LUND UNIVERSITY

Light stability of purple corn anthocyanins microencapsulated with different wall materials

by

CAI YANG

A thesis submitted in partial fulfillment for the degree of Master

in the

LTH

Food technology and nutrition

June 2018

Declaration of Authorship

I, CAI YANG, declare that this thesis titled, 'Evaluation of anthocyanin micro-capsules with diverse wall materials' and the work presented in it are my own. I confirm that:

- This work was done wholely or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: ______________________________

Date: ______________________________

"Sometimes you succeed.... and other times you learn."

Robert Kiyosaki

Popular science

The micro-encapsulation decreases the light stability of anthocyanins- a colorants from purple corn

Introduction

It was interesting to find that the micro-encapsulation, which is a process usually used to enhance the stability of materials, actual decrease the light stability rather than increase it!

Nowadays people are more and more desiring "perfect food", flavor, color, smell and healthy benefits are all in the requests. To fit these requests, natural colorants are widely used as they could offer blazing color and possibly some health benefits as well. Anthocyanin is one of the most typical natural colorants. It is the most widely distributed plant-based colorants in the nature. It gives nice color, purple or blue, and a lot of health benefits like anti-oxidation and anti-inflammation. However, anthocyanins are not stable and easily degrade during storage and transportation, which might cause color fading or color change. Thus, a lot of researches were done to find a increase the stability of anthocyanins. Micro-encapsulation is one of the rapidly developing method to increase the stability of materials. Micro-encapsulation is to protect the materials with wall materials and form a structure like egg in the molecular level. The wall materials protect the aiming materials like the eggshells protecting the egg from the outside environments. This work was firstly aiming at evaluating the protective ability of micro-capsules of anthocyanins with 3 different wall materials, based on the speculation that the micro-encapsulation will increase the light stability of anthocyanins with all 3 different wall materials. However, the results showed that instead of increasing the light stability of anthocyanins, micro-encapsulation actual decrease the light stability of anthocyanins for some reason. This research is interesting and meaningful as many researches proved that the micro-encapsulation increases the color stability of anthocyanins, this research shows that the mechanism of degrading under lights and color fading are different, further researches are needed to understand the mechanism of the degrading under lights and a more effective process needs to be discovered.

Acknowledgement

This thesis and its corresponding research work were conducted with the assistance from many people. First of all I must express my sincere gratitude to Professor Björn Bergenståhl to allow me to join his lab and work with him. He is a wise, earnest and humorous gentleman. During these 6 months, he always patiently encouraged me and helped me to get my ideas into shape with his endless knowledge. It was an enjoyable time to discuss with him, when he could always easily point out the key among perplexing data, help me to analysis all the possibilities and make everything lucid. Here I also want to thank Dr. Cecilia, who is my sub-supervisor. She is very nice and taught me a great deal. She encouraged me a lot while I was in trouble. I need to thank Dr. Atmas, who helped me a lot while Cecilia was in Bolivia. And I need to apologize to her as well because I always messed up the lab. I cannot thank Dan and Hans more for their assistance during my work. I would like to thank Professor Federico as well for his encouragements. I would not be possible to finish this thesis without the support of the department of food science and technology and Lund University, thanks a lot for all the equipment and lab access that the department and university gave to me. I also what to thank Nico in Architectural department who gave me the access to the polar lamp and my faithful friends Hongyu and Cui

yuzhao who helped me a lot with my experiments

In addition, I need to thank all my friends in Lund University who cared about me a lot and give me an unforgettable happy time.

Lastly, I want to thank my dear parents, who support me to study and live in a foreign country, and always care about me， calm me down when I was too stressful and motivate me up when I was upset. I love you!

Abstract

Anthocyanin, a subgroup of flavonoids, is one of the most extensively distributed plant-based components (Pedreschi R,2007). What impedes a broader industrial adoption of anthocyanins is its poor stability. Micro-encapsulation is one of the most popular techniques to protect the anthocyanins from oxidation. Micro-encapsules consist of a selected encapsulating material surrounding the anthocyanins. (Mahdavi S,2014) The objective of this work was to define a proper method to prove if the spray dried microencapsulated systems could increase the light stability of anthocyanins from purple corn. To achieve this, microcapsules of anthocyanins were obtained by spray drying anthocyanins with 3 different wall materials. The microstructure of the microcapsules was analyzed by SEM. Furthermore; a method for measuring the encapsulation efficiency was developed. Finally, the protective capacity or the wall materials under light exposure was investigated by measuring the degradation rate of the anthocyanins during 2 hours using HPLC analysis.. Conditions such as the time before injection, and the pH of the samples that could affect the absorbance of the anthohcyanins in HPLC analysis were assessed. This work finally found that the micro-encapsulation decreases the light stability of anthocyanins rather than increase, and a further research is needed to explain the mechanism behind this interesting phenomenon

Key words: Anthocyanin; micro-encapsulation; SEM; micro-encapsulation efficiency; HPLC; degrading rate

Table of Contents

Acknowledgement........ 1
Abstract........ 3
Table of Contents........ 4
1. Introduction........ 5
1.1 Food colorant........ 5
1.2 Anthocyanin........ 6
1.3 Micro-encapsulation........ 9
1.4 SEM........ 11
1.5 HPLC........ 11
2.1 Preparation of purple corn cob extraction........ 12
2.2 Micro-encapsulation formation........ 13
2.3 Micro-encapsulation Efficiency........ 13
2.4 SEM........ 15
2.5 HPLC........ 15
2.5.1 Degrading ratio inside the HPLC machine........ 16
2.5.2 Anthocyanin photo-degradation under low intensity light exposure........ 17
2.5.3 Anthocyanins photodegradation under high intensity light exposure........ 18
3. Result and discussion........ 19
3.1 Micro-encapsulation efficiency........ 19
3.2 SEM........ 21
3.3 HPLC........ 24
3.3.1 Degrading ratio inside the HPLC machine........ 30
3.3.2 Anthocyanin photo-degradation under low intensity light exposure........ 31
Conclusion........ 34
Appendix........ 36
References........ 37

1. Introduction

1.1 Food colorant

In the food industry today, color appearance is becoming one of the primary focuses for both the companies and the customers. As a characteristic to evaluate the overall quality of a food product, as well as flavor, color is somehow likely to act as “fingerprints”.(Yang W,2018). Compared to flavor, color is the first distinctiveness of food products that consumers use to evaluate standards of quality in most cases.

As a consequence, many colorant related studies have been made to improve the stability of food color or its “shelf life” as usually the colorants don’t have the desired stability and may easily fade. Furthermore, there is an increasing interest in the food industry on developing new products with more appealing attributes. This encourages more researches in improving existing colorants or introducing new ones.

The purpose of the food colorant is to try to preserve the natural color of the components of the food product before processing to make the final food appealing, appetizing and healthy. Also, the color of the food products are well-matched with its flavor (e.g. the color of orange juice should always be orange).

The food colorants fall into three categories (Henry, 1996): Natural colors, organic colorants that are directly extracted from natural sources (e.g. anthocyanins); natural identical colors, usually artificially synthesized colorants that could be found in nature as well (e.g. β-carotene), and synthetic colors, artificially synthesized colorants that do not exist in nature (e.g. carmoisine).

During the last decades, synthetic food colorants have been widely used due to their stability and cheapness. However, with the improvement of health consciousness, natural colors are being positioned in consumers' perceptions and demands as better alternatives to synthetic ones (Sigurdson G, 2017).

1.2 Anthocyanin

Mostly, the purple color of vegetables, flowers, and some cereal grains like purple corns are given by anthocyanins. Thus, anthocyanins are widely used as food colorants in food industry. Besides their coloring properties, anthocyanins have different health benefits such as antioxidant functions and anti-inflammation. Generally, anthocyanins are promising natural colorants and healthy food additives (Gould K,

2014).Anthocyanins extracted from purple corn have dark purple color as pure powders, and give bright and intense colors in low concentration solutions.

