CHAPTER 4

Results and discussion

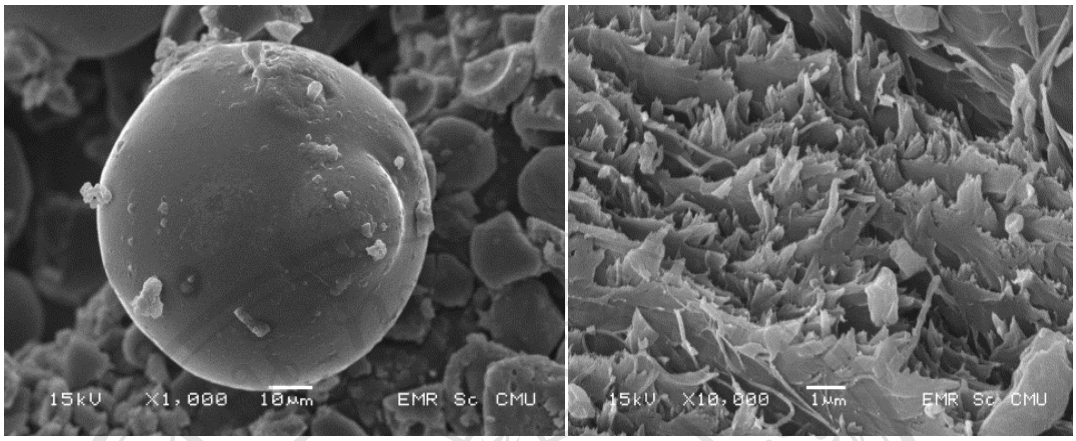
4.1 Physical characteristics of the carotenoids encapsulated in chitosan-TPP

The effects of quantities of carotenoids and TPP on the appearance, particle size and interstitial pore fraction of the carotenoids encapsulated in chitosan-TPP were observed under the SEM. The characteristics of the commercial carotenoids were also compared.

4.1.1 Appearance, particle size and interstitial pore fraction of the carotenoids encapsulated in chitosan-TPP

1) Appearance

Appearances of commercial carotenoids, chitosan and encapsulated carotenoids noticeably. The commercial carotenoids appeared in the spherical shape with smooth surfaces, while the chitosan itself had the spike shape (Figures 4.1a and 4.1b). All samples of the encapsulated carotenoids appeared in orange color with their regular elongated shape and rough surface (AppendixC2). For example, the matrixes loaded with 2.0% (w/v) carotenoids encapsulated by chitosan cross-linked 1.0% (w/v) TPP appeared as shown in Figure 4.2. Changing of the appearance from the smaller, needle shape of chitosan to bigger and rounder shape of the carotenoids encapsulated in chitosan-TPP was due to the fact that the carotenoids particles were hold inside the chitosan-TPP matrix.



a b

Figure 4.1 SEM photograph of commercial carotenoids at X 1,000 (a) and commercial chitosan at X10,000 (b)

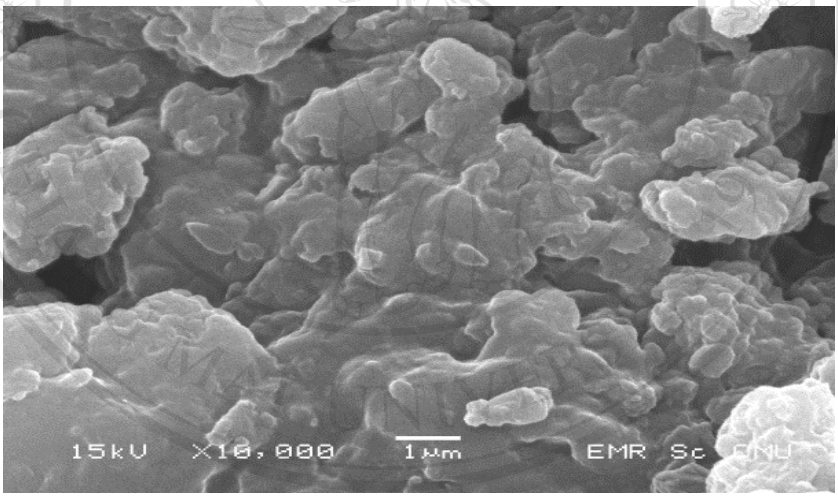


Figure 4.2 SEM images of carotenoids encapsulated in chitosan-TPP prepared using 2.0% (w/v) carotenoids and 1.0% (w/v) TPP

2) Particle size observed under SEM

Diameters of the carotenoids encapsulated in chitosan-TPP were 230-500 nm. The TPP concentration affected the particle size considerably. Adding higher concentration of TPP resulted in decreasing size of the particles, especially with the 4.0 and 5.0% carotenoids samples (Figure 4.3). The smaller size of the carotenoids

encapsulated in chitosan-TPP would assist the application in delivery of carotenoids to various food products.

The decreasing of the particles size with the increasing concentration of TTP was agreed with previous report that the nanoparticles size could be controlled by varying the chitosan: TPP ratio (Hu *et al*., 2008; Luangtana-anan *et al*., 2005; Tsai *et al*., 2008). Another study on preparation of chitosan-carrageenan-tripolyphosphate nanoparticles by polyelectrolyte complexation/ionic gelation indicated that the cross-linker or the TTP decreased size of nanoparticles from 450-500 nm to 150-300 nm (Rodrigues *et al*., 2012).

The recent study of Chattopadhyay and Inamdar (2012) confirmed that the TPP concentration affected the particle size. The TPP which was categorized as a major ingredient for cross linking process exhibited a pronounced effect on the properties of the nanochitosan dispersion. Chattopadhyay and Inamdar (2012) found that at concentration of TPP below 5.0 % (w/w), very few phosphate ions presented to produce effective ionic linkages with the chitosan amino groups. However, the excessive cross linking through TPP bridging caused precipitation (Gordon *et al*., 2011).Accordingly, the optimal concentration of TTP would be determined by optimizing various properties including physical properties, encapsulated efficiencies and storage stability.

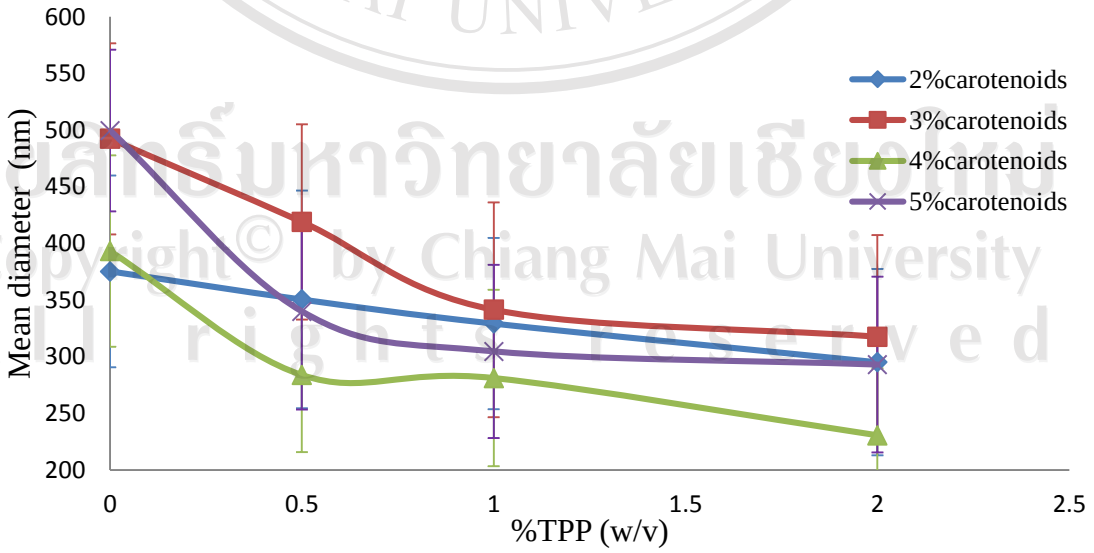


Figure 4.3 Mean diameter of carotenoids encapsulated in chitosan-TPP

2.1) Particle size determined by the Mastersizer

The encapsulated carotenoids particles observed under the SEM were irregular shape. Then, the sample with the highest encapsulation efficiency obtained from the 3.0% (w/v) carotenoids encapsulated in the chitosan crosslinked with 2.0% (w/v) TPP (section 4.1.2) was selected to recheck the particle size using the Mastersizer. It was found that the diameters of samples were between 0.05 to 8.06 µm. The mean diameters (D 4,3) was 399.59±0.71 nm. The particle size of the encapsulated carotenoids particles determined by the Mastersizer were slightly bigger than those obtained by the SEM.

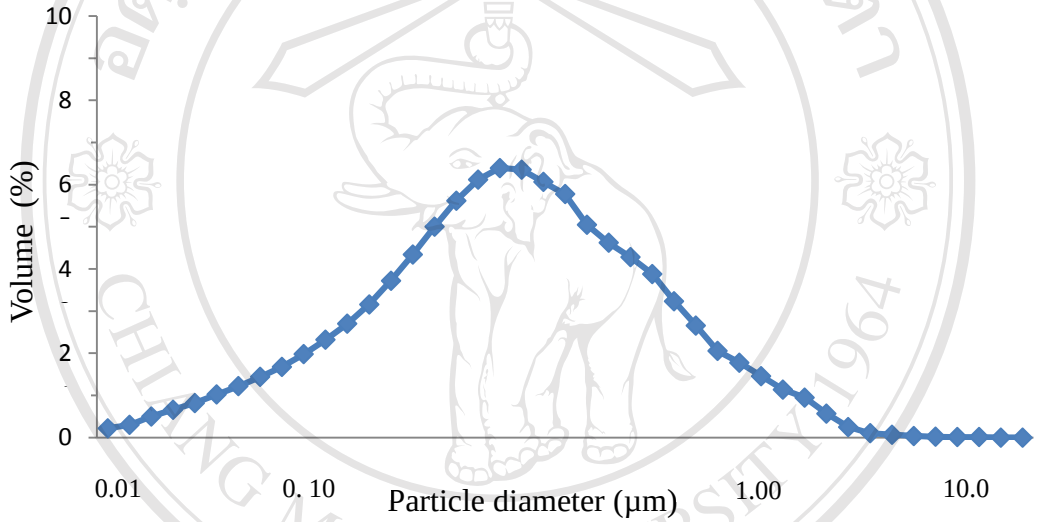


Figure 4.4 Particle size distributions of the 3.0% (w/v) carotenoids encapsulated in chitosan with 2.0% (w/v) TPP

3) Interstitial pore fraction

The effect of the TPP concentration was noticed on the study of interstitial pore fraction. Addition of higher concentration of TTP resulted in greater aggregation of the particles and consequently decreasing the interstitial pore fractions (Figure 4.5). The SEM images codified by Image J program indicated that the interstitial pore fractions were decreased from 14.79 to 8.29, 19.50 to 10.18, 14.17 to 3.52 and 17.20 to 7.06% for the matrixes loaded with 2.0, 3.0, 4.0 and 5.0% (w/v)

carotenoids, respectively. The effect of the TPP on decreasing the porous matrixes was confirmed by the SEM images (Appendix C2).

Increasing the TTP concentration induced the smaller size of the particles as shown in Figure 4.5. Then, the smaller size would induce a greater decrease in the interstitial pore fraction values. This might be because smaller size of the nanoparticles would increase ability in occupying the empty spaces of the porous chitosan matrix. This observation was in accordance with the study of Marcia *et al* (2009) who studied improving barrier and mechanical properties of the hydroxypropyl methylcellulose (HPMC) edible films with chitosan/tripolyphosphate nanoparticles. Marcia *et al* reported that the nanoparticles tend to occupy the empty spaces in the pores of the HPMC matrix and increasing the collapse of the pores.

