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Critical Appraisal of the Collaborative Testing of Analytical Methods. Determination of the Total and Soluble Lead Content of Dry Paint Films

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The results of a collaborative trial are critically examined and a new approach is recommended for the evaluation of a precision data. Proposals are also made for the application of such data in quality assurance schemes.

An evaluation of the determination of the total and soluble lead content of dry paint film has been carried out[1]. Results were obtained from eight laboratories for comparison purposes and the precision calculated in accordance with internationally agreed procedure[2].

The levels tested for total lead were in the range 2000–13,000 mg/kg, the legal limit for dry paint film on toys being 2500 mg/kg. The range of soluble lead tested was between 40 and 220 mg/kg, the limit on paint coatings for pencils and graphic instruments being 250 mg/kg.

Coefficients of variation were found to be up to 7% for total lead and up to 17 per cent. for soluble lead. Consideration is given to the effect of these variations on the enforcement of the statutory limits. The conclusion is reached that the degree of precision found shows that well defined methods should be specified, particularly for enforcement purposes.

This paper is an examination of some of the data obtained in these investigations so that principles can be derived for general application to collaborative trials, especially regarding method specification. The considerations also apply to the proficiency testing of analytical performance.

Experimental Design

The described co-operative trials for the determination of lead in dry paint films by atomic absorption spectroscopy include collaborative trials which were designed to obtain precision data applicable to the use of a standardised procedure. As with many such analytical methods, the conditions of testing are not completely specified, in order to permit measurement over a wider range of analyte concentration than would otherwise be possible.

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Standardised procedures frequently require dilutions to be made and aliquot portions to be taken according to the expected concentration in the sample material. Since this concentration is not known until the completion of the analysis, collaborative trials are frequently carried out under varying conditions of measurement. Furthermore, in the trials to be considered, different optical wavelengths are available for the spectrophotometric determination, having different sensitivities and optimum measurement ranges. Where such variations are permitted in the standardised method, a statement is included that the operator should prepare the final dilution of the test portion to obtain optimum conditions. In these circumstances, guidance should be given as to what these conditions are.

In order to obtain information necessary for this purpose, collaborators agreed to provide procedural details including the actual sample sizes and measurements made, so that the digest ratios used could be calculated. These values were found to be different in every case, from which it may be expected that each determination will have been carried out under different repeatability and reproducibility conditions. This does not invalidate the precision values obtained from the trial, since these do reflect the general performance obtained. Anomalies, however, must be expected and different values could be obtained if the trials were to be repeated.

Significance of the Digest Ratio

The digest ratio is a factor which converts the measurement units into sample concentration units. The larger the digest ratio for a given sample, the smaller is the measurement. The random error in the measurement is then multiplied, in the case of these trials, by differing factors leading to widely differing errors in the reported results.

In order to assist the analysts carrying out the tests, the approximate concentration of each sample material was given. For sample A, the range of total Pb content was given as 1500–2500 mg/kg. If the optimum measuring range were assumed to be 2·5–5·0 μg/ml for the determination at 217 nm wavelength, then the optimum digest ratio would be 1 : 500.

This is achieved by taking the 0·2 g sample into the specified 100 ml of solution without futher dilution. Most laboratories followed this procedure but one did not, further diluting by a factor of five and 10 times respectively in the duplicate determinations, bringing the measurements into the 0–2 μg/ml range. This laboratory showed the greatest deviation from the mean result as well as revealing that the replicates were not determined under comparable repeatability conditions. This difference in procedure between replicates occurred several times with other samples and other laboratories in these trials.

For sample B, the optimum digest ratio could be calculated as 1 : 2500 involving a further ×5 dilution in the specified method. Dilutions actually used gave rise to a wide range of digest ratios from 1 : 900 to 1 : 10,000.

For sample C an optimum 1 : 1250 would be appropriate for the digest ratio. Values used were again wide, varying from 1 : 500 to 1 : 5000 giving rise to measurements which differed by a factor of 10.

Homogeneity of Variance

Conventional procedures for the calculation of precision of analytical methods require the results to be homogeneous and normally distributed about a mean value. Attempts are made to identify abnormal results from different populations by classifying them as "stragglers" or "outliers". In the present trial under discussion, due to the presence of different digest ratios as an additional source of variation, all the results are from different populations, and alternative procedures need to be adopted in order to calculate the random error.

The results of the specified method (total Pb) for sample A can be examined for homogeneity by plotting the frequency of result differences, of which there are 120 from the eight sets of duplicate results. The distribution is shown in Fig. 1(a) where the shape of the curve is clearly not Gaussian. The R value as calculated in the trial is indicated as 400 mg/kg, together with the normal Gaussian curve which would be associated with that figure.

When the laboratory mean results are examined by Dixon's Test, laboratory 7 is indicated as a "straggler" but would not normally be excluded from the precision calculations. If the results from this laboratory, however, are removed, then the R value is halved, becoming only 200 mg/kg. The frequency curve then appears to be normally distributed as shown in Fig. 1(b) with all the remaining laboratories using approximately the same digest ratios. The abnormal differences are then shown in Fig. 1(c) to be due exclusively to laboratory 7, where, due to the high digest ratios used, the R values would correspond to 5 and 10 times the values of the other laboratories.

There was no evidence to suggest any abnormality in the work of laboratory 7. The only difference apparent was the fact that the measurements were obtained in the 0–2 μg/ml range instead of the higher range used by the other laboratories. This is not an immediately obvious fault, especially when considering the determination of soluble lead at a lower level, since all laboratories had of necessity to work in this range with the associated higher errors involved (see Fig. 3). In this latter case, the minimum digest ratio is prescribed in the statutory method.

The use of different digest ratios in a collaborative trial not only results in a lack of homogeneity with its consequent effect on the precision calculations but it also distorts the value obtained for the arithmetic mean of the results.

A New Approach to Collaborative Trial Evaluation

In order to avoid the effect of varying digest ratios on precision calculations it is necessary to express the analytical errors in terms of the analyte amount or concentration at the measurement stage. The first requirement is to estimate the mean concentration in the sample in a manner other than using the arithmetic mean of the reported results. From this value, using the appropriate digest ratio, the predicted measurement for each determination can be made so that all the results as sample concentration would be the same, i.e. without the random error.

The differences of the measured amount from the predicted measurement then provide a population of differences from the assumed theoretical value. The reproducibility is then $2\sqrt{2}$ standard deviations of this population.

A similar population of within laboratory measurement differences can be obtained by subtracting the differences corresponding to each laboratory replicate, taking into account the sign of such deviations. The repeatability can then be estimated as two standard deviations of this population.

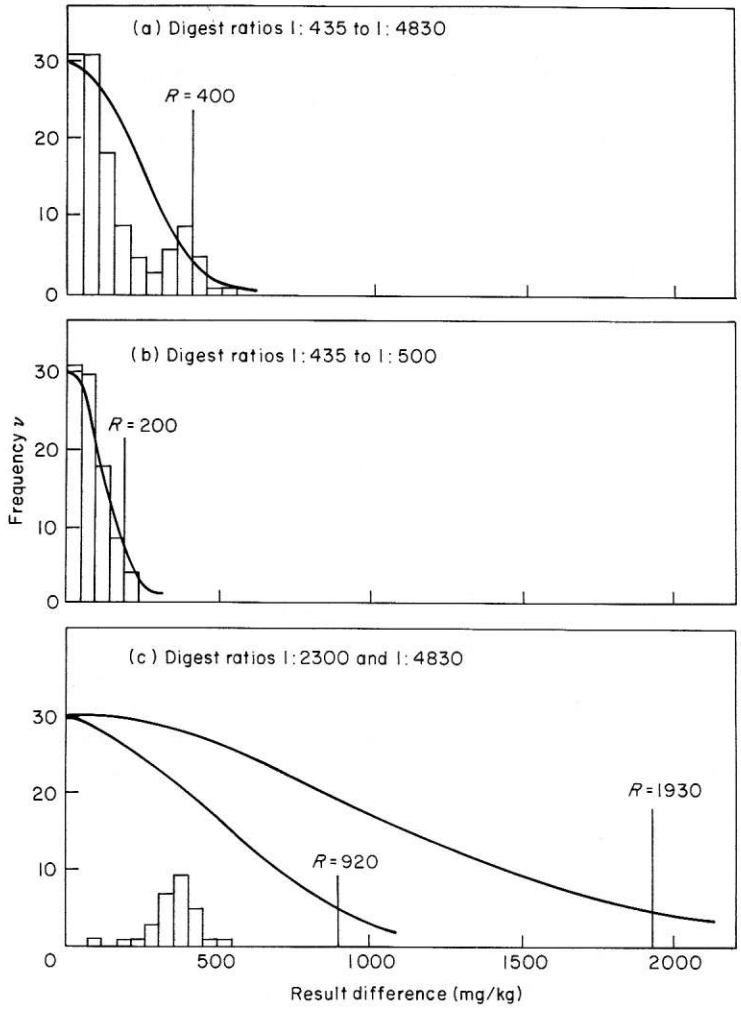

Fig. 1. Sample A: total lead (specified method). Frequency distributions showing lack of homogeneity in result differences. (a) all results (b) omitting Laboratory 7 (c) laboratory 7 alone.

In this method of evaluation the total error is shown as a variation about a regression line which passes through the origin of a graphical plot. This graph is obtained by plotting the measurements obtained in each determination as ordinate (y) against the reciprocal of the digest ratio used (DR), or an appropriate multiple of that reciprocal. In the example shown (Table I) the value 1000/DR is used as abscissa (x).

TABLE I

DETERMINATION OF LEAD IN DRY PAINT FILMS. ESTIMATION OF MEAN AND RANDOM ERROR. TOTAL LEAD (SPECIFIED METHOD) SAMPLE A.

Laboratory	Digest ratio (DR)	(x) 1000/DR	(y) Measurement (μg/ml)	Predicted measurement (μg/ml)	Random error (μg/ml)	
1A	1:497	2·014	4·68	4·40	+0·28	
B	1:498	2·007	4·50	4·39	+0·11	0·17
2A	1:488	2·049	4·20	4·48	−0·28	
B	1:489	2·047	4·40	4·47	−0·07	0·21
3A	1:492	2·034	4·46	4·45	+0·01	
B	1:449	2·227	5·03	4·87	+0·16	0·15
4A	1:491	2·038	4·32	4·46	−0·14	
B	1:483	2·072	4·42	4·53	−0·11	0·03
5A	1:444	2·250	5·02	4·92	+0·10	
B	1:481	2·080	4·57	4·55	+0·02	0·08
6A	1:499	2·005	4·24	4·38	−0·14	
B	1:465	2·153	4·65	4·71	−0·06	0·08
7A	1:2,309	0·434	1·11	0·95	+0·16	
B	1:4,833	0·207	0·52	0·45	−0·07	0·09
8A	1:501	1·999	4·39	4·37	+0·02	
B	1:435	2·302	5·06	5·03	+0·03	0·01
				Sum of squares:	0·2966	0·1174

Regression through the origin:

$$\text{Gradient } \frac{y}{x} = \frac{\Sigma(x.y)}{\Sigma x^2} = \frac{134{\cdot}63}{61{\cdot}587} = 2{\cdot}186$$

$$\text{Mean result} = y.\text{DR} = \frac{y}{x}1000 = 2186 \text{ mg/kg}$$

$$\left(\text{Predicted measurement} = \frac{2186}{\text{DR}} \text{ μg/ml}\right)$$

$$\text{Standard deviation: } \sqrt{\frac{0{\cdot}2966}{15}} = 0{\cdot}141 \qquad \sqrt{\frac{0{\cdot}1174}{8}} = 0{\cdot}121$$

$$R = 2\sqrt{2}(0{\cdot}141) = 0{\cdot}40 \text{ μg/ml} \qquad r = 2(0{\cdot}121) = 0{\cdot}24 \text{ μg/ml}$$

Note. The mean result in mg/kg is calculated from the gradient of the least squares regression line through the origin. The precision values are calculated from measurement differences in μg/ml.

