Setting standards
in analytical science

Presentation at NRL Meeting

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Agenda

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- EU Study on measuring physiological water content in poultrymeat
  - Objective
  - Legal Basis
  - Background
  - Key Features of EU Poultry Production
  - Samples & Sampling
  - Analysis
  - Reporting
  - Timescales
- Inter-laboratory trial of a method for the detection of previously frozen meat (HADH)

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EU Study on measuring physiological water content in poultrymeat

Objective

To analyse the physiological water content in breast filets and legs of chicken raised and slaughtered in the EU

- The results of this study will be compared with the results of the study made in 1993 to evaluate whether the limits of technically unavoidable water uptake (extraneous water) as a result of preparation and cooling of poultrymeat given in Commission Regulation (EC) No. 543/2008 are still relevant or whether they need to be revised.

Legal Basis

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- Consolidated marketing regulation for all agricultural products – Council Regulation (EC) No 1234/2007.
- Detailed rules for the poultrymeat standard are given in Commission Regulation (EC) No 543/2008.
- The standard gives limits of technically unavoidable water uptake (extraneous water) as a result of preparation and cooling:
  - 2% for air chilling
  - 4% for air spray chilling
  - 6% for immersion chilling
- Enforcement of these limits ensures that
  - poultrymeat is prepared according to good manufacturing and hygienic practice
  - consumers are not being disadvantaged by excess “added water” in the fresh poultrymeat they purchased

Background

- Poultry production in the EU is an intensive agricultural activity.
- Last study was published in 1993
- Since 1993, developments in respect of breeds, age at slaughter and the weight at slaughter have taken place across the EU
- Studies performed in the UK and Germany indicate a change in the proportion between physiological water and extraneous water in poultrymeat.
- Control data from NRLs
- Hence the rationale to re-examine W/P ratios across the EU.

Key Features of EU Poultry Production

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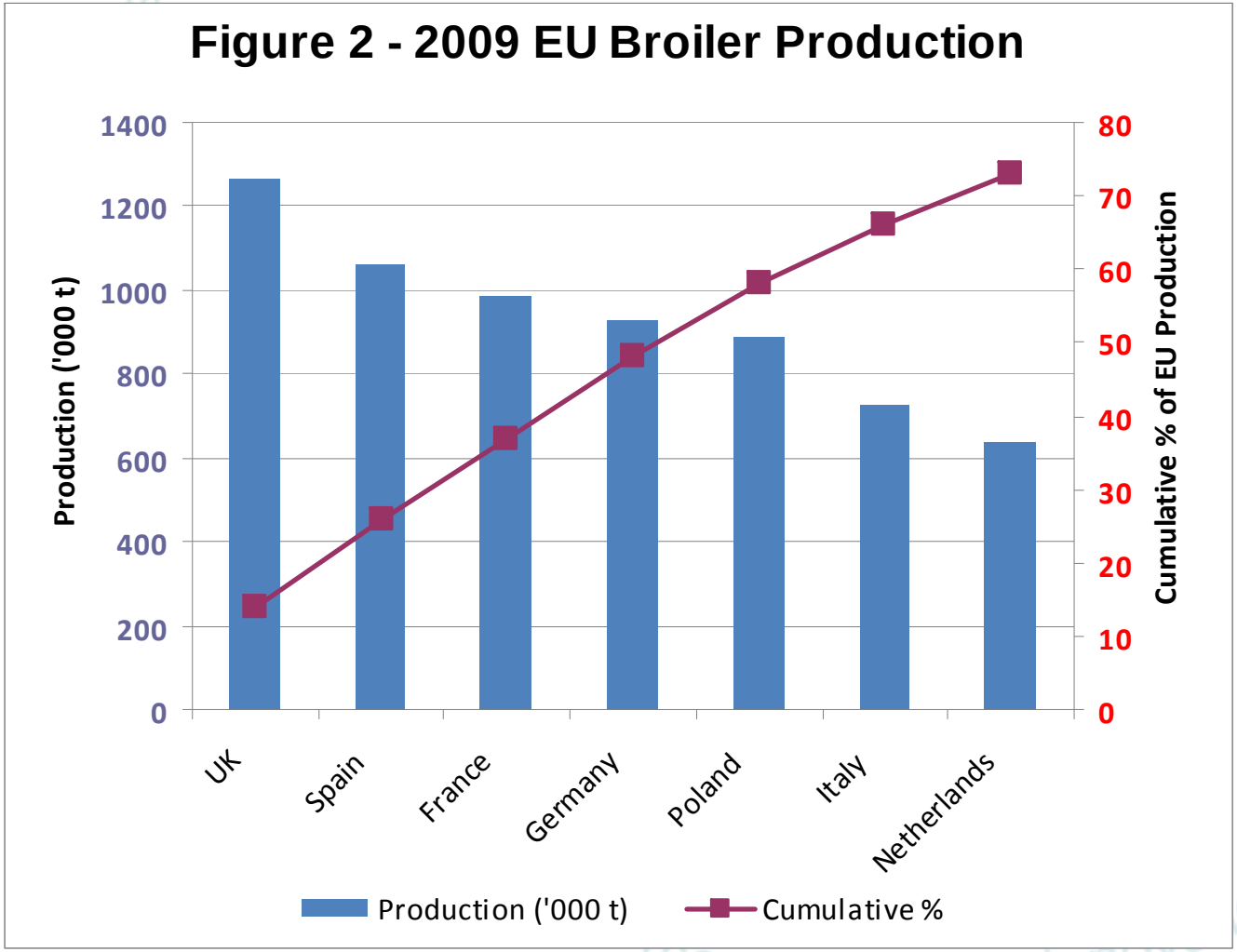
Discussions with UK poultry industry, German and Italian NRLs:

- Most common breeds of poultry produced in the EU:
  - Ross
  - Cobb
- Two class of weights, based on age;
  - Light (approximately 1.3-1.6 kg for 5-5.5 weeks)
  - Heavy (approximately 2.5-2.7 kg for 7-8 weeks)
  - Equal numbers will be taken
- Gender – both males and females important at both weights
  - evidence to suggest that 'Heavy females' may not be available in some Member States e.g. UK and Italy as they mature more quickly than males and reach commercial carcase weights earlier
- Most commonly consumed cuts chosen:
  - Breast (no skin)
  - Leg with skin

Sampling Countries



7 countries constituted just over 74% of the total production

Sampling Points

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- Samples will be collected from significant poultry producers in each of the 7 MS to allow all samples to be collected in one day
- In all MS except Poland:
  - samples will be collected by Slaughterhouse staff under the supervision of the NRL and UK NRL / UK expert external consultant.
- Poland
  - samples will be collected by Slaughterhouse staff under the supervision of the UK NRL / UK expert external consultant.
- A member from the Steering group committee may also attend each MS to witness sampling

Sampling protocol – per country

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Full Factorial Balanced Design of the Key Variables Associated with Commercial Poultry Production in the EU

Breed:	**Ross**				**Cobb**				**Total**
Flock Number:	1		2		2		4		
Weight:	Light	Heavy	Light	Heavy	Light	Heavy	Light	Heavy	
Female:	***3***	***3***	***3***	***3***	***3***	***3***	***3***	***3***	***24***
Resultant Breast:	3	3	3	3	3	3	3	3	24
Resultant Leg:	3	3	3	3	3	3	3	3	24
Male:	***3***	***3***	***3***	***3***	***3***	***3***	***3***	***3***	***24***
Resultant Breast:	3	3	3	3	3	3	3	3	24
Resultant Leg:	3	3	3	3	3	3	3	3	24

Sampling protocol – total

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Per Member State:

Total number of birds:	**48**
Total number of breast samples for analysis:	48
Total number of leg samples for analysis:	48

From 7 Member States:

Total number of birds:	**336**
Total number of breast samples for analysis:	336
Total number of leg samples for analysis:	336

Samples

- Samples taken from two defined flocks / batches in each country.
- Equal numbers of light and heavy birds.
- Equal numbers of male and female birds.
- The samples will consist of chicken breast filets without skin and chicken legs with bones and skin.
- Breasts samples will be removed by cutting the skin (without plucking), whilst leg samples will be plucked by hand.
- **The sampling will be performed without the use of water.**
- LGC will provide individual air-tight bags and labels for every sample to be collected.
- Once sampled, samples will be placed in individual air-tight bags and sealed. Each bag will be labeled with an identification number stating country, origin, breed, weight, age, cut and date of sampling.
- All samples taken in each country will be frozen (18°C or below) and sent by overnight courier to LGC.