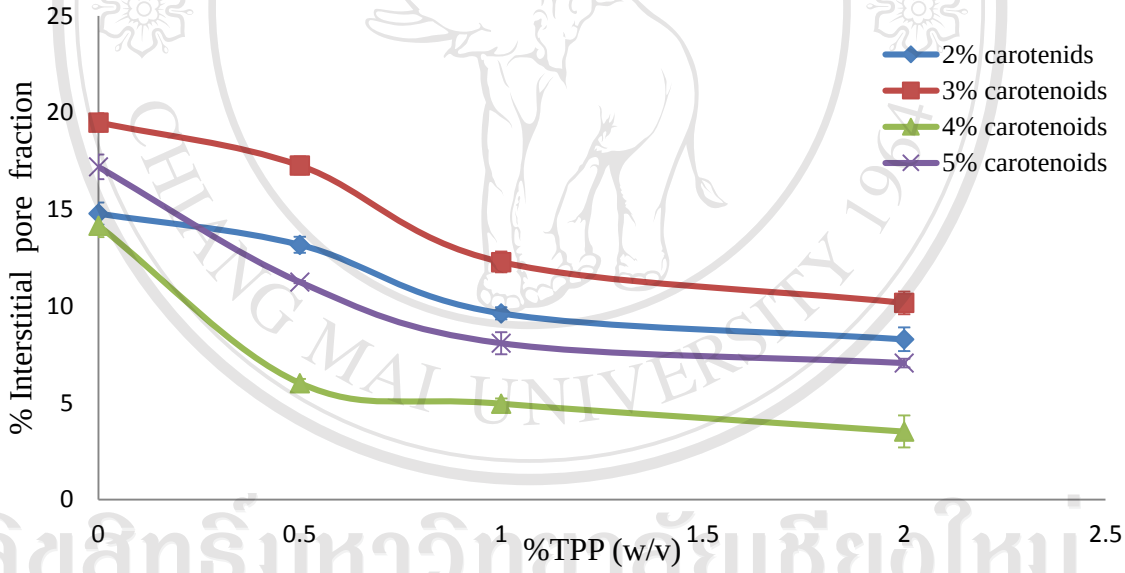


Figure 4.5 Interstitial pore fraction of carotenoids encapsulated chitosan-TPP

4.1.2 Encapsulation efficiencies

The encapsulation efficiencies of the chitosan matrixes with different percentage ratio of the encapsulated carotenoids were increased with the concentration of the TTP (Figure 4.6). The highest encapsulation efficiency of 89.09% was obtained with loading of 3.0% (w/v) carotenoids into the 2.0% (w/v) chitosan-TPP. The higher concentration

of the loading carotenoids did not provide higher encapsulation efficiency as expected. This phenomenon was also observed in other polymeric matrices that over loading of encapsulated material should cause a decrease of encapsulation efficiency (Liu and Park, 2009; Luo *et al.*, 2011; Shah *et al.*, 2009).The higher encapsulation efficiency resulted from an appropriate ratio of the interaction of hydrogen bonding between charged of NH_3^+ group in chitosan and carotenoids. When the concentration of carotenoids become restricted the amount of un-neutralized NH_3^+ groups presented, no further increased in the encapsulation efficiency (Konecsni *et al.*, 2012; Zhang and Kosaraju, 2007).

Ko *et al* (2002) reported the preparation of TPP with chitosan at 1.0, 5.0 and 10.0% (w/v) TPP to entrap felodipine, a drug used to control hypertension. The highest encapsulation efficiency of felodipine encapsulated chitosan-TPP was found with 10.0% (w/v) treatment. The highest encapsulation efficiency was 98.20% when 1.94% (w/w) felodipine was loaded. The lesser encapsulation efficiency of this study as compared to that found in the study of Ko and his team was possibly due to higher concentration of TPP used and the ratio of felodipine to the matrix (1:10) was less than that of carotenoids to the matrix in this study (1:5).

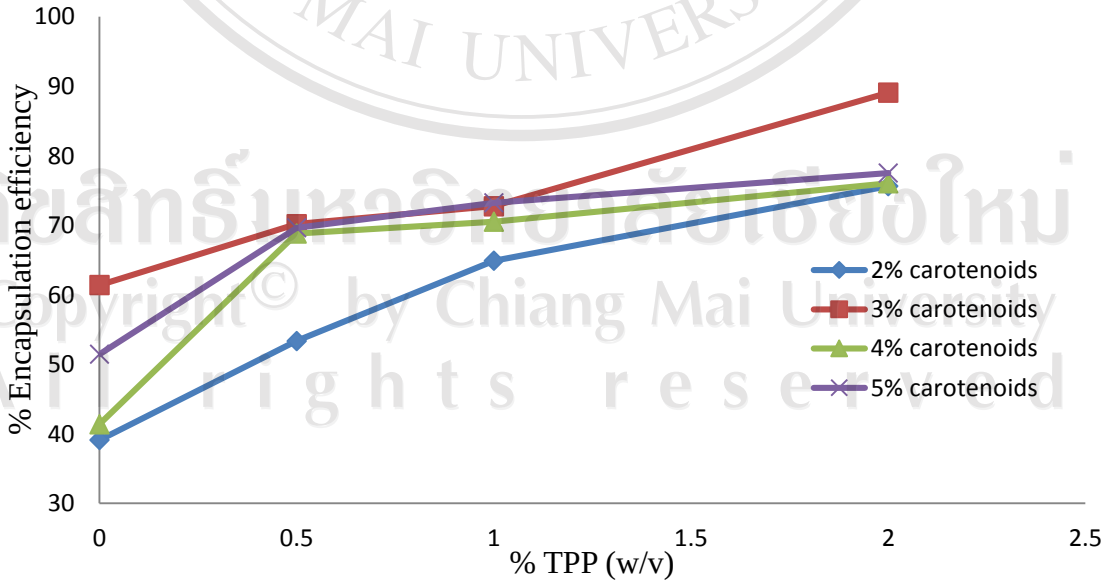


Figure 4.6 Encapsulation efficiencies of carotenoids encapsulated in chitosan-TPP

4.1.3 Color

The L*, a*, and b* values obtained from the chromameter were showed in Figure 4.7, 4.8 and4.9, respectively. All color values of all concentrations of carotenoids tend to increase with the concentration of TPP. According to the description, the L* was the lightness, while the positive a* and b* values was referred to red and yellow color, respectively. Thus, the chitosan-TPP prepared using 2.0% TPP and loaded 3.0% carotenoids exhibited the deepest orange shade. This result was in accordance with the encapsulation efficiency study, the highest load of carotenoids in chitosan-TPP matrix resulted in increasing of color value. Hence, color of the encapsulated sample could be used as the visual guidance for indicating encapsulation efficiency of carotenoids in the matrix.

Although the decrease of interstitial pore fractions was expected to decrease the light exposure between the particles and the L* values should be decreased. However, increasing L* values was observed in this study. The reason of increasing L* values could be due to the profound effect of the chitosan yellow colour.

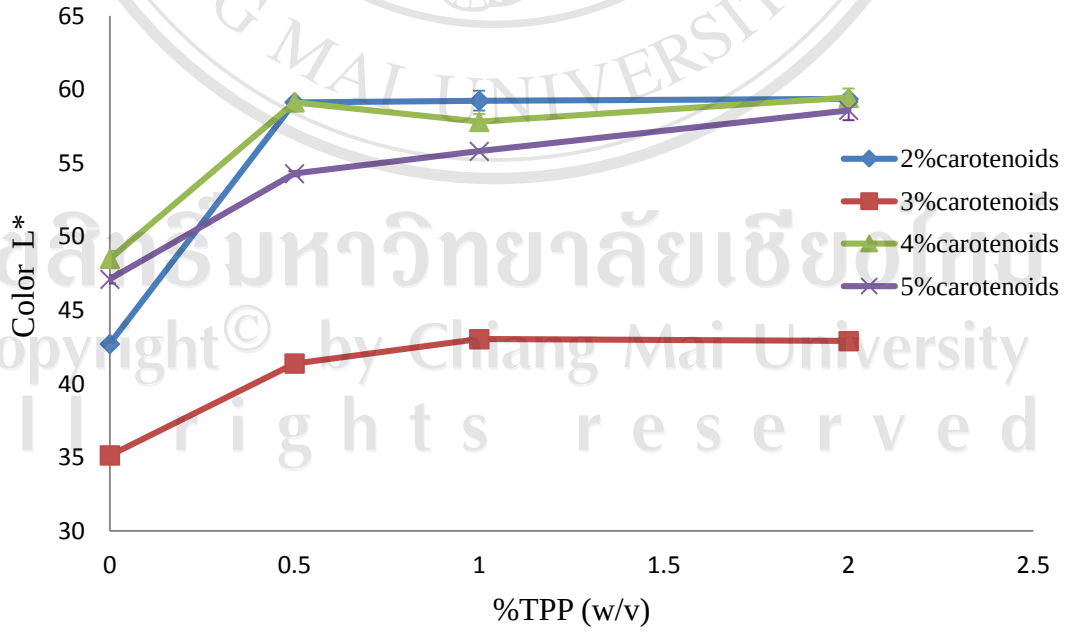


Figure 4.7 The L* values of carotenoids encapsulated in chitosan-TPP

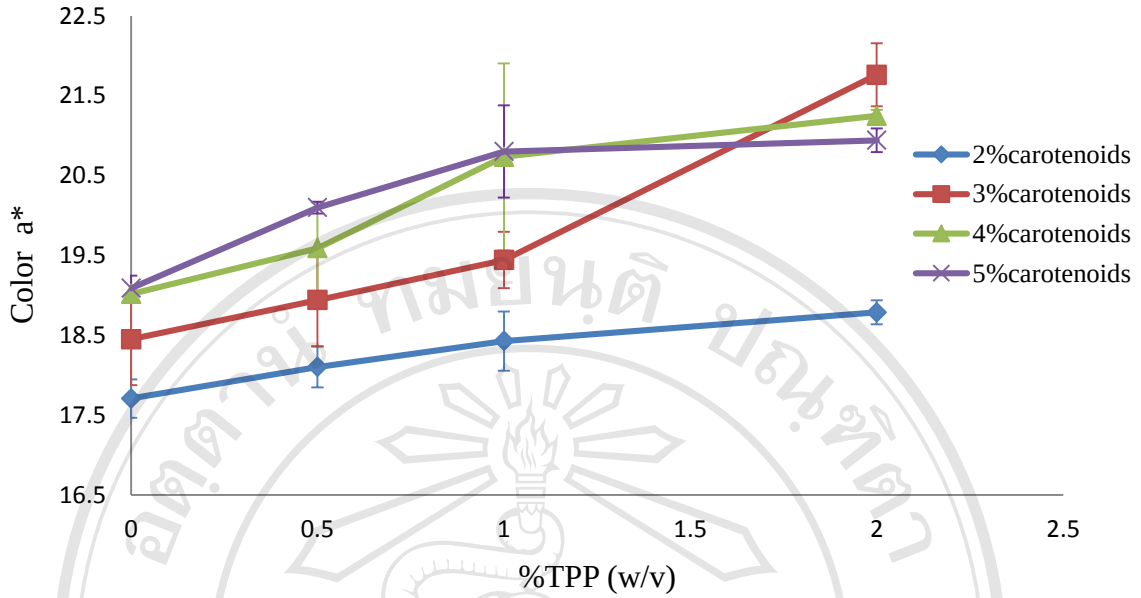


Figure 4.8 The a* value of carotenoids encapsulated in chitosan-TPP

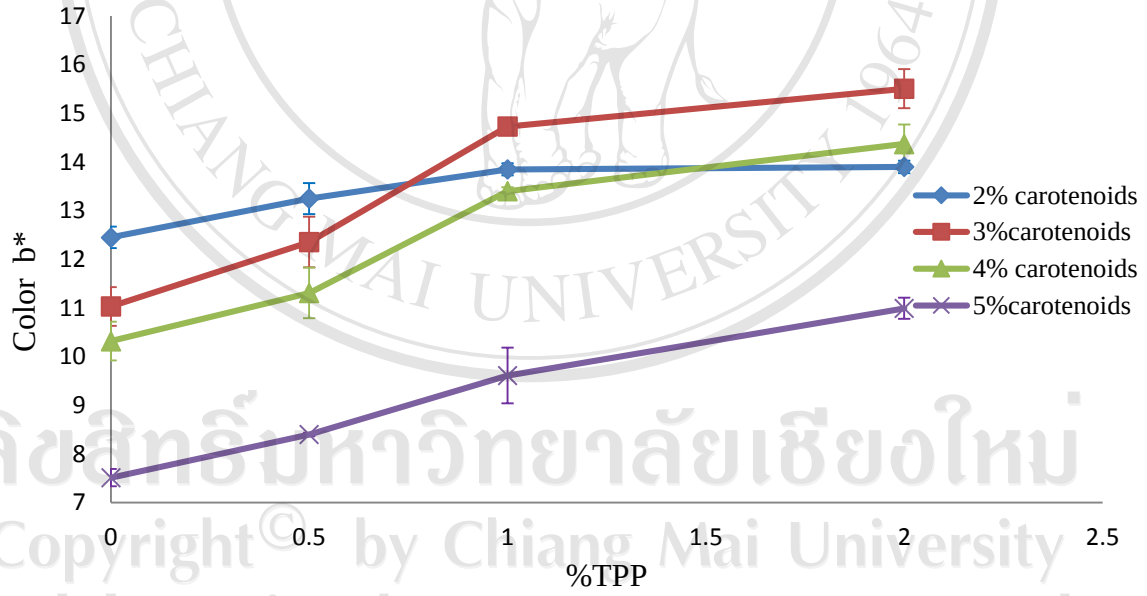


Figure 4.9 The b* value of carotenoids encapsulated in chitosan-TPP

4.1.4 Solubility

Figure 4.10 presented the solubility in ethanol of the 2.0, 3.0, 4.0 and 5.0% (w/v) carotenoids loaded into the 0.0-2.0% chitosan-TPP matrixes. It was found that those solubility values increased from 71.21-76.44%, 72.12-78.56%, 68.51- 77.42% and 68.85-75.40%, respective. The similar tendency was found for solubility test in water where the values of the 2.0, 3.0, 4.0 and 5.0% (w/v) carotenoids samples increased from 55.08-59.13%, 58.64-63.42%, 53.65-60.20% and 54.27-57.92%, respectively (Figure 4.11). The highest solubility percentage of both solvents was achieved with the 3.0% (w/v) carotenoids and the 2.0% (w/v) chitosan-TPP. This result indicated that solubility was affected by the particle size. The smaller size that obtained from adding higher amount of TPP increased the solubility.

