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:107 年 5 月 30 日

碩博士組

名次	獲獎人姓名	指導教師
食品科學系		
第一名	劉康佑	許成光
第二名	Nguyen Xuan Hoang(阮宣宏)	許成光
第三名	倪彥綉	吳思敬
生物資源學系		
第一名	郭晉緯	方引平
第二名	黃羽萱	方引平
第三名	李昱緯	蔡若詩
生化科技學系		
第一名	李汶鈴	陳瑞傑
第二名	黃雅晨	魏佳俐
第三名	王榆婷	魏佳俐
微生物免疫與生物藥學系		
第一名	楊芳俞	吳進益
第二名	陳德宇	謝佳雯
第三名	戴元昌	劉怡文

食品科學系

黑豆機能性茶包開發

劉康佑*、高嘉凰、鄭子菁、尚娟吟、鍾澤斌
、吳奕蓉、楊開丞、黃少傑、王錦昇、許成光

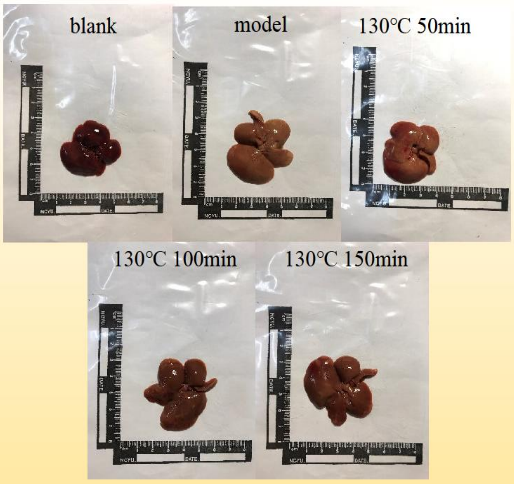
國立嘉義大學　食品科學系

Abstract

黑豆中含有豐富的花青素、黃酮類以及多醣類化合物，具有良好的抗氧化與降血脂等等有益於人體的功效，近年來黑豆的營養價值逐漸被發現，使其成為重點農作物之一，而黑豆水及黑豆茶飲等等飲品也逐年增加，消費者對於具有保健功效的飲品也越來越重視。先前已有實驗對黑豆以 80、120、150℃ 分別焙炒 50、100、150 分鐘探討其中抗氧化物質含量變化，其結果以 120℃ 及 150℃ 有最佳之抗氧化物質含量，本實驗於中間加入 130℃ 以尋找含有良好抗氧化物質與風味之條件，結果顯示以 130℃-50 分鐘之條件有最佳之結果，其後再以 130℃ 之焙炒條件進行降血脂功效之試驗，經焙炒之黑豆茶包茶湯可減少倉鼠血液中膽固醇及三酸甘油脂之含量，且其抗氧化物質可提高肝臟之抗氧化能力及減緩肝臟損傷。黑豆具有良好的抗氧化物質含量與降血脂之效果，經焙炒後沖泡之茶湯同樣含有相同之功效，因此可以此結果為基礎，開發具有良好風味及保健功效之黑豆茶包。

