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Photodecomposition of aspartame in aqueous solutions

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Photodecomposition of aspartame in aqueous solutions under different conditions of light intensity and pH were studied. The effects of photosensitizers (riboflavin and methylene blue) and ascorbic acid on aspartame stability in aqueous solutions at different pH under light were also studied. Light illumination significantly increased aspartame degradation in an aqueous solution (pH 7), indicating that aspartame was very unstable under the illuminated conditions. In the dark, 91% of aspartame in an aqueous solution at pH 7 remained after 10 h of storage. Under 5500 lx of light, however, 39% of aspartame in the solution was destroyed after 10 h of storage. Aspartame degradation under light followed simple zero-order reaction kinetics. The higher the light intensity, the greater the degradation of aspartame. The relative reaction rate for the destruction of aspartame under 0, 1100, 3300 and 5500 lx was 1:1·42:2·80:4·61. The photodecomposition rate of aspartame varied with the pH of the system. Aspartame degradation was fastest at pH 7·0, followed by pH 4·0 and pH 6·0, in decreasing order. Addition of 4·8 ppm riboflavin or 4·8 ppm methylene blue significantly accelerated the aspartame decomposition at pH 7 in the presence of light. There were, however, no significant photosensitizing effects of these sensitizers on aspartame destruction at pH 6 and pH 4. Addition of $1{\cdot}2\times10^{-4}$ M ascorbic acid greatly increased the aspartame degradation at pH 7·0, but did not affect the destruction rate of aspartame at pH 6·0 and pH 4·0. © 1996 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Aspartame, the methyl ester of the dipeptide aspartylphenylalanine, is a nutritive substance with intensely sweet taste and flavour-enhancing properties. Aspartame is about 180 times sweeter than sucrose and exhibits a sugar-like taste without bitter or metallic after-taste. The stability of aspartame in dry-product applications is relatively good. However, aspartame has limited stability in aqueous solutions. It was reported that the decomposition of aspartame followed simple first-order kinetics and the decomposition products had no sweet taste (Prudel & Davidkova, 1981; Holmer, 1984; Bell & Labuza, 1991).

The pH of the system greatly affected the thermal stability of aspartame in aqueous solutions (Prudel & Davidkova, 1981; Özol, 1986). Prudel and Davidkova (1981) showed that aspartame in citrate–phosphate buffer solutions was most stable over the pH range 4–5, becoming less stable as the pH increased or decreased. Özol (1986) reported that aspartame was most stable between pH 4 and 5, decreasing in stability under more acidic and neutral conditions. Bell and Labuza (1991) reported that aspartame in intermediate and low moisture model systems was most stable at pH 5 and become less stable as the pH decreased or increased.

Temperature was also an important factor for the stability of aspartame during storage. As the temperature increased for a given storage time, the amount of aspartame remaining unconverted decreased (Holmer, 1984; Prudel *et al.*, 1986; Fellows *et al.*, 1991; Tsoubeli & Labuza, 1991). Fellows *et al.* (1991) reported that increased temperature caused fast degradation of aspartame in fruit preparations used in yoghurt.

Aromatic amino acids (phenylalanine, tryptophan, tyrosine), histidine and sulphur-containing amino acids (cysteine, cystine and methionine) in both free and peptide forms are reportedly sensitive to photosensitized oxidation (Weil, 1965; Matheson & Lee, 1979; Rosen-

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thal, 1985; Kanner & Fennema, 1987). Many foods naturally contain riboflavin, chlorophylls and their derivatives, which are effective photosensitizers for the formation of singlet oxygen under light (Jung & Min, 1991; Jung *et al.*, 1995*a*). Thus, we expected that aspartame, a methyl ester of aspartidylphenylalanine, might be easily destroyed and its sweet taste would be decreased by light illumination. No research, however, has been reported on the destructive properties of aspartame under light, even though the effects of pH and temperature on aspartame stability under dark conditions have been well documented. The photodegradation of aspartame might be affected by the presence of ascorbic acid in the system since ascorbic acid is easily oxidized under photosensitizing conditions and thus effectively quenches singlet oxygen (Rooney, 1983; Jung *et al.*, 1995*a*,*b*).

The objectives of this research were to study (1) the effects of different light intensity and pH on the aspartame stability in aqueous solutions during storage under light and (2) the effects of photosensitizers (riboflavin and methylene blue) and ascorbic acid on the photodestruction of aspartame in aqueous solutions.