The carotenoids in nature are lipophilic compounds which cannot dissolve in aqueous media. Advantageously, this study indicated the chitosan crosslinked TPP could improve the solubility of carotenoids in aqueous solution effectively. The improving solubility caused by the crosslinking between group I and NH_4^+ (cations) of chitosan with PO_4^{3-} (anions) of TPP (Harold and Charles, 2001; Ralph *et al.*, 2011).

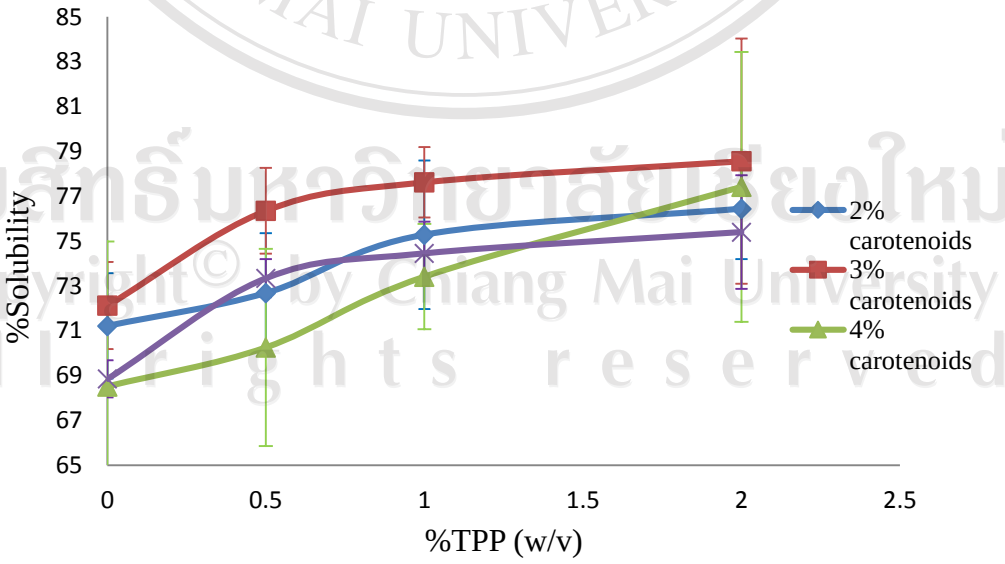


Figure 4.10 Solubility of the carotenoids encapsulated chitosan-TPP in ethanol

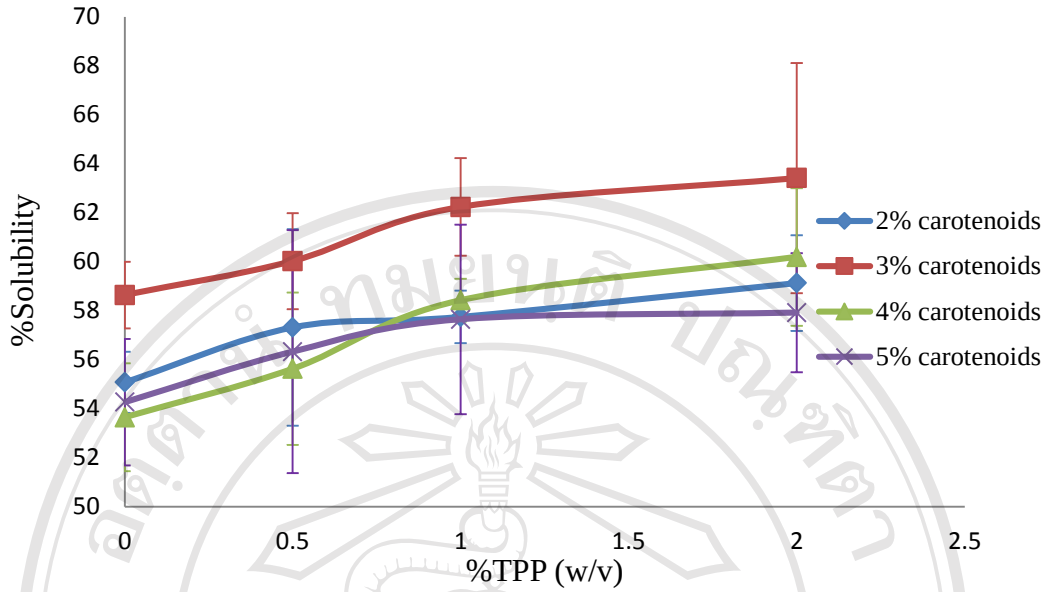


Figure 4.11 Solubility of the carotenoids encapsulated chitosan-TPP in water

4.2 Analysis of chemical composition of carotenoids encapsulated in chitosan-TPP

The complexes of carotenoids encapsulated in chitosan (CCS) and carotenoids encapsulated in chitosan-TPP (CCSTPP) were compared using the FTIR. Figure 4.12 showed peaks at 1739 cm^{-1} in CCS and 1738 cm^{-1} in CCSTPP which attributed to C=O of carbonyl group that was the important characteristic of β-carotene. In addition, the peaks at 2919 cm^{-1} in CCS and at 2920 cm^{-1} in CCSTPP attributed to C-H aliphatic, while those at 2321 cm^{-1} in CCS and 2105 cm^{-1} in CCSTPP attributed to the conjugated phenol group. This observation was in accordance with the study of Zaibunnisa *et al* (2011) who studied characterization of β-carotene complex and found the peak of β-carotene at 1718, 2928 and 2340 cm^{-1}.Chitosan demonstrated the peak at 2850, 1631.8, 1541, 1380 and 1068cm^{-1} in CCS and 2853, 1631.3, 1545, 1379 and 1069cm^{-1} in CCSTPP which attributed to CH_3 group, amide I groups, N–H, C–O of the primary alcoholic groups (–CH_2–OH), and C-O-C group, respectively. These results agreed with the findings of Aydin and Pulat, 2012; Hosseini *et al.*, 2013; Kafshgari *et al.*, 2011 and Lin *et al*., 2007.

The shift of peak at 1541 cm^{-1} in CCS to 1545 cm^{-1} in CCSTPP could be the result of the crosslinking process by phosphoric group of TPP and ammonium group of chitosan (Knaul *et al.*, 1999; Sowasod *et al.*, 2006). In addition, the peak of the conjugated phenol group in the CCS was increased from 2105 to 2321 cm^{-1} in the CCSTPP. This increase verified the higher concentration of carotenoids in the CCSTPP than that in the CCS. This result confirmed that the TTP could enhance the encapsulation of carotenoids effectively.

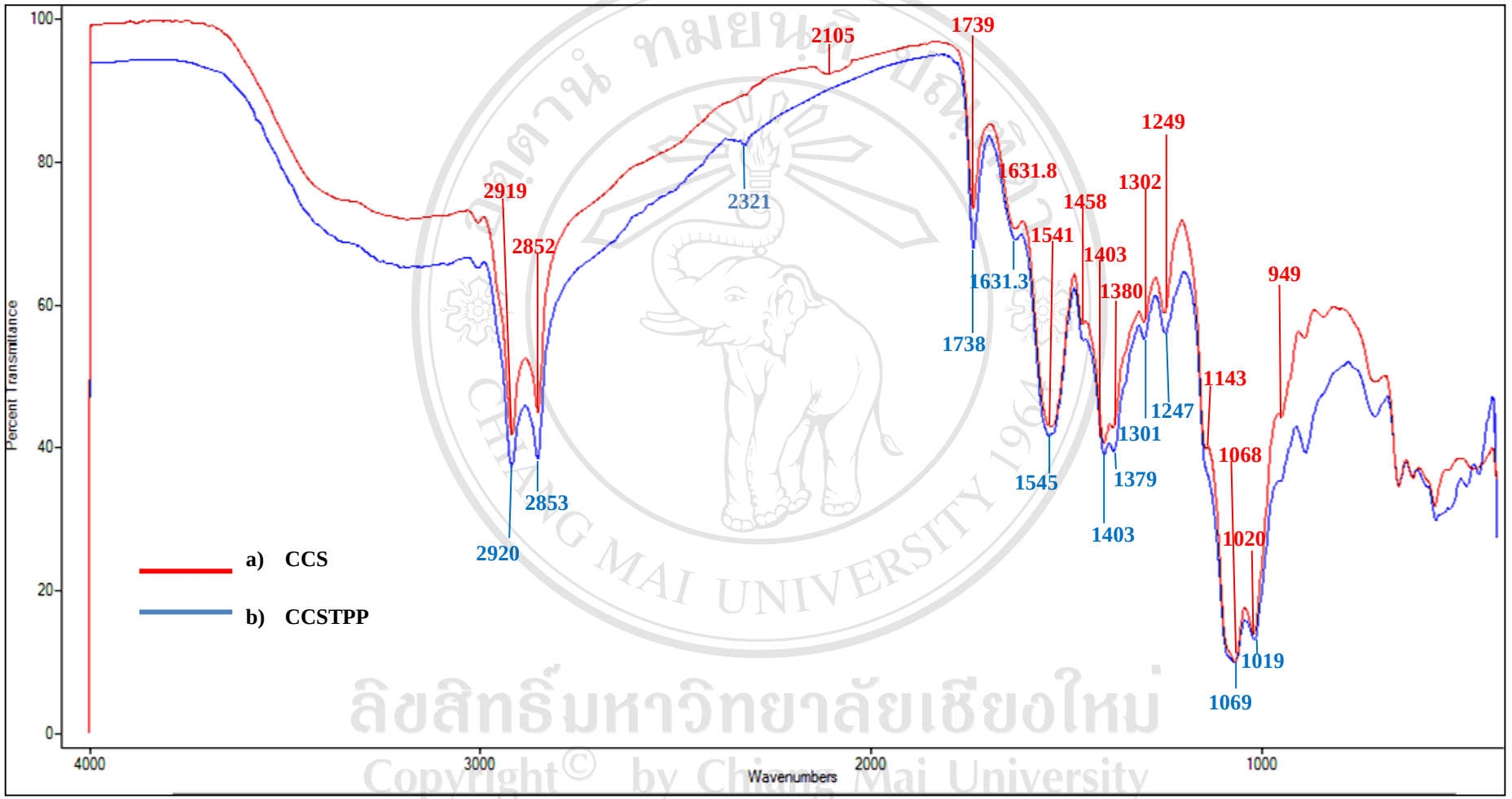


Figure 4.12 FTIR spectrums of carotenoids encapsulated in chitosan-TPP a) without TPP (CCS) b) with 2.0% (w/v) TPP (CCSTPP)

4.3 Releaseing property of carotenoids encapsulated in chitosan-TPP

The releasing properties of carotenoids encapsulated in chitosan-TPP were evaluated using coconut oil, ethanol and phosphate buffer (PBS) at pH 7.4. The sampling procedure was carried out continuously until the concentrations of carotenoids in the tested solvents were constant, which were taken about 420 min. As shown in Figure 4.13 and 4.14, releasing patterns of control (commercial carotenoids; not encapsulated) and encapsulated carotenoids in all solvents were relatively similar. The maximum releasing of 6.57 ppm of the control carotenoids and 6.55 ppm of the encapsulated carotenoids were observed in coconut oil at 180 min. In ethanol, the release of control carotenoids was 5.42 ppm, while that of the encapsulated carotenoids was 5.24 ppm at 180 and 240 min, respectively. In PBS, the release of control carotenoids was 2.08 ppm, and that of the encapsulated carotenoids was1.95 ppm at 300 min. This result demonstrated that the releasing efficiency in oil was superior to ethanol and PBS. However, the releasing properties of the carotenoids encapsulated in chiotsan-TPP and the commercial carotenoids were rather similar. The discrepancy could be due to the effect of the encapsulation matrix. Matalanis *et al.*, (2011) reported that the primary factor that controlled the releasing characteristic was the encapsulation matrix.

