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Verification of the Labelling of Previously Frozen Meat and Poultry by Measurement of ß-Hydroxyacyl-Co A Dehydrogenase (HADH) Activity

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This paper is presented in two parts. Part I collates and introduces the findings from several MAFF-funded studies which investigated improvements to existing methodology for determining whether meat has been previously frozen. Part II presents the results of a survey carried out on behalf of the Working Party on Food Authenticity to investigate whether meat and poultry which was being presented for sale in a chilled or ambient condition, through a number of retail outlet types, was correctly labelled when it had been previously frozen.

ß-hydroxyacyl-Co A dehydrogenase (HADH) is naturally present in muscle mitochondria and is released when these organelles are damaged during freezing and thawing. Measurement of increased amounts of HADH activity in juice expressed from a meat sample gives an indication as to whether the meat has been previously frozen. Standardisation of existing methodology to include different cuts of meat and different species (bovine, porcine, ovine and avian) was carried out to produce an analytical protocol for use in Part II. A fixed method for meat juice extraction was developed and it was concluded that it was possible to differentiate fresh meat and poultry from that which had been previously frozen to -18°C for selected cuts of beef, pork, lamb and breast of chicken, turkey and duck. It was not possible to use the developed method on liver or kidney from any of the red meat species.

Part II details a survey in which a total of 534 fresh meat samples was collected and analysed from supermarkets, butcher shops, market stalls and farm shops during December 1995 and January 1996. The samples, collected by Trading Standards and Environmental Health Departments from 15 different geographical areas were analysed using

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the protocol developed in Part I and were classified as 'authentic' (i.e. not previously frozen) or 'non-authentic' using data from the pre-surveillance study.

It was found that most of the 534 fresh meat samples surveyed had not been previously frozen but that 44 (8%) had been incorrectly labelled. Mislabelling accounted for 16% of lamb, 15% of turkey, 5% of pork, 4% of beef and 3% of chicken samples analysed. These mislabelled samples were purchased from butcher shops (24 samples), supermarkets (10 samples), market stalls (7 samples) and farm shops (3 samples).

GLOSSARY OF TERMS, ABBREVIATIONS AND UNITS

Glossary of terms used in this paper:

In relation to meat or poultry meat:

Authentic	Product which has never been frozen.
Chilled	Product at a temperature of between -2° and 10°C.
Fresh	Product which is not frozen.
Frozen	Product which is at a temperature of -9°C or colder.
Frozen-thawed	Product which has been previously frozen and is currently chilled.
Non-authentic	Product which has been previously frozen and not labelled as such.

Abbreviations used in this paper:

CSL	Central Science Laboratory
HADH	β-hydroxyacyl co-enzyme A dehydrogenase
LGC	Laboratory of the Government Chemist
Log	Natural logarithm, or $\log_e$
MAFF	Ministry of Agriculture, Fisheries and Food
MMPSG	Meat and Meat Products Sub-group of the Working Party on Fooc Authenticity
MSG	Methodology Sub-group of the Working Party on Food Authenticity
NADH	Reduced form of nicotinamide adenine dinucleotide
PSG	Projects Sub-group of the Working Party on Food Authenticity
s.d.	Standard Deviation
VEMS	Validated Enforcement Method Series
WPFA	Working Party on Food Authenticity

Units used in this paper:

°C	degrees Celsius
kg (kilogram)	one thousand gram
nm (nanometre)	10 Ångstroms or 10^{-9}m

Introduction

Fresh or chilled meat and poultry often command higher prices than their frozen counterparts. Dishonest traders, therefore, are provided with the opportunity to make improper financial gains by thawing frozen meat or poultry and presenting it for sale in a chilled condition without informing the consumer that it has been previously frozen[1]. Freezing is used extensively throughout the red meat and poultry industries as a means of preserving product. Inappropriate freezing and thawing techniques can lead to an inferior quality product, particularly from a consumer perspective, if not performed with due care. Although not regarded as posing a significant health threat to consumers, it is recognised that previously frozen meat or poultry may be more susceptible to adverse effects arising from temperature abuse and thus require more careful handling and storage than fresh meat and poultry.

Part I
Pre-surveillance development

The Methodology Sub-group (MSG) of MAFF's Working Party on Food Authenticity (WPFA) considered the various methods currently developed to detect previously frozen meat[2-6], both non-enzymic and enzymic methods. Non-enzymic methods have included light microscopy to examine the muscle structure of meat and to determine the rate of freezing by changes in the configuration and arrangement of muscle tissue. However, the success rate with this method is not good as the techniques used to cryosection and stain the tissue could adversely affect the quality of the resulting slides making assessment difficult.

As cellular structures become damaged during the freezing process then the electrical resistance to migration of ions becomes lower. Some workers have tried to exploit this effect and have used dielectric spectroscopy to determine if meat has been previously frozen. This technique is, perhaps, too sensitive and responds to very slight changes in the structure of the meat. In some instances even temperatures of -2°C, used for deep-chilling of meat, can affect the results. Furthermore, the technique is not able to discriminate between the deterioration in cellular structure caused by maturation or spoilage from the damage caused by freezing.

Two other non-enzymic methods which have been tested for red meats are the determination of the haematocrit value, i.e. the ratio, by volume, of blood cells to plasma, and the examination of erythrocytes. Both of these methods rely on the destruction of red blood cells due to freezing but neither has produced consistent correlation with the degree of freezing to enable the methods to be used as a diagnostic test.

Enzymic methods rely on the fact that when meat freezes ice crystals form within the cells and vesicles, eventually rupturing the membranes and releasing the soluble contents into the cytosol. Many enzyme systems have been evaluated with the view to using their level of activity as an indicator to determine whether or not meat has been previously frozen[5,7,8]. However, few have been able to satisfy the requirements that the enzyme should only be released on freezing and not under normal chilled refrigerated storage, nor that the total enzyme activity should not decrease markedly during storage of the meat in either the chilled or frozen state. One of the main disadvantages with lysosomal enzymes such as α-glucosidase, cathepsin D or lysosomal β-N-acetylglucosaminidase is that there tends to be high levels of soluble enzyme activity present post mortem, probably due to lysis of the lysosomes by the lower pH post mortem.

Of all the enzyme systems investigated the measurement of β-hydroxyacyl-CoA dehydrogenase (HADH) activity appears to give the most consistent results across a number of different species. In common with other enzymic methods, the HADH method is based on the increased activity of the enzyme in the sarcoplasm resulting from the release of the enzyme from muscle mitochondria which have been damaged when the meat or poultry has been frozen and thawed. Increased HADH activity in juice expressed from a sample is an indication that the muscle has previously been frozen. The rationale for developing a method based on HADH was that this enzyme is localised in the mitochondria so that electrophoresis to separate various iso-enzymes is not necessary, it is released by freezing and thawing but not by ageing, activity does not markedly decrease during storage of muscle (either fresh or frozen) and it is easy to detect in muscle tissue. Nevertheless, since the release of HADH into the sarcoplasm from the mitochondria results from mitochondrial damage, the method is not suitable for differentiating between fresh and frozen/thawed comminuted or minced meat.

Meat juice, or press juice, is obtained by applying pressure to the intact muscle tissue. Some mitochondria will be damaged at the surfaces where the meat sample has been cut and some HADH will leak out of these damaged mitochondria into the press juice. The press juice of unfrozen muscle is, therefore, not completely free of HADH activity and thus necessitates a

comparative test. HADH activity is then measured using a spectrometric technique by determining the rate of conversion in the following reaction:

$$\text{acetoacetyl-coenzyme A} + \text{NADH} + \text{H}^{+} \xleftrightarrow{\text{HADH}} \beta\text{-hydroxybutyryl-coenzyme A} + \text{NAD}^{+}$$

The activity of HADH is measured by the decrease in the absorption of the reaction solution at 340nm.

The HADH method has been used successfully to differentiate frozen-thawed meat from the equivalent fresh beef, veal, pork, mutton, poultry and game when the meat was frozen at -12°C or colder[7]. A modification of the spectrometric techniques has been collaboratively tested for differentiating fresh and frozen-thawed poultry[9]. The results of this undertaking indicated that the method, as tested, permits the differentiation of fresh and frozen-thawed chicken breast meat, since significantly higher enzyme activities were obtained in the frozen-thawed samples, provided the freezing process has been undertaken at temperatures colder than -12°C. Samples pre-frozen at -6°C or chill-stored did not exhibit significantly different enzyme activities to fresh samples so the method cannot be used to differentiate such samples from fresh poultry meat.

Since the HADH method had previously only been collaboratively tested on chicken breast meat, the MSG recommended that further testing of the method on different cuts of meat and offal from a number of carcases and from different species was required in order to obtain more comprehensive data and to define more precisely the procedures for sample preparation (e.g. the pressure used to obtain the press juice). The MSG regarded the provision of this information as necessary before conducting any surveillance exercise and to help in assessing the suitability of the method for routine testing.

The preliminary work on the HADH method was designed to determine the limitations of the method on a wide range of species and cuts of meat and to devise equipment capable of consistently producing meat press juice. In particular, this trial addressed the following issues:

a) optimisation of the pressing operation;

b) determination of inter-laboratory variation;

c) determination of inter-carcase variation for particular species;

d) determination of the difference between different cuts from the same species;

e) determination of the effects of ageing of meat on the level of HADH activity;

f) determination of the effects of frozen storage temperature on the level of HADH activity;

g) establishment of a database of HADH activity levels in authentic fresh and frozen/thawed red meat and poultry meat samples.

Methods and Materials - Part I

Meat press standardisation

A meat press was developed in conjunction with the Laboratory of the Government Chemist (LGC) with assistance from the Force and Pressure Group at the National Physical Laboratory (see Appendix 1) which enabled each laboratory in the trial to achieve the same degree of pressing for the different meat samples. Subsequent slight modifications were made to the press and the analytical protocol following investigation of the pre-surveillance data by CSL Torry.

