

The NutraSweet Company

Box 730, 1751 Lake Cook Road, Deerfield, Illinois 60015-5239

Telephone: 708/940-9800



7/3/90

Carl Giannetta, Ph.D.
Center for Food Safety & Applied Nutrition
Food & Drug Administration
200 "C" Street, S.W.
Washington, D.C. 20204

RE: Food Additive Petition No. 7A4044

Dear Dr. Giannetta:

We are responding to your communication dated April 25, 1989 requesting additional information on the use of encapsulated aspartame (APM) in baked goods.

Our responses to your specific inquiries are as follows:

1.(a) Provide basis for calculating
diketopiperazine (DKP)

As presented in the petition, DKP recovery data was reported as a percent of initial aspartame calculated as follows:

$$\frac{\text{Quantity DKP in product}}{\text{Quantity of initial APM}} \times 100$$

Each data point represented an average of at least two analytical results. An aspartame-spiked placebo recovery correction factor was included in the final calculations for both APM and DKP.

The analytical values for DKP, originally submitted in 1987, were corrected using the encapsulated APM-spiked placebo since the extraction efficiencies for APM and DKP spike placebos were similar (greater than 90%), as demonstrated in our method validation.

The spiked placebo was prepared by adding a known quantity of APM to an unsweetened baked sample. The analytical recovery was defined as the amount of APM detected in the spiked placebo expressed as percentage of the amount of APM added. This value, referred to as the placebo spike recovery, was used as a correction factor in subsequent calculations so that final values would reflect only the impact of baking upon degradation. The placebo spike recovery values averaged over 90% for the data presented in Attachments 1, 2, & 4.

In light of our consistently high APM recoveries and therefore low DKP values, and our consistently high placebo spike recovery values, we no longer consider the application of a correction factor as supplying any additional relevant accuracy to the DKP data. Thus the practice has been discontinued and the data presented in Attachment 2 does not include the correction factor for the DKP data.

1.(b) Provide concentrations of DKP in mg/gram

Included in Attachment 1 to this submission is an amended Appendix V which now contains an additional column specifying the DKP content in mg/gram of product. When calculating these values typographical/calculation errors were noted which have been corrected.

2.(a) Provide additional analytical data on products after expected storage

Prototype products were stored under different temperature conditions for extended periods of time.

At specified intervals, samples were removed for aspartame and diketopiperazine analyses according to the methods described in Appendix III of the original petition. Additionally, pH, moisture and water activity were also recorded.

The length of time for stability determination was chosen based upon industry data that tracks product from date of manufacture to the date when product should no longer be sold due to both microbial spoilage and/or texture changes. The shelf-life ranges by product category are: fruit pies 4-7 days; cakes 2-9 days; cookies 2-6 months; and fruit-filled cookies 2-4 months.

The products represented a broad range of moisture content, from 7.5% for the cookies to 53% for the cheesecake and pie filling.

As suggested, an additional prototype product, i.e. fig bar, was formulated as an example of a high moisture cookie. The fig bar consisted of a filling, into which the encapsulated aspartame was incorporated, and an outer cookie jacket. The filling had a pH of 4.5 and moisture content of approximately 15%. The prototype formulation, description of process and analytical method for APM and DKP determination are included in Attachment 3.

The results from the fig bars (Attachment 2) are variable due to the differences in manufacturing (i.e. filling) the cookie. The process used was a commercial line that did not uniformly deposit exact amounts of filling in the cookie.

The analytical results for the various products tested are presented in Attachment 2. The pH, moisture and water activity did not exhibit any appreciable changes over time and are not included.

The stability of aspartame in the final products after baking, appears consistent with other food systems. It is dependent on the pH, moisture and temperature, with chocolate cake presenting the most adverse conditions.

The stability of aspartame in a specific product type exceeds the industry shelf-life requirements.

2.(b) Provide data on reheating.

The standard chocolate cake and the lemon pie filling were baked, cooled and then stored at either room temperature or refrigerated overnight. Both product types were subjected to reheating in a conventional oven and a microwave for different times of exposure. The methods for determining aspartame and DKP are contained in the petition.

The pie filling was chosen as a prototype representing the product category most likely to be reheated after refrigerator storage. Others such as a cheesecake or a cookie would not routinely be heated a second time. Due to loss of moisture, cakes are typically not subjected to reheating. However, we chose the chocolate cake as an example of the most adverse condition for aspartame's stability. The results are presented in Attachment 4.

In conclusion, aspartame-sweetened baked goods can be reheated in either a microwave or conventional oven without compromising APM stability.

We trust that the enclosed information adequately addresses your questions. Please contact me if I can be of further assistance.

