The Physicochemical Properties of *α-Sweet*

Kiyotaka OYAMA
Shigeaki IRINO
Eiji IWAMOTO

The physicochemical properties of *α-sweet*, a dipeptide sweetener aspartame produced by Toyo Soda, have been studied. The sweetener is most stable near pH 4, where the time required to lose 20% of initial sweetness is estimated to be about 1.6 years at 5°C on the basis of kinetic data. The activation energy and entropy of activation are 17 kcal/mol and −36 e. u., respectively. The latter value suggests the sweetener decomposes through a cyclic transition state, which is also consistent with the fact that the major decomposition product is a diketopiperazine (5-benzyl-3, 6-dioxo-2-piperazineacetic acid). The solubility of the sweetener in water is dependent on pH and it exhibits a maximum solubility near pH 2.3.

1. Introduction

α-Sweet is the Toyo Soda's trade name of aspartame (the generic name of α-L-aspartyl-L-phenylalanine methyl ester), the intense sweetener which is approximately 200 times sweeter than sugar. It has a pleasant sweetness without a bitter aftertaste. Because of the intense sweetness, *α-sweet* does not contribute significantly to a caloric intake, thus is very useful as a low caloric sweetener. The sweetener is composed of two naturally occuring essential amino acids, *i. e.*, aspartic acid and phenylalanine, the common ingredients of meat, milk, and other proteineous food stuffs. In the human body, the sweetener is digested and metabolized in the same way as proteins containing these amino acids. Although the safety of the sweetener may be reasonably predicted from the above stated facts, the toxicological studies, which are recognized to be the most extensive and comprehensive in the history of the approval procedure of food additives, have been conducted.[1] As the result, the safety was scientifically well proved, and the sweetener is now approved in more than 35 countries, including U. S. A., Canada, most of European nations, Australia, Japan, etc.

Since almost a decade ago, Toyo Soda has been carrying out the study to develop a new technology to produce the sweetener. Recently we succeeded to establish the highly sophisticated industrial process utilizing the biotechnology based on totally novel enzymatic reaction,[2] and we have started manufacturing and marketing our product under the trade name of *α-sweet*. In this paper, we report some physicochemical properties of *α-sweet*.

2. Experimental

[1] Materials

α-Sweet is obtained by Toyo Soda's enzymatic process. It is free from β-aspartame and contains 0.2% of diketopiperazine and a trace amount of α-L-aspartyl-L-phenylalanine. Reagents used as buffer systems are purchased from Wako Pure Chemicals and are analytical grades.

[2] Measurement of stability of *α-sweet*

A stoppered flask containing approximately 50 mg of *α-sweet* in 50 ml of aqueous buffer was placed in incubator thermostated at an appropriate temperature. An aliquot of the solution was withdrawn at intervals and was subjected to the measurement on a high performance liquid chromatography (h. p. l. c) to determine the amount of remaining *α-sweet.* A CH_3CO_2Na-HCl buffer system was employed for the pH range between 1 and 2, and a Na_2HPO_4-citric acid buffer system for the pH range between 3 and 8. The decomposition products were determined with h. p. l. c. using authentic compounds as standard.

[3] Measurement of solubility of *α-sweet*

The aqueous slurry containing *α-sweet* was subjected to the pH adjustment at a desired pH by the addition of dilute aqueous HCl or $NaHCO_3$ solution under stirring for 1 h. The slurry was filtered through a sintered glass filter (G2) and the amount of *α-sweet* in the filtrate was determined by h. p. l. c.

[4] H. p. l. c. analysis

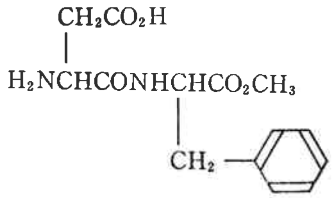
H. p. l. c. analyses were done on a HLC-803 (Toyo Soda Manufacturing Co.) equipped with a UV detector (254 nm) using a column packed with TSK-GEL G 2000 SW.

