Jurnal Teknologi

Full paper

Evaluation of *Capsaicinoids* Extracts as Bioactive Substance for Antimicrobial Films

Tan Wee Leng[a], Ida Idayu Muhamad[a,b*], Dayang Norulfairuz Abang Zaidel[a], Nozieana Khairuddin[a]

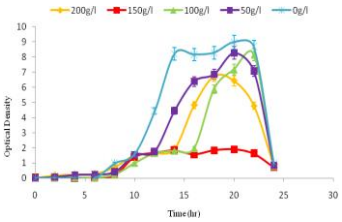
[a]*Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[b]*Cardiovascular Engineering Centre, IJN-UTM, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: idayu@cheme.utm.my

Article history

Received :29 July 2013
Received in revised form :
23 September 2013
Accepted :29 September 2013

Graphical abstract



Abstract

Capsaicinoid, a naturally occurring alkaloid group and active components which can be found in chili peppers variety (*Capsicum spp*.), contributes to the pungency, taste, and aroma of chili peppers. Apart from their culinary and medicinal properties, *capsaicinoid* functions as antioxidant agent in food preservation. In the current research, the evaluation of bio-active substance of *capsaicinoid* was carried out to determine its potential in development of antimicrobial film for packaging. A mixture of acetone: petroleum ether (1:1) had been used in extracting the *capsaicinoids* and carotenoids (natural red pigment).The four types of chili pepper studied were *Green Malagueta Salvador*, *Red Malagueta Salvador*, *red Thai Capsicum Frutescens* and *red Cayenne.* The optimum *capsaicinoids* extraction time and amount of carotenoids (color intensity) for each variety of chili peppers were studied. Lastly, antimicrobial properties of AM films were determined as they inhibited both gram-positive (*Streptococcus* and *B. subtilis*) and gram-negative (*E. coli*) bacteria successfully through agar diffusion test and liquid culture test.

Keywords: *Capsaicinoids*; carotenoid; antimicrobial agent; bioactive substances; antimicrobial film; antioxidant

Abstrak

Capsaicinoids, salah satu jenis kumpulan alkaloid semula jadi merupakan komponen aktif yang terdapat di dalam varieti cili-cili (*Capsicum spp*.) yang menyumbang terhadap sifat pedas, perisa dan aroma cili. Selain dari kegunaan masakan dan nilai perubatan, *capsaicinoids* mempunyai potensi bahan antioksidan bagi membantu penyimpanan makanan dalam tempoh masa yang lebih panjang. Kajian ini membuat penilaian ke atas sifat bahan aktif *capsaicinoids* terhadap perencatan mikro organisma bagi membangunkan aplikasi filem pembungkusan antimikrob. Campuran yang terdiri dari pada aseton dan petroleum (1:1) telah digunakan sebagai pelarut bagi mengekstrak *capsaicinoids* dan karotenoid iaitu bahan warna semula jadi dalam cili. Sebanyak empat varieti cili telah dikaji iaitu *Green Malagueta Salvador*, *Red Malagueta Salvador*, *red Thai Capsicum Frutescens* dan *red Cayenne.* Masa optimum untuk pengekstrakan *capsaicinoid* dan intensiti warna karotenoid dari pada cili-cili berkenaan telah dikaji. Manakala kesan perencatan bahan aktif ekstrak cili ke atas bakteria Gram positif (*Streptococcus* dan *B. subtilis*) .dan bakteria Gram negatif (*E. coli*) dikaji menggunakan kaedah difusi agar dan kultur kaldu.

Kata kunci: *Capsaicinoids*; karotenoid; bahan bioaktif; filem antimikrob; antioksidan

1.0 INTRODUCTION

Active packaging interacts with packed product or the headspace between the package and the food system to obtain a desired outcome Likewise, antimicrobial (AM) food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself [1]. Application of antimicrobial substances can control the microbial population and target specific microorganisms to provide higher safety and quality products. The additives which are commonly used in AM packaging system are potassium sorbate, nisin, imazalil, allylisothiocyanate, triclosan and chitosan. Current efforts focus on obtaining and utilizing natural components such as plant extracts that have antimicrobial properties and antioxidants for wider use and applications in AM films [1].

