Stability of Functional Constituents in Canned Tea Drinks During Processing and Storage †

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To establish an appropriate manufacturing condition of canned tea drinks, the behavior of tea caffeine and catechins during processing and storage was examined.

Caffeine was entirely stable throughout the processing and prolonged storage; however, catechins were relatively unstable, and the higher the pH of infusion caused the larger change.

An addition of a minor amount of L-ascorbic acid was effective to stabilize natural type catechins in the tea drinks. Nitrogen gas flushing at the time of cover-seaming was also effective in protecting catechins to some extent.

A dominant change of catechins seemed to be stereochemical isomerization to corresponding epimer. Pure (−)-epicatechin and (+)-catechin changed to corresponding isomer in tea simulating media by heating. The most essential factor to affect isomerization was pH of solution. A fast isomerization accompanied with browning of solution occured in the region of 6.0 or more of pH. The reaction scarcely proceeded in media less than 5.0 of pH.

The stability of catechins was dependent upon the kind of tea. Catechins were the most stable in fermented tea (black tea), relatively stable in semi-fermented tea (oolong tea), but the least stable in unfermented tea (green tea). Therefore, careful adjustment of pH of infusion, nitrogen gas flushing to headspace of cans at the seaming, avoiding excess heat processing and careful handling during the distribution are required to supply quality-stable canned unfermented tea drinks.

Key words: canned tea drinks, black tea, oolong tea, green tea, caffeine, catechins, epimer, isomerization, tertiary function, HPLC.

The initial market growth of canned tea drinks in Japan started from oolong tea; black tea and green tea followed this. The production volume of these canned tea drinks showed an annual increment because of suiting consumers' favor in Japan. The total production volume reached more than 3 billion cans in 1990.

Most of canned tea drinks are low-acid beverages, and are kept warm in a vending machine during the cold season. Therefore, it is necessary to sterilize the spores of thermophilic anaerobes. Sterilization needs high temperature heat processing at

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a fixed time. Therefore, it is important to prevent the quality change of canned tea drinks during the production and distribution.

Caffeine and catechins, the important constituents of tea, were chosen as indicative substances of the quality change. Recently, catechins received attention as an important tertiary functional constituent of tea[1-5].

The purpose of this series of studies[6,7] is to establish an appropriate manufacturing condition of canned tea drinks by finding out changes of these important constituents during the extraction, filling, heat processing and storage.

In this paper, the effect of pH of infusion on retention of catechins and the behavior of functional constituents in canned tea drinks at the condition of elevated temperature and prolonged storage were described.

Materials and Methods

pH and catechins in canned green tea drink

The effect of manufacturing condition on changes of functional constituents in tea drinks was examined. According to the standard production process of canned tea drinks shown in **Fig. 1**, canned green tea drink was prepared by modifying a producing factor, *i. e.*, pH of infusion. It was selected by reason of relation between pH and the ratio of catechins to caffeine in commercial tea drinks mentioned in the previous paper[7]. Dried green tea leaves ('Yabukita') of a weight of 1% for a weight of treated water were extracted. The treated water used was ion-exchanged, UV-sterilized and activated charcoal-treated potable water. Extraction was carried out for 5 min at 80℃. After cooling to 30℃, pH of the infusion was carefully adjusted at 3.88, 4.58, 5.38 and 6.12, respectively, by adding citric acid. The infusion was filtered with 250 mesh nylon cloth, filled at 95℃ into the tin free steel (TFS) can (Toyoseam™) of 200g capacity and cover-seamed under introgen gas flushing at a rate of 7 ℓ/min to remove residual oxygen gas in headspace of the can. The seamed cans were sterilized for 6 min at 121℃ and immediately cooled. The canned green tea drink was analyzed to determine caffeine and catechins.

