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The Differentiation of Fresh and Frozen-thawed Poultry Meat by the Determination of the ß-Hydroxyacyl-CoA-Dehydrogenase (HADH) Activity of Chicken Breast Press Juice: Collaborative Trial

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The results of a collaborative trial involving eight participants to validate a method for the determination of the ß-Hydroxyacyl-CoA-Dehydrogenase (HADH) activity of chicken breast press juice and to assess its use in differentiating between fresh and frozen-thawed poultry meat are reported. The chicken breast press juice was analysed by a spectrophotometric enzyme assay.

The precision (repeatability and reproducibility) of the method on a range of samples obtained after pre-treatment of chicken breasts under a variety of temperature conditions was poor. It is shown that using this method, differentiation of frozen-thawed chicken meat and fresh chicken meat was possible when freezing had been carried out at temperatures below -12°C: this resulted in a significant ($P<0.01$) increase in HADH activity in press juice as compared to fresh meat samples. However, when chicken breasts were frozen at -6°C, enzyme activity was not significantly increased and therefore these samples after thawing could not be differentiated from fresh.

The Food Labelling Regulations (1984)[(1)] state that frozen-thawed meat may not be offered for sale without a statement that the meat was previously frozen and that it should not be re-frozen. The Food Safety Act (1990)[(2)], the main provisions of which came into effect on the 1st January 1991, has substantially updated and strengthened the primary legislative provisions for the UK in terms of food safety and consumer protection. The Poultry Hygiene Regulations (1976)[(3)], amended (1979)[(4)], deal with protecting the consumer from infections that can be caused by improper treatment of the poultry. Re-freezing and re-thawing of meat is an improper treatment of meat which can increase the populations of micro-organisms such as salmonellae and thus pose a serious health threat.

In addition, the consumer may be disadvantaged because fresh meat usually commands a higher price than frozen meat and thus it is important that differentiation may be made. Thus there is clearly a need for a validated and practical method of differentiating between fresh and

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frozen-thawed poultry meat if the consumer is to be protected from fraud and from possible risks of infection. There are various methods that have been used to achieve this differentiation, the majority of them involve measuring the activities of mitochondrial enzymes that are released when the meat is frozen and then thawed. The freezing and thawing of meat damages muscle mitochondria which results in partial release of the mitochondrial enzymes into the sarcoplasm or muscle juice. The enzymes released are usually either ß-Hydroxyacyl-CoEnzyme A Dehydrogenase (HADH) or glutamic oxalacetic transaminase (GOT). Gottesman and Hamm[5] developed a biochemical technique for measuring HADH activity in meat press juice by means of a spectrophotometric enzyme assay. The test is simple to carry out and has a short analysis time. The activity of HADH can be measured by determining the rate at which nicotinamide-adenine-dinucleotide (reduced)(NADH) is converted to nicotinamide-adenine-dinucleotide (NAD^+) which is dependent on the level of HADH activity, this is achieved by measuring the decrease in the absorption of the reaction solution at 340nm. The enzyme activity is then calculated by using a simple formula. Demmer and Werkmeister[6] also studied HADH activity. They used "gentle homogenisation" instead of a press method, as they claimed it would disintegrate the cells of the tissue without affecting intact mitochondria. After sedimentation of the mitochondria with high speed centrifugation the liberated HADH activity was measured in the supernatant. The work on the enzyme GOT was carried out by Vizzani[7] and involved measuring the activity of GOT in the muscle press juice by means of an electrophoretic method. These enzyme methods are only applicable to whole meat samples, not to comminuted meats.

Other non-enzymic methods for differentiation of fresh and frozen-thawed poultry that have been investigated include Juola and Pekkanen's[8] method, which is based on a colour reaction between malachite green and oxyhaemoglobin, which results in malachite green impregnation of filter paper; Baker and Darfler's[9] method which is based on sensory analysis; Abdallah's[10] method which investigated cellulose acetate electrophoretic patterns of the sarcoplasmic proteins of fresh and frozen-thawed meat. None of these methods have been validated by collaborative trial.

In this report a collaborative trial to validate a modified enzyme assay for the determination of the ß-Hydroxyacyl-CoA-DeHydrogenase (HADH) activity of chicken breast press juice is described.

Method of Analysis being Collaboratively Tested

The method studied in this trial is the spectrophotometric enzyme assay of Gottesman & Hamm[5] method with two minor alterations as proposed by the Birmingham Analytical Laboratory. The modifications require an increased reaction time (6 minutes instead of 3 minutes) and a higher reaction temperature (37°C instead of 25°C).

The method used by participants is given in Appendix I. It was sent to participants in advance of the trial to allow familiarisation with the protocol.

Collaborative Trial Organisation

Participants

Eight laboratories participated in the trial comprising seven UK Public Analyst Laboratories, and the Laboratory of the Government Chemist.

Sample Preparation

All samples were prepared by the Campden Food and Drink Research Association, Chipping Campden, Glos.

Three separate batches of chicken breasts from the same supplier (24h after slaughter) were used. The first batch were sub-divided and placed in frozen storage at -18/20°C, -12°C and -6°C for a period of one month. In addition, a second batch of chicken breasts were obtained and stored at 5°C for 5 days to simulate chilled poultry and a third batch obtained immediately prior to the trial for use as fresh control samples.

Immediately prior to dispatch, three frozen samples were thawed and distributed to participants in cool boxes (approx. 5°C) together with the fresh and chill-stored samples. All samples were dispatched as blind duplicates: thus participants received 10 separate samples in total for analysis.

Results

The results obtained in the trial are reported in Tables I - V

Statistical Analysis of the Results

The trial results were examined for evidence of individual systematic error ($p<0.01$) using Cochran's and Grubb's tests progressively, by procedures described in the internationally agreed Protocol for the Design, Conduct and Interpretation of Collaborative Studies[11].

Calculations for repeatability (r) and reproducibility (R) as defined by the Protocol[11] were carried out on those results remaining after removal of outliers. The resulting values are given in Tables I-V and are summarised in Table VI. In addition, a two tailed t-test was carried out to establish the significance of differences in mean enzyme activities between the fresh and the frozen-thawed samples.

Discussion

The HADH activity increased with the severity of freezing as anticipated. No difference in enzyme activity was observed for fresh or chilled chicken breasts (5.8 & 5.7 U/ml press juice respectively)(Tables I & II). This level is assumed to be a base level where no mitochondrial damage

has taken place and no release of HADH into the sarcoplasm has occurred. As the severity of the freeze temperature increased from -6°C to -18/20°C, the mean activity of HADH increased from 8 U/ml to 23 U/ml with a doubling in activity from -12°C to -18/20°C (Table VI). The values for within-laboratory precision, repeatability (r), were acceptable except where chicken breasts were pre-frozen at -18/20°C: however, the relative standard deviation of the repeatability (RSD_r) was comparable over the whole temperature range (Table VI).

The values for between-laboratory precision, reproducibility (R), were poor for this method. This was probably partly due to laboratory number 3 consistently producing results that were lower than those produced by the other laboratories but insufficiently so as to be an outlier using the statistical tests employed for this trial. However, the values obtained for the relative standard deviation of reproducibility (RSD_R) demonstrate an improvement in precision of this method with increasing enzyme activity e.g. > 11 U/ml. It is unclear why the precision of this method should be poor at low enzyme concentrations but it is suggested that it could be related to the accuracy of measurement of a decrease in absorbance of the reaction solution when enzyme activity is slow. It is also considered likely that intra-batch and inter-batch variation in enzyme levels in chicken press juice affected results as it was not possible to use a single homogeneous matrix for this trial.

The purpose of this trial was to evaluate an enzymatic method to differentiate between fresh and frozen-thawed poultry. The results indicate that the method as tested is imprecise but does allow differentiation of fresh and frozen-thawed poultry meat provided the freezing process has been undertaken at temperatures below -12°C prior to re-thawing, when significantly ($P<0.01$) higher enzyme activities are obtained. For samples which were pre-frozen at -6°C or chill-stored, HADH activity was not significantly different from fresh samples and therefore it was not possible to differentiate such samples from fresh meat.

It is not possible to establish from these trial data whether it is the initial freezing time/temperature process or the freezer storage temperature and length of storage which is the important factor in increasing HADH enzyme activity. This point may be worthy of further investigation as most commercially frozen poultry are blast frozen at -30°C for ca 3 h prior to storage at transport at ca -12°C and thus this may affect the performance of the method.