MATERIALS AND METHODS

Effects of light intensity and pH on aspartame stability

To study the effects of light on aspartame stability, $1{\cdot}2\times10^{-3}$ M aspartame in 0·01 M phosphate buffer (pH 7·0) was prepared, and 20 ml portions were transferred to 30 ml serum bottles. The bottles were covered with rubber septa and placed in a light storage box which has been previously described in detail (Fakourelis *et al.*, 1987; Jung *et al.*, 1991, 1995*a*). To study the effects of light intensities, the prepared samples were stored under different light intensities (0, 1100, 2200, 3300 and 5500 lx). The light intensity was controlled by adjusting the number of fluorescent lamps that were switched on and the distance between the sample level and the fluorescent lamps within the light storage box. The temperature within the light storage box was 25 ± 3°C. To study the effects of pH of the solution on aspartame stability under light, $1{\cdot}2\times10^{-3}$ M aspartame solutions in 0·01 M phosphate buffers (pH 7·0, pH 6·0 and pH 4·0) were prepared and stored under fluorescent light (5500 lx). Aspartame stability in the solutions was monitored using high-performance liquid chromatography (HPLC) (Fellows *et al.*, 1991).

Effects of photosensitizers on aspartame stability under light

To study the effects of photosensitizers on aspartame stabilities under light, 0 ppm and 4·8 ppm solutions of riboflavin or methylene blue and $1{\cdot}2\times10^{-3}$ M aspartame in 0·01 M phosphate buffer (pH 7·0, 6·0 or 4·0) were prepared. As above, 20 ml amounts of prepared sample were transferred to 30 ml serum bottles. The bottles were covered with rubber septa and placed in the light box as described before. The light intensity at the sample level was 5500 lx.

Effects of ascorbic acid on the photodecomposition of aspartame

To study the effects of ascorbic acid on the photodecomposition of aspartame, solutions of $1{\cdot}2\times10^{-4}$ M ascorbic acid and $1{\cdot}2\times10^{-3}$ M aspartame in 0·01 M phosphate buffer (pH 7·0, 6·0 or 4·0) containing 0 ppm and 4·8 ppm riboflavin or methylene blue were prepared. As above, 20 ml amounts of the prepared samples were transferred to serum bottles and covered with rubber septa. The bottles were placed in the light storage box. The light intensity at the sample level was 5500 lx.

HPLC analysis of aspartame

Aspartame was determined by the HPLC method of Fellows *et al.* (1991). The column used was μ-Bondapak C-18 (30 cm×3·9 mm; Waters Associates, Milford, MA). The mobile phase (flow rate 1·0 ml min^{-1}) was aqueous 0·0125 M monosodium phosphate buffer (adjusted to pH 3·5 with 85% phosphoric acid) and acetonitrile 10:90 (v/v). The mixture was passed through a 0·45 μm membrane filter and then degassed by sonication under vacuum. At predetermined time intervals, aliquots (2 ml) of each sample solution were removed and immediately cooled for 2 min in an ice box, and then stored at 4°C to inhibit further destruction until analysis was completed. The aspartame contents in solution were calculated using a standard curve.

Statistical analysis

All the experiments were done in duplicate. Statistical analysis was done using SAS methods (Statistical Analysis Systems Institute, Inc., 1985). Duncan's multiple range test was used to ascertain the treatment effects on aspartame stability (Kim *et al.*, 1995).

RESULTS AND DISCUSSION

Effects of light intensity on the photodecomposition of aspartame

Figure 1 shows the effects of light intensity on the photodecomposition of aspartame during storage. The results clearly showed that light illumination greatly increased the degradation of aspartame during storage and that aspartame stability significantly decreased as the light intensity increased ($P<0{\cdot}05$). These data

clearly demonstrate, for the first time, the self-sensitized photodegradable properties of aspartame. The instability of aspartame under light has not been previously reported in the literature. As judged by all the authors, there was noticeable decrease in the sweet taste in the samples stored under light, even though no systematic sensory analyses of the samples by the trained members of panel were conducted to accurately measure the changes in intensity. This result indicated that photodecomposed products of aspartame had less or no sweet taste.

The degradation of aspartame followed a zero-order reaction kinetics as shown in Fig. 1. It is interesting to note that the kinetics for aspartame degradation under light were different from those reported under dark and high temperature conditions. It has been reported that aspartame degradation, as a function of temperature, followed first-order reaction kinetics (Tsoubeli & Labuza, 1991; Bell & Labuza, 1991; Prudel *et al.*, 1986). The present results suggest that the photodestruction of aspartame might follow a different pathway from thermal destruction. The involvement of active oxygen species such as singlet oxygen or superoxide anion radicals might be possible, but this was not clear in this self-sensitized photodecomposition of aspartame. However, further investigation of the decomposition products of aspartame after light storage should be followed to verify this hypothesis.