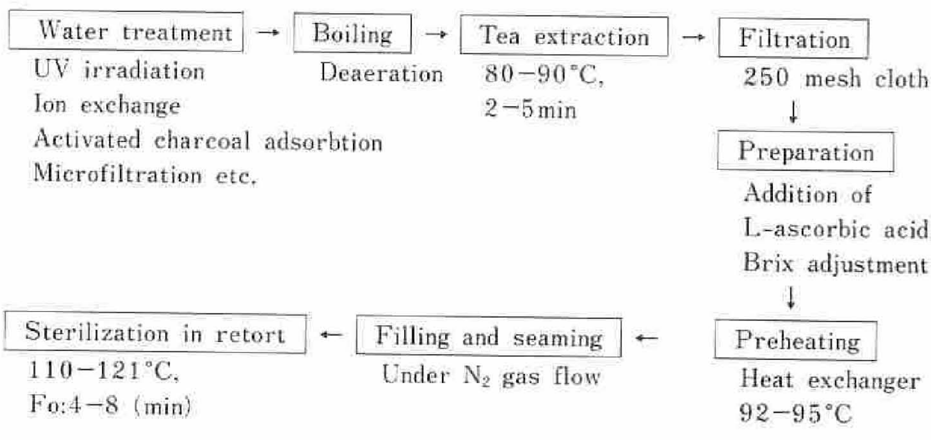

Fig.1. A standard production process of canned tea drinks.

Storage test of canned tea drinks

Various canned tea drinks were prepared by modifying two production factors. One factor was an addition of L-ascorbic acid (AsA) to the infusion, and another was nitrogen gas flushing at the time of cover-seaming of the hot-filled can. Tea leaves used were 'Tieguanyin' (imported) for oolong tea, 'Darjeeling' (imported) for black tea and 'Yabukita' (domestic) for green tea, respectively.

To a part of the infusion extracted by a method similar to the above-mentioned method without the adjustment of pH, L-ascorbic acid was added at a rate of 20 mg per 100g of the infusion. The filtered infusion was filled into the TFS can by a method similar to the above-mentioned method. A part of the filled cans was cover-seamed without the nitrogen gas flushing. Canned green tea, oolong tea and black tea drinks were prepared by the procedure. These canned tea drinks were kept for various periods from 1 month to 6 months at a room temperature and at 55℃, respectively, and were used to simultaneous analysis of caffeine and catechins.

Isomerization of (−)-epicatechin and (+)-catechin in tea simulating solution

Standard reagents of (−)-epicatechin (99.64% purity) and (+)-catechin (100.0% purity) obtained from Kurita Kogyo Co. Ltd. were dissolved to deionized, deaerated water at the concentration of 8mg/100mℓ individually. The pH of each medium was previously adjusted at the fixed values in the range of 3.01 to 7.10 by addition of citric acid and sodium citrate, respectively. 10mℓ of the solution was transferred into pressure-resistant hermetical glass tubes. The tubes were sealed with gas-tight screw-caps under nitrogen gas flushing to headspace of the filled tubes. The sealed tubes were heated in an oil-bath at 121℃ for 6 min, then immediately cooled in a cold water flow. Catechins in the solution were immediately determined and the absorbance of solution at 430nm was measured to evaluate browning by a spectrophotometer (Shimadzu UV-160A) with 10 mm quartz cell.

Analysis of caffeine and catechins

Caffeine and catechins were simultaneously determined by the HPLC method described by Terada *et al.*[9], with a few modifications[6].

The HPLC system (Shimadzu LC-6A) fitted with UV detector (measured at 280nm) and an ODS column(Ultron N-C18, 150mm×4.6mm i. d.) was used. The temperature of the column was maintained at 43℃. The sample solution was diluted with a same volume of acetonitrile, then filtered by a membrane filter (0.45μm pore); 10μℓ of the solution was injected. Analysis was performed by a gradient system. Phosphoric acid (0.1v/v%) containing acetonitrile (0.1 v/v%) and N,N-dimethylformamide (5v/v%) as A solution, and acetonitrile as B solution were used as the mobile phase with a flow rate of 1mℓ/min. Standard reagents for the quantitative determination of tea catechins were (−)-epicatechin (−EC, 99.64% purity), (−)-epigallocatechin (−EGC, 99.59%), (−)-epicatechin gallate (−ECg, 98.43%), (−)-epigallocatechin gallate (−EGCg, 99.64%) and (+)-catechin (+C, 100.0%), obtained from Kurita Kogyo Co. Ltd.