Since: result = measurement (y) . digest ratio (DR)
$= y$. DR $= (y/x)$. 1000
Hence: result = gradient . 1000

The result is therefore a multiple of the gradient as shown in Fig. 2. The gradient of the "least squares" or "best-fit" regression line through the origin can be calculated as[3]:

$$y/x = \Sigma\,(x\,.\,y)/\Sigma\,(x^2)$$

The calculations shown in Table I give a result of 2186 mg/kg instead of the arithmetic mean result of 2228 mg/kg reported in the trial. Precision results calculated in terms of measurement concentration are as follows:

Reproducibility (R) = 0·40 μg/ml
Repeatability (r) = 0.24 μg/ml

Figure 2 shows all the experimental measurements to be within ±2 standard deviations (±0.28 μg/ml) of the predicted values. This error range is for practical purposes independent of the digest ratios used, and is similar to relationships derived from the use of any linear calibration plot.

What is most significant, however, is the effect of the digest ratio on this error when it is used to calculate the result of the determination. The frequency distribution of results shown in Fig. 2(a), where the digest ratios are about 1 : 500, corresponds to an error of about ±6 per cent. Fig. 2(b) shows the effect of the same magnitude of error multiplied to a potential error of about ±30 per cent. using a digest ratio of 1 : 2310. Using a digest ratio of 1 : 4830 the error is further increased to a potential value of ±60 per cent.

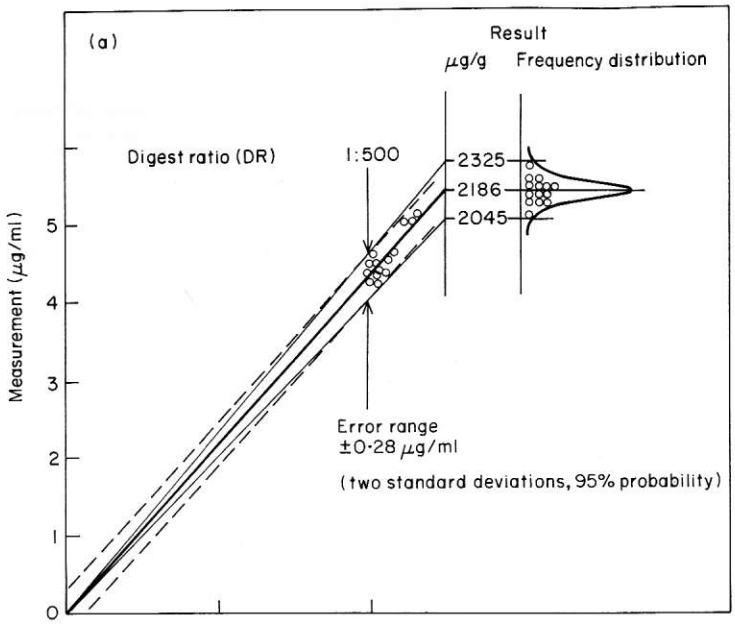

Fig. 2. (a) Sample A: total lead (specified method). Graph showing range of results obtainable using a digest ratio not exceeding 1 : 500, 4·37 ± 0·28 μg/ml. When multiplied by 500 this becomes a relatively narrow range: 2186 mg/kg ± 6·5 per cent. (results from Laboratory 7 excluded as "stragglers").

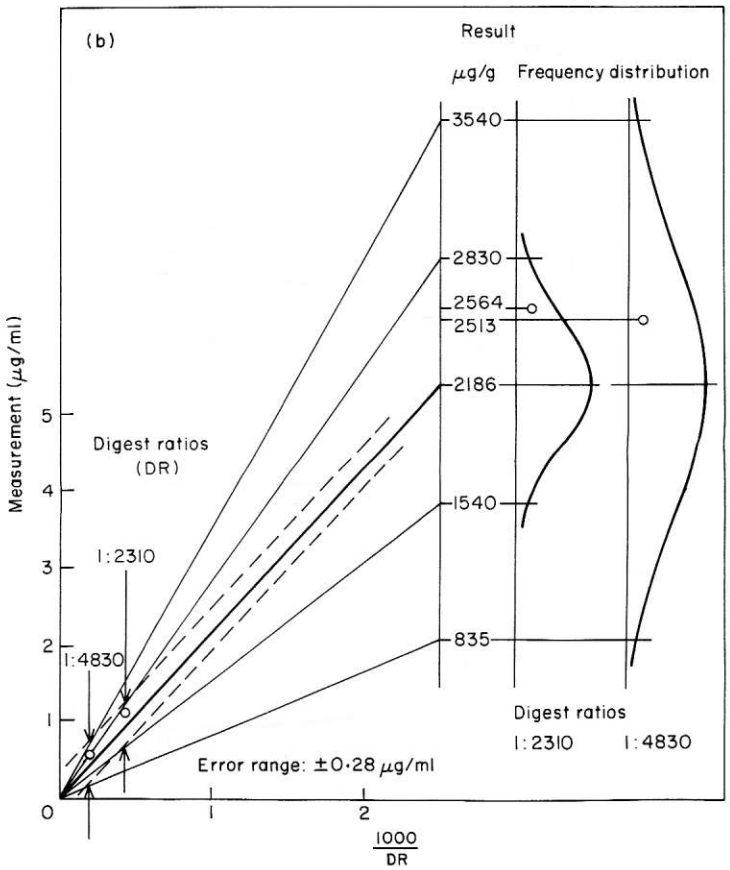

Fig. 2. (b) Sample A: total lead (specified method). Results from Laboratory 7. Graph showing ranges of results obtainable using a digest ratio as high as 1 : 4830, 0·45 ± 0·28 μg/ml. When multiplied by 4830 this becomes a very wide range: 2186 mg/kg ± 62 per cent.

Use of the Analytical System Error

The precision error expressed in terms of the measured amount or concentration units, under conditions of repeatability or reproducibility, can be described as an analytical system error for a standardised method applied to the appropriate matrix, or similar group of matrices, tested in the trial used for measurement of the precision.

This analytical system error, when expressed as a fraction of the measurement made in the determination, gives the appropriate potential relative error.

When multiplied by the appropriate digest ratio used, values of repeatability or reproducibility are given in terms of concentration units of analyte in the sample material. Fig. 3 shows the observed and potential relative error according to the chosen digest ratios and measurement concentrations. Fig. 3(a) refers to sample A when examined for total lead by the specified method. Fig. 3(b) gives the results for the same sample examined for soluble lead by the official method where a digest ratio of not less than 1 : 50 is specified.

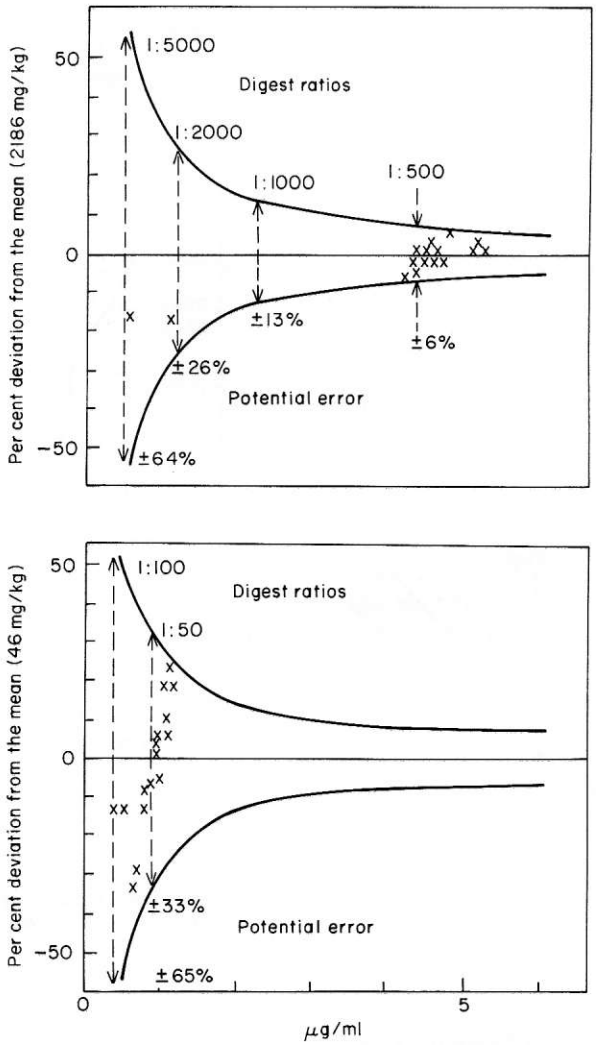

Fig. 3. Relative error curves showing exponential increase as measurements become lower as a result of using higher digest ratios. The observed results both for total [(a), specified method] and soluble [(b), official method] lead lie within the calculated potential error curves.

The errors for soluble lead, when calculated in the same manner as for the total lead, give values of 0·42 μg/ml and 0.27 μg/ml respectively for the reproducibility and repeatability. These are approximately the same for both methods, and these values have been used to calculate the potential errors shown in Fig. 3.

Application for Quality Control Purposes

In order to utilise precision data, international standards recommend plotting values of repeatability and reproducibility against the concentration of analyte

in the sample, to establish whether there is a functional relationship. The values obtained from such a relationship can then be applied for specification and quality control purposes.

Fig. 4 shows the calculated repeatability values for the three levels of total lead determined by the specified method. It also shows the ranges of these values corresponding to the digest ratios used in the tests.

Rather than demonstrating a functional relationship, the plot reveals the need to consider a "target area", corresponding to a range of measurement concentrations with their associated relative errors. The results with the least potential error will be those at the maximum level of measurement on the linear portion of the calibration relationship. Such a target area is shown in Fig. 4, based on the use of the upper half of the calibration range and would be appropriate if a maximum repeatability error of 10 per cent. were to be acceptable.

