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TITLE PAGE
- Korean Journal for Food Science of Animal Resources -
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ARTICLE INFORMATION	Fill in information in each box below
Article Title	Antioxidant properties and diet-related a-glucosidase and lipase inhibitory activities of yogurt supplemented with safflower petal extract
Running Title (within 10 words)	Properties of Yogurt with Safflower Petal Extract
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	We would like to thank Ju Hee Kim and Woong Lae Kim in our laboratory for their support for the analysis of experiments.
Author contributions (This field may be published.)	# These authors contributed equally to this work. Conceptualization: Hong H, Lim JM, Kwon SH, Kim SK. Data curation: Hong H, Lim JM, Kwon SH, Kim SK. Formal analysis: Lim JM, Kwon SH. Methodology: Hong H, Lim JM, Kothari D, Kwon SH. Software: Lim JM, Kwon SH. Validation: Hong H, Kim SK. Investigation: Hong H, Lim JM, Kothari D, Kwon SH, Kim SK. Writing - original draft: Hong H, Lim JM, Kim SK. Writing - review & editing: Hong H, Kim SK.
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

5

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10 Recently, yogurt has been extensively studied to further enhance its functions using edible
11 plant extracts. This study was conducted to investigate whether safflower petal as a natural food
12 additive can be used to develop functional yogurt with improved health benefits. Safflower
13 petals were extracted with ethanol (SPE) and hot water (SPW), and then safflower yogurt was
14 prepared by adding 0%–1.0% of those extracts to plain yogurt. With an increase in the
15 fermentation duration, the pH of SPE and SPW yogurt samples was decreased, whereas
16 titratable acidity and microbial counts were increased. The concentration of total polyphenols
17 and total flavonoids, the activity of antioxidants, and the inhibitory effect on reactive oxygen
18 species (ROS) were higher in SPW yogurt than SPE yogurt. Furthermore, α -glucosidase and
19 lipase activity inhibitory effects of SPW yogurt were higher than those of SPE yogurt. In
20 particular, free radical-scavenging activities, ROS inhibitory effect and α -glucosidase activity
21 inhibitory effects were significantly increased in SPW yogurt in a dose-dependent manner.
22 Overall, these results suggest that safflower petal extract possesses antioxidant activities and
23 that it can downregulate α -glucosidase and lipase activities. The safflower petal extract may
24 have potential benefits as a natural food additive for the development of functional yogurt.

25 Keywords: yogurt, safflower petal, α -glucosidase, lipase, antioxidant activity

26

27 Introduction

28 Diabetes mellitus is a serious chronic metabolic disorder, associated with life-threatening
29 complications (Elbashir et al., 2018). It is characterized by a state of chronically elevated blood
30 glucose levels (hyperglycemia) with altered nutrient metabolism, such as carbohydrates, fats,
31 and proteins, in the body (American Diabetes Association, 2014). Moreover, the oxidative
32 stress state associated with the generation of reactive oxygen species (ROS) increases in both

33 type I and type II diabetes (Omari et al., 2019; Sabu and Kuttan, 2002). ROS generated during
34 oxidative metabolism can cause various disorders.

35 α -Glucosidase enzyme inhibitors, as antidiabetic drugs, can lower blood glucose levels by
36 reducing the intestinal absorption of carbohydrates (Buchholz and Melzig, 2016). Additionally,
37 hyperlipidemia is a common feature in patients with diabetes mellitus and is one of the factors
38 that is associated with considerably increases the risk of premature atherosclerosis (Schofield
39 et al., 2016). It is known that pancreatic lipase regulates the digestion and absorption of dietary
40 lipids (Mhatre et al., 2019). Therefore, inhibition of this enzyme may help prevent
41 complications by regulating lipid levels in the blood of diabetic patients through reducing
42 dietary fat absorption into the body.

43 Despite the growing interest in functional foods that modulate physiological activities by
44 inhibiting these enzymes, the possibility of developing successful and targeted natural products
45 to safely manage these diseases remains unexplored.

46 Yogurt, obtained by fermenting milk using lactic acid bacteria, contains proteins, vitamins such
47 as vitamins A and B, and minerals such as calcium and manganese. Recently, yogurt has been
48 extensively studied to further enhance its functions using bioactive substances from edible
49 plants. Numerous studies have indicated that the addition of these substances increases the
50 concentration of polyphenols and flavonoids, thus increasing the antioxidant and antimicrobial
51 activities of yogurt (Halah and Nayra, 2011; Kang et al., 2018; Lim, 2018).

52 *Carthamus tinctorius* L., commonly known as safflower, belonging to the Asteraceae family
53 (Delshad et al., 2018), is an annual herbaceous crop. It has been cultivated in India, Iran, and
54 China for a long time (Asgarpanah and Kazemivash, 2013). Safflower is used as a traditional
55 herbal medicine to promote blood circulation, regulate menstruation, and alleviate pain of joints
56 (Delshad et al., 2018). Some studies have reported that safflower has more than 200 compounds
57 including flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids, and

58 polysaccharides (Asgarpanah and Kazemivash, 2013). Furthermore, in in vitro and in vivo
59 studies, hydroxysafflower yellow A, a major bioactive compound in safflower, has been found
60 to have a strong oxidative radical-scavenging effect (Tian et al., 2008; Zhao et al., 2018).

61 The present study has focused on whether the addition of safflower extract containing various
62 phytochemicals to yogurt is effective in the antioxidant activity and inhibition of α -glucosidase
63 and pancreatic lipase. Therefore, this study was conducted to investigate the biochemical,
64 microbiological, sensory characteristics, and physiological effects including α -glucosidase and
65 pancreatic lipase activity inhibitory effects of yogurt supplemented with safflower petal (SP)
66 extract in order to enhance the functionality of yogurt.

