involved experimentation to evaluate the antimicrobial effects of LPOS-containing films and coatings. Researchers quantify microbial counts, monitor the shelf life extension of packaged goods, and assess the overall impact on product quality and safety. Furthermore, these materials are part of ongoing efforts to develop sustainable and eco-friendly packaging options. Incorporating lactoperoxidase into packaging materials aligns with the aim of reducing food waste by preserving perishable items for longer periods. Overall, LPOS-containing films and coatings represent an innovative approach in food packaging technology, leveraging the natural antimicrobial properties of lactoperoxidase to safeguard products and contribute to the development of more efficient and sustainable packaging solutions. Table 4 indicates the stability and antibacterial characteistics of LPOS before and after using various specific films and coatings (Gruden et al., 2023; Magacz et al., 2023).

Table 4: The stability and antibacterial properties of LPOS with/without different coatings and films

Pathogenes	**Impacts**	**Films and Coatings Containing Lactoperoxidase**	**References**
Escherichia coli	LPOS showed the greatest reduction of bacteria		Al-Baarri et al 2019
S. mutans *Lactobacilli*	The LPO-system-lozenge inhibiting plaque regrowth and reducing cariogenic bacteria		Welk et al 2021
Salmonella typhimurium DT 104	Inhibitory effect against bacteria	Soybean meal + LPOS	Lee and Min 2013
Salmonella enterica *E. coli* O157:H7	The films inhibited *S. enterica* and *E. coli* O157:H7	Whey protein films + LPOS	Min et al 2005a
L. monocytogenes	Whey protein coating containing LPOS inhibited *L. monocytogenes* in smoked salmon	LPOS+whey protein coating	Min et al 2005b
Pseudomonas spp. *Shewanella spp*.	Chitosan-LPOS films had the highest inhibitory activity against all bacterial growth	Chitosan-LPOS coating	Ehsani et al 2020
Gram-negative and Gram-positive bacteria	LPOS with essential oil to achieve an antibacterial activity for both Gram-negative and Gram-positive bacteria. The lowest count of *L. monocytogenes* was observed	Whey protein coating +LPOS (5% v/v) + Bunium persicum essential oil	Saravani et al. 2019
E. coli (NRRL B-3008), *Listeria innocua* (NRRL B-33314), *Pseudomonas fluorescens* (NRRLB-253)	The LPOS inhibited *L. Innocua* & *P. Fluorescens* for 24-h incubation. The most resistant bacteria to LPOS were *E. coli* and *P. fluorescens*.	Alginate-LPOS coating	Yener et al 2009
Enterobacteriaceae, *Pseudomonas aeruginosa*, aerobic mesophilic bacteria	The antimicrobial activity of coating containing LPOS was attributed to the interaction of $OSCN^-$ and HOSCN with SH groups of proteins in the cell membrane	Alginate-LPOS coating	Yousefi et al 2018
Aerobic mesophilic bacteria, *Enterobacteriaceae*, and *P. aeruginosa*	LPOS enhanced an antibacterial activity	Whey protein+alginate +lactoperoxidase	Molayi et al 2018
S. putrefaciens *P. fluorescens*	Whey protein coating + LPOS + MAP showed lowest bacterial count.	Whey protein coating + LPOS + MAP	Rostami et al 2017

