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Influence of the comsumption of casein, or tuna in the raw, cooked or canned form, on the utilization of iron in the diet of weanling rats

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Der Einfluß einer Aufnahme von Casein bzw. von rohem, gekochtem oder in Konserven sterilisiertem weißem Thunfisch auf die Verwertung des Eisengehalts der Rationen für wachsende Ratten

Summary: A study was made of the influence of the consumption of white tuna (subjected to various thermal treatments) on the bioavailability of dietary iron. Biological assays were carried out on Wistar rats fed semi-synthetic diets varying only in the protein source, casein-methionine, or tuna provided in the following forms: raw, cooked in brine, sterilized with or without soybean oil, and canned and stored for a period of 1 or 3 years. Feed intake, the fecal and urinary excretion of iron, and the iron content of the liver were monitored. Absorption of iron was enhanced by consuming the diet containing raw white tuna. However, the beneficial effect of raw tuna was greatly reduced by cooking it in brine, and even more so by sterilization, especially in the presence of oil. The benefit was partly restored by storing the conserves for a period of 1 or 3 years. It is hypothesized that structural alterations to the protein caused by thermal processes can affect the solubility and bioavailability of iron.

Zusammenfassung: Wachstums- und Bilanzversuche mit Wistar-Ratten auf der Basis semisynthetischer Rationen sollten den Einfluß von Casein oder Thunfisch auf die Verwertung des Eisens (überwiegend in Form von Eisensulfat) feststellen. Der Thunfisch wurde roh, gekocht, in Dosen mit oder ohne Öl sterilisiert bzw. sterilisiert und 1–3 Jahre gelagert eingesetzt. Gemessen wurden das Wachstum, die Eisenbilanz und die Eisengehalte in der Leber. Die Bioverfügbarkeit des Eisens war höher in der Ration mit rohem Thunfisch, nahm aber mit zunehmendem Erhitzungsgrad ab. Nach Lagerung hatte sich die Verfügbarkeit des Eisens wieder etwas verbessert. Es wird geschlossen, daß durch die Hitzebehandlung strukturelle Änderungen im Protein auftreten, wodurch die Eisenverwertung beeinträchtigt wird.

Key words: Iron bioavailability – canned tuna – cooking – sterilization – storage

Schlüsselwörter: Eisen-Bioverfügbarkeit – Thunfisch-Konserven – Kochen – Sterilisation – Lagerung

Introduction

The solubility of iron in the intraluminal medium of the gastro-intestinal tract is a prerequisite for its absorption. Any condition that maintains it in a soluble form will therefore enhance its availability (1).

The bioavailability of iron is affected not only by its chemical and physical forms, but also by other dietary factors. The "meat factor", present in meat, fish, and poultry, enhances its absorption. This is due not only to the fact that the iron contained in these foods is itself very well absorbed, but also because animal-derived products greatly enhance the absorption of non-heme iron (2). The protein of these foods and/or their

digestion products can modify the utilization of dietary iron in the same way that certain peptides and amino acids released during digestion increase its solubility and enhance its absorption (3, 4). It has been shown in rats that lysine, histidine, and cysteine increase the utilization of inorganic iron (5).

In the context of this protein-iron bioavailability interaction, and in the particular instance of fish, one must recognize that thermal processes and easy fat oxidation can produce aldehydes and other carbonyl groups which are able to react with the epsilon group of lysine and other amino acids. These reactions can result in the synthesis of non-absorbable products of high molecular weight, which might bind mineral elements. More specifically, they could interfere with iron uptake (6). These changes could be important if, as indicated by Finch et al. (7), the rate of protein degradation has even more significance than amino acid composition for the absorption of non-heme iron.

The purpose of the present study was not to study the bioavailability of the iron in fish, but rather to analyze the effects of the consumption of raw, cooked, and several kinds of canned tuna on dietary iron utilization.

Material and methods

1. Samples

Twelve Albacore tuna (Thunnus alalunga), each weighing between 4.6 and 5.0 Kg, were caught by a commercial tuna vessel near the point 43° N and 27° W during the month of June 1986. Transportation to port took 10 days, during which time the fish were kept in boxes packed in ice. On arrival, they were frozen to –40° C and stored at –18° C for 4 months. The different stages of sample preparation, described in the following took place at the pilot plant of the Instituto de Investigaciones Marinas de Vigo (CSIC).

