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Effect of cooking methods on total and heme iron contents of anchovy (*Engraulis encrasicholus*)

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Abstract

The effects of cooking methods (electric oven, grill, microwave, and boiling) on total and heme iron contents of anchovy (*Engraulis encrasicholus*) were investigated. Effects of cooking methods on total and heme iron contents of anchovy were statistically significant ($P < 0.05$). The highest total and heme iron losses were found in grilled samples (52.6%, 70.4%), and the lowest were found in boiled samples (11.2%, 30.4%). Boiling was the most suitable method in terms of both total and heme iron contents of anchovy.

Keywords: Total iron; Heme iron; Cooking methods; Anchovy

1. Introduction

Seafoods, especially darker flesh fish, are reasonably good sources of iron, supplying 1–2 mg/100 g muscle (Kinsella, 1988). There are two types of dietary iron, based on different mechanisms of absorption: nonheme and heme. Heme iron is found only in meat, fish and poultry, while nonheme iron is found in all plant and animal products (Clark, Mahoney, & Carpenter, 1997; Kalpalathika, Clark, & Mahoney, 1991; Schricker, Miller, & Stouffer, 1982). The importance of meat iron depends on its heme iron content. Heme iron has a high bioavailability, ranging from 15% to 35% (Kalpalathika et al., 1991) and is not affected by other dietary constituents (Carpenter & Mahoney, 1992; Clark et al., 1997; Schricker et al., 1982). Nonheme iron, on the other hand, has a low bioavailability, ranging from 2% to 20% (Kalpalathika et al., 1991) and is influenced greatly by a variety of enhancing and inhibiting components in the diet (Carpenter & Mahoney, 1992; Clark et al., 1997; Schricker et al., 1982). However, cooking degrades the highly available heme iron into the less available nonheme iron (Buchowski, Mahoney, Carpenter, & Cornforth, 1988; Carpenter & Mahoney, 1992; Carpenter & Clark, 1995; Clark et al., 1997; Han et al., 1993; Jansuittivechakul, Mahoney, Cornforth, Hendricks, & Sisson, 1986; Kalpalathika et al., 1991; Schricker & Miller, 1983). Not much information is available on the degradation mechanism of heme iron, but it is suggested that increases in nonheme iron may be due to release of iron from heme iron complexes by oxidative cleavage of the porphyrin ring (Buchowski et al., 1988; Schricker & Miller, 1983). Besides, its lower bioavailability, nonheme iron is of interest, because it acts as a catalyst for the rapid development of oxidized flavour in cooked meats (Chen, Pearson, Gray, Fooladi, & Ku, 1984; Igene, King, Pearson, & Gray, 1979).

Some researchers (Buchowski et al., 1988; Carpenter & Clark, 1995; Gall, Otwell, Koburger, & Appledorf, 1983; Han et al., 1993; Kongkachuichai, Napatthalung, & Charoensiri, 2002; Lombardi-Boccia, Dominguez, & Aguzzi, 2002; Schricker & Miller, 1983) have studied the effects of heat on amounts of total and heme iron in meat. Gall et al. (1983) reported that cooking (baking, broiling, deep frying, microwaving) did not significantly affect the concentration of iron. According to Chen et al. (1984), both final temperature and rate of heating influenced release of nonheme iron from meat pigment

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extract which was intitally 1.31 μg/g meat. After 5 min heating at 62 °C, final temperature, 10 min heating at 73 °C, final temperature and 20 min heating at 88 °C, the nonheme iron content increased to 1.78 μg/g meat, 2.25 μg/g meat and 2.34 μg/g meat, respectively. Buchowski et al. (1988) found that heating meat in a boiling water bath decreased the amounts of total and heme iron. The concentrations of total and heme iron in unheated meat were 83.9 and 56.9 μg/g D.M., respectively. The decreases in total and heme iron when heating at 60, 77, 97 and 121 °C (autoclaved at 15 psi for 1 h) were 72.5 and 41.8 μg/g D.M., 69.7 and 37.5 μg/g D.M., 79.6 and 41.4 μg/g D.M. and 69.2 and 28.9 μg/g D.M., respectively. Han et al. (1993) reported that total iron in beef *longissimus* and chicken thigh muscles did not change ($P < 0.01$) with increased temperature, and concentrations of total heme iron at 100 °C in beef decreased to 18.8%, and in chicken 23.4%, of the values in raw samples. The results of the study performed by Kongkachuichai et al. (2002) showed that heme iron content in foods decreased during the cooking process, while nonheme iron increased. From the results of this study, it can be calculated that percent total and heme iron decrease ranged from 6.3% to 14.0% and 35.2% to 37.8% in freshwater fishes, and from 9.3% to 10.7% and 33.1% to 61.2% in marine fishes, respectively. According to Lombardi-Boccia et al. (2002), heating procedures caused a decrease in the heme iron content in meat. Their findings showed that the reductions in heme iron contents, on a dry weight basis, were 28% for chicken, 18% for turkey, 11% for beef, and 7% for pork.

