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STUDIES ON THE STABILITY OF L-ASCORBIC ACID (II)

By

Susumu FUJIE*

Introduction

It has been studies by several groups of workers that some kinds of amino acids played the important roles as the stimulators or the inhibitors on the autoxidation of L-ascorbic acid (ASA) (1)~(4). ASA is universally distributed in the diets, especially in vegetables and fruits. At present, ASA is added to many diets not only for the purpose of the vitamin-enrichment, but for the another one of the protection of oxidation and the prevention of browning, because it has two actions, namely, the vitamin-action and the antioxidant-one.

On the other hand, amino acids are present in every diets as the components of protein or the free amino acids or the food additives. It appeared of interest to investigate, therefore, the effects of amino acids on the autoxidation of ASA containing in the natural or synthetic diets. In an earlier paper dealing with the autoxidation and the photoxidation of ASA, it was shown that the stability of ASA was greatly affected by pH (5).

The purpose of the work reported here has been to see the effects of every kinds of amino acids on the autoxidation of ASA under the various pH conditions, and to see the synergism of amino acids.

Experimental

Materials

All chemicals were analytical grade commercial materials and used without further purification.

* Department of Home Economics, Faculty of Education, Shimane University, Matsue, JAPAN

Determination method of ASA

ASA was determined by indophenol-buthanol method (6).

Procedures

The effects of amino acids on the oxidation of ASA under the various pH conditious ; Five milliliters of 12 mg% ASA in aqueous solution was added to 10 ml of each buffer solution ranging from pH 3 to 10, in which a prescribed amino acid was previously dissolved. The final concentration of each amino acid was 2×10^{-2} M. The mixture was allowed to stand by aeration at 50° for 60 minutes in the range from pH 3 to 7, or for 30 minutes in the case of pH 8 or above. Then, 5 ml of this mixture was added to 5 ml of 4 % methaphosphoric acid. After stirring sufficiently, 5 ml of this solution was taken as a sample solution and the remaining ASA was determined by measuring the optical density at 536 nm.

At the same time, the remaining ASA in non-amino acid group was determined with the same prodedure as a control experiment 1.

On the other hand, 5 ml of 12 mg% ASA in aqueous solution was diluted 3 times with buffer solution, and after 5 ml of this mixture was added to 5 ml of 4 % methaphosphoric acid the optical density was determined with the same procedure as a control experiment 2.

Moreover, the optical density was determined as a control experiment 3, when 5 ml of each buffer solution was used in stead of 5 ml of 12 mg% ASA in aqueous solution.

The standard curve of ASA at 536 nm. was prepared just the same as the previous paper.

The synergism of amino acids for the oxidation of ASA ; The mutual relations between the essential amino acids and the other ones were observed on the oxidation of ASA. To 10 ml of each buffer solution n which an essential amino acid and another non-essential one were previously dissolved, 5 ml of 12 mg% ASA in aqueous solution was added. The final concentration of each amino acid was 1×10^{-2} M, respectively. Then the remaining ASA was determined with the same procedure as mentioned above.

Results and Discussion

Effects of amino acids on the oxidation of ASA under the various pH conditions

The remaining ratios of ASA in the presence of the amino acid were determined under the various pH conditions, as the oxidation of ASA closely related to pH. It was already found that ASA was remarkably stable in the case of pH 2 or below (7), and unstable in the case of pH 11 or above (1). In this experiment, therefore, the effects of amino acids on the oxidation of ASA were observed in the pH range from 3 to 10. The results at the acid pH are given in Table 1.

It was recognized that some kinds of amino acids had accelerated the oxidation of ASA and the other kinds of them restrained as shown in Table 1. That is, aspartic acid, glutamic acid, methionine and tryptophan showed clearly the restraining-action for the oxidation of ASA, and in contrast with this, glycine, leucine, arginine, lysine and tyrosine showed fairly the accelerating-action for the oxidation.

