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New Laboratory Method for Expressing Fish Fluid

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Introduction

Fish consists almost exclusively of protein and water; fatty species also contain variable quantities of lipids. As most biochemical processes take place in the liquid phase or have an immediate influence upon its composition (e.g. by absorption of degradation products), the study of expressible fluid is most important. In the laboratory it can be used to carry out numerous analyses, e.g. determination of total volatile bases (TVB), trimethylamine (TMA), and volatile reducing substances (VRS) by microdiffusion, bacteriological tests, assessment of mineral content, etc. Recently it was also utilized for the differentiation of fish species by electrophoresis (Lane *et al.* 1966). It is further of interest for the study of the quality of frozen fish.

A distinction must be made between free drip, which exudes in a natural way from the fish, and expressible fluid obtained when an external force is exerted. The latter procedure is generally applied as it gives considerably more yield. The fluid may be extracted by means of a hydraulic press, handpresses (household types) or electrical centrifuges (fruit juice centrifuges). The hydraulic press is generally used. A quantity of fish is wrapped in non-absorbent cloth, placed into the cylinder and covered by a tight-fitting piston. Holes are pierced into the bottom of the cylinder so that the fluid may flow out freely.

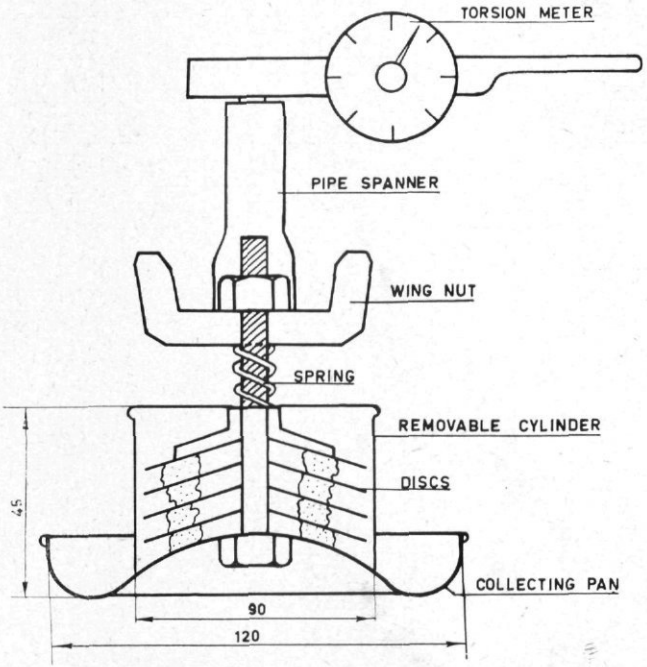

Figure 1. Press for the extraction of expressible fluid.

Banks (1955) designed a special apparatus, consisting of a perforated metal cylinder against which the fish sample is pressed by a rubber bag inflated to the desired pressure. The rubber bag allows a precise adjustment of this pressure.

Preliminary experiments showed that these methods are rather tedious. Moreover for fatty fish (e.g. herring) and semi-fatty fish (e.g. redfish) an insufficient quantity of expressible fluid is obtained. The use of an electrical centrifuge, as proposed by Wittfogel and Gebhardt (1957), gave good results with lean fish (e.g. cod) but was impracticable with fatty and semi-fatty fish.

For this reason an attempt was made to develop a simple technique suitable for all fish.

After numerous tentative experiments a small handpress with discs seemed to fulfil the requirements excellently. In order to determine the possible influence of varying pressure, pressing time, etc., an exhaustive series of tests was carried out to study the fish fluid expressed with this apparatus. Different quantitative aspects, *viz.* amount of expressible fluid, refractive index (RI), TVB, TMA, VRS and total solids, were examined.

Experimental

Apparatus and procedure

The press used (Figure 1) is a modified small commercial handpress (Presse médicale AS, Paris, France) made of galvanized iron with slightly conical discs of 8.3 cm diameter. Pressure is applied by tightening a spring by means of a wing nut. In order to adjust the desired pressure, the press was fitted with a torque meter (Torquometer type TQC-1-FU, Snap-on Tools Corp., Kenosha, USA) connected *via* a pipe-spanner to the wing nut; on the latter a nut of 17 mm was welded.

