FERMENTATION CHARACTERISTICS OF CAMPBELL EARLY GRAPE WINE INOCULATED WITH INDIGENOUS KOREAN WINE YEASTS ENCAPSULATED IN CA-ALGINATE BEADS AFTER AIR-BLAST DRYING

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ABSTRACT

The aim of this study was to test the possibility of using yeast cells encapsulated in calcium alginate (Ca-alginate) beads as a starter for wine fermentation. Characteristics of Korean Campbell Early wines fermented by five free yeast cell types and those encapsulated in 2% Ca-alginate beads were compared using physicochemical analyses and sensory evaluation tests. The encapsulated yeast cells were shown to ferment Korean Campbell Early grapes with a similar efficiency as that exhibited by the five free yeast cell-types. After fermentation, the characteristics of free cells and encapsulated cells did not show significant differences in terms of content of reducing sugars, soluble solids, total acids, organic acids, and free sugars, as well as in terms of viable cell numbers and other physicochemical properties. The encapsulated cells did, however, produce more alcohol than the free cells. Encapsulation in 2% Ca-alginate beads was furthermore found to decrease the production of negative volatile compounds. The sensory evaluation of wines fermented by free cells compared with those fermented by Ca-alginate bead-encapsulated cells yielded similar scores for the following properties: color, taste, flavor, and overall preference. Overall, no significant differences were observed between the two grape wines, and yeast cells encapsulated in 2% Ca-alginate beads therefore showed high stability and served as an effective yeast starter for wine fermentation.

Keywords: Campbell Early grape, wine, calcium alginate bead, yeast cell immobilization, air-blast drying

1. INTRODUCTION

The Campbell Early grape (*Vitis labrusca* cultivar) is a major grape type used in winemaking and constitutes about 70% of the total grape production in Korea (SEO *et al*., 2007; HONG and PARK, 2013). Although the demand for domestic wine in Korea is increasing, the quality of Korean wines remains unreliable owing to high acidity and low sugar content in the wines as well as color weakness of the Campbell Early grape (KIM *et al*., 2017; LEE and KIM 2006; PARK *et al*., 2004). Owing to the short history of wine consumption and the wine industry in Korea, studies on these wines are in the preliminary stages (LEE *et al*., 2006; SEO and YOOK, 2007). Several researchers have recently attempted to address the problems associated with indigenous yeast and winemaking using Campbell Early grapes that have adapted to the Korean environment (HONG and PARK, 2013; LEE *et al*., 2004; PARK *et al*., 2004).

The wine fermentation process depends on the ability of yeast to convert grape sugars into alcohol and other compounds (PADILLA *et al*., 2016; ROMANO *et al*., 2003). Several studies have reported that indigenous yeasts can improve the sensory properties and the quality of local wines (CHAROENCHAI *et al*., 1997; ESTEVE-ZARZOSO *et al*., 1988; HONG and PARK, 2013; LEE *et al*., 2016). However, most Korean wines are fermented using an imported yeast starter (CHOI *et al*., 2011; KIM *et al*., 2007). The identification of a suitable Korean indigenous yeast in winemaking is an important goal for the local wine industry. Previously, *Saccharomyces cerevisiae* D8, M12, S13, *Hanseniaspora uvarum* S6 (previously SS6), and *Issatchenkia orientalis* KMBL5774 were isolated from Korean grapes to improve the wine quality and were found to enhance the local wine quality (HONG and PARK, 2013; KIM, 2006; SEO *et al*., 2007).

Techniques for drying microorganisms include freeze-drying, spray-drying, and fluidized bed-drying (BARBOSA *et al*., 2015; PODDAR *et al*., 2014). However, each of these techniques is associated with reduced microorganism viability. Recently, alternative drying processes have gained interest owing to their lower costs and faster processing times, as compared to freeze-drying (PODDAR *et al*., 2014; SANTIVARANGKNA *et al*., 2007). Immobilized yeast strains have been optimized based on properties such as survival rate, fermentation ability, viability, and ease of handling without the need for specialized laboratories (CAYLAK and SUKAN, 1998). A potential application of such immobilized yeasts is the use of Korean indigenous yeasts as starter cultures in the local wine industry. Different encapsulation techniques have been studied for the preservation and subsequent application of yeast cells (AKIN, 1987; CASSIDY *et al*., 1996; COLAGRANDE *et al*., 1994). Over the last three decades, cell immobilization specific to winemaking has been extensively studied owing to the technical and economic advantages such systems may offer over free cell systems (MARGARITIS *et al*., 1983; STEWART and RUSSELL, 1986; TSAKIRIS *et al*., 2004). The use of yeast immobilized in gel-forming materials such as calcium alginate (Ca-alginate), agar, carrageenan, cellulosic materials, and pectic acid in winemaking has been well documented (COLAGRANDE *et al*., 1994), and of the various reported methods, immobilization of microbial cells by entrapment in Ca-alginate gels is the most widely used approach. This is an attractive technique for various biotechnology, biomedicine, and food technology applications (DE VOS *et al*., 2009; KREGIEL *et al*., 2013; ROKSTAD *et al*., 2014). Ca-alginate encapsulation offers several advantages over the use of free cells: increased functional efficiency with high cell concentrations in the reactor, easy separation of the immobilized cells in the settling tank, short fermentation lag period, and increased stability of the fermentation system (BARDI *et al*., 1996). Several studies in yeast have reported improved survival rates after reduction of moisture content (BEKER and RAPOPORT, 1987; LIEVENSE *et al*., 1992; LIEVENSE *et al*., 1994), indicating that reduced moisture content enhances the preservation of yeast cells.

We previously showed that the immobilization of yeast cells via encapsulation in Ca-alginate beads yielded high survival rates and excellent storability (KIM *et al*., 2017). The aim of this study was thus to investigate the feasibility of using air-blast-dried Ca-alginate bead-encapsulated cells compared to the use of free yeast cells in wine fermentation.

2. MATERIALS AND METHODS

2.1. Strains and medium

Saccharomyces cerevisiae D8 (KACC 93245P), M12 (KACC 93246P), and S13 (KACC 93247P); *Hanseniaspora uvarum* S6 (previously, SS6 and KACC 93248P); and *Issatchenkia orientalis* KMBL5774 (*Pichia kudriavzevii* KMBL5774 and KACC 93124P) were obtained from the Food Microbial Biotechnology Laboratory, Department of Food Science and Biotechnology, Kyungpook National University (Daegu, South Korea). The yeasts were cultured aseptically in 100 mL YPD broth [2% yeast extract, 1% peptone, and 2% glucose (w/v); 2% (w/v) agar was added for solid medium] in a rotary shaker (JSSI-300C, JS Research Inc., Gongju, South Korea) at 30°C for 24 h. All yeast strains were stored in 15% glycerol at -80°C. Ca-alginate beads were prepared using a previously described method (KIM *et al*., 2017). In this study, 2% Ca-alginate bead-encapsulated cells were used and the encapsulated cells showed at least a 51% survival rate when stored at 4°C for 3 months (KIM *et al*., 2017).

2.2. Release and viability of encapsulated Ca-alginate beads

Beads containing yeast cells were rehydrated in 10 mL YPD broth and the release of the yeast cells from the 2% Ca-alginate beads was monitored for 48 h using an optical microscope (Nikon Eclipse, TE2000-U, Melville, NY, USA). After the yeast cells were released from the beads, a 1 mL sample of the resulting yeast culture was serially diluted in a 0.85% (w/v) NaCl solution.

2.3. Viable cell count

Samples were serially diluted with 0.85% NaCl using the appropriate dilution factors. Each sample was spread onto YPD agar medium plates. The plates were cultured at 30°C for 48 h, and the number of white colonies that formed on the YPD agar was counted to provide the number of colony forming units (CFU) (dilutions gave 30 to 300 CFU/mL).

2.4. Vinification processing

Campbell Early grapes (*Vitis labrusca* cultivar) were obtained from Sangju, Kyungpook province, South Korea in 2016. The vinification process was assessed using a general wine-making method (HONG and PARK, 2013). The grapes were washed, stemmed, crushed, and treated with potassium metabisulfite ($K_2S_2O_5$; 200 ppm) to inhibit harmful bacterial and yeast growth. The sugar content off the grape must was raised from 16.5° Brix (w/v; pH 3.2) to 24° Brix by the addition of sucrose. Following inoculation with the two types of yeast cells, namely a 24 h yeast pre-culture or cells immobilized in Ca-alginate beads [5% inoculum (w/v)], fermentation was carried out at 18°C for 14 days in glass bottles (5,000 mL volume) equipped with an airlock using 3,000 mL of grape must. Post fermentation,

free yeast cells, 2% Ca-alginate bead-encapsulated cells, and other lees were eliminated by centrifugation at 6,000 × *g* for 10 min.

2.5. Standard chemical analysis

The pH of the wine was measured using a pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland). The soluble solid content (°Brix) was measured using a refractometer (RA250, ATAGO, Tokyo, Japan), the alcohol content was measured at 15°C using a vinometer, and the total acid content and reducing sugars were quantified using the AOAC method (CAPUTI, 1995). Total phenolic compounds (TPC) were assessed using the Folin-Ciocalteau phenol reagent method (SINGLETON and ROSSI, 1965) and the free sugar content was determined by high-performance liquid chromatography (HPLC) using a Sugar-Pak I column (⌀ 6.5 × 300 mm, Waters, Milford, MA) and a Ca-EDTA buffer (50 mg/L) at a flow rate of 0.5 mL/min. Organic acids were quantified by HPLC using a Shodex RSpak KC-811 column (⌀ 8 × 300 mm, Showa Denko KK, Kawasaki, Japan). The column was run with a mobile phase of 0.1% phosphoric acid at a flow rate of 1 mL/min at 40°C. Organic acids were detected using a refractive-index detector. Acetaldehyde, methanol, and various alcohols (fusel oil) were assessed using gas chromatography (6890N GC; Agilent, Santa Clara, CA, USA) and a flame ionization detector (FID). After distillation, samples were filtered through a membrane filter (Millex-HV, 0.45 μm, Millipore Co., Bedford, MA, USA) before injection. Separation was performed with an HP-FFAP column (⌀ 0.25 mm × 30 m, film thickness = 0.25 μm; Agilent Technologies, Santa Clara, CA, USA) using helium as a carrier gas with a constant flow of 1 mL/min. The chromatographic oven temperature was initially kept at 60°C for 4 min, was then increased to 210°C, at 6°C/min, and was then maintained at 210°C for 2 min. The quantitative determination of volatile compounds was performed using the relative area calculated as the ratio. Samples were assessed in terms of Hunter's color values (L*, lightness; a*, red-green; and b*, yellow-blue) using a vertical-type spectrophotometer (CM-3600d, Konica Minolta, Inc., Tokyo, Japan). All measurements were replicated three times and average values (n = 3) were calculated.

2.6. Sensory evaluation

Sensory evaluation of wines was performed by a panel of twenty trained experts. The color, flavor, taste, and overall preference of the wines were evaluated on a scale of 1 to 5, where 5 was the best score. Overall preference according to the taste and flavor was evaluated using the mean value of a hedonic scale from 1 (very poor, dislike extremely) to 5 (excellent, like extremely) (PIGGOTT, 1988).

2.7. Statistical analysis

Data were expressed as mean±standard deviation (SD) of triplicate experiments. Statistical significance was determined by a Student's t-test for independent means, using Microsoft Excel (Microsoft, Redmond, WA, USA). A one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine differences between means. The critical level for statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Characteristics of grapes fermented by free cells and Ca-alginate bead-encapsulated yeast cells

We previously investigated the effects of Ca-alginate beads and protective agents on the survival rates of five yeast strains using an air-blast drying method, which demonstrated that 2% Ca-alginate beads soaked in protective agents (10% skimmed milk and 10% solutions of various sugars) helped to protect free cells from the environment. Specifically, in this study, *S. cerevisiae* D8 cells with 10% sucrose, *S. cerevisiae* M12 cells with 10% raffinose, *S. cerevisiae* S13 cells with 10% trehalose, *H. uvarum* S6 cells with 10% trehalose, and *I. orientalis* KMBL5774 cells with 10% glucose, all encapsulated in Ca-alginate beads, exhibited the highest survival rates (90.67%, 87.73%, 92.05%, 90.81%, and 87.16% viability, respectively) after air-blast drying at 37°C for 5 h (KIM *et al*., 2017).
Changes in the pH of wine fermented by encapsulated cells compared to those of wines fermented by free yeast cells were shown in Fig. 1. pH has a marked effect on microorganisms: in a study by BAE (2002), it was suggested that a pH <3.2 results in sourness during wine fermentation, while a pH <4.0 is recommended for preventing contamination by other harmful bacteria. In this study, the pH of all samples ranged from 3.41 to 3.65 during fermentation. At the beginning of fermentation, the pH values of all samples were 3.59–3.63 and decreased slowly during fermentation to final values of 3.47–3.52. The changes in total acid content in encapsulated cell-fermented wine compared to free cell-fermented wine were also shown in Fig. 1, where it can be seen that the total acid content in all wines increased from 0.41–0.43 to 0.61–0.69 during fermentation. The total acid content of wines fermented using free cells was found to increase slightly after two days, while that of the wines fermented using 2% Ca-alginate bead-encapsulated cells was found to increase only slightly after three days.

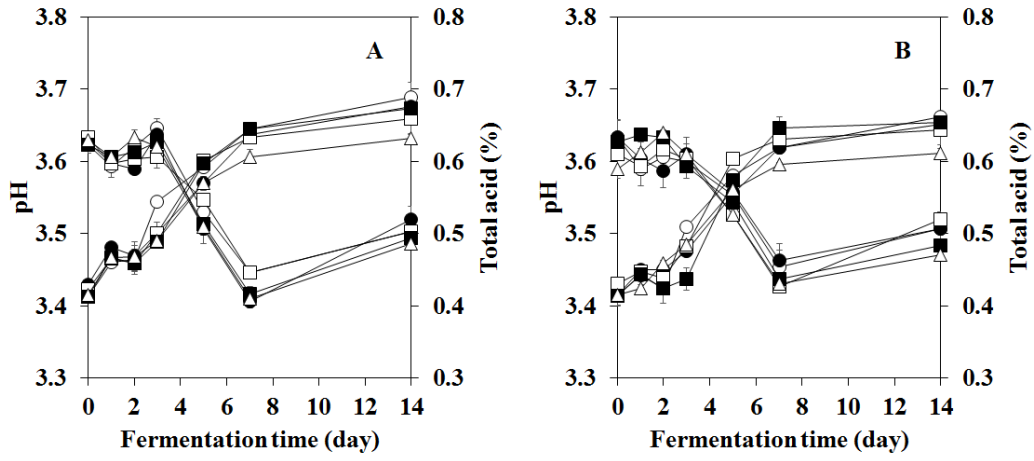

Figure 1. Changes in pH and total acid content during fermentation. (A) Free cells, and (B) cells encapsulated in 2% Ca-alginate beads. Open circles, *S. cerevisiae* D8; filled circles, *S. cerevisiae* M12; open squares, *S. cerevisiae* S13; filled squares, *H. uvarum* S6; open triangles, *I. orientalis* KMBL5774.

SEO and YOOK (2007) have reported that the total acid content during fifteen days of Campbell Early wine fermentation with a commercial wine dry yeast starter was 0.44–0.81. Similar to this, our study demonstrated low total acidity levels in wine fermented using

encapsulated cells for fourteen days. LEE and KIM (2006) reported that the Korean Campbell Early grape has a high level of acidity due to the presence of malic acid and tartaric acid, and investigated six different fermentation processes in terms of their capacity to reduce the levels of acidity. Based on the findings of the study, both carbonic maceration and precipitation methods were recommended for obtaining high quality wines.

The levels of soluble solids in the wines fermented using encapsulated cells were shown to decrease sharply after three days. In contrast, the soluble solid levels in the wines fermented by free cells decreased after two days (Fig. 2). We previously showed that the free and immobilized cell populations exhibited similar growth patterns; however, slight differences were observed at the early stage of the fermentation (KIM *et al*., 2017). It should be noted that, as the cell population in the beads increased, the diameter of the beads increased significantly due to the elastic properties of the alginate gel (VIVES *et al*., 1993). KIM *et al*., (2017) studied characteristics of red wine fermentation using Campbell Early and different sugars. The results revealed that wine fermentation with added glucose was faster, and it yielded a higher alcohol content, than that obtained with any other sugars. The wine fermented by the addition of sucrose and high fructose corn syrup showed results similar to that obtained by the addition of glucose. Similar results were obtained in our experiments; the soluble solid content of the two wines used in this study decreased upon the addition of sucrose, although the fermentation starting points differed. Due to conversion into alcohol, the reducing sugar content of all wine samples decreased sharply (from 25° Brix to 0.17° Brix) during fermentation. The reducing sugar content and the soluble solid content of samples fermented using 2% Ca-alginate bead-encapsulated cells decreased more slowly than that of samples fermented using free cells owing to pore size and different fermentation starting points (Figs. 2 and 3) (KIM *et al*., 2017).

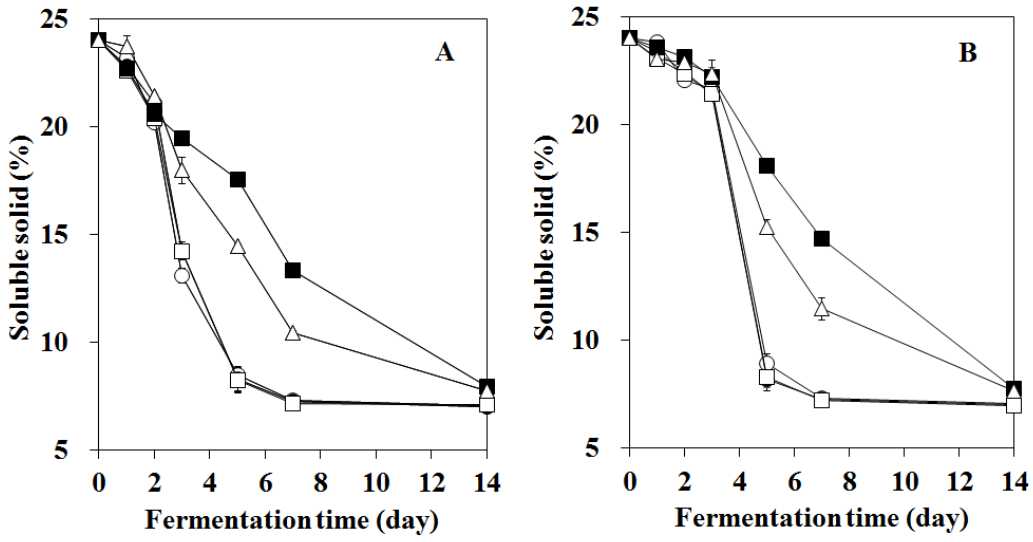

Figure 2. Changes in soluble solid contents during fermentation. (A) Free cells, and (B) cells encapsulated in 2% Ca-alginate beads. Open circles, *S. cerevisiae* D8; filled circles, *S. cerevisiae* M12; open squares, *S. cerevisiae* S13; filled squares, *H. uvarum* S6; open triangles, *I. orientalis* KMBL5774.

Most of the reducing sugar content and soluble solid content were consumed during the fermentation. Changes in alcohol content in the presence of bead-encapsulated cells compared to that in free cells during fermentation were shown in Fig. 3. The alcohol

content of wines fermented using free cells ranged from 9.20±0.06% to 12.97±0.09%, while that of wines fermented using encapsulated cells ranged from 9.75±0.04% to 13.43±0.24%. Although Ca-alginate bead-encapsulated cells fermented at a slightly slower rate than the free cells, their alcohol production was higher than that of free cells. ROUKAS *et al*., (1991) reported that free and immobilized *S. cerevisiae* cells produce the same maximum ethanol concentration under similar fermentation conditions. SINGH *et al*., (1998) also reported that the concentrations of ethanol produced in free cell and immobilized cell (Ca-alginate) batch fermentations were comparable. However, HOLCBERG and MARALITH (1981) reported that the rate of ethanol production by cells entrapped in agar, alginate, and polyacrylamide gels was higher than that of free cells. NORTON *et al*., (1995) reported that the resistance of yeast to ethanol was significantly higher in immobilized cells than in free cells. The findings reported here show that alcohol production using 2% Ca-alginate bead-encapsulated cells was higher than that using free cells. In both free cell and encapsulated cell systems, *H. uvarum* S6 and *I. orientalis* KMBL5774 exhibited slower alcohol production rates and lower maximal alcohol levels compared with *S. cerevisiae* D8, M12, and S13 (Fig. 3).

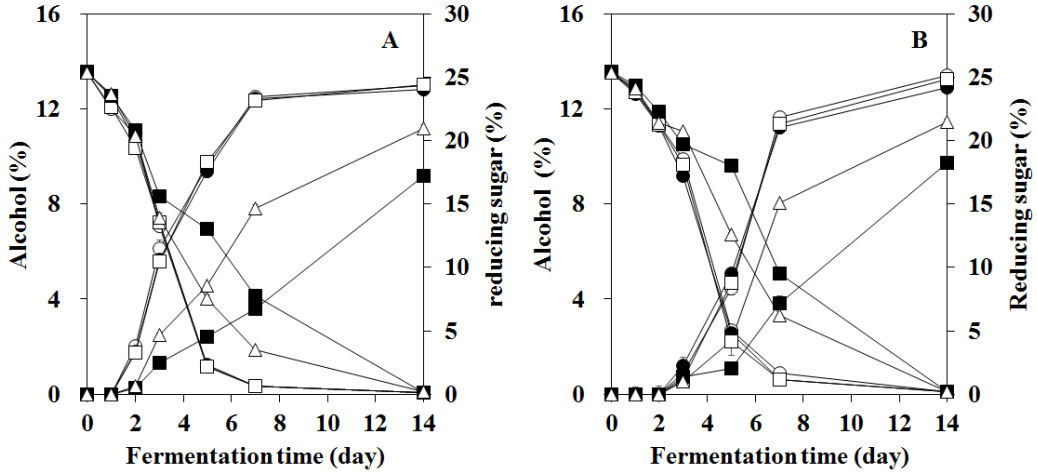

Figure 3. Changes in alcohol and reducing sugar content during fermentation. (A) Free cells, and (B) cells encapsulated in 2% Ca-alginate beads. Open circles, *S. cerevisiae* D8; filled circles, *S. cerevisiae* M12; open squares, *S. cerevisiae* S13; filled squares, *H. uvarum* S6; open triangles, *I. orientalis* KMBL5774.

A high cell density of a non-*Saccharomyces* (*H. uvarum*) yeast has been identified in *V. vinifera* grape must during the first 4-6 days of fermentation, until the ethanol content reached 4-7% (v/v). Accordingly, it has been suggested that the inoculation with pure cultures of *H. uvarum* cells may enhance ethanol production during fermentation (ROJAS *et al*., 2003). In agreement with these reports (MOREIRA *et al*., 2008; ROJAS *et al*., 2003), our findings revealed that *H. uvarum* S6 produced approximately 9.2±0.06 to 9.75±0.04% (v/v) alcohol. In a previous study, the effects of co-fermentation with various inoculation ratios (*S. cerevisiae* W-3: non-*S. cerevisiae; I. orientalis* KMBL5774) were investigated and led to the use of a mixed culture being recommended for better wine quality owing to the low alcohol production capacity of *I. orientalis* KMBL5774 (KIM *et al*., 2008). In our study, however, a low maximal alcohol content [11.20±0.06 to 11.47±0.29% (v/v)] was achieved by single fermentation and the use of Ca-alginate beads encapsulating a single strain (*I. orientalis* KMBL5774) was therefore studied.

The total phenolic compound levels in all the samples ranged from 0.13% to 0.15% (Fig. 4), while LEE *et al*., (2006) reported that the total content of phenolic compounds of Campbell Early wine in Korea was approximately 0.12%. The markedly higher total phenolic compound levels observed in the present study may be attributed to different conditions such as vintage, area, and climate (HUGLIN, 1978; HUGLIN and SCHNEIDER, 1998; SEGUIN, 1975; WINKLER *et al*., 1975). No major differences in total phenolic content were observed between free cell and encapsulated cell fermentations.

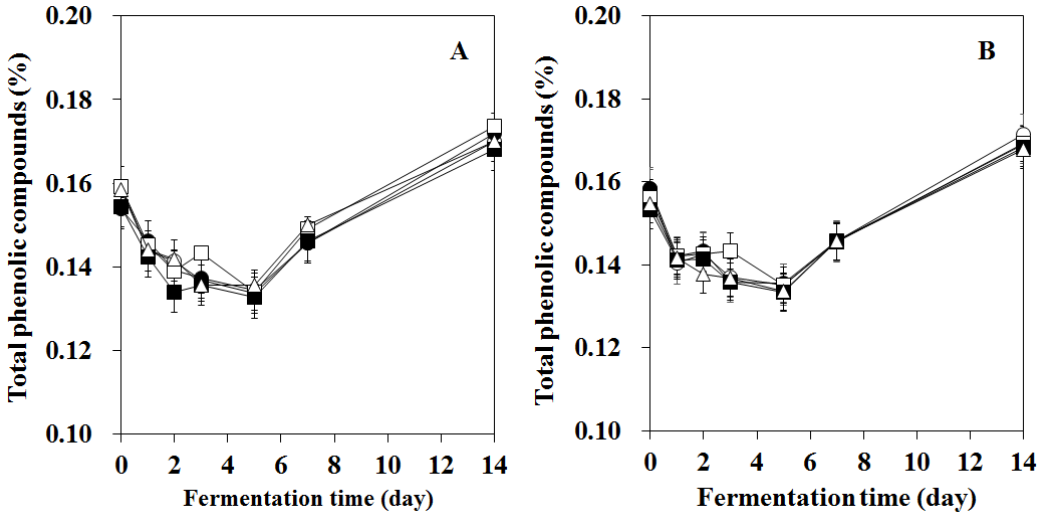

Figure 4. Changes in total phenolic compound contents during fermentation. (A) Free cells, and (B) cells encapsulated in 2% Ca-alginate beads. Open circles, *S. cerevisiae* D8; filled circles, *S. cerevisiae* M12; open squares, *S. cerevisiae* S13; filled squares, *H. uvarum* S6; open triangles, *I. orientalis* KMBL5774.

3.2. Yeast cell release from 2% Ca-alginate beads and viable cell count during fermentation

Encapsulated cells (initial cell concentration: 1.0±0.1 × 10^8 CFU/mL; stored at 4°C for 3 months) and cultured free cells (initial cell concentration: 1.0±0.2 × 10^8 CFU/mL) were inoculated into 5% (w/v) grape must. At the beginning of the fermentation, the free cells began to grow immediately, while the narrow and complex interior structure of the 2% Ca-alginate beads hindered the release of yeast cells from encapsulation following budding from 0 h (Figs. 5 and 6) (KIM *et al*., 2017). Accordingly, viable cell numbers in the 2% Ca-alginate bead samples increased more slowly than those in the free cell samples. As the cell population grows within the beads, the bead diameters increase significantly due to the elastic properties of the alginate gel (BABU *et al*., 1992; VIVES *et al*., 1993). This effect results in a delay in the rate at which the maximal cell population (10^8 CFU/mL) is reached (Fig. 6). KLINKENBERG *et al*. (2001) reported that the viable cell count (*Lactobacillus lactis* ssp. *lactis*) inside beads coated with alginate during milk fermentation remained significant at the beginning of fermentation, and that the alginate beads had a significant effect on the rate of cell release after 48 h of fermentation. In our study, however, the encapsulated cells were released slowly after 6 h and the release rate increased sharply after 48 h. In five to six days, the wines fermented by cells encapsulated in 2% Ca-alginate beads exhibited maximal populations similar to those exhibited by the free cell-fermented wines (Fig. 5).

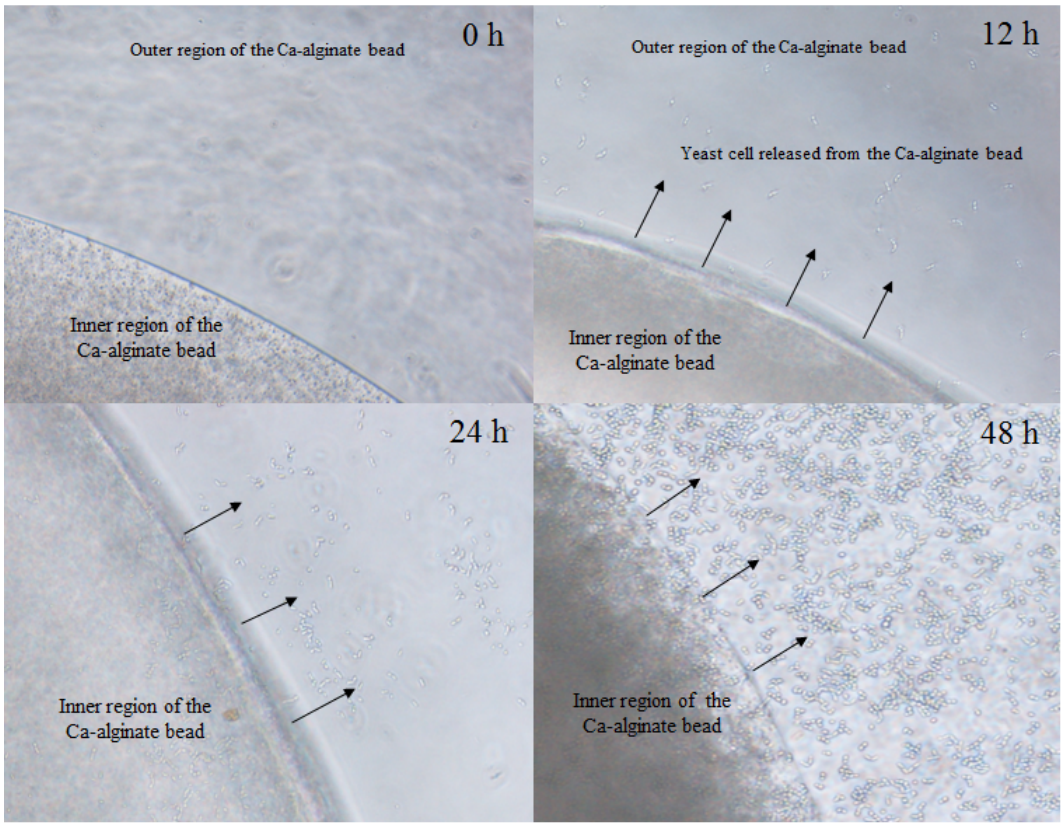

Figure 5. Optical microphotograph (×100 magnification) of yeast cells released from 2% Ca-alginate beads at different time intervals up to 48 h.

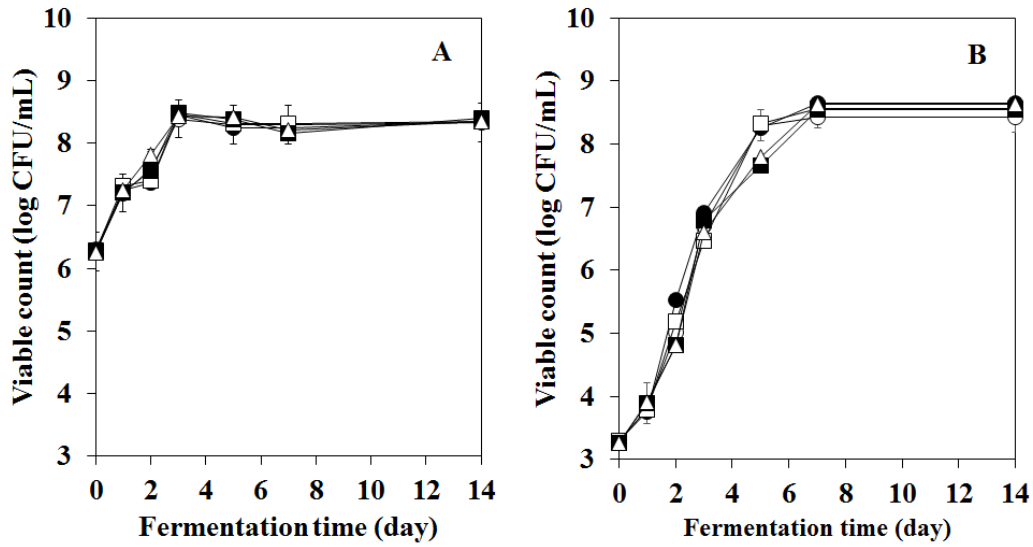

Figure 6. Changes in viable cell count during fermentation. (A) Free cells, and (B) cells encapsulated in 2% Ca-alginate beads. Open circles, *S. cerevisiae* D8; filled circles, *S. cerevisiae* M12; open squares, *S. cerevisiae* S13; filled squares, *H. uvarum* S6; open triangles, *I. orientalis* KMBL5774.

3.3. Physicochemical properties of wines fermented by free cells and encapsulated cells

After fermentation, sucrose, glucose, galactose, and fructose were identified as the sugar components in the wines and of these sugars, sucrose was found to be present at the highest concentration (1.32±0.01 g/L to 2.05±0.43 g/L) in both wine samples (Table 1). The two different fermentation processes were therefore found to yield similar sugar profiles. Free sugars are the major components of grape soluble solids and are related to the final alcohol content of wine (CONDE *et al*., 2007). Using HPLC, SHIRAISHI *et al*., (2010) showed that the major free sugars in grape wine (*Vitis* spp.) were glucose, fructose, and sucrose. KLIEWER (1966) have also reported the presence of glucose, fructose, galactose, sucrose, maltose, melibiose, raffinose, and stachyose in *Vitis* spp.

To determine the organic acid composition of the wines, the levels of malic acid, tartaric acid, citric acid, succinic acid, and acetic acid were assessed in the wines (Table 2). Malic acid and tartaric acid, which contribute 70–90% of the total acidity of grapes, significantly influence the sensory properties of wine (BEELMAN and GALLANDER, 1979; RUFFNER, 1982). Differences in organic acid content of both wines were negligible, nevertheless wines fermented using encapsulated cells exhibited slightly lower levels than those fermented using free cells. The wine fermented by *I. orientalis* KMBL5774, which has previously been shown to be able to rapidly degrade malic acid in medium where it represents the sole carbon and energy source (SEO *et al*., 2007), exhibited lower levels of malic acid than the other wines. As expected, the malic acid concentrations were lower in wines fermented with *I. orientalis* KMBL5774 than in wines fermented with other strains; however, other than malic acid, the levels of other organic acids did not differ markedly between free cell- and encapsulated cell-fermented wines. LEE and KIM (2006) studied the de-acidification of wine made from Campbell Early grapes, and recommended carbonic maceration and cold fermentation to decrease the organic acid content of Campbell Early wine. Several studies have shown that the process of malolactic fermentation results in the degradation of malic acid into lactic acid and carbon dioxide, the consequence of which is a reduction in total acidity (de-acidification) in the wine by strains of lactic acid bacteria (LAB) of the genera *Oenococcus*, *Leuconostoc*, *Lactobacillus,* and *Pediococcus* (BOULTON *et al*., 2013; VOLSCHENK *et al*., 1997; VILJAKAINEN and LAASO, 2000).

As shown in Table 3, the aldehyde content of wines fermented using free and encapsulated cells ranged from 44.88±0.49 to 65.28±4.32 mg/L and from 41.28±2.11 to 65.28±4.32 mg/L, respectively. Only small differences in aldehyde levels (lower in those fermented using encapsulated cells), if any, were observed between the free cell- and encapsulated cell-fermented wines. Acetaldehyde is considered to be a leakage product of alcohol fermentation by yeast, and *S. cerevisiae* and *Kloeckera apiculata* have been shown to produce 0.5–286 mg/L and 9.5–66 mg/L acetaldehyde (GEROYIANNAKI *et al*., 2007). GEROYIANNAKI *et al*., (2007) also reported that white and red grape pomace yielded 345 mg/L and 317 mg/L acetaldehyde, respectively. The lowest reported acetaldehyde level in wine was for cagaita wine produced using encapsulated cells (1.031 mg/L; *S. cerevisiae* UFLA CA11), whereas the free cell equivalent yielded 1.378 mg/L acetaldehyde (OLIVEIRA *et al*., 2011). All wines in this study (both free cell- and encapsulated cell-fermented) yielded acetaldehyde levels well below the official limit of 700 mg/L (KOREA, 2012). During wine production, methanol arises as a result of pectin methyl esterase activity during grape crushing (MASINO *et al*., 2008).

Table 1. Free sugar contents (g/L) in wines fermented using 2% Ca-alginate bead cells compared to those in wines fermented using free cells after fermentation.

Type	*S. cerevisiae* D8			*S. cerevisiae* M12			*S. cerevisiae* S13			*H. uvarum* S6			*I. orientalis* KMBL5774		
	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*- value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value
Sucrose	1.87±0.03	1.80±0.02	0.0420	1.71±0.01	1.72±0.05	0.6413	1.32±0.01	1.34±0.01	0.0512	1.45±0.05	1.32±0.06	0.0396*	2.05±0.13	1.96±0.07	0.3489
Glucose	1.11±0.08	1.09±0.04	0.7200	1.22±0.12	1.11±0.09	0.2321	1.07±0.08	1.01±0.10	0.4266	1.45±0.11	1.25±0.05	0.0477*	1.17±0.06	1.13±0.04	0.6547
Galactose	1.31±0.25*	1.10±0.08*	0.2459	1.22±0.07	1.10±0.08	0.1382	1.25±0.13	1.22±0.07	0.7296	1.29±0.06*	1.03±0.10*	0.0185*	1.32±0.07*	1.10±0.10*	0.0347*
Fructose	0.11±0.01	0.13±0.01	0.1340	0.13±0.01	0.13±0.03	0.8383	0.15±0.01	0.15±0.03	0.9068	0.11±0.00	0.12±0.00	0.0181*	0.10±0.01	0.10±0.02	0.8731
Total free sugars	4.40±0.26	4.12±0.11	0.1662	4.28±0.17	4.06±0.11	0.1382	3.79±0.13	3.72±0.18	0.5917	4.30±0.09	3.71±0.12	0.0026**	4.60±0.05	4.29±0.07	0.0021**

All data are expressed as mean±SD (n = 3).
*$p < 0.05$ and **$p < 0.01$ are considered to be statistically significant by student's *t*-test.

Table 2. Organic acid contents (g/L) in wines fermented by 2% Ca-alginate bead cells compared to those in wines fermented using free cells after fermentation.

Type	*S. cerevisiae* D8			*S. cerevisiae* M12			*S. cerevisiae* S13			*H. uvarum* S6			*I. orientalis* KMBL5774		
	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*- value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value
Lactic acid	0.78±0.02	0.77±0.05	0.7145	0.75±0.04	0.79±0.04	0.3038	0.74±0.05	0.85±0.03	0.0342*	0.82± 0.03	0.89±0.04	0.0598	0.82±0.02	0.94±0.05	0.0238*
Citric acid	0.14±0.06	0.20±0.02	0.2028	0.14±0.03	0.16±0.03	0.6491	0.21±0.04	0.16±0.03	0.1406	0.26±0.03	0.14±0.05*	0.0224*	0.21±0.01	0.16±0.04	0.1133
Tartaric acid	0.91±0.05	0.90±0.07	0.8561	0.83±0.02	0.86±0.03	0.1907	0.89±0.02	0.85±0.02	0.0920	0.95±0.07	0.93±0.09	0.8188	0.93±0.07*	0.92±0.10*	0.9261
Malic acid	1.72±0.01	1.70±0.01	0.0296*	1.89±0.04	1.71±0.06	0.0120*	1.63±0.01	1.65±0.03	0.4034	1.70±0.04	1.69±0.03	0.7625	1.33±0.03	1.03±0.06	0.0013**
Succinic acid	0.13±0.02	0.11±0.07	0.6800	0.12±0.03	0.11±0.01	0.4822	0.13±0.03	0.10±0.04	0.3218	0.09±0.06	0.09±0.04	0.9776	0.09±0.04	0.10±0.03	0.8686
Acetic acid	0.16±0.01	0.15±0.03	0.7050	0.11±0.01	0.13±0.04	0.4306	0.10±0.01	0.13±0.09	0.6319	0.16±0.01	0.12±0.03	0.1030	0.15±0.03	0.10±0.02	0.0714

All data are expressed as mean±SD (n = 3).
*$p < 0.05$ and **$p < 0.01$ are considered to be statistically significant by student's *t*-test.

Table 3. Aldehyde, methanol, and fusel oil contents (mg/L) in wines fermented by 2% Ca-alginate bead cells compared to those in wines fermented using free cells after fermentation.

Type	*S. cerevisiae* D8			*S. cerevisiae* M12			*S. cerevisiae* S13			*H. uvarum* S6			*I. orientalis* KMBL5774		
	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value
Aldehyde	64.17±2.13	64.27±3.32	0.9670	61.90±3.11	53.07±3.15	0.0259	65.28±4.32	54.66±3.45	0.0292*	44.88±0.49	41.28±2.11	0.0451*	52.01±1.26	47.56±1.65	0.0206*
Methanol	112.84±2.11	108.69±3.21	0.1346	110.26±3.22	110.28±2.01	0.9932	109.77±4.13	105.17±3.89	0.2329	105.39±1.25	106.30±1.02	0.3839	105.33±3.28	108.53±1.57	0.2021
Ethyl acetate	121.79±1.36	119.12±1.27	0.0678	126.2±2.14	115.74±2.35	0.0047**	125.33±1.46	113.35±1.43	0.0006$^{\#}$	158.06±3.52	148.61±3.29	0.0274*	132.19±1.14	130.09±3.21	0.3458
1-Propanol	191.67±5.21	155.83±2.74	0.0005$^{\#}$	172.78±3.22	106.20±1.25	0.0000$^{\#}$	155.05±2.14	97.98±1.98	0.0000$^{\#}$	158.25±1.58	121.10±2.04	0.0000$^{\#}$	139.07±2.95	117.35±3.05	0.0003$^{\#}$
Iso-butanol	361.79±2.56	389.12±1.07	0.0001$^{\#}$	346.22±3.14	345.74±2.65	0.8495	385.33±2.36	373.35±2.47	0.0037**	458.06±4.02	448.61±2.69	0.0277*	432.19±1.02	435.09±1.24	0.0352*
Iso-amyl alcohol	391.81±3.56	379.17±1.32	0.0045**	348.16±2.14	333.12±1.96	0.0009$^{\#}$	387.61±3.02	348.65±2.58	0.0001$^{\#}$	443.71±2.04	396.66±2.65	0.0000$^{\#}$	417.87±3.02	405.28±2.79	0.0061**

All the data are expressed as mean±SD (n = 3).
$^{*}p < 0.05$, $^{**}p < 0.01$ and $^{\#}p < 0.001$are considered to be statistically significant by student's *t*-test.

The methanol contents in all the wine samples in this study were found to range from 105.17±3.89 mg/L to 112.84±2.11 mg/L (Table 3), which are well below the standard maximal value for alcoholic beverages (1,000 mg/L), and were not affected by the Ca-alginate bead system. According to MATEOS *et al*., (2006), the major compounds that contribute to the overall volatile effects defining wine aroma are ethyl acetate and higher alcohols such as 1-propanol, isobutyl alcohol, and iso-amyl alcohol. As shown in Table 3, the fusel oil analysis conducted in this study revealed that iso-amyl alcohol, which contributes to wine quality (KOURKOUTAS *et al*., 2001) was present at 333.12±1.96 to 443.71±2.04 mg/L in the wines, and was the most prevalent fusel oil component. The ethyl acetate, 1-propanol, and iso-butanol concentrations ranged from 113.35±1.43 to 158.06±3.52 mg/L, from 97.98±1.98 to 191.67±5.21 mg/L, and from 346.22±1.96 to 443.71±2.04 mg/L, respectively. *H. uvarum* S6 cells (both free and encapsulated cells) yielded the highest levels of iso-butanol and iso-amyl alcohol during fermentation compared with the other yeast types. OLIVEIRA *et al*., (2011) have reported *Hanseniaspora* and *Issatchenkia* (non-*Saccharomyces*) as high ester producers. In this study, the wines fermented using encapsulated cells generally contained lower levels of fusel oil components than the wines fermented using the free cells.

In terms of Hunter's color values, there were no marked differences between the free cell and encapsulated cell fermented wines. The L* values ranged from 38.15±0.01 to 38.97±0.01, the a* values ranged from 7.24±0.04 to 7.66±0.16, and the b* values ranged from 0.00±0.02 to 0.20±0.02 (Table 4). There were no marked differences between the free cells and 2% Ca-alginate bead cells-fermented wines.

3.4. Sensory evaluation

The sensory characteristics of wines fermented using free and encapsulated cells were evaluated by a panel of twenty assessors. Preferences in terms of color, flavor, taste, and overall preference were determined on a scale of 1 (poorest) to 5 (best) (Fig. 7).

In terms of flavor and taste, the wines fermented with *H. uvarum S6* and *I. orientalis* KMBL5774 (both free and encapsulated cell fermentations) obtained the highest scores, while the wines fermented with *S. cerevisiae* D8, M12, and S13 obtained the lowest scores. In agreement with previous reports (MATEOS *et al*., 2006; TORRENS *et al*., 2008), differences in volatile compound levels between the wines seemed to correlate with the sensory evaluation. Among the wines in this study, high levels of acetaldehyde and low levels of ethyl acetate resulted in lower wine quality scores. When comparing wines fermented by free cells with those fermented by encapsulated cells, however, scores for color, taste, flavor, and overall preference did not differ, which indicates that encapsulated yeast cells represent suitable alternative starters for wine fermentation.

Table 4. Hunter's color values for wines fermented by 2% Ca-alginate bead cells compared to those for wines fermented using free cells after fermentation.

Type	*S. cerevisiae* D8			*S. cerevisiae* M12			*S. cerevisiae* S13			*H. uvarum* S6			*I. orientalis* KMBL5774		
	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*- value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value	Free cells	Bead cells	*p*-value
L*	38.97±0.01	38.97±0.02	1.0000	38.69±0.14	38.61±0.01	0.4276	38.44±0.03	38.44±0.01	1.0000	38.17±0.04	38.15±0.01	0.4481	38.28±0.02	38.28±0.01	1.0000
a*	7.66±0.16	7.58±0.03	0.4426	7.24±0.04	7.26±0.03	0.5265	7.46±0.13	7.32±0.03	0.1433	7.55±0.01	7.52±0.03	0.1757	7.47±0.04	7.47±0.03	1.0000
b*	0.01±0.02	0.00±0.02	0.5734	0.20±0.02	0.20±0.01	1.0000	0.13±0.02	0.14±0.02	0.5734	0.20±0.02	0.20±0.01	1.0000	0.05±0.21	0.07±0.12	0.8930
ΔE	38.92±0.01	39.72±0.03	0.0000$^{\#}$	38.64±0.01	39.12±0.13	0.0237*	38.46±0.02	39.15±0.04	0.0000$^{\#}$	38.19±0.02	38.91±0.03	0.0000$^{\#}$	38.30±0.01	39.00±0.02	0.0000$^{\#}$

All the data are expressed as mean±SD (n = 3).
$^{*}p < 0.05$ and $^{\#}p < 0.001$ are considered to be statistically significant by student's *t*-test.

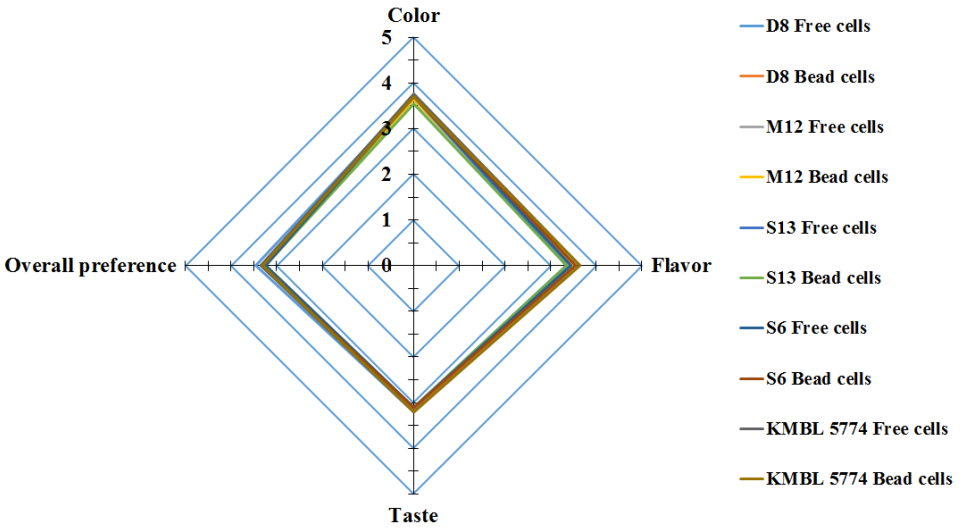

Figure 7. Radar plot of the sensory evaluation scores for wine fermented by 2% Ca-alginate bead cells compared to those for wines fermented by free cells after fermentation. The results reflects the means of scores from 20 semi-trained panelist.

4. CONCLUSIONS

In this study, fermentation characteristics of free yeast cell and yeast encapsulated in Ca-alginate beads (immobilized yeast) were compared. There was no significant difference between free yeast cell and immobilized yeast in terms of reducing sugars content, soluble solids, total acids, organic acids, free sugars, viable cell count and other physiochemical properties. However, immobilized yeast produced slightly higher alcohols and a lower total concentration of volatile acids (compound) than free yeast cell. These results suggest that yeast encapsulated in Ca-alginate beads have a potential to be used as alternative yeast for wine fermentation as it is cost effective compared to freeze-dried yeast.

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