These tendencies in both restraining- and accelerating- action were clearly recognized

Table 1 The remaining ASA under acidic solution in the presence of amino acids

Amino acid (L-form)	pH							
	3		4		5		6	
	(%)*	pH**	(%)*	pH**	(%)*	pH**	(%)*	pH**
Control	95.2	2.90	90.3	4.02	81.8	5.00	74.2	6.01
Gly.	92.4	3.17	88.2	4.07	72.3	5.08	69.1	6.01
Ala.	90.4	3.16	89.0	4.07	78.1	5.09	72.5	6.09
Ser.	88.2	3.10	80.2	4.05	78.2	5.06	70.5	6.07
Thr.	94.6	3.12	92.6	4.06	80.5	5.07	75.8	6.08
Val.	94.3	3.13	93.2	4.05	81.2	5.06	71.6	6.01
Leu.	90.3	3.15	85.2	4.05	73.2	5.05	68.2	6.00
Iso-leu.	92.6	3.10	88.7	4.08	80.0	5.06	73.2	6.08
Asp.	94.7	3.02	92.2	3.84	89.7	4.78	82.1	4.92
Glu.	96.2	3.10	91.0	3.85	85.2	4.78	78.5	5.00
Arg.	82.3	3.86	76.5	4.52	59.3	5.35	53.7	6.82
Lys.	88.5	3.10	82.3	4.04	70.6	5.03	60.9	6.00
His.	90.3	3.99	88.2	4.66	79.3	5.35	70.3	6.48
Pro.	90.6	3.11	86.4	4.18	82.3	5.09	72.7	6.11
Oxy-pro.	95.2	3.03	91.6	4.08	83.2	5.01	77.3	6.08
Cys.	95.6	3.01	90.6	4.01	82.7	5.08	76.5	6.09
Met.	93.9	3.11	92.2	4.03	88.6	5.07	80.2	6.04
Phe-ala.	94.2	3.13	91.3	4.09	80.7	5.08	65.3	6.07
Tyr.	92.6	3.01	81.3	4.03	64.6	5.03	59.3	6.04
Try.	97.3	3.10	95.2	4.08	88.3	5.08	82.7	6.07

* Remaining % ** Final pH

rather in the higher pH level than in lower one. It seemed that is the natural thing to be expected, because ASA was considerably stable at the strong acid pH. Arginine and tryptophan, neverthless, showed strongly an accelerating-action and a restraining-action, respectively, even at pH 3. It can therefore be presumed that these 2 amino acids have very strong activities. And aspartic acid showed strongly a restraining-action at higher pH level, as well as methionine and tryptophan. Aspartic acid is an acidic amino acid, so the final pH was lowered by adding this one. Glutamic acid is similary an acidic one, and restrained to some extent for the oxidation of ASA, too. It can be presumed, therefore, that the restraining-action of the amino acids which are belonging to mono-amino dicarboxylic acid group are responsible for the acidic characters.

On the other hand, the accelerating-action of arginine and lysine may be contributed by its alkaline character according to this reasoning. However, histidine little showed a such action, neverthless it is an alkaline amino acid. The effect of lysine on the oxidation of ASA was intermediate between that of arginine and histidine. It may not be concluded, therefore, that the amino acids belonging to diamino monocarboxylic acid group have necessary the accelerating-action for the oxidation of ASA.

There is no any regular tendency on the other amino acids. For example, while tyrosine belonging to aromatic amino acid group showed an accelerating-action, tryptophan belonging to the same group did entirely the reverse action.

The results at the neutral and alkaline pH were shown in Table 2.

Table 2 The remaining ASA under neutral and alkaline solution in the presence of amino acid

Amino acid (L-form)	pH							
	7*		8**		9**		10**	
	(%)	pH	(%)	pH	(%)	pH	(%)	pH
Control	70.9	7.03	52.7	7.85	40.3	8.90	12.0	9.99
Gly.	63.5	7.04	30.2	7.83	19.6	8.96	0	9.84
Ala.	60.3	7.08	40.3	7.83	17.2	8.98	0	9.88
Ser.	65.7	7.06	37.7	7.71	24.5	8.86	0	9.80
Thr.	73.2	7.07	48.5	7.70	30.4	8.84	0.5	9.74
Val.	65.1	7.05	30.3	7.79	6.7	8.86	0	9.82
Leu.	53.8	7.05	27.3	7.79	2.1	8.87	0	9.86
Iso-leu.	65.3	7.08	41.6	7.83	25.7	8.97	0	9.81
Asp.	80.5	6.52	60.2	6.80	43.7	8.40	4.2	9.68
Glu.	79.3	6.22	58.5	6.31	40.5	7.10	2.7	9.62
Arg.	42.1	8.16	3.1	8.90	0.2	9.24	0	10.08
Lys.	56.3	7.02	30.2	7.76	20.3	8.86	0	9.80
His.	69.3	7.17	40.6	7.82	25.3	8.88	10.2	9.61
Pro.	65.2	7.09	46.3	7.91	36.5	9.00	7.2	9.99
Oxy-pro.	69.8	7.06	48.2	7.80	38.7	8.96	8.5	9.70
Cys.	70.3	7.08	45.2	7.86	30.3	8.95	10.3	9.67
Met.	71.3	7.04	43.7	7.68	27.2	8.84	0	9.80
Phe-ala.	67.8	7.07	36.3	7.79	20.3	8.90	0	9.80
Tyr.	54.2	7.05	26.3	7.70	19.7	8.90	0	9.84
Try.	77.5	7.07	49.9	7.80	35.7	8.93	3.2	9.78