This study was undertaken to evaluate the influence of different cooking methods (baking, grilling, microwave cooking and boiling) on the contents of total and heme iron in anchovy, consumed especially in the Black Sea region of Turkey.

2. Materials and methods

2.1. Preparation of fish samples

Fresh anchovies (*Engraulis encrasicholus*), 8–12 cm in length, were used. These were collected from a coastal area in the Black Sea. Triplicate 4 kg samples of fish were beheaded, eviscerated and the edible parts separated and washed with tap water and drained on a plastic sieve for 10 min. Each sample was then divided into five portions. One portion was analyzed as a fresh sample, the second portion was baked in a 170 °C electrical oven (Arcelik Mini Firin ARMF 4, 1000 W, 220 v, 50 Hz) for 30 min, the third portion was grilled by using an electrical grill (Arcelik Mini Firin ARMF 4, 2000 W, 220 v, 50 Hz) at 300 °C (the distance between heat source and the samples was 4 cm) for a total of 11 and 6 min one side and 5 min the other side, the fourth portion was cooked in a microwave oven (Arcelik MD 581, 2550 W, 230 v, 50 Hz, 2450 ± 50 MHz) with 750 W available cooking power for 8 min and the fifth portion was boiled in a stainless steel pan for 6 min. The fresh and cooked samples were hand-deboned and ground in a glass mortar to ensure homogeneity and representative samples taken for analysis. Samples were packed in glass jars and analyzed for total iron, heme iron and dry matter contents on the same day as sample preparation. The homogenized samples of each fresh and cooked fish were individually analyzed in triplicate for moisture, total iron and heme iron contents.

2.2. Chemical analyses

2.2.1. Total iron

Total iron was determined by the spectrophotometric method. Four grammes of fish were weighed into a Pyrex beherglass, placed in a muffle furnace (Nuve MF 120),with the temperature gradually raised to 450 °C, and ashed for 12 h. After cooling to room temperature, the beherglasses were placed on a hot plate and 1.0 ml concentrated nitric acid added to each and they were heated at 80 °C for 12 h. The beherglasses again transferred to the furnace and the samples were ashed at 450 °C for approximately 2 h and cooled to room temperature. The ash had a white colour; 0.5 ml concentrated HCl was added. The samples were mixed in a vortex mixer (Nuve SL 350) and left for 2 h. Water (2 ml double-distilled) was added and they were again mixed (vortex). More double-distilled water was added to bring the final volume to 10 ml and remixed. Two ml of each digest were placed in borosilicate tubes, followed by the addition of 1 ml of 3% (w/v) hydroxylamine hydrochloride solution and 1.5 ml of total iron colour reagent. The tubes were mixed and left to stand for 10 min. After this the absorbance was read at 533 nm in a Jasco V-530 UV/VIS spectrophotometer. Total iron colour reagent consisted of bathophenanthroline disulfonic acid sodium salt, 30 mg, dissolved in a small volume of double-distilled water and diluted to 100 ml with 3 M sodium acetate solution. Total iron values were calculated from the iron Standard curve (0.00–0.22 mg/100 ml) (Gomez-Basauri & Regenstein, 1992).

2.2.2. Heme iron

Heme iron was determined, using the acidified acetone extraction explained by Hornsey (1956) and Clark et al. (1997). Approximately 10 g of ground sample were weighed into 50 ml centrifuge tubes. To each was added 20 ml of acid–acetone mixture (40 ml of acetone, 9 ml of water, and 1 ml of concentrated hydrochloric acid). Each sample was homogenized for 30 s with a blender (Cat X120, Germany). Then, an additional 20 ml of acid–acetone mixture were added, and the samples were mixed thoroughly; the tubes were capped tightly and kept in the dark for 1 h. The extract was centrifuged at 2200*g* for 10 min. The supernatant was filtered through glass microfiber filters (Whatman GF/A) and the absorbance was measured at 640 nm against a reagent blank. The absorbance was multiplied by the factor 6800 and then divided by the sample weight to give the concentration of total pigments in the meat as μg hematin/g meat. The iron content was calculated with the factor 0.0882 μg iron/μg hematin (Merc, 1989).

2.2.3. Dry matter

Dry matter contents of ground fish samples were determined by drying in an oven at 105 °C to constant weight (AOAC, 1990).

2.3. Statistical analysis

Data were statistically evaluated by analysis of variance and comparisions between cooking method means were made by Duncan's multiple range test using the MSTAT-C programme.

