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Quality of Vietnamese cocoa liquor and butter

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PREFACE

Another year has passed, another period nearing its end. At the conclusion of several happy, memorable years here at the UGent, finishing this master thesis is my final work. But first of all, I want to express my gratitude to a number of people:

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LIST OF ABBREVIATIONS

a*	red/green
AV	acid value
b*	yellow/blue
DAG	diglyceride
DSC	differential scanning calorimetry
FA	fatty acid
FFA	free fatty acid
GC	gas chromatography
Gh	Ghanaian sample
HPLC	high-performance liquid chromatography
L*	lightness
MUFA	mono-unsaturated fatty acid
NMR	nuclear magnetic resonance
pAV	p-anisidine value
PGP	sample from Puratos Grand-Place
POP	1,3-dipalmitoyl-2-oleoyl-glycerol
POS	palmitoyl-stearoyl-2-oleoyl-glycerol
PUFA	poly-unsaturated fatty acid
PSD	particle size distribution
PV	peroxide value
QDA	quantitative descriptive analysis
SFA	saturated fatty acid
SFC	solid fat content
SOS	1,3-distearoyl-2-oleoyl-glycerol
SSS	tri-saturated triacylglycerol
S ₂ U	mono-unsaturated triacylglycerol
SU ₂	di-unsaturated triacylglycerol
TAG	triacylglycerol
TD _x , with x = number	sample from Thu Duc
TD	sample from Trong Duc
TDC	sample from Trong Duc, conched
TDU	sample from Trong Duc, unconched

SAMENVATTING

Cacaoproductie in Vietnam is zich aan het ontwikkelen, en is aan het groeien. Studies naar chemische composities en karakteristieke eigenschappen van de cacaobonen zijn echter nog niet uitgevoerd, en daarom is de kwaliteit van Vietnamese cacaobonen t.o.v. de rest van de wereld nog onbekend. De bedoeling van deze studie is het nagaan van deze eigenschappen.

In het eerste gedeelte werden de physico-chemische eigenschappen van cacao boters van tien clonees afkomstig uit de Mekong Delta in Vietnam geanalyseerd, samen met stalen uit Ghana, Ivoorkust en Indonesië. Chemische karakterisatie werd uitgevoerd door het bepalen van het vetzuur –en triglyceridenprofiel.

Binnen de Vietnamese clonees hadden TD8, TD9 en TD10 een hogere verzadigd vetzuurpercentage. Gemiddelde vetzuurpercentages van de Vietnamese stalen waren vergelijkbaar met het Indonesische staal. Stalen uit Ghana en Ivoorkust waren minder verzadigd ten voordele van een hoger percentage aan mono-onverzadigde vetzuren. Binnen de Vietnamese clonees hadden TD8, TD9, TD10 en TD6 een hoger percentage aan S₂U. Dit resultaat was te zien in latere fysische analyses van de cacao boters, zoals solid fat profils, smelt- en kristallisatiegedrag. Van 25°C tot 35°C vertoonden TD8 en TD9 hogere SFC gehalten terwijl TD2 en TD3 er lagere hadden, dit in overeenkomst met hun chemische compositie. Tijdens niet-isotherme kristallisatie, kristalliseerden TD8 en TD9 eveneens aan een hogere temperatuur. Voor isotherme kristallisatie bij 20°C vertoonden TD13, TD8 en TD9 de hoogste kristallisatiewarmte. Voor TD9, TD11, TD6 en TD3 was de inductietijd de hoogste.

In het tweede gedeelte werden drie Vietnamese en een Ghanese cacaomassa gebruikt om verschillende chocolades te produceren. De cacao boters van de massa's werden eveneens physicochemisch gekarakteriseerd. De chemische compositie van de cacao boters werden bepaald via vetzuur-en triaglycerol profielen, waarbij het Ghanese staal vergelijkbare composities vertoonde met de Vietnamese stalen. De fysische eigenschappen werden nagegaan d.m.v solid fat profile en isotherme crystallisatie.

Twee van de Vietnamese stalen werden vooraf geconcheerd om de hoeveelheid organische zuren aanwezig in de massa te reduceren. De voornaamste niet-vluchtige organische zuren aanwezig waren citroen-, barnsteen- en melkzuur. Vluchtige zuren bestonden voornamelijk uit propionzuur en azijnzuur. Na het conchen was de hoeveelheid azijnzuur significant gedaald.

Nadat de chocolades geproduceerd werden, werden de eigenschappen van de stalen geanalyseerd. Reologie van de stalen werd gekenmerkt door de viscositeit en de vloeigrens van gesmolten chocolade. De chocoladestalen met massa's die vooraf een concheerbehandeling hadden ondergaan, hadden bijgevolg ook een lagere viscositeit. De stalen geproduceerd met Vietnamese cacamassa's vertoonden een gelijkaardig smeltgedrag met het Ghanese staal, waarbij een deel van de Vietnamese stalen op een iets hogere temperatuur smolt. Verschillen in zuurheid die eerder

werden waargenomen bij de cacaomassa's, waren eveneens terug te vinden in de chocoladeproducten, zelfs na het concheren van de cacaomassa's.

Consumenten waren wel in staat om verschillende stalen van elkaar te onderscheiden, maar namen het verschil tussen producten met geconcheerde en hun ongeconcheerde tegenhangers niet waar. Van de specifieke verschillen tussen de chocoladestalen werd enkel het verschil in zuurheid en nasmaak waargenomen door een getraind panel.

SUMMARY

Nowadays, cocoa production in Vietnam is developing and expanding its market to many countries. However, no study towards chemical composition and characteristic properties of the cocoa beans has been conducted, thus making the quality of Vietnamese cocoa beans compared to the other world leading cocoa producing countries remain unknown. The objective of this study is to verify these properties, thereby positioning Vietnamese cocoa beans toward a better understanding .

In the first part of this study, physicochemical properties of cocoa butters of ten clones from the Mekong Delta in Vietnam, and samples from Ghana, Ivory Coast and Indonesia were characterised. Chemical characterisation was performed by describing their fatty acid and triacylglycerol profile.

TD8, TD9 and TD10 of the Vietnamese clones consisted of higher SFA. Average saturated fatty acid levels of the Vietnamese samples were comparable to the Indonesian sample. Samples from Ghana and Ivory Coast were lower in saturated, in favour of mono-unsaturated fatty acids. Within the Vietnamese clones TD8, TD9, TD10 and TD6 contained higher amounts of S₂U. This result was in good correlation with physical analysis of the cocoa butters which were characterised by solid fat profiles, melting and crystallization behaviour. From 25°C to 35°C, TD8 and TD9 displayed higher SFC while TD2 and TD3 presented lower SFC. During non-isothermal crystallisation, TD8 and TD9 crystallised at a higher temperature. For isothermal crystallisation at 20°C TD13, TD8 and TD9 had the highest crystallisation heat. Induction time was highest for TD9, TD11, TD6 and TD3.

In the second part, three Vietnamese and one Ghanaian cocoa liquor were used to produce different chocolate products. The cocoa butters of those liquors were characterised as well. The chemical composition of the cocoa butters was determined with fatty acid and triacylglycerol profiles. The Ghanaian sample displayed comparable chemical composition with the Vietnamese samples. Physical properties were examined by means of solid fat profile and isothermal crystallisation.

Two of the Vietnamese liquors were conched prior to production to reduce liquor acidity. The main non-volatile organic acids were citric, succinic and lactic acid. Volatile acid consisted mainly of propionic and acetic acid. After conching, acetic acid content was significantly decreased. After chocolate production, the characteristics of the chocolates were analysed. Rheology of the chocolate was determined by yield stress and viscosity. The chocolates with pre-conched liquors, had a lower viscosity because of that pre-treatment. The chocolates produced with Vietnamese cocoa liquors displayed comparable melting behaviours to the Ghanaian sample, with two of the Vietnamese samples having a higher peak temperature. Differences in acidity observed between the cocoa liquors were found between the chocolate products as well, even after liquor conching.

Consumers were able to distinguish different samples from one another, but did not detect the difference between chocolate products made from pre-conched liquor and their conched counterpart. From the specific differences between the chocolate, only the sourness and aftertaste were perceived as different by a trained panel.

INTRODUCTION

Cocoa plantation is budding in Vietnam with its importance in the world's market share. From the early stages on, Vietnam has developed its cocoa bean quality via education, seedling selection, fermentation, and so on. Although Vietnamese cocoa beans were tested on the more agricultural quality standards, no study was found on the more detailed composition and characteristics of Vietnamese cocoa beans. This study aims to get a clearer indication of the quality position of Vietnamese cocoa beans in the world.

This study consists of two main parts, the first being the extraction and subsequent analysis of cocoa samples from Vietnam and from Ghana, Ivory Coast and Indonesia, three of the most prominent cocoa exporting countries in the world. The chemical composition of the cocoa butters, such as fatty acid composition, triacylglycerol composition and free fatty acid content was determined. Physical characterisation of the butters was prepared by analysing their solid fat profile, melt and crystallisation.

In the second part, physicochemical characteristics and functionality in chocolate of three Vietnamese cocoa liquors were compared to a Ghanaian sample. Macroscopic composition of the liquors such as moisture content, fat content etc. was analysed. Quality of the liquors was verified by measuring FFA content and oxidation products. Acidity and organic acid content was determined. Chemical description of the cocoa butter was presented by fatty acid and TAG profile. Physical characteristics of the cocoa butters were examined through particle size of the liquors, and SFC profile and isothermal crystallization of the butters.

Prior to production, two of the Vietnamese cocoa liquors were refined using a ball-mill in order to obtain a particle size suitable for production and conched to decrease acidity. The characteristics of the resulting chocolates, such as particle size distribution, flow behaviour, colour, texture and melting range were then determined. Sensory analysis was performed in order to investigate consumer perception.

1. LITERATURE

1.1 COCOA IN VIETNAM

1.1.1 COCOA PRODUCTION AND TRADE

Suitable growing conditions for cocoa plants (*Theobroma cacao* L.) are found in areas between 20° south and 20° north to the equator with an average yearly temperature equal or higher than 27°C and high humidity. As strong winds may damage the plants, altitudes of less than 700m are preferred. The three main cocoa growing regions (Fig. 1.1) are West Africa, South-East Asia and South America (Beckett, 2008).

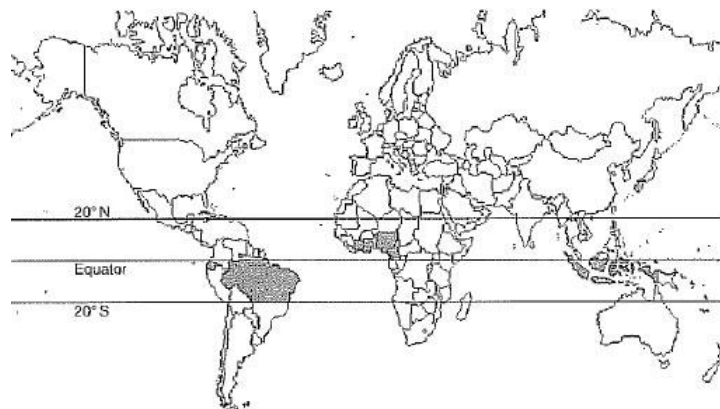


Figure 1.1: Cocoa growing regions (Beckett, 2009)

From the period of 2002/2003 to 2011/2012, daily cocoa prices ranged from 1360 US\$ to 3730 US\$ per ton (ICCO, 2012). During this time frame, total cocoa production has increased from 3.2 to 4.0 million ton. The region with the highest growth rate regarding cocoa production is Africa. From 2002 to 2012, its world production share has increased from 69% to 72%. On average, Africa's cocoa bean production has risen at an annual rate of 3.7%, whereas that of Americas has grown at 3.1%, resulting in a contribution of 14%. Meanwhile, annual growth rate in Asia and Oceania was substantially lower, namely 1.5% growth with a production share decreasing from 17% to 15% from the season of 2002/2003 till 2011/2012. The main growing countries in cocoa production were Ghana (438 000 ton increase), Ivory Coast (112 000 ton increase) and Indonesia (78 000 ton increase) (ICCO, 2012).

Cocoa production in Vietnam was, in the 1980's, advanced to a commercial scale. Back then, the processing capacity was not developed enough to enable selling on the world market until the 1990's. Between 1993 and 2004, hundreds of cocoa clones were imported to inspect whether or not they were suitable to Vietnamese growing conditions (Pham *et al.*, 2008). Vietnamese cocoa

production represents only a small part of Asian cocoa production. From the period of 2006/2007 till 2010/2011, Vietnamese cocoa accounted for 0.07% to 0.48% of Asian cocoa production (ICCO, 2012).

Cocoa can be grown in several regions of Vietnam, but is most suitable in the Mekong Delta and the central highlands. In the central highlands, cocoa is grown at an altitude of 500-800 meters in the provinces of Dak Nong and Dak Lak occupying 650 ha and 1850 ha respectively, while in the Mekong Delta the cocoa trees are cultivated in the Ben Tre (2000 ha) and Tien Giang province. Plans are being made to grow an additional 300 hectares of cocoa in the Lam Dong province. On the central coast, cocoa was once grown in plain of Phu Yen province, however, cocoa plantation is now mostly used for household consumption (Pham *et al.*, 2008).

The main supplier of world market cocoa is Africa, providing 77% of the world cocoa export. Asia and Oceania represent 16%, while the Americas represent 6%. As more and more cocoa is processed in the originating regions, cocoa export will decrease in favor of cocoa product trade. From 2006/2007 till 2010/2011, 87% of the total cocoa export originates from the five highest exporting countries in which Ivory Coast takes the lead with 37% followed by Ghana representing 22%. Indonesia accounts for 15% of the world cocoa export. From 2006/2007 to 2010/2011, Vietnam accounted for 0.01% to 0.34% of the exported cocoa in Asia (ICCO, 2012). Because of the high quality of Ghanaian cocoa beans, these beans are often considered as a reference (Beckett, 2008).

From 2006/2007 until 2010/2011, Europe is the main importer of cocoa, accounting for 58%, followed by the Americas (27%), Asia (14%) and Africa (2%). The US is the primary cocoa importing nation, representing 20%. Germany is the second largest importer (13%), followed by Belgium (7%) (ICCO, 2012).

Cocoa consumption worldwide, which is measured by industrial cocoa grinding, has increased by an average of 2.9% *per annum*, rising from 3.1 to over 3.9 million ton from 2002/2003 to 2011/2011. During this period, demand has fallen only in the cocoa year of 2008/2009 by 6% due to global economic conditions and cocoa bean price increases (ICCO, 2012). Consumption of cocoa based products in Asia is emerging, thereby increasing the demand for cocoa powder, which caused cocoa butter demand be overtaken by cocoa powder demand. After all, the combination of a hot climate and shortage of refrigeration complicates the storage of butter-based products. Moreover, since emerging markets are less accustomed to chocolate taste, milder powder based products are more preferable (ICCO, 2012).

Between 2002/2003 and 2011/2012, worldwide grinding has grown by 2.9% in which Europe's world share has declined from 43% to 40%, however still remained the main processing region. Over this timeframe, Europe's average annual growth was 2.1%. America's share also experienced a decrease from 26% to 21%, with an average annual growth of 0.5%. In contrast, processing in Africa has seen an increase from 14% to 18%, with an average annual growth of 5.7%. In Asia and Oceania, a growth rate of 5.6% resulted in an increase in grinding from 16% to 20%. This growth primarily owes to increase of grindings in Indonesia and Malaysia (ICCO, 2012). Despite of this rapid increase in cocoa

production, Indonesia and Malaysia beans exporters are dealing with the deficiency in consistency and quality (USAID, 2006). Taking this lesson and being beneficial from many international project interventions, Vietnam has carefully developed its cocoa bean quality from the very early stage including agriculture practice, education, seedling selection, fermentation etc. No study has been found on the composition and characteristics of Vietnamese cocoa beans, thus leaving a question mark on its quality position in the world market.

The chocolate market is still mainly led by Western Europe and North America, and additionally expanded to countries as Brazil, India, the Russian Federation, and especially China (ICCO, 2012). Nowadays, consumption has shifted more and more towards organic and fair-trade chocolate, single-origin chocolate, and premium chocolate with exotic ingredients (Afoakwa, 2010).

1.1.2 COCOA VARIETIES AND CLONES

Theobroma cacao L. has four main varieties: *Forastero*, *Criollo*, *Trinitario* (Afoakwa, 2011) and *Nacional* (Beckett, 2008). *Forastero*, mostly grown in West Africa and Brazil (Amoye, 2006) makes 70% of the cocoa bean production and is a bulk grade variety (Hoskin, 1994), which has stronger and harsher flavours (Kattenberg *et al.*, 1993). The other three varieties are viewed as fine grade (Hoskin, 1994) and are considered to have more aromatic or smoother tastes (Kattenberg *et al.*, 1993). *Criollo* cocoa beans are mostly grown in Central and South America (Amoye, 2006) and have a milder flavour than *Forastero* beans (Beckett, 2008) but the trees are lower yielding (Awua, 2002). *Trinitario* is a clone of *Forastero* and *Criollo* (Beckett, 2008). *Nacional*, a variety only grown in Ecuador (Déspreaux, 1998), is known for its fruity-raisin flavour (Counet *et al.*, 2004).

Up till the 1980's, seedlings in Vietnam were selected from trees with large pods. However, since cocoa is cross-pollinating, the seedlings had different characteristics from the mother trees, making this method ineffective. Thus, yield and quality of the cocoa's offspring was unpredictable. When high quality Malaysian clones were imported, a small part of the first generation of seedlings already gave poor yield and quality, although they were carefully selected (Pham *et al.*, 2008). Nowadays, two sources for cocoa planting are available: either from approved seeds of identified parents tested for quality, adaptability and yield or from monoculture clones of qualified trees and possibly identified or unidentified parents. The latter are long-term tested in different growing regions and developed into popular commercial clones which are widely grown and meet 1A Vietnamese standards on cocoa quality published in 2006 according to analysis results of exported Vietnamese cocoa beans in 2006-2007 by Cargill. Nowadays, monoculture clones include TD1-11, TD13, TD14, TD17, TD20, TD33, TD36, TD38, TD39, TD54, TD 55, TD62, TD63 and TD64 with the obtained productivity of 2-5 tonnes/ha (Pham, 2009). TD stands for Thu Duc, the province where Vietnams National Cocoa Seed Centre is located.

Vietnams Ministry of Agriculture and Rural Development has approved 8 commercial clones for usage throughout its country: TD1, TD2, TD3, TD5, TD6, TD8, TD10 and TD14. Although not officially approved, TD9 and TD11 have been used by farmers because of their high yield. Commercial clones

account for 80% of the Mekong Delta cocoa production (Pham *et al.*, 2008). Current available cocoa plants in Vietnam are originated from Forastero and clones of *Forastero* and *Trinitario* (Pham, 2009). Most of them are monoculture of identified trees:

TD 2: *Forastero* x *Forastero*

TD 3: *Trinitario* x *Forastero*

TD 5: *Trinitario* x *Forastero*

TD 6: clone from unidentified parents of *Trinitario* resulting from investigation

TD 7: *Trinitario* x *Forastero*

TD 8: clone from unidentified parents of *Forastero* resulting from investigation

TD 9: clone from unidentified parents of *Trinitario* and *Forastero* resulting from investigation

TD 10: *Trinitario* x *Forastero*

TD 11: *Forastero* x *Forastero*

TD 13: *Trinitario* x *Forastero*

Up to date, according to Phuoc (2009), TD13 will be a high potential commercial clone in the future in terms of bean quality and productivity, hence being selected for this study. TD1, TD4, TD12 and TD14, on the other hand, have their draw backs in resistance to *Phytophthora palmivora* disease after long time growing and are predicted to be eliminated in the future. Currently, TD3 and TD5 are the most dominant clones produced in Vietnam, followed by TD6, TD8, TD9. However, despite its excellent adaptability and high quality beans, defects from *Phytophthora palmivora* will hinder TD5 from being recommended to be cultivated in regions with high rainfall such as Daklak and Lam Dong provinces.

1.2 FROM COCOA BEANS TO LIQUOR AND BUTTER

1.2.1 COCOA BEAN COMPOSITION

Cocoa pods contain 30-45 beans covered in white pulp (Beckett, 2008). The beans consist of two nibs (87.1%), shell (12%), and germ (0.9%) (Dewettinck, 2012). The nibs consist for over half of the dry weight of cocoa butter (Beckett, 2008). Raw cocoa nibs measured by Aremu *et al.* consisted of 62.9% lipids, 17.5% proteins, 4.4% ash, and 5.9% fiber (Aremu *et al.*, 1995).

1.2.2 COCOA BEAN PROCESSING

From the harvest of the cocoa beans, several subsequent processing steps transform the raw materials into cocoa ingredients.

1.2.2.1 FERMENTATION

After harvesting, the cocoa beans and the pulp are removed from the cocoa pod and heaped into piles or placed into boxes to ferment (Wood, 1997). This takes 2 to 8 days, depending on the fermentation conditions and the variety of the cocoa (Hii *et al.*, 2009). Fermentation is performed to develop flavour precursors inside the cocoa beans (Minifie, 1989). The colour of the cotyledons

transforms from purple to brown, and the astringency and bitterness of the cocoa beans is reduced (Lagunes-Gálvez *et al.*, 2007). Bean death plays important role in this process, since it prevents spoilage by germination (Beckett, 2008).

The contents within a cocoa pod are sterile. Only when the pod is opened, its contents are contaminated with micro-organisms from its immediate surroundings (Schwan and Wheals, 2004). The pulp, which contains 10-15% sugars, is a good medium for micro-organisms to grow and is broken down. Cocoa bean fermentation can be divided into three overlapping phases (Wood, 1997). The first phase is characterized by anaerobic yeasts which convert glucose into ethanol in low oxygen and low pH conditions. Anaerobic conditions are created by tight packing while the acidic conditions are caused by the citric acid present in the pulp. The yeasts break down the pulp pectins the pulp, resulting in pulp liquefying and subsequent leaking (Ardhana and Fleet, 2003). As the pulp is drained, air comes into the heap of beans (Lefeber *et al.*, 2011). Furthermore, yeasts generate volatile flavour components and organic acids (Schwan and Wheals, 2004). At higher ethanol concentration, the yeasts become inhibited (Wood, 1997). The second phase is characterized by facultative anaerobic lactic acid bacteria: glucose and fructose are converted into lactic acid, acetic acid and ethanol. Likewise, citric acid is converted to lactic acid, acetic acid and flavour components like diacetyl and 2,3-butanediol (Lefeber *et al.*, 2011). The resulting acidity increase accelerates bean death (Beckett, 2008). In the third phase aerobic acetic acid bacteria are active. Ethanol produced earlier is converted into acetic acid in an exothermic reaction (Wood, 1997), which is further oxidized into carbon dioxide and water (Moens *et al.*, 2013). During this reaction temperature can reach 50°C, accelerating bean death as well (Wood, 1997). At the end, the beans have a moisture content of around 60% (Dand, 1999).

The most common fermentation techniques are heap and box fermentation. Both techniques are commonly used in West-Africa. Since Vietnam strives to obtain cocoa beans comparable to those produced in West-Africa, both techniques are used in Vietnam as well, although basket fermentation exists as well (AusAID, s.d.).

1.2.2.2 DRYING

During drying the moisture content of the fermented cocoa beans is reduced to 7-8% (Beckett, 2008). This can be done either naturally or artificially. The natural method refers to sun drying and takes 5 to 10 days. The artificial way refers to heating the surface over which the beans are spread using gases from a fire or a heat exchanger (Dand, 1999). The latter method is often applied in Asia, where the air humidity is too high (Beckett, 2008). When fire is used, contamination with smoke has to be avoided (Dand, 1999). During the drying process, control is necessary, because moulds can cause unpleasant flavours. In contrast, the beans may not be over-dried (<6%), or they will become more brittle and are more difficult to handle in the further processes (Beckett, 2008). Drying requires time. When dried too quickly, the remnant acidity due to volatile acids such as acetic, propionic, butyric, iso-butyric and iso-valeric acid may be too high (Paramo *et al.*, 2010), which causes acid and astringent flavours (Dand, 1999).

