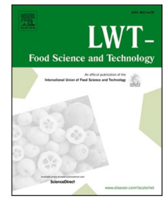
LWT - Food Science and Technology 139 (2021) 110603

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Short communication

Application of DNA mini-barcoding and infrared spectroscopy for the authentication of the Italian product "*bottarga*"

Jessica Frigerio [a,b], Claudio Marchesi [c], Chiara Magoni [b], Francesco Saliu [d,*], Davide Ballabio [d], Viviana Consonni [d], Tommaso Gorini [a], Fabrizio De Mattia [a], Paolo Galli [d], Massimo Labra [b]

[a] *FEM2-Ambiente, Piazza Della Scienza 2, I-20126 Milano, Italy*
[b] *Biotechnology and Bioscience Department, University of Milano-Bicocca, Piazza Della Scienza 2, 20126, Milano, Italy*
[c] *Department of Mechanical and Industrial Engineering, University of Brescia, Via Branze, 38 - 25123, Brescia, Italy*
[d] *Department of Earth and Environmental Science, University of Milano-Bicocca, Piazza Della Scienza 1, 20126, Milano, Italy*

ARTICLE INFO

Keywords:
Tuna
Food authentication
Biomolecular analysis
PCA
PLS-DA
Seafood

ABSTRACT

Tuna "Bottarga" is an artisanal food speciality prepared from the roe of Bluefin Tuna (*Thunnus* thynnus). Due to the recent restriction of Tuna catches fixed by EU, cheaper substitutes from yellowfin tuna (*Thunnus* albacares) are currently available in the mass distribution. Since the product is sold sliced or grated, morphological distinctive features may be lost, and fraudulent substitution is possible. In this study we tested DNA mini-barcoding and infrared spectroscopy as tools to trace its authenticity. A certified chemical standard, fresh (n = 4) and processed (n = 11) ovarian identified by the morphological observation of the tuna catches, commercial bottargasamples (n = 5) and artificial aged aliquots of all these samples (n = 50) were used in the experiments. The results showed that infrared spectroscopy failed in the taxonomic distinction, whereas enabled PLS-DA classification of aged samples (and thus the identification of fresh prepared artisanal products) with model non-error rate in fitting equal to 0.96, and 0.89 in cross-validation. Conversely, DNA mini-barcoding with mtDNA control region, success in species identification (100% maximum identity by the standard comparison BLAST approach against GenBank), regardless the processing state of the product.

1. Introduction

Nowadays the declaration of specific quality attributes to food products has a great interest. Due to the globalization of food markets and the resulting increase in variability and availability of foods from other countries, consumers are interested about the geographical origin and the assumed quality of the consumed products (Bonanno, 2019). Food authentication is the process that verifies that a food is in compliance with its label description. This may include the determination of the declared biological species, the geographical provenance, the production method and/or processing technology employed (Frigerio et al., 2020; van Ruth, Luning, Silvis, Yang; Huisman, 2018).

Under this view, European Union legislation displays a regulatory framework that links products to the defined geographical area where they are produced to recognize food quality and authenticity, by protecting the reputation of the regional foods and guaranteeing relevant prices (Regulation EU, 2012) . A variety of tools were created in order to protect quality and geographic products, i.e. PDO and PGI indications ('Protected Designation of Origin' 'Protected Geographical Indication'). Some Italian products that constitute an expression of Italian cultural heritage but are not included under those certifications are considered for the Italian agricultural products list (MIPAAF, 1999), that is approved and updated every year by the Italian Ministry of Agricultural, Food and Forestry Policies (Ministero delle Politiche Agricole,).

The salted and dried roe of tuna is a specialty appreciated in Italian cuisine under the name "Bottarga" and is listed as an Italian agricultural product (Saliu, Magoni, Lasagni, Della Pergola & Labra, 2019).

Most of the marketed "Bottarga" is obtained from yellowfin tuna (*Thunnus* albacares) specimens fished in Indian and Pacific ocean fishing zones (Di Natale; Addis, 2010), than processed in Italy and presented to the consumers in the form of a powdered "Bottarga di Tonno" (Tuna Bottarga). Gonads are therefore frozen after catching and thawed on-site before preparation.

Conversely, the artisanal product is prepared from Atlantic Bluefin tuna (*Thunnus* thynnus) and it is available only in local restaurants and markets near the italian fishing areas (Catarci, Simeone; Scarpato, 2007)

* Corresponding author.
E-mail address: francesco.saliu@unimib.it (F. Saliu).

https://doi.org/10.1016/j.lwt.2020.110603
Received 14 May 2020; Received in revised form 5 November 2020; Accepted 15 November 2020
Available online 21 November 2020
0023-6438/© 2020 Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

under the name "Bottarga di Tonno Rosso" (Bluefin tuna "Bottarga"). It is sold in slices at higher price than the yellowfin due to a combination of the assumed higher sensory/organoleptic quality (Scano et al., 2013) the recognized geographical origin and the employment of the traditional artisanal preparation which requires a skilled handling (Emery; Katherine, 2010). As powdered "Bottarga" is available in the market composed of different Tuna species and with different origin. The taxonomic identification starting from the morphological features is difficult, and consequently fraud is possible.

In addition, the European regulation (Regulation EU, 2013) declare that processed fish products (i.e. caviar) are excluded from the additional information requirements, i.e. FAO fishing area label, reducing the chances of the consumer to consume authentic bottarga products.

For the best of our knowledge, no analytical methods for the authentication of "Bottarga" are available and, specifically, the distinction of specialties prepared with bluefin tuna roe from the yellowfin substitutes is not described. Moreover, even if the labels report the Italian geographical provenance (claimed as added value), there are no completed studies regarding its organoleptic distinctiveness that helps producers to promote and protect the original "Bottarga".

Starting from this basis, in this study we evaluated the performance of DNA mini-barcoding and infrared spectroscopy in discriminating the product marketed as "Bottarga di Tonno Rosso" and we researched for the molecular features that are distinctively related to its "authenticity".

2. Materials and methods

2.1. Sampling

Fourteen sliced samples of roe from three tuna species and at three processing stage (fresh, frozen, dried), and six powdered samples purchased in local supermarkets were submitted to DNA barcoding analysis (Table 1).

Specifically, the sliced samples (from FEM_B_001 to FEM_B_014) were used as reference samples to validate DNA mini-barcoding analysis, after being morphologically identified at the species level by a veterinary surgeon of our group (RM) using identification key (ISSF). The powdered samples were used as test samples (blinds) instead, since no distinctive morphological feature enabling the verification of the label declaration was displayed.

2.2. Artificial aging

A total of 50 aged sample was obtained from aliquots of the fresh samples described in the previous section. Sixteen replicates were obtained by storing aliquots at room temperature (RT), fourteen by storing at 4 °C and two at −20 °C. Finally, eighteen samples were obtained by aliquots placed in a stove at 60 °C to simulate accelerated aging. Storage time (expressed in days) was normalized considering 1 day at 60 °C as 30 days at RT, 1 day at 4 °C as 0.1 day at RT, and 1 day at −20 °C as 0.03 day at RT (Michotte et al., 2011; Saliu et al., 2013). The list of samples with associated experimental storage conditions, days of storage, normalized days of storage is available (see Supplementary, S1).

2.3. DNA extractions, PCR conditions, DNA sequencing

Extraction of gDNA was obtained starting from 20 mg of roe sample by using DNA easy Blood; Tissue Kit (QIAGEN, Hilden, Germany). Purified gDNA was checked for concentration and purity by using NanoDrop™ One/OneC Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific™, Waltham, Massachusetts, US). The genomic locus selected was the mitochondrial control region (CR) using the couple of primer (i.e., 236 bp region) designed by Mitchell and Hellberg (Mitchell & Hellberg, 2016) A standard PCR amplification was performed using puReTaq Ready-To-Go PCR beads (GE Healthcare Life Sciences, Italy) by following the manufacturer instructions (Frigerio et al., 2019). PCR cycles consisted of an initial denaturation step for 5 min at 95 °C, followed by 35 cycles of denaturation (45 s at 95 °C), annealing (45 s at 49 °C) and extension (1 min at 72 °C), and, hence, a final extension at 72 °C for 7 min. Amplicon occurrence was assessed by electrophoresis on agarose gel using 1.5% agarose TAE gel and amplicon length was measured by comparison against 100bp ladder. Purified amplicons were bidirectionally sequenced using an ABI 3730XL automated sequencing machine (Applied Biosystems, ThermoFisher Scientific, Milano, Italy). The 3′ and 5′ terminal portions of each sequence were clipped to generate consensus sequences for each sample. After manual editing, primer removal and pairwise alignment, the sequences obtained were subjected to an individual Basic Local Alignment Search Tool (BLAST) in GenBank to verify their taxonomic identity.

Table 1
List of reference samples submitted to analysis. In the table are indicated ID specimen, morphological identified species, DNA barcoding identified species, genomic DNA (ng/μl), percentage of identity, sample typology, processing stage and collection site.

ID specimen	Morphological Identified Species	DNA barcoding Identified Species	gDNA (ng/μl)	Percentage identity	Sample typology	Processing stage	Collection site
FEM_B_001	*Thunnus thynnus*	*Thunnus thynnus*	40.2	100%	Reference	Dried	Sardinia (Italy)
FEM_B_002	*Thunnus thynnus*	*Thunnus thynnus*	38.5	100%	Reference	Dried	Sardinia (Italy)
FEM_B_003	*Thunnus thynnus*	*Thunnus thynnus*	39.6	100%	Reference	Dried	Sardinia (Italy)
FEM_B_004	*Thunnus thynnus*	*Thunnus thynnus*	35.4	100%	Reference	Fresh	Sardinia (Italy)
FEM_B_005	*Thunnus thynnus*	*Thunnus thynnus*	30.7	100%	Reference	Fresh	Sardinia (Italy)
FEM_B_006	*Thunnus thynnus*	*Thunnus thynnus*	33.5	100%	Reference	Fresh	Sardinia (Italy)
FEM_B_007	*Thunnus thynnus*	*Thunnus thynnus*	35.7	100%	Reference	Frozen	Sardinia (Italy)
FEM_B_008	*Thunnus thynnus*	*Thunnus thynnus*	29.7	100%	Reference	Frozen	Sardinia (Italy)
FEM_B_009	*Thunnus thynnus*	*Thunnus thynnus*	30.6	100%	Reference	Frozen	Sardinia (Italy)
FEM_B_010	*Thunnus alalunga*	*Thunnus alalunga*	31.1	100%	Reference	Fresh	Sicily (Italy)
FEM_B_011	*Thunnus albacares*	*Thunnus albacares*	29.5	100%	Reference	Frozen	Maldives
FEM_B_012	*Thunnus albacares*	*Thunnus albacares*	40.3	100%	Reference	Frozen	Maldives
FEM_B_013	*Thunnus albacares*	*Thunnus albacares*	31.4	100%	Reference	Frozen	Maldives
FEM_B_014	*Thunnus albacares*	*Thunnus albacares*	28.6	100%	Reference	Frozen	Maldives

2.4. ATR/FTIR analysis

ATR/FTIR analysis was carried out with a Nicolet In10 instrument (Thermo Scientific, Milano, Italy) equipped with a DTGS detector. Each spectrum was recorded in triplicate at 128 scans at 4 cm-1 of resolution in the range of 4000–700 cm-1. Spectra were examined and manipulated with OMNIC software (ThermoFisher Scientific, Milano, Italy).

2.5. Multivariate statistical analysis on ATR/FTIR spectra

Data processing and statistical analysis was performed with MATLAB (MATLAB R2018b, The MathWorks, Inc.: Natick, Massachusetts, United States). Spectral pre-treatments based on Standard Normal Variate and mean centering were used to normalize ATR/IR spectra (Fearn, Riccioli, Garrido-Varo; Guerrero-Ginel, 2009).

The exploratory data analysis was undertaken by means of the PCA toolbox for MATLAB (Eigenvector Research Inc., Manson, WA), while Partial Least Squares Discriminant Analysis (PLS-DA) by the classification toolbox as described in a previous paper (Ballabio, 2015).

3. Results and discussion

3.1. 1 DNA mini-barcoding

DNA mini-barcoding approach is based on the analysis of the variability within a standard region of the genome. An ideal DNA barcode requires two fundamental characteristics: high taxonomic coverage and high resolution (Hebert, Cywinska, Ball; deWaard, 2003). As a general principle, DNA barcode regions should have an high interspecific and low intraspecific variability. COI region is the standard barcode marker first presented by Hebert and colleagues usually used for animal taxonomy (Hebert, Cywinska, Ball; deWaard, 2003) but for tuna the mtDNA control region described by Vinas and Tudela (Viñas; Tudela, 2009) resulted to be the most useful marker to discriminate Thunnus species. Furthermore this marker was used successfully on canned tuna products, developing a DNA mini-barcoding system (i.e., 236 bp region) (Mitchell & Hellberg, 2016). Good DNA yield (20–40 ng/μl) was obtained from all the collected samples and all resulted in a 100% amplification success (Tables 1 and 2). This indicates that different typologies of samples (fresh, frozen and dried) are suitable for DNA mini-barcoding analysis. After that, the sequences obtained from the fourteen fresh samples (from FEM_B_001 to FEM_B_014, Table 1) were used as references to evaluate the reliability of DNA mini-barcoding identification by using the mtDNA control region. The species assignments were obtained by the application of a standard comparison approach against a GenBank database with BLASTn (Altschul, Gish, Miller, Myers, & Lipman, 1990). According to this approach, each barcode sequence was taxonomically assigned to the animal species with the nearest matches (maximum identity 99% and query coverage of 100%). All the reference samples returned 100% maximum identity (with 100% query coverage) notwithstanding the different processing status of the samples (frozen, fresh and dried). Results (Table 1) indicate that DNA mini-barcoding is a powerful tool for species identification even with aged and processed seafood samples. We then submitted the six commercial samples to the analysis (from FEM_B_015 to FEM_B_020, Table 2). Again, DNA mini-barcoding analysis returned 100% maximum identity, indicating no fraudulent misconduct in the product labelling.

3.2. ATR/FTIR analysis

As shown in literature, ATR-FTIR enables a fast non-destructive, sensible determination of the biomolecular profile of fish oil and tissues (Kaitaranta; Ackman, 1981; Saliu, Longhin, Salanti, Degano; Della Pergola, 2016; (Saliu et al., 2017)). However, since "Bottarga" is a processed product, the acquisition of quali-quantitative information may be a difficult task. Initially, for validation purposes, seven replicated spectra of Eicosapentaenoic acid ethyl ester from an USP reference standard (Sigma Aldrich, product number 1234307) were analyzed with the following results: 100% match with the library spectrum, S/N = 2′525, and CV = 1,3% (obtained from normalized spectra using the peak at 2′925 cm-1 as base for the normalization and considering the integrated area from tangential baseline for the carbonyl group at 1735 cm-1). Then, ATR/IR spectra were collected from all the "Bottarga" samples, which showed the typical peaks of lipid (Plans et al., 2015), specifically from the carbonyl of ester bond at 1′743 cm-1, and from C–H stretching of the related acyl chains at 3′009, 2′932, and 2′850 cm-1 (Fig. 1). In addition, peaks related to the protein fraction (Barth, 2007) were detected: two peaks at 3′338 and 3′297 cm-1 (NH stretching, namely amide A band), one peak at 1635 cm-1 (carbonyl stretching, amide I), one peak at 1′540 cm-1 (out-of-phase combination of the NH in plane bend and the CN stretching vibration, amide II) and various signals in the 1400-1200 cm-1 region (amide III mode). After that, PCA was carried out to explore data patterns and identify existing relationships between "Bottarga" biochemical composition encoded by the IR spectra and its observed properties: the shelf-life (expressed as normalized days of storage), the conservation condition and the declared geographical origin The results showed that the aging of "Bottarga" over induced the samples to converge towards similar biochemical profiles, while fresh samples conserved more biochemical diversity, which emerged from a higher spectral variability (in Fig. S2 fresh samples are more scattered indicating higher variability in composition). Fresh and aged samples were well differentiated along the first principal component (PC1), which explained most of the data variance (91%). On the contrary, no clear relationship was found out between the IR spectra and the storage condition applied (Fig. S4) or the geographical origin (Fig. S5).

To further investigate the relationship between the IR spectra and the shelf-life of Bottarga samples, a supervised pattern recognition PLS-DA model (Ballabio & Consonni, 2013; Prado-Fernández, Rodríguez-Vázquez, Tojo; Andrade, 2003) was calculated by using the shelf-life as the response, which was transformed into a categorical two-fold variable able to distinguish fresh and aged (Table S1): class 1 was assigned to the samples stored between 0 and 14 days (26 fresh samples), while class 2 to the samples stored between 120 and 930 days (23 degraded samples). More details about model calibration, validation and performance can be found in the supplementary materials (PLS-DA Section). Thanks to PLS-DA the spectral regions (and thus the related chemical features) more relevant for determining the shelf life of the samples were identified (Fig. 2). Specifically, the analysis of the score plot (Fig. 2a) showed that a good discrimination between degraded samples (positive scores) and fresh samples (negative scores) was

Table 2
List of blind samples submitted to analysis. In the table are indicated ID specimen, declared species, DNA barcoding identified species, genomic DNA (ng/μl), percentage of identity, sample typology, processing stage and collection site.

ID specimen	Declared species	DNA barcoding Identified Species	gDNA (ng/μl)	Percentage identity	Sample typology	Processing stage	Collection site
FEM_B_015	*Thunnus thynnus*	*Thunnus thynnus*	20.5	100%	Blind	Dried	Italian market
FEM_B_016	*Thunnus albacares*	*Thunnus albacares*	22.7	100%	Blind	Dried	Italian market
FEM_B_017	*Thunnus albacares*	*Thunnus albacares*	25.9	100%	Blind	Dried	Italian market
FEM_B_018	*Thunnus albacares*	*Thunnus albacares*	26.7	100%	Blind	Dried	Italian market
FEM_B_019	*Thunnus albacares*	*Thunnus albacares*	26.1	100%	Blind	Dried	Italian market
FEM_B_020	*Thunnus albacares*	*Thunnus albacares*	25.7	100%	Blind	Dried	Italian market

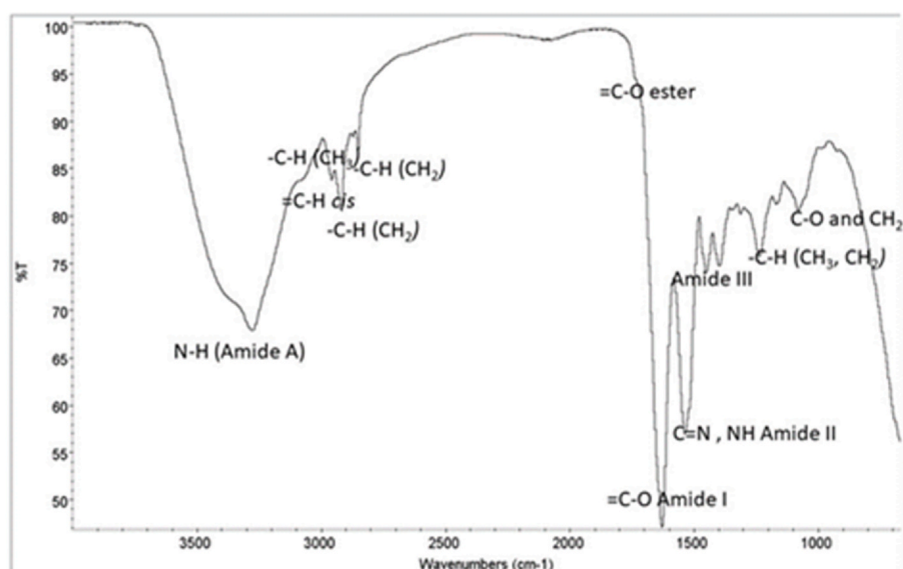


Fig. 1. A typical ATR/FTIR spectra obtained from bluefin "*Bottarga*" with tentative assignments of the main peaks, based on literature data (Barth, 2007; Plans, Wenstrup, & Rodriguez-Saona, 2015) and comparison with reference standard.

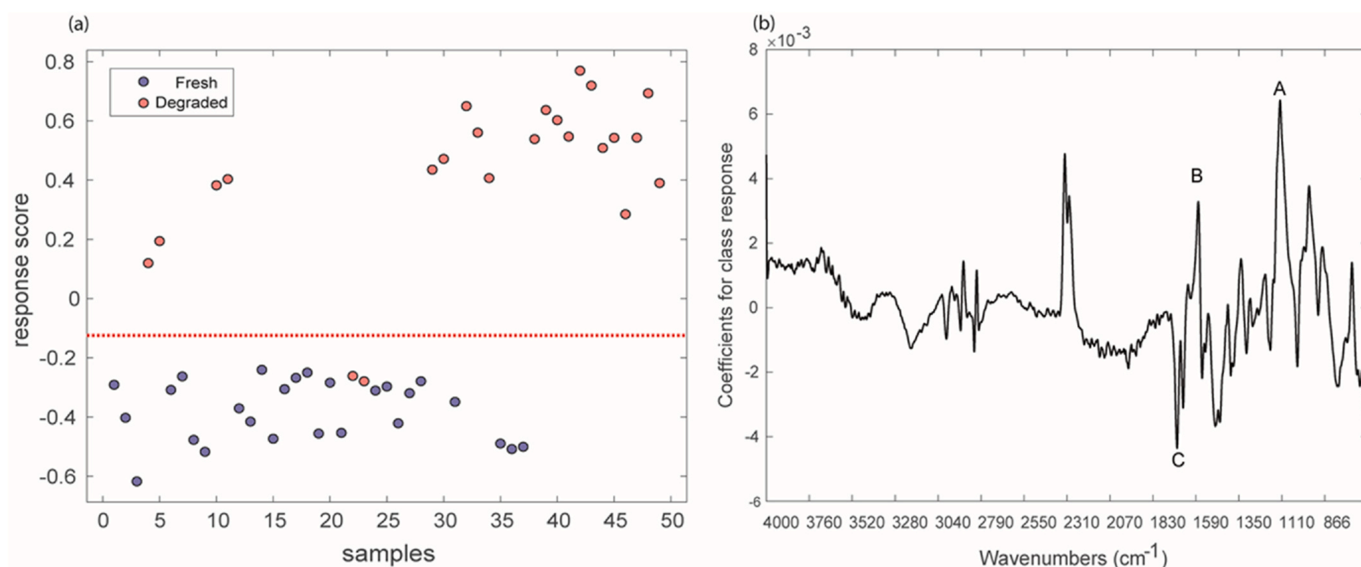


Fig. 2. Scores and loadings of PLS-DA model: (a) plot of the class response score*vs* sample ID; the dotted red line indicates the threshold value for the class assignment: samples with score higher than the threshold are classified as degraded, otherwise as fresh. (b) Plot of the loadings: the x-axis represents the variables (i.e., IR signals at different wavenumbers), while the y-axis indicates the loadings (i.e. model coefficients): variables with high positive coefficient are the most important for the classification of degraded samples. In fact, almost all of the degraded samples have high score (located in the upper region of the (a) plot). On the other hand, variables with low coefficient are the most important for the identification of fresh samples, which are located in the lower region of the score plot (a). The spectral windows, labelled as A, B, and C, are thus the most important for the classification of samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

achieved. This pattern was then interpreted by looking at the corresponding loading plot (Fig. 2b), where the loadings (i.e., the PLS-DA model coefficients) indicated the spectral windows that determined the discrimination between fresh and degraded samples. Specifically, the spectral windows A (1′050-1′150 cm-1), corresponding to the stretching of C–O bond in alcoholic compounds, and B (1600-1650 cm-1), corresponding to the stretching of C=O bond in carboxylic acids, were identified as the most relevant for the characterization of degraded samples. On the contrary, window C (1′735-1′750 cm- 1), corresponding to the stretching of C=O bond in esters, resulted to be useful to characterize fresh samples.

In summary, the depletion of polyunsaturated triglycerides, which can be observed in the 1050–950 cm −1 region related to = C–H out-of-plane bending (Plans et al., 2015), was the most consistent change that occurred during the processing and storage of Bottarga, together with the depletion of the signals related to sugars and free amino acids, probably due to Maillard reaction (Williams; Norris, 2001).

4. Conclusions

Twenty samples of tuna roe from three different tuna species (*Thunnus* thynnus, *Thunnus* albacares and *Thunnus* alalunga) at three different processing stages (fresh, frozen and dried) were submitted to DNA mini-barcoding and infrared analysis. Results showed that DNA

mini-barcoding performed well in the taxonomical authentication of the products, with 100% maximum identity on the reference samples regardless of the processing applied by the manufacturer. On the other hand, infrared spectroscopy failed in the distinction of species, but provided useful fingerprint related to the aging, enabling PLS-DA classification with model non-error rate (NER) in fitting equal to 0.96, and 0.89 in cross-validation. Since Bluefin Tuna "Bottarga" is freshly prepared on site, the sensitivity towards the bio-chemical composition changes induced by storage makes the infrared spectroscopy also a useful tool for its authentication. Overall, the study demonstrates how the integration of the two methods would allow the producers to define traceability and assess the quality of "Bottarga" products, with a simple and rapid procedure. Moreover, the chemical assignment of the bands described in this work and the previous knowledge of tuna roe composition provided an additional chemical support to FTIR spectroscopy for seafood application.

4.1. ANNEX supplementary data

Supplementary data to this article can be found online at https://www.editorialmanager.com/LWT/download.aspx?id=1301723&guid=d9194b31-79f3-44db-a3ad-3279d779.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: FEM2-Ambiente s.r.l., provided support in the form of a salary for authors J.F. and F.D.M. The company only provided financial support in the form of research materials.

Acknowledgements

We thank Andrea Feola for the information regarding the traditional bottarga preparation method.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110603.

Funding

The work was supported by University of Milano Bicocca - FAR 2014. This work was also partially supported by Regione Lombardia, grant E47F17000020009 in the framework of the Project "Food Social Sensor Network - Call Accordi per la Ricerca e l'Innovazione" co-funded by the Italian Government action "POR FESR 2014–2020".

Authors' contributions

Conceptualization: Francesco Saliu; Methodology: Jessica Frigerio, Tommaso Gorini, Davide Ballabio; Formal analysis and investigation: Jessica Frigerio, Tommaso Gorini; Funding acquisition: Fabrizio De Mattia; Supervision: Viviana Consonni, Massimo Labra; Writing – original draft: Francesco Saliu, Claudio Marchesi, Jessica Frigerio, Chiara Magoni; Writing – review & editing Jessica Frigerio, Chiara Magoni. Multivariate analysis (PCA & PLS-DA): Davide Ballabio, Claudio Marchesi. Sampling: Francesco Saliu, Paolo Galli. DNA barcoding analysis: Jessica Frigerio.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Ballabio, D. (2015). A MATLAB toolbox for Principal Component Analysis and unsupervised exploration of data structure. *Chemometrics and Intelligent Laboratory Systems, 149*, 1–9. https://doi.org/10.1016/j.chemolab.2015.10.003

Ballabio, D., & Consonni, V. (2013). Classification tools in chemistry. Part 1: Linear models. PLS-DA. *Analytical Methods, 5*, 3790–3798. https://doi.org/10.1039/c3ay40582f

Barth, A. (2007). Infrared spectroscopy of proteins. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1767*(9), 1073–1101. https://doi.org/10.1016/j.bbabio.2007.06.004

Bonanno, A. (2019). *Geographical indication and global agri-food: Development and democratization*.

Catarci, C., Scarpato, D., & Simeone, M. (2007). *Sostenibilità ambientale ed economia nel mercato del tonno rosso* (Vol. 547). FrancoAngeli.

Di Natale, A., & Addis, P. (2010). The first certification of bluefin tuna issued in the mediterranean sea: The ecocrest© label provided for the 2009 productions of two traditional tuna traps in sardinia. In *Sci. Pap. ICCAT* (Vol. 65). www.ecocrest.org.

Emery, K. (2010). Tonnare in Italy: Science, history and culture of Sardinian tuna fishing. *California Italian Studies, 378*(1), 1–40.

Fearn, T., Riccioli, C., Garrido-Varo, A., & Guerrero-Ginel, J. E. (2009). On the geometry of SNV and MSC. *Chemometrics and Intelligent Laboratory Systems, 96*(1), 22–26. https://doi.org/10.1016/j.chemolab.2008.11.006

Frigerio, J., Agostinetto, G., Sandionigi, A., Mezzasalma, V., Berterame, N. M., Casiraghi, M., et al. (2020). The hidden 'plant side' of insect novel foods: A DNA-based assessment. *Food Research International, 128*, Article 108751. https://doi.org/10.1016/j.foodres.2019.108751

Frigerio, J., Gorini, T., Galimberti, A., Bruni, I., Tommasi, N., Mezzasalma, V., et al. (2019). DNA barcoding to trace medicinal and aromatic plants from the field to the food supplement. *Journal of Applied Botany and Food Quality, 92*, 33–38. https://doi.org/10.5073/JABFQ.2019.092.005

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London - Series B: Biological Sciences, 270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Kaitaranta, J. K., & Ackman, R. G. (1981). Total lipids and lipid classes of fish roe. *Comparative Biochemistry and Physiology – Part B: Biochemistry And, 69*(4), 725–729. https://doi.org/10.1016/0305-0491(81)90376-X

Michotte, D., Rogez, H., Chirinos, R., Mignolet, E., Campos, D., & Larondelle, Y. (2011). Linseed oil stabilisation with pure natural phenolic compounds. *Food Chemistry, 129*(3), 1228–1231. https://doi.org/10.1016/j.foodchem.2011.05.108

Ministero delle Politiche Agricole, M.. www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/398.

Mitchell, J. K., & Hellberg, R. S. (2016). Use of the mitochondrial control region as a potential DNA mini-barcoding target for the identification of canned tuna species. *Food Analytical Methods, 9*(10), 2711–2720. https://doi.org/10.1007/s12161-016-0460-3

Plans, M., Wenstrup, M. J., & Rodriguez-Saona, L. E. (2015). Application of infrared spectroscopy for characterization of dietary omega-3 oil supplements. *Journal of the American Oil Chemists' Society, 92*(7), 957–966. https://doi.org/10.1007/s11746-015-2666-8

Prado-Fernández, J., Rodríguez-Vázquez, J. A., Tojo, E., & Andrade, J. M. (2003). Quantitation of κ-, ι- and λ-carrageenans by mid-infrared spectroscopy and PLS regression. *Analytica Chimica Acta, 480*(1), 23–37. https://doi.org/10.1016/S0003-2670(02)01592-1

Regulation EU. (2013). No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products. https://eur-lex.europa.eu/eli/reg/2013/1379/oj.

Regulation EU. (2012). No 11151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R1151&from=IT.

van Ruth, S. M., Luning, P. A., Silvis, I. C. J., Yang, Y., & Huisman, W. (2018). Differences in fraud vulnerability in various food supply chains and their tiers. *Food Control, 84*, 375–381. https://doi.org/10.1016/j.foodcont.2017.08.020

Saliu, F., Leoni, B., & Della Pergola, R. (2017). Lipid classes and fatty acids composition of the roe of wild Silurus glanis from subalpine freshwater. *Food Chemistry, 232*, 163–168. https://doi.org/10.1016/j.foodchem.2017.04.009

Saliu, F., Longhin, E., Salanti, A., Degano, I., & Della Pergola, R. (2016). Sphingoid esters from the molecular distillation of squid oil: A preliminary bioactivity determination. *Food Chemistry, 201*, 23–28. https://doi.org/10.1016/j.foodchem.2016.01.056

Saliu, F., Magoni, C., Lasagni, M., Della Pergola, R., & Labra, M. (2019). Multi-analytical characterization of perigonadal fat in bluefin tuna: From waste to marine lipid source. *Journal of the Science of Food and Agriculture, 99*(10), 4571–4579. https://doi.org/10.1002/jsfa.9696

Saliu, F., & Orlandi, M. (2013). In situ alcoholysis of triacylglycerols by application of switchable-polarity solvents. *A new derivatization procedure for the gas-chromatographic analysis of vegetable oils Analytical and bioanalytical chemistry, 405*(26), 8677–8684. https://doi.org/10.1007/s00216-013-7190-9

Scano, P., Rosa, A., Pisano, M. B., Piras, C., Cosentino, S., & Dessi', M. A. (2013). Lipid components and water soluble metabolites in salted and dried tuna (Thunnus thynnus L.) roes. *Food Chemistry, 138*(4), 2115–2121. https://doi.org/10.1016/j.foodchem.2012.11.095

Viñas, J., & Tudela, S. (2009). A validated methodology for genetic identification of tuna species (genus Thunnus). *PloS One, 4*(10), Article e7606. https://doi.org/10.1371/journal.pone.0007606