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The Determination of Reducing Sugars by Classical Procedures: Collaborative Trial

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Summary

The results of a collaborative trial involving 19 participants on the determination of reducing sugars in sugar solutions (syrups) by the Lane and Eynon Constant Volume method, the Luff-Schoorl procedure and a modified Luff-Schoorl procedure using standardised reagents, are reported. Significant differences in the mean levels of reducing sugar were observed between the Lane & Eynon and the Luff-Schoorl method and between the Lane & Eynon and modified Luff-Schoorl methods. Significant differences were found in precision between the modified Luff-Schoorl method when compared with both the Lane and Eynon Constant Volume and the original Luff-Schoorl methods. The modified Luff-Schoorl procedure performed with better precision for the samples analysed.

Introduction

Methods of analysis for sugars have been agreed by the European Community and are given in the Commission Directive 79/796/EEC laying down Community methods of analysis for testing certain sugars intended for human consumption[1]. This Directive has been incorporated into UK legislation through The Specified Sugar Products (Amendment) Regulations (1982)[2].

The Directive specifies methods for the determination of reducing sugars expressed as 'invert sugar' using the Lane and Eynon Constant Volume procedure (method 7) or the Luff-Schoorl procedure (method 6). Member States may choose one of these methods for incorporation into their national legislation. The UK has chosen the former.

The Lane and Eynon Constant Volume method has traditionally been preferred to the Luff-Schoorl procedure by UK analysts for the determination of reducing sugars. Among the reasons given for preference of the Lane & Eynon method to the Luff-Schoorl procedure, is that errors are known to occur in the preparation and use of the Luff-Schoorl reagent. In an attempt to rectify this, the International Commission on Uniform Methods of Sugar Analysis (ICUMSA)[3] has recommended the incorporation of a reagent standardisation step in the Luff-Schoorl procedure.

The modified Luff-Schoorl method was tested in a recent collaborative trial conducted by MAFF and the Lancashire County Analysts Laboratory[4] on the analysis of honeys, which included analysis for

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apparent reducing sugar content using a modified Luff-Schoorl procedure. Errors in the estimation of apparent reducing sugar content were thought to have given rise to the observed variabilities in the trial results. Several arguments were put forward as to the cause, notably that of poor attention to proper reagent preparation and storage and especially in the use of freshly boiled distilled water and accurately standardised sodium thiosulphate solution.

In the light of this it was decided that a trial to assess the relative merits of three methods of analysis for the determination of reducing sugars in sugar solutions be carried out. It was anticipated that the results would show which of the methods is the most precise.

Methods of Analysis being Collaboratively Tested

The methods tested in this trial were as follows:

(1) Method 7 of EC Directive 79/796/EEC (Lane and Eynon constant volume modification method): 'LE'. [See Appendix I for outline of method]

(2) Method 6 of EC Directive 79/796/EEC (Luff-Schoorl method): 'LS' [See Appendix II for outline of method]

(3) A modified Luff-Schoorl procedure including a standardisation step, proposed by the British National Committee (BNC) of ICUMSA: 'BNC' [See Appendix III for outline of method]

(4) Method 2 of EC Directive 79/796/EEC for dry matter (or an equivalent method if difficulties are experienced with this method, stating where differences arise). [See Appendix IV for outline of method]

Familiarisation of the methods by participants through the analysis of a pre-trial sample was not carried out as participating analysts carry out these, or similar analyses, on a routine basis.

Collaborative Trial Organisation, Samples and Results

Nineteen laboratories agreed to participate in the collaborative trial (15 UK Public Analyst Laboratories, the Government Laboratories of Jersey and the Isle of Man, The British Sugar Corporation and Tate and Lyle plc).

Samples

The samples comprised four typical commercial products prepared in bulk by Tate and Lyle plc London and consisted of the following:

Sample A: Glucose Syrup - with an estimated dextrose equivalent value of 42

Sample B: Glucose Syrup - with an estimated dextrose equivalent value of 63

Sample C: Black Treacle - commercial product

Sample D: Golden Syrup - commercial product

Sub-samples were prepared by warming each bulk sample in its container in hot water and pouring the warm solutions into 250 ml wide-mouthed pvc screw-topped bottles. Approximately 200 ml of each sample was despatched to participants with instructions to analyse each in duplicate. Differences in the visual characteristics of each sample were so great as to effectively prevent provision of the samples as blind duplicates.

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 Grubbs tests progressively, by procedures described in the internationally agreed Protocol for the Design, Conduct and Interpretation of Collaborative Studies[5].

Repeatability and Reproducibility

Calculations for repeatability (r) and reproducibility (R) as defined by that Protocol[5] were carried out on those results remaining after removal of outliers for each method/sample calculation. These are also given in Tables I - VI and have been summarised in Table V. The results from the analysis for dry matter content have been included primarily as a check for the expression of the reducing sugar content on a dry matter basis.

The values of the within-laboratory precision parameter, the repeatability (r), calculated for the methods under examination in this trial, are of the order that would be anticipated for this type of determination (in the range 0.63 - 1.64). The values for r predicted by the Horwitz equation[6] (using the approximation $r = 0.66R$) are in the range 1.5 - 2.2 .

Analysis of within-laboratory variance ratios (calculated as r_1^2 / r_2^2) using a 2-sided F-test show that only the observed difference in r between LS/BNC for sample B is significant ($P<0.01$). There are no significant differences in the repeatability values for samples A, C, and D between any of the three methods.

The values of the between-laboratory precision parameter, the reproducibility,(R), calculated for all three methods are marginally higher than would be anticipated for this type of determination (in the range 1.88 - 4.38). The values of R derived from the Horwitz equation[6] are in the range 2.3-3.3 .

Analysis of the between-laboratory variance ratios (calculated as $(2R^2_1-r^2_1)/(2R^2_2-r^2_2)$) using a 2-sided F-test, show that no significant differences in R exist between any of the methods for the results of the analyses of samples A and B. However, the results from the analyses of samples C and D show some significant differences in R. Significant differences were found between BNC/LE ($p<0.05$) from the analysis of

sample C and between BNC/LS ($p<0.05$) and LE/LS ($p<0.01$) for the analysis of sample D.

The results of the analyses suggest that the modified Luff-Schoorl method (BNC) exhibits better precision than the other two methods for samples C and D.

Comparison of means

The mean values obtained from each method/sample set were compared by significance testing using a 2-sided Student t-test. From the analysis of the results from samples A and B significant differences in mean values obtained

were found between the LE/LS and the LE/BNC (in all cases at the $p<0.01$ level), but not between the LS/BNC methods. Significant differences in mean values were found between the LE/LS and between LE/BNC methods from analysis of sample C ($p<0.05$). Results of the analysis of sample D showed a significant difference ($p<0.05$) between LE/LS methods.

The results indicate that there are significant differences in results between LE/LS and between LE/BNC methods, the LE method giving higher results, but not between LS/BNC methods.

Dry Matter Content

The respective dry matter contents were used to calculate the Dextrose Equivalents of samples A and B, and thus compare with the anticipated values. The Dextrose Equivalents of samples A and B were expected to be around 42 and 63 % respectively; these agreed with the found Dextrose Equivalents (average of values obtained by the three methods) which were 44 and 64 % respectively.

The data in tables III-VIII were converted to 100 % dry matter basis and re-analysed for statistical outliers. Analysis of the converted data did not then include the original outliers as valid data, these outliers were therefore assumed not to have been caused by variations in the samples' respective dry matter contents as received in the laboratory.