The 12 whole thawed tuna were beheaded, eviscerated, washed and cooked in brine (130 g salt/l water) until a final backbone temperature of 65° C (90 min) was reached. Afterwards, the tuna were cooled to 14° C for about 5 h and the skin, bones, backbone, and red meat removed. Approximately 3 Kg of the product were set aside and used as "the cooked in brine" sample (B).

The rest of the fish, approximately 20 Kg of cooked white tuna, was canned. White meat (80 g) mixed with 35 ml of soybean oil were put into each of about 250 tins (OL-120). The tins were sealed and sterilized at 115° C for 55 min (enough time from the microbiologic point of view) or 90 min (the time employed by industry). The tins were left to "age" for 30 days, allowing the coating liquid and salt to be distributed equally and absorbed by the solid tissue. After this period of time, a mixture of the contents of 12 cans was made for each treatment (C550 and C900).

The remaining tins were stored for a 1- or 3-year period at room temperature (approximately 20° C). After these periods a mixture of the contents of 12 tins representing each storage time was also made (C551, C901, C553 and C903).

As alternative treatments an amount of about 1 Kg of tuna cooked in brine was pressed and introduced into OL-120 80 g capacity cans, and sterilized at 115° C for 55 or 90 min (C55 and C90). No soybean oil was added to these samples. This was done in order to study the characteristics of oil-free preservation, avoiding the effects caused by the presence of oil.

Finally, an amount of 5 Kg of raw white tuna meat, which had been cut into pieces, was chosen at random from different fish and different body parts and used as a reference sample (R).

Each one of the samples was lyophilized and stored at -20 °C, awaiting analysis and use a protein source in animal assays.

Identification key:
R: Raw tuna;
B: Tuna cooked in brine;
C55: Canned tuna sterilized for 55 min;
C90: Canned tuna sterilized for 90 min;
C550: Canned tuna with soybean oil sterilized for 55 min;
C900: Canned tuna with soybean oil sterilized for 90 min;
C551: Canned tuna with soybean oil sterilized for 55 min and stored for 1 year;
C901: Canned tuna with soybean oil sterilized for 90 min and stored for 1 year;
C553: Canned tuna with soybean oil sterilized for 55 min and stored for 3 years;
C903: Canned tuna with soybean oil sterilized for 90 min and stored for 3 years.

2. Biological experiments

2 a. Animals and diets: 120 Wistar weanling rats weighing 39–41 g were used for balance assays. Each group of 10 rats (5 male and 5 female) was placed in individual metabolism cages in an environmentally controlled room maintained at 20–22 °C, with a 12 h light – 12 h dark cycle and 55–70 % humidity.

Following the recommendations of the National Research Council (8), isocaloric and semi-synthetic diets, were prepared with the following theoretical composition: 39.5 % starch (Central Ibérica de Drogas, S.A., Madrid), 39.5 % sugar (Confisa, S.A., Madrid), 7.5 % fat (soybean oil), 5 % non-nutritive fiber (Central Ibérica de Drogas, S.A., Madrid), 3.34 % of a mineral mixture (E. Merck, Darmstadt) and 0.12 % of a vitamin mixture (Roche). The only varied component in the diets was the protein source (10 %): casein supplemented with DL-methionine (0.2 %) (E. Merck, Darmstadt, FRG) or raw tuna, tuna cooked in brine, C55, C90, C550, C900, C551, C553, C901, and C903.

When raw and processed tuna were used as a protein source, fat and minerals were included simultaneously in the diet. Thus, the amount of oil and mineral mixture added to the diet was adjusted for the amount of fat and minerals supplied by the tuna.

For moisture determination homogeneous mixtures of 3–5 g were dried at 100 °C to constant weight by standard methods (9). Total protein was calculated by the Kjeldahl procedure using a 6.25 conversion factor. The total lipid was extracted from lyophilized samples with petroleum ether 40–60 ° in an extraction Unit Soxtec System 1040 Tecator. The ether extract was gravimetrically evaluated. Samples for ash were incinerated using a muffle furnace at 500 °C according to methods outlined in (9). The macronutrient content of the diets is given in Table 1.

