Oil Diffusion in Different Cocoa Butters Using Magnetic Resonance Imaging

Thesis

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ABSTRACT

In a multi-component chocolate product, oil migration, from high oil content filling into the chocolate is one of the major contributors to quality loss. Five different cocoa butter samples were used to model and study the effects of chemical composition and processing conditions on oil migration. Samples were crystalized under two different processing conditions. Control samples were crystalized under 0 shear rate and 0.5 ℃/min cooling rate. Processed samples were crystalized under $250s^{-1}$ shear rate and 4.5 ℃/min cooling rate. Crystalized cocoa butter samples were placed in contact with a cream as a source of liquid oil. Using magnetic resonance imaging, the movements of liquid oil into samples were investigated. The sample oil diffusivity was analyzed based on the Fickian diffusion model. Slightly different chemical composition affected cocoa butter's melting point, solid fat content, microstructure, and resulted in different oil migration rate in the samples from five different origins. Additionally, processing conditions significantly retarded the oil migration rate in cocoa butter system. However, identical trends observed in control and processed samples indicated that different processing conditions would not eliminate the effects of chemical composition in cocoa butter on oil migration kinetics.

Overall, minor differences in chemical composition of cocoa butters from different origins affect the oil diffusivity. Processing conditions can help slow down the rate of oil migration in cocoa butter system. However, it would not overcome the effects of chemical composition on oil migration kinetics.

Keywords: Oil migration, Chemical composition, Cocoa butter

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INTRODUCTION

Oil migration is a major issue in confectionery products and can cause a decrease in the product's quality. For example, fat bloom as a result of oil migration, leads to quality reduction and has negative effects on sensory properties of chocolate products. Cocoa butter, a porous fat material, is one of the main ingredients in chocolate and provides the texture and structure of the products. Several studies have related oil diffusivity to different properties of cocoa butter (Maleky et al., 2012; Marty et al., 2005; Sonwai & Mackley, 2006). Studies have also shown that many factors can affect oil diffusivity in cocoa butter systems. These include factors such as formulation, processing conditions, and storage temperature (Clercq et al., 2014; Maleky et al., 2012; Maleky & Marangoni, 2011; Maleky et al., 2011; McCarthy & McCarthy, 2008; Svanberg et al., 2011). The formulation of confectionary fat significantly impacts the rate of oil migration in cocoa butter by changing the solid fat content (McCarthy & McCarthy, 2008) and structure of the fat network (Svanberg et al., 2011). Additionally, studies have shown that processing conditions can influence the oil diffusivity because changes to the structure of the fat network can occur (Acevedo et al., 2012; Hachiya et al., 1989; Maleky et al., 2012; Maleky & Marangoni, 2008; Maleky et al., 2011; Mazzanti et al., 2011; Sato, 2001; Sonwai & Mackley, 2006; Stapley et al., 1999; Svanberg et al., 2013; Svanberg et al., 2011). Maleky (2011)

and Sonwai (2006) showed the crystal micrographs of cocoa butter samples crystallized with and without shearing and pointed out that shearing was associated with the formation of small and homogeneous particles. Shearing resulted in a lower oil migration rate (Maleky & Marangoni, 2011; Maleky et al., 2012). Moreover, storage temperature is another factor that affects oil migration rate in cocoa butter systems (Depypere et al., 2009; Khan & Rousseau, 2006; McCarthy & McCarthy, 2008; Miquel et al., 2001). Khan studied the effects of three storage temperatures (11℃, 20℃, 26℃) on migration kinetics of oil and showed that oil migration in fat network would be accelerated at higher storage temperatures (Khan & Rousseau, 2006).

However, the effects from the differences in chemical characteristics of cocoa butter on oil diffusivity are still unclear. Cocoa butter from different origins are shown to have different chemical compositions due to environmental growth conditions and harvest treatments in different regions (Dimick, 1989; Marty & Marangoni, 2009).

Marty and Marangoni studied the effects of cocoa butter origins on oil migration kinetics using the Scanner Imaging Technique (Marty & Marangoni, 2009). A lipid soluble dye (Nile Red Stain) was used as a tracker for the a Scanner Imaging Technique. A flatbed scanner and image analysis software were used to monitor the movement of the dye and to quantify migration of the oil through the confectionary fats. They hypothesized that the dye had the same migration rate as the oil and this was comfirmed by Marty (Marty et al., 2005; Marty et al., 2009). Moreover, they also pointed out that the distance travelled by the oil had a linear

corelation with the square root of time, which was then used to quantify the oil migration rate. Marty and Marangoni also investigated the oil migration kinetics of cocoa butter from six different origins (Brazil, China, Ecuador, Ivory Coast, Malaysia and Nigeria) (Marty & Marangoni, 2009). They showed that cocoa butter origins had a strong effect on the oil migration rate. Brazilian and Nigerian samples had the highest oil migration rate and Malaysian cocoa butters exhibited the third highiest rate. In the meantime, the lowest oil migration rates were found in the cocoa butter samples from China, Ecuador, and Ivory Coast. They also pointed out that the amount of oleic acid and triunsaturated triglycerides exerted great influence on the oil migration rate in cocoa butter systems. Moreover, in this study, they also examined the effects of tempering on the oil migration rate. The results showed that the rate of oil migration in tempered samples were 10 to 50 times lower than those in nontempered cocoa butter samples. Overall, Marty and Maragoni demonstrated the different oil migration rates in cocoa butter from different origins using the scanner imaging technique (Marty & Marangoni, 2009).

In our study, five cocoa butters from different origins were investigated as well but using Magenetic Resonance Imaging (MRI) technique. This MRI technique was shown to be a novel and reliable method to examine three-dimensional oil distribution within confectionary fat (Choi, McCarthy, & McCarthy, 2005; Maleky, Mccarthy, et al., 2012; Rumsey & McCarthy, 2012). This technique quantifies the migration of oil by measuring changes in the solid fat content and by mornitoring the movement of liquid oil (Thierry et al., 1997).

One of the advantages of MRI is the visualization of the migration process. Therefore, with this method scientists are able to monitor the migration process in a single sample at different time points and this reduces experimental errors by eliminating sample to sample variation (Thierry et al., 1997).

To understand the behaviors of oil in migration and diffusion mechanism in chocolate systems, the movement of oil needs to be monitored accurately and the diffusional behaviors should be studied. A MRI technique was used in this study to percisely monitor the movement of oil and comprehensive analyse were conducted to quantify the oil migration. The objective of this study is to investigate the effects of minor differences in the chemical composition of cocoa butter on the kinetics of oil diffusivity. In addition, we also aimed to examine the effects of processing conditions on oil migration rate and study the influence of processing conditions on the effects of chemical composition.

CHAPTER 1: LITERATURE REVIEW

1.1 Overview of chocolate

1.1.1 Consumption of chocolate

Chocolate is one of the most popular food products throughout the world. In the per capita consumption report in 2014 (Nieburg, 2014), Switzerland was the nation with the top consumption of 9.0 kg/person, followed by the Germany and Austria with 7.9 kg/person and 7.8 kg/person, respectively. The United States, with a chocolate consumption of 4.3 kg/person, is one of the top 20 chocolate consuming nations in the world (Nieburg, 2014). Chocolate is popular not only because it provides energy and healthy flavonoids, but also offers pure pleasure.

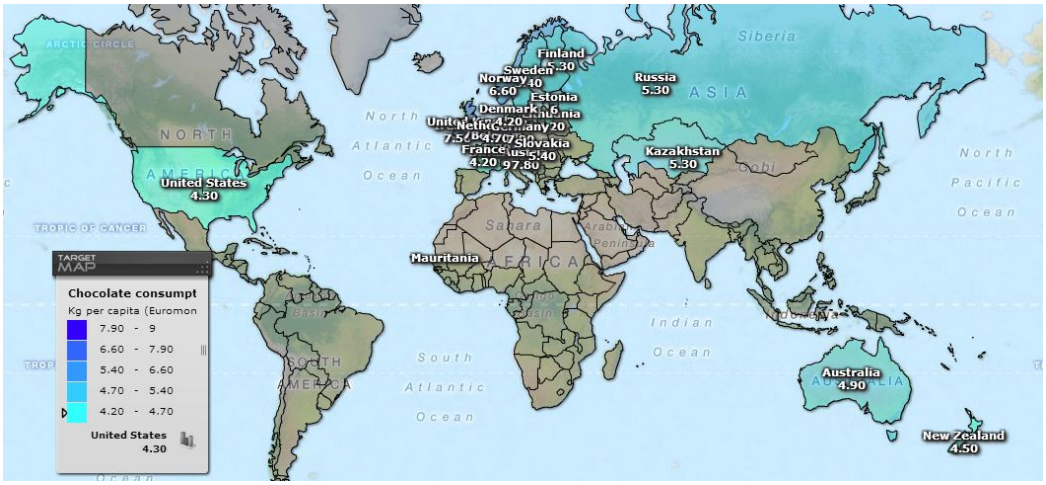


Figure 1. Top 20 chocolate consuming nations in 2014 and their distribution. (Source: Annual Statistic Report from Confectionernews.com)

The pleasure comes from the attractive flavor of cocoa powder and satisfying smooth melted taste of cocoa butter. In order to have a better quality of chocolate with a longer shelf life, scientists devote themselves to the studies of chocolate properties and preventing or slowing down the loss of quality (Altan et al., 2011; Maleky et al., 2012; Marty & Marangoni, 2009; McCarthy & McCarthy, 2008; Ziegler, 2009).

1.1.2 Chocolate products and ingredients

Chocolate can nominally be divided into three kinds: dark chocolate, milk chocolate, and white chocolate (Beckett, 2000). The ingredients for confectionery chocolate include cocoa butter, cocoa liquor, cocoa powder, sugar, emulsifier and milk powder (Geoff Talbot, 2012b). Cocoa butter is the major fat ingredient and is used to both provide the structure and optimize the rheological properties for the chocolate (Wells, 2009).

Besides pure chocolate products, there are many multi-component chocolate products such as enrobed and filled chocolate products (Geoff Talbot & Consultant, 2003). Multi-component confectionery products with a chocolate coating are not new. One of the reasons for its popularity is the continual consumer demand for new taste and textural sensations. Enrobing chocolate is an edible product that is then covered with a chocolate coating (G Talbot, 2009). Typical examples are coated biscuits, coated ice cream bars, coated cakes, coated fruits, and nuts. Filled chocolate is usually achieved by first making a shell of chocolate and then filling the shell with other products such as wine and cream(Birkett & S, 2003). Nuts and cream, which are rich in oil, are popularly used as ingredients in multi-component chocolate products (Yates, 2009).

1.2 Cocoa butter

1.2.1. Chemical composition

Fatty acid and Triacylglycerols composition

The main fatty acid compounds in cocoa butter are palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) (Beckett, 2000; Geoff Talbot, 2012b). There are also small amounts of arachidic acid (C20:0), linoleic acid (C18:2), palmitoleic acid (C16:1), and other fatty acids (Beckett, 2000). Timms and Stewart determined the fatty acid compositions of 140 cocoa butters from different origins. The content of palmitic acid, stearic acid, and oleic acid are about 26.9 %, 36.6% and 31.7%, respectively (Timms & Stewart, 1999).

Cocoa butter is a complex mixture of fatty acids esterified onto a glycerol skeleton to form triacylglycerols (TAG). Most of the triglycerides in cocoa butter are symmetrical. The fatty acid compositions are very simple in cocoa butter, which result in an equally simple triglyceride compositions with mainly 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1-palmitoyl-2- oleoyl-3-estearoyl-glycerol (POSt), and 1,3-diestearoil-2-oleoyl-glycerol (StOSt) (Beckett, 2000; Geoff Talbot, 2012a). Chaiseri and Dimick studied the TAGs content of different sources of cocoa butters and reported the average contents of POP, POSt, and StOSt at around 20%, 40%, and 27%, respectively (Chaiseri & Dimick, 1989).

Free Fatty Acids

Cocoa butter typically has a maximum specified free fatty acid level of 1.75% (Folayan, 2010; Timms & Stewart, 1999). Twenty cocoa butter samples were analyzed

by Foubert et al. (2004). They found that the samples cocoa butter had an average free fatty acid level (oleic acid) at about 1.71% (SD 0.41) and also pointed out that when the free fatty acid level was larger than 1.75% samples showed unexpected problems during crystallization.

Phospholipids

Phospholipids are one the minor compounds in cocoa butter. The content of phospholipids in cocoa butter is quantified by the level of phosphorus (Talbot, 2012a). Foubert et al. determined that the phosphorus levels from 2.3 ppm up to 63 ppm equaled to a phospholipid range of 0.006% up to 0.16% (Foubert and Vanrolleghem, 2004). Dimick and Davis reported 0.0037% phospholipids in the whole cocoa butter and pointed out the positive effects of phospholipid on cocoa butter solidification (Dimick, 1989). Foubert (2004) and Marty (2009) illustrated that phosphorus content of cocoa butter was positively correlated to nucleation induction time.

1.2.2. Origins of cocoa butter

Cocoa butter is one of the derivative products from cocoa beans, the seed of the cocoa tree. Cocoa trees prefer to grow in countries near the equator, such as Ivory Coast, Ghana, and Nigeria (Beckett, 2000). The production of cocoa beans by country in 2014/2015 is shown in Figure 2 (ICCO, 2015).

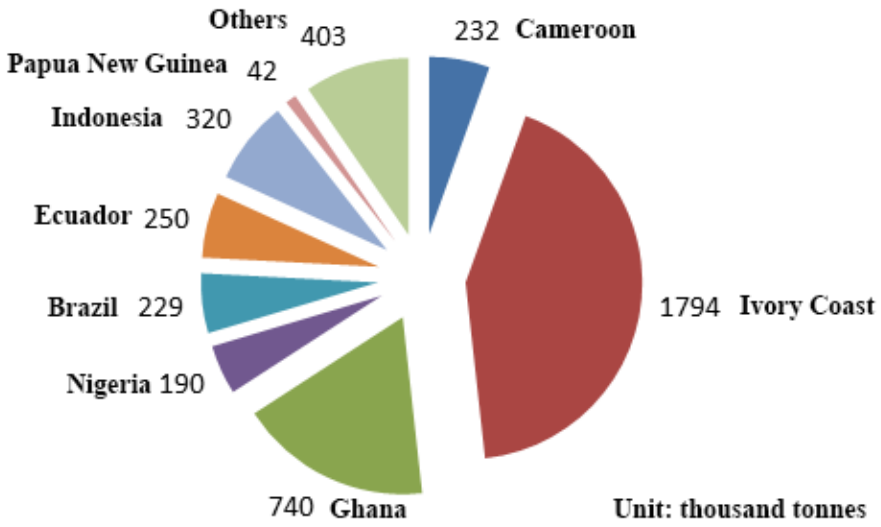


Figure 2. Production of cocoa beans (thousand tons) in year 2014-2015 (source: annual statistic report from ICCO)

Studies have shown that chemical composition varies on both major and minor components of cocoa butter from different origins. TAG compositions of cocoa butter from 20 countries were summarized in Table 1, according to previous studies (Dimick, 1989; Marty & Marangoni, 2009).

Table 1 Triacylglycerol profile and free fatty acid content of cocoa butters from different origins

		Triacylglycerol (%)									Free fatty acid in % oleic acid*
	Cocoa butter origin	PLiP	POO	PLiS	POP	SOO	SLiS	POS	SOS	SOA	
Central and South America	Bolivia	1.1	3.3	3.5	22.6	4	2.1	40.4	22.8	0.5	0.84 (1)
	Brazil	0.9	3.9	3.7	17.9	6.7	3.2	37.1	26	0.04	0.91 ± 0.05 (4)
	Colombia	1.1	3.3	3.6	20.4	4.4	2.3	39.4	25	0.6	1.07 ± 0.29 (2)
	Ecuador	1.2	3	3.2	19.2	5.4	2.3	38.4	26.9	0.4	1.42 ± 0.73 (2)
	Peru	1.5	4.3	3.9	18.3	7.4	3.7	35.8	24.6	0.4	3.11 (1)
	Venezuela	0.9	1	3.1	20.4	2.8	1.9	40.4	28.8	0.8	n.a.
	Costa Rica	1	2.6	3.5	17.8	5.5	3	38.7	27.4	0.4	0.72 (1)
	Dominican Republic	1.1	3.3	3.2	18.4	6.1	2.7	38.2	26.5	0.6	0.88 ± 0.01 (2)
	Guatemala	1	2.3	3.4	19.3	4.9	2.2	39	27.5	0.4	n.a.
	Mexico	1.1	2.4	3.5	19.1	4.1	3	38.8	27.8	0.6	0.42 (1)
	Panama	1	1.5	3	19.1	3.1	2.7	41.4	27.3	0.8	1.07 (1)
Africa	Cameroon	1	3	3.4	17.9	5.8	2.5	38.3	27.7	0.5	n.a.
	Gabon	0.9	3.7	3.5	17.5	7.3	3	37.1	26.5	0.4	n.a.
	Ghana	1.2	2.2	3.4	17.8	4.9	2.2	39	27.5	0.4	n.a.
	Ivory Coast	1	1.9	3	19	3.9	2.5	39.6	28.5	0.6	1.36 ± 0.18 (5)
	Nigeria	1	2.3	3.6	17.9	5.2	3	38.8	27.8	0.5	1.21 (1)
Oceania	Indonesia	1.1	1.6	3	19.9	3.6	1.7	40.6	28.1	0.5	n.a.
	Malaysia	0.7	1.2	2.8	18.4	2.9	2.2	40	31.1	0.8	1.44 ± 0.42 (3)
	Solomon Island	1	0.9	3	19.3	2.8	2	40.7	29.5	0.7	n.a.

n.a.: data not available

*Number in brackets indicates the number of sample analyzed to determine the free fatty acid content of the corresponding cocoa butter.

