

Physical and oxidative stability of fish oil-in-water emulsions prepared with preheated whey protein and maltodextrins

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Sutida Watthanpimol

December 2011

Copyright © 2011 by Sutida Watthanapimol
All rights reserved.

ACKNOWLEDGEMENTS

It is my pleasure to thank many people who made my M.S. degree and this thesis completed. I would like to extremely thank my major professor, Dr.Qixin Zhong. With his expertise, inspiration, ideas, kindness, and great efforts to explain things, he helped me throughout the hard time of doing my research and writing thesis. I am grateful to Dr.John Mount and Dr.Federico Harte who served on my M.S. committee members.

Furthermore, I would like to thank all faculties, secretaries, and student colleagues from the Department of Food Science and Technology for kind assistance and creating friendly work environment.

Lastly, and most importantly, I deeply thank to beloved people who I dedicated my thesis to. I would like to give special thanks to my parents, Mr.Thumrong and Mrs.Sunee Watthanapimol. With their precious love, care, and dedication, I can get to where I am now. Especially, I wish to thank my lovely sisters, Ms.Suvida Watthanapimol, Ms.Jirayus Woraratphoka, and Ms.Jihyeon Oh, for their wise advice, encouragement, and emotional support.

ABSTRACT

Fish oil has abundant polyunsaturated fatty acids that are important to health but are oxidized easily during shelf-life storage. Engineering oil/water interfaces of emulsions has been studied extensively, but practical technologies are still demanded by the food industry. Whey proteins aggregate during heating, and the preheated whey protein/aggregate may increase the robustness of interfaces and in turn improve oxidative and physical stability of fish oil emulsions. In this study, whey protein isolate (WPI) solutions were prepared at 0-100 mM NaCl and pH 7.0 and preheated at 85 °C for 5-30 min. The preheated WPI was used to prepare fish oil emulsions that were evaluated for oxidative stability, droplet size, and zeta-potential during storage at 37 °C and 33.3% relative humidity for 35 days. The emulsions were also spray-dried for evaluation of oxidative stability of powdered products, which were also reconstituted for assessment of physical and oxidative stability. Further, because maltodextrins (MD) improve spray drying performance, emulsions were prepared at WPI:MD mass ratios of 1:2, 1:4, and 1:8. The best oxidative stability for both liquid and spray-dried emulsions was observed for emulsions prepared with WPI preheated at an intermediate NaCl concentration (25 mM), WPI:MD mass ratio (1:4), and heating time (15 min). For these emulsions, the highest extent of lipid oxidation was observed at day 20, with more significant reduction in lipid hydroperoxide (121.55 mEq/kg oil) and less in thiobarbituric-acid-reactive-substances - TBARS (7.10 mmol/kg oil) when compared to bulk fish oil (lipid hydroperoxide of 220.91 mEq/kg oil and TBARS of 8.55 mmol/kg oil). Emulsions prepared with preheated WPI had bigger droplets with less negative zeta-potential that changed more significantly during storage. . Our work demonstrated

an efficient approach to improve oxidative stability of fish oil, but much work is needed to improve both physical and oxidative stability of emulsions.

TABLE OF CONTENTS

CHAPTER I	Introduction.....	1
CHAPTER I	Literature review.....	4
	Omega-3 polyunsaturated fatty acids and their significance to health.....	5
	Oxidation of Omega-3 PUFAs and prevention strategies.....	6
	Spray-drying for production of powdered Omega-3 PUFAs products.....	7
	Whey proteins as emulsifiers and wall materials.....	9
CHAPTER III	Materials and Methods	
	Materials.....	11
	Methods.....	12
	Characterization of preheated WPI solutions.....	13
	Particle size.....	13
	Zeta potential.....	13
	Surface hydrophobicity.....	14
	Preparation of emulsions.....	15
	Stability of emulsions during storage.....	17
CHAPTER IV	Results and Discussions.....	18
	Physical properties of whey protein isolate after preheating.....	19
	Oxidative stability of WPI-stabilized emulsions during storage.....	22
	Emulsions prepared with WPI preheated at different durations.....	22
	Emulsions prepared with WPI preheated at different ionic strengths.....	23
	Emulsions prepared with different WPI:MD ratios.....	23
	Physical stability of emulsions.....	24
	Droplet size.....	24
	Zeta potential.....	26

CHAPTER V	Conclusions and Recommendations	27
REFERENCES		29
APPENDIXES		42
VITA		55

LIST OF FIGURES

Figure 1.	Development of (a) lipid hydroperoxides and (b) TBARS of fish oil emulsions stored at 37 °C, 33% relative humidity for 35 days. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing (loaded with 2 w/v% WPI, 4 w/v% MD, maltodextrin and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 0-30 min before emulsion preparation.....	42
Figure 2.	Development of (a) lipid hydroperoxides and (b) TBARS of spray-dried fish-oil powder stored at 37 °C, 33% relative humidity for 35 days. Fresh emulsions were prepared as those in Figure 1 before spray drying.....	43
Figure 3.	Development of (a) lipid hydroperoxides and (b) TBARS of fish-oil emulsions stored at 37 °C, 33% relative humidity for 35 days. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 4%w/v maltodextrin, 0-100 mM NaCl and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation.....	44
Figure 4.	Development of (a) lipid hydroperoxides and (b) TBARS of spray-dried fish-oil powder stored at 37 °C, 33% relative humidity for 35 days. Fresh emulsions were prepared as those in Figure 3 before spray drying.....	45
Figure 5.	Development of (a) lipid hydroperoxides and (b) TBARS of fish-oil emulsions stored at 37 °C, 33% relative humidity for 35 days. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, maltodextrin at a WPI:MD ratio of 1:2, 1:4, and 1:8, 25 mM NaCl, and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation.....	46
Figure 6.	Development of (a) lipid hydroperoxides (b) TBARS of spray-dried fish-oil powder stored at 37 °C, 33% relative humidity for 35 days. Fresh emulsions were prepared as those in Figure 5 before spray drying.....	47
Figure 7.	Changes of droplet sizes during incubation influence at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 4%w/v maltodextrin and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 0-30 min before emulsion preparation.....	48

Figure 8. Changes of droplet sizes during incubation influence at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 0-100 mM NaCl, 4%w/v maltodextrin and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation.....	49
Figure 9. Changes of droplet sizes during incubation influence at 37°C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, maltodextrin at a WPI:MD ratio of 1:2, 1:4, and 1:8, 25 mM NaCl, and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation.....	50
Figure 10. Changes of zeta-potential of droplets during incubation influence at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 4%w/v maltodextrin and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 0-30 min before emulsion preparation.....	51
Figure 11. Changes of zeta-potential of droplets during incubation influence at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 0-100 mM NaCl, 4%w/v maltodextrin and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation.....	52
Figure 12. Changes of zeta-potential of droplets during incubation influence at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, maltodextrin at a WPI:MD ratio of 1:2, 1:4, and 1:8, 25 mM NaCl, and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation.....	53

LIST OF TABLES

Table 1. Properties of major whey proteins and their compositions in a typical isolate product (Byant & McClements, 1998).....	10
Table 2. Physic properties* of WPI dispersions at pH 7.0 and 0 mM NaCl after preheating at 85 °C for different durations.....	20
Table 3. Physic properties* of WPI dispersions at pH 7.0 with 0-100 mM NaCl after heating at 85 °C for 15 min.....	21

CHAPTER I

INTRODUCTION

Omega-3 polyunsaturated fatty acids (PUFAs), especially EPA (eicosapentaenoic acid, C20:5n3) and DHA (docosahexaenoic acid, C22:6n3), have been shown to maintain good health and prevent several human diseases and disorders (Shibasaki et al., 1999; Uauy & Valenzuela, 2000; Wanasundara & Shahidi, 1998). Fish oil contains considerable amounts of omega-3 PUFAs (Klinkesorn, H-Kittikun, Chinachoti, & Sophanodora, 2004) and may be a useful dietary supplement. However, long-chain PUFAs of fish oils are highly unsaturated and are very susceptible to oxidation. Lipid oxidation can be reduced by antioxidant addition or by microencapsulation of the oil (Heinzelmann, Franke, Velasco, & Marquez-Ruiz, 2000; Kagami et al., 2003; Lin, Lin, & Hwang, 1995; Velasco, Dobarganes, & Marquez-Ruiz, 2003).

Whey proteins are common choices in preparation of food emulsions because of their availability and excellent interfacial properties (Dickinson, 2001), which are dependent on pH, ionic strength and processing conditions (Dalgleish, Dickinson, & Lorient, 1995). Emulsions of PUFAs prepared with whey proteins were also observed to have improved oxidative stability (Coupland & McClements, 1996). It is also well known that whey proteins aggregate during heating, and aggregated whey proteins may provide a thicker interfacial layer than individual proteins and thus improve physical and oxidative stability of fish oil emulsions.

The first objective of this work was to determine the effect of heat treatment on whey protein properties. The second objective was to evaluate physical and oxidative stability of fish-oil emulsions emulsified by preheated whey protein isolate (WPI). Three types of emulsions were studied during storage at 37 °C and 33.3 % relative humidity up to 35 days: fresh emulsions, spray-dried emulsion powders, and emulsions reconstituted from spray-dried

powders. Because maltodextrins (MD) facilitate wall structure of capsules, MD was used at different mass ratios to WPI in preparation of emulsions.

CHAPTER II

LITERATURE REVIEW

Omega-3 polyunsaturated fatty acids and their significance to health

There are two categories of fatty acids saturated and unsaturated, with the latter consisting of one or multiple carbon to carbon double bonds. Polyunsaturated fatty acids (PUFAs) are those with two or more double bonds, and those two or more double bonds, and those with a double bond with a double bond the third carbon from the methyl end of carbon chain are called Omega-3 PUFAs, also named n-3 or ω -3 (Mcmanus, Merga, & Newton, 2011). Omega-3 PUFAs are essential fatty acids because they cannot be synthesized by mammals. Marine foods are good sources of Omega-3 PUFAs that can be further converted into DHA (docosahexaenoic acids) and EPA (eicosapentaenoic acids) in human body (McManus, Merga, Newton, 2011). Dietary supplement products of Omega-3 PUFAs are currently valued at over US \$700 million worldwide, with a growth rate estimated at 8% annually (Clough, 2008). Among recognized health benefits of Omega-3 PUFAs include promotion of infant-growth prevention of cardiovascular diseases reduction of platelet aggregation lowering of hyper-tension protection of cancer and Alzheimer's disease depression release, and inflammation recovery (McManus, Howieson, & Nicholson, 2009; Riediger, Othman, Suh, & Moghadasian, 2009; Ruxton & Derbyshire, 2009). Studies employing Omega-3 PUFAs demonstrated results impacted by factors such as sample size, subject characteristics, duration of study, dosages, form, methods of analysis resulting in development of different hypothesis and conclusions. Marine-sourced of were observed to perform better than those from other sources (McManus, Merga, & Newton, 2011). For example, Wang and others (2006) reported the effective prevention of cardiovascular disease for patients consuming Omega 3 PUFAs from fish or fish oil prevention, but not those consuming α -linoleic acid. In addition to specific types of fatty acids, the

effectiveness of promoting health for foods fortified with Omega -3 PUFAs is impacted by the composition, production, and storage conditions of food products (Kolanowski, 2005).

Oxidation of Omega-3 PUFAs and prevention strategies

With multiple double bonds, Omega-3 PUFAs are easily oxidized by environmental stresses, as reviewed by Cho, Decker, & McClements, (2002) and Nuchi, Decker, & McClements, (2004). Oxidation changes appearance, nutritional quality, taste, and texture, eventually shortening shelf-life. Lipid oxidation is a challenge in fortify food products with Omega-3 PUFAs, especially in emulsion-based foods or beverages where the small size of emulsion droplets presents large surface areas. The rate of lipid oxidation in food emulsions depends on molecular structures of lipids, heat, light, characteristics of droplets, processing conditions, and the presence of antioxidants or pro-oxidants (transition metals). Transition metals, particularly iron, facilitate decomposition of lipid hydroperoxide to aldehyde and ketone that cause off-flavor and off-odor of foods. Preventing iron in the aqueous phase from contacting the oil phase (Cho, Decker, & McClements, 2002; Nuchi, Decker, & McClements, 2004) or incorporating chelating agents such as ethylenediaminetetraacetic acid (EDTA) or antioxidants can potentially reduce the decomposition of hydroperoxides, primary products in lipid oxidation (Mancuso, McClements, & Decker, 1999; McClements & Decker, 2000). As for antioxidants natural ones such as proteins, polysaccharides, and citric acid are desired by food manufacturers. Anti-oxidation properties of milk proteins were speculated to have originated from sulfhydryl groups (Faraji, McClements, & Decker, 2004). In addition, interfacial layer of droplets is a physical barrier that can be engineered to prevent the contact between hydroperoxide residues in

the oil phase and pro-oxidants in the continuous aqueous phase (Tong, Sasaki, McClements, & Decker, 2000; Chaiyasit, Silverstre, McClements, & Decker, 2000). One example of engineering interfaces is the layer-by-layer deposition technique where multiple types of charged emulsifiers can sequentially adsorb to oil droplet surfaces with opposite charges (Guzey & McClements, 2006). Another example of generating thick interfaces is to apply particles to form ‘Pickering emulsions,’ as firstly revealed by Pickering (1907). Particles strongly adsorbed onto the droplet surface strengthens the ability of droplet against instability mechanisms such as coalescence (Melle, Lask, Fuller, & Langmuir, 2000).

