LWT - Food Science and Technology 73 (2016) 251–258

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Relationship between chemical characteristics and bacterial community of a Korean salted-fermented anchovy sauce, *Myeolchi-Aekjeot*

Hae-Won Lee, Yun-Jeong Choi, In Min Hwang, Sung Wook Hong, Mi-Ai Lee*

World Institute of Kimchi, Gwangju 61755, Republic of Korea

ARTICLE INFO

Article history:
Received 17 January 2016
Received in revised form
25 April 2016
Accepted 3 June 2016
Available online 4 June 2016

Keywords:
Myeolchi-Aekjeot
Anchovy sauce
Bacterial diversity
Tetragenococcus
Nitrogen contents

ABSTRACT

Little is known of the relationship between the chemical characteristics and bacterial community of *Myeolchi-Aekjeot*, a traditional Korean fermented anchovy sauce. Therefore, we explored this relationship by determining the salinity, pH, nitrogen content and inorganic content of nine *Myeolchi-Aekjeot* samples. Major resident bacteria in *Myeolchi-Aekjeot* samples were from the genus *Halanaerobium* and *Tetragenococcus*. Interestingly, in samples with a high ratio of total and amino nitrogen content, *Tetragenococcus*, comprising halophilic lactic acid bacteria, was the dominant genus. Furthermore, high total and amino nitrogen contents were found to correlate with the genus *Tetragenococcus*, with R^2 values of 0.85 and 0.89, respectively. Total nitrogen content is generally considered a quality parameter for fish sauces. We therefore conclude that the presence of *Tetragenococcus* bacteria can be an important quality parameter in future studies of salted-fermented anchovy sauces. Our results suggest a relationship between the chemical characteristics and a dominant bacterial community of *Myeolchi-Aekjeot*.

1. Introduction

In Korea, salted-fermented seafoods called *Jeotgal* or *Jeot* are manufactured by salting various aquatic ingredients (150 different sea animals including anchovy, squid, shrimp, oyster, and damselfish) followed by a long fermentation period (Hur, 1996). These fermented foods are applied as seasoning to improve taste (Roh et al., 2010). Salting and fermentation of seafoods to prevent spoilage by decreasing water activity is a widespread practice globally. Moreover, salted-fermented seafoods produce distinctive flavors and tastes because of the fermentation products of diverse microorganisms during the fermentation (Lee, Jung, & Jeon, 2014).

Myeolchi-Aekjeot, a traditional Korean salted-fermented anchovy sauce, has been consumed as a umami-tasting, seasoning ingredient (Moon et al., 2013). The umami of *Myeolchi-Aekjeot* is formed by exogenous enzymes of microorganisms (Yongsawatdigul, Rodtong, & Raksakulthai, 2007). Anchovy (*Engraulis japonicus*), the main ingredient of this salted-fermented sauce, is a fine marine ingredient, rich in amino acids and polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid (Lee, Asaduzzaman, & Chun, 2012). Approximately 221,000 tons of anchovy were harvested in Korea in 2014, and it is an important fish species in Korean local markets because *Myeolchi-Aekjeot* is mainly used to replace salt in cooking. However, it also brings a distinct, characteristic flavor to the food. Specifically, salted-fermented anchovy sauces rich in amino acids and peptides are used to reinforce the nutrition, flavor, and taste of Korean traditional foods including *Kimchi*.

Despite the large amounts (20%–30%) of salt used in fish sauces in general, including *Myeolchi-Aekjeot*, various halotolerant bacteria, such as *Achromobacter*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Tetragenococcus*, and *Vibrio*, are present in fish sauces (Lee, Jung, & Jeon, 2015), providing a distinct flavor and protecting against contamination with spoiling yeasts (Margesin & Schinner, 2001).

Therefore, we investigated the relationships between the chemical characteristics and bacterial community of nine *Myeolchi-Aekjeot* samples to determine quality criteria. The bacterial diversity of the samples was compared with other chemical parameters used to index the quality of salted-fermented anchovy sauces.

* Corresponding author.
E-mail address: leemae@wikim.re.kr (M.-A. Lee).

http://dx.doi.org/10.1016/j.lwt.2016.06.007
0023-6438/

2. Materials and methods

2.1. Samples

Nine samples of commercial *Myeolchi-Aekjeot* were purchased from various local markets in Korea, each manufactured in different provinces: Jeon-nam (A-1, A-4), Chung-nam (A-2, A-3, A-6, A-7, A-8), Jeon-buk (A-5), and Gyeong-buk (A-9). The samples were ripened for at least 10 months, and following their purchase they were stored in the dark at 4 °C.

2.2. Salinity and pH

The salinity and pH of the samples were measured in triplicate using the Mohr method and a pH meter (TitroLine easy; SCHOTT Instruments, Mainz, Germany), respectively.