Other factors suspected of affecting the performance of this method include control of the precision with which the absorbance measurements of the reaction solution are made. It is possible that the marginal differences in HADH activity, produced by a greater precision of measurement, might in some circumstances be sufficient to change the criteria by which frozen-thawed meat is judged. Whilst such differences should be noted, during this trial all results were reported to the same number of decimal places.

One laboratory (No.3) submitted data that was consistently below the levels reported by other participants. It is known that the storage conditions under which the NADH reagent is kept are critical to the performance of the reagent. If moisture is allowed to penetrate the dry NADH, the activity of the enzyme is reduced and there is an increased formation of dehydrogenase inhibitors. The increase of inhibitors slows down the reaction critically affecting a timed assay. It is possible that the NADH reagent used by this laboratory was not anhydrous thereby causing slower reaction times and thus consistently lower results. Additionally, slightly acidic conditions can be destructive to ß-NADH and thus reduce activity. Ideal conditions under which the ß-NADH is stable is a pH of 7.5 which may not have been achieved by this laboratory in this trial.

Conclusions

The determination of HADH activity in chicken press juice can be used to differentiate fresh and frozen-thawed poultry meat provided the freezing process has been carried out at -12°C or below. Further investigation is required to improve precision of the method and to obtain a threshold level of enzyme activity (which may vary according to batch and type of poultry meat), to allow the method to be used in the absence of control samples as encountered in an enforcement situation.

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APPENDIX I

Determination of the ß-hydroxyacyl-CoA-dehyrogenase (HADH) Activity of Chicken Breast Press Juice.

1 Scope and Field of Application

1.1 The freezing and thawing of meat causes damage to muscle mitochondria resulting in a partial release of certain mitochondrial enzymes into the sarcoplasm. Freeze damage to chicken breast meat muscle can be assessed via levels of the enzyme HADH.

1.2 The method describes the determination of the enzyme ß-hydroxyacyl-CoA-dehydrogenase (HADH) in chicken breast press juice by means of a photometric enzyme test using Nicotinamide-adenine dinucleotide (reduced), disodium salt, (NADH).

1.3 The method is applicable to intact chicken breast meat.

1.4 The method is not applicable to minced chicken breast meat.

2 Definition

2.1 The method has no legal status.

2.2 HADH activity is expressed in the equivalent of International units per millilitre of meat press juice (U/ml) under the conditions specified.

1U represents 1 micromole of substrate converted per minute at pH 6.0 and at 37°C.

3 Principle

3.1 The press juice is expressed from the chicken breast sample and diluted with a phosphate buffer.

3.2 Determination of HADH activity is based on the following reaction:-

$$\text{Acetoacetyl-CoA} + \text{NADH} + \text{H}^+ \xleftrightarrow{HADH} \text{ß-Hydroxybutyryl-CoA} + \text{NAD}^+$$

3.3 The rate of conversion of NADH to NAD^+, which is dependent on the level of HADH activity, is measured by the decrease in absorption of the reaction solution at 340 nm.

4 Reagents

(Water should be of de-ionised, distilled or similar quality).

4.1 Phosphate buffer 0.1M (pH 6.0).

4.1.1 Potassium dihydrogen phosphate (KH_2PO_4; AR quality) 13.6g (± 0.1g) made up to one litre with water.

4.1.2 Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$; AR quality) 17.8g (± 0.1g) made up to one litre with water.

4.1.3 To one litre of KH_2PO_4 solution (**4.1.1**) add the Na_2HPO_4 solution (**4.1.2**) until a pH of 6.0 is obtained.

The solution can be stored under refrigeration (less than 5°C) for several months.

4.2 EDTA (disodium salt) solution 10mg/ml.

4.2.1 Accurately weigh 500mg (± 1mg) ethylenediamine tetra-acetic acid (disodium salt; AR quality). Transfer quantitatively to a small 50ml volumetric flask with water. Swirl to dissolve. Make up to the 50 ml mark with water, stopper and invert several times to mix thoroughly.

This solution can be stored under refrigeration (less than 5°C) for several months.

4.3 Standardised NADH solution (nominally 5mg/ml). (To be determined for each batch number of NADH).

4.3.1 Prepare stock NADH solution (10mg/ml) as follows:-

Accurately weigh 250mg (± 1mg) Nicotinamide-adenine dinucleotide (reduced), disodium salt.

($C_{21}H_{27}N_7O_{14}P_2Na_2+H_2O$; BDH Chemicals Ltd.,Poole, Dorset, England; Product 10804 ONLY; REAGENT MUST BE STORED UNDER STRICT ANHYDROUS CONDITIONS).

Transfer quantitatively to a 25ml volumetric flask with water. Swirl to dissolve. Make up to the mark with water, stopper and invert several times to mix thoroughly.

The solution can be stored under refrigeration (less than 5°C) for several days.

4.3.2 Prepare intermediate 5mg/ml NADH solution as follows:-

To 1.0ml of stock NADH solution (10mg/ml) (**4.3.1**) in a stopperable glass tube (**5.6**), add 1.0ml water and mix thoroughly.

4.3.3 Standardisation procedure:-

To a 10mm silica or glass spectrophotometer cell (**5.3**) add the following reagents:-

2.75ml phosphate buffer (**4.1**);

0.20ml (200 microlitres) EDTA disodium salt solution (**4.2**)

Place the cell in a thermostat controlled water bath (**5.2**) maintained at 37°C and allow cell contents to attain 37°C.

Add 0.05ml (50 microlitres) of intermediate 5mg/ml NADH solution (**4.3.2**). Stopper the cell, invert several times to mix and quickly place in cell holder (maintained at 37°C) of the U.V/Visible spectrophotometer (**5.1**).

Ensure the absence of air bubbles.

Measure the absorbance (extinction) of the cell contents at 37°C at 340nm against air.

The required absorbance of the cell contents under the above conditions is 0.720.

Calculation example:-

e.g. Extinction of cell contents = 0.652

∴ required concentration of NADH solution to give 0.720 is given by

$$\frac{0.720 \times 5}{0.652} = 5.52 \text{ mg/ml}$$

This concentration will be provided by mixing together

$$\frac{2 \times 5.52}{2 \times 5.00} = 1.104 \text{ ml stock NADH (4.3.1)}$$

and

$$(2 - 1.104) = 0.896 \text{ water.}$$

Stored under refrigeration (less than 5°C) this solution is stable for several days.

4.4 Acetoacetyl-CoA solution (5mg/ml).

4.4.1 Accurately weigh 5.0mg Acetoacetyl Coenzyme-A, sodium salt (Sigma Chemical Company; Product No. A-1625, stored desiccated below 0°C) to a glass tube (**5.6**)

Add 1.0ml water, swirl to dissolve, stopper and mix thoroughly. This volume will be sufficient for determinations on at least 19 samples.

Stored under refrigeration (less than 5°C) this solution is stable for several days.

(note: On receipt of Acetoacetyl Coenzyme-A, sodium salt, it is advisable to divide the material into accurately weighed 5mg portions ready for later use).

5 Apparatus

5.1 U.V./Visible spectrophotometer, capable of constant temperature control of the cell holder at 37°C.

5.2 Thermostatically controlled water bath suitable for use at 37±0.5°C.

5.3 Silica or glass cells, path length 10mm, e.g. from Hellma (England) Ltd.

5.4 Cast Iron Press (347195; W.H.Smith, Do-it-All by Victor Cast Ware Ltd.), with either porcelain or plastic dish and approximately 2cm thick rigid plastic disc insert; or equivalent alternative.

5.5 Pipettes or syringes capable of accurate delivery of the following volumes

2.60 millilitres (ml);
2.75 millilitres (ml);
200 microlitres (µl);
100 microlitres (µl);
50 microlitres (µl);

5.6 Stoppered glass tubes (capacity >2ml).

5.7 Refrigerator capable of maintaining temperature of < 5°C.

5.8 Volumetric flasks 20ml (Grade B).

5.9 Stop Watch.

5.10 Scalpel (holder and disposable blades).

6 Procedure

6.1 The chicken breast (from one side of a chicken carcass) is cut with a scalpel, transversely rather than longitudinally, to produce two halves to be labelled (a) and (b). Each of the halves are processed separately and as follows:-

6.2 Place the flesh centrally in the porcelain or plastic dish of the cast iron press (**5.4**) or equivalent alternative. Place the metal plate on top of the flesh. Lower the piston screw by rotating the handle until the latter is "hand tight". (This presses on the metal and "squashes" the sample beneath to produce "press juice").

