



綠豆芽脂氧合酶之性質及水產香氣之形成

Characterization of Lipoxygenase from Mung Bean Seedlings and Its Effect on Sea Food Flavor Formation

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一、中文摘要

本研究乃探討綠豆於發芽過程中脂氧合酶之變化及其特性。將綠豆浸水4~5小時後，於自動培育機中發芽4天，於發芽第1.5天之脂氧合酶活性達到最高，約為原來酵素活性之5倍。將發芽1.5天之綠豆芽製備為粗抽出液再經20-50%硫酸銨分劃、Hydroxyapatite管柱層析及Superdex pg 200膠過濾分離後，脂氧合酶活性(LOX)純化了18倍，回收率為4.3%。該部分純化的LOX之最適溫度及pH分別為40°C及pH 6.4，於溫度40°C以下及pH 5.5~7.5下綠豆芽LOX之活性穩定。該LOX對於不同脂肪酸之反應性以18:2最高，其次為20:5、18:3、20:4、22:6。利用正相高效液相層析分析得知，綠豆芽LOX對18:2之氧化產物為9-及13-HODE，比率為6.3:1；對18:3為13-HOTE (100%)。綠豆芽酵素抽出液與不同過氧化物反應後，可形成濃厚之green, cucumber, melon等風味。

關鍵詞：脂氧合酶、綠豆芽、純化、香氣

Abstract

Lipoxygenase (LOX) (linoleate:oxygen oxidoreductase, E.C. 1.13.11.12) activity of mung bean during germinating process was studied. The mung bean seedlings were cultivated over a period of 4 days and LOX activity increased and reached a maximum about 5-folds at day 1.5. LOX from mung bean seedlings was further isolated and purified using 20-50% saturation of ammonium

sulfate fractionation, hydroxyapatite column chromatography and gel filtration on Superdex pg 200. The optimal pH of the partially purified LOX was 6.4, and optimal temperature was 40 °C. At pH ranged 5.5 to 7.5 and temperature below 40 °C, the LOX activity was stable. The LOX from mung bean seedlings showed the highest reactivity toward 18:2 followed by 20:5, 18:3, 20:4 and 22:6. Based on retention time in normal phase HPLC, the products of 18:2 reacting with LOX of mung bean seedlings were 9- and 13-HpODE (hydroperoxyoctadecadienoic acid) at a ratio of 6.3:1, that from 18:3 was 13-HpOTE (hydroperoxyoctadecatrienoic acid). Mung bean extract treated with 13-HpODE or 13-HpOTE developed strong green odor, while those treated with 9-HpODE or 9-HpOTE produced sweet, cucumber or melon-like aroma.

Keywords: *Lipoxygenase, mung bean seedlings, purification, flavor*

二、緣由與目的

市售水產香料之香氣物質較不足，如何強化此等水產香料使其更接近海鮮香氣，仍值得研究。為強化市售水產香料之香氣，探討具水產或海鮮香氣之化合物或形成機制，可能是未來此一領域之研究發展的重點。(Pan and Kuo, 1994)

水產香氣成分之形成與部分水果香氣之產生極為類似，主要參與之酵素為脂氧合酶(lipoxygenase, LOX)與過氧化物水解酶(hydroperoxide lyase, HPLS)。利用脂氧合酶途徑相關酵素來產生此類天然香氣成

分被認為是較有效率、便宜且具有環保概念的方法(Fabre and Goma, 1999)。因此最近許多利用 LOX 的專利 (Patent USN4806379、USN9526413、NEP597069、EP0481147、NFR2696192)也相繼被發展出來。

於前幾年之研究中發現大型藻類之 LOX 活性極高以及香蕉葉中 LOX 具親油性，其對水產香氣形成之影響也已探討 (Pan and Kuo, 1994, Kuo, et al., 1994, 1996 a & b, 1997, 胡, 1998, Kuo et al., 2000, 2001)。近幾年來，有關機能性食品之研究大受重視。因此若脂氧合酶之來源具有機能特性時，不但可以修飾、形成水產風味外，並可獲得保健上之意義。例如，以此用於魚漿製品時，不但加強其鮮魚味外，並可提高魚漿製品之機能特性。故嘗試從“生機飲食”之素材中（亦即芽菜類，例如苜蓿芽，綠豆芽、黃豆芽、豌豆芽、小麥草等），篩選具有脂氧合酶活性之材料，初步發現綠豆芽菜（亦即市售之豆芽菜）不但脂氧合酶活性相當高。將綠豆發芽後，脂氧合酶活性大為提高；於發芽後2-3天，脂氧合酶活性達到最高，其比活性約為海藻之20倍，魚鰓之1000-1200倍左右 (Kuo et al., 2000)。