Purple corn is an abundant source of anthocyanins. . In Peru and Bolivia, and some other Latin American countries, there is a millennium-old history of purple corn cultivation (Pedreschi R, 2007). Many traditional drinks and desserts are prepared with it. However, the purple cobs that contain a high amount of anthocyanins, are hardly ever utilized, which is a regrettable waste.

The anthocyanins have a high sensitivity to light and temperature and may degrade by oxidation and photochemical reactions. This rather fast degradation prevents the anthocyanins from being used in food products. Therefore, microencapsulation is suggested to prevent degradation of anthocyanins.

As a sub group of flavonoid, anthocyanins have a basic structure of C-6 C-3 C-6 configuration of a 15 carbon skeleton, which is referred to be anthocyanidin. Those two six carbon atoms both form phenolic rings respectively referred to A and B ring. There are more than 18 different anthocyanidins in the nature, 6 of them most occur in food materials,

which are: cyanidin, pelargonidin, peonidin, petunidin, malvdin and delphinidin. The substitution are shown in Table 1.1 and the structure are displayed in Figure 1.1

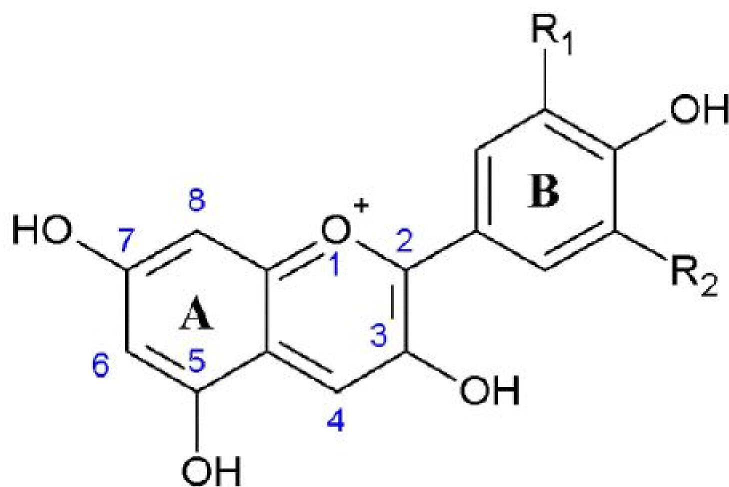


Figure 1.1, Besic structure of anthocyanidins

Name	Substitution		λmax(nm)	Molar Mass(g)
	R_1	R_2		
pelargonidin	H	H	494	271
cyanidin	OH	H	506	287
peonidin	OCH3	H	506	301
delphinidin	OH	OH	508	303
petunidin	OCH3	OH	508	317
malvdin	OCH3	OCH3	510	331

Table 1.1 Substitutions, maximum absorbance, and molar mass of major anthocyanidins

As acylations and glycosylation are essential for the color presenting of anthocyanin, the most dramatic color changes might be pH dependent.(Dangels, 1993) Flavylium cation is the predominant form of anthocyanins at pH 1, when pH goes up to 4.5, this structure will change to carbinol, pseudo-base, or chalcone, which both cause the change of the molecules chromophore and result in the color fading. And when pH rises to above 7, anthocyanins will finally form quinonoidal base, which is commonly blue. Also the type of anthocyanin pigment, light, temperature will influence the stability of anthocyanins as well. The presence of hydroxyl or methoxyl groups will decrease the stability of anthocyanins.(KHOO, 2017)

1.3 Micro-encapsulation

Microencapsulation is a method used to protect components against thermal and photo-degradation. For example, it is shown that the micro-encapsulation of iodine makes it stable for 12 minths under 40 ℃ and a high relative humidity. (Gharsallaoui,2007) The microencapsulation process consists on generating micro-capsules by surrounding a component like anthocyanins (core) with an encapsulating material such as a low molecular weight carbohydrates, proteins or polysaccharides (wall material). . The wall materials may protect the sensitive material in the core by preventing its contact with oxygen and thus, avoid or reduce

the oxidizing reactions (Guo J, 2018). Spray drying is the most common encapsulation method used while freeze drying, fluid bed coating and some other methods are also used. An aqueous solution of sensitive core material and a high molecular weight polysaccharide is atomized into small droplets by a spray nozzle into a heated chamber. The water in the small droplets gets evaporated by a hot air flow. As soon as the water evaporates, the call material get entrapped into the protective wall material and a microcapsule is formed and collected in a container. The comparatively low temperature and splitting dehydration process are advantages for encapsulizing anthocyanins as they are unstable under high temperature.(Gharsallaoui A ,2007) The Figure 1.2 shows the morphology of micro-capsules

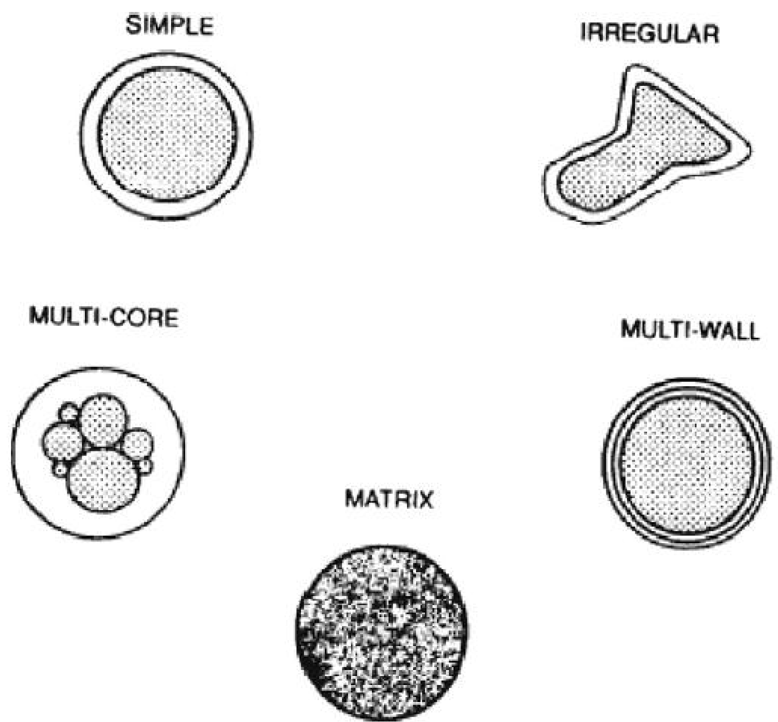


Figure 1.2 Morphology of different micro-capsules (Gibbs, 1999)

1.4 SEM

SEM, the abbreviation that stands for scanning electron microscope, it is a technique used to obtain images of the surface of micro-encapsulated samples. SEM is a microscopic topographic observation method whose magnitude of resolution relies between the transmission electron microscope and the classical optical microscope. It can perform microscopic images directly based on the surface materials of samples. Some advantages of SEM include: High magnification, continuous adjustment, a large depth of field, a large field of vision, and a rich three-dimensional image. It can directly observe the micro-structures of uneven surfaces of various samples. The sample preparation is simple, usually includes a metal sputtering operation.(Khursheed A, 2011)

1.5 HPLC

Reversed phase high performance liquid chromatography（HPLC）is utilized for seperation followed by qualitative identification and quantitative measurement of low molecular weight molecules like anthocyanins. The components in a sample will be separated in the chromatographic column due to their differences in affinity (polarity) between the mobile phase (liquid) and the stationary phase (solid). Single solvent or aqueous/solvent mixtures as the mobile phase, will be pumped into a column by high pressure infusion systems. The components of the

sample are carried through the column by the mobile phase, and then analyzed by a UV-VIS detector. The time a component is retained inside the column before its detected is called the retention time. A chromatogram will display a peak every time a component is detected by the detector. , Thereby the analysis of the components of a samples is implemented (Papadoyannis I, 1990). The UV-VIS spectra can also be obtained among the whole process, thus the spectra of each separated peak can help with the component identification.