2 b. Experimental procedure: The test involved a preliminary 5-day adaptation period during which feed intake and body weight changes were monitored. This was followed by a second period, lasting 7 days, in which iron balances were carried out. Diets and deionized water were available ad libitum. In order to evaluate iron balances, feed intake was monitored during the last week of the trial and urine and feces collected and pooled separately for this period. The animals were then killed and their livers removed, weighed, and frozen at -20 °C for iron composition analysis.

Urine was collected in 5 ‰ V/V HCl solution and later, filtered and diluted. Feces were dried, weighed, and homogenized. Feces, diets, and livers were incinerated and the ash dissolved in $HCl/HNO_3/H_2O$ (1/1/2). The iron content of all samples was deter-

Table 1. Macronutrient composition and iron content in diets of rat experiment[x]

Dietary protein	Moisture	Protein	Fat	Ash	Iron (µg/g dry matter)
		(g/100 g dry matter)			
Casein + DL	6.41 ± 0.03	9.7 ± 0.1	8.0 ± 0.1	2.8 ± 0.1	44.4 ± 3.0
Methionine					
R: Raw tuna	5.72 ± 0.03	10.0 ± 0.1	7.8 ± 0.1	3.4 ± 0.1	46.9 ± 2.9
B: Cooked tuna	5.81 ± 0.06	10.0 ± 0.1	7.7 ± 0.2	3.3 ± 0.1	40.1 ± 3.0
C55: Canned tuna, sterilized 55 min	6.90 ± 0.06	10.1 ± 0.1	8.2 ± 0.1	3.2 ± 0.1	46.5 ± 3.4
C90: Canned tuna, sterilized 90 min	6.45 ± 0.09	9.1 ± 0.1	8.2 ± 0.1	3.2 ± 0.1	39.0 ± 3.2
C550: Canned tuna with oil, sterilized 55 min	6.90 ± 0.07	9.2 ± 0.1	7.8 ± 0.1	3.4 ± 0.1	39.3 ± 2.9
C900: Canned tuna with oil, sterilized 90 min	7.00 ± 0.09	9.6 ± 0.1	8.3 ± 0.1	3.3 ± 0.1	33.5 ± 2.1
C551: Canned tuna with oil, sterilized 55 min, stored 1 year	5.20 ± 0.14	10.6 ± 0.1	7.8 ± 0.3	3.2 ± 0.1	47.4 ± 3.5
C901: Canned tuna with oil, sterilized 90 min, stored 1 year	4.90 ± 0.07	10.4 ± 0.1	7.5 ± 0.1	3.3 ± 0.1	41.3 ± 2.2
C553: Canned tuna with oil, sterilized 55 min, stored 3 year	5.10 ± 0.02	10.2 ± 0.1	7.9 ± 0.1	2.9 ± 0.1	87.6 ± 5.1
C903: Canned tuna with oil, sterilized 90 min, stored 3 year	4.85 ± 0.01	10.3 ± 0.1	8.5 ± 0.2	2.9 ± 0.2	65.0 ± 5.6

[x] Values are means ± standard deviations of four samples

mined by atomic absorption spectrophotometry in a Perkin-Elmer 420 device. Bovine liver (Community Bureau of Reference, BCR, 185 Commission of the European Communities, Brussels) was used as a reference pattern (certified Fe: 214 ± 5 µg/g). Analysis of this standard yielded a value of 218 ± 7 µg/g of iron/g. Interassay coefficients of variation for iron in the diet, feces, and urine were 6.2 % or less.

All glassware and the polyethylene sample bottles were washed with 10N nitric acid. Glass was avoided as much as possible and distilled-deionized water was used throughout. To control possible contamination in the collection of feces and urine, blank-capsules were manipulated in the same way as those actually used for the animals.