Adapted from Chaiseri & Dimick, 1989.

The differences in chemical composition of the cocoa butter samples from different origins are affected by environmental variables, harvesting conventions, and post-harvest treatments (Beckett, 2000; Marty & Marangoni, 2009).

The environmental growth conditions, including temperature, humidity, soil conditions, and sunshine, are varying in different geographic regions (Dimick, 1989; Lehrian et al., 1980; Lehrian & Keeney, 1980; Marty & Marangoni, 2009; Wright et al., 1982). Lehrian et al. documented that cocoa tree growth in areas with higher temperature

had larger amounts of palmitic acid, stearic acid, and mono-unsaturated TAGs in cocoa butter (Lehrian et al., 1980; Lehrian & Keeney, 1980). Dimick studied the cocoa butter from different origins and discovered that the hardness of cocoa butter was affected by the temperature variation in different regions (Dimick, 1989). Moreover, harvest conventions, including cocoa harvest period, cocoa tree type, and cocoa pod maturation, can affect the chemical compositions of cocoa butter. For example, Lehrian (1980) and Wright (1982) showed that lipid compositions of cocoa beans were different when harvested on different maturity level of the respective cocoa pods.

Different regions have their special post-harvest treatments which affect cocoa butter chemical components. The post-harvest treatments include different processing conditions in fermentation and drying. Even though there is no significant effect on the contents of free fatty acid (FFA), DAGs, and TAGs by roasting, Marty suggested that fermentation might have influence on the amount of FFA and type of phospholipids (Marty & Marangoni, 2009).

Overall, considering climate, pre-, and post-harvest treatments, there are many variables that impact the chemical composition of cocoa butter in different origins.

1.2.3. Structure

Cocoa butter, as a fat material, can be crystallized in a number of different structures. It is important to understand the structural organization present in cocoa butter, because it can guide the development of specific properties desired and demanded in confectionary products. A structural hierarchy of fat crystal networks as understood from current knowledge (Deman & Beers, 1987; Heertje, 1993; Marangoni, 2012; Tempel,

1961) is shown in Figure 3. Figure 3 shows the structure of edible fats from TAG molecules to macroscopic structures.

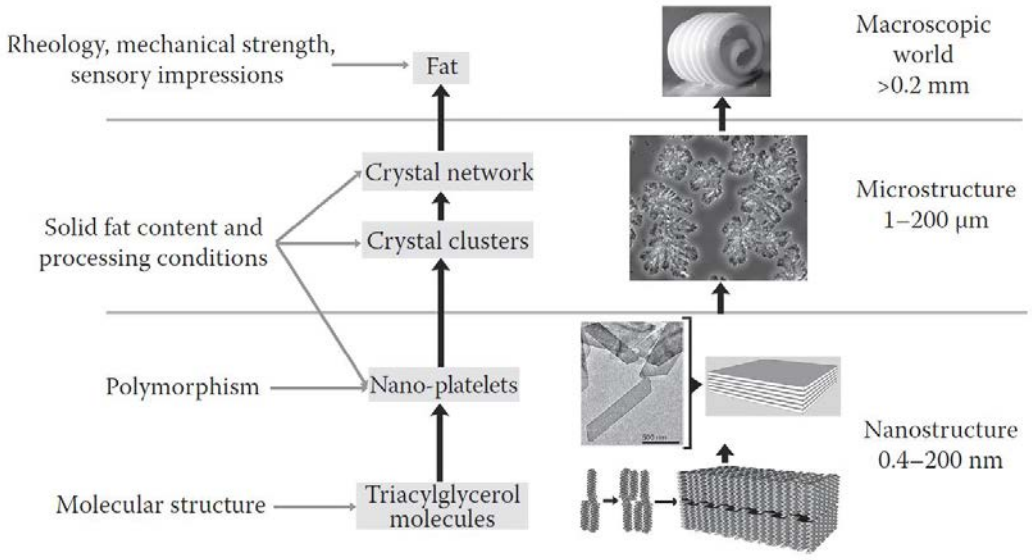


Figure 3. Structural hierarchy in a fat crystal network --- from molecules to material (Marangoni, 2010)

TAG molecules in cocoa butter consist of a glycerol backbone with three fatty acids esterified to the three fatty acid at specific locations referred to as sn-1, sn-2, and sn-3 (Beckett, 2000; Marangoni & Wesdorp, 2013) , as seen in Figure 4a. In cocoa butter, 80% of the TAGs are symmetrical mono-unsaturated with oleic acid at sn-2 position. The fatty acid in the sn-2 position always comes off the glycerol backbone on the opposite side to the other two fatty acids; there is not enough space for three fatty acid chains to be on the same side of the glycerol backbone. The TAGs pack in a ‘chair’ or ‘tuning fork’

structure under different ways: double-chain length packing (Fig. 4b) and triple-chain length packing (Fig. 4c) (Beckett, 2000; Geoff Talbot, 2012b).

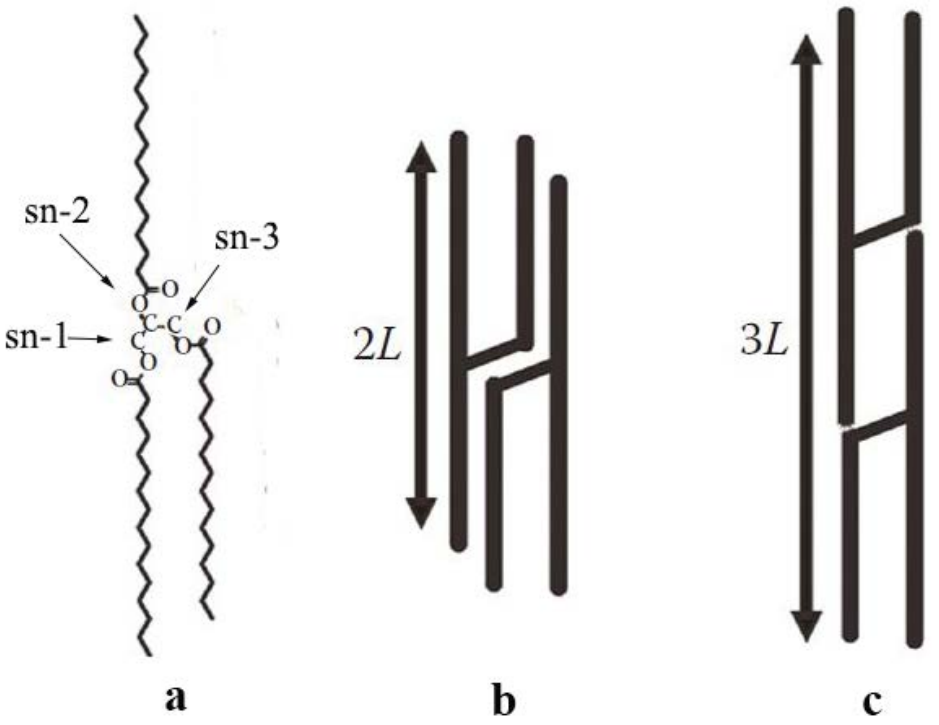


Figure 4. a) Structure of triglycerides; b) Double-chain length packing configuration; c) Triple-chain length packing configuration (Marangoni & Wesdorp, 2013)

In double-chain length packing, the fatty acid in the sn-2 position of one triglyceride molecule is adjacent to fatty acids in the sn-1 or sn-3 positions of the next triglyceride molecule. In many fats, this is a very stable structure but not in cocoa butter. This is because the oleic acid in the sn-2 position is a *cis*-monounsaturated fatty acid and

has a bend in the middle of the fatty acid chain. The bends next to the straight chain will loosen the structure. Alternatively, in the triple-chain structure, the unsaturated fatty acids (oleic acid) in the sn-2 position lie next to each other and the bends fit together. This gives a much more stable configuration of the network (Marangoni et al., 2012; Talbot, 2012a).

As well as crystallizing in a particular chain length configuration, cocoa butter can crystallize in a variety of different polymorphic forms. The basic polymorphic forms are called alpha (α), beta-prime (β′), and beta (β) (Beckett, 2000). The arrangement of TAG chains for α, β′, and β polymorphs of cocoa butters are shown in Figure 5. In cocoa butter, α and β′ polymorphic forms are double chain packing, while β polymorphic phase is triple chain packing. Form α with the chains stacked straight up is more unstable compared to β′ polymorphic phase. On the other hand, the triple-chain packing in β form provides a more stable structure compare to the other two polymorphs.

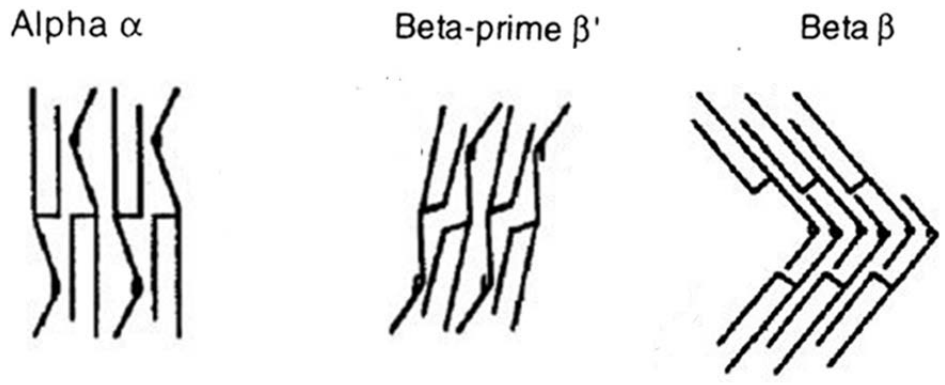


Figure 5. The arrangement of TAG chains for α, β′ and β polymorphs of cocoa butters

However, cocoa butter can crystallize in more than these three basic forms. For many years there were considered to be six polymorphic forms of cocoa butter. This was based on work done by Wille and Lutton (1966b) and Larsson (1966). Wille and Lutton gave the six polymorphic forms the number I through VI (Wille & Lutton, 1966b).Polymorphism α is easier to be formed than β′ and β because it has the lowest energy required. Aging causes a change in the polymorphism form and it is always in the direction of $\alpha - \beta' - \beta$ (Aronhime et al., 1988; Wille & Lutton, 1966a). Cocoa butter is a beta-stable fat. There are two types of β form which are β_V and β_{VI} . β_V is the desired polymorphic phase, while β_{VI} can be transformed from β_V, and responds for fat blooming (James & Smith, 2009; Wille & Lutton, 1966b).

1.3 Oil migration

1.3.1 Fat blooming and oil migration

Chocolate is the combination of fat and not-fat ingredients. Chocolate products melt smoothly in the mouth and release pleasant tastes and aroma when they are produced and stored under appropriate conditions. During storage, chemical interactions or physical changes can result in an unwanted quality of chocolate. There are many factors that can cause quality loss, including lipid oxidation, moisture migration, and fat blooming (Ghosh et al., 2002; Kinta & Hatta, 2012; Minifie, 2012; Subramaniam, 2009; Talbot, 2003; Ziegler, 2009).

Fat blooming, studied in this work, is one of the major reasons of chocolate quality deterioration. When chocolate is produced under the appropriate conditions, both

sugar and fat are present in fine texture and are dispersed uniformly at the macroscopic level. Bloom is a condition in which the fine texture of sugar and fat crystals is lost and the chocolate becomes non-uniform (Kinta & Hatta, 2012). The shelf life of enrobed and filled chocolate is often limited by the changes in the fat crystal network. The transport of foreign oils into chocolate products has influence on accelerating fat blooming (Ziegler, 2009).Therefore, in order to slow down or prevent fat blooming in chocolate products, studies are needed to understand its mechanism.

Migration of oil is the movement of liquid from the side with higher concentration of oil to the side with lower concentration of oil. Previous studies showed that the rate of oil transporting in cocoa butter system is affected by the formulations, composition of cocoa butter, processing conditions, structure of fat network, and storage conditions (Clercq et al., 2014; Maleky, et al., 2012; Maleky & Marangoni, 2011; Maleky et al., 2011; McCarthy & McCarthy, 2008; Svanberg et al., 2011).

1.3.2 Factors affecting oil migration

1.3.2.1 Formulation

Different formulations of chocolate and different chemical compositions of cocoa butter can affect the compound interaction and fat crystallization within the system (Lee et al., 2010; Marty & Marangoni, 2009; McCarthy & McCarthy, 2008; Motwani et al., 2011). Sugar, milk fat, and emulsifier are ingredients besides cocoa butter that showed great influence on oil migration kinetics (McCarthy & McCarthy, 2008; McClements & Demetriades, 1998; Svanberg et al., 2011; Vernier, 1997). As for chemical composition, Clercq et al. studied the influence of cocoa butter diacylglycerols (DAGs) on migration

and pointed out that the presence of DAGs did not delay oil migration nor prevented the appearance of migration induced fat bloom (Clercq et al., 2014). Marty et al. documented the significant effects of oleic acid and tri-unsaturated TAGs on oil diffusivity in cocoa butter system (Marty & Marangoni, 2009).

1.3.2.2 Processing condition

With different processing conditions, chocolate or cocoa butter can vary in rheology properties and fat structures, which results in different viable shelf lives. Studies showed that appropriate processing conditions, such as tempering, shearing, and seeding, could successfully retard oil migration (Dhonsi & Stapley, 2006; Kinta & Hartel, 2010; Maleky & Marangoni, 2011; Marty & Marangoni, 2009; Richter & Sollich, 2009). For example, Maleky and Marangoni found that the structure of cocoa butter crystallized under shearing conditions creates a narrow and circuitous path for liquid oil, which reduces the rate and amount of oil migration in cocoa butter (Maleky & Marangoni, 2011).

Overall, better management of processing conditions during manufacturing can help the formation of better structures in cocoa butter for preventing quality loss in chocolate products (Marty & Marangoni, 2009; Sonwai & Mackley, 2006).

1.3.2.3 Storage temperature

An appropriate storage temperature is important to product quality. The storage temperature can affect the liquid fraction in the crystallized cocoa butter system. Many studies have shown significant effects of storage temperature on oil migration (Ali et al., 2001; Jinap et ak., 2000; Khan & Rousseau, 2006; McCarthy & McCarthy, 2008; Miquel

et al., 2001; Vernier, 1997; Ziegleder & Moser, 1996). The most common storage temperature for cocoa butter is around 18℃-23℃. In most of the studies, the analyzed temperatures ranged from 18℃-35℃ in order to investigate the acceleration of oil migration in higher storage temperature (Guiheneuf et al., 1997; Jinap et al., 2000; Miquel et al., 2001). However, some studies worked on the impact of low temperature on oil diffusivity (Khan & Rousseau, 2006; Ziegleder & Moser, 1996) and showed that low temperature could effectively prevent or slow down oil migration.

1.3.3 Measurement

In order to study the mechanism of oil migration in fat systems, accurate measurements for migrated oil is needed. Many methods have been developed to monitor the migration of oil, including chromatographic techniques, scanner imaging techniques (dyes-tracker method), and Magnetic Resonance Imaging (MRI) measurement. In this study, Magnetic Resonance Imaging technique is utilized.

MRI is one of the applications of magnetic energy. It is widely used in medical treatment for human brain. Moreover, MRI can be also considered as a semi-quantitative technique to monitor and observe the oil migration in same sample by time (Choi et al., 2005; Maleky et al., 2012; Rumsey & McCarthy, 2012). Conventional spin-echo MRI is recognized as an indirect means to measure. Under the magnetic field, the signal of H^1 protons are very short in solid cocoa butter fat because the spin-echo relaxation is strongly enhanced by slow rotational motion of TAG molecules(Guiheneuf et al., 1997). MRI provides a reliable way to non-destructively measure oil migration and to track the real-time movement of oil with reduced variation (Guiheneuf et al., 1997).

MRI has been successfully used in many studies to accurately monitor the oil movement in fat systems (Choi et al., 2005; Guiheneuf et al., 1997; Lee et al., 2010; Maleky et al., 2012; McCarthy & McCarthy, 2008; Rumsey & McCarthy, 2012). For example, the oil migration between a layer of hazelnut oil filling and a layer of dark chocolate was investigated using MRI by Guiheneuf et. al. (1997). They observed substantial differences of migration between the storage conditions of 19 ℃ and 28℃. Paluri and others pointed out that MRI can be used to analyze the moisture uptake in lipid based products (Paluri et al., 2015). Rumsey (2012) and Choi (2005) reported a reasonably good agreement between the Fickian mathematic model curves and MRI experimental data. McCarthy and others studied the diffusivity of oil from peanut butter paste to the chocolate with different formulations and documented the significant impact of chocolate formulations (McCarthy & McCarthy, 2008).

