



岐阜大学機関リポジトリ

Gifu University Institutional Repository

Kinetic Analysis of Freeze-thaw Stability of Mayonnaise

メタデータ	言語: eng 出版者: 公開日: 2019-01-10 キーワード (Ja): キーワード (En): 作成者: ISLAM MUHAMMAD SHARIFUL メールアドレス: 所属:
URL	http://hdl.handle.net/20.500.12099/77262

Kinetic Analysis of Freeze-thaw Stability of Mayonnaise

(マヨネーズの冷解凍安定性に関する動力的解析)

2018

The United Graduate School of Agricultural Science,
Gifu University
Science of Biological Resources
(Gifu University)

ISLAM MUHAMMAD SHARIFUL

Kinetic Analysis of Freeze-thaw Stability of Mayonnaise

(マヨネーズの冷解凍安定性に関する動力的解析)

ISLAM MUHAMMAD SHARIFUL

Acknowledgement

Firstly, the author pays his deepest gratitude to his supervisor, Dr. Takahisa Nishizu, Professor, Department of Applied Life Science, Gifu University for his support and proper guideline for the progress of this works. His immense guidance, precious advice, valuable suggestions throughout the entire process and fruitful comments on the research works has helped a lot. The author will always be grateful for his consistent inspiration and guidance to complete the work successfully.

The author also pays his gratitude to Dr. Masaya Kato, Professor, Department of Biological and Environmental Sciences, Shizuoka University for his important comments and helpful suggestions in the midterm presentation. Also, the author is thankful to Dr. Nakako Katsuno, Assistant Professor, Department of Applied Life Science, Gifu University for her kind support and sharing the valuable knowledge in critical stages of research works.

The author is grateful to Dr. Toshiya Hayashi, Professor, Meijo University for providing Cryo-SEM of his laboratory. The author is also very thankful to all members of laboratory of Food Process and Chemistry in Gifu University for their supports, encouragements and sharing various experiences to reach the final goal. The author would like to thanks particularly to Mr. Sultan Mahomud, Achmad Ridwan Ariyantoro, Wang Xuangpeng, Jia Xiwu, Ryuma Tamaru, Daisuke Mori, Chihiro Tsuji and Yuki Houmon for sharing many important ideas, necessary research materials and active cooperation to strive towards the final destination. The author is also thankful to the office staffs at the international student affair section and Renno Office of Gifu University for their support in the entire period of Japan.

The author is particularly indebted to acknowledge authority of Department of

Agricultural Extension under Ministry of Agriculture in Bangladesh to facilitate a scope of higher studies in a foreign country.

The author would like to express his loving thanks to his wife, son and daughter who made many sacrifices during this work. Without their sacrifices and understanding it would impossible to finish this work. Also, author expresses his heartfelt gratitude to his respected mother, beloved sister and brother in Bangladesh for their patience, blessing, incessant prayer and inspiration to build up this wonderful work.

The Author

Gifu University

September 2018

ABSTRACT

Mayonnaise is semi-solid oil in water emulsion and consumed all over the world. Usually it contains higher oil phase (70% or more) than water phase. As oil in water emulsion destabilization during freeze storage is common. Crystallization of ice and fat has considered the cause for destabilization. The role of oil and ice crystal on destabilization is an essential aspect during freeze storage. Thus the objective of this study was to understand the influence of both water and fat crystal on destabilization. Ice crystal influence had been studied through limiting ice crystal growth and kinetic analysis of destabilization and crystallization had been done to clarify the effect of fat crystal.

In 2nd chapter, coalescence of oil droplets during freezing and destabilization of mayonnaise during freeze-thaw has been studied. Cryo-SEM images of frozen rapeseed oil mayonnaise showed the oil droplets coalesced at temperature -20 °C for 24 h. The freeze-thaw stability of rapeseed oil mayonnaise had been studied with bulky sample at temperature -20 °C. It showed that, separated oil increased with increasing freezing time. Oil separation had been measured from 8 h to 48 h. This study confirm that, coalescence happen during freezing and coalesced oil droplets appear as separated oil while thawing.

In 3rd chapter, influence of ice crystal on destabilization of mayonnaise has been studied. Coalescence of oil droplets using RoM had been studied through limiting the moisture by dry air blowing. The median diameter of oil droplets at the initial was 1.09 µm and after drying by 3 min the median diameter reached at 2.09 µm showed the coalescence by limiting moisture content. The oil droplets also showed the deformation with drying time. Simulated icing results in room temperature also showed increase of oil separation. The freeze-thaw stability of mayonnaise with limiting ice crystal had been done with

anti-frozen polysaccharides (APS). Oil separation decreased with increasing APS concentration. The crystallinity of ice has been studied through XRD measurement. The XRD measurement showed the crystallinity decreased with increasing APS concentration. This study revealed that generation of ice crystal during freezing has significant influence on destabilization.

In 4th chapter, mayonnaise with rapeseed oil and soybean oil was stored at temperature ranging from -20 to -40 °C. Separated oil had been measured with time and destabilization kinetic parameters had been calculated. The destabilization rate constant k_d was found increased with decreasing temperature and highest value was $1.283 \times 10^{-3} \text{ min}^{-1}$ at -40 °C for RoM and lowest value was $1.95 \times 10^{-6} \text{ min}^{-1}$ at -20 °C for SoM. The k_d value found higher in RoM than SoM at each temperature observed. However, the order of destabilization n did not follow any specific pattern. Furthermore, an empirical equation has been derived and found to adequately evaluate the mayonnaise stability under the condition of the investigation.

In 5th chapter crystallization kinetics of fat had been studied in isothermal condition using Avrami model. The crystallization rate constant k_c differed significantly from RoM to SoM and it increased with decreasing temperature. The highest value of k_c observed at -40 °C in RoM and it was 1.14×10^{-2} and the lowest value was found at -20 in SoM and it was 7.87×10^{-5} . The Avrami constant n found increased with decreasing temperature. The n value increased from 0.778 to 1.457 and from 0.763 to 1.340 in RoM and SoM respectively. The crystallization rate constant k_c showed the nucleation and the growth of fat crystal on the other hand Avrami exponent, n reflects the crystal form. The Avrami exponent n also found increased with decreasing temperature indicating transition of crystal growth from needle-like of instantaneous nuclei to a

plate-like form with high nucleation rate. The change of crystal form with temperature found insignificant from RoM to SoM but the rate of crystal growth differed significantly. Importantly, an empirical relation has been found between the rate of fat crystal growth and rate of destabilization from previous chapter.

Lastly in 6th chapter the future suggestions on freeze-thaw stability of mayonnaise have been proposed. The influence of ice crystal on destabilization is poorly known and the mechanism is not yet clear. Moreover, the influence of different additives on ice and fat crystal growth as well as their effect on destabilization during freezing seeks more research.

CONTENTS

ACKNOWLEDGEMENT	iv
ABSTRACT	vi
LIST OF CONTENTS	ix
LIST OF PUBLICATIONS	xii
LIST OF CONFERENCES	xiii
LIST OF TABLES	xiv
LIST OF FIGURES	xv
CHAPTER 1	1-16
General introduction	
1.1 Mayonnaise	2
1.2 Motivation and objectives	3
1.3 Destabilization of mayonnaise	4
1.3.1 Destabilization mechanism	5
1.3.2 Role of ice and fat crystal	7
1.3.3 Destabilization kinetics	10
1.4 Outline of this thesis	12
References	13
CHAPTER 2	17-23
Confirmation of oil droplets coalescence and destabilization of mayonnaise during freeze-thawing	
2.1 Introduction	17
2.2 Materials and Method	18
2.2.1 Materials	18
2.2.2 Preparation of mayonnaise sample	18
2.2.3 Observation of oil droplets coalescence of mayonnaise during freezing using cryo-SEM	19
2.2.4 Destabilization of mayonnaise during freeze-thawing	19
2.2.5 Statistical analysis	19
2.3 Results	20
2.3.1 Observation of oil droplets coalescence of mayonnaise during freezing using cryo-SEM	20
2.3.2 Destabilization of mayonnaise during freeze-thawing	21
2.4 Discussion	21
2.5 Conclusion	22
References	22

	CHAPTER 3	24-37
	Influence of ice crystal on destabilization of mayonnaise.	
3.1	Introduction	24
3.2	Materials and Method	25
	3.2.1 Materials	25
	3.2.2 Preparation of mayonnaise sample	25
	3.2.3 Oil droplets coalescence by dry air blowing	25
	3.2.4 Simulation of ice crystal using desiccant	26
	3.2.5 Destabilization of mayonnaise with limiting water crystal using Anti-frozen Proteins (APS)	26
	3.2.6 Evaluation of water crystallinity with X-ray Diffraction (XRD) measurement	27
	3.2.7 Statistical analysis	27
3.3	Results	28
	3.3.1 Coalescence of oil droplets	28
	3.3.2 Destabilization by simulated ice crystal	30
	3.3.3 Evaluation of water crystallinity with X-ray Diffraction (XRD)	31
	3.3.4 Destabilization rate of mayonnaise with limiting water crystal using APS	33
3.4	Discussion	34
3.5	Conclusion	35
	References	35
	CHAPTER 4	38-50
	Kinetic analysis of freeze-thaw stability of mayonnaise using capillary	
4.1	Introduction	38
4.2	Materials and Methods	39
	4.2.1 Materials	39
	4.2.2 Preparation of mayonnaise sample	40
	4.2.3 Selection of tube for freeze-thaw stability test	40
	4.2.4 Freeze-thaw stability of mayonnaise using capillary tube	40
	4.2.5 Theoretical consideration for destabilization kinetic analysis	41
	4.2.6 Statistical analysis	42
4.3	Results	42
	4.3.1 Influence of sample size on induction time and	42

	destabilization rate constant, k_d	
	4.3.2 Freeze-thaw stability of mayonnaise using capillary	44
	4.3.3 Destabilization kinetic parameters with different temperature	45
4.4	Discussion	47
4.5	Conclusion	48
	References	48
	CHAPTER 5	51-66
	Iso-thermal crystallization kinetic analysis of frozen mayonnaise	
5.1	Introduction	51
5.2	Materials and methods	52
	5.2.1 Materials	52
	5.2.2 Preparation of mayonnaise sample	52
	5.2.3 The model for crystallization kinetics	53
	5.2.4 Polarized light microscopy for observing fat crystal	53
	5.2.5 Thermal behaviors of mayonnaise and oil phase with Differential Scanning Calorimetry (DSC)	54
	5.2.6 Fat crystal structure analysis using Small Angle X-ray Scattering (SAXS)	54
5.3	Results	55
	5.3.1 Fat crystal with temperature	55
	5.3.2 Crystallization kinetics due to temperature change	57
	5.3.3 Thermal behavior of mayonnaise and oil phase	59
	5.3.4 Fat crystal structure change with temperature using SAXS	60
5.4	Relationship between fat crystal growth rate with destabilization rate	62
5.5	Discussion	62
5.6	Conclusion	64
	References	64
	CHAPTER 6	67-70
	Conclusion	
6.1	Conclusions from this work	67
6.2	Future suggestions	68

List of Publications

1. Muhammad Shariful, I., Katsuno, N., and Nishizu, T. (2018). Kinetic analysis of freeze-thaw stability of mayonnaise. *Foods*.7(5), 75 doi: 10.3390/foods7050075
2. Muhammad Shariful, I., Katsuno, N., and Nishizu, T. (2018). Factors affecting mayonnaise destabilization during freezing. *Reviews in Agricultural Sciences*, 6: 72-80. doi: doi.org/ 10.7831/ras.6.72

List of Conferences

1. Islam MS, Katsuno, N., and Nishizu, T. “Kinetic evaluation of mayonnaise destabilization during freezing” 64th Japan Food Science and Technology Conference, Nihon University, Kanagawa, Japan 28-30 August, 2017.
2. Islam MS, Katsuno, N., and Nishizu, T. “Influence of Water Crystallization on Freeze-thaw Stability of Mayonnaise.” 2018 Annual Conference of Japan Society of Refrigerating and Air Conditioning Engineers, College of Engineering, Nihon University (Koriyama), Fukushima, Japan, 4-7 September, 2018

List of Tables

Table 2.1 Composition of mayonnaise

Table 3.1 Induction time and destabilization rate constant of mayonnaise with APS

Table 4.1 Fatty acid composition of oil phases

Table 4.2 Induction time of oil separation for RoM and SoM

Table 4.3 Variation of destabilization kinetic parameters with freezing temperatures for RoM and SoM

Table 5.1 Induction time for generation of fat crystal in RoM and SoM

Table 5.2 Variation of crystallization kinetic parameters with temperature; Avrami exponent, n and crystallization rate constant, K_c

Table 5.3 Variation of crystallization rate constant, K_c with temperature recalculated using an Avrami exponent, n , of 1

List of Figures

Fig. 1.1 (A-D) The probable process of coalescence of mayonnaise type emulsions. (A) Emulsified oil droplets (B) Deformation of oil droplets (C) Coalescence of deformed oil droplets (D) Separated oil while thawing

Fig. 1.2 (A-C) The probable roles of ice crystal on coalescence. (A) Emulsified oil droplets (B) Generation of ice crystal and deformation of oil droplets started (C) Enlargement of ice crystal and deformation of oil droplets close contact of deformed oil droplets and coalescence

Fig. 1.3 (A-C) The probable roles of fat crystal on coalescence. (A) Emulsified oil droplets (B) Generation of fat crystal and deformation of oil droplets started (C) Increase the number of fat crystal and deformation of oil droplets and penetration of fat crystal to the neighboring oil droplets and coalescence

Fig. 1.4 (A-C) The probable roles of both ice and fat crystal on coalescence. (A) Emulsified oil droplets (B) Generation of ice and fat crystal and deformation of oil droplets started (C) Enlargement of ice crystal and increase the number of fat crystal and deformation of oil droplets and close contact of deformed oil droplets and penetration of fat crystal to the neighboring oil droplets and coalescence

Fig. 2.1 (A-D) Cryo-SEM images of RoM showing coalescence of oil droplets (A) and (B) scale bar 50 μm (C) scale bar 10 μm (D) scale bar 25 μm

Fig. 2.2 Freeze-thaw stability of mayonnaise with time at temperature $-20\text{ }^{\circ}\text{C}$ in bulky samples

Fig. 3.1 (A-F) showed microscopic image of RoM Oil droplets at (A) 0 min dry (B) 1 min dry (C) 1.5 min dry (D) 2 min dry (E) 2.5 min dry (F) 3 min dry. Scale bar 20 μm

Fig. 3.2 (A-D) Change of oil droplets diameter of RoM at (A) 0 min dry (B) 1 min dry (C) 2 min dry (D) 3 min dry

Fig. 3.3 (A-D) Change of oil droplets volume of RoM at (A) 0 min dry (B) 1 min dry (C) 2 min dry (D) 3 min dry

Fig. 3.4 (A-D) Deformation of oil droplets of RoM at (A) 0 min dry (B) 1 min dry (C) 2 min dry (D) 3 min dry

Fig. 3.5 Weights of absorbed water and resulted separated oil of RoM. The line indicates the total oil content.

Fig. 3.6 XRD pattern of water phase at temperature $-40\text{ }^{\circ}\text{C}$ at (A) control, (B) 0.05% (C) 0.1% and (D) 0.2% APS

Fig. 3.7 XRD pattern of mayonnaise at temperature $-40\text{ }^{\circ}\text{C}$ at (A) control, (B) 0.05% (C) 0.1% and (D) 0.2% APS

Fig. 3.8 Water phase crystallinity with Anti-frozen Polysaccharides (APS) at temperature $-40\text{ }^{\circ}\text{C}$ in (A) mayonnaise and (B) water phase.

Fig. 3.9 Freeze-thaw stability of mayonnaise with Anti-frozen Polysaccharides (APS) with a concentration of 0.05, 0.1 and 0.2 % at temperature (A) $-20\text{ }^{\circ}\text{C}$ and (B) $-40\text{ }^{\circ}\text{C}$

Fig. 4.1 Induction time with different tube. (A) at $-20\text{ }^{\circ}\text{C}$ (B) at $-30\text{ }^{\circ}\text{C}$ (C) at $-40\text{ }^{\circ}\text{C}$. Inserts presents the magnified graph of (B) and (C)

Fig. 4.2 Destabilization rate constant k_d with different tube. (A) at $-40\text{ }^{\circ}\text{C}$ (B) at $-30\text{ }^{\circ}\text{C}$ (C) at $-20\text{ }^{\circ}\text{C}$. Inserts presents the magnified graph of (C)

Fig. 4.3 The stability of mayonnaise showed remaining oil ratio versus time after fitting the data. (A) RoM and (B) SoM for freezing at temperatures of $-20\text{ }^{\circ}\text{C}$, $-25\text{ }^{\circ}\text{C}$, $-30\text{ }^{\circ}\text{C}$, $-35\text{ }^{\circ}\text{C}$, $-40\text{ }^{\circ}\text{C}$. Points on the fitted curves indicate the experimental data. Results are expressed as the mean of four trials.

Fig. 5.1 Microscopic images of RoM fat crystals (white dots) using PLM. Images (A) to (C) during storage at $-20\text{ }^{\circ}\text{C}$ for $6\text{ h} \pm 5\text{ min}$, $6.5\text{ h} \pm 5\text{ min}$ and $7\text{ h} \pm 5\text{ min}$ respectively. And images (D) to (F) during storage at $-30\text{ }^{\circ}\text{C}$ for $2\text{ h} \pm 5\text{ min}$, $2.5\text{ h} \pm 5\text{ min}$ and $3\text{ h} \pm 5\text{ min}$ respectively and images (G) to (I) $-40\text{ }^{\circ}\text{C}$ for $50\text{ min} \pm 5\text{ min}$, $1.5\text{ h} \pm 5\text{ min}$ and 3

$h \pm 5$ min respectively. Scale bar is 20 μm .

Fig. 5.2 Microscopic images of SoM fat crystals (white dots) using PLM. Images (A) to (C) during storage at -20°C for 14 h 20 min \pm 5 min, 15 h 50 min \pm 5 min and 16 h 35 min \pm 5 min respectively. And images (D) to (F) during storage at -30°C for 11 h 50 min \pm 5 min, 12 h 50 min \pm 5 min and 13 h 20 min \pm 5 min respectively and images (G) to (I) -40°C for 4 h 20 min \pm 5 min, 4 h 50 min \pm 5 min and 5 h 5 min \pm 5 min respectively. Scale bar is 20 μm .

Fig. 5.3 Plots of $\ln\{-\ln(1-X_t)\}$ versus $\ln t$ for isothermal crystallization of (A) RoM and (B) SoM at -20 , -30 and -40°C

Fig. 5.4 (A) Thermal behavior with DSC during cooling at a rate of $5^\circ\text{C}/\text{min}$ (a) RoM, (b) Rapeseed oil, (c) Soybean oil and (d) SoM in the interval of 0 to -75°C . (B) During melting at a rate of $5^\circ\text{C}/\text{min}$ (a) Rapeseed oil, (b) RoM, (c) SoM and (d) Soybean oil in the interval of -75 to 0°C .

Fig. 5.5 (A) Small angle X-ray diffraction of RoM and its oil and water phase showed crystal form changing with temperature (a) water phase at -45°C , (b) RoM at -41°C , (c) RoM at -25°C , (d) Rapeseed oil at -20°C and (e) RoM at -12°C . (B) Small angle X-ray diffraction of SoM and its oil phase showed crystal form changing with temperature (a) Soybean oil at -26°C , (b) SoM at -25°C , (c) Soybean oil at -25°C , and (d) SoM -40°C .