1.2.2.3 ROASTING, WINNOWING AND GRINDING

Roasting of fermented, dried cocoa beans is carried out to further develop flavour and aroma compounds (Minifie, 1989). The heating temperature depends on cocoa origin and desired end product. The lower the temperature, the less cocoa butter from the nib is transferred to the shell, in case of whole bean roasting, reducing cocoa butter losses (Dand, 1999). The applied high temperature will kill the remaining contaminating micro-organisms like *Salmonella* (Beckett, 2008). During roasting the flavour and aroma compounds resulting from the fermentation are further developed (Minifie, 1989), and non-enzymatic browning due to Maillard reaction occurs, darkening the colour. The moisture content is further reduced to 1.5-3% (Dand, 1999), and volatile acids such as acetic acid evaporate while non-volatile acids such as lactic and oxalic acid mostly remain (Beckett, 2008).

When whole beans are roasted, the shell becomes looser (Minifie, 1989). This makes the later shell removal easier. A first disadvantage of whole bean roasting is that the treatment of differently sized beans leads to a less controlled and heterogeneous level of roasting. Secondly, during whole bean roasting cocoa butter melts and diffuses into the shell. When the shell is removed, this part of the cocoa butter is lost. Thirdly, when heating the shell together with the nibs, the energy needed to heat the shell is wasted (Beckett, 2008). Alternatively, nib roasting or even cocoa liquor roasting is applied (Ziegleder, 2009).

After roasting, the shells are removed from the nibs during the so-called winnowing. After grinding fermented, dried, roasted cocoa nibs, cocoa liquor is obtained. During this processing step, particle size is reduced and fat is liberated from the cells of the cocoa nibs, which improves the flow and handling properties (Beckett, 2008). Cocoa butter is removed by pressing cocoa nibs into cocoa cake. This cake is then ground, resulting in cocoa powder (Paoletti *et al.*, 2011).

1.2.3 COCOA LIQUOR

The composition of cocoa liquor is seen in the table below:

Table 1.1: Composition of cocoa nibs measured by Knapp and Churchman (1937), Fincke (1965) and Pearson (1981)

Composition of cocoa liquor	
Water	2.1% - 5%
Fat	48% - 57%
Ash	2.6% - 4.2%
Total nitrogen	2.1% - 2.5%
Starch	6% - 9%
Pectins	4.1%
Crude fiber	2.1% - 3.2%
Cellulose	1.9% - 9%
Tannins	5.8% - 6.2%
Organic acids	0.4% - 2.5%

Total nitrogen includes nitrogen present in proteins, theobromine and caffeine. The water and organic acid content of cocoa liquor depend greatly on drying and roasting conditions.

1.2.3.1 AROMA COMPOUNDS

Cocoa liquor contains several types of aromatic compounds: polyphenols, alcohols, aldehydes, ketones, esters, pyrazines and acids. Polyphenols causes bitterness and astringency (Clapperton *et al.*, 1994). The amount of soluble polyphenols decreases during fermentation, which reduces bitterness and astringency (Villeneuve *et al.*, 1989). Alcohols cause candy and flowery notes, which is desirable in cocoa products (Aculey *et al.*, 2010). Aldehydes and ketones in cocoa are desirable as well, since they produce flowery and fruity notes (Serra-Bonheví, 2005). Both 2-methylbutanal and 3-methylbutanal produce chocolate and malty flavour notes (Jinap *et al.*, 1994). The presence of esters in cocoa is correlated to fruity flavour notes (Jinap *et al.*, 1998). Pyrazines represent 40% of the aroma compounds in roasted cocoa and are some of the most important aroma components in cocoa products (Serra-Bonheví, 2005). The main pyrazine is tetramethylpyrazine, which produces roasted, nutty and chocolate flavour notes and is one of the most important components of roasted cocoa aroma (Afoakwa *et al.*, 2009²). During fermentation acids are formed, and several of them cause sourness and off-flavours (Rodriguez-Campos *et al.*, 2011).

Asian cocoa liquors are known to be highly acidic. South-East Asian and South Pacific cocoa liquors contain higher levels of organic acids than their West African counterparts (Holm *et al.*, 1993), which leads to a higher acid taste (Baigrie and Rumbelow, 1987). Chaudhary and Weber (1990) have also found that cocoa from Central America and South America except Brazil were least acidic while cocoa from Brazil and Asia were very acidic. Cocoa from West Africa was found to have medium acidity. Highly acidic cocoa contained high levels of lactic and acetic acids, which was found by Baigrie and Rumbelow (1987) as well. Luna *et al.* (2002) have reported that acetic acid has an essential role in cocoa liquor acidity. On the other hand, compared to different regions, highly acidic samples contained smaller quantities of citric and oxalic acid (Baigrie and Rumbelow, 1987). Various volatile and non-volatile acids present in fermented cocoa are seen in the table below (Table 2).

Table 1.2: Acids in fermented cocoa (Maarse and van Straten, 1983; Weissberger *et al.*, 1971)

Volatile	Non-volatile (water soluble)
Formic acid	Citric acid
Acetic acid	Phosphoric acid
Propionic acid	Lactic acid
2-Methylpropionic acid	Tartaric acid
Butyric acid	Oxalic acid
2-Methylbutyric acid	Malic acid
3-Methylbutyric acid	Succinic acid
Valeric acid	Pyruvic acid
Caproic acid	
Enanthic acid	
Caprylic acid	

Citric acid (0.37-0.86% on the total weight) is the primary non-volatile acid, followed by lactic acid (0.21%-0.5%). Acetic acid (0.42%-0.81%) is the main volatile acid (Chaudhary and Weber, 1990), and represents 95% of the volatile fatty acids in cocoa liquor (Hoskin and Dimick, 1979). It is produced during the fermentation by oxidation of ethanol (Schwan and Wheals, 2004). It is mostly responsible for high acidity in cocoa (Chaudhary and Weber, 1990) and produces vinegar-like and sour notes (Afaokwa *et al.*, 2009²). C₃ - C₅ acids such as propanoic acid, butanoic acid etc. cause off-flavours in the beans as well (Lopez and Quesnel, 1973). On the other hand, oxalic acid has a positive influence on chocolate taste (Holm *et al.*, 1993).

Apart from the growing region, different fermentation techniques influence the acidity in cocoa as well. Beans that have gone through a high degree of fermentation are likely to be more acid (Chaudhary and Weber, 1990). To reduce acidity in cocoa liquor, manufacturing processes can be altered in order to lower the acid content in fermented beans: drying, roasting and conching can be optimized to reduce the volatile acid content (Manniere and Dimick, 1979; Rapp, 1981).

1.2.4 COCOA BUTTER

1.2.4.1 CHEMICAL PROPERTIES

1.2.4.1.1 FATTY ACID COMPOSITION

Cocoa butter is composed of mainly 3 fatty acids: about 34-36% stearic acid (C18.0), 34-35% oleic acid (C18.1) and 25-27% palmitic acid (C16.0). Linoleic acid (C18.2) and arachidic acid (C20.0) are present in small amounts. The remaining fatty acids are particularly polyunsaturated (Beckett, 2008 and Ribeiro *et al.*, 2012). High unsaturated fatty acid content is related to a softer texture (Chaiseri and Dimick, 1989).

Cocoa pods grown at higher temperatures contain less unsaturated fatty acids than the ones developed at lower temperatures, causing less interruption in the molecular packing of mono-unsaturated triglycerides (TAGs). This has an impact on the crystallization behaviour and, thus, the melting point of cocoa butter (Lehrian *et al.*, 1980).

A research of Lipp and Anklam (1998) showed that cocoa butter produced in South America contains significantly higher levels of oleic acid than those from Africa and Asia. Brazilian cocoa butter contained higher levels di-unsaturated TAGs and lower levels mono-unsaturated TAGs, which results in more unsatisfactory crystallization.

1.2.4.1.2 TRIGLYCERIDE COMPOSITION

The triglyceride profile of cocoa butter is responsible for a highly specific characteristic of chocolate, namely the narrow melting range near below human body temperature (32-35°C).

Cocoa butter is composed of more than 80% of mono-unsaturated TAGs, which consist for 95% out POP, POS and SOS (Loisel *et al.*, 1998). These TAGs have unsaturated oleic acid (O) in the *sn*-2-position and saturated palmitic acid (P) and/or stearic acid (S) in the *sn*-1- and *sn*-3-position. POP,

POS and SOS amount to 17.5%-22.6%, 35.8%-41.4% and 22.8%-31.3%, respectively, in cocoa butter (Chaiseri and Dimick, 1989).

Unsymmetrical mono-unsaturated TAGs such as PPO, SSO and PSO are present in small amounts (Erickson, 1990). Further, di-unsaturated and poly-unsaturated TAGs can add up to 13% and trisaturated TAGs up to 3% (Loisel *et al.*, 1998).

Changes in the triglyceride composition of cocoa butter affect its crystallization behaviour. This, in turn, affects its functionality (Campos *et al.*, 2010). Simultaneously, changes in TAG profile affect the melting behaviour (Lehrian *et al.*, 1980).

The hardness of cocoa butter depends partly on its composition, which depends on the growing area. A study of Chaiseri and Dimick (1989) shows that soft cocoa butter contains higher amounts of di-unsaturated TAGs POO and SOO and lower SOS content than hard cocoa butter. In addition, it was observed that cocoa butters from South America were the softest, containing 9.1% POO and SOO in total, and lowest POS, SOS and SOA content. South American cocoa butters from Bahia and Brazil were especially soft.

1.2.4.1.3 MINOR COMPONENTS

Other than triglycerides, cocoa butter also contains components in minor quantities such as mono- and diglycerides, unsaponifiable matter, free fatty acids and glycol- and phospholipids.

Cocoa butter contains diglycerides in the range of 0.59%-2.8% (Foubert *et al.*, 2004; Shukla, 1995) and traces of monoglycerides (Pontillon, 1998). Unsaponifiable matter such as sterols, pigments, higher aliphatic alcohols and hydrocarbons were present in the range of 0.3% to 1.5% (Schlichter-Aronhime and Garti, 1988; Foubert, 2004). Free fatty acids (FFA's) in cocoa butter have a legal maximum limit of 1.75% according to EU Directive 2000/36/EC. FFA's are formed due to hydrolysis by lipases originating from moulds (Chaiseri and Dimick, 1989). The amount of phospholipids ranges from 0.1% to 0.9%, the values depending on the author (Chaiseri and Dimick, 1995; Shukla, 1995). Glycolipid content in cocoa butter ranges from 0.3% to 0.8% (Chaiseri and Dimick, 1995).

1.2.4.2 PHYSICAL PROPERTIES

1.2.4.2.1 SOLID FAT PROFILE

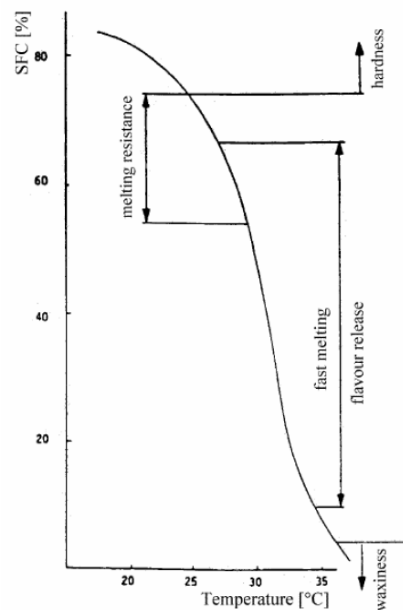


Figure 1.2:Typical SFC-profile of cocoa butter (Foubert, 2003)

The shape of the SFC-profile of cocoa butter gives indications about its physical characteristics (Fig. 1.2). SFC-values between 20° and 25°C are related to cocoa butter hardness. The temperature range where a rapid decline in SFC is observed indicates the heating resistance. In cocoa butter, this temperature range is from 25°C to 35°C. The melting of cocoa butter crystals between 32° and 35°C produces the cooling and creaminess sensation during consumption (Beckett, 2008). Above 35°C, solid fat is responsible for a waxy mouthfeel during tasting (Luccas, 2001).

Cocoa butter destined for chocolate production in tropical regions should be hard at room temperature, meaning an SFC higher than 50% at 25°C. Meanwhile, it still has to melt in the mouth with no waxy residuals left, therefore above 35°C the cocoa butter should be completely melted (Luccas, 2001).

The hardness of a product used to be determined by measuring the resistance to breaking, scratching or flattening, but determination of the SFC by Pulse Nuclear Magnetic Resonance can be used to follow up the SFC (Rutledge *et al.*, 1988) of a sample as a function of time and gives much faster results (Hernandez and Rutledge, 1993).

1.2.4.2.2 CRYSTALLIZATION BEHAVIOUR

1.2.4.2.2.1 POLYMORPHISM

Depending on the polymorphic form, cocoa butter melts over a temperature range from 15°C to 36°C (Huyghebeart, 1971). In fact, cocoa butter can crystallize into different crystal forms and recrystallizes into different polymorphs as well. The six different polymorphs were given the name Form I to form VI by Wille and Lutton in 1966 (Beckett, 2011). In 1999, however, van Malssen *et al.* (1999) showed that there were only 5 polymorphs of cocoa butter which were termed γ , α , β' , β^V and β^{VI} . The desired polymorph for cocoa butter in chocolate is the β^V polymorph, since this polymorph has the best properties for chocolate such as gloss, colour, hardness, snap and shelf life (Dewettinck, 2012).

The γ -form is the most unstable one and has a melting point of 17°C (Talbot, 1999). It transforms rapidly into the α polymorphic form (Beckett, 2011), which can also be formed directly from the melt. This form is more stable than γ , but will still transform into β' within an hour when stored above 6°C. The β' phase exists as a phase range with a variety of melting characteristics rather than an individual phase. The melting and crystallization characteristics of this phase depend on the TAG composition of the cocoa butter. The β polymorphs are formed by transformation from the β' -phase (van Malssen *et al.*, 1999). Direct formation from the melt does not occur unless β seed crystals are present; this phenomenon is called crystal memory.

Two types of β crystal forms exist: β^V and β^{VI} , both of which can be directly formed out of the β' phase. When formed via phase transition, the β^V form can only be formed at temperatures lower than 25°C. β phase formation from the melt due to memory effect is completed faster than phase transformation from the β' phase. Formation from the melt occurs within half an hour whereas phase transition starts after 13 hours and takes 26 hours to complete (van Malssen *et al.*, 1999).

1.2.4.2.2.2 ISOTHERMAL CRYSTALLIZATION

The polymorphism of cocoa butter has a large influence on the quality of chocolate products. In order to ensure product quality and optimize production processes, it is important to understand this crystallization behavior.

Isothermal crystallization of cocoa butter at 20°C consists of 2 steps: the first is the formation of α -crystals from the melt, which is then followed by the second step: the polymorphic transition of α -crystals into β' -crystals. Extra α -crystals are then formed, which are transformed again into β' -crystals (Foubert *et al.*, 2004). In the time frame of the experiment, β' -crystals are not directly formed from the melt, and no polymorphic transition to β -crystals took place. No transformation to β^V -crystals took place, seeing as the duration of the experiment was not long enough. The main crystallization peak resulting from DSC-measurements was fitted to the Foubert (Foubert *et al.*, 2004) model with the following equation:

$$f(t) = a_F x \left[1 - \left(1 + (0.99^{1-n} - 1) x e^{-(1-n)xKx(t-t_{ind,x})} \right)^{\frac{1}{n}} \right] \quad (\text{Eq. 1.1})$$

Parameter a_F (J/g) is the total amount of heat released and is related to the solid fat content (SFC) at equilibrium. This value decreases when the content of unsaturated fatty acids and fraction of di-unsaturated TAGs increase (Foubert *et al.*, 2004). This can be explained by the double bond of di-unsaturated TAGs in the *sn*-3 position, which interrupts the molecular packing of mono-unsaturated TAGs. The higher the percentage of di-unsaturated TAGs, the lower the equilibrium solid fat content. Hence, Asian and Oceanian cocoa butters, containing less of these TAGs (Chaiseri and Dimick, 1989), would have a higher a_F -value than those from Africa and America. Another possible explanation for the lower solid fat content is that di-unsaturated TAGs do not crystallize at 20°C, but at 2.5°C (POO) and 8.6 (SOO) (Hagemann, 1988). Free fatty acids and diacylglycerols are also negatively correlated with solid fat content (Foubert *et al.*, 2004).

Parameter $t_{ind,x}$ is the induction time of the conversion from x percentage of α -crystals, x being 1, to β' -crystals, in the case of the second isothermal crystallization step of cocoa butter at 20°C. The higher the amount of saturated fatty acids, mono-unsaturated and trisaturated TAGs, the lower the induction time. In contrast, higher percentages of diacylglycerols, phosphorus, and free fatty acids are positively correlated with induction time (Foubert *et al.*, 2004).

Parameter K (1/h) is the rate constant of the crystallization process. Higher amounts of unsaturated fatty acids and di-unsaturated TAGs lower the rate constant (Foubert *et al.*, 2004), which was also stated by Chaiseri and Dimick (1989), who observed that di-unsaturated TAGs delay cocoa butter crystallization. Diacylglycerols and free fatty acids also lower the crystallization rate, which was noticed by Pontillon (1998) and Shukla (1995). Because of the absence of a third fatty acid chain, the incorporation of DAGs in the fat crystals is obstructed (Wright *et al.*, 2000).

Parameter n is the order of the reverse reaction. When cocoa butter crystallizes, latent heat is released. This heat is able to remelt neighbouring crystals (Smith *et al.*, 2001). The higher n , the shorter the influence of the reverse reaction on the crystallization process. Higher amounts of mono-unsaturated TAGs and saturated fatty acids give an increase in n . High percentages of mono-unsaturated TAGs and saturated fatty acids increase the melting point of cocoa butter. This means that more heat is needed to remelt the formed crystals, thereby reducing the influence of the reverse reaction. For instance South American cocoa butters, containing the lowest amount of mono-unsaturated TAGs (Chaiseri and Dimick, 1989), would have a higher n -value than African and especially Asian cocoa butters. The presence of diacylglycerols lowers the value of n by inducing a larger number of crystal nuclei, thereby producing smaller crystals. These crystals have more surfaces per volume, and dissolve more easily, which increases the influence of the reverse reaction (Foubert *et al.*, 2004).

1.3 FROM COCOA INGREDIENTS TO CHOCOLATE

1.3.1 REFINING

After mixing the solid ingredients and part of the fat phase, the particles in the chocolate mass are reduced in size by roll refiners.

Particle size influences the rheological properties of chocolate, which in turn influences the sensory properties. According to Beckett (2000) the larger particles are responsible for the gritty mouth feel, while the smaller ones are important to the chocolate flow properties, such as viscosity. Smaller particles have a creamier taste and texture than large particles, and since the maximum particle size for which the tongue detects a smooth texture is 30µm (Afoakwa, 2011), particles larger than 30 µm cause a gritty mouthfeel. European chocolate usually has a fineness of 15-22 µm, while North American chocolate has a fineness of 20-30 µm (Jackson, 1999).

1.3.2 CONCHING

Conching is a combination of mixing and shearing. It is performed to improve chocolate flavour, texture and flow behaviour. Flavour molecules that are initially concentrated on the cocoa particles are redistributed throughout the whole mixture (Ziegleder, 2009). Unwanted astringent and acid flavour compounds formed through roasting and fermentation are evaporated while new flavour components are formed through Maillard reaction causing browning as well. Moisture is removed mainly during the early stages of the conching process. Acids which evaporation temperatures lie above conching temperature, are removed through the evaporating steam. However, over-conched chocolate may have little flavour left (Beckett, 2008). To even further reduce acid levels, mainly acetic acid, cocoa liquor can be pre-treated by a temperature treatment at 70-90°C (Kleinert-Zollinger, 1988).

Conching separates agglomerated particles and makes sure the particles are evenly covered with cocoa butter, thereby turning the flaky structure into a flowing liquid. Coating the particles with fat improves flow properties and reduces viscosity. The higher the shear rate used, the lower the resulting viscosity of the chocolate (Beckett, 2008).

The conching process includes two sub-steps: the dry and wet conching phase. During the dry phase the chocolate has a flaky structure, and particles are partly still uncoated with fat, which makes it easier for the moisture to evaporate. When the temperature increases, the cocoa butter melts and the chocolate becomes a dense paste. During the wet conching phase, cocoa butter and lecithin are added to the mixture, further lowering the viscosity (Beckett, 2008). Lecithin is an emulsifier which is commonly added to chocolate to control its flow properties in a more economical way than adding cocoa butter. As molten chocolate is a suspension of hydrophilic (sugar crystals and milk solids) and more lipophilic (cocoa solids) in a continuous fat phase, it exhibits shear-thinning thixotropic behaviour beyond a yield stress value (Ghorbel *et al.*, 2011). The active components in lecithin are amphiphilic consisting of a hydrophilic and a lipophilic part. The hydrophilic head groups adsorb to

the solid particles-fat interfaces promoting the dispersion of the particles in the continuous phase (lowering plastic viscosity) and preventing their aggregation (lowering yield stress). However, when lecithin is added in excess, the surface-active phospholipids (and glycolipid) can form micelles or bilayers on the covered sugar particles, which will result in a yield stress increase. Chocolate flow data are often fitted to the Casson model expressing Casson yield stress σ_{CA} (Pa) and Casson viscosity η_{CA} (Pa.s) (Beckett, 2000).

1.3.3 TEMPERING

As mentioned above, the desired polymorph for cocoa butter in chocolate is β^V , since this crystal form provides chocolate desired quality properties, such as gloss, colour, hardness, snap and shelf life. Because β^V crystal cannot be formed directly from the melt, a pre-crystallization process called tempering is needed (Dewettinck, 2012).

Aside from the temperature, shear plays a role in the tempering process. For a fat crystal to grow, it needs a seed crystal to expand around. Therefore, a larger crystal has much less influence than several smaller crystals well distributed in the mixture. A high shear rate helps with breaking off the larger crystals and distributing its fragments through the chocolate (Ziegleder, 1985). Using this method, about 1%-3% v/v of seed crystals is needed to temper chocolate (Stapley *et al.*, 1999). Another way to temper the chocolate is to cool it to around 30°C and seed it with pre-tempered chocolate or seed it with β^V or β^{IV} crystals (Beckett, 2008). When properly tempered, the chocolate contracts during cooling, which makes the moulding process easier (Nestius-Svensson, 2011).

Afoakwa *et al.* (2008^b) studied the influence of tempering degree and particle size on the textural properties of dark chocolate. Hardness was highest in under-tempered chocolate (Fig. 5). This is due to the recrystallization of the fat phase which results in intense hardening. Over-tempered chocolate had medium hardness while well-tempered chocolate had the lowest hardness. This is due to the recrystallization of the fat phase which results in intense hardening. Further, it was observed that chocolate hardness increased with decreasing particle size.

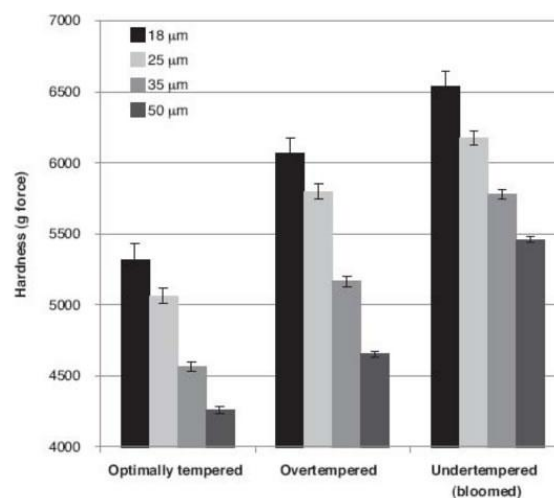


Figure 1.3: Hardness of dark chocolate in function of temper-degree and PSD (Afoakwa *et al.*, 2008b)

2 MATERIALS AND METHODS

2.1 COCOA BUTTER

The origin of the three first cocoa butter samples, retrieved from commercial seeds from: Ghana, Ivory Coast and Indonesia, were randomly selected.

