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參展科別　工程學

作品名稱　Eco-friendly fungal-based protein wood adhesives: A non-toxic and effective alternative application

得獎獎項　四等獎

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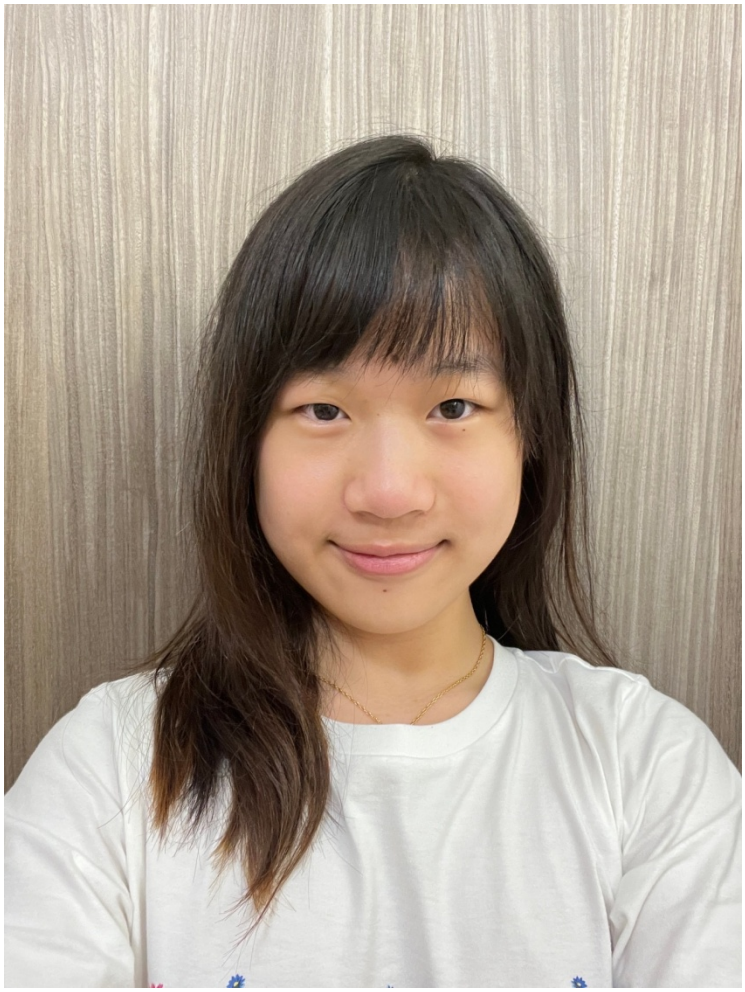
關鍵詞　Fungal-basedprotein、緩衝液、Bradford Protein Assay

作者照片



Abstract

In fungal fermentation for producing alcohol, vinegar, soy sauce, or miso, waste is generated after the process, such as liquor meal or distillers' grains. Some researchers have used beer spent grain (BSG) to extract proteins, modified their properties, and tested their potential as wood adhesives. Using six fungal strains, and protein gels were produced through processes such as centrifugation, alkali hydrolysis, acid neutralization, and centrifugal concentration. The best results in the small-scale adhesive tests were those of the protein extracted from red yeast (*Monascus purpureus*) and koji rice bacteria (*Aspergillus oryzae*). Using the compressive load and tension load methods specified in the ASTM (American Society for Testing and Materials) as reference, the adhesive properties of the wood panels were tested by the Taiwan Testing and Certification Center (ETC).

1. The results of the compressive load method (ASTM D905) showed that the shear strength of red yeast protein gel is 156.1 kgf, while the koji rice protein gel is 51.3 kgf, which are 82.2% and 27.0% of the control group, respectively.
2. The results of the tension load method (ASTM D2339) revealed that the shear strength of red yeast protein gel is 88.1 kgf, while the koji rice protein gel is 40.2 kgf, which are 195.1% and 89.0% of the control group, respectively.

The experimental results indicate that the shear strength of the red yeast protein gel is similar to those of the control group (NanPao Resin White Glue adhesive). In the compressive load method (ASTM D905), the shear strength in the tension load method (ASTM D2339) is significantly better than those of the control group (common glue). This suggests that red yeast protein gel provides a natural and non-toxic alternative choice for wood adhesive applications.

摘要

在真菌釀造過程中除了釀出需要的酒精、醋、醬油，味增，釀造後都會產生廢料：酒糟或酒粕改良性質後測試有作為木質黏合劑的潛力。我們選出 6 種菌種，透過破碎、離心、鹼裂解、酸中和與離心濃縮做出蛋白質膠，小量黏合能力測試結果選最佳的紅麴蛋白質與米麴蛋白質。參照 ASTM 標準壓縮負荷與拉力負荷法。

1. 壓縮負荷法結果顯示紅麴蛋白膠抗剪強度為 156.1kgf，米麴蛋白膠抗剪強度為 51.3kgf，為對照組 82.2%與 27.0%。

2. 拉力負荷法結果顯示紅麴蛋白膠抗剪強度為 88.1kgf，米麴蛋白膠抗剪強度為 40.2kgf，為對照組 195.1%與 89.0%。

實驗結果顯示紅麴蛋白膠在壓縮負荷法強度接近對照組，拉力負荷法強度顯優於對照組，在作為木材黏合劑選擇上提供一種天然且無毒的選擇。

1. Introduction, Purpose, and Literature Review

1.1 Research Background and Introduction:

Formaldehyde (CH_2O, formalin) is an organic compound classified as a Group 1 carcinogen. It has been widely used in industrial production for over a century,

primarily as a key raw material in adhesives for specimen preservation, paint coatings, industrial resins, synthetic fibers, and furniture and construction materials. Formaldehyde has a volatility period of 3 to 15 years.