Fig. 5.6 Relationship between crystallization rate constant K_c and destabilization rate constant k_d . (A) Rapeseed oil mayonnaise and (B) Soybean oil mayonnaise.

CHAPTER 1

General introduction

1.1 Mayonnaise

Mayonnaise is a semi solid oil-water emulsion which is comprising with vegetable oil as oil phase and acidifying ingredient (vinegar), egg yolk (emulsifying agent), salt, flavor, sweetening as water phase (Maud, L., et al., 1999). The regular mayonnaise contains the oil accounts for approximately 75% or more of the total volume (Depree, et al., 2001). It is widely consumed all over the world as food. Like many other food emulsion, it needs to freeze with other food gradients. But while thawing it loses its texture and finally the oil phase separates. This problem associated with freeze storage of mayonnaise.

Being mayonnaise an emulsion, it is thermodynamically unstable system that can be breakdown with a variety of physicochemical process depending on their composition, microstructures and environmental stresses through coalescence, partial coalescence, etc. (McClements, 1999). It is usual that, this instability depends on its composition and environmental factors. So, the proneness to coalescence is the function of lipid composition, solid fat content (SFC), emulsifier type, particle size, temperature, shearing rate and various other factors (Boode & Walstra, 1993; Boode et al., 1993; McClements, 1999; van Boekel, 1981, Walstra, 2003). Understanding the influence of freezing and thawing conditions on the stability of O/W emulsions is required to stop or minimize the destabilization of many types of sauces and food emulsion (McClements 2004; Ghosh et al., 2006; Ghosh and Coupland 2008; Ghosh and Rousseau, 2009; Magnusson et al. 2011).

In food industry, the stability of emulsion depends on their composition (including oils,

emulsifiers, thickening agents, salts, and cryo-protectants) and processing conditions (homogenization, freezing, defrosting conditions, etc). For successful design of stable emulsion therefore depends on a good understanding of the behavior of emulsions during freezing, storage, and thawing, and of the various factors that influence their properties. In this research we examined the influence of crystallization of water and oil phase on mayonnaise stability through limiting ice crystal growth and kinetic analysis respectively. This information would help to gain a more fundamental understanding the crystallization properties that influence the stability of mayonnaise.

1.2 Motivation and objectives

The present study deals with the stability of mayonnaise during freeze-thawing where both oil and water phase crystallized. Many processes are known in which the stability of oil in water emulsion is influenced by the presence of fat crystal. For example, the churning of cream, to produce butter is impossible without crystals into oil phase (Mulder, H. & Walstra, P., 1974.) and fat crystal are necessary for clumping of fat globules in ice cream (Berger and White, 1980). But emulsion like mayonnaise or salad dressing should remain stable during storage and transport. This study focused on the destabilization of mayonnaise that influenced by the crystallization of fat and water.

Ghosh and Coupland, 2008 reported that a high volume fraction and expanding ice press the droplets together and the high sugar or other solute can affect the inter-droplet forces in 20% oil in water emulsion. A combination of these effects can lead to the membrane separating the droplets rupturing and is responsible for coalescence. The contact among oil droplets increased due to reduction of volume fraction of unfrozen part that contain solids and unfrozen water causing emulsion breakdown due to rupturing of membranes that surrounds dispersed particles (Saito et al., 1999; van Aken, Blijdenstein, & Hotrum, 2003). However, emulsion like mayonnaise that contains 30%

water phase may have effect on destabilization during freeze-thawing. For that, a brief study has been made on destabilization considering ice crystal.

On the other hand several workers have studied coalescence in partially crystallized emulsions. Van Boekel, 1981 investigated the influence of crystals in the oil phase on the stability of paraffin-in-water emulsions that were stable in the absence of crystal. McClements et al, 1999 reported on the behavior of hydrocarbon emulsions containing a mixture of solid and liquid globules. Recently Ishibashi et al, 2016 reported the polymorphic change of fat crystal brings change in the mechanism of droplets coalescence of mayonnaise. Miyagawa et al, 2016 reported the volumetric expansion of crystal cause destabilization of mayonnaise during freeze-storage. E. Magnusson et al., (2011) found a strong correlation between the amounts of mono-unsaturated fatty acids (oleic acid) with freeze-thaw stability and the high content of oleic acid was negative for the freeze-thaw stability of mayonnaise type oil in water emulsion. Control of the coalescence process is, therefore, a necessity and a basic understanding of the principles that underlie destabilization of mayonnaise, caused by crystallization of ice and fat, is required.

The above mentioned investigations have clearly shown that the course of coalescence process and the rate at which coalescence occurs depends on the type of emulsion used. The crystals play a key role in it. Therefore, the objective of the present study was to investigate the effect of water and fat crystal properties on emulsion stability and to gain a more fundamental understanding of the variables that influence these properties. Special attention was paid to the kinetic analysis of the process in an attempt to better differentiate among factors.

1.3 Destabilization of mayonnaise

Stability of mayonnaise as well as other food emulsions is an important consideration

during preparation, development and preservation. In case of mayonnaise, stability means the resistance of the droplets to coalesce. Thanasukarn et al. (2004) studied that the emulsifier type (Tween 20, whey protein isolate or casein) influenced the physical properties of oil in water emulsion stability. It was found that most destabilization happened at fat crystallization point. Other than various factors interfacial phenomena also can affect the stability of emulsion. That includes viscosity and visco-elasticity of continuous phase, the volume fraction of disperse phase, droplets size and distributions. The concentration of emulsifying agent can also play role on emulsion destabilization by lowering steric stabilization at low concentration (Dickinson and Ritzoulis, 2000). To stabilize food emulsions protein, casein, etc may be used to prevent droplets coalescence (Dalgleish, 1996). Mayonnaise stability during freezing would depend on oil phase composition as well as water phase composition and their behavior.

1.3.1 Destabilization mechanism

The breakdown of food emulsion prior to consumption is undesirable as it generates negative quality attributes, such as an undesirable appearance, texture, or flavor. Thus, it is important to maintain the stability of food emulsion throughout the shelf life upon reconstitution by the consumer. An emulsion can be considered to be stable if there is no visible change in its overall appearance and contains a homogeneous distribution of oil phase as droplets throughout the entire aqueous phase. In general, there are various mechanisms that can promote the physical instability of O/W emulsions including flocculation, gravitational separation, coalescence, partial coalescence, phase inversion, and Ostwald ripening (van Aken and others 2003; Tcholakova and others 2006). Usually, in mayonnaise destabilization happen through coalescence and/or partial coalescence.

Coalescence is the process where two or more droplets merge together to form a bigger droplet that can eventually leading to “oiling off” and phase separation. The rate and

extent of coalescence closely depends on the nature of the layer of surface-active molecules that surrounds the fat droplets (Goff and Jordan, 1989). The fat droplets may be relatively stable to coalescence when the droplets come into contact if the interfacial layer is able to generate a strong repulsive force and provide mechanical support that resist disruption.

Some proteins and polysaccharides are able to build interfacial layers that are highly resistant to coalescence, compared with some small molecule surfactants (van Aken and others 2003). These researchers also reported that droplets stabilized by proteins such as whey proteins by forming cross-links at the oil-water interface. Chung and others, (2012) found that whey protein-coated fat droplets in model emulsion were much more stable to droplet coalescence than surfactant-coated fat droplets, particularly at higher fat contents.

On the other hand, in case of partial coalescence two or more partially crystallize oil droplets aggregate together to form an irregular shaped flock (Fredrick and others 2010). But partial coalescence strongly depends on the type, amount and location of fat crystal within the oil droplets as well as the nature of interfacial layer along with shearing conditions (Walstra 2003). Partial coalescence increases the product viscosity through forming visible clumps and if fat droplets melt during thawing then phase separation occurs that leads formation of an oil layer on the top of emulsion (Brian, M.D., 2014). When low melting point oil phase is used, the droplets will not crystallize prior to the water phase. If the system is cooled sufficiently below its melting point, the water phase starts to crystallize. The precise crystallization temperature depends on the initial solute concentration in the water phase, since solute tends to depress the melting point. As the progress of water phase crystallization the fat droplets are increasingly concentrated into region of non-frozen aqueous phase between the ice crystals. These freeze concentration

may promote droplets flocculation and or coalescence (Thanasukrn et al, 2004). The probable mechanism of destabilization of mayonnaise type emulsion had been shown in Fig. 1.1.

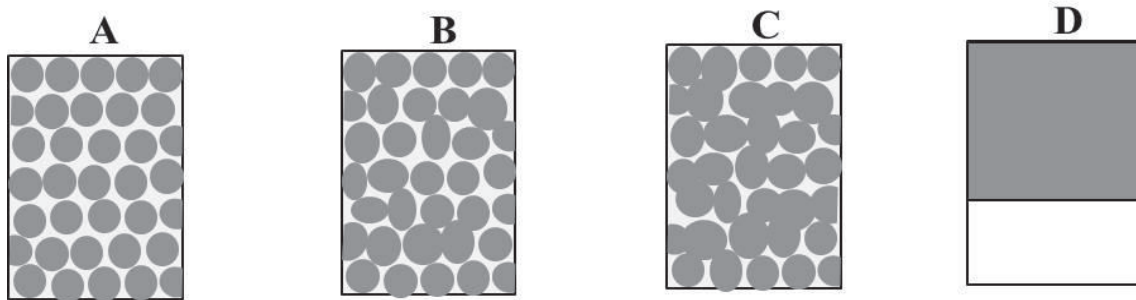


Fig. 1.1 (A-D) The probable process of coalescence of mayonnaise type emulsions. (A) Emulsified oil droplets (B) Deformation of oil droplets (C) Coalescence of deformed oil droplets (D) Separated oil while thawing

1.3.2 Role of ice and fat crystal

Ice crystal increases the lipid droplets concentration in unfrozen solute phase where oil droplets remain in a close pack. The contact among oil droplets and in between ice and oil droplets increased due to reduction of volume fraction of unfrozen part that contain solids and unfrozen water causing emulsion breakdown due to rupturing of membranes that surrounds dispersed particles (Saito et al., 1999; van Aken, Blijdenstein, & Hotrum, 2003). Ice crystal also can cause the increase of stress due to volumetric expansion and also it can alter the viscosity (Miyagawa et al, 2016). Generation of ice crystal is also related with emulsifying properties of emulsifier, p^H status, etc. But little work has been reported till date considering ice crystal effect on emulsion destabilization. The probable ice crystal effect has been shown in Fig. 1.2.

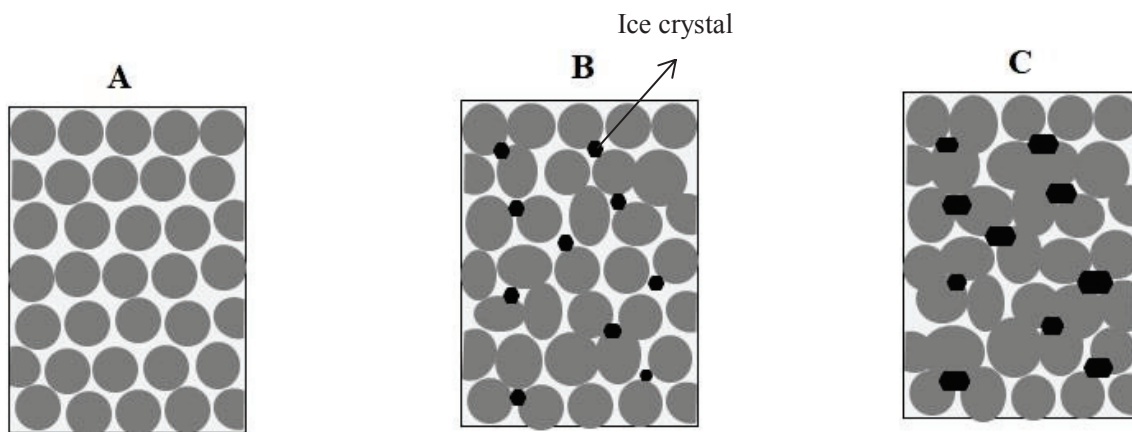


Fig. 1.2 (A-C) The probable roles of ice crystal on coalescence. (A) Emulsified oil droplets (B) Generation of ice crystal and deformation of oil droplets started (C) Enlargement of ice crystal and deformation of oil droplets and close contact of deformed oil droplets and coalescence

Emulsion stability greatly influenced by its oil phase and physico-chemical properties of that oil like crystallization behavior, crystal structure (polymorphic form) crystallization kinetics, crystal location (at the interface or deeper inside the droplet), and crystal growth (leading to a single crystal mass or a number of small crystals) of the dispersed lipid phase. It regulates the resistance of droplets to mechanical stress (e.g. deformation caused by collisions due to Brownian and hydrodynamic motion or due to ice crystals growth) whether partial or total coalescence occurs (Coupland, 2002).

If fat crystal adsorb at the surface of droplet, that enhance emulsion stability by coming in contact with the interface (Tambe & Sharma, 1993). However, recently the opposite result observed by Ishibashi et al., 2016. They showed direct partial coalescence happen due to generation of fat crystal. They also showed the polymorphic change of canola and soybean fat crystal destabilizes mayonnaise following different mechanism.

Emulsion like mayonnaise which contains oil phase more than 70%, where the oil droplets remain very dense. The fat crystal might generate on the surface of droplet. Upon any change in water phase the crystallized oil droplets come in close proximity

then the fat crystal penetrate neighboring oil droplets. The mutual sharing of liquid oil happens and results a coalesced droplet. The probable role of fat crystal on coalescence has been shown in Fig. 1.3.

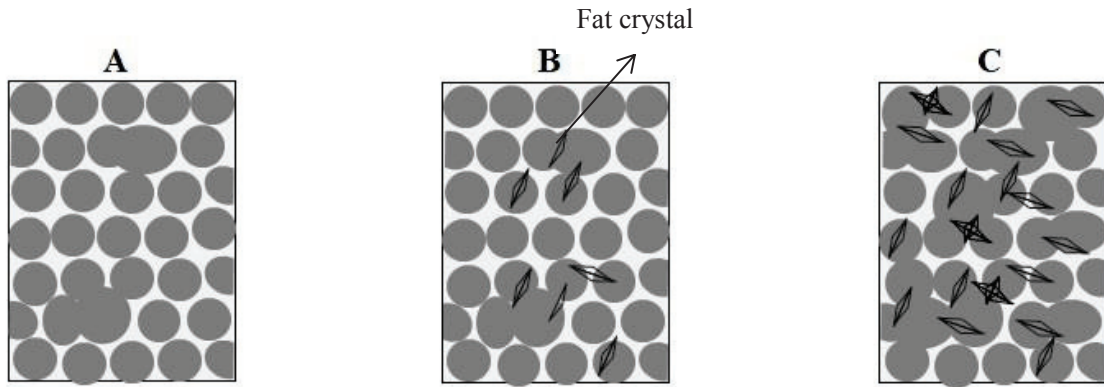


Fig. 1.3 (A-C) The probable roles of fat crystal on coalescence. (A) Emulsified oil droplets (B) Generation of fat crystal and deformation of oil droplets started (C) Increase the number of fat crystal and deformation of oil droplets and penetration of fat crystal to the neighboring oil droplets and coalescence

The effect of ice and fat crystal effect on coalescence has been shown in Fig. 1.2 and 1.3. But during freezing both ice and fat crystallize. Their combined effect of coalescence has been shown if Fig. 1.4.

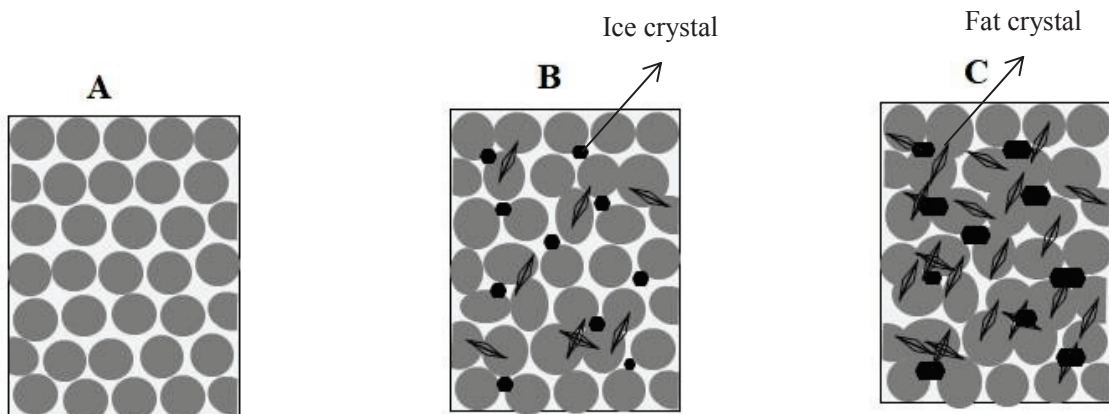


Fig. 1.4 (A-C) The probable roles of both ice and fat crystal on coalescence. (A) Emulsified oil droplets (B) Generation of ice and fat crystal and deformation of oil droplets started (C) Enlargement of ice crystal and increase the number of fat crystal and deformation of oil droplets and close contact of deformed oil droplets and

penetration of fat crystal to the neighboring oil droplets and coalescence

1.3.3 Destabilization kinetics

Kinetic stability refers to stability in terms of time. While an emulsion is never entirely stable according to the kinetic definition, it may be considered to be stable if the material is sufficiently stable within the use time. There is no standard convention for considering stability time. Some authors use half-life, similar to radiation, which is appropriate because most processes that destabilize emulsions are exponential or analogous functions, with respect to process. On the other hand destabilization kinetics refers to the rate at which destabilization happen. In this research destabilization has been considered as separated oil.

Several authors reported different model for describing stability of emulsion based on the nature of emulsion. Lye and Stuckey, 1998 describes a standard kinetics for emulsion stability considering first-order that is by the concentration of the starting product alone. They described the reaction by

$$\frac{V_c(t)}{V_c(0)} = \exp(-kt)$$

Where $V_c(t)$ is the volume of the dispersed phase at time t ,

$V_c(0)$ is the concentration of the dispersed phase at time 0, or starting concentration,

k is the first order rate constant and t is the time elapsed

Taylor, 1998 had been tested the Lifshitz, Slezov and Wagner theory of Ostwald ripening against several model systems. It is generally conceded that this is the best available model to describe Ostwald ripening. One end result is a description of the aging rate:

$$\omega = \frac{dr_c^3}{dt} = \frac{4}{9}\alpha = \frac{8DC_\infty\gamma M}{9\rho^2RT}$$

Where ω is the aging rate, the rate of increase of the cube of the critical radius,

r_c is the critical radius at which the rate of change of droplet radius is zero, that is the droplet is in equilibrium with the bulk phase- at this point all droplets below this radius will decrease in size and above this size, will grow as a result of Ostwald ripening,

t is the time at the point of consideration, and

α is the rate fraction or: $\alpha = K \frac{DC_\infty}{\rho}$

Where K is the rate constant of an Ostwald ripening situation,

D is the diffusional flux of the soluble components,

C_∞ is the bulk solubility of the oil, and

ρ is the viscosity of the oil,

γ is the interfacial tension between the oil and the water,

M is molar mass of the oil,

R is the universal gas constant, and

T is the temperature in Kelvin.

However, Civan et al, (2004) developed a new stabilization model that covers both oil-in-water and water-in-oil emulsions. They begin the model formation study by describing the emulsion decomposition as an irreversible reaction as:



Where, R_E is the stable emulsion

P_D is the dispersed phase, and

P_C is the continuous phase.

