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Tin Binding in Canned Green Beans

Martine Debost and J. Claude Cheftel*

Tin distribution was studied in green beans from detinned cans and in tin-free green bean puree incubated, under nitrogen, with stannous citrate. Tin was determined by colorimetry of a phenylflurorone–Sn^{4+} complex. Canned beans were drained, homogenized, and centrifuged. Approximately 90% of the total tin remained in the drained beans. Ninety percent of this tin was recovered in the centrifugation sediment (up to 21 mg of tin/g dry weight) and could not be extracted from it by acid, alkaline, or saline solutions. Ethylenediaminetetraacetic (0.2 M) and 0.05 M cysteine solutions released respectively 39 and 30% of this bound tin. Pectinases plus cellulases, or α-amylase plus glucoamylase, released no tin; proteases released up to 13%. The model system yielded similar results. In both cases, stannous ions appear to be strongly bound to insoluble bean constituents otherwise than by electrostatic attraction or physical adsorption. Such bindings may account for the absence of toxicity of tin in solid canned foods.

The level of tin in vegetable and animal tissues, and therefore in most human foods, is generally less than 2 mg/kg (Schroeder et al., 1964). Canned foods, however, usually contain 10–100 mg of tin/kg and sometimes several hundred mg/kg (Kolb, 1975) as a result of the corrosion of the tin plate container. The tin dissolves as stannous ions (Sn^{2+}), and the corrosion depends on many factors (Kamm et al., 1961; Hoare et al., 1965; Willey, 1972).

Tin levels up to 200–250 mg/kg have come to be regarded as normal in canned foods (unlacquered cans), and the experience of the large consumption of canned foods shows indeed that the small amounts of tin which they add to the daily diet do not represent a risk. Schroeder et al. (1964) calculated that the daily intake of tin for an adult man in the United States is about 4 mg; the level in various organs was found to be less than 1 mg/kg. Tin does not accumulate in organs of rabbits or rats (Kutzner and Brod, 1971; Fritsch et al., 1977a,b). Absorption studies in man and animals have shown that most of the ingested tin is excreted in the feces (Calloway and McMullen, 1966; Benoy et al., 1971; Hiles, 1974; Fritsch et al., 1977a,b). Tin has been shown to be an essential element for the rat, at a level of 1–2 mg/kg of food (Schwartz et al., 1970, 1974).

The ingestion of liquids (fruit beverages, solutions of stannous chloride or citrate) containing high (250–2000 mg/L) levels of tin may, however, elicit acute temporary gastrointestinal troubles in man, monkey, dog, and cat (Calvery, 1942; Benoy et al., 1971; Cheftel and Truffert, 1972).

Laboratoire de Biochimie et Technologie Alimentaires, Université des Sciences et Techniques, 34060 Montpellier, France.

Short-term toxicity studies (De Groot et al., 1973a,b,c; De Groot and Willems, 1974) have essentially shown that the toxicity of tin varies greatly with the solubility and is apparently related to the supply and metabolism of iron (De Groot et al., 1973c; Kappas and Maines, 1976). Long-term studies with $SnCl_2$ and Na_2SnCl_2 have shown no adverse effects in mice and only slight ones in rats (Roe et al., 1965; Schroeder and Balassa, 1967).

Little is known, however, regarding the chemical form in which stannous ions may be bound in certain foods and thereby become less toxic.

In fruit-based beverages packed in plain tinplate cans, 75–90% of the dissolved tin is present in soluble and dialyzable form, possibly as organic acid chelates (Sherlock and Britton, 1972; Willey, 1972; Albu-Yaron and Semel, 1976). In many solid or partly solid canned foods, including fruits and vegetables, most of the tin appears to be bound to insoluble constituents of the food (Heintze, 1959, 1960; Horio et al., 1966, 1970, 1972; Woidich and Pfannhauser, 1973). Proteins (Gruenwedel and Hao, 1973), polyphenols (Heintze, 1959, 1960), and perhaps chlorophyll and pectins, are able to complex stannous ions in the form of insoluble or soluble chelates.

In order to investigate these points, we have studied the distribution of tin in canned green beans, as well as in a model system (green bean puree incubated with stannous citrate), and the capacity of some fractions of the material to bind stannous tin and to retain it when submitted to extraction with various solvents. Model system studies are difficult because: (a) stannous ions, which remain as Sn^{2+} in the anaerobic conditions of the food in the sealed can, quickly oxidize into Sn^{4+} in the presence of oxygen; (b) both stannous and stannic ions, in an aqueous solution free of complexing agents, between pH 2 and 11 give colloidal

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precipitates of their respective hydroxides.

