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科　　別：生物化學科

作品名稱：解開神秘果的奧秘-檸檬變柳丁的原因

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學　　校：國立臺中女子高級中學

作　　者：吳佩珊

# 作 者 簡 介

我現在就讀台中女中二年級，很喜歡參加比賽、活動，像是即席演說、相聲、歌唱、或是科展、科學趣味競賽等等。倒不是爲了得獎或什麼「要有些收穫」的目的，我只是很純粹的喜歡參加比賽，不想讓自己的生活淹沒在考試和書本中，除了念書之外就一無所有而已。我希望自己的生活是多采多姿的。這次能參加國際科展，真的積聚了太多太多人的幫忙及緣分！我感激交大的許千樹教授、李耀坤教授、心怡學姊、衷核學長，還有聖果農場林美玲小姐的鼎力相助！沒有你們幫忙，我不可能辦到這一切！

# 研究報告

## 摘要

原產於西非的「神秘果」，嘗了之後，30~200 分內，所有酸苦的東西嘗起來都是甜的。在深入蒐集相關資料後，我們發現神秘果有多種特殊效果，僅擷取以下幾種感興趣之方向來研究。〈1〉**使酸苦的感覺變甜〈2〉解酒〈3〉消除蚊蟲叮咬之腫、癢〈4〉抗氧化能力極強。**用食鹽水可萃取出 miraculin 這種醣蛋白，經由生化實驗，推測使酸味變甜爲其 cover 舌尖甜味味蕾之結果，分子量約爲 40000 左右；但在檢測過程中，發現對咖啡、黃連和肉桂，都沒有太顯著的效果，只有酸味有顯著的改變，和以往所閱讀的研究報告有出入，因此懷疑有氧化還原等其他化學效果，將再做深一層研究。消除蚊蟲叮咬之腫癢的成分確定爲小分子所致。經由 Prolox 當量測定法檢測神秘果抗氧化能力數值高達 4974g/nmol，比一般中草藥及蔬菜多 3000 左右。

使酸變甜的原因若深入研究對糖尿病患者和減肥者都是一大福音，塗抹蚊蟲叮咬藥膏也可用天然物質製作，而抗氧化能力高更對人體健康有所幫助。當台灣已大量栽植，相對於日本及美國因地寒而無法培育成功，神秘果研究可成爲另一項產業發展契機。

# Abstract

"Miracle fruit" is a fruit from West Africa. Though it's not sweet itself, if you eat anything that is sour or bitter after eating miracle fruit, the taste will turn sweet. After researching further material, we discovered that there are many amazing functions in miracle fruit, and decided to pick up some of which to study. (1) Turning the sour and bitter tastes into sweetness (2) Relieving alcohol (3) Relieving the hurt from mosquitoes and bugs (4) An excellent antioxidant. We can extract the miraculin that changes the taste from NaCl (aq), and through the biological experiment, we guess that's because miraculin covers the sweet sensor. The molecular weight of miraculin is about 40000.According to the experiment, we found out that miraculin doesn't have a great effect on the taste other than sourness, such as the bitterness of black coffee, *Coptis chinensis*, and cinnamon. . It is much more different from the former report we read. So we doubt that there are some other reactions. The thing, which relieves the hurt from mosquitoes and bugs, are sure to be a simple molecule, not a protein. By the Prolox equivalent weight experiment, we found that the ability of antioxidation got to 4974g/nmol, which is much higher than the normal vegetables and fruits.

The effect of taste changing is really good news for diabetics and weight reducers. And the medicine can also be made by natural material. The excellent antioxidation is helpful for our health, too. Since Japan and America cannot grow the miracle fruit because of the cold weather, developing the functions of miracle fruit seems to be another chance for Taiwan to stand out in the world.

# 一、前言

## （一） 研究動機

### 1.緣起：

基礎化學中提到「人工甜味」物質，例如阿斯巴甜之甜度爲蔗糖的 180 倍，糖精爲 300 倍。而老師在上課的時候，曾提到一種產於西非地區的植物果實，「神秘果」，嘗了之後，30 分到 200 分鐘內，所有酸苦的東西嘗起來都是甜的。當時聽了極感興趣，便進一步翻查相關文獻資料，意外發現神秘果中許多神秘的特殊的功能和人體息息相關，對人體有極大助益，但原因卻神秘未知，因此想從事相關研究，萃取神秘果的化學成分，以解開神秘果之謎。

### 2.神秘果簡介：

〈1〉學　名：*Synsepalum dulcificum Denill*
〈2〉俗　名：神秘果 (Miracle Fruit)
〈3〉原產地：西非
〈4〉可將酸澀的水果轉化成甜美的口感，此種效果可保持 30～200 分鐘。
〈5〉食用方法：拔除蒂頭→整顆放入口中→咬破果皮→咀嚼→充分翻滾 2-3 分鐘→滲入舌頭的味蕾和口腔內壁細胞→吐出種子

＊神秘果之圖片請見附錄

### 3.蚊蟲叮咬

在果園的農民平常飽受蚊蟲盯咬之苦，隨手摘下果園中結實累累的神秘果隨意塗抹，發現其果汁有清涼效果，還能消炎消腫，在 1-2 小時間及完全復原，且不會有麻癢的感覺。

## （二）研究目的

因爲對神秘果產生了興趣，上網檢索，發現許多美國及日本教授做了一些相關的研究，但仍有一些謎題尚未解開，因此想再既有的基礎上做更深一步的探討，分析神秘果內各種化學成分，看看前述的成分是否相同，找出其原因何在。而美國和日本因地處溫帶或寒帶，神秘果樹無法生長，但在台灣卻已成功培植出幾萬棵神秘果樹，且生長良好，倘若能分析出其中對人體有益的成分，可以幫助我們吃下某些對人體有助益卻難以入口的食物，或在醫療上有所貢獻，在世界上有其經濟價值，可以成爲台灣的另一商業契機。主要的研究方向有以下幾點：

1.分析純化出台灣種的神秘果素(Miraculin)，並測試其對各種不同味覺所產生的改變效果差別，是否如期刊 *Biochimica et Biophysica Acta*,1079(1991) 303-307 中寫的，也爲一種醣蛋白。

2.朝基因轉殖方向發展，也許能發展出種植甜的檸檬的技術，使我們能輕鬆吃檸檬並吸收大量的維他命 C。

3.分析純化出消炎止痛之成分，是否爲相同之醣蛋白。

# 二、研究想法及方法

## （一）研究想法

神秘果爲可食用的水果，因爲可以感覺到甜味，表示其成分可能爲水溶性的，應可利用水溶液將其萃取出來；而此種成分關係到人的味覺，所以不能用一般的化學實驗方式測試結果。即當發現某種成分因反應失去了，也不能證明其爲使酸味變甜的物質。所以將採用人體實驗的方式，親自去測試萃取出來的物質，是否有活性。

實驗比照以前美國、日本教授所做過的相關研究，修改其實驗過程，萃取出實驗用的醣蛋白，並測試其他報告中所不曾提過的味道，以找出真正神秘果使酸變甜的原因。另外，再以一些初步的實驗，檢測蚊蟲盯咬、抗氧化等成分的特性，確定其是否爲同樣的蛋白質。

## （二）實驗步驟

### 〈初步實驗--甜味部分〉

**1.確定分子(Miraculin)之性質，及其改變各種味道之程度**

〈1〉材料：神秘果、檸檬片、黑咖啡、黃連粉、肉桂粉、無糖綠茶

〈2〉步驟：

1.嘗原味(檸檬片、黑咖啡、黃連粉、肉桂粉、無糖綠茶)
2.吃半顆神秘果。
3.分“剛吃”、“十分”、“三十分”檢測味覺之改變。
4.煮熟半顆神秘果(3—5 分)。
5.重複 2.3.之步驟，檢測其效果是否相同。
6.無法改變味覺—大分子
可以改變味覺—小分子

## 〈初步實驗—蚊蟲叮咬〉

**確定此成分之性質及效果**

〈1〉 將粉末 1g 加水稀釋至 10mL，還原至近似於原本之果汁。
〈2〉 塗抹於蚊蟲叮咬之部位，觀察其 1~2 小時內之效果。
〈3〉 另配〈1〉之溶液一次，加熱至 100℃，放冷後再塗抹於另一處蚊蟲叮咬之部位視其效果。
〈4〉 若皆有消炎止癢之效—小分子；步驟〈2〉有而〈3〉無—可能爲蛋白質等大分子

## 〈純化 miraculin〉(此實驗均需於 4℃以下進行)

### 1. 萃取神秘果素〈Extraction of miraculin〉

〈1〉 取 3g 粉末〈由聖果農場冷凍乾縮果汁後的粉末〉置於 30ml 水中均勻震盪 2 分鐘。
〈2〉 將此均勻液體離心 4℃，13000rpm，30 分鐘。
〈3〉 將含粉紅色懸浮物（無甜味誘導性質的）的上層液丟棄。
〈4〉 仿照〈2〉〈3〉以清水將沈澱物完全的洗淨，使看起來呈白色，無任何紅色懸浮物殘留。
〈5〉 加入 0.5M 的 $NaCl_{(aq)}$18ml,均勻震盪 2 分鐘，並放於冰箱中萃取過夜。
〈6〉 隔天將此均勻液體以 4℃，13000rpm 離心 30 分。
〈7〉 將上澄清液(有高甜味誘導活性)收集起來,此收集後的溶液爲無色且 pH=4(第一次萃取)。
〈8〉 仿照〈5〉~〈7〉再對沉澱物作 2 次 NaCl(aq)萃取，即可得含 miraculin 的 $NaCl_{(aq)}$

### 2.銨鹽沉澱—分離蛋白質

〈1〉 將 1.—〈7〉的上澄清液至於冰浴中，加入硫酸銨至 50%的飽和度(即每公升加入 314 克硫酸銨)，在 4℃下分次緩緩加入並攪拌(冰浴中進行)，至硫酸銨完全溶解後，並多攪拌 30 分鐘使蛋白質完全鹽析出。

〈2〉 以 13000×g 轉速離心 40 分鐘至沉澱物與上層液完全分開，丟棄上層液，沉澱物即爲蛋白質，加入最少的無菌水完全溶解蛋白質，於 4℃冰存。

3.除鹽〈爲了防止蛋白質在濃度過高的鹽中變形分解，將蛋白質除鹽可保存較久〉:

〈1〉 2.〈2〉之沉澱後的蛋白質以 Mini-Q 水溶解。

〈2〉 將溶液導入預先以 Mini-Q 水平衡之除鹽管柱(desalting colume) 中去除鹽類(以 Mini-Q 水約 18 毫升洗 Desalting column )。

〈3〉 將 3.〈1〉之 1.5mL 溶解液體，注入 Desalting column 中，然後注入 2mL 的水將液體流洗出來，收集於試管中。

4.純化 miraculin—利用 FPLC 進行 HiTrap SP 陽離子交換樹脂管柱的層析

〈1〉 除鹽過後 3.之〈1〉溶液導入預先以磷酸納緩衝液($Na_2HPO4$(aq)0.005M + KH2PO4(aq)0.005M)平衡 HiTrap SP 陽離子交換樹脂，進行層析分離。

〈2〉 以流速 2mL/min，NaCl 梯度爲 0mM~1000mM。

〈3〉 收集方式爲每管收集 2mL 流出液。

〈4〉 收集完畢後，則以下節活性測試方法測各管活性。

## 〈檢測純化 miraculin 之特性〉

1.酸味→甜味檢測

〈1〉 配置 0.1M 檸檬酸水溶液 250mL。

〈2〉 將 FPLC 圖中 $OD_{280}$ 檢測有吸收峰〈蛋白質上苯環結構造成〉的部分取出測試。

〈3〉 先嘗檸檬酸水溶液，再將 200ul 的試液滴於舌尖 1 分鐘後，然後嘗檸檬酸水溶液 200ul，視其是否改變味覺。

〈4〉 確定爲所找尋之蛋白質之後，仿上述萃取純化之步驟，以 20g 粉末萃取出較大量之蛋白質，再作檢測。

〈5〉 配置 0.1M~1.0M 之蔗糖水溶液共十瓶，在〈4〉之後嘗 200ul，視其甜度約分布於何種濃度之間，而得其相對活性。

2.SDS PAGE 電泳測定分子量

SDS-PAGE 決定法原理：

〈1〉 量測各蛋白質標準品的相對移動距離，相對移動距離的定義是自電　泳跑到終點線爲原點，與到各蛋白質帶的中心之直線距離。
〈2〉 同法量測預測蛋白質的相對移動距離。
〈3〉 以各蛋白質標準品的相對移動距離對分子量的對數值作圖，舉分子量與預測蛋白質相鄰的二個蛋白質標準品爲標準，內插求欲測蛋白質分子量。