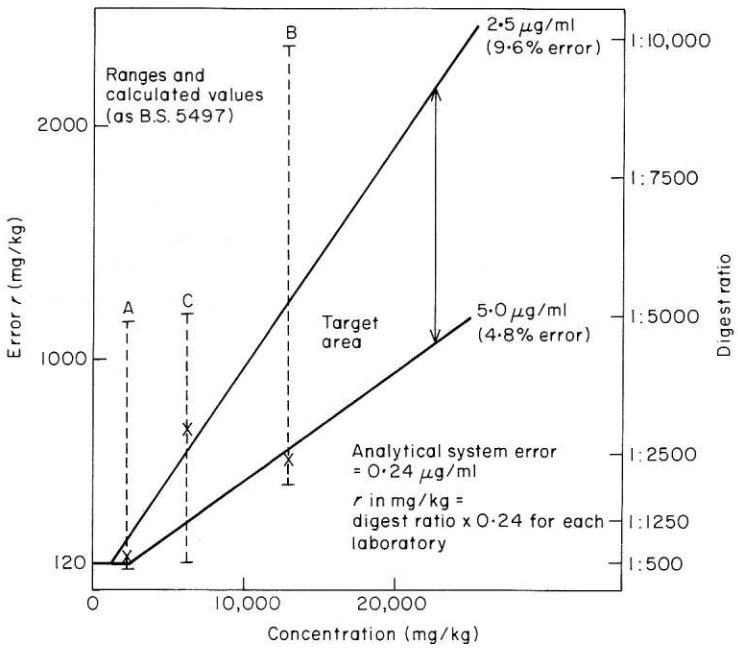

Fig. 4. Total lead (specified method). Repeatability plot calculated in accordance with B.S. 5497, Part 1. The range of values due to each laboratory using different digest ratios is shown. The target area for a measurement range 2·5–5·0 μg/ml corresponds with a maximum error range of 4·8–9·6 per cent.

The quality control procedure would involve the analysis of *identical* amounts of sample material in duplicate, extracting the analyte into the *specified volume* of solution for measurement. For optimised performance in the example being considered, the solution to be analysed should contain between 2·5 and 5·0 μg/ml of Pb. The measurements should not then differ by more than the analytical system repeatability error of 0·24 μg/ml derived from the collaborative trial.

The two results of analysis can then be required *not to differ by more than 10 per cent. of their mean value,* using a digest ratio of approximately 1 : 500. The *r* value will be about 120 mg/kg and will be applicable to concentrations in the sample up to 2500 mg/kg. Above this level, further *identical* dilutions of the prepared solutions will be required and the *r* values will increase proportionally, but providing the dilution is carried out to give measurements in the same concentration range, the same relative repeatability criteria will apply giving the results in the target area of performance in the repeatability plot.

Since the minimum digest ratio of 1 : 500 is specified in the method, the recommended performance range will not be achieved with concentrations in the sample material below 1250 mg/kg. In this case the absolute value of *r* (about 120 mg/kg) will apply to the result of the analysis and the maximum relative error will exceed 10 per cent.

Meeting the repeatability requirement will not necessarily mean that the average result will be within the reproducibility limits for the method. It is therefore recommended that as an additional quality assurance procedure, the actual measurement of each determination be examined to ensure that it is within the recommended measurement range for optimum performance.

Prescribed Method Variants

Statutory methods[4] specify method variants according to the expected concentration of analyte present. If uniformity of variance is to be achieved in a collaborative trial then the appropriate variant must be used by all participating laboratories. The variant to be used must be specified in the protocol and it is likely to be different for each level of analyte to be tested.

The variants are prescribed for bands of concentration and these should be chosen to correspond with an acceptable range of precision performance, ascertained by means of collaborative trial. The method of collaborative trial evaluation proposed in this paper enables concentration ranges to be calculated for each band together with appropriate precision values.

Figure 3 shows how the potential relative error varies with measurement concentration and that it is least at the maximum value on the linear part of the calibration curve. A method requiring the greatest degree of precision should specify taking sufficient sample and using the appropriate variant to obtain a measurement at this maximum value. This is not usually practicable and for general use, it is recommended that the upper half of the calibration range be defined as optimum, permitting the error at the middle of the range to be up to twice the value obtained at the maximum measurement value.

Calculated limits of error corresponding with this recommended measuring range are shown in Table II. The reproducibility and repeatability limits are calculated as 16 per cent. and 10 per cent. respectively. The limits of variation about the true value, in the absence of method bias, are the precision values $\div \sqrt{2}$ for 95 per cent. probability.

Table III shows the precision values calculated on this basis together with values calculated from the collaborative trials in the conventional manner, for comparison purposes.

TABLE II

DETERMINATION OF LEAD IN DRY PAINT FILMS. CALCULATION OF MAXIMUM RELATIVE ERROR WITH ASSOCIATED RANGE OF MEASUREMENT CONCENTRATIONS. BASED ON RESULTS CALCULATED IN TABLE I.

	Reproducibility (R)	Repeatability (r)
Absolute error:	0·40 μg/ml	0·24 μg/ml
Relative error:	$\frac{R}{m} \cdot 100$	$\frac{r}{m} \cdot 100$
at measurement (m)		
from 2·5 μg/ml	16%	10%
to 5·0 μg/ml	8%	5%

TABLE III

DETERMINATION OF LEAD IN DRY PAINT FILMS. COMPARISON BETWEEN PRECISION VALUES CALCULATED BY THE PROPOSED METHOD AND THOSE OBTAINED IN THE COLLABORATIVE TRIAL FOR TOTAL AND SOLUBLE LEAD

A. TOTAL LEAD

Pb content (mg/kg)	Digest ratio	Measurement range (μg/ml)	R (mg/kg)	r (mg/kg)
1250–2500	1:500	2·5–5·0	200	120
2500–5000	1:1000	2·5–5·0	400	240
5000–10,000	1:2000	2·5–5·0	800	480
10,000–20,000	1:4000	2·5–5·0	1600	960

Collaborative trial results

Pb content (mg/kg)	Mean digest ratio	Mean measurement range (μg/ml)	R (mg/kg)	r (mg/kg)
2228	1:540	4·1	400	116
6104	1:1420	4·3	1250	703
12,915	1:3100	4·2	1578	565

B. SOLUBLE LEAD

Pb content (mg/kg)	Digest ratio	Measurement range (μg/ml)	R (mg/kg)	r (mg/kg)
125–250	1:50	2·5–5·0	20	15
250–500	1:100	2·5–5·0	45	30

N.B. If the concentration is less than 125 mg/kg, the potential error will exceed the target range. If greater precision is required, then concentration by evaporation or solvent extraction is necessary.

Collaborative trial results

Pb content (mg/kg)	Mean digest ratio	Mean measurement range (μg/ml)	R (mg/kg)	r (mg/kg)
45	1:50	0·9	21	13
214	1:50	4·2	40	14
220	1:50	4·3	100	35

For the determination of total lead the method should specify taking 0·2 g of sample ±0·001 g, diluting to 100 ml according to the specified method. If it is not possible to weigh this amount accurately or if less than this amount is available, then dilution should be to the appropriate volume so that the digest ratio is 1 : 500.

For the determination of soluble lead the solution should be prepared as specified by the addition of 50 times the weight of solvent to the sample. In both methods, further dilution should be made if necessary, in stages of a factor ×2 according to the expected concentration bands in Table III.

Where a particular variant is chosen and the result obtained is outside the range applicable to that variant procedure, then the analysis should be repeated using the appropriate variant.

Summary and Recommendations

1. The necessity for prescribing procedural variants in a standardised analytical method must be recognised. To avoid lack of homogeneity of variance in a collaborative trial, any such variant must be specified for the appropriate level of analyte to be tested, so that all participating laboratories are carrying out identical procedure.
2. The results of a collaborative trial should not only be used for method evaluation but should also be applicable to quality control procedures. The precision values obtained should be related to the particular method variants and not just to analyte concentration in the sample material.
3. A method of collaborative trial evaluation is proposed so that an analytical system error can be calculated in terms of measurement units, which in the case of instrumental measurement, represents a proportion of full-scale deflection. In the case of a gravimetric determination it represents a proportion of the weight measurement. The appropriate error values can then be calculated and applied to each variant in the prescribed method.
4. For any prescribed method, relative errors obtained can vary from zero to infinity according to random variation and the concentration of analyte present in the material being analysed. It is therefore necessary to prescribe a limited measuring range corresponding with acceptable limits of error. A proposal is made to restrict measurements to the upper half of the measuring range, and in the case of the use of a calibration curve, to the upper half of the linear portion or similar upper part of a curved portion having corresponding error characteristics.
5. For quality control purposes it is recommended that all results be examined to confirm that measurements are made within the specified range. When it is not possible to obtain results within the appropriate range, then the report should include the statement that the result was not obtained under optimum conditions.

It is to be hoped that all analysts will benefit from the extensive work that has been carried out and be more conscious of the necessity of planning their very skilled art of analytical chemistry with only the minimum of error. It is also to be hoped that the considerations will be of benefit to other organisers of collaborative trials and those responsible for proficiency testing of laboratories.

The author thanks Mr B. McLean for his work in initiating and organising the collaborative testing referred to in this paper, as well as for the preparation of his detailed report. Grateful thanks must also be extended to the collaborators who have willingly provided raw data to make this critical appraisal and recommendations possible.

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Methods of Analysis for the Determination of Ice-glaze on Fish Products: Collaborative Trial

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Twelve laboratories participated in a collaborative trial to evaluate four methods of analysis to determine the ice-glaze content of fish and seafood products. Samples of cod and plaice fish fillets, cold and warm water prawns (large and small), cockles, scampi and scallops containing controlled amounts of glaze were analysed. Even under the near ideal conditions used in the trial, the methods for estimating the amount of glaze exhibited considerable variation with respect to accuracy and precision. However, when the data are presented in terms of fish content (ie the parameter of major interest) the performance of the Codex and Lancashire County Council methods is such that they may be recommended for use on an interim basis for fish fillets and prawns.

Glazing is the application of water to the surface of a frozen product, so that a layer of ice is formed. This protects the product from the effects of dehydration and oxidation during storage. The practice is widely used by the frozen seafood industry.

In March, 1987, the Food Advisory Committee (FAC) published a report on coated and ice-glazed fish products[1]. It had considered evidence of abuse in the ice-glazing of fish products and concluded that the problem could be addressed either by a fish content declaration for such products or by a declaration of the weight of product net of glaze. It was advised by its working group on analytical methods, that existing methodology was adequate to enable effective enforcement of both options. However, it considered that the Food Labelling Regulations, 1984[2], should provide sufficient controls to deal with abuses related to the core of ice-glazed products and recommended that "All glazed fish products should bear an indication of net weight of fish core prior to glazing".

Subsequently it was decided that the most appropriate way to implement this recommendation was not via the Regulations made under the Food Act[3], but rather by modification of the Weights and Measures Act 1985[4].

For uniform in-factory enforcement of this proposal, it was considered that a single method of analysis for ice-glaze should be specified. A variety of different methods for ice-glaze determination had been developed. But data, to enable valid comparisons to be made between different procedures, are scarce.

Therefore, it was decided to carry out a collaborative trial to compare the most widely used and accepted procedures, so that the best could be chosen for

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the operation of the proposed controls. To this end, four methods of analysis for the determination of ice-glaze on fish products were investigated.

Organisation of the Trial

The trial was organised by the Food Science Division, MAFF, in conjunction with Ross-Young Limited of Grimsby, who supplied the initial samples (apart from the warm water prawns, supplied by Lyons Seafood, Wiltshire), and carried out the glazing operation.

Participants

Nine public analysts, one food manufacturer's laboratory, the Laboratory of the Government Chemist and the Food Science Laboratory, MAFF, participated in the trial.

Methods, apparatus and reagents used

The methods used in the trial are given in Appendices I–IV; they were:

Method 1–Codex Alimentarius Commission: Determination of Net Contents of Quick-Frozen Fish Fillets[5].

Method 2–Codex Alimentarius Commission: Determination of Net Contents of Quick-Frozen Shrimps and Prawns[6]. Separate procedures for raw and cooked products are described.

