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A New Technique for Objective Evaluation of Marbling in Beef

Elke Albrecht, Jochen Wegner and Klaus Ender

Abstract
The recently developed technique for objective evaluation of marbling using automatic image analysis which is described here makes it possible to measure image analysis parameters which represent essential characteristics of the intramuscular fat deposits in meat. This permits detailed comparisons of different samples which provide much more information than the marbling points which have previously been established subjectively or the fat content determined by chemical means. As an initial area of application, this technique was used to investigate the intramuscular fat deposits in samples of beef from different breeds of cattle.

Intramuscular fatty tissue is an important factor for meat quality. Fat as a vehicle for taste has a crucia1 effect on the enjoyment value of the meat. The lower value placed on fat with increasing prosperity has resulted, via marketing and breeding, in adverse effects on the intramuscular fat content (HOFMANN, 1993). On the one hand, the consumer would prefer no fat if possible but, on the other hand, within the optimum range (2.5 to 4.5% intramuscular fat), fat has a positive effect on the essential meat quality factors.
Many scientific studies discuss connections between intramuscular fat content and smell, taste, tenderness and juiciness. However, it is not just the intramuscular fat content which is important for meat quality, but the fat distribution in the muscle tissue as well. Studies which focus only on the fat content often produce controversia1 information concerning the connections with tenderness. DUFEY (1989), OLDIGS et al. (1990), CUNDIFF et al. (1990) and CHRISTENSEN et al. (1991) found that the intramuscular fat content had no effect on tenderness while LEE and SCHÖN (1986), RISTIC (1988), BERRY and LEDDY (1990) and MONIN and OUALI (1991) did find connections.
Intramuscular fatty tissue consists of accumulations of 1 to over 1000 fat cells within the connective tissue cords between the bundles of muscle fibres (Fig. 1).

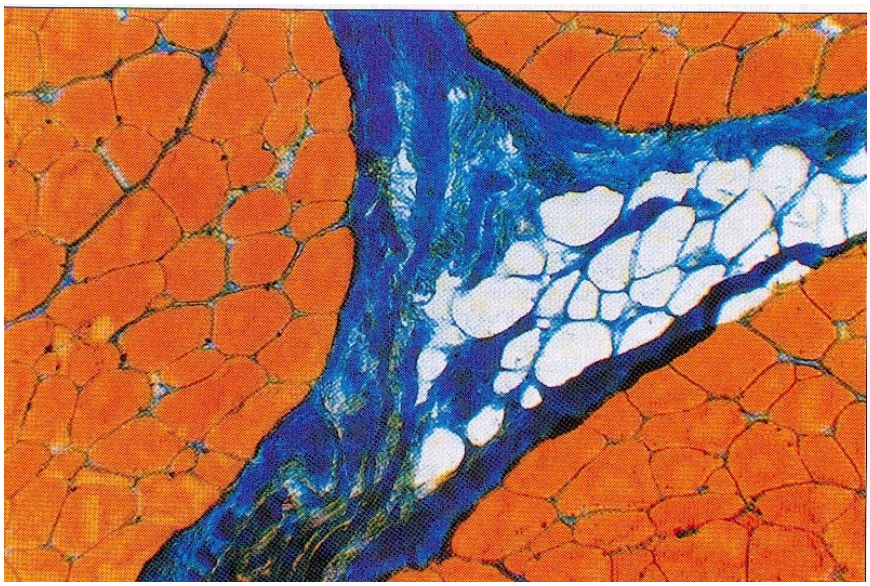


Fig. 1: Microscropic image of a group of intramuscular fat cells, white-fat cells, blue-connective tissue, orange-muscle fibres (approx. 200x magnification)

