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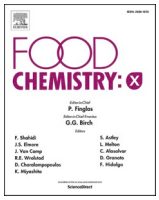

Mechanism of polyhydroxy alcohol-mediated curing on moisture migration of minced pork tenderloin: On the basis of molecular docking

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ABSTRACT

This study investigated the mechanism of glycerol, xylitol, and sorbitol-mediated curing of cured minced pork tenderloin. The use of polyhydroxy alcohol during mediated curing significantly reduced the salt content ($p < 0.01$) and water activity (aw) of the cured pork tenderloin. Low-field nuclear magnetic resonance (LFNMR) revealed that 1 % glycerol, 1 % xylitol, 1 % sorbitol, and 10 % glycerol-mediated curing decreased water mobility, and improved water holding capacity (WHC), and produced uniform dense microstructures. Raman spectroscopy and molecular docking indicated that polyhydroxy alcohols formed hydrogen bonds with myosin, as well as hydrogen bonds with free water molecules to convert free water into bound water to reduce aw, and altered the hydrophobic environment of myosin surface to reduce structural damage caused by high salt content. In conclusion, using polyhydroxy alcohol to mediate curing can effectively reduce the salt content of cured meat and provide a theoretical basis for its application in the cured meat industry.

1. Introduction

Sodium chloride (NaCl) is an important food processing ingredient widely used in cured meat products as a marinade. Most cured meat products contain high levels of NaCl to reduce water activity (aw) and provide unique organoleptic characteristics (Desmond, 2006). However, the World Health Organization (WHO) recommends that adults limit their daily sodium intake to no more than 2 g (5 g NaCl). An excessive NaCl intake increases the risk of osteoporosis, cardiovascular disease, stroke, and other diseases in severe cases (Du, Wang, Zhang, & Popkin, 2020; Leyvraz et al., 2018). Therefore, there is an urgent need to reduce the content of NaCl in foods. The methods for reducing NaCl in cured meats have been reported in some studies, which included utilizing potassium salt instead of sodium salt (Vidal, Lorenzo, Munekata, & Pollonio, 2021), using natural flavor enhancers such as yeast extract and monosodium glutamate (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017), changing NaCl crystals to various size and shapes (Rios-Mera et al., 2019; Rios-Mera, Selani, Patinho, Saldaña, & Contreras-Castillo, 2021), and using emerging non-thermal processing technology, including ultra-high pressure (Bolumar et al., 2021) and pulsed electric field (Arshad et al., 2021). However, the application and actual effects of the above methods are limited. There are only a few studies on exogenous additions in the curing system that can alter the curing process, aside from meat matrix and traditional curing agents.

Mediated curing is the systematic development of low-sodium cured meat products using exogenous food additives as the medium to influence salt permeation and diffusion and moisture migration behavior in the matrix (Gong et al., 2022). Polyhydroxy alcohols (Chen, Li, Song, Weng, & Zhang, 2012; Liu, Wan, et al., 2022) can combine with NaCl as penetrants during low-salt curing; they can reduce the final salt content of meat products by reducing the water activity of the meat and inhibiting the rate of salt diffusion. Glycerol can enhance mass transfer and significantly reduce the water activity of cured meat products while maintaining the edible quality and safety (Semenoglou, Dimopoulos, Tsironi, & Taoukis, 2020). Iseya, Kubo, and Saeki (2000) found that Japanese squid cured in 0.5–1.5 M sorbitol significantly reduces critical moisture content and prevents excessive hardening.

Current work on polyhydroxy alcohol mostly focuses on quality and

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drying. Moreover, there are only a few studies on the intrinsically relevant molecular behavior and mechanisms of moisture migration and exogenous substance behavior mediators during curing. Molecular docking techniques have been widely used to examine the structural characteristics of dietary proteins (Vidal-Limon, Aguilar-Toalá, & Liceaga, 2022), mainly to investigate the interaction between small molecular ligands and biological macromolecules (Liu, Wei, et al., 2022). Eslami-Farsani, Shareghi, Farhadian, and Momeni (2020) reported that glycerol binds to myoglobin via hydrogen bonds; this interaction may alter the structure of myoglobin. The hydroxyl groups of xylitol participate in hydrogen bonding with bovine serum albumin (BSA), strongly regulate the three-dimensional spatial position of xylitol in the binding pocket, and facilitate other forces' action (Kong et al., 2020). However, studies on mediators exhibiting muscle protein interactions in polyhydroxy alcohol-mediated curing are scarce.

The aim of this study was to evaluate the moisture migration and mechanism of polyhydroxy alcohol-mediated curing. The effect of different polyhydroxy alcohols on the moisture migration of cured minced pork tenderloin was explored by measuring moisture content, aw, salt content, water holding capacity (WHC), and low-field nuclear magnetic resonance (LFNMR) technique. In addition, Raman spectroscopy and molecular docking were used to investigate the effects of different polyhydroxy alcohols on muscle protein structure and binding mechanisms. This work can contribute to expanding the classic curing theory and the investigation of the polyhydroxy alcohol-mediated curing process.

2. Materials and methods

2.1. Materials

Pork (Guizhou local pig breed, China) tenderloin was provided by Fuzhiyuan Food Co., Ltd. (Guizhou, China) and shipped fresh to the lab in ice boxes and used within 24 h for all investigations. The salt was purchased from Chongqing Hechuan Salt Chemical Co., Ltd. (Chongqing, China). Food-grade glycerol, sorbitol, and xylitol were purchased from Tyco Palm Chemical Co., Ltd. (Zhangjiagang, China), Shandong Tianli Pharmaceutical Co., Ltd. (Shandong, China), and Zhejiang Huakang Pharmaceutical Co., Ltd. (Zhejiang, China), respectively. Other chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China).

2.2. Cured minced pork tenderloin sample preparation

Each group was provided with 100 g of pork tenderloin, which had been sliced and processed using a mincer (MJ-LZ225, Midea Co., Guangzhou, China) for 1 min. The control group contained 4 % NaCl, while the experimental groups consisted of 4 % NaCl and glycerol (1 %, 10 %), xylitol (1 %, 10 %), and sorbitol (1 %, 10 %), respectively. The samples were vacuum packaged and incubated at 0–4 °C for 24 h to ensure that the curing agents fully penetrated the minced pork tenderloin and then analyzed within 24 h (Liu et al., 2022).