MATERIALS AND METHODS

Colorimetric Determination of Tin with Phenylfluorone. Tin determinations were carried out according to the method of the International Standards Organization (1974). This method is based on the reaction of stannic ions with phenylfluorone, at low pH's, to give an orange colored complex (see Smith, 1970).

Samples of about 5 g of green beans, containing 0.1–1.2 mg of tin, are heated with 20 mL of 36 N H_2SO_4 and 10 mL of 16 N HNO_3 at boiling temperature for ca. 1 h until white fumes appear and the liquid becomes clear. After cooling, the digest is diluted with water to 100 mL.

Tin standards are similarly prepared from metallic tin sheets (Merck No. 7826, Darmstadt, Germany; 500 mg heated in 50 mL of 36 N H_2SO_4 plus 10 mL of 16 N HNO_3) or from a stannous citrate solution (0.5 M citrate; pH 7.9, 200–1200 μg of tin/mL). The latter solution is prepared under nitrogen from $SnCl_2$ and 0.5 M sodium citrate.

To 5–10 mL of a solution containing 10–110 μg of tin are added the following (in order): 10 mL of buffer solution (450 g of sodium acetate + 240 mL of acetic acid/L), 1 mL of ascorbic acid solution (50 g/L), 5 mL of a freshly made polyvinyl alcohol solution (16 g/L) (dissolve in hot water, cool, filter on Whatman No. 2 paper), 5 mL of alcoholic phenylfluorone solution (0.2 g + 20 mL of methanol + 2 mL of 12 N HCl + 95% v/v ethanol to 1 L) (keep in the dark at 4 °C for less than a week).

The mixture is stirred, let stand for 5 min, and diluted with water to 50 mL. After precisely 30 additional min, the absorbance is read at 505 nm. The extinction coefficient $\epsilon_{m\ Atom\ Sn}^{1cm} = 78$. In order to avoid turbidity, the final solution must be close to 1 N in H_2SO_4 (pH about 1.2).

Under the above conditions, the absorbance is proportional to the tin concentration from 0.2 to 2.2 μg of tin/mL of final solution; the percent ratio standard deviation/mean absorbance varies from 4 to 1.4.

The sulfo-nitric digestion assures both the mineralization of the biological material and the necessary oxidation of stannous to stannic ions. By comparison with slow digestion at room temperature it was confirmed that the usual "hot" digestion did not cause a loss of volatile $SnCl_4$, even when the amount of chloride ions was increased by adding HCl to the sample before the digestion (Cl/Sn ratio up to 10).

The results were also not affected when either up to 0.5 g of centrifugation sediment from tin-free green bean puree or up to 2 g of tin-free green bean puree was added prior to the digestion per milliliter of the stannous citrate solution used as standards.

Besides, it was ascertained that neither ferric nor cupric ions, at a concentration seven times as high as that of tin, interfered with the determination.

In addition, a number of determinations were carried out for comparison by neutron activation (Commissariat à l'Energie Atomique, Grenoble, France) and by emission spectroscopy; the colorimetric phenylfluorone method was selected because it was simpler and at least as precise as the other ones.

Preparation of Experimental Material. *1. Canned Green Beans (Detinned Cans).* Canned green beans rich in tin were kindly supplied by CARNAUD S.A. (Boulogne-Sur-Seine, France). The beans, packed in 1/1 (850 mL) plain tin plate cans, had been stored 2–4 years at room temperature (plus eventually up to 2 years at 4 °C). The can contents had the following characteristics: average net weight, 840 g; average "drained weight", 480 g; pH 6.4.

Since the inside surface of the can had initially a tin coating of 11 g/m², the complete solubilization of the tin coating would supply about 570 mg of tin/kg of total product.

The beans were drained, then comminuted into a puree with an "Ultraturrax" homogenizer working at maximum speed, with impulsions of 5 s for a total of 1 min.

2. Model Systems: Green Bean Puree Incubated with Stannous Citrate. Green beans packed in glass jars were bought in a local supermarket. The jar contents had the following characteristics: net weight, about 450 g; pH 5.8; tin content, negligible.

The beans were drained and comminuted; 20 g (19.3 mL) of the resulting puree was then suspended in 80 mL of stannous citrate solution (0.5 M in citrate), pH 7.9, and incubated 17 h at room temperature, under nitrogen and constant stirring, in the presence of 50 μL of toluene in order to prevent microbial growth. The tin content of the stannous citrate solution was adjusted so as to have 100–770 μg of tin/mL of final mixture; final pH 6.6–6.9.

