Molecular docking studies and physicochemical properties on the interaction of xanthone with whey protein (β-lactoglobulin and α-lactalbumin)

P P Rahayu¹, R D Andriani¹ and J M Maligan²

¹Animal Product Technology Department, Faculty of Animal Science, University of Brawijaya, Malang-65145, Indonesia
²Agricultural Product Technology Department, Faculty of Agriculture Technology, University of Brawijaya, Malang-65145, Indonesia

Email: premypuspita@ub.ac.id

Abstract. Whey protein (WP) is a good encapsulating material of food applications and can act as a delivery vehicle for bioactive compounds, including xanthone. The objective of this research is to analyze the interaction of WP with xanthone using molecular modeling and investigate the effect of whey protein at different concentration of xanthone (0.025%; 0.05% and 0.1% (v/v)) on emulsion stability, antioxidant, and chemical structure by FTIR (Fourier Transform Infrared). Each treatment was replicated four times. The docking studies were performed with Programme Autodock Vina PyRx 0.8. Molecular docking studies revealed that interaction of xanthone and whey protein are stabilized by hydrogen bonds, hydrophobic interaction, and van der walls bond. The different concentrations of xanthone gave a highly significant effect on emulsion stability and didn’t give a significant difference between the three treatments on antioxidant activity. FTIR result showed that wave numbers 690-900 and 3010-3100 cm⁻¹ indicate the presence of CH groups and 1500-1600 cm⁻¹ which may indicate the presence of C = C aromatic ring groups, respectively. The highest emulsion stability was at 0.1% with 99.23%. The highest antioxidant was at 0.1% with 86.40%.

1. Introduction
Whey protein is natural vehicles, which evolved to deliver essential micronutrients, as well as immune system components (β-lactoglobulin and α-lactalbumin). Particularly, whey proteins are important food ingredients because of their functional and nutritional properties and have been extensively used as emulsifiers in a food product. Recent studies have indicated the potential of whey protein as emulsifiers in nanoeumulsions that have been tailored for food applications. β-lactoglobulin which is a component of whey protein has the ability to protect unstable bioactive compounds against extreme conditions [1]. The size of β-lactoglobulin of 282 ± 72 nm and 973 ± 106 nm can allow it to the emulsion, at pH 7.0 and 3.0 respectively [2].

Xanthone is one of the bioactive compounds in the mangosteen pericarp which size at 261 nm [3] that acts as an antioxidant, anti-proliferative (inhibitor of cancerous growth), anti-inflammatory, and antimicrobial. Xanthone is the main class phenolic compounds in plants which also include mangostin, mangostenol, mangostinin A, mangostenon B, trapezifolixanthone, α-mangostin, β-mangostin, gacrinon B, mangostanol, flavonoid, epicatechin, and gartanin. These compounds are very useful for
our health [4]. The advantages possessed by xanthone compounds can be utilized as functional foods through fortification into dairy-based foods.

Polyphenol compounds like those in xanthone are most likely to form complexes with milk protein, especially whey proteins [5]. This binding can affect the electron donation capacity of xanthone by reducing the number of hydroxyl groups available in the solution. Studies in the past have shown the effects of milk protein on the antioxidant activity of tea polyphenols, whilst the effect of polyphenol complexation on the stability and conformation of milk proteins has not been addressed [6].

Fortification of bioactive components in dairy products can be done by nanoparticle technology. Whey proteins may be used as a nano delivery system of bioactive compounds. According to Chen and Subirade [7], the application of nanoparticles in milk can be done by encapsulation to protect the active components during processing and storage. Nanoparticles have the advantage of being easily dispersed in food systems, making it easier to absorb in the digestive system. Xanthone is a compound that is resistant to heat and is not damaged at high temperatures. The fortification process of xanthone requires the delivery system of proteins to make the bioactive component stable. Such stability will allow the component to be homogeneously dispersed in the food system and easily absorbed. The result of this study provides the characteristic of WP and xanthone’s interaction on molecular docking studies, emulsion stability, antioxidant, and chemical structure by FTIR.

