國立嘉義大學生命科學院 105 年度
學生學術研究成果優良海報評選獲獎名單

時間：105 年 6 月 1 日

大學部

食品科學系					
第二名	吳峻瑋、謝鈞任	第三名	施凱瀞、張嘉瑄		
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食品科學系學士班

探索飲料代工廠

吳峻瑋　謝鈞任

原物料
- 原料：茶葉、添加物…等
- 物料：紙箱、標籤、瓶胚、瓶蓋

製程
- 測PAA、H_2O_2、pH、Brix、看日印、噴印、液位、外觀、旋蓋角度、扭力還有頂壓及落地測試

品質管理
- 開罐：測扭力、重量、Brix、pH、品評風味、看外觀、檢測保溫品
- 驗水：測導電度、pH、濁度、殘餘氯、總硬度、M鹼
- 數菌、寄貨、測茶多酚和兒茶素、萃茶

微生物
- 配培養基和接菌

翻檢
- 檢查瓶身日印、瓶頸、汙蓋、瓶身、內容物、其他

成品

不接受不良品
不製造不良品
不良品不外流

品質管控：IQC(原物料)、PQC(製程)、FQC(成品)

優良食品工業有限公司暑期實習成果

We are good!

報告者：1022427施凱瀞 1023205張嘉瑄

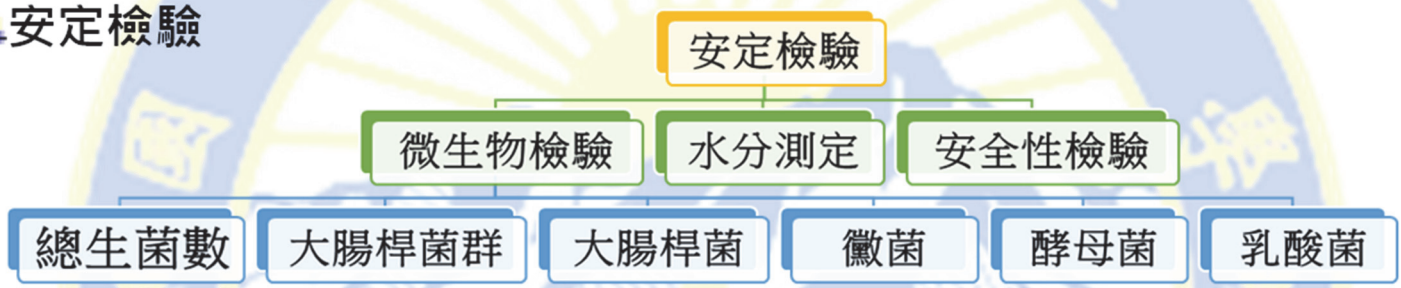
營業項目

- 錠片食品(單及雙層錠片)
- 膠囊、微膠囊食品
- 粉/液劑/顆粒保健食品
- ODM及OEM製造
- 各式軟糖、硬糖
- PTP、雙鋁箔包裝

實習內容

- 主要協助品管研發課
- 生產前協助品研課試做
- 依客戶需求研發新產品
- 調配粉包、液劑與錠片
- 原料歸檔
- 安定檢驗-微生物檢驗

安定檢驗



液劑填充製程

軟糖製程

實習心得

在我們的學群裡主要分為四個部分：微生物、加工、保健、分析，各個學群裡都有能和優良互相結合或者是改進的地方。例如優良在微生物檢驗方面和所學有滿多地方能應用，例如各個菌種在不同培養基上的樣子、菌種檢驗方法；加工方面如液劑的殺菌條件、成品包裝流程，也能應用所學；保健方面，我們認為學校課程可在保健原料萃取、功效探討部分多加強，以應用在生產保健食品；而分析方面，雖然優良屬於OEM公司，但是我們認為如果有餘力，可以多一項分析檢驗項目，這樣一來在原料檢驗方面可以更方便。

食品科學系碩士班

Evaluation of two-stage method for moromi fermentation of soy sauce and optimization of the initial moromi fermentation to improve the soy sauce quality

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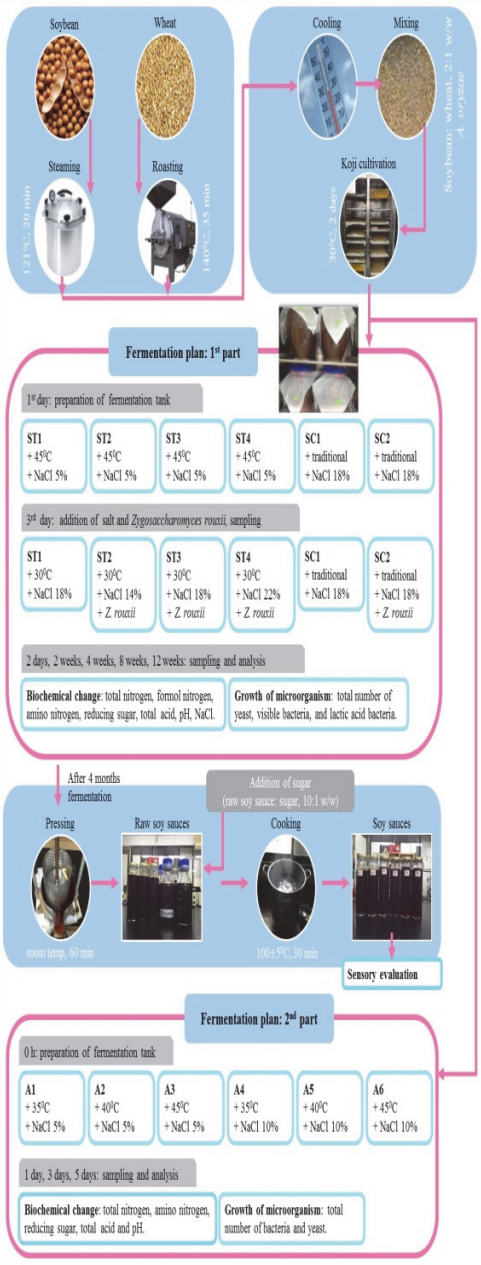
國立嘉義大學 食品科學系[1]、機械與能源工程學系[2]、生物機電工程學系[3]

