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Determination of ethylenediaminetetraacetic acid and its salts in canned mushrooms by reversed-phase ion-pair liquid chromatography

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ABSTRACT

A method for the determination of ethylenediaminetetraacetic acid (EDTA) in the surrounding liquid of canned mushrooms, based on reversed-phase ion-pair high-performance liquid chromatography (HPLC), was developed. Sample pretreatment simply consists in addition of copper(II) chloride and ascorbic acid to the filtered sample and microfiltration prior to injection into the HPLC system. The copper(II)–EDTA complex thus formed is separated with a water–methanol mobile phase containing tetrabutylammonium as the counter ion at pH 4.0 and UV detection is applied at 300 nm. Iron(III) concentrations up to 33 mg/l do not interfere. The recovery is 105.6 ± 3.0% at a level of 18 mg/l EDTA and 101.5 ± 0.8% at a level of 178 mg/l EDTA. The method is linear in the range 7–178 mg/l EDTA. The repeatability of the procedure is 5 mg/l EDTA (n = 20). The limit of determination is 10 mg/l EDTA. The day-to-day (n = 10) relative standard deviation is 2.3%.

INTRODUCTION

Enzymatic browning is a negative quality aspect of edible mushrooms. It is an indirect result of the action of the copper-containing enzyme polyphenol oxidase (PPO), which can react with some hormones to form melanin. EDTA is a complexing agent which may inactivate the action of PPO by sequestering copper [1]. Consequently, enzymatic browning may be inhibited by the addition of EDTA. However, in the Netherlands the addition of EDTA to canned mushrooms is prohibited except for export to the U.S.A.

Various methods have been described for the determination of EDTA in different matrices, including colorimetry [2], titrimetry [3], anion-exchange high-performance liquid chromatography (HPLC) [4] and reversed-phase ion-pair HPLC [5–7]. In the last technique, tetrabutylammonium was used as the counter ion and EDTA was separated as its copper complex [5] or as its iron(III) complex [6,7], which was detected by UV absorption at 254 nm [5,6], 245 or 300 nm [7].

In our hands, the colorimetric method that was described for food products, including canned mushrooms [2], yielded very disappointing results, even if applied to standard solutions, and with the titrimetric method discrimination between EDTA and other chelates is not possible. For this reason, a reversed-phase ion-pair HPLC method was developed, based on the method described by De Kleijn *et al.* [7].

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EXPERIMENTAL

Chemicals and reagents

Ethylenediaminetetraacetic acid, calcium disodium salt dihydrate (Ca-EDTA), was obtained from Sigma. Tetrabutylammonium hydroxide (20% aqueous solution) was purchased from Merck. All other chemicals were of analytical or reagent grade (Merck) and were used without further purification. Water was purified with a Milli-Q water-purification system (Millipore). Copper(II) chloride solution was prepared by dissolving 110 mg of copper(II) chloride dihydrate in 30 ml of glacial acetic acid and diluting with water to a final volume of 100 ml. Iron(III) chloride solution was prepared by dissolving 175 mg of iron(III) chloride hexahydrate in 30 ml of glacial acetic acid and diluting with water to a final volume of 100 ml. A stock standard solution was prepared by dissolving 1 g of Ca-EDTA in 100 ml of water. From this stock solution calibration standards were prepared in water containing 10, 50, 100 and 250 mg/l Ca-EDTA.

Chromatography

The apparatus consisted of the following parts: a Waters Model 6000 A liquid chromatographic pump, a Merck/Hitachi 655A UV–VIS detector, a Rheodyne Model 7125 syringe-loading sample injector, a Kontron Model 480 oven controller and an Olivetti personal computer with Nelson Integration software.

The analytical column was LiChrosorb 5-RP-18 (150 × 4.6 mm I.D.) (Chrompack). The precolumn was Chromguard C_{18} (10 × 3.0 mm I.D.) (Chrompack). The mobile phase was the same as that applied by De Kleijn *et al.* [7] and was prepared by mixing 1 l of 0.03 *M* sodium acetate–acetic acid buffer (pH 4) containing 20 m*M* tetrabutylammonium hydroxide with 100 ml of methanol. Before use the mobile phase was filtered using a Millipore solvent clarification kit equipped with a 0.45-μm HV filter.

The chromatographic conditions used throughout this study were: flow-rate 1.0 ml/min, injection volume 20 μl and column temperature 50°C. Unless stated otherwise, the detection wavelength was 300 nm. Prior to analysis the chromatographic system was conditioned overnight with the mobile phase.

Sample preparation

The surrounding liquid of canned mushrooms was filtered through Schleicher & Schüll paper (595½). Unless stated otherwise, to 22.5 ml of the filtered sample 2.5 ml of the copper(II) chloride solution and 20 mg of L-(+)-ascorbic acid were added. After thorough mixing and microfiltration over a 0.45-μm Acrodisc disposable filter assembly (Gelman Sciences), the filtrate was ready for injection into the HPLC system.

