

# 國立嘉義大學生命科學院

## 學生學術研究成果優良海報評選獲獎名單

時間:106年5月24日


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The background is a bright, clear blue sky filled with numerous white birds in flight, scattered across the frame. At the bottom, a stylized cityscape is visible, featuring several modern buildings with glass facades and a Ferris wheel on the right side. The overall aesthetic is clean, modern, and optimistic.

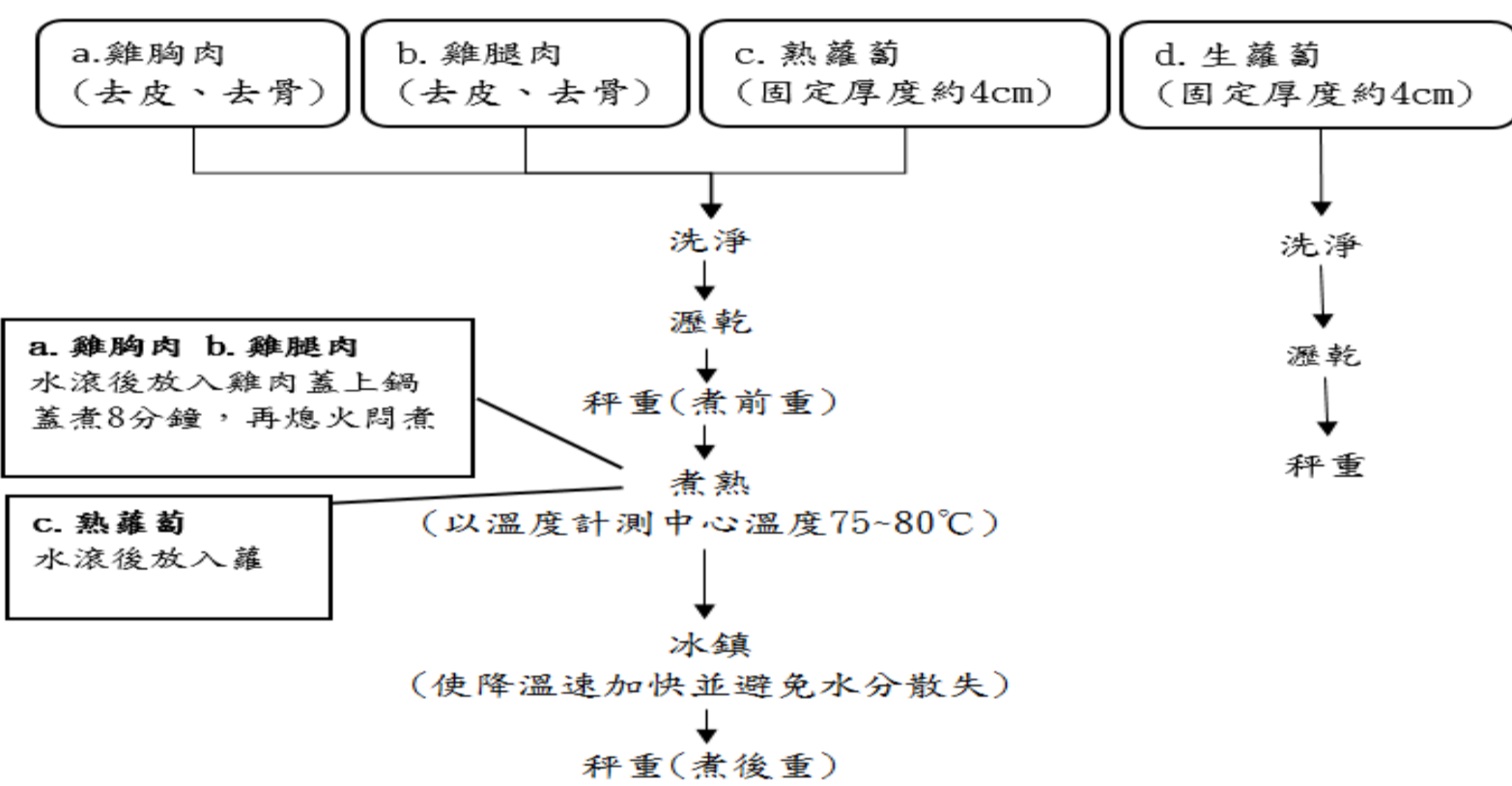
食品科學系  
學士組



非熱製程單元

食品浸泡酒液之方法建立及濃度控制

- 一、實驗目的  
建立食品浸漬酒液的方法，以控制樣品酒精濃度的吸收程度並開發加工流程。
- 二、實驗前處理

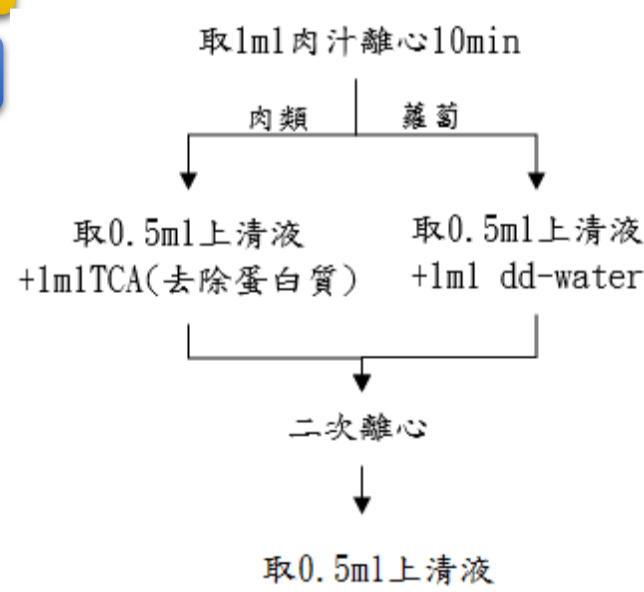


三、測定方法

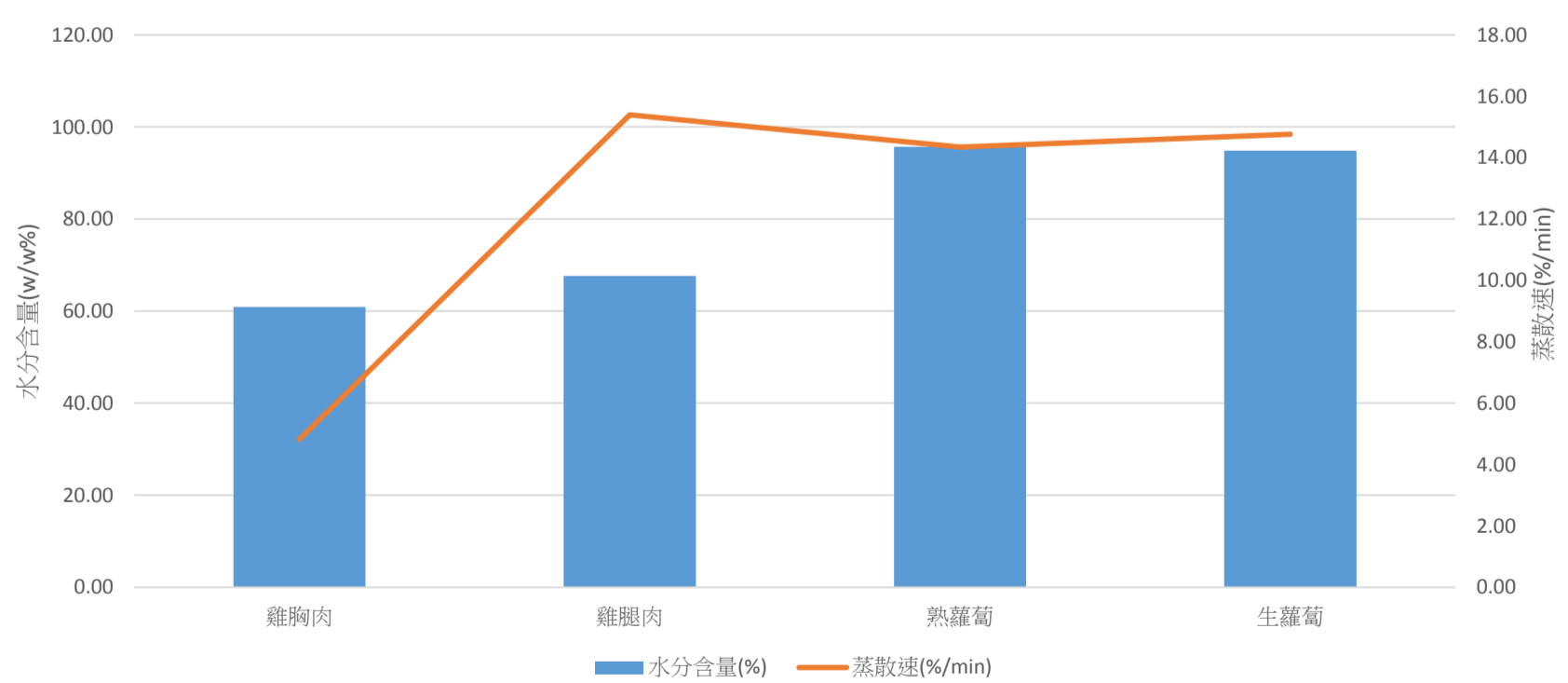
紅外線水分測定儀

Elisa Reader

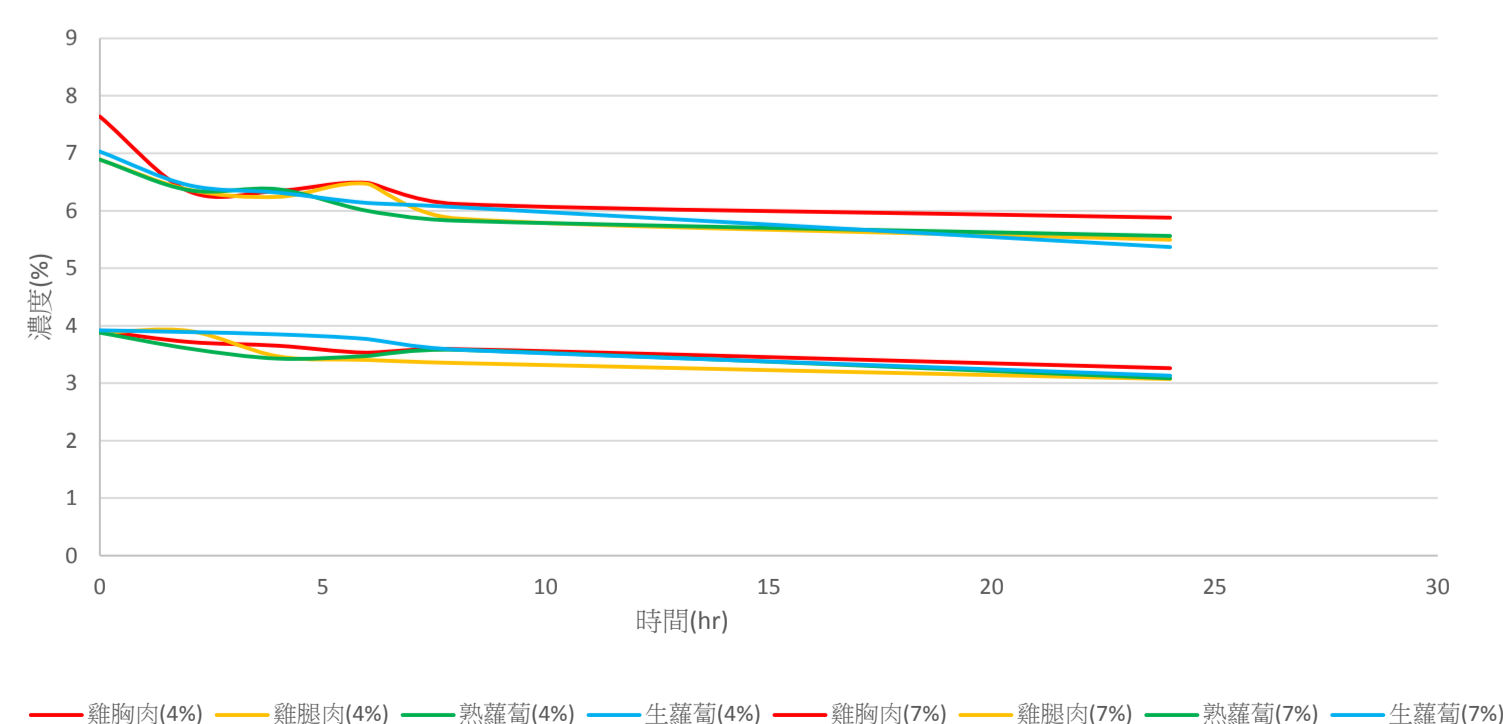
- 浸泡液取樣
- 樣品破碎取樣



四、實驗結果



(圖一) 不同樣品水分含量及蒸散速之關係

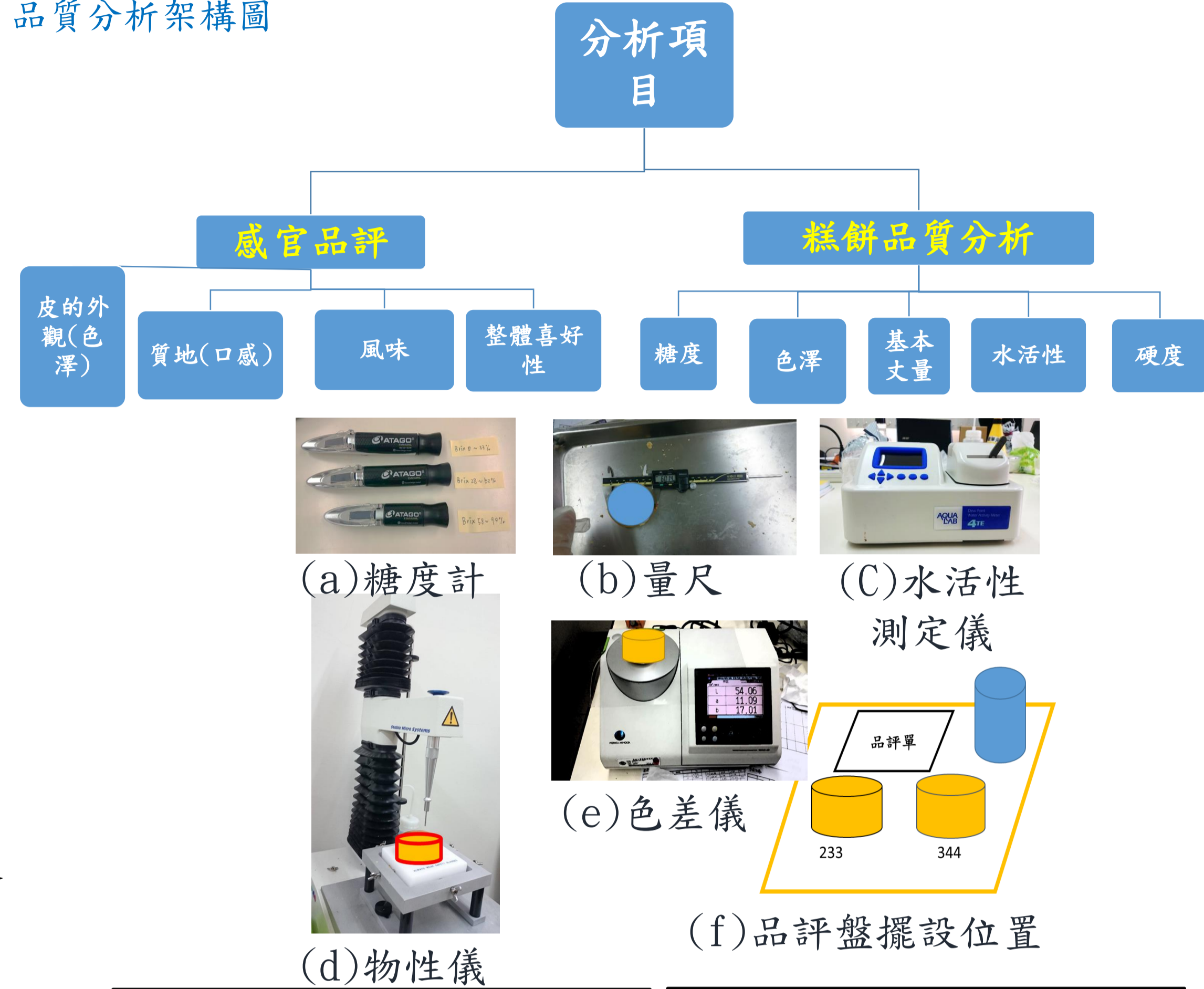


(圖二) 各樣品浸泡液濃度與浸泡時間之變化趨勢

綠豆椪品質分析