**方法：**

〈1〉 製作 stacking gel 爲 7%，running gel 爲 17.5%之電泳膠片，規格爲 10cm*7.4cm*1mm。
〈2〉 各步驟純化之蛋白質溶液、與 FPLC 純化過產生 peak 的試管溶液取適量與適量 Sample loading buffer (5uL 0.1% bromopnenol blue) 混合後，在 100 ℃水浴中 2 分鐘，置入電泳膠片上端之樣品槽。
〈3〉 以 150 伏特固定電壓，Tris-glycine running buffer 的系統通電約 1.5~2 小時。
〈2〉 將膠片取出，浸泡於適量 stain 溶液中(醋酸 200mL、異丙醇 500mL、0.6g comassie blue、水 1.3L)1~2 小時後，換成 destain 顯影，觀測其結果。

## 3.檢測 miraculin 之含量及其分布

〈1〉 測以水、食鹽水萃取、銨鹽沉澱後等各步驟之水溶液，以 1.的方式檢測是否有 miraculin 之存在。

## 4.測試 miraculin 對各種不同的味覺改變之效果

〈1〉 用黑咖啡、黃連粉、肉桂粉、無糖綠茶、胡椒粉作實驗。
〈2〉 以 2.中測出有反應之試液檢測黑咖啡、黃連粉、肉桂粉、無糖綠茶。

# 三、研究結果及討論

## 〈一〉研究結果

### 〈初步實驗-甜味部分〉

1.神秘果本身嘗起來酸味頗重，有一種水果的清香，但幾乎吃不出什麼甜味。

2.食用各種食物之效果：

| 時間 / 食物 | 剛吃 | 10 分鐘後 | 30 分鐘後 |
|---|---|---|---|
| 檸檬片—約 1cm 薄片<br>(極酸，嘴角差點破皮) | 非常明顯的變甜，有點像葡萄柚的味道。 | 可以感覺到一些酸味，甜味不似之前重。 | 還有一點甜，但效力已很薄弱。 |
| 黑咖啡—20c.c.<br>(非常的苦，偶而有酸味後勁) | 咖啡的酸味還在，但比較不苦，有一絲甜，較易入口。 | 比剛吃時苦一些，但快感覺不出有甜味。 | 越來越苦，但比未吃時仍好一些。 |
| 黃連粉—水溶液 20c.c.<br>(非常非常的苦！) | 沒有太大改變，仍會嘗到苦味，也不太有甜味感。 | 就像是本來的黃連粉一樣。 | 就像是本來的黃連粉一樣。 |
| 肉桂粉--水溶液 20c.c.<br>(肉桂的味道) | 肉桂的味道很濃，不太有什麼改變。 | 就像是本來的肉桂粉一樣。 | 就像是本來的肉桂粉一樣。 |
| 無糖綠茶—20c.c<br>(微澀，不甜，有回甘) | 喝起來像是含糖綠茶，但並非糖水，仍有茶味。 | 比剛吃時不甜，但仍比未吃時好多了。 | 慢慢無甜味，回到無糖綠茶的感覺。 |

3.若用沸水煮過，神秘果吃起來更酸，有一種變質的味道，且無上列表格的效果，顯示食物並未發生改變，而此種欺騙味蕾的物質必爲大分子〈蛋白質〉，顯示必須朝向抽取蛋白質邁進。

4.若用神秘果直接浸入檸檬汁等溶液中，反而無效，並不能有變甜之功用。

### 〈初步實驗—蚊蟲叮咬〉

1. 效果：擦起來涼涼的，無果汁的黏膩感，且在 1 小時內不癢不痛，紅腫自然慢慢褪去。

2. 煮沸放涼後：塗抹仍有其效果，和 1.所示差不多，證明其爲小分子，不受溫度影響而變性。

## 〈萃取純化 miraculin〉

1.萃取

〈1〉 用水萃取後溶液成淡粉紅色，有些白色、淡紅色懸浮物，聞起來有果香。

〈2〉 以食鹽水萃取可萃出大部分之 miraculin，期刊上說三次加起來約可得 98%以上的萃取量。

2.銨鹽沉澱離心之後有極少的沉澱物，需用最少量倍數的水(1.5mL 之整數倍)予以溶解。

3.除鹽後混濁液轉爲澄清，以味覺測試步驟檢測確有其活性。

4.純化 miraculin

〈1〉 由 FPLC 之圖中，分離時間 80 分鐘，收集 80 管，於 6-10、28-34 間有明顯大 UV 吸收，取出檢測其是否有活性。

〈35 管〉4-6，23-24 間有明顯大 peak，需取出檢測。

〈80 管〉10-12，28-34 間有明顯大 peak，需取出檢測。

## 〈檢測 miraculin 之特性〉

### 1.FPLC 圖中 $OD_{280}$ 檢測數據〈供參考〉

| mira1_ConcB_01 | | mira1_M1_UV_280nm_01 | | |
|---|---|---|---|---|
| | | | | |
| min | % | min | AU | |
| 0.000 | 0.000000 | 0.000 | 0.001000 | |
| 0.320 | 0.000000 | 0.320 | 0.001000 | |
| 0.640 | 0.000000 | 0.640 | 0.000900 | |
| 0.960 | 0.000000 | 0.960 | 0.001100 | |
| 1.280 | 0.000000 | 1.280 | 0.001200 | |
| 1.600 | 0.000000 | 1.600 | 0.001100 | |
| 1.920 | 0.000000 | 1.920 | 0.001000 | |
| 2.240 | 0.000000 | 2.240 | 0.001000 | |
| 2.560 | 0.000000 | 2.560 | 0.001100 | |
| 2.880 | 0.000000 | 2.880 | 0.001300 | |
| 3.200 | 0.000000 | 3.200 | 0.001500 | |
| 3.520 | 0.000000 | 3.520 | 0.001600 | |

| | | | | |
|---|---|---|---|---|
| 3.840 | 0.000000 | 3.840 | 0.001400 | |
| 4.160 | 0.000000 | 4.160 | 0.001300 | |
| 4.480 | 0.000000 | 4.480 | 0.001200 | |
| 4.800 | 0.000000 | 4.800 | 0.001100 | |
| 5.120 | 0.000000 | 5.120 | 0.001000 | |
| 5.440 | 0.000000 | 5.440 | 0.001000 | |
| 5.760 | 0.000000 | 5.760 | 0.001000 | |
| 6.080 | 0.000000 | 6.080 | 0.001000 | |
| 6.400 | 0.000000 | 6.400 | 0.001000 | |
| 6.720 | 0.000000 | 6.720 | 0.001000 | |
| 7.040 | 0.000000 | 7.040 | 0.001000 | |
| 7.360 | 0.000000 | 7.360 | 0.000900 | |
| 7.680 | 0.000000 | 7.680 | 0.000900 | |
| 8.000 | 0.000000 | 8.000 | 0.000900 | |
| 8.320 | 0.000000 | 8.320 | 0.000900 | |
| 8.640 | 0.000000 | 8.640 | 0.000900 | |
| 8.960 | 0.000000 | 8.960 | 0.000900 | |
| 9.280 | 0.000000 | 9.280 | 0.000900 | |
| 9.600 | 0.000000 | 9.600 | 0.000900 | |
| 9.920 | 0.000000 | 9.920 | 0.000900 | |
| 10.240 | 0.000000 | 10.240 | 0.000900 | |
| 10.560 | 0.000000 | 10.560 | 0.000900 | |
| 10.880 | 0.000000 | 10.880 | 0.000800 | |
| 11.200 | 0.000000 | 11.200 | 0.000800 | |
| 11.520 | 0.000000 | 11.520 | 0.000800 | |
| 11.840 | 0.000000 | 11.840 | 0.000800 | |
| 12.160 | 0.000000 | 12.160 | 0.000800 | |
| 12.480 | 0.000000 | 12.480 | 0.000800 | |
| 12.800 | 0.000000 | 12.800 | 0.000800 | |
| 13.120 | 0.000000 | 13.120 | 0.000700 | |
| 13.440 | 0.000000 | 13.440 | 0.000700 | |
| 13.760 | 0.000000 | 13.760 | 0.000700 | |
| 14.080 | 0.000000 | 14.080 | 0.000700 | |
| 14.400 | 0.000000 | 14.400 | 0.000700 | |
| 14.720 | 0.000000 | 14.720 | 0.000700 | |

| | | | |
|---|---|---|---|
| 15.040 | 0.600000 | 15.040 | 0.000700 | |
| 15.360 | 3.700000 | 15.360 | 0.000700 | |
| 15.680 | 7.000000 | 15.680 | 0.000700 | |
| 16.000 | 10.200000 | 16.000 | 0.000600 | |
| 16.320 | 13.300000 | 16.320 | 0.000600 | |
| 16.640 | 16.600000 | 16.640 | 0.000600 | |
| 16.960 | 19.700000 | 16.960 | 0.000600 | |
| 17.280 | 22.900000 | 17.280 | 0.000600 | |
| 17.600 | 26.100000 | 17.600 | 0.000600 | |
| 17.920 | 29.300000 | 17.920 | 0.000600 | |
| 18.240 | 32.500000 | 18.240 | 0.000600 | |
| 18.560 | 35.800000 | 18.560 | 0.000600 | |
| 18.880 | 38.900000 | 18.880 | 0.000500 | |
| 19.200 | 42.100000 | 19.200 | 0.000500 | |
| 19.520 | 45.300000 | 19.520 | 0.000400 | |
| 19.840 | 48.600000 | 19.840 | 0.000300 | |
| 20.160 | 51.800000 | 20.160 | 0.000300 | |
| 20.480 | 54.900000 | 20.480 | 0.000300 | |
| 20.800 | 58.200000 | 20.800 | 0.000500 | |
| 21.120 | 61.300000 | 21.120 | 0.000900 | |
| 21.440 | 64.500000 | 21.440 | 0.001600 | |
| 21.760 | 67.700000 | 21.760 | 0.002700 | |
| 22.080 | 70.900000 | 22.080 | 0.003800 | |
| 22.400 | 74.200000 | 22.400 | 0.003900 | |
| 22.720 | 77.300000 | 22.720 | 0.003000 | |
| 23.040 | 80.500000 | 23.040 | 0.001900 | |
| 23.360 | 83.700000 | 23.360 | 0.001000 | |
| 23.680 | 86.900000 | 23.680 | 0.000500 | |
| 24.000 | 90.100000 | 24.000 | 0.000200 | |
| 24.320 | 93.300000 | 24.320 | -0.000100 | |
| 24.640 | 96.600000 | 24.640 | -0.000200 | |
| 24.960 | 99.700000 | 24.960 | -0.000300 | |
| 25.280 | 100.000000 | 25.280 | -0.000400 | |
| 25.600 | 100.000000 | 25.600 | -0.000500 | |
| 25.920 | 100.000000 | 25.920 | -0.000600 | |

| | | |
|---|---|---|
| 26.240 100.000000 | 26.240 -0.000700 | |
| 26.560 100.000000 | 26.560 -0.000700 | |
| 26.880 100.000000 | 26.880 -0.000800 | |
| 27.200 100.000000 | 27.200 -0.000800 | |
| 27.520 100.000000 | 27.520 -0.000900 | |
| 27.840 100.000000 | 27.840 -0.000900 | |
| 28.160 100.000000 | 28.160 -0.001000 | |
| 28.480 100.000000 | 28.480 -0.001100 | |
| 28.800 100.000000 | 28.800 -0.001100 | |
| 29.120 100.000000 | 29.120 -0.001100 | |
| 29.440 100.000000 | 29.440 -0.001300 | |
| 29.760 100.000000 | 29.760 -0.001200 | |
| 30.080 100.000000 | 30.080 -0.001200 | |
| 30.400 100.000000 | 30.400 -0.001200 | |
| 30.720 100.000000 | 30.720 -0.001200 | |
| 31.040 100.000000 | 31.040 -0.001200 | |
| 31.360 100.000000 | 31.360 -0.001200 | |
| 31.680 100.000000 | 31.680 -0.001300 | |
| 32.000 100.000000 | 32.000 -0.001200 | |
| 32.320 100.000000 | 32.320 -0.001300 | |
| 32.640 100.000000 | 32.640 -0.001300 | |
| 32.960 100.000000 | 32.960 -0.001300 | |
| 33.280 100.000000 | 33.280 -0.001300 | |
| 33.600 100.000000 | 33.600 -0.001300 | |
| 33.920 100.000000 | 33.920 -0.001300 | |
| 34.240 100.000000 | 34.240 -0.001300 | |
| 34.560 100.000000 | 34.560 -0.001400 | |
| 34.880 100.000000 | 34.880 -0.001400 | |

| mira_2_ConcB_01 | | mira_2_M1_UV_280nm_01 | | |
|---|---|---|---|---|
| | | | | |
| min<br>% | | min<br>AU | | |
| 0.000 | 0.000000 | 0.000 | 0.000000 | |

