Fish Composition: Effects of Fish Preparation and Analytical Methods

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It is important to determine the nutrient content of fish fed to piscivorous species to ensure the animals are receiving an appropriate diet. The results of chemical analyses for nutrients can be affected by various factors including collection of a representative sample, sample preparation methodology, and laboratory and instrument variation. The objective of this project was to determine the effects of sample handling and preparation on dry matter, protein, fat and energy content in rainbow trout *(Oncorhynchus mykiss)*. Variation among laboratories using the same preparation methods was also investigated. Five different methods of preparation were utilized: thawed pureed, frozen pureed, thawed pureed with water, ground with dry ice, and ground with liquid nitrogen. Subsamples from each preparation method were submitted to two laboratories for analyses. It was found that method of preparation does not significantly affect chemical analysis. However, variation among laboratories can be an important source of variation in analysis.

Key Words: fish, proximate analysis, sample preparation

INTRODUCTION

The nutritional status of piscivorous species is dependent upon the quality and composition of the fish they consume. Nutrient concentrations for many fish species have been published, but these data pertain primarily to fillets and other fish parts consumed by humans and captive piscivores are generally fed whole fish.

Although information on the composition of whole fish are scarce, the data that are available demonstrate a considerable variability in nutrient content (Table 1).[1,4,6] Variations in composition of whole fish occur by species, individuals within a species, age, sex, diet, season and year. [1-5,7] Substantial seasonal differences have been found in salmon *(Salmo salar)* , which increase in lipids during the winter, and in herring, which are higher in lipids during the fall. Although the same species, capelin *(Mallotus villosus)* caught off the coast of Iceland during the winter months differ in nutrient content from those caught near Canada in the summer months.

These variations, difficulties in maintaining sufficient supplies of fish, and the likelihood that alternate species or sources of fish will need to be used, provide justification for routine sampling and analysis. Without routine testing, zoos may be dependent on the supplier to provide information on nutrient composition, which is often not specific for a particular shipment or not available. Knowing the actual nutrient concentrations of the fish can aid in determining the most appropriate replacement when ordering new batches, while maintaining continuity in the nutrient composition of the overall diet.

Some zoos feed piscivorous species based on the gross energy content of the diet. While gross energy does not indicate the degree to which an animal is able to utilize the energy in its diet, it

does provide a guide upon which diets may be based. Without appropriate data on the gross energy content of the fish, in conjunction with alterations in dietary intake, animals may undergo body mass fluctuations.

It is also important that data on nutrient content be accurate. Many factors can influence the accuracy of data including sampling technique, sample preparation, and variations between laboratories. Sampling technique and lack of homogeneity can be a great source of error in food analyses. An ideal sample should be homogeneous and identical to the bulk of matter from which it was taken. Additionally, sample preparation can alter results before the analyses begin. Whole fish are often difficult to analyze properly because the entire fish, including bones, skin and viscera, must be combined to have an accurate and homogeneous representation.

METHODS

A study at Brookfield Zoo examining the effects of sample preparation on dry matter, crude protein, crude fat, and gross energy concentrations in rainbow trout *(Oncorhynchus mykiss)* was conducted. In addition, variations between laboratories running the same analyses were evaluated. Medium sized (10-15 cm) trout were subjected to five sample preparation techniques: 1) thawed pureed, 2) frozen pureed, 3) thawed pureed with water, 4) ground with dry ice, and 5) ground with liquid nitrogen.

Sampling Fish were purchased and held at -29°C for 3 weeks. Five 11 kg cases of IQF trout were opened and combined into a single container. Frozen trout (454g) were placed in labeled plastic freezer bags and stored at -29°C until sample preparation, approximately 1 week later. Twenty-four hours prior to processing, the samples were moved to a -80°C freezer. Fish were physically broken into pieces before processing.

Sample Preparation

All samples were blended in a 3.8 L (1 gal) commercial Waring blender (Waring Products, New Hartford, CT) which was thoroughly cleaned and dried between samples. Each of the five treatments was replicated five times to yield a total of 25 samples. Each of the replicates was divided into duplicate sub samples for analysis of dry matter (DM), crude protein (CP), crude fat (CF), and gross energy (GE).

Treatment 1. Thawed pureed. Samples were thawed at 2°C overnight in a covered plastic container. The fish were then blended using a pulse action. A slight increase in the temperature of the puree is probable, but was not measured.

Treatment 2. Frozen pureed. Samples taken directly from the freezer were blended into a coarse powder using a pulse action. Some thawing of the sample was noted during processing, but samples were placed immediately in -80°C freezer .

Treatment 3. Thawed pureed with water. Samples thawed at 2°C overnight in a covered plastic container. The fish were then blended with ISOg cold water using a pulse action. There may have been a slight increase in the temperature of the puree during processing, but was not measured.

Treatment 4. Ground with dry ice. Samples taken directly from the freezer were blended with pieces of dry ice using a pulse action. Additional dry ice was added (totaling 454g) as needed to maintain a coarse frozen powder. Samples did not appear to thaw during processing and carbon dioxide was allowed to evaporate at -80°C.

Treatment 5. Ground with liquid nitrogen. Samples were placed in the blender , covered with liquid nitrogen and blended using a pulse action until a frozen powder was formed. Samples did not appear to thaw during processing and nitrogen was allowed to evaporate at -80°C.

