Determination of Ethylenediaminetetra-acetic Acid in Foods by Colorimetry

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A method is described for the determination of ethylenediaminetetra-acetic acid (EDTA) and its metal chelates in foods. The principle of the method is that free copper ion (Cu^{2+}) reacts with bathocuproine but EDTA-Cu does not under definite condition. Food samples were homogenized with 0.1 M NaOH, then subjected to equilibrium dialysis against 0.02 M NaOH. EDTA and its metal chelates in the dialyzate were converted into EDTA-Cu at pH 2.5 by the addition of CuSO_4 . This solution was then divided into two portions of equal volume. One portion was subjected to assay of free Cu^{2+} , and the other to assay of total (the sum of free and chelated) Cu^{2+} . EDTA was calculated from the difference between the absorbances (477 nm) obtained with free and total Cu^{2+} . Analyses of several foods with EDTA-Ca added showed the recovery of 88.7~99.4% for 50 $\mu\text{g/g}$ and 92.7~99.9% for 250 $\mu\text{g/g}$. Food ingredients such as organic acids, proteins, amino acids, peptides, phenolic compounds and emulsifying agents were without interferences.

Ethylenediaminetetra-acetic acid (EDTA), as its disodium or calcium disodium salt, is frequently used in foods such as mayonnaise, salad dressing and canned crab meat. The primary reason for use of EDTA is its sequestering effect on contaminating metal ions. In 1983, The Ministry of Health and Welfare of Japan permitted the use of EDTA as an antioxidant in commercial foods with maximal level of 250 $\mu\text{g/g}$ for canned foods. Consequently, a simple and reliable method is required for routine inspection of EDTA.

Previous methods for the determination of EDTA have involved gas chromatographic¹⁾ and high-performance liquid chromatographic²⁾ procedures. These methods, however, require elaborate derivatization step or the check of column efficiency at intervals for retaining their best performance. We therefore have sought for an alternative method.

In the literatures, Cu^{2+} in alcoholic beverage

has been determined by simple colorimetry following the formation of a chromophore by reaction with a specific reagent³⁾. Free Cu^{2+} reacts with such reagent but EDTA-Cu does not under definite conditions. We now report the simple colorimetric method for the determination of EDTA in foods by adopting the above reaction mechanism.

Materials and Method

Food samples

Mayonnaise, salad dressing and canned foods were purchased from local outlets in Kobe City. Solid foods were minced into pieces less than 2 mm in length and width for extraction of EDTA.

Reagents

Bathocuproine, EDTA-2 Na and several metal chelates of EDTA were obtained from Wako Pure Chemicals. Cellophane tubing (36/32) for dialysis was also from Wako Pure Chemicals.

All other reagents were of analytical grade.

Preparation of food extract

Food sample (10~20 g) was homogenized with 30 ml of 0.1 M NaOH containing 10% (w/w) NaCl to make 50 ml. The homogenate (25 ml) was introduced into a cellophane tubing of which one end had been closed. After the other end was closed, it was subjected to equilibrium dialysis against 175 ml of 0.02 M NaOH for 15 h at 38°C. The dialyzate was referred to as extract solution.

Assay

The extract solution (40 ml) was measured into a 100-ml beaker. After adjusting the pH to about 2.5 with 0.5 M HCl, 4 ml of 0.1 mM CuSO_4 was added to the solution in order to form EDTA-Cu, and then the volume was made to 50 ml with deionized water. This solution was divided into two portions of equal volume (25 ml each). One portion was introduced into a 100-ml separatory funnel containing 5 ml of 0.4 M potassium phosphate buffer (pH 7.0). After the addition of 3 ml of 10 mM ascorbic acid, 5 ml of color producing reagent which consisted of 40 mg bathocuproine in 100 ml of chloroform was also added. After the funnel had been shaken on a mechanical shaker for 10 min, the chloroform layer was taken for measuring the absorbance at 477 nm. The absorbance obtained corresponded to free Cu^{2+} that had not participate in chelate formation with EDTA.

The other portion was treated in the same manner as above, except that 0.4 M Tris-HCl buffer (pH 8.5) containing 1 mM CoCl_2 replaced the phosphate buffer. The absorbance obtained corresponded to total (the sum of free and chelated) Cu^{2+} . EDTA content was then calculated from the difference between the absorbances obtained with free and total Cu^{2+} by means of a calibration curve.

Results and Discussion

Establishment of reaction conditions

It was shown that color development reaction of Cu^{2+} with bathocuproine should be done in the pH range of 4 to 10³⁾. We have therefore sought the conditions where EDTA-Cu does not

react with bathocuproine within the above pH range. As shown in Fig. 1, EDTA-Cu did not react with bathocuproine in the pH range of 6.5 to 7.5 in the presence of Zn^{2+} . EDTA-Cu reacted with this reagent to some extent even at pH 7.0 when potassium phosphate buffer was replaced with other buffers such as Tris-HCl or acetate buffer (data not shown). Accordingly, we selected potassium phosphate buffer (pH 7.0) for assay of free Cu^{2+} .