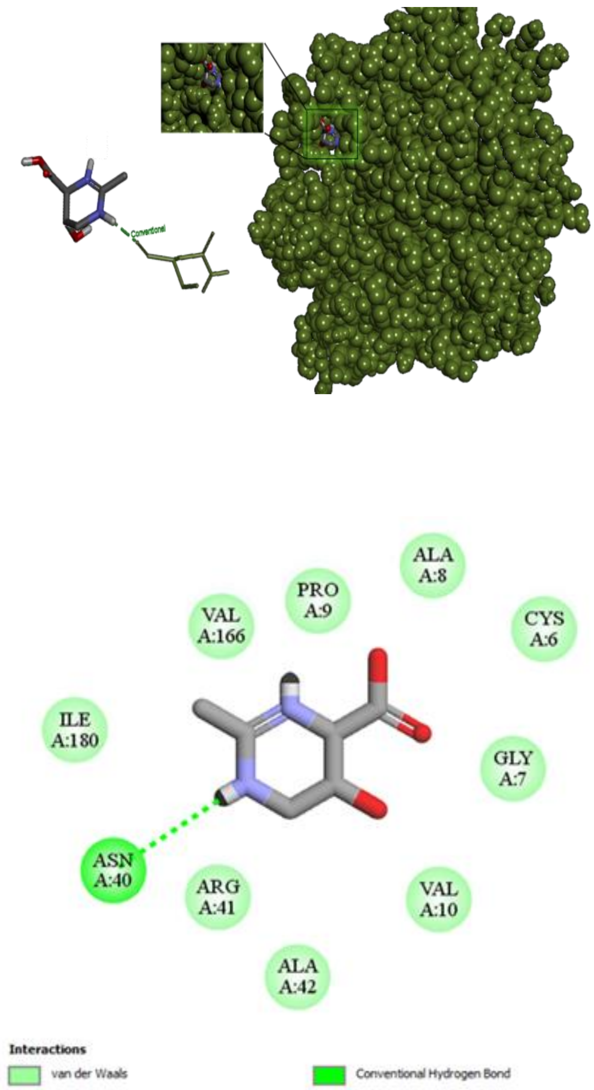
Continue table 4

Aeromonas hydrophila ATCC 7966, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatis RUT,* *Bacillus subtilis* IMG 22, *Pseudomonas pyocyanea,* *Bacillus subtilis var. niger* ATCC 10, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 15753, *Bacillus brevis* FMC3, *Klebsiella pneumoniae* FMC 5, *Corynebacterium xerosis* UC 9165, *Bacillus cereus* EU, *Bacillus megaterium* NRS, *Yersinia enterocolytica,* *Listeria monocytogenes* scoot A, *Bacillus megaterium* EU, *Bacillus megaterium* DSM32, *Klebsiella oxytocica,* *Staphylococcus aerogenes,* *Streptococcus faecalis,* *Mycobacterium smegmatis* CCM 2067	The LPO--100 mM thiocyanate--100 mM H_2O_2 system was effective agent against many of the organisms	Bovine LPO	Uğuz and Ozdemir 2005
Brevibacillus centrosaurus, *B. choshinensis,* *B. lyticum,* *Cedecea davisae,* *Chryseobacterium indoltheticum,* *Clavibacter michiganense pv. insidiosum,* *Kocuria erythromyxa,* *K. kristinae,* *K. rosea,* *K. varians,* *Paenibacillus validus,* *Pseudomonas syringae pv. populans,* *Ralstonia pickettii,* *Rhodococcus wratislaviensis,* *Serratia fonticola,* *Streptomyces violaceusniger,* *Vibrio cholerae-nonO1*	LPO system has inhibition effects on all type bacteria	Bovine lactoperoxidase (LPO)	Cankaya et al 2010

6. Docking of hydroxyectoine into the binding site of LPO

The Auto dock tools (ADT) are applied for molecular docking of proteins and enzymes. The crystal structure of LPO from RCSB Protein Data Bank (PDB) is used for further analysis. The three-dimensional structure of hydroxyectoine (as a ligand) is retrieved as the MOL_2 format from ZINC library. Docking of hydroxyectoine into the binding site of LPO has been performed with Auto Dock 4.0 Software. LGA (Lamarckian Genetic Algorithm) has been used to perform a hundred runs of docking. Docking study can predict the exact binding site for hydroxyectoine on the enzyme. The predicted binding models for LPO-hydroxyectoine complex with the lowest docking energy is shown in (Fig. 3a). As shown, there was a single binding site for hydroxyectoine on LPO that confirms the fluorescence spectroscopy outcomes. The 3D structural models of the LPO- hydroxyectoine complex showed that Asn 40 of LPO join the hydrogen bonding interaction with the hydroxyectoine. Moreover, van der Waals interactions also exist (Fig. 3b). The docking results were in coincident with fluorescence studies that showed static quenching mechanism (Borjian-Boroujeni et al. 2021; Borjian-Boroujeni et al., 2018).

Figure 3: Docking of hydroxyectoine in the binding site of LPO (a), The involved amino acid in formation of H-bond (dash-line) and hydrophobic interaction in LPO-hydroxyectoine complex (b) (Borjian-Boroujeni et al., 2021; 2018).



A

B

8. Conclusion

This study elucidated the potential application of lactoperoxidase, a naturally occurring antimicrobial enzyme found in the LPOS, as a viable substitute for chemical preservatives in order to enhance the overall quality, activity and stability of LPO. The antimicrobial effect of LPO is hypothesized to result from the oxidation of sulfhydryl (SH) groups found in microbial proteins and enzymes. This oxidation process involves the formation of intermediate oxidizing products, which subsequently triggers alterations in various cellular functions, including membrane integrity, passage systems, and metabolic enzymes. This system exhibits bacteriostatic and bactericidal capabilities against Gram-positive and Gram-negative microorganisms, consecutively. Moreover, it exhibits noteworthy antifungal and antiviral properties. LPOS-containing films and coatings has exhibited significant antimicrobial activity, leading to improved shelf-life of food products. However, the selection of film types and coatings incorporating LPOS was restricted to alginate, chitosan, gelatin, and whey protein. Ectoine, an osmolyte, affects LPO, finding higher ectoine concentrations improved LPO's activity, binding to LPO influenced its stability and thermodynamics, showcasing ectoine's potential as a stabilizing agent for lactoperoxidase in industrial and medical contexts. Also, the presence of stabilizers appears to enhance the bactericidal efficacy of the enzyme by increasing its enzymatic activity. This observation indicates that the immobilization process offers hope in terms of stabilizing enzymes and managing their inherent instability. Consequently, additional investigations are imperative to assess the antimicrobial attributes of alternative categories of edible films and coatings that encompass LPOS. The results of kinetic studies of LPO can be used for comparative analysis of the properties of lactoperoxidases from different animal species, which will contribute to the development and the introduction of new ways of using these enzymes in the food and pharmaceutical industries as well as for quality control in the production of food and cosmetic products containing LPO. Furthermore, more research is needed to explore the potential effectiveness of the LPOS in combination with other natural antimicrobial agents and hurdle technology.

Author contribution:

All authors contributed to the study's conception and design. Marziyeh Borjian Boroujeni and Mahmoud Reza Aghamaali, Hashem Nayeri, and Keyvan Beheshti Maal experimented. Marziyeh Borjian Boroujeni wrote the manuscript with support from Mahmoud Reza Aghamaali, Hashem Nayeri, and Keivan Beheshti Maal. Hashem Nayeri and Keyvan Beheshti Maal conceived the original idea. Mahmoud Reza Aghamaali supervised the project.

Conflict of interest

The authors declare no conflict of interest.

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