The calculated rate constants (k) for the photodegradation of aspartame under 0, 1100, 3300 and 5500 lx were $-1{\cdot}057\times10^{-5}$, $-1{\cdot}500\times10^{-5}$, $-2{\cdot}957\times10^{-5}$ and $-4{\cdot}871\times10^{-5}$ mol litre^{-1} h^{-1}, respectively. The relative reaction rates for the degradation of aspartame under 0, 1100, 3300 and 5500 lx were 1:1·42:2·80:4·61. The calculated times required to destroy half of the initial aspartame content ($1{\cdot}2\times10^{-3}$ mol litre^{-1}) under 0, 1100, 3300 and 5500 lx were 113·81, 88·47, 40·78 and 12·41 h, respectively.

Effects of pH on the photodecomposition of aspartame

Figure 2 shows the effects of pH on the photodecomposition of aspartame during storage. Aspartame degradation under the conditions of all tested pHs followed zero-order reaction kinetics. The rate constants (k) for the photodegradation of aspartame at pH 4·0, 6·0 and 7·0 under 5500 lx were $-2{\cdot}143\times10^{-5}$, $-1{\cdot}886\times10^{-5}$ and $-4{\cdot}871\times10^{-5}$ mol litre^{-1} h^{-1}, respectively. That is, the stability of aspartame against photodestruction is greatly dependent on the pH of the system, the stability of aspartame under light being greatest at pH 6, followed by pH 4 and 7, in a decreasing order. It is interesting to note that the photodecomposition of aspartame was greatly accelerated by slightly changing the pH of the system from pH 6 to 7. The photodegradation of aspartame at pH 7 was 2·58 times faster than at pH 6. It was also interesting to note that the pH-dependent properties of aspartame stability under light were the same as under high temperature but dark conditions. However, the thermal stability of aspartame in aqueous solutions was reportedly greater at pH 4 than at pH 6 (Prudel & Davidkova, 1981; Özol, 1986). Prudel and Davidkova (1981) showed that aspartame in citrate–phosphate buffer solutions was most stable over the pH range 4–5, becoming less stable as pH increased or decreased. Özol (1986) demonstrated that aspartame was most stable between pH 4 and 5, decreasing in stability under more acidic and neutral conditions.

Effects of photosensitizers

The photodecomposition of $1{\cdot}2\times10^{-3}$ M aspartame in phosphate buffer (pH 7·0) with/without riboflavin or methylene blue is shown in Fig. 3. The results clearly show that the addition of 4·8 ppm riboflavin or 4·8 ppm methylene blue greatly increased the photodestruction of aspartame in an aqueous solution at pH 7. The

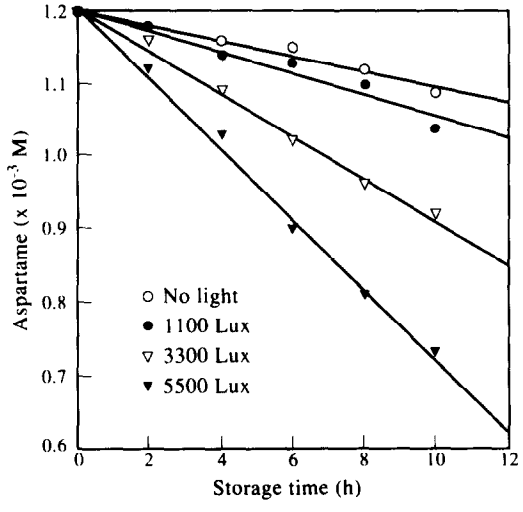

Fig. 1. Effects of light intensity on the stability of aspartame in phosphate buffer (pH 7) during light storage.