Civan et al. (2004) then considered this breakdown to be a power-law function of the instantaneous emulsion fraction available for decomposition according to

$$\frac{dX}{dt} = k_d(X_f - X)^n$$

Where X is the instantaneous decomposed fraction of emulsion

X_f is the maximum achievable fraction of emulsion decomposition or limiting emulsion fraction

n is the order of reaction,

t is time, and

k_d is the decomposition rate constant

In this research the kinetic model proposed by Civan et al. 2004 has been utilized for kinetic analysis.

Outline of this thesis

The thesis has been divided into six chapters organized as follows:

Chapter 1

In this chapter a discussion has been made on mayonnaise and the problem associated with freeze storage. The core objective and motivation has also been explained. It also includes a brief overview of the key factors as well as the kinetics and mechanism of destabilization.

Chapter 2

This chapter confirmed the coalescence of oil droplets during freezing and destabilization while freeze-thawing. To observe coalescence during freezing the frozen mayonnaise has been observed using cryo-SEM. For understanding destabilization mayonnaise has been frozen and separated oil has been measured with time. These results confirm that coalescence happen during freezing and after thawing the coalesced oil droplets appear as separated oil.

Chapter 3

This chapter described the coalescence of oil droplets influenced by ice crystal. Firstly an artificial destabilization condition for coalescence has been made using dry air blowing then ice crystal has been simulated using desiccant. The comparative separation ratio of mayonnaise with limiting ice crystal growth has been shown using anti-frozen polysaccharides (APS). XRD measurement has been conducted to show the crystallinity

difference with APS concentration.

Chapter 4

This chapter introduced a new method for determine the kinetic parameter using capillary. Destabilization kinetic parameter like destabilization rate constant, k_d and order of destabilization, n of mayonnaise has been analyzed using power law function. The induction time and half-life of mayonnaise destabilization has also been discussed. An empirical equation regarding temperature dependent destabilization rate constant has been showed in this chapter.

Chapter 5

Iso-thermal crystallization kinetic analysis of fat crystal has been discussed in this chapter. The crystallization kinetic parameters includes crystallization rate constant, k_c and the Avrami exponent, n . These crystallization kinetic parameters further evaluated through Differential Scanning Calorimetry (DSC) and Small Angle X-ray Scattering (SAXS) measurement. It also attempted to explain the destabilization rate constant, k_d through crystallization kinetics and evaluated the influence of fat crystal growth rate on destabilization.

Chapter 6

This chapter summarizes the research presented in this thesis. It also discussed the applicability of the method used for analyzing induction time and kinetic parameters for understanding the destabilization of mayonnaise during freeze-thawing along with future suggestions.

References

- Berger, K. G. and White, G. W., (1971) An electron microscopical investigation of fat destabilization in ice cream. *J. Food Technol.* 6: 285-294.
- Boode K and Walstra P (1993) Partial coalescence in oil-in-water emulsions 1: Nature

- of the aggregation. *Colloids and Surfaces A—Physicochemical and Engineering Aspects*, 81(1): 121–137.
- Boode K, Walstra, P and Degrootmostert AEA (1993) Partial coalescence in oil-in-water emulsions 2: Influence of the properties of the fat. *Colloids and Surfaces A—Physicochemical and Engineering Aspects*, 81(1): 139–151.
- Brian MD, Cheryl C, Vicki S, Hutkins R and McClements DJ (2014) Factors influencing the freeze –thaw stability of emulsion based foods. *Comprehensive Reviews in Food Science and Food Safety*. 13: 98-113.
- Chung C, Degner B, McClements DJ. (2012) Rheology and microstructure of bimodal particulate dispersions: model for foods containing fat droplets and starch granules. *Food Res Int* 48: 641–9.
- Civan, F., L.J. Alarcon, and S.E. Campbell. (2004) Laboratory Confirmation of New Emulsion Stability Model, *Journal of Petroleum Science and Engineering*, 43: 25-34.
- Coupland JN (2002) Crystallization in emulsions. *Current Opinion in Colloid & Interface Science*, 7(5-6): 445-450.
- Dalgleish DG (1996) Food Emulsions, Chapter 5 in *emulsions and emulsion Stability*, Johan Sjoblom, (ed), Marcel Dekker, New York, 287-323.
- Depree JA and Savage GP (2001) Physical and Flavour Stability of Mayonnaise. *Trends in Food Science and Technology*. 12: 162-171.
- Dickinson E and Ritzoulis C (2000) Creaming and Rheology of Oil in Water Emulsions Containing Sodium Dodecyl Sulfate and Sodium Caseinate, *Journal of Colloid and Interface Science*, 224: 148-154.
- Fredrick E, Walstra P and Dewettinck K. (2010) Factors governing partial coalescence in oil-in-water emulsions. *Adv Colloid Interface Sci*. 153: 30–42.

- Ghosh S, Cramp GL and Coupland JN (2006) Effect of aqueous composition on the freeze-thaw stability of emulsions. *Colloids Surf A Physicochem Eng Asp* 272: 82–8
- Ghosh S, Coupland JN (2008) Factors affecting the freeze–thaw stability of emulsions. *Food Hydrocolloids* 22:105–11.
- Ghosh S, Rousseau D. (2009) Freeze–thaw stability of water-in-oil emulsions. *J Colloid Interface Sci* 339: 91–102.
- Goff H, Jordan W (1989) Action of emulsifiers in promoting fat destabilization during the manufacture of ice cream. *J Dairy Sci* 72:18–29.
- Ishibashi, C., Hondoh, H., and Ueno, S. (2016). Influence of morphology and polymorphic transformation of fat crystal on the freeze thaw stability of mayonnaise type oil in water emulsion. *Food Research International*, 89: 604-613.
- Lye, G. L. and Stuckey, D. C. (1998) Structure and stability of colloidal liquid aphrons. *Colloids and surfaces A: Physiochemical and engineering aspects*. 131: 119-136.
- Magnusson, E., Rosen, C and Nilsson, L. (2011). Freeze-thaw stability of mayonnaise type oil-in-water emulsions. *Food Hydrocolloids* 25 : 707-715.
- Maud L, Jordansson E, Altskar A and Sorensen C (1999) Microstructure and Image Analysis of Mayonnaise. *Food Hydrocolloids*. 13: 113-125.
- McClements DJ (1999) *Food emulsions: Principles, practice, and techniques*. 2nd ed. Boca Raton: CRC Press. London
- McClements DJ (2004) Protein-stabilized emulsions. *Curr. Opin. in Colloid Interface Sci*. 9: 305–13.
- Miyagawa, Y., Ogawa, T., Nakagawa, K. & Adachi, S. (2016). Destabilization of mayonnaise induced by lipid crystallization upon freezing. *Bioscience, Biotechnology, and Biochemistry*, 80(4):786-90.
- Mulder, H. & Walstra, P. *The milk fat globule: emulsion science as applied to milk*

- products and comparable foods, Commonwealth Agricultural Bureaux, Wageningen 1974
- Saito, H., Kawagishi, A., Tanaka, M., Tanimoto, T., Okada, S., Komatsu, H., & Handa, T. (1999). Coalescence of lipid emulsions in floating and freeze-thawing processes: Examination of the coalescence transition state theory. *J. of Colloid and Interface Sci.* 219(1): 129–134.
- Tambe DE and Sharma MM (1993) Factors controlling the stability of colloid-stabilized emulsions. I. An experimental investigation. *Journal of Colloid and Interface Sci.* 157:244-253.
- Taylor, P (1998) Ostwald ripening in emulsions. *Advances in colloid and interface science*, 75:107-163
- Tcholakova S, Denkov ND, Ivanov IB, Campbell B. (2006) Coalescence stability of emulsions containing globular milk proteins. *Adv Colloid Interface Sci* 123–126: 259–93.
- Thanasukarn, P., Pongsawatmanit, R. and McClements, D.J. (2004) Influence of Emulsifier Type on Freeze-thaw Stability of Hydrogenated Palm Oil-in-water Emulsions, *Food Hydrocolloids*. 18: 1033-1043.
- van Boekel MAJS (1981) Estimation of solid liquid ratios in bulk fats and emulsions by pulsed NMR. *Journal of the American Oil Chemists Society.* 58(7): 768–772.
- van Aken G, Blijdenstein TBJ and Hotrum NE (2003) Colloidal destabilization mechanisms in protein-stabilised emulsions. *Current Opinion in Colloid & Interface Sci.* 8(4): 371-379.
- Walstra P. (2003) *Physical chemistry of foods*. Marcel Dekker. New York.

CHAPTER 2

Confirmation of oil droplets coalescence and destabilization of mayonnaise during freezing

2.1 Introduction

Mayonnaise is a semi solid oil-water emulsion which is comprising with vegetable oil as oil phase and acidifying ingredient (vinegar), egg yolk (emulsifying agent), salt, flavor, sweetening as water phase (Maud et al., 1999). The regular mayonnaise contains the oil accounts for approximately 75% or more of the total volume (Depree & Savage, 2001) where usual oil in water emulsion contains less oil phase than continuous phase. It is widely consumed all over the world as food. Oil separation is a problem of mayonnaise associated with freeze storage. All emulsions are thermodynamically unstable system that can be breakdown with a variety of physicochemical process depending on their composition, microstructures and environmental stresses through coalescence, partial coalescence, etc. (McClements, 1999). The proneness to coalescence is the function of lipid composition, solid fat content (SFC), emulsifier type, particle size, temperature, shearing rate and various other factors (Boode & Walstra, 1993; Boode et al., 1993; McClements, 1999; van Boekel, 1981, Walstra, 2003).

Changes in environmental conditions e.g. freezing and freeze-drying can cause changes in physical properties and stability of oil-in-water emulsions that may be related to phase changes of their components (Stadelman & Cotterill, 1994). Usually, environmental stresses make some physical changes (generation of crystals, deformation of disperse droplets, change in emulsifier activity, etc.) in emulsion that influence the stability. We assumed that, the generation of crystal of respective ice and fat phases cause coalescence during freezing and the coalesced oil appeared as separated oil while thawing. This research aims to confirm the destabilization and coalescence of

mayonnaise during freezing.

2.2 Materials and method

2.2.1 Materials

Mayonnaise sample, mayonnaise oil and water phase was obtained from Oriental Yeast Company, (Tokyo, Japan). The oil phase comprised only rapeseed oil and water phase comprised with egg yolk as emulsifier, salt, sugar, acetic acid and vinegar. The composition of water and oil phase showed in Table 2.1.

Table 2.1 Ingredients of the two mayonnaises (prepared in batches of 32.6 g).

Water phase		
Ingredient	% Weight	Weight (g)
Egg yolk	14.1	4.6
Vinegar	7.7	2.5
Egg Albumen	3.7	1.2
Salt	2.1	0.7
Sugar	0.3	0.1
Water	2.1	0.7
Total	30	9.8
Oil phase		
Rapeseed oil/ Soybean oil	70	22.8

2.2.2 Preparation of mayonnaise samples

To prepare 32.6 g of mayonnaise, 22.8 g of the oil phase and 9.8 g of the water phase were used at a ratio of 7:3. The water phase was placed in a 50 ml centrifuge tube then the oil phase was added and mixed in eight stages. Each time one eighth of the oil phase was added then homogenized for 45 s at 25,000 rpm concluding with homogenization for 1 min (McGee, 2004). A Polytron Pt 1200e homogenizer was used for preparing the mayonnaise (Kinematica AG, Lucerne, Switzerland). The median diameter of the oil

droplets was 1.1 μm that has been calculated from the microscopic image of oil droplets through image processing using Image J software.

2.2.3 Observation of oil droplets coalescence of mayonnaise during freezing using cryo-SEM

For observing coalesced droplets during freezing Scanning Electron Microscopy (S-800, Hitachi Co., Ltd., Tokyo, Japan) with cooling (liquid N_2) system has been utilized. For that the mayonnaise samples had been frozen at $-20\text{ }^\circ\text{C}$ for 48 h. Before observing the droplets the frozen mayonnaise sample was soaked in liquid nitrogen. After that the sample had been sliced for getting clear image of oil droplets. For temperature control liquid nitrogen also applied. The samples have been frozen for 24 hours prior to observation. The frozen samples are then immersed into the liquid nitrogen.

2.2.4 Destabilization of mayonnaise during freeze-thawing

To clarify destabilization during freeze-thawing, a rapeseed oil mayonnaise (RoM) has been frozen at $-20\text{ }^\circ\text{C}$ for 8 to 48 h. For that 45 g mayonnaise has been poured in 50 ml centrifuge tube and kept in freezer for 8, 16, 24, 32 and 48 h. After freezing the samples had been thawed at room temperature ($25\text{ }^\circ\text{C}$) for 30 min and then kept in a hot water bath at $60\text{ }^\circ\text{C}$ for 20 min. Then the samples had been centrifuged at $1250\times g$ in a KN-70 table top centrifuge (Kubota Corporation, Tokyo, Japan). The weight separated oil has been measured using a calibration graph. The rest oil in water phase had been measured by using Soxhlet extraction method.

2.2.5 Statistical analysis

All experiments were repeated four times. Significant difference between means (from

replicates) was determined with Tukey's honest significant difference test at a significance level of $P < 0.05$ using Kaleida Graph (Version: 4.1.1, Synergy Software, Reading, PA, USA).

2.3 Results

2.3.1 Observation of oil droplets coalescence of mayonnaise during freezing using cryo-SEM

Fig. 2.1 showed oil droplets status during freezing condition. This figure showed the wall of oil droplets was broken, it might result from the penetration of fat crystal and the droplets seem coalesced. This coalesced oil droplets turn into separated oil while thawing. These results agreed that coalescence happen during freezing condition. It is obvious that the physical changes of oil and water phases (crystallization) may results the coalescence of oil droplets.

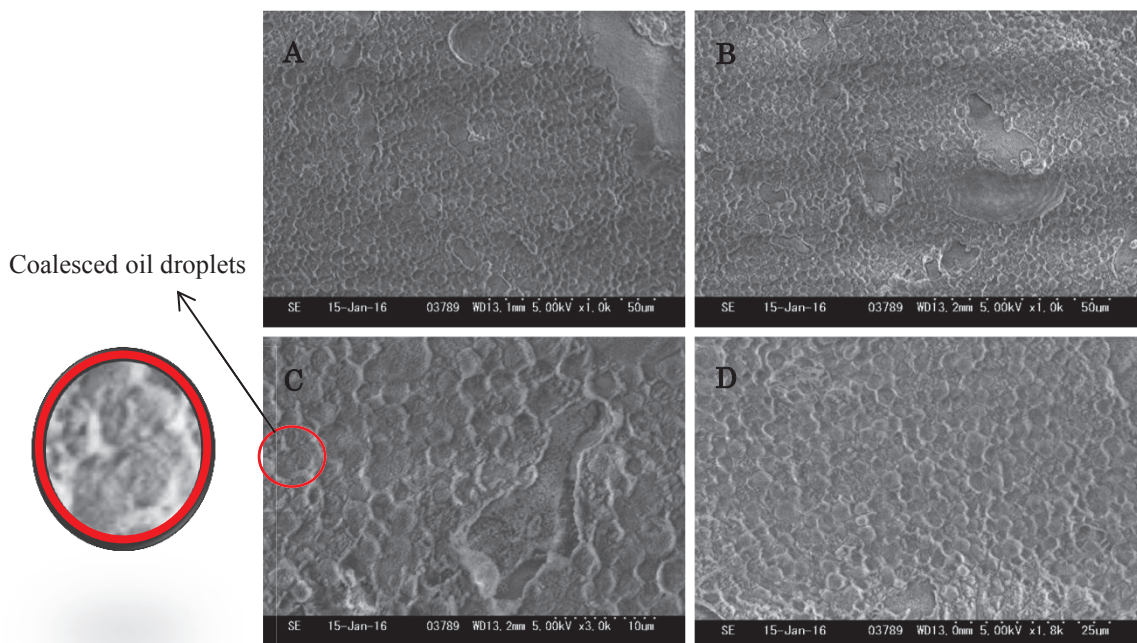


Fig. 2.1 (A-D) Cryo-SEM images of RoM showing coalescence of oil droplets (A) and (B) scale bar 50 μm (C) scale bar 10 μm (D) scale bar 25 μm . Red circle showed the enlargement of coalesced droplets.

2.3.2 Destabilization of mayonnaise during freeze-thawing

The separated oil with time showed that with increasing freezing time the upper part (separated oil) increased (Fig. 2.2). The oil concentration in middle part (creamy part-complex of oil phase and water phase) decreased with freezing time. It might be due to most of the oil separated. Freezing at 8 h did not show oil separation. Oil separation start after freezing 16 h and increased with increasing freezing time. The oil content in lower part (mostly water and other ingredients of water phase) showed increased with increasing freezing time and contains very less separated oil.

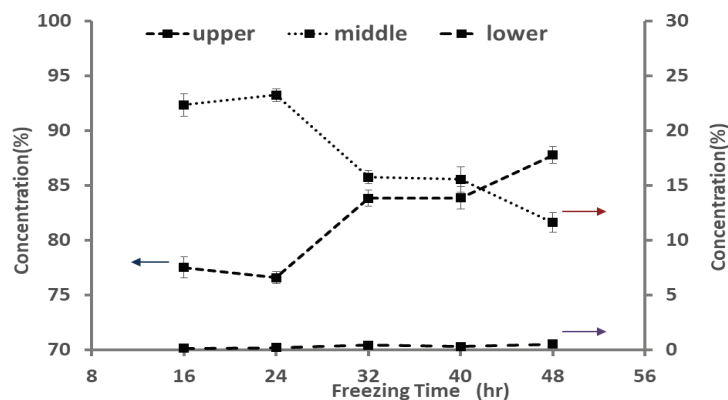


Fig. 2.2 Freeze-thaw stability of rapeseed oil mayonnaise with time at temperature -20°C in bulky samples

2.4 Discussion

Cryo-SEM images of oil droplets showed that coalescence happen during freezing as the border of oil droplets was not distinct. This coalescence might results from the physical change that happen during freezing at -20°C . The most common physical changes that may happen would be crystallization of water and fat. Miyagawa et. al. (2016) and Ishibashi et. al. (2016) reported the crystallization of both water and fat happen at this temperature with rapeseed oil mayonnaise and cause destabilization. The

crystallization of both phases might cause the coalescence of oil droplets at this temperature.

The freeze-thaw stability graph in Fig. 2.2 showed that the separated oil increased with freezing time. The oil content in the middle part decreased with time that means more oil separated from water phase. The oil content in lower part was lowest than other parts showed increased with freezing time. Considering the coalescence, it can be said that the coalescence rate might be increased with freezing time for that separated oil found higher with freezing time. Based on Miyagawa et. al. (2016) and Ishibashi et. al. (2016), if crystallization of water and fat phase responsible for destabilization we can think that the rate of crystallization of water and fat would also be increased with freezing time that has been discussed in the following chapters. However the increased separated oil with longer freezing time was due to higher coalescence rate as the coalesced oil droplets during freezing appeared as separated oil.

2.5 Conclusion

Oil droplets has been observed and found that coalescence happen during freezing. The coalesced oil droplets appeared as separated oil while thawing. Separated oil of mayonnaise during freeze-thawing found increased with time. This might be related with the rate of coalescence of oil droplets that depends on the crystallization of water and fat.

References

Boode K and Walstra P (1993) Partial coalescence in oil-in-water emulsions 1: Nature of the aggregation. *Colloids and Surfaces A—Physicochemical and Engineering Aspects*, 81(1): 121– 137.