The more regularly distributed the intramuscular fat in the muscle, the more the connective tissue is broken up. In order to take this aspect into account, AUGUSTINI et al. (1993) evaluated meat quality by supplementing marbling with the subjective evaluation of the fat fibre strength and fat distribution. Subjective evaluation of marbling at certain points primarily takes account of the quantity of visible fat, ie. accumulations of more than 100 fat cells. This does not include the distribution of microscopic fat deposits.
A complex evaluation of marbling requires the application of new techniques. The combining of video technology, microscopy and computers, ie. automatic image analysis, can open up entirely new opportunities here. SCHOLZ and GREGOR (1993) developed a technique for estimating the intramuscular fat content in fresh pig loin with the aid of video image analysis. However, they did not obtain any information on the fat distribution. HOSHINO et al. (1990) used automatic image analysis to distinguish two types of fat deposits in intramuscular fat. They described punctiform deposits within the bundles of muscle fibres and reticular deposits between them. The authors established connections between the size of the arterioles and the related fat deposits in steers. Their investigations give an indication of how automatic image analysis can be used to explain the mechanism of intramuscular fat storage and to find connections between the structure of the fat deposits and other meat quality parameters. The aim of our study was to develop a technique to permit a quantitative description of the visual appearance of the intramuscular fat by means of automatic image analysis. This technique demonstrates differences in fat deposition between different breeds of cattle.

Automatic image analsis

Automatic image analysis is a technique which has undergone considerable development in recent years and is now indispensable in many areas, from space research to medicine. Where quantitative data are to be ascertained from visual information, ie. information in the form of images, image analysis can be used. Images produced in the microscope are an important source of information in biological research in particular. Evaluating the images produced is very time-consuming by the previous techniques and only a few image contents can be accessed. The use of a system consisting of a camera and a computer is intended to reconstruct the process which takes place in the human eye/brain complex. However, there are differences between the two in that a person has experiences which allow him to recognise things which a machine fails to register. On the other hand, the computer can provide objective, quantitative and reproducible results and generate a large number of new parameters.
The general scheme of an image analysis is shown in Fig. 2. Each image analysis starts by recording the image. A defined area of the image is broken down into picture elements (pixels) and the information concerning the position and the grey or colour value of each pixel is committed to the computer's image memory. After appropriate processing, this information yields data on the image content which is of interest, eg. the size of the total or individual areas, the length of structures, distribution within the image, etc. The parameters for each sample calculated on the basis of that data can be evaluated using appropriate statistical software (SAS).
Image analysis can be carried out in both the microscopic and macroscopic range. If the camera is fitted appropriately with a macroscopic lens or microscope adapter and the system calibrated accordingly, any sample can be processed. An appropriate measurement algorithm is worked out for each application and is then available in the form of a user program. Besides the evaluation of intramuscular fat deposits by image analysis, our working group already has experience of measuring muscle fibres, bundles of muscle fibres, sarcomas, cell nuclei, intercellular sarcomas, cell nuclei, intercellular spaces, adrenal layers, hairs and skin layers.

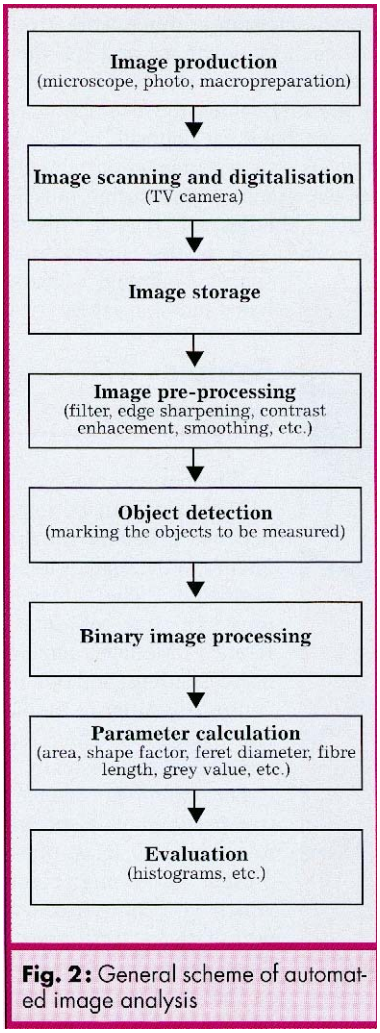


Fig. 2: General scheme of automated image analysis

Sample preparation

Whether automatic image analysis can be used depends crucially on the quality of the images to be evaluated. The images must be free of interference (artefacts, camera noise, shading) and rich in contrast in order to facilitate evaluation using the image analysis system.