Prolonged exposure to high concentrations of formaldehyde can lead to various health issues, including skin cancer, nasopharyngeal cancer, leukemia, respiratory diseases, liver and kidney disorders, early-onset puberty in children, fetal deformities and miscarriages, skin allergies, and eye irritation. Of particular concern, especially in children and adolescents, are the effects on the reproductive system and growth and development, including mechanisms involving chromosomal and DNA damage (genotoxicity), oxidative stress, alterations in enzyme, hormone, and protein levels and functions, cell apoptosis, and toxicogenomic and epigenomic effects such as DNA methylation. The number of cases of early-onset puberty in children in Taiwan exceeded 30,000 in 2022, and one significant factor contributing to early-onset puberty is exogenous environmental hormones, with formaldehyde being a common hazardous chemical substance.

Protein adhesives, also known as natural or bio-based adhesives, offer an eco-friendly alternative to synthetic adhesives. The most common protein adhesive is derived from animal sources, particularly collagen or gelatin. The bonding theory behind protein adhesives involves:

1.1.1 Wetting and Penetration: Protein adhesives wet the wood surface and penetrate into the porous structure, similar to synthetic adhesives.

1.1.2 Chemical Bonding: Protein adhesives contain amino acid residues with functional groups such as amine and carboxyl groups. These groups can form chemical bonds with the hydroxyl groups on wood fibers. Hydrogen bonds, electrostatic interactions, and covalent bonding can occur between the adhesive and wood.

1.1.3 Cross-linking: Protein adhesives can be further crosslinked using heat, moisture, or chemical agents. This cross-linking reinforces adhesive bonding and enhances its resistance to moisture and environmental factors.

Recent efforts have been made to create adhesives with similar purposes to formaldehyde by using soy protein or plant-based proteins as alternatives. However, the production of plant-based proteins typically requires large-scale agriculture, involving water irrigation, pesticide and fertilizer usage, which can result in habitat loss, water pollution, and carbon emissions. The production process is also energy-intensive. Therefore, there is a need to explore the extraction of fungal proteins from discarded biological materials to develop natural adhesives and test their strength. This exploration aims to assess the feasibility of using such alternative solutions. By implementing effective and non-toxic alternatives, it is possible to reduce or eliminate the use of formaldehyde, thereby mitigating the adverse environmental and health impacts associated with its use. This approach promotes sustainable, low-carbon, and energy-efficient green production and consumption.

1.2 Purpose:

Our goal is to experimentally extract proteins from by-products or waste materials produced during fungal fermentation or brewing processes. By using these low-value or unused materials and a simple extraction method, we intend to obtain proteins that can be used as adhesives. We believe that this approach may be more energy- and resource-efficient than directly using plant materials to extract plant protein adhesives. It can also help avoid the hazards of formaldehyde in industrial adhesives.

Experiment 1: Extract proteins from six selected fungal materials and measure their concentrations to calculate protein extraction rates. This will help assess overall efficiency and provide cost data for future production.

Experiment 2: Conduct small-scale adhesive and tension strength tests on the extracted fungal proteins to determine the most suitable option for making fungal protein glue.

Experiment 3: For the most promising results from the small-scale tests, conduct the compressive load (D905) and tension load methods (D2339) from the standard ASTM for adhesive performance on wood boards. This will help identify the most suitable fungal material for making glue.

Literature Reviews:

1.2.1 Reproductive and developmental toxicity of formaldehyde: A systematic review

1.2.1.1 Systematically evaluated the evidence of the association between formaldehyde exposure and adverse reproductive and developmental effects in human populations and *in vivo* animal studies in the peer-reviewed literature;

1.2.1.2 Maternal exposure had adverse reproductive and developmental effects including increased risk of spontaneous abortion (HR/RR = 1.76, 95% CI 1.20–2.59, p=0.002);

1.2.1.3 It had positive associations between formaldehyde exposure and reproductive toxicity, mostly in males.

1.2.1.4 Chromosomal and DNA damage (genotoxicity), oxidative stress, altered level and/or function of enzymes, hormones and proteins, apoptosis, toxicogenomic and epigenomic effects (such as DNA methylation) were identified.

1.2.2 Biomimetic lignin-protein adhesive with dynamic covalent/hydrogen hybrid networks enables high bonding performance and wood-based panel recycling

1.2.2.1 Inspired by the adhesion and de-adhesion behavior of snail slime, we built dynamic covalent/hydrogen hybrid networks into adhesive system for achieving both high bonding performance and reusability;

1.2.2.2 Softwood lignin was purified and pretreated by ultrasonication to form a catechol structure (UAL) and then combined with soybean protein to develop a 100 % bio-based wood adhesive.

1.2.2.3 It also exhibited flame retardancy (LOI = 37.7 %), mildew resistance (60 h), and antibacterial performance (inhibition zone = 8 mm).

1.2.3 Adhesive property and mechanism of silkworm egg glue protein

1.2.3.1 Glycosylated native EGP and recombinant EGP from Pichia pastoris were found to have better adhesive strength than non-glycosylated recombinant EGP from E. coli.

1.2.3.2 It was found to be the first complete sequence of insect oval proteins, thus highlighting their potential for future applications in biomedicine and technology.

1.2.4 Novel Bionic Soy Protein-Based Adhesive with Excellent Prepressing Adhesion, Flame Retardancy, and Mildew Resistance

1.2.4.1 Soy protein (SP)-based adhesives can replace traditional aldehyde-based adhesives for the manufacture of artificial panels.

1.2.4.2 Inspired by the "secretion-hardening" process of mussel adhesion protein and the organic-inorganic hybrid adhesion system of oysters, an inorganic crystal cross-linked hybrid SP adhesive was developed.

1.2.4.3 Prepared plywood samples bonded with the hybrid adhesive gel showed an excellent preload bond strength of 544 kPa, a significant increase compared to pure SP adhesive (19 kPa).

1.2.5 Protein and Polysaccharide-Based Electroactive and Conductive Materials for Biomedical Applications

1.2.5.1 The goal of this review was to establish methods for the fabrication of protein- and polysaccharide-based materials displaying biocompatibility and modular electrical properties;

1.2.5.2 In addition to being renewable and biocompatible, it could also be manufactured cost-effectively through marketable production strategies.