Thus, MRI is a non-distortive and accurate technique that can monitor oil migration of samples over time. It's also the method used in this study.

1.3.4 Mechanism

Migration of oil in chocolate products can be considered as a diffusive process of oil among multiple layers (Maleky et al., 2012). A diffusive process is the movement of molecules from a region of high chemical potential (high concentration) to a region of low chemical potential (low concentration) (Crank, 1975). Oil diffusion is driven by the TAGs concentration gradient between two phases (Clercq et al., 2014; Ghosh et al., 2002; Ziegler, 2009). Fick's second law is a partial differential equation, describing how diffusion causes the concentration to change with time:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (1.1)$$

where C is the foreign oil content in the case of oil migration and D is the diffusion coefficient. This partial differential equation (eq. 1.1) is subject to appropriate initial and boundary conditions for diffusion into a fat layer. With the appropriate initial conditions and boundary conditions offered by Peppas (1994) in a similar situation (binary mixture), the ratio of oil uptake and the uptake as time approaches infinity could be approximated to the following Fickian model:

$$\frac{M_t}{M_\infty} = 1 - \sum_{q-0}^{\infty} \frac{8}{(2q+1)^2 \times \pi^2} \exp\left[\frac{-D(2q+1)^2 \times \pi^2}{l^2} t\right] \qquad (1.2)$$

where M_t is defined as the oil uptake at time t and M_∞ is the maximum oil uptake after the migration reaches equilibrium, t (seconds) is time, D_{eff} ($m^2 s^{-1}$) is the effective diffusion coefficient among the migration behavior, and l (m^2) is the thickness in migrated media where oil migration took place.

Ziegleder proposed another Fickian approximate equation that describes the oil migration in chocolate system (1998):

$$\frac{M_t}{M_\infty} = \frac{KA\sqrt{D_{eff} t}}{V} \qquad (1.3)$$

where M_t and M_∞ are mass migrated at time t and at saturation respectively. A (m^2) is the contact area between two phases while V (m^3) represents the volume through which the diffusion takes place; t is the migration time (seconds). D_{eff} ($m^2 s^{-1}$) is the effective diffusion coefficient among the migration behavior. K is a constant specific to two phases.

Both Fickian diffusion models were successfully utilized to understand this 2-way diffusive process in fat systems (Choi et al., 2005; Clercq et al., 2014; Galdámez et al., 2009; Ghosh et al., 2002; Lee et al., 2010; Maleky & Marangoni, 2011; Maleky et al., 2012; Marty & Marangoni, 2009; McCarthy & McCarthy, 2008; Peppas & Brannon-Peppas, 1994; Rumsey & McCarthy, 2012).

CHAPTER 2. MATERIAL AND METHOD

2.1. Material

Five different cocoa butter samples from five different origins (Ivory Coast (IVC), Indonesia (IND), Brazil (BRZ), Venezuela (VEN), and Ecuador (ECU)) were donated by ADM Cocoa (Milwaukee, WI, USA) and Mars Chocolate (Hackettstown, New Jersey, USA). Interesterified hydrogenated palm oil (IHPO) was donated by Bunge Canada (Toronto, Canada). Peanut oil was purchased from a local retailer.

2.2. Sample preparation

Cocoa butter samples were prepared in triplicate through two processing conditions: static crystallization (control, marked as C) and processed crystallization (mixture of shearing and different cooling rate were applied, marked as P). Samples were heated to 70 ℃ first for 10 min and held at 65 ℃ for 45 min until completely melted in order to destroy crystal memory. For the control samples, the molten cocoa butter at 65 ℃ were transferred to a plastic mold with a dimension of 30 mm x 30 mm x 30 mm. Samples were stored at 23 ℃ for 24 h to complete the crystallization. For processed samples, the molten cocoa butter was sheared by a 3-blade propeller (with a radius of 39 mm) at a shear rate of 250 s^{-1}. While shearing, an iso-temperature water bath was used

to control the sample temperature at 23 ℃. After 10 minutes of shearing, samples were transferred to the plastic mold and stored at 23 ℃ for 24 h.

A soft fat layer (acting as the source of liquid oil for the oil migration experiment) was prepared by blending peanut oil and IHPO at the ratio of 60:40 w/w (S Marty et al., 2005). The cream was prepared five days prior and was stored in a 5 °C incubator (MIR-154 Panasonic, Gunma, Japan).

In the oil migration experiment, a two-layer model (shown in Figure 6), with crystallized cocoa butter on the top and cream at the bottom was used to mimic the multiple-compound chocolate product. Care was taken to ensure that the surface of cocoa butter and cream was flat. Intimate contacts were obtained at the interface between cocoa butter layer and the cream layer. 2-layer samples were visualized under Magnetic Resonance Imaging (MRI) and intimate contracts were examined. Samples with bad contact (shown in Figure 7) were removed. Oil migration was monitored at the interface of this two-layer model.

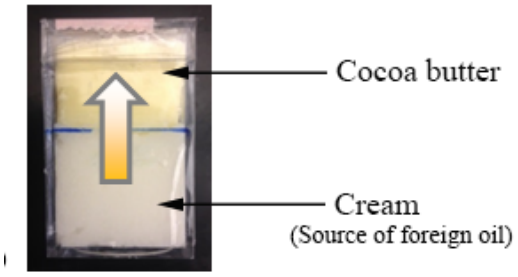


Figure 6. Sample mold setup for the MRI experiment (The upper part of mold was the cocoa butter mixtures from different origins with different processing; the bottom part was filled with 30 grams of the cream)

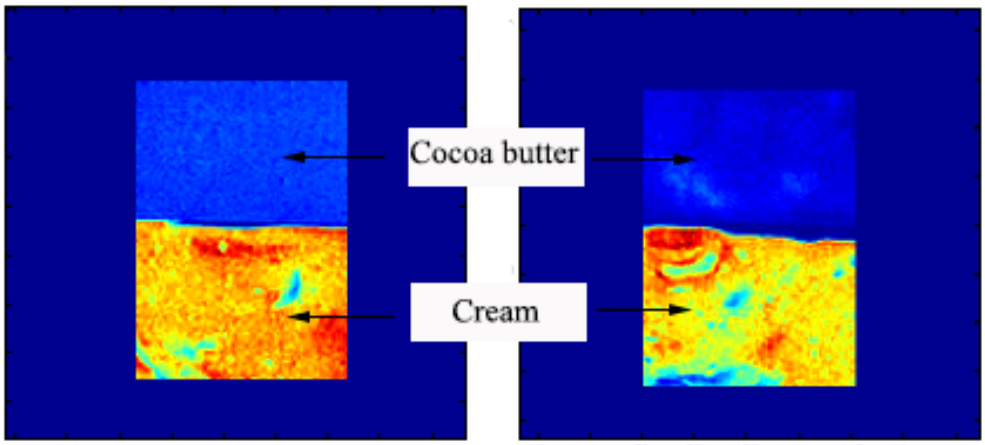


Figure 7. Representative MRI images for the sample with bad contact

2.3. Method

2.3.1. Fatty acid composition evaluation

The fatty acid profile of cream and five different cocoa butter samples were determined by an Agilent 6890-series Gas Chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) with 7683-series auto-sampler. Fatty acid methyl esters (FAME) were obtained according to a method described by Christie (1982). An amount of 20 mg of the fat (cocoa butter) were weighed and transferred into vials. Samples were mixed with sodium-dried diethyl ether and methyl acetate until it was completely dissolved. Then 1 M sodium methoxide in dry methanol was added to the mix. After 5 minutes, a saturated solution of oxalic acid in diethyl ether was added and then samples were centrifuged at about 1500 g for 2 minutes. The supernatants were transferred to a 1.5 ml Agilent vial. Nitrogen was used to gently evaporate the solvent. At the end, 1 ml of

hexane was added to the material left in the vial. An Agilent 6890 GC (Agilent Technologies Inc., Santa Clara, CA) was used with a split injection, a flame ionization detector (FID), auto sampler (Agilent, model 7683), and BPX70 column which has 60 m x 0.22 mm internal diameter with 0.25 µm film thickness (SGE Inc., Austin, TX, USA). Oven temperature was programmed to increase from 110 to 230 °C at a rate of 4 °C/min and maintained at 230 °C for 18 min. The injector was operated at 20.2 psi flow of 17.7 ml/min at a temperature of 250 °C. Helium was used as a carrier gas at an average velocity of 25 cm/s. The FAMEs were identified based on their retention times in comparison with fatty acid methyl ester standards purchased from Sigma Aldrich.

2.3.2. Triacylglyceride Composition

The triacylglyceride composition of cocoa butter was determined by high performance liquid chromatography (HPLC) using a Waters Alliance model 2690 (Waters Millipore Co., Milford, MA) with refractive index detector (Waters model 2410). The columns were Waters xbridge C18 (5 µm, 4.6 X 250 mm).

An amount of 30 mg fat was first dissolved in 600 µl of chloroform followed by1.5 ml of 60/40 acetone/acetonitrile v/v. 10 µl of the sample was injected at once. The temperature for the sample chamber, column, and detector was set at 40 °C. Measurement was taken under isocratic flow rate at 1 ml/min with the mobile phase of acetone/acetonitrile at the ratio of 60/40 v/v.

The assignment of TAG peaks and their concentrations in cocoa butter were determined by the retention time of TAG standards from Sigma (Sigma Chemical Co., St.

Louis, MO) and both data collection and peak integration was performed with the Millennium software (Waters Corporation, Milford MA).

2.3.3. Solid Fat Content measurement

The solid fat contents (SFC) of all the prepared cocoa butter samples were measured after a 24-hour storage using Bruker Minispec spectrometer (Bruker Optics Ltd., ON, Canada). Approximately 3 grams of crystallized cocoa butter was cut into small pieces and placed into NMR glass tubes (10 mm diameter, 1 mm thickness, and 180 mm height). Measurements were taken at 23 ℃.

2.3.4. Polymorphic phase analysis

All of the samples' polymorphic behavior was studied using powder X-ray diffraction (XRD) techniques. Rigaku Multiplex powder X-ray diffractometer (Rigaku, Japan) set at reflection mode with a 1.25deg divergence slit, a 1.25deg scatter slit, and a 0.3 mm receiving slit was used. Approximately 0.3 g of the crystallized sample was placed onto a 1 mm glass X-ray slide with 0.5 mm sample cell. Angular scanning was performed at 2 deg/min from 10 to 30 deg 2-theta at 40 kV accelerating voltage and 15 mA current. Measurement was performed in triplicate. The results were analyzed using PDXL version 2.0 (Rigaku, Japan).

2.3.5. Melting profile analysis

The melting profiles of crystallized cocoa butter samples were studied by Differential Scanning Calorimetry Q2000 (DSC) (TA Instrument, New Castle, DE, USA). Approximately 10 mg of cocoa butter samples was placed in Tzero sealed aluminum pans

and then transferred to the DSC cell. A sealed empty pan was weighed and placed into the DSC cell as reference. The cell was heated from 15 ℃ to 45 ℃ at a rate of 1 ℃/min. Melting properties were analyzed by TA Instruments Universal Analysis 2000 (TA Instrument, New Castle, DE, USA). The apex of the melting peak was considered as the melting point of the sample.

2.3.6. Microstructure analysis

A polarized light microscope (PLM) was used to investigate the microstructures of the samples. A small piece of molten cocoa butter (control) or processed semi-solid fat (processed) was placed on a pre-heated (T= 65 ℃) glass slice. Cover slip was laid over the drop to remove the air and spread the fat to a thinner layer (a thinner fat layer enabled better observation of microstructure). Microscope slices were transferred to 23 ℃ incubators and stored for 24h. The images of samples were taken using a Carl Zeiss microscope (Carl Zeiss Microscopy GmbH, Germany) equipped with a digital color and monochrome camera. Images were acquired using 20X and 50X objective lens with AxioImager software (Carl Zeiss). Images under 20X were used in particle analysis. Particle diameter was obtained by analyzing the PLM images using Image J 1.49 (National Institutes of Health) and PeakFit v4.12 (SigmaPlot, Systat Software Inc. U.S.). Particle distributions were fitted in Gaussian smoothing (Chantrell, Popplewell, & Charles, 1978; Maleky & Marangoni, 2008; Marty et al., 2005; Peters, 2001). The fractal dimension of cocoa butter crystals were acquired by the analysis of PLM images. Adobe Photoshop CS6 software (Adobe System Inc., San Jose, CA, USA) was utilized for the threshold of the image. Then the 2d fraction dimension was calculated by Benoit 1.3

(TruSoft International Inc. St. Petersburg, FL, USA) using the box counting method. For the purpose of further analysis, 2d fractal dimension obtained from the box-counting method was converted to a 3d fractal dimension: $D_{3d} = D_{2d} + 1$(Maleky & Marangoni, 2011).

2.3.7. Magnetic Resonance Imaging measurement

Magnetic resonance imaging (MRI) was used to measure oil migration from the cream to the cocoa butter layer. The advantages of MRI include the ability to measure migration non-destructively and to track the transport of oil in a single sample, avoiding problems of sample-to-sample variation. Conventional spin-echo MRI detects signals from liquid lipid protons only (Thierry M Guiheneuf et al., 1997) and hence it is an indirect measure of oil migration.

The MRI measurement was performed using a 7 Tesla human MRI Philips (Cleveland, Ohio) scanner. The images were acquired with a 32-channel head coil. A multi-slice T1 weighted spin-echo method was selected for the acquisition with the following parameters: relaxation/echo time 1000/9.5 ms, field of view 170 x 75 mm, acquisition matrix 680 x 308, in-plane resolution 0.25 x 0.24 mm, slice thickness 2 mm without inter-slice gap, total acquisition time 60 min. 62 slices were obtained from the sample cell with 12 slices for one sample. Among the 12 slices of each sample, the slices at the edge of the sample were not be used. Thus, about 6 slices with clear interface were used for image analysis. Measurement was taken at 0, 3, 7, 14, 21, 56, 77, 98, and 134 days to monitor oil migration during storage. In order to compare day-to-day variations, any spatial difference in the position of the samples were fixed to the reference image

using FLIRT (which is a part of the FMRIB Software Library) (Jenkinson, Bannister, Brady, & Smith, 2002; Jenkinson & Smith, 2001). Image analysis and data analysis were performed with Matlab 2013b (The Mathworks, Inc., Natick, MA, USA).

Representative MRI images of one sample (IVC) at different time points were shown in Figure 8. Specifically, Figure 8a-c show the two-dimensional images of this 2-layer sample taken at day 0, day 14, and day 134 respectively. The color bar on the right side indicates the signal intensity. The higher signal intensity represents the cream and the lower signal intensity represents the crystallized fat system.

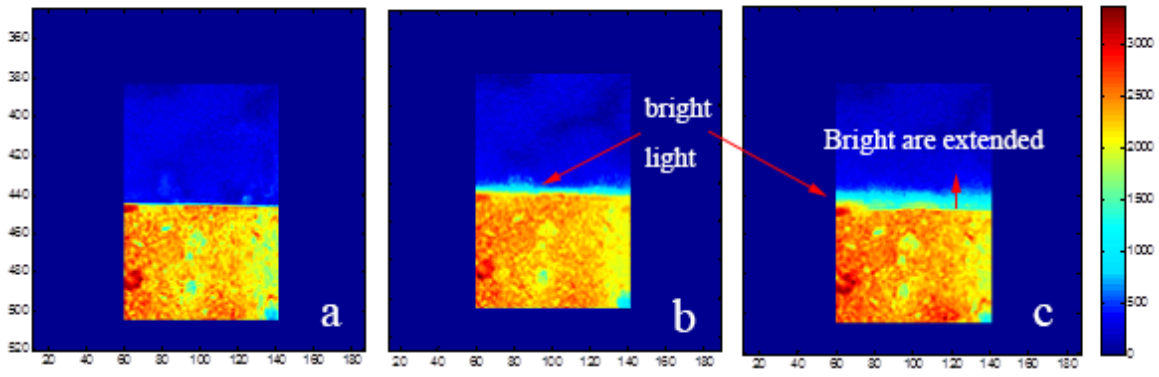


Figure 8. Representative MRI image for the sample scanned at day 0 (a), day 14 (b) and day 134 (c) of storage at 23 ºC

As shown in Figure 8a, the clear interface between the 2 layers indicates that no oil migration occurred at day 0. On day 14 (Fig. 8b), a bright light was observed near the interface on the side of cocoa butter (top layer). The bright light indicates an increase in signal intensity at the contact area of two layers and is related to the occurrence of oil migration. Over the experimental period of 134 days (Fig. 8c), the bright area became broader and extended from the interface toward the crystalized cocoa butter. This extension of the bright area indicates further oil migration over time.

Acquired signal intensity (SI) values for all the samples were rotated counter-clockwise by 90° and plotted in Figure 9. This figure shows the SI changed over time during storage at 23 ℃. X-axes stand for the distance in sample from cocoa butter (0–31 pixels) to cream (31–45 pixels) for each sample. As seen in Figure 9a, the two regions are distinguishable by the vertical line at the 31st pixels. Using the area under the curve (AUC) of the signal intensity in the crystallized fat region, the extent of the sample's oil migration was characterized. The difference between the value of AUC at t=0 and t=t were quantified as the oil mass uptake (M_t) (Ziegleder & Moser, 1996; Ziegleder & Schwingshandl, 1998). Figure 9b shows the one-dimension signal intensity profile of a representative sample during the experimental period over 134 days. As shown in this figure, the area gradually increased over time, which indicates an increase in degree of liquid oil migration into the crystallized fat.