For example, connective tissue plays a special role in beef. It is often present in strong cords which are not streaked with fat. In fresh meat, these appear just as white as the fat. To distinguish between pure connective tissue and fat, a histological staining method has been developed for slices of meat in which the fat is stained a rich red and the connective tissue stays white (Fig. 3). This produces a good contrast for the image analysis.

For the investigations on cattle of different ages and bred for different purposes, a slice 1.5 to 2 cm thick is taken from the muscles *M. longissimus dorsi* (mld.) and *M. semitendinosus* (mst.) 24 hours post mortem (p.m.). The mld. slice is taken at the 12th rib and the mst. slice, after full preparation of the muscle from the round of beef, from the thickest part of the belly of the muscle. These slices are fixed in 5% formaldehyde/ calcium for several days and stored in 5% formaldehyde until further processing. A universal cutting machine (Graef A2501) is used to cut the slices into smaller slices 1 to 2 mm thick which are then rinsed for a minimum of 12 hours. The slices are stained with oil red by simply placing them in the stain solution for 6 to 8 hours. The parent solution of oil red is prepared with 0.5 g of stain in 100 ml of pure isopropanol. This is diluted with distilled water in the ratio of 3 to 2 to obtain a working solution, and filtered. The stained slices are rinsed overnight. The next day, they are differentiated for 2 to 4 hours with 70% isopropanol under constant motion

(motion apparatus from VETEC) and then rinsed for a minimum of 12 hours. The samples can be stored permanently in 5% formaldehyde.

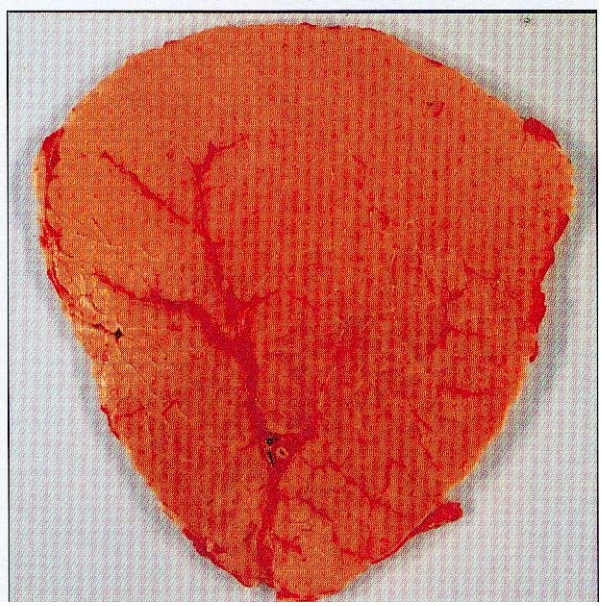


Fig. 3: Stained muscle slice of M. semitendinosus with clearly visible fat deposits (red) and connective tissue cords (white) as well as blood vessels.

Evaluating the samples using the image analysis system

The measurements are carried out using the Quantimet 570 image analysis system from LEICA. First, an image of the meat slice is stored in the image analysis system's image memory via the colour TV camera. To be able to measure an object, it must be larger than the camera's resolution, which means that the areas of fat should measure at least four pixels. The mld. is therefore measured in two sections in order to be able to reproduce the areas of fat large enough. Then the area of the meat slice is marked and measured using threshold value detection.