TABLE I
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method: EC DRY MATTER (g/100g)

Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	81.08(a)	77.84(a)	82.48 (a)	80.33 (a)	80.66	80.89	83.91	84.06
2	81.81	81.47	83.08	82.97	81.77	81.82	84.46	84.49
3	81.62	81.04	82.81	81.71	81.37	81.81	84.46	84.12
4	80.70	80.80	82.40	82.60	81.60	81.50	83.80	84.10
5	82.16	82.08	83.39	83.28	82.10	81.89	84.28	84.54
6	84.70	85.0 *	85.30	85.1 *	81.60	80.6 *	85.70	85.2 *
7	79.50	78.50	82.40	81.10	79.60 (b)	78.80 (b)	82.30 (b)	81.6 (b)
8	81.67	81.02	82.88	82.41	81.64	82.02	84.58	84.47
9	84.21	84.47	85.03 (b)	84.91 (b)	83.31 (b)	83.46 (b)	84.80	84.90
10	81.20	81.10	82.57	82.49	81.39	81.33	84.05	84.00
11	80.90	80.91	82.42	82.42	81.15	81.24	84.00	84.00
12	83.94	83.11	89.40 (b)	89.12 (b)	81.70 (a)	79.83 (a)	84.54	83.84
13	88.84 (b)	88.94 (b)	88.93 (b)	89.43 (b)	82.12	82.03	92.86 (b)	92.87 (b)
14	81.47	81.33	82.84	82.70	81.73	81.51	83.27	83.42
15	81.81	81.40	82.90	82.90	81.30	81.30	84.20 (a)	83.1 (a)
16	81.10	81.00	82.30	82.50	80.50	80.50	83.40	83.50
17	81.10	81.10	82.30	82.50	81.40	81.30	84.50	84.50
18	81.40	81.50	82.90	82.90	81.20	81.20	84.50	84.30
19	91.30	91.4 *	90.30	90.9 *	83.80	83.8 *	89.20	89.8 *
Mean	**81.51**		**82.60**		**81.44**		**84.17**	
r	**0.87**		**1.00**		**0.38**		**0.50**	
S_r	**0.309**		**0.356**		**0.137**		**0.178**	
RSD_r %	**0.38**		**0.43**		**0.16**		**0.21**	
R	**3.43**		**1.32**		**1.27**		**1.22**	
S_R	**1.224**		**0.470**		**0.452**		**0.434**	
RSD_R %	**1.50**		**0.57**		**0.56**		**0.52**	

For Key See Table VI Note : Sample B, 23.5% of original data removed as outliers

TABLE II
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method: EC Lane and Eynon — Invert Sugar(g/100g)

Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	36.47	36.61	54.06	54.06	35.98	35.98	49.84	49.65
2	36.44	36.25	53.53	53.84	35.73	36.15	49.39	49.58
3	36.61	36.16	54.62	54.90	37.06	37.03	50.41	50.44
4	36.50	36.30	55.00	54.10	37.60	37.10	50.20	49.70
5	37.29	36.94	53.97	54.41	36.90	36.92	49.44	49.69
6	36.70	35.90	54.60	54.47	36.29	36.49	49.88	49.97
7	36.70	35.90	54.30	54.60	35.50	36.20	49.1 (a)	51.50 (a)
8	36.88	36.69	54.20	55.05	38.40	38.80	50.29	50.29
9	36.96	37.35	55.03	55.06	36.22	36.44	50.27	50.12
10	35.90	36.00	52.97	52.79	36.90	37.20	48.90	48.80
11	35.50	35.40	52.40	53.20	40.40	39.70	48.70	49.40
12	34.20	35.20	52.70	51.30	34.70	34.90	51.50	52.00
13	36.86	36.13	54.94	54.54	43.91	44.79	49.39	49.76
14	36.70	37.10	55.20	55.20	36.10	36.40	50.10	50.00
15	42.0 (b)	42.8 (b)	62.4 (b)	62.80 (b)	41.10	41.50	57.80	57.60
16	36.70	36.60	54.30	54.40	37.50	37.40	49.90	50.00
17	36.10	36.20	54.10	54.10	36.90	37.00	50.10	50.20
18	37.00	37.30	54.70	54.80	38.00	37.90	50.90	50.40
19	36.60	36.10	53.60	53.30	36.90	36.40	49.90	49.90
Mean	**36.42**		**54.12**		**37.16**		**49.97**	
r	**0.84**		**1.03**		**0.71**		**0.60**	
S_r	**0.300**		**0.369**		**0.254**		**0.216**	
RSD_r %	**0.82**		**0.68**		**0.68**		**0.43**	
R	**1.79**		**2.50**		**4.38**		**1.87**	
S_R	**0.64**		**0.893**		**1.563**		**0.667**	
RSD_R %	**1.76**		**1.65**		**4.21**		**1.335**	

For Key See Table VI

TABLE III
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method: EC Luff-Schoorl

Invert Sugar (g/100g)

Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	35.34	35.40	52.32	51.93	35.30 (a)	32.50 (a)	48.34	48.26
2	35.32	34.82	52.24	52.37	36.09	36.07	52.34	52.10
3	35.18	34.87	51.32	51.99	36.59	36.45	47.62	47.63
4	34.60	34.90	52.40	52.70	35.60	36.10	48.20	49.40
5	36.40	36.02	52.61	52.91	36.02	36.29	49.29	49.12
6	34.79	34.33	51.88	51.02	34.34	34.33	49.39	49.60
7	37.70	36.70	54.20	54.30	36.30	35.70	50.90	51.30
8	34.82	35.12	51.03	51.29	36.11	35.89	49.00	49.06
9	36.79	37.22	52.95	53.10	37.10	36.95	49.69	49.70
10	36.20	35.70	52.40	52.70	35.90	36.40	50.00	49.70
11	35.20	34.30	50.30	50.50	34.60	33.90	45.60	45.60
12	35.30	35.70	52.20	52.70	34.60	35.40	50.60	51.30
13	34.23	34.51	51.05	50.86	37.75	38.41	48.07	47.66
14	35.10	33.40	51.30	51.00	35.80	36.80	48.40	47.90
15	43.30 (b)	43.80 (b)	63.90 (b)	64.20 (b)	42.00 (b)	42.40 (b)	59.10 (b)	58.90 (b)
16	35.20	35.10	52.00	52.10	36.20	36.00	49.20	49.50
17	35.60	36.00	52.20	52.60	36.60	36.20	49.00	49.30
18	35.30	34.90	51.70	51.30	39.10	39.20	49.80	49.50
19	32.90	33.70	50.10	50.20	35.10	34.50	47.10	47.30
Mean	**35.24**		**51.94**		**36.13**		**49.07**	
r	**1.25**		**0.74**		**0.98**		**0.82**	
S_r	**0.448**		**0.263**		**0.350**		**0.29**	
RSD_r %	**1.27**		**0.51**		**0.97**		**0.59**	
R	**2.81**		**2.84**		**3.49**		**4.31**	
S_R	**1.004**		**1.013**		**1.247**		**1.54**	
RSD_R %	**2.85**		**1.95**		**3.45**		**3.14**	

For Key See Table VI

TABLE IV
CLASSICAL SUGARS COLLABORATIVE TRIAL :RESULTS

Method Modified Luff-Schoorl (BNC) — Invert Sugar (g/100g)

Laboratory No.	Sample A		Sample B		Sample C		Sample D	
1	34.94	35.88	53.50	52.58	36.32	36.17	50.54	50.18
2	35.27	35.50	52.46	52.48	35.89	36.27	50.30	50.97
3	34.83	35.83	52.37	53.08	36.11	36.67	50.04	50.45
4	34.90	34.30	52.50	53.00	37.30	36.40	50.20	50.60
5	34.49	34.05	52.12	51.95	35.23	35.37	49.53	49.13
6	34.64	34.22	52.12	50.98	35.22	35.22	56.77 (a)	50.77 (a)
7	36.10	34.30	53.80	51.70	36.30	36.40	50.90	51.10
8	35.01	35.40	51.27	51.27	36.27	35.68	48.86	49.08
9	37.09	37.50	53.42	53.37	37.40	37.26	50.10	49.96
10	35.90	36.30	52.40	52.90	35.80	35.80	48.80	48.60
11	34.60	34.30	51.70	52.90	35.20	36.10	47.30 (a)	49.60 (a)
12	35.10	34.20	52.90	51.70	35.20	36.30	49.50	49.90
13	34.86	34.65	50.67	50.41	36.65	36.14	50.45	51.04
14	35.60	34.70	52.70	53.80	35.50	34.90	49.00	48.50
15	44.90 (b)	44.80 (b)	64.80 (b)	64.40 (b)	42.70 (b)	42.20 (b)	58.00 (b)	57.70 (b)
16	35.80	35.70	52.80	53.00	36.50	36.80	50.20	50.40
17	34.50	34.60	52.80	52.80	35.40	34.80	50.20	49.90
18	34.00	34.40	53.60	53.00	35.70	36.50	48.50	47.30
19	34.80	34.20	51.60	52.00	39.00	38.50	50.20	50.40
Mean	**35.07**		**52.43**		**36.17**		**49.84**	
r	**1.37**		**1.64**		**1.11**		**0.94**	
S_r	**0.491**		**0.585**		**0.397**		**0.335**	
RSD_r %	**1.40**		**1.12**		**1.10**		**0.67**	
R	**2.37**		**2.41**		**2.59**		**2.52**	
S_R	**0.845**		**0.862**		**0.925**		**0.901**	
RSD_R %	**2.41**		**1.64**		**2.56**		**2.52**	

