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Mechanically separated "meat"

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The present paper shows the results of a comparison of four different histochemical methods for measuring level of degradation of muscle structure. The analyses were performed on mixtures of emulsified and coarsely minced breast meat. A total of 11 different mixtures were used ranging from 100% emulsified and 0% coarsely chopped to 0% emulsified to 100% coarsely chopped in intervals of 10%. A similar series of mixtures were produced with thigh meat. Based on results from both muscles it was the staining method using an antibody for laminin which displayed an overall better accuracy in comparison to the other method. As it in addition is a fully automated analysis procedure, it is the one with the highest degree of objectivity and was for those reasons considered the better of the four and will for the same reasons comply with any legislation, where level of degradation of muscle structure is considered of prime importance, as is the case for the use of mechanically separated meat.

Keywords: MSM; Mechanical separated meat; Histochemistry; Methods;

Introduction

According to present legislation any "meat" obtained by mechanically removing residual meat from carcasses or bones after the primal cuts have been removed, has to be considered as MSM and is not allowed to be included in the meat content of a product, but should be included in the list of ingredients. Consequently this has implied a general downgrading of such products and of course also influenced the price of these products.
The reason for this is historical, in the sense that initially this type of production systems and "meat" appeared in a time with limited access to protein and lesser attention to hygiene and food safety. The goal was to recover as much protein and fat as possible, and what was obtained became from that point of view a rather important product, but by present standards this type of "meat" would definitely be considered of rather low quality. In order to protect consumers from such products the commission issued a regulation as described above. Reg (EC) 853/2004. Laying down specific hygiene rules for on the hygiene of foodstuffs. In annex 1 Definitions of that regulation no. 1.14, MSM is defined as "a 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 muscle fibre structure". However, the commission did not indicate any method by which level of degradation could be measured and for the same reason could not indicate any threshold values, by which intact muscle fibre structure could be separated from modified or lost muscle fibre structure. This has implied, that method of recovery has become more important than level of degradation or quality from a legal point of view and leaves consumers with an impression, that level of degradation is the most important quality trait. From our point of view this could be misleading the consumers and does not provide them the necessary information to make informed choices of the food they want to consume. Apparently this was not the intention of the Commission. In the same regulation Reg (EC) 853/2004, in section "whereas" number 20 the European Parliament and the Counsel of the European Union actually has expressed a wish on the definitions of MSM. It is stated that "the definition of Mechanically Separated Meat should be a generic one covering all methods of mechanical separation. Rapid technological developments in this

area mean that a flexible definition is appropriate". Consequently one might argue that present legislation is not in line with the intentions of the Commission on that regulative.
Since the first generation of machine were introduced, there has been huge improvements both in handling and storing of product from these machines, primarily to improve food safety, and there has also been large developments in machines for separation. New principles of separation has been introduced and implemented, and also changes in sieve construction have taken place and by reducing working pressure of older type machinery, varied levels of degradation can be achieved. The industry has now reached a state, which enables them to provide products, which in terms of quality, hardly, if at all, can be distinguished from regular minced meat (Henckel et al., 2011). Consequently there seems to be no objective reasons for maintaining the ban or downgrading of higher qualities of MSM. These evident improvements of the products most likely are the reason why regulations are interpreted and implemented differently in different member states. This was clearly demonstrated by a number of audit reports on the topic of interpretation and implementation of current legislation on MSM, as well as the scientific opinion of EFSA on the subject of MSM (EFSA Panel on Biological Hazards, 1913). This development appears more in line with the current status of the products and the intentions of the Parliament. Furthermore it may also be worthwhile to draw attention to the aspects of sustainability of the production. In the present situation lots of apparently high quality chicken or poultry meat is wasted, simple because it is not feasible from an economical point of view to produce in a way that would comply with present legislation. This applies specifically to smaller production with breeds different from the standard breeds used in the production. And also the use of meat from spent hens for human consumption is very limited because of the present legal limitations on its production. I should mention that practical problems of having these breeds slaughtered also contribute to the waste.
The legal text refers exclusively to the level of degradation of muscle tissue as the key factor for the quality. Generally the level of degradation rarely if ever is considered nor included in standard quality assessments. When used it is an entity, used to describe processability, and it is often in association with water-holding or -binding capacity. We thus have very limited knowledge on the influence of level of degradation on general meat quality or product quality, which may represent a problem particularly, if threshold values are to be decided upon.
The EFSA report concludes that the prerequisite for changes in the present situation is that methods are available by which degradation of muscle fibre structure can be measured accurately and objectively and that threshold values for different classes can be decided upon (if needed). They also stated that microscopic examination of tissue structure was at present one of the most promising methods for characterizing levels of degradation.
This is exactly the scope for part of the MACSYS project which is a project funded by the European Union's Seventh Framework Programme. Further information on the project can be found on the website (www.macsysproject.eu)