Method 3–Lancashire County Council: Determination of Ice-Glaze for IQF cooked and peeled prawns[7].

Method 4–British Frozen Food Federation: Determination of Ice-Glaze for IQF Cooked and Peeled Prawns[8].

Participants were also asked to carry out nitrogen content determinations on the deglazed samples, using an appropriate reference method of their choice.

Samples and glazing procedures used

The amount of ice-glaze on a product is affected by several factors, including surface area/volume ratio, number of glaze applications, initial temperatures of product and glazing water, and the residence time of the product in the glazing equipment. Therefore, it was considered that the various methods should be tested on samples selected to cover as wide a range as possible of the different sizes and shapes of product likely to be ice-glazed in normal commercial practice, and also on samples of products glazed to different levels.

To some extent, the choice of products was restricted by seasonal availability and by difficulties in obtaining authentic, unglazed products, which could then be glazed to accurate levels under controlled conditions.

In Table I, the final selection of products is shown, together with the target levels for glazing, the actual amount of glaze applied expressed on the final product and the nitrogen content of the sample before glazing.

Preliminary checks were carried out using the Codex methods to ensure that the products were free from added glaze before treatment. Prior to glazing all of the products were stored in a cold store operating at −23°C. Products were

removed from store and glazed in one of two ways to form the individual samples sent to trial participants.

(i) *Glazing procedure for products* 1–6 (Table I). The products were accurately weighed and then passed through a water-filled trough and a falling curtain of water, achieved by using a pilot scale batter-enrober with chilled water circulating. Care was taken to ensure that as little material as possible was lost during the operation. After each product had passed through the equipment, residual material was collected and weighed so that the final figure for glaze application could be corrected.

Product 2 required two passes through the procedure to obtain a level of glaze suitable for the trial.

Following each application of glaze, the products were immediately spread out on plastic sheets and returned to a blast-freezer operating at lower than −40°C for at least 30 min. in order to harden the glaze. The glazed products were then weighed again to give the overall glaze uptake. Quantities of 250 g of the glazed product were sealed in polythene bags and labelled with sample numbers to form the individual analytical samples before returning them to the original cold store, prior to distribution.

(ii) *Glazing procedure for products* 7–9 (Table I). The products were removed from cold store as required, weighed and placed into plastic baskets. These were immersed in chilled water for 10 sec. and the products were then immediately removed, spread onto plastic sheets and placed for at least 30 min. in a blast freezer operating at lower than −40°C to harden the glaze. They were then reweighed to give the glaze uptake. Products 8 and 9 (the uncooked fish fillets)

TABLE 1

PRODUCTS, SAMPLE CODES, TARGET GLAZING LEVELS AND NITROGEN CONTENTS OF CONTROLS USED IN THE COLLABORATIVE TRIAL FOR THE COMPARISON OF METHODS FOR THE DETERMINATION OF ICE-GLAZE

Product number	Product type	Sample codes	Target glaze level *g/100 g*	Glaze applied *g/100 g*	Nitrogen content of unglazed sample *g/100 g*
1	Cold water prawns—cooked and small	07/18	15	13·3	2·72
2	Cold water prawns—cooked and small	01/05	25	20·7	2·72
3	Warm water prawns—cooked and small	12/16	20	22·9	1·96
4	Warm, water prawns—	10/13	15	16·9	1·96
5	Cooked cockles	04/08	—	10·7	2.00
6	Uncooked scampi	03/06	—	7·8	2·75
7	Uncooked scallops	14/17	—	5·7	1·85
8	Cod fillets—uncooked and small	09/15	10	3·7	2·87
9	Plaice fillets—uncooked and large	02/11	5	5·2	2·46

were passed twice through this procedure in order to attempt to achieve the target glaze levels specified. However, only about an extra 1 per cent. glaze uptake was obtained by the second application, as melting of the glazing water of the first layer may also have been taking place. Time did not allow further glaze applications but it was decided, in retrospect, that the immersion time of 10 sec. may have been too long. The samples were sealed in polythene bags (250 g portions in the case of scallops and 2 or 3 fillets in the case of cod and plaice) and returned to cold store at −23°C, prior to distribution.

All the glazed products were sub-divided to form individual blind duplicate samples.

Samples were dispatched to participants in expanded polystyrene insulated containers containing dry ice. Each participant was required to analyse the samples using the appropriate methods as follows:

Products 1–7 (*cooked prawns, cooked cockles, uncooked scampi and uncooked scallops*): Methods 2, 3 and 4 were prescribed for glaze determination, and the nitrogen content of the deglazed individual samples was requested.

Samples codes used are given in Table I. Three packets of each sample code were supplied. Analysts were asked to analyse the whole of the contents of each packet by the appropriate method.

Products 8 and 9 (*uncooked fillets*): Method 1 was prescribed for glaze determination and the nitrogen content of the deglazed individual samples was requested.

Samples codes used are given in Table I. One packet of each sample code was supplied. Analysts were asked to analyse the whole of the contents of each packet by the appropriate method.

Results

Participants were asked to report glaze contents and nitrogen contents, expressed both on deglazed sample and on sample as received.

The results obtained by participants for the glaze contents (as g/100g on the sample as received) and nitrogen contents as (g/100g on the deglazed sample are given in Tables II–XVIII. The results from methods 2, 3 and 4 are summarized in Table XIX.

Statistical Analysis of the Results

The results obtained were statistically analysed by procedures described by the British Standards Institution[9]. Significant differences between pairs of individual results were identified using Cochran's Test ($P<0{\cdot}05$), and the extremes of magnitude of pairs of results were identified using Dixon's Test ($P<0{\cdot}05$). Outlying results are marked in the tables of results.

The values of the mean, repeatability (r) and reproducibility (R) (the precision parameters being defined by the procedures given by the British Standards Institution[9]) were calculated and are given in the results tables.

Discussion of Results

If a given method for the determination of the ice-glaze is accurate then the amount of glaze determined by the method should be equivalent to the amount of glaze added to the sample. Assuming that the amount of nitrogen in the sample is unchanged by the method, the ratio of these glaze values should indicate whether the method exhibits poor recovery or whether water, in excess of the amount used for glazing, has also been removed.

An estimation of whether a poor (less than 100 per cent.) or excessive (greater than 100 per cent.) recovery of added glaze was determined by participants was also obtained by consideration of the nitrogen content of the samples before glazing and after being deglazed. This estimation was important to obtain as the deglazing procedures are, to some extent, subjective in nature.

A portion of each of the original, unglazed products was analysed in one reference laboratory for total nitrogen. The figures obtained are given in Table I and are used as the reference values for the nitrogen content.

Participants will have correctly identified the end-point for each determination when the two nitrogen contents (reference value and deglazed value) are the same. All the glaze will then have been quantitatively removed, or there will have been a balance of errors within the analytical procedure to give that result.

METHOD 1

Products 8 and 9, plaice and cod fillets. The subjective nature of this method is reflected in the degree of variation in results found for the analysis of both plaice and cod fillets. Statistical evaluation of the results from analysis of the cod fillets shows relatively better accuracy and precision compared with those from the plaice fillets, where the level of recovered glaze was some 42 per cent. in excess of added glaze (Table II). This may be due to the relatively large surface area to weight ratio of the plaice fillets. During deglazing, this property could aid heat dissipation and total defrosting over the whole of the sample with subsequent loss of physiological water. This is not observed in the results from analysis of the cod, where the relatively thicker fillets could maintain a colder core temperature during deglazing than the plaice fillets. However, this property may have allowed the surface wash water to refreeze during analysis thereby preventing proper deglazing.

The values of repeatability and reproducibility for these two samples (see Table II) are larger than would be considered acceptable in a potential statutory method.

The nitrogen contents of the control samples were lower than that of the test samples after deglazing for both cod and plaice, suggesting that there was some loss of physiological water during the deglazing process (see Table III).

METHODS 2, 3 AND 4

The results from each of these methods of glaze determination exhibit wide variation. In general terms, method 3 produced the more precise results but was often not as accurate as the other methods.

TABLE II

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS

Cod and plaice fillets (Method 1)

Sample code	Ice glaze *g/100 g* (2, 11) uncooked plaice fillet	(9, 15) uncooked cod fillet
Lab no.		
1	7·8, 4·9	1·8, 4·2
2	4·2, 4·5	3·0, 2·7
3	8·0, 7·7	5·1, 5·1
4	7·3, 7·6	3·9, 4·1
5	7·3, 8·6	2·8, 4·5
6	4·7, 4·5	2·6, 2·6
7	6·1, 8·3	5·7, 4·0
8	8·6, 8·3	4·5, 4·5
9	11·6, 7·2	3·9, 3·7
10	9·4, 10.6	n.a., 4.2[a]
11	6·6, 4·3	3·1, 2·3
12	9·7, 10·1	7·6, 5·6
Mean glaze	7·4	4·0
Added glaze	5·2	3·7
r	3·7	2·4
R	6·1	3·9

For key see Table XX.

TABLE III

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED SAMPLES

Cod and Plaice fillets (Method 1)

Sample code	Nitrogen content *g/100 g* (2, 11) uncooked plaice fillet	(9, 15) uncooked cod fillet
Lab no.		
1#	2·19[c], 3·04[c]	3·10, 3·14
2	n.a., n.a.	n.a., n.a.
3	2·66, 2·62	2·99, 3·01
4	2·64, 2·64	3·13, 2·99
5	2·67, 2·45	3·01, 3·08
6*	2·96, 2·86	3·15, 2·97
7	2·85, 2·29	3·08, 2·86
8#	2·40, 2·41	3·04, 2·92
9	2·43, 2·37	2·73, 2·82
10	2·44, 2·63	2·99, 3·00
11	2·27, 2·43	2·69, 2·64
12	3·0, 2·70	3·20, 2·90
Mean	2·59	2·98
Control[b]	2·46	2·87
r	0·46	0·29
R	0·62	0·43

For key see Table XX.
Mean of two results.
* Mean of three results.

Products 1 and 2, cold water prawns. Glaze recovery figures of greater than 100 per cent. were found for samples of small cold-water prawns glazed at levels of 20·7 per cent. (product 2) and 13·3 per cent. (product 1), where the mean determined levels were 25·9 per cent. and 21·4 per cent, respectively (Tables IV and VI). In each case, method 3 gave a relatively higher, less accurate, result but performed marginally better with respect to r and R values. The mean nitrogen contents of the deglazed samples were similar to those of the controls (Tables V and VII].

TABLE IV

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS

Small cold-water prawns (Sample codes 1, 5)

Lab no.	Ice glaze *g/100 g* [Method 2]†	[Method 3]†	[Method 4]†
1	23·8, 23·5	26·1, 25·0	26·0, 25·1
2	21·7, 21·8	28·1, 27·5	25·9, 25·5
3*	28·0, 24·4	26·4, 27·6	28·3, 27·2
4*	26·6, 24·4	26·9, 28·3	23·7, 25·1
5*	27·0, 24·9	27·9, 28·0	25·0, 24·5
6	25·4, 23·7	27·8, 27·1	26·7, 25·4
7	25·3, 24·9	25·7, 26·7	27·7, 26·3
8*	25·5, 24·9	27·6, 26·7	24·1, 24·1
9	26·8, 26·1	27·5, 24·9	26·5, 25·4
10	28·5, 27·7	26·4, 28·7	26·0, 24·0
11	25·9, 25·2	26·3, 26·0	24·5, 22·4
12	22·9, 23·9	28·0, 24·2	28·9, 28·1
Mean	25·1	26·9	25·7
Added glaze	20·7	20·7	20·7
r	3·1	3·4	2·5
R	5·1	3·3	4·6

For key see Table XX.
* Slight modifications to method 2.
† See text.

