



Characterization of taste and micronutrient content of rock oysters (*Crassostrea nippona*) and Pacific oysters (*Crassostrea gigas*) in Japan

Masahiro Yuasa^{a,*}, Koji Kawabeta^b, Ayaka Eguchi^b, Haruka Abe^a, Emi Yamashita^c, Kazunori Koba^a, Mihoko Tominaga^{a,d}

^a Department of Nutrition, Faculty of Nursing and Nutrition, University of Nagasaki, Siebold, 1-1-1 Manabino, Nagayo-cho, Nishisonogi-gun, Nagasaki 851-2195, Japan

^b Graduate School of Human Health Science, University of Nagasaki, Siebold, 1-1-1 Manabino, Nagayo-cho, Nishisonogi-gun, Nagasaki 851-2195, Japan

^c Department of Applied Food Science, Higashiosaka Junior College, 3-1-1 Nishidutsumigakuen-cho, Higashiosaka city, Osaka 577-0044, Japan

^d Graduate School of Education, Hiroshima University, 1-1-1 Kagamiyama, Higashi-Hiroshima city, Hiroshima 739-8524, Japan

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ABSTRACT

In this study, we characterized, for the first time, the taste and typical micronutrient content of rock oysters (*Crassostrea nippona*) and compared these to those of Pacific oysters (*Crassostrea gigas*). Rock oysters were collected in July and August and Pacific oysters in December 2015 in Japan. All samples were homogenized using a blender and Polytron homogenizer. In the rock oysters, some taste qualities, such as initial tastes (acidic bitterness, umami, and saltiness) and richness of aftertaste, were higher than in Pacific oysters (determined by Taste Sensing System) ($P < 0.05$). In contrast, 5'-inosinate and succinic acid did not differ between Pacific and rock oysters. Glycine and some bitter tasting amino acids were more abundant in rock oysters than in Pacific oysters ($P < 0.05$). The concentration of palmitic acid (C16:0), heptadecenoic acid (C17:1), and stearic acid (C18:0) in rock oysters were higher than in Pacific oysters ($P < 0.05$). Moreover, vitamin B₂, vitamin B₁₂, zinc, and taurine content in rock oysters were higher than in Pacific oysters ($P < 0.05$). We conclude that rock oysters may be richer in taste and micronutrients content than Pacific oysters.

Introduction

Oysters are considered a delicacy around the world, and they are eaten raw, broiled, steamed, or fried. Recently, some physiological and biochemical benefits of dietary intake of oysters have been proposed in experimental animal models. For example, dietary intake of oyster extract led to antihypertensive and antihyperglycemic effects in spontaneously hypertensive or diabetic rats (Tanaka et al., 2006). In addition, dietary oyster extract intake decreased liver triacylglycerol (Toda et al., 2015) and total cholesterol (Matsudo et al., 2016) content in rats. Oyster extracts can alleviate ovarian functional disorders by decreasing follicle stimulating hormone receptor expression in ovaries of bisphenol-A treated female rats (Zhou et al., 2014). Therefore, oysters have received considerable attention in the health field.

In Japan, oysters are classified into two: rock oysters (*Crassostrea nippona*), which are available in summer, and Pacific oysters (*Crassostrea gigas*), which are available in autumn and winter. The spawning season of these oysters is summer (June to September) in Japan. In a previous study, the gonad index in summer (June to

September) was higher than other seasons in Japan (Tanaka, 2004; Kobayashi et al., 1997). Glycogen contents of oysters and other shells differed according to seawater temperature (Mann, 1979) or wave action (Wells et al., 1998). In addition, glycogen content of oysters (Okumura et al., 2005; Li et al., 2000) and pen shell, ark shell, and manila clams (Yurimoto, 2015) changed with the seasons. In summer, protein and fat content of Pacific oysters in the ovary were higher than in autumn (Li et al., 2000). In rock oysters of Iwate Prefecture, Japan, free amino acid content in the high gonad index group was higher than in the low gonad index group (Tanaka, 2004). Glycogen content of oyster (Ishihara et al., 1966) or some shells (Yurimoto, 2015) varied according to the kind of tissue. High levels of zinc were observed in oysters in low salinity marine conditions (Mo and Neilson, 1993; Meiller and Bradley, 2002). On the other hand, seasonal changes of seawater temperature and/or salinity were observed in Japan (Kubo et al., 2015; Haraguchi and Sekida, 2008; Okumura et al., 2005). These previous studies suggest that the differences in nutrient content in oysters are a consequence of changing seasons and environments.

