



HEALTH SCIENCES

Antioxidant activity of *Lactobacillus plantarum*, *Lactobacillus sake* and *Lactobacillus curvatus* strains isolated from fermented Turkish Sucuk

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Abstract: In recent years, it is very important to find more safe and natural antioxidants than biological sources to replace synthetic antioxidants. The antioxidant properties of 22 lactic acid bacteria were investigated *in vitro* in the present study. The conducted *in vitro* antioxidant assays included scavenging the 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical, metal (Fe^{2+}) ion chelation, hydroxyl radical and superoxide radical scavenging properties, and anti-lipid peroxidation. Analysis of DPPH free radical scavenging property of microorganisms demonstrated that *Lactobacillus plantarum* IH14L ($90.34 \pm 0.40\%$) strain exhibited the highest activity. The highest Fe^{2+} chelation activity was observed with *Lactobacillus curvatus* GH5L ($75.98 \pm 5.35\%$), while the lowest chelation activity was observed with *L. plantarum* IH14L (19.80 ± 0.05). The highest and lowest hydroxyl radical scavenging strains were *L. plantarum* IH16L ($82.25 \pm 1.60\%$) and *L. plantarum* IH26L (35.60 ± 4.50) strains, respectively. Comparison of superoxide radical scavenging activities of the microorganisms demonstrated that these activities ranged between $21.63 \pm 1.32\%$ and $7.22 \pm 0.04\%$. A positive correlation was identified between the hydroxyl radical scavenging activity of the microorganisms and the anti-lipid peroxidation property. It was found that they had the potential for use in nutritional and probiotic applications as protective and natural antioxidants.

Key words: Antioxidative property, DPPH, Lactic acid bacteria, metal ions, oxidative stress.

INTRODUCTION

The availability of probiotic microorganisms as natural antioxidants has also been investigated in recent years. Lactobacilli make up a large proportion of probiotics and lactic acid bacteria (LAB) antioxidant potential has been reported in several studies (AlKalbani et al. 2019, Antognoni et al. 2019, Song et al. 2019). Antioxidants are chemical compounds that could prevent, halt or reduce oxidative damage. Antioxidants could protect the human body from free radicals and retard the progression of several diseases. Due to the limited antioxidant capacity of the endogenous antioxidant system, the body

requires exogenous regulation and supplements to reduce oxidative stress. Thus, it is required to develop and use effective antioxidants (Kinsella et al. 1993, Lai et al. 2001). Dietary antioxidant supplements became an important instrument in controlling oxidative stress. Microorganisms possess antioxidant systems to maintain free radical levels (Farr & Kogoma 1991). In recent years, there is an interest in the antioxidant effects of microorganisms and their role in health and diseases. Previous studies reported antioxidant properties of LAB and the beneficial effects of these bacteria species on oxidative stress (Kim et al. 2006, Abubakr et al. 2012, Wu et al. 2014, Tang et al. 2017). Various

types of lactobacilli such as *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Lactobacillus rhamnosus* were considered useful probiotics and these microorganisms are commercially available for human consumption (Mishra et al. 2015).

Synthetic antioxidants such as butylated hydroxy-anisole (BHA), butylated hydroxytoluene (BHT), and n-propyl gallate (PG) exhibit strong antioxidant activities against various oxidation systems; however, they also have adverse effects such as liver damage and carcinogenic effects, and their use in food was restricted or prohibited in certain countries (Osuntoki & Korie 2010).

Thus, in recent years, it became very important to find safer and natural antioxidants using biological resources to replace synthetic antioxidants. The present study aimed to determine the antioxidant activities in certain *Lactobacillus* bacteria as natural antioxidants using five different methods.