The ten other samples originate from the Cocoa Seed Centre in Nong Lam University, Thu Duc District, Vietnam. Different clones of cocoa beans were divided into groups based on species as well as similar external appearances. The emphasis lies especially on TD3 and TD5, considering their larger use Vietnam:

- TD 2: Forastero x Forastero
- TD 3: Trinitario x Forastero
- TD 5: Trinitario x Forastero
- TD 6: Trinitario
- TD 7: Trinitario x Forastero
- TD 8: Forastero
- TD 9: Trinitario x Forastero
- TD 10: Trinitario x Forastero
- TD 11: Forastero x Forastero
- TD 13: Trinitario x Forastero

Approximately 120g of fermented cocoa beans were roasted by using an oven with air circulation by a fan. The oven was preheated at 145°C, and then the beans were roasted for 15 minutes at 145°C. The shell was manually removed from the nib. The nibs were stored in a plastic container at room temperature. Afterwards, the beans were grounded with a kitchen blender until powdery material.

2.2 PHYSICOCHEMICAL CHARACTERISATION OF COCOA BUTTER

2.2.1 FAT EXTRACTION

Pearson (1981)

2.2.2 FREE FATTY ACIDS

AOCS Official Method Ca 5a-40, (1966)

2.2.3 FATTY ACID COMPOSITION

AOCS Official Methods Ce 1-62, (1990) & Ce 2-66 (1989)

2.2.4 TRIGLYCERIDE PROFILE

AOCS Official Methods Ce 5b-89 (1997)

2.2.5 MELTING AND CRYSTALLISATION BEHAVIOUR

Equipment

- Q1000 DSC (TA Instruments, New Castle, Delaware, USA)
- Refrigerated Cooling System, (TA Instruments, New Castle, Delaware, USA)
- Purge: Nitrogen gas (Air Liquide, Liege, Belgium)
- Calibration: indium (TA Instruments, New Castle, Delaware, USA), azobenzene (Sigma-Aldrich, Bornem, Belgium) and undecane (Acros Organics, Geel, Belgium)
- Aluminum hermetic pans and lids (TA Instruments, New Castle, Delaware, USA)
- Software: Universal Analysis (TA Instruments, New Castle, Delaware, USA) and SigmaPlot 11.0 (SPSS Inc., Chicago, Illinois, USA)

Procedure

Cocoa butter was melted in an oven at 70°C and 8 to 14 mg sample was inserted and sealed into hermetic aluminum DSC cups. The cups were cleaned with ethanol prior to analysis. The reference used was an empty aluminum pan.

The following time-temperature procedure for non-isothermal crystallisation was used:

1. Equilibrate at 65.0°C
2. Isothermal for 10 min
3. Ramp 5.0°C/min to -20.0°C
4. Isothermal for 5 min
5. Ramp 5.0°C/min to 65.0°C

The following time-temperature procedure for isothermal crystallisation was used:

1. Equilibrate at 65.0°C
2. Isothermal for 10 min
3. Ramp 10.0°C/min to 20.0°C
4. Isothermal for 230 min

Data processing

Non-Isothermal: Integration of the crystallisation and melting peak were completed using a horizontal sigmoid baseline. Constant begin and end points were used at -10°C and 40°C. Onset and offset points were determined using visually selected points.

Isothermal: Isothermal crystallisation yielded two peaks. This was plotted as heat flow (W/g) versus time (min). The first peak was integrated using a horizontal sigmoidal baseline from 10.0 min to a visually selected end point, after which the peak was split starting from 14.6mins (end of cooling step). Onset and offset points were determined using visually selected points.

The second peak was integrated using a horizontal sigmoidal baseline starting from a visually selected point, usually 27 mins, to 150 mins. Offset points were determined using visually selected points. This peak was further processed by integration and fitting the data to the Foubert model (Foubert *et al.*, 2002) using nonlinear regression.

$$f(t) = a_F x \left[1 - \left(1 + (0.99^{1-n} - 1) x e^{-(1-n)xKx(t-t_{ind_x})} \right)^{1/n} \right] \quad (\text{Eq. 1.1})$$

With: f (J/g) = amount of crystallization at time t

t (min) = time

a_F (J/g) = total amount of heat released during 2nd crystallization step = mass fraction

β' crystals formed during 2nd step

n (-) = order of reverse reaction

K (1/min) = rate constant

t_{ind_x} = time needed to polymorph $x\%$ α to β' crystals

Since n is negatively correlated with parameter K , the value of n was fixed to 6 in order to compare the crystallization kinetics of all samples (Sichien, 2007).

2.2.6 SOLID FAT CONTENT

Equipment

- Maran Ultra NMR (Oxford Instruments, Oxfordshire, UK).
- NMR tubes, diameter 10 mm (Bruker, Karlsruhe, Germany)
- Water bath (Julabo, Seelbach, Germany)

Procedure

An amount of 3.5 ml of molten cocoa butter (70°C) was inserted in a glass NMR tube. SFC measurements were done according to the AOCS Official method Cd 16b-93. Firstly, crystal memory was erased. The next step was crystal formation by cooling the fat. Because the polymorphic form of 2-oleo-disaturated glycerides should be stabilized to create reproducible results, the sample was

tempered for 40 h at 26°C. During this period, β^V crystals are formed from the β' polymorph. After tempering, the samples were cooled to 0°C and SFC was measured from 5°C to higher temperature at 5°C intervals.

2.3 COCOA LIQUOR

The following cocoa liquor samples were characterized physicochemically and applied in dark chocolate:

- TD3/5: 50/50 mixture of TD3 and TD5 from Bentre province in Vietnam.
- TD: A mixture with ratio 60/40 of TD3, TD5 and TD6, TD7, TD11, delivered by the Trong Duc Company from Dong Nai, Vietnam.
- PGP: Commercial cocoa liquor from Vietnam delivered by Puratos Grand-Place.
- Gh: Commercial cocoa liquor from Ghana supplied by Cargill, Mechelen, Belgium.

2.4 PHYSICOCHEMICAL CHARACTERISATION OF COCOA LIQUOR

2.4.1 MOISTURE CONTENT

AOCS Ca 2e-84: Karl Fischer method.

2.4.2 DRY MATTER (DM)

AOAC method 935.29: Gravimetric method.

2.4.3 FAT CONTENT

Weibull method (Pearson, 1981).

2.4.4 PROTEIN CONTENT

Kjeldahl method (Pearson, 1981).

2.4.5 FIBER CONTENT

Fiber content was determined using an enzyme kit (Merck KGaA, Downstadt, Germany).

2.4.6 ASH CONTENT

AOAC Official Methods of Analysis: Ash (Williams, 1984)

2.4.7 ORGANIC ACID ANALYSIS

Equipment

- Thermo Finnigan Surveyor HPLC (Thermo Electron Corporation, Brussels, Belgium) with four solvent lines, degasser, autosampler.
- Nebulising gas: Nitrogen.

Procedure

5g cocoa liquor is suspended in 50ml boiling HPLC-grade water. The suspension is then filtered and the residue is washed with HPLC-grade water until 50ml has been collected. 5ml filtrate is diluted to 25ml with acetonitrile.

The mobile phase is 0.01N H₂SO₄ in HPLC-grade water, filtered over a 0.20µm or 0.45µm membrane.

2.4.8 PH

AOAC Official method 13.010.

2.4.9 PARTICLE SIZE DISTRIBUTION (PSD)

0.5g sample was dissolved in isopropanol and analysed through laser light scattering using a Mastersizer (Malvern, Worcestershire, UK). Light colliding with a particle in dispersion scatters at various angles, depending on its size. By means of the Mie theory of light scattering, the angular scattering data can be used to calculate the size distribution of the particles in the analysed dispersion. The particle sizes mentioned in later chapters is expressed as a volume equivalent sphere diameter.

2.4.10 PEROXIDE VALUE

AOCS Official Method Cd 8b-90 (1996)

2.4.11 P-ANISIDINE VALUE

AOCS Official Method Cd 18-90.(1996).

2.5 PROCESSING OF COCOA LIQUOR

2.5.1 GRINDING

The mixture of TD3 and 5 with ratio 50/50 was initially not in the form of cocoa liquor, thus requiring being ground in a Stephan mixer (Stephan, Nazareth, Belgium) before further processing.

The ground mixture of TD3 and 5 with ratio 50/50 and cocoa liquor supplied by Trong Duc Company were refined using a ball-mill (Wienier) in NatraAllcrump (Oostmalle, Belgium) as these chocolate ingredients were too coarse.

2.5.1 CONCHING

In order to reduce the acidity of the mixture of TD3 and 5 with ratio 50/50 and cocoa liquor supplied by Trong Duc Company, these were conched with a Bühler ELK'olino conche (Richard Frisse GmbH, Bad Salzflen, Germany).

The cocoa liquors were conched as follows:

- 12 hours at 60°C
- 12 hours at 70°C
- 12 hours at 80°C
- 12 hours at 85°C

Before and after each conching step a sample was taken to determine the moisture content, pH and organic acids. For TD3/TD5 only a sample prior and after the whole conching process was taken due to insufficient sample material.

2.6 DARK CHOCOLATE

2.6.1 RECIPE

The different cocoa liquors were modified by adding extra cocoa butter until their fat content was identical. With these modified liquors, five chocolate products were created using the same recipe which is presented below:

Table 2.1: Ingredients and suppliers of the chocolate components

Ingredient	Quantity	Supplier
Sugar	48.00%	Barry Callebaut, Wieze, Belgium
Cocoa liquor	40.00%	See below
Cocoa butter	11.60%	Cargill, Mechelen, Belgium
Soy lecithin	0.40%	Barry Callebaut, Wieze, Belgium

Five dark chocolates, each with different cocoa liquors' origin and acidity were produced:

- Gh: low acidity
- TD unconched (TDU): high acidity
- TD conched (TDC): moderate acidity
- TD3/5 conched (TD3/5C): high acidity
- PGP: low acidity

2.6.2 PRODUCTION PROCESS

Table 2.2 describes the dark chocolate production process. Because each cocoa liquor had a different fat content, adjustment to the cocoa liquor using free cocoa butter have been made to result in end products with similar fat contents. Cocoa liquor, sugar, and part of the cocoa butter were mixed and refined to a particle size of <30um. After that, the conching process was performed with two phases: the first is the dry phase where cocoa butter is added till the mixture is 27.72% fat. The second phase is the liquid phase where the mixture is liquefied by addition of the rest of the cocoa butter and lecithin is added. The chocolate was subsequently tempered mechanically to obtain the desired polymorph with a verified temper degree. The tempered chocolate is moulded into bars and kept in a 40% relative humidity cooling cabinet at 18°C. Chocolate bars intended for sensory analysis were wrapped in aluminum foil.

Table 2.2: Production process and parameters.

Chocolate production process	
Mixing	
<u>Equipment:</u>	Planetary mixer VEMA BM30/20 (Machinery Verhoest NV/Vema Construct, Izegem, Belgium)
<u>Procedure:</u>	Rotation speed 3, T = 45 °C, t = 20 min
Refining	
<u>Equipment:</u>	Exact 80S 3-roll mill (E & R Chemicals & Equipment B.V., Vlaardingen, Netherlands)
<u>Procedure:</u>	Roller speed = 400 rpm, T = 35 °C
Conching	
<u>Equipment:</u>	Bühler ELK'olino conche (Richard Frisse GmbH, Bad Salzuflen, Germany)
<u>Procedure:</u>	Dry conching: 1200 rpm clockwise, T = 60°C, t = 120 min 1200 rpm anti-clockwise, T = 70°C, t = 240 min Wet conching: 2400 rpm clockwise, T = 45°C, t = 15 min 2400 rpm anti-clockwise, T = 45°C, t = 15 min
Tempering	
<u>Equipment:</u>	Selmi One Tempering Unit (ECD, Wilrijk, Belgium) Chocometer (Aasted Mikroverk, Farum, Denmark)
<u>Procedure:</u>	Tempering at different temperatures until satisfactory temper degree (3.5 -5).
Moulding	
<u>Equipment:</u>	Chocolate bar mould (Chocolate world, Antwerp, Belgium)
<u>Procedure:</u>	Moulds are filled. Cooling: T = 11°C, t = 30min Demoulding.
Storing	
<u>Equipment:</u>	Cooling cabinet (Chocolate World, Antwerp, Belgium)
<u>Procedure:</u>	Relative humidity = 40%, T = 18 °C Chocolate bars intended for sensory analysis were wrapped in aluminum foil

2.7 QUALITY PARAMETERS OF DARK CHOCOLATE

2.7.1 TEXTURE ANALYSIS

Equipment

- TA500 Texture Analyzer (Instron, High Wycombe, UK)
- 500N load cell
- Penetration probe (needle)

Procedure

The chocolate bars were measured two weeks after production. They were penetrated at 20°C and 30°C over a distance of 10mm by a needle at a speed of 10mm/min. The maximum load (N) used to penetrate the sample is defined as the hardness of the sample.

2.7.2 RHEOLOGY

Equipment

- AR2000 rheometer (TA Instruments, Brussels, Belgium)
- Concentric DIN cylinder:
 - Gap: 5920 μm
 - Cylinder immersed height: 42.00 mm
 - Rotor outer radius: 14.00mm
 - Stator inner radius: 15.00 mm

Procedure

Official ICA method (ICA, 2000): chocolate viscosity was measured using rotational concentric cylinders, also named bob and cup. Pre-shear was 5 sec^{-1} for 15 min, viscosity and stress of the sample were measured at a shear rate of 2 sec^{-1} increasing to 50 sec^{-1} , and at 50 sec^{-1} decreasing to 2 sec^{-1} . Flow behavior was measured as shear stress in function of shear rate and was fitted to the Casson equation. Although ICA has advised against the use of the Casson model, it is still widely used. Afoakwa *et al.* (2009) has compared the Casson equation against the new standard on dark chocolate and concluded that both models were highly able to quantify chocolate flow parameters.

2.7.3 COLOUR

Colour of tempered chocolate bars was measured using a Minolta Model CM-2500D colorimeter (Konica Minolta Sensing Inc., Japan). The chocolate samples were measured after calibration on a white surface.

2.7.4 SENSORY ANALYSIS

The sensory analysis consists of a triangle test and a Quantitative Descriptive Analysis (QDA). The objective of the triangle test is to determine sensorial differences between the samples observed by non-trained consumers. The chocolate samples for both sensory tests were wrapped in aluminum foil and kept at 18 °C. Chocolate bars were cut into equal pieces and presented to the participant on a colourless, odourless plate.

2.7.4.1 TRIANGLE TEST

For this test, three number-coded chocolate samples are offered: two identical samples and one odd sample, in six randomized serving orders. The participant had to distinguish the odd sample. The sessions were taken place at the SensoLab (UGent, Belgium), where participants tasted the samples in individual booths under a red light, which eliminates colour differences. When no differences are discerned, the probability of selecting the correct sample is 1/3. If the samples are distinguishable the proportion of correct answers will be larger than 1/3 (Lawless and Heymann, 2010):

$$H_0: P = 1/3$$

$$H_A: P > 1/3$$

This test determines if the samples are perceived differently by consumers, but does not describe the differences themselves.

2.7.4.2 QUANTITATIVE DESCRIPTIVE ANALYSIS (QDA)

Several aspects of the three main factors used to evaluate chocolate (appearance, flavour and texture) were evaluated by a trained panel. The characteristics concerning these three factors were described by means of a 9-point hedonic scale. The questionnaire itself is to be found in Appendix 4. The sessions were located at the SensoLab, under normal light conditions.

2.8 STATISTICAL ANALYSIS

Statistical analysis was performed with Spotfire S+ 8.2. All testing was done at a significance level of 0.05. One-way ANOVA was done to investigate any significant differences within the samples. Testing for equal variance was executed with the Modified Levene Test. When conditions for equal variance were fulfilled, the Tukey test was used to determine differences between samples when variances were equal. If variances were not equal, Kruskal-Wallis was performed.

3 RESULTS AND DISCUSSION

3.1 INTRODUCTION

This research consists of two main parts. The first part focuses on the physicochemical properties of multiple cocoa butters originating from Vietnam and cocoa butter from the three leading cocoa producing countries in the world (Ghana, Ivory Coast and Indonesia). The aim was to correlate the physical properties (SFC, melting range, non-isothermal and isothermal crystallization) to important chemical characteristics (fatty acid composition, triacylglycerol profile and free fatty acids).

In the second part, the physicochemical properties of three Vietnamese cocoa liquors were compared to one Ghanaian sample. Prior to this, two of the former were refined using a ball-mill to obtain a suitable particle size distribution and conched in order to decrease the acidity. As a matter of fact, Vietnamese cocoa liquor is characterised by an excessive acidic flavour which might jeopardise its acceptance by consumers worldwide. Next, the functionality of the (post-treated) Vietnamese cocoa liquors was compared with that of Ghana in dark chocolate using both instrumental and sensorial evaluation.

3.2 PHYSICOCHEMICAL PROPERTIES OF COCOA BUTTER

In this section, the physicochemical properties of cocoa butter extracted from Vietnamese cocoa beans were determined and compared to that of sample from Ghana, Ivory Coast and Indonesia. Ten clones grown Thu Duc, in the Mekong Delta of Vietnam were sampled.

3.2.1 CHEMICAL PROPERTIES

3.2.1.1 FATTY ACID COMPOSITION

Table 3.1 shows the fatty acid composition (%) of the cocoa butters extracted from ten clones from Vietnam, Ghana, Ivory Coast and Indonesia. In terms of its fatty acid profile, cocoa butter is a rather simple fat. The three main fatty acids in cocoa butter were palmitic acid (C16.0), stearic acid (C18.0) and oleic acid (C18.1), and account for about 95% of the fatty acids. Linoleic acid (C18.2) and arachidic acid (C20.0) are dominating the minor fraction of fatty acids. Fatty acids, such as myristic acid (C14.0) and margaric acid (C17.0), were present in trace amounts and are grouped in the category 'Others'.

Table 3.1: Fatty acid profile (%) of the cocoa butters

FA %	C16.0	C18.0	C18.1	C18.2	C20.0	Others	SFA	MUFA	PUFA
Vietnam	27.3 ± 1.8	35.8 ± 1.9	31.8 ± 0.6	2.7 ± 0.4	1.1 ± 0.0	1.1 ± 0.1	64.9 ± 0.4	32.2 ± 0.6	3.0 ± 0.4
TD2	28.7 ± 0.1	34.0 ± 0.1	32.3 ± 0.1	2.6 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	64.5 ± 0.0	32.6 ± 0.1	2.9 ± 0.0
TD3	27.9 ± 0.2	34.6 ± 0.0	32.5 ± 0.3	2.5 ± 0.0	1.2 ± 0.0	1.1 ± 0.1	64.4 ± 0.1	32.8 ± 0.2	2.7 ± 0.0
TD5	26.8 ± 0.2	36.5 ± 0.1	31.6 ± 0.2	2.8 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	65.0 ± 0.1	32.0 ± 0.1	3.0 ± 0.0
TD6	28.9 ± 0.1	34.2 ± 0.1	32.5 ± 0.1	2.0 ± 0.0	1.2 ± 0.0	1.0 ± 0.1	65.0 ± 0.0	32.9 ± 0.0	2.2 ± 0.0
TD7	26.3 ± 0.1	36.8 ± 0.3	32.0 ± 0.1	2.5 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	64.8 ± 0.1	32.3 ± 0.1	2.9 ± 0.1
TD8	25.2 ± 0.2	38.2 ± 0.0	30.7 ± 0.4	3.6 ± 0.0	1.2 ± 0.0	1.0 ± 0.2	65.3 ± 0.1	31.0 ± 0.2	3.8 ± 0.0
TD9	24.6 ± 0.1	38.8 ± 0.2	31.1 ± 0.3	3.0 ± 0.0	1.2 ± 0.0	1.0 ± 0.1	65.3 ± 0.1	31.5 ± 0.2	3.2 ± 0.0
TD10	30.4 ± 0.1	32.9 ± 0.0	31.4 ± 0.1	2.7 ± 0.0	1.1 ± 0.0	1.2 ± 0.1	65.2 ± 0.1	31.8 ± 0.0	3.0 ± 0.1
TD11	26.9 ± 0.0	36.3 ± 0.0	31.6 ± 0.0	2.8 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	65.0 ± 0.0	32.0 ± 0.0	3.1 ± 0.0
TD13	26.8 ± 0.1	35.8 ± 0.0	32.4 ± 0.0	2.6 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	64.3 ± 0.0	32.7 ± 0.0	3.0 ± 0.0
Ghana	26.7 ± 0.1	36.0 ± 0.1	32.1 ± 0.1	2.8 ± 0.0	1.0 ± 0.0	1.4 ± 0.1	64.3 ± 0.1	32.4 ± 0.0	3.3 ± 0.0
Ivory Coast	28.0 ± 0.2	34.8 ± 0.1	32.3 ± 0.3	2.7 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	64.5 ± 0.1	32.6 ± 0.2	2.9 ± 0.0
Indonesia	27.1 ± 0.1	36.0 ± 0.2	32.4 ± 0.4	2.2 ± 0.0	1.2 ± 0.0	1.0 ± 0.2	64.9 ± 0.1	32.7 ± 0.2	2.4 ± 0.0

Values of Vietnam are the averages of the ten clones.

Within the group of different clones from Vietnam, a relatively large variability in palmitic and stearic acid was observed with ranges of 24.6% - 30.4% and 32.9% - 38.8%, respectively. However, the variability in the total content of saturated fatty acids (SFA) was narrower with 64.4% - 65.3%. TD2, TD3, TD7 and TD13 had a somewhat lower SFA content than the other clones, mainly due to an increased content in oleic acid or by extension in mono-unsaturated fatty acids (MUFA). In terms of its content in linoleic acid or by extension in poly-unsaturated fatty acids, TD8 was highest. The average fatty acid composition of the cocoa butters from the Vietnam clones was similar to that of Indonesia in terms of the palmitic and stearic acid content. The somewhat lower average content in oleic acid for the sampled cocoa butters from Vietnam was compensated by their average higher content in linoleic acid content. The SFA content of the cocoa butter extracted from beans of Ghana and Ivory Coast was somewhat lower than those of the Asian samples because of a decreased content in palmitic and stearic acid, respectively.

Shukla (1995) reported the fatty acid composition of cocoa butters from different origins:

- Ghana: 25.1-25.4% C16.0; 37.6-37.8% C18.0; 32.8-33.6% C18.1, 2.6-2.8% C18.2 and 1.1-1.2% C20.0;
- Ivory Coast: 25.7-25.9% C16.0, 35.4-37% C18.0, 33-34.5% C18.1, 2.9-3.4% C18.2 and 1.2% C20.0;
- Indonesia: 24.2% C16.0, 37.4% C18.0, 34.4% C18.1, 2.8% C18.2 and 1.2% C20.0.

These reference values (Shukla, 1995) are somewhat different than the ones of the sampled cocoa butters from Ghana, Ivory Coast and Indonesia, reflected in higher contents of palmitic acid and lower contents of stearic and oleic acid. This observation can be due to differences in growing conditions, age of the plant (Schlichter-Aronhime & Garti, 1988), the cacao variety (Chaiseri & Dimick, 1989) and the cocoa butter extraction process (Pontillon, 1998).

Lehrian *et al.* (1980) stated that cocoa pods grown at higher temperature contain more saturated fatty acids than the ones developing at lower temperatures. Cocoa pods were grown at different controlled temperatures. Cocoa butters from plants grown at higher temperatures contained more SFA than the ones growing at lower temperatures. Due to the increased temperature, Asian and Oceanian cocoa butter are on average the hardest (Chaiseri and Dimick, 1989). The higher SFA content in cocoa butters from Indonesia and Vietnam in comparison to the sampled African butters was confirmed in this thesis research.

3.2.1.2 TRIACYLGLYCEROL PROFILE

The triacylglycerol (TAG) profiles of the sampled cocoa butters are given in Table 3.2. These results obtained using HPLC-ELSD must be interpreted with caution, as due to the non-linearity of the detection system the small peaks are underestimated for the benefit of the large peaks of POP, POS and SOS. As a result, this technique can only be used for comparison purposes. In contrast, determination of the fatty acid composition using GC can be considered as accurate.