目的:以兩種分析方法來測量樣品其品質。

品質分析架構圖



喜好性品評問卷

姓名: \_\_\_\_\_ 日期: \_\_\_\_\_

樣品名稱: \_\_\_\_\_

說明: 下列有 2 個樣品, 請相互比較並就其外觀(色澤)、質地(口感)、風味及整體喜好性, 依個人喜好予以評分。

~~~在品嚐各樣品間隔中, 請以杯水漱口以維持準確度~~~

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|   |   |   |   |   |   |   |   |   |
| 極 | 非 | 不 | 有 | 不 | 有 | 喜 | 非 | 極 |
| 不 | 常 | 喜 | 不 | 喜 | 不 | 歡 | 常 | 喜 |
| 喜 | 不 | 歡 | 喜 | 歡 | 喜 | 歡 | 喜 | 歡 |
| 歡 | 喜 | 歡 | 歡 | 歡 | 歡 | 歡 | 歡 | 歡 |

意見或評語:

~~~請多多給予我們關於樣品的建議~~~

感謝 配合!

五、結論

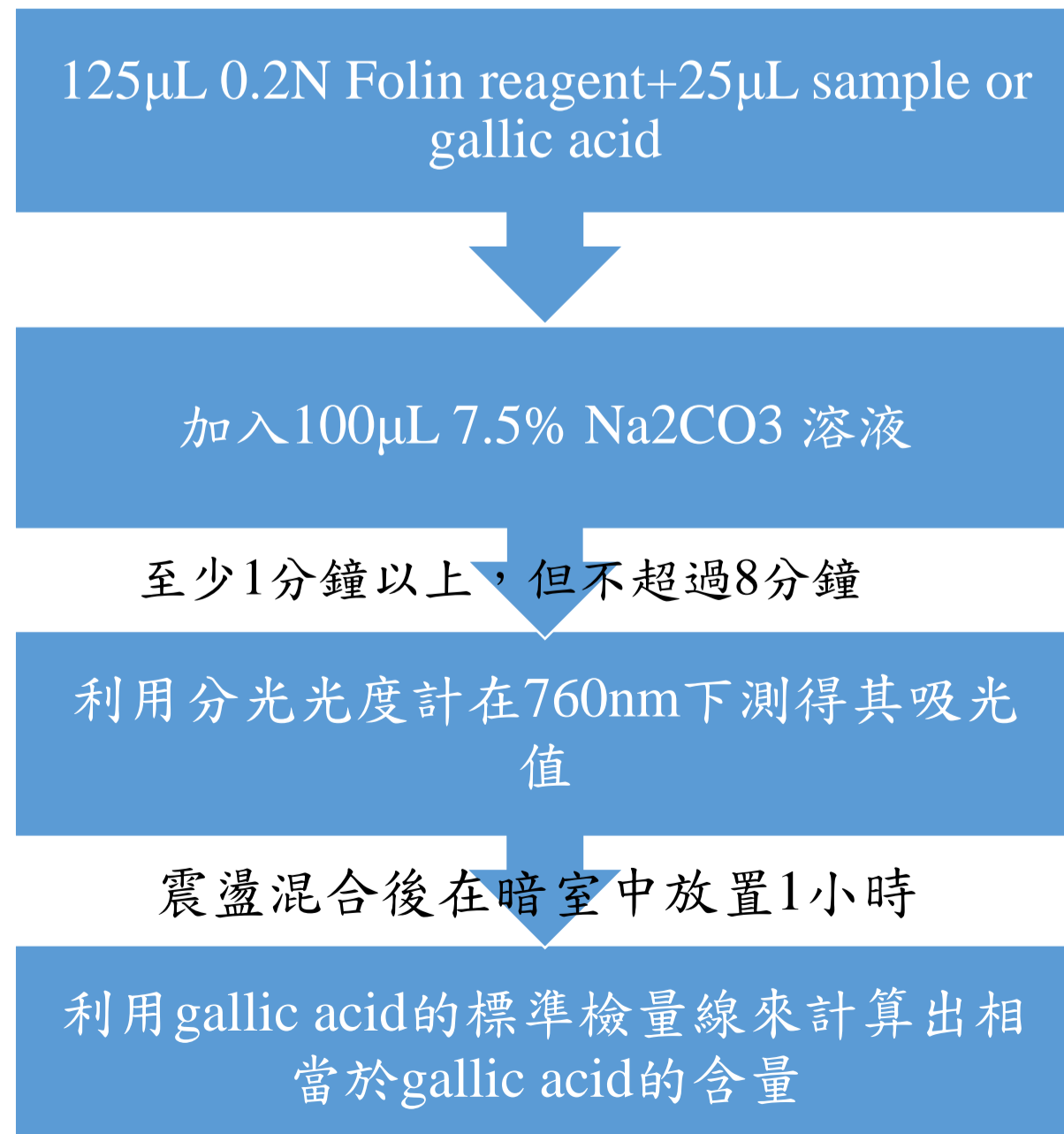
由浸漬液濃度對時間關係變化做討論, 可看出濃度隨時間增加而減少的趨勢, 其中在0hr到2hr間浸漬液濃度改變最大, 可推測此時間斷之浸漬液與樣品酒精濃度相差最大, 濃度梯度越大, 擴散速率也會越快。然而原本預測四種樣品浸漬液會因樣品不同而有一定濃度變化的差異, 但實際實驗過後發現浸漬液濃度趨勢線的變化並無明顯差異, 因此可解釋成浸漬液濃度不會因不同部位、不同煮熟程度、或不同種類的樣品而有所不同。

綠色製程單元

總多酚類分析

原理:

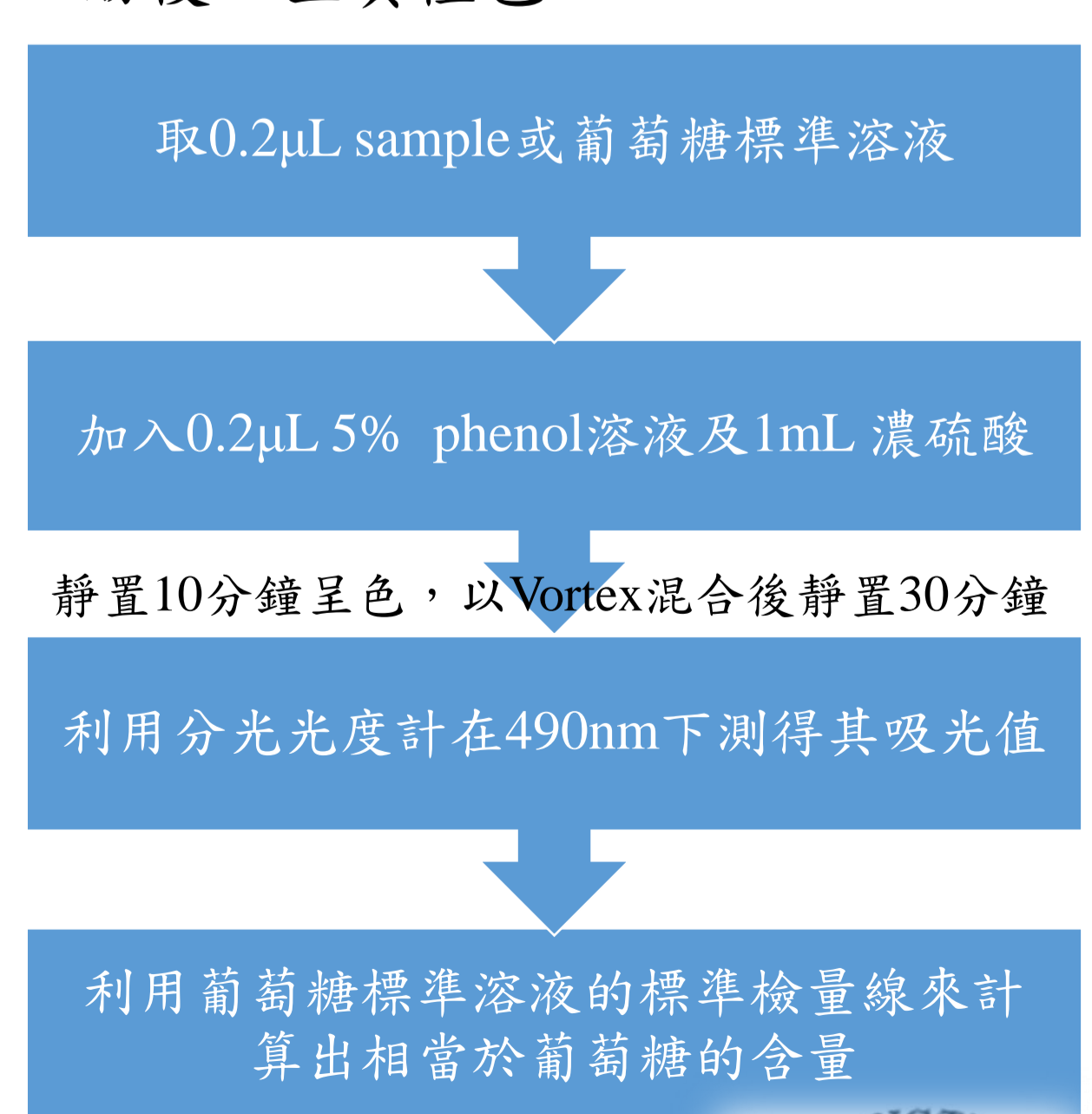
Folin reagent+OH group(酚類), 在 Na<sub>2</sub>CO<sub>3</sub> 溶液催化下, 呈藍紫色。