MATERIALS AND METHODS

Bacterial strains

Twenty-two lactic acid bacteria (12 *Lactobacillus plantarum*, 6 *Lactobacillus sake*, 4 *Lactobacillus curvatus*) were used in the study (Demirel & Gürler 2018). Lactic acid bacteria were isolated from traditional sucuk batters (Montel et al. 1998, Papamanoli et al. 2003, Urso et al. 2006). Then, they were analysed in point of gas production from glucose (Facklam et al. 2002), growth at different temperature (Gürakan et al. 1995), growth at different salt concentrations (Gürakan et al. 1995), growth ability at 3.9 pH (Gürakan et al. 1995), the Voges-Proskauer test, metil red test (Iversen et al. 2006), arginine test (Coppola et al. 1998) and carbohydrate fermentation test (API CHL 50, Biomerieux). Finally, they were molecularly identified by PCR (Polymerase Chain

Reaction) (Berthier & Ehrlich 1998, Aymerich et al. 2003). Microorganisms were incubated in De Man-Ragosa Sharpe (MRS, LAB094) broth at 37°C for 48 hours in anaerobic medium. After incubation, LAB were passed on to MRS agar (LAB223). Fresh cultures were diluted with physiological saline solution until the desired cfu/g level was obtained based on the McFarland turbidity standard (Oztürk & Sagdıç 2013). After determination of McFarland turbidity, which is 11 log cfu / mL, microorganism suspensions were prepared. Antioxidant activity was determined on the prepared bacterial suspensions.

Preparation of intact cells

For each analysis, 0.5 mL microorganism adjusted to McFarland 5 (11 log cfu/mL) was transferred into Eppendorf tubes and centrifuged at 13,000 rpm for 15 min. After the centrifuge, the obtained supernatant was used in the analyses after the pellet was suspended in 1 mL Phosphate buffered saline solution (PBS, Sigma-AldrichP4417).

Determination of the DPPH radical scavenging effect

The DPPH radical scavenging activity was determined with modified Li et al. (2012) method. 1 mL fresh DPPH solution (0.05 mM, in ethanol) was added to the intact cell samples prepared with suspension in 1 mL PBS in Eppendorf tubes and the samples were stored in darkness for 1 hour. Blank was developed by replacing the sample with PBS. 1 mg/mL ascorbic acid (Sigma-Aldrich, A92902) and BHA (Acros: 235231000) were used as positive controls. Samples were centrifuged at 13,000 rpm for 10 minutes and the supernatants were measured spectrophotometrically at 517 nm.

Calculation of the DPPH radical scavenging activity (%) of strains:

$$\text{Scavenging Activity (\%)} = \left[1 - \frac{(A_{\text{sample}} - A_{\text{blind}})}{A_{\text{blank}}} \right] \times 100$$

Blind= PBS solution; Blank= PBS and DPPH solutions

Determination of Fe⁺² ion chelating activity

Fe⁺² ion chelating activity was determined with the method recommended by Decker & Welch (1990). On intact cell samples prepared in 1 mL of PBS suspension in Eppendorf tubes, 2 mM 0.05 mL FeCl₂ was added. The reaction was then initiated with 5 mM 0.2 mL ferrozine and the samples were stored in darkness for 10 minutes. EDTA (1 mg/mL) was used as positive control for comparison. After 10 minutes, the samples were centrifuged at 13,000 rpm for 10 minutes. The chelating activity was measured at a wavelength of 562 nm by determining the reduction in optic density.

Calculation of the chelating activity:

$$\text{Ferric ion chelating activity (\%)} = \left[1 - \frac{(OD_1 / OD_2)}{OD_2} \right] \times 100$$

OD₁: Sample, OD₂: Control

Hydroxyl radical scavenging activity

The study was conducted with the modified Wang et al. (2015) method. The intact cell samples prepared in 1 mL of PBS suspension in Eppendorf tubes were transferred to glass test tubes and 1 mL brilliant blue (0.435 mM), 2 mL FeSO₄ (0.5 mM), 1.5 mL H₂O₂ (3%, w / v) were added and the product was incubated at 37°C for 1 hour. 1 mg/mL ascorbic acid (Sigma, Germany) and BHA (Acros: 235231000) were used as positive controls for comparison. Samples were centrifuged at 3,000 rpm for 5 minutes and their absorbance was measured at 624 nm. The hydroxyl radical scavenging effect of the bacteria was calculated with the following formula:

Calculation of hydroxyl radical scavenging activity:

$$\text{Hydroxyl Radical Scavenging Activity (\%)} = \left[\frac{(A_0 - A_1)}{(A - A_1)} \right] \times 100$$

A₀: The absorbance of the solution that contains a certain concentration of the sample

A₁: The absorbance of the solution when the sample was not present

A: The absorbance of the solution that did not contain the sample and the Fenton reaction system.

