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Abstract

Tunas are among the most traded and valued fish species, and good traceability of tuna products in the world market is needed to protect both consumers and tuna stocks. To that purpose, high-resolution proton nuclear magnetic resonance (^{1}H NMR) spectroscopy combined with multivariate data analysis was used to investigate the molecular components of the aqueous extract of white and red muscles in three species of wild tropical tuna species, namely yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*) and bigeye tuna (*T. obesus*). Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) applied to the processed ^{1}H NMR spectra showed significant separation according to the species and size category (i.e., small tunas < 80 cm fork length *vs* large tunas >80 cm fork length), the storage conditions on-board the purse-seine vessels (i.e., brine- *vs* deep-freezing), and the geographical origin, i.e., where the tuna was caught (Mozambique Channel *vs* western-central Indian Ocean). The major groups of metabolites responsible for differentiation in PLS-DA score plots were the dipeptides (anserine, carnosine) and organic acids (lactate, creatine/phosphocreatine) in the white muscle, and the free amino acids, essential nutrients (choline and its derivatives, phosphatidylethanolamine), dipeptides and organic acids in the red muscle. Our results show that NMR-based metabolomics is a powerful tool to efficiently discriminate specific profiles among wild tuna species, raw muscle tissues, fish storage conditions and tuna geographical origin.

Keywords: *Metabolomics, High-Resolution ^{1}H NMR; Highly migratory large pelagic fish; Purse seine tuna fisheries; western Indian Ocean; Raw white and red muscles.*

1 Introduction

Sustainable fisheries management relies on the availability of accurate species-specific catch data and may suffer when reported data of species or fishing area are erroneous or inaccurate (Watson & Pauly, 2001). Moreover, ensuring traceability of fishery products "from ocean to plate" is a major concern for instances and food markets worldwide. For example, with the objectives to achieve a high level of consumers' health protection and to guarantee their right to information, the European Regulation (No 1169/2011) imposes labelling of fishery products with the commercial and scientific name of the species, the area where the organism was caught, the production methods, and the product's nutritional characteristics. Nevertheless, differences in quality and price according to species, geographical origin, production/fishing modes, or the life history of the live species and product may lead to mislabelling. Efficient methods to assess fishery products authenticity and origin are thus required to help improving fisheries traceability and sustainability, guaranteeing market transparency, and protecting consumers' safety and rights (Nielsen et al., 2012).

The quality and/or origin of fishery product is mostly determined by its biochemical composition, i.e., metabolite profile. Over the past twenty years, Nuclear Magnetic Resonance (NMR) spectroscopy coupled with multivariate analysis has been applied for determining the metabolite profiles of various kinds of food including fish (Martinez et al., 2005; Picone et al., 2011; Wei et al., 2020). The metabolite content of a given sample constitutes a unique profile for the product of all low molecular weight molecules present in, or excreted by, cells or bacteria. Low molecular weight metabolites in fish tissues include free amino acids, nucleotides and related compounds, peptides, organic bases, sugars and inorganic constituents (Piñeiro et al., 2003). In general, the levels of these metabolites vary according to species, environmental conditions, diet, fish tissue, freshness, stress, and post-mortem storage, handling and processing (Piñeiro et al., 2003; Samuelsson & Larsson, 2008). Moreover, compared to other techniques widely used for metabolic profiling such as mass spectrometry, the use of NMR allows a simpler sample preparation and a higher analysis throughput (Jung et al., 2010). Because NMR analyses produce highly complex datasets, multivariate analyses such as Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) have been designed specifically to analyse NMR derived data (Hatzakis, 2019). ^{1}H NMR spectroscopy is therefore a powerful non-specific high-throughput analytical technique to produce a metabolic profile and identify potential compounds useful in characterizing, discriminating and authenticating the metabolic specifications of a particular fishery product and its quality (Hatzakis, 2019).

Tunas are among the most traded and valued fish species, with annual global catch of around 6 million tonnes, i.e. 10% of the world's international seafood trade (FAO, 2020). While bluefin tunas are the most highly valued species although representing about 1.2% of total tuna catch, the global tuna fisheries are dominated by three tropical tuna species, i.e. skipjack tuna (*Katsuwonus pelamis* -SKJ), yellowfin tuna (*Thunnus albacares* -YFT), and bigeye tuna (*T. obesus* -BET) with 51%, 26%, and 8% of the total tuna catch, respectively (FAO, 2020). From the different fishing gears targeting tropical tunas, industrial purse-seine contributes most to global catch, i.e. 69% compared to 12% for pole-and-line, 10% for longline, and 9% for other gears during the period 2014 to 2018 (FAO, 2020). Purse seine is the less selective gear both in terms of size and species composition. Indeed, purse-seiners catch a mix of free-swimming schools and schools associated with drifting floating objects leading to the simultaneous catch of the three co-occurring tropical tunas, from early juveniles (<30 cm) to the largest and oldest individuals (>150 cm for YFT and BET) (Fonteneau et al., 2013). While tunas are mixed in the wells in the case of brine-freezing (i.e., salted water refrigerated at around -18°C), they are sorted according to species, size category and quality appearance in the case of deep-freezing (i.e., -40°C dry well) storage. The duration of fish storage follows the length of the purse seiner's trips, which is 6 to 9 weeks in the Indian Ocean. Purse seine tropical tunas are supplied to the factories (i) in deep-frozen form for large YFT and BET (i.e., > 10 kg) classified on board with "high quality grade" and sold mainly in pre-cooked loins, or (ii) in brined form for SKJ, small tunas (i.e., < 10 kg), and the remaining large YFT and BET that are being processed and sold as cans (Table S1). Misidentification of tuna can occur when fish are sorted onboard purse-seine vessels, at unloading and/or at the factories, especially in the case of small YFT and BET with similar morphological features (Pecoraro et al., 2016). This raises the possibility of accidental mislabelling, intentional species substitution, or mixing of valuable fish by less valuable ones in the commercial tuna market, motivated by the difference of market prices between species and/or when catch restrictions on overfished specific tuna species exist such as YFT in the Indian Ocean (IOTC Secretariat, 2019). Moreover, the purse-seine fishery catching tropical tunas throughout the year, the meat quality is likely to be subject to changes depending on the geographical area where the tuna was caught, the related diet available and consumed by the tuna in the area, as well as its age (i.e., size) and reproductive status, all these factors known to influence tuna's muscle biochemical composition (Chassot et al., 2019; Pecoraro et al., 2016; Sardenne et al., 2016a; Sardenne et al., 2017). The changes in tuna meat quality may thus affect the efficiency of processing and/or the nutritional benefits for consumers.

Although NMR spectrometry was applied on tuna as early as the 90's, only few studies remain available in the literature, especially dealing with tuna muscles (Jääskeläinen et al., 2019; Medina et al., 2000; Sacchi et al., 1993). In this context, the present study aimed at characterizing and discriminating the ^{1}H NMR low molecular weight metabolic profiles of the muscles of three co-occurring tropical tuna species, namely skipjack, yellowfin and bigeye tunas, targeted by the purse-seine fishery in the western Indian Ocean. The ^{1}H NMR spectra of tuna muscles' aqueous extracts were then further analysed using PCA and PLS-DA to test for metabolic differences according to tuna size, on-board freezing storage conditions (brine- *vs* deep-freezing) and geographical origin. Tuna muscle consists of white (or light) and red (or dark) muscles, the latter representing higher proportions in tuna species compared to other fish (e.g., 6-8% of total body mass) (Dickson, 1995); this is because of the high migratory abilities of tunas and their related high needs for fat, glycogen and myoglobin. Both tuna muscles were thus taken into account in the present study: their ^{1}H NMR metabolic profiles were characterised and compared, and the influence of each of the factors listed above was tested.

2 Material and methods

2.1 Chemicals

Methanol, dichloromethane, ethylene glycol tetra-acetic acid (EgTA) and potassium chloride (KCl) were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Deuterium oxide (D_2O) and sodium 3-trimethylsilyl-2,2,3,3-tetradeuteriopropionate (TMSP) were obtained from Eurisotop (Saint-Aubin, France).

2.2 Tuna sampling

A one-year multi-species sampling was carried out throughout 2013 in the western Indian Ocean. A total of 194 tropical tunas including 60 YFT, 62 BET and 72 SKJ were collected during the unloading of purse seiners at Victoria, Seychelles (Table S1). Fishing date and accurate GPS fishing position from purse seiners' logbooks allowed for the distinction of two main geographical origin areas that differ according to their oceanographic specificities (Chassot et al., 2019): Mozambique Channel (MOZ, n=52) and western-central Indian Ocean (WCIO, n=142) (Fig.1). The onboard storage condition was also recorded during sampling, allowing for the separation between fish stored in brine freezing wells (-18°C) versus those stored in dry deep-freezing wells (-40°C) (Table S1). All sampled fish were weighed (kg to the nearest 0.1 kg), and measured in fork length, which refers to the length from the tip of the snout to the fork of the tail

(FL, cm to the nearest 0.5 cm). Then, one sample of around 2 g on a wet weight (ww) basis was taken from the dorsal white muscle (under the dorsal spine on the left side) and a second one from the red muscle (under the pectoral fin on the left side) of each tuna. All samples were stored at –80 °C until further analyses.

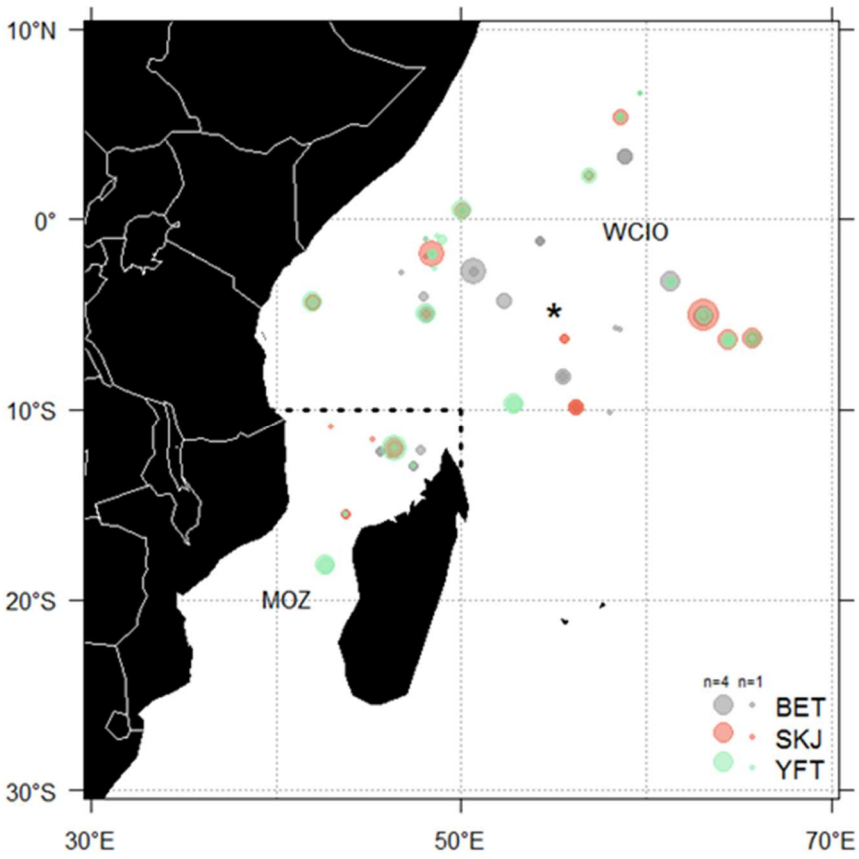

Fig. 1. Location of yellowfin (YFT, n=60), bigeye (BET, n=62) and skipjack (SKJ, n=72) tuna caught by purse seine vessels in the western Indian Ocean throughout 2013. The limit of the two geographical origin areas, Mozambique Channel (MOZ) and western-central Indian Ocean (WCIO) is indicated with dashed line. Star indicates Port Victoria, Seychelles.

2.3 Sample preparation

A total of 388 samples of tunas' white and red muscles (from 194 tunas) were processed for the analysis of ^{1}H NMR Spectroscopy. Approximately 100±0.1 mg ww of each tissue was weighed, crushed with 1 mL of methanol:ethylene glycol tetraacetic acid (EgTA) (5 mM in water) (2/1, v/v) using a FastPrep System® (MP Biomedicals, Illkirch, France), and extracted with 2 mL of dichloromethane:methanol (2:1, v/v). After addition of 0.2 volume of aqueous potassium chloride (KCl) (0.9% w/v), the extracts were further vortexed and centrifuged at 5000g for 10 min, then the lipid supernatants were removed, while the aqueous extracts were freeze-dried. The resulting powders were reconstituted in 700 μL of deuteriumoxide (D_2O) in which sodium 3-trimethylsilyl-2,2,3,3-tetradeuteriopropionate (TMSP; as a chemical shift reference at 0 ppm; 1 mM) was added. Each sample was vortexed and centrifuged for 10 min, 4°C at 5000g to remove any precipitate. Then, 600 µL aliquots were transferred to standard 5 mm - NMR tubes (Norell ST 500, Landisville, NJ) for analysis.

2.4 ^{1}H NMR Analysis

The ^{1}H-NMR spectra were obtained on a Bruker DRX-600-Avance NMR spectrometer (Bruker, Wissembourg, France) operating at 600.13 MHz for ^{1}H resonance frequency using an inverse detection 5mm ^{1}H-^{13}C-^{15}N cryoprobe attached to a CryoPlatform (the preamplifier cooling unit). The ^{1}H-NMR spectra were acquired at 300K using the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence with pre-saturation, with a total spin echo delay (2nτ) of 240ms to attenuate broad signals from proteins and lipoproteins. A total of 128 transients were collected in 32,000 data points using a spectral width of 20 ppm, a relaxation delay of 2 sec, and an acquisition time of 1.36 sec. The spectra were Fourier transformed by multiplication of the FIDs by an exponential weighting function corresponding to a line-broadening of 0.3Hz. All spectra were manually phased and baseline corrected and referenced to TMSP using Bruker TopSpin 2.1 software (Bruker, GMBH, Karlsruhe, Germany). To confirm the chemical structure of metabolites of interest, 2D ^{1}H-^{1}H COSY (Correlation Spectroscopy) and 2D ^{1}H-^{13}C-HSQC (Heteronuclear Single Quantum Coherence Spectroscopy) NMR experiments were performed on selected samples. Spectral assignment was based on matching 1D and 2D data to reference spectra in a home-made reference database, as well as with other databases (http://www.brmb.wisc.edu and http://www.hmdb.ca), and reports in the literature. Absolute concentrations of the metabolites were calculated from the integrals of the TMSP signal (known concentration standard) and, the metabolite signal (unknown concentration) as described in (Amiel et al., 2020).

2.5 Data reduction and multivariate statistical analyses

Data were reduced using the AMIX software (version 3.9, Bruker, Rheinstetten, Germany) to integrate variable wide regions corresponding to the δ 9.0-0.90 ppm region for aqueous white and red muscle extracts. A total of 64 and 78 NMR buckets were selected for white muscle and red muscle respectively, according to resonance signals and multiplicity between 9.0 and 0.9 ppm. Regions corresponding to noise or residual water, methanol and dichloromethane were not selected. To account for differences in sample amount, each integrated region was normalized to the total spectral area.

Multivariate analyses were used to study the effect of *Species* on the metabolome of white muscle and red muscle separately. The effects of *Storage condition* on-board purse-seiners (i.e., brine- *vs* deep-freezing) and *Geographical origin* (i.e., MOZ *vs* WCIO) were also investigated for each species and muscle type. Finally, the effect of *Size category* was considered for YFT and BET

only: two distinct size categories were taken into account, based on YFT and BET life history traits (i.e., small tunas < 80 cm FL *vs* large tunas > 80 cm FL, with FL=80 cm corresponding to the size at which YFT and BET reach their size-at-first maturity, change their aggregative behaviour and habitat from surface to deeper waters and thus their diet; (Chassot et al., 2019, 2015; Sardenne, Bodin, et al., 2016)). SKJ is a smaller tuna species than YFT and BET (maximum length of 110 cm for SKJ compared to 230 cm for YFT and BET), and does not show specific behavioural changes with size being aggregated in schools near the surface through their lifespan. For this reason, the factor Size category was not considered for SKJ. A PCA was first performed to reveal intrinsic clusters and detect potential outliers. PLS-DA was then used to model the relationship between groups and spectral data. Before analysis, the orthogonal signal correction filtering was applied to remove variation not linked to the factors of interest (physiological, experimental, or instrumental variation). Filtered data were mean-centred and scaled (unit variance or Pareto scaling). For the figures, we used Hotelling's T2 statistics to construct 95% confidence ellipses. The goodness-of-fit parameter R^2Y parameter represents the explained variance. Seven-fold cross-validation was used to determine the number of latent variables to include in the PLS-DA model and to estimate the predictive ability (Q^2 parameter) of the adjusted model. The robustness of the PLS-DA regression models was assessed by comparing R^2Y and Q^2: R^2Y varies between 0 and 1, where 1 indicates a perfect fit between the model and the data; When Q^2 is greater than 0.5, the model is considered to have good predictability, and if Q^2 is greater than 0.9, then the model is considered to have excellent predictability.

In addition, the robustness of the PLS-DA model was assessed using a permutation test (200 permutations). Discriminant variables were determined using the VIP (variable importance in the projection) value, a global measure of the influence of each variable on the PLS components. Variables with VIP > 0.8 were considered discriminant. A non-parametric Kruskal–Wallis test was then used to determine which metabolites were significantly different between groups. NMR variables with a p-value <0.05 were considered significantly different. SIMCA-P software (V12; Umetrics AB, Umea, Sweden) was used to perform the multivariate analyses and R software (version 2.12, http://www.r-project.org) was used to perform univariate Kruskal-Wallis test.

3 Results and discussion

3.1 Identification of tropical tunas' white and red muscle metabolite profiles

Both muscles (388 samples) displayed similar metabolites, although changes in the relative intensities of some resonances were observed. Figures 2A and 2B show representative ^{1}H NMR spectra from white and red muscles of tuna respectively. The assignment of the peaks to specific

metabolites was achieved based on the literature and confirmed by 2D COSY and HSQC spectroscopy. The ^{1}H NMR spectra of tuna muscle samples contained a number of assignable amino acids, organic acids, glucose and lipids, as observed in fish muscle in general (Konosu & Yamaguchi, 1982; Piñeiro et al., 2003). Twenty-five metabolites were identified based on their 1D and 2D spectra, and Table 1 lists the compounds assigned. The comparison of tuna muscles spectra allowed for the identification of four groups of signals and related metabolites:

Group I – degradation biomarkers: The low-field region (8.6-4.4 ppm) of the NMR spectra displays signals belonging to adenosine triphosphate (ATP) degradation products, i.e., inosine, inosine-5-monophosphate (IMP), and hypoxanthine. ATP predominates the nucleotides in muscle of live animals under normal conditions, but after death a series of enzymatic reactions leads to the decomposition of ATP into adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine, hypoxanthine, xanthine and ultimately uric acid. When the level of ATP has dropped under a critical level, the muscle enters *rigor mortis*. Moreover, hypoxanthine is a known contributor to the bitter off-flavour of spoiled fish while IMP is usually associated to the desirable taste of fresh fish. The degree of freshness is thus often expressed as the K-value, defined as the ratio of the sum of inosine and hypoxanthine concentrations to the total concentration of ATP metabolites (Karube et al., 1984).

Group II – essential metabolites: In the mid-field region (5.2-3.2 ppm) of the NMR spectra, the signals of choline and its derivative metabolites, namely phosphoethanolamine (PE) and glycerophosphorylcholine (GPC) were detected. The spectral region is also characterized by the presence of sugar signals (glucose). Choline and its derivatives result from the hydrolysis of phospholipids, in particular of phosphatidylcholine, which are found in high proportions in the lipid fraction of SKJ, YFT and BET (Sardenne et al., 2016b). Involvement of these compounds in aging of samples due to storage conditions has already been pointed out (Scano et al., 2012). Hydrolysis of the ester bond that links the glycerol backbone of lipid molecules to fatty acids results in the release of the latter in their free form, making them more prone to oxidation (Refsgaard et al., 2000) which can be very fast in highly unsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) present in tuna muscle in high quantity. Therefore, hydrolysis of the lipid molecules makes the food matrix more exposed to oxidation.

Group III – energy expenditure: The signals with the highest intensity were observed in the low-field and mid-field NMR spectra regions, and assigned to anserine, lactate, creatine and phosphocreatine. Low-intensity signals belonging to carnosine, succinate, and trimethylamine (TMA) were also identified. Creatine and phosphocreatine represent an important energy deposit

261 in skeletal muscle. In fact, most of the creatine is phosphorylated in resting muscle and supplies
262 energy in the form of high energy phosphate for muscular contraction. Fish exposed to stress
263 prior to death have lower values of phosphocreatine and ATP than unstressed fish (Erikson et al.,
264 1997). Moreover, degradation of creatine/phosphocreatine into creatinine/phospho-creatinine
265 through intramolecular cyclization has been reported to be favoured by different chemical–
266 physical conditions including temperature (Chan et al., 1994). Distinct peaks from anserine (β-
267 alanyl-1-methylhistidine) and carnosine (β-alanyl-histidine), the most frequently dipeptides
268 occurring in fish (Abe, 1983; Konosu & Yamaguchi, 1982) were visible in all samples. The high
269 level of anserine observed in tuna muscle is typical for migratory pelagic species (Abe et al.,
270 1986; Konosu & Yamaguchi, 1982). These dipeptides are believed to act as a buffer during
271 anaerobic metabolism, a fact reflected by the high levels in muscles used for burst activity
272 (Boldyrev & Severin, 1990). In addition, anserine and carnosine have been proposed to play
273 additional roles in controlling enzyme activity, inhibiting oxidative reactions, and as
274 neurotransmitter (Boldyrev, 2001; Boldyrev & Severin, 1990). Anserine decomposes into its
275 constituents β-alanine and 1-methylhistidine by hydrolysis. This permitted to estimate the loss of
276 quality during ice storage by measuring the levels of 1-methylhistidine, β-alanine, anserine and
277 tryptophan (Ruiz-Capillas & Moral, 2001). Lactate is typically found in high abundance in tuna
278 muscle as it is involved in burst swimming activity: glucose is broken down and oxidized to yield
279 pyruvate, and lactate is then produced from the pyruvate faster than the body can process it,
280 causing lactate concentrations to rise by a factor 7-20 in tuna muscle after fast swimming/hunting
281 (Guppy et al., 1979). Lactate also plays an important role both in the evolution of the freshness
282 degree and in the formation of an agreeable odour or not (Bramstedt, 1962). Its concentration
283 reflects the initial glycogen stores before death, the fish handling (e.g., duration of the fishing
284 operation), and the extraction procedure.
285 *Group IV – muscle structure and its degradation:* The remaining signals in the high-field (3.0-0.9
286 ppm), mid-field (4.2-3.2 ppm) and low-field (6.8-7.5 ppm) NMR spectra regions belongs to the
287 aliphatic groups, with 12 amino acids identified. Tropical tuna muscles are characterized by a
288 relatively low lipid concentration, and a high concentration of proteins and thus their constituents,
289 amino acids (Sardenne et al., 2016b). Indeed, the ^{1}H-NMR allowed to detect leucine, isoleucine,
290 valine, lysine, alanine, methionine, glycine, taurine, proline, tyrosine, tryptophan, and
291 phenylalanine. Most of these amino acids are free although some might be part of peptides. Large
292 proteins were not visible because their slow tumbling leads to broad signals. Proteins play
293 important roles in physiological functions including osmoregulation and buffering capacity (Van
294 Waarde, 1988); in addition, they contribute to the aroma and flavour of the fish (Konosu &

295 Yamaguchi, 1982), and increase its antioxidant capacity (Chan et al., 1994). During storage and
296 processing, proteins are degraded, and the level of free amino acids and peptides in muscle
297 changes.
298 It is important to underline a low presence of trimetylamine (TMA, peak at 2.89 ppm) in spectra
299 acquired in this study which is in accordance with previous study on Indian Ocean YFT
300 (Jääskeläinen et al., 2019). TMA is a microbial metabolite and it can only be used as an index of
301 spoilage and not as an index of freshness (Jääskeläinen et al., 2019). Development of TMA in
302 seafood depends primarily on the content of the substrate trimethylamine-oxide (TMAO, peak at
303 3.27 ppm) in the fish raw material, which is reduced into TMA by some species in the
304 bacteriological flora of spoiling fish as *Shewanella putrefaciens*, *Photobacterium phosforeum*,
305 and Vibrionaceae (Gram & Huss, 1996). In frozen fish, this reaction can be replaced by a slow
306 enzymatic transformation to dimethylamine (DMA) and formaldehyde. TMA can also result from
307 the breakdown of choline from phospholipids.
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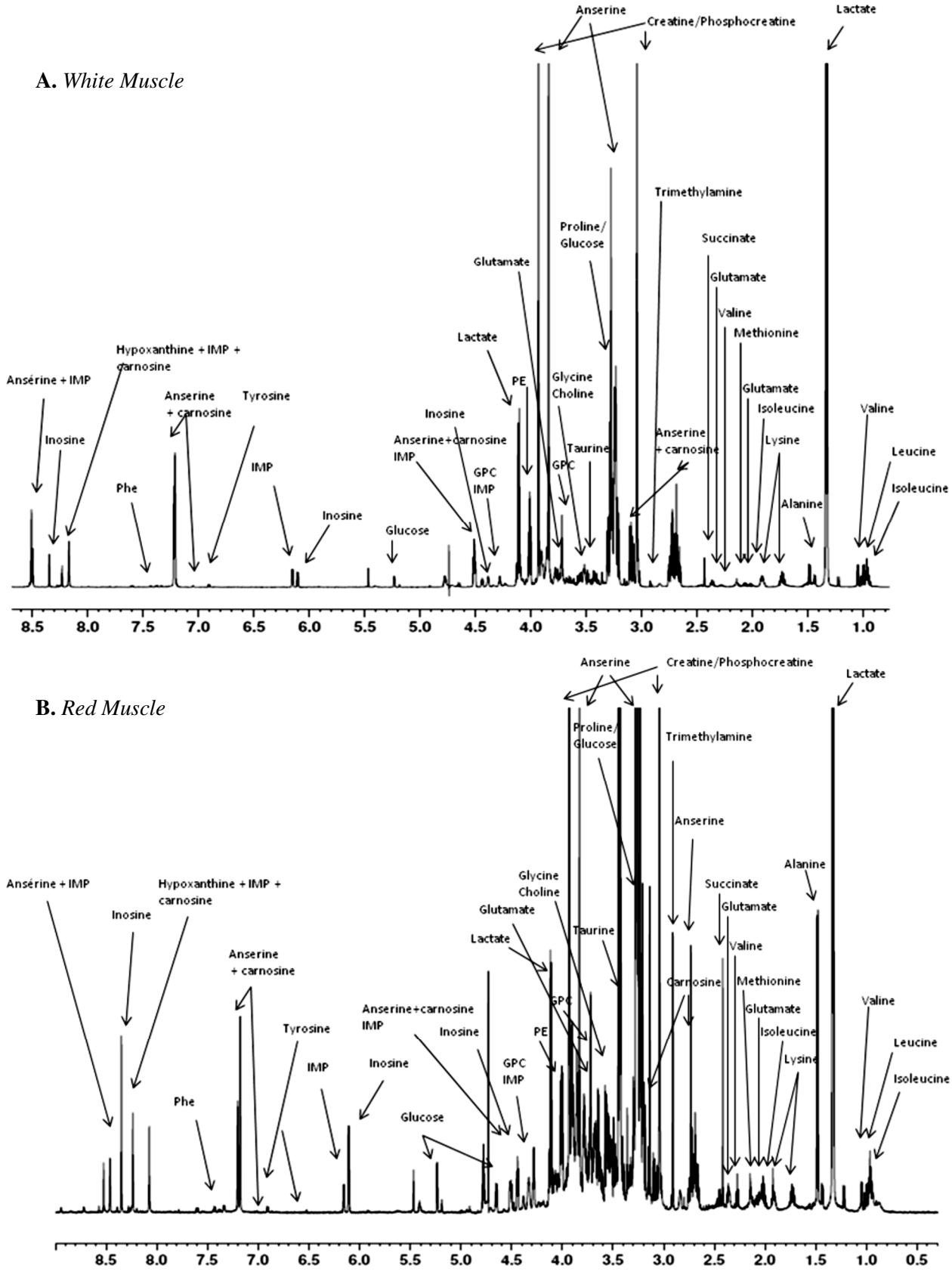

Fig. 2. Typical 600 MHz ^{1}H NMR CPMG spectra from (A) white muscle and (B) red muscle of wild tropical tuna. GPC: Glycerophosphorylcholine; IMP: Inosine-5-monophosphate; Phe: Phenylalanine; PE: Phosphatidylethanolamine.

Table 1. Representative ^{1}H-NMR assignments for white and red muscles of wild tropical tuna at 600 MHz. Group indicates the four groups of signals and related metabolites identified. I – degradation biomarkers; II – essential metabolites; III – energy expenditure; IV – muscle structure and its degradation. Concentration ranges of metabolites were indicated in blue for white muscle, and in red for red muscle. Concentrations were calculated from proton NMR spectra. NQ means "non quantifiable"

Metabolite	Concentration range (mM/g muscle)	δ_H ppm (multiplicity, coupling constant, assignment)/ δ_C ppm	Group	Type of metabolite
Inosine (Ino)	0.1-32; 0.1-25	4.29 (m, CH)/88.2; 4.44 (m, CH)/73.1; 6.10 (d, J=5.7 Hz, CH)/90.9; 8.24 (s, CH)/148.9; 8.35 (s, CH)/142.7	I	Nucleoside
Hypoxanthine (Hyp)	NQ	8.21 (s, CH)/135.0; 8.24 (s, CH)/146.2	I	Purine
Inosine-5-monophosphate (IMP)	0.1-34; 0.1-10	8.21 (s, CH)/148.9; 8.54 (s, CH)/142.6; 6.15 (d, J=5.7 Hz, CH)/89.9; 4.38 (m,CH)/87.1	I	Enzyme
Lactate (Lac)	47-778 ; 32-608	1.32 (d, J=6.9 Hz, CH_3)/22.9; 4.11 (q, J=6.9 Hz, CH)/71.4	II	Organic Acid
Glycerophosphorylcholine (GPC)	0.1-15; 1-47	3.21 (s, $N(CH_3)_3$)/56.6; 3.64 (m, CH_2)/64.6; 4.30(m, CH_2)/62.1	II	Essential nutrient
Choline (Cho)	2-32; 2-38	3.22 (s, $N(CH_3)_3$)/56.7; 3.52 (m, CH_2)/70.1; 4.08 (m, CH_2)/58.5	II	Essential nutrient
Glucose (Glu)	0.3-35; 0.2-58	3.24 (m, CH) /76.9; 3.40 (m, CH)/72.3; 3.50 (m, CH)/74.2; 3.73 (m, CH)/63.3; 3.91 (m, CH_2)/63.4; 4.65 (d, J=7.9 Hz, CH)/98.7; 5.24 (d, J=3.7 Hz, CH)/94.9	II	Monosaccharide
Phosphatidylethanolamine (PE)	5-165 ; 3-90	4.02 (m)	II	Phospholipids
Succinate (Suc)	0.1-16 ; 0.7-21	2.42 (s, CH_2)/36.8	III	Organic Acid
Anserine (Ans)	4-235; 4-106	2.67 (m, CH_2)/34.8; 3.08 (dd, CH_2)/28.3; 3.24 (m, CH_2)/38.3; 3.84 (s, CH_3)/35.2; 4.51 (dd, CH)/56.1; 7.16 (s, CH)/122.8; 8.35 (s, CH)/138.4	III	Dipeptide
Carnosine (Car)	0-60; 0-33	2.68 (m, CH_2)/34.8; 3.08 (dd, CH_2)/30.8; 3.24 (m, CH_2)/38.6; 4.51 (m, CH, ring)/57.6; 7.06 (s, CH, ring)/119.9; 8.17(s CH, ring)/137.4	III	Dipeptide
Trimethylamine (TMA)	0-1 ; 0.5-14	2.91 (s, $N(CH_3)_3$)/47.5	III	Amine
Creatine/phosphocreatine (Crt/P-crt)	13-213 ; 10-137	3.04 (s, CH_3)/39.5; 3.93 (s, CH_2)/66.4	III	Organic Acid
Isoleucine (Ile)	0.1-21 ; 0.4-18	0.94 (t, J=7.4 Hz, CH_3)/13.9	IV	Amino acid
Leucine (Leu)	0.1-42 ; 0.8-37	0.96 (t, J=5.9 Hz, CH_3)/23.6	IV	Amino acid
Valine (Val)	0.2-34; 0.7-23	0.99 (d, J=7.0 Hz, CH_3)/19.4; 1.05 (d, J=7.0 Hz, CH_3)/20.8; 2.28 (m, CH)/31.9	IV	Amino acid
Alanine (Ala)	0.5-47 ; 3-57	1.48 (d, J=7.1 Hz, CH_3)/18.9; 3.78 (q, J=7.2 Hz, CH)/53.1	IV	Amino acid
Lysine (Lys)	0.1-17 ; 0.8-28	1.73 (m, CH_2)/29.2; 1.92 (m, CH_2)/32.7	IV	Amino acid
Proline (Pro)	NQ	2.04 (m, CH_2)/32.1; 2.36 (m, CH_2)/31.7; 3.35 (m, CH_2)/48.9; 4.13 (m,	IV	Amino acid

		CH)/64.0		
Glutamate (Glu)	0.2-31 ; 2-34	2.07 (m, CH_2)/29.6; 2.37 (m, CH_2)/36.1; 3.78 (m, CH)/56.7	IV	Amino acid
Methionine (Met)	NQ	2.13 (m, CH_3 / CH_2)/32.7	IV	Amino acid
Taurine (Tau)	0.7-34 ; 22-337	3.26 (t, J=7.3 Hz, CH_2)/50; 3.44 (t, J=7.3 Hz, CH_2)/38.1	IV	Amino acid
Glycine (Gly)	0.8-27 ; 5-88	3.57 (s, CH_2)/44.3	IV	Amino Acid
Tyrosine (Tyr)	0-9; 0.2-7	6.91 (m, CH ring)/118.9; 7.20 (m, CH ring)/133.5	IV	Amino acid
Phenylalanine (Phe)	0-12; 0-12	7.43 (m, CH ring)/131.8; 7.34 (d, J=7 Hz, CH ring)/132.1 7.39 (m, CH, ring)/130.4	IV	Amino acid

318 3.2 Discrimination between tropical tuna species

319 *3.2.1. Effect of Species on ^{1}H NMR profiles of tuna muscles.*

320

321 The PLS-DA model derived from tunas' OSC-filtered white muscle spectra revealed R^2Y and Q^2
322 values of 0.70 and 0.70 for two latent components, respectively, indicating good fit and
323 prediction abilities for the PLS regression model. The three tropical tuna species were separately
324 grouped on the PLS-DA score plot with six compounds or types of molecules, responsible for the
325 inter-species discrimination (Fig. S1A, Table S2). Dipeptides (anserine and carnosine) were the
326 highest in BET followed by SKJ and finally YFT. Phospholipids (PE) had the highest levels in
327 SKJ and the lowest levels in BET. Finally, organic acids (creatine, phosphocreatine and
328 succinate) were predominant in YFT, excepted for lactate that showed the highest and lowest
329 levels in SKJ and BET, respectively.
330 Better fits and prediction abilities were obtained with the PLS-DA model derived from tunas'
331 OSC-filtered red muscle spectra ($R^2Y = 0.84$ and $Q^2 = 0.79$ for four latent components) showing
332 a clear separation along the first latent component (t[1] axis) between the three tropical tuna
333 species (Fig. S1B). 27 NMR variables were identified, corresponding to 16 compounds or classes
334 of molecules (Table S2), and three NMR variables were not identified (i.e., "unknown").
335 Metabolites from group III (anserine, carnosine, creatine, phosphocreatine, lactate and succinate)
336 and phospholipids (PE) showed similar inter-species differences in the red muscle as previously
337 observed in the white muscle. Additionally, six amino acids contributed to the discrimination of
338 the tropical tuna species: SKJ had the highest levels of isoleucine, leucine, and lysine and the
339 lowest levels of glutamate and valine; BET had the highest levels of glutamate and valine and the
340 lowest levels of taurine; and finally, YFT had the highest levels of taurine and the lowest levels of
341 isoleucine, leucine and lysine. The essential nutrient GPC (group II) was also discriminant with
342 higher levels observed in SKJ than in BET and YFT red muscle. Lastly, fish quality biomarkers
343 (group I: inosine, IMP and hypoxanthine) were predominant in SKJ red muscle followed by YFT
344 and BET.

345

346 *3.2.2. Effect of Species and Size category on ^{1}H NMR profiles derived from tuna muscles*
347 The PCA score plots derived from the white and red muscle profiles were investigated to
348 differentiate the three tropical tuna species, in particular YFT and BET, taking into account the
349 size category (i.e., small tunas ≤ 80 cm FL versus large tunas ≥ 100 cm FL) of the studied fish
350 (data not shown). Supervised PLS-DA models applied on OSC-filtered revealed R^2Y of 0.39 and
351 0.59, Q^2 values of 0.36 and 0.50 with three and four latent components for the tuna's white and

red muscle spectra, respectively, indicating poor fit but good prediction abilities for these PLS regression models. The PLS-DA score plot derived from the OSC-filtered white muscle allowed for the discrimination of SKJ and small YFT only (Fig. 3A). The PLS-DA score plot derived from the red muscle was more discriminant with a clear separation of SKJ, small YFT and large BET, while small BET and large YFT remained confounded (Fig. 3B).

Supervised PLS-DA models applied on OSC-filtered data were then applied on the white and red muscle datasets after excluding SKJ data. The derived PLS-DA score plot did not give any valid model ($Q^2 < 0.30$), whereas it allowed for the separation of small and large YFT and BET (R^2Y = 0.43 and Q^2 = 0.41 for two latent components; Fig. 3C). A total of 15 compounds or classes of molecules were identified and one remained unknown, were responsible for the differences between *Species* and *Size category* in the red muscle (Table S3). From the 15 identified chemical species, 13 were common with those responsible for the discrimination of the three tropical species only (Table S2). Differences between SKJ and the two other species were specified when taking into account YFT and BET size categories. Anserine, lactate, succinate, glutamate, and methionine showed similar levels between SKJ and the small YFT and BET but different ones with large tunas; the opposite trends were observed for TMA. Regarding BET versus YFT, valine, anserine and carnosine were predominant in BET and in large individuals compared to small ones for both species. Creatine / phosphocreatine, taurine and lactate were higher in SKJ and in small individuals compared to large ones for YFT, BET and both species respectively. Finally, although no difference was observed between YFT and BET, methionine, TMA and GPC were affected by *Size category* with the large tunas having the highest levels of methionine and TMA and the lowest levels of GPC (for YFT only).

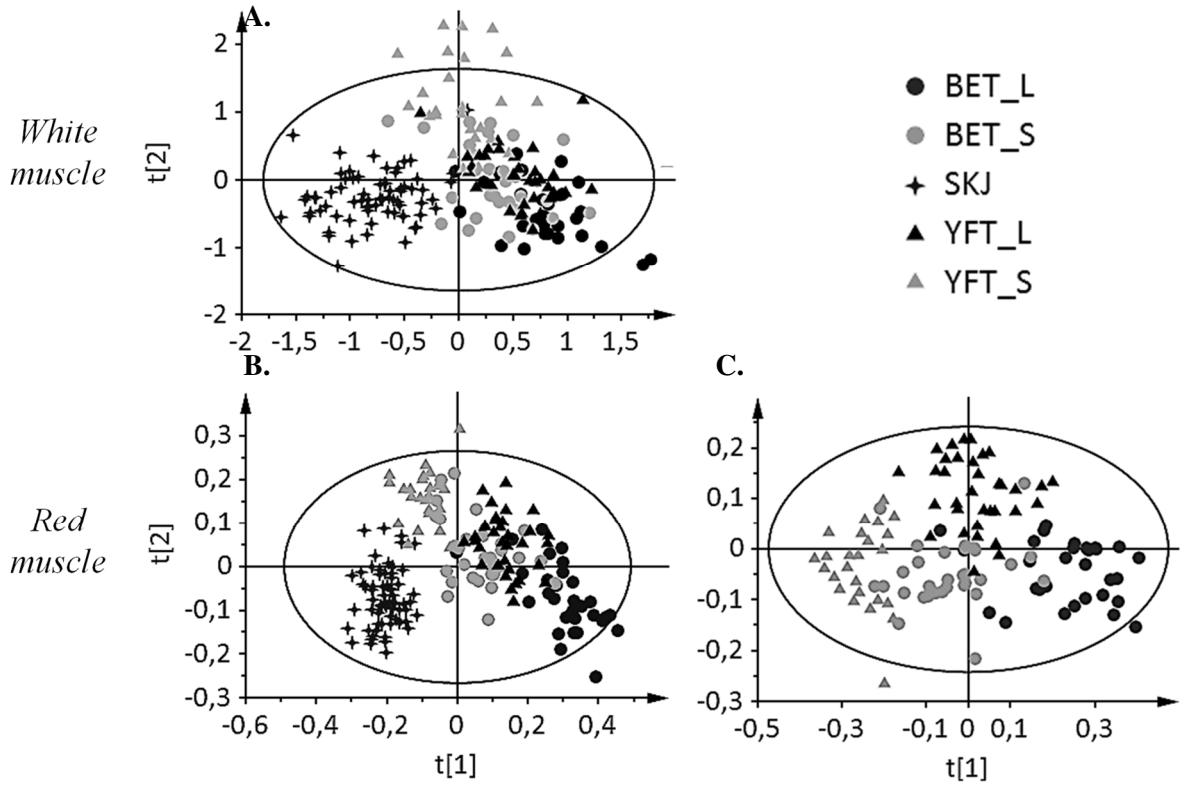

Fig. 3 Two-dimensional Partial Least Squares-Discriminant Analysis (PLS-DA) score plots based on the metabolic profile from white and red muscle aqueous extracts of the three tropical tuna species categorized by size, i.e., small yellowfin tuna (YFT_S, n=25), large yellowfin tuna (YFT_L, n=35), small bigeye tuna (BET_S, n=34), large bigeye tuna (BET_L, n=28) and skipjack tuna (SKJ, n=72). (**A**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of white muscle extracts for all species/size categories, using three components (R^2Y = 0.39 and Q^2= 0.36); (**B**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for all species/size categories, using five components (R^2Y = 0.59 and Q^2 = 0.50); (**C**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for four species/size categories only (excluding skipjack), using two components (R^2Y = 0.43 and Q^2 = 0.41).

3.3 Discrimination of tropical tunas according to storage condition on-board purse seiners

The traceability of the tunas' storage conditions on-board purse seiners allowed for the PLS-DA analysis of the differences in NMR metabolomic profiles from tropical tunas white and red muscle extracts.

The PLS-DA score plot derived from the SKJ and large tuna's OSC-filtered white muscle spectra as well as from the large tuna's red OSC-filtered muscle spectra did not give any valid model (Q^2 < 0.30), suggesting that both freezing modes affect similarly these size class and species. Only the white muscle of small tunas (YFT and BET) showed differences between brine-freezing and deep-freezing storage conditions (R^2Y = 0.69 and Q^2 = 0.46 for two latent components; Fig. 4A), with 14 metabolites identified (Table S4). Among them, nine metabolites corresponded to amino acids that exhibited higher levels in deep-frozen small tunas, most likely reflecting a lower level of protein degradation during this storage mode. Deep-frozen small tunas were also characterised

by lower IMP and higher creatine/phosphocreatine levels compared to brine-frozen ones, which could reflect a slower process of ATP and creatine/phosphocreatine degradation (Chan et al., 1994) .

In the red muscle, the freezing storage condition was a discriminant factor for small YFT and BET ($R^2Y = 0.91$ and $Q^2 = 0.82$ for three latent components; Fig. 4B), and SKJ ($R^2Y = 0.79$ and $Q^2 = 0.73$ for one latent component; Fig. 4C). Deep-frozen small tuna's red muscle had higher levels of four amino acids, glycogen and PE, as a result of lower process of degradation; also, from group III, anserine/carnosine and creatine/phosphocreatine were higher in deep-frozen small tunas compared to brine-frozen tunas, while opposite trends were observed for lactate and succinate. Finally, a lower number of metabolites was identified as responsible for the differences between freezing modes (nine against 12 compounds in small tunas' muscles), with PE, anserine, carnosine and glutamate showing higher levels in deep-frozen but lower levels of succinate and taurine compared to brine-frozen SKJ red muscle.

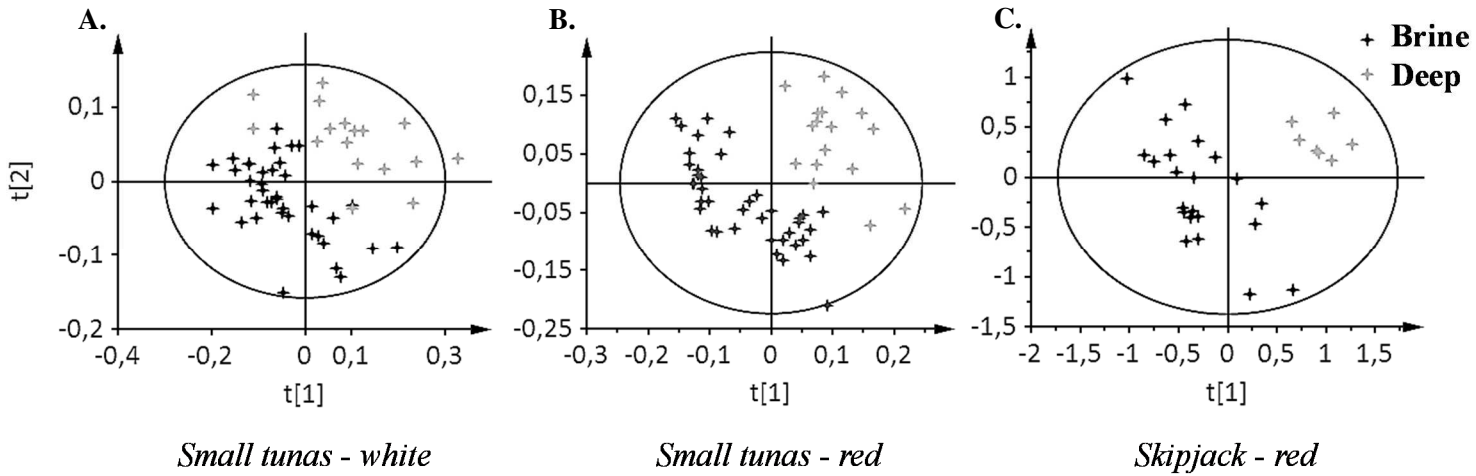

Fig. 4. Two-dimensional Partial Least Squares-Discriminant Analysis (PLS-DA) score plots based on the metabolic profile from white and red muscle aqueous extracts of the tropical tuna species depending on the storage conditions onboard the purse-seine vessels, i.e., brine-freezing and deep-freezing. (**A**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of white muscle extracts for small tunas (n = 83 and 48 stored in brine- and deep-freezing wells, respectively), using two components (R^2Y = 0.69 and Q^2 = 0.46); (**B**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for small tunas (n = 83 and 48 stored in brine- and deep-freezing wells, respectively), using three components (R^2Y = 0.91 and Q^2 = 0.82); (**C**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for SKJ (n = 42 and 17 stored in brine- and deep-freezing wells, respectively), using one component (R^2Y = 0.79 and Q^2 = 0.73).

3.4 Discrimination of the purse seine tropical tunas according to their geographical origin

The information on the fishing locations retrieved from the purse-seine logbooks was merged with the NMR metabolomic profiles from tropical tunas white and red muscle extracts and analysed with PLS-DA.

Fig. 5A, 5B and 5C represent the PLS-DA score plots derived from the white muscle of small YFT and BET ($R^2Y = 0.82$ and $Q^2 = 0.73$ for two latent components), SKJ ($R^2Y = 0.74$ and $Q^2 = 0.70$ for two latent component) and large YFT and BET ($R^2Y = 0.79$ and $Q^2 = 0.77$ for one latent component), respectively. Fig. 5D, 5E and 5F represent the PLS-DA score plots derived from the red muscle of small YFT and BET ($R^2Y = 0.84$ and $Q^2 = 0.73$ for two latent components), SKJ ($R^2Y = 0.89$ and $Q^2 = 0.83$ for one latent component) and large YFT and BET ($R^2Y = 0.85$ and $Q^2 = 0.77$ for three latent component), respectively. All PLS regression models gave good fit and prediction abilities.

Nine metabolites were identified in the white muscle of small and large tunas as responsible for the differences between *Geographical origin*, against only six in the white muscle of SKJ. For all species and size categories, most metabolites were pertaining to the groups II and III. Based on the identified and level changes in metabolites, a greater resemblance of NMR profiles was however observed between SKJ and small tunas compared to large tunas. For instance, the geographical origin of the small tunas and SKJ was well distinguished with small tunas from WCIO having higher levels of essential nutrients (choline and PE), lactate, hypoxanthine, IMP and carnosine in white muscle, but lower levels of anserine compared to fish from MOZ (Table S5). Regarding large tunas, the white muscle of WCIO fish was characterised by lower anserine, PE, creatine/phosphocreatine, IMP and carnosine.

In the red muscle, 18, 16 and 10 metabolites were contributing to the discrimination of large tunas, small tunas and SKJ respectively, according to the *Geographical origin*. Metabolites belong to groups II, III, and IV for small tunas and SKJ, and to all groups for large tunas. In particular, WCIO large tunas had higher GPC, creatine/phosphocreatine, IMP and inosine but lower TMA, methionine and alanine, and WCIO SKJ had higher choline, isoleucine and leucine, hypoxanthine+IMP+carnosine than their congeners from MOZ. Furthermore, large and small tunas exhibited similar variations for 10 metabolites corresponding to higher taurine and anserine+PE+carnosine, and lower glucose, glycogen, glutamate, isoleucine, leucine, lysine, valine and hypoxanthine+IMP+carnosine in WCIO fish. Fewer similitudes were observed between small tunas and SKJ and between large tunas and SKJ with only four (PE, anserine,

carnosine and creatine/phosphocreatine) and one (lactate) similar metabolites variations, respectively.

The discrimination of WCIO and MOZ tunas observed in this study may result from differences of trophic and behavioural ecology. Indeed, while tropical tunas feed mainly on fish and squids when inhabiting WCIO waters, crustaceans predominate their diet in MOZ (Chassot et al., 2019). Such "red feed" is often related to fish tissue softening in pelagic species (Huss et al., 1995) and may favour proteolytic breakdown producing low molecular weight peptides and free amino acids. The high occurrence of such metabolites may reduce the commercial acceptability of fish, but also accelerate bacterial spoilage and produce biogenic amines (Biji et al., 2016). Moreover, the lower levels of burst activity markers in MOZ tuna noted in this study could be related to differences of habitat size and schooling behaviour between the two studied areas. In MOZ, tropical tunas (all species/size categories) are mainly associated with fish aggregating devices (FADs) and present a relatively restricted habitat, whereas they are found both in free-swimming schools and aggregated under FADs in WCIO (especially large tunas) where they are able to cover large distances to search for potential preys (Chassot et al., 2019; Fonteneau et al., 2013).

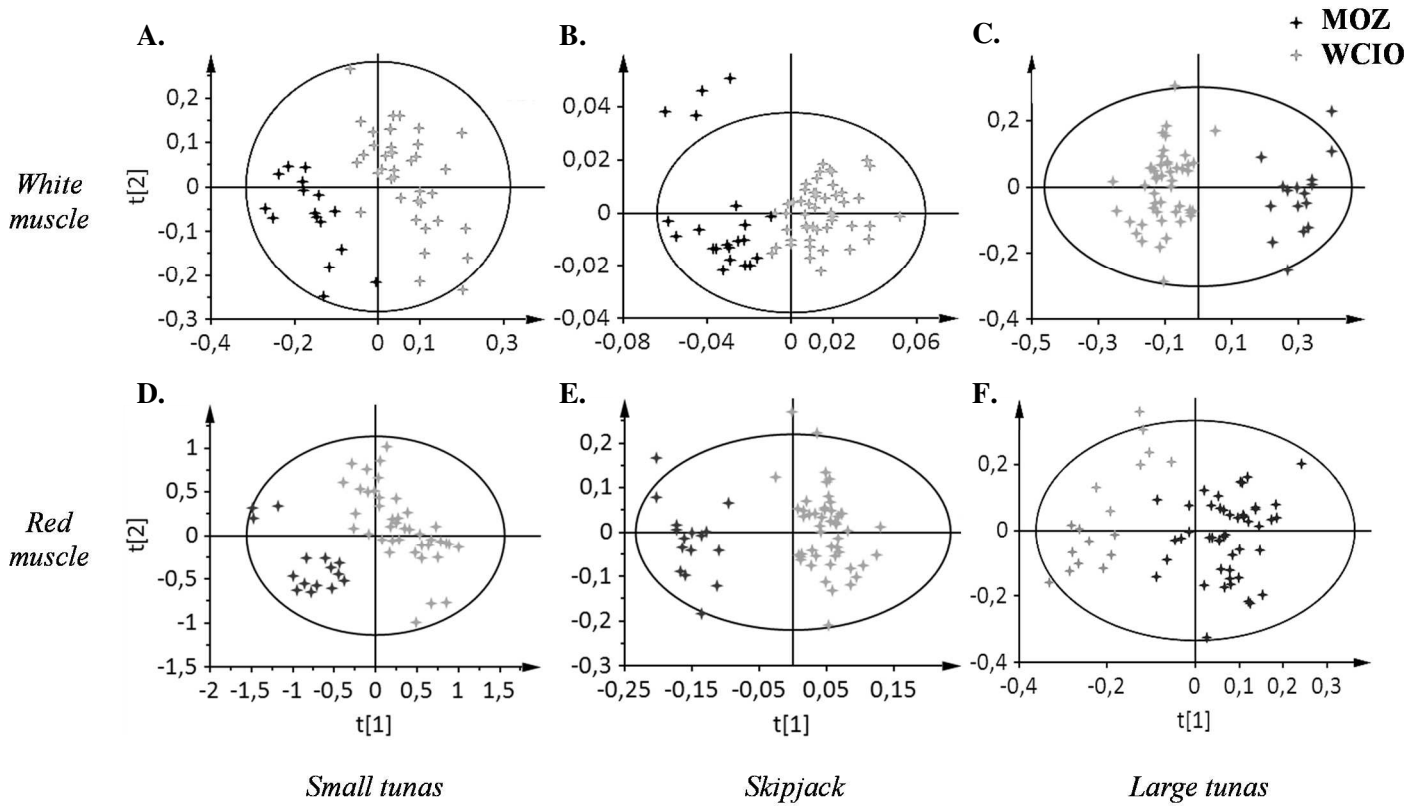

Fig. 5. Two-dimensional Partial Least Squares-Discriminant Analysis (PLS-DA) score plots based on the metabolic profile from white and red muscle aqueous extracts of the tropical tuna species per geographical origin, i.e., Mozambique Channel (MOZ) and western-central Indian Ocean (WCIO). (**A**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of white muscle extracts for small yellowfin and bigeye tunas (n = 18 and 40 in MOZ and WCIO, respectively), using two components ($R^2Y = 0.82$ and $Q^2 = 0.73$); (**B**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of white muscle extracts for skipjack (n = 23 and 48 in MOZ and WCIO, respectively), using two components ($R^2Y = 0.74$ and $Q^2 = 0.70$); (**C**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of white muscle extracts for large yellowfin and bigeye tunas (n = 11 and 54 in MOZ and WCIO, respectively), using one component ($R^2Y = 0.79$ and $Q^2 = 0.77$); (**D**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for small yellowfin and bigeye tunas (n = 18 and 40 in MOZ and WCIO, respectively), using two components ($R^2Y = 0.84$ and $Q^2 = 0.73$); (**E**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for skipjack tuna (n = 23 and 48 in MOZ and WCIO, respectively), using one component ($R^2Y = 0.89$ and $Q^2 = 0.83$); (**F**) PLS-DA score plot of OSC-filtered integrated ^{1}H NMR spectra of red muscle extracts for large yellowfin and bigeye tunas (n = 11 and 54 in MOZ and WCIO, respectively), using three components ($R^2Y = 0.85$ and $Q^2 = 0.77$).

4 Conclusion

In conclusion, high-resolution ^{1}H NMR spectroscopy of aqueous extracts from white and red muscles of tropical tunas proved to be very efficient for evidencing rich metabolic information (amino acids, dipeptides, organic acids, and other essential nutrients) allowing to discriminate species and size categories, and in a lesser extent on-board storage modes. Discrimination with respect to geographical origin for small tropical tunas was also possible (mainly based on burst activity markers and essential nutrients), which could improve traceability of tuna products in world market. Considering the global scale of this market, geographical comparisons should be extended to the global ocean.

Authors' contribution

Conceptualization, NB and HG; Funding Acquisition, NB and EC; Project Administration, NB, HG, LD; Sampling and Data Acquisition, AA, CC, EF, FS and NB; Data Curation and Analysis, AA, CC and MT-F; Writing – Original Draft Preparation, AA and NB; Writing – Review & Editing, All co-authors.

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References

Abe, H. (1983). Distribution of free l-histidine and related dipeptides in the muscle of fresh-water fishes. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *76*(1), 35–39. https://doi.org/10.1016/0305-0491(83)90167-0

Abe, H., Brill, R. W., & Hochachka, P. W. (1986). Metabolism of L-Histidine, Carnosine, and Anserine in Skipjack Tuna. *Physiological Zoology*, *59*(4), 439–450.

Amiel, A., Tremblay-Franco, M., Gautier, R., Ducheix, S., Montagner, A., Polizzi, A., Debrauwer, L., Guillou, H., Bertrand-Michel, J., & Canlet, C. (2020). Proton NMR Enables the Absolute Quantification of Aqueous Metabolites and Lipid Classes in Unique Mouse Liver Samples. *Metabolites*, *10*(1), 9. https://doi.org/10.3390/metabo10010009

Biji, K. B., Ravishankar, C. N., Venkateswarlu, R., Mohan, C. O., & Gopal, T. K. S. (2016). Biogenic amines in seafood: A review. *Journal of Food Science and Technology*, *53*(5), 2210–2218. https://doi.org/10.1007/s13197-016-2224-x

Boldyrev, A. A. (2001). Carnosine as a modulator of endogenous Zn^{2+} effects. *Trends in Pharmacological Sciences*, *22*(3), 112–113. https://doi.org/10.1016/S0165-6147(00)01648-5

Boldyrev, A. A., & Severin, S. E. (1990). The histidine-containing dipeptides, carnosine and anserine: Distribution, properties and biological significance. *Advances in Enzyme Regulation*, *30*, 175–194. https://doi.org/10.1016/0065-2571(90)90017-v

Bramstedt, F. (1962). Amino acid composition of fresh fish and influence of storage and processing. In *Fish in Nutrition* (E. Heen, R. Kreuzer, pp. 61–67).

Chan, K. M., Decker, E. A., & Feustman, D. C. (1994). Endogenous skeletal muscle antioxidants. *Critical Reviews in Food Science and Nutrition*, *34*(4), 403–426. https://doi.org/10.1080/10408399409527669

Chassot, E., Assan, C., Soto, M., Damiano, A., Delgado de Molina, A., Joachim, L. D., Cauquil, P., Lesperance, F., Curpen, M., Lucas, J., & Floch, L. (2015). *Statistics of the European Union and associated flags purse seine fishing fleet targeting tropical tunas in the Indian Ocean 1981-2014* (Centre IRD de Bondy). *IOTC-2015-WPTT17-12*. http://www.documentation.ird.fr/hor/fdi:010063283

Chassot, E., Bodin, N., Sardenne, F., & Obura, D. (2019). The key role of the Northern Mozambique Channel for Indian Ocean tropical tuna fisheries. *Reviews in Fish Biology and Fisheries*, *29*(3), 613–638. https://doi.org/10.1007/s11160-019-09569-9

