INFLUENCE TO VITAMIN C BY OXIDATION BEHAVIOR WITH COMMERCIALLY AVAILABLE SALTS

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1. Introduction

"Asazuke" is a traditional Japanese food which is one of pickle made by salting a vegetable for a night and has been eaten for a long time as a supplying source of vitamin C. In vitamin C, there are reduced type (Ascorbic acid; AsA) and oxidized type (Dehydro acorbic acid; DAsA) ascorbic acids[1], in general, raw vegetable richly contains AsA compared with DAsA[2]. As we efficiently ingest Vitamin C, it is necessary to inhibit oxidation of AsA when "Asazuke" is made. There are Miyamoto's report et al which have carried out on the examination of oxidization behavior of vitamin C, but the study on an influence of the inorganic salts in detail is not found.

So, in order to clarify the effect of salts on oxidation of Vitamin C in "Asazuke", we have studied the effect of some commercially available salts whose quality are different. Moreover, the difference of the behavior that had been obtained by this research was examined by changing of the kinds of inorganic salts, pH, added sodium chloride (NaCl) amount and AsA concentration.

2. Experiments

2.1 Oxidation behavior of AsA using some commercially available salts whose quality is different

2.1.1 Quality of commercially available salts

In experimental, we used 5 kinds of commercially available salt, in which content of bittern component and other additives are different. Table 1 shows purity of NaCl, bittern component concentration, amount of additives and pH of the salts.

Table 1 NaCl purity, concentration of bittern component, amount of additives and pH of commercially available salts

(%)	A	B	C	D	E
NaCl	99.97	95.56	72.44	97.35	99.57
Bittern component	0	1.1	19.9	0.3	0
Additives	-	-	-	Malic acid Citric acid	Basic $MgCO_3$
pH	4.9	5.5	8.8	2.4	10.0

Salt A has high purity of NaCl with little acid side because the bittern component such as magnesium chloride ($MgCl_2$) and potassium chloride (KCl) et al are not included. Salt B is wet type salt containing small amount of bitten with little bit higher pH compared with one of salt A. Salt C is directly dried sea water, containing much amount of bittern with higher pH. Salt D is specially prepared one for pickle use, made from crushed natural salt added malic acid and citric acid with low pH. Salt E is made from salt A with basic magnesium carbonate (Basic $MgCO_3$) as anti-blocking agent, having higher pH compared with those of another samples.

2.1.2 Experimental procedure

As prepared 100mg/l concentration of AsA aqueous solution in advance, each salt was added to the AsA solution as approximately 0.5M of all the salt concentration. 10ml of the obtained sample solution was introduced to a test glass tube which volume was 20ml. The tube was capped, and then dipped in the constant temperature bath adjusted to 25 for fixed hours (0-120hrs).

2.1.3 Composition analysis

The AsA concentration of the sample solution was measured by HPLC (High Performance Liquid Chromatography, Shimazu Seisakusho, Japan).

Water and inorganic content were determined by the salt test method[6]. Each component was determined as follows; water content was measured by the weight loss of dried residue method, chloride content was measured by the titration using silver nitrate solution, calcium and magnesium content were measured by ICP-AES analysis (inductively-coupled plasma atomic emission spectrometry, Shimazu Seisakusho, Japan), sulfuric acid (SO_4) content was measured by IC method (ion chromatography 2000, Dionex, Japan), potassium (K) content was measured by AAS (flame photometry S series, Thermo, Japan) and sodium (Na) content was obtained by the bond calculation method. pH measurement was carried out by the glass electrode method, using pH meter (DKK-TOA products).

2.2 Effected factor for oxidation behavior of AsA

2.2.1 Experimental procedure

1) pH

We prepared 2 samples, including AsA (100mg/l) solution without NaCl and AsA solution added 0.5M of NaCl. pH of these samples were adjusted to fixed pH value (2-9) by adding hydrochloric acid (HCl) solution and sodium hydroxide (NaOH) solution.

2) Bittern components

Various salts were added to AsA (100mg/L) solution to become the salt concentration 0.5M. The salts used here are NaCl, $MgCl_2$, calcium chloride ($CaCl_2$), KCl, sodium sulfate (Na_2SO_4) and magnesium sulfate ($MgSO_4$). To evaluate the effect of SO_4 containing the commercially available salt, NaCl 0.458M added to Na_2SO_4 0.042M sample were prepared to give 0.5M of total salt concentration. These samples were measured AsA concentration every fixed time by means of the same method described 2.1.2.

3) NaCl concentration

NaCl was added to AsA (100mg/l) solution to give fixed concentration of NaCl 0-2M. These samples were also measured their changing AsA concentration every fixed time.