For each determination 100 g of fish cut into pieces of 5 to 10 g was used.

Species

Cod (*Gadus morhua* L.), redfish (*Sebastes marinus* L.) and herring (*Clupea harengus* L.) were used. When necessary fish of varying degree of freshness were taken.

Methods

The following analyses were carried out on the expressible fluid:

Total solids: 5 g was mixed with sand and dried at 105° C until constant weight.

RI: determination on two drops of fluid at 20° C, in an Abbé refractometer.

TVB and *TMA:* according to the diffusion method of Conway (1962).

VRS: according to the method of Farber and Ferro (1956). The expressible fluid was neither filtered nor centrifuged before analysis.

Statistical tests

All experiments were repeated 20 to 30 times and the results were tested statistically by the method of the paired comparisons. The significance of the observed differences was each time examined by means of the t-test according to:

$$t = \frac{d_a}{s_a / \sqrt{n}}, \text{ with } n - 1 \text{ degrees of freedom}$$

d_a = mean difference between paired experiments
s_a = standard deviation of the differences
n = number of pairs

A significant difference was indicated in the tables by the conventional signs and (respectively 95 and 99 per cent probability).

Results and discussion

Factors proper to the method and to the raw material respectively were investigated.

Factors proper to the method

Influence of pressure

The effect of two different pressures (0.35 and 0.50 kg/cm²) was examined. These particular pressures were chosen as at ca. 0.35 kg/cm² the first drops of expressible fluid were expressed, and at a pressure higher than 0.50 kg/cm² pieces of flesh were extruded. The two pressures were adjusted by means of the torque meter. The corresponding moments were 0.10 and 0.15 kgm (8 discs were used). Owing to the loss of fluid pressure decreased gradually and after 10 and 20 minutes respectively the spring was tightened again. After 30 minutes pressing was interrupted as practically no more fluid was liberated.

The results for cod are given in Table I and indicate that the amount of expressible fluid is markedly higher at a pressure of 0.50 kg/cm². The average difference was 24.4 per cent.

Total solids, RI, TVB and TMA appeared not to be influenced, whereas for VRS a very significant difference of 3.2 μeq or 20 per cent was observed. An increase in pressure results in relatively more volatile reducing substances being liberated in the expressible fluid. The reason for this was not investigated in the present work. Banks (1955b), Love (1955) and Connell (1957) indicated that free drip as well as press drip consist mainly of intracellular fluid. Connell reports a value of 90 per cent. However as pressure is increased more and more intracellular fluid mixes with the expressible fluid owing to the rupture of cell walls or by ultrafiltration. The possibility exists that under the conditions described certain components of this intracellular fluid only affect VRS and not TVB nor TMA.

The further values obtained are similar to those of Banks (1955) who also ascertained that for cod more expressible fluid was liberated as the pressure was increased, but that total solids remained nearly constant up to a pressure of ca. 0.50 kg/cm². With higher pressures however more and more water was squeezed out.

Herring and redfish were also subjected to the same experiments and gave analogous results.

It may thus be concluded that pressure need be adjusted accurately only for the VRS determination; for the other determinations this seems to be of no consequence.

TABLE I

Influence of two pressures (0.35 and 0.50 kg/cm²) on the expressible fluid of cod

Determination	*Difference between paired experiments*			*Average values*	
	Min.	*Max.*	*Average*	0.35	0.50
Expressible fluid (ml)	+ 2.0	+10.5	+5.3	21.7	27.0
Total solids	− 0.24	+ 0.19	0	7.86	7.86
RI	− 0.0003	+ 0.0006	−0.0001	1.3474	1.3473
TVB (mg N per 100 ml)	− 1.4	+ 4.4	+0.5	23.5	24.0
TMA	− 0.9	+ 2.1	+0.3	6.2	6.5
VRS (μeq per 5 ml)	0	+ 7.5	+3.2	16.2	19.4

TABLE II

Influence of mincing the fish on the expressible fluid of cod

Determination	*Difference between paired experiments*			*Average values*	
	Min.	*Max.*	*Average*	*Control*	*Minced*
ml	0	+4.0	+2.1	25.5	27.6
Total solids (%)	−0.02	+0.97	+0.50	7.53	8.03
RI	−0.0002	+0.0026	+0.0015	1.3468	1.3483
TVB (mg N %)	−0.1	+3.5	+0.4	31.8	32.2
TMA (mg N %)	−0.1	+2.4	+0.2	6.1	6.3
VRS (μeq/5 ml)	−3.0	+1.5	−0.5	18.8	18.3

As the higher pressure (0.50 kg/cm²) liberates more expressible fluid it was chosen for further experiments.