2. Materials and method

The first stage of this study was using molecular modeling. The docking studies were performed with Programme Autodock Vina PyRx 0.8. The β-lactoglobulin and α-lactalbumin structure were obtained from the Protein data bank (PDB) /http://www.rcsb.org/, and the xanthone structure was generated from PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov).

The second stage of this study was to investigate the effect of whey protein with xanthone. Whey protein isolate (Merck) was dissolved in aqueous solution (9 mg/ml). Xanthone (TCI) was prepared in three concentrations (0.025%; 0.05% and 0.1%). The WPI solution was heat-treated at 60°C for 10 minutes. After that, xanthone was subsequently added to the solution. Stirring was done continuously for 2 hours to achieve complete solubility.

2.1. FTIR spectroscopic

The chemical structure was determined using the procedure described by Moela et al [8] and it was modified. Infrared spectra were recorded on FTIR spectrometer, which was equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter. The reading was processed using ZnSe windows. A solution of polyphenol was added dropwise to the protein solution with constant stirring to ensure the formation of a homogeneous solution and to reach the target. Spectra were collected after 2 h incubation. When producing different spectra, this band was adjusted to the baseline level, in order to normalize these differences spectra.

2.2. Emulsion stability

Emulsion stability index (ESI) of whey protein-xanthone samples was determined using the procedure described by Nagarajan et al [9]. Soybean oil (5 ml) and whey protein-xanthone solution (15 ml) were homogenized using a homogenizer for 1 min. 0.1 ml emulsions were pipetted out at 0 and 10 min and then 0.1% SDS (10 ml) was added to the emulsion. The mixture was mixed thoroughly for 10 s using a vortex mixer. The resulting dispersion was measured using a spectrophotometer at 500 nm wavelength. EAI and ESI were calculated using equation (1):

$$\text{ESI} \% = \frac{A_{10}}{A_0} \times 100$$

Where; $A_0$ = $A_{500}$ at time of 0 minutes, $A_{10}$ = $A_{500}$ at the time of 10 minutes
2.3. Antioxidant activity
The scavenging of DPPH free radicals was used to measure the antioxidant activity of the extracts [10]. Briefly, the sample solutions were thoroughly mixed with freshly prepared 0.05% DPPH ethanol solutions at the ratio of 1:1 and kept for 30 min in the dark at room temperature. The amount of the reaction was determined using UV-VIS spectrophotometer at 517 nm. Neutralization of DPPH radical was calculated using equation (2):

\[
\text{DPPH inhibition} \, \% \, = \, 100 \times \frac{A_0 - A_s}{A_0}
\]

where \(A_0\) is the absorbance of the control and \(A_s\) is the absorbance of the tested sample.

3. Results and discussion

3.1. Molecular docking analysis
\(\alpha\)-Lactalbumin and \(\beta\)-lactoglobulin are the major components in whey protein (± 70%). \(\beta\)-lactoglobulin is widely used in food processing due to its functional properties and nutritious value [11]. Whey proteins have the ability as nano delivery in food systems because they can form nanocapsules. The potential use of \(\beta\)-lactoglobulin as a delivery agent for polyphenols is of special interest. Xanthone is expected to form a complex with whey protein, so it can be dispersed uniformly in food. The complex formation of xanthone with whey protein can be seen through the molecular docking simulation. Molecular docking studies revealed that interaction of xanthone and whey protein are stabilized by hydrogen bonds, hydrophobic interaction, and van der walls bond. In the study, structural modeling was used to show the participation of several amino acid residues in xanthone-\(\alpha\)-lactalbumin and xanthone-\(\beta\)-lactoglobulin complexation (figure 1 and figure 2). The hydrogen bond has an important role in increasing the affinity of the interaction between xanthone molecules and whey proteins (\(\alpha\)-lactalbumin and \(\beta\)-lactoglobulin). The results showed that the affinity value of xanthone binding with \(\alpha\)-lactalbumin and xanthone binding with \(\beta\)-lactoglobulin was -5.7 and -6.6 kcal/mol, respectively. The analysis of molecular docking interaction between lactoglobulin and lactalbumin with xanthone indicated that this complex was dominated by van der walls and hydrophobic bonds.

