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Fatty acid dynamics of the adductor muscle of live cockles (*Cerastoderma edule*) during their shelf-life and its relevance for traceability of geographic origin

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ABSTRACT

Cockles (*Cerastoderma edule*) are commercially important bivalves that support several fisheries in European waters. The fatty acid (FA) profile of the adductor muscle (AM) of freshly collected live cockles can be used to reliably confirm their geographic origin. This approach is paramount for traceability, expose fraud and ensure food safety. However, no study has ever addressed if the FA profile of the AM of live cockles remains stable during shelf-life, as significant shifts may blur FA signatures recorded at harvest. The present study investigated the FA dynamics of the AM of live cockles during their shelf-life (seven days post-harvest under a refrigerated environment). Fatty acid methyl esters were obtained after transesterification of lipid extracts and analyzed through gas chromatography–mass spectrometry (GC–MS). Results indicated that FA profiles remained stable until the third day post-harvest. After this period cockles started to exhibit contrasting FA profiles on their AM, namely a higher percentage of heptadecanoic acid (17:0). This FA is associated with the growth of pathogenic microorganisms responsible for food spoilage. In this way, the FA profile of the AM of live cockles can be used to reliably trace geographic origin up to three days post-harvest, as long as specimens are stored under refrigerated conditions during shelf-life.

1. Introduction

Marine bivalves (e.g. oysters, clams, mussels and cockles) are a highly-valued source of minerals and vitamins (Karnjanapratum, Benjakul, Kishimura, & Tsai, 2013; Orban et al., 2007; Rittenschober, Nowak, & Charrondiere, 2013), being known to be low in fats and rich in omega 3 highly unsaturated fatty acids (HUFAs) that are beneficial to human immune and cardiovascular systems (Adebayo-Tayo, Odu, Anyamele, Igwiloh, & Okonko, 2012; Damsgaard et al., 2007; Sousa et al., 2016). However, these molluscan shellfish are filter feeders and, consequently, pathogenic microorganisms that occur in the environment can accumulate in their digestive tract/glands. As microbiologically contaminated bivalves may not always be properly depurated, or even be depurated at all, and are often consumed raw or lightly cooked, their ingestion can present a serious threat to human health. Indeed, the ingestion of microbiologically contaminated bivalves has already been shown to be associated with outbreaks of a number of diseases, namely typhoid fever, hepatitis A, severe gastroenteritis and cholera (Iwamoto, Ayers, Mahon, & Swerdlow, 2010; Lees, 2000; Potasman, Paz, & Odeh, 2002).

During handling and storage, contamination of bivalves by enteric bacteria of human origin may also occur (Oliveira, Cunha, Castilho, Romalde, & Pereira, 2011). In this way, these processes should be performed under controlled conditions to avoid contamination and growth of pathogenic microorganisms responsible for food spoilage (Emikpe, Adebisi, & Adedeji, 2011). Any change on the taste, smell, appearance or texture of bivalves can turn the product unacceptable and/or unsafe for the consumer (Costa, Conte, & Del Nobile, 2014). It is well known that the quality

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of live bivalves can be rapidly lost post-harvest, which ultimately conditions the shelf-life of these highly priced products (Lee, Lovatelli, & Ababouch, 2008). As live bivalves kept at ambient temperatures post-harvest often display a shorter shelf-life (<48 h), they are most commonly stored on ice or refrigerated environments (0–4 °C) to maintain their quality and safety for human consumption (Ashie, Smith, Simpson, & Haard, 1996; Rey, Miranda, Aubourg, & Barros-Velázquez, 2012). Under optimal storage conditions, live cockles maintain a fresh-like texture for approximately days post-harvesting (Ricardo et al., 2015). Nevertheless, storage at lower temperatures does not impair biochemical reactions (e.g. enzymatic autolysis, lipid oxidation) or microbial growth that can affect the level of freshness of live bivalves (Ashie et al., 1996). About 4–5 days post-harvest live bivalves start to become slimy, produce an off odor, display an increase in pH, loose their water holding capacity and display a sharp decrease in their organoleptic acceptability (Ashie et al., 1996; Parveen et al., 2008). Specific fatty acids (FA) (e.g. those with odd carbon numbers) are recorded in microorganisms and are often used as lipid markers for bacterial contamination (Budge, Parrish, & McKenzie, 2001; Gonçalves, Azeiteiro, Pardal, & De Troch, 2012; Harvey & Macko, 1997). Bacterial FA markers have been used not only to study microbial communities in clinical (Brevik, Veierød, Drevon, & Andersen, 2005) and environmental samples (Mahanty, Ranjan Maji, Ganguly, & Mohanty, 2015), but also as an indicator of the quality of bivalves during storage (Khan, Parrish, & Shahidi, 2005).

The FA profile of the adductor muscle (AM) of live bivalves has also been successfully used to trace the geographic origin of specimens collected in different production areas (Grahl-Nielsen, Jacobsen, Christophersen, & Magnesen, 2010; Olsen, Grahl-Nielsen, & Schander, 2009; Perez et al., 2013; Ricardo et al., 2015). This approach is highly relevant to improve seafood traceability in a globalized market with recurring food safety alerts and, consequently, with a growing consumer awareness level on the geographic origin of traded goods (Leal, Pimentel, Ricardo, Rosa, & Calado, 2015). However, it remains unknown whether this approach can still be used during the shelf-life of bivalves, as bacterial lipid markers and other catabolic pathways associated with spoilage may blur the initial FA signature of the AM displayed immediately after their harvest.