Results

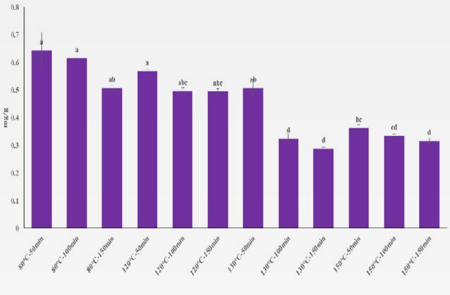
圖一、黑豆茶包成品



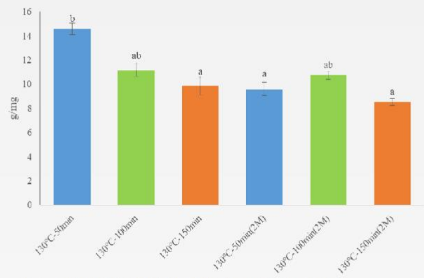
圖二、倉鼠犧牲後隻肝臟外觀

圖三、各組別總酚含量測定結果

圖四、各組別類黃酮含量測定結果



圖五、各組別花青素含量測定結果



圖六、儲藏試驗前後花青素含量測定結果

表一、黑豆茶包對高脂飲食倉鼠血脂之影響

	blank	model	130℃ 50min	130℃ 100min	130℃ 150min
Total Cholesterol (mg/dl)	135.0±19.1 ab	146.6±23.6 b	146.0±20.7 b	130.3±13.5 ab	120.7±8.4 a
Triglyceride (mg/dl)	125.3±47.1 a	225.1±87.9 bc	246.2±105.2 c	236.7±95.2 c	155.7±56.8 ab
LDL (mg/dl)	14.0±3.3 a	21.6±6.2 bc	24.1±4.5 c	18.0±6.7 ab	15.3±2.8 a
HDL (mg/dl)	123.9±22.0 c	118.6±13.5 bc	116.2±16.8 ab	119.3±10.0 ab	103.0±9.9 a

表二、黑豆茶包對高脂飲食倉鼠之肝臟抗氧化能力與脂質過氧化物之影響

	blank	model	130℃ 50min	130℃ 100min	130℃ 150min
TEAC (μg/mg protein)	397.89±38.00 ab	367.29±85.59 a	427.89±59.15 bc	480.72±55.82 c	423.25±62.94 ab
TBARS (Unit/L)	0.347±0.067 a	0.356±0.101 a	0.369±0.037 ab	0.453±0.061 b	0.433±0.131 ab

Conclusions

1. 黑豆茶包含有豐富的抗氧化物質，且其包裝可在儲藏期間內降低抗氧化物質降解之程度，即使放置兩個月後依舊含有與最初有相近的抗氧化物質含量。

2. 黑豆茶包可降低高脂飲食倉鼠血清中膽固醇與三酸甘油酯的含量，特別是 130℃ 150 min 的組別有較佳的功效，並可提高肝臟之抗氧化能力及減緩肝臟損傷，且不會造成體重及食慾不正常下降，由結果可得知黑豆茶包之茶湯對於高脂飲食具有改善及調節血脂之功效。

Acknowledgement

感謝經濟部經費協助實驗完成
計畫編號: E10500006010;E10500006014-022-1

Optimization of protease and amylase activities derived from Chinese and Japanese-type soy sauce koji

Nguyen Xuan Hoang[a], Sophia Ferng[a], Ching-Hua Ting[b], Robin Yih-Yuan Chiou[a], Ying-Chen Lu[a], Jyh-Cheng Chen[a], Yu-Fong Yeh[a], Yi-Ru Lai[a], Huai-wen Yang[a], Jean-Yu Hwang[c], Cheng-Kuang Hsu[a]*
[a]Department of Food Science, National Chiayi University, Chiayi City 60004, Taiwan (ROC)
[b]Department of Mechanical and Energy Engineering, National Chiayi University, Chiayi City 60004, Taiwan (ROC)
[c]Department of Food Science and Technology, Chung-Hwa University of Medical Technology, 89, Wenhwa 1st St., Rende Shiang, Tainan 717, Taiwan (ROC)

Abstract

This research was undertaken to identify the fermentation conditions optimal for the koji productions and to investigate the effects of pH, temperature and salt content on the enzymatic hydrolysis of koji protease and amylase. The experiments were carried out by inoculating the different ratios of soybean to wheat with the spores of *Aspergillus oryzae* using response surface methodology (RSM). The ratios of soybean to wheat were 2:1 and 1:1, corresponding to Chinese-type (BA1) and Japanese-type (BA2), respectively. The optimum fermentation time for both protease and amylase were found at 60 h for both BA1 and BA2. Both protease and amylase activities observed in BA1 were higher than those in BA2. The protease produced by *A. oryzae* was most active and stable in the pH range 7.0-7.7, indicative of a neutral protease. The optimum temperature for protease activity was 52-55 °C, and the enzyme was stable at around 40 °C. On the contrary, the acidic region, pH 5, was preferred to amylase activity and stability. Additionally, the amylase, which was most active at 60 °C and stable at around 48 °C, was more tolerant to heat and salt as compared to the protease.