2.3. Moisture and aw measurements

The moisture content of the 3 g sample was weighed and measured at 24 ± 1 °C using a meat moisture tester (Guanya SFY-30, Shenzhen, China). Aw was determined after reaching equilibrium at 24 ± 1 °C using a portable aw meter (Huake HD-4B, Wuxi, China). The aw instrument was calibrated using saturated NaCl and saturated magnesium chloride solutions. The aw of the sample (5 g) was measured in the meter measurement chamber.

2.4. NaCl content and electronic tongue measurements

The NaCl content of each sample was determined in triplicate using a digital salt meter (ES-421, ATAGO, Tokyo, Japan) according to the method of Zhang, Zhang, Zhou, Wang, and Zhang (2021) with slight modifications. One gram of meat sample was diluted ten times with deionized water and homogenized for 10 s. The NaCl content was expressed in grams per 100 g of meat.

A total of 10 g of meat sample was mixed with 40 mL of distilled water (37 °C) and then homogenized at 3000 rpm for 60 s using a high-speed homogenizer (XHF-DY, Xinzhi Biotechnology Co., Ltd., Ningbo, China). The homogenate was centrifuged at 2000 g for 10 min at 4 °C, and the supernatant was filtered through three layers of gauze. To assess taste perception, 10 mL of filtrate was mixed with 50 mL of ultrapure water using the two-step cleaning method (Tian et al., 2022) of the SA402B electronic tongue (Insent, Kanagawa, Japan). The reference solution contained 0.3 mM tartaric acid and 30 mM potassium chloride.

2.5. Determination of WHC

The WHC of different polyhydroxy alcohols during mediated curing of pork tenderloin mince was determined by centrifugal losses according to the method described by Zhao et al. (2021) with slight modifications. The meat sample weighing approximately 5 g (W_1) was wrapped in filter paper and centrifuged for 10 min (4000 × g, 4 °C). The meat sample weighed after centrifugation was denoted as W_2. The centrifugal loss was calculated as the ratio of the lost weight to the total initial weight.

2.6. Texture profile analysis (TPA)

The samples (1 cm^3) were cooked in an 80 °C water bath pot, the surface moisture was then absorbed by filter paper, and the hardness and chewiness of the products were determined using a TA.TOUCH texture analyzer (Bao Sheng Technology Co., Ltd., Shanghai, China). The probe used was TA/36, and the parameters were set as follows: pre-test rate of 1 mm/s, test rate of 1 mm/s, post-test rate of 1 mm/s, and 50 % compression ratio.

2.7. LF NMR and magnetic resonance imaging (MRI) measurements

The moisture distributions were determined using an LF NMR analyzer (NMI20-040 V-I, Niumag Analytical Instrument Corporation, Suzhou, China) having a proton frequency of 21 MHz at 32 °C. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to assess the spin–spin relaxation time of a 1 g sample in a 25 mm diameter NMR tube (T_2) (Huang, Shi, Ren, Hao, & Weng, 2022). The parameters were set as follows: SF_1 = 22 MHz. NS = 8, TW = 3000 ms, NECH = 6000.

The spatial distribution of water in meat samples was analyzed using MRI. MRI sequences were obtained from hydrogen proton density imaging. The measurement conditions were: SF1 = 22 MHz, TW = 3000 ms, echo time of 20 ms, and 50 mm longitudinal and 50 mm transverse field of view. Using Ramel's law ($V = \gamma B_0/2\pi$), the images were obtained, and then the signal-to-noise ratio was adjusted by a filtering process, and the final images were processed with pseudo-color.

2.8. Scanning electron microscope (SEM) measurements

The microstructure of each sample was measured according to the method of Zhang et al. (2020) with slight modifications. Meat samples were cut into 1 mm × 10 mm × 10 mm slices, fixed in 2.5 % glutaraldehyde solution, washed with 0.1 mmol·L^{-1} phosphate buffer (pH 7), and placed at room temperature (24–25 °C) for 2 h. The samples were washed with distilled water, followed by two times of gradient dehydration with different concentrations of 25 %, 50 %, 70 %, 95 %, and anhydrous ethanol for 1 h each time. The samples were frozen and fractured in liquid nitrogen and then dried in a freeze dryer. Next, gold was sprayed, and then observed and photographed using an SEM (COXEM EM-30, Seoul, Korea) with an acceleration voltage of 20 kV.

2.9. Raman spectra measurements

The Raman spectra were collected using a high-resolution Raman spectrometer/microscope (Lab RAM HR EVO, Horiba Jobin Yvon S.A.S, Paris, France) with a 532 nm laser source. The measurement of the cured pork mince was done by cutting 1 mm × 10 mm × 10 mm slices from the center and placing them on a slide. The laser was first focused on the sample with a 50 × long focal length lens, and then Raman signals were acquired in the range of 400–2000 cm^{-1}. The measurement parameters were: the resolution of 2 cm^{-1}, exposure time of 60 s, and 5 scans for each sample. The obtained spectra were smoothed using Labspec6 analysis software with multi-point baseline correction to remove the fluorescent background. The spectra were normalized to the vibrational intensity of the phenylalanine ring at a 1003 cm^{-1} wave number (Pan, Guo, Li, Song, & Ren, 2017).

2.10. Myosin model prediction and molecular docking

To investigate the intermolecular interactions between the major proteins in muscle and exogenous substances, a three-dimensional structural model of the major proteins in muscle needs to be established. The main muscle protein, myofibrillar protein, is complex and large in size. Hence, myosin, the main protein in myofibrillar protein, was chosen as the modeling object. The amino acid sequence Q9TV61 of myosin was obtained by searching the database UniProtKB. This sequence was then used to screen the template proteins 6ysy (96.1 %) and 2mys (74.8 %) with the best homology in the NCBI database using Protein BLAST. The myosin head model construction was done using Modeller 10.1 (University of California, San Francisco, CA), while the myosin tail model was constructed using a special modeling program named CCBuilder 2.0 (coiledcoils.chm.bris.ac.uk/ccbuilder2/builder) since it has a particular superhelix structure (Wood & Woolfson, 2018). The model was then optimized using GROMACS 2018.8 software, and model evaluation was performed using ERATT and Ramachandran plot. The three-dimensional structures of glycerol, xylitol, and sorbitol were obtained by searching the database PubChem (https://pubchem.ncbi.nlm.nih.gov/) and the structure and energy optimization were completed using Gaussian 09 software.