RESULTS AND DISCUSSION

Sample preparation

Grinding with liquid nitrogen produced the most homogenous sample based on visual examination, while the homogeneity of each of the other processes appeared variable. Grinding with dry ice and both processes that involved thawing appeared to incompletely homogenize skin.

Sample analysis

Using standard deviations of averaged analytical values as an indicator of sample homogeneity , there was little difference among sample preparation methods for the analyses performed at Brookfield Zoo, despite apparent differences upon visual examination (Table 3). In a comparison of nutrient analyses among laboratories (Tables 4 and 5), grinding fish with liquid nitrogen proved to result in the least variation. Yet, it is important to consider all analytical methods and laboratory variation when evaluating the results of fish analyses.

CONCLUSION

Routine analysis of fish is important in determining the nutrient composition of diets consumed by piscivorous species. While no single process was found to be significantly better than another for dry matter, protein, fat, or energy, grinding fish with liquid nitrogen appeared to provide the most homogenous mixture and the most consistent analytical results. Additionally, thawing of fish samples is not appropriate for analysis of any volatile nutrients (e.g. vitamins and fatty acids). Sample preparation for nutrient analyses should provide a homogenous mixture and be consistent over time. Analyses should be completed at the same laboratory for appropriate comparisons.

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Table 1 Range of nutrient composition of fish at Brookfield Zoo between 1992 and 1997[a] (Dry matter basis)

Fish Species	Dry Matter	Crude Protein	Crude Fat	Gross Energy
	----------	---------%---------	----------	kcal/g
Trout (O. my kiss)	21.7-31.8	55.7-63.1	26.0-35.0	5.75-65.1
Capelin (M. villosus)[B]	19.4-25.9	53.9-70.3	17.2-39.0	5.59-6.16
Herring (C. harengus)	23.0-33.6	44.6-81.1	13.7-48.8	5.35-6.63

[a] Analyses completed at least once yearly on new fish shipments of fish.
[B] Capelin were not available or analyzed during portions of 1994-1995.

Table 2. Methods of analysis at three laboratories

Nutrient	Brookfield Zoo	Covance	DHIA
DM	Forced draft oven (60^oC)	Vacuum Oven (100^oC)	Forced draft oven (60^oC)
CP	Nitrogen by Kjeldahl x 6.25	Nitrogen by Kjeldahl x 6.25	Nitrogen by Kjeldahl x 6.25
CF	Soxhlet ether extraction[a]	Chloroform-methanol extraction[b]	Acid hydrolysis and ether extraction[c]
GE	Bomb calorimetry	Calculated difference	Bomb calorimetry

[a] AOAC Method 960.39, modified
[b] AOAC Method 983.23 modified
[c] AOAC Method 954.02

Table 3. Nutrient analysis of fish at the Brookfield Zoo following five preparation methods (dry matter basis).

Trt.	Method	Dry Matter	Crude Protein	Crude fat	Gross Energy
		----------	------%--------	----------	kcal/g
1	Thawed Pureed	27.2± 1.9	53.9 ± 4.9	27.5 ± 3.6	5.35 ± 0.14 [ab]
2	Frozen Pureed	27.4 ± 1.7	54.2 ± 4.5	28.6 ± 4.7	5.27 ± 0.25 [b]
3	Thawed Pureed with water	21.9 ± 0.5	48.2 ± 4.3	33.5 ± 3.1	5.60 ± 0.17 [a]
4	Ground with dry ice	28.6 ± 0.8	52.4 ± 4.6	35.3 ± 11.0	5.41 ± 0.19 [ab]
5	Ground with liquid nitrogen	28.7 ± 1.1	52.2 ± 4.9	30.0 ± 2.4	5.31 ± 0.08 [b]

[ab] Different superscripts within a column differ significantly at $P< 0.05$. Statistical analysis by SPSS 6.1 for Windows (SPSS, Inc., Chicago, IL) by one way ANOVA and Scheffe's test for Post Hoc multiple comparisons.

Table 4. Comparisons of nutrient analysis on thawed pureed fish completed by three laboratories (dry matter basis)

Laboratory	Dry Matter	Crude Protein	Crude Fat	Gross Energy
	----------	---------%---------	----------	kcal/g
Brookfield Zoo	27.2	53.9	27.5	5.35
Covance [a]	28.1	63.0	28.1	5.05
DHIA [a]	27.1	59.1	30.6	5.53
DHIA [b]	30.7	53.1	35.9	5.85

[a] Sample processed by Brookfield Zoo.
[b] Sample Processed by DHIA.

Table 5. Comparisons of nutrient analysis on fish ground with liquid nitrogen completed by three laboratories (dry matter basis)

Laboratory	Dry Matter	Crude Protein	Crude Fat	Gross Energy
	----------	---------%---------	----------	kcal/g
Brookfield Zoo	28.7	52.2	30.0	5.31
Covance [a]	29.4	53.7	36.1	5.48
Covance [b]	27.0	57.5	35.2	5.50
DHIA [b]	29.1	56.0	35.8	5.91

[a] Sample processed by Brookfield Zoo. Covance
[b] Sample Processed by Covance.