Authentic sample collection and preparation

Samples of beef (rump, silverside, topside, sirloin, kidney and liver), pork (leg, chops, liver and kidney), lamb (leg, chops, liver and kidney) were obtained directly from the abattoir by the Meat and Livestock Commission (MLC) after conditioning and verified never to have been frozen. Breast samples of chicken, turkey and duck were authenticated and collected from poultry slaughterhouses by Sovereign Foods Limited.

All the samples were prepared by MLC according to a set protocol and despatched to the specific laboratories corresponding to the sampling plan outlined in Table 1. Duplicates of all samples were prepared and a total of 512 samples was despatched in insulated containers containing a cold pack attached to the lid (to prevent contact with the samples) and sent by overnight courier to arrive at the laboratories by 10:00 am the following morning. The condition and temperature of the samples were checked by each laboratory on arrival to ensure temperature abuse had not occurred.

For the 'X' samples in Table 1 the rump was taken from a single carcase. In the case of the chicken and duck the breast was taken from a minimum number of carcases, each selected from consecutive carcases from the slaughter line and from the same flock of birds. The results from these 'X' samples are used to determine inter-laboratory variation of the method.

Table 1 Sampling plan for HADH trial

	Beef						Pork				Lamb				Chicken	Turkey	Duck	Total
Lab	Rump	Silverside	Topside	Sirloin	Liver	Kidney	Leg	Chops	Liver	Kidney	Leg	Chops	Liver	Kidney	Breast	Breast	Breast	
A	X[1]			5H		5C[2]							6T[3]		X		X+6T	25
B	X					6T	5C					6T			X+5H[4]		X	25
C	X					5C		5C	5C						X+4C		X	22
D	X			5C					5C			5C			X		X	18
P	X			5C			5C			5C					X+5H		X	23
F	X			5H				5C		5C		5C			X		X	23
G	X		6T										5C		X	6T	X+4C	24
H	X		5C										5C		X	5C	X	18
Q	X		5C								5C			5C	X		X	18
J	X	5C												5C	X	5C	X	18
K	X	5C			5C						5C				X		X	18
L	X+4C				5C			6T							X+6T		X	24
	16	10	16	20	10	16	10	16	10	10	10	16	16	10	32	16	22	256
Tot	88						46				52				32	16	22	256

[1] X = analysis of one sample from the same carcase, where possible.

[2] C = analyses of samples from different carcases.

[3] T = analyses of samples from the same carcase, where possible, frozen to -6°C, -9°C and -12°C.

[4] H = analyses of samples from the same carcase, where possible, aged for periods from 5 to 28 days.

Sample preparation: Duplicate samples were prepared and distributed to all 12 laboratories.

To determine inter-carcase variation the samples marked 'C' in Table 1 were selected from different carcases.

The effect of storage temperature was determined on 'T' samples which were stored at -6°C, -9°C or -12°C instead of -18°C as required in the analytical protocol for the comparative test. All other samples were stored at -18°C.

The effect of ageing on the level of HADH activity was determined on 'H' samples by storing sirloin and chicken breast at between 0° and +5°C and analysing samples after 5, 12, 19, 24 and 28 days.

Analytical procedure

The method used in this trial is the VEMS HADH spectrophotometric enzyme assay based on that of Gottesmann and Hamm[7]. The full revised analytical protocol is given in Appendix 2.

Statistical analysis of results

For each cut of meat the results were subjected to appropriate analyses of variance (ANOVA) to estimate both the mean difference between fresh and previously frozen samples, and the extent of the random variation between carcases, between laboratories (reproducibility) and between duplicate assessments by the same laboratory (repeatability). The ANOVA's were also used to test for the statistical significance of the systematically varied factors in the trial design: temperature of storage and duration of storage. By combining the variance components in the correct way it was possible to assess the ability of the method to discriminate between fresh and previously frozen meat for each cut. This was done by comparing the mean difference between fresh and previously frozen samples with the standard error of the difference.

Participating laboratories

The twelve laboratories taking part in the trial (Appendix 3) consisted of nine UK Public Analyst Laboratories, two government agency laboratories, and one industrial laboratory and were chosen following a request to tender.

Results and Discussion - Part I

Meat press standardisation

The variation in the results of the previous collaborative trial[9] on chicken breast meat may have been exacerbated by the lack of uniformity in the method of expressing the meat juice. Therefore, for the purpose of determining 'authentic data' it was decided that in order to overcome this problem it would be necessary to standardise, as far as possible, the procedure for pressing the meat.

Basic testing of this new piece of equipment was carried out by LGC to determine the size of sample required, operator variability and the effect of meat temperature on press. Results are given in Tables 2-4 respectively.

For cutting the samples of meat it was found that using a scalpel was quicker and easier than using a cork borer which produced samples uneven in both size and weight, and which often fell apart along the meat fibres. The results (Table 2) suggest that initial size of sample does not affect enzyme activity in the meat press juice. However, with the larger sample sizes it was not possible to fully compress the equipment to its full extent. Although not specifically tested directly in this investigation, it is thought unlikely that whether meat fibres are presented longitudinally or transversely there will be any significant effect on the enzyme activity in the resulting press juice. Subsequent work at CSL Torry indicated that for poultry it was preferable if the sample consisted of six cut surfaces. The protocol therefore advocates using a scalpel to cut the meat into a cuboid approximately 30 × 30 × 20 (height) mm.

Table 2

Enzyme activity results comparing different sizes, weights and mode of preparation.

Mode of Preparation	Dimensions (mm)	Weight (g)	Enzyme Activity (U/ml)*
Cork borer	28 (diameter) × 45 (length)	19.7	2.2
Cork borer	25 (diameter) × 40 (length)	14.1	2.6
Scalpel	15 × 25 × 20	9.0	2.4
Scalpel	30 × 30 × 20	20.2	2.3
Scalpel	40 × 40 × 25	39.8	2.2

* Direct measurement of enzyme activity determined on meat press juice and not full comparative test.

For testing operator variability with the meat press, 3 operators tested 5 different joints of topside of beef, obtained from a retail outlet. The HADH activity of each press juice sample was determined by one operator (Table 3).

Table 3

Effect of operator on level of enzyme activity.

Operator	Joint Number	Sample Weight (g)*	Enzyme Activity (U/ml)
1	1	21.4	3.7
	2	21.1	3.1
	3	21.0	3.1
	4	25.6	16.1
	5	23.9	3.4
2	1	24.5	5.6
	2	18.9	3.2
	3	25.5	3.4
	4	21.4	15.4
	5	25.3	3.0
3	1	20.1	4.4
	2	18.4	5.5
	3	18.8	3.8
	4	23.6	5.4
	5	27.7	4.0

* Sample size used: 30 × 30 × 20mm

Although some differences between operators were noted they are not as great as the expected change in the level of enzyme activity resulting from freeze-thawing. It is not known why a high result was obtained for joint 4 by two of the operators, but since the full history of these samples is not known it may be that breakdown of cells with release of enzyme may have occurred by some other means (e.g. microbiologically), or that localised freezing of the joint had taken place at some point during its distribution and retail cycle.

Five retail joints of topside of beef at refrigerator temperature (approximately 4°C) and room temperature (approximately 18°C) were tested to determine if the temperature of the meat at pressing had any effect on the level of enzyme activity in the resultant press juice. The temperatures recorded were those measured immediately before pressing commenced, *i.e.* after the sample preparation stage using the scalpel. The results are given in Table 4. Owing to the small sample size it was difficult to obtain juice at 4°C. The differences in the level of enzyme activity at the different temperatures was not considered to be significant compared with the differences expected due to freeze-thawing.

Table 4

Effect of meat temperature on juice expression.

Temperature (°C)	Joint Number	Enzyme Activity (U/ml)*
20	1	3.8
20	2	2.4
20	3	2.6
20	4	5.4
20	5	4.2
13	1	5.2
10	2	2.5
12	3	2.2
7	4	5.5
4	5	2.8

Analyses of authentic samples

Many laboratories reported difficulties in using the meat press, particularly with the offal samples. However, several also experienced problems in trying to express sufficient juice from fresh chicken, turkey and duck samples, although once these samples had been frozen and thawed there did not appear to be any great difficulties in this respect. Care has to be exercised in using the press to prevent juice from being drawn back into the sample when the pressure is released. This is particularly important for those samples which yield minimal juice.

The problem with the offal samples is slightly different as disintegration of the sample often occurred. This suggests that the exerted pressure is too great. Although it may be possible to incorporate a centrifugation step into the analytical protocol to remove the tissue debris, the degree of damage incurred by the mitochondria due to the action of the press is unknown and may be such as to invalidate the results. What does appear to be important is not so much the applied pressure but applying sufficient pressure to enable the juice to be expelled from the sample without undue damage to the sample. It should be considered that uniform pressure for all samples is not as important as obtaining a clear meat juice for analysis.

Inter-laboratory variability

Table 5 gives the results of the analyses of levels of HADH activity, before and after freezing, in beef rump, chicken breast and duck breast as measured by each of the laboratories. The absolute levels of the difference in HADH

activity between the fresh and frozen samples are distinctly different for each of the species, particularly for the duck breast which showed significantly higher levels of HADH activity after freezing. The reproducibility of the method is species dependent. However, with all species the difference in enzyme activity level after freezing was sufficiently greater than that measured in the fresh state for it to be statistically significant and thus the method could be used to determine whether beef rump, chicken breast or duck breast had been previously frozen. It is not possible to determine an absolute limit to enable a single 'fresh' determination to be made and the method needs to include the comparative 'fresh' versus 'frozen' analysis. Billington *et al.*[9] suggested increasing the reaction time from 3 minutes to 6 minutes, and having the higher reaction temperature of 37°C instead of 25°C but this may be more appropriate for samples which exhibit a relatively small difference in HADH activity between the fresh and frozen/thawed samples. These modifications were not tested.

