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Quality-related changes in frozen fish muscle

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Abstract
Frozen storage offers a means of preserving fish; however, during frozen storage, quality is lost due to a deterioration of texture, flavour and colour, especially after long periods of storage, when poor freezing practices are employed or when the initial fish quality is low. The main problem is the change of texture which reduces consumer acceptability. The quality of fish products after freezing and frozen storage is affected by factors such as fish species, temperature and handling before slaughter, slaughtering stress, the biological status of the fish, temperature of the pre-rigor storage, freezing rate, frozen storage temperature and time, temperature fluctuations, thawing procedure and prevention against oxidation (light and oxygen).

Keywords: Frozen storage, Water binding, Dehydration, protein

Introduction

Frozen storage

Frozen storage offers a means of preserving fish; however, during frozen storage, quality is lost due to a deterioration of texture, flavour and colour, especially after long periods of storage, when poor freezing practices are employed or when the initial fish quality is low. The main problem is the change of texture which reduces consumer acceptability (Sikorski *et al.* 1976; Shenouda, 1980) [17, 14]. Texture changes are the result of denaturation of muscle proteins, particularly those in the myofibrillar fraction (Haard, 1992) [15]. For these reasons, different methods of measuring protein denaturation have been used to follow textural deterioration. Among them, protein solubility is one of the most often chosen, because of its simplicity and relatively good correlations with textural characteristics (Shenouda, 1980; De Koning & Mol, 1991) [44, 45, 9,].

Water binding, mobility and distribution

Water in muscle is distributed throughout the tissue with approximately 90 % located intracellular and 10 % extra-cellular (Schnepf, 1989) [41]. The water is physically separated by cellular structures such as membranes, but as these are water permeable, the intra- and extra-cellular water can exchange, for example due to changes in osmotic pressure. Intra-cellular water or muscle water is thought to be distributed between different states or populations of water (‘pools’) characterised by differences in water mobility due to different degrees of binding or association to proteins (Ruan and Chen, 1998). A small portion of the water molecules (less than 0.3 g water/100 g protein) are structurally bound to proteins and show a very different behaviour than that of bulk water (Schnepf, 1989; Isengard *et al.* 2008) [41, 19,]. The main fraction of water interacts with proteins to different degrees and the physical retention of this water is dominated by the association with the myofibrillar structure (Schnepf, 1989) [41]. As described above the rotational mobility of water molecules is typically measured by relaxation NMR. Based on the relaxation curves, the number and size of different water poolscan be determined by the use of different two- or three-way chemometric methods. Changes in the water distribution of a certain sample can indicate quality-related changes due to for example storage temperature or time (Jepsen *et al.* 1999; Jensen *et al.* 2002) [22, 21]. In porcine meat, three different water pools have been identified, the fastest relaxing reflecting water tightly associated with macromolecules, the intermediate reflecting water located within highly organized protein structures and the slowest relaxing reflecting the extra-myofibrillar water containing the sarcoplasmic protein fraction (Bertram *et al.* 2001) [5]. In fresh and pre-frozen cod, respectively, two to three and three (intact, minced and centrifuged cod) and four (minced cod) different water pools have been identified, the number depending on storage conditions (Andersen and Rinnan, 2002; Jensen *et al.* 2002; Andersen and Jorgensen, 2004) [21].

Dehydration and the effect of inorganic salts

The removal of water from solution as a result of ice crystal formation leads to dehydration of the cells (see section 4.4) and the intra-cellular protein molecules. The three-dimensional structure of proteins is stabilised by a network of hydrogen bonds and as many of these are water mediated, they will be disrupted when water is removed. This can result in an exposure of hydrophilic and hydrophobic regions which can interact with other exposed regions, either in the same or in adjacent proteins, resulting in aggregation (Sikorski, 1978; Shenouda, 1980) [44, 45]. Increased solute concentration also affects protein denaturation and aggregation. Upon the freezing of fish muscle at -30° C, potassium and sodium chlorides may form a solution of up to 7 % compared to about 0.5 % in the unfrozen muscle. These and other salt ions can interfere with secondary forces (electrostatic, van der Waals, hydrogen and hydrophobic) that stabilise the tertiary and quaternary structure of the proteins. At low ionic strength, many salts have a solubilising effect on proteins, but at higher ionic strength the inorganic salts compete for water with the hydrophilic groups. This may result in a salting-out effect and decreased solubility of the protein (Sikorski *et al.* 1976; Shenouda, 1980) [44, 45].

Unfrozen muscle water

Freezing is an effective way of preservation because the crystallisation of water results in a more concentrated solution and thus a lowering of the water activity in the food. Water associated with macromolecules, membranes and other ultra-structural elements in cells and tissues can, however, remain unfrozen at tens of degrees Celsius below the equilibrium freezing point of a bulk solution. Even in the presence of ice crystals, this water remains unfrozen due to a combination of the hydration effect, the presence of small solutes, very high viscosity and small dimensions between membranes and macromolecules (Wolfe *et al.* 2002) [49]. It is therefore frequently named 'unfreezable' water, but in compliance with the recommendations of Franks (1986) [13]. and Wolfe *et al.* (2002) [49]. it is referred to as 'unfrozen water' throughout this thesis. The existence of an unfrozen water fraction in frozen foods is the main reason why chemical quality deteriorating processes occur in frozen foods. Several experimental values for the amount of unfrozen water in different muscle foods are reportedin the literature: 9.5 % in cod muscle at -40 °C (Riedel, 1956) [36], 11.0 % in haddock muscle at -40 ° C (Charm and Moody, 1966), 15.1 % in reindeer meat at -80 °C (Roos, 1986) [37]. 26.8 % in cod at -20 and -60 °C (Paper III), 31.0 % in fresh grounded beef meat at -40 °C (Aktas *et al.* 1997a) and 36.7 % in king fish at -90 °C (Sablani *et al.* 2007) [39]. The relatively big differences between the reported values are probably due to differences between species and methodological differences.