Chili pepper (*Capsicum frutescens*) is one of the famous and significant food additives and ingredients which favored and consumed by people all over the world due to their mouth-watering and appetite tempting of flavor, attracting color and their hot pungency. The hot, spicy flavor of chili peppers is contributed by *capsaicinoids*, which is an alkaloid group found in the Capsicum family. Figure 1 and 2 shows the *Capsaicin* (trans-8-methyl-N-vanillylnonenamide) and *dihydrocapsaicin* (trans-8-methyl-N-vanillynonanamide) which are two major compounds among natural *capsaicinoids* [2].*Capsaicin* is a fat soluble, odourless, pungent tasting, off-white solid with a melting point of 62-65°C and a molecular weight of 305.4 kDa [3]. Figure 3 shows the Carotenoids, one of the most general and significant natural pigments, are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria [4].

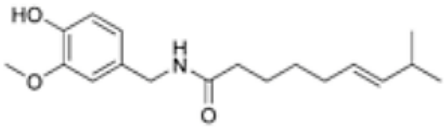


Figure 1 *Capsaicin* [2]

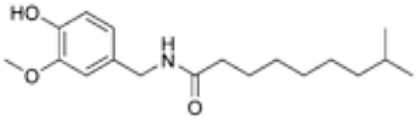


Figure 2 *Dihydrocapsaicin* [2]

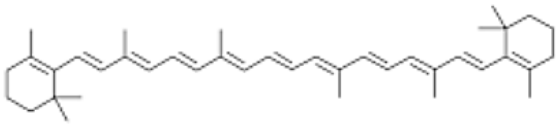


Figure 3 β-carotene [4]

Apart from the culinary properties, *capsaicinoids* act as powerful antioxidants [5-6], antimicrobial properties, present anti-mutagenic and anti-tumoral properties [7-8]. In addition, the carotenoids that can be found in chili pepper are β-carotene, cryphoxanthin, zeaxanthin, antheraxanthin, violaxanthin, capsanthin, capsorubin, and neoxanthin.

The objectives of this study are to evaluate the ability of *capsaicinoids* extracts from four types of chili peppers (*Red Malagueta Salvador*, *Green Malagueta Salvador*, *red Thai Capsicum Frutescen* and *red Cayenne*) as bioactive substance for antimicrobial films.

■2.0 EXPERIMENTAL

2.1 Determination on the Presence of *Capsaicinoid* and Carotenoid in Chili Peppers and Their Optimum Extraction Time [8]

Four types of chili peppers (*Red Malagueta Salvador*, *Green Malagueta Salvador*, red *Thai Capsicum Frutescen* and *red Cayenne*) with 160 g respectively were homogenized with acetone. The homogenized chili peppers were added with the mixture of 400 ml acetone: 400 ml petroleum ether (1:1) to prepare 200 g/l of extracted *capsaicinoids* from 4 kinds of chili peppers. The mixture was stirred by hot plate at room temperature. 5 ml of ether layer was titrated from each beaker at 1 hour interval hour for 7 hours totally. The obtained ether layer was dried with anhydrous Na_2SO_4 before used for HPLC determination.10% of acetonitrile in water was prepared as solvent system. For HPLC determination, the Knauer chromatograph equipped with a computer control system, data processor and UV detector was used. Nova-Pak C18 column was used with 100 μ L of sample, wavelength (A = 280 nm, B=310nm), mobile phase with 90% water and 10% acetonitrile with 5 minute of run time. The rest of ether layer (mixture of *capsaicinoid* and carotenoid) was diluted to 150 g/l, 100 g/l and 50 g/l with mixture of acetone: petroleum ether (1:1).

2.2 Preparation of Film Solution [9]

100 ml of deionized water, 7.31 g of starch, and 3.8 ml of glycerin were mixed and prepared for control film making. On the other hand, 100 ml of deionized water, 7.31 g of starch, 3.8 ml of glycerin and 60% w/w mixture of *capsaicinoid* and carotenoid (200 g/l, 150 g/l, 100 g/l and 50 g/l respectively) were mixed and prepared for four different concentration of antimicrobial film making. These 5 film solutions were heated while homogenized to around 70°C for roughly 40 minute until a homogen and viscous solution was obtained.10 ml of each viscous solution was poured into petri plate and dried at room temperature for 2 days. The films were peeled out after they have dried completely.