Abstract

A two-stages fermentation method was used to process soy sauces and the quality properties of the sauces were evaluated. At the first stage, the koji was mixed with 5% brine solution at 1:2 ratio (w/w) and incubated at 45°C for 2 days. And then at the second stage, the fermentation temperature decreased to 30°C but the brine concentration increased to 14, 18, and 22% with or without the addition of *Zygosaccharomyces rouxii*. At the first stage, the application of high temperature and low salt was effective in promoting the protease activities, thus the total nitrogen, formol nitrogen, and amino nitrogen in the soy sauce increased about 60%, 120% and 130%, respectively, than those in the control (soy sauce made by setting outdoors without temperature control). However, fermentable sugars were also rapid consumed by the microbes and resulted in lower reducing sugar content and poor sensory properties compared to the control.

Thereafter, the objective of further study was to optimize the process parameters for preparation of first fermentation stage of soy sauce, which was considered as an enzymatic hydrolysis for the further fermentation stage. Temperature (35, 40 and 45°C), fermentation time (1, 3 and 5 days) and brine content (5 and 10% w/w) were studied as independent variables. The responses evaluated for deciding the optimum conditions were biochemical properties of total nitrogen, amino nitrogen, reducing sugar and total acid contents and pH, as well as microbial properties of yeast and total bacterial counts. Experimental data were fitted well into second-order polynomial models. The simultaneously optimal conditions were 40.7°C temperature, 4.6 days fermentation time and 10% w/w brine content. The optimized response values for total nitrogen, amino nitrogen, reducing sugar and total acid contents and pH were 1.43, 0.65, 2.79, and 1.28% and 5.48, respectively. These conditions could obtain total nitrogen (> 1.4%) and amino nitrogen (> 0.56%) contents satisfying the requirement of first-grade soy sauce within 5 days fermentation.

Materials and Methods



Results (1st part)

Table 1. Changes in indices of samples at various periods of fermentation*.

