Energy Dispersive X-Ray Fluorescence Spectrometry of Major Tissues of Silky Fowls

Akitoshi NOZAKI and Takashi MAKITA

Department of Veterinary Anatomy, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753, Japan

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ABSTRACT. This report is to survey elements in frozen or freeze-dried samples of major organs of silky fowls with energy dispersive X-ray fluorescence spectrometer (EDXRF). There was not essential difference between frozen and freeze-dried samples. Of major elements, P, S, K, and Cl were always detected and Mn, Fe, Ca, and Zn were detectable in certain organs. Iodine was detected in thyroid glands. Occasionally Br was detected. The duration of freeze drying, 24 hr or 7 days, did not make significant difference in elemental analysis.

— **KEY WORDS:** energy dispersive X-ray fluorescence spectrometer (EDXRF), freeze-dried sample, frozen sample, silky fowl, zinc content.

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X-ray microanalysis and energy loss spectroscopy of ultrathin sections of biological sample have been limited in number and most of results so far published are only encouraging because of limited amount of elements in a section of 40–60 nm and also of dislocation of elements during specimen preparation including fixation, dehydration and embedding. Therefore, even Fe in red blood cell or I in thyroid gland is often failed to evaluate. Some attempts have been conducted to detect elements in bulk specimen with combination of scanning electron microscope (SEM) and X-ray microanalyser. Again dehydration and metallic and carbon coating of specimen required to SEM interfere the efficiency of elemental analysis.

Analysis of frozen or freeze-dried samples with X-ray spectrometry is another choice of approaches. This report is a trial to detect elements in biological specimen and to compare the efficiency of frozen and the freeze dried samples in their elemental detection.

Further to our previous reports on silky fowls [6, 8], X-ray microanalysis [4] and energy loss spectrometry [1, 7, 9] of their pigment granules, this report surveys major elements in frozen or freeze-dried tissues from silky fowls with the energy dispersive X-ray fluorescence spectrometer (EDXRF) [10]. Silky fowls are Chinese origin and their feather is silky touch. Their almost all organs contain specific granules. This is not biocondensation of elements in those organs but specific distribution of pigments in connective tissue. Meat, blood, and egg of this fowl have been evaluated as healthy food or medicine. Metallic elements might contribute the effect.

MATERIALS AND METHODS

A total of 6 silky fowls (three females and three males) were used in this study. Tissue blocks from each organ were either freeze dried or simply frozen. Frozen samples were taken from the following 33 tissue blocks: feather, egg shell, shell membrane, yolk, egg white, blood, liver, kidney, pancreas, spleen, cardiac muscle (atrium), cardiac muscle (ventricle), lung, adrenal gland, eye ball, lens, retina,

esophagus, crop, duodenum, jejunioileum, bone marrow, cerebrum, cerebellum, thyroid gland, chick comb, trachea, breast skin, testis, M. pectoralis major, M. sartorius, femur and gizzard. Freeze dried samples were taken from the liver, kidney, pancreas, spleen, cardiac muscle (ventricle), lung, proventriculus, and gizzard. Freeze drying was conducted for 24 hr or 7 days. The size of each tissue was variable but approximately same size of a given organ in both frozen and freeze drying samples for comparison.

Operating conditions of the energy dispersive fluorescence spectrometer (element analyser JSX-3300ED, JEOL, Tokyo) were 20 kV, 15 μ A, Rh target with Si (Li) semiconductor detector. Vacuum condition of specimen chamber was 10^{-2} Torr. For semi-quantitative analysis, the fundamental parameter method (standard less method) was employed. Results were analysed under the assumption that elements are in the form of oxidative compounds or carbon compounds in this instrument because it was programmed in this EDXRF. They were calculated as weight %, but when the amount of a given element was very small it was recorded as parts per million (ppm).

RESULTS

Major elements in all tissues examined were P, S, K, and Cl, Fe, Ca, Mn, Zn, and occasionally Br were also detectable, though their amount was less than P, S, K, and Cl (Table 1). In the thyroid glands, iodine was the characteristic element (Fig. 1).

There were no essential differences in the elements of the frozen samples (Table 1) and those of the freeze-dried samples (Tables 2 and 3). The duration of freeze drying, 24 hr or 7 days, did not make a significant difference in elemental analysis. Therefore, 24 hr was sufficient in this survey. There was no difference in elements of male and female silky fowls.

