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酵素處理於食用包裝原料性質及食品保鮮影響之綜論 (1)

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摘　　要

傳統包裝使用的石油基聚合物材料存在環境污染等問題，因此食品包裝行業面臨開發兼具環境友善和經濟性包材的挑戰，可生物降解和可再生特性的替代包裝材料彰顯其重要性。本文回顧酵素在食用包裝中的應用，包括轉麩醯胺酸酶 (transglutaminase, TG)、乳過氧化酶系統 (lactoperoxidase system, LPOS) 及溶菌酶 (lysozyme, LY) 等，可以改善包裝性能。TG 可催化蛋白質交聯作用，提高蛋白薄膜的機械性能及改善薄膜水蒸氣透過率等。LPOS 與 LY 為天然的抗菌劑，於食用包裝中展示出抗菌特性，能有效延長食品的保鮮期。另結合高強度超音波預處理，可提升 TG 對於薄膜性能影響；本文也探討其他添加劑，例如抗壞血酸 (ascorbic acid, AA)、鷹嘴豆白蛋白萃取物 (chickpea albumin extract, CPAE) 及 α －生育醇等，在食品包裝中的協同作用或影響。本篇報告回顧近年酵素應用於食品包裝研究，期讓讀者了解酵素處理在食用包裝和延長食品保鮮期方面的應用潛力，供未來研究依循之參考。

關鍵詞：食用薄膜、塗層、酵素。

緒　　言

傳統的包裝材料主要來源為石油衍生的聚合物，這些材料存在諸多問題，例如環境污染、資源枯竭、一次性廢物的產生、化學物質滲入食品及回收侷限等，隨著食品行業致力於減少對環境影響，鼓勵變革以採取有效的可持續食品包裝方法，而現代消費者越來越關注這些合成聚合物對環境的影響和健康危害，食品行業面臨的主要挑戰之一是開發環保、經濟及永續的包裝系統，具可生物降解和可再生特性的替代包裝材料的需求變得迫切 (Kumar *et al.*, 2022; Khandeparkar *et al.*, 2024)。一般而言，蛋白質與多醣薄膜具有較強的機械特性，在中低相對濕度下對氧氣有很強的屏障作用，但由於其親水性質，對水蒸氣的屏障作用較弱 (Devi *et al.*, 2024)。蛋白質結構和蛋白質之間相互作用可以通過物理、化學和酵素處理進行修飾 (Cruz-Diaza *et al.*, 2019)。可食用包裝在食品保鮮方面被特別重視，因為它們能提高整體食品品質，將天然抗菌劑 (包括細菌素、酵素及水果提取物) 納入食用包裝中是研發的重點 (Cissé *et al.*, 2012)。在保護環境的迫切需求下，促使尋求可生物降解的食用包裝成為一大挑戰，酵素被廣泛使用作為提升食用包裝性能的添加物之一。因此，本研究以「可食用薄膜」及「酵素」作為主要關鍵詞，進行文獻檢索，依據酵素類別分類整理，旨在探討數種酵素在食用包裝之應用，以提升其應用性能。

食用包裝

食品包裝的主要目的在於維護食品品質與安全，同時提升運輸及儲存的便利性。因此，所選用的包裝材料應具備良好的阻隔性，能有效防止水分、氧氣、二氧化碳與光線等，隔絕可能導致食品劣變的因子，具有良好的保鮮、抗菌、防潮及防氧化功能。近年來，食品級原料製成的可食性包裝具備生物可降解性，逐漸受到重視，食品級原料可以形成可食性薄層，用於塗覆食品或作為可生物降解的包裝，可食性塗層 (edible coatings) 和可食性薄膜 (edible films) 的術語相似，有時會引起混淆，因此，常使用可食性包裝 (edible packages) 來表示這兩種產品 (Ribeiro *et al.*,

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2024)。可食用包裝分為兩種應用形式：I. 直接塗覆於食品上的可食用塗層或 II. 包裹食品的預成型薄膜 (Suhag *et al.*, 2020)。由不同基材材料製成的可食用薄膜的功能特性也各不相同，單一基材的可食用薄膜在某些方面存在缺陷，通常在可食用薄膜中添加功能性成分，如蛋白質、多醣類、精油、天然產品、納米材料、乳化劑等，以改善其功能特性 (Li *et al.*, 2024)。各種可食用薄膜和塗層使用碳水化合物、脂質、蠟、蛋白質及其混合物等為主要成分配製而成 (Ribeiro *et al.*, 2024)。選擇合適的塗層方法不僅影響食品產品上形成的保護效果，生產成本和製程效率亦有差異 (Suhag *et al.*, 2020)。相關文獻對於可食用包裝之製備如表 1 所示。

轉麩醯胺酸酶

I. 介紹

轉麩醯胺酸酶 (transglutaminase, TG) 在自然界中廣泛存在，它們存在於哺乳動物組織、許多無脊椎動物及微生物細胞中，TG 也存在於大豆、菊芋、飼料甜菜及蘋果的植物組織中 (Kieliszek and Misiewicz, 2014)。研究發現一種產生 TG 的微生物，該微生物的 TG 從菌株 S － 8112 的培養濾液中純化出來，該菌株被認為屬於鏈黴菌屬，其 TG 的活性不需要鈣離子，它與已知的哺乳動物器官中萃取的 TG 明顯不同，後者被定義為鈣依賴性酶 (Ando *et al.*, 1989)。後來，微生物來源的 TG (mTG) 逐漸成為商業應用的主要來源，隨著基因工程技術的發展，不斷提高其活性、穩定性及產量 (Zhang *et al.*, 2023)。mTG 催化三種反應類型 (i) 醯基轉移反應 (acyletransfer reaction)：醯基轉移反應發生在 γ-carboxamide group 或蛋白結合麩醯胺 (protein-bound glutamine) (醯基供體) 與一級胺 (醯基受體) 之間，(ii) 蛋白質交聯反應 (cross-linking reaction)：當醯基受體是蛋白質結合離胺酸的 ε- 氨基團 (ε-amino group of protein-bound lysine) 時，會形成分子內和 / 或分子間的交聯，進而使蛋白質聚合，(iii) 脫醯胺作用 (deamidation)：如果胺基物無法作為醯基受體，mTG 可以催化 glutamyl residues 的脫醯胺作用，使用水作為醯基受體；就乳蛋白而言，由於酪蛋白中離胺酸和甘胺酸含量豐富，交聯更有可能發生 (Velazquez-Domingues *et al.*, 2023)。

