行政院國家科學委員會專題研究計畫 成果報告

高壓處理對牡蠣閉殼肌變性及鮮度保持之影響
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2313-B-040-001-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：中山醫學大學健康餐飲管理學系

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 10 月 26 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

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成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：中山醫學大學健康餐飲管理學系

中　華　民　國　96　年　07　月　31　日

目錄

中文摘要

本研究是以高靜水壓 (100-500 MPa；4℃；0-10 min) 處理牡蠣後觀察其脫殼之情形，並置於 4℃下 貯藏 6 週以觀察鮮度之變化。結果發現以愈高壓力處理之牡蠣其 pH 值及白色度較低壓力處理者為高，且高壓處理後，牡蠣呈現半煮熟、體積較大且較為多汁感。在脫殼效果方面，以 200 MPa 處理 2 min 即有 50% 之牡蠣完全脫殼；300 MPa 處理 0 min 後，超過 90% 完成脫殼；壓力達 400-500 MPa，則達 100% 脫殼的效果。高壓處理並無法抑制脂解酶之活性，且高壓處理後約減少約 2-3 log 生菌數，在貯藏 6 週後仍保持在較低的生菌數，因此以高壓技術進行牡蠣加工之條件建議為 300 MPa/4℃/2 min。
關鍵字：高壓、牡蠣、脫殼、保存期限

ABSTRACT

The effects of high pressure (HP) treatments (100-500 MPa for 0-10 min at 4℃) on shucking and freshness of oysters via storage at 4℃ for 6 weeks were investigated. HP-treated oysters had higher pH and lightness (*L* value) than untreated oysters; the magnitude of changes increased with treatment pressure. HP-induced changes in color generally imparted a cooked, more voluminous and juicy appearance to the raw oyster tissue. After a treatment at 200 MPa for 2 min, 50% oyster muscles became detached from their shells. When the pressure increased to 300 MPa, more than 90% oyster muscles were shucked, and beyond 300 MPa, all oysters were shucked. Pressure treatment did not significantly inhibit lipase activity during the shelf-life study. HP reduced initial microbial load by 2-3 logs and counts remained at a reduced level through the storage study. We suggested that 300 MPa/4℃/2 min would be the best situation for oyster shucking and extending their shelf-life.
Key-words: pressure, oyster, shucking, shelf-life

壹、研究目的

本研究目的為提高牡蠣之保存性及食用之安全性，且考量學者們研究上加工處理條件選擇之紛紜，因此利用不同之樣品處理及貯藏條件，以期能延長牡蠣之保存期限、維持其良好外觀及口感。實驗方法採用兩種不同之樣品處理：(1) 樣品直接進行真空包裝；(2) 樣品加入無菌 3.5% 海鹽溶液 (模擬海水) 再行真空包裝，在 4℃ 下進行 100～500 MPa/0～10 min 之高壓處理，觀察牡蠣脫殼情形外，亦測定其肉體組織及閉殼肌的變性情形。另一方面以原包裝狀態直接進行貯藏 (4℃; 0-6 週)，以觀察微生物生長情形、鮮度及肉體組織變化情形。

貳、文獻探討

近年來，消費者需求安全、低加工性、無添加物及天然之食品，因可保持食物之營養成分、外觀、風味及色澤。牡蠣 (oyster; *Crassostrea gigas*) 因美味與富含營養價值，在世界各地常以半殼 (half-shell) 活體狀態食用，為一高經濟價值食品。牡蠣是濾水性生物 (water-filtering organisms)，因此易累積病原菌微生物，不易藉加工方法去除；且具有閉殼肌 (adductor muscle) 緊密連結左殼 (left valve) 及右殼 (right valve)，造成在食用時將牡蠣肉時脫殼 (shucking) 之困難 (Martin and Hall, 2005)，過去到現在多還是使用牡蠣刀 (oyster knife) 切斷閉殼肌來取肉，但易造成污染及刺破肉體 (Lofland, 1923)。

脫殼技術在近 150 年來大幅地改良，包含人工脫殼、機械脫殼 (Torsch and Parker, 1907)、蒸汽脫殼 (Doxsee and Cook, 1935)、微波脫殼 (Spracklin, 1971)、高周波脫殼 (Paparella and Allen, 1970) 及雷射脫殼 (Singh, 1972) 等。雖然脫殼的效果顯著，但同時失去牡蠣肉的口感，如破壞及煮熟肉體，導致品質降低。

另一方面，牡蠣生食的衛生安全問題在 1970 年代開始受到重視 (Acton, 1970; Beecham et al., 1991; Lee et al., 2003)，研究指出，牡蠣分別在 5℃、7℃ 冷藏或 9℃ 海水浴中保藏第 7 、5 及 12 日時，微生物即生長至不可食狀態，且風味及組織皆不受消費者接受 (Aaraas et al., 2004; López-Caballero et al., 2000)；牡蠣使用抗菌膜包裝 (nisin 或 lacticin NK24 塗抹在低密度聚乙烯膜) 在 10℃ 下冷藏，保存期限可至 10 日 (Kim et al., 2002)。因此牡蠣保存期限的延長是食品學家重要的課題之一。

近幾年高壓技術漸漸受到牡蠣加工業者重視，因高壓可殺滅牡蠣產品中 *Vibrio parahaemolyticus* 及其他病原菌 (Berlin et al., 1999; Mermelstein, 2000)，345 MPa 處理 90 s 可使牡蠣中 *V. parahaemolyticus* 至無法檢測程度；牡蠣在 7℃ 下以 400 MPa 處理 10 min 可完全殺滅 coliforms，高壓處理後之牡蠣以 2℃ 貯藏 41 日後其揮發性鹽基態氮含量仍低於 30 mg/100 g，顯示仍在可食狀態 (López-Caballero et al., 2000)；牡蠣經高壓處理後可將總生菌數減少 2～3 logs，且在 3℃ 下貯藏 27 日後總生菌數仍低於生鮮之牡蠣 (He et al., 2002)；牡蠣以 2℃ 貯藏隨時間增

加其肉體剪切應力 (shear strength) 會減低 10%，以高壓處理 (400 MPa/10 min/7℃) 後組織之剪切應力會增加，且以 2℃ 貯藏 41 日後仍不會降低 (López-Caballero et al., 2000)；牡蠣在 20℃ 下以 100～800 MPa 加壓 10 min，pH 及色澤之 L 值皆上升，且隨壓力增加而增加，顯示肉體蛋白質在越高壓力處理下變性情形愈加明顯 (Cruz-Romero et al., 2004)。另一方面，高壓處理對牡蠣具有良好的脫殼效果，以 242 MPa/2 min、276 MPa/0 min、311 MPa/0 min 處理可分別使 88%、80% 及 100% 之牡蠣完全脫殼 (He et al., 2002)，且脫殼之牡蠣肉體保持良好外觀及形狀，且體積略為增大，並較未高壓處理者更為多汁感 (juicy) (Cruz-Romero et al., 2004; López-Caballero et al., 2000; He et al., 2002)。因此高壓技術應用在牡蠣加工業確為一發展趨勢。但目前相關研究極少，且多半著眼於高壓對微生物之影響，另外處理條件大不相同，如使用真空包裝進行高壓處理 (Cruz-Romero et al., 2004; López-Caballero et al., 2000; He et al., 2002)，亦有添加水後再行真空包裝者