Molecular docking was used to investigate the interactions between myosin and polyhydroxy alcohol using AutoDock Vina software (Scripps 177 Research Institute, La Jolla, CA, USA). Gasteiger charges and missing hydrogen atoms were added to the protein using AutoDockTools-1.5.7. The "Lamarckian genetic algorithm" was chosen to search for docking conformations, and the maximum number of docked conformations was chosen as 9. After the docking was completed, the best docking conformation was evaluated and determined based on the combined score of the scoring function and its root mean square deviation (RMSD). The results were visually analyzed using Discovery Studio 2019 (Accelrys Inc., San Diego, CA, USA) to obtain the confirmation of the 2D and 3D interaction modes.

2.11. Statistical analysis

The values are presented as the mean ± standard deviation for all the experiments. Statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software Inc., California, USA) by one-way ANOVA. The correlations were further analyzed using Origin 2021 (OriginLab Corporation, MA, USA).

3. Results and discussion

3.1. Moisture and aw analysis

Fig. 1a shows the changes in moisture content of minced pork tenderloin with different concentrations of polyhydroxy alcohols. The addition of 1 % glycerol, xylitol, and sorbitol reduced the moisture content of minced pork tenderloin. Polyhydroxy alcohols are commonly used as dehydrating agents in food. A ternary solution of salt-water-alcohol can produce stronger osmosis than a binary solution of salt-water, rapidly stripping free water from the interior of cured meat (Cui et al., 2013). The addition of 10 % glycerol reduced the moisture content of the minced pork tenderloin, but that of 10 % xylitol and sorbitol increased it. The reason may be that xylitol and sorbitol have significant hygroscopic properties. As salt continues to penetrate, the osmotic pressure between cells increases. The high concentration of xylitol and sorbitol can strongly bind the water escaping from the meat due to the osmotic pressure generated by salt (Jang et al., 2015).

The aw reflects the water-binding degree of minced pork tenderloin, and a low aw value indicates a high binding degree. As shown in Fig. 1b, adding different polyhydroxy alcohols can reduce the aw of minced pork tenderloin. The addition of 10 % polyhydroxy alcohols had a significant ability to reduce the aw ($p < 0.0001$). The reason may be that polyhydroxy alcohol contains a large number of hydroxyl groups, which can combine with protein and fat in cured minced pork tenderloin to

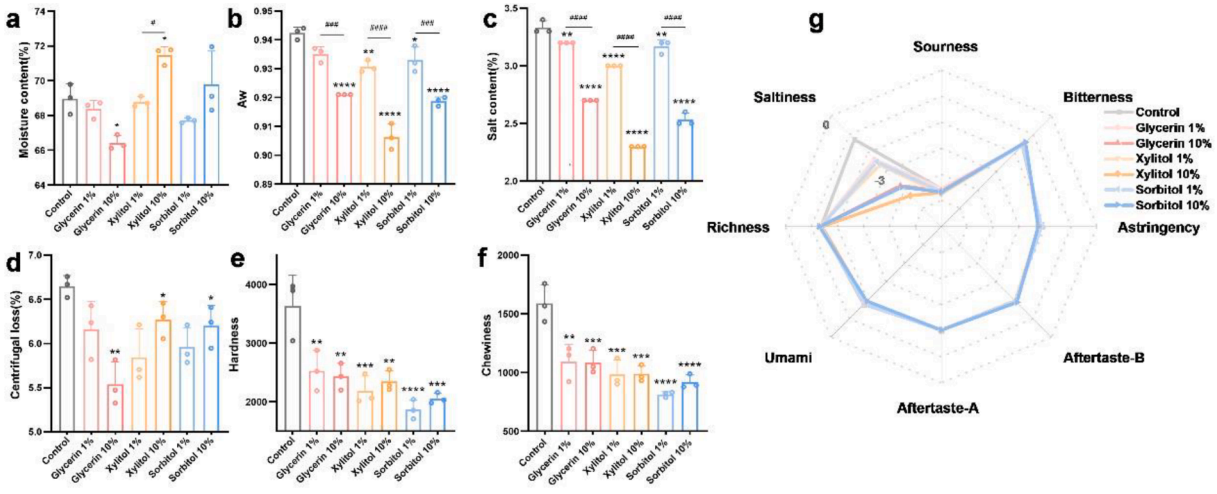

Fig. 1. Effects of different polyhydroxy alcohol-mediated curing on water content (a), aw (b), salt content (c), WHC (d), hardness (e), chewiness (f), and electronic tongue sensory score (g) of minced pork tenderloin (mean ± SD, n = 3). Statistical differences were determined using one-way ANOVA with Tukey's test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ as control group compared with different polyhydroxy alcohol group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ and $^{\#\#\#\#}p < 0.0001$ as 1 % polyhydroxy alcohol compared with 10 % polyhydroxy alcohol).

increase the polarity of some groups, and turn some free water into bound water, thus reducing the aw (Chai et al., 2022; Chen, Liu, Chen, & Ockerman, 2000). Among the three different polyhydroxy alcohols, xylitol and sorbitol showed a stronger ability to reduce aw than glycerol. Xylitol exhibited the strongest ability to reduce aw. This can be attributed to a large number of hydrophilic hydroxyl groups in it that decreases the free water activity of the cured ground pork loin, thus reducing aw. Moreover, since the molecular weight of sorbitol (182.17 Da) is larger than that of xylitol (158.15 Da), its osmotic effect with the ternary solution formed by water and salt is smaller, which may be the reason for the stronger aw reduction effect of xylitol-mediated curing than that of sorbitol (Chen et al., 2012).

