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Journal Name	Food Science and Biotechnology	
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Schedule
Received 1 November 2016
Revised 27 March 2017
Accepted 27 March 2017

Abstract The purpose of this study was to evaluate and compare the antioxidant activities, and their contents, in grape juices prepared by various household juicers, and grape flesh (GF). The grape juices were prepared using a low-speed masticating (LSM) juicer, a high-speed centrifugal (HSC) juicer, and a blender (BLD). The total polyphenol, total flavonoid, total monomeric anthocyanin, and vitamin C contents were highest in the LSM grape juice, and decreased in the order: LSM > BLD > HSC > GF. The antioxidant activities such as DPPH radical scavenging activity, and SOD-like activity were significantly higher in the LSM juice than in other juices and grape flesh. The antioxidant activities and the quality of grape juices were significantly affected by the household juicing method used, and an LSM juicer is strongly recommended for making healthy grape juice, rich in antioxidants.

Keywords (separated by '-') Grape juice - Grape flesh - Low-speed masticating household juicer - Antioxidant content - Antioxidant activity

Footnote Information

3 Antioxidant activities of fresh grape juices prepared using various 4 household processing methods

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7 Received: 1 November 2016 / Revised: 27 March 2017 / Accepted: 27 March 2017
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9 **Abstract** The purpose of this study was to evaluate and
10 compare the antioxidant activities, and their contents, in
11 grape juices prepared by various household juicers, and
12 grape flesh (GF). The grape juices were prepared using a
13 low-speed masticating (LSM) juicer, a high-speed cen-
14 trifugal (HSC) juicer, and a blender (BLD). The total
15 polyphenol, total flavonoid, total monomeric anthocyanin,
16 and vitamin C contents were highest in the LSM grape
17 juice, and decreased in the order: LSM > BLD > HSC >
18 GF. The antioxidant activities such as DPPH radical
19 scavenging activity, and SOD-like activity were signifi-
20 cantly higher in the LSM juice than in other juices and
21 grape flesh. The antioxidant activities and the quality of
22 grape juices were significantly affected by the household
23 juicing method used, and an LSM juicer is strongly rec-
24 ommended for making healthy grape juice, rich in
25 antioxidants.

27 **Keywords** Grape juice · Grape flesh · Low-speed
28 masticating household juicer · Antioxidant content ·
29 Antioxidant activity

Introduction 30

Free radicals can cause oxidative stress, and oxidative 31
damage plays a significant pathological role in chronic 32
diseases including cardiovascular diseases, cancer, diabetes 33
mellitus, and hypertension. Antioxidants, including phe- 34
nolic compounds, flavonoids, and carotenoids, can reduce 35
the risk of oxidative damage by scavenging free radicals 36
and oxygen, and chelating catalytic metals. It is well 37
known that fruits and vegetables are major sources of 38
antioxidants. 39

The grape is one such fruits consumed world-wide, and 40
can be eaten raw (as table fruit), or in processed products 41
such as wine, juice, and sauce among others [1, 2]. Grapes 42
are a good natural source of antioxidants, containing many 43
phytochemicals such as anthocyanin, catechin, epicatechin, 44
resveratrol, and proanthocyanidin, and therefore have 45
strong activity for scavenging free radicals [3]. Besides, 46
these polyphenols are known to have anticarcinogenic, 47
anti-inflammatory, and antiproliferative properties. Most 48
grape phenolic antioxidants are located in the skin and 49
seeds. The estimated polyphenol content is approximately 50
60–70% in grape seeds, 30% in the skin, and only 6% in 51
the flesh [1, 4]. Resveratrols, anthocyanins, and catechins 52
are present in grape skins, while procyanidins are con- 53
centrated in the seeds [1]. Notably, grape seeds contain 54
approximately 20-fold more proanthocyanidins compared 55
with grape skins [5]. The purple colors of grapes and red 56
wines are attributed to anthocyanins, which seem to be 57
significantly correlated with antioxidant properties [4]. 58

Generally, table grapes are consumed after removal of 59
the seeds and skins, which are rich in polyphenols. 60
Therefore, the consumption of grape seeds and skins is 61
very helpful for the intake of bioactive components. Fresh 62
grape juice, directly squeezed from whole grapes using a 63

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64 juicer, has an advantage over the consumption of
65 table grapes, since large quantities of the seeds and skins
66 are included in the juice, and the nutritional value of grapes
67 is significantly affected by processing conditions [6, 7].
68 The phenolic composition and antioxidant activities of
69 grape juices can also change depending on the type of
70 household juicer used [8].