The following indices were calculated from the data obtained for the intake and fecal and urinary excretion of iron:

- Absorbed Fe (A) = Ingested Fe (I) – Fecal Fe;
- Retained Fe (R) = (A) – Urinary Fe;
- Apparent digestibility (% A/I) = A/I x 100;
- Proportion of ingested iron retained in the body (% R/I) = R/I x 100;
- Proportion of absorbed iron retained in the body (% R/A) = R/A x 100;

When urinary and fecal iron excretions were higher than the iron intake, the absorption and retention results were negative (-). In such cases the % A/I, the % R/A and the % R/I of C550 and C900 were considered to be O in Fig. 1.

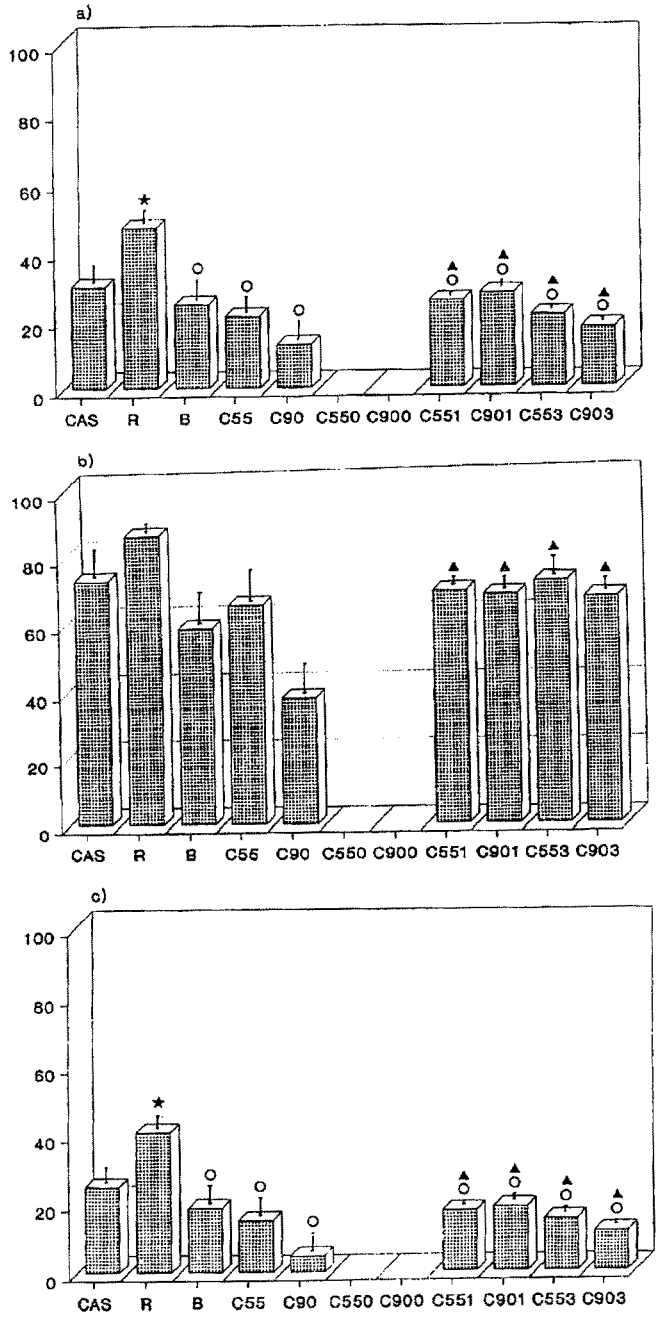

Fig. 1 Nutritional iron utilization in diets containing casein or raw/or processed tuna as a protein source. a) % absorbed/ingested; b) % retained/absorbed; c) % retained/ingested. Statistically significant ($p < 0.05$) (∗) relative to casein; (○) relative to raw tuna; (▲) compared with corresponding initial canned.

2 c. Statistical analysis

The current data were analyzed using the one-way analysis of variance program (ANOVA) currently used to study the effects of dietary protein sources in rats.

The Scheffe test was used to compare means when a significant variation was highlighted by ANOVA. The significance of the results was established at $p < 0.05$ level.

Results

The daily of intake iron by rats fed on canned tuna C900, C901, and C553 was significantly different from that of rats fed on casein or raw tuna (R).

