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Research Article

Influence of Pectin on the Stability of Whey Protein Isolate Stabilized Emulsion for Encapsulating Lutein

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Abstract: The effect of pH, thermal treatment and storage stability of lutein-loaded emulsion prepared with whey protein isolate and stabilized by low and high methyl pectin was investigated. Results showed that emulsions prepared in the absence of pectin were highly unstable and flocculated with increasing temperature (60-120°C) and this was attributed to protein denaturation. On the other hand, the additional of second layer of high methyl or low methyl pectin improved the stability of the emulsion against the environmental stresses. This was due to formation of steric barrier onto droplets. Furthermore, high methyl pectin showed a better stability than low methyl which was attributed to increased viscosity of water phase. The double layer emulsions of whey protein isolate and high methyl pectin exhibited a better physical and chemical stability than single layer emulsions. Additionally, high methyl pectin double layer increased lutein retention during the 5 weeks storage at different temperatures. Therefore, these findings could be useful for preparation of stable lutein emulsion.

Keywords: Emulsion stability, lutein, pectin, whey protein isolate

INTRODUCTION

Proteins and polysaccharides are natural biopolymer widely used in food products, pharmaceutical and cosmetics. In some food system biopolymers are used in making and stabilization of two or more immiscible phases in the form of emulsions (Rodríguez Patino and Pilosof, 2011). Generally, emulsions are not stable and stabilization using biopolymers is achieved through formation and adsorption of a thick layer onto emulsion droplets (Paximada *et al*., 2016a).

Proteins are used as ingredients in different food products because of their high nutritive value, functionality including stabilization of emulsion through a combination of steric and electrostatic mechanism due to their high surface activity (Fioramonti *et al*., 2014). Whey protein isolate is milk protein and consist of beta lactoglobulin (50%), bovine serum albumin, alpha lactalbumin and immunoglobulins (Smithers, 2015). Whey protein isolate is used to stabilize emulsion system because it is amphiphilic and has a tendency to adsorb at oil water interfaces therefore stabilizing oil in water emulsion (Singh, 2011; Tavares *et al*., 2014). Emulsions stabilized by whey protein isolate are sensitive to pH. Whey protein isolate has previously been reported by various researchers to stabilize oil in water emulsions consequently at pH closer to isoelectric point coalescence, aggregation flocculation occurred due to a reduced steric repulsion between protein molecules (Kulmyrzaev *et al*., 2000; Davidov-Pardo *et al*., 2016; Qiu *et al*., 2015).

Polysaccharides are mostly used as stabilizing agents due to their ability to increase emulsions viscosity and reduce surface tension between oil and water or to generate texture (Vianna-Filho *et al*., 2013). Pectins are plant polysaccharides and their structure are composed of a mixture of methyl esterified galacturonan, galactan and araban. They have a pKa between 2 to 4 and are classified as high methyl or low methyl pectin depending on the degree of esterification and their degree of etherification is greater or less than 50% (Munarin *et al*., 2012). Several researches had been conducted to study the performance of pectin with different degree of esterification to emulsions stability

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(Guo *et al*., 2014; Leroux *et al.,* 2003; Schmidt *et al*., 2015). Pectin emulsion stability was mostly associated to droplets size distribution, viscosity and less droplets movements (Lutz *et al*., 2009a).

Previous studies showed that the emulsions made only by proteins such as WPI are highly unstable against different environmental stresses. The addition of polysaccharides to protein stabilized emulsion showed more improved stability (Evans *et al*., 2013). Proteins carry a positive charge below their isoelectric points and can easily interact with anionic polysaccharides. Proteins and polysaccharides interaction can lead to the formation of complexes through attraction or repulsion (Sarika *et al*., 2015). Studies on complex coacervation and electrostatic interaction between proteins and polysaccharides were successfully conducted by Lv *et al*. (2012). The interaction between protein and polysaccharides during the complex formation is mostly affected by factors such concentration, charge density, mixing ratio, pH and ionic strengths (Thongkaew *et al*., 2015).

Addition of pectin to the system stabilized by protein may be done to control rheology and structure hence retarding coalescence, Oswald ripening and phase separation during long period storage (Dickinson, 2011). Addition of pectin to whey protein isolate at pH in proximities of isoelectric point increased stability to coalescence and aggregation through formation of multilayer onto oil droplets (Lutz *et al*., 2009b; Salminen and Weiss, 2014; Surh *et al*., 2006). Similar findings were reported when pectin of different degree of esterification were added to acidified milk (Krongsin *et al*., 2015; Laurent and Boulenguer, 2003; Tromp *et al*., 2004).

Lutein is used as natural pigment in food and their incorporation improve color and nutritional value. Granado-Lorencio *et al*. (2010) and Indyk *et al*. (2014) reported that lutein could be used to fortify different food products. Lutein is carotenoids found in some fruits, vegetables and eggs yolk. Research findings indicated that lutein has antioxidant, anticancer and can act as filter of light that may damage the skin and eye tissues (Saini *et al*., 2015). The use and bioaccessibility of lutein is limited by its poor solubility and sensitivity to light, acid and heat therefore there is a need to increase its solubility and protect lutein against harsh environment condition to increase its uses (Kamil *et al*., 2016).