Materials and methods

The histochemical part (Microscopic examination of tissue structure) of the project is collaboration between Max Rubner Institut (Germany), Leatherhead Food Research (England) and the University of Aarhus, Department of Food Science (Denmark).
Within Europe more methods are now available that allows for such quantitative or semiquantitative (graded scales) assessment of the degradation of muscle structure, although none of these for obvious reasons have gained a wider acceptance. Two German histochemical method one based on toluidine blue and another based on haematoxylin-eosin staining's developed and conducted at the Max Rubner Institute (Branscheid et al.,2011), an English method also based on a toluidine blue stain but with a higher concentration of the dye developed and conducted at the Leatherhead Food Research and a Danish method based on staining's with monoclonal antibodies for myosin (Bader et al. 1982) and laminin (Bayne et al.,1984) for visualization of muscle tissue and level of degradation of muscle structure respectively, developed at the University of Aarhus. The German and the English methods are both largely subjective, whereas the Danish method is objective and fully automated. Also a French method, developed by Histalim, exists, but as this is based on an index (the MDI value)

(www.histalim.com) calculated from sensory evaluation, that are strongly influenced by combination of characteristics, it is not directly comparable to the other methods.
The aforementioned institutions in Germany, Great Britain and Denmark are in the MACSYS project collaborating to test the available methods for capacity and accuracy to quantify the level of degradation and to develop standard procedures for sampling, handling, preparing, staining and cutting of the sections for the finally selected method. Finally in collaboration with SoftCrit in Spain they will develop a fully automated image analysis system, which eventually will reduce or completely eliminate any subjective element of the histochemical analysis.
The reference samples have been produced in the following way. 60 kg of freshly produced deboned thigh muscles was collected from a local Danish Poultry Slaughterhouse and brought to the experimental slaughter house at the University of Aarhus, Department of Food Science in Foulum. Immediately upon arrival remains of bones and visible cartilage mostly from joints was removed manually. The batch was then divided in 2. One of the batches was exposed to 8 minutes of emulsification (Mado), the other was coarsely chopped in a mincer (Bankeryd) sufficed with a plate with 6mm holes. 11 different mixtures were then produced from 100% emulsified and 0% coarsely chopped to 0% emulsified to 100% coarsely chopped in intervals of 10%. Similar procedures and mixtures were produced from pure breast meat where the inner fillets were removed. Five samples from each mixture were measured by each method.

Results and discussion

These reference samples are produced to test the capacity of the different methods to accurately determine the level of degradation or how good correlations can be established between the hypothetical line and the means from the measured samples. Figure 1 and 2 shows the results from the German Toluidine Blue method for breast and thigh meat respectively. The number of marks in the vertical direction indicates the number of analyzed subsamples from the individual mixtures. There appears to be some small difference in range between the sample types, with a somewhat larger range from the breast muscle. The correlation seems fairly good but large variations within individual mixtures are observed. Figures 3 and 4 shows the result of the haematoxylin eosin stain of breast and thigh muscles respectively. A lower range and a larger variation within samples seem to exist in the thigh muscle samples (Fig 4) particularly the most degraded ones. Figure 5 and 6 shows the results by the laminin method. Samples from the breast as well as from the thigh display a larger range and a smaller within sample variation than seen in the previous figures. We also measured the amount of muscle tissue in the samples, these were originally intended to be used to correct for differences in muscle content in the samples. This turned out, however, not to be needed and values of these are not shown here. Figures 7 and 8 displays the results of the English TB method. The breast samples show the highest correlation of all whereas the thigh results are substantially lower. Based on these data we were able to calculate the R and the R^2 value (Table 1). The value R describes the correlation, i.e. the linearity between the relative amount of emulsified and coarsely chopped in the samples and the measured amount of intact fiber structure by the histological method used, whereas R^2 explains how much of the variation between the samples can be described by the relative amount of the two products in mixtures. Thus, the closer these two values are to 1.00 the better prediction of content of structured muscles in the samples by the histological method. These results indicate that overall, the laminin method is the safest and most accurate staining methods, by which level of degradation of muscle tissue can be quantified. That the laminin method further is completely objective and select and measure areas within a section at random further speaks in favour of the laminin method as the reference method. We are currently processing samples taken from poultry slaughter houses in Denmark, which include different principles of separation, different pressures and different raw material. We have also sampled hand deboned meat from back and breast parts which by definition is considered meat. Results from these samples will be given at the presentation.