Calibration and quantification

Calibration graphs were obtained by least-squares analysis using calibration standards containing 10, 50, 100 and 250 mg/l Ca-EDTA. Prior to injection into the HPLC system, these standards were pretreated in the same way as the samples except for paper filtration and addition of L-(+)-ascorbic acid.

In daily use, quantification was performed by comparison of the peak area of a sample with that of a calibration standard containing 100 mg/l Ca-EDTA. All

concentrations are expressed in mg/l EDTA by multiplying the concentration of Ca-EDTA by a factor of 0.712.

RESULTS AND DISCUSSION

Complexation with iron(III) or copper(II)

EDTA can be complexed with, among other cations, iron(III) and copper(II) ions [7,8]. In reversed-phase ion-pair chromatography, the retention of the Cu(II)–EDTA complex is larger than that of the Fe(III)–EDTA complex [7]. Under the conditions described under Experimental, the retention time of the former complex was about 8.5 min (see Fig. 1a) while that of the latter was about 3.5 min.

Using the same mobile phase and a similar type of analytical column, De Kleijn *et al.* [7] determined EDTA in mayonnaise as the Fe(III)–EDTA complex after extraction with dichloromethane. Applying the sample preparation described under Experimental, in most of the samples of canned mushrooms the Fe(III)–EDTA peak was not completely separated from one or more matrix peaks. In our hands, it was not possible to improve this separation by changing the composition of the mobile phase, *i.e.*, by decreasing the methanol content or by increasing the tetrabutylammonium concentration.

For the Cu(II)–EDTA peak no interferences from matrix peaks were observed (see Fig. 1b), so copper(II) was selected for complexation.

Wavelength selection

Investigations on wavelength selection were performed through analysis of several samples of canned mushrooms, applying detection at 245, 270 or 300 nm. Although the molar absorptivity of the Cu(II)–EDTA complex is smaller at 300 nm than at 245 or 270 nm, the best results were obtained at 300 nm with regard to selectivity (see Fig. 1b) and consequently 300 nm was selected as the detection wavelength.

Interference from iron(III) ions

The stability constant of the Fe(III)–EDTA complex ($\log k_1 = 25.1$) is higher than that of the Cu(II)–EDTA complex ($\log k_1 = 18.8$) [8]. Consequently, the determination of EDTA suffers interference if iron(III) ions from the surrounding liquid of canned mushrooms and/or from the HPLC system are present. This interference can be overcome by the addition of L-(+)-ascorbic acid to the surrounding liquid of canned mushrooms.

L-(+)-Ascorbic acid reduces iron(III) to iron(II), which forms a weaker complex with EDTA ($\log k_1 = 14.3$) [8] than copper(II).

In order to check the effect of L-(+)-ascorbic acid, the following experiment was performed. After complexation of a standard containing 18 mg/l EDTA with copper(II) according to the normal procedure, iron(III) ions were added at a concentration of 33 mg/l [about five times the maximum concentration in the liquid of canned mushrooms ($n = 20$)], resulting in total disappearance of the Cu(II)–EDTA peak after a waiting time of 20 min (the waiting time is necessary because of the relatively slow reaction rates of the complex formation and dissociation reactions [8]). After addition of L-(+)-ascorbic acid (20 mg to 25 ml) the Cu(II)–EDTA complex was formed again quantitatively.

