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The content and nutritional significance of minerals on fish flesh in the presence and absence of bone

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Abstract

The aim of the present study was to evaluate the variations in the mineral contents (Fe, Zn, Cu, Mn, Na, K, Ca, Mg and P) and nutritional significance of the minerals of Mediterranean fish, as a function of the presence of bone. The three commercial species analysed were blue whiting (*Micromessistius poutassou;R)*, hake (*Merluccius merluccius, L*) and sole (*Solea vulgaris vulgaris,* Q). As small and large species of hake are distinguished commercially, they were analysed as separate types of fish. No variations in the contents Fe, Zn, Cu, Na and K were observed when bone was included, as the content of trace elements were quite low in all the samples assayed. However, significant increases in the Ca and P contents were observed in all the species analysed when bone was included. To ascertain the nutrional significance of the samples they were considered as ingredients of fish-based homogenised weaning foods. According to the Ca and P contents, and to the nutritional density values, the samples could be considered to be good supplements of Ca and P, when bone is present in the flesh.

Keywords: Fish; Fish bone; Mineral content

1. Introduction

Although it is high in digestible protein, fish flesh is also an important source of minerals. The contents of K, Na, Cl, Mg, P and Ca are up to 1 mg/100 g, whereas those of Fe, Zn, Cu and I are less than 1 mg/100g (Kietzman, Priebe, Rakow & Reichstein, 1974; Navarro, 1991; Paul & Southgate, 1978). However, it is difficult to generalise and to establish the mean mineral values, because they depend on several factors such as species, sex, biological cycle and on the portion of fish analysed (Pérez-Martín, 1986), and also on ecological factors, such as season, place of development, nutrient availability, temperature and salinity of the water.

It is well known that fish flesh is a good mineral source of Ca and P, however, it is interesting to take into account that the splinters as well as bony fish skeleton are richer in these elements [National Research Council (NRC), 1991]. Because bone tissue, in its stage of highest crystallisation, it is constituted by the hydroxyapatite salt, which has Ca and P in a 2.15/1 ratio (w/w) (Russell, Caswell, Hearn & Sharrad, 1986), its addition to the flesh could contribute to high Ca and P contents. Fish can not be eaten with bone in its natural state, as it can not be chewed or digested. However, it is technically possible to process some fish with bone by careful prior homogenization, obtaining a fish purée which could be incorporated in some manufactured foods, increasing the Ca and P contents and the Ca/P ratio of the meal. Examples are the homogenized fish-based weaning foods, which are sold in jars and are the main meal of babies from 6 to 12 months old and young children from 1 to 3 years old.

Ca and P are necessary to maintain an optimal bone development (Cruz & Tsang, 1992), more of both minerals being required during childhood and growing stages (NRC, 1990) to prevent rickets and osteomalacia. Related to the diet, it is necessary to take into account the Ca/P ratio, because it has important consequences for bony development. Theoretically, the same amount of both minerals should be ingested, though ratios

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between 1 and 1.5 could be accepted. However, when this ratio changes from 1 to 2 (Ca/P: 2/1), harmful effects can appear (Aranda & Llopis, 1993). Moreover, it is known that several aberrations in bone mineral homeostasis and bone metabolism result as a consequence of severe Mg deficiency in experimental rats. These include reduced bone growth and bone volume (Carpenter, Mackowiak, Troiano & Gundberg, 1992; Wallach, 1990), and incresed skeletal fragility (Creedon, Flynn & Cashman, 1999; Kenny, McCoy & Williams, 1994). Although Ca, Mg and P are important in bone metabolism and development, other minerals such as Fe, Cu, Zn and Mn are considered to be essential for normal growth and for avoiding several pathologies (Prassad, 1991; Sherman, 1992).

In previous studies, we concluded that the addition of bone to homogenized fish-based weaning foods increased the content and availailability of Ca and P (Martínez, Santaella, Periago & Ros, 1998). For this reason, it could be interesting to prepare fish pasta as a food ingredient. The aim of the present study was to ascertain the influence of incorporating fish bone on the content and nutritional significance of Fe, Zn, Cu, Mn, Mg, Ca, P, Na and K in four commercial types of fish added as ingredients of weaning foods.