4) AsA concentration

0.5M of NaCl was added to AsA solution prepared fixed concentration between 50-250mg/l. These samples, after dipping in the constant temperature bath for fixed time, were also measured their AsA concentration by HPLC as the same method as 2.1.2.

3. Result and discussion

3.1 Oxidation behavior of AsA using some commercially available salt whose quality is different

Fig.1 shows oxidation behavior of AsA when adding 5 kinds of the salt. There are small amount of oxidized AsA per unit hour when adding Salt A, B and D compared with blank without any salt. Even after 70 hours, there is few oxidization of AsA. As it takes approximately 24 hours to prepare "Asazuke", it is suggested that addition of salt inhibits oxidation of Vitamin C in vegetables. On the other hand, in case of Salt C and E, as time elapsed, AsA content drastically decreases to give higher content of oxidized AsA per hour compared with blank. As shown in Table 1, Salt C contains much bittern components. Moreover, Salt C and D show higher pH level. From these results, it is suggested that the inhibiting effect of oxidation of AsA is different on bittern component, pH and NaCl concentration of the salt when "Asazuke" is made.

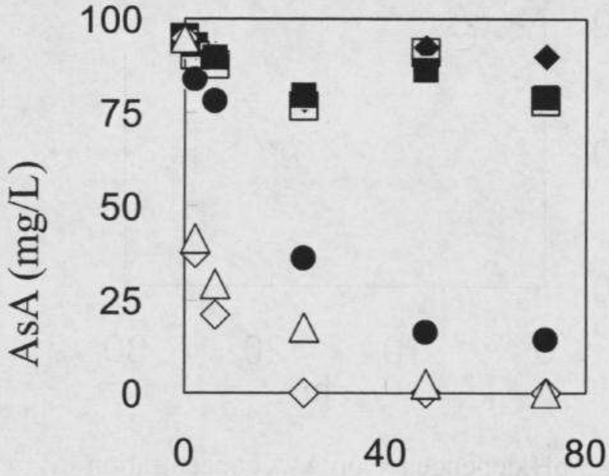


Fig.1 Change AsA concentration with elapsed time
(Salt conc. is approx. 0.5M)
●Blank (pH3.8), ◆SaltA (pH3.7), ■SaltB (pH3.7)
◇SaltC (pH9.4), □SaltD (pH3.6), △SaltE (pH9.3)

3.2 Effected factor for oxidation behavior of AsA

1) Effect of pH

Fig.2 shows the change with time elapsed of AsA concentration on various pH without addition of NaCl. In pH range of 1.8-3.0, AsA is not oxidized even after 24 hours. However, when pH was over 3.8, residual AsA amount decreased below 60% after 24hours.

Fig.3 shows the change of AsA concentration on various pH with addition of NaCl 0.5M. In pH range of 1.8-5.1, there is few oxidation of AsA after 24 hours. It is suggested that addition of NaCl makes oxidation of AsA inhibited wider pH range than one of no addition. However, when pH is neutral to alkaline side, it is observed that AsA tends to be oxidized, even if NaCl adds in. From the above results, if NaCl adds to the system, oxidation of AsA is inhibited at any pH position. On the other hand, in alkaline side, its effect is small. Therefore, we think that pH must be acid side.

Fig.2 pH dependence on AsA concentration (Without NaCl)
◆pH1.8, ■pH2.1, ▲pH3.0, ●pH3.8
◇pH4.8, □pH5.1, △pH8.7, ○pH9.5

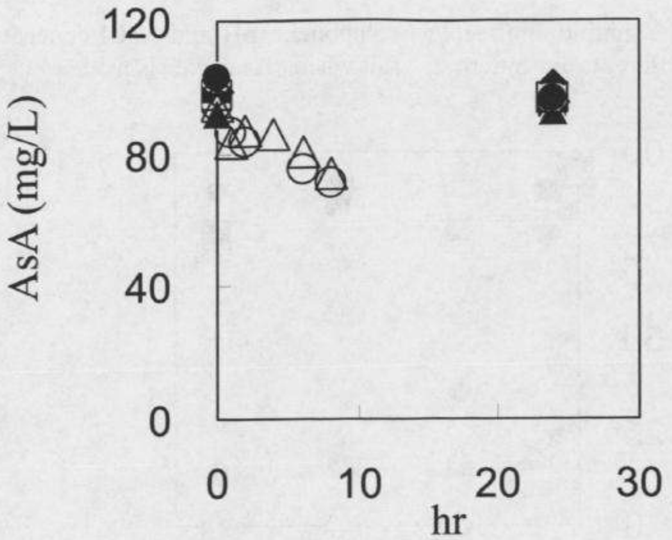


Fig.3 pH dependence on AsA concentration (NaCl 0.5M)