Then the areas of fat are detected after a pre-processing of the image which serves to sharpen the edges, ie. to reinforce the transitions between the background (meat slices) and the object (area of fat). This is followed by an interactive correction of the areas of fat detected. When all of the marked areas of fat are measured, the shape factor of each object is also determined in addition to its size and position. This makes it possible to classify areas of fat as round or long islands. The result is a data file for each slice of meat. The most important measurement values are: (1) area of the slice of muscle, (2) total areas of fat, (3) number of areas of fat, (4) mean value of areas of fat, (5) areas of the three largest areas of fat, (6) proportion of round areas of fat, and (7) proportion of long areas of fat.

The centre of the slice of meat serves as the orientation point for classifying the position of each island of fat. The number of islands of fat in each quarter is counted and the total area of the islands of fat is calculated for each quarter. Depending on the quality of the sample and quantity of stored fat, a measurement takes 5 to 10 minutes.

Objective parameters for marbling

A single parameter cannot fully describe a characteristic as complex as marbling. Consequently, the following parameters are derived from the measurement values referred to above: (1) proportion of areas of fat as the ratio between the total areas of fat and the area of muscle, (2) number of areas of fat per cm^2 as the absolute number of areas of fat related to the area of muscle, (3) size of areas of

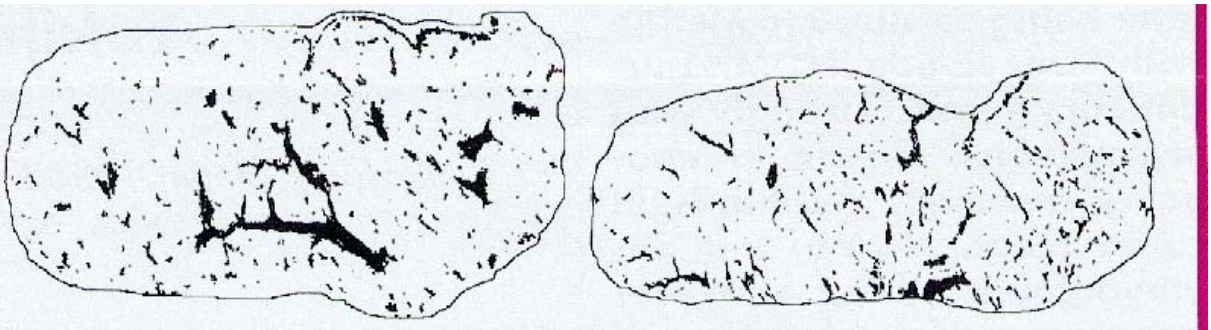
fat as the mean value of all areas of fat, (4) proportion of the three largest areas of fat compared with the total area of fat, (5) proportion of the long areas of fat, (6) distribution of areas of fat as the distribution of the area proportions in each eighth for the mld. and each quarter for the mst.
The proportion of areas of fat is the optical reflection of the intramuscular fat content determined by chemical means, by petroleum ether extraction. The correlation coefficient between the two values is r = 0.82 (Tab. 1).

Tab. 1: Correlation coefficients between parameters of intramuscular fat (cattle, 12 months, n = 37)

	Fat content (chem.)	Proportion of areas of fat	Number of areas of fat/cm²	Size of areas of fat	Proportion (as a percentage) of the three largest areas of fat	Proportion of long areas of fat
Proportion of areas of fat	0.82					
Number of areas of fat/cm²	0.65	0.86				
Size of areas of fat	–0.02	0.02	–0.44			
Proportion (as a percentage) of the three largest areas of fat	0.10	0.09	–0.37	0.84		
Proportion of long areas of fat	0.52	0.57	0.18	0.62	0.69	
Distribution of areas of fat	–0.21	–0.31	–0.62	0.69	0.77	0.34