3. Results and Discussion

[1] General Properties

General Properties of *α-sweet* are as follows:

Structural formula;



; $C_{14}H_{18}N_2O_5$

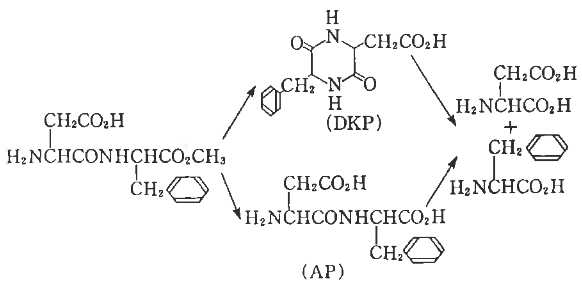
Molecular weight; 294. 3

Isoelectric point; pH 5. 2

Appearance ; Odorless white crystalline powder

[2] Stability

α-Sweet is stable in a dry form on storage at ambient temperature. In solution, however, it tends to decompose first to a cyclic diketopiperazine (DKP, or 5-benzyl-3, 6-dioxo-2-piperazineacetic acid), *α*-L-aspartyl-L-phenylalanine (AP), then to aspartic acid and phenylalanine, two amino acid components of *α-sweet*, as shown in **Scheme 1**.



Scheme 1 Decomposition of *α-Sweet*

The stability of *α-sweet* in solution is a function of pH, temperature, and time. The decomposition rate increases with temperatures. The

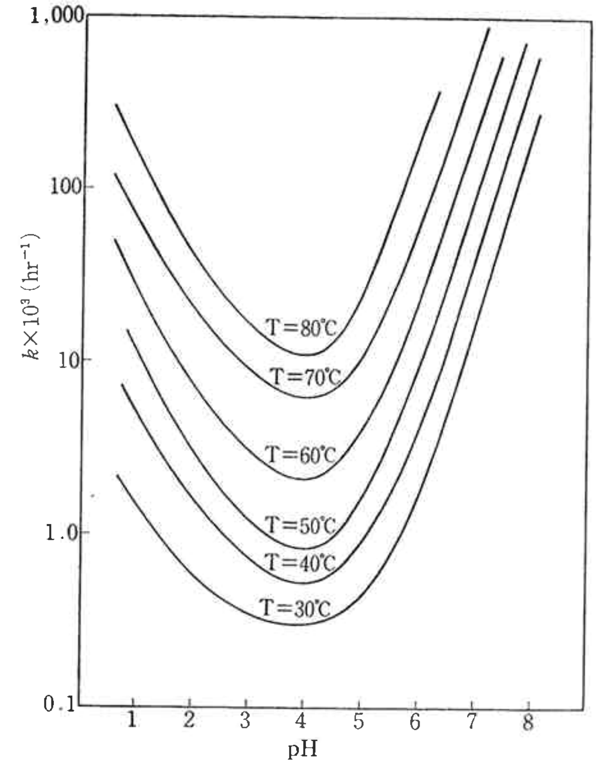


Fig. 1 Rate of *α-Sweet* Decomposition

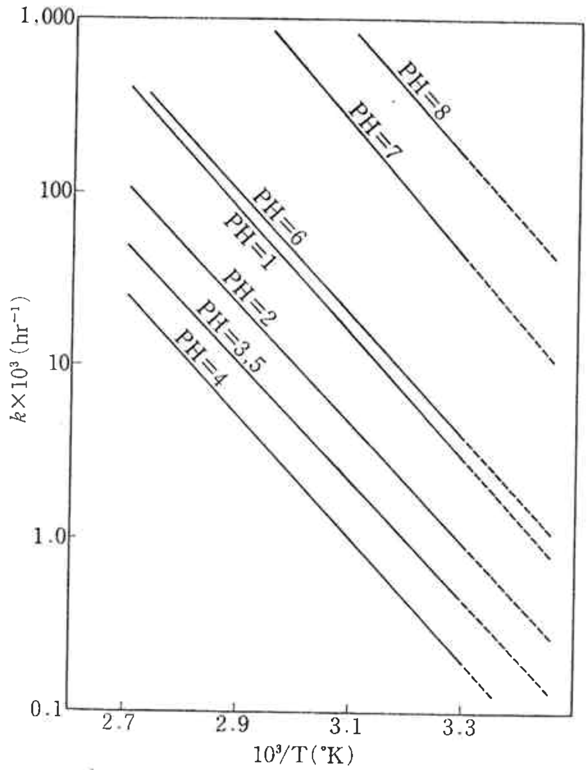
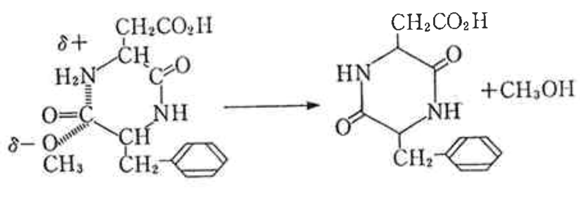