Experimental Design

Physicochemical and microbial properties of koji during the koji making and determination of the optimal time for protease and amylase activities

- Microbial change: total number of bacteria and mold
- Enzyme activity: Protease and amylase
- pH and moisture content

The optimal time for protease and amylase activities: 2.5 days

Effects of pH, temperature and salt content on the activities and stabilities of koji protease and amylase using RSM

Face-centered central composite design

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

The independent variables:
+ pH: 5, 7 and 9
+ Temp: 40, 50 and 60 ℃
+ Salt content: 0, 8 and 16%

Response factors:
+ Protease activity and stability (U/g koji solid)
+ Amylase activity and stability (U/g koji solid)

Results

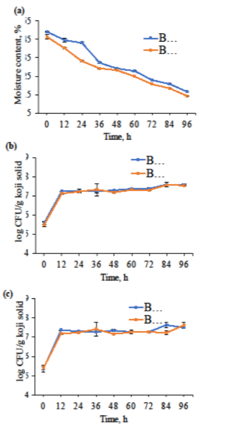


Fig. 1. Changes of moisture content (a), total viable count (b) and total mold (c) of different koji at various periods of koji fermentation.

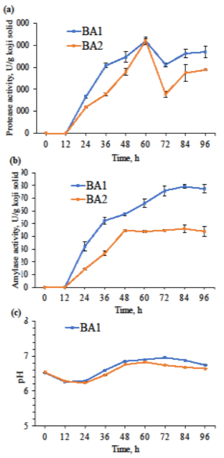


Fig. 2. Changes of protease activity (a), amylase activity (b) and pH (c) of different koji at various periods of fermentation.

Table 1
The treatment combinations and their responses.

Run	pH/X_1	Temperature/X_2, ⁰C	Salt content/X_3, %	BA1 Protease activity (U/g koji solid) P1-10	BA1 Protease activity (U/g koji solid) P1-60	BA1 Amylase activity (U/g koji solid) A1-10	BA1 Amylase activity (U/g koji solid) A1-60	BA2 Protease activity (U/g koji solid) P2-10	BA2 Protease activity (U/g koji solid) P2-60	BA2 Amylase activity (U/g koji solid) A2-10	BA2 Amylase activity (U/g koji solid) A2-60
1	-1 (5.0)	-1 (40)	-1 (0)	1516.33	978.03	78804.74	61805.08	1316.78	877.31	62006.83	50092.67
2	1 (9.0)	-1 (40)	-1 (0)	3178.96	1278.33	3418.26	5034.72	3044.71	1101.09	6434.93	7102.61
3	-1 (5.0)	1 (60)	-1 (0)	2236.37	857.95	171142.25	43002.01	2768.62	639.10	118985.47	40082.45
4	1 (9.0)	1 (60)	-1 (0)	3847.69	847.18	15710.80	3496.79	2430.81	509.60	8245.14	2521.23
5	-1 (5.0)	-1 (40)	1 (16)	65.06	202.18	70367.27	63123.44	119.09	192.85	56329.86	49846.41
6	1 (9.0)	-1 (40)	1 (16)	195.25	281.73	1075.19	472.08	111.53	233.19	2574.71	885.33
7	-1 (5.0)	1 (60)	1 (16)	705.21	442.42	158532.12	46510.97	744.47	404.42	101879.28	30598.86
8	1 (9.0)	1 (60)	1 (16)	157.23	87.58	1916.99	508.13	106.10	28.67	1023.87	407.96
9	-1 (5.0)	0 (50)	0 (8)	1428.40	678.12	150396.24	88206.19	1365.16	660.77	119953.03	75548.68
10	1 (9.0)	0 (50)	0 (8)	1396.82	530.03	3688.24	497.09	913.07	384.61	2858.93	446.82
11	0 (7.0)	-1 (40)	0 (8)	1230.23	969.55	46586.15	42039.13	903.24	869.50	34522.88	32212.10
12	0 (7.0)	1 (60)	0 (8)	2753.80	837.96	78253.67	39065.96	2360.54	726.65	56731.49	27243.65
13	0 (7.0)	0 (50)	-1 (0)	5028.09	1350.68	50923.09	87876.35	4804.09	1273.59	44902.76	77760.88
14	0 (7.0)	0 (50)	1 (16)	643.70	688.01	28156.89	30120.01	784.50	549.78	23059.56	23047.67
15	0 (7.0)	0 (50)	0 (8)	2452.47	1089.10	27034.21	58913.03	2290.34	1032.86	19800.45	46374.70
16	0 (7.0)	0 (50)	0 (8)	2389.12	1119.53	27223.14	60485.11	2175.71	1040.15	20148.19	47162.94
17	0 (7.0)	0 (50)	0 (8)	2412.34	1138.38	27123.19	59123.23	2379.25	1047.76	20102.92	46576.03

P1-10, P1-60, A1-10 and A1-60 were the protease activities with 10 and 60 min reactions and the amylase activities with 10 and 60 min reactions in BA1, respectively; P2-10, P2-60, A2-10 and A2-60 were the protease activities with 10 and 60 min reactions and the amylase activities with 10 and 60 min reactions in BA2, respectively.

