and shell fish from the nonirradiated, provided some (a few milligrams) portion of the exoskeleton is present for analysis.

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Registry No. *N*-Acetyl-D-glucosamine, 3416-24-8; manganese, 7439-96-5.

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Simple Method for Preparing Bone-Free Ash from Fishery Products Analyzed for Mineral Content

Fuad M. Teeny

A simple method was developed for preparing bone-free ash from samples of canned and fresh fish products. The technique employs deionized distilled water to remove bits of bone from the sample ash prior to mineral determination. The addition of bone to the flesh prior to ashing had minimal effect upon the mineral level of the flesh.

A substantial amount of information has been published on the mineral composition of seafoods—raw and processed (Adams, 1975; Sidwell et al., 1973, 1977, 1978; Stansby and Hall, 1967; Thurston, 1958, 1960, 1961a–c). Among these and other published data, there appeared to be a certain amount of conflict regarding the levels of a few elements such as calcium (Ca) and phosphorus (P). Sidwell et al. (1973), in their analysis of several fresh and canned finfish, crustaceans, and mollusks, found great variability in the amounts of Ca and P in the raw flesh. They suggested that is probably due to the method of filleting the fish wherein the smaller fish retained more bone than larger fish. It is generally recognized that Ca and P are the two major minerals found in bone. Gordon and Roberts (1977) reported Ca levels of 5.5 and 252 mg/100 g of the edible portion of fresh and canned sockeye salmon, respectively, and P levels of 175 and 293 mg/100 g. It is likely that the higher levels of Ca and P in the canned samples were due to increased bone level in the canned samples over the fresh. It is rather difficult and time consuming to pick out bone by hand. The inclusion of even very small pieces of bone in the analytical samples can greatly influence the accuracy and precision of elements such as Ca and P.

During the course of our routine mineral analyses of several thousand samples of fresh and canned fishery

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Table I. Mineral Composition of Canned Salmon Flesh Spiked with Broken Pieces of Canned Salmon Bone As Determined by the Established Method and by the Proposed Method

method	spiked with bones	lot no.	replicate	element, μg/g							
				Ca	Fe	K	Mg	Na	P	Sr	Zn
established	no	1	1	213	11.2	3324	289	1565	2263	0.80	27.0
	no		2	185	10.3	3336	289	1572	2257	0.70	26.0
	no		3	191	11.2	3378	290	1588	2280	0.74	26.4
	no		4	148	10.1	3336	288	1590	2264	0.56	25.8
			mean	184	10.7	3344	289	1579	2266	0.70	26.3
			SD	27	0.6	23	1	12	10	0.10	0.5
proposed	no	2	1	56	9.1	3384	282	1591	2210	0.21	25.6
	no		2	61	8.8	3260	280	1575	2193	0.23	26.0
	no		3	57	8.3	3270	276	1545	2168	0.21	25.1
	no		4	60	8.2	3263	278	1563	2180	0.23	25.8
			mean	58	8.6	3294	279	1569	2188	0.22	25.6
			SD	2	0.4	60	3	19	18	0.01	0.4
established	yes	3	1	1268	10.2	3227	334	1579	2817	4.75	33.7
	yes		2	1268	10.7	3292	335	1587	2837	4.70	34.5
	yes		3	1189	11.6	3319	329	1579	2791	4.39	32.3
	yes		4	1461	11.4	3254	343	1588	2933	5.52	36.2
			mean	1296	11.0	3286	335	1583	2844	4.84	34.2
			SD	116	0.6	27	6	5	62	0.48	1.6
proposed	yes	4	1	66	7.4	3291	274	1548	2182	0.24	25.5
	yes		2	68	7.4	3249	270	1531	2154	0.25	25.6
	yes		3	76	8.1	3217	272	1548	2181	0.29	25.3
	yes		4	71	7.3	3187	268	1514	2148	0.26	25.8
			mean	70	7.6	3236	271	1535	2166	0.26	25.6
			SD	5	0.4	45	3	16	18	0.02	0.2

products, we observed that Ca and strontium (Sr) particularly had higher standard deviation among replicates than other elements such as potassium (K) and magnesium (Mg). Occasionally it was observed what appeared to be small bits of bone in the ash, particularly in the ash of canned samples. By trial and error, it was found that by adding water to the ash, the ash went into solution but not the "ashed bones". Preliminary experiments wherein a few pieces of bone were added to the samples prior to ashing showed that the added pieces remained essentially intact and that a simple water wash dissolved all but the bone ash.

On the basis of the above observations and the need for a procedure to determine the mineral level in fish flesh only, a simple method was developed for removal of bone from samples that had been ashed in preparation for mineral determination.