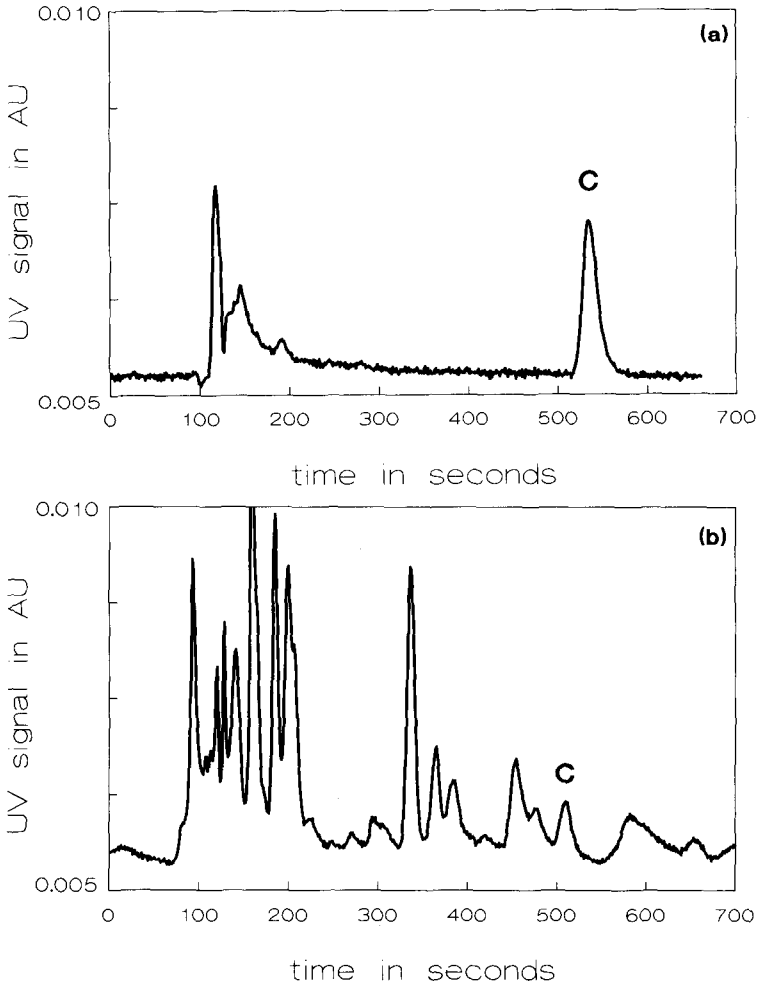

Fig. 1. Complexation of EDTA with copper(II). (a) Standard solution containing 18 mg/l EDTA; (b) sample of surrounding liquid of canned mushrooms containing 8 mg/l EDTA. For sample preparation and chromatographic conditions, see Experimental. C = Cu(II)–EDTA complex.

Confirmation of the presence of EDTA

The difference between the stability constants of the Cu(II)–EDTA and Fe(III)–EDTA complexes can be exploited for confirmation of the presence of EDTA in the surrounding liquid of the canned mushrooms. If, applying the normal sample preparation described under Experimental, a Cu(II)–EDTA peak is detected, this peak will disappear after addition of iron(III) chloride to the filtrate ready for injection into the HPLC system. The volume of the iron(III) chloride solution (see Experimental) to be added has to be adjusted to the presence of L-(+)-ascorbic acid in the filtrate. Assuming complete stability, 35 ml of iron(III) chloride solution would be required for complete oxidation of 20 mg of L-(+)-ascorbic acid [10]. However, owing to the instability of L-(+)-ascorbic acid in aqueous solutions, in our experience the addition

of 5 ml of iron(III) chloride was sufficient for complete dissociation of the Cu(II)–EDTA complex (again a waiting time of 20 min was necessary).

Determination of salts of EDTA

It is assumed that if EDTA is added to canned mushrooms in agro-industrial practice it is applied as the calcium disodium salt. However, if EDTA is added as such or as another salt, it will still be determined with the method described here, provided that the stability constant of the salt is smaller than that of the Cu(II)–EDTA complex. This holds true for most salts, *e.g.*, the Na^+, Li^+ and Ba^{2+} salts [8,9]. For salts with higher stability constants, such as Co(III)–EDTA and Bi(III)–EDTA [8], sequestration of copper in PPO (see Introduction) will not occur and consequently these salts do not prevent enzymatic browning.

Quantitative aspects

The linearity of the method was investigated with calibration standards containing 10, 50, 100 and 250 mg/l Ca-EDTA, corresponding to 7–178 mg/l EDTA. For both peak-area and peak-height measurements linear calibration graphs were obtained over the whole range, with correlation coefficients of 0.9999 and intercepts corresponding to EDTA concentrations <1 mg/l.

The detection limit, based on a signal-to-noise-ratio of 3, was 1.4 mg/l EDTA. The limit of determination, defined as the mean concentration of EDTA in blank samples ($n = 20$), increased with six times the standard deviation ($\bar{x} + 6\sigma_{n-1}$), was 10 mg/l EDTA. The recovery was $105.6 \pm 3.0\%$ ($n = 10$) and $101.5 \pm 0.8\%$ ($n = 9$) at concentrations of 18 and 178 mg/l EDTA, respectively.

The repeatability, as calculated from the differences between the results of duplicate determinations on 20 samples containing EDTA at concentrations in the range 9–114 mg/l, was 5 mg/l. The day-to-day ($n = 10$) relative standard deviation was 2.3% at a concentration of 79 mg/l EDTA.

Determination of EDTA in canned mushrooms

Applying the method described here, the EDTA concentrations in about 150 samples of canned mushrooms were determined. These samples were produced by almost all Dutch suppliers. If EDTA concentrations exceeded the limit of determination (10 mg/l) the results were verified by means of a titrimetric method [3], which yielded corresponding results. During a 3-month period, about 300 sample solutions were injected onto the same chromatographic column; the retention time of the Cu(II)–EDTA peak gradually decreased from about 9 to 6 min. However, the column efficiency and the resolution between the Cu(II)–EDTA peak and surrounding matrix peaks were not negatively affected.

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