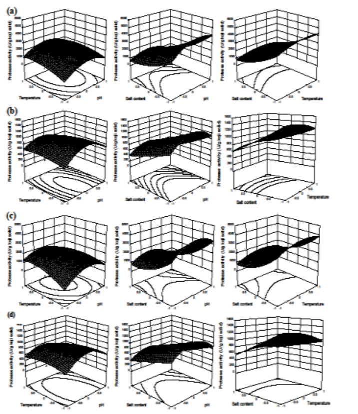


Fig. 3. The response surface plots of protease activity influenced by the reaction parameters of pH, temperature and salt content using coded values. (a), (b): protease activities of BA1 with 10 (P1-10) and 60 min reactions (P1-60), respectively; (c), (d): protease activities of BA2 with 10 (P2-10) and 60 min reactions (P2-60), respectively.

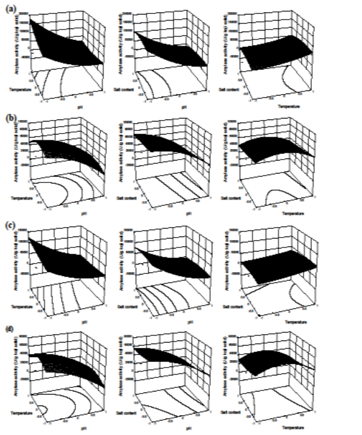


Fig. 4. The response surface plots of amylase activity influenced by the reaction parameters of pH, temperature and salt content using coded values. (a), (b): amylase activities of BA1 with 10 (A1-10) and 60 min reactions (A1-60), respectively; (c), (d): amylase activities of BA2 with 10 (A2-10) and 60 min reactions (A2-60), respectively.

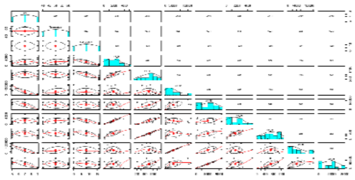


Fig. 5. Correlation between independent variables and responses.

Table 2
Optimal conditions and model validation.

Response	Optimum conditions (actual values) pH	Temperature, ℃	Salt content, %	Predicted response (U/g koji solid)	Experimental response (U/g koji solid)*
P1-10	7.72	55.31	0.00	4506.39	4612.10 ± 102.43
P1-60	7.38	40.64	0.00	1505.59	1453.03 ± 73.50
A1-10	5.00	60.00	5.17	182644.70	176351.15 ± 1418.02
A1-60	5.00	47.55	0.00	80424.15	82288.08 ± 1072.80
P2-10	7.19	52.76	0.00	4157.07	4096.04 ± 100.72
P2-60	7.26	40.00	0.00	1371.26	1295.12 ± 119.11
A2-10	5.00	60.00	3.69	126780.00	124128.64 ± 829.31
A2-60	5.00	48.22	0.00	72047.63	74118.05 ± 999.57

* Data are expressed as the mean ± SD (n = 3).

Conclusions

The protease produced by *A. oryzae* was most active and stable in the pH range 7.0-7.7, indicative of a neutral protease. The optimum temperature for protease activity was 52-55 °C, and the enzyme was stable at around 40 °C. On the contrary, the acidic region, pH 5, was preferred to amylase activity and stability. Additionally, the amylase, which was most active at 60 °C and stable at around 48 °C, was more tolerant to heat and salt as compared to the protease. The appearance of salt adversely affected the protease and amylase activities, resulting in the optimum content of without salt for stabilities of enzymes. Salt content was the most crucial factor contributing to the characteristic of protease, while pH value for amylase. Besides, the protease and amylase had adapt-abilities to salt stress and pH stress, respectively. Our results suggest that the conditions of pH 7.0-7.7 and temperature of 40 °C, and the conditions of pH 5.0 and temperature of 48 °C should be applied in the food industry as the methods of choice to quantify soy sauce koji protease and amylase activities, respectively, derived from *A. oryzae*.