The present study aimed to determine if the FA profile of the AM of live cockles (*C. edule*) displays any significant shifts during the shelf-life of this commercially important species and how long post-harvest can these FA profiles be used to reliably trace the geographic origin of these commercially important bivalves.

2. Material and methods

2.1. Study areas and cockle collection

Fresh cockles *C. edule* ($n = 80$) were collected by hand-raking in Mira Channel (Ria de Aveiro, Portugal; 40°36′ 39.50″ N, 8°44′ 47.40″ W), one of the most important commercial fishing areas for this species in mainland Portugal. All samples collected were immediately stored in aseptic bags and transported to the laboratory within approximately 30 min post-harvest. Packs of ten cockles were placed in mesh-bags and kept in a cold room at 4 °C during seven consecutive days. From the 10 individuals of each mesh-bag were randomly sampled 5 individuals at times T0 (sampling day), T1 (one day post-harvest), T2, T3, T4, T5, T6 and T7 (seven days post-harvest) (1 sampling area X 8 time points X 5 replicates = 40 samples). The adductor muscle (AM) from each cockle specimen was dissected using a sterilized scalpel and stored at −80 °C until FA analysis. The rationale supporting the time frame of the present study (seven days post-harvest) was based upon a preliminary survey performed on five large retail surfaces trading live cockles that consider that the average shelf-life of live bivalves is of only 5 days. By employing a seven days' time frame, it could be expected that spoilage would occur and major shifts in the FA of the AM of live cockles could be recorded.

2.2. Fatty acids analysis

The extraction of total lipids of the AM of each individual cockle was performed according to the Bligh and Dyer (1959) method using methanol/chloroform (2:1, v/v). As phospholipids (PLs) are the main lipids present in the AM of bivalves (Napolitano, Pollero, Gayoso, Macdonald, & Thompson, 1997), PLs were estimated through the phosphorus assay (Bartlett & Lewis, 1970) and an amount of each lipid extract containing 15 µg of PLs was used in the preparation of fatty acid methyl esters (FAMEs) following the procedure described by Aued-Pimentel, Lago, Chaves, and Kumagai (2004). The resulting FAMEs were dissolved in n-hexane (30 µL) and 4 µL of this solution was analyzed by gas chromatography–mass spectrometry (GC–MS) on an Agilent Technologies 6890 N Network (Santa Clara, CA) equipped with a DB-FFAP column with 30 m of length, 0.25 mm of internal diameter, and 0.32 µm of film thickness (J&W Scientific, Folsom, CA). The GC equipment was connected to an Agilent 5973 Network Mass Selective Detector operating with an electron impact mode at 70 eV and scanning the range *m/z* 50–550 in a 1 s cycle in a full scan mode acquisition. The oven temperature was programmed from an initial temperature of 80 °C, with a linear increase to 220 °C at 14.4 °C min^{-1}, followed by

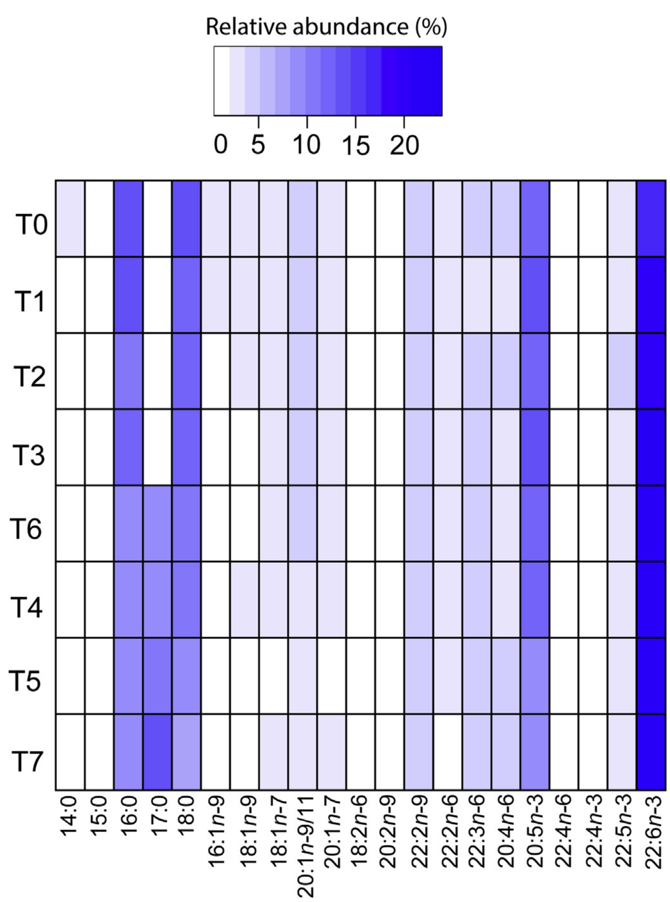

Fig. 1. Heatmap representing the relative abundances (%) of fatty acids (FAs) in the adductor muscle of live common cockles *Cerastoderma edule* during their shelf-life at 4 °C.