2. Material and methods

2.1. Samples

Three commercial species of Mediterranean fish (Table 1), commonly consumed in Spain, were purchased in a local supermarket (Murcia, Spain). The species were: blue whiting (*Micromesistius poutassou, R*), little hake (*Merluccius merluccius, L*), hake (*Merluccius merluccius, L*) and sole (*Solea vulgaris vulgaris, Q*). Although, hake and little hake are scientifically the same fish specie, they are sold as two different commercial types of fish, depending upon their size, as shown in Table 1. From each specie of fish, two samples were obtained, the first was a homogenized flesh, with the bone removed, and the second homogenized flesh containing bone.

2.2. Preparation of samples

The fish were gutted and then cleaned and washed with distilled deionized water. For the boneless sample, the skin and bone were removed prior to homogenization and, for the bone-containing samples, the gutted fish (without head and tail) was skinned and then homogenised in a hand-mincer. Checks were made to ensure that no visible bone fragments were present in the homogenised product. All the samples were minced in a steel hand-mincer to obtain a particle size of 5 mm.

2.3. Mineral composition

The Fe, Zn, Cu, Mn, Mg, Ca, Na and K contents of minced fish were determined after the organic matter of the sample had been destroyed by dry-ashing in a Nabertherm furnace oven, model L3/P (Lilienthal, Bremen, Germany), applying the following mineralization stages to prevent mineral losses by volatilization: 90–250°C (ramp time 2 h, hold time 2 h), 525°C (ramp time 5 h, hold time 7 h) and 525–100°C (ramp time 2 h). To check that there were no losses of mineral elements, recovery studies were made in ashed samples at different temperatures/times and it was verified that, at 525°C, the best recovery percentages were obtained. After cooling, the ash was dissolved in 2 ml of concentrated nitric acid (65% Suprapur®, Merck) and dried on a hot plate. These were subsequently again placed in the furnace at 525°C for 1 h and the recovery of the white ash was carried out with 2 ml of concentrated nitric acid (65% Suprapur®, Merck) and the volume was made up to 50 ml with distilled deionized water. Minerals were measured by a flame atomic absorption spectrophotometer, using a Perkin-Elmer AA spectrophotometer model 3100 (Norwalk, CT, USA) with an air acetylene flame, flow spoiler and corrosion-resistant nebulizer, using a monoelemental hollow cathode lamp for each element. Na and K determinations were perfomed by flame atomic emission spectrophotometry. P analysis was carried out by visible-ultraviolet spectrophoto metry, using the ammonium vanadate-molybdate colorimetric method indicated by the Association of Official Analytical Chemists (AOAC, 1991) and reading the

Table 1
Scientific name, common name, medium size and commercial presentation of the fish samples studied

Scientific name	Common name	Medium size (cm) (range)	Commercial presentation
Merluccius merluccius, L.	Hake	40.1–60.3	Whole fish[a]
Merluccius merluccius, L	Little hake	27.2–34.1	Whole fish
Micromesistius poutassou, L	Blue whiting	19.23	Whole fish
Solea vulgaris vulgaris, L	Sole	5.5–7	Trunk[b]

[a] Whole fish: flesh fish with skin and bony tissue.
[b] Trunk: flesh without tail, skin and back bone.

sample absorbance in a Hitachi-2000 (Tokyo, Japan) double beam molecular spectrophotometer.

2.4. Assay quality control

Appropriate dilutions were used to determine calcium, magnesium, sodium and potassium in all samples, adding to the calcium and magnesium diluted samples lanthanum chloride ($La_3Cl{\cdot}7H_2O$) to obtain a final concentration of 0.27% to overcome potential anionic interferences. Standard solutions (Titrisol-1000 mg l^{-1}) of each mineral element were used to prepare calibration solutions to obtain out the calibration curves. To minimise the risk of contamination, all glassware and crucibles were washed overnight in a 6 M nitric acid acid solution. Distilled deionized water was used to rinse all glassware.