1.2.6 Recent Advances on the Development of Protein-Based Adhesives for Wood Composite Materials—A Review

1.2.6.1 The concerns about the environmental footprint and toxicity of these formulations have prompted researchers to reinvestigate the utilization of bio-based materials to establish safer alternatives;

1.2.6.2 Proteins have triggered new interest in the potential development of adhesives for carpentry application due to their

advantages such as lower toxicity, renewable origin and reduced environmental footprint.

1.2.7 Synthesis and applications of fungal mycelium-based advanced functional materials

1.2.7.1 Mycelium-based composites with tailored structural, physical, chemical, mechanical, and biological properties depending on the strain, feed matrix, and manufacturing process used.

1.2.7.2 This article reviews the synthesis and structural organization of mycelium-based materials. It further discusses the influence of different factors on material properties. Finally, various applications of mycelium-based materials in medicine, cosmetics, packaging, construction and other fields are summarized.

1.2.8 Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients

1.2.8.1 De-oiled sunflower pressed cake is a promising source of edible protein as an alternative to soy and egg proteins, free of toxic substances and low in anti-nutrients;

1.2.8.2 A new method for producing light-colored sunflower protein isolate has been developed, combining weakly acidic protein extraction with subsequent adsorptive removal of phenolic compounds.

1.2.9 Plant protein-based fibers: Fabrication, characterization, and potential food applications

1.2.9.1 This study investigated the emulsifying properties of plant protein glues to prepare plant-based emulsions. The article discusses the preparation, properties and food applications of these emulsions, such as salad dressings and fillings. This review focuses on the fabrication of fibers from plant proteins by self-assembly, electrospinning, solution blowing, wet spinning, and high-temperature shearing, and on several applications of the assembly of these fibrin proteins in high-quality foods.

1.2.9.2 Changes in protein structure and protein-protein interactions during fiber production are discussed in detail, as well as the impact of manufacturing conditions and protein sources on fiber morphology and function.

1.2.10 A Comprehensive Review of Food Hydrogels: Principles, Formation Mechanisms, Microstructure, and Its Applications

1.2.10.1 This article focuses on reviewing the nutritional importance, microstructure, mechanical properties and food hydrogel applications of gels. This article also reviews the structural configuration of hydrogels, which predicts potential future applications in food industry. The results of this review confirm the use of various plant and animal polysaccharide and protein sources as gelling agents.

1.2.11 Recent advances in protein-based emulsions: The key role of cellulose

1.2.11.1 This article reviews the effects of various types of cellulose (i.e., nanocellulose, microcrystalline cellulose (MCC), cellulose derivatives, and regenerated cellulose (RC)) on the stability and interaction mechanisms of protein-based emulsions. The key

role of cellulose in protein emulsions can be summarized as follows, namely strengthening network stability, improving emulsification performance, adjusting emulsion structure, and enhancing environmental stability.

1.2.12 Designing Functional Biomimetic Adhesives: Bringing Nature's Methods to Market

1.2.12.1 A variety of biomimetic polymer adhesives are studied and optimized for various applications, providing next steps towards commercialization. These adhesives are tailored through formulation or polymer design for use on various surfaces and conditions;

1.2.12.2 Structure-function studies demonstrate how surface energy affects optimal adhesion to catechol-containing polymers for bonding different substrates while maintaining required mechanical properties.

1.2.13 Bio-based wood adhesive derived from brewers spent grain

1.2.13.1 This work involves protein extraction by alkali treatment and its subsequent modification, i.e. cross-linking with glyoxal. This cross-linked adhesive was tested by the ABES method and found that the ideal content of glyoxal is 20%. Subsequently, adjusted standard tests for lap shear strength and flexural strength (MOE and MOR values) were used to compare their values with commercially available PVAc adhesives.

1.2.14 Optimization of the conditions required for chemical and biological modification of the yeast waste from beer manufacturing to produce adhesive compositions

1.2.14.1 This paper considers the possible making of environmentally friendly adhesive compositions from such wastes.

1.2.14.2 Chemical crosslinking with glutaric aldehyde and biological cross-linking by enzyme transglutaminase improves the moisture resistance of the adhesive compositions. In terms of their physical and mechanical parameters they are not inferior to glues of natural origin and can be used for bonding paper, cardboard, and wood. The bonding strength of paper is 421.8 N / m, and that of wood is 27.8 MPa.

2. Research Method and Process

1 Materials

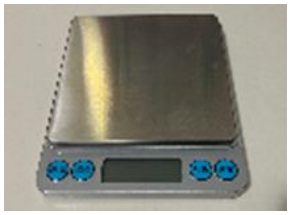
Taiwanese kimchi, Korean kimchi, red yeast, koji rice, bean koji, and sorghum distillers' grains, 1M NaOH, 1M HCl, and Bradford reagent.

			
Taiwanese Kimchi	Korean kimchi	Red yeast	Koji rice

			
Bean koji	Sorghum grains	Bradford reagent	

2 Experimental equipment

Electronic balance, juicer, beaker, 4500 rpm centrifuge, 15 ml centrifuge tube, 1.5 ml centrifuge tube, plastic pipette, pipette and pipette tips, UV-Visible Spectrophotometer, plastic cuvette, commercial glue, spatula, forceps, clamp, tension strength tester

			
Electronic balance	juicer	beaker	4500 rpm centrifuge
			
15 ml centrifuge tube	plastic pipette	UV-Visible Spectrophotometer	plastic cuvette

			
commercial glue	Tongue depressors	clamp	Tension strength tester

3 Experiment 2: Fungal protein adhesive small-scale tension strength testing

Experimental Materials:

