
NEW MODIFIED COTTON FIBER APPLY TO SEPARATE ECG AND EGCG FROM TEA EXTRACT

Tu Ngoc Thach, Pham Thanh Quan, Tong Thanh Danh

University of Technology, VNU-HCM

(Manuscript Received on June 24th, 2010, Manuscript Revised November 01st, 2010)

ABSTRACT: *New technique to modify cotton fiber by esterification with citric acid was developed. The reaction was carried out in the tight flask and applied argon as anti-burning reagent. The optimum condition was 170°C, 5h and 3g of citric acid per 2g of cotton fiber, which result was up to 0.88 mol citric acid grafted on 1 mol glucose. The modified cotton fiber thereafter was applied to purify epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) from green tea polyphenol by column chromatography with the suitable mobile phase.*

Keywords: *ECG, EGCG, tea extract.*

1. INTRODUCTION

Cellulose ion exchange materials are created primarily by attaching a functional groups on different cellulose structure by chemical method such as esterification [1,2,3], etherification [4] or rely on free radical grafting reactions of various monomers on cellulose structure [5,6,7], cellulose materials after processing are required to retain its fiber structure and creates products insoluble or not to be excessive expansion in the various solvent.

Although ionic exchange properties of this material is like other ion exchange resins, but it has some special characteristics that ion exchange resins do not have such as they are very fine with open structure, the porous systems are very different in size so cationic cellulose has a surface area higher than the normal ion exchange resins.

Thanks to the properties above the cationic cellulose have capacity as well as the ion exchange rate higher than the other ion exchange resins and therefore it is proper for chromatography process, in addition to the open structure systems and different porous size allows such a great molecules as protein, enzymes can go into the adsorption site and was thereby able to separate of these compounds [8]. Another reason make it more favorable to particular application is easy to regenerate [9].

EGCG is the most valuable component in tea polyphenol for its pharmaceutical property [10], until now there are various method to separate polyphenol from tea extract [11,12,13] but separation EGCG from tea polyphenol is difficult so there are some HPLC method for analysis [14,15] and separation [16] and there are no industrial process to purify this component from abundance tea polyphenol so

isolation EGCG from the other compound in the mass production is necessary.

2. MATERIALS AND METHOD

2.1. Preparation of cotton

Rude cotton fiber was sank within 18h in solution of alcohol 75% (each 80ml of alcohol contains 0.3ml of H₂SO₄ 98%), the ratio between cotton fiber and solution was 1/20. After treatment, 2g of cotton fiber was sunk in solution of 50ml citric acid which had the concentration depend on condition of investigation. After 1 hour of sinking for completely penetration, the mixer of cotton fiber and solution of citric acid was vaporized almost water at 70°C. After vaporization, cotton fiber was putted into double neck flask for esterification. The investigated parameters were temperature, time, and different dose of citric acid, temperature was controlled indirectly through the temperature of batch, tight system and argon were pumped into to limit natural burning reaction of cotton fibers.

Cotton fiber after reaction was washed out of citric acid with 500ml of water, drying, weighing, and measuring moisture, respectively then continual washing with alcohol 96% in soxhlet extractor within 8h.

2.2. Preparation of tea extract

Tea after extraction with alcohol 60% was vaporized out of alcohol and cooled to remove sediment. The water phase thereafter was extracted with dichloromethane to remove caffeine. Raffinate was extracted with ethyl acetate to get polyphenol. Ethyl acetate layer

after extraction was dried with Na₂SO₄ and allowed to adsorb onto neutralized modified cotton fiber (NMCF), continuing process was vaporized out of solvent, then the dried NMCF was added to the column and washing with suitable cooperation solvent.

2.3. Analysis

Result of esterification was evaluated by titration of modified cotton fiber (MCF), in this process 0.5g of MCF was orderly added 20ml of NaOH 0.09M, 100ml of distillation water. The mixture thereafter was stirred 60 minutes and titrated every 10 minutes with H₂SO₄ 0.02N until got the same results at 2 times of titration.

