sciendo
10.2478/aucft-2023-0007

EFFECTS OF NITROGEN SOURCES AND INORGANIC SALTS ON ANTIOXIDANT ACTIVITY OF GOAT MILK FERMENTED BY *LACTOBACILLUS PLANTARUM* L60

– Research paper –

Wenjing HU[1], Guowei SHU [1], Jiansheng WANG[2*], Chunji DAI[1], Yixin HUI[1], Yueyue MA[2]

[1]*School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, 710021, China*
[2]*Shaanxi Hongxing Meiing dairy Co.,ltd, Weinan, 711799, China*

Abstract: This study investigated the effects of various nitrogen sources (peptone, casein hydrolysate) and inorganic salts (KH_2PO_4, $MgSO_4$ and NaCl) on the antioxidant activity (specifically, DPPH and superoxide anion scavenging rate), acidity, and pH of peptides in goat milk (GM) fermented by *Lactobacillus plantarum* L60 by individual factor experiments. The results indicated that nitrogen sources and inorganic salts significantly affected *L. plantarum* L60's antioxidant and acid-producing abilities, and when the supplemental levels of peptone, casein hydrolysate, KH_2PO_4, $MgSO_4$ and NaCl were 0.7%, 0.3%, 0.3%, 0.15% and 0.9%, respectively, the scavenging ability of antioxidant peptides on DPPH radical and superoxide anion reached the maximum.

Key words: *Lactobacillus plantarum* L60; goat milk; antioxidant; nitrogen sources; inorganic salts.

INTRODUCTION

Antioxidant peptides are short peptides with small molecular weights, which have physiological functions such as chelating metal ions, scavenging free radicals and inhibiting lipid peroxidation (Song et al.,2019; Yang et al., 2017). When compared to amino acids, which are the hydrolyzed products of proteins, antioxidant peptides are easier for the body to absorb and utilize (Chen et al., 2021).

Nowadays, protein-rich plant and animal materials, such as beans (Xie et al., 2019), edible fungi (Li et al., 2019), milk (Aguilar et al., 2017; Keyur et al., 2022; Patel et al., 2021) and marine organisms (Kim et al, 2018), are used as raw materials to prepare polypeptides through microbial fermentation, enzyme hydrolysis and other methods in industrial production. Yang et al. (2020) obtained duck blood antioxidant peptides with strong antioxidant activity and reasonable amino acid composition by enzymatic hydrolysis of duck blood. Maryam et al. (2011) found that β-casein of camel milk had a high antioxidant activity after hydrolyzed by chymotrypsin. According to Abbasi et al. (2022), the highest antioxidant activity was found in the peptide generated by alkaline enzyme hydrolysis of quinoa protein for 0.5 hours. Zanutto et al. (2018) obtained antioxidant peptides and antimicrobial peptides by enzymatic hydrolysis of cow milk with fungal proteases. Chen et al. (2021) used the new wild *Lactobacillus plantarum* L60 isolated from Kefir to ferment goat milk. The goat milk had a 70.8% DPPH scavenging ability after 12 hours fermentation. The maximum antioxidant activity was observed at 52°C for 27 hours in goat milk fermented with *Lactobacillus fermentum* (M4) by Gauravkumar et al. (2020).

When using microbial fermentation to produce antioxidant peptides, the peptidase produced in the process of microbial metabolism can hydrolyze bitter hydrophobic amino acids. As opposed to enzymatic hydrolysis, fermentation can effectively remove the bitter taste of peptides. At the same time, the peptide chain can be also modified and reorganized. And most of the polypeptides have a unique flavor, so it is widely favored by researchers (Caleb et al., 2018).

In recent years, researchers have conducted a lot of studies on the preparation of antioxidant peptides from different raw materials, but the factors affecting the preparation of antioxidant peptides are less explored. In this study, GM was selected as raw material to produce antioxidant peptides. GM is rich in nutrition. When compared to cow milk and human milk, GM has a shorter fatty acid chain length. This makes it easier for the human body to absorb (Clark et al., 2017), and its reasonable amino acid composition helps reduce its sensitization (Hodgkinson et al., 2018). On the one hand, fermentation increases the variety and content of

Received: 11.03.2023
Accepted in revised form: 27.05.2023

* Corresponding author.
E-Mail address: wangjshhx@gmail.com

antioxidant peptides in dairy products. On the other hand, adding live bacteria to dairy products can enhance their nutritional value and taste, the probiotics can also play a role in the body directly or indirectly by producing cell metabolites.
In previous study, our lab found that products of GM fermented by *L. plantarum* L60 exhibit strong antioxidant activity and investigated the effect of prebiotics on it (Chen et al., 2015; Shu et al., 2017). In this study, we used *Lactobacillus plantarum* L60 to ferment the GM, and the effects of adding nitrogen sources and inorganic salts to the medium on the antioxidant activity, pH and acidity of it were explored, which provided a basis for the subsequent medium optimization experiment.