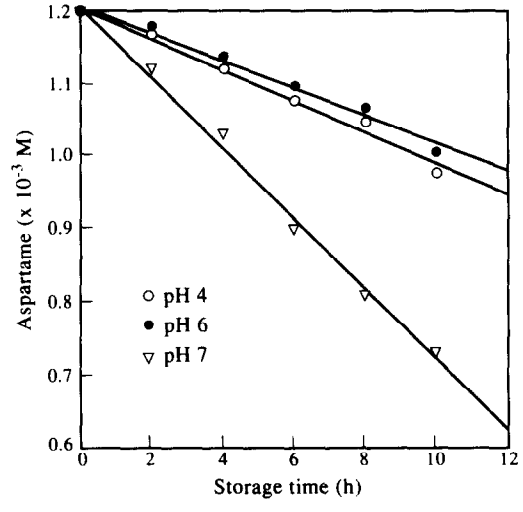

Fig. 2. Effects of different pHs on the stability of aspartame in phosphate buffer during light storage at 5500 lx.

aspartame contents in solutions at pH 7·0 after 10 h of light storage with 0 ppm sensitizer, 4·8 ppm riboflavin and 4·8 ppm methylene blue, decreased from $1·2\times10^{-3}$ M to $0·73\times10^{-3}$, $0·43\times10^{-4}$ and $0·38\times10^{-4}$ M, respectively. That is, riboflavin and methylene blue were effective sensitizers for the destruction of aspartame in aqueous solution at pH 7, and methylene blue had a better sensitizing activity for the destruction of aspartame than riboflavin under the same conditions.

Figures 4 and 5 show the effects of riboflavin or methylene blue on the photodestruction of $1·2\times10^{-3}$ M aspartame in phosphate buffer of pH 6·0 and 4·0, respectively. Riboflavin and methylene blue slightly increased the photodestruction rates of aspartame in solution at pH 6·0 and 4·0. The contents of aspartame in solutions at pH 6·0 containing 0 ppm sensitizer, 4·8 ppm riboflavin and 4·8 ppm methylene blue after 15 h of light storage were $1·01\times10^{-3}$, $0·97\times10^{-3}$ and $0·99\times10^{-3}$ M, respectively. The contents of aspartame in solutions at pH 4·0 containing 0 ppm sensitizer, 4·8 ppm riboflavin and 4·8 ppm methylene blue were $0·97\times10^{-3}$, $0·95\times10^{-3}$ and $0·97\times10^{-3}$ M, respectively. Even though the addition of sensitizers slightly increased the destruction of aspartame, there were no significant differences between the tested samples ($P>0·05$). That is, addition of ascorbic acid did not significantly increase the photodestruction of aspartame in aqueous solutions at pH 4 and 6.

The present results clearly show that the photosensitizing activities of riboflavin and methylene blue were greatly dependent on the pH of the system. The photosensitizing activities of both riboflavin and methylene blue for the destruction of aspartame were high at pH 7, but no significant photosensitizing activities were found at pH 6 and 4.

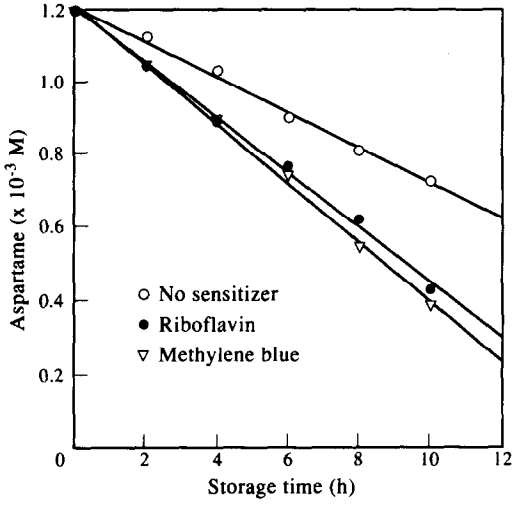

Fig. 3. Effects of 4.8 ppm riboflavin and 4.8 ppm methylene blue on the stability of aspartame in phosphate buffer (pH 7) during light storage at 5500 lx.

Effects of ascorbic acid on the photodecomposition of aspartame

The effect of ascorbic acid on the photodecomposition of aspartame was studied since the sweetener is commonly used as a substitute for sugar in beverages that contain ascorbic acid. Ascorbic acid, due to its singlet oxygen quenching ability, was expected to lower the photodecomposition of aspartame. However, this was not the case. The effects of $1·2\times10^{-4}$ M ascorbic acid on the photodegradation of $1·2\times10^{-3}$ M aspartame in phosphate buffer pH 7·0 containing 0 ppm sensitizer, 4·8 ppm riboflavin or 4·8 ppm methylene blue during light storage are shown in Table 1. These results show that the addition of ascorbic acid accelerated the decomposition of aspartame, especially at the initial decomposition stage at pH 7·0. After 4 h of light storage in the absence of sensitizer, for example, the destruction of aspartame in solutions containing 0 and $1·2\times10^{-4}$ M ascorbic acid was 14·2% and 31·7%,