Spray-drying for production of powdered Omega-3 PUFAs products

Encapsulation is a process by entrapping oil droplets or bioactive compounds within coating materials that act as a protective wall preventing the core components from deterioration and changes in both quality and functionality. In general, capsules are referred to spherical particles composed of an internal phase of an encapsulated ingredient, called ‘the core,’ and an outer coating of an encapsulating agent, called ‘shell’, ‘wall,’ or ‘membrane’. Both the core and shell can be composed of more than one ingredient. Shahidi and Han (1993) listed the benefits of encapsulation process in food applications such as reduced interaction between core compounds and environment, easier handling controlled release of the core components, masking of unpleasant taste of the core and facilitated dispersion of (particularly hydrophobic) core materials in aqueous medium. A large variety of core compounds, wall materials, and encapsulation techniques have been studied to produce a wide range of encapsulation products with varying

shape, particle size, release properties, and re-dispersion ability (Desai & Park, 2005; Gibbs, Kermasha, Alli, & Mulligan, 1999; Gouin, 2004; King, 1995; Shahidi & Han, 1993).

Among the techniques developed to encapsulate food ingredients, spray-drying is the most commonly used because it is a low cost process and does not require complex steps and equipment (Gouin, 2004; Shahidi & Han, 1993). Conventionally, spray-drying is used to convert a liquid product to a powdered one so as to protect core compounds against degradation and oxidation, reduce flashing off of volatile flavors, reduce the expensive ingredients, and facilitate transportation and storage (McClements, 2011).

Examples of wall materials include of polysaccharides (such as gum arabic, alginates, carragenans, maltodextrins) and proteins (such as dairy proteins, gelatin) and low molecular weight carbohydrates (Reineccius, Ward, Whorten, & Andon, 1995; Thevenet, 1995). Other low cost materials such as mesquite gum were also studied (Beristain & Vernon-Carter, 1994; Beristain, Garcí'a, & Vernon- Carter, 2001) interfacial engineering strategies have recently been developed in order to improve quality of emulsions and subsequent spray-dried powders. Particularly relevant to this work is an example of tuna oil emulsions prepared by the layer-by-layer deposition technique before spray drying (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006). The tuna-oil emulsions prepared to possess multiple layers of coatings were observed to have much improved physical and oxidative stability when compared to those with a single layer, in both powdered and reconstituted forms.

Whey proteins as emulsifiers and wall materials

Whey proteins refer to a group of proteins recovered from liquid whey, typically as a byproduct of cheese-manufacturing. Whey protein isolates (WPI) are commercial products with a protein content higher than 90%. β -lactoglobulin (more than 50%) and α -lactalbumin (10-15%) are major whey proteins (Elfagm & Wheelock, 1978). Because of ability to emulsify and stabilize interfaces whey proteins are widely used in food emulsion systems. Recent studies (Livney et al., 2003; Nicorescu et al., 2008; Raikos, 2010) also revealed the ability of whey proteins to adsorb onto oil droplets during heat treatment, which provides additional stability to emulsions.

The ability of whey proteins to form aggregates has been extensively studied. As listed in Table 1, whey proteins denature at different temperature, and the denatured proteins aggregate due to combination of physical and chemical forces. Based on the model of Nicolai et al. (2011), the aggregation of whey protein and eventually possible gel formation can be described by several steps. Native proteins exist as monomers and dimers. During heating, hidden hydrophobic and thiol groups of whey proteins are exposed, allowing inter-molecular interactions. Initially, small oligomers, dimers and trimmers, are linked together by disulfide bonds. When the amount of oligomers exceeds a critical point, primary aggregates are formed. The size and shape of aggregates are affected by several factors such as pH, heating temperature and duration, protein concentration, and ionic strength, eventually resulting in gels with varying strength and appearance. However, the exact mechanisms leading to specific morphology and stability of whey protein aggregates remain complex and require careful interpretation.

Table 1. Properties of major whey proteins and their compositions in a typical isolate product

(Byant & McClements, 1998).

Protein	pI	M.W. (Da)	T_d (°C)^a	% in WPI
β-lactoglobulin	5.2	18400	78	60
α-lactalbumin	4.8 - 5.1	14200	62	22
Bovine serum albumin	4.8 - 5.1	66000	64	5.5
Immunoglobulins	5.5 - 6.8	$15-96 \times 10^4$	72	9.1

^aDenaturation temperature.

Nevertheless, preheating whey proteins to form aggregates as emulsifiers has been studied by several researchers (Demetriades, Coupland & McClements, 1997; Hunt & Dalgleish, 1995; Jost, Baechler & Masson, 1986; Masson & Jost, 1989; Monohan, McClements & German, 1996; Yamauchi, Shimizu & Kamiya, 1980; Yost & Kinsella, 1992). As expected, the stability of emulsions is impacted by preheating conditions such as pH, ionic strength, temperature, and protein content (Demetriades et al., 1997; Hunt & Dalgleish, 1995).

CHAPTER III

MATERIALS AND METHODS

Materials

MEG-3TM Fish oil, labeled as “Omega 30 TG food grade fish oil”, was kindly supplied by Ocean Nutrition Canada (Dartmouth, Nova Scotia, Canada). According to the manufacturer, the product contained 30% omega-3 fatty acids. Fish oil was weighted in 150 ml screw cap bottles and store in a -18°C freezer and thawed right before use; after each use, the head space of container was flushed by nitrogen before being returned to the -18°C freezer. Whey protein isolate (WPI) was provided by Hilmar Ingredients (Hilmar, CA, USA). Maltodextrin (MD 180) was from Grain Processing Corporation (Muscatine, IA). Dialysis tubing with a molecular weight cut-off of 3,500 Da was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other chemicals used were analytical grade and purchased from Fisher Scientific or Sigma Chemical Co. (St. Louis, MO, USA).

Methods

Preparation of WPI solutions

WPI solution was prepared by hydrating 4 %w/v WPI in deionized water. A series of MD solutions were prepared separately at 8, 16 and 32 %w/v in deionized water. The WPI and MD solutions were mixed at an equal volume to obtain a final solution containing 2%w/v WPI and WPI:MD ratios of 1:2, 1:4, and 1:8. The mixture was stirred for about 3 h to ensure complete dissolution of WPI and was kept at 4 °C for 12-15 h before preparing preheated solutions for characterization or emulsification.

Characterization of preheated WPI solutions

The above 2% WPI solutions were adjusted to pH 7.0 and 0-100 mM NaCl using 1 N HCl or NaOH. Solutions were heated in 85 °C water bath up to 30 min and cooled in a room temperature water bath immediately. The preheated solutions were characterized for following properties.

Particle size

The particle size of the WPI solutions was measured using a laser light scattering instrument (model DelsaTM Nano C, Beckman, Coulter, Inc, Fullerton, CA). Volume-weighted mean diameters, $D_{4,3}$, were calculated according to Equation 1. All measurements were conducted on 2 freshly prepared samples, each measured twice.

$$D_{4,3} = \frac{\sum_i Z_i D_i^4}{\sum_i Z_i D_i^3} \quad \text{—————} \quad (1)$$

where Z_i is the number of particles with diameter D_i .

Zeta potential

The zeta potential of the dispersions was analyzed using the above DelsaTM Nano C instrument. The measurements were determined from duplicate samples, with each measured two times for a total of 4 readings. For preheated WPI dispersion prepared with NaCl, dialysis was applied after heating. 3 ml of dispersion sample was taken into dialysis tube and soaked in 400 ml of deionized water for 3 h. Then the solvent was changed 4 times before zeta potential measurement was performed.

Surface hydrophobicity

Surface hydrophobicity of WPI was estimated by the method of Hayakawa and Nakai (1985) with modification. After preheating, the WPI solutions were centrifuged at 10,000g for 15 min at 4 °C and 4 ml of the supernatant was taken to react with 20 µL of a 1-anilino-8-naphthalenesulfonate magnesium salt (ANS) stock solution prepared at 8 mM in 0.1 M phosphate buffer and adjusted to pH 7.0. The reaction was allowed at room temperature for 15 min, followed by measurement for the relative fluorescent intensity (RFI) using a photon-counting FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) with excitation (390 nm) and emission (470 nm) slits set at 5 nm to obtain the fluorospectrum. The RFI values acquired from samples with different protein concentrations were used for linear regression to obtain a slope ($S_o = \text{RFI}/\% \text{protein}$) as surface hydrophobicity. Similar to other measurements, two sample replicates were measured for twice each.

Preparation of emulsions

The 2 wt% WPI solutions with different amounts of MD were adjusted for pH and ionic strength and preheated as above. The same solution without preheating was used as a control. Fish oil-in-water emulsions were prepared by emulsifying 10% v/v fish oil in WPI solutions at 1.5×10^4 rpm for 3 min using a high speed homogenizer (Cyclone I.Q.², The VirTis Company, Inc., Gardiner, NY). Sodium azide was applied to liquid emulsions at 0.1% to prevent microbial growth. The liquid emulsions were kept at storage conditions detailed below for evaluation of physical and oxidative stability. In separate experiments, the prepared O/W emulsions were

spray-dried using a B-290 Mini Spray Dryer (BÜCHI Labortechnik AG, Postfach, Switzerland) at a feed rate of 8.00 ml/min. The inlet and outlet temperatures were at 160 °C and 70 – 80 °C, respectively. The spray-dried powders were collected and stored at -18°C until oxidative stability analysis. The spray-dried powders were also reconstituted in deionized water and adjusted to pH 7.0 at a concentration equivalent to 0.8 mg fish oil/ml. Reconstituted emulsions were placed into capped vials and incubated at the below storage conditions before analyses for physical and chemical stability.

Stability of emulsions during storage

To evaluate emulsion stability, emulsion samples were stored above a saturated solution of magnesium chloride (to achieve 33.3 % relative humidity) and incubated at 37 °C for accelerated oxidation up to 35 days (Shaw, McClements, & Decker, 2007). Evaluation of emulsion stability was conducted at 5-day intervals for lipid oxidation detailed below and also for droplet diameter and zeta potential using the methods detailed above for WPI solutions.

Oxidative changes during storage were monitored by analyzing peroxide value (Shanta & Decker, 1994) and thiobarbituric acid-reactive substances (TBARS) (Ronald, 2001) as indicators of primary and secondary lipid oxidation products, respectively. Hydroperoxide concentration was determined by following the method of Shanta and Decker (1994) who modified the IDF standard method 74A: 1991 (International Dairy Federation, 19). 0.012 g of sample was added with 10 ml of a ternary mixture of 1-butanol, isopropanol, and 0.5 M HCl (in deionized water) at a volume ratio of 2: 2: 1 by vortexing three times for 10 s each. The samples were then centrifuged for 5 min at 10,000g (Sorval RC 5B Plus centrifuge, Kendro laboratory products).

The supernatant was transferred and mixed with 50 μ l of a 3.94 M ammonium thiocyanate solution followed by addition of 50 μ l of a ferrous iron stock solution that was prepared by dissolving 0.132 M BaCl_2 and 0.144 M FeSO_4 in 0.4 M HCl. The final solution was vortexed and incubated at room temperature for 5 min before measurement of absorbance at 500 nm using a UV/Vis spectrophotometer (model BioMate 5, Thermo Fisher Scientific, Pittsburgh, PA). A mixture of all reagents except the sample was used as a blank. Lipid hydroperoxide concentration was determined using a standard curve where standard solutions of ferric chloride were used to react with ammonium thiocyanate directly.