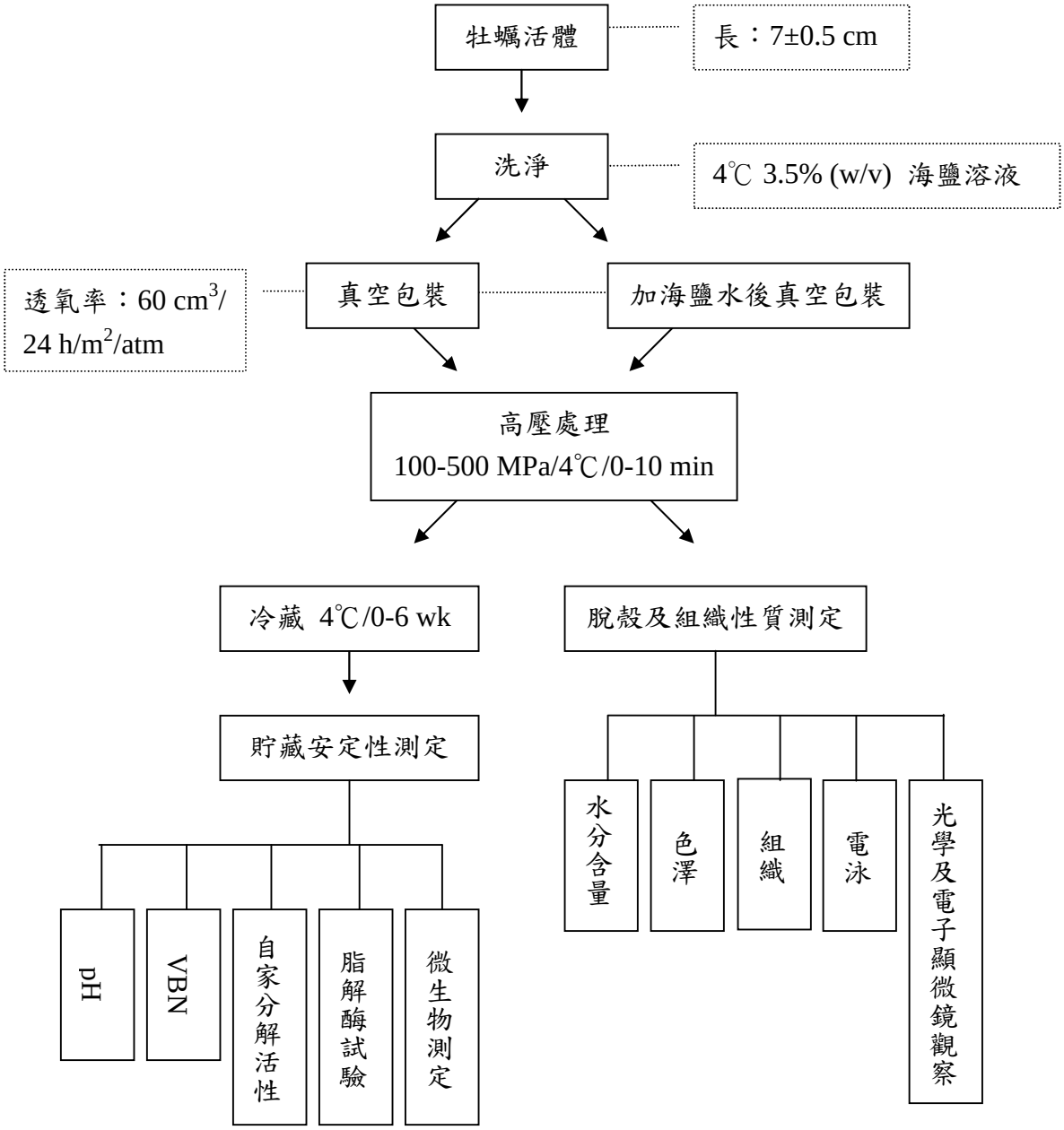
(Smiddy et al., 2005)；貯藏條件亦不相同，有部分學者是將高壓處理後之樣品，直接以真空包裝狀態進行冷藏 (López-Caballero et al., 2000)，部分學者則是將脫殼後之牡蠣移除真空包裝，置入冷水槽中貯藏 (He et al., 2002)。因此欲尋求最佳的高壓處理條件，必須將各種處理方法及貯藏條件統整，除作為牡蠣加工業者之參考外，更能進一步提供鮮食之餐飲業者及消費者更安全、更有效益的選擇。

參、研究方法

1. 材料

取自雲林縣口湖鄉養殖之帶殼活牡蠣 (shelled oyster)，著眼於該地產量居全台之最，品質最佳，大小以 7±0.5 cm 為主以便於高壓處理，在 10℃ 冷藏運回實驗室維持牡蠣之生命力，並立即以 4℃ 3.5% (w/v) 海鹽溶液浸洗 30 min，瀝乾後進行兩種包裝處理：(1) 取 2 個牡蠣置於透氧率為 60 cm^3/24 h/m^2/atm (23℃) 之真空包裝袋 (Cryovac BB-1, Grace, Barcelona, Spain) 中行真空包裝；(2) 取 2 個牡蠣置於真空包裝袋中加入 4℃ 100 ml 3.5% 無菌海鹽水，再行真空包裝。以上兩組進行高壓處理。對照組則為同以上處理但不經高壓處理且置於 4℃ 冷藏者。

2. 實驗流程及方法



肆、結果與討論

不同壓力下對牡蠣脫殼的效果，隨壓力之增加與處理時間之延長而脫殼效果愈佳 (Table 1)，但與處理之壓力大小有較明顯之影響，時間的延長並無顯著的影響，此與高壓之效果是瞬間作用所致 (Jao et al., 2007)。以 100 MPa 處理時，幾乎對牡蠣之閉殼肌無變性之影響，以致無脫殼效果；以 200 MPa 處理時，完全脫殼比例已達約 50%；當壓力提升至 300 MPa 時，完全脫殼比例已達 90% 以上；以 400-500 MPa 處理時，則 100% 均已完全脫殼，顯示牡蠣閉殼肌在 300 MPa 以上時，會發生明顯變性的情形。高壓處理後之牡蠣組織之形狀較為固定，視覺上體積也較為龐大且肉質具有多汁感 (Lopez-Caballero et al., 2000; He et al., 2002)。隨壓力的增加，牡蠣組織的水分含量亦隨之增加 (Table 2)，此與在高壓下會增加蛋白質水合的含量所致 (Cioni and Strambini, 1994; Vidugiris et al., 1995; Silva et al., 2001)，因此也使加壓後的牡蠣組織較具有彈性。在外觀色澤的變化上 (Fig. 1)，100-300 MPa 處理後之牡蠣，其總色差 (△*E*) 不無顯著差異，但明顯較控制組為高，隨壓力增加至 400-500 MPa 時，總色差快速增加，顯示牡蠣組織蛋白質等應都發生明顯變性情形。