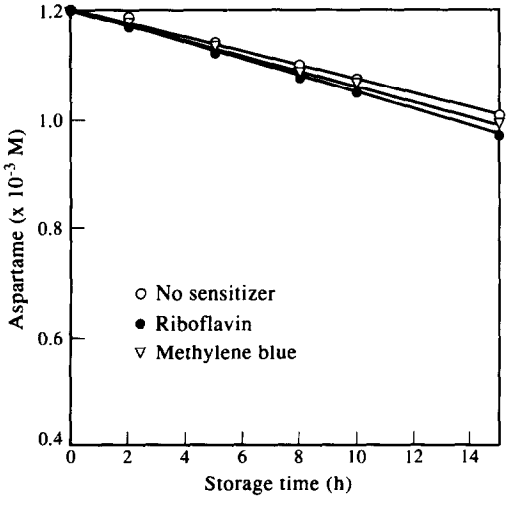

Fig. 4. Effects of 4.8 ppm riboflavin and 4.8 ppm methylene blue on the stability of aspartame in phosphate buffer (pH 6) during light storage at 5500 lx.

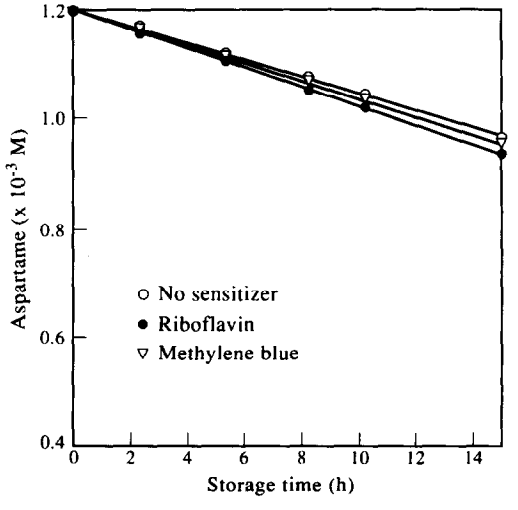

Fig. 5. Effects of 4.8 ppm riboflavin and 4.8 ppm methylene blue on the stability of aspartame in phosphate buffer (pH 4) during light storage at 5500 lx.

respectively. At the later stage of light storage, however, the aspartame contents in both systems became similar regardless of the presence of ascorbic acid in the system. That is, ascorbic acid greatly accelerated photodestruction of aspartame at the initial stage of light storage, but not at the later stage. The accelerated photodecomposition of aspartame by ascorbic acid was unexpected since ascorbic acid is reportedly an effective singlet oxygen quencher (Chou & Khan, 1983; Bodannes & Chan, 1979; Jung *et al.*, 1995*a*). Kanner and Fennema (1987) also reported that riboflavin-sensitized photo-oxidation of tryptophan was greatly reduced by ascorbic acid. The possible explanation that can be offered is that ascorbic acid was rapidly oxidized under light illumination conditions and thus produced radicals. These radicals might accelerate aspartame destruction at the initial stages of light storage. But, at the later stage, no ascorbic acid was available to produce radicals that were very reactive. Jung *et al.* (1995*a,b*) reported that ascorbic acid in aqueous solution was easily oxidized in the presence of light and sensitizers (riboflavin or methylene blue), and that the oxidation rates of ascorbic acid increased greatly as the pH increased from pH 4·5 to 7·5. The authors also reported that ascorbic acid ($1{\cdot}2\times10^{-4}$ mol litre^{-1}) in an aqueous solution at pH 7 is completely destroyed within 15 min in the presence of 3·6 ppm riboflavin and 3300 lx of fluorescence light. It is interesting to note that aspartame photodegradation in an aqueous solution (pH 7) in the presence of ascorbic acid did not follow zero-order reaction kinetics due to the greatly increased photodegradation of aspartame by the ascorbic acid at the initial stage only.

The addition of ascorbic acid at lower pH, however, did not affect the photodestruction rate of aspartame during 10 h of storage under 5500 lx. Tables 2 and 3 show the effects of $1{\cdot}2\times10^{-4}$ M ascorbic acid on the photodegradation of $1{\cdot}2\times10^{-3}$ M aspartame in phosphate buffer of pH 6·0 and 4·0, respectively. The results in Tables 1–3 indicate that the effects of ascorbic acid on the photodestruction of aspartame is also dependent on the pH. Ascorbic acid accelerated the photodestruction of aspartame at pH 7·0, but did not affect the photodestructive stability of aspartame at pH 6·0 and 4·0. This result might be explained by the fact that relatively slow oxidation of ascorbic acid at pH 6·0 and 4·0 might not produce enough radicals to promote aspartame destruction in the system (Jung *et al.*, 1995*a,b*).