Results and Discussion

The concentration of each constituent

determined before and after heat processing was shown in Table 1 for different pH of green tea infusion. Here, 'total catechins' were shown as the sum of (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate and (+)-catechin. The variation of caffeine and catechins in the infusion of different pH after heat processing of canned green tea drink was shown in Fig, 2.

Caffeine was extremely stable, no influence of pH and heat processing was observed on its stability. In contrast, catechins were relatively unstable on heat processing and the higher the pH of infusion caused the larger change. Particularly, (+)-catechin remarkably increased under higher pH and its isomer, (−)-epicatechin, decreased. As the sum of decreased (−)-epicatechin and increased (+)-catechin

Table 1. Concentration of caffeine and catechins in canned green tea drink before and after heat processing in relation to pH of infusion.

	pH	Caffeine	−EGC	+C	−EC	−EGCg	−ECg	Total catechins
			mg/100mℓ					mg/100mℓ
Before processing	6.24	25.62	45.36	1.82	17.59	54.18	14.82	133.77
After processing	6.12	25.52	28.00	4.54	13.68	35.19	11.52	92.93
	5.38	25.50	34.35	3.11	15.52	42.05	12.93	107.96
	4.58	25.40	39.50	2.16	17.19	48.25	14.07	121.17
	3.88	25.45	41.98	1.95	16.53	50.89	14.48	125.83

