Leaching of the Plasticizer, Acetyl Tributyl Citrate: (ATBC) from Plastic Kitchen Wrap

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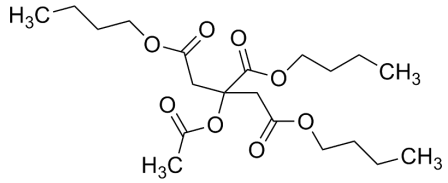
The leaching of acetyl tributyl citrate (ATBC) from plastic kitchen wrap into water, aqueous acetic acid solution, and aqueous skim milk solution heated to 60°C was investigated. A new extraction method was developed to determine the leaching rate. It was found that ATBC is prone to migrate into protein liquids, such as aqueous skim milk solution.

Key words —— acetyl tributyl citrate (ATBC), kitchen wrap, leakage, skim milk, extraction

INTRODUCTION

Acetyl tributyl citrate (ATBC, Fig. 1) is one of the most widely used plasticizers. Plastic kitchen wrap [polyvinylidene chloride (Saran Wrap) films], which is commonly used in the packaging of a large variety of foodstuffs, contains ATBC. The wrap is being used increasingly in microwave ovens to cover foods while cooking and heating. During heating, ATBC along with other plasticizers and stabilizers may leach from the wrap into food. The leaching of plasticizers into aqueous or organic solvent has been examined. Especially, the migration of phthalate plasticizers from blood containers is well documented in the literature[1] and it is known that a use of citrate instead of phthalate produces a reduction in leaching.[2] It is also reported that the leaching of ATBC into aqueous water is low in general.[3] However, the citrates have a higher toxicity to HeLa cells than phthalates.[4] Added to this, the toxicity of ATBC in laboratory animals is well known[5] and recent results have shown that ATBC is toxic to human KB cells,[6] and that ATBC inhibits the proliferation of Lympho node T cells.[7] In consideration of above, we launched to examine the leaching of ATBC into food with actually employing foodstuffs. We examined the amount of ATBC which leaches from kitchen wrap in contact with aqueous solutions of acetic acid, skim milk, and powdered milk.



ATBC: acetyl tributyl citrate

Fig. 1. Structure of ATBC

MATERIALS AND METHODS

Materials —— ATBC, acetic acid, and skim milk powder were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Although the ingredients of skim milk are not disclosed by Wako Pure Chemical Industries, Ltd., skim milk generally contains proteins and saccharides but not lipids. Hagukumi powdered milk was purchased from Morinaga Milk Industry Co., Ltd. (Tokyo, Japan). The ingredients of powdered milk (100 g) are: milk constituent (72.2 g), conditioned lipid (24.3 g), dextrin (3.0 g), and oligosaccharide (0.5 g). Saran Wrap was purchased from Asahi Kasei Home Products Corp. (Tokyo, Japan). Analytical and preparative TLC was conducted on precoated TLC plates (silica gel 60 F_{254}, Merck, Whitehouse Station, NJ, U.S.A.). Column chromatography was performed using Merck silica gel 60N (100–210 μm). All anhydrous solvents were purified following standard methods.

Experimental in General —— ^{1}H-NMR spectra were determined on a JEOL 400 MHz spectrometer (JNM-LD400: JEOL, Tokyo, Japan) using $CDCl_3$ [with tetramethylsilane (TMS) as the internal reference] solution. High Resolution Mass Spectra (HRMS) were obtained on [Liquid Chromatography Mass Spectrometry (LCMS)-IT (Ion-Trap)-

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TOF (Time-of-Flight): Shimadzu, Kyoto, Japan]. Elemental analysis was performed using a Perkin-Elmer 2400 CHN analyzer, Perkin-Elmer, Waltham, MA, U.S.A.). We carried out three independent runs for each experiment, and typical data are shown.

Determination of the ATBC Recovery Rate from Aqueous Solution of Skim Milk/Powdered Milk —— A solution of ATBC (1.08 g) in 3% (w/w) aqueous solution of skim milk (50 g) was stirred at 60°C for 24 hr, and then 50 ml of acetone was added to the solution to precipitate the protein. After centrifugation, the supernatant was extracted with Ethyl acetate (AcOEt). The precipitated protein was stirred vigorously in AcOEt and again centrifuged so that the ATBC was transferred from the protein to AcOEt. The combined organic layers were dried with Na_2SO_4 and evaporated. The residue was purified by silica-gel chromatography (AcOEt: hexane = 1 : 8) to recover ATBC, which weighed 1.00 g (recovery rate: 93%). Spectroscopic data (^{1}H-NMR and HRMS) for the recovered ATBC (Anal. Calcd for $C_{20}H_{34}O_8$: C, 59.68, H, 8.51. Found: C, 59.75, H, 8.32.) were identical with those for the purchased one.

The same procedure as that described above was also followed for powdered milk.

Extraction of ATBC from Aqueous Solution of Acetic Acid/Distilled Water after Contact with Kitchen Wrap —— Glass plates covered with kitchen wrap (4.56 g, total area of 2388 cm^2) were wet thoroughly with 4% (v/v) aqueous acetic acid (2500 ml) and heated at 60°C with stirring for 9 hr. The aqueous acetic acid was extracted with AcOEt. The combined extracts were dried with Na_2SO_4 and evaporated. The residue was purified by silica-gel chromatography (AcOEt : hexane = 1 : 8) to give ATBC, which weighed 12.6 mg. Spectroscopic data (^{1}H-NMR and HRMS) for the obtained ATBC were identical with those for the purchased one.

The same procedure as that described above was also followed for distilled water.