2.3 Broth Medium

2.3.1 Preparation of Nutrient Agar [10]

Firstly, 10 g tryptone, 5 g yeast extract, 10 g NaCl and 15 g agar powder are added into 800 ml H_2O in 1 L scott bottle. The pH of the prepared solution was adjusted to 7.5 with sodium hydroxide. The total volume was adjusted to 1 L with deionized water. It was autoclaved for 15 minutes (screw-caps was only closed loosely while sterilizing).The agar was cooled inside the laminar flow before it was poured into 40 petri plates. The plastic bags of petri plates were cut carefully as they will be used for resealing the poured plates. The agar was poured onto petri plates when the bottles are very warm (can be handheld). Each plate was filled to about half-way carefully to avoid any contamination on the dish or agar. The bubbles which were trapped within the agar were removed by heating gently at the agar surface with a flamed burner was used. The plates were stacked carefully in even, straight piles and kept in cool room before using in order to prevent contamination.

2.3.2 Preparation of Luria Broth [11]

Firstly, 10 g tryptone, 5 g yeast extracts and 10 g NaCl are added to 800 ml water in 1 L Scott bottle. The pH of the Luria agar was adjusted to 7.5 with sodium hydroxide. The total volume was adjusted to 1 L with deionized water. The mixture was mixed and dispensed immediately into 15 conical flasks evenly. Cottons were used to plug at the openings of each conical flask and were then covered with aluminum foils. The conical flasks with the content of Luria Broth were autoclaved for 15 minutes for sterilization purpose. The conical flasks were stored in cool room to avoid contamination.

2.4 Color Measurement

This research was aimed to produce a film packaging with a natural colourant, therefore the stability on colour of the extracts was evaluated corresponding to extraction time. A colorimeter

(Minolta Color Reader: CR-10, Japan) was used to measure the color of the chilies extract. Analytical data were expressed as Hunter *L* (brightness), *a* (greenness/redness) and *b* (yellowness/blueness) values.

2.5 Bacteria Inhibition Test [12]

2.5.1 Agar Diffusion Test

E. coli and *Streptococcus* were used as strain tests. Each type of bacteria was spread evenly on 20 agar plates by using cotton sticks.2 rectangles (2x2cm) were cut and peeled out from films which made up of 0 g/l, 50 g/l, 100 g/l, 150 g/l and 200 g/l of *capsaicinoid* and carotenoid which were extracted from *Red Malagueta Salvador*. Step 3 was repeated for films which made by mixture of *capsaicinoid* and carotenoid extracted from *Green Malagueta Salvador*, red *Thai Capsicum Frutescen* and *red Cayenne*. 5 pieces of films with 0 g/l, 50 g/l, 100 g/l, 150 g/l and 200 g/l of *capsaicinoid* and carotenoid (*Red Malagueta Salvador*) were pasted on agar plates which were inoculated with *E. coli*. Another 5 piece of films with 0 g/l, 50 g/l, 100 g/l, 150 g/l and 200 g/l of *capsaicinoid* and carotenoid (*Red Malagueta Salvador*) were pasted on agar plates which were inoculated with *Streptococcus*. Step 5 and 6 were repeated for film which made by mixture of *capsaicinoid* and carotenoid extracted from *Green Malagueta Salvador*, *red Thai Capsicum Frutescen* and *red Cayenne*. The above inoculated agar plates were incubated at 37°C for 1 day. Inhibition diameter for each sample was determined manually.

2.5.2 Liquid Culture Test

For liquid culture test, only films which made of *red Thai Capsicum Frutescen* were used (0 g/l, 50 g/l, 100 g/l, 150 g/l and 200 g/l of mixture of *capsaicinoid* and carotenoid). 3 pieces of each concentration (0 g/l, 50 g/l, 100 g/l, 150 g/l and 200 g/l) antimicrobial films were peeled out from each petri plate and added into 15 conical flasks respectively.3 sets of 5 conical flasks which contained 0 g/l, 50 g/l, 100 g/l, 150 g/l and 200 g/l antimicrobial films were inoculated with *E. coli*, *B. subtilis*, and *Streptococcus* respectively. All the conical flasks were then transferred to shaking incubator for 24 hours at150 rpm. OD tests were conducted for each 15 samples in time interval of 2 hours for 1 day testing.

2.6 Statistical Analysis

The statistical analysis of the data was performed through one-way analysis of variance (ANOVA) using EXCEL 2010. The data were ranked and statistical differences were evaluated on the ranks with a one-way analysis of variance (ANOVA). In all cases, a value of $p < 0.05$ was considered to be significant.