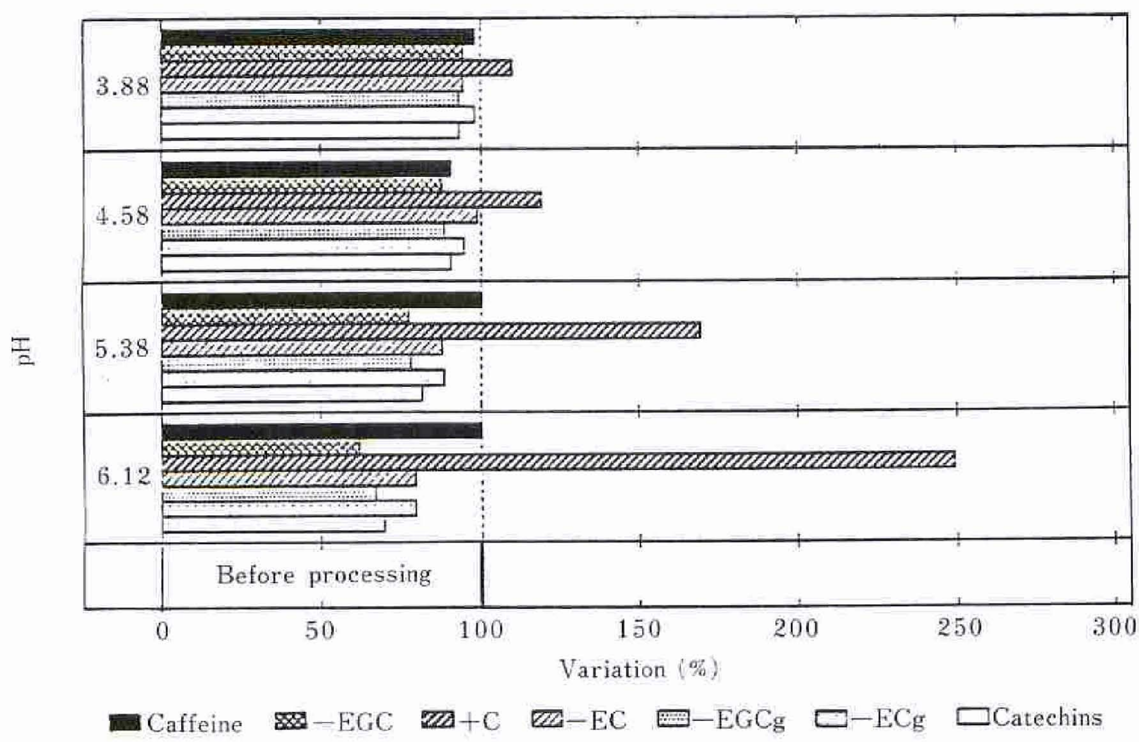

Fig.2 Effect of pH of infusion on variation of caffeine and catechins in canned green tea drink after heat processing at 121°C for 6 min.

seemed to be constant and independent of pH, an intimate relationship between these two catechins was suggested.

The effects of an addition of L-ascorbic acid, nitrogen gas flushing at the time of cover-seaming and the storage condition of various canned tea drinks on changes of these functional constituents were shown in Figs. 3, 4 and 5. Here, the variation of constituents was calculated as the percentage to the concentration before heat processing. In comparison with storage condition at room temperature and 55℃, caffeine was entirely stable in all cases. In contrast, catechins were considerably unstable during heat processing, prolonged or an elevated temperature storage. It was confirmed that (+)-catechin once increased was also decreased in the period of prolonged storage. An addition of L-ascorbic acid was effective to prevent change of catechins during heat processing and storage. Nitrogen gas flushing at cover-seaming was also effective to protect catechins to some extent, but the effectiveness was less than that of the addition of L-ascorbic acid.