II. 使用限制及加工食品應用範圍

TG 自 1998 年起被美國食品藥物管理局 (FDA) 認定為公認安全物質 (generally recognized as safe, GRAS) (Romeih and Walker, 2017)，被廣泛應用於食品加工領域。主要作用機制為促進蛋白質分子間的交聯反應，改善蛋白質的結構穩定性。mTG 的最佳反應溫度範圍是 45 至 55℃，並且根據微生物的不同會略有變化；其活性在 pH4.0 至 9.0 之間保持穩定，而應用於不同蛋白基質，最適交聯反應的 pH 條件會有所變化 (Zhang *et al.*, 2023)。通常在交聯作用結束後，透過 75℃ 加熱 10 至 15 分鐘作為 TG 失活處理 (Jiang *et al.*, 2020; Ahammed *et al.*, 2021)。在乳製品應用方面，可提升優格的附著力、硬度與保水能力；在冷凍品方面，有助於強化冰淇淋的結構穩定性、改善流變現象並減少冰晶形成；此外，亦能增強豆腐的凝膠強度及提升其保水能力 (Akin *et al.*, 2019; Zhang *et al.*, 2019; Ziarno and Zaręba, 2020)。TG 還可以增強肉塊的凝聚力，而無須進行熱處理或添加鹽或磷酸鹽 (Kieliszek and Misiewicz, 2014)。顯示其能在多種蛋白質主原料之加工食品中廣泛應用。在工業生產中，為了提升經濟效益，應積極尋找能夠合成大量高活性酵素並應用低成本培養基原料的新型菌株 (Kieliszek and Misiewicz, 2014)。

III. TG 於食用包裝的應用

(i) 改善薄膜機械性能

蛋白質基薄膜具有生物降解的優點，但因含有高比例的親水性胺基酸，在機械性能和水阻隔性方面不如傳統塑膠薄膜，透過改善機械性能，可使其在食品包裝中更具實用性和競爭力。

Jiang *et al.* (2016) 研究以不同體積比例的濃縮乳清蛋白 (whey protein concentrates, WPC) 與羧甲基化幾丁聚醣 (carboxymethylated chitosan, CMC) 製備複合膜，結果顯示，當體積比 WPC：CMC = 75：25 時，mTG 顯著提升膜材的伸長率，並略提升膜熱性 (thermal properties)，對透光率影響不大，顯示 mTG 有助於改善 WPC － CMC 複合膜的機械性能。

Jiang *et al.* (2020) 發現先將 TG 添加於 WPC 中催化交聯後再混合羧甲基幾丁聚醣 (carboxymethyl chitosan, CMCS) 較 TG 直接添加於 WPC － CMCS 溶液中，薄膜的斷裂伸長率由 21% 提升至 35%；當 TG 添加於已混合的 WPC － CMCS 溶液中進行催化 (WPC － CMCS － TG)，其斷裂伸長率顯著下降，推測可能因 CMCS 抑制 TG 催化 WPC 交聯反應。透過 TG 增強薄膜機械性能，使薄膜不易破裂或損壞，進而提升使用耐久性，同時可提升薄膜的水不溶性，使其在高濕度環境中維持結構與功能性。

表 1.　可食用包裝之製備

Table 1.　Preparation of edible packages

Preparation method	Preparation process	Advantages and disadvantages	References
Film - casting method (wet procedure)	Biopolymers are dissolved in a solvent, then undergo vacuum degassing before being placed in a mold for dehydration and membrane formation.	Advantages: Easy to manufacture, low cost, etc. Disadvantages：Limited form and production yield, variations in evaporation rates, etc.	Cha *et al.*, 2003; Jensen *et al.*, 2015; Suhag *et al.*, 2020; Kumar *et al.*, 2022
Film - extrusion method (dry procedure)	The film forming solution (FFS) is heated and either passed through the slit die for calendaring (slit - die extrusion) or blown through circle die.	Advantages: No solvent required, handling high viscosity polymers easily, etc. Disadvantages：High equipment cost, limits the use of certain polymers, etc.	Skurtys *et al.*, 2010; Jebalia *et al.*, 2019; Ochoa - Yepes *et al.*, 2019; Suhag *et al.*, 2020; Kumar *et al.*, 2022
Coating - dipping method	Dip the product in the coating solution, then heat and dry to form a stable layer.	Advantages: Good coating uniformity on rough surfaces. Disadvantages: Dilution of the coating solution , dirt accumulation and microbial growth in the dipping vat, etc.	Andrade *et al.*, 2012; Valdés *et al.*, 2017; Suhag *et al.*, 2020
Coating -spraying method	Coating solution is distributed onto the surface of the food in droplet form through a set of nozzles. The atomization pressure is a critical parameter in the spraying technique.	Advantages: Provides a uniform thickness coating and for multilayer applications, such as alternating the use of sodium chloride and calcium chloride solutions. Disadvantages: High - viscosity solutions caused the higher thickness of the coating material on the surface.	Suhag *et al.*, 2020
Coating -fluidized - bed processing method	The coating solution is sprayed through multiple nozzles onto the surface of fluidized powder, forming a shell-like structure.	Advantages: Compared to the panning method, it reduces excessive aggregation of coated products or the formation of uneven deposits. It demonstrates efficient drying effectiveness and allows to utilize a lower dimension of surfactant option in examination with dipping and panning forms. Disadvantages: The cost is higher compared to other coating methods.	Suhag *et al.*, 2020
Coating -panning method	Placing products that to be coated in a large rotating pan, sprinkling the coating solution, and ensure the products are evenly coated through rotate and tumble within the pan.	Advantages: This method produces a highly transparent edible coating with excellent flexibility and a glossy solution, and can be applied to extruded food products of various shapes. Disadvantages: Frequent evaporation of water is required to prevent food products from sticking together, large amounts of solution or suspension cannot be freely sprayed on food products. The operation time is long, and the economic efficiency is relatively low.	Suhag *et al.*, 2020