A lot of efforts have been made by researchers to improve lutein solubility and stability against light, heat and acid. Arunkumar *et al*. (2013) and Qv *et al*. (2011) used different protein and polysaccharides to produce lutein nano and micro particles with improved stability against light and temperature. Beicht *et al*. (2013) produced a multilayer emulsion using laccase to induce the crosslinking of beet pectin and whey protein isolate and improve lutein release. While a number of studies have focused on stabilization of lutein using different proteins and polysaccharides, no work has been reported on preparation of lutein loaded emulsions stabilized by whey protein isolate and the influence of high and low methyl pectin addition at pH in close proximity to isoelectric point.

The first objective of this current study was to investigate the influence of high and low methyl pectin on stability of lutein-loaded emulsion stabilized by whey protein isolate at pH around isoelectric points. Secondly, the study aimed at assessing whether emulsions with HMP or LMP could improve stress and storage stability of emulsion with whey protein isolate at pH around protein isoelectric point. Lastly, the study aimed at determining the effect of addition of pectin to retention and degradation of lutein-loaded emulsion stabilized by whey protein isolate.

MATERIALS AND METHODS

Materials: Whey protein isolate was obtained from Hilmar Ingredients (Hilmar, California, USA). The total solid, protein and ash in the dry power were 95.6%, 88.7% and 2.7%, respectively. High methyl pectin with degree of esterification of 67% and low methyl pectin degree of esterification of 35% were supplied by CP Kelco, Shanghai, China). Lutein (purity 90%) was provided by Zhejiang Medicine Co., Ltd (Zhejiang, China). Medium Chain Triglyceride oil (MCT) having (Composition: C8: 57%, C10: 40%, C6: 2% and C12: <1%) was a product of Lonza Inc. (Allendale, NJ, USA).

All other chemicals used were of analytical grade and obtained from Sinopharm Chemical Reagent Company (China).

Methods:

Preparation of single biopolymer solution: Whey protein isolate 1g/100 mL) low or high methyl pectin 1g/100 mL) solutions were prepared separately by dispersing in deionized water and some droplets of sodium azide (0.03 g/100 mL) were added. The solutions were then stirred at 250 rpm at 25°C for 3 h and kept at 4°C overnight to allow complete hydration of the biopolymers prior to further uses (Thongkaew *et al*., 2015).

Preparation of Lutein emulsion stabilized by biopolymers: Oil phase were prepared by heating MCT at 150°C for 2 min followed by addition of 3g/100 mL crystal lutein and further magnetic stirred for 2 min to allow total dissolution. The oil phase contained 3g/100 mL of lutein. Emulsion was prepared by mixing 10 mL of oil phase with 90 mL (1g/100 mL) of whey protein isolate solution. Oil phase and protein solution were mixed at 12, 000 rpm for 5 min using ultraturax (T-25, IKA Works Inc., Wilmington, NC, USA) to obtain

first emulsion. Secondary emulsions were prepared by mixing primary emulsion with low or high methyl pectin solution and homogenized at 12, 000 rpm for 5 minutes using ultraturax T-25according to the method by Beicht *et al*. (2013) with a slight modification. Coarse emulsion samples, 50 mL each, were subjected to high intensity sonication using 1200W ultrasonic processor (JY98-IIIDN, 20 kHz, volume processing capacity: 50-1000 mL, Ningbo Scientz Biotechnology Co., Ningbo, China) equipped with 20 mm diameter probe. Temperature variations in the sample during sonication were monitored with a digital thermometer attached to a thermocouple. Applied power was 40% of the maximal equipment power while sonication time was 6 min. Work time and the rest time for sonication were set at 5s and 2s, respectively, in order to avoid the overheating. Cold water circulating through the containers jacket helped to maintain the samples temperature at 20-30°C (Abbas *et al*., 2014).

Viscosity measurement: The viscosities of biopolymer solutions and emulsions were determined according to Damianou and Kiosseoglou (2006). The apparent viscosities were determined at 25°C using a Brookfield DVII viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass., U.S.A.). SC4-18 spindle was used at 30 rpm. The data were acquired using Brookfield RheocalcT software (Brookfield Engineering Lab Inc., Stoughton, Mass., U.S.A.). One hundred data points were averaged per replication. Three readings were taken per replication.

Thermal stability: The influence of thermal treatment (60, 75, 90, 105 and 120°C) on the stability of lutein emulsion stabilized by biopolymers at pH around protein isoelectric point was investigated (Niu *et al*., 2015). Emulsions were stored overnight at 25°C and then placed in a preheated water bath for 30 min at 60, 75, 90, 105 and 120°C. The tubes were cooled to 25°C using an ice bath and stored overnight at 25°C prior to further analysis.