The previous releasing study of carotenoids from the chitosan-TPP has not been reported in the literature. However, releasing rate of any bioactive core material generally should be effected by many factors including properties of coating materials such as molecular weight/molar ratio of chitosan, quantity of TPP and the assay employed. Recently, McConnell *et al*. (2008) reported that chitosan films prepared without TPP were completely degraded when incubated at 37˚C with pancreatin enzymes, whereas in its presence, only partial degradation occurred to a degree and it depended upon the level of TPP added. Moreover, Lin *et al*. (2008) reported that chitosan-TPP particles become unstable and start breaking down at pH > 7.2 due to the deprotonation of the amino group of chitosan.

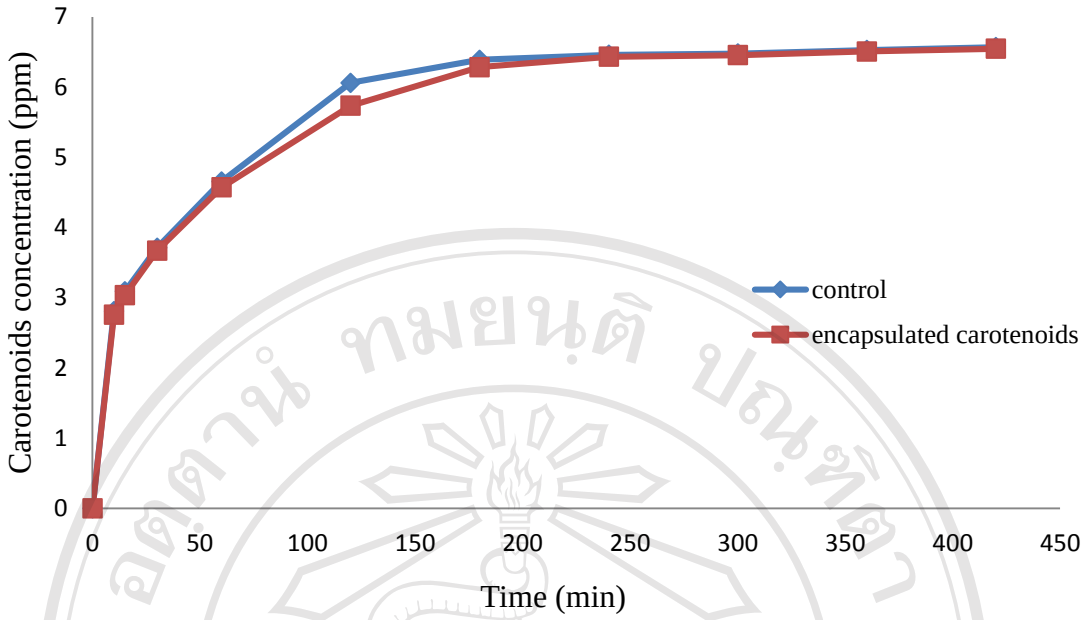


Figure 4.13 Releases of carotenoids encapsulated in chitosan-TPP in oil

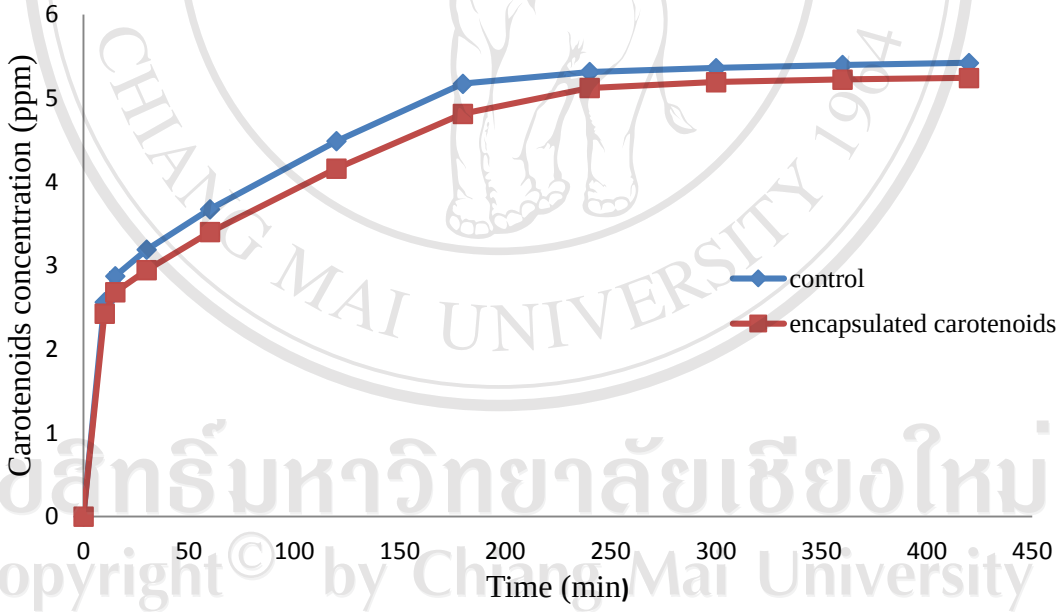


Figure 4.14 Releases of carotenoids encapsulated in chitosan-TPP in ethanol

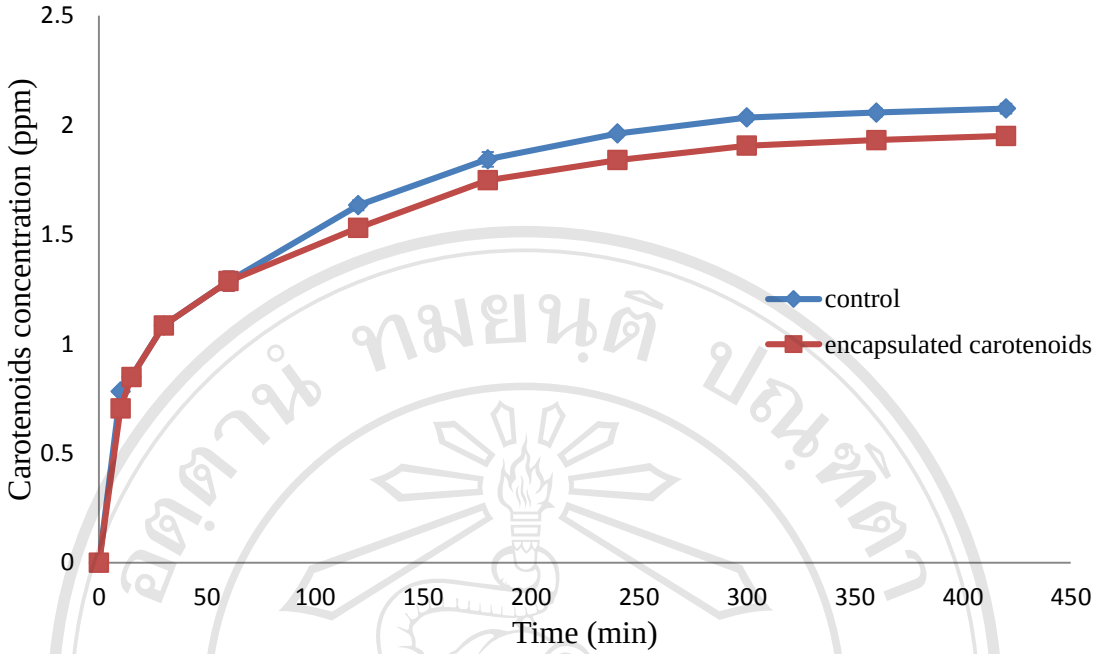


Figure 4.15 Releases of carotenoids encapsulated in chitosan-TPP in PBS

4.4 Storage stability of the carotenoids encapsulated in chitosan-TPP

The 3.0% (w/v) carotenoids encapsulated in chitosan cross linked with 2.0% (w/v) TPP was selected for this study because this formula provided the highest encapsulation efficiency ($P \leq 0.05$) as shown in section 4.1.2.

4.4.1 The retention of carotenoids encapsulated in chitosan-TPP during storage

Retentions of the carotenoids encapsulated in the chitosan-TPP were observed at 5, 25, and 40°C for 60 days and it was found that the concentration of 9.69 ppm decreased to 6.29, 4.95 and 2.69 ppm, respectively. This result suggested that keeping the encapsulated carotenoids at lower temperature would prolong shelf-life of the product considerably.

The correlation coefficient of the plots was used as a parameter to determine the reaction order (Table4.1). The zero order obtained from the plot of the retention of carotenoids concentration versus time (Figure 4.16). The first order obtained from the plot of the natural log of the retention of carotenoids concentration versus time (Figure 4.17). The seconds order obtained from the plot of the 1/ retention of carotenoids concentration versus time (Figure 4.18). The third order obtained from the plot of the 1/ (retention of carotenoids concentration)^2 versus time (Figure 4.19), and the square root order obtained from the plot of the retention of carotenoids concentration versus square root time (Figure 4.20).

All data at different temperature were best fit by a first order kinetic model ($\ln C = \ln Co - k\,(t)$). Degradation rate constants (k) were obtained from the slope of a plot of the natural log of the retention of carotenoids concentration versus time (Figure 4.17). The activation energy (E_a) and frequency factor (A) were determined from the Arrhenius model $k = Ae^{-(Ea/R)/T}$, where E_a/R was the slope and lnA was the intercept of the relationship between the natural log$k$ and (1/T) in degrees Kelvin (Table 4.2).

For a first order reaction, the half-life was determined at a specific temperature by the equation $t1/2 = \ln 2/k$. The enthalpy of activation ($\Delta H^{\neq}$) was obtained by plotting ln (k/T) vs. (1/T), and the entropy of activation ($\Delta S^{\neq}$) was obtained from Equation $\ln (k/T) = \ln (k_B/h) + \Delta S{\neq}/R - \Delta H{\neq}/RT$, based on the transition state theory, Where k_B is the Boltzmann constant, h is Planck's constant and R is gas constant.

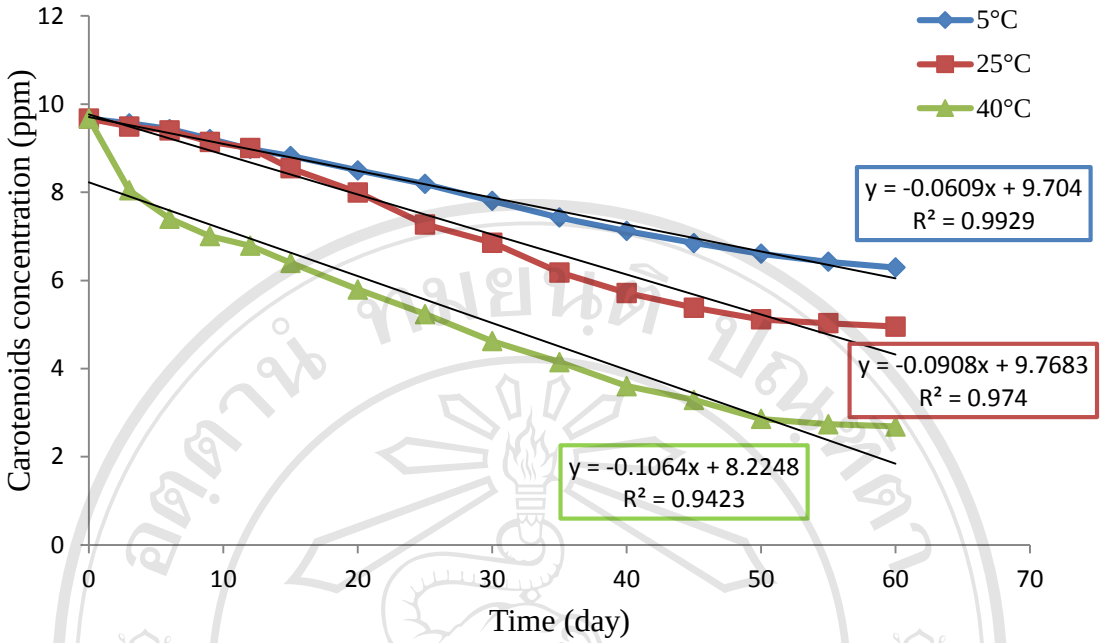


Figure 4.16 Zero order degradation plots of carotenoids encapsulated in chitosan-TPP

