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A GC-MS-based metabolomics investigation on scallop (*Chlamys farreri*) during semi-anhydrous living-preservation

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Lactic acid (PubChem CID: 612)
Malic acid (PubChem CID: 525)
Fumaric acid (PubChem CID: 444972)
Succinic acid (PubChem CID: 1110)
Octadecanoic acid (PubChem CID: 5281)
Hexadecanoic acid (PubChem CID: 985)
Phenylalanine (PubChem CID: 994)
Docosahexaenoic acid (PubChem CID: 445580)
Glutamic acid (PubChem CID: 611)
Aspartic acid (PubChem CID: 424)
Isoleucine (PubChem CID: 6306)
Glycine (PubChem CID: 750)
Pyroglutamic acid (PubChem CID: 499)
Proline (PubChem CID: 145742)
(PubChem CID:)
Leucine (PubChem CID: 857)
2-aminoadipic acid (PubChem CID: 469)

ABSTRACT

We investigated the physiological changes in scallops (*Chlamys farreri*) during semi-anhydrous living-preservation using metabolomics method to obtain a guiding theory in optimizing the preservation conditions. Glycogen, lactic acid, and crude protein levels were measured in 1-day intervals for 6 days, the median lethal time.

The metabolite profiling of scallops after the preservation duration of 10% lethal time (3 days) was achieved based on methyl chloroformate derivation before GC-MS analysis. Carboxyl acids related to respiration (malic, fumaric, and succinic acids), fatty acids (C18:0, C16:0, and C22:6), and amino acids (phenylalanine, glutamic acid, aspartic acid, isoleucine, glycine, pyroglutamic acid, proline, leucine, and 2-aminoadipic acid) were identified as biomarkers. Our results demonstrated that scallops performed an elevated anaerobiosis and depressed aerobiosis, which were not caused by oxygen insufficiency. The switching of energy metabolism patterns and disorder of the osmotic regulation system were also observed, suggesting that oxygen supply is less important for optimizing semi-anhydrous living-preservation.

Industrial relevance: In China, live aquatic products are far more appreciated by consumers than frozen ones. For example, live scallops are more than ten times as expensive as frozen ones in non-local restaurants. However, there is little profit in providing live scallops instead of frozen scallops, due to the high cost of preservation and transportation. Besides the mortality loss, the high costs of running and building the life-preserving condition are the main costs. Thus, optimizing the preservation conditions can reduce the cost. In our case, we introduced metabolomics instead of non-targeted parameter selecting such as the orthogonal test. Based on the elucidated metabolic pathway, we found that oxygen supplementation is not critical. The high cost of building and running oxygen-supplying module in semi-anhydrous living-preservation can be eliminated.

1. Introduction

Scallops are now the major economic shellfish of the world with a capture production of 861,382 t in 2011(FAO, 2014) and the top economic values of commercial shellfishes (1900$/t and 1788$/t for capture and aquaculture, respectively (FAO, 2014)). Over 300 species

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of bivalves worldwide are identified as scallop. However, only 4 species are economically available as aquatic products in China. *Chlamys farreri*, known as the Zhikong Scallop, is the most important economic scallop species in China and is native to the coasts of the Shandong and Liaoning provinces (FAO, 1991).

As the result of development in food supply chains, increasing demands for higher safety and quality in preservation and transportation never end. Aquatic products are not an exception, but even more restricted regarding freshness. Living-preservation and transportation have become hotspots of investigation to increase the quality and value of the aquatic product supply chain (Anacleto et al., 2013; Barrento, Lupatsch, Keay, & Christophersen, 2013; Barrento, Marques, Pedro, Vaz-Pires, & Nunes, 2008; Fotedar & Evans, 2011). Transportation and preservation of live aquatic animals in waterless conditions were developed (Barrento et al., 2013; Cho, Kim, Lee, & Choi, 1994; Mi, Qian, & Mao, 2012), taking advantage of the lower costs and mortality of waterless, compared with submersion, conditions. From capture or aquaculture to consumers, scallops are subjected to various stressors in waterless preservation conditions; thus, the responses of shellfishes are a complex chain, and optimization of preservation and transportation is, therefore, vital to establish waterless preservation technology. Conventional optimizing methods, based on limited information revealed by physiology indexes (Anacleto et al., 2013; Barrento et al., 2008, 2013; Fotedar & Evans, 2011; Funatsu et al., 2007), are usually superficial and aimless. We utilized a metabolomics approach to establish practical guidance for optimization of preservation and transportation parameters and discover the mechanisms of the stresses the scallops suffered. Metabolomics, a subset of systems biology, is a powerful tool for elucidating the answers of what is happening or will happen in organisms through investigating changes in small-molecular-weight metabolites as influenced by environmental stressors (Goodacre, 2005). Currently, metabolomics is part of many fields, such as toxicological assessments (Feng, Li, Wu, & Chen, 2013; Hasegawa, Ide, Kuwamura, Yamate, & Takenaka, 2010), disease diagnosis (Rujescu, 2011; Slocum, Heung, & Pennathur, 2012), and food quality control (Fotakis et al., 2013; Surowiec, Fraser, Patel, Halket, & Bramley, 2011); metabolomics-based investigations of the preservation of post-harvest fruits (Hertog et al., 2011; Lee, Mattheis, & Rudell, 2012), meat products(Argyri, Doulgeraki, Blana, Panagou, & Nychas, 2011), and beverages (Heuberger et al., 2012) were reported several times, though we know of no such research on bivalves. Several reports discussed metabolomics on bivalves (Ji, Wu, Wei, Zhao, & Yu, 2013; Kwon et al., 2012; Spann, Aldridge, Griffin, & Jones, 2011; Wu & Wang, 2010; Wu et al., 2013a,2013b), but most of these reports considered bivalves are detectors of pollution due to their sensitivity to changes in the environment, not as aquatic products or food material.

