and "doses", the terms sweeteners, sweet
response, iso-sweet, and concentrations are relevant to
5 the sweetener industry.

The isobole methodology provides a means to detect
synergy of sweeteners at or below the simple additive
level of current industry practice. Thus, synergy can
10 now be determined for instances that would have been
considered non-synergistic or antagonistic (referred to
as "mixture suppression" in the sweetener industry).
The steps of the isobole method as applied to sweet
taste synergy are described below.
15

The first step in assessing sweetness synergy is to
establish the concentration response relationships for
each of the individual sweeteners. The concentration
response relationship can be conveniently expressed as
20 a plot of sweetener response vs. concentration. This
involves generating sensory data on the sweetness
intensity of the sweetener at several concentrations,
and then modeling the results. Several mathematical
models are available for fitting sweetness intensity
25 data, but will be referred to generically as a
"function" below.

The equation for the concentration response curve of
sweetener "a" is
30

$$R = f_a(d_a) \tag{4}$$

where d_a is now the concentration of sweetener "a" that
produces a sweetness taste intensity of "R", and "f_a"
35 represents the functional form of the "a" curve.

Similarly, the equation for the concentration response
curve of sweetener "b" is

$$R = f_b(d_b) \tag{5}$$

where d_b is now the concentration of sweetener "b" that
produces a sweetness taste intensity of "R", and "f_b"
represents the functional form of the "b" curve.

The second step entails using equation 1 to calculate
the "expected isobole sweetness", R_{ab}, of a given blend
(d_a, d_b).

The concentrations of individual sweeteners that are
iso-sweet to a given sweetness intensity, R_{ab}, are the
inverses of the individual functions. From equation 4,
the equation for the inverse of the sweetness taste
response of sweetener "a" is

$$D_a = f_a^{-1}(R_{ab}) \tag{6}$$

where D_a is now the specific concentration of "a" that
is iso-sweet to sweetness intensity R_{ab}. Similarly,
from equation 5, the sweetener "b" inverse is

$$D_b = f_b^{-1}(R_{ab}) \tag{7}$$

After substituting equations 6 and 7 for the iso-sweet
concentrations, equation 1 can be written as

$$\left(\frac{d_a}{f_a^{-1}(R_{ab})} \right) + \left(\frac{d_b}{f_b^{-1}(R_{ab})} \right) = 1 \tag{8}$$

The expected isobole sweetness intensity R_{ab} for blend (d_a, d_b) can then be obtained from equation 8. Depending upon the forms of the inverse functions, R_{ab} may be solved for algebraically. For example, if f_a and f_b are both Beidler equations, the expression for R_{ab} is the Beidler mixture equation (Beidler, L.M., 1962, "Taste receptor stimulation," Progress in Biophysics 12, 109-151). In those instances where an algebraic solution is not available, R_{ab} can be obtained by numerical methods widely available in computer software programs.

The "expected additive sweetness" R_{a+b} of a blend of sweeteners (d_a, d_b) is directly obtained from the individual concentration response functions by adding them together. Thus, the equation for the expected additive sweetness is

$$R_{a+b} = f_a(d_a) + f_b(d_b) \tag{9}$$

The final step in assessing synergy is to compare the observed sweetness intensity of a blend with its expected sweetness intensity, R_{ab} or R_{a+b}. Data from sensory panels can be compared to the expected results, and whether the difference is statistically significant can be checked using conventional statistical analysis of variance methods, for example, contrast t-tests.

The interaction between two or more physiologically active agents may vary considerably depending upon the weight ratio of each agent. Thus, a given blend may be synergistic at one weight ratio and non-interactive (or even antagonistic) at another weight ratio. The sweeteners in the blends of this invention are present in an amount effective to provide an isobole synergistic effect.

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The N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester used herein may take any
form. For example, it may be in the form of a salt or
complex such as disclosed in U.S. Patent Application

5   No. 09/146,963, U.S. Patent Application No. 09/146,964,
U.S. Patent Application No. 09/148,134 and U.S. Patent
Application No. 09/146,965, all filed September 4,
1998, and all of which are incorporated by reference
herein. Yet another example includes N-[N-

10 (3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine
1-methyl ester cyclodextrin complexes such as described
in U.S. Provisional Patent Application No. 60/100,867,
filed September 17, 1998, which is incorporated by
reference herein.

15

The ratio of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester to sweetener that may be
used in the blends of this invention depend upon the
particular sweetener chosen. For example, the weight

20 ratio of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester to saccharin may vary from
about 1:1.18 to about 1:517. Preferably, the weight
ratio is between about 1:7.5 to about 1:121.