■3.0 RESULTS AND DISCUSSION

3.1 Extraction Work and Optimum Time

The presences of capsaicinoids in four types of chili peppers (Green Malagueta Salvador, Red Malagueta Salvador, Red Thai Capsicum and Red Cayenne) were determined through HPLC analysis. Previous study on Red Thai Capsicum as shown in the chromatogram (Figure 4) shows that *capsaicin* and *dihydrocapsaicin* were detected at 17th and 21st minute respectively compared to the standard solution where the retention times for the *capsaicin* and *dihydrocapsaicin* were 20th and 21st minute [13]. The areas under the peaks in each chromatogram were used to estimate the optimum extraction time to obtain maximum amount of *capsaicinoids* as illustrated in Figure 5 and the optimum extraction time for each variety of chili pepper was summarized in Table 1.

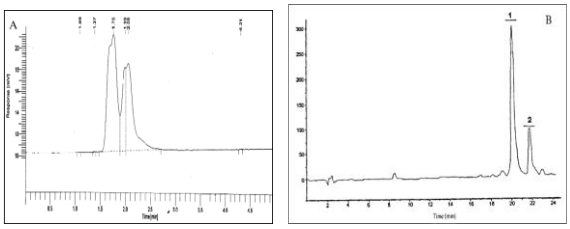


Figure 4 HPLC Chromatogram (A) of Extract of Hot Pepper Fruits (experimentally), (B) of Standard Solution, 1-*capsaicin*, 2-*dihydrocapsaicin* [13]

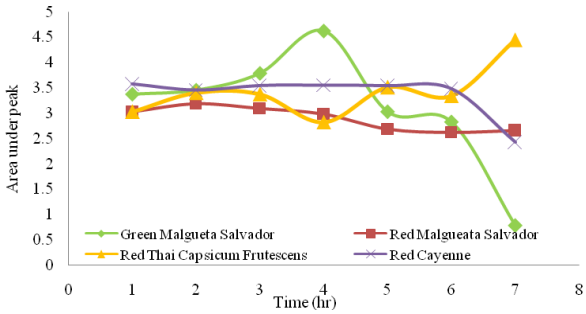


Figure 5 Comparison of optimum extraction time among four types of chili peppers

Table 1 Optimum extraction times for different types of chili peppers

Chili varieties	Optimum extraction time (hr)
Red Cayenne	1
Red Malagueta Salvador	2
Green Malagueta Salvador	4
Red Thai Capsicum frutescens	7

3.2 Determination on the Colour of the Extract

The colour of the extracts from four types of chili peppers was analyzed by using colorimeter corresponding to 7 hours of extraction process where three kinds of parameters (Negative values indicate blue and positive value indicate yellow were measured in this case. They are Lightness, *L* (L=0 yields black and L=100 indicates diffuse white), *a* value (negative values indicate green while positive value indicate magenta) and *b* value (negative values indicate blue and positive value indicate yellow).

The graph with lightness (*L*) versus extraction time (hr), concluded that the lightness of the chili pepper extracts is independent of the extraction time (Figure 6). In other words, lightness of each type of chili pepper extract did not significantly change in corresponding to the extraction time ($p>0.05$).

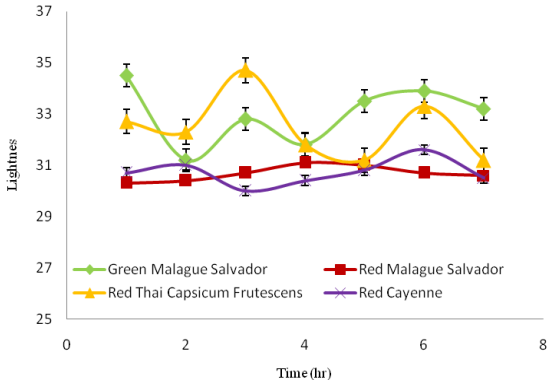


Figure 6 Lightness of the chili pepper extracts corresponding to extraction time (hr)

According to Figure 7, all the red colour chili peppers (*Red Malagueta Salvador*, *Red Thai Capsicum Frutescens* and *Red Cayenne)* gave positive *a* value which is corresponding to the colour indication where positive *a* value show magenta colour content. On the other hand, only *Green Malagueta Salvador* gave negative a value as it consists of green colour content.

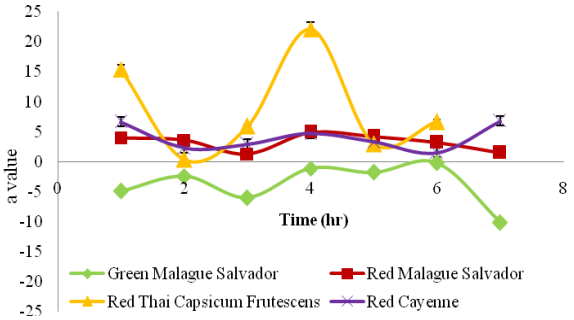


Figure 7 *a* Value of the chili pepper extracts against extraction time

Referring to Figure 8, generally all the *b* value are positive which brings the meaning of yellowish content among all types of chili pepper extracts. The *b* values are not stable and fluctuate throughout seven hours of extraction process.

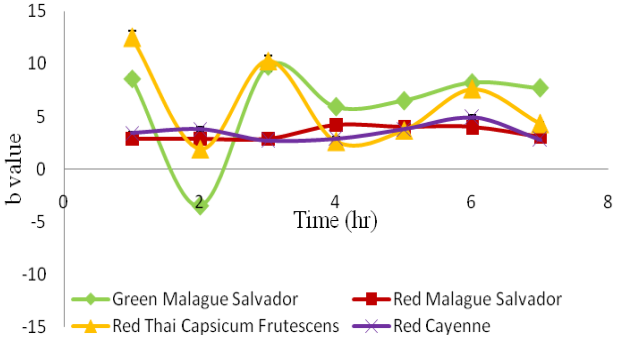


Figure 8 *b* Value of the chili pepper extracts against extraction time

3.3 Agar Diffusion Test

3.3.1 Summaries on the Comparisons Among E. Coli (Gram Negative) Inhibition Zone Diameters in the Presence of Capsaicinoid from Different Type of Chili Peppers

Referring to Figure 9, it is obviously that the *Red Malagueta Salvador* imposed a significant impact in hindering the growth of *E. coli* throughout the range of concentration from 50 g/l to 200 g/l of *capsaicinoid*-films ($p<0.05$). Its maximum inhibition zone diameter was 5 cm. As overall, for all types of chili peppers, the higher concentration of *capsaicinoid* the stronger the inhibitory effect which led to larger inhibition zone diameter. The control films showed no inhibition area and colonies were formed all over the plate. The result is in agreement with previous the study that showed the ability of *capsaicin* as the main compound for antimicrobial activity against *E. coli* [14].

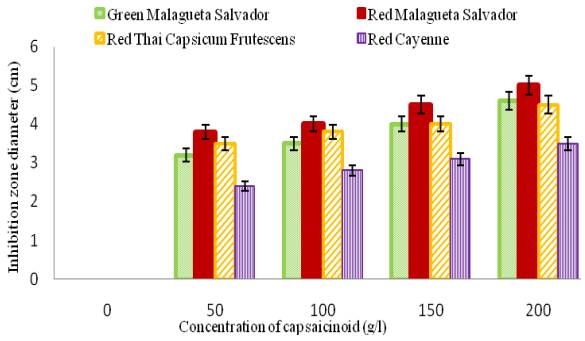


Figure 9 *E. coli* inhibition zone diameter (cm) versus concentration of *capsaicinoid* (g/l) extracted from different type of chili peppers

3.3.2 Comparisons among Streptococcus (Gram positive) Inhibition Zone Diameters in the Presence of Capsaicinoid from Different Type of Chili Peppers

Figure 10 shows comparison of the effect of *capsaicinoid* from four types of chili peppers on the inhibition of *Streptococcus*. It can be observed that the *capsaicinoid* from each type of chili pepper demonstrated larger inhibition zone at higher concentration of *capsaicinoid* (g/l). In general, the diameters achieved were in the range of 3 to 4.5 cm. In the aspect of antimicrobial effect, 200 g/l of Red Thai Capsicum *Capsaicinoid* film significantly gave the largest inhibition zone diameter compared to other types of chilies ($p<0.05$). The control films showed no inhibition area and colonies were formed all over the plate.

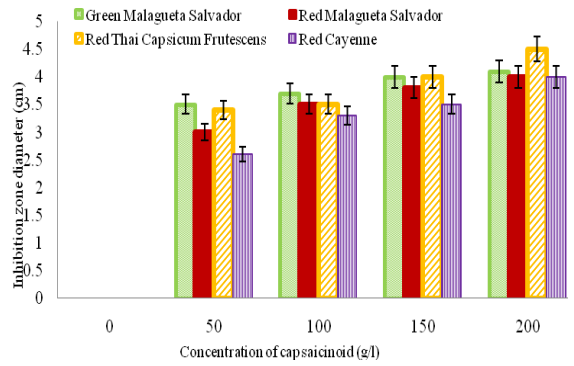


Figure10 *Streptococcus* inhibition zone diameter (cm) versus concentration of *capsaicinoid* (g/l) extracted from different type of chili peppers

3.4 Comparison of Gram Positive and Gram Negative Bacteria Growth in the Presence of *Red Thai Capsicum Frutescens Capsaicinoid* AM film in Liquid Culture Test

3.4.1 Inhibition of E. coli (Gram Negative Bacteria) in Liquid Culture Test

According to Figure 11, control film (0 g/l *capsaicinoid*) did not show any inhibitory effect on *E. coli*. The *E. coli* undergoes 6 hours of lag phase, 10 hours of exponential phase, 6 hours of stationary phase and 2 hourss of death phase. The film which was incorporated with 50 g/l of *capsaicinoid* was added into another conical flask which was inoculated with *E. coli*. From figure 11, the lag phase of *E. coli* maintained at 6 hours, 8 hours taken for exponential phase, 2 hours for stationary and death phase respectively. It shows that the *capsaicinoid* gave impact to block the growth of *E. coli* by shortening its stationary phase and extending its death phase. The concentration of *capsaicinoid* was increased to 100 g/l, 150 g/l and 200 g/l for the next replicates. Based on figure 11, there was not much difference in inhibitory strength among 150 g/l and 200 g/l with 100 g/l of *capsaicinoid*. They all show 6 hours of lag phase, 12 hours of acceleration phase and 6 hour of death phase. The *E. coli* population maintained (stationary phase) at 18th hour (a peak). This phenomenon can be explained through the un-stabilization effect by the addition of 100 g/l, 150 g/l and 200 g/l of *capsaicinoid*. However, 200 g/l of *capsaicinoid* gave weaker inhibitory effect on *E. coli*. Briefly, films with *capsaicinoid* concentration within the range of 100 g/l gave the strongest inhibitory effect towards *E. coli*.

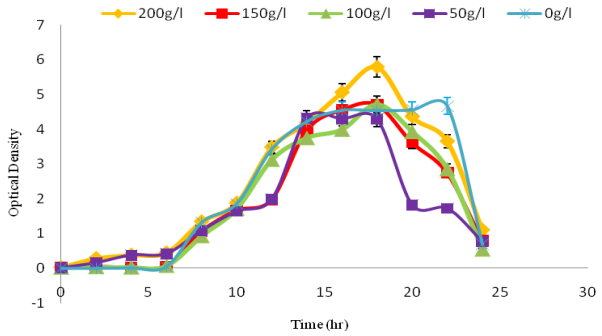


Figure 11 Optical Density (OD) of *E. coli* growth versus time at different concentration of *Red Thai Capsicum Frutescens Capsaicinoid* extract

3.4.2 Inhibition of Streptococcus (Gram Positive Bacteria) in Liquid Culture Test

Based on Figure 12, control film gave normal shape of growth curve where 4 hours was taken by *Streptococcus* to complete it lag phase and 12 hours, 6 hours and 2 hours for exponential phase, stationary phase and death phase respectively. On the other hand, 50 g/l *capsaicinoid*-film gave slightly inhibitory effect on the *Streptococcus* where the stationary phase was shorten to 2 hours whereas the exponential phase was prolonged to 14 hours and both of lag phase and death phase remained at 4 hours and 2 hours respectively. As the overall view, 100 g/l *capsaicinoid* antimicrobial film gave the strongest inhibitory effect on the *Streptococcus* throughout the 24 hours inoculation process.

