Rapid Multiparameters Approach to Differentiate Fresh Skinless Sea Bass (Dicentrarchus labrax) Fillets from Frozen-Thawed Ones

Sylvain Marlard¹,²,³,⁴,⁵,⁶,*, Périne Doyen¹,*, and Thierry Grard¹

¹Univ. Littoral Côte d’Opale, Convention ANSES, EA 7394, ICV – Institut Charles Viollette, Boulogne-sur-Mer, France; ²INRA, France; ³University of Lille, Lille, France; ⁴ISA, Lille, France; ⁵University of Artois, Arras, France; ⁶Univ. du Maine Laboratoire mer, molécules, santé, EA 2160, IUT de Laval, Laval, France

ABSTRACT

Food authenticity is one of the major issues in the mind of today’s consumers. The sale of frozen-thawed fish fillets under the “fresh fillet” label is considered as a commercial fraud. However, their close sensory properties complicate the differentiation. This study focused on analyzing the composition of exudate (pressed flesh juice) in order to rapidly differentiate between fresh and frozen-thawed skinless fillets of sea bass (Dicentrarchus labrax). Protein concentration, α-D-glucosidase specific activity, nucleotides and related compounds (NRCs) concentration, and free calcium concentration were measured in exudates corresponding to fresh or frozen-thawed sea bass fillets. Significant increases of these four parameters were observed in exudates from frozen-thawed fillets, especially with a twofold increase in NRCs and free calcium concentrations. These results suggest that NRCs and free calcium concentrations can be promising indicators to rapidly detect mislabeling of fresh fillets.

KEYWORDS

Sea bass; frozen-thawed fillet rapid detection; α-D-glucosidase; nucleotides and related compounds; free calcium

Introduction

The consumption of fish has increased recently; in France for example, there was an increase of 24.9 kg per habitant in 2015, an increase of 0.9 kg in 5 years (FranceAgrimer, 2016). Consumers prefer to eat freshly caught fish, but frozen storage is widely used to protect these products against microorganism growth during the long transportation to consumers (Pavlov, 2007). Some businessmen are tempted to cheat and label thawed fillets as “fresh fillets” in the market. Indeed, such exercise could be very lucrative when thawed fish are sold out of the fishing season. Nevertheless, the final consumer should be appropriately informed when fishery products have been defrosted, according to article 28 of Regulation EU 1169 (2011).

Several methods, such as the observation of eye lens opacity (Love, 1956; Yoshioka and Kitamikado, 1983) and sensory analysis (Parisi et al., 2002) can be used to differentiate frozen-thawed whole fish from fresh. A large amount of fish is sold in the form of fillet, and several instrumental methods have been tested to differentiate a fresh fillet from a frozen-thawed one. For example, the use of spectroscopy in visible and near-infrared has shown conclusive results on grass carp (Ctenopharyngodon idella) (Cheng et al., 2015). Utilizing the front face fluorescence spectroscopy to specifically measure the Nicotinamide-Adenine Dinucleotide (NADH) fluorescence spectra might also be considered as a promising tool for testing whiting (Merlangius merlangus) (Karoui et al., 2006). The use of impedance spectroscopy to identify European sea bass (Dicentrarchus labrax), Atlantic salmon, and sea bream (Sparus aurata) frozen samples (Fernández-Segovia et al., 2012; Fuentes et al., 2013; Vidaček et al., 2008) has also been investigated. Others techniques such as the analysis of salmon DNA degradation by a Comet Assay have been used to distinguish frozen-
thawed fillets (Le Grandois et al., 2013). Certain volatile compounds were also identified as potential markers to differentiate between fresh and frozen-thawed counterpart sea bream, cod (Gadus morhua), and European sea bass (Leduc et al., 2012). Elevated enzymatic activities of α-D-glucosidase (AG) (Duflos et al., 2002) and lactate dehydrogenase (Diop et al., 2016) in species like whiting (Merlangus merlangus), plaice (Pleuronectes platessa), mackerel (Scomber scombrus), and sea bream (Sparus aurata) also have been reported to highlight the thawing of fish. Among these methods, however, few tests have been carried out with the fish fillet exudate. The freezing of fish results in the leakage of enzymes into the exudate, and an increase in AG activity could then be measured (Duflos et al., 2002). For example, the protein composition of sea bass fillet exudate has been tested, and changes led to a differentiation of frozen-thawed fillets (Ethuin et al., 2015).