25 The weight ratio of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester to acesulfame-
K may vary from about 1:2.48 to about 1:1497.
Preferably, the weight ratio is between about 1:14 to
about 1:294.

30

The weight ratio of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester to aspartame
may vary from about 1:3.97 to about 1:564. Preferably,
the weight ratio is between about 1:13 to about 1:238.

35

The weight ratio of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester to sucrose may

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vary from about 1:746 to about 1:94,115. Preferably,
the weight ratio is between about 1:1786 to about
1:33,333.

5   The sweetener blend of this invention may be used in
    any food or beverage composition. Such compositions
    include baked goods, dairy products, carbonated and
    non-carbonated beverages, confections, gums and the
    like. The blend may be packaged in individual serving
10 sachets or such larger packaging as desired.

    One example of a particularly preferred blend is
    sucrose and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
    phenylalanine 1-methyl ester. Such a blend may be
15 advantageously employed as a synergistic sucrose
    extender which could be particularly beneficial in
    economies in which sucrose demands a premium price.
    This invention is also directed to an aqueous solution
    comprising the sweetener blend described above. The
20 aqueous solution of this invention includes beverages,
    e.g., carbonated and non-carbonated soft drinks, and
    liquid table top sweeteners. The aqueous solutions of
    this invention contain the sweetener blend in a
    concentration range effective to provide isobole
25 synergistic sweetening to the solution. The
    concentrations of N-[N-(3,3-dimethylbutyl)-L-α-
    aspartyl]-L-phenylalanine 1-methyl ester and the other
    sweetener will vary depending on the application and
    the blend weight ratio. Once the blend ratio of the
30 sweetener blend has been determined, the concentration
    of the sweetener blend used in an aqueous solution that
    provides an isobole synergistic effect can be readily
    determined without undue experimentation. One skilled
    in the art can use the invention described herein to
35 formulate various products having isobole synergistic
    sweetness.

Examples of the advantages of this invention may
include: real cost savings realized from overall
reduction in total sweetener use, the ability to
sweeten products to a higher sweetness level than would
5   be possible with certain single sweeteners alone, due
to presence of bitter or other side tastes, and an
overall improvement in sweet taste character and time-
intensity or temporal profile.

10 The examples which follow are intended as an
illustration of certain preferred embodiments of the
invention, and no limitation of the invention is
implied.

15   EXAMPLE 1
    Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
      L-phenylalanine 1-methyl ester/saccharin Synergistic
                  Blends (1:7.5 weight ratio)

20 Five different aqueous solution of neotame/saccharin
blends were prepared. Each solution had a blend weight
ratio of 1:7.5, but different sweetener concentration
levels as noted in Table 1.

25   The sweetness intensities of each of the five
solutions were measured by a trained taste panel of 10-
15 individuals using a 15 point intensity scale
measured in sucrose equivalency (% SE), i.e., 2SE = 2%
sucrose, 5SE = 5% sucrose, 7.5SE = 7.5% sucrose, 10SE =
30 10% sucrose and 12SE = 12% sucrose. The results of
these measurements are set forth in Table 1.

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EXAMPLE 2

Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/saccharin Synergistic
Blends (1:29 weight ratio)

5

Five blends of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester and saccharin
were prepared as described in Example 1, except that in
each of these blends the ratio of N-[N-(3,3-
10   dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester to saccharin was 1:29.

The sweetness intensities of each of the five
solutions were measured as previously described and the
15   results of these measurements are set forth in Table 1.

EXAMPLE 3

Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/saccharin Synergistic
20   Blends (1:121 weight ratio)

Five blends of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester and saccharin
were prepared as described in Example 1, except that in
25   each of these blends the ratio of N-[N-(3,3-
dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester to saccharin was 1:121.

The sweetness intensities of each of the five
30   solutions were measured as previously described and the
results of these measurements are set forth in Table 1.
Table 1 also shows the predicted isobole expected
sweetness and predicted additive expected sweetness
values for each of the blends.

