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RESEARCH NOTE

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The Effects of Wash Solutions and Freezing Temperatures on the Microbial Growth and Physical Properties of *Capsosiphon fulvescens*

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Abstract In order to determine the optimal storage conditions of *Capsosiphon fulvescens* (*maesaengi*), 2 types of wash solutions (distilled water and seawater) and storage temperatures (-20 and -80°C) were evaluated for the effectiveness of microbial growth inhibition and the changes of texture, color, and proximate composition. Thawed samples that had been washed with seawater and stored at -20°C for 50 days showed a 1.1-fold increase in hardness compared to the initial hardness of the sample (1.9×10^5 dyne/cm^2). There was no change in moisture, ash, or crude lipid during storage at -20 and -80°C for 60 days, while there was a 1±0.2% decrease in crude protein content for the control during storage at both -20 and -80°C for 60 days. In conclusion, the recommended optimal storage conditions for retaining the quality of *C. fulvescens* are: temperatures at or below -20°C and washings with either distilled water or seawater for inhibiting microbial growth, temperatures at or below -20°C and a washing with seawater to prevent reductions in hardness, and a temperature of -80°C and washings with either distilled water or seawater to protect against color changes.

Keywords: *Capsosiphon fulvescens*, microbial growth, storage condition

Introduction

Capsosiphon fulvescens (*maesaengi*) is green algae cultivated on the Southern coast of Korea. The cells typically exist in groups of 2 and 4 enclosed in a cell wall in a tubular thallus. They are less than 10 cm long and 2 cm wide and are reproduced by biflagellated isogametes released from the bisexual gametophytes (1). Several studies have reported on the effects of *C. fulvescens* extract on lipid metabolism and melanogenesis (2, 3). Recently, considerable attention has been given to *C. fulvescens* as an alternative resource to create new economic opportunities. Approximately 1,000 tons (wet weight) of *C. fulvescens* are produced per year in South Korea. Its market has continued to increase with the increasing consumer preference for healthy foods. In order to meet the demand for *C. fulvescens* during harvest period (December to February), this seaweed was freezed at -70°C in a cold storage room for annual seaweed supply after minimal processing such as washing, weighing, and packaging. Few works have been carried out on the effects of storage temperature and washing solution to increase the shelf-life with cost effective way. In this study, *C. fulvescens* was evaluated based on the effects of wash solutions and storage temperatures on microbial growth inhibition and the changes of texture, color, and proximate composition, which may be used as indicators for determining storage conditions.

Materials and Methods

We obtained *C. fulvescens* specimens from Jangheung, a county located on the Southwest coast of Korea during February, 2006. The frozen samples were allowed to thaw at 4°C for 24 hr prior to analyses.

Microbiological analysis To begin, *C. fulvescens* was washed with seawater or distilled water and then a 10 g sample was homogenized in a homogenizer (Omni Macro Homogenizer, Omni international, Waterbury, CT, USA) for 30 sec in 90 mL of phosphate buffered saline (PBS, pH 7.2). The PBS was plated on nutrient agar, violet bile red agar, MacConkey agar, and thiosulfate citrate bile salts sucrose agar (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37°C for 24 hr in triplicate prior to the counting of microorganisms (4-6).

Bacterial identification To identify a bacterium based on its 16S ribosomal gene sequence, genomic DNA was extracted using an Accuprep Genomic DNA extraction kit according to the manufacturer's protocol using a bacterium grown overnight on marine agar (Difco Laboratories) (7). DNA amplification was carried out in the following manner: 5 min of pre-soaking followed by 30 cycles at 94°C, 30 sec at 55°C for denaturing, 30 sec at 72°C for annealing, 40 sec for extension, and finishing with an incubation at 72°C for 5 min using the universal primers 27F (5-AGAGTTTGAT CCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTA CGACTT-3') (8). The closest known relatives of the new isolates were determined by performing sequence database searches and the sequences of closely related strains were retrieved from GenBank or the Ribosomal Database Project (RDP) libraries. Nucleotide (NT) sequence similarities were calculated using the PHYDIT program (8, 9).

Texture and colour analysis After washing *C. fulvescens* with seawater or distilled water, the samples were stored at -20 and -80°C for 50 days. They were then placed in a holder that consisted of a 50 mL centrifuge

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tube without a bottom and containing a cap to support *C. fulvescens*. The hardness of the samples was measured in triplicate with a Rheometer (Sun Scientific Co., Ltd., Tokyo, Japan). The surface color of *C. fulvescens* was measured in a Hunter color system using a colorimeter (JC 801S; Color Techno System Co., Tokyo, Japan). The samples were standardized with black and white standard plates (X=78.86, Y=80.11, Z=90.58).