Ahammed *et al.* (2021) 製備玉米醇溶蛋白與明膠複合膜，透過 1% TG 處理下，TG 顯著提升複合膜抗拉應力 (tensile stress, TS)、斷裂伸長率 (elongation at break, EAB) 和楊氏模數 (Young's Modulus, YM) 等機械性能，與交聯程度呈正相關，同時，亦大幅改善水不溶性，使複合膜在水中 98% 不溶於水，優於以戊二醛交聯處理之效果，主要歸因於 TG 改變蛋白質的 α 螺旋次級結構。

(ii) 改善薄膜水蒸氣阻隔性

蔬果收穫後的水分損失導致產品的收縮和重量減少。Marquez *et al.* (2017) 將分離乳清蛋白 (whey protein isolate, WPI) 與山梨醇、果膠混合製備複合塗層，添加 TG 後，應用於新鮮的蘋果切片、馬鈴薯及胡蘿蔔表面，進行 10 天冷藏觀察。結果顯示，添加 TG 複合塗層在儲存 6 天內能可防止重量損失，10 天後略有減重；相較之下，未添加 TG 的蛋白質塗層在 6 天後與未塗層的損失重量幾乎相等。此與 Jianga *et al.* (2016)、Ahammed *et al.* (2021)、Seiwert *et al.* (2021) 研究結果一致，經 TG 處理後，降低 WPC－CMC 複合膜、木聚醣－WPI 複合膜及明膠－玉米醇溶蛋白複合膜之水蒸氣滲透率 (water vapor permeability)。顯示 TG 可改善多種蛋白基質可食用塗層或薄膜之水蒸氣阻隔性。

(iii) 超音波預處理對 TG 的影響

研究顯示，超音波 (ultrasound, US) 預處理可有效改變蛋白質結構，使其容易受到 TG 作用，進而增強交聯效率。Ahmadi *et al.* (2017) 證實，超音波處理乳清蛋白溶液 2.5 分鐘，顯著降低流動行為指數 (flow behavior index, n)；在沒有超音波情況下添加 TG 對硬度值沒有影響，但經 US 處理樣品的硬度顯著增加，這顯示 US 對蛋白質結構的關鍵影響，但超音波處理時間達到 7.5 分鐘時，硬度顯著下降，可能是過度交聯阻礙均勻結構的形成。傳統上，乳清蛋白需要經過熱變性，使其結構容易被酵素作用，然而熱處理不僅需要較高的能源成本，還可能降低蛋白質的營養價值 (Phillips and Williams, 2011)。超音波預處理能部分取代熱變性，使蛋白質結構更易受酵素作用，使薄膜生產製程上可以達到更高效率和更低的成本。

酸、鹼或交聯劑等化學處理方法已被廣泛用於改善薄膜性能，用於蛋白質共價交聯的化學試劑包括戊二醛、乙二醛或甲醛等醛類，由於醛類化合物具有毒性，因此許多研究探討使用天然交聯劑來改善蛋白質薄膜的性能 (Wittaya, 2012)。Wang and Xiong (2021) 利用天然多酚－氧化阿魏酸 (oxidized ferulic acid, OFA) 與氧化單寧酸 (oxidized tannic acid, OTA) 改善乳清蛋白薄膜理化性質，結果顯示 OFA 僅在較高的處理濃度 (5.0%) 下提高了膜的拉伸強度和楊氏模數，但在 2.5 和 5.0% 的濃度下均提高了膜的延展性；OTA 在 2.5 和 5.0% 的濃度下均顯著提高了拉伸強度和彈性，但不影響膜的延展性。綜合而言，TG 作為天然交聯劑前提下，能夠改善蛋白質薄膜的性能，在實際應用時需控制反應條件，且相較於化學交聯劑成本較高，具成本效益驗證挑戰。

乳過氧化酶

I. 介紹

乳過氧化酶系統 (lactoperoxidase system, LPOS) 是一種存在於牛奶、淚液及唾液中的天然抗菌酶系統，系統包含三個主要元素：(I) 乳過氧化酶 (lactoperoxidase, LPO)、(II) 過氧化氫 (hydrogen peroxide, H_2O_2) 和 (Ⅲ) 硫氰酸鹽 (thiocyanate, SCN^-)。LPO 催化 SCN^- 的氧化，在 H_2O_2 的幫助下，生成氧化產物如次氫氰酸根 (hypothiocyanite, $OSCN^-$) 和次氫氰酸 (hypothiocyanous acid, HOSCN)，這些活性化合物透過氧化微生物蛋白質和酶中存在的巰基 (sulfhydryl, SH)，最終導致細胞死亡 (Yousefi *et al.*, 2022)。LPOS 具有廣泛的抗菌範圍，可用於食品包裝 (Mohamed *et al.*, 2013)。