Figure 1. Interaction between \(\alpha\)-lactalbumin and xanthone.
Figure 2. Interaction between \( \beta \)-lactoglobulin and xanthone.

Table 1. Interaction between xanthone and whey protein (\( \alpha \)-lactalbumin dan \( \beta \)-lactoglobulin).

| Complex                                      | Interaction types      | 
|----------------------------------------------|------------------------|
|                                              | Van der walls          | Hydrogen bond          | Hydrophobic interaction |
| Xanthone- \( \alpha \)-lactalbumin           | LEU105, TRP104, THR33, HIS32, GLN54, GLU49 | GLN43                  | ALA106, ILE41, VAL42    |
| Xanthone- \( \beta \)-lactoglobulin          | TRP61, TYR20, SER21    | GLN59                  | PHE151, TYR42, CYS66    |

Table 1 showed the amino acid residues that interacted with Xanthone and \( \alpha \)-lactalbumin by Van der Waals interaction were LEU105, TRP104, THR33, HIS32, GLN54, GLU49. Hydrogen bond was in GLN43 and hydrophobic interactions were in ALA106, ILE41, and VAL42. The interaction between Xanthones and \( \beta \)-lactoglobulin was stabilized by Van der walls in amino acids TRP61, TYR20, SER21. Hydrogen bond was in GLN59 and hydrophobic interactions were in PHE151, TYR42, CYS66, respectively. Nonevalent interactions such as hydrophobic interaction \([12,13]\), hydrogen bonding \([13,14]\) and van der Waals \([14]\) have been reported to dominate the interaction between \( \beta \)-lactoglobulin with phenolic.

Milk protein is composed of amino acids. Proline is one of the amino acids in milk protein, which has an open structure \([15]\). It can cause a bond between xanthone and whey protein. Sahihi et al \([16]\) explained that \( \beta \)-lactoglobulin has a single polypeptide chain with 162 amino acids, with a three-dimensional structure such as of 1 \( \alpha \)-helix, 9 anti-parallel \( \beta \)-strands, 8 \( \beta \) sheets with hydrophobic bonds. This result is supported by Wu et al \([17]\), the hydrophobic interaction of the phenolic compound with a hydrophobic group of proteins will form a hydrogen bond. It formed a hydrogen bond between OH group of the phenolic compound with the polar group (NH\(_2\), NH, OH, and the SH group) on the protein surface.

Polyphenols can also interact covalently or non-covalently with protein \([12,13]\). Murray et al \([18]\) stated that non-covalent interactions between phenolic compounds and hydrophobic proteins would be stabilized by hydrogen bonds. Interaction between milk protein and phenolic compound forms van der walls bonds in complex formation, and the protein structure isn’t changed \([19]\).
Proline group has a strong affinity for the hydroxyl (-OH) group in catechins [20]. Casein has a tendency to interact with other proteins and some ligands, based on the hydrophobic character of casein micelles. The interaction of polyphenol-proteins is dominated by non-covalent interactions. It is hydrophobic interactions. It is stabilized by hydrogen bonds [21]. The structure and molecular weight of polyphenols have an important role in the interaction of protein-polyphenols, high molecular weight polyphenols can more strongly bind proteins [22]. The interaction of polyphenols with globular proteins can result in changes in protein structure and conformation. The binding affinity depends on the molecular size of the polyphenols. The greater the size of the polyphenol molecule, the greater the tendency to form complexes with proteins [23]. The interaction of milk protein with polyphenols induces structural changes in whey and casein proteins [12,24,25].

