Characterization of cottage cheese using *Weissella cibaria* D30: Physicochemical, antioxidant, and antilisterial properties


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**ABSTRACT**

This study aimed to evaluate the potential of *Weissella cibaria* D30 as an adjunct culture in cottage cheese, including an assessment of antioxidant, antilisterial, and compositional parameters. Cottage cheese samples were manufactured using a commercial starter culture and probiotic strains *Lactobacillus rhamnosus* GG (GG) or *W. cibaria* D30 (W) and without probiotic (control). Samples were stored at 4 ± 1°C for 28 d. Bacterial cell counts (log cfu/g) of control, GG, and W samples were counted at 0, 7, 14, 21, and 28 d. Counts of *W. cibaria* D30 in the W samples remained at 6.85 log cfu/g after 28 d. Total solids, fat, protein, ash, and pH were measured and no significant differences were observed in compositional parameters or pH after 28 d of storage in all cheeses except those inoculated to *Listeria monocytogenes*. To measure the antilisterial effect, *Listeria monocytogenes* was inoculated into the cottage cheese samples and bacterial cell counts were obtained at 0, 6, 12, 24, 48, 72, 96, 120, and 144 h. *Listeria monocytogenes* counts were less than the analytical limit of detection (<10 cfu/g) in the inoculated GG and W samples, whereas the counts of *L. monocytogenes* in the inoculated control sample remained at 3.0 log cfu/g after 144 h. We used the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity assays to assess antioxidant activity: GG and W samples exhibited significant increases in antioxidant activity compared with the control sample. These results indicate that *W. cibaria* D30 has potential as an adjunct culture in the dairy industry.

**Key words:** probiotic, cottage cheese, antioxidant, antilisterial effect

**INTRODUCTION**

Probiotics are defined as “live microorganisms which when administered in adequate numbers confer a health benefit to the host” (FAO/WHO, 2001). Although probiotics must be able to survive adequately in food carriers, their viability after passing through the gastrointestinal tract is also crucial. Various dairy products have been investigated for their effect on probiotic viability after exposure to the gastrointestinal tract, and cheese has been identified as a good carrier of probiotics (Karimi et al., 2011). In this regard, cheese has certain beneficial characteristics, including physical and chemical properties. High fat content, high water activity, and the pH of cheese may offer added protection to probiotics and help them maintain higher viability throughout gastric transit (Phillips et al., 2006; Makelainen et al., 2009). Furthermore, cheese has a higher buffering capacity, which controls the high acidic environment of the gastrointestinal tract and helps to create a favorable environment for probiotic viability (Karimi et al., 2011).

Fresh cheeses are particularly vulnerable to contamination by foodborne pathogens such as *Listeria monocytogenes*, which is considered a postprocessing contaminant. This bacterium is usually associated with refrigerated, ready-to-eat (RTE) foods and can grow within wide temperature (0 to 42°C) and pH (4 to 9) ranges and under high salt concentrations (10%; Mysak et al., 2009). *Listeria monocytogenes* is frequently associated with listeriosis outbreaks; thus, the European Union has stipulated microbial criteria for RTE foods (EC regulation 2073), stating that in those foods “able to support the growth of *L. monocytogenes*,” the pathogen must be absent in 25 g of product during their shelf life (European Commission, 2005). Hence, control of *L. monocytogenes* growth and multiplication in fresh cheese has been demanded. Some studies have focused on the addition of adjunct cultures isolated from herbs, fruits, and vegetables to prevent the growth of *L. monocytogenes* in cheese (Ho et al., 2018).

Korean kimchi is emblematic of Korean culture and is a famous side dish popular in Korea; it is made of salt-
ed cabbage with a variety of seasonings. Many studies have generated plausible evidence of the antimicrobial, anti-inflammatory, and antiadhesive activity against foodborne pathogens and the antioxidant and antitumor effects of probiotics isolated from Korean kimchi (Lee et al., 2015; Khan and Kang, 2016; Son et al., 2017). Weissella species are reported to be prominent during kimchi fermentation and have antioxidative, antifungal, antibacterial, anticancer, anti-inflammatory, and immune-boosting potential (Kang et al., 2016). Lactic acid bacteria (LAB) isolated from fermented foods are generally recognized as safe; thus, extensive investigations have been carried out to use these novel strains as adjunct cultures in the cheese industry (Ho et al., 2018; Papadopoulou et al., 2018). Therefore, the aim of the present study was to investigate the potential of W. cibaria D30, isolated from Korean kimchi, as an adjunct culture in cottage cheese and to examine its characteristics with respect to physicochemical properties, antioxidant activity, and antilisterial activity in cheese.