67

68 Materials and Methods

69 **Plant materials and extract preparation**

70 Dried safflower petals were purchased from a local market (Daejodong, Seoul, Korea). For
71 ethanol extraction (SPE), 100 g of dried safflower petals was precipitated with 900 mL of 99.9%
72 ethanol in a shaking incubator at room temperature for 15 h. For water extraction (SPW), 100
73 g of dried safflower petals was soaked in 900 mL of 100°C hot water for 1 h using a reflux
74 condenser (Reflux-A, Reservoir; LK Lab Korea Inc., Namyangju, Korea). These extracts were
75 centrifuged at 13 000 rpm for 15 min; then, the supernatant was collected and passed through
76 qualitative filter paper No. 1 (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and 0.22 μ m syringe
77 filter (Sartorius, Goettingen, Germany). The aqueous extract was concentrated using a vacuum
78 freeze-dryer at -80°C (Unifreez™ FD-8; Daihan Scientific, Wonju, Korea). The concentration
79 of SPE and SPW was 50 mg/mL.

80

81 **Preparation of yogurt supplemented with SP extract**

82 SPE and SPW were added to sterilized milk (Maeil Dairies Co., Ltd. Seoul, Korea) at
83 concentrations of 0%–1.0% and homogenized using Homogenizer T 25 (Ika, Staufen, Germany)
84 at 25°C for 10 min, and then pasteurized at 85°C for 15 min. Thereafter, the samples were
85 immediately cooled to 43°C and inoculated with a commercial yogurt starter culture (0.02%,
86 v/v) of lactic acid bacteria (Lyofast YAB 450 AB; Sacco s.r.l., Codaragok, Italy) containing
87 *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Lactobacillus*
88 *acidophilus*, and *Bifidobacterium animalis* spp. *lactis*. Yogurt added with 0% safflower extract
89 was used as control. These yogurt samples were fermented at 43°C for 6 h and stored at 4°C
90 until further analysis.

91

92 **Proximate composition, pH, titratable acidity, and viscosity**

93 Moisture and total ash content was measured using the methods of Association of Official
94 Analytical Chemists (2000). The concentration of proteins, crude fats, lactose, and total solids
95 was measured using Mikloscan (Milkoscan Minor 78110; Foss Co., Hillerød, Denmark). The
96 pH of yogurt samples was measured using a digital pH meter (Model 735P; IS Technology Co.,
97 Incheon si, Korea). Titratable acidity (TA) was determined by adding 9 mL of dH₂O to 1 g of
98 yogurt sample and then titrating the yogurt mixture to pH 8.3 with 0.1 N NaOH. The amount
99 of acid produced was calculated as follows:

$$100 \text{ TA (\% lactic acid)} = \text{dilution factor} \times V_{\text{NaOH}} \times 0.1 \text{ N} \times 0.009 \times 100$$

101 where, V_{NaOH} is the volume of NaOH required to neutralize the acid.

102 Viscosity of yogurt supplemented with SP was measured using a viscometer (Model LV DV 1+;
103 Brookfield Engineering Laboratories, Inc., Middleboro, USA) with spindle No. 63 at 50 rpm
104 for 5–8 min.

105

106 **Viable cell count**

107 Viable cell count in yogurt was determined on a *Streptococcus thermophilus* agar (Sigma-
108 Aldrich, St. Louis, USA) plate after incubating at 37°C for 48 h. The results are expressed as
109 CFU/mL.

110

111 **2,2-Diphenyl-2-picrylhydrazyl radical-scavenging activity assay**

112 2,2-Diphenyl-2-picrylhydrazyl (DPPH) inhibition was determined as described previously
113 (Kang et al., 2018). Briefly, each yogurt extract (16 µL) was mixed with 320 µL of 0.15 mM
114 DPPH solution (Sigma-Aldrich). The mixture was shaken and incubated at room temperature
115 for 15 min. The absorbance of the solution was measured at 515 nm using an ELISA reader
116 (Synergy 2; BioTek Instruments Inc.). Inhibition of DPPH oxidation (%) was calculated as
117 follows:

$$118 \quad \% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

119 where, *A* is the absorbance at 515 nm.

120

121 **2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging activity assay**

122 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging activity
123 was determined using the method of Kang et al. (2018). The stock solution comprised 7.4 mM
124 ABTS (Sigma-Aldrich) and 2.5 mM potassium persulfate solutions. The mixture was prepared
125 by mixing the two stock solutions at a ratio of 1:1 (v/v) and allowed to react for 16 h in dark.
126 The ABTS solution was diluted with tertiary dH₂O to the appropriate absorbance (7.00 ± 0.05),
127 which was measured at 734 nm. The ABTS solution (180 µL) was mixed with 20 µL of yogurt
128 extract, and the mixture was covered with aluminum foil and placed in the dark at room
129 temperature for 30 min. The absorbance of the mixture was measured at 734 nm using an
130 ELISA reader (Synergy 2; BioTek Instruments Inc.). Each sample was measured in triplicate,
131 and percent inhibition was calculated using the following equation:

$$132 \quad \% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

133 where, A is the absorbance at 734 nm
134

135 **Total polyphenol and total flavonoid content**

136 Total phenolic content (TPC) was determined using the method of Wei et al. (2011) with
137 modifications. Briefly, each yogurt extract (100 μ L) was mixed with 100 μ L of 1 N Folin–
138 Ciocalteu reagent (Sigma-Aldrich); this mixture was thoroughly mixed and incubated at room
139 temperature for 3 min. Na_2CO_3 (200 μ L, 1 N) was added to the mixture, which was allowed to
140 react at room temperature for 90 min. The developed color in the mixture was measured using
141 an ELISA reader (Synergy 2; BioTek Instruments Inc., Winooski, USA) at 725 nm. The TPC
142 is expressed as gallic acid equivalent (Sigma-Aldrich). The regression line of the gallic acid
143 standard was used to determine the TPC in yogurt extract as micrograms of gallic acid
144 equivalent per milliliter (μg GAE/mL).