* 60 minutes at 50° ** 30 minutes at 50°

It was recognized, in the case of the neutral, that aspartic acid, glutamic acid and tryptophan showed the restraining-action, and on the other hand, leucine, arginine, lysine and tyrosine did accelerating-action. These tendencies were essentially identical to that found at the acid pH.

In the case of the alkaline pH, a large portion of the amino acids showed generally the accelerating-action and the higher the pH, the more effective the action, except some kinds of amino acids. These tendencies were very significantly recognized in the case of glycine, valine, leucine, arginine and tyrosine. It was found, on the other hand, that aspartic acid, glutamic acid, proline, hydroxyproline and tryptophan did not exceptionally show such action. Moreover, though the accelerating-action was little found at pH 8 in the case of threonine and methionine, recognized obviously at pH 9 or above. There was not any amino acid which raised the remaining ratio of ASA.

In view of the above facts it was contributed that aspartic acid, glutamic acid and tryptophan had clearly the restraining-action for the oxidation of ASA, whereas leucine, arginine, lysine and tyrosine aecelerated considerably the oxidation. And it was found that a large portion of the amino acids had accelerated the oxidation of ASA at alkaline pH, except some kinds of amino acids which restraint the oxidation at the neutral or acid pH. And it seemed that the amino acids having the accelerating-action at the neutral or acid pH accelerate more strongly the oxidation of ASA at the alkaline pH.

The synergism of amino acids for the oxidation of ASA

As the independent effect of amino acid was observed on the oxidation of ASA, a further search was made for the synergism of them. That is, the interactions between all of the amino acids and the essential amino acids which play very important parts in the

Table 3 The synergism effect of amino acid at the acid pH

(Remaining %)

Amino acid	Non	Thr.	Val.	Leu.	IsO-leu.	Lys.	Met.	Phe-ala.	Try.
Control	81.8	80.5	81.2	73.2	80.0	70.6	88.6	80.7	88.3
Gly.	72.3	77.4	80.9	76.2	69.8	69.3	82.3	75.3	84.1
Ala.	78.1	76.1	79.5	80.5	71.7	74.2	84.6	75.8	78.1
Ser.	78.2	80.6	83.2	76.6	77.5	70.5	80.7	74.7	84.2
Thr.	80.5	—	83.6	81.1	70.3	72.3	79.1	70.1	81.4
Val.	81.2	83.6	—	80.5	79.2	68.8	82.6	68.3	86.4
Leu.	73.2	81.1	80.5	—	75.3	80.5	81.5	80.4	86.3
Iso-leu.	80.0	70.3	79.2	75.3	—	73.3	80.3	79.7	86.1
Asp.	89.7	86.2	78.7	85.6	86.7	87.7	88.9	88.5	89.3
Glu.	85.2	83.4	80.1	83.7	77.6	80.9	89.9	75.6	87.9
Arg.	59.3	81.7	70.4	67.1	70.5	64.4	66.3	77.1	71.5
Lys.	70.6	72.3	68.8	80.5	73.3	—	84.4	75.3	81.5
His.	79.3	76.9	82.7	70.5	75.0	73.4	80.6	78.4	87.2
Pro.	82.3	80.0	79.8	85.4	77.5	80.5	87.7	70.2	85.4
Oxy-pro.	83.2	84.5	80.6	78.7	81.3	77.6	85.6	80.5	89.0
Cys.	82.7	80.3	78.6	77.9	71.3	75.7	83.2	71.6	83.1
Met.	88.6	79.1	82.6	81.5	80.3	84.4	—	81.5	85.7
Phe-ala.	80.7	70.1	68.3	80.4	79.7	75.3	81.5	—	86.5
Tyr.	64.6	71.7	73.5	72.5	70.2	60.3	78.3	60.5	76.7
Try.	88.3	81.4	86.4	86.3	86.1	81.5	85.7	86.5	—

animal nutrition were investigated at the acid, neutral and alkaline pH, respectively. These results are given in Table 3~5.

