THE FATTY ACID COMPOSITION OF DIFFERENT BOVINE ADIPOSE TISSUES IN RELATION TO THE PROTON PULSE NMR RELAXATION MEASUREMENTS

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Background

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful analytical techniques available in the grading of meat products. The general features of proton relaxation in muscle are characterised by longitudinal relaxation time (T_1) and transverse relaxation time (T_2). In rigor muscle, a multicomponent T_2 relaxation behaviour is observed, which gives information about dynamics and. distribution of water. The longest T_2 (T_{21}) was regarded as free water while the shortest T_2 (T_{22}) was considered as mainly held in myofibrils. The previous year on ICoMST the applications of the ^{1}H NMR was reviewed by Tornberg (2001) to analyse the meat quality traits. Close correlation was found between drip loss and T_{22} relaxation time (r=0.60-0.77). There is a significant negative relationship between T_{21} relaxation time and sensory determined tenderness. Bertram et al. (2002) has suggested superior performance of continuous distribution analysis of T_2 relaxation as an alternative method for determination of water holding capacity. Gunstone (1993) has published comprehensive reviews that deal specifically with the use of ^{13}C NMR techniques in the analysis of lipid mixtures. Lie Ken Jie & Mustafa (1997) also used ^{13}C NMR spectroscopy to analyses unsaturated fatty acids and triglycerides.

Objectives

The aim of this study was to examine if there is any relationship between the fatty acid composition of fat samples taken from different locations of carcass and that of relaxation times obtained by proton pulse NMR spectroscopy.

Methods

Altogether 6 Holstein-Friesian fattening bull calves were slaughtered on the average 187 of days and 225 kg of live weight. The average cold carcass weight was 115 kg and the average dressing percentage 51,21 %. In addition to slaughter records, 2-3 g fat samples were taken from three locations (subcutaneous fat from rump region, perinephric-, and internal fat) in a subsequent trial for proton pulse NMR spectroscopy and fatty acid analysis. The proton relaxation times were measured with a MINISPECT PC 140 (Brucker, Germany) NMR system (operating frequency: 40 MHz, 1 Tesla). A T_1 relaxation time was determined by an inversion recovery method with eight different time intervals between the 180° and 90° pulses. Repetition time was 5 times T_1. The transverse relaxation, T_2, was measured using the Carr-Purcell-Meilboom-Gill (CPMG) sequence: the echo time was 1 ms, the number of echos detected was 1000. Multiexponential behaviour of T_2 curves was analysed by least squares statistical procedure. Water content of fat samples was determined gravimetrically. Fresh fat samples were weighted before and after drying at 104 °C for 48 h, the water content was expressed in percent of wet weight. The determination of fatty acid composition in samples was analysed simultaneously using CHROMPACK CP 900 gas chromatography. When analysing the fatty acid content the results relating to the unknown sample were given as the relative mass percentage of the fatty acid methyl esters. For statistical analysis software of SPSS 8.0 computer package were used.

Results, discussion

In the *Table 1.*, the results of the spectroscopy examinations of the samples taken from the subcutaneous, perinephric and internal fat can be seen on the bases of the multiexponential analysis. The T_1 relaxation time was changed during the monoexponential analysis between 240 and 260 ms. The longest T_1 monoexponential relaxation time was measured in perinephric fat, while the longest T_2 monoexponential relaxation time in the subcutaneous fat. There were significant differences between the groups, neither in T_1 nor for T_2 monoexponential relaxation times. The biexponential analysis of T_2 relaxation times and curves gave an alternative for separating and determination of proportion of water fraction with different mobility. The shorter relaxation times characterise the bounded water fraction, while the longer relaxation refers to free water. From the results, it seems that, the proportion of the free water is more significant, in a case of all the three fat samples, in cases of the subcutaneous and the internal fat, more than 93%, in turn in the perinephric fat 88%. This proves that, the water can be found mainly in the extracellular field, and relatively small quantity in the intracellular field. The component shows fast T_2 relaxation time (T_{22}), has the shortest time in the internal fat, but considering its proportion the highest (11,9%) in perinephric fat. By examination of the water content in fat from different locations, it can be seen that, the water content of perinephric fat is the highest, which is differ significant ($P<0.01$) from the water content in the subcutaneous and the internal fat. Within this, the proportion of bounded water is the highest, and the proportion of the free water with slow T_2 (T_{21}) relaxation time is less. Close and significant correlation (r=0,67; $P<0,001$) was established between the water content and the fast component of the biexponential T_2 relaxation time, which supports the tendencies mentioned above. The *Table 2.* shows the fatty acid composition of fat samples from different locations. According to the statistical analysis the perinephric fat contained significantly ($P<0,01$) higher amounts of saturated fatty acids due to higher palmitic acid (C 16:0) and stearic acid (C 18:0) content ($P<0,005$ and $P<0,001$) than that of subcutaneous, and internal fat. In addition, it contained higher amounts of myristic acid ($P<0,05$) in comparison with subcutaneous fat. These three saturated fatty acids contributed more than 90% of the total saturated fatty acids. In case of monounsaturated fatty acid significant difference ($P<0,05$) there was shown for the quantity of palmitoleic acid (C 16:1) between the perinephric and subcutaneous fat. Comparing the result of the ^{1}H NMR examination and the fatty acid composition of the fat samples, it can be seen in both cases that, the perinephric fat significantly differed either from the internal and subcutaneous samples. According to the biexponential analysis of T_2 relaxation time of perinephric fat, statistical differences were established between the proportion of fast, short relaxation components, and the fatty acid composition in the monounsaturated fatty acid proportion. This provided evidence that, the differences shown in the fatty acid composition resulted in variation of the relaxation times or in the proportion of components with fast or slow relaxation components.