Based on the present results, it is not recommended that riboflavin-containing beverages with pH 7 be sweetened with aspartame as a sugar substitute, especially when the products are sold in transparent glass bottles, because light exposure of the products during manufacturing and handling is inevitable. If there are no alternatives to aspartame as a sugar substitute for the beverages with neutral pH, products on the shelves should be protected from direct sunlight or fluorescent light by using coloured glass bottles or other light-protected packaging materials. It might also be beneficial to food manufacturers in related fields to note that the stability of aspartame under light could be dramatically increased just by slightly changing the pH of the food system from pH 7 to pH 6. Further investigations are needed on the mechanism of the photodestruction of

Table 1. Effects of $1{\cdot}2\times10^{-4}$ M ascorbic acid on the photodestruction of $1{\cdot}2\times10^{-3}$ M aspartame in phosphate buffer (pH 7·0) containing no sensitizer, 4·8 ppm riboflavin or 4·8 ppm methylene blue during light storage

Storage	Aspartame content ($\times10^{-3}$ M) during light storage					
	No sensitizer		Riboflavin		Methylene blue	
Time (h)	None[a]	AA[b]	None[a]	AA[b]	None[a]	AA[b]
0	1·20	1·20	1·20	1·20	1·20	1·20
2	1·12	0·96	1·04	0·93	1·03	0·88
4	1·03	0·82	0·90	0·78	0·88	0·80
6	0·90	0·77	0·77	0·70	0·74	0·71
8	0·81	0·74	0·62	0·56	0·54	0·58
10	0·73	0·71	0·43	0·45	0·38	0·43

[a]None: system containing no ascorbic acid.
[b]AA: system containing $1{\cdot}2\times10^{-4}$ M ascorbic acid.

Table 2. Effects of $1{\cdot}2\times10^{-4}$ M ascorbic acid on the photodestruction of $1{\cdot}2\times10^{-3}$ M aspartame in phosphate buffer (pH 6·0) containing no sensitizer, 4·8 ppm riboflavin or 4·8 ppm methylene blue during light storage

Storage	Aspartame content ($\times10^{-3}$ M) during light storage					
	No sensitizer		Riboflavin		Methylene blue	
Time (h)	None[a]	AA[b]	None[a]	AA[b]	None[a]	AA[b]
0	1·20	1·20	1·20	1·20	1·20	1·20
2	1·18	1·14	1·17	1·15	1·17	1·14
4	1·14	1·10	1·12	1·10	1·13	1·09
6	1·10	1·07	1·08	1·06	1·08	1·05
8	1·07	1·06	1·05	1·03	1·06	1·03
10	1·01	1·02	0·97	0·98	0·99	0·97

[a]None: system containing no ascorbic acid.
[b]AA: system containing $1{\cdot}2\times10^{-4}$ M ascorbic acid.

Table 3. Effects of $1{\cdot}2\times10^{-4}$ M ascorbic acid on the photodestruction of $1{\cdot}2\times10^{-3}$ M aspartame in phosphate buffer (pH 4·0) containing no sensitizer, 4·8 ppm riboflavin or 4·8 ppm methylene blue during light storage

Storage	Aspartame content ($\times10^{-3}$ M) during light storage					
	No sensitizer		Riboflavin		Methylene blue	
Time (h)	None[a]	AA[b]	None[a]	AA[b]	None[a]	AA[b]
0	1·20	1·20	1·20	1·20	1·20	1·20
2	1·17	1·19	1·16	1·18	1·17	1·18
4	1·12	1·14	1·12	1·14	1·12	1·14
6	1·08	1·09	1·06	1·08	1·07	1·09
8	1·05	1·06	1·03	1·05	1·05	1·05
10	0·98	0·97	0·95	0·95	0·96	0·96

[a]None: system containing no ascorbic acid.
[b]AA: system containing $1{\cdot}2\times10^{-4}$ M ascorbic acid.

aspartame in aqueous solutions in the absence and presence of sensitizers. Nevertheless, the results from this study should aid the food industry in the design and formulation of low-calorie beverages.

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