總多醣類分析

原理:

多醣加硫酸後會脫水水解成單醣, 形成醣醛類化合物, 加入苯酚後, 呈黃橙色。





# 統一企業實習心得分享

食品科學系三年甲班 吳苡珊、賴怡欣

## 鮮乳製作流程



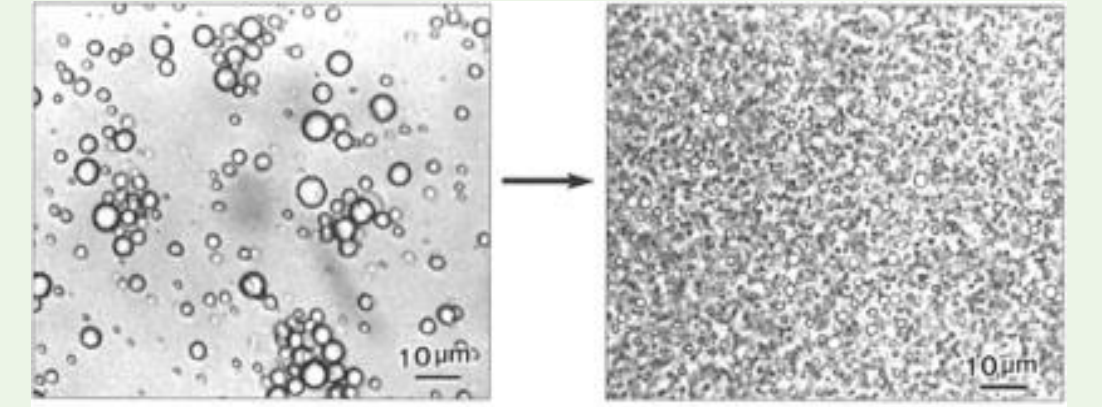
1. 運送：生乳全程 4°C 低溫保鮮送至乳廠



2. 保存：冷循環裝置讓生乳儲存於 7°C 新鮮狀態



3. 清淨：強力的遠心分離裝置(清淨機)將生乳及雜質分離



4. 均質化：透過強力施壓，使靜止於表面如乳霜般的薄膜乳脂肪球，變為細緻的粒子。利於鮮乳中的養分更好吸收，口感更細膩



5. 殺菌：130°C 超高溫瞬間殺菌(瑞穗鮮乳)；72°C 低溫殺菌技術(瑞穗極製鮮乳)



6. 充填包裝：採 E S L 無菌充填包裝技術



7. 成品檢驗運送至各通路

## 麵包管制流程

### 原料檢驗

水-水質

麵包體  
麵粉、植物油-黃麴毒素  
蛋液-水分、抗生素、沙門氏菌

### 微生物檢驗

總生菌數  
大腸桿菌  
大腸桿菌群

### 成品檢驗

規格  
水分  
外包裝  
官能品評




麵包體

餡料

蛋液-沙門氏菌  
植物油-黃麴毒素

總生菌數  
大腸桿菌  
大腸桿菌群  
沙門氏菌

奶油軟法

The background is a bright, clear blue sky filled with numerous white birds in flight, scattered across the frame. At the bottom, a stylized cityscape is visible, featuring several modern buildings with glass facades and a Ferris wheel on the right side. The overall aesthetic is clean, modern, and optimistic.

# 食品科學系 碩博士組



# Analysis on bioavailability and metabolite profile of anti-hyperuricemic fraction of alfalfa extract in a cell co-culture model coupled with UHPLC-ESI-Orbitrap-MS/MS

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## Abstract

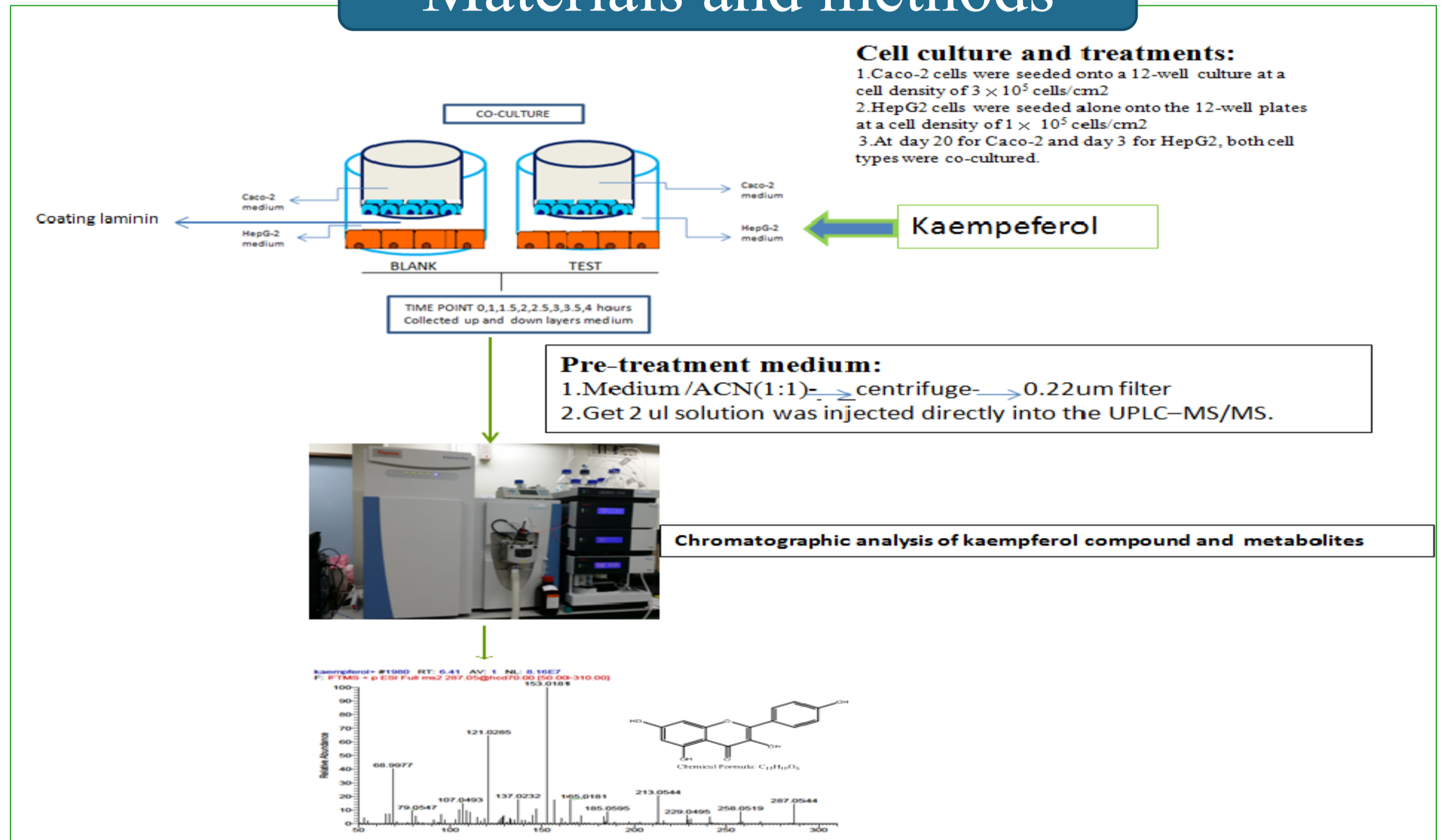
*Medicago sativa* Linn. (Leguminosae) is commonly known as Alfalfa which means “father of all foods”. Alfalfa is not only a good source of animal feed but also a therapeutic agent used in anecdotal medicine. It has been demonstrated to contain various active compounds that are beneficial for human health [1]. Recent pharmacological studies show that alfalfa extract exhibits neuroprotective, hypocholesterolemic, antioxidant, antiulcer, antimicrobial, hypolipidemic, and estrogenic activity, and is effective for treating atherosclerosis, heart disease, stroke, cancer, diabetes and menopausal symptoms. We found that alfalfa leaves have anti-hyperuricemic potential because of its xanthine oxidase inhibitory activity. By using biochemical analysis in conjunction with liquid chromatography tandem mass spectrometry (HPLC-DAD-ESI-MS) technique, we verified that the anti-hyperuricemic fraction of alfalfa extract contain various functional ingredients, including salicylic acid, sinapic acid, *p*-coumaric acid, apigenin, tricrin, kaempferol, and chrysoeriol. The aim of this study the anti-hyperuricemic fraction was further subjected for the examination of bioavailability and metabolite profile produced by intestine cells and hepatocytes in a transwell cell co-culture model in conjunction with UHPLC-ESI-Orbitrap MS/MS analysis. Anti-hyperuricemic fraction of alfalfa extract was included in the medium of the upper layer in the cell culture model comprising Caco-2 cells in upper layer (transwell) and HepG2 cell in bottom layer. The cell medium of the both layers were collected separately at different time points after the treatment, followed by UHPLC-ESI-Orbitrap MS/MS analysis to detect the distribution of functional components and verify the active compounds as well as the corresponding metabolite profile generated by intestinal and hepatic metabolism. Results indicated that in the Caco-2/HepG2 co-culture model, the kaempferol was detectable in the upper layer (Caco-2) medium until 120 min, and fourteen related metabolite in the bottom layer (HepG2 cell) medium compounds including C<sub>15</sub>H<sub>10</sub>O<sub>9</sub>S (Sulfation), C<sub>25</sub>H<sub>31</sub>N<sub>3</sub>O<sub>11</sub>S (GSH Conjugation 2), C<sub>21</sub>H<sub>14</sub>O<sub>10</sub> (Glucuronide Conjugation) derivatives were detected after 90 min. Our result demonstrated the bioavailability and potential metabolite profile of kaempferol produced by intestine and hepatocytes. The established model can be useful for rapid evaluation of bioavailability and metabolite profile of natural dietary products

Keywords: UPLC-ESI-MS/MS, co-culture, bioavailability

## Introduction

Kaempferol, a major flavonoid compound in the diet that widely existed in daily food such as vegetables and fruits. Investigations have shown that kaempferol exhibits a wide range of biological activities including antioxidant, anti-inflammatory, anticoagulant, and cardiovascular-protective effects [3]. Previous our studies have also found that kaempferol possesses significant XO inhibitory activity in vitro. However, the anti-hyperuricemic fraction which one of kaempferol further subjected for the examination of bioavailability and metabolite profile produced by intestine cells and hepatocytes in a transwell cell co-culture model in conjunction with UHPLC-ESI-MS/MS analysis is still obscure. Anti-hyperuricemic fraction of alfalfa extract was included in the medium of the cell culture model comprising Caco-2 cells in upper layer (transwell) and HepG2 cell in bottom layer. The cell medium of the both layers were analyzed at different time points after the treatment, followed by UHPLC-ESI-MS/MS analysis to detect the distribution of functional components and verify the active compounds as well as the corresponding metabolite profile generated by intestinal and hepatic metabolism. The established model can also be useful for rapid evaluation of bioavailability, metabolite profile and hepatic protection activity of orally administered natural products.