The aim of this work was to study the composition of the exudate in order to develop reliable, complementary, and fast tools to identify frozen-thawed sea bass fillets. Protein concentration and AG specific activity, already tested in other fish species exudates (Table 1), were measured in sea bass exudates. Furthermore, two additional parameters, nucleotides and related compounds (NRCs) concentration and free calcium concentration, were measured in this study. The use of these concentrations in order to differentiate fresh and frozen-thawed fillets was, to our knowledge, investigated for the first time (Table 1). The use of these two parameters would allow fish industries to easily implement quick evaluation methods. This set of four indicators was also investigated in order to support industry for the rapid identification of potential fraudulent labelling of frozen-thawed fish fillet.

Materials and methods

Fish material

The sea bass (Dicentrarchus labrax) sample was acquired from Aquanord sea farm (Gravelines, France). This local supplier of farmed sea bass allowed for a model with controlled environmental conditions and freshness. Strictly controlled growing and breeding conditions were: temperature 18 ± 5°C, pH 8.2, total ammonia <30 mol/L, and dissolved oxygen level over 99% (v/v) saturation (7°ppm). Sea bass (average body weight 500 ± 150 g) were slaughtered via asphyxia/hypothermia and were kept on ice (0–2°C). Fish samples were rapidly skinned (less than 90 min after slaughtering) and filleted by the Centre de Formation des Produits de la Mer et de la Terre (CFPMT: Training Center for sea and land products) (Boulogne-sur-Mer, France). Cling-film protected fillets were stored in polystyrene boxes with crushed ice and kept at 0–2°C. Ten fish produced 20 fillets that were divided in two groups: 10 fillets corresponding to the fresh condition were subjected to immediate analysis; 10 fillets corresponding to the frozen-thawed condition were frozen at −30°C, stored at −20°C for 40 days, then removed from the freezer, and stored between 0°C and +4°C for 24 h to thaw completely. Each analysis was performed on a fresh and a frozen-thawed fillet provided from the same fish.