Influence of the number of discs
Preliminary experiments showed that it is more convenient to operate with from 5 to 8 discs. In order to determine if changing the number of discs (and thus the contact surface) influences the composition of the expressible fluid, a series of comparative tests with 5 and 8 discs respectively was carried out on cod.

Owing to the larger contact surface with eight discs the amount of expressible fluid was distinctly higher and differed significantly at the 95 per cent level. The average difference was 5.7 ml or 26.7 per cent.

The other determinations were almost uninfluenced and no significant difference was recorded. This indicates that the composition of the expressible fluid is not modified by changing the number of discs. As eight discs give distinctly more expressible fluid, this number is to be preferred for practical reasons. Similar results were obtained for herring and redfish.

Influence of pressing time
Pressing times of 10 and 30 min. respectively were tested. Preliminary experiments had shown that an almost maximum quantity of expressible fluid was obtained in 30 min. Between 30 and 60 min. only an additional 2 or 3 ml were squeezed out.

As expected, a larger quantity of fluid was obtained with cod after 30 min; on average this was 28.5 ml against 23.7 ml for 10 min.; i.e. 20.2 per cent more. Total solids, RI, TVB, TMA and VRS showed a slight decrease, which appeared not to be at all significant. For herring and redfish analogous results were obtained. The pressing time is of no importance and needs not to be strictly adhered to. As after 30 minutes the amount of expressible fluid is distinctly higher, it is preferable to take this time approximately into account.

Influence of quantity of fish
Usually 100 g of fish was introduced into the press, and in order to check if this quantity had to be weighed accurately a series of tests with 100 and 110 g of fish was carried out. The amount of fluid from 110 g was higher for the three species; the difference however was only significant at the 95 per cent level for cod and averaged 3.6 ml. This is certainly due to the fact that lean fish have a greater moisture content than redfish or herring and that their firmer consistency liberates the expressible fluid more easily. The other determinations showed nowhere any significant differences.

The conclusion may thus be drawn that the quantity of fish need not be weighed accurately. This offers an important practical advantage and allows the use of the press aboard fishery research vessels, where weighing generally causes serious difficulties.

Influence of mincing the fish
Instead of pieces of fish for the extraction of expressible fluid, minced fish may also be used. Comparative experiments were carried out with both techniques. An electrical meat grinder was used. Minced redfish and herring caused serious difficulties as, owing to the softer consistency of these fish, a large quantity of fish tissue was extruded when pressure was applied. The amount of expressible fluid decreased considerably and had to be filtered but this method was suitable for cod. The results of the comparative tests are given in Table II.

It was not only noticed that more expressible fluid was liberated (on the average 8.2 per cent), but also that the fluid contained more dissolved substances: total solids were on the average 7.1 per cent higher and RI increased by 0.0015 units. This is probably due to the presence of a higher percentage of intracellular fluid as mincing certainly damages more cells. It does not appear to affect TVB, TMA and VRS-determinations significantly.