Storage stability: Lutein emulsion were stored for 5 weeks at different temperatures of 4, 25 and 40°C and the pH was adjusted to 4 and 5 using1M HCl and NaOH.

Physical stability: Physical stability was assessed by measuring the mean droplets size and zeta potential of emulsions for 5 weeks of storage (Abbas *et al*., 2015). The z-average diameter (Dz), zeta potential was measured using a Malvern Zetasizer Nano ZS analyzer (Malvern Instruments Ltd., Malvern, UK) with a He-Ne laser at 633 nm. The measurements were performed at 25°C and 173° scattering angle. The electrical charge (zeta potential) was determined from measurements of the direction and velocity that the droplets or particles moved in the applied electric field using the Smoluchowski model. The solution was used to derive the particle size distribution by a laser particle size analyzer. The refraction index applied was 1.59 for material and 1.33 for water dispersant.

Lutein retention: The retention of Lutein was calculated by extraction method (Matos *et al*., 2015). Five milliliters of Lutein-loaded emulsion were mixed by 15 milliliters hexane and vortexed for 8 minutes at 25°C. Free lutein were extracted from emulsion and hexane were evaporated using a rotary evaporator. Ethanol was added to free lutein followed by centrifugation at 5,000 rpm for 15 minutes and filtration to determine the component retained during separation processes. A standard curve of lutein in ethanol was used to determine the amount of free lutein using UV-Vis spectrophotometer at the absorbance of 446 nm (UV-1600; Mapada Instruments Co., Ltd., China).This calculation was used to determine the lutein retention during 5 weeks storage period.

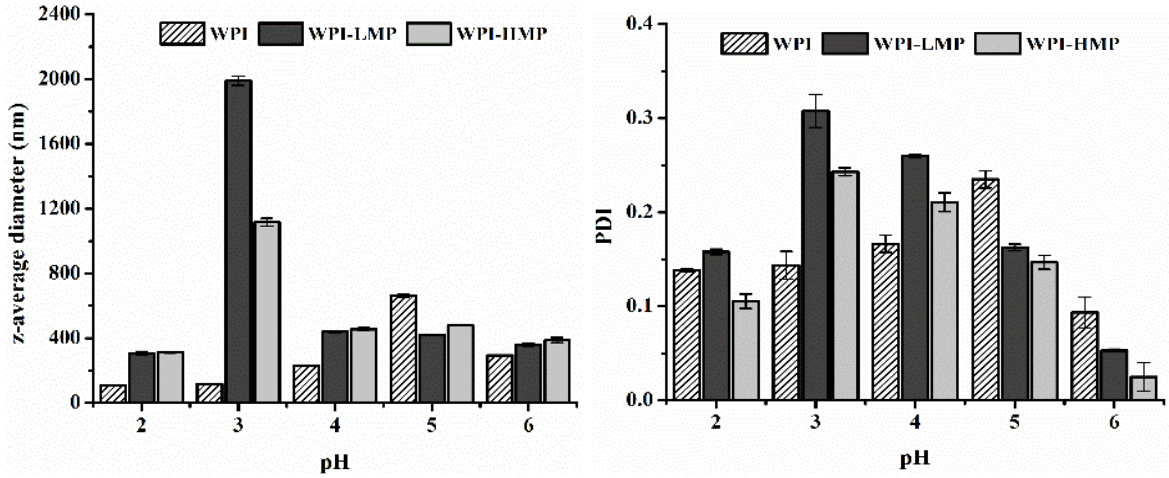
Lutein retention efficiency was calculated as follows:

$$\text{Lutein Retention Efficiency (\%)} = \frac{\text{Loaded Lutein} - \text{Free Lutein}}{\text{Loaded Lutein}} \times 100$$

Statistical Analysis: Each experiment was repeated in triplicate under the same conditions. A one-way analysis of variance (ANOVA) was applied to estimate the statistical difference. Significant differences ($p<0.05$) between means were determined using Tuckey's tests. Statistical analyses were evaluated with SPSS software (version 19.0, SPSS Inc., Chicago, Illinois).