Table 3.2: TAG profile (area%) of the cocoa butters

	POP	POS	SOS	others	SSS	S ₂ U	SU ₂	S ₂ U/SU ₂
Vietnam	18.9 ± 0.3	45.1 ± 0.3	30.2 ± 0.2	5.8 ± 0.2	0.4	97.4	2.0	52.5
TD2	21.1	44.5	27.6	6.8	0.5	96.4	3.0	32.7
TD3	22.1 ± 0.1	45.1 ± 0.1	28.3 ± 0.0	4.6 ± 0.0	0.5	96.7	2.8	35.1
TD5	17.4 ± 0.0	44.6 ± 0.1	31.2 ± 0.0	6.9 ± 0.0	0.4	97.2	2.4	41.1
TD6	21.5	45.4	28.5	4.7	0.6	97.5	1.8	54.0
TD7	17.8 ± 0.4	44.1 ± 0.2	32.3 ± 0.4	5.8 ± 0.1	0.5	97.1	2.3	41.8
TD8	15.9 ± 0.4	44.8 ± 0.0	32.4 ± 0.0	6.9 ± 0.1	0.4	98.3	1.3	75.3
TD9	14.6 ± 0.4	45.0 ± 0.5	34.5 ± 0.2	5.9 ± 0.1	0.4	98.3	1.3	73.8
TD10	21.9	47.0	25.5	5.6	0.4	98.0	1.5	67.1
TD11	18.1 ± 0.2	45.9 ± 0.3	31.1 ± 0.2	4.9 ± 0.0	0.4	97.9	1.5	65.1
TD13	19.0 ± 0.3	44.4 ± 0.1	30.5 ± 0.2	6.1 ± 0.1	0.4	97.0	2.5	38.9
Ghana	16.6 ± 0.1	44.0 ± 0.1	32.1 ± 0.2	7.4 ± 0.0	0.4	96.5	3.1	31.5
Ivory Coast	17.8	46.3	29.4	6.5	0.4	96.9	2.5	39.5
Indonesia	18.3 ± 0.2	44.9 ± 0.1	30.7 ± 0.1	6.0 ± 0.0	0.5	96.8	2.6	36.6

Values of Vietnam are the averages of the ten clones.

Within the group of different clones from Vietnam, a relatively large variability in POP and SOS was observed, with ranges of 14.6% - 22.1% and 25.5% - 34.5%, respectively. This was expected based on the broad ranges in palmitic and stearic acid. In contrast, the POS and disaturated TAGs (S₂U) content of the different clones fell within a relative narrow range, namely 44.1% - 45.9% and 96.4% - 98.3%. The trisaturated TAGs (SSS) content, represented by PPP, PPS and PSS, is very low for all clones but this may, as already mentioned, be due to the non-linearity of the detector. The monosaturated TAGs (SU₂), represented by PLO, POO and SOO, varied from 1.3% to 3.0%. In cocoa butter, no or only trace amounts of triunsaturated TAGs (UUU) are present. TD2, TD3, TD5, TD7 and TD13 had a somewhat lower S₂U content than the other clones, due to their relatively lower content in saturated fatty acids. In TD 8 and TD 9, the highest values for S₂U were observed, resulting from their high SFA content.

The average TAG composition of the cocoa butters from the Vietnam clones was very similar to that of Indonesia. In comparison to the Indonesian sample, the Vietnamese cocoa butters was on average somewhat richer in POP at the expense of SOS, following the trend of palmitic and stearic acid content. The SU₂ content of the cocoa butter extracted from beans of Ghana and Ivory Coast was somewhat lower than those of the Vietnamese samples because of their lower SFA content. Surprisingly, the SU₂ content of the cocoa butter of Ivory Coast was similar to that of Indonesia. This might rather be attributed to the measuring technique than to the sample. Indeed, the peak intensity in the chromatogram of the Ivory Coast sample was clearly lower than that of Indonesia. Thus, the small peaks might be more underestimated in the cocoa butter from Ivory Coast than that of Indonesia which explains a similar SU₂ content. Due to the limitations of HPLC-ELSD, it's also difficult to compare triacylglycerol compositions reported in literature. Shukla (1995), Chaiseri and Dimick, (1989) and Foubert *et al.* (2004) reported the following values for POP, POS and SOS, though the values for each country differ per study:

- Ghana: 15.3%-17.8% POP, 39.0%-40.1% POS, 27.5% SOS
- Ivory Coast: 15.2%-19.0% POP, 39.0%-41.9% POS, 25.1%-28.5% SOS
- Indonesia: 17.5%-19.9% POP, 40.6%-41.8% POS, 25.8%- 28.1% SOS

The ratio of S_2U/SU_2 (monosaturated-diunsaturated) in cocoa butter is associated with sensorial properties and technological attributes (Bessler and Orthoefer, 1983). Cocoa butters with a low S_2U/SU_2 ratio may display unsatisfactory crystallization (Lipp and Anklam, 1998). The S_2U group contains predominantly POP, POS and SOS, which have melting points ranging from 35.3°C to 41.2°C in their pure and stable crystal form. In contrast, the SU_2 group contains mostly SOO and POO, which melts at 23.7°C and 19.2°C resp., making them liquid around room temperature (Bockisch, 1998). Thereby, the higher the SU_2 fraction in the cocoa butter, the lower its melting point and hardness. The hardness of cocoa butter depends partly on its triacylglycerol composition, which is influenced by the growing region. Softer cocoa butters have higher levels of POO and SOO, and lower S_2U content (Chaiserer and Dimick, 1989; Lipp and Anklam, 1998).

When comparing the sampled cocoa butters, it is apparent those from Vietnam had the highest average S_2U/SU_2 ratio. However, it must be noted that the variability within the Vietnamese clones is very large which makes direct comparison with the other origins difficult. Within the Vietnamese samples TD2, TD3, TD5, TD7 and TD13 had a relatively low S_2U/SU_2 . These samples exhibited a somewhat lower SFA content. On the contrary, TD8 and TD9, which exhibited the lowest MUFA and highest SFA content, showed clearly the highest S_2U/SU_2 ratio, followed by TD10 and TD11.

3.2.1.3 FREE FATTY ACID CONTENT

Table 3.3 shows the free fatty acid (FFA) content (%) of the cocoa butters extracted from the Vietnamese clones, Ghana, Ivory Coast and Indonesia. Within the group of different clones from Vietnam, FFA content varied from 0.74% to 1.06%. The average FFA content of the butters from Vietnam was higher, slightly higher and lower than that of Ivory Coast, Indonesia and Ghana, respectively.

Table 3.3: FFA content (%) of the cocoa butter

Sample	FFA (%)	Sample	FFA (%)
Ghana	1.03	TD2	0.91
Ivory Coast	0.62	TD3	0.90
Indonesia	0.84	TD5	0.88
Vietnam	0.90	TD6	0.89
		TD7	1.06
		TD8	0.75
		TD9	0.74
		TD10	0.96
		TD11	0.87
		TD13	1.04

Values of Vietnam are the averages of the ten clones.

According to EU Directive 2000/36/EC the maximum FFA content of cocoa butter may not exceed 1.75%. It can be concluded that all sampled cocoa butters were compliant with this criterion. Elevated FFA levels can result from mould contamination where lipases cause hydrolysis, or from diseased pods. Mould growth can occur due to extended storage, prolonged fermentation, or inadequate drying of the beans (Chaiseri and Dimick, 1989). Apart from their pronounced influence on cocoa butter crystallization (Foubert *et al.*, 2004), elevated FFA levels can result in a rancid off-flavour.

3.2.2 PHYSICAL PROPERTIES

3.2.2.1 SOLID FAT PROFILE

The SFC-profile of cocoa butter affects the macroscopic attributes of chocolate, such as hardness, heat resistance and overall sensorial quality (Beckett, 2008). From such a profile, different textural characteristics can be deduced (Beckett, 2008; Luccas, 2001):

- SFC between 20°C and 25°C reflects hardness;
- SFC between 25°C and 35°C reflects heat resistance;
- SFC between 32°C and 35°C reflects cooling and creamy sensation;
- SFC at 35°C reflects waxy mouthfeel.

Figure 3.1 shows the SFC-profiles (%) of the tempered cocoa butters extracted from ten clones from Vietnam. A large variability in SFC-values is present in the range 25°C – 35°C between the different clones. In Figure 3.2, the average SFC-profile of the Vietnam and those of Ghana, Ivory Coast and

Indonesia are presented. In general, the Ghana sample showed lower SFC-values in the range 5°C – 30°C, due to its higher SU₂ content, and a higher SFC at 35°C, due to its higher SOS content, in comparison with the other origins.

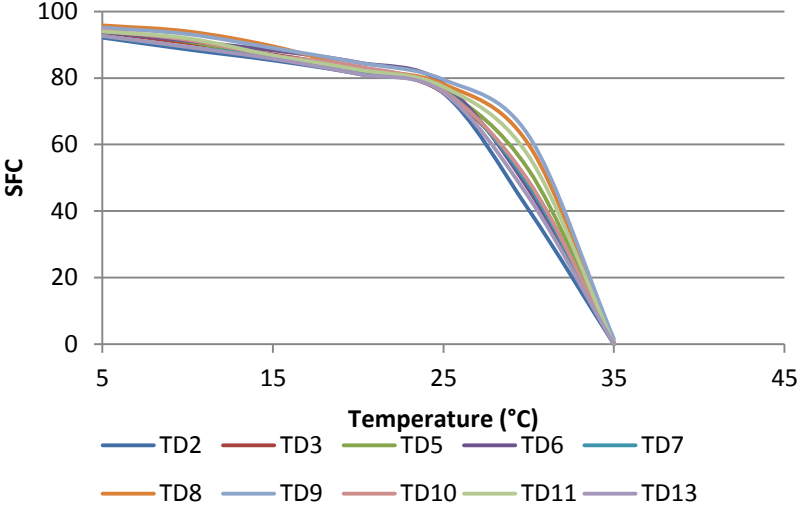


Figure 3.1: SFC-profile of the cocoa butters from the Vietnamese clones

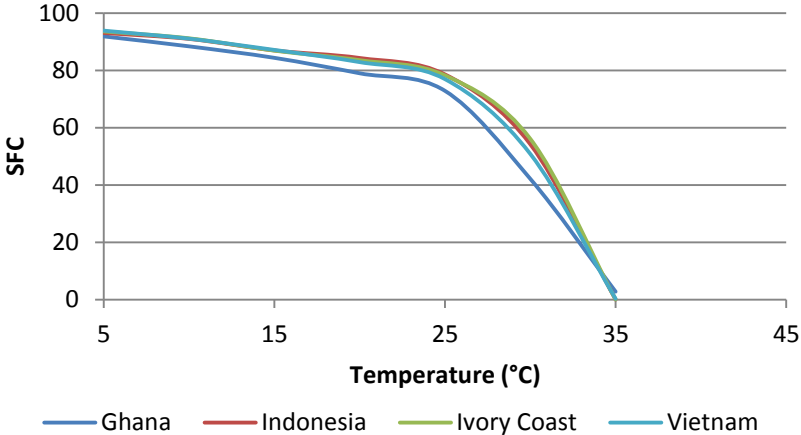


Figure 3.2: SFC-profile of the cocoa butters from Vietnam, Indonesia, Ghana and Ivory Coast

At 5°C, the SFC of the Vietnamese samples varied between 92.1% and 95.8%. The SFC of the butter from Indonesia and Ivory Coast fell within that range, while the Ghanaian sample started at a lower SFC. As the temperature increases, the SFC-value gradually decreased until 25°C. From then on, the tempered fat melted rapidly. Significant differences between the samples were present at all temperatures.

In terms of its functionality, the SFC-values in the range 20°C – 35°C of cocoa butter are important (Table 3.4). The full data table is in Appendix 1.

Table 3.4: SFC-profile of the cocoa butters

	20 C°	25 C°	30 C°	35 C°
Vietnam	82.9 ± 1.2	76.9 ± 1.4	50.8 ± 7.0	0.3 ± 0.5
TD2	81.2 ^b ± 0.1	75.4 ^b ± 0.6	40.4 ^a ± 0.5	0.0 ^a ± 0.0
TD3	82.7 ^{cd} ± 0.1	75.6 ^{bc} ± 1.4	48.8 ^e ± 1.4	0.0 ^a ± 0.0
TD5	83.0 ^{def} ± 0.1	76.4 ^{bcd} ± 0.7	52.4 ^f ± 0.7	0.0 ^a ± 0.0
TD6	84.7 ^g ± 0.3	78.3 ^{ef} ± 0.3	46.5 ^d ± 0.3	0.3 ^{ab} ± 0.3
TD7	82.3 ^c ± 0.1	76.6 ^{cd} ± 0.1	47.9 ^e ± 0.1	0.1 ^a ± 0.1
TD8	83.0 ^{de} ± 0.6	78.2 ^{ef} ± 0.98	60.0 ⁱ ± 0.9	0.8 ^b ± 0.5
TD9	84.6 ^g ± 0.4	79.5 ^g ± 0.4	62.7 ^j ± 0.4	1.6 ^c ± 0.7
TD10	83.5 ^{ef} ± 0.5	76.2 ^{bc} ± 0.2	49.0 ^e ± 0.2	0.0 ^a ± 0.0
TD11	82.5 ^{cd} ± 0.3	77.4 ^{de} ± 0.9	56.5 ^h ± 0.9	0.0 ^a ± 0.0
TD13	81.2 ^b ± 0.1	75.6 ^{bc} ± 0.7	44.2 ^c ± 0.7	0.0 ^a ± 0.0
Ghana	79.0 ^a ± 0.1	72.8 ^a ± 0.2	42.1 ^b ± 0.2	2.8 ^d ± 1.2
Ivory Coast	83.5 ^f ± 0.3	78.2 ^{ef} ± 0.8	56.1 ^h ± 1.4	0.0 ^a ± 0.0
Indonesia	84.3 ^g ± 0.4	78.6 ^{fg} ± 0.2	54.2 ^g ± 0.4	0.0 ^a ± 0.0

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Values of Vietnam are the averages of the ten clones.

The SFC between 20°C and 25°C relates to the **hardness** of the cocoa butter (Gunstone, 2009). Within the group of different clones from Vietnam, the SFC varied from 81.2% to 84.7% and from 75.4% to 79.5%, respectively. Moreover, in terms of the hardness of the different Vietnamese clones, TD9 and TD6 were the hardest. In contrast, TD2 and TD13 were the softest samples. When comparing the origins, the cocoa butter from Indonesia was the hardest, followed by that of Ivory Coast. The samples from Vietnam were somewhat softer, and the Ghanaian cocoa butter was clearly the softest. Shukla (2006) reported that cocoa butters from Ivory Coast, Ghana, Nigeria, Malaysia, India and Sri Lanka exhibited SFC-values ranging from 74.8% to 83.7% at 25°C. The data of the sampled cocoa butters fell within this range, except for the Ghanaian sample (72.8%).

A quality parameter wielded in the chocolate industry, is the SFC difference between 25°C and 35°C. Cocoa butter destined for chocolate production in tropical regions should be hard at room temperature, meaning an SFC higher than 50% at 25°C or higher. Meanwhile, it has to melt in the mouth with no waxy residuals left, therefore above 35°C no SFC should remain (Luccas, 2001). The temperature range (25°C – 35°C) in which a strong decline in SFC is observed, represents the **heating resistance** (Beckett, 2008). Within the butters from Vietnam, TD9 exhibited the highest heat resistance, followed by TD8. In contrast, TD2 and TD13 showed the lowest heat resistance. Regarding the different origins, the samples from Indonesia, Ivory Coast and Vietnam had higher SFC-values than the Ghanaian sample, while the latter exhibited the highest SFC at 35°C.

In tempered cocoa butter, the SFC between 30°C and 35°C commonly goes from about 45% to about 1% and this melting behaviour is responsible for the typical chocolate **mouthfeel** (Gunstone, 2009). For the sampled cocoa butter, the SFC at 30°C was generally higher than 45% and the SFC at 35°C was lower than 1%, except the Ghanaian sample. Within the group of different clones from Vietnam, the SFC at 30°C and 35°C varied from 40.4% to 62.7% and from 0.0% to 1.6%, respectively. The low SFC-value at 30°C of TD2 is likely to a combined effect from the relatively low S₂U content (96.4%), low SOS content (27.6%) and relatively high FFA content (0.91%). In contrast, the high SFC-value of TD9 can be attributed to a combined effect from the relatively high S₂U content (98.3%), high SOS content (34.5%) and relatively low FFA content (0.74%). Comparing the origins, Ivory Coast exhibited the highest SFC value at 30°C before the sample of Indonesia, those of Vietnam on average and that of Ghana. This trend confirms the importance of FFA on the heat resistance of chocolate, as a negative correlation (-0.77) between FFA content and SFC at 30°C. This observation may directly be valorized by chocolate producers which target export to tropical countries. Further, positive correlations between the SFC at 30°C and S₂U content (0.73) and S₂U/SU₂ ratio (0.68), and a negative correlation with the SU₂ (-0.69) were found. The latter was also found by Ribeiro *et al.* (2012), even at all temperatures of the SFC-profile. Indeed, diunsaturated TAGs have low melting points, namely 2.5°C for POO and 8.6°C for SOO (Hagemann, 1988), decreasing SFC. At 35°C, only the tempered cocoa butters from the Vietnamese clones TD6, TD7, TD8, TD9 and the one from Ghana were not fully melted. These samples were the richest in SOS (TD7, TD8, TD9 and Ghana) or trisaturated TAGs (TD6). According to Vereecken *et al.* (2008), this higher SFC at higher temperature is caused by SSS, which forms solid fat when the other TAGs are melted. A significant SFC at 35°C is responsible for a waxy mouthfeel but this will be very limited for the samples mentioned above.

3.2.2.2 NON-ISOTHERM CRYSTALLISATION BEHAVIOUR

This part focuses on the non-isothermal crystallisation behaviour and the subsequent melting of the sampled cocoa butters from Vietnam and that of Ghana, Ivory Coast and Indonesia. A typical crystallization curve recorded using DSC at a cooling rate of 5°C/min can be seen in Fig. 3.3 and is characterized by a large peak resulting from the crystallisation of mainly POP, POS and SOS, and surrounded by a shoulder at the high (mainly trisaturated TAGs) and low temperature side (monosaturated TAGs). Here, the crystallisation (or melting) curves were described using following parameters:

- **T_{onset} (°C):** temperature at which the crystallisation (or melting) starts;
- **T_{max} (°C):** temperature at which the crystallisation (or melting) occurs the fastest, observed as a maximum;
- **Enthalpy (J/g):** The total energy which is released (or absorbed) during crystallizing (or melting).

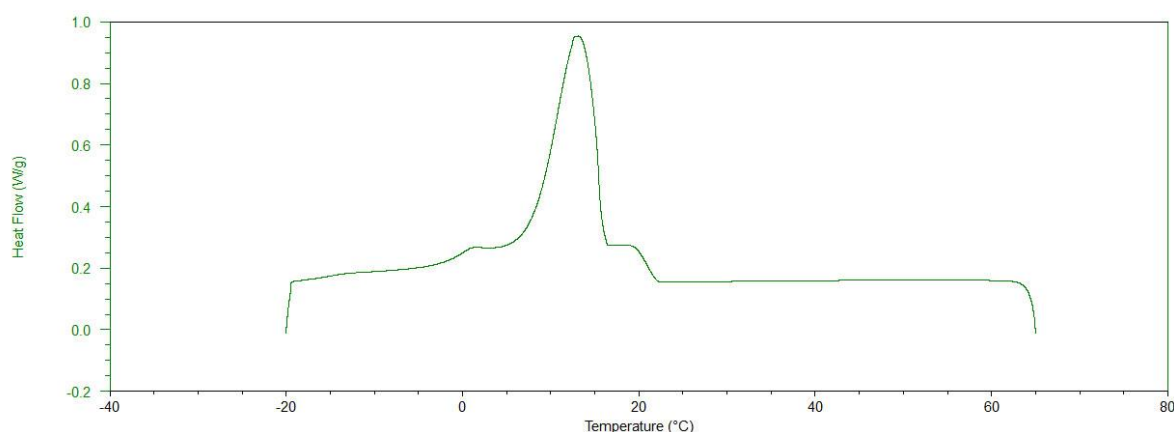


Figure 3.3: Non-isothermal crystallisation profile of cocoa butter (TD3)

Table 3.5 shows the characteristic parameters of the non-isothermal crystallisation of the cocoa butters extracted from ten clones from Thu Duc in Vietnam, Ghana, Ivory Coast and Indonesia.

Table 3.5: Non-isothermal crystallization parameters of the cocoa butters following cooling at a rate of 5°C/min

	$T_{\text{onset}} (^{\circ}\text{C})$	$T_{\text{max}} (^{\circ}\text{C})$	Enthalpy (J/g)
Vietnam	21.8 ± 0.6	13.3 ± 0.6	68.2 ± 4.4
TD2	$21.4^{\text{abc}} \pm 0.3$	$12.7^{\text{ab}} \pm 0.4$	$72.1^{\text{e}} \pm 3.6$
TD3	$22.0^{\text{bcd}} \pm 0.2$	$12.8^{\text{ab}} \pm 0.3$	$67.6^{\text{cd}} \pm 1.0$
TD5	$21.9^{\text{bcd}} \pm 0.6$	$13.1^{\text{bc}} \pm 0.2$	$65.8^{\text{abc}} \pm 1.9$
TD6	$21.4^{\text{abc}} \pm 0.2$	$12.9^{\text{ab}} \pm 0.0$	$77.7^{\text{f}} \pm 1.3$
TD7	$21.4^{\text{abc}} \pm 0.3$	$13.6^{\text{cd}} \pm 0.3$	$69.4^{\text{cde}} \pm 1.6$
TD8	$20.7^{\text{a}} \pm 0.2$	$14.3^{\text{e}} \pm 0.2$	$62.4^{\text{a}} \pm 3.4$
TD9	$22.2^{\text{cd}} \pm 0.3$	$13.9^{\text{de}} \pm 0.5$	$67.0^{\text{bc}} \pm 2.8$
TD10	$21.8^{\text{abcd}} \pm 0.1$	$12.5^{\text{a}} \pm 0.1$	$68.4^{\text{cde}} \pm 1.0$
TD11	$22.6^{\text{d}} \pm 0.2$	$13.1^{\text{bc}} \pm 0.7$	$63.8^{\text{ab}} \pm 1.4$
TD13	$22.6^{\text{d}} \pm 1.5$	$13.1^{\text{bc}} \pm 0.3$	$66.1^{\text{abc}} \pm 3.1$
Ghana	$22.6^{\text{ef}} \pm 0.6$	$13.7^{\text{cd}} \pm 0.0$	$69.5^{\text{cde}} \pm 1.7$
Ivory Coast	$21.1^{\text{ab}} \pm 0.2$	$12.9^{\text{ab}} \pm 0.2$	$70.8^{\text{de}} \pm 1.3$
Indonesia	$22.7^{\text{d}} \pm 0.8$	$13.6^{\text{cd}} \pm 0.4$	$69.4^{\text{cde}} \pm 2.9$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Values of Vietnam are the averages of the ten clones.

Within the cocoa butter group extracted from Vietnamese clones, T_{onset} varied in the range 20.7°C – 22.6°C, being lowest for TD8 and highest for TD11 and TD13. The butter from Ghana and Indonesia exhibited a similar high T_{onset} , while that of Ivory Coast was significantly lower. No correlations were found between T_{onset} and the chemical parameters, suggesting that not one specific parameter has a dominating impact on the starting temperature of the non-isothermal crystallisation but rather a combination of parameters. In addition, other chemical properties than those determined might have a significant impact.

Regarding T_{max} , TD10 exhibited the lowest value, while TD8 and TD9 showed the highest. There was a strong negative correlation between T_{max} and POP content ($R = -0.84$) and a positive correlations with SOS ($R = 0.87$). This is due to the lower melting point of POP *versus* SOS.

A large variability of crystallisation enthalpy was observed within the Vietnamese cocoa butters, ranging from 62.4 J/g to 77.7 J/g. The average enthalpy of the Vietnamese cocoa butters was somewhat lower than those from the three other origins. No correlations were found between this melting parameters and the chemical properties described in the above section.

Fig. 3.4 shows a typical melting profile recorded using DSC at a heating rate of 5°C/min following non-isothermal crystallisation. The curve is characterized by a large peak with a shoulder at the high temperature side.

