

Supplementary Information

Species Identification of Animal Glue Adhesives on Polychrome Cultural Heritage: An Integrated Analytical Approach Combining Gel Sampling, ATR-FTIR Spectroscopy, and Machine Learning

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hydrogel sampling

Section 1. Performance evaluation of three supervised learning algorithm models

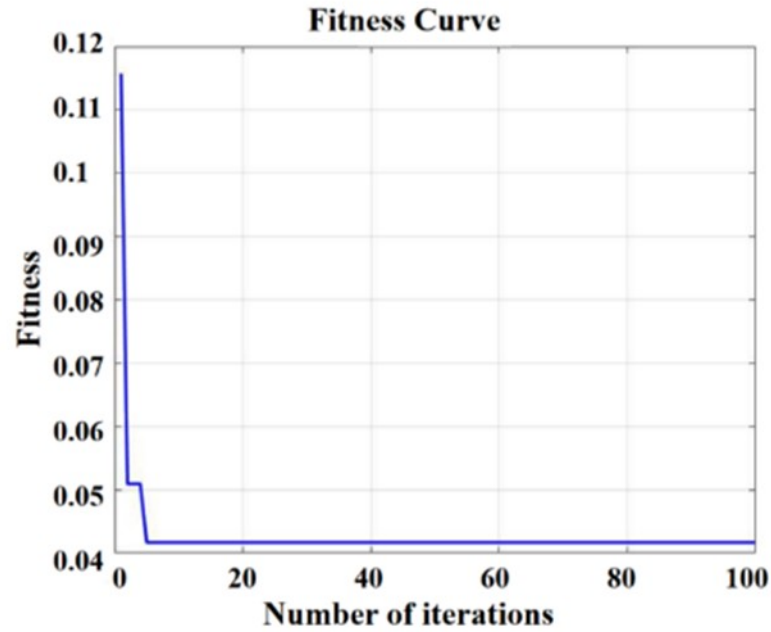


Fig. S1 Fitness curve of the SVM model, illustrating the optimization process and convergence behavior over 100 iterations. The monotonic decrease in the fitness value indicates progressive improvement of the model's performance metric.

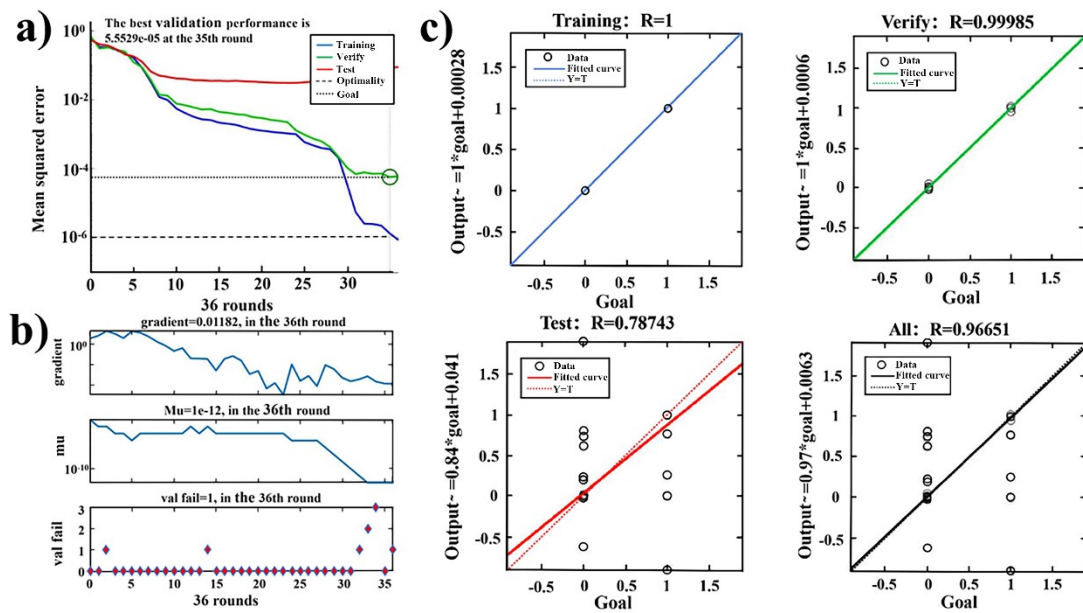


Fig. S2 Classification performance of the BNN model. (a) Training mean squared error (MSE) over epochs. (b) Final optimization parameters. (c) Correlation between predicted and actual values for the training, validation, and test sets.