Extraction of ATBC from Aqueous Solution of Skim Milk after Contact with Kitchen Wrap —— Glass plates covered with kitchen wrap (3.32 g, total area of 1800 cm^2) were wet thoroughly with 3% (w/w) aqueous skim milk (2.1 kg, 1750 ml) and heated at 60°C with stirring for 9 hr. To the aqueous skim milk, 1750 ml of acetone was added to precipitate protein from the solution. After centrifugation, the supernatant (3000 ml) was extracted with AcOEt (6000 ml). The precipitated protein was stirred vigorously in AcOEt and again centrifuged so that the ATBC was transferred from the protein to AcOEt. The combined extracts were dried with Na_2SO_4 and evaporated. The residue was purified by silica-gel chromatography (AcOEt: hexane = 1 : 8) to give ATBC, which weighed 27.9 mg. Spectroscopic data (^{1}H-NMR and HRMS) for the obtained ATBC (Anal. Calcd for $C_{20}H_{34}O_8$: C, 59.68, H, 8.51. Found: C, 59.71, H, 8.69.) were identical with those for the purchased one.

RESULTS AND DISCUSSION

Determination of the Recovery Rate of ATBC from Aqueous Solution of Skim Milk and Powdered Milk

To the best of our knowledge, few attempts have so far been made to establish a reliable method for the extraction of ATBC from edible liquids. It was anticipated that the constituents of skim milk and powdered milk (protein, lipid, carbohydrate, *etc.*) would hamper the complete extraction of ATBC. Thus we examined the reliability of our extraction method before performing the extraction experiments. A given quantity of ATBC was added to aqueous solutions of skim milk (3%, 15%) and of powdered milk (3%, 15%) and recovered using our extraction method. The results are shown in Table 1. The recovery rates were not satisfactory except in the case of 3% aqueous skim milk. It was found that ATBC is prone to transfer into protein and lipids contained in skim milk and powdered milk, making the extraction of ATBC very difficult. Thus, the recovery rates were low (75%, 85%, 67%). We therefore abandoned the examination of aqueous powdered milk, and turned our attention to water, acetic solution, and skim milk solution (3%).

Leaching of ATBC from Kitchen Wrap into Water, Acetic Solution, and Aqueous Solution of Skim Milk

The amount of ATBC that leached from the plastic kitchen wrap into water, acetic acid solution (4%), and aqueous solution of skim milk (3%) was examined using our extraction method. It is known that the content of ATBC in kitchen wrap is about 5%.[8] Our preliminary experiments also confirmed that extraction with CH_2Cl_2 from the wrap (2.71 g) yielded all of the ATBC contained (136 mg, 5%). Thus we determined the leaching rate based on the reported data that 5% of ATBC is contained in kitchen wrap. The detailed results are given in

Table 1. Recovery Rate of ATBC from Aqueous Skim Milk and Aqueous Powdered Milk

	Skim milk solution		Powdered milk solution	
ATBC	3%	15%	3%	15%
Added (g)	1.08 g	1.08 g	1.00 g	1.01 g
Recovered (g)	1.00 g	0.75 g	0.85 g	0.67 g
Recovery rate (%)[a)]	93%	75%	85%	67%
	(91%, 96%)	(70%, 76%)	(85%, 89%)	(59%, 69%)

a) Since we carried out three independent runs for each experiment, typical data are shown and data of other two runs are shown in parentheses.

Table 2. Leaching of ATBC from Kitchen Wrap into Water, Acetic Acid Solution, and Aqueous Solution of Skim Milk

		Wrap (g)	Conditions	ATBC contained (mg)[a)]	Leaching of ATBC (mg)	Leaching rate (%)[b)]
Entry 1	In water (2000 ml)	4.58	60°C, 24 hr	229	4.5	2.0 (1.8, 2.0)
Entry 2	In acetic acid (4%, 2500 ml)	4.56	60°C, 9 hr	228	12.6	5.5 (5.5, 5.6)
Entry 3	In skim milk (3%, 1750 ml)	3.32	60°C, 9 hr	166	27.9	16.8 (18.7, 15.1)

a) Based on the reported data that the content of ATBC in kitchen wrap is 5%. *b*) Since we carried out three independent runs for each experiment, typical data are shown and data of other two runs are shown in parentheses.

Table 2. After 24-hr contact with water at 60°C (Our preliminary experiments showed that a contact with aqueous solution at 60°C for 9–24 hr provided the best conditions for examining the leaching of ATBC. A higher or lower temperature did not give a reliable reproducibility), 2.0% of the ATBC contained in the kitchen wrap had leached into water (Table 2, Entry 1). After 9-hr contact with 4% aqueous acetic acid at 60°C, 5.5% of the ATBC had leached into the solution (Table 2, Entry 2). Surprisingly, 16.8% of the ATBC had leached into aqueous skim milk solution after 9-hr contact at 60°C (Table 2, Entry 3). Considering the recovery rate (93%) shown in Table 1, the actual leaching rate into aqueous skim milk should be 18%. What should be stressed is that ATBC is prone to migrate into liquid protein, such as aqueous skim milk solution. Although the amount of ATBC leached into skim milk is very low as toxic agents [LD_{50} for ATBC is 31.5 grams/kilograms body weight (g/kg bwt)],[9)] it is widely known that ATBC is not volatile (boiling point of ATBC is 173°C/1 mmHg), and stable under normal conditions, and hence, the contaminant in skim milk might have harmful effects on infants, who drink it.

In conclusion, the present results show that ATBC leaches from kitchen wrap into aqueous liquids. It is noteworthy that the highest leaching rate was observed in protein-containing aqueous skim milk solution. It appears reasonable to state that plastic kitchen wrap containing ATBC should be used carefully when it will come into direct contact with liquid protein such as milk. Further studies on the leaching of ATBC from kitchen wrap into foodstuffs under various conditions will be reported later.

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