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Project Name: An evidence based review of the state of knowledge on methods for distinguishing mechanically separated meat (MSM) from desinewed meat (DSM)

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Executive Summary

Regulation (EC) No 853/2004 defines mechanically separated meat (MSM) as the product from mechanical separation of residual flesh from bones where there has been loss or modification of the muscle fibre structure. MSM cannot count towards the meat content of products for the purposes of Quantitative Ingredient Declaration (QUID) requirements in EU Food Labelling legislation.

In a previous study carried out by Leatherhead Food Research, low pressure MSM (previously referred to as desinewed meat (DSM) or 3mm meat) has been shown to have a considerable amount of intact muscle fibre structure similar to some meat preparations (made from hand deboned meat (HDM)) and was very different to high pressure MSM. Based on this research and analytical evidence in the literature, DSM was considered in the UK to fall within the definition of 'meat preparations' in EU food law rather than that of MSM.

An audit by the Food and Veterinary Office of the European Commission (FVO) was conducted in March 2012 and led to a change in UK policy to align with the Commission's interpretation that DSM was treated in all respects as MSM, including for the purposes of QUID. This has significant economic implications as the value of the low pressure MSM is considerably reduced. It is accepted that there is no evidence of any increased food safety risks associated with low pressure MSM (DSM).

This document is an evidence-based review of the state of the art of methods for the identification of mechanically desinewed or 'low pressure' meat (DSM) and MSM and methods that might be used to distinguish between DSM, HDM and MSM. It is intended to provide support for making a case at EU level for a separate EU definition and associated hygiene requirements for DSM obtained from non-ruminant bones.

The document summarises key points from a large number of publications and also from the European Food Safety Authority (EFSA) scientific opinion on the public health

risks related to MSM derived from poultry and swine¹ which was published on 27 March 2013. The literature search for this review and the EFSA report were extensive and covered a wide range of techniques and approaches. The bulk of the publications covered composition of samples produced under different conditions and from different cuts of meat, identification of markers for MSM and the use of histology as a means of assessing MSM.

Despite the large amount of research published, no one individual parameter was identified as being suitable as an indicator of mechanical separation per se. This review has shown that there is no single method or approach that is available that can be used to distinguish between low pressure MSM and a meat preparation made from HDM. Histology can distinguish between high pressure MSM and low pressure MSM / HDM. EFSA identified calcium and cholesterol as potential markers for MSM with histology also being considered as promising; however, as EFSA did conclude, none of these markers differentiated DSM from MSM and HDM.

In summary from the literature there were very few published studies that compared DSM with MSM and HDM and these were mostly histology based comparisons. The main aim was to see whether it was possible to distinguish high pressure MSM from DSM and to see whether DSM was similar to HDM. If it is a requirement to differentiate DSM from HDM then it will be extremely difficult to do this analytically. The research to date has shown that low pressure MSM (DSM) is similar to HDM which has been minced or processed. There is nothing that is published either from this review or from the EFSA report that clearly distinguishes low pressure MSM (DSM) from hand deboned material. Methods that rely on chemical or compositional changes are not consistent indicators and the levels in DSM and HDM vary to such an extent that overlaps preclude these as being suitable for distinguishing DSM from HDM. Muscle fibre structure is modified in HDM if minced or frozen and so can be similar to DSM. The presence of bone can often be higher in HDM depending on the skill of the deboning so that is unreliable as a marker.

¹ <http://www.efsa.europa.eu/en/efsajournal/pub/3137.htm>

An area that has not been covered in the literature and not considered in the EFSA report is the effects of desinewing on the structure of the muscle fibre. HDM preparations and machine deboned products often involve a desinewing stage, which is used to remove sinew and connective tissue, and this will damage the muscle fibre structure in a similar manner to the mechanical separation itself. This means that HDM could be minced then desinewed and have a similar structure to low pressure machine deboned products (i.e. low pressure MSM (DSM)).

Although no one method has been identified to distinguish between the types of products, in considering the research available and the EFSA report, one approach to distinguish DSM from HDM could involve a multivariate analytical approach with a cascade or decision tree as the best method. The decision tree would include levels of calcium, fat, oxidation behaviour, nuclei damage, muscle fibre integrity and a measure of texture. There should be categories where the sample fits high confidence limits that it is hand deboned, and categories where it is a high confidence that it is low pressure MSM (DSM) or high pressure MSM. Overlap or “grey” areas should also be included and a decision taken as to how to label these for legislative purposes.

Recommendations for future research to assist in development of a robust test for distinguishing low pressure MSM (DSM) from MSM and HDM include the evaluation of a quantitative histological method based on the UK and German technique. The histology method developed by the UK (Groves, 2011) is similar to that used in Germany and is simple enough to be used in many companies and enforcement laboratories. It clearly relates to the current legislation and can clearly distinguish between high pressure MSM and low pressure MSM (DSM). It therefore could be considered as a suitable method for a measure of the quality of the sample in terms of loss or modification of muscle fibre structure. It lacks quantification so future research should look at this aspect, especially the use of “free” good quality image analysis software that is available. The advantage of this approach is that it is in alignment with both the UK and German system and similar to the French system. A common definition of the level of muscle loss or modification would need to be agreed.

In addition to a histology approach recommendations include investigations of the potential of spectroscopy techniques, antibody methods and an approach that combines composition, structure (muscle fibre, nuclei and fatty tissue) and texture. It might be possible to combine some aspects of future research with a new EU FP7 funded project looking at development of an in-line method for assessing meat quality on machine deboned material. The workplan has been agreed and practical work is about to commence. This EU project will provide many samples of chicken from different cuts of meat and these could be used to evaluate any differences between HDM and DSM using methods that are not included in the project such as texture and hyperspectral imaging.

Abbreviations

AMR	Advanced meat recovery (machined deboning systems)
CNS	Central nervous system
DSM	Desinewed meat (low pressure MSM)
HDM	Hand deboned meat
MSM	Mechanically separated (removed, deboned) meat / material
MRM	
MDM	
PUFA	Poly-unsaturated fatty acids
TSE	Transmissible spongiform encephalopathies

Background

Legislative context

MSM is defined in EU food hygiene legislation (Regulation (EC) No 853/2004 of the European Parliament and of the Council) as follows:

“Mechanically separated meat’ or ‘MSM’ means the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure”.

If a product falls within the definition of mechanically separated meat, it falls outside the definition of “meat” laid down in Commission Directive 2001/101/EC (Commission Directive 2001/101/EC amending Directive 2000/13/EC relating to the labelling, presentation and advertising of foodstuffs) .

As such, MSM cannot count towards the mandatory meat content declaration for the purposes of Quantitative Ingredient Declaration (QUID) required under Directive 2000/13/EC (implemented by the Food Labelling Regulations 1996 as amended).

Therefore, the presence of MSM (and the animal species from which it comes) must be declared separately in the ingredients list.

When Regulation (EC) No. 853/2004 was adopted, the technology for an objective assessment of loss or modification of the muscle fibre structure of meat was not available. Hence, determining whether a product satisfies the MSM definition in that regard is usually based on the processing method, or method of production.

Regulation (EC) 853/2004 provides a definition for meat preparations as follows:

“Meat preparations’ means fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.

Therefore, according to the Regulation, muscle fibre structure is highly relevant in differentiating between MSM and meat preparations.

Regulation (EC) No. 853/2004 also sets down different rules for MSM produced by techniques that do not alter the structure of the bones and those that do. This is based on whether the product has a calcium content that is not significantly higher than that of minced meat, for which a limit is set down in Regulation (EC) No. 2074/2005.

Difficulties in interpreting the MSM definition within EU Member States have been acknowledged at EU level. A European Commission Communication to the European Parliament and Council published in late 2010

(http://ec.europa.eu/dgs/health_consumer/docs/msm_report_20101202_en.pdf)

acknowledged inconsistency in interpretation and application across the EU. The Commission’s Food and Veterinary Office (FVO) identified many instances of non-compliance during their audits of official controls undertaken in several Member States in 2012 and 2013.

It was a difference in interpretation of the MSM definition in EU food law between the UK and the European Commission that led to the introduction of the UK DSM moratorium. An FVO audit of UK official controls on MSM conducted in March 2012 concluded that the UK's interpretation that DSM obtained from bones does not fall within the definition of MSM and instead falls within the definition of 'meat preparations' was not in line with that of the Commission whose interpretation is that it is MSM.

UK policy was subsequently changed to align with the Commission's interpretation and the policy change was introduced by the Food Standards Agency (FSA) by means of a moratorium. Under the moratorium the production of DSM from ruminant (cattle, sheep and goats) bones was prohibited from 28 April 2012 and from 26 May 2012, DSM from non-ruminant (poultry and pork) bones has been required to be produced in accordance with the hygiene requirements for MSM and treated in all other respects as MSM, including for the purposes of labelling .