Urinary and fecal iron excretion was similar in all groups, with two exceptions: first, animals that ingested raw tuna, for whom elimination via the feces was lower, and second, those that ate canned tuna stored for 3 years (C553 and C903), for whom fecal iron losses were higher (Table 2). The positive effect of raw tuna on iron absorption decreased after thermal treatment: after cooking (B), more so after sterilization (C55), and in relation to the period of sterilization (C90). Furthermore, the effect of the sterilization process was even more negative when carried out in the presence of oil. In groups C550 and C900 the decrease in iron absorption proved to be significant, not only with respect to group R, but also in relation to group B.

Storing the preserves over a period of 1 (C551 and C901) and 3 years (C553 and C903) seemed to attenuate the negative effect of the thermal processes (Table 2).

Iron retention showed similar changes to those already described for its absorption.

When raw tuna was included in the diet, the digestibility (% A/I), the retained percentage of the iron absorbed (% R/A), and the overall nutritive utilization (% R/I) of dietary iron were maximum and superior to those of the casein standard diet. However, these coefficients decreased sequentially when B, C55, C90, C550 and C900, processed tunas were included in the diets (Fig. 1).

The digestive and metabolic effectiveness of utilization of dietary iron improved, however, when the rats ingested preserves which had been stored for a period of 1 or 3 years (Fig. 1).

The absolute and concentrations of hepatic iron decreased in animals fed on diets that included sterilized preserves without oil and those from group C900. In contrast, these concentrations increased in animals that ingested diets with preserves stored for 3 years

Throughout the experiment the weight of the animals fed on diets containing raw tuna, tuna cooked in brine or the tuna preserves stored for a period of 1 or 3 years developed similarly to that of rats fed casein. On the other hand, excluding C55, the increase in weight of rats given preserves prepared a short time beforehand, was significantly inferior to that of the others, though of little quantitative importance (Table 4).

Discussion

It must be recognized that this study focuses mainly on the influence of the consumption of white tuna (subjected to various thermal treatments) on non-heme iron utilization. In the test diets, almost all the iron was of non-heme origin and came from the mineral mixture (FeSO4).The amount of Fe supplied by white tuna amounted to about only 7 % of the total dietary iron.

The differences in iron consumption, described in Table 2, were due to slight variations in feed intake and some variability in the iron content of diets, especially for the groups fed C553 and C903. These dietary variations were not attributable to a higher

Table 2. Iron utilization by rats fed on diets containing raw or processed tuna as a protein source (μg/day)[x]

Dietary protein	Ingested Fe	Fecal Fe	Urinary Fe	Absorbed Fe	Retained Fe
Casein + DL-Methionine	443.9 ± 18.9	313.1 ± 20.4	25.9 ± 1.8	137.0 ± 26.5	113.6 ± 23.9
R: Raw tuna	449.0 ± 20.9	240.8 ± 21.5*	28.3 ± 4.7	208.2 ± 17.5*	180.0 ± 16.8*
B: Cooked tuna	392.4 ± 20.6	302.7 ± 20.9	27.4 ± 2.2	103.5 ± 25.9★	79.2 ± 24.5★
C55: Canned tuna, sterilized 55 min	430.2 ± 23.4	339.9 ± 17.1	22.4 ± 2.4	90.5 ± 23.5★	68.1 ± 23.6★
C90: Canned tuna, sterilized 90 min	366.9 ± 30.2	320.8 ± 30.5	28.8 ± 4.1	46.1 ± 38.1★	17.3 ± 37.9★
C550: Canned tuna with oil, sterilized 55 min	348.2 ± 12.8	350.6 ± 34.9	33.8 ± 6.7	-2.4 ± 15.8**△	-36.2 ± 17.7**△
C900: Canned tuna with oil, sterilized 90 min	296.9 ± 11.2**	319.7 ± 13.7	24.9 ± 1.5	-20.9 ± 13.5**•△	-45.7 ± 14.3**•△
C551: Canned tuna with oil, sterilized 55 min, stored 1 year	358.2 ± 14.0	267.6 ± 8.5	27.1 ± 1.0	90.6 ± 6.0★▲	63.5 ± 6.1★▲
C901: Canned tuna with oil, sterilized 90 min, stored 1 year	327.1 ± 20.7**	237.8 ± 17.1	28.1 ± 2.8	89.3 ± 7.5★▲	61.2 ± 7.7★▲
C553: Canned tuna with oil, sterilized 55 min, stored 3 year	620.9 ± 17.9△▲□ **•	492.5 ± 19.0△▲□ **•	36.0 ± 6.6	128.4 ± 7.3▲	92.5 ± 10.0▲
C903: Canned tuna with oil, sterilized 90 min, stored 3 year	476.7 ± 21.6▲□	396.1 ± 21.7★□	26.9 ± 3.0	80.6 ± 5.0★▲	53.7 ± 5.3★▲