DISCUSSION

Although not found in the high amount, Zn in almost all

Fig. 1. Elemental analysis of frozen thyroid. Thyroid glands contain iodine (arrows).

samples was characteristic to the silky fowl. Zn is detectable in oyster, eels, terrapin, and other Chinese medicines [11]. The silky fowl is also sometimes used as a material substance in Chinese medicine. Analysis of one Chinese medicine made of the tissues of silky fowls, Wuchi Paifeng Wan, detected P (0.958%), Fe (0.0357%), Ca (0.0447%), Mg (0.0628%), S (0.71%), Cu (10.8ppm), Zn (107ppm), and Mn (6.13ppm) [personal communication].

Electron energy loss spectrometry (EELS) of pigment granules in the skin of silky fowls also detected Zn and Ca [1, 7, 9].

The major elements P, S, K, and Cl were also detected by the wet scanning electron microscope (WET-SEM) in hydrated specimens [3, 5].

The overall elemental analysis employed in this survey is versatile in estimating approximate amounts of elements in frozen or freeze-dried samples. There are two types of element analyser: energy dispersive (as used in this survey) and wave length dispersive. The later type is more sensitive but requires electrons in the amount of hundreds or thousands times for fluorescent X-ray images. Therefore, the energy dispersive type is preferred for biological specimens.

Table 1. Elemental analysis of hydrated frozen tissues of silky fowls detected with EDXRF

	Na	P	S	Cl	K	Ca	Mn	Fe	Zn
Feather	<5.99 ^{a)}	1.07	25.60	4.35	10.10	9.69	0.11	0.34	0.12
Egg shell	<2.62 [*]	3.81	1.30	n.d.	0.16	60.60	<119 ppm [*]	139 ppm	n.d.
Shell membrane	<6.28 [*]	0.91	21.60	11.50	15.50	2.74	<453 ppm [*]	531 ppm	n.d.
Yolk	n.d. ^{b)}	16.20	8.85	6.35	9.07	14.60	<612 ppm [*]	0.67	0.32
Egg white	n.d.	1.05	15.80	17.70	27.60	1.90	<665 ppm [*]	0.14	n.d.
Blood	n.d.	3.39	9.84	28.70	20.90	1.97	<475 ppm [*]	3.13	346 ppm
Liver	n.d.	12.70	12.40	3.25	25.90	1.18	0.13	1.22	0.40
Kidney	8.21	11.20	9.84	9.82	19.20	1.23	528 ppm	0.77	0.14
Pancreas	<4.90 [*]	15.70	8.39	5.64	24.47	1.43	741 ppm	0.19	0.17
Spleen	n.d.	9.57	16.30	n.d.	22.60	2.57	<0.15 [*]	3.19	0.27
Atrium	n.d.	9.23	14.30	5.40	26.90	1.60	998 ppm	0.74	0.29
Ventricle	<7.97 [*]	8.65	13.30	5.11	27.30	1.71	<719 ppm [*]	0.79	0.35
Lung	<5.11 [*]	8.80	9.77	12.30	25.70	1.64	<484 ppm [*]	2.37	0.11
Adrenal glands	n.d.	16.90	11.70	<1.55 [*]	17.70	5.62	<0.17 [*]	0.69	0.15
Eye ball	<4.64 [*]	4.07	5.09	29.00	12.50	18.60	<291 ppm [*]	920 ppm	481 ppm
Lens	<9.95 [*]	3.94	4.63	42.80	6.55	4.10	<419 ppm [*]	575 ppm	n.d.
Retina	n.d.	6.53	4.55	8.26	3.91	41.80	<417 ppm [*]	0.17	521 ppm
Esophagus	n.d.	8.81	16.20	1.86	22.90	5.14	<0.12	0.54	0.40
Crop	n.d.	6.80	11.30	14.00	29.10	2.35	<591 ppm [*]	0.32	0.19
Duodenum	<7.06 [*]	9.74	13.80	5.35	19.10	4.52	892 ppm	0.48	0.26
Jejunioileum	<22.6 [*]	10.70	14.90	n.d.	15.10	3.70	<0.30 [*]	1.20	0.29
Bone marrow	n.d.	10.60	11.00	5.09	22.50	6.60	<982 ppm [*]	3.43	<808 ppm [*]
Cerebrum	<5.75 [*]	14.90	9.33	3.72	25.90	1.11	637 ppm	0.30	409 ppm
Cerebellum	<5.81 [*]	15.00	9.14	3.44	24.90	1.09	745 ppm	0.29	866 ppm
Thyroid glands	24.90	8.00	12.10	n.d.	7.43	4.35	<0.22 ppm [*]	0.76	<0.18 [*]
Chick comb	<14.6 [*]	5.49	14.80	13.20	13.30	6.92	<0.13 [*]	0.61	<0.13 [*]
Trachea	<7.15 [*]	7.96	14.30	11.30	11.60	8.00	649 ppm	0.27	907 ppm
Breast skin	<8.61 [*]	4.54	13.20	14.90	17.70	2.55	<853 ppm [*]	0.33	0.22
Testis	<5.43 [*]	12.20	9.40	8.64	26.40	1.29	<480 ppm [*]	0.17	0.14
M. pectoralis major	n.d.	11.30	10.10	n.d.	37.50	1.50	<0.11 [*]	0.20	<965 ppm [*]
M. sartorius	n.d.	9.88	13.40	2.59	31.80	0.87	<699 ppm [*]	0.34	0.21
Femur	<11.7 [*]	9.30	6.34	1.55	2.65	33.60	<643 ppm [*]	0.19	<604 ppm [*]
Gizzard	n.d.	9.45	9.92	11.60	27.30	3.30	<764 ppm [*]	0.47	0.15

