X-RAY MICROTOMOGRAPHY FOR BEEF INTRAMUSCULAR FAT ASSESMENT

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ABSTRACT

In the present research, the X-ray microtomography (µCT) technique was used to quantify intramuscular fat content and to study fat distribution in different breed and commercial meat joint. Two different breeds, Podolian *vs* Charolaise, chosen to exhibit variability in terms of visible structure of fat, were used. High Pearson correlation coefficients (r= 0.92 - 0.99, P<0.001) were found between fat content, expressed as percentage object volume (POV) determined by µCT and fat content analyzed by official method. Useful informations were provided from quantitative three dimensional parameters describing the fat structure, such as the structure model index (SMI), the object structure/ volume ratio (OSVR) and the structure separation (SS). Charolaise breed showed higher POV and SS (P<0.01) values than Podolian. X-ray microtomography allows a rapid estimation of intramuscular fat of meat and provides a more accurate description of the fat microstructure and meat quality.

Index Terms— intramuscular fat, meat microstructure, quantitative analysis, X-ray microtomography,.

I. INTRODUCTION

Intramuscular fat content in meat is a very important compound influencing the palatability properties such as taste, juiciness and texture. There are several methods to analyse quantitative intramuscular fat content (Monin 1998), although, the reference method (AOAC, 1995), which is commonly used, is based on chemical analysis. This technique is time consuming, destructive and uses harmful, flammable solvents with health and environmental hazards. X-ray microtomography (µCT), on the contrary, is a non-destructive technique with several advantages compared with other methods, including the ability to image low moisture materials. The series of slices, covering the entire sample, can be rendered into a 3-D image that can either be presented as a whole or as virtual slices of the sample at different depths and in different directions. Commonly used methods for the analysis of fat do not provide information on the spatial distribution of fat but provide only its percentage volume. µCT on the other hand, provides the percentage volume and also the spatial distribution of the fat. The aim of the present research is to demonstrate the capability of X-ray microtomography as a useful technique to quantify intramuscular fat content and to study fat distribution in different breed and commercial meat joint

II. MATERIALS AND METHODS

The meat slices (150) analysed came from 30 animals of two different breed (Podolian - P *vs* Charolaise - CH) provided by two farm situated in the Gargano National Park 50 km Northwest of Foggia, Southern Italy. Animals were slaughtered at 18 months of age at the end of the finishing period. Five different commercial joints were collected, in order to exhibit variability in terms of the visible structure of fat: tenderloin (TL), top sirloin (TS), top side (TSi), chuck tender (CT),eyeround (ER) removed by the following muscles *psoas major*, *biceps femoris*, *semimembranosus*, *supraspinatus*, *semitendinosus* respectively. Three samples were prepared for each type of meat, each 28mm in diameter and a thickness of 18mm. Each sample used for x-ray microtomography (µCT) analysis was wrapped with parafilm to avoid dispersion of moisture; the parafilm does not interfere with the x-rays. The same samples used for µCT analysis were also used for chemical analysis. Analysis of intramuscular fat content was performed according to AOAC (1995) method, using the FOSS SoxCap 2047 System in combination with Soxtec extraction systems using petroleum ether .

For X-ray microtomography (µCT) the samples were imaged under the same conditions, using the Skyscan 1172 high-resolution desktop X-ray microtomography system (Skyscan, Belgium). A meat sample was placed on a rational plate, the source and the detector were fixed, while the sample was rotated during measurement. Power settings of 60 kVp and 167 µA were used. A CCD camera with 2000 x 1048 pixels was used to record the transmission of the conical X-ray beam through all samples. An exposure time of 1475 ms were chosen to minimize the noise. Smoothing and beam-hardening correction steps were applied to suppress noise and beam hardening artifacts, respectively. Scan time, on average, required 20 minutes. A set of flat cross section images, was obtained for each sample after tomographical reconstruction by the reconstruction software NRecon. Three-dimensional reconstructions of samples were created by effectively stacking all 2D tomographs, a total of 125 slice images with a slice spacing of 0.069mm.