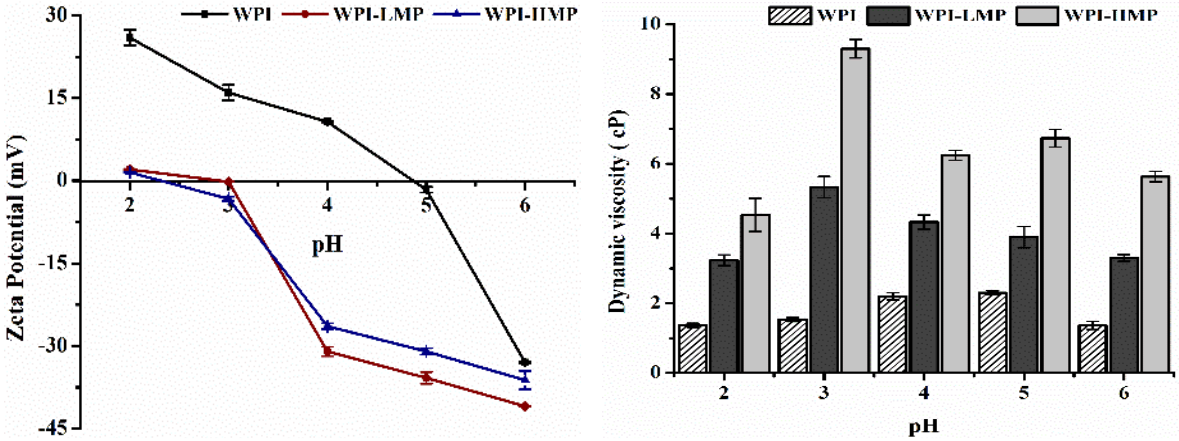
RESULTS AND DISCUSSION

Effect of pH on z-average diameter and polydispersity of fresh emulsion: Food is subjected to different pH during digestion therefore in our study the influence of pH on z-average diameter and PDI was investigated in pH range between 2 to 6. WPI emulsion prepared at pH 5 showed the z-average diameter of 662 nm with PDI 0.1385 (Fig. 1a and 1b). This might be attributed to a large electrostatic interaction between whey protein isolate coated droplets. The z-average diameter and PDI were high at pH 5 due to the aggregation of WPI coated droplets around isoelectric point. Drastic increase in size at pH near isoelectric point as a result of electrostatic repulsion between whey protein isolate coated oil droplets correlated with findings of Qian *et al*. (2012). The addition of LMP and HMP to the system significantly increased the size



(a) (b)

Fig. 1: PH dependence of emulsions with WPI 1g/100 mL, LMP 1g/100 mL or HMP 1g/100 mL and 10 g/100 mL MCT with 3g/100 mL of lutein; (a): Z-average size; (b): Polydispersity index



(a) (b)

Fig. 2: PH dependence of emulsions with WPI 1g/100 mL, LMP 1g/100 mL or HMP 1g/100 mL and 10 g/100 mL of MCT with 3g/100 mL of lutein; (a): Zeta Potential; (b): Dynamic viscosity

diameter and PDI atpH 3. The z-average diameter and PDI reached 1990.41 nm and 1116.35 nm in LMP and HMP respectively, while their PDI were 0.3 and 0.25 (Fig. 1a and 1b). The addition of LMP and HMP promoted aggregation due to the bridging and charge neutralization between whey protein isolate and both pectin. However, the addition of LMP and HMP to the system did not significantly affect the size diameter of the emulsion at pH from 4 to 6. This might be attributed to the prevention of droplets aggregation as evidenced by small z-average diameter. In addition, there was no flocculation observed at this pH range and this was attributed to the pectin that adsorbed onto droplets surface. There could be an increased electrostatic and steric repulsion between droplets due to adsorbed LMP and HMP layer that were strong enough to prevent droplets aggregation.

Effect of pH on zeta potential and viscosity of fresh emulsion: Our previous experiments pH change affected z-average size and PDI, in these series of experiments we wanted to investigate the effect of pH on emulsion zeta potential and viscosity. The results indicated that the zeta potential of WPI emulsion varied from+26 mV to -31mVand had zeta potential of almost zero at pH around 5 (Fig. 2a). Whey protein isolate solution null zeta potential value was around 5 and concluded that it was the isoelectric point which is accordance with Lamoudi *et al*. (2015). The addition of HMP or LMP to WPI stabilized emulsion showed significant changes in zeta potential (Fig. 2a). Comparing WPI-LMP to WPI-HMP fresh emulsions, results showed no significant difference in zeta potential and were closer to zero at pH between 2 and 3. At pH 3, the zeta potential of whey protein emulsion

was +18 mV after high methyl and low methyl pectin addition the value of zeta potential reached almost zero. This was attributed to ability of pectin to cause charge neutralization and similar results were reported by Qiu *et al*. (2015) where addition of different amount of pectin to wheat protein resulted in charge neutralization at given pH. However, zeta potential was significantly different at pH between 4 and 6. Zeta potential of WPI-LMP and WPI-HMP emulsions were between -31 and -40 and -26 and -36 mV, respectively. The reduction of zeta potential after the addition of LMP or HMP was attributed to a strong interaction or electrostatic attraction that occurred between a protein and polysaccharide below the isoelectric points of whey protein, since at these pH values proteins were highly positive charged and polysaccharides negatively charged and therefore there was a strong interaction that led to a decreased value of zeta potential. These results are in agreement with the findings of Paximada *et al*. (2016b) who reported that addition of polysaccharide to protein emulsion resulted in reduced values of zeta potential. The viscosity of whey protein isolate emulsions increased with increasing pH and reached higher value at pH around protein isoelectric point (pH 4 and 5; 2.2 cP and 2.3 cP respectively). This was attributed to droplets flocculation and aggregation at this point (Fig. 2b).On the other side, the addition of LMP and HMP to the system significantly increased the emulsions viscosity with increasing pH**.** However, the emulsions viscosity was not significantly different from pH 4 to 6 after addition of LMP and HMP to the system. LMP emulsion systems showed lower viscosity value compared with HMP emulsion systems. However at pH 3 of systems with WPI-LMP and WPI-HMP the viscosity was higher as results of complex formation between protein and polysaccharide. The viscosity of protein/polysaccharide mixture could increase as a result of complex or coacervate formation at given pH in accordance with Schmitt *et al*. (2009). The above results indicated that HMP and LMP (1%, w/v) at pH around protein isoelectric point (4-5) resulted in increased emulsion stability against coalescence. Therefore, further works were carried out around that pH range.