Table I. Detinned Cans of Green Beans (Composition of Green Bean Puree and of Fractions Obtained by Centrifugation)

	puree	supernatant	sediment (S_1)
wet wt, % of puree	100	70	30
dry wt, %	6.4	4.0	12
protein, % of wet wt of each fraction	1.45	0.35	4.0
protein, g in each fraction issued from 100 g of wet puree	1.45	0.24	1.2

Fractionation of Green Bean Purees and Extraction of Tin. *1. Fractionation.* The green bean purees both from the detinned cans and from the incubated model systems were centrifuged at 27000*g* for 20 min at room temperature, giving sediments S_1. Table I shows the dry solids and the protein content of the different fractions from canned green beans; it can be calculated that about 83% of the protein is recovered in the centrifugation sediment.

2. Extraction of Tin with Water and Various Solutions. The sediments S_1, both from the canned beans and from the model systems, were separately resuspended in water or in various extraction solutions (volume equal to volume of supernatant), let stand 30 min at room temperature, and again centrifuged. This treatment was repeated once, giving sediments S_2. The solutions used for the extraction were the following: sodium chloride, 0.5 M; sodium carbonate, monobasic, 0.5 M, pH 8.1; sodium hydroxide, 1 M; hydrochloric acid, 1 M; sodium citrate, 0.5 M, pH 7.6; ethylenediaminotetraacetic acid, disodium salt, 0.2 M, pH 4.3; cysteine, 0.05 M, pH 3.7. At each step, the tin was determined in the supernatants and in the sediments.

3. Release of Tin with Enzymes. In addition, the water-washed sediments S_2, both from the canned beans and from the model systems, were submitted to the action of the various hydrolytic enzymes indicated in Table II; the sediment was suspended in the corresponding buffer solution (a) in the presence of the enzyme, (b) without the enzyme, and (c) with the heat-inactivated enzyme. After incubation, the suspensions were centrifuged as described above and gave sediments S_3.

RESULTS

Canned Green Beans. *1. Distribution of Tin.* Table III records the amounts of tin found in the various fractions of green beans from each of six cans. The drained beans account only for 55–60% of the can contents, but

Table II. Enzymes Used for Tin Release Tests and Conditions of Use[a]

type	origin	reference	μg of enzyme/g of dry wt of sediment S_2	incubation buffer	incubation time, h	incubation T, °C
α-amylase[b]	*Bacillus subtilis*	Merck, 1329	107	0.1 M sodium acetate, pH 5.0	24	40
glucoamylase[b]	*Aspergillus niger*	Merck, 1330	2870	0.1 M sodium acetate, pH 5.0	24	40
cellulase[c]	*Aspergillus niger*	Calbiochem, 21947	7460	0.05 M sodium acetate, pH 4.7	24	28
pectinase[c]	*Aspergillus niger*	Sigma, P.4625	1495	0.05 M sodium acetate, pH 4.7	24	28
protease[d]	*Streptomyces griseus*	Sigma, P.5130	4820	0.1 M sodium phosphate, pH 8.0	3.5	45

[a] All release tests were performed in centrifugation tubes with 14–20 mL of incubation mixture. Thirty microliters of toluene is added to each tube. The incubation mixture contained the following amounts of S_2 sediments (from centrifuged green bean puree or model mixture): 37 mg dry wt/mL (detinned cans); 6.4 mg dry wt/mL (model systems). [b] α-Amylase and glucoamylase were added together. It was checked by addition of 2 mg of corn starch/mg of dry weight sediment that complete hydrolysis of this added starch occurred. [c] Cellulase and pectinase were added together. [d] Enzyme/substrate ratio was 1:100 in protease tests.

Table III. Detinned Cans of Green Beans (Distribution of Tin in Green Bean Fractions (Each Data Corresponds to One Can))

	can no. 1	2	3	4	5	6
storage time at room temperature (year)	2	4	4	4	4	4
additional storage time at 4 °C (year)	0.5	0	0	0.5	1	2
total content, g	845	841	842	830	849	845
brine, g	364	371	360	350	330	351
drained green beans, g	481	470	482	480	519	494
mg of tin/kg of brine	11	80	120	20	70	74
mg of tin/kg of drained green beans (puree)	120	560	600	720	750	900
% of total tin in drained green beans	94	90	87	98	94	94
mg of tin present in a sample of puree	5	15	17	18.5	20.5	21
mg of tin present in:						
centrifugation supernatant	0.39	1.3	0.55	0.69	1.02	0.6
1st wash (water) supernatant	<0.02	0.24	0.89	0.28	0.41	0.39
2nd wash (water) supernatant	<0.02	0.1	0.11	0.13	0.2	0.21
mg of tin present in centrifugation sediment S_2	4.43	13.3	15.4	17.4	18.8	19.8
% of tin from puree sample recovered in S_2	89	89	91	94	92	94
mg of tin/g dry wt of sediment S_2	3.1	13.9	15.1	17.6	19.1	21.6