MATERIALS AND METHODS

Bacterial strain

The strain *Lactobacillus plantarum* L60 was procured from the School of Food and Biological Engineering, Shaanxi University of Science and Technology.
The strain *Lactobacillus plantarum* L60 was inoculated into MRS Medium, cultured at 37℃ for 24h, activated twice, and then cultured at 37℃ for 18h to obtain the 3rd generation bacterial suspension. The resulting suspension was then refrigerated at 4℃.

Fermentation of goat mike and preparation of whey sample to be tested

Firstly, a concentration of 14% GM should be prepared from reconstituted GM, pasteurized it at 90℃ for 15 min and cool down, and then inoculated the 3rd generation bacterial suspension with 5% inoculum, cultivated it in a water bath to curd at 42°C, kept it in the refrigerator for later use.
After shaking the fermented GM well, determined its pH value. The fermented milk was adjusted to a pH of 3.4-3.6 using a solution of 1M HCl, and the supernatant was collected after being centrifuged at 8000 r/min for 15min. The pH of fermented milk was then adjusted to 8.3 using a solution of 1M NaOH. After centrifugation again at 8000 r/min for 15 min, the supernatants were collected as samples for testing.

Scavenging rate of DPPH free radical

The DPPH radical solution and the samples to be tested were prepared following the method described by Unal et al. (2013). Allowed each group to sit in the dark for 30 minutes before measuring the absorbance at 517nm. The scavenging rate can be calculated using the formula (1). During the experiment, a 0.25mg/mL Trolox solution was used as a control.

$$\text{DPPH free radical scavenging rate}(\%) = \left(1 - \frac{A_i - A_j}{A_0}\right) \times 100\% \quad (1)$$

The formula consists of three groups:
A_0: the control group, A_i: the experimental group, A_j: the blank group.

Scavenging rate of superoxide

To get the Tris-HCl- EDTA-2Na buffer, prepared a Tris-HCl solution with a concentration of 50mM and adjusted the pH to 8.2 by 1mM EDTA-2Na. 5.7mL of the buffer mentioned earlier, 0.2 mL of the sample and 0.1 mL of a solution containing 5mmol/L of pyrogallic acid were added in an anaerobic tube with a capacity of 20mL. Mixed the contents thoroughly and began timing immediately. Over the next 5 minutes, measured the absorbance at 325nm every 30 seconds. As a control group, distilled water was used instead of the sample. The formula (2) below was used to calculate the scavenging rate (Jiang et al., 2014).

$$\text{Superoxide scavenging rate}(\%) = \frac{\Delta A_0/min - \Delta A_1/min}{\Delta A_0/min} \times 100\% \quad (2)$$

The formula can be expressed as follows:
ΔA_0/min: minutely change in absorbance of the control group, ΔA_1/min: minutely change in absorbance of the experimental group.

Test for acidity and pH

The acidity and pH values were determined according to the previous methods in our laboratory (Chen et al., 2015).

Analyses of statistics

All experiments and analyses were conducted in triplicate, and the resulting were reported as average values. The data were processed using the Origin 2021 software package and Microsoft Excel 2010.

RESULTS

In this experiment, several factors affecting *Lactobacillus plantarum* L60's growth were selected, which were categorized into nitrogen sources and inorganic salts. Under the premise of optimal fermentation conditions, we examined how fermented GM's antioxidant activity was affected by these factors.

Effect of peptone addition on antioxidant activity of L60 fermented GM

To conduct the experiment, various concentrations of peptone were added to the reconstituted GM, including 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%. As indicated in figure 1(a), the trend of DPPH free scavenging rate and superoxide anion scavenging rate was slightly different. The antioxidant activity changed slowly when the dosage was 0.1-0.5%. When the dosage was 0.7%, it reached its maximum DPPH and superoxide anion scavenging rate. As peptone concentrations increased continually, antioxidant activity gradually decreased.

As shown in figure 1(b), fermented GM's pH value and acidity behaved oppositely. With the increase of peptone concentration, pH value decreased and acidity value increased gradually.

Effect of casein hydrolysate on antioxidant activity of L60 fermented GM

To conduct the experiment, various concentrations of casein hydrolysate were added to the reconstituted GM, including 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%. Data in figure 2(a) suggested that the trend of DPPH free scavenging rate and superoxide anion scavenging rate was the same, both showed an increase at first, a rapid decline, and then a certain increase.