Determination of TBARS followed the method of Ronald (2001) and Hu & Zhong (2010). A thiobarbituric acid stock solution was prepared to contain 15 g trichloroacetic acid, 0.75 g thiobarbituric acid, and 0.8 % 2, 6-di-tert-butyl-4-methylphenol (BHT) in 100 ml of the above ternary solvent mixture of 1-butanol, isopropanol, and 0.5 M HCl. A powder or liquid emulsion sample was added with the thiobarbituric acid stock solution to a total volume of 10 ml. After vortexing, samples were centrifuged 10000g for 5 min, the supernatant taken and incubated in a 95 °C water bath for 2 h. After cooling in a room temperature water bath, the absorbance at 532 nm was measured. The ternary solvent mixture without emulsion sample added was treated identically to obtain absorbance as a sample blank. TBARS values were estimated using a standard curve constructed using 1, 1, 3, 3 – tetramethoxypropane (TMP) as a standard.

Experimental design

Two replicate samples were prepared, and two measurement replicates were performed. Averages and standard deviations from 4 measurements were reported. To analyze differences among treatments, a one-way ANOVA was conducted at a significance level of 0.005. If significant differences were detected, a multiple comparison test (Duncan test) was applied. The software SAS[®] version 9.22 (SAS Institute, Cary, NC) was used in all analyses.

CHAPTER IV
RESULTS AND DISCUSSION

Physical properties of whey protein isolate after preheating

Table 2 summarizes physicochemical characteristics observed for preheated WPI dispersions in the absence of NaCl after heating at 85 °C for different durations. Bigger particles formed after heating for a longer time, from 82.45 nm before heating to 116.60 nm after heating for 30 min. These results agree with a report of larger denatured β -lactoglobulin aggregates formed at a higher temperature or a longer heating time (Jang & Swaisgood, 1990). An increase in heating time also corresponded to a progressive change of zeta potential of WPI particle to be less negative. When WPI was not heated, the zeta-potential was -29.65 mV that decreased its magnitude -22.50 mV after being heated for 30 min. Native whey proteins have hydrophilic, charged amino acids on surface. Upon heating, proteins are unfolded, with some embedded hydrophobic amino acids now exposed to the surface, and the end result is the decreased number of charged amino acids on the surface. Heat-induced increase in abundance of hydrophobic amino acids on protein surface is well-known (Bryant & McClements, 1998) and is verified by results of surface hydrophobicity in Table 2.

Table 2. Physic properties* of WPI dispersions at pH 7.0 and 0 mM NaCl after preheating at 85 °C for different durations.

Heating time (min)	Particle diameter (nm)	Zeta-potential (mv)	Surface-hydrophobicity index (S ₀)
0	82.45 ± 5.73 ^e	-29.65 ± 1.06 ^a	54.40 ± 1.70 ^e
5	99.60 ± 1.41 ^d	-28.35 ± 1.20 ^a	59.20 ± 1.56 ^d
15	104.50 ± 1.13 ^c	-27.70 ± 1.27 ^a	65.35 ± 1.20 ^c
20	108.45 ± 2.90 ^b	-25.05 ± 0.35 ^b	69.05 ± 2.19 ^c
25	110.85 ± 3.75 ^b	-23.02 ± 1.25 ^b	75.50 ± 1.70 ^b
30	116.60 ± 3.25 ^a	-22.50 ± 1.27 ^b	82.50 ± 2.40 ^a

*Results are reported as means ± standard deviation and values with different superscript letters in each column are significantly different ($P \leq 0.05$).

Table 3 shows properties of WPI after heating at pH 7.0 and 0-100 mM NaCl at 85 °C for 15 min. There was no difference between diameters of WPI dispersions preheated at 0 or 10 mM NaCl. After heating, the particle size increased from 122.8 nm at 25 mM NaCl to 246.65 nm at 100 mM NaCl. The larger size of WPI particles formed at a higher concentration of added NaCl is due to reduced Debye length and thus increased likelihood of protein aggregation.

At a higher ionic strength (higher NaCl concentration), zeta-potential of preheated WPI became less negative due to a higher extent of protein unfolding and aggregation, as indicated by

a higher surface hydrophobicity (Table 3). Zeta potential dialysis are also reported in Table 3 and was less negative than the corresponding sample after dialysis. This indicates that NaCl impacts measurement of zeta potential of preheated WPI dispersions and dialysis is needed to properly assess this parameter.

As for surface hydrophobicity, it is measured based on the quantity of ANS specific hydrophobic groups exposed to an aqueous phase, which is increased after heating for a longer time and/or at a higher ionic strength (Demetriades et al., 1997). Impacts of heating on whey protein surface hydrophobicity were also verified for emulsions stabilized by WPI (Euston et al., 2000).

Table 3. Physic properties* of WPI dispersions at pH 7.0 with 0-100 mM NaCl after heating at 85 °C for 15 min.

NaCl concentration (mM)	Mean diameter (nm)	Zeta-potential before dialysis (mv)	Zeta-potential after dialysis (mv)	Surface-hydrophobicity index (S_0)
0	105.4 ± 1.56 ^d	-25.95 ± 0.64 ^a	-	68.55 ± 2.62 ^e
10	111.95 ± 2.19 ^d	-23.85 ± 0.92 ^a	-27.41 ± 0.45 ^a	82.05 ± 2.19 ^d
25	122.8 ± 3.68 ^c	-21.85 ± 0.78 ^b	-26.35 ± 0.32 ^b	93.4 ± 3.11 ^c
50	162.35 ± 8.70 ^b	-19.85 ± 1.06 ^b	-24.65 ± 0.18 ^b	122.85 ± 6.58 ^b
100	246.65 ± 22.70 ^a	-15.4 ± 1.56 ^c	-21.12 ± 0.66 ^c	141.2 ± 6.51 ^a

*Results are reported as means ± standard deviation and values with different superscript letters in each column are significantly different ($P \leq 0.05$).

Oxidative Stability of WPI-stabilized emulsions during storage

The first set of experiments was carried out to identify the optimum preheating time for WPI that was the most effective in inhibiting lipid oxidation in corresponding emulsions. The second set of samples was tested for WPI preheated using the optimized heating time but varying ionic strengths. The third set of samples was prepared using different MD concentrations and same heating time and ionic strength selected from the first and second sets of samples (all figures were showed in appendix).

Emulsions prepared with WPI preheated at different durations

Oxidative stability of emulsions prepared with WPI preheated at 85 °C for different durations is presented in this section. All samples oxidized very rapidly with increases in lipid hydroperoxides and TBARS from day 0 to day 20, followed by decreased oxidation rates at longer incubation times tested up to day 35, for both liquid (Figure 1) and dried emulsions (Figure 2). At day 20, lipid hydroperoxides of liquid emulsions prepared with preheated WPI were lower than those of the emulsion prepared with unheated WPI and bulk fish oil (Figure 1a), but the impact of preheating time on lipid hydroperoxides was not significant. TBARS was not significantly different for all samples (Figure 1b). The preheating time of 15 min was chosen for subsequent studies because of the lowest average lipid hydroperoxide value (Figure 1a). For this emulsion, lipid hydroperoxide and TBARS values at day 20 were 169.32 mEq/ kg oil and 8.16 mmol/ kg oil, respectively, that were respectively improved from 220.91 mEq/ kg oil and 8.55 mmol/kg oil for the bulk fish oil. Spray-dried powders (Figure 2) showed similar oxidative stability to liquid emulsions (Figure 1). The powders prepared with WPI preheated for 15 min

had the lowest lipid hydroperoxide (153.23 mEq/ kg oil) and TBARS (7.41 mmol/ kg oil) values at day 20 of storage.

Emulsions prepared with WPI preheated at different ionic strengths

In this set of experiments, WPI solutions at pH 7.0 were dissolved with different concentrations of NaCl and preheated at 85 °C for 15 min before being applied to prepare emulsions. Figure 3 shows the reduced formation of lipid hydroperoxides and TBARS in liquid emulsions for samples prepared with WPI preheated with NaCl. The emulsions prepared with 25 mM NaCl exhibited the lowest lipid hydroperoxide concentration (137.65 mEq/kg oil) and TBARS (7.48 mmol/kg oil) after 20 days of oxidation, while samples with other ionic strengths were statistically similar. Similar trends were observed for spray-dried powders (Figure 4): the sample prepared with 25 mM NaCl demonstrated the lowest amount of hydroperoxides (127.44 mEq/kg oil) and TBARS (7.18 mmol/kg oil) at day 20.

Emulsions prepared with different WPI:MD ratios

The above identified preheating conditions, i.e., at 85 °C for 15 min and with 25 mM NaCl, were selected to study the impact of WPI:MD ratio on oxidative stability of resultant emulsions. Figure 5 shows oxidative stability of liquid emulsions stabilized by preheated WPI with different maltodextrin concentrations was significantly improved when compared with bulk fish oil. Formations of hydroperoxides and TBARS showed similar trends for emulsions and bulk fish oil, with the maximum values detected after 20 days of storage. At day 20, the sample prepared with a WPI:MD ratio of 1:4 showed significantly lower lipid hydroperoxide (121.55

mEq/kg oil) and TBARS (7.10 mmol/kg oil) than other treatments. The observations were similar for spray-dried powders (Figure 6), with the sample prepared with a WPI: MD ratio of 1: 4 demonstrating lipid hydroperoxide value of 108.99 mEq/ kg oil and TBARS value of 6.98 mmol/kg oil.

Physical stability of emulsions

Droplet size

Physical stability was tested for both fresh and reconstituted emulsions by measuring droplet diameter changes during storage at 37°C. Changes in particle diameters of WPI-stabilized emulsions prepared by WPI preheated in the absence of NaCl for different durations are shown in Figure 7a for fresh emulsions and Figure 7b for reconstituted emulsions. Overall, bigger droplets were observed for emulsions prepared with WPI preheated for a longer time, and reconstituted emulsions had bigger droplets than the corresponding fresh emulsions. It was also observed that mean droplet sizes of emulsions stabilized by unheated WPI were significantly smaller than those stabilized by preheated WPI throughout storage time. Droplet sizes increased during storage, indicating possible occurrence of aggregation or coalescence, but the exact mechanism was not studied.

Figure 8 shows changes in droplet size for emulsions prepared from WPI preheated with different NaCl concentrations at 85 °C for 15 min. The measured droplet size was greater for emulsions prepared with WPI preheated with a higher concentration of NaCl. All samples showed an increase in droplet size during storage for both fresh and reconstituted emulsions. The

emulsions prepared with 100 mM NaCl had significantly larger particle sizes comparable to those with lower NaCl concentration. The Debye length decreases with an increase in ionic strength and this in turn increases the possibility of droplet aggregation. (McClements, 2004). However, whether the bigger droplet sizes at a higher ionic strength were caused by aggregation or coalescence is a future research topic.

Figure 9 presents mean droplet diameters of emulsions prepared with preheated WPI and three MD concentrations. When MD concentration was increased, the emulsion droplet sizes increased correspondingly. The droplet sizes of emulsions made with WPI:MD ratios of 1:8, 1:4, and 1:2 were slightly different from day 0 to day 20 of storage. After 25 days or longer, droplet sizes of emulsions prepared with a WPI:MD ratio of 1:4 and 1:8 were significantly larger than that prepared with a WPI:MD ratio of 1:2. Influence of MD concentration on emulsion droplet sizes may be explained by depletion flocculation due to non-adsorbing biopolymers. Physically, when the concentration of non-adsorbing biopolymers in the aqueous phase and thus osmotic pressure is sufficiently high, droplets diffusing to each other exclude biopolymers from the inter-droplet regime that subsequently has a lower osmotic pressure. The higher osmotic pressure in other regime then forces the two droplets to aggregate. The aggregation is reversible upon dilution, and therefore the phenomenon is called depletion flocculation. The extent and rate of flocculation are higher at a higher concentration of non-adsorbing biopolymers (Dickinson, 2003).

Zeta potential

Zeta potentials of emulsion droplets prepared with WPI heated at 85 °C from 5 to 30 min were recorded during storage at 37 °C. The zeta potential of the droplets in fresh emulsions was less negative for emulsions prepared with WPI preheated for a longer time (Figure 10), similar to observations from preheated WPI dispersions (Table 2). This is expected because WPI is the only ionizable constituent in emulsions. During storage, zeta-potential of all samples became less negative (Figure 8).