3.2. Emulsion stability and antioxidant activity

3.2.1. Emulsion stability. The different concentrations of xanthone had a highly significant effect \((p<0.01)\) on emulsion stability (table 2). The average emulsion stability in whey protein-xanthone emulsion with the addition of xanthone concentration 0.025\%, 0.05\% and 0.1\% increased. The highest emulsion stability was in 0.1\% xanthone concentration. This means that the concentration produces the most stable emulsion.

| Xanthone concentration (%) | Emulsion stability (%) | Antioxidant (%) |
|----------------------------|------------------------|-----------------|
| 0.025                      | 89.14\(^a\)            | 74.51\(^a\)     |
| 0.05                       | 94.02\(^b\)            | 77.12\(^a\)     |
| 0.1                        | 99.23\(^c\)            | 86.40\(^a\)     |

Note: Different uppercase letters in the same column indicated a highly significant effect \((p<0.01)\)

The binding of whey protein-xanthone increased emulsion stability. This is due to the existence of several bonds that play a role in the interaction, such as hydrogen bond, hydrophobic interaction, and van der walls bond. This is in accordance with molecular docking results that there is some interaction between whey protein and xanthone. This is consistent with the results of the study that proteins that interact with phenolic compounds will produce high emulsion values. Proteins have a stable emulsion if they are bound to phenolics [26]. This is indicated by high emulsion stability value. A number of studies explained that the interaction between milk protein and phenolic compounds changes the stability, structure, digestibility and functional properties of the protein. The emulsion stability has an important role in determining the properties of emulsions in food emulsion systems [27,28]. The emulsion is a suitable way of delivering functional ingredients into food systems.

3.2.2. Antioxidant activity. In this experiment, the total antioxidant interaction between WP and xanthone used 3 treatments of 0.1\%; 0.05\%; and 0.025\%. Statistically, there was no significant difference between the three treatments on antioxidant activity. However, the highest total antioxidant was at 0.1\% concentration of xanthone (table 2). Based on total antioxidants, it indicates that whey protein could be used as a carrier vehicle of xanthone. This is in accordance with Milani \textit{et al} [29], which explains that whey protein is suitable as a transfer system of bioactive compounds. It means that plant-based bioactive compounds carried by WP still possess their antioxidant activity.

Several studies have shown that xanthones obtained from mangosteen pericarp have remarkable biological activities such as antioxidant, antitumor, anti-inflammatory, anti-allergy, antibacterial, antifungal, and antiviral activities. Antioxidant activity of xanthone is more than vitamin E and vitamin C [30]. Xanthones are also known for their potential to inhibit several stages in the carcinogenesis process, as well as tumor cells including kinase, cyclooxygenase, ribonucleotide reductase and DNA polymerase [31]. An increase in antioxidant activity that resulted from the combination of WP-xanthone makes this material suitable as an antioxidant protein delivery system.
Figure 3. Peaks of FTIR spectroscopic.
3.2.3. FTIR spectroscopic measurement. Peaks of FTIR spectroscopic measurements are shown in figure 3. The irradiate spectra of the interaction between whey protein and xanthone using 3 treatments revealed that treatments 0.1% and 0.025% had similar peaks, while 0.05% treatment resulted in different peaks, especially at a wavelength of 2500-2700 cm\(^{-1}\). It indicates the presence of OH groups Carboxylic acids with hydrogen bonds. This is in accordance with the results of molecular docking analysis that α-lactalalbumin-xanthone were stabilized by hydrogen in GLN43, while β-lactoglobulin-xanthone was stabilized by hydrogen bond in GLN59. Some studies also show that interaction between WP and phenolic forms hydrogen bond, van der Waals attraction and Hydrophobic interaction [13-14]. The study revealed that wave numbers 690-900 and 3010-3100 cm\(^{-1}\) indicated the presence of CH groups, which are the groups of xanthone compounds. Wavenumber 1500-1600 cm\(^{-1}\) indicated the presence of C = C aromatic ring groups. Wavenumber 675-995, 1610-1680 and 3010-3095 cm\(^{-1}\) indicated the presence of C-H Alkene groups. Wavenumber 2850-2970 and 1340-1470 cm\(^{-1}\) indicated the presence of C-H Alkane groups. Wavenumber 2100-2260 cm\(^{-1}\) indicated the presence of C=C Alkane groups. Wavenumber 1500-1570 cm\(^{-1}\) indicated the presence of NO\(_2\) groups. Wavenumber 1180-1360 cm\(^{-1}\) indicated the presence of C-N Amina/amida groups. Wavenumber 1050-1300 cm\(^{-1}\) indicated the presence of C-O Alkohol/eter/asam karboksilat/ester groups. According to Wu et al [17], the hydrophobic interaction of the phenolic compound with proteins will form a hydrogen bond. It is formed the interaction between OH group (phenolic) and polar group (NH\(_2\), NH, OH, and the SH group) on the protein surface. The emergence of the xanthone functional group on the irradiate spectra indicates that the process has been done correctly, thus it doesn't eliminate xanthone content.