3.2. NaCl content and electronic tongue analysis

Fig. 1c shows the effect of adding different concentrations of polyhydroxy alcohol-mediated curing on salt content. The addition of different concentrations of polyhydroxy alcohols significantly reduced the salt content of cured meat ($p < 0.01$). Moreover, the salt reduction effect by the addition of a 10 % concentration of glycerol, xylitol, and sorbitol was significant ($p < 0.0001$), and xylitol had the best effect on reducing salt content. This decrease in salt content may be due to several alcohol-water hydrogen bonds formed in the ternary solution of higher molecular weight polyhydroxy alcohols containing water and salt instead of water-water hydrogen bonds, which prevent the diffusion of salt in osmotic solvents (Chen et al., 2012; Dimakopoulou-Papazoglou & Katsanidis, 2016). At the same time, a high concentration of alditol solution can be adsorbed on the surface of cured meat to form a "barrier effect" to prevent salt infiltration (Gong et al., 2022).

The electronic tongue sensory score based on the taste sensing system for minced pork tenderloin with different polyhydroxy alcohols during mediated curing is shown in Fig. 1g. The only difference between different treatment groups was in terms of saltiness. Consistent with the results of the salt content, the 10 % xylitol-mediated group tasted the least salty, indicating that polyhydroxy alcohol-mediated curing could reduce the salt content of minced pork tenderloin.

3.3. WHC analysis

The effect of different polyhydroxy alcohol-mediated curing on the WHC of minced pork tenderloin was quantified by centrifugal loss. As depicted in Fig. 1d, the addition of 1 % of different polyhydroxy alcohols reduced the centrifugal loss of minced meat; polyhydroxy alcohol can bind the free water in the cured minced pork tenderloin, thus reducing the water activity, decreasing the water loss of meat caused by centrifugation, and improving the WHC of the cured minced pork tenderloin (Chai et al., 2022). However, for the curing group with 10 % different polyhydroxy alcohols, the change in WHC was different. The addition of 10 % glycerol obtained better WHC than that of 1 % glycerol. However, the centrifugal losses by the addition of 10 % xylitol and sorbitol were higher than that of 1 % xylitol and sorbitol. Han, Wang, Xu, and Zhou (2014) elucidated that the microstructure change during curing was closely related to the WHC of cured minced pork tenderloin. The changes in WHC in cured minced pork tenderloin mediated by high-concentration xylitol and sorbitol may be due to the destruction of protein structure caused by a large amount of xylitol and sorbitol, resulting in changes in microstructure. This could result in loss of water bound by high-concentration xylitol and sorbitol, and a decrease in WHC of cured minced pork mediated by 10 % xylitol and sorbitol.

3.4. TPA analysis

Hardness and chewiness are often used to evaluate texture. As depicted in Fig. 1e and f, the hardness and chewiness of minced pork tenderloin decreased with an increase in concentration. The addition of 10 % xylitol and sorbitol significantly reduced the hardness and chewiness of minced pork tenderloin; however, the reduction effect of the 1 % concentration group of polyhydroxy alcohol was greater than those of the 10 % concentration, suggesting that the addition of higher concentration of xylitol and sorbitol might have an adverse effect on the texture. This may be because the minced pork tenderloin with a high concentration of xylitol and sorbitol contains more moisture and loses more moisture after cooking, resulting in increased hardness and chewiness of the product (Chai et al., 2022).

3.5. LF NMR and MRI analysis

To investigate the reason for the alteration of water content and water activity by different polyhydroxy alcohols during mediated curing, LF NMR was used to characterize the water distribution and flow. As depicted in Fig. 2a, there are three different states of water in minced pork tenderloin, among which T_{2b} (0–10 ms) corresponds to bound water tightly bound with macromolecules, T_{21} (10–100 ms) represents immobilized water trapped in myofibrillar protein network, and T_{22} (100–1000 ms) refers to free water existing between myofibrillar bundles (Song et al., 2021). The ratio and relaxation time of bound water, immobilized water, and free water are shown in Fig. 2b and c. The free water ratio (PT_{22}) of the glycerol group is slightly lower than that of the control group, and T_{21} and T_{22} of the glycerol group decreased, indicating that glycerol-mediated curing reduced the free water content, increased the bound water content, and decreased the water activity of cured meat, which is consistent with the previous data of water activity. The water changes in the 1 % xylitol and sorbitol group were consistent with those in the glycerol group, but PT_{22} increased significantly in the 10 % concentration xylitol and sorbitol group, particularly in the 10 % xylitol group, where the percentage of free water reached 10 %. In addition, T_{2b}, T_{21}, and T_{22} of the 10 % xylitol and sorbitol group were higher than the 1 % xylitol and sorbitol group but lower than the control group, indicating that their water was transitioning to the free state. This may be because the high concentration of xylitol and sorbitol makes it difficult to penetrate the cured meat inside, and its attachment to the surface of cured meat, in conjunction with the continuous seepage of water molecules, forms a layer of xylitol and sorbitol-water binary solution on the surface of cured meat, preventing the seepage of water molecules. At the same time, high concentrations of xylitol and sorbitol weaken the ability of water molecules to bind to proteins, allowing and filling the gaps between protein molecules and binding to free water molecules in the cured meat, which may explain the decrease in water activity but increase in free water content (Bigelow & Lee, 2007).

MRI is a non-destructive method widely used to study the water distribution in food (Tan, Lin, Zu, Zhu, & Cheng, 2018). T_2 weighted imaging of MRI was used to evaluate the effect of different polyhydroxy alcohols during mediated curing on the water distribution of minced pork tenderloin. The red and blue colors in T_2 false-color images represent higher and lower intensities, respectively. As shown in Fig. 2b, compared to the control group, the 1 % and 10 % glycerol, 1 % xylitol, and 1 % sorbitol images were bluer, indicating a decrease in water content. However, the images of the 1 % xylitol and sorbitol group were redder than that of the control group, and there were more red dots within, indicating a higher water content than the control group. This result agrees with the previous data on moisture content and LF NMR.