脂解酶為一種分布極廣的酵素，作用為水解脂肪成游離脂肪酸，因此導致食品的異味及變質 (Chai et al., 1984)。若酵素發生些微結構上的改變，可使酵素失去活性，高壓可使蛋白質發生變性，亦可使蛋白質的結構發生顯著改變，但高壓對酵素活性之作用可能有 2 種結果：一為在較低壓力下會活化酵素，提升酵素活性；二為在較高壓力下會使酵素失活。Table 3 顯示牡蠣脂解酶經高壓處理後貯藏於 4℃ 下 6 週之活性變化，結果顯示脂解酶在貯藏第一週較為穩定，而隨貯藏時間之延長，其活性快速增加，而較高的壓力反而使脂解酶有活化的現象，此結果推測為脂解酶的分子量較小，因此在高壓下反而有活化其活化位置的作用所致。

牡蠣較其他水產品比較起來，具有較高含量的肝醣，因此在牡蠣腐敗時會發生發酵作用而降低組織的 pH 值 (Cook, 1991)，因此測定牡蠣之 pH 值為測定其腐敗與否的良好指標之一。高壓處理牡蠣在冷藏過程中 pH 值的變化如 Fig. 2 所示，隨著壓力的增加，pH 的下降甚為緩慢，較控制組在第 3 週起即快速下降，顯示有良好的微生物抑制效果。在好氧性總生菌數 (aerobic plate coung; APC) 的測定方面，美國 FDA 對於新鮮加工的雙殼肉類之微生物數量標準為 ＜500,000 CFU/g，超過 1,000,000 CFU/g 者則被定義為不合格。在控制組與 100 MPa 處理者方面 (Fig. 3)，發現在貯藏第 2 週後即已發生腐敗現象，隨壓力增加至 200-300 MPa 時即顯示，儘管貯藏至第 6 週結束，APC 仍在良好等級，當壓力增加至 400-500 MPa 時，APC 已無法測定到，因此顯示在愈高壓力處理下，牡蠣的生菌數方面在冷藏 6 週後仍保持可食狀態，但未經高壓處理者 (控制組)，則在第 2-3 週即已發生腐敗情形。

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Table 1 HP treatment shucking effect on oysters

Pressure (MPa)	100			200			300			400			500		
Time (min)	0	2	10	0	2	10	0	2	10	0	2	10	0	2	10
N (%)	100	98	92	10	8	7									
P (%)		2	8	45	41	38	8	2	1						
F (%)				45	51	55	92	98	99	100	100	100	100	100	100

N means no release of adductor muscle after HP treatment
P means partial release of adductor muscle after HP treatment
F means partial release of adductor muscle after HP treatment
48 oysters were used for each treatment

Table 2 Effect of HP treatment (2 min) on moisture contents of oyster tissue

Pressure (MPa)	Control	100	200	300	400	500
Moisture Contents (%)	78.6	79.1	80.3	81.0	81.6	82.3

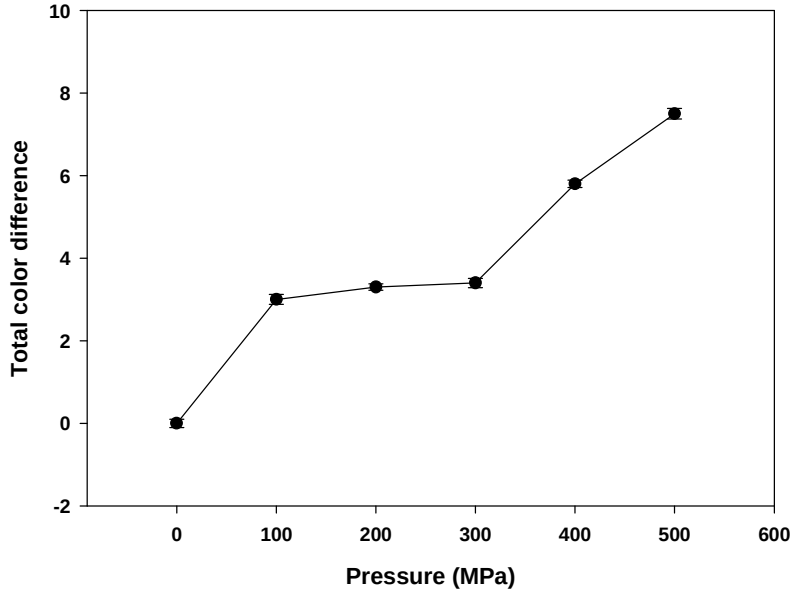

Fig. 1 Effect of HP treatment (2 min) on total color difference ($\triangle E$) of oyster body tissue.

Table 3 Effect of HP treatment (2 min) on lipase activity (nmol 4-MU/min/mg protein)

Pressure (MPa) / Weeks	Control	100	200	300	400	500
0	4.2	3.1	2.9	4.1	4.1	4.8
1	7.6	6.8	4.3	4.6	5.8	6.2
2	14.1	13.2	19.0	11.0	9.8	8.6
3	30.4	26.9	19.3	31.2	35.7	33.8
4	40.1	38.7	36.9	53.0	31.4	36.4
5	44.8	42.6	39.6	56.4	33.9	38.4
6	50.2	48.6	44.6	59.8	38.6	43.8

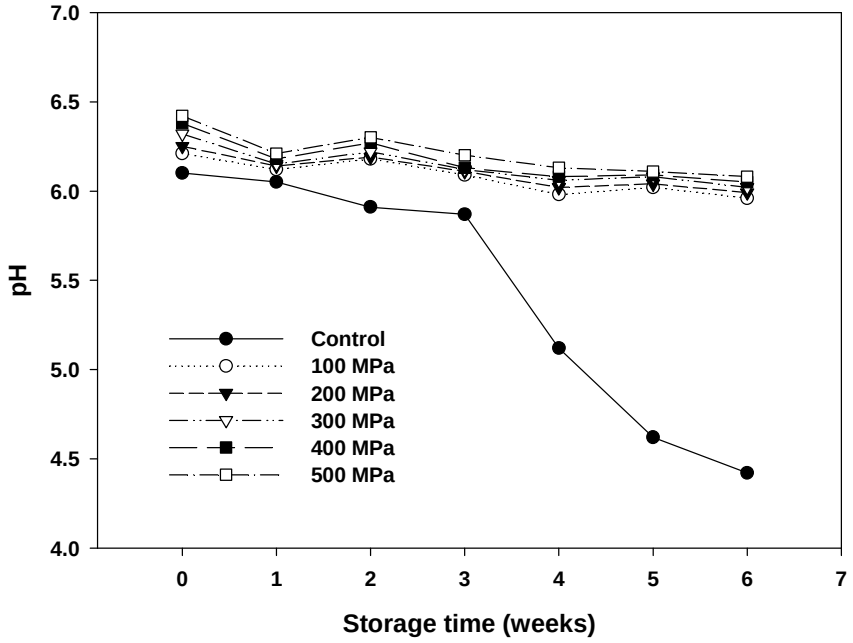

Fig. 2　Changes in pH for HP-treated oysters during refrigerated storage.