35

Table 1.
    Synergy of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
    L-phenylalanine 1-methyl ester/saccharin Blends

Blend Ratio Wt:Wt	Neotame (ppm)	Saccharin (ppm)	Observed Sweetness Intensity	Predicted Isobole Expected Sweetness	Predicted Additive Expected Sweetness
1:7.5	1.15	8.60	1.90	2.0	2
1:7.5	2.50	18.64	4.95	3.85 **	4 **
1:7.5	4.10	30.65	6.84	5.46 **	6 **
1:7.5	6.05	45.20	8.14	6.88 **	8
1:7.5	8.44	63.06	9.69	8.12 **	10
1:29	0.73	21.16	1.78	1.98	2
1:29	1.48	43.01	4.65	3.67 **	4
1:29	2.33	67.55	6.20	5.13 **	6
1:29	3.31	96.04	7.48	6.41 **	8
1:29	4.48	130.09	8.78	7.54 **	10
1:121	0.29	34.99	2.68	1.96 *	2 *
1:121	0.59	71.06	4.89	3.73 **	4 **
1:121	0.95	114.16	6.71	5.34 **	6 *
1:121	1.40	168.39	8.24	6.78 **	8
1:121	1.99	239.31	8.93	8.05 **	10
Statistical Significance: Statistical results are from t - tests comparing the observed sweeteners intensity with the expected intensity * 10% significance level ** 5% significance level					

A comparison of the observed sweetness intensity values
to the isobole expected sweetness values
in Table 1 shows that the blends of N-[N-(3,3-
dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester with saccharin displayed isobole and additive
synergy at blend ratios ranging from 1:7.5 to 1:121.

EXAMPLE 4

Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/acesulfame-K Synergistic
Blends (1:14 weight ratio)

Five different aqueous solutions of neotame/acesulfame-K blends were prepared. Each solution had a blend weight ratio of 1:14, but different sweetener concentration levels as noted in Table 2.

The sweetness intensities of each of the five solutions were measured as previously described and the results of these measurements are set forth in Table 2.

EXAMPLE 5
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester/acesulfame-K Synergistic Blends (1:64 weight ratio)

Five blends of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and acesulfame-K were prepared as described in Example 4, except that in each of these blends the ratio of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester to acesulfame-K was 1:64.

The sweetness intensities of each of the five solutions were measured as previously described and the results of these measurements are set forth in Table 2.

EXAMPLE 6
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester/acesulfame-K Synergistic Blends (1:294 weight ratio)

Five blends of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and acesulfame-K were prepared as described in Example 4, except that in each of these blends the ratio of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester to acesulfame-K was 1:294.

The sweetness intensities of each of the five
solutions were measured and the results of these
measurements are set forth in Table 2. Table 2 also
shows the predicted isobole expected sweetness and
5 predicted additive expected sweetness values for each
of the blends.

Table 2.
        Synergy of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
10   L-phenylalanine 1-methyl ester/acesulfame-K Blends

Blend Ratio Wt:Wt	Neotame (ppm)	Ace-K (ppm)	Observed Sweetness Intensity	Predicted Isobole Expected Sweetness	Predicted Additive Expected Sweetness
1:14	1.08	15.29	1.79	1.91	2
1:14	2.39	33.91	4.27	3.65 **	4
1:14	4.02	56.90	6.10	5.24 **	6
1:14	6.05	85.71	7.92	6.67 **	8
1:14	8.65	122.49	9.18	7.96 **	10
1:64	0.63	40.38	1.52	1.86	2
1:64	1.37	87.71	3.60	3.49	4
1:64	2.25	143.93	5.73	4.92 **	6
1:64	3.31	211.76	7.45	6.19 **	8
1:64	4.61	295.17	9.10	7.31 **	10
1:294	0.23	67.65	2.34	1.92	2
1:294	0.52	154.20	3.97	3.67	4
1:294	0.90	266.73	5.68	5.26	6
1:294	1.40	415.38	7.71	6.67 **	8
1:294	2.07	614.74	8.81	7.90 **	10
Statistical Significance: Statistical results are from t - tests comparing the observed sweetness intensity with the expected intensity ** 5% significance level					

35 A comparison of the observed sweetness intensity values
    to the isobole expected sweetness values
    in Table 2 shows that the blends of N-[N-(3,3-
    dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
    ester with acesulfame-K displayed isobole synergy at
40 blend ratios ranging from 1:14 to 1:294.

EXAMPLE 7
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/aspartame Synergistic
Blends (1:13 weight ratio)

5

Five different aqueous solutions of neotame/aspartame
blends were prepared. Each solution had a blend weight
ratio of 1:13, but different sweetness concentration
levels as noted in Table 3.

10

The sweetness intensities of each of the five
solutions were measured as previously described and the
results of these measurements are set forth in Table 3.