Sampling SOP

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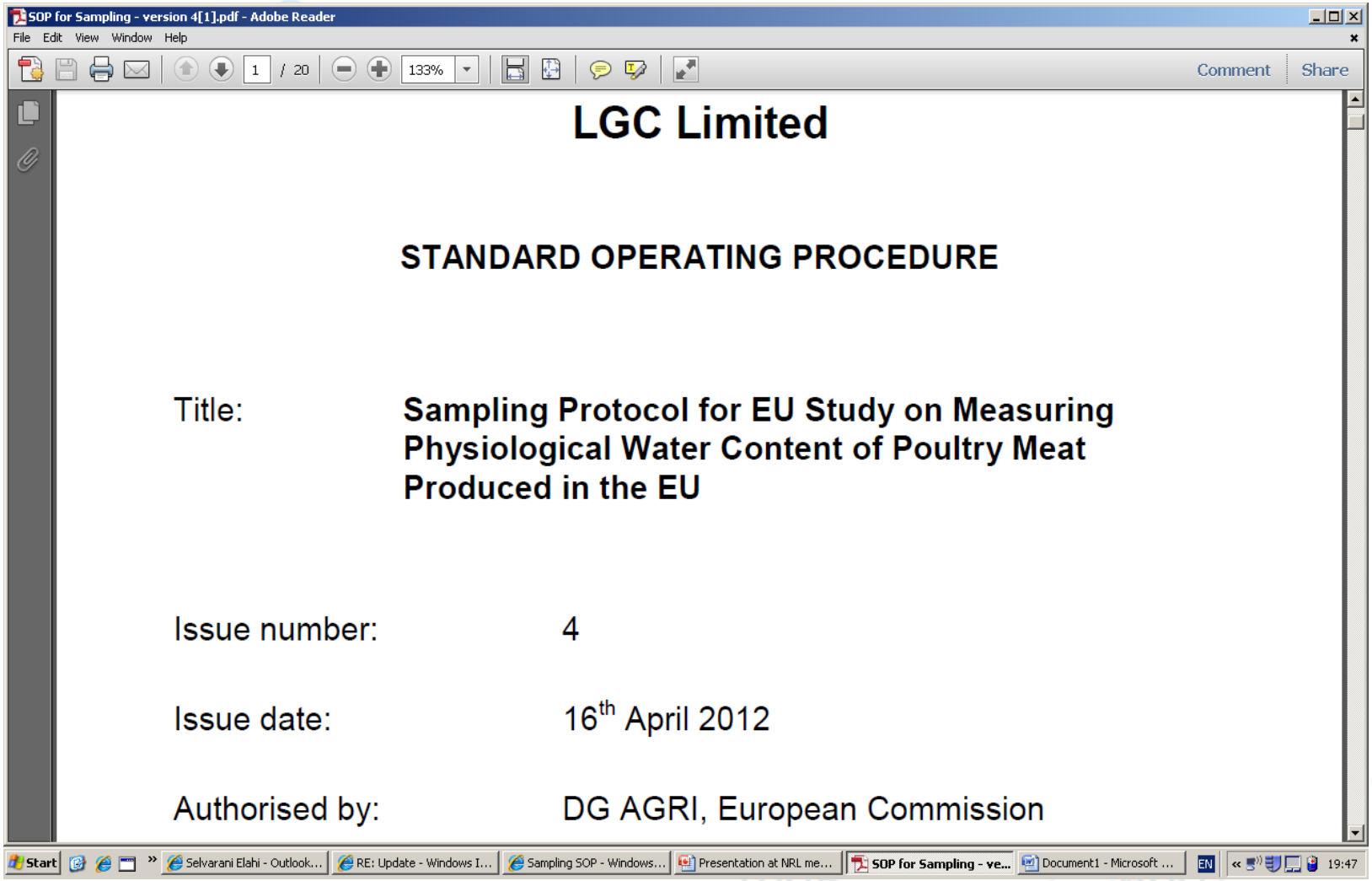