II. 使用限制及加工食品應用範圍

LPOS 具抗菌特性，且被公認為安全物質 GRAS，因此常被作為天然的食品防腐劑進行研究 (Elliot *et al.*, 2004)。國際乳品聯盟 (International Dairy Federation) 發布在缺乏冷藏設備的情況下，應用 LPOS 作為生乳保存的指引方針 (IDF, 1988; Seifu *et al.*, 2005)，顯示 LPOS 的使用獲得國際乳業組織的認可，以及其在乳品冷鏈缺乏環境下之應用價值。LPO 在 78℃ 加熱 15 sec 內完全失活，在 pH 3 至 5 酸性條件下熱穩定性差，在 pH 3 時會失去活性 (de Wit and Hooydonk, 1996; Kussendrager and van Hooijdonk, 2000)。根據國際食品法典委員會 (Codex Alimentarius Commission, CAC) 的規定，新鮮生乳中添加約 10 mg 的 SCN^- 可外源性地活化 LPOS (Yousefi *et al.*, 2022)。LPOS 價格昂貴可能是導致其與海藻酸鈉塗層應用在雞胸肉研究不足的原因之一 (Yousefi *et al.*, 2018)。由於 $OSCN^-$ 的生成通常受限於 SCN^- 濃度，需依賴人工合成的 SCN^- 添加，Golmohamadi *et al.* (2016) 研究指出可望利用低經濟價值白芥種子粕農副產物作為 SCN^- 萃取來源，用於製備 GRAS 之 LPOS。LPOS 在食品工業中的

主要是作為天然的抗菌系統，用於延長食品的保存期限。在乳品產業，特別是在生乳儲存或運輸至加工廠過程中做為保鮮用途 (Seifu *et al.*, 2005)。在發酵乳製品方面，LPOS 對於發酵菌種造成影響，其能降低乳酸菌之酸生產速率，延遲優格製作的凝乳時間 (Basaga and Dik, 1994)；以 LPOS 處理乳源製作切達起司呈現較弱凝乳結構，產率略低於未經處理製作的生乳源，以 LPOS 處理山羊乳製作高達起司之脂肪分解程度較低，有助於減輕山羊起司濃烈風味，大腸桿菌及葡萄球菌數量亦較低 (Zall *et al.*, 1983; Seifu *et al.*, 2004)。在肉品方面，可減少豬肉火腿與肉醬氧化程度 (Beňo *et al.*, 2024)。將 LPOS 食品應用上，可能有降低產品製成率、影響發酵菌株、價格昂貴之限制，而將 LPOS 結合包裝，可避免對產品之影響，然而必須考量酵素的生產成本、有效載體等經濟及技術的因素。

III. LPOS 於食用包裝的應用

與直接應用抗菌劑相比，抗菌的可食用薄膜更受青睞，因為可以控制擴散到食品表面的劑量 (Yousefi *et al.*, 2022)。表 3 列出 LPOS 於食用包裝之抗菌效果。

多項研究證實含有 LOPS 的食用薄膜於海鮮食品具抗菌性。Min *et al.* (2005) 研究顯示，在 4 與 10℃的冷藏條件下，含有 LPOS 的乳清蛋白塗層可有效抑制冷燻鮭魚表面之李斯特單胞菌 (*Listeria monocytogenes*) 生長，約降低 1.5 log CFU/cm^2 的抑菌效果。相比之下，於瓊脂培養基中抑菌效果更顯著，達 4.2 log CFU/cm^2，被歸因培養基中水活性較高，促進 $OSCN^-$, HOSCN 等氧化產物擴散並發揮抗菌作用。相對地，煙燻鮭魚中含有較多 SH 化合物，易與氧化產物反應，降低 LPOS 抗菌效率。Rostami *et al.* (2017) 研究結果顯示，鱸魚片在 16 天貯存期間，乳清蛋白－ LPOS 塗層結合 MAP 相較於單一乳清蛋白塗層，具有最低的細菌數量 [腐敗希瓦氏菌 (*Shewanella putrefaciens*) 和螢光假單胞菌 (*Pseudomonas fluorescens*) 的總活菌數與低溫細菌數在貯存結束時均低於 7 log CFU/g]，總揮發性基氮 (total volatile basic nitrogen, TVB-N) 值最低。Ehsani *et al.* (2019) 研究使用含 LPOS 或鼠尾草精油 (sage essential oil, SEO) 的 3 種可生物降解薄膜，幾丁聚醣、藻酸鹽及明膠，對冷藏溫度 (4 ± 2℃) 下儲存 20 天的魚漢堡之品質影響，結果顯示含有 LPOS 幾丁聚醣薄膜，具有最低的細菌數量，而藻酸鹽薄膜的抑菌效果優於明膠薄膜，此外含有 LPOS 或 SEO 的幾丁聚醣薄膜具有良好的感官特性。進一步研究顯示 α －生育醇 (α-tocopherol) 可能影響 LPOS 的抗菌效果。Shokri and Ehsani (2017) 研究顯示含有 LPOS (2.5%) 和不同濃度 α －生育醇 (1.5 和 3%) 的乳清蛋白塗層溶液 (whey protein coating solution, WPS)。在 4℃冷藏期間對鱸魚片保質期 (16 天) 之初始總菌數 (total viable counts, TVC) 及嗜冷菌 (psychrotrophic bacteria) 數量顯著低於未添加 LPOS 組別 ($P < 0.05$)，而 LPOS + WPS 塗層對 4℃貯存下的鱸魚片對於 TVC、嗜冷菌、螢光假單胞菌及腐敗希瓦氏菌抑制效果最高外，同時發現，含有 α －生育醇 (1.5 和 3%) 會降低塗層的抗菌效果，依據此細菌學結果推論 α －生育醇可能會影響 LPOS 的抗菌效果，然而，需要更多研究來確認 LPOS 和 α －生育醇之間的相互作用機制。