Acknowledgements

This research was partially supported by the Ministry of Science and Technology in Taiwan, R.O.C. (MOST 105-2320-B-415-005 -)

機能性樹豆漿抗氧化活性與改善 HepG2 細胞脂質蓄積評估

倪彥綉、吳芸臻、吳思敬*

國立嘉義大學食品科學

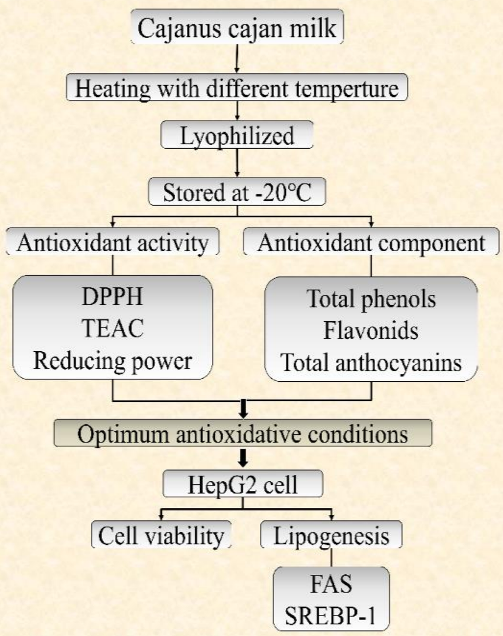
摘 要

樹豆 (*Cajanus cajan* Linn. Millsp.)，為豆科 (Leguminosae)、樹豆屬 (Cajanus)，是原住民重要的傳統糧食作物之一。近年市面上已有以不同豆類為基質之豆漿產品出現，如：黑豆漿、毛豆漿及紅豆漿等。因此本研究以樹豆 (臺東 3 號) 取代傳統黃豆製成樹豆漿 (Cajanus cajan milk, CCM)，探討樹豆漿之理化性質、抗氧化活性及機能性成分並進一步以油酸鹽與棕櫚酸鹽 (Oleate 與 Palmitate) 誘導 HepG2 細胞脂質蓄積模式探討樹豆漿對肝損傷之保護效應。結果顯示，於理化性質部分，不同加熱溫度之樹豆漿各組間皆無顯著差異，但與黃豆漿皆有顯著差異。抗氧化活性部分，90°C 樹豆漿組與黃豆漿及其他加熱組相比，於 DPPH 自由基清除能力、還原力有較好之抗氧化活性且含有較高之總酚含量。於細胞實驗部分，經 Oleate 與 Palmitate 以及 1 ~ 5mg/mL 樹豆漿共處理後 HepG2 細胞中 FAS 及 SREBP-1 的表現量皆呈現下降趨勢，表示樹豆漿能抑制 FAS 及 SREBP-1 兩脂質合成相關蛋白表現量。綜合上述，樹豆漿與黃豆漿相比整體上具較好之抗氧化活性且具有特殊風味。其中 90°C 樹豆漿具較高抗氧化能力及含有較多的總酚含量，並可能具有改善肝臟脂質蓄積的潛力，期能以此加工條件作為基礎，進一步開發出適合工業生產之機能性樹豆漿新產品。

前言

隨著國人飲食習慣改變，高脂飲食導致肥胖人口逐年增加，研究指出高脂飲食會導致高血脂症，而長期高血脂症可能引起非酒精性脂肪性肝病 (non-alcoholic fatty liver disease, NAFLD)，包括肝臟脂質蓄積和肝損傷，且流行病學和臨床研究證實血液中高量的脂質與心血管疾病呈正相關性 ，因此，自飲食中攝取植化物質以達日常保健為現今所必需。樹豆 (*Cajanus cajan* Linn. Millsp.)，為豆科 (Leguminosae)、樹豆屬 (Cajanus)，為多年生矮灌木，具調節血糖、抗菌及抗氧化等功效。本研究室先前證實樹豆 (臺東 3 號)之乙醇萃取物具有高的抗氧化活性與調節血脂功效，且於細胞實驗中可有效提升抗氧化酵素 (CAT、GPx、GRd 及 SOD) 活性，減緩脂質過氧化，並抑制 NO、IL-6、TNF-α 及 IL-1β 等細胞激素釋放，減緩發炎反應。故本研究以樹豆 (臺東 3 號) 取代傳統黃豆製成樹豆漿 (Cajanus cajan milk, CCM)，探討樹豆漿加工製程對其理化性質、抗氧化活性之影響，並測定機能性成分，以及初步評估樹豆漿對肝損傷之保護效應。

實驗架構



結果

表一、不同溫度加熱樹豆漿萃取率

Table 1. The extraction of Cajanus cajan milk

	SM-90℃	CCM-85℃	CCM-90℃	CCM-95℃	CCM-121℃	CCM-UH
*Extract yield (%)	34.24^{b}	18.50±2.12^{d}	22.66±0.48^{c}	20.59±2.24^{c}	21.50±2.12^{c}	42.35±1.07^{a}

*Extract yield (%) = dry weight of water extract/raw weight of sample × 100

Each value is expressed as mean ± S.D. (n=3).

