



Improved extraction of bioactive compounds by cold storage of spirulina fermented by *Kazachstnia servazzii*

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스피루리나의 *Kazachstnia servazzii* 호모발효 및 자가소화를 통한 생리활성 물질 추출 향상

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Abstract

In this study, alcohol fermentation and cold storage were conducted to enhance the extraction of bioactive compounds in spirulina. After alcohol fermentation of 20% spirulina paste with 5% glucose and 1% yeast extract using *Kazachstnia servazzii* KM2017 for 2 days, the pH and acidity were 6.91 and 0.26%, respectively. Viable yeast cells increased from 1.21×10^7 CFU/mL to 2.51×10^8 CFU/mL. In addition, the protein content of spirulina doubled to 423.98 µg/mL. Polyphenols and flavonoids were also increased to 350.12 µg/mL and 28.04 µg/mL, respectively. Notably, the inherent off-odor of spirulina paste was partially removed with production of 1.3% alcohol. After cold-storage of yeast fermented spirulina paste at 1°C for 5 days, the extraction of bioactive compounds was further enhanced, resulting in 464.09 µg/mL protein, 420.21 µg/mL polyphenol and 34.41 µg/mL flavonoids. On the other hand, the viable yeast cell count decreased to 4.39×10^6 CFU/mL, revealing partial disruption of the spirulina. Thus, it was concluded that the odor and extraction of biologically active substance were improved by both yeast fermentation and cold storage of spirulina.

Key words : spirulina, *Kazachstnia servazzii*, cold-storage, biologically active compounds

Introduction

Spirulina (*Oscillatoria spirulina*) is one of the oldest algae on earth. Spirulina has been designated as a future food by the United Nations Food and Agriculture Organization because it contains a variety of physiologically active substances, such as carbohydrates, protein, vitamins, minerals, and gamma-linolenic acid (1). Spirulina is also classified as

an alkaline super-food that provide health and prevention of chronic disease. It is a valuable food ingredient for illness, old age, and in infants, because of its high absorption rate of over 95% (2). The biological activity of spirulina was investigated. The nutritional content of spirulina, and the anti-oxidant, anti-aging, and anti-proliferating effects of spirulina extract were reported (3).

Spirulina has an excellent immune activity, and has been patented in Russia for use in radiotherapy. Furthermore, the National Aeronautics and Space Administration (NASA) has been developing spirulina-based space food (4). Spirulina has diverse physiological effects, including strong antioxidant effect, tumor cell suppression activities, inhibition of the production of lipid peroxides, improved immune function and

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circulation (5), suppressed aging and prevention of disease in adults. The increasing recognition of the physiological benefits of spirulina has led to its use as a high-value natural functional food ingredient.

Fermentation has been used as a classical method for preservation in Eastern and Western cultures. Yeast is an important organism in brewing industry because it provides valuable nutrient ingredient and various wine. Yeasts can obtain energy by breaking down hexose through anaerobic respiration (6). During anaerobic respiration, pyruvic acid is broken down and alcohol and carbon dioxide are released. Yeasts can grow between 10°C and 37°C, showing limited growth at lower and higher temperatures. Yeast fermentation provided alcohol as well as condiments with pleasant flavor for food industry. β -Glucans as biologically active compound could be extracted from yeast cell wall. It could strengthen innate immunity in the elderly, and anti-cancer activity (7).

In spite of health promoting effect, the spirulina had some limitations as food ingredients because of indigenous odor. The removal of the distinctive smell and optimizing the production of physiologically active substances from spirulina by yeast fermentation has not been studied. Particularly, the strain *K. servazzii* KM 2017 has a low alcohol producing ability but a good gas generating ability, when comparing the strains *S. cerevisiae*, *K. serazzii*, and *W. hamomyces* (8,9). In addition, the *K. servazzii* strain has not only excellent gas generating ability but also fruit flavor during the growth period (10).

Therefore, it was judged that *K. servazzii* KM2017 is optimal for the strain which increases physiologically active substance while eliminating the odor of spirulina.

