BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 51 (12), 3649—3650 (1978)

Preparation of Shark Powder and Shark Paste as Biological Standard Reference Materials

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(Received July 4, 1978)

Synopsis. As a possible biological standard reference material for metal analysis, shark powder has been prepared from the muscle of sharks (*Squalus mitsukurii*) and compared with shark paste which was prepared from the same raw material adopting the procedure of Japanese fish paste production. With respect to the homogeneity of metal contents and to the stability during storage, both samples were found to be similar.

As a possible biological standard reference for metal analysis the authors have suggested the preparation of powdered Japanese tea leaves.[1] Since there has been no fish reference material available, a search for a suitable fish reference material was commenced. A wet fish reference material, shark paste, has been proposed by the present authors as a possible candidate for a new biological reference material.[2] In this report, a comparison is made between the wet fish reference material and the dry powder, shark powder, prepared from the same raw material, from the standpoint of practical utilization.

Experimental

Preparation of Shark Powder. The procedure for preparation of shark powder in comparison with shark paste, is outlined in Fig. 1. Two equal amounts of the white muscle was dissected from eight sharks (*squalus mitsukurii*) and one was used for shark powder and the other was used for shark paste. The freeze-dried muscle was pulverized with an agate ball mill (Fritsch Co., Ltd.) followed by sieving with a polyvinylidene chloride fiber sieve (50 mesh). The powder obtained was bottled in polystyrene bottles of 20 ml and kept in a desiccator containing silica gel.

Other Reference Material. Tuna powder was obtained from NBS. This material is packed in a polyethylene pouch which is sealed in an aluminium can and it is necessary to use immediately after opening the can. The metal contents are not certified yet for this material.

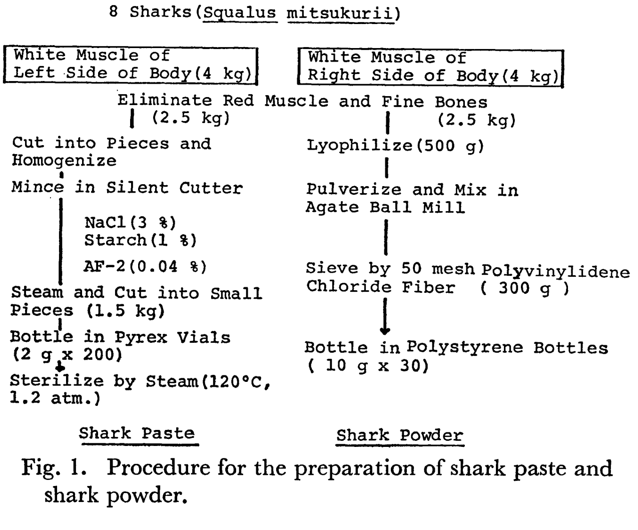

Fig. 1. Procedure for the preparation of shark paste and shark powder.

Determination of Water and Metal Content. Five lots of 0.1—0.5 g were taken from the bottles and dried at 90 °C in dry oven for 24 h to determine the water content. The powder was then used for the determination of metals by atomic absorption and flame emission spectrometry.

For mercury determination, 5 additional portions were taken and digested with HNO_3 and H_2O_2 in the same manner as previously reported.[3]

Results and Discussion

Homogeneity of Shark Powder. The content of metals in the shark powder and NBS-tuna is shown in Table 1. The homogeneity with respect to K, Na, Mg, and Hg is similar for both samples. However, with respect to Ca, the results for shark powder indicated a better homogeneity compared to NBS-tuna. According to Rook,[4] the comparatively poor homogeneity of Tuna with respect to Ca might be attributed to the bone meals in tuna meat. In this study, the raw materials, shark meat, which contains less bones than tuna meat was utilized. In addition, sieving with the polyvinylidene chloride fiber would eliminate the comparatively heterogeneous part which was considered to be mainly the fisca containing a higher amount of Ca than the muscle part.

According to Barnes,[5] the variation in calcium determinations for NBS-tuna can be controlled to within

Table 1. Metal content in shark powder and tuna powder (μg/g-dry)

	Shark powder	NBS-tuna[a]		Shark powder	NBS-tuna[a]
K	17300	12300	Ca	185	253
	17100	12000		208	385
	17400	12000		210	331
	17100	12700		200	493
	17300	12000		199	338
Na	4000	1240	Hg	7.68	0.59
	4080	1280		7.72	0.60
	4090	1310		7.88	0.57
	4080	1290		8.29	0.57
	4070	1270		7.75	0.50
Mg	1090	1080			
	1080	1080			
	1090	1060			
	1070	1100			
	1100	1110			

a) Determined by present authors.