Indices	Samples	Fermentation time 2 days	14 days	28 days	56 days	84 days
TN (g/100 ml)	SC1	0.79 ± 0.03^{bD**}	1.49 ± 0.01^{aC}	1.55 ± 0.01^{aB}	1.59 ± 0.01^{aA}	1.56 ± 0.02^{aB}
	SC2	0.78 ± 0.03^{bD}	1.48 ± 0.03^{aBC}	1.58 ± 0.02^{aA}	1.52 ± 0.01^{bBC}	1.54 ± 0.02^{aAB}
	ST1	1.26 ± 0.02^{aC}	1.41 ± 0.03^{cB}	1.46 ± 0.01^{bA}	1.41 ± 0.02^{cB}	1.41 ± 0.02^{bB}
	ST2	1.25 ± 0.02^{aB}	1.42 ± 0.01^{cA}	1.44 ± 0.01^{cA}	1.43 ± 0.02^{cbA}	1.44 ± 0.02^{bA}
	ST3	1.25 ± 0.02^{aC}	1.41 ± 0.01^{cB}	1.41 ± 0.01^{dB}	1.44 ± 0.02^{cA}	1.44 ± 0.01^{bA}
	ST4	1.25 ± 0.03^{aC}	1.46 ± 0.01^{bB}	1.49 ± 0.01^{cA}	1.50 ± 0.01^{bA}	1.44 ± 0.01^{bB}
FN (g/100 ml)	SC1	0.37 ± 0.01^{bE}	0.76 ± 0.00^{cD}	0.92 ± 0.00^{bA}	0.90 ± 0.01^{aAB}	0.87 ± 0.00^{bC}
	SC2	0.37 ± 0.01^{bD}	0.77 ± 0.00^{cC}	0.86 ± 0.01^{bB}	0.91 ± 0.01^{aA}	0.87 ± 0.00^{bB}
	ST1	0.81 ± 0.01^{aE}	0.83 ± 0.01^{bB}	0.83 ± 0.01^{dB}	0.86 ± 0.02^{cA}	0.86 ± 0.00^{aA}
	ST2	0.81 ± 0.00^{aA}	0.84 ± 0.00^{aB}	0.87 ± 0.01^{bA}	0.86 ± 0.01^{cA}	0.86 ± 0.01^{aA}
	ST3	0.81 ± 0.01^{aC}	0.84 ± 0.01^{bB}	0.84 ± 0.00^{cB}	0.85 ± 0.02^{cAB}	0.86 ± 0.00^{aA}
	ST4	0.81 ± 0.00^{aB}	0.86 ± 0.01^{aA}	0.86 ± 0.00^{bA}	0.87 ± 0.03^{bcA}	0.87 ± 0.00^{aA}
AN (g/100 ml)	SC1	0.25 ± 0.01^{aE}	0.60 ± 0.01^{bD}	0.74 ± 0.01^{aA}	0.71 ± 0.01^{aB}	0.67 ± 0.01^{aC}
	SC2	0.25 ± 0.00^{aE}	0.61 ± 0.00^{aD}	0.68 ± 0.01^{bB}	0.71 ± 0.01^{aA}	0.67 ± 0.00^{aC}
	ST1	0.57 ± 0.03^{bB}	0.59 ± 0.00^{cAB}	0.59 ± 0.01^{dAB}	0.61 ± 0.01^{cbA}	0.59 ± 0.01^{bAB}
	ST2	0.57 ± 0.01^{bD}	0.61 ± 0.00^{aB}	0.64 ± 0.00^{bA}	0.62 ± 0.00^{cbB}	0.60 ± 0.00^{bC}
	ST3	0.57 ± 0.02^{bE}	0.60 ± 0.00^{bA}	0.60 ± 0.02^{dA}	0.60 ± 0.01^{dA}	0.59 ± 0.01^{bA}
	ST4	0.58 ± 0.00^{aD}	0.62 ± 0.00^{aBC}	0.63 ± 0.01^{cA}	0.62 ± 0.00^{cbAB}	0.61 ± 0.00^{aC}
RS (g/100 ml)	SC1	2.03 ± 0.03^{aE}	4.88 ± 0.02^{aD}	5.53 ± 0.11^{aC}	6.91 ± 0.02^{aB}	7.05 ± 0.09^{aA}
	SC2	2.04 ± 0.01^{aE}	4.83 ± 0.05^{aD}	5.51 ± 0.08^{aC}	6.40 ± 0.05^{bB}	6.80 ± 0.05^{bA}
	ST1	0.63 ± 0.01^{bE}	1.02 ± 0.00^{cD}	1.08 ± 0.03^{dC}	1.13 ± 0.01^{dB}	1.17 ± 0.01^{dA}
	ST2	0.63 ± 0.01^{bE}	1.34 ± 0.02^{cD}	1.48 ± 0.02^{cC}	1.61 ± 0.02^{cB}	1.66 ± 0.01^{cA}
	ST3	0.63 ± 0.01^{bD}	1.24 ± 0.01^{cC}	1.29 ± 0.02^{dB}	1.43 ± 0.03^{dA}	1.45 ± 0.01^{dA}
	ST4	0.63 ± 0.06^{bC}	0.98 ± 0.01^{dB}	0.99 ± 0.02^{eB}	1.03 ± 0.02^{eA}	1.02 ± 0.01^{eA}
TA (g/100 ml)	SC1	0.47 ± 0.00^{bD}	1.13 ± 0.02^{aBC}	1.12 ± 0.00^{aC}	1.15 ± 0.00^{aB}	1.33 ± 0.04^{aA}
	SC2	0.48 ± 0.02^{bD}	1.19 ± 0.00^{aB}	1.12 ± 0.04^{aC}	1.15 ± 0.00^{aBC}	1.31 ± 0.02^{aA}
	ST1	2.59 ± 0.04^{aC}	2.90 ± 0.02^{aA}	2.72 ± 0.02^{aB}	2.54 ± 0.02^{bC}	2.74 ± 0.04^{aB}
	ST2	2.58 ± 0.02^{aB}	2.76 ± 0.04^{bA}	2.58 ± 0.02^{bB}	2.40 ± 0.02^{cC}	2.56 ± 0.00^{bB}
	ST3	2.59 ± 0.04^{aB}	2.81 ± 0.04^{bA}	2.57 ± 0.02^{bBC}	2.52 ± 0.00^{bC}	2.53 ± 0.04^{bBC}
	ST4	2.58 ± 0.02^{aC}	2.93 ± 0.02^{aA}	2.76 ± 0.04^{aB}	2.75 ± 0.02^{aB}	2.72 ± 0.02^{aB}
pH	SC1	6.19 ± 0.00^{bA}	5.70 ± 0.01^{aB}	5.67 ± 0.01^{aC}	5.36 ± 0.01^{bD}	5.30 ± 0.01^{bE}
	SC2	6.20 ± 0.00^{aA}	5.66 ± 0.00^{bB}	5.65 ± 0.00^{bC}	5.40 ± 0.01^{aD}	5.32 ± 0.01^{aE}
	ST1	4.30 ± 0.01^{cC}	4.32 ± 0.00^{cB}	4.38 ± 0.01^{cA}	4.26 ± 0.01^{cD}	4.20 ± 0.01^{cE}
	ST2	4.30 ± 0.00^{cC}	4.32 ± 0.01^{cB}	4.39 ± 0.01^{cA}	4.30 ± 0.01^{cC}	4.25 ± 0.00^{cD}
	ST3	4.31 ± 0.01^{cC}	4.33 ± 0.00^{cB}	4.39 ± 0.00^{cA}	4.30 ± 0.00^{cD}	4.25 ± 0.00^{cE}
	ST4	4.30 ± 0.00^{cB}	4.30 ± 0.01^{cB}	4.36 ± 0.01^{dA}	4.28 ± 0.01^{dC}	4.21 ± 0.01^{dD}
NaCl (% w/v)	SC1	17.89 ± 0.11^{bA}	16.59 ± 0.11^{bB}	15.76 ± 0.06^{bC}	15.76 ± 0.06^{bC}	15.65 ± 0.06^{bC}
	SC2	17.82 ± 0.06^{aA}	16.55 ± 0.06^{aB}	15.65 ± 0.06^{bC}	15.58 ± 0.06^{bC}	15.54 ± 0.06^{bC}
	ST1	16.84 ± 0.06^{cA}	16.16 ± 0.11^{cB}	15.72 ± 0.11^{bC}	15.69 ± 0.06^{abC}	15.65 ± 0.06^{bC}
	ST2	13.05 ± 0.06^{dA}	12.25 ± 0.00^{dB}	11.96 ± 0.06^{cC}	11.96 ± 0.06^{dC}	11.93 ± 0.00^{cC}
	ST3	16.81 ± 0.11^{cA}	16.19 ± 0.13^{cB}	15.69 ± 0.06^{bC}	15.65 ± 0.06^{bcC}	15.61 ± 0.00^{bC}
	ST4	21.25 ± 0.11^{aA}	20.13 ± 0.06^{eB}	19.59 ± 0.06^{eC}	19.45 ± 0.06^{eCD}	19.41 ± 0.11^{eD}

