Temperature and pH Effects on the Release of Chromium from Stainless Steel into Water and Fruit Juices

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The effects of pH and temperature on the release of Cr into water and juices exposed to stainless steel were measured in order to investigate whether such incidental Cr in acid foods may have nutritional significance. By use of graphite furnace atomic absorption spectrometry, Cr was determined in acidified water (HCl) and in processed and hand-squeezed fruit juices, before and after heat treatment in stainless steelware. No Cr was released into unacidified water. After 1 h at 90–95 °C, Cr was 54 ppb in pH 2.5 water and 16 ppb in pH 3.0 water; amounts of Cr released into juices were 31–50 ppb (3–5 μg/100 mL). Juice Cr concentration did not vary with pH, probably due to differing acid components. The released Cr passed through a 0.2-μm filter. Since the recommended range for daily Cr intake is 50–200 μg, stainless steelware may contribute to human Cr nutrition if incidental inorganic Cr is absorbed and converted to its biologically active form.

Dietary chromium (Cr) is needed in microgram amounts by animals and man for normal insulin activity and glucose metabolism (Mertz, 1981). Until recently, the ultratrace amounts of Cr detected in acid foods exposed to stainless steel during industrial processing and home preparation were considered too low to be of nutritional consequence. Except for a study two decades ago showing significant Cr in rhubarb and tomatoes cooked in stainless steel pots (Schroeder et al., 1962), Cr-containing equipment, drugs, pigments, and other ingested nonfood materials have been largely ignored as potential sources of dietary Cr. This judgement is now recognized to have been based on erroneously high measurements of Cr in human blood and urine (Guthrie et al., 1978b). Recently, using clean room techniques to reduce environmental contamination and new analytical instrumentation capable of detecting picogram amounts of Cr, investigators have measured <0.5 ppb of Cr in human blood and urine (Guthrie et al., 1978a; Kayne et al., 1978; Versieck et al., 1978; Veillon et al., 1979). This is more than 10-fold lower than previous determinations. In view of these extremely low Cr levels in the body, even very small amounts of incidental Cr released into foods merit investigation for potential nutritional significance.

The experiments reported here were undertaken, therefore, to investigate the conditions favoring Cr release from stainless steelware when home cooking procedures are simulated and to assess the incidental Cr "contamination" likely to result from the commercial processing of acid foods. The purposes of these experiments were (1) to measure Cr release from stainless steel pots into water at pH levels characteristic of acid foods and at temperature and time intervals which are common in cooking and (2) to measure Cr release into fruit juices exposed to the same pots under the same conditions, to assess to probable contribution of equipment and containers used during processing of acid juices, and to determine whether the Cr released into acid juices from stainless steel is within the submicron range so that it has a potential for intestinal absorption.

EXPERIMENT 1: CHROMIUM IN ACIDIFIED WATER

Materials and Methods. *Sample Preparation.* Cr-free water (Milli-Q System, Millipore, Bedford, MA) was acidified with Cr-free HCl to pH 2.5 and pH 3.0. These acidities were selected to represent pHs commonly encountered in foods. Acid food pHs vary from 1.8 in undiluted lemon and lime juice to 2.5 in a 10% lemon juice solution and from 3.5 for samples of pineapple juice to 4.3 for tomato juice (Handy and Gould, 1962; Table I). Unacidified Cr-free water served as a blank. All laboratory procedures were conducted in a clean air class 100 laminar flow work station (EACI, Hagerstown, MD). Previously used 300-mL covered stainless steel teapots (Vollrath 46214, No. 304 steel, 18–20% Cr) without deformations, pitting, or staining were selected as representative of stainless steelware used in food processing and preparation.

The teapots were washed in a mechanical stainless steel dishwasher prior to use, soaked overnight in Cr-free detergent, and rinsed 3 times before use. Three sets of pots were prepared. Each set consisted of three pots containing 250 mL of either unacidified water or pH 3.0 water or pH 2.5 water. Temperature treatments were chosen to simulate typical food preparation procedures. Set I was (a) brought to a boil (98–100 °C) for 10 mins, (b) held at a simmer (90–95 °C) for the remainder of a 2-h period, (c) allowed to cool for 1 h at room temperature (22 °C) and then (d) refrigerated (5 °C). Set II received treatments b–d, and set III received treatments c and d. Before and after each treatment, 3-mL aliquots were decanted into polypropylene tubes (Falcon 2063) and stored at room temperature. Aliquots from each pot of water were also withdrawn after 1 and 4 h of refrigeration.