Products 3 and 4, warm water prawns. The precision of the methods with small warm-water prawns was similar to that obtained with (small) cold-water prawns. However, the mean recovery figures for glaze determination ranged between 98 and 110 per cent., method 3 producing the highest figure (Table VIII). The mean nitrogen figures for the deglazed samples were again in good agreement with the controls (Table IX).

In the analysis of the large warm-water prawns glazed at a level of 16·9 per cent. the mean recovery figures for methods 2, 3 and 4 were 96, 110 and 93 per cent. respectively (Table X). The mean values for the nitrogen content of the deglazed samples were again close to the control value (Table XI).

Overall, the glaze results from the large prawns were less variable than those of the small prawns, irrespective of glaze level. Method 3 was slightly more precise than methods 2 and 4 for the analysis of these samples.

TABLE V

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF THE ICE GLAZE ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Small cold-water prawns (Sample codes 1, 5)

Lab no.	Nitrogen content *g/100 g* [Method 2]†	[Method 3]†	[Method 4]†
1	2·60[c], 2·85[c]	3·06[d], 2·95[d]	2·85, 2·76
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	2·71, 2·62	2·80, 2·64	2·60, 2·59
4*	2·70, 2·68	2·62, 2·68	2·70, 2·65
5*	2·56, 2·58	2·67, 2·67	2·61, 2·57
6	2·68, 2·75	2·68, 2·67	2·65, 2·60
7	2·63, 2·73	2·77, 2·70	2·62, 2·63
8*	2·58, 2·59	2·72·2·65	2·57, 2·57
9	2·67, 2·73	2·69, 2·57	2·88, 2·64
10	2·67, 2·65	2·70, 2·70	2·65, 2·66
11	2·61[c], 2·24[c]	2·55, 2·64	2·68, 2·62
12	2·40, 2·50	2·50, 2·50	2·70, 2·50
Mean	2·64	2·66	2·65
Control[b]	2·72	2·72	2·72
r	0·13	0·16	0·21
R	0.26	0.22	0.25

For key see Table XX.
* Slight modifications to method 2.
† See text.

TABLE VI

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS

Small cold-water prawns (Sample codes 7, 18)

Lab no.	Ice glaze *g/100 g* [Method 2]†	[Method 3]†	[Method 4]†
1	18·5, 18·7	21·9, 21·0	20·5, 21·4
2	13·6, 17·1	22·5, 24·0	21·0, 21·3
3*	19·1, 21·1	22·9, 24·2	24·2, 24·2
4*	21·1, 23·1	23·4, 25·1	20·8, 20·0
5*	19·0, 19·1	22·4, 22·7	20·2, 21·4
6	20·6, 19·7	22·3, 24·1	19·2, 20·0
7	21·2, 19·9	22·2, 21·2	21·9, 16·0
8*	20·8, 19·9	22·8, 22·3	18·8, 20·7
9	23·3, 20·6	22·4, 21·5	24·0, 21·3
10	23·4, 23·4	24·2, 23·6	20·4, 17·7
11	22·5, 22·2	21·8, 22·8	17·9, 20·1
12	19·8, 21·3	22·8, 23·2	25·5, 22·5
Mean	20·6	22·8	20·9
Added glaze	13·3	13·3	13·3
r	3·3	2·2	4·9
R	5·2	2·9	6·3

For key see Table XX.
* Slight modifications to method 2.
† See text.

TABLE VII

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Small cold-water prawns (Sample codes 7, 18)

Lab no.	Nitrogen content *g/100 g* [Method 2]†	[Method 3]†	[Method 4]†
1	2·91, 2·75	2·82, 2·80	2·90, 2·81
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	2·77, 2·62	2·68, 2·74	2·72, 2·72
4*	2·81, 2·72	2·76, 2·73	2·66, 2·70
5*	2·61, 2·71	2·72, 2·59	2·72, 2·64
6	2·80, 2·83	2·78, 2·69	2·72, 2·64
7	2·77, 2·74	2·79, 2·76	2·72[c], 2·00[c]
8*	2·74, 2·68	2·76, 2·71	2·69, 2·68
9	2·82, 2·71	2·83, 2·81	2·85, 2·80
10	2·74, 2·77	2·78, 2·82	2·69, 2·73
11	2·40, 2·72	2·79, 2·66	2·64, 2·64
12	2·60, 2·50	2·60, 2·60	2.70, 2·70
Mean	2·72	2·74	2·72
Control[b]	2·72	2·72	2·72
r	0·27	0·14	0·10
R	0·32	0·21	0·21

For key see Table XX.
* Slight modifications to method 2.
† See text.

TABLE VIII

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS

Small warm-water prawns (Sample codes 12, 16)

Lab no.	Ice glaze *g/100 g* [Method 2]†	[Method 3]†	[Method 4]†
1	20·9, 20·2	26·2, 23·4	23·7, 20·6
2	17·4, 16·8	25·7, 24·4	23·6, 22·6
3*	22·5, 21·1	25·1[c], 35·3[c]	35·4[d], 35·4[d]
4*	24·8, 22·3	26·3, 23·9	24·1, 23·1
5*	23·1, 23·5	27·7, 25·8	21·5, 21·5
6	22·1, 21·6	24·3, 23·7	21·1, 21·9
7	22·8, 21·1	25·4, 23·0	23·5, 21·5
8*	24·7, 23·1	26·2, 24·0	21·6, 20·9
9	24·3, 24·5	25·5, 22·8	24·1, 21·4
10	24·6, 24·3	25·1, 24·2	21·8, 21·2
11	22·7, 24·9	27·7, 27·3	22·7, 24·1
12	25·3, 22·4	26·7, 25·2	28·2[d], 29·3[d]
Mean	22·6	25·2	22·3
Added glaze	22·9	22·9	22·9
r	3·1	3·8	3·2
R	5·7	4·2	3·4

For key see Table XX.
* Slight modifications to method 2.
† See text.

TABLE IX

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Small warm-water prawns (Sample codes 12, 16)

Lab no.	Nitrogen content *g/100g* [Method 2]†	[Method 3]†	[Method 4]†
1	2·05, 2·05	2·11, 2·16	2·09, 2·00
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	2·00, 2·02	1·92, 1·92	2·41[d], 2·33[d]
4*	1·96, 1·99	1·97, 1·92	1·92, 1·92
5*	2·06, 1·96	1·93, 2·04	2·05[c], 1·84[c]
6	2·01, 2·07	1·93, 1·89	1·88, 2·00
7	1·94, 1·96	1·98, 2·06	1·92, 1·91
8*	1·99, 2·06	1·97, 1·99	2·05, 2·01
9	1·89, 1·93	2·38, 2·25	1·91, 1·89
10	1·96, 2·06	2·14, 2·10	1·97, 1·97
11	1·91, 1·96	2·00, 2·00	1·95, 1·91
12	2·10, 2·10	2·00, 1·90	2·10, 2·10
Mean	2·00	2·03	1·97
Control[b]	1·96	1·96	1·96
r	0·11	0·14	0·11
R	0·18	0·36	0·21

For key see Table XX.
* Slight modifications to method 2.
† See text.

TABLE X

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS

Large warm-water prawns (Sample codes 10, 13)

Lab no.	Ice glaze *g/100g* [Method 2]†	[Method 3]†	[Method 4]†
1	14·5, 12·9	17·4, 17·6	15·2, 16·4
2	11·5, 12·3	18·7, 17·8	15·4, 15·9
3*	15·6, 15·5	19·2, 19·6	26·4[d], 26·3[d]
4*	16·5, 14·8	19·2, 18·1	15·4, 13·1
5*	15·5, 15·4	18·5, 19·6	16·2, 15·7
6	16·2, 15·4	18·9, 18·8	15·4, 14·7
7	16·6, 15·0	21·2[c], 17·3[c]	17·8, 15·6
8*	17·0, 16·9	18·2, 17·9	15·6, 16·1
9	20·3, 17·9	18·4, 17·7	15·9, 15·7
10	18·1, 18·2	18·8, 17·9	16·5, 15·2
11	17·5, 15·9	19·4, 18·2	17·3, 17·0
12	20·9, 17·1	19·9, 20·9	22·7[c], 28·5[c]
Mean	16·2	18·7	15·8
Added glaze	16·9	16·9	16·9
r	3·3	1·6	2·4
R	6·3	2·5	2·8

For key see Table XX.
* Slight modifications to method 2.
† See text.

TABLE XI

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT OF FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Large warm-water prawns (Sample codes 10, 13)

Lab no.	Nitrogen content *g/100g* [Method 2]†	[Method 3]†	[Method 4]†
1	2·13, 2·05	2·06, 2·19	2·13, 2·16
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	1·96, 2·04	2·02, 2·00	2·16, 2·23
4*	1·96, 2·07	2·00, 2·06	1·90, 1·87
5*	1·93, 2·18	2·31, 2·04	2·10, 2·03
6	2·03, 2·05	1·98, 1·98	1·93, 1·94
7	1·97, 2·00	2·78[c], 2·00[c]	1·99, 1·99
8*	1·98, 2·02	2·05, 2·06	1·98, 2·03
9	2·18, 2·10	2·41, 2·30	1·91, 1·97
10	2·05, 2·06	1·98, 2·02	2·13, 1·99
11	1·76[d], 1·93[d]	2·04, 2·03	2·01, 1·97
12	2·00, 2·10	1·90, 2·10	2·00[c], 2·30[c]
Mean	2·04	2·08	2·02
Control[b]	1·96	1·96	1·96
r	0·21	0·24	0·13
R	0·21	0·37	0·29

For key see Table XX.
* Slight modifications to method 2.
† See text.

Product 5, cockles. All of the methods were inaccurate with these samples. The mean recovery figures for methods 2, 3 and 4 were 48, 59 and 64 per cent. respectively of the actual added glaze of 10·7 per cent. (Table XII). Overall, methods 3 and 4 exhibited marginally better precision than method 2. The mean nitrogen contents of the deglazed samples ranged from 2·07 to 2·14 per cent. i.e. all greater than the control figure of 2·00 per cent. (Table XIII). This suggests that some loss of physiological water may have occurred.

Product 6, scampi. The percentage glaze recovery figures for the samples glazed at a level of 8·5 per cent. were 65, 88 and 77 per cent. respectively for methods 2, 3 and 4 (Table XIV). All three methods exhibited quite wide variation in results for glaze determination, shown by relatively large values for r and R. Methods 3 and 4 were slightly more precise than method 2. The nitrogen figures of the deglazed samples were all somewhat lower than the control figure of 2·75 per cent. (Table XV) and this may be indicative of incomplete glaze removal.

Product 7, scallops. A low mean percentage recovery figure for glaze was found for method 2 (33 per cent.) compared to methods 3 (58 per cent.) and 4 (58 per cent.) at a glaze level of 5·7 per cent. (Table XVI). One laboratory even reported a **negative** glaze figure for this sample. Method 2 exhibited the poorest precision. The precision of methods 3 and 4 was similar but still rather poor. The respective mean deglazed nitrogen figures for methods 2, 3 and 4 were high (2·62, 2·63 and 2·67) in comparison to the control figure of 1·85 (Table XVII).