Encapsulation efficiency (%) of lutein in emulsion: Lutein encapsulation efficiency in WPI, WPI-LMP and WPI-HMP were evaluated at pH around protein isoelectric point range (4-5). As shown in Table 1, the encapsulation efficiency was significantly high in WPI emulsion compared with other emulsion systems. However, the increase in pH from 4 to 5 decreased the encapsulation efficiency in all systems. The lutein encapsulation efficiency in WPI, WPI-LMP and WPI-HMP was reduced by 25, 10 and 6% respectively. This was due to addition of pectin that decreased emulsifying ability these findings are in agreement with Noshad *et al*. (2015) who reported that addition of second layer to protein emulsion resulted in lower encapsulation efficiency to due to modification of surface morphology.

Table 1: Lutein encapsulation efficiency (%) of lutein in emulsion system

pH	WPI	WPI-LMP	WPI-HMP
4	89.31±2.52^{f}	85.76±0.51^{d}	86.25±2.46^{e}
5	67.12±0.03^{a}	77.55±0.03^{b}	81.34±0.07^{c}

Sample with different letters are significantly different at $p< 0.05$

Effect of heat treatment on the emulsion stability: It is widely recognized that food products may be subjected to high temperature during processing and cooking. Against this background, it was necessary to investigate the effect of different high temperature on lutein emulsions. The effect of high temperature treatment (60°, 75°, 90°, 120°C) on WPI, WPI-LMP, WPI-HMP emulsions prepared at pH 4 and 5 for 30 min was evaluated. As shown in (Fig. 3), heating at 60^{o}C didn't show much effect on zeta potential and z-average diameter of all emulsions. Further increase in heating temperature slightly increased the z-average diameter while decreased the zeta potential of WPI-LMP and WPI-HMP emulsions compared with WPI emulsions. The z-average diameter of WPI emulsions increased to 4625.15 nm and 7878 nm when heated at 120^{0}C, at pH 4 and 5 respectively (Fig. 3a and 3b). Moreover, the electrical charge shifted from + 3.13 mV to -10.34 mV at 120°C at pH4 while at pH5, the zeta potential varied from -5.12 mV to -12.99 mV (Fig. 3c and 3d). These results can be correlated with protein thermal denaturation, which induced the conformation change and exposure of reactive groups located at interior protein parts. These results are in agreement with the findings of Lamoudi *et al*. (2015) and Xiang *et al*. (2015). However, WPI-LMP and WPI-HMP emulsion were not significantly affected by heat treatment. Better stability was due to the pectin's ability to adsorb and form thick layer onto the oil droplet and increased aqueous phase viscosity. Addition of LMP and HMP could prevent oil droplets aggregation in emulsion (Qiu *et al*., 2015). In addition, WPI-HMP had better thermal stability than WPI-LMP at both pH. This might be attributed to their viscosity difference as shown in Fig. (3b). The higher viscosity the greater emulsion stability. These results are in accordance with the findings of Guo *et al*. (2014). In addition, the effect of high temperature treatment (60°, 75°, 90°, 120°C) on WPI, WPI-LMP and WPI-HMP on lutein retention at pH 4 and 5 was also evaluated. As shown in Table 2, heating at 60°C didn't show significant effect on lutein retention efficiency in all emulsions. However, the increase in heating temperature significantly decreased lutein retention efficiency in WPI emulsions compared

