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Quantification of mass fat fraction in fish using water–fat separation MRI

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ABSTRACT

Selection of fish with appropriate fat content and anatomic distribution is searched in fish industry. This necessitates fast and accurate measurements of mass fat fraction maps on a large number of fish. The objective of this work is to assess the relevance of MRI water–fat separation for this purpose. For the separation of the water and fat images we rely on a single T_2^* and a multiple peak fat spectrum model, the parameters of which are estimated using the "Varpro" method. The difference of proton density between fat and water and the lack of the signal from the macromolecules are taken into account to convert the obtained proton density fat fraction into mass fat fraction. We used 0.23T NMR to validate the method on 30 salmon steaks. The fat fraction values were in the range of 5% to 25%. Very good accordance was found between 1.5T MRI and NMR although MRI slightly overestimated the mass fat fraction. The R^2 of the linear regression was equal to 0.96 ($P<10^{-5}$), the slope to 1.12 ($CI_{95} = 0.03$). These results demonstrate that a good accuracy can be achieved. We also show that high throughput can be achieved since the measurements do not depend on the position and we conclude that, for example, it is feasible to quantify the mass fat fraction in fish steaks within about one minute per sample.

1. Introduction

The quantity as well as the distribution of lipid in fish are important factors that influence the quality of the product. Indeed the quantity of lipid in flesh highly influences the attributes of eating quality, such as texture and flavour [1], and the overall consumer acceptability of fish [2]. On its side, subcutaneous adipose tissue is discarded during fillet trimming and thus negatively linked to fillet yields.

In order to optimize the quality, improvement by genetic selection is a promising strategy [3]. Genetic selection benefits from the advances of DNA fingerprinting. Typical programs use sib selection: different families are grown together. A part of the fish is analysed in order to select the best families, the remaining fish being identified with a posteriori DNA-pedigreed and used for reproduction [4]. Hence, by measuring fat content and its distribution on a large number of fish, it is possible to select families with desirable properties such as fat deposition in valuable parts. Considering the several thousands of individuals to phenotype in such a selective breeding program, a rapid and accurate method for the quantification of lipid content and its distribution mapping is needed.

Several methods are available to measure lipid content in fish. Chemical extraction methods such as Folch and Soxhlet provide accurate measurements but are time consuming and necessitate the use of several chemical products like solvent. Faster methods have been developed such as analysis based on microwaves [5] or NMR [6]. However these methods require sample preparation such as drying and thus need several manual operations. Moreover all these analytical methods are invasive and destructive, and can only provide information on the distribution at the cost of multiple spatial samplings which would be more time consuming. Noninvasive and rapid methods such as Fatmeter [7], DXA [8], EchoMRI [9] or ultrasounds [10] are quite accurate for the measurement of total body composition. However they only measure a global fat content and cannot be used to determine the distribution.

The lipid content and its distribution may be evaluated on only one representative steak cut at a specific anatomic location in the fish. In this case computer vision may be used to measure subcutaneous and intramuscular fat thanks to the visual contrast between fat and muscle [11,12]. However it is restricted to fish with pigmented flesh and only intermediate correlations (0.77) were estimated between visual traits measured and the muscle fat content [11].

MRI, in particular the water–fat separation approach, and X-ray computer tomography (CT) may provide the basis of non invasive and rapid quantification of the lipid content and its distribution. The contrast in CT images is good enough to separate lean muscle from subcutaneous fat, however comparative studies have demonstrated

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that water–fat separation MRI has a higher sensitivity than CT for the detection of low intramuscular fat [13,14] which is commonly encountered in fish. The large field-of-view (FOV) of MRI allows the analysis of several samples together and with no extra-time for sample preparation. Thus, even if the initial cost of the system is high, the possibility to analyze a high number of samples per hour would highly reduce the cost and make MRI competitive with other analytical methods. Finally, beyond the interest for fish industry, and since it uses no radiations, MRI could also be used in vivo for researches on fish physiology, for example to study seasonal variations of fat deposition.

To our knowledge, water–fat separation MRI has been used only once in the case of fish, for the quantification and localization of fat in Atlantic mackerel at 1.5T [15]. The authors succeeded in globally visualizing the fat. However, they found no agreement between fat quantified by MRI and gas chromatography analysis and could not clearly explain why.

Our contribution is to reexamine the relevance of water–fat separation MRI in the case of fish and to evaluate the possibility of high throughput measurements for the handling of a high number of samples. We have also investigated the conversion from proton density fat fraction (*PDFF*), which is the metric typically measured by water–fat separation MRI, to a mass fat fraction (*MFF*). The latter can be more easily compared with output measurements from chemical analysis methods.

We tested our approach on salmons. Indeed this specie has been widely studied especially regarding fat characteristics and relevant data such as fat spectrum can be found in the literature. We validated our results by comparison with NMR measurements.