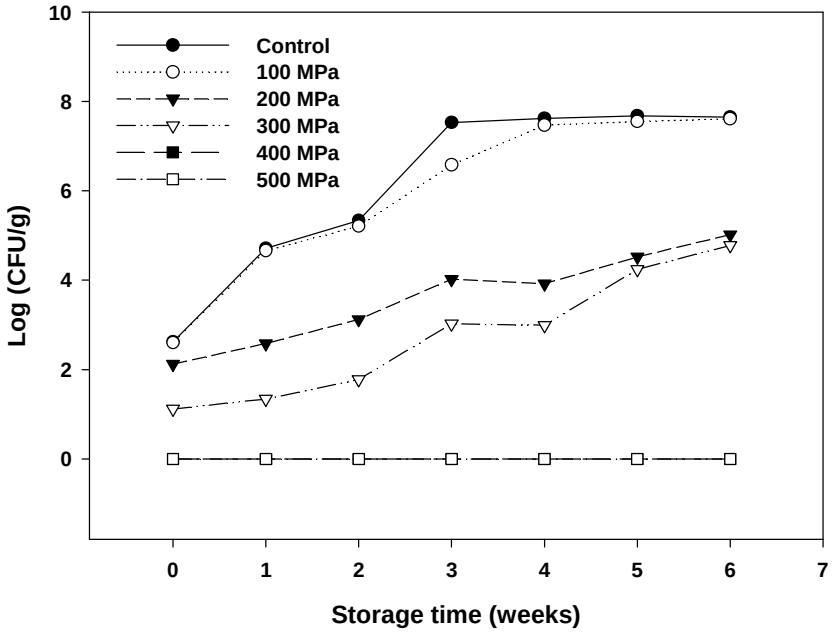

Fig. 3　Effect of HP on APC of oysters during refrigerated storage.

陸、研究成果自評

本研究按照計畫申請之內容進行，研究成果本人認為良好，且已投稿至國外學術期刊「Food Chemistry」，該期刊為排名前 20% 之優良期刊，希望能有良好成果。所有研究方法均依序進行。亦期望未來能加強水產高壓加工之研究基礎，並強化其應用性，如此研究成果方能圓滿。

出席國際學術會議心得報告

計畫編號	95-2313-B-040-001-
計畫名稱	高壓處理對牡蠣閉殼肌變性及鮮度保持之影響
出國人員姓名 服務機關及職稱	徐國強 中山醫學大學健康餐飲管理學系助理教授
會議時間地點	96.06.24-27；Segovia, Spain
會議名稱	5th International Symposium in Chemical Engineering and High Pressure Processes
發表論文題目	Comparative Applications of Thermal and Hydrostatic Pressure Treatments to Tomato Juice: Carotenoids and Lycopene, Radical-Scavenging Capacity, Inactivation of Pectin Methylesterase and Polygalacturonase

一、參加會議經過

本次活動在西班牙當地時間 6 月 24 日起舉辦 4 天，本人搭乘台北時間 6 月 21 日晚上 11 時 10 分班機前往，經法蘭克福轉機後，於當地時間 6 月 22 日下午抵達馬德里市，並進駐當地旅館，於隔天中午搭乘火車前往 Segovia，車程約 2 小時餘。當天晚上即參加主辦單位舉辦之歡迎酒會，並和與會人士有短暫交談及交流。第二天即為正式研討會議程，於上午完成報到後，即參與研討會討論，本人參與的是應用類的研討會，會中討論高壓加工技術在現在食品加工領域，無論是學術或產業領域，均有深入之討論。會議於 6 月 27 日下午 4 時結束。

二、與會心得

本次活動參與國家以歐洲及美國國家為主，亞洲只有台灣、日本及菲律賓三國有代表與會，顯示歐美在高壓及分析領域之用心與專注。另外，由於高壓設備受限於廠商技術的不成熟以及費用極高等問題，使高壓在產業界一直無法順利拓展，因此本次研討會亦邀集相關設備廠商參與，一同檢討改善設備研發問題。而此次研討會之研究水準及討論風氣均具有相當程度，因此在此新穎技術尚在研發之時，對於基礎理論及未來應用等方面均有良好的結合，也使亞洲國家可以學習歐美國家無論在理論或實務上之研究與應用，此次研討會加入了高壓分析相關技術，例如超臨界萃取技術等，也瞭解高壓技術的應用範圍相當廣泛。對我國而言，可在此歐美等已經在市場上有產品問世的國家主導下，發表我國從事高壓食品加工技術的研究成果，也使其他國家重視台灣的學術研究地位，是相當值得的。

Title:

Comparative Applications of Thermal and Hydrostatic Pressure Treatments to Tomato Juice: Carotenoids and Lycopene, Radical-Scavenging Capacity, Inactivation of Pectin Methylesterase and Polygalacturonase

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Running head:

Application of Thermal and Pressure to Tomato Juice

Abstract

Effects of thermal (hot- and cold-break) and high pressure treatments (100-500 MPa/4, 25 and 50℃) on the processing qualities of tomato juice were investigated. Both thermal treatments induced significant changes of color, viscosity and radical-scavenging capacity of tomato juice compared with control (fresh tomato juice), moreover, hot-break processing (92℃/2 min) almost completely inactivated PME and PG. High pressure treatments behind 200 MPa at 4 and 25℃ maintained the color, extractable total carotenoids and lycopene, and radical-scavenging capacity, further, those beyond 400 MPa at the same temperatures could improve all the quality attributes in this study except inactivation of PME. However, high pressure treatments at 50℃ had less efficiency or even contrary effects compared with those at

lower temperatures. At 500 MPa/4 and 25℃, the total carotenoids and lycopene contents increased about 60%; viscosity increased 20%; and PG activity decreased to 10%. The most efficiency of pressure treatments on inactivation of PME in tomato juice was observed at 200 MPa/25℃. This research clearly shows that it is possible to selectively produce good tomato juice products by high pressure processing at ambient temperature.