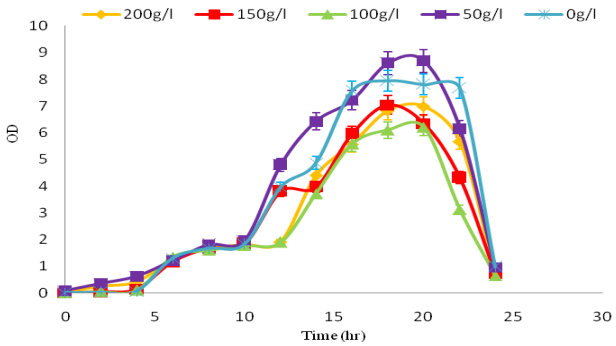


Figure 12 Optical Density (OD) of *Streptococcus* growth at different concentration of *Red Thai Capsicum Frutescens Capsaicinoid* extract

3.4.3 Inhibition of B. subtilis (Gram Positive Bacteria) in Liquid Culture Test

Referring to Figure 13, *B. subtilis* consumed 6 hours for its lag phase, 8 hours for exponential phase, 8 hours for stationary phase and 4 hours for death phase under the presence of the control film (0 g/l of *capsaicinoid* content) which reflects that the control film did not impose inhibitory effect on the *B. subtilis*. Unexpectedly, 50 g/l of *capsaicinoid* has stronger strength of inhibitory property compared to 100g/l, 150 g/l and 200 g/l of *capsaicinoid*. It was shown by the delaying of lag phase to 8 hours as the *B. subtilis* struggled longer time to adapt itself in this cultured environment. In addition, *B. subtilis* did not achieve stationary phase as it was unstabilized by 50 g/l of *capsaicinoid*. At the same time, the death phase had been extended to 4 hours. For 100 g/l, the lag phase of *B. subtilis* was delayed to 8 hours which is similar to 50 g/l. but only 2 hours was taken for death phase which means that *B. subtilis* survived longer in 100 g/l *capsaicinoid* of Luria broth.150 g/l *capsaicinoid* did not affect *B. subtilis* much as longer stationary phase was achieved (8 hours). 150 g/l was recommended to be used to inhibit the *B. subtilis* as it showed the highest inhibitory when incorporated into the films [15]. Besides, 150 g/l gave a better inhibition effect on *B. subtilis* in liquid culture test compared to agar diffusion test probably due to the difference in the mobility of the bacterial cells within the two systems as suggested by previous study [16]. The agar diffusion test allows little or no mobility of the non-motile bacteria, whereas the liquid culture test uses a liquid broth under constant agitation which enables cell movement and exposure to the film despite the non-motile characteristic of the strain [16]. Previous study by Soetarno *et al.*, suggest that all kinds of Capsicum fruits are useful as antibacterial and anti candidal agents and not necessarily the most pungent pepper as in the traditional use [14]. This explains the inhibition of *E. coli*, *Streptococcus* and *B. subtilis* tested in this study.

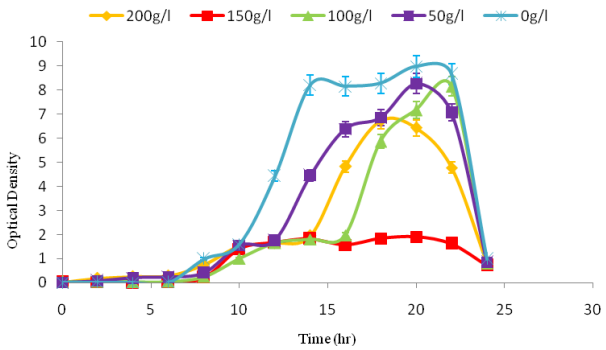


Figure 13 Optical Density (OD) of *B. subtilis* growth at different concentration of *capsaicinoid*

4.0 CONCLUSION

Capsaicinoids and carotenoid were successfully extracted simultaneously by conventional solvent extraction method with mixture of solvent which composes of acetone: petroleum ether (1:1). The presence of *capsaicinoids* (*capsaicin* and *dihydrocapsaicin*) had been proved experimentally and the optimum extraction time for *Green Malagueta Salvador*, *Red Malagueta Salvador*, *Red Thai Capsicum Frutescens* and *Red Cayenne* have been determined through HPLC analysis. Among others, it was found that *Red Thai Capsicum Frutescens Capsaicinoid* gave the highest inhibitory effect towards all microbes tested. The Red Thai Capsicum *Capsaicinoid* was further incorporated into developed film to determine the inhibitory effects. When immobilized in film, the *Red Thai Capsicum Frutescens Capsaicinoid* inhibited gram negative bacteria *E. coli*, Gram positive bacteria *Streptococcus* and *B. subtilis* at 50 g/l, 100 g/l and 150 g/l of optimum concentration respectively through liquid culture test.