Next, in order to assay total Cu^{2+} , the condition must be found where the complete reaction of EDTA-Cu with bathocuproine took place as well as free Cu^{2+} . It follows from the results in Fig. 2 that reaction pH gave a great effect on the reaction of EDTA-Cu with bathocuproine. When the reaction of EDTA-Cu with this reagent was carried out below pH 6.5, the complete reaction was not achieved even in the presence of foreign metal ion (Co^{2+}). In contrast, the reaction of EDTA-Cu with bathocuproine was almost stoichiometric when the reaction was done above pH 8.0 in the presence of Co^{2+} . The use of other metal ions instead of Co^{2+} resulted in incomplete reaction of EDTA-Cu with bathocuproine (Table 1). These results suggest the prerequisite of Co^{2+} for complete reaction of EDTA-Cu with bathocup-

Fig. 1 Reaction of EDTA-Cu with bathocuproine as the function of pH

EDTA-Cu ($0.2 \mu\text{mol}$), dissolved in buffers with different pH, was reacted with bathocuproine in the presence of Zn^{2+} ($1 \mu\text{mol}$). Buffers used were as follows: 0.1 M acetate (pH 5~6), 0.1 M potassium phosphate (pH 6~7.5), 0.1 M Tris-HCl (pH 7.5~9), 0.1 M carbonate (pH 9~10).

Fig. 2 Reaction of EDTA-Cu with bathocuproine in the presence of Co^{2+} as the function of pH

EDTA-Cu ($0.2 \mu\text{mol}$), dissolved in buffers with different pH, was reacted with bathocuproine in the presence of CoCl_2 ($2 \mu\text{mol}$). Buffers used were as follows: 0.1M acetate (pH 5~6), 0.1M potassium phosphate (pH 6~7.5), 0.1M Tris-HCl (pH 7.5~9), 0.1M carbonate (pH 9~10). The optical intensity of equimolar CuSO_4 ($0.2 \mu\text{mol}$) was 0.56.

Table 1 Reactivity of EDTA-Cu toward bathocuproine in the presence of foreign metal ion

Metal ion	Absorbance at 477 nm
Co^{2+}	0.56
Sn^{2+}	0.26
Zn^{2+}	0.23
Pb^{2+}	0.29

Twenty ml of EDTA-Cu solution ($10 \mu\text{M}$) was reacted with bathocuproine at pH 8.5 using Tris-HCl buffer (0.1M) in the presence of foreign metal ions indicated. The amount of each metal ion used was $1 \mu\text{mol}$. The absorbance of equimolar CuSO_4 ($0.2 \mu\text{mol}$) was 0.56.

roine. The use of CoCl_2 for assay of total Cu^{2+} is reasonable because its presence did not interfere with Cu^{2+} -bathocuproine reaction and its content in commercial foods could be negligible. Accordingly, Tris-HCl buffer containing CoCl_2 was adopted for assay of total Cu^{2+} .

Whether the optical intensities obtained at pH 7.0 and pH 8.5 in Cu^{2+} -bathocuproine reaction were identical was also critical for successful application of the present method for determination of EDTA. Fortunately, the

optical intensity was constant in the pH range of 4 to 10, and independent of component of buffers used (data not shown).

Calibration curve

A series of standard EDTA solution, prepared by dissolving EDTA-2 Na in deionized water, were subjected to assay of both free and total Cu^{2+} . Then, the difference between the absorbance at 477 nm obtained by both assays was plotted against the amount of EDTA (as calcium disodium salt). The straight line fitting to 5 points had a slope of 0.98 and an intercept of 0.02 with a correlation coefficient of 0.996. The lowest level for linearity was $0.374 \mu\text{g/ml}$. Therefore, the present method had a minimum quantitative level of about $7 \mu\text{g/g}$ when 10 g of sample was extracted and diluted to 200 ml.

Conditions for EDTA-Cu formation

In the present method, EDTA and its metal chelates must be converted into EDTA-Cu prior to assay. Fig. 3 shows examples of the conversion of representative metal chelates into EDTA-Cu as the function of pH. The formation of EDTA-Cu from EDTA-Ca was achieved below pH 8.0 (Fig. 3). However, pH value below 3.0 was necessary for the conversion of EDTA-Zn. Table 2 shows the formation ratio of EDTA-Cu from several metal chelates. The range of metal chelates examined were limited to those expected to occur in most food sam-

Fig. 3 Conversion of metal chelates of EDTA into EDTA-Cu as the function of pH

—△—: EDTA-Ca; —▲—: EDTA-Zn

Table 2 Formation ratio of EDTA-Cu from several kinds of EDTA metal chelates at pH 2.5