Key Words: Tomato juice; Hydrostatic pressure; Thermal treatment; Lycopene, Radical-scavenging capacity

1. Introduction

Besides microbial safety, important quality aspects of such tomato products are color, flavor, and consistency (Hayes, Smith, & Morris, 1998). In tomato products, an important reaction is the degradation of the red pigment lycopene, originally in the trans form, that isomerizes to the cis structure during thermal process, resulting in changes of the color (Rodrigo, van Loey, & Hendrickx, 2006). Moreover, in the thermal-stabilized tomato juice products, the changes of color and flavor can also be resulted from nonenzymatic browning (Porretta, 1991; Servili, Selvaggini, Taticchi, Begliomini, & Montedoro, 2000). Consistency of tomato products refers to their viscosity and the ability of their solid portion to remain in suspension throughout the shelf-life of the product. Consistency is normally improved using technological processes which minimize pectin-breakdown by enzymes [pectin methylesterase (PME) and polygalacturonase (PG)] in pectin- and cellulose-rich tomato cultivars, can normally be reduced by using hot- or cold-break techniques (Anthon, Sekine, Watanabe, & Barrett, 2002), which represent that tomatoes are pre-heated at 90-95℃ and 60℃, respectively (Moresi & Liverotti, 1982). The consumption of tomato products has been associated with a lower risk of developing digestive tract and prostate cancers (Giovannucci, Rimm, Liu,

Stampfer, & Willett, 2002) due to the ability of lycopene and other antioxidant components to prevent cell damage through synergistic interactions (Friedman, 2002; George, Kaur, Khurdiya, & Kappor, 2004). From the methodological point of view, the widespread use of the stable 2,2-dipheyl-1-picrylhydrazyl (DPPH˙) radical-scavenging model in recommended as fast, easy and accurate, for measuring the antioxidant activity of plant foods (Da Porto, Calligaris, Celotti, & Nicoli, 2000; Espín, Soler-Rivas, Wichers, & García-Viguera, 2000).

Effect of thermal processing on the processing qualities of tomato juices were widely investigated (Sieso & Crouzet, 1977; Sánchez-Moreno, Plaza, de Ancos, & Cano, 2006a). The volatile components and vitamin C of canned tomato juice can be reduced by treatments at 100℃ for 10 min (Sieso & Crouzet, 1977; Youssef & Rahman, 1982). The color of tomato juice degraded more rapidly with increasing temperature, therefore, one of the advantages of cold break over hot break is that the final product had more natural color (Goodman, Fawcett, & Barringer, 2002; Sánchez-Moreno et al., 2006a). However, heating causes an increase in the overall antioxidant potential of the tomato juice coincide with the positive effect of the temperature on the extractability of lycopene (Anese, Manzocco, Nicoli, & Lerici, 1999; Anese, Falcone, Fogliano, Nicoli, & Massini, 2002; Sánchez-Moreno et al., 2006a). Besides, lycopene in tomato is relative resistant to degradation, whereas other antioxidants (ascorbic acid, tocopherol and β-carotene) decrease as a function of thermal processing (Abushita, Daood, & Biacs, 2000). At temperatures higher than 78℃ and 90℃, respectively, PME and PG in tomato juices could be completely inactivated (De Sio, Dipollina, Villari, Loiudice, Laratta, & Castaldo, 1995; Fachin, van Loey, Nguyen, Verlent, Indrawati, & Hendrickx, 2003).

Thermal processing is conventionally used to inactivate microorganisms and enzymes and extend the shelf life of juice products. However, thermal processing can adversely affect the sensory and nutritive qualities of tomato juices (Goodman et al., 2002; Youssef & Rahman, 1982). The consumers demand safe, fresh and minimally processed foods, therefore, a nonthermal food processing such as hydrostatic pressure has developed (Popper & Knorr, 1990; Knorr, 1993).

Hydrostatic pressure processing is a technology with the objective to process and/or preserve foods by inactivation of vegetative microorganisms (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989) and quality related enzymes (Weemaes, Ludikhuyze, Van de Broeck, & Hendrickx, 1998). Due to the ability to maintain the quality attributes of the fresh material because mainly non-covalent bonds are affected by pressure, high pressure processing can be an alternative for heat treatment in the context of food preservation (Cheftel, 1992). Some researchers have demonstrated that high pressure processing improved viscosity and color properties in comparison with their conventional heat-processed counterparts (Porretta et al., 1995). PG in tomato-based products could be totally inactivated at some pressure/temperature combinations: 550 MPa/20℃ (Fachin et al., 2003); 500 MPa/60℃ (Crelier, Robert, & Juillerat, 1999) and 800 MPa/25℃ (Shook, Shellhammer, & Schwartz, 2001). Tomato PME, a heat-labile enzyme at ambient pressure, was dramatically stabilized against thermal denaturation at pressures above atmosphere and up to 500-600 MPa, and was completely inactivated at 800 MPa/70℃ over 20 min (Crelier, Robert, Claude, & Juillerat, 2001).

Surprisingly, few researchers have reported that the effect of high pressure treatments on the processing quality of tomato juice, such as carotenoids and antioxidant properties, in comparison with that of thermal treatments (hot- and cold-break). Therefore, the main objective of this research was to compare the effects between thermal and high pressure treatments on the factors that affect the processing quality of tomato juice, such as carotenoids, radical-scavenging capacity, PME and PG activities. This work could offer the information of application of hydrostatic pressure processing in food industries.

2. Materials and methods

2.1 Tomatoes and juice preparation

Red daydream tomatoes were purchased from Yenshui Farmer's Association in Tainan County, Taiwan. Daydream tomatoes were stored at 7℃ before treatments within 7 days. Washed

tomatoes (200 g), equilibrated at room temperature, were homogenized twice by a Warring-blender with the high speed for 10 sec and sieving (0.8 mm holes) to remove pieces of skin and any seeds. The tomato juice was obtained as the control (adjusting pH to 4.5 with 10 M NaOH) in this study and used for the following treatments and measurements immediately.

2.2 Thermal treatment (hot-break and cold-break)

Tomato juice (150 g) was poured into double polyethylene bags (250×360 mm, thickness: 50 micron, Medisch Labo Service, Menen, Belgium) and vacuum sealed followed by hot-break (92℃; 2 min) or cold-break (60℃; 2 min). Afterward, the tomato juice was immediately cooled in ice water for 2 min and equilibrated at room temperature for 10 min.

2.3 Combined pressure-temperature treatments

Tomato juice was filled into polyethylene pouches (10 cm×13 cm, capacity 200 ml) which were heat-sealed after removal of the air and subjected to 100 to 500 MPa in combination with temperatures at 4, 25 and 50℃. Afterward, the tomato juice was immediately cooled in ice water for 2 min and equilibrated at room temperature for 10 min.

2.4 High-pressure equipment

A high-pressure apparatus (CIP UNIT, Mitsubishi Heavy Industries Ltd., Japan) with an oil-pressure generator and a compressing vessel, in which the internal portion (diameter: 50 mm; height: 120 mm) was a flat-bottomed cylindrical shape, was employed. The vessel temperatures during pressure treatments were controlled by a circulator.