Figure 1 Toluidine blue staining from Max Rubner Institut (MRI), Germany of standards originating from breast muscle. Meat (X-axis) is indicating relative amounts of coarsely minced meat relative to emulsified meat. TB_MRI (Y-axis) is % of structured muscle in sample.

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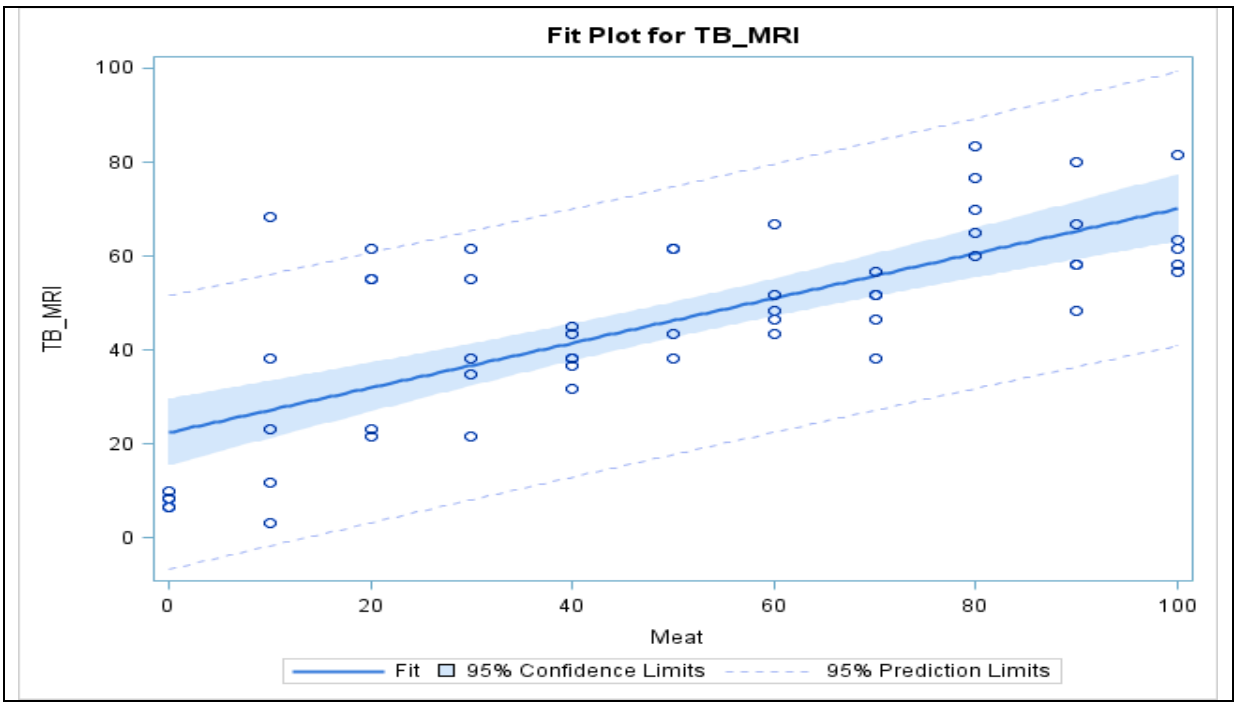

Figure 2 TB staining of references from thigh muscle (MRI-method). Meat (X-axis) is indicating relative amounts of coarsely minced meat relative to emulsified meat. TB_MRI (Y-axis) is % of structured muscle in sample.