Figure 11 shows zeta-potential of fresh and reconstituted emulsions for those prepared with WPI preheated at different concentrations of NaCl. The zeta potential was less negative for emulsions prepared with WPI preheated at a higher NaCl concentration, similar to observations from WPI dispersions (Table 3). Again, electrostatic screening effects of ions reduce electrophoretic mobility and thus the measured zeta-potential of colloidal particles (Hunter, 1986; Israelachvili, 1992), but these emulsions were not treated by dialysis. During storage, zeta potential of all samples became less negative, more significant for emulsions prepared with a lower NaCl concentration. Similar magnitudes and trends were observed for reconstituted emulsions (Figure 11b).

For emulsions prepared with different concentrations of MD, zeta-potential was similar at day 0 and remained similar during storage (Figure 12a). MD are hydrophilic nonionic ingredients and are not expected to be surface-active or to adsorb to charged surfaces. Therefore, a higher concentration of MD did not influence zeta potential of emulsions. Zeta potentials observed for fresh emulsions had similar trends to those of reconstituted emulsions (Figure 12b) and became less negative for both sets of emulsions during storage.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

Preheating WPI to modify interfacial properties of whey proteins was observed to have improved oxidative stability of fish oil in the three types of emulsions tested: fresh emulsions, spray-dried powders, and reconstituted emulsions. Emulsions showing the best oxidative stability among the studied conditions were observed for those prepared with WPI preheated at 85 °C for 15 min and in the presence of MD at 4 times mass of WPI and 25 mM NaCl. The improvement was more significant in reduced formation of primary lipid oxidation products and less in secondary oxidation products. Preheating changed dimension and zeta-potential of whey proteins and the corresponding emulsions, and the degree of changes was influenced by preheating conditions, including heating duration, NaCl concentration and MD concentration. All treatments showed an increase in droplet size during storage based on light scattering experiments, indicating the possible occurrence of coalescence that requires additional techniques such as microscopy to identify exact instability mechanisms. Combination of other surface engineering approaches such as adsorption of a polyelectrolyte with opposite charges may reduce coalescence. The engineered interfacial structures may also improve oxidative stability of fish oil emulsions. Additionally, emulsion pH may impact physical and oxidative stability. Eventually, emulsions with excellent physical and oxidative stability during processing and storage can be used to improve healthfulness of food products.

LIST OF REFERENCES

Alamed, J., McClements D.J., Decker, E.A. (2006). Influence of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in oil-in-water emulsions containing omega-3 fatty acids, *Food Chemistry*, 95, 585.

Baussay, K., Le Bon, C., Nicolai, T., Durand, D., Busnel, J. (2004). Influence of the ionic strength on the heat-induced aggregation of the globular protein blactoglobulin at pH7. *Journal of Biological Macromolecules Int.*, 34, 21-28.

Beristain, C.I., Garcia, H.S., Vernon-Carter, E.J. (1994). Mesquite gum (*Prosopis juliflora*) and maltodextrin blends as wall materials for spray-dried encapsulated orange peel oil. *Drying Technology*, 12, 1727–1733.

Bos, M.A., Vliet, T.V. (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: a review. *Adv. Colloid Interface Sci.*, 91, 437–471.

Cho, Y.J., McClements, D.J., Decker, E.A. (2002). Ability of surfactant micelles to alter the physical location and reactivity of iron in oil-in-water emulsions. *Journal of Ag. Food Chem.*, 50, 5704-5710.

Coupland, J.N., McClements, D.J. (1996). Lipid oxidation in food emulsions review. *Trends Food Sci. Technol.*, 7, 83–91.

Clough, P. (2008). Marketing omega-3 products as nutraceuticals, *International Symposium on Fatty Acids – Opportunities for Health Education and Investment*, 19(3), 233–234.

Coupland., J., McClement, D.J. (1997). Physical properties of liquid edible oils. *Journal of Am. Oil Chem. Soc.*, 74, 1559-1564.

Corredig, M., Douglas, G., Dalgleish, D.G. (1995). A differential microcalorimetric study of whey proteins and their behaviour in oil-in-water emulsions . *Colloids and Surfaces B: Biointerface*, 4(6), 411-422.

Dalgleish, D.G. (1996). *Emulsions and emulsion stability*. Marcel Dekker, New York, 287–325.

Demetriades, K., McClements, D.J. (1998). Influence of pH and heating on physicochemical properties of whey protein-stabilized emulsions containing a nonionic surfactant. *Journal of Agriculture and Food Chemistry*, 46, pp. 3936–3942.

Dalgleish, D.G., Dickinson, E., Lorient, D. (1995). Food Macromolecules and Colloids. *The Royal Society of Chemistry, Cambridge*, 23.

Demetriades, K., Coupland, J. N., McClements, D. J. (1997). Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. *Journal of Food Science*, 62, 462-467.

Desai, K.G.H., Park, H.J. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology*, 23, 1361–1394.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed system, *Food Hydrocolloids*, 17, 25– 39.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, 17(1), 25–39.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and surfaces B: Biointerfaces*, 20, 197-210.

Durand, D., Gimel, J. C., Nicolai, T. (2002). Aggregation, gelation and phase separation of heat denatured globular proteins. *Physica A: Statistical Mechanics and Its Applications*, 304, 253-265.

Dziezak, J.D. (1988). Microencapsulation and encapsulated ingredients. *Food Technology*(April), 136–151.

Dybowska, E.A (2011). Whey protein-stabilized emulsion properties in relation to thermal modification of the continuous phase. *Journal of Food Engineering*, 104(1), 81-88.

Elfagm, A.A, Wheelock, J.V. (1978). Heat Interaction Between α -Lactalbumin, β -Lactoglobulin and Casein in Bovine Milk *Journal of Dairy Science*, 61(2), 159-163

El-Badry, A.M., Graf, R., Clavien, P.A. (2010). Omega 3 – Omega 6: What is right for the liver. *Journal of Hepatology*, 47, 718–725.

Euston, S.R., et al. (2000). Aggregation kinetics of heated whey protein-stabilized emulsions. *Food Hydrocolloids*, 14, 156-161.

Faraji, H., McClements, Decker, E.A. (2004). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. *J. Agric. Food Chem.*, 52, 4558.

Farrell, K.T. (1985). Spices, Condiments and Seasonings. *The AVI Pub. Co. Westport, CN, USA*.

Gibbs, B.F, Kermasha, S., Alli, I., Mulligan, C.N. (1999). Encapsulation in the food industry: a review. *Int. J. Food Sci. Nutr.*, 50, 213–224.

Gharsallaoui, A., Roudaut, O., Chambin, O., Voilley, A., Saurel, R., (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, 40(9), 1107-1121.

Gouin, S. (2004). Micro-encapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science and Technology*. (15), 330–347.

Guzey, D., McClements, D.J., Weiss, J. (2003). Adsorption kinetics of BSA at air-sugar solution interfaces as affected by sugar type and concentration. *Food Res. Int.*, 36, 649-660.

Hunter, R.J. (1998). *Foundation of Colloid Science*; Oxford University Press: Oxford, England; Vol 1.

Hunt, J., Dalgeieish, D.G., (1995). Heat Stability of Oil-in-Water Emulsions Containing Milk Proteins: Effect of Ionic Strength and pH, *Journal of Food Science*, 60(5), 1120-1123.

Jost, R., Baechler, R., Masson, G. (1986). Heat gelation of oil-in-water emulsions stabilized by whey protein. *Journal of Food Sci.*, 51, 440-449.

Klinkesorn, U., Sophanodora, P., Chinachoti, P., McClements, D.J. (2004). Stability and rheology of corn oil-in-water emulsions containing maltodextrin. *Food Res. Int.*, 37, 851-859.

Klinkerson, U., Sophanodora, P., Chinachoti, P., Decker, E.A., McClements, D.J., Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. *Food Research International*, 39(4), 449-457.

Kolanowski, W. (2005). Bioavailability of omega-3 PUFA from foods enriched with fish oil. A mini review, *Polish Journal of Food and Nutrition Sciences*, 14/55(4), 335–340.

Mancuso, J. R., McClements, D. J., Decker, E. A. (1999). The effects of surfactant type, pH and chelators on the oxidation of salmon oil-in- water emulsions. *Journal of Agric. Food Chem.*, 47, 4112–4116.

Mancuso, J.R., McClements, D.J., Decker, E.A (1999). Ability of iron to promote surfactant. *Journal of Ag. Food Chem.*, 47, 4146–4149.

Mancuso, J.R, McClements, D.J., Decker, E.A. (2000). The effects of surfactant type, pH, and chelators on the oxidation of the antioxidant activity of a high molecular weight fraction of whey, *Journal of Ag. Food Chem.*, 48, 1473-1478.

McClements, D.J. (2000). Comments on viscosity enhancement and depletion flocculation by polysaccharides, *Food Hydrocolloids*, 14, 173–177.

McClements, D.J. (2004). Food Emulsions: Principles, Practice, and Techniques. *CRC Press, Boca Raton, FL, USA.*, 53-339.

McClements, D.J., Decker, E. (2000). Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science*, 65(8), 1270-1282.

McManus, A., Merga, M., Newton, W. (2011). *Omega-3 fatty acids. What consumers need to know. Appetite*, Apr 8, 57(1), 80-83.

McManus, A., Howieson, J., and Nicholson, C. (2009). *Review of literature and resources relating to the health benefit of regular consumption of seafood as part of a healthy diet*, Centre of Excellence for Science, Seafood and Health, Curtin Health Innovation Research Institute, Curtin University of Technology, Perth.

Mei, L., McClements, D. J., Decker, E. A. (1999). Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsion droplets. *Journal of Agric. Food Chem.*, 47, 2267–2273.

Monohan, F.J., McClements, D.J., German, J.B. (1996). Disulfide-mediated polymerization reactions and physical properties of heated WPI-stabilized emulsions, *Journal of Food Science*, 61(3), 504-509.

Nuchi, C.D., Hernandez, P., McClements, D.J., Decker, E.A. (2002). Ability of lipid hydroperoxides to partition into surfactant micelles and alter lipid oxidation rates in emulsions. *Journal of Ag. Food Chem.*, 50, 5445-5449.

Ogawa, S., Decker, E. A., McClements, D.J. (2003). Influence of environmental conditions on the stability of oil in water emulsions containing droplets stabilized by lecithin droplets stabilized by lecithin-chitosan membranes. *Journal of Agric. Food Chem.*, 51, 5522–5527.

Ostdal, H., Daneshvar, B., Skibsted, L.H. (1996). Reduction of ferrylmyoglobin by α -lactoglobulin. *Free Radical Res.*, 24, 429-438.

Pouzot, M., Durand, D., Nicolai, T. (2004). Influence of the ionic strength on the structure of heat set globular protein gels of β -lactoglobulin. *Macromolecules*, 37, 614-620.

Pouzot, M., Nicolai, T., Visschers, R. W., Weijers, M. (2005). X-ray and light scattering study of the structure of large protein aggregates at neutral pH. *Food Hydrocolloids*, 19, 231-238.

Reineccius, G.A., Ward, F.M., Whorton, C., Andon, S.A. (1995). Developments in gum acacia for the encapsulation of flavors., 161-168.

Renard, D. (1994). Etude de l'aggrégation et de la gélfication des protéines globulaires: application à la beta-lactoglobuline. *Ph.D. Dissertation. University of Nantes, France.*

Riediger et al., (2009). A systematic review of the roles of n-3 fatty acids in health and disease, *Journal of the American Dietetic Association*, 109(4), 668–679.

Ronald, B.P. (2001). Spectrophotometric measurement of secondary lipid oxidation products. *Current Protocols in Food Analytical Chemistry*, D2.4.1-D2.4.8.

Ruxton, C. H. S., Calder, P. C., Reed, S. C., & Simpson, M. J. A. (2005). The impact of long-chain n-3 polyunsaturated fatty acids on human health. *Nutrition Research Reviews*, 18(1), 113–129.

Ruxton, H.S., Derbyshire, E. (2009). Latest evidence on omega-3 fatty acids and health, *Nutrition and Food Science*, 39(4), 423–438.

Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A., Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of Human Nutrition and Dietetics*, 17(5), 449–459.

Shahidi, F., Han, X.Q. (1993). Encapsulation of food ingredients. *Critical Review in Food Science and Nutrition*, 33, 501–547. 11.

Shahidi, F., Miraliakbari, H. (2005). Omega-3 fatty acids in health and disease: Part 2-health effects of omega-3 fatty acids in autoimmune diseases, mental health, and gene expression. *Journal of Medicinal Food*, 8(2), 133–148.

Shanta, N. C., Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC Int.*, 77 (2), 421–424.