The objective of this work was to obtain the surface characters of of the micro-capsules and develop the methodology to evaluate the micro-capsules of purple corn anthocyanins with 3 different wall materials based on the micro-encapsulation efficiency as well as light stability

.

2. Method

2.1 Preparation of purple corn cob extraction

The dried purple corn cobs from Bolivia were steeped in 60% aqueous ethanol solution for 3 hours. The solution with the extracted anthocyanins was filtered and collected into a round bottom flask. A rotary evaporator

was used at 40℃ until the ethanol were removed. Then the concentrate was freeze dried to get the anthocyanin powders. Those powders were stored in the fridge at -30℃.

2.2 Micro-encapsulation formation

Spray drying is the method used to encapsulate the anthocyanins. Anthocyanin powders are dissolved in water aqueous solution of the wall materials of the proportions given in Table 2.1. After homogenization, those 3 different solution were spray dried at 150 ℃ and 3 different micro-encapsulations were obtained.

MD	GA	SC
40% maltodextrin 60% anthocyanin	34% maltodextrin+6% gum arabic 60% anthocyanin	50% sodium caseinate 50% anthocyanin

Table 2.1 The components and proportion of each micro-capsules

2.3 Micro-encapsulation Efficiency

It is assumed that the Micro-encapsulation efficiency (ME) is the ratio between the anthocyanins inside the micro-capsule and the total anthocyanins (AT). The anthocyanins is distributed as both anthocyanins inside the wall material (AR) and a fraction that is attached at the surface

of the micro-capsule (AS). Thus the Micro-encapsulation efficiency percentage can be calculated by the Equation 2.1:

$$ME = \frac{AR}{AT} \times 100\% = \frac{AT - AS}{AT} \times 100\% \qquad \text{Equation 2.1}$$

A solution was used to wash out the anthocyanins attached on the surface. For the determination of the best mixture of solvents for extracting the anthocyanins in the surface of the microcapsules, methanol was the first choice, but it does not have enough dissolving capacity, and probably dissolve some sugar as well. However, methanol does dissolve some of the anthocyanins at the same time does not dissolve the wall materials, thus the objective solvent have similar properties to methanol, but it would be better if it have higher dissolving capacity for anthocyanins and lower influences on sugar fractions.According to Hansen’s theory, the solution most probably have similar three dimensional solubility parameters to methanol. (Hansen C, 2012) Thus this solution should have a suitable polarity that gives it a proper solvent powder that is enough to dissolve all the anthocyanins on the surface but will not destroy the structure of micro-capsules.

Once the best mixture of solvents was identified. to quantify the AS, 100mg of MD-GA and SC were mixed with the solution, vortexed for 1 min and ultra-sonicated for 1 min at room temperature. The content of

anthocyanins in the solution after filtration, which is AS, was determined by UV (VARIAN Palo Alto, CA) according to the calibration curve of anthocyanins in this solution made before. All analyses were performed in triplicate.

2.4 SEM

Scanning electron microscope (SEM) was used to look into the surface morphology of the micro-capsules. All three different micro-capsules were firstly set on aluminum stubs, followed by a gold coating process. Then, they were scanned under an accelerating voltage with magnifications of ×250, ×1000, ×5000 times

2.5 HPLC

All the anthocyanin extraction (AN) and the micro-capsules were analyzed by a high performance liquid chromatography (HPLC) (Agilent Technologies Santa Clara, CA). All the analyzed data was graphically represented using ths software Agilent HPLC online. A C-18 column (Purospher STAR RP-18 endcapped (3 μ m)) was used. 0.2μm RC membrane syringe filters were used to filter the solvents and sample solutions. A gradient method as Table 2.2 below was used to separate the micro-capsules:

Time(min)	A(%)(formic acid)	B(%)(acetonitrile)	D(%)(water)
0.00	10.0	5.8	84.2
20.00	10.0	12.4	77.6
35.00	10.0	21.8	68.2
40.00	10.0	31.2	58.8
45.00	10.0	45.3	44.7
55.00	10.0	5.8	84.2

Table 2.2 HPLC method time table

In this method, the flow rate was kept as 0.8ml/s, the injection volume was set to be 25µl, and the analyzed spectra was 200nm to 600nm. The wavelength used for detecting the peaks separated from antyocyanins were 520nm.

2.5.1 Degrading ratio inside the HPLC machine

As the degrading rate of anthocyanins in solution is quite high, there was a possibility that the samples in the HPLC degraded significantly while waiting for the auto-injection that may cause a significant error. To define the degrading ratio, the anthocyanin was dissolved in an aqueous solution, in a pH 3 buffer and in a pH7 buffer and analyzed by HPLC with sample injections after 0, 1, 2 and 4 hours of waiting time.

The change of area of the highest peak was used to represent the degrading curve, thus the degrading ratio could be calculated equation by the Equation 2.2 below:

$$R_d = \frac{A_0 - A_t}{A_0} \times \frac{1}{t} \times 100 \quad \% \qquad \text{Equation 2.2}$$

R_d is the degrading ratio, t is the time that sample kept in HPLC (hour), A_0 is the area of the highest peak that kept in HPLC for 0 hour, A_t is the area of the highest peak that kept in HPLC for t hours

2.5.2 Anthocyanin photo-degradation under low intensity light exposure

In order to evaluate the protective ability of the different wall materials, a rough model of the degradation progress under low intensity illumination conditions was assesed. A comparison between the different wall material and the pure anthocyanin extract was made. The changes of each sample under continuous illumination were tracked and analyzded by HPLC at different periods of time. .

The different periods were samples were collected after illumination were 1day, 3 days, 7 days, 14 days and 21 days.

The area of peak 1 was used to illustrate the anthocyanin change by the

Equation 2.3 below:

$$W_1 = \text{v} \times c = \frac{v \times A}{\varepsilon} \qquad \text{Equation 2.3}$$

Where W_1 is the weight of the component that gave peak 1 on the chromatograph, v is the volume of the water that samples were diluted before analysis in HPLC, c is the concentration of the component that gave peak 1 on the chromatograph in the solution for HPLC analysis, A is the absorbance 1, Ɛ is the molar absorptivity of the component that gave peak 1 on the chromatograph, which is got from the Ana's research (GOUVÊA, 2012)

2.5.3 Anthocyanins photodegradation under high intensity light exposure

A further evaluation was made by the HPLC analysis of high intensity periodically illumination treated samples, anthocyanin extraction and three micro-capsules were evenly encased in special small transparent glass containers. Each container will load 50 mg samples spread on the whole provided surface. Those containers with samples were put under a polar lamp for periodical time. This polar lamp gave the radiation that has the same wavelength and intensity as day light. The containers was set on special points and the intensity of light at each point was determined. The

total energy that the samples received from the polar lamp per unit area was calculated through Equation 2.4

$$E = {}^{I \times t}\!/\!_{A} \qquad \text{Equation 2.4}$$

E is the energy that the samples received from the polar lamp per unit area, I is the intensity at each point t is the time that each sample exposed to the light, A is the area of the container. The period was set to be in the Table 2.3 below:

Rack number	1	2	3	4	5	6
Time	15min	30min	45min	60min	90min	120min

Table 2.3 Illumination time for periodically measurement

All the samples after illumination were analyzed by HPLC.

3. Result and discussion

3.1 Micro-encapsulation efficiency

This solution is defined as a mix of acetic acid, methanol, acetonitrile at the proportion of 2:10:13 after a couple of trials.

The anthocyanin content calibration curve is made as Figure 3.1

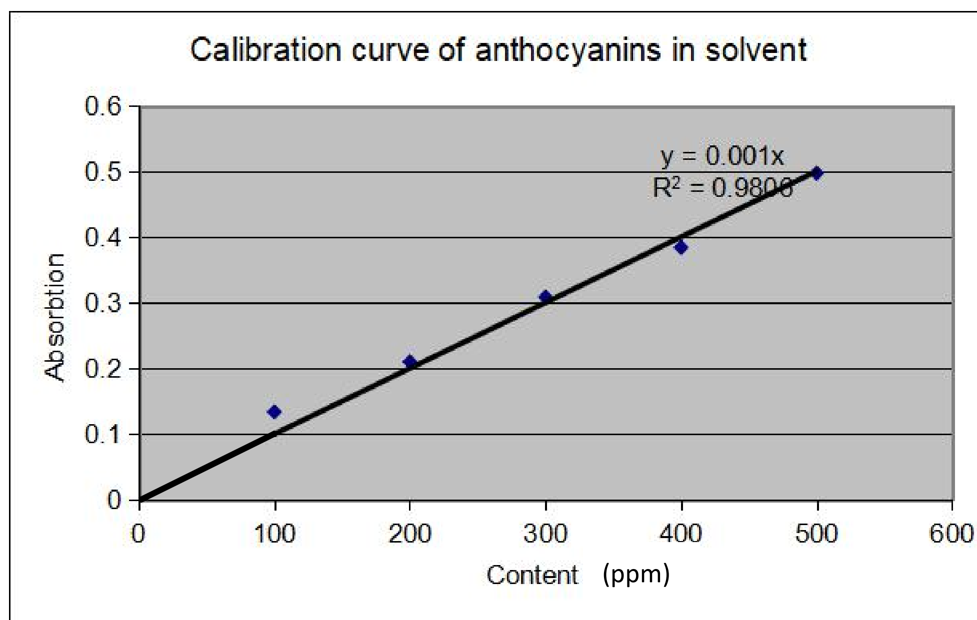


Figure 3.1 Calibration curve of anthocyanins in solvents

According to this calibration curve, the micro-encapsulation efficiency of these 3 different micro-capsules were calculated shown in Table 3.1.

Sample	GA	MD	SC
Absorbance of filtered Liquid after wash (LAW)	0.210	0.200	0.453
	0.196	0.208	0.480
	0.210	0.208	0.500
Experiment error(standard deviation)	0.008	0.014	0.080
Average absorbance of LAW	0.205	0.205	0.478
Anthocyanin content in LAW(ppm)	205.3	205.2	477.6

AS(mg)	4.11	4.10	9.55
AT(mg)	40.0	40.0	50.0
ME	0.897	0.897	0.809

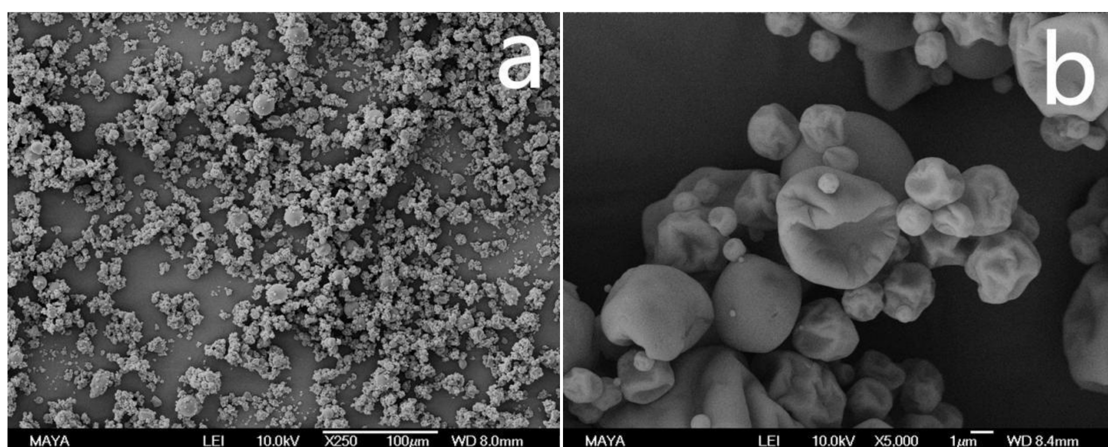
Table 3.1 Micro-encapsulation Efficiency
All the data was analyzed by t-test, for GA and MD, P=0.981, for GA and SC, P= 0.0011, for MD and SC, P=0.0024, which means there is not a significant difference between GA and MD, but there is significant between GA and SC, MD and SC, AS is the weight of the anthocyanins on the surface of the micro-capsules, AT is the weight of total anthocyanins, ME is the micro-encapsulation efficiency

From the table we can see that the ME of GA and MD are all most the same, bothe around 90%, while efficiency of SC is comparatively lower, around 81%, which is still an acceptable efficiency for micro-encapsulation. Thus which one is the best wall material for the micro-encapsulation of anthocyanins still can not be decided. This need further research about stability and color stability of encapsulated anthocyanins, micro-capsules physical and chemical stability.

3.2 SEM

Surface characterization of the three micro-capsules that encapsulated with different wall materials was given by SEM. The photo (figure) showed inwards spheroidal dented structures of the particles forming agglomerated. Some of the puny particles are embed in the dents of the surfaces of the big ones, which was also observed by Silva et al. (2014).

These characteristic are expected because the powders are produced by spray drying, which will force the micro-capsules to form spheroidal structures due to the surface tension. The moisture will be evaporated during a short time that cause the inwards dents and forms the ridges. Figure 3.2 a-i show that comparing to MD and GA, the dents of SC are deeper and larger, while the dents of MD and GA are smaller and shallower. The dents would give a larger surface area which probably give more area for the anthocyanin molecular adhere to causing a lower micro-encapsulation efficiency. Figure 3.2 also shows that there are some imperfections on the surfaces of micro-capsules, which could be the reason to reduce the protective ability. Figure3.2 shows more fractured particles of the GA sample than of the MD sample, which possibly gives MD a stronger protective ability.



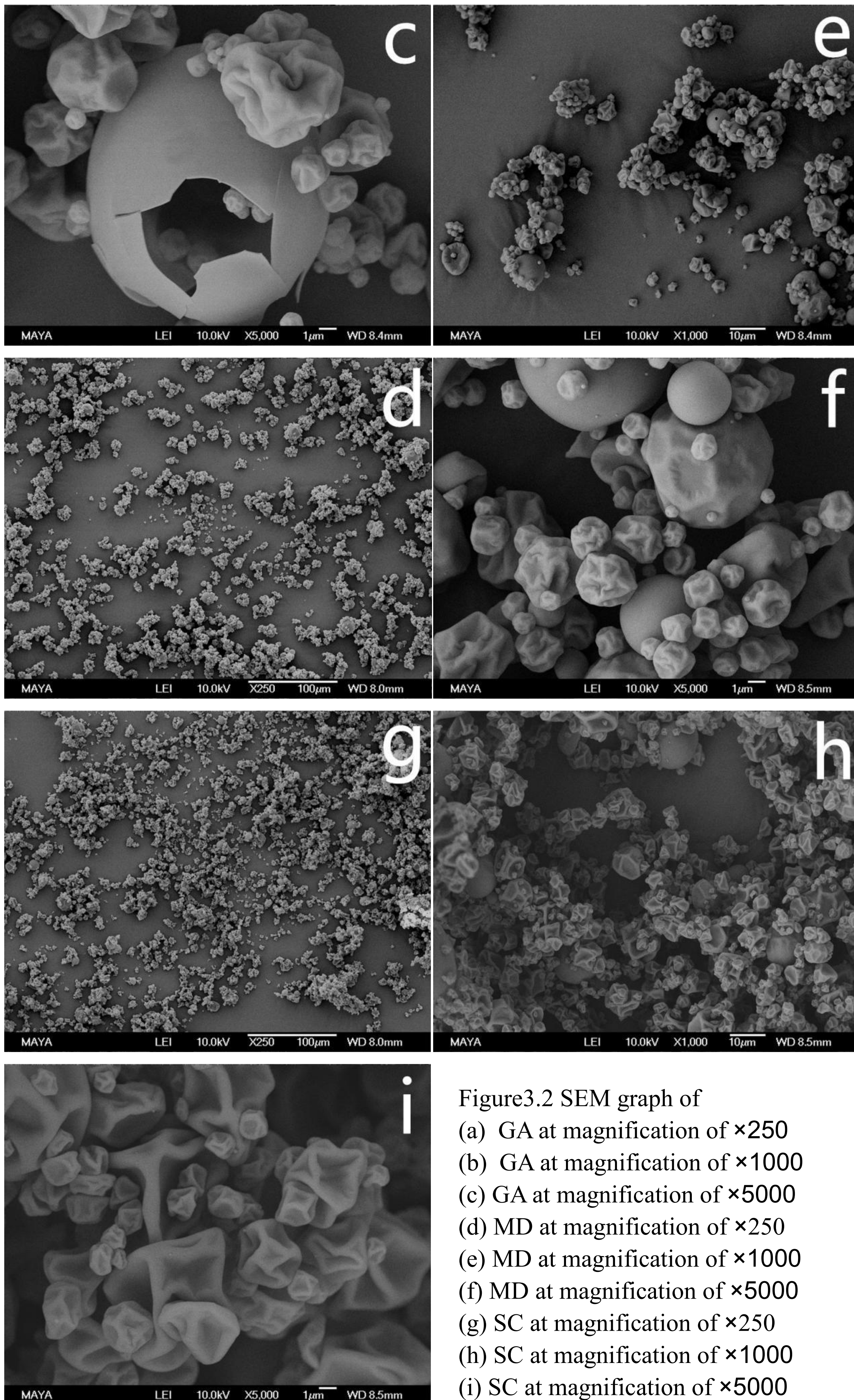


Figure3.2 SEM graph of
(a) GA at magnification of ×250
(b) GA at magnification of ×1000
(c) GA at magnification of ×5000
(d) MD at magnification of ×250
(e) MD at magnification of ×1000
(f) MD at magnification of ×5000
(g) SC at magnification of ×250
(h) SC at magnification of ×1000
(i) SC at magnification of ×5000

3.3 HPLC

The composition of the anthocyanin extraction is mainly ancyanindin-based non- and monoacylated derivatives (soluble and bound 16-18). Figure 3.3 is the HPLC chromatogram at 520 nm of anthocyanin extraction.

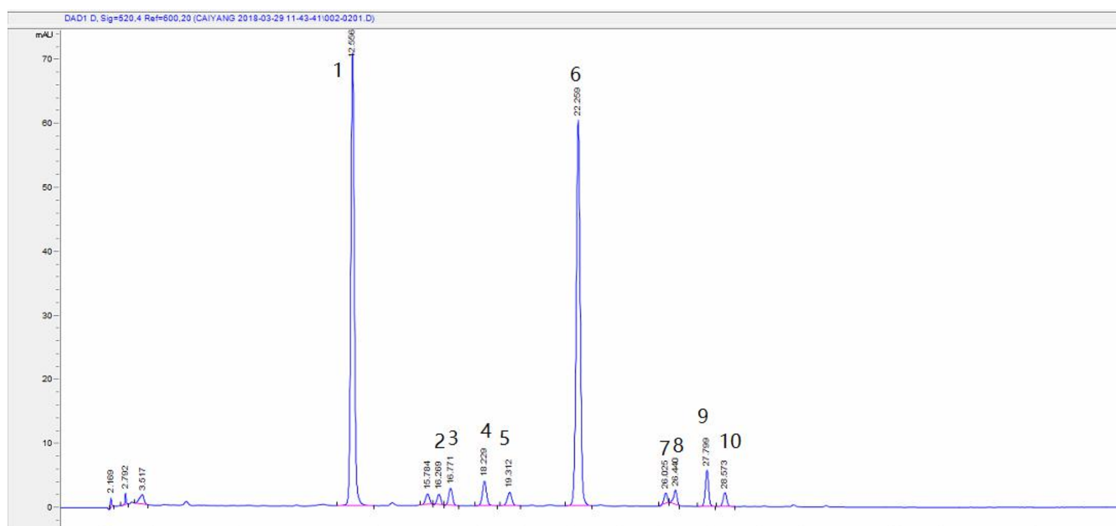


Figure 3.3 HPLC chromatogram of anthocyanin at 520nm

Using the HPLC method for this lab, 10 main separated peaks were obtained from the extraction with the visible UV spectra. According to Barry et al , the purple corn has 6 primary anthocyanins below in table

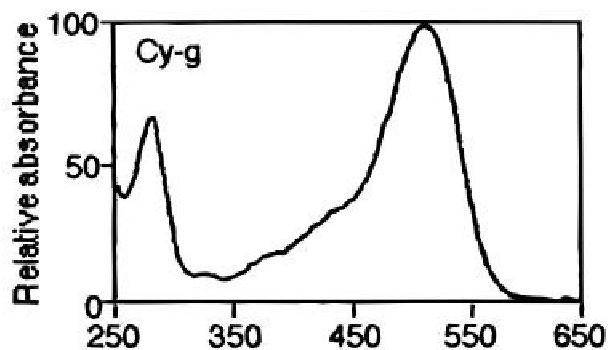
Anthocyanins	%	Lambda max (nm)	Total m/z	Aglycone m/z
Cyanidin-3-glucoside	55.3	516	449	287
Pelargonidin-3-glucoside	3.3	503	433	271
Peonidin-3-glucoside	15.0	516	463	301
Cyanidin-3-(6"-malonylglucoside)	20.5	519	535	287
Pelargonidin-3-(6"-malonylglucoside)	1.1	507	519	271
Peonidin-3-(6"-malonylglucoside)	4.8	519	549	301

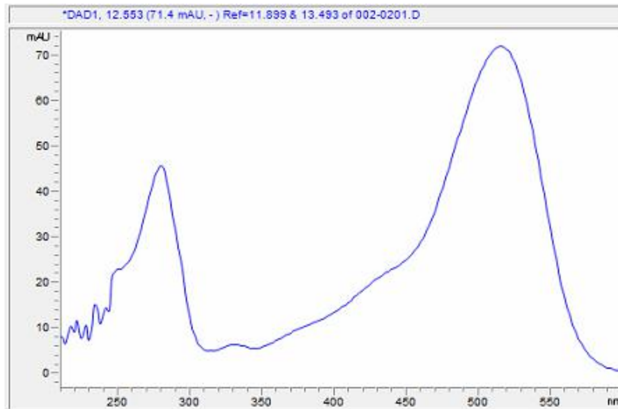
TABLE 3.2 Anthocyanins identified from the source purple corn[25]

It is obvious that those anthocyanins are based on 3 different anthocyanidins, thus it is speculated that those 10 peaks are respectively based on one of these 3 anthocyanidins (cyanidin, pelargonidin and peonidin). On the strength of the UV spectra of these 10 peaks and reference UV spectra of these 3 anthocyanidins, those 10 peaks are divided in to 3 groups. They are:

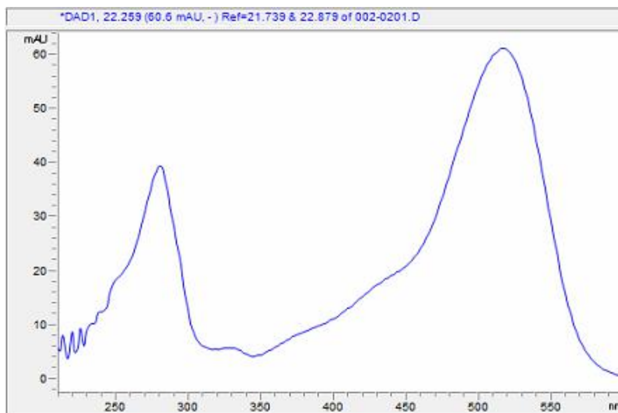
Group 1.cyanidin based:Peak 1,6,9

Cyanidin reference UV spectra[9]:

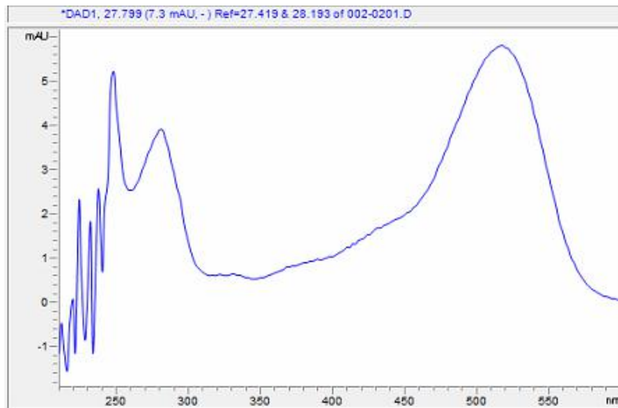




Peak1



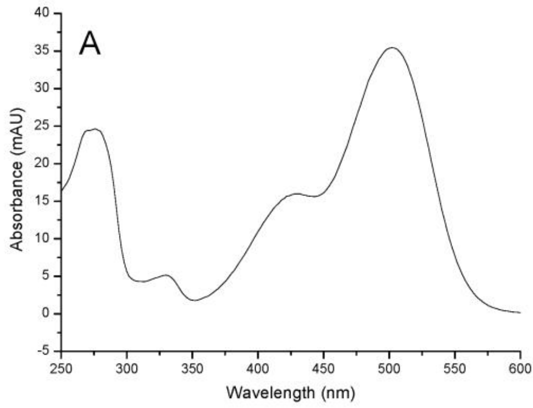
Peak6



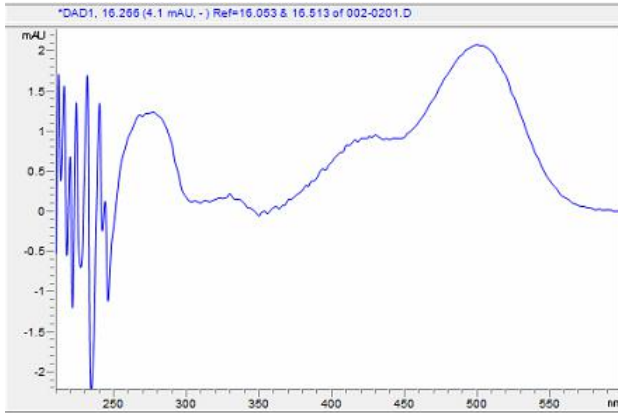
Peak 9

Group 2. pelargonidin based: Peak 2,8

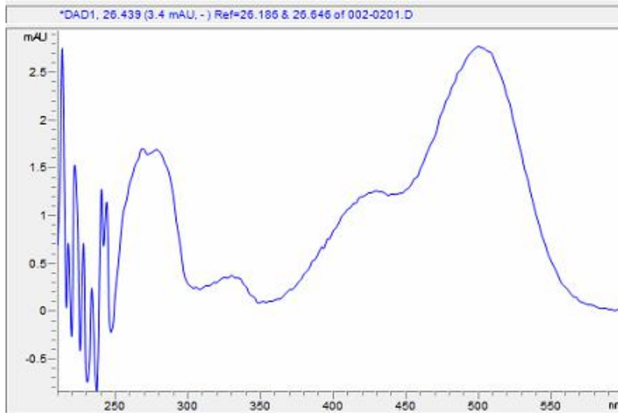
Reference of pelargonidin[10]:



(From LUIGIA LONGO,2005)



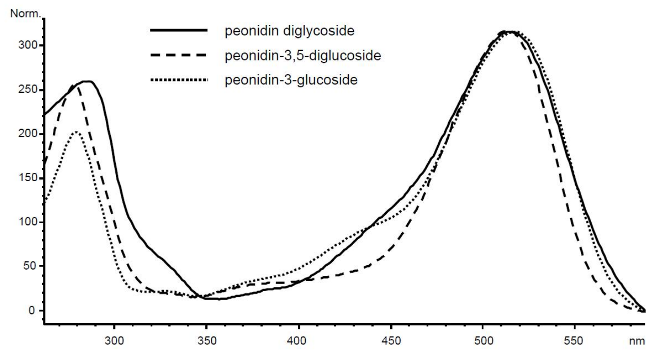
Peak 2

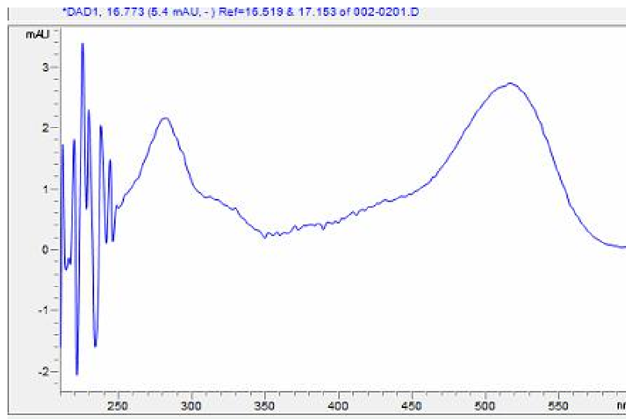


Peak 8

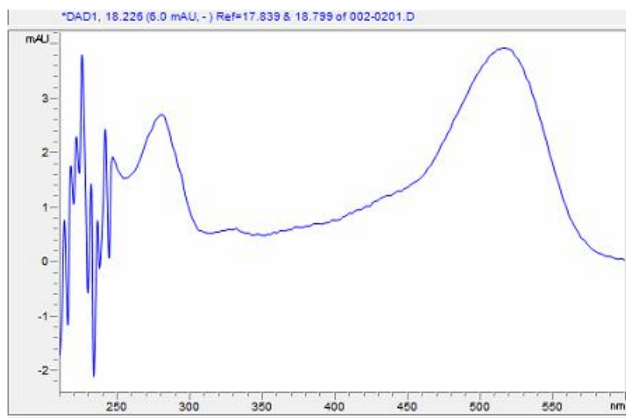
Group 3. peonidin based: peak 3,4,5,7,10

Reference of peonidin[11]:

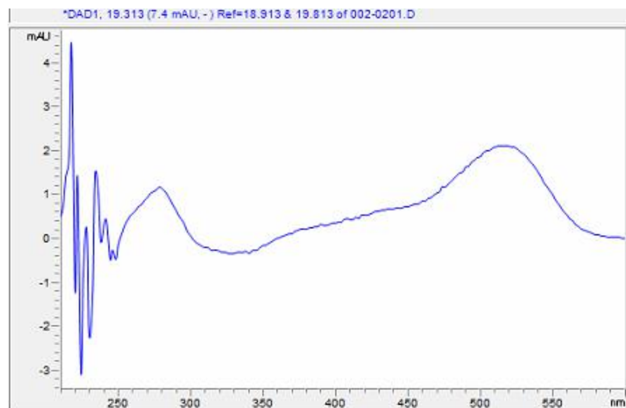




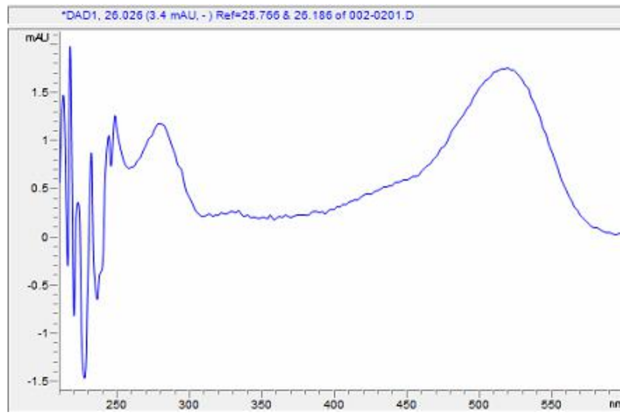
Peak3



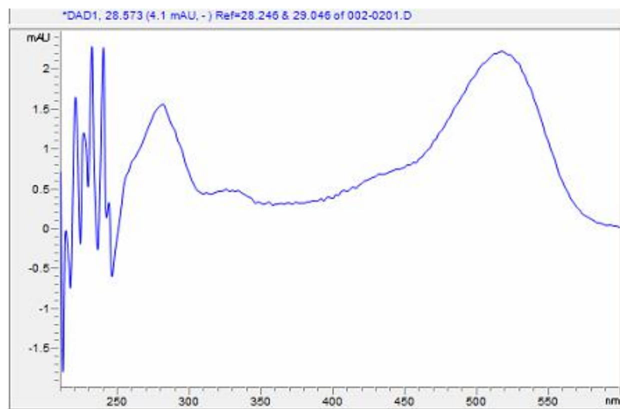
peak4



Peak 5



Peak 7



peak 10

From the grouping of UV spectra and retention time, we can assume that peak3,4,5 are from the same substance, and a tentative identification could be made in Table 3.3. This speculation could eventually be affirmed by a mass-spectra in the further research.

Peak	Possible base anthocyanidin	Possible anthocyanin
1	cyanidin	cyanidin-3-glucoside
2	pelargonidin	pelargonidin-3-glucoside
3,4,5	peonidin	peonidin-3-glucoside
6	cyanidin	cyanidin-3-(6-malonylglucoside)

7	peonidin	peonidin-3-(6-malonylglucoside)
8	pelargonidin	pelargonidin-3-(6-malonylglucoside)
9	cyanidin	cyanidin-3-(dimalonylglucoside)
10	peonidin	peonidin-3-(dimalonylglucoside)

Table 3.3 Speculation of identification of anthocyanin extraction

3.3.1 Degrading ratio inside the HPLC machine

From Table 3.3 peak 1 is speculated to be a cyanindin based component. The areas of peak 1 of the samples in different solvents and the degrading ration per unit time are shown in the Table 3.4, It illustrates that in this experiment, the degrading ratio of the samples in water is low and queuing int the machine will not influence too much on the results. Thus the results that got from the water solutions are reliable.

Time(hours)		0	1	3	Dr
Area of peak 1	water	829	783	772	2.26%
	ph3 buffer	871	853	852	0.73%
	ph7 buffer	830	789	749	3.29%

Table 3.4 Degrading rate of anthocyanins inside the HPLC machine

3.3.2 Anthocyanin photo-degradation under low intensity light exposure

The mass fraction of component that gave peak 1 on the chromotagraph (component 1) was calculated by Equation 2.3 and are shown in the Figure 3.4

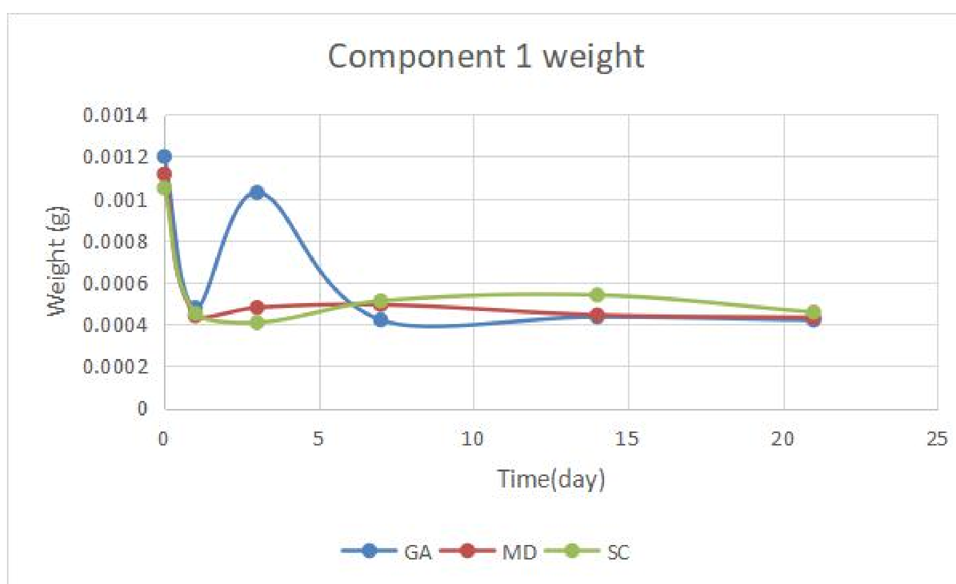


Figure 3.4 Component 1 weight change by time for 3 samples

The figure illustrates degrading process over the 21 days. There was a significant drop in the first day in all the 3 samples. After that, the areas kept constant and low for all 3 samples except one point of GA at day 3. This means that even the anthocyanins were encapsulated, the degrading happens rapidly and the majority happened in the first day, In addition, the data from the time shorter than one day were not collected, there is a possibility that the degrading was finished in a few hours, which means the protective capacity of encapsulation seems not to be very powerful. Thus a further experiment of illumination experiment under higher

intensity light and shorter time was done after this. The mechanism of the abnormal increase of GA is unclear, it could possible be an error.

3.3.3 Anthocyanins photodegradation under high intensity light exposure

The area of peak 1 was used to illustrate the degrading process in the 2 hours under high intensity light and are shown in figure 3.5:

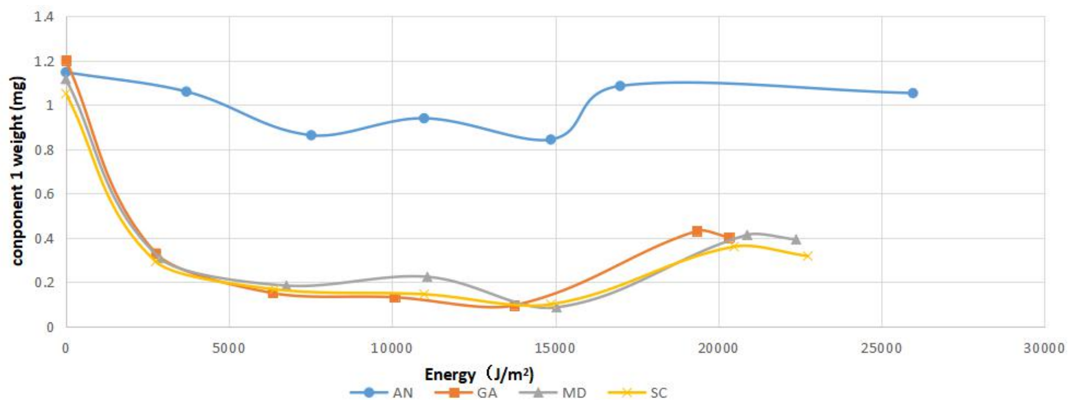


Figure 3.5 Component 1 weight change under high intensity light

This figure illustrated that generally in the whole experiment process, the weight change of the anthocyanin extract (AN) is much lower than the 3 encapsulated ones, which means rather than improve the light stability, micro-encapsulation decreased the light stability of the anthocyanins for some reason. In the first 15 min, there is just a small decrease for AN,while in the 3 other samples there is a sharp drop. This means that there was a rapid degrading process happening in the first 15 minutes for the 3 micro-capsules under the radiation simulating the wavelength and intensity of daylight, which confirmed to the results got from the