Table 5

Results of HADH activity for beef rump, chicken breast and duck breast showing inter-laboratory variation

	Beef Rump	Chicken Breast	Duck Breast
Reproducibility σ^2:	9.84	3.9	172.1
Repeatability σ^2:	2.33	7.26	151.7
Mean Difference :	5.8	8.0	35.3

Inter-carcase variability

Inter-carcase variation was tested for all species and cuts of meat and poultry and the results are presented in Table 6. Ignoring the data for liver and kidney for all the red meat species, this trial would suggest that the degree of variability between carcases was less than that recorded between laboratories for the method itself.

The results for both liver and kidney of beef, pork and lamb were very variable, with many of the laboratories reporting difficulties especially with the fresh offal samples. This is probably due to the problems of the presence of fine tissue particles in the press juice which would have interfered with the subsequent enzyme assay.

Table 6

Results of HADH activity for beef samples showing inter-carcase variation

	Sample	Carcase Variance σ^2	Repeatability σ^2	Overall Mean Difference	Standard Deviation σ	Number of Standard Deviations from Zero
Beef:	Rump	0.35	1.94	5.60	1.15	4.87
	Silverside	0.06	2.71	6.50	1.19	5.46
	Topside	0.00	4.30	6.80	1.47	4.63
	Sirloin	0.13	0.30	2.80	0.53	5.28
	Liver	469.60	145.70	52.70	23.30	2.26
	Kidney	0.00	184.10	36.40	9.59	3.80
Pork:	Chops	12.26	2.06	12.4	3.65	3.40
	Leg	20.48	76.88	17.0	7.68	2.21
	Kidney	122.9	56.7	37.9	12.30	3.08
	Liver	455	1309	63.5	33.31	1.97
Lamb:	Leg	16.03	14.98	19.40	4.84	4.01
	Chops	5.35	2.80	15.40	2.60	5.92
	Kidney	0.00	1961.00	27.60	31.31	0.88
	Liver	0.00	1927.00	-42.60	31.4	-1.36
Chicken:	Breast	5.64	6.86	9.10	3.01	3.02
Duck:	Breast	19.20	89.82	27.60	8.01	3.45
Turkey:	Breast	0.00	53.49	7.70	5.17	1.49

Effect of ageing of meat

The effect of ageing on the level of HADH activity was investigated in beef sirloin and chicken breast samples. The results are given in Table 7 and graphically displayed in Figure 1. Although the number of samples in this study was relatively small it can be concluded that there was no statistical significant difference in the level of HADH activity in the sirloin or chicken breast due to ageing of the samples up to 28 days. Following discussion at a MSG meeting it transpired that there is some meat on the market which is matured for considerably longer (up to 14 weeks in vacuum pack). Thus further work was conducted by CSL Torry which confirmed that the ageing of meat up to 14 weeks did not affect the efficacy of the HADH method as a means of determining whether or not meat or poultry had been previously frozen.

Effect of storage temperature

The effect on the level of HADH activity of storage at -6°C, -9°C and -12°C was studied for beef topside, pork chops, lamb chops, and breast of chicken, turkey and duck. The results are presented in Table 8. Unfortunately there is no scope for a direct statistical comparison of the -18°C data with the higher temperature data (except for chicken breast and duck breast) since the respective analyses were conducted by different laboratories. The results indicate, however, that for chicken and duck breast there is a significant difference in the HADH activities between -6°C and -9°C, and for chicken breast a further significant difference in HADH activity between -12°C and -18°C. However, for all the other samples there is no significant difference in the HADH activities measured when stored at warmer than or equal to -12°C. This would suggest for all samples tested that slight 'case hardening' or 'accidental' lowering of temperature should not produce any false positive results. All samples would have to have been frozen to colder than -12°C (-9° C in the case of chicken and duck breast) before a sample could be identified as having been previously frozen. Researchers at CSL Torry have suggested that the rate of freezing may be important and thus this factor was included in the analytical protocol for the surveillance exercise.

Table 7

Effect of ageing on HADH activity level in beef sirloin and chicken breast

	Mean Age (days)							
	5	12	19	24	28	Reproducibility σ^2	Repeatability σ^2	F.Value (Age)
Chicken	-9.7	-8.8	-10.1	-9.9	-9.1	2.43	9.08	0.08 (n.s.)
Beef	-3.3	-2.9	-3.2	-4	-3.3	1.41	0.79	0.18 (n.s.)

Figure 1

Effect of ageing on HADH activity level in beef sirloin and chicken breast

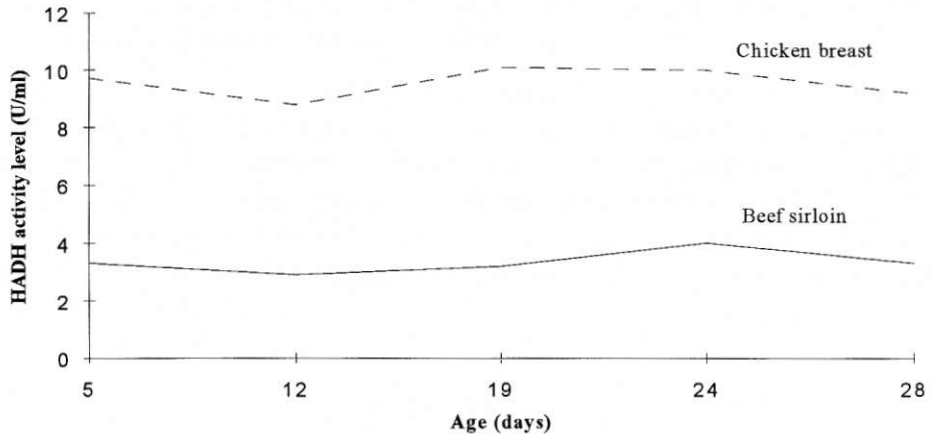

Table 8

Effect of frozen storage temperature on HADH activity level on various species and cuts of meat and poultry

			Mean Temperature			
Species/Cut	**Repeatability σ^2**	**F-Value (Temp)**	**-18°C**	**-12°C**	**-9°C**	**-6°C**
Beef Topside	0.67	2.66 (n.s.)		-0.8	-0.6	0.5
Pork Chops	1.46	1.05 (n.s.)		-8.6	-8.3	-7.4
Lamb Chops	14.58	2.49 (n.s.)		-13.5	-13.2	-8.1
Chicken (Breast)	6.57	9.70 (**)	-11.04	-5.2	-8.6	-0.7
Turkey Breast	0.74	1.96 (n.s.)		-0.7	0.4	0.3
Duck Breast	6.25	13.35 (***)	-15.5	-12.0	-13.4	-4.2

Conclusions - Part I

Overall, the pre-surveillance work showed that it was possible to differentiate fresh meat which had never been frozen from thawed previously frozen meat of beef rump, silverside, topside and sirloin; pork and lamb leg and chops; and breast of chicken, turkey and duck. The degree of variation in the difference of HADH activity in the comparative test between carcases of the same species was found to be less than the variation within the method itself. It was not possible, using the existing press and analytical protocol to differentiate between fresh and previously frozen liver or kidney of bovine, porcine or ovine origin.

Age of meat does not appear to be a major factor in the measured values of HADH activity. There was no significant difference in HADH activity in chicken breast or beef sirloin with the age of the sample up to 28 days old (Figure 1) and further work by CSL Torry corroborated these findings on beef which had been commercially stored for 14 weeks.

It was also found that the temperature to which a piece of meat was frozen was important. There was no significant difference in HADH activity in the beef, pork or turkey samples tested at frozen storage temperatures down to -12°C. For duck and chicken breast there is a significant difference in HADH activity in samples stored at temperatures colder than -9°C, and for chicken breast there was also a significant difference in samples stored at -12°C compared with those stored at -18°C. The *rate* of freezing is thought to be important and thus the analytical protocol in the survey emphasises that slow freezing should be used for the comparative analysis.

Although it was decided to use the meat press for extracting the meat juice from the surveillance samples in Part II, in the context of maintaining uniformity between laboratories, it is recognised that this may not be necessary in routine analyses. What appears to be important is that a method should be used which produces sufficient pressure to allow the juice to flow but which does not break down the structure of the sample such that pieces of tissue are contained within the exudate.

Part II
Surveillance Exercise

Structure of survey

Meat is the largest of the retail sectors in the UK with an annual turnover in the region of £10bn. Although beef is the most popular carcase meat at retail

this sector has been in decline for many years and poultry, and particularly chicken, continues to take an increasing share of the meat supplied in the UK. However, within both the beef and poultry markets the trend is shifting towards further processed products. Fresh meat and poultry are essentially commodity products and, as such, little branding is carried out. Although large supermarkets are taking an increasing proportion of the retail trade in fresh meat and poultry the individual butcher shop is still regarded as important, with much smaller volumes sold through market stalls and farm shops.

Sampling strategy

The Meat and Meat Products Sub-group (MMPSG) of the WPFA devised the sampling strategy using as a basis experience developed during the pre-surveillance trial and their own knowledge of the products and market. Only the retail sector was considered and outlet types were divided into five broad categories: large multiple supermarkets (floor space $\geq$ 20,000 sq.ft.), convenience stores (floor space 4,000 - 10,000 sq.ft.), butcher shops, market stalls and farm shops. Although the sampling plan was devised to give a wide national spread of products it was not considered to be representative of the UK retail market in terms of market share of the various types of outlets and sampling was weighted to provide, for example, a greater number of samples from butcher shops with respect to their market share.

Samples collected

Only products which were offered for sale in a chilled condition and were not labelled as having been previously frozen were collected for the survey. Following the pre-trial study the MMPSG decided that the survey should concentrate on samples of beef rump, lamb and pork chops, and chicken and turkey breast meat, with the poultry from either whole carcases, appropriate portions or fillets. The treatment of samples after collection and prior to analysis was regarded as an important criterion in the survey and precautions to avoid freezing of samples or excessive delays before collection of the meat juice were incorporated into the protocol.