The values of the proportion of areas of fat are larger than the fat content determined by chemical means since the intramuscular fatty tissue, which is emphasised by staining, contains more than just the fat which can be extracted by chemical means. The fat is stored in cells which are surrounded by connective tissue, so the stained areas also consist of areas of connective tissue and water (Fig. 1).
The number of areas of fat gives an important indication of the "quality of marbling": the more areas of fat the better. The size of each area of fat should be as small as possible. This is closely related to the proportion of the three largest areas of fat compared with the total area of fat. The larger this value, the more the appearance is marked by large fat cords.
A lower value for the proportion of 1ong fat islands means more punctiform fat deposits and fewer large deposits, which are usually present as long cords. The distribution of the islands of fat over the muscle area is reflected in the distribution of the area proportions over the individua1 areas of the muscle. The smaller the value for the distribution, the more regu1arly the fat is distributed.
Tab. 1 shows the phenotypical correlation coefficients of the characteristics of the intramuscular fat of 12-month-old cattle. The values show that there are close connections between the number of areas of fat per cm^2 and the proportion of areas of fat, but no connection with the size of the areas of fat, which means that a higher fat content is achieved in this age range mainly by a larger number of fat deposits rather than by larger fat deposits (r = 0.86). A larger number of areas of fat is associated with a lower value for the distribution of areas of fat (r=-0.62). Larger areas of fat are associated with a higher proportion of the three largest areas of fat compared with the total area of fat (r = 0.84), more long areas of fat (r=0.62) and a poorer distribution of areas of fat (r=0.69).
To illustrate the additional, more accurate information which can be obtained by image analysis, Fig.. 4 compares two samples with the same proportion of areas of fat. The samples show a different pattern and distribution of fat deposits. The marbling of sample 1 was evaluated subjectively with 3 points and that of sample 2 with 2 points, although the samples' chemical fat content was found to be 3.49% and 4.22% respectively. The data obtained by image analysis reveal the cause of this discrepancy. The average size of the areas of fat in sample 1 is double that in sample 2. Sample l shows large fat cords, which give values of 34% for the proportion of the three largest areas of fat. At 77.4%, the proportion of long fat deposits is markedly higher than the value

for sample 2. Sample 2 was found to contain a larger number of smaller and more evenly distributed areas of fat. The parameters obtained from the image analysis thus describe the more regular intramuscular fat distribution and finer structure of sample 2, which could not be determined in the fresh meat by estimating the marbling.



Sample 1 (no. 254/DA 24 mon.)		Sample 2 (no. 259/SR 24 mon.)
3	Marbling [points]	2
3.49	Fat content (chem.) [%]	4.22
8.67	Proportion of areas of fat [%]	8.54
3.32	Number of areas of fat / cm²	6.72
2.49	Size of areas of fat [mm²]	1.24
34.0	Proportion of 3 largest areas of fat [%]	16.4
77.4	Proportion of long areas of fat [%]	58.0
9.4	Distribution of areas of fat [%]	5.0

Fig. 4: Areas of fat detected in two muscle sections with parameters of intramuscular fat

Differences in fat distribution between breeds

Nine or ten 12-month-old bulls of the breeds Belgian Blue (WBB), German Angus (DA), Galloway (Ga) and Friesian (SR) were available for the investigation. Tab. 2 and 3 show the results of the evaluation of the intramuscular fat by image analysis. The young Belgian Blue bulls, as a heavily muscled breed with double muscling, had very few deposits of intramuscular fat. The fat parameters for the WBB should therefore be interpreted bearing in mind the lower fat content.
The values for the *M. longissimus dorsi* in Tab. 2 indicate that the DA, Ga and SR showed similar proportions of areas of fat but differences in the fineness and distribution of the fat. For the Ga, the largest value for the number of areas of fat per cm^2 and the smallest values for the size of the areas of fat, the proportion (as a percentage) of the three largest areas of fat, the proportion of long areas of fat and for the distribution of areas of fat were determined. The results indicated a finer and more regular deposition of fat for the Galloway. The Friesians did not differ significantly, as regards the number and size of the areas of fat or the distribution of areas of fat, from the Galloway or the Angus. As regards the proportion of the three largest areas of fat and the proportion of long areas of fat, the values were significantly higher than for the Galloway, so they had more long and larger fat deposits than the Galloway and therefore occupy an intermediate position.
The fat distribution was also investigated in the *M. semitendinosus*, although there was no subjective assessment of the marbling. When the muscles were compared (Tab. 3), the mst. showed a coarser, less even fat deposition than the mld. Between the breeds, there were differences in the quantity, fineness and distribution of the intramuscular fat which were similar to the differences in the mld., ie. the Galloway showed the smallest values for the fat distribution parameters, associated