## Materials and methods



## Results

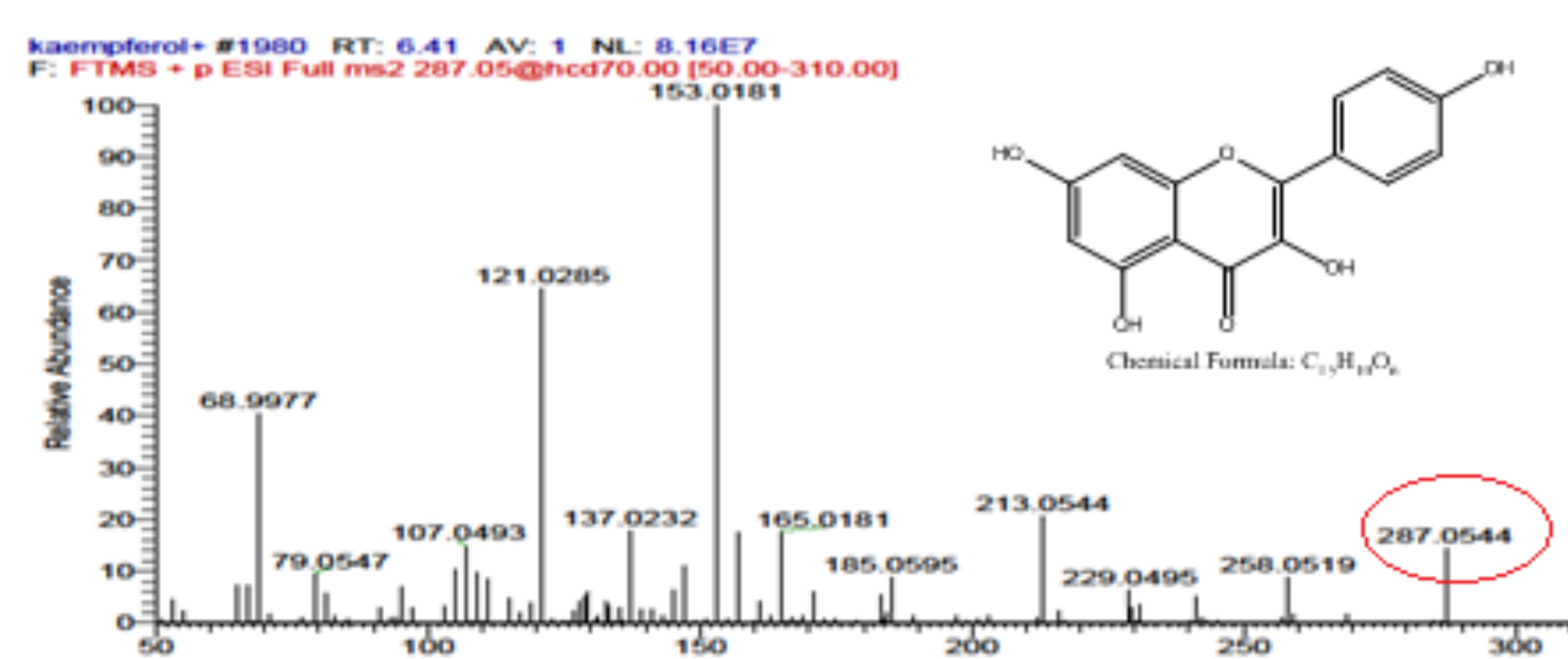


Figure 1. ESI-HRMS spectra of Kaempferol

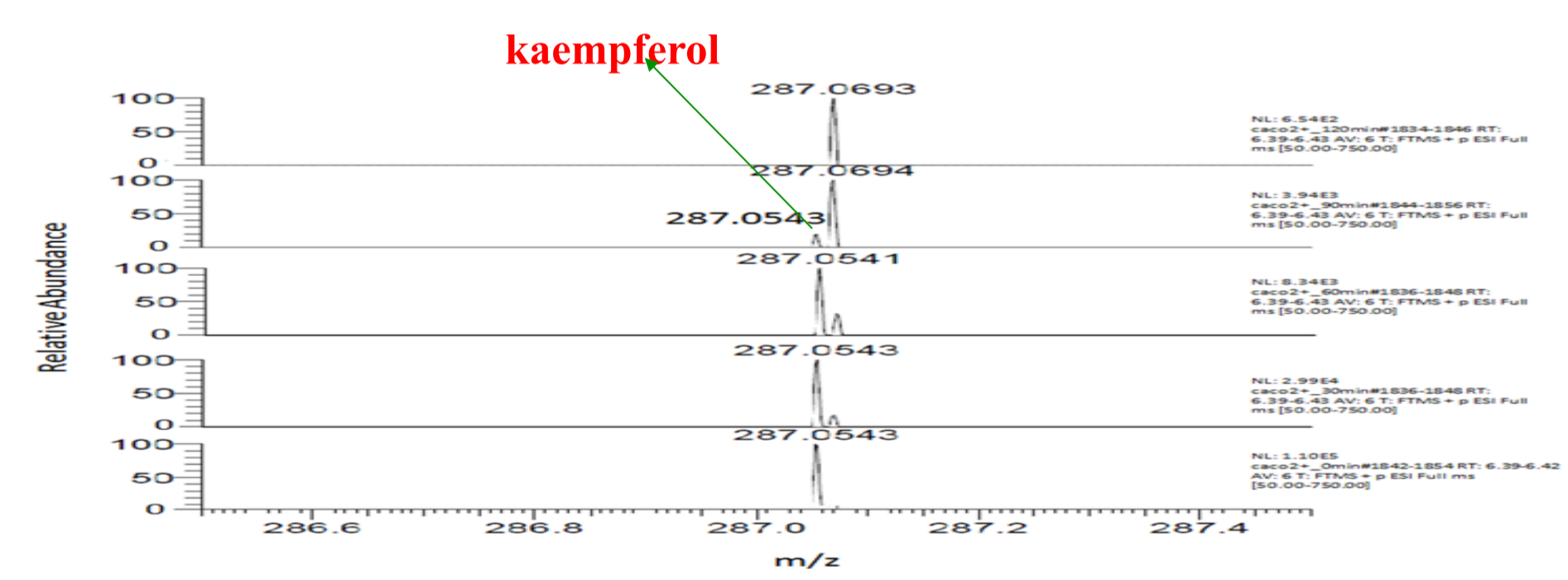


Figure 3. The UHPLC-ESI-MS spectrum of kaempferol in caco2 medium at different time (positive ion mode)

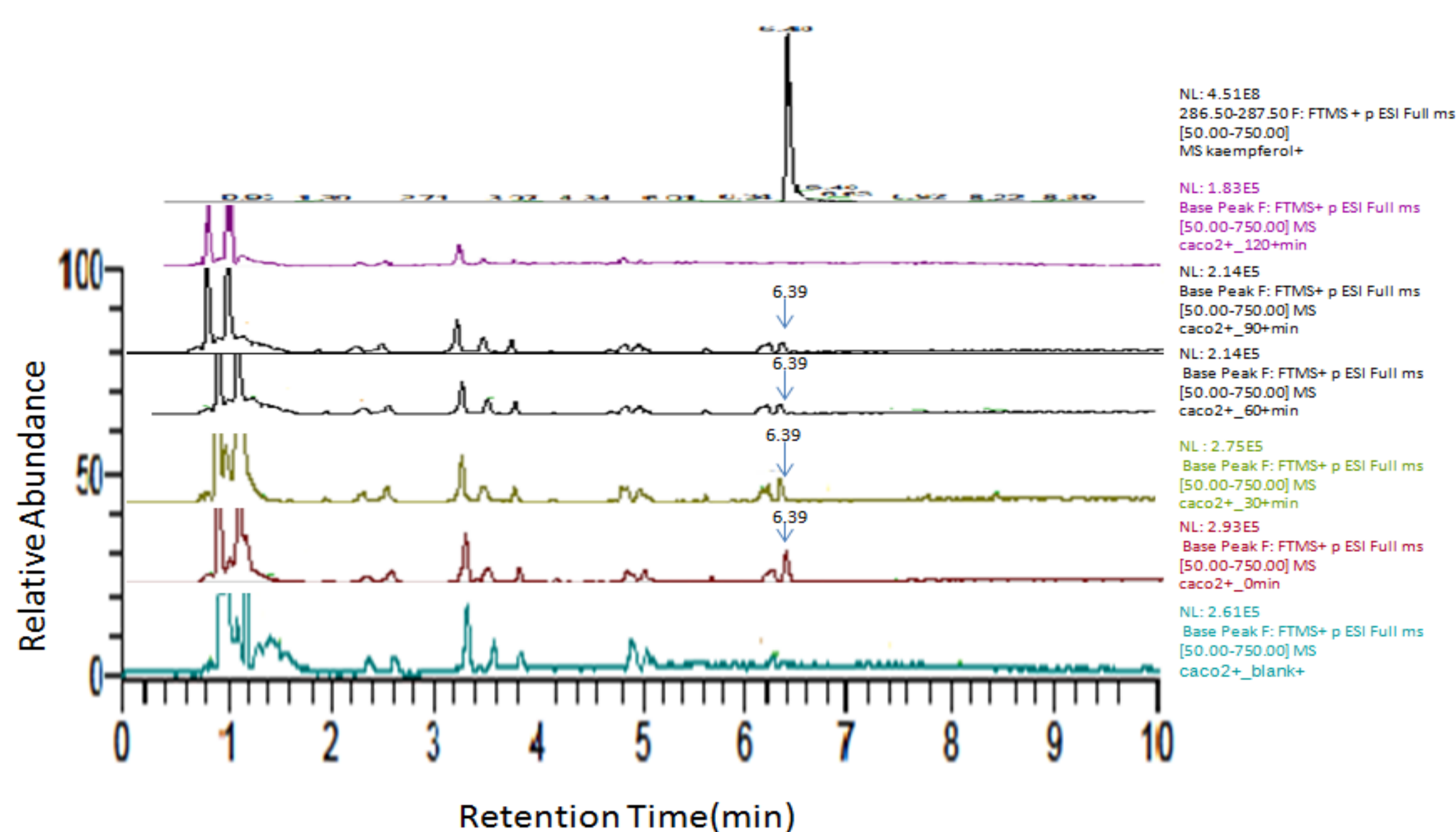


Figure 2. LC/MS chromatograms of Caco2 medium at different time (positive ion mode)

