Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v47i1.74807

CC BY

In vitro study of antioxidant activity of lactic acid bacterial strains isolated from Algerian fermented products

Nabila Benhamada[1,2*], Ouahiba Benhamada[1,3], Sara Mellit[2], Imène Moussaoui[2] and Tayeb Idoui[1,3]

[1]Laboratory of Biotechnology, Environment and Health, Nature and Life Sciences Faculty, University of Mohamed Seddik Ben Yahia, Jijel 18000, Algeria. [2]Molecular and Cell Biology Department, Nature and Life Sciences Faculty, University of Mohamed Seddik Ben Yahia, Jijel 18000, Algeria. [3]Applied Microbiology and Food Sciences Department, Nature and Life Sciences Faculty, University of Mohamed Seddik Ben Yahia, Jijel 18000, Algeria. *Author for correspondence. E-mail: n.benhamada@yahoo.fr

ABSTRACT. The aim of this study was to evaluate the antioxidant activity of 17 strains of lactic acid bacteria isolated from traditionally fermented durum wheat. *In vitro* tests were caried out: resistance to hydrogen peroxide, total phenolic compounds, DPPH and superoxide anion radicals scavenging assay, and finally, ferric reducing antioxidant power. The greatest resistance to hydrogen peroxide was observed in LS09, LS10, and LS17 strains. Among these three strains, the highest content of phenolic compounds was registered in LS17, this strain presented also the highest reducing power activity. Furthermore, the highest ability to scavenge the DPPH radical was observed in LS10, to scavenge superoxide anion radical was in LS09. Moreover, the use of natural antioxidant can be used in food processing to limit the use of chemical antioxidants.

Keywords: LAB; antioxidant property; DPPH; natural antioxidants.

Received on November 30, 2024
Accepted on May 19, 2025

Introduction

An imbalance between increased creation of reactive oxygen species (ROS) and/or free radicals and their elimination by antioxidant systems is the cause of oxidative stress, which is a situation in which an organism exhibits excessive ROS activity (Jomova et al., 2023; Zhang et al., 2023; Averill-Bates, 2024). Known as oxidative eustress or "good stress," a physiological level of ROS is defined by low to moderate concentrations of oxidants that are involved in the control of numerous metabolic processes. An unhealthy state known as oxidative stress, or "bad stress," is brought on by elevated amounts of ROS, which can be produced by endogenous or exogenous sources (Jomova et al., 2023).

ROS are molecules with one or more unpaired electrons and at least one oxygen atom that can exist on their own. This category includes oxygen free radicals such as superoxide anion radical, hydroxyl radical, hydroperoxyl radical (Jakubczyk et al., 2020). On the other hand, excessive ROS production causes redox equilibrium to be upset, which then causes oxidative stress and ROS-mediated damage to all significant molecules, particularly proteins, DNA, and membranes (Ghosh et al., 2018; Liguori et al., 2018; Checa & Aran, 2020; Juan et al., 2021). Conversely, in stressful situations, ROS produce pro-inflammatory molecules, this leads to inflammation, which is a major factor in aging and the development of various diseases (Srivastava & Kumar, 2015). These include cancer, liver and vascular disorders, and cardiac, autoimmune, neurodegenerative, and respiratory diseases (Aldosari et al., 2018; Glennon-Alty et al., 2018; Yang & Lian, 2020; Zuo & Wijegunawardana, 2021; Rudrapal et al., 2022).

Cells have an efficient endogenous antioxidant system which is made up of enzymatic antioxidants like superoxide dismutases (SODs), catalase (CAT), glutathione peroxidases (GPXs) and thioredoxin (Trx) (Halliwell, 2022) and non enzymatic molecules, such as: proteins with thiol or phenolic groups (glutathione), melatonin, coenzyme Q10 (Sharifi-Rad et al., 2020; Losada-Barreiro et al., 2022; Sangouni et al., 2022).

Exogenous antioxidant compounds like vitamin C, vitamin E, and carotenoids, play a crucial role in many antioxidant processes in living organisms. They act synergistically with endogenous antioxidants to preserve or restore redox equilibrium (Sharifi-Rad et al., 2020). Other natural antioxidants like: phenolic compounds, protect the cell from the damage caused by free radicals (Losada-Barreiro et al., 2022).

The rise in diseases that plague society has made it necessary to look for safe antioxidants that can boost the body's antioxidant reserves. According to scientific studies, some lactic acid bacteria (LAB) have a potent

antioxidant effect and lessen the oxidative damage that stress causes to the body (Noureen et al., 2019; Yang et al., 2021).

The antioxidant activity of LAB has attracted a lot of attention in recent years (Hu et al., 2023; Łepecka et al., 2023; Rwubuzizi et al., 2023; Vougiouklaki et al., 2023). Although the precise antioxidant mechanism of LAB is still unclear, it was shown that these LAB possess antioxidant properties. They can scavenge a certain amount of ROS, protect against free radicals, and control the oxidative stress by upregulating the activity of antioxidant enzymes in the host and downregulating the activity of enzymes linked to the formation of ROS (Amaretti et al., 2013; Tang et al., 2017; Shi et al., 2019).

The aim of this study is to determine the antioxidant activities for some LAB strains isolated from underground stored durum wheat by using different *in vitro* tests.

Material and methods

Lactic acid bacteria

A total of 17 strains of LAB isolated from traditionally fermented wheat were used in this study. They were previously assessed for Gram staining and catalase testing. The strains were kept at - 20°C in presence of 20% glycerol and sub-cultured in MRS broth at 37°C for 24 hours right before every experimental procedure.

Intact cells and free cell supernatant preparation

For each analysis, 0.5 mL of the lactic acid strain adjusted to McFarland 5 (11 log CFU mL^{-1}) was transferred to Eppendorf tubes and centrifuged at 13,000 rpm for 15 min. After the centrifugation, the pellet suspended in 1 mL of phosphate-buffered saline (PBS) was used to carry out the various tests. While the supernatant obtained has been used for the determination of total phenolic content (Düz et al., 2020).

In vitro determination of antioxidant potency

Hydrogen peroxide (H_2O_2) resistance test

The method described by Li et al. (2012) was used, with some modifications. The overnight cultures of lactic strains were inoculated at 2% (vol/vol) into MRS broth as a control group, and MRS broth containing 0.5 or 1.0 mM of H_2O_2 separately as a sample group. Both control and sample groups were incubated at 37°C for 8 hours. The growth of LAB was measured by a spectrophotometer at 600 nm. The test was carried out in triplicate. The following formula was used to determine the survival rate (%) of lactic acid strains damaged by H_2O_2:

Survival rate (%) = (As/Ac) ×100 %;

As: The absorbance of the sample group; Ac: The control absorbance.

Scavenging of DPPH free radical

The DPPH radical scavenging activity of lactic strains was determined according to the procedure of Düz et al. (2020). Briefly, 1 mL of a freshly prepared DPPH solution (0.05 mM in ethanol) was added to the intact cell samples prepared with suspension in 1 mL of PBS in Eppendorf tubes. Samples were kept in darkness at room temperature for 1 hour. After incubation, samples were centrifuged at 13,000 rpm for 10 minutes and the percentage of DPPH radical scavenging capacity was measured spectrophotometrically at 517 nm. 1 mg mL^{-1} ascorbic acid was used as a positive control. The DPPH radical scavenging activity (%) of strains was calculated as follows: Scavenging activity (%) = $[1- (A_{sample} - A_{blind}) / A_{blank}] \times 100$,

Blind: PBS solution; Blank: PBS and DPPH solutions.

Scavenging activity of superoxide anion radical

The superoxide anion radical scavenging activity was determined using pyrogallol autoxidation method (Wu et al., 2014). First, 4.5 mL of Tris-HCl solution (0.05 M, pH 8.2) was mixed with 0.1 mL of the strain, adjusted to McFarland 5 concentration. The reaction mixture was then incubated for 20 min. at 25°C in a water bath. After that, 0.4 mL of 0.25 M pyrogallol (preheated to 25°C) was added, and the mixture was incubated for 4 min. at 25°C. Finally, to stop the reaction, 0.1 mL of HCl (8 M) was added. Absorbance was measured at 320 nm. The control included an equal quantity of 0.05 M Tris-HCl buffer (pH 8.2) to replace the sample. 1 mg mL^{-1} ascorbic acid was used as a positive control. Scavenging activity of the superoxide anion radical was calculated as follows:

Superoxide anion radical scavenging (%) = [(A_0 – A_1) / A_0] x 100;

A_0: Absorbance of the sample; A_1: Absorbance of the solution without the sample.

Determination of total phenolic content

An aliquot (1 mL) of the previously prepared lactic acid strain supernatant was introduced in a test tube with 5 mL of 0.5 M Na_2CO_3 solution and mixed briefly with 1 ml of Folin-Ciocalteu reagent. The mixture was left to rest for 30 min. The supernatant's absorbance was measured at 725 nm, and the results were expressed as micrograms gallic acid equivalent (GAE)/g (Livinska et al., 2016).

Ferric reducing antioxidant power (FRAP)

The reducing power was measured using the method described by Zhang et al. (2017). A mixture of 0.5 mL of the lactic acid strain, 0.5 mL of potassium ferricyanide solution (1%, W/V), and 0.5 mL of phosphate-buffered saline (PBS, 0.2 M; pH 6.6) was prepared. Then incubated in a water bath at 50°C for 20 min. The mixture was quickly cooled to room temperature and a solution of trichloroacetic acid (10%, p/v) was added. After centrifugation at 1399 × g for 5 min, an aliquot of the supernatant (1 mL) was combined with 1 mL of distilled water and 0.2 mL of ferric trichloride (0.1%, w/v). The mixture's absorbance (A_s) at 700 nm was calculated by comparing it to a blank sample (A_b) that was replaced with PBS. The percentage of reducing power was calculated as follows:

Reducing power (%) = (A_s – A_b)/A_b×100;

As: The absorbance of the sample group; Ab: The absorbance of the blank.

Statistical analysis

The results were presented as a mean ± standard deviation (SD), performed with Microsoft Excel (Microsoft Corporation, 2019). The correlation between the values obtained with the measurement methods was analyzed by Pearson correlation analysis at $p < 0.05$. The correlation matrices were assessed by Microsoft Excel (Microsoft Corporation, 2019). In addition, comparison of the measurements obtained by microorganism type was conducted with one-way ANOVA using SPSS software version 22.0 for Windows.

Results and discussion

Resistance to hydrogen peroxide

The results of the lactic strains' resistance to hydrogen peroxide are shown in Table 1.

Table 1. The survival rate of 17 strains of lactic acid bacteria (LS01-LS17) in MRS medium at 0.5 and 1mM of H_2O_2 solution.

Lactic strain	Survival at 0.5 mM H_2O_2 (%) (***)	Survival at 1.0 mM H_2O_2 (%) (***)
LS01	48.02 ± 1.20	31.17 ± 0.29
LS02	40.11 ± 0.53	32.00 ± 0.43
LS03	41.32 ± 4.33	31.55 ± 0.26
LS04	42.96 ± 1.14	31.45 ± 0.00
LS05	39.49 ± 0.83	28.36 ± 1.01
LS06	42.44 ± 0.40	33.03 ± 6.09
LS07	35.79 ± 1.29	30.31 ± 1.23
LS08	39.46 ± 1.35	28.81 ± 0.68
LS09	50.58 ± 1.03	36.09 ± 2.67
LS10	64.44 ± 0.59	36.01 ± 1.80
LS11	39.75 ± 0.37	25.59 ± 6.32
LS12	45.31 ± 0.34	33.26 ± 0.18
LS13	33.21 ± 0.04	26.69 ± 0.24
LS14	40.2 ± 1.15	32.76 ± 0.56
LS15	26.59 ± 0.34	21.11 ± 0.36
LS16	38.82 ± 3.05	27.75 ± 0.75
LS17	52.21 ± 0.00	33.9 ± 0.00

*** P = 0.000.

In this study, all strains were tested for survival under two concentrations of hydrogen peroxide (Table 1). When strains were exposed to 0.5 mM H_2O_2. Survival rates varied between 26.59 ± 0.34% and 52.21± 0.00%. When they were exposed to 1.0 mM H_2O_2, survival rates varied between 21.11 ± 0.36% and 36.09 ± 2.67% (P = 0.000***).

A previous study by Mu et al. (2018) showed that different concentrations of H_2O_2 might inhibit the growth of six strains of lactobacilli. Indeed, for the strains exposed to 0.5 mM H_2O_2, survival rates ranged from 72.67 to 91.05%. And when they were exposed to 1.0 mM H_2O_2, survival rates ranged from 30 to 50%.

Hydrogen peroxide may easily pass through cell membranes and damage DNA, proteins, and lipids causing oxidative stress (Mishra et al., 2015). Despite its low toxicity, it is involved in the formation of ROS such as hydroxyl radicals that cause damage in cells. Bacteria that have a catalase are highly resistant to H_2O_2, but LAB in general, and as our study shows, have a low resistance to H_2O_2 due to the absence of this activity (Tang et al., 2017). Decreased growth rates indicate that the presence of peroxide causes damage to the bacterial cell (Wang et al., 2006).

Strains with high resistance to hydrogen peroxide were selected for the other tests of antioxidant activity, these were the coded strains LS09, LS10, and LS17.

Determination of phenolic content

The determination of total phenolic content produced by lactic acid bacterial strains was carried out according to the Folin-Ciocalteu method. Phenolic compounds reduce the Folin-Ciocalteu reagent by giving a blue coloration proportional to the phenolic compounds present in the reaction medium.

The obtained results are shown in Figure 1. According to this figure, we found that the three strains produced phenolic substances with different rates ranging from 1.24 ± 0.002 µg GAE g^{-1} to 1.28 ± 0.04 µg GAE g^{-1} for LS10 and LS17, respectively.

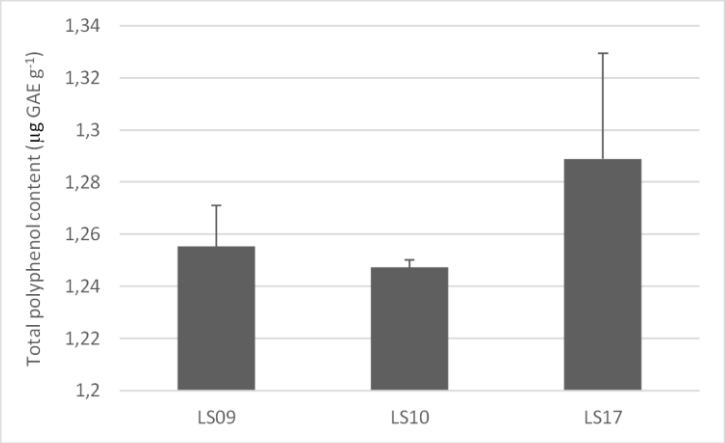

Figure 1. Results of phenolic content of lactic strains (LS09, LS10 and LS17) using Folin-Ciocalteu assay.

According to Skorokhod and Kurdysh (2014), only plants and microorganisms are capable of synthesizing precursors of phenolic compounds. Livinska et al. (2016) investigated the production of polyphenols by some lactic acid bacteria in different media. They showed that the majority of them (> 90%) produced phenolic acids in cucumber juice. Only three strains were able to produce phenolic acids in all the tested media. These strains were also characterized by high antioxidant activity. Plant-derived strains have been found to produce phenolic compounds only when grown on cucumber but were not capable of producing these compounds in milk or MRS medium. According to these researchers, the production of phenolic compounds depends on the strain itself as well as its origin.

LAB are capable of producing phenolic compounds as an end product during fermentation; this ability is strain-specific. The increase of phenolic compounds during enzymatic hydrolysis of lactic acid bacteria during fermentation leads to an increase in antioxidant activities (Muñoz et al., 2016).

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging test

For the evaluation of the antioxidant activity of a compound or bacterial cell, several methods are highlighted, all based on radical trapping. The most widely used is the radical DPPH because of its ease, speed, sensitivity and reproducibility compared to other methods (Prior et al., 2005). The DPPH method is commonly used in antioxidant activity studies, where an increase in activity is proportional to the suppression of the purple color formed when the DPPH radical is added to the medium.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical results show a highly significant difference (p = 0.000***) between samples. These results are shown in Figure 2. According to this figure, the three strains had the ability to scavenge the radical DPPH, the rate of this scavenging activity varied between 55.00 ± 0.02% and 93.75 ± 0.77%. The strain LS10 showed the highest radical scavenging activity (93.75 ± 0.77%) followed by LS17 (92.0 ± 1.27%). The LS09 strain has the lowest radical scavenging activity with a percentage of 55 ± 0.02%. Scavenging activities of LS10 and LS17 strains are very close to that of ascorbic acid (93.91 ± 0.00%). Our results were in agreement with those from the study of Düz et al. (2020), who showed that DPPH radical scavenging activities in lactic acid bacterial strains varied between 90.34 and 58.38%. Thus, *L. plantarum* IH14L showed the highest activity (90.34 ± 0.40%).

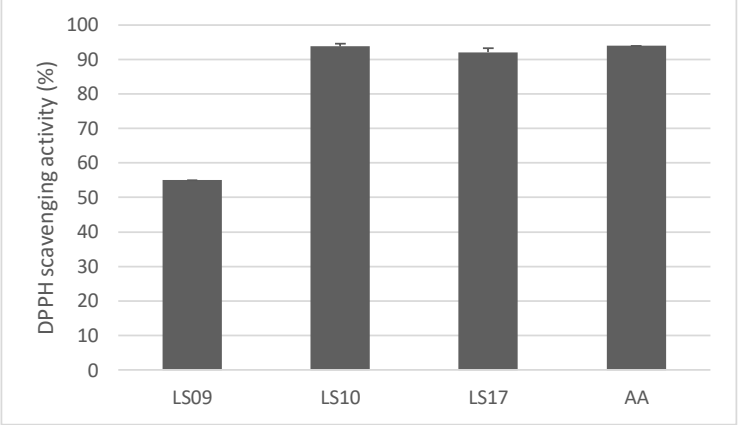

Figure 2. Results of DPPH scavenging activity of lactic acid bacterial strains (LS09, LS10 and LS17). AA: ascorbic acid.

On the other hand, our study's results confirm that the lactic acid strains demonstrated significant antioxidant activity. This contrasts with the findings of Zhang et al. (2017), who reported DPPH radical scavenging percentages ranging from 49.48 to 59.67% for the strains *L. curvatus* SR6 and *L. paracasei* SR10-1, respectively. Similarly, the results reported by Łepecka et al. (2023) were much lower, ranging from 1.19 to 36.05%. Additionally, Arasu et al. (2016) observed a scavenging activity of only 48.63% for *L. brevis* P68, isolated from gherkins. The highest scavenging activity, 85.24%, was recorded by Rwubuzizi et al. (2023) for *Streptococcus salivarius* strain ST59HK.

Previous studies have demonstrated that the antioxidant activity of some lactic strains may be associated with the production of cell surface compounds such as exopolysaccharides (Feng & Wang, 2020), bioactive peptides, antioxidant enzymes, and manganese ions (Davis & Milner, 2009). In addition, Talib et al. (2019) reported that the DPPH radical scavenging of *Lactobacillus* strains isolated from kefir is related to the content of total phenolic and total flavonoid. Recent studies confirmed that the significant increase in phenolic compounds of LAB during fermentation of healthy drinks increase the ability to capture DPPH radical (Kuo et al., 2021; Li et al., 2021). Furthermore, Alkalbani et al. (2019) reported that the DPPH levels can be associated with peptides released as a consequence of proteolysis.

Superoxide anion radical scavenging activity

The results of the superoxide anion scavenging activity are represented in Figure 3. According to this figure, the three lactic strains have the ability to capture the superoxide anion radical. We noted a scavenging rate ranged from 53.62 to 55.71% attributed to the strains coded BL17 and BL09, respectively. These results are very close to that of vitamin C (61.08 ± 2.55%).

Ji et al. (2015) investigated the antioxidant activity of some strains of *Leuconostoc* sp. They determined that these strains had greater than 35% scavenging activity. Düz et al. (2020) noted percentages of superoxide anion scavenging ranging from 7.22 ± 0.04% and 21.63 ± 1.32%. Based on these results, it is clear that the three strains tested in our work presented high antioxidant activity. The ability to trap the superoxide anion by the lactic strains could be related to their production of exopolysaccharides (EPS). Indeed, these EPSs are known by their antioxidant activity because they exhibit free radical scavenging and metal chelation activities (Feng & Wang, 2020).

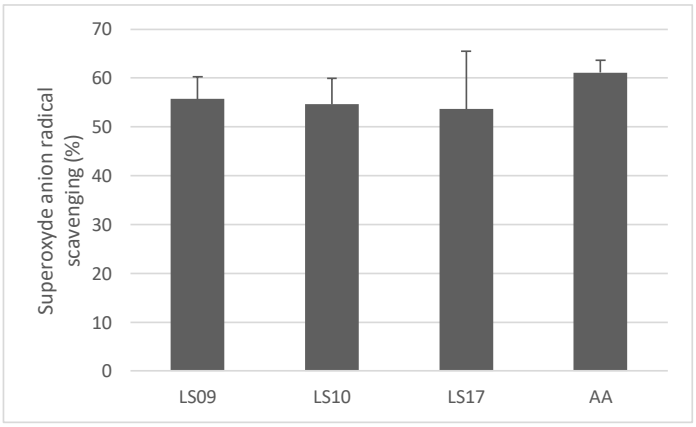

Figure 3. Results of superoxide anion scavenging rate of the lactic strains (LS09, LS10, and LS17). AA: ascorbic acid.

Reducing power

This method is based on the ability of the tested strains to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The mechanism is known as an indicator of giving electrons activity, characteristic of the antioxidants action. The obtained results in this work (Figure 4) were significantly different (P = 0.001**). According to this figure, the three lactic strains were able to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The best result was recorded in the LS17 strain (33.95%) followed by LS09 (31.21%) and LS10% (23.09%). The levels of reducing power registered in our work were lower to those found by Zhang et al. (2017) which demonstrated that *L. curvatus* SR6 and *L. paracasei* SR10-1 exhibited a reducing power of about 47.31 and 44.24%, respectively. Furthermore, Düz et al. (2020) reported metal (Fe^{+2}) ion chelating effects between 20 and 75% for all tested strains. Also, values between 66 and 87% were recorded in the study of Rwubuzizi et al. (2023).

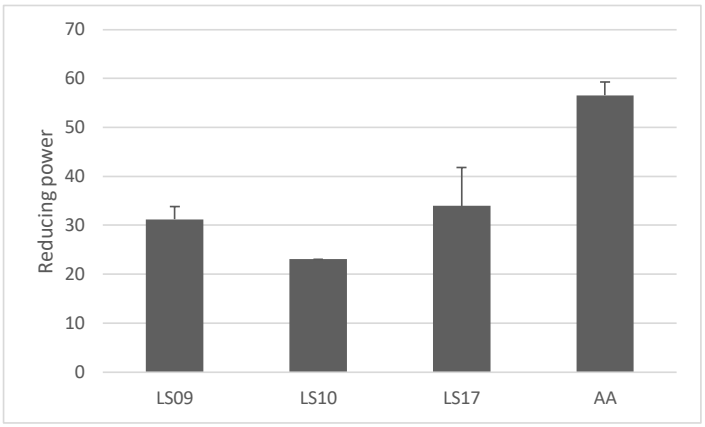

Figure 4. Results of reducing power of lactic strains (LS09, LS10 and LS17). AA: ascorbic acid.

Iron is involved in the formation of free radicals because of Fenton reactions, which lead to the production of the extremely toxic radical ($HO^{\cdot}$) (Talib et al., 2019). The chelating activities of LAB may be associated with the physiological chelators mapped on the bacterial cell wall (Lin & Yen, 1999). It was reported that the antioxidant activity in LAB is associated with the expression of iron binding protein (Yamamoto et al., 2002).

Relationship between phenolic compounds and antioxidant activity of lactic acid bacteria

The relationship between total phenolic content (TPC) and antioxidant capacity (DPPH and superoxide anion radical (SAR) scavenging activity and reducing power (RP)) was evaluated by the Pearson correlation (Table 2). TPC is positively and significantly correlated with RP (** r = 0.81). Indeed, negative correlations were registered between TPC and survival at 1 mM H_2O_2 and scavenging activity of SAR (r = -0.97; r = -0.74, respectively).

According to Dobrinas et al. (2021), Osman et al. (2021), and Lyu et al. (2022), the types and quantities of phenolic compounds might contribute to the varying antioxidant activity. The relationship between antioxidant activity and total phenolic content can be influenced by various factors. Total phenolic content,

in fact, does not account for all present antioxidants. It is important to consider the synergistic interactions among the antioxidants in a mixture, as antioxidant activity depends not only on concentration but also on the structure and interactions between the different antioxidants (Piluzza & Bullitta, 2011).

Some relationships were also recorded between some parameters. For instance: high positive correlation between survival at 1 mM H_2O_2 and SAR scavenging activity (r = 0.87), and between survival at 0.5 mM H_2O_2 and DPPH (r = 0.62). Also, survival at 0.5 mM and 1 mM H_2O_2 correlated negatively with RP (r = *-0.93 and -0.67, respectively). DPPH presented significant negative correlation with SAR scavenging activity (r = -0.85).

Table 2. Pearson's correlations between antioxidant activities measured using different assays and total phenolic contents.

	TPC	0.5 mM H_2O_2	1 mM H_2O_2	DPPH	SAR	RP
TPC	1					
0.5 mM H_2O_2	ns-0.56301	1				
1 mM H_2O_2	ns-0.97709786	ns0.3742552	1			
DPPH	ns0.29684677	ns0.62207025	ns-0.49324727	1		
SAR	ns-0.74839973	ns-0.12678478	ns0.8723926	ns-0.85551217	1	
RP	**0.81390088	*-0.93840493	ns-0.67162877	ns-0.31321123	ns-0.22377366	1

TPC: total phenolic content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, SAR: Superoxide anion radical, RP: ferric reducing power, ** indicates significant difference at P < 0.01, * indicates significant difference at P < 0.1, ns: not significant.

Conclusion

This study demonstrated that LAB strains isolated from fermented wheat products exhibited varying levels of antioxidant activity. These antioxidant properties could potentially be applied in the future to inhibit food oxidation processes. As a result, the use of natural antioxidants may help reduce or even eliminate the need for chemical antioxidants. Additionally, it would be valuable to extend this research through an *in vivo* study using animal models subjected to oxidative stress. By treating these animals with LAB and assessing their oxidative stress levels, further insights could be gained into the potential therapeutic effects of LAB in mitigating oxidative damage.

References

Aldosari, S., Awad, M., Harrington, E. O., Sellke, F. W., & Abid, M. R. (2018). Subcellular reactive oxygen species (ROS) in cardiovascular pathophysiology. *Antioxidants*, *7*(1), 1-16. https://doi.org/10.3390/antiox7010014

Alkalbani, N. S., Turner, M. S., & Ayyash, M. M. (2019). Isolation, identification, and potential probiotic characterization of isolated lactic acid bacteria and *in vitro* investigation of the cytotoxicity, antioxidant, and antidiabetic activities in fermented sausage. *Microbial Cell Factories*, *18*(1), 1-12. https://doi.org/10.1186/s12934-019-1239-1

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M., & Bordoni, A. (2013). Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. *Applied Microbiology and Biotechnology*, *97*, 809–817. https://doi.org/10.1007/s00253-012-4241-7

Arasu, M. V., Al-Dhabi, N. A., Ilavenil, S., Choi, K. C., & Srigopalram, S. (2016). *In vitro* importance of probiotic *Lactobacillus plantarum* related to medical field. *Saudi Journal of Biological Sciences*, *23*(1), 6-10. https://doi.org/10.1016/j.sjbs.2015.09.022

Averill-Bates, D. (2024). Reactive oxygen species and cell signaling. Review. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1871*(2), 1-11. https://doi.org/10.1016/j.bbamcr.2023.119573

Checa, J., & Aran, J. M. (2020). Reactive oxygen species: Drivers of physiological and pathological processes. *Journal of Inflammation Research*, *2020*(13), 1057-1073. https://doi.org/10.2147/JIR.S275595

Davis, C. D., & Milner, J. A. (2009). Gastrointestinal microflora, food components and colon cancer prevention. *Journal of Nutritional Biochemistry*, *20*(10), 743-752. https://doi.org/10.1016/j.jnutbio.2009.06.001

Dobrinas, S., Soceanu, A., Popescu, V., Popovici, I. C., & Jitariu, D. (2021). Relationship between total phenolic content, antioxidant capacity, Fe and Cu content from tea plant samples at different brewing times. *Processes*, *9*(8), 1-11. https://doi.org/10.3390/pr9081311

Düz, M., Doğan, Y. N., & Doğan, İ. (2020). Antioxidant activity of *Lactobacillus plantarum, Lactobacillus sake* and *Lactobacillus curvatus* strains isolated from fermented Turkish Sucuk. *Anais da Academia Brasileira Ciências*, *92*(4), 1-13. https://doi.org/10.1590/0001-3765202020200105

Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes*, *12*(1), 1-24. https://doi.org/10.1080/19490976.2020.1801944

Ghosh, N., Das, A., Chaffee, S., Roy, S., & Sen, C. K. (2018). Reactive oxygen species, oxidative damage and cell death. In S. Chatterjee, W. Jungraithmayr, & D. Bagchi (Eds.), *Immunity and inflammation in health and disease: Emerging roles of nutraceuticals and functional foods in immune support* (pp. 45-55). https://doi.org/10.1016/B978-0-12-805417-8.00004-4

Glennon-Alty, L., Hackett, A. P., Chapman, E. A., & Wright, H. L. (2018). Neutrophils and redox stress in the pathogenesis of autoimmune disease. *Free Radical Biology and Medicine*, *125*, 25-35. https://doi.org/10.1016/j. freeradbiomed.2018.03.049

Halliwell, B. (2022). Reactive oxygen species (ROS), oxygen radicals and antioxidants: where are we now, where is the field going and where should we go? *Biochemistry and Biophysical Research Communications*, *633*, 17-19. https://doi.org/10.1016/j.bbrc.2022.08.098

Hu, Y., Zhao, Y., Jia, X., Liu, D., Huang, X., Wang, C., Zhu, Y., Yue, C., Deng, S., & Lyu, Y. (2023). Lactic acid bacteria with a strong antioxidant function isolated from "Jiangshui," pickles, and feces. *Frontiers in Microbiology*, *14*, 1-13. https://doi.org/10.3389/fmicb.2023.1163662

Jakubczyk, K., Dec, K., Kałduńska, J., Kawczuga, D., Kochman, J., & Janda, K. (2020). Reactive oxygen species - sources, functions, oxidative damage. *Polski Merkuriusz Lekarski*, *48*(284), 124-127.

Ji, K., Jang, N. Y., & Kim, Y. T. (2015). Isolation of lactic acid bacteria showing antioxidative and probiotic activities from kimchi and infant feces. *Journal of Microbiology and Biotechnology*, *25*(9), 1568-1577. https://doi.org/10.4014/jmb.1501.01077

Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Archives of Toxicology*, *97*(10), 2499–2574. https://doi.org/10.1007/s00204-023-03562-9

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences, 22*(9), 1-21. https://doi.org/10.3390/ijms 22094642

Kuo, H.-C., Kwong, H. K., Chen, H. Y., Hsu, H.-Y., Yu, S.-H., Hsieh, C.-W., Lin, H.-W., Chu, Y.-L., & Cheng, K.-C. (2021). Enhanced antioxidant activity of *Chenopodium formosanum Koidz*. by lactic acid bacteria: Optimization of fermentation conditions. *PLoS ONE*, *16*(5), 1-13. https://doi.org/10.1371/journal.pone.0249250

Łepecka, A., Szymanski, P., Okon, A., & Zielinska, D. (2023). Antioxidant activity of environmental lactic acid bacteria strains isolated from organic raw fermented meat products. *LWT - Food Science and Technology*, *174*, 1-7. https://doi.org/10.1016/j.lwt.2023.114440

Li, S., Zhao, Y., Zhang, L., Zhang, X., Huang, L., Li, D., Niu, C., Yang, Z., & Wang, Q. (2012). Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. *Food Chemistry*, *135*(3), 1914-1919. https://doi.org/10.1016/j.foodchem .2012.06.048

Li, T., Jiang, T., Liu, N., Wu, C., Xu, H., & Lei, H. (2021). Biotransformation of phenolic profiles and improvement of antioxidant capacities in jujube juice by select lactic acid bacteria. *Food Chemistry*, *339*, 127859. https://doi.org/10.1016/j. foodchem.2020.127859

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757-772. https://doi.org/10.2147/CIA.S158513

Lin, M.-Y., & Yen, C.-L. (1999). Antioxidative ability of lactic acid bacteria, *Journal of Agricultural and Food Chemistry*, *47*(4), 1460-1466. https://doi.org/10.1021/jf981149l

Livinska, O., Ivaschenko, O., Garmasheva, I., & Kovalenko, N. 2016. The screening of lactic acid bacteria with antioxidant properties. *AIMS Microbiology*, *2*(4), 447-459. https://dx.doi.org/10.3934/microbiol.2016.4.447

Losada-Barreiro, S., Sezgin-Bayindir, Z., Paiva-Martins, F., & Bravo-Díaz, C. (2022). Biochemistry of antioxidants: Mechanisms and pharmaceutical applications. *Biomedicines*, *10*(12), 1-47. https://doi.org/10.3390/biomedicines10123051

Lyu, J. I., Ryu, J., Seo, K.-S., Kang, K.-Y., Park, S. H., Ha, T.-H., Ahn, J.-W., & Kang, S.-Y. (2022). Comparative study on phenolic compounds and antioxidant activities of Hop (*Humulus lupulus* L.) strobile extracts. *Plants, 11*(1), 1-10. https://doi.org/ 10.3390/plants11010135

Microsoft Corporation. (2019). *Microsoft Excel* (Versão 2019) [Software]. Microsoft Corporation.

Mishra, V., Shah, C., Mokashe, N., Chavan, R., Yadav, H., & Prajapati, J. (2015). Probiotics as potential antioxidants: a systematic review. *Journal of Agricultural and Food Chemistry*, *63*(14), 3615-3626. https://doi.org/10.1021/jf506326t

Mu, G., Gao, Y., Tuo, Y., Li, H., Zhang, Y., Qian, F., & Jiang, S. (2018). Assessing and comparing antioxidant activities of lactobacilli strains by using different chemical and cellular antioxidant methods. *Journal of Dairy Science*, *101*(12), 10792-10806. https://doi.org/10.3168/jds.2018-14989

Muñoz, R., de las Rivas, B., López de Felipe, F., Reverón, I., Santamaría, L., Esteban-Torres, M., Curiel, J. A., Rodríguez, H., & Landete, J. M. (2016). Biotransformation of phenolics by *Lactobacillus plantarum* in fermented foods. In J. Farias, C. Martínez-Villaluenga, & E. Peñas (Eds.), *Fermented Foods in Health and Disease Prevention* (pp. 63-83). Academic Press. https://doi.org/10.1016/B978-0-12-802309-9.00004-2

Noureen, S., Riaz, A., Arshad, M., & Arshad, N. (2019). *In vitro* selection and in vivo confirmation of the antioxidant ability of *Lactobacillus brevis MG 000874*. *Journal of Applied Microbiology*, *126*(4), 1221-1232. https://doi.org/10.1111/jam.14189

Osman, M. A., Mahmoud, G. I., & Shoman, S. S. (2021). Correlation between total phenols content, antioxidant power and cytotoxicity. *Biointerface Research in Applied Chemistry*, *11*(3), 10640-10653. https://doi.org/10.33263/BRIAC113.1064010653

Piluzza, G., & Bullitta, S. (2011). Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharmaceutical Biology, 49*(3), 240-247. https://doi.org/10.3109/13880209.2010.501083

Prior, R., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302. https://doi.org/10.1021/jf0502698

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. *Frontiers in Pharmacology*, *13*(806470), 1-15. https://doi.org/10.3389/fphar.2022.806470

Rwubuzizi, R., Kim, H., Holzapfel, W. H., & Todorov, S. D. (2023). Beneficial, safety, and antioxidant properties of lactic acid bacteria: A next step in their evaluation as potential probiotics. *Heliyon*, *9*(4), 1-12. https://doi.org/10.1016/j.heliyon.2023.e15610

Sangouni, A. A., Taghdir, M., Mirahmadi, J., Sepandi, M., & Parastouei, K. (2022). Effects of curcumin and/or coenzyme Q10 supplementation on metabolic control in subjects with metabolic syndrome: A randomized clinical trial. *Nutrition Journal*, *21*(62), 1-10. https://doi.ord/10.1186/s12937-022-00816-7

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., ... Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology, 11*(694), 1-21. https://doi.org/10.3389/fphys.2020.00694

Shi, Y., Cui, X., Gu, S., Yan, X., Li, R., Xia, S., Chen, H., & Ge, J. (2019). Antioxidative and probiotic activities of lactic acid bacteria isolated from traditional artisanal milk cheese from Northeast China. *Probiotics and Antimicrobial Proteins*, *11*(4), 1086-1099. https://doi.org/10.1007/s12602-018-9452-5

Skorokhod, I., & Kurdysh, I. (2014). The low-molecular weight antioxidants of microorganisms. *Mikrobiolohichnyi Zhurnal*, *76*(3), 48-59.

Srivastava, K. K., & Kumar, R. (2015). Stress, oxidative injury and disease. *Indian Journal of Clinical Biochemistry*, *30*(1), 3-10. https://doi.org/10.1007/s12291-014-0441-5

Talib, N., Mohamad, N. E., Yeap, S. K., Hussin, Y., Aziz, M. N. M., Masarudin, M. J., Sharifuddin, S. A., Hui, Y. W., Ho, C. L., & Alitheen, N. B. (2019). Isolation and characterization of *Lactobacillus* spp. from kefir samples in Malaysia. *Molecules, 24*(14), 1-18. https://doi.org/10.3390/molecules24142606

Tang, W., Xing, Z., Li, C., Wang, J., & Wang, Y., (2017). Molecular mechanisms and *in vitro* antioxidant effects of *Lactobacillus plantarum* MA2. *Food Chemistry*, *221*, 1642-1649. https://doi.org/10.1016/j.foodchem.2016.10.124

Vougiouklaki, D., Tsironi, T., Tsantes, A. G., Tsakali, E., Van Impe, J. F. M., & Houhoula, D. (2023). Probiotic properties and antioxidant activity *in vitro* of lactic acid bacteria. *Microorganisms*, *11*(5), 1-13. https://doi.org/10.3390/microorganisms11051264

Wang, Y.-C., Yu, R.-C., & Chou, C.-C. (2006). Antioxidative activities of soymilk fermented with lactic acid bacteria and bifid bacteria. *Food Microbiology*, *23*(2), 128-135. https://doi.org/10.1016/j.fm.2005.01.020

Wu, D., Sun, M. Z., Zhang, C., & Xin, Y. (2014). Antioxidant properties of *Lactobacillus* and its protecting effects to oxidative stress caco-2 cells. *Journal of Animal and Plant Sciences*, *24*(6), 1766-1771.

Yamamoto, Y., Poole, L. B., Hantgan, R. R., & Kamio, Y. (2002). An iron-binding protein, dpr, from *Streptococcus mutans* prevents iron dependent hydroxyl radical formation *in vitro*. *Journal of Bacteriology*, *184*(11), 2931–2939. https://doi.org/10.1128/JB.184.11.2931-2939.2002

Yang, J., Dong, C., Ren, F., Xie, Y., Liu, H., Zhang, H., & Jin, J. (2021). *Lactobacillus paracasei* M11-4 isolated from fermented rice demonstrates good antioxidant properties *in vitro* and in vivo. *Journal of the Science of Food and Agriculture*, *102*(88), 3107-3118. https://doi.org/10.1002/jsfa.11652

Yang, S., & Lian, G. (2020). ROS and diseases: role in metabolism and energy supply. *Molecular and Cellular Biochemistry*, *467*, 1-12. https://doi.org/10.1007/s11010-019-03667-9

Zhang, X., Yu, S., Li, X., Wen, X., Liu, S., Zu, R., Ren, H., Li, T., Yang, C., & Luo, H. (2023). Research progress on the interaction between oxidative stress and platelets: Another avenue for cancer? *Pharmacological Research*, *191*, 1-12. https://doi.org/10.1016/j.phrs.2023.106777

Zhang, Y., Hu, P., Lou, L., Zhan, J., Fan, M., Li, D., & Liao, Q. (2017). Antioxidant activities of lactic acid bacteria for quality improvement of fermented sausage. *Journal of Food Science*, *82*(12), 2960-2967. https://doi.org/10.1111/1750-3841.13975

Zuo, L., & Wijegunawardana, D. (2021). Redox Role of ROS and Inflammation in pulmonary diseases. In Y. X. Wang (Ed.), *Advances in experimental medicine and biology* (v. 1304) (pp. 187-204). Springer. https://doi.org/10.1007/978-3-030-68748-9_11