Fish exudates preparation and protein extraction

Fish exudates were obtained from flesh juice (Morel, 1979) after centrifugation (12,500°rpm at 5°C) (Ayala et al., 2005; Duflos et al., 2002; Tironi et al., 2010), according to the method of Ethuin et al. (2015). Each exudate was prepared from a single fillet, and totally 10 exudates from fresh fillets and 10 exudates from frozen-thawed fillets were obtained. Protein concentration in exudate was determined with the Bradford method (Bradford, 1976) using the Bio-Rad reagent (Bio-Rad, Marnes-la-Coquette, France) and bovine serum albumin as standards. All analyses were performed in triplicate. Total analyzing time for fish exudates preparation and protein extraction was less than 1 h.
| Methods | Fish species | Material | References |
|---------|--------------|----------|------------|
| **Morphological and sensorial** | North sea cod (Gadus callarias) | Eyes | Love (1956) |
| Observation of eye lens opacity | Carp (Cyprinus carpio), Japanese horse mackerel (Trachurus japonicus), Pacific mackerel (Pneumatophaus japonicus), red sea bream (Pogonias major), yellowtail (Seriola quinquergadati) | Eyes | Yoshioka and Kitamikado (1983) |
| Morphometric analyses by light microscopy | Sea bass (Dicentrarchus labrax) | Axial muscle | Ayala et al. (2005) |
| Sensory analysis | Sea bass | Whole fish | Parisi et al. (2002) |
| **Biochemical** | Gilthead sea bream (Sparus aurata) | White muscle | Iglesias et al. (2009) |
| Volatile compounds | Sea bass, gilthead sea bream, cod (Gadus morhua) and salmon (Salmo salar) | Fillet | Leduc et al. (2012) |
| Comet assay | Salmon | Cell suspension | Le Grandois et al. (2013) |
| Protein composition | Sea bass | Exudate | Ethuin et al. (2015) |
| Protein concentration | Sea bass | Exudate | This study |
| α-glucosidase, β-N-acetyl-glucosaminidase | Cod, saithe (Gadus vires), red fish (Sebastes marinus), haddock (Gadus aeglefinus) | Press juice | Rehbein et al. (1978) |
| α-glucosidase, β-glucuronidase, β-galactosidase, β-N-acetyl-glucosaminidase | Cod | Press juice | Rehbein (1979) |
| β-hydroxyacyl-CoA-dehydrogenase | Carp (Cyprinus carpio), red sea bream | Blood | Kitamikado et al. (1990) |
| β-hydroxyacyl-CoA-dehydrogenase | Crawfish (Procambarus clarkii), trout (Salmo gairdneri) | Fillet | Hoz et al. (1992) |
| β-hydroxyacyl-CoA-dehydrogenase | Giltheaded sea bream, salmon, blackspot sea bream (Pagefius centrodonus), sole (Solea solea) | Meat | Fernández et al. (1999) |
| α-glucosidase | Plaice (Pleuronectes platessa), whiting (Merlangus merlangus), mackerel (Scomber scombrus) | Exudate | Duflos et al. (2002) |
| lactate dehydrogenase | Gilthead sea bream | Exudate | This study |
| NRCs concentration | Sea bass | Exudate | This study |
| free calcium concentration | Sea bass | Exudate | This study |
| **Physical** | Plaice, whiting, mackerel (Scomber scombrus) | Whole fish | Duflos et al. (2002) |
| Torrymeter | Japanese horse mackerel | Meat juice or natural drip juice | Uddin and Okazaki (2004) |
| NIR | Gilthead sea bream, red mullet (Mullus barbatus), sole (Solea vulgaris), swordfish (Xiphias gladius) | Epaxial white muscle | Ottavian et al. (2013) |
| VIS/NIR | Red Sea bream | Whole fish | Uddin et al. (2005) |
| | Cod | Whole fish | Sivertsen et al. (2011) |
| | Halibut (Psetta maxima) | Fillet | Zhu et al. (2012) |
| | Grass carp (Ctenopharyngodon idella) | Fillet | Cheng et al. (2015) |
| | Rainbow trout (Oncorhynchus mykiss) | Meat sample (head or body) | Hall et al. (1998) |
| ¹H NMR | Cod | Fillet | Martinez et al. (2005) |
| Front-face fluorescence spectroscopy | Whiting | Fillet | Karoui et al. (2006) |
| Mid-infrared spectroscopy | Whiting | Fillet | Karoui et al. (2007) |
| Impedance spectroscopy | Sea bass | Fillet | Vidaček et al. (2008) |
| | Grass carp, tilapia (Oreochromis niloticus) | Whole fish | Zhang et al. (2012) |
| | Salmon | Fillet | Fernández-Segovia et al. (2012) |
| Raman spectroscopy | Gilthead sea bream | Fillet | Fuentes et al. (2013) |
| | Horse mackerel (Trachurus trachurus), European anchovy (Engraulis encrasicolus), red mullet (Mullus surmuletus), Bluefish (Pomatomus saltatrix), salmon, flying gurnard (Trigla lucema) | Fillet | Veligolu et al. (2015) |

NRCs: Nucleotides and Related Compounds; NIR: Near-InfraRed spectroscopy; VIS/NIR: visible/Near-InfraRed spectroscopy; ¹H NMR: Proton Nuclear Magnetic Resonance spectroscopy
**Determination of AG specific activity**

AG (EC 3.2.1.20) specific activity was assayed in accordance with the method of Duflos et al. (2002). The reaction mixture was incubated at 37°C for 2 h and followed by the addition of 1 mL potassium hydroxide (0.2 M) to terminate the reaction. Control reactions were performed in the same way, but the exudate was added after the stopping reagent. The absorbance value was measured at 405 nm, and the specific enzyme activity was quantified in mg/protein/h. The analysis time was less than 3 h.

**Detection of free calcium concentration**

Free calcium concentration in the exudate was estimated with the Calcium Detection Kit (Abcam Company, Cambridge, UK) according to the manufacturer’s protocol and previous report (Ansari et al., 2017). The analysis time was less than 30 min.

**Determination of NRCs**

The concentration of NRCs in exudates was assessed with UV spectrophotometry at 260 nm according to the method of Barcelo et al. (1986). Calibration curve was generated with denatured herring sperm DNA (ThermoFisher, Waltham, MA, USA). The analysis time was less than 30 min.

**Statistical analysis**

All experiments were performed in triplicate, and all values are given as means ± standard deviation. Statistical analyses were performed using “XLSTAT-Pro” 2014 (Addinsoft, Paris, France). Differences of means between fresh and frozen-thawed fillets, concerning the four studied parameters, were compared using the independent t-test (significance was defined at $p < 0.05$).