Sample Analysis. Cr concentrations were determined by using a Perkin-Elmer 4000 atomic absorption spectrophotometer equipped with an HGA 500 graphite furnace, tungsten–halogen background corrector and a Model 56 chart recorder. Duplicate 25-μL samples were injected into pyrolytically coated graphite furnace tubes. Three or more injections were made when duplicates differed by 1.0 ppb or more. Samples were read to the first decimal, in the concentration mode, against standards of 5, 15, and 30 ppb or of 10, 30, and 50 ppb. Selected samples of HCl–water and of juices were also measured by method of standard addition. Instrument conditions were as follows: wavelength 357.9 nm; integration time 5 s; argon gas flow 300 mL/min, reduced to 50 mL/min during atomization. The furnace program, selected as appropriate for both the water and the fruit juices is presented in Table II.

Results. Since most duplicate injections of the HCl–water samples differed by <0.5 ppb, results of the HCl–water experiments are presented as averages in Figure 1.

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0021-8561/83/1431-0089$01.50/0

Table I. Cr Release from Stainless Steelware and Processing Equipment

sample (250 mL)	pH	container	Cr content, ppb ± SD[c] unheated	heated	increase
tomato juice					
canned	4.3	stainless steel teapot[a]	18	50 ± 2.2	32
fresh	4.3	polypropylene tube	2		
HCl-water	2.5	tomato juice can[a]	0	<1	<1
pineapple juice					
canned	3.5	stainless steel teapot[a]	56 ± 1.8	57	1
bottled	3.5	stainless steel teapot[a]	10	60 ± 1.2	50
fresh	3.5	polypropylene tube	<1		
HCl-water	2.5	pineapple juice can[a]	0	23 ± 1.1	23
lemon juice fresh, 10%	2.5	stainless steel 1-qt pot[b]	<1	34 ± 1.6	33
lime juice bottled	2.0	stainless steel teapot[a]	15	46	31

[a] Heated for 1 h at 90–95 °C. [b] Heated for 5 min at 98–100 °C and then held for 2 h at 22 °C. [c] Standard deviations are presented where three or more determinations were made. All other measurements represent the average of duplicate injections which differed by <1.0 ppb.

Table II. Graphite Furnace Settings

step	temp, °C	time, s ramp	hold
dry	120	40	5
dry	200	50	5
char	600	35	5
atomize	2700	0	4
clean out	2700	1	4

The ability of water to stimulate Cr release from stainless steel was primarily dependent on pH. Unacidified water averaged 0.1 ppb of Cr (range 0.0–0.5). Cr release occurred at both pH 2.5 and pH 3.0, as shown in Figure 1, but at least twice as much Cr was released at pH 2.5 than at 3.0, regardless of sample treatment. After cooking, cooling, and refrigeration, pH 2.5 samples I and II contained 75 and 59 ppb, respectively. At pH 3.0, samples I and II contained only 37 and 21 ppb, respectively. Much less Cr was released into the unheated III samples. Cr concentration after 1 h at room temperature and 4 h of refrigeration was 22 ppb in pH 2.5 water and 10 ppb in pH 3.0 water. While heating accelerated Cr release at both acidities, it had a greater effect on the more acid water. After 1 h at 98–100 °C, pH 2.5 treatment I water contained nearly 3 times as much Cr, 59 ppb, as pH 3.0 water, 23 ppb. However, during the hour when the pots were removed from the heat and allowed to return to room temperature, the rate of Cr release was the same for both pHs, each adding 15 ppb.