幾丁聚醣由於帶正電荷的氨基 ($-NH_2$)，容易與多陰離子相互作用，形成聚電解質複合物 (Ardean *et al.*, 2021)，其亦可與其他抗菌性物質結合增加抗菌效果。Mohamed *et al.* (2013) 探討不同濃度的幾丁聚醣膜 (0.5、1 及 1.5%) 與乳過氧化酶系統 (LPOS) 的結合，對於多種病原菌的抑制效果，結果顯示 1 和 1.5% w/w 的幾丁聚醣結合 LPOS 和 / 或碘對 *Xanthomonas campestris* pv. *mangifera indica* 的抑制效果高於單獨使用的幾丁聚醣膜或低濃度 (0.5%) 的幾丁聚醣。另 Cissé *et al.* (2012) 製備幾丁聚醣－ LPOS 複合膜，抗菌效果受溫度和貯存時間的影響，在 25℃下製作並存放 5 天時，抗菌效果最佳，$OSCN^-$ 與 OI^- 濃度隨著溫度增加至 25℃達到最高值，此乃化學反應速率隨溫度升高而增加，使分子獲得的動能增加，從而提高有效碰撞的次數，導致反應產物的生成。

Yousefi *et al.* (2018) 研究含有不同濃度 LPOS 的藻酸鹽塗層對雞胸肉片保質期的影響，結果顯示 4 和 6% 的 LPOS 濃度對綠膿桿菌 (*Pseudomonas aeruginosa*) 有效的抗菌效果，感官評估中含 6% LPOS 塗層的評分最高與不含 LPOS 的藻酸鹽塗層分數有顯著差異 ($P < 0.05$)，顯示高濃度 LOPS 對於雞胸肉片的整體感官特性具有正面的影響。

綜合上述多項研究結果，LPOS 應用於食用包裝中，展現出良好的天然抗菌潛力，可於魚肉與禽肉等高蛋白、高水活性食品的保鮮應用，主要機制來自於 LPOS 系統中活性氧化產物 (如 $OSCN^-$、HOSCN) 對微生物的抑制作用。然而，LPOS 的抗菌效果受多項因素影響，包括基質中 SH 化合物的中和作用、膜製備條件、貯存溫度及與其他添加劑 (如 α- 生育醇) 的拮抗性。因此，雖然 LPOS 作為食品包裝中天然抑菌技術的應用潛力廣泛，仍需針對其與不同材料、環境條件及活性成分間的交互作用進行深入探討與改善其實用性。

表 2.　轉麩醯胺酸酶於食用包裝對食品保鮮之效用

Table 2.　The effects of transglutaminase on edible packages for food preservation

Edible packages	Activity of TG	Food	Function	References
Whey protein -carboxymethylated chitosan composite films	10 U/g protein	—	Improved the water vapor barrier properties and mechanical properties, and there was no impairment of thermal stability of composite films.	Jiang *et al.*, 2016
Whey proteins film	10 U/g protein	Cheese slices	Increased puncture deformation values and made films less green, more yellow and darker than non - added films.	Cruz - Diaza *et al.*, 2019
Cardoon seed oil cake proteins film	100 mU/mL	Peanuts	Improved Young's modulus and tensile strength, less hydrophilic and permeable to gases likely due to the formation of a more compact structure due to isopeptide bonds formed by TG.	Mirpoor *et al.*, 2024
Zein - oleic acid composite films	—	—	Under low pH and high drying temperatures with TG treatment improves tensile strength, solubility, and water vapor permeability.	Masamba *et al.*, 2016
Gelatin - zein composite films	—	—	Improved water insolubility, water vaper permeability and mechanical properties are potential candidates for high moisture food packaging application.	Ahammed *et al.*, 2021
Xylan - whey protein isolate composite films	30 IU/100g WPI	—	With the addition of xylan and TG, water vapor permeability and oxygen permeability decreased, and tensile stress increased.	Seiwert *et al.*, 2021
Whey protein - pectin edible coating	8 U/g protein	Apples, carrots, potatoes	Reduction of weight loss, microbial growth prevention, antioxidant activity preservation and no change in fruit and vegetable hardness and chewiness.	Marquez *et al.*, 2017
Whey protein concentrate carboxymethyl - chitosan composite films	6 U/g protein	—	Improved the film's elongation at break, tensile strength, and crystallinity.	Jiang *et al.*, 2020