Each value on the top with different letters in the columns is significantly ($p<0.05$).

SM = soybean milk ；CCM = Cajanus cajan milk. ；CCM-UH = Unheated Cajanus cajan milk

表二、不同加熱溫度對樹豆漿理化性質之影響

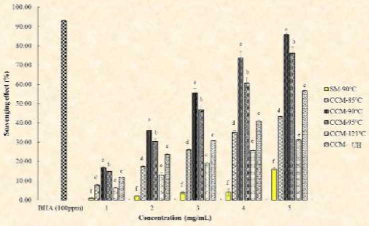
Table 2. Effect of Cajanus cajan milk with different heating time on physiochemical analysis

	L	Hunter a	Hunter b	pH value	Soluble solids (° Brix)
SM-90℃	82.44±0.49^{a}	-1.71±0.23^{f}	13.16±0.44^{a}	6.50 ± 0.01^{d}	4.73±0.06^{a}
CCM-85℃	37.05±0.05^{cd}	0.60±0.01^{e}	0.88±0.06^{d}	6.67 ± 0.04^{c}	2.45±0.37^{b}
CCM-90℃	37.41±0.04^{c}	1.02±0.00^{d}	1.03±0.04^{d}	6.82 ± 0.03^{a}	2.45±0.37^{b}
CCM-95℃	36.79±0.01^{d}	1.59±0.05^{b}	1.06±0.02^{d}	6.74 ± 0.01^{b}	2.23±0.15^{b}
CCM-121℃	36.19±0.05^{e}	1.41±0.05^{c}	1.78±0.02^{c}	6.72 ± 0.05^{bc}	2.45±0.37^{b}
CCM-UH	58.48±0.63^{b}	2.70±0.04^{a}	3.44±0.06^{b}	5.82 ± 0.02^{c}	2.03±0.06^{b}

Each value is expressed as mean ± S.D. (n=3).

Each value on the top with different letters in the columns is significantly ($p<0.05$).

SM = soybean milk ；CCM = Cajanus cajan milk. ；CCM-UH = Unheated Cajanus cajan milk



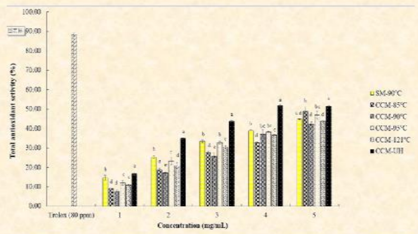
圖一、不同加熱溫度樹豆漿之清除 DPPH 自由基能力

Fig 1. Scavenging effect of Cajanus cajan milk with different heating times on the DPPH radical.

Each value is expressed as mean ± S.D. (n=3).

Each value on the top with different letters in the columns is significantly ($p<0.05$).

SM = soybean milk ；CCM = Cajanus cajan milk. ；CCM-UH = Unheated Cajanus cajan milk



圖二、不同加熱溫度樹豆漿之總抗氧化能力

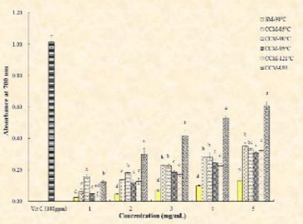
Fig2. Total antioxidant activity of Cajanus cajan milk with different heating times.

Each value is expressed as mean ± S.D. (n=3).

Each value on the top with different letters in the columns is significantly ($p<0.05$).

SM = soybean milk ；CCM = Cajanus cajan milk. ；

CCM-UH = Unheated Cajanus cajan milk



圖三、樹豆漿經不同加熱溫度之還原力

Fig 3. Reducing power of Cajanus cajan milk with different heating times.

Each value is expressed as mean ± S.D. (n=3).

Each value on the top with different letters in the columns is significantly ($p<0.05$).