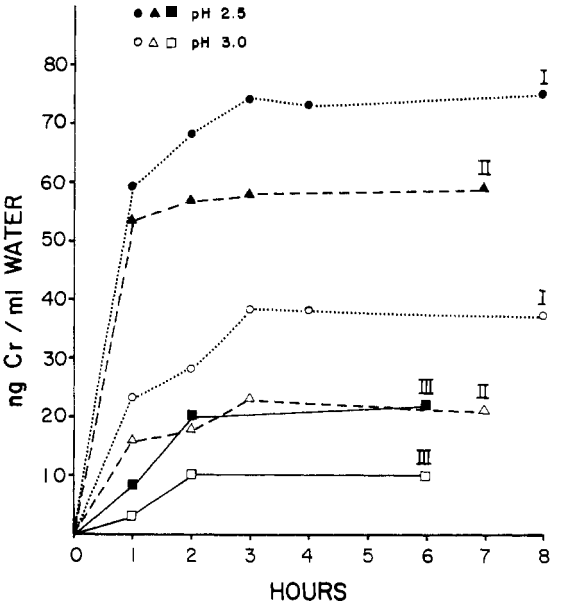

Figure 1. Effect of temperature on Cr release from stainless steel into acidified water. (I) Hour 1: heated to 98 °C and held for 10 min and then reduced to 90–95 °C. Hour 2: held at 90–95 °C. Hour 3: cooled at 22 °C. Hour 4: refrigerated at 5 °C. (II) Hour 1: heated to 90–95 °C. Hour 2: cooled at 22 °C. Hour 3: refrigerated at 5 °C. (III) Hour 1: held at 22 °C. Hour 2: refrigerated at 5 °C.

EXPERIMENT 2: CHROMIUM IN JUICES

Materials and Methods. The pHs of canned tomato and pineapple juices, bottled pineapple and lime juices, and fresh hand-squeezed pineapple, lemon and tomato juices were measured. Aliquots were stored in polypropylene tubes for Cr determination. In order to compare Cr release into acid juices with release into HCl-acidified water, 250 mL of each of the commercial juices was heated to 90–95 °C for 1 h in stainless steel teapots. For assessment of whether cans might be Cr sources, the emptied tomato and pineapple juice cans were washed and 250 mL of pH 2.5 water was heated in each for 1 h at 90–95 °C. So that a home cooking procedure could be more closely simulated, 250 mL of the hand-squeezed lemon juice, diluted 10-fold with tap water (0.3 ppb of Cr), was heated at 90–100 °C for 5 min in a 1-qt, covered stainless steel pot (Farberware) and held at room temperature for 2 h. This diluted lemon juice was filtered through a 0.2-μm filter (Nalge 450-0020). Cr was determined in each of the samples by the method described.

Results. The Cr content of the juices, before and after heating in stainless steel, and of the water heated in the juice cans is presented in Table II. Standard deviations are reported for those samples which were injected into the graphite furnace 3 or more times because differences between duplicate injections were 1.0 ppb or more. All other samples differed less than 1.0 ppb and are presented as averages of duplicate injections. The processed fruit juices, as purchased, contained widely varying amounts of Cr. All were considerably higher than the amounts in the hand-squeezed juices. After heating in stainless steelware, the amounts of Cr increased in all the juices except for the canned pineapple juice, which was found to contain nearly as much Cr before heating as it did afterwards; when pH 2.5 water was heated in the can in which the pineapple juice had been purchased, 23 ppb of Cr was released, implicating the can as a major Cr source.

The different Cr increments in these acid juices did not parallel pH differences. The greatest increment, 50 ppb, was in the pH 3.5 bottled pineapple juice. Although the other juices ranged from pH 2.0 to 4.3, they induced almost identical increments of 31–33 ppb. This included the lemon juice treated in the 1-qt pot rather than in the smaller teapots. Filtration of the heated lemon juice did

not result in any loss of Cr. The Cr particles released from the pot appear, therefore, to be smaller than 0.2 μm.

DISCUSSION

When heated in stainless steel at 90–95 °C for 1 h, water and acid juices released comparable levels of Cr: 54 ppb in pH 2.5 water, 16 ppb in pH 3.0 water, and from 31 to 50 ppb in the juices. But the acid juices behaved differently from the HCl–water in two respects: (1) At a given pH, juices induced greater Cr release than HCl–water. The Cr increment in pH 4.3 tomato juice, 32 ppb, was double that in pH 3.0 water, 16 ppb; it was nearly the same in pH 3.5 pineapple juice, 50 ppb, as in pH 2.5 water, 54 ppb. (2) Cr release was increased when the water was more acid, but juice pH, alone, could not account for the variations in the juices. These differences are probably due to the characteristic organic acids which constitute a particular juice. In tomatoes, 65–80% of the acid is citric acid (Handy and Gould, 1962; Villarreal et al., 1960), an effective chelator (Stoewsand et al., 1979). The amount of citric acid is greater, for example, in firm ripe than in soft ripe tomatoes and tomato pH varies from pH 4.3 to 4.6 (Villarreal et al., 1960). The acid composition of plants will also vary with geography, growing and harvesting conditions, plant variety, and even the height of the plant above the ground, maturity, size, and season (Mertz, 1969; Paul and Palmer, 1972). Hence, organic acid constituents may cause not only species differences but also batch to batch differences in the amount of Cr released and may account, in large measure, for the similar Cr increments found in juices as different in pH as lime (2.0) and tomato (4.3).