Based on figure 2(b), as casein hydrolysate concentration increased, the pH value increased first and then decreased, but the range of change was small. The acidity was opposite to pH, and the variation range was large, which might be due to casein hydrolysate that can change the buffering capacity of GM.

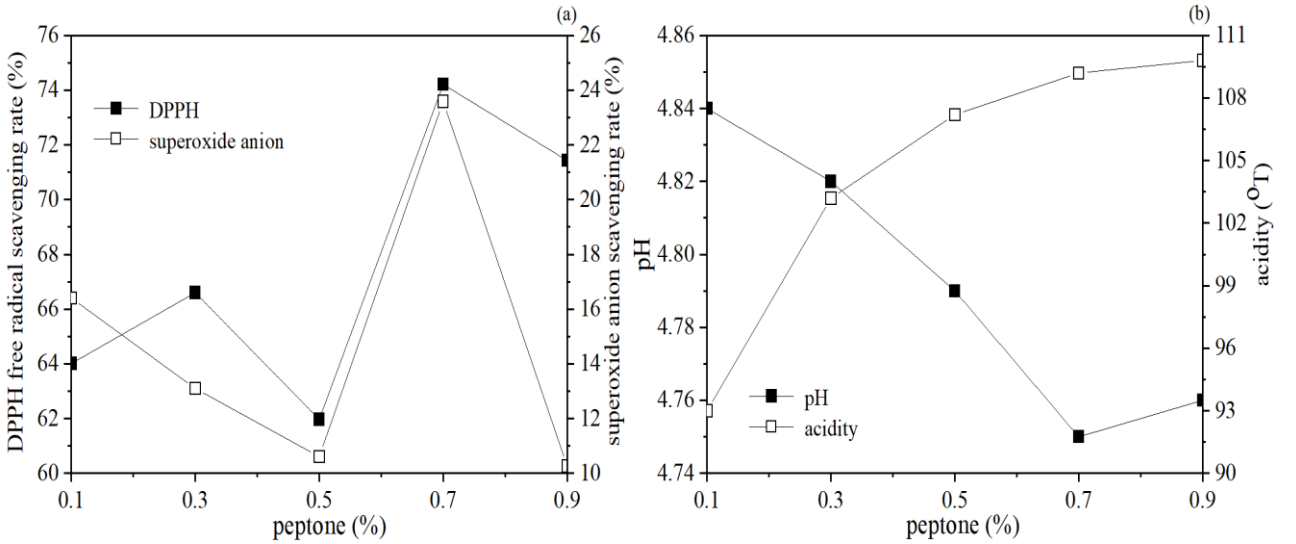

Figure 1 Effect of peptone on antioxidant (a), acidity and pH (b) of GM fermented by *L. plantarum* L60

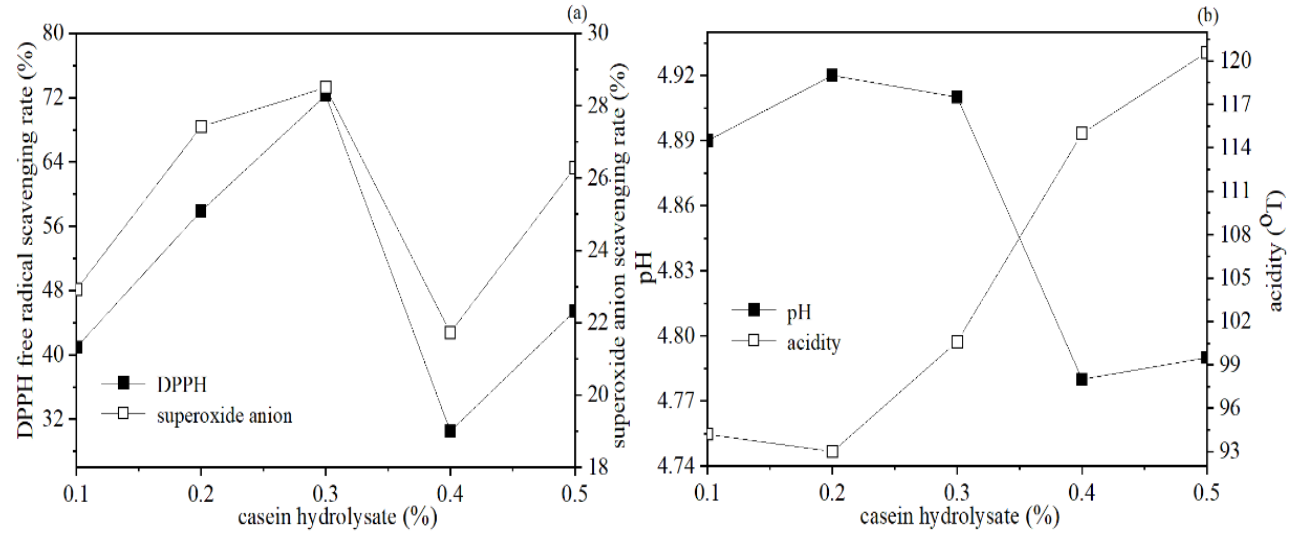

Figure 2 Effect of casein hydrolysate on antioxidant (a), acidity and pH (b) of GM fermented by *L. plantarum* L60

