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The effects of pre-salting methods on water distribution and protein denaturation of dry salted and rehydrated cod – A low-field NMR study

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ABSTRACT

Low field Nuclear Magnetic Resonance (LF-NMR) relaxation time measurements were used to evaluate the effect of different pre-salting methods (brine injection of salt and/or phosphates followed by brining, solely brining, pickling and kench salting) on the protein denaturation and change in muscle properties during the production steps of dry salted cod fillets followed by rehydration. The NMR relaxation curves were affected by the salting method and represented well the structural differences between the salting methods at each processing step. Significant correlations were observed between the NMR relaxation parameters and all physicochemical quality properties measured, except the cooking yield, when samples from all processing stages were analyzed together. The longitudinal relaxation time T_1, and the faster relaxing transverse relaxation time T_{21} were shown to be especially sensitive to protein denaturation in the fillets. The water distribution indicated that the salting and rehydration processes changed the cells irreversibly. The study indicated that pre-brining by brine injection followed by brining, with low salt concentrations, led to the least protein denaturation during the dry salting and rehydration process.

1. Introduction

Salting of fish is a traditional preservation method, which has been used for centuries. Dry salted Atlantic cod (*Gadus morhua*) is one of the largest export products from Iceland and Norway. The major markets are in Southern Europe and Latin America, where rehydrated cod is commonly used in bacalao dishes. During the production of dry salted cod the fish is filleted or butterfly split and then heavily salted. Traditionally the salting has been done by various combinations of pickling, brining and/or kench salting. In kench salting the fish is piled into stacks in alternating layers of fish and salt. The fish takes up salt while liquid diffusing from the muscle is allowed to drain away. In pickle salting a similar procedure is performed, but in closed vats. The liquid diffusing from the muscle during salting therefore forms a saturated brine solution as the salt dissolves (van Klaveren and Legrende, 1965). The use of lower salt concentrations has however been shown to lead to increased water holding capacity and higher yields (Offer and Trinick, 1983; Wilding et al., 1986; Slabyj et al., 1987; Barat et al., 2002). In recent years pre-salting by brining, followed by dry salting, has therefore become the most popular method in the production of heavily salted cod products and experiments with pre-salting by a combination of brine injection and immersion have recently been made. For brine pre-salted fish, the fillets are submerged in brine for 1–4 days before being stacked in alternating layers of salt, where the fish is kept for 10–13 days before packaging and export. The fish is rehydrated prior to consumption, but this step is usually performed by the consumer.

Low field Nuclear Magnetic Resonance (LF-NMR) proton relaxation behavior has been widely used to gain further insight into muscle behavior. The relaxation behavior observed corresponds to water and fat molecules in the muscle. However, in lean species such as cod, the fat content can be ignored. In meat and fish muscle at least two water populations can be identified. The interpretation of the relaxation times is however controversial. According to Bertram and Andersen (2007) and Bertram et al. (2009) three water populations were identified in pork muscle; the first with a fast relaxation time, T_{2B}, at 1–3 ms, suggested to correspond to water closely associated with macromolecules, the second relaxation time T_{21} at 40–80 ms, suggested to correspond to myofibrillar water and the slowest relaxation time T_{22} at 200–400 ms, believed to correspond to extra-myofibrillar water. Other studies have suggested that the shorter relaxation time T_{21} relates to water located within organized protein structures and the longer relaxation time T_{22} relates to water in the space between the myofibrils (Bertram et al., 2001; Erikson et al., 2004). The NMR parameters have been shown to correlate well with various physicochemical properties of meat and fish muscle. Andersen and Rinnan (2002) showed that

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good correlations between moisture content and the NMR relaxation times could be obtained using the SLICING method and according to Jepsen et al. (1999) the water holding capacity (WHC) can be predicted using LF-NMR within the range of 30–90%. Bertram et al. (2000) showed that T_2 relaxation times were an efficient method to determine the WHC in pork as well as indicate pH-induced structural changes occurring in the muscle postmortem.

The aim of the present study was to follow the structural changes in cod muscle occurring during dry salting and rehydration, as affected by various pre-salting methods, by means of LF-NMR relaxation time measurements. The NMR parameters obtained were compared to physicochemical measurements of moisture, salt, protein and non-protein nitrogen content of the muscle, as well as WHC and muscle pH as presented by Thorarinsdottir et al. (2010) for validation. The NMR parameters were then compared to electrophoresis (SDS–PAGE) and differential scanning calorimetry (DSC) measurements, as presented by Thorarinsdottir et al. (2011). The aim was also to show the applicability of LF-NMR in optimization of quality control within the dry salting process.