The diversity among fresh, bottled, and canned tomato and pineapple juices suggests Cr release into foods from extraction and processing equipment. The 1 ppb in fresh pineapple juice and the 2 ppb in fresh tomato juice are indicative of very low endogenous Cr levels in these juices. (The hand-squeezed pineapple and tomato juices in this experiment were obtained from single pieces of fruit and may, of course, differ from pooled juice samples.) The 10 ppb of Cr in bottled pineapple juice and the 18 ppb of Cr in tomato juice from a can which did not release Cr are probably derived from extraction and/or processing equipment; likewise, a major portion of the Cr found in the canned pineapple juice may be ascribed to the can in which it was heat processed.

While these experiments provide evidence that pH, temperature, and time determine the extent to which a food may induce release of Cr from stainless steelware, the amount of incidental Cr in a particular serving of food will be "individualized" by the surface area in contact with the food, the viscosity of the food, and its mechanical and chemical interactions with added ingredients. In a recent survey of acid foods exposed to stainless steel during harvesting, processing, and/or preparation, Stoewsand et al. (1979) found 10 ppm of Cr in red cabbage brine but only 0.2 ppm in sauerkraut brine, although pH was nearly the same in each.

It has been estimated that U.S. dietary Cr intakes range from 5 to 150 μg/day, with an average of 50–60 μg (Kumpulainen et al., 1979). This is at the low end of the 50–200-μg range which has been recommended as "safe and adequate" (Mertz, 1981; National Academy of Sciences, 1980). The amounts released into heated acid juices and water in the experiments reported here ranged from 30 to 75 ppb, 3.0 to 7.5 μg of Cr in 100 mL. Longer heating and storage would be expected to increase these levels. Acid foods such as pineapple and tomato juices are popular beverages while tomato, pineapple, and lemon products and vinegar are staple recipe ingredients. Citric acid predominates in many fruits and is added commercially and at home to a variety of foods. If acid foods which have contacted stainless steel during cooking are used in generous amounts, a fair amount of Cr may be ingested. It is also apparent that the incidental Cr content of foods which have come in contact with stainless steel in processing or home cooking may be much higher than in the raw foods.

However, it cannot be assumed that such "gains" would offset the sizable losses reported in food processing (Schroeder, 1971, 1974). Although Cr was less than 0.2 μm, we do not know the size limits or conditions necessary for the absorption of this incidental inorganic Cr (Mertz, 1981). Furthermore, biologically active Cr is an organically complexed molecule which may be obtained from the diet or from the conversion of inorganic Cr into this active molecule. The synthesis within the body of the active molecule requires time, especially for adults, and may be impaired in some individuals (Mertz, 1969). For these reasons, the usefulness to the body of incidental inorganic Cr depends first on whether it is absorbed at all and second on whether it is "activated" and stored or excreted.

Nevertheless, it appears that the levels of Cr which may be ingested from a number of nonfood sources by some individuals do have the potential to contribute measurably to total Cr intake. Hichwa et al. (1981) found a range of 0–253 ppm of Cr in 16 brands of aspirin and 0–839 ppm in 17 samples of lipstick. Other drugs and cosmetics, as well as food pigments, may also contribute Cr. Thus, the further investigation of the ingestion and fate of incidental Cr from food processing and preparation and from other sources may provide important clues to the understanding of Cr balance and nutritional status, with implications for other essential and toxic trace elements to which foods may be exposed.

ACKNOWLEDGMENT

We gratefully acknowledge the generous advice and valuable suggestions given by C. Veillon and the cooperation of K. Patterson, Beltsville Human Nutrition Research Center, U.S. Department of Agriculture. We also thank Y. Dam for graphic and technical assistance and E. L. Offenbacher for suggestions on preparing the manuscript.

Registry No. Cr, 7440-47-3; stainless steel, 12597-68-1.

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Received for review February 5, 1982. Revised manuscript received September 10, 1982. Accepted September 29, 1982. Supported by U.S. Department of Agriculture Competitive Research Grant 59-2369-0-1-517-0. This work was presented in part at the 66th Annual Meeting of the Federation of American Societies of Experimental Biology, New Orleans, LA, April 1982.