| | | | | |
|---|---|---|---|---|
| 0.640 | 0.000000 | 0.640 | -0.000100 | |
| 1.280 | 0.000000 | 1.280 | -0.000100 | |
| 1.920 | 0.000000 | 1.920 | 0.000000 | |
| 2.560 | 0.000000 | 2.560 | 0.000100 | |
| 3.200 | 0.000000 | 3.200 | 0.000100 | |
| 3.840 | 0.000000 | 3.840 | 0.000200 | |
| 4.480 | 0.000000 | 4.480 | 0.000400 | |
| 5.120 | 0.000000 | 5.120 | 0.000700 | |
| 5.760 | 0.000000 | 5.760 | 0.001100 | |
| 6.400 | 0.000000 | 6.400 | 0.001500 | |
| 7.040 | 0.000000 | 7.040 | 0.001900 | |
| 7.680 | 0.000000 | 7.680 | 0.001900 | |
| 8.320 | 0.000000 | 8.320 | 0.001600 | |
| 8.960 | 0.000000 | 8.960 | 0.001400 | |
| 9.600 | 0.000000 | 9.600 | 0.000900 | |
| 10.240 | 0.430000 | 10.240 | 0.000700 | |
| 10.880 | 1.480000 | 10.880 | 0.000500 | |
| 11.520 | 2.540000 | 11.520 | 0.000400 | |
| 12.160 | 3.630000 | 12.160 | 0.000200 | |
| 12.800 | 4.680000 | 12.800 | 0.000100 | |
| 13.440 | 5.740000 | 13.440 | 0.000100 | |
| 14.080 | 6.810000 | 14.080 | 0.000100 | |
| 14.720 | 7.860000 | 14.720 | 0.000000 | |
| 15.360 | 8.940000 | 15.360 | 0.000000 | |
| 16.000 | 10.010000 | 16.000 | 0.000000 | |
| 16.640 | 11.080000 | 16.640 | 0.000000 | |
| 17.280 | 12.160000 | 17.280 | 0.000000 | |
| 17.920 | 13.230000 | 17.920 | 0.000000 | |
| 18.560 | 14.280000 | 18.560 | 0.000000 | |
| 19.200 | 15.340000 | 19.200 | -0.000100 | |
| 19.840 | 16.410000 | 19.840 | -0.000100 | |
| 20.480 | 17.460000 | 20.480 | -0.000100 | |
| 21.120 | 18.540000 | 21.120 | -0.000100 | |
| 21.760 | 19.630000 | 21.760 | 0.000000 | |
| 22.400 | 20.680000 | 22.400 | 0.000000 | |
| 23.040 | 21.760000 | 23.040 | 0.000000 | |
| 23.680 | 22.830000 | 23.680 | 0.000000 | |

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|---|---|---|---|---|
| 24.320 | 23.890000 | 24.320 | 0.000100 | |
| 24.960 | 24.960000 | 24.960 | 0.000100 | |
| 25.600 | 26.030000 | 25.600 | 0.000400 | |
| 26.240 | 27.090000 | 26.240 | 0.000600 | |
| 26.880 | 28.160000 | 26.880 | 0.000600 | |
| 27.520 | 29.210000 | 27.520 | 0.000800 | |
| 28.160 | 30.290000 | 28.160 | 0.001000 | |
| 28.800 | 31.360000 | 28.800 | 0.001300 | |
| 29.440 | 32.430000 | 29.440 | 0.001600 | |
| 30.080 | 33.480000 | 30.080 | 0.001900 | |
| 30.720 | 34.560000 | 30.720 | 0.001900 | |
| 31.360 | 35.630000 | 31.360 | 0.001800 | |
| 32.000 | 36.690000 | 32.000 | 0.001600 | |
| 32.640 | 37.760000 | 32.640 | 0.001100 | |
| 33.280 | 38.830000 | 33.280 | 0.000800 | |
| 33.920 | 39.890000 | 33.920 | 0.000500 | |
| 34.560 | 40.960000 | 34.560 | 0.000300 | |
| 35.200 | 42.030000 | 35.200 | 0.000200 | |
| 35.840 | 43.110000 | 35.840 | 0.000100 | |
| 36.480 | 44.160000 | 36.480 | 0.000000 | |
| 37.120 | 45.240000 | 37.120 | -0.000100 | |
| 37.760 | 46.310000 | 37.760 | -0.000200 | |
| 38.400 | 47.380000 | 38.400 | -0.000300 | |
| 39.040 | 48.440000 | 39.040 | -0.000300 | |
| 39.680 | 49.510000 | 39.680 | -0.000300 | |
| 40.320 | 50.580000 | 40.320 | -0.000400 | |
| 40.960 | 51.640000 | 40.960 | -0.000400 | |
| 41.600 | 52.710000 | 41.600 | -0.000400 | |
| 42.240 | 53.760000 | 42.240 | -0.000400 | |
| 42.880 | 54.830000 | 42.880 | -0.000400 | |
| 43.520 | 55.910000 | 43.520 | -0.000200 | |
| 44.160 | 56.960000 | 44.160 | -0.000400 | |
| 44.800 | 58.040000 | 44.800 | -0.000300 | |
| 45.440 | 59.110000 | 45.440 | -0.000400 | |
| 46.080 | 60.180000 | 46.080 | -0.000400 | |
| 46.720 | 61.240000 | 46.720 | -0.000500 | |
| 47.360 | 62.310000 | 47.360 | -0.000600 | |

| | | | |
|---|---|---|---|
| 48.000 | 63.380000 | 48.000 -0.000600 | |
| 48.640 | 64.440000 | 48.640 -0.000600 | |
| 49.280 | 65.510000 | 49.280 -0.000700 | |
| 49.920 | 66.560000 | 49.920 -0.000700 | |
| 50.560 | 67.630000 | 50.560 -0.000700 | |
| 51.200 | 68.710000 | 51.200 -0.000700 | |
| 51.840 | 69.780000 | 51.840 -0.000800 | |
| 52.480 | 70.840000 | 52.480 -0.000800 | |
| 53.120 | 71.910000 | 53.120 -0.000800 | |
| 53.760 | 72.980000 | 53.760 -0.000800 | |
| 54.400 | 74.040000 | 54.400 -0.000800 | |
| 55.040 | 75.110000 | 55.040 -0.000900 | |
| 55.680 | 76.160000 | 55.680 -0.000900 | |
| 56.320 | 77.240000 | 56.320 -0.000900 | |
| 56.960 | 78.310000 | 56.960 -0.000900 | |
| 57.600 | 79.360000 | 57.600 -0.000600 | |
| 58.240 | 80.440000 | 58.240 -0.000800 | |
| 58.880 | 81.510000 | 58.880 -0.000800 | |
| 59.520 | 82.580000 | 59.520 -0.000900 | |
| 60.160 | 83.660000 | 60.160 -0.000900 | |
| 60.800 | 84.720000 | 60.800 -0.001000 | |
| 61.440 | 85.770000 | 61.440 -0.001100 | |
| 62.080 | 86.820000 | 62.080 -0.001100 | |
| 62.720 | 87.910000 | 62.720 -0.001200 | |
| 63.360 | 88.990000 | 63.360 -0.001200 | |
| 64.000 | 90.040000 | 64.000 -0.001200 | |
| 64.640 | 91.110000 | 64.640 -0.001200 | |
| 65.280 | 92.190000 | 65.280 -0.001200 | |
| 65.920 | 93.260000 | 65.920 -0.001300 | |
| 66.560 | 94.320000 | 66.560 -0.001300 | |
| 67.200 | 95.410000 | 67.200 -0.001300 | |
| 67.840 | 96.460000 | 67.840 -0.001300 | |
| 68.480 | 97.520000 | 68.480 -0.001000 | |
| 69.120 | 98.590000 | 69.120 -0.001200 | |
| 69.760 | 99.660000 | 69.760 -0.001200 | |
| 70.400 | 100.000000 | 70.400 -0.001300 | |
| 71.040 | 100.000000 | 71.040 -0.001400 | |

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|---|---|---|
| 71.680 100.000000 | 71.680 -0.001400 | |
| 72.320 100.000000 | 72.320 -0.001400 | |
| 72.960 100.000000 | 72.960 -0.001400 | |
| 73.600 100.000000 | 73.600 -0.001400 | |
| 74.240 100.000000 | 74.240 -0.001500 | |
| 74.880 100.000000 | 74.880 -0.001500 | |
| 75.520 100.000000 | 75.520 -0.001500 | |
| 76.160 100.000000 | 76.160 -0.001500 | |
| 76.800 100.000000 | 76.800 -0.001500 | |
| 77.440 100.000000 | 77.440 -0.001500 | |
| 78.080 100.000000 | 78.080 -0.001400 | |
| 78.720 100.000000 | 78.720 -0.001500 | |
| 79.360 100.000000 | 79.360 -0.001500 | |

| mira_3_ConcB_01 | | mira_3_M1_UV_280nm_01 | | |
|---|---|---|---|---|
| | | | | |
| min<br>% | | min<br>AU | | |
| 0.000 | 0.000000 | 0.000 | -0.001200 | |
| 0.640 | 0.000000 | 0.656 | -0.001000 | |
| 1.280 | 0.000000 | 1.312 | -0.001100 | |
| 1.920 | 0.000000 | 1.968 | -0.001100 | |
| 2.560 | 0.000000 | 2.624 | 0.000600 | |
| 3.200 | 0.000000 | 3.280 | 0.001000 | |
| 3.840 | 0.000000 | 3.936 | -0.000500 | |
| 4.480 | 0.000000 | 4.592 | -0.000800 | |
| 5.120 | 0.000000 | 5.248 | -0.000500 | |
| 5.760 | 0.000000 | 5.904 | -0.000100 | |
| 6.400 | 0.000000 | 6.560 | 0.000300 | |
| 7.040 | 0.000000 | 7.216 | 0.001000 | |
| 7.680 | 0.000000 | 7.872 | 0.001500 | |
| 8.320 | 0.000000 | 8.528 | 0.000700 | |
| 8.960 | 0.000000 | 9.184 | -0.000100 | |

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|---|---|---|---|---|
| 9.600 | 0.000000 | 9.840 | -0.000600 | |
| 10.240 | 0.390000 | 10.496 | -0.000800 | |
| 10.880 | 1.480000 | 11.152 | -0.000900 | |
| 11.520 | 2.530000 | 11.808 | -0.000900 | |
| 12.160 | 3.590000 | 12.464 | -0.001000 | |
| 12.800 | 4.680000 | 13.120 | -0.001000 | |
| 13.440 | 5.730000 | 13.776 | -0.001000 | |
| 14.080 | 6.810000 | 14.432 | -0.001000 | |
| 14.720 | 7.860000 | 15.088 | -0.001000 | |
| 15.360 | 8.930000 | 15.744 | -0.001000 | |
| 16.000 | 10.010000 | 16.400 | -0.001000 | |
| 16.640 | 11.060000 | 17.056 | -0.001000 | |
| 17.280 | 12.140000 | 17.712 | -0.001000 | |
| 17.920 | 13.190000 | 18.368 | -0.000900 | |
| 18.560 | 14.280000 | 19.024 | -0.001000 | |
| 19.200 | 15.330000 | 19.680 | -0.000900 | |
| 19.840 | 16.390000 | 20.336 | -0.000900 | |
| 20.480 | 17.480000 | 20.992 | -0.000800 | |
| 21.120 | 18.530000 | 21.648 | -0.000700 | |
| 21.760 | 19.590000 | 22.304 | -0.000500 | |
| 22.400 | 20.660000 | 22.960 | -0.000500 | |
| 23.040 | 21.730000 | 23.616 | -0.000500 | |
| 23.680 | 22.790000 | 24.272 | -0.000600 | |
| 24.320 | 23.860000 | 24.928 | -0.000600 | |
| 24.960 | 24.930000 | 25.584 | -0.000500 | |
| 25.600 | 25.990000 | 26.240 | -0.000100 | |
| 26.240 | 27.080000 | 26.896 | 0.000000 | |
| 26.880 | 28.130000 | 27.552 | -0.000100 | |
| 27.520 | 29.190000 | 28.208 | -0.000100 | |
| 28.160 | 30.280000 | 28.864 | -0.000100 | |
| 28.800 | 31.330000 | 29.520 | -0.000100 | |
| 29.440 | 32.410000 | 30.176 | 0.000000 | |
| 30.080 | 33.460000 | 30.832 | 0.000000 | |
| 30.720 | 34.530000 | 31.488 | -0.000100 | |
| 31.360 | 35.590000 | 32.144 | -0.000100 | |
| 32.000 | 36.660000 | 32.800 | -0.000300 | |
| 32.640 | 37.740000 | 33.456 | -0.000400 | |