故本計畫中將研究綠豆芽脂氧合酶之特性，後續並將利用固定化酵素方法探討形成水產香氣之可行性。藉綠豆芽脂氧合酶形成水產香氣，將可大為提高其利用價值。由水產香氣物質形成之途徑及最適條件，或可作為未來發展水產香料之參考。

### 三、結果與討論

將綠豆浸水4~5小時後，於自動培育機中發芽4天。於發芽第1.5天之脂氧合酶活性達到最高，約為原來酵素活性之5倍(圖一)。將發芽1.5天之綠豆芽製備為粗抽出液再經20-50%硫酸銨分割、Hydroxyapatite 管柱層析及 Superdex pg 200 膠過濾分離後，脂氧合酶活性(LOX)純化了18倍，回收率為4.3% (表1)。純化過程之 Hydroxyapatite 管柱層析圖如圖二所示，Superdex pg 200 膠過濾如圖三所示。該部分純化的 LOX 經不連續電泳分析仍有三個電泳帶，以膠過濾分析其分子量約為91KD。其最適溫度及 pH 分別為40°C (圖四)及 pH 6.4 (圖五)，於溫度40°C以下 (圖六)及 pH 5.5~7.5 下 (圖七)綠豆芽 LOX 之活性穩定。該 LOX 對於不同脂肪酸之反應性以18:2最高，18:3、20:4、20:5、22:6之反應性差異不大(表2)。利用正相高效液相層析分析得知，綠豆芽 LOX 對18:2之氧化產物為9-及13-HODE，比率為6.3:1；對18:3為9-HOTE (圖八)。若將綠豆芽酵素抽出液再與9 or 13-HpODE、9 or 13-HpOTE 反應30分鐘，則會產生哈密瓜味、香瓜味、甜瓜味、青草味、小黃瓜等風味(表3)，該風味成份正利用 GC-MS 加以探討。

### 四、謝辭

感謝本校校長王昭雄博士於研究經費及實驗環境之支持以及海洋大學水產食品科學研究所孫寶年教授之提攜與幫忙，使得實驗得以順利進行。

Table.1 Purification of lipoygenase from mung bean seedlings

stage	total activity ( $\mu\text{mole}/\text{min}$ )	total protein (mg)	specific activity ( $\mu\text{mole}/\text{mg}\cdot\text{min}$ )	recovery (%)	purification (fold)
crude extract	1972.6	1120.8	1.76	100	1.0
20-50% (NH <sub>4</sub> )SO <sub>4</sub>	982.4	328.6	2.99	49.8	1.7
Hydroxyapatite	159.8	18.9	8.45	8.1	4.8
Superdex pg 200	84.8	2.7	31.42	4.3	17.9

Table 2. Formation of hydroperoxide from polyunsaturated fatty acid reacted with partially purified lipoxygenase from mung bean seedlings.

fatty acids	hydroperoxide ( $\mu\text{mole/mg}$ protein-min)	relative activity (%)
18:2 ( $\omega$ -6)	0.56	100
18:3 ( $\omega$ -3)	0.42	74.1
20:4 ( $\omega$ -6)	0.41	72.7
20:5 ( $\omega$ -3)	0.43	77.1
22:6 ( $\omega$ -3)	0.40	71.8

表三. 綠豆芽酵素抽出液與不同過氧化物反應後之香氣描述

基質	香氣描述
18:2-9OOH	哈密瓜味、青草味、香瓜味、甜瓜味
18:2-13OOH	濃青草味
18:3-9OOH	小黃瓜味、香瓜味、哈密瓜味
18:3-13OOH	濃郁的青草味、小黃瓜味