Concerning the kind of tea, catechins

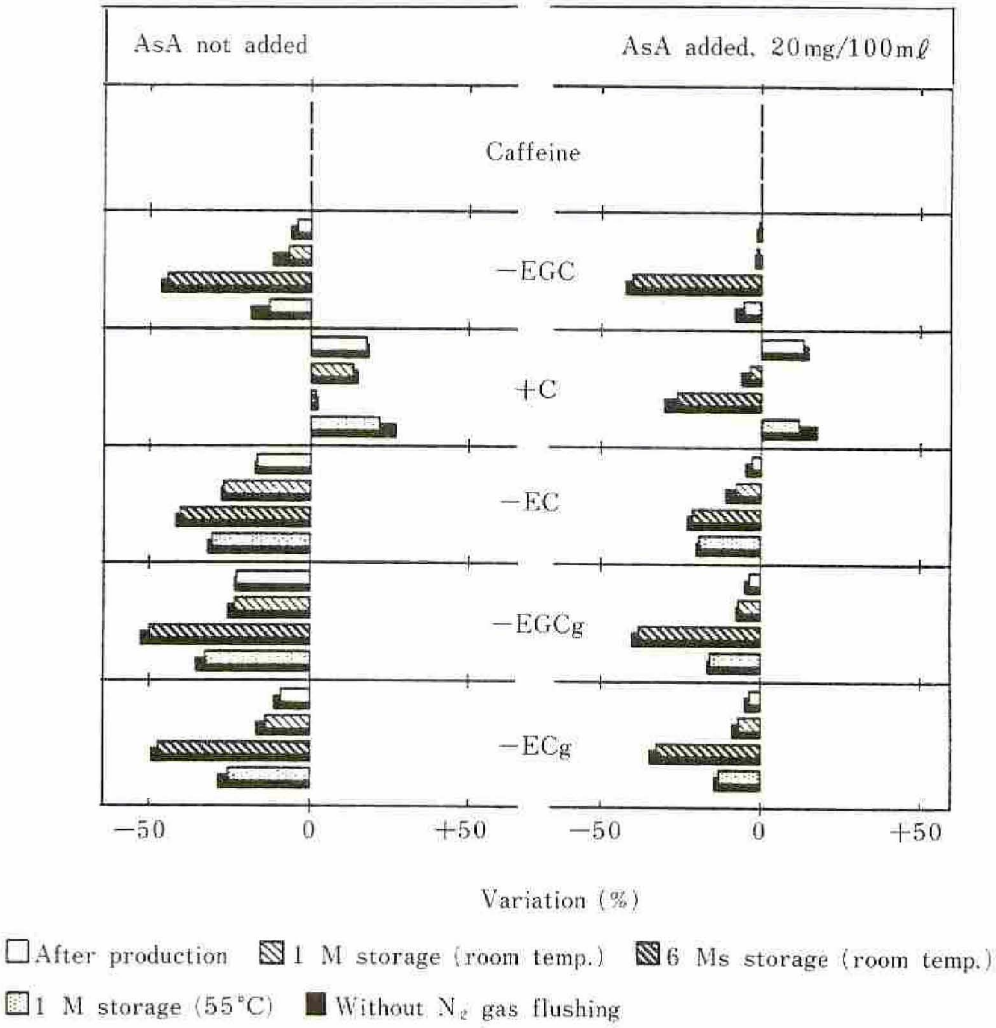

Fig.3. Variation of caffeine and catechins in canned black tea drink during storage with or without L-ascorbic acid.

were the most stable in black tea (fermented tea), relatively stable in oolong tea, but were the least stable in unfermented green tea from a viewpoint of the formation of (+)-catechin.

From the decrease of (−)-epicatechin and the increase of (+)-catechin during heat processing, a dominant change of catechins in tea drinks seemed to be an isomerization to corresponding isomer because of the difference of stereochemical conformation. The only difference was the configuration on the asymmetric carbon atom of the second or the third position of 3-hydroxyfuravanon.

The relation of pH of solution to the isomerization of pure (−)-epicatechin to corresponding isomer was shown in Fig, 6. The same relation to the isomerization of pure (+)-catechin to corresponding isomer was shown in Fig, 7.

Nakagawa[10] studied the effect of heating on changes of catechins in roasted green tea and mentioned that the thermal product from (−)- epicatechin or (+)-catechin was (−)- catechin or (+)-

