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Investigation of production method, geographical origin and species authentication in commercially relevant shrimps using stable isotope ratio and/or multi-element analyses combined with chemometrics: An exploratory analysis

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A B S T R A C T

Three factors defining the traceability of a food product are production method (wild or farmed), geographical origin and biological species, which have to be checked and guaranteed, not only in order to avoid mislabelling and commercial fraud, but also to address food safety issues and to comply with legal regulations. The aim of this study was to determine whether these three factors could be differentiated in shrimps using stable isotope ratio analysis of carbon and nitrogen and/or multi-element composition. Different multivariate statistics methods were applied to different data subsets in order to evaluate their performance in terms of classification or predictive ability. Although the success rates varied depending on the dataset used, the combination of both techniques allowed the correct classification of 100% of the samples according to their actual origin and method of production, and 93.5% according to biological species. Even though further studies including a larger number of samples in each group are needed in order to validate these findings, we can conclude that these methodologies should be considered for studies regarding seafood product authenticity.

1. Introduction

Fishery products, especially Decapoda crustaceans, are among the most relevant food commodities in terms of trading figures. Among them, marine shrimps and prawns account for about 15% of the total value of internationally traded fishery products (FAO Fisheries & Aquaculture Department, 2012) and are important economic resources for many countries. In addition to catches in different areas—mainly the Food and Agriculture Organization of the United Nations (FAO) fishing areas 51 (Western Indian ocean) and 71 (Western Central Pacific ocean)—shrimps and prawns are increasingly being produced in aquaculture facilities, especially in some areas of China, Southeast Asia and Ecuador.

Due to the increasing demand for foodstuffs in general, and for high-quality products in particular, and also due to the globalisation of markets and trade, adulteration can occur through the food chain (Pascoal, Barros-Velázquez, Cepeda, Gallardo, & Calo-Mata, 2008a). A product can be deliberately substituted with a lower quality and cheaper counterpart, or an unintentional error can cause an inadvertent mislabelling of products, leading, in both cases, to commercial fraud that affects both the food industry and the consumers. Adulteration of food products not only has economic implications, but also represents a potential public health risk (Spink & Moyer, 2011). Regulations have appeared all over the world in order to fight against adulteration and misbranding of foods (U.S. Food and Drug Administration, 2013), underlining the need for labelling fishery and aquaculture products with the scientific name of the species used, the production method (caught at sea or from aquaculture), and the place of origin (Council Regulation (EC) No 104/2000 of 17 December, 1999).

Among fish products in general, and crustacean species in particular, the substitution of an appreciated high quality species by another of lower quality and with a lower price is especially frequent (Pascoal et al., 2008a). In these cases, inadvertent or deliberate adulteration leads to mislabelling and commercial fraud. In addition, these adulteration practices can affect the marine conservation programs that protect overexploited species or populations (Rasmussen & Morrisey, 2008).

The place of origin of the components within a food product should also be checked and guaranteed. Consumers demand information about the geographic origin of the food they eat. In addition

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to the perception of higher quality products with geographical indications (Protected Designation of Origin, Protected Geographical Indication, Traditional Specialty Guaranteed), some other geographic-related factors affect consumer habits, and therefore the food industry, trade and regulations. These factors include the consumer's preference for foodstuffs they perceive as having a lower environmental footprint (e.g., local products), the perception of different health safety implications depending on the origin of the product, and even patriotism in the sense that some people feel they should buy food produced in their own country. Origin authenticity assessment is important not only because of the increasing demand for information from consumers and to prevent commercial fraud, but also, in order to ensure the safety of foodstuffs, traceability at all stages is compulsory for all food and feed industries (Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January, 2002). The relevance of traceability for food quality and safety has been clearly demonstrated after recent serious food crises, such as the 2013 scandal of non-beef that was labelled beef mincemeat in Europe (Quinn, 2013); the 2011 *Escherichia coli* outbreak in Germany (Buchhholz et al., 2011); the recurring bird flu events in China (Larson, 2013) and the epidemic of bovine spongiform encephalopathy in the UK (Will et al., 1996). It must be pointed out that seafood in particular is exposed to a wide spectrum of contaminants, such as pathogenic microorganisms, heavy metals and chemical pollutants, and therefore the authentication of its geographical origin is of capital importance, especially when a contaminated product from a particular area must be withdrawn from the market.

Together with the species and the geographical origin, another important factor defining traceability is the production method (e.g., wild or farmed; organic or intensive) (Moretti, Turchini, Bellagamba, & Caprino, 2003). During recent years, farmed fish production has greatly increased compared to wild fish capture. However, organoleptic characteristics, nutritional values and usually price are not the same for fish that is wild-caught and fish that is farmed. Furthermore, the quality of cultured fish depends on the diet they receive.