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|---|---|---|---|
| 33.280 | 38.790000 | 34.112 | -0.000600 |
| 33.920 | 39.880000 | 34.768 | -0.000700 |
| 34.560 | 40.940000 | 35.424 | -0.000700 |
| 35.200 | 42.010000 | 36.080 | -0.000700 |
| 35.840 | 43.080000 | 36.736 | -0.000700 |
| 36.480 | 44.160000 | 37.392 | -0.000800 |
| 37.120 | 45.210000 | 38.048 | -0.000900 |
| 37.760 | 46.280000 | 38.704 | -0.001000 |
| 38.400 | 47.340000 | 39.360 | -0.001100 |
| 39.040 | 48.430000 | 40.016 | -0.001100 |
| 39.680 | 49.480000 | 40.672 | -0.001200 |
| 40.320 | 50.560000 | 41.328 | -0.001200 |
| 40.960 | 51.610000 | 41.984 | -0.001200 |
| 41.600 | 52.680000 | 42.640 | -0.001200 |
| 42.240 | 53.760000 | 43.296 | -0.001200 |
| 42.880 | 54.810000 | 43.952 | -0.001200 |
| 43.520 | 55.890000 | 44.608 | -0.001200 |
| 44.160 | 56.940000 | 45.264 | -0.001200 |
| 44.800 | 58.010000 | 45.920 | -0.001200 |
| 45.440 | 59.080000 | 46.576 | -0.001300 |
| 46.080 | 60.140000 | 47.232 | -0.001300 |
| 46.720 | 61.210000 | 47.888 | -0.001300 |
| 47.360 | 62.280000 | 48.544 | -0.001300 |
| 48.000 | 63.360000 | 49.200 | -0.001300 |
| 48.640 | 64.410000 | 49.856 | -0.001300 |
| 49.280 | 65.480000 | 50.512 | -0.001300 |
| 49.920 | 66.560000 | 51.168 | -0.001300 |
| 50.560 | 67.610000 | 51.824 | -0.001300 |
| 51.200 | 68.690000 | 52.480 | -0.001200 |
| 51.840 | 69.740000 | 53.136 | -0.001300 |
| 52.480 | 70.810000 | 53.792 | -0.001300 |
| 53.120 | 71.880000 | 54.448 | -0.001300 |
| 53.760 | 72.940000 | 55.104 | -0.001300 |
| 54.400 | 74.010000 | 55.760 | -0.001400 |
| 55.040 | 75.080000 | 56.416 | -0.001400 |
| 55.680 | 76.130000 | 57.072 | -0.001400 |
| 56.320 | 77.210000 | 57.728 | -0.001500 |

| | | | |
|---|---|---|---|
| 56.960 | 78.280000 | 58.384 -0.001500 | |
| 57.600 | 79.340000 | 59.040 -0.001500 | |
| 58.240 | 80.410000 | 59.696 -0.001500 | |
| 58.880 | 81.490000 | 60.352 -0.001500 | |
| 59.520 | 82.540000 | 61.008 -0.001500 | |
| 60.160 | 83.590000 | 61.664 -0.001600 | |
| 60.800 | 84.670000 | 62.320 -0.001400 | |
| 61.440 | 85.740000 | 62.976 -0.001600 | |
| 62.080 | 86.810000 | 63.632 -0.001600 | |
| 62.720 | 87.870000 | 64.288 -0.001300 | |
| 63.360 | 88.960000 | 64.944 -0.001400 | |
| 64.000 | 90.010000 | 65.600 -0.001500 | |
| 64.640 | 91.090000 | 66.256 -0.001600 | |
| 65.280 | 92.160000 | 66.912 -0.001600 | |
| 65.920 | 93.240000 | 67.568 -0.001600 | |
| 66.560 | 94.310000 | 68.224 -0.001700 | |
| 67.200 | 95.360000 | 68.880 -0.001700 | |
| 67.840 | 96.440000 | 69.536 -0.001700 | |
| 68.480 | 97.510000 | 70.192 -0.001700 | |
| 69.120 | 98.570000 | 70.848 -0.001700 | |
| 69.760 | 99.640000 | 71.504 -0.001700 | |
| 70.400 | 100.000000 | 72.160 -0.001700 | |
| 71.040 | 100.000000 | 72.816 -0.001700 | |
| 71.680 | 100.000000 | 73.472 -0.001800 | |
| 72.320 | 100.000000 | 74.128 -0.001700 | |
| 72.960 | 100.000000 | 74.784 -0.001700 | |
| 73.600 | 100.000000 | 75.440 -0.001800 | |
| 74.240 | 100.000000 | 76.096 -0.001800 | |
| 74.880 | 100.000000 | 76.752 -0.001800 | |
| 75.520 | 100.000000 | 77.408 -0.001800 | |
| 76.160 | 100.000000 | 78.064 -0.001800 | |
| 76.800 | 100.000000 | 78.720 -0.001800 | |
| 77.440 | 100.000000 | 79.376 -0.001900 | |
| 78.080 | 100.000000 | | |
| 78.720 | 100.000000 | | |
| 79.360 | 100.000000 | | |

| mira4_ConcB_01 | | mira4_M1_UV_280nm_01 | | |
|---|---|---|---|---|
| | | | | |
| min<br>% | | min<br>AU | | |
| 0.000 | 0.000000 | 0.000 | -0.000700 | |
| 0.640 | 0.000000 | 0.640 | -0.000600 | |
| 1.280 | 0.000000 | 1.280 | -0.000300 | |
| 1.920 | 0.000000 | 1.920 | 0.000550 | |
| 2.560 | 0.000000 | 2.560 | 0.000700 | |
| 3.200 | 0.000000 | 3.200 | 0.000450 | |
| 3.840 | 0.000000 | 3.840 | -0.000100 | |
| 4.480 | 0.000000 | 4.480 | -0.000150 | |
| 5.120 | 0.000000 | 5.120 | 0.000050 | |
| 5.760 | 0.000000 | 5.760 | 0.000350 | |
| 6.400 | 0.000000 | 6.400 | 0.000700 | |
| 7.040 | 0.000000 | 7.040 | 0.000800 | |
| 7.680 | 0.000000 | 7.680 | 0.000600 | |
| 8.320 | 0.000000 | 8.320 | 0.000100 | |
| 8.960 | 0.000000 | 8.960 | -0.000250 | |
| 9.600 | 0.000000 | 9.600 | -0.000500 | |
| 10.240 | 0.000000 | 10.240 | -0.000600 | |
| 10.880 | 0.000000 | 10.880 | -0.000600 | |
| 11.520 | 0.000000 | 11.520 | -0.000700 | |
| 12.160 | 0.000000 | 12.160 | -0.000650 | |
| 12.800 | 0.000000 | 12.800 | -0.000650 | |
| 13.440 | 0.000000 | 13.440 | -0.000700 | |
| 14.080 | 0.000000 | 14.080 | -0.000700 | |
| 14.720 | 0.000000 | 14.720 | -0.000700 | |
| 15.360 | 0.610000 | 15.360 | -0.000700 | |
| 16.000 | 1.680000 | 16.000 | -0.000700 | |
| 16.640 | 2.740000 | 16.640 | -0.000750 | |
| 17.280 | 3.810000 | 17.280 | -0.000700 | |
| 17.920 | 4.880000 | 17.920 | -0.000700 | |
| 18.560 | 5.930000 | 18.560 | -0.000750 | |
| 19.200 | 7.010000 | 19.200 | -0.000700 | |
| 19.840 | 8.080000 | 19.840 | -0.000700 | |

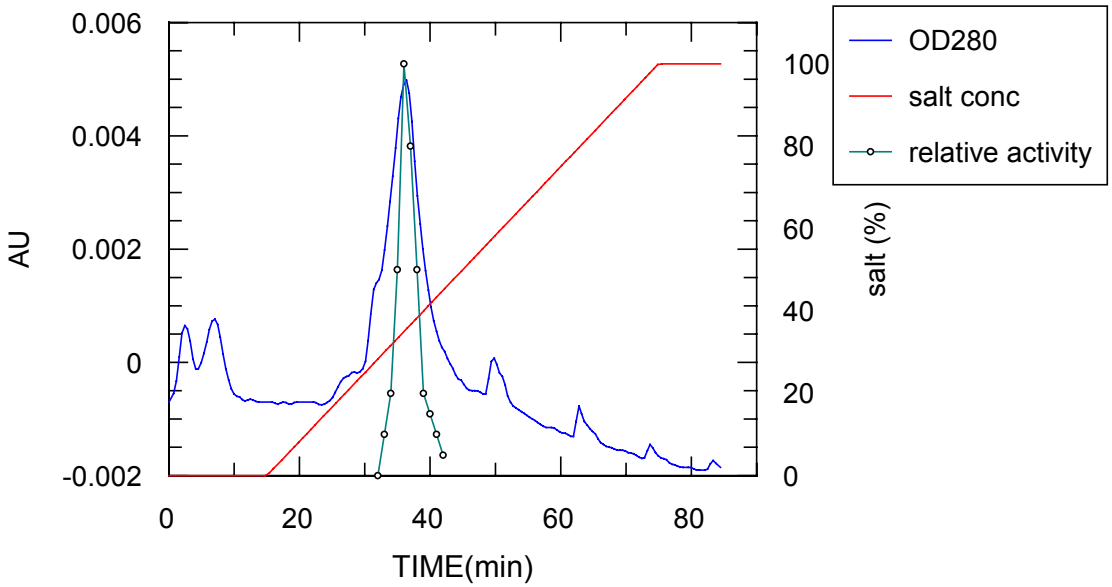
| | | | |
|---|---|---|---|
| 20.480 | 9.140000 | 20.480 | -0.000700 | |
| 21.120 | 10.210000 | 21.120 | -0.000700 | |
| 21.760 | 11.260000 | 21.760 | -0.000700 | |
| 22.400 | 12.340000 | 22.400 | -0.000700 | |
| 23.040 | 13.410000 | 23.040 | -0.000750 | |
| 23.680 | 14.480000 | 23.680 | -0.000750 | |
| 24.320 | 15.540000 | 24.320 | -0.000700 | |
| 24.960 | 16.590000 | 24.960 | -0.000650 | |
| 25.600 | 17.680000 | 25.600 | -0.000500 | |
| 26.240 | 18.740000 | 26.240 | -0.000350 | |
| 26.880 | 19.810000 | 26.880 | -0.000250 | |
| 27.520 | 20.860000 | 27.520 | -0.000250 | |
| 28.160 | 21.940000 | 28.160 | -0.000150 | |
| 28.800 | 23.030000 | 28.800 | -0.000200 | |
| 29.440 | 24.080000 | 29.440 | -0.000150 | |
| 30.080 | 25.140000 | 30.080 | -0.000050 | |
| 30.720 | 26.210000 | 30.720 | 0.000600 | |
| 31.360 | 27.280000 | 31.360 | 0.001400 | |
| 32.000 | 28.340000 | 32.000 | 0.001400 | |
| 32.640 | 29.410000 | 32.640 | 0.001600 | |
| 33.280 | 30.490000 | 33.280 | 0.002250 | |
| 33.920 | 31.540000 | 33.920 | 0.002900 | |
| 34.560 | 32.610000 | 34.560 | 0.003600 | |
| 35.200 | 33.680000 | 35.200 | 0.004450 | |
| 35.840 | 34.740000 | 35.840 | 0.004900 | |
| 36.480 | 35.830000 | 36.480 | 0.005050 | |
| 37.120 | 36.880000 | 37.120 | 0.004450 | |
| 37.760 | 37.960000 | 37.760 | 0.003300 | |
| 38.400 | 39.010000 | 38.400 | 0.002500 | |
| 39.040 | 40.080000 | 39.040 | 0.001850 | |
| 39.680 | 41.140000 | 39.680 | 0.001300 | |
| 40.320 | 42.210000 | 40.320 | 0.000850 | |
| 40.960 | 43.290000 | 40.960 | 0.000550 | |
| 41.600 | 44.340000 | 41.600 | 0.000300 | |
| 42.240 | 45.440000 | 42.240 | 0.000200 | |
| 42.880 | 46.510000 | 42.880 | 0.000000 | |
| 43.520 | 47.580000 | 43.520 | -0.000100 | |

| | | | |
|---|---|---|---|
| 44.160 | 48.640000 | 44.160 -0.000300 | |
| 44.800 | 49.690000 | 44.800 -0.000300 | |
| 45.440 | 50.760000 | 45.440 -0.000450 | |
| 46.080 | 51.830000 | 46.080 -0.000500 | |
| 46.720 | 52.890000 | 46.720 -0.000500 | |
| 47.360 | 53.980000 | 47.360 -0.000500 | |
| 48.000 | 55.030000 | 48.000 -0.000550 | |
| 48.640 | 56.090000 | 48.640 -0.000600 | |
| 49.280 | 57.160000 | 49.280 0.000050 | |
| 49.920 | 58.230000 | 49.920 0.000100 | |
| 50.560 | 59.290000 | 50.560 -0.000200 | |
| 51.200 | 60.360000 | 51.200 -0.000250 | |
| 51.840 | 61.430000 | 51.840 -0.000600 | |
| 52.480 | 62.490000 | 52.480 -0.000750 | |
| 53.120 | 63.560000 | 53.120 -0.000800 | |
| 53.760 | 64.630000 | 53.760 -0.000850 | |
| 54.400 | 65.690000 | 54.400 -0.000900 | |
| 55.040 | 66.760000 | 55.040 -0.000950 | |
| 55.680 | 67.830000 | 55.680 -0.001000 | |
| 56.320 | 68.890000 | 56.320 -0.001050 | |
| 56.960 | 69.980000 | 56.960 -0.001100 | |
| 57.600 | 71.030000 | 57.600 -0.001150 | |
| 58.240 | 72.110000 | 58.240 -0.001150 | |
| 58.880 | 73.160000 | 58.880 -0.001150 | |
| 59.520 | 74.230000 | 59.520 -0.001200 | |
| 60.160 | 75.290000 | 60.160 -0.001250 | |
| 60.800 | 76.380000 | 60.800 -0.001250 | |
| 61.440 | 77.430000 | 61.440 -0.001300 | |
| 62.080 | 78.510000 | 62.080 -0.001350 | |
| 62.720 | 79.580000 | 62.720 -0.000650 | |
| 63.360 | 80.630000 | 63.360 -0.001000 | |
| 64.000 | 81.690000 | 64.000 -0.001100 | |
| 64.640 | 82.760000 | 64.640 -0.001200 | |
| 65.280 | 83.820000 | 65.280 -0.001250 | |
| 65.920 | 84.890000 | 65.920 -0.001400 | |
| 66.560 | 85.960000 | 66.560 -0.001450 | |
| 67.200 | 87.040000 | 67.200 -0.001500 | |