Direct Analysis of Carbofuran and Its Carbamate Metabolites in Rapeseed Plants by Nitrogen–Phosphorus Detector Gas Chromatography

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Carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were extracted from acid-digested rapeseed plants with methylene chloride. The concentrated methylene chloride extract was passed through a column that contained carbon–attaclay and silica gel. The eluant of this column was concentrated and passed through a column containing acid alumina, Florisil, and silica gel. The fractions containing carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were collected and concentrated separately prior to the addition of the internal standard. Carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were determined by gas chromatography with a nitrogen–phosphorus detector using a 15% Apiezon L column. Recoveries of carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran averaged 85% for all three compounds in the 0.03–5-ppm range. The residues of carbofuran in rapeseed plants were maximum in the plants which were collected 6–8 days after seeding. The concentration of the metabolites, 3-ketocarbofuran, and 3-hydroxycarbofuran increased until 11 days after seeding.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is widely used for controlling flea beetles (mainly *Phyllotreta* sp.) on rapeseed crops in Western Canada. Flea beetles may severely damage a rapeseed crop from the time the seedlings emerge until the crop is established in the field a few weeks later. Carbofuran is applied either as an in-furrow granular or a foliar spray treatment to control flea beetle feeding on seedling rapeseed crops. To develop an understanding of how granular carbofuran protects young rapeseed plants, it was imperative that a method be developed to determine the persistence of carbofuran and two of its main metabolites in rapeseed plants.

Carbofuran and its main metabolite, 3-hydroxycarbofuran, have been determined by gas chromatography (GC) either directly or by prior derivatization. Direct analysis of carbofuran and 3-hydroxycarbofuran has been carried out on corn utilizing microcoulometric detection and a 20% SE-30 column (Cook et al., 1969). Williams and Brown (1973) applied a similar method to the analysis of the same two compounds in small fruits but used an electrolytic detector and a 6% OV-210 and 4% OV-101 mixed-phase column.

An analytical method was developed for carbofuran, nonconjugated 3-hydroxycarbofuran, and 3-ketocarbofuran (Figure 1) using a silica column and a mobile phase of trimethylpentane–2-propanol in high-pressure liquid chromatography (HPLC) with an ultraviolet (UV) adsorption detector (Lawrence and Leduc, 1977). Recently Lee and Westcott (1980) developed an analytical method for carbofuran and 3-hydroxycarbofuran in rapeseed plants by reverse-phase HPLC.

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Residues of carbamate insecticides and their metabolites in various crops have been analyzed by several methods (Bowman and Beroza, 1967; Butler and McDonough, 1971; Cassil et al., 1969; Van Middelem et al., 1971). Apparently there are no previous publications on direct quantitative analysis of carbofuran and its carbamate metabolites, 3-ketocarbofuran and 3-hydroxycarbofuran, in rapeseed plants by GC using a nitrogen–phosphorus detector (N–P detector).

This paper describes a direct analytical method for carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran in rapeseed plants by GC using a N–P detector and a nonpolar, nonsilicon liquid phase, Apiezon L. Metalaxyl (Figure 1) was used as an internal standard in this study. The method was used to determine carbofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran in rapeseed plants that grew in plots treated with granular carbofuran.

EXPERIMENTAL SECTION

Apparatus. The GC was a Hewlett-Packard Model 5730A equipped with dual N–P detectors (Model 18789A). The column was a 2 mm i.d. × 1.2 m glass column packed with 15% Apiezon L on Chromosorb W (acid washed, dimethyldichlorosilane treated). The operating conditions were column, injector, and detector temperature 170, 200, and 300 °C, respectively. The carrier gas, helium, flow rate was 30 mL/min and hydrogen and air flow rates were 3 and 50 mL/min, respectively. The sensitivity settings were electrometer range 1 and attenuator 16. The recorder was a Perkin-Elmer Model 56 and chart speed was 5 mm/min. The homogenizer was a Virtis, Model 45.

Reagents. All organic solvents were glass-distilled residue-free grade, and the water was distilled in a metal Barnsted still. Carbofuran (99.5% purity), 3-hydroxycarbofuran (99% purity), and 3-ketocarbofuran (analytical standard) were obtained from FMC Corp., Agricultural

0021-8561/83/1431-0092$01.50/0