Table IV. Stannous Citrate-Green Bean Puree Model Systems[a] (Tin Contents of Insoluble Constituents (Water-Washed Sediments S_2))

mg of tin/kg of incubation mix	98	190	284	304	419	504	550	711
mg of tin/kg of wet puree in incubation mix	512	1009	1500	1618	2080	2500	2740	3530
molar ratio of citrate/tin	459	317	160	146	114	95	87	67
mg of tin present per assay	10.4	20.2	30.0	32.4	41.6	50.0	54.5	70.5
% of total tin[b] recov. in S_2 sediment	39.5	33.7	31.5	30.2	31.2	31.2	29	22.4[c]
mg of tin/g dry wt[b] of S_2 sediment	5.7	9.3	14.3	14.0	17.8	21.7	22	22.5

[a] All mixes contained 20 g of puree and 80 mL of "stannous chloride + 0.5 M sodium citrate" solution (see Materials and Methods section). [b] Each result is the average of three determinations. [c] Tin in sediments S_2 obtained by difference between total tin and tin in supernatant.

for 87–98% of the total tin; moreover, 89–94% of the tin present in the drained beans is bound to insoluble constituents of water-washed sediments S_2.

If bound tin (mg of tin/g dry weight of sediment S_2) is plotted against the tin initially present (g of tin/kg of puree from drained beans), a linear relationship is obtained, up to 21 mg of tin/g (Figure 1). This would indicate that the maximum "tin binding capacity" of the insoluble constituents of green beans has not been reached.

2. Extraction of Tin with Water and Various Solutions. Washing sediments S_1 with water or with the various extraction solutions mentioned in the Materials and Methods section showed that (1) sodium chloride, sodium carbonate, sodium hydroxide, hydrochloric acid, and sodium citrate released no more tin than water and (2) EDTA and cysteine released substantially more tin: the correponding S_2 sediments contained respectively 39 and 30% less tin than the water-washed S_2 sediment (Debost, 1978).

3. Release of Tin with Enzymes. Experiments carried out with the enzymes listed in Table II showed that protease is the only one of the several enzymes tested which released any tin from the water-washed sediments S_2, 8–13% (Debost, 1978).

Model Systems. *1. Distribution of Tin.* Table IV shows the distribution of tin in the different fractions of the model systems prepared as described in the Materials and Methods section from originally tin-free green bean puree. The maximum amount of bound tin (on water-washed sediments S_2) was 39% of the total tin available; increasing the duration of the incubation from 17 to 64 or 210 h did not modify the result. While one (or two) additional wash of sediment S_2 with cold water removed only

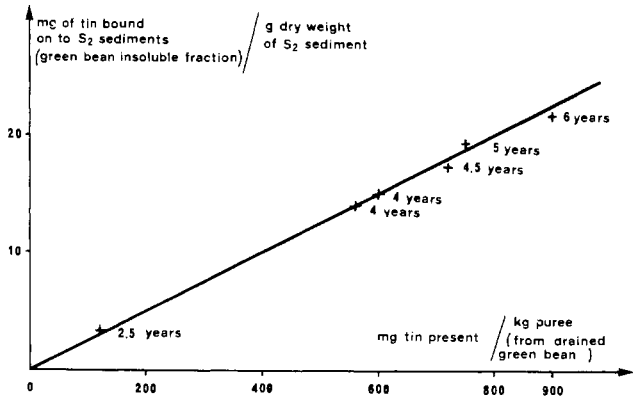

Figure 1. Detinned cans of green beans: Tin content of water-washed S_2 sediments (green bean insoluble fraction) as a function of the tin content of drained green beans at various storage time (years).