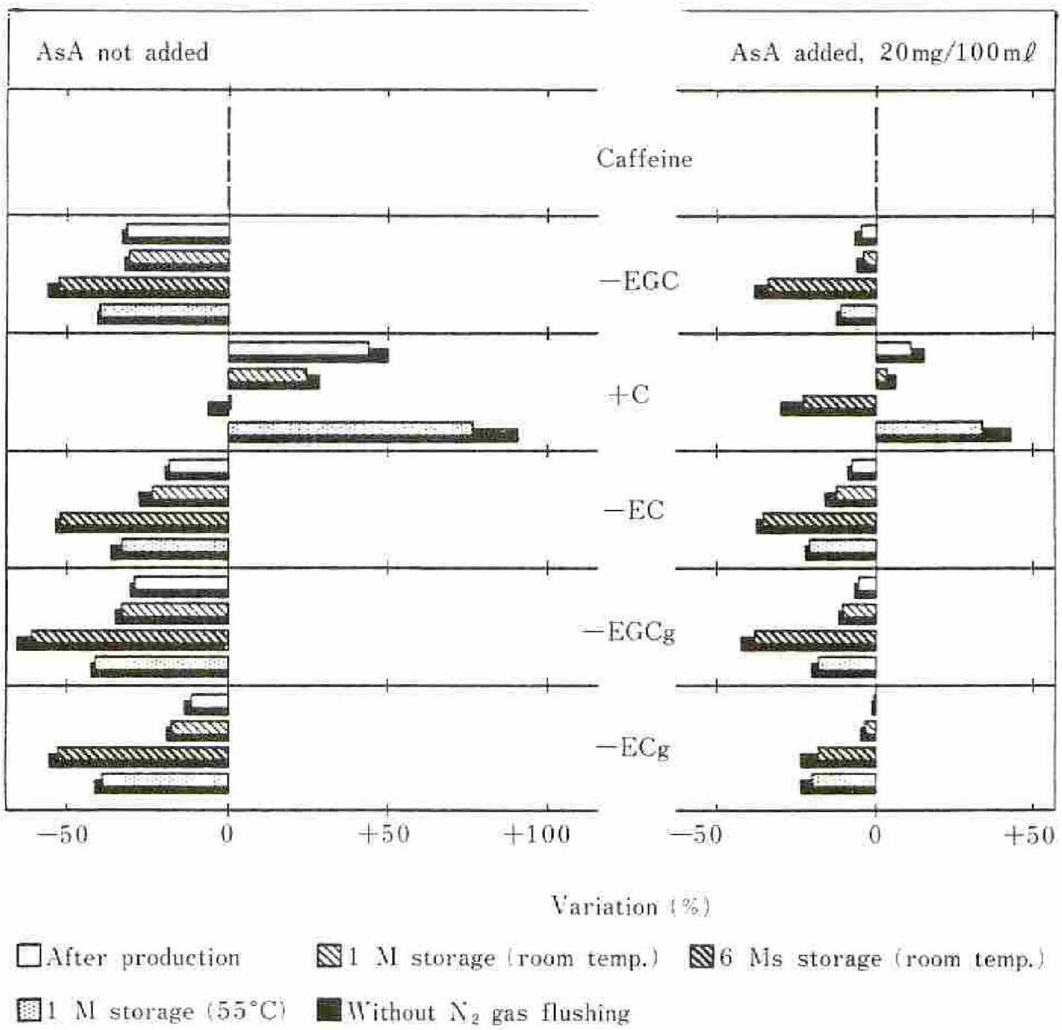

Fig.4. Variation of caffeine and catechins in canned oolong tea drink during storage with or without L-ascorbic acid.