15   EXAMPLE 8
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/aspartame Synergistic
Blends (1:57 weight ratio)

20   Five blends of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester and
acesulfame-K were prepared as described in Example 7,
except that in each of these blends the ratio of N-[N-
(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-
25 methyl ester to aspartame was 1:57.

The sweetness intensities of each of the five
solutions were measured as previously described and the
results of these measurements are set forth in Table 3.

30
                         EXAMPLE 9
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/aspartame Synergistic
Blends (1:294 weight ratio)

35

Five blends of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester and aspartame

were prepared as described in Example 7, except that in each of these blends the ratio of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester to aspartame was 1:238.

The sweetness intensities of each of the five solutions were measured as previously described and the results of these measurements are set forth in Table 3. Table 3 also shows the predicted isobole expected sweetness and predicted additive expected sweetness values for each of the blends.

Table 3.
      Synergy of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
      L-phenylalanine 1-methyl ester/aspartame Blends

Blend Ratio Wt:Wt	Neotame (ppm)	Aspartame (ppm)	Observed Sweetness Intensity	Predicted Isobole Expected Sweetness	Predicted Additive Expected Sweetness
1:13	2.41	32.41	3.52	3.70	4
1:13	4.04	54.24	5.78	5.33	6
1:13	6.05	81.33	7.77	6.82 **	8
1:13	8.58	115.35	9.29	8.16 **	10
1:13	11.8	158.56	10.26	9.36 **	12
1:57	1.42	80.42	2.98	3.56	4
1:57	2.29	129.93	5.38	5.05	6
1:57	3.31	187.69	6.38	6.40	8
1:57	4.51	255.95	8.13	7.62 *	10
1:57	5.95	337.83	9.07	8.72	12
1:238	0.58	136.56	3.58	3.70	4
1:238	0.95	225.30	6.08	5.32 **	6
1:238	1.40	332.73	7.78	6.82 **	8
1:238	1.96	454.36	9.33	8.19 **	10
1:238	2.64	627.87	10.20	9.43 **	12
Statistical Significance: Statistical results are from t - tests comparing the observed sweetness intensity with the expected intensity * 10% significance level ** 5% significance level					

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A comparison of the observed sweetness intensity values to the isobole expected sweetness values in Table 3 shows that the blends of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
5   ester with aspartame displayed isobole synergy at blend ratios ranging from 1:13 to 1:238.

EXAMPLE 10
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
10   L-phenylalanine 1-methyl ester/sucrose Synergistic
Blends (1:1786 weight ratio)

Five different aqueous solutions of neotame/sucrose blends were prepared. Each solution had a blend weight
15   ratio of 1:1786, but different sweetener concentration levels as noted in Table 4.

The sweetness intensities of each of the five solutions were measured as previously described and the
20   results of these measurements are set forth in Table 4.

EXAMPLE 11
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/sucrose Synergistic
25   Blends (1:10,000 weight ratio)

Five blends of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and sucrose were prepared as described in Example 10, except that
30   in each of these blends the ratio of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester to sucrose was 1:10,000.

The sweetness intensities of each of the five
35   solutions were measured as previously described and the results of these measurements are set forth in Table 4.

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EXAMPLE 12
Preparation of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
L-phenylalanine 1-methyl ester/aspartame Synergistic
Blends (1:33,333 weight ratio)

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Five blends of N-[N-(3,3-dimethylbutyl)-L-α-
aspartyl]-L-phenylalanine 1-methyl ester and aspartame
were prepared as described in Example 10, except that
in each of these blends the ratio of N-[N-(3,3-
10   dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester to sucrose was 1:33,333.

The sweetness intensities of each of the five
solutions were measured as previously described and the
15   results of these measurements are set forth in Table 4.
Table 4 also shows the predicted isobole expected
sweetness and predicted additive expected sweetness
values for each of the blends.

Table 4.
    Synergy of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-
    L-phenylalanine 1-methyl ester/sucrose Blends

Blend Ratio Wt:Wt	Neotame (ppm)	Sucrose (% w/w)	Observed Sweetness Intensity	Predicted Isobole Expected Sweetness	Predicted Additive Expected Sweetness
1:1786	1.99	0.356	2.69	3.09	4
1:1786	3.76	0.674	5.33	4.95	6
1:1786	6.05	1.085	7.89	6.72 *	8
1:1786	9.05	1.622	9.06	8.37	10
1:1786	12.95	2.322	10.32	9.84	12
1:10,000	1.28	1.245	2.37	3.31	4
1:10,000	2.25	2.182	5.05	5.02	6
1:10,000	3.31	3.213	6.50	6.61	8
1:10,000	4.47	4.338	8.60	8.07	10
1:10,000	5.72	5.556	10.12	9.39	12
1:33,333	0.59	2.244	2.94	3.63	4
1:33,333	0.99	3.776	5.36	5.39	6
1:33,333	1.40	5.340	7.97	7.06 **	8
1:33,333	1.82	6.937	9.72	8.64 **	10
1:33,333	2.25	8.564	10.87	10.13 **	12
Statistical Significance: Statistical results are from t - tests comparing the observed sweetness intensity with the expected intensity * 10% significance level ** 5% significance level					