For Key See Table VI

TABLE V

Method of	Sample	Mean	r	S_r	RSD_r	R	S_R	RSD_R	n
Lane & Eynon	A	36.43	0.84	0.30	0.82	1.79	0.64	1.76	18
	B	54.12	1.03	0.37	0.68	2.50	0.89	1.65	18
	C	37.15	0.71	0.25	0.68	4.38	1.56	4.21	18
	D	49.97	0.60	0.22	0.43	1.87	0.67	1.33	17
Luff-Schoorl (LS)	A	35.24	1.25	0.45	1.27	2.81	1.00	2.85	18
	B	51.98	0.74	0.26	0.51	2.84	1.01	1.95	18
	C	36.13	0.98	0.35	0.97	3.49	1.25	3.45	17
	D	49.07	0.82	0.29	0.59	4.32	1.54	3.14	18
Luff-Schoorl	A	35.07	1.37	0.49	1.40	2.37	0.85	2.41	18
	B	52.43	1.64	0.59	1.12	2.41	0.86	1.64	18
	C	36.17	1.11	0.40	1.10	2.59	0.93	2.56	18
	D	49.84	0.94	0.34	0.67	2.52	0.90	1.81	16

For key, see Table VI

TABLE VI

Key to results tables I -V

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 % probability.
R	Repeatability (between-lab variation). The value below which the absolute difference between two single test results obtained with the same method on identical test material inder different conditions may be expected to lie with 95 % probability.
S_r	The relative standard deviation of the repeatability.
S_R	The standard deviation of the reproducibility
RSD_r	The relative standard deviation of the repeatability $S_r \times 100/x$
RSD_R	The relative standard deviation of the reproducibility $S_R \times 100/x$
(a)	Cochrans outlier ($p<0.01$) not used in calculation of statitical parameters.
(b)	Grubbs outlier ($p<0.01$) not used in calculation of statistical parameters.
*	Different dry matter method used, results not used in calculation of statistical parameters.

Conclusions

From statistical analyses of the results from this trial, it has been shown that modification of the Luff-Schoorl procedure to incorporate reagent standardisation steps has improved the precision of the method so that it exhibits better precision than both the original Luff-Schoorl procedure and the Lane and Eynon Constant Volume method. Both the Luff-Schoorl and modified Luff-Schoorl methods gave similar values which are lower than the value obtained with the Lane & Eynon method when applied to the same sample. As these determinations are all empirical in nature, it is important that the appropriate method is selected for the estimation of the standard, not withstanding its inherent precision. Nevertheless, in view of the increasing international recognition of the Luff-Schoorl method it is important that only the Luff-Schoorl method with the incorporated reagent standardisation step is prescribed.

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APPENDIX I

Measurement of Reducing Sugars Expressed as Invert Sugar (Lane and Eynon Constant Volume modification)

As described in method 7 of EC Directive 79/796/EEC, in which the reducing sugars content of the sample is determined by titrating a sample solution, at its boiling point, against a specified volume of Fehlings solution, using methylene blue as indicator.

APPENDIX II

Determination of Reducing Sugars Expressed as Invert Sugar (Luff-Schoorl)

As described in method 6 of EC Directive 79/796/EEC, in which the reducing sugars in the sample are heated to boiling point under standardised conditions with a copper II solution, which is partially reduced to copper I. The excess copper II is subsequently determined iodometrically.

APPENDIX III

Determination of Reducing Sugars Expressed as Invert Sugar (modified Luff-Schoorl)

As described in Appendix II above except that the Luff-Schoorl reagent is standardised as follows:

Take 25.0 ml of Luff-Schoorl reagent, transfer to a conical flask and add 25.0 ml of the standard reducing sugar solution (1.25 mg/ml), transfer to a conical flask and add 25.0 ml of the standard sugar solution. Add anti-bumping granules and 1 ml of 3 methylbutan-1-ol. Fit the flask to the reflux condenser and place on to a wire gauze under which a flame has been lit. Adjust the flame so as to bring the contents of the flask to boil in about 2 min., reduce the flame so that the solution boils gently and reflux for exactly 10 min. Cool immediately in cold water and after 5 min. titrate as follows:

Add 10 ml of potassium iodide solution and, with constant swirling of the flask, immediately add 25 ml of sulphuric acid solution carefully in small increments to prevent excessive foaming. Titrate with 0.1 N sodium thiosulphate solution until a dull yellow colour appears; add starch indicator and complete the titration.

Carry out a blank titration (without boiling) on a mixture of 25 ml of Luff-Schoorl reagent and 25 ml of water after adding 10 ml of potassium iodide and 25 ml of sulphuric acid solution.

APPENDIX IV
Determination of Dry Matter

As determined by Method 2 of EC Directive 79/796/EEC in which the dry matter is determined at a temperature of 70 ± 1°C using a vacuum oven at a pressure not exceeding 3.3 KPa (34 mbar). The test portions are prepared by mixing with water and Kieselguhr before drying.

References:

1 Council Directive 79/796/EEC, O J No L239, **22**, 22 September 1979.

2 The Specified Sugar Products (Amendment) Regulations (1982 SI : No 255, 1982, HMSO, London)

3 International Commission on Uniform Methods of Sugar Analysis

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6 Horwitz W., Anal.Chem. 1982, **54**, 67A-76A

J. Assoc. Publ. Analysts, 1992, 28, 63-67

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No.V5

MOISTURE IN COCOA AND CHOCOLATE PRODUCTS

Correspondance 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 moisture content of cocoa mass and powder.

2. Definition

Moisture content: the content of total water as determined by the method specified.

3. Principle

The sample is mixed with sand and dried to constant weight at 103 ± 2°C.

4. Reagents

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

4.1 Sea Sand The size of grains must not exceed 0.5 mm diameter. Before use, clean the sand by preparing a slurry of the sand with 10% hydrochloric acid and then washing well with water until a neutral washing is obtained. Dry and ignite the sand at 575 ± 25°C for 30 min. After ignition allow to cool in a desiccator and, when cool, store in a tightly sealed bottle. Commercially prepared acid- washed sand is also suitable.

5. Apparatus

5.1 Metal weighing dishes, resistant to attack by the sample and the conditions of the test, of minimum diameter 50 mm and minimum height 20 mm, with closely fitting lids.

5.2 Glass rods, of such a length that they lie wholly in the dishes.

5.3 Drying oven, temperature-controlled by thermostat at 103 ± 2°C throughout the volume of the oven.

5.4 Desiccator, containing freshly activated silica gel (with a water content indicator) or an equivalent desiccant.

5.5 Analytical balance

6. Procedure

6.1 Preparation of the dish

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Introduce about 20 g of the treated sand into a weighing dish together with a small glass rod. Place the rod, dish and the upturned lid in the drying oven and dry for 4 hr. at 103 ± 2°C.

Remove the dish and the upturned lid from the oven, immediately place the lid on the dish and the glass rod on top of the lid and then put into the desiccator. Allow the dish to cool to ambient temperature (normally about 1 hr. cooling). Weigh the dish, lid and rod to the nearest 0.2 mg (m_0).

6.2 Test portion

Weigh into the dish, to the nearest 0.2 mg, about 5 g of grated, powdered or otherwise finely divided sample (m_1 = weight of sample, dish, lid and rod). Mix the sample with the sand using the glass rod to achieve as homogeneous a mixture as possible.

Place the dish, contents (including rod) and lid (placed beside the corresponding dish) in the drying oven and dry for 4 hr. at 103 ± 2°C. Remove the dish and lid from the oven, immediately place the lid on the dish and put the covered dish in the desiccator. Allow to cool to ambient temperature and weigh to 0.2 mg. Repeat the drying process, but leave for only 30 min. Repeat until the difference between two successive weighings is no greater than 5 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

The moisture content, calculated as a percentage by mass of the prepared sample, is given by:

$$\% \text{ moisture content} = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

where:

m_0 is the weight (mg) at ambient temperature of the dish, sand, lid and rod after drying in the oven.

m_1 is the weight (mg) of the sample, dish, sand, lid and rod before drying.

m_2 is the weight (mg) of the sample, dish, sand, lid and rod after drying in the oven.

9. References

9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81-101.

9.2 "Determination of Moisture", International Office of Cocoa and Chocolate/International Sugar Confectionery Manufacturers' Association (IOCC/ISCMA), Analytical Method 3/1952 (previously OICC Analytical Method 3/1952). London.

9.3 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 V0 of the series[3].

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. For both types of sample, r may be taken to be 0.4% moisture. This precision corresponds to an overall relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 4% for the cocoa powder, and about 8% for the drier cocoa mass.

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. R may be taken as 0.7% moisture in the cocoa mass samples. In cocoa powder samples its observed value is about twice this, which is unexpectedly high.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method, but its accuracy was not tested by spiking with known amounts of water. However, there is no reason to suspect any systematic bias.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.4% moisture for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six samples of cocoa powder once (three samples in blind duplicate). All these samples had

been alkali-treated and their cocoa butter content ranged from 11% to 21% by mass.

Participants also each analysed six samples of cocoa mass once (three samples in blind duplicate). Each pair of samples had been tempered and moulded into approximately 50 g size bars; only one pair (O/R) had been alkali-treated. The fat content was 56 - 57% by mass.