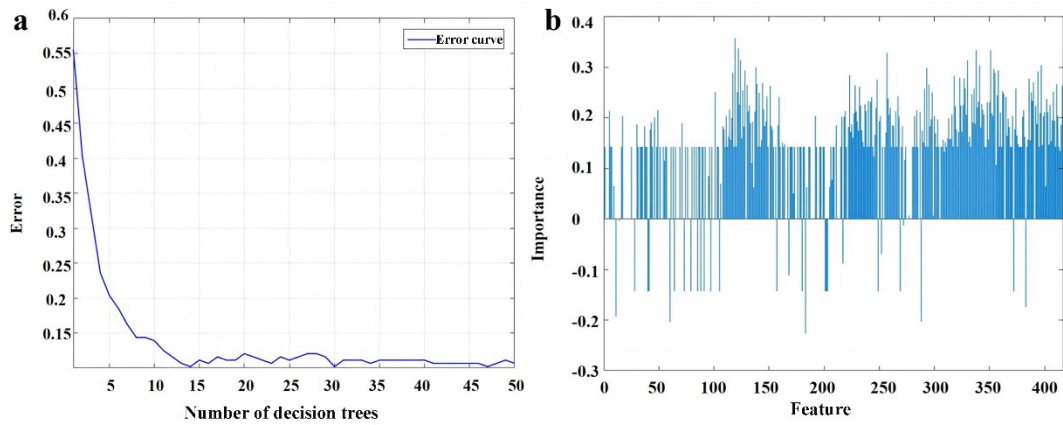


Fig. S3 Performance evaluation of the RF algorithm. (a) Curve illustrating the relationship between the number of decision trees and the model error, showing a gradual decrease and eventual stabilization of error as the number of trees increases; (b) Bar chart of feature importance, displaying the contribution of each feature to the model's predictive outcome, with positive values indicating a positive impact and negative values indicating a negative impact.

Section 2. Validation of classification models for four animal glues adhesives across various scenarios

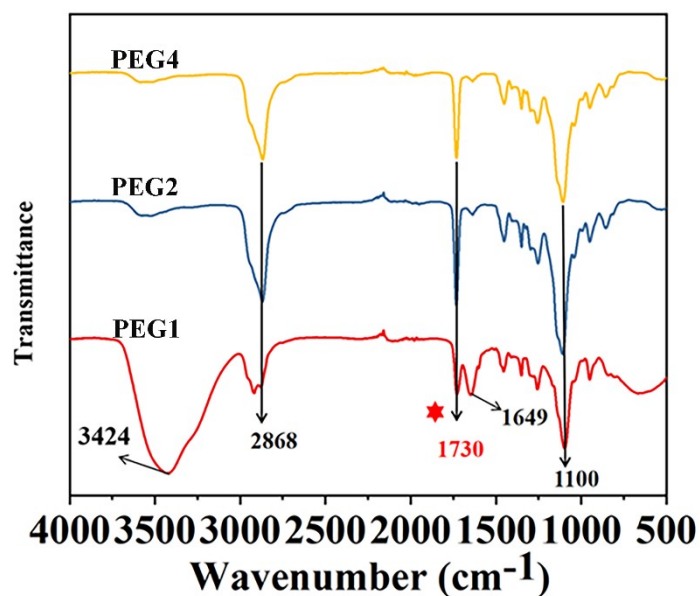


Fig. S4 Infrared spectra of PEG1, PEG2, and PEG4 hydrogels.