A comparison of the observed sweetness intensity values
to the isobole expected sweetness values
in Table 4 shows that the blends of N-[N-(3,3-
dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester with sucrose displayed isobole synergy at blend
ratios ranging from 1:1786 to 1:33,333.

The benefits of this invention include ingredient cost
savings, reduction in sweetener use, overall sweet
taste and temporal profile character improvement are
possible because of the knowledge of isobole synergy.

Other variations and modifications of this invention
will be obvious to those skilled in the art. This
invention is not limited except as set forth in the
claims.

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WHAT IS CLAIMED IS:

1.   A sweetener blend comprising N-[N-(3,
3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl
ester and another sweetener in a combined amount
effective to provide an isobole synergistic sweetening
effect.

2.   A sweetener blend according to claim 1, wherein
said other sweetener is selected from the group
consisting of saccharin, acesulfame-K, aspartame,
sucrose and mixtures thereof.

3.   A sweetener blend according to claim 2, wherein
said other sweetener is saccharin.

4.   A sweetener blend according to claim 3, wherein
said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester and said saccharin are
present in a weight ratio from about 1:1.18 to about
1:517.

5.   A sweetener blend according to claim 4, wherein
said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-
phenylalanine 1-methyl ester and said saccharin are
present in a weight ratio from about 1:7.5 to about
1:121.

6.   A sweetener blend according to claim 3, wherein
said blend provides an additive synergistic sweetening
effect.

7.   A sweetener blend according to claim 2, wherein
said other sweetener is acesulfame-K.

8.   A sweetener blend according to claim 7, wherein
said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-

phenylalanine 1-methyl ester and said acesulfame-K are present in a weight ratio from about 1:2.48 to about 1:1497.

9.   A sweetener blend according to claim 8, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said acesulfame-K are present in a weight ratio from about 1:14 to about 1:294.

10.   A sweetener blend according to claim 7, wherein said blend provides an additive synergistic sweetening effect.

11.   A sweetener blend according to claim 2, wherein said other sweetener is aspartame.

12.   A sweetener blend according to claim 11, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said aspartame are present in a weight ratio from about 1:3.97 to about 1:564.

13.   A sweetener blend according to claim 12, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said aspartame are present in a weight ratio from about 1:13 to about 1:238.

14.   A sweetener blend according to claim 2, wherein said other sweetener is sucrose.

15.   A sweetener blend according to claim 14, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said sucrose are present in a weight ratio from about 1:746 to about 1:94,115.

16. A sweetener blend according to claim 15, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said sucrose are present in a weight ratio from about 1:1786 to about 1:33,333.

17. An aqueous solution comprising N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester, another sweetener and water, wherein N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said another sweetener are present in an amount effective to provide isobole synergistic sweetening of said solution.

18. An aqueous solution according to claim 17, wherein said other sweetener is selected from the group consisting of saccharin, acesulfame-K, aspartame, sucrose and mixtures thereof.

19. An aqueous solution according to claim 18, wherein said other sweetener is saccharin.

20. An aqueous solution according to claim 19, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said saccharin are present in a weight ratio from about 1:1.18 to about 1:517.

21. An aqueous solution according to claim 20, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said saccharin are present in a weight ratio from about 1:7.5 to about 1:121.

22. An aqueous solution according to claim 19, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and saccharin are present

in an amount effective to provide additive synergistic sweetening of said solution.

23. An aqueous solution according to claim 18, wherein said other sweetener is acesulfame-K.

24. An aqueous solution according to claim 23, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said acesulfame-K are present in a weight ratio from about 1:2.48 to about 1:1497.

25. An aqueous solution according to claim 24, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said acesulfame-K are present in a weight ratio from about 1:14 to about 1:294.

26. An aqueous solution according to claim 23, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and acesulfame-K are present in an amount effective to provide additive synergistic sweetening of said solution.

