

以納豆菌發酵生產凝乳酵素之研究

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摘要

近幾年來，由於犢牛凝乳酶產量不足，細菌所生產的凝乳酶經廣泛的被接受成為犢牛凝乳酶產品之一。本論文研究是藉由納豆菌 (*Bacillus subtilis natto* Takahashi) 的固態及液態培養，來獲得環境因子對其生產凝乳酶的影響，並以回應曲面法 (RSM) 探討生產因子之最佳化，同時以超過濃縮裝置、電泳 (SDS-PAGE) 進行部份純化及分子量分析，並進行酵素活性影響因子之影響。由納豆菌的固態培養可以得知，在 37 °C、pH 6、溼度 70% 的環境下，以米殼和基本鹽類來培養 72 小時之後，有最高的凝乳比率。而由納豆菌的液態培養可以得知，在 37 °C、pH 6、轉速 175 rpm 的環境下，以澱粉、玉米抽出物、黃豆粉、乾奶粉以及基本鹽類來培養 72 小時之後，有最高的凝乳比率。由回應曲面法的結果可以得知，在配方組成濃度分別為：澱粉 55.41 g/L、玉米抽出物 1.5 g/L、黃豆粉 2.69 g/L 以及乾奶粉 22.29 g/L 時，有最高的凝乳活性 1048.02 SU/mL。經由超過濃縮裝置以及電泳分析之後，可以得知納豆菌所生產的凝乳酶分子量介於 20000 - 30000 g/mole 之間。納豆菌所生產的凝乳酶在牛奶基質溫度為 60 °C、pH 6 時，有最佳的酵素活性；在 60 °C 加熱 60 分鐘或者 70 °C 加熱 5 分鐘之後，此凝乳酶喪失 80% 以上的酵素活性。而由 pH 對其酵素活性的影響結果中可以得知，在 pH 5 - pH 6 之間，此酵素的凝乳活性則是相當的穩定。由各金屬離子對納豆菌凝乳酶的影響結果中可以得知，一價陽離子對其凝乳活性並無顯著影響，但是二價陽離子卻是有明顯的促進凝乳活性的效果。而在添加汞離子時，此酵素會馬上失去其酵素活性，所以汞離子為此納豆菌凝乳酶的抑制劑。

關鍵詞：凝乳酶、納豆菌、回應曲面法、凝乳比率、酵素活性、抑制劑

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