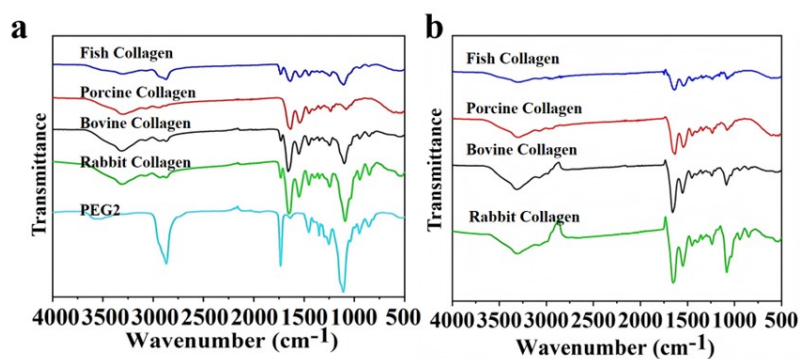


Fig. S5 (a) FTIR spectra of the pristine PEG2 hydrogel and the PEG2 hydrogel after sampling the simulated animal glue sample; (b) FTIR spectra derived by subtracting the spectrum of the PEG2 hydrogel matrix from that of the post-sampling PEG2 hydrogel, highlighting the spectra features of animal glue.

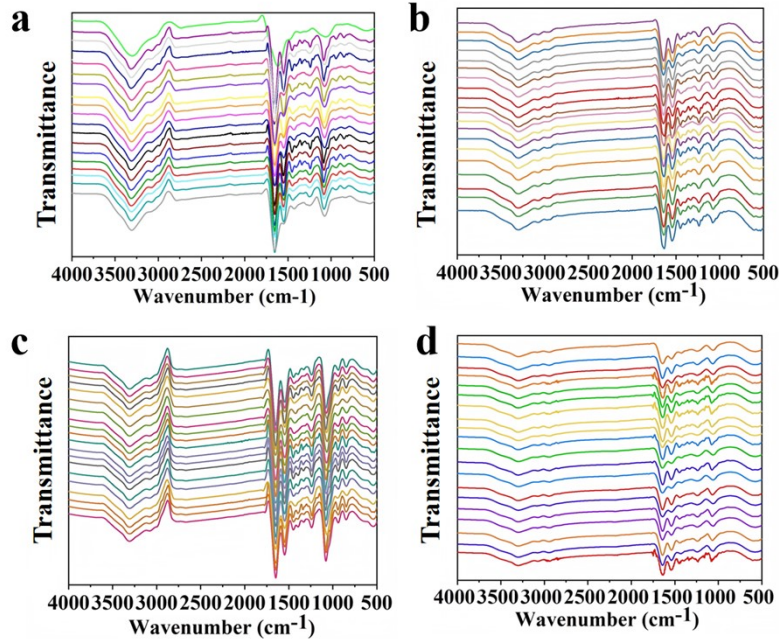


Fig. S6 FTIR spectra obtained by subtracting the spectrum of the PEG2 hydrogel matrix from that of the post-sampling PEG2 hydrogel, for simulated mural samples containing four types of animal glues: (a) bovine; (b) porcine; (c) rabbit; (d) fish.