2.5 Color

Hunter L, a, and b of tomato juice were measured by a HunterLab colorimeter (Color Meter ZE-2000, Nippon Denshoku Co., Japan). The red-yellow ratio (a/b) was reported to indicate the

redness of tomato juice (Min & Zhang, 2003).

2.6 Carotenoids and lycopene (Lin & Chen, 2003; Lin & Chen, 2005)

A 8 g juice sample was mixed with 40 mL of ethanol-hexane (4:3, v/v) and 0.2 g magnesium carbonate. The solution was shaken in a shaker at 140 rev./min for 30 min, which the upper layer was collected in a flask. The lower layer was further extracted with 32 mL ethanol-hexane (4:3, v/v) and shaken for 30 min. Again, the upper layer was collected in the same flask. The lower layer was repeatedly extracted with 15 mL hexane and shaken for 20 min, followed by addition of 5 mL hexane and the solution was homogenized by a polytron (PT-3000, KINEMATICA AG, Switzerland) at 12,000 rpm for 5 min. The mixture was filtered through Whatman No.1, and the filtrates were combined and poured into the same flask. Then, 150 mL distilled water and 100 mL 10% NaCl solution were added to the filtrate for partition, and the upper phase was also collected. The lower layer was again extracted with 20 mL hexane. All the filtrates were pooled and evaporated to dryness under vaccum. The residue was dissolved in 1 mL methylene chloride and filtered through a 0.2 μm membrane filter for HPLC [Model L-5000 LC equipped with a Model L-4000 spectrophotometer and a Model D-2000 chromato-integrator (Hitachi Ltd., Japan)] analysis with a YMC C30 column (250×4.6 mm E.D, 5μm particle; Tokyo, Japan). The injection volume was 20 μL. A gradient mobile phase of 1-butanol/acetonitrile (30:70, v/v) (A) and methylene chloride (B) was used: 99% A and 1% B initially, increased to 4% B in 20 min, 10% B in 50 min and returned to 1% B in 55 min. The detection wavelength was 476 nm and the flow rate was 2.0 mL/min.

2.7 Viscosity

The viscosity of tomato juice was studied using a Brookfield viscometer, springle # 4 at 10 rpm, 25℃ and only the 10th round readings were recorded (mPa・s) (Oke, Ahn, Schofield, & Paliyath, 2005).

2.8 PME activity (Anthon et al., 2002)

A 30-mL aliquot of a solution containing 0.2 M NaCl and 1.0 % pectin was equilibrated and adjusted to pH 7.0. Following the addition of 1.0 mL of the tomato juice, the pH was readjusted to 7.0 and maintained at this pH for 10 min by the addition of either 0.05 or 0.005 N NaOH, depending on the activity of the sample. The rate was calculated as μmol of NaOH consumption by the control being boiled for 20 min was subtracted as a blank. All activities of tomato juice samples are reported as the percentage of the activities of the control.

2.9 PG activity (Anthon et al., 2002; Fachin et al., 2003; Pressey, 1986)

Five mL of tomato juice was centrifuged at 7,500 g for 10 min, the supernatant was replaced by cold distilled water (1:1) adjusted the pH to 3.0 with 0.1 M HCl and mixed for 30 min. After centrifuging at 9,000 g for 20 min, the supernatant was removed and PG was extracted from the pellets with 1.2 M NaCl (1:1) for 1 h. The mixture was centrifuged at 18,200 g for 10 min and the supernatant was assayed for PG activity. All steps were performed at 4℃.

The PG activity assay was based on the release of reducing groups produced by PG and measured using a spectrophotometric method. 0.1 mL of the extracted enzyme solution was incubated with 0.3 mL of 0.2% polygalacturonic acid at 35℃ for 10 min. To terminate the reaction, 2 mL of 0.1 M borate buffer (pH 9.0) and 0.4 mL of 1% cyanoacetamide were added to the reaction mixture and boiled for 10 min. After cooling, the absorbance was measured at 276 nm and room temperature. Blank samples were determined in the same way with the control being boiled for 20 min. Each sample was measured in duplicate. All activities of tomato juice samples are reported as the percentage of the activities of the control.

2.10 Scavenging effect on DPPH˙ radical (Sánchez-Moreno et al., 2006a; Sánchez-Moreno, Plaza, de Ancos, & Cano, 2003a)

Two fractions (hydrophilic and hydrophobic fractions) were prepared from tomato juices and

used in the antioxidant assay. Each tomato juice sample (30 g) were extracted with 10 ml of sodium phosphate buffer (0.1 M, pH 3.0) and centrifuged at 12,000 g for 20 min at 4℃. The pellet was homogenized with 20 mL of sodium phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 g for 15 min at 4℃. Supernatants were combined to yield the hydrophilic fraction. The pellet was then extracted with 20 mL of tetrahydrofuran (THF; Sigma Chemical Co., St. Louis, MO, USA) three times and centrifuged at 10,000 g for 10 min at 4℃. Supernatants were combined to yield the hydrophobic fraction. The solvent was evaporated to dryness and the organic residue was dissolved in 3 mL of a Tween 20 solution (10% THF). An aliquot of sample fraction (0.1 mL) with appropriate dilution was added to 3.9 mL of DPPH· (3.0×10^{-2} g/L, Sigma Chemical Co., St. Louis, MO, USA) in methanol. The decrease in absorbance was determined by a spectrophotometer (Hitachi U-2000, Japan) at 515 nm at 0.5 min intervals until the reaction reached a plateau (time at the steady state). A calibration curve at 515 nm was made with DPPH· to calculate the DPPH· concentration in the reaction medium.

The parameters EC_{50}, which reflects 50% depletion of the initial DPPH· and the time needed to reach the steady state at EC_{50} concentration (T_{EC50}) were calculated. The antiradical efficiency ($AE = 1/EC_{50}T_{EC50}$), a parameter that combines both factors, was also calculated (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998).

2.11 Statistical analysis

The Statistical Analysis System (SAS Institute Inc., Cary, N.C., USA) was adopted to performed data analysis and statistical computations for analysis of variance (ANOVA) and Duncan's test. Significance of differences was defined at $p \leqq 0.05$. The differences among treatments were verified by their least significant difference. Experiments were conducted in triplicate.

3. Results and discussion

3.1 Color

Effects of thermal and pressure processing on the red-yellow ratio, indicating the redness of tomato juices, are shown in Figure 1. An a/b ratio of 1.90 or greater represents a first quality product in terms of color and an a/b ratio of less than 1.80 means that the tomato products may be unacceptable for inclusion in products where a bright red color is desired (Hayes et al., 1998). The a/b value of the control was 3.62 and appreciated more than that of hot-break tomato juice of 3.30 ($p \leqq 0.05$) and cold-break tomato juice of 3.54. The result in a low a/b value represented an orange to brown color due to the breakdown of lycopene and formation of Maillard reaction products by the intensive heat treatment (Shi & Le Maguer, 2000; Krebbers, Matser, Hoogerwerf, Moezelaar, Momassen, & Van den Berg, 2003). All the high pressure treatments except of 100 MPa/50℃ were appreciated more than control and both thermal treatments on the colors of tomato juices. And a/b values of tomato juice increased up to 3.84 with pressure levels elevated to 500 MPa. Results agree that an increase in the red color (a value; data not shown) of high pressure treated tomato juice compared to thermal treatments, attributed to the better homogenization and brightening of the red color (Porretta, Birzi, Ghizzoni, & Vicini, 1995; Krebbers et al., 2003). However, contradictory results can be found in literature about the effect of high pressure on fruits and vegetables. Combined high pressure and thermal treatments at 300-700 MPa/65℃ for 60 min did not significantly change the L*a*/b* parameter to tomato puree (Rodrigo, Cortés, Clynen, Schoofs, van Loey, & Hendrickx, 2006). This might be due to the different parameters and processing conditions being used in different literature.

3.2 Carotenoids and lycopene

The total carotenoids and lycopene contents of control are 212.8 and 145.6 μg/g, respectively (data not shown), which are higher than those of tomato (*Tau-Tai Lan T93*) juices for about 20% probably due to different cultivars (Lin & Chen, 2003). After the cold- and hot-break, both total carotenoids and lycopene contents of tomato juices slightly but insignificantly decreased about 1%

($p > 0.05$) (Figure 2). The positive effect of temperature on the extractability of lycopene is described in the literature, this effect being time-depending (Porrini, Riso, & Testolin, 1998). Changes in lycopene concentration in tomato puree have been shown at 90℃/110 min, and 110℃/1.1 min but not at 120℃/0.1 min (Anese et al., 2002).

This is the first time that a comparison between the impact of high-pressurized tomato juices and tomato juices processed by traditional technologies on carotenoids has been carried out. After high pressure processing beyond 300 MPa at 4 and 25℃, both total carotenoids and lycopene contents significantly increased up to 62% and 56%, respectively, as compared with control (Figure 2). It has been reported that high pressure treatment (500 MPa/20℃/2 min) increased the lycopene content of tomato puree compared with the raw puree (Krebbers et al., 2003). In addition, some researchers have shown increases in extractable carotenoids and lycopene as a result of high pressure treatment (400 MPa/25℃/15 min) of tomato puree (Sánchez-Moreno, Plaza, de Ancos, & Cano, 2006b). The result could explain the increased redness of tomato juice after high pressure treatments (Figure 1). However, high pressure treatments behind 200 MPa at 4 and 25℃ might only slightly modify the protein structure which is bound with carotenoids but could not induce the extraction of the pigments. Total carotenoids and lycopene contents of the tomato juices by high pressure treatments at 50℃ were much lower than those at either 4 or 25℃, probably owing to the preventing effect of pressure on protein thermal denaturation at denaturing temperatures (Heremans & Smeller, 1998). It has been reported that high pressure treatment can affect the membranes in vegetable cells (Shi & Maguer, 2000). In addition, carotenoids are tightly bound to macromolecules, in particular to protein and membrane lipids, and high pressure processing is known to affect macromolecular structures such as proteins and polymer carbohydrates (Gärtner, Stahl, & Sies, 1997).

3.3 Viscosity

The viscosity of fresh tomato juice (control) was 1,875 mPa・s and shown in Figure 3.

Hot-break juice had a significantly higher viscosity (1,986 mPa•s) than control and cold-break juice (1,547 mPa・s) ($p \leqq 0.05$). Some researchers reported that when using hot break method, the temperatures are high enough for pectolytic enzyme inactivation, and this leads to a concentrate of greater viscosity (Goodman et al., 2002; Fito, Clemente, & Sanz, 1983). In addition, break temperature influences the viscosity of tomato products by changing pectin retention (Xu, Shoemaker, & Luh, 1986), but this study eliminated that effect to show that viscosity differences are caused by enzyme activity.

High-pressure treatments at ambient temperature resulted in a more jelly-like, homogeneous structure of the tomato puree due to protein-tissue coagulation and compacting compared to thermal treatments (Porretta et al., 1995; Krebbers et al., 2003; Verlent, Hendrickx, Rovere, Moldenaers, & van Loey, 2006). Viscosity of tomato juice increased linearly with pressure level elevated from 100 to 500 MPa at various temperatures (4, 25 and 50℃), however, the viscosity loss occurred with the pressures at 100 and 200 MPa in comparison with control (Figure 3). 300-MPa treatments at various temperatures resulted in retention of the viscosity. 400- and 500-MPa treatments could improve (increase) the viscosity up to 20% probably due to PG inactivation, compacting effects, or protein-tissue coagulation (Krebbers et al., 2003). Some researchers showed contradictory results that the highest loss in consistency of the tomato homogenate after combined pressure-temperature treatment was found at 300 MPa at all temperatures tested (30-70℃) compared with those at the other pressure levels from 100 to 500 MPa (Verlent et al., 2006). They observed that tomato PME was very active in presence of tomato PG at pressure up to 300 MPa. PME creates a good substrate for PG, which also has a sufficient high activity at 300 MPa. Moreover, high-pressure sterilization (700 MPa/80 or 90℃) of tomato puree brought about a considerable reduction in viscosity may be due to the relative long preheating time applied to reach the starting temperature (80 or 90℃) for pressurization (Krebbers et al., 2003). The results in the loss of viscosity of tomato juice also showed that the treating pressure levels behind 200 MPa could not inactivate both PME and PG.

3.4 PME and PG activity

Effect of the thermal and high pressure treatments on the PME and PG activities in tomato juices were shown in Figure 4. After the cold-break processing, PME and PG activities decreased 30% and 12%, respectively, meanwhile, the residual activities of the both enzymes were lower than 2%. Thermal inactivation of the both enzymes in tomato juices were investigated in some literature. PG in tomato juice (at natural pH value) was completely inactivated by thermal treatment at 93℃ for 3 min (Fachin et al., 2003), moreover, at the temperature ranged from 55-60℃, the residual PG activity was 60-80% due to the presence of heat stable PG1. PME in tomato juice (pH 4.2) was almost completely inactivated by thermal treatment at 88℃ for 20 s, and the residual activity was about 30% after a treatment at 73℃ for 80 s (De Sio et al., 1995).