- Boode K, Walstra, P and Degrootmostert AEA (1993) Partial coalescence in oil-in-water emulsions 2: Influence of the properties of the fat. *Colloids and Surfaces A—Physicochemical and Engineering Aspects*, 81(1): 139–151.
- Depree JA and Savage GP (2001) Physical and Flavour Stability of Mayonnaise. *Trends in Food Science and Technology*. 12: 162-171.
- Ishibashi, C.; Hondoh, H.; Ueno, S. Influence of morphology and polymorphic transformation of fat crystal on the freeze thaw stability of mayonnaise type oil in water emulsion. *Food Res. Int.* 2016, 89, 604–613.
- Maud L, Jordansson E, Altskar A and Sorensen C (1999) Microstructure and Image Analysis of Mayonnaise. *Food Hydrocolloids*. 13: 113-125.
- McClements DJ (1999) *Food emulsions: Principles, practice, and techniques*. 2nd ed. Boca Raton: CRC Press. London
- McGee, H. *On Food and Cooking: The Science and Lore of the Kitchen*; Simon & Schuster: New York, NY, USA, 2004; pp. 633–634. ISBN 0-684-80001-2.
- Miyagawa, Y.; Ogawa, T.; Nakagawa, K.; Adachi, S. Destabilization of mayonnaise induced by lipid crystallization upon freezing. *Biosci. Biotechnol. Biochem.* 2016, 80, 786–790.
- Stadelman WJ and Cotterill OJ (1994) Egg science and technology 105–175. *Food Products Press*, an imprint of the Haworth Press, Inc. New York.
- van Boekel MAJS (1981) Estimation of solid liquid ratios in bulk fats and emulsions by pulsed NMR. *Journal of the American Oil Chemists Society*. 58(7): 768–772.
- Walstra P. (2003) *Physical chemistry of foods*. Marcel Dekker. New York.

CHAPTER 3

Influence of ice crystal on destabilization of mayonnaise

3.1 Introduction

Mayonnaise is an oil-in-water (O/W) emulsion, in which oil droplets are dispersed in water phases is thermodynamically unstable system. Many food emulsions (e.g., mayonnaise, sauces, and beverages) are frozen to improve their shelf life (Degner et al., 2013; Magnusson, Rosén, & Nilsson, 2011) or are commercially supplied as frozen foods. However, most O/W emulsions are easily destabilized after freeze-thawing because of the crystallization of fats and water in emulsions (Degner, Chung, Schlegel, Hutkins, & McClements, 2014; Ghosh & Coupland, 2008).

When an O/W emulsion is stored at low temperatures ($< -20\text{ }^{\circ}\text{C}$) its water and oil phase crystallized (Miyagawa et al., 2016). The formation of ice crystals in an emulsion results in the following: flocculation of oil droplets, increase of ion strength, and pH variation in the unfrozen aqueous phase (Thanasukarn, Pongsawatmanit & McClement, 2004). These changes in the emulsion increase droplet-droplet contact. In addition, the ice crystals become larger due to recrystallization during storage, resulting in interfacial membrane disruption (Fioramonti, Arzeni, Pilosof, Rubiolo & Santiago, 2015). Moreover, emulsifiers that adsorb to the droplet interface may be damaged by water crystallization because emulsifiers lose their functionality with dehydration, e.g., cold denaturation of proteins (Davey, Zabik & Dawson, 1969; Xiong, 1997). Redistribution of emulsifiers to oil droplets and ice surfaces removes emulsifiers from the droplet surface, promoting deterioration of the emulsion (Hillgren, Lindgren & Alden, 2002). These aforesaid studies showed the effect of ice crystal on destabilization of O/W emulsion during freezing. However, the influence of ice crystals on the freeze-thaw stability of mayonnaise type emulsion has not been clarified though it bears importance

for investigation.

The purpose of the present study was to analyze destabilization by freeze-thawing, focusing on the influence of the crystallization of ice. In this study, simulated icing has been made with desiccant and coalescence of oil droplets had been observed artificially with dry air. Furthermore, the crystallinity of mayonnaise and water phase has been investigated using anti-frozen polysaccharides (APS) through XRD measurement.

3.2 Materials and Methods

3.2.1 Materials

Mayonnaise sample, mayonnaise oil and water phase was obtained from Oriental Yeast Company, (Tokyo, Japan). Rapeseed oil mayonnaise (RoM) had been used in this study. The oil phase comprised only rapeseed oil and water phase comprised with egg yolk as emulsifier, salt, sugar, acetic acid and vinegar.

3.2.2 Preparation of mayonnaise sample

To prepare 32.6 g of mayonnaise, 22.8 g of the oil phase and 9.8 g of the water phase were used at a ratio of 7:3. The water phase was placed in a 50-ml centrifuge tube then the oil phase was added and mixed in eight stages. Each time one eighth of the oil phase was added then homogenized for 45 s at 25,000 rpm concluding with homogenization for 1 min (McGee, 2004). A Polytron Pt 1200e homogenizer was used for preparing the mayonnaise (Kinematica AG, Lucerne, Switzerland).

3.2.3 Oil droplets coalescence by dry air blowing

For observing oil droplets coalescence, dry air has been blown using a hair dryer. For that 1 g mayonnaise has been taken in a glass petri dish. Then dry air from hair dryer

has been blown directly to the sample. Sample has been dried for 0, 1, 1.5, 2, 2.5 and 3 mins. After drying the sample has been observed using light microscope. For observing oil droplets glass slide with cover slip has been used. The image of oil droplets has been captured and then droplet size has been calculated by image processing using Image J (Image J 1.43u, Java 1.6.0_10 (32-bit), National Institute of Health, Bethesda, MD, USA). The change of droplets number as well as the change in volume of oil droplets has been measured. The circularity of oil droplets has also been considered for confirming deformation and coalescence.

3.2.4 Simulation of ice crystal using desiccant

Simulated icing condition has been created at room temperature using desiccant for kids pamper. For that, 10 g mayonnaise had been taken in 15 ml centrifuge tube. Then desiccant has been mixed at concentration of 0.01, 0.025, 0.05 and 0.075 g/g mayonnaise sample. Desiccant was mixed with mayonnaise using spatula. After mixing the desiccant the samples were stored at room temperature for 1 h. After that the samples were centrifuged for 5 min at 1250×g in a KN-70 table top centrifuge (Kubota Corporation, Tokyo, Japan). The separated oil had been measured through image processing using Image J. The desiccant with absorbed water has been collected and washed with alcohol. The absorbed water has been calculated from the desiccant by keeping the desiccant in oven for overnight.

3.2.5 Destabilization of mayonnaise with limiting water crystal using Anti-frozen Polysaccharides (APS)

Freeze-thaw stability of mayonnaise has been evaluated with anti-frozen polysaccharides (APS) using capillary. The volume of the capillary was 6.6 μ L, with an

inner diameter of 0.34 mm, an outer diameter of 0.86 mm and a length of 72 mm. Anti-frozen polysaccharides (EL1) can lower the water crystallinity that has been provided by Kaneka Corporation, Tokyo, Japan. Three different concentrations (0.05, 0.1 and 0.2% of water phase) of APS have been used. Stability was evaluated at temperature -20 and -40 °C with different time until 95% or more oil of oil phase had been separated. The detailed procedure for mayonnaise preparation and stability evaluation had been discussed in the previous section.

3.2.6 Evaluation of water crystallinity with X-ray Diffraction (XRD) measurement

The crystallinity of water in mayonnaise as well as in water phase has been measured through XRD measurement. For that mayonnaise and water phase with APS of three different concentrations had been used. Aichi synchrotron center, Aichi, Japan had been privileged us for XRD measurement. X-ray diffraction (XRD) (BL5S2 of the Aichi Synchrotron Radiation Center, Aichi, Japan) was used to determine the water crystallinity in mayonnaise and its water phase at temperature -40 °C. The sample was placed in XRD cells (Length: 80 mm, Outer diameter: 0.4 mm and thickness: 0.01 mm, Hilgenberg quartz glass by TOHO, Japan) and cooled from room temperature to -40 °C at a rate 40 °C min⁻¹ with liquid N₂. The temperature was maintained in entire measurement. Data has been acquired at each 3 sec. The peak area of ice crystal has been measured with time using Origin (OriginPro 2016 (64-bit) b9.3.226., Version: 93E, OriginLab Corporation, Northampton, MA 01060 USA).

3.2.7 Statistical analysis

All experiments were repeated four times. Significant difference between means (from replicates) was determined with Tukey's honest significant difference test at a significance level of $P < 0.05$ using Kaleida Graph (Version: 4.1.1, Synergy Software, Reading, PA, USA).

3.3 Results

3.3.1 Coalescence of oil droplets

Coalescence of oil droplets has been studied by blowing dry air over mayonnaise sample. Fig. 3.1 showed the oil droplet status after drying by hot air. The diameter of oil droplets has been shown in Fig. 3.2. The initial median diameter of oil droplets was 1.0971 μm and after drying for 1, 2 and 3 min the median diameter was 1.1647, 1.4497 and 2.090 μm respectively. It observed that with increasing drying time the median diameter of oil droplets increased. It indicated that oil droplets underwent coalescence. This coalescence might derive from limiting water and denaturation of egg yolk for that decreased emulsifying properties decreased. Fig. 3.4 showed the change of circularity of oil droplets with drying time. These results suggest that with drying time the oil droplets undergoes deformation and coalesced.

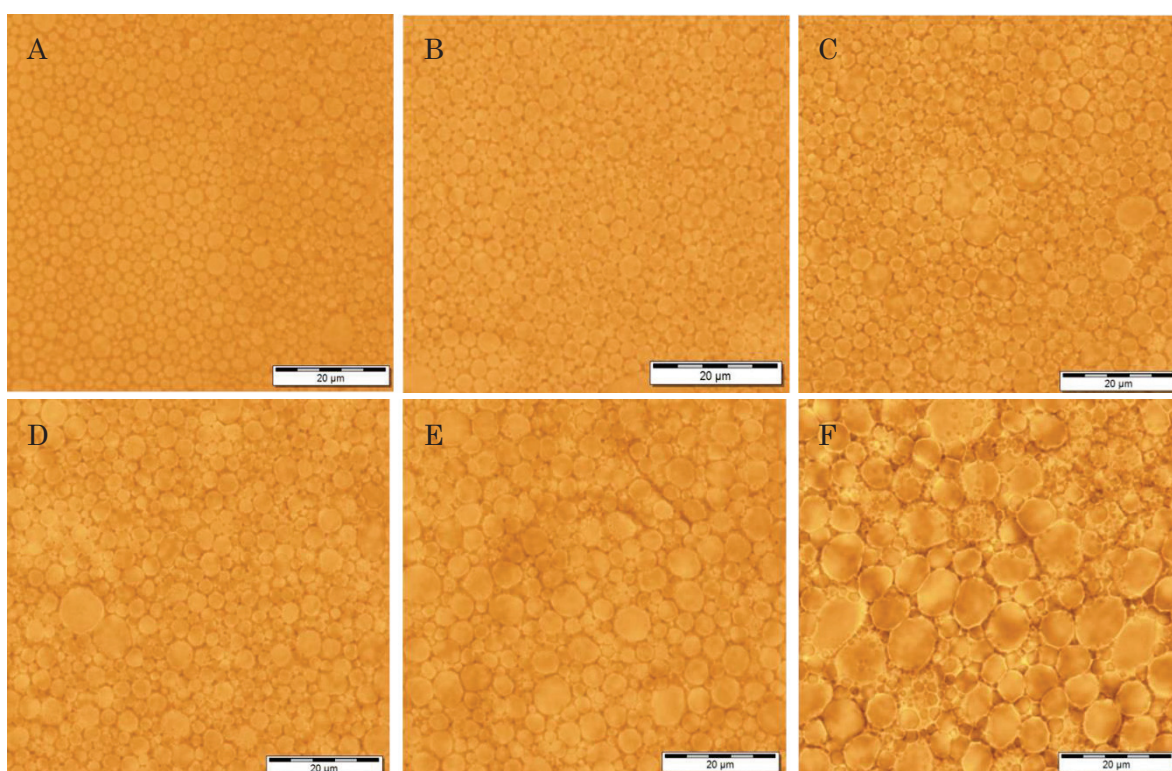


Fig. 3.1 (A-F) showed microscopic image of RoM Oil droplets at (A) 0 min dry (B) 1 min dry (C) 1.5 min dry (D) 2 min dry (E) 2.5 min dry (F) 3 min dry. Scale bar 20 μm .

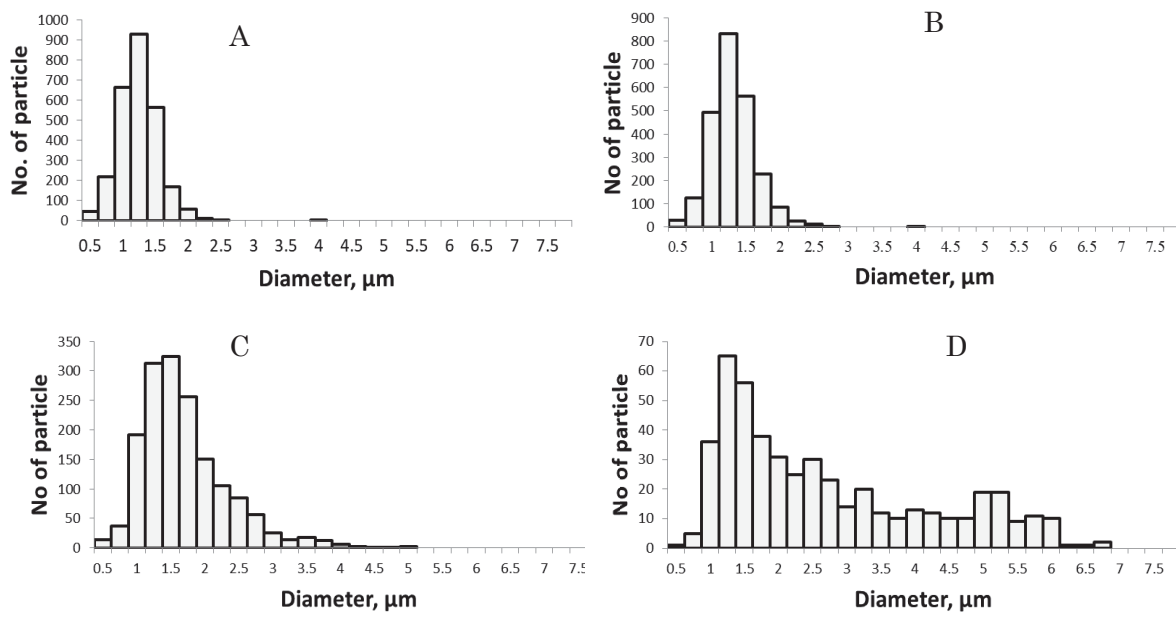


Fig. 3.2 (A-D) Change of oil droplets diameter of RoM at (A) 0 min dry (B) 1 min dry (C) 2 min dry (D) 3 min dry

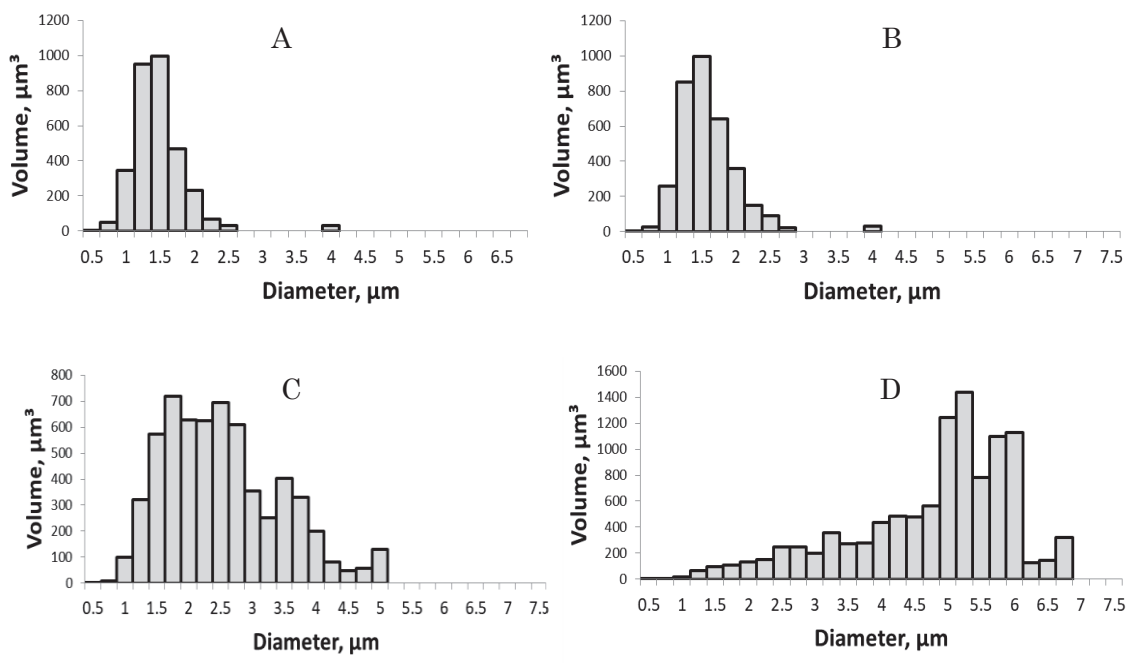


Fig. 3.3 (A-D) Change of oil droplets volume of RoM at (A) 0 min dry (B) 1 min dry (C) 2 min dry (D) 3 min dry

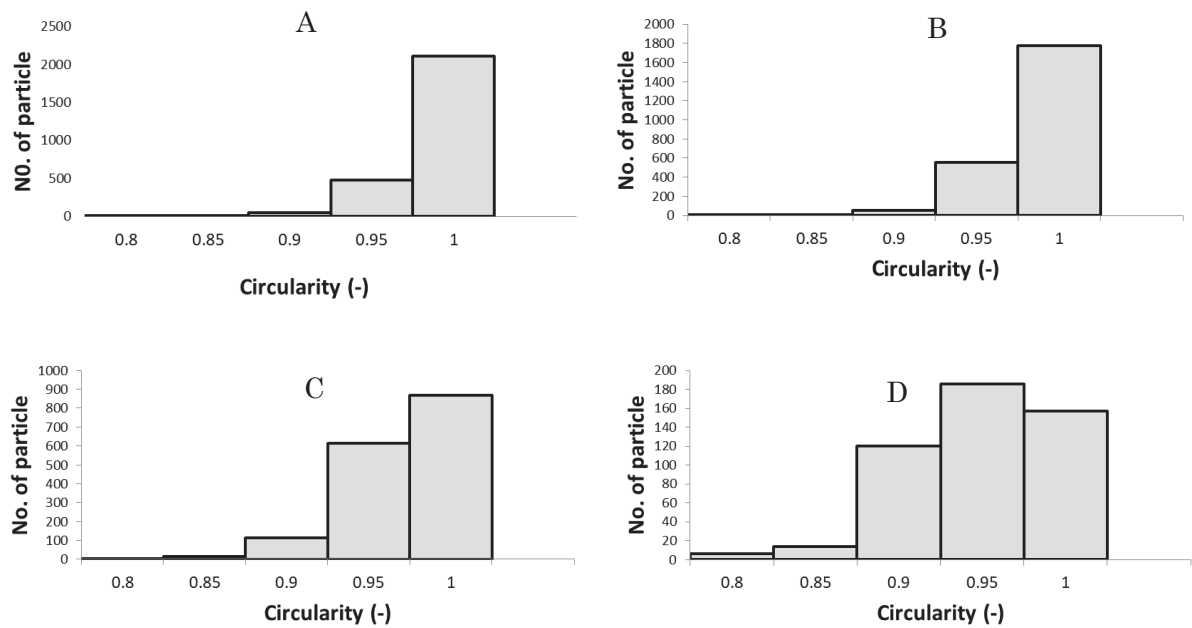


Fig. 3.4 (A-D) Deformation of oil droplets of RoM at (A) 0 min dry (B) 1 min dry (C) 2 min dry (D) 3 min dry

3.3.2 Destabilization by simulated ice crystal

Ice crystal has been simulated using desiccant. Fig 3.5 showed that the increasing amount of absorbed water caused the increased amount of separated oil. The amount of desiccant added into mayonnaise was 0.2, 0.4, 0.6, 0.8 and 1 g/ 10 g mayonnaise. The absorbed water was 0.1304, 0.3234, 0.4768, 0.789 and 0.9143 g respectively. The weight of separated oil by the respective amount of absorbed water was 0.0935, 2.4713, 3.8924, 3.9586 and 5.1197 g respectively. This experiment has been conducted in room temperature. So, the oil phase that separated due to cause of scarcity of water and the extra pressure exerted by the desiccant particle that swelled through absorbing water. The amount of separated oil increased with the increasing amount of absorbed water suggested that the ice crystal during freezing condition brings oil droplets in close proximity. This closeness might result the coalescence of oil droplets. In freezing

condition if both ice and fat crystal generates, that condition would be very prone for coalescence.

