

## Pilot-scale production of *Omija-cheong* by low temperature incubation: An assessment of quality characteristics

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### 저온숙성 방법에 따른 생오미자 당절임 농축액인 오미자청의 파일럿 규모 생산 및 품질특성

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#### Abstract

*Omija-cheong*, concentrated extracts from sugar-treated *Omija* fruit (*Schisandra chinensis* Baillon), is produced by traditional manner in Korea. The quality characteristics of *Omija-cheong* processed at low temperature with a pilot-scale were investigated to optimize the incubation time. With increasing incubation time in processing *Omija-cheong*, the pH level of *Omija-cheong* remained constant, while titratable acidity and organic acids increased. Fresh *Omija* fruits contained citric, malic and succinic acids, most of which were extracted into concentrated extracts after 37 days of incubation and reached to the stable concentration after 47 days of incubation. Titratable acidity in *Omija-cheong* gradually increased from 1.18% to 2.71%, and also was correlated with total concentration of organic acids. About 80% of supplemented sucrose for manufacturing *Omija-cheong* was converted into glucose and fructose until 68 days of incubation, and the composition of free sugars was maintained to be stable up to 138 days of incubation. The contents of total flavonoids and phenolic compounds in *Omija-cheong* were 24.1 mg-GAE/L and 1,635 mg-QE/L at 57 days of incubation, which were more than 9 and 5 times higher than those in *Omija* fruits, respectively. From the quality characteristics in processing *Omija-cheong* by low-temperature incubation, more than 60 days of incubation is required for the constant quality and value-added beverage.

**Key words** : sugar-treated *Omija*, pilot-scale production, *Omija-cheong*, *Schisandra chinensis*

#### INTRODUCTION

*Schisandra chinensis* Baillon, is a deciduous woody vine that is native to forests in eastern Asian countries such as in northwestern China, far-eastern Russia, Korea and Japan. The deep red colored berries have five distinct tastes; sourness, sweetness, bitterness, saltiness and pungency.

Because of the five distinct tastes, *S. chinensis* is known as “*Omija*” in Korean and “*wu wei zi*” in Chinese. The berries are used in traditional Chinese medicine and act as an astringent for the lungs and kidneys, to generate body fluid, and to reduce thirst (1). In Russia, *S. chinensis* has been used as an adaptogen to increases physical working capacity and protect against a broad spectrum of harmful factors such as heat shock, skin burn, cooling, frostbite, aseptic inflammation, irradiation and heavy metal intoxication (2).

The active ingredients of *S. chinensis* are lignans such as schisandrins and gomisins, which have been isolated and quantified from the seeds (3,4). Various biological activities of lignans from *Omija* berries have been investigated such

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as anti-oxidative protection (5,6), improvement of circulation and blood flow for cardiovascular disorder (7), enhancement in memory and learning (8), and hypnotic and sedative effect (9). However, the active ingredients in ethanolic extracts from the air-dried fruit or seed of *S. chinensis* appear to be fat-soluble which limit their bioavailability (10). On the other hand, the color of *Omija* fruit derived from anthocyanins and flavonols has been identified and characterized (11-13). Water-based extracts from the fruit of *S. chinensis* also exhibit a wide range of biological functions such as anti-oxidative activities (12-14), antimicrobial activity (15), alcohol metabolizing activity (16) and stress treatment (17).

Nowadays, the dried fruit of *S. chinensis* has been commercialized as *Omija*-tea worldwide. In Korea, beverages such as *Omija-cheong* and *Omija* wines with fresh and/or dried fruits of *S. chinensis* are popular. *Omija-cheong*, known as a natural extract of *S. chinensis*, is produced in traditional manner, and is used for cold drinks and tea by diluting with drinking water. In order to produce *Omija-cheong* in traditional manner, equal quantities of fresh *Omija* fruits and sucrose are mixed and incubated at around 15°C for 1 to 6 months. The low temperature incubation was conventionally used to prevent microbial spoilage and undesired fermentation (18). However, there is little information on the quality characteristics of *Omija-cheong* as a commercialized beverage in Korea. The aim of this study was to establish the optimal incubation time for manufacturing *Omija-cheong* by pilot-scale in Korean traditional manner, based on the physicochemical characteristics of *Omija-cheong* as a concentrated extract.