In this study, the yeast fermentation of spirulina was optimized using *K. servazzii* KM2017 to enhance the extraction of bioactive compounds. In particular, the effect of cold storage on the morphology and phytochemicals (phycocyanin, β -glucan, phenolics compounds) of yeast fermented spirulina were analyzed. The present study will provide the valuable information for utilizing as a novel food ingredient of spirulina.

Materials and methods

Spirulina pastes and reagents

Spirulina powder was obtained from USA (Nutraceuticals International Group LLC, Paramus, NJ, USA). Yeast extract

from Choheung Corporation (Ansan, Korea) was used as the nutrient in the medium for fermentation of spirulina. All reagents were of high grade.

Yeast strain and starter cultures

The strain *K. servazzii* KM2017 (KCCM 11989P) isolated from fermented apple juice was confirmed by PCR and used for yeast fermentation. The genomic DNA was extracted using the boiling method based on Chelex beads and amplified by PCR (Veriti R™ 96-well Thermal Cycler (ABI).

DNA amplification involved initial denaturation at 95°C for 15 min, 1 cycle; denaturation at 95°C for 20 sec, 30 cycles; annealing at 50°C for 40 sec, 30 cycles; extension and final extension at 72°C for 1 min 30 sec and 5 min, respectively, 1 cycle. Lastly, sequencing was carried out using Big Dye® Terminator v3.1 Cycle sequencing Kits using an ABI prism 3730XL DNA analyzer (capillary 50 cm).

The nucleotide sequence was analyzed by Blast N. Sequence analysis of NCBI was performed to confirm the results. The reliability of NCBI sequence analysis is 99.9% similarity with *K. servazzii*. *K. servazzii* KM2017 was smeared onto medium containing Difco™ Lactobacilli MRS broth agar (Becton, Dickinson and Company, Sparks, MD, USA) and cultured at 30°C in an IS-971R incubator (Jeio Tech., Kimpo, Korea) for 24 h. Next, a single colony was collected for two-time subculture, inoculated onto MRS broth sterilized for 15 min at 121°C, and cultured for 24 h at 30°C to generate the starter.

Yeast fermentation of spirulina paste

To prepare the spirulina paste, 20 g of spirulina powder was added to 45 mL of distilled water and homogenized (Shaking incubator HK-S125C) for 5-10 min. To remove other microorganisms, the solution was heated to 85°C in a water bath for 30 min. Next, yeast fermentation was performed by adding 10 mL of 10% YE and 25 mL of 20% glucose as nutrients, inoculating with 1% *K. servazzii* KM2017, and culturing for 2 days at 30°C with shaking. Yeast fermented spirulina paste was kept for 5 days at 1°C.

Measurement of pH, acidity, and viable cell count

The pH was measured using a model 420+ pH meter (Thermo Scientific, Waltham, MA, USA). Titratable acidity was calculated, as lactic acid content (% v/v), by adding 1 mL of reagent to 9 mL of distilled water, and titrating with 0.1 N NaOH to make the solution pH 8.3, as shown in the following equation:

Viable count was investigated by adding 1 g of the fermentation products to 9 mL of sterilized water followed by serial dilutions to 10^4 , 10^5 , and 10^6 , with 20 μL of each dilution spread onto MRS agar plates. Following culture for 24 h at 30°C in a thermostatic incubator, the colony forming units (CFU/mL) was determined.

Measurement of β -glucan and reducing sugar content

Approximately 5 g of the sample is suspended in 100 mL of distilled water, and then approximately 120 mg of amylase (20 units) was added. pH was adjusted to 6.9 with 0.1 N sodium hydroxide solution, followed by shaking at 20°C for 2 h. The solution was adjusted to pH 5.0 with 0.1 N hydrochloric acid solution. Cellulase (50 units) was added and the mixture was shaken at 37°C for 2 h for enzymatic degradation. After then, 60 mg of aminoglucosidase and 0.1 N hydrochloric acid solution were added. and then mixture was hydrolyzed at 60°C for 2 h. A 400 mL of ethanol (95%) is added to the above enzymatic degradation product, followed by precipitation at 4°C for 12 h, followed by centrifugation (10,000 rpm, 10 min) to obtain a precipitate of the sample. After 500 mL of 80% ethanol was added. The precipitate was obtained by centrifugation (10,000 rpm, 10 min). The final precipitate was homogenized and resuspended in distilled water. Sample solution (1 mL) was mixed with 1 mL of 5% phenol solution and then quickly added 5 mL of sulfuric acid. Absorbance was measured at a wavelength of 470 nm (11).