The method was used for the determination of Ca, iron (Fe), Mg, P, K, sodium (Na), Sr, and zinc (Zn) in canned pink salmon (*Oncorhynchus gorbuscha*), fresh canned sockeye salmon (*Oncorhynchus nerka*), fresh steelhead trout (*Salmo gairdneri*), and fresh black cod (*Anoplopoma fimbria*). The data were compared with those obtained using the method of Teeny et al. (1984), which makes no distinction between samples with and without bones. The data from this study show specifically how bone in the fishery products interferes with the true analysis of the mineral content of the muscle tissue.

MATERIALS AND METHODS

Samples of canned pink salmon steaks were drained, and the flesh was manually cleaned of bones with a forceps to pick out whatever bone pieces were seen with the eye. The bone was air-dried and broken into fine pieces so that a representative portion of it could be added back to the bone-clean flesh when the recovery studies were performed. The bone-clean flesh was thoroughly blended with a hand mixer. Approximately 5 g each was weighed into acid-washed 50-mL Vycor crucibles and placed under an infrared heat lamp to dry and char. The crucibles were transferred to a muffle furnace, and the temperature of the furnace gradually was raised to 500 °C and maintained at this temperature for 24–48 h to obtain a white ash. The crucibles were allowed to cool to room temperature, and 25 mL of deionized distilled water (deionized distilled water was used throughout the study) was added to each crucible. The crucibles were each covered with a watch glass, and the contents were heated almost to boiling. After the mixture was cooled to room temperature, the liquid was carefully decanted into a 100-mL volumetric flask, leaving behind bits and pieces of bone ash residue. Each crucible was rinsed several times with water and decanted into the flask (total volume of 75 mL). The residue was washed out with a fine stream of water and discarded or saved for mineral analysis in a manner similar to that of the water-soluble fraction. Each crucible was then rinsed with 20 mL of 20% nitric acid (v/v), and the rinse was added to the flask and made to volume with water.

A Jarrell-Ash Model 975 inductively coupled argon plasma emission spectrometer was used for elemental analysis. Calibration of the instrument and analysis of the samples were done with background and interelement corrections. Results are expressed in microgram per gram (μg/g) of wet tissue.

RESULTS AND DISCUSSION

Recovery Studies. The effect of bone upon the recovery of minerals from fish flesh was investigated by determining the mineral content of canned salmon flesh with and without added fish bones. For the experiments, a portion of the bones collected earlier was mixed with bone-clean flesh and analyzed for mineral content by the proposed and by the established methods (Teeny et al., 1984).

Analysis of the data presented in Table I showed that the addition of bone to the flesh caused a sharp rise in the levels of Ca, Mg, P, Sr, and Zn when analysis was made by the established method (compare lot 1 vs lot 3), whereas only slight increases in Ca and Sr levels were observed in samples analyzed by the proposed method (compare lots 2 and 4). This difference in mineral levels between the

Table II. Mineral Composition of Air-Dried Salmon Bone by Established and Proposed Methods

	established, total		proposed					
			total		water-soluble		water-insoluble	
element	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Ca	281361	58.58	274593	57.36	3444	1.25	271149	98.75
Fe	454	0.09	549	0.11	66	12.02	483	87.92
K	25240	5.26	33552	7.01	28242	84.17	5310	15.83
Mg	14761	3.0	14430	3.01	982	6.80	13448	93.20
Na	4399	0.92	5967	1.25	2576	43.17	3391	56.83
P	150679	31.37	146069	30.52	3540	2.42	142529	97.58
Sr	1058	0.22	1022	0.21	11	1.08	1011	98.92
Zn	2316	0.48	2491	0.53	20	0.80	2471	99.20
total	480268	99.99	478673	99.99	38881		439792	

Table III. Effect of Heat upon Recovery of Calcium and Phosphorus from Canned Fish Flesh

			sample				
element	heat	replicate	A	B	C	D	E
Ca	near-boiling	1	170	60	59	88	137
		2	174	59	62	98	123
		3	172	58	66	90	124
		mean	172	59	62	92	128
		SD	2.0	1.0	3.5	5.0	7.8
		CV (×100)	1.2	1.7	5.6	5.8	6.1
Ca	room temp	1	170	45	78	91	160
		2	171	51	65	86	155
		3	171	48	54	87	189
		mean	171	48	65	88	168
		SD	0.6	3.0	12.7	2.6	18.4
		CV (×100)	0.4	6.2	18.8	3.0	11.0
P	near-boiling	1	3367	2429	1995	3134	3022
		2	3401	2429	1987	3080	3013
		3	3362	2433	2006	3107	3034
		mean	3377	2430	1996	3097	3032
		SD	21	2	9	15	10
		CV (×100)	0.6	0.1	0.4	0.5	0.3
P	room temp	1	3423	2303	1982	3108	2929
		2	3411	2344	1978	3070	3070
		3	3415	2320	1941	3107	3053
		mean	3416	2322	1967	3095	3017
		SD	6	21	22	22	77
		CV (×100)	0.2	0.9	1.1	0.7	2.6