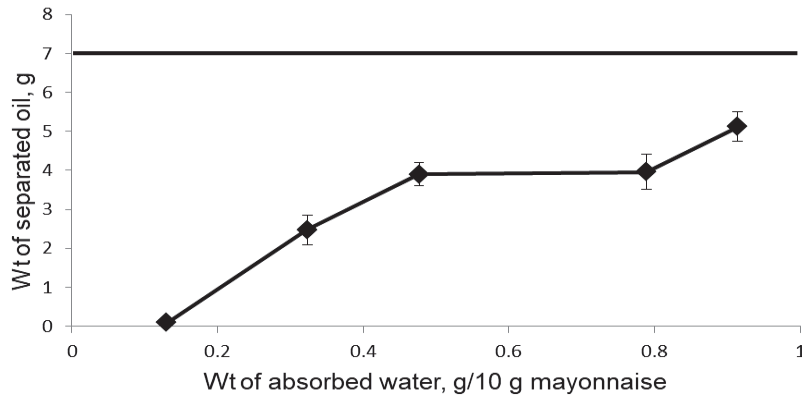


Fig. 3.5 Weights of absorbed water and resulted separated oil of RoM. The line indicates the total oil content.

3.3.3 Evaluation of water crystallinity with X-ray Diffraction (XRD)

Fig. 3.6 and 3.7 showed the XRD pattern of water phase and mayonnaise respectively. The sharp diffraction peaks of d-spacing 3.9, 3.7 and 3.5 Å ($\lambda = d \sin 2\theta, \lambda = 1.0 \text{ \AA}$) were found in both water and oil phase and represent as water crystal and the diffraction peaks of d-spacing 4.2, 4.4 and 4.5 Å represents as fat crystal. The Figures also showed that the diffraction peak increased with time. The growth of peak area decreased with APS concentrations.

From Fig. 3.8 it was observed that the crystallinity (crystal amount) was higher in control samples than APS containing samples. The crystallinity (crystal amount) has been considered as the peak area of ice crystal. In Fig. 3.8 (A) showed the peak area of mayonnaise and (B) showed the peak area of water phases. The crystallinity of mayonnaise sample decreased with increasing APS concentration except 0.2% but in water phases the crystallinity decreased with increasing APS concentration. These crystallinity differences might cause the stability difference during freezing with

capillary.

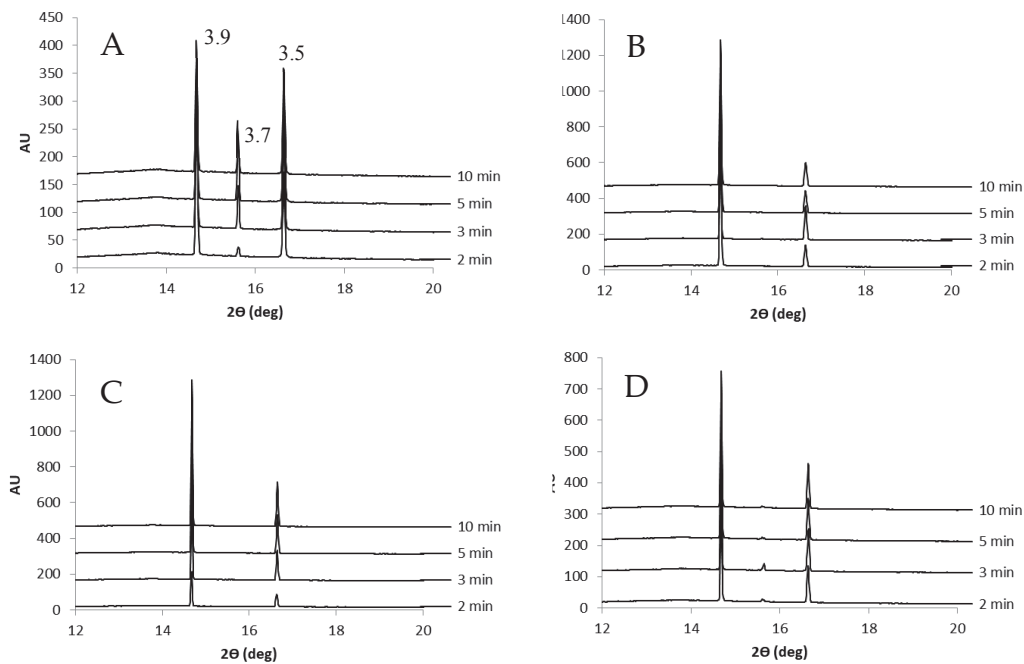


Fig. 3.6 XRD pattern of water phase at temperature -40 °C at (A) control, (B) 0.05% (C) 0.1% and (D) 0.2% APS. D-spacing 3.9, 3.7 and 3.5 Å corresponds to ice crystal.

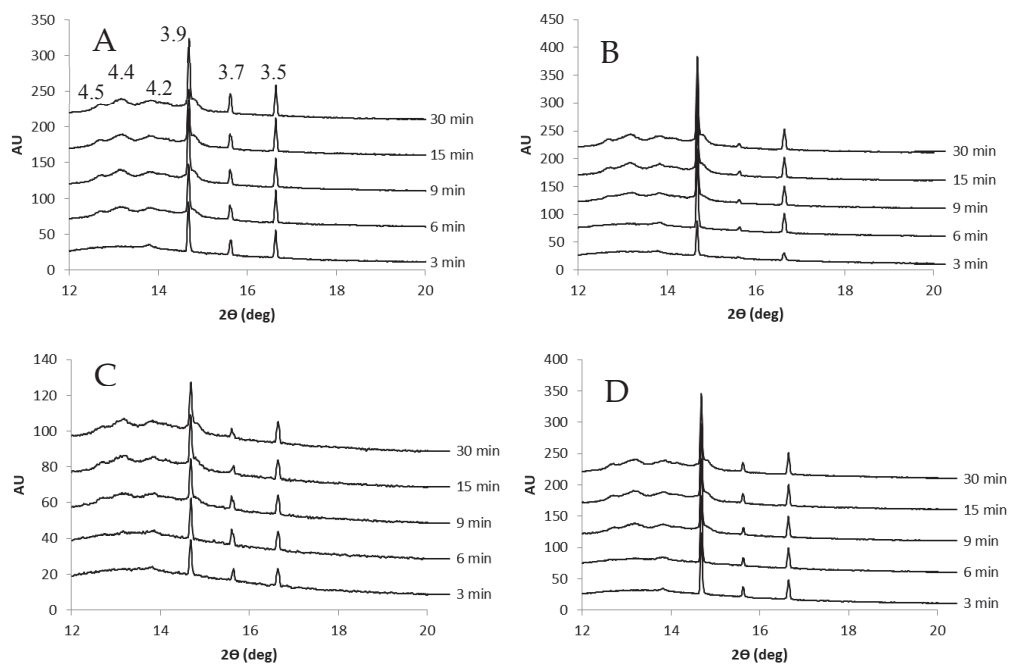


Fig. 3.7 XRD pattern of mayonnaise at temperature -40 °C at (A) control, (B) 0.05% (C) 0.1% and (D) 0.2% APS. D-spacing 4.5, 4.4 and 4.2 Å corresponds to fat crystal.

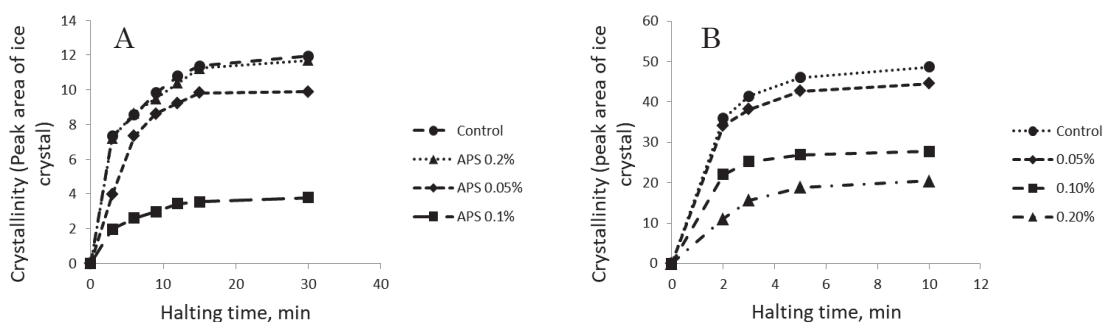


Fig. 3.8 Water phase crystallinity with Anti-frozen Polysaccharides (APS) at temperature -40 °C in (A) mayonnaise and (B) water phase.

3.3.4 Destabilization rate of mayonnaise with limiting water crystal using APS

Fig. 3.9 showed that the stability differed with APS concentration. With increasing APS concentration the stability increased in both -20 and -40 °C. Though, all samples are totally separated after a certain time. The control samples showed lower stability than APS containing samples. All samples contained same oil phase (rapeseed oil) the difference in water phase. From these results, it can be assumed that the stability difference is derived from APS. To clarify the stability difference the crystallinity of APS containing samples XRD measurement has been conducted to evaluate the crystallinity of water. APS increased induction time and lowered destabilization rate constant, k_d (details in page 41) that has been showed in Table 3.1.

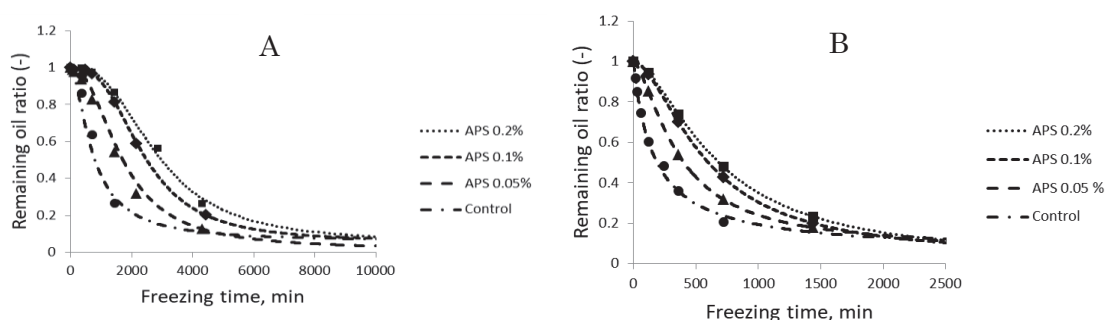


Fig. 3.9 Freeze-thaw stability of mayonnaise with Anti-frozen Polysaccharides (APS) with a concentration of 0.05, 0.1 and 0.2 % at temperature (A) -20 °C and (B) -40 °C

Table 3.1 Induction time and destabilization rate constant of mayonnaise with APS

APS concentration	Induction time, min		Destabilization rate constant, k_d min ⁻¹	
	At -20°C	At -40°C	At -20°C	At -40°C
Control	123	8	0.00117	0.00632
0.05%	446	53	0.00039	0.00211
0.1%	844	104	0.00025	0.00116
0.2%	946	113	0.00020	0.00099

3.4 Discussion

The influence of ice crystal on coalescence has been studied. Fig. 3.1 showed that scarcity of water can cause coalescence. But it might be due to lowering the emulsifying properties of emulsifier or making close proximity of oil droplets that favored coalescence. Scarcity of water causes deformation and coalescence of oil droplets. Due to lowering of water the interfacial layer between two oil droplets become thinner that can easily ruptured for coalescence. To avoid the losing of emulsifier through denaturation of emulsifier we did simulation of ice crystal using desiccant. The desiccant experiment in room temperature suggested that the volumetric expansion of ice crystal can cause scarcity of water as well as create pressure that bring the oil droplets close proximity resulted deformation of oil droplets that favor further coalescence. The deformation and coalescence of oil droplets further agreed by desiccant experiment results showing separated oil increased with increasing desiccant concentration. Moreover, experimental results with anti-frozen polysaccharides showed that the stability increased with increasing APS concentrations (Fig. 3.9). The crystallinity of APS containing samples had been evaluated through XRD measurement. XRD measurement with mayonnaise and water phase showed that the crystallinity decreased with increasing APS concentrations in water phase in Fig. 3.8. The decreasing

crystallinity with increasing APS concentration might cause the stability difference with APS containing samples. The above experimental results explained the influence of ice crystal on destabilization of mayonnaise during freezing condition. It influenced by making the oil droplets close proximity through deformation. Furthermore, due to volumetric expansion might exert pressure that favors coalescence with crystallized oil droplets.

3.5 Conclusion

This research studied the influence of ice crystal of coalescence of oil droplets during freezing. Ice crystal influences the coalescence by lowering the distance of oil droplets. Generation of ice crystal lowers the water content in water phase that make deformation and close proximity of oil droplets. Though mayonnaise type oil in water emulsion contains lower amount of water than oil phase but it has significant effect on stability during freeze storage.

References

- Boode, K., Bisperink, C., & Walstra, P. (1991). Destabilization of O/W emulsions containing fat crystals by temperature cycling. *Colloids and Surfaces*, 61: 55-74.
- Davey, E. M., Zabik, M. E., & Dawson, L. E. (1969). Fresh and frozen egg yolk protein fractions: Emulsion stabilizing power, viscosity and electrographic patterns. *Poultry Science*. 48(1): 251-260.
- Degner, B. M., Chung, C., Schlegel, V., Hutkins, R., & McClements, D. J. (2014). Factors influencing the freeze-thaw stability of emulsion based foods. *Comprehensive Reviews in Food Science and Food Safety*. 13: 98-113.
- Degner, B. M., Olson, K. M., Rose, D., Schlegel, V., Hutkins, R., & McClements, D. J.

- (2013). Influence of freezing rate on the microstructure and physicochemical properties of food emulsions. *Journal of Food Engineering*, 119(2): 244-253.
- Fioramonti, S. A., Arzeni, C., Pilosof, A. M. R., Rubiolo, A. C., & Santiago, L. G. (2015). Influence of freezing temperature and maltodextrin concentration on stability of linseed oil-in-water multilayer emulsions. *Journal of Food Engineering*, 156: 31-38.
- Ghosh, S., & Coupland, J. N., (2008). Factors affecting the freeze-thaw stability of emulsions. *Food Hydrocolloids*, 22: 105-111.
- Hillgren, A., Lindgren, J., & Alden, M. (2002). Protection mechanism of Tween 80 during freeze-thawing of a model protein, LDH. *International Journal of Pharmaceutics*, 237: 57-69.
- Ishibashi, C., Hondoh, H., and Ueno, S. (2016). Influence of morphology and polymorphic transformation of fat crystal on the freeze thaw stability of mayonnaise type oil in water emulsion. *Food Research International*, 89: 604-613.
- Magnusson, E., Rosen, C., & Nilsson, L. (2011). Freeze-thaw stability of mayonnaise type oil-in-water emulsions. *Food hydrocolloids*, 25: 707-715.
- McGee H (2004) *On food and cooking – The science and lore of the kitchen*, Macmillan Library Reference USA, Inc., pp. 633-4. Scribner. NewYork.
- Miyagawa, Y., Ogawa, T., Nakagawa, K. & Adachi, S. (2016). Destabilization of mayonnaise induced by lipid crystallization upon freezing. *Bioscience, Biotechnology, and Biochemistry*, 80(4):786-90.
- Palanuwech, J., & Coupland, J. N. (2003). Effect of surfactant type on the stability of oil-in-water emulsions to dispersed phase crystallization. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 223: 251-262.
- Rousseau, D. (2000). Fat crystals and emulsion stability- A review. *Food Research*

International, 33: 3-14.

Thanasukarn, P., Pongsawatmanit, R., & McClements, D. J. (2004). Impact of fat and water crystallization on the stability of hydrogenated palm oil-in-water emulsions stabilized by whey protein isolate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 246: 49-59.

Vanapalli, S. A., Palanuwech, J., & Coupland, J. N. (2002a). Influence of fat crystallization on the stability of flocculated emulsions. *Journal of Agricultural and Food Chemistry*, 50: 5224-5228.

Vanapalli, S. A., Palanuwech, J., & Coupland, J.N. (2002b). Stability of emulsions to dispersed phase crystallization: Effect of oil type, dispersed phase volume fraction, and cooling rate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 204: 227-237.

Xiong, Y. L. (1997). Protein denaturation and functionality losses. In M. C. Erickson & Y. C. Huang (Eds.), *Quality in frozen foods*. Pp: 111-139. New York: Chapman & Hall.

CHAPTER 4

Destabilization Kinetics of Mayonnaise during Freeze-thawing

4.1 Introduction

Mayonnaise is a semi solid oil in water emulsion which is composed of vegetable oil as oil phase and acidifying ingredient (vinegar), egg yolk (emulsifying agent), salt, flavor, sweetening as water phase (Depree & Savage, 2001). Commercial producers use more than 70 % oil phase and 30 % water phase. Like many others food emulsions it needs to freeze storage with other food gradients. Mayonnaise for being an oil - water emulsion, it is unavoidably destabilize due to thermal stress generates during freezing (Miyagawa et al. 2016). This problem associated with freeze storage with other foods. The destabilization of oil in water emulsion is usually occurred through creaming, coalescence or partial coalescence of oil droplets, separation of oil and water, inversion of oil in water emulsion to water in oil emulsion, etc (Rousseau, 2000; Thanasukarn et al. 2004). If an oil in water emulsion is stored at low temperature ($< 20\text{ }^{\circ}\text{C}$), it's oil and water phase crystalize. This change in water and oil phase leading to destabilization (Miyagawa et al. 2016). Ishibashi et al. (2016) reported that the polymorphic change of fat crystal changes the mechanism of destabilization. But the fat crystal growth have also influence on destabilization that may favor for coalescence by increasing the effective collision among droplets. However, the effect of fat crystal on destabilization is still unclear. To understand the influence of crystallization on destabilization, kinetic analysis would be an effective tool. From this viewpoint, one important consideration is the rate at which the destabilization happens with temperature. The knowledge of rate of destabilization of emulsion would help to take some relevant economic decisions (Krebs et al. 2012).

A rate equation typical of first order has been proposed by Civan et al. (2004) considering destabilization of emulsion is the process of irreversible single reaction. The kinetics of the decomposition was therefore described as a power law function of the instantaneous emulsion fraction available for destabilization.