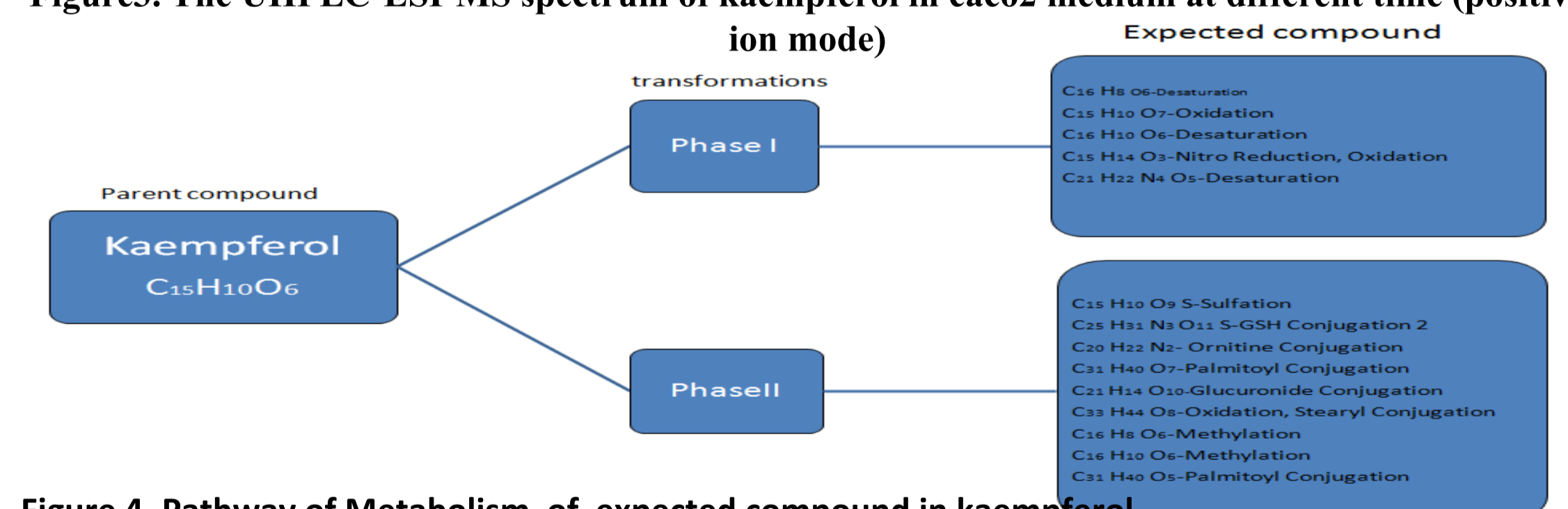


Figure 4. Pathway of Metabolism of expected compound in kaempferol

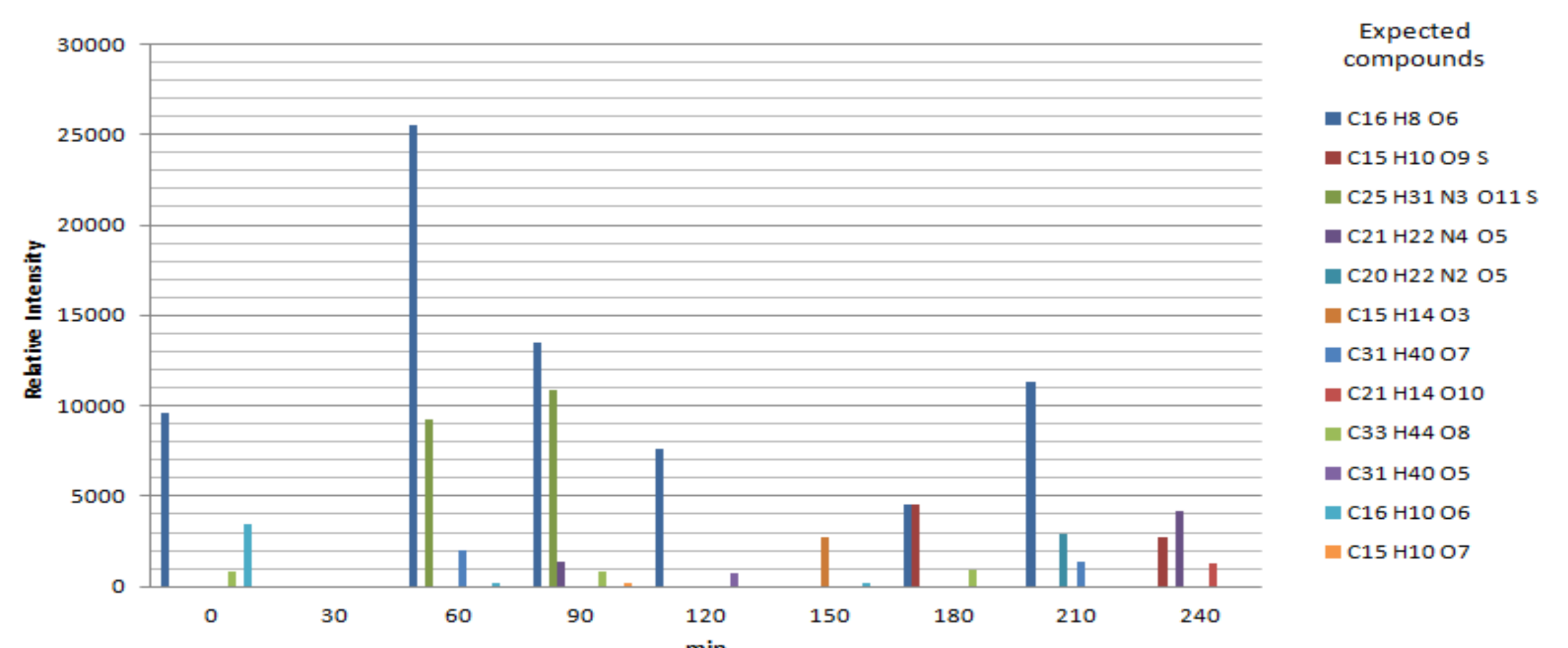


Figure 5. Metabolism products of kaempferol at different time point after co-culture in bottom layer of HepG2 cell medium.

## Conclusion

Our results suggest that kaempferol can be absorbed by the intestinal cells by 90 minutes, and metabolized through phase I, and II enzyme pathways in hepatocytes. The bioavailability by hepatocytes may also support the potential nutraceutical application of alfalfa extract in hyperuricemia prevention that we found previously. Additionally, the established model can also be useful for rapid evaluation of bioavailability, metabolite profile and hepatic protection activity of orally administered natural products.

## Reference

- [1] Bora KS, Anupam S. Phytochemical and pharmacological potential of *Medicago sativa*: A review. *Pharm Biol* 2011; 49: 211–220
- [2] Maria-Paz Romero, Maria-José Motilva. Effect of the co-occurring olive oil and thyme extracts on the phenolic bioaccessibility and bioavailability assessed by in vitro digestion and cell models. *Food Chemistry* 2014; 149:277–284.
- [3] Wang Yajie, Guowen Zhang, Junhui Pan, and Deming Gong. Novel Insights into the Inhibitory Mechanism of Kaempferol on Xanthine Oxidase. *J. Agric. Food Chem* 2015;63, 526–534



# 探討 *Lactobacillus harbinensis* LYC 1050、1052、1053 其益生菌特性並開發蘋果乳酸菌發酵飲品及抗氧化能力

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## 摘要

本項研究首要目的是要探討經由天然水果發酵液篩選的乳酸桿菌，將其以 16S rDNA 親源性分析顯示三種分離菌株與 *L. harbinensis* 和 *L. perolens* 密切相關，再針對益生菌特性包括耐酒精、耐高糖及抑菌試驗，觀察其是否適合做為發酵菌種，之後在蘋果汁中接種 1% 活化菌液於 37°C 發酵 24 小時，分析哪一株具有最高的細菌生長情形，並比較其抗氧化能力並評估其應用性。發酵可以有助於改善食品的功能性，結果顯示，LYC 1052 在發酵 16 小時具有最高的菌數 (5.3-8.3 log CFU/ml)。抗氧化能力方面，清除 DPPH 自由基能力部分並沒有顯著的變化，但是還原力確實具有菌數較高的 LYC 1052 有最好的能力，其含量為 235.58 µg/mL Vit C equivalent。經 LYC 1052 發酵後的蘋果發酵液具較好的抗氧化活性，並具有開發新穎蘋果乳酸菌發酵飲品之潛力，未來需進一步進行爾後之研究。

## 前言

目前功能性食品市場含有益生菌的產品是最被大眾所接受，並且在每年全球保健食品消費金額也是不容小覷，主要是由於現代人外食普遍、精神壓力大與化學添加物的濫用，造成腸道正常菌相失衡，造成容易引起便秘、下痢甚至是腸胃道潰瘍。摘取的水果在內部與表面皆存在許多天然的乳酸菌，再經過適當的溫度和時間培養，篩選出適當的天然發酵菌種，經過 16S rDNA 親源性分析顯示三種分離菌株與 *L. harbinensis* 和 *L. perolens* 密切相關。由於益生菌產品目前大多使用乳製品作為發酵基質，但是近年來發現世界上約有一半的人有乳糖耐受不良的情形，尤其是亞洲及非洲人 (Ana Paula Espirito-Santo *et al.*, 2015)。因此，本研究目的開發水果發酵飲品，透過幫助水果自然發酵的乳酸菌額外添加入果汁中，來增加果汁飲品的保健功效及抗氧化能力，並期望能透過分離的菌株改善製作果汁酵素製程。

## 材料與方法

### 一、實驗材料

1. 澄清蘋果濃縮汁
2. 乳酸菌株經由天然水果發酵液分離

The strains were maintained in MRS broth (Difco) at 37 °C for 16 h.

### 二、實驗方法

1. 將分離出的菌種以聚合酶連鎖反應技術鑑定再經由 NCBI 資料庫比對
2. 經由 MRS 培養基隔夜培養活化後進行耐酒精、耐高糖、抑菌試驗
3. 之後將濃縮汁稀釋後接種菌種進行 24 小時發酵再針對菌數變化、pH 值、可滴定酸進行分析，另外也會對酵素的抗氧化能力以 DPPH 清除自由基能力、還原力等抗氧化能力試驗進行評估。

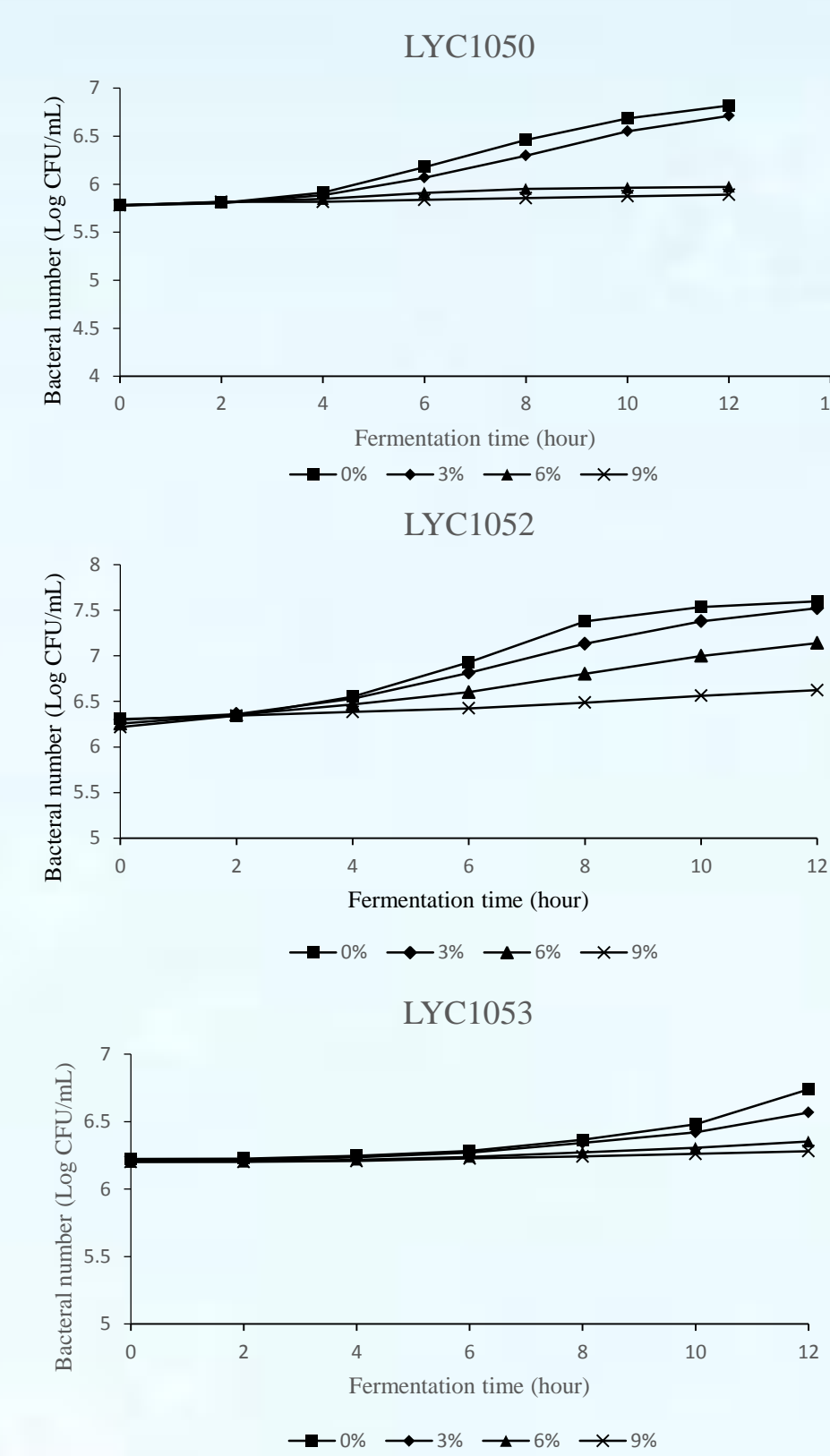


Fig. 2. Alcohol tolerance of LYC1050, LYC1052, LYC1053.