Quality

The quality of fish products after freezing and frozen storage is affected by factors such as fish species, temperature and handling before slaughter, slaughtering stress, the biological status of the fish, temperature of the pre-rigor storage, freezing rate, frozen storage temperature and time, temperature fluctuations, thawing procedure and prevention against oxidation (light and oxygen) (Sorensen *et al.* 1995; Sigholt *et al.* 1997; Erikson *et al.* 1997; Kristoffersen *et al.* 2006; Nielsen and Jessen, 2007) [11, 46, 24, 32,]. If fish is frozen quickly, stored at low, non-fluctuating temperatures and thawed in the best way according to its rigor-state, the quality can be as good as or better than fresh fish stored for a few days at 0 °C (Cappeln *et al.* 1999) [6]. For optimally handled cod, the quality remains as high as for fresh cod for one month at -30 °C. The fish is still suitable for consumption after one year, though the characteristic frozen storage flavour starts to develop after approximately three months. Fatty species, such as trout, are stable during frozen storage and are still suitable for consumption after 18 months, though only of high quality up to six months. Due to a high content of poly unsaturated fatty acids (PUFA), fatty species are susceptible to lipid oxidation, which results in rancid taste and odour, if not packed in an oxygen-free atmosphere (Nielsen and Jessen, 2007) [32]. In lean fish, protein denaturation causes textural and functional changes in the fish muscle, whereas oxidative lipid degradation results in the characteristic cold-store flavour.

Factors affecting protein changes

Several factors are of importance in relation to the protein changes occurring during frozen storage: ice crystal formation, dehydration, increased concentration of salts in the unfrozen water pools, changes in lipids and fatty acids, lipid oxidation, enzymatic breakdown of trimethylamine oxide (TMAO) and interactions between these factors. Protein denaturation in frozen muscle has been reviewed by several authors (Love, 1970; Sikorski *et al.* 1976; Shenouda, 1980; Mackie, 1993) [44, 45].

Denaturation of muscle proteins

Denaturation of muscle proteins has been attributed to different factors, fish species being one of them. Basically, fish species can be divided into two groups based on the possession or not of trimethylamine oxide demethylase (TMAOase), which degrades trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA) during iced and/or frozen storage. The enzyme is mostly found in the viscera and red muscle of fishes belonging to gadoid species (Mackie & Thomson, 1974; Hebard *et al.* 1982; Haard, 1992) [28, 15,]. During frozen storage, a strong relationship between the production and accumulation of FA and the deterioration of texture in muscle of this group of fish has been found (Dingle *et al.* 1977; Gill *et al.* 1979; Matthews *et al.* 1980; Parkin and Hultin, 1982; Kelleher *et al.* 1981; Jahncke *et al.* 1992) [34, 34, 23,]. Although FA might react with proteins during frozen storage, it is still not clear how FA accelerates protein denaturation (Ang & Hultin, 1989). Hake, fish belonging to the Order Gadiformes, are gaining importance as fisheries, as cod are increasingly being depleted (Morrow, 1992) [30]. These species represent the main high-quality white-fleshed fish in some European countries, such as Spain (Whitaker, 1980), where they are very often sold in the frozen state. It is also an important source of derived frozen products. There are some data on storage properties of frozen South Atlantic hake, Merluccius hubbsi and M. gayi (Almandos *et al.* 1984; Ciarlo *et al.* 1985) [2, 8,] M. bilinearis (Hiltz *et al.* 1976; Licciardello *et al.* 1980) [18, 25,], M. productus (Crawford *et al.* 1979) and M. capensis (De Koning & Mol, 1991) [9]. Also, it has been reported that there are changes in some sensory and chemical parameters in European hake (Merluccius merluccius) during ice and frozen storage (Pérez-Villarreal & Howgate, 1987, 1991). However, there are no data on changes in muscle protein solubility of frozen stored European hake.

Changes in functional properties caused by protein changes

- Freezing and frozen storage may, as described above, result in denaturation and aggregation of especially myofibrillar proteins resulting in products with reduced WHC and increased drip loss upon thawing causing a hard, dry and fibrous fish product with altered colour and reduced juiciness (Sikorski *et al.* 1976; Shenouda, 1980; Barroso *et al.* 1998) [44, 45]. The main changes are reported to occur in myosin light-chain, but actin and actinin also degrade during frozen storage (Careche *et al.* 1998; Saeed *et al.* 1999; Saeed and Howell, 1999; Kiran Jasra *et al.* 2001; Badii and Howell, 2002b; Schubring, 2005; Kjaersgard *et al.* 2006b). Some of the changes reported are increases in *ß*-sheet at the expense of -helix structure (Herrero *et al.* 2004). As the main part of muscle water is located within the myofibrillar structure, changes in this typically result in reduced WHC. Numerous studies have shown a relationship between decrease in protein extractability and increased toughness of fish. Protein

solubility and extractability are often used to characterize the degree of protein denaturation during frozen storage. Increased protein aggregation results first in an increased protein insolubility in salt solutions and thereafter in un-extractability in sodium dodecyl sulphate (SDS) and SDS plus s-mercaptoethanol. Storage temperature and time have great impact on the degree of protein denaturation and many authors have shown a relation between storage temperature, time as well as a combined effect and degree of proteindenaturation or muscle toughness. Protein changes or changes in texture are reported to be higher after storage at -10 to -20° C compared to -30° C, temperatures below -30° Care less studied (Licciardello *et al.* 1982; Chapman *et al.* 1993; Herrero *et al.* 2004) [26, 7,].

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