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by

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Optimum Conditions for Fish Lipid Extraction

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ABSTRACT

Lipid contents of six fish species were analyzed based on the method of Bligh & Dyer (1959). Samples ranging from 1 to 9 grams were extracted with 50 ml. of three mixtures of methanol and chloroform (2:1, 1:1 and 1:2 v/v). The aliquot was taken from the bottom layer after adding 30 ml. water and used for lipid determination. The lipid content of fish flesh were found to range 0.6 % to 11.5 %. Significant variation of lipid content of the same species was caused by mixture of solvent used, the ratio of solvent volume and sample size and the interaction effect of these factors. Duncan' s multiple range test showed that the optimum conditions for lipid extraction of fish can be achieved by: 1. use methanol : chloroform 2:1 with lean fish, 2. use methanol : chloroform 1 : 2 with fatty fish and 3. at least 50 folds of solvent volume to the sample size was used. Methanol : chloroform 1:1 may preferentially be used for any fish tissue, in general.

INTRODUCTION

Fish lipid, as well as other animal tissue lipid can be extracted by several methods, namely, Soxhlet method, Folch method (Folch et al., 1957) and Bligh-Dyer method (Bligh and Dyer, 1959). Among these methods, the method developed by Bligh and Dyer has been widely used due to its simplicity and rapidness. The procedure involves an initial extraction of 100 g sample with a solvent mixture of 200 ml methanol and 100 ml chloroform, followed by a second extraction with 100 ml chloroform and addition of 100 ml water to form a biphasic solution. The final ratio of methanol-chloroform-water is 20:20:18 (v/v) when the moisture content of the fish was 80 %. This method was originally developed for lean fish like cod whose muscle tissue contain 80 % moisture and about 1 % lipid, and was required a second extraction. In our preliminary observation, the solvent ratio used in this method did not effectively extract the fat from fatty fish, and the result was subject to the sample size. It was thus decided to determine how the optimum solvent mixture would vary in relation to fish fat content and sample size in order to find out an optimum extraction condition which yields a correct fat content for fish having a wide range of fat.

MATERIALS & METHOD

Sample preparation

Cod (*Gadus morhua*), red hake (*Urophycis chuss*), herring (*Clupea harengus*), Mackerel (*Scomber scombrus*), winter flounder (*Pseudopleuronectes americanus*) and butter fish (*Peprilus triacanthus*) were obtained randomly from Point Judith Fishery Cooperation, Galilee (R.I.). Fish were filleted, ground through a Hobart meat grinder (model A-120) using a 50 mm plate and homogenized for 30 second in a food processor (Black & Decker). The resulting samples were kept in the refrigerator (4 ^{0}C), until analyzed.

Lipid extraction procedure

Samples weighing from 1-9 g were blended with 50 ml of solvent mixture, for 2 min at a moderate speed (rheostat setting at 40) using Waring blender. The homogenate was filtered through a coarse filter paper (Thomas) into a separatory funnel, and 30 ml distilled water added to allow phase separation. When there was difficulty in phase separation due to emulsion formation, small amount of NaCl (approximately 1-2 g) was added to break down the emulsion. Ten ml of the bottom layer (chloroform) was transferred into a preweighed vial and evaporated over mild heat in the hood. After the solvent had been evaporated, it was flushed with nitrogen stream and weighed for the lipid content. The weight difference was used for computing a fat content, which was expressed in g lipid per 100 g sample (%).

Moisture determination

The moisture content was determined by a conventional oven drying method at 110^{0}C, and was expressed as g moisture per 100 g sample (%).

Statistical design and analysis

A factorial design with two replications was used to evaluate the effect of 15 treatments based on the combinations of three different solvent mixtures of methanol and chloroform (2:1, 1:1 and 1:2) and five levels of sample size (1, 3, 5, 7 and 9 g). Analysis of variance (ANOVA) as condduw using the Statistical Analysis System Package (SAS, 1982) to determine the fignificance of variation. The following sources of variation and degree of freedom were used for each fish : solvent (2), sample size (4), solvent x sample size (8) and error (15). Because of significant differences in lipid contents among fish species, the ANOVA was performed separately on 3 groups based on the lipid contents (lean fish below 3%; medium fatty fish 3-8%; and fatty fish above 8%). In addition, the sighificance of differences in means was determined by Duncan's multiple range test.