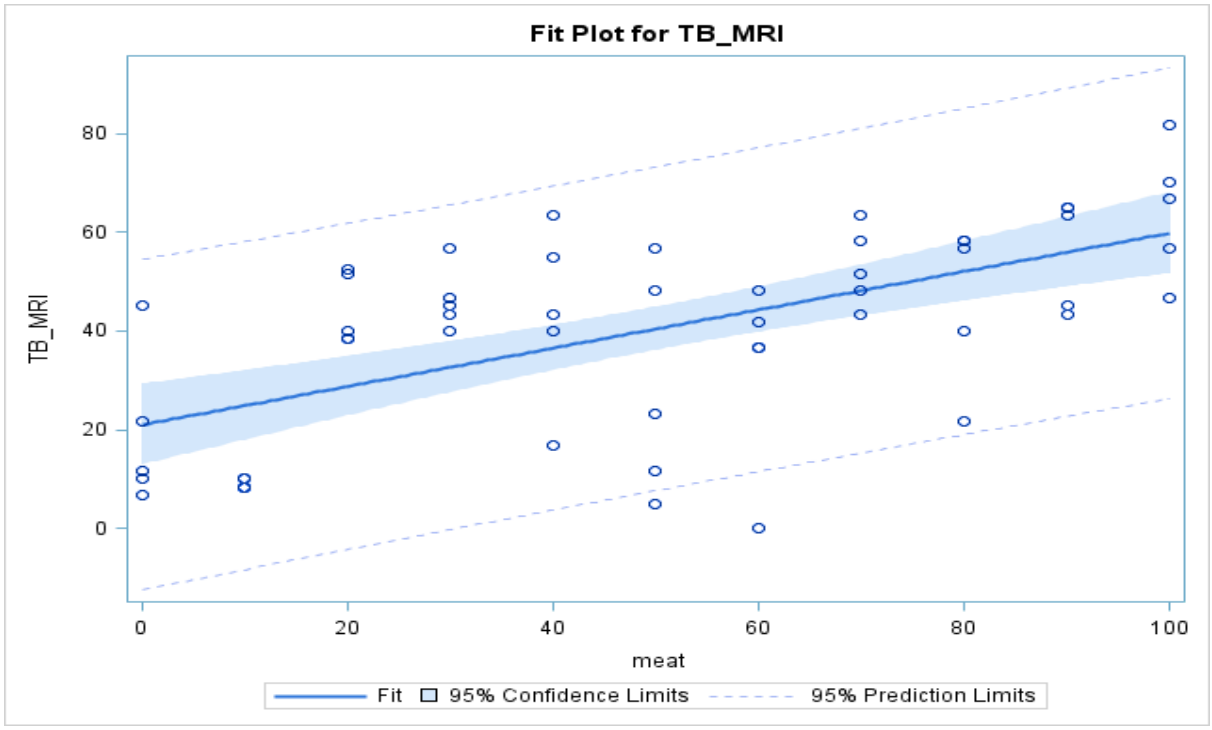

Figure 3 HE staining of references originating from breast muscle. Meat (X-axis) is indicating relative amounts of coarsely minced meat relative to emulsified meat. HE_mri (Y-axis) is % of structured muscle in sample.