* Data are expressed as the mean ± SD (n=3). ** Different small letters indicate significant difference in a column, different capital letters indicate significant difference in a row ($p<0.05$).

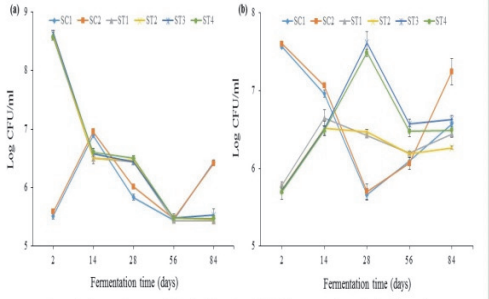


Figure 1. Changes of microorganisms count, (a) lactic acid bacteria and (b) viable yeast, at various periods of fermentation.

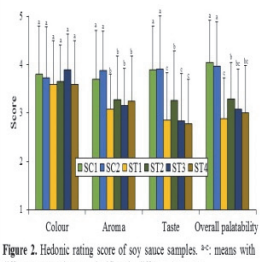


Figure 2. Hedonic rating score of soy sauce samples. a-c: means with different superscripts are significantly different ($p<0.05$).

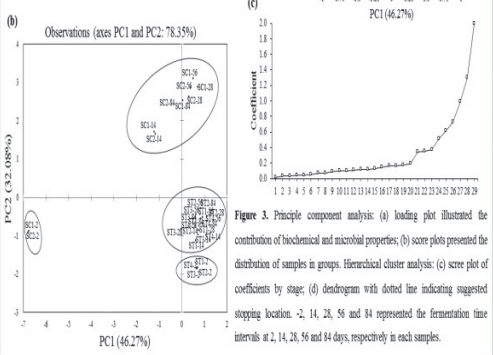


Figure 3. Principle component analysis: (a) loading plot illustrated the contribution of biochemical and microbial properties; (b) score plots presented the distribution of samples in groups. Hierarchical cluster analysis: (c) scree plot of coefficients by stage; (d) dendrogram with dotted line indicating suggested stopping location. -2, 14, 28, 56 and 84 represented the fermentation time intervals at 2, 14, 28, 56 and 84 days, respectively in each samples.

Results (2nd part)

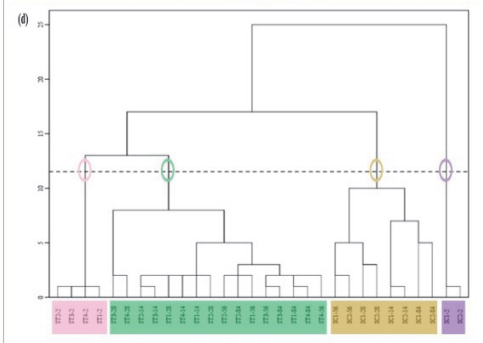


Figure 3. Hierarchical cluster analysis: (c) scree plot of coefficients by stage; (d) dendrogram with dotted line indicating suggested stopping location. -2, 14, 28, 56 and 84 represented the fermentation time intervals at 2, 14, 28, 56 and 84 days, respectively in each samples. (continued)

Table 2. The treatment combinations and their responses.

Run	Independent variable: Temperature (°C)	Time (day)	Brine content (%, w/w)	Response: TN (g/100 ml)	AN (g/100 ml)	RS (g/100 ml)	TA (g/100 ml)	pH	Yeast (log CFU/ml)	TBa (log CFU/ml)
1	35	1	5	0.80	0.28	0.18	0.65	6.17	9.06	9.17
2	35	1	10	0.78	0.28	1.49	0.54	6.23	7.25	7.72
3	35	3	5	1.24	0.52	0.14	1.03	5.62	8.85	9.57
4	35	3	10	1.23	0.50	2.30	0.78	5.96	7.50	7.75
5	35	5	5	1.31	0.57	0.17	1.57	5.13	8.75	9.90
6	35	5	10	1.30	0.56	2.23	1.56	4.99	6.70	7.09
7	40	1	5	0.99	0.40	0.32	0.78	6.03	9.07	9.15
8	40	1	10	0.97	0.39	1.86	0.62	6.18	6.26	7.21
9	40	3	5	1.31	0.56	0.51	1.64	5.23	9.02	9.11
10	40	3	10	1.34	0.58	2.51	1.02	5.86	7.81	7.99
11	40	5	5	1.35	0.64	0.78	1.88	4.89	8.87	8.97
12	40	5	10	1.43	0.64	2.71	1.26	5.24	7.88	8.16
13	45	1	5	1.02	0.44	0.96	1.20	5.31	8.92	9.00
14	45	1	10	0.99	0.42	2.09	0.80	6.01	6.03	7.16
15	45	3	5	1.21	0.55	1.59	1.62	4.98	8.53	8.79
16	45	3	10	1.29	0.58	3.01	1.02	5.81	7.64	7.81
17	45	5	5	1.31	0.61	1.95	1.73	4.80	8.15	8.69
18	45	5	10	1.37	0.67	3.52	1.15	5.73	7.10	7.31

Table 3. Optimization models including the objective function and constrain functions for searching the optimum fermentation condition of soy sauce.