**Results and discussion**

In this study, four parameters were measured in exudates of fresh or frozen-thawed sea bass fillets in order to assess their effectiveness to differentiate these two storage conditions.

**Protein concentration**

Protein assays represented a necessary step for studying AG specific activity. Protein concentration (Figure 1) was about 1.7-fold higher in frozen-thawed fillets than in fresh fillets. Protein denaturation during frozen storage has been highlighted for many years (Dyer, 1951). The formation of large ice crystals during freezing led to cell membrane deterioration (Mazur, 2010). Release of intracellular proteins into the exudate could therefore have occurred. The increase of protein concentration observed in the frozen-thawed fillet exudate could be linked to such release (Ethuin et al., 2015). However, this conventional parameter in biochemistry cannot be used as the sole marker to certify the correctness of fish fillet labeling. Indeed, the protein content may vary according to fish species (Güner et al., 1998; Soriguer et al., 1997) and to the seasons (Albrecht-Ruiz and Salas-Maldonado, 2015; Jan et al., 2012), which could influence the labeling certification.

**AG specific activity**

The result showed an increase of AG specific activity (Figure 2) in frozen-thawed fillets. This increase of AG activity was already observed in thawed fillets of several fish species, including whiting and plaice (Duflos et al., 2002), sardine (*Sardina pilchardus*), horse mackerel, and anchovy (*Engraulis encrasicolus*) (Alberio et al., 2014). These observations indicated the disruption of
membranes during the freeze-thawing. During thawing, the diluted external medium increases hydrostatic pressure in cells and induces the rupture of plasma membrane (Mazur, 2010; Takamatsu and Zawlodzka, 2006). This process leads to an exudate particularly enriched in intracellular enzymes. However, the observed statistically significant ratio of 1.47 between fresh and frozen-thawed fillets of sea bass was marginally highlighted due to important standard deviations.

**Figure 1.** Protein concentration in sea bass fresh fillets and frozen-thawed fillets exudates, quantified in mg/mL. Bars indicate the standard deviation. * reported significant difference ($p < 0.05$).

**Figure 2.** α-D-glucosidase specific activity in sea bass fresh fillets and frozen-thawed fillets exudates, quantified in mg/protein/h. Bars indicate the standard deviation. * reported significant difference ($p < 0.05$).
Indeed, this parameter exhibited greater variations in fresh fillets (Supplementary data), which could potentially minimize the differences between fresh and frozen-thawed fillets. These greater standard deviations for AG activity were already observed in other species such as mackerel (Duflos et al., 2002) and cod (Benjakul et al., 2003). To our knowledge, the increase of AG specific activity in sea bass frozen-thawed fillets was reported for the first time. Nevertheless, the great standard deviation represented a limitation for using AG activity as a reliable indicator. Therefore, the use of other complementary markers will be essential to define a method that allows identification of mislabeled sea bass fillets.

**Nucleotides and related compounds**

NRCs are a group of compounds, including adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine 5’-monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx). After the death of fish, ATP is quickly transformed into IMP, via ADP and AMP degradation (Kasemsarn et al., 1963; Lakshmanam and Gopakumar, 1999). Postmortem nucleotide metabolism has been previously investigated in several fish species using chromatographic methods (Hong et al., 2015). In this study, the results showed NRC concentration (Figure 3) twice as high in frozen-thawed fillets compared to fresh fillets. Özogul et al. (2005, 2010) studied contents of different nucleotide degradation compounds and observed an IMP concentration decrease parallel to an HxR and Hx increase. These measurements were realized in fresh sea bass stored on ice in a cold room maintained at 4 ± 1°C, but not in frozen sea bass. Moreover, the link between NRCs concentration and the freeze-thawing of fish fillets has not been greatly studied. For example, samples of milkfish (*Chanos chanos*) subjected to frozen storage at −20°C for 18 weeks showed a gradual decrease of ATP, ADP, AMP, and IMP levels, while HxR and Hx increased (Jiang et al., 1987). The ATP level also decreased in frozen-thawed gilthead sea bream (Mendes et al., 2001). NRCs concentration obtained in frozen-thawed sea bass fillets in this study could be correlated with results from studies mentioned above. According to current commercial practice, fresh fish can be stored 3–4 days on ice.