表 3.　乳過氧化酶系統於食用包裝之抗菌效果

Table 3.　Antimicrobial effects of lactoperoxidase system on edible packages

Edible packages	Concentration of LPOS	Function	References
Whey protein packages	29 mg/g of coating	Reduced the number of *Listeria monocytogenes* on smoked salmon.	Min *et al.*, 2005
Chitosan film	5% v/v of film	Inhibited Xanthomonas campestris pv. Mangiferae indicae and did not changed the chitosan film permeability to gas and water vapor.	Cissé *et al.*, 2012
Chitosan film	5% v/v of film	Inhibited bacterial and fungal. Presence of a second electron donor such as iodine could positively influence the antimicrobial effect of LPOS against some mango pathogens.	Mohamed *et al.*, 2013
Whey protein coating	1.25, 2.5, 5 or 7.5% v/v	Reduced *Shewanella putrefaciens* and *Pseudomonas fluorescens* of the rainbow trout fillets more than 1.5 log.	Shokri *et al.*, 2014
Whey protein coating	2.5% v/v	Whey protein coating combined LPOS and MAP packaging inhibited microbial growth, reduced the TBARS values and TVB - N formation.	Rostami *et al.*, 2017
Whey protein coating	2.5% v/v	Combination of LPOS and a-tocopherol inhibited bacteria, showed antioxidant properties, and could have a high potential to be applied in the shelf life extension of pike - perch fillets during cold storage.	Shokri and Ehsani., 2017
Alginate coating	2, 4 or 6% v/v	Alginate coatings containing 6% v/v LPOS exhibited better antibacterial effects.	Yousefi *et al.*, 2018
Alginate film, chitosan film, gelatin film	10% v/v of film	Chitosan films containing LPOS significantly suppress *Pseudomonas* spp. and *Shewanella* spp. during 20 - day storage at refrigerated condition compared with other treatments ($P < 0.05$).	Ehsani *et al.*, 2019

溶菌酶

I. 介紹

溶菌酶 (lysozyme, LY) 為天然抗菌劑，也稱為胞壁酸酶，是一種小型水解酶，在自然界中無處不在，幾乎存在於所有分泌物、人類、動物組織及鳥蛋中 (Amara *et al.*, 2016)。它被認為是一種耐高溫酵素，在廣泛的 pH 範圍內具有高穩定性 (Amara *et al.*, 2016)。它的抑菌機制可以分為酵素的溶解及非酵素溶解的抗菌機制 (Wu *et al.*, 2019)。它通過破壞細菌細胞壁肽聚醣聚合物中的 N- 乙醯胞壁酸 (nacetyl muramic acid) 和 N- 乙醯葡萄醣胺 (N-acetyl glucosamine) 的 β 1 － 4 醣苷鍵，對革蘭氏陽性細菌具有很強的抗菌活性 (Amara *et al.*, 2016)。當 LY 與乙二胺四醋酸 (ethylenediaminetetraacetic acid, EDTA) 結合時，EDTA 會破壞革蘭氏陰性菌的外膜，顯著增加抗菌範圍 (Kandemir *et al.*, 2005)。近幾十年來，與生鮮和最少加工產品相關的食源性疾病日益嚴重，抗菌包裝是一個有前景的領域，因為它可以通過使用最少量的抗菌劑來抑制食品表面的致病或腐敗微生物的生長 (Boyacı *et al.*, 2016)。作為一種天然存在且具有抗菌活性的酵素，LY 亦常添加到包裝材料中，增加抑菌性 (Kandemir *et al.*, 2005; Datta *et al.*, 2008)。表 4 列出食用包裝添加 LY 的抗菌效果。

II. 使用限制及加工食品應用範圍

美國食品藥物管理局和歐盟批准 LY 為公認安全的食品添加劑 (Kaewprachu *et al.*, 2015)。溶菌酶在溫度 30 － 60℃、pH 4 － 7 範圍間具有良好的穩定性 (Yao *et al.*, 2022)。然而，在溶入包裝後，其酶活性可能會降低。研究指出，以天然的胞外多醣體基質製備薄膜時，酵素活性回收率僅為理論值的 23 至 70% 之間；此外，薄膜中含有蔗糖，可能會降低其活性穩定性，於 21 天儲藏後，活性損失接近 35% (Kandemir *et al.*, 2005)。LY 與 LPOS 的價格相較於其他食品防腐劑更高，因此有許多研究集中於其固化與改質。為提升溶菌酶對革蘭氏陰性菌的抑菌能力，已有許多透過紅外線光譜、螢光光譜、核磁共振等物理或化學方式改質溶菌酶之研究日益增加 (Wu *et al.*, 2019)。另外，將溶菌酶和與螯合劑併用，可增強對革蘭氏陰性菌的抗菌效果 (Padgett *et al.*, 1998)。但當鈣、鎂、鐵、鈉、鉀離子等多種陽離子的存在時，會降低甚至中和溶菌酶 - 螯合劑組合抗菌效果 (Boland *et al.*, 2004)。在食品加工應用方面，LY 與膠結合可作為防腐劑與乳化劑應用於蛋黃中 (Hashemi *et al.*, 2018)。將梨子浸入溶菌酶溶液中能減少褐變指數和總細菌數，並保持總酚含量 (Xu *et al.*, 2019)。此外，溶菌酶、乳酸菌素與 EDTA 的組合可用於控制影響醃製肉品安全與腐敗的特定菌種，將抗菌劑融入在包裝材料中，可將其濃度集中於微生物活躍區域，可能提升效抑制敏感菌種 (Gill and Holley, 2000)。

III. LY 於食用包裝的應用

Yüceer and Caner (2020) 研究結果發現溶菌酶－幾丁質 (LY － C) 塗層具有提高蛋殼強度的效果，能延長保質期，但臭氧及超音波處理的雞蛋在儲存期間可維持較低的細菌數量，優於 LY － C 塗層。Kaewprachu *et al.* (2015) 研究在冷藏儲存期間 (4℃，7 天)，比較豬絞肉用兒茶素－ LY 混合明膠複合膜 (catechin-lysozyme gelatin film, CLGF) 和商業薄膜 (聚氯乙烯，PVC) 包裹的品質，結果顯示商業薄膜之總菌數、酵母及黴菌等微生物的生長顯著高於 CLGF 包裝 ($P < 0.05$)；CLGF 包裝的 TBARS 略有增加，而商業薄膜的 TBARS 為顯著增加 ($P < 0.05$)，顯示食用薄膜以 LY 塗層處理具有延長豬絞肉保質期之潛力。