2.3. Nitrogen content measurement

2.3.1. Total nitrogen (TN) content

The TN content of the *Myeolchi-Aekjeot* samples was measured in triplicate via the Kjeldahl method. Briefly, 500 mg of each sample on nitrogen-free paper was placed in a digestion tube with 12 mL of concentrated sulfuric acid and 2 g of the catalyst ($CuSO_4$:K_2SO_4, 1:4). Digestion was then performed at 420 °C for 60 min, and the final solution was cooled until its color changed to transparent yellow. It was then distilled in an automatic distillation unit, and the distillate was titrated with 0.1 M hydrochloric acid until a light pink color was achieved. The TN content was calculated as follows:

$$\text{Total nitrogen content (\%)} = \frac{(a-b)\times M\times 14.01}{S}\times 100$$

where a is the titrated volume of the standard acid for the sample (mL), b is the titrated volume of standard acid for the reagent blank (mL), M is the molarity of hydrochloric acid, 14.01 is the molar mass of nitrogen, and S is the sample weight (g).

2.3.2. Volatile basic nitrogen (VBN) content

The VBN content of the *Myeolchi-Aekjeot* samples was measured in triplicate via the Conway microdiffusion method. Briefly, 5 g of each sample was suspended in distilled water to obtain a final volume of 100 mL and then sonicated for 10 min. After filtration, the sample filtrate was neutralized with 5% sulfuric acid, and 1 mL of this sample solution with 1 mL of saturated potassium carbonate was placed in the outer chamber of a Conway dish. Then, 1 mL of 0.01 N sulfuric acid was placed in the inner chamber of the Conway dish. The dishes were incubated at 25 °C for 60 min and then titrated with 0.01 N sodium hydroxide and Brunswik's reagent. The VBN content was calculated as follows:

$$\text{Volatile basic nitrogen content (mg\%)} = \frac{(b-a)\times 0.14\times f\times D}{S}\times 100$$

where a is the titrated volume of 0.01 N sodium hydroxide consumed by the sample (mL), b is the titrated volume of 0.01 N sodium hydroxide consumed by the blank reagent (mL), 0.14 is the VBN content value corresponding to mL of 0.01 N sodium hydroxide, f is the factor of 0.01 N sodium hydroxide, D is the dilution magnification, and S is the sample weight (g).

2.3.3. Amino nitrogen content

The AN content of the *Myeolchi-Aekjeot* samples was measured in triplicate by using the formol method. In total, 2 g of each sample was suspended in distilled water to obtain a final volume of 100 mL and then sonicated for 30 min. Thereafter, 20 mL of this sample solution was mixed with 20 mL of formalin solution, and then titrated to pH 8.3 with 0.1 N sodium hydroxide. The AN content was calculated as follows:

$$\text{Amino nitrogen content (mg\%)} = \frac{(A-B)\times 1.4\times f\times D}{S}\times 100$$

where A is the titrated volume of 0.1 N sodium hydroxide consumed by the sample (mL), B is the titrated volume of 0.1 N sodium hydroxide consumed by the blank reagent (mL), 1.4 is the AN content value corresponding to mL of 0.1 N sodium hydroxide, f is the factor of 0.1 N sodium hydroxide, D is the dilution magnification, and S is the sample weight (g).

2.4. Crude ash content

The crude ash of the *Myeolchi-Aekjeot* samples was measured in triplicate via the AOAC method. In total, 3 g of each sample was ashed by heating at 550 °C for 12 h and then carefully weighed.

2.5. Inorganic element content

Each sample (1–2 g) in a polytetrafluoroethylene (PTFE) digestion vessel was hydrolyzed by a microwave digestion system with 7 mL of nitric acid (HNO_3, purity 70%, electronic grade, Dong Woo Fine Chem. Co. Ltd., South Korea) and 1 mL of hydrogen peroxide (H_2O_2, purity 30%, electronic grade, Dong Woo Fine Chem. Co. Ltd, South Korea). The details regarding the experimental conditions are shown in Table S1. Ultrapure water (18.2 MΩ cm) was obtained from a Milli-Q ultrapure water purification system (Millipore Co., USA). A calibration standard solution was prepared by diluting the Multi-Element Calibration Standard 3 (PerkinElmer, USA).

Calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), zinc (Zn), and sodium (Na) content was analyzed by an ICP-OES system (Optima 8300, PerkinElmer, USA), and lead (Pb) and cadmium (Cd) content was analyzed by an ICP-MS system (NexION 300D, PerkinElmer, USA). The operating conditions of the instruments are provided in Table S2.

2.6. Bacterial diversity analysis

2.6.1. DNA extraction

Total DNA was extracted from the samples using a Power Soil DNA isolation kit (Mo Bio Laboratories, CA, USA), according to the manufacturer's instructions. The total DNA was quantified by the PicoGreen assay (Quant-iT PicoGreen ds-DNA assay kit; Invitrogen, Paisley, UK).

2.6.2. Barcoded PCR amplification and high-throughput sequencing

The total DNA was amplified using a primer set (27F, 5′-GAGTTTGATCMTGGTCAG-3′; 518R, 5′-WTTACCGCGGCTGCTGG-3′) targeting the hypervariable V1–V3 region of the *16S rRNA* gene region, with ten base sample-specific barcode sequences (Table S3). The polymerase chain reaction (PCR) protocol was as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min; and a final extension for 8 min. Purification of the PCR products was performed using AMPure beads (Beckman Coulter, CA, USA). The pooled PCR products were immobilized and amplified through emulsion PCR on DNA capture beads using an emPCR kit (Life Sciences, CT, USA) according to a massively parallel pyrosequencing protocol (Margulies et al., 2005). High-throughput sequencing was