3.6. Microstructure

Fig. 2c shows the microstructure of minced pork tenderloin after different polyhydroxy alcohols during mediated curing. Significant differences in surface morphology and texture were observed between the different polyhydroxy alcohol treatments, with the control group showing a non-uniform pore size and particle distribution and a coarser structure. The addition of 1 % and 10 % glycerol improved the microstructure of the minced meat, with the 1 % glycerol group having a more

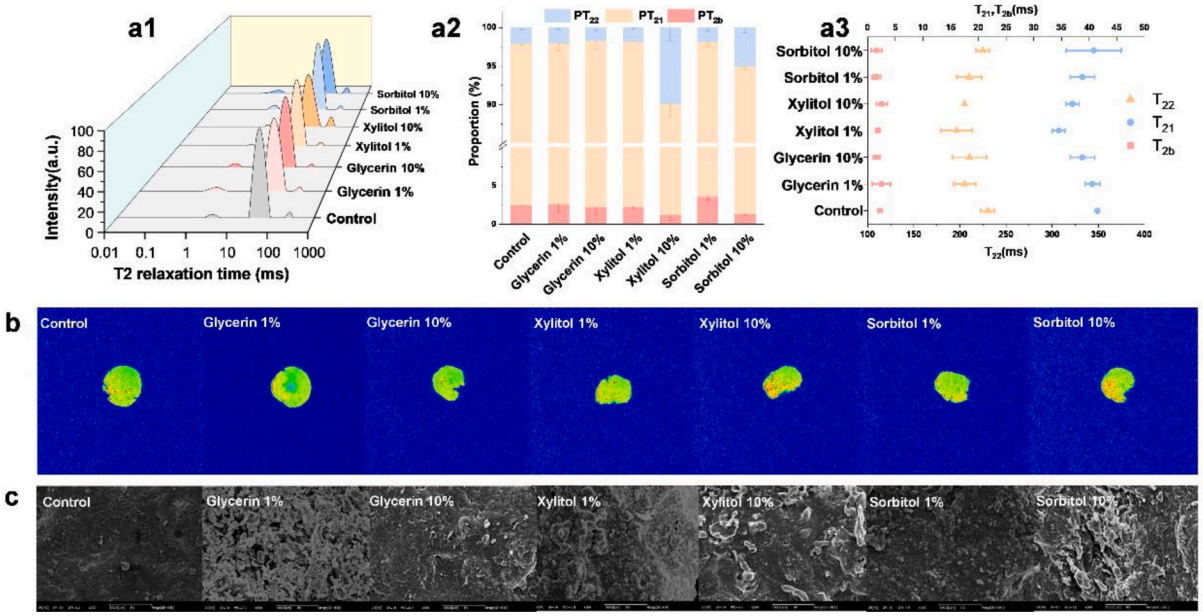

Fig. 2. The curve of T_2 relaxation time (a1), T_{21}, T_{2b}, and T_{22} relaxation percentage (a2) and time (a3), MRI images (b), SEM images (c) of different polyhydroxy alcohol-mediated curing of minced pork tenderloin.

uniform and fine texture. The addition of 1 % xylitol and sorbitol improved the microstructure of the minced meat and formed a gel with uniform particle distribution. In contrast, the microstructure of 10 % xylitol and sorbitol formed coarse aggregates, and the structure became rough and non-homogenous. This may be due to the viscosity barrier formed by the high concentration of xylitol and sorbitol that impedes the exchange of substances inside and outside the cured meat and weakens the ability of proteins and lipids to bind water molecules, affecting protein–protein interactions and thus the formation of a homogeneous gel structure (Walayat et al., 2022).

3.7. Raman spectroscopy analysis

Raman spectroscopy is often used to characterize structural changes in meat proteins. Fig. 3a shows Raman spectra of minced pork tenderloin mediated curing with different polyhydroxy alcohol. The Raman spectral bands of amide I (1600–1700 cm^{-1}) are used to measure the secondary structure. Different polyhydroxy alcohol-mediated curing had no significant effect on the secondary structure of meat protein. This indicates that different polyhydroxy alcohol-mediated curing did not change the helical structure of meat protein. The normalized intensity of proteins at 760 cm^{-1} (I_{760}) in the Raman spectrum is produced by the stretching vibration of the tryptophan ring, which measures the change in the tryptophan microenvironment. When the tryptophan residues transition out of an embedded hydrophobic microenvironment to an exposed polar environment, the intensity of I_{760} decreases, and the greater the degree of exposure, the more the I_{760} decreases (Herrero, 2008). As shown in Fig. 3b, 1 % of different polyhydroxy alcohol-mediated curing groups increased the strength of I_{760}. It indicates that tryptophan residues are embedded in a more hydrophobic microenvironment, and the combination of different polyols with protein protects the structure of meat protein. The 10 % glycerol-mediated curing group significantly increased the strength of I_{760} ($p < 0.001$). However, the I_{760} intensity of 10 % xylitol and sorbitol-mediated curing groups was lower than that of the control group, which indicated that tryptophan residues were exposed to water from the embedded hydrophobic environment. It indicates that the tertiary structure of the protein may be destroyed. This is consistent with the WHC and SEM results in this study.

3.8. Molecular docking analysis

ERRAT is commonly used to assess the quality of protein structures

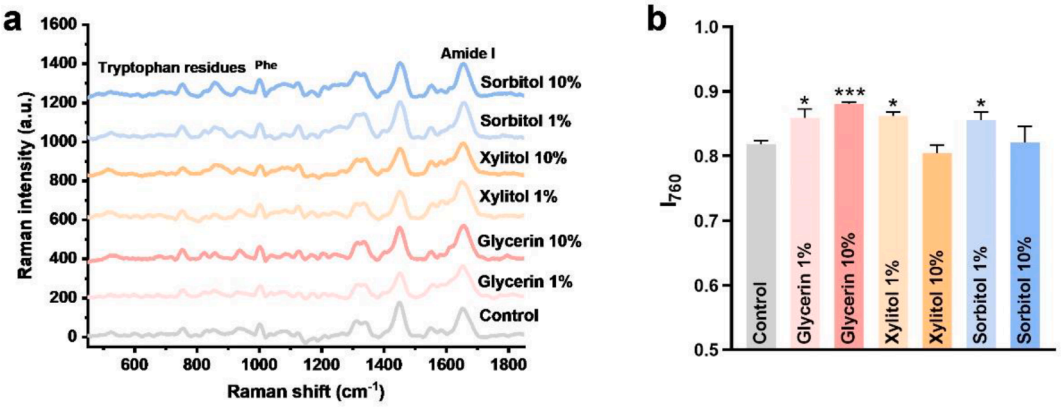

Fig. 3. Effects of different polyhydroxy alcohol-mediated curing on Raman spectra (400–2000 cm^{-1}) (a) and I_{760} normalized intensity of bands, (b) of minced pork tenderloin (mean ± SD, n = 3). Statistical differences were determined using one-way ANOVA with Tukey's test. *, $P < 0.05$; ***, $P < 0.001$.