Proximate analysis Moisture, crude protein, crude lipid, and ash contents were determined according to the standard methods of the AOAC (10).

Statistic analysis All statistical analyses were performed using the SPSS Version 9.0 for Windows (SPSS Inc., Chicago, IL, USA). ANOVAs were conducted to compare significant changes in proximate compositions of *C. fulvescens* between control and 2 treatment groups. Statistical significance was accepted at $p<0.05$.

Results and Discussion

After incubating the samples (*C. fulvescens*) at 20°C for 2 and 4 days, there were increases of 2.6±0.2 and 5.1±0.1 log CFU/g, respectively, in total viable counts from the initial loads of the control (2.5 log CFU/g) (Fig. 1A). There were no significant differences in microbial growth inhibitions between the wash solution types (distilled water and seawater) or the storage temperatures of -20 and -80°C. The samples washed with either seawater or distilled water and frozen at -20°C for 40 days showed a 0.5±0.1 log CFU/g increase in total viable counts from their initial control load of (2.5 log CFU/g) (Fig. 1B). However, after frozen storage at or below -20°C for 60 days the samples had total viable counts of 2.1±0.1 log CFU/g, suggesting that this storage temperature and time could slow the rate of all chemical reactions and extend product shelf-life by such negative effects as temperature shock, the concentration of extracellular solutes, dehydration, and ice formation that protects against microorganisms (11). Estrada-Flores (12) reported that frozen foods produced microbial growths of 3 to 5 log units per g at -7.5°C. Therefore, maintaining the storage temperature below -20°C is recommended for stopping microbial growth. Table 1 shows that 4 bacteria identified by the 16S rDNA sequence and reference strain comparisons were *Bacillus subtilis* subsp. *subtilis* DSM 10T, *Achromobacter insolitus* LMG 6003T, *Brevundimonas nasdae* DSM 14572T, and *Microbacterium lacticum* DSM 20427T (13-15). Enteric bacteria such as *Escherichia coli*, *Salmonella*, and *Vibrio*

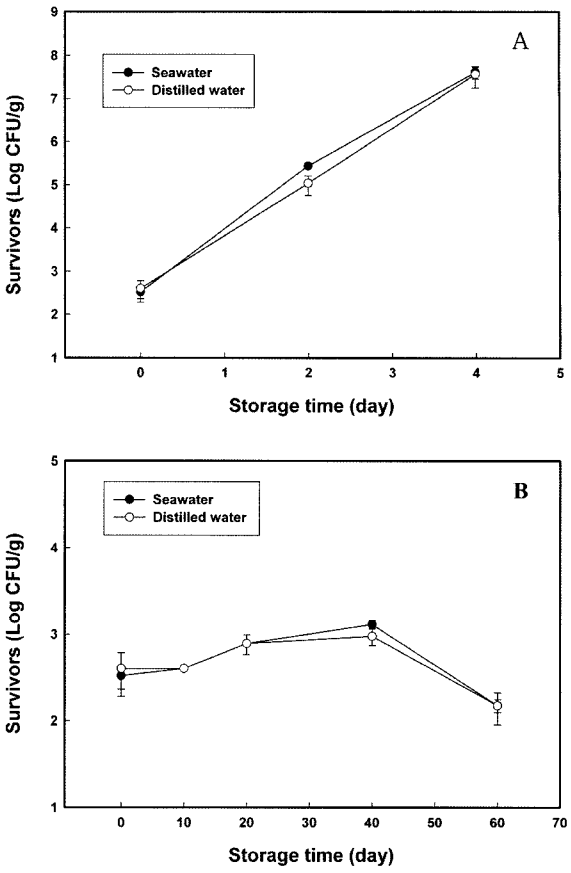

Fig. 1. Changes in the total viable counts for *Capsosiphon fulvescens* during storage at 20°C (A) and -20°C (B). Each sample was tested in triplicate.

species were not detected in the samples.