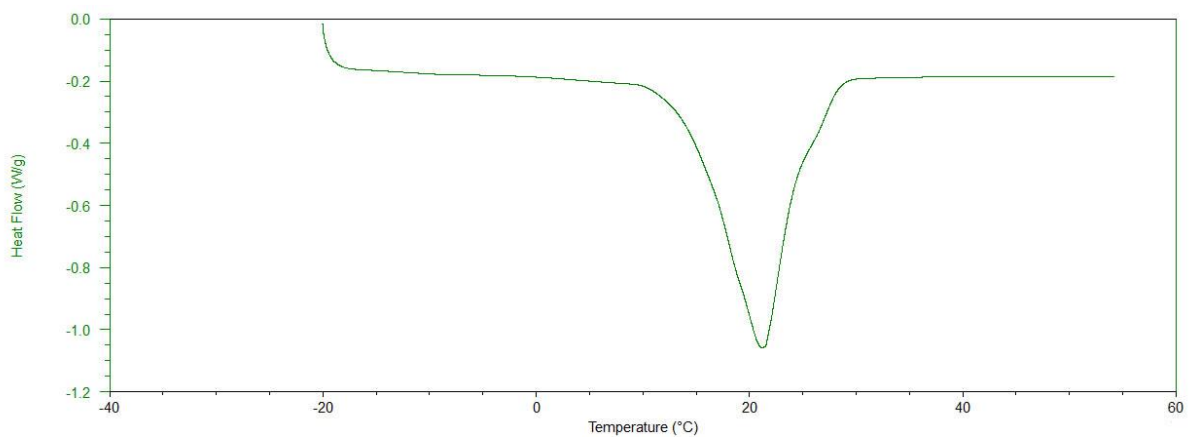


Figure 3.4: Melting profile of cocoa butter (TD3) following non-isothermal crystallisation

Table 3.6: Melting parameters of the cocoa butters following non-isothermal crystallization, both at a rate of 5°C/min

	$T_{\text{onset}}(^{\circ}\text{C})$	$T_{\text{max}}(^{\circ}\text{C})$	Enthalpy (J/g)
Vietnam	12.9 ± 0.5	21.6 ± 0.4	85.5 ± 2.1
TD2	12.6 ^{ab} ± 0.3	21.0 ^{ab} ± 0.3	85.9 ^{bcd} ± 0.8
TD3	12.9 ^{bcd} ± 0.1	21.5 ^{bcd} ± 0.2	83.5 ^{ab} ± 0.9
TD5	13.1 ^{cd} ± 0.3	21.5 ^{bcde} ± 0.3	85.5 ^{bcd} ± 1.4
TD6	13.4 ^{de} ± 0.2	20.9 ^a ± 0.1	89.9 ^e ± 0.9
TD7	13.0 ^{bcd} ± 0.3	21.7 ^{cdef} ± 0.2	87.2 ^d ± 1.5
TD8	14.1 ^f ± 0.2	21.9 ^{ef} ± 0.3	85.0 ^{abcd} ± 1.2
TD9	13.7 ^{ef} ± 0.2	22.0 ^f ± 0.3	86.6 ^{cd} ± 2.6
TD10	12.7 ^{bc} ± 0.2	21.2 ^{abc} ± 0.1	84.1 ^{abc} ± 0.7
TD11	12.9 ^{bcd} ± 0.6	22.1 ^f ± 0.5	82.7 ^a ± 3.2
TD13	12.9 ^{bcd} ± 0.2	21.8 ^{def} ± 0.3	84.7 ^{abcd} ± 1.0
Ghana	12.2 ^a ± 0.3	21.3 ^{abcd} ± 0.1	86.9 ^d ± 0.5
Ivory Coast	12.6 ^{ab} ± 0.2	21.2 ^{abc} ± 0.1	84.7 ^{abcd} ± 0.5
Indonesia	12.9 ^{abcd} ± 0.2	21.3 ^{bcde} ± 0.4	86.3 ^{cd} ± 2.5

Values of Vietnam are the averages of the ten clones.

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

T_{onset} of the Vietnamese cocoa butters varied from 12.7°C to 14.1°C, being lowest for TD2 and TD10, and highest for TD8 and TD9. The melting started at relatively lower temperature in the case of the cocoa butter from Ghana, Ivory Coast and Indonesia. A negative correlation was found between T_{onset} and SU_2 content (-0.72). Thus, the higher the content of TAGs like SOO and POO, the lower the starting temperature of melting. These monosaturated TAGs interrupt the molecular packing of the disaturated TAGs (Foubert *et al.*, 2004)

The lowest T_{max} was observed for TD6 followed by TD2, while TD8 and TD9 exhibited the highest peak maximum temperature. Similar as for the non-isothermal crystallization, T_{max} correlated negatively with the POP content ($R = -0.60$) and positively with the SOS content ($R = 0.68$), although the correlation coefficients are lower.

In a lower extent than for the non-isothermal crystallization data, the melting enthalpy of the Vietnamese cocoa butters showed variability in the range 82.7% - 89.9%. The average value was somewhat higher than that of the Ivory Coast sample but somewhat lower than those of Ghana and Indonesia. No correlations between enthalpy and the determined chemical data were found.

3.2.2.3 ISOTHERMAL CRYSTALLISATION BEHAVIOUR

The isothermal crystallisation behaviour at 20°C of the extracted cocoa butters from beans of Vietnam, Ghana, Ivory Coast and Indonesia was studied. A typical crystallization curve recorded using DSC is shown in Fig. 3.5. The first part of the graph (0 to 10 min) represents the melting at 65°C to erase the thermal history, the second part (10 to 14.5 min) represents the cooling step to 20°C at a rate of 10°C/min and the third part (14.5 min to 245.5 min) represents the isothermal period. The crystallisation behaviour at 20°C of cocoa butter is characterised by a two-step process. In the graph below, crystallisation already starts at the end of the cooling step. The second and main step occurs within the experimental timeframe of 35 min – 80 min. The crystallisation mechanism was elucidated by Foubert (2003). During the first crystallisation peak, α -crystals are formed from the melt. During the second peak, a polymorphic transition from α - into β' -crystals occurs. In addition, extra α -crystals are formed, which are immediately transformed into the more stable β' -crystals.

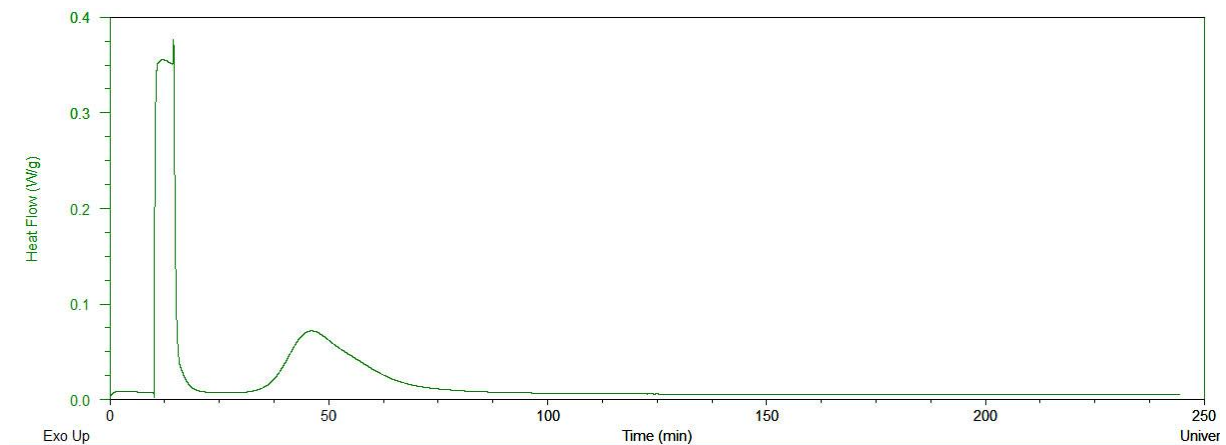


Figure 3.5: Isothermal crystallization profile of cocoa butter (TD3) at 20°C

Here, the focus was on the second step of the crystallisation profile. The integrated data of the second crystallisation peak were fitted to the Foubert model (Foubert *et al.*, 2002). The derived parameters a_F , t_{ind} , and K for the sampled cocoa butters are presented in Table 3.8.

Table 3.8: Isothermal crystallization parameters of the cocoa butters during the second step applying the Foubert model

	Enthalpy ₁ (J/g)	a _F (J/g)	t _{ind} (h)	K (h ⁻¹)
Vietnam	12.2 ± 1.7	83.1 ± 2.9	0.35 ± 0.02	5.7 ± 0.8
TD2	12.5 ^{cd} ± 0.9	82.3 ^{ab} ± 3.4	0.36 ^{cd} ± 0.03	6.3 ^d ± 0.7
TD3	13.5 ^d ± 0.7	79.7 ^a ± 2.5	0.34 ^{bc} ± 0.02	5.3 ^{ab} ± 0.5
TD5	10.5 ^{abc} ± 0.1	81.7 ^{ab} ± 2.9	0.36 ^{cd} ± 0.01	6.0 ^{cd} ± 0.4
TD6	13.6 ^d ± 3.5	84.5 ^{bc} ± 0.4	0.33 ^b ± 0.02	5.9 ^{bcd} ± 0.4
TD7	13.7 ^d ± 0.3	83.6 ^{abc} ± 1.0	0.36 ^{cd} ± 0.01	5.3 ^{abc} ± 0.4
TD8	13.3 ^d ± 1.5	85.0 ^{bc} ± 2.6	0.35 ^{bcd} ± 0.02	6.2 ^d ± 0.2
TD9	12.4 ^{bcd} ± 1.3	84.9 ^{bc} ± 2.8	0.30 ^a ± 0.00	6.1 ^d ± 0.2
TD10	13.1 ^d ± 0.9	79.1 ^a ± 2.3	0.39 ^{ef} ± 0.01	4.7 ^a ± 0.1
TD11	9.9 ^a ± 0.7	82.8 ^{ab} ± 2.7	0.33 ^{bc} ± 0.02	5.6 ^{bcd} ± 0.3
TD13	10.3 ^{ab} ± 0.6	87.8 ^c ± 5.2	0.36 ^{de} ± 0.02	5.3 ^{ab} ± 0.8
Ghana	10.9 ^{abc} ± 1.0	79.5 ^a ± 2.1	0.41 ^f ± 0.01	5.4 ^{abc} ± 0.1
Ivory Coast	12.2 ± 1.4	83.4 ^{abc} ± 0.9	0.30 ^a ± 0.01	7.3 ^e ± 0.5
Indonesia	10.1 ^a ± 1.3	85.4 ^{bc} ± 3.5	0.30 ^a ± 0.01	6.2 ^d ± 0.5

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Values of Vietnam are the averages of the ten clones.

The parameter a_F (J/g) represents the total amount of heat released during the 2nd crystallization step. A significant variability was observed between the Vietnamese cocoa butters, ranging from 79.1 J/g to 87.8 J/g. When comparing different origins, it was apparent that the Ghanaian cocoa butter had the lowest a_F , followed by the average of Vietnam and Ivory Coast. Indonesia exhibited the highest value. The obtained a_F values were higher than those found by Foubert *et al.* (2004). The latter research sampled in total 20 cocoa butters from Africa, South-America and Asia and observed a_F values ranging from 49.1 J/g to 78.9 J/g, with a mean of 70.3 J/g. This difference is likely due to the higher levels of oleic acid and SU₂, and lower levels of S₂U and FFA. Based on multiple linear regression, Foubert *et al.* (2004) concluded that a_F is inversely correlated to the amount of unsaturated fatty acids, monosaturated TAGs, free fatty acids and diacylglycerol content.

The parameter t_{ind} represents the induction time of the conversion of α -crystals into β' -crystals. This parameter varied for the Vietnamese cocoa butters from 0.30 h to 0.39 h, those from Ivory Coast and Indonesia were similar to the minimum of this range, while that of Ghana exceeded the maximum. The observed induction times were shorter than those found by Foubert *et al.*, (2004). This is likely due to lower levels of FFA (between 0.62% and 1.04% for this research and between 1.18% and 2.77% for Foubert *et al.*, 20014). Here, a positive correlation (R = 0.74) was found between t_{ind} and FFA of the sampled cocoa butters from Vietnam, Ghana, Ivory Coast and Indonesia. Following PCA, Foubert *et al.* (2004) stated that the induction time decreases with higher amounts of SFA disaturated and trisaturated TAGs, and lower amounts of diacylglycerols, phosphorus and free fatty acids. The parameter t_{ind} was also inversely correlated (R = -0.70) to the parameter K, since it depends on the crystal growth rate of the cocoa butters (Foubert *et al.*, 2004). The cocoa

butter must release sufficient heat in order to be detected (Toro-Vazquez *et al.*, 2002). Thus, the higher the growth rate, the faster crystal formation is detected and the shorter t_{ind} .

The parameter K (1/h) is the rate constant of the crystallization process and varies significantly between the Vietnamese cocoa butters, ranging from 4.7 h^{-1} to 6.3 h^{-1} . Regarding the origins, the Ghanaian sample exhibited the lowest K -value. Ivory Coast and Indonesia showed a higher K value than the average of the Vietnamese butters. The rate constant is lowered by presence of unsaturated fatty acids, monosaturated TAGs (Foubert *et al.*, 2004), DAGs and free fatty acids (Pontillon, 1998; Shukla, 1995). Here, a negative correlation ($R = -0.82$) between K and FFA was found.

3.3 FUNCTIONALITY OF COCOA LIQUOR

In this part, one reference Ghanaian and three Vietnamese cocoa liquors (GrandPlace Puratos, TD3/5, Trong Duc) in which two of the latter were conched, were used to produce five different cocoa liquors with variation in acidity intensity. They were then processed to produce five different chocolates with the same formulation. The physicochemical characteristics, acidity and sensory profile were examined.

3.3.1 PROCESSING OF COCOA LIQUOR

3.3.1.1 BALL MILL PROCESSING

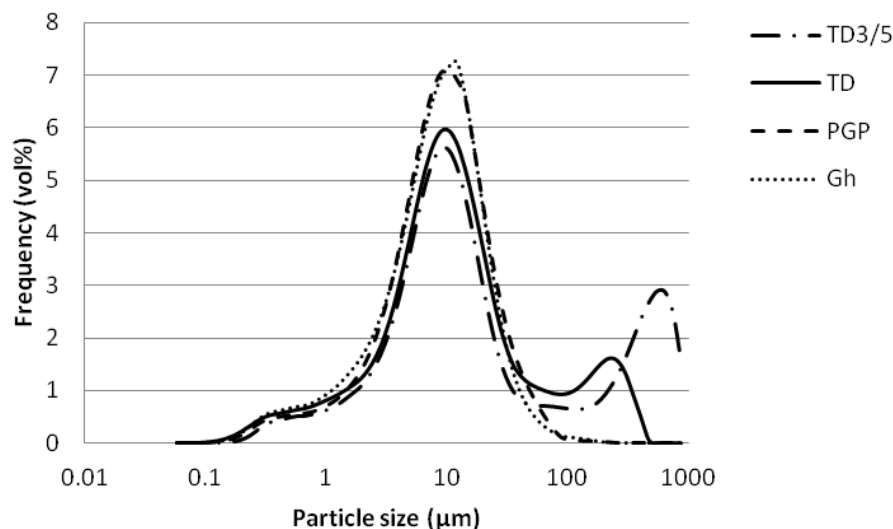


Figure 3.6: Particle size of cocoa liquor before ball milling.

Cocoa liquors supplied by Grand Place Puratos and Ghana possessed proper fineness with particle diameters $D[v,0.9]$ of 23.9 and 21.5 respectively and were suitable for further processing. However, the TD3/5 and Trong Duc liquors initially contained large particles with a $D[v,0.9]$ larger than $100\mu\text{m}$ (Figure 3.6), thus being further milled by ball mill to enable the next process using the 3 roll-refiner. When the particle diameter of the liquor is larger than $100\mu\text{m}$, the rolls on the refiner could be damaged.

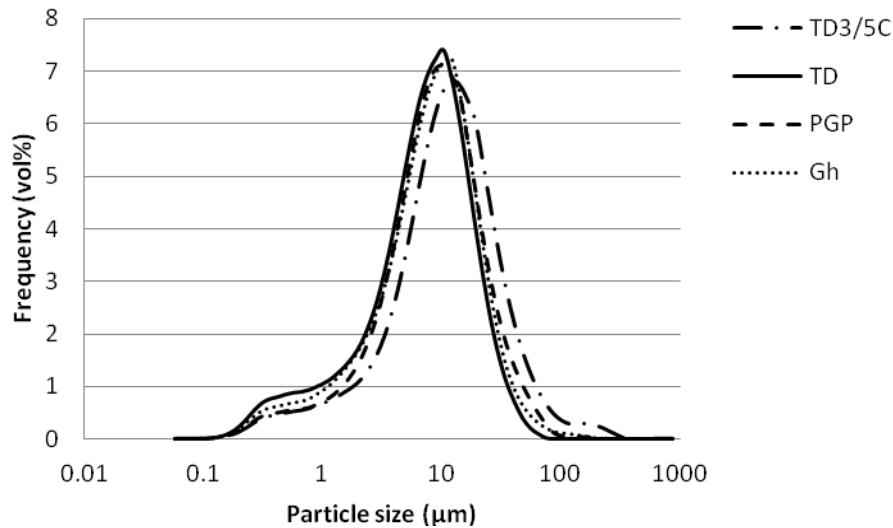


Figure 3.7: Particle size of cocoa liquor after ball milling.

Figure 3.7 shows the PSD of four liquors in which those of Trong Duc and TD3/5 were sufficiently reduced after ball milling to a $D[v,0.9]$ range of 18.8 to $33.4\mu\text{m}$.

Table 3.9: Particle size of the cocoa liquors

	$D[v,0.9](\mu\text{m})$	$D[v,0.5](\mu\text{m})$	$D[v,0.1](\mu\text{m})$	$D[43](\mu\text{m})$	$D[32](\mu\text{m})$	$D[21](\mu\text{m})$
TD3/5	$476.6^c \pm 146.7$	$12.5^d \pm 3.9$	$2.4^e \pm 0.4$	$112.8^c \pm 60.80$	$4.6^e \pm 0.9$	$0.7^d \pm 0.1$
TD3/5U	$33.4^a \pm 0.9$	$10.7^c \pm 0.2$	$2.2^e \pm 0.2$	$16.9^{ab} \pm 0.2$	$3.9^d \pm 0.1$	$0.6^c \pm 0.0$
TD	$119.7^b \pm 27.3$	$9.6^{bc} \pm 0.4$	$1.7^c \pm 0.1$	$35.4^b \pm 4.4$	$3.2^{bc} \pm 0.1$	$0.5^a \pm 0.0$
TDU	$18.8^a \pm 0.4$	$7.1^a \pm 0.1$	$1.2^a \pm 0.1$	$9.0^a \pm 0.2$	$2.6^a \pm 0.1$	$0.5^b \pm 0.0$
PGP	$23.9^a \pm 0.3$	$8.3^{ab} \pm 0.1$	$1.9^d \pm 0.1$	$11.5^a \pm 0.2$	$3.4^c \pm 0.1$	$0.6^c \pm 0.0$
Gh	$21.5^a \pm 0.6$	$7.9^a \pm 0.1$	$1.5^b \pm 0.0$	$10.7^a \pm 0.4$	$3.0^b \pm 0.0$	$0.5^b \pm 0.0$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

3.3.1.2 CONCHING

Asian cocoa liquors are highly acidic leading to acidic taste of the resulting chocolate (Baigrie and Rumbelow, 1987) and other taste defects (Lopez and Quesnel, 1973). During the conching process, moisture and acids having boiling temperature higher than conching temperature are removed with the evaporating steam (Beckett, 2008). In order to reduce the acidity in the Vietnamese samples, the cocoa liquors of which had low pH namely TD3/5 and TD were treated with a conching step prior to chocolate production. The pH of all samples were recorded before and after conching as seen in Table 3.10.

3.3.1.2.1 ACIDITY

Table 3.10: Acidity of the liquor samples.

	pH
TD3/5U*	4.67
TD3/5C*	4.78
TDU*	4.85
TDC*: Conched 60°C	4.86
Conched 60° - 70°C	4.98
Conched 60° - 80°C	4.97
Conched 60° - 85°C	5.00
PGP	5.22
Gh	5.55

*: U stands for unconched, C stands for conched

Cocoa beans with a low pH (4.75-5.19) after fermentation are considered well fermented, while cocoa beans with a higher pH (5.5-5.8) are considered poorly fermented (Afoakwa *et al.*, 2008)¹. Hence, all Vietnamese cocoa liquors appeared to have experienced a good fermentation practice. However, Jinap *et al.* (1995) have compared chocolates produced from cocoa beans with high (5.5-5.8), low (4.75-5.19) and intermediate (5.20-5.49) pH. They have found that cocoa beans with intermediate pH resulted in chocolate with higher notes of chocolate flavour, and lower pH resulted in lower notes of chocolate. Portillo *et al.* (2007) have also reported that cocoa beans with pH lower than 4.5 have decreased aromatic potential. Therefore, PGP liquor had an intermediate pH (5.22) while the Ghanaian sample had a high pH (5.55). Since TD3/5 and TDU samples both had low initial pH values at 4.67 and 4.87 respectively, these two liquors were treated by conching up to the minimum moisture content and reached the pH values of 4.85 and 5 accordingly. Among different conching temperatures, the only remarkable increase in pH was observed when conching at 70°C.

3.3.1.2.2 MOISTURE CONTENT

Table 3.11a: Moisture and dry matter content of cocoa liquor samples.

	Moisture (%)	Dry matter (%)
TD3/5U	3.7 ± 0.0	95.4 ± 0.1
TD3/5C	0.4 ± 0.0	99.6 ± 0.1
TDU	2.0 ± 0.0	97.4 ± 0.1
TDC	0.5 ± 0.0	99.7 ± 0.1
PGP	1.0 ± 0.0	98.5 ± 0.1
Gh	0.9 ± 0.0	98.8 ± 0.1

Moisture content of cocoa liquors was given in Table 3.11a. Among the four samples, TD3/TD5 initially had the high moisture content of 3.7% which exceeds the usual values of 1.5-3% for roasted cocoa beans (Dand,1999). The initial higher moisture and lower fat content of TD3/5U compared with TDU explained the encountered difficulty during earlier processing. TD3/5U displayed poor flow behavior, and proved more difficult to grind. Fat content of the different samples were within usual range of 48%-57% (Pearson, 1981). After liquor conching, moisture content of both the TD3/TD5 and

the Trong Duc sample has decreased due to evaporation during the process especially during the early phases which was also mentioned by Beckett (2008). The moisture content of the conched samples decreased even more than that of the 2 commercial samples namely PGP and Gh. The liquors that initially contained the higher moisture levels also had the lowest pH ($R = -0.88$).

3.3.1.2.3 ORGANIC ACIDS

From the organic acids detected in the cocoa liquors (Table 3.12), the non-volatile acids were oxalic, tartaric, malic, citric, succinic, and lactic acid in which the three latter acids occupied the major proportion. There was no particular order of importance, although the Ghanaian sample contained a lower amount of lactic acid. Citric acid originates from the pulp present in cocoa pods (Ardhana and Fleet, 2003). During the fermentation, glucose and fructose are converted to lactic acid, acetic acid and ethanol while and citric acid is converted to lactic acid acetic acid as well (Lefeber *et al.*, 2011). Afterwards, the earlier produced ethanol is converted to acetic acid (Wood, 1997; Schwan and Wheals, 2004) and remains after drying. Thompson *et al.* (2001) have found that acetic acid bacteria may be present even during the drying process, which even increases acetic acid in cocoa beans. Citric acid that is not converted remains after drying as well (Rodriguez-Campos *et al.*, 2011). Oxalic and malic acid are initially present, and remains throughout fermentation and drying. Succinic acid is formed during fermentation, and remains after drying. Propanoic acid is formed during the later days of the fermentation as well (Rodriguez-Campos *et al.*, 2011).

For most of the non-volatile organic acids, it has been found that not only are there significant differences between the Ghanaian and Vietnamese samples, but differences between Vietnamese liquors themselves are present as well, which could be explained by different fermentation conditions (Jinap and Dimick, 1990).

Table 3.12: Levels of organic acids measured by HPLC.