**Figure 3.** Nucleotides and related compounds (NRCs) concentration in sea bass fresh fillets and frozen-thawed fillets exudates, quantified in mg/mL.

Bars indicate the standard deviation. * reported significant difference ($p < 0.05$)
before sale. In the case of sea bass, such qualification of fish as “fresh” would remain to be investigated with the NRCs measurement described in this study. A twofold higher NRCs concentration in a frozen-thawed fillet could still be observed after storage on ice, even though the complete ATP degradation cycle in ice-stored sea bass proceeds at a slower pace than in most species (Kyrana and Lougovois, 2002). However, the objective of this NRCs measurement was to rapidly detect a freeze-thawing process but not to define the freshness loss of sea bass fillet during storage time. This increase of NRCs concentration in frozen-thawed sea bass fillets was probably due to the liberation of sarcoplasmic nucleotides linked to the DNA damage, which has been reported in thawed salmon (Le Grandois et al., 2013).

**Free calcium concentration**

Calcium (Ca$^{2+}$) is one of the main regulatory and signaling ions in all muscles. Therefore, it is interesting to investigate the free calcium concentration in sea bass fillets. An increase of free calcium concentration (Figure 4) was observed in frozen-thawed fillets with a ratio of 2 as compared with fresh fillets. To our knowledge, this parameter was never tested in exudates or in fish fillets. Calcium is stored in the endoplasmic reticulum, which is mainly responsible for the Ca$^{2+}$ signaling in muscle cells (Berridge, 2002). The membrane integrity of endoplasmic reticulum might have been disrupted during defrosting, and this could lead to a rise of free calcium. Moreover, myofibrillar proteins like tropomyosin are linked to Ca$^{2+}$, and the frozen storage induced the denaturation of tropomyosin (Benjakul et al., 2003). A release of free calcium could result from a conformational change of the tropomyosin during this denaturation. This rapid assay of free calcium detection could be a promising indicator for industries to differentiate visually identical fresh fillets from frozen-thawed ones.

![Figure 4](image-url) Free calcium concentration in sea bass fresh fillets and frozen-thawed fillets exudates, quantified in µg/mL. Bars indicate the standard deviation. * reported significant difference (p < 0.05)
Conclusion

The present study focused on measuring four parameters in the exudate in order to differentiate between fresh and frozen-thawed skinless fillets of sea bass. NRCs concentration and free calcium concentration were tested in sea bass exudates for the first time. Observation of higher value (twofold) in frozen-thawed fillets for both parameters highlights the potential of applying these two rapid and not expensive techniques as tools to differentiate fresh from frozen-thawed sea bass fillets. Moreover, NRCs and free calcium concentrations should be further investigated in other fish species in order to develop a rapid test and to improve the reliability of labeling for the consumer.

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Disclosure statement

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References

Alberio, G. R., Barbagallo, R. N., Todaro, A., Bono, G., and Spagna, G. 2014. Effect of freezing/thawing process in different sizes of blue fish in the Mediterranean through lysosomal enzymatic tests. Food Chem. 148: 47–53. doi:10.1016/j.foodchem.2013.10.013

Albrecht-Ruiz, M., and Salas-Maldonado, A. 2015. Chemical composition of light and dark muscle of Peruvian anchovy (Engraulis ringens) and its seasonal variation. J. Aquat. Food Prod. Technol. 24(2): 191–196. doi:10.1080/10498850.2012.762705

Ansari, M. A., Raish, M., Ahmad, A., et al. 2017. Sinapic acid ameliorate cadmium-induced nephrotoxicity: in vivo possible involvement of oxidative stress, apoptosis, and inflammation via NF-kappaB downregulation. Environ. Toxicol. Pharmacol. 51: 100–107. doi:10.1016/j.etap.2017.02.014

Ayala, M. D., López Albors, O., Blanco, A., et al. 2005. Structural and ultrastructural changes on muscle tissue of sea bass, Dicentrarchus labrax L., after cooking and freezing. Aquaculture 250(1–2): 215–231. doi:10.1016/j.aquaculture.2005.04.057

Barcelo, F., Barcelo, I., Gavilanes, F., Ferragut, J. A., Yanovich, S., and Gonzales-Roth, J. 1986. Interaction of anthracyclines with nucleotides and related compounds studied by spectroscopy. Biochim. Biophys. Acta 884: 172–181.