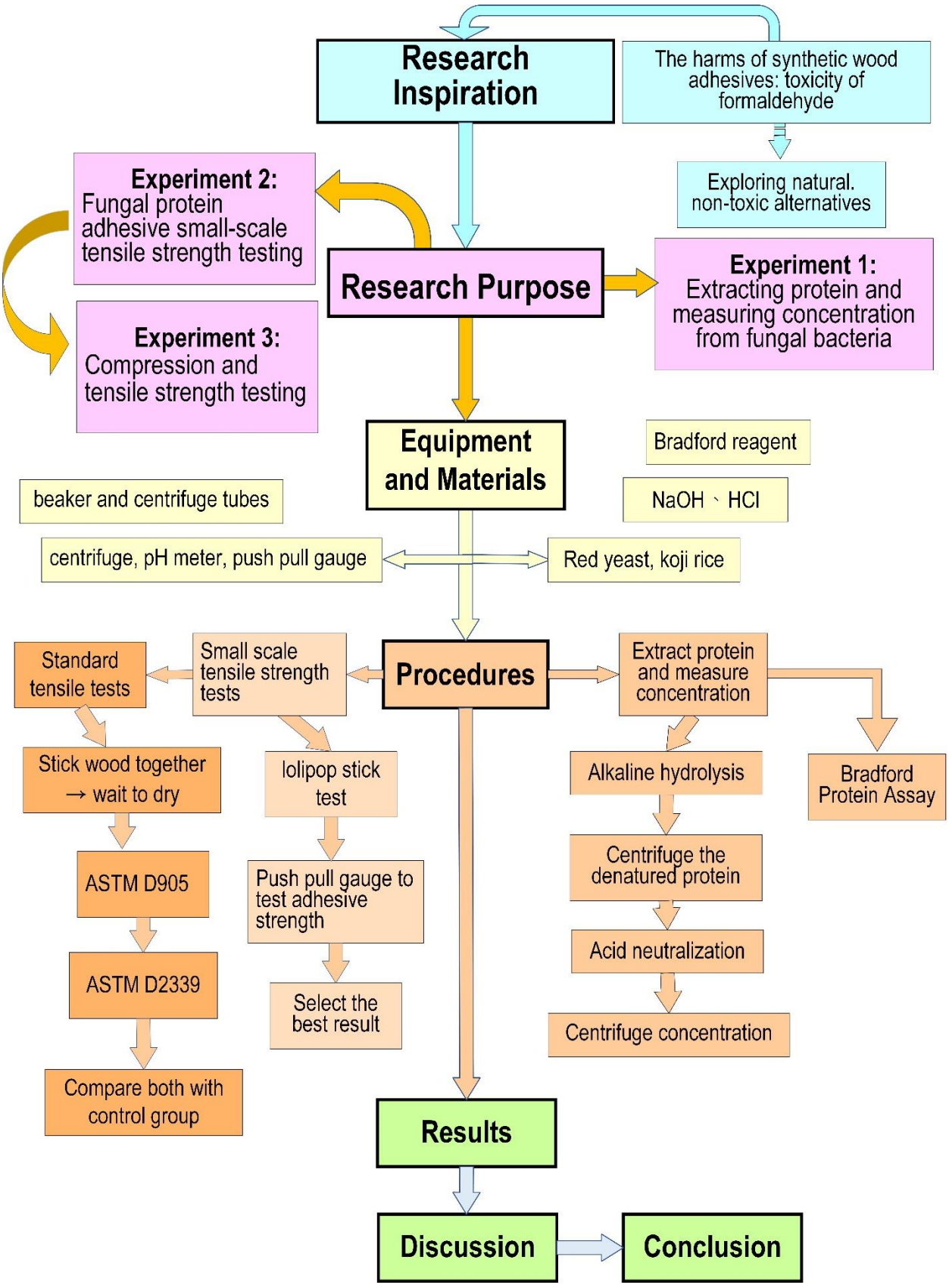
Fungal protein extracts from Taiwanese kimchi, Korean kimchi, red yeast rice, rice koji, soybean koji, and sorghum distillers' grains, tongue depressors, electronic balance, G-type clamp, tension strength tester, commercial glue (control group)

4.Experiment 3: Compression and Tension Strength Testing

Experimental Materials:

Fungal protein extracts from red yeast rice and rice koji, pre-cut wooden boards, electronic balance, commercial glue (control group)

5. Flow diagram of procedures:


Research Inspiration
The harms of synthetic wood adhesives: toxicity of formaldehyde
Exploring natural. non-toxic alternatives
Experiment 2: Fungal protein adhesive small-scale tensile strength testing
Research Purpose
Experiment 1: Extracting protein and measuring concentration from fungal bacteria
Experiment 3: Compression and tensile strength testing
Equipment and Materials
Bradford reagent
beaker and centrifuge tubes
NaOH 、HCl
centrifuge, pH meter, push pull gauge
Red yeast, koji rice
Procedures
Standard tensile tests
Small scale tensile strength tests
Extract protein and measure concentration
Stick wood together → wait to dry
lolipop stick test
Alkaline hydrolysis
Bradford Protein Assay
ASTM D905
Push pull gauge to test adhesive strength
Centrifuge the denatured protein
ASTM D2339
Select the best result
Acid neutralization
Compare both with control group
Centrifuge concentration
Results
Discussion
Conclusion

ASTM is an international standards organization that publishes technical standard agreements for various materials, product, systems and others.
ASTM D905: Standard Test Method for Strength Properties of Adhesive Bonds in Shear by Compression Loading allows for the study of shear strengths of adhesives used for bonding wood and other similar materials.

ASTM D2339: Standard Test Method for Strength Properties of Adhesives in Two-Ply Wood Construction in Shear by Tension Loading. This test method is intended to be applied only to adhesives used in bonding wood to wood.

6.Experiment methods

Experiment 1: Extraction of Fungal Protein and Measurement of Concentration

(A) Experiment Objective:

The objective of this experiment is to extract proteins from six selected fungal materials (Taiwanese kimchi juice, Korean kimchi juice, red yeast rice, soybean koji, rice koji, and sorghum distillers' grains) and measure their concentration. This will help evaluate the overall efficiency and provide a basis for future cost analysis.

(B) Experimental Steps:

1. Weigh 10 grams of each fungal material using an electronic balance and place them in a beaker containing six times their weight of 1M NaOH. Mix the contents in the beaker and then homogenize them in a juicer.