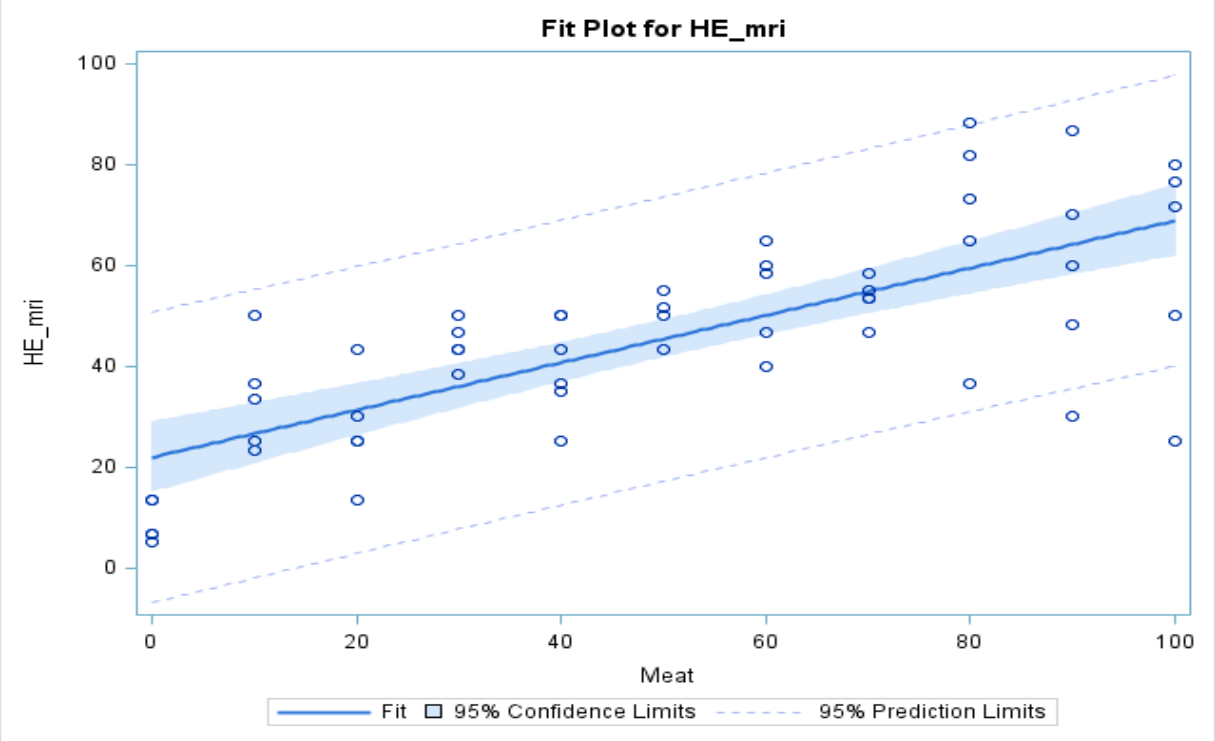

Figure 4 HE staining of references from thigh muscle. Meat (X-axis) is indicating relative amounts of coarsely minced meat relative to emulsified meat. HE_MRI (Y-axis) is % of structured muscle in sample.

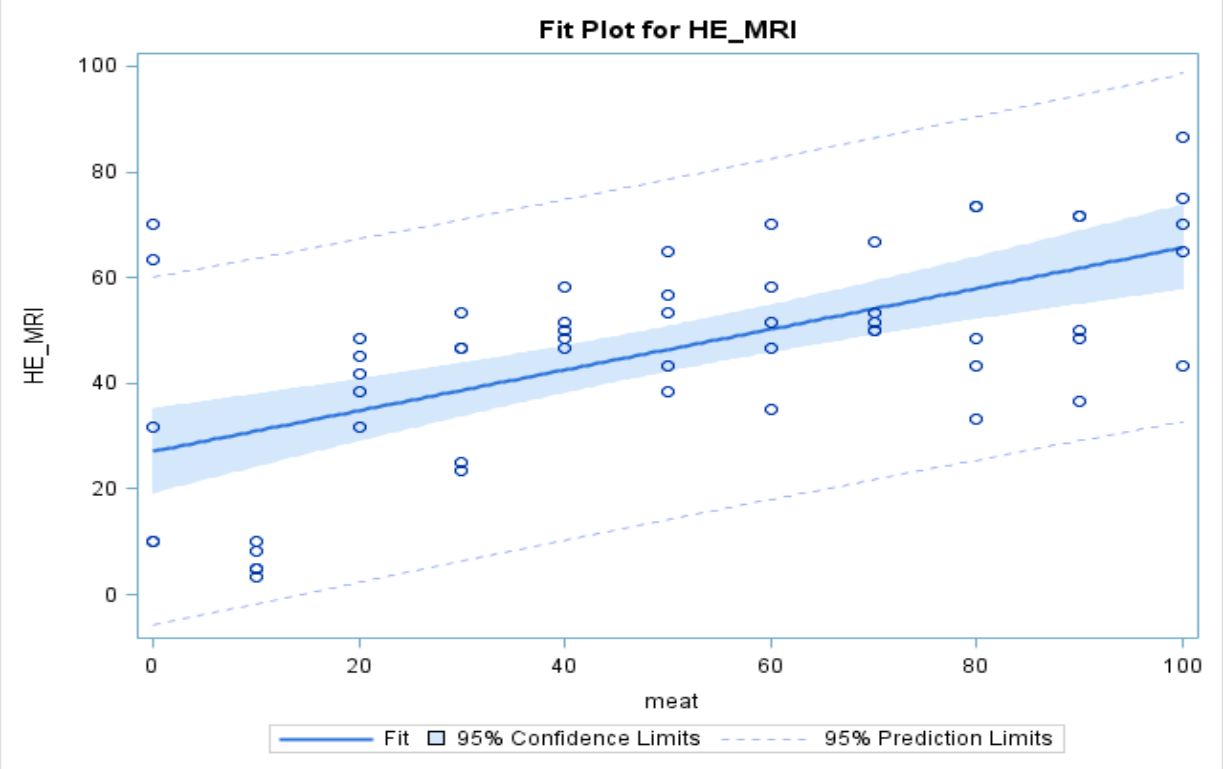

Figure 5 Laminin staining of references from breast. Meat (X-axis) is indicative of relative amounts of coarsely minced meat relative to emulsified meat. Mean (Y-axis) is total amount of structured meat in the samples.