Although a total of 551 fresh meat and poultry samples was collected from the various outlet types during December 1995 and January 1996 only 534 were included in the final analyses. Some samples had to be disregarded as being unsuitable for analysis. Figure 2 shows the breakdown of the samples by species and collection period (before or after Christmas). Figure 3 shows the breakdown of the outlet type sampled compared with the sampling plan. The survey included both domestic and imported meats covering branded and own-label products. Collection of the samples was by Trading Standards and Environmental Health Departments as outlined in Appendix 3.

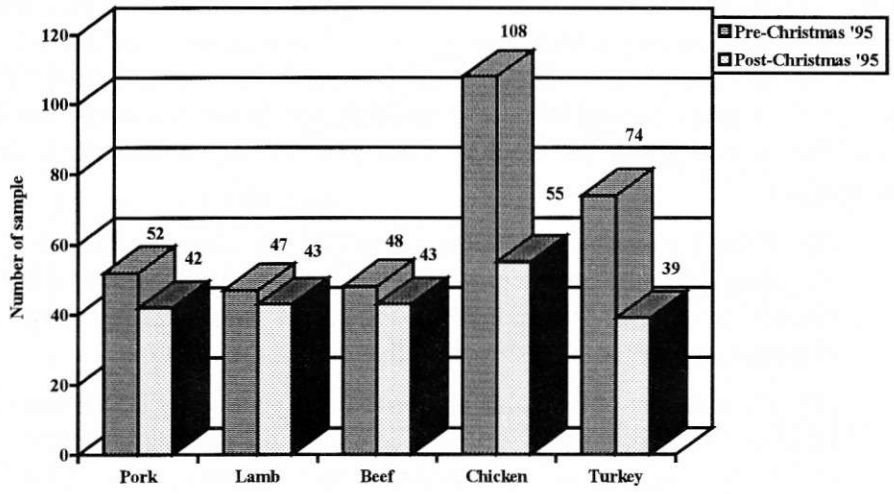

Figure 2 Number of samples collected for each of the different species

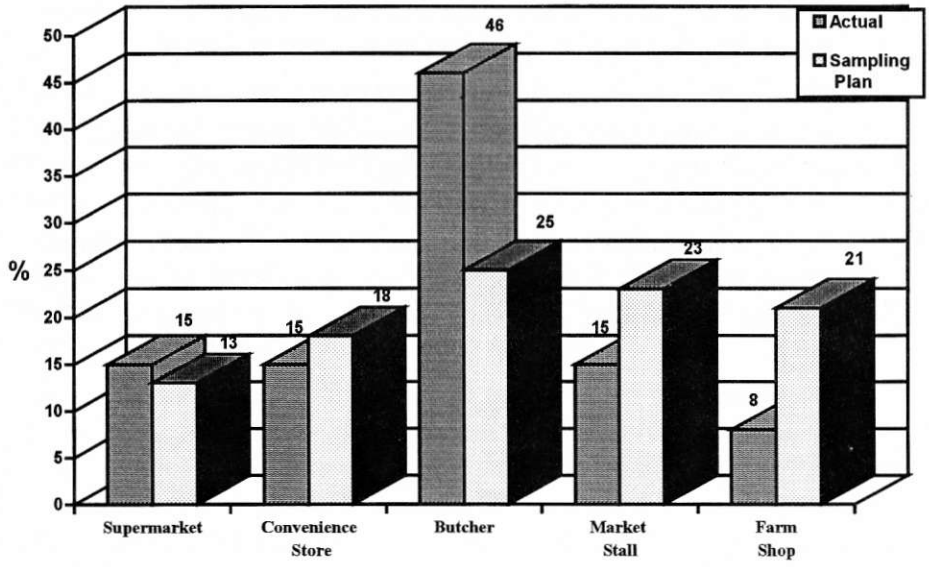

Figure 3 Percentage retail outlets sampled compared with the sampling plan

Interpretation of results

In the pre-surveillance work and in the actual survey the level of HADH activity was determined on the samples as received (X_0) and after being frozen and thawed once (X_1). An additional measurement of HADH activity was made after twice freezing and thawing (X_2) in the samples collected in the

survey. The Analytical Protocol made provision for the analysis of standards of known HADH activity which were to be used as a measure of laboratory performance and comparison. Unfortunately, as can be seen from Figure 4 the standards themselves proved to be too unstable and, therefore, could not be used in the assessment of the results. Four possible approaches were then considered:

(a) Comparison of the X_0 value against the distribution of X_0 and X_1 values obtained for the pre-surveillance data. Mislabelling of a sample would be determined if the value was clearly more characteristic of the X_1 distribution than the X_0 distribution.

(b) Comparison of the value of $D = (X_1 - X_0)$ with the distribution of D obtained for the pre-surveillance data and declaring a sample to be 'non-authentic' if the value is clearly inconsistent with the typical distribution of D exhibited by an authentic, i.e. never been frozen, sample.

(c) Comparison of the value of $R_1 = (X_0/X_1)$ with the distribution obtained for the pre-surveillance data. Mislabelling of a sample would be determined if the value was clearly inconsistent with the typical distribution of R_1 exhibited by an authentic sample.

(d) Determining the value $R_2 = (X_1/X_2)$ in addition to that of R_1 and then using the difference between R_1 and R_2 to gauge the likelihood that a sample is authentic. Theoretically, the more times a sample has been frozen the closer the ratios R_1 and R_2 converge to some limiting value and thus the difference between them converges to zero. Therefore, values close to zero (or even negative values) cast increasing doubt on the authenticity of the sample.

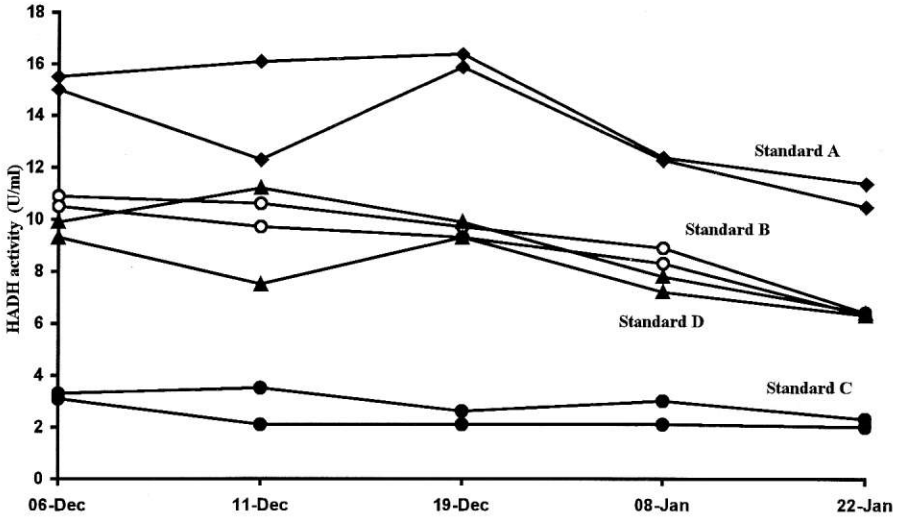

Figure 4 Stability of standards A, B, C and D analysed by CSL Torry

It was considered that approach (c) was the most appropriate since the ratio $R_1 = (X_0/X_1)$ appeared to be a more stable entity than either X_0 or X_1 individually, or the difference between them. Although the approach in (d) is sound its practical application is surrounded by uncertainty owing to the lack of pre-surveillance data from which to obtain a distribution of R_2 values, since these samples were not frozen-thawed twice. Furthermore the R_2 values for the surveillance data are centred around values clearly below unity, for all five meat species, so that it was considered unwise to rely upon the assumption that the ratio converges rapidly to a value of unity.

In order to develop a discriminatory rule based on the ratio R_1, it is important to have a credible statistical distributional model for R_1 to act as a basis for attaching degrees of confidence to any decisions which are derived from it. Histograms for the values of the ratio R_1, computed from the surveillance samples, are shown in Figures 5-9 for each of the five meat species. The raw data, exhibited prior to logarithmic transformation, are universally right-skew. However, after transformation the distributions draw close to a symmetric, normal profile. The main exception to this pattern is seen with lamb, for which the data are either slightly right-skew or else bi-modal, even after transformation. A possible explanation for this is that the heavy upper tail corresponds to a bulge of non-authentic samples, an argument made more plausible by the fact that lamb samples appear to show the greatest non-authenticity problem among those studied.

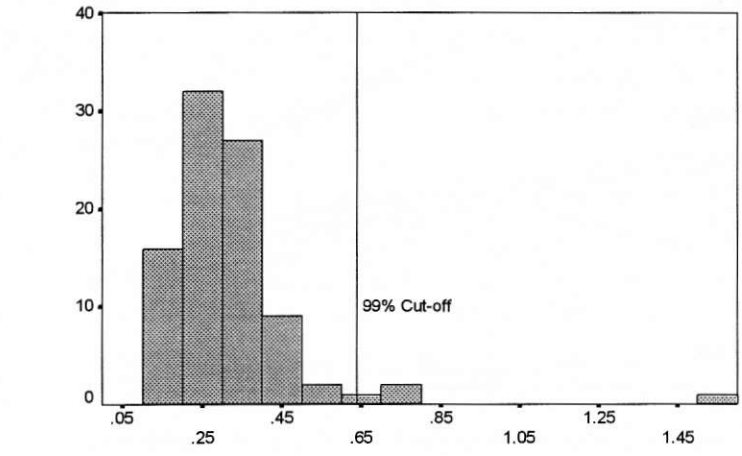

Figure 5

Histogram of R_1 values for the beef samples in the surveillance study. The superimposed reference line indicates the upper 99% cut-off value for authentic R_1 ratios, as estimated from the pre-surveillance data. (s.d. = 0.18; Mean = 0.32; N = 90)