TABLE III
Reproducibility of the method

Fish species	*Number of duplications*	*Average difference (ml)*	*Maximum difference (ml)*	*Standard deviation (ml)*
Cod	150	1.6	4.5	1.38
Redfish	82	1.7	6.0	1.51
Herring	105	1.6	4.5	1.43

TABLE IV
Influence of storage on the amount of expressible fluid and its total solids content

Fish species	*Determination*	*Storage time (in days)*			
		0	2	7	9
Cod	Expressible fluid (ml)	22.7	26.5	30.5	31.4
	Total solids (%)	8.60	7.29	6.40	6.20
	Total solids (g per 100 g fish)	1.95	1.93	1.95	1.94
Redfish	Expressible fluid (ml)	21.2	19.5	19.2	18.9
	Total solids (%)	8.95	8.94	8.83	8.75
	Total solids (g per 100 g fish)	1.90	1.74	1.70	1.65
		0	2	4	6
Herring	Expressible fluid ml)	15.7	15.2	14.5	13.8
	Total solids (%)	11.92	10.74	10.09	9.40
	Total solids (g per 100 g fish)	1.87	1.63	1.46	1.30

The use of minced fish has the advantage that a more homogeneous sample from a greater number of fish can be taken. An important disadvantage is that this method can only be applied to lean fish species.

Factors proper to the used fish

Influence of species

The higher the moisture content of the fish, the larger is the quantity of expressible fluid. For very fresh fish 80 per cent of the observations (about 300 per species) varied between the following limits per 100 g:

cod: 24-28 ml (average: 26.2 ml)
redfish: 17-21 ml (average: 19.5 ml)
herring: 12-15 ml (average: 13.8 ml)

The great advantage of the new method is emphasized when comparing the values obtained with those from other techniques. Banks (1955) with his special equipment collects an average of 7.1 g expressible fluid per 100 g cod after 6 hours at a pressure of 0.5 kg/cm^2. Connell (1957) on the other hand obtained about 10 g expressible fluid with a hydraulic press after 60 minutes at 1,600 kg/cm^2.

When pressing time is rather long the influence of temperature must be taken into account and precautions must be taken to prevent evaporation losses. This is superfluous for the method described as pressure is only applied during a short period (10 to 30 minutes), thus providing a supplementary advantage.

The reproducibility of the method was also determined by means of duplicate analyses carried out during the different stages of experimentation.

The standard deviation (Table III) was determined according to:

$$s = \sqrt{\frac{d^2}{2n}}$$

d = difference between duplicate analyses
n = number of pairs

Application of the F-test showed the standard deviations not to be significantly different. The combined standard deviation was 1.42 ml. The reproducibility of the method may thus be considered very satisfactory.

Influence of the degree of freshness

A series of storage experiments in ice was carried out. For cod and redfish the experiment lasted nine days, the fish being approximately five days old at the start. Herring was approximately three days old and was kept for six days. Ten fish were used for each experiment.

From Table IV it appears that the amount of expressible fluid and total solids are affected by the degree of freshness of the fish. After nine days a considerable increase in the number of ml (38.3 per cent) was observed for cod, whereas total solids proportionally decreased indicating that more water was present in the expressible fluid. As it was observed that the total solids in 100 g of fish remained practically constant (1.93 to 1.95), it may be concluded that the increase in the amount of expressible fluid is primarily due to the liberation of water. This is caused by the fact that the water-holding capacity of the proteins decreases as deterioration progresses.

These data are fairly identical with those of Banks (1955b), who ascertained that after some 12 days the amounts of expressible fluid increased from 7.2 to 10.2 g or 41.6 per cent.

Redfish and herring however gave a different picture. Both the amount of expressible fluid and total solids decreased during storage. The number of ml dropped by 10.8 per cent for redfish and by 12.1 per cent for herring; total solids also decreased. As the percentage of total solids fell, it may also be concluded that for both species the expressible fluid is more and more diluted by exuded water. This phenomenon is thus analogous to that in the lean species (cod). The decrease in the number of ml can undoubtedly be attributed to the fact that the expressible fluid is retained by physical forces due to the softer and more gelatinous consistency of semi-fatty fish (redfish) and fatty fish (herring); this consistency becomes softer and softer as deterioration progresses.

Other advantages of the method

Besides the advantages mentioned above, several additional practical advantages should be emphasized.

The press is very easy to operate, it can be dismounted and remounted quickly without difficulty. For the same reason it is very convenient to clean and easily sterilized. It is moreover very cheap thus allowing a great number of presses to be put into service simultaneously.

Finally, it should be noticed that the press can be used successfully for other fishery products such as smoked fish, cooked shrimps, raw and cooked prawns, etc., and that larger models allowing bigger quantities of fish to be pressed, can also be used.

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