Benjakul, S., Visessanguan, W., Thongkaew, C., and Tanaka, M. 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. Food Res. Int. 36(8): 787–795. doi:10.1016/S0963-9969(03)00073-5

Berridge, M. J. 2002. The endoplasmic reticulum: a multifunctional signaling organelle. Cell Calcium 32(5–6): 235–249.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1–2): 248–254.

Cheng, J.-H., Sun, D.-W., Pu, H.-B., et al. 2015. Integration of classifiers analysis and hyperspectral imaging for rapid discrimination of fresh from cold-stored and frozen-thawed fish fillets. J. Food Eng. 161: 33–39. doi:10.1016/j.jfoodeng.2015.03.011

Diop, M., Wattier, D., Masson, P. Y., et al. 2016. Assessment of freshness and freeze-thawing of sea bream fillets (Sparus aurata) by a cytosolic enzyme: lactate dehydrogenase. Food Chem. 210: 428–434. doi:10.1016/j.foodchem.2016.04.136

Duflos, G., Le Fur, B., Mulak, V., Becel, P., and Malle, P. 2002. Comparison of methods of differentiating between fresh and frozen-thawed fish or fillets. J. Sci. Food Agric. 82(12): 1341–1345. doi:10.1002/(ISSN)1097-0010

Dyer, W. 1951. Protein denaturation in frozen and stored fish. J. Food Sci. 16(1–6): 522–527. doi:10.1111/j.1365-2621.1951.tb17416.x
Ethuín, P., Marlard, S., Deloisière, M., et al. 2015. Differentiation between fresh and frozen-thawed sea bass (Dicentrarchus labrax) fillets using two-dimensional gel electrophoresis. Food Chem. 176: 294–301. doi:10.1016/j.foodchem.2014.12.065

Fernández, M., Mano, S., de Fernando, G. D. G., Ordlómez, J. A., and Hoz, L. 1999. Use of β-hydroxyacyl-CoA-dehydrogenase (HADH) activity to differentiate frozen from unfrozen fish and shellfish. Eur. Food Res. Technol. 209(3–4): 205–208. doi:10.1007/s0021700505481

Fernández-Segovia, I., Fuentes, A., Aliño, M., Masot, R., Alcañiz, M., and Barat, J. M. 2012. Detection of frozen-thawed salmon (Salmo salar) by a rapid low-cost method. J. Food Eng. 113(2): 210–216. doi:10.1016/j.jfoodeng.2012.06.003

FranceAgrimer. 2016. Consommation des produits de la pêche et de l’aquaculture 2015.

Fuentes, A., Masot, R., Fernández-Segovia, I., Ruiz-Rico, M., Alcañiz, M., and Barat, J. M. 2013. Differentiation between fresh and frozen-thawed sea bream (Sparus aurata) using impedance spectroscopy techniques. Innov. Food Sci. Emerg. Technol. 19: 210–217. doi:10.1016/j.ifset.2013.05.001

Güner, S., Dincer, B., Alemdag, N., Colak, A., and Tüfekci, M. 1998. Proximate composition and selected mineral content of commercially important fish species from the Black Sea. J. Food Agric. 7(3): 337–342. doi:10.1002/(ISSN)1097-0010

Hall, L. D., Evans, S. D., and Nott, K. P. 1998. Measurement of textural changes of food by MRI relaxometry. Magn. Reson. Imaging 16(5–6): 485–492.

Hong, H., Regenstein, J. M., and Luo, Y. 2015. The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: a review. Crit. Rev. Food Sci. Nutr. 57(9): 1787–1798.

Hoz, L., Yustes, C., Camara, J. M., Ramos, M. A., and García De Fernando, G. 1992. β-hydroxyacyl-CoA-dehydrogenase (HADH) differentiates unfrozen from frozen-thawed crawfish (Procambarus clarkii) and trout (Salmo gairdneri) meat. Int. J. Food Sci. Technol. 27(2): 133–136. doi:10.1111/j.1365-2621.1992.tb01188.x

Iglesias, J., Medina, I., Bianchi, F., Careri, M., Mangia, A., and Musci, M. 2009. Study of the volatile compounds useful for the characterisation of frozen and frozen-thawed cultured gilthead sea bream fish by solid-phase microextraction gas chromatography–mass spectrometry. Food Chem. 115(4): 1473–1478. doi:10.1016/j.foodchem.2009.01.076

Jan, U., Shah, M., Manzoor, T., and Ganie, S. A. 2012. Variations of protein content in the muscle of fish Schizothorax niger. Am.-Eurasian J. Sci. Res. 7(1): 1–4.