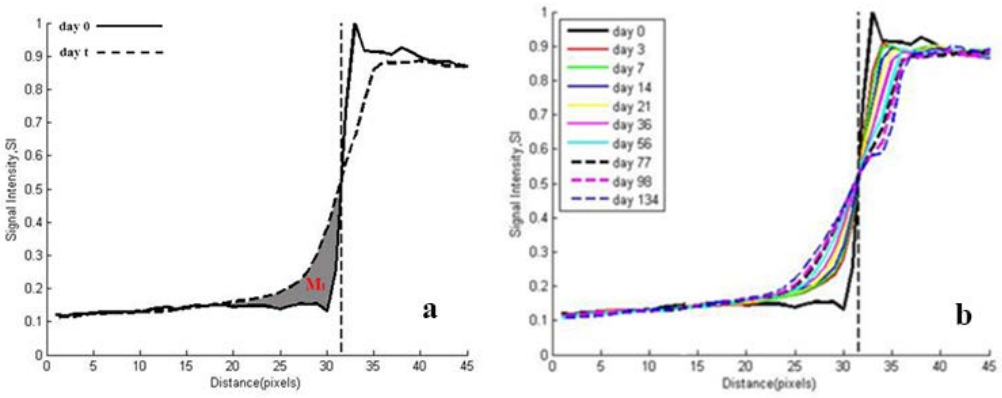


Figure 9. a) Representative 1-D Signal intensity profile of the sample in vertical direction from top (cocoa butter) to bottom (cream); b) 1-D Signal intensity profile of the sample over time

2.4. Statistical analysis

Samples were analyzed in at least triplicate. Data analysis was conducted using Statistic Analysis System (SAS) 9.4 (SAS institute Inc. Cary. NC, U.S.A.). One-way ANOVA and T-test were applied to determine the significance of difference. Differences were considered significant if $p<0.05$.

CHAPTER 3. RESULT AND DISCUSSION

3.1. Result and discussion for different properties of crystallized cocoa butter

According to previous studies (Chapter 1), chemical compositions of cocoa butter vary depending on their geographic locations. In order to understand the effects of small differences in chemical compositions on oil diffusivity in the cocoa butter system, different properties of cocoa butter were studied.

3.1.1. Fatty acid evaluation

Fatty acid profiles for all the samples were measured using gas chromatograph and the results are shown in Table 2. As seen in this table, palmitic acid, stearic acid and oleic acid were the major fatty acids in the cocoa butter samples which account for about 26%, 35% and 33% of total fatty acid, respectively. The content of saturated and unsaturated fats in the cocoa butter samples were around 62% and 36%, respectively. The BRZ sample has the lowest saturated fat content and the highest unsaturated fat content compared to the other samples. It is interesting to note that the saturated fat content of the BRZ and the IND samples were significantly lower ($p<0.05$) compared to the IVC, VEN and ECU samples. Miyasaki (2015) who studied the chemical composition of cocoa butter from Brazil reported that the content of unsaturated and saturated fats were about 60% and 39%, respectively.

From the fatty acid composition analysis, the cocoa butter samples from different origins marked significantly different ($p<0.05$) in the major types of fatty acids. For saturated fatty acids, the ECU sample has the highest palmitic acid content but lowest in stearic acid content compared to other samples. On the other hand, the IVC sample was significantly lower in palmitic acid content but highest in stearic acid content. Interestingly, the VEN sample was second highest in both palmitic and stearic acids. The IND sample has significantly lower palmitic acid but was higher in stearic acid. BRZ sample was significantly lowest in both palmitic and stearic acids. Lehrian and Keeney (1980) stated that cocoa grown at lower temperatures contains less saturated fatty acids than those developed at higher temperatures.

Table 2. Fatty acid composition of the Cocoa butter from Ivory Coast (IVC), Indonesia (IND), Brazil (BRZ), Venezuela (VEN), and Ecuador (ECU)

	Saturated fat (%)	Unsaturated fat (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)
IVC	63.8 ^{a}	35.9 ^{d}	25.6 ^{bc}	36.6 ^{a}	32.7 ^{d}	2.8 ^{d}
IND	62.6 ^{c}	37.0 ^{b}	25.7 ^{bc}	35.2 ^{b}	33.7 ^{b}	2.9 ^{c}
BRZ	59.9 ^{d}	39.7 ^{a}	25.1 ^{c}	33.3 ^{c}	35.9 ^{a}	3.5 ^{a}
VEN	63.5 ^{b}	36.2 ^{c}	26.6 ^{b}	35.2 ^{b}	33.1 ^{c}	2.8 ^{d}
ECU	63.6 ^{ab}	36.1 ^{cd}	29.2 ^{a}	32.9 ^{c}	32.4 ^{e}	3.2 ^{b}

* Low case letters (a, b, c …) represent statistically significant differences of different compounds among the samples from different origins ($p<0.05$).

The BRZ sample also contained superior amounts of oleic (35%) and linoleic acid (3.5%) content. Gunstone and Harwood (2007) also reported that cocoa butter produced in South America contains levels of oleic acid and linoleic acid (generally >3 %) significantly higher than those from Africa and Asia. Cocoa butter from Brazil was often reported unsatisfied crystallization (Lipp & Anklam, 1998). It is interesting to note that, although ECU has the lowest oleic acid content, it has second highest amount of linoleic acid content compared to the other samples. On the other hand, the IND sample was the second highest in oleic acid content but significantly lower in linoleic acid content compared to the other samples. The IVC and VEN samples were among the lowest in both oleic and linoleic acid contents.

3.1.2. Triacylglyceride Composition

Triacylglyceride (TAG) content, as another index of chemical composition of cocoa butter, was studied and reported in Table 3. The TAGs profile of the IVC, IND, BRZ, and ECU samples were summarized from the work of Marty (2009) who shared the same source of raw materials with this study. As shown in Table 3, the cocoa butter samples were mainly composed of symmetrical TAGs with oleic acid in the 2-position: 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1-palmitoyl-2- oleoyl-3-estearoyl-glycerol (POSt), and 1,3-diestearoil-2-oleoyl-glycerol (StOSt). They contained only trace amounts of the unsymmetrical triglycerides (PPO, PStO, and StStO).

The TAGs can be classified into four main groups of TAGs: tri-saturated SSS, symmetric di-saturated-mono-unsaturated SUS, mono-saturated-di-unsaturated UUS, and tri-unsaturated UUU (as summarized in Table 3 (where S = saturated, U = unsaturated)).

The VEN sample was highest in total SSS (2.5%), followed by IND (1.6%), ECU (1.2%), IVC (1.1%), and BRZ (0.8%). Total SUS TAG content were higher in IVC sample (93.0%), IND sample (93.0%), and ECU sample (92.5%), followed by VEN (85.3%) and BRZ (85.2%). Total tri-unsaturated UUU content were highest in the BRZ sample (5.6%) followed by ECU (3.0%), IVC (2.4%), IND (2.4%), and VEN (0.5%). However, the symmetrical TAGs varied differently in different samples. For example, the VEN sample contained unusually high in POSt (41.2%) compared to others in the range of 36.9% (IND) to 37.3% (ECU and IVC).

Table 3. Triacylglyceride (TAG) composition, Phosphorous, and Free fatty acid content of the Cocoa butter from Ivory Coast (IVC), Indonesia (IND), Brazil (BRZ), Venezuela (VEN), and Ecuador (ECU)

	IVC [1]	IND [1]	BRZ [1]	VEN	ECU [1]
OOL (%)	0.6 ± 0.20	1.0 ± 0.30	0.5 ± 0.10	0.0 ± 0.00	1.1 ± 0.50
PLP (%)	1.5 ± 0.50	1.2 ± 0.40	1.5 ± 0.10	1.7 ± 0.14	1.3 ± 0.50
OOO (%)	1.8 ± 0.50	1.4 ± 0.30	5.1 ± 0.50	0.5 ± 0.09	1.9 ± 0.90
POP (%)	24.3 ± 0.60	22.7 ± 0.50	22.8 ± 0.40	17.8 ± 0.03	24.1 ± 1.10
StOO (%)	2.1 ± 0.50	1.8 ± 0.40	7.0 ± 0.20	3.7 ± 0.02	2.1 ± 0.80
POSt (%)	37.3 ± 1.80	36.9 ± 1.90	34.1 ± 0.50	41.2 ± 0.19	37.3 ± 1.80
PPSt (%)	0.1 ± 0.10	0.1 + 0.10	0.1 ± 0.10	0.4 ± 0.05	0.1 ± 0.10
StOSt (%)	31.4 ± 0.80	33.4 ± 0.60	28.3 ± 0.20	26.3 ± 0.03	31.1 ± 1.80
StStP (%)	0.3 ± 0.10	0.4 ± 0.10	0.1 ± 0.10	0.5 ± 0.02	0.3 ± 0.20
StStSt (%)	0.7 ± 0.30	1.1 ± 0.50	0.6 ± 0.10	1.6 ± 0.07	0.8 ± 0.50
SSS (%)	1.1	1.6	0.8	2.5	1.2
SUS (%)	93.0	93.0	85.2	85.4	92.5
UUS (%)	2.1	1.8	7	3.67	2.1
UUU (%)	2.4	2.4	5.6	0.5	3
Phosphorus (ppm)	8.0	11.6	75.0	N. A.	4.3

[1] Marty, S., & Marangoni, A. G. (2009). Effects of cocoa butter origin, tempering procedure, and structure on oil migration kinetics. *Crystal Growth and Design*, *9*(10), 4415–4423.

[2] P = palmitic acid, O = oleic acid, and St = stearic acid.

[3] SSS = tri-saturated TAGs (PPSt + StStP + StStSt); SUS = symmetrical di-saturated-mono-unsaturated TAGs (POP + StOSt + POSt); UUS = mono-saturated-di-unsaturated TAGs (StOO); UUU = tri-unsaturated TAGs (OOO+OOL);

In addition, Lonchampt and Hartel (2004) and Shukla (2006) analyzed cocoa samples from different origins and found that Brazilian cocoa butter showed the highest content of UUS, which corroborates the results presented here. The StOO content of the BRZ sample was obviously highest at 7% compared to other origins that ranged from 1.8% (IND) to 3.7% (VEN). The UUS TAGs have melting points between 27 °C and 42 °C that are responsible for structural and sensorial properties at room temperature. The ratio between SSU and UUS contents in cocoa butter is selectively associated with technological functionality used by the industry (Bessler & Orthoefer, 1983). According to Lipp and Anklam (1998), cocoa butter with lower SSU and higher UUS levels, such as the Brazilian cocoa butter, can lead to unsatisfactory crystallization characteristics.

Beside the fatty acids and the TAGs of cocoa butter, minor compounds like phospholipids also influence the cocoa butter network (Foubert & Vanrolleghem, 2004). Phospholipids are an important minor component in cocoa butter and the contents of phospholipid vary due to the different growth conditions.

3.1.3. Solid fat content study

The solid fat contents (SFC) for cocoa butter samples were measured using pulsed nuclear magnetic resonance (p-NMR) and results are reported in Table 4. The SFC for control samples were in the range of 61%-78%. We observed slight drops in the SFCs of processed samples in comparison with those of control samples, although the difference was not statistically significant. For the control samples, the BRZ sample had the lowest SFC (60.71%) while the VEN sample was highest in SFC on average (78.30%). Moreover, the IVC, VEN, and ECU samples have significantly higher SFC compared to

the IND and BRZ samples. A similar pattern was observed for processed samples but the differences in SFC between the VEN, ECU, and IVC samples were now apparent. The SFC of the VEN sample was significantly higher than ECU, but the SFC of the IVC sample has no significant difference with the VEN and ECU samples. Cocoa butter from South American regions close to the equator, such as Venezuela, were reported to be similar to those of African origin. However, Brazil is a cooler climate area and produces softer cocoa butter (Timms, 2012).

Table 4. Solid Fat Content (SFC) of cocoa butter samples from different origins under different processing conditions

	Control (%)	Processed (%)
IVC	76.56^{a,i}	75.69 $^{AB, i}$
IND	71.34 ^{b,i}	70.17 ^{C,i}
BRZ	60.71 ^{c,i}	59.39 ^{D,i}
VEN	78.30 ^{a,i}	77.95 ^{A,i}
ECU	75.7 ^{a,i}	74.15 ^{B,i}

* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).
** Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).
*** Low case of Roma letters (i, ii) represent statistically significant differences between control samples and processed samples in each origins ($p<0.05$).

The SFC is a parameter that expresses the solid/liquid mass relation of a fat at a specific temperature and is strongly influenced by the chemical composition of cocoa butter (Ribeiro et al., 2012). The relationship of SFC with the saturated fat content of the

samples is shown in Figure 10. The SFC of both the control and processed samples were directly correlated to the saturated fat content of the samples and can be seen across the origins with significantly high correlation coefficients. The correlation coefficient between the SFC and percent saturated fat content were at 0.982 and 0.973 for control and processed samples, respectively.

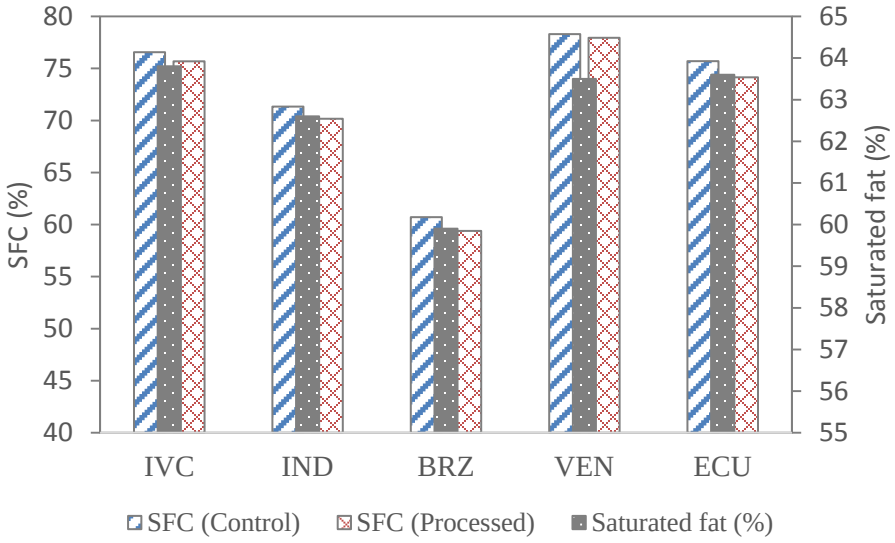


Figure 10. Solid fat content (SFC) of control and processed cocoa butter samples

It is interesting to note that the SFC of the VEN samples for both control and processed samples were significantly higher than others although its saturated fat content was significantly lower than IVC and not significant different than ECU. This observation can be attributed to TAG compositions which can provide more insight into the properties of the fat. The evidently high SFC of the VEN sample can be attributed to

its highest tri-saturated SSS TAG content (2.5%) compared to others in the range of 0.8% (BRZ) to 1.6% (IND). Analysis of the symmetrical SUS TAGs composition revealed that the VEN sample was higher in POSt (41.2%) compared to IVC (37.3%) and ECU (37.3%). According to Shukla (2006), the SFC is correlated with the symmetrical SUS TAGs presented in the cocoa butter composition and tends to increase with greater SSU content. However, this is not evident in this study. The VEN sample was significantly lower in total SSU (85.3%) compared to both IVC at 93% and ECU at 92.5%. Moreover, high SFC of VEN may be attributed to its trace amounts of tri-unsaturated TAGs UUU at only 0.47% compared to others at 2.4% (IVC, IND) to 5.6% (BRZ). In addition, small amount of phosphorus content in the VEN sample compared to others in the range of 4.3 ppm (ECU) to 75 ppm (BRZ) could be associated with the evidently high SFC in VEN sample.

The higher content of tri-unsaturated TAGs (UUU) in the BRZ samples (5.6%) compared to others in the range of 0.47% (VEN) to 3% (ECU) can potentially explain the lower SFC in the BRZ samples compared to others. Marty (2009) also documented a lower value of SFC in the Brazilian samples compared with the cocoa butters from other sources. Lipp and Anklam reported that higher percentages of POO and StOO were characteristic of cocoa butter with superior softness and can lead to unsatisfactory crystallization characteristics (Lipp and Anklam, 1998). The BRZ sample has enormously high StOO (7%) compared to others in the range of 1.8% (IND) to 3.7% (VEN). High content of this mono-saturated TAG can interrupt the molecular packing of the symmetrical TAGs and providing a superior softness to the cocoa butter (Foubert et al., 2004). Moreover, abundant phospholipids were observed in the BRZ samples and may be

related to its evidently low value of SFC. Studies showed that the level of phosphorus has a significant impact on cocoa butter solidification (Arruda & Dimick, 1991; Foubert & Vanrolleghem, 2004; Lechter, 2012; Marty & Marangoni, 2009).

