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Optimization of a multiple headspace sorptive extraction method coupled to gas chromatography-mass spectrometry for the determination of volatile compounds in macroalgae

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ABSTRACT

A novel extraction technique is proposed in which the Multiple Headspace Extraction (MHE) approach is used in conjunction with Headspace Sorptive Extraction (HSSE) and Gas Chromatography-Mass Spectrometry (GC–MS) detection. The extraction method was developed to determine volatile compounds in macroalgae. Optimization of the extraction parameters was carried out using design of experiments to identify factors that affect the extraction: extraction time, temperature, twister length and amount of sample. The results of the optimization led to an extraction of 2 g of sample using a 20 mm Twister® at 66 °C for 180 min. The progression constants (β) were calculated for 43 volatile compounds, 29 of which could be quantified using the method. Linearity was attained with a determination coefficient higher than 0.99 for all studied compounds. Inter-day and inter-twister precisions ranged from 0.22% to 19.01% and from 0.69% to 14.76% respectively, and values below 10% were obtained for the majority of compounds. LOD and LOQ values ranged from the values obtained for diethyl succinate (0.012 μg/L and 0.088 μg/L, respectively) and those obtained for dimethyl sulfide (5.544 μg/L and 40.286 μg/L, respectively). However, for the majority of compounds values obtained were below 1 μg/L (LOD) and 5 μg/L (LOQ). Compounds such as ethyl acetate, hexanal, heptadecane, 2-hexenal, 6-methyl-5-hepten-2-one, dimethyl sulfide, benzyl alcohol, beta ionone, or beta cyclocitral, among others were correctly determined in three species of macroalgae: *Ulva* sp., *Gracillaria* sp. and *Enteromorpha* sp.

1. Introduction

Macroalgae, also known as seaweeds, are a group of photosynthetic non-flowering plant-like organisms that generally live attached to rock or other hard substrata in coastal areas [1]. Macroalgae serve many purposes for humans: as food, fertilizers, soil conditioners, animal feed, fish feed, biomass for fuel, cosmetics, integrated aquaculture and as an agent for wastewater treatment [2]. The diversity of macroalgae functions is due to their ability to produce a wide range of metabolites [3]. A particularly interesting group of beneficial compounds in macroalgae are the volatile compounds.

Volatile compounds in macroalgae provide a mechanism for communication, for defence against predators and the environment and they act as sexual pheromones [4]. For humans, these compounds are known for their function as therapeutic drugs, as a source of flavouring agents in food, a source of odorants in the perfume industry and as an indicator of water pollutants [5]. Most importantly, the volatile compounds affect the aroma and flavour of macroalgae as dietary products [6]. As a consequence, the extraction and determination of volatiles from macroalgae have been carried out for many years using different analytical methods.

Studies into the extraction of volatile compounds from macroalgae have been carried out by conventional methods, for instance liquid-liquid extraction and distillation [7,8]. These methods have several disadvantages such as the use of expensive and hazardous organic solvents, the loss of analytes during extraction and their time-consuming nature [3]. Novel methods such as dynamic headspace extraction, solid phase microextraction (SPME) and supercritical fluid extraction (SFE) in combination with detection by gas chromatography (GC) have also been developed [9–11]. However, the methods developed are mostly for qualitative purposes and they are only applicable to certain species of macroalgae [12–15]. It is acknowledged that none of the analytical methods

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developed to date are suitable for all macroalgae species due to the marked differences between the varieties, which constitute different matrices for analysis [3]. Thus, there is a need to develop a general method for analysis that is applicable to macroalgae regardless of the species.

An extraction method using the headspace mode that can eliminate the matrix effect has been established and this is called multiple headspace extraction (MHE) [16]. The further development of this approach has led to a combination with a novel extraction technique, SPME, to provide multiple headspace solid phase microextraction (MH-SPME) [17]. As a static sorption-based method, the headspace variation for stir bar sorptive extraction (SBSE) called headspace sorptive extraction (HSSE) is more advantageous than SPME due to its higher sensitivity [18]. Despite the fact that HSSE has proven advantages over SPME, there has been no attempt to develop multiple headspace extraction using HSSE although this would allow the possibility of overcoming the matrix effect, which has been a disadvantage in the study of solid samples using HSSE.

The aim of the study described here was to develop and validate an optimized analytical method for the characterization and determination of volatile compounds in macroalgae using multiple headspace sorptive extraction (MHSSE) and gas chromatograhy in tandem with mass spectrometry (GC–MS) detection. This is a new development and it may provide the opportunity to obtain a simple and reliable solid sample extraction method that could be used to analyze macroalgae regardless of species.

