2	Running title: Various properties of quark cheese with ginseng extract
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4	Physiochemical Analysis, Antioxidant Effects, and Sensory
5	Characteristics of Quark Cheese Supplemented with Ginseng
6	Extract
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(Short communication)

24 **Abstract** The objective of this study was to evaluate physicochemical and sensory 25 properties, the texture profile, and antioxidant activity of ginseng extract-supplemented quark cheese as a new cheese product intended to improve public health. After addition 26 27 of less than 1.0% ginseng extract, the moisture content of quark significantly decreased, 28 while fat and protein levels increased, although microbial counts and lactose and ash 29 contents were not affected significantly (p < 0.05). In terms of color, L* values decreased significantly with increasing concentration of ginseng extract, while a* values increased 30 31 significantly (p < 0.05). The results of texture profiling showed that cohesiveness and springiness were unaffected, whereas hardness, gumminess, and chewiness increased 32 significantly. The 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) 33 34 radical-scavenging activities of the cheese fortified with 0, 0.5%, or 1.0% of the ginseng extract were $4.22\% \pm 0.12\%$, $20.14\% \pm 1.34\%$, and $56.32\% \pm 1.54\%$, respectively. The 35 36 results of sensory analysis indicated that bitterness, ginseng odor, and aftertaste 37 significantly improved with increasing concentration of ginseng extract (p < 0.05). 38 However, there was no significant difference in the overall quality attributes of quark 39 cheese between the no-supplement control and samples with less than 0.5% of the 40 ginseng extract (p>0.05), suggesting that these products could help to promote public 41 health as functional foods.

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43 *Keywords:* quark cheese, ginseng extract, antioxidant effect, texture analysis, sensory
44 evaluation

45 Introduction

46 Recently, the development and production of high-value dairy products has increased 47 to meet the demand for biofunctional food products, driven by the increasing interest of 48 consumers in their health and well-being (Swensson et al., 2017). Many biotech 49 companies have developed new dairy products as functional foods with additional 50 biofunctional activity for human health as well as acceptability to consumers.

51 Oxidative stress in the human body can result from several types of reactive oxygen 52 species (ROS), which are continuously produced as byproducts of aerobic metabolism 53 (Biller and Takahashi, 2018; Bury et al., 2018). Some species of reactive oxygen are 54 highly toxic, causing various diseases, and must be rapidly detoxified by antioxidants 55 through various cellular mechanisms (Apel and Hirt, 2004).

The health benefits of dairy products have been known for a long time. In particular, many studies have revealed that cheese has antioxidant effects because it contains polyphenolic compounds (Hilario et al., 2010; Branciari et al., 2015). Some researchers have reported that the antioxidant effects that benefit health are due to the complexation between the phenolics and milk proteins (Park et al., 2018). However, this antioxidant activity is relatively weak due to low concentrations of polyphenolic compounds in cheese products.

Many researchers have studied the diversification of cheese products by adding
various ingredients including herbs to increase biofunctionality (Lee et al., 2016).
However, there is still no research on the effects of ginseng extract-supplemented quark
cheese as a new cheese product.

67 In particular, quark cheese is a type of fresh dairy product manufactured by warming68 of soured milk until curdling, without aging. It is classified as fresh acid-set cheese and

has a relatively soft texture. Traditionally, quark is processed as a dairy food in
Northern European countries. Although quark cheese was originally manufactured
without any protease, producers recently began adding small amounts of rennet. Many
investigators have studied the physiochemical properties of quark cheese during its
processing (Ferreiro et al., 2016).

Ginseng is a traditional medicinal plant used in Northeast Asian countries, particularly in Korea. Ginseng root extracts contain saponin, which is the major active ingredient and is known to have therapeutic activities against various diseases such as cancer, hypertension, and diabetes or to improve weak health (Jung and Jin, 1996). Many studies have reported the effects of red ginseng extract on cheese products such as camembert cheese (Lee and Bae, 2018), but the application of ginseng extract to quark cheese remains unclear.