27. An aqueous solution according to claim 18, wherein said other sweetener is aspartame.

28. An aqueous solution according to claim 27, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said aspartame are present in a weight ratio from about 1:3.97 to about 1:564.

29. An aqueous solution according to claim 28, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said aspartame are

present in a weight ratio from about 1:13 to about 1:238.

30. An aqueous solution according to claim 18, wherein said other sweetener is sucrose.

31. An aqueous solution according to claim 30, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said sucrose are present in a weight ratio from about 1:746 to about 1:94,115.

32. An aqueous solution according to claim 31, wherein said N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester and said sucrose are present in a weight ratio from about 1:1786 to about 1:33,333.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C07K5/06   A23L1/236

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 39979 A (ISHII SHOICHI ;AJINOMOTO KK (JP); SHIMIZU TETSUJI (JP)) 17 September 1998 (1998-09-17)	1,2,6,7, 9-13,17, 18,22-29
Y	claims, tables	1-32

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2000	31/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kronester-Frei, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US MATTERN, RALPH-HEIKO ET AL: "Conformational analysis of potent sweet taste ligands by nuclear magnetic resonance, computer simulations and x-ray diffraction studies" retrieved from STN Database accession no. 127:356297 XP002132936 abstract & J. PEPT. RES. (1997), 50(4), 286-299 ,	1-32
Y	WO 94 11391 A (NOFRE CLAUDE ;TINTI JEAN MARIE (FR)) 26 May 1994 (1994-05-26) Table 1, compound 6, page 7 and 13/14	1-32
Y	WO 98 04156 A (BATEMAN KRISTINE A) 5 February 1998 (1998-02-05) claims, page 23, line 20-page 24, line 12, Table 1	1-32
Y	US 4 915 969 A (BEYTS PAMELA K) 10 April 1990 (1990-04-10) example 1	1-32
P,X	WO 99 30577 A (NUTRASWEET CO) 24 June 1999 (1999-06-24) claims	1-32
P,X	WO 99 30578 A (NUTRASWEET CO) 24 June 1999 (1999-06-24) claims	1-32
P,X	WO 99 57997 A (NAGAI TAKESHI ;AJINOMOTO KK (JP); HIRANO ATSUHIKO (JP); ISHIDA HIR) 18 November 1999 (1999-11-18) claims	1,2,6,7, 9,10,17, 18,22-26
P,X	WO 99 57998 A (NAGAI TAKESHI ;AJINOMOTO KK (JP); HIRANO ATSUHIKO (JP); ISHIDA HIR) 18 November 1999 (1999-11-18) claims	1,2, 11-13, 17,18, 27-29

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Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9839979	A	17-09-1998	JP	10248520 A	22-09-1998
			JP	10248521 A	22-09-1998
			AU	4398097 A	29-09-1998
			EP	0974272 A	26-01-2000
WO 9411391	A	26-05-1994	FR	2697844 A	13-05-1994
			AT	138935 T	15-06-1996
			AU	664663 B	23-11-1995
			AU	5468194 A	08-06-1994
			BG	61609 B	30-01-1998
			BG	99299 A	29-09-1995
			CA	2139233 A	26-05-1994
			CN	1090571 A,B	10-08-1994
			CZ	9403319 A	18-10-1995
			DE	69303032 D	11-07-1996
			DE	69303032 T	02-10-1996
			DK	669935 T	01-07-1996
			EP	0669935 A	06-09-1995
			ES	2091114 T	16-10-1996
			FI	945451 A	22-12-1994
			GR	3020164 T	30-09-1996
			HU	72192 A	28-03-1996
			IL	107551 A	16-08-1998
			JP	2818032 B	30-10-1998
			JP	8503206 T	09-04-1996
			LT	1457 A,B	15-06-1994
			MD	960256 A	31-03-1998
			NO	945090 A	30-12-1994
			NZ	257870 A	26-03-1996
			PL	306841 A	18-04-1995
			RO	112621 A	28-11-1997
			SK	158694 A	10-05-1995
			US	5480668 A	02-01-1996
			ZA	9308430 A	13-06-1994
WO 9804156	A	05-02-1998	AU	3968697 A	20-02-1998
US 4915969	A	10-04-1990	CA	1224962 A	04-08-1987
			GB	2154850 A,B	18-09-1985
WO 9930577	A	24-06-1999	AU	1924899 A	05-07-1999
WO 9930578	A	24-06-1999	AU	1936499 A	05-07-1999
WO 9957997	A	18-11-1999	JP	11318382 A	24-11-1999
WO 9957998	A	18-11-1999	JP	11318381 A	24-11-1999