71 In this study, we tried to determine and compare the
72 antioxidant levels and activities in grape juices prepared
73 using various household processing methods, as well as the
74 consumption of grapes as table fruit without seeds and
75 skins. The grape juices were prepared using a blender
76 (BLD), a conventional juicer (high-speed centrifugal juicer,
77 HSC juicer), and a screw-type juicer (low-speed masticating
78 juicer, LSM juicer). While an HSC juicer grinds and filters
79 the grapes with a flat blade disk rotating at a high speed of
80 8000–12,000 rpm, an LSM juicer squeezes the grapes with
81 a vertical helical screw (auger), rotating at a low speed of
82 approximately 40–80 rpm. Kim et al. [9] reported that
83 tomato juice prepared using an LSM juicer was richer in
84 antioxidant compounds compared with that produced using
85 an HSC juicer, since destruction of the compounds by
86 oxidation could be minimized as heat generation was
87 negligible with a slow-speed rotating auger. From this
88 result, it is expected that the antioxidant levels and
89 activities in grape juices may be significantly affected by
90 the type of household juicer used.

91 A large number of studies have been published on the
92 antioxidant activities of grape juice and grape products, but
93 there are limited reports of fresh grape juices prepared
94 using various household processing methods [8]. The aim
95 of this study is to evaluate and compare the antioxidant
96 activities (focused on free radical scavenging activities),
97 and the quality, of grape juices prepared using various
98 household juicers, and the grape flesh without skin and
99 seeds.

100 Materials and methods

101 Materials

102 The grapes (*Vitis labrusca*, Campbell Early) were obtained
103 from a local market in Gimhae, Korea. Folin-Ciocalteu's
104 reagent, tannic acid, pyrogallol, DL-dithiothreitol (DTT),
105 L-ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH),
106 dimethyl sulfoxide (DMSO), hydrogen peroxide, and fluo-
107 rescein were purchased from Sigma-Aldrich (St. Louis,
108 MO, USA). 2,2'-Azobis (2-methylpropionamide) dihydro-
109 chloride (AAPH) was purchased from Wako Pure
110 Chemical Industries, Ltd. (Osaka, Japan), and copper sul-
111 fate (II) pentahydrate was purchased from Kanto Chemical
112 Co. (Chuo-ku, Tokyo, Japan). All organic solvents and

other chemicals were of analytical grade or complied with
the standards needed for cell culture experiments, or high
performance liquid chromatography (HPLC) grade.

Preparation of grape juices and grape flesh

117 Grapes were detached from a bunch and washed with tap
118 water before juice extraction. The HSC and LSM grape
119 juices were prepared using an HSC juicer (SJ 600, Dongah
120 Co., Ltd., Korea) and a vertical-type LSM juicer (SJ200B,
121 Hurom Co., Ltd., Korea), respectively, according to the
122 manufacturer's instructions. For BLD juice preparation, the
123 same amount of water (1:1, w/w) was added to washed
124 grapes before grinding for 5 min using a blender (HM-
125 1600 PB, Hanil Co., Korea). The grape flesh (GF) was
126 prepared from washed grapes by removing skins and seeds
127 by hand. Each sample was used immediately in experi-
128 ments, or freeze-dried just after sample preparation using a
129 freeze-dryer (FDU-7003, Operon Co., Ltd., Korea) and
130 kept at $-20\text{ }^{\circ}\text{C}$ before analysis.

Physicochemical analyses

132 The pH of grape juices was determined using a pH meter
133 (AG 8603, Mettler Toledo, Schwerzenbach, Switzerland)
134 at $25 \pm 1\text{ }^{\circ}\text{C}$. Total soluble solids (TSS) were measured
135 with a digital refractometer (Atago, PAL-1 digital refrac-
136 tometer, Tokyo, Japan). Titratable acidity (TA) of the
137 grape juice was measured by titrating 100 mL of five-fold
138 diluted juice to pH 8.3 with standardized 0.1 N NaOH. The
139 TA was expressed as the percentage of citric acid per
140 100 mL of juice.

Analysis of total polyphenol content

142 Total polyphenol content was analyzed according to the
143 Folin-Denis method with some modification [10]. A sam-
144 ple (0.1 g) of freeze-dried juice powder was dissolved in
145 10 mL of 80% MeOH, resuspended thoroughly by vor-
146 texing, and kept for 2 h at room temperature in the dark.
147 After centrifuging the solution at $1000\times g$ and $4\text{ }^{\circ}\text{C}$ for
148 20 min, a supernatant (GJME) was collected. First, 0.2 mL
149 of 50% Folin-Ciocalteu's reagent was added to 0.4 mL of
150 properly diluted GJME and mixed well at room tempera-
151 ture. After 3 min, 0.4 mL of 2% Na_2CO_3 was added, and
152 the mixture was incubated for 60 min at room temperature.
153 The absorbance was measured at 750 nm using a spec-
154 trophotometer (Libra S22, Biochrom, Cambridge, Eng-
155 land), and quantified from a calibration curve using tannic
156 acid as a standard. All samples were analyzed in triplicate.
157 Total polyphenol content was expressed as milligrams of
158 tannic acid equivalents (TAE) per 100 mL juice.