Scanning Electron Microscopy (SEM) was analyzed at Institute of Chemistry, the Vietnamese Academy of Science and Technology.

ECG and EGCG was analyzed by HPLC-MS and HPLC-UV at High-Tech Analysis Center Hoan Vu (column XBD C18-150 mm, ID 4.6 mm, H₂O:CH₃CN, flow rate 0.7 ml/min, $\lambda = 280$ nm).

2.4. Result and discussion

2.4.1 Results of esterification respect to temperature

Investigation was carried out orderly at 120°C, 130°C, 140°C, 150°C, 160°C, 170°C with the fixed time, citric acid:cotton fiber (Figure 1). The reaction time and citric acid:cotton was chosen at 420 minutes and 4:2 (g/g) respectively (all of experiments were carried out in double flask 500ml sinking ½ volume in batch of glycerin).

Investigated temperature only carried out up to 170°C due to higher temperature could cause decomposition. From the Figure 2, at 120°C, consumed NaOH was 0.001465 (mol) but at 170°C NaOH consumed up to 0.002627. At low temperature (lower than 150°C), consumed NaOH increased fast and linearly, when temperature transfer from 140°C to

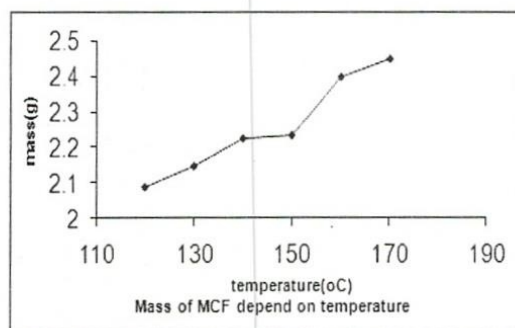


Figure 1. Change of mass with temperature

The consumed NaOH was signal to measure the efficiency of esterification so from the number above we could conclude that productivity of esterification increased when apply higher temperature. The mass showed large difference due to the reaction was promoted by temperature which resulted higher grafted citric acid onto cellulose structure. Higher temperature caused a decrease of difference mass which could easily explain by burning reaction of cellulose, the difference of consumed NaOH was also high due to more effective of grafting. It was also showed that when temperature changed from 140°C to 160°C the efficiency of esterification did not show much differently.

Higher temperature caused increase of esterification because the melting point of citric acid is 153°C so citric acid could deeply

penetrate into cellulose structure. The mass also increased when applied higher temperature, when temperature changed from 120°C to 170°C, the difference of mass (g) was 0.350846g. Difference of mass increased when temperature was changed from 120°C to 160°C but it showed decrease from 160°C to 170°C. From 140°C to 150°C and 150°C to 160°C, difference of mass was 0.009679g and 0.169705g but difference of consumed NaOH was 0.000154mol and 0.0000382mol respectively.

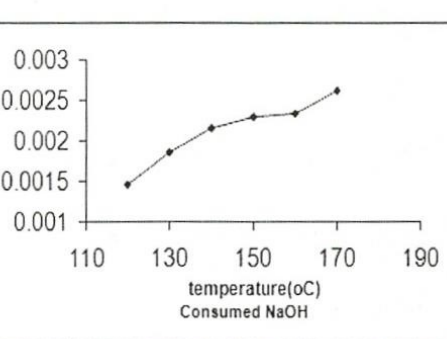


Figure 2. Mol of consumed NaOH to neutralized 1g MCF

In Figure 3, from 150°C to 160°C, large change of mass and very small change of consumed NaOH was recorded so at low temperature, the esterification only occurred at one acidic group of citric acid. Comparing the difference of consumed NaOH when temperature changed from 140°C to 150°C and

160°C, the difference of consumed NaOH was only 0.000192 mol which was so low compared to 0.000689 mol when changed from 120°C to 140°C. Continuing increasing of temperature from 160°C to 170°C caused the big change of consumed NaOH that is 0.000281 mol of citric acid.

150°C to 160°C, we concluded that there was change from one acidic group of esterification to two acidic group of esterification at this range of temperature. If we assumed that one acidic groups of citric acid was esterified at the

temperature lower than 160°C and two acidic groups of citric acid was esterified at the temperature upper than 160°C, the degree of esterification (mol of grafted citric acid per mol of glucose) would be calculated.

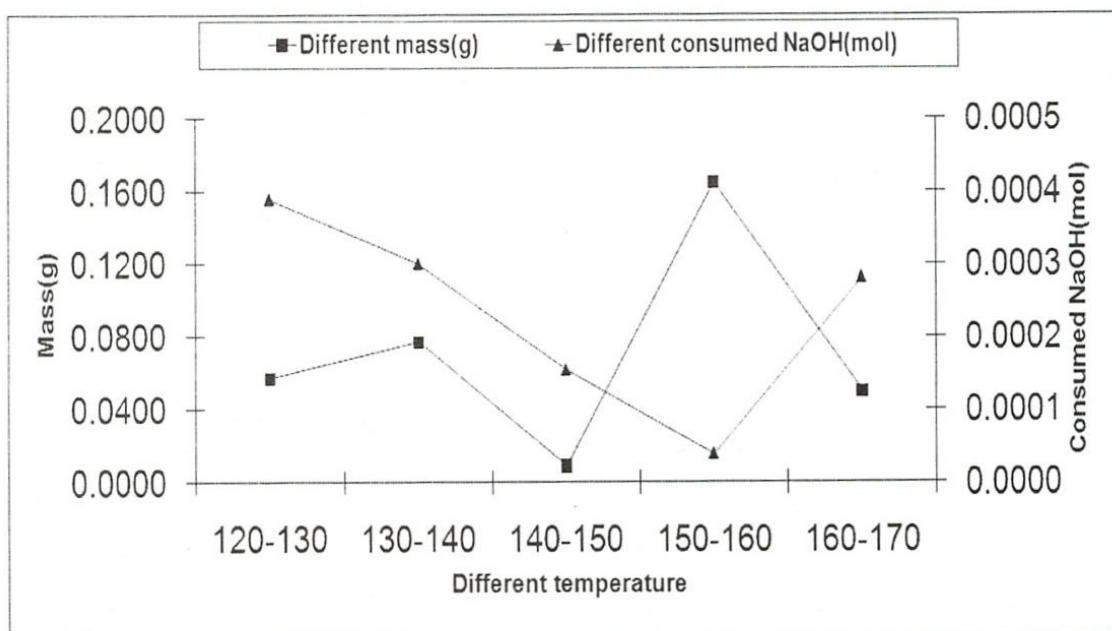


Figure 3. Difference of mass and consumed NaOH with different temperature.

2.4.2 Results of esterification respect to time

Esterification was carried out with different time which were 3h, 4h, 5h, 6h, 7h,

set temperature was 170°C and ratio between cotton fiber and citric acid was 4:2(g/g) (all of experiments were carried out in double flask 500ml sinking ½ volume in batch of glycerin).

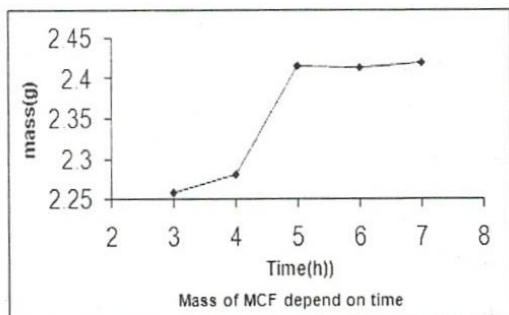


Figure 4. Change of mass with time of esterification

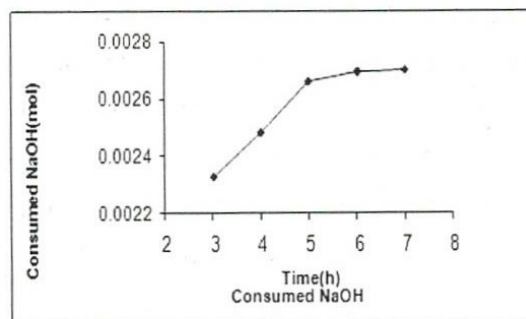


Figure 5. Mol of NaoH to neutralize 1 g MCF

From Figure 4 and Figure 5, we see that from the starting time to 3h, the difference of mass and difference of consumed NaOH were 0.08615g per hour and 0.0007766mol per hour that was too high comparing to difference of mass and difference of consumed NaOH when transferred from 3h to 4h, which were only 0.02274725g and 0.000152576mol. From 4h to 5h, difference of mass is high comparing to the others (0.13481575g) but difference of consumed NaOH (0.000182038mol/g) is little higher comparing to difference of consumed NaOH when transferred from 3h to 4h. Due to mass and consumed NaOH was a measurement of esterification so from the figure above, longer esterified time caused increase of esterified efficiency, this trend showed clearly when transferred from 3h to 5h but nearly standstill after 5h.

In Figure 3, the different mass and consumed NaOH in range time from 0h to 3h was higher than from 3h to 4h, these results suggested that in range time from 0h to 3h, only one acidic group of citric acid was esterified. When time transferred from 3h to 4h, the remained groups of citric acid was esterified but with a small grafting quantity, that caused a slight change of mass and

consumed NaOH. In the range time from 4h to 5h, the above trend was continuing with quick increase of mass and consumed NaOH, which showed a large amount of citric acid was esterified. Changeable speed of mass was quicker comparing to the ranged time from 3h to 4h, consumed NaOH also showed a big change but with the same speed from 3h to 4h. From this point of view, we could conclude that the in ranged time from 4h to 5h, the esterification almost occurred at two acidic group of citric acid. For explanation, from 0h to 3h, there were a lot of active sites on cellulose structure so it is easy for citric acid to graft on cellulose structure. The mass and consumed NaOH did not show much change when time of reaction reached 5h due to almost active sites on cellulose structure was occupied, another reasons are citric acid which in the liquid form could gradually vaporize and stuck on the wall of flask or the slowly decomposition of citric acid so the productivity did not improve clearly after 5h. From the table if we accepted that only one group of citric acid was esterified at temperature lower than 5h and both group of citric acid was esterified at the temperature higher 5h, the degree of esterification could relatively calculate

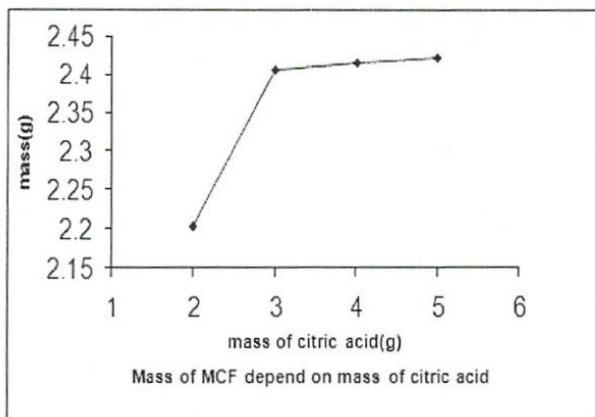


Figure 6. Change of mass with time of citric acid

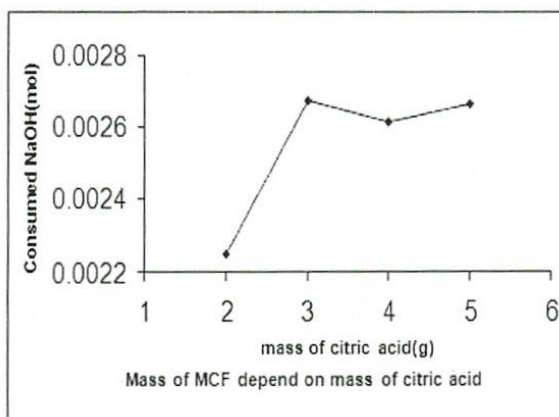


Figure 7. Mol of NaOH to neutralize 1 g MCF

From the Figure 6 and Figure 7, increasing of citric acid caused an increase of mass and consumed NaOH. Increasing of used citric acid from 2g to 3g caused a significant increase of mass and consumed NaOH (which was 0.2027g and 0.000421013 mol) but the mass seemed to be standstill when mass of citric acid reached the value of 3g per 2g of cotton fiber. For explanation, when mass of citric acid reached 3g, the productivity of reaction did not show increase due to almost active sites on cellulose structure was occupied and a large

amount of citric acid gradually vaporized and crystallized on the wall of flask which caused the lost of citric acid, another reason could be named was the lost of citric acid because of decomposition at high temperature.

Due to temperature and time of reaction were high, we assumed that the esterification occurred at two acidic group of citric acid and from the figure above, the yields of esterification could be calculated (Figure 8). The suitable condition was chosen at 3g of citric acid per 2g of cotton fiber.

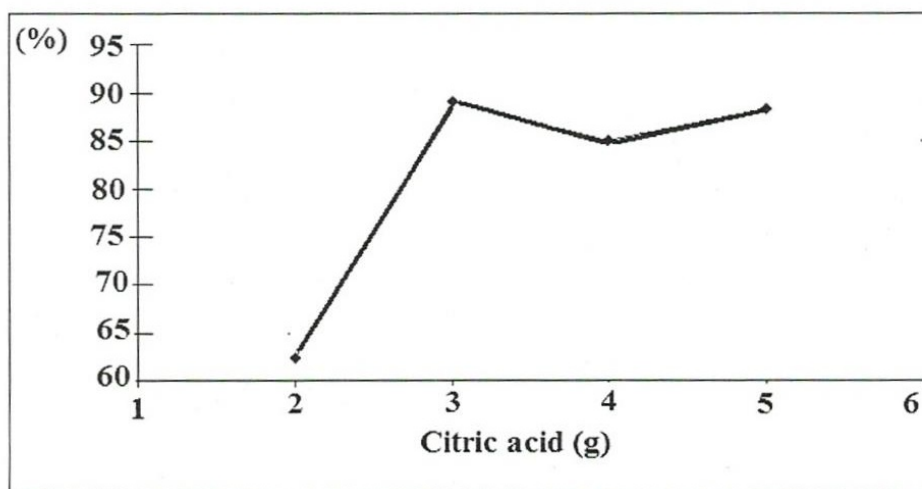
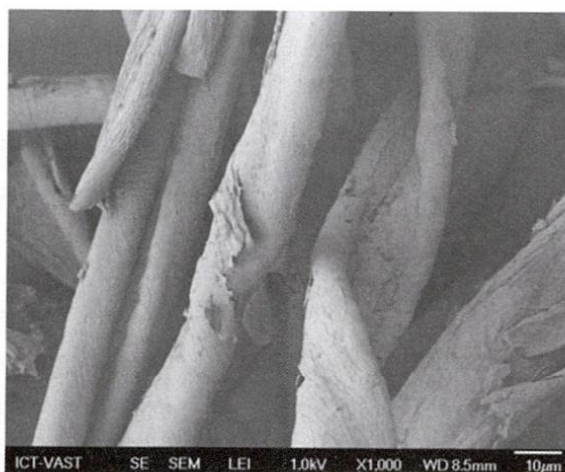
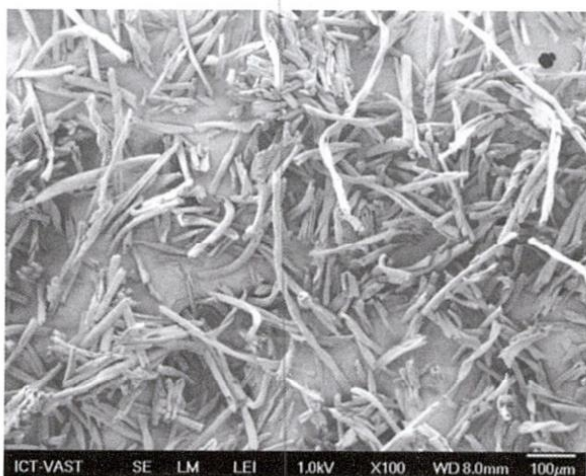


Figure 8. The yields of esterification with mass of citric acid

2.4.3. Property analysis of MCF

2.4.3.1 SEM analysis

The picture below showed that MCF is a oval rope with the diameter about 20 μm , the distribution of length is pretty wide from tens to several hundred μm . The surface of this fiber is rugged and there are also a lot of scratches points especially at the starting and ending. The



Picture 1. SEM with magnification of 100 and 1000.

2.4.3.2 HPLC-MS analysis of ECG

HPLC-MS was carried out to define the components in this fraction and specially identify ECG, the result was showed 4 big peaks, the peak at retention time 12.61 is the most intensive. we encoded the peak at 10.65, 12.06, 12.61, 12.94 as peak 1, 2, 3, 4 respectively for further convenience. In MS analysis, the most intensive peak is 441 which is the main part of ECG so the peak at retention time 12.61 is peak of ECG. Found out both MS is likely but the retention time is very different, which could conclude that they are possible the optical isomers. The main peaks in

MS are 209.08 and 209.09 which suggested as catechin and epicatechin. The MS of Peak 4 is unknown components, the comparing with three MS above was carried out which showed some peak with the same position. The peak 335.20 in MS suggested the some transformation stage was the same in ionization process of three components. From the comparing, the peak at retention time 12.94 is possible a derivative of catechin. The HPLC showed that this fraction mainly contained ECG and a low concentration of the other catechins.

2.4.3.2 HPLC analyze the purification of EGCG

The only one and sharpened peak in HPLC-UV showed that EGCG was separated in the pretty pure form.

3. CONCLUSION

The suitable condition to remove lignin and impurities: 0.3ml H₂SO₄ 98% per 80 ml of

alcohol 75% per 4 g of cotton fiber and 18 h of reaction. The suitable condition for esterification: 170°C, 5h and 3g of citric acid per 2g of cotton fiber. Apply MCF to separate ECG and EGCG from tea polyphenol: percentage of EGCG up to 78.4%.

NGHIÊN CỨU TÁCH CHIẾT ECG VÀ EGCG TỪ TRÀ BẰNG SỢI BÔNG

Từ Ngọc Thạch, Phạm Thành Quân, Tống Thanh Danh

Trường Đại Học Bách Khoa, ĐHQG-HCM

TÓM TẮT: Phát triển các kỹ thuật mới để hoạt hóa sợi bông bằng phản ứng este hóa với axit citric, phản ứng đã được thực hiện trong các bình kín với khí hiếm Argon. Các điều kiện tối ưu là 170 °C, 5 giờ với tỷ lệ là 3 g của acid citric với mỗi 2 g chất xơ bông và có thể nâng lên mức 0,88 mol acid citric trên 1 mol glucose. Các sợi bông sau khi hoạt hóa đã được áp dụng để chiết tách epigallocatechin gallate (EGCG) và epicatechin gallate (ECG) từ polyphenol trong trà xanh bằng sắc ký cột với các pha động phù hợp. Kết quả phân tích HPLC cho thấy chỉ có một đỉnh cao của EGCG.

Từ khóa: ECG, EGCG, chiết tách trà.

REFERENCES

- [1].Parab Harshala, Joshi Shreeram, Shenoy Niyoti, Lali Arvind, SARMA Sarma U. S., Sudersanan M., *Esterified coir pith as an adsorbent for the removal of Co(II) from aqueous solution*, Bioresource Technology, Vol.99, 2083-2086, (2008).
- [2].Thái, N.H.V., *Chế tạo và sử dụng lignocellulose biến tính để tách caxi, magie, mangan trong xử lý nước cấp*, Luận văn tốt nghiệp cao học trường đại học Bách Khoa Đại Học Quốc Gia HCM, (2008).
- [3].Lê Thanh Hung, Phạm Thành Quân, Lê Minh Tâm, Nguyễn Xuân Thom, *Nghiên cứu khả năng hấp phụ và trao đổi ion của xơ dừa và vỏ trấu biến tính*, Tạp chí phát triển Khoa học & Công nghệ, Đại học Quốc gia TP HCM, tập 11, 5-12, (2008).
- [4].David William O'Connell, Thomas Francis O'Dwyer, *Heavy metal adsorbents prepared from the modification of*