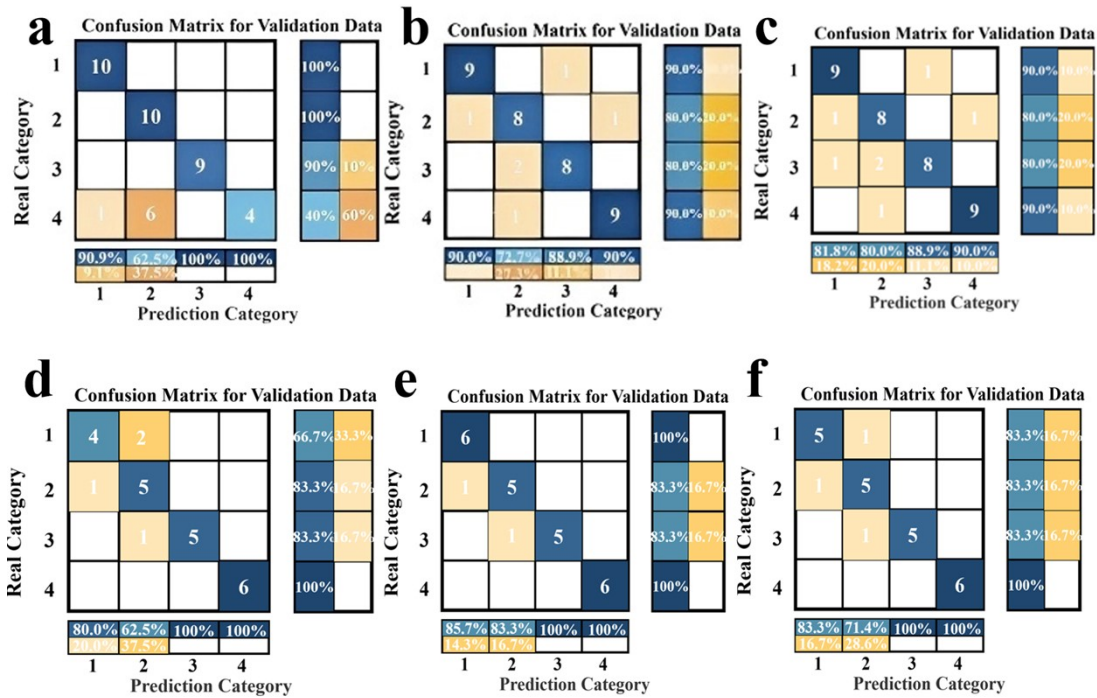


Fig. S7 Confusion matrices for the actual validation sets of three classification models applied to mural simulation samples containing four types of animal glues UV-accelerated aging for 168 hours. (a-c) Validation based on in-situ measurements using a benchtop infrared spectrometer: (a) SVM, (b) BNN, and (c) RF models. (d-f) Validation based on in-situ measurements using a handheld infrared spectrometer: (d) SVM, (e) BNN, and (f) RF models. Categories 1, 2, 3, and 4 correspond to bovine, porcine, rabbit, and fish glues, respectively.

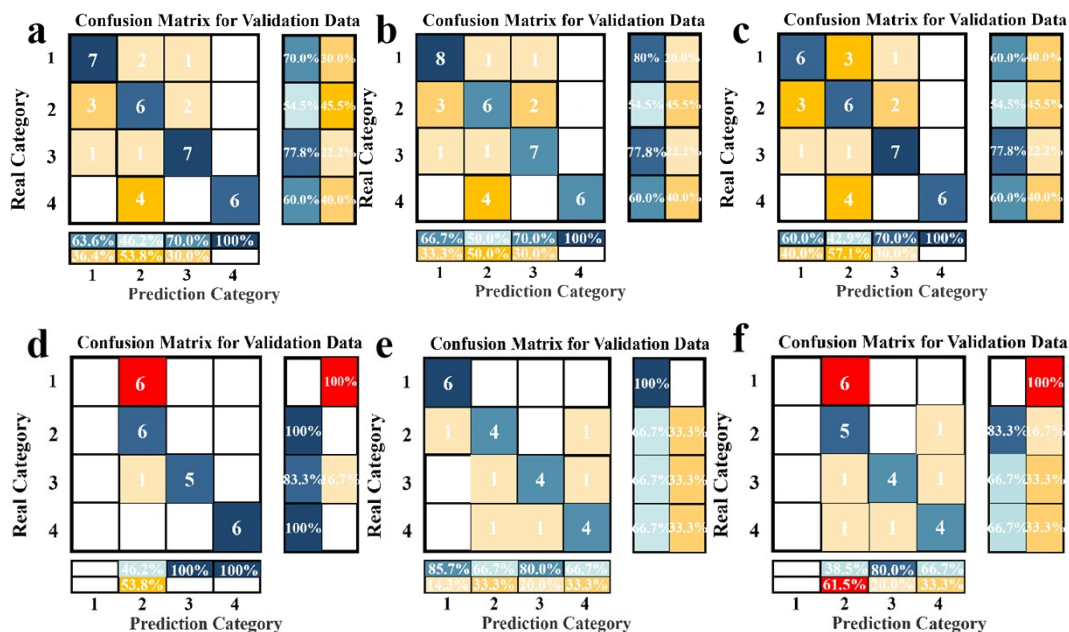


Fig. S8 Confusion matrices of the actual validation sets for three classification models applied to mural simulated samples containing four types of animal glues UV-accelerated aging for 336 hours. (a-c) correspond to in situ detection using a benchtop infrared spectrometer for validating of the (a) SVM, (b) BNN, and (c) RF models. (d-f) correspond to in situ detection using a handheld infrared spectrometer for validating of the (d) SVM, (e) BNN, and (f) RF models. Categories 1, 2, 3, and 4 correspond to bovine, porcine, rabbit, and fish glues, respectively.