Fig. 2 Arrhenius Plots of the Decomposition Rates

maximum stability with respect to pH lies near pH 4. The stability decreases as the solution becomes more acidic or more basic than pH 4, the decomposition rate being faster in the latter region than the former. In an alkaline solution it decomposes considerably rapidly. (**Fig. 1**)

The Arrhenius plots of the decomposition rates are shown in **Fig. 2**, from which one can calculate the activation energy (ΔEa) and entropy of activation ($\Delta S^{\ddagger}$) to be 17 kcal mole^{-1} and −36 e. u., respectively. The large negative value of $\Delta S^{\ddagger}$ suggests that the decomposition reaction may proceed via a cyclic transition state,[3] which is consistent with the fact that the major product



Scheme 2 Transition State of Decomposition of *α-Sweet*

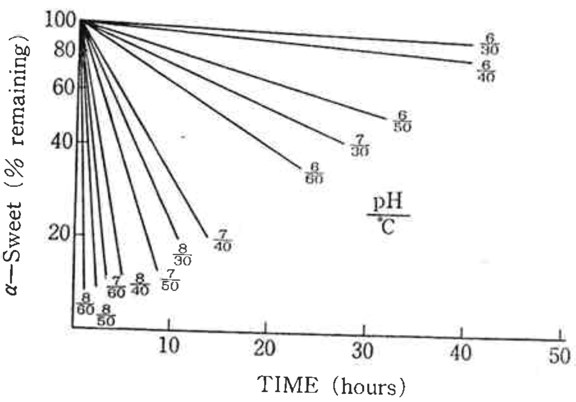


Fig. 3 Stability of *α-Sweet* in Aqueous Buffer

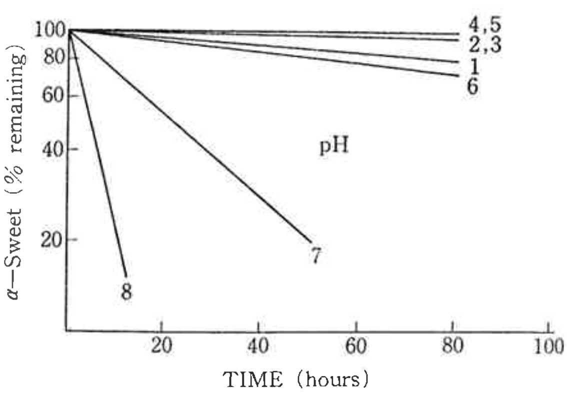


Fig. 4 Stability of *α-Sweet* in Aqueous Buffer at 30°C

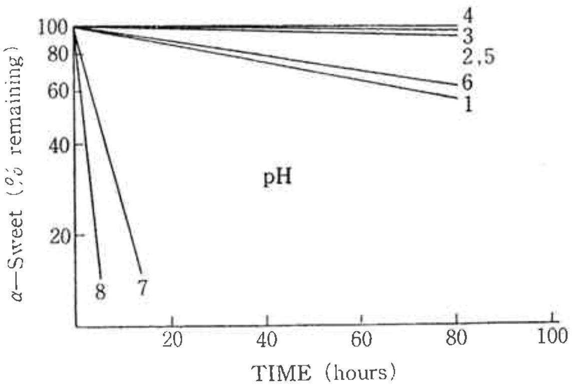


Fig. 5 Stability of *α-Sweet* in Aqueous Buffer at 40°C

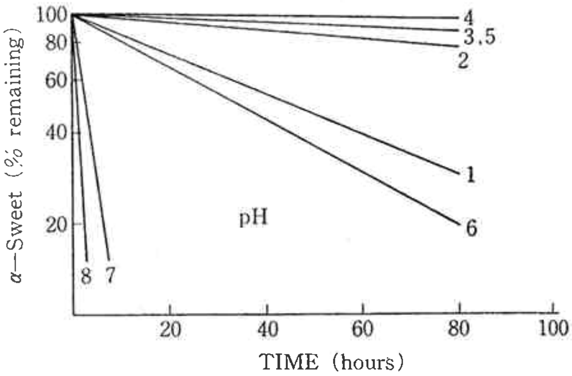


Fig. 6 Stability of *α-Sweet* in Aqueous Buffer at 50°C

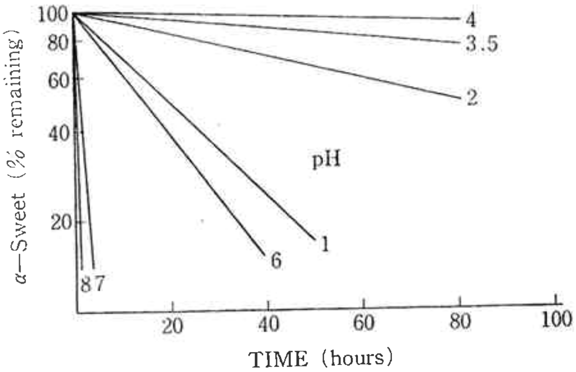


Fig. 7 Stability of *α-Sweet* in Aqueous Buffer at 60°C

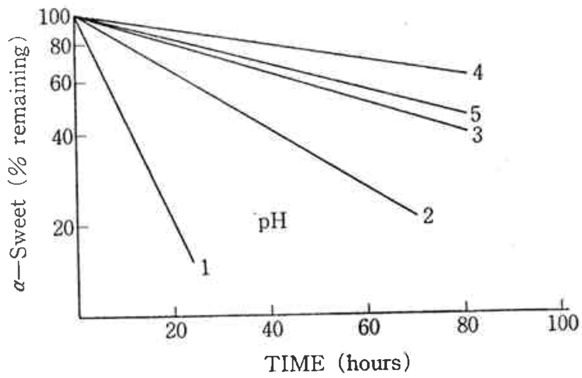


Fig. 8 Stability of *α-Sweet* in Aqueous Buffer at 70°C

is a diketopiperazine (**Scheme 2**).