◆pH1.8, ■pH2.2, ▲pH3.0, ●pH3.8
◇pH4.8, □pH5.1, △pH7.4, ○pH9.2

2) Effect of bittern components

Fig.4 shows effect of oxidation behavior of AsA when bittern component is added. Chloride compound, including NaCl and $MgCl_2$ inhibited oxidation of AsA. It is observed that there is few oxidation of AsA occurred after 24 hours. On the other hand, sulfate such as $MgSO_4$ has no effect on inhibiting oxidation of AsA similar to the previous report[3]. After 24 hours, amount of AsA became below 40%. Then, the solution which contains NaCl 0.458M plus Na_2SO_4 0.042M (total 0.5M) is compared with one of NaCl 0.5M (Fig.5). Fig.5 means that both system inhibited oxidation of AsA. This composition is a model of the highest sulfate content in the salt commercially available in Japan. It is suggested that there is no influence in the oxidation of AsA in the sulfate concentration contained in a commercially available salt.

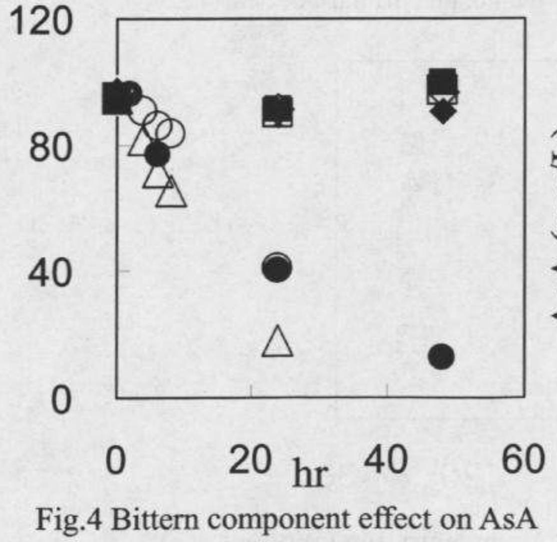


Fig.4 Bittern component effect on AsA concentration (Each 0.5M)

●Blank, ◆NaCl, ■$MgCl_2$, ◇$CaCl_2$
□KCl, △$MgSO_4$, ○Na_2SO_4

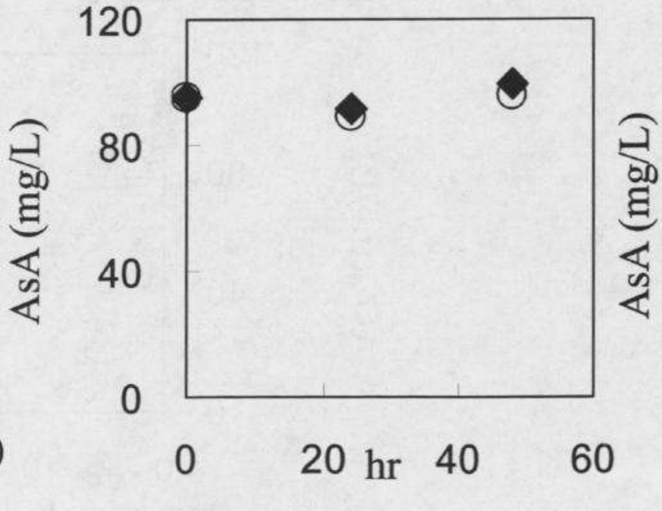


Fig.5 Sulfate effect on AsA concentration (NaCl0.5M, NaCl 0.458M+Na_2SO_4 0.042M)