[x] Values are means ± standard error for 10 animals.
* Significant differences ($p < 0.05$) relative to casein;
★ Significant differences ($p < 0.05$) relative to R;
• Significant differences ($p < 0.05$) relative to B;
△ Significant differences ($p < 0.05$) compared with corresponding oil free preserve;
▲ Significant differences ($p < 0.05$) compared with corresponding initial canned;
□ Significant differences ($p < 0.05$) compared with corresponding 1 year stored preserve.

content of Fe in preserves stored for a period of 3 years. Iron content of these did not increase compared to the freshly prepared preserves: C550: 3.14 ± 0.21 mg Fe/100 g dry matter, C900: 3.81 ± 0.23 mg Fe/100 g dry matter, C553: 3.16 ± 0.22 mg Fe/100 g dry matter, C903: 3.45 ± 0.17 mg Fe/100 g dry matter.

Differences in iron consumption do not seem to affect the interpretation of results greatly, since the rats that ingested most iron (C553 and C903), also showed higher fecal iron excretion than the rest.

Table 3. Iron content in liver of rat[x]

Dietary protein	Weight (g)	µg Fe/Liver	µg Fe/g Liver
Casein + DL methionine	4.7 ± 0.2	433.3 ± 50.3	92.3 ± 9.2
R: Raw tuna	3.8 ± 0.2*	389.8 ± 33.4	103.2 ± 10.0
B: Canned tuna	3.5 ± 0.1*	441.1 ± 30.0	122.4 ± 6.8*
C55: Canned tuna, sterilized 55 min	3.8 ± 0.3*	320.5 ± 21.2•	79.8 ± 5.4•
C90: Canned tuna, sterilized 90 min	3.8 ± 0.3*	265.8 ± 39.2*•	74.8 ± 12.0•
C550: Canned tuna with oil, sterilized 55 min	3.6 ± 0.1*	387.6 ± 21.1	107.3 ± 5.7△
C900: Canned tuna with oil, sterilized 90 min	3.6 ± 0.1*	338.4 ± 31.8●	95.1 ± 9.2•
C551: Canned tuna with oil, sterilized 55 min, stored 1 year	3.9 ± 0.2*	320.2 ± 21.0•	98.0 ± 5.4•
C901: Canned tuna with oil, sterilized 90 min, stored 1 year	3.8 ± 0.3*	363.4 ± 27.3	98.3 ± 12.4
C553: Canned tuna with oil, sterilized 55 min, stored 3 year	3.6 ± 0.8*	445.6 ± 37.1△□	120.5 ± 9.6*△
C903: Canned tuna with oil, sterilized 90 min, stored 3 year	4.1 ± 0.5	468.3 ± 18.8*△▲	114.4 ± 5.3*△

[x] Values are means ± standard error for 10 animals.
* Significant differences ($p < 0.05$) relative to casein;
* Significant differences ($p < 0.05$) relative to R;
• Significant differences ($p < 0.05$) relative to B;
△ Significant differences ($p < 0.05$) compared with corresponding oil free preserve;
▲ Significant differences ($p < 0.05$) relative to C900;
□ Significant differences ($p < 0.05$) relative to C551.

The daily intake of iron by animals that ate the casein and raw tuna diets was similar. However, fecal excretion of iron by the latter group was significantly lower than that of the casein group. It can therefore be seen that these rats absorbed higher quantities of iron and retained much more. One can conclude that, compared to casein, white tuna had a beneficial effect on the dietary utilization of iron (Table 2).