Superoxide anion radical scavenging activity

The superoxide anion (O₂'-) radical scavenging activity assay was conducted spectrophotometrically with improved pyrogallol autoxidation method (Wu et al.2014). Superoxide anion radical was produced with pyrogallol (1, 2, 3-benzotriol) autoxidation systems under alkaline conditions. Initially, 0.1 mL microorganism sample, adjusted to McFarland 5 concentration was added to 4.5 mL Tris-HCl solution (0.05 M, pH 8.2) and the reaction mixture was incubated for 20 minutes in a 25°C water bath. Then, 0.4 mL pyrogallol (0.25 M, preheated to 25°C) was added and the mixture was incubated at 25°C for 4 minutes. 0.1 mL HCl (8M) was added to stop the reaction. Absorbance was measured at 320 nm. The control included an equal amount of 0.05 M Tris-HCl buffer (pH 8.2) to replace the sample. 1 mg/mL ascorbic acid and BHA were used as positive controls. The superoxide radical scavenging activity was calculated as follows:

$$\text{Superoxide anion radical scavenging activity (\%)} = \left[\frac{(A_0 - A_1)}{A_0} \right] \times 100$$

A₁: The absorbance of the samples

A₀: The absorbance of the solution that did not include the sample

Anti-lipid peroxidation

According to Hsu et al. (2008) method, fresh egg yolk was added to an equal volume of PBS (0.2 mol / L; pH 7.2) and the solution was mixed with a magnetic stirrer. The resulting suspension was diluted with PBS (egg yolk / PBS, 1:25, V/V). 1 mL egg yolk suspension, 0.5 mL microorganism sample solution adjusted to McFarland 5, 1 mL PBS, and 1 mL of iron sulfate (25 mmol/L) were mixed and stirred at 37°C for 15 minutes. 1 mL trichloroacetic acid (20%, W/V) was added, kept in static reaction for 10 minutes, and the mixture was centrifuged at 3000xg for 10 minutes. Thiobarbituric acid (2 mL; 0.8%, W/V) was to 3 mL supernatant and the mixture was then heated by stirring in a boiling water bath for 10 minutes and cooled to room temperature. The mixture was centrifuged at 3000 x g for 10 minutes and its absorbance was measured at 532 nm (A_s). The blank included 0.5 mL of PBS to replace the sample (A_0).

$$\text{Lipid peroxide inhibition rate (\%)} = (A_0 - A_s) / A_0 \times 100$$

Statistical analyses

Descriptive statistics (mean and standard deviation) were used in data analysis. Furthermore, the correlation between the values obtained with the measurement methods was analyzed by Pearson correlation analysis. In addition, comparison of the measurements obtained by microorganism type was conducted with one-way ANOVA. All tests were conducted in triplicate.

RESULTS AND DISCUSSION

The antioxidant capacities of LABs were tested using intact cells. Previous studies suggested that lactic acid bacteria may be responsible for the antioxidant effects of cell wall

polysaccharides, peptidoglycan, lipoteichoic acid and proteins (Yi et al. 2009, Liu et al. 2010, Zhang et al. 2011, Li et al. 2012). It was reported that the difference between the antioxidant activities of microorganisms may be associated with proteolytic activity and the resulting peptides, and may vary based on the species, strain and dose (Kudoh et al. 2001, Lin & Chang 2000, Virtanen et al. 2006, Zhang et al. 2013).

All microorganisms used in the present study exhibited antioxidant properties with all tested methods (Table I).

DPPH radical scavenging effect

DPPH radical scavenging method is commonly used in antioxidant activity studies due to its ease, speed, sensitivity and reproducibility when compared to other methods. The antioxidant activity increases in proportion with the removal of purple color formed when DPPH radical was added to the medium. Antioxidant effects differed between strains and radical scavenging activities between 90.34% and 58.38% were determined. *L. plantarum* IH14L exhibited the highest activity (90.34 ± 0.40%), while GH8L (58.38 ± 0.60%) exhibited the lowest activity. It was found that the DPPH radical scavenging activities of ascorbic acid and BHA used as antioxidants in nutrients were 94.31 ± 0.33% and 85.79 ± 0.25%, respectively.

Previous studies demonstrated that antioxidant activities of certain LAB strains could be associated with the production of cell surface compounds such as extracellular polysaccharides produced by strains such as *Lactococcus lactis subsp.lactis* 12 (Pan & Mei 2010) and *Bifidobacterium animalis* RH (Xu et al. 2011) and lipoteichoic acid on bifidobacteria (Yi et al. 2009).

The DPPH radical scavenging activities of the microorganisms determined in the present study were similar to the findings reported

Table I. Free radicle scavenging and inhibition of lipid peroxidation activity (%) of different strains of LAB from sucuk.

Microorganisms Type	Source	Scavenging of DPPH· (%)	Fe ²⁺ chelating effect (%)	Scavenging of ·OH (%)	Scavenging of (O ₂ '-)(%)	Anti-lipid peroxidation (%)
<i>L.plantarum</i> 4L18	Sucuk	80.96 ±2.01	57.63±0.02	51.39±0.02	8.42±0.08	40.18 ±2.23
<i>L.plantarum</i> 4L20	Sucuk	82.81± 1.00	69.87±0.70	36.12±3.22	8.24±1.65	48.57±0.22
<i>L.plantarum</i> 4L2	Sucuk	69.46±0.20	59.75±0.88	81.85±3.49	9.26±4.67	42.08±2.23
<i>L.plantarum</i> 5L2	Sucuk	67.04±0.40	62.25±3.23	71.62±4.18	9.23±0.08	53.32±2.46
<i>L.plantarum</i> GH8L	Sucuk	58.38±0.60	70.86±2.58	77.52±2.63	15.35±3.10	46.20±4.47
<i>L.plantarum</i> GH2.7L	Sucuk	75.42±3.42	64.71±0.11	51.06±3.52	13.95 ±3.34	40.18±0.44
<i>L.plantarum</i> İH16L	Sucuk	75.00 ±0.40	56.51±3.47	82.25±1.60	15.61 ±1.48	53.32±3.35
<i>L.plantarum</i> İH18L	Sucuk	72.30±1.00	48.18±3.94	73.17±1.30	11.55 ±1.94	52.84±2.23
<i>L.plantarum</i> İH14L	Sucuk	90.34±0.40	19.80±0.05	67.63±1.55	19.85±0.62	51.26±2.23
<i>L.plantarum</i> İH26L	Sucuk	87.78±2.81	39.11±1.82	35.60±4.50	10.90±1.36	43.03±1.34
<i>L.plantarum</i> İH28L	Sucuk	71.44±1.41	73.03±3.53	81.18±4.45	21.63 ±1.32	51.89±1.79
<i>L.plantarum</i> İH25L	Sucuk	80.25±2.21	66.87±2.70	45.12±0.29	7.22±0.04	53.16±3.58
<i>L.sake</i> İH17L	Sucuk	86.64±0.40	39.15±0.11	44.06±4.35	13.77±0.86	43.19±1.11
<i>L.sake</i> İH22L	Sucuk	80.82±1.00	71.99±1.11	72.52±4.43	19.88 ±1.15	56.01±2.68
<i>L.sake</i> İH15L	Sucuk	73.15±1.00	53.14±0.47	82.20±1.32	15.70 ±0.86	59.33 ±2.46
<i>L.sake</i> İH23L	Sucuk	81.39±0.60	53.39±0.23	73.53±6.35	15.99 ±2.93	44.14±3.80
<i>L.sake</i> GH6L	Sucuk	63.92±0.80	65.50±5.82	75.66±2.90	15.32 ±0.82	45.56±3.13
<i>L.sake</i> GH7L	Sucuk	68.89±0.20	71.45±1.17	72.98±4.97	14.21 ±0.49	60.60±0.67
<i>L.cruvatus</i> İH1L	Sucuk	80.39±3.62	71.07±0.05	78.86±0.73	7.66±1.65	52.21 ±2.23
<i>L.cruvatus</i> İH4L	Sucuk	77.13±1.41	46.10±3.11	76.35±0.59	9.18±2.81	51.58±6.26
<i>L.cruvatus</i> GH5L	Sucuk	65.19±0.20	75.98±5.35	75.53±1.35	17.39±0.62	63.29±2.69
<i>L.cruvatus</i> GH1L	Sucuk	82.67±1.61	60.75±1.35	59.22±3.15	16.19 ±0.90	46.67±2.90
Ascorbic acid (1mg/mL)		94.31±0.33	-	77.72 ± 0.04	3.71±0.45	58.35 ±0.68
BHA (1mg/mL)		85.79±0.25	-	95.29 ± 0.32	28.22±0.53	65.81±0.32
EDTA(1mg/mL)		-	92.18 ± 0.00	-	-	-

Data represent Mean ± (STD).

in previous studies (Zhang et al. 2011, Ji et al. 2015). Ji et al. (2015) 11 determined that DPPH radical scavenging activity of 11 *Lactobacillus* spp. filtrates was close to 50%, while Zhang et al. (2011) determined the DPPH radical scavenging activities of *L. casei* subsp. *casei* SY13 and *L. delbrueckii* ssp. *bulgaricus* LJJ strains as 23.99% and 27.50%, respectively. Ural (2016)

implemented a bacterial cell density of 10¹¹ cfu/mL and reported the DPPH radical scavenging activity of *L. casei* species (EMP2 strain) as 78.5%, and the DPPH radical scavenging activity of *L. delbrueckii* subsp. *bulgaricus* species between 56.3% and 77.7%. Wang et al. (2009) reported that intact cells exhibited antioxidant capacity in analyzes conducted with various *L. fermentum*

concentrations (10^6 - 10^9 cfu / mL) and the findings demonstrated that the antioxidant capacity increased in a concentration-dependent manner. Alkanbani et al. (2019) report that the increase in DPPH% and ABTS% during storage may be due to antioxidant activities of peptides released as a result of proteolysis. Talib et al. (2019) reported that *Lactobacillus* spp. isolated from kefir showed DPPH radical scavenging activity related to total phenolic content (TPC) and total flavonoid content (TFC) activities. In the present study, it was found that in vitro studied microorganisms exhibited high antioxidant activity against DPPH radical.

Fe²⁺ ion chelating activity

Transition metals are able to catalyze reaction to release reactive oxygen species, such as hydroxyl radical and superoxide anion. Furthermore, ferrous ions can catalyze the breakdown of lipid peroxides which leads to the formation of off flavour during the food shelf life. Iron could form free radicals as a result of Fenton reactions. Thus, the reduction in Fe²⁺ concentration with Fenton reactions has a protective effect on oxidative damage (Rival et al. 2001). The chelating activities of lactic acid bacteria are revealed due to the physiological chelators located on the bacteria cell wall (Lin & Yen 1999). In the present study, it was determined that all tested strains had metal (Fe²⁺) ion chelating effects. The results also demonstrated that *L. cruvatus* GH5L exhibited the highest activity when compared to the positive standard Ethylene diamine tetraacetic acid (EDTA) (1 mg/mL) (92.18 ± 0.00%). Furthermore, it was determined that IH28L strain exhibited the highest activity among *L. plantarum* species and IH22L strain exhibited the highest activity among *L. sake* species.

Apart from the production of antioxidants and free radical scavenging activities, probiotics also exhibit a certain degree of metal chelating

activity (Spyropoulos et al. 2011). Yamamoto et al. (2002) identified a new antioxidant iron binding protein called ferritin-like iron-binding protein (Dpr) in LAB. During the study on Dpr, Fenton reaction inhibiting activity of free cell extracts was determined. It was reported that some LAB species such as *Streptococcus thermophilus* 821 (Lin & Yen 1999), *Bifidobacterium longum* 15708 (Lin & Yen 1999) and *L. casei* KCTC 3260 (Lee et al. 2005) exhibited antioxidant activities by eliminating the transition metal ions and explained this finding by the ability of the strain to withstand high O₂ levels. In the present study, it was found that ferric ion chelating activity of all LABs was 58.96% on average and there was no statistical difference between the species. It was demonstrated that the studied microorganisms exhibited non-enzymatic (Gavin et al. 1998) defense mechanisms such as metal ion chelation. The result of this study showed that the strains from LAB fermented sucuk can chelate metal ions like ferrous ions and inhibiting the catalysis of oxidation by metal ions.

Hydroxyl radical scavenging activity

Since the hydroxyl radical is primarily formed by the Fenton reaction biologically, it is produced with Fe²⁺/H₂O₂ system in in vitro conditions and the hydroxyl radical scavenging activity of the antioxidant is measured (Zhang et al. 2013). Since several antioxidants are also metal chelators, they can alter Fe²⁺ activity. It was reported that chelation of these ions by certain antioxidants may inhibit the formation of hydroxyl radicals (Kao & Chen 2006).

In the present study, it was determined that *L. plantarum* IH16L (82.25 ± 1.60%) exhibited the highest hydroxyl radical scavenging activity, and the hydroxyl radical scavenging activity of *L. sake* IH15L strain was 82.20 ± 1.32 and that of the *L. cruvatus* IH1L strain was 78.86% ± 0.73).

Comparison of the strains to ascorbic acid (1 mg/mL, 77.72 ± 0.04%) and BHA (1 mg/mL, 95.29 ± 0.32%) demonstrated that their scavenging effect was close to the effect of the ascorbic acid.

It was reported that the consumption of LAB in food reduced the risk of ROS accumulation and could degrade hydrogen peroxide and superoxide (Virtanen et al. 2006). The hydroxyl radical scavenging activities of 11 *L. plantarum* species isolated from traditional Chinese fermented food products were investigated and it was determined that *L. plantarum* C88 (10¹⁰ cfu/mL, 44.31%) exhibited high inhibition and hydroxyl radical scavenging activity increased in a dose dependent manner in different concentrations between 10⁸ and 10¹⁰ cfu/mL (Li et al. 2012). The antioxidative properties of 4 *Lactobacillus* spp. strains of human origin obtained from the Korean research institute were investigated in vitro and it was found that the hydroxyl radical scavenging effect of intact cells of the bacteria exhibited lower inhibition when compared to cell lysates (Kim et al. 2006). It is observed in the current study that the intact bacteria cells scavenged the hydroxyl radical at a higher level when compared to previous studies. In the present study, we believe that intact *L. plantarum* IH16L *L. sake* IH15L and *L. cruvatus* IH1L strain cells exhibited strong hydroxyl radical scavenging activity, probably due to their ability to bind metal ions such as Fe²⁺.

Superoxide anion radical scavenging activity

Antioxidant enzymes such as SOD, NADH-oxidase and NADH peroxide and both heterologous non-heterologous enzymes such as catalase are considered important LAB enzymatic defense systems against oxidative stress. These intracellular enzymes can be obtained after degradation of bacterial cells. *In vitro* antioxidant activities of non-cellular extracts of

certain LAB strains confirmed this fact (Lin & Yen 1999, Kullisaar et al. 2002). All strains used in the present study exhibited different superoxide radical scavenging activity rates. Comparison of the scavenging activities of the strains was demonstrated that the findings ranged between 21.63 ± 1.32% and 7.22 ± 0.04%. The highest activity was observed in *L. plantarum* IH28L strain also exhibited high hydroxyl radical scavenging activity (43.6%).

An important *in vivo* enzymatic antioxidant defense mechanism is superoxide dismutase (Ji et al. 2015). MnSOD was identified in lactic acid bacteria (Yamamoto et al. 2002). In the present study, superoxide radical anion scavenging activities demonstrated low inhibition rates when compared to other radical scavenging effects due to the use of intact cells. We considered that this may be due to the fact that the lactic acid bacteria we used removed metal ions instead of increasing the SOD level to block the oxidative chain reaction. Zhang et al. (2011) reported that non-cellular 2 *Lactobacillus* bacteria (SY13 and LJJ, isolated from yogurt) and *L. Rhamnosus* GG (LGG) control strain extracts exhibited superoxide anion scavenging effects, however the results of the analysis conducted with intact cells demonstrated that only SY13 and LGG strains were effective due to the fact that these two bacteria were resistant to superoxide anion. Ji et al. (2015) studied 5 *Lactobacillus* sp. and 6 *Leuconostoc* sp. strains and determined that all *Leuconostoc* sp. except E1 and E2 strains exhibited a scavenging activity of above 35%, and *Lactobacillus* spp. S1 strain exhibited the highest activity.

Anti-lipid peroxidation

The oxidation of cellular membrane phospholipids into peroxide derivatives is described as lipid peroxidation (Yarsan 1998). Inhibition of lipid peroxidation is a general

method used to determine antioxidative activity. In the present study, the lipid peroxidation inhibition rate ranged between 63.29 and 40.18 %. *L. cruvatus* GH1L exhibited the highest inhibition rate, while *L. plantarum* GH2.7L exhibited the lowest activity.

The most significant effect of free oxygen species on biological systems is on lipids. This phenomenon is known as lipid peroxidation. In a study where antioxidative properties of yogurt bacteria were investigated, plasma lipid inhibition rates of *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 strains were determined as 57% and 42%, respectively, while the inhibition rates of the same strains were 61% and 56%, respectively (Ou et al. 2006). Ural (2016) determined lipid peroxidation inhibition of microorganisms with blood plasma that contained biological lipid. The highest inhibition was reported with *L. rhamnosus* SMC6 strain (39.2%) and the lowest inhibition was with *L. delbrueckii* ssp. *bulgaricus* 12L strain (30.2%) (Ural 2016). In that study, egg yolk was used as the lipid source when determining anti-lipid peroxidation activity, and Zhang et al. (2017) who used the same method, found that anti-lipid peroxidation capacities of *L. curvatus* (SR6) and *Lactobacillus paracasei* (SR10-1) isolated from Chinese traditional fermented meat products were $55.00 \pm 5.19\%$ and $63.89 \pm 0.93\%$, respectively. In another method that used linoleic acid emulsion as a lipid source, inhibition of lipid peroxidation in intact cells and cell lysates of certain human bacteria was investigated and it was found that *L. acidophilus* 3111 strain exhibited 65.2% inhibition in cell lysates and 49.7% inhibition in intact cells (Kim et al. 2006). It was reported that lipid peroxidation inhibition rates in *Bifidobacterium longum* (ATCC 15708) and *Lactobacillus acidophilus* (ATCC 4356) bacteria pellets and supernatants varied between 11 and 29% at 10^9 cfu/mL (Lin & Chang

2000). In the present study, the bacteria density was 10^{11} cfu/mL and it was considered that lipid inhibition varied based on the density used.

Analysis of variance (ANOVA) findings demonstrated that there were no statistically significant differences between the mean DPPH radical scavenging activity, ferric (Fe^{+2}) ion chelating activity, hydroxyl and superoxide radical scavenging activities and anti-lipid peroxidation ($p > 0, 05$) (Table II).

The correlations between the findings obtained with antioxidant measurement methods are presented in Table III. Pearson correlation analysis revealed statistically significant, moderate, negative correlations between DPPH radical scavenging activities and iron (Fe^{+2}) ion chelating activities and hydroxyl radical scavenging activities ($p < 0.05$). Thus, it was determined that while the DPPH radical scavenging capacity increased, iron (Fe^{+2}) ion chelating activity and hydroxyl radical scavenging activity decreased. Furthermore, a statistically significant, moderate, positive correlation was found between hydroxyl radical scavenging activity and anti-lipid peroxidation ($p < 0.05$). Thus, as hydroxyl radical scavenging activity increased, anti-lipid peroxidation values increased as well.

The oxygen metabolite, which is effective on lipid peroxidation, includes the superoxide group (ultimately exhibits the effects after it is converted to hydroxyl group) and the hydroxyl group. A positive correlation was determined between the hydroxyl radical scavenging activity of the microorganisms and anti-lipid peroxidation activity. Thus, it could be suggested that microorganisms inhibited lipid peroxidation by scavenging the hydroxyl radical.

One of the action mechanisms of antioxidants is to prevent the onset of peroxidation by removing the products that might bind to hydrogen atoms in the hydroxyl

Table II. Comparison of the results obtained by microorganism type.

Microorganisms		N	Mean	Std. Deviation	F	p
Scavenging of DPPH·	<i>L. plantarum</i>	12	75.93	9.06	0.005	0.995
	<i>L. sake</i>	6	75.80	8.60		
	<i>L. curvatus</i>	4	76.35	7.78		
	Total	22	75.97	8.32		
Fe ²⁺ ion chelating effect (%)	<i>L. plantarum</i>	12	57.39	15.33	0.270	0.766
	<i>L. sake</i>	6	59.11	12.85		
	<i>L. curvatus</i>	4	63.49	13.21		
	Total	22	58.96	13.88		
Scavenging of ·OH (%)	<i>L. plantarum</i>	12	62.88	17.93	0.778	0.474
	<i>L. sake</i>	6	70.16	13.28		
	<i>L. curvatus</i>	4	72.50	8.96		
	Total	22	66.61	15.49		
Scavenging of (O ₂ '-) (%)	<i>L. plantarum</i>	12	12.61	4.71	1.252	0.308
	<i>L. sake</i>	6	15.81	2.17		
	<i>L. curvatus</i>	4	12.61	4.90		
	Total	22	13.48	4.28		
Anti-lipid peroxidation (%)	<i>L. plantarum</i>	12	48.01	5.37	1.302	0.295
	<i>L. sake</i>	6	51.48	8.03		
	<i>L. curvatus</i>	4	53.44	7.02		
	Total	22	49.94	6.53		

N: Number of isolates; p: significance level.

radical structure by adding a hydrogen molecule to free oxygen radicals. It could be suggested that the decrease in hydroxyl radical scavenging activities with the increase in the scavenging activities of DPPH, a commercial radical, could be the result of the reduction of free radical formation by the microorganisms.

CONCLUSION

Furthermore, the antioxidant activity was considered to vary based on the isolation source of the microorganisms. Synthetic antioxidants such as BHA, BHT have been widely used in

the production and maintenance of nutrients and may lead to various health problems. Our in vitro antioxidant studies demonstrated that the antioxidant properties of LAB were strain specific. Furthermore, most in vitro antioxidant assays demonstrated that the mechanism of antioxidant action could occur mainly through ROS scavenging and/or chelating transition metal ions such as iron and copper that play a role in ROS/RNS formation. However, further research is required to identify the precise antioxidant compounds they contain and also to elucidate the molecular mechanisms underlying the antioxidant activities. Thus, considering

Table III. Correlations between the findings obtained with antioxidant measurement methods.

	DPPH·		Fe ⁺²	·OH	O ₂ ^{'-}	Anti-lipid peroxidation
Scavenging of DPPH· (%)	r	1	-.588	-.596	-.090	-.275
	p		.004	.003	.692	.215
	N		22	22	22	22
Fe ⁺² ion chelating effect (%)	r		1	.211	-.026	.275
	p			.346	.910	.216
	N			22	22	22
Scavenging of ·OH (%)	r			1	.381	.449
	p				.080	.036
	N				22	22
Scavenging of (O ₂ ^{'-})(%)	r				1	.261
	p					.241
	N					22

N: Number of isolates; **r:** pearson correlation; **p:** signifiacnce level.

both the benefits of synthetic antioxidants and their health hazards, the potential of the studied microorganisms for use in nutrient and probiotic applications is promising. The properties of *L. plantarum* IH14L, *L. plantarum* IH16L and *L. curvatus* GH5L strains should be examined at molecular level and further in vivo studies should be conducted. We know that fermented meat products are often preferred by consumers. Synthetic antioxidants are added to these products. Considering the toxicity and economic size of synthetic antioxidants our work is promising for the production of new bioactive foods. It has been determined with this study that it provides the antioxidant properties necessary for the evaluation of these bacteria as probiotic bacteria. Potential protective effects of lactic acid bacteria determined by in vitro antioxidant methods should be conducted in vivo using animal models in the next step, and the information collected should ultimately result in human clinical studies.

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MD and YND conceived the original idea. MD and YD was responsible for carry out the experiments. ID made statistical analysis of the data. All authors discussed the results and contributed to the final manuscript.