SM = soybean milk ；CCM = Cajanus cajan milk. ；

CCM-UH = Unheated Cajanus cajan milk

表三、不同加熱溫度對樹豆漿萃取物的花青素、總酚和類黃酮含量之影響

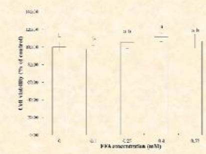
Table 3. Effect of different heating time on total anthocyanins , total phenolics and flavonoids of Cajanus cajan milk extracts

Group	Total anthocyanins	Total phenolics	Flavonoids	Flavonoids
	mg/g dry weight	GAE mg/g dry weight	rutin mg/mL	quercetin mg/mL
SM-90℃	ND	31.83 ± 0.38^{b}	13.46 ± 0.73^{a}	1.45 ± 0.09^{b}
CCM-85℃	0.21 ± 0.02^{b}	31.90 ± 2.38^{b}	6.15 ± 0.16^{c}	0.53 ± 0.02^{c}
CCM-90℃	0.06 ± 0.02^{c}	44.86 ± 4.95^{a}	4.20 ± 0.16^{d}	0.57 ± 0.01^{c}
CCM-95℃	0.16 ± 0.05^{b}	43.19 ± 0.41^{a}	4.57 ± 0.28^{d}	0.53 ± 0.03^{c}
CCM-121℃	ND	42.00 ± 4.46^{a}	5.13 ± 0.28^{cd}	0.48 ± 0.07^{c}
CCM-UH	1.87± 0.04^{a}	43.65 ± 1.45^{a}	8.74 ± 1.11^{b}	1.65 ± 0.04^{a}

Each value is expressed as mean ± S.D. (n=3).

SM = soybean milk ；CCM = Cajanus cajan milk. ；CCM-UH = Unheated Cajanus cajan milk

Each value on the top with different letters in the columns is significantly ($p<0.05$).

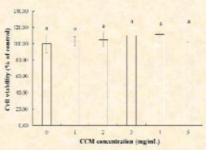


圖四、脂質誘導劑 (free fatty acid, FFA) 對 HepG2 細胞存活率之影響

Fig 4 . Effect of free fatty acid (FFA) on HepG2 cell viability.

Each value is expressed as mean ± S.D. (n = 3).

Each value on the top with different letters in the columns is significantly ($p<0.05$).

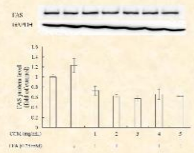


圖五、樹豆漿對 HepG2 細胞存活率之影響

Fig 5. Effect of Cajanus cajan milk. (CCM) on HepG2 cell viability.

Each value is expressed as mean ± S.D. (n = 3).

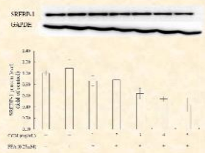
Each value on the top with different letters in the columns is significantly ($p<0.05$).



圖六、於 FFA 誘導 HepG2 細胞內脂質合成作用中樹豆漿對 FAS 表現量之影響

Fig 6. Effect of Cajanus cajan milk on FAS protein level in FFA-induced HepG2 cell.

Each value is expressed as mean ± S.D. (n = 3).



圖七、於 FFA 誘導 HepG2 細胞內脂質合成作用中樹豆漿對 SREBP-1 表現量之影響

Fig 7. Effect of Cajanus cajan milk on SREBP-1 protein level in FFA-induced HepG2 cell.

Each value is expressed as mean ± S.D. (n = 3).

結論

1. 總體而言，樹豆漿 (85°C、90°C、95°C 及 121°C) 抗氧化能力優於 90 °C 黃豆漿，其中，於濃度 5 mg/mL 下，又以 90°C 樹豆漿之 DPPH 自由基清除能力、還原力高於其他加熱樹豆漿組 (85°C、95°C 及 121°C)，且總酚含量為加熱豆漿組中最高。
2. 於脂質誘導 HepG2 細胞內脂質蓄積模式下，樹豆漿可減少 FAS 及 SREBP-1 蛋白表現量，代表樹豆漿可能具減緩肝臟脂質蓄積能力。
3. 綜合上述，樹豆漿具有優於黃豆漿之抗氧化能力，並具有改善肝臟中脂質蓄積潛力，期能以 90°C 加熱作為基礎，加以調整改善生產製程，做為製程優化之機能性樹豆漿之依據。