Another important consideration would be experimental method. Usually the researchers rated destabilization through separated oil using bulky sample (Cornacchia & Roos, 2011; Magnusson et al. 2011; Ghosh & Coupland, 2008). But bulky sample brings size effect for that, the data would not be reliable for kinetic analysis. For that a typical method to analyze kinetic parameters has been approached using capillary.

No efforts have been reported to analyze kinetic parameters with mayonnaise. Therefore, the objective of this research is to analyze the kinetic parameters of mayonnaise during freeze-thawing. For that, the destabilization rate constant, the order of destabilization, half-life and induction time had been calculated through measuring separated oil and a model had been introduced to evaluate stability with temperature.

4.2 Materials and methods

4.2.1 Materials

The ingredients of the oil and water phases for the mayonnaise were obtained from the Oriental Yeast Company (Tokyo, Japan). Two types of mayonnaise were prepared, rapeseed oil mayonnaise (RoM) and soybean oil mayonnaise (SoM). The oil phase consisted of only rapeseed oil or soybean oil and the water phase consisted of egg yolk as the emulsifier, salt, sugar, water and vinegar. Table 4.1 shows fatty acid composition of oil phases respectively. The principal fatty acid in rapeseed oil was oleic acid (18 : 1) and in soybean oil it was linoleic acid (18 : 2).

Table 4.1 Fatty acid composition of oil phases

Oil	Fatty acid %		
	Oleic acid (18 : 1)	Linoleic acid (18 : 2)	Linolenic acid (18 : 3)
Rapeseed	72	13	4
Soybean	18	65	2

4.2.2 Preparation of mayonnaise sample

To prepare 32.6 g of mayonnaise, 22.8 g of the oil phase and 9.8 g of the water phase were used at a ratio of 7:3. The water phase was placed in a 50-ml centrifuge tube then the oil phase was added and mixed in eight stages. Each time one eighth of the oil phase was added then homogenized for 45 s at 25,000 rpm concluding with homogenization for 1 min (McGee, 2004). A Polytron Pt 1200e homogenizer was used for preparing the mayonnaise (Kinematica AG, Lucerne, Switzerland).

4.2.3 Selection of tube for freeze-thaw stability test

For kinetic analysis it was important consideration that the size of sample may affect the temperature and time dependent separation of oil during freeze-thawing. Use of bulky sample may change the separation ratio. Five different glass tubes have been selected for freeze-thaw stability test. The inner diameter of the tube was 0.34, 0.8, 1.8, 3.4 and 12.6 mm. The kinetic parameters as well as the induction time have been calculated using these tubes. All the glass tubes had been supplied by Rikaken corporation, Japan. Oil separation test had been performed by pouring mayonnaise with different temperature and time.

4.2.4 Freeze-thaw stability of mayonnaise

Freeze-thaw stability of mayonnaise was tested using selected tubes. The sample had

been poured using suction pump. To seal the opening of tube Araldite adhesive had been used. Tube with samples directly chilled through hanging in chilling liquid with different temperature ranging from -20 to -40 °C. Data had been collected with time until maximum achievable fraction of oil phase had reached. After chilling, the samples were kept in hot water bath at 60 °C for 5 min then centrifuged for 5 min at 1250×g in a KN-70 table top centrifuge (Kubota Corporation, Tokyo, Japan). With centrifugation the separated oil form a distinct layer in tube. After taking the picture of tube, the weight of separated oil had been calculated using Image J software (Image J 1.43u, Java 1.6.0_10 (32-bit), National Institute of Health, Bethesda, MD, USA). The camera had been used for capturing picture was Canon speed lite 90EX.

4.2.5 Theoretical consideration for destabilization kinetic analysis

According to Civan et al. (2004) the destabilization rate can be written as follows:

$$-\frac{dS_m}{dt} = -R_D = k_d S_m^n \dots\dots\dots(1)$$

Where S_m is remaining oil fraction of mayonnaise, R_D is destabilization rate, k_d is destabilization rate constant, n is the order of destabilization and t is time.

Taking into account that, the initiation of mayonnaise destabilization during freezing may be delayed by a certain time, t_i and stability at that time is S_i , so the initial condition can be defined by

$$S_m = S_i, \quad t = t_i \dots\dots\dots(2)$$

Therefore, the analytical solution of equation (1) at isothermal condition subject to equation (2) it can be obtained as

$$S_m^{1-n} = S_i^{1-n} - (1-n)k_d(t - t_i) \dots\dots\dots(3)$$

Determination of destabilization rate constant, k_d and the order of destabilization, n could be sought using differential method of analysis from the fitted graph using

equation (3) (Levenspiel, 1999). Moreover, the half-life of the mayonnaise based on the maximum decomposable fraction can be determined by substituting $S_m = 0.5S_i$ in equation (3). So the half-life can be written as

$$(t - t_i)_{.5} = \frac{S_i^{1-n} - (0.5S_i)^{1-n}}{(1-n)k_d} \dots\dots\dots(4)$$

Further assumption had been taken that the destabilization rate constant k_d is a function of temperature, T and there would be an empirical relationship between destabilization rate constants with temperature. It is mentionable that, usually in chemical kinetics the rate constant increased with increasing temperature but in case of mayonnaise destabilization the phenomena is opposite as destabilization strongly depends on crystallization of oil and water phase. Hence

$$k_d = f(T) \dots\dots\dots(5)$$

Thus, the kinetic model in equation (3) can be written by following which can be used to evaluate the stability of mayonnaise at different temperature.

$$S_m^{1-n} = S_i^{1-n} - (1 - n)f(T)(t - t_i) \dots\dots\dots(6)$$

4.2.6 Statistical analysis

All experiments were repeated four times. Significant difference between means (from replicates) was determined with Tukey’s honest significant difference test at a significance level of $P < 0.05$ using Kaleida Graph (Synergy Software, Reading, PA, USA).

4.3 Results

4.3.1 Influence of sample size on induction time and destabilization rate constant, k_d

Fig. 4.1 and Fig. 4.2 showed the induction time and destabilization rate constant, k_d

with different size of tube with rapeseed oil mayonnaise. It showed that the induction time increased with increasing the diameter of tube used. The different diameter of tubes contained the different amount of sample. Bulky sample take longer time for heat transmission results every part of the sample took longer time to receive the temperature. The shortest induction time 0.9 min was found at -40 °C in capillary (inner diameter 0.34 mm) where as in 12.6 mm tube the induction time was 16 min. The induction time using capillary at -20 °C was 251 min. On the other hand the induction time using 12.6 mm tube at -20 °C was longest and it was 1420 min. The induction time found increased with increasing diameter of the tube at all temperature observed. This result suggested that the induction time calculated using capillary was more reliable than other bulky tube as capillary can overcome the bulk effect.

From Fig. 4.2 the destabilization rate constant, k_d found decreased with increasing diameter of the tube used. The destabilization rate constant, k_d also affected by the size of sample. Bulky samples took longer time to receive temperature resulted slower separation rate than capillary. The k_d value using capillary at -40 °C was 0.04899 min^{-1} whereas in 12.6 mm tube it was 0.01195 min^{-1} that was four times higher. The k_d value at -20 °C using capillary was 0.0012 min^{-1} and using 12.6 mm tube it was 0.000147 min^{-1} . These results also suggested that the kinetic parameters using capillary influenced by the size of the sample. The kinetic parameter obtained when the tube diameter is zero is an intrinsic parameter without size effect. The induction time and k_d determined using capillary were closed to the intercept values are more reliable than other bulky tube.

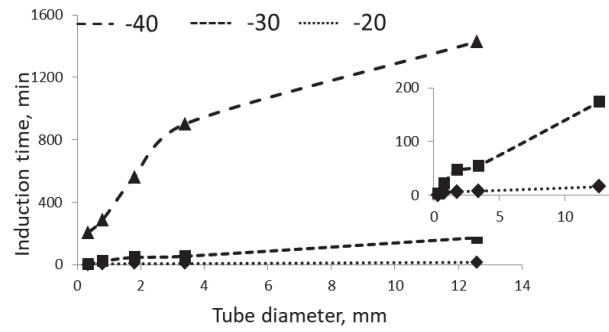


Fig. 4.1 Induction times with different tubes at -40, -30 and -20 °C. Inserts presents the magnified graph of -20 and -30 °C

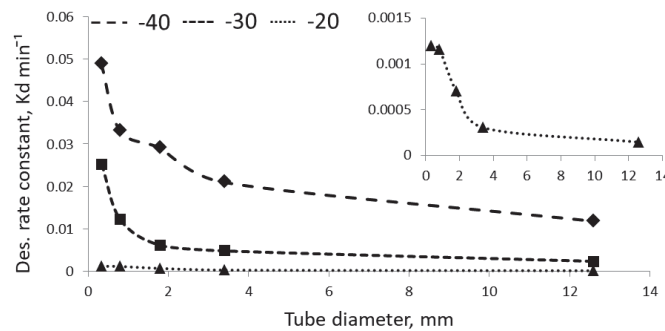


Fig. 4.2 Destabilization rate constant k_d with different tubes at -40, -30 and -20 °C. Inserts presents the magnified graph of at -20 °C

4.3.2 Freeze-thaw stability of mayonnaise using capillary

The remaining oil after freezing with different temperature and time differ significantly that presented in Fig. 4.3 (A) and (B). The graph showed a typical sigmoidal pattern, firstly induction time and then sudden increase the speed of separation and lastly separation slowed down. When the remaining oil ratio was 0.95, that point has been considered as induction time. The induction time of both RoM and SoM has been shown in Table 4.2. The longest induction time at -20 °C for SoM and it was 633.33 min and the shortest one 0.9 min was at -40 °C for RoM and chilling temperature have

significant effect on induction time. This result also agreed with the result of Rousseau, 2000; Ishibashi et al. 2016. While considering the time for kinetic measurement induction time has been excluded.

After induction time the speed of oil separation increased. The speed of separation was differed from RoM to SoM. The time needed to reach the maximum separated fraction was lower in RoM than SoM and it followed the decreasing order of temperature from -20 °C to -40 °C. Mayonnaise sample with -20 °C took longer time to reach its maximum separated fraction. These have been attributed from the crystallization rate of both RoM and SoM (Miyagawa et al. 2016). The stability data has been used to calculate kinetic parameters excluding induction time.

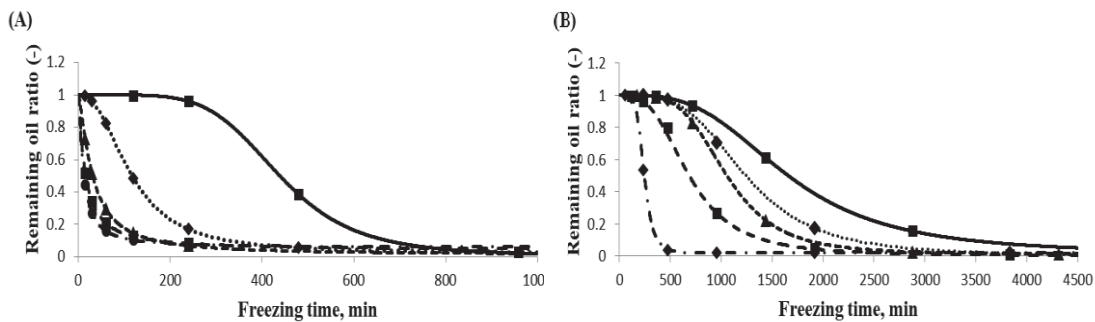


Fig. 4.3 The stability of mayonnaise showed remaining oil ratio versus time after fitting the data. (A) RoM and (B) SoM for freezing at temperatures of -20 °C (—), -25 °C (.....), -30 °C (---), -35 °C (- · -), -40 °C (· · ·). Points on the fitted curves indicate the experimental data. Results are expressed as the mean of four trials.

4.3.3 Destabilization kinetic parameters with different temperature

Table 4.3 presented the values of kinetic parameters at different temperatures for RoM and SoM respectively. It was observed that the destabilization rate constant k_d had minimum value at -20 °C and the maximum value at -40 °C. The k_d value of RoM found higher than SoM in respective each temperature and found increasing with

decreasing temperature. Therefore, providing particle size and other experimental condition are same in the present study, it is suffice to conclude that the major physical condition causing variation in the destabilization rate constant was the temperature. The order of destabilization n however did not follow any specific pattern with respect of temperature in both RoM and SoM. The value ranged from 0.0524 to 1.75356 for RoM and 0.1103 to 1.3462 for SoM. The destabilization rate constant found strongly dependent with temperature.

Table 4.2 Induction time of oil separation for RoM and SoM

Temperature, °C	Induction time, min	
	RoM	SoM
-20	251.9 ^a	633.3 ^a
-25	33.2 ^b	578.4 ^b
-30	3.4 ^c	516.7 ^c
-35	1.5 ^c	267.3 ^d
-40	0.9 ^c	140.5 ^e

Results are expressed as the mean of four trials.

^{a, b, c, d} Mean values with superscripts containing different letters in the same column are significantly different ($p < 0.05$).

Table 4.3 Variation of destabilization kinetic parameters with freezing temperature for RoM and SoM

Temperature, °C	RoM		SoM	
	k_d , min ⁻¹	n	k_d , min ⁻¹	n
-20	1.78×10^{-5}	0.05	1.95×10^{-6}	0.43
-25	6.40×10^{-5}	0.14	4.83×10^{-5}	0.54
-30	4.28×10^{-4}	1.37	8.59×10^{-5}	1.35
-35	8.82×10^{-4}	1.75	3.37×10^{-4}	0.11
-40	1.28×10^{-3}	1.25	7.89×10^{-4}	0.76

Results are expressed as the mean of four trials.

RoM, Rapeseed oil mayonnaise; SoM, Soybean oil mayonnaise; k_d , Destabilization rate constant; n , Order of destabilization

4.4 Discussion

Capillary tube has privileged to get reliable information about induction time as well as kinetic parameters calculated in this research. The induction time has been calculated using different diameter sized tube and found that with increasing the diameter of the tube, induction time increased, showed in Fig. 4.1. The differences in induction time due to the variation of tube come from the size effect. Bulky tube took longer time to receive heat on the other hand the capillary tube can receive heat instantly so, induction time calculated using capillary mostly reflects the properties of the sample. In lower temperature the induction time found lower than that of higher temperature that closely related with fat crystal generation time (Miyagawa *et al.*, 2016). At lower temperature, supersaturation is higher and more nucleation happened that accelerate crystallization. For that, induction time found lower at lower temperature and higher at higher temperature.

The destabilization kinetics of mayonnaise was analyzed at different temperature. The RoM destabilized faster at -40 °C showing highest destabilization rate constant, k_d and SoM kept at -20 °C showed higher stability with lowest destabilization rate constant Table 4.3. The destabilization rate constant was higher in RoM than SoM at each temperature observed and it increased with decreasing temperature. The increasing tendency of destabilization rate constant with decreasing temperature was mostly influenced by crystallization of fat.

The amount of fat crystal depends on the fatty acid composition of rapeseed oil and soybean oil and rapeseed oil contains lower amount of saturated fat than soybean oil. The rapeseed oil is mainly composed of oleic acid whereas soybean oil is linoleic acid dominant oil (Chow, 1992). The melting point of oleic acid is higher than that of linoleic acid. These compositional variations resulted the higher amount of fat crystal in

RoM than SoM that further caused higher k_d in RoM than SoM at each temperature. Magnusson *et al.*, 2011 reported that the high content of oleic acid was negative for the freeze-thaw stability of a mayonnaise type O/W emulsion.

The destabilization order n did not follow any specific pattern that means the mechanism of coalescence differ from each temperature. Recently Ishibashi *et al.*, 2016 reported that canola and soybean oil mayonnaise destabilize differently and the mechanism of coalescence also differ from -15, -20 and -30 °C due to morphological change of fat crystal. Though we don't have direct clear evidence of this explanation but Ishibashi *et al.*, 2016 showed the change of crystal morphology and polymorphic change with XRD. Kaufmann *et al.*, 2013; Kalnin, 2009; Silva *et al.*, 2009 also reported the change of fat crystal form and structure with temperature change.

4.5 Conclusion

The methods found suitable for evaluating the destabilization kinetics through power law function. The destabilization rate constant k_d found increased with decreasing temperature showing temperature dependency. The order of destabilization n varied with temperature showing the mechanism of destabilization.

References

- Chow. C.K. (1992). Fatty acids in foods and their health implications. New York: Marcel Dekker.
- Civan, F., L.J. Alarcon, and S.E. Campbell. (2004) Laboratory Confirmation of New Emulsion Stability Model, Journal of Petroleum Science and Engineering, 43: 25-34.
- Cornacchia, L. and Roos, Y. H. (2011) Lipid and water crystallization in

- protein-stabilised oil-in-water emulsions. *Food Hydrocolloids*, 25: 1726-1736.
- Depree, J.A. and Savage, G.P. (2001) Physical and Flavour Stability of Mayonnaise. *Trends in Food Science and Technology*. 12: 157-163.
- Ghosh, S. and Coupland, J.N. (2008). Factors affecting the freeze-thaw stability of emulsion. *Food Hydrocolloids* 22 : 105-111.
- Ishibashi, C., Hondoh, H., and Ueno, S. (2016). Influence of morphology and polymorphic transformation of fat crystal on the freeze thaw stability of mayonnaise type oil in water emulsion. *Food Research International*, 89: 604-613.
- Kalnin, D.J.E. (2009). Characterization of lipid nanostructures using DSC coupled to SAXS and WAXS. *JEEP*, 00017 : 01-05.
- Kaufmann, N. and Kirkensgaard, J.J.K., Andersen, U., and Wiking, L. (2013). Shear and rapeseed oil addition affect the crystal polymorphic behavior of milk fat. *J. Am. Oil Chem. Soc.* 90(6) : 871-880.
- Krebs, T., Schroen, C. G. P. H., Boom, R. M. (2012). Separation kinetics of an oil - in - water emulsion under enhanced gravity. *Chemical Engineering Science*. 71: 118-125.
- Levenspiel, O. (1999). *Chemical reaction engineering* (3rd ed.). New York, NY : Wiley.
- Magnusson, E., Rosen, C and Nilsson, L. (2011). Freeze-thaw stability of mayonnaise type oil-in-water emulsions. *Food Hydrocolloids* 25 : 707-715.
- McGee H (2004) *On food and cooking – The science and lore of the kitchen*, Macmillan Library Reference USA, Inc., pp. 633-4. Scribner. New York.
- Miyagawa, Y., Ogawa, T., Nakagawa, K. & Adachi, S. (2016). Destabilization of mayonnaise induced by lipid crystallization upon freezing. *Bioscience, Biotechnology, and Biochemistry*, 80(4):786-90.
- Rousseau, D. (2000). Fat crystals and emulsion stability – a review. *Food Research*

International, 33: 3-14.

Silva, J.C., Plivelic, T.S., Herreraa, M.L. and Ruscheinsky, N. (2009). Polymorphic phases of natural fat from Cupuassu (*Theobroma grandiflorum*) beans : A WAXS/SAXS/DSC study. *Crystal Growth Design*. 9 : 5155-5163.

Thanasukarn, P., Pongsawatmanit, R. and McClements, D.J. (2004) Influence of Emulsifier Type on Freeze-thaw Stability of Hydrogenated Palm Oil-in-water Emulsions, *Food Hydrocolloids*. 18: 1033-1043.