2. Materials and methods

2.1. Chemicals and reagents

Fourty three volatile compounds were used in this study. Hexyl acetate, 2-heptenal, eicosane, 1-chloropentane, 1-octen-3-ol, safranal, ethyl acetate, hexanal, 5-methyl furfural, alpha ionone, 2-hexenal, 2,4-heptadienal, 6-methyl-5-hepten-2-one, furfural, isovaleric acid, isoamyl acetate, 2-methylundecane, dodecane, octadecane, tetradecane, heptadecane, 8-heptadecene, ethyl isovalerate, beta cyclocitral and isobutyl acetate were purchased from Sigma Aldrich (St. Louis, MO, USA). Ethyl octanoate, diethyl succinate, benzyl alcohol, phenol, nonanoic acid, hexanoic acid, ethyl butyrate, ethyl hexanoate, 1-butanol, decanoic acid, octanoic acid, benzaldehyde and acetic acid were purchased from Merck KGaA (Darmstadt, Germany). Beta ionone, dimethyl sulfoxide and 2-phenylethyl acetate were purchased from Fluka (Buchs, Switzerland). Acetyl pyridine was purchased from Lancaster Synthesis Inc. (Lancaster, United Kingdom). Acetone, absolute ethanol and cyclohexane were obtained from Panreac Quimica S.L.U. (Barcelona, Spain). All the standards employed presented purities over 99%.

2.2. Preparation of standard solutions

Standard solutions were prepared in absolute ethanol except for the mixture of 1-chloropentane, 2-methyl undecane, dodecane, octadecane, tetradecane, heptadecene and 8-heptadecene, which were prepared in cyclohexane. Concentrations of mother solutions prepared were varied between 250 and 12500 mg/L depending on their concentration level: low, medium and high (Table 1). All standard solutions were stored at 4 °C.

2.3. Macroalgae samples

Macroalgae samples were obtained from the company Suralgas (Vejer de la Frontera, Spain). The products were harvested in San Fernando, Cadiz, Spain (October, 2016). Three species of macroalgae, namely *Ulva* sp., *Gracillaria* sp. and *Enteromorpha* sp., were obtained as fresh macroalgae. All three species were employed, after dehydration, as samples for MHSSE. An additional dehydrated macroalgae sample consisted of 50% *Ulva* sp. and 50% *Gracillaria* sp. (Suralgas, Spain) and this was used for the optimization of HSSE in the determination of volatile compounds.

2.4. Preparation of macroalgae sample for determination of volatile compounds

Dehydrated mixed macroalgae was milled (Carrefour Home Coffee Grinder, Les Ulis, France) and then stored in its original jar.

Fresh macroalgae were weighed on a balance (Ohaus® CS Series, Greifensee, Switzerland) to obtain a wet mass and the sample was rinsed with distilled water five times to remove debris and once with milli Q water. Rinsed algae were dried overnight at room temperature, lyophilized (Virtis K, Warminster, USA) for 48 h and weighed to obtain the dry mass of algae. Lyophilized algae were milled and then placed in a conical tube sealed with parafilm and stored at −25 °C.

2.5. Multiple headspace sorptive extraction

Headspace sorptive extraction was performed using PDMS (polydimethylsiloxane) Twisters® (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) (0.5 mm PDMS film thickness, 20 mm or 10 mm length) in a waterbath (Pselecta Tectron 3473100, Barcelona, Spain). Two grams of milled lyophilized algae were placed in 20 mL vials and then the twister was put in headspace mode and the vials were sealed with 20 mm seals PTFE/Silicone (Symta, Madrid, Spain). For the calculation of the progression constants and calibration curves needed for the quantification, extraction was carried out four consecutive times at 66 °C for 180 min. After each extraction the vials were cooled at −25 °C for 60 min before the seals were opened and the twister was removed prior to carrying out the next extraction. Vials were left to stand to room temperature for 15 min prior to the next extraction. A thermostatic waterbath in which several samples could be placed at the same time, was employed for the analysis of real samples and a single extraction of 180 min was performed for each sample.

2.6. Instrumentation

2.6.1. Sample introduction

The sample introduction system consisted of a Thermal Desorption Unit (TDS-2) equipped with a MultiPurpose Sampler (MPS), which can automatically handle a programme for 98 liners containing twisters and a Programmed-Temperature Vaporization (PTV) Cooled Injector System (CIS-4) by Gerstel (Mülheim an der Ruhr, Germany). A liner containing glass wool was used in the PTV. The Thermal Desorption Unit was operated in splitless mode. The desorption temperature was programmed from 40 °C to 300 °C with 0.5 min delay time and 10 min holding time, while the ramp rate was 60 °C/min and the helium flow was 75 mL/min. The desorbed analytes were then cryofocused in the PTV system using liquid nitrogen at −140 °C. The PTV system was programmed from −140 °C to 300 °C and held for 5 min at 10 °C/s prior to GC–MS analysis.

2.6.2. GC–MS analysis

The sample introduction system was installed on an Agilent Technology 7890 Gas Chromatography System coupled with an Agilent 5975C inert Mass Spectrometry Detector (Agilent Technologies, Palo Alto, CA, USA). The separation was achieved on a GC DB Wax capillary column (J&W Scientific, Folsom, CA, USA),

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