Reducing sugar content was measured using the dinitrosalicylic acid (DNS) method (12). Spirulina paste was centrifuged at 13,200 rpm for 10 min. The supernatant was mixed with 3 mL of DNS and left to react for 5 min at 100°C . After cooling for 40 min at room temperature in a dark place, absorbance was measured at 550 nm. Reducing sugar content was calculated by comparison to a standard curve of reference glucose.

Measurement of protein

Fermented spirulina paste was centrifuged at 10,000 rpm for 10 min. The supernatant was diluted 4-fold and added with 5 mL of Bradford dye reagent (13). Thereafter, the reaction solution was reacted at $20\text{--}30^\circ\text{C}$ for 30 min and the absorbance of reaction solution was measured at 595 nm. Protein content was obtained by comparison to a standard curve of bovine serum albumin.

Measurement of polyphenols and flavonoids

Polyphenol content was measured by the Folin-Denis method (14). A 60 μL of 2-fold diluted Folin reagent was added to cold storage spirulina (60 μL), and the mixture was homogenized and left for 3 min. To this, 60 μL of 10% Na_2CO_3 was added, and the mixture was left to react for 60 min in a thermostatic incubator at 30°C . After the reaction was completed, the absorbance of the sample was measured at 700 nm. Total polyphenol content was calculated by comparison to a standard curve for the reference substance garlic acid.

Flavonoid content was measured as previously described (15). Each sample (125 μL) 1,080 μL of 80% ethanol, 30 μL of 10% aluminum nitrate, and 30 μL of 1 M potassium acetate. After leaving the mixture for 40 min at room temperature, the absorbance was measured at 415 nm. Total flavonoid content was calculated by the comparison to a standard curve of quercetin.

Measurement of α - α -diphenyl- β -picrylhydrazyl free radical (DPPH) scavenging activity

The free radical scavenging activity of fermented spirulina and cold storage product was analyzed by measuring the ability to reduce DPPH. Each sample (160 μL) received 40 μL of 0.15 mM DPPH solution. The mixture was left for 30 min and the absorbance was measured at 517 nm. The free radical scavenging activity was analyzed as the concentration (IC_{50}) that indicated the half inhibition of full activity. Trolox was used as the positive control.

Measurement of phycocyanins

For measurement of green phycocyanin pigments, 10 mL of fermented spirulina product was left for 10 h in a dark room at 5°C . Thereafter, the spirulina paste was centrifuged at 13,200 rpm, and then the supernatant was collected. The absorbance was measured at 562 nm, 620 nm, and 652 nm. Phycocyanin content was obtained using the following equations (16).

$$\text{C-PC (C-phycocyanin): } [A_{620} - 0.474(A_{652})]/5.34$$

$$\text{APC (allophycocyanin): } [A_{652} - 0.208(A_{620})]/5.09$$

$$\text{PE (phycocerythrin): } [A_{562} - (2.41(\text{C-PC}) - 0.849(\text{APC}))]/9.62$$

Chromaticity measurement

The chromaticity of fermented spirulina products was measured using a colorimeter (Color Reader, CR-10, Minolta, Osaka). Color values for lightness (L), redness (a), yellowness (b) were measured. The values of the standard white plate

used were L: 96.12, a: -4.41, and b: 7.20.

Morphology analysis

The fermented spirulina was diluted 2-fold. The morphological changes during fermentation and cold storage of spirulina were observed using a microscope at 500x magnification (LEICA ICC 50 E, Wetzkar, Germany).