CHAPTER 5

Iso-thermal crystallization kinetics of frozen mayonnaise

5.1 Introduction

Mayonnaise is semi-solid oil in water emulsion. As oil in water emulsion, it is thermodynamically unstable system and sensitive to environmental stress, as for example cooling and freezing (Guzey & McClements, 2006). This un-stability problem associated with the freeze storage with other foods. When mayonnaise is kept in freezing condition, crystallization of both oil and water phase may occur. These phase transition may lead to destabilization of mayonnaise. A destabilization mechanism of oil in water emulsion caused by oil phase crystallization is partial coalescence (Palanuwech & Coupland, 2003). The mechanism of mayonnaise destabilization is also partial coalescence. If semi-crystallized oil droplets come in close contact, crystals from one droplet may penetrate the interface to the other. The residual liquid oil in the droplets then flow out and wet the solid fat, thereby maintaining the linkage between the droplets, and when the oil melts true coalescence will occur (Vanapalli & Coupland, 2001).

The amount of solid fat formed in the droplets, as well as the size and morphology of the crystals, may have a great impact on the destabilization (Boode, Walstra, & Degrootmostert, 1993). These factors are in turn affected by the triglyceride composition (Boode et al., 1993), additives to the oil (Kalnin, Schafer, Amenitsch, & Ollivon, 2004) and the freezing conditions, such as the cooling rate and temperature (Tippetts & Martini, 2009). A slow cooling rate generally gives larger crystals than a fast cooling rate. The crystals formed on fast cooling are rigid but unstable, whereas slowly cooled fat generally gives more stable crystals (Campos, Narine, &

Marangoni, 2002).

Several reports on non-isothermal crystallization have been discussed in the previous section. But mayonnaise as well as other food need to storage in isothermal condition. For that, the crystallization, especially the rate of crystal growth is important for freeze storage. Crystallization kinetic analysis of mayonnaise at isothermal condition can express the crystal growth. Recently, Ishibashi et al., 2016 reported the polymorphic change of fat crystal changed the mechanism of coalescence but the polymorphic change of fat crystal have no influence on the stability of mayonnaise during freeze storage. Miyagawa et al., 2016 also reported the volumetric expansion of fat crystal cause destabilization of mayonnaise during freeze-storage and is an important cause of mayonnaise destabilization. Therefore, the purpose of this study was to kinetic analysis of fat crystal in isothermal storage.

5.2 Materials and methods

5.2.1 Materials

Mayonnaise sample, mayonnaise oil and water phase was obtained from Oriental Yeast Company, Japan. Two types of mayonnaise like rapeseed oil mayonnaise (RoM) and soybean oil mayonnaise (SoM) had been used. The oil phase comprised only rapeseed oil or soybean oil and water phase comprised with egg yolk as emulsifier, salt, sugar, acetic acid and vinegar.

5.2.2 Preparation of mayonnaise sample

To prepare 32.6 g of mayonnaise, 22.8 g of the oil phase and 9.8 g of the water phase were used at a ratio of 7:3. The water phase was placed in a 50-ml centrifuge tube then

the oil phase was added and mixed in eight stages. Each time one eighth of the oil phase was added then homogenized for 45 s at 25,000 rpm concluding with homogenization for 1 min (McGee, 2004). A Polytron Pt 1200e homogenizer was used for preparing the mayonnaise (Kinematica AG, Lucerne, Switzerland).

5.2.3 The model for crystallization kinetic analysis

The Avrami equation had been used to quantify crystallization kinetics (Meng *et al.* 2014). According to that model:

$$1 - X_t = \exp(-K_c t^n) \dots \dots \dots (7)$$

Where, X_t is the relative degree of crystallinity at time t , K_c is crystallization rate constant, that depends primarily on temperature and n is the Avrami exponent. The analytical solution of the equation (7) would be:

$$\ln[-\ln(1 - X_t)] = n \ln t + \ln K_c \dots \dots \dots (8)$$

So, plotting $\ln[-\ln(1 - X_t)]$ vs $\ln t$ can give a straight line and the value of K_c and n can be obtained from the intercept and slope respectively.

5.2.4 Polarized light microscopy for observing fat crystal

To calculate crystallization kinetic parameters through observing fat crystal in mayonnaise sample during storage at -20, -30 and -40 °C, polarized light microscope has been used. The temperatures of the samples were controlled using LINKAM (LK-600PM and 10021, LINKAM Scientific Instruments Ltd., UK). The sample was taken in between two cover slips and cooled at temperature -20, -30 and -40 °C at rate of 5 °C/min and then halting for 10 h, 8 h and 5 h at -20, -30 and -40 °C respectively for rapeseed oil mayonnaise. For soybean oil mayonnaise the halting time was 24 h, 18 h and 10 h for temperature -20, -30 and -40 °C respectively. Image had been captured

from the first appearance of crystal. The sample chamber of LINKAM was filled with N₂ gas for preventing water condensation on the surface of the sample (Ishibashi *et al.* 2016). The time related crystal amount had been measured by image processing using image J that further used to calculate crystallization rate constant.

5.2.5 Thermal behavior of mayonnaise and oil phase with Differential Scanning Calorimetry (DSC)

A heat flux type SEIKO EXSTAR SII-6200 DSC, manufactured by SEIKO Instruments Incorporation had been used for investing the thermal behavior of mayonnaise, oil and water phase. 10 mg samples were taken in an aluminum pan and sealed it properly. An empty aluminum pan was used as reference. A temperature profile from 0 °C to -75 °C, halting for 5 min and cooling or heating rate is 5 °C/min. The crystallization temperature was determined from the onset temperature of peaks and the melting temperature was determined from the peak top temperature on the thermograms.

5.2.6 Fat crystal structure analysis using Small Angle X-ray Scattering

SAXS (Aichi Synchrotron Radiation Center, Aichi, Japan) was used to determine the structure of fat crystal during temperature change. The X-ray wavelength used was 1.5 Å with light energy 8.2 keV. The temperature profile had been used was similar to that of DSC. For that, the samples (mayonnaise, oil and water phase) had been frozen to -90 °C at a rate of 50 °C/ min then the sample melted at a rate of 5 °C/ min. Data has been recorded considering the endothermic peak of DSC i.e for RoM at -41, -25 and -12 °C, for rapeseed oil at -20 °C, for SoM at -25 and -40 °C, for soybean oil at -26 and -39 °C and for water phase at -25 °C.

5.3 Results

5.3.1 Fat crystal with temperature

Fat crystal of both RoM and SoM has been observed using polarized light microscopy at temperature -20, -30 and -40 °C in isothermal condition. Fig. 5.1 and Fig. 5.2 showed the fat crystal of RoM and SoM respectively. The time for fat crystal appearance in RoM at -20, -30 and -40 °C was 360, 120 and 50 min respectively and fat crystal appearance time for SoM was 860, 710 and 260 min respectively. Considering temperature, the crystal appearance time was longer at higher temperature and with decreasing temperature the time decreased. The time for fat crystal appearance in RoM was lower than SoM at each temperature observed. Fig. 5.1 and Fig. 5.2 also showed the rate of crystal growth was higher in RoM than SoM. These results also agreed with the result of Ishibashi et al., (2016). Further, Magnusson et al., (2011) reported soybean oil is high linoleic acid containing oil and rapeseed oil is high oleic acid containing oil. This crystal generation time varied due to the fatty acid composition of oils.

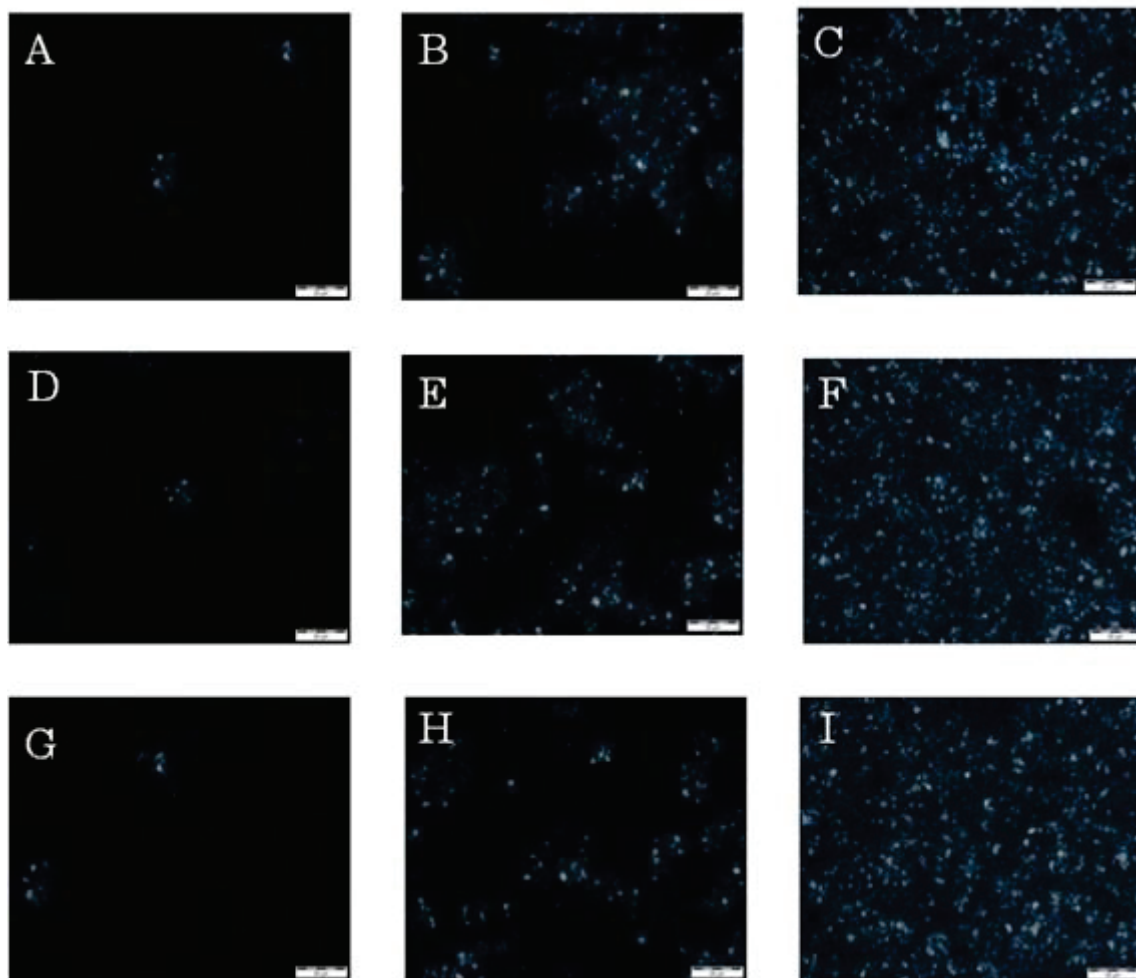


Fig. 5.1 Microscopic images of RoM fat crystals (white dots) using PLM. Images (A) to (C) during storage at $-20\text{ }^{\circ}\text{C}$ for $6\text{ h} \pm 5\text{ min}$, $6.5\text{ h} \pm 5\text{ min}$ and $7\text{ h} \pm 5\text{ min}$ respectively. And images (D) to (F) during storage at $-30\text{ }^{\circ}\text{C}$ for $2\text{ h} \pm 5\text{ min}$, $2.5\text{ h} \pm 5\text{ min}$ and $3\text{ h} \pm 5\text{ min}$ respectively and images (G) to (I) $-40\text{ }^{\circ}\text{C}$ for $50\text{ min} \pm 5\text{ min}$, $1.5\text{ h} \pm 5\text{ min}$ and $3\text{ h} \pm 5\text{ min}$ respectively. Scale bar is $20\text{ }\mu\text{m}$.

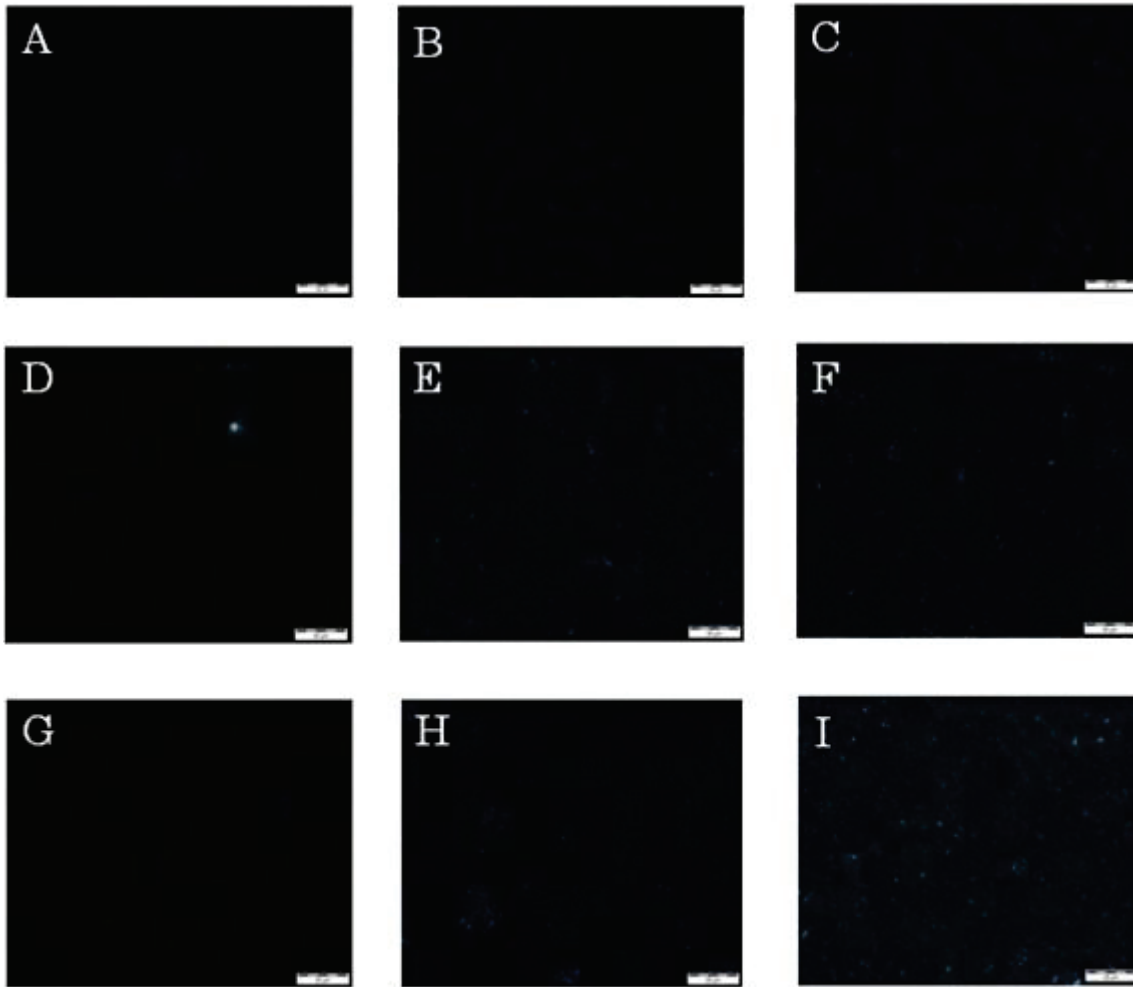


Fig. 5.2 Microscopic images of SoM fat crystals (white dots) using PLM. Images (A) to (C) during storage at $-20\text{ }^{\circ}\text{C}$ for 14 h 20 min \pm 5 min, 15 h 50 min \pm 5 min and 16 h 35 min \pm 5 min respectively. And images (D) to (F) during storage at $-30\text{ }^{\circ}\text{C}$ for 11 h 50 min \pm 5 min, 12 h 50 min \pm 5 min and 13 h 20 min \pm 5 min respectively and images (G) to (I) $-40\text{ }^{\circ}\text{C}$ for 4 h 20 min \pm 5 min, 4 h 50 min \pm 5 min and 5 h 5 min \pm 5 min respectively. Scale bar is 20 μm .

5.3.2 Crystallization kinetics due to temperature change

To quantify differences in the crystallization behaviors of both RoM and SoM at different temperatures the fat crystal data from PLM images in Fig. 5.1 and 5.2 were

fitted separately by Avrami equation for crystallization kinetic analysis. According to equation (8), the value of $\ln[-\ln(1 - X_t)]$ against $\ln t$ gives a linear regression line of fat crystal at different crystallization temperature; the slope for n and the intercept for $\ln k_c$ as shown in Fig. 5.3. The induction time for fat crystal generation and crystallization rate constant k_c and Avrami exponent, n showed in Table 5.1 and 5.2 respectively. The Avrami exponent n expresses the change of crystal dimension with temperature change. In this research both RoM and SoM showed higher Avrami exponent, n with decreasing temperature that means with temperature change the dimension of fat crystal changed. The difference of n value considering each temperature observed between RoM and SoM is not significant. But the difference of n value considering temperature -20 and -40 °C was about one that indicated the change of form of fat crystal happened at these temperatures.

The crystallization rate constant, k_c showed the growth rate of fat crystal with temperature. It was found the k_c value increased with decreasing temperature like destabilization rate constant, k_d and in RoM it was higher than SoM. The k_c values that calculated, considering unified n value as 1 showed in Table 5.3 also increased with decreasing temperatures.

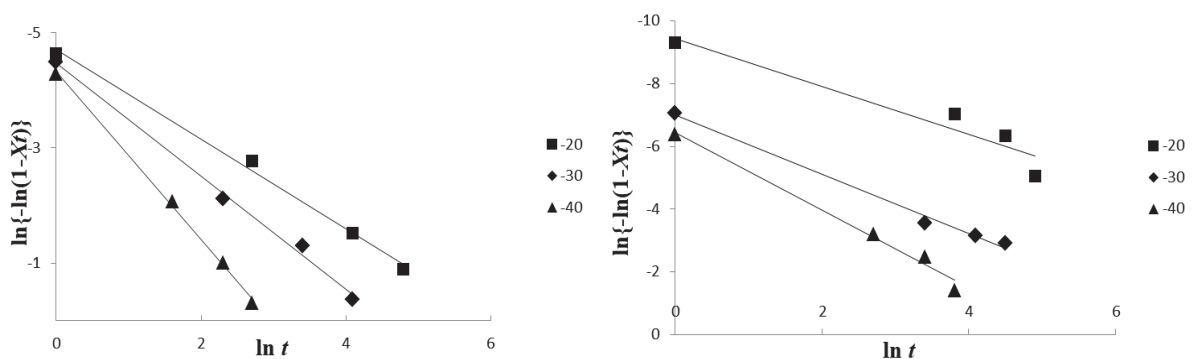


Fig. 5.3 Plots of $\ln\{-\ln(1 - X_t)\}$ versus $\ln t$ for isothermal crystallization of (A) RoM and (B) SoM at -20, -30 and -40 °C

Table 5.1 Induction time for generation of fat crystals in RoM and SoM

Temperature, °C	RoM Induction time, min	SoM Induction time, min
-20	360	860
-30	120	710
-40	50	260

Table 5.2 Variation of crystallization kinetic parameters with temperature; Avrami exponent, n and crystallization rate constant, K_c

Temperature, °C	n		K_c	
	RoM	SoM	RoM	SoM
-20	0.78	0.76	9.01×10^{-3}	7.87×10^{-5}
-30	0.98	0.94	1.11×10^{-2}	9.07×10^{-4}
-40	1.46	1.34	1.14×10^{-2}	1.87×10^{-3}

Table 5.3 Variation of crystallization rate constant, K_c with temperature recalculated using an Avrami exponent, n , of 1

Temperature, °C	n	$K_c \text{ min}^{-1}$			
		RoM	R^2	SoM	R^2
-20	1	4.73×10^{-3}	0.91	3.60×10^{-5}	0.83
-30	1	1.09×10^{-2}	0.99	7.68×10^{-4}	0.99
-40	1	2.80×10^{-2}	0.90	2.88×10^{-3}	0.95

5.3.3 Thermal behavior of mayonnaise and oil phase

The thermal behavior of mayonnaise as well as their oil phase was investigated using DSC. The DSC thermograms of crystallization and melting of both RoM and SoM and their oil phases were presented in Fig. 5.4 (A) during cooling from 0 to -75 °C at a rate of 5 °C. The RoM showed three exothermic peaks during cooling. The sharp, long exothermic peak at temperature -31.78 °C represented the crystallization of water of mayonnaise (Magnusson *et al*, 2011). The small exothermic peak (marked by arrow in Fig. 5.4 (A) before water crystallization at -29.05 °C indicated as crystallization of high melting saturated fat and another exothermic peak after water crystallization at

-58.13 °C indicated low melting unsaturated fat fraction of mayonnaise. The exothermic peak of rapeseed oil also showed the crystallization of low melting unsaturated fat. The exothermic peaks of saturated fat and water were distinct in SoM and indicated at -17.63 °C and -31.78 °C respectively. The broad exothermic peaks at -70.84 °C and -53.38 °C indicated unsaturated fat and -21.63 °C indicated saturated fat of soybean oil. DSC results indicated that in both mayonnaise samples the high melting saturated fat fraction crystallized first followed by water and low melting unsaturated fat.