Tables 1 and 2 summarise the statistical data; the moisture levels were expressed as a percentage by mass of the sample.

A6 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

TABLE 1

Statistical Analysis of the % Moisture in Cocoa Powder Samples

Sample	G/M	H/L	J/K
Number of Laboratories retained after eliminating outliers	17	18	17
Number of Laboratories eliminated as outliers	1	0	1
Number of Results accepted after eliminating Outliers	34	36	34
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	3.12	3.37	3.91
REPEATABILITY			
Standard Deviation S_r	0.14	0.075	0.11
Relative Standard Deviation RSD_r (%)	4.5	2.2	2.8
Repeatability r [$2.8 \times S_r$]	0.39	0.21	0.30
REPRODUCIBILITY			
Standard Deviation S_R	0.49	0.42	0.52
Relative Standard Deviation RSD_R (%)	15.7	12.5	13.3
Reproducibility R [$2.8 \times S_R$]	1.36	1.18	1.45

TABLE 2
Statistical Analysis of the % Moisture in Cocoa Mass Samples

Sample	N/S	O/R	P/Q
Number of Laboratories retained after eliminating outliers	15	15	15
Number of Laboratories eliminated as outliers	3	3	3
Number of results accepted after eliminating Outliers	30	30	30
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	1.62	1.95	1.72
REPEATABILITY			
Standard Deviation S_r	0.143	0.075	0.09
Relative Standard Deviation RSD_r(%)	8.8	3.8	5
Repeatability r [$2.8 \times S_r$]	0.40	0.21	0.24
REPRODUCIBILITY			
Standard Deviation S_R	0.164	0.207	0.246
Relative Standard Deviation RSD_R(%)	10	11	14
Reproducibility R [$2.8 \times S_R$]	0.46	0.58	0.69

J.Assoc. Publ. Analysts, 1992, 28, 69-72

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No.V6

ASH IN COCOA AND CHOCOLATE PRODUCTS

Correspondance 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 ash content of cocoa mass; this is taken as a measure of total mineral content.

2. Definition

Ash content: the content of total ash as determined by the method specified.

3. Principle

The sample is ignited under controlled conditions to an inorganic carbon free residue: this residue is calculated as the total ash content.

4. Reagents

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

4.1 Ethanol, 95% by volume.

4.2 Hydrochloric acid, dilute, approximately 7 g HCl/100 ml. Carefully add, with stirring to 500 ml water, 100 ml concentrated hydrochloric acid (HCl: $\rho_{20} = 1.18$ g/ml), and mix.

5. Apparatus

5.1 Incineration dishes, made of platinum or silica (ie materials not affected by the test conditions), and of 25-50 ml capacity.

5.2 Desiccator, containing freshly activated silica gel (with a water content indicator) or an equivalent desiccant.

5.3 Electric muffle furnace, air-ventilated, temperature controlled by thermostat at 600 ± 25°C, fitted with a pyrometer.

5.4 Infra-red lamp

5.5 Water bath, boiling.

5.6 Analytical balance

6. Procedure

6.1 Preparation of the incineration dish

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Clean the incineration dish, whether new or used, with boiling dilute hydrochloric acid. Rinse it free from acid with a large quantity of water. Heat it for 30 min. in the muffle furnace. Remove it from the furnace, allow it to cool to ambient temperature in the desiccator and weigh it to the nearest 0.1 mg (m_1). Not more than four incineration dishes shall be put in the desiccator at one time.

6.2 Test portion

Weigh into the prepared incineration dish to the nearest 0.1 mg, 2-5 g of the sample (m_0) such that the minimum expected total ash content is between 10 and 50 mg. Carbonise by either of the following procedures.

(i) Place the dish and sample in the cold muffle furnace and slowly bring to temperature (600 ± 25°C) in a fume hood with the exhaust vent of the muffle furnace open or the door incompletely closed.

(ii) Heat the dish and sample under an infra-red lamp until smoking ceases and then transfer to a heated muffle furnace.

Heat for 2 hr. at 600°C. Remove dish and place in desiccator to cool. When cool, remove from desiccator, moisten the ash with ethanol and dry under the infra-red lamp or on the water bath. Replace the dish in the muffle furnace and heat for a further hour. Remove dish and allow to cool to ambient temperature in the desiccator. When cool, weigh to the nearest 0.1 mg. Repeat the final step until the difference in two successive weighings is no greater than 1 mg. Let the final weighing 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 total ash content, calculated as a percentage by mass of the sample, is given by:

$$\% \text{ total ash content} = \frac{m_2 - m_0}{m_1} \times 100$$

Where:

m_0 is the initial weight of sample to the nearest 0.1 mg;

m_1 is the weight of the incineration dish;

m_2 is the final weight of the incineration dish and carbonised sample.

The result is expressed as this percentage to two places of decimals.

9. References

9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81-101

9.2 "Determination of ash in cocoa and chocolate products", International Office of Cocoa and Chocolate/International Sugar Confectionery Manufacturers' Association (IOCC/ISCMA), Analytical Methods 4a/1973 (previously OICC Analytical Method 4a/1973), London

9.3 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 V0 of the series[3].

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 in Table 1. At about 3% ash, r may be taken to be 0.3% ash. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 3.8%.

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 Table 1. R may be taken as 0.5% ash; this precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, among different laboratories of about 6%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method, but its accuracy was not tested by spiking with known amounts of mineral, However, there is no reason to suspect any systematic bias.

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 0.3% ash for a single determination.

A5. Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six samples of cocoa mass once (three samples in blind duplicate). Each pair of samples had

been tempered and moulded into approximately 50 g size bars; only one pair (O/R) had been alkali-treated. The fat content was 56 - 57% by mass.

Table 1 summarises the statistical data; the ash levels were expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of the % Total Ash Cocoa Mass Samples

Sample	N/S	O/R	P/Q
Number of Laboratories retained after eliminating Outliers	17	16	17
Number of Laboratories eliminated as outliers	1	2	1
Number of results accepted after eliminating outliers	34	32	34
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	2.81	3.34	2.83
REPEATABILITY			
Standard Deviation s_r	0.09	0.08	0.11
Relative Standard Deviation RSD_r (%)	3.2	2.4	3.8
Repeatability r [$2.8 \times S_r$]	0.25	0.22	0.30
REPRODUCIBILITY			
Standard Deviation S_R	0.13	0.18	0.16
Relative Standard Deviation RSD_R (%)	4.7	5.2	5.8
Reproducibility R [$2.8 \times S_R$]	0.37	0.49	0.46

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

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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V7

UNSAPONIFIABLE MATTER IN COCOA AND CHOCOLATE PRODUCTS

Correspondance 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 unsaponifiable matter of cocoa butter.

2. Definition

Content of unsaponifiable matter: the content of unsaponifiable matter as determined by the method specified.

3. Principle

The sample is saponified and then extracted with petroleum ether. The extract is dried and then weighed.

4. Reagents

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

4.1 Ethanol, 95% (*V/V*).

4.2 Potassium hydroxide solution: dissolve 60 g of potassium hydroxide in 40 ml water.

4.3 Ethanol, dilute: 50% (*V/V*).

4.4 Petroleum ether: petroleum ether, boiling range 40-60°C, redistilled.

4.5 Phenolphthalein solution, 1% (*m/V*) in ethanol (**4.1**).

4.6 Acetone

5. Apparatus

5.1 Oven, electrically heated, thermostatically controlled at 52.5±2.5°C.

5.2 Water bath, boiling.

5.3 Separators, 250 ml, each fitted with a teflon stopcock and stopper.

5.4 Desiccator, containing freshly activated silica gel (with water content indicator), or an equivalent desiccant.

5.5 Flasks, 250 ml with ground glass joints.

5.6 Flasks, 50 ml.

5.7 Reflux condenser, with ground glass joints.

5.8 Oven, electrically heated, themostatically controlled at 102.5±2.5°C.

5.9 Analytical balance

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All ground glass joints should be free from lubricants; they are to be cleansed with petroleum ether before starting the analysis. Do not handle the flasks by hand but use tongs, etc.

6. Procedure

6.1 Fat saponification

Melt the fat sample in the oven at 52.5±2.5°C and then filter. Accurately weigh to the nearest 0.1 mg about 5 g of the filtered fat into a 250 ml ground glass stoppered flask. Add 50 ml of ethanol and 10 ml of potassium hydroxide solution. Attach a reflux condenser to the flask, place on a boiling water bath and heat for 1 hr. Stop the heating. Add 50 ml water through the top of the condenser, shake and cool to room temperature. Check that the solution does not contain unsaponified fat droplets.