Objective function		
Y_{TN}	$1.321 + 0.045X_1 + 0.210X_2 + 0.008X_3 - 0.043X_1X_2 + 0.012X_1X_3 + 0.017X_2X_3 - 0.077X_1^2 - 0.135X_2^2$	To maximize
Constrain functions		Constrain ranges
Y_{AN}	$0.574 + 0.047X_1 + 0.124X_2 + 0.003X_3 - 0.019X_1X_2 + 0.007X_1X_3 + 0.007X_2X_3 - 0.038X_1^2 - 0.058X_2^2$	≥ 0.60
Y_{RS}	$1.554 - 0.551X_1 + 0.572X_2 + 0.840X_3 + 0.211X_1X_2 - 0.118X_1X_3 + 0.131X_2X_3 + 0.186X_1^2 - 0.156X_2^2$	≥ 2.50
Y_{TA}	$1.229 + 0.116X_1 + 0.380X_2 - 0.186X_3 - 0.134X_1X_2 - 0.100X_1X_3 - 0.046X_2X_3 - 0.064X_1^2 - 0.04X_2^2$	≤ 1.40
Y_{pH}	$5.582 - 0.121X_1 - 0.429X_2 + 0.213X_3 + 0.186X_1X_2 + 0.185X_1X_3 + 0.021X_2X_3 - 0.01X_1^2 - 0.015X_2^2$	≥ 5.00
Constrain of variables		
	X_1, X_2, X_3	≥ -1 and ≤ 1

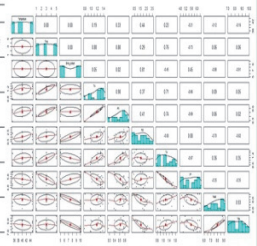


Figure 4. Correlations between independent variables and responses.

Table 4. The optimal fermentation conditions.

Temperature (°C)	Time (day)	Brine content (%, w/w)	TN (g/100 ml)	AN (g/100 ml)	RS (g/100 ml)	TA (g/100 ml)	pH
40.7	4.6	10	1.43	0.65	2.79	1.28	5.48

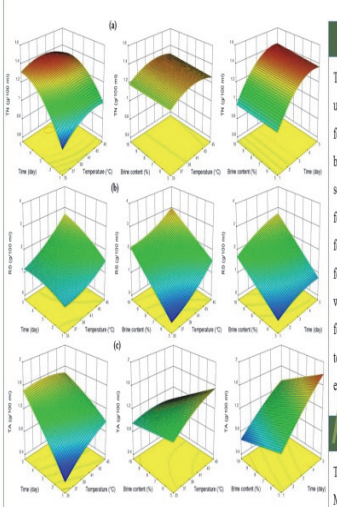


Figure 5. Response surface plots of combined effects of independent variables on (a) total nitrogen content (TN), (b) reducing sugar content (RS) and (c) total acid content (TA).

Conclusions

The present study provided the better understanding of effects of temperature, fermentation time and brine content on biochemical and microbial characteristics of soy sauce during not only two-stage long fermentation but also shortly initial fermentation, which would be very useful for the preparation of enzymatic hydrolysis within short period of soy sauce fermentation and for further study to be able to shorten the fermentation time and enhance the qualities of soy sauce.

Acknowledgements

This research was partially supported by the Ministry of Science and Technology in Taiwan, R.O.C. (MOST 103-2221-E-415 - 016 -).

石蓮花水萃取物對MG-BSA adducts 誘導肝臟星狀細胞活化之影響

Water extract of *Graptopetalum paraguayense* E. Walther inhibits the activation of hepatic stellate cells induced by MG-BSA adducts

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國立嘉義大學食品科學系

摘 要

肝臟星狀細胞 (hepatic stellate cells, HSCs) 在肝臟纖維化發展過程中為關鍵調控角色，當肝臟受損時，HSCs 會活化造成肝纖維化發生。甲基乙二醛 (methylglyoxal, MG) 為一高反應活性之羰基化合物，為形成糖化終產物 (advanced glycation end-products, AGEs) 主要先驅物。研究指出，AGEs 會誘導 HSCs 增生，而肝臟是主要清除 AGEs 之場所。本研究室先前已證實石蓮花 (*Graptopetalum paraguayense* E. Walther) 水萃取物具抗氧化、抗糖化、保護四氯化碳及甲基乙二醛誘導之肝損傷等功效，並鑑定其活性成分。故本研究以 MG-BSA adducts 誘導 HSCs 模式評估石蓮花水萃取物對其保護效應。以 MTT 法分析 MG-BSA adducts 及樣品對細胞之安全劑量範圍。結果顯示，MG-BSA adducts 於 100 μg/mL 濃度下細胞具 85% 以上存活率。另外，隨著石蓮花水萃取物濃度上升 HSCs 存活率下降，於 1000 μg/mL 濃度下細胞存活率達 80% 以上，得知石蓮花水萃取物可抑制 HSCs 增生，並利用 dihydroethidium (DHE) 測定 AGEs 與石蓮花水萃取物對 HSCs 產生胞內活性氧 (reactive oxygen species, ROS) 之影響。結果得知，石蓮花水萃取物有效降低胞內氧化壓力生成。另外，以西方墨點法分析 HSCs 活化指標因子 transforming growth factor beta (TGF-β) 之表現量。結果顯示，石蓮花水萃取物具降低細胞中 TGF-β 表現量之能力。綜合上述，石蓮花水萃取物具改善 MG-BSA adducts 誘導 HSCs 活化之潛力，預防肝臟纖維化發生，期能將結果應用於開發石蓮花相關保健產品。