- cellulose: A review*, Bioresource Technology, Vol.99, 6709-6724, (2008).
- [5]. Hesham H. Sokker, Sayed M. Badawy, Ehab M. Zayed, Faten A. Nour Eldien, Ahmad M. Farag, *Radiation-induced grafting of glycidyl methacrylate onto cotton fabric waste and its modification for anchoring hazardous wastes from their solutions*, Journal of Hazardous Materials, 168(1):137-44, (2009).
- [6]. T.S. Anirudhan, L.Divya, M. Ramachandran, *Mercury (II) removal from aqueous solutions and wastewaters using a novel cation exchanger derived from coconut coir pith and its recovery*, Journal of Hazardous Materials, Vol. 157, 620-627, (2008).
- [7]. T.S. Anirudhan, P.G. Radhakrishnan, *Improved performance of a biomaterial-based cation exchanger for the adsorption of uranium(VI) from water and nuclear industry wastewater*, Environmental Radioactivity, Vol.100, 250-257, (2009).
- [8]. S. V. Wal and J. F. K. Huber, *High-pressure liquid chromatography with ion-exchange cellulose and its application to the separation of estrogen glucuronides*, Journal of Chromatography, Vol.102, 353-374, (1974).
- [9]. Fadhel Aloulou, Sami Boufia, Jalel Labidi, *Modified cellulose fibres for adsorption of organic compound in aqueous solution*, Separation and Purification Technology, Vol.52, 332-342, (2006).
- [10]. Ha, N. H. Nghiên cứu trích ly Polyphenol từ trà *Camellia sinensis* (L.), *Luận văn tốt nghiệp cao học trường đại học Bách Khoa Đại Học Quốc Gia Tp.HCM*, (2006).
- [11]. Hyong Seok Park, Hee Jin Lee, Min Hye Shin, Kwang-Won Lee, Hojoung Lee, Young-Suk Kim, Kwang Ok Kim, Kyoung Heon Kim, *Effects of cosolvents on the decaffeination of green tea by supercritical carbon dioxide*, Food Chemistry, Vol.105, 1011-1017, (2007).
- [12]. Huiling Liang, Yuerong Liang, Junjie Dong, Jianliang Lu, Hairong Xu, Hui Wang. *Decaffeination of fresh green tea leaf (Camellia sinensis) by hot water treatment*, Food Chemistry, Vol.101, 1451-1456, (2006).
- [13]. Jian-Liang Lu, Ming-Yan Wu, Xiao-Li Yang, Zhan-Bo Dong, Jian-Hui Ye, Devajit Borthakur, Qing-Lei Sun, Yue-Rong Liang, *Decaffeination of tea extracts by using poly (acrylamide-co-ethylene glycol dimethylacrylate) as adsorbent*, Journal of Food Engineering, Vol.97, 555-562, (2009).
- [14]. Bing Hu, Lin Wang, Bei Zhou, Xin Zhang, Yi Sun, Hong Ye, Liyan Zhao, Qiuhui Hu, Guoxiang Wang, Xiaoxiong Zeng, *Efficient procedure for isolating methylated catechins from green tea and effective simultaneous analysis of ten catechins, three purine alkaloids, and gallic acid in tea by high-performance liquid chromatography with diode array*

- detection*, Journal of Chromatography, 1216(15), 3223-31, (2009).
- [15]. Lihu Yao, Yueming Jiang, Nivedita Datta, Riantong Singanusong, Xu Liu, Jun Duan, Katherine Raymont, Alan Lisle, Ying Xu, *HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (Camellia sinensis) grown in Australia*, Food Chemistry, Vol.84, 253-263, (2004).
- [16]. Jun Xu, Tianwei Tan, Jan-Christer Janson, *One-step purification of epigallocatechin gallate from crude green tea extracts by mixed-mode adsorption chromatography on highly cross-linked agarose media*, Journal of Chromatography A, Vol.1169, 235-238, (2007).