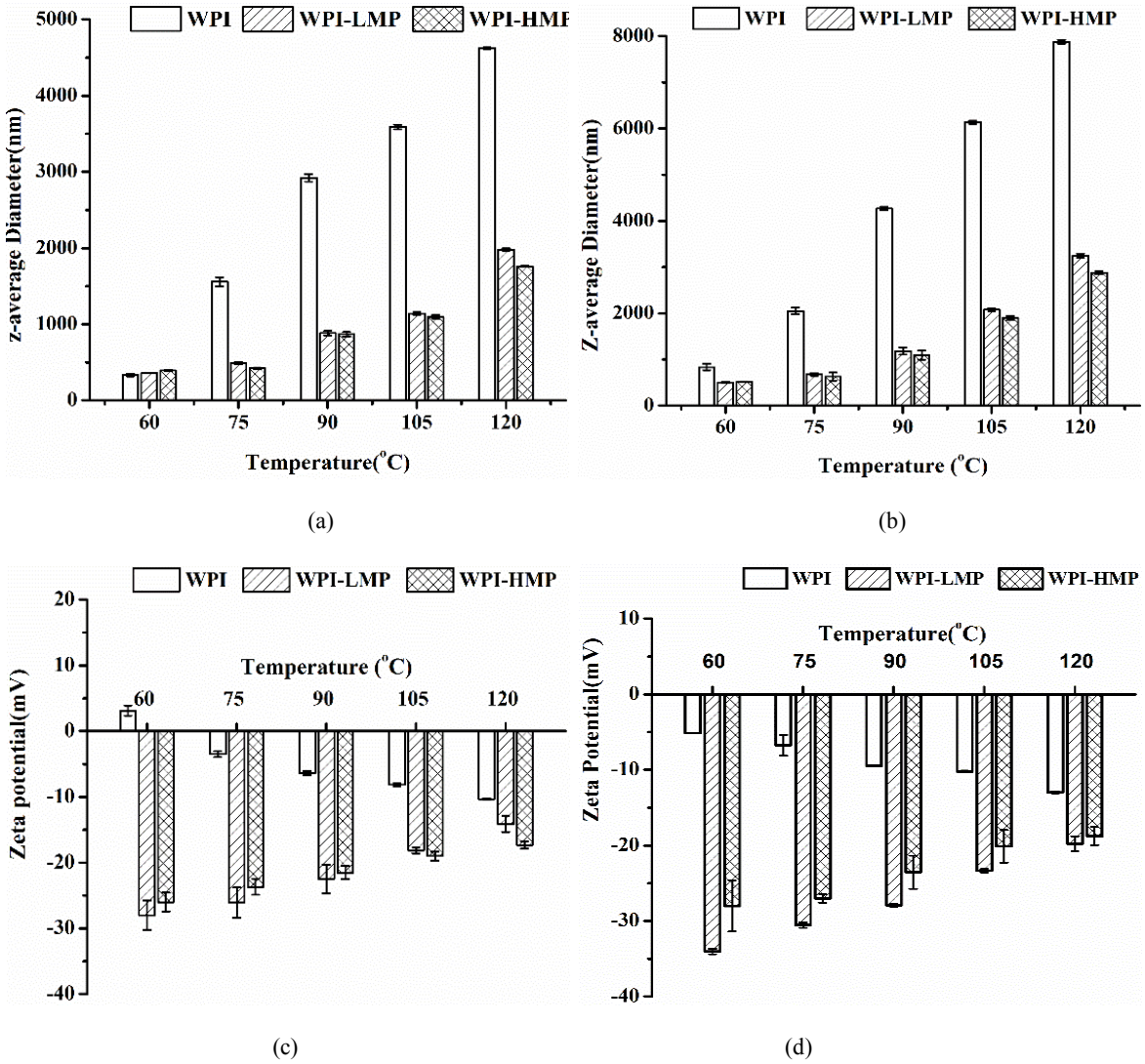


Fig. 3: Effect of thermal treatment on; (a): Z average size at pH4; (b): Z-average size at pH5; (c): Zeta potential at pH4; (d): Zeta potential at pH5

Table 2: Effect of heating on lutein retention (%) in emulsion

	pH 4			pH 5		
Temperature (ºC)	WPI	WPI-LMP	WPI-HMP	WPI	WPI-LMP	WPI-HMP
Fresh emulsion	89.31±2.52	85.76±0.51	86.25±2.46	67.12±0.03	77.55±0.03	81.34±0.07
60	82.11±0.27	80.74±0.42	81.33±0.68	62.09±0.32	74.67±0.07	78.04±0.78
75	70.42±0.63	74.06±0.19	77.97±0.69	47.02±0.98	65.56±1.13	74.22±0.44
90	40.57±0.51	63.07±1.17	68.08±0.11	18.21±0.77	54.15±1.03	60.13±0.06
120	15.30±1.23	47.03±0.44	53.25±0.55	7.13±0.29	32.05±0.99	40.77±1.76

with WPI-LMP and WPI-HMP emulsions. The lutein retention efficiency of WPI emulsions decreased by 83% and 89% when heated at 120°C, at pH 4 and 5 respectively. While, lutein retention efficiency in WPI-LMP and WPI-HMP heated at 120°C decreased by 45, 38 and 59, 50% at pH4 and 5 respectively. This might be due to the thermal protein denaturation. These results are in accordance with the findings of z-average diameter (Fig. 1a).

