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Original Research Article

Composition of food grade Atlantic salts regarding triacylglycerides, polysaccharides and protein

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ABSTRACT

The present work investigates, for the first time, the presence of triacylglycerides, polysaccharides, and protein of 16 food grade sea salts. Triacylglycerides, obtained by Soxhlet extraction with *n*-hexane, and representing a median content of 1.5 mg/kg of dry salt, were mainly composed by palmitic (42.8 mol%), stearic (13.3), linolenic (12.5), oleic (12.0), and linoleic acid (9.1) residues. Also, a dialysis-based methodology was developed to isolate the polymeric material from sea salt in amounts to allow its characterisation. The polymeric material accounted for 144 mg/kg of dry salt, mainly composed by sulfated polysaccharides and also containing protein. Polysaccharides were rich in uronic acid residues (21 mol%), glucose (18), galactose (15), and fucose (13), whereas the protein was composed by the hydrophobic amino acids alanine (25 mol%), leucine (14), and valine (14). These biomolecules arise from the surrounding environment of saltpans. Their characterisation contributes to the pursuit of parameters of origin.

1. Introduction

Sodium chloride is largely used in food industry for conservation as well as to confer pleasant sensory properties to foods. Sea salt is the main source of sodium chloride for use in food. It is a natural product obtained by evaporation of seawater in saltpans. Saltpans are man-made systems where seawater gives rise to sea salt due to the combined effect of wind and sunlight. Essential for life in small quantities, the salt is one of the oldest, most ubiquitous, food seasonings.

Besides the well-known composition of sea salt regarding its inorganic elements (Galvis-Sánchez et al., 2013), nothing was known about the organic components of sea salt until Silva et al. (2009) revealed the presence of volatile organic compounds in this natural product. This and following studies regarding the presence of volatile compounds in sea salt concluded that they should arise from three main sources: algae (Donadio et al., 2011; Silva et al., 2010a,b) surrounding bacterial community (Donadio et al., 2011; Silva et al., 2010a,b), and anthropogenic activity (Serrano et al., 2011; Silva et al., 2010b). These compounds, depending on their odour threshold, may confer aroma to the salts, depending on their origin. For example, sea salt violet odour, attributed to β-ionone, seems to be diagnostic of salts from Aveiro, in Portugal, and Guérande, in France, harvested in environments surrounded by a large number of plant and algae diversity (Silva et al., 2010a).

If it was possible to find hydrophobic volatile compounds in sea salt, it should also be possible that hydrophobic non-volatile compounds such as triacylglycerides could be found in this natural product. Representing the major constituents of every living cell (Napolitano et al., 1997), fatty acids (FAs), namely saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated (PUFAs), are known constituents of the marine biota, including algae (van Ginneken et al., 2011), phytoplankton (Chen, 2012; Napolitano et al., 1997), and cyanobacteria (Guedes et al., 2011).

Seawater is also known to contain polymeric material, often reported as marine mucilage, coming from marine organisms such as algae (Leppard, 1995), phytoplankton (Pistocchi et al., 2005a,b), and bacteria (Leppard, 1995; Pistocchi et al., 2005b). These include polysaccharides (Sartoni et al., 2008) and protein (Metaxatos et al., 2003). These polymeric materials may also be associated to sea salt.

The aim of this study was to explore the presence of triacylglycerides and polymeric material, namely polysaccharides and protein in food grade sea salt, thus contributing to a more detailed knowledge of the sea salt molecular composition. This knowledge could be a useful tool in the pursuit of a recognised

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product qualification, as foreseen by the European legislation. In 2012, marine salt producers of Guérande, France, received a protected geographical indication (PGI) label, confirming the authentic origin of this sea salt (Dufossé et al., 2013).

2. Materials and methods

2.1. Samples

In this study a set of 16 samples of food grade sea salt coming from different saltpans was analysed. These samples, all with origin in the Atlantic Ocean, came from Aveiro and Figueira da Foz (FF), in the North Coast of Portugal; Castro Marim (CM), in Algarve, South of Portugal; Cádiz (CD), in Andalucia, southwestern Spain; Île de Ré (IR), in the Western Coast of France; La Palma island (LP), in Canarias Islands; and Sal island (S), in Cape Verde. Sea salt from Aveiro came from three different saltpans, namely *Peijota* (PJ), *18 dos Caramonetes* (18C), and *Grã Caravela* (GCa). For some of these origins, sea salt samples with different years of production were analysed. The information of the year was included in the sea salt identification (e.g. PJ04 refers to *Peijota* salt produced in 2004). These samples were supplied by the participants of project SAL – Sal do Atlântico – "Revalorisation of the Atlantic traditional saltpans identity – Recovery and promotion of the biological, economical, and cultural potential of the humid zones from the coast", supported by the European Commission (INTERREG IIIB Programme). Samples were stored in glass bottles until analysis. Each analysis described was performed at the same time for all sea salts under study.