Kind of EDTA metal chelate	Formation ratio of EDTA-Cu (%)
EDTA-Sn	99.8
EDTA-Zn	99.7
EDTA-Pb	99.6
EDTA-Fe	1.8

The amount of each metal chelate used was 0.2 μ mol.

ples. All the metal chelates examined could be converted into EDTA-Cu, with exception of EDTA-Fe. It was shown that EDTA-Fe has a stability constant higher than that of EDTA-Cu⁴⁾. This may account for the little conversion of EDTA-Fe into EDTA-Cu. Thus, it is recommended that EDTA-Cu formation should be done below pH 3.0. Once the metal chelates were converted into EDTA-Cu, the reverse reaction could not take place even when pH was brought to about 7.0 with potassium phosphate buffer. This aspect is beneficial to the present method, in which the assay of free Cu²⁺ was carried out at pH 7.0. We previously reported that EDTA-Fe was recovered as EDTA-2 Na through dissociation when dialysis was carried out at pH value above 12⁵⁾. In the present method, dialysis of the sample as the extraction procedure was done above pH 12. Accordingly, EDTA-Fe could be determined by the present method even if this chelate remained unchanged at the EDTA-Cu formation step (Table 2).

Recovery test

The utility of the present method was tested in 16 samples of 4 kinds of foods. EDTA was added to each food at levels of 50 and 250 μ g/g as its calcium disodium salt. The food samples were then analyzed according to the method described in Materials and Method, the results being presented in Table 3. Recovery of EDTA from mayonnaise and salad dressing were appreciably lower than those from other food samples, as is obvious from Table 3. Thus, the recovery of EDTA is dependent on the food to be analyzed. Food samples examined were

Table 3 Recovery of EDTA from several foods

Food	Added (μ g/g)	Recovery* (%)
Mayonnaise	50	89.2 $\pm$ 5.4
	250	93.4 $\pm$ 4.8
Salad dressing (separate type)	50	88.7 $\pm$ 6.1
	250	92.7 $\pm$ 5.7
Canned crab meat	50	99.4 $\pm$ 3.9
	250	99.6 $\pm$ 3.7
Canned bamboo shoot	50	98.8 $\pm$ 5.2
	250	99.9 $\pm$ 4.5

* Data represent mean value and standard deviation of four trials.

EDTA as its calcium disodium salt was added to each food indicated.

found to contain no EDTA (data not shown).

Interfering substances in foods

An attempt was made to evaluate the interference of some food ingredients on the present method. The interfering substances examined were limited to their possible ranges in foods examined. Such organic acids as citric, tartaric and oxalic did not interfere with the present method even at levels in excess of 1% (w/w). Amino acids (glycine, glutamic acid) and peptide (glycylglycine) were also without interferences at levels of 0.01% (w/w). Gallic acid, a class of phenolic compounds, did not affect the present method at levels of 0.01% (w/w). These results may be ascribed to the fact that Cu²⁺ shows a preference of EDTA to these compounds examined with respect to complex formation. When assay of EDTA was directly applied to real sample such as mayonnaise, rich in protein and emulsifying agents, the value obtained was less than one-tenth of the added level (50 μ g/g). This may be due to the formation of Cu²⁺-protein complex and/or to emulsification of the reaction mixture. The use of equilibrium dialysis at the extraction step could effectively eliminate their interferences, as is obvious from the results in Table 3. Thus, an important feature of the present method is that extraction of EDTA is performed by using equilibrium dialysis, which allows some interfering substances such as proteins and emul-

sifying agents to be removed.

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食品中のエチレンジアミン四酢酸の比色定量

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本報では、食品中のエチレンジアミン四酢酸 (EDTA) とその金属キレート の定量法について報告する。定量法の原理は、一定の条件下で、銅イオン (Cu^{2+}) はバソクプロインと反応するが EDTA-Cu は反応しないことである。食品を 0.1 M の NaOH でホモジナイズし、0.02 M の NaOH に対し透析する。透析液の一定量に、既知量の CuSO_4 を加え pH 2.5 で EDTA およびその金属キレートを EDTA-Cu に変換する。その後、溶液を 2 等分し、一方を遊離 Cu^{2+} の測定に供する。もう一方を EDTA-Cu を含め総 Cu^{2+} の測定に供する。EDTA 量は、総 Cu^{2+} より得られた吸光度 (477 nm) より遊離 Cu^{2+} より得られた吸光度を差し引いた値から検量線より求める。数種の食品に添加した EDTA-Ca の回収率は 50 $\mu\text{g/g}$ で 88.7~99.4%, 250 $\mu\text{g/g}$ で 92.7~99.9% であった。有機酸、アミノ酸、ペプチド、蛋白質および乳化剤などは、本定量法を妨害しなかった。