Therefore, the aim of this study was to evaluate the physiochemical properties, changes in color and texture, sensory properties, and antioxidant effects of quark cheese supplemented with ginseng extract *in vitro*, as compared with regular quark cheese (control). Our hypothesis was that quark cheese with the added ginseng extract would have higher antioxidant concentrations as compared to controls. The results of this study can be practically applied by biofunctional-dairy-food manufacturers to help maintain public health.

88

89 Materials and Methods

90 Materials

Ginseng extract was obtained from Hwain Korea Co. (Seoul, South Korea). The
commercial starter was purchased from the New England Cheese-making Supply Co.
(South Deerfield, MA, USA), whereas Man-Rogosa-Sharpe (MRS) agar was obtained
from Difco Laboratories (Detroit, MI, USA).

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Preparation of quark cheese

To prepare quark cheese supplemented with the ginseng extract, 5 L of pasteurized 96 97 milk (Pasteur Milk Co., Ltd., Seoul, Korea) was supplemented with different 98 concentrations of the ginseng extract (0, 0.1%, 0.5%, or 1%). The commercial starter (Streptococcus lactis and S. cremoris) was inoculated at 0.002% (w/v) into milk 99 samples mixed with the ginseng extract, and the mixture was incubated in a cheese vat 100 (Sunil Instrument Co., Daejeon, South Korea) at 35°C for 220 min. After cultivation, 101 102 rennet was added (0.2 mL/L), and the mixture was allowed to stand at 4°C for 19 h for 103 coagulation. The curds were packaged into sacks and allowed to stand for 18 h to drain out the remaining whey. 104

105 Enumeration of microbial cells

Samples from every stage of the cheese making process (inoculation, fermentation,
cooling, and storage) were collected, and microbial growth was measured by the
standard plate-counting method on MRS agar plates.

109 Physicochemical analysis

110 During fermentation, pH was determined with a pH meter (Inolab pH 720, Weihein,111 Germany). Proximate analyses of the contents of moisture, crude fat, protein, and

112 lactose were performed by AOAC methods. The total solids, protein, fat, and ash113 contents were measured according to the methods of AOAC International (2000).

114 Antioxidant activity

Each cheese sample was added to twice its volume of methanol (cheese:methanol ratio of 1:2) and kept for 1 h at 30°C in a shaking incubator (SI-900R, Jeio Tech, Kimpo, Korea), centrifuged at 1,900 g for 10 min (Combi-514R, Hanil Co., Ltd., Seoul, Korea), and passed through Whatman No. 2 filter paper. The filtrates were used as samples for the analysis of antioxidant activity.

2,2'-azino-bis-3-120 Radical-scavenging activity determined was by а 121 ethylbenzothiazoline-6-sulphonic acid (ABTS; Sigma, St. Louis, MO, USA) assay. We mixed 14 mM ABTS and 5 mM potassium persulfate in 0.1 M potassium phosphate 122 123 buffer (pH 7.4) in a 1:1 ratio and incubated them for 16 h in a dark room at 25°C. The mixture was diluted with 0.1 M potassium phosphate buffer (pH 7.4) until the 124 125 absorbance at 734 nm wavelength reached 0.7 ± 0.02 on a spectrophotometer (X-ma 3200, Human Co., Ltd., Seoul, Korea). A 20 µL sample was then added to 980 µL of the 126 127 above solution, and the mixture was incubated for 5 min in 37°C. Absorbance was 128 measured at 734 nm. The antioxidant activity was calculated as follows:

129 Antioxidant activity (%) =
$$\left(1 - \frac{A_S}{A_C}\right) \times 100$$

130 A_c: absorbance values of the negative control

131 A_s: absorbance values of an experimental sample

Textural analysis

133 Texture profile analysis was performed using a TA-XT2 texture analyzer (Texture 134 Technologies, Surrey, UK). Quark cheeses were prepared in a cube shape $(30 \times 30 \times 30)$ mm) and tempered at 10°C. The textural analysis was carried out at room temperature. 135 The data acquisition rate was 200 pps. The force threshold and contact force were 10 136 and 5 g, respectively, and the samples were compressed to 50% of their height. The P75 137 probe was employed, and the speed of the probe was 2.5 mm/s during the analysis. In 138 terms of color, L*, a*, and b* values of each sample were determined with a chroma 139 140 meter (CR-400 head, Konica Minolta, Japan).