In the case of acidic condition, it was found that the acceleration effects of such amino acids as leucine or arginine or lysine or tyrosine were generally lowered by adding of the other ones having no the accelerating-action, as can be seen from Table 3. And these tendencies were more clearly recognized in the case of the combinations with the amino acids having the restraining-action. But this phenomena did not necessary occur in all cases. There were some exceptions, that is, the combinations of leucine and iso-leucine, tyrosine and phenyl-alanine, etc., to this tendency.

On the other hand, it was found that the amino acids, such as aspartic acid or glutamic acid or methionine or tryptophan which restrained the oxidation of ASA, had raised the ASA remaining ratios of the other ones as a general rule, except a few kinds of cases.

It was recognized, moreover, that there were several combinations in which the ASA remaining ratio showed lower value than that in each case, and that there were many examples in the combinations of phenyl-alanine or iso-leucine and the other amino acids. It can be presumed, therefore, that these amino acids might have any special action accelerating the oxidation of ASA in the presence of the other ones.

The synergism effects of amino acids at the neutral pH are given in Table 4.

The depression effects of the some kinds of amino acids on the accelerating-actions of lysine, leucine, arginine and tyrosine were found to a certain extent at the neutral, too, as shown in Table 4. But it was not so obvious tendency as recognized at the acid pH. Because, it was observed that there were a fair number of amino acids by which the accelerating-actions of the 4 amino acids mentioned above were scarcely influenced.

Table 4 The synergism effect of amino acid at the neutral pH

(Remaining %)

Amino acid	Non	Thr.	Val.	Leu.	Iso-leu.	Lys.	Met.	Phe-ala.	Try.
Control	70.9	73.2	65.1	53.8	65.3	56.3	71.3	67.8	77.5
Gly.	63.5	69.8	60.4	55.2	60.8	50.9	60.7	66.0	70.4
Ala.	60.3	71.6	58.2	50.4	64.7	54.3	61.3	63.5	71.6
Ser.	65.7	68.4	60.3	51.5	63.3	60.1	63.5	64.7	70.1
Thr.	73.2	—	70.0	56.3	74.7	65.7	67.0	60.3	74.3
Val.	65.1	70.0	—	61.7	63.2	50.4	60.8	62.2	66.9
Leu.	53.8	56.3	61.7	—	57.8	41.2	55.5	52.7	60.8
IsO-leu.	65.3	74.7	63.2	57.8	—	55.3	62.7	65.9	64.7
Asp.	80.5	75.9	72.4	62.9	71.0	70.2	77.3	76.8	79.0
Glu.	79.3	77.2	69.8	60.6	68.2	60.1	73.6	76.2	77.1
Arg.	42.1	60.5	53.5	48.7	52.7	49.8	56.4	51.0	62.6
Lys.	56.3	65.7	50.4	41.2	55.3	—	61.2	66.1	66.7
His.	69.3	69.9	66.3	60.7	67.7	65.4	70.9	70.4	78.3
Pro.	65.2	67.8	64.2	55.3	61.9	59.5	60.3	66.5	76.4
Oxy-pro.	69.8	68.1	64.7	58.2	66.8	64.0	65.3	67.3	71.2
Cys.	70.3	70.4	66.1	51.6	60.4	60.4	62.7	63.2	73.9
Met.	71.3	67.0	60.8	55.5	62.7	61.2	—	69.0	72.0
Phe-ala.	67.8	60.3	62.2	52.7	65.9	66.1	69.0	—	70.3
Tyr.	54.2	68.7	60.3	50.1	56.2	53.3	56.2	58.7	68.5
Try.	77.5	74.3	66.9	60.8	64.7	66.7	72.0	70.3	—

Among them, the combinations of lysine and glycine or valine or leucine showed significantly lower remaining ratios than that in each case, respectively. It seemed consequently that lysine might have any special action strengthening the accelerating-action of the amino acids for the oxidation of ASA at the neutral, in a similar manner as the case of phenyl-alanine or iso-leucine at the acid pH.

On the other hand, aspartic acid, glutamic acid and tryptophan which had the restraining-action depressed fairly the oxidation of ASA in the presence of the other amino acids. But this tendency was not so clearly recognized as observed at the acid pH.

The synergism effects of amino acids at the alkaline pH are summarized in Table 5.