Leave for approximately 5 minutes to allow press juice to accumulate in the base of the dish.

6.3 Transfer the press juice to an appropriately labelled stopperable glass tube (**5.6**) by means of a disposable Pasteur pipette.

A minimum volume of 0.5ml is required.

(If the volume collected is insufficient then repeat step 6.2).

The press juice can be stored under refrigeration (less than 5°C) for a maximum of four days if necessary.

6.4 Transfer 100 microlitres (µl) of the press juice into a 20 ml volumetric flask (**5.8**). Make up to the mark with phosphate buffer 0.1M (**4.1**), stopper and invert several times to mix thoroughly.

6.5 To a 10mm spectrophotometer cell (**5.3**) placed in a thermostatted water bath (**5.2**) maintained at 37°C add the following:-

2.60ml phosphate buffer 0.1M (**4.1**)
200µl (microlitres) EDTA (disodium salt) solution (**4.2**)
100µl (microlitres) diluted press juice

Allow the cell contents to attain 37°C then add

50µl (microlitres) standardised NADH solution (**4.3**)

Place a stopper on the cell and invert several times to mix the contents.

Dry the cell faces quickly with a tissue.

6.6 Place the cell in the cell holder (at 37°C) of the U.V./Visible spectrophotometer (**5.1**).

6.7 Add 50µl (microlitres) Acetoacetyl-CoA solution (**4.4**) to the cell and mix to start the reaction. Ensure the absence of air bubbles.

6.8 Immediately measure the absorbance/extinction at 340nm (against air) and start the stop watch (**5.9**).

Leave the cell in the spectrophotometer.

6.9 After six minutes measure the absorbance/extinction again at 340nm.

The difference between the two readings ΔE is the decrease in absorption at 340nm, over a six minute reaction time.

7 Expression of Results

7.1 Formula and Method of Calculation

$$\text{HADH activity (to nearest 0.1U/ml)} = \frac{v}{-xdxa} \times \Delta E/\text{min} \times \textit{dilution factor}$$

where:-

ΔE = is the decrease in absorption at 340nm, over a six minute reaction time.

v = volume of test mixture (3.0ml)

_ = extinction coefficient NADH 340nm (**4.3**)

d = cell path length (1.0cm)

a = volume of press juice dilution (0.1ml)

$$\text{U/ml} = \frac{3 \times \Delta E \times 200}{6.3 \times 1 \times 0.1 \times 6}$$

$$= \Delta E \times 158.73$$

TABLE I

HADH activity (U/ml) in chicken press juice from fresh chicken breasts.

	U/ml	
Laboratory	a	b
1	6.50	4.25
2	7.70	6.50
3	2.45	3.10
4	4.00	4.35
5	5.55	6.45
6	5.40	4.40
7	9.35	11.55
8	5.85	5.80
Mean	5.8	
r	2.6	
SD_r	0.93	
RSD_r	15.9	
R	6.6	
SD_R	2.36	
RSD_R	40.6	

For key, see table VII

TABLE II

HADH activity (U/ml) in chicken press juice from chicken breasts stored at 5°C for 5d

	U/ml	
Laboratory	a	b
1	4.60	7.60
2	5.90	4.80
3	2.85	2.85
4	4.25	4.20
5	6.55	6.90
6	5.00	5.20
7	9.15	11.30
8	4.50	4.75
Mean	5.7	
r	2.7	
SD_r	0.97	
RSD_r	17.2	
R	6.4	
SD_R	2.30	
RSD_R	40.6	

For key, see table VII

TABLE III

HADH activity (U/ml) in chicken press juice from chicken breasts pre-frozen at -6°C for 1 month then thawed.

	U/ml	
Laboratory	a	b
1	10.90	10.40
2	9.75	8.60
3	1.50	4.45
4	7.65	6.25
5	7.30	7.00
6	8.65	7.05
7	11.35	14.75
8	5.95	8.90
Mean	8.2	
r	4.1	
SD_r	1.48	
RSD_r	18.2	
R	8.8	
SD_R	3.14	
RSD_R	38.5	

For key, see table VII

TABLE IV

HADH activity (U/ml) in chicken press juice from chicken breasts pre-frozen at -12°C for 1 month then thawed.

	U/ml	
Laboratory	a	b
1	11.70	14.55
2	11.25	11.35
3	7.05	6.50
4	10.95	12.30
5	11.20	10.70
6	8.85	11.75
7	13.65	14.45
8	9.25	11.40
Mean	11.1	
r	3.4	
SD_r	1.23	
RSD_r	11.1	
R	6.6	
SD_R	2.34	
RSD_R	21.2	

For key, see table VII

TABLE V

HADH activity (U/ml) in chicken press juice chicken breasts pre-frozen at -18/20°C for 1 month then thawed

	U/ml	
Laboratory	a	b
1	20.65	27.50
2	20.00	28.70
3	16.50	16.40
4	18.95	25.45
5	20.10	28.50
6	15.80	23.70
7	30.00	24.65
8	20.70	25.95
Mean	22.7	
r	13.2	
SD_r	4.70	
RSD_r	20.7	
R	13.2	
SD_R	4.70	
RSD_R	20.7	

For key, see table VII

TABLE VI

Summary of precision characteristics of HADH method

Temp (°C)	Mean U/ml	n	r	S_r	RSD_r %	R	S_R	RSD_R %
Fresh	5.8	8	2.6	0.93	15.9	6.6	2.36	40.6
+ 5	5.7	8	2.7	0.97	17.2	6.4	2.30	40.6
- 6	8.2	8	4.1	1.48	18.2	8.8	3.14	38.5
- 12	11.1	8	3.4	1.23	11.1	6.6	2.34	21.2
- 18/20	22.7	8	13.2	4.70	20.7	13.2	4.70	20.7

For key, see table VII

Table VII

Key to Tables I to VI

Symbol	Definition
SD_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability (within-laboratory variation) The value below which the absolute difference between two single test results obtained with the same method on the identical test material under different conditions may be expected to lie with 95% probability.
SD_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility (between-laboratory variation). The value below which the absolute difference between two single test results obtained with the same method on the identical test material under different conditions may be expected to lie with 95% probability.

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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 13

ICE-GLAZE ON QUICK FROZEN PRAWNS

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

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

2. Definition

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 tap-water is introduced (in the case of the raw product), or immersed in tap-water maintained at 27°C until thawing is deemed to be completed (in the case of the cooked product). The weight loss is assumed to be loss of ice-glaze.

4. Reagents

None

5. Apparatus

5.1 Analytical balance

5.2 Sieve: Clean and dry, with woven wire cloth of nominal square aperture size 2.8 mm and conforming to the requirements of ISO R565, or of aperture size 2.38 mm and conforming to the requirements of US No 8 Standard Screen. Sieves are to be of diameter 200 mm or 300 mm.

5.3 Container with inlet/overflow, into which fresh tap-water at room temperature can be introduced at the bottom of the container at the rate of approximately 25 l/min.

5.4 Water bath: A vessel containing tap-water at 27 ± 1°C equal in weight to 8 times the weight of sample taken (**6.1**).

0004-5780/93 +5 $20.00

6. Procedure

6.1 Place the sample in a freezer of temperature -18 ± 2°C and allow to equilibrate. For analysis, remove the sample from low temperature storage, open immediately, accurately weigh in g to one decimal place (m_0)

6.2 Weigh a clean dry sieve (**5.2**), using a 200 mm diameter sieve if the sample weight is 500 g or less, or 300 mm if greater than 500 g. Let the weight in g of the sieve, to one decimal place, be m_1.

6.3 Transfer the weighed portion to the sieve. Deglaze by one of the following methods:

6.3.1 Frozen Raw Products: immerse the sieve and test sample in the container with running water (**5.3**).

6.3.2 Frozen Cooked Products: immerse the sieve and test sample in the water bath containing the specified quantity of tap-water (**5.4**).

6.4 Leave the product immersed until all the ice is melted. After all the 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 the sieve and test sample, incline the sieve at an angle of about 20° and drain for two minutes.

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

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

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

$$\% \text{ ice-glaze content} = 100 \times (m_0 + m_1 - m_2) / m_0$$

where:

m_0 is the initial frozen weight taken (**6.1**);
m_1 is the initial weight of sieve alone (**6.2**);
m_2 is the observed deglazed weight with sieve (**6.5**).