preliminary radiation trial with a wolfram hot wire lamp that the majority of the degrading process finished in the first day. Even though the component weight changes of encapsulated ones are much lower than the unencapsulated anthocyanin, but the component weight of all the samples have similar changing trend while the energy they got was increasing: decreased at the beginning while the accumulating energy lower than $5kj/m^2$, but got a slight upswing, while the accumulating energy is more than $20kj/m^2$, it fluctuated to decrease a bit and finally rise again. A speculation of the mechanism of the degrading process was made to explain this trend, which is that the degrading process is a two-stage process, in the first stage, it's a decomposition process, molecular broke down and component decreased or disappeared, followed by a second stage that the broken down component were synthesized. This second stage may start before the first stage was totally finished. But to verify this speculation, further researches are needed.

Conclusion

In conclusion, a mix of acetic acid, methanol, acetonitrile at the proportion of 2:10:13 is a good solvent for the micro-encapsulation efficiency determination as it has a good dissolve ability for anthocyanins but does not dissolve the wall materials. The micro-capsules of anthocyanins with the wall material of maltodextrin and maltodextrin mixed with gum arabic have better micro-encapsulation efficiency than with sodium caseinate, but the micro-encapsulation of these three micro-capsules are above 80%, which is an acceptable efficiency comparing with the micro-encapsulation with other methods. And the micro-capsules encapsulated by maltodextrin has the best surface characters that have the shallowest dents and less imperfections on the surface. The micro-capsules encapsulated by sodium caseinate has the least imperfections but has the deepest dents. The illumination experiments shows that micro-encapsulation actual decreases the stability of anthocyanins under light exposure. The reason for this result is still unclear, but a speculation is given that the homogenization process in the micro-capsules formation might decrease the particle sizes of anthocyanins which might have a negative influence on the light stability. Also, HPLC analysis is a good method for the components concentration

changes detection even though the analysis process of anthocyanins in HPLC is long , as the degrading during waiting time before HPLC injection does not influence the determination. A HPLC and MS analysis of the anthocyanindin standards will help for the identification of anthocyanins' components in order to understand the mechanism behind degrading process.

Appendix

Color difference of anthocyanin aqueous solution before and after light experiment

Before light experiment **After light experiment**

References

1. Yang W, Kortesniemi M, Yang B, Zheng J. Enzymatic Acylation of Anthocyanins Isolated from Alpine Bearberry (Arctostaphylos alpina) and Lipophilic Properties, Thermostability, and Antioxidant Capacity of the Derivatives. Journal of Agricultural and Food Chemistry. 2018;66(11):2909-2916.
2. Gould K. Anthocyanins: Springer; 2014.
3. Sigurdson G, Tang P, Giusti M. Natural Colorants: Food Colorants from Natural Sources. Annual Review of Food Science and Technology. 2017;8(1):261-280.
4. Khursheed A. Scanning electron microscope optics and spectrometers. Singapore: World Scientific Pub. Co.; 2011.
5. Guo J, Giusti M, Kaletunç G. Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: Impact of processing and storage parameters on encapsulation efficiency. Food Research International. 2018;107:414-422.
6. Pedreschi R, Cisneros-Zevallos L. Phenolic profiles of Andean purple corn (*Zea mays L.*). Food Chemistry. 2007;100(3):956-963.

7. Mahdavi S, Jafari S, Ghorbani M, Assadpoor E. Spray-Drying Microencapsulation of Anthocyanins by Natural Biopolymers: A Review. Drying Technology. 2014;32(5):509-518.
8. Stoll L, Costa T, Jablonski A, Flôres S, de Oliveira Rios A. Microencapsulation of Anthocyanins with Different Wall Materials and Its Application in Active Biodegradable Films. Food and Bioprocess Technology. 2015;9(1):172-181.
9. Miyazawa T, Nakagawa K, Kudo M, Muraishi K, Someya K. Direct Intestinal Absorption of Red Fruit Anthocyanins, Cyanidin-3-glucoside and Cyanidin-3,5-diglucoside, into Rats and Humans. Journal of Agricultural and Food Chemistry. 1999;47(3):1083-1091.
10. LONGO L. Determination of Anthocyanins in Ruscus aculeatus L. Berries. Journal of Agricultural and Food Chemistry. 2005;:475-479.
11. Castillo-Muñoz N, Fernández-González M, Gómez-Alonso S, García-Romero E, Hermosín-Gutiérrez I. Red-Color Related Phenolic Composition of Garnacha Tintorera (Vitis viniferaL.) Grapes and Red Wines. Journal of Agricultural and Food Chemistry. 2009;57(17):7883-7891.
12. Sinela A, Mertz C, Achir N, Rawat N, Vidot K, Fulcrand H et al. Exploration of reaction mechanisms of anthocyanin degradation in a

roselle extract through kinetic studies on formulated model media. Food Chemistry. 2017;235:67-75.

13. Gouvêa A, Araujo M, Schulz D, Pacheco S, Godoy R, Cabral L. Anthocyanins standards (cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside) isolation from freeze-dried açaí (Euterpe oleraceae Mart.) by HPLC. Food Science and Technology. 2012;32(1):43-46.

14. Jorgensen K, Skibsted L. Light sensitivity of carotenoids used as food colours. Zeitschrift f�r Lebensmittel-Untersuchung und -Forschung. 1990;190(4):306-313.

15. Cavalcanti R, Santos D, Meireles M. Non-thermal stabilization mechanisms of anthocyanins in model and food systems—An overview. Food Research International. 2011;44(2):499-509.

16. Weber F, Boch K, Schieber A. Influence of copigmentation on the stability of spray dried anthocyanins from blackberry. LWT - Food Science and Technology. 2017;75:72-77.

17. KOPJAR M, JAKŠIĆ K, PILIŽOTA V.Influence of sugars and chlorogenic acid addition on anthocyanin content, antioxidant activity and color of blackberry juice during storage. Journal of Food Processing and Preservation. 2012;36(6):545-552.

18. Pacheco-Palencia L, Talcott S. Chemical stability of açai fruit (*Euterpe oleracea Mart*.) anthocyanins as influenced by naturally occurring and externally added polyphenolic cofactors in model systems. Food Chemistry. 2010;118(1):17-25.

19. Buchowski H, Khiat A. Solubility of solids in liquids: one-parameter solubility equation. Fluid Phase Equilibria. 1986;25(3):273-278.

20. Hendry G. Natural food colorants. Bishopbriggs: Blackie Academic & Professional; 1996.

21. Khursheed. Scanning Electron Microscope Optics and Spectrometers. 2nd ed. Hackensack: World Scientific Publishing Company, Incorporated; 2010.

22. Papadoyannis I. HPLC in clinical chemistry. 1st ed. New York: Dekker; 1990.

23. Khoo H, Azlan A, Tang S, Lim S. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & Nutrition Research. 2017;61(1):1361779.

24. Hansen C. Hansen Solubility Parameters. 1st ed. Hoboken: Taylor and Francis; 2012.

25. Encapsulation, Color Stability, and Distribution of Anthocyanins from Purple Corn (Zea mays L.), Blueberry (Vaccinium sp.), and Red Radish (Raphanus sativus) in a Cold-Setting Pectin-Alginate Gel.