As previously described, the oxidation of ASA was remarkably accelerated by adding of the amino acids at the alkaline pH, except the 5 ones (i. e., aspartic acid, glutamic acid, proline, hydroxyproline and tryptophan), and this tendency was clearly observed in the case of valine, leucine and arginine. Consequently, there is a strong presumption that these 3 amino acids will influence on the ASA remaining ratios in the presence of the other ones. The results obtained in this experiment, as can be seen in Table 5, was in accord with this expectation. In particular, ASA was completely oxidized in the combinations of valine and arginine or lysine or proline or tyrosine, leucine and serine or arginine or lysine or phenyl-alanine or some other amino acids, respectively. And there was no any combination which had raised the ASA remaining ratio. The remaining ratios in the combinations of leucine and the other amino acids showed especially lower values than that in the other cases. And even the amino acids having the restraining-action for the oxidation could not inhibit the accelerating-action of leucine.

The foregoing amino acids having the restraining-action at the acid or neutral pH

Table 5 The synergism effect of amino acid at the alkaline pH

(Remaining %)

Amino acid	Non	Thr.	Val.	Leu.	Iso-leu.	Lys.	Met.	Phe-ala.	Try.
Control	40.3	30.4	6.7	2.1	25.7	20.3	27.2	20.3	35.7
Gly.	19.6	20.3	4.6	7.9	20.4	18.7	20.4	18.7	26.3
Ala.	17.2	19.6	4.5	3.2	21.3	19.1	20.1	19.2	21.4
Ser.	24.5	27.7	10.2	0	25.0	21.5	24.7	17.2	23.1
Thr.	30.4	—	18.8	5.8	30.3	23.6	30.5	30.6	20.4
Val.	6.7	18.8	—	4.2	10.5	0	2.1	9.8	12.9
Leu.	2.1	5.8	4.2	—	1.0	0	3.7	0.8	7.4
Iso-leu.	25.7	30.3	10.5	1.0	—	22.6	25.0	24.7	30.9
Asp.	43.7	40.6	20.2	11.2	31.7	39.7	24.7	26.4	33.5
Glu.	40.5	39.8	9.3	7.6	30.3	31.4	25.4	22.3	37.3
Arg.	0	20.7	0	0	17.2	6.6	20.3	16.2	9.2
Lys.	20.3	23.6	0	0	22.6	—	21.9	19.5	21.7
His.	25.3	28.8	15.2	4.6	21.7	23.3	26.6	22.1	22.7
Pro.	36.5	32.6	0	3.7	31.5	20.0	30.3	21.6	20.4
Oxy-pro.	38.7	30.5	14.4	7.4	35.4	35.7	25.7	27.4	31.2
Cys.	30.3	30.0	13.2	1.3	24.1	26.5	28.5	30.3	31.4
Met.	27.2	30.5	2.1	3.7	25.0	21.9	—	24.6	25.8
Phe-ala.	20.3	30.6	9.8	0.8	24.7	19.5	24.6	—	18.7
Tyr.	19.7	23.1	0	3.5	23.5	16.3	20.8	18.9	27.0
Try.	35.7	20.4	12.9	7.4	30.9	21.7	25.8	18.7	—

showed the same values as control and little influenced the ASA remaining ratios of the other ones. These results differed from those obtained in the case of acid or neutral pH. It may be concluded from these results, therefore, that there is no combination of amino acids raising the ASA remaining ratio under the alkaline condition at which the existence of the amino acids accelerate the oxidation of ASA.

Summary

The present investigation was undertaken to see the the effects of the amino acids on the oxidation of ASA under the various pH conditions and to see the synergism of the amino acids. The following results were obtained.

At the acid pH, aspartic acid, glutamic acid, methionine and tryptophan showed the restraining-action for the oxidation of ASA. It was found, in contrast with this, that glycine, leucine, arginine, lysine and tyrosine showed the accelerating-action. And these tendencies were clearly recognized rather in the higher pH level than in lower one.

The aspects of the amino acids in the case of the neutral pH were essentially identical to that found at the acid pH.

In the case of the alkaline pH, a large portion of the amino acids showed the accelerating-action and the higher the pH, the more effective the action, except some kinds of the amino acids (i. e., asprtic acid, glutamic acid, proline, hydroxyproline and tryptophan). And the accelerating-action was significantly recognized in the case of glycine, valine, leucine, arginine and tyrosine. There was not any amino acid which raised the remaining ratio of ASA.

The acceleration effects of such amino acids as leucine or arginine or lysine or tyrosine were generally lowered by adding of the other ones having the restraining-action for the oxidation of ASA, except some examples. And it was found that the amino acids, such as aspartic acid or glutamic acid or methionine or tryptophan which restrained the oxidation of ASA, have raised the ASA remaining ratios of the other ones as a general rule, except a few kinds of cases. But these tendencies were not recognized in the case of alkaline condition, but in the case of acidic or neutral one.

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