To calculate the detection limit ($X_{\text{blank}} + 3\text{S.D.}$), the definition and criteria established by the IUPAC were followed (Analytical Methods Committee, 1987; Long & Winefordner, 1983), as the lowest concentration of a substance that can reliably be detected by the analytical process using a confidence limit for 1-α of 0.99 (α, significance level or probability of committing a Type I error) and the concentration limits obtained (minimum detectable concentration) were calculated. The entire analytical procedure was tested for both measurement precision and accuracy in order to assess the degree of reliability which can be allocated to the data generated by this investigation.

The precision of the method was established by a calculation of the between-assay variation coefficients from data of ten independent analyses, including the pre-treatment steps, carried out at different times (Alegría, Barberá & Farré, 1988; Barberá, Farre & Roig, 1990). The level of accuracy was continuously monitored by two types of studies: an analysis of "Cod muscle" (certified reference material, CRM422), supplied by Community Bureau of Reference Material, and a spiked recovery test. For both studies, analyses were done on five replicates of 2 g and these samples were analyzed in parallel, following the analytical procedure used in this work. The spiked test gave the recovery percentages showed in Table 2. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications and are shown in Table 2, together with the results for the detection limits, sensitivity, precision and analysis of "Cod muscle" (certified reference material, CRM422).

2.5. Nutrient density

The nutrient density for each mineral in the four samples of fish, has been shown to be an index to obtain the evaluation of the nutritional significance of minerals. The nutrient densidity (ND) value for each mineral in the four samples of fish was obtained in the following way:

$$ND(\%) = [(Np/Ep)/(Nr/Er)] \times 100$$

Where Np = nutrient concentration (mineral element) in the sample, Ep = energy supplied by food, Nr = recommended daily intake of the nutrient (mineral element) and Er = recommended energy intake for humans. Since the samples of fish were evaluated as ingredients for weaning foods, the data used to calculate the ND were the recommended dietary allowances (RDA) of minerals and energy for babies from 6 to 12 months old and young children from 1 to 3 years old, according to the recomendations of the National Research Council (NRC, 1989).

Table 2
Instrumental conditions and results of concentration limits, sensitivity, precision and analysis of "Cod muscle" (CRM#422)

Element	λ/slit(nm)	Detection limits (mg/kg dry weight)	Precision (%)	Sensitivity (mg/l)	"Cod muscle"(CRM#422)			
					Certified[a]	Found[a]	I.C. (95%)[b]	% Recovery
Cu	324.8/0.7	0.075	1.53	0.085	1.05±0.007	1.01±0.01	0.99–1.03	96.2
Fe	248.3/0.2	0.937	1.08	0.029	5.46±0.30	5.53±0.26	5.02–6.03	101
Zn	213.9/0.7	0.871	2.16	0.002	19.6±0.5	20.1±0.42	19.3–20.9	103
Mn	279.5/0.2	0.302	2.35		0.543±0.028	0.531±0.03	0.472–0.598	97.7
Ca	422.7/0.7	12.01	1.98		(330)[c]	(323±7)	309–337	(97.9)
Mg	285.2/0.7	16.86	2.20		(1.4)	(1.38±0.2)	0.99–1.77	(98.6)
Na	589/0.2	–	3.90		(2200)	(2301±256)	1799–2803	(105)
K	766.5/0.2	–	2.55		(21700)	(22190±723)	20773–23607	(103)
P	400/-	0.97	1.96					

[a] Each mean value is accompanied by its standard deviation.
[b] Intervals of confidence (95%).
[c] Values in brackets are not certified.

2.6. Statistical analysis

Statistical analysis was performed using SYSTAT software, version 5.0 (Wilkinson and Howe, 1992). An analysis of variance (ANOVA) was applied to ascertain the effect of the type of fish and the effect of the bone addition on the mineral content. Tukey's test with a significance level of 95% was used to compare individual pairs of means.

3. Results and discussion

3.1. Mineral content

Analyses of variance for the mineral contents of the different species of fish and for their presence and absence are shown in Table 3. Mineral contents, except for Cu and Mn, depended on the type fish. The addition of bone determined Mg, Ca and P contents with the higher significance ($P > 0.001$) and also Fe and Zn ($P < 0.01$), but Cu, Mn, Na and K contents were not affected by the addition of bone ($P > 0.05$).