Statistical analyses

The differences between the groups were statistically evaluated using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test or Student's t-test using SPSS (Chicago, IL, USA). P values <0.05 were considered to be statistically significant.

Results and discussion

pH and acidity of fermented spirulina

After yeast fermentation for 2 days at 30°C, 20% spirulina

storage of yeast led to the production of neutral amino acids, proteins, and organic compounds (19).

Viability cell count

On yeast fermentation day 1, the viable count of yeast in the spirulina paste increased from 3.24×10^6 CFU/mL to 1.21×10^8 CFU/mL. Although the production of bioactive compounds increased with yeast fermentation, the spirulina paste fermented for 2 days maintained a viable count of 10^7 CFU/mL indicating yeast viable cell of 5.33×10^7 CFU/mL. This is similar to the result that when yeast cultivation was carried out for about 3 days, the number of yeast cells was decreased (20). It was confirmed that the number of viable cells of yeast decreased as cold storage progressed as shown in Table 1. During cold storage of fermented spirulina the viable cell of *K. servazzii* decreased from 5.33×10^7 CFU/mL. Although the spirulina paste provided a good growth environment for yeast, the cold storage of fermented spirulina resulted in the cold storage, indicating the viable cell counts of 4.39×10^7 CFU/mL for 5 days.

Table 1. Physicochemical properties during cold storage and yeast fermentation of spirulina

| | Time (days) | pH | Acidity (%) | Reducing sugar (%) | β-Glucan (mg/g) | Protein (μg/mL) | Viable cell count (yeast) (CFU/mL) × 10 ⁶ |
|--------------------|-------------|--------------------------|------------------------|------------------------|------------------------|--------------------------|--|
| Yeast fermentation | 0 | 6.92±0.01 ^{a1)} | 0.26±0.02 ^c | 4.97±0.02 ^a | 0.04±0.02 ^a | 242.98±2.54 ^c | 3.24±2.61 ^c |
| | 1 | 6.22±0.01 ^b | 0.38±0.01 ^d | 1.72±0.01 ^b | 2.01±0.06 ^b | 250.76±3.12 ^c | 121±8.24 ^b |
| | 2 | 6.09±0.02 ^c | 0.51±0.01 ^a | 0.68±0.03 ^f | 4.75±0.14 ^c | 423.98±3.65 ^b | 53.3±8.66 ^a |
| Cold storage | 1 | 6.09±0.02 ^c | 0.52±0.01 ^a | 0.88±0.02 ^c | 5.12±0.08 ^d | 453.65±4.41 ^a | 21.3±3.24 ^b |
| | 3 | 6.13±0.01 ^d | 0.47±0.01 ^b | 1.39±0.01 ^d | 6.21±0.09 ^e | 459.43±3.42 ^a | 7.41±5.21 ^c |
| | 5 | 6.16±0.01 ^c | 0.41±0.02 ^c | 1.53±0.02 ^c | 8.53±0.12 ^f | 464.09±2.51 ^a | 4.39±4.21 ^c |

¹⁾Values with different letters in the same row significantly different (analyzed using ANOVA and Duncan's multiple range test: p<0.05, a>b>c>d>e>f).

paste (w/v) was subjected to cold storage for 5 days in a refrigerator. The initial pH of spirulina paste was 6.92±0.01. Thereafter, pH decreased slightly and acidity increased from 0.26 to 0.51% for 2 days due to the organic acids produced by the yeast. This may reflect the production of oxalic acid, malic acid, acetic acid, citric acid, succinic acid, and lactic acid by yeast (17).