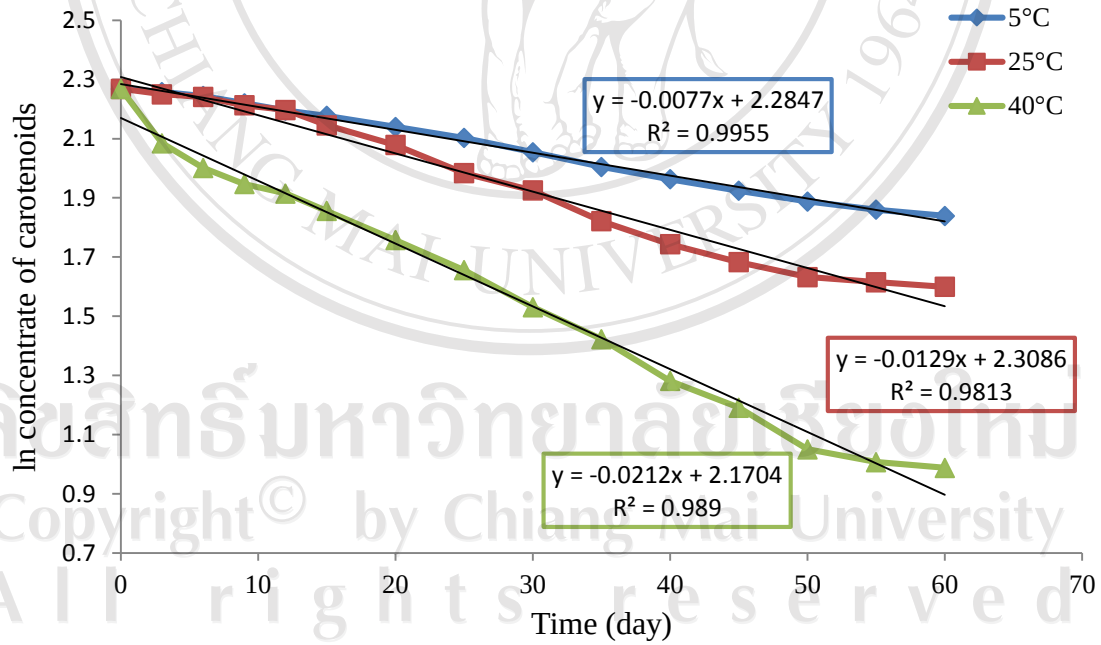


Figure 4.17 First order degradation plots of carotenoids encapsulated in chitosan-TPP

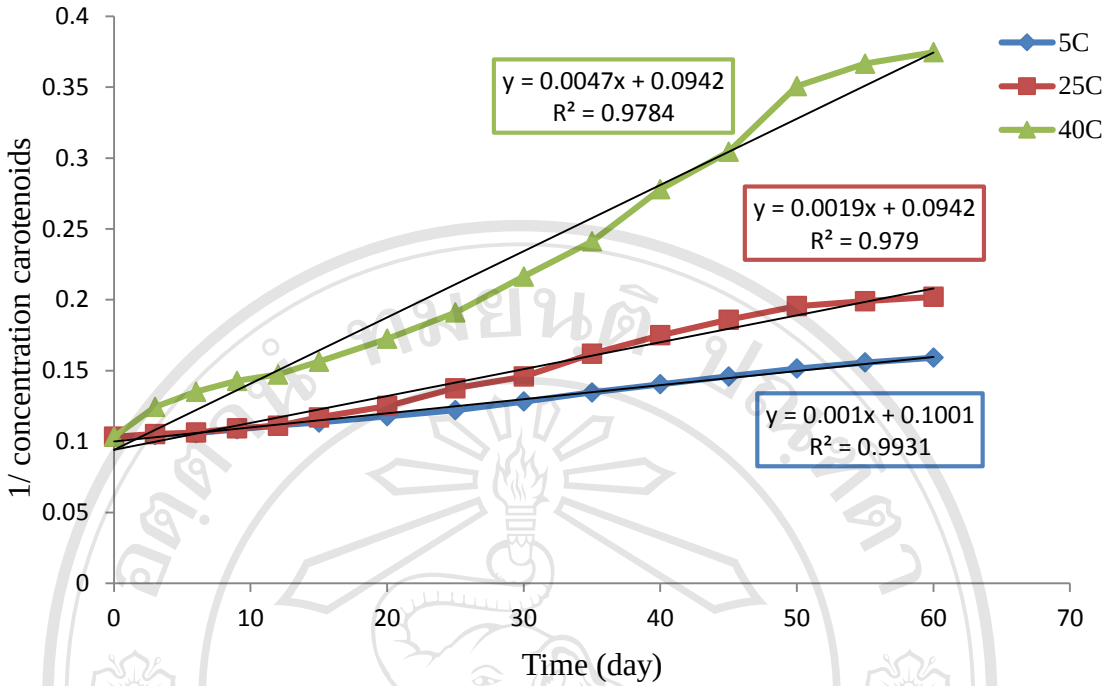


Figure 4.18 Second order degradation plots of carotenoids encapsulated in chitosan-TPP

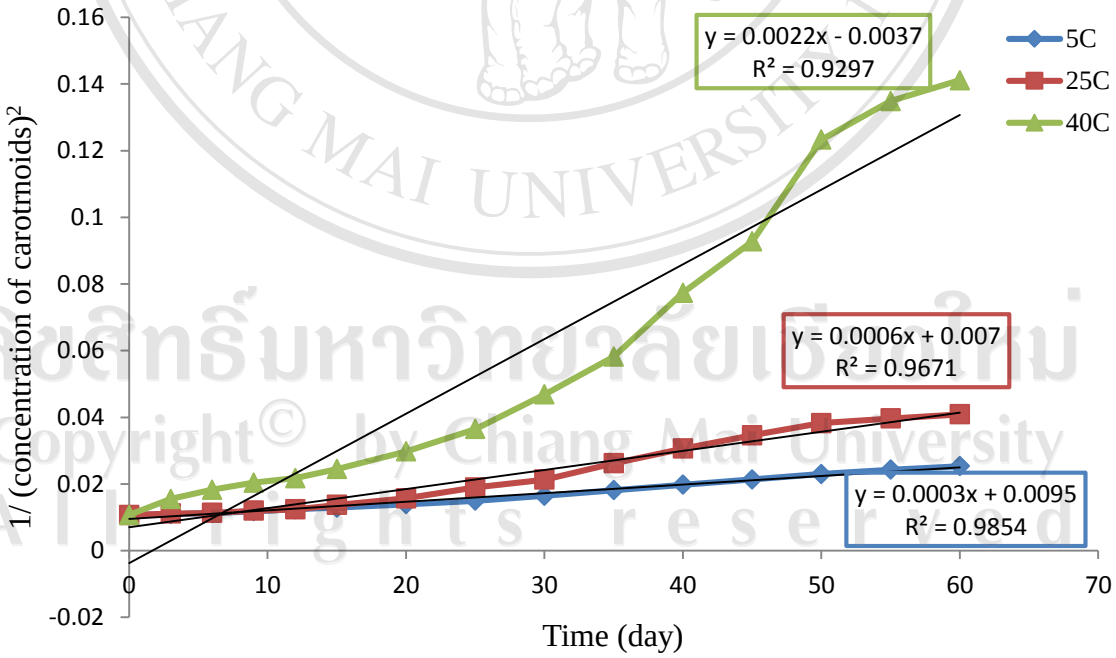


Figure 4.19 Third order degradation plots of carotenoids encapsulated in chitosan-TPP

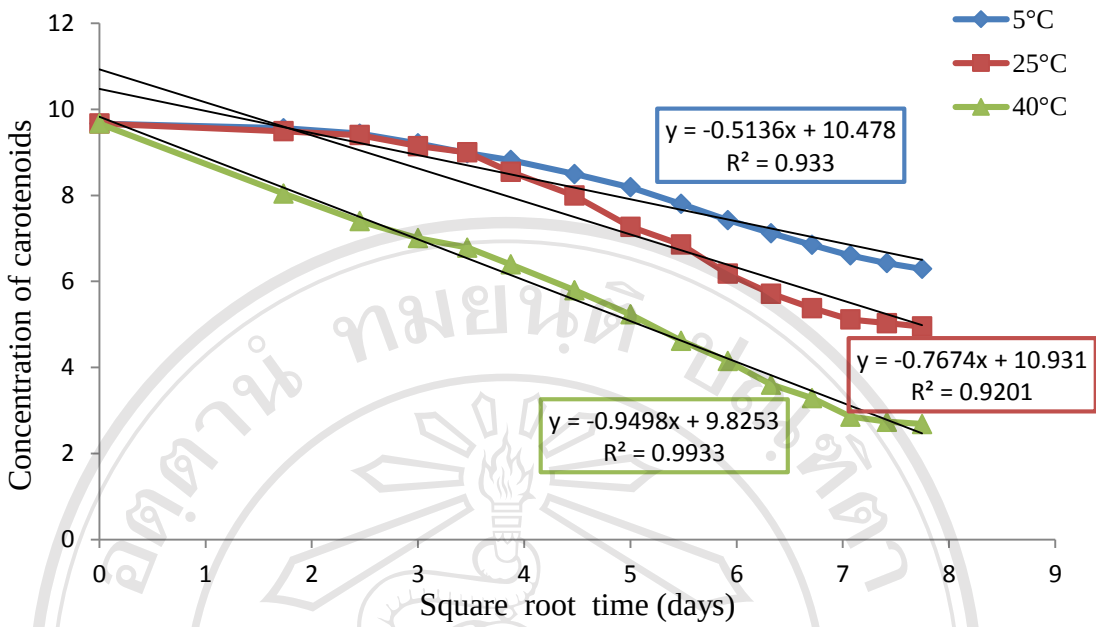


Figure 4.20 Square order degradation plots of carotenoids encapsulated in chitosan-TPP

Table 4.1 The values of R-square obtained from the trend lines of zero order, first order, second order, third order and square root order plots of carotenoids degradation during storage at 5, 25 or 40°C for 60 days

Temperature (°C)	R-square (R^2)				
	Zero order	First order	Second order	Third order	Square root order
5	0.992	0.995	0.993	0.985	0.933
25	0.974	0.981	0.979	0.967	0.920
40	0.942	0.989	0.978	0.929	0.993

Table 4.2 Arrhenius parameters and thermodynamic parameters of carotenoids encapsulated chitosan-TPP

Activation energy[a] : E_a (kJ/mol)	Intercept : In*A*(day^{-1})	R^2 (Arrhenius plot of In*k*/T versus 1/T)	Enthalpy of activation[b] : $\Delta H^{\neq}$(kJ/mol)	Entropy of activation[b] :$\Delta S^{\neq}$(J/mol·K)	R^2 (Arrhenius plot of carotenoids concentrations in encapsulated sampleversus Time)
20.66 ± 1.27	4.0460 ± 0.5342	0.9886	18.21 ± 1.26	-219.49 ± 4.44	0.9864

[a]Values were obtained from slopes of Arrhenius plots.(In*k*/T versus 1/T)

[b]Values were obtained from transition state theory equations. *A*, frequency factor.

Degradation rate constants (*k*) is the reaction rate depends on temperature. The speed of reaction for a reactant or product in a particular reaction is intuitively defined as how fast or slow a reaction takes place. As expected, the increased storage temperatures lead to increasing *k* value. Previous researchers have showed *k* value at 25°C of β-carotene without encapsulation was 0.069 day^{-1} (Spada *et al*, 2012). Vera *et al* (2007) reported *k* values at 40°C of β- and α-carotene in the dehydrated carrot with 0.0520 to 0.7520 a_w were between 0.1300 to 0.0400 and 0.1230 to 0.0310 day^{-1}, respectively. Spada *et al* (2012) reported *k* values at 25°C of β-carotene encapsulated in native starch, 6DE starch and 12DE starch by freeze drying which were 0.0760, 0.0310 and 0.0170 day^{-1}, respectively. In addition, the *k* value at 25°C of β-carotene encapsulated by freeze drying in maltodextrin was 0.440 day^{-1} (Desobry *et al*, 1997).

The *k* values at various temperature of carotenoids encapsulated in chitosan in this study were relatively less than those obtained from the previous study. This suggested that encapsulation of carotenoids in the chitosan-TPP was a novel potential method that could improve the stability of carotenoids effectively.

Half-life is the amount of time required for a quantity to fall to half its value as measured at the beginning of the time period. Thus, half-life can be used to describe any quantity which follows an exponential decay. The previous works indicated that there were several factors influenced shelf-life of encapsulated carotenoids. For example, the presence of lipid in the encapsulated product would promote their degradation because carotenoids act as antioxidants through free radical scavengers (Robert *et al*, 2003). In the difference temperature, the half-life values of encapsulated carotenoids were 89.63, 49.44 and 32.66 days at 5, 25 and 40°C, respectively. The half-life values at 25°C of β-carotene without encapsulation was 10 days (Spada *et al*, 2012) and that of the encapsulated β-carotene obtained from freeze drying in 25DE maltodextrin, was 34.5 days at 25°C (Desobry *et al*, 1997). This study found the half-life value at 25°C of carotenoids encapsulated in chitosan-TPP was more than those of the β-carotene without encapsulation process and the β-carotene encapsulated by freeze drying in 25DEmaltodextrin.