Sincerely,



Robert C. Peterson
Director, Regulatory Compliance

ASPARTAME IN
BAKED GOODS

Appendix V
Page 1

Analytical Results in Percent for
Aspartame and Diketopiperazine
Recovery from Baked Goods

Product/ Bake Conditions	Formulated AFM in percent	Calculated ¹ AFM in percent	Percent Recovery		DKP ⁴ (Mg/g)
			AFM ²	DKP ³	
A. Cheesecake					
250°F/30 min.	0.07	0.076	96.0	3.0	0.02
	0.10	0.111	91.1	4.1	0.04
	0.15	0.158	95.4	2.5	0.01
	0.22	0.236	87.5	0.9	0.02
	0.30	0.319	89.9	1.5	0.04
250°F/35 min.	0.15	0.162	92.6	6.7	0.04
250°F/50 min.	0.15	0.172	84.0	6.4	0.01
300°F/40 min.	0.15	0.186	82.1	14.6	0.02
B. Cookie					
410°F/10 min.	0.15	0.168	100.1	2.1	0.03
	0.25	0.276	97.5	2.4	0.06
	0.35	0.376	96.0	2.4	0.09
	0.45	0.510	94.9	2.6	0.13
	0.55	0.609	93.3	2.7	0.16
	0.40	0.455	98.3	2.5	0.11
	0.50	0.557	98.2	1.9	0.11
410°F/12 min.	0.35	0.411	78.9	15.5	0.61
360°F/18 min.	0.35	0.423	73.1	20.7	0.83
450°F/9 min.	0.35	0.409	81.6	13.3	0.51

(continued)

ASPARTAME IN
BAKED GOODS

Appendix V
Page 2

Analytical Results in Percent for
Aspartame and Diketopiperazine
Recovery from Baked Goods

Product/ Bake Conditions	Formulated AFM in percent	Calculated ¹ AFM in percent	Percent Recovery		DKP ⁴ (Mg/g)
			AFM ²	DKP ³	
C. Yellow Cake					
350°F/22 min.	0.08	0.089	95.7	8.1	0.07
	0.15	0.167	94.0	7.4	0.12
	0.20	0.221	94.5	8.5	0.17
	0.30	0.331	89.1	7.9	0.24
	0.40	0.439	84.9	7.2	0.28
	0.40	0.435	87.7	3.9	0.16
	0.55	0.599	90.1	4.4	0.25
350°F/30 min.	0.20	0.231	75.7	11.8	0.26
350°F/45 min.	0.20	0.245	61.1	24.0	0.54
400°F/25 min.	0.20	0.226	86.6	8.8	0.19
310°F/50 min.	0.20	0.239	70.0	21.0	0.48
D. Modified Yellow Cake					
350°F/30 min.	0.20	0.221	94.1	4.0	0.08
	0.35	0.383	94.3	3.4	0.12
	0.50	0.544	97.3	3.1	0.15
350°F/40 min.	0.35	0.42	81.5	10.4	0.40
	0.35	0.42	80.6	10.7	0.42
390°F/30 min.	0.35	0.40	92.0	5.5	0.20
	0.35	0.40	89.5	6.3	0.23
310°F/50 min.	0.35	0.41	85.0	10.0	0.38

(continued)

ASPARTAME IN
BAKED GOODS

Appendix V
Page 3

Analytical Results in Percent for
Aspartame and Diketopiperazine
Recovery from Baked Goods

Product Bake Conditions	Formulated APM in percent	Calculated ¹ APM in percent	Percent Recovery		DKP ⁴ (Mg/g)
			APM ²	DKP ³	
E. Chocolate Cake					
350°F/25 min.	0.25	0.298	75.7	16.2	0.45
	0.35	0.411	82.1	11.9	0.45
	0.45	0.528	76.9	16.1	0.78
	0.55	0.648	78.1	17.1	1.02
	0.65	0.761	77.5	14.3	0.97
	0.45	0.532	79.1	13.9	0.68
	0.65	0.773	85.5	12.4	0.88
	0.85	1.025	78.9	13.1	1.24
350°F/35 min.	0.45	0.535	75.7	15.2	0.74
390°F/25 min.	0.45	0.544	76.4	13.5	0.67
390°F/20 min.	0.45	0.552	82.2	16.5	0.83
310°F/35 min.	0.45	0.534	74.1	17.3	0.85
390°F/30 min.	0.45	0.577	65.9	23.6	1.26
F. Modified Chocolate Cake					
350°F/25 min.	0.20	0.215	91.9	9.6	0.19
	0.35	0.375	101.3	9.7	0.33
	0.50	0.536	88.7	9.5	0.46
360°F/35 min.	0.35	0.40	66.3	27.1	0.98
	0.35	0.40	64.1	24.7	0.89
400°F/27 min.	0.35	0.39	81.6	17.6	0.62
	0.35	0.39	82.1	17.7	0.62
310°F/40 min.	0.35	0.38	72.1	25.3	0.88

(continued)