159 **Analysis of total flavonoid content**

160 Total flavonoid content was measured according to the
 161 method described by Zhishen with some modification [11].
 162 First, 0.03 mL of 5% NaNO₂ was added to 0.4 mL of
 163 properly diluted GJME. After 5 min, 0.3 mL of 1% AlCl₃
 164 was added to the mixture and incubated for 6 min at room
 165 temperature, before 0.2 mL of 1 M NaOH and 0.07 mL of
 166 deionized water were added sequentially. The absorbance
 167 of the mixture was measured at 510 nm using a spec-
 168 trophotometer, and quantified using quercetin as a stan-
 169 dard. All samples were analyzed in triplicate. Total
 170 flavonoid content was expressed as milligrams of quercetin
 171 equivalents (QE) per 100 mL juice.

172 **Analysis of total monomeric anthocyanin**

173 Anthocyanin content was determined using the method
 174 described by Lee et al. [12], with some modification. A sample
 175 (0.2 g) of freeze-dried juice powder was dissolved in 3 mL of
 176 deionized water and kept for 3 h at 4 °C. An aliquot of
 177 supernatant was collected by centrifugation at 850×g
 178 and 4 °C for 20 min. Then, 0.1 mL of the sample was added
 179 to 0.9 mL of pH 1.0 buffer (0.2 M KCl + 0.2 M HCl) or
 180 0.9 mL of pH 4.5 buffer (0.02 M sodium acetate + 0.1 M
 181 citric acid) and incubated for 20 min. The absorbance of each
 182 solution was measured at 520 nm and 700 nm, respectively,
 183 using a spectrophotometer. The content of total monomeric
 184 anthocyanin was calculated from a molecular extinction
 185 coefficient (26,900 L/cm·mol) of cyanidine-3-glucoside and
 186 expressed as milligrams of cyanidine-3-glucoside equivalents
 187 (CE) per 100 mL juice using the following equation. Analyses
 188 were conducted in triplicate.

Total monomeric anthocyanin content (mg CE/100 mL)

$$= \frac{A \times ME \times SC \times 150 \times 100}{\epsilon \times L}$$

190 where $A = \{(A_{520\text{nm}} - A_{700\text{nm}}) \text{ at pH}1.0\} - \{(A_{520\text{nm}}$
 191 $- A_{700\text{nm}}) \text{ at pH}4.5\}$, MW is the molecular weight of
 192 cyanidine-3-glucoside (449.2 g/mol), SC is the solid con-
 193 tent of grape juice (g/mL), ϵ is the molecular extinction
 194 coefficient of cyanidine-3-glucoside (26,900 l/cm·mol),
 195 and L is the cell path length (1 cm).

196 **Analysis of vitamin C content**

197 Vitamin C (L-ascorbic acid) content was analyzed using the
 198 method described by Furusawa with some modification [13].
 199 A sample (0.1 g) of freeze-dried juice powder was dissolved
 200 in 1 mL of 2 mg/mL DTT in 2% acetic acid, and stored for
 201 3 h at 4 °C in the dark, in order to reduce dehydroascorbic
 202 acid to ascorbic acid. The supernatant was analyzed by
 203 HPLC (Ultimate 3000, Dionex, Sunnyvale, CA, USA) after

centrifugation at 1000×g and 4 °C for 20 min. Each sample
 was filtered through a 0.45 μm membrane filter (Millipore
 Corp., Bedford, MA, USA) before injection. L-Ascorbic acid
 was separated using a C₁₈ column (Gemini 5 μm C18,
 250 × 4.6 mm; Phenomenex, Torrance, CA, USA) and 2%
 acetic acid was used as the mobile phase, in isocratic mode
 with a flow rate of 1 mL/min. The peaks were monitored
 using a photodiode array detector (PDA, Dionex, Sunnyvale,
 CA, USA) at 254 nm. The content of vitamin C was cal-
 culated from the peak area corresponding to L-ascorbic acid
 using a calibration curve of authentic L-ascorbic acid.
 Analyses were performed in triplicate.

216 **Analysis of DPPH radical scavenging activity**

217 DPPH radical scavenging activity was assayed based on the
 218 method described by Blois with some modification [14].
 219 Briefly, 0.2 mL of the properly diluted sample (GJME) was
 220 added to 0.8 mL of 0.3 mM DPPH solution in 80% etha-
 221 nol, and incubated in the dark for 10 min at room tem-
 222 perature. **The radical scavenging activity (%) was**
 223 **calculated** from the decrease in the absorbance measured at
 224 517 nm using a spectrophotometer. The results were
 225 expressed as the amount of juice (mg of dry weight) which
 226 is required to scavenge 50% of DPPH radical (IC₅₀). Dis-
 227 tilled water was used as a control.