Table 4.3 Degradation rate constants (*k*) and half-life values of carotenoids encapsulated chitosan-TPP

Temperature (°C)	Degradation rate constants : *k* (day^{-1})	Half-life (days)
5	0.0077 ± 0.0001	89.63 ± 1.35
25	0.0129 ± 0.0004	49.44 ± 1.57
40	0.0213 ± 0.0012	32.66 ± 1.91

The Arrhenius parameters and thermodynamic parameters for carotenoids encapsulated in chitosan were showed in table 4.2. The activation energy (E_a) is defined as the minimum energy that must be put into a chemical system, containing potential reactants, in order for a chemical reaction to occur. In this study the E_a of carotenoids encapsulated in chitosan-TPP at 5 to 40°C was 20.66 kJ/mol. Robert *et al* (2003) reported the E_a at 25 to 55°C of carotenoids encapsulated in starch was 52.16 kJ/mol. The possible explanation that the E_a of this study revealed less than that reported by Robert *et al* (2003)might due to the difference of the studied temperature as well as alteration of the wall particles.

The enthalpy of activation ($\Delta H\neq$) and entropy of activation ($\Delta S\neq$) were obtained from the slope and intercept value, respectively, from a plot of ln (k/T) versus $1/T$. In general $\Delta H\neq$ is a measure of energy barrier that must be overcome by reacting molecules and is related to the strength of the bonds, which are broken and made in the formation of the transition state from the reactants (Vikram *et al.*, 2005). The $\Delta H\neq$ of carotenoids encapsulated in chitosan-TPP was 18.21 kJ/mol, which was less than those of β-carotene encapsulated in starch 52.70 kJ/mol and gelatin77.79kJ/mol (Robert *et al*., 2003).

The $\Delta S\neq$ of carotenoids encapsulated in chitosan- TPP had a negative value of -219.49 J/mol. In general $\Delta S\neq$ is related to the number of molecules with appropriate energy that can actually react. The value of $\Delta S\neq$ also includes steric and orientation requirements along with solvent effects (Vikram *et al.,* 2005). The negative value of $\Delta S\neq$ indicated that entropy decreased upon achieving the transition state, which often

indicated an associative mechanism. Robert *et al* (2003) reported the entropy of activation of β-carotene encapsulated by spry drying in starch and gelatin of -175.66 and -104.56 kJ/mol, respectively. The entropy of activation of carotenoids encapsulated in this study was less than those obtained by Robert *et al* (2003). This result suggested that the chitosan-TPP matrix could protect carotenoids better than the starch and gelatin.

4.4.2 Antioxidant properties of carotenoids encapsulated chitosan-TPP during storage

The antioxidant properties of encapsulated carotenoids during storage were tested by the $ABTS^+$ method (Roberta *et al.,* 1999). The results were presented as the percent $ABTS^+$ inhibition. On the first day, the inhibition of the encapsulated carotenoids was 50.34%. Then, the inhibitions of all samples storage at all various temperatures were gradually decreased. On the 60 days, the inhibitions of samples storage at 5, 25 and 40 °C were decreased to 41.50, 29.00 and 18.62%, respectively (Figure 4.21).

Degradations of the encapsulated carotenoids followed the first order kinetic as shown in Figure 4.22. The degradation rate constants (k) of $ABTS^+$ inhibition of the encapsulated carotenoids were increased with increasing temperature (Table 4.4). Although, the chitosan-TTP matrix would act as a permeability barrier, depressing oxygen transfer from the surrounding environment onto the carotenoids, however, temperature seemed to have the pronounce effect on the property of carotenoids. This study suggested that the encapsulated carotenoids should be kept at the refrigeration temperature.

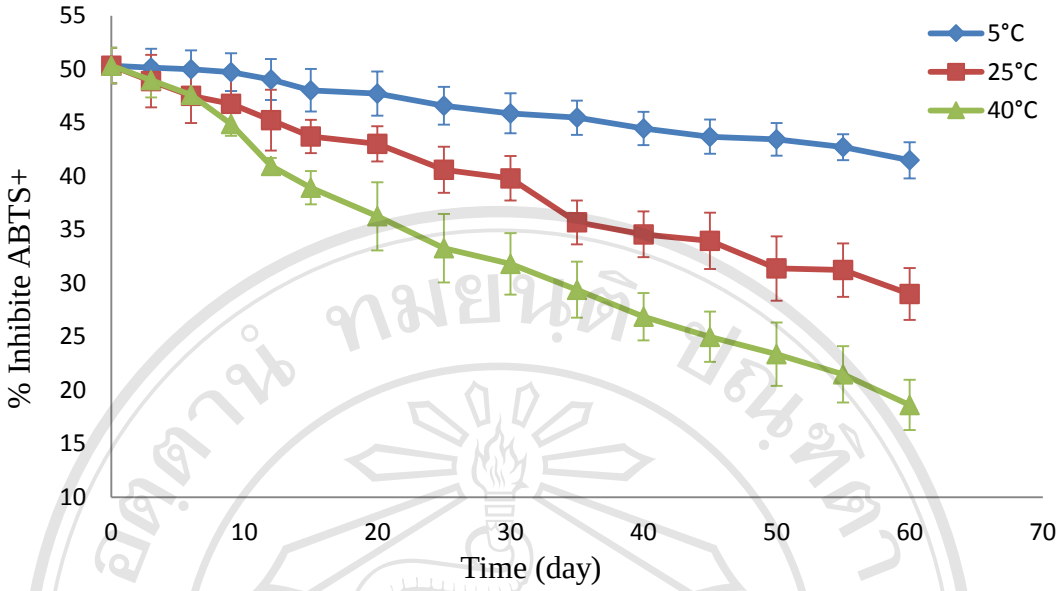


Figure 4.21 ABTS^{+} inhibition of carotenoids encapsulated in chitosan-TPP

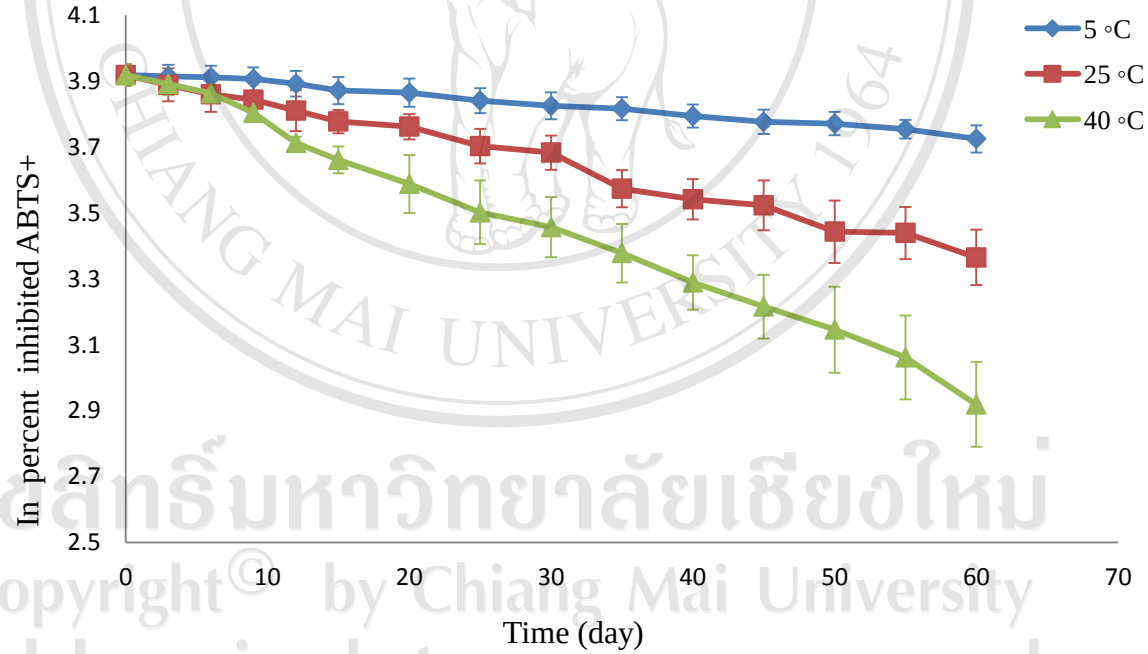


Figure 4.22 First order plots of ABTS^{+} inhibition of carotenoids encapsulated in chitosan-TPP

Table 4.4 Degradation rate constants (*k*) of $ABTS^{+}$ inhibition of carotenoids encapsulated chitosan-TPP

Temperature(°C)	*k* (day^{-1})
5	0.0033 ± 0.0002
25	0.0092 ± 0.0007
40	0.0161 ± 0.0025

4.4.3 Color stability of carotenoids encapsulated in chitosan-TPP during storage

During 60 days storage, the L* values (ranging from 0 to 100) or lightness increased with time and the increasing was more pronounced at higher storage temperature. In contrast, the a* and b* values which the value was positive for red and yellow colors, respectively, showed increasing with storage time (Figure 4.23, 4.25 and 4.27).Changes of the color parameters could be due to the oxidation of carotenoids during storage at various temperatures. As expect, the changes of color parameters would follow the first order kinetic (Figure4.24, 4.26 and 4.28). Table 4.5 presented the degradation rate constants (*k*) as well as the *k* values of L*, a* and b*. Sonia *et al* (2007) prepared β-carotene encapsulated in a mannitol matrix by freeze drying and stored at 25°C at several relative humidities (RH). The color of sample at RH of 11% and 44% consisted of an initial phase of fast loss. The degradation of color was best fit in the first order kinetic. The *k* value of a* value were $0.111day^{-1}$ at 11% RH and 0.061 day^{-1} at 44% RH. In this study, the *k* value of a* value of the carotenoids encapsulated in chitosan-TPP was 0.0056 day^{-1} at 25°C. These information confirmed the greater stability of carotenoids in the chitosan-TPP matrix.

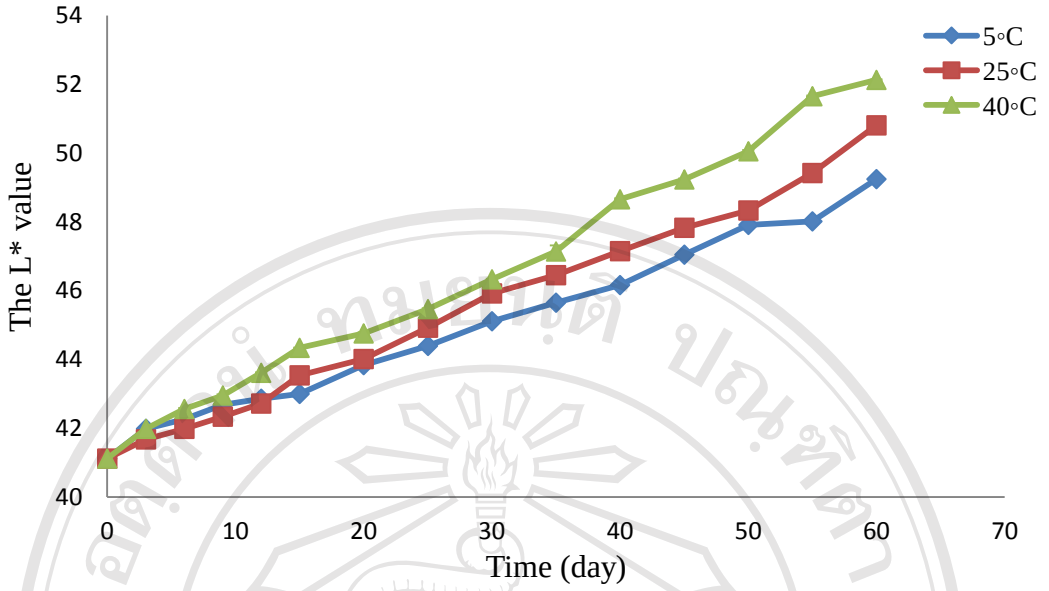


Figure 4.23 The L* values of carotenoids encapsulated in chitosan-TPP during storage at various temperatures