Pertinent literature

Bertram, H. C., Donstrup, S., Karlsson, A. H., Andersen, H. J. (2002): Meat Science. 60. 279-285.
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Table 1. Characteristics of mono and biexponential analysis in adipose samples

	Fat sample	Mean	SD	Min.	Max.
Monoexponential (sec)	subcutaneous	0.24	0.02	0.21	0.27
	perinephric	0.26	0.03	0.22	0.29
	internal	0.24	0.02	0.21	0.26
	Combined mean	**0.25**	**0.02**	**0.21**	**0.29**
Monoexponential (msec)	subcutaneous	131.86	5.11	124.22	137.93
	perinephric	128.88	8.42	112.23	134.41
	internal	130.13	2.16	126.58	132.63
	Combined mean	**130.29**	**5.62**	**112.23**	**137.93**
Biexponential T_{21} fast (msec)	subcutaneous	22.83	2.93	19.11	26.01
	perinephric	21.58	2.17	18.91	24.50
	internal	19.91	1.10	19.04	21.59
	Combined mean	**21.44**	**2.404**	**18.91**	**26.01**
Biexponential T_{21} fast (%)	subcutaneous	6.63	2.34	2.98	9.22
	perinephric	11.91	6.87	2.66	23.24
	internal	6.20	3.20	3.97	12.65
	Combined mean	**8.24**	**5.07**	**2.66**	**23.24**
Biexponential T_{22} slow (msec)	subcutaneous	135.39	5.89	125.47	142.25
	perinephric	135.61	9.13	118.06	144.30
	internal	132.94	2.58	129.03	136.24
	Combined mean	**134.69**	**6.19**	**118.06**	**144.30**
Biexponential T_{22} slow (%)	subcutaneous	93.38	2.347	90.78	97.02
	perinephric	88.09	6.87	76.76	97.34
	internal	93.81	3.20	87.35	96.03
	Combined mean	**91.76**	**5.07**	**76.76**	**97.34**

Table 2. Fatty acid composition of adipose samples

Fatty acids (%)	Subcutaneous		Perinephric		Internal		Combined	
	mean	SD	mean	SD	mean	SD	mean	SD
C14:0	3.44	0.83	2.57	0.46	3.30	0.64	**3.10**	**0.74**
C15:0	0.32	0.36	0.39	0.09	0.42	0.21	**0.38**	**0.23**
C16:0	22.32	1.84	19.17	1.38	23.08	0.95	**21.52**	**2.21**
C16:1	2.31	0.38	1.75	0.34	1.96	0.38	**2.01**	**0.42**
C17:0	1.77	0.22	1.67	0.13	1.38	0.71	**1.61**	**0.44**
C18:0	17.36	1.43	25.94	1.62	19.64	3.08	**20.98**	**4.25**
C18:1	40.60	2.48	38.52	1.88	38.67	2.63	**39.26**	**2.42**
C18:2n-6	4.30	1.81	5.57	1.36	5.21	1.06	**5.03**	**1.47**
C20:0	0.10	0.08	0.18	0.09	0.17	0.03	**0.15**	**0.08**
C18:3n-6	1.03	2.52	0.00	0.00	0.00	0.00	**0.34**	**1.46**
C20:1	0.12	0.11	0.15	0.06	0.13	0.04	**0.13**	**0.07**
C18:3n-3	0.29	0.15	0.22	0.06	0.25	0.06	**0.25**	**0.1**
C22:0	0.00	0.00	0.06	0.05	0.02	0.04	**0.03**	**0.04**
C22:1n-11	0.00	0.00	0.02	0.04	0.02	0.05	**0.01**	**0.04**
C22:1n-9	0.00	0.00	0.02	0.05	0.02	0.04	**0.01**	**0.03**
SAFA	45.32	3.21	49.97	3.09	47.99	4.02	**47.76**	**3.80**
MUFA	43.03	2.45	40.44	1.62	40.79	2.7297	**41.42**	**2.47**
PUFA	5.62	1.69	5.8	1.40	5.46	1.11	**5.62**	**1.34**