乳酸菌可以增加起司酸度，有助於風味提升，而 LY 潛在作用可能對參與起司熟成的乳酸菌造成影響。D'Incecco *et al.* (2016) 研究 Grana Padano cheeses (GPC) 中常檢出的菌種：*Lactobacillus helveticus, Lb. delbrueckii* 和 *Lb. rhamnosus*，及游離胺基酸在添加或不添加 LY 製作的起司之間的差異性，結果顯示游離胺基酸總含量在添加或不添加 LY 的 GPC 之間無顯著差異 ($P = 0.48$)，顯示蛋白水解以相同速率進行；幾丁聚醣－溶菌酶 (CL) 和幾丁聚醣－鏈黴菌素 (CN) 塗層對 Halloumi cheese 的感官性質均無不良影響，而在 3℃貯存 35 天期間 2 種塗層對於 Halloumi cheese 浸泡在 10 和 15% 鹽水中之微生物數量沒有顯著差異，顯示 CL 和 CN 塗層在 10 和 15% 鹽水中對測試的微生物具有相似的抑制效果，建議鹽水溶液的鹽含量可以從 15% 降至 10% (Mehyar *et al.*, 2017)。

Boyacı *et al.* (2016) 研究，通過酸化來活化薄膜抗菌功效，結果顯示，當 pH 值降低到 5.5 以下時，LY 開始釋放，隨著 pH 值從 5.5 逐漸降低到 3.0，釋放的 LY 含量和釋放速率增加。Wei *et al.* (2017) 研究發現抗壞血酸 (ascorbic acid, AA) 的添加濃度小於 3.1 $mg.cm^{-2}$ 時， LY 釋放速率顯著降低 33.7% ($P < 0.05$)，並增加總釋放 LY 約 29.8 到 89.3% ($P < 0.05$)，且抗菌活性隨 AA 濃度的增加而增強。Güçbilmez *et al.* (2007) 在玉米醇溶蛋白薄膜中加入鷹嘴豆白蛋白萃取物 (chickpea albumin extract, CPAE)，結果顯示改善部分純化的 LY 在玉米蛋白薄膜中的分佈，並減緩 LY 釋放速度 1.5 到 3.5 倍，主要取決於 CPAE 添加濃度。Fabra *et al.* (2013) 研究發現豌豆蛋白和

玉米澱粉膜中，添加 LY，在 10 與 25℃環境下，均能擴散到食物模擬物表面 (瓊脂)，但最高的 LY 量仍然停留在食品模擬物的表面，LY 主要在食品表面發揮作用，是由於高分子量限制其在食品模型中的擴散。以上研究顯示 LY 在薄膜或塗層中的釋放受多種因素影響，包括薄膜材料的類型、CPAE 及 AA 等其他添加物協同作用、pH 及溫度等環境條件，了解這些因素如何影響 LY 釋放，有助於設計可控制釋放速率的抗菌膜。

傳統食品保存方法如冷藏、冷凍及化學防腐劑，但隱藏損傷食品品質及化學防腐劑對於健康影響等缺點，因此消費者傾向選擇天然的防腐劑 (Farshidi *et al.*, 2018)。近年有研究將天然的精油、有機酸及細菌素等結合至包裝材料中，然而精油與有機酸往往伴隨氣味添加 (Catarino *et al.*, 2017; Ehsani *et al.*, 2019; Venkatachalam and Lekjing, 2020)，可能影響食品風味。LPOS 與 LY 被公認為安全物質或安全添加劑，能夠提升可食用包裝抗菌性，然而其價格相較於其他食品防腐劑更高，限制在大規模中的應用。

其他酵素於可食用包裝之應用

蛋白質交聯是指在多肽鏈內部或蛋白質分子之間形成共價鍵，這個過程可以透過化學試劑或生物催化劑的作用下進行，多種轉移酶和氧化還原酶都能引發蛋白質交聯。其中，TG 在蛋白質交聯方面的應用已被廣泛研究和認可。此外，小漆酶 (small laccase, SLAC) 和山葵過氧化酶 (horseradish peroxidase, HRP) 等氧化還原酶也能促進蛋白質的共價交聯並改變其性質，酵素在改善和優化食用薄膜性能方面具有重要意義。在此整理其他數種酵素應用在可食用包裝的研究成果，以提供更全面的見解。

Yang and Zhao (2022) 研究指出在 H_2O_2 的存在下，HRP 可以氧化蛋白質中的酪胺酸殘基，促使蛋白質交聯，葡萄糖氧化酶 (glucose oxidase) 可以催化 D- 葡萄糖的氧化，即 H_2O_2 和葡萄糖酸，HRP、葡萄糖氧化酶及 D- 葡萄糖組成的三元系統可能誘導蛋白質交聯，這種三元系統可通過一步法和兩步法進行交聯：(i) 一步法：HRP、葡萄糖氧化酶和 D- 葡萄糖同時加入蛋白質受質中，整個反應系統在一定時間內進行葡萄糖氧化和蛋白質交聯；(ii) 兩步法：葡萄糖氧化酶和 D- 葡萄糖同時加入蛋白質受質中進行葡萄糖氧化後，再將 HRP 加到反應系統中進行蛋白質交聯。整體而言，一步法比兩步法更有效地誘導蛋白質交聯，因 HRP 對形成 H_2O_2 的利用效率更高。

