# 開發微藻脂肪酸快速分析方法

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摘要

微藻中含有豐富營養素且生長快速，其中富含對人體有益之 EPA、DHA 以及 ARA 等，故分析微藻脂肪酸組成具有營養上意義。然而，微藻的細胞壁厚需經細胞破壁才能完整的萃取脂肪，而超音波法是眾多細胞破碎中具快速、方便且安全的技術；此外，現今 GC-MS (gas chromatography-mass spectrometry) 的應用性廣泛，且普遍存在於各分析場所，因此本研究將探討超音波細胞破壁輔助萃取方式用於改善傳統索氏與研磨珠法對微藻脂肪萃取步驟，並結合 GC-MS 進行脂肪酸分析。藉由 GC-MS 定量脂肪酸需先建立以質譜作為偵測器之相對反應係數，結果顯示用於驗證係數正確性的樣品於不同偵測器下之分析結果，經反應係數校正後其脂肪酸組成相近，各脂肪酸標準品與內部標準品在質譜中各濃度比值的線性迴歸之相關係數皆大於 0.99，顯示本研究建立的反應係數正確。另外，微藻以索氏法萃取 6 個小時得到脂肪酸量為 4.99±1.77 mg/g，而使用常用於破碎微藻之研磨珠法在乙醇-正己烷的輔助萃取下，總含量可達 18.64±1.62 mg/g，顯示索氏法萃取效果不佳，然而藉由超音波前處理僅需 20 分鐘，其脂肪酸含量與研磨珠法具有相同的結果，但相較於超音波，研磨珠法需特殊設備且整體操作耗費 2 小時以上。本研究使用之超音波法在試驗時經超音波處理之樣品未分離脂肪，徑直將萃取液連同藻渣直接進行後續衍生化，結果顯示 0-30 分鐘的超音波作用下脂肪含量並無差異，表明不經超音波也可在後續的皂化步驟萃取脂肪，而經進一步的確認，遂將超音波萃取後之萃取液分離，得到脂肪含量僅 4.11±2.43 mg/g，因而發現超音波並不能增加微藻脂肪萃取率。其後為了證明直接以脂肪酸分析皂化步驟，使用氫氧化鈉-甲醇溶液於 80℃下加熱 15 分鐘，即可達到良好脂肪萃取作用，遂開發微藻脂肪酸之快速分析方法，該方法可以有效地從學術界公認細胞壁厚且堅固的小球藻中萃取脂肪，並分析其脂肪酸組成。除此之外，微藻一同以此法進行分析，並與文獻比較結果相符，顯示本方法具有良好的萃取與分析效果。綜合上述，微藻不經萃取，直接以本法進行皂化後衍生化再搭配 GC-MS 分析得以將傳統脂肪酸分析前處理 6 小時的時間縮短至 40 分鐘。

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