關鍵字：肝纖維化、星狀細胞、石蓮花、糖化終產物

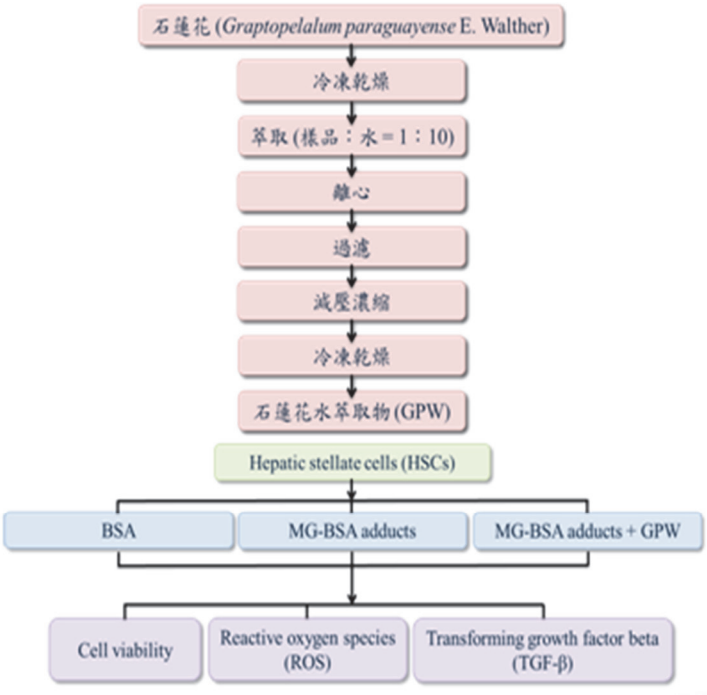
前 言

肥胖為近年來公眾健康議題，肥胖衍生之疾病以非酒精性脂肪肝病發展成肝癌之相關性為最高。糖化終產物 (advanced glycation end-products, AGEs) 在肝臟中會引起氧化壓力，肝臟為主要清除 AGEs 之場所，也是 AGEs 攻擊的器官。甲基乙二醛 (methylglyoxal, MG) 為一高反應活性之羰基化合物，為形成 AGEs 主要先驅物，並導致氧化壓力，MG adducts 結合蛋與血清低密度脂蛋白 (LDL cholesterol) 及三酸甘油酯 (triglycerides) 具相關性。

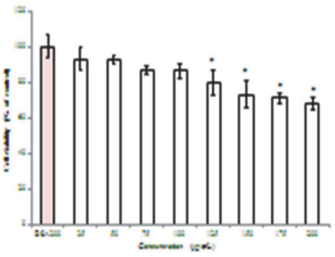
石蓮花 (*Graptopetalum paraguayense* E. Walther) 屬於景天科 (Crassulaceae) 植物，研究指出石蓮花在生物體上可降低血壓、提升抗氧化活性及預防癌症，且其乾燥葉將性與萃取自 CCl_4 誘導化學性肝損傷之功效。

本研究以 HSCs 為探討對象，經 MG-BSA adducts 誘發後評估石蓮花水萃物對其保護效應。

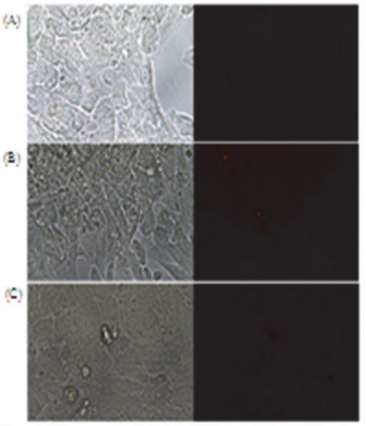
實驗架構



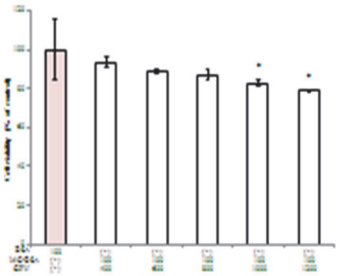
結 果



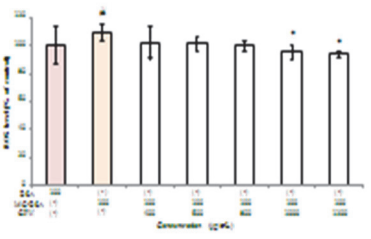
圖一、不同濃度 MG-BSA adducts 對 HSCs 細胞存活率之影響
Fig 1. Effect of MG-BSA adducts on the cell viability in HSCs.