Zhou *et al.* (2020) 探討 SLAC 對乳清分離蛋白－果膠複合膜的影響，結果顯示，SLAC 的加入對膜的結晶度影響不大，不同濃度的 SLAC 對膜的顏色影響不顯著，然而，使用 4 U/100 mL 濃度的 SLAC，膜的透明度最好，使用 6 U/100 mL 濃度的 SLAC 具最佳的交聯效果，薄膜之含水量、水蒸氣穿透率及透氧性最低，顯示 SLAC 在 WPI-果膠複合膜具降低含水量和提高機械性能方面應用潛力。

Tarhan and Sen (2022) 比較熱變性與鹼酶 (alcalase enzyme) 對含有馬鬱蘭精油 (marjoram essential oil) 和百里香 (thyme) 萃取物的乳清和大豆蛋白食用包裝的影響，與熱變性薄膜相比，酵素水解處理後的薄膜顯得更薄、硬度較低及黏著性較高，這可能是酵素水解導致蛋白質分子重組和聚集減少，另鹼酶水解薄膜的水蒸氣滲透性低於熱變性薄膜，使其在塗層應用中具有更好的屏障性能，因此，在起司塗層應用結果顯示，鹼酶能提供更好的防潮保護，相較於熱變性能顯著減少起司硬度的增加。

結　論

可食用包裝廣泛應用在保鮮食品，其具備環境友善與功能性優勢，近年來逐漸受到重視，美國 Apeel Sciences 公司開發以植物源的可食性塗層，藉由防止水分流失和氧化延長酪梨、柑橘類等鮮果之保鮮期，並已進入美國 Costco 與 Kroger 等大型零售通路販售；美國 Mori 公司所研發之蠶絲蛋白塗層技術，則應用於生鮮肉品、魚類與蔬果，具有減緩水分散失與氧化作用；英國 Notpla 公司開發海藻為基質製作可食用薄膜，作為容器裝盛水並可直接食用。可食用包裝技術逐漸已進入市場商品化應用。包裝的基質常使用碳水化合物、脂質、蠟及蛋白質等生物聚合物，通過功能性成分的添加以提升食品包裝應用性能，酵素被廣泛使用作為提升食用薄膜性能的添加物之一，在前述研究成果中，展現出酵素在提升食品薄膜性能之潛力。TG、LPOS 及 LY 等酵素被公認為安全物質或安全添加劑，然而其在工業化應用仍受生產成本限制。為降低成本，已有研究利用低經濟副產物作為萃取來源，並符合永續理念；同時，透過物理或化學改質提升酵素功能效率，也是可行途徑。綜合而言，若能兼顧低成本開發與功能提升，將有助於酵素在可食用包裝上應用，滿足食品保存的需求與產業發展。

表 4.　溶菌酶於食用包裝之抗菌效果

Table 4.　Antimicrobial effects of lysozyme on edible packages

Edible packages	Concentration of LY	Function	References
Zein film	1,400 -2,800 U/cm²	The combinational incorporation of partially purified lysozyme with disodium EDTA·$2H_2O$ or CPAE and disodium EDTA·$2H_2O$ gave zein film effective on *Escherichia coli* and *Bacillus subtilis*.	Güçbilmez *et al.*, 2007
Isolate pea protein, starch based films	0, 50, 75 and 100 mg/g hydrocolloid	Pea protein and starch films containing LY exhibited against *Listeria monocytogenes*.	Fabra *et al.*, 2013
Gelatin film	100 g	Gelatin film containing LY - catechin applied to minced pork exhibited lower microbial growth compared to commercial film.	Kaewprachu *et al.*, 2015
Whey protein - oleic acid films	7.87 mg/g film solution	The smoked salmon slices coated by activated LY containing whey protein - oleic acid films showed significantly lower (almost 0.6 decimal) *Listeria innocua* counts than controls after 1 week at 4°C.	Boyacı *et al.*, 2016
Zein based films	16.5 mg/g film solution	AA effectively sustained the release of LY and antimicrobial activity increased with the increase of AA concentration.	Wei *et al.*, 2017
Chitosan coating	60% w/w film solution	Reduced psychrotrophs, anaerobes, lactic acid bacteria, and yeasts and molds by 1.8 - 2.2 log in 10 - 15% NaCl brined cheese.	Mehyar *et al.*, 2017
Chitosan coating	20% dry weight film solution	Enhanced the antibacterial characteristics of eggs while prolong the storage period at ambient temperature.	Yüceer and Caner, 2020

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Review of enzymatic treatment on the properties of edible packaging materials and the impact on food preservation [1]

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Abstract

The use of petroleum-based polymer materials in traditional packaging poses significant environmental pollution concerns. As a result, the food packaging industry is faced with the challenge in developing materials that are both environmentally friendly and economically viable. Biodegradability and renewable properties of alternatives packaging materials are becoming increasingly important. The paper reviews the application of enzymes in edible packaging, including transglutaminase (TG), the lactoperoxidase system (LPOS), and lysozyme (LY), which could improve the packaging performance. TG catalyzes protein cross-linking, enhancing the mechanical properties of protein films and improving water vapor permeability. Both LPOS and LY are natural antimicrobial agents that exhibit strong antimicrobial properties in edible packaging, in order to extend the shelf life of food. Furthermore, the combination of TG and high-intensity ultrasound pretreatment can significantly enhance the impact on film performance. The paper also analyzes the synergistic effects or influence of other additives, such as ascorbic acid (AA), chickpea albumin extract (CPAE), and α-tocopherol, on food packaging. In summary, this review highlights recent research on the application of enzymes in food packaging, providing insights to the potential of enzymes in edible packaging and extension of shelf life, and offering valuable references for future studies.

Key words: Edible Films, Coatings, Enzymes.

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