Interestingly, the SFC of the IND sample was rather low compared to others, regardless of the BRZ sample. The TAG analysis revealed that its total tri-saturated TAG content was rather high (SSS at 1.6%) and its total SSU (93%) was highest among others. Further analysis of the tri-unsaturated TAGs showed similar content with the IVC sample at 2.4%. The low amount of SFC could be attributed to much a higher content of phosphorus in the IND sample at 11.6 ppm compared to IVC (8 ppm) and ECU (4.3 ppm).

IVC and the ECU samples showed comparable content of SSS, SSU, and UUS TAGs. This can be explained by the similar SFC in both control and processed samples.

3.1.4 Polymorphic phase

Polymorphism behaviors of the samples were studied using X-ray diffraction (XRD) analysis. The representative XRD patterns for control and processed samples after crystallization for 24 hours are shown in Figure 11a and 11b, respectively.

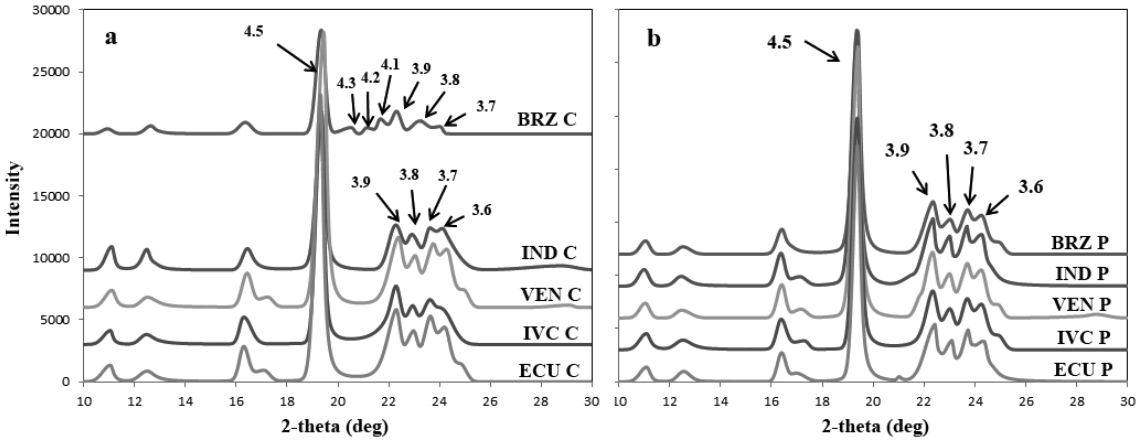


Figure 11. X-ray diffraction (XRD) patterns of control (a) and processed (b) cocoa butters samples crystallized at 23℃ for 24 h

For control samples except for the BRA sample, one strong peak at 4.5 Å and four small peaks at 3.9 Å, 3.8 Å, 3.7 Å, 3.6 Å were observed, which indicate the β_V polymorphic form (Wille & Lutton, 1966b). β_V is a stable polymorphic phase for cocoa butter which provides desirable thermal properties and rheological properties (Beckett, 2000; Talbot, 2012a).

For BRZ control samples, a strong peak at 4.5 Å (19.3 2θ) and six small peaks at 4.3Å, 4.2 Å, 4.1 Å, 3.9 Å, 3.8 Å, and 3.7 Å were observed. This pattern indicates a

mixture of β′(form III and form IV) and β_V polymorphic form (Ali, 2013; Jun-Hyun & Swanson, 2006; Wille & Lutton, 1966b). Previous studies illustrated that cocoa butter, in the absence of processing, initially crystallizes in unstable polymorphic forms, β'_{III} and β'_{IV}, and then transforms to a more stable form, β_V (Sonwai & Mackley, 2006; Wille & Lutton, 1966b). As shown in Figure 11, the Brazilian control sample in this experiment was still in the transition from β′ to β_V. The slow crystallization of the Brazilian sample could be due to low saturated fat content and high unsaturated fat content as discussed earlier. Chaiseri and Dimick also reported slow crystallization in Brazilian cocoa butter compared to the cocoa butter from Ivory Coast and Malaysia. (Chaiseri & Dimick, 1995) The transition from unstable form (β′) to stable form (β_V) as the crystallization continues results in changes in SFC and crystalline alignment (Beckett, 2000; Bouzidi & Narine, 2012; Alvarado et al., 2004; Sasaki et al., 2012). The unstable crystalline phase can promote the oil migration process (Hartel, 1999; Wille & Lutton, 1966b). However, the effects of polymorphic forms on oil diffusivity are complicated and will not be investigated in this work. Thus, with a distinctly unstable polymorphic form, the Brazilian control samples will not be discussed in the oil migration analysis.

For processed samples, all of them were in β_V polymorphic form regardless of origins. This shows that processing accelerates crystallization process of cocoa butter and promotes stable polymorphic transition. Processing having similar effects on polymorphic transitions of cocoa butter has been reported by previous researches (Shi & Maleky, 2015).

3.1.5. Melting point

In order to study the samples' melting point, the melting profiles of samples were studied using differential scanning calorimetry (DSC). The representative melting profiles for both control and processed samples after 24-hours crystallization are shown in Figure 12a and 12b, respectively.

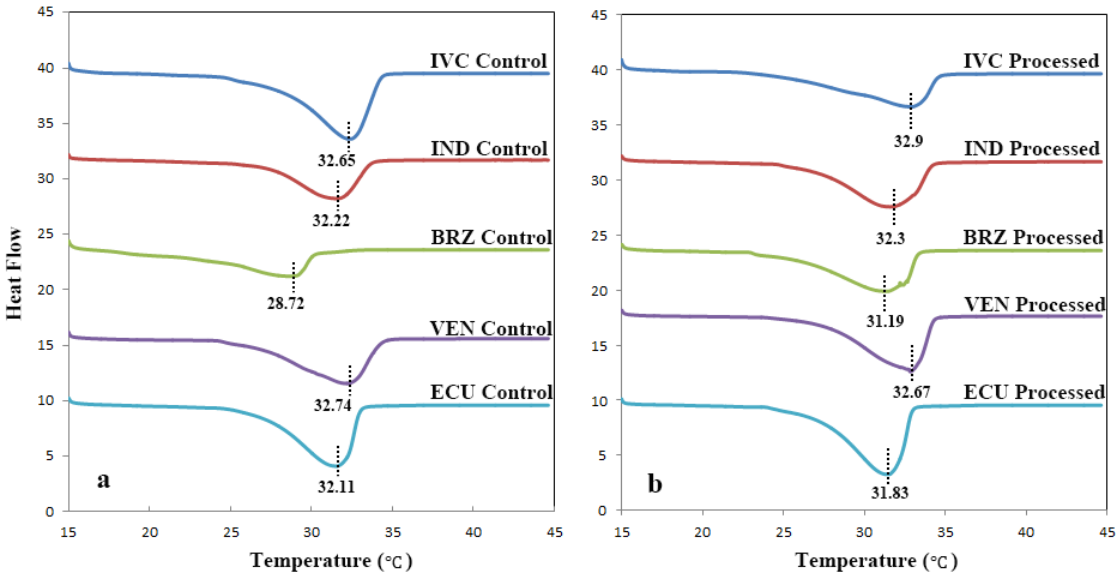
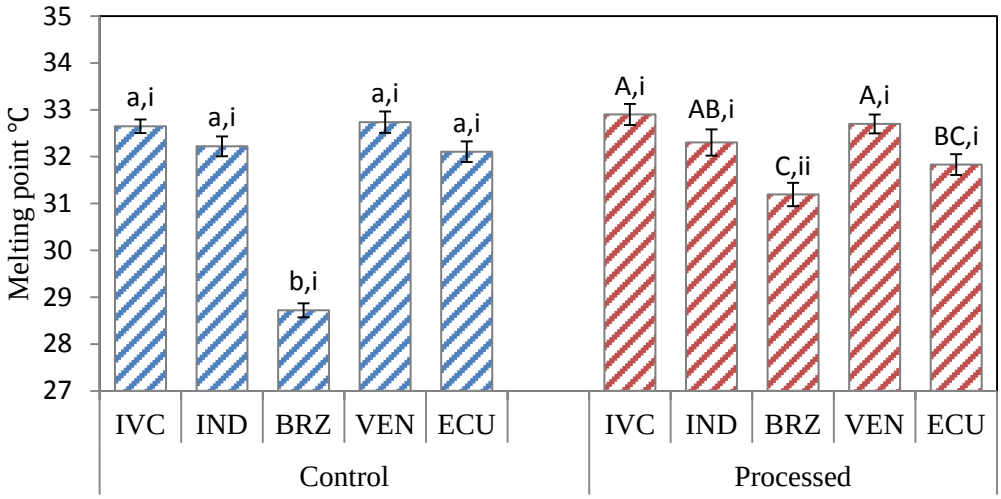


Figure 12. DSC heating curve of control (a) and processed (b) cocoa butter samples

The Brazilian control samples showed a lower melting point at 28.72 ℃ (Fig. 12a). However, the rest of the control samples (Fig. 12a) and all of the processed samples (Fig. 12b) have a melting point at around 32 ℃.

Melting points for all the samples were plotted and compared in Figure 13. As shown in Figure 13, the melting point of the BRZ control sample was significantly lower compared to the rest of control samples. For processed samples, the VEN and IVC samples had significantly higher melting points compared with the BRZ and ECU cocoa butters. The melting points for the IND samples were similar to those of IVC, VEN, and ECU, but significantly higher than those of the BRZ samples. The BRZ samples had the lowest melting points, possibly due to the higher content of unsaturated fat and lower content of saturated fat (seen in Table 2). Moreover, there was no significant difference between control and processed cocoa butters from IVC, IND, VEN, and ECU. The significant differences between the control BRZ samples and the processed BRZ samples was due to the different polymorphic phases.



* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).
** Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).
*** Low case of Roma letters (i, ii) represent statistically significant differences between control samples and processed samples in each origins ($p<0.05$).
Figure 13. Melting points for control and processed cocoa butter samples

3.1.6. Microstructure investigation

Previous studies have shown that a difference in SFC could result in a different microstructure of the fat crystal network (Narine & Marangoni, 1999). Regardless of processing conditions, SFCs are different in cocoa butters from different origins due to the effects of chemical composition (shown in Table 2). Moreover, studies have suggested that processing applied during cocoa butter crystallization can induce significant changes in the structure of cocoa butter (Acevedo et al., 2012; Sonwai & Mackley, 2006). In order to explore the effects of chemical characteristics and the impacts of processing conditions on the structure of cocoa butter, polarized light microscopy (PLM) was employed. PLM images showing the microstructure of cocoa butters are presented in Figure 14.

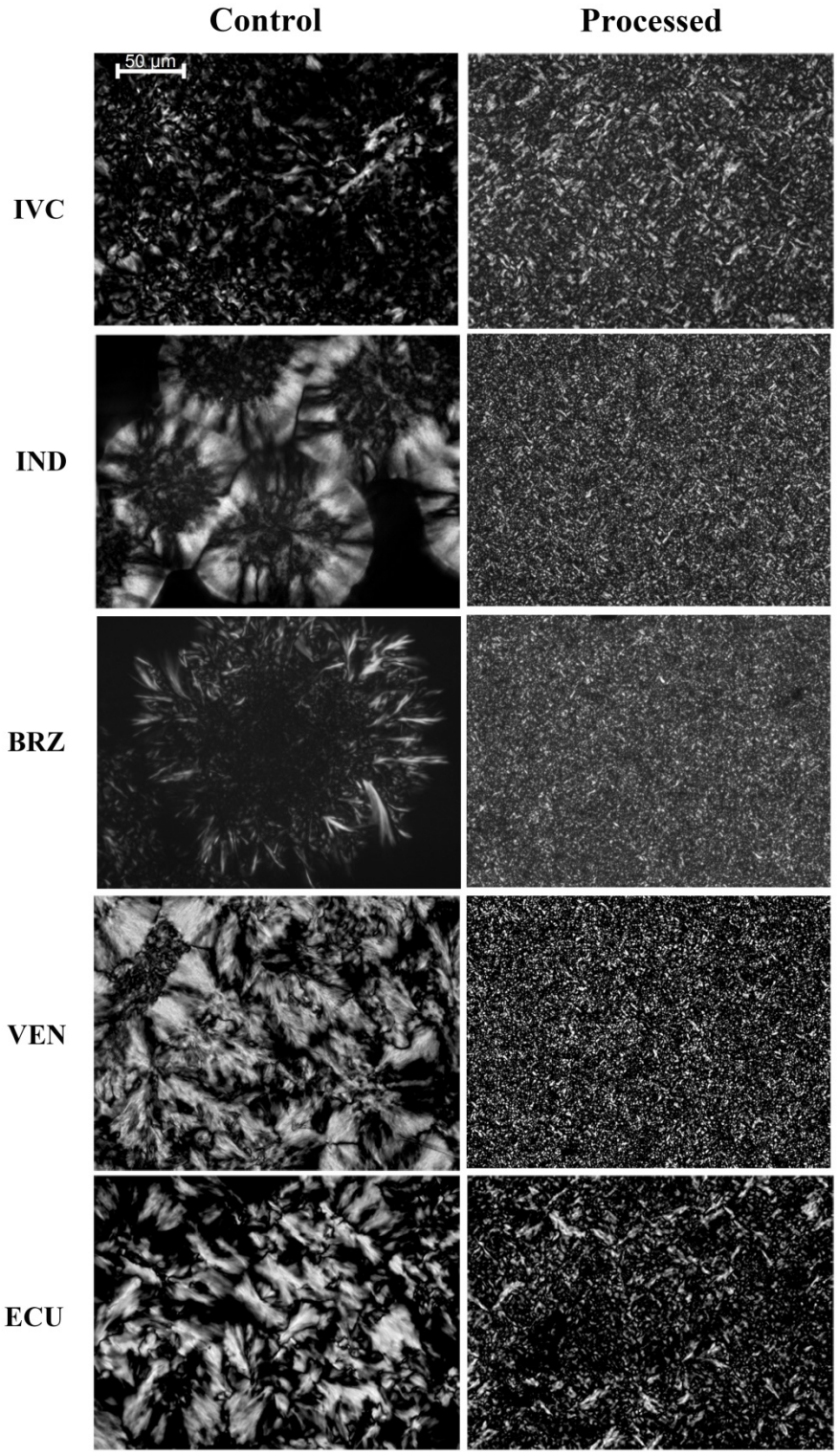


Figure 14. PLM micrographs for control and processed cocoa butter samples

In control samples, differences in microstructure were observed among the cocoa butters from different origins. The IVC sample, reported with the nucleation type of sporadic (Marty & Marangoni, 2009), exhibited heterogeneous microstructure with both small round crystals and medium size needle-like crystals. In the IND sample, large bulk crystals aggregated into spherical cluster; The BRZ sample exhibited needle-like crystal which joined together to form clusters. Less dense crystals were observed in the BRZ samples compared with the rest of control samples. VEN and ECU exhibited both large and small particles with bow-tie structure and spherical cluster.

However, as shown in Figure 14, processing applied during crystallization significantly changed the structure of the cocoa butter crystalline network into micro scale. Smaller particles with more homogeneous distribution can be observed in processed samples compared to the control samples. The shearing process during cocoa butter crystallization breaks the newly formed crystals and distributes them evenly throughout the system (Maleky & Marangoni, 2008; Stapley et al., 1999). They also attributed the high density of small and homogeneous networks in the sheared system to high rate of nucleation.

Within the processed samples, larger crystals were observed in the IVC and ECU samples compared with the others. On the other hand, no apparent morphological difference was observed among the IND, BRZ and VEN samples.

Besides morphological analysis, the PLM micrographs were quantified for crystal dimensions and frequency distribution, as reported in Figure 15. Wider crystal size distributions were observed in the control samples compared to the processed samples for all cocoa butter origins. The broad distribution of crystal diameters in the control samples

can be explained from the heterogeneous arrangement of the crystal network. On the other hand, narrower frequency distribution of crystal diameters in the processed samples suggests that the size of crystals centralized in smaller ranges and this match the observation of homogeneous distribution in Figure 14.