Acknowledgement

The authors would like to thank the Ministry of Science and Innovation (MOSTI) Malaysia, the Ministry of Higher Education (MOHE) and Research Grant (03H98) from Research Management Centre UTM for their support of this study.

References

[1] Appendini, P., and J. H. Hotchkis. 2002. Review of Antimicrobial Food Packaging. *Innovative Food Science & Emerging Technologies*. 3(13): 113–126.
[2] Pena-Alvarez, A., E. Ramirez-Maya, and L. A. Alvarado-Suarez. 2009. Analysis of *Capsaicin* and *Dihydrocapsaicin* in Peppers and Pepper Sauces by Solid Phase Microextraction-gas Chromatography-mass Spectrometry. *Journal of Chromatography. A*. 1216(4): 2843–2847.
[3] Hayman, M., and P. C. A. Kam. 2008. *Capsaicin*: A Review of Its Pharmacology and Clinical Applications. *Current Anaesthesia & Critical Care*. 19(5): 338–343.
[4] Dong, S. L., K. C. Syung, and L. Y. Kit. 1992. Carotenoid Loss in Dried Red Pepper Products. *International Journal of Food Science and Technology*. 21: 179–185.
[5] Henderson, D. E., and S. K. Hendersor. 1992. Thermal Decomposition of *Capsaicin*. 1. Interactions with Oleic Acid at High Temperatures. *Journal of Agricultural and Food Chemistry*. 40: 2263–2268.
[6] Suhaj, M. 2006. Spice Antioxidants Isolation and Their Antiradical Activity: A Review. *Journal of Food Composition and Analysis*. 19(6): 531–537.
[7] Cichewicz, R. H, and P. A. Thorpe. 1996. The Antimicrobial Properties of Chile Peppers (Capsicum Species) and Their Uses in Mayan Medicine. *Journal of Ethnopharmacology*. 52: 61–70.
[8] Ejechi, B. O., O. E. Nwafor, and F. J. Okoko. 1999. Growth Inhibition of Tomato-rot Fungi by Phenolic Acids and Essential Oil Extracts of Pepper Fruit (Dennetiatripetala). *Food Research International*. 32(4): 395–399.
[9] Quintavalla, S., and L. Vicini. 2002. Antimicrobial Food Packaging in Meat Industry. *Meat Science*. 62(7): 373–380.
[10] Kerr, T., and M. Barbara. 2001. *Applications in General Microbiology: A Laboratory Manual*. 6th Edition. Published by Hunter Textbook, Inc.
[11] Thitilertdecha, N., T. Aphiwat, and R. Nuansri. 2008. Antioxidant and Antibacterial Activities of *Nepheliumlappaceum L*. Extracts. *Food Science and Technology*. 1–7.
[12] Sudharameshwari, K., and J. Radhika. 2007. Antibacterial Screening of Aegle Marmelos, lawsonia Inermis and Albizzia Libbeck. *Complementary and Alternative Medicine*. 4 (2): 199–204.
[13] Perucka, I., and W. Oleszek. 2000. Extraction and Determination of *capsaicin*oids in Fruit of Hot Pepper Capsicum Annum L. by Spectrophotometry and High-performance Liquid Chromatography. *Food Chemistry*. 4: 287–291.
[14] Soetarno, S., Sukrasno, E. Y., and Sylvia. 1997. Antimicrobial Activities of the Ethanol Extracts of Capsicum Fruits with Different Levels of Pungency *Jurnal Matematika & Sains*. 2(2): 57–63.
[15] Dainelli, D. 2008. Active and Intelligent Food Packaging: Legal Aspects and Safety Concerns. *Trends in Food Science & Technology*. 19(9): 103–112.
[16] Ida I. M., Khairuddin, N., and Ling, L. H. 2011. Effect of Lauric Acid Addition on the Microbial Efficacy of Chitosan-based Film. *Jurnal Teknologi*. 54(Sains & Kejuruteraan): 101–109.