(Bowie, Lüthy, & Eisenberg, 1991). Fig. 4a1 and b1 show the overall quality factors of the myosin head and tail models. ERRAT scores were 95.238 and 97.106. PROCHECK describes the protein dihedral angle of amino acid residues in the structure of the mass ψ and φ within a reasonable range (Laskowski, MacArthur, Moss, & Thornton, 1993). In addition, it can also reflect whether the conformation of the protein is reasonable or not. The results of the PROCHECK evaluation were shown through the Ramachandran diagram. As shown in Fig. 4a2, the Ramachandran plot for the myosin head model displayed that 92.3 % of residues were in the permissive region, and only 0.5 % of residues were in the forbidden region. The dihedral angle of 99.5 % amino acid residues in the MHC head model was within a reasonable range. The Ramachandran plot for the myosin tail model (Fig. 4b2) showed that 100 % of the amino acid residues were in the permissive region. The above evaluation diagram showed that the three-dimensional model of myosin (Fig. 4a3 and b3) constructed in this experiment had high quality and reasonable spatial structure. Thus, it can be used for molecular docking research.

Molecular docking was used to predict the binding sites of polyhydroxy alcohol-myosin interactions to analyze the main molecular mechanism of action of proteins in minced pork tenderloin cured with different polyhydroxy alcohols. The best docking poses of glycerol-myosin, xylitol-myosin, and sorbitol-myosin are outlined in Fig. 5a–f. As reported in Fig. 5a1–a4 and d1–d4, the binding energy of the myosin head and glycerol was −4.8 kJ/mol^{-1}, and the amino acid residues Ser181, Gly184, Glu180, Ser245, and Gly467 of myosin head interacted with glycerol through H-bonds, and the corresponding distances were 3.64, 2.42, 2.72, 1.98, and 2.84 Å. The binding energy of the myosin tail and glycerol was −3.6 kJ/mol^{-1}. The AspB:194, GluB:197, and GluB:201 residues of the myosin tail were the main contributors to the formation of H-bonds with glycerol, and the corresponding distances were 2.27, 2.64, and 2.15 Å. As reported in Fig. 5b1–b4 and e1–e4, the binding energy of the myosin head and xylitol was −5.0 kJ/mol^{-1}, and the myosin head interacted with xylitol using residues Lys273, Ser274, Asp598, Lys601, and Asp602 through H-bonds, and the corresponding distances were 4.47, 3.49,5.10,5.31, and 3.74 Å. In addition, an acceptor–acceptor interaction was observed between xylitol and the Ser274 residue of the myosin head, which was unfavorable for binding. The binding energy of the myosin tail and xylitol was −4.1 kJ/mol^{-1}. The AspB:195, GluB:197, and GluB:201 residues of the myosin tail were the main contributors to the formation of H-bonds with glycerol, and the corresponding distances were 3.45, 4.19, and 4.64 Å. As reported in Fig. 5c1–c4 and f1–f4, the binding energy of the myosin head and sorbitol was −5.2 kJ/mol^{-1}, and the myosin head interacted with sorbitol using residues Ser274, Glu477, and Lys599 through H-bonds, and the corresponding distances were 2.25, 2.41, 2.38, 2.02, and 2.05 Å. The binding energy of the myosin tail and sorbitol was −4.9 kJ/mol^{-1}, and the myosin tail interacted with sorbitol using GluA:197 and GluB:201 through H-bonds, and the corresponding distances were 5.02 and 4.78 Å. Similar to xylitol, sorbitol produced unfavorable acceptor–acceptor interactions with Lys273 and Lys432 in the head of the myosin and with GluA:197 and AspB:195 in the tail of myosin.

The analysis of the docking results shows that the main force generated by the different polyhydroxy alcohols with myosin is hydrogen bonding. Polyhydric alcohol can reduce the damage caused by salt ions to the protein structure and stabilize the protein structure. Several studies have shown that polyhydroxy alcohol can form many hydrogen bonds with protein to protect protein (Chen, Zhang, Hemar, Li, & Zhou, 2020; Eslami-Farsani et al., 2020; Mohammadi, Shareghi, Farhadian, & Saboury, 2021). The binding energy of the head was higher than that of the tail, indicating that different polyhydroxy alcohols could bind more tightly to the myosin head. It indicated that the myosin head was the main binding site for different polyhydroxy alcohols. At the same time, the combination of xylitol and sorbitol with