One must not forget that casein is not a very suitable protein for the utilization of iron (10). The present results are in accordance with the stimulating effect of whole fish or fish protein on non-heme iron absorption. Some authors (11, 12) attribute this effect to its amino acid composition and/or the nature of its protein degradation products.

Table 4. Body weight increase in rats fed on diets containing raw or processed white tuna as protein source(x)

Dietary protein	Initial weight	Final weight
	g/rat	
Casein + DL-methionine	41.5 ± 0.6	77.5 ± 2.2
R: Raw tuna	41.4 ± 0.5	74.1 ± 1.7
B: Cooked tuna	41.6 ± 0.5	74.5 ± 2.3
C55: Canned tuna, sterilized 55 min	41.6 ± 0.8	68.3 ± 2.5
C90: Canned tuna, sterilized 90 min	41.5 ± 0.7	62.2 ± 2.6*
C550: Canned tuna with oil, sterilized 55 min	41.0 ± 0.3	67.2 ± 1.7*
C900: Canned tuna with oil, sterilized 90 min	41.7 ± 0.3	67.4 ± 1.8*
C551: Canned tuna with oil, sterilized 55 min, stored 1 year	39.7 ± 0.6	72.5 ± 1.5
C901: Canned tuna with oil, sterilized 90 min, stored 1 year	40.5 ± 0.5	71.8 ± 3.2
C553: Canned tuna with oil, sterilized 55 min, stored 3 year	39.6 ± 0.3	69.9 ± 1.6
C903: Canned tuna with oil, sterilized 90 min, stored 3 year	39.5 ± 0.3	71.6 ± 1.7

(x) Values are means of 10 animals ± standard error
* Significant differences ($p < 0.05$) relative to casein group.

The thermal treatments obviously produced changes in the tuna which reduced or eliminated its stimulatory effect on the utilization of dietary iron. This negative influence was more marked when two processes were combined – cooking + sterilization – and when the latter process was carried out for a longer period of time or in the presence of oil. In this case, most of the animals that ingested preserves C550 and C900 had final body weights which were significantly inferior to those of the control group and which also showed a negative iron balance (80 % of the animals in C550 and 90 % in C900). This negative balance in the C900 group was partially due to a lower iron intake, but in both groups (C550 and C900) fecal iron excretions were equal to or even exceeded the iron intakes. These observations could be explained by the presence in the lumen of digestion products of the preserves, which enhanced the fecal elimination of endogenous iron.

This effect cannot be due to variations in the total amino acid content of the tuna protein, since the latter was not modified (13). Nevertheless, it is likely that the thermal processes created structural alterations to the protein or were the cause of new digestion products which, according to Finch et al. (7), could have modified the bonds and stability of the iron and, consequently, exerted influence on its bioavailability.

This hypothesis is supported by the suggestion that the above-mentioned structural modifications are more pronounced by heating in the presence of oil (6). In fact, the most negative iron absorption occurred in rats for which the cans of tuna were sterilized with oil (C550 and C900). After the storage of these preserves, iron bioavailability was partially improved. Similar responses of lower bioavailability as a result of sterilization and the recuperation of bioavailability after storage of the cans have also been observed for zinc (14). Other studies have shown that low available lysine values in white tuna

protein following sterilization were counteracted by storage (15). As has been described, lysine increases the absorption of iron through the formation of tridentate chelates; stable complexes which keep iron in a soluble state (16). It is suspected that both changes, i.e., greater Fe absorption and superior lysine availability, are related. The increase of Fe bioavailability in the diets containing the stored preserves was not due to enrichment of the tuna preserve through the solubilization of Fe from the walls of the cans, as has been mentioned.

Finally, it can be seen that no conclusions can be drawn from these results which are directly applicable to human nutrition. However, since the rat utilizes inorganic iron better that man (17, 18) and the changes described in this paper relate fundamentally to non-heme iron, it would appear that the effects of the consumption of processed tuna on the bioavailability of non-heme iron could be of greater interest to human nutrition. The subject deserves further study in relation to protein modifications.

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