DSC thermograms of mayonnaise and their oil phase during melting from -75 °C to 0 °C have been shown in Fig. 5.4 (B). The endothermic peaks at -10.04 ± 1 °C showed the melting of ice of both mayonnaise samples. The endothermic peaks at -24.76 and -15.72 °C of rapeseed oil and RoM respectively correspond to the melting of unsaturated fat. The broad endothermic peaks at -37.47 and -23.94 °C of soybean oil and SoM correspond to the unsaturated fat. The above results agreed by Ishibashi *et al.* 2016.

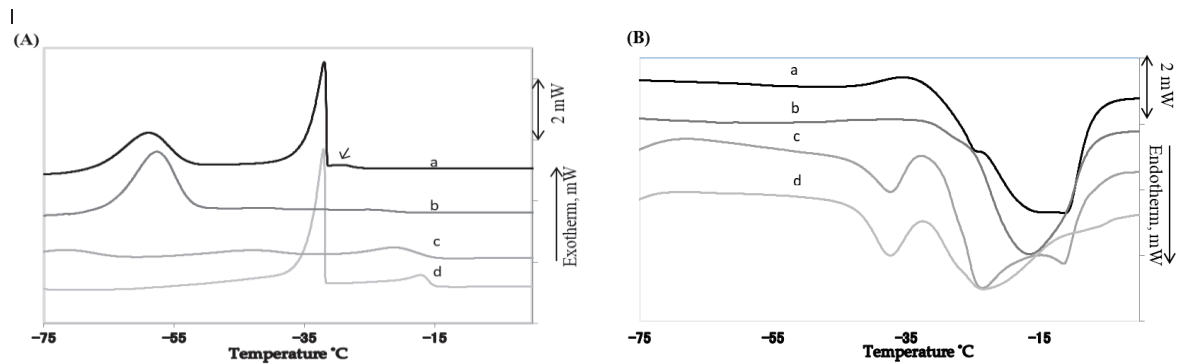


Fig. 5.4 (A). Thermal behavior with DSC during cooling at a rate of 5 °C/min (a) RoM, (b) Rapeseed oil, (c) Soybean oil and (d) SoM from 0 to -75 °C. **(B)** Thermal behavior with DSC during melting at a rate of 5 °C/min (a) Rapeseed oil, (b) RoM, (c) SoM and (d) Soybean oil from -75 to 0 °C.

5.3.4 Fat crystal structure change with temperature using SAXS

Fig. 5.5 showed the X-ray pattern obtained with temperature of RoM and SoM and their

respective oil phases. A new peak was observed for RoM at -41 °C (arrow marked) revealed increased the lamellar spacing of 5.56 nm. Silva et al., (2009) also reported the change of lamellar spacing from -20 to -40 °C with natural fat from Cupuassu. It might be due to further rearrangement of triglycerides chains at this temperature. At temperature -20 and -25 °C the diffraction peak found same in both rapeseed oil and RoM. It seems that the crystal form changed at -41 °C than -20 and -25 °C. The Avrami exponent increased by 0.7 at -40 °C from -20 °C results the change in crystal type from needle like to plate like shape (Kaufmann et. al., 2013).

On the other hand a new diffraction peak observed in soybean oil and SoM at temperature -39 and -40 °C respectively resulting swelling of crystal lamella packing sized 1.4 nm. Like RoM the SoM also showed the structural change at temperature -40 °C (Kalnin, 2009; Silva *et al.*, 2009). These results also agreed with the results of Avrami exponent, n of crystallization kinetics showed in Table 5.2.

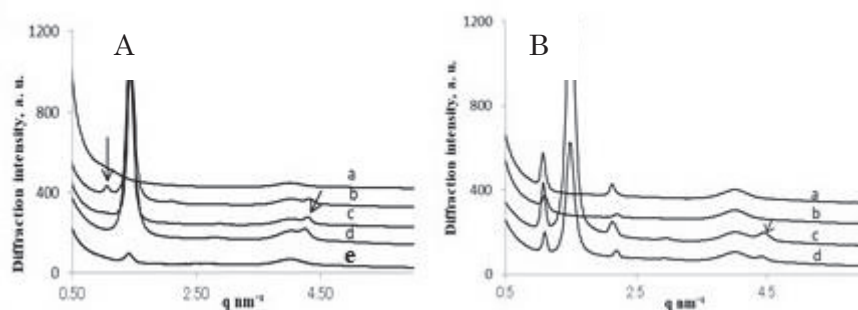


Fig. 5.5 (A) Small angle X-ray diffraction of RoM and its oil and water phase showed crystal form changing with temperature (a) water phase at -45 °C, (b) RoM at -41 °C, (c) RoM at -25 °C, (d) Rapeseed oil at -20 °C and (e) RoM at -12 °C. **(B)** Small angle X-ray diffraction of SoM and its oil phase showed crystal form changing with temperature (a) Soybean oil at -26 °C, (b) SoM at -25 °C, (c) Soybean oil at -25 °C, and (d) SoM -40 °C.

5.4 Relationship between fat crystal growth rate with destabilization rate

Fig. 5.6 (A) and (B) show the relationship between the crystallization rate constant, K_c , and the destabilization rate constant, k_d , of RoM and SoM respectively. For both types of mayonnaise, there were strong correlations between k_d and K_c , with R^2 values of 0.96 and 0.99 for RoM and SoM, respectively. These results show that both parameters were strongly dependent on temperature and increased with decreasing temperature. The increasing K_c value at a lower temperature indicated that changes in the nucleation and/or growth rates of the fat crystals might influence the separation rate.

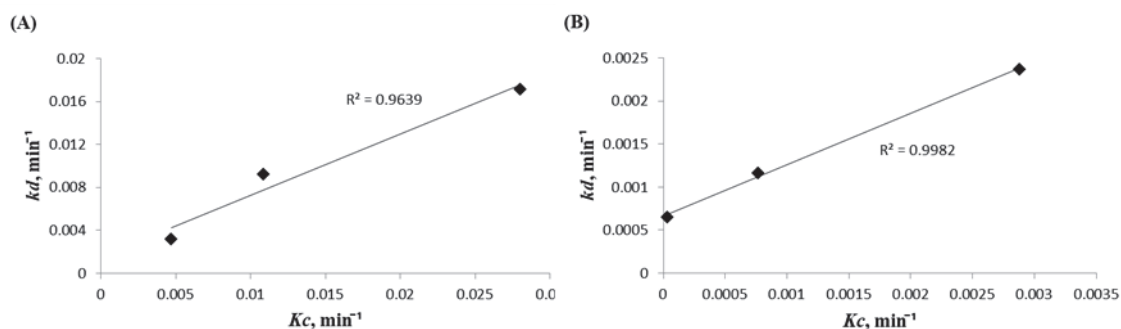


Fig. 5.6 Relationship between crystallization rate constant K_c and destabilization rate constant k_d . (A) Rapeseed oil mayonnaise and (B) Soybean oil mayonnaise.

5.5 Discussion

Avrami model has been privileged to analyze kinetic parameters of crystallization during isothermal condition. The fat crystal image from PLM has been used to calculate crystallization rate constant, k_c and Avrami exponent, n . The crystallization rate constant indicated the growth rate of fat crystal with temperature. It also indicates the nucleation rate of fat crystal with temperature change. However, the crystallization rate constant, k_c differed from RoM to SoM and in RoM it was higher than SoM at each temperature observed. The k_c value was also influenced by the temperature. In isothermal condition, at lower temperature the growth rate was higher than higher

temperature. The Avrami exponent n also showed increased with decreasing temperature indicating the type of fat crystal change with temperature in isothermal condition. From -20 to -40 °C the n value increased by 0.7 indicated the type of crystal from needle like to plate like structure. It is difficult to distinguish the change of fat crystal type using microscopic image but the SAXS results in Fig. 5.5 showed some additional peak at temperature -40 °C in both RoM and SoM. These peaks were not observed at temperature -20 and -30 °C. It was also not direct proved of change of fat crystal type but the generation of new lamellar structure might have influence on the type of fat crystal. The DSC results in Fig. 5.4 showed the crystallization temperature of oil phase and water phase of RoM and SoM. The crystallization temperature of oil phase and oil phase in mayonnaise sample found different. The endothermic peaks at -24.76 and -15.72 °C of rapeseed oil and RoM respectively correspond to the melting of unsaturated fat and the broad endothermic peaks at -37.47 and -23.94 °C of soybean oil and SoM corresponding unsaturated fat of soybean oil. One important thing is that, this DSC result obtained from non-isothermal condition but in isothermal condition both the oil crystallized at other temperatures like -20, -30 and -40 °C. The fat crystal appearance time was longer when the freezing temperature was higher than crystallization temperature and it found shorter when freezing temperature was similar to the crystallization temperature. And the rate of fat crystal growth also found slower at higher temperature and faster in lower temperature. This indicated that low temperature favored the nucleation. The induction time of destabilization calculated in the previous chapter have little difference with the time needed for appearing fat crystals. However, the crystallization rate constant, k_c influenced by the freezing temperature and the type of oil used and the type of fat crystal also changed with temperature. But the change of fat crystal type was not significant from RoM to SoM. Fig. 5.6 showed that there was a

linear relationship between fat crystal growth rate and destabilization rate. It indicated that increasing fat crystal amount caused increasing destabilization.

5.6 Conclusion

Crystallization kinetic analysis has been conducted to understand the growth and change of form of fat crystal using Avrami model. The crystallization rate constant k_c differed significantly from RoM to SoM and it increased with decreasing temperature. The nucleation rate in isothermal condition differed from each temperature and found higher at lower temperature and vice-versa. The Avrami exponent n also found increased with decreasing temperature indicating transition of crystal growth from needle-like of instantaneous nuclei to a plate-like form with high nucleation rate. The change of crystal form with temperature found insignificant from RoM to SoM but the rate of crystal growth differed significantly and there was a linear relationship between fat crystal growth rate and destabilization. The change of crystal growth rate with temperature might influence the rate of oil separation that has been discussed in previous chapter.

References

- Boode, K., Walstra, P., & Degrootmostert, A. E. A. (1993). Partial coalescence in oil-in-water emulsions. 2. Influence of the properties of the fat. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 81, 139-151.
- Campos, R., Narine, S. S., & Marangoni, A. G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Research International*, 35(10), 971-981.
- Coupland, J. N. (2002). Crystallization in emulsions. *Current Opinion in*

- Colloid & Interface Science, 7(5-6), 445-450.
- Cramp, G. L., Docking, A. M., Ghosh, S., & Coupland, J. N. (2004). On the stability of oil-in-water emulsions to freezing. *Food Hydrocolloids*, 18(6), 899-905.
- Ghosh, S., & Coupland, J. N. (2008). Factors affecting the freeze-thaw stability of emulsions. *Food Hydrocolloids*, 22(1), 105-111.
- Guzey, D., & McClements, D. J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Advances in Colloid and Interface Science*, 128, 227-248.
- Ishibashi, C., Hondoh, H., and Ueno, S. (2016). Influence of morphology and polymorphic transformation of fat crystal on the freeze thaw stability of mayonnaise type oil in water emulsion. *Food Research International*, 89: 604-613.
- Kalnin, D.J.E. (2009). Characterization of lipid nanostructures using DSC coupled to SAXS and WAXS. *JEEP*, 00017 : 01-05.
- Kalnin, D., Schafer, O., Amenitsch, H., & Ollivon, M. (2004). Fat crystallization in emulsion: influence of emulsifier concentration on triacylglycerol crystal growth and polymorphism. *Crystal Growth & Design*, 4(6), 1283-1293.
- Kaufmann, N. and Kirkensgaard, J.J.K., Andersen, U., and Wiking, L. (2013). Shear and rapeseed oil addition affect the crystal polymorphic behavior of milk fat. *J. Am. Oil Chem. Soc.* 90(6) : 871-880.
- Magnusson, E., Rosen, C and Nilsson, L. (2011). Freeze-thaw stability of mayonnaise type oil-in-water emulsions. *Food Hydrocolloids* 25 : 707-715.
- McGee H (2004) *On food and cooking – The science and lore of the kitchen*, Macmillan Library Reference USA, Inc., pp. 633-4. Scribner. New York.

- Meng, Z., Yang, L., Geng, W., Yao, Y., Wang, X. and Liu, Y. (2014). Kinetic study on the isothermal and nonisothermal crystallization of monoglyceride organogels. *The Scientific World Journal*. Article ID 149753, pp : 1-7.
- Miyagawa, Y., Ogawa, T., Nakagawa, K. & Adachi, S. (2016). Destabilization of mayonnaise induced by lipid crystallization upon freezing. *Bioscience, Biotechnology, and Biochemistry*, 80(4):786-90.
- Palanuwech, J., & Coupland, J. N. (2003). Effect of surfactant type on the stability of oil-in-water emulsions to dispersed phase crystallization. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 223(1-3), 251-262.
- Silva, J.C., Plivelic, T.S., Herreraa, M.L. and Ruscheinsky, N. (2009). Polymorphic phases of natural fat from Cupuassu (*Theobroma grandiflorum*) beans : A WAXS/SAXS/DSC study. *Crystal Growth Design*. 9 : 5155-5163.
- Tippetts, M., & Martini, S. (2009). Effect of cooling rate on lipid crystallization in oil- in-water emulsions. *Food Research International*, 42(7), 847-855.
- Vanapalli, S. A., & Coupland, J. N. (2001). Emulsions under shear-the formation and properties of partially coalesced lipid structures. *Food Hydrocolloids*, 15(4-6), 507-512.
- Vanapalli, S. A., Palanuwech, J., & Coupland, J. N. (2002). Stability of emulsions to dispersed phase crystallization: effect of oil type, dispersed phase volume fraction, and cooling rate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 204(1-3):227-237.

CHAPTER 6

Conclusion

6.1 Conclusions from this works

Mayonnaise is a semi solid oil-in-water emulsion and destabilize during freezing. This problem associated with the freeze storage with other foods. Generation of ice and fat crystal during freezing are the cause of destabilization. This study focused on the influence of both ice and fat crystal on destabilization.

Chapter 2 confirmed the coalescence and destabilization during freezing. Cryo-SEM images of oil droplets of frozen mayonnaise showed that coalescence happen during freezing. Oil separation with bulky sample showed that separated oil increased with time. These results suggested that coalescence happen during freezing and coalesced droplets appeared as separated oil while thawing and this phenomena might depends on crystallization of water and oil phases.

Chapter 3 studied the probable influence of ice crystal of coalescence of oil droplets. Coalescence of oil droplets had been observed by dry air blowing. Mayonnaise contains higher oil phase ratio than water phase so, shortage of water lower the distance among oil droplets and made deformation. Simulated icing experiment showed relation of water absorbed by desiccant with separation of oil. Oil separation increased when higher amount of water absorbed by desiccant. Stability with limiting water crystallinity using APS showed that the stability increased with increasing APS concentration. The crystallinity of water had further evaluated with XRD measurement. The XRD results showed, with the increasing APS concentration the crystallinity decreased in water phase. From these results it can be conclude that ice crystal influences the coalescence by lowering the distance of oil droplets. Though mayonnaise type oil in water emulsion

is oil phase dominating emulsion but water phase has significant effect on stability during freeze storage.

Chapter 4 described kinetic analysis of destabilization of mayonnaise using capillary. The method found suitable for evaluating the destabilization kinetics through power law function. The destabilization rate constant k_d found increased with decreasing temperature showing temperature dependency. The order of destabilization n varied with temperature showing the mechanism of destabilization was different at each temperature observed. Moreover, the kinetic model was found to adequately evaluate the mayonnaise stability under the condition of the investigation. The induction time and kinetic parameters; destabilization rate constant k_d , and the order of destabilization n , were reliable because capillary can overcome the size effect from bulky sample.

In chapter 5 kinetic analyses has been conducted to understand the growth and change of form of fat crystal using Avrami model. The crystallization rate constant k_c differed significantly from RoM to SoM and it increased with decreasing temperature. The nucleation rate in isothermal condition differed from each temperature and found higher at lower temperature and vice-versa. The Avrami exponent n also found increased with decreasing temperature indicating transition of crystal growth from needle-like of instantaneous nuclei to a plate-like form with high nucleation rate. The change of crystal form with temperature found insignificant from RoM to SoM but the rate of crystal growth differed significantly. Importantly, a linear relationship found between rate of fat crystal growth with destabilization rate.

6.2 Future suggestions

Mayonnaise is most common oil-in-water emulsion that consumed all over the world.

Destabilization during freezing with other food is an important problem. This study enumerates the possible mechanism of coalescence in relation with water scarcity and/or by reducing the emulsifying activity. The coalescence of mayonnaise oil droplets is different than other oil-in-water emulsion. Mayonnaise contains about 70-80% oil phase while usual oil-in-water emulsion contains higher amount of water phase than oil phase. Usually emulsion with higher water phase, the oil droplets flocculates prior to coalesce. But in mayonnaise, the distance between oil droplets is very small, so it deforms rather than flocculate. In this research this deformation of oil droplets in relation with moisture has been studied by drying as well as by simulating the ice crystal. But in freezing condition how the ice crystal influence the deformation is still need for further investigation.

Another important initiative has been taken for kinetic analysis of mayonnaise destabilization during freeze thawing. Destabilization rate constant, k_d and the order of destabilization, n has been calculated from the separated oil. Destabilization rate constant, k_d showed dependency on temperature and an empirical equation has been derived to evaluate mayonnaise destabilization. But the order of destabilization did not follow any specific pattern. However, further study is needed to explain the kinetic parameters as well as their utility in mayonnaise stability.

Several authors worked on the influential factors related to the destabilization of oil-in-water emulsion during freezing. They suggested that fat and ice crystallization are the important factors of destabilization. In this study the growth of fat crystal in isothermal condition has been studied through kinetic analysis using Avrami model. The crystallization rate constant, k_c and the Avrami exponent, n increased with decreasing temperature. The growth of fat crystal depends on temperature and the properties of oil

used. In this research RoM and SoM were used for crystallization kinetic analysis and found the crystallization rate constant differed significantly but the change of crystal type was insignificant. These results suggested the growth of fat crystal influence on destabilization rather than crystal form. Further study is needed to understand crystallization kinetic parameters and influential factors effect on it. That can help to lower destabilization of mayonnaise during freezing.