During the cold storage at 1°C, pH increased slightly and the acidity decreased slightly. After cold storage for 5 days, the final product showed pH 6.16 and acidity of 0.41%. The pH increase during cold storage may be the release of neutral amino acids and proteins from spirulina degradation. This result is similar to a study in which β-glucan and protein increased, resulting in decreased acidity during cold storage of yeast cell at low temperatures (18). In another study, cold

Reducing sugars

Reducing sugars have an aldehyde group or form an aldehyde group in solution (21). Glucose was used as the reference substance to measure reducing sugars. The initial reducing sugar concentration was 4.97%. As yeast fermentation progressed, the reducing sugar concentration decreased, reaching 0.68% on fermentation day 2. This is similar to reports that yeasts decrease level of reducing sugars by breaking down sugars during fermentation. After fermentation was complete, cold storage at 1°C showed the increasing trend of reducing sugar content, reaching 1.53% for 5 days. The increase in reducing sugar content during cold storage is due to the cell lysis of yeast as well as cell disruption of spirulina. This is similar to another study, in which yeast extract was prepared by cold storage and

enzymatic hydrolysis, and there was a slight increase in reducing sugar concentration during cold storage (22,23).

β -Glucan

During the yeast fermentation of spirulina, β -glucan content increased from was 0.04 mg/g to 4.75 mg/g for 2 days as shown in Table 1. The results of the physicochemical analysis after yeast growth showed a somewhat similar result to that of 0.09 mg/g of β -glucan. As the yeast fermentation proceeded, it was confirmed that the amount of β -glucan was increased. It was concluded that β -glucan of yeast was eluted with decreasing viable cell count by yeast fermentation for 2 days. Yeast cell lysis is depending on the fermentation date during yeast fermentation (24). The content of β -glucan increased by cold storage of yeast fermented spirulina. The content of β -glucan was 6.21 mg/g after 3 days. It was judged that various physiologically active substances were also eluted due to the cold storage of yeast cells while the sample was stored at a refrigeration temperature of 1°C. The β -glucan content was 8.53 mg/g after the cold storage for 7 days.

It has been reported that the lower temperature or higher temperature of 50°C resulted in the collapse of the cell wall of the yeast, resulting in the elution of physiologically active substances (25). However, during the storage at high temperature, physiologically active substance of yeast was increased. But spirulina could change its unique color (26).

Protein

The initial protein content in 20% spirulina paste was 242.98 μ g/mL as shown in Table 1. This result is similar to the values of 1,102.88 μ g/mL and 1,200.04 μ g/mL in raw spirulina in another study (27), considering 5-fold dilution of fermented spirulina paste. The protein content increased during yeast fermentation, reaching 423.98 μ g/mL on fermentation for 2 days. The increase may be because of the release of protein as the increased viable cell count on fermentation day 1 decreased on day 2. Also, it is due to the lysis of spirulina cells. Another study reported the similar results increasing in protein, where the initial protein content was 0.03 g/g and increased to 0.25 g/g after yogurt fermentation for 3 days (28).

It was concluded that the yeast fermentation of spirulina and its cold storage higher content of bioactive compounds due to the cold storage through cell death and cell licking,

Analysis of polyphenols, flavonoids, and DPPH

Antioxidant activity of fermented spirulina paste was

determined by DPPH radical scavenging activity, and analysis of polyphenol content and flavonoid content analyses. The results for polyphenol and flavonoid content are shown in Fig. 1. Polyphenolic compounds are widely distributed in nature, and mostly exist in vacuolar. Anthocyanins, flavonoids, pro (pancho) cyanidin, remunerators, ligands, and tannin are included (29).

Polyphenol have various physiological and pharmaceutical effects, including anticancer, antihypertensive, anti-inflammatory, antidiabetic, and anti-aging activities. The polyphenol and flavonoid contents before yeast fermentation were value 213.79 μ g/mL, 23.26 μ g/mL, respectively. In particular, the flavonoid content was similar to the value flavonoid content of 120.24 μ g/mL previously measured in pure spirulina extract, considering the 5 fold dilution of fermented spirulina paste.

During yeast fermentation, the polyphenol and flavonoid contents increased gradually. On fermentation for 2 days, their content were 350.84 μ g/mL and 28.41 μ g/mL, respectively, with a large increase in the polyphenol content.

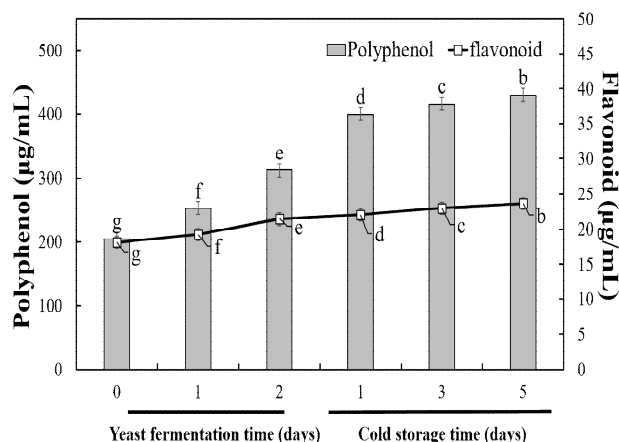


Fig. 1. Polyphenol and flavonoid after yeast fermentation and cold storage of spirulina.

Values with different letters in the same bar significantly different (analyzed using ANOVA and Duncan's multiple range test: $p < 0.05$, $a > b > c > d > e > f$).

This may be because the alcohol produced during yeast fermentation causes a large amount of the total polyphenol to be released (30). On fermentation day 2, antioxidant substances are thought to be released due to the cell disruption of spirulina.

During low-temperature storage, the polyphenol and flavonoid contents increased gradually, reaching 420.55 μ g/mL and 34.01 μ g/mL, respectively, for 5 days. After the copious release of protein, organic compounds, and phenolics compounds during yeast fermentation, storage at

low-temperature resulted in the death of yeast cell and accompanied by the release of bioactive compounds from both yeast and spirulina cells. In effect of temperature on growth and cold storage of *Spirulina pliantness* the low temperature increased the autolysis (31). This is similar to the description of increased polyphenol and flavonoid content, as indicator of antioxidant ability, during yeast fermentation of wine (32).

Table 2 shows the DPPH radical scavenging activity, which was an indirect measure of the antioxidant activity of the fermented spirulina. In the similar analysis, an alcohol-free wine is reacted with the stable free radical DPPH⁺, with the decrease in DPPH radical measured by spectrophotometric (33). The spirulina without yeast fermentation showed the IC₅₀ value of 2.48 mg/mL and decreased to 1.21 mg/mL after yeast fermentation for 2 days at 30°C. After the end of fermentation and cold storage for 5 days at 1°C, the IC₅₀ value decreased to 0.88 mg/mL, which was even lower than the value after 2 days of yeast fermentation. Conclusively, the cold storage of fermented spirulina may decreased IC₅₀ value because the degradation of both yeast cells and spirulina during cold storage led to the release of bioactive compounds.

Table 2. DPPH radical scavenging activity after cold storage and yeast fermentation of spirulina

| Time (days) | IC ₅₀ |
|--------------------|----------------------------------|
| Yeast fermentation | |
| 0 | 2.48±0.41 ¹⁾ mg/mL |
| 2 | 1.21±0.23 mg/mL ^{***2)} |
| Cold storage | |
| 5 | 0.88±0.11 mg/mL ^{***} |
| BHA (standard) | 4.62±0.18 µg/mL |

¹⁾Values represent the mean±SD (n=3).

²⁾Significant differences were observed before and after fermentation using Student's t-test at *** p<0.0018.53.

Phycocyanin

The phycocyanin content was also found to increase during yeast fermentation and cold storage. As shown in Figure 2, Table 3, the C-PC, APC and PE contents before yeast fermentation were value 0.078±0.003 mg/g, 0.055±0.003 mg/g, 0.150±0.001 mg/g. Especially, picocyanin, which is one of the specific coloring components of spirulina, is contained in a great number of cyanobacteria (34). These pigments are responsible for photosynthesis and have a great effect on nutrient supply and antioxidant effect (35). The C-PC value of the yeast fermentation day 2 was 0.121 mg/g and the APC value was 0.096 mg/g. Finally the second day PE value of yeast fermentation was 0.169 mg/g. It was

confirmed that this value was increased by yeast fermentation. This is similar to the results of studies that various pigment components elute with partial lysis of spirulina (36). After cold storage, the C-PC, APC, and PE values of spirulina increased. The cold storage 5 day, C-PC value was 0.144 mg/g, APC value was 0.126 mg/g and the PE value was 0.192 mg/g. Especially, it was confirmed that the color became darker after cold storage. This is similar to the study that the color changes as the spirulina pigment elutes (37).

Table 3. Phycocyanin content extracted from spirulina during yeast fermentation and cold storage

| Time (day) | C-PC (mg/g) | APC (mg/g) | PE (mg/g) |
|--------------------|---------------------------|--------------------------|--------------------------|
| Yeast fermentation | | | |
| 0 | 0.078±0.003 ¹⁾ | 0.055±0.003 ^c | 0.150±0.001 ^f |
| 1 | 0.096±0.002 ^e | 0.098±0.004 ^d | 0.161±0.002 ^e |
| 2 | 0.121±0.003 ^d | 0.096±0.003 ^d | 0.169±0.002 ^d |
| Cold storage | | | |
| 1 | 0.126±0.001 ^e | 0.104±0.004 ^c | 0.175±0.003 ^c |
| 3 | 0.135±0.002 ^b | 0.116±0.003 ^b | 0.182±0.004 ^b |
| 5 | 0.144±0.001 ^a | 0.126±0.002 ^a | 0.192±0.003 ^a |

¹⁾Values with different letters in the same column significantly different (analyzed using ANOVA and Duncan's multiple range test: p<0.05, a>b>c>d>e>f).

Chromaticity measurement

Table 4 shows the changes in the chromaticity of spirulina. The color value of the initial spirulina fermented product was L: 63.12, a: 0.23, b: 23.14. This is similar result to the low a value of red color in a study of the quality characteristics of tofu using spirulina powder (38). As the yeast fermentation, the L value decreased while the a and b values increased. Afterwards, the brightness dimmed as cold storage progressed. This is similar to the results of the lysis of spirulina, showing the dark color due to the physiologically active substance and chlorophyll-a, phycocyanin eluted (24). In particular, the value of a, which represents the red value, was also increased. It was reported that the spirulina analysis

Table 4. Chromaticity measurement of spirulina during yeast fermentation and cold storage

| Time (day) | L value | a value | b value |
|--------------------|--------------------------|------------------------|-------------------------|
| Yeast fermentation | | | |
| 0 | 63.12±0.09 ¹⁾ | 0.23±0.09 ^f | 23.14±0.08 ^f |
| 1 | 60.21±0.18 ^b | 0.31±0.10 ^e | 24.51±0.07 ^e |
| 2 | 57.69±0.15 ^c | 0.41±0.19 ^d | 25.25±0.04 ^d |
| Cold storage | | | |
| 1 | 55.05±0.08 ^d | 0.89±0.05 ^e | 28.83±0.09 ^e |
| 3 | 54.12±0.12 ^e | 1.25±0.11 ^b | 31.76±0.11 ^b |
| 5 | 53.95±0.08 ^e | 1.69±0.09 ^a | 33.24±0.12 ^a |

¹⁾Values with different letters in the same column significantly different (analyzed using ANOVA and Duncan's multiple range test: p<0.05, a>b>c>d>e>f).

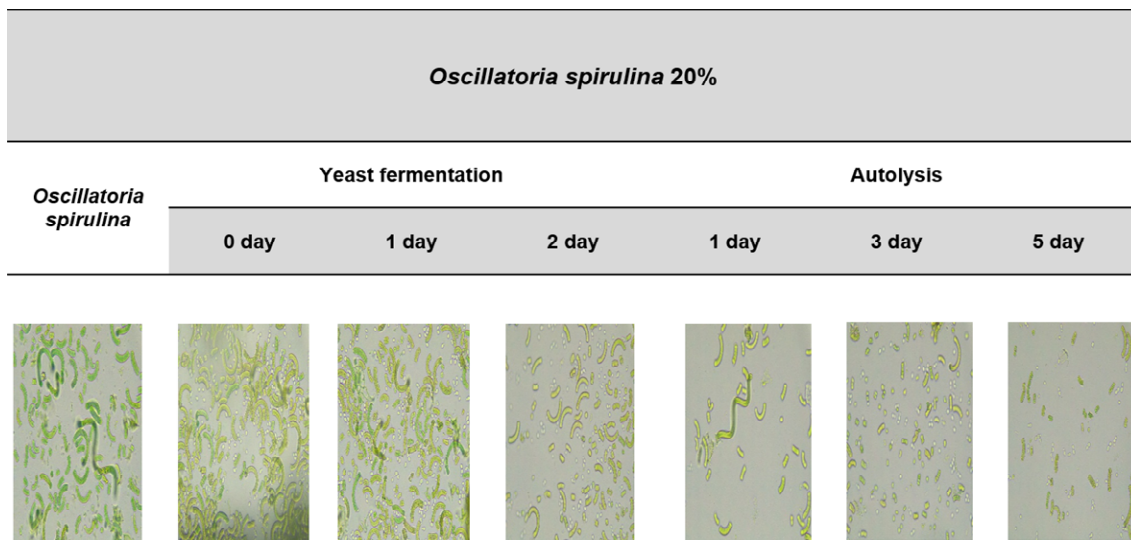


Fig. 2. Morphology of spirulina during yeast fermentation and cold storage.

showed that the anthocyanin in spirulina was eluted and reddish (39).

Morphology

Fig. 2 shows spirulina morphology during yeast fermentation and cold storage process of spirulina paste. As the yeast fermentation proceeded spirulina could not maintain the spiral shape, showing fragmented shape. It is inferred that spirulina was destroyed by shaking culture of yeast fermentation (24). It was also confirmed that spirulina was destroyed more by cold storage at refrigeration temperature. This is similar to the results of a study that cold storage of algae was performed at refrigeration temperature (40). Conclusively, it was judged that the morphological change of spirulina by yeast fermentation and cold storage could enhance the production of physiologically active substances.

요 약

스피루리나 paste (20%)에 *K. servazzii* KM2017 효모를 접종하여 30°C에서 2일간 진탕배양 한 후 1°C에서 5일 동안 보관하면서 생리활성물질의 변화를 측정하였다. 스피루리나 발효물의 pH는 효모 발효 2일에 감소한 후 저온 숙성과정에서 약간 증가하였다. 초기 산도는 0.26%에서 효모 발효 2일 동안 0.51%로 증가한 후 저온 숙성과정에서 산도가 감소하였다. 수용성 고형분 함량은 9.4 °Brix에서 효모 발효 중에 감소한 후 저온숙성과정에서 약간 증가하였다. 환원 당은 초기 4.97%에서 효모 발효 2일에 0.68%로 급격하게

감소한 후 저온숙성 5일에 1.53%로 증가하였다. 항산화활성은 DPPH 라디칼 소거능 측정에서 초기 IC₅₀ 값이 2.48 mg/mL에서 효모발효와 저온숙성 후에 각각 1.21 mg/mL, 0.88 mg/mL로 감소하였다. Polyphenol함량은 초기 213.79 µg/mL에서 효모 발효 후에 350.84 µg/mL로 증가하였으며, 5일 동안 저온숙성에 의해서 420.55 µg/mL로 증가하였다. Flavonoid함량은 초기 23.26 µg/mL에서 5일 동안 저온숙성에 의해서 34.01 µg/mL로 증가하였다. 스피루리나 발효물의 색도 값 측정에서 L값은 효모발효와 저온 숙성중에 감소하였으며, 반면에 a, b 값은 증가하였다. C-phycoyanin, allophycocyanin, phycoerythrin색소의 초기 함량은 각각 0.078, 0.055, 0.150 mg/g에서 5일 동안 저온 숙성 후에 0.144, 0.126, 0.192 mg/g로 증가하였다. 스피루리나 발효물은 초기 효모 생균수 3.24×10⁶ CFU/mL에서 발효 1일에 1.21×10⁸ CFU/mL로 증가한 후 발효 2일에 5.33×10⁷ CFU/mL로 감소하였으며, 저온 숙성 5일에 효모 생균수는 4.39×10⁶ CFU/mL로 크게 감소하였다.

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