We conducted a time series assay of glycogen, lactic acid, and protein as energy consumption indicators of scallops subjected to simulated semi-anhydrous living-preservation. The end point of the time series was set as the 50% lethal time, LT50, because many individuals would then be in the sublethal state. The 10% lethal time, LT10, was set as the end point of preservation treatment for the metabolomics experiment, according to the commercial requirement of the native market. GC-MS-based metabolite profiling and pattern recognition were conducted, followed by pathway elucidation focused on the regulation of respiration and energy metabolic pattern-related substances, such as succinic acid, malic acid and lactic acid.

2. Materials and methods

2.1. Reagents

Methyl chloroformate (MCF), pyridine, anhydrous methanol, sodium hydroxide, chloroform, dodecylamine, anhydrous sodium sulfate, and sodium chloride were from the China National Pharmaceutical Group Corporation (Shanghai, China) of analytical grade. All the water used was distilled water prepared by our laboratory.

2.2. Scallop preservation and survival recognition.

The scallops (*Chlamys farreri*) were harvested from the Bohai Sea in China and reared in a recirculating aquaculture and depuration system in our laboratory for at least 1 week. The scallops were chilled from room temperature to 5 °C at 10 °C/hour before preservation. The preserving process was emulated in SANYO MIR-154 low-temperature incubators (SANYO Electric Co., Ltd., Moriguchi, Osaka, Japan), set at 5 °C, with wet paper, humidified every 12 hours covering the scallops. For survival rate and time series analyses, 750 scallops were separated into 50 subgroups, 15 individuals per subgroup. Preservation survival rates of 5 subgroups, a total of 75 individuals, were observed every day without retrieval. Among the 75 individuals, 15 living individuals, 3 per subgroup, were sampled for biochemistry analysis conducted after termination of the preservation survival study. The preservation was terminated, and the remaining groups were discarded once the LT50 was determined and reached. For metabolomics investigation, another 20 individuals in baseline state just after stresses eliminating were set as the control group and held in the depuration system, and 20 baseline individuals were set as the preserved group and preserved in the conditions mentioned above for 3 days.

The survival recognition was conducted by observing the closing of shell valves while stimulating the soft tissue with a stick (Paukstis, Janzen, & Tucker, 1997). If the shell valve closed in 30 seconds, the individual was recognized as alive.

2.3. Glycogen level analysis

Glycogen levels were analyzed with a commercial glycogen analysis kit (Jiancheng, Nanjing, Jiangsu, China). Briefly, all the soft tissue was homogenized and placed in a boiling bath with 1 mL alkaline solution for 20 minutes. An additional boiling bath for 5 minutes was conducted after the kit's chromogenic reagent was added. Glycogen concentration was calculated by the absorbance at 620 nm of samples with an equivalent glucose standard.

2.4. Lactic acid level analysis

Lactic acid level was analyzed with a commercial lactic acid analysis kit (Jiancheng, Nanjing, Jiangsu, China). The homogenates were centrifuged in 4000 rpm, and the supernatants were in a tube and set into a solution of NAD^+, lactate dehydrogenase, and chromogenic reagent in 37 °C for 5 minutes. Reaction terminator was added before measurement of the absorbance at 530 nm. The lactic acid level was calculated with the external standard from the analytical kit.

2.5. Protein level analysis

The protein level was analyzed by the Bradford method (Bradford, 1976). The supernatants of the tissue homogenates were diluted 100 times and mixed with 0.1 mg/mL coomassie blue solution. Absorbance at 595 nm was measured after allowing the reaction to proceed for 2 minutes. The external standard calculation was conducted with bovine serum albumin (BSA) as the standard.

2.6. Metabolomics sample pretreatment and MCF derivatization

The GC-MS based metabolomics chemical analysis was modified from a previous report (Smart, Aggio, Van Houtte, & Villas-Bôas, 2010).