Silvestre, MCP, Chaiyasit, W., Brannan, RG, McClements, DJ, and Decker, E.A., (2000). Ability of surfactant headgroup size of the antioxidant activity of a high molecular weight fraction of whey, *Journal of Ag. Food Chem.*, 48, 1473-1478.

Tcholakova, S., Denkov, N.D., Ivanov, I.B., Campbell, B. (2002). Coalescence in h-lactoglobulin-stabilized emulsions: effects of protein adsorption and drop size, *Langmuir*, 18, 8960– 8969.

Tong, L. M., et al. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *Journal of Agric. Food Chem.*, 48, 1473-1478.

Tong, L.M., Sasaki, S., McClements, DJ, and Decker, E.A. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey, *J. Agric. Food Chem.*, 48, 1473.

Thevenet, F. (1995). Acacia gums: Natural encapsulation agent for food products, *Encapsulation and controlled release of food ingredients, ACS symposium series*, American Chemical Society, Washington, DC, 590, 51–59.

Wang et al., (2006). N-3 fatty acids from fish or fish-oil supplements, but not α -linoleic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies. A systematic review, *American Journal of Clinical Nutrition*, 84, 5–17.

Walstra, P. (2003). *Physical Chemistry of Foods*, Marcel Decker, New York, NY.

Wilde, P., Mackie, A., Husband, F., Gunning P., Morris V. (2004). Proteins and emulsifiers at liquid interfaces, *Adv. Colloid Interface Sci.* 108–09, 63– 71.

Yamauchi, K., Shimizu, K., Kamiya, T. (1980). Emulsifying properties of whey protein. *Journal of Food Science*, 45, 1237–1242.

Ye, A. (2010). Surface protein composition and concentration of whey protein isolate-stabilized oil-in-water emulsions: Effect of heat treatment. *Colloid and Surface B: Biointerfaces*. 78, 24-29.

Ye, A., Singh, H. (2006). Heat stability of oil-in-water emulsions formed with intact or hydrolysed whey proteins: influence of polysaccharides. *Food Hydrocolloids*, 20(2-3), 269-276.

Ye, A., Taylor, S. (2009). Characterization of cold-set gels produced from heated emulsions stabilized by whey protein. *Int. Dairy Journal*, 19(12), 721-727.

Yost, R., Kinsella, J.E. (1992). Microstructure of whey protein isolate gels containing emulsified butter fat droplets. *Journal of Food Science*, 57(4), 892-897.

APPENDIXES

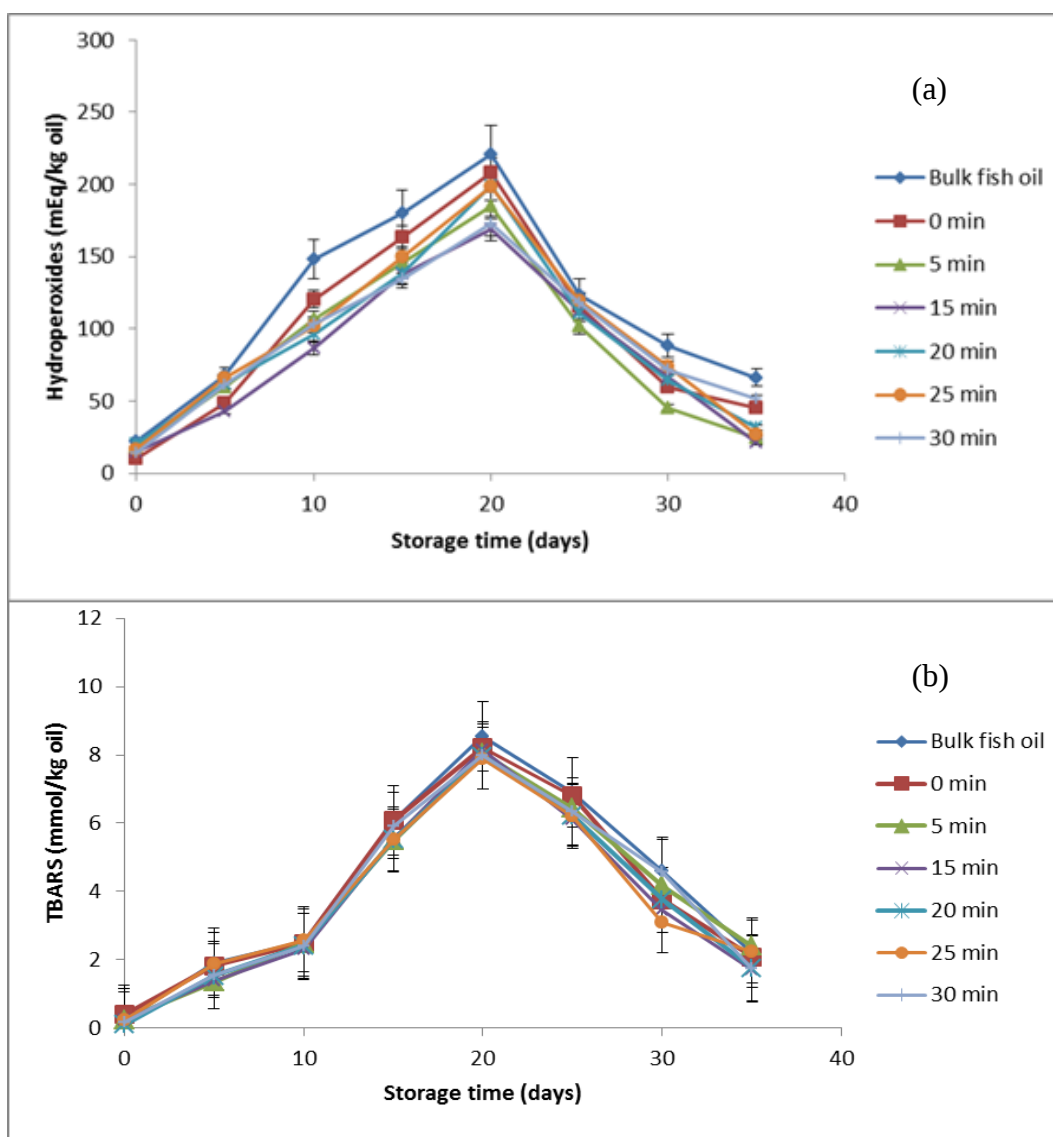


Figure 1. Development of (a) lipid hydroperoxides and (b) TBARS of fish oil emulsions stored at 37 °C and 33% relative humidity for 35 days. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2 w/v% WPI, and 4 w/v% maltodextrin. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 0-30 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

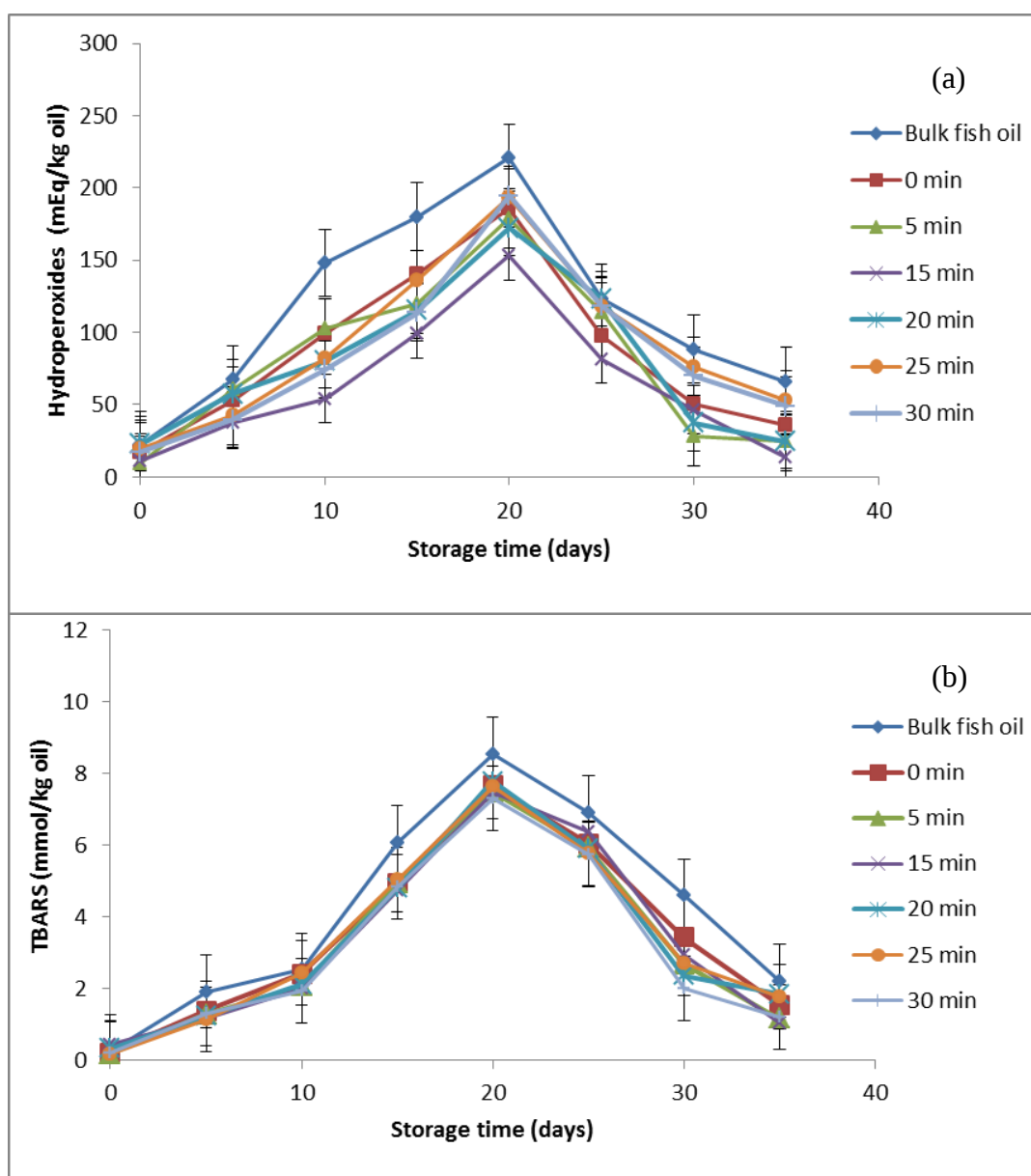


Figure 2. Development of (a) lipid hydroperoxides and (b) TBARS of spray-dried fish-oil powder stored at 37 °C and 33% relative humidity for 35 days. Fresh emulsions were prepared as those in Figure 1 before spray drying. Error bars are standard deviations from two replicates, each with two measurements.