Effect of KH_2PO_4 on antioxidant activity of L60 fermented GM

To conduct the experiment, various concentrations of KH_2PO_4 were added to the reconstituted GM, including 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%. As detailed in figure 3(a), when the supplemental level of KH_2PO_4 was low (0.1%-0.3%), the scavenging rate of DPPH and superoxide anion increased rapidly, and the highest value was reached when the supplemental level was 0.3%. However, the antioxidant activity decreased rapidly and remained stable as KH_2PO_4 concentration increased.

According to figure 3(b), with an increase in KH_2PO_4 concentration, fermented GM's pH values decreased continuously, but its acidity increased.

Effect of $MgSO_4$ on antioxidant activity of L60 fermented GM

To conduct the experiment, various concentrations of $MgSO_4$ were added to the reconstituted GM, including 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%. The results depicted in figure 4(a) demonstrate that the antioxidant activity of fermented GM exhibited an initial increase followed by a subsequent decrease. The antioxidant activity of fermented GM was the highest when the supplemental level of $MgSO_4$ was 0.15%.

From figure 4(b), with an increase in $MgSO_4$ concentration, the pH value decreased rapidly first and then increased slowly. The change of acidity value was opposite to that of pH value. Therefore, low concentration of $MgSO_4$ can promote the acid production of L60, while high concentration can inhibit the acid production of bacterial fermentation.

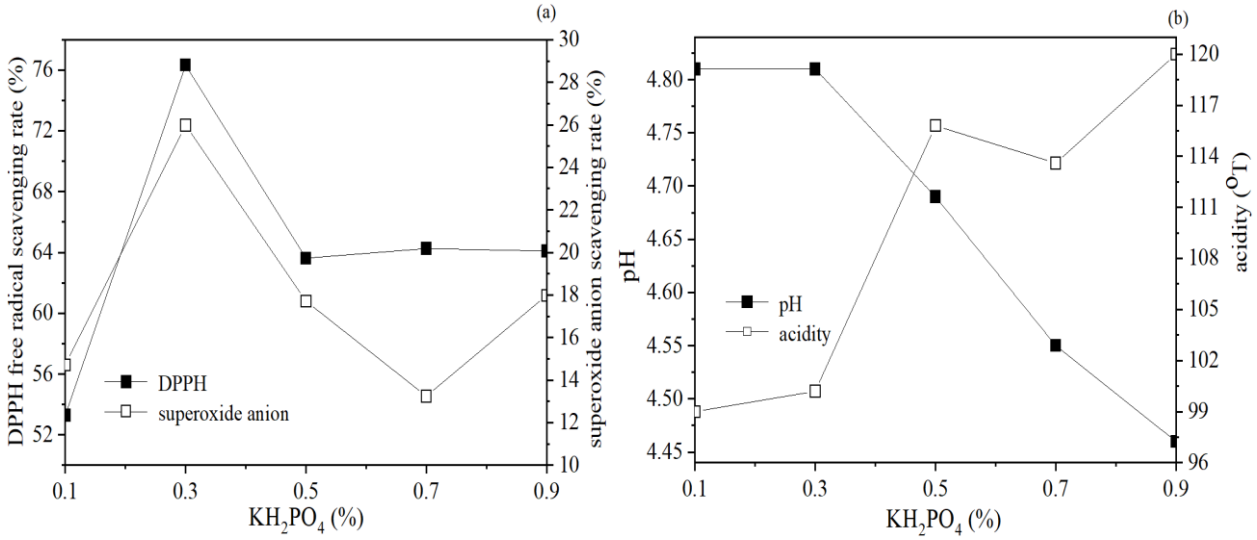

Figure 3 Effect of KH_2PO_4 on antioxidant (a), acidity and pH (b) of GM fermented by *L. plantarum* L60

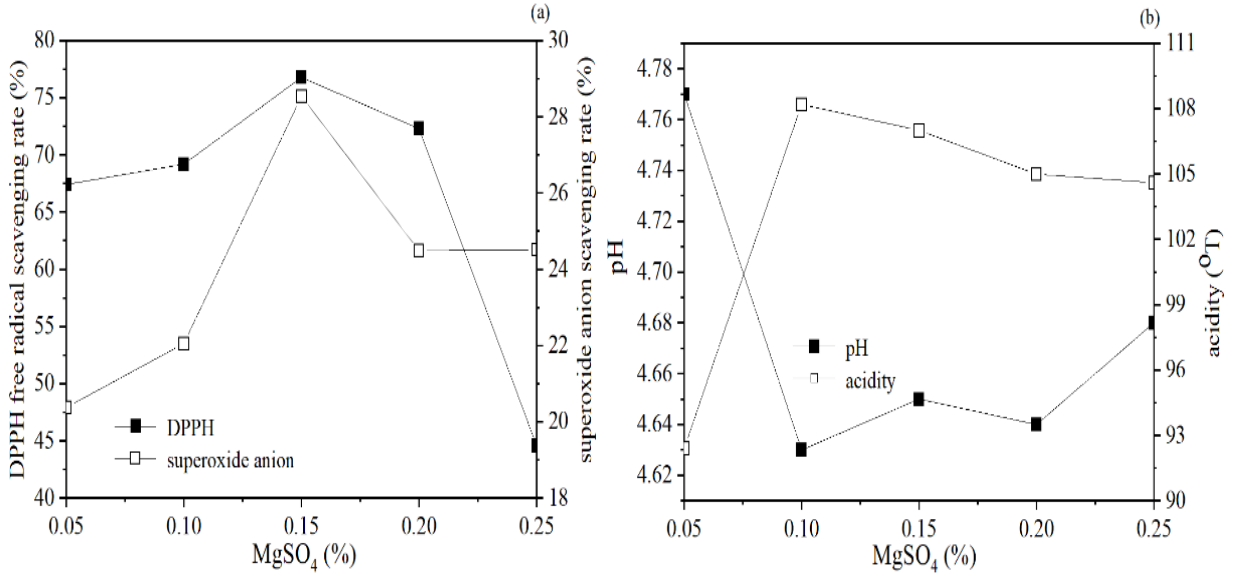

Figure 4 Effect of $MgSO_4$ on antioxidant (a), acidity and pH (b) of GM fermented by *L. plantarum* L60

Effect of NaCl on antioxidant activity of L60 fermented GM

To conduct the experiment, various concentrations of NaCl were added to the reconstituted GM, including 0.3%, 0.6%, 0.9%, 1.2%, and 1.5%. As shown in figure 5(a), fermented milk's antioxidant activity first increased slowly to maximum value, and then decreased rapidly, and was the highest when the supplemental level of NaCl was 0.9%, the DPPH free scavenging rate was 76.05% and the superoxide anion scavenging rate was 28.90%.

As illustrated by figure 5(b), the acidity of fermented GM increased firstly and then decreased. The pH was opposite to the acidity. It can be seen from the figure that NaCl can promote the fermentation capacity of bacteria to produce acid when the dosage was 0.3%-0.9%. However, when the concentration of NaCl was higher than 0.9%, the acidity began to decline, and the pH value rose rapidly.

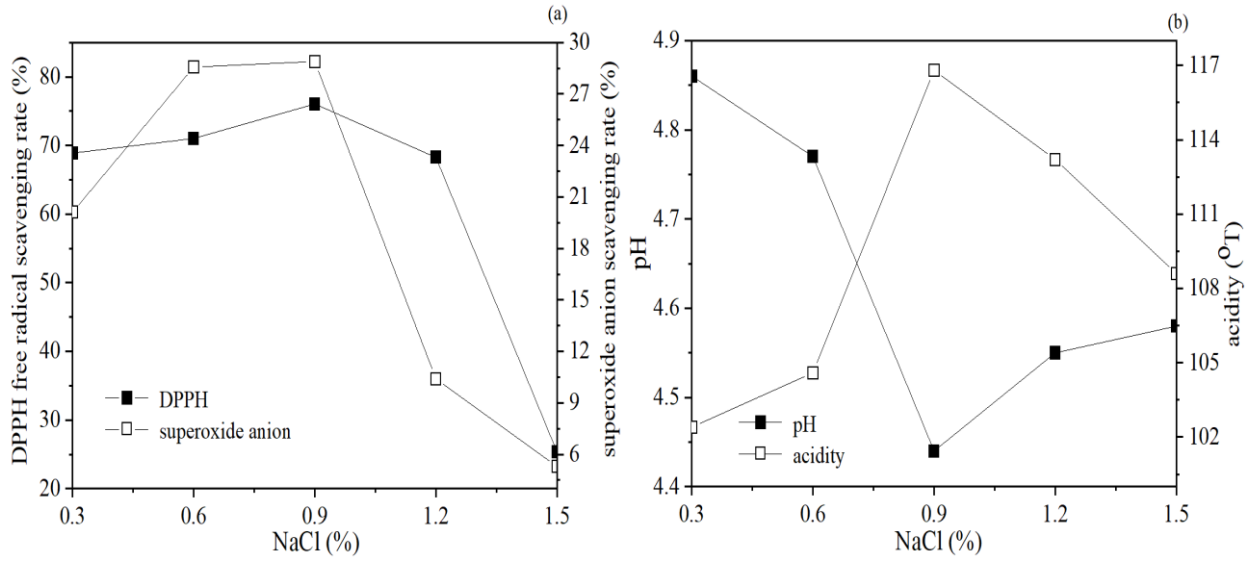

Figure 5 Effect of NaCl on antioxidant (a), acidity and pH (b) of GM fermented by *L. plantarum* L60

DISCUSSION

As a widely used starter culture, lactic acid bacteria play a crucial role in the production of bioactive peptides in dairy products. The production of peptides is affected by culture medium and fermentation conditions. In this paper, DPPH radical and superoxide anion scavenging rate were used to determine the antioxidant activities of fermented GM. We found that different nitrogen sources and inorganic salts had different contributions to the antioxidant activities of fermented milk. The optimal mixing ratio of nitrogen sources and inorganic salts and other nutrient elements, or the effect of other nitrogen sources and inorganic salts on antioxidant activity of fermented milk can be further explored.

It has been reported that the antioxidant activity of lactic acid bacteria shows a good correlation with the level of protein hydrolysis, and the more casein hydrolysates prepared from the cell-free extracts of *lactobacillus* after hydrolysis, the stronger the antioxidant activity (Ramesh et al., 2012). The antioxidant activity of fermented GM is mainly due to the proteolytic system of lactic acid bacteria, which can decompose the protein in GM to produce antioxidant peptides, and the hydrolyzed protein is derived from the action of cell envelope proteinase (CEP). CEP is a special proteolytic enzyme obtained from lactic acid bacteria. In the food industry, it can release bioactive peptides in milk proteins, such as ACE inhibitory peptides, antioxidant peptides, etc (Ren et al., 2013).

The results of this experiment found that the addition of a specific amount of nitrogen sources could enhance the antioxidant activity and reduce the pH value of fermented milk, which may be because the addition of nutrients promoted the growth of bacteria (Xiang et al., 2023), which made lactic acid bacteria produce more protease, thus promoting the decomposition of milk protein and producing more antioxidant peptides, which increased the antioxidant activity. The results of Cheng et al. (2019) also showed that adding peptone could improve the CEP activity and specific activity of *Lactobacillus plantarum* LP69 fermentation medium. At the same time, it may also be because adding nitrogen sources increased the total protein content in GM, so the amount of protein acted by hydrolase also increased, resulting in the increase of antioxidant peptides content.

The antioxidant activity of fermented milk

exhibited an initial increase followed by a subsequent decrease in response to increasing concentrations of KH_2PO_4, concomitant with a decline in pH, it possibly because PO_4^{3-} can act as a buffer to neutralize the large amount of lactic acid produced by lactic acid bacteria during metabolism, thus changing the pH of fermentation medium. At the same time, the appropriate concentration of K^+ plays an important role in the process of enzyme catalysis, which can promote enzyme activity, but with the increase of concentration, it will lead to protein denaturation and decrease of enzyme activity (Cheng et al., 2019; Na et al., 2010). It can be seen that, as an important salt in the growth process of *Lactobacillus*, the addition of KH_2PO_4 has a certain promoting effect on the acid production capacity of *Lactobacillus plantarum* L60, which is consistent with the research results of Ji (2013) on the fermentation performance of *Lactobacillus Bulgaricus* LB6.

Mg^{2+} has a great effect on the growth of gram-positive bacteria, it can promote the growth of bacteria and the generation of primary metabolites at an appropriate concentration (Wu, 2009).

The highest antioxidant activity of fermented GM was observed at a supplemental level of 0.9% NaCl. On the one hand, 0.9% NaCl is of great significance for the maintenance of the osmotic pressure of bacterial cells, and this concentration is the most suitable concentration to ensure the survival rate of bacterial cells, so it is more favorable to accumulate the antioxidant peptides at this concentration. On the other hand, suitable concentration of NaCl can promote the growth of bacteria, but the excessive Na^+ and Cl^- can cover on the surface of the peptides through electrostatic effect, which reduces the hydration and potential of the peptides, and promotes the aggregation, coagulation and precipitation of the peptides, thus resulting in the decrease of antioxidant activity of GM fermentation products (Zhang, 2016).

CONCLUSIONS

This study investigated the impact of various nitrogen sources and inorganic salts on both antioxidant activity and acid production of *Lactobacillus plantarum* L60 fermented GM. It is possible to increase the antioxidant activity of GM by adding appropriate additives. The results showed that the optimal dosage of peptone, casein hydrolysate, KH_2PO_4, $MgSO_4$ and NaCl were respectively 0.7%, 0.3%, 0.3%, 0.15%, 0.9%. The scavenging rates of DPPH free radical were 74.21%, 72.29%, 76.32%, 76.78%, 76.05%, respectively, and the superoxide anion scavenging rates reached to 23.59%, 28.5%, 25.98%, 28.54%, 28.9%, respectively. The study results can provide a basis to develop functional dairy products.

ACKNOWLEDGMENTS

The work was partially supported by the Scientific Research Program Funded by Shaanxi Provincial Education Department (No.22JC022), Department-city linkage key projects sub-topic of Shaanxi Province (No.2022GD-TSLD-58-2), Key R&D Projects in Shaanxi Province (No. 2023-YBNY-182).

REFERENCES

1. Abbasi, S., Moslehishad, M. & Salami, M. (2022). Antioxidant and alpha-glucosidase enzyme inhibitory properties of hydrolyzed protein and bioactive peptides of quinoa. *International Journal of Biological Macromolecules,* 213, 602-609. DOI: 10.1016/j.ijbiomac.2022.05.189.
2. Acquah, C., Di Stefano, E. & Udenigwe, C. C. (2018). Role of hydrophobicity in food peptide functionality and bioactivity. *Journal of Food Bioactives,* 4, 88–98. DOI: 10.31665/JFB.2018.4164.
3. Aguilar-Toalá, J. E., Santiago-López, L. & Peres, C. M. (2017). Assessment of multifunctional activity of bioactive peptides derived from fermented milk by specific Lactobacillus plantarum strains. *Journal of dairy science,* 100(1), 65-75. DOI: 10.3168/jds.2016-11846.
4. Ashokbhai, J. K., Basaiawmoit, B. & Das, S. (2022). Antioxidative, antimicrobial and anti-inflammatory activities and release of ultra-filtered antioxidative and antimicrobial peptides during fermentation of sheep milk: In-vitro, in-silico and molecular interaction studies. *Food Bioscience,* 47, 101666. DOI: 10.1016/J.FBIO.2022.101666.
5. Chen, H., Hui, Y. & Chen, L. (2015). Effect of probiotic *Lactobacillus* strains on antioxidant activity from fermented goat milk. *Carpathian Journal of Food Science & Technology,* 7(2), 109-114.
6. Chen, L., Hui, Y. & Gao, T. (2021). Function and characterization of novel antioxidant peptides by fermentation with a wild Lactobacillus plantarum 60. *LWT,* 135, 110162. DOI: 10.1016/j.lwt.2020.110162.
7. Chen, M., Ning, P. & Jiao, Y. (2021). Extraction of antioxidant peptides from rice dreg protein hydrolysate via an angling method. *Food Chemistry,* 337, 128069. DOI: 10.1016/j.foodchem.2020.128069.

8. Cheng, F., Chen, H. & Lei, N. (2019). Effect of prebiotics, inorganic salts and amino acids for cell envelope proteinase production from Lactobacillus plantarum LP69. *Acta Scientiarum Polonorum Technologia Alimentaria,* 18(3), 269-278. DOI: 10.17306/J.AFS.0656.
9. Cheng, F., Chen, H. & Lei, N. (2019). Effects of Carbon and Nitrogen Sources on Activity of Cell Envelope Proteinase Produced by LP69. *Acta Universitatis Cibiniensis. Series E: Food Technology,* 23(1), 11-18. DOI: 10.2478/aucft-2019-0002.
10. Clark, S. & García, M. B. M. (2017). A 100-year review: Advances in goat milk research. *Journal of dairy science,* 100(12), 10026-10044. DOI: 10.3168/jds.2017-13287.
11. Dharmisthaben, P., Basaiawmoit, B. & Sakure, A. (2021). Exploring potentials of antioxidative, anti-inflammatory activities and production of bioactive peptides in lactic fermented camel milk. *Food Bioscience,* 44, 101404. DOI: 10.1016/J.FBIO.2021.101404.
12. Ding, X., Qian, F. & Mu, G. (2023). Optimization of medium composition of Lactobacillus plantarum Y44 using Plackett–Burman and Box–Behnken designs. *Preparative Biochemistry & Biotechnology,* 1-9. DOI: 10.1080/10826068.2023.2166957.
13. Hodgkinson, A. J., Wallace, O. A. M. & Boggs, I. (2018). Gastric digestion of cow and goat milk: Impact of infant and young child in vitro digestion conditions. *Food Chemistry,* 245, 275-281. DOI: 10.1016/j.foodchem.2017.10.028.
14. Ji, Z. (2013). *Screening and optimization of lactobacillus sp. fermenting goat milk for production ace-inhibitory peptides.* Shaanxi University of Science and Technology, Xi'an.
15. Jiang, H., Tong, T. & Sun, J. (2014). Purification and characterization of antioxidative peptides from round scad (Decapterus maruadsi) muscle protein hydrolysate. *Food Chemistry,* 154, 158-163. DOI: 10.1016/j.foodchem.2013.12.074.
16. Kim, S. S., Ahn, C. B. & Moon, S. (2018). W. Purification and antioxidant activities of peptides from sea squirt (Halocynthia roretzi) protein hydrolysates using pepsin hydrolysis. *Food Bioscience,* 25, 128-133. DOI: 10.1016/j.fbio.2018.08.010.
17. Li, M., Jiang, Y. & Zhang, X. (2019). Preparation and antioxidant activity of protein peptides by enzymatic from Hohenbuehelia serotina. *Food & Machinery,* 35(11), 176-181. DOI: 10.13652/j.issn.1003-5788.2019.11.035.
18. Liu, N., Zhang, M. & Wang, Y. (2010). Effect of different concentrations of metal ions on the acid protease activity. *Journal of Anhui Agricultural Sciences,* 38(32), 18043-18044. DOI:10.13989/j.cnki.0517-6611.2010.32.022.
19. Panchal, G., Hati, S. & Sakure, A. (2020). Characterization and production of novel antioxidative peptides derived from fermented goat milk by L. fermentum. *LWT,* 119, 108887. DOI: 10.1016/j.lwt.2019.108887.
20. Ren, X., Pan, D. & Zeng, X. (2013). Effects of enzymatic hydrolysis with cell wall proteinase (cep) on structural and functional properties of casein. *Modern Food Science and Technology,* 29(11), 2643-2648. DOI: 10.13982/j.mfst.1673-9078.2013.11.027.
21. Salami, M., Moosavi-Movahedi, A. A. & Moosavi-Movahedi, F. (2011). Biological activity of camel milk casein following enzymatic digestion. *Journal of dairy research,* 78(4), 471-478. DOI: 10.1017/S0022029911000628.
22. Shu, G., He, Y. & Wan, H. (2017). Effects of Prebiotics on Antioxidant Activity of Goat Milk Fermented by L60. *Acta Universitatis Cibiniensis. Series E: Food Technology,* 21(2), 11-18. DOI: 10.1515/aucft-2017-0010.
23. Singh, R., Kaushik, J. & Man, N. (2012). Comparative evaluation of selected strains of lactobacilli for the development of antioxidant activity in milk. *Dairy science & technology,* 92(2), 179-188. DOI: 10.1007/s13594-011-0048-z.
24. Song, X., Liang, Q. & Zhang, Y. (2019). Review on the research progress of antioxidant peptides in Cheese. *China Dairy Industry,* 47(5), 42-45. DOI:10.3969/j.issn.1001-2230.2019.05.010.
25. Unal, G., El, S. N. & Akalin, A. S. (2013). Antioxidant activity of probiotic yoghurt fortified with milk protein based ingredients. *Italian Journal of Food Science,* 25(1), 63-69.
26. Wu, Z. (2009). *Studies on producing corn peptide by germ hydrolysis zein*. Heilongjiang Bayi Agricultural University, Daqing.
27. Xie, J., Du, M. & Shen, M. (2019). Physico-chemical properties, antioxidant activities and angiotensin-I converting enzyme inhibitory of protein hydrolysates from Mung bean (Vigna radiate). *Food chemistry,* 270, 243-250. DOI: 10.1016/j.foodchem.2018.07.103.
28. Yang, J., Huang, J. & Zhu, Z. (2020). Investigation of optimal conditions for production of antioxidant peptides from duck blood plasma: response surface methodology. *Poultry science,* 99(12), 7159-7168. DOI: 10.1016/j.psj.2020.08.060.
29. Yang, L. (2017). *Study on the antioxidant peptides of products fermented from different base of goat milk and its functionalities,* Northwest A & F University, Xianyang.

30. Zanutto-Elgui, M. R., Vieira, J. C. S. & do Prado, D. Z. (2019). Production of milk peptides with antimicrobial and antioxidant properties through fungal proteases. *Food chemistry,* 278, 823-831. DOI: 10.1016/j.foodchem.2018.11.119.
31. Zhang, C. (2016). Study on preparation of antioxidant peptides by enzymolysis of large yellow croaker *(Pseudosciaena crocea)* and its properties and functional mechanism. Fujian Agriculture And Forestry University, Fuzhou.