## Materials and methods

### Omija fruits and other materials

Fresh *Omija* fruits were provided by Jangsu County (Jeonbuk, Korea), which were cultivated in Jangsu County and harvested in 2014. Food-grade white sucrose was supplied from CJ CheilJedang (Seoul, Korea). Reference standards of fructose, glucose, sucrose, maltose, oxalic acid, citric acid, malic acid, succinic acid, and gallic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and quercetin was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Folin-Ciocalteu's phenol reagent was purchased from Sigma-Aldrich Chemical Co. The solvents for high-performance liquid chromatography (HPLC) and other chemicals were analytical grade reagents. Manufacturing

equipment for *Omija-cheong* such as a bubble washer, a conveyor with air blower, 1,000 kg-scale incubation tanks with temperature control (Motiontec, Daejeon, Korea) were kindly provided by the Jangsu Center for Agricultural Technology (Jeonbuk, Korea).

### Preparation of crude extract and soluble extracts from *Omija* fruits

The harvested fresh fruits were washed with tap and deionized water, blended with an electrical blender, and then used as crude samples. The crude samples were centrifuged and the supernatant were used as crude extracts for analyzing physicochemical characteristics. Soluble extracts were prepared by first removing the stems and seeds from washed *Omija* fruits manually, and 3 g of the flesh was soaked in 3 mL of deionized water, and sonicated for 30 min. Insoluble materials were removed by centrifugation and the supernatant was filtered for preparing soluble extracts. The soluble extracts of *Omija* fruits were used for determining the compositions of free sugars and organic acids using HPLC.

### Manufacturing *Omija-cheong* and preparation of concentrated extracts

Fresh *Omija* fruits were washed with tap water using a bubble washer (Samjin Plant, Gyeonggi, Korea). Excess water was drained and dried using the mesh-belt conveyor with an air blower. The washed *Omija* fruits (500 kg) were weighed and put into a 1,000 kg-scale incubation tank alternating with sucrose (500 kg) in equal quantities. For this study, we used a water-jacketed type incubation tank for a temperature control and an agitation system for intermittent mixing. The temperature of the incubation tanks was maintained at 15°C. The samples collected from the incubation tank were centrifuged to remove insoluble materials and used as concentrated extracts.

### Determination of moisture content, pH, total sugar content, and titratable acidity

Moisture content of *Omija* fruits was measured using a moisture analyzer (ML-50, AND Co., Ltd., Tokyo, Japan). The soluble extracts and concentrated extracts were diluted 5 times with deionized water and pH levels were measured using a pH meter (Orion Star A211, Thermo Scientific, Waltham, MA, USA). Measurement of total sugar content (°Brix) was carried out with soluble extracts and concentrated extracts by using a refractometer with an accuracy of  $\pm 0.2$  °Brix (PAL-1, ATAGO, Tokyo, Japan). According to the

detection range of the refractometer, the concentrated extracts were diluted 2 times with deionized water. For measuring titratable acidity, the samples were diluted 10 times with deionized water and followed by titration with 0.1 N sodium hydroxide, using phenolphthalein as an end-point indicator. Titratable acidity was calculated and expressed in % (w/v) of acetic acid.