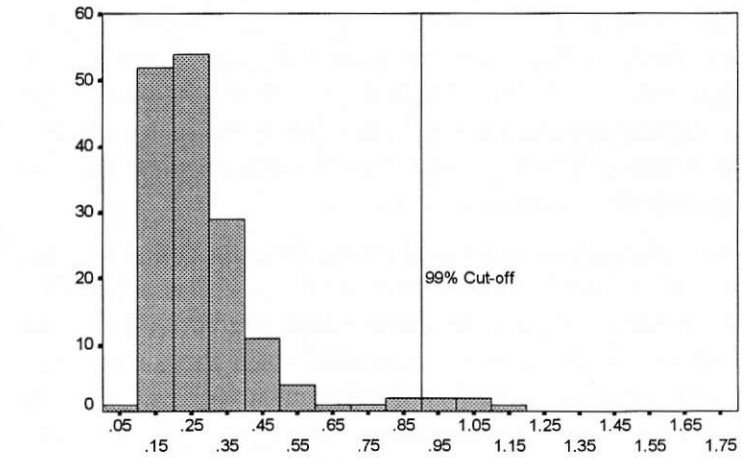

Figure 6

Histogram of R_1 values for the chicken samples in the surveillance study. The superimposed reference line indicates the upper 99% cut-off value for authentic R_1 ratios, as estimated from the pre-surveillance data. (s.d. = 0.18; Mean = 0.30; N = 160)

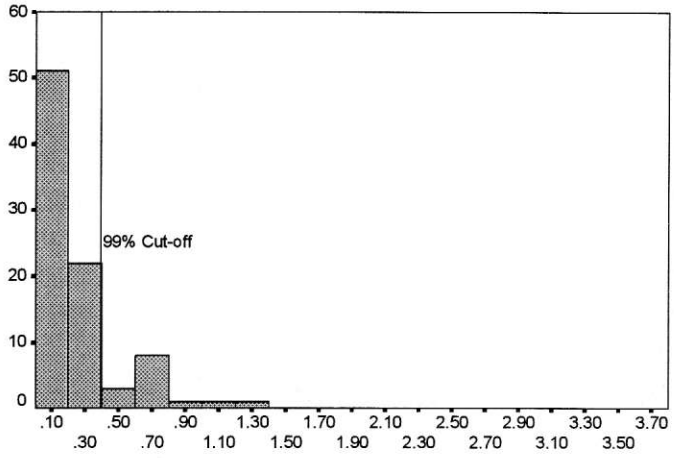

Figure 7

Histogram of R_1 values for the lamb samples in the surveillance study. The superimposed reference line indicates the upper 99% cut-off value for authentic R_1 ratios, as estimated from the pre-surveillance data. (s.d. = 0.23; Mean = 0.26; N = 87)

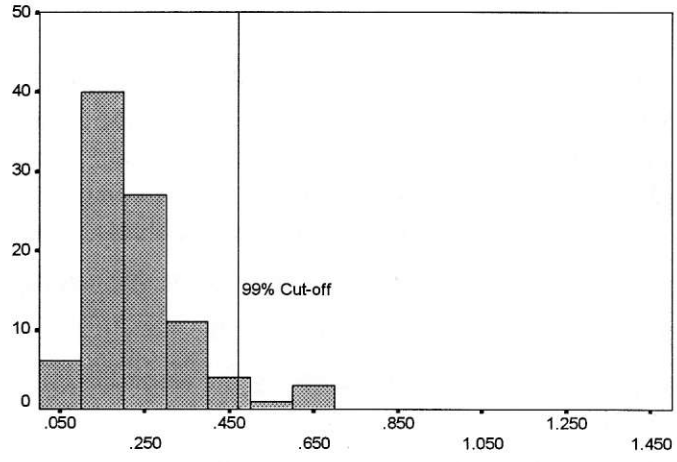

Figure 8

Histogram of R_1 values for the pork samples in the surveillance study. The superimposed reference line indicates the upper 99% cut-off value for authentic R_1 ratios, as estimated from the pre-surveillance data. (s.d. = 0.12; Mean = 0.23; N = 92)

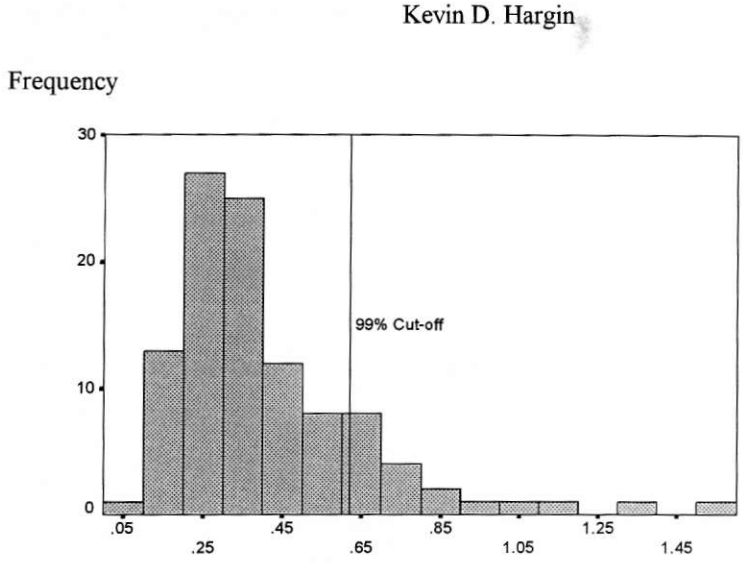

Figure 9

Histogram of R_1 values for the turkey samples in the surveillance study. The superimposed reference line indicates the upper 99% cut-off value for authentic R_1 ratios, as estimated from the pre-surveillance data. (s.d. = 0.25; Mean = 0.41; N = 105)

Having decided that the normal distribution provided a reasonable model for the variation in the value of log R_1 among authentic samples, the mean and standard deviation (s.d.) was estimated for each of the five meats. The s.d. was estimated by adding together the following three random components of variation, each separately estimable from the pre-surveillance data:

- variation between different meat carcases of the same species/cut;
- variation between laboratories on samples from the same carcase;
- variation between replicate measurements by the same laboratory on the same sample.

The mean of the log R_1 distribution for each meat was estimated by averaging the mean values for each laboratory which had provided values in the pre-surveillance study. The results are shown in Table 9, which also shows the simple mean obtained from the surveillance study. In comparing the pre-surveillance and surveillance estimates of the mean the latter should be expected to exhibit greater values if there are non-authentic samples present.

Assuming a normal distribution of log R_1 among authentic samples, with the s.d. and means displayed in Tables 9 and 10, then it is possible to calculate ranges of values within which it would be expected an authentic sample should fall with any prescribed level of probability. Thus a sample from the survey with log R_1 exceeding a suitably chosen high percentile of the distribution, e.g. 99%, is quantifiably uncharacteristic of authenticity and may, with some justification, be said to be more likely to be 'non-authentic'. Table 11 shows the computed value of the upper 99% cut-off point for log R_1 for authentic meat samples for each species. The corresponding value of R_1 is also shown, along with an approximate 95% confidence interval for the value of the upper 99% cut-off.

Table 9

Mean log (R_1) among authentic samples of meat in the pre-surveillance study and among all samples (authenticity not known) from the survey.

Meat	Pre-surveillance	Surveillance
Beef	-1.25	-1.23
Chicken	-1.32	-1.35
Lamb	-1.78	-1.60
Pork	-1.84	-1.59
Turkey	-1.29	-1.04

False positives / false negatives

The probability of a false positive is defined, by the nature of the discriminatory rule, to be 1%, or at least believed to be 1%, based on the evidence of the pre-surveillance data available to devise the rule. The probability of a false negative cannot be determined quite so elegantly, because no part of the study was deliberately designed to provide data on the distribution of R_1 values among non-authentic samples. However, the R_2 values from the survey may provide a good approximation to this unavailable data if it is assumed that all (or nearly all) of the samples are originally authentic and only become non-authentic after being once-frozen and/or that even if some of the samples are non-authentic, i.e. have been frozen at least once more than suspected, the value of the ratio approaches an equilibrium sufficiently quickly for the value of R_2 not to be too dissimilar to the value of

R_1 for a typical non-authentic sample. Applying this reasoning the estimate of the likely probability of false negatives is given in Table 12.

Table 10

Calculation of the s.d. of log (R_1) among authentic samples in the pre-surveillance and surveillance trials

(The figures in brackets are degrees of freedom for estimation of the variance components)

	Variance Components			Pre-Surveillance	Surveillance
Meat	**Carcase**	**Lab**	**Within Lab**	**s.d.**	**s.d.**
Beef	0.00 (4)	0.06 (10)	0.06 (16)	0.34	0.40
Chicken	0.01 (4)	0.15 (14)	0.12 (48)	0.52	0.49
Lamb	0.01 (4)	0.05 (1)	0.07 (20)	0.36	0.68
Pork	0.06 (4)	0.09 (1)	0.07 (20)	0.47	0.49
Turkey	0.04 (4)	0.04* (0)	0.04 (5)	0.35	0.55

* Note: For turkey, reliable data were obtained for only a single laboratory. Since this made it impossible to estimate the between-lab component of variance, the within-lab variance was imputed, based on the observation that these two components are very similar for each of the other meat types.

Table 11.

Estimated 99% cut-off point for log (R_1) or R_1 for authentic samples.

(Bracketed figures represent the limits of an approximate 95% confidence interval for the upper 99% point for R_1).

Meat	log (R_1) scale	R_1 scale
Beef	-0.45	0.63 (0.49, 0.83)
Chicken	-0.11	0.90 (0.64, 1.25)
Lamb	-0.95	0.39 (0.20, 0.75)
Pork	-0.75	0.47 (0.20, 1.11)
Turkey	-0.48	0.62 (0.28, 1.38)

Table 12

Empirical derivation of the probability of false negatives when using the 99% cut-off rule to discriminate between authentic and non-authentic samples.