Table S1. Validation overall accuracy of protein classification models based on benchtop and handheld infrared spectrometer

Conditions	Validation set overall accuracy (%) ^a					
	SVM		BNN		RF	
	handheld	benchtop	handheld	benchtop	handheld	benchtop
Unaged Simulated samples after hydrogel sampling	/	90.1	/	90.0	/	86.0
Simulated samples aged for 0 hours (unaged)	100	100	100	98.9	100	91.8
Simulated samples aged for 72 hours	100	100	100	100	100	97.5
Simulated samples aged for 168 hours	83.3	82.5	91.7	85.0	87.5	82.9
Simulated samples aged for 336 hours	70.8	65.0	75.0	67.5	54.2	62.5

$$^a \text{Overall accuracy} = \frac{\text{The number of correctly distinguished samples}}{\text{Total number of samples}}$$

Section 3. Classification of animal glues in mural replica by hydrogel sampling-ATR-FTIR analysis

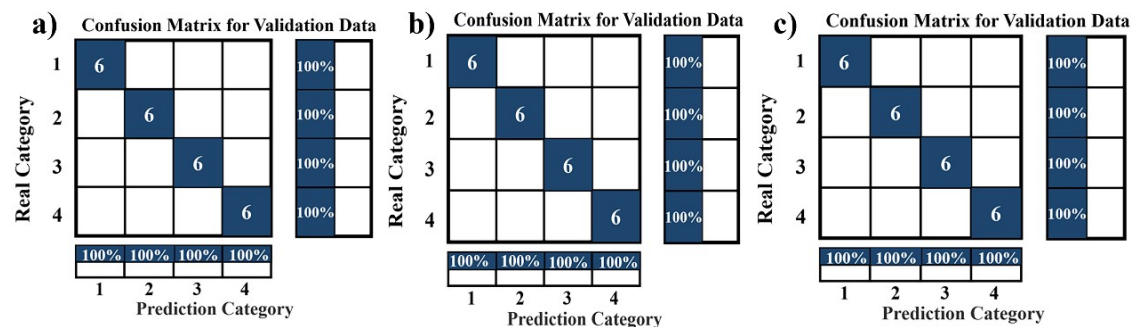


Fig. S9 Confusion matrices of the actual validation sets for three classification models applied to unaged mural replica: (a) SVM, (b) BNN, and (c) RF models. Categories 1- 4 represent bovine, porcine, rabbit, and fish glues, respectively.

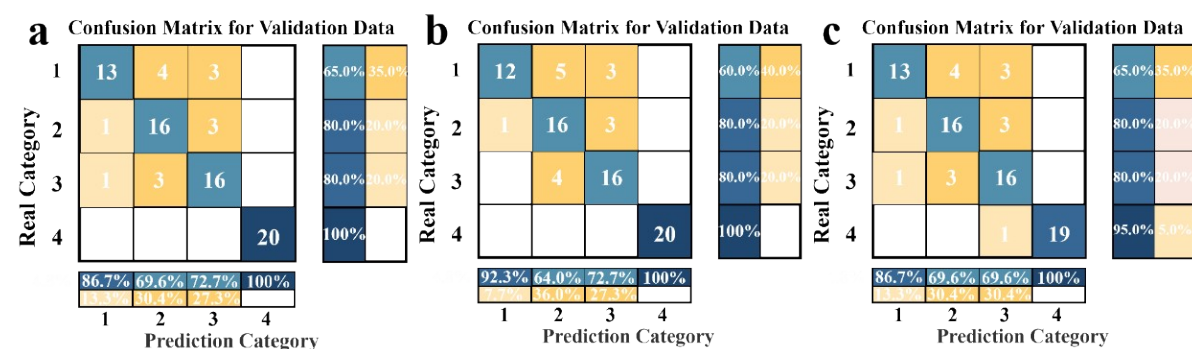


Fig. S10 Confusion matrices of the classification results for the mural replica sample after 168 hours UV-accelerated aging, obtained using hydrogel sampling combined with ATR-FTIR spectroscopy. Results are shown for (a) SVM, (b) BNN, (c) RF models. Categories 1- 4 represent bovine, porcine, rabbit, and fish glues, respectively.