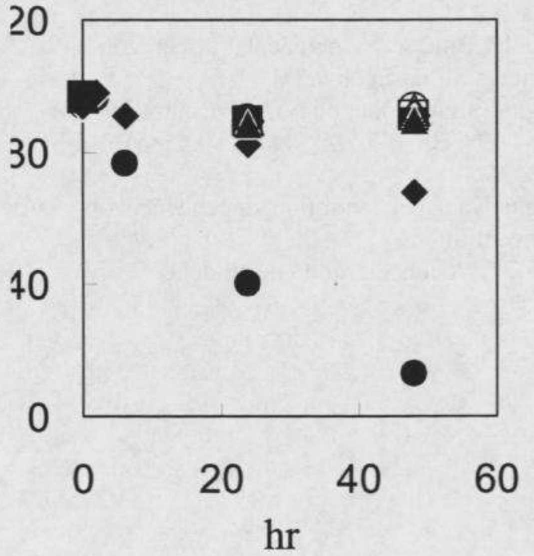
◆NaCl, ○NaCl + Na_2SO_4

3) Effect of NaCl concentration

Fig.6 shows effect of NaCl concentration on oxidation behavior of AsA. Over 0.1M of NaCl, oxidation of AsA is negligible after 50 hours. However, in case of 0.05M of NaCl concentration in the sample, AsA can be oxidized as time to become approximately 70% of AsA after 50 hours. However, In general, to make "Asazuke", added concentration of NaCl is around 0.5M. So, the oxidation of AsA can be inhibited.

4) Effect of AsA concentration

Fig.7 shows effect of AsA concentration on AsA oxidation behavior. It is reported that it is easy to be oxidized when AsA concentration is low[4]. In case of 0.5M addition of NaCl, there is not oxidized in any AsA concentration till about 120 hours. As NaCl concentration in "Asazuke" is approximately 0.5M, it is believed that oxidation behavior of AsA can be inhibited even if AsA concentration in vegetable changes.



g.6 NaCl concentration dependence on ;A concentration

Blank, ◆0.05M, ■0.1M, ▲0.2M
0.4M, □0.5M, △1M, ○2M

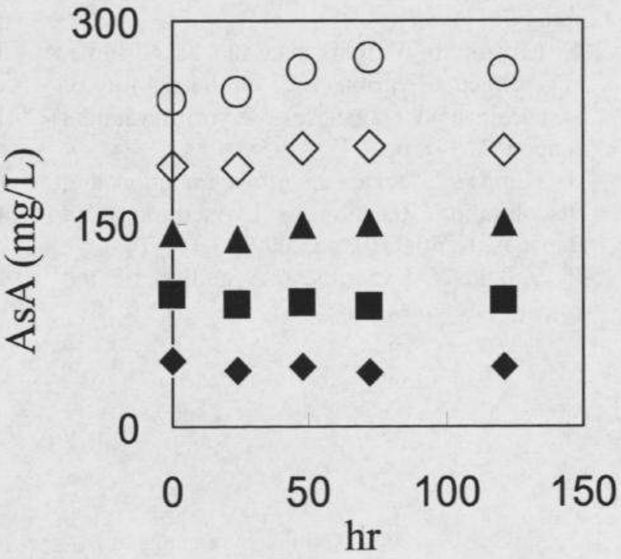


Fig.7 AsA concentration dependence

◆50mg/L, ■100mg/L, ▲150mg/L
◇200mg/L, ○250mg/L

4. Conclusion

We studied oxidation behavior of AsA, an important form of Vitamin C, to select proper commercially available salt for production of "Asazuke", which is one of supplying sources of Vitamin C.

Among 5 kinds of the salts with different quality, it is clarified that AsA is easily oxidized with some salts including higher pH salt and bittern component richly contained salt having high pH. Furthermore, by an evaluation for the effect of pH and bittern component on oxidation behavior of AsA, it is cleared that AsA is easily oxidized in higher pH. So pH is preferably acid side to inhibit AsA oxidation. As sulfate salt, for example $MgSO_4$ which is one of bittern component, adds to AsA solution instead of NaCl, we found that oxidation of AsA can not be inhibited. However, a small amount of sulfate, as ordinarily containing the commercially available salt, does not inhibit oxidation of AsA because of the effect of NaCl.

We studied more about the effect of NaCl concentration and AsA concentration on oxidation behavior of AsA. As NaCl content is below 0.05M, inhibiting effect of NaCl for oxidation of AsA becomes small. However, as NaCl concentration is around 0.5M for producing "Asazuke" in general, we found that this concentration level inhibits oxidation of AsA. As NaCl concentration is around 0.5M, we found that AsA concentration does not effect on inhibiting oxidation of AsA.

In the future, we will study further investigation using vegetables in order to select proper commercially available salt to control oxidation behavior of Vitamin C in "Asazuke".

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Fig.1 Change AsA concentration with elapsed time (Salt conc. is approx. 0.5M)

Fig.2 pH dependence on AsA concentration (without NaCl)

Fig.3 pH dependence on AsA concentration (NaCl 0.5M)

Fig.4 Bittern component effect on AsA concentration (Each 0.5M)

Fig.5 Sulfate effect on AsA concentration (NaC l 0.5M, NaCl 0.458M+Na_2SO_4 0.042M)

Fig.6 Nacl concentration dependence on AsA concentration

Fig.7 AsA concentration dependence