| | | | |
|---|---|---|---|
| 67.840 | 88.090000 | 67.840 -0.001500 | |
| 68.480 | 89.170000 | 68.480 -0.001550 | |
| 69.120 | 90.220000 | 69.120 -0.001550 | |
| 69.760 | 91.310000 | 69.760 -0.001550 | |
| 70.400 | 92.360000 | 70.400 -0.001600 | |
| 71.040 | 93.440000 | 71.040 -0.001600 | |
| 71.680 | 94.510000 | 71.680 -0.001650 | |
| 72.320 | 95.570000 | 72.320 -0.001700 | |
| 72.960 | 96.640000 | 72.960 -0.001700 | |
| 73.600 | 97.710000 | 73.600 -0.001400 | |
| 74.240 | 98.770000 | 74.240 -0.001550 | |
| 74.880 | 99.840000 | 74.880 -0.001650 | |
| 75.520 | 100.000000 | 75.520 -0.001700 | |
| 76.160 | 100.000000 | 76.160 -0.001700 | |
| 76.800 | 100.000000 | 76.800 -0.001800 | |
| 77.440 | 100.000000 | 77.440 -0.001800 | |
| 78.080 | 100.000000 | 78.080 -0.001850 | |
| 78.720 | 100.000000 | 78.720 -0.001850 | |
| 79.360 | 100.000000 | 79.360 -0.001850 | |
| 80.000 | 100.000000 | 80.000 -0.001850 | |
| 80.640 | 100.000000 | 80.640 -0.001900 | |
| 81.280 | 100.000000 | 81.280 -0.001900 | |
| 81.920 | 100.000000 | 81.920 -0.001900 | |
| 82.560 | 100.000000 | 82.560 -0.001900 | |
| 83.200 | 100.000000 | 83.200 -0.001700 | |
| 83.840 | 100.000000 | 83.840 -0.001800 | |
| 84.480 | 100.000000 | 84.480 -0.001850 | |

## 2.酸味→甜味檢測

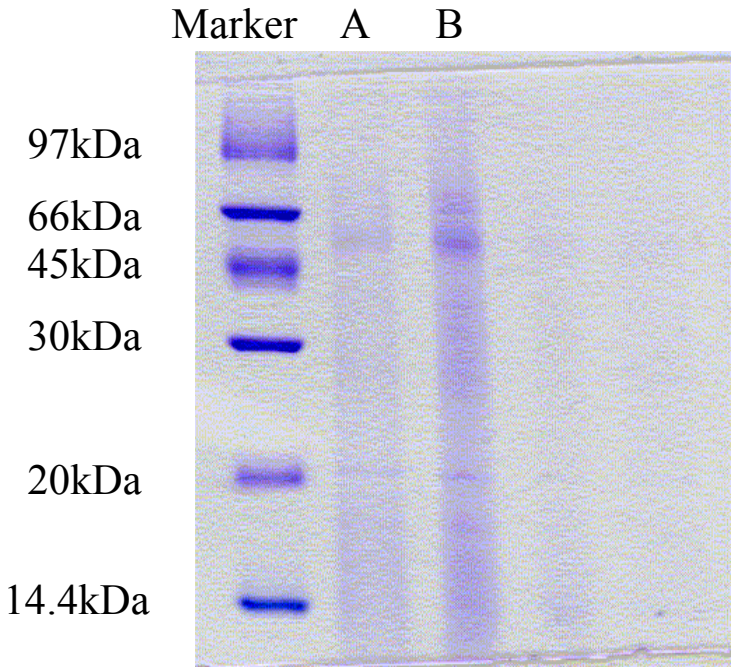
〈1〉 下圖爲純化出之 FPLC 和其活性區之相對活性結果，在第二個 peak 處會有甜味，於 28-34 分鐘間，可測出活性，一滴在舌尖就感覺變甜。




活性與 miraculin 分布圖

## 3.SDS PAGE 電泳測定分子量

(1)最初的神秘果粉末

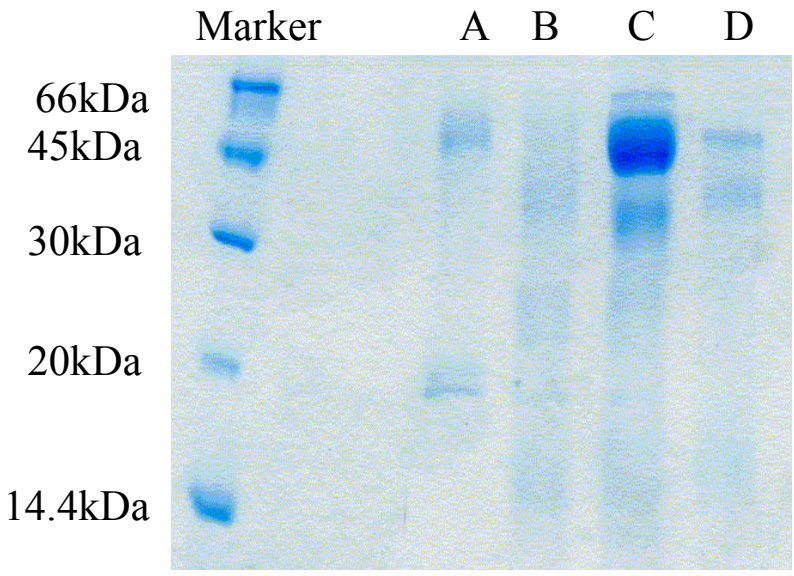



A：1g 粉末加水溶解之液體部分

B：3g 粉末加水溶解部分

〈說明〉可看見本來的果汁部分含有許多不同的蛋白質和其他成分，濃度越濃，看得越明顯。

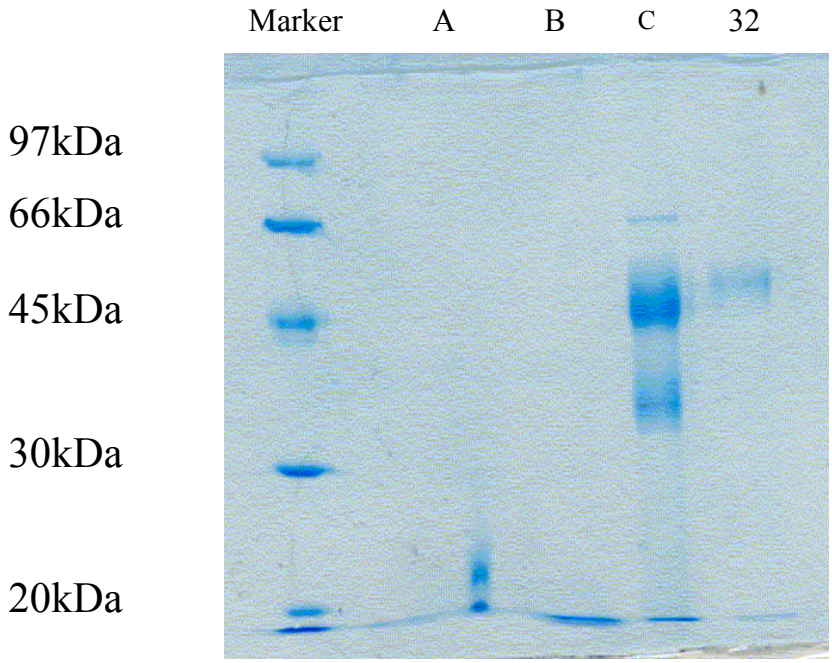
(2)萃取各步驟所得成分之電泳圖-1




| | |
|---|---|
| A | 用水萃取 |
| B | 用 NaCl 萃取(未濃縮) |
| C | 銨鹽沉澱後(已除鹽並濃縮) |
| D | 第 31 管(80) |

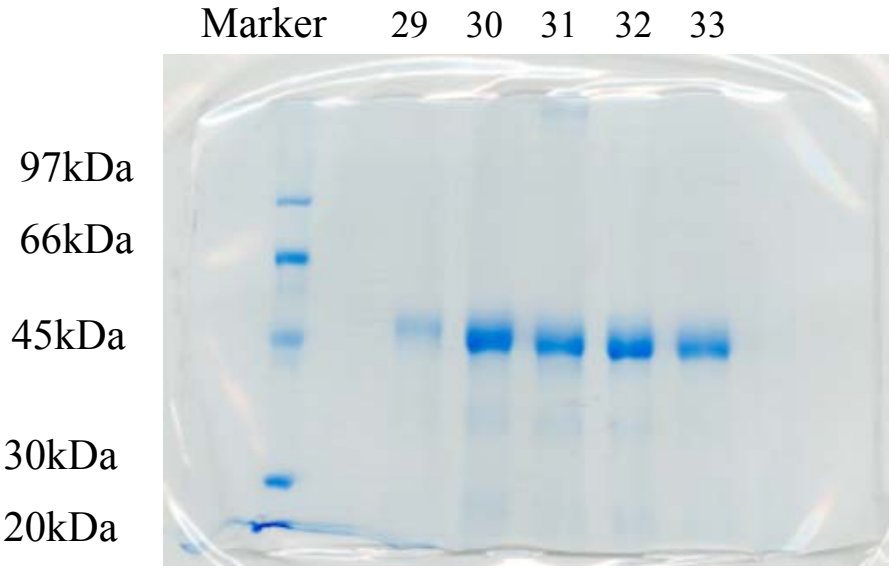
〈說明〉由此可見水萃取部分含少量的 miraculin，而在分子量 20000 左右有另一個不爲 miraculin 的蛋白質，可在萃取的過程中先行被去除掉。而以食鹽水萃取部分因 sample 未濃縮，故 miraculin 看得不甚明顯。但在經銨鹽沉澱過後的 sample，又經過真空濃縮 40 倍，故有相當明顯的 band 落在 40000 左右。以〈80 管〉萃取出的 28-34 管爲 miraculin zp 分布範圍，故取出 31 互相印證，可知其分子量的確落在 40000 左右。

(3)萃取各步驟所得成分之電泳圖-2




〈說明〉此爲另一張萃取各步驟所得成分之電泳圖，A、B、C 皆同上一張電泳圖，但其間距拉得較開，可發現銨鹽沉澱後的 sample 在 66kDa 的部分還有一條很細的 band，但在 FPLC 層析過後的 32 管並不見它，故推測其爲層析時另一個 peak 所代表的蛋白質。而因食鹽水的部分未除鹽，鹽的濃度太高而擠壓到水的 band，在實驗技巧上也獲得“需注意鹽的濃度”之經驗。

(4)FPLC 層析後含 miraculin 之試液




〈說明〉此爲〈80 管〉FPLC 層析出來，經由味覺測試後，確有味覺改變之管數，可知 miraculin 分子量約爲 40000 左右，並知其蛋白質分布範圍頗廣〈橫跨的管數不少〉。

### 4.檢測 miraculin 之含量及其分布

| 溶液 / 溶液 | 水萃取之上層液 | 食鹽水萃取之上層液 | 銨鹽沉澱後之上層液 | FPLC 之試液 |
|---|---|---|---|---|
| 本身食用效果 | 喝起來甜甜的，反倒沒有神秘果的酸味，有果香 | 鹹鹹的，不甜，無特別酸味或其他味道。 | 嘗起來苦苦的難以入口。 | 前 10 多管無鹽，像白開水，越往後越有鹽的苦味。 |
| 改變檸檬酸水溶液之效果 | 可以覺察得出一點點變甜的效果，但並不明顯。 | 變甜的效果非常明顯！尤其是第一次濃度極高！ | 無任何效果。 | 除了第 2 段 peak 外，其他皆無變甜之效。 |

### 5.測試 miraculin 對各種不同的味覺改變之效果

同上〈初步實驗—甜味檢測〉2.之結果。

## 〈二〉實驗討論

### 〈初步實驗-甜味部分〉

1. 咖啡、黃連和肉桂，都沒有太顯著的效果。而黑咖啡會消除苦味,卻無法感覺到甜味；就算有，也非常非常的稀少！並非每一種味覺都能改變。只有檸檬酸有顯著的酸變甜的效果。