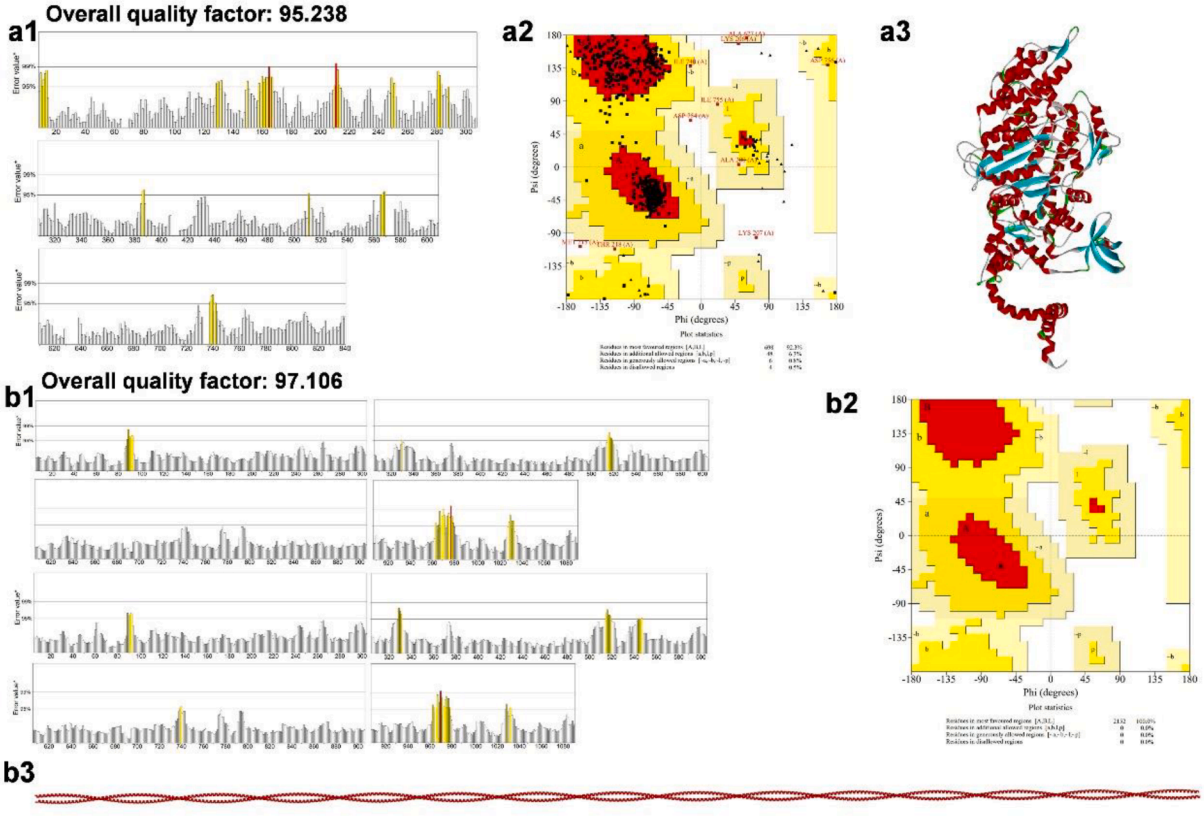

Fig. 4. ERRAT analysis for the myosin head (a1) and myosin tail (b1), Ramachandran plots for the myosin head (a2) and myosin tail (b2). Three-dimensional structure of the myosin head (a3) and myosin tail (b3).

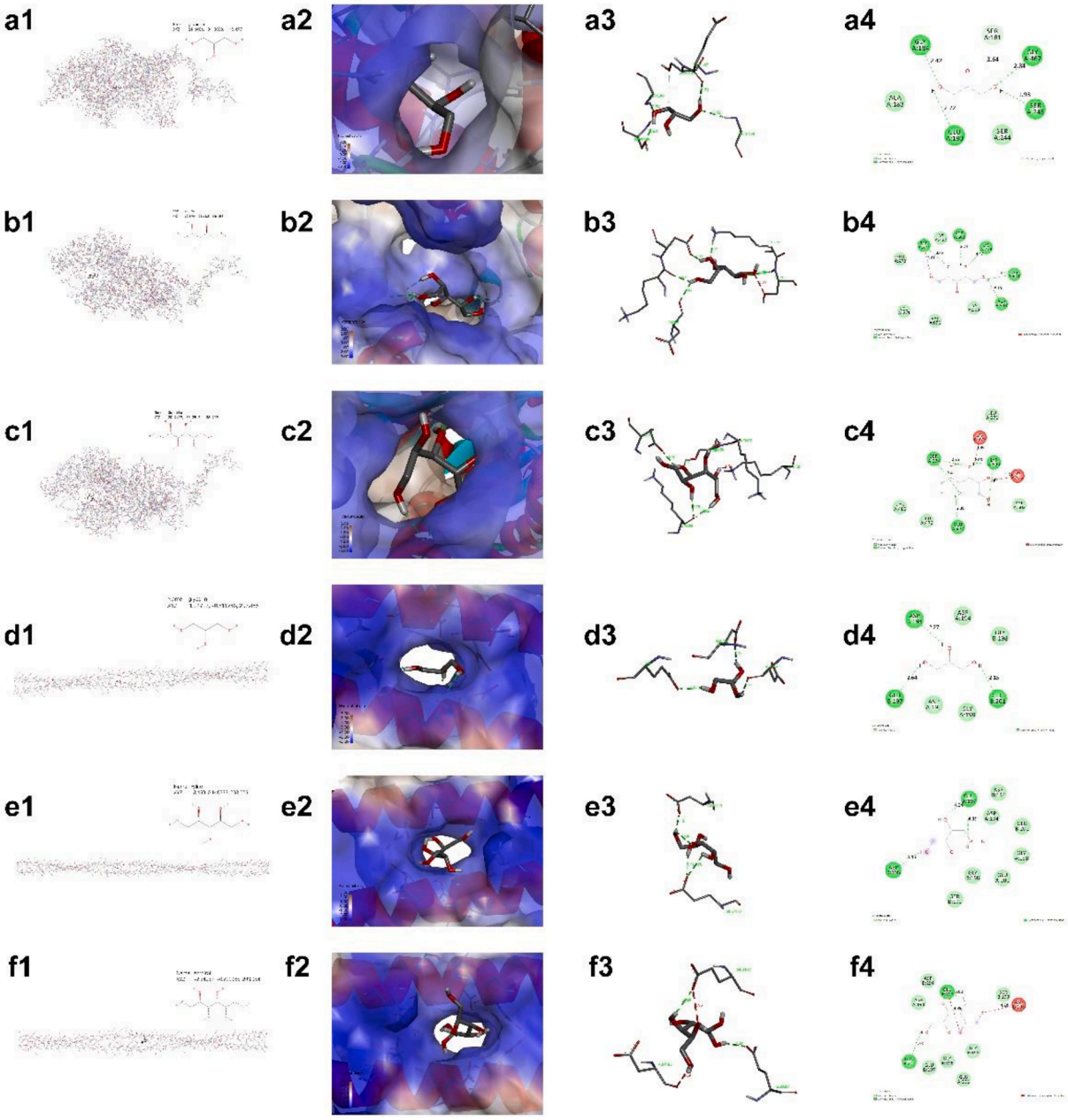

Fig. 5. The schematic diagram generated using the diagram of discovery studio shows the interactions of myosin with different polyhydroxy alcohols. Overall structure (a1-f1), combination pocket (a2-f2), three-dimensional schematic diagram (a3-f3) and two-dimensional schematic diagram (a4-f4).

myosin had an unfavorable interaction, which might explain the adverse effects of high concentrations of xylitol and sorbitol-mediated curing on the microstructure and water production in cured minced meat.