As expected, the levels of trace elements (Table 4), expressed as mg/100 g on a wet weight basis, were quite low within the samples, since it is known that the content of these minerals in fish are lower than 1 mg/100 g (Navarro, 1991). However, the Fe content in blue whiting (*Micromesistius poutassou*) increased significantly when bone was present (from 0.40 to 2.70 mg/100 g). This may be due to unavoidable fragments of blood remaining in samples after cutting and portioning the fish. In any case, these portion reflect food samples as normally harvested for preparation. Sole (*Solea vulgaris vulgaris*) flesh samples showed the highest Fe levels (0.8 mg/100 g) among all the samples assayed, as well as flesh with bone samples (0.9 mg/100 g).

Zn contents in flesh were lower than 1 mg/100 g, but showed a variability that ranged from 0.41 (hake) to 0.70 (little hake) mg/100 g. These values were comparable to those described in Spanish Food Tables (Moreiras, Carbajal & Cabrera, 1992) for these commercial types of fish (0.4, 0.3, 0.4, and 0.7 mg/100 g respectively). Cu levels found in hake flesh and in bleu whiting flesh were too low, though higher than the levels reported by Holland, Brown and Buss (1993) for these commercial types of fish (0.27 and 0.29, respectively). Mn contents were not shown because they were lower than the detection limit in all the samples assayed.

Table 3
Variance analysis and F values of contents of Fe, Zn, Cu, Mn, Na, K, Mg, Ca and P for the type of fish and for the presence of bone

	Source of variation	
Minerals	Type of fish[a]	Presence of bone
Fe	74.77***	10.08**
Zn	88.94***	25.16**
Cu	9.64 NS	1.31 NS
Mn	4.16 NS	8.77 NS
Na	110.70***	0.09 NS
K	18.99***	0.02 NS
Mg	31.80***	19.86***
Ca	128.20***	537. 12***
P	75.22***	62. 90***

[a] Significant differences for: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS: no significant differences for $P > 0.05$.

As shown in Table 3 neither Na nor K were affected by the presence of bone ($P > 0.05$, Table 2) whereas the levels of these elements depend upon the type fish. As reported by Holland et al. (1993), the levels of Na and K found in blue whiting are 90 and 330 mg/100 g, respectively. The levels observed in the present study in the flesh of blue whiting were slightly higher (136 mg/100 g for Na and 388 mg/100 g for K), and in the flesh with bone sample. Na content was also higher (142 mg/100 g) but not K (104 mg /100 g). Moreover, it was observed that Na and K levels decreased when bone was present, blue whiting being the only exception, which showed a significant increase ($P < 0.01$). Moreover, the Na content of fish flesh is low, and for this reason it is suitable food for people needing a low Na content diet.

The addition of bone to fish flesh, in order to obtain an homogenised food such as weaning food, is expected to increase mainly Ca and P (and maybe Mg) contents. Mg contents in all the samples without bone were close to 38 mg /100 g of edible part, as was reported by Huss (1988). These levels increased slightly after bone addition except for sole samples, since bony tissue has a considerable amount of this mineral as it reported by Wetherlay and Gais (1987) who noted that Mg content of bony tissue, of the striped bass was high (88.3 g of mg / 100 g of scales). Therefore, the scales can supply a considerable amount of this mineral.

All samples showed Ca values higher than 25 mg/100 g, which is the medium Ca value in fish as reported by Kietzman (1974). Moreover, bone addition increased Ca content because fish skeleton is constituted of bone tissue (Kietzman et al., 1974), Ca and P being important constituents of it (Prentice & Bates, 1994). In the present study, very high Ca values were observed in all the commercial types assayed with bone (351 mg/100 g for blue whiting and 476 mg/100 g for sole). Samples without bone also gave high values of P, ranging from 421 mg/100 g for hake to 604 mg/100 g for blue whiting. These values were higher than those observed by Oehlenschläger (1990) for these commercial types (193 and 168 mg/100 g, respectively). P content increased significantly after bone addition, being duplicated in some case, with a range from 731 mg/100 g for hake to 1249 mg/100 g for sole. Finally, due to the importance of maintaining a good ratio of Ca/P to obtain an optimal bone development, the molar ratio of Ca/P was calcu-

lated. The values obtained in all the samples were in the range 0.03–0.7 reported by Navarro (1991), all the samples without bone being close to the lowest limit in the range and samples with bone closer to the highest limit.