For image processing and analysis, the skyscan software, CT-Analyser (CTAn) was used. Image segmentation was firstly carried out on the smoothed 8-bit grey-scale images obtained from the reconstruction step, using CTAn (Skyscan) software. For data analysis, prior to 3D reconstruction, a component-labelling algorithm, available within

CTAn, was used to isolate the largest 3D connected structures. All reconstructions where created in CTAn (Skyscan) using an adaptive rendering (locality 10 and tolerance 0.25) algorithm and saved as P3G surface model (SkyScan model format). P3G models were then imported into CT vol software (Skyscan) for visualization.
The following seven geometric parameters were measured using the CTAn software (Skyscan):
i) the Percent object volume (POV) which is the proportion of the volume of interest (VOI) occupied by binarised solid objects (e.g. fat);
ii) the object surface/volume ratio (OSVR) which is the basic parameter in order to characterise the complexity of the structure and to estimate the thickness (e.g. the size and distribution of fat in each sample);
iii) the fragmentation index (FI), developed and defined by Hahn, Vogel, Pompesius-Kempa and Delling (1992) as the index of the structural connectivity. It calculates the relative convexity or concavity of the sample surface;
iv) the structure model index (SMI) which estimates the shape of the structure (0=ideal flat, 3=cylindrical and 4=spherical);
v) the structure separation index (SS) which is the thickness of the spaces as defined by binarisation within volume of interest (i.e. fat) and it can be calculated either from 2D or 3D images;
vi) the degree of anisotropy (DA) which measures the preferential alignment of fat.
The value for the total fat content obtained from chemical analysis is the percentage of wf/ws where ws is the weight of the sample and wf is the weight of fat present in the sample, whereas the value obtained from µCT analysis is the percentage of Vf/Vtot, where Vf is the volume of fat and Vtot is the total volume, and it is equal to the Percent object volume parameter of the CTAn software (Skyscan). In order to have the same unit values for each type of analysis the following equations were used to convert the data acquired from chemical analysis (Toledo 1999):

$$\rho_p = 1329{,}9 - 0{,}51814 \cdot T \quad (1)$$

$$\rho_f = 925{,}59 - 0{,}41757 \cdot T \quad (2)$$

where: rp is protein density (kg/m3), rf is fat density (kg/m3170), T is the temperature (25°C).
Data were subjected to analysis of variance, using the GLM procedure of the SAS statistical software (1999).

III. RESULTS AND DISCUSSION

Figure 1a shows an example of the grey level reconstructed cross section images acquired by µCT for the CT samples for the CH. The contrasts in these images are based on the differences in absorption of X-rays by the constituents of the sample (e.g fat and protein). This contrast is produced by a variation of density and a change in composition of the sample and is based exclusively on the detection of an amplitude variation of X-rays transmitted through the sample itself. The obtained image is a map of the spatial distribution of the X-rays in which the brighter regions correspond to the higher level of attenuation, i.e. higher density region. It can be assumed from these figures that the dark grey areas represent fat as it has a lower absorption coefficient with respect to protein. Further information cannot be acquired from these images, as these are only 2D representations of the samples. Figure 1b shows as an example the set of flat cross sections that were obtained for these samples after binarisation of the images using the reconstruction software NRecon (Skyscan), from these images the fat/protein/air matrix is clearly visible. On the other hand, figure 1c shows as an example the three-dimensional reconstructions of the CT samples for both breeds from which the geometrical parameters were calculated using the CTAn software (Skyscan). Table 1 shows the Pearson correlation coefficients for fat determined by chemical analysis and POV in the five different commercial joints of both breeds, it can be noted that the POV is highly correlated with fat determined by chemical analysis, in both genotype and in all joints analysed in a range of r= 0.92 - 0.99 ($P<0.001$). The high correlations between the two methods for determination of fat percentage highlight the validity of µCT, therefore this analyzing technique allows an accurate quantification of the visual appearance of fat in meat. Previous research (Pipeck, Jelenikova & Sarnovsky, 2004) found positive correlation between fat and fat content determined by video image analysis. However this analysis showed some inaccuracies in fat determination as consequence of interfering influence of connective tissue and bones. On the contrary, the present study verified the high level of reliability and accuracy of the X-ray microtomography technique. In addition, it seems useful in determining the spatial distribution of muscular fat.
Table 2 shows the Pearson correlation coefficients for fat content and all µCT parameters in Charolaise and Podolian breed. It can be noted that fat content and POV are negatively correlated with OSVR ($P<0.001$), FI ($P<0.001$) and SS ($P<0.01$) in both breeds. OSVR indicates the fat distribution within the sample: the higher the value, the more finely distributed is the fat present in the sample. The FI parameter is the index of connectivity and is a measure of relative convexity or concavity of the total solid surface, based on the principle that concavity indicates connectivity, and convexity indicates isolated disconnected structures (Lim & Barigou, 2004), while, SS indicate distance of fleck of fat within the samples. These results highlighted that the meat with higher content of fat have smaller flecks of fat that are closer to each other and with better connected lattices. The POV is not correlated with SMI and DA.