Jiang, S. T., Hwang, B. S., and Tsao, C. Y. 1987. Protein denaturation and changes in nucleotides of fish muscle during frozen storage. J. Agric. Food Chem. 35(1): 22–27. doi:10.1021/jf00073a006

Karoui, R., Lefur, B., Grondon, C., et al. 2007. Mid-infrared spectroscopy as a new tool for the evaluation of fish freshness. Int. J. Food Sci. Technol. 42(1): 57–64. doi:10.1111/j.1365-2621.2006.01208.x

Karoui, R., Thomas, E., and Dufour, E. 2006. Utilisation of a rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen-thawed fish fillets. Food Res. Int. 39(3): 349–355. doi:10.1016/j.foodres.2005.08.007

Kassemsarn, B. O., Perez, B., Murray, J., and Jones, N. 1963. Nucleotide degradation in the muscle of iced haddock (Gadus aeglefinus), lemon sole (Pleuronectes microphalus), and plaice (Pleuronectes platessa). J. Food Sci. 28(1): 28–37. doi:10.1111/j.1365-2621.1963.tb00155.x

Kitamikado, N., Yuan, C. S., and Ueno, R. 1990. An enzymatic method designed to differentiate between fresh and frozen-thawed fish. J. Food Sci. 55(1): 74–76. doi:10.1111/j.1365-2621.1990.tb06019.x

Kyrana, V. R., and Lougovois, V. P. 2002. Sensory, chemical and microbiological assessment of farm-raised European sea bass (Dicentrarchus labrax) stored in melting ice. Int. J. Food Sci. Technol. 37(3): 319–328. doi:10.1046/j.1365-2621.2002.00572.x

Lakshmanam, P., and Gopakumar, K. 1999. K-value, an index for estimating fish freshness and quality. Curr. Sci. 76(3): 400–404.

Le Grandois, F., Krzewinski, F., Le Fur, B., et al. 2012. Differentiation of fresh and frozen/thawed fish, European sea bass (Dicentrarchus labrax), gilthead seabream (Sparus aurata), cod (Gadus morhua) and salmon (Salmo salar), using volatile compounds by SPME/GC/MS. J. Sci. Food Agric. 92(12): 2560–2568. doi:10.1002/jsfa.5673

Love, R. 1956. Post-mortem changes in the lenses of fish eyes. II.—effects of freezing, and their usefulness in determining the past history of the fish. J. Sci. Food Agric. 7(3): 220–226. doi:10.1002/jsfa.1097-0010

Martinez, I., Bathen, T., Strandal, I. B., et al. 2005. Bioactive compounds in cod (Gadus morhua) products and suitability of 1H NMR metabolite profiling for classification of the products using multivariate data analyses. J. Agric. Food Chem. 53(17): 6889–6895. doi:10.1021/jf0507902

Mazur, P. 2010. A biologists’ view of the relevance of thermodynamics and physical chemistry to cryobiology. Cryobiology. 60(1): 4–10. doi:10.1016/j.cryobiol.2009.12.001

Mendes, R., Quinta, R., and Nunes, M. L. 2001. Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast. Eur. Food Res. Technol. 212(2): 141–146. doi:10.1007/s002170000222

Morel, M. 1979. Une méthode pour déterminer si le poisson est à l’état frais ou décongelé. Sci. Peche. 288: 13–17.
Ottavian, M., Fasolato, L., Facco, P., and Barolo, M. 2013. Foodstuff authentication from spectral data: toward a species-independent discrimination between fresh and frozen–thawed fish samples. J. Food Eng. 119(4): 765–775. doi:10.1016/j.jfoodeng.2013.07.005

Özogul, F., Gokbulut, C., Özyurt, G., Özogul, Y., and Dural, M. 2005. Quality assessment of gutted wild sea bass (Dicentrarchus Labrax) stored in ice, cling film and aluminium foil. Eur. Food Res. Technol. 220(3–4): 292–298. doi:10.1007/s00217-004-1029-8

Özogul, F., Özden, Ö., Özoğul, Y., and Erkan, N. 2010. The effects of gamma-irradiation on the nucleotide degradation compounds in sea bass (Dicentrarchus labrax) stored in ice. Food Chem. 122(3): 789–794. doi:10.1016/j.foodchem.2010.03.054

Parisi, G., Franci, O., and Poli, B. M. 2002. Application of multivariate analysis to sensorial and instrumental parameters of freshness in refrigerated sea bass (Dicentrarchus labrax) during shelf life. Aquaculture 214(1–4): 153–167. doi:10.1016/S0044-8486(02)00058-3

Pavlov, A. 2007. Changes in the meat from aquaculture species during storage at low temperature and attempts for differentiation between thawed-frozen and fresh chilled meat. A review. BJVM 10(2): 67–75.