The samples that were thawed after being washed with seawater and stored at -20°C for 50 days showed a 1.1-fold increase in hardness from their initial hardness (1.9×10^5 dyne/cm^2), while the samples washed with distilled water had a 0.7-fold decrease from their initial hardness (2.2×10^5 dyne/cm^2). The samples that were thawed after being washed with seawater and stored at -80°C for 50 days produced a 1.4-fold increase in hardness from their initial hardness (1.9×10^5 dyne/cm^2), while the samples washed with distilled water had a 1.6-fold decrease in hardness from their initial hardness (2.2×10^5 dyne/cm^2) (Fig. 2). Therefore, the samples washed with seawater tended to increased in hardness. Chung and Shyu (16)

Table 1. The identification of bacteria isolated from *Capsosiphon fulvescens* by 16S rDNA sequencing analysis using an NCBI database

Strain	Gene bank accession number	Similarity (%)
Bacillus subtilis subsp. *subtilis* DSM 10T	AJ276351	100
Achromobacter insolitus LMG 6003T	AY170847	99.64
Brevundimonas nasdae DSM 14572T	AB071954	99.63
Microbacterium lacticum DSM 20427T	X77441	99.29

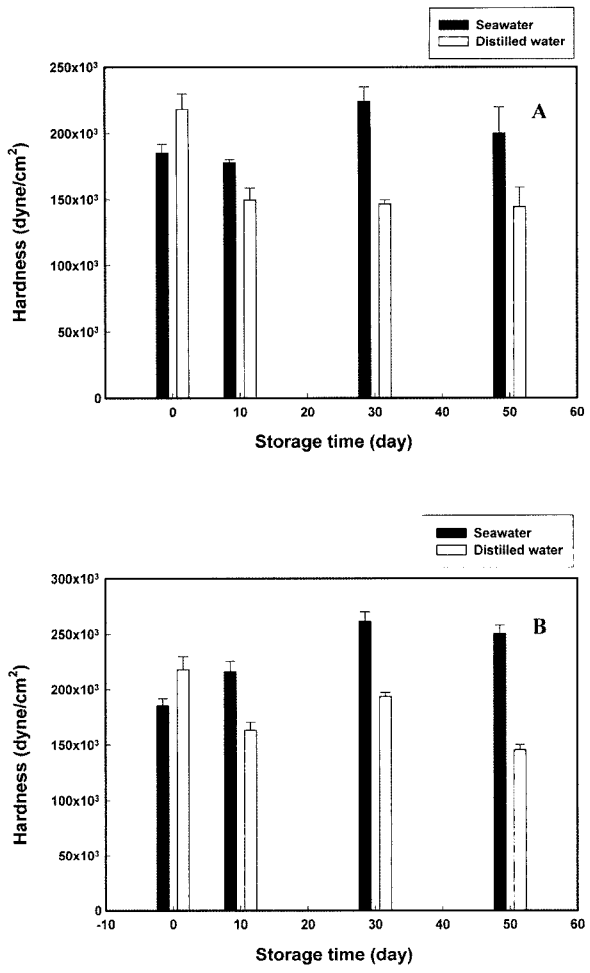

Fig. 2. The changes in texture (hardness) of *Capsosiphon fulvescens* by wash solutions during storage at -20°C (A) and -80°C (B). Hardness was measured in triplicate using a rheometer.

stated that an increase in hardness was the direct effect of the NaCl solution causing a decrease in moisture content.

For samples stored 50 days at -80°C, the Hunter color scale 'L' values (luminosity) did not change significantly from the initial value of 9.51, whereas the samples stored at -20°C had lower 'L' values for the same length of storage time (data not shown). After 10-days of storage at -20 and -80°C, the samples changed from their initial 'a' value (red-green color) of -4.1 to values of -10.1, and -9, respectively. After this time the 'a' values remained almost constant until the completed 50 days of storage at both temperatures. The 'b' values (yellow-blue color) showed no significant changes from their initial value of 10.5. No significant differences in 'L', 'a', and 'b' values existed between the distilled and seawater wash solutions (data not shown). Color changes have been correlated with nutritional losses and are often used to control storage conditions (17). The results from this study indicated that the 'L' value was a sensitive parameter for evaluating color changes during a long-term storage at low temperatures. There were no significant changes in proximate compositions, such as moisture, fiber, ash, and crude lipid between the controls, the samples washed before storage, and the samples stored at -20 and -80°C. However, there was a 1±0.2% decrease in crude protein content for the control during storage at both -20 and -80°C for 60 days (data not shown).

In conclusion, the recommended optimal storage conditions for retaining the quality of *C. fulvescens* are: temperatures at or below -20°C and washings with either distilled water or seawater for inhibiting microbial growth, temperatures at or below -20°C and a washing with seawater to prevent reductions in hardness, and a temperature of -80°C and washings with either distilled water or seawater to protect against color changes.

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