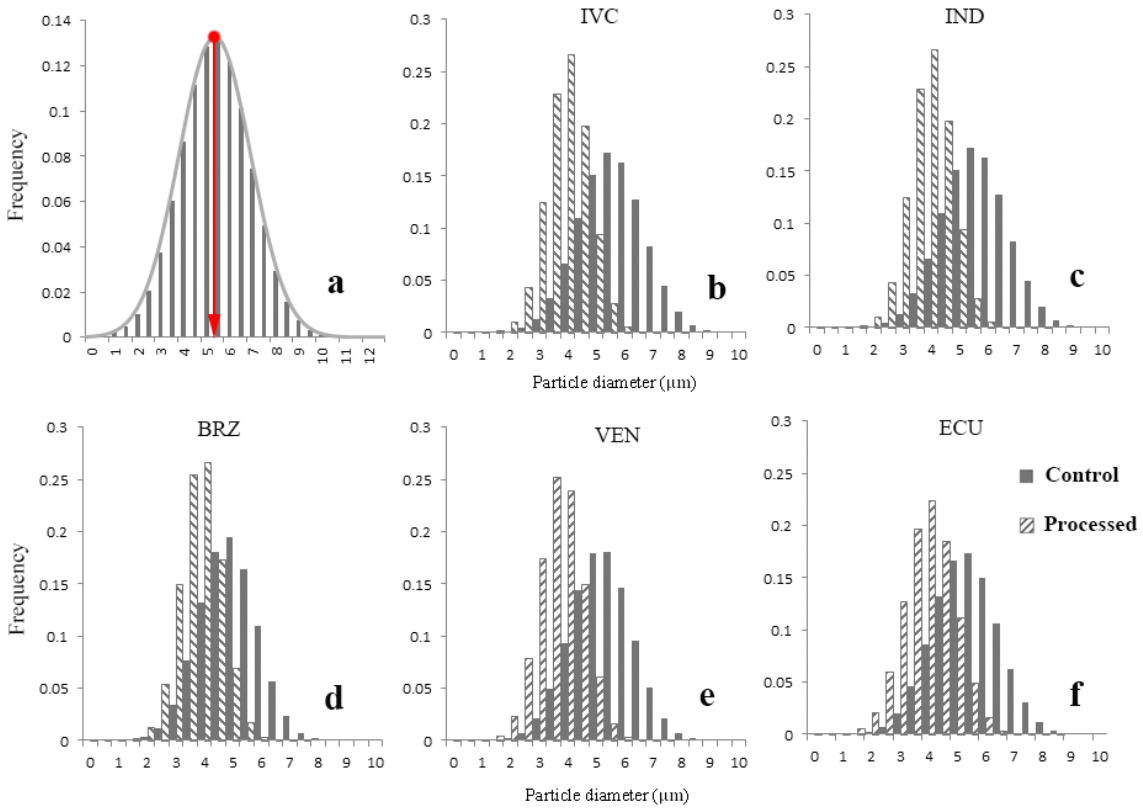


Figure 15. a) Representation of frequency distribution of particle diameter and mean crystal diameter (marked with arrow) c)-f) Particle diameter distribution of control and processed cocoa butter sample from Ivory Coast (IVC), Indonesia (IND), Brazil (BRZ), Venezuela (VEN), and Ecuador (ECU), respectively

Moreover, both control and processed samples were further analyzed using Image J and PeakFit software. As shown in Figure 15a, the particle diameter with highest frequency is considered as mean crystal diameter. From visual analysis of Figure 15b-f, the mean diameters were obviously smaller in processed samples compared to control samples for all the samples from different origins. The measured mean crystal diameters are reported in Table 5.

Table 5. Mean crystal diameter (a) of crystalized cocoa butters

	a (μm)	
	Control	Processed
IVC	$5.00 \pm 0.09^{b,i}$	$3.72 \pm 0.06^{A,ii}$
IND	$5.43 \pm 0.10^{a,i}$	$3.75 \pm 0.06^{AB,ii}$
BRZ	$4.97 \pm 0.10^{b,i}$	$3.82 \pm 0.06^{AB,ii}$
VEN	$5.25 \pm 0.12^{ab,i}$	$3.65 \pm 0.07^{B,ii}$
ECU	$5.46 \pm 0.11^{a,i}$	$3.94 \pm 0.07^{A,ii}$

* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).
** Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).
*** Low case of Roma letters (i, ii) represent statistically significant differences between control samples and processed samples in each origins ($p<0.05$).

As expected, the mean particle diameters of the processed samples were significantly ($p<0.05$) smaller compared to the control samples for all origins. Among the control samples, mean particle diameters of BRZ and IVC were significantly smaller than the ECU and IND samples. For processed samples, although less morphological differences were observed within samples analyzed in Figure 14, the mean diameter of

the samples were significantly different among different origins. The processed IVC and ECU samples have significantly ($p<0.05$) larger mean particles. Interestingly, the mean crystal diameters of VEN samples were significantly smaller among the origins. Therefore, slight differences in chemical compositions of cocoa butter among different origins greatly impacted the micro-structure of both control and processed samples.

3.2. Result for oil migration experiment and relative discussion

The migration of oil was monitored using MRI over 100 days and oil uptake by cocoa butter was quantified by image analysis which had been introduced in the previous section.

3.2.1. Control samples

The kinetics of oil migration in the control samples was studied to understand the effects of minor differences in chemical compositions of cocoa butter among different origins without the influence of processing. The oil uptake graphs for control samples from different origins are shown in Figure 16a. As shown in this figure, all of the samples reached saturation point after 55 days (around 8 weeks). The oil uptake by the cocoa butter from cream followed a similar pattern which can be represented as the curve shown in Figure 16b. The oil uptake increased linearly over time during the first 8 weeks and then the slope of the curve decreased dramatically when it reached the saturation point. For better quantification, the curve was divided into two regions based on the specific duration (before 8 weeks and after 8 weeks), as shown in Figure 16b. The solid

line in region 1 is the linear regression of the experimental data before 8 weeks while dash line in region 2 represents the linear curve fitting for the data after 8 weeks.

The slope values of the solid line and the dash line of the control samples from different origins are reported in Table 6 as slope 1 and slope 2, respectively. Slope 2 of all of the samples were prominently lower than slope 1. Ranged between 0.0003 and 0.0037, the small value of slope 2 approximating to zero indicates the achievement of the saturation point as the curve reached a plateau.

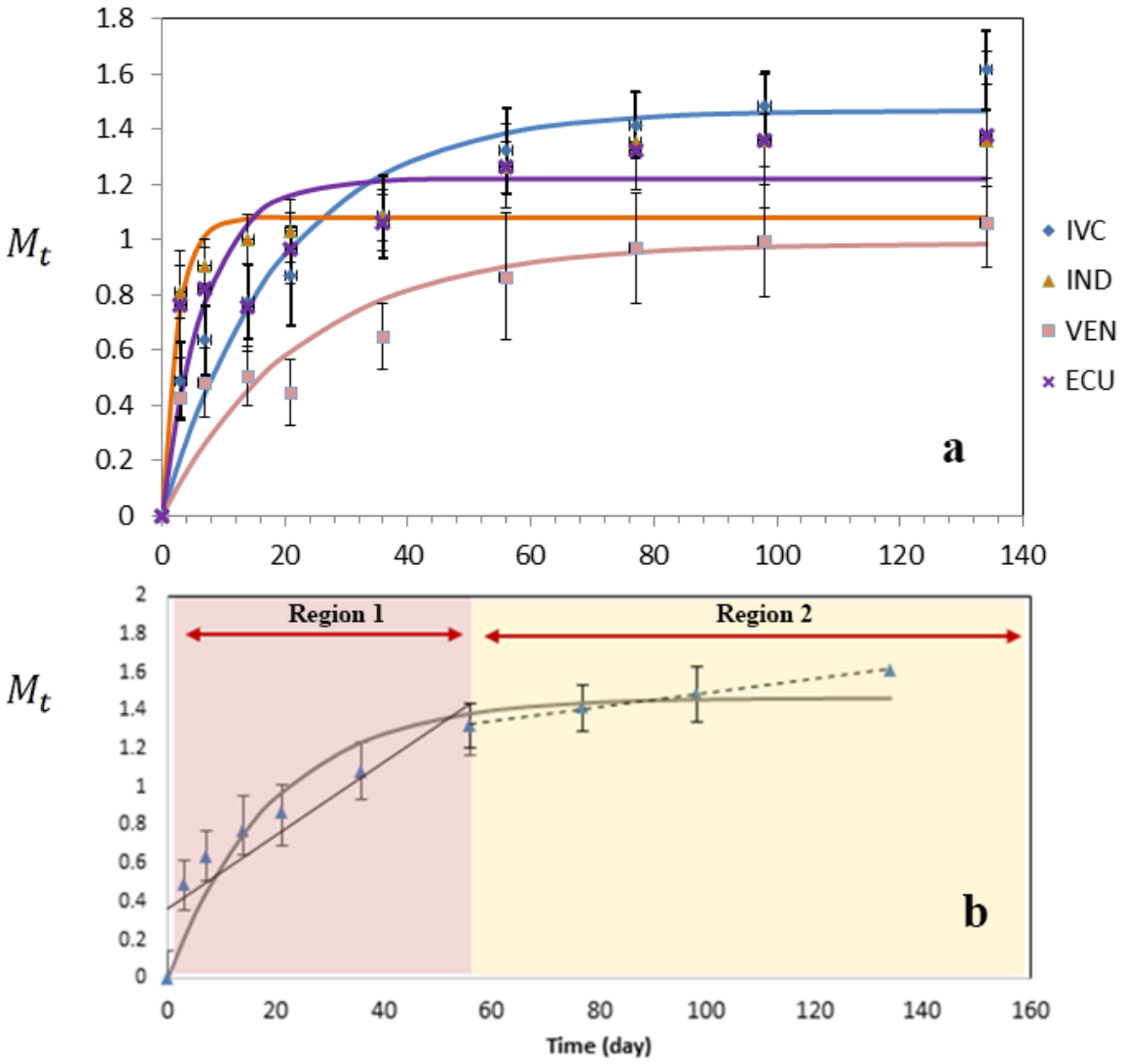


Figure 16. a) Oil uptakes curve for one representative samples; b) Oil uptakes (in arbitrary unit) over experimental period of control samples from IVC, IND, VEN and ECU

Table 6. Slope change in mass transfer curves (oil uptake as a function of time) of control samples

	IVC		**IND**		**BRZ**		**VEN**		**ECU**	
	slope	R^2	slope	R^2	slope	R^2	slope	R^2	slope	R^2
Slope 1	0.019	0.82	0.014	0.50	N.D.	N.D.	0.011	0.71	0.015	0.56
Slope 2	0.0037	0.99	0.0003	0.98	N.D.	N.D.	0.0023	0.91	0.0014	0.85

Further, a curve fitting method was also conducted to determine the saturation point. The experimental data were fit into an algebraic expression as follows:

$$M_t = \mathrm{M}_\infty{}'(1 - e^{-bt}) \qquad (3.1)$$

where $\mathrm{M_t}$ is defined as the oil uptake at time t, M_∞ is the maximum oil uptake after the migration reach equilibrium, b is the rate constant. This equation describes the exponential rise of the oil uptake, $\mathrm{M_t}$ from zero at t=0 and reaching maximum oil uptake, M_∞ at the saturation point. Similar approaches have been used in previous studies to describe the uptake of liquid material in lipid systems over time (McCarthy & McCarthy, 2008; Paluri et al., 2015). Figure 17 shows the curve fitting of control samples from different origins with respective to sum of square of error (SSE). The low values of the SSE (SSE<0.4) indicate that the models were adequate to describe the experimental data. The models were further used to determine the saturation point of the samples since both the slope-changing method and the fitting-curve method made evident that the oil uptake in all samples have reached plateau after 8 weeks.

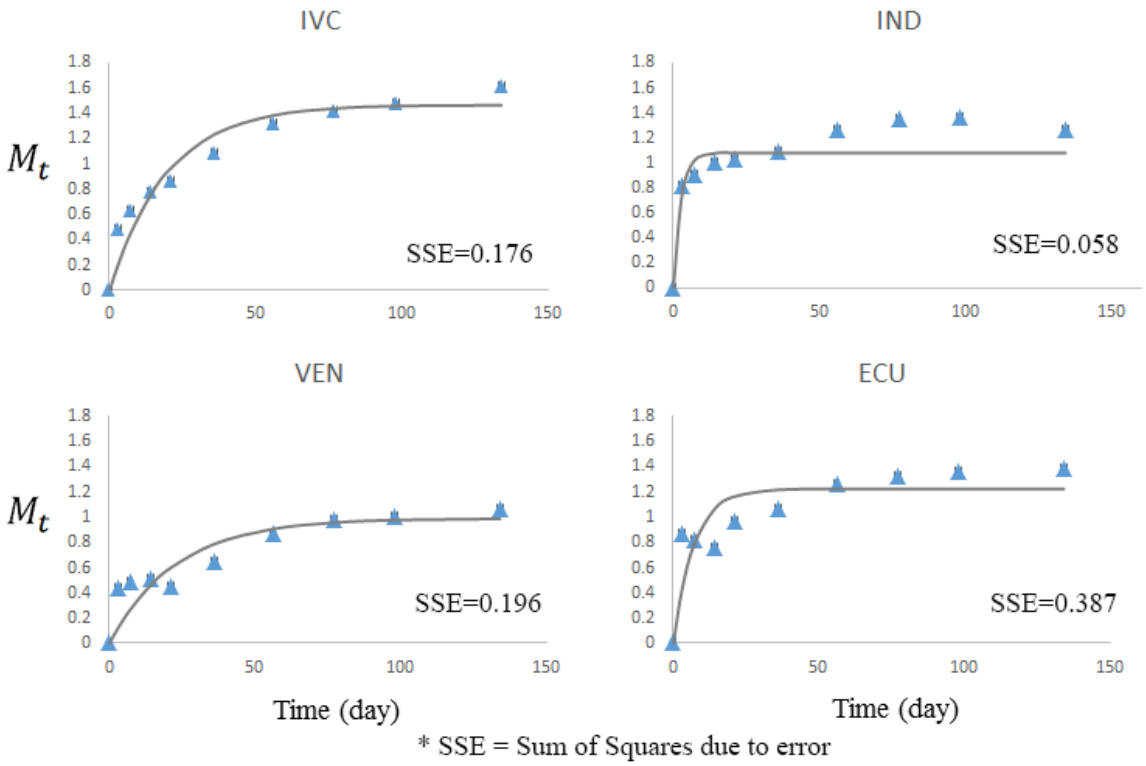
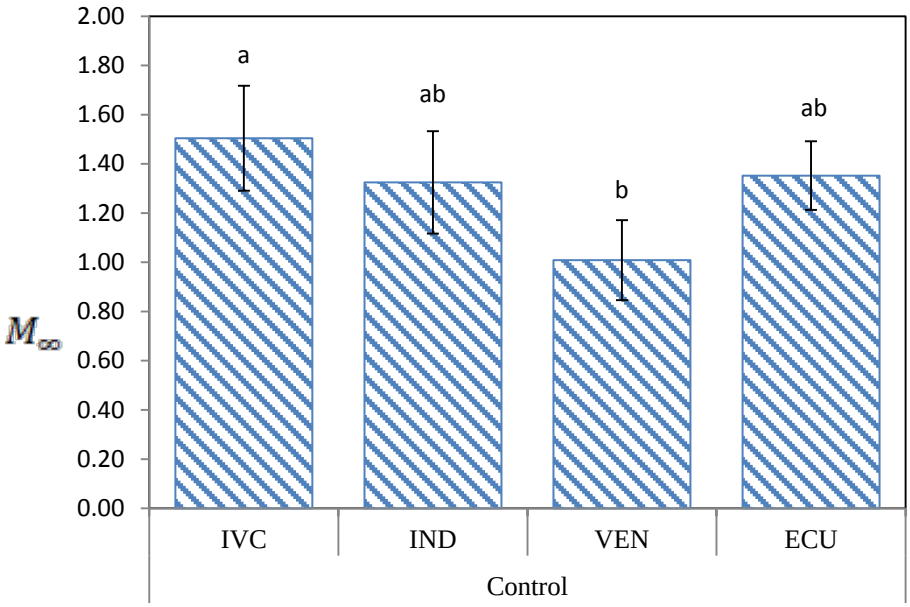


Figure 17. Curve fitting for control samples in algebraic expression $M_t = \mathrm{M}_\infty'(1 - e^{-bt})$

Maximum oil uptake (M_∞) is the amount of oil uptake at saturation point when sample have been contacted with a foreign oil source for a sufficiently long time that can be used to describe the extent of migration. The M_∞ of the control samples from different origins are plotted in Figure 18. The maximum oil uptake of the control samples were higher in IVC samples, followed by IND and ECU samples, and lower in the VEN sample. Interestingly, the maximum oil uptake for IVC, IND, and ECU were not significantly different from each other.



* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).

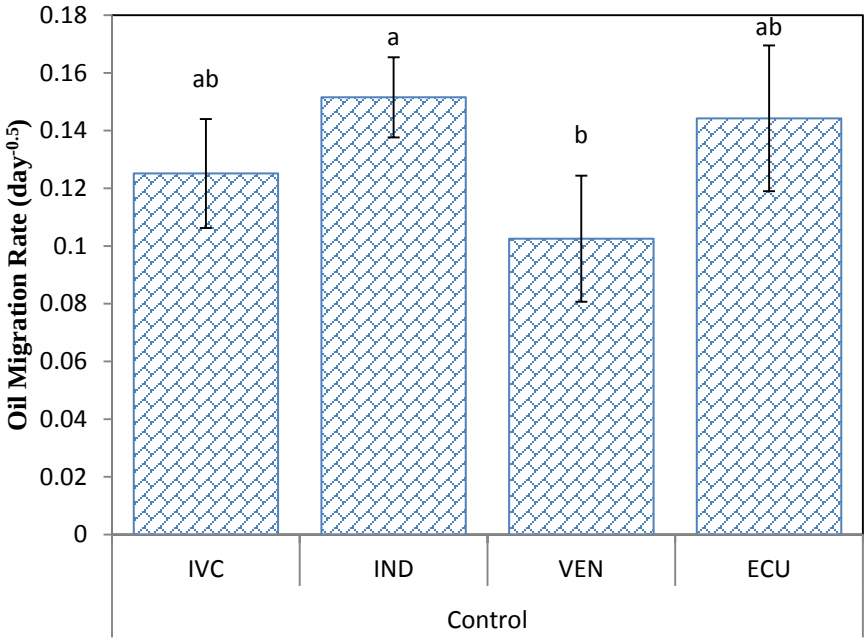
Figure 18. *Maximum oil uptake for the control samples from different origins*

Even though M_∞ is a good indicator for oil migration, studies have documented the importance of the oil migration rate over M_∞ to characterize the mass transfer of oil during the experimental period for a different fat system (Altan et al., 2011; Ghosh et al., 2002). For example, Altan studied the rate and extent of oil migration in chocolate and almond products, highlighted that the M_∞ can be higher in samples with lower oil migration rate (Altan et al., 2011).