#### Measurement of color and color difference

The color and color difference were analyzed with a Chroma Meter (CM-5, Konica Minolta, Tokyo, Japan) using the Hunter system, which identifies color using three attributes: L (white=100, black=0), a (red=positive, green=negative), and b (yellow=positive, blue=negative). The color difference ( $\Delta E$ ), a measure of the distance in color space between two colors, was determined by comparison to a deionized water with colorimeter values of L=99.98, a=-0.03, and b=0.01, using the following relationship:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

#### Analyses of free sugars and organic acids by HPLC

For quantification of free sugar, the HPLC (Waters, Milford, MA, USA) system combined with a 410 differential refractometer was used with a LC-NH<sub>2</sub> column (Supercosil™, 4.6  $\Phi$ ×250 mm, Supelco, Bellefonte, PA, USA). The solvent system, consisting of an 80:20 acetonitrile to water ratio, was run isocratically at a flow rate of 0.8 mL/min. Fructose, glucose, sucrose and maltose were used for establishing standard curves. For analyzing organic acids, the HPLC system combined with a 486 Tunable absorbance detector ( $\lambda$ =210 nm) was used with a HPX-87H column (Aminex®, 7.8  $\Phi$ ×300 mm, Bio-Rad Lab, Richmond, CA, USA). The solvent system, consisting of 0.01 M sulfuric acid, was run isocratically at a flow rate of 0.4 mL/min. Citric, oxalic, malic, succinic and acetic acid were used for establishing standard curves. The soluble extracts and concentrated extracts were injected to a HPLC system after filtration with a 0.45  $\mu$ m membrane filter (Millipore Co., Billerica, MA, USA).

#### Total flavonoids content

Total flavonoid contents of *Omija* fruits and *Omija-cheong* were determined using an aluminum nitrate colorimetric method (19) with minor modifications. An aliquot of the sample diluted with deionized water (0.5 mL) was mixed with 0.75 mL of 95% of ethyl alcohol and 0.75 mL of deionized water. Then 0.1 mL of 10% aluminum nitrate, 0.1

mL of 1 M potassium acetate, and 2.8 mL of deionized water were added and mixed. After incubation for 40 min at room temperature, the absorbance at 415 nm was measured using a spectrophotometer (Optizen POP, Mecasys Co., Daejeon, Korea). The standard curve was prepared with quercetin solutions at 0~300 mg/L in 95% ethyl alcohol, and total flavonoid values were expressed in terms of quercetin equivalent (QE).

#### Total content of phenolic compounds

Total phenolic compound contents of *Omija* fruits and *Omija-cheong* were determined by the Folin-Ciocalteu colorimetric method (20) with minor modifications. An aliquot of the sample diluted with deionized water (0.1 mL) was mixed with 0.5 mL of Folin-Ciocalteu's phenol reagent and 6.0 mL of deionized water. After incubation for 2 min at room temperature, 2 mL of 15% sodium carbonate and 1.4 mL of deionized water were added and mixed. After incubation for 120 min at room temperature, the absorbance was measured at 755 nm. The calibration curve was prepared with 0~170 mg/L solutions of gallic acid in 95% ethyl alcohol, and total phenolic values were expressed in terms of gallic acid equivalent (GAE).

#### Statistical analysis

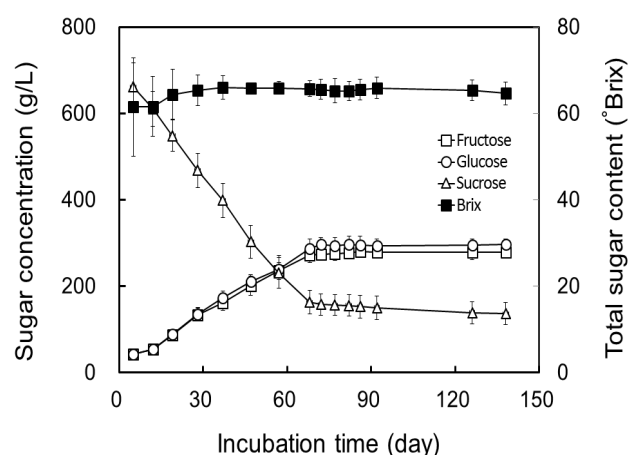
All experiments were performed in triplicates and mean values and standard deviations were reported. Analysis of variance was conducted and the mean separations were analyzed using the Duncan's multiple range tests ( $p < 0.05$ ). The statistical analyses were conducted using the SPSS for Windows 12.0 software (SPSS Inc., Chicago, IL, USA).

## Result and discussion

#### Total sugar content and composition of free sugars in *Omija* fruits and *Omija-cheong*

Moisture contents of fresh *Omija* fruits was measured to be 80.0%. Previous studies have reported moisture content of fresh fruits had in the range of 79.6% to 86.4% (21,22) which are similar with the results in this study. Total sugar content of crude extract from fresh *Omija* fruits was determined to 9.7 °Brix, which was slightly lower than previously reported (22). As shown in Fig. 1, total sugar contents of *Omija-cheong* ranged from 61.5 to 65.3 °Brix during 28 days of incubation, and stabilized at around 66.0 °Brix after 37 days of incubation. Based on a traditional

manner, equal quantities of fresh *Omija* fruits (500 kg) and sucrose (500 kg) were alternatively mixed in 1,000 kg-scale incubation tank for preparing *Omija-cheong*. High content of sucrose in processing *Omija-cheong* at low temperature needs time to not only solubilize sucrose but also extract the soluble portions of *Omija* fruits by osmotic pressure of sucrose. The status of sucrose at initial stage in processing *Omija-cheong* was a major limit in mixing, and caused the fluctuation in total sugar content. The composition of free sugars in *Omija* fruits were determined to 18.5 g/L of fructose, 16.6 g/L of glucose, and 15.1 g/L of sucrose by using soluble extracts. The major sugar components of fresh fruits harvested in 2005 from Jangsu County (Korea) were well matched with the results of our study (23). Decreases in sucrose content with increases in both fructose and glucose contents in fresh *Omija* fruits also have been previously reported, which depend on the postharvest ripening conditions (23). The concentration of supplemented sucrose decreased with increases in both fructose and glucose according to the incubation time in processing *Omija-cheong* (Fig. 1). In our study, the conversion of sucrose into glucose and fructose continued to 68 days of incubation, and was minimized after 68 days of incubation. The composition of free sugars in *Omija-cheong* was maintained until 138 days of incubation, and almost 80% of supplemented sucrose was converted into glucose and fructose. Changes of sugar composition in fermented *Omija* with sucrose have been reported, where 50.3% of the supplemented sucrose was exhausted and has been converted into 22.9% of glucose and 21.5% of fructose during 6 months of fermentation (24). Based on the changes of free sugars in manufacturing *Omija-cheong*, it requires at least 68 days of incubation at a given condition.



**Fig. 1.** Changes of free sugars in processing *Omija-cheong* according to the incubation time.

#### pH, titratable acidity and composition of organic acids in *Omija* fruits and *Omija-cheong*

pH, titratable acidity, and composition of organic acids in *Omija* fruits and *Omija-cheong* are summarized in Table 1. The pH levels of *Omija* fruits was 3.07, which was slightly higher than reported in previous studies with fresh fruits (22). The pH of *Omija* fruits was also affected by postharvest conditions and gradually decreased from 2.81 to 2.68 according to the postharvest time (23). The pH level at initial stage (5 days of incubation) in processing *Omija-cheong* was 2.96 which slightly lower than that of *Omija* fruits, and slightly decreased to 2.90 at 92 days of incubation. Titratable acidity of fresh *Omija* fruits was determined to be 1.16% (w/v). According to the processing *Omija-cheong*, titratable acidities gradually increased from 1.18% (w/v) at 5 days, to 2.71% at 138 days of incubation. The organic acids in *Omija* fruits were 19.2 g/L of citric acid, 6.30 g/L of malic acid, and 159 g/L of succinic acid with the soluble extracts.

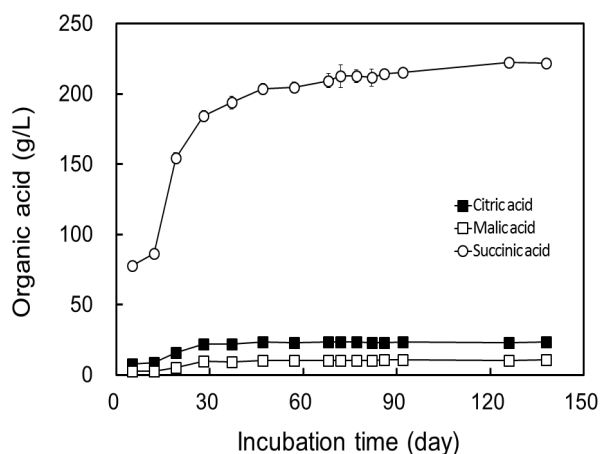
**Table 1.** pH, titratable acidity and composition of organic acids in *Omija* fruits and *Omija-cheong*

Day	pH	Titratable acidity (%)	Organic acid (g/L)		
			Citric acid	Malic acid	Succinic acid
Fresh fruit <sup>1)</sup>	3.07±0.02 <sup>b2)</sup>	1.16±0.02 <sup>a</sup>	19.2±1.62 <sup>c</sup>	6.30±0.63 <sup>c</sup>	159±5.40 <sup>b</sup>
5	2.96±0.08 <sup>a</sup>	1.18±0.06 <sup>a</sup>	7.91±0.16 <sup>a</sup>	2.64±0.23 <sup>a</sup>	77.6±1.31 <sup>a</sup>
19	2.93±0.04 <sup>a</sup>	1.90±0.34 <sup>b</sup>	15.7±1.55 <sup>b</sup>	5.26±0.81 <sup>b</sup>	154±3.85 <sup>b</sup>
37	2.92±0.04 <sup>a</sup>	2.26±0.19 <sup>c</sup>	22.2±0.95 <sup>d</sup>	9.25±0.32 <sup>d</sup>	194±4.45 <sup>c</sup>
68	2.87±0.05 <sup>a</sup>	2.67±0.11 <sup>d</sup>	23.2±0.49 <sup>d</sup>	10.5±0.36 <sup>c</sup>	209±4.91 <sup>d</sup>
92	2.90±0.05 <sup>a</sup>	2.70±0.13 <sup>d</sup>	23.4±0.46 <sup>d</sup>	10.8±0.68 <sup>c</sup>	215±0.85 <sup>d</sup>
138	2.88±0.03 <sup>a</sup>	2.71±0.12 <sup>d</sup>	23.2±0.53 <sup>d</sup>	10.2±0.48 <sup>c</sup>	222±2.05 <sup>e</sup>

<sup>1)</sup>Soluble extract from fresh *Omija* fruits, a raw material for *Omija-cheong* processing.

<sup>2)</sup>The values with different superscripts within a column are significantly different ( $p < 0.05$ ) by Duncan's multiple range tests.

Succinic acid was a major organic acid in this study, which is well matched with the previous report (23). The contents of organic acids were also affected by the postharvest ripening conditions, and the quantities of organic acids increased two-fold after 8 days of postharvest storage. On the other hand, fresh *Omija* fruit purchased from Hongcheon County (Korea) in 1998 showed a different composition of organic acids with 0.33% citric acid and 3.87% malic acid (25). Water-based extracts from dried *Omija* fruits contained 3.9~16.1% of citric acid as a major organic acid with 0.023~4.6% of succinic acid as a minor organic acid (12,25,26). The differences in composition of organic acids might be dependent on the varieties of *S. chinensis*, conditions of cultivation and postharvest, status of dehydration and experimental conditions. The changes of organic acids in concentrated extracts from processing *Omija-cheong* are shown in Fig. 2. Most of the citric, malic, and succinic acids in fresh *Omija* fruits were extracted into *Omija-cheong* rapidly within 37 days of incubation and reached to the stable concentrations after 47 days of incubation. The increase in titratable acidity was directly dependent on the contents of total organic acids in *Omija-cheong* during processing ( $r=0.97$ ,  $p<0.01$ ). Based on the changes of organic acids contents during *Omija-cheong* processing, it requires at least 47 days of incubation for constant quality at a given condition.



**Fig. 2.** Changes of organic acids in processing *Omija-cheong* according to the incubation time.

### Color and color difference

The Hunter index for color characteristics of *Omija* fruits and *Omija-cheong* were quantified by lightness (L), redness (a), yellowness (b), and summarized in Table 2. As a major color of *Omija* fruits with soluble extracts, the value of redness was 49.2, whereas the values of lightness and yellowness

were 19.0 and 32.8, respectively. In processing *Omija-cheong*, the value of redness was around 68.0 until 19 days of incubation, and gradually decreased to 60.1 after 68 days of incubation. The Hunter index for lightness, redness, and yellowness were stabilized and maintained at around 43.0, 60.1, and 25.9 after 68 days of incubation, respectively. The variation in Hunter index at early stage of *Omija-cheong* processing might be related to the status of sucrose which was not dissolved yet. The colors of anthocyanins from *S. chinensis* fruits were reported to be depended on both thermal degradation and ultraviolet radiation (13,27). However, the Hunter index of *Omija-cheong* in this study was remained to be constant because the incubation was carried out with stainless tank which prevents sun-light. Based on the color characteristics, it requires more than 68 days of incubation for *Omija-cheong* manufacture at a given condition.

**Table 2.** Color and color differences of *Omija* fruits and *Omija-cheong*

Day	Hunter Index			
	Lightness (L)	Redness (a)	Yellowness (b)	Color difference ( $\Delta E$ )
Fresh fruit <sup>1)</sup>	19.0±0.10 <sup>2d)</sup>	49.2±0.10 <sup>a</sup>	32.8±0.15 <sup>c</sup>	62.1±0.08 <sup>a</sup>
5	62.9±1.00 <sup>c</sup>	66.2±1.80 <sup>c</sup>	18.2±0.87 <sup>a</sup>	93.1±1.83 <sup>d</sup>
19	56.4±1.93 <sup>d</sup>	68.0±1.93 <sup>c</sup>	24.4±2.15 <sup>b</sup>	91.7±2.47 <sup>d</sup>
37	49.2±1.60 <sup>c</sup>	65.9±1.53 <sup>c</sup>	24.2±1.05 <sup>b</sup>	85.7±1.59 <sup>c</sup>
68	43.0±1.81 <sup>b</sup>	60.1±1.45 <sup>b</sup>	25.9±1.36 <sup>b</sup>	78.3±2.17 <sup>b</sup>
92	44.8±1.56 <sup>b</sup>	60.4±1.56 <sup>b</sup>	25.8±1.30 <sup>b</sup>	79.6±0.78 <sup>b</sup>
138	43.8±1.55 <sup>b</sup>	60.9±1.21 <sup>b</sup>	25.3±1.57 <sup>b</sup>	79.2±1.13 <sup>b</sup>

<sup>1)</sup>Soluble extract from fresh *Omija* fruits, a raw material for *Omija-cheong* processing

<sup>2)</sup>The values with different superscripts within a column are significantly different ( $p<0.05$ ) by Duncan's multiple range tests.

### Flavonoids and phenolic compounds in *Omija* fruits and *Omija-cheong*

As bioactive ingredients, total flavonoids and phenolic compounds in *Omija* fruits and *Omija-cheong* were analyzed with gallic acid and quercetin as standards, and are shown in Fig. 3. The flavonoid concentration in crude extracts from *Omija* fruits was quantified to be 2.91 mg-GAE/L. The concentration of flavonoids in *Omija-cheong* was 6.50 mg-GAE/L at 5 days of incubation, and dramatically increased to 22.2 mg-GAE/L at 37 days of incubation. The concentration of flavonoids in *Omija-cheong* was maintained at around 24.1 mg-GAE/L after 57 days of incubation. The content of flavonoids in *Omija-cheong* was more than 8 times higher than that in *Omija* fruits. The concentration of phenolic compounds was 297 mg-QE/L in *Omija* fruits. The

concentration of phenolic compounds in *Omija-cheong* was 439 mg-QE/L at 5 days of incubation, and increased to 1,322 mg-QE/L at 28 days of incubation. The concentration of phenolic compounds in *Omija-cheong* was maintained at around 1,635 mg-QE/L after 57 days of incubation. The content of phenolic compounds in *Omija-cheong* was more than 5 times higher than that in *Omija* fruits. The increases in both flavonoids and phenolic compounds during *Omija-cheong* processing might be due to the extended incubation time with sucrose. The wide ranges in the contents of flavonoids and phenolic compounds depended on extraction methods with solvents from *S. chinensis* (13,28-30). The changes of phenolic compounds showed a similar profile as that of flavonoids during *Omija-cheong* processing (Fig. 3). Based on the changes of flavonoids and phenolic compounds in manufacturing *Omija-cheong*, it requires at least 57 days of incubation for stabilized concentrations at a given condition.

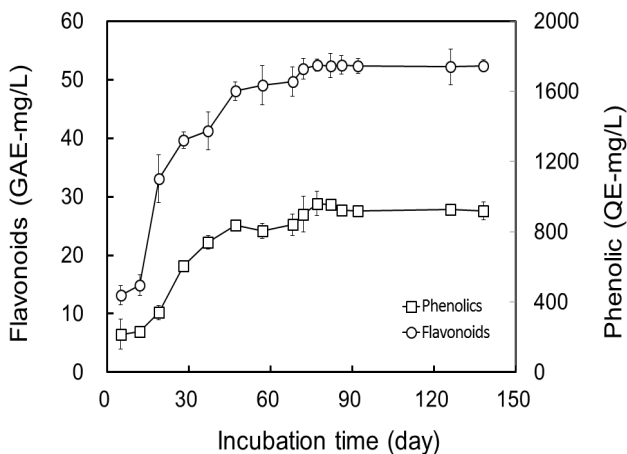


Fig. 3. Contents of flavonoids and phenolic compounds in manufacturing *Omija-cheong*.

## 요 약

전통적인 저온숙성 당절임 방법을 이용한 오미자청의 제조과정에서 최적의 배양시간을 찾기 위하여 파일럿 규모 (1톤)의 연구를 시행하였다. 생오미자와 동일한 량으로 투입된 설탕은 배양시간에 따라 과당과 포도당으로 전환되었으며 오미자청에 존재하는 유리당의 조성은 68일 이상의 배양기간에서는 일정하게 유지되었다. 배양기간에 따른 오미자청의 pH는 일정한 수준을 유지한 반면, 적정 산도와 유기산의 함량은 배양시간 37일까지 지속적으로 증가한 이후 68일 이후에는 일정한 조성을 유지하였다. 생오미자에 존재하는 주요 유기산은 숙신산으로 확인되었으며 오미

자청에 존재하는 유기산의 조성 역시 생오미자와 유사하였다. 오미자에 존재하는 주요 생리활성물질인 총 플라보노이드와 폴리페놀화합물은 60일 이상 배양한 오미자청에서 높은 함량을 보이고 138일까지 일정한 수준을 유지하였다. 특히, 오미자청에 함유된 총 플라보노이드와 폴리페놀화합물의 함량은 생오미자 과육에 비하여 각각 9배와 5배 정도 높은 수준으로 증가하였다. 생오미자를 저온숙성 당절임 방법을 적용하여 오미자청으로 제조·가공하는 과정에서 유리당, 유기산 및 생리활성물질 등에 대한 일정한 품질을 확보를 위해서는 최소 60일 이상의 배양기간이 필요한 것으로 확인되었다. 이러한 연구결과는 전통적인 저온숙성 당절임 방법으로 제조·가공되는 오미자청의 고품질화와 기능성 음료 개발을 위한 제조·가공공정의 표준화에 유용한 정보가 될 것으로 판단된다.

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