## 五、參考文獻

- Kuo, J.M. and Pan, B.S. Effect of lipoxygenase on formation of cooked shrimp flavor compound- 5,8,11-tetradecatrien-2-one. *Agric. Biol. Chem.* 1991, 55, 847-848.
- Kuo, J.M. and Pan, B.S. Occurrence and properties of 12- lipoxygenase in the hemolymph of shrimp (*Penaeus japonicus* Bate) *J. Chinese Biochem. Soc.*, 1992, 21, 9-16.
- Kuo, J.M., Pan, B.S., Zhang, H. and German, J.B. Identification of 12-lipoxygenase in the hemolymph of tiger shrimp (*Penaeus japonicus* Bate), *J. Agric. Food Chem.*, 1994, 42, 1620-1623.
- Kuo, J.M., Hwang, A., Hsu, H.H., and Pan, B.S. Preliminary identification of lipoxygenase in algae (*Enteromorpha intestinalis*) for aroma formation, *J. Agric. Food Chem.*, 1996a, 44, 2073-2077.
- Kuo, J.M., Hwang, A., Hsu, H.H., and Pan, B.S. Identification of lipoxygenase isozymes in marine green algae for aroma formation, Volunteered paper of IFT Meeting on 1996, 1996b, section No: 80D-18.
- Kuo, J.M., Hwang, A. and Yeh, D.B. 1997 Purification, Substrate Specificity and Products of a  $\text{Ca}^{2+}$ -Stimulating Lipoxygenase from Sea Algae (*Ulva lactuca*) *J. Agric. Food Chem.*, 45(6), 2055-2060.
- Kuo, J.M. (郭建民), Lin, A.C. (林安琪), Lin, Y.C. (林雅純), Yuan, L.L. (袁巧玲), Chen, L.S. (陳麗淑) and Wu, C.H. (吳佳慧) 2000 Characterization of lipoxygenase from mung bean seedlings, 第 30 屆食品科技學會年會論文, Sec. No.: PE-21
- Kuo, J.M., Hwang, A., Wu, H.C., Chu, H.L. and Yeh, D.B. 2001 Lipoxygenase from Banana Leaf: Purification of an enzyme that catalyzed the oxygenation of linoleic acid at 9-position IFT Annu. Meet., Abstract 44C-2.
- Pan, B.S. and Kuo, J.M. Flavor of shellfish and kamaboko flavorants, In *Seafoods: Chemistry, Processing, Technology and Quality*; F. Shahidi and J.R. Botta Eds.; Blackie Academic & Professional, London, 1994; pp.85-114.
- Pan, B.S. and Kuo J.M 2000 Lipoxygenase in Seafood Enzymes edited by Norman F. Haard and Benjamin K. Simpson pp.317-336, Marcel Dekker, Inc, New York.

## 六、自評

本研究已順利完成計畫之目標，不但將酵素純化，並且探討其特性。初步發現以綠豆芽酵素確可形成良好之香氣成分。然而綠豆芽酵素穩定性不佳，於純化過程酵素漸漸失活，此為後續研究需克服之問題。目前正嘗試瞭解其失活之原因，並探討固定化後酵素之穩定性。

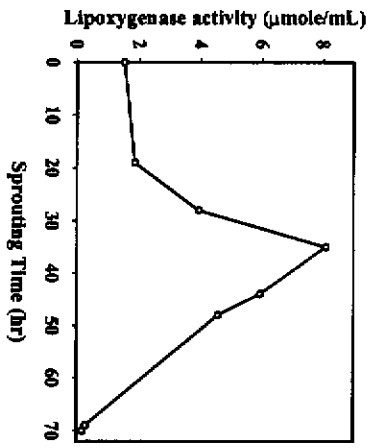


Figure 1. Change of LOX activity during germinating process of mang bean.

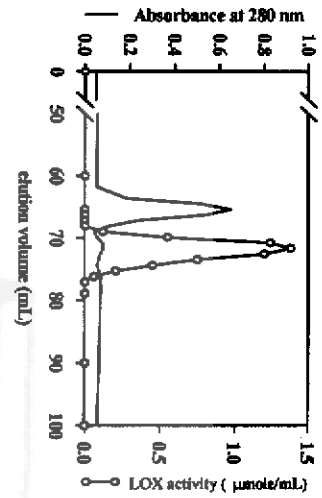


Figure 2. Elution profile of mang bean seedling LOX on Superdex pg 200 column (1.6x60 cm). Column was equilibrated with 50 mM phosphate buffer (pH 6.3) and eluted with the same buffer at a flow rate of 0.6 mL/min. Fractions of 3 mL were collected and assayed for protein as absorbance at 280 nm and for LOX activity.

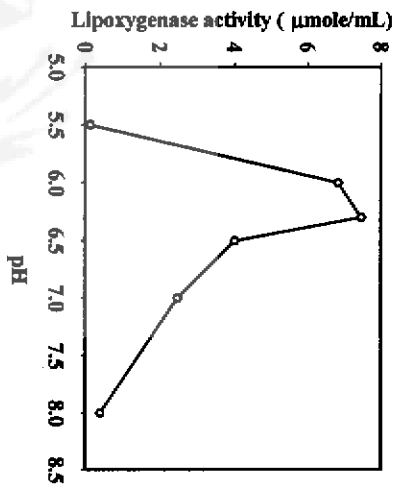


Figure 3. pH profile of LOX activity of mang bean seedling.