2. 因爲神秘果本身不太甜，且有酸味，所以可推得之並非因其過甜而蓋掉其他味覺而使檸檬之味道發生改變。

## 〈蚊蟲叮咬〉

1. 已確定爲小分子，萃取之步驟便不能如同 miraculin 之方式。

2. 聖果農場曾拿冷凍乾縮處理過後的錠劑去測酸鹼值，是 11.13，可能因爲能酸鹼中和蟻酸等物質，而達到消腫止癢之效，但令人存疑的是，實驗時 miraculin 應該是在 pH6.82 的 buffer 中做的，並不是鹼性。

## 〈萃取純化 miraculin〉

1. 萃取出的爲一種名爲 miraculin，且經過日本厚生省認證的蛋白質，在吃的時候可以快速 cover 甜味及酸味味蕾，而使舌頭甜味的感覺被放大，並縮小其他味道的效果。

2. 就相關文獻而言，如果 miraculin 只是純粹的 cover 甜味味蕾,應該可以明顯的感覺到甜味，但在甜味測試中，可發現除了酸味→甜味效果顯著，其他只能稍作改善。因此我們猜測，可能同時還有其他的化學反應作用產生，或是蛋白質結構只能欺騙酸味細胞。

3. 若把神秘果放進檸檬汁中，就無法產生改變味覺的效果，因此可知其不爲普通化學反應，且蛋白質 pH 值呈中性，在過酸的環境中會降解掉。

4. 由電泳圖可知，原本在神秘果內有不少蛋白質，但經過萃取和實驗檢測後可得單一的蛋白質，判斷應爲改變味覺的主因。

# 四、結論與應用

## 〈甜味部分〉

1. 將 miraculin 大量提煉後，可作成丸劑出售〈現已有西非一公司從事此種生產〉，取代可能致癌的代糖，可攝取甜味又不至於攝取過多糖分，爲糖尿病患者之一大福音。

2. 可幫助食用不加糖的蛋糕、冰淇淋、或是原味優酪乳，和檸檬、酸梅等酸味高，富含維他命 C 的水果，減少糖分攝取有可平衡體內酸鹼值，收減肥之效。現甚至有某些高級餐廳推出神秘果大餐，讓顧客可以輕鬆吃下許多不同的水果。

3. 日本、美國雖有從事相關之研究，但因地處溫、寒帶，無法大量種植，光原料要價就極其昂桂，倘若台灣能研發萃取技術，必可成爲競爭力極高的產業。

## 〈蚊蟲叮咬〉

此種天然清涼且不黏膩的消腫止癢成分，倘若能作成噴劑或軟膏，可成爲另一天然的擦蚊蟲叮咬之藥品，對人體較無副作用或損傷。

# 五、展望

## 神秘果的其他特殊效果：

### 〈1〉抗氧化能力極強

神秘果本身食用期大約一個禮拜左右，但將果汁冷凍乾縮後，其使酸澀味變甜能力的效果，可長達幾年之久；而將神秘果的果汁塗抹在鮮豬肉上，和另一塊沒塗抹的放在室外陽光下做對照實驗，發現後者很快就發臭，吸引許多蒼蠅、螞蟻，但塗抹神秘果果汁的豬肉塊，卻可以放好幾天不致腐壞，且神秘果的甜味並未吸引螞蟻。

聖果農場將冷凍乾縮後的果汁粉末，摻入 3 倍的玉米澱粉，製成錠劑，送往食品工業研究所，以 Trolox 當量測定法檢測其抗氧化能力數值。

送測結果此錠劑之抗氧化值高達 4974n mole Trolox/g！一般抗氧化能力高的食品，其值皆可到達 4 位數左右，能力強的如某些中草藥、蔬果和維他命 C 也只到 1000 多而已。

這樣的成分不多見，將做後續深入研究，而其不黏膩及抗氧化之效，可能可以成爲天然的化妝品成分，或在防腐劑上有所貢獻。

### 〈2〉解酒

在高雄曾有高階警官，召開記者會證明，喝酒後，酒精檢測爲 1 以上，但吃了神秘果後，酒精濃度驟降，再測只在 0.2 以下（0.25 以下即爲標準值）。但身體內的酒精卻未被分解，僅限於口中偵測不到，而逃過檢測。若先食用神秘果後再喝酒，可以將酒的酸澀轉化成香醇可口，亦可提昇酒量，隔天也較無宿醉情形。

### 〈3〉種子的神秘效果

神秘果的種子又稱陰陽子，有一半邊被一層白白的膜緊緊的包覆著，嚼食時有苦味，會使舌頭發麻，但能紓解心絞痛、頭痛、喉嚨痛，甚至治療關節炎，和降低類固醇用量等醫療上極具價值的效果。想分析種子的化學成分，是否與市面上所販售之藥劑相同，或是對上述的病痛有更佳的治療效果。

# 六、附錄

## 1.buffer 溶液配製

buffer A：$Na_2HPO4$(aq)0.005M+KH2PO4(aq)0.005M

buffer B：NaCl(aq) 1M + Na2HPO4 0.005M + KH2PO4 0.005M

## 2.電泳膠片配製成分表

| | Running gel (17.5%)x2 | Stacking gel (1.0%)x2 |
|---|---|---|
| Acrylamide(50%) Bisacrylamide 高分子聚合物 | 3.062mL | 0.69mL |
| $H_2O$ | 3.92mL | 3.5595mL |
| 2M Tris-$H_3PO_4$ | 1.645mL | |
| 10%SDS 陰離子界面活性劑 | 88.166uL | 49.5uL |
| TEMED 催化劑 | 13.34uL | 7uL |
| 1M Tris-$H_3PO_4$ pH=6.5 | | 625.5uL |
| APS(10%) 誘導劑 | 43.75uL | 25.5uL |

## 3.儀器、材料

材料：藥品購自 Merck、Aldrich 以 Mini-Q 配製。

儀器：

搖動培養箱（Firstek, Scientific, orbital shaking incubator Model-s302R）

高速離心機（Kabata 7820）

微量離心機（Hermle）

真空濃縮機（EYELA rotary vacuum evaporator N-N series）

HiTrap Desalting column（Pharmacia, 5mL）

EPLC system（Pharmacia Biotech EPLC System）

SDS-PAGE 系統材料：

Acylamide-Biscrylamide 購自 Aldrich

Tris-$H_3PO_4$ 購自 STRATAGENE

SDS(Dodecyl sulfate sodium salt)購自 Aldrich

APS(Ammonium persulfate)購自 Aldrich

THEMD(N,N,N',N'-tetramethylethylene)購自 BDH

Glycine 購自 Lancaster

## 4.神秘果照片

神秘果樹，果實叢生於葉腋下。

市面推出之神秘果禮盒，一顆要價 100 元。

大量的神秘果採收。

# 七、參考文獻

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# 評 語

能夠以實驗證明純化的蛋白質成分具有與複合物相當的反應現象，具備科學家追根究底的精神。應該深入瞭解該蛋白質的分子量與成份，以及相關的生化反應，方能進一步研究其與味蕾的作用及反應機制。

# Isolation and Characterization of the Sour Taste-modifying Protein, Miraculin, from *Richadella dulcifica*

Pei-Shan Wu
National Taichung Girls' Senior High School, Taichung, Taiwan
42, Hsing Chung Street, Taichung, Taiwan
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**TABLE OF CONTENTS** **PAGE**

# Abstract

*Richadella dulcifica*, a native plant in tropical West Africa, bears red berries which contain protein assigned as miraculin and has the unusual property of modifying a sour taste into a sweet taste.

Miraculin is extracted with 0.5 M sodium chloride solution, and purified from the extracts by 50% ammonium sulfate fractionation, sulfopropyl ion-exchange column chromatography, and concanavalin A-Sepharose affinity column chromatography.

Previously, primary structure analysis of miraculin revealed that it consists of 191 amino acid residues with 7 cysteine residues and was found to be a basic protein with PI value of ~ 9. This taste-modifying protein, miraculin, is also a glycoprotein with Asn-linked oligosaccharide moieties located at Asn-42 and Asn-186.

It is interesting to find that the M.W. of miraculin is 28,000 by nucleotide sequence analysis of its cDNA as reported previously, but the present investigation showed that the M.W. of purified miraculin is 55,000 by sodium dodecyl sulfate-polyacrylamiade gel electrophoresis. By reduction of the miraculin with 5%  -mercaptoethanol, the M.W. of miraculin is reduced to 28,000, and it suggests that miraculin is a homodimer linked by disulfide bond(s). To investigate whether the dimeric form is essential to its function of modifying the sourness into sweetness, the miraculin was reduced with  -mercaptoethanol and then alkylated with iodoacetate to address this issue.

Furthermore, in order to study whether the carbohydrate moiety of miraculin, is essential for its function, the cDNA of miraculin was cloned into pGEX-2T vector and expressed in E.Coli..

# Introduction

In chemistry class of my senior high school, the teacher has mentioned that there is a kind of fruit, which can modifying your taste. I was really interested in that.

The fruit's name is *Richadella dulcifica*, a native plant in tropical West Africa, bears red berries that contain miraculin. Another name of *Richdella dulcifica* is miracle fruit. According to the materials, the reason of taste-modifying function is because miraculin can cover your sour sensor and let the sourness turn into sweetness. The function works obviously when tasting lemons. The crude extracts of fruits have been used as sour-taste modifying agent and are commercially available.

After literature survey of miraculin, it indicates that there were two different molecular weight (M.W.) of miraculin. One is 48 kDa, and another one is 28 kDa. It drew my attention to syudy it.

Miracle fruit is not a plant that is not easy to take care of. It can only grow well under high temperature and enough sunshine. And the fact affects the price very much. Miracle fruit is expensive and hard to buy in normal market. If recombinant miraculin can still has the biological function, the price will be lower a d it is truly a welfare fore people. The effect of taste modifying function is really good news for diabetics and weight reducers. They can reduce the ingestion of sugar. And the medicine can also be made by natural material.

The photograph of miracle fruit which is grown in southern Taiwan.

## Problems

1. In my preliminary experiments, I found that miraculin is a dimeric form, and that will explain why previous paper reported two different M.W.. Therefore, it is interesting to study whether the dimeric form is essential for its biological activity of modifying the sourness into sweetness.
2. The proposal is to study whether miraculin an be produced by recombinant technology in *E. coli* to study whether the carbohydrate moieties of miraculin are indispensable for its taste modifying activity.

## Hypothesis

1. The dimeric form of native miraculin is essential for its biological function.
2. Carbohydrate moieties of miraculin are indispensable for its biological function.

# Materials and Methods

## Materials:

1. The crude extract of miracle fruit was from Sen Yuh Farm Science Co., LTD., Taiwan.
2. Disodium hydrogenphosphate, potassium dihydrogenphosphate, ammonium sulfate, citric acid anhydrous (Showa Chemical Co. Ltd, Japan), and sodium chloride (WakoChemical Industries, Ltd, Japan) were used for purification of miraculin. Distilled water was used for all the aqueous solution.
3. Acrylamide, N,N,N',N'-rtetramethylethylene (TEMED), Coomassie brilliant blue (GIBCO BRL), Tris, ammonium persulfate (APS), SDS, β-mercaptoethanol, and bromophenol blue (Merck) were used for SDS-PAGE.
4. Sulfopropyl (SP) ion-exchange column chromatography, Hitrap Desalting column and concanavalin A-Sepharose (Con A) affinity column chromatography were obtained from Pharmacia Biotech (Uppsala, Sweden). And RNeasy column was purchased from Qiagen Inc. (Valenicia, CA).
5. Isopropyl-D-thiogalactopyranoside (IPTG), glutathione, restriction enzymes and ampicillin were acquired from Boehringer Mannheim Co..P-GEX-2T vector was from Pharmacia. Bacto-tryptone, bacto-yeast-extract and agar were obtained from Difco Co..
6. Equipments:
    1. Beckman J2/21 M/E centrifuge
    2. Beckman L8-80M centrifuge
    3. Sigma 2K15 centrifuge
    4. Pharmacia FPLC
    5. Hitachi U-3200 spectrophotometer
    6. Jasco Model 715 Spectropolarimeter

## Purification of miraculin:

5g of the crude extracts of miracle fruits crude was homogenized for 2 min in 30 ml of 0.5 M NaCl solution and then extracted overnight. The homogenate was centrifuged at 13,000 rpm for 40 min. The supernatants which showed high sweet-reducing activity were collected. Ammonium sulfate fractionation was carried out by addition of solid ammonium sulfate to the pooled solution to bring about 50% saturation. The precipitate from the solution was collected by centrifugation at 13,000 rpm for 40 min and suspended in 0.01M $KH_2PO_4$-$Na_2HPO_4$ buffer (pH 6.8).