Storage stability at different temperature: Fresh emulsion were stored at different temperatures (4, 25 and 40°C) for 5 weeks. The z-average diameter change significantly increased with increasing storage temperature and time (Fig. 4). The z-average diameter of WPI emulsion stored at different temperature was significantly higher compared to WPI-LMP and WPI-HMP emulsions. Emulsion are thermal unstable system and during storage they have a tendency to form

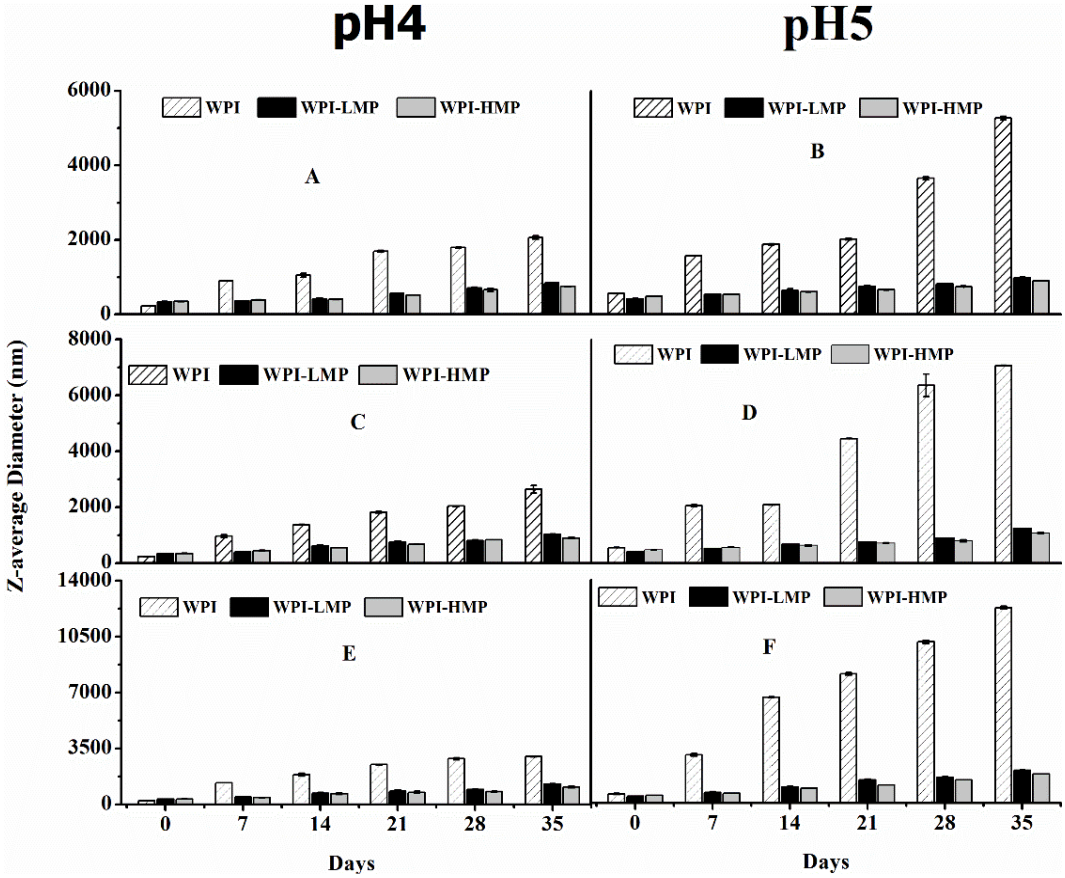


Fig. 4: Effect of storage temperature on Z-average diameter; (a): Emulsion pH4 at 4°C; (b): emulsion pH5 at 4°C; (c): emulsion pH4 at 25°C; (d): emulsion pH5 at 25°C; (e): Emulsion pH4 at 40°C; (f): Emulsion pH5 at 40°C

aggregate or flocculate due to Brownian movement. They also might be due to loss or reduction the electrostatic repulsion interaction between droplets prevent Van der walls and hydrophobic attraction that led to aggregation. These results are in accordance with the findings of Teo *et al*. (2016). Milk protein was used to stabilize beta carotene oil in water emulsion and the zeta potential decreased during storage time at pH 5 and was concluded that there was a suppressed electrostatic repulsion at pH near isoelectric point (Xu *et al*., 2013). However, the addition of LMP or HMP to WPI emulsions system increased the electrostatic repulsion between droplets and the water phase viscosity leading to a stable emulsion system. The storage at 40^0C accelerated the droplets aggregation in all emulsions. This change was more pronounced in WPI emulsion compared with WPI-LMP and WPI-HMP emulsions after 5 weeks storage (Fig. 4). This phenomena was attributed to the increase in oil solubility and migration of oil out particle known as Oswald ripening (Hategekimana *et al*., 2015). A pectin thick layer was formed on to droplet thus preventing aggregation and reduced van der walls attraction (Zhao *et al*., 2014). In addition, HMP system showed better emulsion stabilization capacity than LMP system during storage due to its high viscosity that formed a gel network in the system. The degree of esterification did not have a significant impact on storage stability. The stability of emulsions with LMP and HMP was found to be significantly affected by viscosity (Leroux *et al*., 2003).