141 Sensory analysis

142 Consumer sensory analysis was performed by 22 panelists (12 females, 10 males, age 26-30 years) who were screened according to accepted international standards (ISO 143 144 13299:2003). Quantitative descriptive analysis was performed to evaluate the differences in the sensory characteristics among ginseng-supplemented quark cheese 145 samples (Ng et al., 2012). A continuous scale from 0 to 8 was used to measure the 146 147 following characteristics: creamy odor, acid odor, ginseng odor, acid taste, bitter taste, aftertaste, and overall quality. Water and plain bread were provided between samples as 148 149 a palette cleanser and quark cheese without the ginseng extract served as the reference 150 standard.

151 Statistical analysis

The data were expressed as a mean \pm standard deviation. For statistical comparisons, the results were subjected to one-way analysis of variance (at *p*<0.05) and Duncan's multiple-range test in IBM SPSS 22 software (IBM, Armonk, NY, USA).

156 Results and Discussion

157

Physicochemical analysis

158 The composition of cheese samples supplemented with different concentrations (0.1%, 0.5%, or 1.0%) of the ginseng extract is presented in Table 1. The concentration 159 of solids in the ginseng extract was $61\% \pm 1.0\%$ (w/v). The moisture content of ginseng 160 161 extract-supplemented quark cheese was significantly lower than that of the control. Lee 162 et al. (2016) reported that this effect might be due to the influence of ginseng extract on the water-holding capacity of cheese. The ginseng extract, like Inula britannica extract, 163 164 seemed to facilitate contraction of the cheese matrix by binding particles together and 165 via expulsion of whey, thereby lowering the amount of entrapped water in the protein network. In addition, fat and protein contents were significantly increased by the 166 addition of the ginseng extract. As for lactose and ash contents, these were not affected 167 168 significantly by the addition of the ginseng extract at a concentration of less than 1.0% (*p* >0.05). 169

The ginseng extract did not significantly influence microbial counts of quark cheese (*p*>0.05). Kim et al. (2008) also reported that the addition of red ginseng extract to yogurt does not change lactic acid bacteria counts in the yogurt. At a concentration of less than 1.0%, the added glycoside-rich ginseng extract seemed to have no advantageous effects on the growth of lactic acid bacteria in our study.

In terms of the color parameters at different concentrations (0.1%, 0.5%, and 1.0%)
of the ginseng extract in quark samples, L* values decreased significantly with the
increasing concentration of the ginseng extract, while a* values significantly increased

178 (p < 0.05). b* values also increased with the increasing concentration of the ginseng 179 extract. In this study, the dark brown color of the ginseng extract itself might have 180 affected the color of the final quark cheese product. These results are supported by the 181 study by Kim et al. (2008), who demonstrated that the addition of red ginseng extract to 182 yogurt decreases L* values but increases b* values.

183 Changes in the texture profile of ginseng extract-supplemented quark cheese were 184 assessed too. Regarding hardness, all the samples showed an increase with the 185 increasing concentration of the ginseng extract (0.1% to 1.0%; p < 0.05). This 186 phenomenon may be due to the lower moisture content of ginseng extract-supplemented 187 cheese. In all the samples, cohesiveness and springiness were measured and were found to range from 0.73 to 0.80 and from 0.35 to 0.41, respectively. However, the differences 188 189 were not significant (p>0.05). Gumminess and chewiness significantly increased with the increasing concentration of ginseng extract. These results can be explained by the 190 high hygroscopicity of ginseng extract. Song et al. (2007) reported that ginseng extract 191 192 has high water-absorbing capacity and causes weakness of the dough of white bread.

193 Antioxidant activity

The ABTS assay is a standard method for measuring antioxidant activities. The radical of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), i.e., ABTS⁺⁺, is produced via oxidation of ABTS by potassium persulfate. The mechanism of this assay is reduction of ABTS⁺⁺ in the presence of an antioxidant (Re et al., 1999). Some researchers have found that ginseng contains various antioxidant compounds (Chung et al., 2017). Fig. 1 depicts the results of ABTS radical-scavenging assays of ginseng extract-supplemented quark cheese. Increasing concentration of the ginseng extract caused a concomitant increase in the ABTS radical-scavenging activity (r = 0.803, p < 0.01). The ABTS radical-scavenging activities of the cheeses fortified with 0, 0.5%, or 1.0% of the ginseng extract were 4.22% \pm 0.12%, 20.14% \pm 1.34%, and 56.32% \pm 1.54%, respectively. This effect may be due to polyphenolic compounds such as flavonoids as well as various ginsenosides found in the ginseng extract (Chen et al., 2006; Ramesh et al., 2012; Jung et al., 2016).

207 Sensory evaluation

208 The sensory attributes of quark cheese supplemented with different concentrations (0.1%, 0.5%, or 1.0%) of the ginseng extract are presented in Fig. 2. The ginseng flavor 209 210 and taste increased significantly with the increasing concentration of the ginseng extract 211 in quark cheese (p < 0.05). Flavor properties such as bitterness and ginseng odor 212 significantly increased with the increasing concentration of the ginseng extract (p < 0.05). 213 In addition, the score of the aftertaste increased with the concentration of the ginseng 214 extract. In terms of total quality, there was no significant difference between the no-215 supplement control and the samples with less than 0.5% of added ginseng extract in quark cheese (p>0.05). Many researchers have noted that the addition of ginseng extract 216 217 to other food products generally has negative sensory scores, despite the biofunctional 218 activities of this ingredient toward human health (Lee et al., 2008; Lee et al., 2011). In 219 contrast, our study revealed that the addition of the ginseng extract at a concentration of 220 less than 0.5% did not significantly affect the total quality score.

221

222 Conclusion

223 This study was designed to develop ginseng extract-supplemented quark cheese and 224 to evaluate its antioxidant effects (as possible benefits for human health), 225 physicochemical changes (including color and texture), and sensory properties, as 226 compared to a no-supplement control. The data on lactic acid bacterial counts, color, texture, and sensory evaluation from this study indicate that ginseng extract 227 228 concentrations of 0.5% could be applicable to the development of quark cheese with 229 biofunctional activities, such as antioxidant effects. It is known that ginseng extract 230 contains various ginsenosides with biofunctional properties toward human health. In 231 addition, some adult diseases are caused by oxidative stress in the human body. 232 Therefore, ginseng extract-supplemented cheese products may help to maintain human health and prevent such diseases. Furthermore, the production of quark cheese that 233 contains ginseng extract may broaden the applications of ginseng and increase the 234 demand for cheese products. 235

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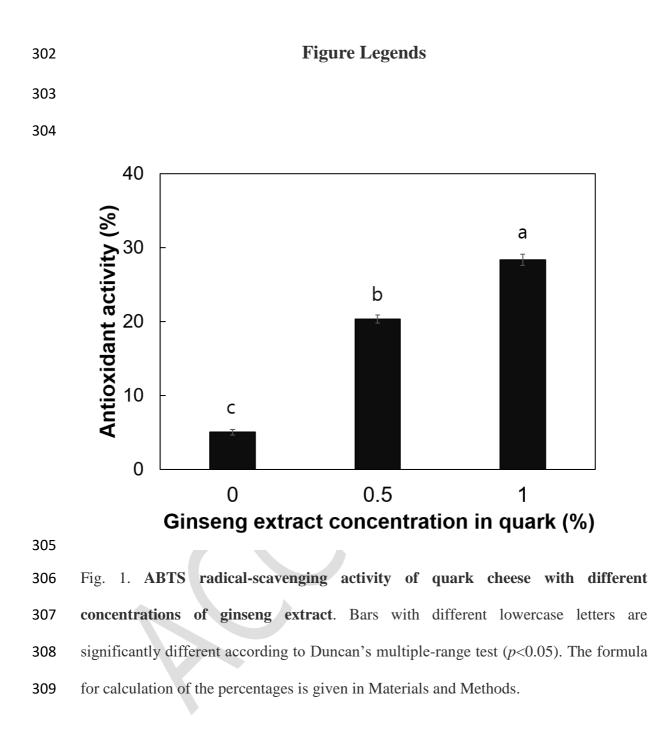
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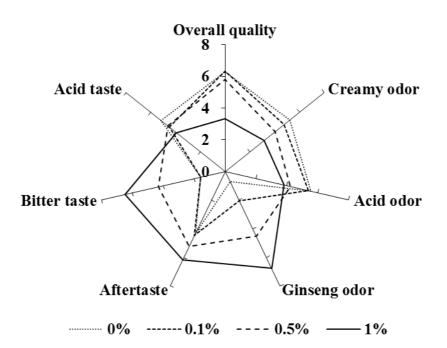
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311 Fig. 2. The mean sensory intensity rating of ginseng-supplemented cheese. There

312 were 22 judges. Each sensory attribute was tested in triplicate, and a quark cheese

313 sample without the ginseng extract served as the reference standard.

315 Table 1. Physicochemical, color, and textural analysis of quark cheese supplemented with ginseng extract

Items		Ginseng extract concentration			
		Control	0.1%	0.5%	1.0%
Moisture (%, w/w)		69.77±0.83 ^a	66.54 ± 0.34^{b}	66.68±0.79 ^b	$64.82 \pm 0.34^{\circ}$
Fat (%)		1.63 ± 0.26^{d}	$3.20 \pm 0.07^{\circ}$	6.36 ± 0.32^{b}	$9.90{\pm}0.04^{a}$
Protein (%)		14.31 ± 0.34^{b}	16.89 ± 0.40^{ab}	16.94±0.22 ^{ab}	18.75 ± 0.47^{a}
Lactose (%)		2.50 ± 0.39^{a}	2.00 ± 0.29^{a}	2.25 ± 0.40^{a}	2.50 ± 0.33^{a}
Ash (%)		2.08 ± 0.08^{a}	2.06 ± 0.06^{a}	$2.04{\pm}0.05^{a}$	1.93 ± 0.09^{a}
pH		4.41	4.30	4.37	4.44
Microorg	ganisms (CFU/g)	9.06 ± 0.08^{a}	9.19±0.08 ^a	$9.04{\pm}0.08^{a}$	$9.01{\pm}0.08^{a}$
	L*	88.13±0.13 ^a	87.12±0.22 ^b	84.38±1.00 ^c	81.58±0.13 ^d
Color	a*	-1.48 ± 0.07^{d}	$-0.97 \pm 0.07^{\circ}$	-0.09 ± 0.02^{b}	$0.55 {\pm} 0.04^{a}$
	b*	6.09 ± 0.13^{d}	$7.52 \pm 0.10^{\circ}$	11.06 ± 0.04^{b}	14.23 ± 0.10^{a}
	Springiness (mm)	0.73 ± 0.08^{a}	$0.75 {\pm} 0.05^{a}$	$0.79{\pm}0.06^{a}$	$0.80{\pm}0.05^{a}$
	Gumminess (N)	146.23 ± 2.55^{d}	198.63±1.56 ^c	273.13±3.32 ^b	270.87 ± 4.25^{a}
Texture	Cohesiveness	0.35 ± 0.13^{a}	0.47 ± 0.14^{a}	0.35 ± 0.14^{a}	0.41 ± 0.12^{a}
	Adhesiveness (N)	285.82 ± 7.98^{d}	416.05±3.25°	484.09±5.89 ^b	568.61±9.95 ^a
	Hardness (N)	425.00 ± 6.89^{d}	$477.00 \pm 8.28^{\circ}$	515.10 ± 9.68^{b}	667.10 ± 7.89^{a}
	Chewiness (N)	$99.18 {\pm} 8.72^{d}$	$149.76 \pm 5.68^{\circ}$	216.54 ± 3.45^{b}	237.41 ± 9.79^{a}

316 during cheese production as well as viable cell counts.

317 ^{a-d} Means within a row with different superscript letters are significantly different, at p < 0.05.

318 L* value (lightness): the shades from black (-) to white (+), a* value (redness): the hue from green (-) to red

319 (+), b* value (yellowness): the hue from blue (-) to yellow (+), CFU: colony-forming units.