TABLE XII

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS

Cockles (Sample codes 4, 8)

Lab no.	Ice glaze *g/100* [Method 2]‡	[Method 3]‡	[Method 4]‡
1	4·5†, 5·5†	6·6, 4·0	6·1, 6·1
2	2·1, 3·3	6·6, 6·1	6·9, 6·3
3*	4·1, 4·5	6·1, 7·1	10·2, 8·6
4*	5·6, 4·8	7·1, 8·5	6·6, 6·9
5*	5·0, 8·5	8·8, 6·1	7·7, 6·6
6	n.a., n.a.	7·0, 6·9	5·5, 5·4
7	2·4, 4·0	4·5, 5·5	7·0, 7·7
8*	5·3, 3·6	5·5, 4·8	5·1, 6·5
9	5·5, 6·5	5·9, 5·0	7·3, 7·9
10	7·8, 6·6	6·8, 8·6	5·9, 6·0
11	5·8, 5·0	5·7, 5·4	4·4, 6·2
12	4·7, 5·5	5·8, 6·4	10·2, 8·3
Mean	5·0	6·3	6·9
Added glaze	10·7	10·7	10·7
r	3·1	2·8	2·2
R	4·6	3·5	4·1

For key see Table XX.
* Slight modifications to method 2.
† Figures analysed as cooked, not included.
‡ See text.

TABLE XIII

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Cockles (Sample codes 4, 8)

Lab no.	Nitrogen content *g/100* [Method 2]‡	[Method 3]‡	[Method 4]‡
1	2·19†, 2·21†	2·30, 2·03	2·18, 2·12
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	2·16, 2·04	2·07, 2·03	2·16, 2·22
4*	2·05, 2·07	2·17, 1·97	2·07, 2·12
5*	2·02, 2·21	2·07, 2·23	2·26, 2·14
6	n.a., n.a.	2·12, 2·13	2·09, 2·11
7	2·09, 2·01	2·16, 2·14	2·10, 2·12
8*	2·27[d], 2·23[d]	2·17, 2·18	2·28, 2·20
9	1·96, 2·21	2·22, 1·98	2·13, 2·06
10	2·10, 2·09	2·14, 2·09	2·08, 2·10
11	1·95, 1·97	2·10, 2·07	2·05, 2·15
12	2·20, 2·00	2·20, 2·10	2·20, 2·00
Mean	2·07	2·12	2·14
Control[b]	2·00	2·00	2·00
r	0·28	0·28	0·18
R	0·28	0·28	0·20

For key see Table XX.
* Slight modifications to method 2.
† Figures analysed as cooked, not included.
‡ See text.

TABLE XIV

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT ON FISH PRODUCTS

Scampi (Sample codes 3, 6)

Lab no.	Ice glaze *g/100* [Method 2]‡	[Method 3]‡	[Method 4]‡
1	4·1†, 4·6†	5·5, 5·5	5·3, 6·2
2	1·5, 1·9	6·6, 6·6	6·8, 5·6
3*	4·6, 4·9	11·7[d], 9·4[d]	7·1, 8·9
4*	5·0, 8·4	8·0, 7·0	4·0, 4·2
5*	3·6, 3·8	9·1, 5·4	6·5, 6·3
6	n.a., n.a.	5·6, 5·2	3·7, 5·2
7	5·5, 4·1	5·4, 6·1	11·5[c], 7·0[c]
8*	3·8, 5·1	6·9, 7·0	4·8, 7·3
9	5·9, 4·5	4·5, 4·8	5·7, 6·3
10	7·0, 5·0	6·3, 6·5	4·6, 4·3
11	6·3, 5·8	5·7, 8·6	5·8, 5·7
12	7·9, 6·5	7·0, 8·6	8·3, 8·3
Mean	5·1	6·9	6·0
Added glaze	7·8	7·8	7·8
r	3·1	3·1	2·3
R	5·0	3·6	4·1

For key see Table XX.
* Slight modifications to method 2.
† Figures analysed as cooked, not included.
‡ See text.

TABLE XV

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Scampi (Sample codes 3, 6)

Lab no.	Nitrogen content *g/100* [Method 2]‡	[Method 3]‡	[Method 4]‡
1	2·79†, 2·64†	2·73, 2·71	2·73, 2·99
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	2·60, 2·65	2·57, 2·54	2·63, 2·68
4*	2·59, 2·58	2·62, 2·64	2·65, 2·70
5*	2·74, 2·55	2·64, 2·66	2·58, 2·54
6	n.a., n.a.	2·58, 2·58	2·62, 2·61
7	2·64, 2·81	2·52, 2·57	2·72, 2·99
8*	2·63, 2·57	2·54, 2·53	2·59, 2·65
9	2·59, 2·55	2·57, 2·63	2·69, 2·56
10	2·59, 2·55	2·62, 2·60	2·64, 2·63
11	2·58, 2·59	2·61[c], 2·49[c]	2·56, 2·65
12	2·70, 2·50	2·70[c], 2·40[c]	2·80, 2·60
Mean	2·61	2·60	2·67
Control[b]	2·75	2·75	2·75
r	0·23	0·06	0·28
R	0·23	0·17	0·34

For key see Table XX.
* Slight modifications to method 2.
† Figures analysed as cooked, not included.
‡ See text.

TABLE XVI

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT ON FISH PRODUCTS

Scallops (Sample codes 14, 17)

Lab no.	Ice glaze *g/100* [Method 2]‡	[Method 3]‡	[Method 4]‡
1	2·5†, 0·7†	2·7, 1·9	2·8, 3·4
2	−1·1, −1·5	2·3, 3·1	3·4, 3·2
3*	2·1, 1·1	5·2, 2·8	5·9[e], 4·3[e]
4*	3·5, 4·0	4·1, 4·8	3·7, 3·9
5*	2·4, 1·2	2·8, 3·2	2·9, 3·3
6	n.a., n.a.	3·4, 3·2	2·1, 2·4
7	1·1, 1·3	1·6, 2·4	3·0, 3·7
8*	2·4, 1·6	3·6, 4·7	3·5, 3·8
9	1·5, 2·3	2·3, 2·8	3·4, 3·4
10	2·7, 3·5	3·6, 3·6	2·8, 3·1
11	4·3, 2·9	4·3, 3·3	3·6, 4·3
12	1·3, 1·6	4·4, 3·8	5·5[d], 5·8[d]
Mean	1·9	3·3	3·3
Added glaze	5·7	5·7	5·7
r	1·7	1·9	0·9
R	4·3	2·7	1·5

For key see Table XX.
* Slight modifications to method 2.
† Figures analysed as cooked, not included.
‡ See text.

TABLE XVII

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZED FISH PRODUCTS

Scallops (Sample codes 14, 17)

Lab no.	Nitrogen content *g/100 g* [Method 2]‡	[Method 3]‡	[Method 4]‡
1	2·77†, 2·73†	2·83[d], 2·80[d]	2·81, 2·74
2	n.a., n.a.	n.a., n.a.	n.a., n.a.
3*	2·60, 2·61	2·57, 2·64	2·66, 2·64
4*	2·62, 2·63	2·62, 2·68	2·69, 2·70
5*	2·84, 2·66	2·60, 2·62	2·65, 2·63
6	n.a., n.a.	2·65, 2·67	2·65, 2·65
7	2·68, 2·69	2·72, 2·67	2·69, 2·72
8*	2·63, 2·68	2·67, 2·65	2·71, 2·70
9	2·59, 2·51	2·70, 2·59	2·67, 2·61
10	2·66, 2·61	2·64, 2·64	2·68, 2·65
11	2·58, 2·41	2·62, 2·60	2·57, 2·62
12	2·60, 2·60	2·60, 2·50	2·60, 2·70
Mean	2·62	2·63	2·67
Control[b]	1·85	1·85	1·85
r	0·18	0·12	0·09
R	0·25	0·14	0·15

For key see Table XX.
* Slight modifications to method 2.
† Figures analysed as cooked, not included.
‡ See text.

General Discussion

Method 1. For the determination of ice-glaze in plaice and cod fillets, method 1 is considered to be unsuitable as an enforcement method. The subjective nature of this method is reflected in the poor levels of accuracy and precision obtained.

Methods 2, 3 and 4. All three methods gave quite variable results. No single procedure could be applied with success to all the seafoods in this trial. The suitability of a method for use with a given product is determined by several factors, some of which are discussed below.

(A) SAMPLE CHARACTERISTICS

(i) Surface area to weight ratio. Comments received from participants indicated apparent inconsistencies in the deglazing procedures. In certain cases, total defrosting was observed for samples with a relatively large surface area to weight ratio (small prawns and cockles), whereas only partial defrosting (i.e. approaching a deglazing situation) was observed for samples with a relatively low surface area to weight ratio (large prawns, scampi and scallops). The risk of extracting intrinsic water from the sample obviously increases with the extent of deglazing until the sample is thawed. Samples such as small prawns and cockles may therefore give falsely high results.

(ii) The potential to absorb excess water. Water absorption appears to have had a marked effect on the analysis of scallops, even though steps were taken by the processors to minimise the problems. The potential for absorption to occur depends on whether the product is cooked or not, and may also be influenced by the method of freezing used (blast freezing, cryogenic freezing etc.)

(iii) Level of glaze added. It is not clear to what extent the actual level of glaze on a sample affects the rate of deglazing, though a sample with a relatively higher level of glaze might be expected to deglaze at a slower rate than a similar sample with a relatively lower level of glaze.

(B) METHOD CHARACTERISTICS

Many participants expressed concern about certain aspects of the methods. These included steps that could be controlled more strictly (e.g., deglazing times and temperatures, method of agitation and draining techniques).

(i) Deglazing time. Critical timing of the deglazing procedure is obviously important in terms of repeatability and reproducibility of the methods. A deglazing time that would be just sufficient to remove the glaze should be the aim but this would be highly dependent upon sample type and expected glaze level. However, if samples were allowed to thaw totally, there would be a danger of excessive water absorption, particularly for scallops.

(ii) Deglazing temperature. It is not clear from the results of this trial what effect deglazing temperature had. Both method 2 (where appropriate) and method 3 specified a bath temperature of 27°C but is it not possible to compare these methods critically because of their inherent differences overall. Similarly, comparison of method 4 (20°C bath temperature) with the other methods is not feasible. A lower bath temperature may allow more control over the rate of deglazing. However, some participants found it more difficult to maintain the lower bath temperature stipulated in method 4, even though twice the volume of water was used compared with method 3.