Meat	R_1 cut-off	No. R_2 values below cut-off	Empirical probability
Beef	0.64	21/90	23%
Chicken	0.90	105/160	66%
Lamb	0.39	1/87	1%
Pork	0.47	2/92	2%
Turkey	0.62	10/105	10%

The principal rationale for the discrimination rule implied by Table 11 is to gauge the prevalence of mislabelling of meat in the UK retail market. Having established that simple application of the rule will incur two different types of misclassification error, with probabilities of occurrence which can be quantified, this information is used to refine the estimates of the prevalence of non-authenticity. This is achieved by ensuring that the final prediction of the number of authentic (A) and non-authentic (N) samples in the survey dataset is consistent not only with initial estimates, based on the number (a) above the cut-off in Table 11 and the number (n) below, but also with the estimated probabilities of misclassification. The following two equations need to be solved:

$$n = A(p_1) + N(1 - p_2) \qquad (1)$$

$$a = A(1 - p_1) + N(p_2) \qquad (2)$$

where p_1 and p_2 are the respective probabilities of false positives and false negatives, the former being believed to be 0.01 and estimates of the latter being displayed in Table 12.

Results and Discussion - Part II

Application of the discrimination rule developed in the preceding paragraphs results in the estimated mislabelled samples as outlined in Table 13. Figures 10, 11 and 12 graphically illustrate the number of mislabelled sample identified in the survey in relation to the species and retail outlet type.

Table 13.

Estimated percentage of mislabelled meat using the refined 99% cut-off discrimination rule. The figures in brackets provide an approximate 95% confidence interval for the percent mislabelled samples.

Meat	R_1 cut-off	No. R_1 values above cut-off	% above cut-off	Adjusted % mislabelled
Beef	0.64	4/90	4	5 (0-6%)
Chicken	0.90	5/160	3	6 (0-4%)
Lamb	0.39	14/87	16	15 (4-41%)
Pork	0.47	5/92	5	5 (0-49%)
Turkey	0.62	16/105	15	16 (0-66%)

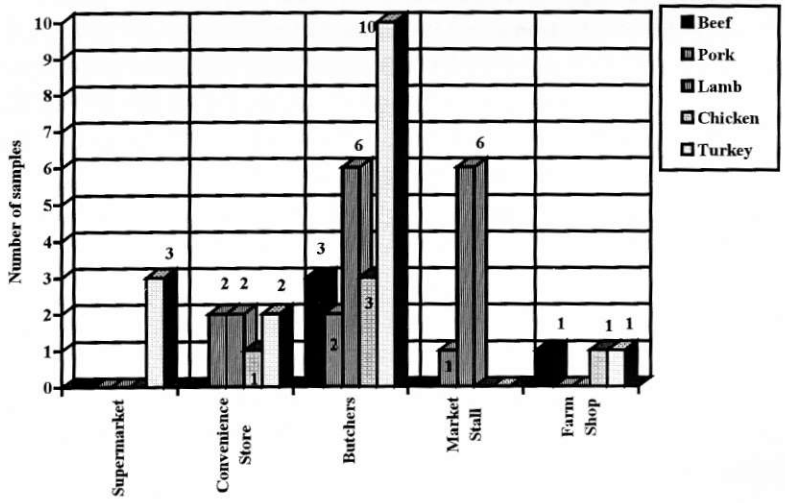

Figure 10 Numbers of mislabelled samples by species and retail outlet type

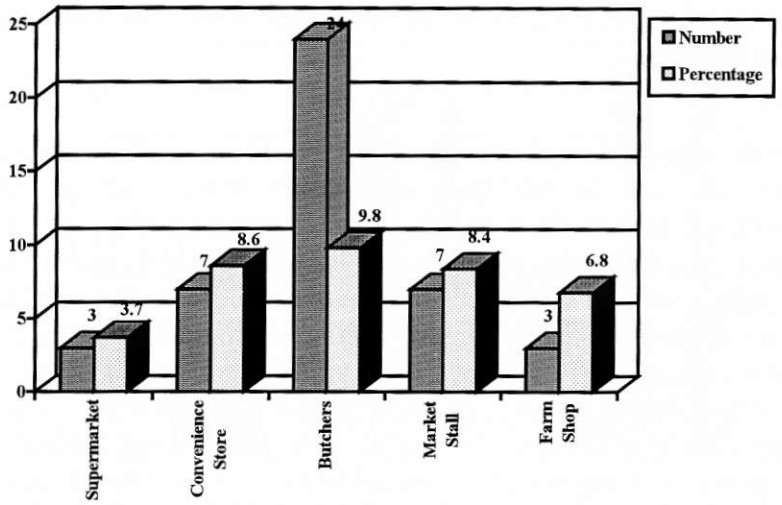

Figure 11 Number and percentage of mislabelled samples by outlet type

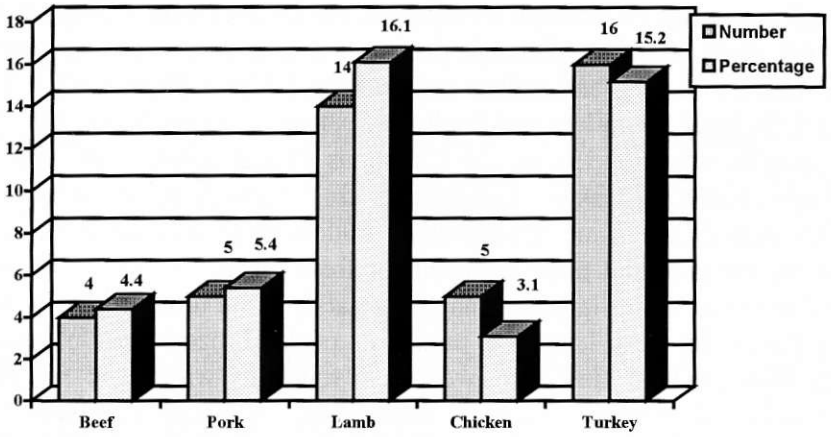

Figure 12 Number and percentage of mislabelled samples by species

The approximate confidence interval for the estimated prevalence of non-authenticity in meat is much wider for those three species, i.e. lamb, pigs and turkey, with relatively scant pre-surveillance data with respect to between-laboratory variation. However, the cut-off limits for the various species are felt to offer sufficient safeguard against either false positives or false negatives

that the level of mislabelling identified accurately reflects the situation at the time of the survey.

Although the analytical protocol required a second freezing/thawing cycle this data was not used in the final assessments because of the lack of corresponding data from the authentic pre-surveillance samples. However, it did produce some interesting results in that the level of HADH activity in some of the samples increased significantly after the second freeze/thaw cycle, while with others the level remained almost unchanged from the first cycle. This was surprising since it was generally assumed that the ratio of HADH activity of the first and second freeze/thaw cycles would approach unity. There was no obvious difference between the species. One possible explanation may be that the degree of disruption of the mitochondria is related to the age of the meat, with very fresh meat exhibiting tougher membrane systems than older meat which has undergone a certain degree of maturation. If this is so, it is one reason why it is not so reliable to depend on an absolute measurement of HADH activity within an unknown sample, but that R_1 should be established.

The overall proportion of samples identified as mislabelled was 8.2% (using a 99% cut-off point criterion) although this was split unevenly between beef, pork and chicken (3-5%) and turkey and lamb at 15-16% (Figure 12). The relatively high level for turkey and lamb may reflect to some degree the fact that the samples were taken over the Christmas period which is the time of year of highest sales of turkey. Demand for fresh turkeys is extremely high during this period and some unscrupulous traders may have been keen to capitalise on the premium price obtained for fresh birds. As far as lamb is concerned the level of mislabelling may relate also to the time of year in that the home season for fresh lamb was passed its peak. Frozen home-produced or frozen New Zealand lamb may well have found its way on to the chill counter without the requisite labelling declaration. Although provision was made within the sampling plan to ascertain the country of origin for all samples this information was not always available. Consequently, it is not possible to draw any firm conclusions regarding this aspect of the mislabelled samples. Apart from pork there appeared to be no difference in the level of mislabelling pre- or post-Christmas (Figure 13).

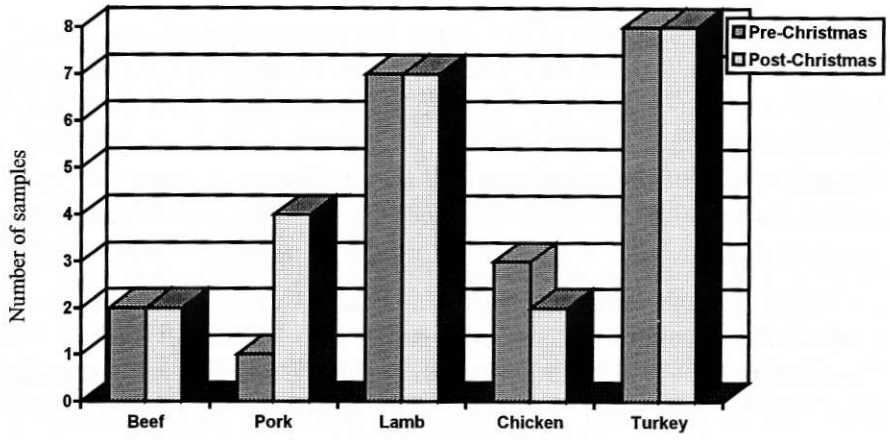

Figure 13 Number of mislabelled samples Pre- and Post-Christmas 1995

While mislabelled samples were identified in all retail outlet types it is interesting to note that only 3.7% of samples originating from supermarkets were mislabelled whereas for all other outlet types the level was between 6.8-9.8% (Figure 11). Only turkeys were found to be mislabelled from the supermarkets while butcher shops had non-authentic samples of all species. However, it should be stressed that the sampling plan did not reflect the retail sector in terms of market share for each outlet type.

Likewise, the sampling plan was not designed to indicate regional variation, but a more national picture, and therefore no conclusions can be drawn regarding the geographic distribution of mislabelled samples.

Conclusions - Part II

The overall incidence within the survey of mislabelling of previously frozen meat and poultry of 8.2% of the samples from this survey indicates that while most samples were correctly labelled a significant number of samples were misdescribed. Of particular concern were the levels of lamb (16%) and turkey (15%) which were found to be mislabelled.