The sample was applied to SP ion-exchange column equilibrated with 0.01 M $KH_2PO_4$-$Na_2HPO_4$ buffer. The column was eluted first with the phosphate buffer, and the adsorbed substances were eluted with a liner gradient of NaCl (0-0.5 M) in the buffer. The total volume of the gradient solution was 80 ml and fractionations of 10 ml were collected with a flow rate of 1 ml/min. The active fractions were pooled and concentrated. The concentrated solution was subjected to the following affinity chromatography.

The sample obtained above was applied to Con A-Sepharose 4B column equilibrated with 0.01 M $KH_2PO_4$-$Na_2HPO_4$ buffer containing 0.5 M NaCl. The column was eluted first with 0.01 M $KH_2PO_4$-$Na_2HPO_4$ buffer, and the adsorbed substances were eluted with a liner gradient of methyl-α-D-glucoside (0-0.15 M) in the starting buffer at a flow rate of 1ml/min. The active fractions were pooled.

The sweet-inducing activity of miraculin was assayed by tongue myself. 400 ul of miraculin solution were held in the mouth for 2 minutes and spat out. The mouth was rinsed with water and then 0.02 M citric acid was tasted. The protein which had the biological function can be confirmed to be miraculin.

The homogeneity and molecular weight ( M.W. ) of purified miraculin were determined by sodium dodecyl sulfate-polyacrylamiade gel electrophoresis (SDS-PAGE).

## Biological function test of dimer and monomer miraculin:

The SDS-PAGE was used to analyze which is the best concentration of β-mercaptoethanol to break the disulfide bond that is only between molecules but not in the molecular structure of miraculin. After finding the most appropriate concentrate, β-mercaptoethanol was added into native miraculin to break the disulfide bond. Then the same method that has metioned above was used to assay the biological function of dimmer and monomer miraculin.

## Preparation of recombinant miraculin:

The pulp and seed of miraculin were grinded under liquid nitrogen to a fine powder using a mortar and pestle. The sample was lysed by adding 20 ml Buffer RLT. The lysate was centrifuged at room temperature at 3000 rpm for 10 min. 0.5 volumes of 96-100% ethanol was added to the cleared lysate. The sample was applied to an RNeasy maxi column and centrifuged for 5 min at 5000 rpm. The flow-through was discarded. 15 ml Buffer RW1 was added to the RNeasy column and centrifuged for 5 min at 5000 rpm. The flow-through was discarded. 10 ml Buffer RPE was added to the RNeasy column and centrifuged for 10 min at 5000 rpm to wash the column. The flow-through was discarded. The appropriate volume of RNase-free water was pipeted directly onto the RNeasy silica-gel membrane. The sample was stood for 1 min, and then centrifuged for 3 min at 3000-5000 rpm. The elution step was repeated as described with a second volume of RNase-free water. The total RNA was isolated in the solution.

The reverse transcriptase was used to produce the first strand cDNA from the total RNA. And the cDNA of miraculin was obtained by Polymerize Chain Reaction (PCR) with forward and reversed primers.

Forward primer: 5’-GGA TCC GAT TCG GCA CCC AAT CCC GTT -3’

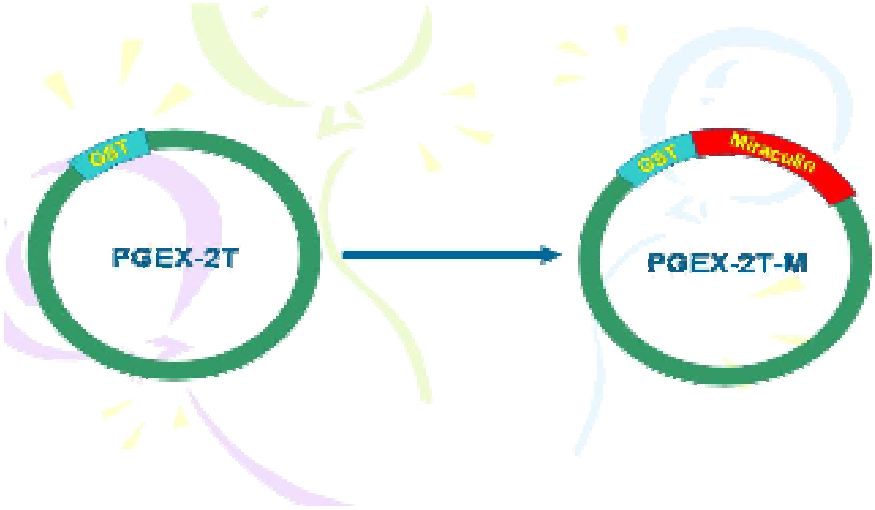
Reversed primer: 5’-GAA TTA TTA GAA GTA TAC GGT TTT GTT GAA -3’

5 ul 10 x PCR buffer (100 mM Tris-HCl, pH 9.0, 25 mM $MgCl_2$, 500 mM KCl, 0.1 % Gelatin), 2 ul 10 mM dNTPs, specific primers, 0.5 ul *Taq* DNA polymerase, and distilled

water were added to 2 ng template cDNA for total 50ul.

Reaction program: 95℃ for 1 min, 45 ℃ for 1 min, 65℃ for 2 min. After 35 cycles were finished, the sample reacted at 65℃ for 7 min.

The plasmid from competent *E. coli* was reacted with restriction enzymes *Bam*H I and *Eco*R I. The cDNA of miraculin was cloned into pGEX-2T vector to get pGEX-2T-M, and transformed into *E. coli*, incubating at 37℃. When the optical density at 600 nm of culture reached 0.5; the culture was induced with 0.5mM IPTG, and incubated at 4℃ for 16 hr. The E. coli was centrifuged at 4000 rpm for 10 min. Resuspension buffer (10 mM Tris-HCl, pH7.5, 100 mM NaCl, 1 mM $MgCl_2$ and 1mM DTT) and 100 ul lysozyme (10 mg/ml) were added. The cells were lysed with sonicator.




**Construction of pGEX-2T vector and pGEX-2T-M.**

The sample was applied to Glutathione-Sepharose 4B column equilibrated with PBS (150 mM NaCl, 16 mM $Na_2HPO_4$, 4 mM $NaH_2PO_4$, pH7.3) and 1 mM DTT. The column was eluted first with PBS buffer, and the fusion protein of recombinant glutathione-S-transferase (GST)-miraculin was eluted with Elution buffer (5 mM Glutathione, 50 mM Tris-HCl, pH 8.0) at a flow rate of 1ml/min.

Periodic acid-Schiff stain (PAS) was used to confirm that fusion protein, GST-miraculin, which was not a glycoprotein. Then the same method that has metioned above was used to assay the biological function of GST-miraculin.

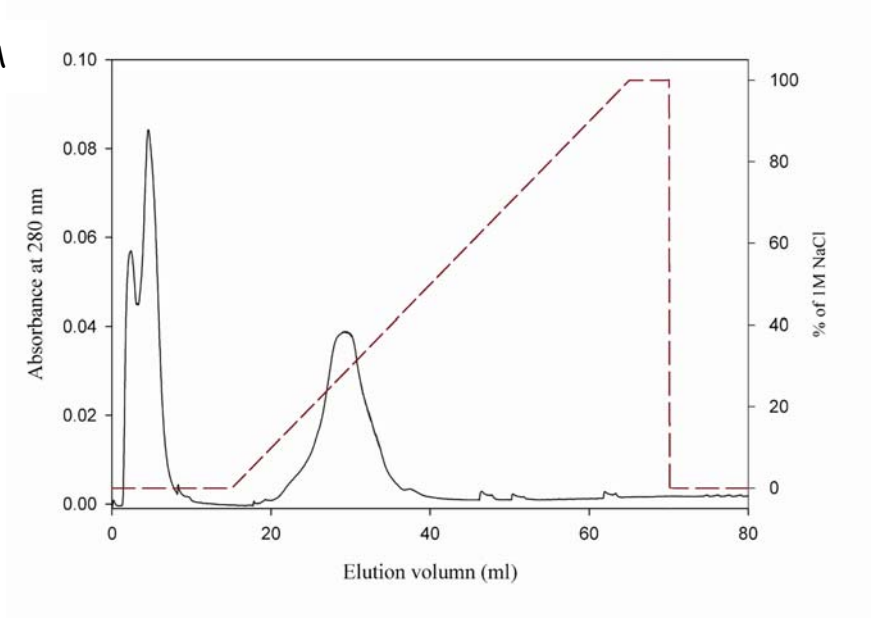
# Results and discussion

## I. Purification of miraclin from the red berries of *Richadella dulcifica*

Miraculin was isolated and purified from the red berries of *Richadella dulcifica*. The Sp ion-exchange column and Con affinity column graphs from FPLC were shown as 1A and B.

Miraculin was purified and shown to be homogenized, and the apparent M.W. of miraculin was shown to be 55,000 (Fig. 2). The yield is 2.2 mg/kg from red berries.

1A




2A

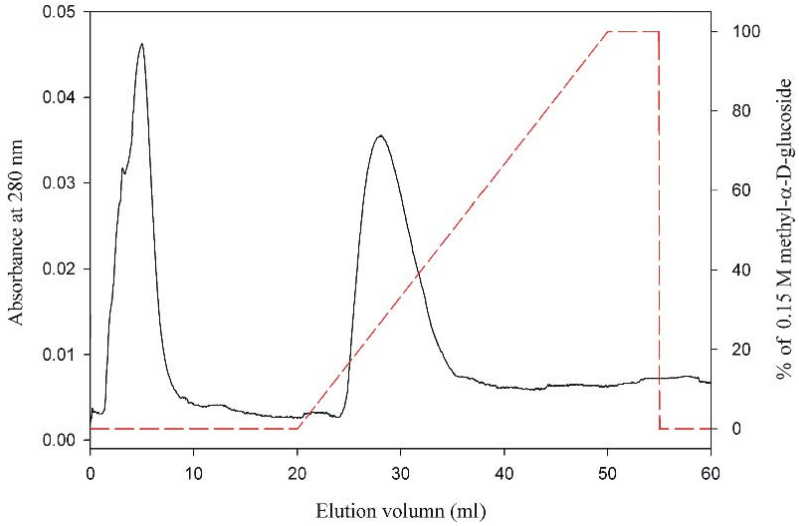



Fig. 1A: Purification of miraculin by SP ion-exchange column chromatography.
1B: Purification of miraculin by Con A affinity column chromatography.

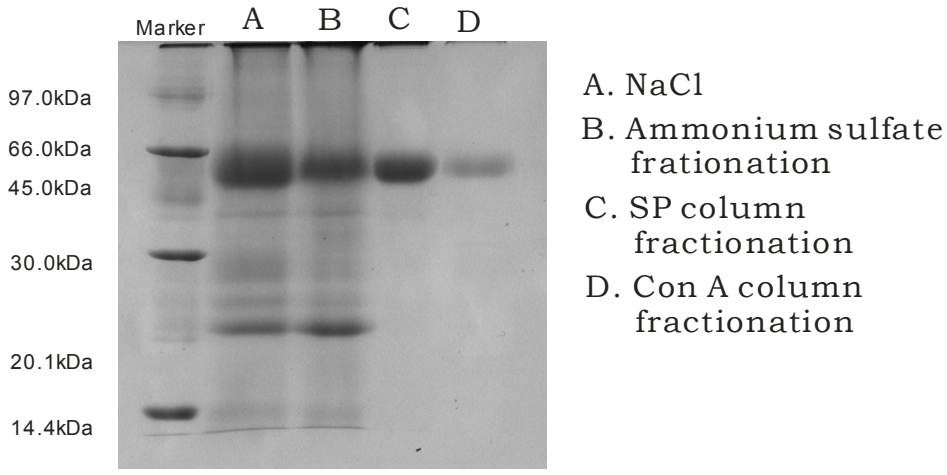



Fig. 2: Purification of miraculin analyzed by SDS-PAGE.

## II. Miraculin is a homo-dimmer

When the purified miraculin was reduced with 5% β-mercaptoethanol, the apparent M.W. of miraculin was reduced to 25,000 (Fig. 3). It indicates that miraculin is a homodimer. 0.1% of β– mercaptoethanol was the most appropriate concentration to break the disulfide bond of dimmer miraculin, and miraculin can just become a monomer (Fig. 4).

When miraculin was reduced with 5% β-mercaptoethanol and alkylated with iodoacetate, the reduced alkylated miraculin lost its sour taste-modifying activity, and it suggested that the dimeric form is essential for its biological activity.