9. References

9.1 GC Hodson, MJ Scotter and R Wood, J. Assoc. Publ. Analysts, 1989, **27**, 85-108.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[(2)].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised in Tables 1 and 2. Overall, r may be taken to be 3.3% glaze at levels between 15% and 30% glaze. This corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of 4 - 8%.

A2 Reproducibility

The absolute difference between two test results carried out under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised in Tables 1 and 2. Overall, R may be taken to be about 6% glaze at the appropriate levels; this corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 7 - 14%.

A3 Trueness (Bias)

The observed accuracy of the method may be assessed by comparing the overall mean of the results with the expected values given in Tables 1 and 2. The cold-water prawns gave a recovery of 140% glaze or more, while the larger warm-water prawns gave almost quantitative yields.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggest that levels of ice glaze lower than 3% cannot be detected with confidence.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed four samples of quick frozen cooked coldwater prawns once, and four samples of quick frozen cooked warm-water prawns once. These comprised small whole cold-water prawns analysed in blind duplicate (1/5; 7/18) and whole warm-water prawns analysed similarly (small, 12/16; large, 10/13).

Tables 1 and 2 summarise the statistical data; no outlying results were reported. The ice-glaze levels were expressed as a percentage by mass of the sample.

A6 Interpretation of Observed levels

The subjective nature of the method is reflected in the poor overall levels of accuracy and precision established by the results of the collaborative trial; there is a distinct tendency towards overestimation, and the observed values of repeatability and reproducibility (Tables 1 and 2) are larger than would be considered acceptable in a conventional chemical method. Nevertheless the method is recommended for the analysis of quick frozen shrimps and prawns until a more precise method is established.

The shellfish content of the original sample, expressed as a percentage by weight, is given by subtracting the glaze content from one hundred. It is recommended that the results should normally be interpreted in terms of shellfish content, since this is the parameter of interest to the consumer.

TABLE 1

Statistical Analysis of the % Ice-glaze in Quick-frozen Cooked Cold-water Prawn Samples

Sample	1/5 Small	7/18 Small
Number of Laboratories	12	12
Number of results accepted	24	24
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	25.1	20.6
Actual (target) value	20.7	13.3
REPEATABILITY		
Standard Deviation S_r	1.1	1.2
Relative Standard Deviation RSD_r (%)	4.4	5.7
Repeatability r [2.8 x S_r]	3.1	3.3
REPRODUCIBILITY		
Standard Deviation S_R	1.8	1.9
Relative Standard Deviation RSD_R(%)	7.3	9.0
Reproducibility R [2.8 x S_R]	5.1	5.2

TABLE 2

Statistical Analysis of the % Ice-glaze in Quick-frozen Cooked Warm-water Prawn Samples

Sample	12/16 Small	10/13 Large
Number of Laboratories	12	12
Number of results accepted	24	24
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	22.6	16.2
Actual (target) value	22.9	16.9
REPEATABILITY		
Standard Deviation S_r	1.1	1.2
Relative Standard Deviation RSD_r(%)	4.9	7.3
Repeatability r [2.8 x S_r]	3.1	3.3
REPRODUCIBILITY		
Standard Deviation S_R	2.0	2.2
Relative Standard Deviation RSD_R(%)	9.0	13.9
Reproducibility R [2.8 x S_R]	5.7	6.3

A7 Key to Tables 1 and 2

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

J. Assoc. Publ. Analysts, 28, 123-127

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 14

ICE-GLAZE ON QUICK FROZEN PRAWNS

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

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

2. Definition

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

3. Principle

The sample is thawed by immersion in tap-water at 27°C for a set time, during which it is gently agitated. It is then drained in a sieve and weighed. The weight loss is assumed to be loss of ice-glaze.

4. Reagents

None

5. Apparatus

5.1 Analytical balance

5.2 Sieve: Clean and dry, of diameter 200 mm and nominal aperture size 2.8 mm (conforming to the requirements of ISO R565), or of aperture size 2.38 mm (conforming to the requirements of US No 8 Standard Screen).

5.3 Water bath: a vessel containing tap-water at 27 ± 1°C equal in weight to 8 times the weight of sample taken (**6.1**).

6. Procedure

6.1 Place the sample in a freezer of temperature -18°C ± 2°C and allow to equilibrate. For analysis, remove the sample from low temperature storage, open immediately and accurately weigh in g to one decimal place (m_0).

6.2 Transfer the weighed portion to the water bath (**5.3**) and leave it in the water for two min. with occasional gentle stirring.

6.3 Empty the contents of the water bath into a sieve (**5.2**); incline the sieve at an angle of about 20° and allow to drain for two minutes.

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6.4 Remove the sample from the sieve and reweigh. Let the final weight in g, to one decimal place, be m_1.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

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

$$\% \text{ ice-glazed content} = 100 \times (m_0 - m_1) / m_0$$

where:

m_0 is the initial frozen weight taken (**6.1**);

m_1 is the observed deglazed weight (**6.4**).

9. Reference

9.1 GC Hodson, MJ Scotter and R Wood, J. Assoc. Publ. Analysts, 1989,**27**, 85-108.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised in Tables 1 and 2. Overall, r may be taken to be 3.8% glaze at levels between 15% and 30% glaze. This corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of 3 - 6%. The analysis of large prawns may be expected to be more precise, with a lower target for r (1.6% glaze).

A2 Reproducibility

The absolute difference between two test results carried out under reproducibility conditions should not be greater than the reproducibility, R deduced from the collaborative trial data summarised in Tables 1 and 2. Overall, R may be taken as about 4% glaze at the appropriate levels; this corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 4 - 6%.

A3 Trueness (Bias)

The observed accuracy of the method may be assessed by comparing the overall mean of the somewhat imprecise results with the expected values given in Tables 1 and 2. The cold-water prawns gave a recovery of 150% glaze or more, while the larger warm-water prawns gave more satisfactory results (110%).

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggest that levels of ice glaze lower than 4% cannot be detected with confidence. The limit of detection in large prawns may be lower (2% glaze).

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed four samples of quick frozen cooked cold-water prawns once, and four samples of quick frozen cooked warm-water prawns once. These comprised small whole cold-water prawns analysed in blind duplicate (1/5; 7/18) and whole warm-water prawns analysed similarly (small, 12/16; large, 10/13).

Tables 1 and 2 summarise the statistical data; four outlying results were reported. The ice-glaze levels were expressed as a percentage by mass of the sample.

A6 Interpretation of Observed Levels

The subjective nature of the method is reflected in the poor overall levels of accuracy and precision established by the results of the collaborative trial; there is a distinct tendency towards massive overestimation, and the observed values of repeatability and reproducibility (Tables 1 and 2) are larger than would be considered acceptable in a conventional chemical method. Nevertheless the method is recommended for the analysis of quick frozen shrimps and prawns until a more precise method is established.

The shellfish content of the original sample, expressed as a percentage by weight, is given by subtracting the glaze content from one hundred. It is recommended that the results should normally be interpreted in terms of shellfish content, since this is the parameter of interest to the consumer.

TABLE 1
Statistical Analysis of the % Ice-glaze in Quick-frozen Cooked Cold-water Prawn Samples

Sample	1/5 Small	7/18 Small
Number of Laboratories	12	12
Number of results accepted	24	24
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	26.9	22.8
Actual (target) value	20.7	13.3
REPEATABILITY		
Standard Deviation S_r	1.2	0.79
Relative Standard Deviation RSD_r (%)	4.5	3.4
Repeatability r [2.8 x S_r]	3.4	2.2
REPRODUCIBILITY		
Standard Deviation S_R	1.2	1.0
Relative Standard Deviation RSD_R(%)	4.4	4.5
Reproducibility R [2.8 x S_R]	3.3	2.9

TABLE 2
Statistical Analysis of the % Ice-glaze in Quick-frozen Cooked Warm-water Prawn Samples

Sample	12/16 Small	10/13 Large
Number of Laboratories	12	12
Number of results accepted	22	22
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	25.2	18.7
Actual (target) value	22.9	16.9
REPEATABILITY		
Standard Deviation S_r	1.4	0.57
Relative Standard Deviation RSD_r(%)	5.4	3.1
Repeatability r [2.8 x S_r]	3.8	1.6
REPRODUCIBILITY		
Standard Deviation S_R	1.5	0.89
Relative Standard Deviation RSD_R(%)	6.0	4.8
Reproducibility R [2.8 x S_R]	4.2	2.5

A7 Key to Tables 1 and 2

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

J. Assoc. Publ. Analysts, 28, 129-132

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V15

SOLUBLE SOLIDS IN VINEGAR

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

The method determines the loss of mass on drying of vinegar

2. Definition

Total soluble solids: the weight of matter remaining after drying by the method specified.