All types of retail outlets sampled within the survey produced some degree of mislabelling. However, the incidence was less prevalent in large supermarkets, and about equal in the rest. Although in itself the practice of freezing and defrosting meat or poultry does not constitute a health risk it should be recognised that previously frozen meat or poultry is more susceptible to adverse effects arising from poor handling and storage regimes.

Follow-up action

A summary of the results of the survey has previously been made available to the public through the MAFF/Department of Health *Food Safety Information Bulletin*[10]. The individual Trading Standards and Environmental Health Departments which participated in the survey have been informed of the detailed results of the samples which they collected in order that appropriate action can be initiated. Relevant trade associations and retailers have been informed of the results of the survey through the membership of the MMPSG.

Following formal sampling Norfolk County Council has already taken a successful prosecution against one retailer under Sections 14 and 15 of the Food Safety Act 1990. Further action against retailers is being considered by other Local Authorities, including Cambridgeshire and Shropshire County Councils, as a result of subsequent sampling by these authorities.

Acknowledgements

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Thanks are due also to Dr Jeremy Hall, British Poultry Meat Federation, for help in supplying the authentic poultry samples, and to Mr Michael Fogden and Mr Mike Owen, Meat and Livestock Commission, for arranging supply of the red meat samples and for organising the collection, preparation and distribution of all the samples to the participating laboratories in the pre-surveillance trial. Also, to Whitbread plc for supplying meat samples for further method development work.

The author would also like to thank Dr Clifton Gay (MAFF) for his expertise and advice with the statistical analyses of the pre-surveillance and surveillance data.

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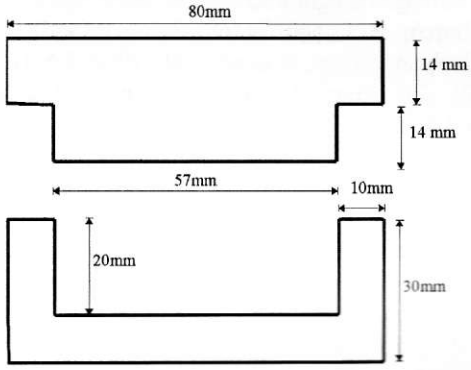
Appendix 1

MEAT PRESS

DESIGN

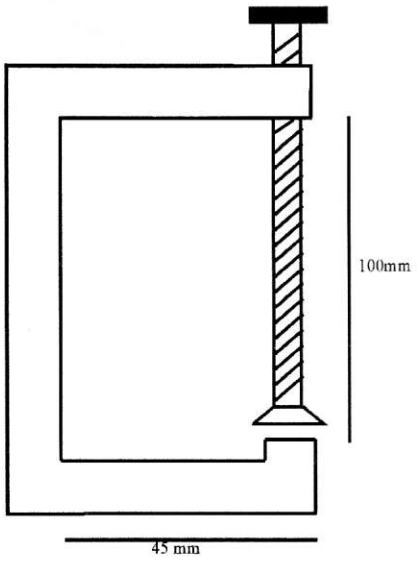
The meat press consists of a perspex container comprising a circular base unit and lid, and a G-clamp. The dimensions of each are given below in Figure 1.

Figure 1 Dimensions of meat press and G-clamp



Base Unit and Lid

G-clamp

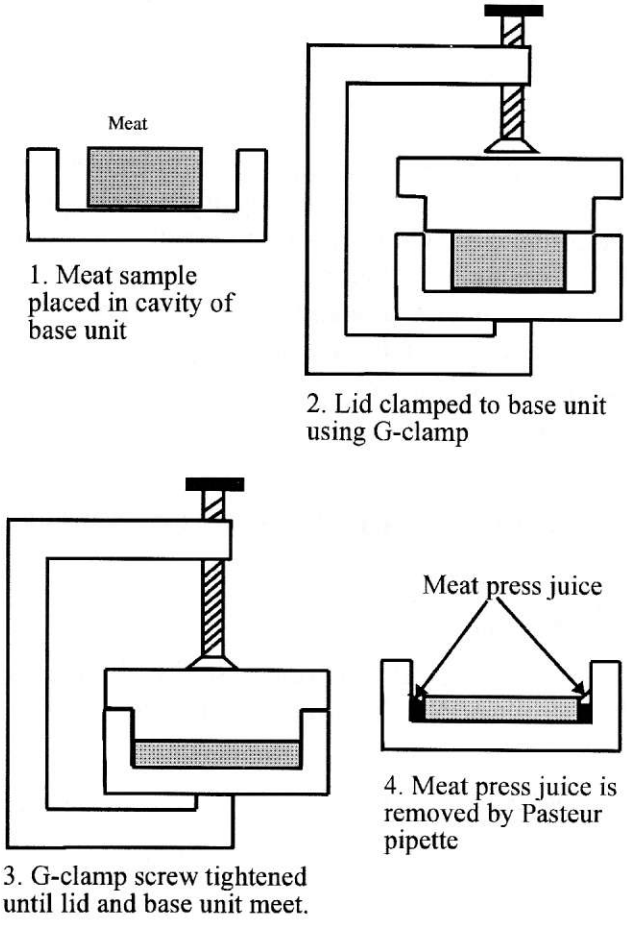


SYSTEM OF OPERATION

A sample of meat is cut to the required dimensions (30 × 30 × 20 (height) mm) and placed in the cavity of the base unit. The lid is applied to the base unit and clamped in place using the G-clamp. The G-clamp screw is then tightened until the lid and base unit meet and additional turns of the screw do not compress the meat further, i.e. at this point the meat sample has been compressed by an amount equivalent to the depth of the lid insert.

The G-clamp screw and lid are removed and the meat press juice which collects in the base unit is then removed using a Pasteur pipette (note that when the G-clamp is removed, hand pressure on the lid is still required to prevent the meat acting like a sponge resulting in the meat press juice being absorbed. These various stages are illustrated in Figure 2 below.

Figure 2 Operation of meat press



APPENDIX 2

ANALYTICAL PROTOCOL:

PROCEDURE TO BE FOLLOWED TO DIFFERENTIATE BETWEEN FRESH AND FROZEN/THAWED MEAT

Notes:

Before the survey commences and to ensure compliance with this protocol, laboratories will be responsible for liaising with the designated local authority with respect to:

1. *the number of retail samples which can be handled by the laboratory at any one time;*
2. *timing of sample collection to ensure maximum time from arrival at the laboratory to analysis does not exceed 24 hours.*

The samples will be despatched to the laboratories in insulated containers. Chilled conditions will be maintained by means of ice-packs. On arrival at the laboratory, the samples should be examined carefully to check for any evidence of surface freezing. If there are concerns about the condition of the samples, product temperature measurements should be made and recorded and any other relevant information noted in the report to MAFF. Retail samples may be stored at the laboratory (between 0° and +5°C) for a maximum of 24 hours prior to analysis.

METHOD FOR DETERMINING HADH ACTIVITY

1. Scope and field of application

1.1 The method is a photometric enzyme test for determining the β-hydroxyacyl co-enzyme A dehydrogenase (HADH) activity of meat press juice.

1.2 The method is not applicable to minced meat.

2. Definition

2.1 HADH activity is expressed in International Units per ml of meat press juice (U/ml). 1 U represents 1 micromole of substrate converted per minute at pH 6.0 and 25°C.

3. Principle

3.1 Three sub-samples should be prepared for analysis. The first sub-sample should be analysed directly (see 3.2 to 3.5). The second and third sub-samples should be slowly frozen for at least 2 days at -18°C ± 2°C. Following thawing the second sub-sample should be analysed and the third sub-sample re-frozen for at least 2 days at -18°C ± 2°C and then analysed (3.2 to 3.5).

3.2 The meat juice is expressed from the sample and diluted with a phosphate buffer.

3.3 Determination of the HADH activity is based on the following reaction:

$$\text{acetoacetyl-coenzyme A} + \text{NADH} + \text{H}^{+} \xleftrightarrow{\text{HADH}} \beta\text{-hydroxybutyryl-coenzyme A} + \text{NAD}^{+}$$

3.4 Aliquots of reagents and diluted press juice are pipetted into a quartz spectrophotometer cuvette.

3.5 Using a U.V. spectrophotometer the rate of conversion of NADH to NAD^{+} is measured by the rate of decrease in the absorption of the solution at 340 nm. The difference in HADH activities between the fresh and laboratory frozen sub-samples is then used to determine whether the meat sample has previously been frozen.

4. Health and Safety

4.1 EYE PROTECTION SHOULD NORMALLY BE WORN AT ALL TIMES.

4.2 CARE SHOULD BE TAKEN WHEN HANDLING THE MEAT PRESS.

4.3 CARE SHOULD BE TAKEN WHEN HANDLING SYRINGES.

5. Pre-training requirements

5.1 Use of meat press.

5.2 Use of U.V. Spectrophotometer.

5.3 Use of a pH meter.

5.4 Use of an analytical balance.

6. Reagents

6.1 Laboratory reagent grade (GPR) or analytical reagent grade (AR) reagents are suitable unless otherwise stated. Water should be de-ionised, distilled or of similar quality.

Note: *Laboratories are advised to check with suppliers on the availability of reagents since demand may be particularly high due to this survey.*

6.2 Phosphate Buffer (pH 6.0) (± 0.05).

6.2.1 Potassium di-hydrogen phosphate (KH_2PO_4) 13.6g (± 0.1g) made up to one litre with water.

6.2.2 Disodium hydrogen phosphate ($Na_2HPO_4.2H_20$) 17.8g (± 0.1g) made up to one litre with water.

6.2.3 To 1 litre of potassium di-hydrogen phosphate solution, (**6.2.1**) add the disodium hydrogen phosphate solution (**6.2.2**) slowly until a pH of 6.0 (± 0.05) is obtained. The solution can be stored under refrigeration (approximately 4°C) for at least one month. The pH should be checked before use.

6.3 EDTA solution (26.9 mMol/l) - Weigh out 0.500g (± 5 mg) ethylenediamine tetra-acetic acid disodium salt into a 50 ml beaker, dissolve in approximately 20 ml of water and transfer to a 50 ml volumetric flask with water, make up to the mark and mix. This solution can be stored under refrigeration for several months.