In Japan, some suggest that rock oysters have a richer taste and

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* Corresponding author.

E-mail address: yuasa@sun.ac.jp (M. Yuasa).

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more nutrients than Pacific oysters. However, there is no evidence for these contentions. Furthermore, previous studies (Mann, 1979; Wells et al., 1998; Li et al., 2000; Meiller and Bradley, 2002; Yurimoto, 2015) have examined flavor components and/or some nutrients content in Pacific oysters, but these have not been characterized in rock oysters. Therefore, in this study, we characterized the taste (taste qualities, and some flavor components of oysters) and typical micronutrient (glycogen, vitamin B₂, vitamin B₁₂, zinc, and magnesium) content of rock oysters and compared these to those of Pacific oysters.

Materials and methods

Sample preparation

Rock and Pacific oysters were collected in some regions of Japan. In July and August 2015, rock oysters were collected in nine production regions [Nagasaki (Sasebo and Goto city), Kumamoto, Oita, Mie, Kyoto, Tottori, Ishikawa, and Yamagata prefecture]. Three oysters in each production region were used ($n = 27$). In December 2015, Pacific oysters were collected in 11 production regions [Nagasaki (Sasebo and Goto city), Oita, Fukuoka, Hiroshima (Etajima and Kure city), Hyogo, Mie, Miyagi, Iwate, Hokkaido prefecture]. Three oysters in each production region were used ($n = 33$). All samples were homogenized using a blender (magic BULLET MB-1001) (OAK LAWN MARKETING, INC., Nagoya, Japan) and Polytron homogenizer PT 10/35 (KINEMATICA AG, Luzern, Switzerland), and stored at -25°C until further use.

Measurement of taste quality

The taste quality was determined using the Taste Sensing System TS-5000Z, which is equipped with ceramic reference electrodes, artificial lipid-based membrane sensors [C00 (acidic bitterness), AAE (umami), and CTO (saltiness)], an autosampler, an electronic unit for data acquisition, and an analysis application (version 1.6.5) (Kobayashi et al., 2010; Phat et al., 2016). It mimics the human taste sense, with each sensor playing the role of the lipid-based membrane of the human tongue. The sensors interact with various taste materials in the food samples via electrostatic and hydrophobic interactions, resulting in changes in the potential of the lipid-based membranes in the sensors. The changes are detected by a computer as sensor output and evaluated in terms of taste quality.

The taste quality was measured by immersing the sensors in a standard solution (30 mmol/L of KCl and 0.3 mmol/L of tartaric acid) to obtain the membrane potential (V_r). The standard solution had negligible taste and was used in this system as an alternative to human saliva. Next, the taste sensors were immersed in the sample solution to obtain the potential (V_s). The difference in potential ($V_s - V_r$), which is called the initial taste quality, should be approximate to the initial taste at sensory evaluation. Following this, the sensors were rinsed lightly with the standard solution and were immersed in the standard solution again to obtain the potential (V_r'). The difference in potential ($V_r' - V_r$), which is called the aftertaste quality [change of membrane potential caused by adsorption (CPA)], provides data on the adsorption of bitter and astringent substances.

In this study, acidic bitterness-A, umami, saltiness (the initial taste qualities), acidic bitterness-B, and richness (the aftertaste qualities) were measured. Measurements on samples were repeated four times, and the results were analyzed by analysis application in basic process mode. Homogenized samples were diluted 20-fold with ultrapure water. The taste qualities of the control sample were determined for each assay, and all results were calibrated and compared with those of oysters for each taste quality.

Determination of taste and flavor components

Salt equivalents measurement

Salt equivalents were determined using salinometer (Pocket Salt Meter PAL-SALT) (Atago Co., Ltd., Tokyo, Japan), and expressed as g per 100 g.