For all the reasons stated above, the authentication and origin of foodstuffs must be guaranteed, and accurate and reliable analytical methods are needed in order to verify the production method, geographical origin and species of the components used in a food product. Many different instrumental techniques have been proposed for food authentication (Drivelos & Georgiou, 2012): high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy, infrared spectroscopy (IR), capillary electrophoresis, and, more recently, DNA-based and proteomic methods (Ortea et al., 2012). Over the last decade, the isotope ratio and the composition of selected elements have provided measurements revealing a unique isotopic fingerprint for the sample being analysed, so stable isotope ratio and multi-element analysis have become two of the most frequently used techniques for assessing authenticity and traceability in food products (Drivelos & Georgiou, 2012).

The content and availability of elements in soils depends on different factors, such as pH, humidity and clay-humus complex (Kim & Thornton, 1993). The elemental composition of vegetation reflects the bio-available and mobilised nutrients present in the soils where plants are growing, which also determines the multi-element composition of the animals consuming that vegetation. Therefore, the elemental composition profile may be used as a unique marker for characterising the diet and geographical origin of food products (Kelly, Heaton, & Hoogewerff, 2005). Due to the differential distribution of C_3 and C_4 plants from the equator to the poles, and due to the fact that C_3 plants have lower $^{13}C/^{12}C$ ratios than C_4 plants, differences in $^{13}C/^{12}C$ ratios can be found in plant material according to geographical origin (Kelly et al., 2005). Differences in $^{15}N/^{14}N$ ratios are more related to local agricultural practices (Oulhote, Le Bot, Deguen, & Glorennec, 2011). Since carbon and nitrogen propagate from prey to predator through the trophic chain, $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios yield a history of feeding relationships. They are the two most informative parameters for analysing the diet of animals, and, therefore, they can be used as a proxy for determining geographical origin.

Stable isotope ratio (SIR) and multi-element analyses have been used extensively for authenticating wines, fruit juices, olive oil, honey, milk and dairy products, coffee, tea, cereal crops and meat (Drivelos & Georgiou, 2012; Gonzálvez, Armenta, & de la Guardia, 2009; Kelly et al. 2005; Vinci, Preti, Tieri, & Vieri, 2013), but have scarcely been used in fish and shellfish studies. In seafood species, most of these studies have applied SIR analysis to discriminate economically important wild and farmed fish species, such as Atlantic salmon (Dempson & Power, 2004), gilthead sea bream (Moreno-Rojas et al., 2007; Serrano, Blanes, & Orero, 2007), turbot (Busetto et al., 2008) and sea bass (Gordon Bell et al., 2007), and even to differentiate farmed trout fed with plant- or fish-protein-based diets (Moreno-Rojas, Tulli, Messina, Tibaldi, & Guillou, 2008). Few reports are available regarding the use of element profiling to establish the geographical origin of marine organisms. Smith and Watts (2009) determined the origin of farm-raised shrimp by trace metal profiling and multivariate statistics using a database on the composition of shrimp from different countries, including white leg shrimp (*Penaeus vannamei*) and black tiger shrimp (*Penaeus monodon*). Wild and farm-raised salmon were differentiated using elemental analysis and different classification modelling (Anderson, Hobbie, & Smith, 2010), and sea cucumber samples from three water environments of China were identified using multi-element analysis and chemometric techniques (Liu et al., 2012). Regarding species identification, the SIRs of carbon and nitrogen were recently used to differentiate the gadoid fish species Atlantic cod and saithe (Monteiro Oliveira, Sant'Ana, Ducatti, Denadai & de Souza Kruliski, 2011). Due to the large number of samples and variables usually measured in these profiling strategies, the application of the appropriate chemometric tools is essential to extract relevant information and establish patterns between different samples. Multivariate data analysis methods are highlighted as the most frequently used, especially principal component analysis (PCA) and discriminant analysis (Drivelos & Georgiou, 2012; Oulhote et al., 2011).

The aim of this study was to determine whether shrimp species, geographical origin and production method (wild vs. farmed) could be differentiated using either SIR analysis of carbon and nitrogen or multi-element composition (Pb, Cd, As, P, S) or a combination of both. Results obtained using different datasets for the element composition analysis (only Pb, Cd and As; only P and S; and the five elements together) were compared. To assess for the effect of different sample sizes when a relatively low number of samples are analysed by SIR, as it is done in many authenticity studies found in bibliography, we added a new set of specimens to the initial set and compared the results obtained ($n = 31$ vs $n = 45$).

At each of the three levels (species, origin and production method), different multivariate statistics methods were applied in order to evaluate their performance.

2. Materials and methods

2.1. Shrimp samples

Specimens analysed according to production method, geographical origin and species are shown in Table 1. They were collected using either extractive fishing procedures or from aquaculture farms from different geographical localisations worldwide. Shrimps, whole animals, were frozen on board and shipped to our