The pH dependence of stability at different temperatures (30-80°C) are shown in **Fig. 3**–**Fig. 9** The time to lose 20% and 50% of initial sweetness of *α-sweet* at different pHs and temperatures are listed in Table 1 and 2, respectively. *α-Sweet* exhibits good stability at pH ranges of most carbonated soft drinks (pH 2-5). Under the conditions of storage in a refrigerator (5°C and pH 4), time to lose 20% and 50% of initial sweetness are 1.6 and 4.9 years respectively.

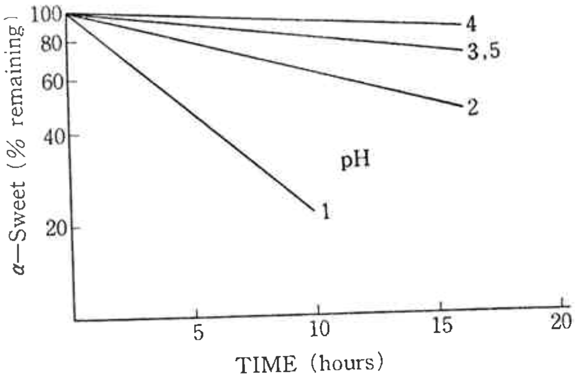


Fig. 9 Stability of *α-Sweet* in Aqueous Buffer at 80°C

[3] Solubility

Solubility of *α-sweet* in water is also pH and temperature dependent (**Fig. 10**). It exhibits a minimum solubility at pH 5.2 (isoelectric point) and a maximum at pH 2.3 as shown in **Fig. 11**.

4. Conclusion

As is apparent from the data shown above, *α-sweet* is satisfactorily stable under the conditions

Table 1 Time to lose 20% of the initial sweetness at varions pH and temperature (Hr)

pH	Temperature (°C)							
	5	15	30	40	50	60	70	80
1	890 (1.2M)	300 (13D)	150 (6.2D)	45 (1.9D)	20	8.6	3.3	1.4
2	2600 (3.6M)	890 (1.2M)	380 (16D)	140 (5.8D)	74 (3.1D)	31 (1.3D)	11	5.6
3	5600 (7.7M)	1900 (2.6M)	640 (27D)	300 (13D)	190 (7.7D)	74 (3.1D)	25 (1.0D)	14
4	14000 (1.6Y)	4600 (6.5M)	740 (1.0M)	420 (18D)	260 (11D)	100 (4.4D)	36 (1.5D)	20
5	5600 (7.7M)	1900 (2.6M)	500 (21D)	240 (10D)	130 (5.5D)	56 (2.3D)	20	9.3
6	620 (26D)	230 (9.4D)	130 (5.5D)	57 (2.4D)	24 (1.0D)	10	3.7	1.2
7	66 (2.7D)	22	15	5.7	2.4	10	0.37 (22 MIN)	
8	16	5.6	1.1	0.48 (29 MIN)				

Y; year, M; month, D; day, MIN; minute. The data at 5°C and 15°C are calculated from the Arrhenius plots (Fig. 2)