After the high pressure treatments at 100 MPa and all temperatures, the residual activities of PME unchanged or insignificantly reduced compared with control (Figure 4). The initial activity was reduced to 27.8% using the treatment of 200 MPa at 25℃, and this combination was the most efficient in terms of PME inactivation. The higher efficiency of low-pressure/mild-temperature treatments on tomato puree PME was also reported (Hernández & Cano, 1998). Several studies have pointed out that polymeric proteins, stabilized by non-covalent bonds, are dissociated at low pressures (Balny & Masson, 1993). However, an activation effects were observed in the cases of higher pressure (beyond 300 MPa) treatments at all temperatures in this study, could be attributed to reversible configuration and/or conformation changes of the enzyme and/or substrate molecules (Ogawa, Fukuhisa, Kubo, & Fukumoto, 1990). Maximal PME activity was observed at 300 MPa and 50℃ and almost 1.7 times as control.

PG activity was strongly reduced up to 90% by the high pressure treatments beyond 400 MPa at ambient and low temperatures (25 and 4℃). However, pressure from 100-300 MPa had slight or insignificant effects on inactivation of PG up to 14%, demonstrating the pressure resistance of PG (Krebbers et al., 2003). At 50℃, PG was more resistant to high pressure treatments probably

due to reversible configuration of the enzyme, and the result was confirmed in literature (Fachin et al., 2003). The increased activity of PME and the inactivation of PG may to some extent be an explanation for the observed increase in viscosity compared to control and thermal treating juices. However, this can not explain the tendency of increased viscosity at higher pressures. The similar results were reported in literature (Krebbers et al., 2003).

3.5 Scavenging effect on DPPH· radical

The scavenging effect on DPPH· radical measurement can estimate the capacity of the most reactive compounds against a reference radical (Anese et al., 2002), therefore, we adopted this method instead of other measurements of antioxidant activity, such as redox potential. In tomato products, vitamin C and polyphenols (flavonolids and hydroxycinnamic acids) are reported to be the major antioxidant hydrophilic components, and vitamin E and carotenoids mainly constitute the hydrophobic fraction (Takeoka, Dao, Flessa, Gillespie, Jewell, Huebner, Bertow, & Ebeler, 2001; Martínez-Valverde, Periago, Provan, & Chesson, 2002). The radical-scavenging capacities of hydrophilic and hydrophobic fractions of tomato juices by different treatments were respectively evaluated (Figure 5a and b). Until now, no data have been available about the radical-scavenging capacity of tomato juices by high-pressure/temperature combinations.

3.6 Hydrophilic fraction

The EC_{50} and T_{EC50} values of the hydrophilic fraction of control were 68.2 g/g DPPH· and 20.8 min, respectively (Figure 5a). After hot- and cold-break treatments, both EC_{50} and T_{EC50} values significantly increased ($p \leqq 0.05$) due to the depletion in vitamin C (Dewanto, Wu, Adom, & Liu, 2002; Sánchez-Moreno et al., 2006b). Thermal processing at 88℃ for 2, 15 and 30 min decreased the vitamin C content in tomatoes, however, there was no loss or gain in content of both total phenolics and flavonoids (Dewanto et al., 2002). The results showed that the radical-scavenging capacity of tomato juice decreased by thermal treatments at 60 and 92℃ for 2

min.

Pressure processing beyond 300 MPa at 4 and 25℃ maintained the radical-scavenging capacity of tomato juice compared with control due to the values of EC_{50}, T_{EC50} and AE with no significant differences ($p > 0.05$). As the pressures were behind 200 MPa at 4℃ only, the radical-scavenging capacity of tomato juices were unchanged ($p > 0.05$). Pressure processing (100-500 MPa) at 50℃ significantly decreased the AE values of tomato juices, which indicated that the loss of radical-scavenging capacity was mainly due to the high temperature treatment. Previous studies in orange juices showed that the depletion of vitamin C after combined treatment of high-pressure/temperature was dependent mainly on temperature intensity, showing losses after 400 MPa/40℃/1 min, but not after 350 MPa/30℃/2.5 min (Sánchez-Moreno, Plaza, de Ancos, & Cano, 2003b). The results in this study showed that pressures beyond 300 MPa at 4 and 25℃ had positively protective effect on the radical-scavenging capacity of tomato juices, and those are in agreement with Fernandez Garcia et al. (Fernandez Garcia, Butz, & Tauscher, 2001), who reported that the water-soluble antioxidative capacity [2,2'-azino-bis-(ethylbenzothiazoline-6-sulfonic acid) diammonium salt; $ABTS^+$ assay] of tomato puree processed by high-pressure treatments (500 and 800 MPa/20℃/5 min) was not, or only insignificantly, reduced compared to that of untreated puree. On the contrary, probably due to the different tomato cultivars, a high-pressure treatment (400 MPa/25℃/15 min) applied to tomato puree resulted in decreases of vitamin C content and AE value compared with the untreated puree (Sánchez-Moreno et al., 2006b).

3.7 Hydrophobic fraction

The EC_{50} and T_{EC50} values of the hydrophobic fraction of control were 364 g/g $DPPH^{\cdot}$ and 39.0 min, respectively (Figure 5b). After the cold-break treatment, both EC_{50} and T_{EC50} values significantly increased 30% and 17%, respectively ($p \leqq 0.05$). The hot-break treatment induced a decrease of EC_{50} value, meanwhile T_{EC50} value was unchanged ($p > 0.05$). Some authors have

found that lycopene is the most important compound in the hydrophobic fraction in tomatoes in the ABTS/H_2O_2/HRP and DPPH· radical scavenging systems (Cano, Acosta, & Arnao, 2003; Sánchez-Moreno et al., 2006b).

Pressure processing behind 300 MPa at 4 and 25℃ maintained the radical-scavenging capacity of tomato juice compared with control due to the values of EC_{50}, T_{EC50} and AE with no significant differences ($p > 0.05$). As the pressures were beyond 300 MPa at 4 and 25℃, the AE values of tomato juices significantly increased up to 21% ($p \leqq 0.05$). Pressure processing (100-500 MPa) at 50℃ significantly decreased the AE values of tomato juices compared with that at lower temperatures (4 and 25℃), which indicated that the loss of radical-scavenging capacity was mainly due to the high temperature treatment.

4. Conclusion

Based on the extraction of carotenoids and lycopene, radical-scavenging capacity and inactivation of PME and PG of tomato juice, high pressure processing can be an alternative for hot- or cold-break processing. High pressure processing at 4 and 25℃ has almost the same effects on all the processing qualities of tomato juice, however, that at 50℃ has less efficiency or even contrary effects. Besides PME and PG inactivation, a 300-MPa treatment improves the extractable carotenoids and lycopene contents and retains the other properties; moreover, pressure treatments at 400 and 500 MPa can improve all the quality attributes in this study. Therefore, a 500-MPa treatment at ambient temperature can be useful in processing tomato juice in considering the inactivation of the enzymes.

This opens new perspectives to produce improved processing qualities of tomato juice products as an alternative for hot- and cold-break processes. The applied conditions require further optimization to mild pressure/temperature combinations, assuring inactivation of microbial and optimal flavor for storage.

Acknowledgments

This study was financially supported by National Science Council, ROC, No. NSC 94-2313-B-040-005.

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