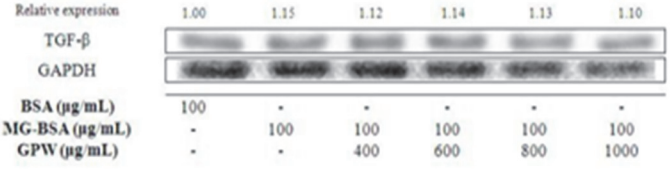
圖二、HSCs 細胞活性氧分子以 DHE 染色螢光 (A) BSA (B) MG-BSA adducts (C) MG-BSA 與石蓮花水萃物共處理
Fig 2. Representative images of HSCs stained with the ROS indicator dye DHE (A) BSA (B) MG-BSA adducts (C) MG-BSA adducts with GPW.



圖三、MG-BSA adducts 與 GPW 共處理對 HSCs 細胞存活率之影響
Fig 3. Effect of MG-BSA adducts and *Graptopetalum paraguayense* E. Walther on water extracts concentrations on the cell viability in HSCs.



圖四、石蓮花水萃物對於 MG-BSA adducts 誘發 HSCs 細胞活性氧分子之清除影響
Fig 4. Scavenging effects of *Graptopetalum paraguayense* E. Walther on reactive oxygen species (ROS) in MG-BSA adducts induced HSCs.



圖五、石蓮花水萃取物對 MG-BSA adducts 誘發 HSCs 轉型生長因子 (TGF-β) 表現之影響
Fig 5. Effect of GPW on transforming growth factor beta (TGF-β) expressions in MG-BSA adducts induced HSCs.

結 論

1. 隨著石蓮花水萃取物之濃度上升，肝臟星狀細胞 HSCs 細胞存活率隨之下降，顯示石蓮花水萃取物可抑制 HSCs 增生。
2. HSCs 經 MG-BSA adducts 誘導後增加其胞內活性氧之生成，而與石蓮花水萃取物共處理則減緩細胞之氧化壓力。
3. MG-BSA adducts 誘導會增加 HSCs 細胞中 TGF-β 之表現量，而石蓮花水萃取物具降低細胞中 TGF-β 表現量之能力。

Fractionation of bioactive components from *Ganoderma amboinense* using supercritical carbon dioxide technology

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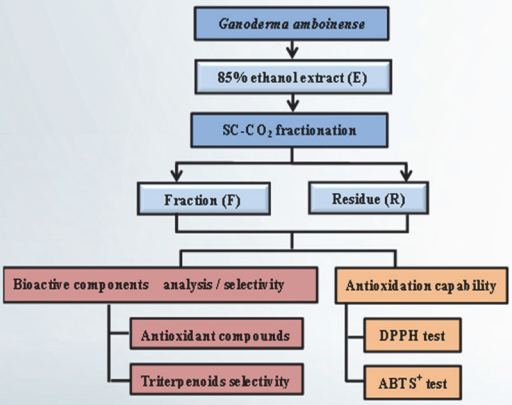
ABSTRACT

The objectives of this study were to investigate the optimal operation process for yielding bioactive components-rich fractions from *G. amboinense* extract using supercritical carbon dioxide (SC-CO_2) fractionation technique as well as antioxidant activity of obtained fractions. SC-CO_2 fractionation was performed using an orthogonal design to obtain optimal fractionation parameters. The operation conditions were: three different temperatures (40, 50, and 60℃), three different pressure levels (20, 25, and 30 MPa), three flow rates of *Ganoderma* extract liquor (1, 2, and 3 mL/min), and SC-CO_2 flow rate at 5 ml/min. The results showed that much higher level of the bioactive compounds, including triterpenoids, flavonoids, polyphenols, and adenosine, was detected in either the extract (F) or residue (R) fraction compared to those in alcohol extract (E) of *G. amboinens*. The highest concentrations of antioxidant compounds, including triterpenoids, polyphenols and flavonoids were obtained in the fraction (F) at the operation condition of 20MPa, 50℃ and 3 ml/min sample flow rate; while the highest concentrations of polysaccharides were yielded in the fraction (R) at the operation condition of 20MPa, 50℃ and 3 ml/min sample flow rate. DPPH radicals scavenging activity was increased in SC-CO_2 fractions. There is high correlation between antioxidant activity and the contents of antioxidant compounds ($P < 0.01$). The correlation coefficient between the contents of antioxidant compounds and DPPH and ABTS radicals scavenging activity were 0.825 and 0.733, respectively.

EXPERIMENTAL DESIGN



SELECTIVITY DEFINITION

$$\text{Selectivity} = \frac{F_{\text{Triterpenoids (mg/g)}}}{R_{\text{Triterpenoids (mg/g)}}}$$

PREDICTED SELECTIVITY BY REGRESSION EQUATION

Selectivity = C_1*pressure + C_2*temperature + C_3* Injection flow rate
Where C_1, C_2 and C_3 are linear regression coefficients.

RESULTS

Table 1. Experimental determined and predicted values of selectivity.