4. Conclusion
This study showed that the interaction of xanthone and whey protein is stabilized by hydrogen bonds, hydrophobic interaction, and van der walls bond. FTIR spectroscopic measurements revealed that the wavenumbers 690-900 and 3010-3100 cm\(^{-1}\) indicated the presence of CH groups and 1500-1600 cm\(^{-1}\) indicated the presence of C = C aromatic ring groups. The highest emulsion stability was at 0.1% with 99.23%. The highest antioxidant was at 0.1% with 86.40%.

References
[1] Caillard R, Boutin Y and Subirade M 2011 Characterization of Succinylated β-Lactoglobulin and Its Application as The Excipient in Novel Delayed Release Tablets Int. Dairy J. 21 27–33
[2] Mounsey J S, O’Kennedy B T, Fenelon M A and Brodkro A 2008 The Effect of Heating on B-Lactoglobulin–Chitosan Mixtures as Influenced by pH and Ionic Strength Food Hydrocoll. 22 65–75
[3] Teixeira M, Alonso M J, Pinto M M M and Barbosa C M 2005 Development and characterization of PLGA nanospheres and nanocapsules containing xanthone and 3-methoxyxanthone European Journal of Pharmaceutics and Biopharmaceutics 59(3) 491–500
[4] Obolskiy D, Ivo P, Nisarat S and Michael H 2009 Garcinia mangostana L.: A Phytochemical and Pharmacological Review Phytother. Res. 23(8) 1047–65
[5] Vegarud G E, Langsrud T and Svenning C 2000 Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics Brit. J. Nutr. 84 91–8
[6] Yan Y, Hu J and Yao P 2009 Effects of Casein, Ovalbumin, and Dextran on the Astringecy of Tea Polyphenols Determined by Quartz Crystal Microbalance With Dissipation Langmuir 25 397–402
[7] Chen L and Subirade M 2005 Chitosan/β-lactoglobulin Core-Shell Nanoparticles as Nutraceutical Carriers Biomaterials 26 6041–53
[8] Maoela M S, Arotiba O A, Baker P G L, Mbusela W T, Jahed N, Songa E A and Iwuoha E I 2009 Electroanalytical Determination of Catechin Flavonoid in Ethyl Acetate Extracts of Medicinal Plants J. Electrochem. 4 1497–510
[9] Nagarajan M, Benjakul S, Prodpran T, Songtipya P and Kishimura H 2012 Characteristics and Functional Properties of Gelatin from Splendid Squid (Loligo formosana) Skin as Affected by
Extraction Temperatures Food Hydrocoll. 29(2) 389–97

[10] Raghavendra M, Reddy A M, Yadav P R, Raju A S and Kumar L S 2013 Comparative studies on the in vitro antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of india. Asian J. Pharm. Clin. Res. 6(3) 96–99

[11] Perez M D and Calvo M 1995 Interaction of B-Lactoglobulin with Retinol and Fatty Acids and Its Role as A Possible Biological Function for This Protein: A Review J. Dairy Sci. 78 978–88

[12] Kanakis C D, Hasni I, Bourassa P, Hamdani S, Tarantilis P A and Tajmir-Riahi H A 2011 Milk B-Lactoglobulin Complexes with Tea Polyphenols Food Chem. 127 1046–55

[13] Jauregi P, Olatujoye J B, Cabezudo I, Frazier R A and Gordon M H 2016 Astringency Reduction in Red Wine by Whey Proteins Food Chem. 199 547–55

[14] Wu X, Wu H, Liu M, Liu Z, Xu H and Lai F 2011 Analysis of Binding Interaction between (−)-Epigallocatechin (EGC) and B-Lactoglobulin by Multi-Spectroscopic Method. Spectrochim. Acta A 82 164–8