MATERIALS AND METHODS

Microorganisms and Culture Conditions

*Lactobacillus rhamnosus* GG (KCTC 12202 BP) and *W. cibaria* D30 were used as probiotic strains in the present study. The *W. cibaria* D30 strain was isolated from Korean kimchi and has demonstrated probiotic characteristics (Yu et al., 2018). The cheese starter culture was supplied by Culture Systems Inc. (Mishawaka, IN) in freeze-dried form and comprised *Lactobacillus acidophilus, Bifidobacterium longum,* and *Streptococcus thermophilus.* The probiotic strains were streaked onto de Man, Rogosa, and Sharpe (MRS; BD BBL, Franklin Lakes, NJ) agar plates directly from the −80°C stock and incubated at 37°C for 24 h. One colony from each strain was inoculated into 10 mL of MRS broth and incubated under the same conditions as above. Subsequently, a 5-mL aliquot was inoculated into 500 mL of MRS broth and incubated in a shaking incubator at 37°C and 150 rpm for 10 to 12 h, followed by centrifugation at 5,000 × g for 10 min at 4°C. The supernatant was discarded and the pellet was resuspended in 0.1% peptone water. Cell suspensions of each strain were mixed in the same volume and serially diluted to achieve a count of 5 to 5.5 log cfu/g in cottage cheese. The colony count of the inoculum was determined by spread plating on listeria selective agar (Oxoid Ltd., Basingstoke, UK) at required dilutions and then incubating at 37°C for 48 h.

Preparation of Cottage Cheese

Commercially available pasteurized low-fat milk (4% fat, Seoul, South Korea) was used to produce cottage cheese. Cheese was manufactured according to Jeon et al. (2016) with some modifications. The milk was preheated to 37°C, starter culture and liquid animal rennet (Maysa, Istanbul, Turkey) were added at 0.2% (about 10⁹ cfu/g) and 100 μL/L, respectively. Cheese milk was incubated at 37°C until the pH reached approximately 4.7 to 4.5. When the curd was produced, it was cut manually with aid of a cheese wire knife into 1-cm³ pieces. Subsequently, the whey was removed by 2 to 3 repetitive washings with cold water (4°C).

Probiotic was added at 10⁹ cfu/g after the addition of the starter culture. The control cheese sample was prepared following the same procedure without adding probiotics. For antilisterial activity, cheese samples were cut into small slices (3 × 6 cm), and 100 μL of inoculum was spread over cheese slices by using a bent glass rod. Inoculated individual pieces were vacuum-packed and stored at 4°C.

Six types of cheese were prepared and labeled as follows: cheese inoculated with starter culture and *L. rhamnosus* GG as adjunct (GG); W = cheese inoculated with starter culture and *W. cibaria* D30 as adjunct (W); cheese inoculated with starter culture only (control, C); GG cheese inoculated with *L. monocytogenes* (GGL); W cheese inoculated with *L. monocytogenes* (WL); and C cheese inoculated with *L. monocytogenes* (CL).

Composition and pH

Cheese samples were analyzed for protein, fat, total solids contents, and ash. Total protein and fat were analyzed by the Kjeldahl method (method 991.20; AOAC International, 2006) and Soxhlet extraction method, respectively. Total solids content was analyzed by oven drying a known weight of samples at 102°C for 3 to 4 h until at a constant weight. Ash content was obtained by heating appropriate weights of samples in a muffle furnace at 550°C overnight. The pH of all
cottage cheese samples was measured using a digital pH meter (WTW-720, WTW, Weilheim, Germany) equipped with a glass electrode, which was immersed in cheese samples homogenized with distilled water. All analyses were conducted in triplicate.

**Viability and Verification of LAB**

Lactic acid bacteria counts of cheese samples were obtained at 0, 7, 14, 21, and 28 d of storage according to the method of Abadia-Garcia et al. (2013). Briefly, cheese samples (10 g) were homogenized in 90 mL of 0.1% peptone water and subsequently macerated in a stomacher for 1 min. The LAB were enumerated by spread plating on MRS agar at required dilutions followed by incubating at 37°C for 72 h. The count of *W. cibaria* D30 in the W sample was taken at the end of shelf life (28 d) and verification was done based on morphology of the strain.

**Antioxidant Activity**

**Water-Soluble Extracts.** Water-soluble extracts (WSE) were prepared using the method of Perna et al. (2015) with some modifications. Briefly, 10 g of cheese sample was suspended in 30 mL of distilled water and kept at 40°C under gentle stirring for approximately 1 h. The homogenates were centrifuged at 5,000 × g at 4°C for 30 min. Subsequently, the uppermost fat layer was removed, the supernatant was filtered through Whatman No. 2 filter paper, and the WSE were further used to analyze antioxidant activity.

**Antioxidant Activity Assays.** Total antioxidant activity was determined according to the method of Savikin et al. (2009). Two hundred microliters of WSE was added to 1 mL of freshly prepared 100 μM 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) and allowed to stand in the dark for 15 to 20 min. Absorbance was measured by spectrophotometer at 517 nm. Three replicates were carried out for each sample. The absorbance of the blank was measured by using distilled water and following the same procedure as above. The results were expressed as percentage of scavenging activity, which was calculated as follows:

\[
\text{DPPH radical scavenging activity (\%) = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100,}
\]

where *A*<sub>control</sub> and *A*<sub>sample</sub> represent the absorbance of control (distilled water) and WSE, respectively.

The ABTS radical solution was prepared by reacting 14 mM ABTS and 5 mM potassium persulfate in the dark at room temperature for 12 to 16 h. Before the assay, the ABTS•⁺ radical cation solution was diluted with 0.1 M ethanol at a ratio of 1:10 to obtain absorbance of 0.7 ± 0.02 at 734 nm. Then, 20 μL of sample (WSE) was suspended in 980 μL of the prepared ABTS•⁺ radical cation solution and incubated at 37°C for 5 min. The absorbance was then measured at 734 nm. This experiment was conducted in 3 replicates. The formula used to calculate the scavenging activity was as follows:

\[
\text{ABTS radical scavenging activity (\%) = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100,}
\]

where *A*<sub>control</sub> and *A*<sub>sample</sub> represent the absorbance of control (distilled water) and WSE, respectively.

**Viability of *L. monocytogenes***

The viability of *L. monocytogenes* in cheese samples was evaluated at 0, 6, 12, 24, 48, 72, 96, 120, and 144 h. Enumeration was carried out according to the method of Jesus et al. (2016). Briefly, 10 g of cheese sample was homogenized with 90 mL of 0.1% sterile peptone water, followed by maceration in a stomacher for 1 min. Decimal dilutions were spread-plated on listeria selective agar (Oxoid Ltd.) and incubated at 37°C for 48 h.

**Statistical Analysis**

The results were obtained for each treatment in triplicate and are presented as means ± standard deviations. Statistical analyses were conducted using IBM SPSS statistics 20 (SPSS/IBM Corp., Chicago, IL). The data were assessed using one-way ANOVA. A difference was considered significant at *P* ≤ 0.05 using Duncan’s multiple range test.

**RESULTS AND DISCUSSION**

**Viability of LAB in Cottage Cheese at Refrigerated Storage (4°C)**

The changes in microflora during 28 d of storage at 4°C are illustrated in Figure 1. On d 0, total LAB counts were 8.5 ± 0.01, 9.02 ± 0.05, and 8.99 ± 0.06 log cfu/g in C, GG, and W samples, respectively. At 7 d of storage, LAB count increased in all cheese samples and reached 8.76 ± 0.04, 9.34 ± 0.05, and 9.03 ± 0.05 log cfu/g in C, GG, and W samples, respectively. The LAB count continued to increase until 14 d of storage in the GG and W samples, whereas a significant reduc-
tion (1.2 log cfu/g) was observed in the C sample \( (P < 0.05) \). The declining LAB in the C sample after 7 d can be explained by the high vulnerability of starter LAB to the harsh cheese environment such as high pH (pH 4.4–4.5), no residual lactose, and low temperature (4°C; Steele et al., 2013). In contrast, the steady increase in LAB until 14 d in the GG and W samples can be attributed partially to high tolerance of nonstarter LAB, such as \( L. \) rhamnosus \( GG \) and \( W. \) cibaria D 30 (Lazzi et al., 2014). In particular, the survivability of \( L. \) rhamnosus to pyruvate degradation and ribose catabolism has been demonstrated in several cheeses (Lazzi et al., 2014), and \( W. \) cibaria D30 shows high tolerance at pH 3 (Yu et al., 2018).

Nonetheless, the LAB count decreased in all samples after 14 d of storage \( (P < 0.05) \). At 21 d of storage, LAB counts were 6.93 ± 0.07, 8.38 ± 0.17, and 8.03 ± 0.05 log cfu/g in the C, GG, and W samples, respectively. At 28 d of storage, LAB counts reached 6.84 ± 0.05, 7.51 ± 0.053, and 7.24 ± 0.015 log cfu/g in the C, GG, and W samples, respectively. Demirci et al. (2017) showed a decline in viable count of \( Lactobacillus \) casei 431 in yogurt beyond 14 d of storage. This behavior could be due to the inhibitory activities of LAB, such as production of organic acids (lactic acid), hydrogen peroxide, nutrient competition, bacteriocin, diacetyl, and alcoholic compounds (Shah, 2000; Karimi et al., 2011). However, the rate of decline of viable cell count was lower in the cottage cheese samples with probiotics (GG and W) than in the C sample. In addition, we detected no difference in LAB viability in the GG and W samples \( (P > 0.05) \).

The general shelf life of fresh cheese stored in a refrigerator is 10 to 12 d. Nevertheless, at the end of the study period (after 28 d), the viable count of \( W. \) cibaria D30 in W cheese was 6.85 log cfu/g.

### Compositional Analysis and pH

The compositional data of all samples after 28 d of storage are shown in Table 1. Values of experimental cheese samples were compared with those of the control cheese. The addition of probiotics did not have a significant effect on protein, TS, or ash content. The addition of probiotics reduced the fat content of the experimental samples but not significantly \( (P > 0.05) \).

Abadia-Garcia et al. (2013) reported the influence of \( Lactobacillus \) casei ATCC 373 and \( L. \) rhamnosus GG ATCC 53103 added to cottage cheese. We did not detect a significant difference \( (P > 0.05) \) between experimental samples in pH levels at 28 d of storage. The pH values were 4.58 ± 0.01, 4.57 ± 0.01, 4.56 ± 0.01, 4.52 ± 0.02, and 4.54 ± 0.02 in C, GG, W, GGL, and WL samples, respectively.

### Antioxidant Activity of WSE During Storage Period

The antioxidant activity of cottage cheese samples during storage at 4°C was analyzed at 0, 7, 14, 21, and

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**Table 1.** Composition and pH of cottage cheese samples after 28 d of storage at 4°C

<table>
<thead>
<tr>
<th>Item</th>
<th>C</th>
<th>GG</th>
<th>W</th>
<th>GGL</th>
<th>WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (%)</td>
<td>24.05 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.04 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.02 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.02 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.0 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20.70 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.94 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.93 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.84 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.91 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.94 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.74 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.79 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.79 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.68 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.83 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.84 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.80 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.79 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.74 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>4.58 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.57 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.56 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.52 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.54 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

*Means within a row with different superscripts differ \( (P < 0.05) \).

<sup>a</sup> All values are means of 3 replicates \( (± \) SD).
activities in C, GG, and W samples were, respectively, 26.04 ± 0.98%, 41.71 ± 1.02%, and 44.6 ± 0.93% in the DPPH assay, and 42.43 ± 1.07%, 62.05 ± 1.65%, and 65.10 ± 1.03% in the ABTS assay. There was no difference in antioxidant activity among cheese samples with different strains of probiotics (P > 0.05). These findings are in agreement with those of Mushtaq et al. (2016), who studied the antioxidant activity of Lactobacillus casei 279, Lactobacillus brevis 021, and Lactobacillus plantarum 01 strains added to Kalari cheese. This can be explained by the fact that cheese contains a high amount of protein and these proteins (especially casein) are subjected to proteolysis by enzymes present in milk, such as plasmin. In addition, the residuals of rennet added during the cheese-making process and microbial proteolytic enzymes result in the production of bioactive peptides. These bioactive peptides are responsible for antioxidant activity by inhibiting the formation of free radicals such as oxygen and nitrogen, for example, via different mechanisms (Sarmadi and Ismail, 2010). However, samples with probiotics showed an increase (P < 0.05) in antioxidant activities, which may be explained by the fact that some LAB have specific abilities to produce antioxidant enzymes (i.e., superoxide dismutase, catalase, glutathione reductases, and thioredoxin) to scavenge free radicals, thereby resulting in oxidative defense (Ianniello et al., 2015). Overall, results showed that the antioxidant activity of fresh cheese was enhanced by the addition of probiotic strains.

Viability and Behavior of Inoculated L. monocytogenes in Cottage Cheese During Storage

When pasteurized milk is used for cheese manufacturing, a major cause of cheese spoilage is cross-contamination due to improper handling, which mainly affects the surface of cheese (Carminati et al., 2000; Frye and Donnelly, 2005). In this study, the surface of the cottage cheese was inoculated with a multi-strain cocktail of L. monocytogenes to simulate this postprocessing contamination. Figure 4 shows the behavior of L. monocytogenes throughout storage. The initial counts of L. monocytogenes were 5 to 5.5 log cfu/g in all 3 samples (CL, GGL, and WL). We detected no significant difference in L. monocytogenes count in the control sample (CL) at 6 h (P > 0.05), whereas the counts of GGL and WL samples were significantly reduced by 0.87 and 0.77 log cfu/g, respectively (P < 0.05). Beyond 6 h, L. monocytogenes counts declined steadily in all 3 samples, and at 120 h the count reached 2.0 log cfu/g in the GGL and WL samples and 3.4 log cfu/g in the CL sample. At 144 h, the L. monocytogenes counts in...
acetic acid, lactic acid, and bacteriocin. The potential formation of antimicrobial compounds was demonstrated by the use of W. cibaria D 30 enhanced antioxidant activity and did not affect the compositional parameters or pH, which ensures its potential use as an adjunct culture in cottage cheese production to improve quality. The viability of W. cibaria D 30 above the minimum threshold level of 10^5 cfu/g until the end of the shelf life shows its potential to deliver probiotic health benefits to the consumer at the time of consumption.

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REFERENCES


Figure 4. Bacterial cell counts of Listeria monocytogenes during storage at 4°C. Results are expressed as mean ± SD (n = 3). CL, GGL, and WL = C, GG, and W cheeses inoculated with L. monocytogenes, where C = cheese inoculated with starter culture (control); GG = cheese inoculated with starter culture and Lactobacillus rhamnosus GG as adjunct; W = cheese inoculated with starter culture and Weissella cibaria D30 as adjunct.

GGL and WL samples were below the limit of detection (<10 cfu/g), whereas that of the CL sample was 3.0 log cfu/g.

These results demonstrated that L. monocytogenes was able to survive for a longer period in CL (15 d) than in the GGL and WL samples. These findings are in agreement with results from other studies in Galotyri cheese, a soft acid curd cheese (Rogga et al., 2005) and Greek Graviera hard cheese (Giannou et al., 2009). Rogga et al. (2005) and Giannou et al. (2009) explained that the combined hurdle effect of lactic acid, pH, and water activity inhibited the growth of the pathogen but that it survived for a longer period with a low death rate under refrigerated storage. Thus, faster inhibition (144 h) of L. monocytogenes in the GGL and WL samples demonstrated that L. rhamnosus GG and W. cibaria D30 act as protective adjunct cultures through the potential formation of antimicrobial compounds such as acetic acid, lactic acid, and bacteriocin.

CONCLUSIONS

The present study revealed that the W. cibaria D 30 strain isolated from Korean kimchi has potential for use as an adjunct culture in cheese manufacture. In particular, W. cibaria D 30 could provide additional hurdle effects to prevent the growth of L. monocytogenes and ensure the microbial safety of RTE soft cheeses. Ascertaining the long-term survival of L. monocytogenes in RTE soft cheese is crucial because of the potential risk of transmittance of the pathogen from cheese slicers to other RTE foods that might support its growth. In ad-