with the largest number of fat deposits.The estimated fat distribution of the breeds was correct only for the age range of 12 months.
Due to its simple structure, the mst. is an ideal model for studying the connections between muscle structure and the deposition of intramuscular fat and growth-related changes in fat deposits. The results of this study will be published at a later date.

Tab. 2: Structure of intramuscular fat in M. longissimus dorsi

Age range: 12 months		Belgian Blue	German Angus	Galloway	Friesian
	n	9	9	10	9
Marbling (point)	Mean	1.0^{a}	1.6^{bc}	1.5^{b}	1.9^{c}
	STD	0.0	0.5	0.5	0.3
Fat content (chem.) [%]	Mean	0.27^{a}	1.34^{b}	1.26^{b}	2.18
	STD	0.11	0.56	0.38	0.80
Proportion of areas of fat [%]	Mean	0.58^{a}	3.22^{b}	3.51	3.92^{b}
	STD0.24	0.69	1.46	1.02	
Number of areas of fat / cm^2	Mean	0.5^{a}	2.5^{b}	4.5^{c}	4.0^{c}
	STD	0.2	0.7	1.8	0.9
Size of areas of fat [mm^2]	Mean	1.12^{a}	1.32^{ab}	0.82^{c}	0.99^{ac}
	STD	0.26	0.23	0.29	0.22
Proportion of the 3 largest areas of fat [%]	Mean	28.7^{a}	36.7^{ab}	20.6^{ac}	29.2^{ab}
	STD	6.4	8.0	13.0	6.6
Proportion of long areas of fat [%]	Mean	50.5^{a}	68.3^{b}	57.2^{a}	66.6^{b}
	STD	12.1	1.9	11.4	5.4
Distribution of areas of fat [%]	Mean	11.5^{a}	10.5^{ac}	6.2^{b}	8.7^{bc}
	STD	3.3	3.2	2.9	0.9

Values with dissimilar exponents are significantly different (< 0.05)

Tab. 3: Structure of intramuscular fat in M. semitendinosus

Age range: 12 months		Belgian Blue	German Angus	Galloway	Friesian
	n	9	9	10	9
Fat content (chem.) [%]	Mean	0.39^{a}	0.93^{b}	1.11^{bc}	1.41^{c}
	STD	0.12	0.24	0.37	0.54
Proportion of areas of fat [%]	Mean	0.74^{a}	2.38^{b}	2.22^{b}	2.60^{b}
	STD	0.30	0.91	1.07	1.16
Number of areas of fat / cm^2	Mean	0.4^{a}	1.2^{b}	2.6^{c}	1.7^{b}
	STD	0.1	0.3	0.9	0.7
Size of areas of fat [mm^2]	Mean	2.05^{a}	1.96^{ac}	0.85^{b}	1.53^{c}
	STD	0.68	0.77	0.22	0.31
Proportion of the 3 largest areas of fat [%]	Mean	39.8^{a}	47.8^{a}	26.7^{b}	40.8^{a}
	STD	6.9	12.5	8.5	10.1
Proportion of long areas of fat [%]	Mean	56.9^{a}	76.2^{b}	53.6^{a}	75.9^{b}
	STD	14.1	6.2	13.0	6.6
Distribution of areas of fat [%]	Mean	27.2^{a}	20.3^{b}	16.9^{b}	19.8^{b}
	STD	4.8	5.5	5.7	5.3

Values with dissimilar exponents are significantly different (< 0.05)

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Corresponding author:

Dr. J. Wegner, Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany