The origin of mussels (Mytilus galloprovincialis): NIRS explanatory identification and the effect on consumers

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ABSTRACT

This study aimed to explore the possibility of using Near-infrared spectroscopy (NIRS) for the authentication of mussels from six different geographical areas (Campania (South-Italy), Marche (Central-Italy), Veneto (North-Italy), Greece, Ireland and Spain). Secondly, the influence of mussels origin information on liking and willingness to buy was investigated on 130 consumers (from Campania region) by conducting an online consumer test, under blind and informed conditions.

NIRS-based classification method clearly identified six clusters corresponding to mussels with different origins. Also, consumers resulted strongly influenced by the origin information, preferring the familiar and local mussels compared to those from other geographic regions.

Comparing to methods based on chemical analysis, NIRS identification does not require reagents and is simpler, faster, more economical, and environmentally safer. Therefore, considering the importance of origin information perceived by consumers, NIRS could be a useful analytical method for mussels control to guarantee their authentication and traceability.

Introduction

Over the last five decades, world production of marine bivalves, particularly mussels (Mytilus galloprovincialis), has rapidly increased, reaching >17 million tonnes in 2020. (FAO, 2020). The increase in production has led to many food safety alerts mainly due to false labelling declarations (Galimberti, 2013). Consequently, to protect consumer interests and guarantee the food safety, authentication and traceability are critical (Li, Boyd, & Sun, 2016; Zhao et al., 2019). The EU Regulation, 2065/2001 regulates the traceability of sea products along the entire supply chain and the label information that must be provided to the consumer: the commercial name of the species, the origin and the production method (fished or farmed). Accordingly, traceability along the fish supply chain is a requirement shared by consumers, producers, and distributors.

However, despite the evolution of the regulatory framework for seafood traceability, there are still several issues related to the authenticity and traceability of the sea-food area. According to the studies conducted by Charlebois (2014), and later by Costa Leal, Pimentel, Ricardo, Rosa, and Calado (2015) seafood traceability mainly suffer from a lack of information on routine audits of traceability procedures.

Secondly, those studies highlighted the still need for cheap and easy-to-use methods to address the seafood origin. Currently, the available methods used in traceability systems are geochemical (trace element fingerprinting (TEF), Carson, 2013), biochemical (fatty acids composition, Kelly et al., 2012), and/or molecular (polymerase chain reaction (PCR) for DNA amplification (Galimberti, 2013) tools.

These techniques need to be performed by experts in the lab and cannot be applied at multiple trading locations throughout the supply chain. On the contrary, an ideal tool for tracing seafood products should be easy to perform, quick and cheap.

Near-infrared spectroscopy (NIRS) is perfectly in line with these characteristics. Indeed, as largely reported, NIRS has been used for food and beverage authentication showing to be particularly simple, cheap, versatile and also faster than many other techniques (Ottavian et al., 2012). In particular, the studies conducted by Novelli et al. (2009) and later by Ottavian et al. (2012) showed how NIRS successfully discriminated wild and farmed sea bass, with the same classification performance of the chemical and morphometric methods.

The geographical origin of marine bivalves is a cue of quality and needs to be guaranteed to protect both consumers and producers. However, to the best of our knowledge, the NIRS has not been applied to
mussels.

Moreover, the origin of food products influences consumer choice in different ways. First, representing a cue for other quality properties such as sensory characteristics, the origin drives the expected quality (Ver- legh & Van Ittersum, 2001). Secondly, the origin of a food product plays a pivotal role in determining consumer choices led by symbolic, affective or ethical reasons (Stefani, Romano, & Cavicchi, 2006). As a result, any reference to the origin of food conveys a sense of tradition and authenticity (Bell & Valentine, 1997).

The aim of this study was twofold. First, the possibility of using NIRS for the authentication of mussels from different geographical areas was explored. Secondly, the influence of mussels origin information on liking and willingness to buy was investigated on 130 consumers by conducting an online consumer test. Image analysis (length and colour parameters) was also conducted to physically characterise the mussels of different origins.

Materials and methods

Samples

Mussels were purchased from a local retailer which guaranteed the different provenance origins (detailed samples information are reported as supplementary data, in Table S1). Mussels from Campania (South-Italy), Marche (Central-Italy), Veneto (North-Italy), Greece, Ireland and Spain were collected for both the consumer test and NIRS analysis. Samples were eventually stored and refrigerated temperature (4 ± 1 °C) and used for analysis within 24 h of the collection time. The length (cm) of all the samples was measured with a millimetre calibre.

NIRS analysis

After cleaning and shelling, three aliquots of 15 individuals (approximately 15 g each) each were drained for 30 s on a filter paper and immediately placed in a 10 cm diameter Petri dish and scanned (10 times each) in reflectance mode at 4 cm-1 intervals from 4000 to 10000 cm-1 (NIRFLEX N-500, Buchi, Italy).

Image analysis

The image analysis was performed by a software-managed electronic eye (IRIS - Alpha M.O.S. Visual Analyzer VA400 – 500x, France). After the automatic calibration with the colour checker, samples were placed inside the capture chamber and pictures were taken. The software available in the instrument (Alphasoft, version 14.0) was used for the image (colour tones screening) and statistical analyses.

Consumer test

An online questionnaire was created using the Google Moduli platform and filled in by 130 consumers (females = 78; age average = 34 y. o.) from Campania region. The questionnaire was constituted of three sections, as presented in Table S2 of supplementary data. In the first section, a blind test was conducted, and consumers were asked to look at six pictures of fresh mussels with different origins (from Campania, Marche, Veneto, Greece, Ireland and Spain) identified with random three-digit codes. Willingness to buy (Yes/No answer) and expected liking (9-point Likert scale) were asked for each blind sample. In the second section, an informed test was conducted. Therefore, the procedure was the same (willingness to buy and global liking), but the samples were identified with their origin. In the last section, consumers were asked to indicate their gender, age, the consumption frequency of fresh mussels (responses were recorded on a 5-point category scale: never, 1 time/month, 2 time–3 times/month, 1 time/week, more) and the general liking for fresh mussels (9-point Likert scale: 1 = totally disliked; 9 = totally liked). Consumers who declared to not consume fresh mussels (“never” on the 5-point scale) or not like the fresh mussels were excluded from the dataset.

Participants signed written informed consent according to the principles of the Declaration of Helsinki and the ethical standards of the University of Naples Federico II. The privacy rights of human subjects were always observed and names and surnames were not collected. The extended content of the questionnaire is reported as supplementary data (S3).

Data analysis

Regarding the NIRS data, first, a comparison among all raw spectral data of mussels samples was performed by one-way analysis of variance (ANOVA). A mean spectrum, calculated by NIRFLEX N-500 software deriving from all spectra acquired for each sample of mussel, was used in a Principal Component Analysis (PCA) and the factor loadings of the first two components were extrapolated. Secondly, to reduce the light scattering and to remove the additional variation in base-line shift typically present in diffused reflectance spectra, mathematical pre-treatments were applied, as suggested by de la Roza-Delgado et al. (2007). The standard normal variate (SNV), first and second derivatives were calculated using Unscrambler X (version 10.4, CAMO) software to determine the best identification strategy. The models were evaluated considering the percentage of correctness extrapolated from the confusion matrix of the estimation sample and the cross-validation results.

PCA was run on pre-treated data and the new scores were then analysed by Discriminant Factor Analysis (DFA) using XLSTAT (version 2016.02, Addinsoft).

Finally, the predictive power of PCoA axes for the prediction of mussels origin was assessed using a supervised logistic regression for a 2-class classification model. The construction, parameter tuning, and validation of the model were performed using the R caret package (http s://CRAN.R-project.org/package=caret). The predictive model was used to determine the Campania or Spain origins based on the PCoA axes (from 1 to 7) as input data and the random forest as method for classification (the number of trees was set to 500 by default). Training input data was restricted to the samples with the status “Campania” (n = 6), or “Spain” (n = 2) representing 80 % of the cohort, while the test data was represented by “Campania” (n = 2, that is the 20 % of the cohort), randomly drawn. The global robustness of the model was evaluated through a Leave-One-Out Cross-Validation resampling method, while the performance was estimated on the test cohort with the area under the curve, sensitivity, and specificity.

To study the effect of origin on the willingness to buy, blind and informed data were analysed by Mann-Whitney (two-tailed) test. With the same aim, blind and informed liking data were analysed by t-test. XLSTAT (version 2016.02, Addinsoft) was used to analyse the consumer test data and the significance level was set at p < 0.05.

From the image analysis, 98 colour tones came out. Hierarchical clustering analysis was carried out to group the different tones in colour families, obtaining in this way 18 clusters (Alphasoft, version 14.0).

A Principal Component Analysis was run using the consumer data (willingness to buy and liking scores, in blind and informed conditions) as active variables. The 18 colour families and the length (cm) of the samples were used as supplementary variables.

Results and discussion

NIRS analysis

Fig. 1 shows the average spectra of the six examined mussels (Absorbance vs Wavelengths) and the distribution of the F-Fisher values extrapolated from the ANOVA model, in function of the explored wavelengths (nm). The F-Fisher distribution helps the reader to identify the wavelength ranges where the spectra are significantly different from each other. Below the dotted baseline (F = 2.261) the spectra are not
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Fig. 1. Average NIR spectra of mussels from six different origins (Campingia; Greece; Venice; Ireland; Spain; Marche). The dotted straight line (baseline) is the level of significance (p ≤ 0.05) for the Fisher value (F) obtained in the ANOVA. Below the dotted baseline, the F values do not show significant differences among spectra (p > 0.05).

significantly different (p > 0.05).

Considering the untreated NIR spectra, the main statistical differences (p ≤ 0.05) between the samples were observed between 1000 and 2000 nm. As it can be seen from Fig. 1, the dominating absorption bands of mussels' spectra were at 1933 nm 1 and 1454 nm, and at 1792 nm. According to the study conducted by Zhao, Chen, Huang, and Fang (2006), these bands could be mainly attributable to the 2nd and 1st overtone and the combination absorption band, of free and trapped water, respectively. This is in agreement with the high water content of these samples (p > 0.05).

Moreover, as can be observed in Fig. 2, the wavenumbers of 1160 nm and 1360 nm resulted in the highest contributions (loadings) on PC1. These signals correspond to the band at 1164 nm and 1369 nm in mussels' spectra ascribable to methyl, methylene, and methoxy absorptions. This outcome suggests that the differences in alkyl chains mainly drive the discriminating property of mussels' spectra on PC1. Considering the PC2, the most significant loadings were observed at 1454 nm and 1792 nm frequencies, corresponding to the OH signals of water and polysaccharides on mussels' spectra.

Since mussels are filter-feeders whose diet consists mainly of phytoplankton (Alkanani, Parrish, Thompson, & McKenzie, 2007) to acquire proteins, lipids, carbohydrates, and other components, the chemical composition of mussels is primarily dependent on the aquatic environment quality (Orban et al., 2002). Moreover, the meat yield, chemical composition, and lipid profile of mussels are strongly influenced by water temperature and food availability. Consequently, different production sites, and especially different origins, with different conditions, promote changes in the growth and nutritional composition of mussels (Olivera et al., 2015). In particular, several studies demonstrated the influence of the different environmental and nutritional conditions on the composition of mussels (Astorga-España, Rodríguez-Rodríguez, & Díaz-Romero, 2007; Khan, Parrish, & Shahidi, 2006), especially including the free amino-acid profile, the fatty acid composition or the volatile fraction (McLean & Bulling, 2005; Fuentes, Fernández-Segovia, Escriche, & Serra, 2009). Also, the identified differences could be related to different aromatic profiles. Some aromatic compounds identified in mussels could be due to petroleum contamination since the uptake of aromatic hydrocarbons has previously been reported in the study of Ruiz et al. (2011). Accordingly, different areas may have different contamination levels as reported by Fuentes et al., 2009. Another main factor influencing the biochemical composition of mussels is the water temperature. Indeed, Ventrella et al. (2008) and later Fernández et al. (2015) concluded that the seasonal variations observed for the biochemical parameters studied (especially for glycogen, proteins and fatty acids) were closely linked to the water temperature. In particular, the highest accumulation of glycogen was observed during spring and summer, when the temperature is warmer, while a depletion was mainly observed during autumn and winter. Therefore, the differences noted in the present study could be also due to the differences in temperature of the different origin sites.

Furthermore, as explained above, mathematical pre-treatments were applied to spectra data to remove the additional variation in the baseline shift typical of the reflectance spectra. Table 1 shows the confusion matrix for the estimation sample and the cross-validation results, considering the standard normal variate (SNV), first and second derivatives pre-treatments.

A confusion matrix was generated to summarize and quickly examine the % of well-reclassified observations, corresponding to the ratio of the number of observations well-reclassified over the total number of observations. Simultaneously, the resulting cross-validation was also examined to verify what would happen if a certain observation was excluded from the estimation sample. As can be observed in Table 1, SNV and first derivative pre-treatments produced the highest percentages of estimation (82.65 % and 83.63 %, respectively) and cross-validation (75.22 % and 77.04 %, respectively).

Fig. 3 shows the score plot from DFA (run on the first-derivate pre-treated data) representing the observations on the factor axes. The total explained variance is equal to 91.30 %. This high value could suggest

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Fig. 2. Loadings line plots (PC1 and –PC2) of raw NIR spectra.
and frozen-thawed swordfish (Fasolato et al., 2012) successfully identified (81.1% of accuracy) fresh mussels from different geographical origin, but weight, dimensions, and spatial-environmental factors (Brunner, Eugster, Trenka, Bergamin-Strotz, & Blust, 2007) are also influenced by the multi-elemental composition of mussel shells assessed by inductively-coupled plasma mass spectrometry. In turn, bioaccumulation of trace elements in mussels is influenced not only by several biological characteristics (such as sex, size, sexual maturity, reproduction stages, etc.) but also by environmental factors (including pH, temperature, salinity, dissolved oxygen, organic carbon, etc.) (Fattorini et al., 2008; Mubiana & Blust, 2007). Therefore, the levels of trace elements in mussels can be used to establish the geographical differences (Fattorini et al., 2008).

Considering all the influencing factors on the multi-elemental composition, Zhao et al. (2019) used the neodymium isotopic ratio (143Nd/144Nd ratios) to assess the geographical traceability of mussels collected in Japanese and Chinese coastal waters. They successfully discriminated the mussels in function of their origin, observing that the Nd distribution and variability perfectly followed the geological characteristics of the watersheds.

Both the multi-elemental composition and the radiogenic isotopic ratios of the heavy elements technique resulted in successful and reliable geographical traceability of mussels. However, these techniques require expert knowledge and lab work time resulting inefficient for on-line applications. On the contrary, NIRS-based identification methods do not need to be necessarily performed by experts, being simpler, faster and also more economical, and environmentally safer. Therefore, NIRS could be an easy and fast method to guarantee the consumers’ quality and traceability, protecting the consumers and producers’ interests.

### Consumer test

Consumers were asked to evaluate their willingness to buy six samples of mussels first in blind condition and then with the origin information. Fig. 4 shows the frequencies (%) of positive and negative answers given by 130 consumers from Campania region.

As expected and according to the literature (Felzensztein, Hibbert, & Vong, 2004; Di Monaco, Di Marzo, Cavella, & Masi, 2005), going from blind to informed evaluation, the number of negative answers increased for all the evaluated samples, except for the Campania ones. The willingness to buy resulted significantly affected by origin information only for the samples from Campania, Ireland and Spain (p ≤ 0.05). In particular, by considering samples from Ireland, when they were evaluated under blind conditions, 63% of consumers would buy the represented mussels; when consumers were provided with the origin information, the percentage of positive answers significantly decreases from 63% to 42%. Also, considering samples from Spain, 77% of consumers would buy the represented mussels in blind conditions; when consumers were provided with the origin information, the percentage of positive answers significantly decreased to 65%. On the contrary, positive answers significantly increased (from 54 to 73%) for the mussels from Campania region.

Consumers were also asked to score the expected liking of the six samples, first under blind conditions and then with the origin information.
information.

No significant differences in terms of expected liking were observed for all the evaluated samples, except for mussels from Campania region. In that case, the average expected liking score significantly increases when consumers are informed about the mussels’ origin (blind = 4.3 ± 0.2 vS info = 5.3 ± 0.2).

The present results clearly show that signalling of origin influences the willingness to buy and expressed liking, demonstrating that local products are preferred over the same products from elsewhere. These outcomes are in line with the literature which reports how liking is driven by expectations. Indeed, the influence of consumers’ origin is enhanced by the fact that belonging to a specific geographical area generates such positive feelings towards food from that same area (van der Lans et al., 2001).

As well known, food liking and choice are dynamic phenomena influenced by many interacting factors (with physiological, psychological, socio-cultural and economic meanings) related to both the consumers and food products (Hirschman, Carscadden, Fleischauer, Hasak, & Mitchell, 2004). Food origin plays a pivotal role in consumers’ decision, influencing the individual attitudes, the willingness to buy and finally the choice (Van Ittersum, Candel, & Meulenberg, 2003). In the study conducted by Dransfield et al. (2005), the involved subjects declared to prefer pork labelled as raised in their own country compared to imports. Similarly, in the Swedish study conducted by Ekelund, Fernqvist, and Tjärnemo (2007), consumers significantly preferred tomatoes labelled “native” compared to imports (Ekelund et al., 2007). Also, origin information showed similar effects in the studies conducted by Resano, Sanjuán, and Albisu (2007) and Iaccarino, Di Monaco, Mincione, Cavella, and Masi (2006), in which local cured ham and local salami, respectively, resulted significantly preferred compared to imports.

However, food preferences are also moved by other food characteristics, such as the aspect which may lead consumers to anticipate differences in product properties and the consumers’ purchase probability (Spence, Levitan, Shankar, & Zampini, 2010; Schifferstein, Wehrle, & Carbon, 2019). Food acceptability, intended as perceived sensory quality and preference, is affected by colour (Fernández-Vázquez, Stinco, Meléndez-Martínez, Heredia, & Vicario, 2011).

Fig. 4. Frequencies (%) of positive (Yes) and negative (No) answers collected for blind and informed samples of six different mussels. *Significant differences (p ≤ 0.05).

Fig. 5. PCA of consumer test and physical data of six different mussels. Observations (samples); Active variables (consumer data) Supplementary variables (physical data).
In the present study, consumer test and physical (image analysis and length) data were analyzed by Principal Component Analysis (PCA). Fig. 5 shows the resulting biplot obtained from PCA, including both observations (samples) and variables (active and supplementary), on the first two dimensions accounting for the 94.47 % of the variance.

As it can be observed, the six different samples were characterised by different shadows of colours whose chromatic codes are reported on the graph. Secondly, mussels from Spain were mainly characterised by length and resulted in the most preferred samples when evaluated under blind conditions. On the other hand, if evaluated together with the origin information, mussels from Campania region resulted more associated with willingness to buy.

Therefore, colour features and size influence the liking and willingness to buy mussels, especially if evaluated in blind conditions. Indeed, liking and willingness to buy in blind conditions resulted positively correlated with mussels from Spain. It is worth noting that mussels from Spain are very common in the Campania region market, therefore the specific colour variety is reasonably familiar to consumers, leading to certainty about what qualities experiences to expect.

Strengths and limitations

The strength of this study refers to the practical characteristics of the NIR spectroscopy. This technique is indeed low-cost, non-destructive and not time-consuming. However, our findings should be interpreted under the limitations of this research. Indeed, the study was somewhat limited by the restricted time of analysis. Mussels were collected and analysed for a limited period of the year. Considering that this limit might impact the validity of NIRS prediction, these specific results should not be generalised to the yearly population. Nevertheless, the manuscript may offer to the readers a procedure that can be easily repeated and validated with a higher number of samples from different origins and seasonability.

Conclusions

The present study aimed to investigate the possibility of using NIRS for the authentication of mussels from different geographical areas and highlight the importance of the origin information felt by consumers living in Campania region (Italy).

NIR spectroscopy perfectly allows to identify mussels from different origins. This technique resulted in easy-to-perform, reliable and mostly, not time-consuming. NIRS could be used for on-line applications along the supply chain, which could help the monitoring and control systems. Consumers resulted influenced by the origin information both in the willingness to buy and global liking. Therefore, the origin guarantee is a pivotal need for consumers and needs to be a just as pivotal objective for producers, retailers and, ultimately, authorities.

Institutional Review Board Statement.

The study was conducted in agreement with the guidelines of the Declaration of Helsinki and the Italian ethical requirements on research activities and personal data protection (D.L. 30.6.03n. 196). Informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Sharon Puleo: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. Rossella Di Monaco: Conceptualization, Methodology, Writing – review & editing, Supervision. Antonio Luca Langelotti: Resources, Supervision, Project administration. Paolo Masi: Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfoodch.2022.100497.

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