2. Materials and methods

2.1. Experimental design

Wild Atlantic Cod (*G. morhua*) was caught by long-line in February 2006. The fish was bled and gutted on board and stored in tubs on flake ice until processed 3–4 days post catch. The fish was beheaded and filleted at the processing plant. The fillets were divided into five groups of 45–50 fillets and each fillet was identified with a numbered plastic tag. Brine injections of salt (IS) and a combination of salt and phosphates (ISP) were performed in a FOMACO FMG 64/256F (FOMACO Food Machinery Company, Koga, Denmark) injection machine, using an injection pressure of 1.1 bar. The injected fillets were immersed in brine for 2 days (12% salt) in a 1:1 fish-to-brine ratio. Fillets from an additional group were immersed in brine for 2 days without prior injection (brined, Br). The fourth pre-salting method was pickling (Pi), where fillets were covered by alternating layers of fish and salt in closed tubs for 3 days, causing liquid extracted from the muscle due to the strong salting to form a saturated brine. After pre-salting, fillets from all groups were dry salted for 22–23 days at 3–5 °C by stacking them in alternating layers in open tubs. The liquid extracted from the flesh by the salt was allowed to drain away. Cod kench salted for 26 days was held as a reference group (Ke). Commercial coarse salt from Tunisia and the Bahamas were used for pre-salting and dry salting, respectively. A mixture of sodium and potassium pyrophosphates and sodium and potassium tripolyphosphates (Carnal 2110, CFB Budenheim, Budenheim, Germany) was used in the phosphate injected group.

2.2. Low-field NMR measurements

A low field Bruker mq 20 benchtop NMR analyzer (Bruker Optics GmbH, Rheinstetten, Germany) with 20 MHz and 0.47 T magnetic field was used for measurements of proton longitudinal (T_1) and transversal (T_2) relaxation times. Samples were taken from the middle part of the fillet and successively minced and placed in 10 mm sample tubes. Six replicates were made from each sample group and all measurements were performed at 2 °C. The sample compartment was cooled to the desired temperature by pumping compressed air through liquid nitrogen and finally through the sample compartment. The flow and temperature was controlled by a variable temperature control unit (BTV3000, Bruker Optic GmbH, Rheinstetten, Germany). The Receiver Gain (RG) was set to 70 dB, the Receiver Delay (RD) was 4 s, the Number of Scans (NS) was 4 and no dummy shots were used. Longitudinal relaxation times were measured with an Inversion Recovery (IR) pulse sequence measuring 30 points. Transverse relaxation times were measured with a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Carr and Purcell, 1954; Meiboom and Gill, 1958), with an interpulse spacing τ of 1 ms and the number of collected echoes was 200.

The NMR data was collected with the Bruker Minispec software and successively maximum normalized to allow comparison of samples with different size and water content. This was done by giving the strongest echo signal a value of 100 and scaling the following echo signals accordingly. Transversal relaxation data was fitted to a multi-exponential curve by using the Low-field NMR toolbox for Matlab (The Mathworks Inc. Natric, MA), as described by Pedersen et al. (2002). Residual analysis of the exponential fittings indicated that two exponentials were sufficient to describe the system for all samples. A bi-exponential fitting thus resulted in two water populations A_{21} and A_{22}, with corresponding relaxation times T_{21} and T_{22}. The weighted amount of water in each population compared to the total amount of observed water ($A_{21} + A_{22}$) was calculated. Longitudinal relaxation times (spin–lattice), measured with an Inversion Recovery (IR) pulse sequence, were fitted with a mono-exponential function.

2.3. Physicochemical reference measurements

All fillets were weighed individually before salting as well as after each processing step. The total processing yield was found by comparing the weight after each processing step to the original weight prior to salting. The cooking yield of the rehydrated fillets was estimated by steam cooking middle pieces from three fillets from each group for 12 min at 95–100 °C in a Convostar steam oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). The samples were weighed and cooked on a grid whereby the water extruded from the muscle during cooking could leak away. Samples were allowed to cool down to 25 °C before being weighed again (Mettler Toledo SB 16001 DR, ±0.01 g, Mettler Instruments AB, Greifensee, Switzerland).

The moisture content was measured by drying 5 g of minced muscle mixed with sand in a ceramic bowl for 4 h at 103 ± 2 °C. The moisture content was based on the weight differences before and after the drying of three replicates for each sample (ISO-6496, 1999). The salt content was measured with the Volhard Titrino method (AOAC, 2000) and the total protein content was obtained from the total nitrogen content (TN $*$ 6.25) and analyzed with the Kjeldahl method (ISO-5983, 2005). Trichloroacetic acid (TCA)-soluble nitrogen was measured to estimate amounts of non-protein nitrogen by the Kjeldahl method as described in Thorarinsdottir et al. (2004). Water holding capacity (WHC) was determined with the centrifugal method described by Eide et al. (1982). Approximately 2 g of the samples were weighed precisely into a vial and centrifuged (Sorvall RC-5B, Dupoint Company, USA) at 210g (1300 rpm) and temperatures in the range of 2–5 °C for 5 min. The WHC (%) is calculated as the ratio of water in the sample after centrifugation to water in the sample before centrifugation. Results are presented as an average of three measurements. pH measurements were performed with a pH electrode (SE 104 Mettler Toledo GmbH, Greifensee, die Schweiz) connected to a Knick pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was immersed in the minced samples at 20 ± 2 °C. The results from the physicochemical and yield measurements were described by Thorarinsdottir et al. (2010).

Measurements and results from electrophoresis (SDS–PAGE) and differential scanning calorimetry (DSC) analysis in the fillets