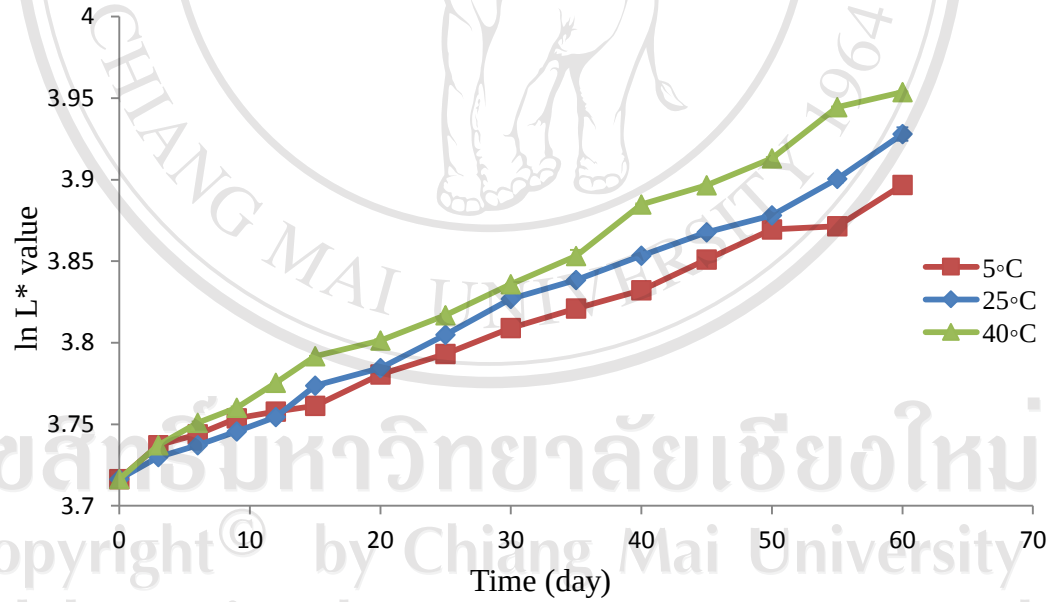


Figure 4.24 First order degradation plots of L* values of carotenoids encapsulated in chitosan-TPP

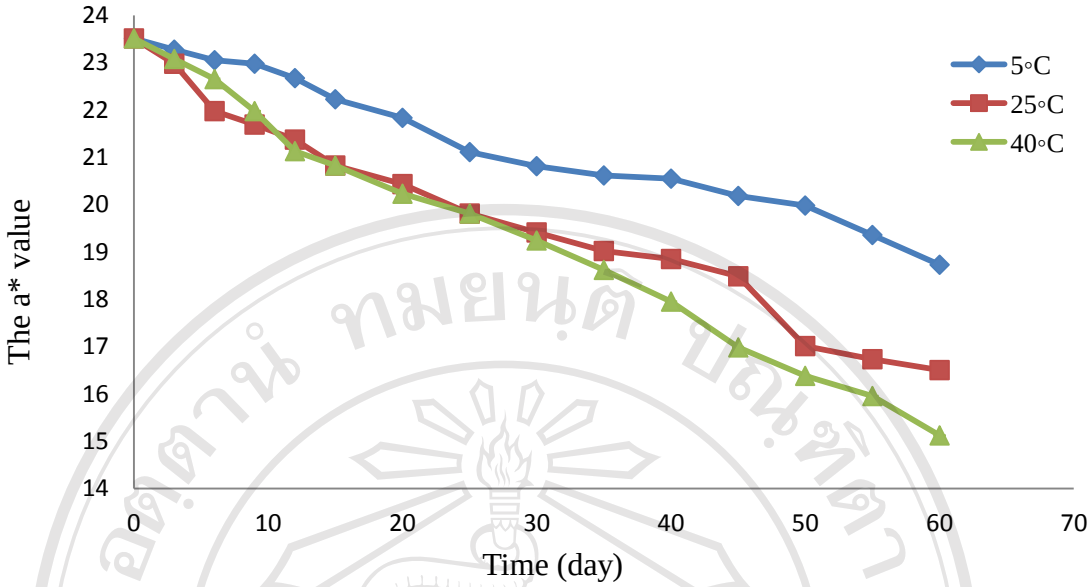


Figure 4.25 The a* values of carotenoids encapsulated in chitosan-TPP during storage at various temperatures

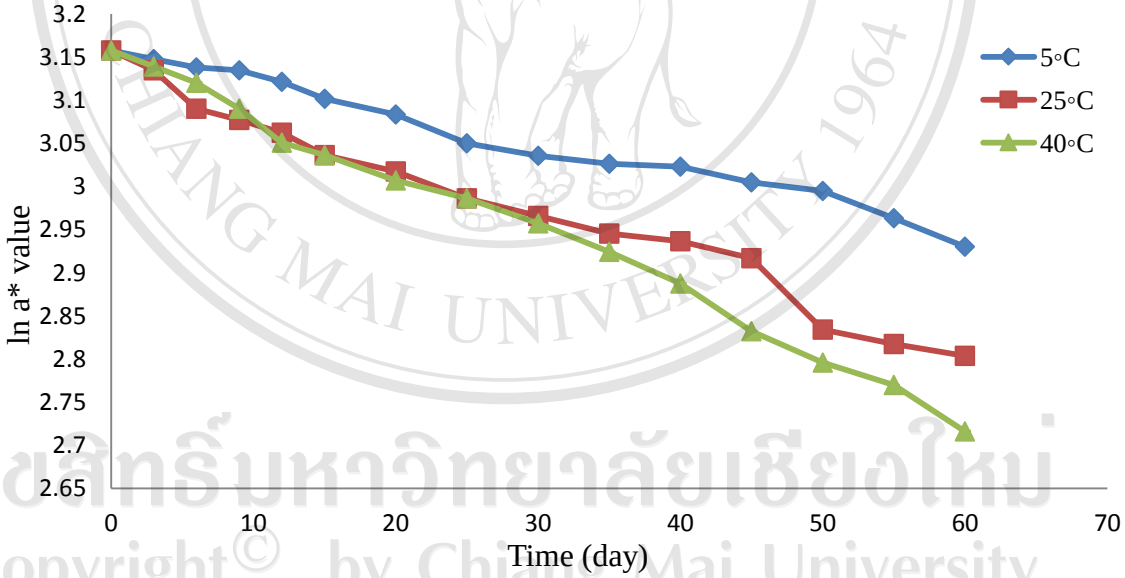


Figure 4.26 First order degradation plots of a* value of carotenoids encapsulated in chitosan-TPP

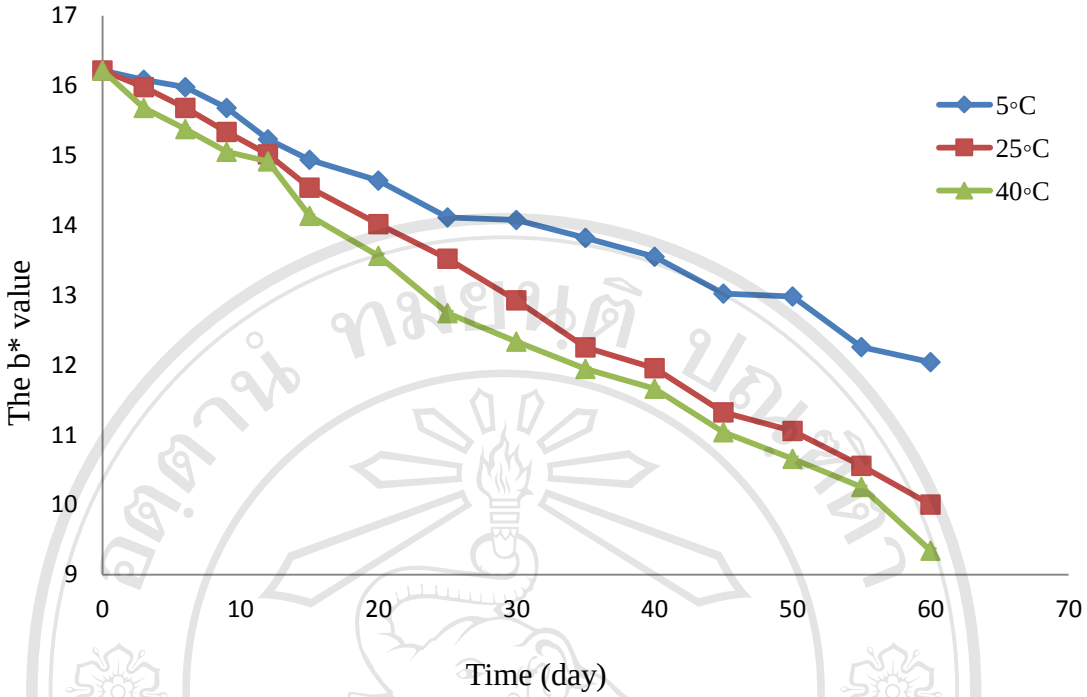


Figure 4.27 The b* values of carotenoids encapsulated in chitosan-TPP during storage at various temperatures

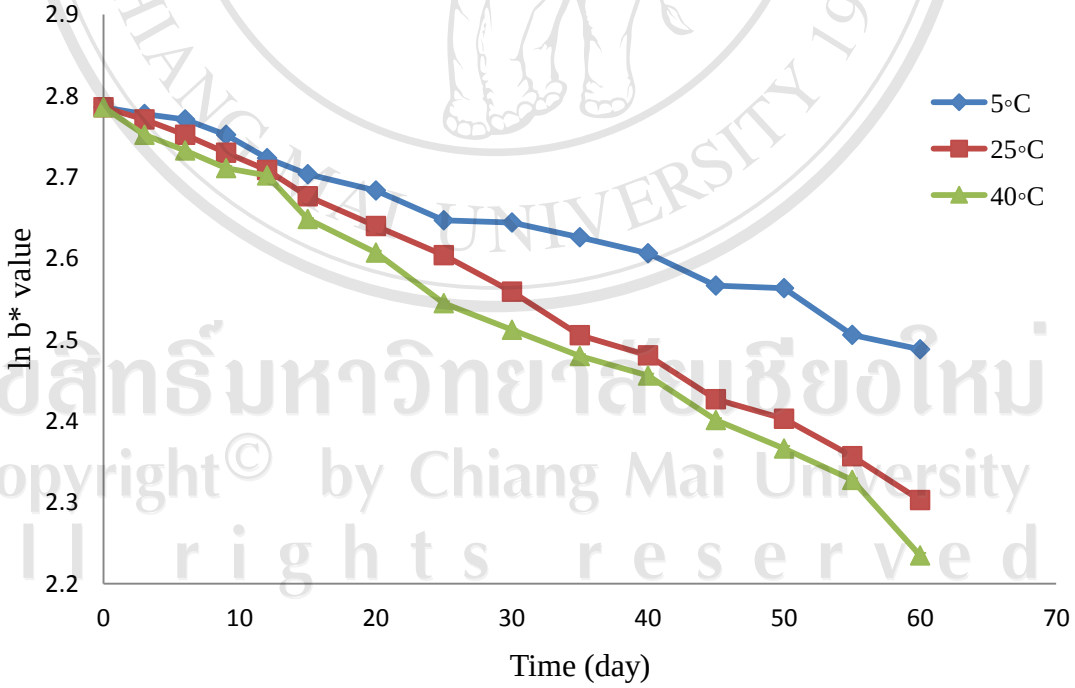


Figure 4.28 First order degradation plots of b* values of carotenoids encapsulated in chitosan-TPP

Table 4.5 Degradation rate constants (*k*) of color parameters carotenoids encapsulated in chitosan-TPP

Temperature (C°)	*k* of L* value (day^{-1})	*k* of a* value (day^{-1})	*k*ofb* value (day^{-1})
5	0.0028 ± 0.0000	0.0036 ± 0.0000	0.0049 ± 0.0000
25	0.0034 ± 0.0000	0.0056 ± 0.0000	0.0081 ± 0.0000
40	0.0038 ± 0.0001	0.0071 ± 0.0000	0.0087 ± 0.0001

4.5 Application of carotenoids encapsulated in chitosan-TPP as food colorant

The major purpose of this research was to improve stability of carotenoids by encapsulation with the novel chitosan-TPP and then determining the application in food product. For food colorant study, the 3.0% (w/v) carotenoids encapsulated in 2.0% (w/v) chitosan-TPP was selected as colorant in the white commercial salad cream and the commercial drink made from the clear concentrated grape juice. The salad cream was selected as the lipid based model food, while the commercial drink was used as the aqueous based model food.

4.5.1 Salad cream

The Food and Drug Administration (2010) has controlled the maximum quantity of carotenoids in salad cream and mayonnaise of 155.8 ppm. In this experiment, 133.34 ppm of carotenoids was adequate to provide the color of salad cream. The visual colors of the commercial salad cream, salad cream mixed with the encapsulated carotenoids and salad cream mixed with the control carotenoids were white, orange and yellow as presented in Figure 4.29. Therefore, the encapsulated carotenoids achieved higher a* and less b* as compared to those values of the control sample (Table 4.6).