Table 2 Time to lose 50% of the initial sweetness at varions pH and temperature (Hr)

pH	Temperature (°C)							
	5	15	30	40	50	60	70	80
1	2800 (3.9M)	940 (1.3M)	460 (19D)	140 (5.8D)	63 (2.7D)	27 (1.1D)	10	4.3
2	8100 (11M)	2800 (3.9M)	1200 (1.7M)	430 (18D)	230 (9.6D)	95 (4D)	35 (1.4D)	17
3	17000 (2.0Y)	5800 (8.0M)	2000 (2.8M)	940 (1.3M)	580 (24D)	230 (9.7D)	77 (3.2D)	43 (1.8D)
4	43000 (4.9Y)	14000 (1.6Y)	2300 (3.2M)	1300 (1.8M)	830 (1.1M)	330 (14D)	110 (4.7D)	63 (2.6D)
5	17000 (2.0Y)	5800 (8.0M)	1500 (2.1M)	750 (1.0M)	410 (17D)	170 (7.2D)	63 (2.6D)	29 (1.2D)
6	1900 (2.6M)	700 (29D)	410 (17D)	180 (7.4D)	74 (3.1D)	32 (1.8D)	12	3.9
7	200 (8.5D)	69 (2.9D)	46 (1.9D)	17.8	7.4	3.2	1.2	0.39 (23 MIN)
8	50 (2.1D)	17	3.5	1.5	0.83 (50 MIN)	0.41 (24 MIN)		

Y; year, M; month, D; day, MIN; minute. The data at 5°C are calculated from the Arrhenius plots (Fig. 2)

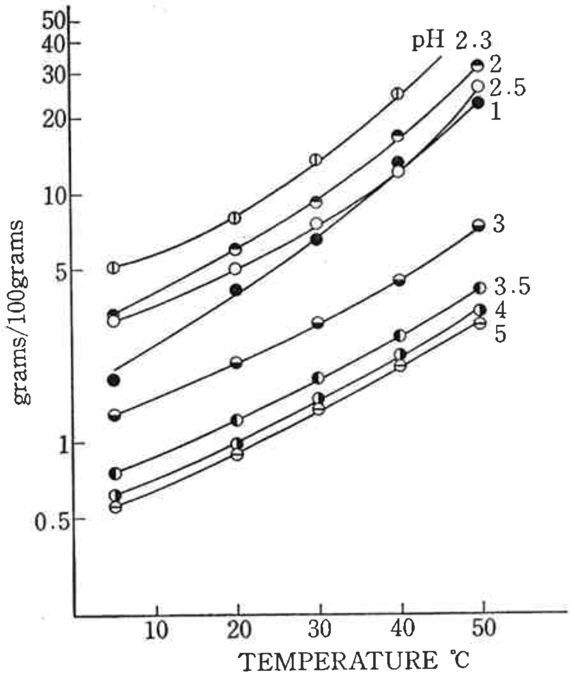


Fig10. Temperature Effect on *α-Sweet* Solubility

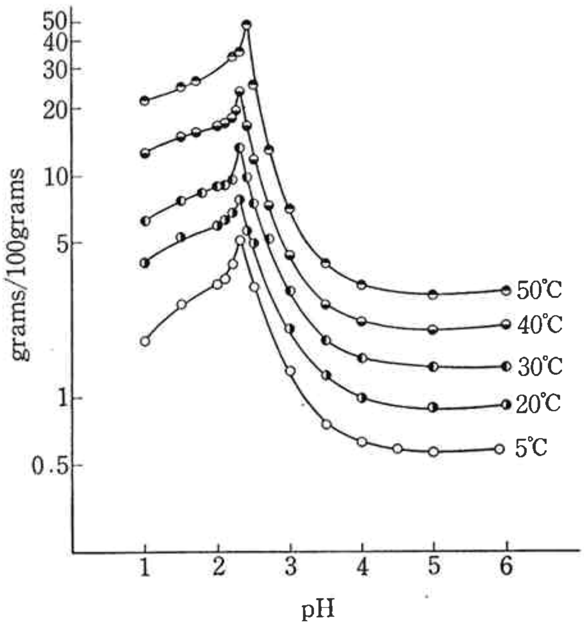


Fig.11 pH Effect on *α-Sweet* Solubility

encountered in many foods. Therefore, it can be said that *α-sweet* will find many applications in a variety of dry food, beverages, frozen and refrigerated desserts, dairy products, confections, etc. However, the use in cooking or baking that requires long heating is not recommened, since it tends to decompose in solution at higher temperatures. It can be satisfactorily used in applications where the duration of heating is relatively short.

References

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