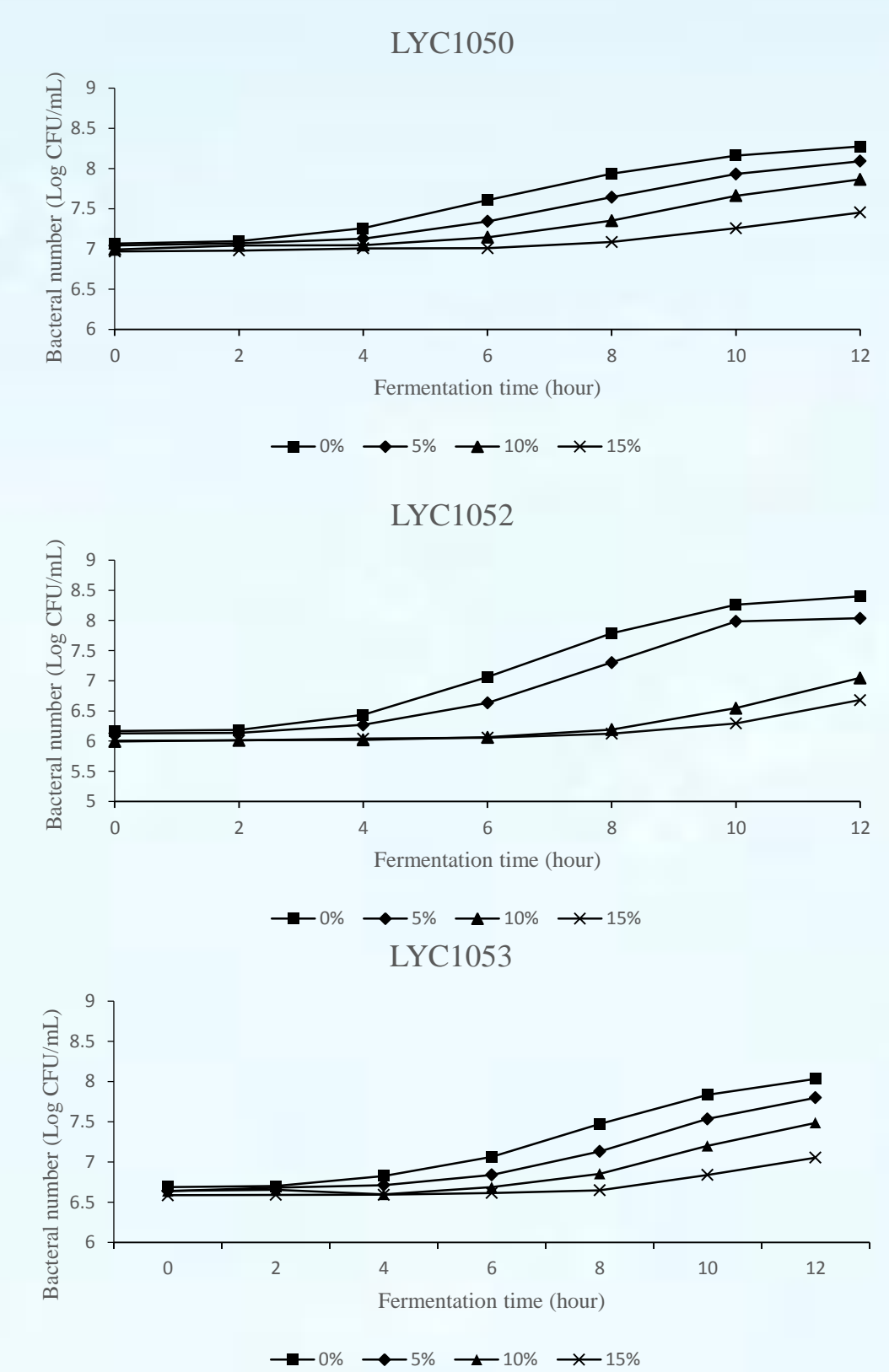


Fig. 3. The growth curve of the strains LYC1050, LYC1052, LYC1053 which in different sugar levels.

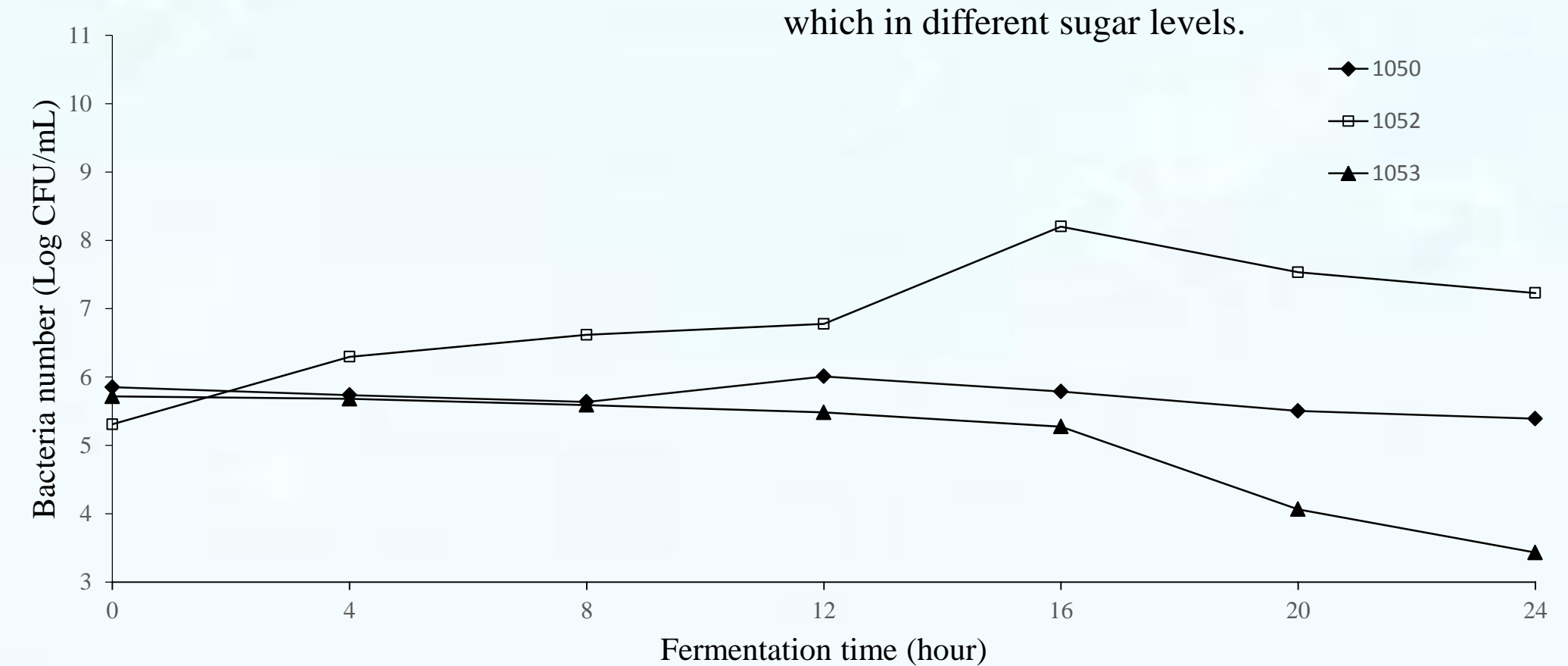


Fig. 4. Growth curves of LYC1050, LYC1052, LYC1053 in apple juice

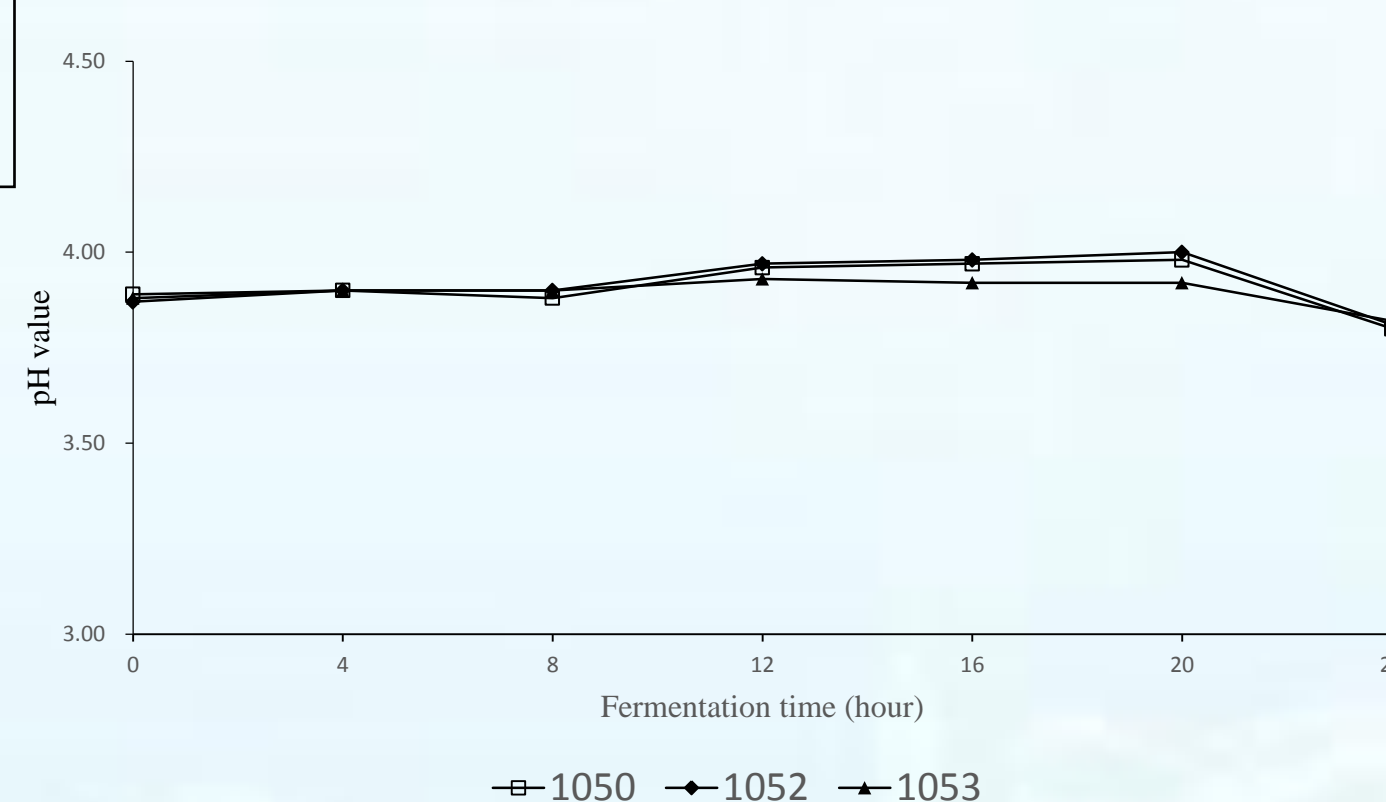


Fig. 5. pH value of LYC1050, LYC1052, LYC1053 in apple juice.