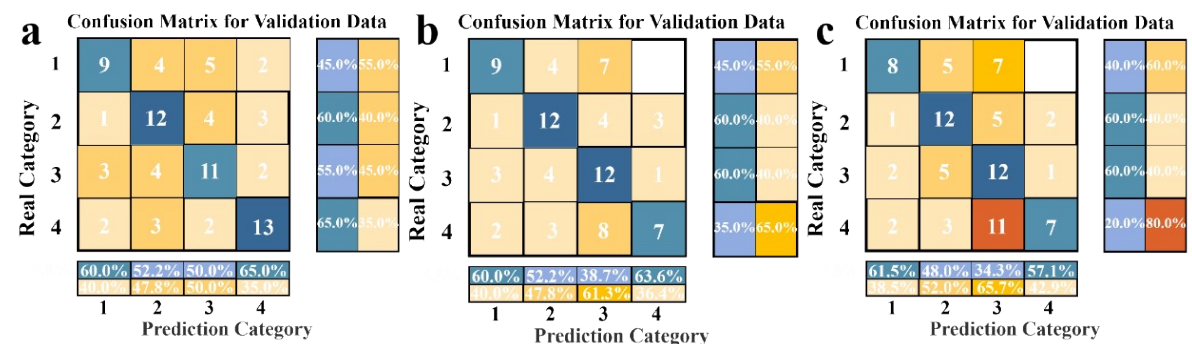


Fig. S11 Confusion matrices of the classification results for the mural replica sample after 336 hours UV-accelerated aging, obtained using hydrogel sampling combined with ATR-FTIR spectroscopy. Results are shown for (a) SVM, (b) BNN, (c) RF models. Categories 1- 4 represent bovine, porcine, rabbit, and fish glues, respectively.

Section 4. Non-Invasive evaluation of mural simulated samples via hydrogel sampling

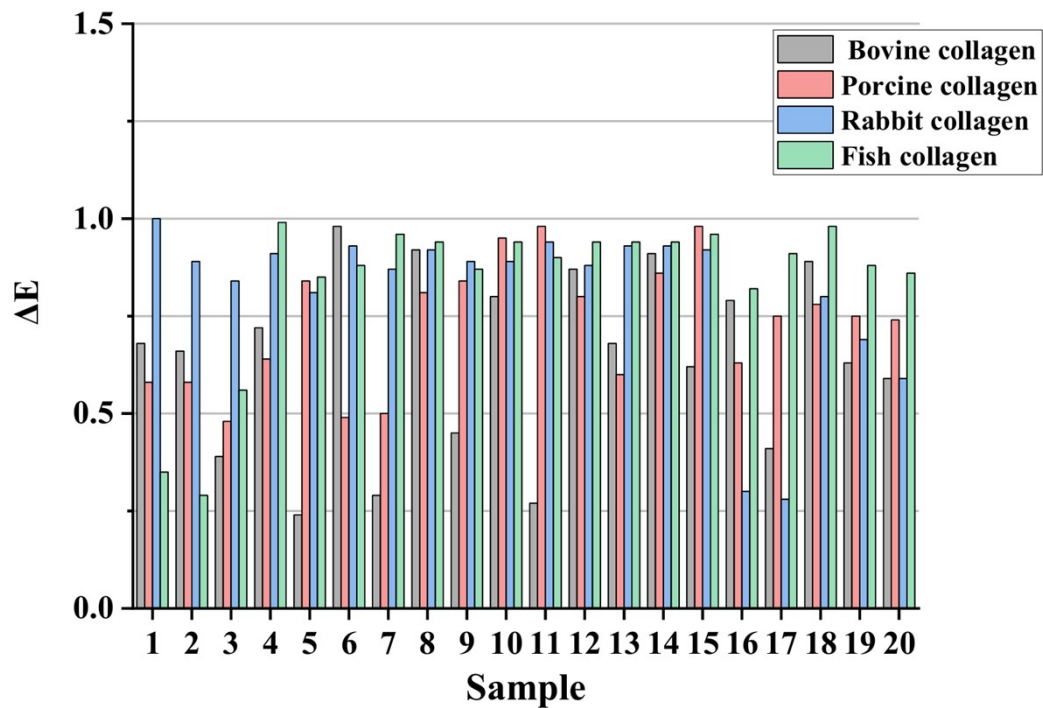


Fig. S12 Color difference values (ΔE) of the mural simulated samples before and after protein extraction via hydrogel sampling.