Table IV. Effect of Sample Weight upon Recovery of Calcium and Phosphorus from Canned Fish Flesh

			sample weight, g			
element	heat	replicate	1–2	2–4	4–6	6–10
Ca	near-boiling	1	62	58	59	56
		2	63	59	57	58
		3		59	61	65
		4		58	60	
		5			60	
		mean	62	58	59	60
		SD	0.7	0.6	1.5	4.7
		CV (×100)	1.1	1.0	2.5	7.8
P	near-boiling	1	2751	2731	2718	2878
		2	2703	2703	2694	2793
		3		2767	2880	2799
		4		2713	2857	
		5			2899	
		mean	2727	2728	2810	2823
		SD	34	28	96	47
		CV (×100)	1.2	1.0	3.4	1.7

two methods as a result of the bone addition shows that the proposed method is effective in removing bone from the sample. In contrast, the established method does not differentiate between mineral source and includes minerals from bone and flesh that are soluble in 4% HNO_3.

The hypothesis that bone-ash residue is primarily bone and was similar in composition to bone was tested as follows: The mineral composition of bone was determined by both established and proposed methods. The proposed method measured the mineral composition of the water-soluble and water-insoluble fractions of the bone ash separately, and mineral data were combined for comparison purposes. Analysis of the data (Table II) showed that the mineral composition of the bone by both methods was in total agreement. A total mineral content of 480268 μg/g of edible flesh was found by the established method compared to 478673 μg/g by the proposed method. Ca and P constituted about 58 and 31%, respectively, of the total

Table V. Recovery of Calcium, Sodium, and Phosphorus Added to Canned Salmon Flesh

compd added	mineral added, μg/g			mineral found,[a] μg/g			recovery, %		
	Ca	Na	P	Ca	Na	P	Ca	Na	P
NaCl		12700			12690			99.9	
		12700			12590			99.1	
		12700			12689			99.9	
$CaCl_2$	5272			5120			97.1		
	5272			5019			95.2		
	5272			5092			96.5		
NaTPP		5060	3820		5078	3844		100.4	100.6
		5060	3820		4890	3620		96.6	94.8
		5060	3820		4982	3727		98.5	97.6
NaCl, $CaCl_2$	5272	12700		5194	12793		98.5	100.7	
	5272	12700		5248	12694		99.5	100.0	
	5272	12700		5019	12194		95.2	96.0	
NaCl, NaTPP		17760	3820		16693	3429		94.0	89.8
		17760	3820		16396	3439		92.3	90.0
		17760	3820		17802	4056		100.2	106.2
$CaCl_2$, NaTPP	5272	5060	3820	5211	5321	3952	98.8	105.2	103.5
	5272	5060	3820	5182	5282	3927	98.3	104.3	102.8
	5272	5060	3820	5298	5323	4029	100.5	105.2	105.5
NaCl, $CaCl_2$, NaTPP	5272	17760	3820	5213	18091	4022	98.9	101.9	105.3
	5272	17760	3820	5461	18302	4256	103.6	103.1	111.4
	5272	17760	3820	5386	18292	4327	102.2	103.0	113.3

[a] Calcium, phosphorus, and sodium values of 97.5, 721, and 2113 μg/g, respectively, initially found in canned salmon were subtracted from the tabulated values.

Table VI. Mineral Content of Canned Pink and Sockeye Salmon, Fresh Steelhead, and Black Cod Samples As Determined by the Established Method and by the Proposed Method