Exp. No.	Pressure (Mpa)	Temperature (°C)	Injection flow rate (ml/min)	Selectivity Exp.	Selectivity Pre.
1	30	60	1	1.61	2.42
2	20	50	3	3.24	2.45
3	30	40	3	2.97	3.08
4	20	60	2	1.51	2.12
5	25	60	3	2.49	2.80
6	30	50	2	2.91	2.75
7	25	50	1	3.15	2.08
8	25	40	2	1.74	2.41
9	20	40	1	2.53	1.73

Predicted electivity regression equation: 0.065*Pressure + 0.002*Temperature + 0.351*Injection flow rate

Table 2. Component contents of ethanol extract, fraction, and residue from *G. amboinense*

Sample	Bioactive components (mg/g)			
	Triterpenoids	Polysaccharides	Flavonoids	Polyphenols
Extract (E)	105.79 ± 8.97	190.32 ± 7.89	41.25 ± 2.17	20.15 ± 1.45
Fraction (F)				
1	160.94 ± 16.91*	142.72 ± 14.65*	23.24 ± 5.52*	10.61 ± 1.30*
2	334.34 ± 11.13*	154.13 ± 12.62*	59.92 ± 1.31*	41.06 ± 2.36*
3	284.68 ± 11.93*	105.44 ± 4.41*	38.88 ± 3.11	28.19 ± 1.40*
4	170.38 ± 7.89*	87.79± 5.32*	20.52 ± 1.26*	4.37 ± 0.33*
5	329.6 ± 9.10*	125.97 ± 0.41*	46.91 ± 3.92	22.32 ± 3.20
6	174.56 ± 8.43*	120.96 ± 13.61*	29.76 ± 0.99*	20.78 ± 2.53
7	304.75 ± 4.20*	112.55 ± 6.58*	44.76 ± 2.18	25.33 ± 1.66*
8	174.73 ± 9.88*	110.73 ± 6.17*	33.88 ± 4.87*	13.21 ± 0.16*
9	190.42 ± 16.13*	92.50 ± 2.44*	55.77 ± 6.63*	22.10 ± 2.48
Residue (R)				
1	99.74 ± 3.89*	195.94 ± 13.9	20.64 ± 1.60*	10.12 ± 0.76*
2	103.27 ± 13.14	201.82 ± 9.93	31.20 ± 5.14*	31.98 ± 2.35*
3	95.87 ± 4.23*	182.36 ± 8.13	26.07 ± 2.89*	31.88 ± 1.39*
4	113.05 ± 6.59	166.24 ± 7.02*	29.37 ± 3.10*	10.23 ± 0.16*
5	132.14 ± 15.65*	201.76 ± 9.94	27.38 ± 2.17*	4.88 ± 0.18*
6	59.98 ± 2.49*	198.05 ± 9.65	25.80 ± 5.56*	6.29 ± 0.68*
7	96.53 ± 0.92*	198.34 ± 9.45	14.41 ± 1.01*	8.25 ± 2.49*
8	100.23 ± 6.32	200.44 ± 5.08*	22.87 ± 0.97*	1.87 ± 0.29*
9	75.2 ± 1.85*	185.41 ± 9.27	18.67 ± 1.54*	8.09 ± 2.64*

Marked in red indicates the fraction exerted the highest antioxidant compounds contents.
Data are mean ± S.D. of triplicate determinations.
*Significant difference between fraction and extract by t-test ($p<0.05$).

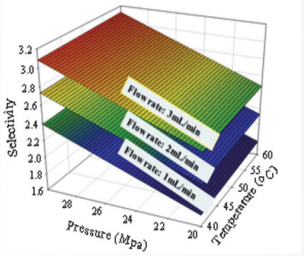


Figure 1. Effect of termperature and pressure on the selectivity at various sample flow rate.

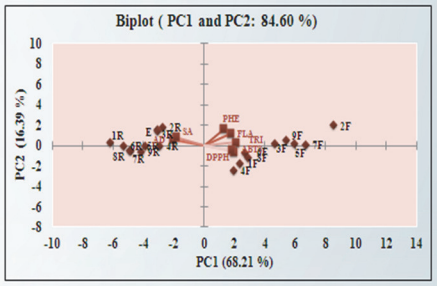


Figure 2. Principal components analysis based on different bioactive components in (F), (R) and (E) fraction of *G. amboinense* and their antioxidant activity (DPPH and ABTS). PHE: Polyphenols; FLA: Flavonoids; TRI: Triterpenoids; SA: Polysaccharides; AD: Adenosine.

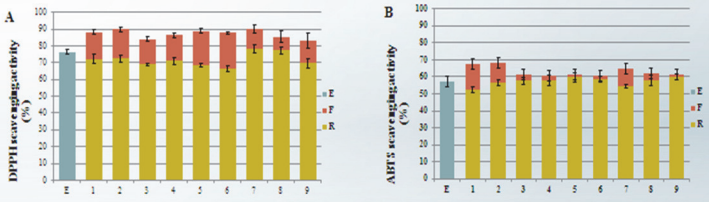


Figure 3. DPPH scavenging activity (A) and ABTS scavenging activity (B) of the extract and SC-CO_2 fractions. Data are mean ± S.D. of triplicate determinations.

Table3. Pearson's product moment correlation analyses between antioxidant ability and contents of antioxidant compounds

Antioxidant assay	Correlation coefficient (*r*) Antioxidant compounds
DPPH	0.825
ABTS	0.733

CONCLUSIONS

This study showed that fractionation of *G. amboinense* extract using continuous SC-CO_2 partition technology was able to effectively concentrate bioactive constituents. In addition, SC-CO_2 technology could increase the antioxidant activities of the fractions.

The optimal operation condition was performed at 20Mpa, 50°C and 3 ml/min injection flow rate, exerting the highest content of bioactive components and the highest selectivity of triterpenoids.

The contribution of operated parameters on selectivity was injection flow rate > pressure > temperature.