Has been agreed with the steering group and will be supplied to the NRLs and Slaughterhouses in advance of sampling

Samples at LGC

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LGC will:

- Record arrival on LIMs
- Keep the samples frozen until required for analysis.
- Only process samples from one country at any one time.
- Prepare a SOP for sample preparation
- Train two dedicated technicians for sample preparation
- Homogenize all the samples for this study in their frozen state in accordance with the requirements Regulation 543/2008/EC.
- NOT composite samples . Analysis of individual samples will permit calculation of the variation due to Country, Breed, Weight, Laboratory, Individual test sample.
- Place each individual homogenized sample in a minimum of two samples pots (pot 1 - Analysis by NRL, pot 2 - Reference pot)
- Store all homogenized samples frozen until required for analysis.

Samples at LGC

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- Once samples from all 7 countries have been homogenized
- A stratified sampling plan will be used to select samples for each laboratory so that participating laboratories analyse a carefully selected set of materials from all sampled countries. This will:
  - prevent 'confounding' of laboratory and country effects which would otherwise make it impossible to distinguish genuine differences between countries of origin from spurious differences due to laboratory procedures.
  - Suitable statistical analysis will then permit separate tests for significance of between-country differences in test items independently of laboratory effects.
  - In addition, it will allow any anomalous laboratory effects to be identified. The distribution plan will be produced in consultation with the LGC Statistics team.

Participating Laboratories

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1	UK
2	Germany
3	Italy
4	Netherlands
5	Spain
6	France
7	Ireland
8	Denmark

Analysis

- The samples and a quality control material (e.g. LGC RM 7152, Processed Pork) will be sent frozen by overnight courier to the participating countries.
- A SOP for analysis will be prepared which will detail requirements for analysis. This will include:

a) No. of replicates required per sample – 3.
b) Agreement required between the three replicates.
c) Requirement to analyse the RM in duplicate in every analytical batch and assess performance against the limits supplied.
d) Instructions for repeats to be performed if b and or c above result in failures.
e) Details of the methods to be used:
   - The water content by the oven drying method as described in Regulation 543/2008 (ISO 1442:1997, Meat and meat products - Determination of moisture content (Reference method).
   - The amount of nitrogen for the protein calculation shall be determined by the Kjeldahl or equivalent method according to Regulation 543/2008 (ISO 937, Meat and meat products - for the determination of protein content or equivalent).

- A standard reporting sheet for completion by each participating laboratory

Statistical Analysis

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- The results will be analysed by LGC's statistics team. The data will be analysed to detect differences between:
  - Breed, Weight, Gender, Country, Cut & Laboratory effects

Statistical analysis will include:

a) outlier identification methods of ISO 5725 and additional techniques as appropriate. Identified outliers will be reviewed, checked and corrected if appropriate. It is not foreseen that outliers will be removed from the data set unless deemed so extreme as to indicate serious technical error in measurement.

b) Multi-way analysis of variance to test for the significance of the above effects compared to measurement variance

c) Variance component extraction to establish the individual variance contributions.

d) Estimation of the mean Water content, protein content and W-P ratio

e) Calculation of confidence limits appropriate for the sample size used in current regulations.

f) Comparison of results with the results of the 1993 study to evaluate whether the limits of technically unavoidable water uptake (extraneous water) as a result of preparation and cooling of poultry meat given in Commission Regulation (EC) No. 543/2008 are still relevant or whether they need to be revised .

Draft Report

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- The preliminary final report will be submitted end of ‘Sep 2012
- A leaflet / paper for submission to a peer reviewed journal
- A PowerPoint presentation of the study and its results
- Study report
- Draft final report will be submitted end of ‘Nov 2012

Gantt Chart

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Key Tasks	Month 1 Dec 2011	Month 2 Jan 2012	Month 3 Feb 2012	Month 4 Mar 2012	Month 5 April 2012	Month 6 May 2012	Month 7 Jun 2012	Month 8 Jul 2012	Month 9 Sep 2012	Month 10 Oct 2012	Month 11 Nov 2012
Task 1: Project initiation meeting with steering group in Brussels	⇨										
Task 2: Finalisation of sampling protocol including poultry producers to sample from in the 7 Ms	⇨	⇨									
Task 3: Preparation of sample bags and identification numbers and dispatch to participating NRLs / participating poultry processors	⇨	⇨									
Task 4: Sequential sampling of poultry from 7 Member States and dispatch of frozen samples to LGC.		⇨	⇨	⇨							
Task 5: Finalisation of analytical protocol and agreement by the Steering Group via teleconference.			⇨	⇨	⇨						
Task 6: Sample preparation and frozen dispatch of homogenised portions to eight participating laboratories.					⇨	⇨					
Task 7: Laboratories to analyse samples and QC material and report back to LGC.					⇨	⇨	⇨	⇨			
Task 8: LGC statistics team to analyse results from the 7 MS and produce a statistical report.							⇨	⇨	⇨		
Task 9: Submit the preliminary final report to the steering group in a									⇨	⇨	
Task 10: Discuss results of the study with steering group in a meeting in Brussels										⇨	⇨
Task 11: LGC to send final report to the Steering Group and DGAGRI.										⇨	⇨

Progress to date

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- 50% samples taken from UK
- 100% samples taken from Germany
- Spanish sampling dates confirmed
- Italian sampling week confirmed
- French sampling week confirmed
- Netherlands – awaiting confirmation
- Poland – awaiting decision on slaughterhouse request to keep identity of farms anonymous

Issues Encountered

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- Breed differences
- Sub breeds e.g. Ross 308, 708 etc..
- Weight differences
- Slaughterhouse schedules only set a week in advance at most!
- Availability of breeds and required weights within 2 days
- Gender identification on the line
- Ideally 4 staff required on sampling days (min 3)
- UK NRL staff prepared samples
- Poland – request to keep identity of farms anonymous
- Sampling SOP has been refined as a result of above
- UK NRL staff are fast becoming poultry experts!

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Method to Detect Previously Frozen Poultry (HADH)

Background

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- Poultry is a highly perishable food
- At 0°C shelf life is ~10 days
- Consumers perceive ‘fresh’ poultry to be superior to frozen
- Attracts a higher retail price
- Fresh/chilled poultry for the EU market can only be achieved by EU production
- Third country poultry enters the EU as frozen usually for further processing.

Legislation

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Council Regulation 1234/2007

- Defines poultrymeat
- Poultrymeat (whole birds and parts) can only be marketed as:
  - 'Fresh' or 'Chilled' – stored between -2°C and +4°C
  - Frozen – stored -12°C or less
  - Quick frozen – stored at not higher than -18°C
- Previously frozen poultry after thawing cannot be marketed as 'fresh' or 'chilled'

HADH method

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Rationale

β- Hydroxyacyl-Co A dehydrogenase (HADH)

- Enzyme naturally present in muscle mitochondria
- It is released when cells are damaged as part of the freeze – thaw process
- Increased amounts of HADH in juice expressed from a meat sample indicates that the meat had been previously frozen
- Measured colorimetrically :-

Original MAFF Study 1997 – Key Conclusions

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1. It was possible to differentiate fresh meat from that which had been previously frozen to -18°C for the following species and cuts:

- Beef: Rump, Topside, Silverside, Sirloin
- Pork: Leg, Chops
- Lamb: Leg, Chops
- Chicken Breast
- Turkey Breast
- Duck Breast

Original MAFF Study 1997 – Key Conclusions

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2. Does not work for liver or kidney from beef, pork or lamb.
3. No significant variation between carcases within a species.
4. No significant variation due to age of meat in chicken breast or beef sirloin up to 28 days old.
5. No significant difference in HADH activity in the beef, pork, lamb and turkey samples above -12°C.
6. For chicken breast there was a significant difference in samples below - 9°C and some difference between -12°C and -18°C.
7. Participants had difficulty using the press with chicken breast and soft meats.