3. Principle

The residual mass of a test portion is determined after evaporation on a water bath followed by drying at atmospheric pressure in an oven at 103 ± 2°C. To ensure the total volatilisation of the acetic acid, the evaporation step is repeated three times after restoration to at least the original volume with distilled water.

4. Reagents

Wherever the use of water is required, distilled or water of equivalent purity is to be used.

5. Apparatus

5.1 Pipettes, 10 ml.

5.2 Filter and filter papers, slow-filtering paper.

5.3 Dishes, of platinum, ceramic or glass. The dishes must have lids which fit very well but which can be readily removed. The dishes should be 75 mm in diameter.

5.4 Water bath

5.5 Atmospheric pressure drying oven, well ventilated and thermostatically controlled with temperature regulation at 103 ± 2°C. The temperature should be uniform throughout the oven.

5.6 Desiccator, containing freshly activated silica gel with a water content indicator, or equivalent desiccant.

5.7 Analytical balance, capable of weighing to at least 0.1 mg.

5.8 Glass stirring rod

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6. Procedure

6.1 Uncover the dish and place it and its lid in the oven at 103°C for 1 hr.

6.2 Place the lid on the dish and transfer the covered dish to the desiccator.

6.3 Allow the dish to cool to room temperature and accurately weigh to the nearest 0.1 mg (m_1).

6.4 Shake the sample, and filter it through the filter paper.

6.5 Pipette 10 ml of sample into the dish.

6.6 Place the dish on a boiling water bath and evaporate almost to dryness.

6.7 Add 15 ml of distilled water to the dish and stir.

6.8 Wash the stirring rod into the dish with a small quantity of distilled water.

6.9 Evaporate almost to dryness on a boiling water bath.

6.10 Repeat processes 6.7 to 6.9 a further two times.

6.11 Place uncovered dish and its lid in the oven at 103°C for 3 hr.

6.12 Cover the dish and transfer the covered dish to the desiccator.

6.13 Allow the dish to cool to room temperature and accurately weigh to the nearest 0.1 mg as quickly as possible (m_2).

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

Calculate the total soluble solids of the sample, expressed as a percentage mass to volume, by the formula:

$$\% \text{ Total soluble solids} = (m_2 - m_1) \times 10$$

where

m_1 is the weight in grams of the empty dish and lid after process 6.3;

m_2 is the weight in grams of the dish, its lid and the final dried sample after process 6.13.

9. References

9.1 MJ Scotter and R Wood, J. Assoc. Publ. Analysts, 1985, **23**, 107-117.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised in Table 1. For untreated vinegar (and for the fortified vinegars), r may be taken to be 0.10 g per 100 ml; this corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 4%.

A2 Reproducibility

The absolute difference between two test results carried out under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised in Table 1.
R may be taken as 0.17 g per 100 ml, which corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of about 8%.

A3 Trueness (Bias)

The results of the collaborative trial demonstrate that the presence of additional substances in the stock vinegar does not affect the performance of the procedure. In particular, added acetic acid is removed quantitatively during the drying process, while added sodium chloride and added citric acid is recovered quantitatively. The extent of any systematic bias due to the occlusion of acetic acid in the soluble solids residue obtained after drying remains unknown, but is unlikely to be significant; in any case it is demonstrably less than that associated with the corresponding AOAC procedure, which was also tested during the collaborative trial.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggest an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.1 g per 100 ml for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed sixteen subsamples of vinegar once (eight samples in blind duplicate). Sample 2/10 was untreated vinegar; the other samples were the same vinegar containing in

addition 2 g glacial acetic acid per 100 ml (A), 0.5g sodium chloride per 100 ml (S) and/or 0.5 g citric acid per 100 ml (C), as follows: sample 4/6, A; sample 8/11, S; sample 1/16, C; sample 3/15, A+S; sample 5/13, A+C; sample 7/9, S+C; sample 12/14, A+S+C.

Table 1 summarises the statistical data, expressed as g soluble solids per 100 ml vinegar.

TABLE 1

Statistical Analysis of the % Soluble Solids in Vinegar Samples

Sample	2/10	4/6	8/1	11/16	3/15	5/13	7/9	12/14
Number of Laboratories retained after eliminating outliers	18	18	17	18	15	18	18	18
Number of Laboratories eliminated as outliers	0	0	1	0	3	0	0	0
Number of results accepted after eliminating outliers	36	36	34	36	30	36	36	36
LEVEL OF ANALYTE								
Mean observed value $\bar{x}$	0.77	0.75	1.27	1.22	1.25	1.20	1.72	1.70
REPEATABILITY								
Standard Deviation S_r	0.03	0.02	0.02	0.03	0.02	0.06	0.03	0.04
Relative Standard Deviation RSD_r(%)	4	3	2	2	2	5	2	2
Repeatability r [2.8 x S_r]	0.09	0.06	0.05	0.08	0.06	0.16	0.07	0.11
REPRODUCIBILITY								
Standard Deviation S_R	0.06	0.06	0.05	0.06	0.05	0.08	0.05	0.07
Relative Standard Deviation RSD_R(%)	8	8	4	5	4	7	3	4
Reproducibility R [2.8 x S_R]	0.17	0.18	0.14	0.16	0.15	0.22	0.15	0.19

A7 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

J. Assoc. Publ. Analysts, 28, 133-138

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V16

TOTAL FAT IN MAYONNAISE

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

The method allows the determination of the total fat content of mayonnaise and other emulsified sauces.

2. Definition

Fat content: the total content of fat as determined by the method specified.

3. Principle

The well-mixed sample is digested with hydrochloric acid and the resulting liquid filtered through two moistened pleated filter papers. The residue remaining on the filter papers is dried and extracted for 4 hr. with petroleum ether or *n*-hexane. The solvent is distilled off and the residual fat is dried at 103 ± 2°C under atmospheric pressure, cooled and weighed. The fat content is calculated from the weight obtained.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise.

4.1 Indicator paper

4.2 Petroleum ether, boiling range 40 - 60°C, or *n*-hexane.

4.3 Hydrochloric acid, approximately 4 mol/l.

4.4 Silver nitrate solution, 0.1 mol/l.

4.5 Water, distilled or demineralised.

4.6 Cotton wool, defatted.

5. Apparatus

5.1 Ceramic wire gauze, for Bunsen burner and tripod.

5.2 Beakers, 600 ml, tall form.

5.3 Desiccator, containing silica gel or other suitable drying agent.

5.4 Soxhlet extractor with siphon, capacity about 100 ml, fitted with ground glass joints and a flat-bottomed 250 ml flask.

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5.5 Extraction thimbles, defatted (e.g. Schleicher & Schull No 603 or Macherey & Nagel No 645F) to fit the Soxhlet extractor.

5.6 Double pleated filter papers, 150 - 200 mm diameter with average pore diameter 5 µm maximum (e.g. Schleicher & Schull No 597 1/2 or No 595 1/2, or Macherey & Nagel No 616 1/4 or No 615 1/4).

5.7 Glass rod

5.8 Glass funnel, 100 mm diameter minimum.

5.9 Sand or water bath, with suitable means of controlling heating.

5.10 Anti-bumping granules

5.11 Watch-glass cover, 100 mm diameter.

5.12 Drying oven, electrically heated and thermostatically controlled at 103 ± 2°C.

6. Procedure

6.1 Sample Preparation and Storage

Take the contents of an entire package or several packages to provide a subsample of at least 200 g. Store in a tightly closed container at 2 - 6°C in the dark to prevent any alteration. Allow the sample to reach uniform room temperature before analysis, stirring if necessary.

6.2 Procedure for fat determination

6.2.1 Dry a flat-bottomed extraction flask, containing an anti-bumping granule, in the oven for 1hr. at 103 ± 2°C, cool in a desiccator to room temperature, and weigh; designate as weight *A*.

6.2.2 Weigh (to the nearest mg) 3 - 5 g of the well-mixed sample (depending upon the weight of fat expected, which should not exceed 3 g) into a 600 ml beaker (**5.2**); designate weight of sample as *C*.

6.2.3 Add 150 ml of hydrochloric acid (**4.3**) to the beaker and stir with the glass rod. Add a few anti-bumping granules, cover the beaker with a watch-glass, and heat to boiling. Keep the contents boiling gently on a low heat for 1 hr, stirring frequently.

6.2.4 Add 150 ml of hot water to the beaker. Place the fluted filter papers in the funnel and moisten thoroughly with hot water. Filter the hot digested liquid quickly, and wash the beaker, watch-glass cover and glass rod three times with hot water, passing each successive washing through the filter papers. Use a Celite filter aid if necessary.

Test the washings for absence of acidity, using indicator paper (**4.1**), or for the absence of chloride, using silver nitrate solution (**4.4**). Continue washing the filters until the filtrate is free of acid.

6.2.5 Place the funnel containing the filter papers in the beaker with the watch-glass and glass rod, and dry in the oven for 1 hr.

6.2.6 Transfer the dry filter papers to an extraction thimble. Remove any traces of fat present in the beaker with a piece of cotton wool damped with extraction solvent (**4.2**), and add this to the extraction thimble. Place the thimble in the extraction apparatus, add solvent to the extraction flask, and assemble the extractor. Rinse the beaker, watch-glass cover and glass rod with solvent and add the rinsings to the extraction apparatus. Heat the extraction flask on a sand or water bath, and allow the extraction to proceed continuously for 4 hr.

6.2.7 Remove the bulk of the solvent by distillation, and any traces of solvent remaining with a gentle stream of air. Dry the flask in a horizontal position in the oven for 1 hr. at 103 ± 2°C, cool in the desiccator and weigh to the nearest mg. Repeat the drying, cooling and weighing process until successive weights differ by no more than 1 mg; designate this weight as *B*.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

The total fat content, expressed as a percentage by mass of the sample (i.e. in g/100 g), is given by:

$$\% \text{ total fat content} = 100 \times (B - A) / C$$

where

A is the weight in g of the empty flask and granule (**6.2.1**);

B is the weight in g of the flask with extracted fat after drying (**6.2.7**);

C is the weight in g of sample taken (**6.2.2**).

9. References

9.1 MJ Scotter, V Staniforth and R Wood, J. Assoc. Publ. Analysts, 1989,**26**, 103-115.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability limit, r, deduced from the collaborative trial data summarised below (Table 1). When analysing mayonnaise containing about 75% total fat, the value of r may be taken as 1.1%. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 0.5%.

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised below (Table 1). For total fat contents of about 75%, R may be taken as 2.0%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of less than 1%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method. Comparison in Table 1 between the observed means and the calculated recipe values of total fat content suggests satisfactory accuracy. The mean observed values were always somewhat higher than the "expected" values, but any possible systematic bias may be neglected; the differences were never more than 1.1 g/100 g. Such slight bias could be caused by the occlusion of traces of solvent in the fat residues after drying.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggest an accuracy which, if maintained, corresponds to an extrapolated theoretical lower limit of roughly 1.1 g/100 g for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six subsamples of mayonnaise once (three different samples in blind duplicate). The total fat content of each sample was also calculated from its recipe.

Table 1 summarises the statistical data; the total fat content is expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of the % Total Fat in Mayonnaise Samples

Sample	1/3	4/6	2/5
Number of laboratories retained after eliminating outliers	16	19	20
Number of laboratories eliminated as outliers	4	1	0
Number of results accepted after eliminating outliers	32	38	40
LEVEL OF ANALYTE			
"Expected" recipe value	75.5	76.6	78.7
Mean observed value	76.6	77.4	79.3
REPEATABILITY			
Standard Deviation S_r	0.37	0.40	0.57
Relative Standard Deviation RSD_r (%)	0.48	0.52	0.72
Repeatability r [2.8 x S_r]	1.03	1.12	1.60
REPRODUCIBILITY			
Standard Deviation S_R	0.68	0.72	1.07
Relative Standard Deviation RSD_R(%)	0.89	0.93	1.35
Reproducibility r [2.8 x S_R]	1.89	2.03	2.99

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

J. Assoc. Publ. Analysts, 28, 139-143

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 17

EGG-YOLK IN MAYONNAISE

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

The method allows the determination of the egg-yolk content of mayonnaise and other emulsified sauces.

2. Definition

Egg-yolk content: the content of egg-yolk as determined by the method specified.

3. Principle

The phospholipids are extracted together with fat using a mixture of chloroform and ethanol. After ashing, the phosphate content is determined gravimetrically as the quinoline phosphomolybdate.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise.

4.1 Ethanol, 96% (*V/V*).

4.2 Chloroform

4.3 Chloroform-ethanol mixture, 3:2 by volume.

4.4 Acetone

4.5 Sulphuric acid, density 1.84 g/ml.

4.6 Nitric acid, density 1.40 g/ml.

4.7 Magnesium acetate, $Mg(CH_3COO)_2.4H_2O$, low in phosphorus.

4.8 Quinoline molybdate solution

4.8.1 Sodium molybdate

4.8.2 Distilled water

4.8.3 Citric acid

4.8.4 Quinoline, freshly distilled.

4.8.5 Dissolve 70 g of sodium molybdate, $Na_2MoO_4.2H_2O$, in 150 ml of distilled water.

4.8.6 Dissolve 60 g of citric acid in 150 ml of distilled water and add 85 ml of nitric acid.

4.8.7 Slowly pour solution (**4.8.5**) into solution (**4.8.6**), stirring constantly.

4.8.8 To 100 ml of distilled water, carefully add 35 ml of nitric acid (**4.6**) and 5 ml of quinoline (**4.8.4**). Pour this solution into solution (**4.8.7**) stirring continuously. Allow to stand for 24 hr. at room temperature. If a precipitate forms, remove it by filtration. Add 280 ml of acetone and then dilute to 1 litre with water. Keep the molybdate reagent (**4.8**) in a well-closed plastic container in a dark place.

5. Apparatus

5.1 Electrical hotplate, with magnetic stirrer.

5.2 Erlenmeyer flask, 300 ml, with reflux condenser.

5.3 Pleated filter paper, 15 cm diameter.

5.4 Volumetric flask, 250 ml.

5.5 Platinum dish, approximately 130 ml capacity.

5.6 Sintered glass filter crucible, G4.

5.7 Muffle furnace, maintained at 800°C.

5.8 Water bath

5.9 Desiccator

5.10 Erlenmeyer flask, 250 ml.

5.11 Watch-glass

5.12 Glass rod

5.13 Filter paper, ashless.

5.14 Hotplate, electrical.

5.15 Buchner flask

5.16 Drying oven, electrically heated and thermostatically controlled at 260 ± 20°C.

6. Procedure

6.1 Sample Preparation and Storage

Take the contents of an entire package or several packages to provide a subsample of at least 200 g. Store in a tightly closed container at 2-6°C in the dark to prevent any alteration. Allow the sample to reach uniform room temperature before analysis, stirring if necessary.

6.2 Separation of phospholipids

6.2.1 Weigh (to the nearest 10 mg) 12-13 g of the well-mixed sample (m_0) into a 300 ml Erlenmeyer flask (**5.2**).

6.2.2 Add 100 ml of chloroform and 75 ml of ethanol to the flask, and mix thoroughly using the magnetic stirrer until a homogeneous suspension is obtained. Heat for 1 hr. under reflux with continuous stirring.

6.2.3 Allow the flask to cool and stand overnight. Filter the contents of the flask through a pleated filter paper, previously moistened with chloroform-ethanol mixture (**4.3**), into a 250 ml volumetric flask.

Rinse the Erlenmeyer flask and the filter with more chloroform-ethanol solvent, and add to the volumetric flask, finally diluting with the same solvent to 250 ml.

6.2.4 Pipette 100 ml of the solution (**6.2.3**) into a platinum dish, cover with an ashless filter paper and evaporate off the solvent cautiously over a water bath to dryness. Add 3.5 g of magnesium acetate to the dish. Cut the filter paper into pieces and add to the contents of the dish. Cover the dish with another ashless filter paper. Calcine the residue gently over a flame and then in a muffle furnace at 800°C until a white powder is obtained (about 1 hr.).

6.2.5 Dissolve the ash (**6.2.4**) carefully in 15 ml of nitric acid (by allowing the acid to flow along a glass rod) and transfer to a 250 ml Erlenmeyer flask. Rinse the dish several times with water, adding the rinsings to the flask. Dilute the flask contents to 50 ml and allow to cool to room temperature.

6.2.6 Add 50 ml of quinoline molybdate reagent (**4.8**) to the flask with continual stirring. Cover the flask with a watch-glass and boil on the hotplate for 1 min. Allow the flask to cool to room temperature, stirring 2-3 times.