6.4 NADH solution (7.05 mMol/l) - As beta-nicotinamide adenine dinucleotide, reduced form disodium salt (anhydrous molecular weight 709.4):- Weigh the equivalent of 0.025g (± 2 mg) beta-nicotinamide adenine dinucleotide, reduced form disodium salt (Boehringer or Sigma) taking into account purity, moles of water and ethanol present, as given by the suppliers information sheet, into a 5 ml volumetric flask. Make up to the mark with water and mix. The solution can be stored for several days in a refrigerator.

6.5 Acetoacetyl - Coenzyme A solution (5.7 mMol/l) - Dissolve 0.005g acetoacetyl-Coenzyme A Na salt (Sigma, code A1625) in 1 ml of water.

Note: In practice, 5 mg of acetoacetyl-Coenzyme A is purchased, and is supplied in a small brown vial. Pipette 1.0 ml of water into the supplied container and mix. The solution can be stored for two days.

7. Apparatus

7.1 Normal laboratory glassware and apparatus.

7.2 Meat press: the press consists of a perspex container comprising a circular base unit and lid, and a G clamp and is illustrated in Appendix 1, Figure 1. The perspex container has been specially designed for this survey and is available at a cost of approximately £125 (including postage and packaging) from Dr Paul Lawrence, Laboratory of the Government Chemist, Queens Road, Teddington, Middlesex TW11 0LY (☎ 0181 943 7443).

7.3 Syringes and pipettes (e.g. Pipetteman) capable of the accurate measurement of 0.05 ml, 0.1 ml, 0.2 ml and 2.6 ml.

7.4 UV Spectrophotometer with a thermostat controlled cell holder at 25°C ± 1°C.

7.5 Stop watch.

7.6 Analytical balance capable of weighing to 0.001g.

7.7 Water bath at 25°C ± 1°C.

8. Procedure

8.1 MAFF will arrange for a series of 3 known standards of HADH activity (labelled 'A', 'B' and 'C') and 1 'blind' standard to be sent to each laboratory. The 3 standards labelled 'A', 'B' and 'C', should be analysed to test the procedure prior to testing any retail samples. The 'blind' sample should be included in the first batch of retail samples. Sufficient quantity of standard labelled 'B' will be supplied and it should be included in each batch of retail samples. The standards are to be analysed using the procedure set out below in sections 8.8 to 8.11 and following any additional instructions accompanying them.

8.2 Prepare three sub-samples from each sample using a scalpel, ensuring that only muscle is taken and that the sub-samples are as similar as possible. The sample must not be cut more than is necessary or comminuted as this will increase the HADH content of the press juice.

8.3 Two of the sub-samples from **8.2** should be cut to the required size for pressing (see **8.4**), sealed in separate bags, then placed in -18°C freezers on a plastic tray previously held at room temperature. The samples should be stored in the freezer for a minimum of 2 days before thawing. Do not fast freeze as slow freezing is important to ensure maximum disruption of the mitochondria for the release of HADH.

8.4 From the remaining sub-sample cut (using the scalpel) a cuboid shaped portion of flesh with base dimensions of approximately 30 × 30mm and a height of 20mm to be used for the HADH test. For the poultry cuboid it is necessary to have six cut surfaces. Wipe off any excess surface liquid on the sample with a tissue.

Note: It may be necessary to increase the height of poultry samples to 30mm to ensure adequate compression in order to obtain sufficient juice. If it is not possible to obtain a height of 30mm from a single cube cut from one breast fillet, then two cuboids stacked together to give a height of 30mm can be used. (Each cuboid must have six cut surfaces).

8.5 Place the test portion in the cavity of the base unit of the perspex container (Appendix 1. Figure 2). Insert the lid of the container into the base unit and clamp it in place using the G-clamp. Tighten the G-clamp screw until the lid and base unit meet such that the meat sample has been compressed by an amount equivalent to the depth of the lid insert and the press juice flows from the meat.

8.6 Remove the G-clamp screw and lid of the container. When the G-clamp is removed, hand pressure is required on the lid to prevent the meat acting like a sponge resulting in the meat press juice being absorbed. (Holding the press at a slight angle during and after compression helps the juice to flow away from the meat and minimises reabsorption). Recover the meat press juice which has collected in the base unit into a vial using an appropriate pipette.

8.7 If the juice cannot be analysed immediately it can be stored in the frozen state

(-18°C ± 2°C) for at least one week. Ensure that no pieces of flesh are present in the press juice if it is to be frozen.

8.8 The meat juice is diluted 1:200 with the phosphate buffer (pH 6.0) (**6.2**) to give a working solution.

8.9 To a 1 cm quartz spectrophotometer cell, add accurately by pipette 2.6 ml phosphate buffer (pH 6.0) (**6.2**) and 0.20 ml EDTA solution (**6.3**). Adjust the temperature to 25°C ± 1°C by placing the cell in a water bath (**7.7**). Add, by syringe, 50µl (± 2µl) NADH solution (**6.4**) and 100µl (± 2µl) of the diluted meat juice. Mix the contents of the cell by inversion.

8.10 To start the reaction add, by syringe, 50µl (±2µl) acetoacetyl-Coenzyme A solution (**6.5**) to the cell and mix by several inversions. Ensure no air bubbles are present.

8.11 Immediately wipe the cell with a tissue, place the cell in the spectrophotometer thermostat controlled cell holder (25°C ± 1°C) and measure the extinction at 340 nm (against air). Start the stop watch. Leave the cell in the spectrophotometer and after exactly three minutes again read the extinction at 340nm. The difference between the two readings Δ E is the decrease in absorption at 340nm.

Note: Should the time slightly exceed three minutes, as long as the time elapsed has been noted when the extinction is re-measured Δ E/min can still be calculated.

8.12 Repeat the spectrophotometer measurement for a second 100µl (± 2µl) aliquot of the diluted meat juice (**8.8 to 8.10**)

8.13 The two frozen sub-samples should be thawed for 24 hours (overnight) in a refrigerator (0°C to +5°C) after a freezing period of at least 2 days. Repeat the HADH test (**8.5 to 8.11**) on one of these samples.

8.14 The third sub-sample should be re-frozen (to -18°C ± 2°C) for a period of at least 2 days then thawed for 24 hours (overnight) in a refrigerator (0°C to +5°C). Repeat the HADH test (**8.5 to 8.11**) on this sample.

Note: For the frozen/thawed samples the 'drip' from the thawing process must be combined with the juice extracted by the meat press in paragraph 8.5.

9. Calculation

$$\text{HADH activity U/ml} = \frac{V}{C \times d \times a} \times \Delta E / \min \times \textit{dilution factor of meat juice}$$

where V = volume of test mixture = 3 ml

C = extinction co-efficient for NADH at 340 nm = 6.3

d = light path of cell = 1 cm

a = volume of diluted meat juice = 0.1 ml

T = time over which decrease in absorption is measured in minutes

$$\Delta E/min = \frac{\text{Extinction at start of reaction - extinction at T min}}{T}$$

The equation becomes:-

$$\text{HADH activity U/ml} = \frac{3}{6.3 \times 1 \times 0.1} \times \Delta E / \min \times \textit{dilution factor of meat juice} *$$

*(e.g. 200 for beef)

10. Expression of results

10.1 The individual HADH measurements and any other information required should be recorded on the attached form. Additional sheets may be photocopied as required.

10.2 Results should be quoted to the nearest 0.1 U/ml HADH activity.

10.3 All results should be sent to Dr Kevin Hargin by 16 February 1996 at the following address:

Ministry of Agriculture, Fisheries and Food
Food Labelling and Standards Division
Room 320, Ergon House
c/o Nobel House
17 Smith Square
London SW1P 3JR

10.4 All raw data should be retained by the laboratories until a final report of the work has been published by MAFF.

10.5 All invoices must be submitted to MAFF before the end of February 1996.

11. Disposal

11.1 No specific problems.

12. References

Gottesmann P and Hamm R (1982). *Fleischwirtschaft* **62,** 1301 - 1305.

Gottesmann P and Hamm R (1985). *Fleischwirtschaft* **65,** 591 -592

Billington M *et al* (1992). *J Assoc Publ Analysts* **28**, 103-116.

13. Analytical quality assurance

13.1 Performance characteristics

13.1.1 Limit of detection: not applicable

13.1.2 Bias (WRM and WRS): not applicable

13.1.3 Precision (Wp)

Wp_{12} (absolute) = 0.5 at 1.0 U/ml level. Separate dilutions of press juice used.

Wp_{12} (absolute) = 3.5 at 12.8 U/ml level.

13.2 Internal quality control

13.2.1 Instrument calibration: consult equipment manuals

13.2.2 Blank determinations: not applicable

13.2.3 Recovery check: not applicable

13.2.4 Reference material check: not applicable

Meat and Meat Products Sub-group Secretariat

November 1995

Appendix 3

The following laboratories participated in the analyses of samples for Part I and/or Part II of this paper:

Avon Scientific Services (I&II)
Birmingham City Council Envir. Services (I)
CSL Food Science Laboratory, Torry (I&II)
Derbyshire County Council PA Dept. (I&II)
Greater Manchester Scientific Services (II)
Hampshire Scientific Services (I&II)
Hereford & Worcester Scientific Services (I&II)
Humberside Scientific services (I&II)
Laboratory of the Government Chemist (I&II)
Lincolne, Sutton & Wood Ltd (I&II)
Strathclyde Regional Council, Glasgow (I)
Tickle & Reynolds PA Laboratory (I&II)
Unilever Research (I&II)

The following local authorities participated in the collection of samples for the survey:

Association of Greater Manchester Authorities
Avon County Council
Ballymena Borough Council
Cambridge County Council
City of Aberdeen District Council
City of Edinburgh District Council
City of Glasgow District Council
Derbyshire County Council
Devon County Council
London Borough of Kingston
London Borough of Sutton
Norfolk County Council
North Yorkshire County Council
Shropshire County Council
Surrey County Council