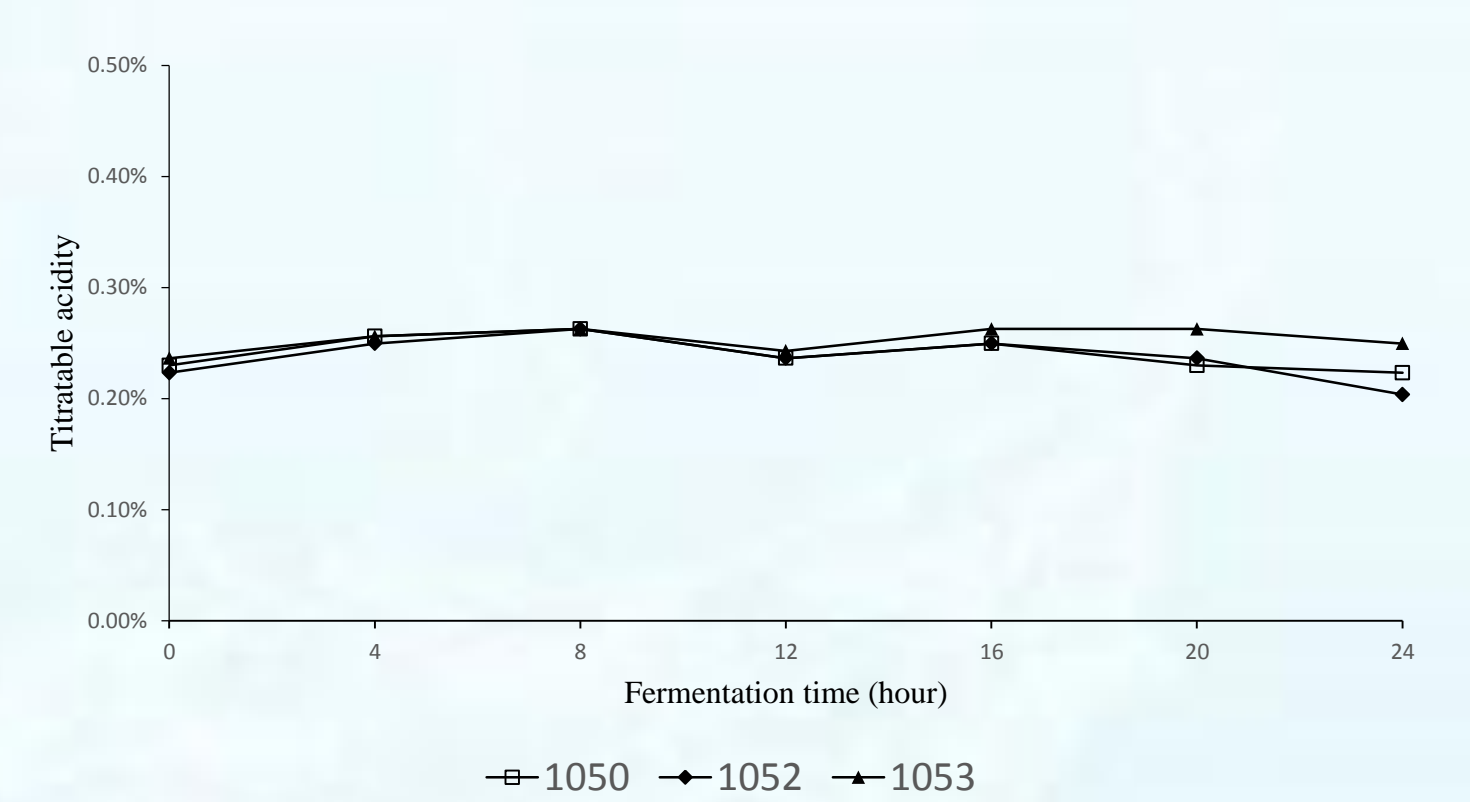


Fig. 6. Titratable acidity of LYC1050, LYC1052, LYC1053 in apple juice.

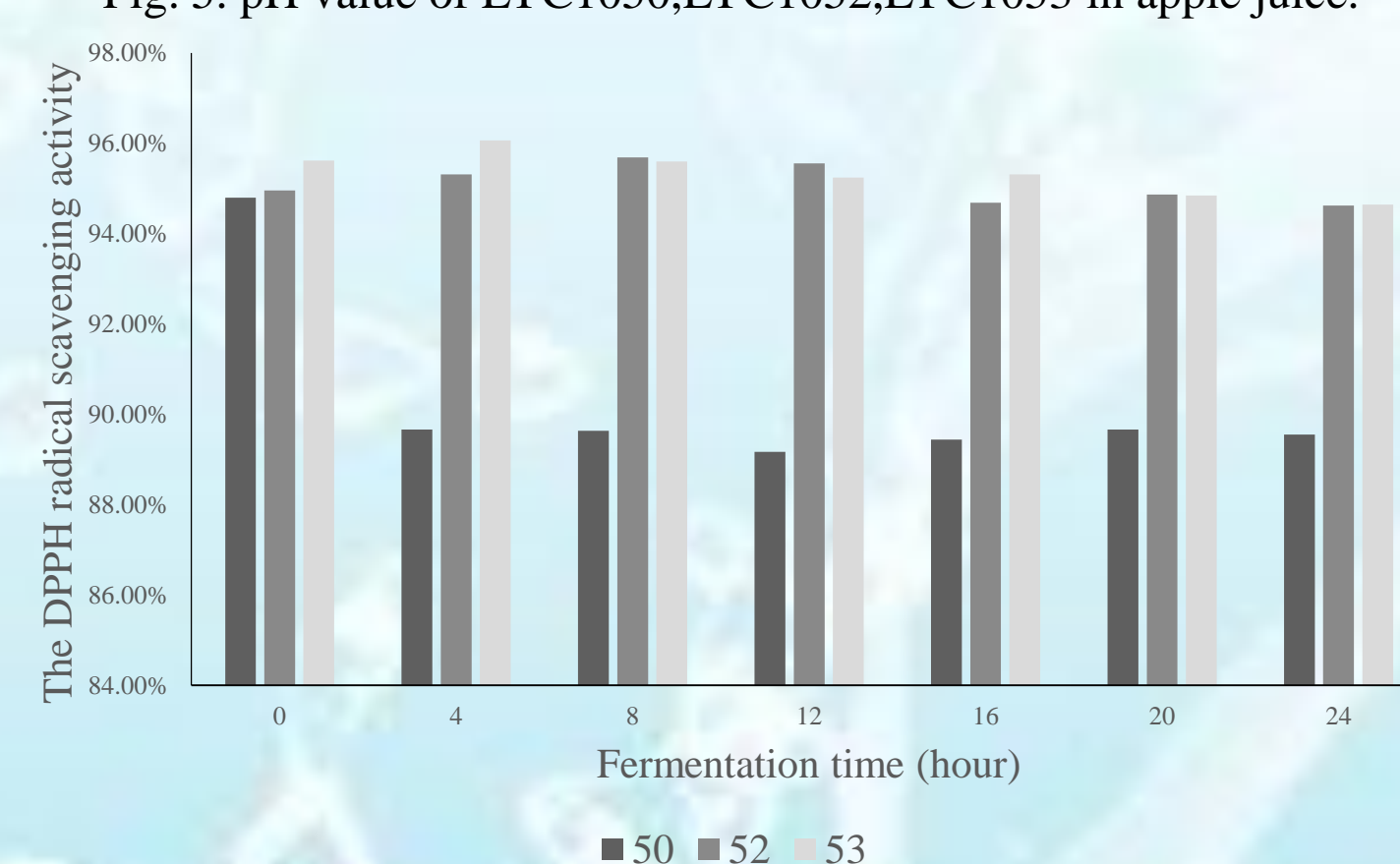


Fig. 7. The DPPH radical scavenging activity of fermentation time in different groups

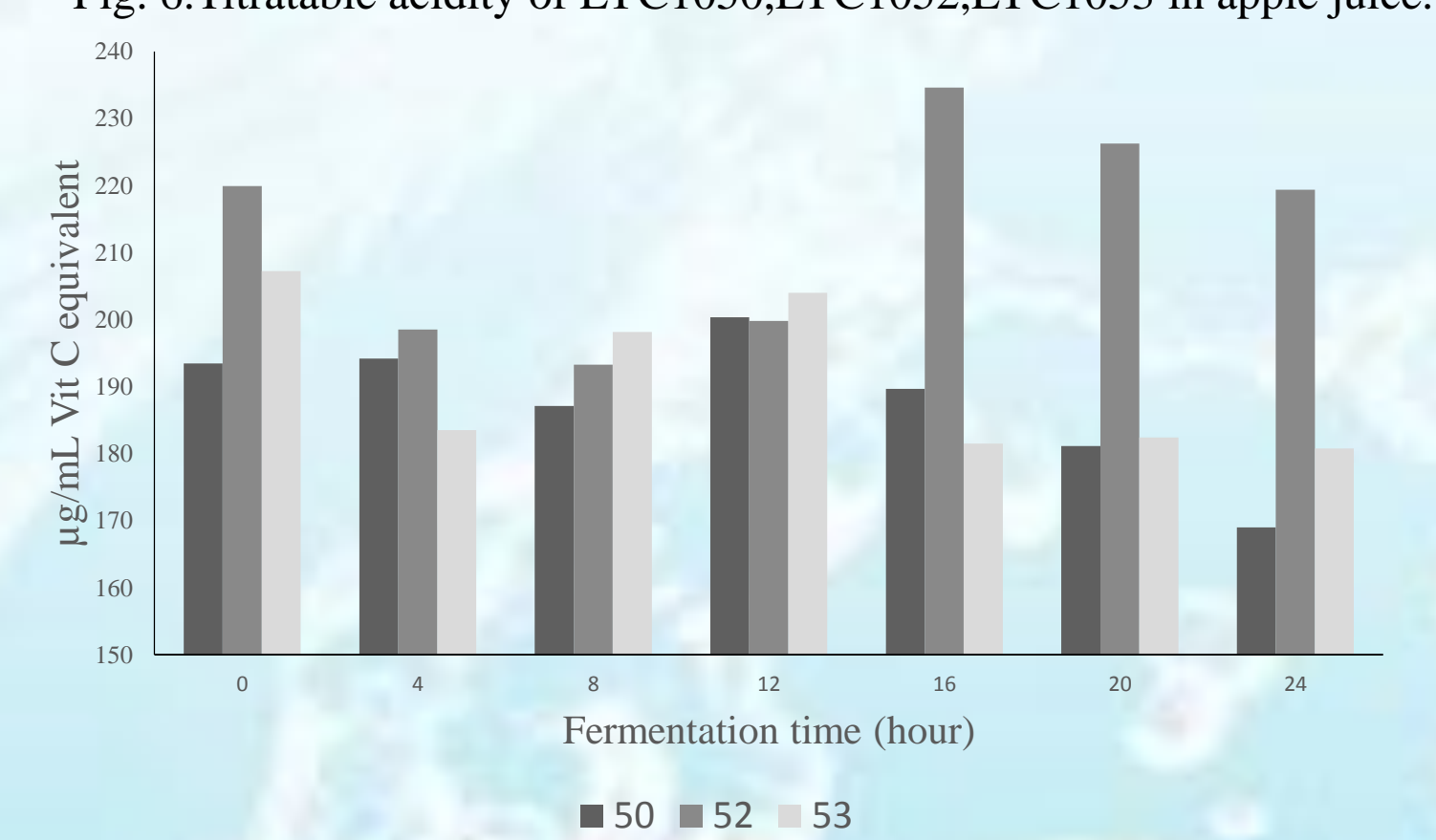


Fig. 8. Reducing power of fermentation time in different groups

## 結果

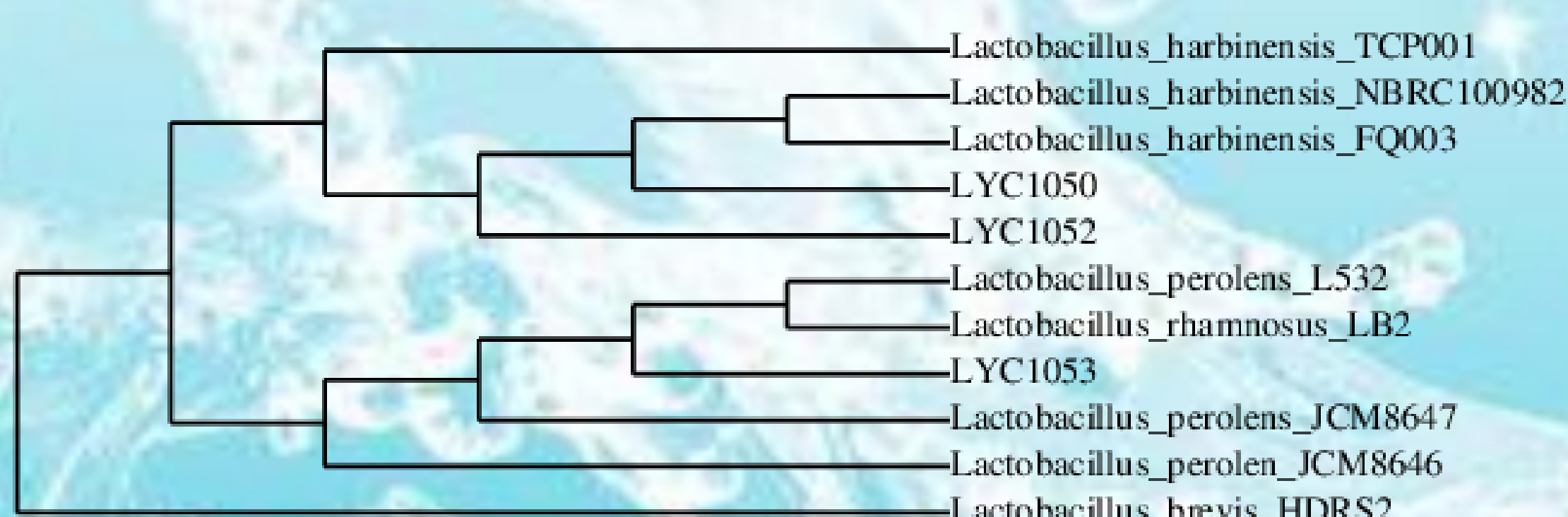


Fig. 1. Neighbor-joining analysis based on sequences of the 16S rDNA gene in 8 isolates.