3.9. Correlation analysis

As shown in Fig. 6, the correlation between physical and chemical indices, water distribution, and protein structural changes was analyzed. The aw showed a very significant positive correlation with salt content, saltiness, and PT_{21} ($p < 0.001$), and a significant positive correlation with the chewiness and PT_{2b} ($p < 0.05$), Furthermore, there was a very significant negative correlation with PT_{22} ($p < 0.001$), and a significant negative correlation with moisture content ($p < 0.05$), The results showed that the salt content is closely related to aw and migration of water; different polyhydroxy alcohols changed the salt content in minced pork tenderloin by changing the activity and distribution of water. Thus, it provides a theoretical basis for application in the curing meat industry.

4. Conclusions

This study explores the possibility of different polyhydroxy alcohol-mediated curing in moisture migration, microstructural changes, and protein-polyhydroxy alcohol interactions. The results showed that 1 % of glycerol, xylitol, and sorbitol could reduce the salt content of minced pork tenderloin and improve the quality of cured meat. However, the effect of xylitol was the most significant. Although the addition of 10 % glycerol, xylitol, and sorbitol could reduce the salt content of minced pork tenderloin, high concentrations of xylitol and sorbitol significantly negatively influenced the quality of minced pork tenderloin, whereas the addition of 10 % glycerol had no negative effect. Therefore, xylitol and glycerol can be suitable for low-concentration and high-

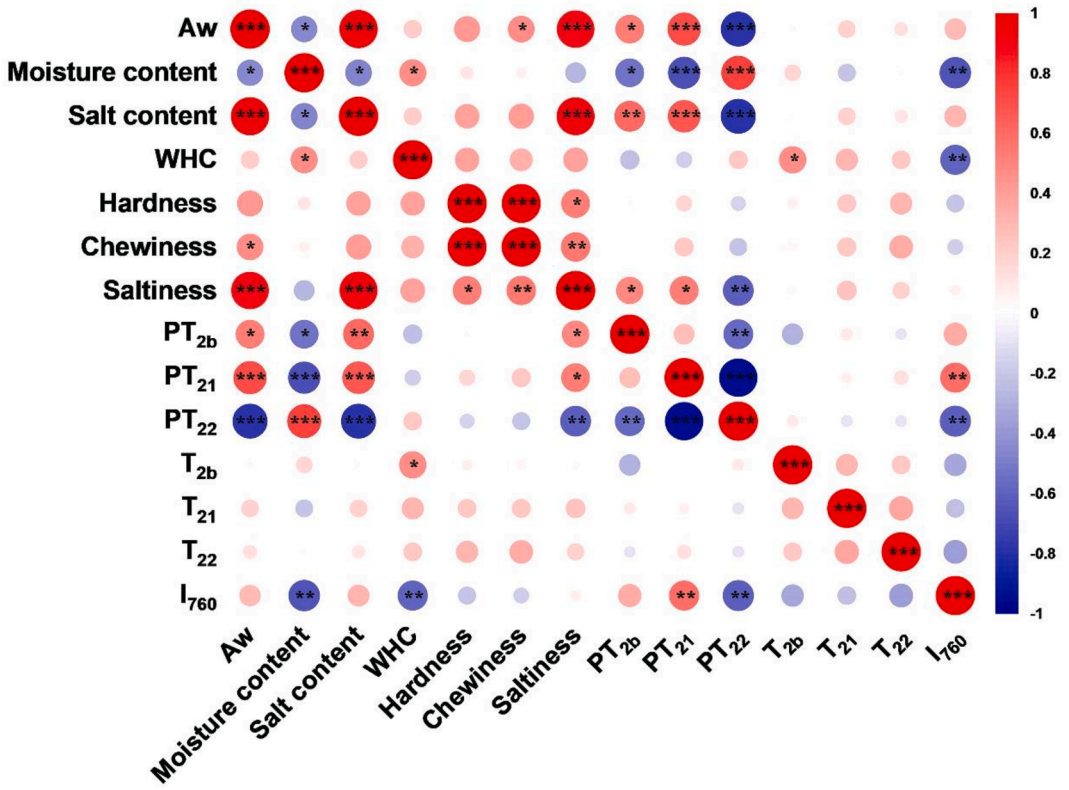

Fig. 6. Pearson's correlation analysis. Heat map of the correlation between physical and chemical indices, water distribution, and protein structural change data (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

concentration polyhydroxy alcohol-mediated curing, respectively. According to the results of Raman spectroscopy and molecular docking, polyhydroxy alcohols could form hydrogen bonds with myosin as well as hydrogen bonds with free water molecules, converting free water into bound water to reduce aw and altering the hydrophobic environment of the myosin surface to reduce structural damages caused by high salt content. However, further research into the mechanism of action of polyhydroxy alcohol-mediated curing at a more specific molecular level is required.

CRediT authorship contribution statement

Linggao Liu: Conceptualization, Visualization, Formal analysis, Writing – original draft. **Ying Zhou:** Conceptualization, Formal analysis, Writing – review & editing. **Jing Wan:** Conceptualization, Formal analysis, Writing – review & editing. **Qiujin Zhu:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Shenghui Bi:** Conceptualization, Writing – review & editing. **Yeling Zhou:** . **Sha Gu:** . **Dan Chen:** . **Yanpei Huang:** Writing – review & editing. **Bokai Hu:** Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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