DPPH radical scavenging activity (%)

$$= \left(1 - \frac{\text{Abs. of sample}}{\text{Abs. of control}} \right) \times 100$$

229 **Analysis of SOD-like activity** 230

231 Superoxide dismutase (SOD)-like activity was analyzed
 232 using the method described by Murklund [15]. Briefly,
 233 0.65 mL of 50 mM Tris-HCl buffer (pH 8.7) and 0.1 mL
 234 of 7.2 mM pyrogallol were added sequentially to 0.1 mL
 235 of the properly diluted sample (GJME) and the mixture was
 236 incubated for 10 min at 25 °C. SOD-like activity (%) was
 237 assayed by measuring superoxide anion radical inhibition
 238 at 420 nm using a spectrophotometer. Distilled water was
 239 used as a control. The results were expressed as the amount
 240 of juice (μg of dry weight) required to reduce 50% of the
 241 superoxide anion (IC₅₀).

$$\text{SOD - like activity (\%)} = \left(1 - \frac{\text{Abs. of sample}}{\text{Abs. of control}} \right) \times 100$$

243 **Oxygen radical absorbance capacity (ORAC) assay** 244

245 The ORAC assay was performed on the freeze-dried
 246 samples as described by Kurihara et al. [16]. Peroxyl

247 radical was generated using AAPH (20 mM) and fluores-
 248 cein (40 nM) was used as a target of free radical attack.
 249 The decay of fluorescence was measured using a multi-
 250 functional plate reader (GENios; Tecan Trading AG,
 251 Grödig, Austria) with fluorescent filters (excitation wave-
 252 length: 485 nm and emission wavelength: 535 nm). The
 253 blank-corrected area under the fluorescence decay curve
 254 for each sample was plotted against the Trolox concen-
 255 trations, and the ORAC values were expressed as μM of
 256 trolox equivalents ($\mu\text{M TE}$).

257 Statistical analysis

258 All data are presented as mean \pm SD. The mean values
 259 were compared using one-way analysis of variance
 260 (ANOVA) followed by Duncan's multiple range tests
 261 (SPSS, version 19). Significance was accepted as a prob-
 262 ability of 5% and was defined as $p < 0.05$.

263 Results and discussion

264 Physicochemical properties

265 The general physicochemical properties of grape juices
 266 prepared by various household methods and grape flesh
 267 were shown in Table 1. The yield of the LSM juicer was
 268 79.1%, which was better than that of the HSC juicer
 269 (45.0%). The difference in yield is caused by the different
 270 juice extracting mechanisms, and a similar result was
 271 obtained when preparing tomato juice [9]. The TSS of the
 272 grape juices ranged from 13.8 to 14.4. The TA of the GF
 273 and the LSM juice were higher than those of the HSC and
 274 BLD juices. Higher TA in grape juice is known to produce
 275 better sensory attributes, associated with its characteristic
 276 flavor and astringency [17]. While the pH of LSM and HSC
 277 grape juices, and GF, was similar and ranged from 3.20 to
 278 3.23, BLD juice showed the highest pH of 3.34. Aguilar-
 279 Rosas et al. [18] reported that the pH of juice was directly
 280 related to temperature. Heat generation during blender

operation seems to be one of reasons for the high pH. 281
 Maintaining a low pH also helps to prevent the growth of 282
 pathogenic microorganisms in the fruit juice [18]. 283

Total polyphenol content 284

Polyphenols are grouped into flavonoids, phenolic acids, 285
 and phenolic compounds in plants, and are known to have 286
 antioxidant effects in vitro and in vivo [1]. Since the 287
 antioxidant activities of plant extracts are closely related 288
 with their polyphenol content, extracts containing high- 289
 level of polyphenols have a great importance as natural 290
 antioxidants [19]. Phytochemicals, especially phenolics in 291
 fruits and vegetables, are major bioactive compounds 292
 known for their health benefits [4]. In particular, grape 293
 skins and seeds are reported to be abundant in polyphenols 294
 and exert potent free radical scavenging effects by con- 295
 verting radicals into stable compounds to stop radical- 296
 mediated reactions [1]. Total polyphenol contents of grape 297
 juices produced using various household methods are 298
 shown in Fig. 1(A). These results indicated that grape 299
 juices were rich in polyphenols, but the contents were quite 300
 different according to the juicing methods. While total 301
 polyphenol content of the LSM grape juice ($326.8 \pm$ 302
 $1.6 \text{ mg TAE}/100 \text{ mL}$) was the highest, that of GF (con- 303
 sumption of fresh grapes after removal of skins and seeds) 304
 was the lowest ($47.1 \pm 0.4 \text{ mg TAE}/100 \text{ mL}$) among the 305
 samples, as expected. Xu et al. [2] reported that grape skins 306
 and seeds were rich in phenolic compounds, and that the 307
 seeds contained higher levels than the skins. Total 308
 polyphenol content of the HSC grape juice ($90.3 \pm 1.4 \text{ mg}$ 309
 $\text{TAE}/100 \text{ mL}$) was significantly lower compared with that 310
 of the LSM grape juice. This result is due to the different 311
 extraction mechanisms of the juicers. The flat blade disk 312
 rotating at a high speed (8000–12,000 rpm), causes 313
 deflection of a considerable amount of the grape to waste, 314
 and therefore the extraction of polyphenols from grape 315
 skins and seeds is insufficient [9]. Although a blender 316
 ground all parts of the grape without loss during the juicing 317
 process, the total polyphenol content of BLD juice was 318

Table 1 Yields, total soluble solids (TSS), titratable acidity (TA) and pH of grape juices prepared using various household processing methods, and grape flesh

	LSM	HSC	BLD	GF
Yield (%)	79.1	45.0	100.0	44.6
TSS ($^{\circ}\text{Brix}$)	$14.3 \pm 0.1^{\text{a}}$	$14.4 \pm 0.1^{\text{a3}}$	$13.8 \pm 0.1^{\text{b}}$	$13.7 \pm 0.1^{\text{b}}$
TA (%)	$0.32 \pm 0.00^{\text{b}}$	$0.29 \pm 0.00^{\text{c}}$	$0.20 \pm 0.01^{\text{d}}$	$0.35 \pm 0.00^{\text{a}}$
pH	$3.22 \pm 0.01^{\text{b}}$	$3.20 \pm 0.01^{\text{c}}$	$3.34 \pm 0.01^{\text{a}}$	$3.23 \pm 0.01^{\text{b}}$

Grape juices were prepared using a low-speed masticating juicer (LSM), a high-speed masticating juicer (HSC), and a blender (BLD). Grape flesh (GF) was prepared by removing seeds and skins from whole grapes

¹ The results are expressed as mean \pm SD ($n = 3$)

³ Values with different letters in the same column are significantly different, $p < 0.05$

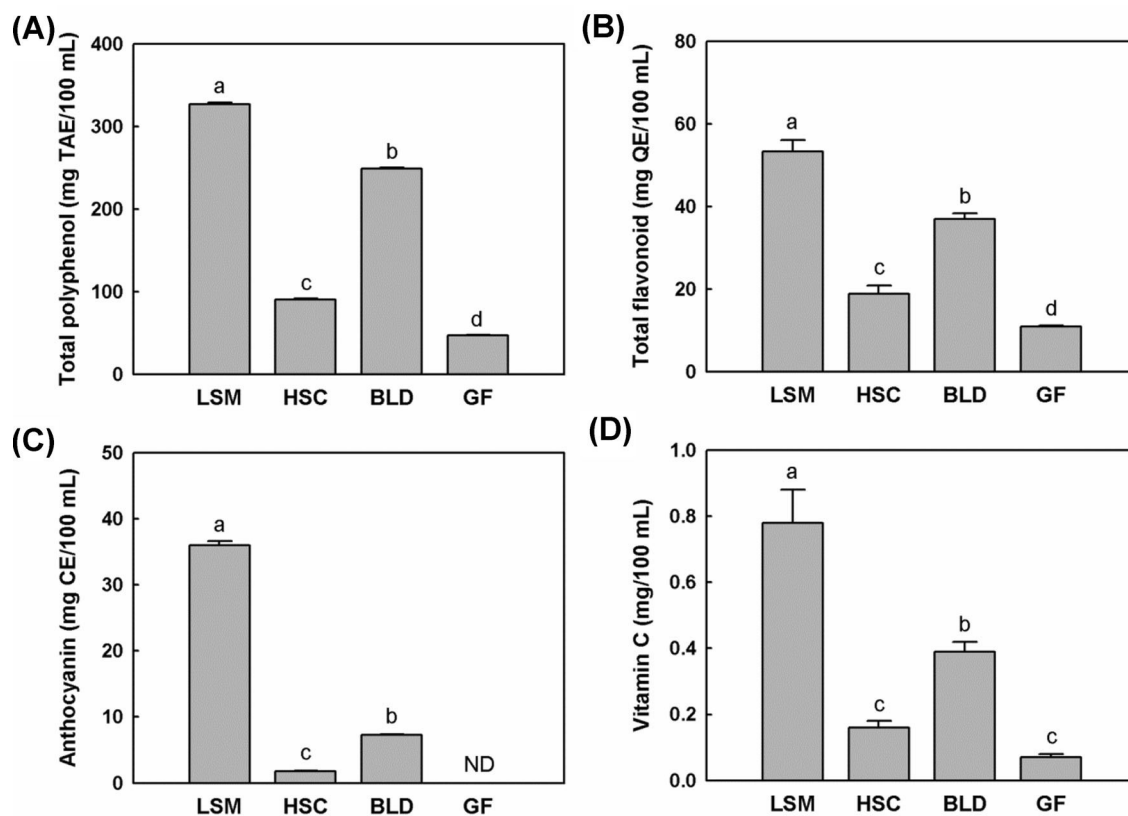


Fig. 1 The antioxidant contents of grape juices and grape flesh. (A) Total polyphenol, (B) total flavonoid, (C) total monomeric anthocyanin, and (D) vitamin C. Grape juices were prepared using a low-speed masticating juicer (LSM), a high-speed masticating juicer (HSC), and a blender (BLD). Grape flesh (GF) was prepared by

removing seeds and skins from whole grapes. TAE tannic acid equivalents; QE quercetin equivalents; and CE cyanidine-3-glucoside equivalents. The results are expressed as mean \pm SD ($n = 3$). Different superscripts signify significant differences ($p < 0.05$) by Duncan's multiple range test. ND not detected

319 lower than that of LSM juice. Since oxidation was accel- 338
 320 erated by the blender, due to heat generated by the high- 339
 321 speed rotating blade, the polyphenols were more easily 340
 322 destroyed than when using the LSM juicer. Also, BLD 341
 323 juice showed higher polyphenol oxidase (PPO) activity 342
 324 than LSM juice, which could be one of the reasons for the 343
 325 low polyphenol content (data not shown). In a similar study 344
 326 by Burin et al. [8] on total polyphenol contents in grape 345
 327 juices using the Folin–Ciocalteu method, the results for 346
 328 homemade, commercial, and organic grape juices ranged 347
 329 between 111.7 and 343.3 mg GAE/100 mL. They sug- 348
 330 gested that the differences come from juice processing 349
 331 techniques, including extraction type, temperature, and 350
 332 addition of enzymes. Previous study with tomato juices 351
 333 prepared using different household extraction methods 352
 334 showed similar results as with grape juices [9]. These 353
 335 results suggest that, from the view point of polyphenol 354
 336 uptake, the consumption of fresh, whole grape juice is 355
 337 better than that of grape flesh without seeds and skins, and

an LSM juicer was more effective for preparation of grape 338
 juice rich in polyphenols, than an HSC juicer or a blender. 339

Total flavonoid content 340

341 Flavonoids are a group of polyphenols, consisting of a C6– 342
 343 C3–C6 flavone skeleton, with a three-carbon bridge 344
 345 cyclized with oxygen between the phenyl groups, and have 346
 347 been shown to inhibit tumor growth, NF- κ B activation, and 348
 349 cytokine synthesis [20]. Flavonoids are widely distributed 350
 351 in fruits and vegetables. In particular, grapes are known to 352
 353 be rich in several flavonoids including resveratrol, antho- 354
 355 cyanin, kaempferol, myricetin, and quercetin [2, 21]. Total 356
 flavonoid contents of fresh grape juices, produced using 357
 household juicers, ranged from 18.8 ± 2.1 for HSC juice, 358
 to 53.3 ± 2.8 mg QE/100 mL for LSM juice (Fig. 1(B)). 359
 The GF showed the lowest total flavonoid content. The 360
 results showed a similar trend to total polyphenol contents 361
 as discussed above. However, the difference in the total 362
 363
 364

Author Proof

355 flavonoid contents among the juices was lower than that in
356 the total polyphenol contents.

357 **Total monomeric anthocyanin content**

358 Monomeric anthocyanin content, determined by the struc-
359 tural transformation that occurs when pH changes, is also
360 known as the total anthocyanin content. Anthocyanin is
361 highly pigmented in many red, purple, and blue flowers,
362 fruits, and vegetables, especially in grapes and berries. The
363 most abundant anthocyanins in grape juices were noted as
364 cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and
365 malvidin-3-*O*-glucoside [22]. Anthocyanin is the major
366 contributor to the free radical scavenging activity of red
367 grapes [4]. The total anthocyanin contents of grape juices
368 and GF are shown in Fig. 1(C). The anthocyanin content in
369 LSM grape juice was significantly higher than those of
370 other juices ($p < 0.05$). Interestingly, the LSM grape juice
371 (36.0 ± 0.6 mg CE/100 mL) showed about 20-fold higher
372 total anthocyanin content than HSC grape juice
373 (1.8 ± 0.1 mg CE/100 mL). The BLD juice also showed a
374 low total anthocyanin content (7.3 ± 0.1 mg CE/100 mL).
375 Many studies reported that anthocyanins are unsta-
376 ble compounds, and easily susceptible to degradation
377 through factors such as light, pH, oxygen, enzymes, and
378 temperature during storage, and especially during heat
379 processing [22, 23]. Therefore, the anthocyanins in BLD
380 juices were supposed to be destroyed due to the enhanced
381 oxidation caused by the relatively high temperature and the
382 action of PPO. Tiwari et al. [22] reported that total
383 anthocyanin was 15.8 mg/100 mL in ozonated grape juice,
384 which was lower than that of LSM juice in this study. The
385 differences seem to be caused by various factors such as
386 grape species and the processing method. These results
387 indicated that the LSM juicer was suitable for the extrac-
388 tion of anthocyanin from grapes, with minimum degrada-
389 tion. As expected, when we have fresh grapes, after
390 removal of skins and seeds (GF), most of the anthocyanins
391 are also discarded together with skins and seeds.

392 **Vitamin C contents**

393 Vitamin C reduces the initial quinone formed by PPO
394 enzymes to the original diphenol, preventing the process
395 that leads to browning [24]. Therefore, vitamin C has a
396 positive effect on color during juice making. Fig-
397 ure 1(D) shows vitamin C contents of the grape juices
398 produced using various household juicers, and the grape
399 flesh. The results were similar to those for polyphenols,
400 flavonoids, and anthocyanins. The content of vitamin C in
401 LSM grape juice was the highest, and decreased in the
402 order: LSM > BLD > HSC > GF with a range between
403 0.07 ± 0.01 and 0.78 ± 0.10 mg/100 mL. The lower

404 contents in BLD and HSC juices were supposed to be
405 mainly attributed to the thermolysis and oxidation of
406 ascorbic acid, due to grinding with high-speed rotating
407 blades [25]. We can consume greater than tenfold more
408 vitamin C with LSM juice, compared with grape flesh
409 without seeds and skins.

DPPH radical scavenging activity

DPPH radical scavenging activity was measured by
detecting reduction of DPPH free radicals after electron
transfer from antioxidants, a widely used test of free-rad-
ical scavenging ability [14]. The DPPH radical scavenging
activities (IC_{50}) of the grape juices prepared using various
juicing methods, ranged from 0.27 ± 0.01 to 3.81 ± 0.16
mg (Fig. 2(A)). The IC_{50} values of LSM and BLD grape
juices were the lowest, that is, the DPPH radical scav-
enging activity of both juices was better than that of other
samples. Higher antioxidant contents, such as total
polyphenol, total flavonoid, vitamin C, and total antho-
cyanin, in LSM and BLD juices seems to be one of the
major reasons for this result, which is in agreement with
other studies [26]. The similar results were observed as
tomato juices prepared using different household extraction
methods among which LSM juicer showed lower DPPH
radical scavenging activity than HSC juicer [9].

SOD-like activity

SOD-like activity was measured by elimination of super-
oxide, a kind of active oxygen. Among the samples, the
LSM grape juice showed the highest SOD-like activity,
with the lowest IC_{50} value of 3.9 ± 0.5 μ g (Fig. 2(B)). The
other three samples showed similar activities. Iwasawa
et al. [27] noted that the main candidate for SOD-like
activity was polyphenol rather than vitamin C in fruits. The
SOD-like activities of the samples in this study were higher
than those reported by Dani et al. [28]. This may be due to
the use of different grape cultivars and sample preparation
methods (fresh vs. freeze-dried samples).

Oxygen radical absorbance capacity (ORAC)

The ORAC assay, which is recommended as the standard
method for determining both the antioxidant activity of
food, and human plasma antioxidant capacity [29, 30], was
adapted in this study. The ORAC values of grape juices
prepared using various household processing methods are
shown in Fig. 3. Concordant with the results of total
polyphenols, flavonoids, anthocyanins, vitamin C, and
DPPH radical scavenging activity, the grape juices pre-
pared using the blender (BLD) and the LSM juicer showed
the highest ORAC values (2.78 ± 0.03 and

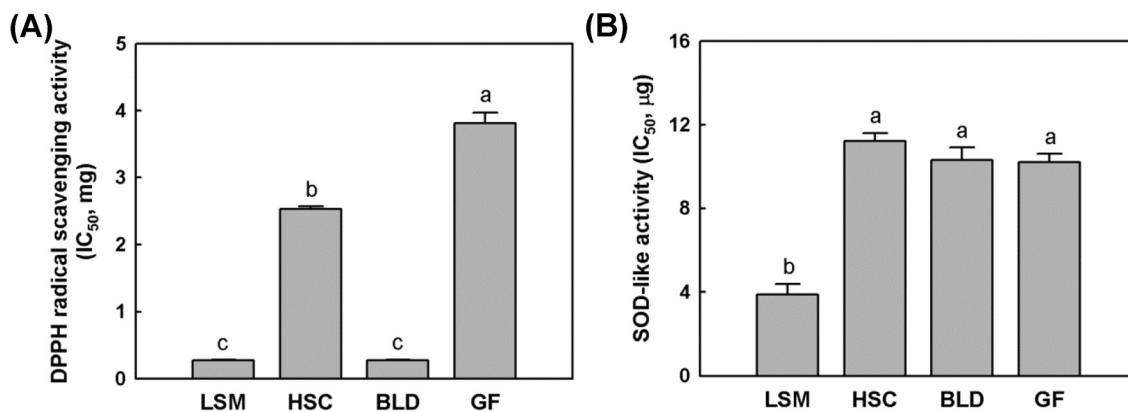


Fig. 2 The antioxidant activities of grape juices and grape flesh. (A) DPPH radical scavenging activity and (B) SOD-like activity. Grape juices were prepared using a low-speed masticating juicer (LSM), a high-speed masticating juicer (HSC), and a blender (BLD). Grape flesh (GF) was prepared by removing seeds and skins from

whole grapes. IC₅₀: the amount of juice (dry weight) required for 50% reduction of DPPH radical or superoxide anion radical. The results are expressed as mean ± SD (*n* = 3). Different superscripts signify significant differences (*p* < 0.05) by Duncan's multiple range test

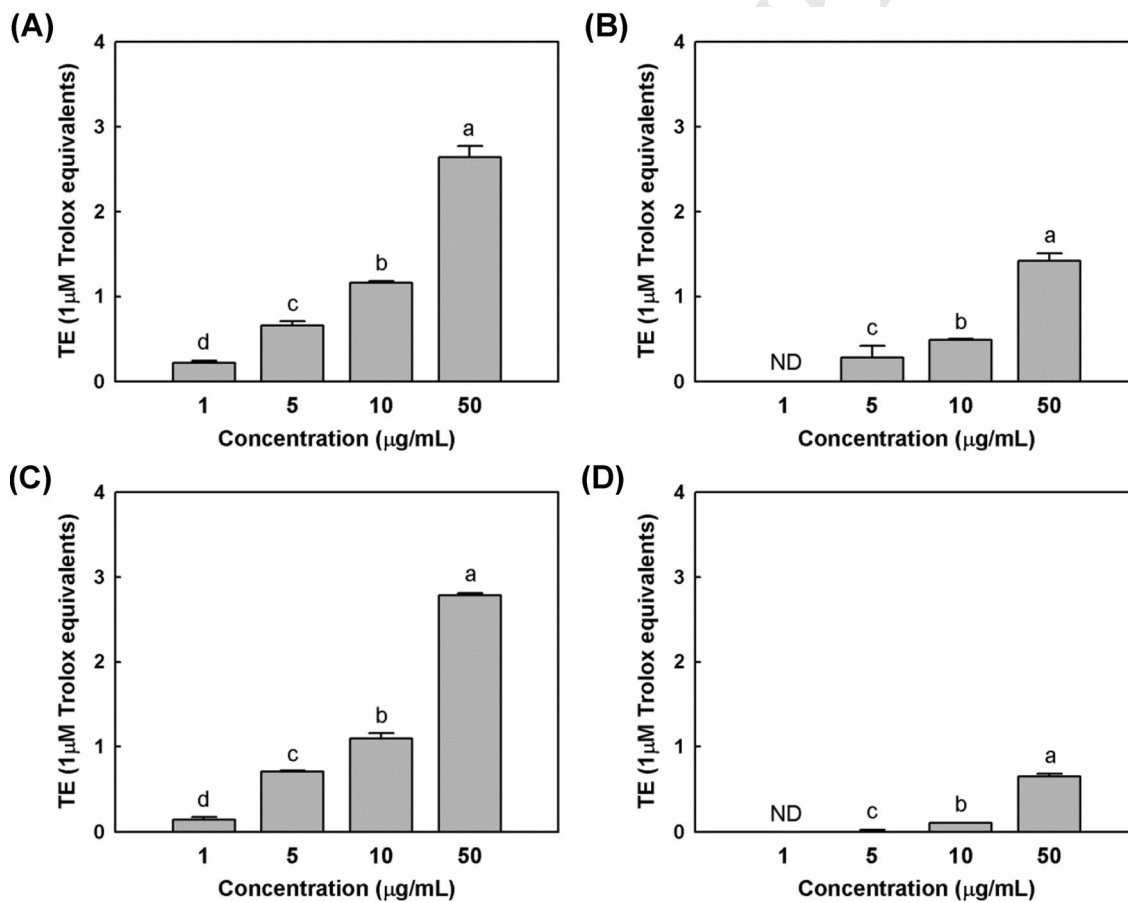


Fig. 3 Oxygen radical absorbance capacity (ORACROO-) values of grape juices prepared using various household processing methods, and grape flesh. (A) Low-speed masticating juicer (LSM); (B) high-speed masticating juicer (HSC); (C) blender (BLD); and (D) grape

flesh (GF). The results are expressed as mean ± SD (*n* = 3). For all the bars of each sample, different superscripts signify significant differences (*p* < 0.05) by Duncan's multiple range test. ND: Not detected

451 2.64 ± 0.13 μM TE at the concentration of 50 μg/mL,
452 respectively), followed by grape juices using the HSC
453 juicer (1.42 ± 0.09 μM TE at 50 μg/mL). The raw grape
454 flesh showed the lowest ORAC value (0.65 ± 0.03 μM TE
455 at 50 μg/mL). Dávalos et al. [31] reported that the ORAC
456 value could be changed according to their phenolic con-
457 tent contents among red and white grape juices. It seems that the
458 higher contents of total polyphenol and total flavonoid in
459 LSM and BLD made a positive contribution to higher
460 ORAC values.

461 Our research demonstrated that the antioxidant activi-
462 ties, and the quality of grape juices, were significantly
463 affected by the household juicing method used. Antioxi-
464 dant activities and nutritional properties of LSM grape
465 juice were the highest, compared to those of other grape
466 juices. Also, the consumption of whole grape juice is more
467 beneficial to health than that of grape flesh because we can
468 take more antioxidants from grape skins and seeds in whole
469 grape juice. Therefore, an LSM juicer is strongly recom-
470 mended for making healthy grape juice rich in
471 antioxidants.

472 **Acknowledgements** This work was supported by the 2015 Inje
473 University research grant.

474 **Compliance with ethical standards**

475 **Conflict of interest** The authors declare no conflict of interest.

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