IV. CONCLUSION

X-ray microtomography analysis is suitable for rapid estimation of intramuscular fat of meat. The comparison of this method with the classical Soxhlet extraction showed a high correlation between fat contents obtained by both methods in all samples. X-ray microtomography allows a more accurate study of the fat in meat, than the visual appearance. It shows the microstructure of fleck of fat measuring the size, shape, networking/connectivity and distribution of various phases. Although this procedure could appear expensive, X-ray microtomography analysis provide more information on the fat content and on its spatial distribution inside the sample in order to obtain a more accurate description of meat quality and its expected palatability.

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Table 1-Pearson correlation coefficients for fat and percentage objective volume (POV) in five different commercial joints of Podolian and Charolaise breed (n=450)

	POV	
	Podolian	Charolaise
Top side	0.99***	0.98***
Eye of round	0.92***	0.95***
Chuck tender	0.99***	0.93***
Top sirloin	0.98***	0.98***
Tenderloin	0.99***	0.99***

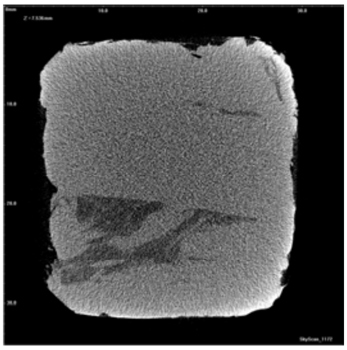
*** =P<0.001

Table 2-Pearson correlation coefficients for µCT indexes[1] in Podolian and Charolaise breed (n=450).

		OSVR	FI	SMI	SS	DA
POV	Podolian	-0.75***	-0.98***	-0.01	-0.73**	-0.36
	Charolaise	-0.77***	-0.94***	0.03	-0.70**	-0.14
OSVR	Podolian		0.79***	0.01	0.24	-0.11
	Charolaise		0.84***	0.20	0.51*	-0.25
FI	Podolian			0.02	0.74**	0.37
	Charolaise			0.02	0.63**	0.20
SMI	Podolian				0.12	0.06
	Charolaise				-0.06	-0.11
SS	Podolian					0.07
	Charolaise					0.09

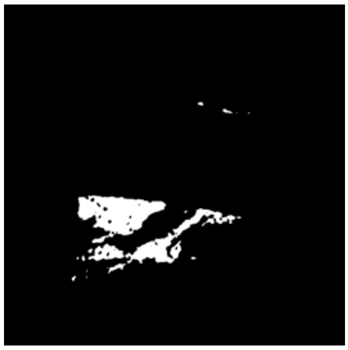
* P<0.05; **P<0.01; ***P<0.001
[1]POV=percent object volume, OSVR=object surface/volume ratio, FI=fragmentation index, SMI=structure model index, SS= structure separation, DA= degree of anisotropy

Figure 1. a) Grey level reconstructed cross section images acquired by μCT for the chuck tender sample for the Charolaise breed; b) Set of flat cross sections for the chuck tender sample for Charolaise breed after binarisation; c) Three-dimensional reconstructions of the chuck tender sample for Charolaise breed.



a)

10 mm



b)

10 mm

c)

10 mm