6.2 Extraction

Transfer the solution obtained to a 250 ml separator. Rinse the condenser and flask with five 10 ml portions of petroleum ether and add to separator. Shake vigorously for 1 min., periodically releasing the pressure by inverting the separator and opening the stopcock. Allow to stand until phase separation is nearly complete (usually about 10 min.). Drain the soap solution as completely as possible into a second 250 ml separator ensuring that no petroleum ether is transferred. If an emulsion forms, break it by the addition of a few millilitres of ethanol.

Repeat the extraction of the soap solution twice with two 50 ml portions of petroleum ether; combine all the petroleum ether extracts and wash three times with 50 ml portions of dilute ethanol (50% *V/V*).

Drain each wash solution to about 2 ml, ensuring that no petroleum ether is transferred. Then gently rotate the separator and let the layers separate for 5 - 10 min. Drain the remaining wash solution, closing the stopcock when the petroleum ether starts to pass the bore of the stopcock. Check the last wash with phenolphthalein: if alkali is present, wash again and recheck.

6.3 Weighing of unsaponifiable matter

Transfer the petroleum ether solution into a 250 ml flask through the top of the separator. Rinse the separator and its pouring edge twice with 10 ml of petroleum ether and add the rinsings to the main solution. Evaporate to approximately 5 ml on the water bath. Transfer quantitatively, rinsing three times with 3- 5 ml portions of petroleum ether, to a 50 ml flask which has been previously dried by heating for not less than 1 hr. in the oven at 102.5±2.5°C, cooled in a desiccator for not less than 1 hr. and then weighed to 0.1 mg.

Place the flask with its contents on the water bath and evaporate to dryness. Add 2 - 3 ml acetone and remove all solvent on the boiling water bath with a gentle stream of air or nitrogen passing through the flask. Place the flask horizontally in the oven at 102.5±2.5°C and dry for 30 min. Remove and cool in a desiccator for not less than 1hr.; then weigh.

Repeat the drying, cooling and weighing process until the change in weight is not more than 1.5 mg. Discard the sample if the weight after the third weighing varies by more than 1.5 mg from the second.

6.4 Blank

Repeat the whole procedure but omitting the fat sample to obtain the blank weight

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 percentage of unsaponifiable matter content is given by:

$$\% \text{ unsaponifiable matter content} = 100 \times (m_1 - m_2) / m_0$$

Where:

m_1 is the mass in grams of the residue;
m_2 is the mass in grams of the blank;
m_0 is the mass in grams of the sample.

9. References

9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81- 101.

9.2 "Determination of unsaponifiable matter in cocoa butter", International Office of Cocoa and Chocolate/International Sugar Confectionery Manufacturers' Association (IOCC/ISCMA) Analytical Method 102B, 1977. London.

9.3 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 V0 of the series[3].

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). For unsaponifiable contents within the range 0.27% - 0.55%, r may be taken as 0.10 - 0.15%. This precision corresponds to a relative standard

deviation of repeatability (coefficient of variance of repeatability), RSD_r, of 10 - 14%.

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 unsaponifiable contents within the range 0.27 - 0.55%, R may be taken as 0.09 - 0.21%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 12 - 14%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method, but its accuracy was not tested by spiking with known amounts of authentic unsaponifiable matter. However, there is no reason to suspect systematic bias.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.1% unsaponifiable matter for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six samples of cocoa butter once (three samples in blind duplicate). One pair of samples (V/X) had been pressed from roasted nib; another (T/Z) was V/X spiked with 1% m/m stearic acid; the third (W/Y) pair was solvent-extracted cocoa butter, expected to contain higher amounts of unsaponifiable matter.

Table 1 summarises the statistical data; the unsaponifiable matter content was expressed as a percentage by mass of the sample.

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

TABLE 1
Statistical Analysis of the % of Unsaponifiable Matter in Cocoa Butter Samples

Sample	T/Z	V/X	W/Y
Number of Laboratories retained after eliminating outliers	16	16	18
Number of Laboratories eliminated as outliers	2	2	0
Number of results accepted after eliminating outliers	32	32	36
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	0.28	0.27	0.55
REPEATABILITY			
Standard Deviation S_r	0.39	0.32	0.57
Relative Standard Deviation RSD_r (%)	14	12	10
Repeatability r $[2.8 \times S_r]$	0.11	0.09	0.16
REPRODUCIBILITY			
Standard Deviation S_R	0.039	0.032	0.075
Relative Standard Deviation RSD_R (%)	14	12	14
Reproducibility R $[2.8 \times S_R]$	0.11	0.09	0.21

J. Assoc. Publ. Analysts, 28, 79-82

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V8

ACIDITY IN COCOA AND CHOCOLATE PRODUCTS

Correspondance 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 acidity of cocoa butter.

2. Definition

Acidity: the content of acid (calculated as oleic acid) as determined by the method specified.

3. Principle

The fat sample is dissolved in a neutral mixture of ethanol and diethyl ether; the free fatty acids then in solution are titrated against standard ethanolic potassium hydroxide solution and calculated as oleic acid.

4. Reagents

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

4.1 Standard potassium hydroxide solutions

Potassium hydroxide solutions, 0.1 mol/l and 0.5 mol/l in 95% *(V/V)* ethanol, are stored in brown or yellow glass bottles furnished with rubber stoppers and then decanted for use. Each solution should be colourless or straw yellow. Stable colourless solutions can be prepared by either of the following procedures.

4.1.1 Solution in distilled solvent

Reflux 1 litre of ethanol with 8 g of potassium hydroxide and 5 g of aluminium pellets for one hr., then distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow the whole to stand for several days and decant off the clear and colourless supernatant liquid from the deposited potassium carbonate.

4.1.2 Solution in undistilled solvent

Add 4 ml of aluminium to 1 litre of ethanol and allow the mixture to stand for several days. Decant off the supernatant liquid and dissolve therein the necessary amount of potassium hydroxide. After allowing to stand 1 or 2 days, it may be used as such.

The required amount of potassium hydroxide is 5.61 g for a 0.1 mol/l solution and 28.05 g for a 0.5 mol/l solution.

4.2 Phenolphthalein, 1% *(m/V)* in 95% *(V/V)* ethanol.

4.3 Diethyl ether/ethanol mixed solvent, equal volumes of diethyl ether and 95% *(V/V)* ethanol containing 0.3 ml of phenolphthalein solution (**4.2**) in 100 ml. Neutralise immediately before use by means of 0.1 mol/l ethanolic KOH solution (**4.1**).

5. Apparatus

5.1 Volumetric equipment

5.2 Analytical balance

6. Procedure

Weigh (to the nearest 0.01 g) 5 - 10 g fat into a 250 ml conical flask, and add 50 - 150 ml of the mixed solvent (**4.3**) to dissolve the fat. Titrate, while stirring, the fat solution against the ethanolic potassium hydroxide solution 0.5 mol/l (or 0.1 mol/l for acidities less than 2%) using phenolphthalein as indicator. The end point occurs when a pink colour remains for at least ten seconds.

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 acidity, expressed as a percentage of oleic acid, is given by:

$$\% \text{ acid content } = \frac{a \times M \times 282}{10 \times m_o}$$

Where:

m_o is the mass in grams of the fat sample taken;

a is the number of millilitres of ethanolic potassium hydroxide needed for neutralisation;

M is the strength, in mol/l, of ethanolic potassium hydroxide used;

the number 282 is the molecular weight of oleic acid.

9. References

9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81- 101.

9.2 "Determination of the acid value (A.V.) and acidity", International Union of Pure and Applied Chemistry, Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th Edition, method 2.201.

9.3 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[3].

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). At acidities corresponding to 1- 2.5% oleic acid, r may be taken as 0.08%. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of less than 3%.

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 acidities within the range 1- 2.5%, R may be taken to be 0.3 - 0.8%, corresponding to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 11%.

A3 Trueness (Bias)

The collaborative trial included the analysis of a sample of cocoa butter before and after spiking with 1% of stearic acid. An overall mean difference of 0.65% was found, suggesting a recovery of 65%, but no correction should be made.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.1% acid for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six samples of cocoa butter once (three samples in blind duplicate). One pair of samples (V/X) had been pressed from roasted nib; another (T/Z) was V/X spiked with 1% m/m stearic acid; the third pair (W/Y) was solvent-extracted cocoa butter.

Table 1 summarises the statistical data; the acidity was calculated as oleic acid and expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of Acidity expressed as the % of Oleic Acid in Cocoa Butter Sample

Sample	T/Z	V/X	W/Y
Number of Laboratories retained after eliminating outliers	13	18	16
Number of Laboratories eliminated as outliers	5	0	2
Number of results accepted after eliminating outliers	26	36	32
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	1.68	1.03	2.48
REPEATABILITY			
Standard Deviation S_r	0.018	0.029	0.018
Relative Standard Deviation RSD_r (%)	1.1	2.8	0.7
Repeatability r $[2.8 \times S_r]$	0.05	0.08	0.05
REPRODUCIBILITY			
Standard Deviation S_R	0.046	0.11	0.28
Relative Standard Deviation RSD_R (%)	2.7	10.7	11.3
Reproducibility R $[2.8 \times S_R]$	0.13	0.32	0.78

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, 83-88

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No V9

TOTAL FAT IN COCOA AND CHOCOLATE PRODUCTS

Correspondance on this method may be set 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 milk chocolate, cocoa mass and cocoa powder.

2. Definition

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

3. Principle

The product is hydrolysed with diluted hydrochloric acid and filtered. The dried mass containing the fat is extracted with petroleum ether, the solvent evaporated and the residue weighed.

4. Reagents

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

4.1 Hydrochloric acid, 25% (*m/m*) (ρ_{20}= 1.12). Mix two volumes of concentrated hydrochloric acid (ρ_{20}= 1.118) with one volume of water.

4.2 Petroleum ether, boiling range within the range 30-60°C. Dry and redistil if necessary.

4.3 Silver nitrate solution, 0.1 mol/l.

4.4 Anti-bumping granules, e.g. powdered pumice which has been previously defatted.

5. Apparatus

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

5.2 Condenser

5.3 Filter paper, fluted and of pore size to allow the filtration of the digest at a reasonable speed.

5.4 Glass wool

5.5 Extraction thimble, double thickness, fat-free, to fit the Soxhlet extractor.

5.6 Analytical balance

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5.7 Water-bath, electrically heated.

5.8 Oven, at 100-101°C.

6. Procedure

6.1 Digestion of the sample

Grind material such as cocoa nibs, cocoa shell, press cake, expeller cake, etc, to a maximum of 150 μm (check using a wire mesh), care being taken to avoid liberation of fat during the grinding operation. Weigh the ground sample to the nearest 1 mg into a 300-500 ml beaker: take sufficient sample to yield about 1 g of fat on extraction. Add, while stirring continuously, 45 ml of boiling water followed by 55 ml hydrochloric acid. Add anti-bumping agent. Cover the beaker with a watch glass and boil gently for exactly 15 min., keeping the volume approximately constant by adding water if necessary.

Rinse the watch glass into the beaker with 100 ml of water. Filter the digest through a wet fat-free fluted filter paper, using a Celite filter aid if necessary.

Rinse the beaker three times with hot water, passing the washings through the filter; continue to wash until the filtrate ceases to react on addition of silver nitrate solution. Transfer the wet filter paper and residue to a fat-free extraction thimble. Place a glass wool plug over the filter paper and dry for 6-18 hr. in a small beaker in an oven at 100-101°C. Also dry the first beaker and the watch glass.

6.2 Extraction of fat

Dry the flat-bottomed 250 ml flask containing a few anti-bumping granules in an oven at 100-101°C for 1 hr. Cool, weigh to the nearest 0.1 mg and connect to the Soxhlet extractor. Support the thimble on a spiral or on glass balls to ensure efficient working of the siphon. Wash both beakers and the watch glass with about 150 ml petrol, and pour the solvent gradually into the thimble. Extract under reflux until extraction is complete: this may be verified by replacing the weighed flask with a second dry weighed flask and repeating the extraction of fat operation, extracting for about 2 hr. There should be less than 1 mg of fat recovered in the second flask.

Evaporate the solvent over a boiling water-bath and dry the flask containing the fat in an oven at 100-101°C. Weigh and repeat the drying operation until the variation in weight of two consecutive weighings is less than 1 mg.

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 prepared sample is given by:

$$\% \text{ total fat} = 100 \times (m_2 - m_1)/m_0$$

where

m_0 is the mass in g of the sample;

m_1 is the mass in g of the dried flask;

m_2 is the mass in g of the flask and the extracted fat.

9. References

9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81- 101.

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 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 (Tables 1-3). For total fat contents within the range 10%-60%, r may be taken as 0.4-0.9%, though the observed value of r was somewhat greater (1.5) with one of the cocoa mass samples. The relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, was less than 1% when analysing chocolate or cocoa mass samples, but 1-3% with cocoa powder.

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 (Tables 1-3). For total fat contents within the range 10-60%, R may be taken as 0.8-1.9%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of about 1% (2-5% with cocoa powder).

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method. Comparison in Tables 1-3 between the observed mean and the approximate recipe values of total fat content suggests satisfactory accuracy. While observed values tended to be low, they rarely differed by more than 2% fat from the expected value, which was itself subject to uncertainty.

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 1% fat for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six subsamples of milk chocolate once (three different samples in blind duplicate). They similarly analysed three different samples of cocoa mass and three of cocoa powder, each in blind duplicate. The approximate nominal total fat content was known from the recipe of each sample.

Tables 1, 2 and 3 summarise the statistical data for the milk chocolate, cocoa mass and cocoa powder respectively; the total fat content is expressed as a percentage by mass of the sample.

TABLE 1
Statistical Analysis of the % Fat in Milk Chocolate Samples

Sample	A/D	B/E	C/F
Number of Laboratories retained after eliminating outliers	16	17	16
Number of Laboratories eliminated as outliers	2	1	2
Number of results accepted after eliminating outliers	32	34	32
LEVEL OF ANALYTE			
Nominal Recipe Value	27.5	30.0	37.5
Mean observed value $\bar{x}$	29.29	29.43	35.64
REPEATABILITY			
Standard Deviation S_r	0.17	0.15	0.15
Relative Standard Deviation RSD_r (%)	0.58	0.51	0.42
Repeatability r [2.8 × S_r]	0.47	0.43	0.42
REPRODUCIBILITY			
Standard Deviation S_R	0.31	0.29	0.36
Relative Standard Deviation RSD_R (%)	1.06	0.99	1.01
Repeatability R [2.8 × S_R]	0.87	0.82	1.02

TABLE 2

Statisitical Analysis of the % Fat in Cocoa Mass Samples

Sample	N/S	O/R	P/Q
Number of Laboratories retained after eliminating outliers	16	15	17
Number of Laboratories eliminated as outliers	2	3	1
Number of results accepted after eliminating outliers	32	30	34
LEVEL OF ANALYTE			
Nominal Recipe Value	56	56	57
Mean observed value $\bar{x}$	55.17	54.25	54.88
REPEATABILITY			
Standard Deviation S_r	0.32	0.26	0.52
Relative Standard Deviation RSD_r (%)	0.58	0.48	0.95
Repeatability r [2.8 × S_r]	0.91	0.73	1.47
REPRODUCIBILITY			
Standard Deviation S_R	0.59	0.39	0.67
Relative Standard Deviation RSD_R (%)	1.07	0.72	1.22
Repeatability R [2.8 × S_R]	1.66	1.10	1.88

TABLE 3

Statistical Analysis of the % Fat in Cocoa Powder Samples

Sample	G/M	H/L	J/K
Number of Laboratories retained after eliminating outliers	15	15	18
Number of Laboratories eliminated as outliers	3	3	0
Number of results accepted after eliminating outliers	30	30	36
LEVEL OF ANALYTE			
Nominal Recipe Value	16	21	11
Mean observed value $\bar{x}$	14.91	19.11	10.37
REPEATABILITY			
Standard Deviation S_r	0.20	0.22	0.32
Relative Standard Deviation RSD_r (%)	1.43	1.15	3.09
Repeatability r [2.8 × S_r]	0.55	0.61	0.90
REPRODUCIBILITY			
Standard Deviation S_R	0.42	0.40	0.55
Relative Standard Deviation RSD_R (%)	2.82	2.09	5.30
Repeatability R [2.8 × S_R]	1.18	1.12	1.53

A6 Key to Tables 1, 2 and 3

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, 89-94

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V10

MILK FAT IN COCOA AND CHOCOLATE PRODUCTS

Correspondance on this method may be set 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 milk fat content of milk chocolate.

2. Definition

Milk fat: the content of milk fat as determined by the method specified.

3. Principle

The method relies on the fact that milk fat is unique amongst the commonly occurring fats in that it contains *n*-butyric acid.

The fat, extracted from the milk chocolate sample, is saponified. The water-soluble fatty acids are separated by isothermal gas-liquid chromatography in the presence of an internal standard. The *n*-butyric acid is thus determined and used as an index of the milk fat content.

4. Reagents

During the analysis, unless otherwise stated, only use reagents of a recognised analytical grade and distilled water or water of equivalent purity.

4.1 Anti-bumping granules, defatted powdered pumice.

4.2 Ethanolic potassium hydroxide, 0.5 mol/l.

Dissolve potassium hydroxide (**4.2.1**) in ethanol (**4.2.2**).

4.2.1 Potassium hydroxide, pellets.

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

4.3 *ortho*-Phosphoric acid solution, 5% (*m/V*).

Dissolve *ortho*-Phosphoric acid (**4.3.1**) in water.

4.3.1 *ortho*-Phosphoric acid, 85% (*m/m*)

4.4 *n*-Valeric acid, stock solution

Weigh accurately between 240 and 260 mg of *n*-valeric acid (**4.4.1**) and dilute to 100 ml with water.

4.4.1 *n*-Valeric acid, chromatographically pure.

4.5 *n*-Butyric acid, stock solution

Weigh accurately between 380 and 420 mg of *n*-butyric acid (**4.5.1**) and dilute to 100 ml with water.

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4.5.1 *n*-Butyric acid, chromatographically pure and preferably assayed by titration.

4.6 Internal standard solution

Make an accurate 10 fold dilution of an aliquot of the *n*-valeric acid stock solution (**4.4**), to give an aqueous standard solution containing approximately 0.25 mg/ml. 200 ml of this solution should be adequate.

4.7 *n*-Butyric acid standard solution

Make an accurate 10 fold dilution of an aliquot of the *n*-butyric acid stock solution (**4.5**), to give an aqueous standard solution containing approximately 0.4 mg/ml. 100 ml of this solution should be adequate.

4.8 Mixed standard solutions

Prepare a series of seven standard mixtures of *n*-valeric and *n*-butyric acids by mixing 2.0 ml of internal standard solution (**4.6**) with 5.0, 3.5, 2.0, 1.00, 0.50, 0.20 and 0.00 ml of *n*-butyric acid standard solution (**4.7**), and making each up to 7.0 ml by adding the calculated volume of water. Calculate the actual concentrations of *n*-valeric and *n*-butyric acids from the accurate weight of the acids taken in the preparation of the stock solutions (**4.4, 4.5**); the nominal weights per 7 ml of each mixed standard solution are 0.50 mg *n*-valeric acid and respectively 2.00, 1.40, 0.80, 0.40, 0.20, 0.08, and 0.00 mg *n*-butyric acid. These solutions should be stored in a refrigerator and will be stable for up to a month.

5. Apparatus

5.1 Water-bath, electrically heated or steam-bath.

5.2 Beakers, 50 ml, with watch glasses to cover.

5.3 Analytical balance, sensitive to 0.1 mg

5.4 Test-tubes, 10 ml, with ground-glass stoppers.

5.5 Graduated pipettes, 5 ml and 1 ml.

5.6 Volumetric pipettes, 5 ml and 2 ml.

5.7 Fluted filter papers, diameter 90 mm, slow.

5.8 Filter funnels, to accept filter paper (**5.10**).

5.9 Oven, at 100-101°C

5.10 Gas chromatograph, able to accept dual 1.5 m x 6 mm (o.d.) (5 ft × 1/4") glass columns with on-column injection, and fitted with flame ionisation detectors and a potentiometric recorder with chart width preferably not less than 200 mm.

5.10.1 Specified chromatographic conditions

1.5 m x 6 mm (o.d.) (5 ft × 1/4") glass column packed with 5% Carbowax 20M and 0.5% terephthalic acid on 100-120 mesh, acid-washed Supasorb; nitrogen flow rate, 50 ml/min; column temperature 125°C (isothermal); flame ionisation detectors: hydrogen flow rate, 50 ml/min; air flow rate, 500 ml/min.

5.10.2 Preparation of recommended column packing

Reflux 100 ml of ethanol with 4 g of Carbowax 20M and 0.4 g of terephthalic acid until dissolved. Add 20 g of Supersorb (eg BDH) and boil under reflux to remove air. Filter rapidly at the pump (approximately 25 ml of the solution is retained by the Supersorb) and dry the residue under vacuum. After packing the columns, purge with nitrogen at 220°C for 24 hr.

6. Procedure

6.1 Extraction of fat

Extract the fat using an appropriate procedure.

6.2 Saponification

Ensure that the fat is homogeneous by melting and mixing it before taking a sample aliquot. Weigh 100-110 mg of this fat sample accurately (W mg) into a 50 ml beaker. Add 3 ml of ethanolic potassium hydroxide (**4.2**), cover the beaker with a watch glass and immediately place on a boiling water-bath or steam-bath. Heat for at least 10 min, or until no fat globules are visible on the surface of the liquid. Remove the watch glass and continue heating until the ethanol has completely evaporated. Allow the beaker to cool and add 5.0 ml of water, cover with a watch glass and shake carefully to dissolve the soap completely. It may occasionally be necessary to warm the mixture gently to encourage the dissolution of the soaps.

6.3 Preparation of acids

Add 5.0 ml of *ortho*-phosphoric acid solution (**4.3**) to the beaker and swirl gently to coagulate the precipitated higher fatty acids. Filter through a fluted filter paper. Pipette 5.0 ml of filtrate and 2.0 ml of internal standard solution into a test tube and mix.

6.4 Injection of acids

Inject a nominal 1 µl of the final solution onto the GLC column, using an on-column injection technique. Measure the peak heights corresponding to *n*-butyric and *n*-valeric acids to the nearest 0.5 mm. The micro-syringe should be flushed occasionally with distilled water to prevent corrosion of the plunger by residual phosphoric acid.

6.5 Construction of the calibration curve

Inject a nominal 1 µl of each of the seven mixed standard solutions (**4.8**) and measure peak heights as in 6.4. Plot a calibration curve relating the weight of *n*-butyric acid (mg) in 7 ml of mixed standard solution (**4.8**) to the corresponding peak height ratio, PHR, defined in Section 8.

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

For each chromatogram, calculate the peak height ratio, PHR, defined by:

$$\text{PHR} = \frac{\text{Peak Height corresponding to } n\text{-butyric acid (mm)}}{\text{Peak Height corresponding to } n\text{-valeric acid (mm)}}$$

From the calibration curve (**6.5**), read off the weight of *n*-butyric acid, *M* mg, corresponding to the peak height ratio calculated from the sample chromatogram (**6.4**).

The original sample of fat (of weight *W* mg) therefore contains $2 \times M$ mg of *n*-butyric acid; the level of *n*-butyric acid in the extracted fat is:

$$\%\ n\text{-butyric acid content of fat} = 100 \times (2 \times M/W)$$

If a concentration of 3.60 mg of n-butyric acid in 100 mg of any milk fat is taken as an arbitrary average value when determined by this method, then the concentration of milk fat in the extracted fat is given by:

$$\%\ \text{milk fat content of fat} = 100 \times 100 \times 2 \times M/(W \times 3.60)$$

The milk fat content of the original sample of milk chocolate is then given by:

$$\%\ \text{milk fat content of chocolate} = A \times 100 \times 2 \times M/(W \times 3.60)$$

where:

A is the % total fat content of original sample of cocoa or chocolate product. (This value can be determined using Method No. V9 "Total fat in cocoa and chocolate products" of the MAFF Validated Methods for the Analysis of Foodstuffs series.)

9. References

9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Pub. Analysts, 1984, **22**, 81-101.

9.2 DO Biltcliffe and R Wood, J. Assoc. Pub. Analysts, 1982, 20, 69-88.

9.3 AR Phillips and BJ Sanders, J. Assoc. Pub. Analysts, 1968, 6, 89-95.

9.4 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 protocol V.0 of the series[4].

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). At levels of milk fat of 6-7%, r may be taken as 0.6%. At lower levels the repeatability deteriorates. The overall relative standard deviation of

repeatability (coefficient of variance of repeatability), RSD_r, for levels of milk fat of 6-7%, may be taken as less than 3%.

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). The observed reproducibility was not as satisfactory as expected; R may be taken as 2.0%. This precision corresponds to an overall relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of up to 13%.

A3 Trueness (Bias)

The collaborative trial established precision parameters for the method. Its accuracy depends crucially on the factor chosen to convert *n*-butyric acid into milk fat levels; considerable bias can be introduced by the use of an inappropriate conversion factor. The arbitrary choice of 3.60 arises from the milk fat used for a preliminary study (**8.2**), and does not necessarily represent the figure of choice for a statutory procedure. Nevertheless results based on it will be useful if treated with caution. A previous study of this method (**8.3**) reported the observed levels of *n*-butyric acid in 19 different samples of butter fat; the average level was 3.62% m/m, but the extremes were 3.38-4.00% (*m/m*). The use of this average value as a basis for milk fat estimation appears unlikely to introduce an error of much more than 10% of the observed value.

Comparison in Table 1 between the observed mean and the approximate recipe values of total fat content suggests satisfactory accuracy at 6-7% milk fat levels. Using an arbitrary factor of 3.60, recoveries with samples B/E and C/F were 92-94%. At lower levels, the observed recovery was unexpectedly high (145%), suggesting that this factor did not correspond to the milk ingredient of sample A/D.

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 2% milk fat for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six subsamples of milk chocolate once (three different samples in blind duplicate). The approximate nominal milk fat content was known from the recipe of each sample.

The stipulated conditions of gas-liquid chromatography (**5.12.1**) were recommended and specified by MAFF (**8.1**) to the participants in the collaborative trial. Other similar conditions may be suitable (**8.2, 8.3**).

Table 1 summarises the statistical data; the milk fat contents are expressed as a percentage by mass of the sample.

TABLE 1
Statistical Analysis of the % Milk Fat in Milk Chocolate Samples

Sample	A/D	B/E	C/F
Number of Laboratories retained after eliminating outliers	16	12	14
Number of Laboratories eliminated as outliers	2	6	4
LEVEL OF ANALYTE			
Nominal Recipe Value	3.7	7.5	6.7
Mean observed value $\bar{x}$	5.36	6.91	6.31
REPEATABILITY			
Standard Deviation S_r	0.56	0.20	0.14
Relative Standard Deviation RSD_r (%)	10.5	2.9	2.3
Repeatability r [2.8 × S_r]	1.57	0.56	0.40
REPRODUCIBILITY			
Standard Deviation S_R	0.69	0.40	0.66
Relative Standard Deviation RSD_R (%)	12.9	5.7	10.5
Reproducibility R [2.8 × S_R]	1.93	1.11	1.85

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, 95-98

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V11

LOSS OF MASS ON DRYING OF QUICK FROZEN FRENCH FRIED POTATOES

Correspondance 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 moisture content of quick frozen french fried potatoes, as estimated by the loss of mass on drying.

2. Definition

Loss of mass on drying: the loss of mass on drying under the conditions specified.

3. Principle

The sample is dried to constant weight at 103 ± 2°C.

4. Reagents

None.

5. Apparatus

5.1 Blender

5.2 Weighing dishes, resistant to attack by the sample and the conditions of the test, preferably made of nickel, aluminium, stainless steel or glass, and of diameter 60-80 mm and 25 mm deep, with well fitting but easily removable lids.

5.3 Convection drying oven, temperature-controlled by thermostat at 103 ± 2°C throughout the volume of the oven.

5.4 Desiccator, containing freshly activated silica gel (with a water content indicator) or an equivalent desiccant.

5.5 Analytical balance

6. Procedure

Thoroughly homogenise the frozen sample in a blender. Weigh about 10 g in duplicate, to the nearest 0.1 mg, into desiccated, tared weighing dishes. These procedures should be carried out in such a way as to avoid condensation of water vapour from the atmosphere onto the sample.

Place the uncovered dishes containing the samples, with their lids, in the convection drying oven for 16 hr. Replace the corresponding lids and transfer to the desiccator to cool. When cool, weigh as quickly as possible to 0.1 mg. Uncover and place the dishes with their lids in the

oven for a further 2 hr. Replace the lids, cool in the desiccator, and re-weigh. Repeat the 2 hr. drying, desiccation and weighing steps until the decrease in mass between successive weighings does not exceed 0.5 mg or until increase in mass is recorded.

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 moisture content, calculated as a percentage by mass of the prepared sample, should be reported to two decimal places, and is given by:

$$\% \text{ Moisture content } = \frac{(M_1 - M_2)}{(M_1 - M_0)} \times 100$$

where:

M_0 is mass in g of dried, tarred dish and lid;

M_1 is mass in g of dried, tarred dish and lid plus undried sample;

M_2 is (lowest) mass in g of dried, tarred dish and lid plus dried sample.

9. References

9.1 DO Biltcliffe, HJ Judd and R Wood, J. Assoc. Off. Analyt. Chem., 1984, **67**, 635- 636.

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 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. For both types of sample, r may be taken to be 0.3% moisture; this corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 0.10% for the uncooked frozen product, and somewhat hi- gher (0.16%) for the oven- ready product.

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. R may be taken as 2.0% moisture in the uncooked frozen product, and about 3.3% moisture in the oven-ready product. This corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of 0.8-1.8% between laboratories.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method, but its accuracy was not tested by spiking with known amounts of water. However, there is no reason to suspect any systematic bias. The possible loss of mass due to the loss of volatiles other than water may introduce bias only if the result is interpreted as water content.

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 0.3% moisture for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed two samples (A and B) of quick- frozen uncooked french fried potatoes once, each in known duplicate. They also analysed two samples (C and D) of "oven-ready" quick-frozen partly cooked french fried potatoes ("oven chips") once, each in known duplicate. The ingredients of the oven chips included vegetable fat. All the samples were stored in a freezer and macerated while still frozen.

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

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

TABLE 1

Statistical Analysis of the % Moisture in Quick Frozen Uncooked Potato Samples

Sample	A	B
Number of Laboratories retained after eliminating outliers	14	14
Number of results accepted after eliminating outliers	28	28
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	74.71	72.35
REPEATABILITY		
Standard Deviation S_r	0.08	0.08
Relative Standard Deviation RSD_r (%)	0.10	0.10
Repeatability r [2.8 × S_r]	0.21	0.21
REPRODUCIBILITY		
Standard Deviation S_R	0.71	0.56
Relative Standard Deviation RSD_R (%)	0.95	0.77
Reproducibility R [2.8 × S_R]	1.99	1.58

TABLE 2

Statistical Analysis of the % Moisture in Quick-frozen Partly Cooked ("Oven-ready") Potato Samples

Sample	C	D
Number of Laboratories retained after eliminating outliers	13	13
Number of results accepted after eliminating outliers	26	26
LEVEL OF ANALYTE		
Mean observed value $\bar{x}$	63.06	64.93
REPEATABILITY		
Standard Deviation S_r	0.10	0.10
Relative Standard Deviation RSD_r (%)	0.16	0.16
Repeatability r [2.8 × S_r]	0.29	0.29
REPRODUCIBILITY		
Standard Deviation S_R	0.76	1.17
Relative Standard Deviation RSD_R (%)	1.2	1.8
Reproducibility R [2.8 × S_R]	2.14	3.27

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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V12

ICE-GLAZE ON QUICK FROZEN FISH FILLETS

Correspondance 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 fish fillets 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 applying a gentle spray of cold tap-water and then drained, dried and weighed. The weight loss is assumed to be loss of ice-glaze.

4. Reagents

None

5. Apparatus

5.1 Analytical balance

5.2 Spray: a gentle spray of cold tap-water.

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, accurately weigh in g to one decimal place (m_0) and put it under a gentle spray of cold tap-water.

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

6.3 Allow the sample to drain; remove adhering water by the use of a paper towel and weigh the deglazed product. Let the final weight 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:

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

where:

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

m_1 is the observed deglazed weight (**6.3**).

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 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 both species, r may be taken to be 2.4% glaze at 4% levels, and 4% glaze at 8% levels. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 20%.

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. For both species, R may be taken as 6% glaze at 8% levels; this corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of over 30%.

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 Table 1. The cod fillets gave a recovery of 108% glaze, while the plaice fillets

(which had a larger surface area to weight ratio) gave 142%. The overestimates must be due to loss of physiological water, and are unsatisfactory for plaice fillets.

A4 Limit of Detection

This limit has not been established, but the poor accuracy demonstrated by the collaborative trial suggests that levels of ice glaze lower than 2.5% cannot be detected with confidence, and that even higher observed levels may not indicate glaze in samples with inappropriate physical properties.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed four samples of quick frozen whole uncooked fish fillets once. These comprised two species, each analysed in blind duplicate: "large" plaice fillets (2/11) and "small" cod fillets (9/15). The selected cod fillets were in fact thicker than the plaice fillets.

Table 1 summarises the statistical data; no outlying results were reported, though one sample was not analysed and its duplicate not accepted. The ice-glaze levels were expressed as a percentage by mass of the sample.

TABLE 1
Statistical Analysis of the % Ice-glaze in Quick-frozen Fish Samples

Sample	2/11	9/15
Number of laboratories	12	12
Number of results accepted	24	22
LEVEL OF ANALYTE		
Mean observed value	7.4	4.0
Actual (target) value	5.2	3.7
REPEATABILiTY		
Standard Deviation S_r	1.32	0.86
Relative Standard Deviation RSD_r (%)	18	22
Repeatability r [2.8 × S_r]	3.7	2.4
REPRODUCIBILITY		
Standard Deviation S_R	2.18	1.39
Relative Standard Deviation RSD_R (%)	28	35
Reproducibility r [2.8 × S_R]	6.1	3.9

A6 Interpretation of observed levels

The subjective nature of the method is reflected in the poor 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 (Table 1) are larger than would

be considered acceptable in a conventional chemical method. Nevertheless the method is recommended for the analysis of quick frozen fish fillets until a more precise method is established.

The fish 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 fish content, since this is the parameter of interest to the consumer.

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