## 結論

綜合以上結果，三種篩選菌株 LYC 1050、LYC 1052、LYC 1053，經由耐糖與耐酒精試驗，結果認為以 LYC 1052 最適合在蘋果汁中生長，再經過發酵觀察生長曲線與先前試驗相符 LYC 1052 菌數增加，而 LYC 1050 能在其中維持菌數，而 LYC 1053 則無法在酸性較高的果汁環境下存活。而 pH 值在發酵中期有些微上升，有文獻認為乳酸菌將蘋果汁中的蘋果酸、琥珀酸被代謝成較弱的乳酸，而後持續發酵則乳酸大量增加則 pH 值逐漸下降。抗氧化活性方面，DPPH 自由基清除能力並沒有顯著性的變化。而在 LYC 1052 發酵到菌數最高時具有較佳之還原力。

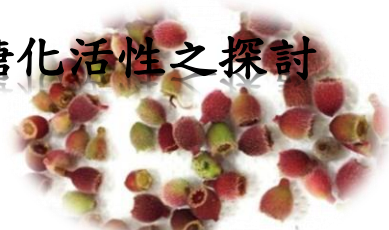




# 豔紫野牡丹果實萃取物抗氧化及抗糖化活性之探討

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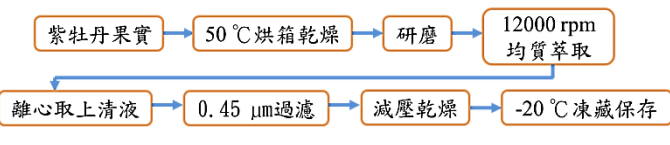


## 壹、前言：

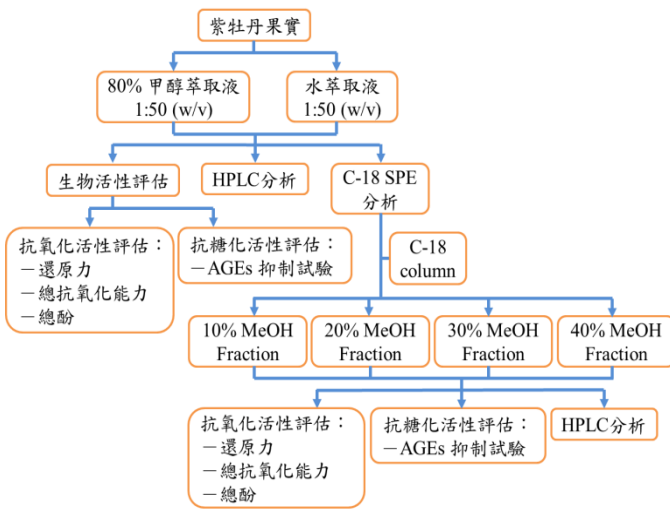
隨著時代變遷，預防醫學意識抬頭，現代人開始重視健康自然，對於人工合成的化學藥品所造成的副作用產生疑慮，因此轉而從天然物中尋求藥物及療法。本篇試驗是採用豔紫野牡丹 (*Tibouchina semidecandra* Cogn.) 作為試驗原料，其屬野牡丹科，為一種觀賞型的灌木植株，因其具有鮮豔的紫色花瓣而得名。除觀賞之外，常見為中藥材，其富含豐富的丹寧、花青素及類黃酮。是一種良好的天然素材，藉由生物活性的評估，進而鑑定其所含的活性物質，可作為一個具有開發潛力的天然原料。

## 貳、材料與方法：

### 一、萃取方法：



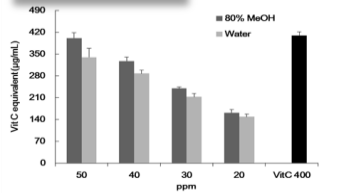
### 二、實驗架構：



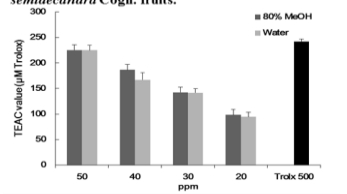
### 三、實驗方法：

- 還原力測定：**取萃取液 200  $\mu$ l 加入 200  $\mu$ l 1%  $K_3Fe(CN)_6$  溶液，於 50°C 水浴加熱 20 分鐘後，立即冷卻，再加入 200  $\mu$ l 10% Trichloroacetic acid 溶液，離心取上清液 400  $\mu$ l 加入等量的去離子水及 80  $\mu$ l 0.1%  $FeCl_3$  溶液，反應 10 分鐘後，於 700 nm 測其吸光值，並以 VitC 做為正對照組。
- 總抗氧化能力測定：**取萃取液 50  $\mu$ l 加入 950  $\mu$ l 之稀釋  $ABTS^{+}$ ，反應 6 分鐘後，於 734 nm 測其吸光值，並以沒食子酸做為正對照組。
- 總酚測定：**萃取液 0.1 ml 加入 0.5 ml Folin-Ciocalteu's phenol reagent，混合後反應 3 分鐘，加入 0.4 ml 7.5% sodium carbonate 水溶液，反應 30 分鐘後於 750 nm 測其吸光值。
- 進階糖化終產物生成抑制試驗：**先將 80% 甲醇萃取液以真空濃縮乾燥機去除甲醇後，再以 1% DMSO 回溶，取萃取液 200  $\mu$ l 加入 200  $\mu$ l 以磷酸緩衝液配置含有 0.06%  $NaN_3$  之 BSA 及 1.5 M D(-)-Fructose，利用螢光偵測儀於激發光 360 nm 與散色光 460 nm 測定反應 0 小時及 24 小時，並以 3 mM Aminoguanidine 做為正對照組。

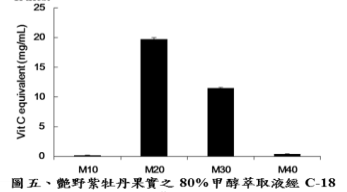
## 參、結果：



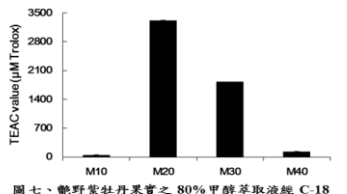
圖一、豔紫野牡丹果實之 80% 甲醇萃取液於不同濃度下對總還原力之影響  
Figure 1. Reducing power of 80% methanol extract at different concentrations from *Tibouchina semidecandra* Cogn. fruits.



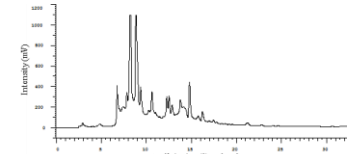
圖三、豔紫野牡丹果實之 80% 甲醇萃取液於不同濃度下對總抗氧化能力之影響  
Figure 3. Trolox equivalent antioxidant capacity (TEAC) of 80% methanol extract at different concentrations from *Tibouchina semidecandra* Cogn. fruits.



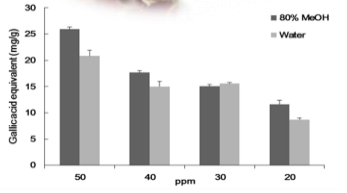
圖五、豔紫野牡丹果實之 80% 甲醇萃取液經 C-18 沖提區段對還原力之影響  
Figure 5. Reducing power of 80% methanol extract from *Tibouchina semidecandra* Cogn. fruits eluted fractions of SPE C-18 column.



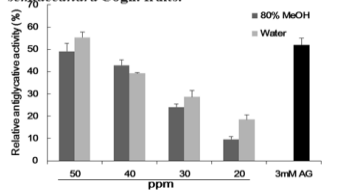
圖七、豔紫野牡丹果實之 80% 甲醇萃取液經 C-18 沖提區段對總抗氧化能力之影響  
Figure 7. Trolox equivalent antioxidant capacity (TEAC) of 80% methanol extract from *Tibouchina semidecandra* Cogn. fruits eluted fractions of SPE C-18 column.



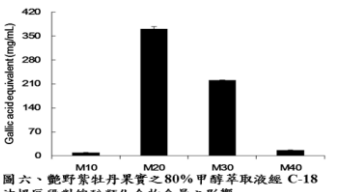
圖九、豔紫野牡丹果實 80% 甲醇萃取液 HPLC 層析圖譜  
Figure 9. HPLC chromatograms monitored at 254 nm of 80% methanol extracts from *Tibouchina semidecandra* Cogn. fruits.



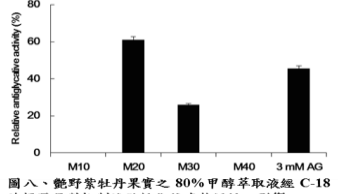
圖二、豔紫野牡丹果實之 80% 甲醇萃取液於不同濃度下對總酚類化合物含量之影響  
Figure 2. Total phenolic content of 80% methanol extract at different concentrations from *Tibouchina semidecandra* Cogn. fruits.



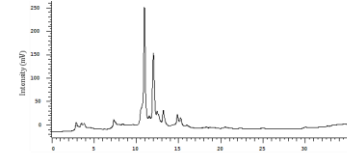
圖四、豔紫野牡丹果實之 80% 甲醇萃取液於不同濃度下對抑制進階糖化終產物活性之影響  
Figure 4. Relative inhibitory activities against AGEs formation of 80% methanol extract at different concentrations from *Tibouchina semidecandra* Cogn. fruits.



圖六、豔紫野牡丹果實之 80% 甲醇萃取液經 C-18 沖提區段對總酚類化合物含量之影響  
Figure 6. Total phenolic content of 80% methanol extract from *Tibouchina semidecandra* Cogn. fruits eluted fractions of SPE C-18 column.



圖八、豔紫野牡丹果實之 80% 甲醇萃取液經 C-18 沖提區段對抑制進階糖化終產物活性之影響  
Figure 8. Relative inhibitory activities against AGEs formation of 80% methanol extract from *Tibouchina semidecandra* Cogn. fruits eluted fractions of SPE C-18 column.



圖十、豔紫野牡丹果實 80% 甲醇萃取液經 C-18 管柱之 20% 甲醇沖提區段 HPLC 層析圖譜  
Figure 10. HPLC chromatograms monitored at 254 nm of 20% methanol eluted fraction from *Tibouchina semidecandra* Cogn. fruits 80% methanol extract.

## 肆、結論：

豔紫野牡丹果實之 80% 甲醇萃取液在抗氧化能力的表現上較水萃組佳，且與濃度呈正相關，進一步的以 SPE C-18 固相萃取管柱進行區分，結果顯示，以 20% 甲醇沖提區段具有較佳之抗氧化活性及總酚類化合物，且其進階糖化終產物抑制率相較於對照組 Aminoguanidine 有較好的抑制率。若可將豔紫野牡丹果實之生物活性物質進一步分離純化，具有開發作為降血糖相關保健品之潛力。

## 陸、參考文獻：

16at H. M., Rezali M. F., Ujang Z. 2010. Isolation and identification of radical scavenging and tyrosinase inhibition of polyphenols from *Tibouchina semidecandra* L. *J. Agric. Food Chem* 58, 10404–10409.