a): deviation 2 σ . b) n.d.: not detectable; cncentration: weight % or ppm.

Table 2. Comparison of elemental analysis of 7 days or 24 hr freeze-dried samples of silky fowls detected with EDXRF

		P	S	Cl	K	Ca	Fe	Zn	Br
Liver	7 days	12.80	12.61	3.82	23.17	0.97	1.45	0.38	995 ppm
	24 hr	11.67	12.36	3.48	25.17	1.50	1.39	0.21	0.10
Kidney	7 days	11.64	10.31	7.45	25.60	2.79	1.03	0.30	893 ppm
	24 hr	10.56	10.80	7.41	27.42	2.35	1.55	0.31	0.36
Ventricle	7 days	8.92	12.91	6.82	28.42	1.49	0.73	0.17	664 ppm
	24 hrs	7.69	12.78	6.97	29.89	2.47	0.97	0.19	0.34
Spleen	7 days	11.86	10.17	5.67	29.05	0.67	2.64	0.11	609 ppm
	24 hr	12.14	10.39	4.77	28.89	1.23	1.89	0.13	0.14
Pancreas	7 days	14.48	8.98	5.70	24.70	3.46	0.29	0.33	533 ppm
	24 hr	15.19	10.59	2.20	24.80	3.18	0.74	0.21	0.19

concentration: weight % or ppm.

Table 3. Comparison of elemental analysis of frozen and freeze-dried samples

		P	S	Cl	K	Ca	Fe	Zn	Br
Lung	1 ^{a)}	8.80	9.77	12.30	25.70	1.64	2.37	0.11	n.d. ^{d)}
	2 ^{b)}	8.42	10.57	13.65	25.93	1.98	2.30	824 ppm	0.16
	3 ^{c)}	10.83	13.58	17.68	33.97	2.62	3.07	0.11	0.21
Trachea	1	7.96	14.30	11.30	11.60	8.00	0.27	907 ppm	640 ppm
	2	7.88	15.35	7.92	15.72	6.51	0.46	1000 ppm	0.14
	3	10.44	20.65	10.72	21.92	9.37	0.67	0.15	0.21
Esophagus	1	8.81	16.20	1.86	22.90	5.14	0.54	0.40	n.d.
	2	7.13	12.48	11.50	26.68	3.70	0.31	0.14	0.18
	3	9.30	16.12	14.82	35.23	5.01	0.42	0.19	0.25
Duodenum	1	9.74	13.80	5.35	19.10	4.52	0.48	0.26	n.d.
	2	9.93	11.54	4.75	26.11	6.83	0.58	0.20	0.12
	3	12.95	15.13	6.11	35.03	9.45	0.81	0.28	0.17
Jejunioileum	1	10.70	14.90	n.d.	15.10	3.70	1.20	0.29	n.d.
	2	7.11	13.05	5.40	27.00	6.47	0.46	0.26	0.14
	3	9.24	17.03	7.00	36.08	8.85	0.63	0.35	0.20

a) Frozen sample, b) Freeze dried sample, c) Freeze dried sample, d) n.d.: not detectable; concentration: weight % or ppm.

However, the wave-length dispersive element analyser should be reevaluated when using bulk frozen or freeze dried specimen as samples because they can resist considerably strong electron beams.

A combination of the present analyser (EDXRF) and the scanning electron microscope, especially the WET-SEM and the environmental SEM (ESEM) [2, 3, 5], is available. The wave length dispersive type (WDXRF) used in concert with the electron microscope is also available.

Elements in 33 frozen tissues of silky fowls were analysed with an energy dispersive X-ray fluorescence spectrometer. Also analysed were elements in 5 tissues freeze-dried for 24 hr or 7 days.

Major elements detected were P, S, Cl, and K. Ca, Fe, Mn, and Zn were also detectable but minor elements. Comparisons of frozen and freeze-dried samples and also comparisons between tissues freeze-dried for 24 hr and 7 days did not find essential differences.

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