Organic acids ($\mu\text{g/g}$)	Oxalic	Citric	Tartaric	Malic	Succinic	Lactic	Acetic	Propionic	Total organic acids
TD3/5U	1146 ^a ± 51	3328 ^{bc} ± 154	87 ^{ab} ± 8	1266 ^e ± 149	2339 ^{ab} ± 175	3920 ^e ± 200	2687 ^b ± 162	5355 ^d ± 248	20129
TD3/5C	1003 ^a ± 41	2949 ^{ab} ± 113	37 ^a ± 13	674 ^{abcd} ± 88	2162 ^a ± 129	3466 ^d ± 99	1130 ^a ± 95	4880 ^c ± 157	16301
TDU	1824 ^{bc} ± 63	3114 ^{bc} ± 90	113 ^{abc} ± 18	804 ^{bcd} ± 37	2414 ^{ab} ± 163	1646 ^b ± 24	3839 ^d ± 219	3498 ^a ± 80	17250
TDC: 60°C	1964 ^c ± 39	3334 ^{bc} ± 83	125 ^{bc} ± 15	920 ^d ± 55	2615 ^{bc} ± 85	1738 ^b ± 67	3600 ^d ± 150	3734 ^{ab} ± 99	18030
60°-	2031 ^c	3458 ^c	190 ^{cd}	565 ^{abc}	2872 ^c	1518 ^b \pm	3019 ^c	4752 ^c	18405
70°C	± 229	± 412	± 33	± 105	± 277	128	± 143	± 57	
60°-	2090 ^c	3500 ^c	219 ^d	545 ^{ab}	2861 ^c	1526 ^b	2885 ^{bc}	4792 ^c	18417
80°C	± 64	± 100	± 10	± 37	± 84	± 79	± 5	± 101	
60°-	2077 ^c	3522 ^c	195 ^{cd}	740 ^{bcd}	2908 ^c	1462 ^b	2704 ^{bc}	4820 ^c	18429
85°C	± 23	± 41	± 86	± 220	± 41	± 42	± 33	± 148	
PGP	1558 ^b ± 84	2943 ^{ab} ± 60	65 ^{ab} ± 19	831 ^{cd} ± 154	2916 ^c ± 125	2889 ^c ± 157	1481 ^a ± 55	5368 ^d ± 49	18051
Gh	2040 ^c ± 243	2672 ^a ± 170	121 ^{abc} ± 28	446 ^a ± 62	2838 ^c ± 229	651 ^a ± 66	1179 ^a ± 83	3938 ^b ± 200	13886

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Volatile organic acids identified by HPLC-ELSD were propionic and acetic acid. Propionic acid was recorded as the main volatile organic acid. Higher amounts of acetic acid was initially found in Trong Duc sample, however this trend was reversed after the conching treatment. These findings are contrary to Hoskin and Dimick (1979) and Jinap and Dimick (1990), who stated that acetic acid represents 95-99% of the volatile fatty acids in cocoa liquor. Luna *et al.* (2002) stated that acetic acid has an essential role in cocoa liquor acidity and causes vinegar-like notes in chocolate (Afaokwa *et al.*, 2009²). However, in this study, there was only a weak correlation between acetic acid content and liquor acidity ($R = -0.39$). Instead, a stronger correlations was found for lactic acid ($R = -0.63$) as lactic acid formed during the fermentation remains during further processing steps (Thompson *et al.*, 2001). Correlations were found between pH and malic ($R = -0.58$) and citric acid ($R = -0.44$) as well. pH of the liquors decreased when total organic acid content decreased ($R = -0.49$).

Higher levels of organic acids were found in the Vietnamese samples in comparison with the Ghanaian samples. With exception of oxalic, succinic, and tartaric acid, all other major acids in Vietnamese liquors dominated over those in Ghana samples. The finding concurs with Holm *et al.* (1993) who stated that South-East Asian cocoa liquors contain higher amounts of organic acids than West African liquors. According to Baigrie and Rumbelow (1987), highly acidic samples contained smaller amounts of citric and oxalic acid. The results obtained was partly in accordance with the measurements in this thesis. Higher citric acid levels in Vietnamese samples might be due to the

higher thickness of the pulp containing high level of citric acid or the difference in fermentation length where conversion of citric acid to lactic and acetic acid took place.

The changes in organic acid content while conching the Trong Duc and TD3/5 liquors can be seen in Figure 3.8 and 3.9 respectively.

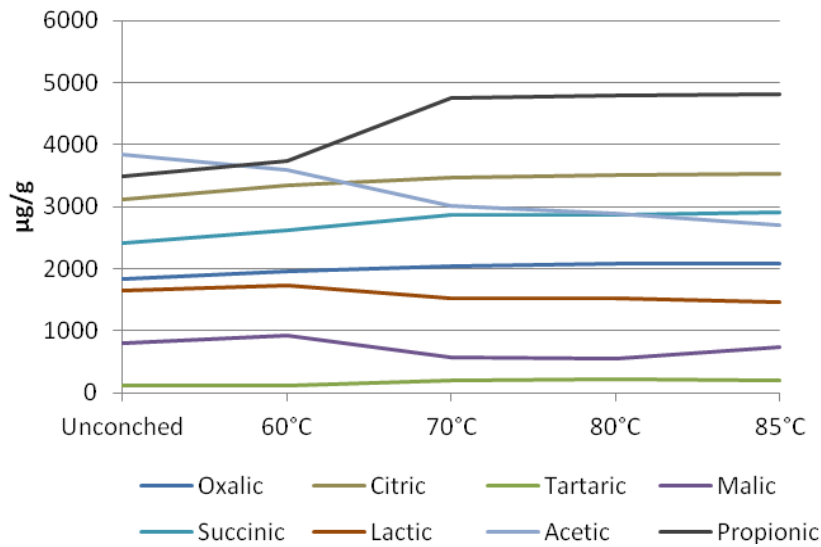


Figure 3.8: Organic acid content of the Trong Duc liquor while conching

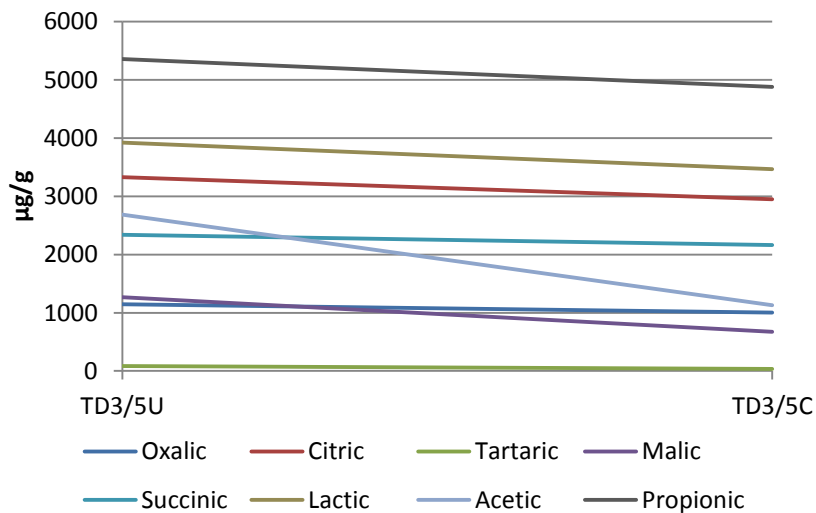


Figure 3.9: Organic acid content of the TD3/5 liquor while conching

Acetic acid decreased significantly during conching in both samples (Table 3.12). Lactic acid and malic acid did not decrease significantly after conching for Trong Duc, but there was a significant decrease after conching for TD3/5. Propionic acid decreased significantly in TD3/5 after conching, but did not decrease in Trong Duc. This result is in agreement with Kleinert-Zollinger (1988), who proved that the conching pre-treatment of cocoa liquor mainly removes acetic acid. The other acids do not evaporate as much, and while moisture does evaporate, the some acid's concentration increases.

3.3.2 COMPOSITION OF COCOA LIQUOR

3.3.2.1 MACRO-COMPOSITION

Table 3.11b: Macrocomposition of the cocoa liquors.

	Dry matter (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)
TD3/5U	95.4 ± 0.1	48.3±0.4	12.8± 0.1	16.7 ± 0.8	3.1 ± 0.0
TD3/5C	99.6 ± 0.1	51.3 ± 0.1	13.2 ± 0.3	15.3 ± 0.8	3.1 ± 0.0
TDU	97.4 ± 0.1	53.6 ± 0.2	13.6 ± 0.2	16.4 ± 0.9	3.2 ± 0.0
TDC	99.7 ± 0.1	55.1 ± 0.1	13.7 ± 0.1	16.9 ± 0.1	3.3 ± 0.0
PGP	98.5 ± 0.1	52.6 ± 0.5	13.5 ± 0.0	17.3 ± 0.6	3.7 ± 0.0
Gh	98.8 ± 0.1	54.8 ± 0.2	14.1 ± 0.0	16.4 ± 0.4	3.3 ± 0.0

The macro-composition of the cocoa liquors is listed in Table 3.11. Of the unconched samples, the Ghanaian commercial sample had the highest fat content. Conching improved the total fat content, especially with the TD3/TD5 sample. This is explained by the moisture and organic acid removal, increasing the fraction of remaining fat.

3.3.2.2 FAT COMPOSITION AND FUNCTIONALITY

3.3.2.2.1 QUALITY PARAMETERS

Due to oxidation, lipids can turn rancid, which deteriorates the food product. Unsaturated fatty acids are especially sensitive. Oxidation of fats results in primary oxidation products that, in turn, react to form secondary oxidation products.

Table 3.13: Quality parameters of the extracted cocoa butter

	PV (meq O ₂ /kg)	pAV (adsorbance/g)	Totox	FFA (g/100g)
TD3/5U	1.15 ± 0.14	1.48 ± 0.02	3.78	1.60 ± 0.01
TD3/5C	1.01 ± 0.04	1.56 ± 0.02	3.58	1.03 ± 0.01
TD	1.69 ± 0.02	1.46 ± 0.02	4.84	0.75 ± 0.00
TDU	1.41 ± 0.1	1.65 ± 0.09	4.47	0.78 ± 0.00
TDC	1.60 ± 0.10	1.00 ± 0.00	4.20	0.60 ± 0.00
PGP	0.63 ± 0.00	1.51 ± 0.03	2.77	1.69 ± 0.01
Gh	1.52 ± 0.13	1.15 ± 0.04	4.19	1.90 ± 0.01

The peroxide value (PV) is a measure for primary oxidation products, which consists of peroxides and hydroperoxides. The p-anisidine (pAV) value is used to describe the amount of secondary oxidation products. Depending on the fat, off-flavours develop around a PV higher than 10. Fats of acceptable quality should have a p-AV lower than 10 as well (Rossell, 1994). Results (Table 3.13) show that all the tested samples had PV's lower than 10.

The totox value is an indication of total oxidation of a food product, incorporating both PV and p-AV. It is defined as:

$$totox = pAV + (2 * PV) \quad (\text{Eq.3.1})$$

Calculated tottox values show that all samples have tottox values lower than 10, thereby complying with the standards (Nielsen, 2009).

According to EU Directive 2000/36/EC, FFA of cocoa butter must not exceed 1.75%. Results in the table show that all samples comply with this value except the Ghanaian commercial sample.

3.3.2.2.2 FATTY ACID PROFILE

Table 3.14 shows the results for fatty acid analysis of extracted cocoa butters from four liquors. Fatty acids which had an area $\geq 0,05\%$ were included in the results for the calculation. The three main fatty acids in cocoa butter were palmitic acid (C16.0), stearic acid (C18.0) and oleic acid (C18.1). Linoleic acid (C18.2) and arachidic acid (C20.0) were present in smaller amounts.

Table 3.14: Fatty acid profile of the extracted cocoa butters measured by GC

	C 16.0	C 18.0	C18.1	C 18.2	C20.0	Others	SFA	MUFA	PUFA
TD3/5C	27.1 ± 0.0	33.3 ± 0.0	32.8 ± 0.0	3.5 ± 0.0	1.4 ± 0.0	1.9	62.5	33.3	4.2
TDU	26.5 ± 0.1	35.3 ± 0.1	32.9 ± 0.0	2.6 ± 0.0	1.2 ± 0.0	1.5	63.8	33.3	2.9
TDC	26.6 ± 0.0	35.2 ± 0.0	32.8 ± 0.0	2.6 ± 0.0	1.2 ± 0.0	1.6	63.7	33.3	3.0
PGP	27.2 ± 0.0	35.3 ± 0.0	32.2 ± 0.1	2.6 ± 0.0	1.2 ± 0.0	1.5	64.3	32.6	3.1
Gh	26.5 ± 0.1	36.1 ± 0.0	32.2 ± 0.0	2.8 ± 0.0	1.0 ± 0.0	1.3	64.2	32.6	3.2

The Vietnamese cocoa butters contained three main fatty acids, namely palmitic acid (26.5-27.2%), stearic acid (33.3-35.3%), and oleic acid (32.2-32.9%). Linoleic acid and arachidic were also present with minor amounts of 2.6-3.5% and 1.2-1.4% respectively. The two commercial samples (PGP and Gh) were both highest in SFA. SFA from Ghanaian cocoa butter was comparable with the previous part about cocoa butter analysis. In contrast, those were lower in Vietnamese samples which might be due to differences in growing conditions. FA compositions of conched samples and their unconched counterparts were similar (data not shown for TD3/5U).

Compared to the cocoa butters from the previous chapter (3.1.1.1), palmitic and stearic acid content in the Vietnamese samples were lower while oleic acid content was higher. The Vietnamese samples contained lower amounts of SFA and higher amounts of both MUFA and PUFA. TD3/5 had the lowest SFA content and was rich in MUFA as well as PUFA. Palmitic acid content of PGP and TD3/5U was slightly higher than palmitic acid content for Asian cocoa butter analysed by Foubert *et al.* (2004). Stearic acid of the Vietnamese samples was lower. The rest of the FAs of the Vietnamese samples were comparable to what was found for Asian samples by Foubert *et al.* (2004).

3.3.2.2.3 TAG PROFILE

The triglyceride composition of a cocoa butter is responsible for its specific melting characteristic, which melts in the mouth (Lehrian *et al.*, 1980). Cocoa butter comprises 3 main TAGs, as seen previously, namely POP, POS and SOS. The TAG profile for the liquors are in Table 3.15 below:

Table 3.15: TAG composition of the extracted cocoa butters measured by HPLC.

	POP	POS	SOS	others	SU ₂	S ₂ U	SSS	S ₂ U /SU ₂
TD3/5U	18.3 ± 0.1	45.8 ± 0.1	30.5 ± 0.3	5.3 ± 0.3	1.42	97.68	0.90	68.9
TD3/5C	18.2 ± 0.3	46.0 ± 0.4	30.6 ± 0.1	5.1 ± 0.6	1.32	97.82	0.86	73.9
TDU	17.4 ± 0.0	46.1 ± 0.2	30.5 ± 0.3	6.0 ± 0.2	2.27	97.21	0.52	42.7
TDC	17.3 ± 0.1	45.5 ± 0.1	30.9 ± 0.2	6.4 ± 0.5	2.53	96.83	0.64	38.3
PGP	18.0 ± 0.3	45.8 ± 0.4	30.6 ± 0.1	5.6 ± 0.1	2.20	97.31	0.49	44.3
Gh	16.2 ± 0.2	45.1 ± 0.3	32.5 ± 0.2	6.1 ± 0.3	2.48	97.09	0.42	39.1

The Vietnamese cocoa butters had a POP range of 17.4%-18.2%, POS from 45.4% to 46.1% and SOS from 30.2% to 30.5%. TAGs profiles were comparable referring to the Vietnamese and Ghanaian cocoa samples from Chapter 3.1.1.2.

Differences in TAGs composition among the Vietnamese liquors are pronounced. From this analysis, liquor from the mixture of TD3/5 contained higher amounts of S₂U than that of TD and PGP which was contrary to its FA profile.

As with Chapter 3.1.1.2, compared to a study of Chaiseri and Dimick (1989), who analysed cocoa butters from different countries in Africa, Asia and South America, the Vietnamese samples had higher POS and comparable POP and SOS content than those in that study. The Ghanaian sample in this study had lower POP, higher POS and SOS suggesting the harder butter than the average value of representative African beans. This could again be due to the non-linearity of the HPLC-ELSD detector.

The ratio of S₂U to SU₂ TAGs is a measure of sensory and technological attributes of a cocoa butter. Low S₂U to SU₂ ratios may give rise to inadequate crystallization (Lipp and Anklam, 1998), since the SU₂ group melts at a lower temperature range (see chapter 3.1.3). The Ghanaian reference had the lowest S₂U /SU₂ ratio, predicting the softer cocoa butter than Vietnamese samples. Although the difference was not very dominant, it is in good agreement with previous studies (Chaiseri and Dimick, 1989).

3.3.2.2.4 SFC PROFILE

The SFC profile of a cocoa butter indicates its melting behavior at different temperatures (Figure 3.10). SFC of a cocoa butter influences the heat stability, consistency and sensorial qualities of a chocolate (Beckett, 2008).

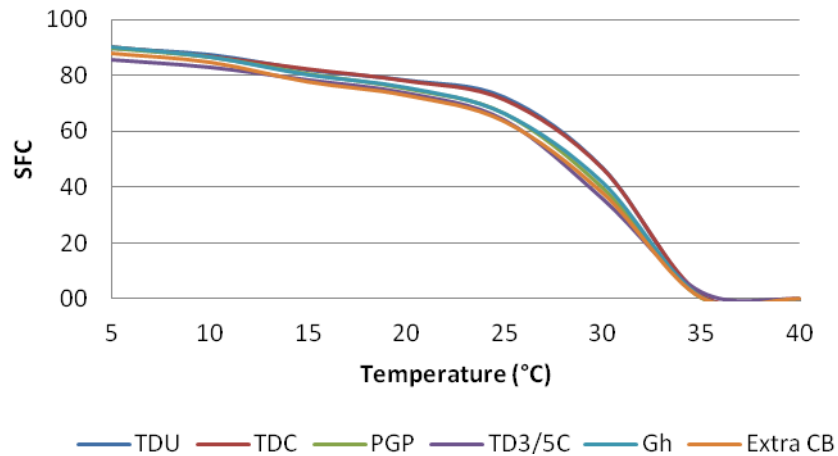


Figure 3.10: SFC profile of the extracted cocoa butters measure by NMR.

Table 3.10 shows the SFC of cocoa liquors at different temperatures. SFC of the samples decreased slowly from 5-25°C, then continued that trend rapidly until 35°C. At the starting temperature at of 5°C, TD3/5 had the lowest SFC than other samples (85%).

Temperature from 20°C to 25°C indicates cocoa butter hardness (Beckett, 2008) which is negatively influenced by free fatty acid content (Foubert *et al.*, 2004). It was apparent that the two Trong Duc samples were the hardest while TD3/5C and the additional cocoa butter are the softest. PGP and Ghanaian samples were intermediate in hardness and showed no significant difference in SFC at this temperature. This phenomenon could be because TD3/5 had the lowest SFA (62.5%), highest PUFA (4.2%) and higher FFA (1.03%) content. The Trong Duc samples had intermediate (63.7%-63.8%) SFA, but the lowest FFA (0.60%-0.78%), while PGP and Gh had the highest SFA (64.3% and 64.2% resp.) content, but also higher FFA content (1.69%-1.90% respectively). SFC at 20°C was positively correlated with the amount of SFA and negatively correlated with the amount of PUFA and FFA (R = 0.44, -0.84 and -0.53 respectively).

The temperature range in which a rapid decline in SFC is observed, meaning 25° to 35°C, indicates the heating resistance of a cocoa butter (Beckett, 2008). The cocoa butters all had a SFC far above 50% at 25°C and were nearly completely melted at 35°C with exception of TD3/5C. At 35°C, small amounts of SFC were still detected, which would indicate a very small amount of waxy mouthfeel. This makes the cocoa butters suitable for use within tropical regions, where they would be still hard at room temperature while leaving no waxy residues in the mouth (Luccas, 2001). When comparing

samples in terms of heat resistance, a similar trend was found as described in temperature range of 20-25°C. The cocoa butters with the highest ΔSFC_{25-35} were the Trong Duc samples. TD3/5C had the lowest ΔSFC_{25-35} , having a lower SFC at 25°C and the highest solid fat residue at 35°C. PGP and Gh had the intermediate value. This was also apparent during the texture analysis of the chocolates at 30°C, where TDC and TDU were the hardest while TD3/5C was the softest. SFC at 30°C was positively correlated to SFA and negatively correlated to PUFA and FFA ($R = 0.47, -0.85$ and -0.48 respectively).

Table 3.16: SFC profile of extracted cocoa butters measured by NMR.

SFC profile	5°C	10°C	15°C	20°C	25°C	30°C	35°C
TD3/5C	85.5 ^a ± 0.2	82.9 ^a ± 0.2	78.3 ^a ± 0.3	73.6 ^a ± 0.2	64.0 ^a ± 0.2	35.9 ^a ± 0.9	2.7 ^c ± 0.4
TDU	89.6 ^c ± 0.5	87.3 ^c ± 0.5	81.8 ^c ± 0.4	78.2 ^c ± 0.6	72.1 ^c ± 0.3	46.8 ^e ± 0.3	1.2 ^{ab} ± 0.1
TDC	90.0 ^c ± 0.4	86.7 ^c ± 0.2	82.1 ^c ± 0.1	77.9 ^c ± 0.3	71.1 ^c ± 0.2	46.5 ^e ± 0.8	1.5 ^b ± 0.3
PGP	89.8 ^c ± 0.3	86.6 ^c ± 0.7	80.6 ^b ± 0.3	75.3 ^b ± 0.7	66.4 ^b ± 0.3	39.8 ^c ± 0.6	0.6 ^{ab} ± 0.6
Gh	90.0 ^c ± 0.5	86.4 ^c ± 0.2	80.1 ^b ± 0.2	75.5 ^b ± 0.2	66.2 ^b ± 0.4	41.6 ^d ± 0.7	0.8 ^{ab} ± 0.0
Extra CB	88.0 ^b ± 0.1	84.8 ^b ± 0.4	77.8 ^a ± 0.5	72.9 ^a ± 0.4	63.6 ^a ± 0.5	37.9 ^b ± 0.5	0.5 ^a ± 0.4

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Over the whole temperature trajectory, except at 35°C, SFC of the Vietnamese samples were lower than compared to those in Chapter 3.1.2.1, which could be explained by their lower SFA and higher MUFA content. TD3/5C had a higher SFC at 35°C, which could be explained by its higher SSS (0.86%) content. SSS is responsible for solid fat at a temperature where a large part of the other TAGs are melted (Vereecken *et al.*, 2008).

Negative correlations were present between SFC and the amount of palmitic acid present ($R = -0.72, -0.66$ and -0.78 for 20°C, 25°C and 30°C respectively), since these have a lower melting point than stearic acid. A positive correlation was found between stearic acid and SFC of the cocoa butters ($R = 0.54, 0.42$ and 0.6 for 20°C, 25°C and 30°C respectively).

3.3.2.2.5 ISOTHERMAL CRYSTALLISATION OF THE COCOA BUTTERS

The parameters describing the crystallization behavior of the cocoa butters extracted from the liquors are in the table below.

Table 3.17: Parameters a_F , t_{ind} and K derived from fitting with the Foubert equation.

	a_F (J/g)	t_{ind} (h)	K (h ⁻¹)
TD3/5C	74.3 ^{ab} ± 1.5	0.39 ^b ± 0.01	4.03 ^a ± 0.22
TDU	79.7 ^c ± 2.3	0.30 ^a ± 0.00	6.30 ^c ± 0.06
TDC	79.7 ^c ± 0.3	0.29 ^a ± 0.01	6.50 ^c ± 0.44
PGP	73.9 ^{ab} ± 2.1	0.41 ^b ± 0.01	4.51 ^a ± 0.08
Gh	75.6 ^{bc} ± 1.3	0.40 ^b ± 0.02	5.35 ^b ± 0.31
CB extra	71.2 ^a ± 1.2	0.62 ^c ± 0.01	4.29 ^a ± 0.18

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Parameter a_f (J/g) represents the total amount of heat released during the second crystallisation step. TDU and TDC had the highest a_f which followed observations made of its SFC profile in Chapter 3.2.2.2.4. TDU and TDC had the lowest FFA content. Compared to Chapter 3.1.2.3, crystallization heat of the Vietnamese samples lies lower, mostly because of the higher content of their higher MUFA and FFA content. Gh also had a higher FFA level, and a lower a_f value. Correlations have been found between decrease of a_f and the presence of di-unsaturated TAGs, which disturbs the molecular packing of mono-unsaturated TAGs. A weak inverse correlation has been found between a_f and POP, which has a lower melting point than SOS (Foubert et al., 2004).