(iii) Agitation. The technique of agitation during deglazing is also important since vigorous agitation could increase the risk of loss of sample matter—a point made by several participants—resulting in falsely high glaze levels. Though it would be virtually impossible to prevent loss of sample matter completely, strict control should be exercised over the agitation procedure. Participants were critical of the technique used in method 2(i) because of the high flow rate used and subsequent turbulence within the container. Analysis of the wash water from method 3 showed that the levels of nitrogen lost to the washing were between 0·02 and 0·06 per cent. of the mean deglazed sample weight (Table XVIII). However, the corresponding figure for the washings from the deglazing of scampi was 0·13 per cent. It was noticeable that the scampi tissue had a relatively gelatinous texture, which upon deglazing was more prone to breakup and removal than the scallop tissue which was also uncooked. The cooked

TABLE XVIII

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE GLAZE CONTENT ON FISH PRODUCTS: NITROGEN CONTENT OF DEGLAZING WATER USED IN METHOD 3

	Nitrogen content *g/100 g* Sample codes						
Lab no.	(1, 5) SCW-prawns	(3, 6) Scampi	(4, 8) Cockles	(7, 18) SCW-prawns	(10, 13) LWW-prawns	(12, 16) SWW-prawns	(14, 17) Scallops
1	0·03, 0·04	0·10, 0·08	0·03, 0·02	0·04, 0·02	0·02, 0·02	0·02, 0·02	0·04, 0·06
2	n.a., n.a.	n.a., n.a.	n.a., n.a.	n.a., n.a.	n.a., n.a.	n.a., n.a.	n.a., n.a.
3	0·03, 0·06	0·13, 0·21	0·05, 0·05	0·07, 0·07	0·02, 0·02	0·02, 0·02	0·07, 0·07
4	0·03, 0·05	0·14, 0·11	0·05, 0·04	0·04, 0·05	0·02, 0·02	0·02, 0·02	0·05, 0·08
5	0·06, 0·07	0·18, 0·12	0·07, 0·05	0·05, 0·06	0·02, 0·03	0·03, 0·03	0·07, 0·07
6	0·04, 0·02	0·09, 0·04	0·03, 0·03	0·03, 0·04	0·01, 0·01	0·01, 0·01	0·04, 0·03
7	0·05, 0·07	0·15, 0·20	0·08, 0·07	0·07, 0·06	0·06, 0·02	0·03, 0·04	0·07, 0·07
8	0·05, 0·05	0·18, 0·11	0·04, 0·03	0·05, 0·04	0·02, 0·02	0·01, 0·02	0·06, 0·06
9	0·05, 0·06	0·13, 0·08	0·05, 0·05	0·06, 0·06	0·04, 0·02	0·03, 0·02	0·06, 0·05
10	0·05, 0·06	0·14, 0·15	0·05, 0·05	0·06, 0·06	0·02, 0·01	0·02, 0·02	0·05, 0·05
11	0·03, 0·03	0·09, 0·07	0·03, 0·04	0·04, 0·04	0·01, 0·02	0·02, 0·02	0·05, 0·04
12	0·06, 0·08	0·16, 0·27	0·08, 0·06	0·09, 0·09	0·02, 0·03	0·02, 0·02	0·07, 0·08
Mean (1)	0·05	0·13	0·05	0·05	0·02	0·02	0·06
Mean (2)	0·07	0·14	0·06	0·06	0·03	0·03	0·06

(1) Calculated on sample as received basis.
(2) Calculated on de-glazed sample weight basis.
All results rounded to two decimal points. No outlier tests were performed.

samples of prawns and to some extent the cockles, were less prone to tissue loss under the same conditions. Nevertheless, it was clear from these results that the quantity of nitrogen lost to the wash water in method 3 is usually small and is unlikely to have a significant affect on the results obtained. For routine use, therefore, nitrogen analysis could be excluded from this method.

(iv) Draining procedures. Much criticism was received about the draining procedures used on the samples following glaze removal. In all of the methods the deglazed sample is drained for 2 min. on a sieve inclined at an angle of about 20 degrees to the horizontal. In method 2, the sample is reweighed in the sieve, which allows for the retention of microparticulate sample material trapped in the sieve mesh, but also involves weighing any water carried in the sieve. In methods 3 and 4, on the other hand, the sample is removed from the sieve before weighing. Here, loss of sample material in the sieve is possible but the problem of water retention in the sieve is overcome. None of the methods stipulated any procedure for removal of carried-over water, such as blotting the base of the sieve on absorbent paper or even shaking most of the water off. One participant was particularly concerned about this problem and calculated that, from a mean excess weight due to carried over water of about 5 g, an error in the region of 2 per cent. of the total sample weight was incurred.

Conclusions

None of the methods tested in this trial appear, at first sight, to be suitable for recommendation for use as an enforcement method. There seems to be wide variation with respect to accuracy and precision; it should, however, be

TABLE XIX

COLLABORATIVE TRIAL OF METHODS FOR DETERMINATION OF ICE-GLAZE CONTENT ON FISH PRODUCTS: SUMMARY OF RESULTS

	Ice glaze *g/100 g*						
Sample codes	(1,5) SCW-prawns	(3, 6) Scampi	(4, 8) Cockles	(7, 18) SCW-prawns	(10, 13) LWW-prawns	(12, 16) SWW-prawns	(14, 17) Scallops
Added glaze	20·7	7·8	10·7	13·3	16·9	22·9	5·7
Meth. 2							
$\bar{x}$	25·1	5·1	5·0	20·6	16·2	22·6	1·9
r	3·1	3·1	3·1	3·3	3·3	3·1	1·7
R	5·1	5·0	4·6	5·2	6·3	5·7	4·3
n	12	10	10	12	12	12	10
Meth. 3							
$\bar{x}$	26·9	6·5	6·3	22·8	18·7	25·2	3·3
r	3·4	3·1	2·8	2·2	1·6	3·8	1·9
R	3·3	3·6	3·5	2·9	2·5	4·2	2·7
n	12	11	12	10	11	11	12
Meth. 4							
$\bar{x}$	25·7	6·0	6·9	20·9	15·8	22·3	3·3
r	2·5	2·3	2·2	4·9	2·4	3·2	0·9
R	4·6	4·1	4·1	6·3	2·8	3·4	1·5
n	12	11	12	12	10	10	10

appreciated that the preparation of samples for this trial is also inherently variable and would also contribute to the observed variation. That is so, although the samples in this trial had been glazed under strictly controlled conditions and therefore may not be exhibiting the full variability in glaze content that would be typical in commercial samples.

Nevertheless, it should be recognized that the analytical results given in this report have all been expressed as added glaze on a sample as analysed basis. Any regulations which may be made to enact the provisions of the FAC recommendation will require an indication of the net weight of fish core to be made. For simple arithmetical reasons, and provided the glaze is less than 50 per cent. of the total product weight, the recovery of fish will always be nearer 100 per cent. than will be the recovery of glaze. Expressing the results in terms of fish content, i.e. as the FAC recommended, will improve both the accuracy and apparent precision and will represent the parameter of interest to the consumer.

On this basis it is possible to make the following observations from the trial:

(1) For fish fillets, method 1 (the Codex procedure) is recommended for the present.

(2) For prawns, methods 2 (the Codex procedure) and 3 (the Lancashire County Council procedure) are recommended for the present. The nitrogen determination of the wash water in method 3 need not be carried out. The former method appears to be the more accurate, the latter the more precise.

(3) A method cannot, at present, be recommended for the determination of ice-glaze in molluscs such as scallops, mussels and cockles, and further work needs to be carried out in this area.

TABLE XX

KEY TO RESULTS TABLES

a	Single result reported, values not used in calculation of mean, repeatability and reproducibility.
b	Control result: analysis of sample before glazing by expert laboratory.
c	Result rejected as outlier by Cochran's test ($p<0{\cdot}05$), values not used in calculation of mean, repeatability and reproducibility.
d	Result rejected as outlier by Dixon's Test ($p<0{\cdot}05$), values not used in calculation of mean, repeatability and reproducibility.
n.a.	Not analysed.
r	Repeatability (within-lab variation). The value below which the absolute difference between two single test results obtained with the same method on identical test material under the **same conditions** may be expected to lie with 95 per cent. probability.
R	Reproducibility (between-labs variation). The value below which the absolute difference between two single test results obtained with the same method on identical test material under **different conditions** may be expected to lie with 95 per cent. probability.

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Appendix I: Codex Alimentarius Commission
Determination of Net Contents of Quick Frozen Fish Fillets

1. SCOPE AND FIELD OF APPLICATION

The method is designed to determine the net contents of raw quick frozen fish fillets covered by glaze.

2. DEFINITION

The content of ice-glaze: the weight of ice-glaze as determined by the method specified.

3. PRINCIPLE

The sample is thawed by applying a gentle spray of cold water and then drained, dried and weighed. [Weight loss is assumed to be loss of ice-glaze.]

4. APPARATUS

4.1 *Analytical balance*

4.2 *Gentle spray of cold tap water*

5. PROCEDURE

5.1 Place sample in a freezer of temperature $-18°C \pm 2°C$ and allow to equilibrate. For analysis remove and sample from low temperature storage, open immediately, accurately weigh the sample, to one decimal place (m_0 g). Place under a gentle spray of cold water.

5.2 Agitate carefully so that the product is not broken. Spray until all the ice-glaze that can be seen or felt is removed.

5.3 Allow the sample to drain; remove adhering water by the use of a paper towel and weigh the deglazed product. Let the final weight, to one decimal place, be m_1 g.

6. EXPRESSION OF RESULTS

6.1 *Calculation of ice glaze*: The ice-glaze content of the original sample, expressed as a percentage by weight, is given by:

$$\frac{m_0 - m_1}{m_0} \times 100$$

Where m_0 = the initial frozen weight taken (5.1), m_1 = the determined deglazed weight (5.3).

6.2 *Repeatability*: To be assessed from the results of the collaborative trial.

6.3 *Reproducibility*: To be assessed from the results of the collaborative trial.

7. REFERENCE

Codex Alimentarius Commission CAC/RM 93–1981 (Hake Standard) [note that although a number of procedures are described in the Codex Standards for this determination, the above is the latest recommended procedure].

Appendix II: Codex Alimentarius Commission
Determination of Net Contents of Quick Frozen Shrimps and Prawns

1. SCOPE AND FIELD OF APPLICATION

The method is designed to determine the net contents of raw and cooked quick frozen shrimps and prawns covered by glaze.

2. DEFINITION

The content of ice-glaze: the percentage weight of ice-glaze as determined by the method specified.

3. PRINCIPLE

The sample is thawed by immersion into a container into which running water is introduced in the case of raw product, or immersed in water maintained at 27°C until thawing is deemed to be completed in the case of cooked product. [Weight loss is assumed to be loss of ice glaze.]

4. APPARATUS

4.1 *Analytical balance*

4.2 *Sieve*: Clean and dry, with woven wire cloth with nominal square aperture size 2·8 mm, and conforming to requirements of ISO R565, or of aperture size 2·38 nm, and conforming to the requirements of U.S. No. 8 Standard Screen.

Sieves are to be of diameter 20 cm or 30 cm.

4..3 *Container*: Into which fresh water at room temperature can be introduced at the bottom of the container at the rate of approximately 25 litres per min.

4.4 *Container and water*: Of temperature 27°C ± 1°C. The amount of water should be equal to 8 times the weight of sample taken (see 5.2).

5. PROCEDURE

5.1 Place sample in a freezer of temperature −18°C ± 2°C and allow to equilibrate.

5.2 Accurately weigh the sample to one decimal place. Let initial weight be m_o g.

5.3 Weigh a clean dry sieve (4.2). Use a 20 cm diameter sieve if sample weight m_o g is 500 g or less, or 30 cm if greater than 500 g. Let weight of sieve, to one decimal place, be m_1 g.

5.4 Transfer weighed portion to sieve (4.2).

5.5 *Raw products*: Immerse sieve and test sample in container with running water (4.3).

Cooked products: Immerse sieve and test sample in a vessel containing the specified quantity of water (4.4).