2. Heat the mixture in a water bath at 60°C for 1 hour. After heating, draw the liquid from the homogenized fungal material into 15 ml centrifuge tubes and centrifuge them at 4500 rpm for 30 minutes.

3. Use a plastic pipette to transfer the supernatant to another beaker. Slowly add 1M HCl to the supernatant and mix it until neutral to slightly acidic as indicated by litmus paper, at which point it may appear to have suspended particles.

4. Transfer the neutralized solution into 15 ml centrifuge tubes and centrifuge them at 4500 rpm for 30 minutes to remove the supernatant, leaving behind the precipitated fungal protein.

5. Concentrate the fungal protein precipitate as much as possible in the same centrifuge tube, and take a small amount of protein for appropriate dilution to measure protein concentration.

6. Measure the protein concentration using Bradford reagent. First, dilute the standard solution of bovine serum albumin (BSA) to the specified concentration mentioned in the manufacturer's instructions.

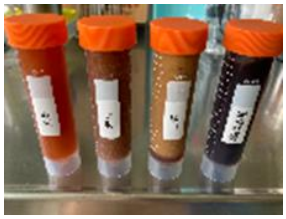
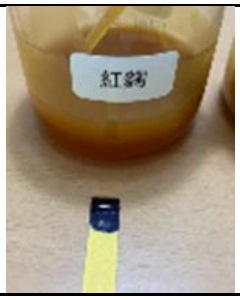
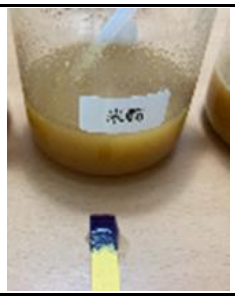
7. Transfer 20 µl of the BSA protein solution into a 1.5 ml centrifuge tube and add 1000 µl of Bradford Reagent. Mix well and allow it to stand at room temperature for 5 minutes.

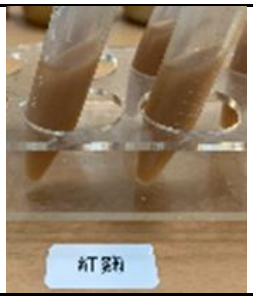
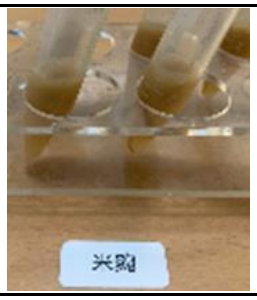
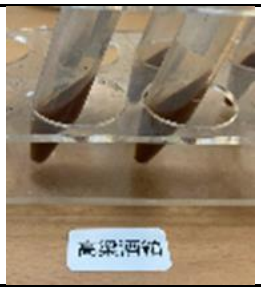
8. Transfer 20 µl of the diluted fungal protein solution into a 1.5 ml centrifuge tube and add 1000 µl of Bradford Reagent. Mix well and allow it to stand at room temperature for 5 minutes. If the protein solution is too concentrated, further dilute it by a factor of 10 or more.

9. Set the wavelength of the spectrophotometer at 595 nm. First, aspirate 1000 µl of water into a plastic cuvette for calibration.

10. Aspirate 1000 µl of the reaction mixture of the BSA protein standard into the plastic cuvette. Gently tap to remove air bubbles, place it in the spectrophotometer, measure the absorbance, and record the value. Enter the value into Excel to create an XY scatter plot and determine the trendline (standard curve).

11. Aspirate 1000 μl of the reaction mixture of the fungal protein into the plastic cuvette. Gently tap to remove air bubbles, place it in the spectrophotometer, measure the absorbance, and record the value. This value was introduced into the standard curve for BSA protein to calculate the X value, which represents the protein concentration of the solution. If it's a protein dilution, multiply it by the dilution factor to obtain the original fungal protein solution's protein concentration.

			
Crushed materials	Red yeast supernatant is a strong base.	Koji rice supernatant is a strong base.	Bean koji supernatant is a strong base.
			
Distiller grains' supernatant is a strong base.	Neutralized red yeast	Neutralzed koji rice	Neutralized bean koji
			
Neutralized distillers' grains	Centrifuged red yeast	Centrifuged koji rice	Centrifuged bean koji

			
Centrifuged distillers' grains	Precipitated red yeast	Precipitated koji rice	Precipitated bean koji
			
Precipitated distillers' grains			

Experiment 2: Fungal Protein Adhesive and Tension Strength Testing

(A) Experiment Objective: The objective of this experiment is to apply fungal protein extracted from six selected fungal materials onto a tongue depressor for small-scale adhesive testing and tension strength measurement. The aim is to identify the fungal species suitable for further production of fungal protein adhesive.

(B) Experimental Steps:

1. Measure and mark a line 2 centimeters from one end of the tongue depressor to indicate the area for adhesive bonding.
2. Place the tongue depressor on an electronic balance and tare it to zero. Weigh 0.25 grams of concentrated fungal protein solution onto the bonded area.
3. Evenly spread the concentrated fungal protein solution onto the bonded area. Place another tongue depressor on top, secure both sides with a clamp, and align the two tongue depressors into a straight line.

4. Allow the protein adhesive to sit for a few days until completely dried for secure bonding. Subsequently, attach the bonded tongue depressor to a sturdy doorframe using a nylon rope and connect it to a tension strength gauge. Ensure that it forms a straight line. Measure the force required to pull apart the bonded tongue depressors.

		
Secured depressors	Using a G-Clamp for securing in a straight line.	Apparatus after adding the gauge

Experiment 3: Fungal Protein Adhesive and Tension Strength Testing

(A) Experiment Objective: To precisely evaluate its adhesive properties, the service of the Taiwan Commodity Inspection and Verification Center was sought for standardized tension strength testing. The objective was to identify the most suitable fungal material for producing adhesive. The testing followed two American standards: ASTM D905 (Standard Test Method for Strength Properties of Adhesive Bonds in Shear by Compression Loading) and ASTM D2339 (Standard Test Method for Strength Properties of Adhesives in Two-Ply Wood Construction in Shear by Tension Loading), both of which are used for measuring wood adhesion.