2. Materials and methods

2.1. Water and fat image computation

We used a signal model which includes multiple spectral peaks of the fat and a single-T_2^*. It was found to give better results over a range of clinical relevant signal-to-noise ratio (SNR) [16] and is written as:

$$s(n,\rho_w,\rho_f,T_2^*,f_b)=\left(\rho_w+\rho_f\sum_{p=1}^{P}\alpha_p e^{j2\pi f_{fp}TE(n)}\right)e^{-\frac{TE(n)}{T_2^*}}e^{j2\pi f_b TE(n)}+b(n) \quad (1)$$

where f_b is the local frequency shift due to the static field inhomogeneity, ρ_w et ρ_f are respectively the signals of the water and fat components at $t=0$, α_p and f_{fp} are the relative amplitudes and frequencies of each fat spectral peak p, $TE(n)$ is the echo time n. $b(n)$ is a complex noise component, which is assumed Gaussian.

For the fat spectrum we relied on values found in the literature: we deduced average fatty acid carbon chain length (*CL*), unsaturation degree (*UD*) and polyunsaturation degree (*PUD*) from fatty acids composition measured on a variety of salmons [17]. The frequencies f_{fp} and the relative amplitudes α_p were then deduced as described by Hamilton et al [18].

Estimation of the variables ρ_w, ρ_f, f_b and T_2^* was done using "Varpro" algorithm [19]. Moreover, we applied the post-correction scheme proposed in [20] in order to get rid of the noise effect. We slightly modified it by imposing a fat fraction between 0 and 1.

2.2. Mass fat fraction computation

Regarding fish application, the fat fraction of interest is the mass fat fraction. However, ρ_w and ρ_f are not proportional to a mass but to a quantity of protons.

For the conversion of the MRI signal in mass we used the formula described in [21]: the signal ρ_x, with x = f or w is proportional to $m_x\frac{\mathcal{N}N_x}{M_x}$ where m_x, M_x and N_x are respectively the total mass, the molar mass and the number of hydrogen protons of molecule x, and $\mathcal{N}$ is the Avogadro number. The total mass is required to compute the mass fat fraction. However macromolecules do not give any signal in MRI because of their too short relaxation times compared to the sampling times of MRI signal. In order to overcome this issue, we assumed that the percentage of water in pure muscle in g/g is known and does not differ significantly neither within nor between samples. Thus, noting H this value, the total mass is equal to $m_f+\frac{1}{H}m_w$. Consequently, the MRI mass fat fraction in each voxel is expressed as:

$$MFF=\frac{\frac{M_f}{N_f}|\rho_f|}{\frac{M_f}{N_f}|\rho_f|+\frac{1}{H}\frac{M_w}{N_w}|\rho_w|} \quad (2)$$

For sake of comparison we also computed the proton density fat fraction expressed as:

$$PDFF=\frac{|\rho_f|}{|\rho_f|+\left|\rho_w\right|} \quad (3)$$

M_w and N_w are respectively equal to 18 and 2. M_f and N_f depend on the fatty acids composition. They were computed as the weighted average molar mass and number of protons of each fatty acid. The value of H is discussed in Section 2.4.

2.3. MRI sequence

Images were acquired on a Siemens Avanto (Siemens AG Medical Solutions, Erlangen, Germany) 1.5-T scanner. The parameters common to all the experiments were as follows: we used a 2D monopolar gradient echo sequence, 6 echoes, $TE(1)=2.35$ ms, echo spacing of 2.5 ms, flip angle = 90. We tuned TR to 3000 ms, 5 times the value of the highest T_1 in order to avoid T_1 confounding effects [22] (the T_1 of fat and water had been previously estimated to 380 and 600 ms). The steaks were maintained at 4 °C in order to avoid water loss. It is to be noted that, even at low temperature, fat in fish is not crystallized [23] and thus gives signal. Values of f_{fp} were computed for 4 °C [24]. Their values as well as the values of the corresponding amplitudes α_p are presented in Table 1. Indeed it was shown that not taking this into account could lead up to 5% bias in fat fraction [25]. The other parameters for the different experiments are presented in Table 2.

2.4. MRI/NMR comparison

Three Scottish farmed salmons were cut in steaks, skin and bones were removed so that the subsequent NMR analysis, which requires samples easy to be grinded, could be performed on exactly the same material. 27 steaks were used and 3 samples were made of an assembly of the leanest parts of the fish in order to get a larger variability. This finally led to 30 samples. The steaks were arranged horizontally in trays one above the other so that four of them were put together in the MR system.

Table 1
Relative amplitudes of each spectral peak, α_p, in function of their frequency, f_{fp}, at 4 °C. The frequencies are expressed relative to the one of water.

f_{fp} (Hz)	−10.2	−3.83	59.4	152	185	199	226	245	270
α_p (%)	10.10	0.92	3.68	10.1	5.51	8.76	5.51	47.1	8.27