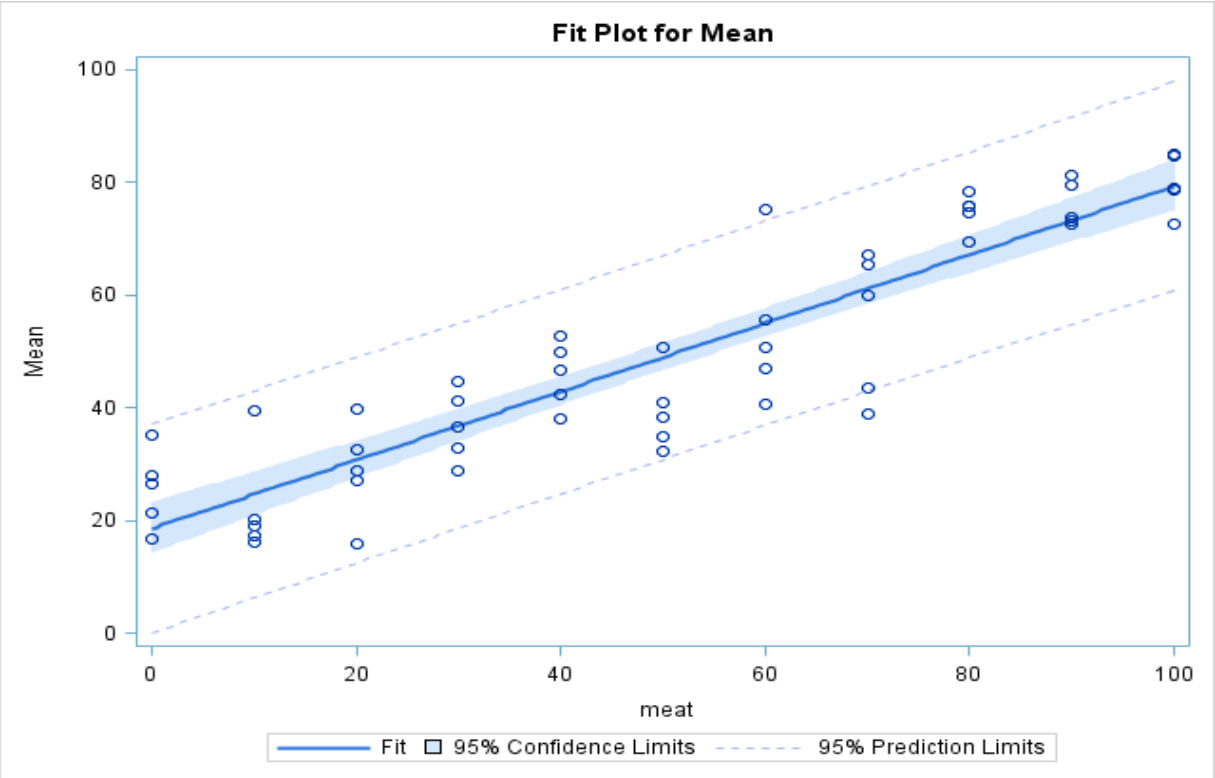

Figure 6 Laminin staining of references from thigh. Meat (X-axis) is indicative of relative amounts of coarsely minced meat relative to emulsified meat. Mean (Y-axis) is total amount of structured meat in the samples.