In this study, oil migration rate was calculated based on an approximate equation from Crank (1957). According to Crank's study, uptake is considered as a dimensionless quantity, M_t/M_∞, which could be approximated by the following equation:

$$\frac{M_t}{M_\infty} = kt^{0.5} \qquad (3.2)$$

where k (day $^{-0.5}$) represents the migration rate, M_t is the oil uptake at the time t, and M_∞ is the uptake at a sufficiently long time. Eq. 3.2 shows a linear correlation between the dimensionless uptake and the square root of the time. The ratio of M_t/M_∞ can range from zero at the initial time to approximately one after a long time (Peppas & Brannon-Peppas, 1994). However, Peppas suggested that this equation is used when M_t/M_∞ is less than 0.67 (Peppas & Brannon-Peppas, 1994). The oil migration rates (k) of the control samples are shown in Figure 19.



* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).

Figure 19. Oil migration rate for the control samples from different origins

As shown in Figure 19, among samples from different origins, the IND sample showed a higher migration rate among others, which was 0.15. On the other hand, the migration rate of the VEN sample was significantly lower (0.10) compared to the IND sample (0.15). The rates of oil migration were similar in the IVC, IND, and ECU samples. Marty et al (2005), who used a similar fat system (cocoa butter/cream model) also reported similar oil migration rates ranging from 0.17 to 0.20 quantified using Crank's equation (Crank, 1957) (eq. 3.2). Moreover, Marty and Marangoni reported that the oil

migration in IVC and ECU samples were not significantly different.(Marty & Marangoni, 2009)

In order to better understand the effects of chemical composition on oil migration in cocoa butter systems, fatty acids composition (Table 2) and TAGs profile of the cocoa butters were investigated. TAG crystallization forms the basis for the development of a fat crystal network. About 20% of the triglycerides in cocoa butter are liquid at room temperature, therefore variation of chemical composition can result in different SFC in cocoa butters (Marty & Marangoni, 2009). Small changes in TAG composition can have a big impact on the crystallization properties of cocoa butter (Timms, 2012). Lohman (1994) and Marty (2009) who studied oil diffusivity of cocoa butter also proposed that lower SFC in cocoa butter promoted the migration of oil into the fat network. The diffusive process of the oil in the cocoa butter is driven by different TAGs concentration between a oil-rich phase (cream) and a crystalline fat-rich phase (cocoa butter) (Galdámez et al., 2009; Ghosh et al., 2002). The higher the solid fat content, the less the percentage of liquid oil present in the samples. As the migration takes place through the liquid portion of cocoa butter, a higher SFC decreases the rate of oil transporting (Ghosh et al., 2002).

As expected, the oil migration rates of the cocoa butter samples from different origins were inversely related to the SFC (Table 4). The significantly lower oil migration rate in the VEN sample can be attributed to high SFC (78.3%) compared to other samples. High solid content in the VEN sample retarded the foreign oil from passing through cocoa butter matrix. As discussed in previous section, the VEN sample has high saturated fat content (63.5%) comparable to the IVC and ECU samples. However, the significantly

higher SFC and lower oil migration rate of the VEN sample in particular may be associated with the relatively high content of both stearic and palmitic acids. In comparison, the IVC sample was the highest in stearic acid but lower in palmitic acid, while ECU sample was highest in palmitic acid but lowest in stearic acid, compared to other samples.

The analysis of TAG profiles reveal that the VEN sample had the highest content of tri-saturated TAGs (by a significant amount), highest content of symmetrical POSt TAG, the lowest content of tri-unsaturated TAGs (UUU), and a negligible amount of phosphorus compared to samples from other origins. These properties may contribute to the superiority of the VEN sample over the others in SFC content and the correspondingly lower oil migration rate. Moreover, the oil migration rates of the IVC and ECU samples were as comparable as their TAG profiles were similar, in particular the SSS, SUS, and UUS TAGs contents. Thus, the TAGs compositions of cocoa butter not only influence the SFC but also directly affect the oil migration rate.

On the other hand, the oil migration rate of the IND sample was significantly higher ($p<0.05$) as it has the lowest SFC (71.34%). The IND sample has a higher oleic acid content but is significantly lower in linoleic acid compared to the ECU sample. The high content of tri-unsaturated TAGs in the IND sample compared to others may also contribute to its high oil migration rate (Figure 19). This result agreed with the conclusion from the study by Marty and Marangoni (2009) which evaluated the significant correlations of oil migration rate with the content of tri-unsaturated TAGs. In addition, the high oil migration in the IND sample could be attributed to high phosphorus content compared to others. Minor compounds like phospholipids also influenced the

structure of cocoa butter network (Foubert.I, Vanrolleghem P.A., 2004; Savage & Dimick, 1995). Phospholipids are an important minor component in cocoa butter as studies showed that the level of phosphorus has significant impact on cocoa butter solidification (Arruda & Dimick, 1991; Foubert.I, Vanrolleghem P.A., 2004; Lechter, 2012; Stéphanie Marty & Marangoni, 2009).

Overall, significant differences in the oil migration rate of the samples can be attributed to differences in chemical composition observed among samples from different origins. In particular, fatty acid, TAGs, and phospholipid levels are significantly affected by the SFC of cocoa butter and directly contribute to the variations in the oil migration rate.

3.2.2. Processed samples

Similar analysis as employed in the controls samples were performed on the processed samples to evaluate the effect of processing on oil migration rate of cocoa butter samples from different origins. As shown in Figure 20, it took longer for the oil uptakes from cream to coca butter to reach the plateau, which was about 77 days (11 weeks). The maximum oil uptake (M_∞) of the processed samples from different origins were plotted and compared in Figure 21. The maximum oil uptake (M_∞) of the processed samples, ranged from 0.4 to 1.1, were also lower than the M_∞ in the control samples (0.8-1.4). This shows that the shearing during crystallization slowed down oil migration rate and decreased the maximum amount of oil uptake by the cocoa butter samples from all origins. The processing also allowed the BRZ sample to achieved stable β_V polymorphic form for the oil migration behaviors to be studied.

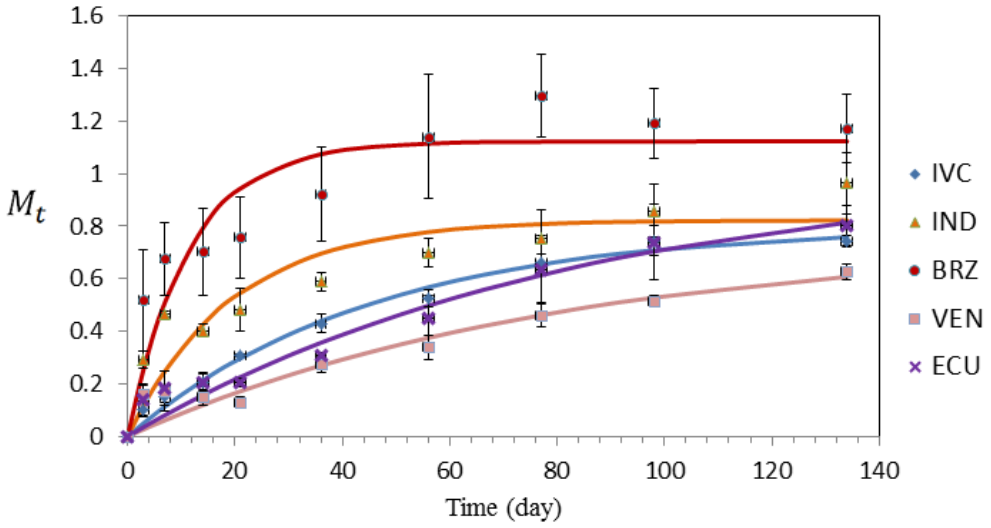
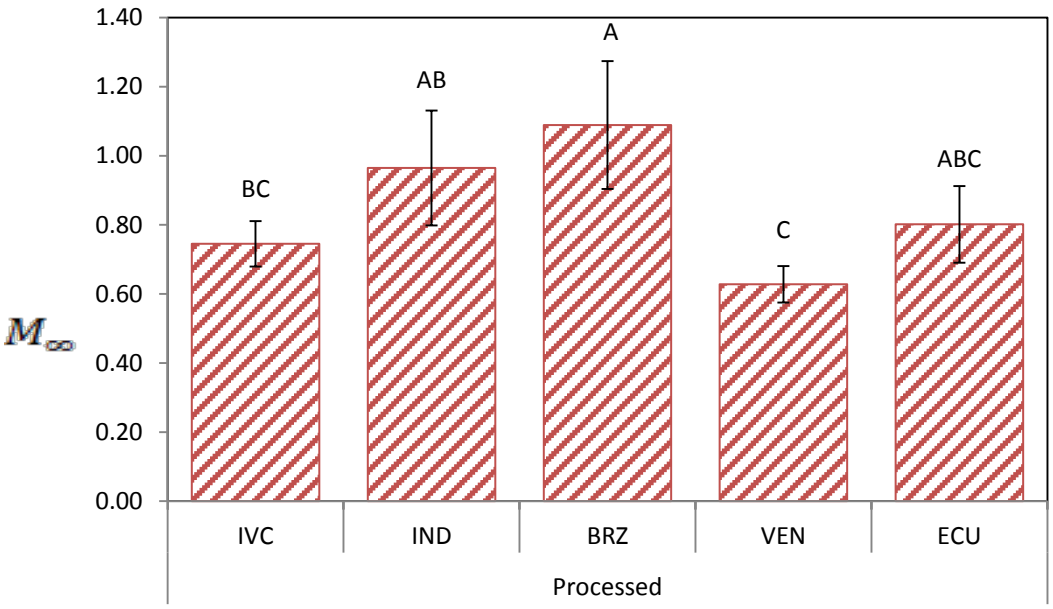


Figure 20. Oil uptakes (in arbitrary unit) over experimental period of processed samples from IVC, IND, BRZ, VEN and ECU

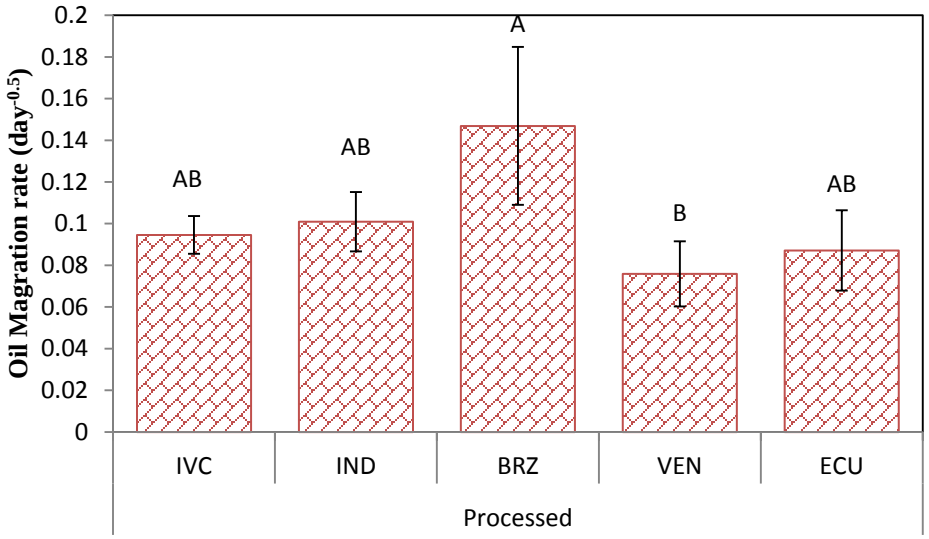
The M_∞ were significantly different among samples from different origins. As expected, the M_∞ was significantly ($p<0.05$) highest in the BRZ sample and lowest in the VEN sample compared to others. On the other hand, the M_∞ of the IND sample has no significant difference with BRZ sample. The M_∞ of the ECU sample was lower than the IND sample but higher than the IVC sample. However, there was no significance difference between M_∞ of these samples.



* Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).

Figure 21. Maximum oil uptake for the processed samples from different origins

The oil migration rates of the processed samples, calculated based on eq. 3.2 were plotted in Figure 22. The oil migration rates among samples from different origins were significantly different ($p<0.05$). Notably, the trend for oil migration rates of the processed samples was similar to the control samples. This clearly shows the effects of chemical composition for different cocoa butter origins on the oil migration kinetics. The BRZ processed sample had the highest oil migration rate and the lowest oil migration rate was found in the VEN processed sample. The oil migration rate of the BRZ samples was 0.14, which was significantly higher than the VEN samples (0.076). There was no significant difference in the rates of oil migration between the IVC, IND, and ECU processed samples. This result agreed with the observation in Marty's study (Marty & Marangoni, 2009) which reported similar oil migration rates in tempered ECU, IVC and IND cocoa butter.



* Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).

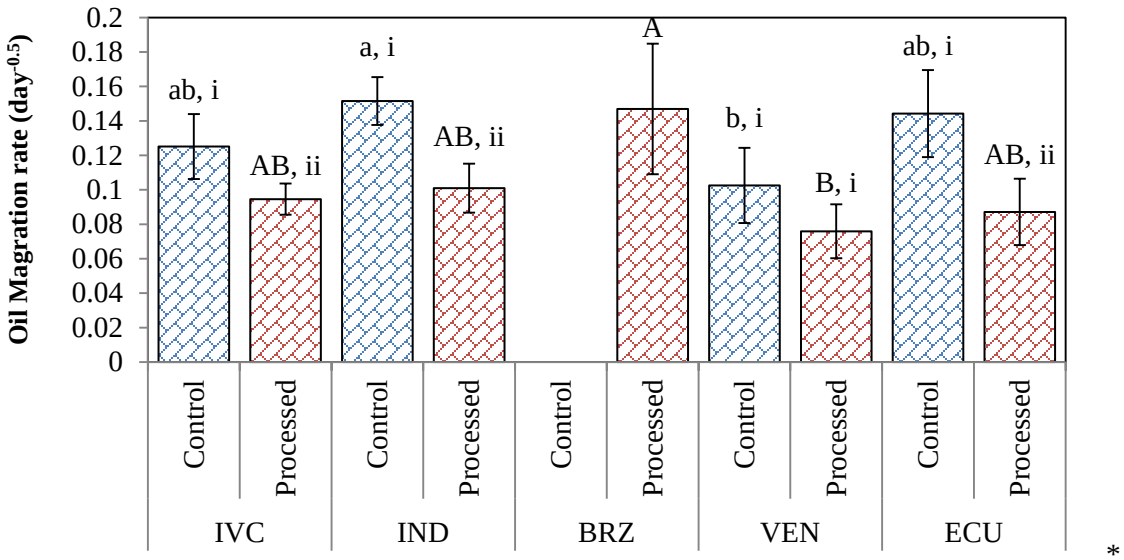
Figure 22. Oil migration rate for the processed samples from different origins

Higher oil migration rates in the BRZ samples can be attributed to its high unsaturated fatty acids (59.9%) content and low saturated fatty acids (39.7%) content (Table 2). This is also evident from its lowest SFC (59.9%) (Table 4) compared to samples from other origins. Thus, the effects of chemical composition significantly ($p<0.05$) influenced the oil migration rate of the processed samples.

3.2.3. Comparison of control and processed samples

3.2.3.1. Oil migration rate

In order to understand the effects of processing conditions on oil diffusivity in cocoa butter from different origins, the migration rate of control and processed samples were compared in Figure 23. The processing condition applied during cocoa butter crystallization significantly delayed the oil migration in the IVC, IND, and ECU samples. Interestingly, the VEN sample showed no significant difference between control and processed samples. The shearing process during the crystallization of cocoa butter has been reported to retard oil migration as a result of significant modification on the structure of cocoa butter networks (Acevedo et al., 2012; Maleky & Marangoni, 2008; Maleky et al., 2012; Maleky et al., 2011; Shi & Maleky, 2015; Sonwai & Mackley, 2006; Stapley et al., 1999).



Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).
** Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).
*** Low case of Roma letters (i, ii) represent statistically significant differences between control samples and processed samples in each origins ($p<0.05$).

Figure 23. Comparison of Oil migration rate for the control and processed samples from different origins

Even though applying shearing during crystallization could slow down the transport of oil, similar trends of oil migration rate were observed among the samples from different origins for both control and processed samples. Thus, the processing during cocoa butter crystallization decreased oil migration in the cocoa butter system. However, the influence of processing on oil diffusivity could not overcome the effect of minor differences in chemical composition.