sample	method	element, μg/g							
		Ca	Fe	K	Mg	Na	P	Sr	Zn
salmon									
pink	estab	347	7.8	2986	340	4381	2798	1.19	5.6
		377	7.5	2948	330	4236	2745	1.29	5.3
	prop.	94	7.6	2926	313	4128	2745	0.40	5.2
		97	6.7	2851	307	4036	2514	0.40	5.0
pink	estab	52	10.1	3347	290	3286	2256	0.21	6.8
		54	9.9	3377	292	3322	2273	0.22	6.6
	prop.	52	9.8	3158	303	3649	2269	0.23	5.9
		50	9.8	3171	303	3685	2272	0.22	5.9
pink	estab	561	10.6	3584	275	5843	3573	1.53	16.2
		813	10.9	3587	280	5776	3668	2.33	18.0
	prop.	170	10.0	3569	257	5757	3367	0.39	14.2
		172	10.1	3608	256	5731	3362	0.38	14.3
sockeye	estab	235	10.2	2890	262	713	2129	0.88	19.8
		341	11.4	2962	270	738	2221	1.23	20.0
	prop.	59	9.4	2858	251	698	1995	0.20	17.6
		62	9.7	2797	249	696	1987	0.22	17.6
sockeye	estab	433	14.7	2869	278	713	2210	1.77	20.0
		182	13.3	2836	265	699	2055	0.74	18.3
	prop.	73	12.9	2776	258	688	1995	0.29	18.2
		76	13.2	2817	258	698	2008	0.30	18.3
sockeye	estab	251	10.9	2937	276	662	2111	0.95	19.1
		159	11.8	3067	285	689	2163	0.62	19.3
	prop.	55	4.1	2878	281	687	1996	0.21	18.2
		48	3.9	2865	285	686	1987	0.18	18.7
steelhead	estab	95	4.1	4078	333	592	2367	0.19	4.1
		75	4.6	4248	343	611	2452	0.12	4.3
	prop.	76	4.3	4067	301	529	2316	0.12	4.2
		77	3.9	4050	303	527	2306	0.12	4.1
black cod	estab	45	2.6	3380	231	963	1636	0.24	2.1
		46	2.2	3414	235	974	1651	0.25	2.0
	prop.	47	1.9	3389	241	1021	1701	0.25	1.8
		48	1.9	3435	242	1022	1709	0.26	2.0

mineral contents of the bone and were predominantly (about 98%) in the water-insoluble fraction of the bone ash. By contrast, K and Na were very soluble in water and were present at the 84 and 43% levels, respectively, in the water-soluble fraction of the bone ash. However, K and Na represented only about 7% of the total mineral content of the bone.

Optimum Parameters. On the basis of our observations that the levels of Ca and P in fish samples analyzed for their mineral content fluctuate significantly with the presence or absence of fish bone, we used Ca and P as a barometer in determining the optimum parameters for the method.

The effect of heat upon the recovery of Ca and P was studied by adding 25 mL of water to the sample ash and maintaining the samples at room temperature or heating to near boiling. Results (Table III) showed that the samples that were heated to near boiling had slightly more consistent values from replicate to replicate (generally lower coefficient of variance) than those samples held at

room temperature. Extending the heating time to 2 h showed no advantage over the shorter period. Moreover, a decrease in water volume to 15 mL had no effect upon the results. Thus, the author settled on the use of 25 mL of water heated to near boiling.

Experiments using 1- to 10-g fish samples were conducted, and data showed that sample weight had no apparent effect upon Ca and P recoveries (Table IV).

Matrix Effect. Canned fishery products often contain NaCl added during processing for flavor enhancement. They may also contain some form of polyphosphate added for various reasons such as retardation of curd (Wekell and Teeny, 1988) and inhibition of struvite formation (Gillies, 1975). Therefore, the effect of sample matrix upon the recovery of Ca, Na, and P from canned fish samples containing these three compounds was determined by the proposed method. For the experiments, solutions of $CaCl_2$, NaCl, and NaTPP were added singularly and in combination to bone-clean flesh samples and mineral recovery was determined. The data (Table V) showed that recoveries were 95.2–103.6% for Ca, 92.3–105.2% for Na, and 89.8–113.3% for P regardless of whether the three compounds were added singularly or in combination.

Several canned salmon, fresh steelhead, and fresh black cod samples were analyzed for mineral content, and the results were compared with the data obtained using the established method. The data (Table VI) clearly show that for fresh fish, where bone is normally excluded from the sample prior to analysis, the mineral levels found by both methods were in good agreement and variations among replicates were rather small. However, for the canned samples, where bone is likely to be present at various levels, analysis by the established method resulted in higher mineral values and greater variations among replicates than by the proposed method. This was especially evident for elements such as Ca and Sr. Therefore, in order to get more consistent and uniform mineral data in fish flesh, bone fragments must be excluded prior to the final analysis of the samples. This task has been accomplished by the development of this simple method that separates between the flesh ash that is water-soluble and the bone ash is primarily water-insoluble.

CONCLUSION

A simple method was developed for separating bits of bone from the ash of canned and fresh fish samples prior to determining the mineral content in the samples. The method is very simple, employing only water for separating the bone from the ashed samples.

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Registry No. Ca, 7440-70-2; P, 7723-14-0; Sr, 7440-24-6; K, 7440-09-7; Mg, 7439-95-4; Fe, 7439-89-6; Na, 7440-23-5; Zn, 7440-66-6.

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