ASPARTAME IN
BAKED GOODS

Analytical Results in Percent for
Aspartame and Diketopiperazine
Recovery from Baked Goods

Product/ Bake Conditions	Formulated AEM in percent	Calculated ¹ AEM in percent	Percent Recovery		DKP ⁴ (Mg/g)
			AEM ²	DKP ³	
G. Lemon Pie Filling					
375°F/35 min.	0.25	0.294	95.2	1.9	0.05
	0.25	0.292	97.1	2.4	0.06

- 1 The formulated percent adjusted for the weight difference between batter and baked goods due to moisture loss.
- 2 The amount of AEM detected in final product relative to amount added before baking (corrected for AEM placebo spike recovery).
- 3 The amount of DKP detected in final product relative to amount of AEM added before baking (corrected for AEM placebo spike recovery and, as applicable, for chromatographic interference).
- 4 The concentration of DKP in mg/g.

Analytical Results for Aspartame and Diketopiperazine
From Baked Goods after Differing Storage Conditions

Product/ Storage Conditions	Formulated ARM in percent	Percent Recovery		DKP ³ (Mg/g)
		ARM ¹	DKP ²	
A. Cheesecake	0.25			
Initial		95.3	4.0	0.09
45°F 5 days		103.1	5.8	0.14
9 days		89.8	1.5	0.10
15 days		87.9	4.2	0.10
70°F 5 days		99.0	8.0	0.19
9 days		86.1	3.4	0.15
15 days		80.0	8.6	0.20
90°F 5 days		92.5	12.2	0.29
B. Cookie	0.35			
Initial		95.7	2.7	0.09
-5°F 4 weeks		103.0	3.0	0.10
12 weeks		92.0	3.5	0.12
32 weeks		99.3	2.8	0.10
36 weeks		99.1	3.2	0.11
70°F 4 weeks		103.8	2.9	0.10
8 weeks		100.1	3.1	0.11
12 weeks		96.4	3.6	0.12
16 weeks		97.4	3.0	0.11
20 weeks		92.8	3.0	0.11
32 weeks		91.0	4.3	0.15
36 weeks		96.8	3.3	0.11
90°F 4 weeks		101.7	3.6	0.12
8 weeks		103.5	4.2	0.14
12 weeks		94.3	4.6	0.15
16 weeks		100.4	4.6	0.15
20 weeks		97.1	4.5	0.15
32 weeks		96.2	4.5	0.15
36 weeks		99.5	4.5	0.15

(continued)

Analytical Results for Aspartame and Diketopiperazine
From Baked Goods after Differing Storage Conditions

Product/ Storage Conditions	Formulated AFM in percent	Percent Recovery		DKP ³ (Mg/g)
		AFM ¹	DKP ²	
<hr/>				
C. Yellow Cake	0.25			
Initial		90.8	6.8	0.17
45°F 4 days		87.5	10.4	0.26
6 days		89.9	9.1	0.22
8 days		88.5	7.7	0.19
11 days		88.4	7.9	0.20
14 days		81.8	10.2	0.25
18 days		86.2	9.7	0.24
70°F 4 days		84.4	9.9	0.25
6 days		84.6	12.3	0.31
8 days		83.6	12.5	0.31
11 days		80.3	13.7	0.34
14 days		73.2	14.8	0.36
80°F 4 days		75.0	17.3	0.43
6 days		72.6	15.3	0.38
D. Chocolate Cake	0.25			
Initial		83.7	10.3	0.24
45°F 4 days		78.4	24.9	0.60
10 days		77.5	16.8	0.40
15 days		73.3	18.3	0.44
70°F 4 days		74.0	30.2	0.73
10 days		72.3	28.2	0.68
15 days		55.8	39.6	0.95
90°F 4 days		73.0	11.8	0.73
E. Lemon Pie Filling	0.25			
Initial		107.2	2.7	0.07
45°F 8 days		98.7	3.2	0.08
13 days		103.9	3.4	0.09
70°F 8 days		101.1	3.9	0.10
13 days		103.7	4.4	0.11
90°F 8 days		95.2	6.5	0.16

(continued)

Analytical Results for Aspartame and Diketopiperazine
From Baked Goods after Differing Storage Conditions