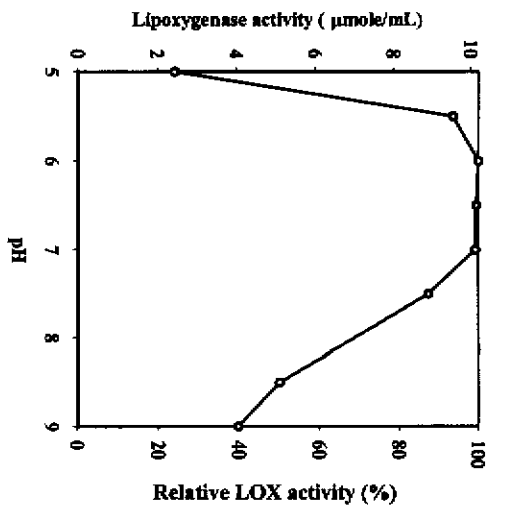


Figure 4. pH stability of LOX activity from mang bean seedlings.

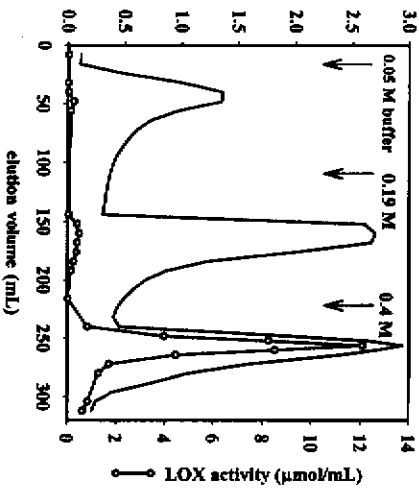


Figure 5. Elution profile of mang bean seedling LOX on hydroxyapatite column (2.6x15 cm). Column was equilibrated with 50 mM potassium buffer (pH 6.3) and eluted with 0.19 or 0.4 M phosphate buffer at a flow rate of 1 mL/min. Fractions of 4 mL were collected and assayed for protein as absorbance at 280 nm and for LOX activity.

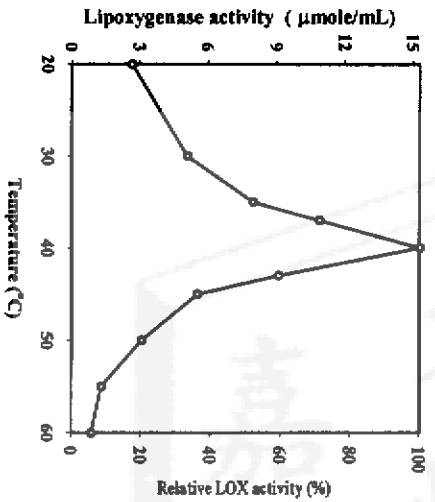


Figure 6. Temperature profile of LOX activity from mang bean seedlings.

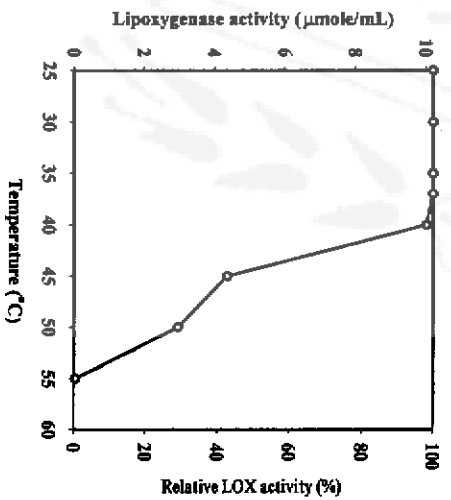


Figure 7. Thermal stability of LOX activity from mang bean seedlings.

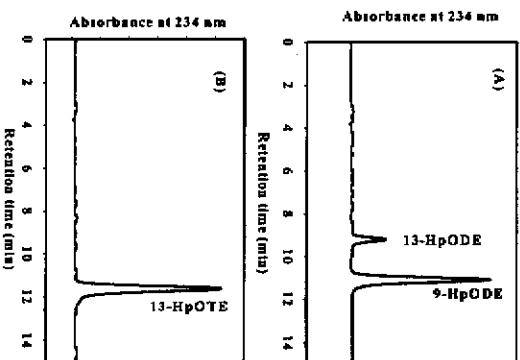


Figure 8. Normal phase HPLC chromatograms of products derived from (A) thiole (B) thiole acid reacted with the partially purified LOX of mang bean seedlings.