Leave the product in the water until all ice is melted.

After all glaze that can be seen or felt has been removed (i.e. when the external surface of the sample becomes soft) and the shrimps or prawns separate easily, remove sieve and test sample, incline the sieve at an angle of about 20° and drain for two minutes.

5.6 Weigh the sieve containing the drained product. Let the final weight, to one decimal place, be m_2 g.

6. EXPRESSION OF RESULTS

6.1 *Calculation of ice glaze*: The ice-glaze content of the original sample, expressed as a percentage by weight, is given by:

$$\frac{m_0 + m_1 - m_2}{m_0} \times 100$$

Where m_0 = the initial frozen weight taken (5.2), m_1 = the initial weight of sieve (5.3), m_2 = the determined de-glazed weight with sieve (5.6).

6.2 *Repeatability*: To be assessed from the results of the collaborative trial.

6.3 *Reproducibility*: To be assessed from the results of the collaborative trial.

Appendix III: Lancashire County Council
Determination of Ice Glaze for IQF Cooked and Peeled Prawns

1. SCOPE AND FIELD OF APPLICATION

The method is designed to determine the ice-glaze content of IQF cooked and peeled prawns.

2. DEFINITION

The content of ice-glaze: the percentage weight of ice glaze as determined by the method specified.

3. PRINCIPLE

The sample is immersed in water at 27°C for a set time, during which it is gently agitated. Sample is then drained in a sieve and weighed. [Weight loss is assumed to be a loss of ice-glaze].

4. APPARATUS

4.1 *Analytical balance*

4.2 *Sieve*: Clean and dry, of aperture size 2·8 mm, diameter 20 cm and conforming to requirements of ISO R565, or of aperture 2·38 mm, and conforming to the requirements of U.S. No. 8 Standard Screen.

4.3 *Container and water*: Of temperature 27°C ± 1°C. The amount of water should be equal to 8 times the weight of sample taken (see 5.2).

5. PROCEDURE

5.1 Place sample in a freezer of temperature of −18°C ± 2°C and allow to equilibrate.

5.2 Remove the pack from low temperature storage, open and accurately weigh a sample. Let initial weight, to one decimal place, be m_0 g.

5.3 Transfer weighed portion to water container (4.2) and leave in water for 2 min with occasional gentle stirring.

5.4 Empty the contents of water container onto the sieve (4.3), saving the water for subsequent analysis.

5.5 Incline the sieve at about 20° and allow to drain for 2 min. Remove the sample from the sieve and reweigh. Let final weight, to one decimal place, be m_1 g.

5.6 Carry out a nitrogen content determination on the final water in the container used to remove the ice-glaze.

6. EXPRESSION OF RESULTS

6.1 *Calculation of ice-glaze*: The ice-glaze content of the original sample, expressed as a percentage by weight, is given by:

$$\frac{m_o - m_1}{m_o} \times 100$$

Where m_0 = the initial frozen weight taken (5.1), m_1 = the determined defrosted weight (5.6).

6.2 *Repeatability*: To be assessed from the results of the collaborative trial.

6.3 *Reproducibility*: To be assessed from the results of the collaborative trial.

7. REFERENCE

D. W. Lord, M. S. Green, and J. T. Rhodes, *Environmental Health*, 1986, **94(6)**, 143.

Appendix IV: British Frozen Food Federation
Determination of Ice-Glaze for IQF Cooked and Peeled Prawns

1. SCOPE AND FIELD OF APPLICATION

The method is designed to determine the ice-glaze content of IQF cooked and peeled prawns.

2. DEFINITION

The content of ice-glaze: the percentage weight of ice-glaze as determined by the method specified.

3. PRINCIPLE

The sample is immersed in water maintained at 20°C for a set time, during which it is continuously agitated. Sample is then drained in a sieve and weighed. [Weight loss is assumed to be loss of ice-glaze.]

4. APPARATUS

4.1 *Analytical balance*

4.2 *Sieve:* Clean and dry, of aperture size 2.8 mm, and conforming to requirements of ISO R565, or of aperture 2·38 mm, and conforming to the requirements of U.S. No. 8 Standard Screen.

4.3 *Container and water*: Of temperature 20°C ± 1°C. The container is fitted with a thermostatically controlled heater capable of maintaining temperature of water at 20°C ± 1°C. The amount of water should be equal to 16 times the weight of sample taken (5.2).

5. PROCEDURE

5.1 Place sample in a freezer of temperature −18°C ± 2°C and allow to equilibrate.

5.2 Accurately weigh the sample. Let initial weight, to one decimal place, be m_0 g.

5.3 Transfer weighed portion to sieve (4.2). Immerse sieve and test sample in water (4.3).

5.4 Leave sieve and sample in water at 20°C ± 1°C for 30 seconds. Agitate the water and sample continuously during that time.

5.5 Remove sieve and sample after the 30 s., incline, and allow sample to drain for 2 min.

5.6 Transfer sample by fingers to a dry pre-tared container and reweigh. Let final weight, to one decimal place, be m_1 g.

6. EXPRESSION OF RESULTS

6.1 *Calculation of ice-glaze*: The ice-glaze content of the original sample, expressed as a percentage by weight, is given by:

$$\frac{m_0 - m_1}{m_0} \times 100$$

Where m_0 = the initial frozen weight taken (5.1), m_1 = the determined defrosted weight (5.6).

6.2 *Repeatability*: To be assessed from the results of the collaborative trial.

6.3 *Reproducibility*: To be assessed from the results of the collaborative trial.

J. Assoc. Publ. Analysts, 1989, **27**, 109–112

Differentiation between Food Grade and Non–food Grade Mineral Hydrocarbons by Thin Layer Chromatography

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One of the most important criteria for checking the purity of food grade mineral hydrocarbons is to test for the presence of polycyclic aromatic hydrocarbons (PAH's). The blue fluorescence test for the detection of PAH's, using a long wave UV lamp, after thin layer chromatography on a silica gel G plate, can be used to differentiate between food grade and non-food grade mineral hydrocarbons. The method is simple and can be used for routine analysis.

Mineral hydrocarbons are used in foods for lubrication, polishing, and as release agents. A committee appointed by the Ministry of Agriculture, Fisheries and Food (UK) reviewed the use of mineral hydrocarbons in foods, and concluded[1] that their use could be permitted if technologically required, provided that the mineral hydrocarbon used was free from PAH's, which are reported to be carcinogenic[2]. The Food and Drug Administration (FDA) of the USA also permitted the use of mineral hydrocarbons (white food grade) in certain products[3], while in India, the Prevention of Food Adulteration Act, 1954 permits the use of food grade mineral hydrocarbons in hard-boiled sugar confectionery, chewing gum, and bubble gum[4].

Mineral hydrocarbons are not readily absorbed across the intestinal wall, since they are not affected by lipolysis and do not form a diffusible complex with bile salts[5].

Various methods have been published for the detection of mineral hydrocarbons in foods, e.g. in oils and fats[6], bakery products[7], smoked foods[8], bread[9,10], whole black pepper[11], raisins[12], dried fruits[13], and chillies[14]. The purity of mineral hydrocarbons, with regard to suitability for food use, can be tested by methods described in the British Pharmacopoeia[15], Indian Pharmacopoeia[16], UK food regulations[17], FDA (USA) regulations[3], and Bureau of Indian Standards regulations[18].

For the quantitative extraction of PAH's from mineral hydrocarbons, various solvents such as furfural and phenol have been recommended, as has preferential extraction with dimethylsulphoxide in hexane. Propylene carbonate is said to be a superior solvent for this purpose since it does not mix with linear or cyclic hydrocarbons, fat, or water[19].

Methods used for the detection of PAH's have included GLC[19], UV spectrophotometry[7,20,21,22,23,24], IR spectrometry[25,26], and, for some PAH's, β-induced fluorescence spectrometry after TLC[27]. Methods for the estimation of PAH's in basic foodstuffs[28] and in milk[29] are also reported. However, these methods are not suitable for routine analysis as the amount of mineral

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hydrocarbon extracted is very small. For routine analysis, a TLC method can be adopted after clean-up on a basic alumina column. Any interference due to volatile oils in the sample can be removed by keeping it in an oven at 100°C for 1 hr before spotting, there being no danger of loss of light or heavy mineral hydrocarbons as they have high boiling points. Solvents investigated for the extraction of mineral hydrocarbons from food products during the present investigation included petroleum ether, chloroform, benzene, octane and cyclohexane. Of these, chloroform was found to be the best.

Apparatus

1. Chromatographic column 1 cm in dia. × 25 cm height.
2. Chromatographic plate 20 cm × 10 cm × 2·5 mm.
3. UV lamp (long wave, 365 nm).

Reagents

1. *Chloroform A.R.*
2. *Silica gel G for TLC.*
3. *Petroleum ether (40° ~ 60°).*
4. *Alumina, basic active.*
5. *Cyclohexane A.R.*
6. *Iso-octane.*
7. *Sodium sulphate, anhydrous.*
8. *Mineral oil (liquid paraffin I.P. and machine oil).*
9. *2:7 Dichlorofluorescein solution (0·2% in ethanol).*

Method

EXTRACTION

Weigh approximately 25 g of powdered food into a beaker, add 25 ml of chloroform, mix, allow to stand for 15 min. and filter. Repeat the extraction three times, collecting the extracts in a beaker. Concentrate the combined extracts to small volume by evaporation on a water bath.

Prepare a slurry of basic alumina in petroleum ether, and transfer it to the chromatographic column. Add approximately 2 g of anhydrous sodium sulphate to the top of the column, and transfer the concentrated extract on to the column with the aid of 10 ml of petroleum ether. Elute with 50 ml of petroleum ether. Evaporate the eluate, and keep the residue in an oven at 100°C for 1 hr. Dissolve the residue in 0·5 ml of chloroform.

THIN LAYER CHROMATOGRAPHY

Spot approximately 20 µl of the solution of the residue on to a previously activated silica gel G plate (thickness 0·25 mm), together with solutions of known food grade and non-food grade hydrocarbons (i.e. liquid paraffin and machine oil respectively). Develop the plate in any one of the solvents (petroleum ether, cyclohexane or *iso*-octane) to a distance of 15 cm from the base line. Dry the plate at room temperature and then in the oven to remove traces of solvent.

Examine the plate under a long wave UV lamp (365 nm). Blue or greenish-blue fluorescent spots indicate the presence of PAH's. The R_f values cannot be reported as they depend on the source of the non-food grade mineral hydrocarbon, different PAH's being derived from different petroleum sources. Also, the intensity of fluorescence increases as the mid-boiling point of the mineral oil increases. At the same time, the product becomes less soluble in the mobile phase, giving rise to a reduced R_f. Thus, PAH's in heavy lubricating oil give an intense blue fluorescent spot near the starting point, whereas PAH's in light lubricating oil give a less intense blue spot near the solvent front[30].

The fluorescent spots originating from aliphatic hydrocarbons in food grade and non-food grade mineral hydrocarbons can be observed at the solvent front, or at $R_f = 0{\cdot}7$ (when the chamber atmosphere is saturated by use of a filter paper lining), after spraying with 2 : 7 dichlorofluorescein. After such spraying, the blue or greenish-blue fluorecence observed at the mid-point with non-food grade mineral hydrocarbons is quenched due to the shift in wavelength.

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