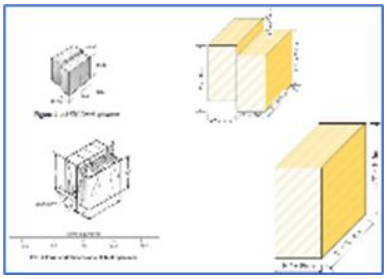
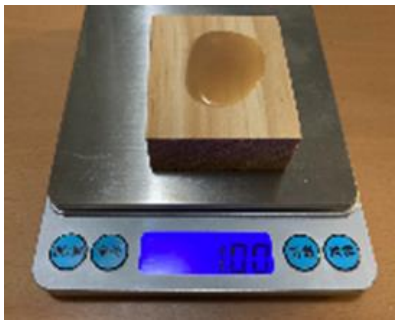
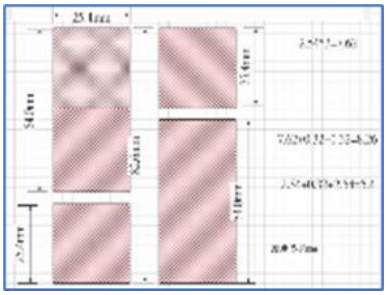
(B) Experimental Steps:

By referencing ASTM D905, a standard method for measuring the shear strength of adhesives used in wood was implemented.

ASTM D905 Compression Load Method: The wooden blocks used were 50.8 mm by 44.4 mm and 19.0 mm thick. The bonded area measured 44.4 mm by 44.4 mm, and the fungal protein applied to the bonding area was 1.0 gram.

ASTM D2339 Tension Load Method: The wooden blocks used were 54.0 mm by 25.4 mm and were 5 to 10 mm thick. The bonded area measured 25.4 mm by 25.4 mm, with 0.5 grams of fungal protein applied to the bonding area. Finally, square wooden blocks measuring 25.4 mm by 25.4 mm were adhered to both ends.

After allowing the adhesive to dry and cure for a few days, the bonded wooden boards were sent to the Taiwan Commodity Inspection and Verification Center, where specialized measuring instruments were used to determine the shear force required to separate them.

		
Compression load method apparatus	The protein amount was 1g in the compression load method	Prepared wood pieces for the compression load method
		
Tension strength apparatus	The protein amount was 0.5g in the tension strength method	Prepared wood pieces for the tension strength method

Test Procedure for both ASTM tests:

a. Prepare and condition the test sample in accordance with the test standard. The sample consists of two blocks of wood bonded to each other with a slight offset.
b. Place the sample in a shearing tool mounted in a test machine capable of applying a compressive load. The dimensions of the shearing jig required for this test are defined within the standard.
c. Apply a compressive load to the sample at the constant position rate specified within the standard.
d. Continue loading until failure.

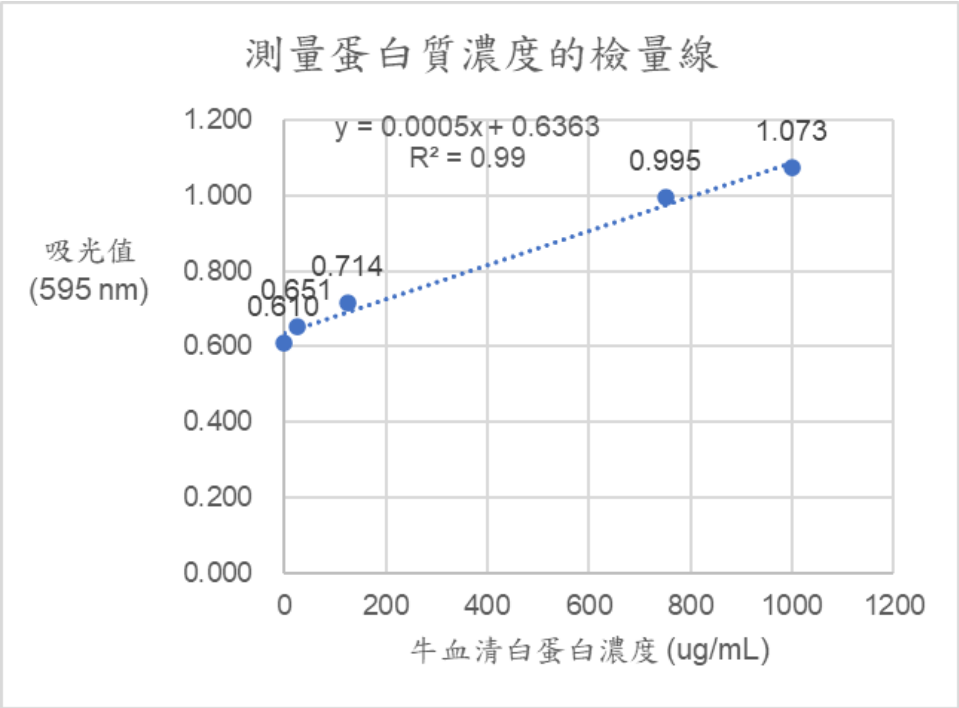
3. Results and Discussion

Experiment 1: Extraction of Fungal Protein and Measurement of Concentration

1. The absorbance values obtained from the standard solution of bovine serum albumin (BSA) at 595 nm wavelength were obtained and inserted into Excel. Create an XY scatter plot and obtain the trendline, which represents the calibration curve for protein concentration.

Calibration Curve for Protein Concentration:	
Standard Solution: Bovine Serum Albumin (BSA)	
µg/mL	Absorbance Value (595 nm)
1000	1.073
750	0.995
500	0.899
250	0.802
125	0.714
25	0.651
0	0.610

(1) The measured absorbance values for the diluted fungal protein solutions were introduced into the calibration curve to obtain the corresponding Y values. Calculating the X value from this allows us to determine the protein concentration of the diluted solution. Multiplying by the dilution factor yields the protein concentration of the original solution, as shown in the table below.