RESULTS & DISCUSSION

Lipid content and relationship to moisture content

The lipid content of the tested fish varied due to the different treatment applied (Table 1); 0.67–1.29 % in cod, 0.75–1.50 % in winter flounder, 0.71–1.27 % in red-hake, 1.68–5.99 in herring, 1.87–6.33 % in mackerel, and 1.90–11.56 in butter fish. Such variations in the lipid content are seen in the reported values for cod which vary frow 0.6 % (Stanby, 1962), 0.73 % (Lands, 1986), 0.31–0.70 % (Bligh and Dyer, 1959), and 0.67–1.29 % from this laboratory. The differences in the reported values can be attributed to the use of different extraction condition in addition to biological and seasonal variability. The moisture content was less in herring, mackerel and butter fish which contain high fat, but it was high in the lean fish species like cod, winter flounder and red hake (Table 1). This confirms that the moisture content shows an inverse relationship with lipid content in the muscle.

Effect of solvent mixture

Regarding the mixture of solvent used, the amount of lipid extracted from cod, winter flounder and red hake was higher when the 2:1 ratio of methanol to chloroform was used. This is in agreement with the result of the fat analysis on cod muscle by Bligh and Dyer (1959). However, when mackerel, herring and butter fish were analyzed, the 1:2 ratio of methanol to chloform yielded higher lipid contents than the 2:1 ratio. On the other hand the 1:1 ratio of methanol chloroform was found to be moderatly effective to all species tested. The differences in lipid contents caused by varying solvent ratio was highly significant ($p<0.01$) in all species of fish tested.

According to ANOVA by fish category, the differences of lipid content found in fish within the same category was not significant at $p<0.05$. Based on the result of the Duncan's multiple range test, the 2:1 ratio of methanol to chloroform was the best in terms of extraction efficiency for lean fish (cod, winter flounder and red hake), while the 1:2 ratio was the best for medium fatty fish (mackerel and herring) and fatty fish (butter fish) ($p<0.05$) (Table 2).

Results of this experiment indicated that the 2:1 ratio of methanol to chloroform is not suitable for all species, and that the proper ratio of solvents depend upon the fat content of fish. Bligh and Dyer (1959) found the 1:2

ratio of methanol to chloroform is the most suitable solvent system. The results of the analysis of cod in this study at the 2:1 ratio were in agreement with those reported by Bligh & Dyer (1959). Furthermore, the other lean fish species namely winter flounder and red hake were also preferentialy extracted by the mixture of methanol-chloroform 2:1. On the other hand, the mixture of methanol-chloroform 1:2 was perceivable to be the best solvent for fatty fish. Although little information about lipid class of fish is available, Levern (1962) listed examples of fish in which the flesh depot lipid consisted essentially of triglycerides. They were herring, sardine, pilchard, menhaden, anchovy, mackerel, and tuna. These species are fatty fish. On the other hand, the lean fish whose lipid is compose of primarily phospholipid. The reason for the greater extraction efficiency of the solvent mixture having a high proportional of chloroform for fatty fish can be thus explained by the above finding that the lipid of fatty fish is composed of triglycerides as a major class lipid which is preferentially extracted by chloroform over methanol.

Effect of sample size

At the constant volume of solvent mixtures used, the amount of lipid extracted was decreased with an increase in the amout of sample (Table 3). At 1 g sample to 50 ml solvent, the maximum amount of lipid was extracted in all fish samples for all solvent mixtures used. The result of the ANOVA based on the fish group showed that these were highly significant differences, in fat extraction among different sample sizes. The Duncan's multiple range test showed that the maximun lipid was extracted at 1 g of sample to 50 ml solvent for all fish groups and solvent mixtures (Table 3).

It was evident from the result that the sample size of 1 g per 50 ml solvent was most appropriate. This indicates that the proportion of solvent to sample size should be at least 50-fold. However, the solvent mixture to a final dilution 20-fold the volume of tissue sample was used by Folch, *et al.* (1956). It was noted that when the sample of 5 g or greater was used, there occurred an interfacial fluff. Salt is often added to prevent the emulsion formation at the interfacial phase of the solvent.

Partitioning interaction effect

The maximum amount of lipid content was obtained at the sample size of 1 g. The same result was observed in every fish category (Table 4.) This could be perceivable that the main factor affected lipid extraction is the amount of solvent rather than the solvent mixture itself. The least variations in the amount of lipid extracted were seen when the 2:1 ration of methanol to chloroform for the lean fish, the 1:2 ratio for the medium fatty fish and fatty fish.

Table 1. Lipid and moisture of fish flesh as affected by species and extraction[1] condition.