3.2. Nutritional significance of mineral content

To ascertain the nutritional significance of the observed variations of mineral content in the samples of fish flesh and fish flesh with bone, the nutrient density (*ND*) was calculated. This nutritional quality index has the advantage of being independent of the amount of food consumed and an *ND* value of 100% or more indicates that the food, if it is consumed in an adequate serving, contributes substantially to the intake of that particular nutrient.

The *ND* values of flesh and flesh with bone samples for babies of 6–12 months and 1–3 years old are shown in Tables 5 and 6. Table 5 shows the *ND* values obtained for 100 g of fish samples, whereas Table 6 shows the *ND* values for the amount of fish used to prepare a 250 g jar of the commercial fish-based weaning foods, according to the recommendations of the ESPGAN (1981), which is 45 g of fish sample.

Table 4
Mineral content expressed as mg/100 g on a wet weight basis in four commercial types of fish

Type of fish	Minerals elements[a]								
	Cu	Fe	Zn	Na	K	Mg	Ca	P	Ca/P
Blue whiting									
Flesh	0.29±0.03a	0.40±0.04cd	0.53±0.02de	136±10.28b	388±17.77a	36.7±1.90bc	17.7±1.45d	604±60.46cd	0.02
Flesh and bone	0.14±0.02bc	2.70±0.12a	0.82±0.20ab	142±7.17ab	104±7.14c	83.7±5.95a	351±46.95c	882±6.27b	0.39
Little hake									
Flesh	0.04±0.02c	0.33±0.04d	0.70±0.08bc	124±8.75b	446±4.26a	36.7±0.63bc	38.3±13.69d	533±10.30cde	0.07
Flesh and bone	0.10±0.01c	0.57±0.09bcd	0.84±0.03a	64.89±4.76d	327±13.90a	50.0±3.20b	435±60.27ab	1047±176.31b	0.41
Hake									
Flesh	0.07±0.02c	0.51±0.07bcd	0.41±0.04e	143±10.69ab	320±47.52a	36.9±2.08bc	25.6±4.91d	421±41.38e	0.06
Flesh and bone	0.03±0.01c	0.33±0.02d	0.65±0.03cd	90.7±13.11cd	470±43.09cd	53.8±8.89b	360±75.94bc	731±101.64c	0.49
Sole									
Flesh	0.07±0.02	0.80±0.40bc	0.59±0.03cd	160±9.83a ±	286±121.68ab	35.3±2.41cd	80.1±4.36cd	519±26.17cd	0.15
Flesh and bone	0.26±0.18ab	0.90±0.18b	0.68±0.05c	138±8.75ab	121±34.24bc	28.3±4.85b	476±20.67a	1249±91.44a	0.35

[a] Different letters in the same column are significantly different ($P < 0.05$).

Table 5
Nutrient density (*ND*) in 45 g of sample, which is the amount of fish in a 250 jar of fish-based weaning food

			Cu	Fe	Zn	Mg	Ca	P	Na	K
Nutrient density (%)										
Blue whiting	Flesh	6–12 months	472–243	23.4	62.1	349	17.3	708	398	55.3
		1–3 years	256–260	36	47.7	411	19.8	677	487	59.2
	Flesh with bone	6–12 months	137–117	158	96.3	818	345	1034	338	14.7
		1–3 years	179–126	242	73.3	938	393	988	509	15.8
Little hake	Flesh	6–12 months	37.8–32.4	18.6	13.4	346	36.1	604	351	361
		1–3 years	49.5–34.6	28.5	10.3	397	41.4	577	429	387
	Flesh with bone	6–12 months	94–81	32.2	16.2	472	410	1187	184	265
		1–3 years	124–86.8	49.5	12.3	455	471	954	225	285
Hake	Flesh	6–12 months	70.6–60.7	30.9	8.4	373	25.9	511	434	277
		1–3 years	92.7–64.8	47.2	6.4	428	29.7	487	531	29.7
	Flesh with bone	6–12 months	30.1–26.1	20	13.3	544	364	888	275	408
		1–3 years	39.6–27.9	38.1	10.2	624	418	848.7	337	437
Sole	Flesh	6–12 months	55.3–47.7	58.5	9.5	280	63.4	494	382	195
		1–3 years	72.9–50.8	58.5	6.8	322	72.9	550.3	467	208
	Flesh with bone	6–12 months	206–177	42.8	7.7	225	465	1190	329	81.9
		1–3 years	270–190	65.7	8.4	258	434	1138	402	87.7