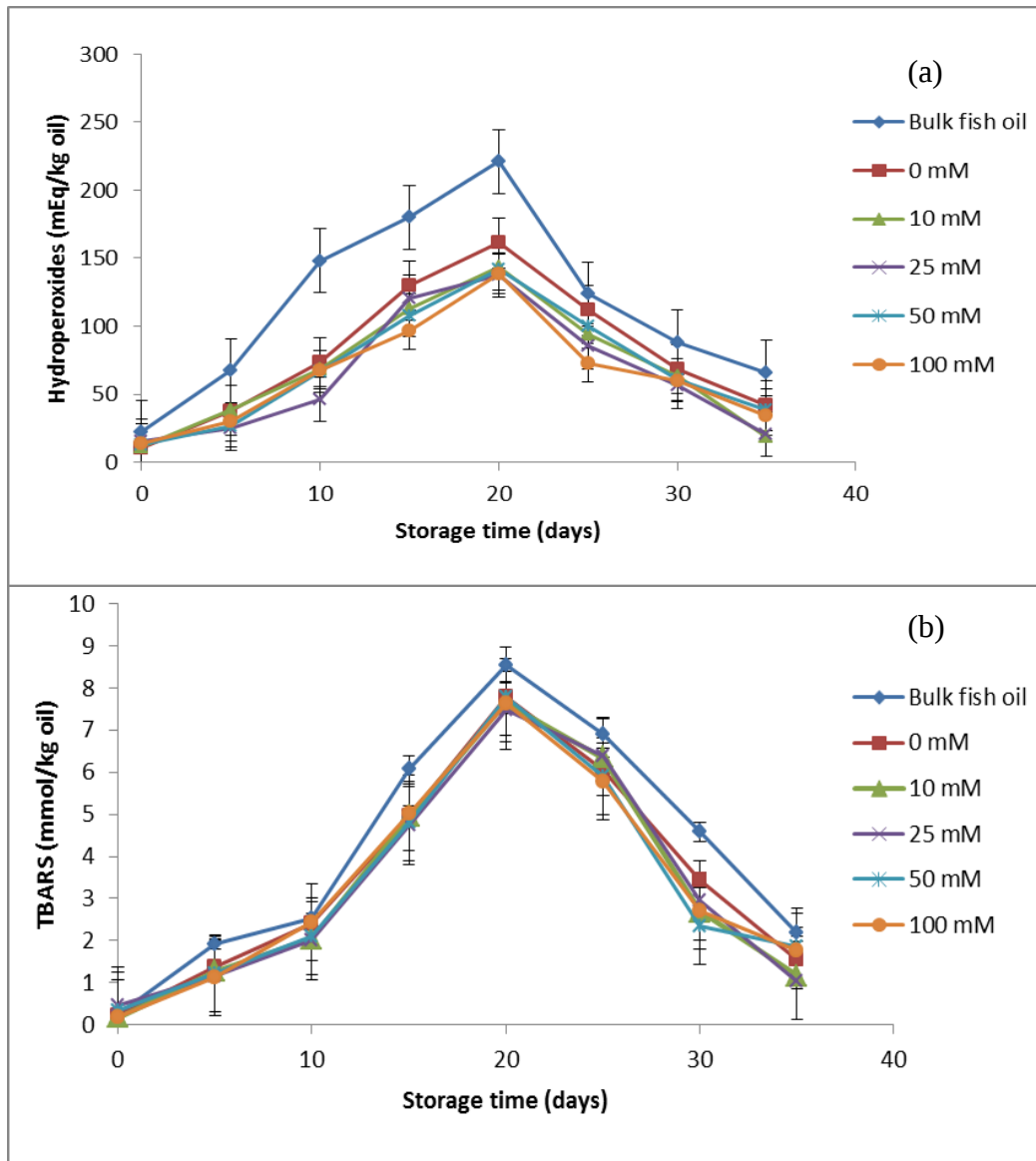


Figure 3. Development of (a) lipid hydroperoxides and (b) TBARS of fish-oil emulsions stored at 37 °C and 33% relative humidity for 35 days. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 4%w/v maltodextrin, and 0-100 mM NaCl. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 15 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

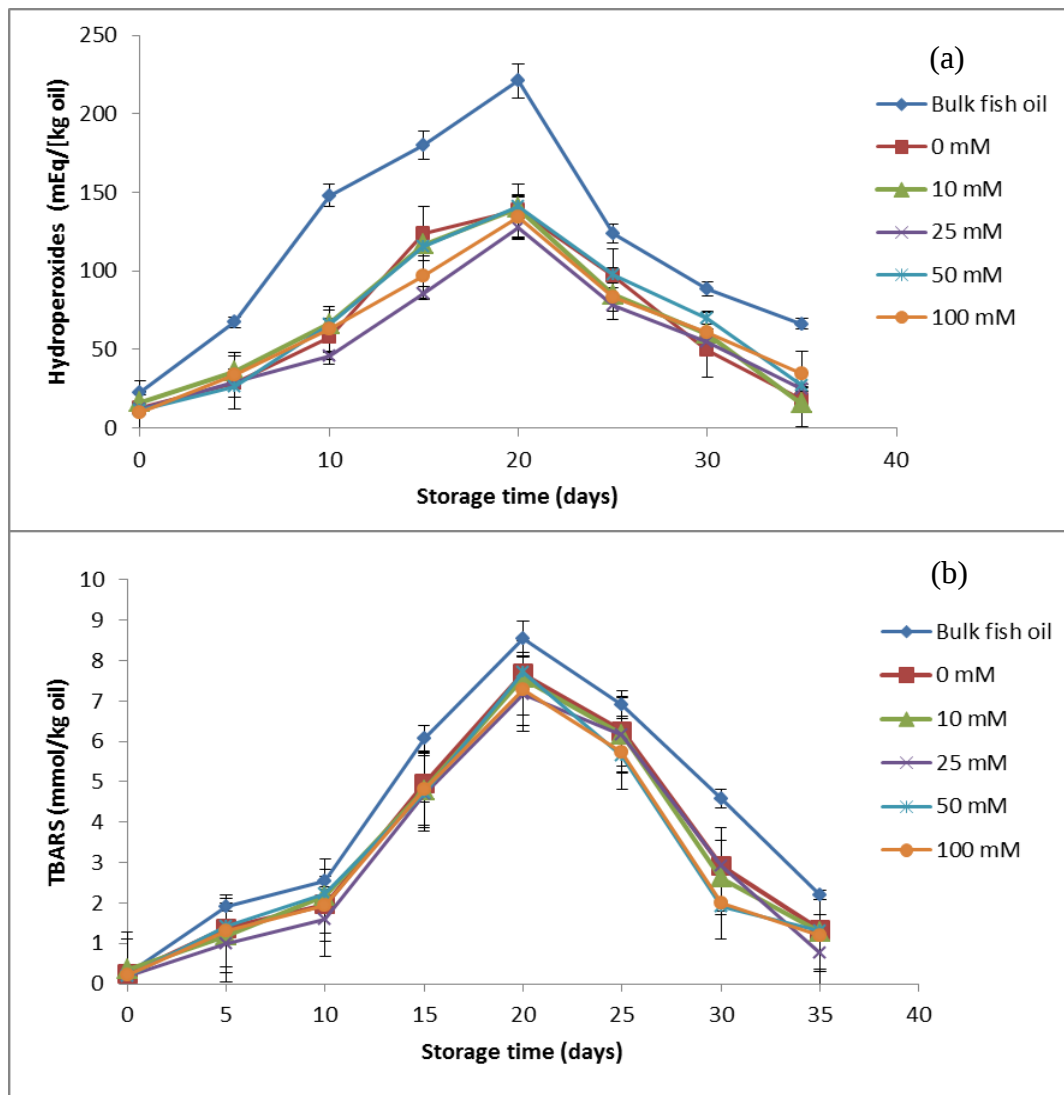


Figure 4. Development of (a) lipid hydroperoxides and (b) TBARS of spray-dried fish-oil powder stored at 37 °C and 33% relative humidity for 35 days. Fresh emulsions were prepared as those in Figure 3 before spray drying. Error bars are standard deviations from two replicates, each with two measurements.

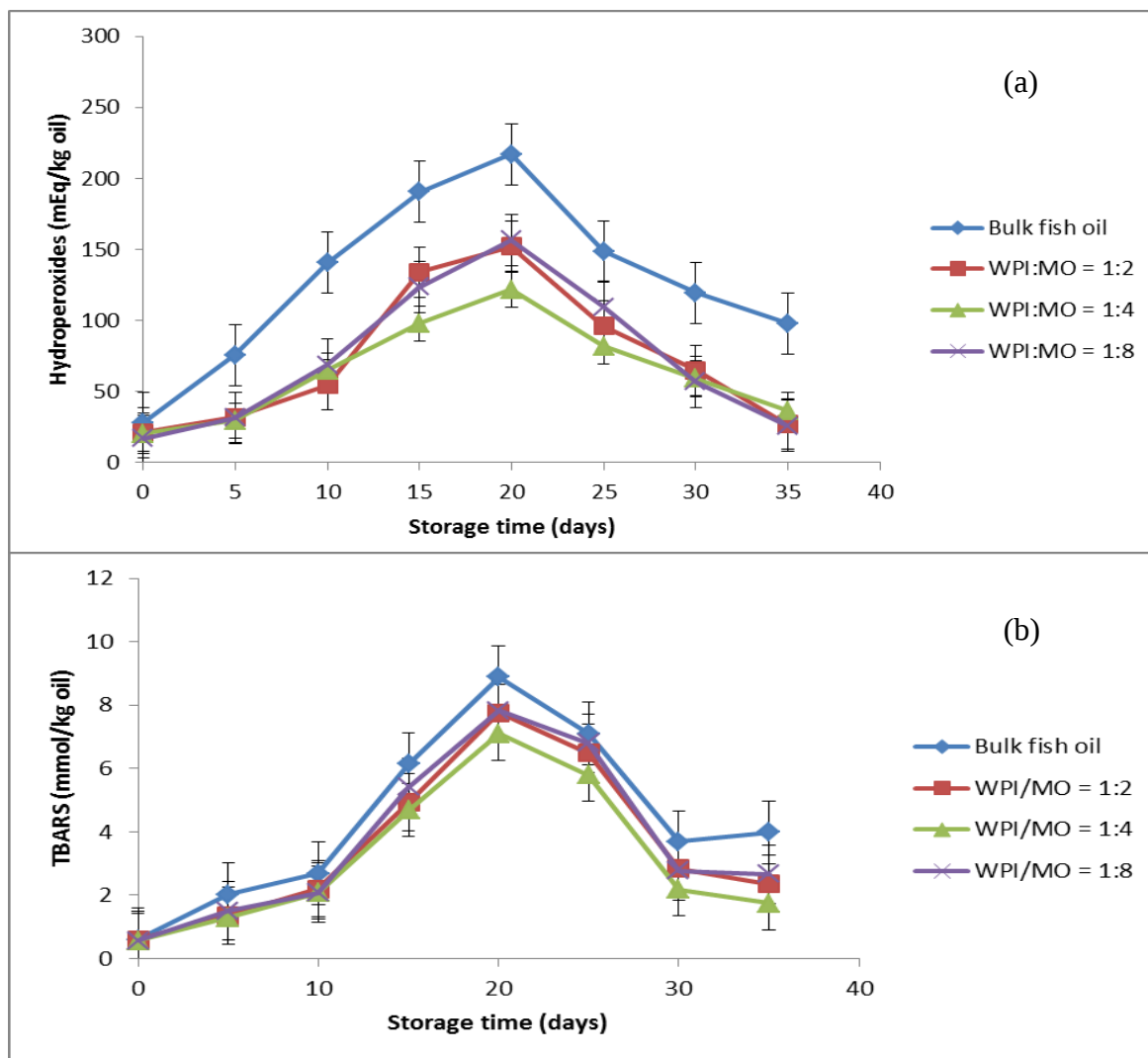


Figure 5. Development of (a) lipid hydroperoxides and (b) TBARS of fish-oil emulsions stored at 37 °C and 33% relative humidity for 35 days. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, maltodextrin (MD) at a WPI:MD ratio of 1:2, 1:4, and 1:8, and 25 mM NaCl. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 15 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