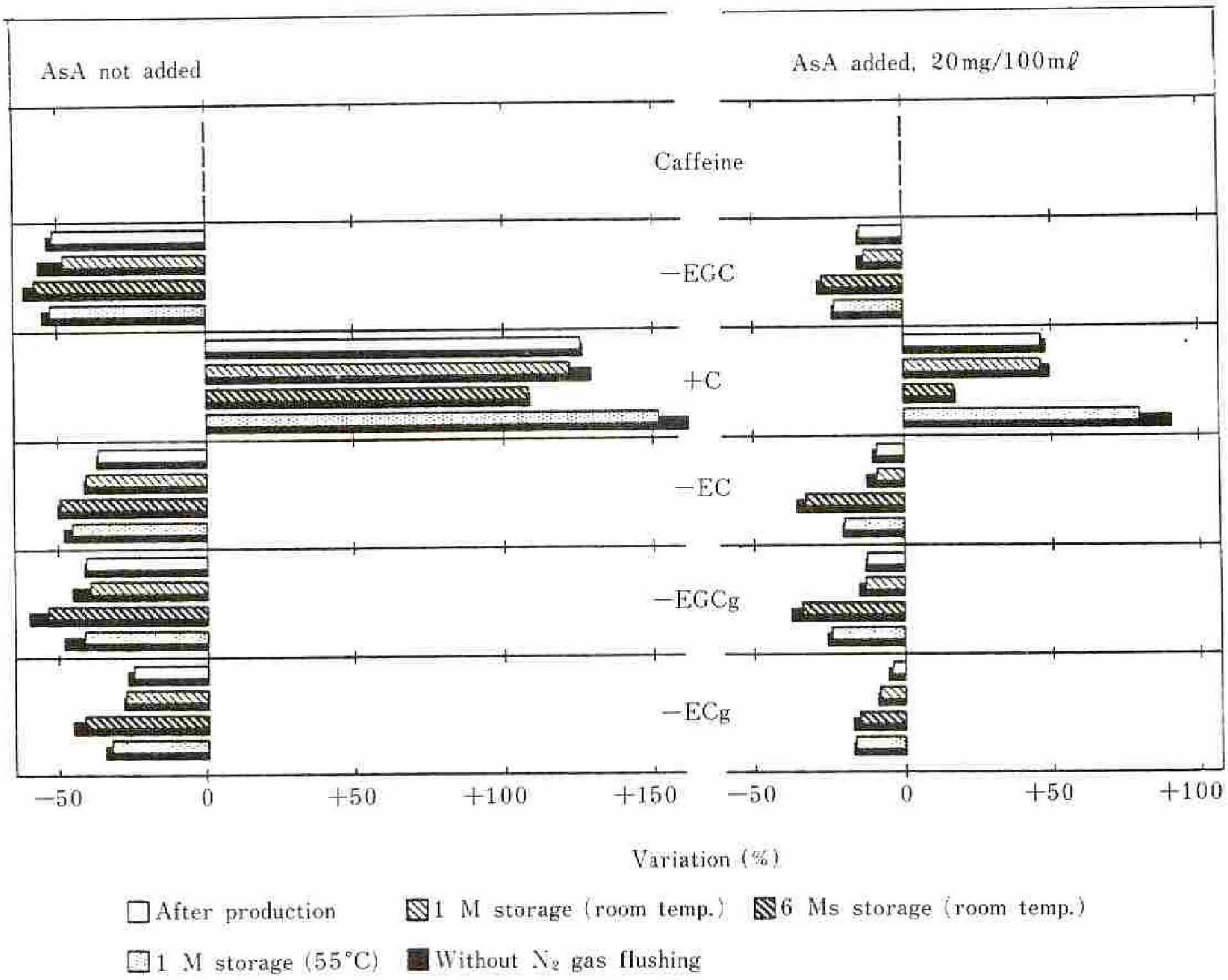

Fig.5. Variation of caffeine and catechins in canned green tea drink during storage with or without L-ascorbic acid.

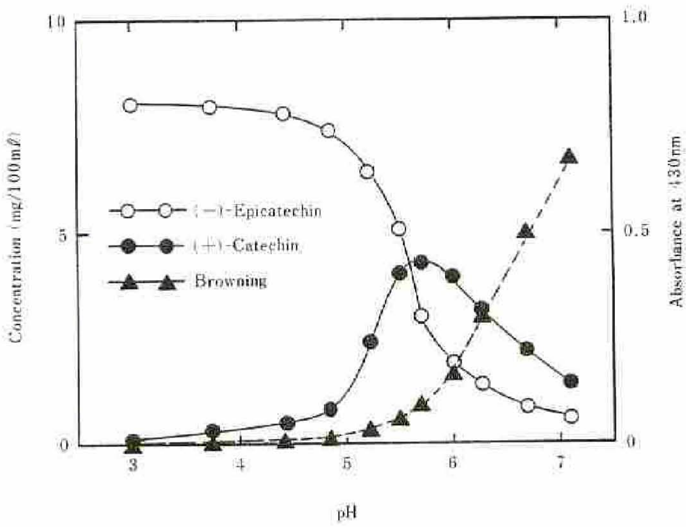

Fig.6. Relation of pH to isomerization from (−)-epicatechin to (+)-catechin and browning of citrate buffer solution heat processed at 121°C for 6 min.