Cut-off Ratios Established

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- The average HADH activity levels in meat which had never been frozen were determined prior to the survey.
- From the comparative analysis of the survey samples, a ratio of the HADH activity levels before and after freezing was determined for each species.
- The ratio above which a sample was considered to have been previously frozen was calculated for each species:
  - beef rump 0.64
  - chicken breast 0.90
  - lamb chops 0.39
  - pork chops 0.47
  - turkey breast 0.63

FSA/LGC study 2006 – Key Conclusions

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Chicken

- Significant difference between fresh or superchilled chicken and previously frozen chicken
- But very high R1 cut-off value (0.9):
  - Would result in a large number of false negatives
  - i.e. previously frozen chicken would have an R1 value lower than 0.9, and thus could not be distinguished from fresh chicken
- This cut-off value was obtained using the original pressing protocol
  - was found to cause highly variable results in the current study.
  - several laboratories reported difficulties in using the pressing protocol, particularly with soft tissue such as offal and fresh chicken
  - increased variability resulting from problems in obtaining reproducible samples of meat juice from fresh chicken may have led to the higher R1 cut-off value obtained.

FSA/LGC study 2006 – Key Conclusions

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- Fresh chicken data for chilled chicken breast gave:
  - Mean R1 value for fresh chicken of 0.18
  - With a standard deviation (SD) of 0.109
  - Using a factor of 2.8*SD to estimate the 99% cut-off limit
  - The new recommended R1 limit for fresh chicken should be 0.5.

<u>Turkey</u>

- Possible to distinguish between fresh or superchilled turkey and previously frozen turkey (regardless of which freezing process is used)
- Existing R1 value (0.62) continues to be appropriate for survey and enforcement purposes.

Effectiveness of Method to Deal with New Technologies

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- Superchilling – chicken and turkey is indistinguishable from fresh
- Arkansas or Rapid Freezing – chicken and turkey can be differentiated from fresh or superchilled
- Conventional Freezing - Possible to distinguish between fresh or superchilled

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Inter-laboratory Study of Improved HADH Method 2012

Aim

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To carry-out an inter-laboratory trial of an improved HADH method for detection previously frozen poultry

Laboratories:

- 12 UK OCLs – funded by Defra
- 12 NRLs – funded by European Commission

Timescale:

- April – December 2012

Objectives

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1. Study Design
2. Manufacture of Presses
3. Practice Analysis by Laboratories
4. Sample Collection
5. Sample transport
6. Inter-laboratory trial

1. Draft Study Design

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- Each laboratory will be sent 6 chilled chicken breasts and 6 previously frozen and defrosted chicken breasts.
- A duplicate analysis on each chicken breast will be requested.
- The HADH method requires one portion of a sample to be analysed as received and the other after freezing and defrosting
- Therefore 12 chicken breasts are required for each participating laboratory.
- UK: 12 laboratories = 12 x 12 = 144 chicken breasts.
- EU: 12 NRLS (for added water in poultry) = 12 x 12 = 144 chicken breasts
- Total number of samples required = 288
    - 144 chilled chicken breasts.
    - 144 conventionally frozen chicken breasts.

2. Manufacture of Presses

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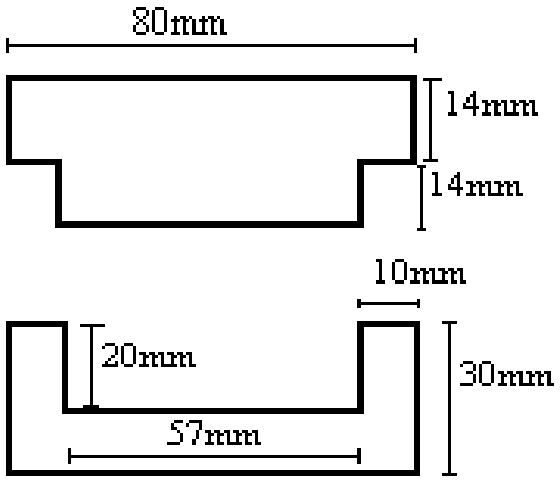
- To ensure that the method is consistently applied by all participating laboratories, presses will be supplied.
- The National Physical Laboratory's workshop will manufacture 30 Perspex presses



Base Unit and Lid

3. Practice Analysis by Laboratories

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LGC will issue:

- a press
- the method, in the form of a SOP
- instructions for a practise analysis to each participating laboratory. Laboratories will be asked to:
  - purchase chilled poultry samples locally
  - freeze one of the practise samples and thaw before analysis
  - Analyse fresh and frozen and thawed sample using the SOP
  - Report results to LGC
  - LGC will analyse data to assess whether all participating laboratories are able to successfully deploy the method before the trial samples are issued.