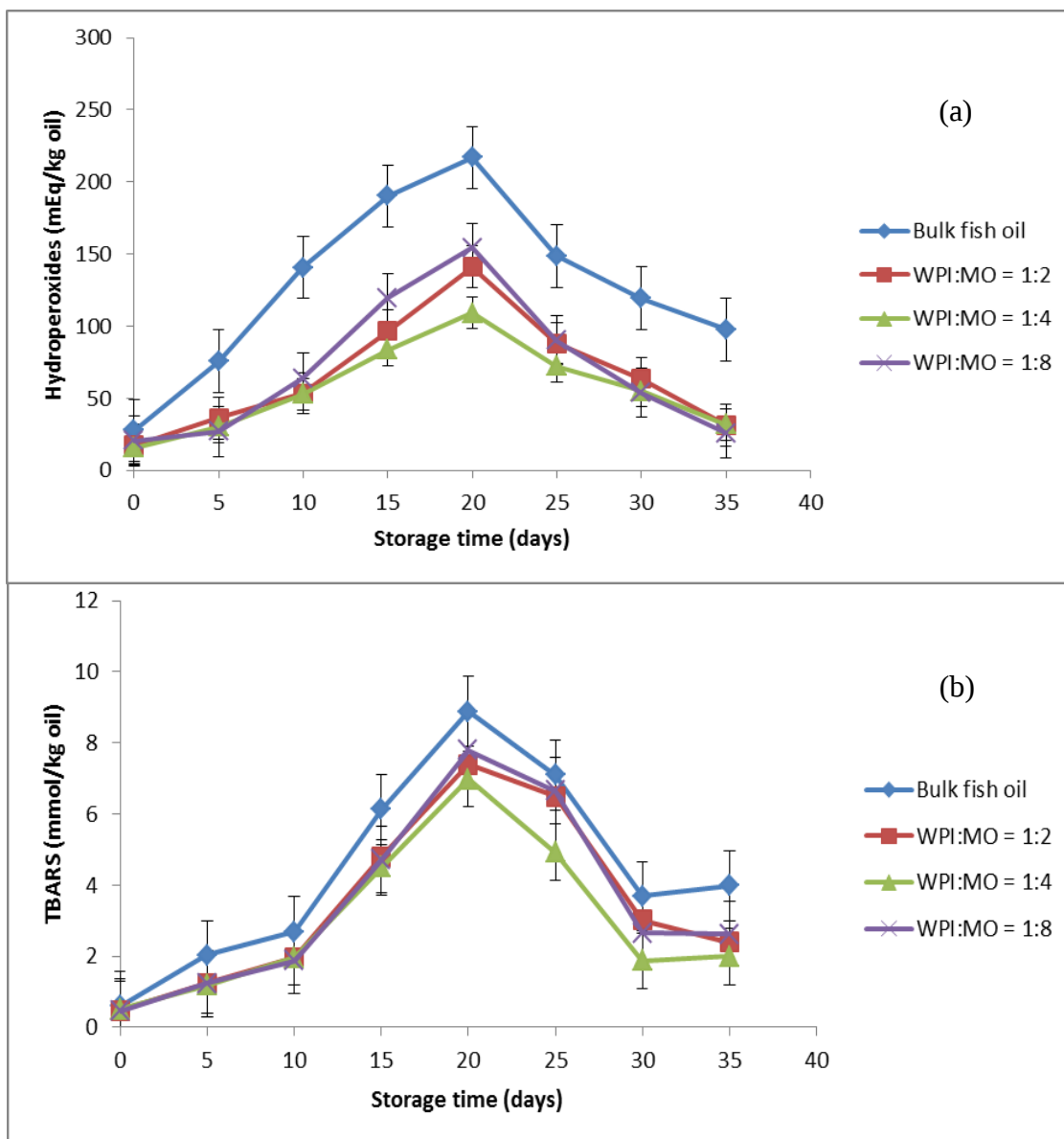


Figure 6. Development of (a) lipid hydroperoxides (b) TBARS of spray-dried fish-oil powder stored at 37 °C and 33% relative humidity for 35 days. Fresh emulsions were prepared as those in Figure 5 before spray drying. Error bars are standard deviations from two replicates, each with two measurements.

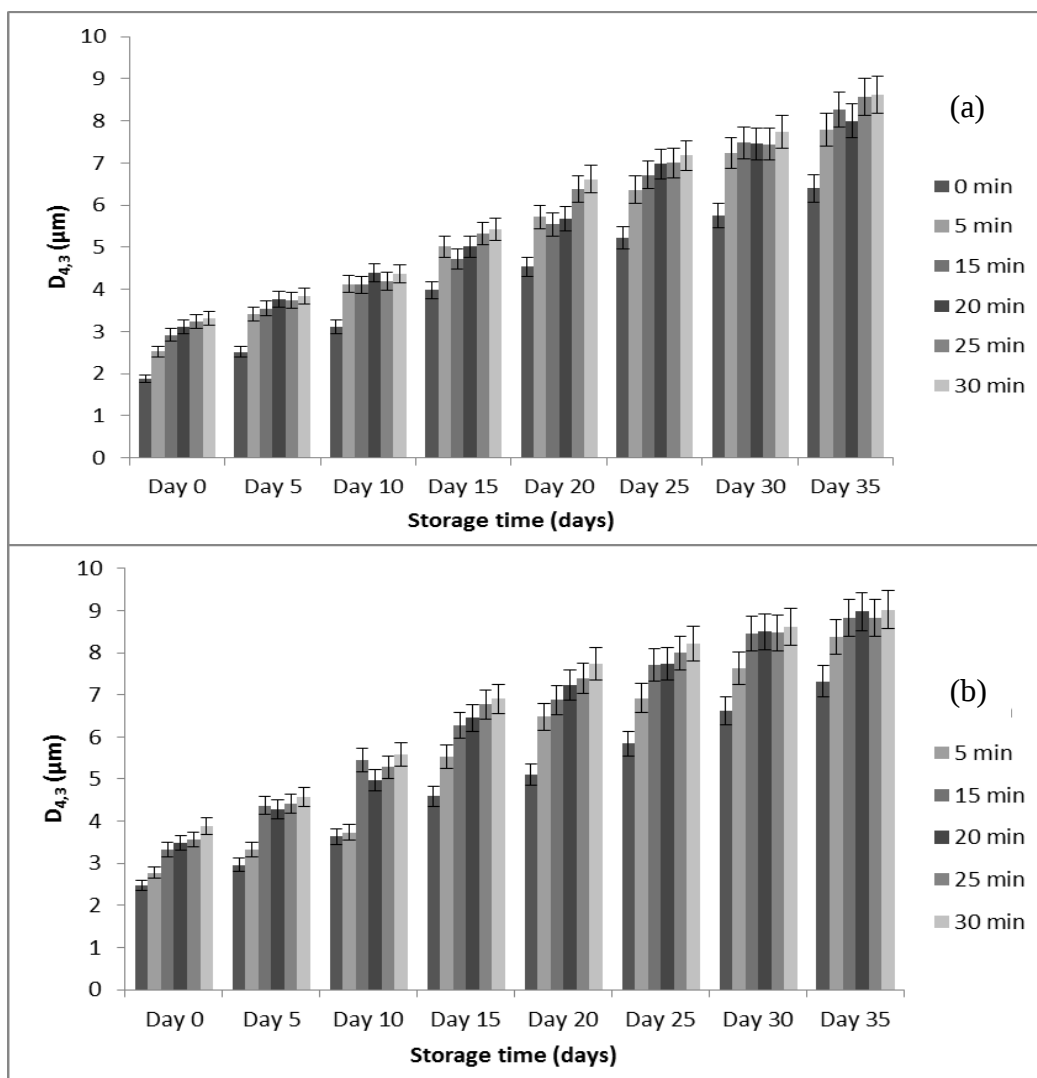


Figure 7. Changes of droplet sizes during incubation at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, and 4%w/v maltodextrin. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 0-30 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

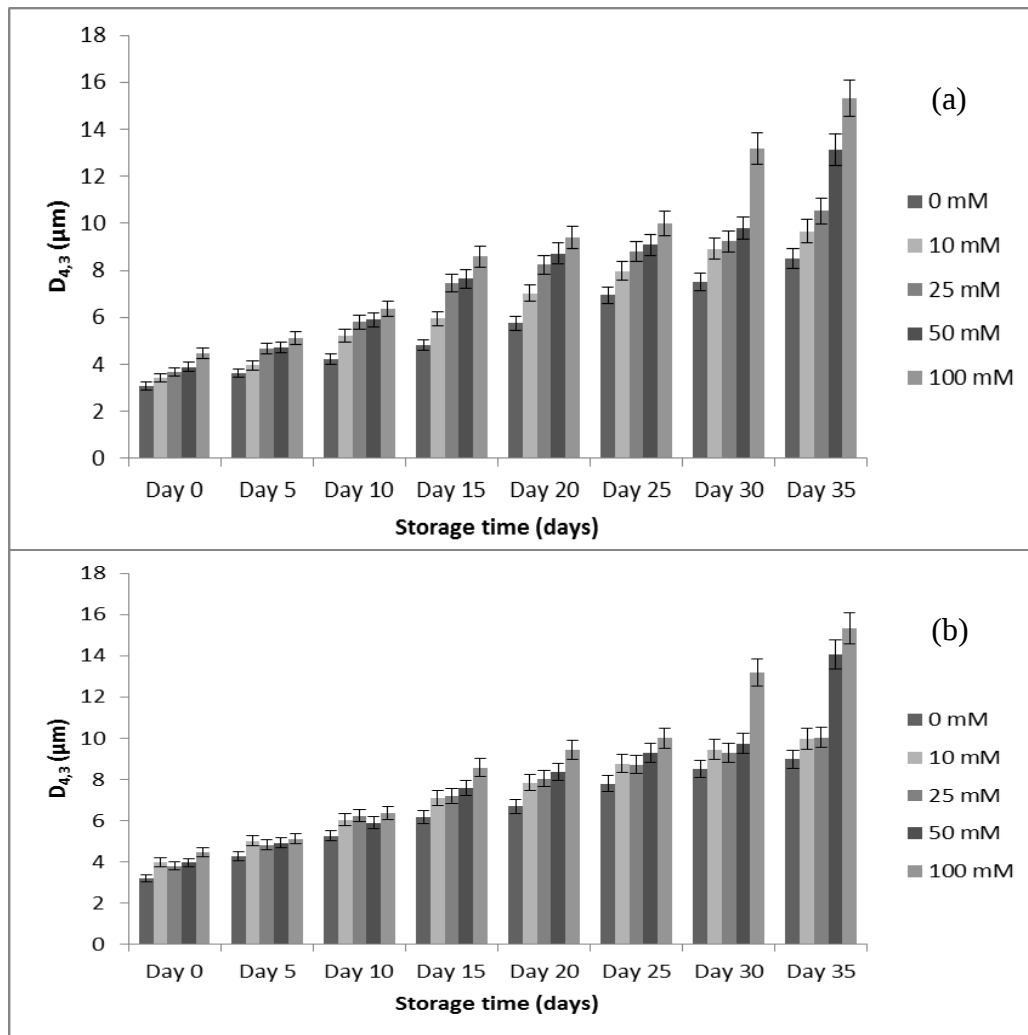


Figure 8. Changes of droplet sizes during incubation at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 0-100 mM NaCl, and 4%w/v maltodextrin. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 15 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

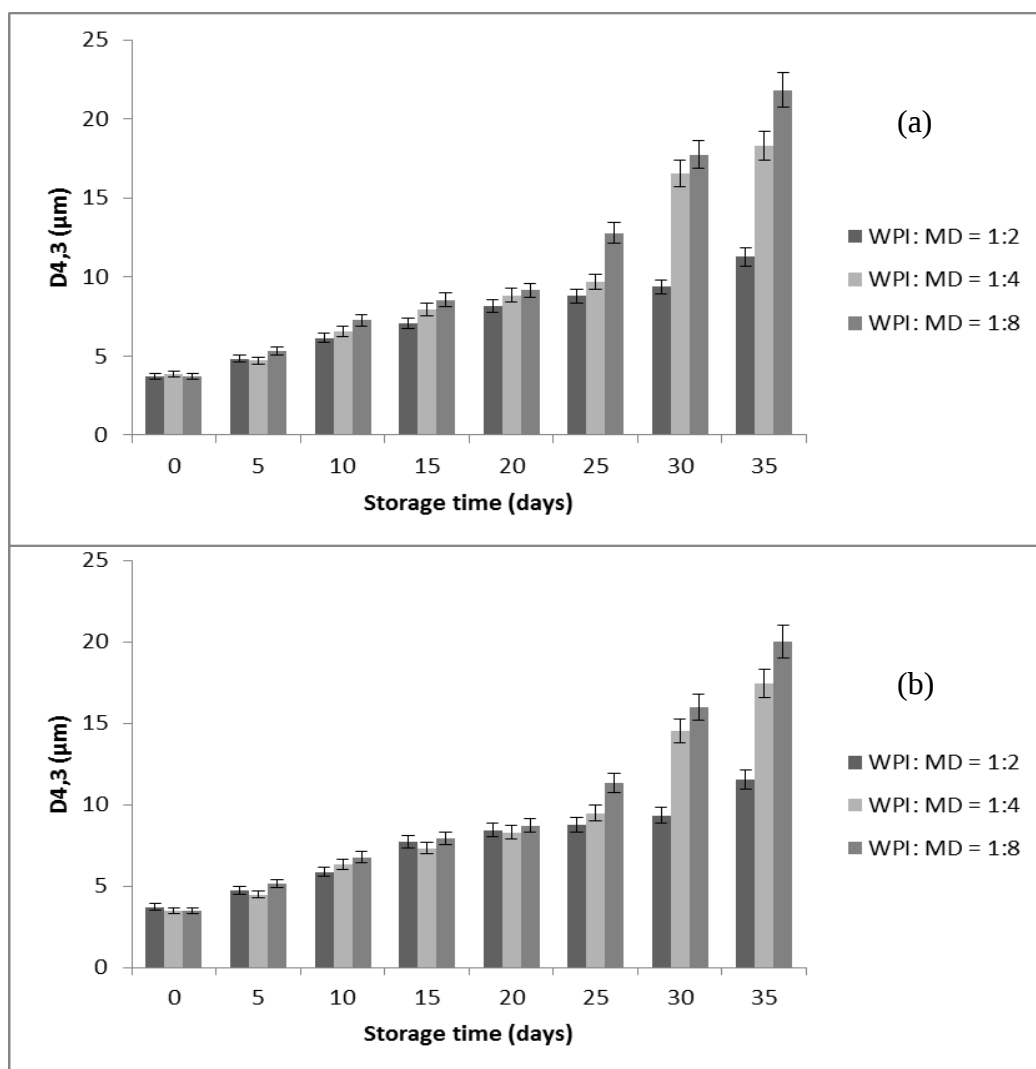


Figure 9. Changes of droplet sizes during incubation at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, maltodextrin (MD) at a WPI:MD ratio of 1:2, 1:4, and 1:8, and 25 mM NaCl. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 15 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