Parameter t_{ind} is the induction time for conversion of α -crystals to β' -crystals. A strong positive correlation ($R^2 = 0.78$) has been found between FFA content of the fat and t_{ind} . No clear correlations have been detected for t_{ind} and diverse TAGs. It is apparent that the induction time is considerably higher for the extra added cocoa butter than for the rest of the samples. The induction time for TDU and TDC are significantly lower than that of the Ghanaian sample, probably because of its lower FFA content. Other Vietnamese samples do not differ from the Ghanaian cocoa butter by much. Values found for t_{ind} were comparable to those from Chapter 3.1.2.3.

Parameter K (1/h) is the rate constant of the crystallization process. TDU and TDC have the highest rate constant while TD3/5C has the lowest. TD3/5C had higher amounts of unsaturated fatty acids, which lowers the crystallization rate (Foubert *et al.*, 2004). A negative correlation has been found between FFA content and K , which has also been presented by Pontillon (1998) and Shukla (1995). Values found for K were comparable to those found in 3.1.2.3.

3.3.3 CHOCOLATE QUALITY ATTRIBUTES

3.3.3.1 MOISTURE CONTENT

The moisture content of the dark chocolates (Table 3.18) was low and similar for all samples, except for TDU which was slightly higher. This can be attributed to the higher moisture content of that particular cocoa liquor (2.0g/100g). Chocolate contains commonly 0.5%-1.5% moisture, mostly in the cocoa solids, without it affecting drastically the flow properties (Afoakwa *et al.*, 2007). Nevertheless, small changes in flow parameters are expected within this range.

Table 3.18: Moisture content of the produced chocolates

	Moisture (g/100g)
TD3/5C	$0.4^a \pm 0.0$
TDU	$0.7^b \pm 0.1$
TDC	$0.4^a \pm 0.0$
PGP	$0.4^a \pm 0.0$
Gh	$0.4^a \pm 0.1$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

3.3.3.2 ACIDITY

The pH of chocolate can be regarded as a measure for its acidity. The values are shown in Table 3.19 and show high correlation ($R = 0.99$) with the pH of the cocoa liquors. In fact, the pH of the chocolate is very similar with that of the respective chocolate, being highest for Ghana and lowest for the conched TD3/5.

Table 3.19: Acidity of the produced chocolate samples.

	pH
TD3/5C	4.7
TDU	4.9
TDC	5.0
PGP	5.1
Gh	5.4

3.3.3.3 PARTICLE SIZE DISTRIBUTION

Since dark chocolate consists of 65%-75% solid particles suspended in a continuous cocoa butter matrix (Afoakwa, 2007), the particle characteristics has a large impact on the physical characteristics of chocolate. The particle size distribution has an influence on several quality attributes of chocolate, such as the mouthfeel, hardness and rheology amongst others. The measured particle size distributions of the produced chocolates can be seen in Figure 3.11.

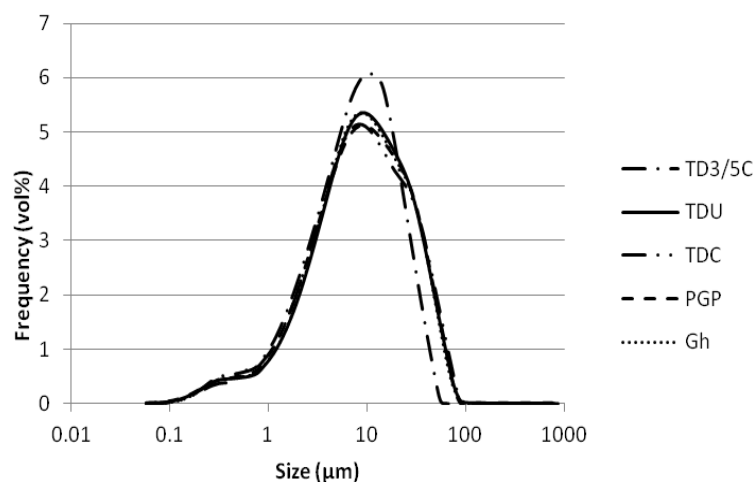


Figure 3.11: PSD of the chocolate product measured by laser diffraction.

The PSDs of TDU, TDC, PGP and Ghana are rather similar. The one of TD3/5C was characterised by a narrower peak due to less large particles. This can be explained by the processing conditions during refining of the latter. TD3/5C underwent more cycles on the refiner because of handling difficulties, finally resulting in a finer chocolate mass. The handling difficulties might be due to the presence of less free fat of than in the other liquors jeopardizing its flowability. Table 3.20 shows that the distribution characteristics of the chocolate are very similar, except for TD3/5C.

When particle size exceeds 30µm, the particles cause a gritty mouth feel (Afoakwa, 2011). Smaller particles have a creamier texture and taste than larger particles (Beckett, 2000). On the other hand, when the particles are too small, a dry mouthfeel is perceived. Four of the five chocolates have a D[v,0.9] close to the threshold of 30 µm, which might cause the perception of graininess consumers. However, trained panel members did not distinguish this (see 3.3.3.8.2).The most European chocolates have usually a fineness of 15-22 µm, determined by a micrometer (Jackson, 1999).

Table 3.20: Particle size distribution characteristics of the produced chocolate

	D[v,0.9](µm)	D[v,0.5](µm)	D[v,0.1](µm)	D[4.3](µm)	D[3.2](µm)	D[2.1](µm)
TD3/5C	22.2 ^a ± 0.5	7.3 ^a ± 0.2	1.4 ^a ± 0.2	9.9 ^a ± 0.2	2.8 ^a ± 0.2	0.5 ^a ± 0.0
TDU	31.3 ^b ± 3.1	8.5 ^a ± 0.4	1.7 ^a ± 0.2	13.0 ^a ± 1.0	3.1 ^{ab} ± 0.3	0.5 ^a ± 0.1
TDC	32.7 ^b ± 1.0	8.1 ^a ± 0.4	1.6 ^a ± 0.2	13.2 ^a ± 0.5	3.2 ^b ± 0.8	0.8 ^b ± 0.5
PGP	31.6 ^b ± 0.7	8.2 ^a ± 0.1	1.6 ^a ± 0.1	12.9 ^a ± 0.3	2.9 ^a ± 0.1	0.4 ^a ± 0.0
Gh	31.0 ^b ± 2.9	8.3 ^a ± 0.4	1.7 ^a ± 0.2	12.8 ^a ± 1.0	3.1 ^{ab} ± 0.3	0.5 ^a ± 0.1

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

3.3.3.4 FLOW BEHAVIOUR

Rheology is an important technological and quality attribute of chocolate. The flow behaviour plays a major role in the effectiveness during production processes and the sensory perception (Afoakwa, 2011). Rheologically, molten chocolate can be described as a non-Newtonian liquid which properties depend on its formulation (*e.g.* fat content and emulsifiers) on one hand, and on the process conditions (such as refining, conching and tempering) on the other hand (Tscheuschner and Wunsche, 1979; Vavreck, 2004; Schantz & Rohm, 2005). Production costs can be controlled by manipulating the fat content, lecithin concentration and PSD (Afoakwa *et al.*, 2009). The two most widely used parameters to describe chocolate flow are Casson yield stress and Casson viscosity (Afoakwa *et al.*, 2007) following the fit of the flow data to the Casson model:

$$\sqrt{\sigma} = \sqrt{\sigma_{CA}} + \sqrt{\eta_{CA}} \cdot \sqrt{\dot{\gamma}} \quad (\text{Eq.3.2})$$

With: σ (Pa) = shear stress
 σ_{CA} (Pa) = Casson yield stress
 η_{CA} (Pa.s) = Casson viscosity
 $\dot{\gamma}$ (s⁻¹) = shear rate

3.3.3.4.1 CASSON YIELD STRESS

The yield value is the stress to trigger chocolate flow. Table 3.21 shows the Casson yield stress of the chocolate samples (upward curve), that for the downward curve showed similar trends and is presented in Appendix 2.

Table 3.21: Casson yield stress of the chocolate samples

	σ_{CA} (Pa)
TD3/5C	$11.7^d \pm 0.2$
TDU	$6.0^c \pm 0.1$
TDC	$5.7^c \pm 0.1$
PGP	$4.1^a \pm 0.1$
Gh	$4.9^b \pm 0.4$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Significant differences in σ_{CA} between the samples were observed. PGP exhibited the lowest σ_{CA} followed by Gh. The σ_{CA} of TDU and TDC was not significantly different, suggesting that a moisture difference of 0.3% in the low moisture range didn't had a clear impact on this flow parameter. A very distinct outlier was TD3/5C, with a yield stress at 11.7 Pa.

Strong correlations were found between σ_{CA} and the particle diameters D[v,0.9] (R = -0.95), D[v,0.5] (R = -0.89), D[v,0.1] (R = -0.85) and D43 (R = -0.97). Prasad *et al.* (2003) stated that chocolate yield value depends on inter-particle interactions, which is determined by its specific surface area.

3.3.3.4.2 VISCOSITY

The plastic viscosity of chocolate is defined as its resistance to flow. Table 3.22 shows the Casson viscosity of the chocolate samples (upward curve). Viscosity parameters for the downward curve showed similar results and are presented in Appendix 2.

Table 3.22: Casson viscosity of the chocolate samples

	η_{CA} (Pa.s)
TD3/5C	$1.71^a \pm 0.01$
TDU	$2.01^c \pm 0.01$
TDC	$1.92^b \pm 0.01$
PGP	$2.10^d \pm 0.01$
Gh	$1.99^c \pm 0.02$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Similar to σ_{CA} , significant differences in η_{CA} between the samples were observed. PGP exhibited the highest σ_{CA} followed by Gh and TDU. The η_{CA} of TDU was significantly higher than that of TDC suggesting that a moisture difference of 0.3% in the low moisture range had an impact on this flow parameter. TD3/5C had the lowest Casson viscosity, suggesting that the longer refining process resulted in more free fat and/or a higher packing efficiency.

Strong positive correlations were found between η_{CA} and the particle diameters D[v,0.9] (R = 0.85), D[v,0.5] (R = 0.88), D[v,0.1] (R = 0.80) and D43 (R = 0.87).

Chocolate viscosity is influenced by its fat content: the higher the fat content, the greater the distance between the solid particles, which cause the viscosity to drop. In this experimental set-up,

however, the chocolate recipe was standardized on fat content. In addition, Beckett (2000) stated that chocolate viscosity is very little altered when fat is added at a fat content higher than 32%. All produced chocolates had a fat content of 33.8%. However, the observed differences in η_{CA} might be (at least partly) due to differences in free fat.

An explanation for the lower viscosity for TD3/5C and TDC could be their longer conching time, since the liquors of these two chocolates were pre-treated by conching 48 hours. Conching separates agglomerated cocoa particles and makes sure the particles are evenly covered with cocoa butter. Coating the particles with fat more evenly improves flow properties and reduces viscosity (Beckett, 2008).

Further, the viscosity is influenced by PSD: when particle size decreases, the particle surface increases, therefore more fat is bound by the particles, leaving less free fat to aid with the chocolate flow (Beckett, 2009). On the other hand, the viscosity is highly dependent on the packing efficiency which means that the smaller particles fill the voids created by the larger particles and promote flow. The higher peak intensity of TD3/5C probably points out the higher packing efficiency in this chocolate.

Finally, chocolate moisture was only slightly correlated with viscosity ($R = 0.25$). In chocolate, moisture at the surface of sugar particles increases friction and apparent viscosity. It also aggregates sugar particles to form gritty lumps (Afoakwa *et al.*, 2007).

3.3.3.4.3 THIXOTROPY

Molten chocolate is a shear-thinning liquid. It is thick when static and becomes less viscous over time when sheared. Afoakwa *et al.* (2008) calculated thixotropy as the absolute difference between shear stress at 5 s^{-1} during ramp up and ramp down. Table 3.23 shows that the chocolate samples were not thixotropic (low values), suggesting that they were well conched (Afoakwa *et al.*, 2008).

Table 3.23: Thixotropy of the chocolate samples

	Thixotropy (Pa)
TDU	$0.09^a \pm 0.04$
TDC	$0.08^a \pm 0.06$
PGP	$0.20^a \pm 0.09$
TD3/5C	$0.21^a \pm 0.09$
Gh	$0.38^a \pm 0.31$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

3.3.3.5 COLOUR

Visual appearances are important features of a product. These characteristics are the first attributes a consumer perceives, and are the first attributes the product is judged by (Gellynck *et al.*, 2011). To represent a colour, the $L^*a^*b^*$ system developed by the Commission Internationale de l'Eclairage (CIE) is commonly used, where colour is regarded as a characteristic based on three parameters, namely lightness, hue and saturation. Lightness describes the light- or darkness of a colour and is

described by L^* , hue classifies the colour (red, blue, yellow...) and is described by the positive or negative sign of a^* and b^* , and saturation describes how far the colour differs from grey and is described by the absolute value of a^* and b^* . The $L^*a^*b^*$ colour space visualizes those parameters.

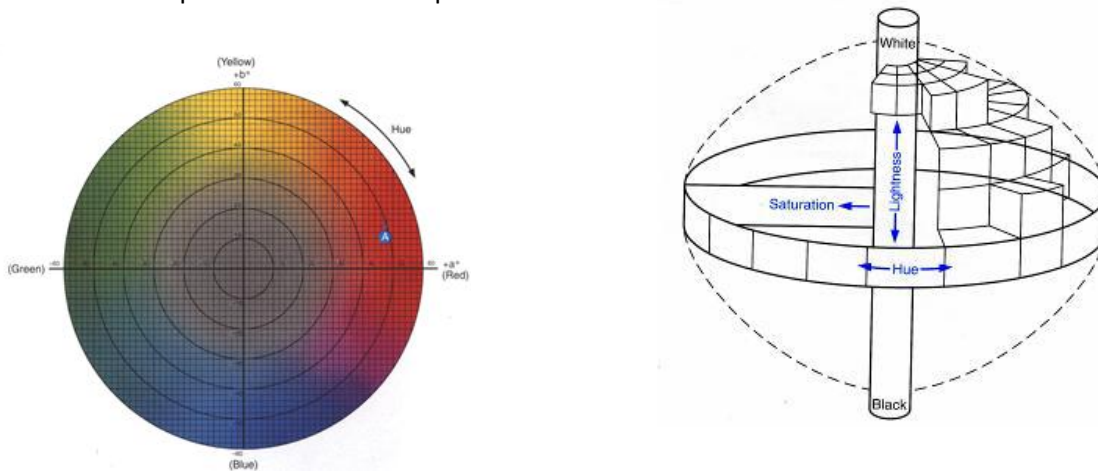


Figure 3.12: Left: a^* , b^* chromaticity diagram (Gellynck *et al.*, 2011)
Right: Three-dimensional colour space (Gellynck *et al.*, 2011)

The letters on the axis are to be interpreted as follows:

<u>L^*: lightness</u>	<u>a^*: red/green</u>	<u>b^*: yellow/blue</u>
$L^* = 0$: black	$a^* = \text{positive}$: red	$b^* = \text{positive}$: yellow
$L^* = 100$: white	$a^* = \text{negative}$: green	$b^* = \text{negative}$: blue

Significant differences in all three colour parameters between the chocolate samples were found (Table 3.24). The chocolate that resembles the Ghanaian reference sample the most in terms of colour parameters is PGP. The chocolate that differs the most from Gh is TD3/5C, which is significantly lighter. TDC is slightly darker than TDU possibly due to Maillard reactions during the extra conching step (Beckett, 2008), but the observed difference was not significant (p -value 0.099).

Table 3.24: Measured colour characteristics of the chocolate samples.

	L^*	a^*	b^*
TDU	$29^b \pm 0.8$	$8.6^c \pm 0.4$	$6.8^c \pm 0.5$
TDC	$28.5^b \pm 0.6$	$7.6^b \pm 0.6$	$5.7^a \pm 0.6$
PGP	$27.1^a \pm 1.0$	$5.9^a \pm 0.4$	$4.6^a \pm 0.4$
TD3/TD5C	$31.3^c \pm 0.6$	$9.1^c \pm 0.3$	$8.4^d \pm 0.4$
Gh	$26.6^a \pm 0.5$	$6.2^a \pm 0.3$	$4.6^a \pm 0.3$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

According to Afoakwa *et al.* (2008), the particle size distribution has an influence on the chocolates' colour. As a matter of fact, the scattering factor in a densely packed medium is inversely related to the particle size (Saguy and Graf, 1991). Finer chocolate particles will scatter more light, and its chocolate colour will be lighter and more saturated than with coarser particles (Afoakwa *et al.*,

2008). Indeed, a negative correlation has been found for the chocolate particle distribution D[v,0.9] (R = -0.80), D[v,0.5] (R = -0.78), D[v,0.1] (R = -0.67), average particle diameter D[43] (R = -0.80) and the L* value. TD3/5C, which has a considerably smaller particle diameter than the rest of the samples, is the lightest sample. The effect of particle size was also visible on both a* (R = -0.61 for D[v,0.9], R = -0.53 for D[v,0.5] and R = -0.58 for D[43]) and b* values (R = -0.80 for D[v,0.9], R = -0.72 for D[v,0.5] and R = -0.79 for D[43]), being highest for TD3/5C.

For the L*a*b* system, the total difference in colour between two samples can be described as follows:

$$\Delta E^* = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (\text{Eq. 3.3})$$

As seen in Table 3.25, there is as much difference between the Vietnamese samples themselves as compared to the Ghanaian sample. On the other hand, the small colour difference between TDC and TDU indicates that conching the cocoa liquor prior to chocolate production does not greatly result in colour alteration.

Table 3.25: Calculated colour differences between chocolate samples

ΔE^*	TDU	TDC	PGP	TD3/5C	Gh
TDU	-	1.61	14.83	17.94	15.20
TDC	1.61	-	13.61	17.13	13.96
PGP	14.83	13.61	-	16.02	12.10
TD3/TD5C	17.94	17.13	16.02	-	16.43
Gh	15.20	13.96	12.10	16.43	-

A study by Kuehni and Marcus (1979) presented that the average ΔE^* for which 50% of the observers were able to perceive a colour difference was $\Delta E^* = 1$. Since ΔE^* values for all sample combinations exceed that level, it is possible to distinguish the different chocolate colours by the naked eye.

3.3.3.6 TEXTURE

The texture of a chocolate is an important property for consumer acceptability (Beckett, 2003). Instrumental measurement enables to characterize this aspect of chocolate in an efficient and objective way (Lawless and Heymann, 1998). Results of the texture analysis at 20°C and 30°C are in Figure 3.13.

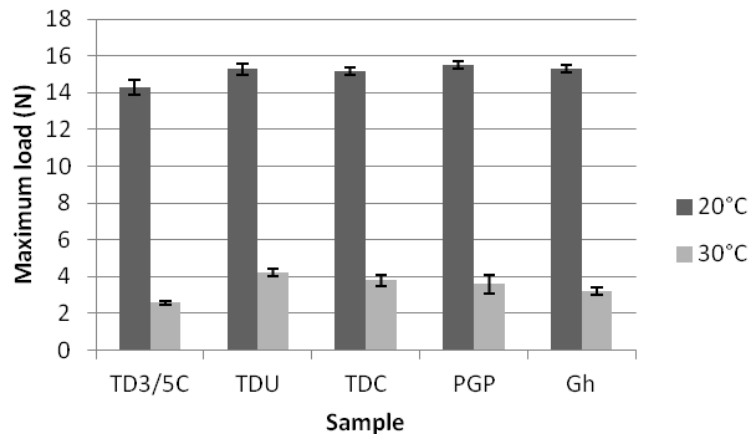


Figure 3.13: Hardness of the chocolate samples

Differences in hardness between the samples were more apparent at 30°C than at room temperature. At 20°C, all samples had a comparable hardness except the softer TD3/5C, which also had lower SFC at that temperature. Hardness at 20°C was correlated to SFC at 20°C ($R = 0.59$), and hardness at 30°C was correlated to SFC at 30°C ($R = 0.90$). At 30°C, TD3/5C was softer as well, and TDU and TDC, which had the highest SFC at that temperature, were hardest.

TD3/5C was the sample containing the least amount of SFA and the highest quantity of unsaturated fatty acids. Hardness at 20°C and 30°C were strongly inversely correlated with PUFA content ($R = 0.93$ and 0.91 respectively). MUFA and PUFA content was negatively correlated to hardness at 20°C ($R = -0.54$ and -0.94 respectively). Hardness of the chocolate samples at 30°C was also inversely correlated with the amount of FFA ($R = -0.82$).

According to Afoakwa et al. (2008), PSD has an influence on chocolate hardness. The smaller the particle sizes, the higher the specific surface areas, which causes more inter-particle interactions, resulting in an increase in chocolate hardness. This effect is more pronounced at lower fat levels (25%)(Afoakwa et al., 2009). No correlation between PSD and hardness was found in this study.

3.3.3.7 MELTING RANGE

To analyse in detail the melting behavior of the chocolate, DSC measurements were performed. Starting from 20°C, the samples were heated till 45°C, and melting heat was recorded. The form of a melting curve of a chocolate sample is visible in Figure 3.14.

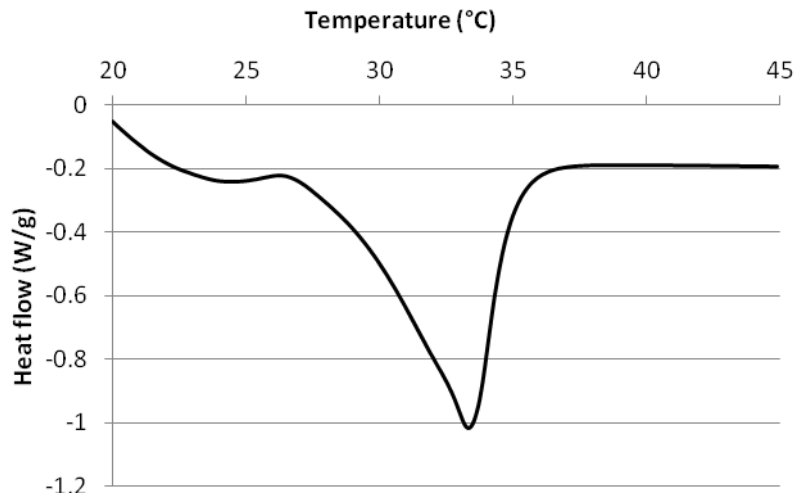


Figure 3.14: Melting curve of a chocolate sample measured by DSC

Melting parameters of the chocolate samples are in Table 3.26. No significant differences between the samples have been found for the peak temperature, but correlations between the **peak temperature** and the chemical composition of the cocoa butter of the sample have been found. T_{max} was inversely correlated with SU_2 ($R = -0.58$) and positively correlated with S_2U ($R = 0.66$), since SU_2 lowers the melting temperature of the cocoa butter (Lipp and Anklam, 1998).

Table 3.26: Melting parameters of the chocolate products measured by DSC.

	T_{max} (°C)	Offset (°C)	Enthalpy (J/g)
TD3/5C	$33,6^a \pm 0.3$	$37,9^b \pm 0.3$	$39,2^a \pm 1.0$
TDU	$33,7^a \pm 0.2$	$37,9^b \pm 0.4$	$40,1^{ab} \pm 1.7$
TDC	$33,3^a \pm 0.2$	$37,3^{ab} \pm 0.4$	$42,2^b \pm 0.7$
PGP	$33,5^a \pm 0.3$	$37,4^{ab} \pm 0.3$	$39,7^{ab} \pm 0.7$
Gh	$33,4^a \pm 0.1$	$36,7^a \pm 0.1$	$37,7^a \pm 0.9$

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

The Ghanaian chocolate had the lowest **offset temperature**, while TD3/5C and TDU had the highest. This could be explained by differences in SSS content, since SSS provides a higher SFC in cocoa butter at higher temperatures (Vereecken *et al.*, 2008). A positive correlation between SSS and offset temperature was indeed found ($R = 0.63$). Offset temperature was also inversely correlated to SU_2 content. SU_2 lowers the melting point of cocoa butter (Lipp and Anklam, 1998).