In terms of food safety, the FSA has publicly stated that there is no evidence of any increased food safety risks associated with DSM obtained by mechanical separation or the process by which it is produced.

Industry implications

Classifying DSM as MSM has economic implications for the meat industry and the consumer within the EU (especially in the UK where DSM has previously been permitted as a meat preparation) as well as for sustainable food manufacture and waste reduction. The British Meat Processors Association estimated a cost of £200 million to the UK economy. Following the Commission's ruling, the Food Standards Agency asked the European Food Safety Authority (EFSA) to deliver a scientific opinion on the health risks associated with MSM obtained from the bones of poultry and swine. The EFSA opinion was published in March 2013 ([EFSA opinion 2013](#)) and describes methods for determining whether a product is MSM, together with recommendations for further research. The EFSA Opinion is expected to provide the basis for EU level working group discussions expected to begin in 2013/2014 and this report will help inform the UK position.

Aims and objectives

Leatherhead Food Research (LFR) was commissioned to conduct an evidence-based review of the state of the art of methods for the identification of DSM/MSM and methods that might be used to distinguish between DSM and MSM and HDM, to support discussions at EU level for making a case for providing a separate EU definition and hygiene requirements for DSM obtained from non-ruminant bones. The objectives were to summarise the main findings from a large body of evidence and to comment on the potential for specific methods that could be developed further. In addition, the objective to critically assess the EFSA report and evaluate any areas for additional research was included.

The scope was to look at all published literature but not to consider ruminant material. Objectives to obtain evidence on the microbiological status of MSM and consequent risks were also beyond the scope of this project but where relevant papers assessed this aspect, these have been included. The FSA has carried out and published a provisional study into the microbiological status of MSM². However, it must be noted that MSM must be produced in line with food hygiene regulations and meet all relevant microbiocriteria set out in Regulation (EC) No. 2073/2005.

The approach looked at all methods that were used to analyse for HDM, DSM and MSM, and an objective was to attempt to identify the key papers in each area of analysis. Another was to consider the need to identify the loss or modification of muscle fibre structure as set out in Regulation (EC) No 853/2004.

This report is the result of that study and is intended to inform policy makers on the current methods for distinguishing between DSM, HDM and MSM and to support making a case at EU level for providing a separate EU definition and hygiene requirements for DSM obtained from non-ruminant bones. This will allow a common understanding from the literature of the best methods to ensure that any future,

² Description of the processes used in the UK to manufacture MSM and former DSM meat products from poultry and pork and an initial assessment of microbiological risk. James, C ; Purnell, G; James, S. http://www.foodbase.org.uk/results.php?f_category_id=&f_report_id=826

proposed legislative changes can be enforced effectively whilst minimizing waste, increasing sustainability and maintaining consumer safety and information.

Current Methods to Classify MSM

There are several current methods used to distinguish MSM, DSM and meat which have developed independently within the EU. These are published methods obtained from the literature but mainly also known to the authors from personal communication. These are summarised below:

UK

The method developed and used in the UK was funded by the Government's Food Authenticity Programme (Groves 2011)³ and is based on light microscopy of frozen sections stained with Toluidine Blue. The method used preparations provided by the meat industry produced with known machine types and pressures. It primarily assesses the level of damage to the muscle and also comments on the state of the fat, the level of connective tissue and hyaline cartilage and overall integrity of tissue components. It was developed as a simple method that could be applied by enforcement laboratories and training has been provided to Public Analysts. The method is currently being considered for submission for UKAS accreditation.

Although a simple semi-quantitative assessment was evaluated, the method is essentially a subjective method relying on the observations of the microscopist/histologist. However, limited blind trials carried out within Leatherhead showed that those with little experience of microscopy could easily follow the method and evaluate the sample structure easily. The results with chicken and pork indicated that the level of disruption was severe in MSM and there was no need for quantification. Two practical-based workshops were funded for Industry and Public Analysts to receive training and evaluate the method, and no difficulties were raised from these.

³<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18019>

Germany

The method used in Germany, which was developed independently from the UK method, also uses staining with Toluidine Blue on frozen sections (Branscheid 2011., Branscheid and Troeger 2012). The method is semi-quantitative. Assessment is made of the level of muscle fibre disintegration within seven grades from completely free of modifications to complete disintegration of structure. Obviously, a clear definition of these grades is crucial for the reliability of the method.

Denmark

In Denmark two approaches were developed based on immunological staining and visualisation by light microscopy (Henckel 2011). Unlike the UK and German methods, the procedure involves chemical fixation of the sample, dehydration and embedding in paraffin wax. This is a classical histology method. Following sectioning of the embedded material, two different staining methods using antibodies were developed. The first method used anti-myosin antibodies (myosin is a main muscle fibre protein) to visualise the muscle content in the section. The second approach used an antibody stain to Laminin which was visualised only when the muscle fibres were structured. Therefore any damage removed the staining. In order to quantify the method, an image analysis technique was used to measure the relative areas of staining (Henckel 1998.). The method was reported to be successful in that the level of muscle fibre integrity could be quantified, but the software and equipment used in these experiments is no longer available.

France

The French company Histalim has developed a method based on chemical fixation, dehydration and paraffin wax embedding and is similar in preparation to the Danish method. The sections are stained with a differential staining method highlighting the muscle and connective tissue (fat is removed in the preparation of the sections, as in the Danish method). The images are then quantified using an image analysis technique (Sifre, Andre et al. 2009) and a level of destructuration (damage to the muscle) as described by the authors is calculated. The development of this technique relied on an agreed level of acceptable loss of structure for the samples used. To achieve this, the

researchers used panels of assessors ascribing a view as to whether each sample was MSM or meat. There are some comments that can be made for and against this method and they are described later in the evaluation section.

Following the development of the above method, which is accredited under the French quality system, a European project under the framework of Eurostars titled LOTIMS (Laboratory and Online Tools to Investigate Meat Structure E! 4309) was launched to validate the method.

Czech Republic

A publication from researchers in the Czech Republic describing the use of histology to examine MSM obtained from different machines has been identified from this search and so might be in use in that country (Komrska, Tremlová et al. 2011). The method was not described clearly in the publication but was most likely a similar method to that used by the French and Danish. It involved the use of different stains and also used image analysis to quantify the results.

The methods described above principally addressed the legislation on muscle fibre structure integrity. No consideration was given to microbiological or TSE risk.

Search Criteria

The search was conducted through Dialog which examined several databases. These are listed at the beginning of Appendix 1 together with the search strategy. To carry out the search key words used:

Mechanically separated meat; mechanically recovered meat; machine recovered meat; machine deboned meat; desinewed meat; 3mm meat; baader meat; distinguish; analyse; chicken; turkey; pork; lamb; beef.

These key words were searched in combinations as described in the search strategy and the results reviewed manually. Once the results showed relevant findings on meat rather than non-food applications the abstracts were downloaded and reviewed.

Search Results

General scope of papers identified

Five hundred and thirty four publications were identified from the search and approximately two hundred were found to be relevant. Of these publications, approximately 140 were not cited in the EFSA report. It could be that they were examined and simply not listed to keep the report succinct. Some 60 papers in the EFSA report were not found in this search and they have been added to this review. These numbers show both the large number of publications on the subject and also the difficulties in finding them all. Both this search and the EFSA report did not identify relevant references to the use of spectroscopic techniques or impedance measurements but these are potentially valuable as suitable methods for evaluation of mechanically separated meat properties. It is likely these references did not appear as they involved meat cuts rather than mechanically separated samples.

The papers reviewed covered the following topics/methods of differentiation: functional and sensory properties; cholesterol values; proximate composition; calcium levels; chemical and microbiological composition; polymerase chain reaction (PCR) methodology; histology and polarized light microscopy; gel electrophoresis; fatty acid composition; application of neural networks; fluoride content; bone marrow content; bone particle content; metabolomics and proteomics; risk assessment and risk markers for CNS and TSE; nucleotide content; oxidation behaviour; immunological detection of keratin; colour parameters; enzyme immunoassay and immunoblotting techniques; spectroscopy techniques; cartilage content; haem pigment content.

It is not intended to report on these methods in detail as many of them were covered in the EFSA report and there is little value in repeating them here. However examples of the main methods and key findings of the publications investigating their use are given in tables A1-5 in Appendix 2. A summary of these methods is given in Table A6 with some comments on the approach used or the results obtained.

Compositional Analysis (Table A1)

Compositional analysis included chemical measurement of levels of water, protein, ash, fat, calcium, iron and other elements. This type of analysis produced a large amount of information. However, many of the results were conflicting as they depended on a number of factors such as type of machine, class of meat used, presence of skin, etc and no clear pattern emerged. An example of the range of approaches used and the results obtained can be seen in Table 1. This variability was confirmed by the EFSA report which collated the results from several publications and evaluated them statistically.

Calcium has long been considered as a marker for MSM, mainly due to the presence of material from marrow and particles from crushed bones after very high pressure treatment. The calcium content is regulated in many countries (EU: 1000mg/100g) and the amount of bone particles is regulated in the USA. The publications generally reported higher levels of calcium in MSM when compared to HDM but the levels were not consistent and this makes distinguishing material based on calcium difficult if not impossible. A survey of calcium in pork samples found variability with levels in meat up to 150ppm and there was a recommendation for a rise in the legal limit to 200ppm (table 1). Other elements, such as iron produced mainly from the marrow by high pressure separation processes, also were investigated as potential markers but again there were conflicting results.

Only 15 publications were found which considered cholesterol and not all of them contained a comparison with HDM. The analyses for fat frequently showed higher levels in MSM but again the results were variable and so fat is not a reliable indicator of quality. However, many papers discussed the role of fat and its subsequent oxidation on storage and commented on the marked increase in fat degradation in high pressure MSM. It is possible that a measure of fatty acid composition after a short stress test could be used as an indicator of MSM.

In summary the additional publications on compositional analysis reviewed here contained similar results to those in the EFSA report. However the table at end of the EFSA report summarizing the compositional analysis takes into account the type of

meat used in the HDM samples but not how they were prepared. Comparisons made from MDM against chicken breast for example could be considered not to be truly comparable. In addition, very few publications considered the two-stage deboning and the effect of removing the sinew/ connective tissue.

Histology (Table A2)

Initial histology studies focused on potential markers for MSM such as bone or cartilage. More recently the studies have focused on the muscle fibre structure, comparing HDM with gentle, 'low pressure' MSM (DSM) and "hard" MSM (high pressure MSM). These studies form the bulk of publications assessing levels of muscle fibre damage. This emphasis was influenced by the wording of the legislation regarding the modification of muscle fibre structure. Different methods have been developed and some element of quantification has been attempted. All investigations that compared DSM and MSM concluded that gentle machine deboning (DSM) gave similar results to HDM.

Whatever technique is selected for evaluation of meat quality there needs at some point to be a separate measure of how that is classified. Due to the variability in the technologies and the meat sources it is likely that this will not be a simple level of muscle structure or composition. The publication of Sifre, Andre et al. (2009) is the only one that has attempted to address this. The failure of their method, according to the EFSA report, was that it was too subjective. Several of the histology publications refer to other observed differences between the HDM, DSM and MSM samples such as disruption of fatty tissue and disruption of nuclei. Although not stated in the regulations, changes to the nuclei are involved in the disruption of the muscle fibre structure and could be considered as a measure of the state of the muscle.

Markers for MSM (Table A3)

Most publications refer to the search for a marker for the presence of MSM in meat products and the approaches used were varied. Many reported haem as a potentially useful marker and others proposed marrow proteins. Electrophoresis and combinations with an immunological approach also were reported as showing promise.

Other methods (Table A4)

Proteomics and metabolomics were two techniques used to compare DSM, MSM and HDM. Proteomic studies did produce some marker proteins that showed promise.

Many publications on functionality were focussed on benefits of using MSM in products and few examined the functional properties of the material produced in itself. Since the older version of the definition of MSM included the description of viscosity as “flowing like a puree” it is a little surprising that this has not been done.

Spectroscopy techniques, particularly Near Infra Red (NIR) have been used to assess properties of meat (composition and other properties such as colour) but not applied to MSM. Hyperspectral imaging is known to be applicable to composition differences and meat quality and could be considered in future research.

Risks from DSM / MSM (Table A5)

The areas of risk considered for MSM are principally believed to be microbiological and BSE related. The publications assume an increased microbiological risk for MSM although many related this to the hygiene conditions during production rather than an intrinsic property of the machine deboning or material. Some considered the degree of comminution affected the level of contamination found. The FSA has performed provisional research in to the microbiological status of MSM with the microbial data provided by current pork and poultry MSM producers showing that the overall microbiological quality of MSM and TVC's, particularly the type of MSM that has a calcium content that is not significantly higher than that of minced meat, is similar to that of both minced meat and meat preparations.⁴ The BSE concerns related to incorporation of MSM in products where the spinal cord and head meat were present. They also refer to the methods used around the time of the BSE crisis so an updated risk assessment and screen for risk material would be useful. However, as described under the aims and objectives, microbiological risks are beyond the scope of this project as MSM production must meet hygiene standards. Similarly the use of material which

⁴ Description of the processes used in the UK to manufacture MSM and former DSM meat products from poultry and pork and an initial assessment of microbiological risk. James, C ; Purnell, G; James, S. http://www.foodbase.org.uk/results.php?f_category_id=&f_report_id=826

poses a potential risk from BSE is prohibited in the production of MSM. This project solely focuses on the need to indentify between types of MSM and meat preparations.

Although out of the scope of this work, future reseach may also wish to consider the potential for other factors that may be considered as adverse constituents of meat, such as elevated calcium, phosphate or PUFAs which are associated with high pressure MSM (but possibly not DSM). However, since they are reported to be elevated in MSM, fatty acids might also be useful as a measure of pressure and quality.

EFSA Report - summary

EFSA considered that all machine deboned material was MSM. For distinction between types of MSM they considered the following areas of analysis: chemical, histological, molecular, textural and rheological.

The EFSA report is extensive and has summarised the publications well. However there are some aspects that deserve comment based on experience of the subject and following the review of publications on which this report is based.

The conclusion in the EFSA report that calcium and possibly cholesterol were the only appropriate chemical parameters was based on a consideration of all the methods cited. The report produced a model to derive probabilities that the material was MSM based on published data for calcium. The report also acknowledged that calcium alone does not allow differentiation between low pressure MSM (DSM) and other meat products and so the conclusion that further work should be around calcium seems contradictory and this report considers that other factors are needed to differentiate between MSM and DSM or meat preparations. Publications reviewed in the preparation of this report did not find calcium to be a factor that could reliably differentiate between MSM and DSM and not at all between DSM and HDM.

The EFSA report also acknowledged that histology was a promising method but required further validation and quantification. This report agrees with that observation. Particularly important however, is the comment that there was no discussion of the

relevance of modification of muscle fibre structure as a measure of MSM when other accepted techniques to process meat are known to modify the muscle structure. This is a difficult area and one which would benefit from further discussion in reviewing any changes.

The EFSA report recommended further studies to collect data based on calcium levels and cholesterol, but also suggested that an approach using combined parameters could be promising. The approach to use antibody labelling of the sample of meat preparation using selected antibodies in some form was considered but not recommended. Recently, methods for assessing meat using antibodies have advanced greatly and offer the advantages of a rapid quantitative method. The report refers to some publications on antibodies (Nakano, Ozimek et al. 2012) and it recommends their use in combination with other methods although these are not specified.

Although texture was discussed in the report it did not consider this approach as suitable for further development. This methodology could be applied to MSM as a measure of quality but the reasons given for not progressing this were not fully clarified.

Spectroscopy techniques were not discussed in the report but have been shown (see table 4) to relate to measures of meat quality. It may be they were omitted because they have not yet been applied to MSM but they should be considered in future research as they have been shown to be a successful approach to meat quality and unpublished observations indicate they might be useful in classifying machine deboned meat.

Finally, risk factors were discussed in the EFSA report but no conclusion on the level of risk associated with mechanically separated products was given.

Discussion, Conclusions and Future Direction

Discussion

The use of MSM is legislated under the hygiene regulations and as such is a permitted ingredient. There is also a labelling requirement for consumer information and

authenticity purposes and as part of this, MSM cannot currently count towards the meat content. This has economic implications and there are discussions as to the perceived value of different MSM-type products, namely the low pressure MSM previously referred to as DSM, and high pressure MSM. For these reasons there is a need to be able to distinguish low pressure MSM and high pressure MSM from a HDM preparation.

Based upon the available literature and the recent comprehensive EFSA scientific opinion (EFSA, 2013) it is apparent that potential methods exist for the differentiation of High Pressure Mechanically Separated Meat (HP MSM). However, the further distinction of Low Pressure (LP) MSM or Desinewed Meat (DSM) produced by modern manufacturing process, from hand-deboned minced meat (HDM) represents a difficult analytical challenge.

The publications that include a comparison of hand deboned meat, desinewed or low pressure MSM and high pressure MSM are summarised in Table 1. Only a relatively few publications described comparisons with different types of MSM. There is extremely little published evidence on the differentiation of DSM and HDM but where it was considered, then DSM was found to be similar to minced HDM. Any method(s) chosen for analysis as to whether it is MSM, DSM or HDM should be easy for laboratories to use across the EU. The use of microscopy as an analytical method does deliver observations that relate directly to the regulations. The methods of Groves and Branscheid (Branscheid, Judas et al. 2008, Groves 2011) are easily used by most laboratories. Those by Sifre, and Henckel (Sifre, Andre et al. 2009) (Henckel 1998) are classic histology methods and generally only available in veterinary or medical laboratories. The latter methods also have drawbacks in that they require regulated chemicals such as formalin and the technique removes fat so changes the sample to some extent. The use of Image analysis to quantify the level of fibre modification requires clear distinction between the components and thresholding to allow the image to be quantified. A method for then assessing the level of structural integrity has to be determined. In the Histalim method, this is based on the analysis of the fat by Sifre et al (2009) but there are consequent variabilities and errors that might develop between different samples.

A factor that needs to be considered when comparing MSM with HDM is that often the type of HDM is not described, or the comparison used against MSM is prime muscle minced through a 3mm plate rather than meat that was directly removed from the residual carcass by hand. There are views that the comparison of MSM and DSM with HDM should use hand deboned material similar to the machine deboned material. There could however be an argument that the comparison should be a typical meat preparation which could involve hand deboned muscle minced through a 3mm mincer. The aim of any future changes to the legislation should factor in these aspects. .

Additionally and importantly, although the methods listed in Table 1 compared samples of hand deboned meat (HDM) with typical MSM and lower pressure material or drum produced vs auger, they have not stated a level of muscle fibre integrity that is acceptable for the sample to be considered meat or a meat preparation. No method other than the sensory panel measurement by Sifre et al (2009) has included the need for a value or level of the required property (in this case muscle fibre integrity) that makes it a meat rather than MSM. The report by Groves (2011) did consider this as an element that would need to be taken into account in future research.

Any intention to base a definition of meat based on the level of intact muscle fibre structure remaining when the flesh is removed from the bone needs to take into account the structure of a meat preparation. Typical processing of hand deboned meat including freezing, mincing and salt and phosphate treatment will disrupt the muscle structure and cause some damage (see Fig 1). It is important to recognise that modification of the muscle fibre structure takes place in HDM for a number of legitimate reasons and therefore any regulations on meat preparations or MSM/DSM should take this into account. The impact of processing was considered in the EFSA report but not discussed as an issue that could impact on the regulation ((EC) 853/2004).

A further area of importance that was not covered in the EFSA report but needs to be considered in any review of legislation is that the process of mechanical separation of the flesh from the bones or carcasse is a two-stage process with the second stage being a desinewing one to remove sinew / connective tissues. This desinewing stage will damage the muscle fibre structure further. Personal experience of chicken produced

from bones by a two-stage process has revealed that the product from the first stage is quite intact and by eye appears to be pieces of chicken, but after the desinewing stage is much more paste-like or minced in appearance. In this area of consideration it should be noted that HDM preparations are also often subjected to a desinewing process after mincing, causing further loss of muscle fibre structure. This desinewing process means that there is a loss of muscle fibre structure after the mechanical separation process.

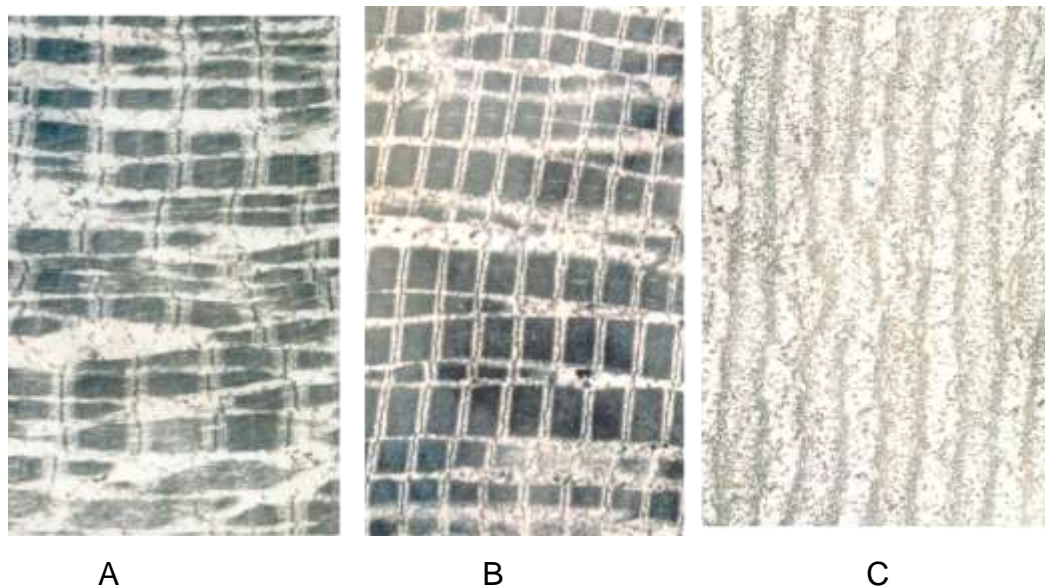


Figure 1 showing typical structure of pork muscle as viewed in the transmission electron microscope. A: raw B: cooked C: cooked after treatment with salt and polyphosphate as for the ham curing process

(taken from *D. F. Lewis, K. H. M. Groves, J. H. Holgate Food Microstructure Vol. 5, Number 1, 1986*)

The structure of the muscle fibre was described in the EFSA report and consideration of this can help in deciding on markers which might vary according to the quality of the meat preparation, DSM or MSM. Obviously, the first level of structure is the integrity of the whole muscle, and this is generally accepted in the literature as being disrupted in a meat preparation, especially if the meat is minced through a 3mm mincer. The second structural level is the whole muscle fibre. This will be modified in a meat preparation, generally to produce fragments of fibres and dispersed muscle fibre protein. The muscle fibre consists of protein filaments arrayed in a very structured striated manner inside a membrane. The muscle fibres are multinucleated cells and the nuclei are situated just

under the membrane at the edges of the fibre. This means that most types of physical damage to the muscle fibre structure will release the nuclei first. Such changes to the nuclei were commented on by both (Branscheid 2011) and (Groves 2011). The histological methods published generally refer to the integrity of the muscle fibres but this is visualised at a level where it is the muscle rather than the individual fibre that is assessed. Branscheid (2011) and Groves (2011) both comment on the level of internal order of the filaments (striation or banding). If this is disrupted then it means the muscle fibre structure has been modified. However the external appearance of the fibre could appear intact. It is a possibility that methods such as spectroscopy could measure the internal fibre integrity.

Since the low pressure MSM (DSM) extraction process is relatively mild compared with HP-MSM it is unlikely that suitable markers specifically for DSM can be identified from bone or marrow. Neither calcium nor cholesterol is likely to serve as a suitable marker for differentiating LP MSM/DSM from minced HDM. If markers do exist then they are more likely to be structural changes in the meat fibres visible by microscopy. Alternatively, more subtle biochemical changes such as damage to nuclei suggest that microscopic examination of nuclei or modifications to nucleic acids might serve as suitable markers for LP-MSM or DSM.

Although cholesterol has been identified by the EFSA review panel as a potential marker for MSM the characterisation of fatty acid profiles and other oxidation products may also provide useful target analytes since marrow derived fat content and cholesterol is generally increased in HP MSM. Additionally, in MSM fatty acid oxidation products thought to be from the marrow fat have been reported to increase upon storage. However, histology studies have indicated damage to the fatty tissue and the published increase in oxidation on storage could be as a result of this. Sample pre-incubation might be used to accelerate oxidation and increase the level of the target analyte.

The EFSA report is extensive and covers many, but not all, of the techniques of interest. It does not include the use of spectroscopy despite references existing for the use of spectroscopic techniques to assess meat structure and quality. However it was difficult

to find these references as they were not found in this current search either and were only discovered from the proposal for the EU FP7 funding for a quantitative method to assess meat quality (Meat Assessment and Classification System (MACSYS) 2014) and from a search on meat quality. The potential for hyperspectral imaging for assessing meat quality (ElMasry G. (2010)) should also be evaluated. Finally, the EFSA report does not define the criteria for distinguishing DSM from high pressure MSM and does not appear to comment on any possible risk, microbiological or otherwise, associated with DSM.

Conclusions

In conclusion the research to date has shown that low pressure MSM (DSM) is similar to HDM which has been removed and minced or processed and both are very different to high pressure MSM. There is nothing that is published in terms of methodology that clearly distinguishes this machine deboned material from hand deboned material. If it is a requirement that there must be a way to differentiate DSM from HDM then it will be extremely difficult to do this. Methods that rely on chemical or compositional changes are not consistent indicators and the levels in DSM and HDM vary to such an extent that overlaps preclude these as being suitable for distinguishing DSM from HDM. Muscle fibre structure is modified in HDM if minced or frozen and so can be similar to DSM. The presence of bone can be higher in HDM depending on the skill of the deboning.

In considering the research available from this report and the EFSA report, one approach to distinguish DSM from HDM could involve a multivariate approach with a cascade or decision tree as the best method. The decision tree would include levels of calcium, fat, oxidation behaviour, nuclei damage, muscle fibre integrity and a measure of texture. There should be categories where the sample fits high confidence limits that it is hand deboned, and categories where it is a high confidence that it is DSM or MSM. Overlap or “grey” areas should also be included and a decision taken as to how to label these for legislative purposes.

There are methods that could be assessed for their ability to distinguish DSM from HDM and these include spectroscopy (NIR / Hyperspectral imaging). A very recent

paper by Clerjon et al indicates that fluorescence polarimetry might well be successful in this area also.⁵

Future research

Future research in this area should also consider the following:

- A comparison of hand deboned residual material vs machine deboned, taking into account the type of meat remaining on the bones so that a true assessment of the property (variation in composition or structure) can be made. This should allow a level of loss or modification of muscle structure to be determined for a meat preparation.
- The histology method developed by the UK (Groves 2011) is similar to that used in Germany Branscheid, W., Bauer, A., Troeger, K., (2011) ((Branscheid, 2011) and is simple enough to be used in many companies and enforcement laboratories. It clearly relates to the current legislation and can clearly distinguish between MSM and DSM. It therefore could be considered as a suitable method for a measure of the quality of the sample. It lacks quantification so future research should look at this aspect, especially the use of “free” good quality image analysis software that is available. The advantage of this approach is that it is in alignment with both the UK and German system and similar to the French system. A common definition of level of modification would need to be agreed.
- Whatever approach is used the research could also usefully evaluate the microbial loads in the different products and the effects of comminution on these. Although this is not an authenticity requirement, information would determine whether microbial loads in MSM were similar to meat preparations. We are aware that the FSA is already taking work forward on the microbial loads of MSM.
- Inter laboratory assessment should be included as part of method assessment.

⁵ {NOTE:Clerjon S, Luca C, Peyrin L F, Lepetit J, Culioli J (notified from research Gate 2014)
"Fluorescence polarimetry for quantification of muscle fibres destructure during grinding}

- The difference between first stage flesh removal and subsequent desinewing should be included or at least considered as to whether it should be reflected in the legislation.
- A consideration of the ways to assess quality issues to help inform a more representative wording for the regulation rather than just loss or modification of fibre structure. This should include a consideration of rheology as a measure of the property of the material itself as this would relate directly to perceived quality. The published literature only considers MSM in terms of use in products and effects on emulsifying, gelling or other textural properties. However a measure of the viscosity or softness of the MSM might provide the required test level.

A newly funded project called MACSYS has been approved under the EU framework 7 programme. This project aims to develop a fast objective method to perform real time in-line quality classification of comminuted poultry meat using spectroscopy. This approach will be correlated with a well-defined histochemical method for the assessment of poultry meat quality quantified with image analysis. There are nine partners in the consortium which includes Aarhus University; Robert Damkjaer (Denmark); Lima (France); Carometec; Softcrits (Spain); University of Copenhagen; Leatherhead Food Research (UK); Max Rubner Institute (Germany); Marel (Netherlands). The project workplan has been agreed and the kick off meeting was held in February 2014. If a wider examination of the classification of MSM, DSM and meat preparations is to be carried out, then the samples generated for this project offer the opportunity to combine results and enhance the EU approach. This would have to be agreed with the project consortium and project officers.

Table 1: Summary of methods/publications that have assessed DSM (low pressure MSM) separately from High pressure MSM and HDM

Area	Reference	Comments
Histology	(Branscheid, Judas et al. 2008)	Compared Baader wishbone, MSM and minced thigh using chemical analysis and histology. Reported that gentle pressure MSM (DSM) similar to HDM in calcium, bone and structure. Only semi-quantitative and mostly differentiating high pressure MSM from the rest.
Histology	(Groves 2011)	Compared chicken pork & turkey HDM, DSM, MSM from known machine conditions using histology. Similar method to Branscheid and not quantitative. Could be made so but what level is set by DSM? Too similar to HDM. Like Branscheid the method is applicable for use in most laboratories as examines frozen sections and a simple stain.
Histology	(Henckel, Vyberg et al. 2004) (Henckel 2011)	Used staining and antibodies towards laminin as well as myosin and image analysis to quantify the results. Authors considered results very promising for assessment of muscle damage. They also recommend that a scale be made of all meat preparations based on biochemical composition and degradation of structure regardless of whether it is hand deboned or machine deboned. Methodology more complex than Groves and Branscheid and not suitable for many laboratories. Image analysis is quantitative but care needs to be taken to ensure that the thresholding is carried out in a consistent and reproducible manner. Efsa considered availability of antibodies too restrictive.

Table 1 contd: Summary of methods/publications that have assessed DSM (low pressure MSM) separately from High pressure MSM and HDM

Area	Reference	Comments
Histology	(Sifre, Andre et al. 2009)	Details on machine conditions and samples not published and consequently difficult to assess results. Does accept that machine deboned meat with good preservation is not classified as MSM for contribution to the meat content. Uses classic histology preparation techniques similar to Henckel and so limited in application to laboratories that can carry these out. Caveats as above and recent publication by authors (2012) refining the conditions for increased reproducibility demonstrates difficulties of technique.
Histology	(Komrska, Tremlová et al. 2011)	Evaluated three different MSM/DSM samples. No HDM comparison. Results showed different machine types gave good muscle structure but that there were differences between them which could be differentiated by microscopy. Methodology similar to Henckel / Sifre and Image analysis used so caveats on those as above.
Risk	(Hajmeer, Cliver et al. 2006)	300 samples examined and tested for central nervous tissue using an ELISA kit. None detected. Limited by LOD of kit but strong indication that material is safe from BSE risk. Not included in Efsa report.

Table 1 contd: Summary of methods/publications that have assessed DSM (low pressure MSM) separately from High pressure MSM and HDM

Area	Reference	Comments
Markers	(Savage, Ian Richardson et al. 1995)	Gel electrophoresis carried out on a wide range of MRM and HDM samples as well as PAD meat normally not considered MRM. Clearly possible to distinguish MRM and HDM raw samples for beef pork and chicken. PAD meat was also different to MRM. Results from mixtures and products were promising.
Composition	(Mayer, Smith et al. 2007)	Although compositional differences were found, including calcium, between HDM and DSM, levels were below legal limit.
Matabolomic	(Surowiec, Koistinen et al. 2011)	Metabolomics approach. Same samples as used by Groves. Molecule measurement and pattern but no conclusions and did not differentiate between DSM and MSM.

Appendix 1 - Search Strategy

The following databases were searched:

Professional Market Research, Abstracts in New Technology & Engineering, Adis Clinical Trials Insight, AdisPharmacoeconomics & Outcomes News, Adis R&D Insight, AdisReactions Database, AGRICOLA, AGRIS, Allied & Complementary Medicine™, Analytical Abstracts, Australian Education Index, BIOSIS® Toxicology, BIOSIS Previews®, British Education Index, British Library Inside Conferences, British Nursing Index, Business & Industry, CAB ABSTRACTS, Chemical Business Newsbase, Chemical Engineering & Biotechnology Abstracts, Chemical Safety Newsbase, Civil Engineering Abstracts, COS Conference Papers Index, Current Contents® Search, Derwent Drug File, Derwent Drug Registry, DH-DATA: Health Administration, Medical Toxicology & Environmental Health, Dialog Global Reporter, DIOGENES® FDA Regulatory Updates, Drug Information Fulltext, Earthquake Engineering Abstracts, Ei Compendex®, Ei EnCompassLIT, Embase®, Embase® Alert, EMCare®, Energy Science and Technology, ERIC, ESPICOM Pharmaceutical & Medical Device News, FDAnews, FLUIDEX (Fluid Engineering Abstracts), Foodline®: MARKET, Foodline®: PRODUCT, Foodline®: SCIENCE, FSTA®, Gale Group Computer Database™, Gale Group Health Periodicals Database, Gale Group New Product Announcements / Plus®, Gale Group Newsletter Database™, Gale Group PharmaBiomed Business Journals, Gale Group PROMT®, Gale Group Trade & Industry Database™, GEOBASE, GeoRef, HSELINE: Health and Safety, ICONDA - International Construction Database, IMS Company Profiles, IMS New Product Focus, IMS Pharma Trademarks, IMS R&D Focus, IMS R&D Focus Drug News, Incidence & Prevalence Database, Inspec®, International Pharmaceutical Abstracts, Jane's Defense & Aerospace News, King's Fund, KOSMET: Cosmetic Science, Lancet Titles, Material Safety Datasheets -OHS™, Mechanical & Transportation Engineering Abstracts, MEDLINE®, New England Journal of Medicine, NTIS: National Technical Information Service, PAIS International, Paperbase, PAPERCHEM, PASCAL, Pharmaceutical and Healthcare Industry News Database, Pharmaprojects, PIRABASE, Polymer Library, ProQuest Advanced Tech & Aerospace Professional, ProQuest Biological & Health Science Professional, ProQuest Dissertations and Theses Professional (Module), ProQuest Environmental Science

Professional, ProQuest Materials Research Professional, ProQuest Newsstand Professional, Prou Science Daily Essentials, Prou Science Drug Data Report, Prou Science Drugs Of The Future™, PsycINFO, Registry of Toxic Effects of Chemical Substances (RTECS®), SciSearch®: a Cited Reference Science Database, Social SciSearch®, Toxfile®, Transport Research International Documentation, TULSA™ (Petroleum Abstracts), UBM Computer Full Text, Weldasearch®, World News Connection, Zoological Record Plus 564°09
In addition PubMed was also searched.

SEARCH SETS

SET 1

“mechanically separated meat*” OR “mechanically recovered meat*” OR “mechanically deboned meat*” OR “machine separated meat*” OR “machine recovered meat*” OR “machine deboned meat*” OR “de sinewed meat*” OR “desinewed meat*” OR “baader meat*” OR “3mm meat*” OR “3 mm meat*” OR “3 millimetre meat*” OR “3mm manufacturing meat*” OR “3 mm manufacturing meat*”

SET 2

“mechanically separated beef*” OR “mechanically recovered beef*” OR “mechanically deboned beef*” OR “machine separated beef*” OR “machine recovered beef*” OR “machine deboned beef*” OR “de sinewed beef*” OR “desinewed beef*” OR “baader beef*” OR “3mm beef*” OR “3 mm beef*” OR “3 millimetre beef*” OR “3mm manufacturing beef*” OR “3 mm manufacturing beef*”

SET 3

“mechanically separated lamb*” OR “mechanically recovered lamb*” OR
“mechanically deboned lamb*” OR “machine separated lamb*” OR “machine
recovered lamb*” OR “machine deboned lamb*” OR “de sinewed lamb*” OR
“desinewed lamb*” OR “baader lamb*” OR “3mm lamb*” OR “3 mm lamb*” OR
“3 millimetre lamb*” OR “3mm manufacturing lamb*” OR “3 mm manufacturing
lamb*”

SET 4

“mechanically separated pork*” OR “mechanically recovered pork*” OR
“mechanically deboned pork*” OR “machine separated pork*” OR “machine
recovered pork*” OR “machine deboned pork*” OR “de sinewed pork*” OR
“desinewed pork*” OR “baader pork*” OR “3mm pork*” OR “3 mm pork*” OR “3
millimetre pork*” OR “3mm manufacturing pork*” OR “3 mm manufacturing
pork*”

SET 5

“mechanically separated pig*” OR “mechanically recovered pig*” OR
“mechanically deboned pig*” OR “machine separated pig*” OR “machine
recovered pig*” OR “machine deboned pig*” OR “de sinewed pig*” OR
“desinewed pig*” OR “baader pig*” OR “3mm pig*” OR “3 mm pig*” OR “3
millimetre pig*” OR “3mm manufacturing pig*” OR “3 mm manufacturing pig*”

SET 6

“mechanically separated poultry*” OR “mechanically recovered poultry*” OR
“mechanically deboned poultry*” OR “machine separated poultry*” OR “machine
recovered poultry*” OR “machine deboned poultry*” OR “de sinewed poultry*”

OR “desinewed poultry*” OR “baader poultry*” OR “3mm poultry*” OR “3 mm poultry*” OR “3 millimetre poultry*” OR “3mm manufacturing poultry*” OR “3 mm manufacturing poultry*”

SET 7

“mechanically separated chicken*” OR “mechanically recovered chicken*” OR “mechanically deboned chicken*” OR “machine separated chicken*” OR “machine recovered chicken*” OR “machine deboned chicken*” OR “de sinewed chicken*” OR “desinewed chicken*” OR “baader chicken*” OR “3mm chicken*” OR “3 mm chicken*” OR “3 millimetre chicken*” OR “3mm manufacturing chicken*” OR “3 mm manufacturing chicken*”

SET 8

“mechanically separated turkey*” OR “mechanically recovered turkey*” OR “mechanically deboned turkey*” OR “machine separated turkey*” OR “machine recovered turkey*” OR “machine deboned turkey*” OR “de sinewed turkey*” OR “desinewed turkey*” OR “baader turkey*” OR “3mm turkey*” OR “3 mm turkey*” OR “3 millimetre turkey*” OR “3mm manufacturing turkey*” OR “3 mm manufacturing turkey*”

SET 9

“mechanically separated mince*” OR “mechanically recovered mince*” OR “mechanically deboned mince*” OR “machine separated mince*” OR “machine recovered mince*” OR “machine deboned mince*” OR “de sinewed mince*” OR “desinewed mince*” OR “baader mince*” OR “3mm mince*” OR “3 mm mince*” OR “3 millimetre mince*” OR “3mm manufacturing mince*” OR “3 mm manufacturing mince*”

SET 10

(S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9)

SET 11

analy* OR identif* OR distinguish* OR differentiat* OR discriminat* OR detect*
OR determ* OR test OR tests OR testing OR recogni* OR assess* OR categoris* OR
categoriz* OR classif* OR characteri* OR assay* OR quantif* OR quantitat* OR
measur* OR examin* OR “tell apart” OR “telling apart” OR composition*

SET 12

(S10 AND S11)

SET 13

Methylsulfonylmethane* OR Methylsulphonylmethane* OR “Methyl
sulfonylmethane*” OR “Methyl sulphonylmethane*” OR “Methyl sulfonyl
methane*” OR “Methyl sulphonyl methane*” OR “Methylsulfonyl methane*” OR
“Methylsulphonyl methane*” OR (men and sex) OR HIV

SET 14

(S12 NOT S13)

Appendix 2

Table A1: Examples of Publications on Composition

Reference	Main Findings
(Field RA 1976)	Evaluated MSM against comparable HDM (same residual bones used to hand trim) ; found the MSM had lower protein, higher fat and variable calcium depending on the origin of the meat, but MSM sometimes similar to HDM.
(Negrao, Mizubuti et al. 2005)	A comparison of MSM flour against HDM flour with fresh chicken breast as control HDM. MSM higher in protein, lower in fat and higher in ash.
(Al-Najdawi and Abdullah 2002)	Comparison of MSM and HDM from similar sources found MSM had higher fat and calcium and lower protein.
(Calhoun, Schnell et al. 1999)	Similar approach and results to Al-Najdawi & Abdullah with the exception of fat which was similar to the HDM.
(Campbell and Hunt 1996) (Kolsarici, Candogan et al. 2010).	Compared MSM from different passes / drum size in Baader against whole tissues. Number of passes/drum hole size affected percentage of moisture, fat and connective tissue.
(Crosland, Patterson et al. 1995)	Compared compositions of beef, lamb, pork, chicken, turkey from different machines (piston and auger) including HDM from similar sources. Variable results depended on species, machine type & source. Fat lower in MSM often, Calcium and iron variable but often higher, purine bases very variable. Concluded differences not

	consistent enough to differentiate HDM and MSM, and that MSM could be considered as meat.
(Nagy, Lenhardt et al. 2007)	Compared Hard separation (Protecon type piston) against soft separation (Baader-type) poultry with breast and thigh as controls. Hydroxyproline, calcium and bone high in hard separated meat with soft separated similar to controls. Concluded soft separated meat properties similar to fresh meat.

Table A1 contd.: Examples of Publications on Composition

Reference	Main Findings
(Windham and Field 2000)	Compared AMR material before and after desinewing stage for iron content, and assessed method of measurement. Results variable and no pattern with pressure and dwell time seen. Recommends sinew removal stage should be considered in evaluating total iron content.
(Kolsarici, Candogan et al. 2010)	Different sources of chicken MDM compared with frozen storage. Source (structural) affected composition greatly. No comparison with HDM.
(Serdaroglu M.)	Beef and Turkey MDM compared with same source HDM. Higher fat, calcium and cholesterol in MDM.
(Trindade, Felício et al. 2004)	Useful review of publications on composition of hen MSM including cholesterol content and fatty acids, however controls used were breast and thigh meat. MSM higher in fat generally, collagen sometimes higher and cholesterol mostly higher.
(Field, Deligeersang et al. 2002)	See Table 3. Analysed composition specifically for markers.
(Mayer, Smith et al. 2007)	Looked at composition of MSM, DSM and HDM in beef with and without the use of a saw to remove spinal cord. DSM with use of the saw gave the highest calcium level and the HDM the lowest. All were below the required limit.
(Stenzel and Hidebrandt 2006)	Following a survey of pork samples authors proposed that the limit for calcium at 100ppm should be raised to 200ppm.

Table A2: Examples of Publications using Histology / Structure

Reference	Main Findings
(Branscheid, Judas et al. 2008)	Composition and histology of chicken furcula (wishbone) deboned by Baader, MSM and minced chicken thighs as HDM control. Baader deboned wishbone meat was found to be similar to thigh meat in calcium, bone and microbiological characteristics. Structurally they were also similar in level of intact muscle fibre whereas MSM had more disrupted muscle fibre structure and large changes to the nuclei.
(Branscheid, Judas et al. 2009)	Simplified method for determining MSM by visualization of bone and cartilage. Commercial MSM samples intended for doner production were used. Minced pork spiked with bone and cartilage was used as controls. DSM not compared.
(Branscheid 2011.)	Compared structure of samples from different MSM systems using similar method to Groves. Four categories of muscle fibre disintegration and nuclei modification identified, varying from no disruption to complete amorphous structure and dense aggregation of nuclei. Not known if final MSM or first stage product.
(Branscheid and Troeger 2012)	Products from different mechanical recovery techniques were compared with minced controls obtained from same source chicken and turkeys. Composition analysis and histology carried out which showed only small differences in chemical composition whereas histology showed clear differences between gentle (DSM) and high shear MSM samples. Standards were developed for the different categories of degradation to be used in future assessments.
(Groves 2011)	Pork chicken and turkey samples from known machine conditions assessed and compared to minced HDM (clean muscle source). Clear differences were seen between high pressure MSM and low pressure DSM, with similar levels of structural breakdown in mince controls and DSM. Nuclei and fatty tissue changes and cartilage levels also good indicators of high pressure MSM. Final desinewed products examined, but later in examination of lamb and beef samples from the first stage deboning and second stage desinewing were compared. Desinewing stage caused increased damage.

Table A2 contd: Examples of Publications using Histology / Structure

Reference	Main Findings
(Henckel, Vyberg et al. 2004)	Compared structure of chicken from auger MSM, Baader and HDM (same source but minced twice for equal comparison). Composition and histology evaluated. Histology using two approaches; immunohistochemistry with myosin antibodies and an alternative complex staining procedure to detect myosin and general tissues. An image analysis system was developed to quantify the staining and structures. Compositionally, fat and water were significantly influenced by method of recovery and type of carcass meat. Calcium in breast MSM (auger) was significantly higher than other samples. MSM Baader and HDM similar calcium. Air present in all samples but higher in breast removed by auger MSM. Concluded that major source of compositional variation was the apt from which the meat was recovered. Bone was not present in most of the samples including the high pressure (auger) MSM. Structurally there were differences between the samples but authors concluded that discrimination between the methods was extremely difficult or impossible. They recommended that given this, the legislation should consider a redefinition of meat into whole meat and minced meat which should include MSM and that the minced meat should be graded according to quality using composition and structure evaluations.
(Henckel 2011)	Similar approach to 2004 but used antibodies towards laminin as well as myosin and image analysis to quantify the results. Authors considered results very promising for assessment of muscle damage.
(Hildebrandt, Rauscher et al. 2006)	Microscopy to detect bone particles. Successful but unable to detect MSM by this method for MSM with low bone particle content.
(Koolmees P A. (1986))	Compared MSM from different production sites using composition and histology to examine pork and chicken. However the approach was to use microscopy to quantify the different tissues and bone present so the sample was defatted and comminuted before sectioning. Therefore no evaluation of the original structure could be carried out (and

this was not the intention of the study).

Table A2 contd: Examples of Publications using Histology / Structure

Reference	Main Findings
(Komrska, Tremlová et al. 2011)	Evaluated three different MSM/DSM samples. No HDM comparison. Results showed different machine types gave good muscle structure but that there were differences between them which could be differentiated by microscopy.
(Botka-Petrak, Hraste et al. 2011)	Evaluated characteristics of Beehive MSM. No comparison with HDM or DSM. Compared histology with chemical composition and found good agreement. No comments made on level of disruption of muscle.
(Pickering, Evans et al. 1995)	Part of a series of publications on methods to detect MSM in products. Materials and methods for production of samples same as given in Table 3 (Savage, Ian Richardson et al. 1995). Concluded that light microscopy to detect hyaline cartilage using Toluidine Blue staining could possibly be a method for detecting MSM in products. Approach more of a marker rather than structural observations of muscle.
(Sifre, Andre et al. 2009)	Method to measure level of disruption of muscle fibre structure using histology and image analysis. Level of damage related to whether MSM or not defined by results obtained from subjective assessment of samples for appearance and consistency by many panels of professionals in the field.
(Sifre, Coton et al. 2013)	Same method as above 2009 but refines the microscope conditions etc for good reproducibility.

Table A3: Examples of Publications Assessing Markers for MSM / DSM

Reference	Main Findings
(Day and Brown 2001)	Capillary gel electrophoresis used. HDM was chicken breast. Some promising results based on level of haemoglobin.
(Field 1999)	A review of methods to measure marrow as a marker for MSM. Most promising were those based on haem pigments or iron.
(Field, Deligeersang et al. 2002)	Analyses of bovine marrow for iron, zinc and α tocopherol as potential markers. Variability in marrow and muscle rules them out as a marker.
(Pickering, Griffin et al. 1995)	Antibodies to chicken bone marrow proteins raised and tested using ELISA methodology to test for MSM in products, both raw and cooked. Good detection of MRM in products but cross reactivity with blood, skin and added proteins such as soya. Additionally it was not sensitive to cooked chicken MRM.
(Pussa, Raudsepp et al. 2009)	Analysed HDM vs MDM for PUFAS (polyunsaturated fatty acids which are thought to be converted into oxylipins during storage. These could be toxic (eg leucotoxin diol) at high concentrations. Postulate that the most abundant oxylipin (9,10,13 THODE) might be a potential marker for MSM.
(Savage, Ian Richardson et al. 1995)	Gel electrophoresis carried out on a wide range of MRM and HDM samples as well as PAD meat normally not considered MRM. Clearly possible to distinguish MRM and HDM raw samples for beef pork and chicken. PAD meat was also different to MRM. Results from mixtures and products were promising.
(Nakano, Ozimek et al. 2012)	Immunodiffusion technique used to detect keratin sulphate, a cartilage glycosoaminoglycan. Tested chicken MSM and products labeled as containing MSM. Found good results suggesting this could be a reliable test for MSM in products.

Table A3 contd: Examples of Publications Assessing Markers for MSM / DSM

Reference	Main Findings
(Scarborough, Jones et al. 1993)	Examined levels of free purines and pyrimidine bases and metabolites as markers for MRM. MRM compared with minced breast and thigh meat. Results indicated too much variability in levels and no correlation with comminution to make these good markers for MRM.
(Skarpeid, Moe et al. 2001)	Multivariate analysis of isoelectric focusing protein profiles used to detect MRM. Results very promising and could differentiate between MRM produced with bones from extremities and ribs and MRM made with backbones, as well as HDM. No examination of DSM.
(Stevenson, Pickering et al. 1992)	Immunological technique and ELISA promising for protein as a marker for MRM.
(Stevenson, Pickering et al. 1994)	ELISA using purified antibodies to chicken red bone marrow showed excellent ability to differentiate between HDM and MRM but not so good at detecting low levels of MRM in products. Western blotting gave superior results for this level of detection.
(Stevenson, Marchioni et al. 1996)	Electron spin resonance spectroscopy used to detect irradiated MRM by using bone fragments.

Table A4: Examples of Publications Assessing Meat / MSM / DSM using Other Techniques

Reference	Main Findings
(Surowiec, Fraser et al. 2011)	Proteomic approach. Same samples as used by Groves. Analysis of proteins by electrophoresis followed by mass spectrometry indicated two potential markers for chicken MRM: haemoglobin subunits and those similar to myosin-binding protein C.
(Surowiec, Koistinen et al. 2011)	Metabolomics approach. Same samples as used by Groves. Molecule measurement and pattern. No conclusive results.
(EIMasry G. (2010))	Hyperspectral imaging as a tool for meat quality. New technique that has been applied to assess meat tenderness, colour, marbling, surface contamination and bone fragment detection. No applications on MSM found.
(Kathirvel, Ermakov et al. 2008)	Resonance Raman Spectroscopy used to follow lipid oxidation in mechanically separated turkey. Successful and showed promise as a method.
(Berzaghi 2005)	NIR Spectroscopy as a method to predict composition of hen breast meat. MSM/DSM not studied. Method successful for dry matter, lipids, protein and major fatty acids.
(Prieto 2009.)	Extensive review on applications of NIR Spectroscopy to predict beef quality with tables of references. MSM/DSM not studied. Compositional analysis most promising potential. Texture and other sensory qualities less so due to variability in meat properties. Possibly different wavelengths might be more successful in these areas.
(Prieto N. 2009)	On-line use of Visible and NIR spectroscopy used to assess beef quality on-line. Good results for beef colour and some indications that results related to sensory characteristics.