4. Sample Collection

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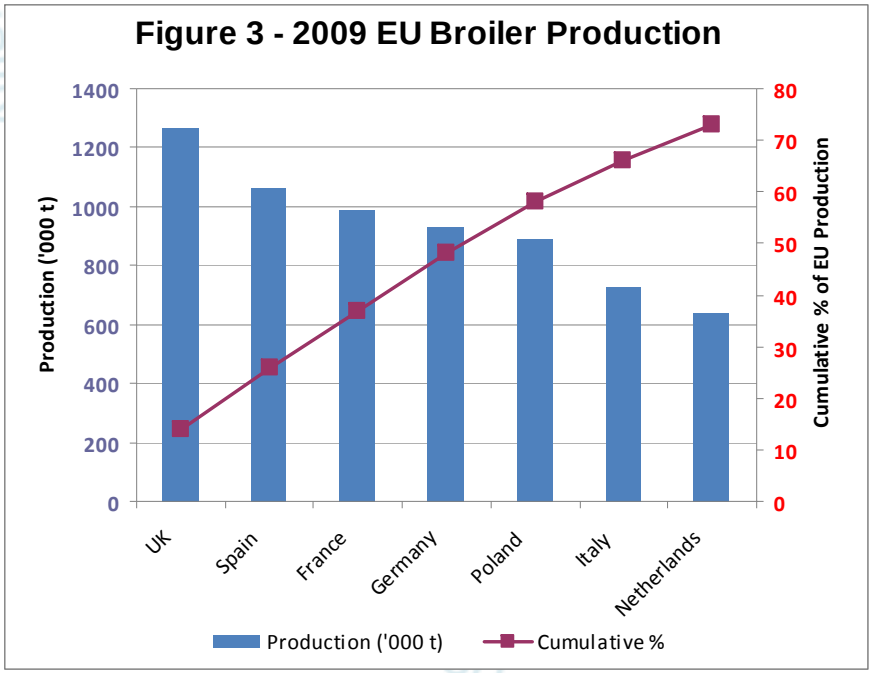
- LGC will be working with a UK company who will provide all the samples for this study.
- They will be packed in an insulated box packed with ice packs at the company under the supervision of LGC staff.

5. Sample transport

- Samples will be transported to:
  - UK OCLs using Bernard Matthews refrigerated vans
  - NRLs in EU by overnight courier



Invitations will be extended to the NRLs in the above countries + an additional 5 NRLs

6. Inter-laboratory trial

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- Before the samples are dispatched, LGC will issue:
  - instructions for the trial
  - including a detailed timetable of when samples are to be analysed.
  - laboratories will be asked to commit to undertaking sample analysis within the period specified as a condition of receiving payment for their services.
- Each laboratory will receive:
  - 6 chilled chicken breasts
  - 6 previously frozen and defrosted chicken breasts.
  - QC sample

6. Inter-laboratory trial – results sheet

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Sample No.	R1 Value[4]			
	Chilled chicken breasts		Previously frozen and defrosted chicken breasts	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Sample 1				
Sample 2				
Sample 3				
Sample 4				
Sample 5				
Sample 6				

6. Inter-laboratory trial – statistical evaluation

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- The final evaluation and results of the inter-laboratory testing performance will be evaluated on the basis of the "International Standard ISO 5725-2:1994 - :2010: Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.“
- "The study will additionally respect relevant provisions of ISO/IEC 17043 where they do not conflict with the primary aim of the study."

6. Inter-laboratory trial – reporting

- The results of this trial will be presented to Defra and EC as a scientific report.
- Once the scientific report has been accepted by Defra and EC, a peer reviewed paper will be published in a relevant journal, ideally one that is open access.


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