(2) By introducing the absorbance values measured for the diluted fungal proteins into the Y values of the calibration curve,

(3) The original concentration of extracted fungal proteins, when multiplied by the volume of the obtained proteins, yields the total amount of extracted proteins. Dividing this by the amount of fungal material used provides the protein extraction rate, as shown in the table below.

Sample	Dilution factor	Absorbance value (595 nm)	Concentration (ug/mL)	Original concentration (mg/mL)	Extracted amount (mg)	Extraction rate
Taiwanese Kimchi	10X	0.883	493.4	4.93	22.2	0.02%
Korean kimchi	10X	0.792	311.4	3.11	15.6	0.03%
Koji rice 1	10X	0.722	171.4	1.71	25.7	0.6%
Koji rice 2	10X	0.749	225.4	2.25	33.8	
Red yeast	10X	0.718	163.4	1.63	24.5	0.2%
Bean koji	10X	1.515	--	--	--	--
Bean koji	50X	0.711	149.4	7.47	112.1	1.1%
Distillers' grains	10X	1.759	--	--	--	--
Distillers' grains	50X	0.687	101.4	5.07	76.1	0.8%

(4) Both the Taiwanese and Korean kimchi extracts were obtained from leftover kimchi juice, which was filtered then centrifuged to extract the fungal biomass. The overall fungal biomass was lower compared to the other four types of fungi, resulting in relatively lower protein extraction. Since it was extracted from residual kimchi juice, which has a high moisture content, the calculated extraction rate was extremely low.

(5) The reason for the two sets of data for rice koji fungal proteins is that during the final centrifugation for concentration, the protein precipitate could not adhere tightly to the bottom of the test tube. Consequently, when removing the supernatant, some of the protein from the bottom was inadvertently sucked up. To resolve this, the volumes of the rice koji protein extracts were increased and then separately analyzed for protein

concentration. Finally, the total extraction amount was summed, and the extraction rate was calculated.

(6) The extraction rates for the four types of fungal proteins were between 0.6% and 1.1%. The higher protein content in bean koji fungal protein may be attributed to the fact that the brewing material of soybeans naturally have a high protein content. Thus, during the extraction process, it is challenging to avoid extracting soybean proteins. In contrast, rice koji and red yeast use white rice as their brewing material, which has a lower protein content, while sorghum has slightly higher protein content. Consequently, the proteins extracted in these three cases are likely to be primarily from the fungi themselves.

(7) The absorbance values for the diluted 10x solutions of bean koji and distillers' grain fungal proteins exceeded the scope of this study's standard curve. Although absorbance values could still be measured, they fell outside the confidence interval. Therefore, these data points were not used to calculate protein concentrations.

(8) The absence of "Red yeast 50X" in the table is due to a very high dilution factor, which resulted in an extremely low absorbance value, making it impossible to calculate the extraction rate. As a result, this data point was excluded.

Experiment 2: Results of Small-Scale Adhesion and Tension Testing of Fungal Protein Adhesives

(1) The results of the small-scale adhesion and tension testing experiment are shown in the table below. Among the tested fungal protein adhesives, rice koji protein glue and red yeast protein glue exhibited adhesive properties closest to the commercial white glue (control group).

Protein adhesive samples	Tested average tension strength	Comparison with control group

Taiwanese Kimchi adhesive	3.3 kg	17.0%
Korean kimchi adhesive	2.6 kg	13.4%
Red yeast adhesive	17.1 kg	88.1%
Koji rice adhesive	18.6 kg	95.8%
Bean koji adhesive	11.3 kg	58.2%
Distillers' grain adhesive	11.2 kg	57.7%
Control group (white glue)	19.4 kg	100%

(2) In the small-scale testing experiment, the fungal protein adhesives that showed tension values closest to the adhesive strength of commercial white glue were red yeast and koji, which were 88.1% and 95.8% as effective as the commercial white glue, respectively. Therefore, these two fungal protein adhesives were selected for further precise testing.

(3) The main purpose of this experiment was to screen 2 to 3 fungal protein adhesives with the potential for advanced testing in subsequent standard and precise tests. In this experiment, it was challenging to fix the bonded tongue depressors in a stable horizontal position while simultaneously attaching it to both the tension meter and the door frame using G-clamps. Therefore, this experiment involved a small quantity of various fungal protein samples and used a simplified tension testing method to preliminary select red yeast and koji rice fungal protein adhesives for further experiments.

Experiment 3: Results of Fungal Protein Adhesive Adhesion and Tension Testing

(1) The experiment followed the testing methods specified in ASTM D905 and ASTM D2339, which are U.S. national standards for adhesive bonds' strength properties in wood.

The standard tension testing was conducted by Taiwan Product Testing and Verification Center, and the results are presented in the table below:

(2) ASTM D905 Compression Load Method:

Sample	Compression strength (kgf)	average strength (kgf)	Standard difference	Comparison with control group
Red yeast adhesive	175.6	156.1	18.5	82.2%
	138.7			
	154.1			
Koji rice adhesive	31.2	51.3	29.9	27.0%
	37.1			
	85.6			
White glue	156.8	189.8	37.6	100%
	230.7			
	182.0			

(3) Results of the Compression Loading Method indicate that the shear strength of Red Yeast Protein Glue is 82.2% compared to the commercial white glue (control group), while the shear strength of koji rice adhesive is 27.0% compared to the commercial white glue.

(4) ASTM D2339 Tension Load Method:

Sample	Tension strength (kgf)	average strength (kgf)	Standard difference	Comparison with control group
Red yeast adhesive	108.0	88.1	19.6	195.1%
	68.8			
	87.6			
Koji rice adhesive	41.9	40.2	1.9	89.0%
	38.2			
	40.5			
White glue	62.3	45.2	15.0	100%

(5) Results of the Tension Loading Method show that the shear strength of Red Yeast Protein Glue is 195.1% compared to the commercial white glue (control group), nearly double the tension strength. The shear strength of Koji Protein is 89.0% compared to the commercial white glue. Red Yeast Protein Glue exhibits more than twice the shear strength of Koji Protein in this experiment, confirming its high adhesive properties and potential for future research and development.