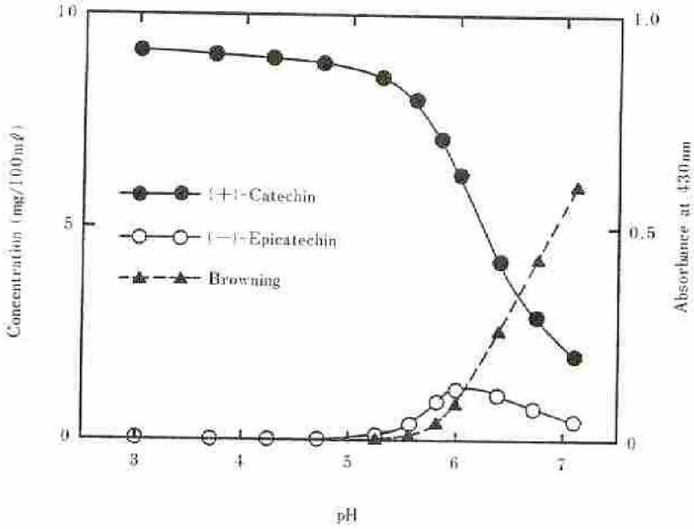

Fig.7. Relation of pH to isomerization from (+)-catechin to (−)-epicatechin and browning of citrate buffer solution heat processed at 121°C for 6 min.

epicatechin due to the epimerization, respectively, and racemization as described in previous research[11] was not observed.

Only two peaks were observed on the chromatograms and a good coincidence of retention time with two corresponding standard reagents were observed throughout the experiments.

Accordingly, a precise qualitative determination was not accomplished to distinguish (+)-catechin from (−)-catechin or (−)-epicatechin from (+)-epicatechin, respectively. Therefore, each reaction product was designated as 'apparent' catechins determined from the peak area of standard (+)-catechin or (−)-epicatechin.

From the results obtained, isomerization of catechins and browning of tea simulating solution scarcely proceeded in weakly acidic media. In contrast, isomerization and browning proceeded in media exceeding pH 5.0, and were accelerated at higher pH near 6.0.

The results suggested that reaction of natural type catechins proceeded via isomerization to corresponding epimer as secondary derivatives finally to polymerization. It was considered that the tendency of remarkable browning at higher pH was a result of formation of quinoid form catechins[12] which induced polymerization.

The similar result obtained by addition of citric acid as an acidifier suggested that the effect of ascorbic acid on prevention of isomerization was not attributed to its reductivity but acidity.

It was well known that secondary function such as taste and odor of natural organic compounds which have chirality tended to change by isomerization. Then the isomerization of catechins in tea drinks might spoil these valuable tertiary functions. This is an undesirable phenomenon and should be prevented as much as possible. Most of the pathological studies[2,3,5] were performed by dosing natural (−)-epi-type catechins to experimental animals as ingredients of diet to prove tertiary function of tea catechins.

Finally, the following conclusions were obtained for manufacturing condition of quality-stable canned tea drinks from these examinations.

1) Caffeine was entirely stable; however, catechins were relatively unstable in various canned tea drinks during the processing and storage.

2) A dominant change of catechins during the processing and storage seemed to be isomerization to corresponding epimer. It was proved by the fact that pure (−)-epicatechin and (+)-catechin stereochemically changed to corresponding isomer by heating in citrate buffer solution.

3) pH of infusion was the most essential factor in stabilizing natural type catechins. A fast isomerization reaction accompanied with browning of solution occured in the region of 6.0 or more of pH. Accordingly, a pH adjustment of infusion or a slightly acidic extraction was preferable in the point of view of protecting natural type catechins.

4) An addition of a minor amount of L-ascorbic acid was effective in stabilizing natural type catechins.

5) Unfermented tea drinks were considerably unstable canned products compared with the fermented one.

Therefore, efficient treatment of water for the extraction, careful adjustment of pH of the infusion, addition of a minor amount of L-ascorbic acid, perfect removal of dissolved oxygen, prompt handling of the extracted solution, nitrogen gas flushing to headspace of cans at the seaming, avoiding excess heating in the sterilization and careful handling during the distribution are required to obtain quality-stable canned unfermented tea drinks.

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