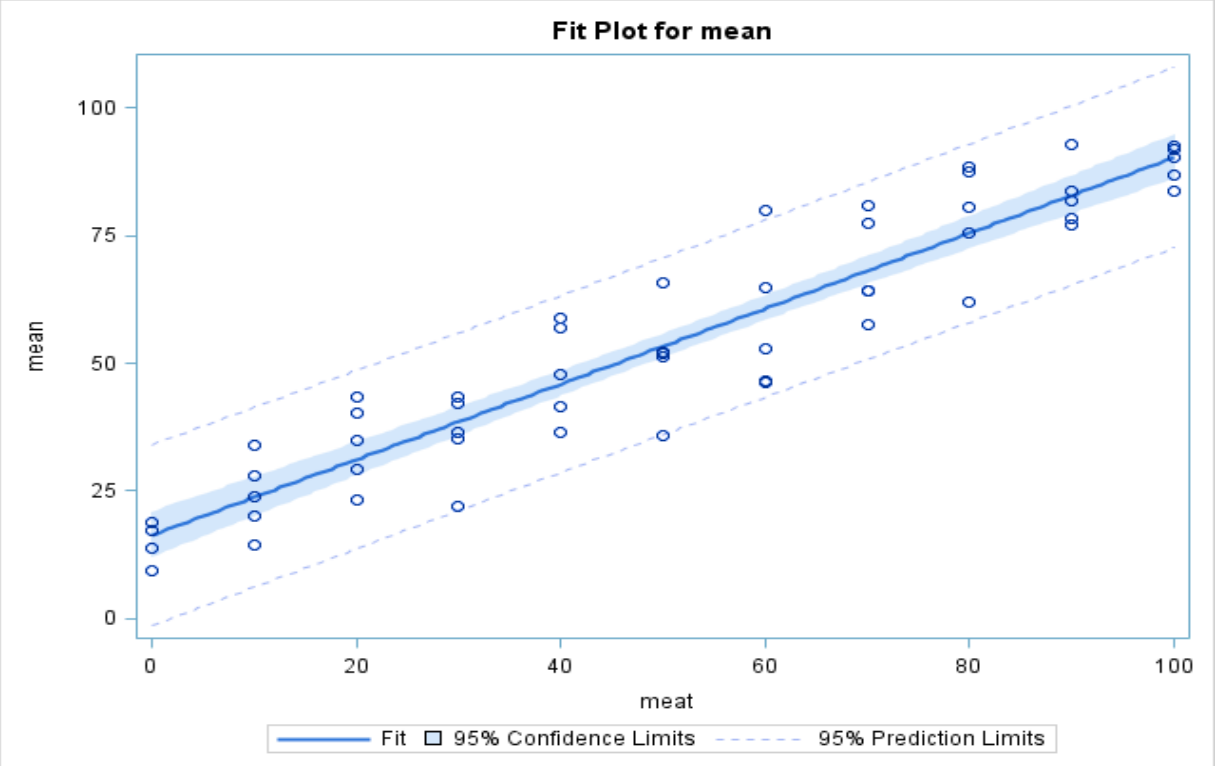

Figure 7 TB staining of references from breast muscle. Meat (x-axis) is indicating relative amounts of coarsely minced meat relative to emulsified meat. LFR_ny (Y-axis) is % of structured muscle in sample

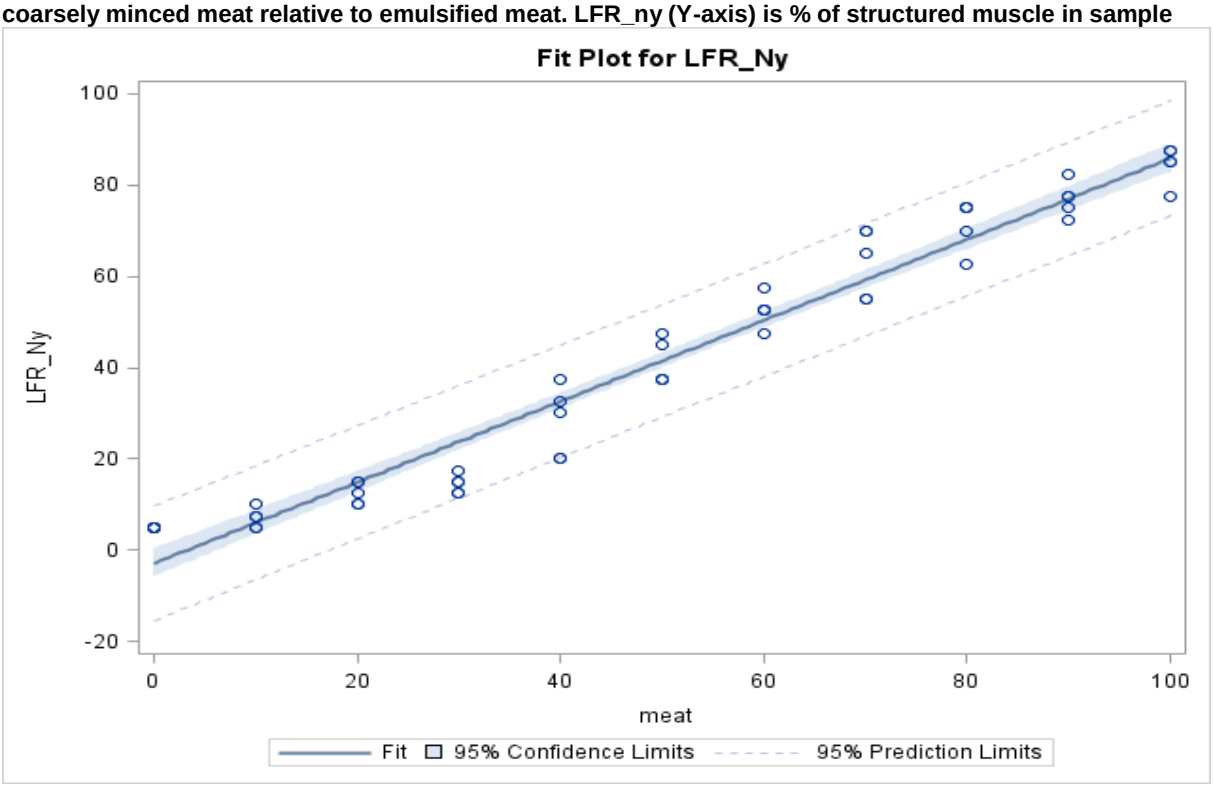

Figure 8 TB staining of references from thigh muscle. Meat (x-axis) is indicating relative amounts of coarsely minced meat relative to emulsified meat. LFR_ny (Y-axis) is % of structured muscle in sample.