6.2.7 Heat a sintered glass filter crucible (**5.6**) at 260 ± 20°C for 30 min, cool in a desiccator and weigh to the nearest mg (m_1).

6.2.8 Transfer the precipitate (**6.2.6**) to the sintered glass filter crucible with gentle suction, and wash five times with 20 ml volumes of water.

6.2.9 Dry the crucible and contents at 260 ± 20°C in the drying oven for 1 hr., cool in a desiccator and weigh to the nearest mg (m_2).

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

8.1 The lipid phosphoric acid (lecithin) content, expressed in terms of P_2O_5 as a percentage by mass of the sample (i.e. in g P_2O_5/100 g sample) is given by:

$$\% \text{ lipid phosphoric acid content} = \frac{2.5 \times (m_2 - m_1) \times 0,03207 \times 100}{m_o}$$

Where:

m_0 is the weight of sample taken;

m_1 is the weight of the empty sintered glass filter crucible;

m_2 is the weight of the sintered glass filter crucible and precipitate.

8.2 The egg-yolk content, expressed as a percentage by mass of the sample (ie in g/100 g) is given by:

% egg-yolk content (g/100 g) = % lipid phosphoric acid content × 102

9. References

9.1 MJ Scotter, V Staniforth and R Wood, J. Assoc. Publ. Analysts, 1989,**26**, 103- 115.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised below (Table 1). When analysing mayonnaise containing about 5% egg-yolk, the value of r may be taken as 0.6%. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of 4-5%

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised below (Table 1). For egg-yolk contents of about 5%, R may be taken as 0.7%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of about 5%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method. Comparison in Table 1 between the observed mean and the calculated recipe values of egg-yolk content suggests satisfactory accuracy. The mean observed values differ by no more than 0.1 g/100 g from the "expected" values.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggest an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.6% egg-yolk for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six subsamples of mayonnaise once (three different samples in blind duplicate). The egg-yolk content of each sample was also calculated from its recipe.

Table 1 summarises the statistical data; the egg-yolk content is expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of the % Egg-yolk in Mayonnaise Samples

Sample	1/3	4/6	2/5
Number of laboratories retained after eliminating outliers	18	19	15
Number of laboratories eliminated as outliers	2	1	5
Number of results accepted	36	38	30
LEVEL OF ANALYTE			
"Expected" recipe value	4.7	5.1	5.6
Mean observed value	4.6	5.1	5.5
REPEATABILiTY			
Standard Deviation S_r	0.26	0.28	0.11
Relative Standard Deviation RSD_r(%)	5.7	5.5	2.0
Repeatability r [2.8 x S_r]	0.74	0.79	0.31
REPRODUCIBILITY			
Standard Deviation S_R	0.30	0.28	0.13
Relative Standard Deviation RSD_R(%)	6.5	5.5	2.4
Reproducibility r [2.8 x S_R]	0.85	0.78	0.37

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

J. Assoc. Publ. Analysts, 28, 145-152

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V18

VINYL CHLORIDE IN FOODS

Correspondence on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

The method allows the determination of the vinyl chloride content of foodstuffs.

2. Definition

Vinyl chloride content: the content of vinyl chloride as determined by the method specified.

3. Principle

The vinyl chloride content of foodstuffs is determined by means of gas chromatography, using the "headspace" method.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise.

4.1 Vinyl chloride, VC, of purity greater than 99.5%.

4.2 *N,N*-Dimethylacetamide, DMA, not containing any impurity with the same chromatographic retention time as VC or as the internal standard (**4.3**), under the conditions of the test.

4.3 Diethyl ether or *cis*-2-butene, in DMA (**4.2**) as the internal standard solution, ISS. These internal standards should not contain any impurity with the same chromatographic retention time as VC, under the conditions of the test.

5. Apparatus

5.1 Gas chromatograph, fitted with automatic headspace sampler or with facilities for manual sample injection.

5.2 Detector, flame ionisation or other suitable detector.

5.3 Gas chromatographic column, (see Appendix 1), meeting the following requirements:

5.3.1 It must be capable of separating the air peak, the VC peak of the standard solution (**6.1**) and the internal standard peak, if this is used;

5.3.2 The signal obtained with a solution containing 0.005 mg VC/litre or 0.005 mg VC/kg (**6.1**) must be equal to at least five times the background noise.

5.4 Sample phials or flasks, fitted with a silicone or butyl rubber septum. When using manual sampling techniques, the taking of a sample in the headspace with a syringe may cause a partial vacuum to form inside the phial or flask. Hence, for manual techniques where the phials are not pressurised before the sample is taken, the use of large phials is recommended.

5.5 Micro-syringes

5.6 Gas-tight syringe, for manual headspace sampling.

6. Procedure

Vinyl chloride is a hazardous substance and a gas at ambient temperature, therefore the preparation of solutions should be carried out in a well ventilated fume cupboard.

Take all the necessary precautions to ensure that no VC or DMA is lost.

It is highly recommended that when employing manual sampling techniques, an internal standard (**4.3**) should be used; when using an internal standard, the same solution should be utilised throughout the procedure.

6.1 Preparation of standard VC solutions

6.1.1 Concentrated standard VC solution, approximately 2000 mg/kg.

Weigh to an accuracy of 0.1 mg a suitable glass vessel and place in it a quantity (eg 50 ml) of DMA (**4.2**). Re- weigh. Add to the DMA a quantity (eg 0.1 g) of VC (**4.1**) in liquid or gas form, injecting it slowly into the DMA. The VC may also be added by bubbling it into the DMA, provided that a device is used which will prevent loss of DMA. Re-weigh to an accuracy of 0.1 mg. Wait 2 hr. to allow equilibrium to be attained. Keep the standard solution in a refrigerator.

6.1.2 Dilute standard VC solution A

Take a weighed amount of concentrated standard solution (**6.1.1**) and dilute to a known volume or a known weight, with ISS (**4.3**) or with DMA (**4.2**). The concentration of resultant diluted solution (Solution A) is expressed as mg/l or mg/kg respectively.

6.1.3 Dilute standard VC solution B

Repeat the procedure described above (**6.1.1 and 6.1.2**) to obtain a second diluted standard solution B with a concentration approximately equal to 0.02 mg of VC/l of ISS or DMA. Dispense this solution into

two phials (**5.4**). Seal the phials and proceed as described under 6.4 below.

6.2 Validation of standard VC solutions A and B

6.2.1 Calibration curve for Solution A

Prepare two series of seven phials (**5.4**): add to each phial, volumes of dilute standard VC solution A (**6.1.2**) and DMA (**4.2**) or ISS (**4.3**) such that the final concentrations of the duplicate solutions will be approximately equal to 0; 0.005; 0.010; 0.020; 0.030; 0.040; 0.050 mg/l of DMA. Seal the phials and proceed as described under 6.4 below. Accept the calibration curve thus obtained if it meets the criteria listed under 6.2.2 below.

6.2.2 Acceptability of calibration curve

(i) The repeatability of the responses as given in recommendation ISO R 5725 should be better than 0.002 mg of VC/l or kg of DMA;

(ii) The curve must be constructed from at least seven pairs of points. The curve should be calculated from these points by least square techniques.

(iii) The curve must be linear; that is, the standard deviation of the responses around the regression line divided by the mean value of all responses must not exceed 0.07.

6.2.3 Validation of Solution A

If the average of two gas chromatographic determinations relating to Solution B (**6.1.3**) do not differ by more than 5% from the corresponding point on the calibration curve for Solution A (**6.2.1, 6.2.2**), then Solution A is accepted. If the difference is greater than 5%, reject all the solutions obtained under 6.1 and 6.2, and repeat the procedure from the beginning.

6.3 Construction of the "addition" curve for samples

6.3.1 Homogeneous foodstuffs

Prepare two series of seven phials (**5.4**): add to each phial a quantity of sample, obtained from the foodstuff under investigation, of not less than 5 g. Try to ensure that an equal quantity is added to each phial. Close the phial immediately. Add to each phial such volumes of diluted standard VC solution (containing the internal standard if considered useful) as will give concentrations of added VC in the phials equal to 0; 0.005; 0.010; 0.020; 0.030; 0.040 and 0.050 mg/kg of foodstuff. Use diluted standard VC solutions (**6.1.2**) such that the ratio between the volume (µl) of this VC solution and the quantity (g) of foodstuff contained in the phial is as low as possible and not more than 5. Seal the phials and proceed as described under 6.4 below. Accept the "addition" curve thus obtained if it meets the criteria under 6.2.2(ii) and 6.2.2(iii) above.