5'-inosinate assay

The 5'-inosinate (IMP) were measured using a Prominence ultra-fast liquid chromatography (UFLC) system equipped with a DGU-20A3 degassing unit, two LC-20AD pump, an SIL-20A HT autosampler, a CTO-20A column oven, an SPD-20A UV-VIS detector, and the software LC Solution version 1.23 SP1 (Shimadzu Co., Ltd., Kyoto, Japan). A Shim-pack WAX-1 (4.0 mm I.D. \times 5.0 mm, 3 μm) (Shimadzu Co., Ltd., Kyoto, Japan) was used for separation (Yuasa et al., 2017; Adachi et al., 2002). 4 mL of iced 10% (v/v) perchloric acid was added to 500 mg of homogenized samples, and supernatants were collected after centrifugation at $3500 \times \text{rpm}$ and 4°C for 10 min. Then 1.5 mL of iced 10% (v/v) perchloric acid was added in pellet, and supernatants were collected after centrifugation at $3500 \times \text{rpm}$ and 4°C for 10 min. The supernatants were combined and adjusted to pH 5.0 with 5 mol/L KOH, incubated on ice for 30 min, and collected after centrifugation at $3500 \times \text{rpm}$ and 4°C for 10 min. These were filled up to 40 mL using ultrapure water. They were then pre-filtered through a 0.45- μm nylon microfilter (Starlab Scientific Co., Ltd., Shaanxi, China), using the following conditions: mobile phase, 50 mmol/L phosphate buffer (pH 3.1); flow rate, 1.0 mL/min; reaction temperature, 40°C ; injection volume, 20 μL ; and monitoring with a UV/VIS detector at 260 nm. The 5'-IMP content was expressed as mg per 100 g.

Succinic acid assay

Succinic acid was measured by Prominence UFLC system equipped with a DGU-20A3 degassing unit, two LC-20AD pump, an SIL-20A HT autosampler, a CTO-20A column oven, an SPD-20A UV-VIS detector, and the software LC Solution version 1.23 SP1 (Shimadzu Co., Ltd., Kyoto, Japan). A YMC-Pack CA column (4.6 mm I.D. \times 250 mm) (YMC Co., Ltd., Kyoto, Japan) was used for separation. 50 mg of homogenized samples was derivatized by dicarboxylic acid labeling reagents for high-performance liquid chromatography (HPLC) (YMC Co., Ltd., Kyoto, Japan). The derivatized samples were pre-filtered through a 0.45- μm nylon microfilter (Starlab Scientific Co., Ltd., Shaanxi, China) using the following conditions: mobile phase, 5 mmol/L potassium dihydrogen phosphate: acetonitrile (88:12 by vol.); flow rate, 1.2 mL/min; reaction temperature, 40°C ; injection volume, 10 μL ; and monitoring with a UV/VIS detector at 230 nm. Succinic acid content was expressed as mg per 100 g.

Free amino acids assay

Free amino acids were measured using a Prominence UFLC system equipped with a DGU-20A3 degassing unit, two LC-20AD pump, an SIL-20A HT autosampler, a CTO-20A column oven, an SPD-20A UV-VIS detector, and the software LC Solution version 1.23 SP1 (Shimadzu Co., Ltd., Kyoto, Japan). An Inertsil ODS-3 (4.6 mm I.D. \times 150 mm, 3 μm) (GL Sciences Inc., Tokyo, Japan) was used for separation. Detection of free amino acids was performed as follows: L-aspartate, L-glutamate, L-serine, glycine, L-histidine, L-arginine, L-alanine, L-proline, L-tyrosine, L-valine, L-methionine, L-cysteine, L-isoleucine, L-leucine, L-phenylalanine, L-lysine, and taurine were derivatized to PTC-amino acids using phenyl isothiocyanate, as described previously (Yuasa et al., 2017; Glevarec et al., 2004). 0.5 mL of 0.1 mol/L HCl was added in 500 mg of homogenized samples, and supernatants were collected after centrifugation at $3000 \times \text{rpm}$ and 4°C for 10 min. Then 0.5 mL of methanol was added in 0.5 mL of the supernatants, and the supernatants were collected after centrifugation at $3000 \times \text{rpm}$ and 4°C for 10 min. 5 μL of these supernatants was transferred to Eppendorf tube and vacuum dried. These were neutralized with 20 μL of ethanol: ultrapure water: triethylamine

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