Method of extraction		Lipid content (%)					
Solvent mix. Meth:chlor	Sample size (g) (with 50 ml solvent)	cod	flounder	red hake	herring	mackerel	butter-fish
2:1	1	1.19	1.15	1.27	5.99	5.70	11.56
2:1	3	1.02	1.08	1.26	5.61	5.96	11.06
2:1	5	0.97	1.01	1.12	3.23	4.18	4.42
2:1	7	1.06	1.01	1.06	1.96	2.46	2.53
2:1	9	1.04	1.01	1.10	1.68	1.87	1.90
1:1	1	1.09	1.20	1.07	4.22	5.53	10.80
1:1	3	0.94	0.94	0.93	5.62	5.29	10.11
1:1	5	0.87	0.91	0.92	5.38	5.54	10.21
1:1	7	0.95	0.91	0.93	5.51	5.24	5.69
1:1	9	0.92	0.91	0.95	3.61	3.53	6.59
1:2	1	1.29	1.50	1.23	4.42	6.33	11.25
1:2	3	1.03	1.14	0.95	5.22	5.26	10.06
1:2	5	0.84	0.85	0.87	5.29	5.31	9.96
1:2	7	0.67	0.79	0.76	5.29	5.60	10.28
1:2	9	0.69	0.75	0.71	5.08	5.48	10.01
moisture (%)		80.98±0.57	79.11±0.31	82.20±0.16	76.84±0.25	75.75±0.34	73.52±0.11

1. Values are means of 2 replications.
2. Significantly different between sample size within each solvent mixture as well as between sample size ($p<0.05$).

Table 2. Effect of solvent ratio on lipid extraction in various fish groups.[1]

methanol–chloroform ratio	fish category I (cod, flounder, hake)	II (herring, mackerel)	III (butter–fish)
2:1	1.09 A	3.86 C	6.30 C
1:1	0.96 B	5.11 B	8.68 B
1:2	0.94 C	5.46 A	10.31 A

1. Values were means of the pooled data from the same fish group.
2. Means within each column with the same letter are not significantly different ($p > 0.05$)
3. Data was based on the pool of different sample sizes at each solvent ratio.

Table 3. Effect of sample size on lipid extraction in fish groups[1].

Sample size (g/50 ml solvent)	Fish category I (cod, flounder, hake)	II (herring, mackerel)	III (butter–fish)
1	1.22 A	5.86 A	11.20 A
3	1.03 B	5.49 B	10.40 B
5	0.93 C	4.82 C	8.19 C
7	0.91 C	4.34 D	6.17 D
9	0.90 C	3.54 E	6.16 D

1. Value were means of pooled data from the same fish group.
2. Means within each column with the same letter are not significantly different ($p > 0.05$).
3. Data were based on the pool of different solvent ratios at each sample size.

Table 4. Effect of solvent ratio and sample size on lipid extraction for various fish groups[1]

Lean fish (cod, flounder, hake)

Solvent mixture	Sample size (g) 1	3	5	7	9	4,25[F]
2:1	1.20±0.90[A]	1.12±0.15[AB]	1.03±0.07[B]	1.04±0.03[B]	1.05±0.04[B] **	4.23
1:1	1.12±0.07[A]	0.94±0.01[B]	0.90±0.03[B]	0.93±0.02[B]	0.93±0.02[B] **	32.79
1:2	1.34±0.19[A]	1.04±0.09[B]	0.86±0.05[C]	0.74±0.08[D]	0.72±0.03[D] **	36.43

Medium fatty fish (herring, mackerel)

Solvent mixture	Sample size (g) 1	3	5	7	9	4,15[F]
2:1	5.84±0.18[A]	5.78±0.38[A]	3.70±0.56[B]	2.21±0.30[C]	1.77±0.14[C] **	123.76
1:1	5.70±0.42[A]	5.46±0.31[A]	5.46±0.30[A]	5.37±0.30[A]	3.57±0.15[B] **	32.17
1:2	6.04±0.38[A]	5.24±0.18[B]	5.30±0.22[B]	5.44±0.38[B]	5.28±0.32[B] **	4.72

Fatty fish (butter-fish)

Solvent mixture	Sample size (g) 1	3	5	7	9	4,5[F]
2:1	11.6±0.33[A]	11.1±0.34[A]	4.42±0.13[B]	2.53±0.01[C]	1.90±0.04[D] **	901.4
1:1	10.8±0.21[A]	10.1±0.25[B]	10.2±0.14[B]	5.65±0.02[C]	6.59±0.05[D] **	432.1
1:2	11.1±0.24[A]	10.1±0.07[B]	9.96±0.14[B]	10.3±0.38[B]	10.0±0.03[B] **	12.84

1. Values are means of 2 replications.
2. Means within each row with different letter are significantly different ($p.< 0.05$).

CONCLUSION

There are at least two factors involving the optimum condition of lipid extraction. The amount of solvent should be sufficient to overcome the sample size and the ration of solvents should be appropriately set based on the fish lipid content. The 50-fold, volume of the solvent to the weight of sample was adequate for efficient extraction. The solvent of high proportion of chloroform is preferential for the fatty tissue, while the solvent mixture of high methanol is favorable for those species of low fat. The solvent mixture of equal ration of methanol to chloroform is suitable for the screening purpose when the lipid level of fish species is not known.

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