3. Results and discussion

Total and heme iron contents for raw and cooked anchovy are given in Table 1, and show the microgrammes of iron per gramme of tissue on a dry weight basis. The effects of cooking methods on the total iron content of anchovy were significant ($P < 0.05$). The total iron contents were found to be 38.4 μg/g for raw anchovy, 34.5 μg/g for boiled anchovy, 25.5 μg/g for baked anchovy, 23.9 μg/g for microwaved cooked anchovy and 18.4 μg/g for grilled anchovy. The differences between total iron contents of anchovies baked in an electrical oven and microwave oven were not significant ($P > 0.05$). The mean total iron losses during cooking were 52.6%, 38.5%, 34.4% and 11.2% for grilling, microwave cooking, baking and boiling, respectively (Table 2). Most of these decrements may be due to moisture losses that occurred upon cooking, because, except for microwave cooking, total iron losses paralelled dry matter contents. Except for the boiled samples, our results are higher than those reported by Buchowski et al. (1988) and Kongkachuichai et al. (2002). Our results are also different from the results of Gall et al. (1983) as cooking did not significantly affect the concentration of iron and Han et al. (1993) reporting that total iron in beef *longissimus* and chicken thigh muscles did not change with increased temperature, but their results are on a wet basis. Cooking method, cooking temperature, cooking period and the type of meat may contribute to different iron contents.

The concentrations of heme iron in the cooked anchovies decreased from 6.5 μg/g in the raw samples to 7.6 μg/g in baking, 4.9 μg/g in grilling, 7.5 μg/g in microwave cooking and 11.5 μg/g in boiling. The effects of cooking methods on heme iron contents were found to be significant ($P < 0.05$) and the highest values of heme iron were observed in raw anchovies. The lowest values of heme iron were observed in grilled anchovies and, again, the differences between heme iron contents of anchovies baked in electrical oven and microwave oven were insignificant ($P > 0.05$). Mean heme iron losses during cooking were 70.4% for grilling, 54.5% for microwave cooking, 53.7% for baking and 30.4% for boiling (Table 2). A number of investigators have demonstrated that nonheme iron is increased and heme iron is decreased by heating (Buchowski et al., 1988; Carpenter & Clark, 1995; Kongkachuichai et al., 2002; Lombardi-Boccia et al., 2002; Schricker & Miller, 1983). This may be due to release of iron from the heme iron complex by oxidative cleavage of the porphyrin ring and conversion of heme to nonheme iron (Buchowski et al., 1988; Schricker & Miller, 1983). Except for the grilled anchovies, our findings on heme iron losses during cooking are consistent with the observations of Buchowski et al. (1988), studied on autoclaved meat, and Kongkachuichai et al. (2002) on steamed marine fish. In our study, heme iron losses during grilling were higher than those reported by previous researchers. This is probably due to the high cooking temperature, because

Table 1
Total iron, heme iron and dry matter contents in raw and cooked anchovy

	Raw	Baked	Grilled	Microwaved	Boiled
Total iron, μg/g D.M.	38.9 ± 1.80 a	25.5 ± 2.33 c	18.4 ± 0.55 d	23.9 ± 1.11 c	34.5 ± 1.82 b
Heme iron, μg/g D.M.	16.5 ± 0.64 a	7.6 ± 0.76 c	4.9 ± 0.59 d	7.5 ± 0.62 c	11.5 ± 0.51 b
Dry matter, %	34.1 ± 0.64 e	40.8 ± 0.75 b	47.4 ± 0.32 a	38.6 ± 0.57 c	36.5 ± 0.71 d

Values are mean ± SD of three replicates. Means in a line with different letters are significantly different ($P < 0.05$).

Table 2
Losses of total and heme iron of cooked anchovy (dry weight basis)

	Raw	Baked	Grilled	Microwaved	Boiled
Loss of total iron (%)	–	34.4	52.6	38.5	11.2
Loss of heme iron (%)	–	53.7	70.4	54.5	30.4

the anchovies were grilled at 300 °C for 11 min. The mean of heme iron losses in boiled anchovies (30.4%) in this study was similar to those reported by Buchowski et al. (1988) who showed that the heme iron losses in meat submersed in a water bath ranged from 26.5% to 34.1%. These were higher than the values reported by Han et al. (1993) and Lombardi-Boccia et al. (2002), but the latter two researchers expressed their results on a wet basis.

As can be seen from Table 1, cooking methods significantly affected the dry matter contents of anchovies ($P < 0.05$). The dry matter content of raw anchovy (34.1%) increased to 36.5%, 38.6%, 40.8% and 47.4% in boiled, microwave cooked, baked and grilled samples, respectively. According to these results, the highest water losses occurred in grilling, and baking, then microwave cooking, followed by boiling. Due to cooking temperature and period, these results would be expected.

When comparing the effects of cooking methods on total iron, heme iron and dry matter contents of anchovies, our results showed significant differences between cooking methods. The highest total and heme iron losses were observed in grilled samples and the lowest were observed in boiled samples. Also, the highest dry matter contents were found for the grilled samples, while boiled samples had the lowest. Therefore, boiling is the most suitable method in terms of total and heme iron contents of anchovies.

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