145 Total flavonoid content (TFC) was measured using the method of Abeysinghe (2007) with
146 modifications. Briefly, each yogurt extract (100 μ L) was mixed with diethylene glycol (500 μ L)
147 and NaOH (50 μ L, 1 N). The mixture was incubated at 37°C for 60 min. The developed color
148 in the mixture measured using an ELISA reader (Synergy 2; BioTek Instruments Inc.) at 420
149 nm. The TFC is expressed as quercetin equivalent (Sigma-Aldrich). A standard curve was
150 generated using the quercetin standard solution, and the results are expressed as quercetin
151 equivalent per milliliter.

152

153 **Cell lines and cell culture**

154 The human colorectal cell line (HT-29) used in this study was purchased from the American
155 Type Culture Collection (Manassas, USA). HT-29 cells were cultured in RPMI 1640 medium
156 (Lonza, Walkersville, USA) supplemented with 10% fetal bovine serum (Atlas Biologicals,
157 Fort Collins, USA), 100 U/mL penicillin (Gibco, Grand Island, USA), and 100 $\mu\text{g}/\text{mL}$
158 streptomycin (Gibco) at 37°C under 5% CO_2 . The cells were re-fed with the medium every 2 d

159 until 80% confluence.

160

161 **Measurement of ROS**

162 ROS were measured using the stain 2',7'-dichlorofluorescein diacetate (DCFDA, Sigma-
163 Aldrich). Briefly, the cells were seeded in six-well plates, incubated overnight, and then treated
164 with diluted yogurt extract for 16 h. Thereafter, the cells were treated with 1 µg/mL
165 lipopolysaccharide (Sigma- Aldrich)-containing medium for 6 h. The cells were then incubated
166 with DCFDA at a final concentration of 10 µM for 30 min and washed twice with cold PBS.
167 The plates were subsequently measured using the fluorescence microscope (Model IX71,
168 Olympus Optical Co., Ltd., Tokyo, Japan). Photographs were captured using the Olympus
169 DP71 camera and DP controller software (Olympus Optical Co., Ltd., Tokyo, Japan). Finally,
170 ROS were quantified using Image J software (National Institute of Health, Bethesda, USA).

171

172 **Measurement of α -glucosidase inhibition**

173 α -Glucosidase activity inhibitory effect was determined using a modified assay reported by
174 Kwon et al. (2006). Briefly, 50 µL of yogurt extract was added to 100 µL of 0.1 M phosphate
175 buffer (pH 6.9) containing α -glucosidase solution (1 U/mL, Sigma-Aldrich) in 96-well plates.
176 The plates were incubated at 25°C for 10 min. After pre-incubation, 50 µL of 5 mM p-
177 nitrophenyl- α -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to
178 each well. The mixture was incubated at 25°C for 5 min. The absorbance of the mixture was
179 measured at 405 nm before and after incubation using an ELISA reader (Synergy 2; BioTek
180 Instruments Inc.). The absorbance of yogurt extract was compared with that of the control,
181 which was 50 µL of buffer solution. The α -glucosidase activity inhibition is expressed as
182 percent inhibition and was calculated using the following equation:

183 $\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}}] \times 100$

184 where, A is the absorbance at 405 nm
185

186 **Measurement of pancreatic lipase inhibition**

187 Pancreatic lipase inhibition was determined using the modified method of Kim et al. (2007)
188 using *p*-nitrophenyl butyrate (NPB). Briefly, the enzyme solution was prepared by adding 30
189 μL of lipase solution from porcine pancrease Type VI-S (Sigma-Aldrich). This solution was
190 composed of 10 mM morpholinepropanesulfonic acid and 1 mM ethylenediaminetetraacetic
191 acid (pH 6.8) in 850 μL of Tris buffer (100 mM Tris-HCl and 5 mM CaCl_2 , pH 7.0). Yogurt
192 extract (100 μL) was mixed with an enzyme substrate solution (880 μL) containing 10 mM *p*-
193 NPB and incubated for 15 min at 37°C. The absorbance of the sample was measured at 400 nm
194 using an ELISA reader (Synergy 2; BioTek Instruments Inc.) and was compared with that of
195 the control buffer solution, without the extract. The lipase activity inhibition is expressed as
196 percent inhibition and was calculated using the following equation:

$$197 \text{ \% Inhibition} = [(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}}] \times 100$$

198 where, A is the absorbance at 400 nm
199

200 **Statistical analysis**

201 All data are expressed as mean \pm standard deviation (SD) of three experiments. Statistical
202 analysis of the data was performed using the one-way analysis of variance (ANOVA; SPSS 25,
203 SPSS Inc., Chicago, USA) followed by Duncan's multiple range test and Student *t*-test for
204 comparison of means. Statistical significance was set at $p < 0.05$.