Table 6
Nutrient density (*ND*) per 100 g of fish samples analysed

			Cu	Fe	Zn	Mg	Ca	P	Na	K
Nutrient Density (%)										
Blue whiting	Flesh	6–12 months	630–539	52.1	138	776	38.4	1574	885	123
		1–3 years	569–577	80	106	913	44	1505	1083	132
	Flesh with bone	6–12 months	304–260	352	214	1817	766	2297	750	32.81
		1–3 years	398–279	538	163	2084	873	2196	1130	35.19
Little hake	Flesh	6–12 months	84–72	41.5	29.9	769	80.4	1342	780	803
		1–3 years	110–77	63.5	22.9	883	92.2	1283	954	860
	Flesh with bone	6–12 months	209–180	71.7	36	1048	912	2637	408	588
		1–3 years	275–193	110	27.5	1011	1046	2119	499	634
Hake	Flesh	6–12 months	157–135	68.8	18.8	828	57.6	1135	964	616
		1–3 years	206–144	105	14.3	950	66.1	1083	1179	66
	Flesh with bone	6–12 months	67–58	44.5	29.7	1209	810	1973	612	906
		1–3 years	88–62	68	22.7	1387	929	1886	748	970
Sole	Flesh	6–12 months	123–106	84.6	21.2	623	141	1097	849	433
		1–3 years	162–113	130	16.2	715	162	1223	1038	463
	Flesh with bone	6–12 months	458–393	95.2	17.2	499	1034	2645	730	182
		1–3 years	601–421	146	18.7	572	964	2528	893	195

Table 5 shows that the *ND* values of Mg, P, Na and K were much higher than 100% and these results confirm that fish flesh and fish flesh with bone, in the four species studied are adequate sources of these elements. Little hake and hake were not good sources of Cu, since their *ND* values were less than 100% in samples of flesh and flesh with bone. Moreover, the *ND* values of Fe and Zn did reach 100% in the majority of samples. A particular observation in the four species was that the flesh with bone can be considered a good source of Ca but not the flesh without bone.

The *ND* values calculated for a 250 g jar of fish-based weaning food, shown in Table 6, showed a similar trend to the *ND* calculated in 100 g of the sample (Table 5). According to the *ND*, the fish-based weaning foods prepared with flesh fish are a good source of Mg, P, Na and K, and a poor source of Cu, Fe, Zn and Ca. However, the addition of bone improved the Ca *ND* values, giving values higher than 100%. For this reason, the addition of bone during the manufacturing process would be of value as it is presumed to increase the Ca content and the nutritional value of the weaning food, with a better Ca/P ratio (Table 4).

Although *ND* values are adequate for some minerals, it is nessessary to take into account that many factors present in foods may affect their bioavailability. So, *ND* values are not by themselves sufficient to identity food as a rich mineral source. In studies of mineral availability in fish-based weaning foods, where the presence or absence or bone was taken into account, it have been established that Ca, Mg and P have good availability, whereas Fe and Zn show low availability (Martínez et al., 1998).

4. Conclusions

The contents of Fe, Cu and Zn, in all the samples assayed, were quite low in the four types of fish, while Ca and P contents were higher than those of the trace elements. Because of the high Ca and P contents of bone, the contents of these mineral were increased when bone were present. From a nutritional point of view and according to the *ND* values, the samples were good bone sources of Mg, Ca and P. So bone addition can be considered an important supplement of the majority of minerals in the diet.

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