Color of the salad cream mixed with the encapsulated carotenoids slightly increased with time. Then, the observed colour was constant at the storage time of 3

hr (Table 4.6). This minor increase suggested the fast releasing of carotenoids from the chitosan-TPP matrix and the result agreed with the releasing property in coconut oil that the carotenoids would take 3 hr to completely release from the particle. In addition, separation of salad cream mixed with carotenoids color was not appeared after keeping at room temperature for 7 hr. Also, addition of the commercial or encapsulated carotenoids did not affect viscosity of the salad cream (Table 4.7). The prepared carotenoids would be used as colorants in food system satisfactory.

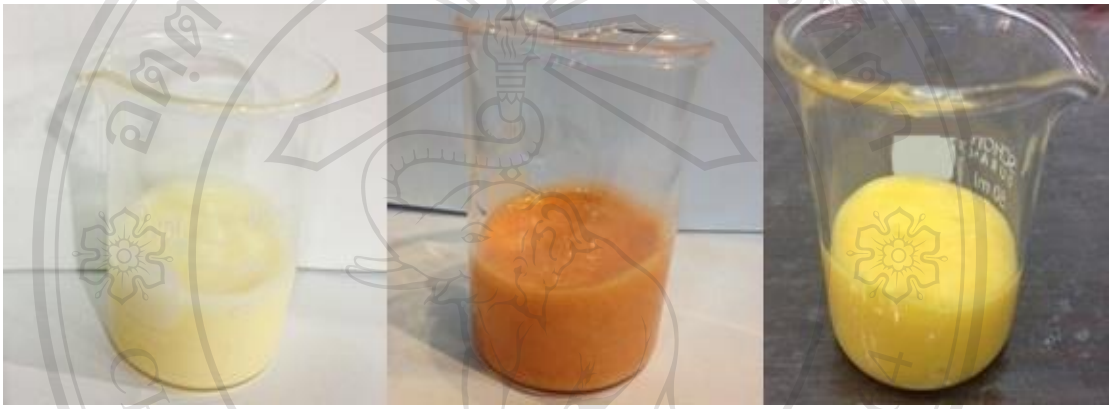


Figure 4.29 Appearances of the original salad cream(left),
salad cream mixed with carotenoids encapsulated chitosan-TPP (middle),
salad cream mixed with the commercial carotenoids (right)

Table 4.6 Color values of the salad cream samples

	L*	a*	b*
Original salad cream	66.57 ± 0.04^{k}	-0.71 ± 0.02^{a}	16.49 ± 0.38^{e}
Control carotenoids	51.66 ± 0.49^{i}	12.35 ± 0.03^{f}	30.81 ± 0.16^{m}
Encapsulated carotenoids	49.19 ± 0.06^{f}	23.30 ± 0.01^{l}	12.26 ± 0.20^{a}
Salad cream mixed with control carotenoids	47.01 ± 0.07^{c}	8.17 ± 0.17^{b}	24.41 ± 0.49^{i}
Salad cream mixed with encapsulated carotenoids	53.34 ± 0.16^{j}	16.61 ± 0.20^{g}	13.08 ± 0.08^{b}

Table 4.6 Color values of the salad cream samples (continuous)

	L*	a*	b*
Salad cream mixed with control carotenoids after keeping for 3 hr	46.63 ± 0.38^{c}	9.74 ± 0.09^{c}	25.94 ± 0.06^{j}
Salad cream mixed with encapsulated carotenoids after keeping for 3 hr	51.04 ± 0.04^{h}	17.77 ± 0.08^{h}	14.30 ± 0.14^{c}
Salad cream mixed with control carotenoids after keeping for 4 hr	45.29 ± 0.21^{b}	10.74 ± 0.14^{d}	26.76 ± 0.22^{k}
Salad cream mixed with encapsulated carotenoids after keeping for 4 hr	49.72 ± 0.16^{g}	18.89 ± 0.05^{i}	15.85 ± 0.04^{d}
Salad cream mixed with control carotenoids after keeping for 5 hr	44.38 ± 0.15^{a}	11.85 ± 0.07^{e}	27.90 ± 0.02^{l}
Salad cream mixed with encapsulated carotenoids after keeping for 5 hr	47.89 ± 0.08^{e}	20.19 ± 0.06^{j}	16.93 ± 0.02^{f}
Salad creammixed with control carotenoids after keeping for 6 hr	44.15 ± 0.08^{a}	11.99 ± 0.09^{e}	28.02 ± 0.08^{l}
Salad cream mixed with encapsulated carotenoids after keeping for 6 hr	47.77 ± 0.10^{d}	20.34 ± 0.07^{jk}	17.13 ± 0.08^{f}
Salad cream mixed with control carotenoids after keeping for 7 hr	44.03 ± 0.02^{a}	12.04 ± 0.12^{e}	28.29 ± 0.15^{l}
Salad cream mixed with encapsulated carotenoids after keeping for 7 hr	47.27 ± 0.23^{d}	20.12 ± 0.10^{k}	17.18 ± 0.06^{f}

Values were mean ± S.D. (n=5). Different letter (a-m) in the same column indicated significant differences ($p<0.05$) between samples.

Table 4.7 Viscosity of salad cream

Hr	Viscosity (centipoises)		
	Salad cream	Salad cream with control carotenoids	Salad cream with encapsulated carotenoids
0	8933.73 ± 2.60[a]	8936.88 ± 2.83[a]	8936.58 ± 3.40[a]
3	8933.96 ± 3.16[a]	8937.78 ± 0.39[a]	8937.60 ± 0.18[a]
4	8934.16 ± 3.29[a]	8938.20 ± 0.18[a]	8938.36 ± 0.09[a]
5	8934.00 ± 3.22[a]	8939.25 ± 0.82[a]	8939.75 ± 0.41[ab]
6	8934.03 ± 3.18[a]	8942.58 ± 0.47[b]	8942.81 ± 0.40[ab]
7	8934.16 ± 3.12[a]	8945.11 ± 0.17[b]	8946.41 ± 0.18[c]

Values were mean ± S.D. (n=5). Different letter (a-c) in the same column indicated significant differences ($p<0.05$) between samples.

4.5.2 Commercial drink

The Food and Drug Administration (2010) has controlled the maximum quantity of carotenoids in drink of 15.58 ppm. In this experiment, 10 ppm of carotenoids was enough to provide color of the commercial drink. The commercial drink made from concentrated grape juice, Sappe Beauti Drink brand, was used for this study because this product was clear and had no color. Changing of color after adding this juice with carotenoids would be easy to observe. Figure 4.30 showed the original appearance of the commercial drink as well as the samples mixed with carotenoids. The sample with the carotenoids encapsulated in chitosan-TPP had the orange color with the positive a* and b*(Table 4.7), while the sample with the control carotenoids contained slightly deeper orange color. The discrepancy of color between carotenoids samples might be due to the difference sources of raw material. The a* and b* values of the tested samples were increased with time and the values were constant at the storage time of 14 hr (Table 4.8).

The sample with the carotenoids encapsulated chitosan-TPP had slightly sedimentation of the particles. However, the sedimentation disappeared at the storage time of 14 hr. The deteriorating particles should explain increasing a* and b* values after keeping the sample for 14 hr.

Addition of the control carotenoids did not change the viscosity of the drink. However, the encapsulated carotenoids resulted in slight increase of the viscosity (Table 4.9). The higher viscosity could cause by the gel-like forming of chitosan-TPP in water (Berger *et al.*, 2004).

Table 4.8 Color values of the drink samples

	L*	a*	b*
Commercial drink	37.43 ± 0.06^{f}	-0.19 ± 0.01^{a}	0.59 ± 0.01^{a}
Control carotenoids	51.66 ± 0.60^{l}	12.35 ± 0.03^{i}	30.81 ± 0.16^{l}
Encapsulated carotenoids	49.19 ± 0.07^{k}	23.30 ± 0.01^{m}	12.26 ± 0.20^{b}
Drink mixed with controlcarotenoids	36.98 ± 0.15^{e}	9.73 ± 0.21^{b}	15.82 ± 0.17^{f}
Drink mixed with encapsulated carotenoids	44.34 ± 1.20^{j}	15.52 ± 0.35^{j}	12.87 ± 0.06^{c}
Drink mixed with control carotenoids and keeping for 5 hr	35.20 ± 0.35^{c}	11.69 ± 0.15^{c}	18.13 ± 0.11^{i}
Drink mixed with encapsulated carotenoids and keeping for 5 hr	43.07 ± 0.99^{j}	16.99 ± 0.11^{k}	13.29 ± 0.21^{d}
Drink mixed with control carotenoids and keeping for 14 hr	32.29 ± 0.26^{b}	11.84 ± 0.10^{de}	18.82 ± 0.07^{j}
Drink mixed with encapsulated carotenoids and keeping for 14 hr	39.72 ± 0.20^{i}	20.11 ± 0.68^{l}	15.01 ± 0.06^{e}
Drink mixed with control carotenoids and keeping for 15 hr	31.82 ± 0.55^{b}	11.91 ± 0.11^{de}	18.90 ± 0.04^{jk}

Table 4.8 Color values of the drink samples (continuous)

	L*	a*	b*
Drink mixed with encapsulated carotenoids and keeping for 15 hr	38.79 ± 0.12^{i}	20.33 ± 0.15^{l}	15.15 ± 0.06^{e}
Drink mixed with control carotenoids and keeping for 16 hr	31.15 ± 0.10^{a}	12.03 ± 0.13^{f}	19.02 ± 0.10^{k}
Drink mixed with encapsulated carotenoids and keeping for 16 hr	38.27 ± 1.19i	20.64 ± 0.07^{l}	15.18 ± 0.03^{e}

Values were mean ± S.D. (n=5). Different letter (a-m) in the same column indicated significant differences ($p \leq 0.05$) between samples.

Table 4.9 Viscosity of the drink samples

Hr	Viscosity (centipoises)		
	Commercial drink	Drink mixed with control carotenoids	Drink mixed with encapsulated carotenoids
0	2.01 ± 0.01^{a}	2.32 ± 0.02^{a}	4.53 ± 0.02^{a}
5	2.02 ± 0.01^{a}	2.34 ± 0.01^{ab}	4.56 ± 0.01^{b}
14	2.02 ± 0.01^{a}	2.36 ± 0.01^{b}	4.58 ± 0.01^{bc}
15	2.03 ± 0.02^{a}	2.37 ± 0.02^{b}	4.59 ± 0.01^{cd}
16	2.03 ± 0.01^{a}	2.37 ± 0.01^{b}	4.60 ± 0.01^{d}

Values were mean ± S.D. (n=5). Different letter (a-d) in the same column indicated significant differences ($p \leq 0.05$) between samples

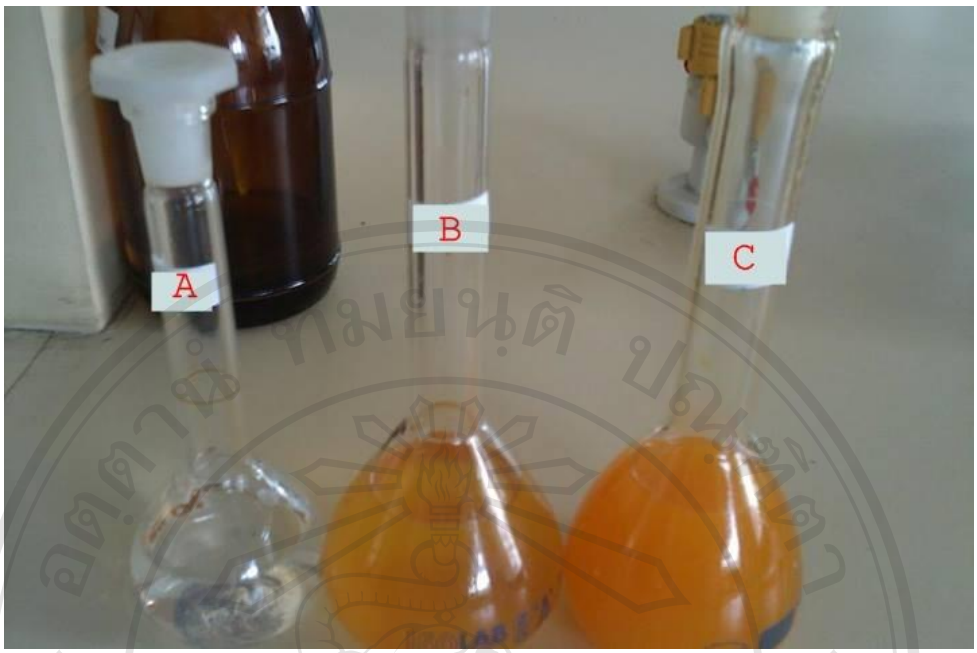


Figure 4.30 Appearances of the original drink (left), commercial drink mixed with carotenoids encapsulated chitosan-TPP (middle), commercial drink mixed with commercial carotenoids (right)