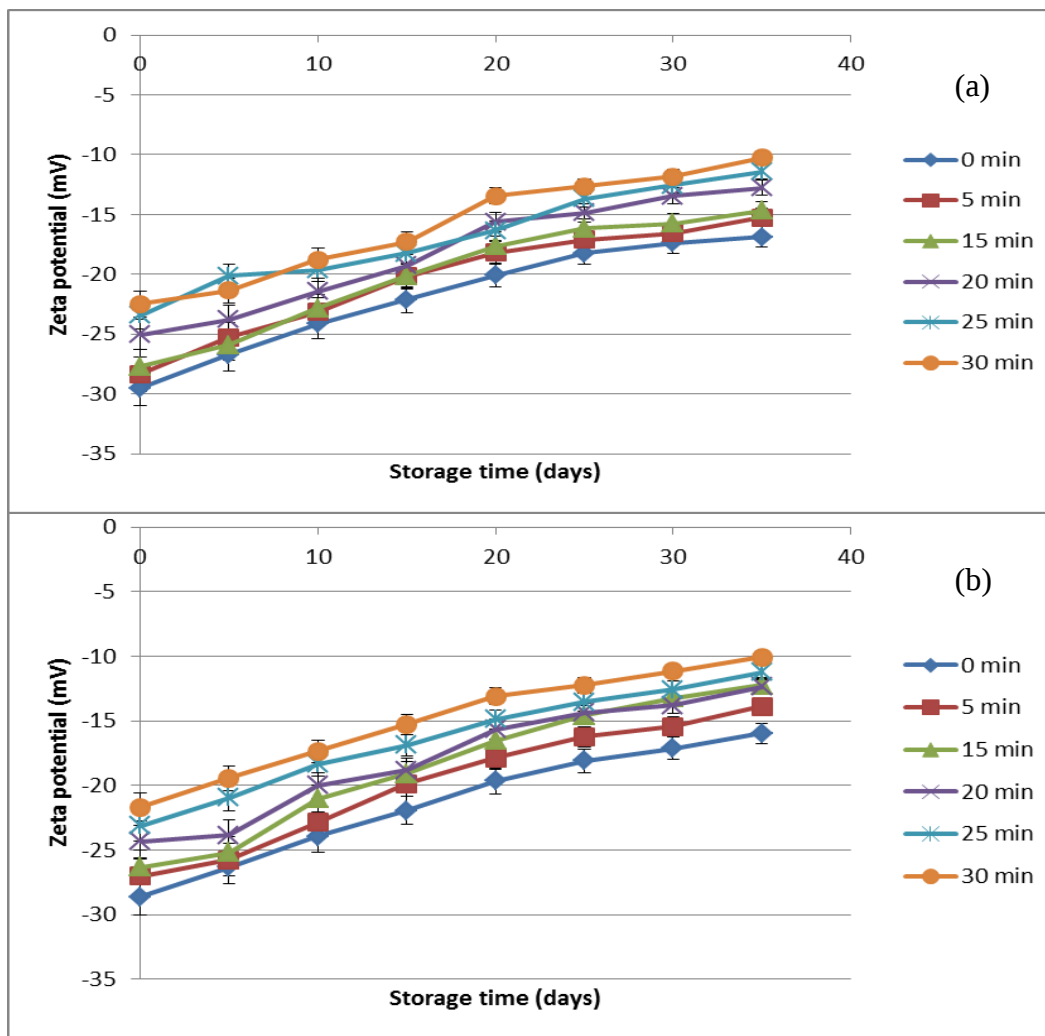


Figure 10. Changes of zeta-potential of droplets during incubation at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI and 4%w/v maltodextrin. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 0-30 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

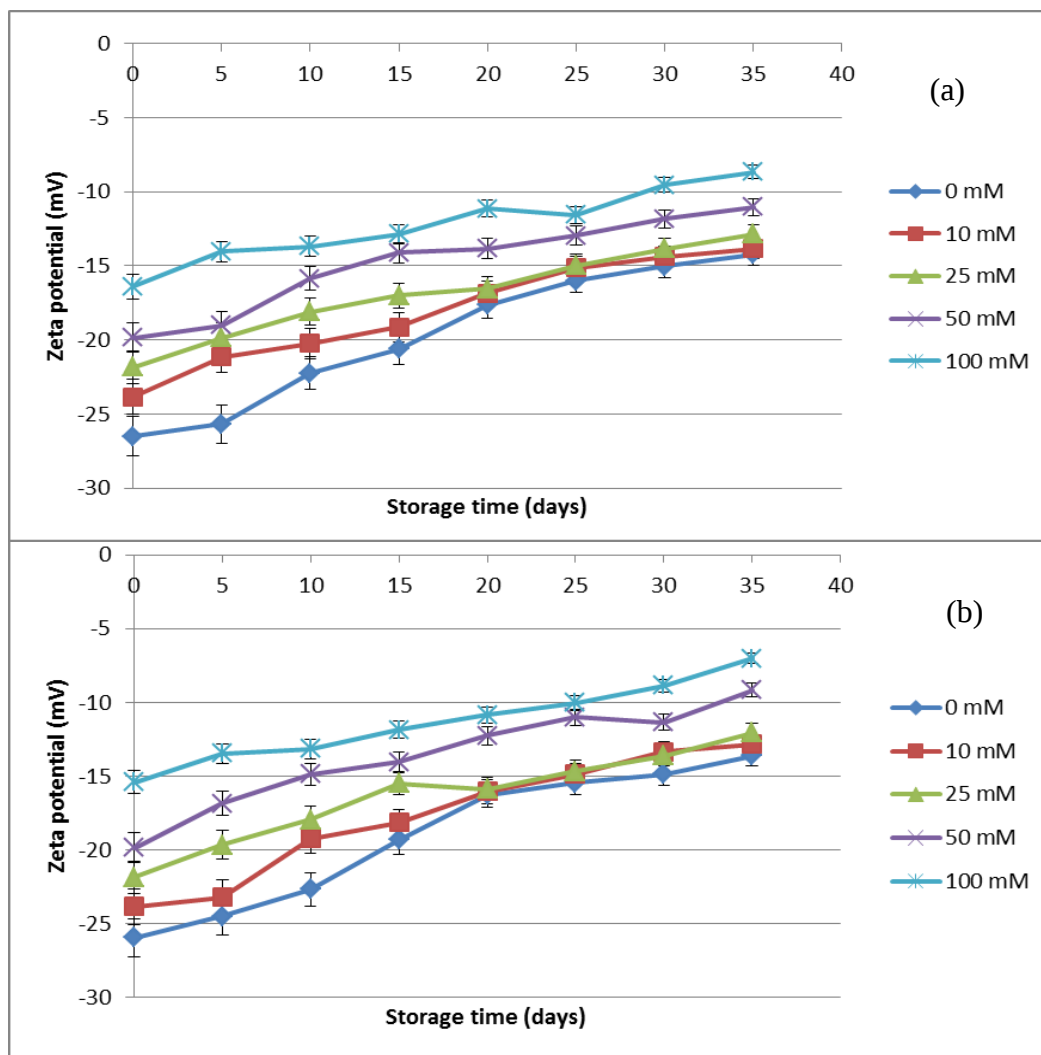


Figure 11. Changes of zeta-potential of droplets during incubation at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, 0-100 mM NaCl, 4%w/v maltodextrin and adjusted to pH 7.0. The aqueous phase was preheated at 85°C for 15 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

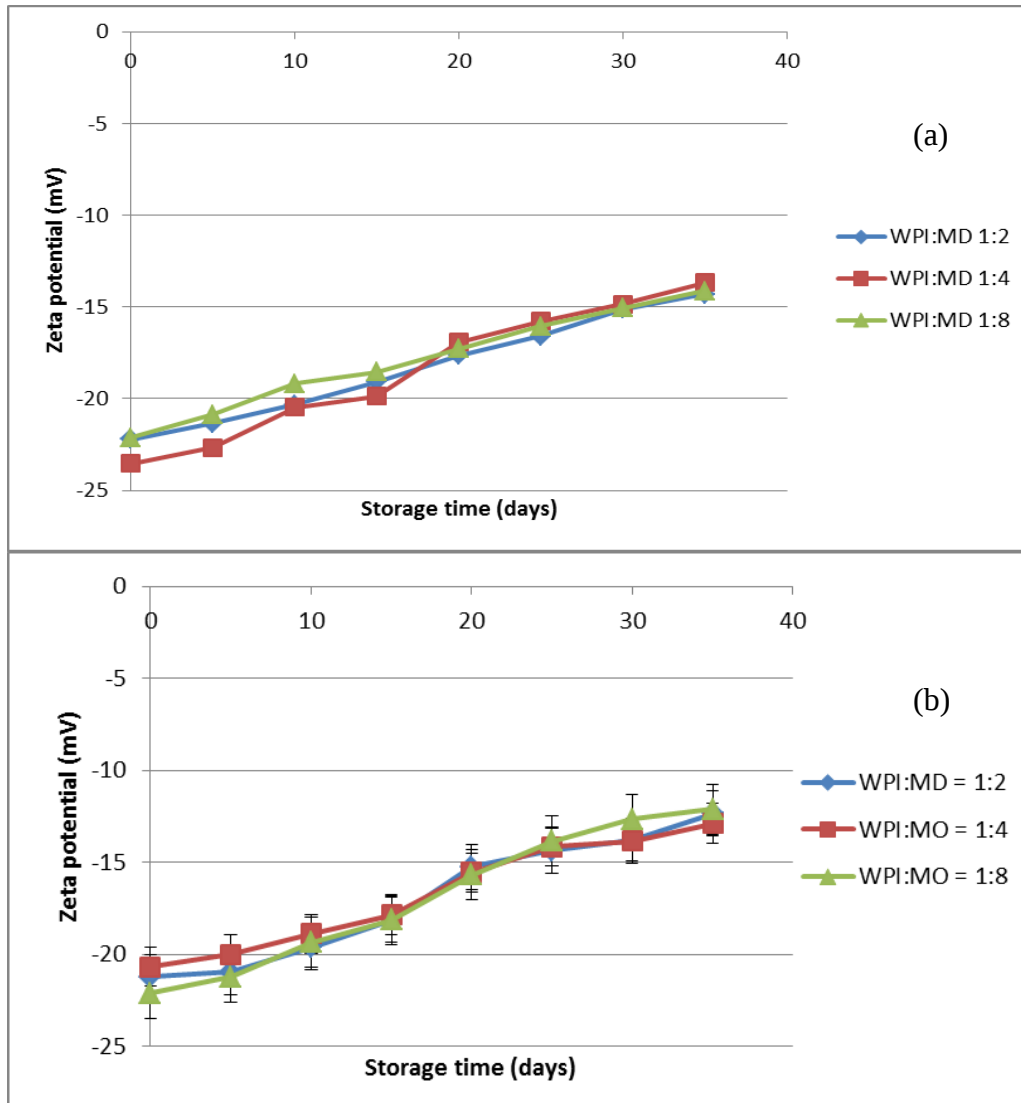


Figure 12. Changes of zeta-potential of droplets during incubation at 37 °C for (a) fresh fish-oil emulsions and (b) the corresponding emulsions reconstituted from spray-dried powders. Emulsions were prepared at 10 v/v% fish oil and an aqueous phase containing 2%w/v WPI, maltodextrin (MD) at a WPI:MD ratio of 1:2, 1:4, and 1:8, and 25 mM NaCl. The aqueous phase was adjusted to pH 7.0 and preheated at 85°C for 15 min before emulsion preparation. Error bars are standard deviations from two replicates, each with two measurements.

VITA

Sutida Watthanapimol was born on March 9, 1984 in Bangkok, Thailand. She received her B.S. in Food Science and Technology from Kasetsart University, Bangkok, Thailand in 2006. She began her M.S. program in Food Science and Technology focusing in Food Chemistry at the University of Tennessee at Knoxville in August 2009, Tennessee and graduated from this program in December 2011.