Product/ Storage Conditions	Formulated AEM in percent	Percent Recovery		DKP ³ (mg/g)
		AEM ¹	DKP ²	
F. Fig Bars	0.30			
Initial		90.8	1.0	0.02
45°F 2 weeks		88.6	0.2	0.003
4 weeks		114.0	2.2	0.03
6 weeks		115.2	2.3	0.03
8 weeks		112.2	1.2	0.02
10 weeks		93.1	1.0	0.01
12 weeks		100.0	0.9	0.01
16 weeks		122.6	1.1	0.02
21 weeks		120.5	1.8	0.03
24 weeks		105.6	2.5	0.04
70°F 2 weeks		91.8	0.3	0.004
4 weeks		95.3	2.3	0.04
6 weeks		94.4	1.9	0.03
8 weeks		98.3	2.0	0.03
10 weeks		93.0	1.5	0.02
12 weeks		99.0	1.4	0.02
16 weeks		81.2	1.8	0.02
21 weeks		83.6	2.6	0.04
24 weeks		89.9	4.3	0.07
90°F 2 weeks		78.7	0.4	0.006
4 weeks		84.3	2.8	0.04
6 weeks		93.1	3.5	0.05
8 weeks		92.3	3.4	0.05
10 weeks		87.2	3.0	0.05
12 weeks		63.0	3.0	0.05
14 weeks		81.4	4.7	0.07
16 weeks		69.4	4.6	0.07
21 weeks		80.6	7.0	0.11
24 weeks		73.1	6.8	0.10

- 1 The amount of AEM detected in final product relative to amount added before baking (corrected for AEM placebo spike recovery).
- 2 The amount of DKP detected in final product relative to amount of AEM added before baking (corrected for AEM placebo spike recovery and, as applicable, for chromatographic interference).
- 3 The concentration of DKP in mg/g.

Typical Formula
Fig Bars

	<u>Ingredients</u>	<u>Approximate Percentage</u>
Filling:		
	Fig paste	55
	Cracker crumbs	3
	Dextrose	15
	Baker's sugar	18
	High fructose corn syrup	8
	Citric acid	0.2
	Salt	0.5
	Encapsulated AFM (21% AFM)	1.4
Cookie:		
	Powdered sugar	19
	Salt, bicarbonates	1
	Shortening	14
	High fructose corn syrup	2
	Whole eggs	6
	Whole milk	9
	Flour	50
Process:		
	o The cookie dough and filling were independently mixed	
	o The fig bars were formed in a pilot size extruder (The extrusion process resulted in random cookie to fig filling ratio)	
	o The fig bars were baked in a tunnel oven for ten minutes	
	o Storage was in bags	

**Analytical Results of Aspartame and Diketopiperazine
from Baked Goods Exposed to Different Heating Conditions**

Product ¹ Storage Temperature	Final ² Temperature	Oven ³ Time	Percent Recovery		DKP ⁶ (Mg/g)
			ARM ⁴	DKP ⁵	
A. Pie Filling					
Initial/RT	RT	N.Ap.	93.7	2.4	0.06
RT	95°F	M 10 sec.	96.7	2.6	0.07
RT	160°F	M 21 sec.	92.7	2.4	0.06
RT	195°F	M 30 sec.	92.1	2.2	0.06
Refrigerator	100°F	M 16 sec.	91.4	2.0	0.05
Refrigerator	160°F	M 29 sec.	96.8	2.2	0.06
Refrigerator	195°F	M 39 sec.	97.1	2.4	0.07
RT	90°F	1.5 min.	93.1	1.7	0.05
RT	160°F	5.5 min.	97.6	2.0	0.06
RT	195°F	8.0 min.	94.3	4.2	0.17
Refrigerator	90°F	2.0 min.	96.5	2.3	0.07
Refrigerator	160°F	6.5 min.	97.0	1.9	0.06
Refrigerator	195°F	9.0 min.	93.7	2.2	0.08
B. Chocolate Cake					
Initial/RT	RT	N.Ap.	87.7	9.4	0.22
RT	96°F	M 6 sec.	92.2	9.9	0.23
RT	163°F	M 17 sec.	82.0	10.8	0.26
RT	194°F	M 24 sec.	91.9	6.8	0.16
Refrigerator	95°F	M 10 sec.	86.2	8.8	0.21
Refrigerator	163°F	M 22 sec.	89.5	11.3	0.27
Refrigerator	195°F	M 31 sec.	84.3	13.9	0.33
Initial/RT	RT	N.Ap.	89.7	2.7	0.06
RT	90°F	2 min.	87.8	2.6	0.06
RT	160°F	3.8 min.	89.6	4.0	0.10
RT	195°F	5.5 min.	88.3	2.8	0.07
Initial/Refrigerator	RT	N.Ap.	85.8	4.0	0.09
Refrigerator	90°F	3.0 min.	83.2	3.8	0.09
Refrigerator	160°F	5.0 min.	84.2	4.0	0.10
Refrigerator	197°F	8.5 min.	80.3	3.0	0.07

1 RT = room temperature

2 Oven temperatures or, for microwave samples, product temperatures

3 M = microwave

4 The amount of ARM detected in final product relative to amount added before baking (corrected for ARM placebo spike recovery).

5 The amount of DKP detected in final product relative to amount of ARM added before baking (corrected for ARM placebo spike recovery and, as applicable, for chromatographic interference).

6 The concentration of DKP in mg/g.