551 Dickson, K. A. (1995). Unique adaptations of the metabolic biochemistry of tunas and billfishes for life in
552 the pelagic environment. *Environmental Biology of Fishes*, *42*(1), 65–97.
553 https://doi.org/10.1007/bf00002352
554 Erikson, U., Beyer, U. R., & Sigholt, T. (1997). Muscle high-energy phosphates and stress affect K-values
555 during ice storage of Atlantic salmon (*Salmo salar*). *Journal of Food Science*, *62*(1), 43–47.
556 https://doi.org/10.1111/j.1365-2621.1997.tb04365.x
557 FAO. (2020). *The State of World Fisheries and Aquaculture 2020. Sustainability in action.* Food and
558 Agriculture Organization of the United Nations. https://doi.org/10.4060/ca9229en
559 Fonteneau, A., Chassot, E., & Bodin, N. (2013). Global spatio-temporal patterns in tropical tuna purse
560 seine fisheries on drifting fish aggregating devices (DFADs): Taking a historical perspective to
561 inform current challenges. *Aquatic Living Resources*, *26*(01), 37–48.
562 https://doi.org/10.1051/alr/2013046
563 Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of*
564 *Food Microbiology*, *33*(1), 121–137. https://doi.org/10.1016/0168-1605(96)01134-8
565 Guppy, M., Hulbert, W. C., & Hochachka, P. W. (1979). Metabolic Sources of Heat and Power in Tuna
566 Muscles: II. Enzyme and Metabolite Profiles. *Journal of Experimental Biology*, *82*(1), 303–320.
567 Hatzakis, E. (2019). Nuclear Magnetic Resonance (NMR) Spectroscopy in Food Science: A Comprehensive
568 Review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 189–220.
569 https://doi.org/10.1111/1541-4337.12408
570 Huss, H. H. (ed), Boerresen, T., Dalgaard, P., Gram, L., Jensen, B., Joergensen, B., Nielsen, J., Baek Olsen,
571 K., Gill, T., & Lupin, H. M. (1995). Quality and quality changes in fresh fish. *FAO Fisheries*
572 *Technical Paper*, *No 348*, 203.
573 IOTC Secretariat. (2019). *Report of the 21st session of the IOTC Working Party on Tropical Tunas*. *IOTC-*
574 *2019-WPTT21-R*, 142.
575 Jääskeläinen, E., Jakobsen, L. M. A., Hultman, J., Eggers, N., Bertram, H. C., & Björkroth, J. (2019).
576 Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and
577 salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage.
578 *International Journal of Food Microbiology*, *293*, 44–52.
579 https://doi.org/10.1016/j.ijfoodmicro.2018.12.021
580 Jung, Y., Lee, J., Kwon, J., Lee, K.-S., Ryu, D. H., & Hwang, G.-S. (2010). Discrimination of the Geographical
581 Origin of Beef by ^{1}H NMR-Based Metabolomics. *Journal of Agricultural and Food Chemistry*,
582 *58*(19), 10458–10466. https://doi.org/10.1021/jf102194t
583 Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E., & Toyama, K. (1984). Determination of fish freshness
584 with an enzyme sensor system. *Journal of Agricultural and Food Chemistry*, *32*(2), 314–319.
585 https://doi.org/10.1021/jf00122a034

Konosu, S., & Yamaguchi, K. (1982). The flavor components in fish and shellfish. In *Chemistry and biochemistry of marine food products* (R. E. Martin, G. J. Flick, C. E. Hebard, & D. R. Ward, pp. 367–404).

Martinez, I., Bathen, T., Standal, I. B., Halvorsen, J., Aursand, M., Gribbestad, I. S., & Axelson, D. E. (2005). Bioactive Compounds in Cod (*Gadus morhua*) Products and Suitability of ^{1}H NMR Metabolite Profiling for Classification of the Products Using Multivariate Data Analyses. *Journal of Agricultural and Food Chemistry*, *53*(17), 6889–6895. https://doi.org/10.1021/jf0507902

Medina, I., Sacchi, R., & Aubourg, S. (2000). Application of ^{13}C NMR to the selection of the thermal processing conditions of canned fatty fish. *European Food Research and Technology*, *210*(3), 176–178. https://doi.org/10.1007/pl00005508

Nielsen, E. E., Cariani, A., Aoidh, E. M., Maes, G. E., Milano, I., Ogden, R., Taylor, M., Hemmer-Hansen, J., Babbucci, M., Bargelloni, L., Bekkevold, D., Diopere, E., Grenfell, L., Helyar, S., Limborg, M. T., Martinsohn, J. T., McEwing, R., Panitz, F., Patarnello, T., … Carvalho, G. R. (2012). Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature Communications*, *3*(1), 851. https://doi.org/10.1038/ncomms1845

Pecoraro, C., Zudaire, I., Bodin, N., Murua, H., Taconet, P., Díaz-Jaimes, P., Cariani, A., Tinti, F., & Chassot, E. (2016). Putting all the pieces together: Integrating current knowledge of the biology, ecology, fisheries status, stock structure and management of yellowfin tuna (*Thunnus albacares*). *Reviews in Fish Biology and Fisheries*, 1–31. https://doi.org/10.1007/s11160-016-9460-z

Picone, G., Balling Engelsen, S., Savorani, F., Testi, S., Badiani, A., & Capozzi, F. (2011). Metabolomics as a Powerful Tool for Molecular Quality Assessment of the Fish *Sparus aurata*. *Nutrients*, *3*(2), 212–227. https://doi.org/10.3390/nu3020212

Piñeiro, C., Barros-Velázquez, J., Vázquez, & Figueras, A. (2003). Proteomics as a Tool for the Investigation of Seafood and Other Marine Products. *Journal of Proteome Research*, *2*(2), 127–135. https://doi.org/10.1021/pr0200083

Refsgaard, H. H. F., Brockhoff, P. M. B., & Jensen, B. (2000). Free Polyunsaturated Fatty Acids Cause Taste Deterioration of Salmon during Frozen Storage. *Journal of Agricultural and Food Chemistry*, *48*(8), 3280–3285. https://doi.org/10.1021/jf000021c

Ruiz-Capillas, C., & Moral, A. (2001). Changes in free amino acids during chilled storage of hake (*Merluccius merluccius* L.) in controlled atmospheres and their use as a quality control index. *European Food Research and Technology*, *212*(3), 302–307. https://doi.org/10.1007/s002170000232

Sacchi, Raffaele., Medina, Isabel., Aubourg, S. P., Giudicianni, Italo., Paolillo, Livio., & Addeo, Francesco. (1993). Quantitative high-resolution carbon-13 NMR analysis of lipids extracted from the white

muscle of Atlantic tuna (*Thunnus alalunga*). *Journal of Agricultural and Food Chemistry*, *41*(8), 1247–1253. https://doi.org/10.1021/jf00032a016

Samuelsson, L. M., & Larsson, D. G. J. (2008). Contributions from metabolomics to fish research. *Molecular BioSystems*, *4*(10), 974–979. https://doi.org/10.1039/B804196B

Sardenne, F., Bodin, N., Chassot, E., Amiel, A., Fouché, E., Degroote, M., Hollanda, S. J., Pethybridge, H., Lebreton, B., Guillou, G., & Ménard, F. (2016a). Trophic niches of sympatric tropical tuna in the Western Indian Ocean inferred by stable isotopes and neutral fatty acids. *Progress in Oceanography*, *146*, 75–88. https://doi.org/10.1016/j.pocean.2016.06.001

Sardenne, F., Chassot, E., Fouché, E., Ménard, F., Lucas, V., & Bodin, N. (2016b). Are condition factors powerful proxies of energy content in wild tropical tunas? *Ecological Indicators*, *71*, 467–476. https://doi.org/10.1016/j.ecolind.2016.06.031

Sardenne, F., Kraffe, E., Amiel, A., Fouché, E., Debrauwer, L., Ménard, F., & Bodin, N. (2017). Biological and environmental influence on tissue fatty acid compositions in wild tropical tunas. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *204*, 17–27. https://doi.org/10.1016/j.cbpa.2016.11.007

Scano, P., Rosa, A., Locci, E., Manzo, G., & Dessì, M. A. (2012). Modifications of the ^{1}H NMR metabolite profile of processed mullet (*Mugil cephalus*) roes under different storage conditions. *Magnetic Resonance in Chemistry*, *50*(6), 436–442. https://doi.org/10.1002/mrc.3819

Van Waarde, A. (1988). Biochemistry of non-protein nitrogenous compounds in fish including the use of amino acids for anaerobic energy production. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *91*(2), 207–228. https://doi.org/10.1016/0305-0491(88)90136-8

Watson, R., & Pauly, D. (2001). Systematic distortions in world fisheries catch trends. *Nature*, *414*(6863), 534–536. https://doi.org/10.1038/35107050

Wei, F., Fukuchi, M., Ito, K., Sakata, K., Asakura, T., Date, Y., & Kikuchi, J. (2020). Large-Scale Evaluation of Major Soluble Macromolecular Components of Fish Muscle from a Conventional ^{1}H-NMR Spectral Database. *Molecules*, *25*(8), 1966. https://doi.org/10.3390/molecules25081966