[15] Stojadinovic M, Radosavljevic J, Ognjenovic J, Vesic J, Prodic I, Stanic-Vucinic D and Cirkovic-Velickovic T 2013 Binding Affinity between Dietary Polyphenols and B-Lactoglobulin Negatively Correlates with the Protein Susceptibility to Digestion and Total Antioxidant Activity of Complexes Formed Food Chem. 136 1263–71

[16] Sahihi M, Bordbar A K and Ghayeb Y 2011 Thermodynamic stability and retinol binding property of β-lactoglobulin in the presence of cationic surfactants J. Chem. Thermodyn. 43 1185-9

[17] Wu X, Liu M, Xia L, Wu H, Liu Z and Xu X 2013 Conjugation of functional oligosaccharides reduced in vitro allergenicity of β-lactoglobulin Food Agricul. Immunol. 24 379–91

[18] Murray R. K, Granner D K and Rodwell V W 2006 Biokimia Harper (Jakarta: Penerbit Buku Kedokteran EGC)

[19] Mehranfar F, Bordbar A K and Parastar H A 2013 Combined Spectroscopic, Molecular Docking and Molecular Dynamic Simulation Study on the Interaction of Quercetin with B-Casein Nanoparticles J. Photochem. Photobiol., B. 127 100–7

[20] Arts M J T J, Haenen G R M M, Wilms L C, Beetstra S A J N, Heijnen C G M, Voss H P and Bast A 2002 Interactions between Flavonoids and Proteins Effect on the Total Antioxidant Capacity J. Agric. Food Chem. 50 1184–7

[21] Yuksel Z, Avci E and Erdem Y K 2010 Characterization of Binding Interactions Be-Tween Green Tea Flavanoids and Milk Proteins Food Chem. 121 450–6

[22] Frazier R A, Papadopoulou A and Green R J 2006 Isothermal Titration Calorimetry Study of Epicatechin Binding to SerumAlbumin J. Pharm. Biomed. Anal. 41 1602–5

[23] De Fretas N and Mateus S 2001 Structural features of procyanidin interactions with salivary proteins J. Agri. Food Chem. 49 940–5

[24] Hasni I, Bourassa P, Hamdani S, Samson G, Carpenter R and Tajmir-Riahi H A 2011 Interaction of milk α- and β-caseins with tea polyphenols Food Chem. 126(2) 630–9

[25] Jobstl E, Howse J R, Fairclough J P A and Williamson M P 2006 Noncovalent cross-linking of casein by epigallocatechin gallate characterized by single molecule force microscopy J. Agri. Food Chem. 54 4077–81

[26] Prommajak T and Ravivan P 2013 Physical Properties of Gelatin Extracted from Skin of Thai Panga Fish 131 (Pangasius bocourti Sauvage) J. Food Appl. Biosci. 3 131–45

[27] Rahayu P P, Purwadi, Radiati L E and Manab A 2015 Physico chemical properties of whey protein and gelatine biopolymer using tea leaf extract as crosslink materials Curr. Res. Nutr. Food Sci. 3 224–36

[28] Yildirim-Elikoglu S and Erdem Y K 2018 Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry Food Rev. Int. 1–32

[29] Milani P G et al 2017 Fortification of the whey protein isolate antioxidant and antidiabetic activity with fraction rich in phenolic compounds obtained from Stevia rebaudiana (Bert.). Bertoni
leaves *J. Food Sci. Technol.* 54(7) 2020–9
[30] Kondo M, Zhang L, Ji H, Kou Y and Ou B 2009 Bioavailability and Antioxidant Effects of a Xanthone-Rich Mangosteen (Garcinia mangostana) Product in Humans *J. Agri. Food. Chem.* 57(19) 8788–92
[31] Shan T, Ma Q, Guo K, Liu J, Li W, Wang F and Wu E 2011 Xanthones from mangosteen extracts as natural chemopreventive agents: Potential anticancer drugs *Curr. Mol. Med.* 11 666–77