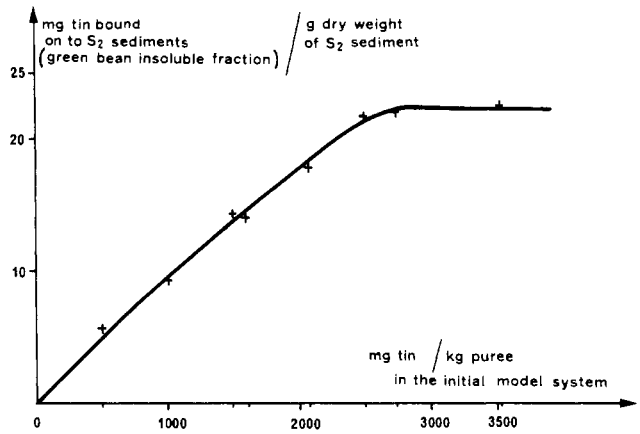

Figure 2. Stannous citrate–green bean puree model systems: Tin content of water-washed S_2 sediments (green bean insoluble fraction) as a function of the initial milligrams of tin/kilogram of green bean puree ratio.

5% (or 6%) of the tin, we found that water above 40 °C weakens the binding (Debost, 1978).

Figure 2 shows the tin content of water-washed sediment S_2 (mg of tin/g dry weight of S_2) plotted as a function of the ratio milligrams of tin/kilogram of green bean puree in the incubation mix. The shape of the curve suggests that a stoichiometric binding takes place, with a maximum of 22.5 mg bound tin/g dry weight of sediment S_2.

2. Extraction of Tin with Water and Various Solutions. The results were close to those observed with the sediments from the canned beans. EDTA and cysteine were the only agents able to remove part of the bound tin, 35 and 26%, respectively (Debost, 1978).

3. Release of Tin with Enzymes. Table V reports the results recorded with the various enzymes; they are almost identical with those obtained with the canned beans.

DISCUSSION

Strong binding of tin by insoluble constituents of green beans takes place both in corroding tin plate cans and in stannous citrate model systems. The maximum amount of bound tin (ca. 20 mg of tin/g dry weight) reaches 90–98% of the available tin in the first case and only 39% in the second. The difference may be ascribed to the fourfold dilution of the puree by the citrate solution in the model system and to the large amount of citrate which is a complexing agent for tin.

In both cases, a notable proportion of tin is bound, and the binding resists acid, alkaline, and saline extraction; this,

Table V. Stannous Citrate–Green Bean Puree Model Systems (Tin Contents of Insoluble Constituents before and after Enzymatic Treatment; Sediments S_2 and S_3)

	α-amylase + glucoamylase	cellulase + pectinase	protease	α-amylase + glucoamylase	cellulase + pectinase	protease	α-amylase + glucoamylase	cellulase + pectinase	protease
mg of tin/kg of puree in initial incubation mix	512		1080	1096	2500			3530	
mg of tin present per assay	10.4		21.6	21.9	50			70.5	
mg of tin/g dry wt of S_2 sediment	5.7		10.9	11	21.9			21.7	
% of tin present in S_2 recov. in S_3	99	99	90.5	85	99	99.5	99.5	99.5	87
mg of tin/g dry wt of S_2 sediment	5.5	5.7	9.9	9.8	22.7	21.4	20.5	21.5	18.6

as well as the shape of the curve of Figure 2, suggests that in both cases tin is bound by a nonreversible reaction other than physical adsorption or electrostatic attraction. The fact that in both cases the binding also resists extraction with a 0.5 M citrate solution further suggests that the binding occurs through stannous ions and not through stannous citrate. The partial release of tin with EDTA and cysteine would indicate that at least 30–40% of the stannous ions are bound to the insoluble bean constituents by way of the formation of complexes of weaker affinity for tin than the tin–EDTA or the tin–cysteine adduct. The experiments with enzymes do not provide much additional information, except to indicate that protein constituents may participate in the binding of tin. However, the extent of nitrogen solubilization has not been ascertained, nor the degree of starch, pectin, or cellulose hydrolysis. Horio (1972) found that neither pectinases or cellulases, nor artificial gastric juice, released any tin from insoluble fractions of canned mandarins, peaches, and asparagus.

Our results also recall the findings of Woidich and Pfannhauser (1973), and the earlier observations of Horio (1966, 1970) of tin binding onto insoluble constituents of fruits and vegetables. In all studies to this date, the chemical form under which tin is bound is not known. Model system experiments with proteins, cysteine, chlorophyll, pectins, and polyphenols in contact with stannous citrate should give additional information. It would also be of interest to prepare vegetable fractions richer in bound tin (in view of investigating their toxicity); accelerated corrosion and electrolysis tests could be useful in this respect.

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