3. Standards

Fatty acid internal standard heptadecanoate methyl ester (C17:0) was purchased from Sigma (Germany). Sugar internal standard 2-deoxyglucose was purchased from Sigma. The 20 L-amino acids standards (alanine – Ala, arginine – Arg, asparagine – Asn, aspartic acid – Asp, cysteine – Cys, glutamic acid – Glu, glutamine – Gln, glycine – Gly, 4-hydroxyproline – Hyp, isoleucine – Ile, leucine – Leu, lysine – Lys, methionine – Met, phenylalanine – Phe, proline – Pro, serine – Ser, threonine – Thr, tryptophan – Trp, tyrosine – Tyr, and valine – Val) and the internal standard L-norleucine were all purchased from Sigma.

3.1. Sea salt moisture content

The moisture content of the salts under study was determined in order to express the content of triacylglycerides and polymeric material (PM), as sea salt dry weight. To determine the moisture content of the sea salts under study, 5 g of each sample were dried in an oven at 110 °C during 12 h (Koloff et al., 1969). The weight of each sea salt was recorded after cooling to room temperature, in a desiccator. Three independent aliquots of each sample were analysed.

3.2. Triacylglycerides extraction and fatty acid composition

Sea salt (20 g) triacylglycerides were extracted with *n*-hexane (150 mL) in a Soxhlet apparatus (250 mL round bottom flask; Soxhlet chamber of 50 mL capacity; 23 mm × 100 mm cartridge) during 4 h (Passos et al., 2009). The organic extracts of each salt were obtained from two independent aliquots. These were combined and evaporated under vacuum until all *n*-hexane was removed. The triacylglycerides obtained were then transesterified to determine fatty acids (FAs) composition by gas chromatography.

Fatty acid methyl esters (FAMEs) were prepared by transesterification with sodium methoxide (Aued-Pimentel et al., 2004). As the amount of triacylglycerides extracted from the 2 × 20 g of sea salt was very small, the sample was directly dissolved in 0.5 mL of an internal standard solution of heptadecanoate methyl ester (C17:0) prepared in *n*-hexane (0.03 mg/mL). Then, a methanolic KOH solution (2 M) was added (0.02 mL) and the resulting solution was mixed vigorously for 30 s in a vortex shaker. After, a saturated NaCl solution (0.2 mL) was added and the sample was centrifuged at 2000 rpm during 5 min. The organic phase was transferred to a clean tube and evaporated under vacuum. FAMEs were dissolved in 0.1 mL of *n*-hexane and aliquots (0.2–0.5 μL) were used for GC–qMS analysis. An Agilent Technologies 6890 N Network gas chromatograph with a split/splitless injector was used, equipped with a 25 m column DB-1 (QUADREX Corporation) with i.d. and film thickness of 0.25 mm and 0.05 μm, respectively, and connected to an Agilent 5973 quadrupole mass selective detector. Samples were injected in splitless mode (5 min) with the injector operating at 220 °C. The GC oven temperature programme was set at an initial temperature of 75 °C, raised to 163 °C at 15 °C/min, then raised to 173 °C at 3 °C/min, holding for 3 min, then raised to 175 °C at 1 °C/min, and then raised to 250 °C at 15 °C/min, holding for 5 min. The flow rate of the carrier gas (He) was set at 1.7 mL/min. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV scanning the range 40–500 *m/z*, in a full scan acquisition mode. Identification of the chromatogram peaks was done comparing all mass spectra with the data base system of the GC–qMS equipment (Wiley 275). FAs quantification was achieved with the internal standard method using $C_{17:0}$ and considering the response factor of all the identified FAs as one. One aliquot of each sea salt extract was analysed.

3.3. Isolation of polymeric material from sea salt

For the isolation of the polymeric material (PM) from sea salt crystals in quantitative amounts in order to allow its analysis, a methodology needed to be developed (Fig. 1). First, dialysis membranes (size 5, 12–14 kDa, Medicell), with a volume of approximately 80 mL, were filled directly with 60 g of sea salt crystals. This procedure was carefully preformed not to damage the membrane due to the crystals sharp edges. Then, distilled water was added to fill the remaining air gaps and the dialysis membranes were sealed avoiding the presence of air inside. For each sea salt, eight membranes were prepared simultaneously. The filled membranes were placed in one jar containing 10 L of distilled water. The dialysis occurred during approximately 1 week, under stirring, with 2–3 times daily water exchanges. Five drops of toluene and five drops of chloroform were added always to the new dialysis water in order to avoid microbial contamination. The dialyses ended when the conductivity of the dialysate water after 6 h was similar to that of the distilled water. Then, the content of the dialysis membranes were pooled and concentrated by rotary evaporation at 30 °C until a volume of 30 mL. To eliminate the presence of any sea salt, the concentrated solution was again dialysed in 10 L of water for more 2 days with 2–3 daily water exchanges, controlling the conductivity of the dialysate. The retentate was then frozen and freeze-dried to obtain the PM from sea salt. Due to its hygroscopicity, it was stored in a desiccator with P_2O_5 until analysis to avoid water absorption.

3.4. Analysis of the polymeric material from sea salt by mid-infrared spectroscopy

The PM isolated from sea salt was characterised by mid-infrared spectroscopy in the 4000–500 cm^{-1} region, with 8 cm^{-1} resolution and 128 co-added scans, using a Golden-Gate single reflectance ATR in a Bruker IFS-55 instrument (Bruker, Karlsruhe, Germany). Five independent aliquots of each sample were analysed (Coimbra et al., 1998). The resulting spectra were transferred through