5% relative standard deviation. Since the calcium determination by flame spectrometry tends to be subjected to chemical interferences, these values obtained in this study might be improved by adopting a more accurate method such as isotope dilution mass spectrometry. In any case it is considered that homogeneity of the prepared shark powder is higher with respect to calcium compared to that for NBS-tuna.

Preservation of Shark Powder. Preservation is one of the most important problems when a biological material is handled. With respect to shark powder and more particularly shark paste, changes of organic constituents by both chemical and biological activities during the preservation period should be considered. ^{60}Co irradiation which is often adopted in plant materials, however, is not suitable for this material, since chemical changes of some trace organic compounds are suspected during the irradiation.

As an index of preservation, the chang of weight was measured in a three month period during summer. The powders were kept in the same polystyrene bottles in a desiccator containing silica gel. The water and ash contents of both samples are shown in Table 2.

TABLE 2. CHANGE OF WATER AND ASH CONTENTS DURING STORAGE

	June, 1977		September, 1977	
	Water	Ash	Water	Ash
Shark powder	7.34%	5.46%	5.27%	5.35%
NBS-tuna	1.04	4.28	1.64	4.04

Shark powder lost a little moisture during storage whereas NBS-tuna powder gained a little moisture. In plant samples, less change of weight was observed as previously reported.[1)] This fact indicates that care should be taken in the preservation of these powders. With respect to the ash content in dry matter, little weight change was observed for shark powder and tuna powder. In addition, no change in flavor was observed with shark powder, which indicated that oxidation of oily materials did not take place during the storage. These observations suggest that NBS-tuna powder should be preserved in a can until it is used as stated by NBS. Shark powder, however, could be preserved in a desiccator for at least 3 months without any significant change of chemical components, provided the dry weight is measured prior to utilization.

TABLE 3. METAL CONTENTS IN SHARK PASTE AND SHARK POWDER

	Shark paste		Shark powder
	μg/g-wet	μg/g-dry	μg/g-dry
K	3650±100	11600+300	17200+200
Na	13700±450	43400±3000	4060±40
Mg	220±6	890±13	1080±10
Ca	59±3	187±13	200±10
Fe	26±5	83±15	12±1
Zn	3.1±0.6	10±2	15±1
Hg	1.96±0.05	6.22±0.16	7.88±0.22

average±s.d.

Comparison of Shark Powder and Shark Paste. In Table 3, the metal contents of shark powder and shark paste are shown. The values of shark paste are shown for a wet weight as well as a dry weight basis (dry weight are calculated from the values on the wet weight basis using the water content of 68.5%).

The Na content is ten times higher in shark paste than in shark powder, due to the addition of NaCl in the preparation process of shark paste. The contents of K and Mg are about 30% smaller in shark paste which might be caused by the addition of starch and by the loss during the steaming process. The high content of Fe in shark paste might be due to contamination from the material of the silent cutter.

Fundamentally, shark powder which was less processed in the preparation step, must be subjected to less contamination and loss of elements. Thus, its elementary composition represents that of the natural shark meat. Shark paste, a manufactured product is somewhat different from the natural fish meat, but the difference in metal contents is sufficiently small for Hg, K, Ca, and Mg (within 30%) to enable to be used as a standard reference material for the metal analysis of fish, because the metal contents are the same order as the natural ones.

With respect to the homogeneity of elements, both samples provided similar result, when 0.5 g, dry lots (*ca.* 2 g wet lots in shark paste) were analyzed.

The preservation test indicated that both samples can be preserved for at least 3 months at room temperature. Longer preservation is now under investigation, and it is thought that they will be preserved for one year or longer.

As a conclusion, both shark powder and shark paste are good candidates for new standard reference materials. Since most of the actual field determinations of metals in fish are performed with "wet" or "fresh" samples, shark paste might be a more suitable standard reference material, for example, in the determination of Hg in fish meat by the combustion-gold trap atomic absorption technique. On the other hand, when metal analysis is performed by X-ray fluorescence spectrometry, shark powder would be more suitable than shark paste. Therefore, these two samples can be utilized according to the fields and requirements of analysis.

This work is supported partially by Japan-U.S.A. Co-operative Project between JSPS and NSF No. 6R023.

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