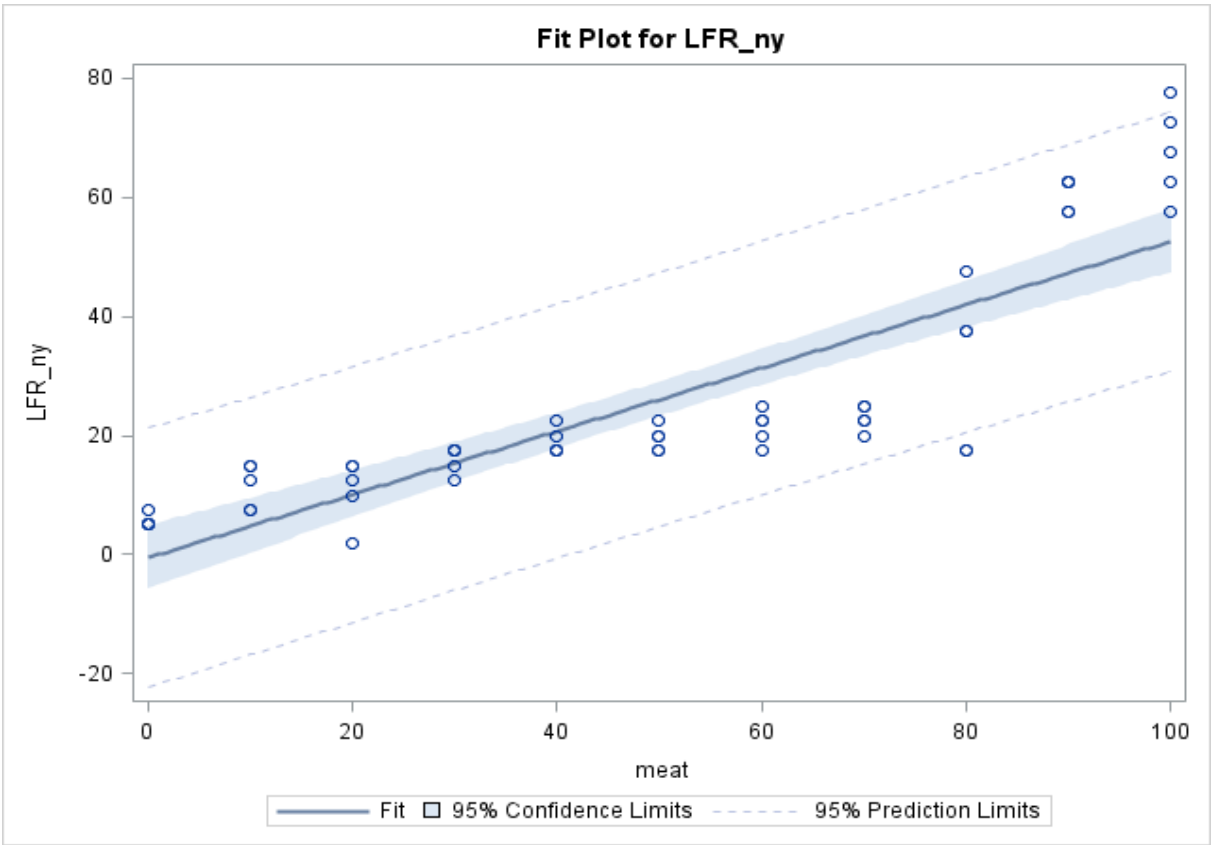

Table 1 Prediction of degree of structured meat in reference samples by four histological methods. #subs – number of subsamples in each quality. For breast and thigh muscle samples.

	AU Laminin Total			MRI HE			MRI TB			LFR TB		
	# subs	R	R^2	# subs	R	R^2	# subs	R	R^2	# subs	R	R^2
Thigh muscles	5	0.94	0.88	5	0.62	0.38	5	0.61	0.37	5	0,86	0,75
Breast	5	0.91	0.83	5	0.73	0.54	5	0.73	0.54	5	0,97	0,95

In conclusion, our present results shows that a method to quantify level of degradation of muscle structure is now available, which is the prerequisite for "quality" differentiation based on level of degradation of mechanically separated meat.

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