205

206 **Results and Discussion**

207 **Proximate composition of yogurt supplemented with SP extract**

208 The nutritional composition of yogurt supplemented with SPE and SPW is shown in Table 1.

209 The moisture content of SPE yogurt increased with an increase in the concentration of SPE
210 added. The moisture content of SPE yogurt was 81.67 - 82.13%, which was significantly lower
211 than that of plain yogurt ($p < 0.05$). In SPE yogurt, the concentration of proteins, fats, lactose,
212 and total solids increased with an increase in the concentration of SPE. The lactose
213 concentrations in 0.5% and 1.0% SPE yogurt were 13.02% and 13.11%, which were
214 significantly higher than those in 0% and 0.1% SPE ($p < 0.05$). The concentration of nutrients in
215 SPE yogurt tended to be higher than that in SPW yogurt. In particular, the protein
216 concentrations in SPE yogurt were 4.33 - 4.40%, which were significantly higher than those of
217 SPW yogurt ($p < 0.05$). The supplementation of yogurt with red ginseng extract changed the
218 composition of yogurt, that is, with an increase in red ginseng extract concentration, the
219 moisture content and protein and lactose concentrations increased (Jung et al., 2016). These
220 findings are similar to our data. According to Pires et al. (2018), the supplementation of various
221 plant petal extracts to yogurt marginally increased the nutritional composition such as protein
222 and lactose concentrations, except the moisture content. Overall, yogurt composition was
223 affected by the addition of SP extract.

224

225 **Changes in pH, TA, microbial count, and viscosity of yogurt with SP extract**

226 As shown in Fig. 1A and 1D, the pH of SPE and SPW yogurt decreased significantly during
227 fermentation ($p < 0.05$). In contrast, the TA level in SPE and SPW yogurt was significantly
228 increased during fermentation as shown in Fig. 1B and 1E ($p < 0.05$). The pH and TA of all
229 yogurt samples were similar during the initial and final stages of fermentation. These results
230 are consistent with those of previous studies, that is, the pH decreased but the TA level increased
231 due to acid production in red ginseng extract- and ginseng extract-supplemented yogurt (Jung
232 et al., 2016; Jang et al., 2018).

233 The viable cell counts in SPE and SPW yogurt during fermentation are shown in Fig. 1C and

234 1F. They ranged from 7.49 to 9.77 Log₁₀ (CFU/g) and 7.43 to 9.99 Log₁₀ (CFU/g), respectively.
235 The number of viable cells in SPE was not significantly different from that in SPW yogurt.
236 However, the number of viable cells in SPE and SPW yogurt was slightly lower than that in
237 plain yogurt at the end of fermentation. It has been reported that in yogurt supplemented with
238 cinnamon ethanol extract, the viable cell counts were marginally decreased at the end of
239 fermentation (Choi et al., 2016). However, yogurt fermented with Korean traditional plant
240 extracts showed an increase in lactic acid bacteria count during fermentation (Joung et al., 2016;
241 Jung et al., 2016).

242 The viscosity of yogurt supplemented with SP extracts at different concentrations is shown in
243 Fig. 1G and H. The viscosity of yogurt with SPE and SPW tended to be slightly lower than that
244 of plain yogurt. The viscosity of 1.0% SPW and 0.5% SPE yogurt was significantly decreased
245 compared with plain yogurt ($p < 0.05$). It is known that viscosity is related to the aggregation of
246 casein micelles (Joyce et al., 2017). Sung et al. (2015) reported that the viscosity of yogurt
247 supplemented with 5% freeze-dried mulberry fruit juice was significantly lower than that of the
248 control. Tseng and Zhao (2013) reported that the viscosity of yogurt supplemented with wine
249 grape pomace was decreased; they suggested that this was probably because the addition of a
250 high concentration of wine grape pomace into yogurt resulted in the breakdown of the
251 coagulated milk. Furthermore, it has been reported that carthamin yellow from safflower
252 significantly decreased whole blood viscosity, plasma viscosity, and erythrocyte aggregation
253 index, in an animal study (Li et al., 2009).

254

255 **Antioxidant activity, TPC, and TFC**

256 As shown in Table 2, the DPPH radical-scavenging activity of SPW yogurt was 5.81%–10.66%,
257 which was considerably higher than that of SPE yogurt, which was 1.03%–3.24%. Moreover,
258 the ABTS radical-scavenging activity of SPW yogurt was 40.00%–49.67%, but the activity was

259 not detected in SPE yogurt. These results are consistent with those of Kang et al. (2018), who
260 reported that the antioxidant activity of yogurt supplemented with water extract of fermented
261 pepper was higher than that of methanol extract. Here, the antioxidant activities of yogurt
262 seemed to increase with the concentration of SPE and SPW. In particular, the antioxidant
263 activity of 1.0% SPW yogurt was the highest, and it was significantly higher than that of plain
264 yogurt ($p < 0.05$). These results can be attributed to the TPC and TFC in the SP. Similar to the
265 antioxidant effects, the TPC and TFC in yogurt increased with the increase in the concentration
266 of SPW and SPE. The TPC and TFC in yogurt supplemented with SPW 1.0% were 393.13 and
267 320.90 $\mu\text{g/mL}$, respectively, which were the highest among the yogurt samples ($p < 0.05$). The
268 TPC in yogurt supplemented with SPE and SPW was 93.55–115.12 and 282.52–393.19 $\mu\text{g/mL}$,
269 respectively, and the TFC was 52.53–77.76 and 180.97–320.90 $\mu\text{g/mL}$, respectively. It is
270 known that edible safflower yellow pigments, which belong to the C-glucosylquinochalcone
271 family of flavonoids, are water soluble (Li et al., 2018). This suggests that hot water extracted
272 more bioactive substances such as polyphenols and flavonoids from SP, in this study. Kruawan
273 and Kangsadalampai (2006) reported that the water extract of *Carthamus tinctorius* L. presented
274 high antioxidant activity and TPC. In summary, the results demonstrated that SP extracted with
275 hot water had greater antioxidant capacity, as evidenced by an increase in the TPC and TFC.

276

277 **Inhibition of ROS production in LPS-stimulated human colon adenocarcinoma**

278 The effect of SPE yogurt on intracellular ROS levels was determined in order to elucidate its
279 antioxidant activity, through which SPE can protect cells against LPS-induced damage. LPS, a
280 major component of bacterial cell walls, induces the production of inflammatory mediators such
281 as ROS (Lee et al., 2015). ROS play an important role in oxidative stress associated with the
282 development of chronic diseases such as cancer and type II diabetes (Lee et al., 2015). Therefore,
283 this study was focused on LPS-induced ROS production in vitro. As shown in Fig. 2, SPE and

284 SPW yogurt significantly reduced ROS production in LPS-induced HT-29 cells ($p < 0.001$). Ma
285 et al. (2016), based on their study in LPS-induced hepatocellular (HepG2) cells, reported that
286 SP yellow B possesses a high ROS-scavenging activity. As mentioned above, edible safflower
287 yellow pigments are water soluble, and therefore, the ROS-scavenging ability of SPW yogurt
288 with a high TPA and TFC was higher than that of SPE yogurt. Thus, these results demonstrated
289 the antioxidant effect of SP extract-supplemented yogurt in vitro.

290

291 **α -Glucosidase and lipase activity inhibitory effects**

292 It is well known that α -glucosidase and pancreatic lipase are involved in the digestion and
293 absorption of carbohydrates and lipids, respectively (Buchholz and Melzig, 2016; Elbashir et
294 al., 2018). In this study, the activities of these enzymes were measured to determine whether
295 SP could regulate enzymes related to diabetic complications such as hyperglycemia and
296 hyperlipidemia. As shown Fig. 3B, SPW yogurt significantly inhibited the α -glucosidase
297 activity in a dose-dependent manner ($p < 0.05$). Furthermore, the inhibitory effect of SPW yogurt
298 on pancreatic lipase activity was the highest at a concentration of 0.5% (Fig. 3D). However,
299 these enzyme inhibitory effects of SPE yogurt were significantly lower than those of plain
300 yogurt, except the α -glucosidase activity inhibitory effect of 0.1% SPE yogurt (Fig. 3A and C;
301 $p < 0.05$). The α -glucosidase activity inhibitory effect of 0.1% SPE yogurt was significantly
302 increased compared with yogurt samples with other concentrations of SPE ($p < 0.05$). Several
303 studies have suggested that various plants inhibit the activities of α -glucosidase and pancreatic
304 lipase (Elbashir et al. 2018; Gholamhoseinian et al., 2008; Yoshioka et al., 2019). Elbashir et
305 al. (2018) suggested that the strong α -glucosidase activity inhibitory effect of these plants is
306 related to their antioxidant capacities due to the presence of bioactive compounds including
307 polyphenols, flavonoids, and tannins. In this study, SPW yogurt had more TPC and TFC, and
308 showed higher free radical-scavenging activities than SPE yogurt. Therefore, SP extract-

309 supplemented yogurt might alleviate hyperglycemia and hyperlipidemia by downregulating the
310 digestion and absorption of carbohydrates and lipids.

311 Taken together, the number of viable LAB in safflower yogurt was similar to that the plain
312 yogurt, which was more than Korea Food Standard (1×10^7 CFU/mL) and the pH was 4.26-4.31,
313 which were similar to that of the plain yogurt (pH 4.3). These results indicated that SP extract-
314 supplemented yogurt had no negative effects on yogurt characteristics compared with plain
315 yogurt. The addition of SP extract resulted in an increase in the TPC and TFC, which increased
316 the antioxidant activity of SP extract-supplemented yogurt. The inhibitory effect on enzymes
317 that are involved in carbohydrate and lipid digestion and absorption resulted in a positive effect
318 on the regulation of hyperglycemia and hyperlipidemia. In addition, although the changes in
319 the storage characteristics of SP extract-supplemented yogurt were not evaluated in this study,
320 they were considered stable because there were no significant negative changes in the quality
321 of yogurt stored at 4°C for 21 d. In conclusion, SP extract has potential application in the
322 production of high value-added products, as it can enhance health benefits of various foods
323 including yogurt. Further animal studies are needed to confirm the action mechanisms and in
324 vivo effects to validate the potential benefits of yogurt supplemented with SP extract in
325 managing weight and blood glucose.

326

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433 Figure legends

434

435 **Fig. 1. Changes in pH, titratable acidity, viable cell counts, and viscosity during**
436 **fermentation of yogurt supplemented with safflower petal extract.** (A, D) pH, (B, E)
437 titratable acidity, (C, F) viable cell counts, and (G, H) viscosity. (A-C) Ethanol extracts:
438 ●, 0%; ○, 0.1%; ▼, 0.5%; △, 1.0%. (D-F) Hot water extracts: ●, 0%; □, 0.1%; ■, 0.5%;
439 ◇, 1.0%. Different uppercase letters indicate a significant difference ($p < 0.05$).

440

441 **Fig. 2. Antioxidant effects of yogurt supplemented with safflower petal extract on**
442 **human colorectal cells.** Cells treated with (A) ethanol extract and (B) hot water extract.
443 SPE: safflower petal ethanol extract; SPW: safflower petal hot water extract.

444

445 **Fig. 3. α -Glucosidase and porcine pancreatic lipase activity inhibitory effects of**
446 **yogurt supplemented with different concentrations of safflower petal extract.** (A,
447 C) safflower petal ethanol extract, (B, D) safflower petal hot water extract. Different
448 uppercase letters indicate a significant difference ($p < 0.05$).

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Table 1. Proximate analysis of yogurt supplemented with safflower petal extract

Component	Concentration of safflower petal extract (%)	Total amount (%)	
		SPE	SPW
Moisture	0.0	83.19 ± 0.34 ^b	83.19 ± 0.34 ^b
	0.1	82.13 ± 0.14 ^a	82.59 ± 0.36 ^b
	0.5	81.99 ± 0.53 ^a	81.41 ± 0.05 ^a
	1.0	81.67 ± 0.32 ^a	81.42 ± 0.06 ^a
Ash	0.0	0.80 ± 0.02	0.80 ± 0.02
	0.1	0.80 ± 0.03	0.81 ± 0.03
	0.5	0.81 ± 0.02	0.82 ± 0.02
	1.0	0.81 ± 0.03	0.81 ± 0.03
Protein	0.0	3.95 ± 0.11 ^a	3.95 ± 0.11
	0.1	4.33 ± 0.15 ^{bB}	3.93 ± 0.25 ^A
	0.5	4.37 ± 0.10 ^{bB}	4.01 ± 0.15 ^A
	1.0	4.40 ± 0.10 ^{bB}	3.98 ± 0.06 ^A
Fat	0.0	3.63 ± 0.06	3.63 ± 0.06 ^b
	0.1	3.63 ± 0.15	3.40 ± 0.10 ^a
	0.5	3.70 ± 0.26	3.73 ± 0.16 ^b
	1.0	3.73 ± 0.21	3.73 ± 0.23 ^b
Lactose	0.0	11.77 ± 0.06 ^a	11.77 ± 0.06 ^a
	0.1	12.01 ± 0.26 ^a	12.03 ± 0.06 ^b
	0.5	13.02 ± 0.06 ^b	13.07 ± 0.12 ^c
	1.0	13.11 ± 0.06 ^{bB}	13.07 ± 0.06 ^{cA}
Total solids	0.0	15.75 ± 0.45	15.75 ± 0.45
	0.1	15.89 ± 0.36	15.76 ± 1.01
	0.5	15.95 ± 0.59	16.04 ± 0.68
	1.0	16.05 ± 0.44	16.02 ± 0.21

Values are mean ± SD (n = 3).

Different small letters in the same column and capitalized letters in the same row are significantly different (p<0.05).

SPE: safflower petal ethanol extract. SPW: safflower petal hot water extract.

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457 **Table 2. Antioxidant activity of, and total polyphenol content (TPC) and total**
 458 **flavonoid content (TFC) in yogurt supplemented with safflower petal extract**

	Concentration of safflower petal extract (%)	Total amount	
		SPE	SPW
TPC ($\mu\text{g/mL}$)	0.0	115.12 \pm 28.47	282.52 \pm 4.47 ^a
	0.1	93.55 \pm 37.28	380.52 \pm 8.60 ^b
	0.5	94.49 \pm 30.49	381.46 \pm 8.61 ^b
	1.0	103.86 \pm 37.17	393.19 \pm 0.81 ^b
TFC ($\mu\text{g/mL}$)	0.0	52.53 \pm 10.00 ^a	180.97 \pm 30.97 ^a
	0.1	57.12 \pm 3.98 ^{ab}	210.80 \pm 2.29 ^b
	0.5	73.17 \pm 4.59 ^{bc}	270.44 \pm 11.92 ^c
	1.0	77.76 \pm 3.97 ^c	320.90 \pm 2.29 ^d
Antioxidant activity DPPH (%)	0.0	3.24 \pm 0.62	5.81 \pm 0.61 ^a
	0.1	1.03 \pm 0.58	6.69 \pm 0.78 ^a
	0.5	2.60 \pm 0.20	7.17 \pm 3.94 ^a
	1.0	2.79 \pm 0.85	10.66 \pm 1.21 ^b
ABTS (%)	0.0	ND	40.00 \pm 1.54 ^a
	0.1	ND	48.00 \pm 3.00 ^b
	0.5	ND	48.33 \pm 1.76 ^b
	1.0	ND	49.67 \pm 0.88 ^b

459 Values are mean \pm SD (n = 3).

460 Different superscripts in the same column are significantly different (p<0.05).

461 SPE: safflower petal ethanol extract; SPW: safflower petal hot water extract; ND: not
 462 detected; DPPH: 2,2-diphenyl-2-picrylhydrazyl; ABTS: 2,2'-azino-bis (3-
 463 ethylbenzothiazoline-6-sulfonic acid)

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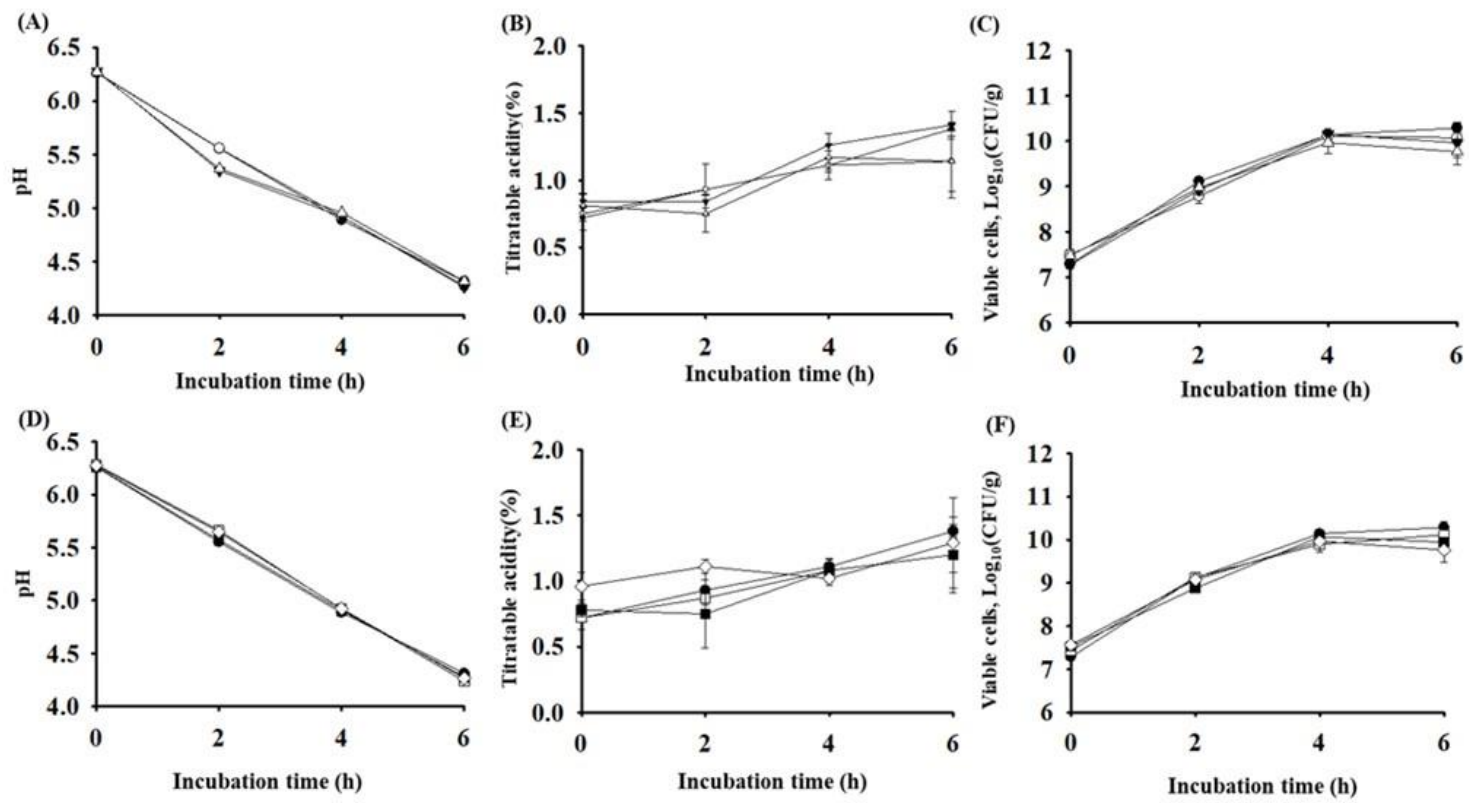


Figure 1

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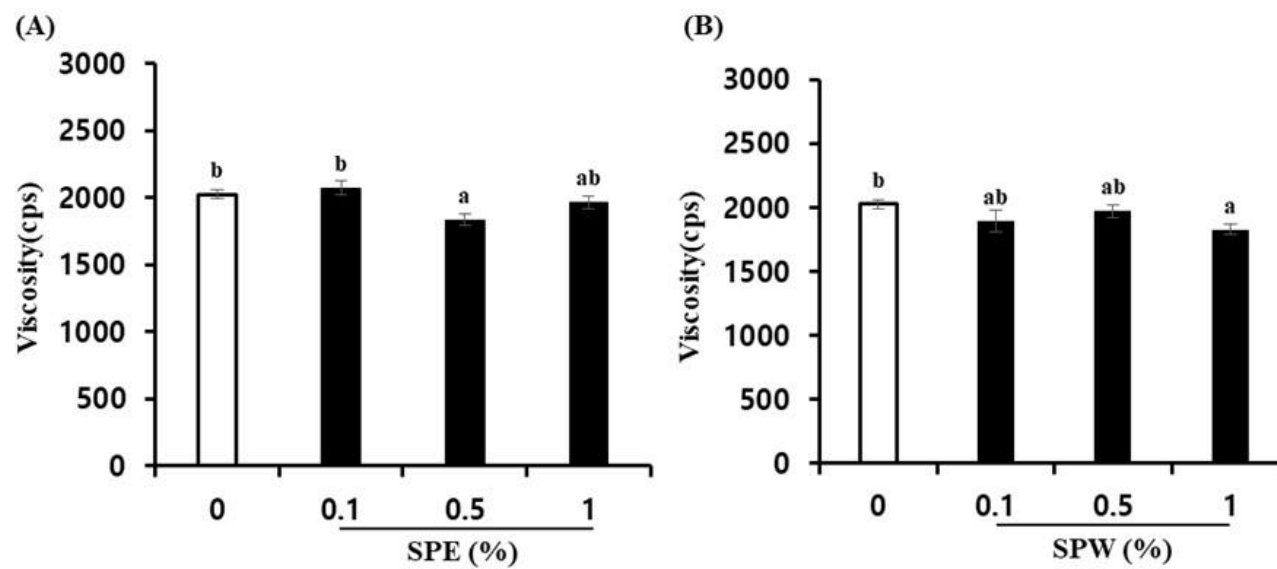
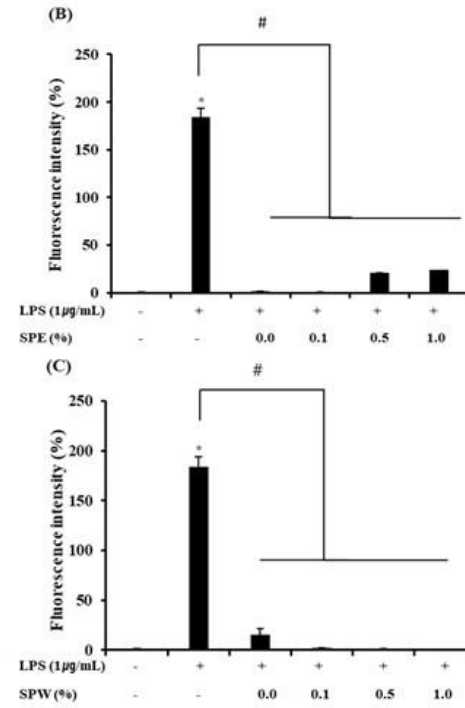
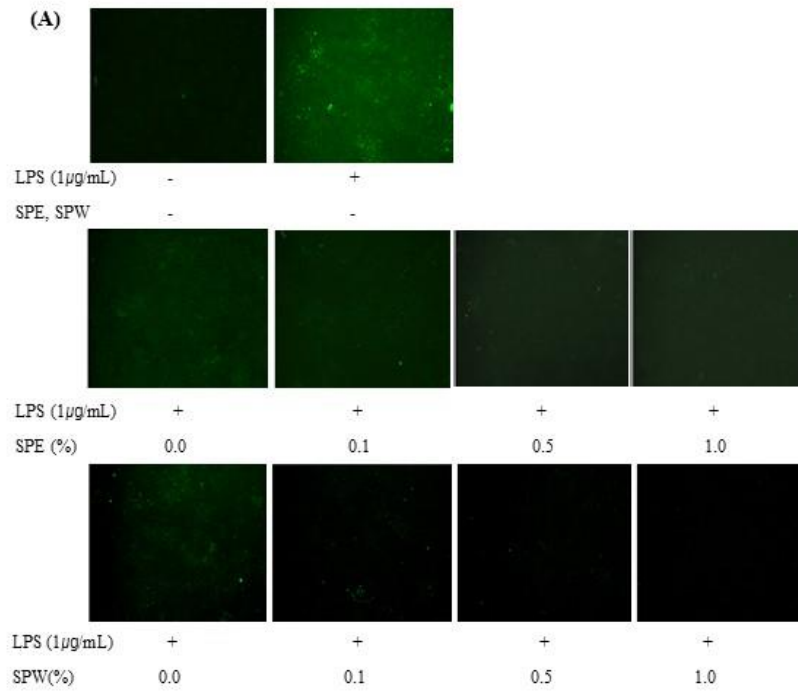


Figure 2

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Figure 3



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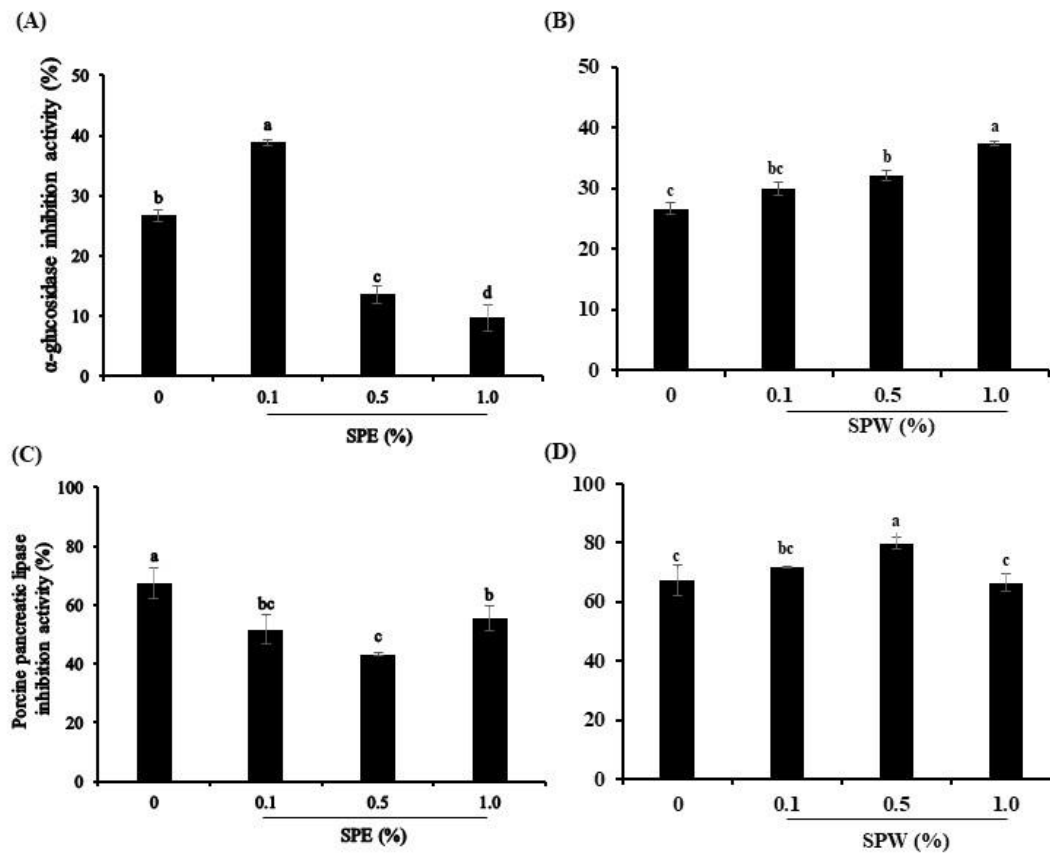


Figure 4

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