6.3.2 Other foodstuffs

Prepare two series of seven phials (**5.4**): add to each phial a quantity of sample, obtained from the foodstuff under investigation, of not less than 5 g. Try to ensure that an equal quantity is added to each phial. Close the phial immediately. Add to each phial for each 5 g of sample 5 ml of an appropriate solvent (preferably distilled or demineralised water) containing internal standard (**4.3**) if considered useful, and such volumes of diluted standard VC solution as will give concentrations of added VC in the phials equal to 0; 0.005; 0.010; 0.020; 0.030; 0.040 and 0.050 mg/kg of foodstuff. Use diluted standard VC solution (**6.1.2**) such that the ratio between the volume (µl) of this VC solution and the quantity (g) of foodstuff contained in the phial is as low as possible and not more than 5. Seal the phials and proceed as described under 6.4 below. Accept the "addition" curve thus obtained if it meets the criteria under 6.2.2(ii) and 6.2.2(iii) above.

6.4 Gas chromatographic determinations

6.4.1 Put all the sealed phials in a waterbath for 2 hr at 60 ± 1°C to allow equilibrium to be attained. Agitate the phials avoiding contact between the contained liquid and the septum (**5.4**) to obtain a solution or a suspension as homogeneous as possible.

6.4.2 Take a sample from the headspace in the phial. When utilising manual sampling techniques, care should be exercised in obtaining a reproducible sample (**5.4**); in particular, the syringe should be prewarmed to the temperature of the sample. Measure the area (or the height) of the peaks corresponding to the VC (and to the internal standard, if used). Construct a graph in which the ordinate value shows the areas (or heights) of the VC peaks or the ratio of the areas (or heights) of VC peaks to the areas (or heights) of the internal standard peaks; and the abscissa value shows the quantities of VC added (mg) relative to the quantities of foodstuff weighed into each phial (kg). Projecting to zero peak area (or height), the intersect on the abscissa axis then shows the unknown concentration of VC in the sample under investigation (**Fig. 1**).

6.4.3 If necessary, remove from the column the excess DMA (4), using appropriate methods as soon as peaks from the DMA appear on the chromatogram.

6.5 Confirmation of the VC content

If the quantity of VC determined exceeds the limit in Annex II, paragraph 2 of the Council Directive 78/142/EEC, the results should be confirmed in one of the three ways 6.5.1-3 outlined below.

Fig 1

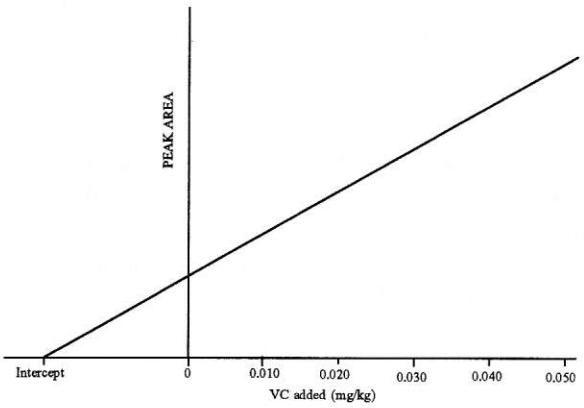

Fig. 1 A graph in which the ordinate value shows the areas of the VC peaks (or the ratio of the areas of VC peaks to the areas of the internal standard peaks); the abscissa value shows the quantities of VC added, related to the quantities of the sample of foodstuff weighed in each phial.

6.5.1 Change of stationary phase

Use at least one other column having a stationary phase of different polarity; this procedure should continue until a chromatogram is obtained with no evidence of overlap of the peaks corresponding to VC and/or internal standard with those corresponding to constituents of the foodstuff.

6.5.2 Change of detector

Use other detectors, e.g. a micro-electrolytic conductivity detector.

6.5.3 Use of mass spectrometry

The finding, that molecular ions with parent masses (m/e) of 62 and 64 are present in the ratio of 3:1, can be regarded with high probability as confirming that VC is present. In case of doubt, the total mass spectrum should be checked.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

From the graph (**6.4.2**), read the observed level of VC and express as mg of VC per kg of foodstuff.

9. References

9.1 D O Biltcliffe and R Wood, The Determination of Vinyl Chloride in Foods : Collaborative Study, J. Assoc. Publ. Analysts, 1982, **20**,55- 65.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

GLC Columns and Conditions

1 Recommended Conditions

The following column and conditions were recommended by MAFF to the participants in the collaborative trial (Appendix 2):

3.0 m x 2 mm i.d. stainless steel column with 25% di- iso-decylphthalate and 0.5% Atpet 80 on 60- 70 mesh Diatomite C- AW HMDS;

carrier gas flow rate, 20 ml/min.;

column temperature 85°C (isothermal).

2 Suitable Condition

The following columns and conditions of gas- liquid chromatography were used by the eight laboratories participating in the collaborative trial[1] (Appendix 2).

Lab.1 3.0 m × 2 mm i.d. stainless steel column with 25% di-iso-decylphthalate and 0.5% Atpet 80 on 60-70 mesh Diatomite C-AW HMDS; nitrogen carrier gas, flow rate 20 ml/min.; column temperature 85°C; automatic injection.

Lab.2 As lab.1, except: manual injection.

Lab.3 As lab.1, except: 60-85 mesh Chromosorb W AW-DMCS; column temperature 50°C.

Lab.4 As lab.1, except: glass column; 60- 80 mesh Diatomite C-AW HMDS; nitrogen carrier gas, flowrate 30 ml/min.; manual injection.

Lab.5 As lab.1, except: 10 ft × 1/4 in i.d. column; 60-85 mesh Chromosorb W AW DMCS; nitrogen carrier gas, flow rate 75 ml/min.; column temperature 45°C; manual injection.

Lab.6 5 ft × 4.5 mm i.d. glass column with 0.2% carbowax 1500 on Carbopak C; nitrogen carrier gas, flow rate 20 ml/min.; column temperature 70°C; manual injection.

Lab.7 As lab.1, except: nitrogen carrier gas, flow rate 22 ml/min.; column temperature 65°C; manual injection.

Lab.8 As lab.1, except: nitrogen carrier gas, flow rate 24 ml/min.; column temperature 83°C; manual injection.

APPENDIX 2

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised below (Table 1). The observed repeatability, r, always fell below the value of 3 μg/kg given in the adopted EC Directive on the method of analysis of vinyl chloride in foodstuffs. Overall, r may be taken to be 3 μg/kg. This corresponds to a standard deviation of repeatability, S_r, of approximately 1 μg/kg. At this precision, the relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, is about 10% at levels of 10 μg/kg.

A2 Reproducibility

The absolute difference between two test results carried out under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised below (Table 1). R may be taken as 7 μg/kg. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility) of about 25% at levels of 10 μg/kg.

A3 Trueness (Bias)

The samples consisted of orange drink spiked with known levels of VC; the recoveries were lower than might have been expected, being of the order of 50%. Poor recovery has been consistently observed from an orange drink matrix, in contrast to oil samples where the measured levels are usually very close to the level of spiking.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to a lower limit of roughly 1 μg/kg for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Three samples of orange drink were prepared by spiking with VC at levels of 5, 15 and 30 µg/kg. Participants in the collaborative trial each analysed the three samples twice.

Table 1 summarises the statistical data; the levels of VC are expressed in µg/kg.

TABLE 1

Statistical Analysis of Vinyl Chloride (µg/kg) in Orange Drink Samples

Orange Drink sample	A	B	C
Number of Laboratories retained after eliminating outliers	6	6	6
Number of Laboratories eliminated as outliers	2	2	2
Number of results accepted after eliminating outliers	6	6	6
LEVEL OF ANALYTE			
Level of added analyte	5	15	30
Mean observed value $\bar{x}$	4.05	6.16	16.09
REPEATABILITY			
Standard Deviation S_r	0.34	0.58	0.93
Relative Standard Deviation RSD_r(%)	8.5	9.3	5.8
Repeatability r [2.8 x S_r]	0.96	1.61	2.6
REPRODUCIBILITY			
Standard Deviation S_R	1.0	2.1	2.5
Relative Standard Deviation RSD_R(%)	25	33	16
Reproducibility R [2.8 x S_R]	2.8	5.8	7.0

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility