Supplementary Material

Probiotic-fermented tomato with hepatic lipid metabolism modulation effects: analysis of physicochemical properties, bioactivities, and potential bioactive compounds

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1. Supplementary materials and methods

1.1 Total phenolic content measurement

Total phenolic content was measured in line with the Folin-Ciocalteu method with modification.[1] In brief, 500 μL of Folin-Ciocalteu reagent (10%, v/v) was added to 100 μL of sample supernatant and then stood for 5 min. Afterward, 400 μL of Na_2CO_3 (75 g/L, w/v) was added to the mixture and stood for another 30 min avoiding light. The absorbance value of the mixture at 765 nm was detected by the microplate reader (Varioskan LUX, Massachusetts, USA). Total phenolic content was calculated from the gallic acid standard curve and presented as milligrams of gallic acid equivalent (GAE) per milliliter of LFT (mg GAE/mL).

1.2 Total flavonoid content measurement

Total flavonoid content was determined following a previous study.[2] Briefly, 100 μL of sample supernatant, 50 μL of $NaNO_2$ (50 g/L, w/v), and 300 μL of distilled water were mixed and stood for 5 min. Subsequently, 100 μL of $AlCl_3$ (100 g/L, w/v) was added to the mixture followed by vortexing and standing for 5 min. Finally, 200 μL of NaOH (2 mol/L) was added to the mixture to react for 5 min and the absorbance value of the mixture was determined at 510 nm via the microplate reader (Varioskan LUX, Massachusetts, USA). Total flavonoid content was calculated from the rutin standard curve and presented as milligrams of rutin equivalent (RE) per milliliter of LFT (mg RE/mL).

1.3 Antioxidant activities assessment

The antioxidant activities of samples were assessed by measuring 2,2’-azinobis(3-ethyl-benzothiazoline)-6-sulphonic acid ($ABTS^{+}\cdot$), 2,2-diphenyl-1-picrylhydrazyl (DPPH·), and hydroxyl (·OH) radical scavenging capacity according to the instructions of commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2. Supplementary results and discussion

2.1 Changes in total phenolic and flavonoid contents of tomato before and after fermentation

Phenols and flavonoids are valuable phytochemicals with beneficial effects on human health. As illustrated in Fig. S2, total phenolic and total flavonoid contents were remarkedly higher in LFT than in UFT ($P < 0.05$), indicating that total phenol and total flavonoid contents were increased after LP fermentation. These were in line with the previous findings that LAB fermentation enhanced the contents of total phenols and flavonoids of plant-based samples.[3, 4] These could be due to the fact that the hydrolytic enzymes (like glycosidases and tannase) produced by LAB during fermentation released more bound and complex phenolic compounds or transformed them into simpler forms.[5, 6] Moreover, the low pH environment created by LAB fermentation may also help avoid phenolic acid degradation.[7, 8] Notably, total phenolic and flavonoid contents were lower in SFT than in LFT, which may be due to the transformation or degradation of several readily degradable or thermally unstable phenols and flavonoids upon sterilization,[9] suggesting that LFT may be more bioactive and healthful than SFT.

2.2 Changes in antioxidant activities of tomato before and after fermentation

It is well known that antioxidant activities contribute to the alleviation or suppression of oxidative stress in the body, which helps to reduce the risk of cancer, inflammation, and metabolic disorders.[10] In this current study, $ABTS^{+}\cdot$, $DPPH\cdot$, and $\cdot OH$ radical scavenging capacities were determined to investigate the effects of fermentation on the antioxidant activity of tomato. As depicted in Fig. S3, $ABTS^{+}\cdot$, $DPPH\cdot$, and $\cdot OH$ radical scavenging rates were notably higher in LFT compared with UFT ($P < 0.05$), and sterilization had no remarkable effect on the scavenging rate except for that of $DPPH\cdot$, demonstrating that LP fermentation improved the antioxidant activity of tomato. Previous studies have pointed out that phenols and flavonoids are natural antioxidants and fermentation facilitates the release of simpler forms of free phenols, which help to improve the antioxidant activity of foods.[6, 10] The findings of this study support this notion, as the contents of total phenols and flavonoids of tomato were significantly increased after fermentation, which partly explained why LFT exhibited enhanced antioxidant activity than UFT. In addition, LAB also had been reported to possess antioxidant activity, and this could also account for the higher antioxidant activity of LFT.[11-13]

References

1. H. Liu, Y. Ni, Q. Yu and L. Fan, Evaluation of co-fermentation of L. plantarum and P. kluyveri of a plant-based fermented beverage: Physicochemical, functional, and sensory properties, *Food Res. Int.*, 2023, **172**, 113060.
2. F. Shi, L. Wang and S. Li, Enhancement in the physicochemical properties, antioxidant activity, volatile compounds, and non-volatile compounds of watermelon juices through Lactobacillus plantarum JHT78 fermentation, *Food Chem.*, 2023, **420**, 136146.
3. J. Nie, X. Fu, L. Wang, J. Xu and X. Gao, Impact of Monascus purpureus fermentation on antioxidant activity, free amino acid profiles and flavor properties of kelp (Saccharina japonica), *Food Chem.*, 2023, **400**, 133990.
4. Z. Li, Y. Dong, Y. Zhang, M. Zheng, Z. Jiang, Y. Zhu, S. Deng, Q. Li and H. Ni, Lactobacillus-fermentation enhances nutritional value and improves the inhibition on pancreatic lipase and oral pathogens of edible red seaweed Bangia fusco-purpurea, *LWT - Food Sci. Technol*, 2023, **179**, 114643.
5. J. Meng, J.-L. Wang, Y.-P. Hao, M.-X. Zhu and J. Wang, Effects of Lactobacillus fermentum GD01 fermentation on the nutritional components and flavor substances of three kinds of bean milk, *LWT - Food Sci. Technol*, 2023, **184**, 115006.
6. Z. Wang, Y. Feng, N. Yang, T. Jiang, H. Xu and H. Lei, Fermentation of kiwifruit juice from two cultivars by probiotic bacteria: Bioactive phenolics, antioxidant activities and flavor volatiles, *Food Chem.*, 2022, **373**, 131455.

7. H. Xie, P. Gao, Z.-M. Lu, F.-Z. Wang, L.-J. Chai, J.-S. Shi, H.-L. Zhang, Y. Geng, X.-J. Zhang and Z.-H. Xu, Changes in physicochemical characteristics and metabolites in the fermentation of goji juice by Lactiplantibacillus plantarum, *Food Biosci.*, 2023, **54**, 102881.

8. M. Friedman and H. S. Jürgens, Effect of pH on the Stability of Plant Phenolic Compounds, *J. Agric. Food Chem.*, 2000, **48**, 2101-2110.

9. Q. Hong, G. Chen, Z. Wang, X. Chen and J. Kan, Effects of different thermal processing methods on bioactive components, phenolic compounds, and antioxidant activities of Qingke (highland hull-less barley), *Food Sci. Hum. Wellness*, 2023, **12**, 119-129.

10. E. Martinelli, D. Granato, L. Azevedo, J. E. Gonçalves, J. M. Lorenzo, P. E. S. Munekata, J. Simal-Gandara, F. J. Barba, C. Carrillo, M. S. Riaz Rajoka and L. Lucini, Current perspectives in cell-based approaches towards the definition of the antioxidant activity in food, *Trends Food Sci. Technol.*, 2021, **116**, 232-243.

11. F. Ghiasi, S. M. B. Hashemi and E. Abedi, Effective enhancement of food oxidative stability induced by Lactobacillus strains: In vitro activity, *Food Control*, 2023, **153**, 109912.

12. S. Wu, Y. Chen, Z. Chen, Q. Zhou, F. Wei, P. Li and Q. Gu, Antioxidant properties and molecular mechanisms of Lactiplantibacillus plantarum ZJ316: A potential probiotic resource, *LWT - Food Sci. Technol*, 2023, **187**, 115269.

13. X. Xu, Y. Qiao, Q. Peng, V. P. Dia and B. Shi, Probiotic activity of ropy Lactiplantibacillus plantarum NA isolated from Chinese northeast sauerkraut

and comparative evaluation of its live and heat-killed cells on antioxidant activity and RAW 264.7 macrophage stimulation, *Food Funct.*, 2023, **14**, 2481-2495.