4. Conclusion and application

The extraction yields of the four fungal protein adhesives used in this experiment range from 0.6% to 1.1%. Although the red yeast glue showed the best performance in the tension tests, its extraction yield was only 0.8%. Further optimization of the extraction process will be needed for future large-scale production. This optimization might involve adjustments in alkaline hydrolysis concentration, heating temperature, and duration to increase the yield and reduce costs.

Fungal Protein: Fungal proteins are typically derived from fungal strains, such as *Aspergillus niger*, and are primarily composed of "mycoprotein". These fungi can produce proteins during various bioprocesses, which are then extracted and purified to become components of protein glue.

In the literature review, it has been mentioned that proteins extracted from beer lees can be combined with other compounds to improve their characteristics. In future experiments, we could consider adjusting the pH of the fungal protein glue or adding natural thickeners, coagulants, or other substances to optimize the adhesive properties of the red yeast protein glue. This could enhance its shear and tension strength, making it more powerful for industrial applications.

The production process of conventional plant protein adhesives often consumes a significant amount of water and energy, leading to potential waste and increased

emissions of pollutants, and carbon dioxide emissions. By using by-products of fungal fermentation or brewing to produce protein glue, it's possible to reduce the consumption of natural resources, decrease environmental impact, and create new products to increase value. In the future, fungal protein adhesives can provide a natural and non-toxic choice for wood bonding.

Furthermore, after confirming the adhesive properties of red yeast protein glue, we can conduct additional tests to further characterize the properties of this adhesive, including:

4.1 **Microbiological Testing:** Microbiological tests could be performed to evaluate the antifungal properties of protein adhesives. This can be done by inoculating the fungus onto a protein gel sample and observing its growth.

4.1.1 **Add antifungal ingredients:** Natural or chemical antifungal agents, such as antioxidants, silver ions or plant extracts, can be added to increase the mildew resistance of the glue.

4.1.2 **Packaging and Storage Conditions:** Assess the impact of different packaging methods and storage conditions on the adhesive's antifungal properties to ensure its quality is maintained during storage.

4.2 A**ntibacterial Properties**: This test evaluates the antibacterial performance of the adhesive using microbiological experiments. For example:

4.2.1 **Adding antibacterial agents**: Natural or chemical antibacterial agents, such as chitosan, or copper sulfate, can be incorporated to enhance antibacterial properties.

4.2 Flame Resistance Testing

4.3.1 Thermal Stability : This could include evaluating flame resistance by exposing the fungal protein adhesive to high temperatures and observing its melting and burning behavior.

4.3.2 Incorporating Flame Retardants: We could be enhancing flame resistance by adding flame retardants like aluminum trihydroxide, magnesium chloride, and others.

5.4 **General Performance Testing**

5.4.1 Conducting tests for properties such as flexibility, heat resistance, and melting point to gain a broader understanding of various fundamental general performance characteristics of protein adhesives, especially when different additives are introduced.

5.5 **Environmental Performance Testing**

5.5.1 **Biodegradability Testing**: Testing the biodegradability of red yeast protein adhesives to ensure its environmental friendliness.

5.5.2 **Biocompatibility Testing**: If the adhesive is intended for use in food or medical fields in the future, biocompatibility testing is required to ensure that it does not cause adverse reactions.

5.Future applications：

This experiment has confirmed the high feasibility of using extracted red yeast protein as a natural and efficient adhesive. Fungal proteins are diverse in structure and function, and beyond their application as wood adhesives in this experiment, they can be explored for various other purposes in the future, such as food gels, drug carriers in biotechnology, and biodegradable packaging materials. This opens

up new possibilities for various industries. Traditional protein adhesive preparation processes often involve significant water and energy consumption and may result in wastes and emissions. Utilizing fungal proteins to produce protein adhesives holds the promise of reducing reliance on natural resources and minimizing environmental impact.

In the future, further research and development of red yeast protein adhesive can also explore the following areas:

Utilizing Nanotechnology: Nanotechnology may contribute to improving the structure and performance of protein adhesives, enhancing their adhesion properties.

Fungal Proteins: Fungal proteins are commonly used in the food industry to create meat substitutes, such as plant-based meat and vegetarian burgers, as well as other food applications. They are also employed in biotechnological applications.

Biotechnology:

1. **Drug Delivery**: Fungal protein adhesives can be used as drug delivery systems to enhance drug stability and delivery efficiency.

2. **Tissue Engineering:** They can be used in tissue engineering and regenerative medicine to support three-dimensional structures and cell cultures.

Packaging Materials:

1. **Biodegradable Packaging:** Fungal protein adhesives can be used to create biodegradable packaging materials, reducing issues related to plastic waste.

2. **Food Packaging:** They can serve as layers and films for food packaging to improve shelf life and freshness.

Medical Devices:

1. Surgical Sutures: Fungal protein adhesives can be used in medical devices like surgical sutures to improve biocompatibility and reduce allergic reactions.

2. Biodegradable Implants: Used for biodegradable implants, such as sutures and scaffolds.

Construction Materials:

1. Structural Materials: Fungal protein adhesives can be used to produce bio-based construction materials, including walls, panels, and structural materials.

Textiles and Fibers:

1. Textile Additives: Used to improve the dyeing, printing, and texture of fibers to produce biodegradable textiles.

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此研究主要以環境友善方式藉由紅麴萃取物與單寧酸提取不同酵素中的蛋白質之結合，並應用於木材貼合時的黏著性質強化研究，整體作品概念深具新穎性，數據分析藉由 ASTM 標準測試方法確實定義出其改善工程之成效。唯建議接著劑與木頭間之結合行為可加以說明，以確立其接合強度提升之學理機制。為體現未來實際使用，保存時間與環境狀態可加以探討。