Regulation EU 1169. 2011. Regulation (EU) no 1169/2011 of the European parliament and of the council of 25 October 2011 on the provision of food information to consumers. Off. J. Eur. Union 50: 18–63.

Rehbein, H. 1979. Development of an enzymatic method to differentiate fresh and sea-frozen and thawed fish fillets. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 169(4): 263–265. doi:10.1007/BF01193791

Rehbein, H., Kress, G., and Schreiber, W. 1978. An enzymic method for differentiating thawed and fresh fish fillets. J. Sci. Food Agric. 29(12): 1076–1082. doi:10.1002/(ISSN)1097-0010

Sivertsen, A. H., Kimiya, T., and Heia, K. 2011. Automatic freshness assessment of cod (Gadus morhua) fillets by Vis/Nir spectroscopy. J. Food Eng. 103(3): 317–323. doi:10.1016/j.jfoodeng.2010.10.030

Soriguer, F., Serna, S., Valverde, E., et al. 1997. Lipid, protein, and calorie content of different Atlantic and Mediterranean fish, shellfish, and molluscs commonly eaten in the south of Spain. Eur. J. Epidemiol. 13(4): 451–463.

Takamatsu, H., and Zawlodzka, S. 2006. Contribution of extracellular ice formation and the solution effects to the freezing injury of PC-3 cells suspended in NaCl solutions. Cryobiology 53(1): 1–11. doi:10.1016/j.cryobiol.2006.03.005

Tironi, V., de Lamballerie, M., and Le-Bail, A. 2010. Quality changes during the frozen storage of sea bass (Dicentrarchus labrax) muscle after pressure shift freezing and pressure assisted thawing. Innovative Food Sci. Emerging Technol. 11(4): 565–573. doi:10.1016/j.ifset.2010.05.001

Uddin, M., and Okazaki, E. 2004. Classification of fresh and frozen-thawed fish by near infrared spectroscopy. J. Food Sci. 69(8): C665–C668. doi:10.1111/j.1750-3841.2004.tb18015.x

Uddin, M., Okazaki, E., Turza, S., Yumiko, Y., Tanaka, M., and Fukuda, Y. 2005. Non-destructive visible/NIR spectroscopy for differentiation of fresh and frozen-thawed fish. J. Food Sci. 70(8). doi:10.1111/j.1365-2621.2005.tb11509.x

Velioglu, H. M., Temiz, H. T., and Boyaci, I. H. 2015. Differentiation of fresh and frozen-thawed fish samples using Raman spectroscopy coupled with chemometric analysis. Food Chem. 172: 283–290. doi:10.1016/j.foodchem.2014.09.073

Vidaček, S., Medić, H., Botka-Petrak, K., Nežak, J., and Petrak, T. 2008. Bioelectrical impedance analysis of frozen sea bass (Dicentrarchus labrax). J. Food Eng. 88(2): 263–271. doi:10.1016/j.jfoodeng.2008.02.010

Yoshioka, K., and Kitamikado, M. 1983. Differentiation of freeze-thawed fish from fresh fish by the examination of medulla of crystalline lens. Bull. Jpn. Soc. Sci. Fish. 49(1): 151.

Zhang, L., Shen, H., and Luo, Y. 2010. Study on the electric conduction properties of fresh and frozen–thawed grass carp (Ctenopharyngodon idellus) and tilapia (Oreochromis niloticus). Int. J. Food Sci. Technol. 45(12): 2560–2564. doi:10.1111/j.1365-2621.2010.02428.x

Zhu, F., Zhang, D., He, Y., Liu, F., and Sun, D.-W. 2012. Application of visible and near infrared hyperspectral imaging to differentiate between fresh and frozen-thawed fish fillets. Food Bioprocess Technol. 6(10): 2931–2937. doi:10.1007/s11947-012-0825-6