Table A4 contd: Examples of Publications Assessing Meat / MSM / DSM using Other Techniques

(Abdullah and Al-Najdawi 2005)	Seems to be the only publication that evaluates MDM functionality alone and not just in products as an ingredient. Compared MDM with HDM from a similar source and measured emulsifying ability, pH, protein, moisture, WHC and sensory. pH of MDM higher than HDM. Sensory found no difference initially between MDM and HDM. Storage frozen showed MDM deteriorated more than HDM in sensory properties.
(Daros, Masson et al. 2005)	Assessed textural properties of a sausage made with MDM. Strong correlation between level of MDM and tensile and compressive strength.
(Perlo, Bonato et al. 2006)	Assessed texture of chicken nuggets made with HDM and MDM. No difference with upto 40%.
(Petracci M. (2011))	Reviews methods for assessing meat quality. Not related to MSM or DSM but does attempt a list of methods for characterization which include texture. Only 1 reference to texture and not for MSM.
Petzet A., Bauer A (2013)	Assessed pH, drip loss and shear force for pork with use of Raman Spectroscopy as a measure inline. Results considered promising. No application to MSM considered by authors but is an alternative rapid method for meat quality.

Table A5: Examples of Publications Assessing Risk Associated with Meat / MSM / DSM

Reference	Main Findings
(Brown 2001)	Concluded that BSE caused Creuzefeldt-Jacob disease most probably through adulteration of cooked meat products with MRM contaminated by compressed spinal cord and paraspinal ganglia as these were not excluded from carcasses and MSM until 1995.
(Collins, Hafner et al. 2000)	Looked at use of microscopy and immunohistochemistry to detect spinal cord in AMR products before and after desinewing. 2 in 196 samples contained spinal cord in product before desinewing including some around the publication date. A combination of three microscopy techniques was able to detect central nervous tissue in samples.
(Consulting 2002)	Study of possible causes of BSE in the UK by survey of practices. Concluded that the most potentially infective material in use at the time was MRM and head meat.
(Hafner, Sutton et al. 2008)	Immunohistochemistry used to detect DRG in AMR products. Successful method with spiked samples but unspiked samples showed no DRG fragments suggesting no infectivity.
(Hajmeer, Cliver et al. 2006)	Compared MSM, DSM and HDM samples and tested for central nervous tissue using ELISA methods. In 300 samples all results were negative (below the limit of detection of 0.1% with the ELISA kit).
(Pussa, Raudsepp et al. 2009)	Analysed HDM vs MDM for PUFAS (polyunsaturated fatty acids which are thought to be converted into oxylipins during storage. These could be toxic (eg leucotoxin diol) at high concentrations. Postulate that the most abundant oxylipin (9,10,13 THODE) might be a potential marker for MSM.
(Wesley, Harmon et al. 2002)	PCR techniques used to assess MSM turkey samples for Listeria. 57% were found to be contaminated with Listeria.

Table A6 Summary of Areas of Publications and Comments

Marker	Limit/Value Found	Comments
Calcium and other elements	MSM >100mg/100g EU limit	Calcium content alone does not distinguish MSM or DSM as it is related to the machine type, pressures used and cut of meat used. Alternative markers including protein ash, iron were not as reliable. Efsa report recommends further studies based on calcium - however p53 of EU report states that calcium does not distinguish LP MSM / DSM.
Phosphorus	0.08 to 1.1% for pork found.	Considerable variation in samples, thought to be due to technological variability. High phosphate might be a concern. Legal limit EC 1333/2008 5000mg/kg.
Ash	0.997 mean for HDM 1.639 for MSM given in Efsa report.	Calcium content is related to ash content but would require specific conversion factors to account for type of bone, age of animal and level of marrow, cartilage and periosteum. Efsa report considered there was a significant overlap statistically and therefore ash could not be used for classifying a product as MSM. Publications found in this survey agree with this.
Iron	70% increase	Elevated by 70% in HP MSM poultry meat. However too variable and overlap between HDM and MSM according to Efsa report. From published research high pressure MSM was found to discolour rapidly on exposure to air (thought to be due to haem pigments), and it would be worth revisiting this in future research.
Lipids, Fatty Acids, Cholesterol		Lipids from structural fat or marrow and lipid oxidation products are potential markers. Post processing formation of toxic fatty acids (PUFAs) could be a concern but inconsistent data found. Cholesterol may also be increased by marrow and also from any skin present. Efsa report considered this could be used to classify MSM as there was a difference between HDM and MSM, however not enough data available. Very few publications were found that measured cholesterol.

Table A6 contd. Summary of Areas of Publications and Comments

Marker	Limit or Value	Comments
Moisture Content	54-73%	Highly variable. Can be less in MSM. Not suitable as a marker or value.
Collagen	Variable muscle to connective tissue ration from 0.3 to 6.9	Usually removed by MSM process (in the second stage). May be elevated if skin is present. Content equivalent to HDM reported by some. Not suitable as a marker but high levels affect quality so could be considered as one area to be measured.
Rheology & texture		Almost solely used to show improved or similar performance in products. Efsa report considered this type of analysis not practicable for finished products due to a lack of homogeneous structure. However, it should be considered as a method for differentiating between MSM / DSM and HDM as it directly relates to perceived quality. This would need to be included with the intention to measure the rheology as a function of the material alone and not in a finished product. Personal experience of DSM and MSM at Leatherhead Food Research has shown that texture alone is not directly related to muscle fibre integrity as some samples were low viscosity but reasonable structure. Speed of discolouration did appear to relate to high pressure MSM and should be considered as a measure.
Chemical		Refers to standard analytical methods for calcium etc. Specific elements covered above.
Bone particles	1.9-2.5%	Can be assessed chemically or by microscopy. Possible marker however MSM does not always contain bone particles and HDM can sometimes contain bone so not suitable.

Table A6 contd. Summary of Areas of Publications and Comments

Marker	Limit or Value	Comments
Cartilage		Related to connective tissue. Thought to be elevated in High pressure MSM by many publications, especially hyaline cartilage. However not related to muscle fibre integrity.
Marrow		Haem pigments or marrow proteins showed promise as indicators of high pressure MSM but not really DSM.
Other tissues		Considered a factor in chicken MSM as whole carcasses deboned, but only present in other species by contamination (deliberate or accidental).
Microscopy Muscle structure and other elements	Meat destructuretion index given by one study (Histalim) as 58.1% above which = MSM. Others qualitative or semi-quantitative only	Possible marker. Efsa describes breakdown in muscle fibre structure due to other treatments such as tumbling, ageing etc. However they still considered fibre structure as relating to MSM which seems contradictory. Publications examined structural studies including nuclei damage but this has not been followed up and could be a promising area for measuring differences between MSM and DSM, HDM. Little quantitative data reported for studies on muscle fibre structure. Efsa report states this is a promising method but available data not quantitative or if quantitative did not provide objective threshold values. The method of Branscheid or Groves is a simple one which can be carried out at many laboratories. However it is not quantitative. It could be made so but would need a separate measure on what constitutes MSM.

Table A6 contd. Summary of Areas of Publications and Comments

Marker	Limit or Value	Comments
Protein electrophoresis	7.5% MRM	Marrow based marker. Heamaglobin: myoglobin ratios. Claimed to detect 7.5% mrm. Little consideration of levels in DSM.
Proteomics		Identification of potential markers haemaglobin subunits and a protein similar to myosin binding protein C (myBP-C). Authors suggest that enzymes released in MRM processing result in increased levels of myBP-C. However the increased disruption of the muscle fibre structure caused by the production of MSM could account for this result.
Metabolomics	Multi-parameter Chemometric modelling. Reported sensitivity as low as 10%	Multivariate chemometric analysis required. No specific marker detected. This could yield a result for a marker for MSM but further work is required.
Spectroscopy		No reference to this in Efsa report. Publications found in this search were discovered from the Macsys proposal so obviously missed in both search strategies. Obvious potential in online assessment of meat quality but not certain at current state if it would relate to the muscle fibre integrity and so fulfil regulations. Is part of the MACSYS project so results should be available in the next two years.

Table A6 contd. Summary of Areas of Publications and Comments

Marker	Limit or Value	Comments
Risk		<p>Microbiological risk assumed and publications report elevated levels in MSM so obviously a concern. However other than the increased level of muscle fibre breakdown is considered to aid growth there is nothing unusual or exceptional in MSM material that is not found in meat generally. EFSA considered this aspect and did not seem overly concerned with it as an increased risk. No results relating to DSM vs HDM available. Phosphate is regulated and not considered a safety or health issue by EFSA. Calcium and bone have been addressed above. Central nervous tissue and BSE concerns are mentioned in the EFSA report but not in great detail. The survey of a large number of publications by (Hajmeer, Cliver et al. 2006) showed very little contamination of MSM by central nervous tissue. However this is an obvious area that would need to be completely safe. DSM needs to be assessed.</p>

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