3.2.3.2. Effective diffusion coefficient analysis

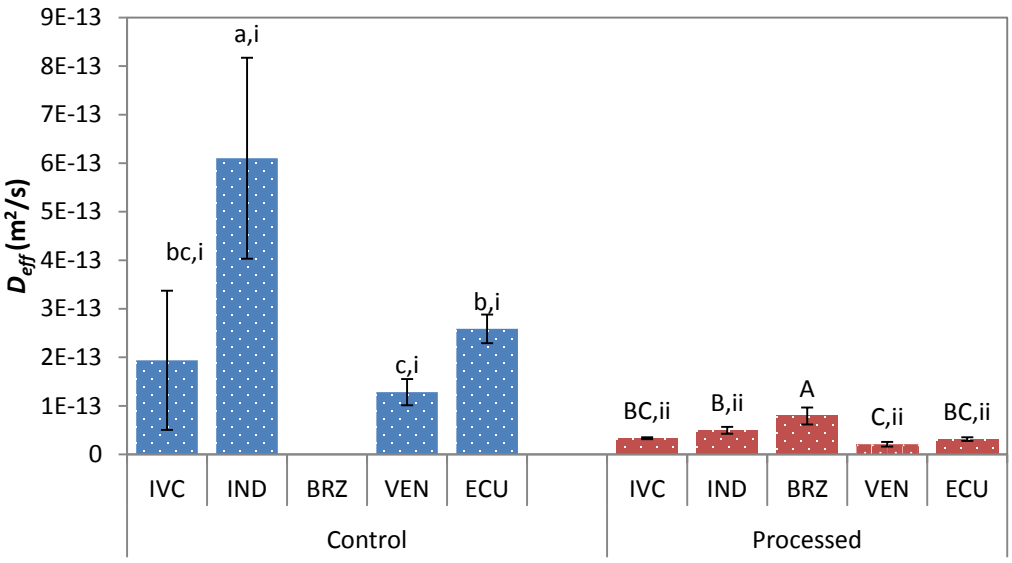
As mentioned earlier, the migration of oil in multiple lipid layers is considered a diffusion process. The rate of oil migration can be better analyzed from a diffusion equation where the diffusion coefficient is a quantitative measurement of the rate at which a diffusion process occurs (Crank, 1975). In lipid systems, which consist of a mixture of solid fat and liquid oil, the diffusion coefficient is also referred to as the effective diffusion coefficient, D_{eff} (Chinachoti, 1997; Ghosh et al., 2002). The effective diffusion coefficient in the lipid phase can be calculated based on two different mathematics models: the Peppas model and the Zigeler model.

Peppas model

With the appropriate initial conditions and boundary conditions offered by Peppas (1994) in similar fat systems, the ratio of M_t and M_∞ could be approximated to the following equation:

$$\frac{M_t}{M_\infty} = 1 - \sum_{q=0}^{\infty} \frac{8}{(2q+1)^2 * \pi^2} \exp\left[\frac{-D_{eff}\,(2q+1)^2 * \pi^2}{l^2} t\right] \qquad (3.3)$$

where M_t is defined as the oil uptake at time t, M_∞ is the maximum oil uptake after the migration reaches equilibrium, t (s) is time, D_{eff} ($m^2 s^{-1}$) is the effective diffusion coefficient among the migration behavior, and l (m) is the thickness of cocoa butter where oil migration took place. The thickness of oil migration, l can be measured as the oil migration front as defined in Marty and Maleky's work (Maleky & Marangoni, 2011; Marty et al., 2009).



* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins ($p<0.05$).
** Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins ($p<0.05$).
*** Low case of Roma letters (i, ii) represent statistically significant differences between control samples and processed samples in each origins ($p<0.05$).
Figure 24. Comparison of effective diffusion coefficient, D_{eff} for the control and processed samples from different origins (Peppas model)

The diffusion coefficients of samples were calculated and shown in Figure 24. The diffusion coefficients of processed samples were significantly lower ($p<0.05$) than the control samples for all the cocoa butter from different origins. Notably, diffusion coefficients for control samples were almost 10 times higher than those in processed samples. This is in agreement with the study by Maleky (2011) who reported that the diffusion coefficients in static samples are 8 times higher than the D_{eff} in sheared samples. However, the trends of D_{eff} across different origins were similar for both control and processed samples regardless of the BRZ sample. For the control samples, the

IND sample has the highest D_{eff} at 6.10×10^{-13} m^2s^{-1}, which was almost 5 times the D_{eff} of the VEN sample. The VEN sample has the lowest D_{eff} at 1.28×10^{-13} m^2s^{-1}. The D_{eff} of the IND sample was almost five times higher than that of the VEN sample. On the other hand, the D_{eff} of the IVC sample (1.94×10^{-13} m^2s^{-1}) was not significantly different from the ECU sample (2.59×10^{-13} m^2s^{-1}) which were in between the VEN and IND samples.

For the processed samples, as expected, the BRZ sample had the highest D_{eff} with 0.79×10^{-13} m^2s^{-1}, while lower values of D_{eff} were obtained in the IVC, IND, VEN and ECU samples at 0.33×10^{-13} m^2s^{-1}, 0.50×10^{-13} m^2s^{-1}, 0.21×10^{-13} m^2s^{-1} and 0.32×10^{-13} m^2s^{-1}, respectively. Similarly, the VEN sample was also reported as the lowest value of D_{eff} of the processed samples. Interestingly, the differences in D_{eff} among the control IND, IVC and ECU samples were minimal compared to the processed samples in oil migration rate.

Zigeler model

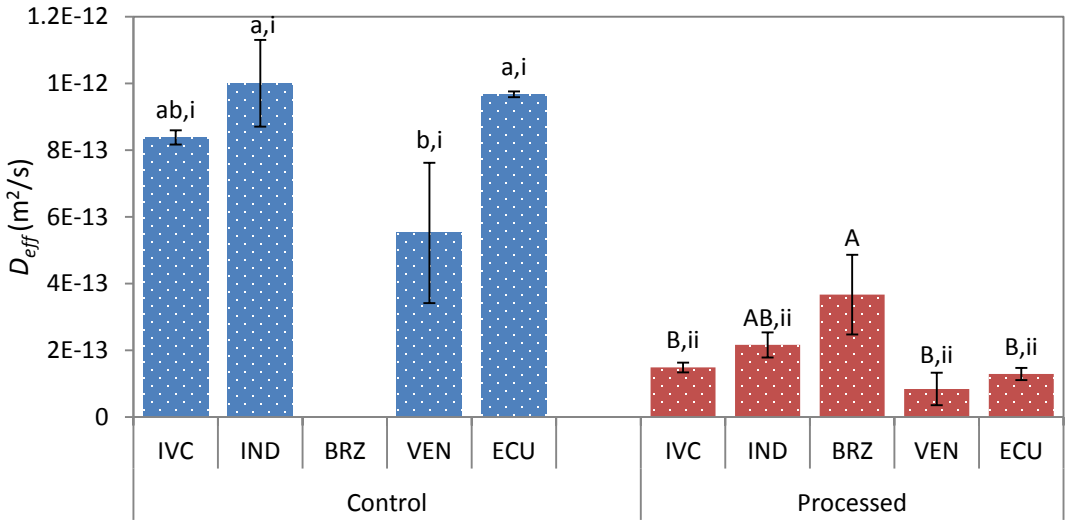
An alternative approximate equation to describes the oil migration in chocolate systems was proposed by Ziegleder(1998) as follows:

$$\frac{M_t}{M_\infty} = \frac{KA\sqrt{D_{eff}t}}{V} \qquad (3.4)$$

where M_t and M_∞ are the mass migrated at time t and at saturation, respectively. A (m^2) is the contact area between two phases, V (m^3) represents the volume through which the diffusion takes place, t (s) is the migration time, D_{eff} (m^2s^{-1}) is the effective diffusion

coefficient among the migration behavior, and K is a constant specific to two phase. In an ideal system K=1. However, K>1 when there are structural changes due to swelling or eutectic effects and K<1 happens when there is insufficient contact between the two phases (Cussler, 1997; Ziegler, 2009).

Equation (3.4) can be simplified to $M_t/M_\infty = \sqrt{D_{eff}t}/l^2$. The *l* is the thickness of fat media where oil migration took place. The D_{eff} using the Ziegler model of the cocoa butter of different origins for control and processed samples were calculated and plotted in Figure 25.



* Low case letters (a, b, c …) represent statistically significant differences among the control samples from different origins (p<0.05).
** Capital letters (A, B, C …) represent statistically significant differences among the processed samples from different origins (p<0.05).
*** Low case of Roma letters (i, ii) represent statistically significant differences between control samples and processed samples in each origins (p<0.05).
Figure 25. *Comparison of effective diffusion coefficient for the control and processed samples from different origins (Zigeler model)*

As shown in Figure 25, the D_{eff} values were slightly higher but similar trends were observed between the samples: the D_{eff} were significantly higher (8 times) in control samples than in processed ones and the trends of D_{eff} were also similar among the IVC, IND, and ECU cocoa butter in both controls and processed samples. Further analysis showed that the D_{eff} of the VEN controlled sample was significantly lower than the IND sample, but less variation was observed for the processed samples. Higher D_{eff} was observed in the BRZ processed sample (3.67×10^{-13} m^2s^{-1}), which was 4 times more than the VEN processed sample (5.52×10^{-13} m^2s^{-1}).

Comparing the Peppas and Zigeler models, the effective diffusion coefficients obtained from the Peppas model were more distinguishable between different origins in both control and processed samples. In the Zigeler model, the K constant in eq. 3.4 is considered as 1 when the contact between two phases is assumed in an ideal condition (Cussler, 1997; Maleky & Marangoni, 2011; Ziegleder & Schwingshandl, 1998). However, this ideal condition is difficult to be proven in this study. Therefore, Peppas model seems to be more credible and reliable in the application of this study and thus, used thereafter.

The effective diffusion coefficients in this study as summarized in Table 7 are also in agreement with the values reported in similar fat migration systems (Lee et al., 2010; Maleky & Marangoni, 2011; McCarthy & McCarthy, 2008). McCarthy (2008) obtained a diffusion coefficient of 2.3×10^{-11}(m^2s^{-1}) in peanut butter paste through the chocolate with different formulation, while Lee et al. (2010) showed a D_{eff} value of $1.1-2.0 \times 10^{-13}$ m^2s^{-1} in another 2-layer fat system. Maleky reported the D_{eff} values

of $8.1 \times 10^{-12}(m^2s^{-1})$ and $1.3 \times 10^{-12}(m^2s^{-1})$ for static and shear samples respectively (Maleky & Marangoni, 2011).

The range of diffusion coefficients reported for liquid through liquids were around approximately 10^{-9} m^2s^{-1} and for liquid through solids were approximately 10^{-14} m^2s^{-1} (Cussler, 2009; Ghosh et al., 2002). The values of D_{eff} in this study ranged from 2.1×10^{-14} m^2s^{-1} to 6.1×10^{-13} m^2s^{-1} and were therefore within the range of liquid and solid media. Thus, we postulated that the migration of foreign liquid oil through the crystalized fat were mainly via the liquid portion of the cocoa butter. The network of cocoa butter is composed of solid crystals that traps and holds liquid oil, where about 20% of the TAGs are in liquid forms at room temperature (Timms, 2012).

While comparing the effects of processing and different origins of cocoa butter on the$D_{eff}$and oil migration rate values, it becomes apparent that the D_{eff} parameter was more sensitive in distinguishing the oil diffusivity behaviors of the samples. In particular, the oil migration rates between the IVC and ECU control samples had no significant difference but their D_{eff} values were significantly different. When considering both oil migration rates and diffusion coefficients, this study shows that the processing condition effectively reduced diffusion of foreign oil through cocoa butter. However, similar trends observed between the control and processed samples for both parameters may conclude that the processing condition was unable to eliminate the effects of chemical compositions between different cocoa butter origins.

3.2.3.3. Permeability analysis

As we discussed above, small changes in chemical composition impact the microstructure of crystallized cocoa butter. And the structure of cocoa butter is important in affecting oil migration rate (Maleky, 2008). To further investigate the relationship between structural factors and the oil migration rate, it is necessary to introduce another parameter, the permeability coefficient. The permeability coefficient is a measure of the ability of a porous material to allow fluids to pass through it (Schlumberger, 2015; Whitaker, 1986) and is widely used in describing the network of fat materials (Dahlenborg et al. 2015; Alvarado et al., 2004; Maleky & Marangoni, 2011; Marty & Marangoni, 2009). The permeability coefficient introduced in Darcy's law was calculated using the following equation (Whitaker, 1986):

$$\mathrm{B} = \frac{a^2}{\tau} \emptyset^{\frac{2}{D-3}} \qquad (3.5)$$

where a is the particle diameter, $\emptyset$ is the solid fat content and D is the 3d fractal dimension of the crystal network (Bremer et al. 1989). τ is tortuosity of the fat network; the tortuosity describes the degree of how much diffusion will be retarded in a porous medium. The value of tortuosity was investigated based on the equation proposed by Cussler (1997) and the results are shown in Table 7:

$$\tau = \varepsilon \frac{D_0}{D_{eff}} \qquad (3.6)$$

where D_0 is the diffusion coefficient in a bulk fluid, D_{eff} represents the effective diffusion coefficient, and ε is the porosity of the porous media fat. Most of the studies that discussed tortuosity (Crossley & Aguilera, 2001; Cussler, 1997) considered the void fraction ε in a fat network can be simplified as 1- SFC.

Table 7. Effective diffusion coefficient ($D_{eff}(m^2s^{-1})$), Tortuosity (τ), and Permeability coefficient (B) of oil in different cocoa butters

	$D_{eff\times10^{13}}(m^2s^{-1})$		$\tau \times D_0 \times 10^{-10}$		$B \times {D_0}^{-1} \times 10^{22}\ (m^2)$	
	Control	Processed	Control	Processed	Control	Processed
IVC	1.94	0.33	0.93	8.76	2.43	0.18
IND	6.10	0.50	0.55	4.69	7.28	0.27
BRZ	N.D.	0.80	N.D.	2.33	N.D.	1.47
VEN	1.28	0.21	2.48	9.39	0.82	0.08
ECU	2.59	0.31	0.83	8.98	3.07	0.18

For the control samples, the permeability coefficient was higher in the IND samples and lower in the VEN samples. The trend matched the observation of higher oil migration rates in the IND controls and lower oil migration rates in the VEN control samples. For the processed sample, the trend of the permeability coefficient was also identical with the trend of oil migration rates observed in Figure 22. Higher oil migration rates were reported in the BRZ sample and lower in the VEN sample.

The permeability coefficient of the processed samples decreased by 10 times compared to the control samples. High permeability coefficients of the control samples indicate that it is easier for oil to pass through the fat crystalline network of control samples compared to the processed ones. According to eq. 3.5, a high permeability coefficient is contributed by high SFC, large crystal sizes, low fractal dimensions, and small tortuosity factors (Maleky & Marangoni, 2011; Marty & Marangoni, 2009). In the processed samples, smaller and more homogeneous fat crystal distribution (Fig. 14) can pack more tightly to form a stronger network, which is reflected by the smaller value in the permeability coefficient. Narrower paths around the crystals retarded the migration of

oil in this crystalline network, which explain the lower oil migration rate and smaller diffusion coefficient in the processed samples compared to the control samples.

Regardless of the BRZ sample, a higher value of permeability coefficient was observed within the IND sample and a lower value of permeability coefficient was observed in the VEN sample, for both control and processed samples. Even though processing significantly changed the structure of cocoa butter in the micro-scale, the effects of the chemical composition remained dominant. The permeability coefficient was positively influenced by the oil diffusivity which means that a higher oil migration rate is associated with a higher permeability. Therefore, slight differences in the chemical composition of cocoa butter significantly affect the oil diffusivity in a cocoa butter system. Although appropriate processing may assist in slowing down the oil migration rate of cocoa butter, the processing applied could not eliminate the inherent effect of chemical composition.

CHAPTER 4. CONCLUSION

This study investigated the effects of minor differences in the chemical composition of cocoa butter on oil diffusion kinetics. Cocoa butters produced in different geographic locations have similar major chemical composition and minor compounds. However, the contents of fatty acid, TAGs, and minor compounds from different regions were significantly varied. Interestingly, small differences in the fatty acids, TAGs, and minor compounds can result in significant difference in oil migration rate among the cocoa butter samples of different origins. Lower percentage of saturated fatty acids in cocoa butter resulted in low SFC. A lower SFC is associated with higher oil diffusivity in fat crystalline networks. Slight differences in chemical composition and minor compounds in cocoa butter influence nucleation and crystal growth, which influence permeability in fat networks. Moreover, processing significantly retarded the diffusion of oil in cocoa butter systems by providing a strong network and narrow, tortuous path. However, similar trend in oil migration rate were observed in both control and processed samples from different origins, indicating that dramatic changes in processing could not overcome the effect of minor variations in their chemical compositions. This research is significantly useful because the effects of chemical composition and crystallization conditions in relation to the oil diffusivity behaviors of cocoa butter from different origins were revealed. It provides useful knowledge to the chocolate industry in regards to prioritizing better

selection of raw materials over processing for manufacturing multi-component chocolate products. For future studies, the effects of small changes in chemical composition and minor compounds on cocoa butter nano-structure should be studied to further understand the mechanism of oil migration in cocoa butter system.

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