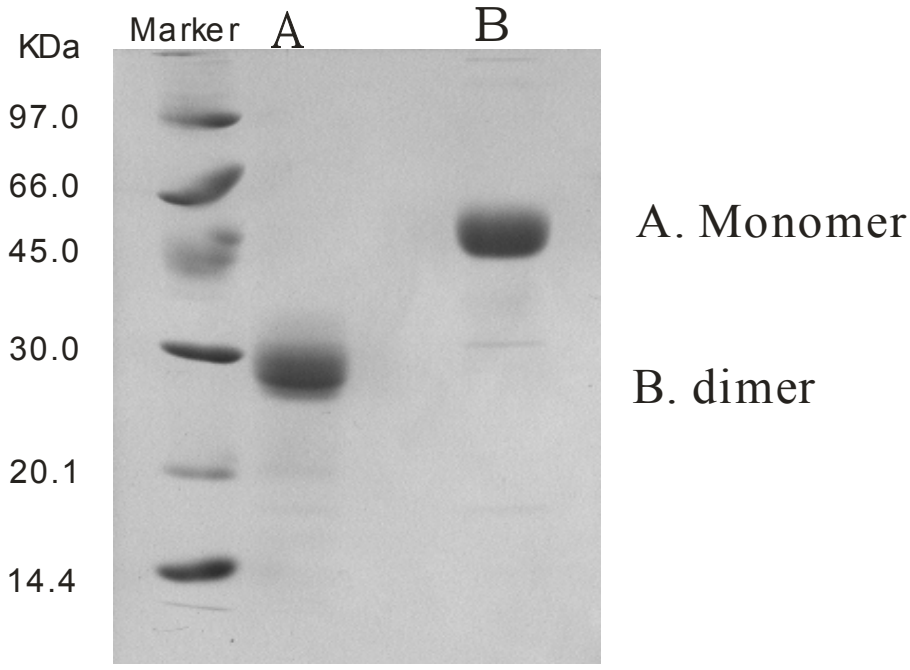



Fig. 3: SDS-PAGE of dimmer and monomer miraculin.

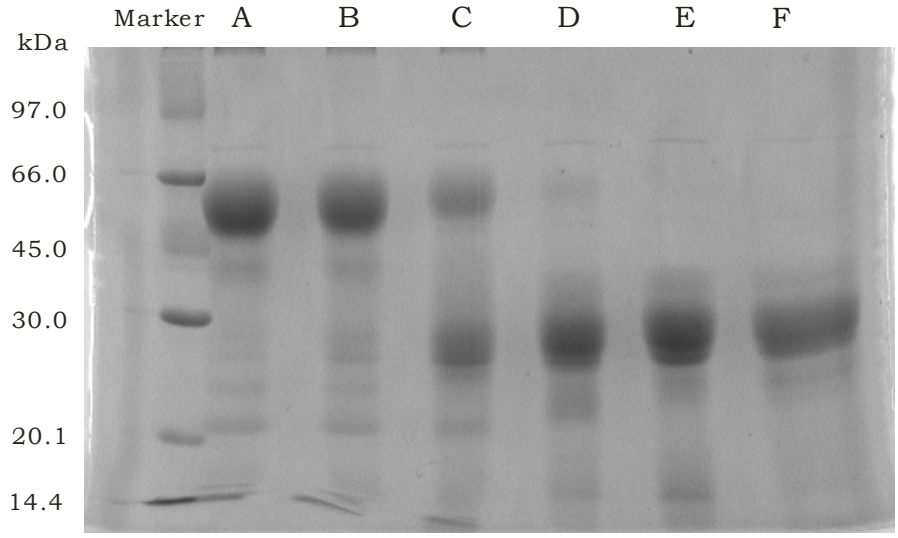



Fig. 4: Demostration of miraculin of different concentrations of β-mercaptoethanol.

## III. Preparation of recombinant miraculin

The cDNA of miraculin was obtained by PCR, and the nucleotide sequence of miraculin cDNA was shown in Fig. 5. The amino acid sequence of miraculin was deduced from the cDNA sequence, and indicates that miraculin contains 191 amino acid with 7 cysteine residues. Two putative glycosylation sites were found at Asn-42 and Asn-186.

The cDNA of miraculin was ligated into the expression vector, pGEX-2T, and expressed in *E. coli.* The fusion protein, GST-miraclin, was purified from the lysate of expressed cells with a glutathione-Sepharose 4B affinity column. The result was shown in Fig. 6. SDS-PAGE of periodic acid-Schiff stain of miraculin and GST-miraculin was shown in Fig. 7. The recombinant miraculin completely lost its taste-modifying activity.

1 GAT TCG GCA CCC AAT CCG GTT CTT GAC ATA GAC GGA GAG AAA CTC CGG ACG GGG ACC AAT
D S A P N P V L D I D G E K L R T G T N 20

61 TAT TAC ATT GTG CCG GTG CTC CGC GAC CAT GGC GGC GGC CTT ACA GTA TCC GCC ACC ACC
Y Y I V P V L R D H G G G L T V S A T T 40

121 CCC AAC GGC ACC TTC GTT TGT CCA CCC AGA GTT GTC CAA ACA CGA AAG GAG GTC GAC CAC

P N G T F V C P P R V V Q T R K E V D H 60

181 GAT CGC CCC CTC GCT TTC TTT CCA GAG AAC CCA AAG GAA GAC GTT GTT CGA GTC TCC ACC

D R P L A F F P E N P K E D V V R V S T 80

241 GAT CTC AAC ATC AAT TTC TCG GCG TTC ATG CCC TGT CGT TGG ACC AGT TCC ACC GTG TGG

D L N I N F S A F M P C R W T S S T V W 100

301 CGG CTC GAC AAA TAC GAT GAA TCC ACG GGG CAG TAC TTC GTG ACC ATC GGC GGT GTC AAA

R L D K Y D E S T G Q Y F V T I G G V K 120

361 GGA AAC CCA GGT CCC GAA ACC ATT AGT AGC TGG TTT AAG ATT GAG GAG TTT TGT GGT AGT

G N P G P E T I S S W F K I E E F C G S 140

421 GGT TTT TAC AAG CTT GTT TTC TGT CCC ACC GTT TGT GGT TCC TGC AAA GTA AAA TGC GGA

G F Y K L V F C P T V C G S C K V K C G 160

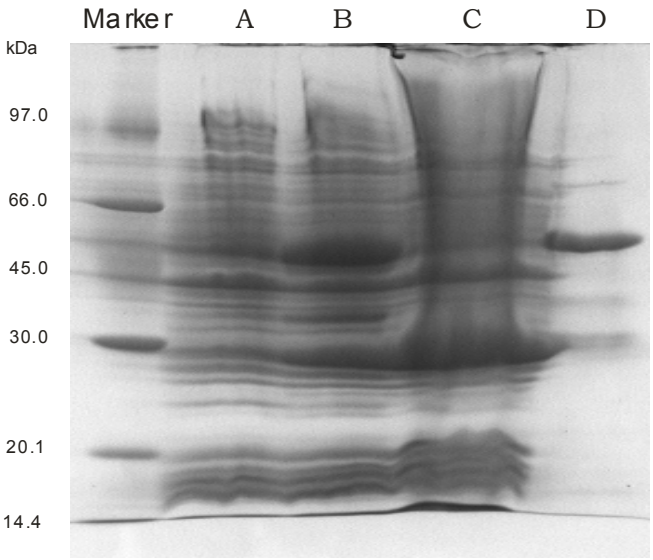
481 GAT GTG GGC ATT TAC ATT GAT CAG AAG GGA AGA AGG CGT TTG GCT CTC AGC GAT AAA CCA

D V G I Y I D Q K G R R R L A L S D K P 180

541 TTC GCA TTC GAG TTC AAC AAA ACC GTA TAC TTC TAA

F A P E P N K T V Y F *

Fig.5: Nucleotide sequence of miraculin cDNA




A. Crude extract from uninduced cells.
B. Crude extract from cells, induced at 4℃ gor 16 hrs.
C. Clarified crude extract from induced cells.
D. Eluent of GST-miraculin.

Fig. 6: Purification of GST-miraculin analyzed by SDS-PAGE.

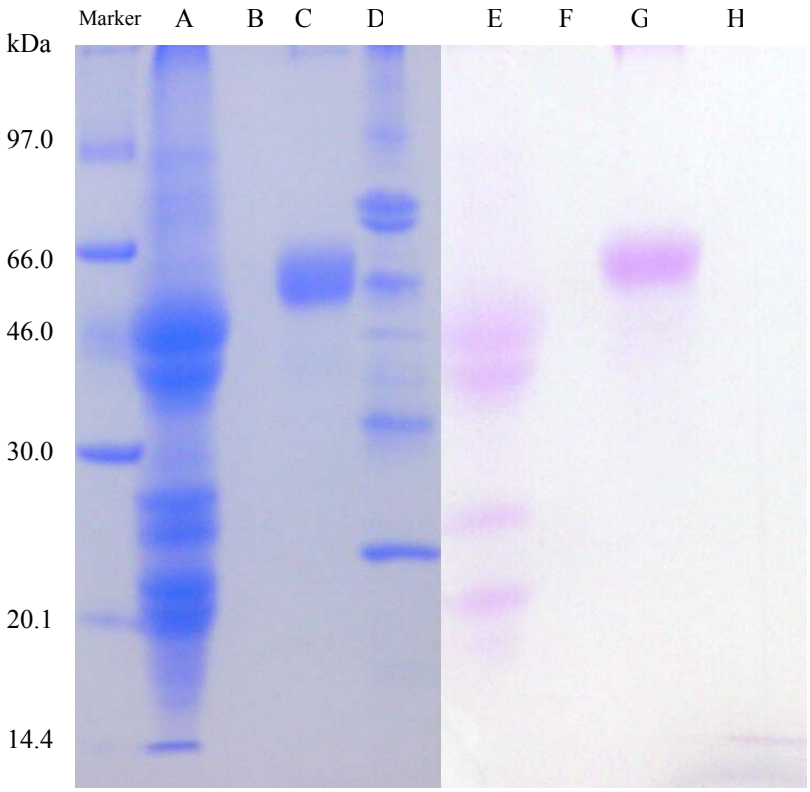
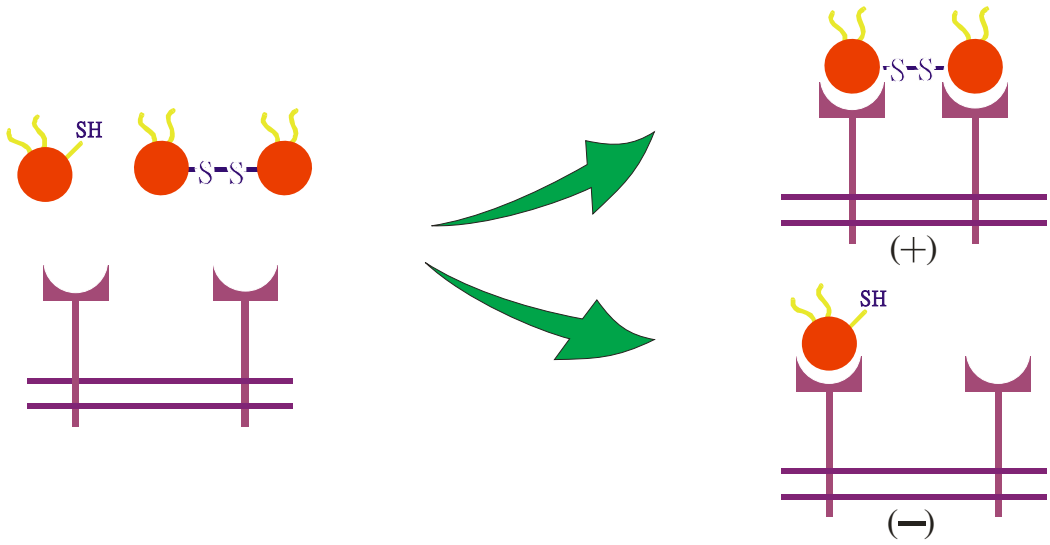



Fig. 7: Staining for carbohydrates and proteins after SDS-PAGE analysis of miraculin and GST-miraculin.

## Conclusion

Miraculin was purified from red berries of *Richadella dulcifica,* and shown to be homogenous by SDS-PAGE analysis. The M.W. of native miraculin was found to be 55,000. After reduction and alkylation of native miraculin, the monomer of miraculin lost its taste-modifying activity. It indicated that the dimeric from is essential to the taste-modifying activity, and suggests that the receptor of the miraculin on the cell membrane of tongue membrane could be a dimmer to exert its activity.




Nucleotide sequence of miraculin showed that it is a glycoprotein with two putative glycosylation sites at Asn-42 and Asn-186. The recombinant protein prepared from *E. coli* express system lost its taste-modifying activity. It suggests that the glycosylation of miraculin is indispensable to its taste-modifying activity.

## Acknowledgement

I would like thank for many people for helping me to do this project about miraculin. The greatest appreciations are for Professor Jung-Yaw Lin and Ms. Yui-Ping Weng. They truly directed my study questions, poster presentation and taught me the experimental technology. Also, I am grateful for Mr. Yi-Da Chung who helped me with the computer skills to present a nice poster and project. Ms. Mai-Ling Lin is another person I thank a lot. She supported me all the materials I need about miracle fruit or its crude extract. I thank to Wen-Hsin Chang, Hong-Ren Lee, Fa-Han Lee and all the members in the lab of professor Jung-Yaw Lin who took care of me during the experiment.

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Your helps made me brave and interested to complete the study about miraculin. And you also let me got involved into the situation easily. I do thank all of you!

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