Effect of storage on lutein retention (%) in emulsion: On the other side, the storage temperatures significantly affected lutein retention on emulsions stabilized by WPI at pH 4 and 5 than WPI-LMP and WPI-HMP. After 5 weeks storage time at 40°C, lutein retention was significantly reduced by 80, 45.2, 40 and 89, 66, 60.94% however, the storage at 4 and 25°C gradually reduced lutein retention in WPI-LMP and WPI-HMP emulsions than in WPI emulsions (Fig. 5). The higher lutein loss at high temperature might be attributed to the Oswald ripening phenomena. Carotene stability under various temperature was studied and higher temperature reduced carotene retention (Ziani *et al*., 2012). The addition of LMP or HMP to WPI emulsion system at pH 4 significantly reduces the loss of lutein during storage. This might be due to the formation of thick layer onto oil droplets and increased viscosity of water phase. Addition of second layer to

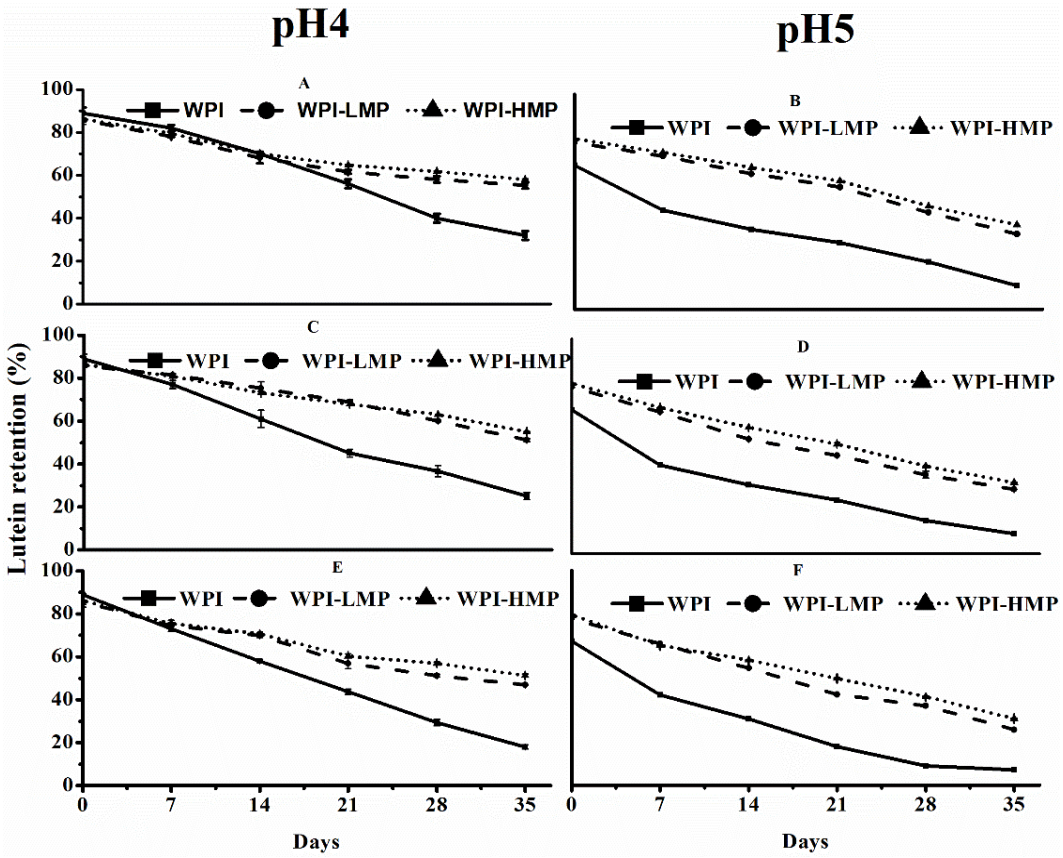


Fig. 5: Lutein retention at different temperature; (a): Emulsion pH4 at 4°C; (b): Emulsion pH5 at 4°C; (c): Emulsion pH4 at 25°C; (d): Emulsion pH5 at 25°C; (e): Emulsion pH4 at 40°C; (f): Emulsion pH5 at 40°C

emulsions could increase lutein retentionat different temperature (Lim *et al*., 2014). HMP system had a better retention than LMP system due to high viscosity of water phase that prevented lutein migration (Leroux *et al*., 2003).

CONCLUSION

During this study, stable lutein-loaded oil in water emulsion was prepared with whey protein isolate and pectin at pH around protein isoelectric point. The physical characteristics of WPI emulsion showed it was more unstable under various environmental stress compared to WPI-LMP and WPI-HMP emulsions at near protein isoelectric point pH (4 and 5). Results also showed that lutein retention during heat treatment and storage period was higher and stable in WPI-LMP and WPI-HMP emulsions than in WPI emulsion. These findings revealed that emulsions based delivery system with better stability could be prepared with WPI-LMP or WPI-HMP around the pH protein isoelectric point.

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