TD3/5C and Gh had the lowest **melting heat**, while TDC had the highest. This could be explained by its FFA and PUFA content since these interrupt the molecular packing of the SU₂ molecules (Foubert *et al.*, 2004). TDU and TDC both had low FFA and PUFA content, and both had a higher melting enthalpy than the other samples, which contained higher FFA and PUFA levels.

3.3.3.8 SENSORY EVALUATION

3.3.3.8.1 TRIANGLE TEST

Triangle tests determine whether or not consumers can distinguish different samples. However, it does not tell us where the differences lie. The results of the triangle test are in Table 3.27 below.

Each combination has been tested at least 23 times. For practical reasons, only a select few comparisons have been made. The first is a comparison between a chocolate made from conched liquor compared to its unconched counterpart. The second combination determines whether or not a difference is detected between a conched non-commercial and a commercial Vietnamese sample. Thirdly, the conched non-commercial sample was compared with the Ghanaian reference. Fourth, a commercial Vietnamese sample was compared against the Ghanaian sample, and lastly, two conched non-commercial samples were tested.

Table 3.27: Fraction of correct answers for each combination.

Comparison	Average	p-value
TDC - TDU	0.47	0.058
TDC – PGP	0.56	0.016
TDC - Gh	0.50	0.044
PGP - Gh	0.50	0.026
TDC – TD3/5C	0.74	0.000

Participants were able to significantly distinguish samples from all combinations except for TDC-TDU. This means that conching the cocoa liquor did not result in distinguishable differences between the conched and non-conched sample. However, participants were able to differentiate chocolate sample resulting from cocoa liquors from different sources. Differences between commercial and non-commercial samples from Vietnam (TDC-PGP) were distinguishable, as well as differences between chocolates resulting from different Vietnamese non-commercial liquor samples (TDC-TD3/5C). Both commercial and non-commercial Vietnamese samples were found to be different from the Ghanaian samples (PGP-Gh and TDC-Gh).

3.3.3.8.2 QUANTITATIVE DESCRIPTIVE ANALYSIS

Scores on a 9-point hedonic scale given by the panel members for chocolate appearance, texture and mouth feel are in the Table 3.28 below. Scores have to be interpreted with caution, since only five panel members were able to participate.

Table 3.28: Appearance, texture and mouth feel scores attributed by a trained panel.

	Fat bloom	Snap	Hardness	Creaminess	Graininess	Dryness	Aftertaste
TD3/5C	7.2 ^a ± 0.7	6.4 ^a ± 1.2	6.5 ^a ± 1.2	3.2 ^a ± 1.2	2.4 ^a ± 1.2	5.0 ^a ± 1.7	7.5 ^b ± 1.1
TDU	6.9 ^a ± 0.8	6.9 ^a ± 1.0	6.8 ^a ± 0.9	3.8 ^a ± 1.3	2.4 ^a ± 1.3	5.1 ^a ± 1.8	6.9 ^{ab} ± 1.2
TDC	7.3 ^a ± 0.6	5.9 ^a ± 2.7	6.5 ^a ± 1.3	2.8 ^a ± 1.1	3.2 ^a ± 1.8	5.4 ^a ± 2.3	6.6 ^{ab} ± 1.1
PGP	7.2 ^a ± 0.4	6.2 ^a ± 2.0	6.5 ^a ± 1.4	3.3 ^a ± 0.9	3.1 ^a ± 2.3	5.0 ^a ± 2.0	6.1 ^a ± 1.3
Gh	7.4 ^a ± 0.7	6.5 ^a ± 2.1	6.8 ^a ± 1.3	3.8 ^a ± 1.4	3.0 ^a ± 2.0	4.8 ^a ± 1.9	5.9 ^a ± 1.4

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

Except for the **aftertaste**, none of the previous characteristics was perceived as different in all five samples. TD3/5C was perceived as more astringent than the two commercial samples (Gh and PGP). According to the texture analysis, TD3/5C was significantly less **hard** than the Ghanaian sample at 20°C, but this was not detected by the panel members.

Table 3.29: Aroma and flavour scores attributed by a trained panel.

	Cocoa smell	Acidic smell	Sweet flavour	Bitter flavour	Sour flavour	Cocoa flavour
TD3/5C	5.5 ^a ± 1.2	2.8 ^a ± 1.5	4.1 ^a ± 1.2	6.1 ^a ± 1.7	6.1 ^b ± 1.6*	5.3 ^a ± 1.1
TDU	6.1 ^a ± 1.2	2.2 ^a ± 1.5	5.0 ^{ab} ± 1.5	6.4 ^a ± 1.2	4.5 ^{ab} ± 2.4	5.8 ^a ± 1.4
TDC	5.2 ^a ± 1.8	3.3 ^a ± 2.3	5.1 ^{ab} ± 1.5	5.8 ^a ± 2.1	5.1 ^b ± 1.7	5.4 ^a ± 1.7
PGP	5.9 ^a ± 1.7	3.1 ^a ± 1.9	5.4 ^{ab} ± 2.0	5.5 ^a ± 1.8	4.5 ^{ab} ± 2.0	5.4 ^a ± 1.2
Gh	5.7 ^a ± 1.6	2.1 ^a ± 1.3	6.2 ^b ± 1.2	5.9 ^a ± 0.9	2.5 ^a ± 1.2	6.3 ^a ± 1.0

^{a,b,c,d,e,f}: different superscripts point out significant differences between the cocoa butters at a significance level of 0.05

The panel did not distinguish significant differences in either **cocoa or acidic aroma** (Table 3.29) between all samples. As for flavour, no statistically significant differences were detected for **bitter and cocoa flavours**.

TD3/5C was perceived as less **sweet** than the Ghanaian reference, and both TDC and TD3/5C had a **sourer flavour** than the Ghanaian sample. This perceived sourness was related to chocolate pH ($R = -0.93$). However, it should be noted that the panel was not trained to describe sour aromas and flavours, and results from those tests must be interpreted with caution. Increase in pH was also correlate to increase in chocolate flavour ($R = 0.68$), which concurs with Jinap *et al.* (1995), who stated that lower pH resulted in lower notes of chocolate flavour. Lactic and malic acid were also inversely correlated to chocolate flavour ($R = -0.82$ and -0.74 respectively). It must also be mentioned that in combination its high acidity, the TD3/5C sample contained low concentrations of oxalic acid as well. This might be the cause of the off-taste of the chocolate sample, which was often remarked by the panel and the consumers. It was found that the amount of oxalic acid present was inversely related to sour notes ($R = -0.67$). The presence of oxalic acid even had a positive correlation with perceived chocolate flavour ($R = 0.58$). Indeed, Holm *et al.* (1993) has presented that oxalic acid has a positive influence on **chocolate taste**. Panel members observed a higher cocoa flavour for the Ghanaian sample.

CONCLUSIONS

In this study, Vietnamese cocoa was characterized and compared to those of other origins. Firstly, cocoa butters of several clones originating from the Mekong Delta in Vietnam were characterized. In the second part, liquor mixtures of several clones, commercial samples from Vietnam as well as Ghana were characterized and compared. Their functionality in dark chocolate was described.

In the first part, the physicochemical properties of cocoa butters from Vietnam, Ghana, Ivory Coast and Indonesia were characterized. Concerning the Vietnamese clones, there was a large variability in the relative fatty acid composition between the different samples, however, the range in SFA was quite narrow. TD8, TD9, TD10 and to a lesser degree, TD5, TD6 and TD11 consisted of higher levels of SFA, to the expense of MUFA. The average SFA content of the Vietnamese samples was comparable to that of the Indonesian cocoa butter. Samples from Ghana and Ivory Coast were somewhat lower in SFA. TD8 and TD9, followed by TD10 and TD6 contained the highest amounts of S_2U , this in accordance with their higher SFA content. The average Vietnamese sample has higher S_2U and lower SU_2 compared to cocoa butter from other regions. FFA content of all samples were well within the legal norms.

The physical characteristics of the cocoa butters were described by their melting and crystallisation behaviour. Within the Vietnamese clones, there was a considerable variability in SFC from 25°C to 35°C. In this temperature range, TD8 and TD9 displayed higher SFC because of their elevated S_2U ($R = 0.73$ at 30°C) and lower free fatty acid content ($R = -0.77$), followed by TD11, TD5, TD10, TD3, TD7 and TD6 in this order while TD2 and TD13 were overall lower in SFC. On average, from 20°C to 35°C, Vietnamese cocoa butter had a higher SFC than the Ghanaian cocoa butter, but lower than those from Ivory Coast and Indonesia, due to higher levels of FFA. Within the Vietnamese clones TD8, TD9, TD7, TD5, TD11 and TD13 had the highest peak temperature for non-isothermal crystallisation while TD10, TD2, TD3 and TD6 had the lowest. On average, Vietnamese cocoa butter presented a higher peak maximum than Ivorian cocoa, but lower than that of Ghana and Indonesia. Peak temperature was positively correlated with SOS and negatively correlated to POP ($R = 0.87$ and -0.84 respectively). During the isothermal crystallisation at 20°C, the focus was on the second crystallisation peak. The amount of solid fat at equilibrium was highest for the Indonesian sample and lowest for the Ghanaian sample. Vietnam and Ivory Coast were intermediate. Within the Vietnamese clones TD13, TD8, TD9 and TD6 had the highest crystallisation heat for the second peak. The induction time of conversion from α -crystals into β' -crystals was highest for the Ghanaian sample and lowest for samples from Indonesia and Ivory Coast. Induction time for the average Vietnamese sample is intermediate. Within the Vietnamese clones, induction time for TD9, TD11, TD6, TD3 and TD8 were the shortest. The main correlation found for t_{ind} and the samples was their FFA levels ($R = 0.74$). Crystallisation rate is lowest for the Ghanaian sample and highest for samples from Ivory Coast and Indonesia. Rate constant K was highest for TD2, TD8, TD9 TD5 and TD6. Between different regions,

the average *K*-value for the Vietnamese samples was intermediate. The main correlation for *K* and the different samples was FFA content ($R = -0.82$).

In the second part, the impact of the cocoa liquor properties on the attributes of dark chocolate was investigated. Three Vietnamese and one Ghanaian liquor sample was used to produce chocolate. Two Vietnamese samples, which had the highest moisture content, had low pH values (4.67 and 4.85) ($R = -0.88$). Low pH values were found to be mainly linked to lactic acid content ($R = -0.63$). In order to reduce their acidity, the liquors underwent a conching treatment, keeping part of the Trong Duc liquor unconched as a reference. Both conched samples obtained after the treatment successfully a higher pH value. However, total organic acid concentration of the Trong Duc sample did not decrease. Chocolate pH was linked to liquor pH ($R = 0.99$), and even after the conching treatment, an acidity difference remained in the finished product.

The five liquors were analysed chemically and physically. Gh and PGP contained the highest amount of SFA to the expense of MUFA. TD3/5C contained the lowest amounts of SFA in favour of PUFA. TDU and TDC were intermediate. TAG analysis showed that TD3/5 contained the lowest amount of SU_2 , and the highest amount of S_2U and SSS. TDC, followed by Gh contained the highest amounts of SU_2 , and the lowest amounts of S_2U . Physical characteristics comprised solid fat profile and isothermal crystallisation. TDU and TDC had the highest SFC over most of tested temperature range; this because of its FA composition and low FFA ($R = -0.53$ and -0.48 at 20°C and 30°C respectively) content. TD3/5C was mainly softer due to low SFA ($R = 0.44$ and 0.47 at 20°C and 30°C respectively), and high FFA levels. Residual SFC at 35°C of TD3/5C could be explained by its higher SSS content ($R = 0.96$). Gh and PGP were intermediate. During isothermal crystallisation at 20°C , TDU and TDC had the highest crystallisation heat, shortest induction time for β' crystallisation, and highest rate constant, mostly because of lower FFA and MUFA levels.

Next, chocolate rheology was analysed. Casson yield stress was particularly high for TD3/5C. TD3/5C and TDC had the lowest Casson viscosity probably because the conching treatment of the cocoa liquors, creating more free fat which reduced their viscosity. Both yield stress and viscosity were related to particle size ($R = -0.95$ and 0.85 respectively). In addition, because of its smaller particle size, TD3/5C was lightest in colour ($R = -0.8$), with the highest saturation ($R = -0.61$ and -0.8 for a^* and b^* respectively). As for the melting range of the chocolate, no significant differences in peak temperature were found, but correlations between peak temperature and S_2U and SU_2 have been found ($R = 0.66$ and -0.58 respectively). The Ghanaian chocolate had the lowest offset temperature because of its lower trisaturated triacylglycerol content ($R = 0.63$), while TD3/5C and TDU exhibited the highest values. TD3/5C and Gh had the lowest melting heat, while TDC had the highest. Hardness of the chocolates at 20°C and 30°C was found to be related to SFC at the corresponding temperatures ($R = 0.59$ and 0.90 respectively).

As for sensory evaluation, consumers could detect the odd sample from the triangle test for most combinations, except for the conched versus unconched sample. Acid reduction via conching was instrumentally measurable, but it was not distinguished by consumers in this study. As for

appearance, texture and mouth feel scores, the trained panel did not detect any differences between the samples except for the aftertaste. Panel members could detect differences in sour flavour between the samples. The Vietnamese samples, especially TD3/5C, were perceived as sourer.

FURTHER RESEARCH

As the non-linearity of the HPLC-ELSD detector prohibits comparison of TAG compositions of the samples studied in this research to those of other origins described in literature, the measuring method should be adjusted in order to be able to compare TAG profiles to one another.

Although two of the liquors were conched, sourness in their final chocolate product was still detected. Further investigations could be made in order to eliminate the present acid taste.

For this research, only five panel members participated in the sensory analysis. With a larger panel, more significant results could be obtained.

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APPENDIX

Appendix 1: Measured SFC of samples from Vietnam, Ghana, Ivory Coast and Indonesia.

	5 C°	10 C°	15 C°	20 C°	25 C°	30 C°	35 C°
Vietnam	93.9 ± 1.1	91.0 ± 1.7	87.1 ± 1.4	82.9 ± 1.2	76.9 ± 1.4	50.8 ± 7.0	0.3 ± 0.5
TD2	92.1 ^{ab} ± 0.3	88.6 ^a ± 0.3	85.3 ^{ab} ± 0.1	81.2 ^b ± 0.1	75.4 ^b ± 0.6	40.4 ^a ± 0.5	0.0 ^a ± 0.0
TD3	93.8 ^{de} ± 1.4	90.5 ^d ± 0.1	87.0 ^d ± 1.9	82.7 ^{cd} ± 0.1	75.6 ^{bc} ± 1.4	48.8 ^e ± 1.4	0.0 ^a ± 0.0
TD5	94.3 ^{ef} ± 0.1	91.0 ^{de} ± 0.3	86.2 ^{bcd} ± 0.4	83.0 ^{def} ± 0.1	76.4 ^{bcd} ± 0.7	52.4 ^f ± 0.7	0.0 ^a ± 0.0
TD6	93.9 ^{de} ± 0.2	91.2 ^d ± 0.3	88.3 ^e ± 0.3	84.7 ^g ± 0.3	78.3 ^{ef} ± 0.3	46.5 ^d ± 0.3	0.3 ^{ab} ± 0.3
TD7	92.7 ^{abc} ± 0.2	89.3 ^{bc} ± 0.1	86.5 ^{cd} ± 0.1	82.3 ^c ± 0.1	76.6 ^{cd} ± 0.1	47.9 ^e ± 0.1	0.1 ^a ± 0.1
TD8	95.8 ^g ± 0.5	94.0 ^g ± 0.5	89.5 ^f ± 0.5	83.0 ^{de} ± 0.6	78.2 ^{ef} ± 0.98	60.0 ⁱ ± 0.9	0.8 ^b ± 0.5
TD9	95.1 ^{fg} ± 0.7	93.2 ^f ± 0.2	89.0 ^{ef} ± 0.2	84.6 ^g ± 0.4	79.5 ^g ± 0.4	62.7 ^j ± 0.4	1.6 ^c ± 0.7
TD10	94.2 ^{ef} ± 0.4	91.5 ^{de} ± 0.3	86.8 ^{cd} ± 0.6	83.5 ^{ef} ± 0.5	76.2 ^{bc} ± 0.2	49.0 ^e ± 0.2	0.0 ^a ± 0.0
TD11	94.0 ^e ± 0.7	91.9 ^e ± 0.4	86.8 ^{cd} ± 0.4	82.5 ^{cd} ± 0.3	77.4 ^{de} ± 0.9	56.5 ^h ± 0.9	0.0 ^a ± 0.0
TD13	92.6 ^{ab} ± 0.2	89.3 ^c ± 0.1	85.8 ^{bc} ± 0.4	81.2 ^b ± 0.1	75.6 ^{bc} ± 0.7	44.2 ^c ± 0.7	0.0 ^a ± 0.0
Ghana	91.8 ^a ± 0.3	88.4 ^a ± 0.1	84.4 ^a ± 0.1	79.0 ^a ± 0.1	72.8 ^a ± 0.2	42.1 ^b ± 0.2	2.8 ^d ± 1.2
Ivory Coast	93.6 ^{cde} ± 0.7	91.2 ^d ± 0.1	86.9 ^d ± 0.7	83.5 ^f ± 0.3	78.2 ^{ef} ± 0.8	56.1 ^h ± 1.4	0.0 ^a ± 0.0
Indonesia	93.0 ^{bcd} ± 0.3	91.0 ^{de} ± 0.5	86.9 ^d ± 0.3	84.3 ^g ± 0.4	78.6 ^{fg} ± 0.2	54.2 ^g ± 0.4	0.0 ^a ± 0.0

Appendix 2: Rheological parameters of the chocolate samples for the downward curve.

Yield stress downwards (Pa)	
TDU	5.97 ± 0.04*
TDC	5.71 ± 0.06*
PGP	4.24 ± 0.03*
TD3/5C	11.83 ± 0.05*
Gh	5.05 ± 0.19

*=significantly different from Ghanaian sample

Viscosity downwards (Pa.s)	
TDU	1.991 ± 0.013*
TDC	1.910 ± 0.010
PGP	2.050 ± 0.013*
TD3/5C	1.695 ± 0.014*
Gh	1.946 ± 0.023

*=significantly different from Ghanaian sample

Name & Surname: _____

nr:

Aim: You are presented with 3 sets of 3 samples. Taste the samples in each set and find the odd (different) one from the three samples. Then, write the codes of these odd samples in the table below.

Set	Odd sample code
1	
2	
3	

Remarks (if any):

Thank you very much for your participation!

Ghent,/...../2013

Dear panelist,

The goal of this session is to teach the procedure for evaluating chocolate and dealing with the scales and constructing consensus on the given sample over all the panelists. For some attributes, references are given.

You have three dark chocolates on the plate in front of you that you should evaluate.

Please follow the procedure closely. Drink water when said and do not hesitate to do the same between the different steps if you think it is necessary.

APPEARANCE

Step 1: Look at the chocolate.

Indicate the degree of the following characteristics.

1. Fat bloom/gloss on the outside of the chocolate.

- 1. Lot of fatbloom, surface almost white, not suitable for consumption.
- 2. Lot of fatbloom, not suitable for consumption.
- 3. Considerable fat bloom. Not suitable for consumption.
- 4. Faint fat bloom. Visible to those who pay attention to it.
- 5. No gloss, but visible fat bloom either.
- 6. Dusty, little or no gloss.
- 7. Acceptable gloss for a dark chocolate.
- 8. Pleasant gloss. Gloss like dark chocolate.
- 9. Very glossy. Perfect chocolate.

AROMA

Step 2: Smell at the broken piece of chocolate (1 half of the chocolate).

Evaluate the intensity of the following aromas.

2. Cocoa smell

1 Not present	2	3	4	5	6	7	8	9 Highly present

3. Acidic smell

1 Not present	2	3	4	5	6	7	8	9 Highly present

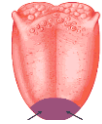
Flavour

Step 3: Place the broken piece (1 half) chocolate on your tongue and let it melt.

Evaluate the intensity of the following flavours. Sweet and bitter are evaluate together.

4. Sweet flavour (on the tip of the tongue)

1 Not present	2	3	4	5	6	7	8	9 Highly present



5. Bitter flavour (at the back of your tongue)

1 Not present	2	3	4	5	6	7	8	9 Highly present



6. Sour flavour (side of the tongue)

1 Not present	2	3	4	5	6	7	8	9 Highly present



7. Cocoa flavour

1 Not present	2	3	4	5	6	7	8	9 Highly present

Step 4: Drink now a sufficient amount of water to wash away the flavour and the remaining chocolate parts.

TEXTURE

Step 5: Bite though the chocolate (1/4 of the total tablet).

Evaluate the intensity of the following texture attributes.

8. Snap (= sound and force necessary to bite trough the chocolate)

1 No snap	2	3	4	5	6	7	8	9 High snap

9. Hardness (= during the chewing/biting on the chocolate)

1 Absolutely not hard	2	3	4	5	6	7	8	9 Very hard

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10. Creaminess

1 Absolutely not creamy	2	3	4	5	6	7	8	9 Very Creamy

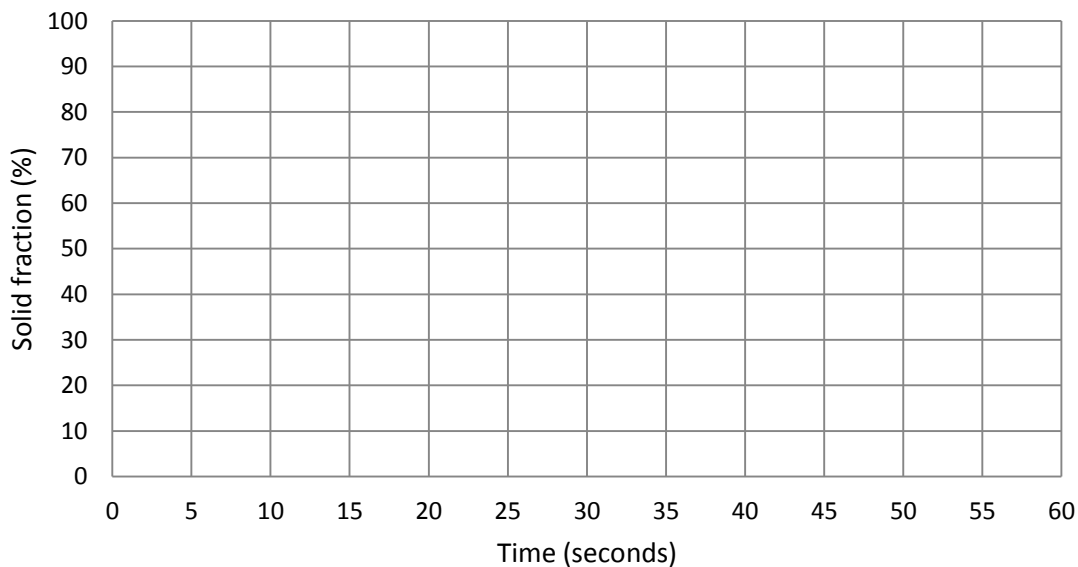
Step 6: Drink now a sufficient amount of water to wash away the flavour and the remaining chocolate parts.

Step 7: Evaluate the melting behaviour of the piece of chocolate (last half piece of chocolate).

This is done by a taking the chocolate in the mouth for 1 minute and touch it slightly with the tongue. Do not bite on the chocolate!!! From the moment that you put the chocolate on your tongue, you need to indicate on the graph every five seconds how you evaluate the hardness off the chocolate.

10. Evaluation of the melting behaviour of the chocolate

Melting behaviour



Step 8: Evaluate the mouthfeel that you experience after evaluating the melting behaviour.

11. Graininess in the mouth = unevenness detected on the tongue ≠ film on the tongue

1 Absolutely not grainy	2	3	4	5	6	7	8	9 Very grainy
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12. Dryness in the mouth: the amount of saliva that is absorbed by the sample during chewing. Give attention to the dryness of the mouth, not the dryness of the sample.

1 Absolutely not dry Butter	2	3	4	5	6	7	8	9 Very dry Rice waffle/Rusk

13. Aftertaste in the mouth

1 Not present Water	2	3	4 Milk	5	6 White wine	7	8	9 Highly present Red wine